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Regioselective One-Pot Protection of D-Glucosamine

Ken-Lien Chang,[†] Medel Manuel L. Zulueta,[†,§] Xin-An Lu,[†] Yong-Qing Zhong,[†] and Shang-Cheng Hung*[,†,‡]

[†]*Genomics Research Center, Academia Sinica, 128, Section 2, Academia Road, Taipei 115, Taiwan,* [‡]*Department of Applied Chemistry, National Chiao Tung University, 1001, Ta Hsueh Road, Hsinchu 300, Taiwan, and* [§]*Institute of Chemistry, University of the Philippines, Diliman, Quezon City 1101, Philippines*

schung@gate.sinica.edu.tw

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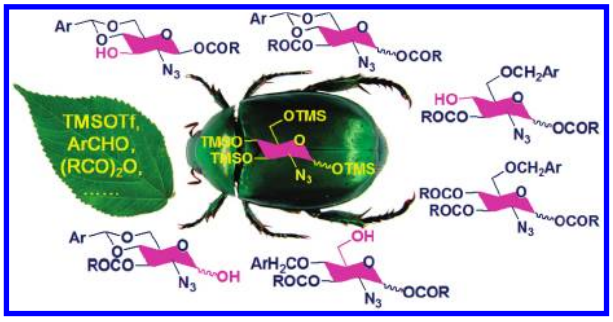
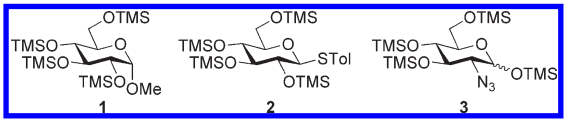


A highly regioselective one-pot transformation of 2-azido-2-deoxy-1,3,4,6-tetra-*O*-trimethylsilyl-D-glucopyranose via sequential additions of various reagents was systematically studied, yielding the fully protected derivatives and the 1-, 3-, 4-, as well as 6-alcohols, respectively.

The chirality of the anomeric center along with the polyhydroxylated character of several monosaccharide units has enabled Nature to generate carbohydrate polymers of staggering complexity.[1] The information embedded in these structures that are exploited in living systems attracted keen interest and fueled the advance of the emerging field of glycomics.[2] Among the widely distributed sugar components, D-glucosamine and its *N*-acetylated and *N*-sulfonated derivatives, are found in numerous biologically potent molecules such as cell surface *N*-glycoproteins, proteoglycans (heparan sulfate, heparin), hyaluronic acid, glycosphingolipids (Lewis a/x), glycosylphosphatidylinositol (GPI) anchors, blood group antigens, bacterial cell wall, lipopolysaccharides, and chitin/chitosan.[2c,3] The 3-O and 4-O positions of the glucosamine residues are typically involved in the recurring linear assemblies as is the case in hyaluronic acid, chitin, and heparan sulfate.[4] The more decorated *N*-glycoproteins and glycolipids have branching structures occupying not only the 3-O and 4-O, but also the 6-O portions as well.[5] The reducing side attachments are frequently β-oriented, although α-linkages are also common.

Carbohydrate structure−activity relationship (SAR) studies require well-defined molecules which may only be reasonably acquired through chemical synthesis.[6] Such preparation necessitates the acquisition of properly protected monosaccharide assembly units. Traditional protocols rely on independent preparative routes and step-by-step protection−deprotection schemes with focus on the careful differentiation of the multiple hydroxyls of an unprotected starting material, often suffering tedious workups and time-consuming purifications. To tackle these problems and efficiently create a library of suitable monosaccharide synthons for glycomics study, a trimethylsilyl trifluoromethanesulfonate (TMSOTf)-mediated regioselective one-pot protection strategy was recently developed by us.[7] There, a variety of D-glucose derivatives with different protecting group patterns was prepared from the simple per-*O*-trimethylsilylated glucopyranosides with anomeric fixed α-OMe (**1**) and β-STol (**2**).



In continuation of this effort, we disclose herein the application of our novel approach on the anomeric nonfixed D-glucosamine unit **3** to synthesize a series of building blocks that include fully protected sugars and 1-, 3-, 4-, as well as 6-alcohols. In this case, the azido group was selected to mask the 2-C position of the glucosamine unit due to its dual utility in forming either α- (by nonparticipating effect)[8] or β-glycosidic linkage (by nitrile solvent effect).[9] Other amino

(1) Voit, B.; Appelhans, D. *Macromol. Chem. Phys.* **2010**, *211*, 727–735.
(2) (a) Shriver, Z.; Raguram, S.; Sasisekharan, R. *Nat. Rev. Drug Discovery* **2004**, *3*, 863–873. (b) Paulson, J. C.; Blixt, O.; Collins, B. E. *Nat. Chem. Biol.* **2006**, *2*, 238–248. (c) Varki, A.; Cummings, D. C.; Esko, J. D.; Freeze, H. H.; Stanley, P.; Bertozzi, C. R.; Hart, G. W.; Etzler, M. E. In *Essentials of Glycobiology*, 2nd ed.; Cold Spring Habor Laboratory Press: New York, 2009. (d) Krishnamoorthy, L.; Mahal, L. K. *ACS Chem. Biol.* **2009**, *4*, 715–732. (e) Hyun, J.-A.; Kronewitter, S. L.; Leoz, M. L.; Lebrilla, C. B. *Curr. Opin. Chem. Biol.* **2009**, *13*, 601–607. (f) Zhang, H.-L.; Ma, Y.; Sun, X.-L. *Med. Res. Rev.* **2010**, *30*, 270–289.
(3) (a) Bryant, C. E.; Spring, D. R.; Gangloff, M.; Gay, N. J. *Nat. Rev. Microbiol.* **2010**, *8*, 8–14. (b) Paulick, M. G.; Bertozzi, C. R. *Biochemistry* **2008**, *47*, 6991–7000. (c) Gandhi, N. S.; Mancera, R. L. *Chem. Biol. Drug Des.* **2008**, *72*, 455–482. (d) Wang, L.-X.; Huang, W. *Curr. Opin. Chem. Biol.* **2009**, *13*, 592–600. (e) Taube, S.; Jiang, M.; Wobus, C. E. *Viruses* **2010**, *2*, 1011–1049.
(4) (a) Esko, J. D.; Lindahl, U. *J. Clin. Invest.* **2001**, *108*, 169–173. (b) Liu, H.; Zhang, Z.; Linhardt, R. J. *Nat. Prod. Rep.* **2009**, *26*, 313–321.
(5) (a) Nyame, A. K.; Kawar, Z. S.; Cummings, R. D. *Arch. Biochem. Biophys.* **2004**, *426*, 182–200. (b) Hokke, C. H.; Deelder, A. M.; Hoffmann, K. F.; Wuhrer, M. *Exp. Parasitol.* **2007**, *117*, 275–283.
(6) (a) Banoub, J. *Chem. Rev.* **1992**, *92*, 1167–1195. (b) Bongat, A. F. G.; Demchenko, A. V. *Carbohydr. Res.* **2007**, *342*, 374–406.
(7) (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.
(8) (a) Hung, S.-C.; Thopate, S. R.; Chi, F.-C.; Chang, S.-W.; Lee, J.-C.; Wang, C.-C.; Wen, Y.-S. *J. Am. Chem. Soc.* **2001**, *123*, 3153–3154. (b) Lee, C.-J.; Lu, X.-A.; Kulkarni, S. S.; Wen, Y.-S.; Hung, S.-C. *J. Am. Chem. Soc.* **2004**, *126*, 476–477. (c) Lee, J.-C.; Chang, S.-W.; Liao, C.-C.; Chi, F.-C.; Chen, C.-S.; Wen, Y.-S.; Wang, C.-C.; Kulkarni, S. S.; Puranik, R.; Liu, Y.-H.; Hung, S.-C. *Chem.—Eur. J.* **2004**, *10*, 399–415. (d) Lu, L.-D.; Shie, C.-R.; Kulkarni, S. S.; Pan, G.-R.; Lu, X.-A.; Hung, S.-C. *Org. Lett.* **2006**, *8*, 5995–5998.

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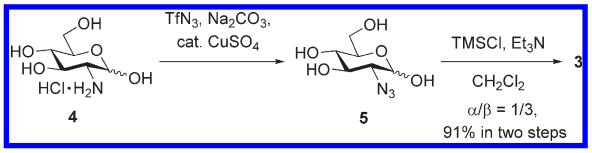
SCHEME 1. Preparation of 2-Azido-2-deoxy-1,3,4,6-tetra-*O*-trimethylsilyl-D-glucopyranose 3

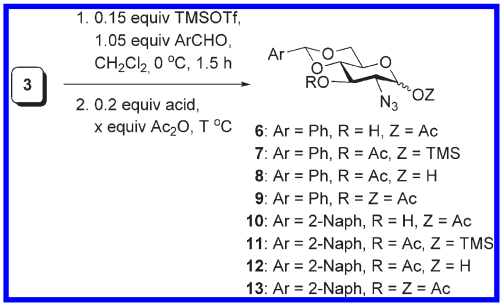


protecting groups, e.g. Boc, Cbz, Troc, Phth, ClAc, CF_3CO, and CCl_3CO, all prefer the β-form because of the participating effect by the carbonyl group.[10] The azido group can also be easily transformed into *N*-acetyl or free amine by using thioacetic acid[11] or a series of reductants, respectively. The next encountered problem is the differentiation of four hydroxy groups on the D-glucosamine unit, which contains one primary alcohol at 6-C and three secondary alcohols at 1-C, 3-C, and 4-C. Conceptually, the 4,6-dihydroxyls can be blocked by arylidene formation, and the remaining 1-OH and 3-OH can be distinguished by their differences in acidity.

To initiate this one-pot approach, the per-*O*-silylated ether **3** needs to be synthesized and the two-step procedure is depicted in Scheme 1. Commercially available D-glucosamine hydrochloride **4** was first reacted with freshly prepared triflic azide (TfN_3) in the presence of copper sulfate as catalyst to afford the corresponding azido derivative **5**.[12] After partial purification, the crude tetraol **5** underwent per-*O*-silylation to furnish the product **3**, which was recrystallized from ethanol[13] as a white solid with a 91% overall yield.

Having acquired the common intermediate **3**, we went forward with the regioselective one-pot protection strategy. The general protocols involved the following: (1) selective protection of 4,6-dihydroxyl groups as arylidene acetals followed by regioselective 1-*O*-acylation to provide the 3-alcohols; (2) 4,6-*O*-arylidenation, 1,3-di-*O*-acylation to obtain the fully protected derivatives; (3) 4,6-*O*-arylidenation, 1,3-di-*O*-acylation, and regioselective ring-opening of arylidene acetals to furnish the 6-alcohols, fully protected derivatives, and 4-alcohols, respectively; and (4) 4,6-*O*-arylidenation, 1,3-di-*O*-acylation, and regioselective removal of the protecting group at 1-O to yield the 1-alcohols. 4,6-*O*-Arylidene formation and the subsequent acylation are core steps in the one-pot process. Treatment of compound **3** with 1.05 equiv of benzaldehyde and catalytic TMSOTf smoothly implemented the first transformation. With the already acid-containing mixture, it is convenient to pursue the acylation stage under acid catalysis. Promoted by TMSOTf, acetylation was attempted with 1.1 equiv of Ac_2O initially at ice bath temperature and then gradually warmed to room temperature. Regrettably, as detailed in entry 1 of Table 1, the one-pot reaction led to the 1-acetylated 3-alcohol **6**, 3-acetylated

TABLE 1. Acid-Promoted Regioselective One-Pot 4,6-*O*-Arylidenation and Acetylation



entry	acid	*x*	*T* (°C)	Ar	product (yield, %)
1	TMSOTf	1.1	0 to rt	Ph	**6** (7) + **7** (36) + **8** (11) + **9** (15)
2	TMSOTf	1.1	0 to rt	2-Naph	**10** (8) + **11** (34) + **12** (13) + **13** (17)
3	$Cu(OTf)_2$	1.1	0 to rt	Ph	**6** (11) + **7** (23) + **8** (15) + **9** (31)
4	$Cu(OTf)_2$	1.1	0 to rt	2-Naph	**10** (9) + **11** (19) + **12** (12) + **13** (25)
5	TMSOTf	2.5	40	Ph	**9** (32)
6	$Cu(OTf)_2$	2.5	40	Ph	**9** (57)
7	$Cu(OTf)_2$	5	40	Ph	**9** (69)
8	$Cu(OTf)_2$	7.5	40	Ph	**9** (74)
9	$Cu(OTf)_2$	10	40	Ph	**9** (77)
10	$Cu(OTf)_2$	10	40	2-Naph	**13** (74)

1-TMS ether **7**, 1-alcohol **8**, and 1,3-diacetate **9** in 7%, 36%, 11%, and 15% yields, respectively. Similar results were obtained in the case of 2-naphthaldehyde (entry 2), and the acetylated derivatives **10**−**13** were individually isolated in 8%, 34%, 13%, and 17% yields. Alternatively, copper(II) trifluoromethanesulfonate [$Cu(OTf)_2$][14] in place of TMSOTf registered marginal improvements under the same conditions (entries 3 and 4). When the amount of Ac_2O was raised to 2.5 equiv and the temperature to 40 °C, a better yield was delivered by $Cu(OTf)_2$ (57%, entry 6) than TMSOTf (32%, entry 5) for the 1,3-diacetate **9**. Further increase in the amount of Ac_2O in a $Cu(OTf)_2$-catalyzed acetylation[15] resulted in yield enhancement (entries 7 and 8) and at 10 equiv, the fully protected derivatives **9** and **13** were generated in 77% (entry 9) and 74% (entry 10) yields, respectively. Notably, mixtures of anomers were afforded in these reactions.

Since the regioselective 1-*O*-acylation could not be efficiently executed with acid catalysis, this second stage conversion was attempted utilizing the basic approach.[16] The reaction conditions and results are outlined in Table 2. After protecting the 4-O and 6-O positions with a benzylidene acetal, the TMS groups at the 1-O and 3-O positions were removed by treatment with tetra-*n*-butylammonium fluoride (TBAF) together with acetic acid to control the pH value of the solution.[17] Without quenching the reaction, the 1-*C*-hydroxy group was regioselectively acetylated employing triethylamine (Et_3N) and 1.1 equiv of Ac_2O to afford only the β-form[18] 3-alcohol **6*β*** in 91% yield (entry 1). Here, the carboxylic acid used to moderate the basicity of TBAF should be the parent acid of the anhydride reagent used to protect 1-O in order to prevent undesirable mixed anhydride

(9) (a) Schmidt, R. R.; Behrendt, M.; Toepfer, A. *Synlett* **1990**, 694–696. (b) Ratcliffe, A. J.; Fraser-Reid, B. *J. Chem. Soc., Perkin Trans.* **1990**, *1*, 747–750.

(10) (a) Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Bassov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734–753. (b) Greene, T. W.; Wuts, P.G. M. In *Protective Groups in Organic Synthesis*, 4th ed.; John Wiley & Sons: Hoboken, NJ, 2007.

(11) (a) Rosen, T.; Lico, I. M.; Chu, D. T. W. *J. Org. Chem.* **1988**, *53*, 1580–1582. (b) Shangguan, N.; Katukojvala, S.; Greenberg, R.; Williams, L. J. *J. Am. Chem. Soc.* **2003**, *125*, 7754–7755.

(12) Alper, P. B.; Hung, S.-C.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 6029–6032.

(13) Wang, C.-C.; Lee, J.-C.; Luo, S.-Y.; Fan, H.-F.; Pai, C.-L.; Yang, W.-C.; Lu, L.-D.; Hung, S.-C. *Angew. Chem., Int. Ed.* **2002**, *41*, 2360–2362.

(14) Kulkarni, S. S.; Hung, S.-C. *Lett. Org. Chem.* **2005**, *2*, 670–677.

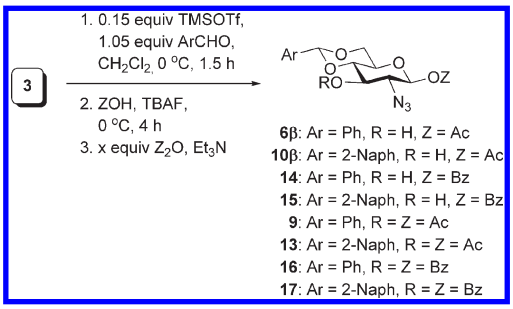
(15) Tai, C.-A.; Kulkarni, S. S.; Hung, S.-C. *J. Org. Chem.* **2003**, *68*, 8719–8722.

(16) Lu, X.-A.; Chou, C.-H.; Wang, C.-C.; Hung, S.-C. *Synlett* **2003**, *9*, 1364–1366.

(17) Lee, Y. J.; Ishiwata, A.; Ito, Y. *Tetrahedron* **2009**, *65*, 6310–6319.

(18) Luo, S.-Y.; Kulkarni, S. S.; Chou, C.-H.; Liao, W.-M.; Hung, S.-C. *J. Org. Chem.* **2006**, *71*, 1226–1229.

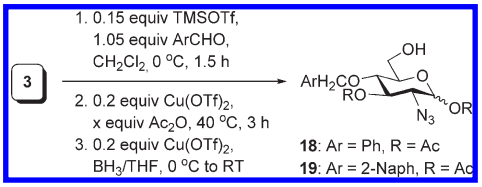
TABLE 2. Regioselective One-Pot 4,6-*O*-Arylidenation Followed by Basic Acylation



entry	Ar	*x*	Z	product	yield (%)
1	Ph	1.1	Ac	**6*β***	91
2	2-Naph	1.1	Ac	**10*β***	90
3	Ph	1.1	Bz	**14**	89
4	2-Naph	1.1	Bz	**15**	87
5	Ph	3	Ac	**9**[a]	94[c]
6	2-Naph	3	Ac	**13**[b]	92[c]
7	Ph	3	Bz	**16**	92[c]
8	2-Naph	3	Bz	**17**	91[c]

[a] α/β = 1/6. [b] α/β = 1/6. [c] Acylation was carried out together with DMAP.

TABLE 3. Regioselective One-Pot Protection to Synthesize the 6-Alcohols



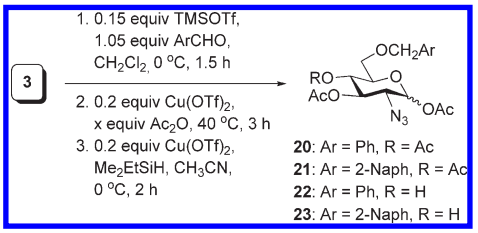
entry	*x*	Ar	product	yield (%)
1	2.5	Ph	**18**	38
2	2.5	2-Naph	**19**	35
3	10	Ph	**18**	59
4	10	2-Naph	**19**	57

formation. By applying this method with other combinations of aryl aldehyde (2-naphthaldehyde, benzaldehyde) and acid anhydrides (Ac_2O, Bz_2O), the target 3-alcohols (**10*β***, **14**, **15**) were obtained as single isomers in excellent yields all in one pot (entries 2−4). To drive the 1,3-di-*O*-acylation, the previously obtained arylidene acetals were treated with Et_3N and 3 equiv of acid anhydride under catalytic 4-*N*,*N*-dimethylaminopyridine (DMAP) at room temperature (entries 5−8). DMAP is necessary to provide excellent product turnover, but in the case of acetylation, addition of this catalyst supplied a mixture of the α- and *β*-anomers **9** (94%) and **13** (92%), whereas only the *β*-isomers **16** (92%) and **17** (91%) were isolated after benzoylation.

We next tackled the preparation of 6-alcohols via the fully protected derivatives in one pot (Table 3). Efforts to regioselectively open the 4,6-*O*-arylidene acetal after full protection using the above-mentioned basic conditions turned out unsuccessful. Following the route through the $Cu(OTf)_2$-mediated 1,3-di-*O*-acetylation, the regioselective 6-*O*-ring-opening of the originally formed arylidene acetal was, then,

(19) (a) Wang, C.-C.; Luo, S.-Y.; Shie, C.-R.; Hung, S.-C. *Org. Lett.* **2002**, *4*, 847–849. (b) Shie, C.-R.; Tzeng, Z.-H.; Kulkarni, S. S.; Uang, B.-J.; Hsu, C.-Y.; Hung, S.-C. *Angew. Chem., Int. Ed.* **2005**, *44*, 1665–1668.

TABLE 4. Regioselective One-Pot Protection To Synthesize the Fully Protected Derivatives and 4-Alcohols



entry	*x*	Ar	product	yield (%)
1	10	Ph	**20**	62
2	10	2-Naph	**21**	60
3	2.5	Ph	**22**	45
4	2.5	2-Naph	**23**	44

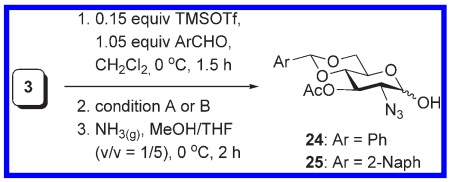
explored by using BH_3/THF[7,19] as the reductant in the presence of 0.2 equiv of $Cu(OTf)_2$ initially at 0 °C and gradually warmed to room temperature. In entries 1 and 2, when 2.5 equiv of Ac_2O was used for acetylation, the desired 6-alcohols **18** and **19** were obtained in 38% and 35% yields, respectively. Increasing the amount of Ac_2O to 10 equiv would benefit the reactions, and the yields of products **18** and **19** were improved to 59% (entry 3) and 57% (entry 4), respectively.

To synthesize the alternate ring-opening product, BH_3/THF was replaced by Me_2EtSiH[20] as the reductant with $Cu(OTf)_2$ still acting as a promoter. The results are summarized in Table 4. Again, the acetylation conditions played a critical role in the outcome of the one-pot process. Following acetylation carried out with 10 equiv of Ac_2O in a $Cu(OTf)_2$-catalyzed reaction, subsequent addition of Me_2EtSiH and $Cu(OTf)_2$ at ice-bath temperature provided the unanticipated fully protected 1,3,4-tri-*O*-acetylated derivatives **20** (entry 1) and **21** (entry 2) in 62% and 60% yields, respectively. The reaction presumably went through regioselective ring-opening at 4-O followed by 4-*O*-acetylation in situ owing to the large excess of Ac_2O. This phenomenon was not observed in the borane-reductive 6-*O*-opening (Table 3, entries 3 and 4). When the amount of acetylating reagent was reduced to 2.5 equiv and then followed by the Me_2EtSiH-mediated regioselective 4-*O*-ring-opening of the arylidene acetals in the same flask, the 4-alcohols **22** (entry 3) and **23** (entry 4) were acquired in 45% and 44% yields, respectively.

In carrying out the one-pot strategy to prepare the 1-alcohols, the basic and acidic conditions used for 1,3-di-*O*-acetylation in generating fully protected derivatives were handy in accessing the target molecules. As illustrated in Table 5, when the arylidene acetals were treated with Ac_2O in the presence of Et_3N and DMAP followed by regioselective 1-*O*-deacetylation with ammonia in MeOH/THF at 0 °C, the expected hemiacetals **24** (entry 1) and **25** (entry 2) were only isolated in 25% and 21% yields, respectively. The side products include an α,*β*-unsaturated aldehyde presumably generated through an open ring elimination reaction involving the 3-*O*-acetate. On the other hand, employing $Cu(OTf)_2$ and 10 equiv of Ac_2O for 1,3-di-*O*-acetylation resulted in the much better 59% yield for **24** (entry 3) and 53% for **25** (entry 4) after 1-*O*-deacetylation.

(20) Yang, W.-C.; Lu, X.-A.; Kulkarni, S. S.; Hung, S.-C. *Tetrahedron Lett.* **2003**, *44*, 7837–7840.

TABLE 5. Regioselective One-Pot Protection To Synthesize the 1-Alcohols



entry	condition[a]	Ar	product	yield (%)
1	A	Ph	**24**	25
2	A	2-Naph	**25**	21
3	B	Ph	**24**	59
4	B	2-Naph	**25**	53

[a]Condition A: AcOH, TBAF, 0 °C, 2 h, then 3 equiv of Ac_2O, Et_3N, DMAP, 0 °C to rt. Condition B: 0.2 equiv of $Cu(OTf)_2$, 10 equiv of Ac_2O, 40 °C, 3 h.

In summary, efficient conversion of D-glucosamine into various synthons bearing chemically differentiable protecting groups was, therefore, demonstrated by employing properly applied one-pot protocols. These synthons can be readily used as glycosyl building blocks in oligosaccharide assembly.

Experimental Section

General Procedure for the One-Pot Synthesis of 3-Alcohols 6*β*, 10*β*, 14, and 15. To a solution of compound **3** (0.265 g, 0.536 mmol) and ArCHO (0.562 mmol) in dichloromethane (3 mL) containing freshly dried 3 Å molecular sieves (300 mg) was slowly added trimethylsilyl trifluoromethanesulfonate (80.4 μmoL) at 0 °C under nitrogen atmosphere. The mixture was stirred at the same temperature for 1.5 h, and acetic acid/benzoic acid (1.17 mmol) and tetra-*n*-butylammonium fluoride (1.17 mmol) were sequentially added to the reaction solution. After stirring for another 4 h at 0 °C, acetic anhydride/benzoic anhydride (0.589 mmol) and Et_3N (5.36 mmol) were added to the solution, and the reaction mixture was stirred overnight at 0 °C. The whole mixture was filtered through a pad of Celite, and the filtrate was consecutively washed by water (3 mL) and saturated $NaHCO_{3(aq)}$ (3 mL). The aqueous layer was extracted with ethyl acetate (3 × 5 mL), and the combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (EtOAc/Hex = 1/3) to provide the desired 3-alcohol. For the preparation of the per-*O*-trimethylsilylated ether **3** and other regioselective one-pot procedures, please see the Supporting Information.

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