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From Solution Phase to "On-Column" Chemistry: Trichloroacetimidate-Based Glycosylation Promoted by Perchloric Acid−Silica

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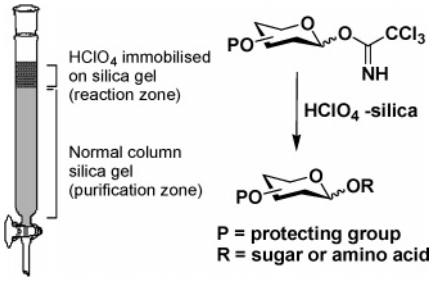

Activation of ester-protected glycosyl trichloroacetimidate donors by perchloric acid immobilized on silica afforded 1,2-*trans* disaccharides in 60−90% yields. Applying this approach to one-pot sequential glycosylation resulted in efficient syntheses of the *N*-linked glycan trimannoside and Le^X and Le^A trisaccharides in very good yield (76%, 62%, and 59% yields, respectively). Solution phase reactions were also translated to a solid phase format; priming the top of a standard silica chromatography column with perchloric acid immobilized on silica facilitated "on-column" glycosylation with subsequent "*in situ*" purification of products. Coupling yields from this approach were comparable to those obtained from the corresponding solution-phase disaccharide couplings. A series of glycosylated amino acids were also synthesized in high yield with use of the on-column approach.

Over the last twenty years or so, study of the biological function of carbohydrates and glycoconjugates, previously a largely neglected dimension of biological complexity, has become one of the fastest growing areas in biochemistry and cell biology.[1,2] It is now clearly understood that carbohydrates are actively involved in biological events involving cell−cell interactions,[3] inflammation,[4] signal transduction,[5] and fertility and development.[6,7] However, the precise detail of carbohydrate action remains to be delineated in many, perhaps most cases.[2] The requirement for rapid, straightforward syntheses of oligosaccharides and glycoconjugates is therefore obvious. Of the various synthetic strategies developed to date, glycoside syntheses based on glycosyl trichloroacetimidates are particularly popular,[8] second only to thioglycoside-based reactions.[9] Glycosyl imidate donor species were first reported by Sinaÿ in 1976,[10] with Schmidt and co-workers subsequently introducing the corresponding trichloroacetimidates in 1980.[11] Recently Yu and co-workers explored the application of *N*-substituted glycosyl trifluoroacetimidates[12] and reported particularly good reactivity for sialic acid glycosylation reactions. Demchenko and co-workers[13] noted that thioimidates are versatile donors for 1,2-*cis* and 1,2-*trans* glycosylation reactions. A number of acids have been exploited as promoters for trichloroacetimidate-based reactions, including TMSOTf,[14] $BF_3.Et_2O$,[15] and TBSOTf.[16] Noting recent reports of the use of perchloric acid immobilized on silica gel as an acid catalyst for various reactions in carbohydrate chemistry, including acetylation of reducing sugars[17] and Ferrier rearrangement[18] of glycals, we were drawn to explore the application of this reagent system.[19] Herein we describe the use of $HClO_4$−silica as an efficient promoter for conventional and two-step/one-pot solution phase glycosylation reactions using glycosyl trichloroacetimidate donors. In addition, we demonstrate the use of silica−perchloric acid in solid-phase, "on-column" glycosylation reactions with both sugar and amino acid alcohol acceptors.

Initial experiments with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate **1** (1.3 mmol) as

(1) (a) Varki, A. *Glycobiology* **1993**, *3*, 97−130 and references therein. (b) *Essentials of Glycobiology*; Varki, A., Cummings, R., Esko, J., Freeze, H., Hart, G., Martin, J., Eds.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1993.

(2) (a) Feizi, T. *Glycoconj. J.* **2000**, *17*, 553−565. (b) Le Poole, I. C.; Gerberi, M. A. T.; Kast W. M. *Curr. Opin. Oncol.* **2002**, *14*, 641−648.

(3) Nissen, P.; Hansen, J.; Ban, N.; Moore, P. B.; Steitz, T. A. *Science* **2000**, *289*, 920−930.

(4) Cach, T. R.; Zang, A. J.; Grabowski, P. J. *Cell* **1981**, *27*, 487−496.

(5) (a) Emilsson, G. M.; Breaker, R. R. *Cell. Mol. Life Sci.* **2002**, *59*, 596−607. (b) Breaker, R. R. *Nat. Biotechnol.* **1997**, *15*, 427−431. (c) Breaker, R. R. *Science* **2000**, *290*, 2095−2096.

(6) Vacquier, V. D.; Moy, G. W. *Dev. Biol.* **1997**, *192*, 125−135.

(7) Tiemeyer, M.; Goodman, C. S. *Development* **1996**, *122*, 925−936.

(8) Schmidt, R. R.; Jung, K.-H. *Carbohydrates in Chemistry and Biology*; Ernst, B., Hart, G. W., Sinaÿ, P., Eds.; Wiley-VCH: Weinheim, Germany, 2000, pp 5−60 and references therein.

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(10) (a) Pougny, J.-R.; Sinaÿ, P. *Tetrahedron Lett.* **1976**, 4073−4076. (b) Pougny, J.-R.; Jacquinet, J.-C.; Nassr, M.; Duchet, D.; Milat, M.-L.; Sinay, P. *J. Am. Chem. Soc.* **1977**, *99*, 6762−6763.

(11) Schmidt, R. R.; Michel, J. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 731−733.

(12) (a) Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, 2405−2407. (b) Yu, B.; Tao, H. *J. Org. Chem.* **2002**, *67*, 9099−9102.

(13) (a) Demchenko, A. V. *Curr. Org. Chem*., **2003**, *7*, 35−79 and references therein. (b) Demchenko, A. V.; Pornsuriyasak, P.; De Meo, C.; Malysheva, N. N. *Angew. Chem., Int. Ed.* **2004**, *43*, 3069−3072.

(14) Schaubach, R.; Hemberger, J.; Kinzy, W. *Liebigs Ann. Chem.* **1991**, 607−614.

(15) (a) Preuss, R.; Schmidt, R. R. *Synthesis* **1988,** 694−697. (b) Zimmermann, P.; Bommer, R.; Bär, T.; Schmidt, R. R. *J. Carbohydr. Chem.* **1988**, *7*, 435−452.

(16) Haase, W.-C.; Seeberger, P. H. *Curr. Org. Chem.* **2000**, *4*, 481−511.

(17) Misra, A. K.; Tiwari, P.; Madhusudan, S. K. *Carbohydr. Res.* **2005**, *340*, 325−329.

(18) (a) Agarwal, A.; Rani, S.; Vankar, Y. D. *J. Org. Chem.* **2004**, *69*, 6137−6140. (b) Misra, A. K.; Tiwari, P.; Agnihotri, G. *Synthesis* **2005**, *2*, 260−266.

(19) (a) Mukhopadhyay, B.; Russell, D. A.; Field, R. A. *Carbohydr. Res.* **2005**, *340*, 1075−1080. (b) Mukhopadhyay, B.; Collet, B.; Field, R. A. *Tetrahedron Lett.* **2005**, *46*, 5923−5925.

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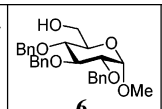
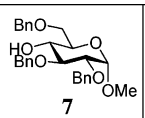
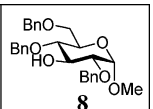
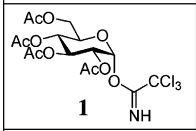
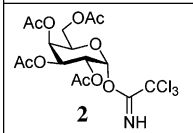
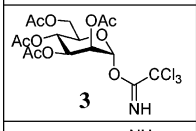
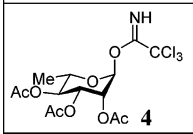
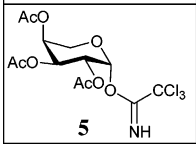
TABLE 1. Glycosylation Reactions of a Range of Trichloroacetimidate Donors and Sugar Alcohol Acceptors Promoted by $HClO_4$–Silica

ACCEPTORS ⇨ / DONORS ⇩	 **6**	 **7**	 **8**
 **1**	**9** (72%)	**10** (55%)	**11** (64%)
 **2**	**12** (89%)	**13** (68%)	**14** (71%)
 **3**	**15** (78%)	**16** (69%)	**17** (73%)
 **4**	**18** (87%)	**19** (81%)	**20** (93%)
 **5**	**21** (94%)	**22** (81%)	**23** (83%)

donor provided 72% of the desired 1,2-*trans* disaccharide **9** when reacted at −10 °C for ca. 1 h in dichloroethane (10 mL) with methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **6** (1.0 mmol) in the presence of $HClO_4$–silica (50 mg) (Table 1). The only other notable components at the end of the reaction period were unreacted acceptor and hydrolyzed donor. Encouraged by this result, a series of ester-protected glycosyl tricholoroacetimidate donors were synthesized and used for glycosylation reactions with a series of primary and secondary alcohol acceptor substrates. Results of these experiments are summarized in Table 1; yields quoted are for the isolated products formed in reactions conducted by undergraduate students with no prior experience in carbohydrate chemistry. The good-to-excellent yields obtained reflect the robustness of the $HClO_4$–silica glycosylation procedure. Due to the greater reactivity of primary alcohols, reactions with primary acceptor **6** gave better yields (72−94%) than reactions employing secondary alcohol acceptors **7** and **8** (55−93%). Excellent glycosylation yields from reactions employing the rhamnosyl trichloroacetimidate **4** and arabinosyl trichloroacetimidate **5** showed that the strategy is also effective for deoxy and pentose sugars, respectively.

The success of $HClO_4$–silica as a promoter for disaccharide syntheses prompted further studies toward trisaccharide syntheses. 3,6-Di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside, the *N*-linked glycan core trimannoside, is the natural ligand for the lectin concanavalin A.[20] There are several reported approaches for the synthesis of this type of trisaccharide.[21] Our approach was to establish a dimannosylation strategy using $HClO_4$–silica as promoter, while also investigating this reagent system in all other steps in the synthesis. Starting from free D-mannose, bromoethyl α-D-mannopyranoside **25** was prepared by a Fischer-type glycosylation with use of bromoethanol and $HClO_4$–silica. Ortho-esterification of the bromoethyl glycoside **25** with trimethyl orthobenzoate[20a] and $HClO_4$–silica gave the corresponding 2,3:4,6-di-ortho ester derivative, which was rearranged by the addition of H_2O to afford the required 2,4-di-*O*-benzoyl-α-D-mannopyranoside **26** along with 2,6-di-*O*-benzoyl-α-D-mannopyranoside **27** in 84% yield (**26**:**27** 1.5:1.0) (Scheme 1). With the monosaccharide diol acceptor **26** in hand, glycosylation was performed with an excess of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate **3** (3.0 equiv with respect to the acceptor), using $HClO_4$–silica as the promoter. The desired trimannoside derivative **28**[22] was obtained in 76% yield (Scheme 1).

Success in the one-pot trimannoside synthesis led us to explore the use of $HClO_4$–silica for the synthesis of more complex oligosaccharides, such as Le^X and Le^A trisaccharide derivatives. Here the relative reactivity of the 3- and 4-*OH* groups[23] of a partially protected *N*-acetylglucosamine building block **29** offers scope for one-pot sequential glycosylation,[24] so limiting the number of protecting group manipulations and removing rounds of chromatographic purification. The acceptor diol **29** was synthesized by protecting the primary hydroxyl group of known allyl 2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside **29**[25] with a *tert*-butyldiphenylsilyl (TBDPS) group, which leaves the 3- and 4-*OH* groups free for glycosylation with fucosyl and galactosyl residues, in either order, to achieve the target Le^X/Le^A trisaccharides (Scheme 2). The TBDPS group not only serves the purpose of selective protection of the primary hydroxyl group but also provides sufficient lipophilicity so that the diol acceptor is soluble in organic solvent (e.g., CH_2Cl_2). Accordingly, acceptor **29** was reacted with fucosyl trichloroacetimidate donor **30**,[26] chosen for its known ability to give α-fucopyranosides in high yield,[27] in the presence of $HClO_4$–silica in DCE−Et_2O (2:1).[28] The exclusive product was the 3-*O*-α-fucosylated disaccharide **31**, which was formed with 1,2-*cis* stereoselectivity. The stereo- and regioselectivity of the reaction was confirmed by isolating a sample of disaccharide from the reaction mixture prior to addition of the second donor. ^{1}H NMR spectroscopic analysis (δ_H 5.26 ppm, J = 3.2 Hz, H-1Fuc)

(20) Naismith, J. H.; Field, R. A. *J. Biol. Chem.* **1996**, *271*, 972−976 and references therein.

(21) (a) Oscarson, S.; Tidén, A.-K. *Carbohydr. Res.* **1993**, *247*, 323−328. (b) Yu, H. N.; Ling, C.-C.; Bundle, D. R. *J. Chem. Soc., Perkin Trans. 1* **2001**, 832−837. (c) Ratner, D. M.; Plante, O. J.; Seeberger, P. H. *Eur. J. Org. Chem.* **2002**, *5*, 826−833.

(22) Karamanska, R.; Mukhopadhyay, B.; Russell, D. A.; Field, R. A. *Chem. Commun.* **2005**, 3334−3336.

(23) Gallo-Rodriguez, C.; Gil-Libarona, M. A.; Mendoza, V. M.; de Lederkremer, R. M. *Tetrahedron* **2002**, *58*, 9373−9380.

(24) (a) Tanaka, H.; Matoba, N.; Tsukamoto, H.; Takimoto, H.; Yamada, H.; Takahashi, T. *Synlett* **2005**, *5*, 824−828. (b) Tanaka, H.; Adachi, M.; Tsukamoto, H.; Ikeda, T.; Yamada, H.; Takahashi, T. *Org. Lett.* **2004**, *4*, 4213−4216.

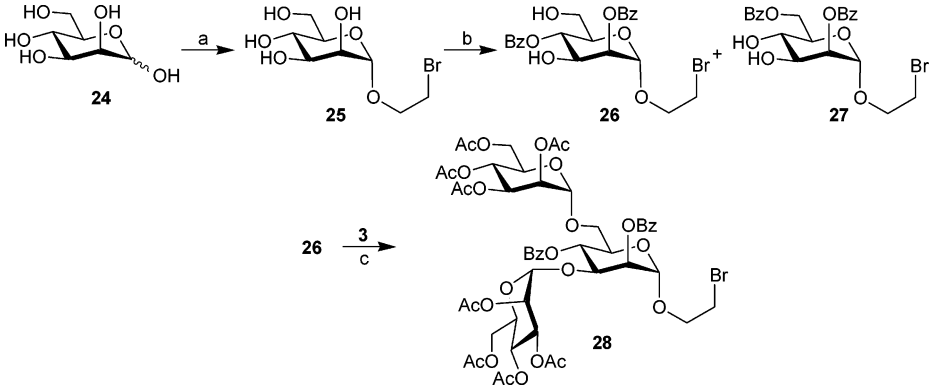
(25) Kusomoto, S.; Yoshimura, H.; Imoto, M.; Shimamoto, T.; Shiba, T. *Tetrahedron Lett.* **1985**, *26*, 909−912.

(26) Manzoni, L.; Castelli, R. *Org. Lett.* **2004**, *6*, 4195−4198.

(27) Windmüller, R.; Schmidt, R. R. *Tetrahedron Lett.* **1994**, *35*, 7927−7930.

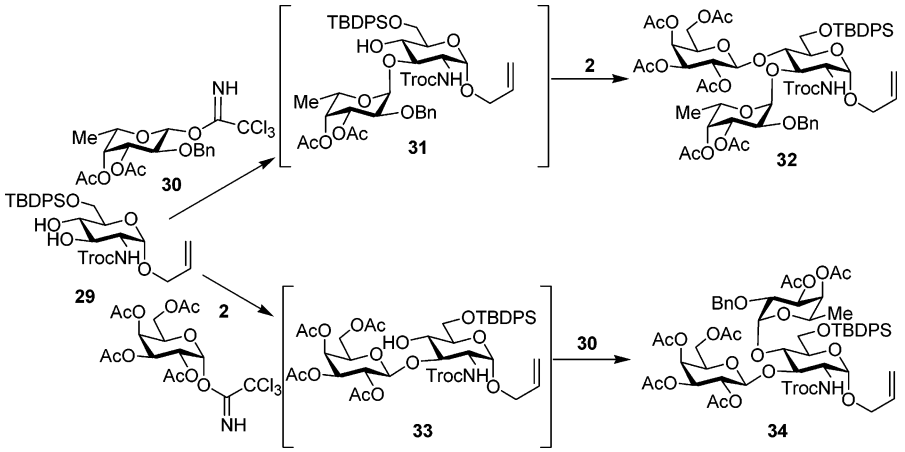
(28) When CH_2Cl_2 was used as solvent at −20 °C, an α/β mixture of the disaccharide was obtained in a ratio of 2:1. However, the same reaction in DCE−Et_2O (2:1) at −40 °C afforded only α-glycoside. The result is consistent with solvent effects on glycosylation reactions: Demchenko, A.; Stauch, T.; Boons, G.-J. *Synlett* **1997**, 818−820.

SCHEME 1. $HClO_4$–Silica-Promoted Trimannoside Synthesis[a]



[a] Reagents and conditions: (a) 2-bromoethanol, $HClO_4$–silica, CH_3CN, 45%; (b) (i) trimethyl orthobenzoate, $HClO_4$–silica, CH_3CN, (ii) H_2O, 84% (**26**:**27** 1.5:1.0); (c) $HClO_4$–silica, CH_2Cl_2, 76%.

SCHEME 2. One-Pot Sequential Glycosylation Reactions, Promoted by Perchloric Acid–Silica, for the Synthesis of Le^X and Le^A Trisaccharide Derivatives



proved consistent with formation of the 1,2-*cis*-α-fucoside linkage; a downfield shift of the *H*-3 signal of the GlcNHTroc moiety (from 3.98 to 4.38 ppm) confirmed the (1→3)-fucosyl linkage. After completion of disaccharide formation, as judged by TLC, galactosyl trichloroacetimidate donor **2** was added to the reaction mixture to afford the protected Le^X trisaccharide derivative **32** in 62% isolated yield (Scheme 2).

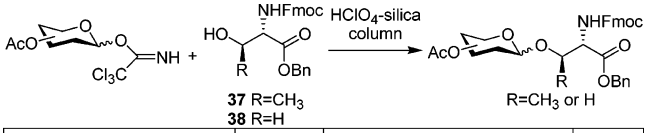
In a similar fashion, reaction of diol acceptor **29** with galactosyl trichloroacetimidate **2**, followed by the fucosyl trichloroacetimidate **30**, gave the Le^A trisaccharide derivative **34** in 59% isolated yield (Scheme 2). The regiospecificity of these reactions is clearly apparent by comparison of the anomeric carbon region of ^{13}C NMR spectra of the Le^X derivative **32** with that of the Le^A derivative **34** (see Figure 1 in the Supporting Information). Although the R_f for both Le^X and Le^A derivatives are the same (R_f 0.25, 2:1 *n*-hexanes–ethyl acetate), only single trisaccharide derivatives were isolated in both sets of reactions. It is important to note that when similar reactions were performed in the presence of TMSOTf as a promoter, the first step (*i.e.*, the formation of the disaccharides) was successful but subsequent addition of a second glycosyl donor to the reaction only gave rise to a mixture of compounds, dominated by hemiacetal arising from hydrolysis of the second donor substrate. It would appear that TMSOTf-mediated activation of trichloroacetimidates is more moisture sensitive than $HClO_4$–silica based glycosylation reactions.

Since the above reactions essentially involve adding silica to a reaction, we next considered adding the reaction to silica. That is, a chromatography column filled with flash silica gel was topped with a band of perchloric acid–silica, with a view to conducting the reactions "on-column", followed by "*in situ*" purification of the products. Useful results were obtained when a mixture of glucosyl trichloroacetimidate donor **1** and glucosyl acceptor **6** was employed. These two reactants were charged onto the column in dry CH_2Cl_2 and left for 30 min. The column was then eluted with 2:1 *n*-hexanes–ethyl acetate and the desired disaccharide **9** was obtained in 64% yield, a yield comparable to that obtained in the corresponding solution phase reaction. Further representative experiments from Table 1 were therefore repeated with the "on-column" approach, again with yields comparable to those obtained from solution phase chemistry (see Table 2 in the Supporting Information).

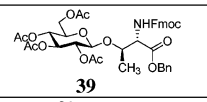
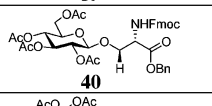
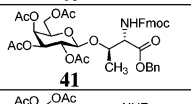
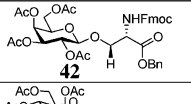
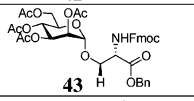
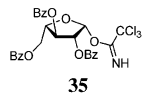
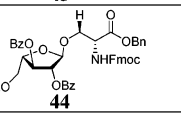
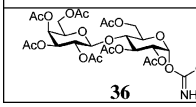
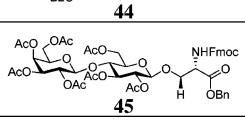
The activity of many proteins is highly dependent on their glycosylation state.[29] Synthesis of homogeneous glycopeptides, and hence glycosylated amino acid building blocks, is therefore topical.[30,31] We therefore focused our attention on the synthesis of *O*-glycosylated amino

(29) Sears, P.; Wong, C.-H. *Cell. Mol. Life Sci.* **1998**, *54*, 223–252.

TABLE 2. "On-Column" Synthesis of Glycosylated Amino Acids



37 R=CH_3
38 R=H

Trichloroacetimidate donor	Glycosyl acceptor	Product	Yield
1	**37**	 **39**	62 %
1	**38**	 **40**	60 %
2	**37**	 **41**	78 %
2	**38**	 **42**	75 %
3	**38**	 **43**	74 %
 **35**	**38**	 **44**	70 %
 **36**	**38**	 **45**	68 %

acids using the "on-column" glycosylation approach. A mixture of Fmoc-threonine benzyl ester **37** or Fmoc-serine benzyl ester **38** and a range of "disarmed" glycosyl trichloroacetimidate donors in dry CH_2Cl_2 were charged onto columns. After 10 min, the columns were eluted with 1:1 *n*-hexanes−EtOAc, giving the 1,2-*trans*-linked *O*-glycosides in good yield (Table 2). Unreacted amino acid derivative and the corresponding hemiacetal of the donor were isolated as byproducts; 1,2-*cis*-glycosides were not isolable in any case.

In summary, perchloric acid−silica serves as a convenient and effective promoter for trichloroacetimidate-based glycosylation reactions with ester-protected glycosyl donors. One- and two-step glycosylation reactions in solution give very good yields of the requisite glycoside products. In addition, use of perchloric acid−silica for "on-column" glycosylation, and subsequent "*in situ*" separation, provides a novel and robust method for glycoside synthesis. Studies to explore the compatibility of the procedures described with other glycosylation building blocks are ongoing.

Experimental Section

Preparation of $HClO_4$ Immobilized on Silica. Immobilized perchloric acid on silica was prepared essentially as described previously[18a] except that it was dried for 2 h at 110 °C instead of 6 h. $HClO_4$ (0.3 mmol, as a 70% aqueous solution) was added to a slurry of silica gel (5 g, 200 mesh) in Et_2O (15 mL) and the solvent was removed under reduced pressure. The resulting powder was kept at 110 °C for 2 h and used directly in reactions.

General Procedure for Solution Phase Glycosylation Reactions. A mixture of glycosyl acceptor (1 mmol), glycosyl trichloroacetimidate (1.3 mmol), and 4 Å MS (1 g) in dry DCE (10 mL) was cooled to −10 °C. $HClO_4$−silica (50 mg) was added and the mixture was allowed to stir until TLC (*n*-hexanes−EtOAc 2:1) showed complete disappearance of the starting materials. The mixture was filtered through a pad of Celite and the filtrate was evaporated in vacuo. The crude product was purified by flash chromatography to afford compounds **9**−**23**, the structures of which were confirmed by ^1H and ^{13}C NMR spectroscopy and mass spectrometry (Supporting Information).

General Procedure for "On-Column" Glycosylation Reactions. A 45 × 1.5 cm^2 glass column was dry-packed with flash silica gel (20 g) and $HClO_4$−silica (5 g) on top. Then a mixture of donor (1.3 mmol) and acceptor (1 mmol) in dry CH_2Cl_2 (1 mL) was carefully charged onto the column, which was allowed to stand at room temperature for 30 min. The column was then eluted with 1:1 *n*-hexanes−EtOAc to afford glycosylated amino acid followed by unreacted amino acid derivative and the corresponding hemiacetal of the donor.

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Supporting Information Available: Literature references for the synthesis of glycosyl donors and acceptors (**1**−**8**); references for known disaccharides (**9**−**13**); references for known glycosylated amino acids (**39**−**45**); specific rotations and ^1H and ^{13}C NMR and high-resolution mass spectral data for all new compounds, along with copies of NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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