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Stereocontrolled Synthesis of β-D-Mannuronic Acid Esters: Synthesis of an Alginate Trisaccharide

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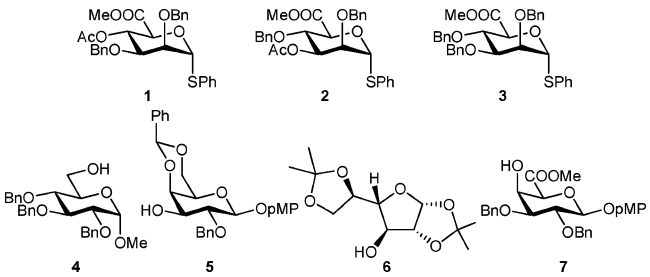
Alginates are linear and polyanionic polysaccharides that occur in Nature and are composed of D-mannuronic acid and L-guluronic acid residues.[1] The uronic acid monomers are interconnected through 1,4-interglycosidic linkages that have a 1,2-cis configuration. Three main alginate polymers have been reported, one containing mainly β-D-mannuronic acid building blocks, one containing mainly α-L-guluronic acid building blocks, and the third composed of alternating sequences of β-D-mannuronic and α-L-guluronic acid motifs. On the basis of their thickening and gel-forming capacities, alginates have found wide and diverse applications as additives in food industry and coating materials.

Recently, it was discovered that mixtures of alginate oligomers have immunomodulating properties by binding to Toll-like receptors (TLRs) 2 and 4.[2] TLRs are key players in mammalian immunity.[3] They recognize molecules, mostly from microbial origin, and instigate activation of inflammatory and antimicrobial innate immune responses. Additionally, recognition of microbial products by TLRs expressed on dendritic cells triggers functional maturation of dendritic cells and leads to initiation of antigen-specific adaptive immune responses. The availability of well-defined fragments of alginates and functionalized derivatives thereof would be highly useful in studying the mechanism of TLR-mediated recognition and signal transduction leading to cytokine production. However, to date, no synthesis of alginate oligomers has been reported.

Both β-D-mannuronic and α-L-guluronic acid oligomers have a 1,2-cis equatorial relationship, which is difficult to attain in a stereocontrolled manner. The group of Crich has found an elegant solution for the introduction of the similarly challenging β-D-mannoside linkage by application of a 4,6-*O*-benzylidene protection at 1-thio and 1-sulfoxide functionalized mannoside donors bearing nonparticipating groups at the O2 and O3 positions.[4,5] Upon activation of these donors, the 4,6-*O*-benzylidene moiety destabilizes the mannosyl oxacarbenium ion relative to the covalent α-mannosyl triflate intermediate.[6] The latter is thought to undergo an S_N2-type displacement with the incoming acceptor. The influence on the reactivity (disarming effect) and the associated stereochemical steering of the benzylidene protective group pattern can be explained by the locking of the mannose C6−O6 bond in the *tg* conformation, leading to torsional strain and more dominant electronic effects.[7] The importance of these electronic effects is further highlighted by the use of nonparticipating, powerfully electron-withdrawing protecting groups for the stereocontrolled introduction of 1,2-cis equatorial glycosidic linkages.[8]

Recently, we disclosed an efficient entry to suitably protected uronic acid donors by the application of 2,2,6,6-tetramethylpiperidinyloxy free radical (TEMPO)/ [bis(acetoxy)iodo]benzene (BAIB), as a regio- and chemoselective oxidation system.[9] The presence of an electron-withdrawing carboxyl moiety at C5 marks such uronic acid donors as highly unreactive. A study toward the glycosylating properties of protected 1-thio glucuronic and galacturonic donors showed that these donors could be applied in a sequential glycosylation strategy in which 1-hydroxyl and 1-thio donors were activated with sulfonium ion based promoter systems.[10] We here demonstrate that 1-thio mannuronic acid esters are viable starting compounds for the construction of β-(1→4)-linked mannuronic acid oligomers.

Table 1. Stereocontrolled Glycosidation of 1-Thio β-Mannuronic Acids Esters Using Ph_2SO/Tf_2O or NIS/TMSOTf



entry	donor	acceptor	Ph_2SO/Tf_2O yield (α/β)	NIS/TMSOTf yield (α/β)	disaccharide
1	**1**	**4**	81% (1/>10)	91% (1/>10)	**8**
2	**2**	**4**	66% (1/0)		**9**
3	**3**	**5**	55% (1/>10)		**10**
4	**1**	**5**	69% (1/>10)	58% (1/>10)	**11**
5	**1**	**6**	71% (1/>10)		**12**
6	**1**	**7**		99% (1/2)	**13**

Thiomannuronic acid derivatives **1**, **2**, and **3** were selected to establish their glycosylating properties in terms of yield and stereoselectivity (Table 1).[11] The 4-*O*-acetyl donor **1** was condensed with primary alcohol acceptor **4** following preactivation of **1** using in situ generated diphenylsulfonium bistriflate at −50 °C and subsequent slow addition of **4**, affording β-linked disaccharide **8** ($^1J_{CH}$ = 156 Hz) in 81% yield. Condensation of the 3-*O*-acetylated donor **2** with acceptor **4** led to α-disaccharide **9** ($^1J_{CH}$ = 175 Hz), whereas condensation of tri-*O*-benzyl donor **3** with the sterically more demanding acceptor **5** furnished β-disaccharide **10** ($^1J_{CH}$ = 159 Hz).[12] The stereochemical outcome of these condensations indicates that the 3-*O*-acetyl moiety may participate through a six-membered transition state, thereby preventing β-side attack.[13] The glycosylating properties of 4-*O*-acetyl donor **1** were further evaluated by executing condensation reactions with the less reactive and therefore more α-directing glycosyl acceptors **5**, **6**, and **7**. The condensations of **1** with **5** and **6** led to the isolation of β-disaccharides **11** (69%) and **12** (71%), respectively. Subjection of the sterically and electronically deactivated acceptor **7** to the glycosylation protocol did not lead to a productive coupling, indicating that the reactivity of the acceptor plays a decisive role on the outcome of a glycosylation.[8c]

The finding that the introduction of 1,2-cis equatorial glycosidic linkages can be attained with different types of donors and promoter systems guided us to explore NIS/TMSOTf[14] mediated glycosylation reactions.[15] In a first attempt, a mixture of donor **1**, acceptor

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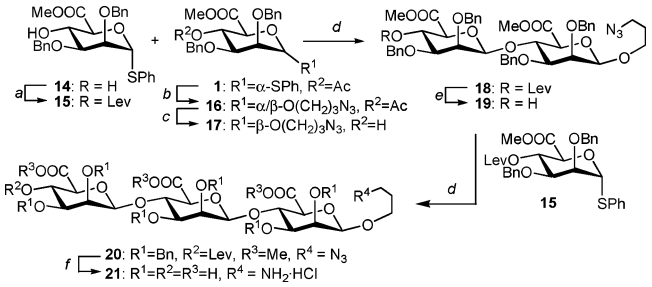
Scheme 1. Synthesis of the Alginate Trisaccharide **21**[a]



[a] Reagents and conditions: (a) Lev_2O, pyridine, rt, 86%; (b) 3-azidopropanol, Ph_2SO, Tf_2O, TTBP, DCM, −75 °C to rt, 80% (α/β = 1/5); (c) cat. NaOMe, MeOH, rt, 60%; (d) NIS, TMSOTf, 0 °C, 2 h, **18**: 78% (α/β = 1/>10), **20**: 50% (α/β = 1/>10); (e) $N_2H_4 \cdot H_2O$, pyridine/AcOH (4/1), rt, 95%; (f) $N_2H_4 \cdot H_2O$, pyridine/AcOH (4/1), rt then KOH, THF/H_2O (1/1) then H_2, Pd(black), MeOH/AcOH (10/1), 35%.

4, and NIS in dichloromethane was cooled to −40 °C and a catalytic amount of TMSOTf was added to afford β-disaccharide **8** in 91% yield. In another experiment, donor **1** was preactivated with NIS and an equimolar amount of TMSOTf.[15a] However, no complete activation of donor **1** could be attained as monitored by TLC analysis. After addition of acceptor **4**, the β-linked disaccharide **8** was isolated in the same yield (91%). These results suggest that, contrary to the Ph_2SO/Tf_2O conditions, the donor is not transformed into an α-triflate intermediate. Presumably, direct S_N2-type displacement of the initially formed iodosulfonium species is at the basis of the observed β-selectivities in the NIS/TMSOTf mediated glycosylation reactions. The standard NIS/TMSOTf protocol was also applied for the condensation of donor **1** and benzylidene protected acceptor **5** to give β-linked disaccharide **11** in 58% yield. Partial cleavage of the acid labile benzylidene functionality explains this moderate yield. Finally, the earlier unproductive coupling of acceptor **7** with donor **1** was executed using NIS/TMSOTf. Disaccharide **13** was isolated in excellent yield as a mixture of anomers (α/β = 1/2), probably as a result of the sterical bias in the acceptor.

The stage was now set for the assembly of the spacer containing mannuronic acid trisaccharide **21** using the NIS/TMSOTf activation system (Scheme 1). Levulinoylation of compound **14** using Lev_2O in pyridine afforded donor **15** in excellent yield. The requisite acceptor **17** was synthesized under Ph_2SO/Tf_2O mediated conditions of **1** with 3-azidopropanol, affording fully protected **16** in 80% yield as an inseparable mixture of anomers.[16] Ensuing basic hydrolysis of the acetate ester using NaOMe/MeOH delivered the target β-linked acceptor **17** in 60% yield. It was found that hydrolysis of the β-anomer **16**β occurred much faster than hydrolysis of the corresponding α-oriented anomer **16**α, leading to facile separation of the anomers. Donor **15** and acceptor **17** were subjected to the NIS/TMSOTf mediated condensation, giving β-linked disaccharide **18** in 78% yield. Standard deprotection of the levulinoyl group using hydrazine hydrate yielded acceptor disaccharide **19**, which was subjected to the same coupling cycle giving trisaccharide **20** in 50% yield. Deprotection of the mannuronic acid trisaccharide **20** was accomplished following levulinoyl deprotection, hydrolysis of the methyl ester, and final reduction of the azide and benzyl groups. HW40 gel filtration afforded deprotected trisaccharide **21** in 35% yield over the three steps.

In summary, a facile synthesis route toward β-linked mannuronic acids is presented. The carboxylic ester function in the mannuronic acid donors sufficiently influences the electronic environment around the anomeric center to allow good to excellent β-selectivities in Ph_2SO/Tf_2O and NIS/TMSOTf mediated glycosylation events. Further research is devoted to the synthesis of extended β-mannuronic acid oligomers, the development of a similar strategy to the assembly of all types of alginate oligomers, and the biological evaluation of these compounds.

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Supporting Information Available: All general procedures, the synthesis and characterizations of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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