Stereoselective Glycosylation of 3-Deoxy-D-manno-2-octulosonic Acid with Batch and Microfluidic Methods

Atsushi Shimoyama, Yukari Fujimoto,* Koichi Fukase

Department of Chemistry, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan
Fax +81(6)68505419; E-mail: yukarif@chem.sci.osaka-u.ac.jp

Received 9 June 2011

Abstract: A practical and efficient stereoselective synthesis of 3-deoxy-D-manno-2-octulosonic acid (Kdo) glycoside was achieved using *N*-trifluoroacetoimidate as the glycosyl donor with both batch and microfluidic methods. The method used a conformationally constrained glycosyl donor, which has a bulky isopropylidene group at the 4,5-O-position. The strained conformation directed the coordination of the acetonitrile solvent, which led to the enhancement of reactivity and α-selectivity in the glycosylation.

Key words: glycosylation, carbohydrates, stereoselective synthesis, carbocation, natural products

3-Deoxy-D-manno-2-octulosonic acid (Kdo) is an acidic 2-ketooctose that is ubiquitously present in Gram-negative bacteria, particularly in the lipopolysaccharide (LPS) of the outer membrane and also in the capsular polysaccharide of the bacterial cell surface. Kdo serves as a linker between the lipophilic terminal component called lipid A and the polysaccharide part of LPS and is considered to affect the interaction of lipid A with toll-like receptor 4 (TLR4), which specifically recognizes LPS and activates the innate immune system. For further detailed investigations on the biological role of Kdo, the chemical syntheses of Kdo-containing glycans are essential. In the glycosylation of Kdo, the following issues should always be considered: 1) The neighboring group effect is not available because of the 2-keto-3-deoxy structure. 2) The presence of the 3-deoxy structure and a C1 carboxyl group easily lead to the β-hydrogen elimination to afford a glycal as the major byproduct.

We have developed a glycosylation using Kdo fluorides as glycosyl donors for the synthesis of *Escherichia coli* Re-LPS, *Helicobacter pylori* Kdo-lipid A, and their derivatives.[1–7] High α-selectivity was obtained using 4,5-*O*-isopropylidene- or *tert*-butyldimethylsilyl (TBS)-protected Kdo fluorides as donors. With these Kdo donors, the undesirable β-side attack by a glycosyl acceptor is prevented by the presence of the bulky isopropylidene or TBS group. Other glycosylations have also been reported; with Kdo chloride, bromide,[8–16] phosphite,[17] and 3-phenylselenyl-Kdo[18] via substitution of 6-*O*-triflate of glucosamine with a Kdo residue[19] and that of 3-iodo intermediates with acyclic saccharide precursors.[20] However, many of these methods are difficult to apply for the synthesis of complex LPS partial structures with many acyl groups and phosphates.

Even in our previous method, we occasionally observed the cleavage or migration of acid-labile protecting groups during the glycosylation because strong Lewis acids were required for the activation of the Kdo fluorides.[4,5] Therefore, the synthetic strategy was restricted, and longer reaction schemes were often required for synthesizing LPS partial structures composed of lipid A and Kdo. Recently, we developed a Kdo glycosylation with an *N*-phenyltrifluoroacetimidate donor and applied it to the synthesis of *H. pylori* Kdo-lipid A.[21] The *N*-phenyltrifluoroacetimidates can be activated by a catalytic amount of Lewis acids under mild conditions and have proven very useful for various oligosaccharide and glycoconjugate syntheses.[22–26] We found that the combination of trimethylsilyltrifluoromethanesulfonate (TMSOTf) as the catalyst and cyclopentyl methyl ether (CPME) as the solvent was efficient for glycosylations with Kdo *N*-phenyltrifluoroacetimidate **1a**. In addition, we observed that microfluidic conditions reduced the formation of the undesired glycal.[21] However, the optimized reaction conditions varied depending on the substrates. Therefore, in the present study, we investigated more efficient methods and found that Kdo glycosylation with **1a** proceeded smoothly with trifluoromethanesulfonic acid (TfOH) in acetonitrile, and α-selectivity was controlled by the donor–acceptor ratio in batch conditions as well as in microfluidic mixing. The present methods were applied to the synthesis of disaccharides and trisaccharides with Kdo moieties, which are derivatives of the LPS partial structures.

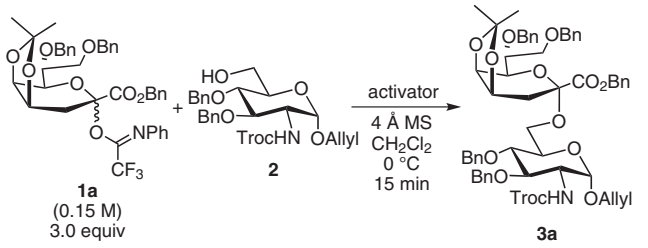
First, we examined various acid catalysts for the glycosylation of **1a** with a glycosyl acceptor **2** in dichloromethane (Table 1). Among the acids tested, TfOH was the most efficient for obtaining the highest yield. In addition, we evaluated the selectivity at lower temperatures. At –78 °C, the α-selectivity decreased to α/β = 43:57 (yield 34%) with TMSOTf in CH_2Cl_2, which is comparable to the conditions in Table 1, entry 1 (at 0 °C, the yield and selectivity were 61%, α/β = 73:27, respectively).

Then, the solvent effects on the glycosylation were investigated using donor **1a** and acceptor **2** (Table 2, entries 1–11). The α-selectivity in MeO*t*-Bu and CPME was higher than that in dichloromethane, but the yield was lower (Table 2, entries 3 and 4). In acetone or EtOAc, the stereoselectivity were scarcely observed (Table 2, entries 5 and 6). On the other hand, nonpolar solvents such as toluene,

SYNLETT 2011, No. 16, pp 2359–2362
Advanced online publication: 13.09.2011
DOI: 10.1055/s-0030-1260313; Art ID: U04811ST
© Georg Thieme Verlag Stuttgart · New York

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

Table 1 Effect of Activators on the Glycosylation of Kdo *N*-Phenyltrifluoroacetimidate **1a**



Entry	Activators (equiv)	Yield of **3a** (%),[a] α/β
1	TMSOTf (0.2)	61, 73:27
2	TBSOTf (0.5)	65, 78:22
3	TfOH (1.0)	75, 72:28
4	$BF_3{\cdot}OEt_2$ (0.2)	trace

[a] The yield was calculated from compound **2**.

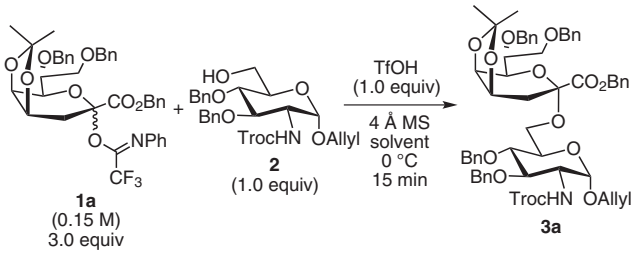
1,2-dichloroethane, and benzene gave higher selectivity than ether-type solvents (Table 2, entries 7–9). Subsequently, acetonitrile was found to give the best yield (86%) with satisfactory selectivity (Table 2, entry 11). The kinetic axial-oriented solvent effect of nitriles has been well-known and used for various oligosaccharide syntheses.[24–29] In the present case, no such effect was observed, indicating that steric hindrance due to the isopropyridene group in **1a**, with a boat-like conformation, prevents the coordination of acetonitrile from the α-face. Next, the stoichiometry of the donor, acceptor, and TfOH were examined in acetonitrile (Table 2, entries 11–14). Reducing the amount of TfOH decreased the selectivity (Table 2, entry 12). Interestingly, only α-glycoside was obtained when 1.5 equivalents of glycosyl acceptor **2** was used with Kdo donor **1a** (Table 2, entry 14), with 62% yield.

The present Kdo glycosylation reaction was then applied to the synthesis of trisaccharide **6a**. Because acceptor **5** possessed the acid-labile *p*-methoxybenzyl group (MPM), and many synthetic steps were required for synthesizing **5**, a catalytic amount of TfOH and a large excess of Kdo donor **1a** were used for the glycosylation. Thus, trisaccharide **6a** was obtained in good yield with moderate selectivity (Table 3, entry 1).

In addition, the Kdo–Kdo disaccharide **7a** was obtained by the glycosylation of Kdo acceptor **4** with **1a** in a good yield with moderate selectivity (Table 3, entry 2).

We also investigated the glycosylation with Kdo *N*-phenyltrifluoroacetimidate **1b** having bulky TBS groups at the 4- and 5-positions. In our previous study on the synthesis of Re-LPS, the TBS-protected Kdo fluoride showed lower reactivity than the 4,5-*O*-isopropylidene-protected Kdo fluoride.[6] On the other hand, the glycosylation with **1b** in CPME in the presence of Lewis acids did not proceed at all. However, the present conditions efficiently promoted the glycosylation of the acceptor **2** with **1b** to

Table 2 Solvent Effects on the Glycosylation Using Kdo *N*-Phenyltrifluoroacetimidate **1a**



Entry	Solvents	Yield of **3a** (%), α/β (calcd from **2**)
1	CH_2Cl_2	75, 72:28
2[a]	1,4-dioxane	13, n.d.
3	CPME	54, 86:14
4	MeO*t*-Bu	57, 95:5
5	acetone	59, 50:50
6	EtOAc	73, 55:45
7	toluene	72, 91:9
8	DCE	66, >95:5
9[a]	benzene	63, 100:0
10	DMF	complex mixture
11	MeCN [**1a** (3.0 equiv), **2** (1.0 equiv)]	86, 85:15
12[b]	MeCN [**1a** (3.0 equiv), **2** (1.0 equiv)]	88, 68:32
13[b]	MeCN [**1a** (1.5 equiv), **2** (1.0 equiv)]	68, 71:29
14[c]	MeCN [**1a** (1.0 equiv), **2** (1.5 equiv)]	62, 100:0

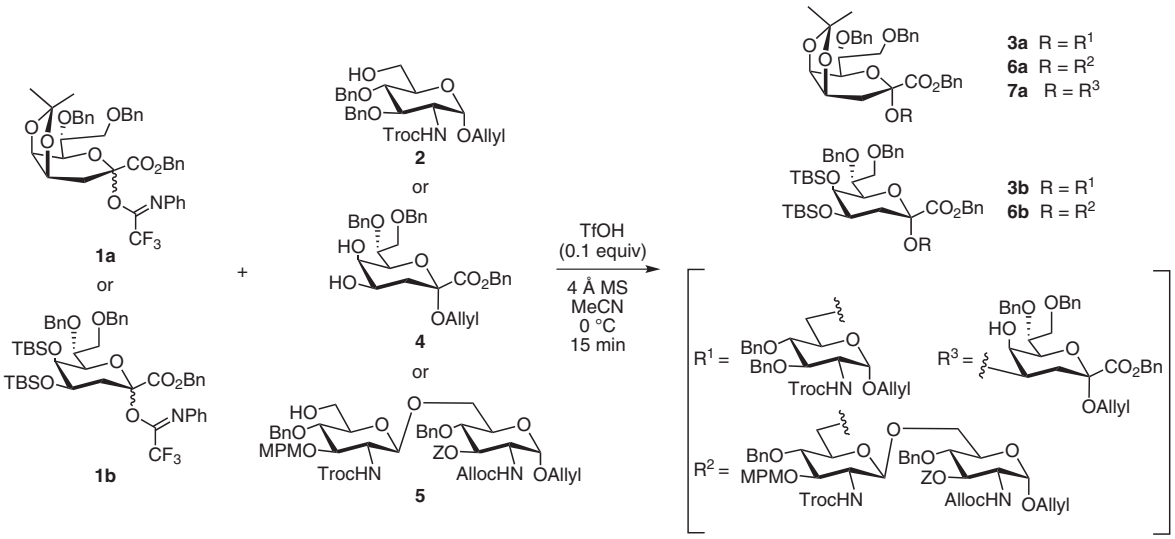
[a] The reaction was performed at 25 °C.
[b] TfOH (0.1 equiv).
[c] TfOH (1.0 equiv). The yield was calculated from donor **1a**.

give the desired disaccharide **3b** in a good yield with moderate selectivity (Table 3, entry 3). The glycosylation of disaccharide acceptor **5** with **1b** gave the desired trisaccharide **6b** in a good yield (Table 3, entry 4). Furthermore, Kdo donor **1b** exhibited higher selectivity than Kdo donor **1a** (Table 3, entries 1 and 4).

As described earlier, the stoichiometry of the donor, acceptor, and TfOH considerably influenced stereoselectivity. Because reducing the amount of TfOH decreased α-selectivity and using an excess of the acceptor had the opposite effect, rapid activation of the donor and fast addition of the acceptor to the intermediate oxocarbenium ion seemed to be important for obtaining high α-selectivity. Although the reason why these kinetic processes are critical for improving stereoselectivity is still uncertain, enhancing the mixing efficiency was expected to provide further improvements. Therefore, we applied this reaction to a microfluidic reaction system (Table 4), which enables efficient mixing and rapid heat transfer.[30] In fact, we have

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

Table 3 Glycosylation Reactions of Kdo *N*-Phenyltrifluoroacetimidates **1a**,**b**



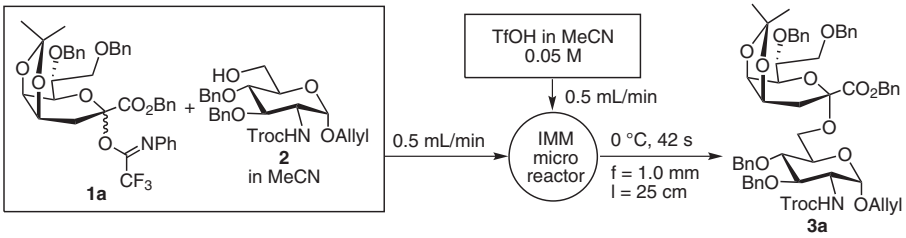
Entry	Donor (equiv)	Acceptor (equiv)	Yield (%),α/β (calcd from acceptor)
1	**1a** (5.0)	**5** (1.0)	**6a** (quant.), 75:25
2	**1a** (3.0)	**4** (1.0)	**7a** (92), 79:21
3	**1b** (3.0)	**2** (1.0)	**3b** (91), 80:20
4	**1b** (5.0)	**5** (1.0)	**6b** (95), 84:16

achieved practical and stereoselective glycosylations such as α-sialylation,[25,26] β-mannosylation,[31] and N-glycosylation of asparagines[32] by using the microfluidic system.

For the present microfluidic Kdo glycosylation, an acetonitrile solution of Kdo donor **1a** and acceptor **2** was mixed with TfOH solution in acetonitrile with various concentrations using an IMM's micromixer[33] at a flow rate of 0.5 mL/min. The reaction mixture was allowed to flow for an additional 42 s through a tubular reactor (Φ = 1.0 mm), and then quenched by adding it to an Et_3N–CH_2Cl_2 solution. As a result, the microfluidic reaction gave noticeably higher α-selectivity than the batch-type reaction (Table 4, entries 2–4). The higher concentrations of the acceptor led to higher selectivity. These results suggested that a reactive species with a relatively shorter lifetime was generated from the donor, and kinetically regulated fast addition of the acceptor resulted in the higher α-selectivity.

In conclusion, we developed a practical α-selective Kdo glycosylation with *N*-phenyltrifluoroacetimidate-functionalized glycosyl donors in acetonitrile under both batch and microfluidic conditions. The use of acetonitrile enhances reactivity and supports high α-selectivity. The present glycosylation method using *N*-phenyltrifluoroacetimidate and TfOH in acetonitrile would be applicable to

Table 4 Microfluidic Methods for the Glycosylation of Kdo *N*-Phenyltrifluoroacetimidate **1a**[a]



Entry	Donor **1a** (equiv)	Acceptor **2** (equiv)	Yield of **3a** (%), α/β (calcd from **2**)
1	37.5 mM (3.0)	12.5 mM (1.0)	67, 80:20
2	37.5 mM (1.5)	25.0 mM (1.0)	60, 95:5
3	75.0 mM (3.0)	25.0 mM (1.0)	83, 92:8
4	75.0 mM (1.5)	50.0 mM (1.0)	73, 95:5

[a] All of the concentrations in Table 4 are the final concentrations. The concentration of TfOH in the reaction mixture was 25.0 mM in all cases.

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

the synthesis of Kdo glycosides as well as other glycosides in nature.

Representative Procedure of Batch Reaction for the Synthesis of Disaccharide 3a

To a mixture of donor **1a** (8.8 mg, 12.2 μmol), acceptor **2** (2.4 mg, 4.08 μmol) and MS 4 Å in anhyd MeCN (70 μL) was added a solution of TfOH (0.36 μL, 4.08 μmol) in MeCN (10 μL) at 0 °C under Ar atmosphere, and the reaction mixture was stirred 15 min. After addition of Et_3N, the mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography (toluene–EtOAc = 20:1 → 10:1 → 3;1) to give **3a** as a colorless amorphous solid (3.7 mg, 85% from acceptor).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.34–7.21 (m, 25 H, $CH_2C_6H_5$), 5.93–5.80 (m, 1 H, OCH_2CH=CH_2), 5.25 (dd, *J* = 17.2, 1.5 Hz, 1 H, OCH_2CH=CH_2), 5.18 (dd, *J* = 10.4, 1.5 Hz, 1 H, OCH_2CH=CH_2), 5.15 (d, J_{gem} = 12.2 Hz, 1 H, $COOCH_2C_6H_5$), 5.04 (d, *J* = 10.0 Hz, 1 H, H-1), 4.95 (d, J_{gem} = 12.2 Hz, 1 H, $COOCH_2C_6H_5$), 4.83–4.60 (m, 10 H, $C_6H_5CH_2$, $COOCH_2CCl_3$), 4.38 (d, *J* = 7.8 Hz, 1 H, H-4′), 4.33 (d, *J* = 12.3 Hz, 1 H, H-5′), 4.15 (dd, *J* = 12.8, 5.3 Hz, 1 H, OCH_2CH=CH_2), 4.03 (dd, *J* = 10.5, 1.6 Hz, 1 H, H-7′), 3.98–3.89 (m, 3 H, OCH_2CH=CH_2, H-2, H-6a), 3.80 (d, *J* = 9.5 Hz, 1 H, H-3), 3.75 (dd, *J* = 10.5, 5.3 Hz, 1 H, H-8a′), 3.69 (dd, *J* = 9.3, 2.0 Hz, 1 H, H-8b′), 3.69 (t, *J* = 8.9 Hz, 1 H, H-4), 3.57 (t, *J* = 9.4 Hz, 1 H, H-5), 3.52 (dd, *J* = 10.6, 6.1 Hz, 1 H, H-6b), 2.34 (dd, *J* = 14.7, 5.3 Hz, 1 H, H-3a′), 2.09 (dd, *J* = 14.6, 6.1 Hz, 1 H, H-3b′), 1.51 (s, 1 H, $CHCH_3$), 1.34 (s, 1 H, $CHCH_3$). HRMS (ESI-QTOF, positive): *m/z* calcd for $C_{58}H_{64}Cl_3NO_{14}$ $[M + Na]^+$: 1126.3290; found: 1126.3285.

Representative Procedure of Microfluidic Reaction for the Synthesis of 3a

Dry MeCN was injected in advance to the micromixer by using a syringe pump and saturated the microfluidic system. Subsequently, a solution of donor **1a** (54.0 mg, 75.0 μmol, 0.15 M) and the acceptor **2** (14.3 mg, 25.0 μmol, 0.05 M) dissolved in MeCN (500 μL) and a solution of TfOH (2.2 μL, 25.0 μmol, 0.05 M) dissolved in MeCN (500 μL) were injected to the IMM micromixer by each syringe pump at the flow rate of 0.5 mL/min and mixed at 0 °C. After the reaction mixture was allowed to flow at 0 °C for 42 s through a stainless reactor tube (Φ = 1.0 mm, l = 25 cm), the mixture was added dropwise to Et_3N–CH_2Cl_2 at 0 °C. The mixture was concentrated in vacuo to give the crude product. The residue was purified by silica gel column chromatography (toluene–EtOAc = 20:1 → 10:1 → 3:1) to give **3a** (51 mg, 72%) and the stereoselectivity was analyzed by ^{1}H NMR (α/β = 92:8).

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

Acknowledgment

This work was supported in part by Grant-in-Aid for Scientific Research (No. 22310136, 21106008, 23241074 and 20-870) from JSPS/MEXT, grants from Osaka University Global COE program (Frontier Biomedical Science Underlying Organelle Network Biology) and the Naito Foundation, and a funding program for Next Generation World-Leading Researchers (NEXT Program; LR025) from JSPS and CSTP.

References and Notes

(1) Imoto, M.; Kusunose, N.; Matsuura, Y.; Kusumoto, S.; Shiba, T. *Tetrahedron Lett.* **1987**, *28*, 6277.
(2) Imoto, M.; Kusunose, N.; Kusumoto, S.; Shiba, T. *Tetrahedron Lett.* **1988**, *29*, 2227.
(3) Kusumoto, S.; Kusunose, N.; Imoto, M.; Shimamoto, T.; Kamikawa, T.; Takada, H.; Kotani, S.; Rietschel, E. T.; Shiba, T. *Pure Appl. Chem.* **1989**, *61*, 461.
(4) Fukase, K.; Kamikawa, T.; Iwai, Y.; Shiba, T.; Rietschel, E. T.; Kusumoto, S. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 3267.
(5) Fukase, K.; Kurosawa, M.; Kusumoto, S. *J. Endotoxin Res.* **1994**, *1*, 149.
(6) Yoshizaki, H.; Fukuda, N.; Sato, K.; Oikawa, M.; Fukase, K.; Suda, Y.; Kusumoto, S. *Angew. Chem. Int. Ed.* **2001**, *40*, 1475.
(7) Fujimoto, Y.; Iwata, M.; Imakita, N.; Shimoyama, A.; Suda, Y.; Kusumoto, S.; Fukase, K. *Tetrahedron Lett.* **2007**, *48*, 6577.
(8) Unger, F. M. *Adv. Carbohydr. Chem. Biochem.* **1981**, *38*, 323.
(9) Paulsen, H.; Stiem, M.; Unger, F. M. *Tetrahedron Lett.* **1986**, *27*, 1135.
(10) Paulsen, H.; Hayauchi, Y.; Unger, F. M. *Liebigs Ann. Chem.* **1984**, 1270.
(11) Paulsen, H.; Schueller, M. *Liebigs Ann. Chem.* **1987**, 249.
(12) Paulsen, H.; Stiem, M.; Unger, F. M. *Liebigs Ann. Chem.* **1987**, 273.
(13) Kiso, M.; Fujita, M.; Tanahashi, M.; Fujishima, Y.; Ogawa, Y.; Hasegawa, A.; Unger, F. M. *Carbohydr. Res.* **1988**, *177*, 51.
(14) D'Souza, F. W.; Kosma, P.; Brade, H. *Carbohydr. Res.* **1994**, *262*, 223.
(15) Kosma, P.; Hofinger, A.; Muller-Loennies, S.; Brade, H. *Carbohydr. Res.* **2010**, *345*, 704.
(16) Van der Klein, P. A. M.; Filemon, W.; Boons, G. J. P. H.; Veeneman, G. H.; Van der Marel, G. A.; Van Boom, J. H. *Tetrahedron* **1992**, *48*, 4649.
(17) Reiner, M.; Schmidt, R. R. *Tetrahedron: Asymmetry* **2000**, *11*, 319.
(18) Ikeda, K.; Akamatsu, S.; Achiwa, K. *Carbohydr. Res.* **1989**, *189*, C1.
(19) Esswein, A.; Rembold, H.; Schmidt, R. R. *Carbohydr. Res.* **1990**, *200*, 287.
(20) Tanaka, H.; Takahashi, D.; Takahashi, T. *Angew. Chem. Int. Ed.* **2006**, *45*, 770.
(21) Shimoyama, A.; Saeki, A.; Tanimura, N.; Tsutsui, H.; Miyake, K.; Suda, Y.; Fujimoto, Y.; Fukase, K. *Chem. Eur. J.* **2011**, *17*, *in press*.
(22) Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405.
(23) Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Tetrahedron Lett.* **2002**, *43*, 5573.
(24) Yu, B.; Sun, J. *Chem. Commun.* **2010**, *46*, 4668.
(25) Goi, T.; Tanaka, K.; Fukase, K. *J. Carbohydr. Chem.* **2007**, *26*, 369.
(26) Tanaka, K.; Fujii, Y.; Tokimoto, H.; Mori, Y.; Tanaka, S.; Bao, G. M.; Siwu, E. R.; Nakayabu, A.; Fukase, K. *Chem. Asian. J.* **2009**, *4*, 574.
(27) Hashimoto, S.; Hayashi, M.; Noyori, R. *Tetrahedron Lett.* **1984**, *25*, 1379.
(28) Murase, T.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1988**, *184*, C1.
(29) Ikami, T.; Ishida, H.; Kiso, M. *Methods Enzymol.* **2000**, *311*, 547.
(30) Suga, S.; Yamada, D.; Yoshida, J. *Chem. Lett.* **2010**, 404.
(31) Tanaka, K.; Mori, Y.; Fukase, K. *J. Carbohydr. Chem.* **2009**, *28*, 1.
(32) Tanaka, K.; Miyagawa, T.; Fukase, K. *Synlett* **2009**, 1571.
(33) IMM's micromixer: http://www.imm-mainz.de.

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

Copyright of Synlett is the property of Georg Thieme Verlag Stuttgart and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.