Organic Process Research & Development **2009**, *13*, 983–990

Renaissance of Traditional Organic Reactions under Microfluidic Conditions: A New Paradigm for Natural Products Synthesis

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Abstract:

Continuous flow synthesis for bioactive natural products is described. Efficient procedures using the microfluidic system were developed for the large-scale synthesis of important synthetic units of asparagine-linked oligosaccharide in glycoprotein. Advantageous aspects of microfluidic conditions, i.e., efficient mixing, fast heat transfer, and residence time control led to cation-mediated reactions, such as α-sialylation, β-mannosylation, and reductive opening of the benzylidene acetal groups in high yields. Microfluidic dehydration was developed for the industrial-scale synthesis of the immunostimulating natural terpenoid, pristane. The base-mediated aldol condensation in an aqueous biphasic system enabled the multigram synthesis of β-hydroxyketones in high yields.

Introduction

A continuous flow microreactor, an innovative technology, has been used to realize efficient mixing and fast heat transfer in organic syntheses.[1,2] The flow system allows the reaction to be quenched immediately after the formation of the unstable products. Furthermore, once the reaction conditions are optimized for a small-scale operation, the same conditions are directly applicable to large-scale synthesis under the flow process. We have been investigating the application of these advantageous features of the microfluidic systems to the "key" but "problematic" organic reactions under the conventional batch apparatus, in particular applying them to bioactive natural product synthesis.[3] Our successful examples are the cation-mediated reactions, such as α-sialylation,[3b,g] β-mannosylation,[3f] and reductive opening of the benzylidene acetal groups,[3d] for which the improved procedure under microfluidic conditions enabled the preparation of the key synthetic intermediates for the oligosaccharides on a multigram scale, eventually leading to the total synthesis of the asparagine-linked oligosaccharide (*N*-glycan).[3g] A significant improvement has also been achieved for the dehydration, which resulted in the industrial-scale synthesis of the immunostimulating natural terpenoid, pristane, 500 kg to 1 ton synthesis in a year.[3c] Alternatively, the microfluidic reaction exerted profound effects even on the base-mediated aldol condensation in an aqueous biphasic system,[3e] which enabled the multigram synthesis of the important intermediate for the chiral auxiliary in the natural alkaloid synthesis. By referring to our microfluidic reactions as the examples in this review, we will discuss the new aspects of using the microfluidic systems for controlling the hitherto difficult reactions in conventional organic synthesis. The microfluidic reactions can offer a direct and practical route to the desired compounds, with mixing efficiency and temperature control not associated with scale-up problems, and therefore be regarded as a new paradigm for the practical synthesis and, in favorable cases, the industrial synthesis of the bioactive natural products.

1. Application of Microfluidic Systems to the Synthesis of Asparagine-Linked Oligosaccharides. Among the various types of oligosaccharide structures, asparagine-linked oligosaccharides (*N*-glycans) are the most prominent in terms of diversity, complexity, and biological activities.[4] The chemical synthesis[5] provides an attractive opportunity to evaluate their biological functions, in consideration of difficulty of isolation and/or scarcity amount of the glycans from the natural sources.

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10.1021/op900084f CCC: $40.75
Published on Web 07/17/2009

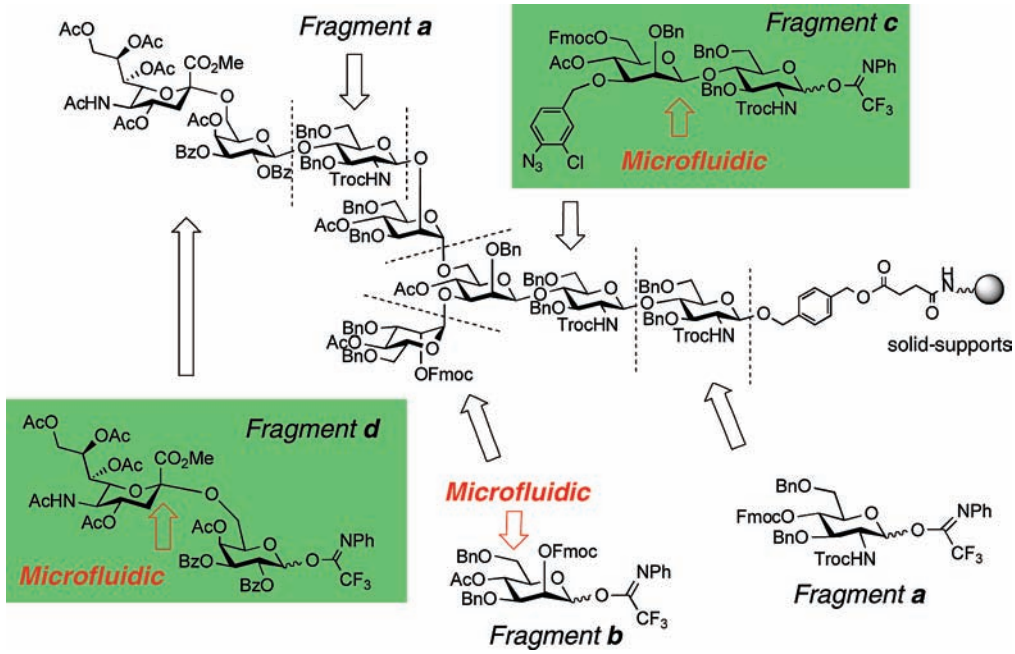

Figure 1. **Synthesis of asparagine-linked oligosaccharide via glycosylations under microfluidic conditions and on solid-supports.**

Our interests in elucidating unknown biological functions of mammalian *N*-glycans of the diverse structures, have motivated us to establish a library-directed synthesis of the complex-type *N*-glycans on the solid-supports;[3g] the initial target of our strategy is sialic acid-containing *N*-glycan with asymmetrical branching chains (Figure 1), which is difficult to obtain from natural sources. To efficiently prepare target *N*-glycan as well as other diverse structures of this family, we designed fragments **a**−**d** with the *N*-phenyltrifluoroacetimidate as the leaving group, which can be efficiently glycosylated on the solid-supports to construct the *N*-glycan structures. Herein, two challenging glycosyl bond formations, i.e., β-mannosylation and α-sialylation, were constructed in advance in solution, by the aid of the microfluidic systems as will be discussed in the following sections (Figure 1).[3b,g,f]

Microfluidic α*-Sialylation.* We have previously developed an efficient α-sialylation by utilizing the highly reactive sialyl donors having C-5 cyclic imides (Table 1), especially *N*-phthalimide (**1a**), by virtue of the "fixed-dipole moment effects" (α-only, 92% on 50 mg scale).[6] The scale-up in batch process, however, significantly decreased the yield and selectivity. Thus, the 100 mg-scale reaction of **1a** gave only 60% of α-sialoside accompanied by a significant amount of a glycal byproduct. The decrease in sialylation efficiency might be due to the high reactivity of the donor **1a**. For such a case, precise reaction control is very difficult under the conventional batch process conditions, especially when the reaction is scaled up. Thus, the disorder of the reaction factors in the scaled up batch reaction, i.e., (i) precise temperature control, (ii) mixing efficiency between acceptor, donor, and Lewis acid, and (iii) reaction time, might lead to the glycal production; in order to circumvent these problems, we used a continuous flow microreactor.

For the present microfluidic sialylation, a propionitrile solution of sialyl donor **1a** and acceptor **2** with various concentrations was mixed with TMSOTf solution in dichloromethane at −78 °C using an IMM micromixer[7] with a channel width of 40 μm at the flow rate of 1.0 mL/min (Table 1). After the reaction mixture was allowed to flow at −78 °C for additional 47 s through a reactor tube ($\Phi = 1.0$ mm, $l = 1.0$ m), the mixture was quenched by another flow of excess triethylamine dissolved in dichloromethane by using a T-shaped mixer at −78 °C. When the concentrations of the donor **1a**, acceptor **2**, and TMSOTf were adjusted to 0.15 M, 0.1 M, and 0.08 M, respectively, disaccharide **3a** was obtained in only 14% yield, and a large amount of the acceptor **2** was recovered (entry 1). However, we were pleased to find that the yield of **3a** dramatically increased (88%) when the concentration of the Lewis acid was increased up to 0.15 M (entry 2). Finally, the desired α-sialoside **3a** was obtained in quantitative yield by increasing the concentration of the donor **1a** to 0.2 M (entry 3). Thus, the microfluidic reaction successfully controlled the high reactivity of the sialyl donor **1a** for α-sialylation. Obviously, vigorous and rapid mixing of the substrates with the high

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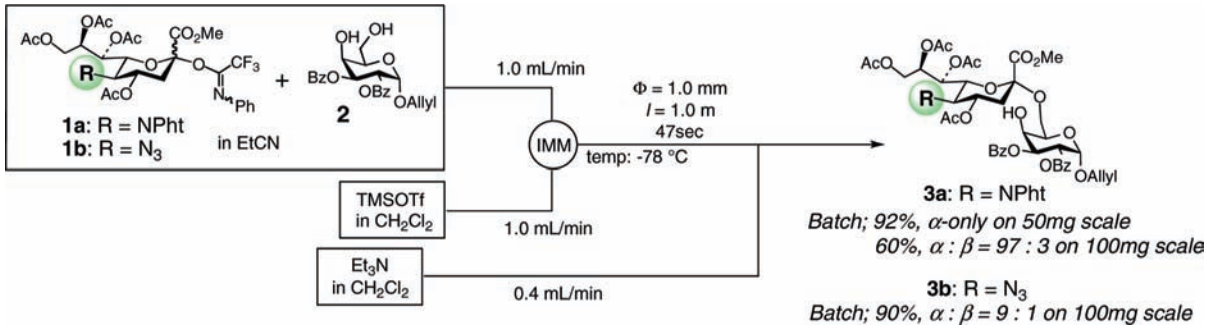
(7) IMM micromixer: http://www.imm-main2.de/.

Table 1. **Optimization of α(2−6)-sialylation using IMM micromixer**



entry	donor **1a,b** (M)	acceptor **2** (M)	TMSOTf (M)	yield of **3a,b** (%)	α:β
1	0.15 (**1a**)	0.1	0.08	14	α only
2	0.15 (**1a**)	0.1	0.15	88	α only
3	0.2 (**1a**)	0.1	0.15	>99	α only
4	0.2 (**1b**)	0.1	0.15	>99	20:1

concentrations of the acids is responsible for the success of the microfluidic sialylation; the trend is completely different from that of the corresponding batch reaction, since the decomposition of the donor has long been regarded as a severe problem under such drastic conditions.[6]

Unfortunately, the selective deprotection of the *N*-phthalyl group in the presence of the C-2 methoxycarbonyl in **3a** was troublesome on the 1−2 g reaction scale. As an alternative to the *N*-phthalyl function, we also employed the C-5 azide group in sialyl donor **1b** because this azide group should direct similar "fixed-dipole moment effects", but should be easier to convert to naturally occurring *N*-substituents of neuraminic acids, i.e., *N*-acetyl or *N*-glycolyl groups (see structure in Figure 1).[3g] As anticipated, sialylation between **1b** and galactosyl acceptor **2** in the presence of TMSOTf as an activator and MS4A in propionitrile provided **3b** in 90% yield with good α-selectivity (α:β = 9:1 on 100 mg scale). Furthermore, applying the continuous microfluidic sialylation, which was established for *N*-phthalyl derivative **1a** (Table 1, entry 3), improved both the yield and α-selectivity (entry 4); **3b** was obtained quantitatively with near perfect α-selectivity (α:β = 20:1). The α and β stereoisomers were easily separated by chromatography on silica gel, and the pure α-isomer was readily converted to the desired imidate fragment **d** (Figure 1) using the general procedure; hence, we successfully prepared fragment **d** on the 5−10 g scale.[3g]

Microfluidic β-Mannosylation. Stereoselective formation of the β-mannoside linkage, a key glycosylation in the synthesis of the Manβ(1−4)GlcNTroc fragment **c** of *N*-linked glycans, is another challenging topic in the oligosaccharides synthesis. Aside from the recently developed protocols by other groups,[3f,5] we also have achieved an excellent β-selectivity in the reaction of 4,6-*O*-benzylidene-mannopyranosyl-*N*-phenyltrifluoroacetimidate **4** with *N*-Troc-glucosamine acceptor **5** (R = Bn, 93% yield, β:α = 95:5 on 20 mg scale) using the bulky and dual Lewis acid/cation trap reagent, TMSB(C_6F_5)$_4$ (Table 2, entry 1).[8]

Nevertheless, it is difficult to apply our β-mannosylation protocol to a few gram-scale syntheses of the Manβ(1−4)-GlcNTroc fragment **c** because the scaled-up glycosylation requires a large quantity of the bulky TMSB(C_6F_5)$_4$ activator, which has limited commercial availability.[8] Therefore, from a practical viewpoint for preparing the fragment **c** as a starting material, we refocused on applying the more common TMSOTf as a glycosyl activator because our earlier experiments indicated that TMSOTf shows a good yield and β-selectivity on a 20 mg scale (90% yield, β:α = 93:7) (entry 2).[8a] However, the efficiency of glycosylation catalyzed by TMSOTf is extremely sensitive to the reaction scale as well as the addition speed of the Lewis acid (entries 2−5). When TMSOTf was added dropwise to a solution of mannosyl donor **4** and acceptor **5** at −78 °C, the yield of β-mannoside **6** gradually decreased as the reaction scale increased (entries 2−4). On a 50 mg scale, 63% of β-disaccharide **6** was isolated, whereas only 27% of β-isomer **6** was obtained on a 500 mg scale (entries 3 and 4). For an unknown reason, slow addition of a Lewis acid in the larger-scale reactions inhibited the glycosylation process at an earlier stage.[3f] Moreover, even the subsequent addition of the TMSOTf catalyst did not activate the glycosylation between the remaining starting materials. On the other hand, when the acid was added to the initial solution of **4** and **5** in one portion, mannosylation proceeded smoothly (entry 5). However, the β-selectivity decreased to 4.9:1, presumably due to the exothermic nature of the reaction, i.e., heat is generated while rapidly mixing, which leads to an overall decrease in the isolated yield of β-disaccharide **6** (61% on 900 mg scale). Therefore, we decided to examine the microfluidic conditions based on the observations shown in Table 2, which indicate that the current glycosylation is sensitive to the addition speed of the Lewis acid, i.e., slow or fast mixing, as well as to the reaction scale.

We initially constructed the microfluidic system such as in Table 2, based on our previous experiences with microfluidic α-sialylation.[3b,g] In addition to the aspects mentioned above, an attractive feature of the microfluidic reaction is that the reaction can be readily optimized under the flow process;[2d] the optimal conditions are rapidly determined using a small quantity

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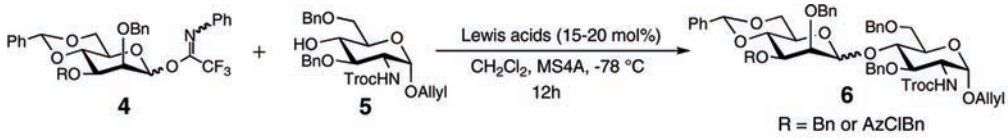
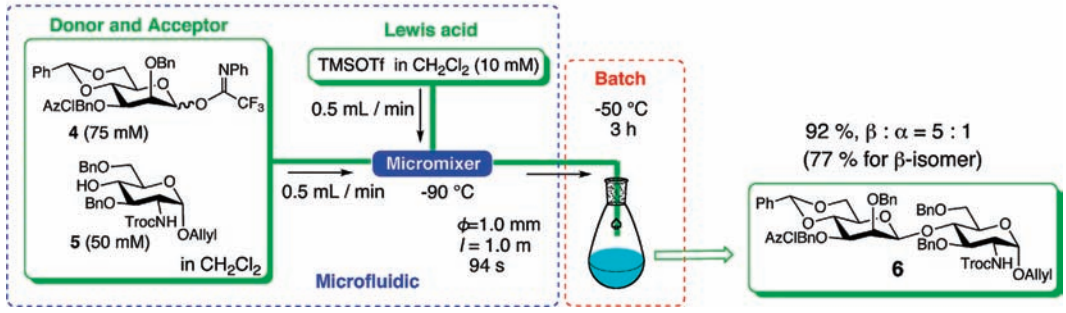
***Table 2. β*-Mannosylation under batch and integrated microfluidic/batch conditions**[3f]



entry	Lewis acids	addition of LA	scale (mg)	yield (β-isomer, %)	β : α
1	$TMSB(C_6F_5)_4$	dropwide	20	88	95 : 5
2	TMSOTf	dropwide	20	84	93 : 7
3	TMSOTf	dropwide	50	63	NA
4	TMSOTf	dropwide	500	27	NA
5	TMSOTf	in one portion	900	61	4.9 : 1



of materials, i.e., concentrations of the substrates, mixing speed, temperature, and residence time. For this optimization, we have used the Comet X-01 micromixer[9] with a channel width of ~500 μm, where the micromixing with IMM mixer (40 μm) caused the significant solution blockage problems due to the low solubility of both the donor **4** and the acceptor **5** in dichloromethane at very low temperature of −78 to −90 °C. Rapid screening of more than 30 conditions in a combinatorial fashion led to a new reaction system where the microfluidic system is integrated with a conventional batch apparatus (Table 2).[3f] Namely, the reaction solution through the efficient micromixing between the reactants at a low temperature, was subsequently inserted into the batch system, and then was conventionally stirred in a flask for a few hours to complete the reaction; although it is theoretically possible to maintain an indefinite residence time by increasing the reactor tube length, in certain conditions, i.e., when the reaction has to proceed for more than an hour, employing an extremely long tube is impractical. The optimal conditions in the integrated microfluidic/batch apparatus as depicted in Table 2, i.e., micromixing at −90 °C and a batch reaction at −50 °C for 3 h, provided α/*β*-mannoside **6** (R = Bn) in 92% yield and with a moderate *β*-selectivity (*β*:α = 5.0:1). It should be noted that, although the *β*-selectivity was somewhat lower than that observed in the small-scale batch reaction (Table 2, entry 2), the isolated *β*-mannoside **6** could be obtained in a similar efficiency (77% for microfluidic reaction versus 84% for 20 mg scale batch reaction).[3f] Moreover, under the established conditions, compound **6** applied to the solid-phase *N*-glycan synthesis was reproducibly obtained even in the scaled-up synthesis by simply preparing stock solutions of substrates and reagents, and then continuously pumping them into the integrated microfluidic/batch system.

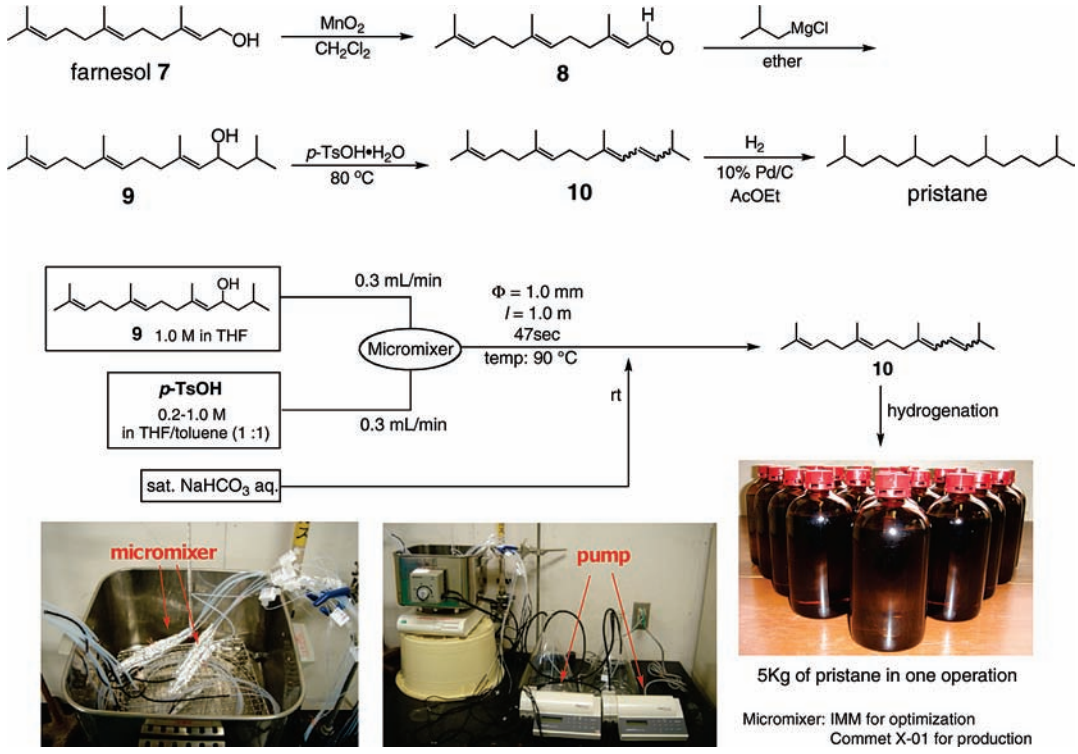
We also applied the microfluidic conditions to the acid-mediated reductive opening of sugar 4,6-*O*-benzylidene acetals, in preparing the fragment **b** in a large scale (Figure 1).[3d] As in the case of the microfluidic α-sialylation described above, the rapid and vigorous mixing with the highly concentrated acid was critical for the reaction to be successful; presumably, the efficient micromixing between substrate, hydride, and high concentration of the acid might accelerate the reaction, while the rapid heat transfer prevents the undesired hydrolysis.

Thus, three formerly "difficult" acid-mediated reactions under the conventional batch conditions significantly facilitated the large-scale preparation of the *N*-glycan fragments **b**−**d**; stock preparations for these key synthetic intermediates eventually led to the first solid-supported synthesis of the sialic acid-containing complex-type *N*-glycan (Figure 1).[3g]

2. Microfluidic Dehydration: A Process Synthesis of Immunoactivating Natural Product, Pristane. 2,6,10,14-Tetramethylpentadecane (pristane) is a saturated isoprenoid isolated from the basking shark, *Cetorhinus maximus*.[10] This hydrocarbon oil is known to induce tumorigenesis in mice and arthritis and lupus nephritis in rats, and has been widely used as an adjuvant for monoclonal antibody production in mouse ascites.[11] However, in 2002, the basking shark was listed on Article II of the Washington Convention (Convention on International Trade in Endangered Species of Wild Fauna and Flora), and since then, the availability of pristane from a

(9) Comet X-01 micromixer: http://homepage3.nifty.com/techno-applications/ or E-mail: yukio-matsubara@nifty.com.

Scheme 1. **Process synthesis of pristane**



natural source has become very limited.[11] Therefore, an efficient chemical synthesis of pristane has been long desired.

When considering the synthesis of this simple hydrocarbon in a nonstereoselective manner, one can immediately come up with a commonplace route, i.e., (1) oxidation of farnesol **7**, (2) alkylation, (3) dehydration, and (4) hydrogenation, as shown in Scheme 1. The synthesis is quite simple when 50 mg of the sample is prepared, but it suddenly becomes difficult when 200 kg of this hydrocarbon is required in a year with more than 98% purity. Namely, the preparation of 5 kg of pristane in about a week is necessary in order to supply enough material in the market.

The challenging step in Scheme 1 is the acid-catalyzed dehydration of allylic alcohol **9**. When the reaction was performed in 100 mg scale using a catalytic amount of *p*-TsOH in benzene at 80 °C, the corresponding diene **10** was obtained in 55% yield as its (*E*)- and (*Z*)-stereoisomers which were transformed to pristane by hydrogenation. However, when the scale was raised to 100 g, various cation-mediated byproducts, such as the cyclized products or the alkyl group migrated compounds were produced. As expected, these hydrocarbons were very difficult to separate from the desired diene **10**, even by repeated distillation or by silica gel chromatography, although the latter is not realistic for kilogram-scale purification.

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Inspired by the successful application of the microfluidic systems to the acid-mediated reactions during the *N*-glycan synthesis described above, we examined the microfluidic dehydration under the following conditions (Scheme 1):[3c] Allylic alcohol **9** (1.0 M in THF) was mixed with a solution of *p*-TsOH (various concentrations of 0.2−1.0 M in THF/toluene = 1:1) at 90 °C by using an IMM micromixer[7] at each flow rate of 0.3 mL/min. After the reaction mixture was allowed to flow for an additional 47 s at 90 °C through a reactor tube (Φ = 1.0 mm, l = 1.0 m), the mixture was quenched by a saturated $NaHCO_3$ solution at room temperature.

When a 0.2 M solution of *p*-TsOH was used, only a trace amount of **10** was obtained, and the starting material **9** was largely recovered. However, we again found that the yield of the dehydrated compound depends on the concentration of the acid; **10** was finally obtained in 80% yield (total yield from farnesol **7**) at the acid concentration of 1.0 M. As opposed to the conventional batch reactions, the common features observed for the microfluidic α-sialylation and the reductive opening of the benzylidene acetals can again be applied to the current dehydration; namely, the success of "very fast" acid-mediated reactions under the microfluidic conditions result from the rapid micromixing with the concentrated acid. It is noted that under the established microfluidic conditions, the formation of other byproducts could not be detected.

Having established the optimal conditions for dehydration, the kilogram synthesis of pristane was examined (Scheme 1).[3c] The crude alcohol **9**, derived from 8 kg of farnesol **7** without any purification process, was subjected to the key microfluidic dehydration under the conditions established in Scheme 1. For such a large-scale microfluidic reaction, we again introduced Comet X-01,[9] which we know from the previous examples exhibited similar mixing efficiency to the IMM micromixer and

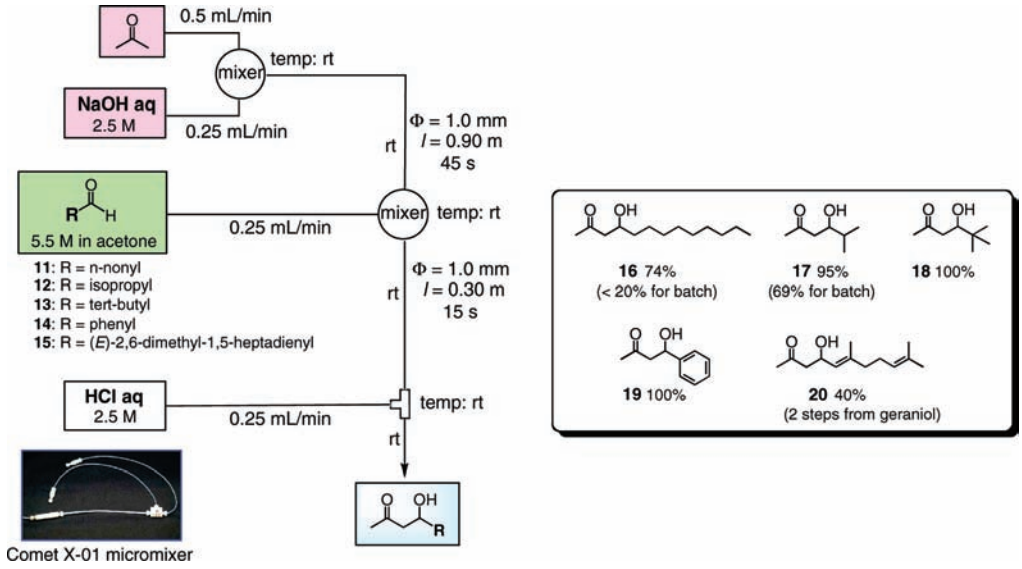

Figure 2. **Biphasic aldol condensation under the microfluidic conditions.**

avoids the stuffing problem by using the relatively large tube hole (~500 μm). This is especially useful for process synthesis, when an easily crystallized material, such as TsOH, is used at high concentration. Therefore, this mixer, together with the availability at a low price, is well suited for establishing a micro-chemical plant. As shown in Scheme 1, we arranged 10 micromixers in a row and achieved the 8-kg-scale dehydration during 3−4 days. It is noted that the present micro-chemical plant does not require any special apparatus or devices, and such a simple system in Scheme 1 continuously performed efficient dehydration. The resulting solution eluted from the micromixing system was quenched by a saturated $NaHCO_3$ solution, extracted by ethyl acetate and concentrated, and the mixtures were shortly passed through a silica gel pad in order to remove the hydrophilic byproduct, affording the pure diene **10**.

Finally, the hydrogenation provided pristane in 50−55% overall yields (~5 kg) with >99% purity based on the gas chromatography analysis. Since the present pristane synthesis involves only one simple purification step by filtration with a silica gel pad, we believe that our protocol is superior to the hitherto known synthesis, purification of which necessitates tedious distillation many times at the final stage. Actually, even repeated distillation cannot purify the pristane completely, and the mixture of the hydrocarbon products shows much decreased activity in the antibody production, and sometimes shows no activity by the "production rot". Indeed, our pristane reproducibly produced by this route is confirmed to induce antibody production in mouse ascites twice as efficiently as natural pristane or the other synthetic products, due to non-negligible contamination of the other hydrophobic compounds.[10]

3. Microfluidic Aldol Condensation in Aqueous Biphasic System. Much improvement on the "traditional" organic reactions under the microfluidic conditions can also be realized for the base-mediated aldol reaction,[3e] one of the most fundamental C−C bond-forming reactions.[12] Particularly, β-hydroxy α-methyl ketones are useful synthetic intermediates which can be prepared by using acetone as a precursor of enolate.[3c] This acetone-based aldol reaction is conveniently performed by slowly adding the aldehydes to a biphasic solution of acetone and sodium hydroxide below 10 °C. For this conventional reaction, acetone is used as a solvent in order to achieve the efficient condensation. However, the efficiency of this protocol under batch conditions significantly decreases when the reaction is performed on large scale since (i) inefficient mixing of the biphasic solution, (ii) difficulty in maintaining the reaction temperature, and (iii) a long time exposure of base-sensitive starting materials and products to the highly basic reaction media lead to the aldehyde polymerization. The situation becomes worse particularly when the reaction is applied to the aldehydes containing the α-protons; e.g., the reaction with decyl aldehyde **11** resulted in the production of a large amount of aldehyde-polymer gel, and the desired 4-hydroxy-2-tridecanone **16** is obtained in only less than 20% yield (Figure 2).

We therefore expected that the aqueous biphasic aldol condensation would be a good case where the advantageous aspects of microfluidic reaction can be featured at their maximum; a microfluidic system was designed in Figure 2 by integrating two micromixers.[9,13] The appropriate flow rates and residence time were optimized for these two micromixing processes. In the first mixer, acetone enolate should be formed from acetone and aqueous NaOH solution,[3e] while the enolate can be rapidly reacted with aldehydes, owing to the efficient mixing by the second micromixer. Undesired polymer formation of the base-sensitive aldehydes was effectively suppressed with this sequential process. In addition, the aldol products, which are also susceptible to the base-mediated polymerization, can

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be readily removed from the system after quenching with the hydrochloric acid by the use of a T-shaped mixer (Figure 2).

We examined the decyl aldehyde **11**, isobutyraldehyde **12**, pivalaldehyde **13**, benzaldehyde **14**, and geranyl aldehyde **15** as the representative aldehydes, which contain the different numbers of α-protons. Gratefully, the reaction with decyl aldehyde **11** under the conditions optimized in Figure 2 resulted in a significant increase of the aldol product formation; 4-hydroxy-2-tridecanone **16** was obtained in 74% yield, which is about a 4-fold increase in yield compared with that of the conventional batch conditions (∼20%).[3e] In comparison, the direct mixing of the 5.5 M aldehyde solutions in acetone and the aqueous 2.5 M NaOH solution gave a significant amount of the polymerized products, similar to the results obtained by the batch reaction.[3e] Furthermore, the use of lower concentrations of the aldehyde solutions than those optimized in Figure 2 resulted in the incomplete conversion. These results clearly show the success for applying the sequential micromixing systems, as well as the importance of the reaction concentration for the efficient micromixing, similar to the acid-mediated microfluidic reactions described above. Not only the efficiency for the aldol process but also the "room temperature-reaction" make the established microfluidic procedure to be quite an attractive method for the β-hydroxyketone derivatives, such as for industrial-scale preparation. The reaction with isobutyraldehyde **12**, which contains a single α-proton, also gave **17** in 95% yield. The comparison experiment under the conventional batch reactions gave 69% of **17**;[14] hence, the efficiency of the microfluidic system for the biphasic aldol reaction can thus be proved. β-Hydroxycarbonyl compound **17** is the important intermediate in preparing the chiral aminoindanol derivatives, useful chiral auxiliaries for highly substituted piperidine alkaloid synthesis via asymmetric azaelectrocyclization.[14] As expected from these two examples, the reaction with the simpler pivalaldehyde **13** and benzaldehyde **14**, which have no α-proton, gave the aldol products **18** and **19** in nearly quantitative yields. It is noted that the dehydrated product of the labile 4-hydroxy-4-phenyl-2-butanone **19** could not be detected from the reaction mixtures since the reaction was quenched immediately after the formation of **19** under the flow process of Figure 2. The relatively unstable (*E*)-3,7-dimethyl-2,6-octadienal **15** (geranyl aldehyde) also reacted with the acetone enolate under the conditions in Figure 2, providing the 40% yield of β-hydroxyketone **20** (two-step yield from geraniol) together with the recovery of the aldehyde **15**. Any other byproducts could not be detected from the reaction mixtures; thus, the general and efficient aldol reaction in biphasic system has been realized by integrating all favorable aspects of microfluidic reactions, namely, efficient mixing, precise temperature control, and the "easy handling" of the reactive intermediate (enolate) and the aldol adducts by controlling the residence time.

Conclusions

In conclusion, we have achieved the efficient glycosylation, dehydration, and aldol reaction under the microfluidic conditions. During our research in applying the microfluidic systems to these acid- and base-mediated reactions, a distinctly different reactivity from that in the conventional batch stirring was found; the vigorous micromixing of the reactants is critical, especially for the "fast" reactions to be successful. Such a common feature might be owed to the integration of all favorable aspects of microfluidic conditions, namely, efficient mixing, precise temperature control, and the easy handling of the reactive intermediate by controlling the residence time. A rapid determination of the reaction conditions is another aspect in using the microfluidic conditions in a combinatorial fashion. The efficiency of our microfluidic reactions exemplified in this review, together with the other examples reported by others, provokes the need to reinvestigate the traditional or imaginary reactions which have so far been performed and evaluated only in batch apparatus, and therefore have not been utilized throughout for organic synthesis. We strongly believe that the microfluidic reactions call a return to the "traditional" organic reactions for natural product synthesis.

Experimental Section

Microfluidic α-Sialylation.[3b] *Allyl 2,3-Di-O-benzoyl-6-O-(methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-phthalimido-D-glycero-α-D-galacto-2-nonulopyranosylonate)-α-D-galactopyranoside (**3a**).* A solution of TMSOTf (54 μL, 0.30 mmol, 0.15 M) in CH_2Cl_2 (2.0 mL) was injected in advance to the IMM micromixer[7] by using a syringe-pump at the flow rate of 1.0 mL/min. Subsequently, a solution of donor **1a** (150 mg, 0.20 mmol, 0.20 M) and the acceptor **2** (43 mg, 0.10 mmol, 0.10 M) dissolved in EtCN (1.0 mL) was also injected to the micromixer by another syringe-pump at the flow rate of 1.0 mL/min and mixed at −78 °C. After the reaction mixture was allowed to flow at −78 °C for an additional 47 s through a stainless reactor tube ($\Phi = 1.0$ mm, $l = 1.0$ m), the mixture was quenched by another flow of an excess triethylamine dissolved in CH_2Cl_2 at −78 °C. It takes about 2−3 min to consume 43 mg of the acceptor **2** under the above conditions. The mixture was extracted with ethyl acetate, washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give the crude product. The excess glycal obtained as a byproduct was removed by preparative TLC on silica gel (4% MeOH in chloroform), and the yield of **3a** was analyzed by ^{1}H NMR. The conditions established herein can be readily applicable to the scale-up synthesis simply by preparing the stock solutions of substrate and reagents and pumping them continuously into the micromixer, i.e., α-sialylation using 2.3 g of the acceptor **2** under the present microfluidic system has been realized for 10 min: HRMS *m/z* calcd for $C_{49}H_{51}NO_{21}Na$ $(M + Na)^+$ 1012.2851, found 1012.2873; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97−7.92 (m, 4H, aromatic), 7.76−7.75 (m, 2H, Phth), 7.66−7.65 (m, 2H, Phth), 7.45−7.42 (m, 4H, aromatic), 7.32−7.28 (m, 2H, aromatic), 5.87 (ddd, $J = 5.51$, 10.7, 22.3 Hz, 1H, $OCH_2C\underline{H}{=}CH_2$), 5.71 (m, 2H, H-2,3), 5.53 (ddd, $J = 5.03$, 10.6, 11.1 Hz, 1H, H-4′), 5.45 (dd, $J = 2.71$, 5.55, 8.42 Hz, 1H, H-8′), 5.34 (m, 1H, H-4), 5.31 (dd, $J = 1.73$, 17.2 Hz, 1H, $OCH_2CH{=}C\underline{H_2}$), 5.18 (dd, $J = 2.42$, 8.31 Hz, 1H, H-7′), 5.15 (dd, $J = 1.44$, 10.4 Hz, 1H, $OCH_2CH{=}C\underline{H_2}$), 5.10 (dd, $J = 2.34$, 10.6 Hz, 1H, H-6′), 4.43 (s, 1H, H-1), 4.29 (dd, $J = 2.73$, 9.76 Hz, 1H, H-9′a), 4.27 (dd, $J = 5.09$, 13.2 Hz, 1H, $OC\underline{H_2}CH{=}CH_2$), 4.23 (t, $J = 10.6$ Hz, 1H, H-5′), 4.19 (t, 1H,

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H-5), 4.00 (dd, J = 6.00, 13.3 Hz, 1H, OC$\underline{H}_2$CH=CH_2), 4.05−4.02 (m, 2H, H-9′b, H-6a), 3.91 (s, 3H, $CO_2C\underline{H}_3$) 3.87 (dd, J = 6.50, 10.0 Hz, 1H, H-6b), 3.03 (s, 1H, O$\underline{H}$), 2.80 (dd, J = 5.14, 12.9 Hz, 1H, H-3′eq), 2.15 (s, 3H, Ac), 2.13 (s, 3H, Ac), 1.88 (s, 3H, Ac), 1.84 (s, 3H, Ac), 2.01 (m, 1H, H-3′ax).

Microfluidic β-Mannosylation.[3f] *Allyl 4-O-[3-O-(4-Azido-3-chlorobenzyl)-4,6-O-bendilidene-2-O-benzyl-α-D-mannopyranosyl]-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (**6**).* A solution of TMSOTf (90.4 μL, 0.500 mmol, 12.5 mM) in CH_2Cl_2 (40.0 mL) was injected, in advance, into the Comet X-01 micromixer[9] by a syringe-pump at a flow rate of 0.50 mL/min. Then a solution of donor **4** (C4-OAzClBn, 1.41 g, 2.03 mmol, 75.0 mM) and acceptor **5** (776 mg, 1.35 mmol, 50.0 mM) dissolved in CH_2Cl_2 (27.0 mL) was injected into the micromixer by another syringe-pump at a flow rate of 0.5 mL/min. The reaction was mixed at −90 °C. After the reaction mixture was allowed to flow at −90 °C for an additional 94 s through a Teflon tube reactor (Φ = 1.0 mm, l = 1.0 m), the mixture was introduced into a flask, which was previously cooled to −50 °C. The reaction mixture was stirred for 3 h at this temperature, and the mixture was quenched by triethylamine at −50 °C. The resulting mixture was extracted with ethyl acetate, washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give the crude product. The residue was purified by column chromatography on silica gel (13% ethyl acetate in toluene) to give β-mannoside **6** as a colorless oil (674 mg, 69%): ESI-MS m/z calcd for $C_{53}H_{55}Cl_4N_4O_{12}$ $(M + H)^+$ 1079.2, found 1079.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40−7.03 (m, 23H, aromatic), 5.95−5.86 (m, 1H, −CH_2−C$\underline{H}$=CH_2), 5.46 (s, 1H, PhC$\underline{H}$=), 5.30 (dd, J = 17.3, 1.5 Hz, 1H, −CH_2−CH=C$\underline{H}_2$), 5.24 (dd, J = 10.3, 1.0 Hz, 1H, −CH_2−CH=C$\underline{H}_2$), 5.04 and 4.67 (each d, J_{gem} = 11.5 Hz, 2H, −C$\underline{H}_2$Ph), 5.01 (d, $J_{N,2}$ = 10.0 Hz, 1H, N$\underline{H}$), 4.92 (d, J = 3.4 Hz, 1H, H-1), 4.82 (s, 2H, −C$\underline{H}_2$Ph), 4.73 and 4.37 (each d, J_{gem} = 12.0 Hz, 2H, −C$\underline{H}_2$Ph), 4.67 and 4.64 [each d, J_{gem} = 12.2 Hz, 2H, −C$\underline{H}_2$−($C_6H_3N_3Cl$)], 4.62 and 4.59 (each d, J_{gem} = 12.7 Hz, 2H, −NH−COO−C$\underline{H}_2$-CCl_3), 4.67 (s, 1H, H-1′), 4.09 (dd, J = 12.3, 5.1 Hz, 1H, −C$\underline{H}_2$−CH=CH_2), 4.06−3.93 (m, 5H, −C$\underline{H}_2$−CH=CH_2, H-2, H-4, H-4′, H-6′a), 3.70−3.57 (m, 5H, H-3, H-4, H-6a, H-6b, H-2′), 3.45 (t, J = 10.3 Hz, 1H, H-6′b), 3.37 (dd, J = 9.5, 2.9 Hz, 1H, H-3′), 3.07 (td, J = 9.5, 4.9 Hz, 1H, H-5′).

Microfluidic Dehydration.[3c] *Preparation of **10**.* A solution of p-toluenesulfonic acid (1.0 M) in a mixed solvent of THF and toluene was injected in advance into the 10 Comet X-01 micromixers[9] arranged in a row, by using a HPLC pump at the flow rate of 0.3−1.0 mL/min. Subsequently, a solution of the alcohol **9** (1.0 M) in THF was also injected into the micromixers by another HPLC pump at the flow rate of 0.3−1.0 mL/min and mixed at 90 °C. After the reaction mixture was allowed to flow at 90 °C for an additional 47 s through a stainless reactor tube (Φ = 1.0 mm, l = 1.0 m), the mixture was quenched by introducing an aqueous $NaHCO_3$ solution at room temperature. It takes about 3−4 days to consume 8 kg of the alcohol **9** under above conditions. The mixture was extracted with ethyl acetate, washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give the crude product. The hydrophilic byproducts were removed by a silica gel pad (eluted by hexane), and the dehydrated compound **10** was then subjected to the hydrogenation for the pristane synthesis.

Microfluidic Aldol Reaction.[3e] *Preparation of **17**.* Acetone and the 2.5 M NaOH solution were injected into the first Comet X-01 micromixer[9] at room temperature, by using syringe-pumps at the flow rate of 0.5 mL/min for acetone and 0.25 mL/min for the NaOH solution. After the mixture was allowed to flow at this temperature for 45 s through a Teflon reactor tube (Φ = 1.0 mm, l = 0.9 m), the intermediary enolate was mixed with a solution of isobutyraldehyde **12** (10 g, 139 mmol, 5.5 M) in acetone (25 mL) through the second micromixer (Comet X-01) by another syringe-pump at the flow rate of 0.25 mL/min at room temperature. After the reaction mixture was allowed to flow at the same temperature for an additional 15 s through a Teflon reactor tube (Φ = 1.0 mm, l = 0.3 m), the mixture was quenched by another flow of 2.5 M HCl solution (0.25 mL/min) using the T-shaped mixer at room temperature. It takes about 20 min to consume 10 g of the substrate **12** under the above conditions. The mixture was extracted with ethyl acetate, washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give the crude product. The residue was purified by distillation under reduced pressure (50−70 °C/20 mmHg) to afford **17**[14] (17 g, 95%).

Acknowledgment

We acknowledge Mr. Koichi Koyama and Mr. Keiichi Watanabe, Kishida Chemical Co., Ltd., for helpful discussion and arrangement for promoting the synthetic pristane in the market. The present work was conducted with the aid of the Micro-Chemical Plant Technology Union (MCPT) for the Project of Micro-Chemical Technology for Production, Analysis, and Measurement Systems financially supported by NEDO. This work was also partially supported by Grants-in-Aid for Scientific Research Nos. 19681024 and 19651095 from the Japan Society for the Promotion of Science, Collaborative Development of Innovative Seeds from Japan Science and Technology Agency (JST), New Energy and Industrial Technology Development Organization (NEDO, project ID: 07A01014a), Research Grants from Yamada Science Foundation as well as Molecular Imaging Research Program, Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

Received for review April 7, 2009.

OP900084F