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Saccharides

Efficient Synthesis of the Disialylated Tetrasaccharide Motif in N-Glycans through an Amide-Protection Strategy

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Abstract: A disialylated tetrasaccharide, Neu5Ac(α2,3)-Gal(β1,3)[Neu5Ac(α2,6)]GlcNAc (**1**), which is found at the termini of some N-glycans, has been synthesized. Compound **1** was obtained through an α-sialylation reaction between a sialic acid donor and a trisaccharide that was synthesized from the glycosylation of a sialylated disaccharide with a glucosaminyl donor. This synthetic route enabled the synthesis of the as-described disialylated structure. A more-convergent route based on the glycosylation of two sialylated disaccharides was also established to scale up the synthesis. Protection of the amide groups in the sialic acid residues significantly increased the yield of the glycosylation reaction between the two sialylated disaccharides, thus suggesting that the presence of hydrogen bonds on the sialic acid residues diminished their reactivity.

Introduction

Asparagine-linked oligosaccharides (N-glycans) are glycans of glycoproteins that possess high diversity and complexity. These compounds are involved in a variety of biological events, such as cell–cell recognition, adhesion, signal transduction, and protein quality control.[1] For example, the presence of sialic acids on N-glycans enhanced the circulatory residence time of the glycoproteins in blood.[2] Some complex N-glycans have characteristic partial structures, such as a core fucose at position 6 of the initial *N*-acetylglucosamine (GlcNAc) and a bisecting GlcNAc moiety in a (β1,4)-linkage to the mannose of the trimannosyl core, both of which have shown particular biological properties.[3]

The structures of disialylated tetrasaccharide (Neu5Ac(α2,3)-Gal(β1,3)[Neu5Ac(α2,6)]GlcNAc) and monosialylated trisaccharide Gal(β1,3)[Neu5Ac(α2,6)]GlcNAc were identified among the N-glycans that were isolated from fetuin and bovine blood coagulation factor X.[4] These structures were also found in the gangliosides of human colonic adenocarcinoma and in human milk.[5] The physiological roles of these oligosaccharide motifs have not yet been elucidated. Therefore, we planned to synthesize these motifs in an effort to investigate their biological functions. In a previous study, we synthesized the trisaccharide motif;[6] herein, we synthesized compound **1**, which contained the disialylated tetrasaccharide motif.

Compound **1** (Figure 1) includes an allyl glycoside moiety, which may be used for bioconjugation to thiol compounds. One of the keys to synthesizing the disialylated motif in compound **1** is the stereoselective α-sialylation reaction. Thus, we developed efficient and highly stereoselective (α2,6)- and (α2,3)-sialylation reactions of galactose derivatives by utilizing a sialyl donor with an *N*-phenyl trifluoroacetimidate leaving group[7] and C5-phthalimide or azide functionalities.[8]

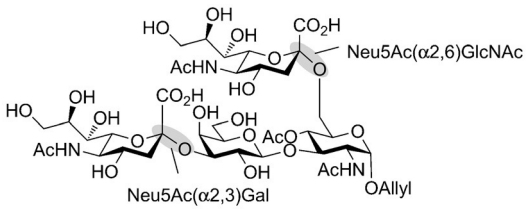

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Figure 1. Structure of Neu5Ac(α2,3)Gal(β1,3)[Neu5Ac(α2,6)]GlcNAc.

We recently reinvestigated the α-sialylation reaction by using a readily available C5–NHAc donor that had been described as possessing "modest to low" reactivity.[9] We found that the key to obtaining high yield and high selectivity with the C5–NHAc donor was strict temperature control (around −80 °C), with the careful addition of trimethylsilyl trifluoromethanesulfonate (TMSOTf). Thus, Sia(α2,6)Gal, Sia(α2,3)Gal, and

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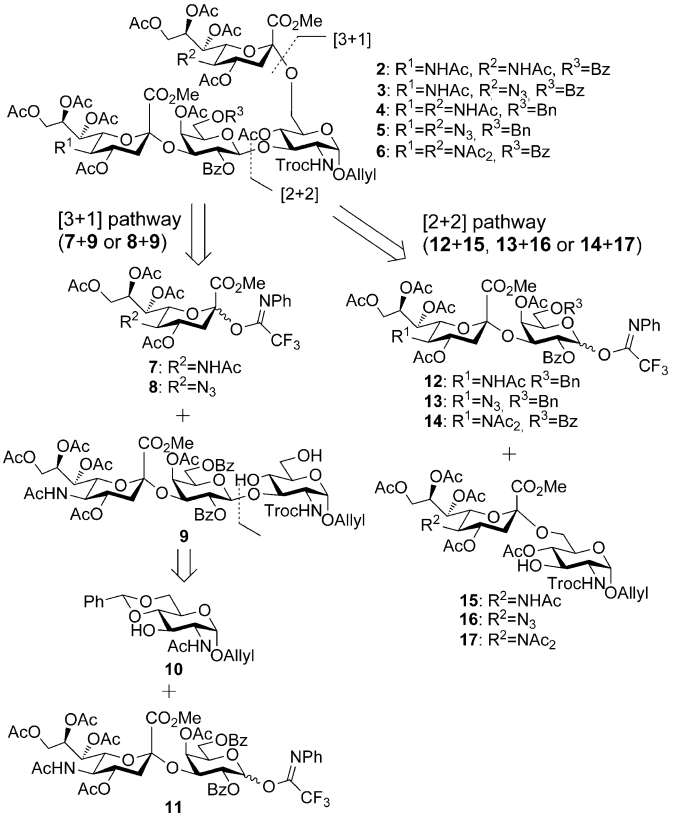
Sia(α2,6)GlcN were obtained in excellent yields with high α-selectivities, and the large-scale preparation of these compounds was achieved by using microfluidic synthesis methods that enabled the efficient mixing and rapid removal of the reaction heat. Herein, we used C5–azide and C5–NHAc donors in the sialylation reactions.

We considered two pathways for the synthesis of compound **1**: "[3+1]" and "[2+2]" pathways (Scheme 1). Ando et al. synthesized (α2,3)/(α2,6)-disialyl lactotetraosyl ceramide, which contained the tetrasaccharide motif. For the synthesis of the hexasaccharide moiety, they employed "[5+1]" and "[4+2]" pathways, which corresponded to our [3+1] and [2+2] pathways, respectively.[10]

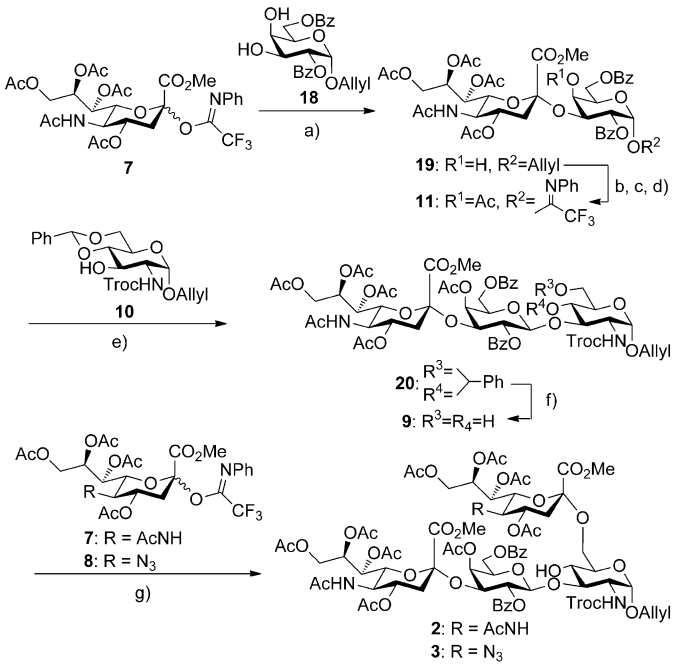


Scheme 1. Planned synthesis of Neu5Ac(α2,3)Gal(β1,3)[Neu5Ac(α2,6)]GlcNAc.

Herein, the target tetrasaccharide (**2** or **3**) was first obtained through a [3+1] pathway by α-sialylation with sialyl donors **7** or **8** by using trisaccharide acceptor **9**. However, the selectivity of the sialylation reaction was low. In the [2+2] pathway, the reactivity of the glycosylation reaction between compounds **12** and **15** was extremely low, whilst the yield of the glycosylation reaction between compounds **13** and **16** was not good. The use of *N,N*-diacetylated sialic acid donor **14** and acceptor **17** constituted another key to the successful synthesis of compound **1** through the [2+2] pathway. Demchenko and Boons previously reported that the *N,N*-diacetylated sialic acid donor had higher reactivity than the monoacetylated donor.[11] Herein, we found that acetyl protection of the C5–NHAc moiety in the sialic acid residue significantly increased the yield of the glycosylation reaction between the two sialylated disaccharides (**14** and **17**) in the [2+2] pathway, thus suggesting that the presence of hydrogen bonds on the sialic acid residues decreased the reactivity. The synthesis of tetrasaccharide **1** is described in detail below.

Results and Discussion

First, we investigated the "[3+1]" pathway (Scheme 2). Stereoselective sialylation of the 2,6-di-*O*-benzoylated galactose acceptor (**18**) with C5–NHAc sialyl donor **7**[9] was performed in a similar manner to the previously reported method, that is, under strict temperature control at −78 °C with the careful addition of TMSOTf.[9] The desired disaccharide (**19**) was obtained in 70% yield with excellent α-selectivity (α/β = 99:1). The disaccharide Neu5Acα(2,3)Gal donor (**11**) was obtained through acetylation of the galactose C4–OH moiety, followed by cleavage of the allyl group by using an iridium complex, (1,5-cyclooctadiene)bis(methyldiphenylphosphine) iridium(I)hexafluorophosphate, and the introduction of phenyltrifluoroacetimidate.[7] Glycosylation of glucosamine acceptor **10** with compound **11** was performed in CH_2Cl_2 by using TMSOTf as an activator to provide trisaccharide Neu5Ac(α2,3)Gal(β1,3)GlcNAc (**20**) in 80% yield. After cleavage of the benzylidene group of compound **20** under acidic conditions, we performed the sialylation of tri-



Scheme 2. Synthesis of tetrasaccharides **2** or **3** by using a "[3+1]" strategy; reagents and conditions: a) compound **18**, TMSOTf, EtCN, −78 °C to RT (70% yield, α/β = 99:1); b) Ac_2O, pyridine (quantitative yield); c) (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I)PF_6, H_2, THF, water, I_2 (quantitative yield); d) *N*-phenyltrifluoroacetimidoyl chloride, K_2CO_3, DMF (quantitative yield); e) compound **10**, TMSOTf, CH_2Cl_2, 4Å molecular sieves (80% yield); f) TFA/water/CH_2Cl_2 = 1:1:10, 0 °C to RT (70% yield); g) compound **7** or **8**, TMSOTf, EtCN, −78 °C to RT (compound **2**: 64% yield, α/β = 2:1; compound **3**: 90% yield, α/β = 1.5:1).

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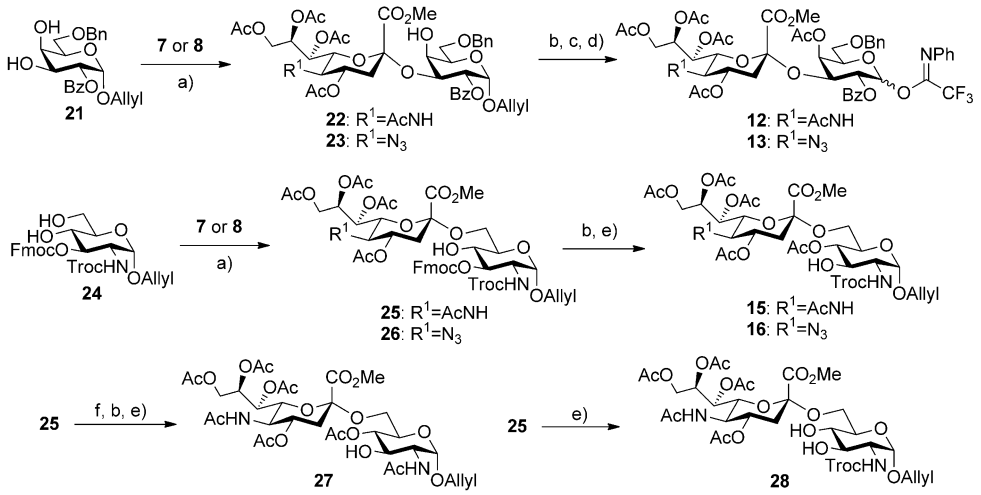
saccharide acceptor **9** by using two different sialyl donors: C5–NHAc sialic acid donor **7** and C5–azide donor **8**.[8b,9] Sialylation with C5–NHAc donor **7** afforded compound **2** in 64% yield, with an α/β selectivity of 2:1. On the other hand, C5–azide donor **8** showed higher reactivity: Sialylation of compound **9** with azidosialic acid donor **8** gave compound **3** in improved yield (90%), with lower α-selectivity (α/β = 1.5:1) compared with the reaction with C5–NHAc sialic acid donor **7**. Separation of the α and β isomers by column chromatography on silica gel was difficult; therefore, we explored a more-efficient method of synthesizing the tetrasaccharide.

Next, we investigated the "[2+2]" pathway, in which the tetrasaccharide structure was constructed through a glycosylation reaction between disaccharides Sia(α2,3)Gal and Sia(α2,6)GlcNAc. First, α-sialylation of 2-*O*-benzoyl-6-*O*-benzyl galactose derivative **21** was performed by using the C5–NHAc and the C5–azide sialyl trifluoroacetimidates (**7** and **8**) as donors.[8b,9] Thus, the desired disaccharides (**22** and **23**) were obtained in satisfactory yields and excellent selectivities (compound **22**: 86% yield, α only; **23**: quantitative yield, α/β = 19:1; Scheme 3). Thus, galactose acceptor **21** showed higher reactivity than 2,6-di-*O*-benzoylated acceptor **18**. Disaccharides **22** and **23** were converted into donors **12** and **13**, respectively.

For the synthesis of the sialyl glucosamine unit, sialylation with glucosamine acceptor **24** was performed by using C5–NHAc and C5–azide sialyl donors **7** and **8** to afford sialylated disaccharides **25** and **26**, respectively, in good yields and selectivities (compound **25**: 95% yield, α only; compound **26**: 89% yield, α/β = 10:1). After protecting the C4 position of glucosamine, the fluorenylmethyloxycarbonyl (Fmoc) group was cleaved to give disaccharide acceptors **15** and **16**. C2–NHAc acceptor **27** was prepared from compound **25** to decrease the steric hindrance introduced by the 2,2,2-trichlorethoxycarbonyl (Troc) group at the C2 position of glucosamine. Acceptor **28**, which possessed two hydroxy groups at the 3- and 4-positions of glucosamine, was also prepared by cleavage of the Fmoc group of compound **25**.

With the donors and acceptors in hand, we next investigated the synthesis of the tetrasaccharide (Table 1). Glycosylation of C5′–NHAc donor **12** and acceptor **15** did not proceed at all (Table 1, entry 1). Glycosylation of compound **12** with the less-hindered C2–NHAc acceptor **27** yielded the desired tetrasaccharide (**29**) in low yield (Table 1, entry 2), whereas the reaction of compound **12** with 3,4-di-OH acceptor **28** only gave trace amounts of tetrasaccharide **30** (Table 1, entry 3). On the other hand, the glycosylation reaction between C5–azide sialylated disaccharide donor **13** and acceptor **16** provided the desired product (**5**) in 50% yield (Table 1, entry 4). However, this yield was unsatisfactory, and the preparation of C5–azide sialic acid required several reaction steps. Thus, we investigated a simpler and more-efficient route to the tetrasaccharide.

These results revealed that modifying the C5-nitrogen atom of the sialic acid dramatically affected the outcome of the glycosylation reaction between the sialylated saccharides. It is possible that the hydrogen-bonding network that was formed by the C5–NHAc moieties of sialic acid decreased their reactivity. Although these hydrogen-bonding effects were reported by Kononov et al.[12] to be essential for the sialylation reaction to proceed, the authors did not report that a hydrogen bond involving NHAc, some distance from the reactive site, also affected the outcome of the glycosylation reaction. In view of these considerations, we investigated a synthesis of the tetrasaccharide through glycosylation of the *N,N*-diacetyl sialyl disaccharide donor (**14**) and acceptor (**17**; Scheme 4). Compounds **14** and **17** were readily prepared from compounds **19** and **25**, respectively, as described below: After the acetylation of compound **19** by using isopropenyl acetate and catalytic amounts of *p*-TsOH·H_2O,[11a] (*N*-phenyl)trifluoroacetimidate was introduced to



Scheme 3. Synthesis of the sialylated disaccharide; reagents and conditions: a) compound **7** or **8**, TMSOTf, EtCN, −78 °C, 4Å molecular sieves (compound **22**: 86% yield, α only; compound **23**: quantitative yield, α/β = 19:1; compound **25**: 95% yield, α only; compound **26**: 89% yield, α/β = 10:1); b) Ac_2O, pyridine; c) (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I)PF_6, H_2, THF, water, I_2; d) *N*-phenyltrifluoroacetimidoyl chloride, K_2CO_3, acetone (compound **12**: quantitative yield in three steps; compound **13**: 84% yield in three steps); e) 15% Et_3N, CH_2Cl_2 (compound **15**: 83% yield in two steps; compound **16**: 83% yield in two steps; compound **27**: 84% yield in three steps; compound **28**: 98% yield); f) zinc–copper couple, 1,4-dioxane, acetic acid. Ac_2O = acetic anhydride, Bn = benzyl.

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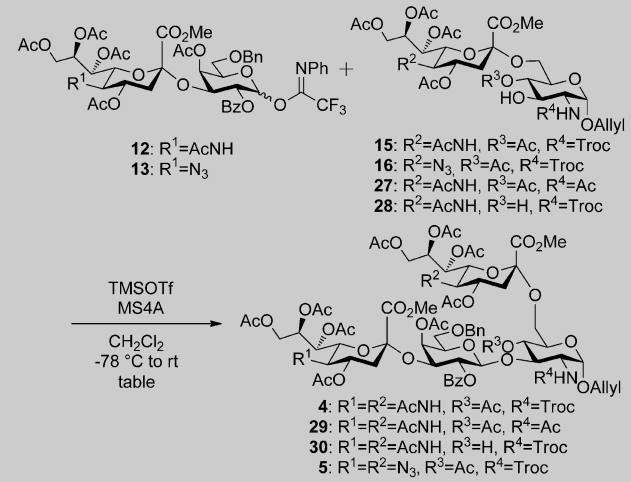
Table 1. Glycosylation reactions between the sialylated donors and acceptors.



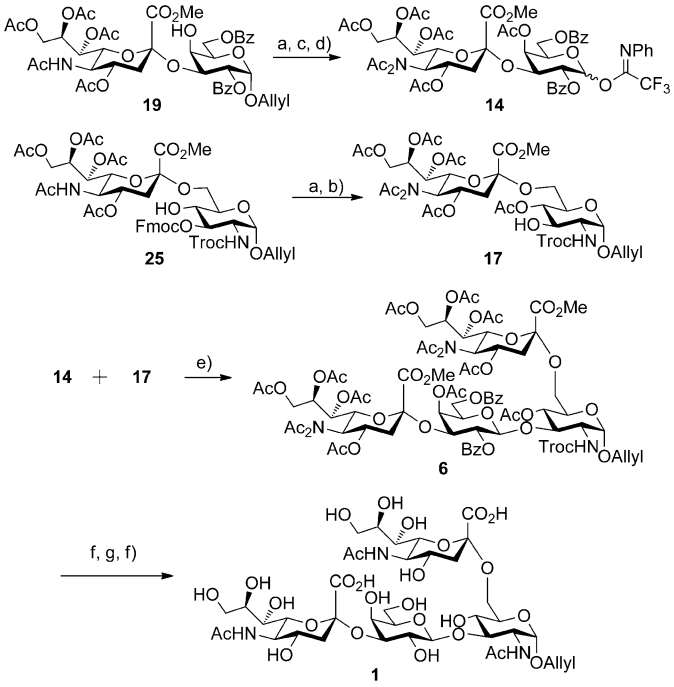
Entry	Donor	Acceptor	Poduct	Result
1	**12**	**15**	**4**	0%
2	**12**	**27**	**29**	26%
3	**12**	**28**	**30**	Trace
4	**13**	**16**	**5**	50%

Entry	Donor	Acceptor	Product	Yield [%]
1	**12**	**15**	**4**	0
2	**12**	**27**	**29**	26
3	**12**	**28**	**30**	trace
4	**13**	**16**	**5**	50

give *N*,*N*-diacetyl donor **14**. Compound **17** was prepared by introducing an acetyl group onto the amide, followed by removal of the Fmoc group. Glycosylation of compound **14** with compound **17** was then carried out as follows: TMSOTf (1 equiv) was added to a solution of compound **14** (2 equiv) and compound **17** (1 equiv) in CH_2Cl_2 in the presence of 4 Å molecular sieves at room temperature and the mixture was stirred overnight. The reaction proceeded smoothly to afford the desired tetrasaccharide (**6**) in quantitative yield. This result indicated that the NHAc group in sialic acid significantly affected the outcome of the glycosylation reaction, presumably by modulating the intermolecular hydrogen-bonding network. The desired tetrasaccharide (**1**) was obtained through deprotection of compound **6**.

Conclusion

Disialylated tetrasaccharide Neu5Ac(α2,3)Gal(β1,3)[Neu5Ac(α2,6)]GlcNAc was synthesized by using two distinct strategies: "[3+1]" and "[2+2]" syntheses. In the [3+1] strategy, a sugar chain was elongated in a stepwise manner, but the stereoselectivity of the second α-sialylation reaction was found to be low. The [2+2] strategy offered a convergent synthetic route in which two sialylated disaccharides were prepared by effectively coupling the α-sialylation reactions. Our investigations into the glycosylation reaction of sialylated disaccharides revealed that the hydrogen-bonding effects of the sialic acid



Scheme 4. Synthesis of tetrasaccharide **1** by using a "[2+2]" strategy; reagents and conditions: a) isopropenyl acetate, *p*-TsOH·H_2O, 95 °C; b) (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I)PF_6, H_2, THF, water, I_2; c) *N*-phenyltrifluoroacetimidoyl chloride, acetone, K_2CO_3, (compound **14**: 98% yield in three steps); d) 15% Et_3N, CH_2Cl_2 (compound **17**: 66% yield in two steps); e) TMSOTf, CH_2Cl_2, 4Å molecular sieves, RT, overnight (quantitative yield); f) LiOH, water/1,4-dioxane/THF = 1:2:4, g) Ac_2O, $NaHCO_3$, water (quantitative yield in three steps). TsOH = *p*-toluenesulfonic acid, Bz = benzoyl.

were essential not only in the α-sialylation reactions, but also in the glycosylation of sialylated disaccharides.

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Keywords: amides · carbohydrates · N-glycans · protecting groups · tetrasaccharides

[1] *Comprehensive Glycoscience: From Chemistry to Systems Biology, Vol. II and III* (Eds.: J. P. Kamerling, G.-J. Boons, Y. C. Lee, A. Suzuki, N. Taniguchi, A. G. J. Voragen), Elsevier, Utrecht, **2007**.

[2] a) A. G. Morell, R. A. Irvine, I. Sternlieb, I. H. Scheinberg, *J. Biol. Chem.* **1968**, *243*, 155—159; b) S. Elliott, T. Lorenzini, S. Asher, K. Aoki, D. Brankow, L. Buck, L. Busse, D. Chang, J. Fuller, J. Grant, N. Hernday, M. Hokum, S. Hu, A. Knudten, N. Levin, R. Komorowski, F. Martin, R. Navarro, T. Osslund, G. Rogers, N. Rogers, G. Trail, J. Egrie, *Nat. Biotechnol.* **2003**, *21*, 414 – 421; c) M. Sato, T. Furuike, R. Sadamoto, N. Fujitani, T. Nakahara, K. Niikura, K. Monde, H. Kondo, S. I. Nishimura, *J. Am. Chem. Soc.* **2004**, *126*, 14013 – 14022.

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[3] M. Takahashi, Y. Kuroki, K. Ohtsubo, N. Taniguchi, *Carbohydr. Res.* **2009**, *344*, 1387 – 1390.
[4] a) S. Takasaki, A. Kobara, *Biochemistry* **1986**, *25*, 5709 – 5715; b) T. Mizuochi, K. Ymashita, K. Fujikawa, K. Titami, A. Kobata, *J. Biol. Chem.* **1980**, *255*, 3526 – 3531.
[5] a) D. F. Smith, D. A. Zopf, V. Ginsburg, *Anal. Biochem.* **1978**, *85*, 602 – 608; b) Y. Fukushi, E. Nudelman, S. B. Levery, T. Higuchi, S. Hakomori, *Biochemistry* **1986**, *25*, 2859 – 2866; c) E. Nudelmant, Y. Fukushi, S. B. Levery, T. Higuchi, S. Hakomori, *J. Biol. Chem.* **1986**, *261*, 5487 – 5496.
[6] G. Bao, K. Tanaka, K. Ikenaka, K. Fukase, *Bioorg. Med. Chem.* **2010**, *18*, 3760 – 3766.
[7] S. Cai, B. Yu, *Org. Lett.* **2003**, *5*, 3827 – 3830.
[8] a) K. Tanaka, T. Goi, K. Fukase, *Synlett* **2005**, 2958 – 2962; b) K. Tanaka, Y. Fujii, H. Tokimoto, Y. Mori, S. Tanaka, G.-m. Bao, E. R. O. Siwu, A. Nakayabu, K. Fukase, *Chem. Asian J.* **2009**, *4*, 574 – 580; c) K. Tanaka, K. Fukase, *Org. Process Res. Dev.* **2009**, *13*, 983 – 990; d) Y. Uchinashi, K. Tanaka, Y. Manabe, Y. Fujimoto, K. Fukase, *J. Carbohydr. Chem.* **2014**, *33*, 55 – 67.
[9] Y. Uchinashi, M. Nagasaki, J. Zhou, K. Tanaka, K. Fukase, *Org. Biomol. Chem.* **2011**, *9*, 7243 – 7248.
[10] T. Ando, H. Ishida, M. Kiso, *Carbohydr. Res.* **2003**, *338*, 503 – 514.
[11] a) A. V. Demchenko, G.-J. Boons, *Tetrahedron Lett.* **1998**, *39*, 3065 – 3068; b) A. V. Demchenko, G.-J. Boons, *Chem. Eur. J.* **1999**, *5*, 1278 – 1283.
[12] a) L. O. Kononov, N. N. Malysheva, E. G. Kononova, A. V. Orlova, *Eur. J. Org. Chem.* **2008**, 3251 – 3255; b) L. O. Kononov, N. N. Malysheva, A. V. Orlova, *Eur. J. Org. Chem.* **2009**, 611 – 616; c) L. O. Kononov, N. N. Malysheva, A. V. Orlova, A. I. Zinin, T. V. Laptinskaya, E. G. Kononova, N. G. Kolotyrkina, *Eur. J. Org. Chem.* **2012**, 1926 – 1934.

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