Continuous Flow

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One-Flow, Multistep Synthesis of Nucleosides by Brønsted Acid-Catalyzed Glycosylation**

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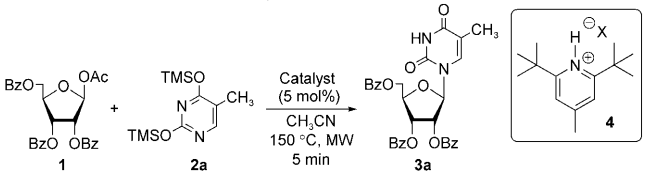
Nucleosides and their structural variants are a well-established and important class of antiviral and anticancer agents.[1] More recently, they have become the chemical centerpiece of the development of genetic therapies, biological probes, and modern DNA sequencing technologies, as well as investigations into the molecular mechanisms of chemical carcinogenesis and DNA repair.[2] Due to this broad utility, the development of more efficient methods for the synthesis of nucleoside analogues remains a high priority. Currently the most widely used method of ribonucleoside synthesis is the Vorbrüggen modification of the silyl-Hilbert–Johnson reaction.[3] This approach assembles the important nucleosidic bond by joining a glycosyl donor with a silylated nucleobase, under Lewis acid promotion.[4] A high-throughput two-step glycosylation/deprotection sequence for the synthesis of nucleoside libraries is a notable recent improvement.[5,6] Nevertheless, despite these advances the stoichiometric use of Lewis acids, e.g., trimethylsilyl triflate (TMSOTf), remains the state of the art. The limitations of this requirement are not insignificant and include the functional group compatibility of the Lewis acid and the quenching, separation, and disposal thereof, particularly on preparative scale.

We now introduce pyridinium triflate salts as efficient glycosylation catalysts for the synthesis of nucleosides and nucleoside analogues. Examples of TsOH-catalyzed glycosylations (in the melt) of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose by purines and other nucleophiles whose conjugated acids are of sufficient acidity have been described, but to the best of our knowledge this is the first general method of nucleoside formation (i.e., purines and pyrimidines) catalyzed by Brønsted acids.[7] Moreover, the melt/fusion conditions are not required for reactivity; our catalyst and the high reaction temperatures readily available to flow synthesis enable this new method and make it practical and expedient in organic solvents.[8] We further report the development of a general and scalable method for the continuous flow synthesis of nucleosides. An in-line deprotection step allows for one-flow multistep synthesis of unprotected nucleosides without intermediate isolation or purification operations.

An important goal at the outset of our studies was the discovery of a catalytic alternative to traditional Lewis acid glycosylation promoters. Continuous flow techniques and microreactor technology are rapidly emerging as complements to traditional batch methods of organic synthesis,[9,10] and we reasoned that the attributes of these tools would provide access to reaction conditions not generally feasible in batch. We also anticipated that an additional advantage of the use of a sub-stoichiometric quantity of the promoter would be that subsequent transformations could be incorporated into the flow system without additional quenching or purification operations.

A critical metric of any new catalyst for nucleoside synthesis would be throughput, i.e., high product yield in a short reaction time, in order to facilitate a single-pass continuous flow approach. Our initial results in batch (microwave irradiation) indicated that pyridinium triflates would satisfy these requirements. A catalytic amount (5 mol%) of several pyridinium triflates effected quantitative conversion and high yield in fewer than 5 min at 150 °C. As such, the commercially available, inexpensive ribofuranose **1** and silyl protected thymine **2a** could be combined to form the desired β anomer of nucleoside **3a** (Table 1, entries 3–6).

Table 1: Selection of the catalyst.[a]



	Catalyst	Yield [%][b]
1	none	0
2	trimethylsilyl triflate	91
3	pyridinium triflate	98
4	2,6-lutidinium triflate	96
5	2,4,6-collidinium triflate	97
6	**4a** (X = triflate)	98
7	**4b** (X = methanosulfonate)	0
8	**4c** (X = trifluoroacetate)	0
9	**4d** (X = chloride)	0
10	pyridinium *p*-toluenesulfonate	0
11	ethyldiisopropylamine triflate	22[c]

[a] Conditions: 0.2 mmol of **1**, 0.22 mmol of **2a**, 1.5 mL of CH_3CN, 5 mol% of catalyst in 0.5–2 mL MW vial (Biotage initiator). Ac = acetyl, Bz = benzoyl, TMS = trimethylsilyl. [b] Yield of isolated product. [c] Recovered 71% of **1**.

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We discovered that both the pyridinium cation and triflate counterion play essential roles in this process. Other pyridinium salts derived from alternative acids failed to show any conversion under these conditions (Table 1, entries 7–10). Moreover, triflate salts derived from alkylamines afforded inferior results; for example, ethyldiisopropylamine provided **3a** in only 22% yield (Table 1, entry 11).

For practical reasons the easy-to-handle salt of 2,6-di-*tert*-butyl-4-methylpyridine was preferred, but for large-scale preparative experiments (see below) we would suggest the use of less expensive and commercially available pyridinium salts, such as pyridinium triflate itself.

It should be noted that even though TMSOTf gave satisfactory yield under microwave conditions (Table 1, entry 2), the flow variant was not feasible to run due to decomposition of the ribofuranose and subsequent clogging of the reactor (see Supporting Information for details). Moreover, even with the use of 1 equivalent of pyridinium triflate, no conversion was observed after 20 h under otherwise identical batch conditions at room temperature, indicating that the high temperature provided by flow synthesis would be necessary for this new catalyst system to be of any practicality.

A significant challenge of converting the batch conditions into a continuous flow process related to the poor solubility of persilylated heterocyclic bases in CH_3CN and required careful design of the reactor setup.[11] We found it was necessary to heat the bases (**2a–i**) to 40 °C prior to flowing them into the reactor in order to prevent their precipitation. In addition, the T-mixer where both reagent streams meet had to be held at 100–150 °C (the reaction temperature) in order to prevent solid formation and subsequent clogging (see the Supporting Information for details of the reactor setup).

The results of preparative scale batch and continuous flow experiments are shown in Table 2. The reaction tolerates a variety of nucleobases including 5-substituted uracils (**2a–f**), cytosine (**2g**), and the purine bases guanine (**2h**) and adenine (**2i**). Notable features of the reaction include low catalyst loadings (typically 5 mol%), short reaction times (0.5–20 min), and high yields following off-line purification (80–99%, with six out of nine examples ≥95%).

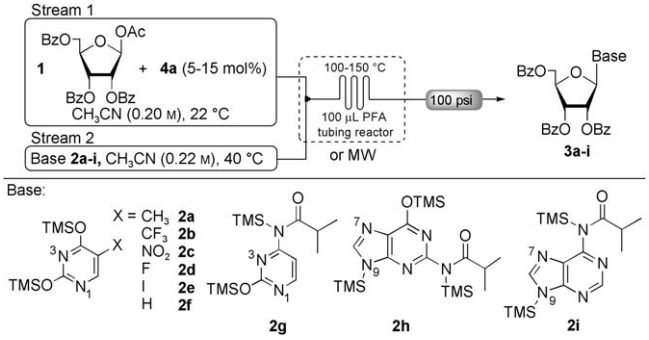
Differences in the required reaction time for the various nucleobases can be attributed to reversible σ-complex formation between the silylated bases and the pyridinium catalyst (weak Brønsted acid).[12] This trend is clearly seen in the series of substituted uracils **2a–c** where reaction time and temperature can be correlated to the basicity of the pyrimidines (**2a** > **2b** > **2c**). In the case of uracils with small substituents in the 5-position (Table 2, entries 4 and 6) and cytosine (Table 2, entry 7), N1,N3-bisnucleoside by-products were observed in both batch and flow experiments. It was also found that the tubing diameter had significant impact on the formation of bisalkylation by-product.

Interestingly, we observed that when tubing with a larger inner diameter (0.75 mm) was used, the amount of bisalkylation by-product increased (and correspondingly the yield of the desired product decreased) when compared to the related batch experiment. These anomalous results could be overcome by simply employing a reactor with a smaller inner diameter (0.5 mm) which promotes a more uniform laminar flow profile and hence increased accuracy of the residence times (see Supporting Information for details).[13] In the case of cytosine, guanine, and adenine (**2g–i**), residual bis(trimethylsilyl)acetamide (BSA) from the silylation step had no negative influence in the glycosylation reaction.

To demonstrate the scalability of the reaction we directly transferred the optimized conditions to the commercially available flow system from Vapourtec (Scheme 1).[14] This reactor comprises two independent HPLC pumps that deliver substrate solutions from reservoirs into the reactor. Mixing occurs in a T-joint connector and the resulting solution is then pumped into a 2 mL PFA tubing (1 mm i.d.) reactor that is heated in a convection chamber.

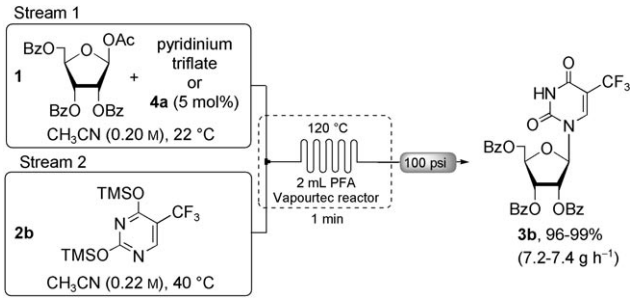
By maintaining all the key parameters from the small-scale flow reactor (concentration, catalyst loading, residence time, and temperature), we obtained 26 g of **3b** (7.4 g h^{-1}) in 3.5 h, without any further optimization. The yield of **3b**

Table 2: Synthesis of nucleosides in batch and continuous flow.



	Base	Method[a]	**4a** (mol%)	*T* [°C]	*t*[b] [min]	Yield[c] [%]
1	**2a**	MW	5	150	3	99
		flow	5	150	5	96
2	**2b**	MW	5	120	1	99
		flow	5	120	1	99
3	**2c**	MW	5	120	0.5	96
		flow[d]	5	120	0.5	99
4	**2d**	MW	10	100	10	85 (10, 3)
		flow[d]	10	100	10	87 (9, 0)
5	**2e**	MW	5	120	1	99
		flow	5	120	3	99
6	**2f**	MW	5	120	5	85 (12, 3)
		flow[d]	10	100	20	88 (11, 0)
7	**2g**	MW	5	150	10	83 (11, 0)
		flow[d]	15	120	5	80 (6, 14)
8	**2h**	MW	5	150	5	95[e]
		flow	5	150	5	95[f]
9	**2i**	MW	5	150	3	99
		flow	5	150	5	95

[a] MW method: 0.2 mmol of **1**, 0.22 mmol of **2a**, 1.5 mL of CH_3CN, catalyst in 0.5–2 mL MW vial (Biotage Initiator). Flow method: Flow reactions were run in 100 µL PFA (perfluoroalkoxy), 0.75 mm i.d. tubing reactor unless specified otherwise. [b] Time of MW reaction refers to hold time (temperature ramp time ca. 1 min and cooling time ca. 45 s); time of flow reaction refers to residence time in the PFA tubing reactor. [c] Yield of isolated product, numbers in parenthesis refer to N1,N3-bisnucleoside by-product and recovered ribofuranose, respectively. [d] 100 µL PFA, 0.5 mm i.d. tubing reactor. [e] Mixture of isomers N9:N7 4.5:1. [f] Mixture of isomers N9:N7 4.7:1.



Scheme 1. Large-scale synthesis of **3b** using the Vapourtec R2 + /R4 flow system.

(99%) was consistent with that attained using the microscale flow reactor. Under similar conditions, reaction with pyridinium triflate (5 mol%) as a catalyst afforded **3b** in 96% yield.

Finally, we turned our attention to the development of a one-flow, multistep synthesis of fully deprotected nucleosides. Such a telescoping strategy is a very effective tactic for truncating a multistep synthesis, particularly when conducted in a continuous flow manner.[15] It eliminates the need for purification and isolation and allows for the drastic changes in reaction conditions from one step to the next.[16] Typically, deprotection of perbenzoylated ribonucleosides is achieved with methanolic ammonia, however, these conditions usually require long reaction times that are generally unsuitable for continuous flow synthesis.[3a,b] Nevertheless, taking advantage of the "extreme" reaction conditions that can be employed in microreactors, we first attempted the single-step ammonolysis of **3i** using methanolic NH_3 (75 equiv, 7 N) at 200 °C for 10 min.[17] However, even under these forcing conditions, the deprotection of **3i** was incomplete, providing adenosine (**5i**) and 5′-*O*-benzoyladenosine in 53 and 33% yields, respectively.

A transesterification approach to benzoyl deprotection using ethanolic sodium ethoxide solved this problem. Toward this end, the reactor configuration depicted in Scheme 2 was assembled, and after minimal optimization, the continuous and uninterrupted two-step process (glycosylation; deprotection) afforded adenosine (**5i**) in 98% overall yield (reactor A: 150 °C, 5 min; reactor B: 75 °C, 12 min). Unfortunately, these reaction conditions proved unfeasible for the synthesis of **5b** due to extensive precipitation of the product in the proximal part of reactor B. Recognizing that the debenzoylation of the ribofuranose was most likely very fast at 75 °C (and that product **5b** has limited solubility in ethanol), we anticipated that lowering the rate of the deprotection might improve the solubility profile of **5b**. By lowering the reaction temperature to 40 °C and increasing the residence time to 20 min, solid formation was minimized and **5b** was obtained in 81% yield (reactor A: 120 °C, 8.3 min; reactor B: 40 °C, 20 min).[18] Utilizing methanolic NaOMe (0.15 M) completely eliminated precipitation of the product in reactor B allowing nucleosides **5b** and **5e** to be successfully obtained in 94 and 93% respectively (reactor A: 120 °C, 3 min; reactor B: 50 °C, 8 min).

Using these conditions, adenosine was also obtained in 95% yield indicating that this continuous one-flow multistep sequence will be of general use for the synthesis of nucleosides.

In summary, the first general method of Brønsted acid-catalyzed nucleoside synthesis described herein was made possible by and is practical because of the flow reaction format. The glycosylation catalysts of choice in this rapid, high-yielding, continuous, one-flow, multistep synthesis of nucleosides are pyridinium triflates, e.g., 2,6-di-*tert*-butyl-4-methylpyridinium triflate. This process was scaled easily and without further optimization using a commercial flow synthesis instrument. By telescoping the glycosylation and deprotection steps into a single, continuous and uninterrupted reactor network, thereby circumventing the need to isolate and purify the intermediate product, the synthesis of nucleosides has been significantly streamlined.

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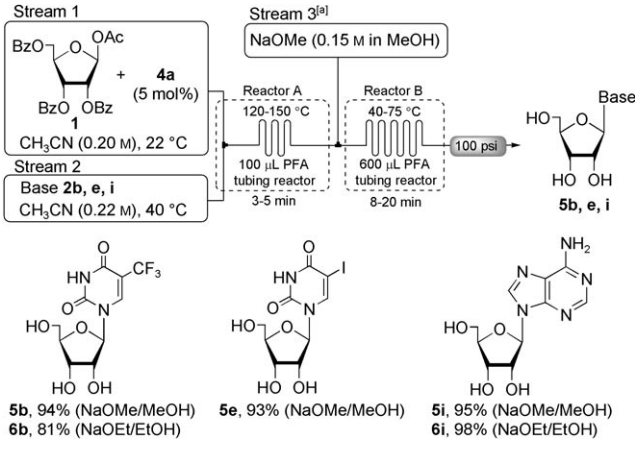

Scheme 2. One-flow multistep synthesis of nucleosides **5**. [a] In the case of **3b** and **3l**, NaOEt (0.125 M in EtOH) can also be used.

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[11] TMS-protected uracils can be stored under argon at room temperature for several weeks, whereas TMS protection of cytosine, adenine, and guanine should be performed immediately prior to glycosylation.

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[17] Conducted using an HPLC pump in conjunction with a stainless steel tube reactor equipped with a 500 psi back pressure regulator.

[18] Even though a fine suspension in reactor B appeared over time, no signs of coating the internal surface of the reactor were observed, thus allowing a continuous flow operation.

