Acyl and Silyl Group Effects in Reactivity-Based One-Pot Glycosylation: Synthesis of Embryonic Stem Cell Surface Carbohydrates Lc(4) and IV(2)Fuc-Lc(4)

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · MARCH 2012

Impact Factor: 12.11 · DOI: 10.1021/ja300284x · Source: PubMed

7 AUTHORS, INCLUDING:

Medel Manuel L Zulueta
Academia Sinica
30 PUBLICATIONS **310** CITATIONS
SEE PROFILE

Kuo-I Lin
Academia Sinica
35 PUBLICATIONS **2,181** CITATIONS
SEE PROFILE

Cheng-Chung Wang
Academia Sinica
41 PUBLICATIONS **1,424** CITATIONS
SEE PROFILE

JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
pubs.acs.org/JACS

Acyl and Silyl Group Effects in Reactivity-Based One-Pot Glycosylation: Synthesis of Embryonic Stem Cell Surface Carbohydrates Lc_4 and $IV^2Fuc\text{-}Lc_4$

Yun Hsu,[†,‡] Xin-An Lu,[†] Medel Manuel L. Zulueta,[†] Chih-Ming Tsai,[†] Kuo-I Lin,[†] Shang-Cheng Hung,*[,†,§] and Chi-Huey Wong*[,†]

[†]Genomics Research Center, Academia Sinica, 128, Section 2, Academia Road, Taipei 115, Taiwan
[‡]Department of Chemistry, National Tsing Hua University, 101, Section 2, Kuang-Fu Road, Hsinchu 300, Taiwan
[§]Department of Applied Chemistry, National Chiao Tung University, 1001, Ta-Hsueh Road, Hsinchu 300, Taiwan

Supporting Information

ABSTRACT: Relative reactivity evaluations showed the graded arming of toluenyl thioglucosides by variously positioned silyl groups but not by their acyl counterparts. These findings were applied in reactivity-based one-pot assembly of linker-attached Lc_4 and $IV^2Fuc\text{-}Lc_4$, which are components of human embryonic stem cell surface. The sugar−galectin-1 binding was also examined.

Carbohydrates are commonly found at the cell surface, aiding recognition, adhesion, and signal transduction events.[1] Particularly abundant are glycosphingolipids (GSLs), which have sugar components attached to ceramide. GSLs are diverse and can be further subdivided into ganglio-, globo-, isoglobo-, lacto-, and neolacto series on the basis of their core sequence and connectivities.[2] The variety and quantity of GSLs differ among cell types at various developmental stages as well as in cancer progression.[3] For example, human embryonic stem cells highly express globo- and lacto-series GSLs, but upon differentiation to embryoid body outgrowth cells, these GSLs are downregulated, and the expressions of gangliosides increase.[4] The lacto-series GSLs explicitly detected are lactotetraosyl (Lc_4) and 2‴-*O*-fucosyl-Lc_4 ($IV^2Fuc\text{-}Lc_4$) ceramide (Figure 1). Lc_4 carries the core sequence common to all

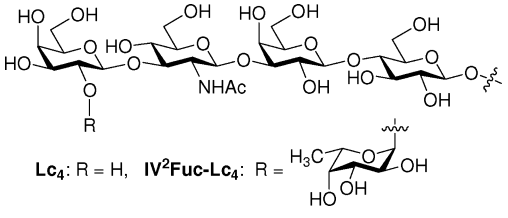


Figure 1. The structures of Lc_4 and $IV^2Fuc\text{-}Lc_4$.

lacto-series GSLs, and $IV^2Fuc\text{-}Lc_4$ contains the H type 1 antigen. We report herein the chemical synthesis of these carbohydrates through a reactivity-based one-pot strategy. Their interactions with galectin-1, a prominent decoder of cell-surface information,[5] were also examined in solution.

The effect of protecting groups on glycosyl donor reactivity is well-known.[6] Initially deduced from the higher reactivity of perbenzylated over peracylated donors, the armed/disarmed concept[7] was expanded into numerical values that define the reactivities as imparted by protecting groups on glycosyl donors.[8,9] Currently, relative reactivity values (RRVs) have been assigned to hundreds of thioglycosides, allowing the one-pot assembly of many important oligosaccharides.[10] While unconventional for acyl groups, Demchenko's group reported that 2-O-benzoylated *S*-benzoxazoyl donors are considerably more reactive than their 2-O-benzylated counterparts.[11] Accordingly, it was proposed that cooperative arming arises from the ability of a 2-*O*-acyl group, via an acyloxonium ion, to stabilize the oxocarbenium ion intermediate formed during glycosylation.

Another new finding is the arming effect of silyl-based protection. Bols and co-workers[12] showed that multiple large silyl protecting groups, which are more inclined to orient axially, increase donor reactivity by minimizing the electronic interaction between the oxygen substituents and the developing positive charge. They also asserted that silyl groups are devoid of intrinsic arming electronic effects and that monosilylation, because of its marginal effect on ring conformation, is insufficient to provide a significant increase in reactivity. Confirmations of reactivity enhancement by acyl and silyl groups, however, have yet to be made using the existing RRV database.

Before moving to the oligosaccharide preparation, we systematically investigated the positional effect of acyl and bulky silyl groups on D-glucose-based thiotoluenyl donors by RRV determination.[13] Drawing on our regioselective one-pot protection strategy,[14] we prepared the full set of monoacetylated, monobenzoylated, monosilylated, and disilylated thioglucosides with benzyl groups masking the other hydroxy positions. Thioglucosides carrying a free hydroxyl at different locations were also synthesized. The 2-O position gave the highest values for the acetyl (**1**; RRV = 983) and benzoyl (**2**; RRV = 1265) groups (Figure 2). Nonetheless, we did not observe a reactivity enhancement by the 2-*O*-acyl group because the 2-alcohol **3** (RRV = 1900) and the tetrabenzylated **4** (RRV = 2656)[9] are still more reactive. Consequently, the 4-

Received: January 11, 2012
Published: March 5, 2012

   dx.doi.org/10.1021/ja300284x | *J. Am. Chem. Soc.* 2012, 134, 4549−4552

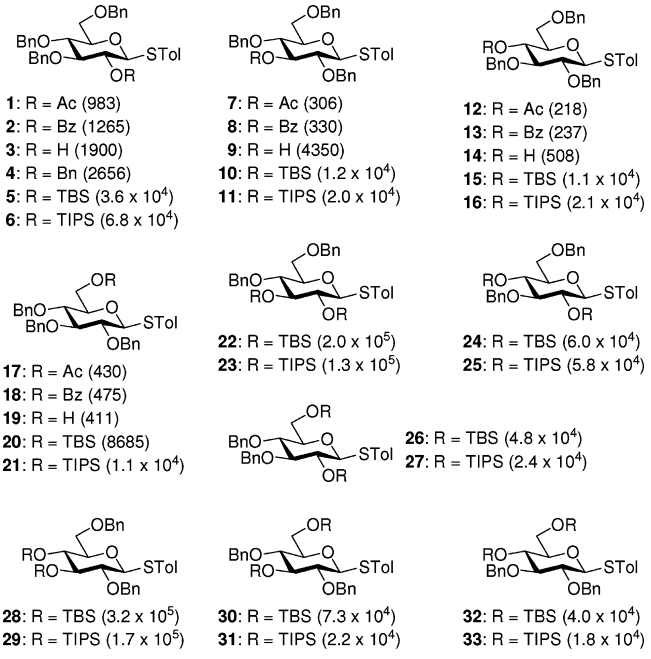


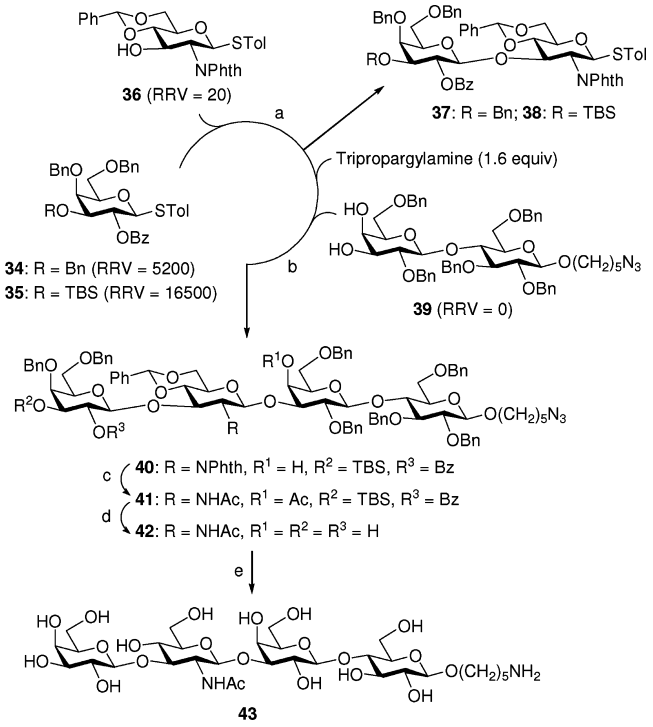
Figure 2. RRVs of different silylated, benzylated, and acylated thioglycosides (Ac, acetyl; Bz, benzoyl; Bn, benzyl; Tol, toluenyl).

O-acylated compounds **12** and **13** possessed the lowest RRVs, with an approximately 5-fold lower reactivity than the corresponding 2-O-acylated glucosides. As the 2-O and 4-O positions are on opposite sides of the pyranosyl oxygen, the presumed stabilization by the 2-*O*-acyl group on the transient oxocarbenium ion is distinct from the destabilizing 4-*O*-acyl electron-withdrawing influence. Notably, work on acid-mediated hydrolysis of methyl glucosides in water detected a minor rate increase attributed to 2-O participation.[15] Recently, the higher reaction rate manifested by 2-O-benzoylated relative to 2-O-benzylated donors was extended from *S*-benzoxazoyl to *S*-ethyl leaving groups, but differentiation was not significant for *O*-pentenyl, *S*-phenyl, *S*-toluenyl, and *S*-thiazolinyl groups.[16] The stereochemical orientation of the *S*-benzoxazoyl group was also found to be vital for rate enhancement.[17] These accounts and our results imply that the 2-*O*-acyl arming tendency is strongly modulated by the leaving group, which, although without proof, may well be extended to the reaction solvent and activator. Thus, a decrease in the leaving group's propensity for departure significantly dampens any rate effect caused by formation of the acyloxonium ion.

Replacement of benzyl with a *tert*-butyldimethylsilyl (TBS) or triisopropylsilyl (TIPS) group at different locations all increased donor reactivity. Moreover, TIPS offered a slightly better enhancement than TBS in all cases. The degree of arming was greatest at 2-O, with approximately 14-fold (**5**) and 26-fold (**6**) increases in reactivity upon exchange of benzyl with TBS and TIPS groups, respectively. As anticipated, the least reactive of the monosilylated donors were found to be the ones in which the silyloxy group is positioned at 6-C (**20** and **21**), where it has a predictably minor influence on ring conformation. Thus, we have shown here that monosilylation certainly does provide substantial reactivity enhancement. Further affirming the bulky group arming effect, disilylated donors were generally more reactive than monosilylated ones. Adjacent silyl groups gave more pronounced enhancements, consistent with the torsional effect. Relative to tetrabenzylated **4**, the reactivity increase ranged from 49-fold for the 2,3-di-*O*-TIPS derivative **23** to 120-fold for the 3,4-di-*O*-TBS derivative **28**.

For the Lc_4 assembly, the 2-O-benzoylated thiogalactoside **34**[10c] (RRV = 5200) could be used to affect the required β-linkage upon glycosylation of alcohol **36** (RRV = 20)[9] (Scheme 1). Like toluenyl thioglucoside, **34** was found to be less reactive

Scheme 1. Synthesis of Compound 43[a]



[a]Reagents and conditions: (a) 3 Å molecular sieves, 1.2 equiv of NIS, 0.4 equiv of TMSOTf, CH_2Cl_2, −55 °C, 2 h; **37**: 30%, **38**: 78%. (b) 1.2 equiv of NIS, 1.0 equiv of AgOTf, 0 °C, 10 min, **40**: 40% (one pot). (c) (1) ethylenediamine, *t*BuOH, reflux, 20 h; (2) Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt, 18 h; 78% (two steps). (d) (1) TBAF, CH_2Cl_2, rt, 18 h; (2) NaOMe, MeOH, rt, 13 h; 70% (two steps). (e) Pd/C, H_2, MeOH with 5% formic acid; 93%. Phth: phthaloyl.

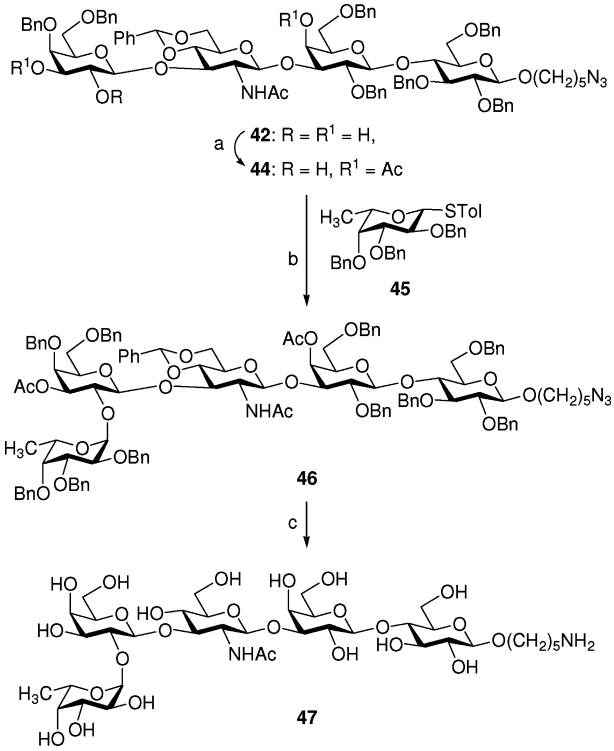
than toluenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactoside (RRV = 17 000).[9] Addition of *N*-iodosuccinimide (NIS) and trimethylsilyl triflate (TMSOTf) to the CH_2Cl_2 solution of building blocks **34** and **36** supplied the adduct **37** in a meager 30% yield. A significant amount of the unreacted alcohol **36** was also recovered. As an alternative to **34**, the 3-O-silylated thiogalactoside **35** was synthesized,[14d] and RRV measurements revealed a higher reactivity comparable in magnitude to that for the similar TBS group installation on the glucose core. Selective activation of **35** in the presence of **36** fortunately gave the disaccharide **38** in a satisfactory 78% yield, consistent with the notion that raising the donor reactivity in the presence of poorly reactive acceptors also increases the glycosylation yield.[18] Without quenching of the initial coupling step, further assembly in one pot was attempted by adding the lactosyl diol **39**[10b] followed by NIS and TMSOTf. Because the equatorial 3′-hydroxyl of **39** is more reactive than the axial 4′-hydroxyl, the β1→3 link should be formed preferentially. Unfortunately, the desired tetrasaccharide **40** was not obtained. We figured that strong acids negatively affect the outcome of the second coupling. Thus, incorporation of tripropargylamine to

dx.doi.org/10.1021/ja300284x | J. Am. Chem. Soc. 2012, 134, 4549−4552

neutralize the activator of the first coupling followed by glycosylation of **39** promoted by the mild NIS/AgOTf conditions delivered **40** in a one-pot yield of 40%. Here we also isolated the disaccharide **38** in 18% yield. Conversion of the phthalimido functionality to acetamido was carried out with ethylenediamine to afford the free amine, which was subsequently acetylated using Ac_2O and *N*,*N*-dimethyl-4-aminopyridine (DMAP). The 4′-hydroxyl was also acetylated in this step, giving the product **41** in a two-step yield of 78%. Stepwise cleavage of the silyl group by tetra-*n*-butylammonium fluoride (TBAF) and the ester moiety by NaOMe/MeOH delivered triol **42** (70%). Global hydrogenolysis led to the linker-attached Lc_4 (**43**) in 93% yield.

To construct the IV^2Fuc-Lc_4 backbone, we started by regioselective acetylation of compound **42** (Scheme 2).

Scheme 2. Synthesis of Compound 47[a]



[a]Reagents and conditions: (a) 2.1 equiv of Ac_2O, pyridine, CH_2Cl_2, rt, 18 h; 70%. (b) 3 Å molecular sieves, 1.2 equiv of NIS, 0.4 equiv of TMSOTf, CH_2Cl_2, −60 to −40 °C, 1 h; 77%. (c) (1) NaOMe, MeOH, rt, 6 h; (2) Pd/C, H_2, MeOH with 5% formic acid; 90% (two steps).

Through the steric effect of the glycosidic linkage, regioselective 3‴,4′-di-O-acetylation of **42** with Ac_2O was achieved, affording 2‴-alcohol **44** (70%). Glycosylation of **44** using fucosyl donor **45**[13] promoted by NIS/TMSOTf formed pentasaccharide **46** (77%). Subsequent deacetylation (NaOMe/MeOH) followed by hydrogenolysis (Pd/C, H_2) successfully furnished compound **47** in 90% yield.

Using isothermal titration calorimetry, we investigated the binding of compounds **43** and **47** with galectin-1, the first mammalian galectin found among 15 members.[5] Galectins are a highly conserved family of β-galactoside-binding proteins that play various roles in cancer progression, immune response, inflammation, and development by interacting with cell-surface carbohydrates.[19] As shown in Table 1, **43** (31.5 μM) and **47** (33.8 μM) gave nearly identical K_d values upon interaction with galectin-1. The synthetic sugars also bound the protein about 2 times more tightly than did lactose (71.4 μM). This implies that the fucosylation of the terminal galactose in Lc_4 does not have any sizable effect on recognition of the sugar by the protein. On the other hand, the higher affinity of **43** and **47** relative to lactose might be due to the lack of acetamido group in the latter sugar.

Table 1. Dissociation Constants (K_d) of Selected Sugars and Galectin-1 Determined Using Isothermal Titration Calorimetry[a]

entry	sugar	K_d (μM)[a]
1	compound **43**	31.5
2	compound **47**	33.8
3	lactose	71.4
4	galactose	–[b]

[a]The actual parameter measured by the technique is the association constant K_a, which is the inverse of K_d. [b]No binding was detected.

In summary, we systematically investigated the effect of acyl and bulky silyloxy groups at different locations on donor reactivity of the toluenyl thioglucoside by RRV analysis. It was found that while silyl groups possess a strong arming influence at all locations, this was not particularly demonstrated by 2-*O*-acyl groups, as originally reported for donors with *S*-benzoxazoyl leaving groups. A similar pattern was observed for toluenyl thiogalactoside. This understanding was successfully implemented in the assembly of linker-attached Lc_4 through a reactivity-based one-pot strategy. IV^2Fuc-Lc_4 was also generated using an intermediate in the Lc_4 transformation. The synthesized sugars bound galectin-1 to the same extent.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and copies of ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

schung@gate.sinica.edu.tw, chwong@gate.sinica.edu.tw

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Science Council (NSC 97-2113-M-001-033-MY3 and NSC 98-2119-M-001-008-MY2) and Academia Sinica.

■ REFERENCES

(1) *Essentials of Glycobiology*, 2nd ed; Varki, A., Cummings, R. D., Esko, J. D., Freeze, H. H., Stanley, P., Bertozzi, C. R., Hart, G. W., Etzler, M. E., Eds.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 2009.

(2) Yu, R. K.; Yanagisawa, M.; Ariga, T. In *Comprehensive Glycoscience: From Chemistry to Systems Biology*; Kamerling, J. P., Ed.; Elsevier: Amsterdam, 2007; pp 73−122.

(3) Hakomori, S. *Biochim. Biophys. Acta* **2008**, *1780*, 325−346.

(4) Liang, Y.-J.; Kuo, H.-H.; Lin, C.-H.; Chen, Y.-Y.; Yang, B.-C.; Cheng, Y.-Y.; Yu, A. L.; Khoo, K.-H.; Yu, J. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 22564−22569.

(5) (a) Camby, I.; Le Mercier, M.; Lefranc, F.; Kiss, R. *Glycobiology* **2006**, *16*, 137R−157R. (b) Boscher, C.; Dennis, J. W.; Nabi, I. R. *Curr. Opin. Cell Biol.* **2011**, *23*, 383−392.
(6) Fraser-Reid, B.; Jayaprakash, K. N.; López, J. C.; Gómez, A. M.; Uriel, C. In *Frontiers in Modern Carbohydrate Chemistry*; Demchenko, A. V., Ed.; American Chemical Society: Washington, DC, 2007; pp 91−117.
(7) Fraser-Reid, B.; Wu, Z.; Udodong, U. E.; Ottosson, H. *J. Org. Chem.* **1990**, *55*, 6068−6070.
(8) Douglas, N. L.; Ley, S. V.; Lücking, U.; Warriner, S. L. *J. Chem. Soc., Perkin Trans. 1* **1998**, 51−65.
(9) Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734−753.
(10) (a) Hsu, C.-H.; Hung, S.-C.; Wu, C.-Y.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2011**, *50*, 11872−11923. (b) Hsu, C.-H.; Chu, K.-C.; Lin, Y.-S.; Han, J.-L.; Peng, Y.-S.; Ren, C.-T.; Wu, C.-Y.; Wong, C.-H. *Chem.—Eur. J.* **2010**, *16*, 1754−1760. (c) Tsai, B.-L.; Han, J.-L.; Ren, C.-T.; Wu, C.-Y.; Wong, C.-H. *Tetrahedron Lett.* **2011**, *52*, 2132−2135.
(11) (a) Kamat, M. N.; Demchenko, A. V. *Org. Lett.* **2005**, *7*, 3215−3218. (b) Mydock, L. K.; Demchenko, A. V. *Org. Lett.* **2008**, *10*, 2103−2106. (c) Mydock, L. K.; Demchenko, A. V. *Org. Lett.* **2008**, *10*, 2107−2110.
(12) (a) Jensen, H. H.; Pedersen, C. M.; Bols, M. *Chem.—Eur. J.* **2007**, *13*, 7576−7582. (b) Pedersen, C. M.; Marinescu, L. G.; Bols, M. *Chem. Commun.* **2008**, 2465−2467. (c) Pedersen, C. M.; Nordstrøm, L. U.; Bols, M. *J. Am. Chem. Soc.* **2007**, *129*, 9222−9235.
(13) Lee, J.-C.; Greenberg, W. A.; Wong, C.-H. *Nat. Protoc.* **2007**, *1*, 3143−3152.
(14) (a) Wang, C.-C.; Lee, J.-C.; Luo, S.-Y.; Kulkarni, S. S.; Huang, Y.-W.; Lee, C.-C.; Chang, K.-L.; Hung, S.-C. *Nature* **2007**, *446*, 896−899. (b) Wang, C.-C.; Kulkarni, S. S.; Lee, J.-C.; Luo, S.-Y.; Hung, S.-C. *Nat. Protoc.* **2008**, *3*, 97−113. (c) Chang, K.-L.; Zulueta, M. M. L.; Lu, X.-A.; Zhong, Y.-Q.; Hung, S.-C. *J. Org. Chem.* **2010**, *75*, 7424−7427. (d) Huang, T.-Y.; Zulueta, M. M. L.; Hung, S.-C. *Org. Lett.* **2011**, *13*, 1506−1509. (e) Hu, Y.-P.; Lin, S.-Y.; Huang, C.-Y.; Zulueta, M. M. L.; Liu, J.-Y.; Chang, W.; Hung, S.-C. *Nat. Chem.* **2011**, *3*, 557−563.
(15) Heuckendorff, M.; Pedersen, C. M.; Bols, M. *Org. Lett.* **2011**, *13*, 5956−5959.
(16) Premathilake, H. D.; Mydock, L. K.; Demchenko, A. V. *J. Org. Chem.* **2010**, *75*, 1095−1100.
(17) Crich, D.; Li, M. *Org. Lett.* **2007**, *9*, 4115−4118.
(18) Zeng, Y.; Wang, Z.; Whitfield, D.; Huang, X. *J. Org. Chem.* **2008**, *73*, 7952−7962.
(19) (a) Leffler, H.; Carlsson, S.; Hedlund, M.; Qian, Y.; Poirier, F. *Glycoconjugate J.* **2004**, *19*, 433−440. (b) Di Lella, S.; Sundblad, V.; Cerliani, J. P.; Guardia, C. M.; Estrin, D. A.; Vasta, G. R.; Rabinovich, G. A. *Biochemistry* **2011**, *50*, 7843−7857.