Total synthesis of alginate and zwitterionic SP1 oligosaccharides

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Synthetic Alginate oligosaccharides

1.1 Introduction

Alginates are linear polysaccharides composed of D-mannuronic acid and L-guluronic acid residues, which are interconnected by (1→4) glycosidic linkages with a 1,2-*cis* configuration. Alginates are not randomly arranged but consist of segments of similar residues, that are homopolymannuronates (-MM-) and homopolyguluronates (-GG-) or segments with alternating residues (-MG-/-GM-).[1] Alginates are produced by marine brown algae, such as laminaria and by bacteria, such as *Azobacter Vinelandii* and *Pseudomonas aeruginosa*. Alginates have found wide application in food industry for their favourable gelling properties and are applied among others as stabilizer and emulsifiers.[2] Alginates are also considered as interesting biomaterials and are used and investigated for

many biomedical applications. In this context it is important that alginates have shown anti-tumour, antiviral and immunomodulatory properties.[1,3] For instance, a mixture of alginate oligomers, in particular those with high (MM) content, has been shown to stimulate cytokine production from human monocytes, a process in which Toll-like receptors (TLRs) may be involved.[1a,3c,4] To establish structure-activity relations and to elucidate the mechanism of this immunomodulation and other important biological processes in which alginates are involved, well-defined fragments of alginates are needed.[5] Organic synthesis is the most straightforward approach to obtain a coherent row of well-defined alginate fragments in sufficient amounts.

The assembly of alginate oligomers by organic synthesis presents several challenges. Alginates are anionic polysaccharides containing solely uronic acids. The introduction of a carboxylic acid in each residue of the alginates chain can be accomplished following two distinct synthetic strategies; a post-glycosylation oxidation approach or a pre-glycosylation oxidation approach. In the post-glycosylation oxidation approach, the C-5 carboxylic acids are introduced at the oligomer stage by selective removal of all the C-6 hydroxyl protecting groups and subsequent oxidation of the liberated primary alcohols. In this approach, besides the need for an orthogonal protecting group that is stable during the glycosylation events and other (protecting) manipulations en route to the oligomer, the simultaneous oxidation of multiple primary alcohols to carboxylates is a major hurdle to take.[7] In the pre- glycosylation oxidation approach, the oligo/polysaccharide chain is assembled using suitably protected uronic acid building blocks. The presence of the C-5 carboxylic ester in these building blocks makes both donor and acceptor less reactive in the glycosylation reactions. Besides, possible side reactions originating from the presence of the C-5

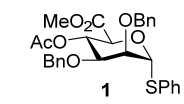
carboxylic ester, such as epimerization and β-elimination can also reduce the yields of the glycosylations.[8] Both approaches share the main challenge to stereoselectively introduce 1,2-*cis* glycosidic linkages.[6] Several methods for the introduction of 1,2-*cis* glycosidic bonds have been developed (see for example the Introductory chapter) and some of these methods have been used for synthesis of alginate oligo/polysaccharides.[9]

1.2 Synthesis of *β*-D-mannuronic acid alginates

The first synthesis of an alginate oligosaccharide, an *β*-D-mannuronic acid trisacharide was reported by Van den Bos *et al*.[10] Three 1-thio-mannuronate esters (donors **1**-**3**, Table 1.1) were selected to establish their glycosylating properties in terms of reactivity and stereoselectivity. Using the pre-activation glycosylation protocol of the group of Crich, both 4-*O*-Ac donor **1** and tri-*O*-Bn donor **3** were coupled with primary and/or secondary alcohol acceptors **4**, **5** and **6** to afford 1,2-*cis* (β) disaccharide products in 55-81% yield with excellent stereoselectivity (Table 1.1, entry 1, 3, 4, 5). It was hypothesized that the anomeric α-triflate was stabilized by the presence of the C5-carboxylic acid ester, and that this species could undergo a S_N2-like substitution to provide the β-products. Contrary, coupling of the 3-*O*-Ac donor **2** with acceptor **4** gave the disaccharide with the opposite anomeric configuration (Table 1.1, entry 2). A possible explanation is the participation of the 3-*O*-Ac moiety thereby shielding attack from the *β*-side. Also, the standard NIS/TMSOTf mediated glycosylation protocol was examined by the coupling of donor **1** with acceptors **4** and **5**, to give the desired disaccharides with excellent stereoselectivity (Table 1.1, entry 6, 7). However, the stereoselectivity of the condensation of donor **1** with galacturonic acceptor **7** was moderate (Table 1.1, entry 8). With this information available,

1-thio-mannuronic acid donor **7** was applied in the synthesis of spacer containing mannuronic acid trisaccharide **13**, using the NIS/TMSOTf mediated glycosylation protocol (Scheme 1.1).

Table 1.1 Glycosylation of 1-thio mannuronic acid donors **1**, **2** and **3**.



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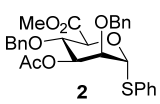

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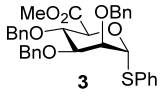

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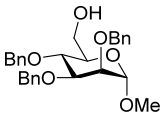

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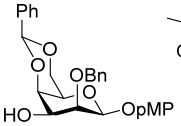

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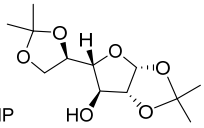

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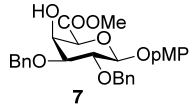
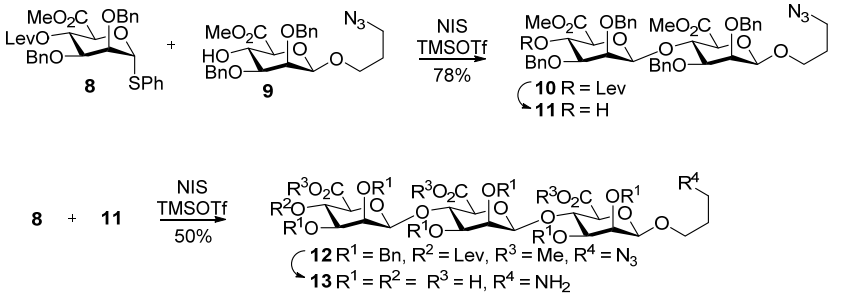


7

entry	donor	acceptor	yield	α/β	entry	donor	acceptor	yield	α/β
1[a]	**1**	**4**	81%	1/>10	5[a]	**1**	**6**	71%	1/>10
2[a]	**2**	**4**	66%	1/0	6[b]	**1**	**4**	91%	1/>10
3[a]	**3**	**5**	55%	1/>10	7[b]	**1**	**5**	58%	1/>10
4[a]	**1**	**5**	69%	1/>10	8[b]	**1**	**7**	99%	1/2

[a] Ph_2SO/Tf_2O; [b] NIS/TMSOTf.

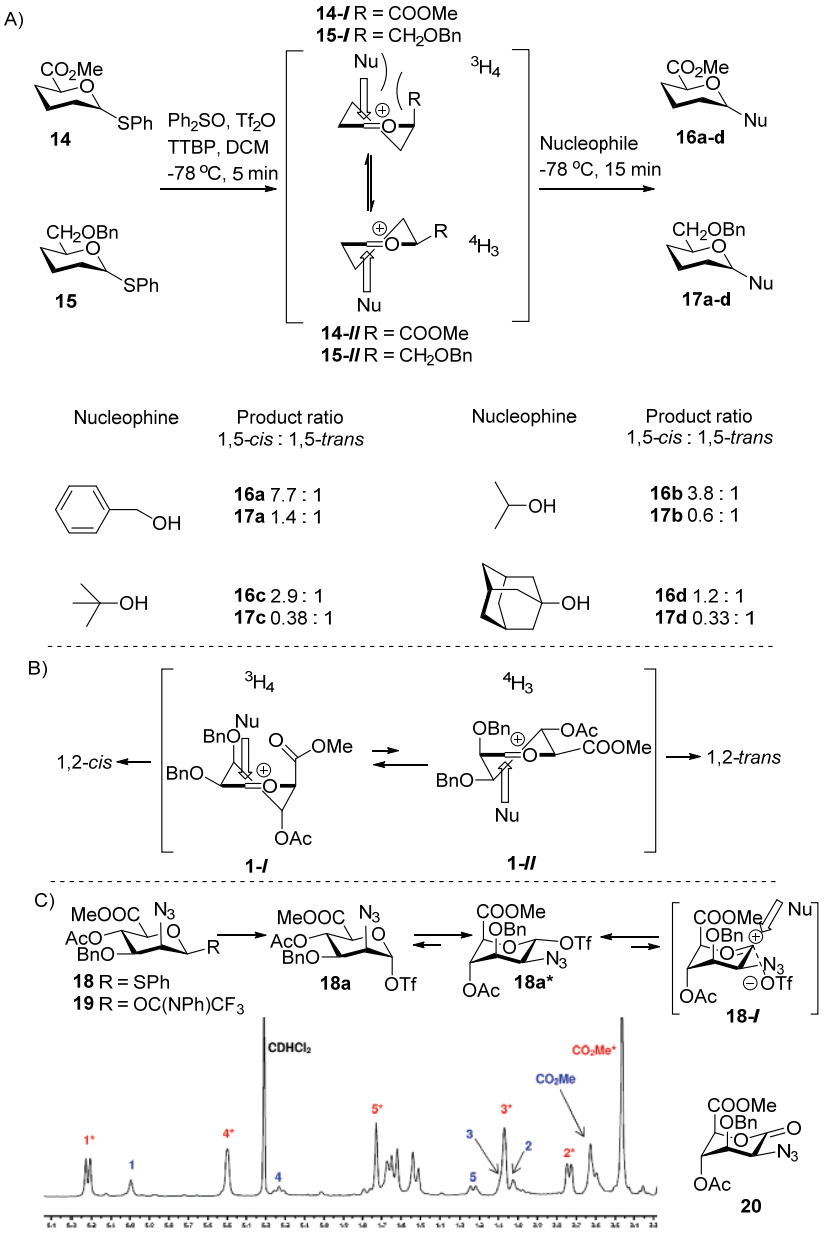
Condensation of 1-thio mannuronic acid donor **8** with acceptor **9** afforded the 1,2-*cis* disaccharide product **10** in 78% yield. Subsequent delevulinoylation delivered disaccharide acceptor **11**, which was coupled with donor **8** to give the all-*cis* trisaccharide **12** in 50%. After global deprotection, target trisaccharide **13** was obtained in 35% yield over the three final steps.



Scheme 1.1 Synthesis of an alginate trisaccharide.

The high stereoselectivity of the mannuronate ester donors to give 1,2-*cis*-linked products initiated several studies to the underlying mechanism.[11] First, it was shown that the stereoselectivity of the glycosylations are independent of the applied donor (thioglycosides, carboxylbenzyl and *N*-phenyl trifluoroacetimidate donors) and the activation protocol, as all condensations proceeded in a 1,2-*cis*-manner.[11a] To establish the stereodirecting effect of the C5-carboxylate ester, the stripped pyranosyl uronate ester donor **14** and its "non-oxidized" counterpart **15** donor were coupled with a range of acceptors of varying size (Scheme 1.2 A). Although the selectivity in the coupling reactions depended on the steric demands of the nucleophile, the highly 1,5-*cis*-directing influence of the C5-carboxylate ester in **14** became obvious. This outcome was explained by postulating that the reaction proceeds via an S_N1-type mechanism, in which two half-chair oxocarbenium ions **14-*I*/15-*I*** and **14-*II*/15-*II*** act as product-forming intermediates. The incoming nucleophile will preferentially attack the half chairs from the face that leads to a chair-like transition state, as opposed to the higher energy twist-boat transition state that would results from attack on the opposite face. The *pseudo*-axially oriented C5-carboxylate ester stabilizes the half-chair oxocarbenium ion intermediate **14-*I*** by through-space

interaction of the electron-rich carbonyl function with the anomeric cation and by minimizing the electron-withdrawing effect of the carboxylate ester. Combining the favorable axial orientation of the C5 carboxylate ester with the preferred orientation of alkoxy substituents at the C2, C3 and C4 positions the most stable half-chair conformer of the mannuronate ester oxacarbenium ion can be estimated. Alkoxy groups at the C3 and C4 preferentially take up axial positions to allow through-space stabilization of the anomeric cation, while C2-alkoxy groups prefer an equatorial position to enable hyperconjugative stabilization by the axial C-H σ bond. On this basis, mannuronate ester oxacarbenium conformer **1-*I*** is much more stable than conformer **1-*II***, because all substituents in the $^{3}H_{4}$ conformer **1-*I*** occupy a preferred position whereas all substituents in the $^{4}H_{3}$ conformer **1-*II*** are in an unfavorable position (Scheme 1.2 B).[11a] Nucleophilic attack on the β-face of the $^{3}H_{4}$ conformer leads to the formation of the 1,2-*cis*-β-mannuronate ester products (Scheme 1.2 B). These findings are supported by low-temperature NMR experiments. Monitoring the activation of mannosazide uronate **18** showed the formation of a conformational mixture of anomeric triflates in which the $^{1}C_{4}$ chair conformer **18a***dominated. Both the electron-withdrawing triflate at C-1 and the methyl ester at C-5 render the anomeric center of the mannuronate triflate so electron-depleted that the structure of the covalent triflate approaches the conformation of the oxacarbenium ion $^{3}H_{4}$ half-chair **18-*I*** (Scheme 1.2 C).[11b,11g] Further support was found in the NMR spectroscopic and X-ray crystallographic analysis of mannuronate lactone **20**, the C-1 of which carries a partial positive charge and which is sp2-hybridized. This lactone adopts a flattened $^{1}C_{4}$ chair, very similar to the $^{3}H_{4}$ half-chair, at room temperature (Scheme 1.2 C).[11b]

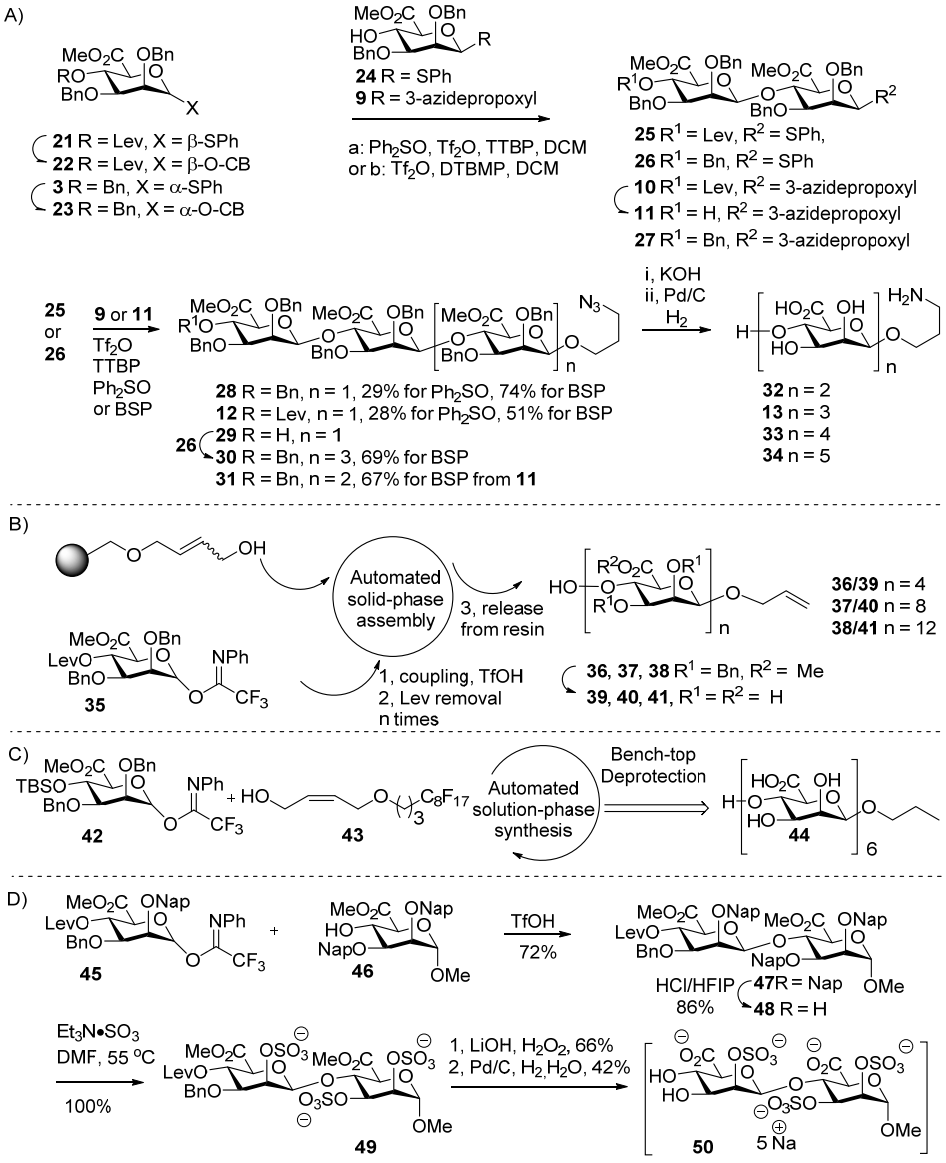


Scheme 1.2 A): Oxocarbenium ions of "stripped" uronate ester **14** and its benzyloxymethyl counterpart **15**; B): the mannuronic acid oxocarbenium ion; C): part of the ^{1}H NMR spectrum obtained after activation of mannuronic acid ester **18** and **19**; and the structure of lactone **20**.

Next, the scope of the method to prepare β-D-mannuronic acid alginate oligosaccharides in solution phase was investigated (Scheme 1.3 A).[11a] In a chemoselective coupling approach, thiomannoside donors **21** and **3** were coupled with thiomannoside acceptor **24** to give disaccharide thiodonors **25** and **26**, respectively. The moderate yield of these glycosylations can be explained by activation of the thiomannosyl acceptor and/or the dimer products. Conversion of thiomannoside donors **21** and **3** into carboxylbenzyl (CB) donors **22** and **23**, allowed for an orthogonal glycosylation approach and the desired disaccharides **25** and **26** were both obtained in 65% yield, with excellent 1,2-*cis*-selectivity. The spacer containing disaccharides **10** and **27** were synthesized in 62% and 67% yield by condensation of **9** with thiomannoside donors **21** and **3**, using the 1-benzenesulfinyl piperidine BSP/Tf_2O couple instead of the Ph_2SO/Tf_2O activator. Removal of the levulinoyl group in dimer **10** provided acceptor **11**. Disaccharide donors **25** and **26** were coupled with monosaccharide acceptor **9** and disaccharide acceptor **11** to yield desired trisaccharides **28** and **12** (74% and 51% yield respectively) and tetrasaccharide **29** (67% yield) with good stereoselectivity again. The pentasaccharide **30** was obtained from the [2+3] coupling in 69% yield and excellent stereoselectivity. Fully protected **27**, **28**, **31** and **30** were subjected to saponification and subsequent hydrogenolysis to give the target di-, tri-, tetra- and pentamer (**32**, **13**, **33** and **34**).

The high 1,2-*cis* selectivity of mannuronate donors was an incentive to explore a solid-phase synthesis. Walvoort *et al*. reported the automated solid-phase synthesis of an alginate tetramer **39**, octamer **40** and dodecamer **41** using monomannuronic acid imidate donor **35.** Three equivalents of donor **35** in combination with TfOH as activator and three repetitive coupling cycles gave an efficiency/yield per coupling step of 90% (Scheme 1.3

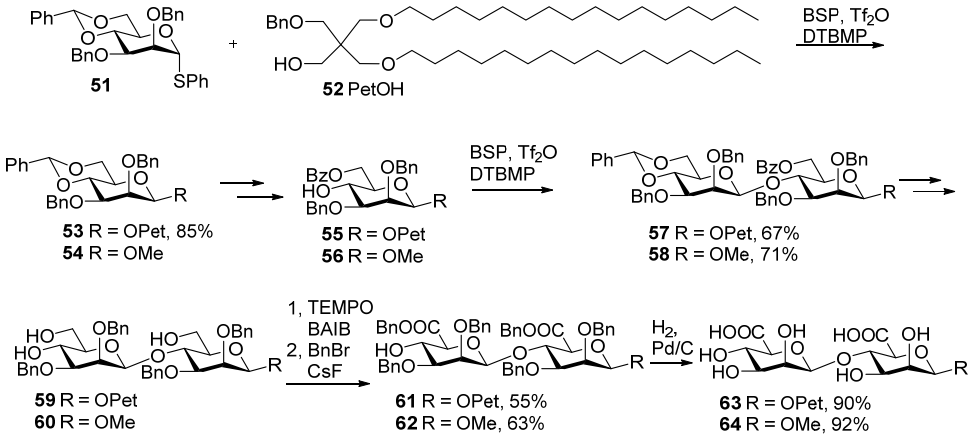
B).[11c] Metathesis mediated cleavage of the oligomers from the solid support led to the isolation of crude reaction mixtures, containing approximately 90%, 55% and 40% of the desired all-*cis*-products of the partially protected alginate tetramer **36**, octamer **37** and dodecamer **38**, respectively.



Scheme 1.3 Synthesis of the alginate oligosaccharides.

Pohl and coworkers synthesized β-D-mannuronic acid alginate oligomers up to the hexasaccharide **44** from monomannuronic acid imidate donor **42** by utilizing a strategy employing fluorous-tag assisting purification in 7% yield over 9 steps (75% average yield per reaction step and 56% efficiency/yield per coupling cycle, see Scheme 1.3C).[11e] Recently, Volbeda *et al.* synthesized a sulfated β-D-mannuronic acid alginate disaccharide **50** by use of mannuronic acid imidate donor **45** (Scheme 1.3D).[11f] The disaccharide **47** was synthesized from donor **45** and acceptor **46** in 72%. The 2-naphthylmethyl (Nap) protecting group was chemoselectively removed by using a new protocol employing a catalytic amount of HCl in hexafluoro-2-propanol. Three sulfate groups were installed using $SO_3 \bullet Et_3N$, and ensuing saponification and final debenzylation gave the desired sulfated β-D-mannuronic acid alginate disaccharide **50**.

Utilizing a post-glycosylation oxidation approach, Huang and Jiang assembled an oligo-β-(1-4)-D-mannuronic acid neoglycolipid (Scheme 1.4).[11h] Building on the stereoselectivity of the benzylidene mannose technology of Crich, **57** and dimer **58** were generated and further functionalized into the neoglycolipid **63** and dimer **64**.



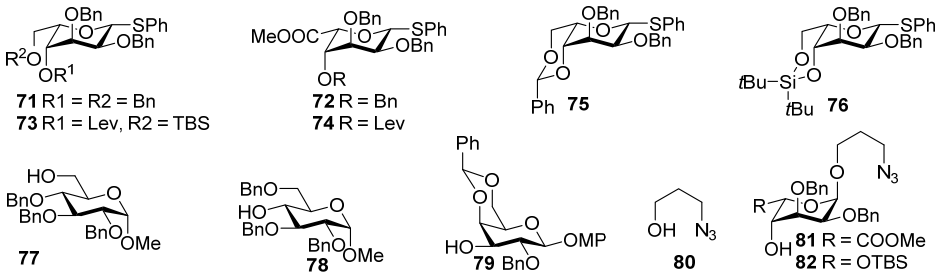
Scheme 1.4 Synthesis of β-(1-4)-oligo-D-mannuronic acid neoglycolipids.

1.3 Synthesis of α-L-guluronic acid alginate

Short oligomers of α-L-guluronic acid alginate have been synthesized by two groups.[12,13] Dinkelaar *et al.* described an evaluation of the glycosylating properties of gulose and guluronate ester donors and the first synthesis of a guluronic acid trimer (Scheme 1.5A and Table 1.2).[12] Key compound β-*S*-phenyl-gulopyranose **70** was obtained from L-gulonic acid γ-lactone **65** on 500 mmol scale (100 g) with only one chromatographic purification. Starting from compound **70**, guluronate donors (**72** and **74**), gulose donors (**71**, **73**, **75** and **76**) and acceptors (**81** and **82**) were obtained (Table 1.2). The glycosylating properties of gluronate donors (**72** and **74**), gulose donors (**71**, **73**, **75** and **76**) were examined using acceptors **77**-**82** (Table 1.2). This study shows that the presence of the carboxylic acid ester at C-5 in the gluronate donors does not contribute favorably to the formation of the 1,2-*cis*-gulosidic linkage. The best α-selectivity was obtained by the 2,3-di-*O*-benzyl-4,6-*O*-silylidine-1-thioguloside donor **76.** It is important to note that the poor nucleophilicity of the hydroxyl group at C-4 in gulose acceptor **82** and especially in guluronic acceptor **81**

reduced the yield of the glycosylations (Table 1.2).

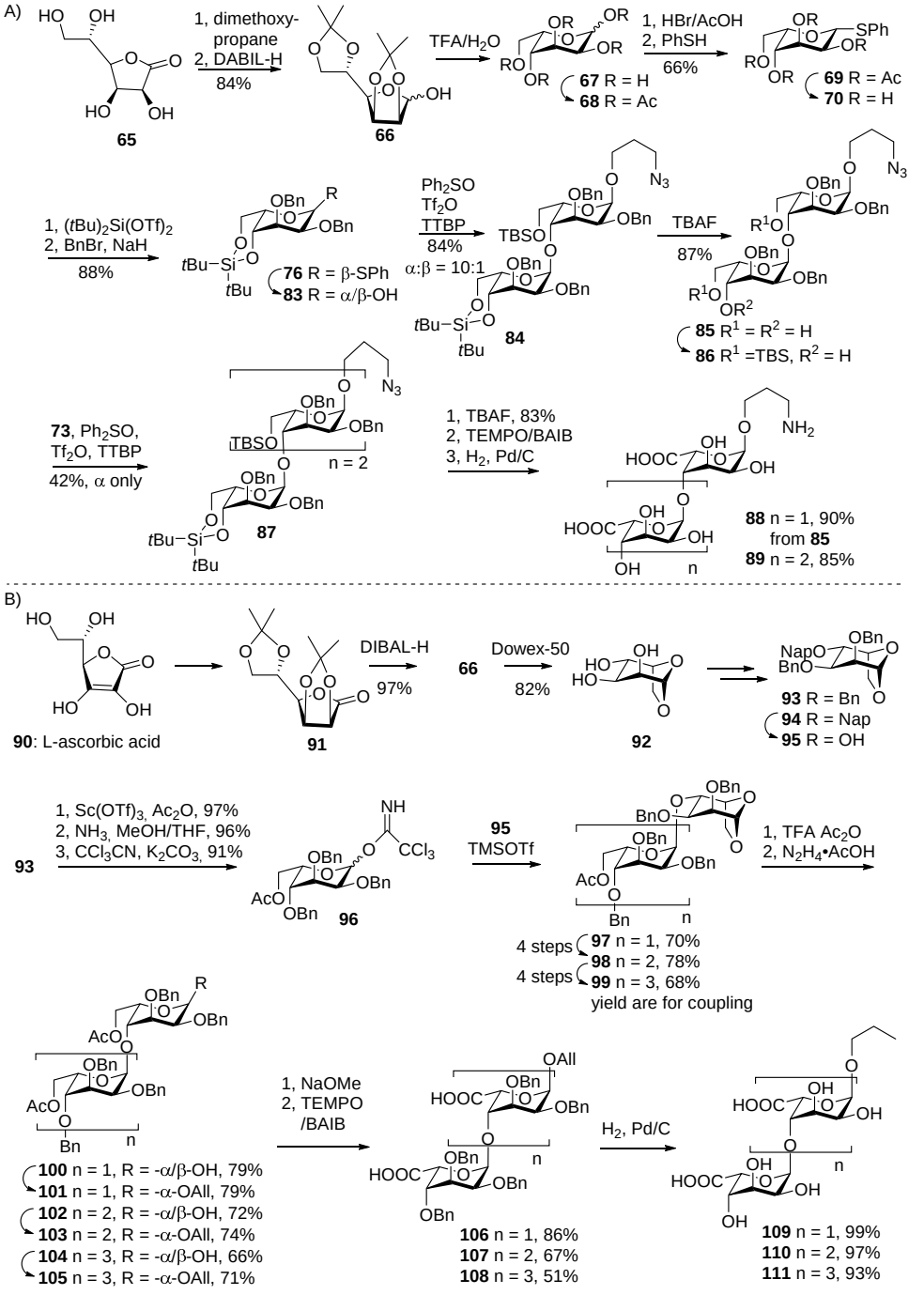
Table 1.2 Glycosylations of guluronate donors (**72** and **74**), gulose donors (**71**, **73**, **75** and **76**).



Entry	Donor	Acceptor	Yield(%)	(α/β)	Entry	Donor	Acceptor	Yield(%)	(α/β)
1	**71**[a]	**77**	71	13:1	9	**74**[b]	**80**	77	1:3
2	**71**[a]	**78**	91	10:1	10	**74**[b]	**81**	34	3:1
3	**71**[a]	**79**	73	>20:1	11	**73**[a]	**80**	86	3:1
4	**72**[b]	**77**	73	3:1	12	**73**[a]	**82**	48	6:1
5	**72**[b]	**78**	94	>20:1	13	**75**[a]	**80**	88	3:1
6	**72**[b]	**79**	79	10:1	14	**75**[a]	**82**	45	6:1
7	**74**[b]	**77**	66	1:3	15	**76**[a]	**80**	75	5:1
8	**74**[b]	**78**	64	>20:1	16	**76**[a]	**82**	48	10:1

[a] Ph_2SO, TTBP, CH_2Cl_2, -78 °C, Tf_2O, 10 min, nucleophile, to 0 °C. [b] Ph_2SO, TTBP, CH_2Cl_2, -45 °C, Tf_2O, 10 min, then -78 °C, nucleophile, to 0 °C.

It was decided to use a dehydrative condensation strategy because this glycosylation approach is well suited to couple unreactive nucleophiles. Hemiacetal donor **83**, prepared by hydrolysis of thiogulose **76**, was coupled with acceptor **82** to yield disaccharide **84** with excellent α-selectivity (Scheme 1.5A). Removal of the 4,6-*O*-silylidine group and protection of C6-hydroxyl gave disaccharide acceptor **86**. Subsequent dehydrative coupling of **86** with hemiacetal donor **83** afforded α-trisaccharide **87** with excellent stereoselectivity but in a moderate 42% yield. Having fully protected dimer **86** and trimer **87** available the target *α*-L-guluronic acid alginate fragments **88** and **89** were prepared by desilylation, subsequent selective oxidation of primary C-6 hydroxyl and final hydrogenolysis.[12]



Scheme 1.5. Synthesis of α-L-guluronic acid alginate.

Hung and coworkers circumvented the low nucleophilicity of the gulose acceptors by the application of 1,6-anhydro-*β*-L-gulopyranosyl-4-alcohol **95**, which places the C-4 hydroxyl in a more accessible position. Acceptor **95** was synthesized from L-ascorbic acid **90** in an efficient way as depicted in Scheme 1.5B.[13] Trichloroacetimidate donor **96** was generated from tri-benzyl 1,6-anhydro-*β*-L-gulopyranose **93** by sequential acetolysis, anomeric deacetylation and trichloroacetimidation. Condensation of **96** with acceptor **95** afforded the expected disaccharide **97** in 70% yield (α:β = 4:1). Conversion of **97** into hemiacetal **100** was achieved by cleavage of the 1,6-anhydro ring in **97** with TFA and Ac_2O followed by anomeric deacetylation with N_2H_4·HOAc. Coupling of the the anomeric hydroxyl in **86** with allyl bromide via Williamson etherification using potassium tert-butoxide as a base furnished the α-linked dimer **101** (79%) as a single product. Contrary, condensation of the imidate donor, obtained by trichloroacetimidation of **100**, with allyl alcohol yielded 8% of the desired α-dimer **101** and 68% of the undesired β-dimer. The synthesis of fully protected trimer **103** and tetramer **105** was completed by the same sequence of events (elongation with donor **96**, 1,6-anhydro ring opening, O1-deacetylation and O1-allylation). Finally, the target *α*-L-guluronic acid alginate dimer **109**, trimer **110** and tetramer **111** were obtained from compounds **101**, **103**, and **105** by deacetylation, oxidation of primary alcohol and hydrogenolysis.[13]

1.4 Conclusion

β-D-mannuronic acid alginate oligosaccharides up to the dodecasaccharide were assembled by use of newly developed mannuronate ester donors. Glycosylations using these mannuronate ester donors proceed with excellent 1,2-*cis*-selectivity both in solution and automated solid phase syntheses. With the objective to explain the origin of this 1,2-

cis-selectivity details of the underlying reaction mechanism have been elucidated. Whereas a pre-glycosylation oxidation approach is the most efficient in the construction of mannuronic acid alginate oligosaccharides, α-L-guluronic acid alginate oligosaccharides are best accessible by a post-glycosylation oxidation approach. Two successful strategies have been developed. One comprises elongation from the non-reducing to the reducing end using 1,6-anhydro-β-L-gulopyranosyl-4-alcohol as a repeating glycosyl acceptor and the other one comprises elongation from the reducing to the non-reducing end using a 2,3-di-*O*-Bn-4,6-*O*-silylidene hemiacetal gulose donor. In chapter 4 of this thesis the first synthesis of mixed-sequence alginate oligosaccharides, featuring both β-D-mannuronic acids (M) and α-L-guluronic acid (G), is described.

1.5 References

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