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Synthesis of Alginate Oligosaccharides Containing L-Guluronic Acids

Fa-Chen Chi,[a] Suvarn S. Kulkarni,[c] Medel Manuel L. Zulueta,[a, d] and Shang-Cheng Hung*[a, b]

Dedicated to Prof. Chun-Chen Liao on the occasion of his 65th birthday and retirement

Alginates are linear unbranched binary polymers, composed of β-D-mannuronic acid (M) and its C5 epimer, α-L-guluronic acid (G). The monomer units are covalently linked together through (1→4)-glycosidic bonds in different sequences -MMMM- (**1**), -MGMG- (**2**), or -GGGG- (**3**, Figure 1).[1] These nontoxic polysaccharides can be isolated from marine brown algae (phaeophyceae) or from different bacteria belonging to the genera Azotobacter and Pseudomonas.[2] Bacterial alginates are additionally acetylated at the O2 and/or O3 positions of the D-mannuronic acid residues.[3] Alginates exhibit potent biological properties including stimulation of growth factors,[4] antitumor[5] and antiviral activities,[6] and immunomodulation through binding with Toll-like receptors in mammalian systems.[7] Their O-sulfonated derivatives display anticoagulant activity.[8] The G-rich alginates can be used for encapsulation of cells and enzymes,[9] and islets of Langerhans immobilized in G-rich alginates have been evaluated as a potential treatment for type-1 diabetes.[10] Besides, alginates have gel-forming properties,[11] and they are widely used in the food industry[12] and medical dressings.[13] Since the structure–activity relationship of alginates remains unclear, procurement of chemically well-defined oligomers is highly desired. To date, a synthesis of an alginate-related trisaccharide containing all β-mannuronic acids has been reported.[14] In continuation with our efforts in the applications of L-form 1,6-anhydrohexopyranoses to the synthesis of biologically potent oligosaccharides and natural products,[15] we describe herein a concise route employing 1,6-anhydro-β-L-gulopyranoses as key synthons to prepare alginate-related di-, tri-, and tetrasaccharides consisting of G blocks with all α1→4-linkages. A strategy towards the synthesis of an MG disaccharide skeleton is also presented.

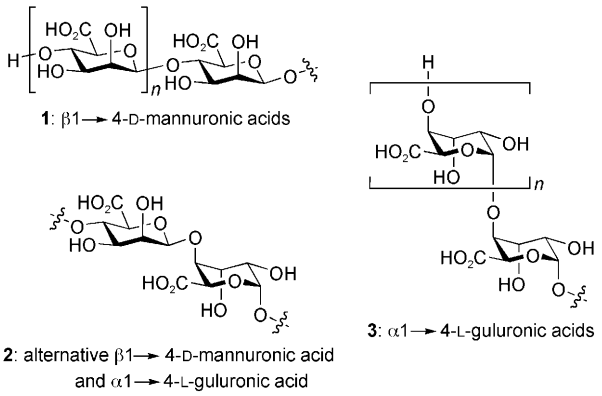

Figure 1. The structures of naturally occurring alginates.

The major challenges for preparing alginate oligosaccharides include the generation of L-gulopyranosyl sugars,[16] the stereoselective formation of a 1,2-*cis*-α-glycosidic bond, and the choice of an appropriate protecting scheme for chain elongation as well as functional-group transformation. As illustrated in Scheme 1, a retrosynthetic plan of the target -GGGG- oligomer **3** revealed two plausible approaches, to construct the sugar chains either "from the reducing end to the nonreducing end" or vice versa. The first route entails Schmidt's glycosylation[17] of an orthogonally protected L-gu-

[a] Dr. F.-C. Chi, M. M. L. Zulueta, Prof. Dr. S.-C. Hung
Department of Chemistry
National Tsing Hua University
101, Section 2, Kuang-Fu Road, Hsinchu 300 (Taiwan)
Fax: (+886) 3-5711082
E-mail: hung@mx.nthu.edu.tw

[b] Prof. Dr. S.-C. Hung
Genomics Research Center
Academia Sinica
128, Section 2, Academia Road, Taipei 115 (Taiwan)

[c] Prof. Dr. S. S. Kulkarni
Department of Organic Chemistry
Indian Association for the Cultivation of Science
Jadavpur, Kolkata 700032 (India)

[d] M. M. L. Zulueta
Institute of Chemistry
University of the Philippines
Diliman, Quezon City 1101 (Philippines)

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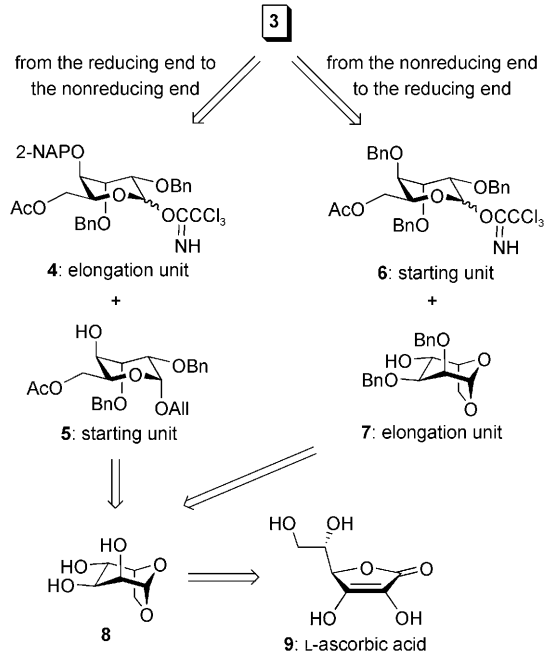
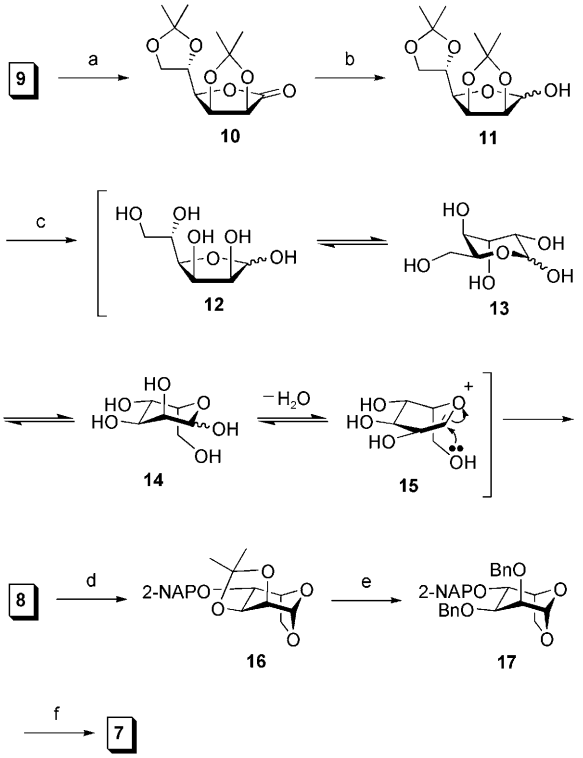


Scheme 1. Retrosynthesis of alginate oligosaccharides.

lopyranosyl trichloroacetimidate **4** (an elongation unit) with the 4-alcohol **5** (a starting unit) to obtain a key GG disaccharide, which could be elaborated by a regioselective O4′-deprotection–glycosylation sequence to give the oligosaccharides with different chain length. Conversely, by the other approach, the O4-differentiated starting unit **6** could be coupled with the 1,6-anhydro-β-L-gulopyranosyl 4-alcohol **7** (an elongation unit) to afford a GG disaccharide that could be extended through consecutive opening of the 1,6-anhydro ring followed by anomeric deprotection–coupling iterations. A temporary protection, the 2-naphthylmethyl group (2-NAP),[18] is needed to mask the 4-hydroxy group in **4** that can be deprotected prior to coupling cycle. In addition, the acetyl group is required to block the primary hydroxy group, which could be oxidized to the corresponding carboxylic acid at the later stage. The installation of the desired α-glycosidic linkage would be ascertained owing to the anomeric effect and the nonparticipating nature of the O2-benzyl group. The L-gulo compounds **4**–**7** could be synthesized from a common intermediate, 1,6-anhydro-β-L-gulopyranose (**8**), which is in turn accessible from abundantly available L-ascorbic acid (**9**).

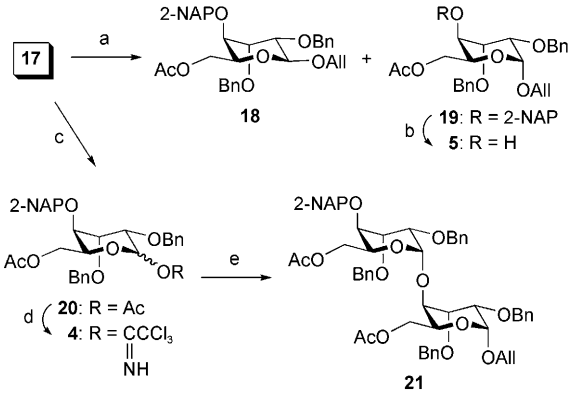
An efficient synthesis of the 1,6-anhydro-β-L-gulopyranosyl sugars **8** and **7** is described in Scheme 2. The L-gulonic γ-lactone **10**, generated from L-ascobic acid **9** by hydrogenation of the C=C bond followed by 2,3:5,6-di-O-isopropylidenation in two steps,[19] underwent DIBAL-H reduction to give the furanosyl lactol **11** in 97% yield. Acidic hydrolysis of compound **11** in a mixture of water and diglyme at 145°C furnished 1,6-anhydro-β-L-gulopyranose **8** in 82% yield. The absolute configuration of **8** was determined by an X-ray diffraction analysis of its single crystal.[20] The reaction probably proceeds through the removal of isopropylidene groups



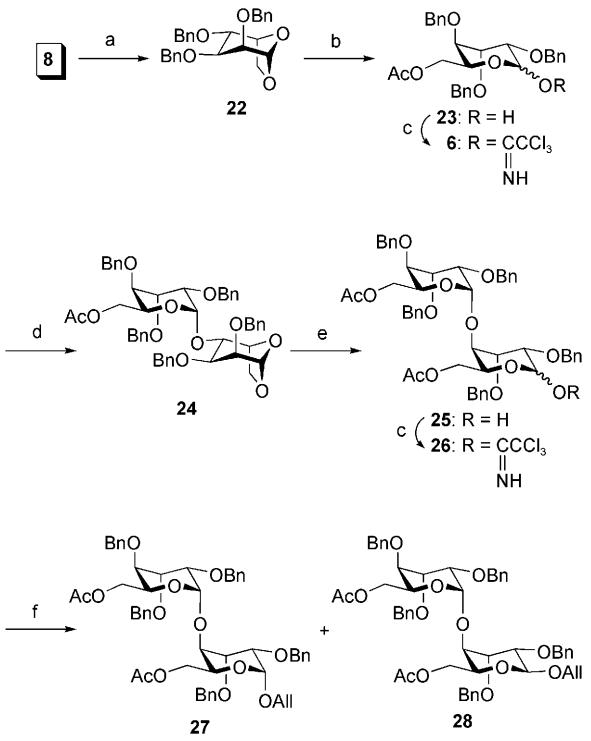
Scheme 2. Reagents and conditions: a) see reference [19]; b) 1. DIBAL-H, THF, −78°C, 40 min, 97%; c) Dowex-50 acidic resin, H_2O, diglyme, 145°C, 5 h, 82%; d) 1. $Me_2C(OMe)_2$, cat. CSA, RT, 20 h, 82%; 2. NaH, 2-$C_{10}H_7CH_2Br$, DMF, 0°C→RT, 14 h, 99%; e) 1. 70% $AcOH_{(aq)}$, 70°C, 16 h, 99%; 2. NaH, BnBr, DMF, 0°C→RT, 2 d, 94%; f) DDQ, CH_2Cl_2/H_2O = 9:1, RT, 2 h, 81%. DIBAL-H = diisobutylaluminum hydride, CSA = camphorsulfonic acid, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

in **11** to give L-gulose in the furanosyl form **12**, which equilibrates with the pyranosyl forms **13** and **14**. The intermediate **14** undergoes elimination of a water molecule under acidic conditions at high temperature to provide the oxocarbenium ion **15**, which can be intramolecularly attacked by the C6-hydroxy group to furnish the triol **8**. Transformation of **8** into the ketal **16** was carried out through consecutive 2,3-O-isopropylidenation (82%) and O4-etherification (99%) in two steps. Acid hydrolysis of **16** yielded the corresponding 2,3-diol (99%), which was di-O-benzylated to provide the ether **17** (94%). Removal of the 2-NAP group in **17** with DDQ afforded the 4-alcohol **7** in 81% yield.

With the 1,6-anhydro-β-L-gulopyranosyl synthons in hand, we first explored the coupling of the sugar chain from the reducing end to the nonreducing end. The synthesis of alginate disaccharide skeleton **21** is depicted in Scheme 3. One-pot $Sc(OTf)_3$-catalyzed acetolysis of compound **17** followed by $BF_3{\cdot}OEt_2$-promoted anomeric coupling with allyl alcohol gave the β-linked product **18** and its α isomer **19** in 37% and 41% yields, respectively. The structural identification of both C1 epimers **18** and **19** was carried out through a series of NMR spectral experiments (see the Supporting Information). The $J_{1,2}$ coupling constant of the former was 8.0 Hz, whereas that of the latter was 3.4 Hz. This fact indicated



Scheme 3. Reagents and conditions: a) cat. $Sc(OTf)_3$, Ac_2O, 0 °C, 5 h, then $H_2C{=}CHCH_2OH$, $BF_3{\cdot}OEt_2$, 3 Å M.S., −16 °C, 18 h, **18**: 37 %, **19**: 41 %; b) DDQ, CH_2Cl_2/H_2O = 18:1, RT, 2 h, 78 %; c) TFA, Ac_2O, 0 °C→RT, 24 h, 97 %; d) 1. $NH_{3(g)}$, MeOH/THF = 1:5, 0 °C, 18 h, 91 %; 2. CCl_3CN, K_2CO_3, CH_2Cl_2, RT, 14 h, 89 %; e) AgOTf, **5**, CH_2Cl_2, −78 °C, 1 h, 14 %. M.S. = molecular sieves, TFA = trifluoroacetic acid.



Scheme 4. Reagents and conditions: a) NaH, BnBr, DMF, 0 °C→RT, 15 h, 96 %; b) 1. $Sc(OTf)_3$, Ac_2O, RT, 2.5 h, 97 %; 2. $NH_{3(g)}$, MeOH/THF = 1:4, 0 °C, 18 h, 96 %; c) CCl_3CN, K_2CO_3, CH_2Cl_2, RT, 5 h, **6**: 91 %, **26**, 89 %; d) cat. TMSOTf, **7**, 4 Å M.S., CH_2Cl_2, −86 °C, 1 h, **24**: 70 %, **24β**: 17 %; e) 1. TFA, Ac_2O, 0 °C, 16 h; 2. $H_2NNH_2{\cdot}AcOH$, DMF, 0 °C→RT, 6 h, 79 % in two steps; f) *t*BuOK, $H_2C{=}CHCH_2Br$, *t*BuOH, 0 °C, 2 h, **27**: 79 %. TMSOTf = trimethylsilyl trifluoromethanesulfonate.

that the H1 and H2 protons of compound **18** possess a *trans*-diaxial relationship. The NOESY spectrum of **18** exhibited a through-space cross-interaction of the H1 proton with the H5 proton, but not with the H2 proton, confirming that the glycosidic bond of **18** is β-linked. The opposite phenomenon, observed in the NOESY spectrum of **19**, showed the stereochemistry to be the α form. These spectral techniques were used to characterize the α or β configuration of the newly formed glycosidic bonds throughout the study. Treatment of compound **19** with DDQ yielded the desired 4-alcohol **5** (78 %), which could be applied as a glycosyl acceptor. For the preparation of the glycosyl donor **4**, three steps were needed for the conversion from the ether **17**. Treatment of **17** under acetolysis conditions afforded the 1,6-diacetate **20** in excellent yield (97 %). Regioselective O1-deacetylation of **20** with ammonia led to the lactol (91 %), which was treated with trichloroacetonitrile and potassium carbonate to furnish the expected product **4** in 89 % yield. To our dismay, TMSOTf-activated coupling of compound **4** with the 4-alcohol **5** failed to give the desired disaccharide **21**, whereas AgOTf as a promoter provided **21** in low yield (14 %), presumably owing to the steric barrier imposed by the axial 4-hydroxy group of **5**.

Alternatively, the other strategy, "from the nonreducing end to the reducing end" using the 1,6-anhydro-β-L-gulopyranose **7** as a repeating glycosyl acceptor, was further investigated. In comparison with compound **5**, the reactivity of the 4-hydroxy group in the rigid bicyclo[3.2.1] system of **7** is enhanced by conformational switching of the pyranosyl ring, thereby changing the orientation of the 4-hydroxy group from the axial to equatorial position.[15c] As summarized in Scheme 4, benzylation of the triol **8** yielded the corresponding ether **22** (96 %), which was subjected to sequential acetolysis (97 %) and O1-deacetylation (96 %) to provide the 1-alcohol **23**. Trichloroacetimidation of **23** led to the donor **6** (91 %), which was coupled with the 4-alcohol **7** in the presence of TMSOTf to afford the expected α disaccharide **24** ($J_{1',2'}$ = 3.4 Hz) and its β isomer ($J_{1',2'}$ = 8.0 Hz) in 70 % and 17 % yields, respectively. Cleavage of the 1,6-anhydro ring in **24** with TFA and Ac_2O followed by removal of the O1-acetyl group with $H_2NNH_2{\cdot}HOAc$ gave the corresponding lactol **25** (79 %, α/β = 1:1) in two steps. Initial attempts for allylation of the imidate **26**, prepared from **25** in 89 % yield, with allyl alcohol employing TMSOTf as the catalyst unfortunately provided the desired α form compound **27** (8 %, $J_{1,2}$ = 3.8 Hz) in a low yield along with the major β isomer **28** (68 %). In sharp contrast, coupling of the 1-alcohol **25** with allyl bromide via Williamson etherification using potassium *tert*-butoxide as a base furnished the α-linked molecule **27** (79 %) as a single product. It should be noted that the acetyl groups remained unaffected under these basic conditions. The high stereoselectivity is perhaps induced by the chelation effect of potassium cation with C1-alkoxide and the lone-pair electrons of O2, preferring the 1,2-*cis* configuration.[21]

Scheme 5 delineates the chain elongation of the GG disaccharide and deprotection sequences to obtain alginate oligosaccharides. TMSOTf-catalyzed coupling of the imidate donor **26** with the 4-alcohol **7** afforded the α-linked trisaccharide **29** (78 %, $J_{1',2'}$ = 3.3 Hz) exclusively. Acetolysis of **29** followed by anomeric deacetylation led to the trisaccharide hemiacetal **30** (72 % overall yield in two steps), which was

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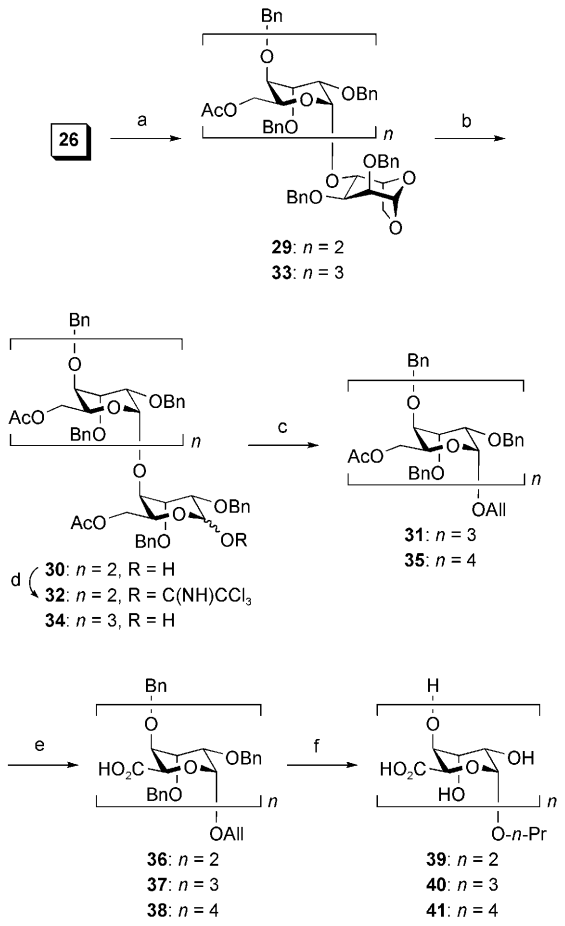
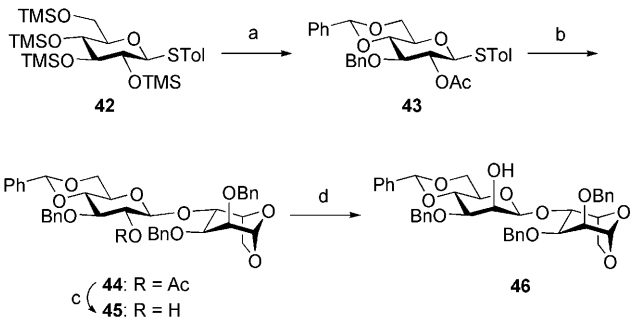


Scheme 5. Reagents and conditions: a) cat. TMSOTf, **7**, 4 Å M.S., CH_2Cl_2, −86 °C, 3 h, **29**: 78 %, **33**: 68 %; b) 1. TFA, Ac_2O, 0 °C, 16 h; 2. $H_2NNH_2{\cdot}AcOH$, DMF, 0 °C→RT, 6 h, **30**: 72 %, **34**: 66 % in two steps; c) *t*BuOK, $H_2C{=}CHCH_2Br$, *t*BuOH, 0 °C→RT, 2 h, **31**: 74 %, **35**: 71 %; d) CCl_3CN, K_2CO_3, CH_2Cl_2, RT , 36 h, 89 %; e) 1. NaOMe, MeOH, RT, 2 h; 2. TEMPO, BAIB, CH_2Cl_2/H_2O = 2:1, RT, 1 h, **36**: 86 %; **37**: 67 %; **38**: 51 % in two steps; f) H_2, 10 % Pd/C, MeOH/H_2O/AcOH = 7:3:1, RT, 12 h, **39**: 99 %; **40**: 97 %; **41**: 93 %. TEMPO = 2,2,6,6-tetra-methyl-1-piperidinyloxy free radical, BAIB = *bis*-acetyloxyiodobenzene.

similarly O-allylated to yield the single α-linked derivative **31** (74 %, $J_{1,2}$ = 3.5 Hz). Likewise, a five-step reiteration starting from the 1-alcohol **30** through the imidate formation (**32**, 89 %), sugar coupling (**33**, 68 %, $J_{1',2'}$ = 1.0 Hz, a sole isomer), 1,6-anhydro ring opening, O1-deacetylation (**34**, 66 % in two steps), and O1-allylation (71 %) provided the expected single tetrasaccharide **35** ($J_{1,2}$ = 3.7 Hz) with all α-glycosidic bonds. Cleavage of the acetyl groups in compounds **27, 31**, and **35** gave the individual primary alcohols, which underwent TEMPO oxidation[22] to furnish the corresponding carboxylic acids **36**–**38**[23] in 86 %, 67 %, and 51 % yields (in two steps), respectively. Global deprotection under hydrogenolysis conditions followed by purification on a Sephadex G25 column afforded the target molecules **39**–**41** in 99 %, 97 %, and 93 % yields, respectively.

The synthesis of a key MG-alginate disaccharide building block is illustrated in Scheme 6. Regioselective one-pot protection of the per-O-trimethylsilylated thioglucoside **42** by



Scheme 6. Reagents and conditions: a) cat. TMSOTf, PhCHO, 3 Å M.S., CH_2Cl_2, −86 °C, 2 h, then Et_3SiH, −86 °C, 5 h, then Ac_2O, −86 °C→0 °C, overnight, 78 % overall yield; b) **7**, DMTST, CH_2Cl_2, 3 Å M.S., 0 °C, 11.5 h, 84 %; c) NaOMe, MeOH, RT, 12 h, 94 %; d) 1. Tf_2O, pyridine, 0 °C→RT, 8 h, 89 %; 2. $NaNO_2$, [15]crown-5, HMPA, RT, 18 h, 62 %. TBAF = tetra-*n*-butylammonium fluoride, DMTST = dimethyl(thiomethyl)sulfonium trifluoromethanesulfonate, Tf_2O = trifluoromethanesulfonic anhydride.

tandem 4,6-O-benzylidenation, 3-O-benzylation, and 2-O-acetylation furnished the glycosyl donor **43** in 78 % yield.[24] DMTST-promoted coupling of **43** with the 4-alcohol **7** cleanly afforded the β-form disaccharide **44** (84 %, $J_{1',2'}$ = 7.8 Hz), which was subjected to deacetylation to give the 2′-alcohol **45** in 94 % yield. A two-step inversion of compound **45** through triflation (89 %) followed by S_N2 substitution with sodium nitrite (62 %) was carried out to obtain the β-mannoside **46** as a single isomer.

In summary, we have developed a facile synthesis of 1,6-anhydro-β-L-gulopyranoses and applied these synthons to prepare the G-linked alginate oligosaccharides **39**–**41** in excellent α selectivity by assembly of sugar chain from the nonreducing end to the reducing end. Using the same glycosyl acceptor, a concise strategy for synthesizing the MG-linked disaccharide **46** is also established. This key disaccharide is fully equipped with appropriate protecting groups for chain elongation and final functional-group transformation that can lead to the -MGMG- series of alginate oligomers.

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