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Fully Automated Continuous Meso-flow Synthesis of 5′-Nucleotides and Deoxynucleotides

Chenjie Zhu,[†,‡] Chenglun Tang,[†,‡] Zhi Cao,[†,‡] Wei He,[†] Yong Chen,[†,‡] Xiaochun Chen,[†,‡] Kai Guo,[†] and Hanjie Ying*[,†,‡]

[†]College of Biotechnology and Pharmaceutical Engineering, Nanjing University of Technology, Nanjing 210009, China

[‡]National Engineering Technique Research Center for Biotechnology, Nanjing 211816, China

Supporting Information

ABSTRACT: The first continuous meso-flow synthesis of natural and non-natural 5′-nucleotides and deoxynucleotides is described, representing a significant advance over the corresponding in-flask method. By means of this meso-flow technique, a synthesis with time consumption and high-energy consumption becomes facile to generate products with great efficiency. An abbreviated duration, satisfactory output, and mild reaction conditions are expected to be realized under the present procedure.

INTRODUCTION

Nucleotides and their derivates are a well-established and important class of antiviral and anticancer drugs.[1] More recently, they have become the chemical centerpiece of the development of genetic therapies, biological probes, and modern DNA sequencing technologies, as well as investigations into the molecular mechanisms of chemical carcinogenesis and DNA repair.[2] Among them, 5′-nucleotides have been widely used in the pharmaceutical and food industries.[3,4] For example, inosine 5′-monophosphate and guanosine 5′-monophosphate are widely used in various foods as flavour potentiators due to their characteristic taste. 5′-Nucleotides are also conditionally essential dietary nutrients in infant formula, which are found to contribute to iron absorption in the gut and to influence desaturation and elongation rates in fatty acid synthesis, in particular long-chain polyunsaturated fatty acids, and have been proven to promote immune function and reduce diarrheal disease in infants.[5]

The widespread interest in 5′-nucleotides has promoted extensive studies on their synthesis. Generally, there are four methods for the synthesis of 5′-nucleotides: (1) fermentation using micro-organisms,[6] where, in this process, nucleotides are usually difficult to get through the cell membrane due to their strong polarity, thus increasing the difficulties of extraction; (2) the enzymatic method,[7] where microbial and plant nucleoside phosphotransferases (EC 2.7.1.77, NPase) are most widely applied as biocatalysts for the synthesis of nucleotides by the transfer of phosphate groups from organic phosphates to nucleosides; however, this method is usually limited by the narrow specificity of the enzyme and the reactions are either reversible or inhibited by the products, which need to use a gross excess of phosphate donor; (3) enzymolysis of RNA,[8] which is the most mature method today but still has some limitations, such as a long production cycle, a complicated separation and refinement process, high processing cost, and easy contamination; and (4) the chemical synthesis method,[9] in which this process can be divided into two stages, first, the use of phosphorylating reagent for the selective phosphorylation of the 5′-hydroxy of the nucleoside, and subsequently, hydrolysis of phosphoryl chloride to yield the corresponding 5′-nucleotides, such as Yoshikawa's procedure.[10] Many industrial chemical syntheses of 5′-nucleotides are performed in batch reactors. However, such processes suffer from several drawbacks from a practical point of view, including (i) a long reaction time, e.g., for the synthesis of uridine 5′-monophosphate, the reaction usually needs up to more than 20 h; (ii) low reaction temperature, i.e., usually subzero temperatures are required in order to suppress formation of side products both in the phosphorylation and hydrolysis process, especially in the latter, which is an extremely exothermic reaction and may cause safety risks; (iii) low efficiency due to start-up and shut-down procedures and batch-to-batch variations; (iv) excess amounts of phosphorylating reagent, where normally, more than 3 equiv are needed; (v) environmental pollution caused by the fuming of $POCl_3$, in which it hydrolyses in moist air, releasing phosphoric acid and choking fumes of hydrogen chloride. From an economic and environmental perspective, the development of a more efficient method for the chemical synthesis of 5′-nucleotides remains a high priority.

Recently, flow techniques and microreactor technology have drawn considerable attention in the fields of chemical synthesis, biologic, and medical sciences.[11] With the advantage of their high mixing and heat transfer rates, easy modulation, and safe operation, the technologies of microreaction are rapidly emerging as complements to traditional batch methods of organic synthesis.[12] Moreover, the potential to run multistage continuous reactions, an uninterrupted microreactor sequence, and easy number-up of microsystems for increasing the scale of production using continuous flow technology is also beneficial in terms of industrial production.[13] Recently significant advances in a flow synthesis of nucleosides[14] prompted us to use these useful tools to improve the synthesis of 5′-nucleotides.

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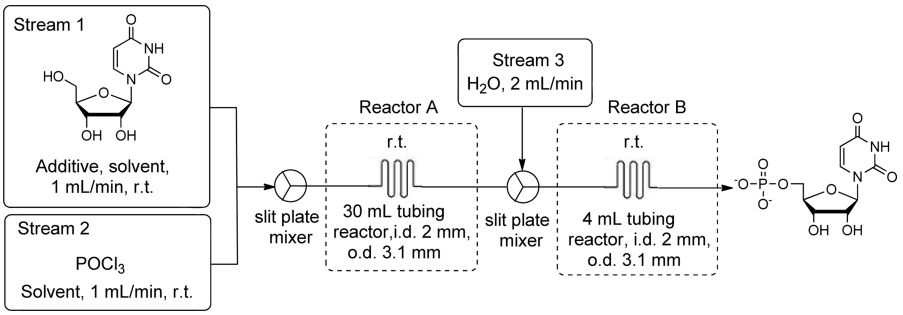

Figure 1. Process diagram for continuous meso-flow synthesis of a nucleotide.

In continuation of our efforts to explore green approaches for synthetic chemistry[15] and continuous interest in nucleic acid chemistry,[16] herein, we report the use of a meso-flow system for the rapid and clean synthesis of natural and non-natural 5′-nucleotides and deoxynucleotides via the combinations of multiple transformations into a single uninterrupted sequence. This strategy maximizes the speed and efficiency of synthesis by eliminating the need for low temperature, reducing excess amounts of phosphorylating reagent, and handling of the intermediates and allows for the drastic changes in reaction conditions.

RESULTS AND DISCUSSION

Initial experiments were carried out using uridine as a model substrate (see Figure 1). The selective phosphorylation of uridine has proven to be particularly difficult using Yoshikawa's procedure,[10] which may be due to the highest occupied molecular orbital energy of uracil.[17] One of the chief concerns in any fluidic experiment is the formation of solids in the reactor. Thus, a significant challenge of converting batch conditions into a flow process is the poor solubility of nucleosides in common organic solvents. Hence, we first examined the effects of solvents under the present meso-flow system (Table 1). The nature of the solvent plays an important role in the 5′-monophosphorylation reaction. It should dissolve all of the components of the reaction: unprotected nucleoside, phosphorylation reagent, and catalyst, and it should be dry, aprotic, and inert to the reagents. A range of polar solvents were tested for this reaction. However, all of them were unsatisfactory except for trimethyl phosphate (TMP) and triethyl phosphate (TEP) (Table 1, entries 1−2). The use of other trialkyl phosphates such as tributyl phosphate (TBP) was not successful (Table 1, entry 3), because the solubility of nucleoside is fairly low. When DMF or DMSO was used as a solvent (Table 1, entry 4 and 5), side product nucleotide 2′,3′-cyclic phosphate was formed via Vilsmeier reaction (Scheme 1). Recently, phosphorylation of nucleosides using acetonitrile as solvent was reported;[18] however, our attempts to dissolve uridine in acetonitrile failed (Table 2, entry 6). The Fischer group reported that reaction of adenosine with $PSCl_3$ is accelerated in pyridine as compared to reaction in triethyl phosphate due to activation of $PSCl_3$ by pyridine,[19] the use of pyridine as solvent in this reaction also did not show satisfactory results (Table 1, entry 7). The use of other aprotic solvents such as dioxane, sulfolane, and hexamethylphosphoramide were also unsuccessful. Among the solvents examined, trimethyl phosphate was found to be the most effective, which may interact with $POCl_3$ to form an active ionized phosphorylating agent[10] and has been suggested to accelerate phosphorylation of nucleosides.[17,20] For these reasons, trimethyl phosphate was selected as the solvent for the meso-flow synthesis system. The uridine−TMP solution cannot exceed a concentration greater than 0.95 M because of solubility issues; the final concentration of uridine was chosen to 0.4 M after parameter optimization (see Table S1 in the Supporting Information).

Table 1. Optimization studies for the meso-flow synthesis of uridine 5′-monophosphate[a]

entry	solvent	additive (mol %)	time (min)[b]	yield (%)[c]
1	TMP		25	73
2	TEP		25	67
3	TBP		25	43
4	DMF		25	27 (36[d])
5	DMSO		25	36 (29[d])
6	MeCN			
7	pyridine		25	51
8[e]	TMP	H_2O	25	47
9	TMP	DMAP	20	79
10	TMP	lutidine	20	78
11	TMP	pyridine	20	74
12	TMP	imidazole	20	76
13	TMP	triethylamine	20	71
14	TMP	proton sponge	15	85

[a]Reaction conditions: flow reactions were run in PFA (perfluoroalkoxy), 2.0 mm i.d. tubing reactor, residence time was controlled by adjusting the length of tubing reactor, flow rate: 1 mL/min, 1:1.5:1 molar ratio of uridine: $POCl_3$: additive, at room temperature unless specified otherwise. [b]Time refers to residence time in reactor A; time of hydrolysis process in the tubing reactor B was ca. 1 min. [c]Isolated yield based on uridine after purification using preparative HPLC. [d]The yield of 2′,3′-cyclic phosphate is shown in parentheses. [e]0.02 equiv of H_2O was added.

However, even using trimethyl phosphate as the solvent, as much as 10% total yield of 2′- and 3′-monophosphate were identified in addition to the product 5′-monophosphate according to the ^{31}P, ^{1}H NMR and LCMS spectrum.[21] In addition, trace amounts of depyrimidination product was also detected, which may be due to the hydrolysis of the glycoside bond under acidic conditions. The Yoshikawa group reported that the formation of 2′- or 3′-monophosphate could be markedly inhibited by the addition of a small amount of water to the reaction mixture.[10b] However, addition of 0.02 equiv H_2O to stream 1 of the meso-flow system gave a poor result (Table 1, entry 8). We reasoned that these side reactions might be promoted by HCl formed in situ during the reaction process and that the use of a base as an acid-neutralising agent might be beneficial for this reaction. Therefore, a range of bases were

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Scheme 1. Formation of 2′,3′-cyclic phosphate using DMF or DMSO as solvents

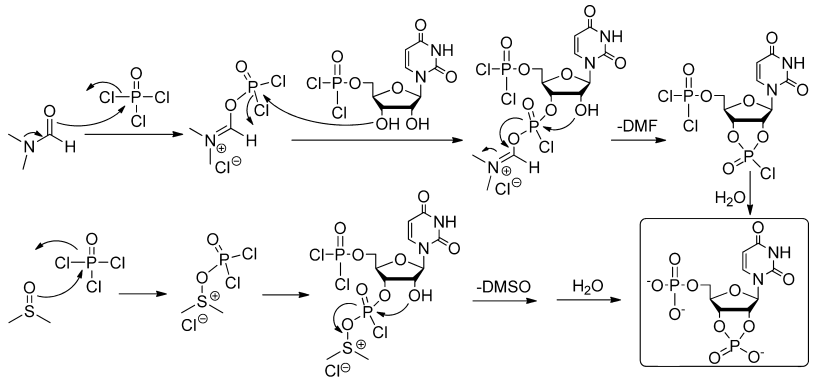

Table 2. Effect of aperture types and inner diameter[a]

entry	aperture type	inner diameter (mm)	yield[b]
1	50 μm−33°	2.0	85.6
2	50 μm−164°	2.0	86.1
3	100 μm−33°	2.0	86.9
4	100 μm−164°	2.0	87.3
5	300 μm−33°	2.0	88.1
6	300 μm−164°	2.0	89.2
7	300 μm−164°	0.75	91.4
8	300 μm−164°	0.5	92.7

[a]Reaction conditions: flow reaction were run in PFA tubing reactor, flow rate: 1 mL/min, 1:1.5:1 molar ration of uridine−$POCl_3$−proton sponge, residence time: 15 min, at room temperature unless specified otherwise. [b]Averaged data of three times.

tested for the reaction (Table 1, entries 9−14). Among the bases examined, proton sponge (*N*,*N*,*N*′,*N*′-tetramethyl-1,8-naphthalenedi-amine) was found to be the most effective (Table 1, entry 14); the reaction proceeded much faster, and the side reaction was strongly inhibited. A further study showed that proton sponge was not necessary for all reactions. For most nucleoside reactants, no addition of a base as catalyst was effective enough to complete the reaction (see Table 3). Encouraged by these results and in order to search for the optimum reaction conditions, we screened a variety of parameters of this reaction, such as concentration, residence time, flow rate, catalyst loading, molar ratio, and temperature (for more details see Table S1 in the Supporting Information).

Besides the presence of a base, another experimental parameter—"low reaction temperature"—has previously been regarded as critical for the step of the hydrolysis process.[10,17,18,21] As discussed above, hydrolyzation of phosphoryl chloride is an extremely exothermic reaction, which can bring the risk of explosion in a traditional batch facility. Maintaining the reaction temperature at a very low temperature (usually at subzero temperatures) is indeed essential in the batch procedure for safety concerns and inhibition of side reactions. One of the most significant features of flow technology is the extremely fast mixing by virtue of short diffusion path. Heat transfer is normally much more efficient in a flow system than in a conventional batch instrument due to the high surface-to-volume ratio. These features are quite advantageous for conducting extremely fast and highly exothermic reactions. Due to this advantage, the present meso-flow system can be operated at remarkably mild conditions in comparison with the batch process, viz., room temperature instead of subzero temperature.

It is well-known that, in the flow system, the reaction rate and yield are greatly restricted by mixing efficiency,[22] which is mainly affected by the micromixer. The slit plant micromixer LH25 used in this system works according to the multi-lamination principle.[23] The mixer is comprised of two microstructured plates is extremely versatile for a wide application in mixing and dispersing. The mixing device is adapted for various mixing tasks by easily adjusting the width and length of the circular slit in the aperture plate, expressed in the format "μm−°". Table 2 shows the effect of different aperture plates on the yield of uridine 5′-monophosphate production. As can be seen in Table 2, the yield increased with the size of aperture plates. Apart from the effect of different aperture plates, interestingly, we observed that the inner diameter of tubing reactor also has an important effect on the reaction. The yield of uridine 5′-monophosphate increases with decreasing the inner diameter of tubing reactor. These results may be explained that an increase in aperture would likely result in poorer mixing,[24] which would mean that the exotherm becomes mass transfer controlled and therefore is better controlled as more heat can be dissipated over a longer length of tubing. Narrow bore tubing also provides increased exotherm control by increasing the surface area to volume ratio, which promotes a more uniform laminar flow profile and hence increased yield.[14a,25]

By maintaining all of the key parameters of this meso-flow system, we applied the procedure for the synthesis of various of nucleotides (MFS in Table 3). The batch synthesis system was also applied for the purpose of comparison (batch in Table 3). As can be seen in Table 3, most unprotected nucleosides underwent smooth transformation to afford the corresponding nucleotides in moderate to excellent yields with this continuous meso-flow synthesis system (Table 3, entries 1−6). Excellent chemoselectivity was observed under this system, no noticeable overphosphorylated byproduct was detected. The reaction tolerates a variety of nucleobases including uracils, cytosine, and the purine bases guanine and adenine. It is noteworthy that xanthosine 5′-phosphate, which was not readily accessible by conventional batch method,[10a] was also obtained in excellent yield (Table 3, entry 6). To assess the feasibility of using this method on a preparative scale, this continuous flow system was then examined for the synthesis of cytidine 5′-monophosphate; as expected, the reaction proceeded smoothly, similar to the smaller-scale case, we obtained 35.7 g of the desired product in 5 h (92% yield, 7.14 g h^{-1}) after purification using our previous methods,[26] without any further optimization (Table 3, entry 2).

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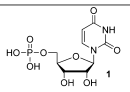
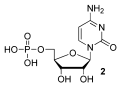
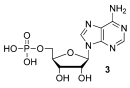
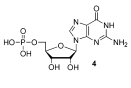
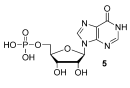
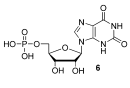
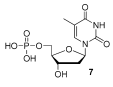
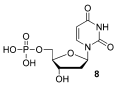
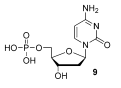
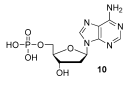
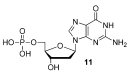
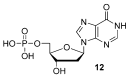
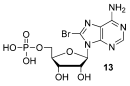
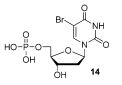
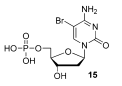
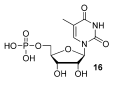
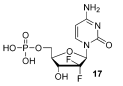
Table 3. Synthesis of nucleotides in a continuous meso-flow system and in batch

Entry	Products	Method	$POCl_3$ (equiv.)	T(℃)	Time (h)	Yield (%)[a]
1[b]		MFS	1.5	r.t.	0.25	89
		Batch	6.0	-5~0	12	78
2		MFS	1.2	r.t.	0.1	94(92[c])
		Batch	2.0	-5~0	3.0	89
3		MFS	1.2	r.t.	0.1	86
		Batch	3.0	-5~0	6.0	80
4		MFS	1.2	r.t.	0.1	88
		Batch	4.0	-5~0	6.0	82
5		MFS	1.2	r.t.	0.1	75
		Batch	3.0	-5~0	3.0	79
6[b]		MFS	1.5	r.t.	0.25	81
		Batch	6.0	-5~0	12	76
7		MFS	1.2	r.t.	0.15	76
		Batch	3.0	-5~0	6.0	72
8[b]		MFS	2.0	r.t.	0.25	85
		Batch	5.0	-5~0	12	72
9		MFS	1.2	r.t.	0.15	87
		Batch	3.0	-5~0	2.0	90
10		MFS	1.2	r.t.	0.15	72
		Batch	3.0	-5~0	6.0	65
11		MFS	1.2	r.t.	0.15	74
		Batch	3.0	-5~0	6.0	66
12		MFS	--	--	--	--
		Batch	3.0	-5~0	4.0	63
13[d]		MFS	2.0	r.t.	0.4	49(63[e])
		Batch	4.0	-5~0	12	38
14[b,d]		MFS	2.0	r.t.	0.4	58
		Batch	6.0	-5~0	12	36(18[f])
15[b,d]		MFS	1.5	r.t.	0.25	53(12[g])
		Batch	4.0	-5~0	6.0	46(17[g])
16[b]		MFS	2.0	r.t.	0.4	64
		Batch	6.0	-5~0	12	75[h]
17		MFS	1.5	r.t.	0.15	78
		Batch	3.0	-5~0	4.0	56

[a]Isolated yield using preparative HPLC unless otherwise noted. [b]Proton sponge was used in this reaction. [c]Isolated yield of continuous production in 5 h after purified by ion-exchange chromatography. [d]Yield was determined by LCMS using chlorpromazine as an internal standard due to its decomposition at room temperature. [e]Proton sponge (2 equiv) and phosphate buffer (0.2 M, pH 7.8) instead of H_2O in the stream 3 during the hydrolysis procedure was used. [f]Yield of 3′,5′,-diphosphate. [g]Yield of 5-Cl-dCMP. [h]Contaminated with cal. 16% of 2′,3′-cyclophosphate.

With the aim to develop and define the scope and limitation of the present method, this continuous flow system was then extended for the synthesis of deoxyribonucleotides (dNTPs). dNTPS are the essential building blocks for the synthesis of DNA molecules and have important therapeutic and diagnostic application. They have been utilized in various molecular biology applications such as PCR, real-time PCR, cDNA synthesis, primer extension, nick translation, DNA sequencing, and DNA labeling.[27] Notably, together with the good results with nucleosides, the yields obtained from the reaction of deoxyribonucleosides using this continuous flow system under similar conditions are also very high (Table 3, entries 7−11). However, the synthesis of dIMP under the present meso-flow system was not successful (Table 3, entry 12); solid formation and subsequent clogging in the tubing reactor occurred during the reaction, which is due to the poor solubility of 2′-deoxyinosine in the TMP even it was heated to 50 °C prior to flowing it into the reactor.

Recently, modified nucleotides attract growing interest due to their great potential for the investigation of important biological processes. Especially for the base-modified nucleotides, which have been used as inhibitors of therapeutically relevant proteins,[28] purine (or pyrimidine) receptor antagonists,[29] or as probes to investigate RNA structure and function.[30] Apart from the natural nucleotides, we further applied this continuous meso-flow system for the synthesis of non-natural modified nucleotides (Table 3, enties 13−17). The reaction of non-natural base-modified nucleosides is more complex compared to the reaction of natural nucleosides. For example, treatment of 8-bromoadenosine under the present procedure led to cleavage of the glycosidic bond and to a partial exchange of the bromo substituent with chlorine from $POCl_3$ (Table 2, entry 13), this observation is in agreement with an earlier report performed in batch.[18] After further optimizing the procedure, we found that increasing the amount of proton sponge and replacing H_2O with 0.2 M phosphate buffer (pH 7.8) in the stream 3 could avoid the depurination and halogen exchange reactions. In view of the fact that the reaction of base-modified nucleosides is much more difficult than the reaction of natural nucleosides,[18,28] the results obtained with the present procedure are also very satisfactory.

One of the applications of the present system is to synthesize 2′-deoxy-2′,2′-difluoro-5′-cytidylic acid (gemcitabine monophosphate). Gemcitabine is a synthetic nucleoside analogue of deoxycytidine with geminal fluorine atoms at the C-2′ carbon. This antineoplastic drug is currently marketed under the name Gemzar and registered as a first-line agent for the treatment of a number of solid tumor types including pancreatic, nonsmall cell lung (NSCL), ovary, bladder, and breast cancer.[31] Gemcitabine acts as an antimetabolite, inhibiting ribonucleotide reductase and DNA synthesis.[32] After entry into the cells, gemcitabine is first phosphorylated to its monophosphorylated form by deoxycytidine kinase. This primary phosphorylation by deoxycytidine kinase is the rate-

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limiting step for the activation of gemcitabine.[33] Poor phosphorylation due to low deoxycytidine kinase expression in the cells represents another important resistance mechanism limiting the activity of gemcitabine.[34] The direct delivery of the gemcitabine monophosphate into the tumour cells has been considered as a potential strategy for overcoming the rate-limiting primary phosphorylation step. This continuous meso-flow system was then examined for the synthesis of gemcitabine monophosphate. As expected, the reaction proceeded smoothly, the desired product 2′-deoxy-2′,2′-difluoro-5′-cytidylic acid was obtained in 78% isolated yield (Table 3, entry 17), which can be further used for the development of gemcitabine derivative lipidic prodrugs or encapsulation in colloidal drug delivery systems.[35]

■ CONCLUSIONS

In conclusion, with the aid of flow technology, a rapid, simple, clean, and efficient method for the synthesis of various natural and non-natural 5′-nucleotides and deoxynucleotides is presented. The advantages of this continuous meso-flow synthesis system are summarized as follows: (1) short reaction times (6−20 min), the period of most reactions are diminished about 30-fold compared to the batch system; (2) low energy consumption of refrigerants, room temperature in flow system vs subzero temperature in batch system; (3) high atom economy and environmental consciousness; $POCl_3$ was kept in a closed system from the beginning to the end to prevent its volatilization, and the amount of $POCl_3$ is greatly reduced compared to the batch system; (4) high reaction efficiency; this convenient two-step flow process simplifies the burdensome and protracted start-up and shut-down procedures and batch-to batch variations; (5) mild reaction conditions and simple operation; there is no need for rigorous exclusion of moisture to effect a clean transformation. Future work about further applications of this procedure are currently under investigation in our group.

■ EXPERIMENTAL SECTION

General Methods. All reagents and solvents were of commercial quality and used without further purification unless stated otherwise. $POCl_3$ was freshly distilled prior to use. MFS experiments were carried out in an experimental setup assembled with two micromixers (slit plate mixer LH25) and three medium pressure constant flow pumps. Thin-layer chromatography (TLC) was performed on precoated aluminum plates (silica gel 60 F_{254}), and the visualization of the spots has been done under UV light (254 nm) or stained with iodine vapor. Melting points were determined in an open capillary tube with a Mel-temp II melting point apparatus. ^1H, ^{13}C, ^{31}P, and ^{19}F NMR spectra were recorded at 400, 100, 162, and 376 MHz on a magnetic resonance spectrometer using D_2O as solvent unless stated. Chemical shifts are reported in parts per million (ppm). Coupling constants J (Hz) were directly taken from the spectra and are not averaged. Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). HRMS spectra were obtained from a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an Infinity cell, a 7.0 T superconducting magnet, an RF-only hexapole ion guide, and an external electrospray ion source (off axis spray) and with ESI(+)-MS and tandem ESI(+)-MS/MS using a hybrid high-resolution and high accuracy MicrOTOF-Q II mass spectrometer. Preparative chromatography was equipped with a peristaltic pump and a 254 nm UV Optics Module.

Typical Experimental Procedure for Meso-flow Synthesis of Cytidine 5′-Monophosphate. The streams of cytidine (0.4 M in TMP, stream 1) and $POCl_3$ (0.48 M in TMP, stream 2) were pumped into the slit plate mixer by two medium pressure constant flow pumps at 1 mL/min each without pressure regulation. The reagent streams were flowed past the reactor coil (reactor A: 12 mL volume, 2.0 mm i.d., 3.1 mm o.d. PFA tubing, 6 min residence time, at room temperature). The combined solution was then met with stream 3 (H_2O, stream 3 was set to 2 mL/min) in another slit plate mixer, which was connected with reactor B (reactor B: 4 mL volume, 2.0 mm i.d., 3.1 mm o.d. PFA tubing, 1 min residence time, at room temperature). Five reactor volumes (5 × 4 mL) were allowed to pass through the reactor in order to achieve steady state before sample collection. The reaction mixture (4 mL) was then collected into a vial equipped with a septum. The crude product was purified by preparative HPLC to give cytidine 5′-monophosphate as a white powder (0.121g, 0.377 mmol, 94%). The analytically pure product was then characterized by ^1H NMR, ^{13}C NMR, ^{31}P NMR, and HR-MS. ^1H NMR (400 MHz, D_2O) δ 8.18 (d, J = 7.4 Hz, 1H), 6.24 (d, J = 7.3 Hz, 1H), 5.90 (s, 1H), 4.23 (dd, J = 41.5, 10.8 Hz, 4H), 4.06 (s, 1H); ^{13}C NMR (100 MHz, D_2O) δ 159.25, 148.62, 143.95, 95.13, 89.57, 83.35, 74.32, 69.05, 63.54; ^{31}P NMR (162 MHz, D_2O): −0.04; HRMS (ESI, M^+): m/z calcd. for $C_9H_{14}N_3O_8P$ 324.0597 $[M + H]^+$, found 324.0593.

Typical Experimental Procedure for Batch Synthesis of Cytidine 5′-Monophosphate. Cytidine (1 mmol, 0.323 g) was added in dry TMP (15 mL); the solution was stirred for 10 min at room temperature and then cooled to −5 °C. $POCl_3$ (2 mmol, 182 μL) was added dropwise while maintaining the temperature at −5 to 0 °C. The reaction was stirred at −5 to 0 °C monitored by HPLC or TLC. Ice-cooled water (2 mL) was then added dropwise carefully while maintaining the temperature below 5 °C; the reaction was further stirred at 0 °C for 0.5 h. After completion, the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The water phase was freeze-dried and purified by preparative HPLC to give the desired product in 89% yield (0.287 g).

■ ASSOCIATED CONTENT

Supporting Information

Complete set of conditions for the reaction optimization and ^1H NMR, ^{13}C NMR, ^{31}P NMR, ^{19}F NMR and HRMS spectra of products. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: yinghanjie@njtech.edu.cn.

Present Address

H.Y.: Puzhu South Road No. 30, Nanjing, Jiangsu, China.

Notes

The authors declare no competing financial interest.

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