Oligosaccharides

Deutsche Ausgabe: DOI: 10.1002/ange.201502581
Internationale Ausgabe: DOI: 10.1002/anie.201502581

Acceptor Reactivity in the Total Synthesis of Alginate Fragments Containing α-L-Guluronic Acid and β-D-Mannuronic Acid**

*Qingju Zhang, Erwin R. van Rijssel, Marthe T. C. Walvoort, Herman S. Overkleeft, Gijsbert A. van der Marel, and Jeroen D. C. Codée**

Abstract: *The total synthesis of mixed-sequence alginate oligosaccharides, featuring both β-D-mannuronic acid (M) and α-L-guluronic acid (G), is reported for the first time. A set of GM, GMG, GMGM, GMGMG, GMGMGM, GMGMGMG, and GMGGMG alginates was assembled using GM building blocks, having a guluronic acid acceptor part and a mannuronic acid donor side to allow the fully stereoselective construction of the cis-glycosidic linkages. It was found that the nature of the reducing-end anomeric center, which is ten atoms away from the reacting alcohol group in the key disaccharide acceptor, had a tremendous effect on the efficiency with which the building blocks were united. This chiral center determines the overall shape of the acceptor and it is revealed that the conformational flexibility of the acceptor is an all-important factor in determining the outcome of a glycosylation reaction.*

Alginates are naturally occurring anionic polysaccharides, and are composed of 1,2-*cis*-linked D-mannuronic acid (M) and L-guluronic acid (G, the C5 epimer of M) residues which are arranged in either homopolymer (polymannuronate -MM-, or polyguluronate -GG-) or heteropolymer -MG- segments[1] (Figure 1).[2] They are found in marine brown algae and various bacteria, including *Pseudomonas aeruginosa*, and have found wide application in the biomaterial and food industry because of their gelling properties.[3] Notably, they have also received attention because of their putative antitumor, antiviral, antigenic, and immunomodulatory activity.[2,4] To firmly establish structure–activity relationships for this class of compounds, well-defined single molecules of a defined length are indispensable.[5] In this framework we have previously reported the fully stereoselective assembly of -MM- fragments employing mannuronic acid donor glycosides for the construction of the β-D-mannosidic linkages.[6] By using an automated solid-phase approach we generated D-mannuronic acid alginate fragments up to the dodecamer level.[7] We, as well as Hung and co-workers, have furthermore described the synthesis of short L-guluronic acid oligomers.[8,9]

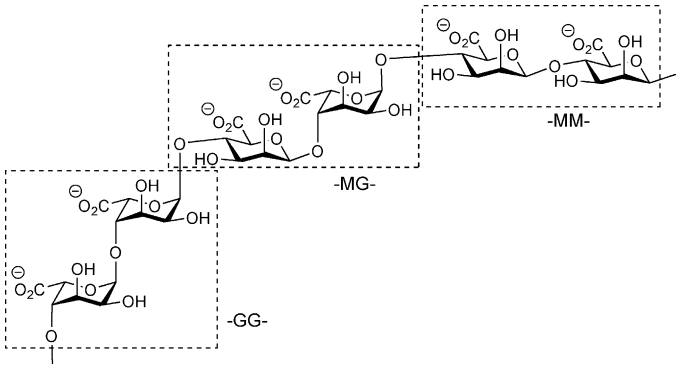


Figure 1. Alginates are composed of -GG-, -MM-, and -MG- blocks.

The assembly of mixed alginate sequences, containing both M and G residues has never been achieved and is particularly challenging because it requires the construction of both β-D-mannuronic acid and α-L-guluronic acid linkages. While D-mannuronic acid donor glycosides can be used for the stereoselective construction of *cis*-glycosidic linkages, L-guluronic acid donors are less stereoselective in glycosylation reactions.[8,10] In addition the guluronic acid C4 hydroxy group is a very poor nucleophile. Hung and co-workers employed 1,6-anhydrogulose synthons to lock the C4–OH in a more accessible environment to increase the reactivity of the alcohol.[9] We herein report the first fully stereoselective synthesis of a set of GM alginate oligosaccharides, thus making these available for the first time for biochemical investigation. The described syntheses reveal that conformational flexibility of the nucleophile can be an all-important factor for the outcome of a glycosylation reaction.

Various approaches can be envisioned for the construction of mixed-sequence alginate oligomers using either monomeric or GM or MG dimer building blocks in a pre-glycosylation oxidation or post-glycosylation oxidation approach.[11] Because of the high fidelity of mannuronic acid donor synthons in the construction of β-mannosidic linkages we opted for an approach using GM building blocks, thus featuring a mannuronic acid donor part. To minimize functional-group manipulation at a late stage of the syntheses we implemented a guluronic acid acceptor part (as opposed to the use of a gulose acceptor) in the GM building blocks.[12]

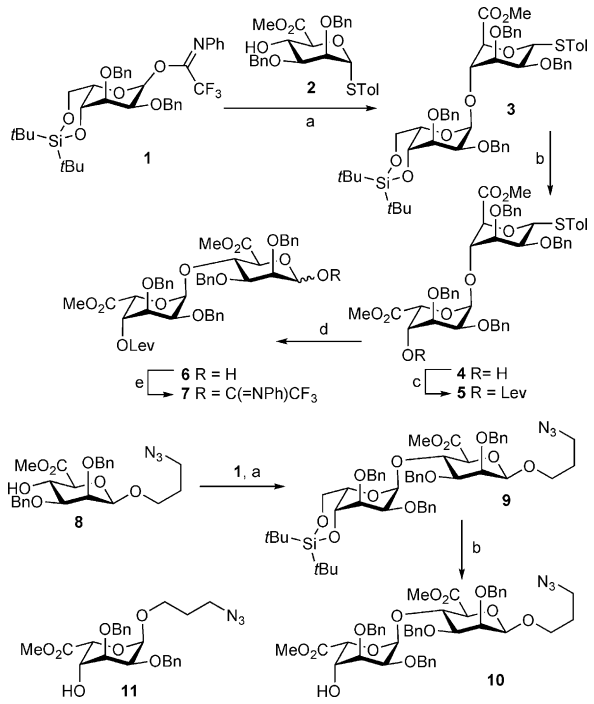
The synthesis of the key GM dimer synthon is depicted in Scheme 1. The silylidene-protected gulose imidate donor **1**[8] and α-*S*-tolyl mannuronic acid acceptor **2**[13] were combined in a fully stereoselective and chemoselective glycosylation event to furnish the dimer **3** in 91% yield. This disaccharide was then transformed into the GM synthon **4** by removal of the

[*] Q. Zhang, E. R. van Rijssel, M. T. C. Walvoort, H. S. Overkleeft, G. A. van der Marel, J. D. C. Codée
Leiden Institute of Chemistry, Leiden University
P.O. Box 9502, 2300 RA Leiden (The Netherlands)
E-mail: jcodee@chem.leidenuniv.nl

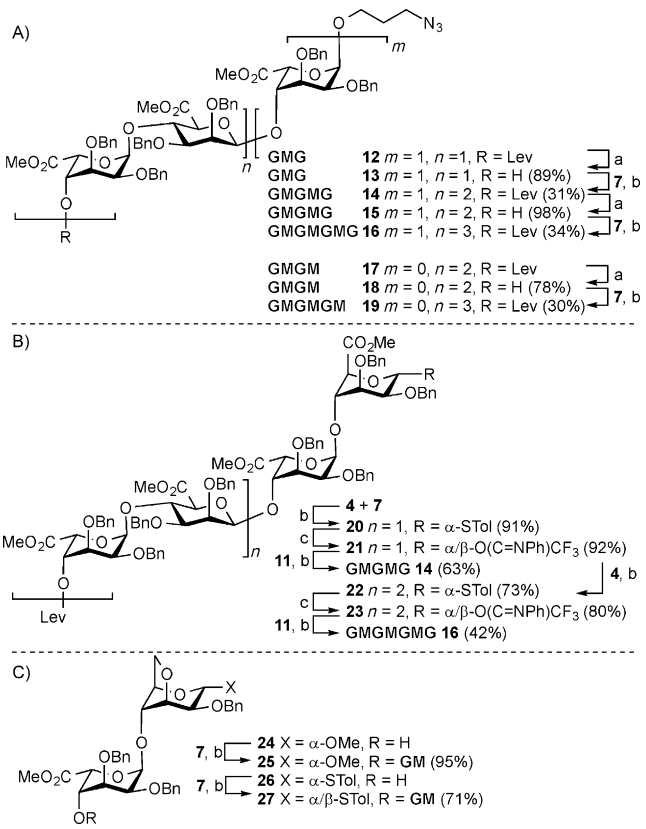
[**] This work was financially supported by The European Research Council (ERC AdG "CHEMBIOSPHING", to HSO).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201502581.

 © 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



Scheme 1. Synthesis of the key disaccharides. a) TMSOTf (cat.), CH_2Cl_2, −78 °C→−20 °C, **3**: 91 %; **9**: 58 %; b) 1. HF·pyridine, pyridine, THF; 2. TEMPO, BAIB, *t*BuOH, THF, H_2O, 3. MeI, K_2CO_3, DMF, **4**: 83 % (3 steps); **10**: 84 % (3 steps); c) LevOH, EDCI, DMAP, CH_2Cl_2, 92 %; d) NIS, TFA, CH_2Cl_2, 91 %; e) $F_3CC(=NPh)Cl$, K_2CO_3, acetone, 98 %. BAIB = [bis(acetoxy)iodo]benzene, DMAP = 4-(*N*,*N*-dimethylamino)pyridine, DMF = *N*,*N*-dimethylformamide, EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, LevOH = levulinoic acid, NIS = *N*-iodosuccinimide, TEMPO = 2,2,6,6-tetramethylpiperidine-1-oxyl, TFA = trifluoroacetic acid, THF = tetrahydrofuran, TMSOTf = trimethylsiyl trifluoromethanesulfonate.



Scheme 2. Synthesis of the oligomers. A) Using the rigid GM acceptors. B) Using the conformationally flexible GM-STol acceptors. C) Using model disaccharides. a) N_2H_4/H_2O, acetic acid, pyridine; b) TBSOTf (cat.), CH_2Cl_2, −78 °C to −45 °C. c) 1. NIS, TFA, CH_2Cl_2; 2. $F_3CC(=NPh)Cl$, K_2CO_3, acetone.

Table 1: Glycosylation reactions of the donors **5**–**7**.

Entry	Donor	Acceptor	Product	Yield [%] (α/β)
1	**7**[a]	**11**	**12**	84 (>20:1)
2	**7**[a]	**10**	**17**	26 (>20:1)
3	**7**[b]	**10**	**17**	45 (>20:1)
4	**6**[c]	**10**	**17**	21 (>20:1)
5	**6**[d]	**10**	**17**	32 (>20:1)
6	**5**[d]	**10**	**17**	20 (>20:1)
7	**7**[e]	**10**	**17**	32 (>20:1)
8	**7**[b]	**24**	**25**	95 (>20:1)
9	**7**[b]	**26**	**27**	71 (>20:1) kl

[a] 0.2 equiv TBSOTf, CH_2Cl_2, −78 °C to −45 °C. [b] 0.6 equiv TBSOTf, CH_2Cl_2, −78 °C to −45 °C. [c] Ph_2SO, TTBP, CH_2Cl_2, −78 °C, Tf_2O, 10 min, nucleophile, to 0 °C. [d] BSP, TTBP, CH_2Cl_2, −78 °C, Tf_2O, 10 min, nucleophile, to 0 °C. [e] 0.6 equiv TBSOTf, thiophene, CH_2Cl_2, −78 °C to −45 °C. BSP = (benzenesulfinyl)piperidine, TBSOTf = *tert*-butyldimethylsiyl trifluoromethanesulfonate, Tf_2O = trifluromethanesulfonic anhydride, TTBP = tri-*tert*-butylpyrimidine.

silylidene moiety, regio- and chemoselective oxidation of the gulose C6, and methylation of the resulting acid. From **4**, the thioglycoside donor **5**, lactol donor **6**, and imidate donor **7** were accessed by levulinoylation (to give **5**), hydrolysis of the thioacetal (to give **6**), and installation of the *N*-phenyltrifluoroacetimidate group (to give **7**), respectively. The GM dimer **9**, bearing an anomeric azidopropyl spacer, was obtained from the silylidene gulose **1** and azidopropyl functionalized mannuronic acid **8**.[9] The condensation of the latter two building blocks was rather slow and the best yield (58 %) was obtained by stirring the glycosylation reaction at low temperature for three days. The dimer **9** was transformed into the GM acceptor **10** using the desilylation/oxidation/methyl ester formation sequence described above.

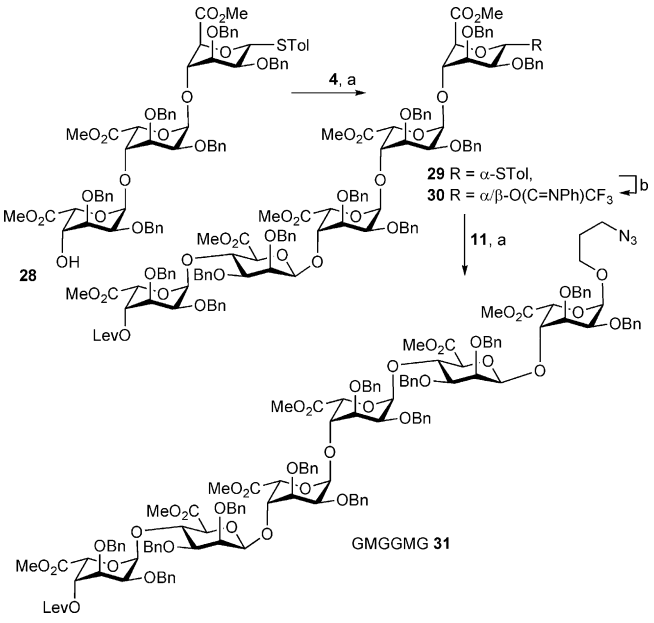
With the GM donors and acceptors in hand we set out to assemble larger oligomers. The condensation of **7** and the monosaccharide acceptor **11**[8] proceeded uneventfully under TBSOTf catalysis to give the GMG trimer **12** in 84 % yield and excellent stereoselectivity (Scheme 2 A and Table 1, entry 1).[14] In sharp contrast, when **7** was condensed with **10** the desired GMGM tetramer **17** was obtained in only 26 % yield (entry 2). Increasing the amount of activator and prolonging reaction time gave **17** in 45 % yield (entry 3). The use of other donor types (**5** or **6**) and a pre-activation strategy to generate a higher concentration of the reactive intermediate anomeric triflate did not lead to a better outcome (entries 4–6). Because we have previously shown that mannuronic acid donors are apt glycosylating agents, we hold the poor guluronic acid C4–OH nucleophile responsible for the low yield in these glycosylations.[8,9] Despite the low yield in the condensation reaction with **10**, we continued with the

 © 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

assembly of longer oligomers as shown in Scheme 2 A. Delevulinoylation of **12** and **17** gave the GMG and GMGM acceptors **13** and **18**, respectively, which were condensed with **7** to give the corresponding pentamer GMGMG **14** and hexamer GMGMGM **19** with excellent stereoselectivity, but again in a low yield (31% and 30% respectively). The pentamer **14** was deprotected (→**15**) to set the stage for another glycosylation with **7**, and led to the GMGMGMG heptamer **16** in 34% yield.

To increase the overall efficiency of the assembly process we reasoned that a more convergent approach, in which the length of both the donor and the acceptor is growing, might be more suitable and we therefore probed the glycosylation between **7** and **4** (Scheme 2 B). This glycosylation proceeded strikingly better than the analogous coupling of **7** and **10**, and the tetrasaccharide **20** was obtained in 91% yield as a single anomer. Remarkably, the large difference in yield for the glycosylations between **7** and the acceptors **4** and **10**, is caused by the difference in the anomeric functionality, a thiocresol and azidopropanol group, respectively, at the reducing end of the disaccharide acceptor, distal from the reacting C4′−OH. To identify the underlying cause for the difference in reactivity of **4** and **10** we performed a set of model couplings. Thioether additives have previously been reported to modulate glycosylation reactions and anomeric sulfonium ions can serve as glycosylating species.[15] To probe whether the anomeric thio function was at the basis of the improved reactivity of **4** we added thiophene[15b] to the condensation of **7** and **10**, to find that this external sulphide had no notable effect on the reaction (Table 1, entry 7). Having established that the thiofunction of the anomeric appendage was not the main contributing factor at play, we reasoned that the conformational flexibility of the acceptor could be the cause for the difference in reactivity between **4** and **10**. Where the β-mannuronic acid moiety in **10** occupies a normal $^4C_1$ chair conformation, the α-mannuronic acid in **4** occurs in an equilibrium of a $^4C_1$ and an inverted $^1C_4$ conformation, with a strong preference for the latter.[13] This conformational flexibility is evident from NMR analysis and originates from the presence of the electron-withdrawing C5-carboxylic acid ester on the mannuronic acid ring combined with the α-anomeric appendage.[16] As a result of the inverted conformation of the reducing-end mannuronic acid, the structure of **4** is more open, thus making the C4′−OH more accessible for an incoming electrophile.

To test this hypothesis we generated two model acceptors having a reducing-end mannoside locked in a $^1C_4$ chair conformation: the locked GM dimer **24**, having an anomeric α-*O*-methyl group, and GM dimer **26**, with an anomeric thiocresol moiety (Scheme 2 C). The acceptors could be condensed with **7** in good to excellent yield (Table 1, entries 8 and 9). The only notable side reaction that took place was the epimerization of the anomeric thioglycoside. From these model experiments we conclude that the overall three-dimensional structure of the acceptor is of decisive influence and that the open shape **4** is at the basis of its apt nucleophilicity. Building on this finding we assembled larger GM oligosaccharides by hydrolysis of the thioacetal in the GMGM tetramer **20** and transformed the resulting hemiacetal into the imidate donor **21**. This donor was condensed with **11** and **4** to give the GMGMG pentamer **14** (63%) and the GMGMGM-STol hexamer **22** (73%), respectively, thus confirming the good nucleophilicity of **4**. Elongation of **22** with another guluronic acid moiety was accomplished by transformation of **22** into the corresponding imidate **23** and ensuing glycosylation with **11** to provide the GMGMGMG heptamer **16** in 42% yield. The decreased yield in this glycosylation is due to partial hydrolysis of the large donor saccharide.[17] It is clear that the approach using the conformational flexible acceptor is overall significantly more effective. Finally we generated a random alginate sequence. The GMGGMG hexasaccharide **31** was synthesized using a [2+3+1] approach in which the trimer **28**, featuring a $^1C_4$ chair guluronic acid residue attached to the acceptor guluronic acid moiety, was first condensed with **7** (Scheme 3). This



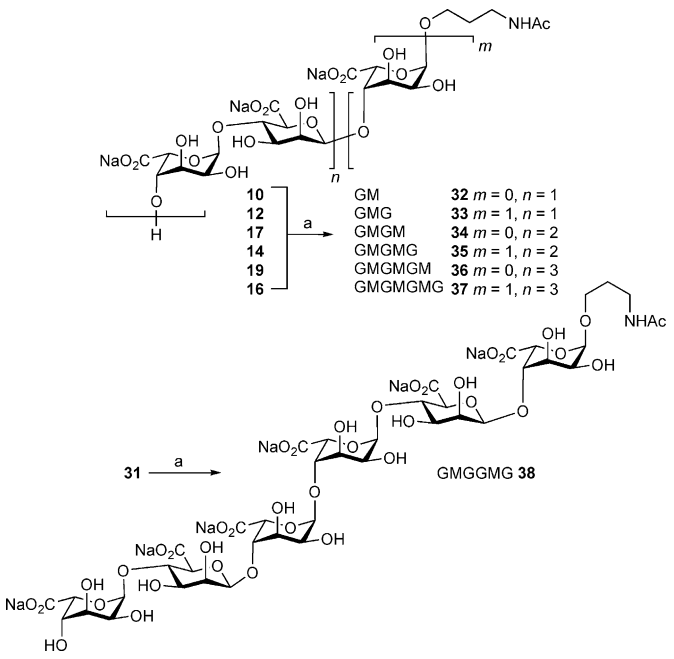
Scheme 3. Assembly of a GMGGMG hexasaccharide. a) TBSOTf (cat.), CH_2Cl_2, −78 °C to −45 °C, **29**: 87%; **31**: 43%. b) 1. NIS, TFA, CH_2Cl_2; 2. $F_3CC(=NPh)Cl$, K_2CO_3, acetone, 82%.

condensation proceeded uneventfully to provide the pentamer GMGGM **29** in 87% yield. This oligosaccharide was transformed into the corresponding imidate donor **30** and then coupled with **11** to give the GMGGMG hexamer **31** in 43% yield.[17]

Finally all prepared oligomers were deprotected by 1) removal of the levulinoyl esters, 2) saponification of the methyl esters, 3) high-pressure debenzylation and azide reduction, and finally 4) acetylation of the formed spacer amine group. Purification of the oligomers was accomplished by HW40 gel size exclusion chromatography, after which the alginate fragments were transformed into the sodium salts (Scheme 4).

In conclusion, we have described for the first time the fully stereoselective assembly of a set of mixed-sequence alginate sequences, thus making them available for biochemical studies. A set of alginate fragments, comprising GM, GMG,

© 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



Scheme 4. Deprotection of the oligosaccharides. a) 1. N_2H_4/H_2O, acetic acid, pyridine; 2. LiOH, H_2O_2, H_2O, THF; 3. *t*BuOH, THF, H_2O, Pd/C, H_2 (4.5 bar); 4. Ac_2O, $NaHCO_3$, THF, H_2O; 5. Dowex-Na^+. **32**: 46%; **33**: 43%; **34**: 50%; **35**: 25%; **36**: 50%; **37**: 60%; **38**: 60%.

GMGM, GMGMG, GMGMGM, GMGMGMG, and GMGGMG sequences, was assembled. During the assembly of the oligomers the conformational flexibility of the GM acceptors was revealed as an all-important factor determining the efficiency of the coupling reactions. While conformational restriction of carbohydrate building blocks has often been used to develop more efficient glycosylation strategies,[18] it is shown here that the use of immobile building blocks can compromise the yield of a glycosylation reaction. The use of conformationally flexible building blocks can be an effective approach to overcome steric interactions in the crowded transition state of a glycosylation reaction by allowing the acceptor to adopt a sterically most favorable shape. In future glycosylations involving poor nucleophiles, care should be taken not to restrict the acceptor in a possibly unfavorable steric environment.

Keywords: carbohydrates · conformation analysis · glycosylation · oligosaccharides · synthetic methods

How to cite: *Angew. Chem. Int. Ed.* **2015**, *54*, 7670–7673
Angew. Chem. **2015**, *127*, 7780–7783

[1] Because of its polymeric nature, mixed sequence alginate can be described, arbitrarily, as being built up from -GM- or -MG- blocks.

[2] a) T. H. Flo, L. Ryan, E. Latz, O. Takeuchi, B. G. Monks, E. Lien, Ø. Halaas, S. Akira, G. Skjåk-Bræk, D. T. Golenbock, T. Espevik, *J. Biol. Chem.* **2002**, *277*, 35489–35495; b) B. H. A. Rehm, S. Valla, *Appl. Microbiol. Biotechnol.* **1997**, *48*, 281–288.

[3] a) S. T. Moe, K. I. Draget, G. Sjåk-Bræk, O. Smidsrød in *Food Polysaccharides and Their Applications* (Ed.: A. M. Stephen), Marcel Dekker, New York, **1995**, pp. 245–286; b) J. Sun, H. Tan, *Materials* **2013**, *6*, 1285–1309.

[4] a) V. L. Campodónico, N. J. Llosa, L. V. Bentancor, T. Maira-Litran, G. B. Pier, *Infect. Immun.* **2011**, *79*, 3455–3464; b) D. M. Ramsey, D. J. Wozniak, *Mol. Microbiol.* **2005**, *56*, 309–322; c) M. Iwamoto, M. Kurachi, T. Nakashima, D. Kim, K. Yamaguchi, T. Oda, Y. Iwamoto, T. Muramatsu, *FEBS Lett.* **2005**, *579*, 4423–4429.

[5] a) F. Wolfram, E. N. Kitova, H. Robinson, M. T. C. Walvoort, J. D. C. Codée, J. S. Klassen, P. L. Howell, *J. Biol. Chem.* **2014**, *289*, 6006–6019; b) P. Baker, T. Ricer, P. J. Moynihan, E. N. Kitova, M. T. C. Walvoort, D. Little, J. C. Whitney, K. Dawson, J. T. Weadge, H. Robinson, D. E. Ohman, J. D. C. Codée, J. S. Klassen, A. J. Clarke, P. L. Howell, *PLoS Pathog.* **2014**, *10*, e1004334.

[6] a) L. J. van den Bos, J. Dinkelaar, H. S. Overkleeft, G. A. van der Marel, *J. Am. Chem. Soc.* **2006**, *128*, 13066–13067; b) J. D. C. Codée, L. J. van den Bos, A. R. de Jong, J. Dinkelaar, G. Lodder, H. S. Overkleeft, G. A. van der Marel, *J. Org. Chem.* **2009**, *74*, 38–47; c) M. T. C. Walvoort, G. Lodder, J. Mazurek, H. S. Overkleeft, G. A. van der Marel, J. D. C. Codée, *J. Am. Chem. Soc.* **2009**, *131*, 12080–12081.

[7] M. T. C. Walvoort, H. van den Elst, O. J. Plante, L. Kröck, P. H. Seeberger, H. S. Overkleeft, G. A. van der Marel, J. D. C. Codée, *Angew. Chem. Int. Ed.* **2012**, *51*, 4393–4396; *Angew. Chem.* **2012**, *124*, 4469–4472.

[8] a) J. Dinkelaar, L. J. van den Bos, W. F. J. Hogendorf, G. Lodder, H. S. Overkleeft, J. D. C. Codée, G. A. van der Marel, *Chem. Eur. J.* **2008**, *14*, 9400–9411; b) see the Supporting Information.

[9] S.-C. Hung, F.-C. Chi, S. S. Kulkarni, M. M. L. Zulueta, *Chem. Asian J.* **2009**, *4*, 386–390.

[10] J. Dinkelaar, A. R. de Jong, R. van Meer, M. Somers, G. Lodder, H. S. Overkleeft, J. D. C. Codée, G. A. van der Marel, *J. Org. Chem.* **2009**, *74*, 4982–4991.

[11] L. J. van den Bos, J. D. C. Codée, R. E. J. N. Litjens, J. Dinkelaar, H. S. Overkleeft, G. A. van der Marel, *Eur. J. Org. Chem.* **2007**, 3963–3976.

[12] In model experiments we have explored a range of orthogonally protected gulose acceptors. From these studies it became clear that the reactivity of a guluronic acid methyl ester C4–OH is not significantly lower than that of a corresponding gulose C4–OH.

[13] M. T. C. Walvoort, W. De Witte, J. Van Dijk, J. Dinkelaar, G. Lodder, J. Mazurek, H. S. Overkleeft, G. A. van der Marel, J. D. C. Codée, *Org. Lett.* **2011**, *13*, 4360–4363.

[14] In preliminary experiments evaluating reaction conditions to construct the ManA-Gul bond, TBSOTF proved to be more effective than TMSOTf and TfOH.

[15] a) S. K. Mulani, W.-C. Hung, A. B. Ingle, K.-S. Shiau, K.-K. Tony Mong, *Org. Biomol. Chem.* **2014**, *12*, 1184–1197; b) J. Park, S. Kawatkar, J. M. Kim, G. J. Boons, *Org. Lett.* **2007**, *9*, 1959–1962.

[16] J. Rönnols, M. T. C. Walvoort, G. A. van der Marel, J. D. C. Codée, G. Widmalm, *Org. Biomol. Chem.* **2013**, *11*, 8127–8134.

[17] We have not made an attempt to recover this lactol, but its identity was indicated by TLC-MS.

[18] See, for example: a) D. Crich, A. U. Vinod, *J. Org. Chem.* **2005**, *70*, 1291–1296; b) H. A. Orgueira, A. Bartolozzi, P. Schell, P. H. Seeberger, *Angew. Chem. Int. Ed.* **2002**, *41*, 2128–2131; *Angew. Chem.* **2002**, *114*, 2232–2235; c) D. Crich, V. Dudkin, *J. Am. Chem. Soc.* **2001**, *123*, 6819–6825.

Received: March 19, 2015
Published online: May 8, 2015

 © 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim