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Accelerated glycosylation under frozen conditions

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Abstract—O-Glycosylations using thiomethyl glycosides as donors were compared under both frozen and unfrozen conditions. In the presence of MeOTf as a promoter, enormous rate acceleration was observed when the glycosylation was conducted in *p*-xylene below its freezing point.

Chemical reactions are believed to proceed most smoothly under freely diffusing conditions, typically in homogeneous solutions. Therefore, it is taken as granted that the reaction temperature must be higher than the melting point of the solvent. In frozen solutions, reactions are considered to be extremely slow, because diffusion of reactants would be severely restricted. However, there have been several reports, which revealed the dramatic rate acceleration of chemical[1] and enzymatic[2] reactions under frozen conditions both in aqueous and nonaqueous systems. These observations were explained by the concentration effect.[3] Below freezing point, but above the eutectic temperature, frozen solution contains a small volume of liquid phase that is in an equilibrium with solid phase. According to the freezing point–composition relationship, it is expected that solutes (reactants and reagents) are highly concentrated in liquid particles. If the effect of concentration overrides the rate reduction caused by cooling, bimolecular reaction would be accelerated in frozen media. Our thought was that freezing would increase the velocity of intermolecular coupling reaction like glycosylation. In this paper, we wish to report the first demonstration of accelerated O-glycosylation in frozen medium.

As part of our effort to develop facile synthetic routes to oligosaccharide molecular probes related to high-mannose type glycoproteins,[4] we planned the preparation of trimannoside **5**, starting from the mannose derivative **1**[5] having lipophilic tether (Scheme 1). Glycosylation of **1** with thioglycoside **2**[5] was attempted in the presence of methyl trifluoromethanesulfonate (MeOTf)[6] and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP). This glycosylation turned out to be quite slow at room temperature. In toluene, it resulted in ca. 60% conversion after 2 days, and elevated temperature (50 °C) was required for completion (Table 1, entry 1). The use of 1,2-dichloroethane facilitated the reaction to an extent that it completes at room temperature, albeit after 19 h (entry 2).

When the same glycosylation was conducted in *p*-xylene (mp 12–13 °C) below freezing temperature, marvelous rate acceleration was observed (entry 3). For instance, at 4 °C, the reaction was complete after 7 h to provide disaccharide **3**[7] in 93% yield ($\alpha:\beta = 8.3:1$). Since solvating properties of *p*-xylene and toluene are likely to be very similar, this result implied that the rate acceleration originates from the unique nature of the frozen environment. After chromatographic separation, anomerically pure **3α** was desilylated (1.5 equiv Bu_4NF, THF, 50 °C, 4 h) to give **4** (quantitative). The latter was then subjected to the second glycosylation, again using **2** and MeOTf/DTBMP as a donor and a promoter, respectively. The effect of freezing was even more prominent in this case (entries 4–6). Namely, the smooth formation of the trisaccharide **5** (77%, $\alpha:\beta = 18:1$) was observed in frozen *p*-xylene (4 °C), while the same reaction in

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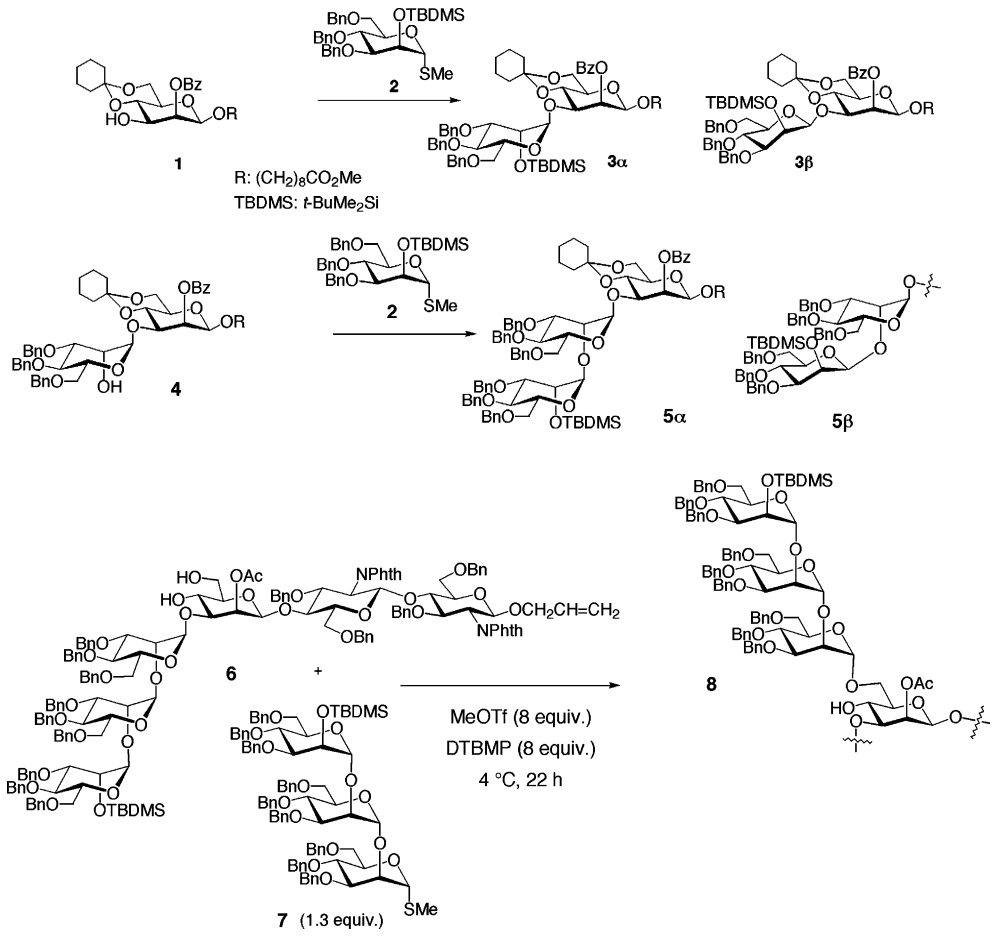


Scheme 1.

Table 1. Glycosylation with thioglycoside **2** or **7**

Entry	Donor (equiv)	Acceptor	Solvent	MeOTf/DTBMP (equiv)	Temperature/ time (°C/h)	Product	Yield (%)	Ratio[a] (α:β)
1	**2** (2.5)	**1**	Toluene	4.5/4.5	50/14	**3**	88	~40:1
2	**2** (2.5)	**1**	$(ClCH_2)_2$	4.5/4.5	rt/19	**3**	91	3.8:1
3	**2** (2.0)	**1**	*p*-Xylene	3.5/2.2	4/7	**3**	93	8.3:1
4	**2** (2.0)	**4**	$(ClCH_2)_2$	4.5/4.5	rt/19, 50/10	**5**	65[b]	15:1
5	**2** (1.5)	**4**	Toluene	2.7/1.6	50/22	**5**	50[c]	~50:1
6	**2** (1.5)	**4**	*p*-Xylene	2.7/1.6	4/18	**5**	77	18:1
7	**7** (1.3)	**6**	Toluene	8.0/8.0	4/22	**8**	6	α[d]
8	**7** (1.3)	**6**	*p*-Xylene	8.0/8.0	4/22	**8**	61	α[d]

[a] Based on the amounts of isolated products.
[b] 18% Recovery of **4**.
[c] 43% Recovery of **4**.
[d] Only α-isomer was isolated.

toluene (22 h) or 1,2-dichloroethane (19 h) was incomplete even at 50 °C.

Block condensation of larger oligosaccharide fragments ($\mathbf{6}^4 + \mathbf{7}^4 \rightarrow \mathbf{8}$) was also accelerated dramatically in frozen *p*-xylene (entries 7, 8).

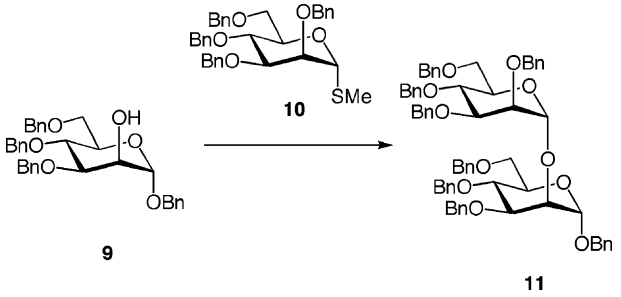
With these results in hand, systematic comparison was made using **9** and **10** as an acceptor and donor pair, and results are summarized in Table 2. In *p*-xylene, both donor (**9**) and acceptor (**10**) were completely consumed after 6 h at 4 °C (entry 7) to give disaccharide **11**[8] in high yield, while conversion was only marginal at room temperature (entry 1). Remarkably, reaction at room temperature (entry 1) was even slower than at −20 °C (entry 8) and comparable with conducted at −40 °C (entry 10). At −20 and −40 °C, complete conversion was observed after 24 h (entry 9) and 48 h (entry 11), respectively, while reactions were extremely sluggish in toluene at these temperatures (entries 3–6) (Scheme 2).

Our results clearly demonstrated that freezing the mixture increases the rate of O-glycosylation. The use of frozen media is technically simple and does not require

Table 2. Glycosylation of **9** with **10**; comparison of frozen and unfrozen systems[a]

	Entry[b]	Solvent[c]	Temperature (°C)	Time (h)	Yield (%)	Ratio (α:β)	Recovery (%)	
							9	**10**
Unfrozen	1	X	rt	6	24	46:1[d]	60	64
	2	T	4	6	11	16:1[e]	73	66
	3	T	−20	6	<1	α[f]	89	87
	4	T	−20	24	5	16:1[e]	78	80
	5	T	−40	6	0[g]	—	88	85
	6	T	−40	48	6	10:1[e]	80	80
Frozen	7	X	4	6	86	3.4:1[d]	ND[g]	
	8	X	−20	6	66	4.5:1[d]	28	34
	9	X	−20	24	81	4.0:1[d]	ND[g]	
	10	X	−40	6	17	10:1[d]	72	74
	11	X	−40	48	82	5.0:1[d]	ND[g]	

[a] Performed by using 0.07–0.12 mmol of **9** (0.03 M in *p*-xylene or toluene), 1.35 equiv of **10**, 2.5 equiv of MeOTf, 1.5 equiv of DTBMP, and molecular sieves 4 Å (ca. 0.3 g/0.1 mmol of **9**).
[b] All reactions were performed in refrigerator (except entry 1) adjusted to the appropriate temperature.
[c] X: *p*-xylene, T: toluene.
[d] Based on the amounts of isolated products.
[e] Determined by ^{1}H NMR (400 MHz, $CDCl_3$).
[f] Only α-isomer could be detected.
[g] Not detectable by TLC.



Scheme 2.

any special devise. It may be suggested as a clue to facilitate glycosylation under certain circumstances. Somewhat unexpectedly, the stereoselectivity seems to be attenuated under frozen conditions (e.g., Table 2, entries 1, 2, 7, 8). In this context, more systematic studies, with various types of glycosyl donors, promoters, and solvents that can be frozen, would be required to clarify the scope and advantage of frozen system in oligosaccharide synthesis.

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References and notes

1. Pincock, R. E.; Kiovsky, T. E. *J. Am. Chem. Soc.* **1965**, *87*, 4100–4107, **1965**, *87*, 2072–2073; **1966**, *88*, 51–55; Grant, N. H.; Alburn, H. E. *Science* **1965**, *150*, 1589–1590.
2. Grant, N. H.; Alburn, H. E. *Nature* **1966**, *212*, 194; Ullmann, G.; Haensler, M.; Gruender, W.; Wagner, M.; Hofmann, H.-J.; Jakubke, H.-D. *Biochim. Biophys. Acta* **1997**, *1338*, 253–258; Wehofsky, N.; Kirbach, S. W.; Haensler, M.; Wissmann, J.-D.; Bordusa, F. *Org. Lett.* **2000**, *2*, 2027–2030; Wehofsky, N.; Haensler, M.; Kirbach, S. W.; Wissmann, J.-D.; Bordusa, F. *Tetrahedron: Asymmetry* **2000**, *11*, 2421–2428; Schuster, M.; Ullmann, G.; Jakubke, H.-D. *Tetrahedron Lett.* **1993**, *34*, 5701–5702; Schuster, M.; Aaviksaar, A.; Jakubke, H.-D. *Tetrahedron* **1990**, *46*, 8093–8102.
3. Pincock, R. E.; Kiovsky, T. E. *J. Chem. Educ.* **1966**, *43*, 358–360.
4. Matsuo, I.; Wada, M.; Manabe, S.; Yamaguchi, Y.; Otake, K.; Kato, K.; Ito, Y. *J. Am. Chem. Soc.* **2003**, *125*, 3402–3403.
5. Preparation of these compounds will be reported in a separate account.
6. Lönn, H. *Carbohydr. Res.* **1985**, *139*, 105–113.
7. Typical experimental procedure (preparation of disaccharide **3**): a solution of compound **1** (21 mg, 0.039 mmol) and **2** (47 mg, 0.079 mmol) in *p*-xylene (3 mL) that contained activated molecular sieves 4 Å (200 mg) and DTBMP (18 mg, 0.086 mmol) was stirred under Ar at room temperature for 0.5 h to ensure dehydration. Subsequently, MeOTf (9.5 μL, 0.141 mmol) was added, and the mixture was rapidly mixed and frozen by liq. N_2. The mixture was stored in refrigerator at 4 °C for 7 h and was defrosted at room temperature. Et_3N (29 mL, 0.212 mmol) was added to quench MeOTf and the mixture was diluted with AcOEt, filtered through Cerite. The filtrate was washed with brine, dried ($MgSO_4$), and evaporated in vacuo. The residue was chromatographed over silica gel (hexane–AcOEt = 3:1) to afford 34.8 mg (83%) of **3α** together with the stereoisomer **3β** (4.2 mg, 10%). Compound **3α**: ^{1}H NMR ($CDCl_3$) δ 8.09 (d, 2H, *J* 7.8 Hz, aromatic), 7.55–7.18 (m, 18H, aromatic), 5.67 (s, 1H, H-2^{I}), 5.08 (s, 1H, H-1^{II}), 4.79 (d, 1H, *J* 11.7 Hz, C*H*HPh), 4.67 (d, 1H, *J* 11.7 Hz, C*H*HPh), 4.38 (s, 1H, H-1^{I}), 4.52 (d, 2H, *J* 11.7 Hz, CH_2Ph), 4.38 (s, 2H, CH_2Ph), 4.07–4.01 (m, 3H, H-3^{I}, 4^{I}, 5^{II}), 3.96–3.87 (m, 4H, H-2^{II}, 4^{II}, 6^{II}), 3.72–3.70 (m, 3H, H-6^{I}, OC*H*H(CH$_2$)$_7$–CO$_2$CH$_3$), 3.65 (s, 3H, CO$_2$CH_3), 3.61 (dd, 1H, *J* 2.2, 9.3 Hz, H-3^{II}), 3.45–3.40 (m, 1H,

OC*H*H(CH$_2$)$_7$–CO$_2$CH$_3$), 3.30–3.24 (m, 1H, H-5^{I}), 2.24 (t, 2 H, *J* 7.6 Hz, C*H*$_2$CO$_2$CH$_3$), 2.28–1.41 (m, 14 H, OCH$_2$C*H*$_2$–(CH$_2$)$_4$C*H*$_2$CH$_2$CO$_2$CH$_3$, cyclohexyl), 1.18 (s, 8 H, O(CH$_2$)$_2$(C*H*$_2$)$_4$(CH$_2$)$_2$CO$_2$CH$_3$), 0.85 (s, 9 H, C(C*H*$_3$)$_3$), 0.00 (s, 3 H, SiC*H*$_3$), −0.04 (s, 3 H, SiC*H*$_3$); ^{13}C NMR (CDCl$_3$) 173.81 (*C*$_2$CH$_3$), 165.36 (O*C*OPh), 100.88 (C-1^{II}, $^{1}J_{CH}$ 175.0 Hz), 99.53 × 2 (C-1^{I}, $^{1}J_{CH}$ 156.7 Hz, cyclohexylidene), 79.72 (C-3^{II}), 73.91 (C-4^{II}), 73.75, 72.71, 72.27 (3 × *C*H$_2$Ph), 72.48 (C-5^{II}), 72.16 (C-3^{I}), 71.44 (C-2^{I}), 71.10 (C-4^{I}), 69.88 (C-2^{II}), 69.81 (O*C*H$_2$(CH$_2$)$_7$CO$_2$CH$_3$), 69.39 (C-6^{II}), 67.91 (C-5^{I}), 61.33 (C-6^{I}), 51.27 (CO$_2$*C*H$_3$), 37.87, 27.89, 25.57, 22.89, 22.62 (cyclohexyl), 33.96 (*C*H$_2$CO$_2$CH$_3$), 29.31 (OCH$_2$*C*H$_2$(CH$_2$)$_6$CO$_2$CH$_3$), 29.00, 28.96, 28.88, 25.65 (O(CH$_2$)$_2$(*C*H$_2$)$_4$(CH$_2$)$_2$CO$_2$CH$_3$), 25.65 (C(*C*H$_3$)$_3$), 24.81 (*C*H$_2$CH$_2$CO$_2$CH$_3$), 18.04 (*C*(CH$_3$)$_3$), −4.51 (Si*C*H$_3$), −5.04 (Si*C*H$_3$). Compound **3β**: ^{1}H NMR (CDCl$_3$) *δ* 8.09 (d, 2H, *J* 7.1 Hz, aromatic), 7.60–7.24 (m, 18H, aromatic), 5.71 (s, 1H, H-2^{I}), 4.85 (d, 1H, *J* 11.1 Hz, C*H*HPh), 4.75 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.68 (d, 2H, *J* 11.5 Hz, C*H*$_2$Ph), 4.62 (s, 1H, H-1^{I}), 4.59 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.57 (d, 1H, *J* 11.1 Hz, C*H*HPh), 4.50 (s, 1H, H-1^{II}), 4.14–4.05 (m, 2H, H-3^{I}, 4^{I}), 3.98–3.74 (m, 7H, H-6^{I}, 2^{II}, 4^{II}, 6^{II}, OC*H*H(CH$_2$)$_7$CO$_2$CH$_3$), 3.64 (s, 3H, CO$_2$C*H*$_3$), 3.52–3.29 (m, 4H, H-5^{I}, 3^{II}, 5^{II}, OC*H*H(CH$_2$)$_7$CO$_2$CH$_3$), 2.39–2.31 (br m, 1H, cyclohexyl), 2.25 (t, 2H, *J* 7.6 Hz, C*H*$_2$CO$_2$CH$_3$), 1.64–1.26 (m, 21H, OCH$_2$(C*H*$_2$)$_6$CH$_2$CO$_2$CH$_3$, cyclohexyl), 0.78 (s, 9H, C(C*H*$_3$)$_3$), −0.18 (s, 3H, SiC*H*$_3$), −0.25 (s, 3H, SiC*H*$_3$); ^{13}C NMR (CDCl$_3$) 173.98 (*C*O$_2$CH$_3$), 165.93 (O*C*OPh), 99.97 (cyclohexylidene), 99.45 (C-1^{I}, $^{1}J_{CH}$ 155.1 Hz), 96.69 (C-1^{II}, $^{1}J_{CH}$ 153.4 Hz), 82.61 (C-3^{II}), 76.61 (C-5^{II}), 74.92, 73.31, 71.56 (3 × *C*H$_2$Ph), 74.50 (C-3^{II}), 74.29 (C-4^{II}), 70.11 (O*C*H$_2$(CH$_2$)$_7$CO$_2$CH$_3$), 69.34 (C-2^{II}), 68.77 (C-5^{I}), 68.66 (C-2^{I}), 68.57 (C-6^{II}), 68.48 (C-4^{I}), 61.45 (C-6^{I}), 51.46 (CO$_2$*C*H$_3$), 38.02, 27.61, 25.79, 22.79, 22.72 (cyclohexyl), 34.14 (*C*H$_2$CO$_2$CH$_3$), 29.45 (OCH$_2$*C*H$_2$(CH$_2$)$_6$CO$_2$CH$_3$), 29.16, 29.07, 25.86 (O(CH$_2$)$_2$(*C*H$_2$)$_4$(CH$_2$)$_2$CO$_2$CH$_3$), 25.95 (C(*C*H$_3$)$_3$), 25.00 (*C*H$_2$CH$_2$CO$_2$CH$_3$), 18. 48 (*C*(CH$_3$)$_3$), −4.20 (Si*C*H$_3$), −5.21 (Si*C*H$_3$).

8. α-Isomer: ^{1}H NMR (CDCl$_3$) *δ* 5.16 (d, 1H, *J* 1.5 Hz, H-1^{II}), 4.95 (d, 1H, *J* 1.7 Hz, H-1^{I}); ^{13}C NMR (CDCl$_3$) *δ* 99.5 (J_{C-H} 170 Hz), 98.2 (J_{C-H} 170 Hz). β-Isomer: ^{1}H NMR (CDCl$_3$) *δ* 4.97 (d, 1H, *J* 1.7 Hz, H-1^{I}), 4.56–4.30 (m, 11H, H-1^{II}, H-2^{I}, PhC*H*$_2$); ^{13}C NMR (CDCl$_3$) *δ* 99.2 ($^{1}J_{C-H}$ 155 Hz), 96.4 ($^{1}J_{C-H}$ 169 Hz).