The Glycosylation Mechanisms of 6,3-Uronic Acid Lactones

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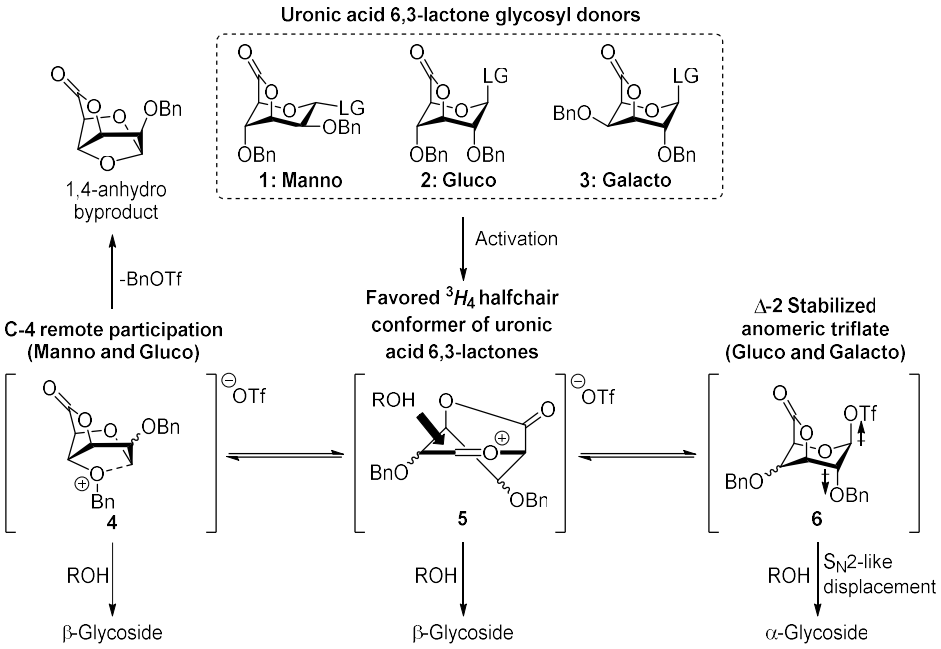
Abstract: Uronic acids are important constituents of polysaccharides found on the cell membranes of different organisms. To prepare uronic acid containing oligosaccharides, uronic acid 6,3-lactones can be employed as they display a fixed conformation and a unique reactivity and stereoselectivity. Herein we report a highly *β*-selective and efficient mannosyl donor based on C-4 acetyl mannuronic acid 6,3-lactone donors. The mechanism of glycosylation is established using a combination of techniques including IR ion spectroscopy combined with quantum-chemical calculations and variable-temperature nuclear magnetic resonance (VT NMR) spectroscopy. The role of these intermediates in glycosylation is assayed by varying the activation protocol and acceptor nucleophilicity. The observed trends show analogy to the well-studied 4,6-benzylidene glycosides and may be used to guide the development of next-generation stereoselective glycosyl donors.

The main challenge in the chemical preparation of oligosaccharides is the stereoselective synthesis of glycosidic bonds, which can exist as *α*- or *β*-diastereomers.[1] The glycosylation reaction is a nucleophilic substitution reaction between an electrophilic glycosyl donor carrying an anomeric leaving group and a glycosyl acceptor containing a nucleophilic alcohol. Depending on the nature of the glycosyl donor, acceptor and reaction parameters, the mechanism of glycosylation is best described as a continuum between S_N1-like and S_N2-like reaction pathways.[2]

Stereocontrol in a glycosylation reaction is difficult to achieve due to two main factors. First, S_N1-like and S_N2-like pathways leading to opposing diastereomers may be simultaneously operative. Secondly, the S_N1-like pathway may not be stereoselective. In order to obtain stereoselective glycosylations, it is therefore essential to ensure that either the reaction takes place *via* one stereoselective pathway (S_N1-like or S_N2-like), or that both pathways lead to the same stereoisomer. In some cases, this can be achieved by the introduction of a bicyclic protecting group on the glycosyl donor.[3] Recently, we reported the use of mannuronic acid 6,3-lactones as glycosyl donors (**1**) and found them to be highly *β*-selective (Scheme 1).[4] The lactone bridge presumably leads to a *β*-selective oxocarbenium ion conformer **5**, but also allows for remote participation of the C-4 benzyl ether (**4**) as evidenced by the formation of a 1,4-anhydrosugar byproduct (Scheme 1). These results contrast those reported for galacturonic acid 6,3-lactone **3**, which is highly α-selective.[5] In this case, reaction of a *β*-triflate intermediate **6** *via* an S_N2-like pathway is presumably responsible for the α-selectivity.



Scheme 1: Proposed glycosylation intermediates of uronic acid 6,3-lactone glycosyl donors (**1**-**3**). LG: Leaving Group

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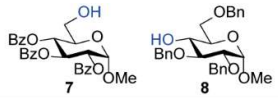
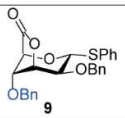
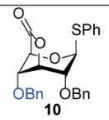
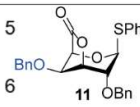
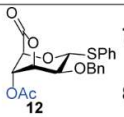
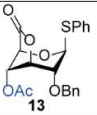
Herein, we report the development of a mannuronic acid 6,3-lactone donor carrying a C-4 acyl group. This donor does not suffer from byproduct formation, whilst still providing good *β*-selectivity. A systematic study of the glycosylation mechanism of uronic acid 6,3-lactones was performed to explain the difference in stereoselectivity and reactivity between manno-, gluco- and galacto-configured donors. To this end, reactive intermediates were characterized using infrared (IR) ion spectroscopy, which provided evidence for remote C-4 acyl participation in the gas phase. Furthermore, glycosyl triflate intermediates were characterized using variable temperature (VT) NMR experiments. Finally, the reactivity of the intermediates was profiled by varying acceptor nucleophilicity. Clear trends emerged that are analogous to those found in other bicyclic donor systems, such as 4,6-benzylidene protected manno- and glucosides.[6] These findings may provide clear directions for designing next-generation stereoselective glycosyl donors.

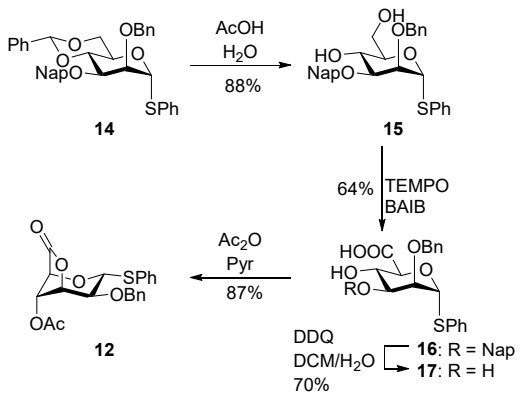
Our previous studies utilized carboxybenzyl (CB) donors which require pre-activation; therefore, we prepared thioglycoside derivatives **9**[3b], **10**[7] and **11**[8] to investigate whether glycosylations under standard (premix) conditions would prevent 1,4-anhydrosugar formation. Glycosylation of **9**-**10** by pre-activation with Ph_2SO/Tf_2O[9] followed by addition of glycosyl acceptor **7** (Table 1, entry 1,3 and 5) mainly led to 1,4-anhydrosugar as expected.[4, 10] Galactoside **11** does not suffer from 1,4-anhydrosugar formation as the C-4 benzyl is positioned

Table 2: Glycosylation of donors **9-13**



Entry	Donor	Acceptor	Promotor	Yield[a] (%)	α/β ratio[b]
1		**7**	Ph_2SO/Tf_2O	7	1/20
2	**9**		NIS/AgOTf	22	1/20
3		**7**	Ph_2SO/Tf_2O	0	--
4	**10**		NIS/AgOTf	0	--
5		**7**	Ph_2SO/Tf_2O	94	20/1
6	**11**		NIS/AgOTf	86	4.6/1
7		**7**	NIS/AgOTf	88	1/20
8	**12**	**8**	NIS/AgOTf	51	1/20
9		**7**	NIS/AgOTf	81	1/2.5
10	**13**	**8**	NIS/AgOTf	53	1/2

[a] Isolated yield of the disaccharide as a mixture of *α*/*β* anomers, [b] ratios were determined in the crude reaction mixture using key integrals in ^{1}H-NMR spectra. Protocols: Ph_2SO, Tf_2O, TTBP, DCM, -78°C then **7**, or **10**/**11**, NIS, DCM AgOTf (cat), -10°C.



Scheme 2: Key steps in the C-4 acetyl 6,3-uronic acid lactone donor-synthesis. Manno-type donor **4** is shown as an example.

equatorially and afforded the *α*-galactoside with excellent stereo-selectivity (*α*/*β* = 20/1, Table 1, entry 5) in line with earlier reports.[5, 7] Glycosylation of **9** using the N-iodosuccinimide (NIS) /AgOTf promoter system under premix conditions led to an improved yield of the *β*-disaccharide (22%), although a significant amount of 1,4-anhydro sugar was still formed (Table 1, entry 2).[11] In case of gluco-lactone **10**, exclusive formation of the 1,4-anhydrosugar was observed under the same conditions. Galactoside **11** provided a much lower α-selectivity under pre-mix conditions as the *β*-triflate intermediate was not pre-formed.

To prevent 1,4-anhydrosugar formation while retaining the stereo-directing capability of the C-4 substituent, we focused on the introduction of a C-4 acetyl ester.[12] The synthesis of manno-type C-4 acetyl donors **12** started from known 4,6-benzylidene protected precursor **14** (Scheme 2).[13] Acidic hydrolysis of the 4,6-benzylidene and subsequent oxidation using TEMPO/BAIB[7, 14] afforded uronic acid **16** in good yield. DDQ oxidation of the 2-methylnaphthyl (Nap) ether afforded the corresponding C-3 alcohol (**17**), which was lactonized using acetic anhydride.[15] Under the same conditions, the C-4 alcohol was acetylated affording the desired uronic acid 6,3-lactone donor **12** in good yields. Gluco-type donor **13** was prepared using an analogous procedure (see supporting information). Notably, the oxidation of a 3,4,6-triol precursor to afford 6,3-uronic acid lactones directly was also successfully explored based on Stahl's procedure for lactonization (see supporting information, scheme S2, **S47**).[3d, 16] Though with a modest yield (19%), this procedure potentially provides access to **12** and **13** and its 4-OH derivatives.

Next, the reactivity and stereoselectivity of donors **12** and **13** was explored (Table 1, entry 7-10). Activation was achieved under premixed conditions with NIS/AgOTf at -10°C. With acceptors **7**-**8**, both donors gave the corresponding disaccharides in a good yield using only a small excess of the donors, showing that the introduction of the C-4 acetyl group prevented byproduct formation as intended. Mannose donor **12** showed excellent *β*-selectivity (*α*/*β* = 1/20), whereas glucose donor **13** formed mostly the *β*-product, albeit with modest selectivity (*α*/*β* = 1/2.5).

To obtain insight in the different stereochemical preference of donors **12** and **13,** we characterized the intermediates likely

responsible for their S_N2-like and S_N1-like pathways. Solvent separated ion pairs (SSIP) such as **4** and **5** are believed to define the S_N1-like pathway and are challenging to characterize due to their intrinsic high reactivity, short life-times and equilibrium with the glycosyl triflate **6**. Recently, we reported the use of collision-induced dissociation tandem mass spectrometry (CID MS/MS) in combination with IR ion-spectroscopy to characterize these elusive glycosyl cations.[17] Electrospray ionization (ESI) of thioglycoside donors was employed to form the precursor ions, which were subsequently fragmented by CID, giving the desired glycosyl cations. Spectral assignments were made by comparing the experimental IR spectra with theoretical IR spectra obtained by high level *ab initio* calculations.[18] Using this technique, glycosyl cations derived from glycosyl donors **9, 10, 12** and **13** were structurally characterized by IR ion spectroscopy in the gas phase. To simplify the IR spectra, the benzyl ethers were replaced by methyl ethers (**18**, **20**, **22** and **24**, see SI p18-22 for preparation details).

The desired fragment cations at *m/z* 187 were formed from protonated methyl ether analogues of lactones **12** and **13** via tandem mass spectrometry. Upon close inspection, a fragment resulting from another fragmentation pathway appeared in the same *m/z* 187 mass channel. (see Figure S7, A and S9, A). To favor oxocarbenium ion formation, the anomeric thioglycosides were oxidized to sulfoxides **18** and **20**. Additionally, the oxidation induced an *m/z* difference of 16 in the parent ion, thereby removing overlap in fragmentation pathways (Figure S7, B and S9, B). Fragment ions of **18** and **20** were mass-isolated in a quadrupole ion trap[19] and subsequently characterized by IR ion-spectroscopy using the FELIX IR free electron laser (IR-FEL) operating in the 700-2100 cm^{-1} region (for details see supporting information S70).

The experimental IR spectra resulting from the cations of lactones **18** and **20** show best agreement with the calculated spectra of the oxocarbenium ions in the $_4E$ conformation (Figure 1, A and B). Interestingly, in both cases the best-match structure does not correspond with the lowest-energy structure, which involves participation of the 4-OMe and is favored by 7.7 kJ/mol and 16.7 kJ/mol in mannuronic acid lactone **19** and glucuronic acid lactone **21**, respectively. Nonetheless, the $_4E$ conformation places the C-4 substituent in pseudo-axial position enabling its remote participation.

In contrast, the IR spectra of lactones **22** and **24** can be assigned to conformers that result from C-4 acetyl participation, which also represent the lowest-energy structures (Figure 1, C and D). Characteristic bands in the measured spectrum of **23** and **25** at ±1550 and 1430 cm^{-1} are in good agreement with the calculated O–C=O$^+$ stretch of the dioxolenium ion and the C_4-H_4 bending mode, respectively (filled). In addition, the presence of only one C=O stretch mode at 1846 cm^{-1} (**23**) and 1851 cm^{-1} (**25**), respectively, rules out the oxocarbenium ion and supports the involvement of the 4-OAc. For the methylated as well as for the acetylated species, no clear differences between mannose

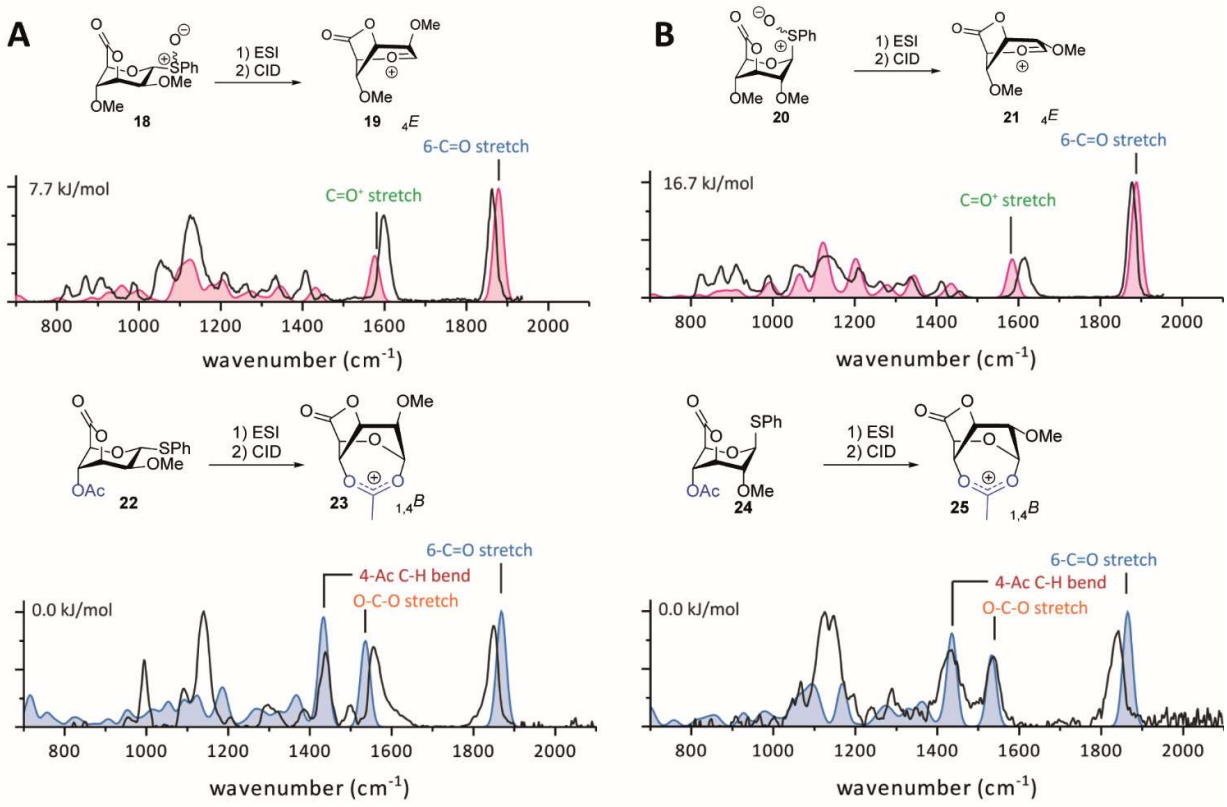


Figure 1: Comparison of the calculated spectra (filled) with the measured spectra of generated 6,3-uronic acid lactone cations **19** (A), **21** (B), **23** (C) and **25** (D). Energies in A and B are relative to the 0.0 kJ/mol structure as reported in figure **S8** and **S10,** respectively.

and glucose are observed in the structures of the glycosyl cations formed.

This may suggest that their S_N1-like pathways are likely very similar and that both the manno- and gluco-type 6,3-uronic acid lactones to be intrinsically *β*-selective in S_N1-like pathways. It should be noted however, that the structure of the glycosyl cations **19**, **21**, **23** and **25** were determined in the gas phase and may differ from those formed in solution.

Next, we set out to characterize the contact-ion pair intermediates in solution by VT-NMR[20]. To this end, we prepared glycosyl sulfoxides **26** and **27** by oxidation of donors **12** and **13** with *m*-CPBA. **26** and **27** were activated at low temperature (-78°C) with Tf_2O in presence of TTBP in CD_2Cl_2 and characterized by VT-NMR. Interestingly, in both cases, the formation of the *β*-triflate (**28** and **29**) as the major species was observed and confirmed by 2D-HOESY NMR (Figure 2). Both samples were allowed to warm up and showed disappearance of the triflate at -10°C (Figure S2) and -4°C (Figure S4) for mannose **28** and glucose **29**, respectively. No *α*-triflate was observed during the experiments. The stability of the β-triflates may result from the electron withdrawing effect of the C-4 acetyl as well as the constrained 6,3-lactone bridge[21]. A VT-experiment to trap the participating acyl group[22] using a C-4 Boc derivative of **12** showed initial β-triflate formation leading to a complex mixture which upon work-up converted to the 1,4-anhydro-byproduct instead of the expected cyclic carbonate (see supporting information, fig. S5-S7).

As both mannoside **12** and glucoside **13** give rise to the same reactive intermediates for the S_N1-like and S_N2-like pathways, the observed difference in stereoselectivity is likely a result of different reactivities of the respective intermediates. To determine the difference in reactivity, a number of glycosylations were performed thereby varying the activation protocol and acceptor nucleophilicity[23] (See Table 2). First, donors **12** and **13** were pre-activated with Ph_2SO and Tf_2O at -60°C to allow for *β*-triflate formation. After full activation, acceptor **7** was added and the mixtures were allowed to slowly warm up. Mannosyl donor **12** retained almost full *β*-selectivity (Table 2, entry 2) indicating that the glycosylation does not take place *via* S_N2-like displacement of the *β*-triflate, but rather *via* an alternative pathway. In contrast, glycosylation of glucosyl donor **13** under pre-activation conditions showed good *α*-selectivity (Table 2, entry 5) suggesting significant reaction *via* the *β*-triflate intermediate similar to galactosyl donor **11** (Table 1, entry 5).To confirm the latter result, glycosylations with allyl-TMS as a nucleophile under pre-activation conditions were performed. Glycosylations with allyl-TMS are irreversible, their steric effects are minimized and its weaker nucleophilicity minimizes reaction *via* an S_N2-like pathway[24]. In these cases, both mannosyl and glucosyl donors displayed high *β*-selectivity, indicating that their S_N1-like pathways lead to the same diastereomer (Table 2, entry 3 and 6 respectively). Apart from the C-2 stereochemistry, mannosyl and glucosyl donor **12** and **13** also differ in the stereochemistry of the leaving group. We synthesized the *α*-SPh glucosyl donor **30** to explore the impact of leaving group orientation (see SI). Glycosylation under premix conditions gave a good yield and showed slight *α*-selectivity (Table 2, entry 7). We hypothesize that activation of the *α*-leaving group may lead to more rapid *β*-triflate formation, which can be displaced by the acceptor.

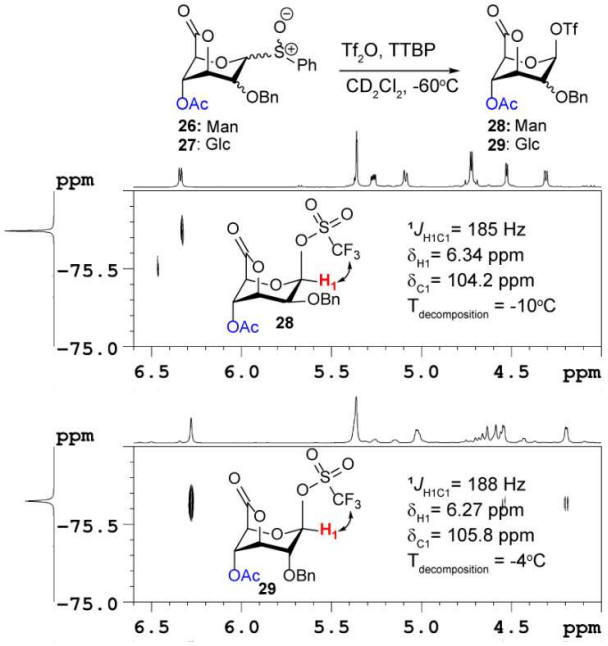
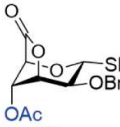
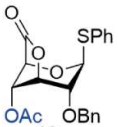
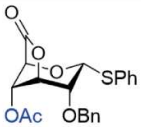


Figure 2: Characterization of glycosyl triflates **28** and **29** at -60 °C in CD_2Cl_2 by VT-NMR.

Table 3: Key-glycosylations to elucidate the mechanism driving the stereoselectivity in 6,3-uronic acid lactone donors **4** and **5**

Entry	Donor	Acceptor	Promotor	Yield[a] (%)	α/β ratio[b]
1		**7**	NIS/AgOTf	88	1/20
2		**7**	Ph_2SO/Tf_2O	79	1/11
3	**12**	AllylTMS	Ph_2SO/Tf_2O	72	<1/10
4		**7**	NIS/AgOTf	81	1/2.5
5		**7**	Ph_2SO/Tf_2O	87	6/1
6	**13**	AllylTMS	Ph_2SO/Tf_2O	55	<1/10
7	 **30**	**7**	NIS/AgOTf	81	2.8/1

[a] Isolated yield of the disaccharide as a mixture of *α*/*β* anomers, [b] ratios were determined in the crude reaction mixture using key integrals in ^{1}H-NMR spectra. Protocols: Ph_2SO, Tf_2O, TTBP, DCM, -78°C then **7**/allylTMS, or **10**/**11**, NIS, DCM AgOTf (cat), -10°C.

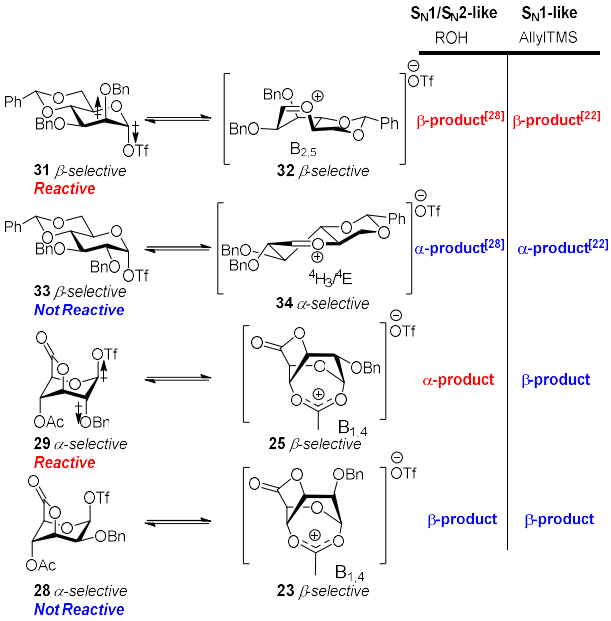


Figure 3: Observed trends in fixed bicyclic glycosyl donor systems. **7** is used as example for ROH. Selected examples in case of the 4,6-benzylidene glycosides for ROH and allyl-TMS are reffered to accordingly.

Overall, these results indicate that the reaction intermediates of both the S_N-1 and S_N-2-like pathways for **12** and **13** are expected to lead to opposing diastereomers (α- and *β*-glycosides, respectively). However, the relative reactivity of the reaction intermediates differs and results in contrasting overall stereo-selectivity. In this respect, glycosylations with uronic acid 6,3-lactones **12** and **13** show a profound analogy with *β*-selective mannosylations utilizing 4,6-benzylidene mannosyl donors, developed by Crich and coworkers (Figure 3).[6, 25] Both are bicyclic systems that stabilize the formation of glycosyl triflates[26] as well as defined glycosyl cation intermediates.[27] S_N2-like pathways are expected to occur *via* glycosyl triflates **28**, **29**, **31**[26] and **33**[28] and S_N1-like reactions are likely to proceed *via* glycosyl cations **23**, **25**, **32**[27] and **34**.[27, 29] The axial triflates of both systems show a comparable trend in reactivity and can be related to the orientation of the C-2 substituent. Both mannoside **31** and glucoside **28** have a Δ-2 effect[30] caused by a neighboring axial C-2 substituent. Both triflate species are reactive in S_N2-like pathways and yield *β*- and α-glycosides with strong nucleophiles, respectively. Counterparts **33** and **28** lacking a Δ-2 effect both lead to triflate species that do not participate in the glycosylation event and selectivity likely arises from alternative pathways. S_N1-like reactions *via* glycosyl cations **34**[29] and **23** would be expected to lead to the observed α- and β-products respectively. However, involvement of a fast equilibrium from the axial-triflate to an equatorial-triflate followed by and S_N2-like displacement cannot be ruled out[31].

In conclusion, we developed a highly *β*-selective and efficient mannosyl donor based on C-4 acetyl mannuronic acid 6,3-lactone donors. The mechanism of glycosylation was established using a combination of analytical techniques and glycosylation experiments. Glycosyl cation intermediates likely involved in the S_N1-like pathways and were characterized using IR ion spectroscopy in the gas phase. Glycosyl triflate intermediates likely involved in the S_N2-like pathways were characterized using VT-NMR. The involvement of these intermediates in glycosylation was assayed by varying the activation protocol and acceptor nucleophilicity. The observed trends showed analogy to the well-studied 4,6-benzylidene glycosides and we believe that these guidelines will enable the design of the next generation of stereoselective glycosyl donors.

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Keywords: Glycosylation • Uronic acids • IR ion spectroscopy • Oxocarbenium ion •

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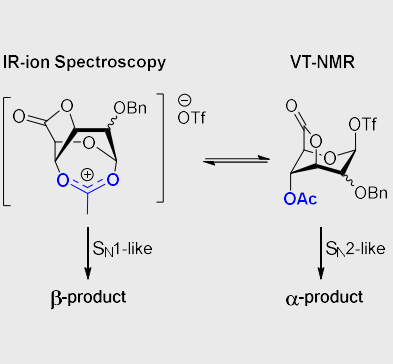
COMMUNICATION

A new type of β-selective mannosylation donors was developed and its glycosylation mechanism was elucidated using an integrated approach combining IR ion spectroscopy and VT NMR.



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