Carbohydrate Research 346 (2011) 2380–2383

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Study of the stereoselectivity of 2-azido-2-deoxygalactosyl donors: relationship to the steric factors of glycosyl acceptors

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ARTICLE INFO

Article history:
Received 13 June 2011
Received in revised form 8 August 2011
Accepted 12 August 2011
Available online 22 August 2011

Keywords:
Glycosylation
Stereoselectivity
Acceptor reactivity

ABSTRACT

The stereoselectivity of a 2-azido-2-deoxygalactosyl ($GalN_3$) donor is found to strongly depend on the nature of the acceptors in glycosylation reactions. The order of the acceptor, the stereochemistry, and the configuration of the monosaccharide all affect the stereochemistry outcome. More reactive acceptors are observed to favor β-products, while less reactive acceptors afford more α-products.

1. Introduction

Chemical synthesis provides one of the most important means to access large quantity of structurally well-defined carbohydrate compounds in homogenous form.[1,2] However, synthesis of oligosaccharides is much more challenging than synthesis of other types of biopolymers (like peptides and nucleotides), largely due to the difficulties in controlling the stereoselectivity and regioselectivity. The control of the stereochemistry (which is not present in cases of peptide linkages and nucleotide linkages) is especially challenging because of the complexity of the contributing factors to the stereoselectivity, such as the configuration of the glycosyl donor,[3–8] the structure of the leaving group,[9] the reaction conditions, the reactivity of the glycosyl acceptor,[10,11] and the protecting groups on the glycosyl donors. Among all glycosidic linkages, 1,2-*cis*-linkages (like α-linkages in glucose or galactose like donors and β-linkages in mannose like donors) are more difficult to form than the 1,2-*trans*-linkages, which are convenient to form in the presence of participating neighboring groups. The α-GalNAc linkage, for example, has been such a linkage of great synthetic interest because of its widespread presence in biological systems.[12,13] Among all the methods people developed for efficient synthesis of α-GalNAc linkages, 2-azido-2-deoxygalactosyl ($GalN_3$) donors have been particularly popular because of their convenience to access and to transform to amino group.[14–16] Our previous research on the remote protecting group effect on the stereoselectivity of a series of $GalN_3$ donors suggest that acetyl groups at 3 and 4 positions are critical for high α-selectivity.[17,18] Higher reaction temperature was also discovered to improve α-selectivity. Other factors, including the acceptors, the leaving groups of donors and activation methods, are also known to affect the stereoselectivity.[19–21,15] In this paper, we report our recent research on the correlation between α-selectivity of a $GalN_3$ donor and the structure of glycosyl acceptors, more specifically, the stereochemistry and the configuration of the acceptors.

2. Results and discussion

2.1. Switch of stereoselectivity of donor 1

During our research of the relationship between protecting groups of glycosyl donors and the stereoselectivity, we accidentally found that one of the donors we made (compound **1,** Scheme 1) showed totally opposite stereoselectivity in reactions with two different acceptors. In a reaction between donor **1** and acceptor **2,** we were surprised to see that only β-disaccharide (compound **3**) was isolated from the reaction, despite the non-participating nature of the azido group and the generally α-selective nature of this donor in our previous study and another literature report.[22] However, when a different acceptor (compound **4**) was applied, under the same reaction conditions, only α disaccharide (compound **5**) was isolated, which is consistent with our previous observation and literature report. It is apparent that the stereoselectivity of donor **1** can switch depending on the nature of the acceptors, even though 2-azido donors are generally considered α-selective. Acceptor dependence of stereoselectivity in glycosylation has been observed and reported by several groups before, but the total inversion of selectivity of a same glycosyl donor under that same reaction conditions is still surprising.[23–25]

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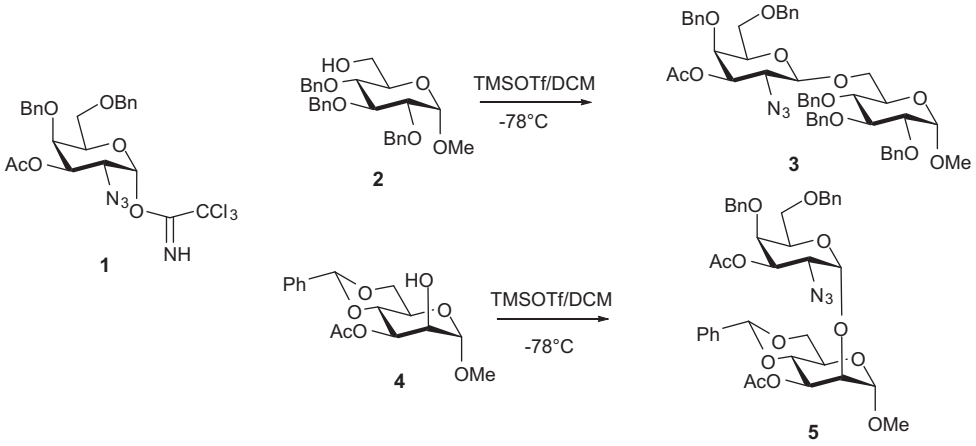
doi:10.1016/j.carres.2011.08.015



Scheme 1. Opposite stereoselectivity of donor **1** in reactions with two acceptors (**2** and **4**).

It is not obvious how this result can be explained. Based on a generally accepted mechanism and our previous experience with similar donors, we propose that there are two competing mechanisms for the formation of disaccharides: an S_N1-like mechanism through an oxocarbenium intermediate and an S_N2-like mechanism through a glycosyl triflate intermediate. Through the S_N1 like mechanism, the stereoselectivity is closely related to the conformation of the oxocarbenium ion intermediate.[26] There are two common conformations for oxocarbenium ions, $^4H_3$ and $^3H_4$ half chairs. $^4H_3$ often favors formation of α-glycoside and $^3H_4$ favors formation of β-glycoside.[10] The preference of the oxocarbenium is therefore the key factor in controlling the stereoselectivity. The preference of the conformation of the oxocarbenium can be affected by many factors.[27,28] Oxocarbenium of galatose donors has been reported to prefer $^4H_3$ halfchair.[29] In the case of donor **1**, our previous study also suggested that $^4H_3$ is the preferred conformation.[17] The S_N1 pathway would therefore most likely afford α-product. The rate determining step of S_N1 pathway should be the rate of the oxocarbenium ion formation, which should be a constant in these two reactions and independent of the reactivity of the acceptor. The S_N2 pathway, on the other hand, will involve glycosyl triflate or oxocarbenium ion pair species as intermediate.[30] The stereoselectivity will be related to the stereochemistry of these intermediates and the activation energy of the S_N2 process. In the case of donor **1**, our hypothesis is that it will favor β product due to the higher population of α-glycosyl triflate intermediate. The rate determining step of the S_N2 pathway should be the bimolecular substitution step, which is dependent on the reactivity of acceptor. Compound **2**, as a primary acceptor, must have very high nucleophilicity and the S_N2 pathway is therefore much faster than the S_N1 pathway, which afforded only β product. Compound **4**, as a secondary axial acceptor, has much lower reactivity in S_N2 reaction and the rate of S_N2 reaction is much lower than that of S_N1 pathway. The reaction of the compound therefore afforded only α-product through the S_N1 pathway. Based on this hypothesis, the stereoselectivity of this reaction totally depends on the reactivity of the acceptors. More reactive acceptor should give more β-product and less reactive acceptor should give more α-product.

The reactivity of acceptors can be affected by a lot of factors, including the stereochemistry, the order of the alcohol, the protecting groups and the hydrogen bonding.[31] It is therefore often very difficult to predict the reactivity of a glycosyl acceptor.[32] There is also lack of experimental method to determine the relative reactivity of glycosyl acceptors in glycosylations. Due to these difficulties, dependency of stereoselectivity of acceptor reactivity has been hard to understand. However, on the other hand, the stereoselectivity of this unique reactivity of donor **1** may be used as an indicator of the reactivity of corresponding acceptors in glycosylation reactions. If we can test the stereoselectivity of various glycosyl acceptors, the information gathered could be used as a reference as to the reactivity of acceptors and used for future prediction of their stereoselectivity in other reactions.

2.2. Test of a series of glycosyl acceptors

We decided to first study how the configuration of the glycosyl acceptors affect the reactivity and then the stereoselectivity of the glycosylation reaction. Eleven more glycosyl acceptors (compound **6**–**16**, Fig. 1) were therefore synthesized from commercially available methyl-α-glycosides through either literature procedures or modified procedures from literature (Fig. 1).[33–43]

All acceptors are protected with benzyl group, so that the steric and electronic effect from protecting group could be normalized. This group of acceptors includes three most common families of monosaccharide building blocks: glucose, galactose and mannose. It also covers all four possible positions of hydroxyl groups in each monosaccharide. The idea was to test and see how the structure of acceptor would affect the stereoselectivity and the relative reactivity. All these acceptors were then tested in reactions with donor **1** under the same reaction conditions. The products were isolated and characterized using NMR with results summarized in Table 1.

The results are generally consistent with our hypothesis. For example, primary acceptors, like **9** and **13**, all favor β-products in the reactions (1:4 and 1:10, respectively). At the same time, axial acceptors, like **10** and **16** all give only α-products. However, the secondary equatorial acceptors showed quite dramatic disparity in the test reactions. In case of glucose acceptors, 2-OH (acceptor **8**) gives only α-product. On the other hand, 3-OH (acceptor **7**) is β selective (1:3.4), while 4-OH (acceptor **6**) shows only low α-selectivity (1.8:1). If our theory stands, these results suggest that the order of reactivity in glucose acceptor is 6-OH > 3-OH > 4-OH > 2-OH. Based on a similar analysis, the order of reactivity of galactose acceptors is determined to be 6-OH > 2-OH > 3-OH > 4-OH. In case of mannose acceptors, the order is 6-OH > 3-OH > 4-OH > 2-OH. The relative reactivity of these acceptors, especially those with secondary equatorial hydroxyl groups, is hard to explain. We believe that it is associated with the relative energy of the transition states of the S_N2 reactions, which should be a combined result from the steric interactions of the donor and the acceptor. The results could reflect a kind of 'matchness' between the donor and the acceptor at the transition states. These results will provide useful information for understanding the relative reactivity of acceptors. It must be

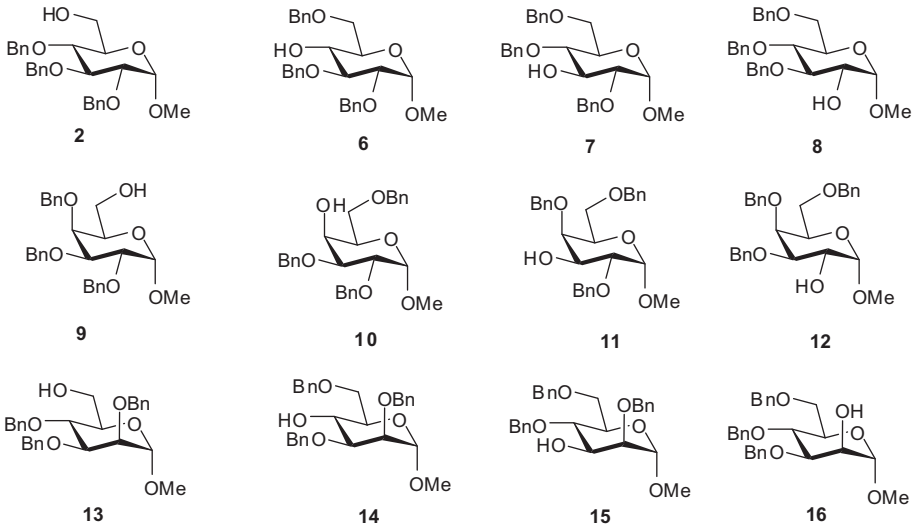
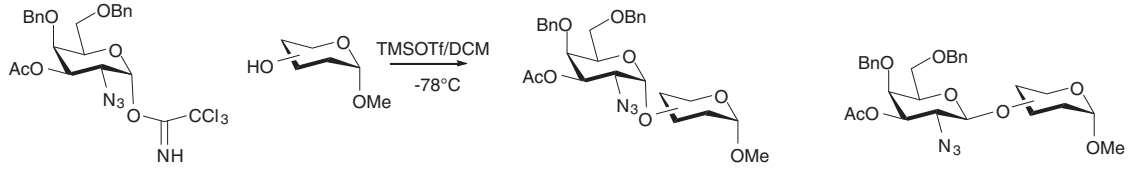


Figure 1.

Table 1



Entry	Acceptor	Sugar	OH #	Product	Yield	α:β ratio
1	**2**	Glc	6	**3**	98	β Only
2	**6**	Glc	4	**17**	56	1.8:1
3	**7**	Glc	3	**18**	53	1:3.4
4	**8**	Glc	2	**19**	68	α Only
5	**9**	Gal	6	**20**	75	1:4
6	**10**	Gal	4	**21**	63	α Only
7	**11**	Gal	3	**22**	65	3:1
8	**12**	Gal	2	**23**	90	1.3:1
9	**13**	Man	6	**24**	90	1:10
10	**14**	Man	4	**25**	81	1.2:1
11	**15**	Man	3	**26**	82	1:4.7
12	**16**	Man	2	**27**	93	α Only

kept in mind that the relative reactivity of the secondary equatorial acceptors is associated with this particular donor. Extra caution must be applied when using this data for glycosylation with other types of donors, especially donors with different configuration from galactose. Further research including both experimental and theoretical approaches to understand the origin of this disparity in stereoselectivity is underway. We are also developing protocols based on these data for more efficient stereoselective synthesis of $GalN_3$ disaccharides. The results will be reported in a timely manner.

3. Conclusion

Our research demonstrated that the stereoselectivity of the $GalN_3$ donor (**1**) is directly associated with the structure of the acceptor. Based on our hypothesis, this disparity is most likely associated with the difference in reactivity of the acceptors derived from the steric factors. These reactions also provide a unique way to characterize the relative reactivity of different acceptors under similar electronic environment. Even though we cannot explain the relative reactivity explicitly, the data obtained in this research could be a good reference for future study of glycosylation reaction of similar acceptors.

4. Experimental

4.1. Materials and general methods

Unless otherwise noted, reagents and solvents were obtained from commercial suppliers and were used without further purification. TLC was performed on pre-coated aluminum plates (Silica Gel F_{254}). Spots were visualized by exposure to UV light or by immersion in *p*-anisaldehyde solution followed by heating. All NMR spectra were recorded on a 360 MHz spectrometer. All proton NMR data were obtained at 360 MHz and all carbon NMR data were obtained at 90 MHz. Proton and carbon chemical shifts are reported in parts per million (ppm) using $CDCl_3$ as an internal reference unless otherwise noted. Coupling constants (*J*) are reported in hertz (Hz) and multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broadened (br).

4.2. General procedure for glycosylation

A solution of the acceptor (1.0 equiv), donor (1.2 equiv) and activated molecular sieves (50 mg/mL solvent) in anhydrous dichloromethane (5.0 mL/mmol donor) was stirred at room temperature for 0.5 h. The reaction was cooled to −78 °C and TMSOTf (0.15 equiv) was added followed by stirring the solution at −78 °C for 15 min, then warming to room temperature for 15 min. It was quenched by addition of Et_3N, concentrated and purified using flash column chromatography to give the products.

4.3. Determination of α/β product ratio

The determination of the ratio of the anomers was achieved in two steps. First, the relative quantity of the α/β anomers was qualitatively determined by characteristic NMR signals, including ^1H NMR of the H1′ and H3′ and ^{13}C NMR of C1′. The ratio was then determined quantitatively using the following diagnostic protons. The identity of these diagnostic protons was established through comparison of the integration with the characteristic protons.

Compound	Diagnostic protons	
	δ_α (ppm)	δ_β (ppm)
17	2.05 (s, 3H, CH_3, OAc)	2.03 (s, 3H, CH_3, OAc)
18	3.29 (s, 3H, OCH_3)	3.28 (s, 3H, OCH_3)
20	2.08 (s, 3H, CH_3, OAc)	2.02 (s, 3H, CH_3, OAc)
22	2.06 (s, 3H, CH_3, OAc)	2.04 (s, 3H, CH_3, OAc)
23	2.06 (s, 3H, CH_3, OAc)	2.02 (s, 3H, CH_3, OAc)
24	2.04 (s, 3H, CH_3, OAc)	2.01 (s, 3H, CH_3, OAc)
25	3.37 (s, 3H, OCH_3)	3.34 (s, 3H, OCH_3)
26	3.35 (s, 3H, OCH_3)	3.30 (s, 3H, OCH_3)

Acknowledgment

The financial support for the research is provided by Binghamton University Research Foundation.

Supplementary data

Supplementary data (synthesis and characterization of acceptors **2**, **6**–**16** and selected disaccharide products) associated with this article can be found, in the online version, at doi:10.1016/j.carres.2011.08.015.

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