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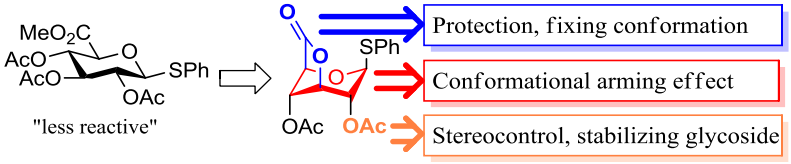

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A Potential Glucuronate Glycosyl Donor with 2-*O*-acyl-6,3-lactone Structure: Efficient Synthesis of Glycosaminoglycan Disaccharides

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ABSTRACT

Development of β-selective glucuronnylation reaction using phenyl 2,4-di-*O*-acetyl-1-thio-β-D-glucopyranosidurono-6,3-lactone was described. Glycosylations of this glycosyl donor with hexosamine derivatives proceeded with excellent yield and β-stereoselectivity to afford glycosaminoglycan-type disaccharides.

Uronic acids are an important class of monosaccharides and are defined as aldohexoses in which primary alcohol is oxidized to a carboxylic acid. Polysaccharides containing uronic acid entities are widespread in nature and display an array of physical properties and biological functions.[1] For example, glycosaminoglycans (GAGs) are ubiquitous components of the extracellular matrix and play essential roles in biological systems.[2] Because of irregular modifications of their skeleton, structurally defined GAG fragments are not easily prepared from natural resources. Thus, novel and efficient methods of the preparation of GAG fragments are crucial to advance our understandings of this important class of biomolecules. Extensive efforts have been made to improve the synthesis of oligosaccharides containing uronic acids.[3] However, the low reactivity of uronic acid derivatives as glycosyl donors in chemical glycosylation steps has impeded progress. This low reactivity results from the electron withdrawing property of the carboxylate group.[4] Currently, the following two strategies have been adopted to overcome this problem: i) "post-glycosylation oxidation[4b,5]": constructing glycosides followed by an oxidation and ii) "arming" the reactivity by the arrangement of protecting groups.[6] Conformational arming effect[7] is also an attractive concept for the activation of uronate donors. Van der Marel *et al.* demonstrated glycosylation reactions of a galacturonate-type donor whose conformation was locked by 6,3-lactone bridge[8] to give 1,2-*cis* glycoside by using the reduced reactivity by the torsional effect[9] of the 6,3-lactone[8a] and high reactivity as glycosyl acceptor[8b]. They also prepared the 6,3-lactone type donor of gluc- and mannuronate.[8] However, they did not demonstrate any conformational arming effect of this class of donor and used common $^4C_1$ type uronate donors in the following studies of glycosylations.[6d,e]

Hence, we designed glucuronate derivative **1** as a key synthon for synthesis of GAGs fragments (Fig. 1), since GAGs contain the β-GlcA moiety in repeating disaccharide units. The conformation of **1** is fixed by a 6,3-lactone bridge in $^1C_4$, with all substituents in an axial orientation.[4b,10] In more axial rich conformation, the reactivity increase is expected due to the different mode of σ-σ* or dipole interactions.[7,11] Also, to enhance 1,2-*trans* stereoselectivity, to suppress side reactions in coupling steps, and to enhance the activation of the thioglycoside, we selected a well-used 2-*O*-acyl protection to employ neighboring group participation.[12] In addition, the rigid 3,6-lactone structure might also activate the orthoester-type intermediate formed by the participation of the 2-*O*-acyl group on donor **1**.

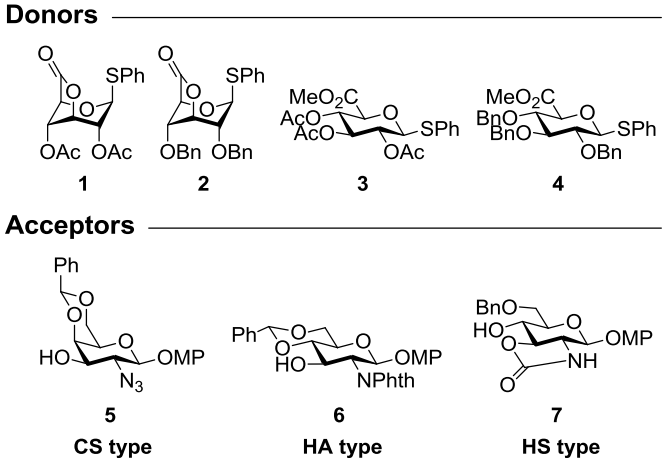
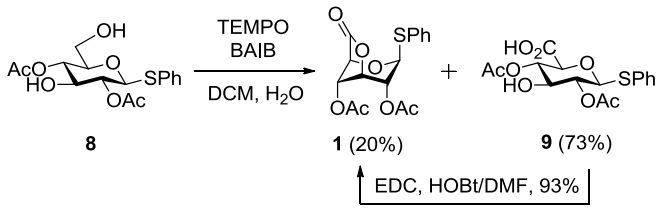


Figure 1. donors and acceptors employed in this study. MP: *p*-methoxy phenyl.

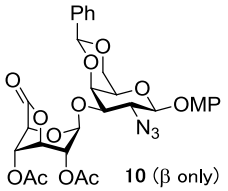
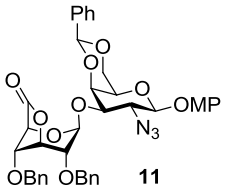
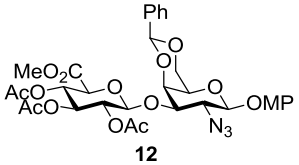
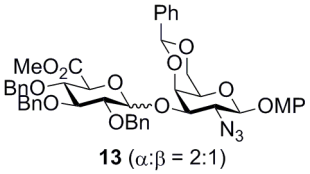
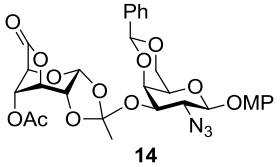
To investigate the efficiency of our strategy, we prepared **1**, *O*-benzyl protected glucuronolactone derivative **2**[8], and a corresponding set of standard methyl glucuronate derivatives **3**[13] and **4**[14] as disarmed and armed counterparts with $^4C_1$ conformation, respectively. As acceptors, hexosamine derivatives **5**[15], **6**[16] and **7**[17] were prepared to afford chondroitin sulfate (CS), hyaluronic acid (HA), and heparan sulfate (HS) type dissaccharide units in GAGs, respectively. (Fig. 1)

The simultaneous lactonization with C6-oxidation[8] of **8** using 2,2,6,6-tetramethylpiperidinyloxy free radical (TEMPO) and [bis(acetoxy)iodo]benzene (BAIB) reagent system[18] afforded 6,3-lactone donor **1** only in 22% but uncyclized derivative **9** in 73% yield. EDC/HOBt system, however, was found to give the lactone **1** in 93% from **9**. (Scheme 1)



Scheme 1. Synthesis of lactone donor **1**.

Table 1. Glycosylations with $GalN_3$ derivative **5** under NIS/TfOH system

#	donor	Conditions[a]	product/α:β ratio[b]	Yield (%)[c]
1	**1**	A	 **10** (β only)	quant.
2	**2**	A	 **11**	trace[d]
3	**3**	A	 **12**	83
4	**4**	A	 **13** (α:β = 2:1)	67
5	**1**	B	 **14**	68
6	**2**	B	**11**	trace[d]
7	**4**	B	**13** (α:β = 2:3)	93

[a](A) donor (1.5 eq.), acceptor (1.0 eq.), NIS (1.5 eq.), TfOH (0.2 eq.), DCM (0.07 M), -40 °C, 1 h, then -10 °C, 1 h; (B) donor (1.2 eq.), acceptor (1.0 eq.), NIS (1.2 eq.), TfOH (0.2 eq.), DCM (0.1 M), -40 °C, 2 h.

[b]Isolated ratio.

[c]Isolated yields based on the acceptor.

[d]Detected only by MALDI-TOF MS.

Table 1 shows the result of glycosylation reactions of the glycosyl donors **1-4** with D-galactosamine derivative **5** as an acceptor substrate. The diacetyl-protected donor **1** under the promotion with NIS/TfOH at -40 °C and stirring at -10 °C for 1 h afforded CS-type disaccharide **10** in a quantitive yield. (# 1) Meanwhile, the glycosylation of dibenzyl-protected donor **2**[8] provided a trace amount of disaccharide **11**. (# 2) Interestingly, this donor **2** was found extremely fragile to be used as a donor substrate even at -40 °C. (# 6) Analyses of the major product from donor **2** suggested that de-benzylation at O-4 seemed to be proceeded, although the exact structure could not be identified. When $^4C_1$ type donor **3** was employed, the corresponding β-linked disaccharide **12** was obtained in 83% yield. (# 3) Unexpectedly, an armed counterpart **4** afforded disaccharide **13** in 67% yield with a slight α-selectivity. (# 4) When the reaction of donor **1** was performed under the conventional condition B (quenched at -40 °C), orthoester **14** was identified as the main product and 24% of acceptor **5** was recovered. (# 5) In the case of donor **4** without elevating temperature, the coupling product **13** was isolated in 93% yield as a slight β-rich mixture. (# 7) The result of # 1 and 5 indicates that a glycosidic bond was formed via orthoester, and product **10** was stable to an acid catalyst at -10 °C. In contrast, from the result of # 4 and 7, disaccharide especially β-glycoside may be unstable under the condition A.

To confirm the versatility of 6,3-lactone and 2-*O*-acyl substituents, we carried out reactions of glucosamine derivative **6** as an acceptor substrate to afford HA type disaccharide. (Table 2, # 1-4) The coupling of donor **1** and acceptor **6** under an optimized condition afforded β-linked disaccharide **15** quantitively. (# 1) Meanwhile, when $^4C_1$ donor **3** was employed, disaccharide **16** was obtained only in 22% yield. (# 2) The glycoside **17** which was obtained from the donor **4** was also unstable at -10 °C. (# 3) By keeping the reaction at -40°C, however, disaccharide **17** was isolated quantitively with a slight increase of β-selectivity. (# 4) Thus, the remarkable high yield, β-selectivity, and stability under acidic conditions of glycoside **15** clarified the advantage of donor **1**.

In the last place, synthesis of HS type disaccharide was evaluated by using a 2,3-carbamate protected glucosamine derivative **7** as an acceptor which can apply to a donor for α-selective glycosylation[19] at farther glycosylation steps. (# 5-8) Glycosylation reaction to 4-OH of *N*-acetylglucosamine derivatives is a well-known challenge because of the poor nucleophilicity.[20] Indeed, the coupling of donor **1** and acceptor **7** at -10 °C gave HS-type disaccharide **18** only in 22% yield. (# 5) Analysis of this reaction revealed the existence of orthoester at -10 °C. This fact suggested that the conversion to the glycoside was incomplete. Tracing the reaction at 0 °C identified that the orthoester disappeared in 3 hours and the β-glycoside **18** was isolated in 63% yield. (# 6) The yield was still moderate but enough for the fragment synthesis as in previous excellent studies.[4b,21] As expected, a donor **3** with acetyl group at O-2 afforded β-linked disaccharide **19** only in 18% yield. (# 7) Interestingly, disaccharide **20** was stable even at 0 °C and the yield was 93% yield as a α-rich mixture. (# 8)

In summary, we designed a novel glucuronate donor with 6,3-lactone and 2-*O*-acyl protection for the construction of glucuronic acid-containing entities. The 6,3-lactone donor **1** demonstrated an excellent versatility via a series of GAGs disaccharide syntheses. In particular, ideal β–selectivities, high yields, and stable glycosides are noteworthy features. Therefore, we believe that donor **1** provides a practical approach to prepare various glycosides and glycoconjugates containing unonate moieties. Currently, further application of this glucuronate donor for longer GAG oligosaccharide synthesis is in progress.

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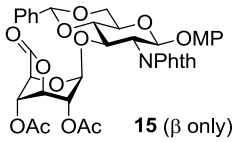
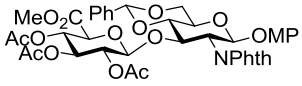
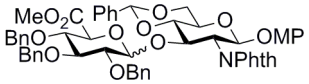
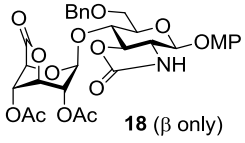
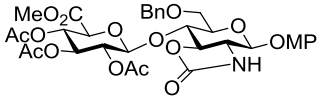
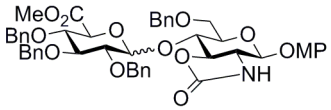
Table 2. Glycosylation with glucosamine derivatives **6** and **7** under NIS/TfOH system

#	donor	acceptor	Conditions[a]	product/α:β ratio[b]	Yield (%)[c]
1	**1**	**6**	A	 **15** (β only)	quant.
2	**3**	**6**	A	 **16** (β only)	22
3	**4**	**6**	A	 **17** (α:β = 5:2)[d]	50
4	**4**	**6**	B	**17** (α:β = 5:3)	quant.
5	**1**	**7**	A	 **18** (β only)	22
6	**1**	**7**	C	**18** (β only)	63
7	**3**	**7**	C	 **19** (β only)	18
8	**4**	**7**	C	 **20** (α:β = 7:3)	93

[a](A) donor (1.5 eq.), acceptor (1.0 eq.), NIS (1.5 eq.), TfOH (0.2 eq.), DCM (0.07 M), -40 °C, 1 h, then -10 °C, 1 h; (B) donor (1.2 eq.), acceptor (1.0 eq.), NIS (1.2 eq.), TfOH (0.2 eq.), DCM (0.1 M), -40 °C, 2 h; (C) donor (1.5 eq.), acceptor (1.0 eq.), NIS (1.5 eq.), TfOH (0.2 eq.), DCM (0.07 M), -40 °C, 2 h, then 0 °C, 3 h.

[b]Isolated ratio.

[c]Isolated yields based on the acceptor.

[d]Determined by ^{1}H-NMR analysis.

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