Glycosidation

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Glycosidation Promoted by a Reusable Solid Superacid in Supercritical Carbon Dioxide**

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Functional roles of glycoconjugates are of growing importance owing to carbohydrate-mediated signal transductions, which are crucial steps for a variety of biological phenomena such as cellular differentiation, aging, immune systems, infections, and malignant alterations of cells. Synthetic studies of carbohydrates are indispensable both for elucidating essential structures of the functional glycoconjugates and for producing therapeutic reagents of carbohydrate-related diseases.[1]

Glycosidation is one of the most important reactions in chemical synthesis of glycoconjugates.[2] In recent years, although there has been an increasing interest in environmentally more-sustainable chemical processes,[3] a practical and cleaner chemical synthetic process of carbohydrates has been discussed very little except for a few pioneering efforts.[4] The advent of a highly efficient, safe, and environmentally benign method for the glycosidation is strongly expected because this key technology will allow for practical and industrial scale production of natural and unnatural glycosides. Enzyme-assisted synthesis has been recognized to be a much more sustainable and efficient strategy than pure chemical protocols in terms of the construction of various seeds libraries for the discovery and research of therapeutic reagents.[5] However, the present potentials are not high enough for large scale synthesis of drug candidates required for the following clinical trials.

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In the present study, our interest is focused on the feasibility of recyclable solid-superacid-promoted glycosidation in supercritical fluids (SCFs). Solid superacids are industrially potent catalysts that can act both as Lewis acids and Brønstead acids. SCFs used as reaction media for homogeneous catalysts or as compressed carriers for heterogeneous catalysts have great potential for manipulation of a variety of catalytic reactions.[3] As CO_2 has a critical point close to ambient temperature ($T_c = 31.3\,°C$, $P_c = 7.4$ MPa) and is nonflammable, nontoxic, and inexpensive, supercritical (sc)CO_2 has received immense interest as an alternative reaction media to petroleum-derived common organic solvents used in large-scale chemical synthesis. However, there has been no example of the glycosidation by means of $scCO_2$ as a reaction media. Although it seemed that this might be owing to the poor solubility of polar biomolecules, such as amino acids and sugars toward $scCO_2$, recent reports[6] on the improved solubility of some sugar acetates in $scCO_2$ greatly motivated us to challenge "green" replacement of conventional synthetic protocols by using hazardous promoters and flammable/toxic organic solvents.[7] We hypothesized that the use of a nonpolar $scCO_2$ may promote glycosidation by increasing the interaction of relatively polar species such as sugar derivatives and alcohols on the surface of solid acid catalysts. Furthermore, this effect will encourage the phase separation of contaminated H_2O, which appears to reduce the yields of the glycosidation due to hydrolysis of the products/intermediates through rehydration at the glycoside bonds. We thought that an original and desired apparatus suited for monitoring glycosidation process must allow for investigating versatile and general conditions of solid-superacid-promoted cleaner glycosidation in $scCO_2$.

To monitor the solubility of typical glycosyl donors and acceptors toward $scCO_2$, a novel apparatus with a quartz-window-equipped reactor was developed and examined in this study. Figure 1 shows schematic view of the apparatus used for glycosidation in supercritical fluids. In addition to the convenient quartz window, it should also be noted that a tailored stirrer connected with the reactor allowed for accelerating heterogeneous solid-acid-promoted glycosidation of various donors and acceptors with different solubility to $scCO_2$. As indicated in Figure 2, the reaction promoted by sulfated zirconia (SO_4/ZrO_2),[8] one of the common and industrially available solid superacid catalysts, was successfully monitored through the quartz window by using 3D digital fine scope. In accordance with the progress of the glycosidation, the reaction mixture exhibited a significant phase-transition state from emulsion to a clear supercritical fluidic phase, suggesting the existence of some sweet spots in the individual reaction condition. The present synthetic system made possible a precise optimization study for searching for suitable reaction conditions. We finally concluded that all the glycosidation reactions between donors **1**,[9] **2**,[10] **3**,[11] and acceptors **4**–**8** by using SO_4/ZrO_2 proceeded smoothly in $scCO_2$ under just over the critical point at 34 °C and 8.0 MPa for 6 h.

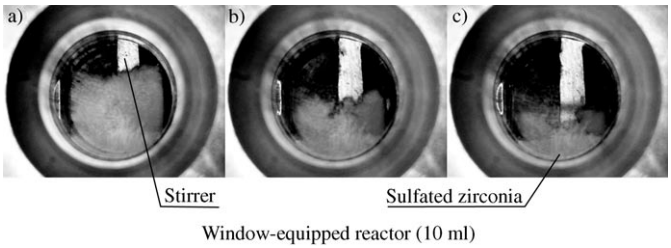


Figure 2. Photographs showing the process of the dissolution of glycosyl donor **1** (126 mg) and acceptor **4** (17 mg) during the glycosidation promoted by sulfated zirconia (126 mg) in $scCO_2$ at 34 °C: a) a mixture of solid materials before addition of CO_2; b) most of the donor and acceptor substrates were dissolved in $scCO_2$ at a pressure of 7.5 MPa; c) at a pressure of 8.0 MPa, only sulfated zirconia remained in the solid state.

Table 1 indicates the results of glycosidation of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate **1** with acceptors **4**–**7**. Coupling of **1** with all acceptors in $scCO_2$ gave only β-glycosides **9**,[12] **10**,[13] **11**, and **12** in 76–87 % yields, suggesting that the reactions proceeded by neighboring

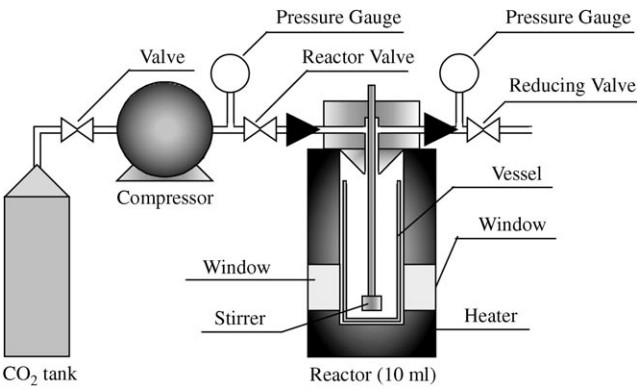
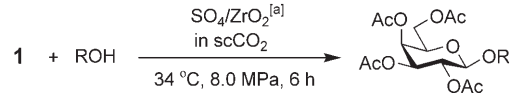


Figure 1. Equipment used for glycosidation reactions in $scCO_2$. The tailored SCF synthesizer is composed of a window-equipped stainless-steel 10-mL reactor with a circulatory heating apparatus, a high pressure pump, a pressure regulator, and a motor-driven stirrer.

Table 1: Glycosidations of glycosyl donor **1** with various glycosyl acceptors promoted by sulfated zirconia in $scCO_2$.[b]

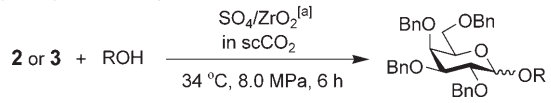


Entry	Acceptor	Products	Yield [%][c]	α/β ratio[d]
1	**4**	**9**	85	0/1
2	**5**	**10**	87	0/1
3	**6**	**11**	76	0/1
4	**7**	**12**	81	0/1

[a] SO_4/ZrO_2 was obtained commercially from Wako Pure Chemical Industries, Ltd. and activated by heating at 650 °C for 2 h before use. [b] Glycosidations were carried out by use of 1.1 equiv of alcohol and 100 wt % of SO_4/ZrO_2 to the donor **1**. [c] Yield of isolated product after purification by SFC. [d] The stereochemistry (α/β ratio) was determined by SFC and ^1H NMR spectroscopy (500 MHz).

group participation mechanism as observed in the reactions of common donors having 2-*O*-acyl protections conducted in common organic solvents. Glycosidation by using glycosyl donors **2** and **3** protected by *O*-benzyl groups were also examined under the same conditions, in $scCO_2$ at 34 °C and 8.0 MPa. The results summarized in Table 2 suggest that both

Table 2: Glycosidations of glycosyl donor **2** or **3** with various glycosyl acceptors promoted by sulfated zirconia in $scCO_2$.[b]



Entry	Donor	Acceptor	Products	Yield [%][c]	α/β ratio[d]
1	**2**	**4**	**13**	86	79/21
2	**2**	**5**	**14**	91	81/19
3	**2**	**7**	**15**	80	72/28
4	**2**	**8**	**16**	54	68/32
5	**3**	**4**	**13**	88	24/76
6	**3**	**5**	**14**	82	45/56
7	**3**	**7**	**15**	78	32/68
8	**3**	**8**	**16**	51	37/63

[a] SO_4/ZrO_2 was obtained commercially from Wako Pure Chemical Industries, Ltd. and activated by heating at 650 °C for 2 h before use. [b] Glycosidations were carried out by use of 1.1 equiv of alcohol and 100 wt% of SO_4/ZrO_2 to the donor **2** or **3**. [c] Yield of isolated product after purification by SFC. [d] The stereochemistry (α/β ratio) was determined by SFC and ^{1}H NMR spectroscopy (500 MHz). Bn = benzyl.

glycosyl donors **2** and **3** were efficiently activated by SO_4/ZrO_2 in $scCO_2$ and afforded the expected glycosides **13**,[14] **14**,[15] **15**,[16] and **16**[17] in 51–91% yields as a mixture of stereoisomers. These results indicate that the combination of SO_4/ZrO_2 with SFC did not work for the improvement of stereoselectivity in the selected glycosidation. The crude products, which contained solid catalyst, were recovered free from any solvent and thus they were dissolved in a small amount of methanol and subjected to simple filtration to separate the reusable catalyst. To minimize the use of organic solvents in the whole synthetic protocols, we decided to employ supercritical fluid chromatography (SFC) with $scCO_2$ as the mobile phase for the final purification of the glycosides instead of common silica gel chromatography. As indicated in Figure 3, it was revealed that SFC, by using $scCO_2$ under methanol-gradient (5–50%) at 30 °C and 14 MPa, allowed for separation of the mixture of α/β glycosides. Yields of the isolated products represent similar characteristics that are observed in the conventional methods with common organic solvents.

In conclusion, we have reported the first example of the cleaner chemical synthesis of glycosides in supercritical fluid ($scCO_2$). Our results show that CO_2 can provide significant advantages in reusable heterogeneous solid acid (SO_4/ZrO_2)-promoted glycosidation of common glycosyl donors with various acceptors over a wide range of temperatures and pressures. All of our glycosidation reactions show that there is a clear preference for glycoside formation over their hydrolytic by-products.[18] Purification of the stereoisomers of the glycosides by means of SFC allowed for totally sustainable and environmentally friendly protocol for the practical and large-scale synthesis of biologically and industrially important glycoconjugates. We are currently investigating the versatility of the present protocols by the combined use of SCF and SFC in the combinatorial synthesis of glycoside-based drug seeds libraries as well as the construction of commercially available SCF synthesizer that can be implemented in existing organic synthesis and carbohydrate chemistry laboratories (compounds used in this study are listed in Scheme 1).

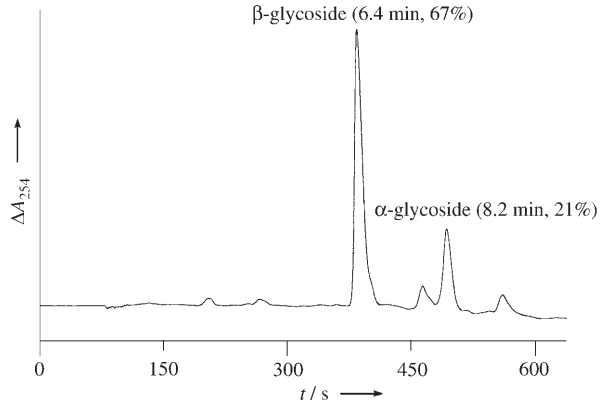


Figure 3. SFC profile for the separation of the anomeric mixture of the glycosides produced by the glycosidation of 2,3,4,6-*tetra*-*O*-benzyl-α-D-galactopyranosyl imidate (**3**) with *n*-octylalcohol (**4**). Purification was performed with a Gilson SF3 SFC system equipped with Chiralpak AD (4.6×250 mm^2, Daicel Chemical Industries, Ltd.) under a $scCO_2$–MeOH gradient program (MeOH, 5% (0 min)→50% (15 min), flow rate = 2.5 mL min^{-1}) at 14 MPa and 30 °C. Monitoring of the compounds was carried out with a UV detector at 254 nm or an evaporative light scattering detector (ELSD) in cases for the purification of O-acetylated products derived from glycosyl donor **1**).

Experimental Section

A quartz vessel charged with glycosyl donor (1 equiv), accepter (1.1 equiv), and sulfated zirconia (100 wt% of the donor) was attached to the reactor (10 mL) as shown in Figure 2. The reactor was heated to 34 °C and CO_2 was introduced successively from a reservoir by a high-pressure pump. The start of the reaction is defined as the time at which the CO_2 pressure reaches 8.0 MPa. After stirring the reaction mixture at 34 °C and 8.0 MPa for 6 h, the reactor was cooled to 20 °C and CO_2 was then slowly leaked out. The residual solid in the vessel was washed out with methanol. Sulfated zirconia was recovered by filtration. The filtrate was evaporated to give a residue that was subjected to SFC for the isolation and analysis (yield and α/β ratio) of the product.

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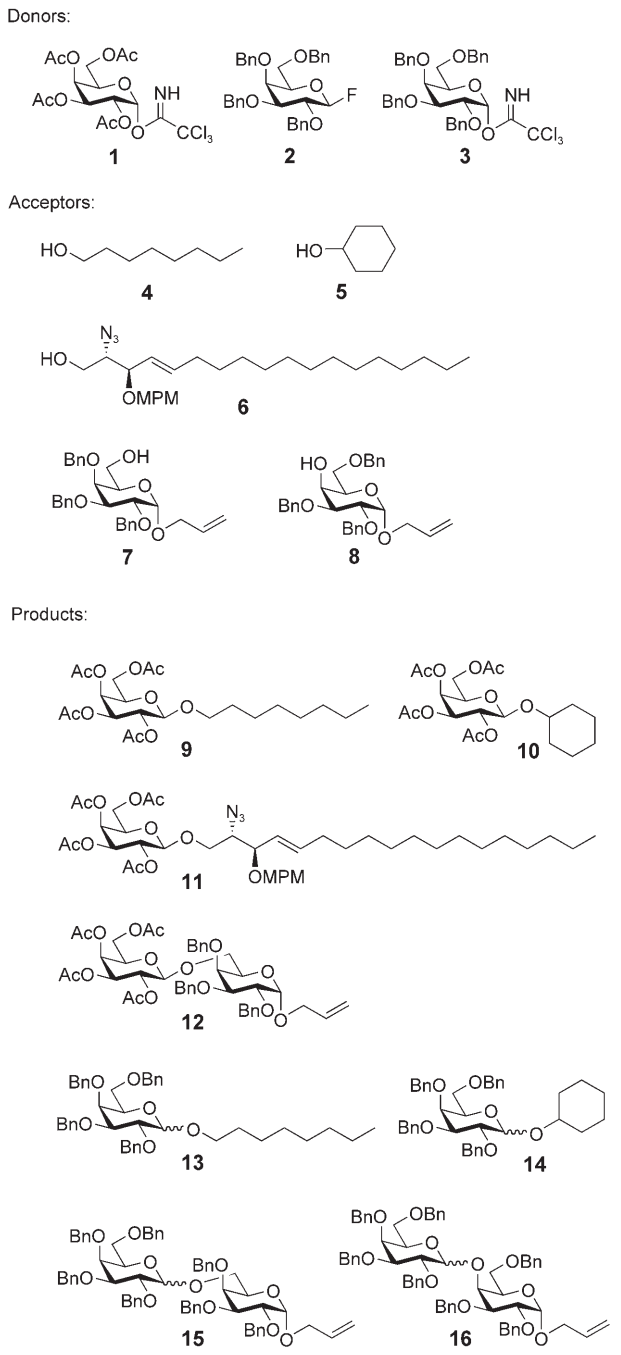

Scheme 1. Chemical structures of glycosyl donors, acceptors, and products used in this study. MPM = 4-methoxybenzyl

[1] R. A. Dwek, *Chem. Rev.* **1996**, *96*, 683.

[2] For selected reviews on chemical glycosidation, see: a) R. R. Schmidt, *Angew. Chem.* **1986**, *98*, 213; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212; b) P. Sinaÿ, *Pure Appl. Chem.* **1991**, *63*, 519; c) K. Toshima, K. Tatsuta, *Chem. Rev.* **1993**, *93*, 1503; d) G.-J. Boons, *Tetrahedron* **1996**, *52*, 1095; e) B. G. Davis, *J. Chem. Soc. Perkin Trans. 1* **2000**, 2137; f) B. Fraser-Reid, J. C. López, A. M. Gómez, C. Uriel, *Eur. J. Org. Chem.* **2004**, 1387.

[3] For example, see: a) P. G. Jessop, T. Ikariya, R. Noyori, *Nature* **1994**, *368*, 231; b) P. T. Anastas, T. C. Williams, *Green Chemistry: Designing Chemistry for the Environment*, Oxford University Press, Oxford, **1998**; c) P. E. Savage, *Chem. Rev.* **1999**, *99*, 603; d) A. Baiker, *Chem. Rev.* **1999**, *99*, 453.

[4] a) K. Sasaki, S. Matsumura, K. Toshima, *Tetrahedron Lett.* **2004**, *45*, 7043; b) H. Nagai, K. Sasaki, S. Matsumura, K. Toshima, *Carbohydr. Res.* **2005**, *340*, 337.

[5] Enzyme-assisted synthesis of glycoconjugates, for example, see: a) C. Unverzagt, H. Kunz, J. C. Paulson, *J. Am. Chem. Soc.* **1990**, *112*, 9308; b) C. Unverzagt, *Angew. Chem.* **1996**, *108*, 2507; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2350; c) K. M. Koeller, C.-H. Wong, *Chem. Rev.* **2000**, *100*, 4465; d) M. Meldal, F.-I. Auzanneau, O. Hindsgaul, M. M. Palcic, *J. Chem. Soc. Chem. Commun.* **1994**, 1849; e) O. Seitz, C.-H. Wong, *J. Am. Chem. Soc.* **1997**, *119*, 8766; f) S. K. George, T. Schwientek, B. Holm, C. A. Reis, H. Clausen, J. Kihlberg, *J. Am. Chem. Soc.* **2001**, *123*, 11117; g) N. Bezay, G. Dudziak, A. Liese, H. Kunz, *Angew. Chem.* **2001**, *113*, 2350; *Angew. Chem. Int. Ed.* **2001**, *40*, 2292; h) M. Fumoto, H. Hinou, H. Ohta, T. Ito, K. Yamada, A. Takimoto, H. Kondo, H. Shimizu, T. Inazu, Y. Nakahara, S. -I. Nishimua, *J. Am. Chem. Soc.* **2005**, *127*, 11804.

[6] a) P. Raveendran, S. L. Wallen, *J. Am. Chem. Soc.* **2002**, *124*, 7274; b) V. K. Potluri, J. Xu, R. Enick, E. Beckman, A. D. Hamilton, *Org. Lett.* **2002**, *4*, 2333.

[7] In general, chemical glycosidation is promoted by means of many hazardous promoters such as trimethylsilyl trifluoromethanesulfonate (TMSOTf), $BF_3{\cdot}Et_2O$, $Cp_2HfCl_2{\cdot}AgClO_4$, AgOTf, and *N*-iodosuccimide/trifluoromethanesulfonic acid (NIS/TfOH) in petroleum-derived common organic solvents such as dichloromethane, dichloroethane, toluene, ether, tetrahydrofuran, and acetonitrile. As for the novel approaches in this area, see: a) K.-S. Ko, F. A. Jaipuri, N. L. Pohl, *J. Am. Chem. Soc.* **2005**, *127*, 13162; b) D. M. Ratner, E. R. Murphy, M. Jhunjhunwala, D. A. Synder, K. F. Jensen, P. H. Seeberger, *Chem. Commun.* **2005**, 578; c) B. Mukhopadhyay, S. V. Maurer, N. Rudolph, R. M. van Well, D. A. Russel, R. A. Field, *J. Org. Chem.* **2005**, *70*, 9059.

[8] a) S.-I. Nishimura, Patent WO PCT/JP99/04261, **1999**; b) H. Nagai, K. Kawahara, S. Matsumura, K. Toshima, *Tetrahedron Lett.* **2001**, *42*, 4159; c) K. Toshima, H. Nagai, K. Kasumi, K. Kawahara, S. Matsumura, *Tetrahedron* **2004**, *60*, 5331.

[9] S. Sato, Y. Ito, T. Nukada, Y. Nakahara, T. Ogawa, *Carbohydr. Res.* **1987**, *167*, 197.

[10] T. Mukaiyama, Y. Murai, S.-I. Shoda, *Chem. Lett.* **1981**, 431.

[11] R. R. Schmidt, J. Michel, *J. Carbohydr. Chem.* **1985**, *4*, 141.

[12] D. S. K. Tsui, P. A. J. Gorin, *Carbohydr. Res.* **1985**, *144*, 137.

[13] H. Kondo, S. Aoki, Y. Ichikawa, R. L. Halcomb, H. Ritzen, C.-H. Wong, *J. Org. Chem.* **1994**, *59*, 864.

[14] A. Bülow, T. Meyer, T. K. Olszewski, M. Bols, *Eur. J. Org. Chem.* **2004**, 323.

[15] H. Uchiro, K. Miyazaki, T. Mukaiyama, *Chem. Lett.* **1997**, 403.

[16] P. A. Gent, R. Gigg, *J. Chem. Soc. Perkin Trans. 1* **1975**, 1972.

[17] Compound **16** was hydrogenated and characterized as known compounds that were reported previously, see: H. A. El-Shenawy, C. Schuerch, *Carbohydr. Res.* **1984**, *131*, 227.

[18] To achieve much higher yields and stereoselective glycosidation by using the present SCF-SFC synthesis, we are now evaluating feasibility of many other solid Lewis acid catalysts and the results will be reported shortly.

