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A New Microfluidic Phase-Transfer Reaction Using HPLC Guard Columns as the Reactor for the N^3-Protection of Uridine Derivatives

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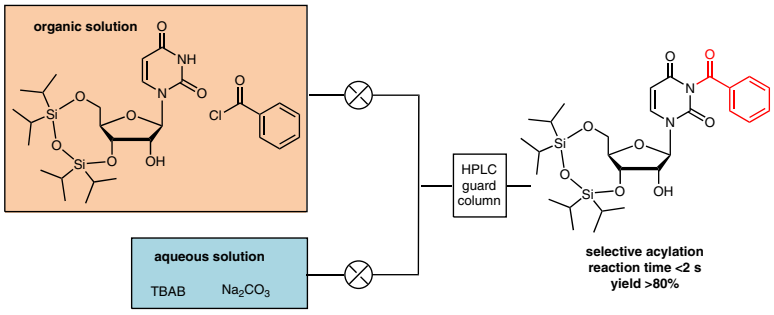

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Abstract N^3-Acylation of uridine derivatives with acyl chlorides, mediated by a phase-transfer reaction, was studied using a new microfluidic device containing an HPLC guard as an effective reactor. The acylated products were obtained in more than 80% yields in very short reaction times of several seconds.

Key words microfluidic system, phase-transfer reaction, nucleoside chemistry, flow chemistry, HPLC guard column

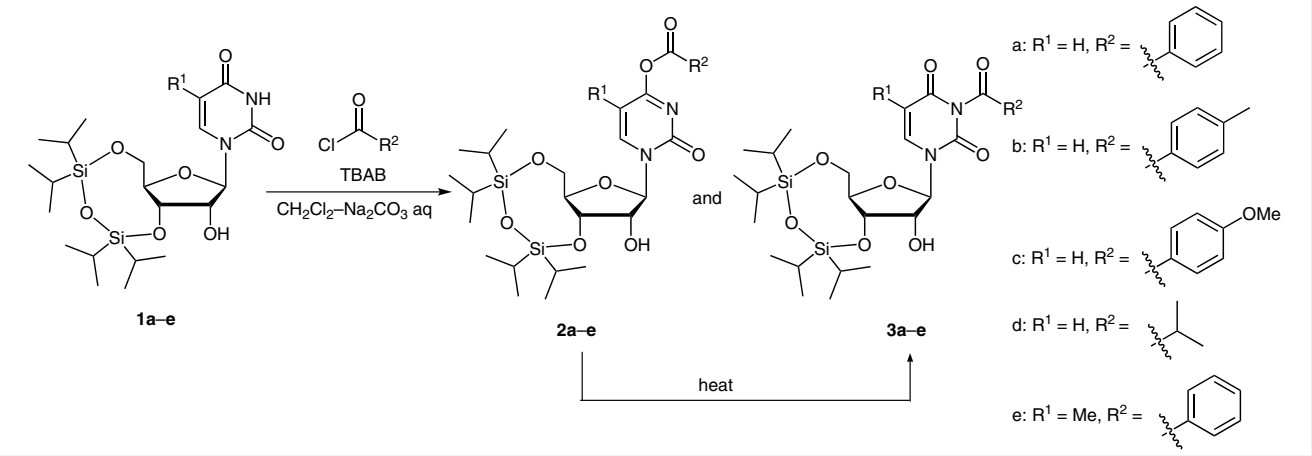
In recent decades, modified nucleic acids have shown potential as gene therapeutic drugs[1] and gene-detection probes.[2] 2′-O-Modification of RNAs has been used to improve the nuclease resistance and binding affinity for target nucleic acids.[3] For the modification of the 2′-hydroxy group of uridine, 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine has been widely used as the intermediate.[4] However, because the N^3- or 4-O-positions are reactive moieties, in some cases, protection of these positions is necessary.[5] Various acyl groups have been used as the protecting group for the uracil base,[6] and we have previously reported that acyl groups can be introduced to the N^3-position very effectively by using a phase-transfer (PT) reaction.[7]

The PT reaction of uridine derivatives is performed as shown in Scheme 1 ($R^1 = R^2 = H$). In these reactions, compound **1** is benzoylated with benzoyl chloride using CH_2Cl_2 as the organic phase and an aqueous solution of sodium carbonate as the aqueous phase. The carbonate anion is transferred into the organic phase by the PT catalyst tetrabutylammonium bromide (TBAB). After the formation of N^3- (**3a**) and 4-O- (**2a**) benzoylated derivatives, compound **2a** is converted into **3a** by heating at 60 °C in 1,2-dichloroethane. Although this reaction is very fast and effective in small-scale preparations, the scaling up of the PT reaction in a batch reactor decreases the reaction rate and lowers the product yields. It is generally accepted that the PT reaction rate is highly dependent on the interfacial area, and also corresponds to the transfer rate of reactants from the aqueous phase to the organic phase promoted by the PT catalyst.[8]

To accelerate PT reactions, several research groups have applied microfluidic devices to achieve the large interfacial areas that are necessary for efficient PT reactions.[8–13] For example, Kitamori et al. reported a notable improvement in the diazo coupling reaction in a microfluidic system. The reaction proceeded with quantitative conversion in the microfluidic system, whereas the yield was 80% with a batch reactor.[9] This result was rationalized in terms of the increased interfacial area in the microfluidic system, which was estimated to be twice that in the batch reactor.

In addition to the expanded interfacial area, Colin et al. and Yuan et al. have reported that the segmented flow pattern formed when the organic and aqueous solutions were mixed at a Y-branch[10] also contributes to the acceleration of the PT reaction, because the efficient mixing inside each solution refreshes the reactants near the interfacial area.[11]

Herein, we report that the use of a microfluidic reaction system improves the reaction rate of N^3-acylation of uridine derivatives under PT conditions. We also show that the insertion of porous materials such as HPLC guard columns into the reaction tubing further increases the reaction rate.



Scheme 1 Phase-transfer N^3-acylation of uridine derivatives

The reactions were carried out by using the microfluidic system shown in Figure 1. We prepared the organic solution by dissolving compound **1** (0.05 M) and benzoyl chloride (1.3 equiv) in dichloromethane, and the aqueous solution by dissolving TBAB (0.01 M) in 0.2 M sodium carbonate solution. The flow system consisted of two plunger pumps and branch unit, linked to polyether ether ketone (PEEK) tubing. Initially, the length of the PEEK tubing was set to 260 cm, and the total flow rate, which is the sum of the flow rate of the organic solution and that of the aqueous solution, was set to 2.0 mL min^{-1}. Under these conditions, the reaction time (i.e., the residence time) was estimated to be 1.9 seconds. Under these conditions, we studied the reaction yields by changing the ratio of the flow rate of the aqueous solution and that of the organic solution (AO ratio) from 0.6 to 5.4. The reaction yield of conversion of **2a** into **3a** was determined by using ^{1}H NMR spectroscopic analysis to compare the residual amount of starting material **1** and product **3a** by reference to well-separated 5-H signals. We studied the effect of the AO ratio, because it has been reported that this ratio is an important factor affecting the reaction yields in microfluidic reactions.[14]

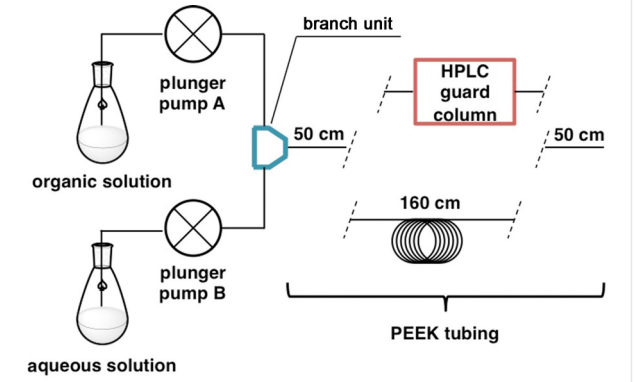


Figure 1 Microfluidic system used in this study

The results are summarized in Figure 2 (A). The highest reaction yield of 84% was obtained when the AO ratio was 5.4. As the AO ratio was decreased from 5.4 to 3.0 and 0.6, the reaction yield also decreased from 84 to 65, and 29%, respectively. Thus, the higher the AO ratio, the higher the reaction yield. These results are consistent with those reported previously by Schouten et al.[14] They reported that an increase in the AO ratio represents an increase in the surface-to-volume ratio of the organic solution when the flow pattern is slug flow. In our experiments, the flow pattern is expected to be slug flow in the PEEK tubing section of the reactor.

We also studied the effect of the length of the PEEK tubing by using tubes of different lengths (65, 130, 260, and 520 cm), with the AO ratio set to 3.0. The results are shown in Figure 2 (B). We expected that the use of longer PEEK tubing would lead to higher yields, because the reaction time is equal to the residence time during which the reaction mixture passes through the PEEK tubing. The reaction yields were found to be 59 ± 5.3%, 57 ± 6.7%, 65 ± 7.4%, and 71 ± 11.6% when the PEEK tube lengths were 65, 130, 260, and 520 cm, respectively [Figure 2 (B)]. Statistically, there was no significant difference between these yields. Thus, these results show that the yields are not affected by the length of PEEK tubing in this reaction, and that it is more important to optimize the AO ratio than to extend the reaction time (i.e., the PEEK tubing length) to obtain better yields.

To improve the yields, we inserted a porous material into the 50-cm-long PEEK tubing. Porous materials have been widely applied in microfluidic systems as catalysts[15] or reagents.[16] In this study, we applied a porous material to expand the interfacial area. The diameter of the PEEK tubing used in the above experiments was 0.25 mm. Therefore, if microporous or nanoporous materials are inserted within

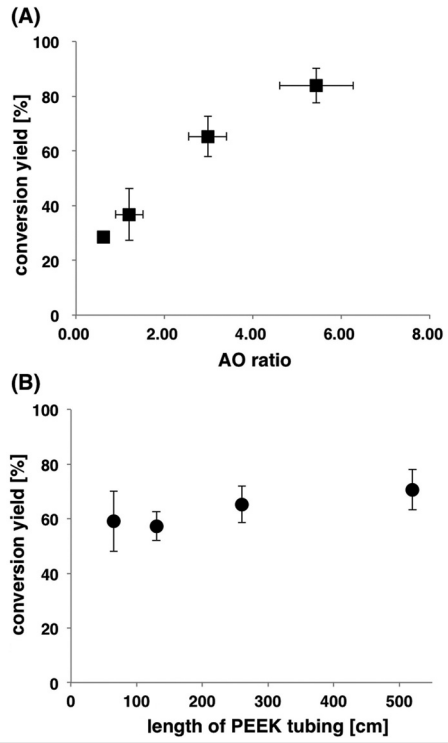


Figure 2 (A) Correlation between conversion yield and the AO ratio. (B) Correlation between the conversion yield and length of PEEK tubing. Error bars indicate standard deviations.

the PEEK tubing, the solutions are forced to pass through narrower pores, resulting in a significantly increased interfacial area. In addition, connecting additional materials changes the flow pattern of the reaction mixture, which could lead to more efficient mixing.

Six types of HPLC guard column with different surface modifications and pore sizes were chosen as the porous materials for these experiments. Details of the columns are shown in Table 1 and Table S1 (Supporting Information). As shown in Table 1, entry 1, the reaction yield was 65% when no guard column was inserted. As expected, the yield improved to 76% when an octadodecyl guard column with 10 nm pores was inserted (Table 1, entry 2). When the surface modification in the column was changed to hydrophilic carbamoyl groups, the yield increased further to 83% (Table 1, entry 3). This result could be because of the nature of the surface modification. Kitamori et al. reported that a hydrophobic surface allowed the organic droplets to merge with the aqueous droplets at the interface,[17] which resulted in a decrease in the AO ratio and a lower yield.

As shown in Table 1, entry 4, the inclusion of an octadodecyl guard column containing a mixture of nanoporous and microporous material (i.e., a nano-microporous material) was tested. The yield in this case was 82%, which is comparable to that of the hydrophilic carbamoyl column (entry 3). Interestingly, this result is better than that obtained with the 10 nm pore-sized octadodecyl column (entry 2), even though the surface modification in both cases is hydrophobic. Thus, the nano-microporous column may be more suitable for the microfluidic PT reaction. In addition, the surface modification by silanol shown in Table 1, entry 5, does not change the effect of the nano-microporous column. Notably, when the pore size of the materials was changed to 2 µm, the yield was comparable to that obtained with the nano-microporous column (entry 6). However, when the pore size was increased to 5 µm, the yield decreased to 74%. Although details of the mechanisms through which the surface modifications and the various sized pores change the yield are unclear, it is apparent that the insertion of an HPLC guard column improves the yields.

Table 1 Details of HPLC Columns and Reaction Yields

Entry	Surface modification	Pore size	Yield (%)
1	–	–	65 (±7.4)
2	octadodecyl	10 nm	76[a] (±4.5)
3	carbamoyl	10 nm	83[b] (±4.5)
4	octadodecyl	11 nm and 1.4 µm	82[b] (±3.0)
5	silanol	11 nm and 1.4 µm	83[b] (±4.7)
6	silanol	2 µm	82[b] (±2.8)
7	silanol	5 µm	74[a] (±1.9)

[a] p value ≤ 0.05
[b] p value ≤ 0.01.

We then examined the applicability of this microfluidic system to other reagents such as isobutyryl chloride (*i*-PrCOCl), toluoyl chloride (TolCl), and *p*-anisoyl chloride (AnCl). In these reactions, the same microfluidic system detailed in Table 1, entry 6, was used. The reactions with BzCl and TolCl gave the same yield of 80% (Table 2, entries 1 and 2). Conversely, AnCl (entry 3) afforded N^3-anisoylated product **3c** in 69% yield, which was lower than that obtained when BzCl and TolCl were used (entries 1 and 2). This is probably because of the relatively low reactivity of AnCl having a strongly electron-donating methoxy group in comparison to that of unsubstituted BzCl and TolCl, which have a less electron-donating methyl group.[18] The highest yield of 89% was obtained when *i*PrCOCl was used as acylating agent (entry 4). In addition, N^3-isobutyrylated compound **3d** was generated without forming intermediate 4-O-acylated compound **2d**. It should be noted that the benzoylation of 5-methyluridine derivative **1e** resulted in a low isolated yield of **3e** (25%; entry 5). This result is probably due to the steric effect of the 5-methyl group, which prevents benzoylation on the 4-O-position.

Table 2 Conversion and Isolated Yields of Reagents Using the Microfluidic System

Entry	Reagent	Product	Isolated yield (%)
1	benzoyl chloride	**3a**	80
2	toluoyl chloride	**3b**	80
3	*p*-anisoyl chloride	**3c**	69
4	isobutyryl chloride	**3d**	89
5	benzoyl chloride	**3e**	25

In conclusion, we have developed a new microfluidic method for the N^3-protection of uridine derivatives by the PT reaction, which was accelerated by the insertion of HPLC guard columns into the flow system.[19] We found that benzoylation succeeded with 83% conversion within a residence time (i.e., reaction time) of 1.9 seconds with the guard column, whereas the yield was 65% without the column. Our data clearly show the usefulness of attaching a microporous guard column to a microflow reaction system for the synthesis of N^3-protected uridine derivatives. This microfluidic method may be applied to other liquid–liquid PT reactions such as alkylation[7] and β-glycosilation.[20] As a potential application, a one-pot sequential synthesis[21] such as a 2′-O-modification following base protection using this microfluidic method is considered to be viable.

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Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0035-1560264.

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(19) **General procedure for the preparation of organic solution:** 3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)uridine (**1a**; 730 mg, 1.46 mmol) and an appropriate acid chloride (BzCl, AnCl, TolCl, *i*PrCOCl; 1.90 mmol) were dissolved in anhydrous dichloromethane (30 mL).

General procedure for the preparation of aqueous solution: Sodium carbonate (4.24 g, 40 mmol) and tetrabutylammonium bromide (645 mg, 2 mmol) were dissolved in degassed H_2O (200 mL).

Procedure for the synthesis of 3c: The flow system was constructed as shown in Figure 1 using a guard column of monolith 2 µm (Table 1 and Table S1, entry 6). The organic solution and the aqueous solution were flowed for 15 min with the flow rate of 0.43 mL min^{-1} and 1.5 mL min^{-1}, respectively. The organic solution was washed three times with saturated aqueous sodium bicarbonate and then dried over sodium sulfate. The resulting solution was evaporated under reduced pressure. The residue was dissolved in 1,2-dichloroethane (3 mL) and the solution was heated at 60 °C for 15 min. The resulting solution was evaporated under reduced pressure and then the residue was purified by column chromatography on silica gel (EtOAc–hexane, 20 to 30%) to give the product (149 mg, 69%) as a white powder. The analytical data of **3c**: ^1H NMR (500 MHz, $CDCl_3$): δ

= 7.88 (d, *J* = 8.5 Hz, 2 H), 7.78 (s, 1 H), 6.95 (d, *J* = 8.7 Hz, 2 H), 5.79 (d, *J* = 8.2 Hz, 1 H), 5.75 (s, 1 H), 4.36 (s, 1 H), 4.20 (d, *J* = 5.0 Hz, 2 H), 4.11 (d, *J* = 8.8 Hz, 1 H), 4.01 (dd, *J* = 13.3, 2.8 Hz, 1 H), 3.87 (s, 3 H), 2.87 (s, 1 H), 1.21–0.93 (m, 27 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 167.6, 165.5, 162.4, 149.2, 139.5, 133.3, 124.3, 114.8, 102.1, 90.9, 82.3, 77.5, 77.3, 77.0, 75.4, 69.1, 60.4, 55.9, 17.7, 17.6, 17.5, 17.5, 17.3, 17.2, 17.2, 17.1, 13.6, 13.2, 13.1, 12.7; HRMS (ESI): *m*/*z* $[M+Na]^+$ calcd for $C_{29}H_{44}N_2O_9Si_2Na$: 643.2478; found: 643.2476. Other procedures and the analytical data are described in the Supporting Information.

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