Accelerated *O*-Glycosylation under Frozen Conditions and Its Application to the Synthesis of Complex Glycans

凍結反応条件下における *O*-グリコシル化反応の加速と複雑な糖鎖の合成への応用

Ishiwata, Akihiro¹; Sakurai, Ayaka¹; and Ito, Yukishige^{1,2}

¹RIKEN Advanced Science Institute, 2-1, Hirosawa, Wako, Saitama 351-0198, Japan

²ERATO Ito Glycotrility Project, JST, 2-1, Hirosawa, Wako, Saitama 351-0198, Japan

FAX: 81-48-462-4680, E-mail: aishiwa@riken.jp & yukito@riken.jp

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Abstract

Enormous rate acceleration was observed when *O*-glycosylation was conducted in *p*-xylene below its freezing point, as determined by comparative studies under both frozen and unfrozen conditions. The mechanistic implications of stereoselectivity on glycosylation under frozen systems were also investigated. Examination of the effects of various factors, such as solvent, concentration, anomeric configuration and protective groups of the donor, revealed that the stereoselectivity was affected by concentration both in liquid and in frozen conditions. The rate acceleration in frozen solvent was caused by the highly concentrated environments found therein. The substrate concentration under frozen conditions was estimated to be about 50 times higher than that before freezing. This manuscript also summarizes the application of this technique to the synthesis of complex glycans as well as bioactive molecules.

要 約

O-グリコシル化反応を *p*-キシレン中凍結条件下で行ったところ、非凍結条件に比べて顕著な反応促進効果があることが見いだされた。凍結反応条件下グリコシル化の立体選択性に関し、反応機構についても、溶媒、濃度、糖供与体のアノマー位の立体化学及び保護基、などの種々の要因の効果を検証し、立体選択性は、凍結、非凍結どちらの反応条件においても基質濃度によって影響をうけることが明らかとなり、凍結条件下における反応加速は高濃度環境によると示唆された。また、溶媒凍結による基質濃縮効果は50倍程度であろうと見積られる。凍結反応を利用した生物活性分子や複雑な糖鎖の合成への応用例についても、あわせてまとめた。

A. Introduction

It is widely perceived that chemical reactions proceed most smoothly in homogeneous solutions, in which molecules diffuse without impediment. Therefore, it is almost a golden rule to maintain the reaction temperature above the melting point of the solvent, and there are few cases where reactions have been conducted under frozen conditions. Most researchers may believe that reactions in frozen solvents barely proceed, because diffusion of reactants must be severely restricted.

However, several reports have shown that certain reactions, both chemical (1–4) and enzymatic (5,6), proceed under frozen conditions both in aqueous and non-aqueous systems. There are some examples for rate acceleration under frozen solvent conditions which should generate a special environment, although this method has not been shown to be a

A. はじめに

グリコシル化反応を含む一般的な化学反応は、均一溶媒系で最もスムーズに進行すると考えられている。そのため、化学反応は通常溶媒として用いられる化学物質の融点以上の温度で行われる。その逆に溶媒の融点以下で反応が行われることは、ほとんどない。これは、凍結した状態では溶媒溶質の運動・拡散が物理的に妨げられるので反応が遅くなる、と考えられるからである。

しかし、溶媒の凍結条件下での反応において、その予想に反して、化学反応や酵素反応の反応速度が、水系及び有機溶媒系の両方で、劇的に増大する例が報告されている (1–6)。一般的な手法として汎用されるに至っていないが、いくつかの反応において、凍結条件下、特殊な反応場が生じ、反応が加速されることが見いだされている。

1964年に Weatherburn と Logan は、フェリシアン化カ

Fig. 1. Recent synthetic applications under frozen conditions. A) 1,4-Addition to the dehydroalanine residue of norcathiasine I, a glycopeptide antibiotic. B) Enzymatic coupling of specific amino acid-containing peptide fragments.

fully versatile tool.

Instability in the freezing of solutions containing both potassium ferricyanide and potassium cyanide was observed by Weatherburn and Logan in 1964 by spectrophotometry and by testing for ferricyanide and ferrocyanide ions (1). Pincock and Kiovsky reported that the decomposition of *t*-butyl performate under basic conditions was accelerated in *p*-xylene below its melting point (2). The non-enzymatic decomposition of performic acid and the oxidation of ascorbic acid were accelerated in a frozen aqueous system, as observed by Grant and Alburn (3). Recently, Naidu *et al.* reported that 1,4-additions of amine and thiol to the dehydroalanine residue of norcathiasine, a glycopeptide antibiotic that shows antibacterial activity against multi-drug resistant Gram-positive bacteria *in vivo*, under various conditions resulted in accelerated reactions and improved yields in a frozen aqueous system (Fig. 1A) (4). In addition, the reverse reactions of enzymatic hydrolysis by peptidic hydrolases have been accelerated in frozen aqueous systems, as reported by Jakubke *et al.* and Bordusa *et al.* (Fig. 1B) (5, 6).

Contrary to popular belief, these reactions were shown to proceed with higher velocity compared to their solution counterparts. These observations may well be explained by the concentration effect (Fig. 2) (7). Since pure solvent freezes as a complete solid, a solution that is a mixture of substrates and reagents should cause depression of the freezing point to the eutectic point (Fig. 2A). During slow freezing of the solvent in the solution, both solid and liquid phases co-exist in the

リウムとシアン化カリウムを含む溶液は、凍結することで不安定になることを、吸光度測定と定性的イオン検出により観測している (1)。Pincock と Kiovsky は、過ギ酸 *t*-ブチル (TBF) の塩基性触媒存在下での分解反応において、*p*-キシレン中、融点以下の凍結条件下で行うと、反応が加速することなどを、報告している (2)。また、Grant と Alburn は、過酸化水素の非酵素的な分解反応や、アスコルビン酸の酸化反応が、水溶液の凍結条件下で加速することを見いだしている (3)。最近、Naidu らのグループは、凍結した水中での有機反応として、多剤耐性グラム陽性菌に対して *in vivo* でも抗菌活性を有するグラニコペプチド系抗生物質の一つであるノカチアシンのデヒドロアラニン部の側鎖へのアミンとチオールのマイケル付加反応を検討したが、凍結条件下反応加速、収率の向上を見いだしている (図 1A) (4)。さらに Jakubke らと Bordusa らは、加水分解酵素の逆反応を利用したペプチド合成においても、水溶液の凍結条件下、反応加速することを見いだしている (図 1B) (5, 6)。

溶媒凍結時に拡散が妨げられている状態での分子間反応において、反応が加速することは異常な現象に思える。しかし、この反応加速の原因は主として基質の濃縮による効果によると説明されている (図 2) (7)。純粋な溶媒は、融点以下の温度で完全に凍結し、固体として存在するが、基質や試薬などの溶質が含まれた溶液では共融点が存在し、凝固点降下が起こる (図 2A)。溶液中の溶媒は融点以下でゆっくりと凍結し、共融点に至るまではその凍結状態には固体相と液体相が共存

Fig. 2. A) Freezing point-composition relationship. B) Schematic models for the effect of freezing of solution of a mixture of substrates for glycosylations. Bold arrow indicated the freezing.

frozen media until the eutectic temperature is reached. Below the freezing point, but above the eutectic temperature, the frozen mixture contains liquid particles that are dispersed in a seemingly solidified mixture (Fig. 2B) (8). The freezing point-composition relationship predicts that solutes (reactants and reagents) are highly concentrated in these liquid particles. Due to this concentration, the reaction rate would be physically accelerated. It is not a general feature of organic solvents that their volumes increase during freezing, like water, to cause a rise in pressure under isochore conditions. In general, reaction rates decrease at lower temperatures. If the effect of concentration overrides the rate reduction caused by reduced temperatures associated with freezing, reactions would be accelerated (9).

In order to accelerate reactions, stirring, heating, concentration and addition of a catalyst are common strategies. Various types of conditions have also proven useful, the most prominent being microwave irradiation (10, 11), ultrasound sonication (12), high pressure compression (13, 14), and microfluidic conditions (15, 16). Compared to these techniques, which require special devices, freezing conditions are readily accessible to nearly all researchers.

B. The First Example of the Rate Acceleration of *O*-Glycosylation in Frozen Medium

Freezing conditions are thought to increase the velocity of intermolecular coupling reactions such as glycosylation. A systematic comparison was conducted using glycosyl acceptor **1** with methyl 1-thioglycoside **2a** as the donor in the presence of methyl trifluoromethanesulfonate (MeOTf) (17) and 2,6-di-*t*-butyl-4-methylpyridine (DTBMP) (Table I, Entries 1–8) (18). This glycosylation was quite slow at room temperature (Table I, Entries 1, 2). When the same glycosylation was conducted in *p*-xylene (m.p. 12–13°C) below its freezing temperature,

することになる。即ち、融点から共融点までの凍結状態では、固体の中に微小な液体領域が分散した状態で含まれている (図 2B) (8)。相図からわかるように、共融点以上の凍結状態に残る液体相に、反応基質や試薬が濃縮される。液体相の基質や試薬の濃度は著しく上昇し、同一温度条件下では、物理的要因にて反応が加速される。水のように、凍結の際に体積が増加するものもあり、等容積条件下圧力があがる場合もあるが、有機溶媒では一般的ではない。

しかし、凍結条件下での反応速度は、通常の溶液条件と同様に温度依存性があり、より低温下では反応速度は低下する。そのため、凍結による基質の濃縮効果が、低温反応での反応速度減少効果に打ち勝つ場合にのみ、反応の加速現象が起ると考えられている (9)。

化学反応を促進する手法として、攪拌、加熱、高濃度、触媒添加などがあり、また、電磁波 (マイクロウェーブ) 照射 (10, 11)、超音波照射 (12)、(超) 高圧縮 (13, 14)、マイクロフロー (15, 16) などの反応場を用いる手法が知られている。特殊な装置が必要でない凍結条件は、古典的な加速法のようにどの研究者にも可能な手法となりうると期待できる。

B. *O*-グリコシル化反応における凍結条件下反応加速現象の最初の例

凍結によってグリコシル化反応のような分子間反応の速度が増加するのではないかと考え、メチルトリフラート (MeOTf) (17) と 2,6-ジ-*t*-ブチル-4-メチルピリジン (DTBMP) の存在下、メチル 1-チオグリコシド (**2a**) を糖供与体とし、糖受容体 (**1**) との凍結、非凍結反応条件下でのグリコシル化反応について系統的に比較検討した (表 I, Entries 1–8) (18)。本反応は室温では非常に遅いが、反応溶媒として融点が 12–13 °C の *p*-キシレンを用い融点以下で反応を行った場合、非常に大

Table I. Glycosylation of 1 with 2 α under frozen conditions^{a)}: Effect of additive solvents, concentration and solvents.

^{a)}The yield and stereoselectivity were determined after gel filtration. ^{b)}Concentration of acceptor before freezing. ^{c)}Frozen conditions. ^{d)}The yield was determined by MALDI-TOF MASS quantitative analysis of crude sample (19). The stereoselectivity was determined by ¹H NMR of crude sample. Methyl 2,3,4,6-tetra-O-[D₇]benzyl-1-thio- α -D-mannopyranoside [D₂₈]2 α was used instead of 2 α (20). ^{e)}For 14 days. ^{f)}The yield and stereoselectivity were determined based on isolated yield. ^{g)}9.4 equiv of methyl 2,3,4,6-tetra-O-benzyl- α -D-mannopyranoside was added as a non-reactive substrate. ^{h)}12.5 equiv. of MeOTf and 7.5 equiv. of DTBMP were used.

Entry	Solvent	Conc. mol/L ^{b)}	Temp	Additive solvents (1%)	Yield /%	Ratio (α : β)
1	<i>p</i> -xylene	0.03	rt	none	33	51:1
2	<i>p</i> -xylene ^{d),e)}	0.03	rt	none	83	55:1
3	toluene	0.03	4 °C	none	25	55:1
4 ^{c)}	<i>p</i> -xylene ^{d)}	0.03	4 °C	none	90	4.3:1
5	toluene ^{f)}	0.03	-20 °C	none	<1	-
6 ^{c)}	<i>p</i> -xylene ^{f)}	0.03	-20 °C	none	66	4.5:1
7	toluene ^{f)}	0.03	-40 °C	none	0	-
8 ^{c)}	<i>p</i> -xylene ^{f)}	0.03	-40 °C	none	17	10:1
9 ^{c)}	<i>p</i> -xylene	0.03	4 °C	toluene	66	7.1:1
10 ^{c)}	<i>p</i> -xylene	0.03	4 °C	CH ₃ CN	71	5.9:1
11 ^{c)}	<i>p</i> -xylene	0.03	4 °C	Et ₂ O	50	5.8:1
12 ^{c)}	<i>p</i> -xylene	0.03	4 °C	CHCl ₃	54	6.6:1
13 ^{c)}	<i>p</i> -xylene	0.03	4 °C	Et ₂ O-CHCl ₃ (1:1)	56	6.1:1
14	toluene ^{d)}	0.15	4 °C	none	78	13:1
15	toluene ^{d)}	0.30	4 °C	none	84	7.9:1
16	toluene ^{d)}	1.5	4 °C	none	90	4.5:1
17	<i>p</i> -xylene ^{d),g)}	0.03	rt	none	15	57:1
18	<i>p</i> -xylene ^{d),h)}	0.03	rt	none	64	12:1
19	<i>m</i> -xylene ^{d)}	0.03	4 °C	none	8	100:1
20 ^{c)}	dioxane ^{d)}	0.03	4 °C	none	87	3.7:1
21	THP ^{d)}	0.03	4 °C	none	61	7.1:1
22 ^{c)}	<i>t</i> -BuCN ^{d)}	0.03	4 °C	none	84	2.5:1
23	CH ₃ CN ^{d)}	0.03	4 °C	none	54	2.1:1

significant rate acceleration was observed (Table I, Entries 4, 6, 8) to give disaccharide 3 in high yield. Remarkably, the reaction at room temperature (Entry 1) was even slower than that at -20 °C (Entry 6) and comparable with that conducted at -40 °C (Entry 8) under frozen conditions. Since the solvating properties of *p*-xylene and toluene which can not be frozen at 4 °C, are likely to be very similar, this result implied that the rate acceleration originates from the unique nature of the frozen environment. On the other hand, rate acceleration in frozen systems was associated with alteration of stereoselectivity. Following these observations, more systematic studies considering various factors, such as solvent, concentration, anomeric configuration and protective groups of the donor, are required to clarify the scope and advantage of frozen systems in oligosaccharide synthesis.

C. Effects of Frozen Conditions on Stereoselectivity and Velocity of O-Glycosylation Reactions

C-1. Effect of Additive Solvents in *p*-Xylene under Frozen Conditions

Examination of the effects of organic co-solvents such as toluene, Et₂O, CH₃CN, CHCl₃, and Et₂O-CHCl₃ (1:1) (19) was conducted, anticipating that these co-solvents will be concentrated in the liquid phase of frozen mixtures and thus will affect the selectivity (Table I, Entries 4, 9–13) (20).

きな反応加速が観測され、2糖 (3) が収率よく得られた (表 I, Entries 4, 6, 8)。注目すべきことは、室温での反応 (Entry 1) は、-20 °Cでの凍結反応よりも遅く (Entry 6)、-40°Cで行ったときと同程度だ (Entry 8) ということである。*p*-キシレンとトルエンの溶媒としての性質はとても似通っているので、得られた結果から考察すると、反応加速は凍結反応場の特殊な性質に起因していると示唆しているように思えた。一方で、凍結条件下における反応加速の際、立体選択性の低下が見られた。この発見に引き続き、溶媒、濃度、糖供与体のアノマー位の立体化学及び保護基、などの種々の要因の効果について更なる系統的な研究を行い、糖鎖合成への適用範囲や利点について明らかにしていく必要があった。

C. O-グリコシル化反応の立体選択性と速度への凍結条件の効果

C-1. *p*-キシレン中凍結条件下添加溶媒の効果

トルエン、エーテル、アセトニトリル、クロロホルム、エーテル-クロロホルム (1 : 1) (19) のような有機系共溶媒の添加効果の検討により、共溶媒は凍結混合物の液体相に濃縮され、立体選択性に影響を及ぼしていることが示唆された (表 I, Entries 4, 9–13) (20)。しかし、その効果はそれほど小さくなく、

However, their effects on stereoselectivity were only marginal. Instead, the rate accelerating effect deteriorated in these cases, indicating that a rigidly ordered frozen state of the mixture is crucial in order to optimally accelerate reactions.

C-2. Effect of Concentration on Stereoselectivity of *O*-Glycosylation

The rate enhancing effect of the frozen system may be explained by its ability to generate highly concentrated micropockets (Fig. 1B). However, it is rather difficult to experimentally assess the substrate concentrations in frozen systems. Our studies have shown that rate acceleration in frozen systems was associated with alteration of stereoselectivity, leading us to explore the correlation between the selectivity and concentration. We expected that, by extrapolating the concentration-selectivity relationship of non-frozen reactions, a reasonable estimate of the degree of concentration in frozen mixtures (Table I) (20) can be made.

To this end, the effect of the concentration of the substrate in toluene (non-frozen) at 4 °C was evaluated (Table I, Entries 3 and 14–16). In toluene, the reaction was accelerated in a concentration dependent manner, while the α -selectivity of the glycoside **3** was reduced at higher concentrations. The result in concentrated toluene solution (1.5 mol/l, Entry 18) was very similar to that in frozen *p*-xylene (0.03 mol/l, Entry 4), in terms of both the product yield (90%) and selectivity (4.5:1 vs. 4.3:1). These results support our hypothesis that the decrease of stereoselectivity in frozen systems was caused by increased concentration, and provided an estimate of substrate concentrations under frozen conditions, which seems to reach as high as ~1.5 mol/l.

However, it is certainly possible that the presence of substrates and reagents in high concentrations had an impact on altered selectivity (Table I, Entries 1, 17 and 18). In *p*-xylene at room temperature, glycosylations under highly crowded environments, such as using an excess amount of reagents, adding a non-reactive substrate (methyl tetra-*O*-benzyl- α -D-mannopyranoside) or using less solvent, were examined. Excess reagents as well as reduced use of solvent were both found to result in reduced α -selectivity.

C-3. Glycosylation in Various Solvents under Frozen Conditions

The frozen effect, which was clearly observed in *p*-xylene (21, 22), was demonstrated to be general by the examination of the glycosylation in other solvents that can be frozen at 4 °C. We compared these solvents with non-freezing solvents possessing similar chemical properties (Table I, Entries 3, 4, 19–23) (20).

In the case of *p*-xylene, rate enhancement was observed relative to the reactions in toluene (Table I) and *m*-xylene (Entry 1). As ethereal and nitrile solvents,

実際、凍結による加速効果は低下した。このことから、最大限に加速効果を引き出すためには凍結混合物が規則正しい凍結状態をとっていることが重要であると推測できる。

C-2. *O*-グリコシル化反応の立体選択性への濃度の影響

凍結反応における反応加速効果は、凍結が持つ高濃縮された微小ポケットの生成能によって説明できると考えられている (図 1B)。しかし、実験的に (凍結状態でなく) その基質濃度の状態を作り上げることは難しい。一方、高濃縮可能と考えられる凍結による反応加速には立体選択性の低下を伴う場合があるため、立体選択性と濃度の関係について調査した。あわせて、凍結していない通常の反応における濃度と選択性の相関を外挿することにより、凍結混合物中の濃度を合理的に評価できると期待した (表 I) (20)。

トルエン中 4 °C で非凍結条件下、基質濃度の効果を評価したところ、反応は濃度依存的に加速したが (表 I, Entries 3, 14–16)、生成物のグリコシド (**3**) の α -選択性は、より高濃度では低下した。基質濃度 1.5 mol/l のトルエン溶液で行った結果 (90%, 4.5 : 1) は、収率、選択性いずれの点においても、*p*-キシレン中 4 °C で凍結条件下で行った結果 (90%, 4.3 : 1) にとても近い。これらの結果は、凍結反応条件下立体選択性の低下は濃度上昇によるものだという仮説を支持し、0.03 mol/l の溶液の凍結では基質濃度が 1.5 mol/l まで到達していると評価できる。

基質そのものや試薬が高濃度に存在していることが、強い影響を持ち、選択性に影響をあたえる可能性もある (表 I, Entries 1, 17, 18)。室温で非凍結条件下、過剰の試薬や疑似基質 (メチルテトラ-*O*-ベンジル- α -D-マンノピラノシド)、溶媒量の減少による高濃度条件でグリコシル化を行い検討したところ、立体選択性の低下は試薬濃度の上昇によっても引き起こされるとわかった。

C-3. 種々の溶媒中凍結条件下でのグリコシル化反応

p-キシレン中観測された凍結効果 (21, 22) が一般的な現象であるかどうかを、4 °C で凍結する溶媒と化学的性質が類似した凍結しない溶媒の他の組み合わせでグリコシル化反応を比較検討した (表 I, Entries 3, 4, 19–23) (20)。

p-キシレンの場合、トルエンや *m*-キシレンに比べ反応加速が見られたが、エーテル系およびニトリル系の凍結溶媒としてジオキサン (m.p. = 10–12 °C) と *t*-BuCN (m.p. = 15–16 °C) を用い、それぞれテトラヒドロフラン (m.p. = –45 °C)、アセトニトリル (m.p. = –48 °C) と比較した。どちらの場合も反応

Table II. Glycosylation of various donors under frozen conditions.^{a),b)}

Acceptor
1

+ Donors
2,4-6

MeOTf (2.5 equiv.)
DTBMP (1.5 equiv.)

MS4A

	R ¹	R ²
3	Bn	Bn
7	Bn	PhCH<
8	Bn	H ₁₀ C ₆ <
9	Bz	Bn

2 β

5

6

Entry	Donor	Solvent	Temp.	Time	Yield /%	Ratio (α : β)
1	2β	<i>p</i> -xylene	rt	1 h	95	37:1
2 ^{c)}	2β	<i>p</i> -xylene	4 °C	1 h	100	8.3:1
3	2β	toluene	4 °C	1 h	73	26:1
4	4	<i>p</i> -xylene	rt	14 d	19	2.4:1
5 ^{c)}	4	<i>p</i> -xylene	4 °C	14 d	51	1.2:1
6	4	toluene	4 °C	14 d	14	1.4:1
7	4^{d)}	<i>p</i> -xylene	rt	14 d	25	2.1:1
8 ^{c)}	4^{d)}	<i>p</i> -xylene	4 °C	14 d	60	1:1.3
9	4^{d)}	toluene	4 °C	14 d	18	1.1:1
10	5^{e)}	<i>p</i> -xylene	rt	5 d	26	3.8:1
11 ^{c)}	5^{e)}	<i>p</i> -xylene	4 °C	5 d	92	1.5:1
12	5^{e)}	toluene	4 °C	5 d	17	2.1:1
13	6	<i>p</i> -xylene	rt	2 d	16	α
14 ^{c)}	6	<i>p</i> -xylene	4 °C	2 d	82	46:1
15	6	toluene	4 °C	2 d	5	α

^{a)}The reactions were carried out in 0.03 mol/L solution of acceptor before freezing. ^{b)}The yield and stereoselectivity were determined after gel filtration. ^{c)}Solvent was freezing. ^{d)}2.5 equiv. of MeOTf and 3.0 equiv. of DTBMP were used. ^{e)}The yield was determined by MALDI-TOF MASS quantitative analysis of crude sample. The stereoselectivity was determined by ¹H NMR of crude sample.

dioxane (m.p. = 10–12 °C) and *t*-BuCN (m.p. = 15–16 °C) were compared with tetrahydropyran (m.p. = –45 °C) and MeCN (m.p. = –48 °C), respectively. In these cases, rate acceleration, albeit small, was also seen. Since the proportion of the β -isomer increased in frozen *p*-xylene, we hoped that the β -directing effect of the nitrile solvent would be augmented. However the selectivity in frozen *t*-BuCN was nearly identical with that in MeCN.

Interestingly, Mong *et al.* reported that glycosylation with 2-*O*-benzylated galactopyranosyl or glucopyranosyl donor under dilute conditions in nitrile solvent gave higher 1,2-*trans* (β) selectivity. They rationalized this intriguing result by enhanced formation of nitrilium ion intermediates with low concentration of substrates (23).

C-4. Effect of Anomeric Stereochemistry of Donor under Frozen Conditions

There are two possible explanations for the reduced α -selectivity of the glycosylation in frozen media. First, higher concentrations of substrates would perturb the nature (*e.g.*, polarity) of the solvent, and affect the stereoselectivity. Second, direct S_N2-type displacement of activated thioglycoside, which produces β -glycoside, might become functional as an alternative pathway that competes with the oxocarbenium ion pathway, when concentrations of **1** and **2 α** are high. To distinguish between these two possibilities, we conducted reactions with β -thioglycoside **2 β** as the donor (20).

As shown in Table II (Entries 1–3), the reaction of the β -isomer **2 β** was much faster than that of the α -isomer (**2 α**), with both reactions proceeding to completion within 1 h at both r.t. and at 4 °C. In frozen *p*-xylene (Entry 2), the α -selectivity was approximately two times higher than the

加速が見られたが、その効果は小さかった。*p*-キシレン中凍結条件下では β 体の生成比が増加したので、当初ニトリル系溶媒の溶媒効果を利用した β -選択性向上を期待していた。しかし、凍結した*t*-BuCN中での立体選択性はアセトニトリルの場合とほぼ一緒であった。

興味深いことに、Mong らは 2-*O*-ベンジルガラクト-、及び同グルコ-ピラノース供与体のグリコシル化において、ニトリル系溶媒中希釈条件下で反応を行うと、1,2-*trans*(β)選択性が向上することを報告している。その中で、この好奇心をそそる結果は、基質の濃度が低いことにより、ニトリリウムイオン中間体の生成が促進するためであると説明している(23)。

C-4. 凍結条件下糖供与体アノマー位の立体化学の影響

グリコシル化の立体選択性が凍結条件下低下した理由は2つ考えられる。まず、基質が高濃度になり基質に対する溶媒分子の数が減少しているので溶媒の極性などの性質が抑えられ、選択性に影響した可能性が挙げられる。また、活性化したチオグリコシドへの直接的 S_N2 反応により β 体を与えるルートは、糖供与体、糖受容体濃度が高い状態では、オキソカルベニウムイオンを経由するルートと競争する別ルートとして機能するかもしれない。

どちらの可能性かを判別する糸口を手に入れるために、 β 体のチオグリコシドを糖供与体として用い、反応を行ったところ、反応は α 体のときより速く、室温でも4 °Cでも1時間以内に完結した(表 II, Entries 1–3) (20)。*p*-キシレン中凍結反

same reaction using the α -thioglycoside **2a** (Table I, Entry 4), while differences in the selectivity under non-frozen conditions were less marked and, instead, shifted slightly to the less α -selective direction (Entries 1 and 3). These results seem to suggest the occurrence of S_N2 -type displacement of the activated intermediate in association with the more dominant oxocarbenium ion-like intermediate under highly concentrated conditions.

C-5. Effect of Protective Groups of Glycosyl Donor under Frozen Conditions

In addition to the perbenzylated thioglycoside **2a**, mannosyl donors with 4,6-*O*-cyclic (**4**, **5**) and 2-*O*-acyl (**6**) protection were studied. In the case of 4,6-*O*-benzylidene protected donor **4**, which has been widely used for direct β -mannosylation (24), the reaction was extremely slow under our standard conditions (Table II, Entries 4–10). However, the rate-enhancing effect of the frozen system was still evident (20). After 14 days under frozen conditions, 51% of the product was obtained with low selectivity ($\alpha:\beta = 1.2:1$) (Entry 5), although use of an excess amount of DTBMP resulted in a slight improvement of the yield (60%) and the selectivity was slightly in favor of the β -glycoside (Entry 8).

Compound **5**, which contained a 4,6-*O*-cyclohexylidene group (25, 26), was revealed to be more reactive than **4** (Entries 10–12). For instance, the disaccharide was obtained in 92% yield ($\alpha:\beta = 1.5:1$) under frozen conditions after 5 days (Entry 11). Since we expected that the 4,6-*O*-cyclohexylidene group would be more deactivating than the 4,6-*O*-benzylidene acetal, because the former would likely confer a higher degree of rigidity to the pyranose ring system, these results were somewhat surprising. In any event, we observed that the proportion of the β -isomer was uniformly increased when 4,6-*O*-cyclic protected donors **4** and **5** were used and that the tendency was more prominent under frozen conditions (Entries 4–12).

The reactivity of 2-*O*-Bz protected donor **6** was revealed to be in between **2a** and **4/5** (Entries 13–15), although much higher activity was initially expected due to the “super arming” effect proposed by Demchenko (27). Significant rate acceleration was observed in reactions with **1** under frozen conditions. In these cases, the α -glycoside was obtained stereoselectively as expected, although formation of a small amount of the β -isomer occurred, possibly due to S_N2 displacement in highly concentrated micropockets (Entry 14).

D. Application to the Synthesis of Various Glycans and Bioactive Molecules

A similar effect was also observed in the synthesis of larger oligosaccharides, extending the practical utility of frozen conditions to the facile preparation of oligomannoside

応条件下、 α -選択性は、 α 体のチオグリコシドを用いた場合と比べ、およそ2倍高かったが、非凍結条件下での選択性の違いはより小さく、実際は α -選択性は若干低下した。このことは、高濃縮条件下、オキソカルベニウムイオン様中間体を經由するより優先的なルートと、活性化したチオグリコシドへの S_N2 反応の経路の存在を示唆する。

C-5. 凍結条件下糖供与体保護基の影響

テトラ-*O*-ベンジル-1-チオグリコシド (**2a**)に加え、4,6-*O*-環状保護基や2-*O*-アシル系保護基を持つマンノース供与体 (**4**, **5**, **6**)について検討を行った。直接的グリコシル化反応 (24) で広く用いられる 4,6-*O*-ベンジリデン保護された供与体 (**4**) の場合、反応は通常の条件では非常に遅かったが、凍結反応条件下、明らかな反応加速が見られた (表 II, Entries 4–10) (20)。14日間反応を行った場合でも、51%の収率で、選択性は低い ($\alpha:\beta = 1.2:1$) 結果となった (Entry 5)。DTBMP を過剰に用いた場合、収率は若干改善し (60%)、 β 体を優先して与えた (Entry 8)。

一方、4,6-*O*-シクロヘキシリデン基 (25, 26) を持つ糖供与体 (**5**, Entries 10–12) は、4,6-*O*-ベンジリデン体 (**4**) と比べ反応性が高く、凍結条件下5日間かかるが、生成物の2糖が92%の収率、 $\alpha:\beta = 1.5:1$ の選択性で得られた (Entry 11)。4,6-*O*-シクロヘキシリデン基は、ピラノース環をよりきつく固定できるため、4,6-*O*-ベンジリデン基に比べ反応性を下げると考えていたので、この結果は想定外であった。4,6-*O*-環状保護基を有する糖供与体 (**4**, **5**) のグリコシル化では、 β 体の生成が一樣に増加し、凍結反応条件下、その傾向は顕著である (Entries 4–12)。

2-*O*-ベンズイル保護された供与体 (**6**) の反応性は、当初 Demchenko らが提唱している “super armed” 効果により (27)、はるかに高い反応性を期待したが、テトラ-*O*-ベンジル保護体 (**2a**) と 4,6-*O*-環状保護体 (**4**, **5**) の丁度中間に位置した (Entries 13–15)。凍結条件下非常に劇的な反応加速が見られ、期待通り α -グリコシドを立体選択的に与えた。凍結反応条件下見られた少量の β 体の生成は、高濃縮された微小空間での S_N2 反応のためであろうと考えている (Entry 14)。

D. 生物活性物質や種々の糖鎖合成への応用

より大きなオリゴ糖の合成においても凍結反応条件下 *O*-グリコシル化反応の加速効果が見いだされ、脂溶性鎖を持つオリゴマンノシドプローブの簡便な合成 (Scheme 1,

Scheme 1. Applications to the synthesis of various glycans under frozen conditions.

Scheme 2. Applications to the synthesis of 2-oxabutylene-linked tri- and penta-mannoside mimics under frozen conditions.

probes having lipophilic tethers (Scheme 1, **10+11**→**12**, **13+11**→**14**) (18), as well as block condensation of oligosaccharide fragments to provide a high-mannose type glycan analogue (**15+16**→**17**) (18, 28).

In addition, this technique was applied to the introduction of a fucose residue to a highly hindered site of an octasaccharide acceptor, completing the synthesis of complex-type glycans derived from *parasitic helminthes* such as *Schistosoma japonicum* (29). Since the separation of the product from unreacted acceptor was impossible on preparative scale, the high conversion of the starting acceptor under frozen conditions resulted in easy isolation (Scheme 1, **18+19**→**20**).

As an additional example, frozen conditions were applied to arabinofuranosylation in order to synthesize a fragment of mycobacterial arabinan (30). A substantial rate-enhancement of glycosylation of the arabinofuranose donor activated by MeOTf was observed in frozen solvent (Scheme 1, **21+22**→**23**).

Very recently, Yuasa *et al.* reported that the reaction of a reactive (methylthio)methoxyethyl α -D-mannopyranoside with aglycon resulted in its decomposition with low yield of the desired product (Scheme 2, **24+25**→**26**), whereas under frozen conditions the reaction afforded the desired internal 2-oxabutylene-linked trisaccharide as well as pentamannoside mimics as the glyco-probe for lectin ligand (**27+25**→**28**, **29**) (31).

E. Conclusion

The results reported in this manuscript clearly demonstrated that freezing reaction mixtures increases the rate of *O*-glycosylation. The origin of the rate enhancing effect of frozen conditions on *O*-glycosylation was revealed by systematic studies. A concentration-selectivity relationship suggested that highly concentrated particles are generated in frozen solvents. The frozen conditions seem to give rise to ~50 times increase of concentration. The rate enhancement effect of frozen *p*-xylene has some generality and was also observed in other solvents and with other donors, although the extent of acceleration was less marked. In addition, the application of this technique was demonstrated in the synthesis of complex glycans including bioactive molecules. Remarkably, the use of frozen media is technically very simple and does not require any special equipment.

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10+11→**12**, **13+11**→**14**) (18) への実験的利用へ拡大した。さらに、オリゴ糖フラグメント間のブロック縮合時にも反応加速が観測され (**15+16**→**17**) (18, 28)、高マンノース型糖鎖アナログの調製が可能となっている。

本手法は日本住血吸虫などの寄生蠕虫類 *parasitic helminthes* から単離、構造決定された複合型糖鎖誘導体の合成に応用された (29)。*P. helminthes* 由来 9 糖の合成の懸案であった、立体的に非常に混んだ位置にヒドロキシ基を有する 8 糖受容体に対するフコース供与体のグリコシル化反応において、凍結反応条件下での反応加速を見いだした (Scheme 1, **18+19**→**20**)。収率の向上に伴い変換効率が上がり、原料と分離困難だった目的物の簡便な単離精製が可能となった。

また、本反応条件は、結核菌などの細胞壁複合糖質の成分多糖であるアラビナンのフラグメント合成の検討において、アラビノフラノシル化へも応用可能であった (30)。すなわち、*p*-キシレン中、メチルトリフラートを活性化する場合、凍結条件下、同様にアラビノフラノース供与体のグリコシル化が著しく加速されることを見いだした (Scheme 1, **21+22**→**23**)。

ごく最近、湯浅らは、メチルチオメトキシ (MTM) エチル α -D-マンノピラノシドとアグリコンとの反応において、MTM 基の反応性が高く分解が見られ目的化合物の収率が低下するが、凍結反応条件下で行うと擬似 3 糖誘導体の収率が向上することを見いだしている (Scheme 2, **24+25**→**26**)。その応用として、2-オキサブチレン架橋された擬似 5 糖誘導体の合成に成功している (**27+25**→**28**, **29**) (31)。

E. まとめ

本論文で紹介した結果は、凍結することで *O*-グリコシル化反応が加速していることを明示している。そして、*O*-グリコシル化反応の加速の要因について系統的に研究され、明らかになってきた。濃度と選択性が相関し、凍結反応条件下では高濃度溶液微小空間が形成されていることを示唆している。その相関から、凍結条件では基質濃度がおよそ 50 倍に上昇していると推測できる。*p*-キシレン中凍結反応条件下見いだされた反応加速現象は、種々の凍結溶媒や、他の糖供与体でも程度に差はあるが観測されたことから、一般性があると考えられる。さらに、生物活性物質を含む複雑な糖鎖などの合成においても凍結反応条件を応用した例を紹介した。注目すべきことは、この凍結条件は、特殊な装置が必要でなく、技術的に非常に簡便だということである。

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Profile of the Authors

and stereoselective glycosylation, bacterial oligosaccharide synthesis, and synthesis of novel glycoconjugate motif.

Ayaka Sakurai graduated from Faculty of Science, Tokyo University of Science in 2009 for BS, and received her M.S. degree from Saitama University in 2011 under supervision of Professor Yukishige Ito. She received a poster award at the annual meeting of the Japanese Society of Carbohydrate Research in 2010. Her current address: Seikagaku Corporation, Higashiyamato, Tokyo 207-0021, Japan.

research interests cover carbohydrate chemistry, glycotechnology and synthetic organic chemistry.

Yukishige Ito graduated from Faculty of Pharmaceutical Sciences, the University of Tokyo, in 1977 and obtained his Ph.D. in 1982 from the same school, under the direction of Professor Masaji Ohno. After two years of postdoctoral training at Department of Chemistry, Massachusetts Institute of Technology in USA under direction of Professor Satoru Masamune, he joined RIKEN and started his career in carbohydrate chemistry under the direction of Dr. Tomoya Ogawa. In the same institute, he became independent as a chief scientist in 1998. He is also a director of the ERATO project of the Japan Science and Technology Agency (JST) (2009~) and holds appointments from Saitama University (1998~) and Rikkyo University (2004~) as a Visiting Professor. His current