OL | Organic Letters

pubs.acs.org/OrgLett Letter

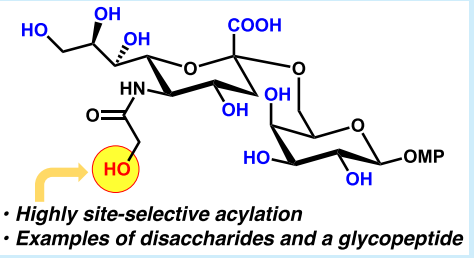
Site-Selective Acylations of α- and β-Hydroxyamides in Complex Molecules: Application of Template-Driven Acylation to Disaccharides and a Glycopeptide

Yasuhiro Nishikawa,* Shione Toda, Takami Matsui, Hanae Takada, Kohei Takemoto, and Osamu Hara*

Cite This: *Org. Lett.* 2021, 23, 2715–2719 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Site-selective acylations of α-and β-hydroxyamides in complex polyols are described. The combination of a pyridine aldoxime ester and $Zn(OTf)_2$ facilitates the acylation of two types of *N*-glycolyl disaccharides, namely, Gal-GlcNGc and Neu5Gc-Gal, both of which are partial structures of polysaccharides responsible for biological actions, with highly site-selective modifications achieved. Furthermore, biotinylation, one of the most important techniques in chemical biology, is used to site-selectively acylate the β-hydroxyl group in a glycopeptide.



Carbohydrates and glycosides are important biological molecules involved in dynamic biological phenomena, including various intercellular signal transduction processes on the surfaces of cells.[1] The key structural feature of this carbohydrate family is the polyol function, which plays complex biological-activity roles through hydrogen-bonding interactions. The ability to chemically modify specific hydroxyl groups in the polyol structure is a powerful tool for exploiting the functions of this class of compound for biological research and pharmaceuticals.[2] In synthetic chemistry, however, the desirable site-selective manipulation of a specific functional group among multiple functional groups has been a formidable challenge that often requires protection−deprotection sequences. Therefore, much effort has been dedicated to achieving direct methods for the site-selective functionalization of nonprotected polyols.[3] Whereas most methods have been developed for the selective functionalizations of relatively simple molecules, such as diols or triols, their applications to more complex molecules, such as natural products and bioactive compounds, are quite limited.[4] As a pioneering work in this field, Miller and coworkers reported the regioselective acylations of erythromycin A with peptide-based organocatalysts.[4h] As another example, Kawabata and coworkers reported the regioselective acylation of digitoxin with a chiral DMAP-based catalyst.[4f] In this context, we successfully site-selectively acylated α- and β-hydroxyamides in polyols **1α** and **1β** by combining Lewis acids and 2-pyridyl oxime esters **2** as mild acylating reagents (Figure 1A).[5] The formation of a metal complex preferentially coordinated by two bidentate molecules, namely, α- and β-hydroxyamide **1** and **2**, facilitates the selective acylation of **1** in preference to other hydroxyl groups due to the proximity effect. Methyl-substituted Cu(I)−ketoxime ester complexes effectively acylate β-hydroxyamides **1β**, probably because the methyl group in **2β** (R^3 in Figure 1A) amplifies the proximity effect by positioning the ester moiety toward the targeted hydroxyl group in the transition state.[6] In nature, the α-hydroxyamide structure has been observed in a variety of *N*-glycolylsaccharides.[7] For example, *N*-glycolylneuraminic acid (Neu5Gc), a representative sialic acid analogue, is not expressed at significant levels on healthy human tissue but is expressed on tumor tissue as glycans; hence Neu5Gc is a potential biomarker for the early diagnosis of cancer and cancer therapy.[8,9] Whereas we examined the site-selective chemical modifications of *N*-glycolyl monosaccharides, there were insufficient examples that demonstrated the utility of our system (Figure 1B).[5] Complex polyols, such as natural polysaccharides, can exhibit unpredictable behavior that interferes with functionalization, which is ascribable to the disposition of the hydroxyl groups in the steric environment, including higher order structures. Developing a robust site-selective modification method requires insight gained by examining new methodologies in complex systems. Herein we report the metal-template-driven acylations of more complex polyol compounds, namely, disaccharides for α-hydroxyamides and an *N*-linked glycopeptide for a β-hydroxyamide.

In previous studies, *N*-glycolylglucosamine (**1a**) and *N*-glycolylneuraminic acid (**1b**) were acylated in contrasting chemical yields (Figure 1B). Prior to investigating the site-

Received: February 20, 2021
Published: March 18, 2021

https://doi.org/10.1021/acs.orglett.1c00612
Org. Lett. 2021, 23, 2715–2719

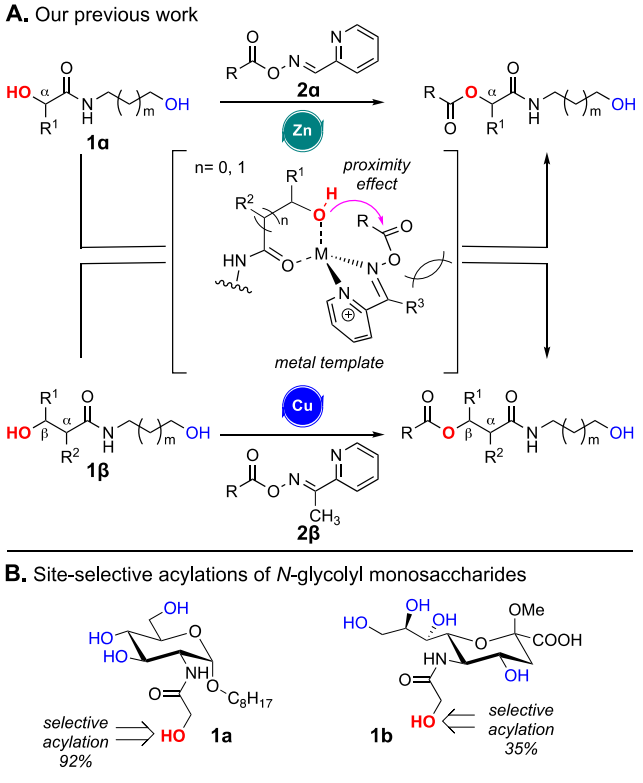
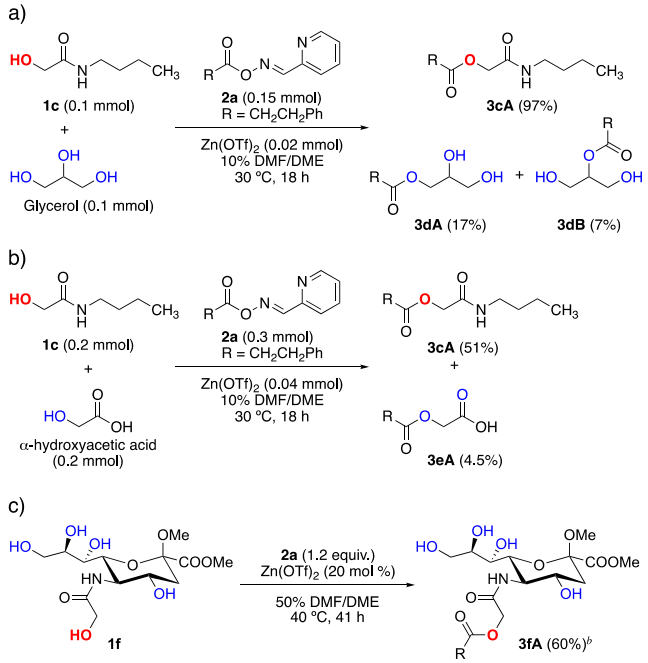


Figure 1. Site-selective acylations of α- and β-hydroxyamides using the metal-template strategy.

selective acylations of disaccharides with the *N*-glycolylglucosamine and *N*-glycolylneuraminic acid units, which are partial polysaccharide structures, we examined the origin of the low yield observed during the acylation of **1b** to gain insight into the reaction mechanism in our system. To evaluate the effect of the 1,2,3-triol in **1b**, we carried out a control experiment using α-hydroxyamide **1c** and glycerol as a model 1,2,3-triol (Scheme 1a). An intermolecular competition reaction using 1.5 equiv of the acylating reagent **2a** in the presence of 0.2 equiv of $Zn(OTf)_2$ resulted in the complete acylation of **1c** to afford **3cA** in 97% yield along with small amounts of **3dA** and **3dB**, which reveals that the α-hydroxyamide is much more rapidly acylated than the 1,2,3-triol. On the contrary, a similar control experiment, in which α-hydroxyacetic acid was used instead of glycerol, led to the incomplete acylation of **1c** (51% yield; Scheme 1b).[10] These results indicate that the low yield obtained from the acylation of **1b** is ascribable to the presence of the carboxylic acid moiety, probably due to ionic interactions with $Zn(OTf)_2$ that inhibit the formation of the desired metal template. In fact, the direct acylation of the methyl ester **1f** of *N*-glycolylneuraminic acid (**1b**) under otherwise identical conditions produced monoacylate **3fA** in higher yield than the product obtained by acylating **1b** (Scheme 1c).

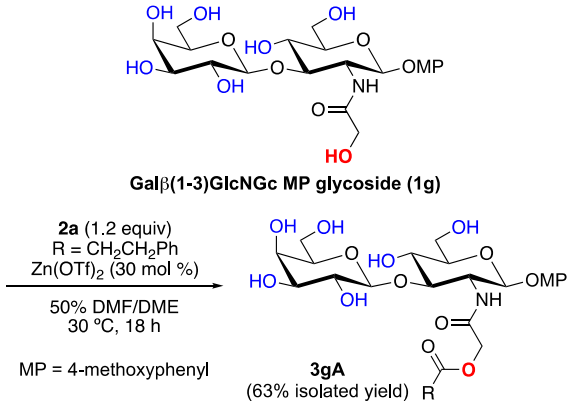
Having obtained complementary results that reveal the preferences of the substrates in our system, we turned our attention to site-selectively acylating disaccharides. First, disaccharide **1g**, an *N*-glycolyl equivalent of Galβ(1−3)-GlcNAc, the partial structure of the terminal oligosaccharides in the blood group antigen, was prepared from a commercially available disaccharide (Scheme 2).[11] The site-selective acylation of **1g** was envisaged to be challenging because the six other hydroxyl groups in **1g** can compete with the targeted

Scheme 1. Control Experiments[a]



[a]Yields were determined by ^{1}H NMR analysis of the unpurified reaction mixtures. [b]Isolated yield.

Scheme 2. Site-Selective Acylation of Disaccharide 1g



α-hydroxyl group during acylation. Accordingly, we investigated the acylation of **1g** under slightly modified standard conditions by analyzing the reaction mixture by ^{1}H NMR spectroscopy. The ^{1}H NMR spectrum of the reaction mixture was almost identical to that of pure **3gA**, which indicates that the acylation of **1g** proceeded in a highly site-selective manner.[12] Overall, monoacylated **3gA** was obtained in 63% isolated yield after high-performance liquid chromatography (HPLC) purification. This result suggests that the reactive primary alcohols in the two carbohydrates do not interfere with our α-acylation method.

We next demonstrated the site-selective acylation of Neu5Gcα(2,6)Galβ methoxyphenyl glycoside (**1h**), a commercially available disaccharide containing the Neu5Gc core (Figure 2A). **1h** corresponds to the partial structure of the oligosaccharide attached to cetuximab, an epidermal growth factor receptor inhibitor and monoclonal antibody produced by murine myeloma cells, in which the terminal Neu5Gc in the glycan is a potential immunogen.[13] The site-selective chemical

https://doi.org/10.1021/acs.orglett.1c00612
Org. Lett. 2021, 23, 2715−2719

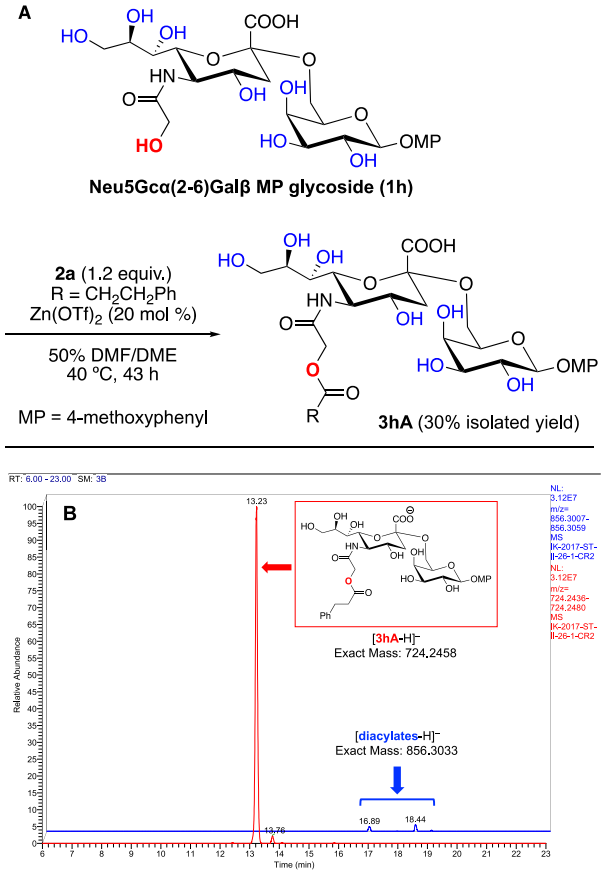


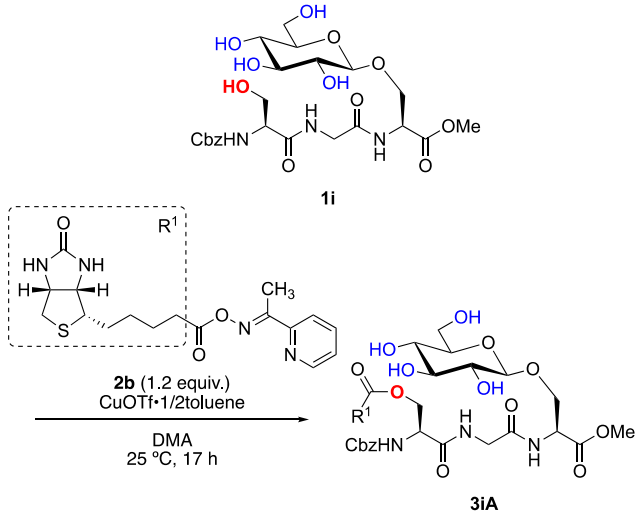
Figure 2. (A) Site-selective acylation of disaccharide **1h** containing the Neu5Gc core. (B) Extracted-ion chromatogram of the reaction of **1h**.

modifications of Neu5Gc-containing oligosaccharides may lead to significant substrate-selective transformations for separation, detection, and chemical modification. Although we expected a low chemical yield from the acylation of **1h** bearing a carboxylic acid moiety, conditions that resulted in incomplete acylation in the control experiment, we believed that the site-selective modification of this class of substrate is highly valuable, even in low yield, because accomplishing this is challenging using the currently available methods. Disaccharide **1h** was acylated under the same conditions used to acylate *N*-glycolyl neuraminic acid (**1f**) to give **3hA**. Preliminary HPLC analysis of the reaction solution revealed low conversion of **1h**, and the NMR spectrum of the reaction mixture was unable to provide site-selectivity information due to its complexity. Accordingly, we used LC-MS followed by analysis of the extracted ion chromatogram (XIC), which is a powerful tool for detecting isomers bearing the same exact mass as the target product that can reveal the site selectivity of the reaction. Surprisingly, the XIC of the monoacylated product ($[M + H]^+$ m/z 724.2458, red trace, Figure 2B), which is highly mass accurate (<3 ppm), revealed the presence of essentially one product. In addition, very small peaks corresponding to diacylated products ($[M + H]^+$ m/z 856.3033, blue trace) were detected in the XIC, confirming that the reaction proceeded in a highly site-selective manner. The prepared monoacylated product was isolated by preparative HPLC using almost the same gradient-elution method used during LC-MS and was unambiguously determined to be **3hA** by 2D NMR spectroscopy.[12]

This template strategy was further applied as a site-selective method for the acylation of β-hydroxyamides because the β-hydroxyl groups in amides are very attractive targets for chemical modification, as evidenced by the side chains of Ser and Thr residues in peptides.[14,15] The introduced acylating motif, which modifies the molecule, must suit the requirements for further applications, which is another important issue. We focused our attention on the biotinylation chemical modification using our strategy because it is one of the most common techniques used to conjugate a range of small molecules and proteins for purification, detection, and various biological assays that utilize strong interactions between streptavidin and biotin.[16,17] To the best of our knowledge, biotin has yet to be site-selectively introduced in organic chemistry, which is probably due to its hydrophilicity that potentially inhibits site-selective control through hydrogen-bonding interactions and Lewis-acid activation. Accordingly, a new pyridylketoxime-type reagent, **2b**, was prepared for site-selective biotinylation.

We first applied the optimized reaction conditions used for the acylation of glycopeptide **1i** to the reaction of **2b**, which led to a low conversion of **1i** and a 16% yield of the monobiotinylated product **3iA** with moderate site selectivity (Table 1, entry 1).[5b] The acylation of **1i** using a ketoxime derivative of **2a** under otherwise identical conditions provided the corresponding acylate in 29% isolated yield; this low yield is possibly ascribable to the chelating properties of the tetrahydrothiophene and imidazolidone cores in biotin that

Table 1. Site-Selective Biotinylation of Glycopeptide 1i



entry	CuOTf·1/2 toluene	conc. (mM)	yield (%)[a] **3iA**	selectivity[a] (**3iA**/isomers[b])
1	0.5 equiv	100	16	62:38
2	0.5 equiv	25	22	67:33
3	1.0 equiv	25	21	64:36
4	1.0 equiv	5	38 (31)[c]	72:28
5[d]	1.0 equiv	5	– (28)[c]	72:28
6	conventional conditions[f]		11	35:65

[a]Yields and selectivities were determined by HPLC with UV detection using an internal standard. [b]Monobiotinylated products. [c]Numbers in parentheses refer to isolated yields. [d]Reaction was performed at 40 °C. [f]Reaction was performed with **1i** and biotin *N*-hydroxysuccinimide ester (1.2 equiv) in pyridine at 40 °C for 67 h. See the SI for details.

https://doi.org/10.1021/acs.orglett.1c00612
Org. Lett. 2021, 23, 2715−2719

can hamper the formation of the appropriate metal template between **1i**, **2b**, and CuOTf. During optimization of the reaction conditions, we observed that dilution to 25 mM slightly improved the yield of **3iA** (entry 2). On the contrary, increasing the amount of CuOTf did not affect the yield, which was unexpected (entry 3). The reaction outcome was further improved under highly dilute conditions, which produced **3iA** in 38% yield with small amounts of monobiotinylated isomers and dibiotinylated byproducts (entry 4). We previously reported that DMA was the best solvent in terms of both the yield and the site selectivity. We reasoned that DMA, as a chelating substrate, engages in the dynamic chelation−dissociation process on the Cu center, with a β-hydroxyamide, a ketoxime ester, and the ketoxime leaving after acylation. As a result, the excess DMA present under dilute conditions may provide opportunities to construct the desired metal complex comprising the β-hydroxyamide and the ketoxime ester by ejecting a ligand on the undesired but relatively strong metal complex, thereby facilitating the site-selective reaction. Unfortunately, a higher reaction temperature did not increase the selectivity or the yield of the isolated product (entry 5). Conventional conditions using the commercially available biotin *N*-hydroxysuccinimide ester in pyridine produced substantial amounts of regioisomers, with unreacted **1i** remaining (entry 6).[18] These results clearly highlight that the reactivities of the five hydroxyl groups in glycopeptide **1i** can be controlled in a desirable fashion to achieve the site-selective reaction of a β-hydroxyl group, even during biotinylation.

In conclusion, we demonstrated the site-selective acylations of α- and β-hydroxyamides in disaccharides and glycopeptides. *N*-Glycolyl disaccharides present in the partial structures of bioactive polysaccharides were acylated with excellent site-selectivities using $Zn(OTf)_2$ and pyridine aldoxime ester **2a** as acylating agents. In addition, site-selective biotinylation, one of the most important techniques in chemical biology and related fields, was accomplished during the site-selective functionalization of the β-hydroxyl group in a glycopeptide bearing an unprotected glucose for the first time. Further applications of this metal-template strategy toward different classes of substrates are currently being explored by our group.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.1c00612.

Experimental procedures, spectroscopic data for new compounds, and HPLC/LC-MS data (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Yasuhiro Nishikawa − *Faculty of Pharmacy, Meijo University, Nagoya, Aichi 468-8503, Japan*; orcid.org/0000-0003-1606-7288; Email: yasuhiro@meijo-u.ac.jp

Osamu Hara − *Faculty of Pharmacy, Meijo University, Nagoya, Aichi 468-8503, Japan*; Email: oshara@meijo-u.ac.jp

Authors

Shione Toda − *Faculty of Pharmacy, Meijo University, Nagoya, Aichi 468-8503, Japan*

Takami Matsui − *Faculty of Pharmacy, Meijo University, Nagoya, Aichi 468-8503, Japan*

Hanae Takada − *Faculty of Pharmacy, Meijo University, Nagoya, Aichi 468-8503, Japan*

Kohei Takemoto − *Faculty of Pharmacy, Meijo University, Nagoya, Aichi 468-8503, Japan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.1c00612

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was partially supported by JSPS KAKENHI grant number JP19K07007 (Y.N.) for Scientific Research (C). Y.N. thanks Meijo University for financial support.

■ REFERENCES

(1) (a) Vong, K.; Yamamoto, T.; Tanaka, K. Artificial Glycoproteins as a Scaffold for Targeted Drug Therapy. *Small* **2020**, *16*, 1906890. (b) Boltje, T. J.; Buskas, T.; Boons, G.-J. Opportunities and challenges in synthetic oligosaccharide and glycoconjugate research. *Nat. Chem.* **2009**, *1*, 611−622.

(2) (a) Cloutier, M.; Gauthier, C. Progress toward the Development of Glycan-Based Vaccines against Campylobacteriosis. *ACS Infect. Dis.* **2020**, DOI: 10.1021/acsinfecdis.0c00332. (b) González-Sabín, J.; Morán-Ramallal, R.; Rebolledo, F. Regioselective enzymatic acylation of complex natural products: expanding molecular diversity. *Chem. Soc. Rev.* **2011**, *40*, 5321−5335.

(3) (a) Wang, T.; Demchenko, A. V. Synthesis of carbohydrate building blocks *via* regioselective uniform protection/deprotection strategies. *Org. Biomol. Chem.* **2019**, *17*, 4934−4950. (b) Blaszczyk, S. A.; Homan, T. C.; Tang, W. Recent advances in site-selective functionalization of carbohydrates mediated by organocatalysts. *Carbohydr. Res.* **2019**, *471*, 64−77. (c) Dimakos, V.; Taylor, M. S. Site-Selective Functionalization of Hydroxyl Groups in Carbohydrate Derivatives. *Chem. Rev.* **2018**, *118*, 11457−11517. (d) Kulkarni, S. S.; Wang, C.-C.; Sabbavarapu, N. M.; Podilapu, A. R.; Liao, P.-H.; Hung, S.-C. One-Pot" Protection, Glycosylation, and Protection−Glycosylation Strategies of Carbohydrates. *Chem. Rev.* **2018**, *118*, 8025−8104. (e) Shugrue, C. R.; Miller, S. J. Applications of Nonenzymatic Catalysts to the Alteration of Natural Products. *Chem. Rev.* **2017**, *117*, 11894−11951.

(4) (a) Li, J.; Grosslight, S.; Miller, S. J.; Sigman, M. S.; Toste, F. D. Site-Selective Acylation of Natural Products with BINOL-Derived Phosphoric Acids. *ACS Catal.* **2019**, *9*, 9794−9799. (b) Li, R.-Z.; Tang, H.; Wan, L.; Zhang, X.; Fu, Z.; Liu, J.; Yang, S.; Jia, Da; Niu, D. Site-Divergent Delivery of Terminal Propargyls to Carbohydrates by Synergistic Catalysis. *Chem.* **2017**, *3*, 834−845. (c) Tay, J.-H.; Argüelles, A. J.; DeMars, M. D., II; Zimmerman, P. M.; Sherman, D. H.; Nagorny, P. Regiodivergent Glycosylations of 6-Deoxy-erythronolide B and Oleandomycin-Derived Macrolactones Enabled by Chiral Acid Catalysis. *J. Am. Chem. Soc.* **2017**, *139*, 8570−8578. (d) Yoganathan, S.; Miller, S. J. *J. Med. Chem.* **2015**, *58*, 2367−2377. (e) Ueda, Y.; Mishiro, K.; Yoshida, K.; Furuta, T.; Kawabata, T. Regioselective Diversification of a Cardiac Glycoside, Lanatoside C, by Organocatalysis. *J. Org. Chem.* **2012**, *77*, 7850−7857. (f) Yoshida, K.; Furuta, T.; Kawabata, T. Perfectly regioselective acylation of a cardiac glycoside, digitoxin, via catalytic amplification of the intrinsic reactivity. *Tetrahedron Lett.* **2010**, *51*, 4830−4832. (g) Lewis, C. A.; Longcore, K. E.; Miller, S. J.; Wender, P. A. An Approach to the Site-Selective Diversification of Apoptolidin A with Peptide-Based Catalysts. *J. Nat. Prod.* **2009**, *72*, 1864−1869. (h) Lewis, C. A.; Miller, S. J. Site-Selective Derivatization and Remodeling of Erythromycin A by Using Simple Peptide-Based Chiral Catalysts. *Angew. Chem., Int. Ed.* **2006**, *45*, 5616−5619.

(5) (a) Nishikawa, Y.; Takemoto, K.; Matsuda, K.; Tanaka, R.; Arashima, A.; Ito, K.; Kamezawa, Y.; Hori, Y.; Hara, O. Metal Template Assisted Proximal Arrangement of a Nucleophile and an

Electrophile: Site-Selective Acylation of α-Hydroxyamides in Polyols. *Org. Lett.* **2018**, *20*, 3367−3371. (b) Takemoto, K.; Nishikawa, Y.; Moriguchi, S.; Hori, Y.; Kamezawa, Y.; Matsui, T.; Hara, O. Site-Selective Esterifications of Polyol β-Hydroxyamides and Applications to Serine-Selective Glycopeptide Modifications. *Org. Lett.* **2019**, *21*, 7534−7538.

(6) (a) Allen, C. L.; Miller, S. J. Chiral Copper(II) Complex-Catalyzed Reactions of Partially Protected Carbohydrates. *Org. Lett.* **2013**, *15*, 6178−6181. (b) Matsumura, Y.; Maki, T.; Tsurumaki, K.; Onomura, O. Kinetic resolution of D,L-*myo*-inositol derivatives catalyzed by chiral Cu (II) complex. *Tetrahedron Lett.* **2004**, *45*, 9131−9134.

(7) (a) Wang, Q.; Matsuo, Y.; Pradipta, A. R.; Inohara, N.; Fujimoto, Y.; Fukase, K. Synthesis of characteristic Mycobacterium peptidoglycan (PGN) fragments utilizing with chemoenzymatic preparation of meso-diaminopimelic acid (DAP), and their modulation of innate immune responses. *Org. Biomol. Chem.* **2016**, *14*, 1013−1023. (b) Melnyk, J. E.; Mohanan, V.; Schaefer, A. K.; Hou, C.-W.; Grimes, C. L. Peptidoglycan Modifications Tune the Stability and Function of the Innate Immune Receptor Nod2. *J. Am. Chem. Soc.* **2015**, *137*, 6987−6990. (c) Raymond, J. B.; Mahapatra, S.; Crick, D. C.; Pavelka, M. S. Identification of the *nam*H gene, encoding the hydroxylase responsible for the *N*-glycolylation of the mycobacterial peptidoglycan. *J. Biol. Chem.* **2005**, *280*, 326−333.

(8) (a) Altman, M. O.; Gagneux, P. Absence of Neu5Gc and Presence of Anti-Neu5Gc Antibodies in Humans—An Evolutionary Perspective. *Front. Immunol.* **2019**, *10*, 789. (b) Adams, O. J.; Stanczak, M. A.; von Gunten, S.; Läubli, H. Targeting sialic acid−Siglec interactions to reverse immune suppression in cancer. *Glycobiology* **2018**, *28* (9), 640−647. (c) Samraj, A. N.; Läubli, H.; Varki, N.; Varki, A. Involvement of a non-human sialic Acid in human cancer. *Front. Oncol.* **2014**, *4*, 33.

(9) (a) Dorvignit, D.; Boligan, K. F.; Relova-Hernández, E.; Clavell, M.; López, A.; Labrada, M.; Simon, H.-U.; López-Requena, A.; Mesa, C.; von Gunten, S. Antitumor effects of the GM3(Neu5Gc) ganglioside-specific humanized antibody 14F7hT against *Cmah*-transfected cancer cells. *Sci. Rep.* **2019**, *9*, 9921. (b) Albertó, M.; Cuello, H.; Gulino, C.; Pifano, M.; Belgorosky, D.; Gabri, M.; Eiján, A.; Segatori, V. Expression of bladder cancer-associated glycans in murine tumor cell lines. *Oncol. Lett.* **2019**, *17* (3), 3141−3150. (c) Shewell, L. K.; Wang, J. J.; Paton, J. C.; Paton, A. W.; Day, C. J.; Jennings, M. P. Detection of *N*-glycolylneuraminic acid biomarkers in sera from patients with ovarian cancer using an engineered *N*-glycolylneuraminic acid-specific lectin SubB2M. *Biochem. Biophys. Res. Commun.* **2018**, *507*, 173−177. (d) Day, C. J.; Paton, A. W.; Higgins, M. A.; Shewell, L. K.; Jen, F. E. C.; Schulz, B. L.; Herdman, B. P.; Paton, J. C.; Jennings, M. P. Structure aided design of a Neu5Gc specific lectin. *Sci. Rep.* **2017**, *7*, 1495. (e) Yu, C.; Gao, K.; Zhu, L.; Wang, W.; Wang, L.; Zhang, F.; Liu, C.; Li, M.; Wormald, M. R.; Rudd, P. M.; Wang, J. At least two Fc Neu5Gc residues of monoclonal antibodies are required for binding to anti-Neu5Gc antibody. *Sci. Rep.* **2016**, *6*, 20029. (f) Malykh, Y. N.; Schauer, R.; Shaw, L. *N*-Glycolylneuraminic acid in human tumours. *Biochimie* **2001**, *83*, 623−634.

(10) A control experiment using α-methoxyacetic acid was conducted. See the Supporting Information for details.

(11) Milland, J.; Sandrin, M. S. ABO blood group and related antigens, natural antibodies and transplantation. *Tissue Antigens* **2006**, *68*, 459−466.

(12) See the Supporting Information for details.

(13) Ghaderi, D.; Taylor, R. E.; Padler-Karavani, V.; Diaz, S.; Varki, A. Implications of the presence of *N*-glycolylneuraminic acid in recombinant therapeutic glycoproteins. *Nat. Biotechnol.* **2010**, *28*, 863−867.

(14) (a) Rawale, D. G.; Thakur, K.; Adusumalli, S. R.; Rai, V. Chemical Methods for Selective Labeling of Proteins. *Eur. J. Org. Chem.* **2019**, *2019* (40), 6749−6763. (b) Krall, N.; da Cruz, F. P.; Boutureira, O.; Bernardes, G. J. L. Site-selective protein-modification chemistry for basic biology and drug development. *Nat. Chem.* **2016**, *8*, 103−113. (c) Boutureira, O.; Bernardes, G. J. L. Advances in chemical protein modification. *Chem. Rev.* **2015**, *115*, 2174−2195.

(15) (a) Vantourout, J. C.; Adusumalli, S. R.; Knouse, K. W.; Flood, D. T.; Ramirez, A.; Padial, N. M.; Istrate, A.; Maziarz, K.; deGruyter, J. N.; Merchant, R. R.; Qiao, J. X.; Schmidt, M. A.; Deery, M. J.; Eastgate, M. D.; Dawson, P. E.; Bernardes, G. J. L.; Baran, P. S. Serine-Selective Bioconjugation. *J. Am. Chem. Soc.* **2020**, *142*, 17236−17242. (b) Saavedra, C. J.; Hernández, D.; Boto, A. Metal-Free, Site-Selective Peptide Modification by Conversion of "Customizable" Units into β-Substituted Dehydroamino Acids. *Chem. - Eur. J.* **2018**, *24*, 599−607. (c) Gimenez, D.; Mooney, C. A.; Dose, A.; Sandford, G.; Coxon, C. R.; Cobb, S. L. The application of perfluoroheteroaromatic reagents in the preparation of modified peptide systems. *Org. Biomol. Chem.* **2017**, *15*, 4086−4095.

(16) (a) Trippier, P. C. Synthetic Strategies for the Biotinylation of Bioactive Small Molecules. *ChemMedChem* **2013**, *8*, 190−203. (b) Elia, G. Biotinylation reagents for the study of cell surface proteins. *Proteomics* **2008**, *8*, 4012−4024.

(17) Miller, B. T.; Collins, T. J.; Nagle, G. T.; Kurosky, A. The occurrence of *O*-acylation during biotinylation of gonadotropin-releasing hormone and analogs. Evidence for a reactive serine. *J. Biol. Chem.* **1992**, *267*, 5060−5069.

(18) The results obtained using our method and the conventional method were compared in terms of site selectivity by HPLC and LC-MS. See the Supporting Information for details.

https://doi.org/10.1021/acs.orglett.1c00612
Org. Lett. 2021, 23, 2715−2719