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Semi-continuous multi-step synthesis of lamivudine

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Abstract

We report the first continuous flow synthesis of lamivudine, an antiretroviral drug used in the treatment of HIV/AIDS and hepatitis B. The key intermediate (5-acetoxy oxathiolane) was prepared by an integrated two step continuous flow process from L-menthyl glyoxalate hydrate in a single solvent, in 95% overall conversion. For the crucial glycosidation reaction, using pyridinium triflate as the novel catalyst, an improved conversion of 95% was obtained. The overall isolated yield of the desired isomer of lamivudine (40%) was improved in the flow synthesis compared to the batch process.

Keywords

Continuous flow synthesis, lamivudine, *N*-glycosidation, micro reactor, pyridinium triflate.

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Introduction:

Continuous flow technology offers many potential advantages compared with traditional batch manufacturing of pharmaceuticals, such as easier reaction optimization and greater control of the process, improved safety and environmental profiles, as well as a reduced manufacturing footprint.[1-3] Pharmaceutical companies, fine-chemical producers, and academia are pursuing continuous flow chemistry in the production of APIs and related products with several interesting developments in this field are emerging. Recently, the safe manufacturing of organic intermediates and APIs under continuous flow conditions has been examined in a review by Kappe and co-workers.[4] As pointed out by the authors, some synthetic steps that were not permitted for safety reasons (*e.g.* use of potentially toxic or explosive intermediates, reactions run under high pressures or above the boiling point of the solvent) can now be performed under flow conditions with minimum risk. For these reasons, flow chemistry can be seen as a novel technology that opens the way for new synthetic routes of valuable molecules.

Nucleosides and their analogues are a well-established and important class of antiviral and anticancer agents.[5] Due to the broad applicability of these compounds, the commercial development of more efficient methods for the synthesis of these drugs remains of high importance. For example, despite a large number of methods in the literature used to prepare antiretroviral drugs such as lamivudine **1** and emtricitabine **2** greener and more efficient processes for the synthesis of such generic drugs is highly desired. Herein, we report the synthesis of lamivudine **1** using flow chemistry; to the best of our knowledge, this is the first general synthesis of lamivudine using continuous flow technology. Lamivudine displays potent activity against HIV-1 and HIV-2 and in the last decade has been one of the most successful drugs for the treatment of HIV as well as chronic hepatitis B.[6] Since the chemical structure of **1** has two chiral centres, it has four stereoisomers of which the (2*R*,5*S*)-isomer (as illustrated in Figure 1) is the most potent in anti-HIV and anti-HBV activities, and importantly its cytotoxicity on cells is lower than its enantiomer.

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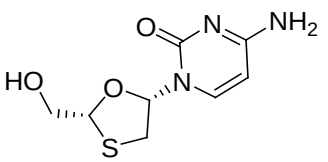
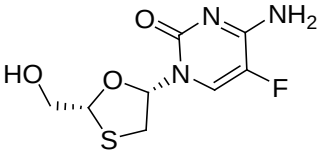


1 (3TC)
Lamivudine



2 (FTC)
Emtricitabine

Figure 1 The structures of oxathiolane nucleoside, lamivudine **1** and emtricitabine **2**.

Efficient syntheses of lamivudine, a drug that is still inaccessible to millions of people in developing countries worldwide, holds great potential both scientifically and socially.[7] This potent nucleoside reverse transcriptase inhibitor (NRTI) is one of the preferred agents used in combination therapy for the first-line treatment of HIV.[8] Although there are quite a few methods stated for the synthesis of lamivudine **1** as a single enantiomer,[9] a novel more efficient route which is suitable for large-scale local manufacture is still needed. Recently, we developed an improved batch method for the synthesis of lamivudine[10] and are now transferring the results into a flow synthesis. South Africa does not have batch manufacturing technology; if batch production was to be implemented the whole infrastructure would have to be established. The fact the flow technology is now well established (here and in many cases) to produce products in higher yield, and in many cases at reduced cost, this is how the process would be implemented locally. The approach of large scale manufacture has been reported in many industrial examples, so the issue of scale up in flow is not problematic.[1-3]

Results & Discussion:

Continuous flow synthesis to 5-acetoxy oxathiolane:

A significant challenge in transferring the batch protocol into a continuous flow process was related to the poor solubility of one of the starting materials, namely 1,4-dithiane-2,5-diol **4**. Initially, we tried to dissolve both starting materials (**3** and **4**) in acetonitrile and discovered that although the menthyl glyoxalate **3** was freely soluble the 1,4-dithiane-2,5-diol **4** was not. We found it necessary to heat the 1,4-dithiane 2,5-diol **4** solution to 40 °C prior to it being fed into the microreactor channel. After preparation of both starting materials **3** (0.43 M) and **4** (0.22 M) in acetonitrile, they were pumped into a 2 mL microreactor using syringes (10 mL), with a flow rate of 0.05 mL/min each (20 min residence time). The microreactor temperature was initially maintained at 110 °C for preliminary screening experiments (Figure

2). The reaction mixture exited the reactor *via* a 10 PSI back pressure regulator, to afford intermediate **5** in 88% conversion.

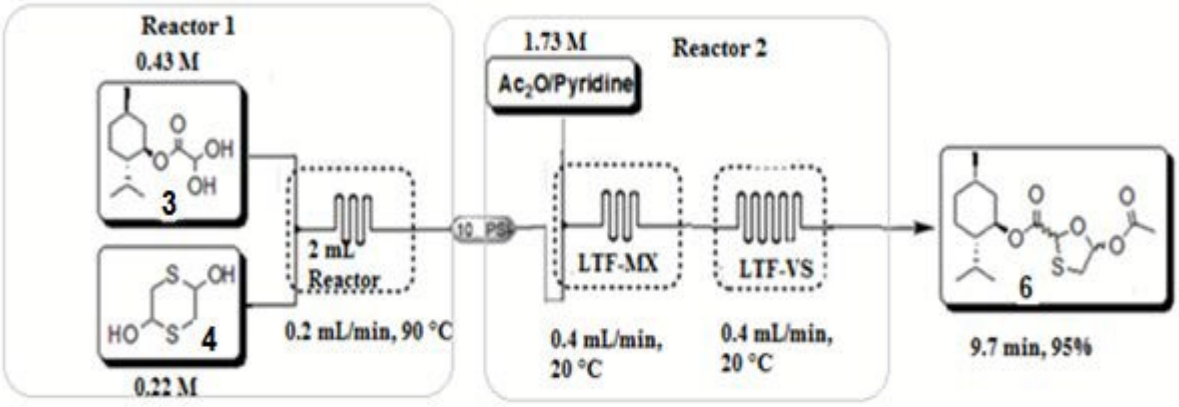


Figure 2 Flow diagram of the synthesis of 5-acetoxy oxathiolane.

After successfully completing the first step, the intermediate 5-hydoxy oxathiolane **5** (0.34 M) was reacted with pyridine and acetic anhydride in acetonitrile (2.17 M) at ambient temperature using two Little Things Factory reactors (0.2 mL LTF-MX + 1.7 mL LTF-V) totalling up to a reactor volume of 1.9 mL to provide the isomer mixture **6** in 95% conversion, which upon recrystallization at -20 °C yielded the desired isomer **7** (48%).

With the knowledge that both steps were feasible, we turned our attention to the development of a telescoped continuous flow synthesis for the two reaction steps (Figure 2). The first two reagents (menthyl glyoxalate hydrate (0.43 M) and 1,4-dithaiane 2,5-diol (0.22 M) in acetonitrile) were pumped into a 2 mL glass reactor (at 110 °C) and the outlet of this reaction was connected to another LTF-MX reactor (0.2 mL, at RT) which was connected to another syringe pump with pyridine and acetic anhydride in acetonitrile (2.17 M). The solution was passed through a LTF-V (1.7 mL, at RT) reactor and the product **6** was collected. Such a telescoping strategy is a very effective tactic for truncating a multistep synthesis, particularly when it is to be performed in a continuous flow manner as it eradicates the need for isolation and purification and allows for drastic changes in reaction conditions from step one to the next.

In this combined two-step continuous flow process, reaction parameters such as residence time, the temperature of the two reaction steps and concentration of the reagents were subsequently investigated to order to maximize the efficiency of the process. We started by determining the appropriate residence time for the continuous flow synthesis of compound

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6 from compounds 3 and 4 at predetermined reaction temperatures and concentrations of 3, 4 and acetic anhydride/pyridine.

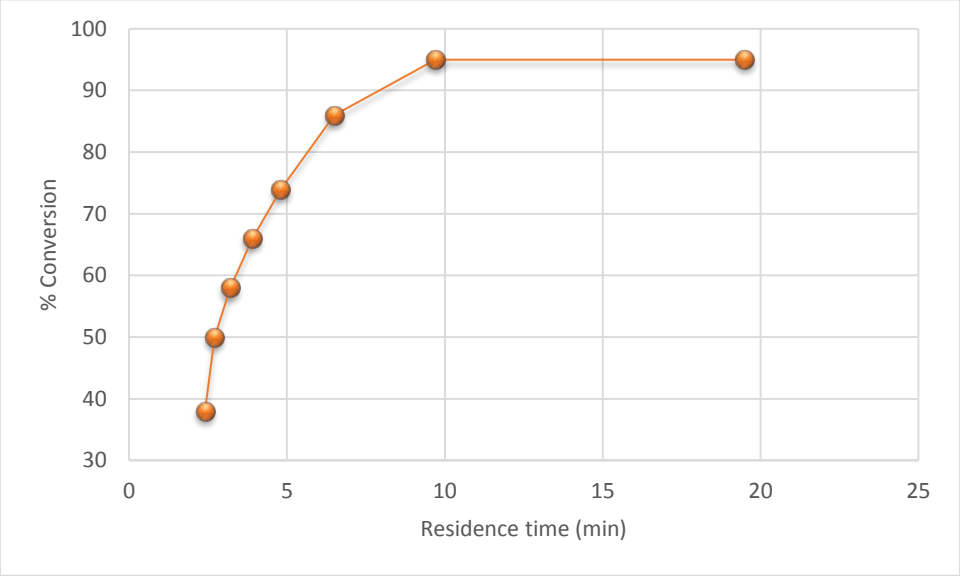

Figure 3 Effect of residence time on conversion to **6** at 0.43 M of **3**, 0.22 M of **4**, 2.17 M of Pyridine/Ac_2O and temperatures (reactor 1 at 90 °C, reactor 2 at 20 °C).

It was found that as the residence time increases, the conversion to **6** increased. The data in the graph shows that it is possible to use reaction times greater than 9.7 min (Figure 3) to achieve maximum conversion, however, the goal is to use as little time as possible in order to gain maximum throughput from the reactor per unit time. As such if one transferred these conditions into an industrial scenario you would select the minimum time of 9.7 min.

Having determined the optimum residence time for the two-step reaction being 9.7 min at the above-mentioned conditions, we then decided to carry out a detailed temperature study on the two reaction steps at this residence time; we started our investigation by varying the temperature of the reaction from 70 °C to 120 °C.

The effect of temperature on the conversion to **6** is illustrated in Figure 4. In summary, it was found that the maximum conversion (95%) was obtained at 90 °C. Then we investigated the temperature of the second step, where the maximum conversion was observed in the range 20-30 °C (Figure 5); by-products were observed at higher temperatures.

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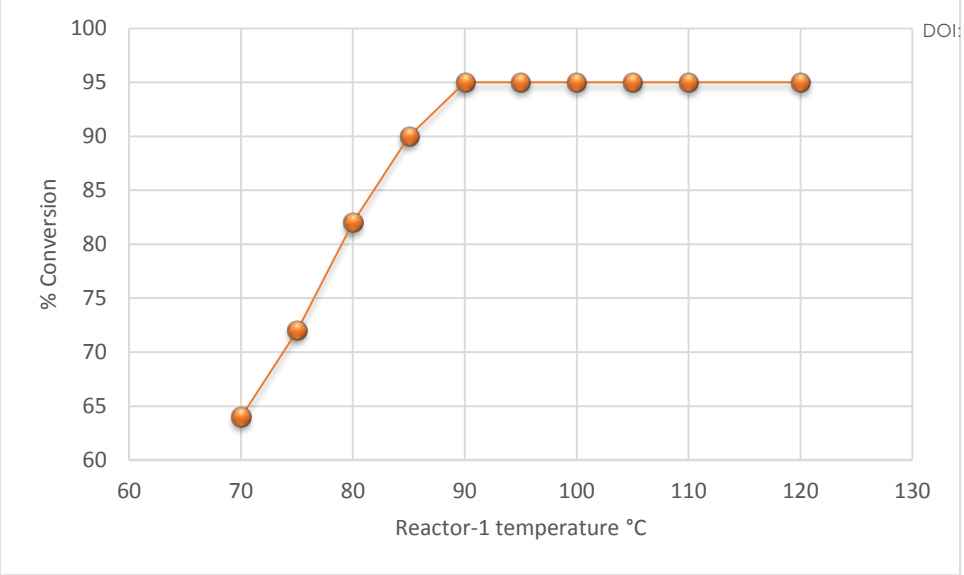

Figure 4 Effect of reactor **1** temperature variations on the conversion to **6** at constant residence time 9.7 min and at 0.43 M of **3**, 0.22 M of **4** and 2.17 M of Pyridine/Ac_2O in CAN.

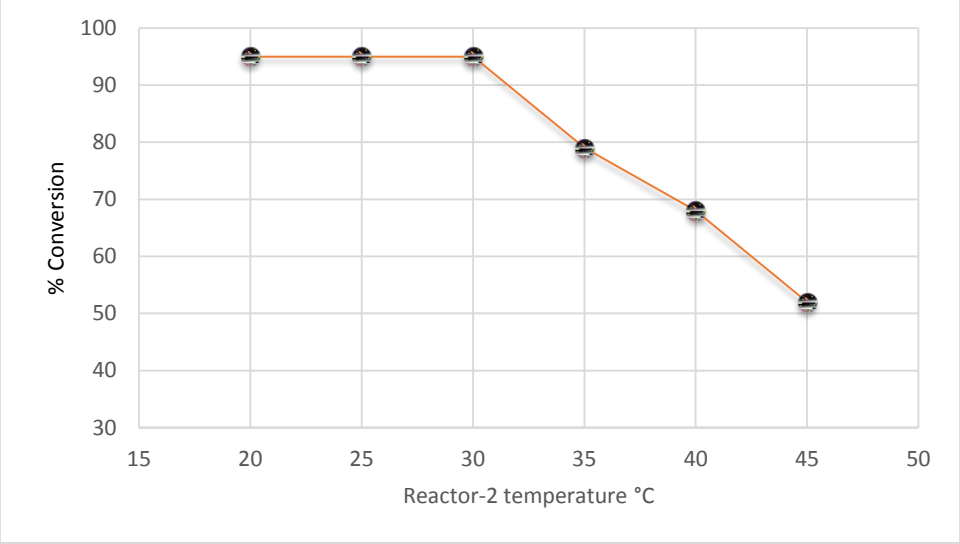

Figure 5 Effect of reactor **2** temperature variations on the conversion to **6** at constant residence time 9.7 min, reactor **1** temperature 90 °C, at 0.43 M of **3**, 0.22 M of **4** and 2.17 M of Pyridine/Ac_2O in acetonitrile.

The residence time (9.7 min) and temperatures of reactors 1 (90 °C) and 2 (20 °C) were kept constant; subsequently, we decided to investigate the concentrations of compound **4** and pyridine/Ac_2O solutions in acetonitrile. We screened different concentrations of compound **4** (0.22 M to 0.41 M) but we found that the conversion was unchanged. Then we turned our

attention to investigating the concentration of Ac_2O/pyridine solution (2.17 M to 1.07 M) and found that over the range 1.73-2.17 M the maximum conversion (95%) remained constant (Figure 6).

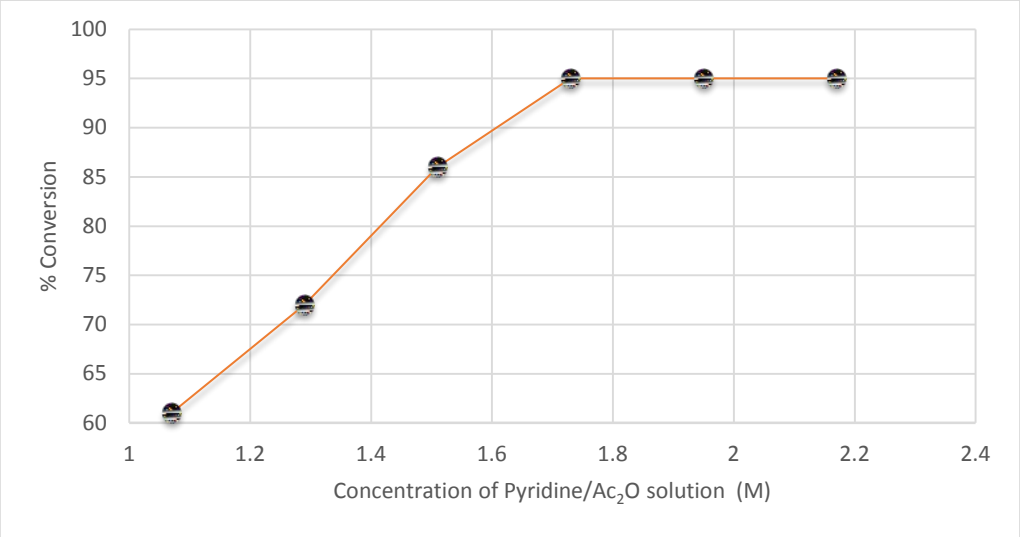

Figure 6 Effect of concentration variations of Pyridine/Ac_2O solution on the conversion to **6** at constant residence time 9.7 min temperatures (90 °C & 20 °C) at 0.43 M of **3** and 0.22 M of compound **4**.

In summary, the optimal condition for flow synthesis of 5-acetoxy oxathiolane **6** is 9.7 min residence time, temperature (reactor one 90 °C, reactor two 20 °C) and concentrations (menthyl glyoxalate hydrate 0.43 M, 1,4-dithane 2,5-diol 0.22 M and Ac_2O/pyridine solution 1.73 M). But when compared with the batch process[10] (reaction time 12 h, temperature 110 °C and concentrations of Ac_2O and pyridine solution 2.17 M) continuous flow gives better results in shorter time. The above detailed optimization conditions, with yields, is shown below (Scheme 1).

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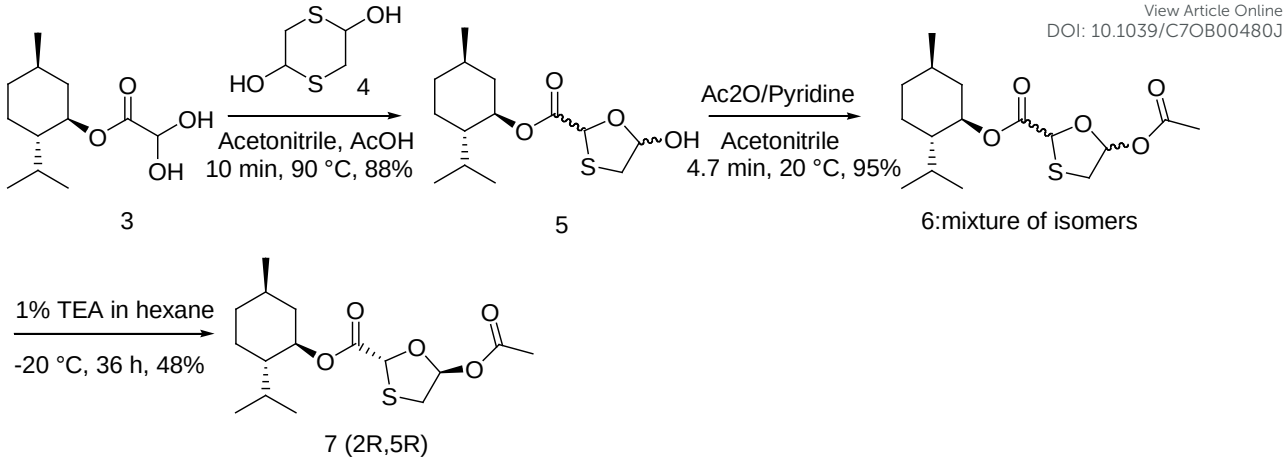

Scheme 1 Synthesis of (2*R*,5*R*)-5-acetoxyoxathiolane **7**.

N-Glycosidation reaction in flow synthesis:

Another substantial challenge in this work is the *N*-glycosidation reaction[11] due to the poor solubility and highly precipitating nature of the silylated nucleobase **8**. The cytosine was silylated with BSA in acetonitrile at 80 °C for about 1 hr in a batch process, after which the solution became clear. This indicated the formation of silylated product **8**. Syringe pumps were subsequently used to deliver the solutions of nucleobase **8** (0.30 M) and a solution of pure isomer 5-acetoxy oxathiolane **7** (0.30 M) and pyridinium triflate (0.30 M) in acetonitrile (Figure 7). Firstly 5-acetoxy oxathiolane **7** and pyridinium triflate were pumped into a 0.2 mL LTF-MS reactor at 20 °C and the product line was subsequently connected to a T-piece mixer where the third syringe delivered the nucleobase **8**, where they reacted at an elevated temperature (80 °C) in PTFA tubing reactor (3.8 mL volume, 0.8 mm i.d.). The isolated products were recrystallized to yield **9** (95%). One experimental detail of significance regarding silylated nucleobase **8**; in order to prevent precipitation and subsequent clogging at the syringe we used thermal controller wrapping to keep the syringe warm (55-60 °C).

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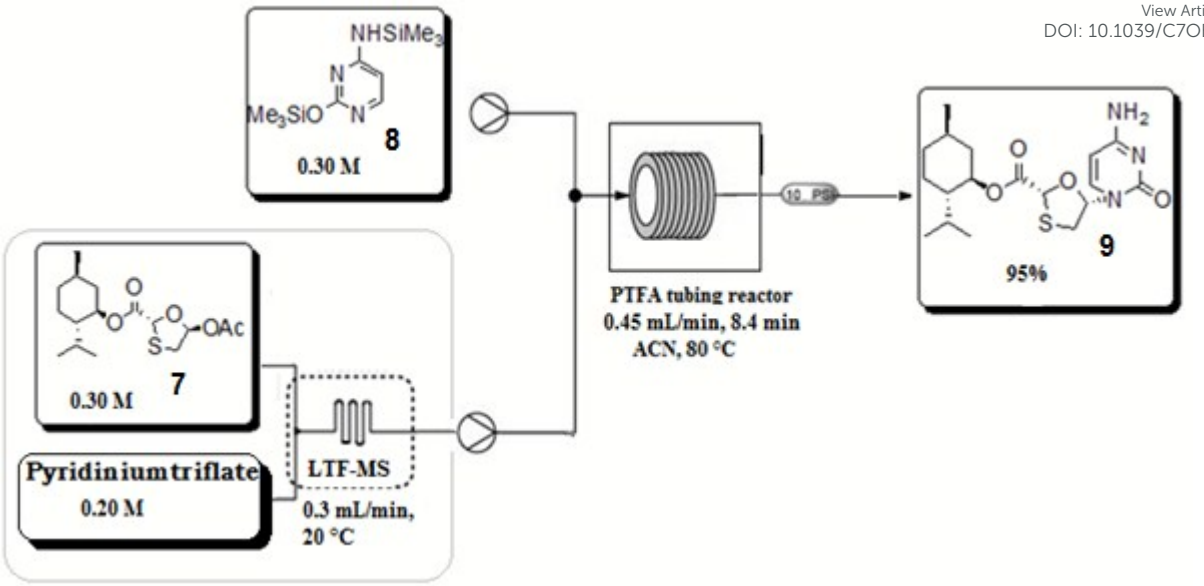

Figure 7 Flow diagram of glycosylation reaction.

After preliminary confirmation of the glycosidation reaction in flow, we further explored the effect of residence time, temperature, and concentration on the conversion of **9**. A residence time investigation on the conversion of **9** was chosen as a first study, where all the reagents concentrations were maintained at 0.30 M and temperature fixed at 80 °C. In this reaction, it was found that as the residence time increases, the conversion of **9** increased (Figure 8). The maximum conversion was observed at a residence time of between 8.4 and 12.6 min.

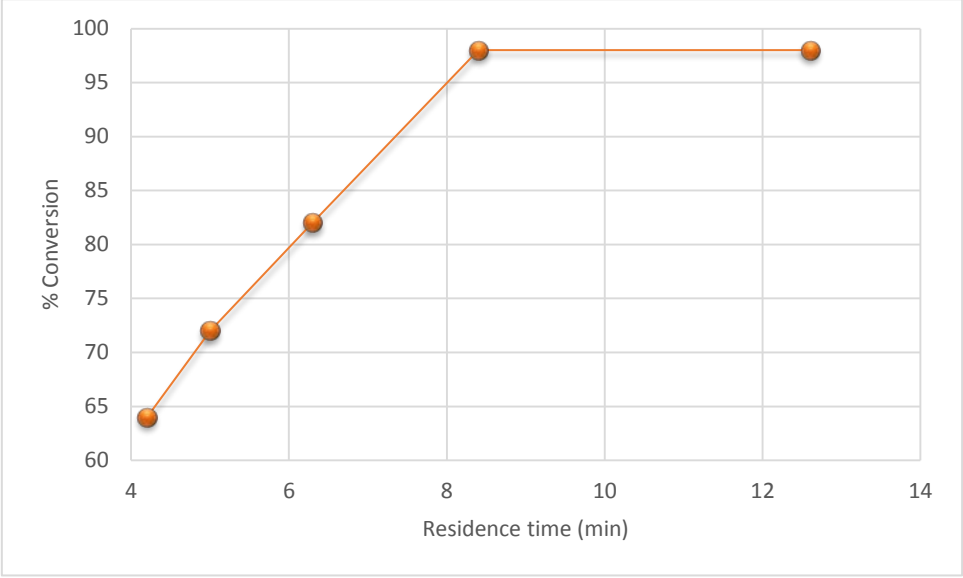

Figure 8 Effect of residence time on conversion to **9** at all reagents concentrations 0.30 M and temperature 80 °C in acetonitrile.

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Next, we turned our attention to studying the temperature effect on the conversion to **9**. The concentrations of all reagents were kept constant throughout the study at 0.30 M and the minimum residence time of 8.4 min was selected. Effect of temperature on the conversion to **9** (Figure 9) shows that the conversion reaches a maximum at 80 to 85 °C.

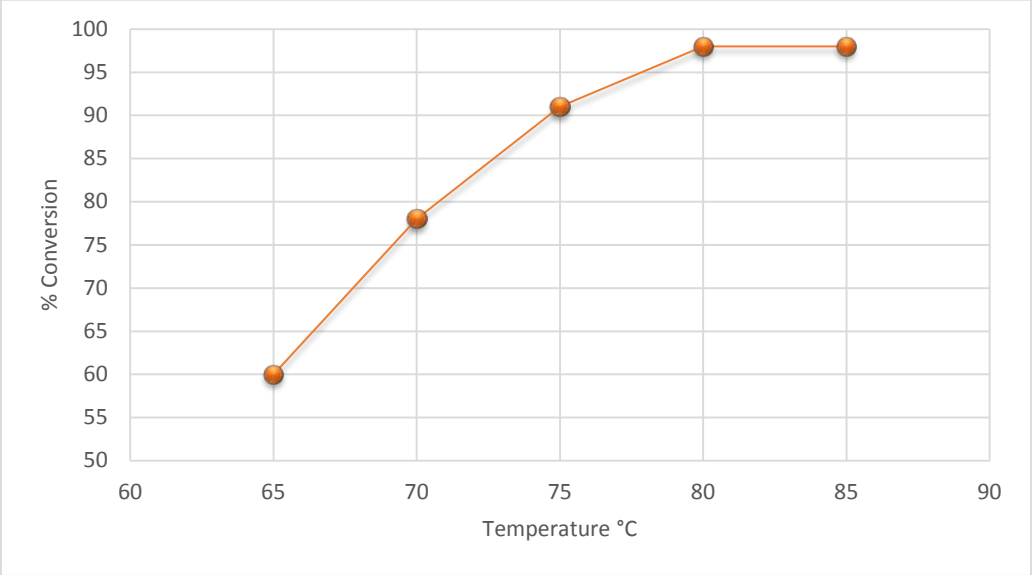

Figure 9 Effect of temperature variations on the conversion to **9** at constant residence time 8.4 min & concentrations of all reagents 0.3 M in ACN.

Finally, we turned our attention towards the effect of reagent concentrations on the conversion to **9**. Firstly we chose silyl product **8** concentrations from 0.20 M to 0.37 M at the optimum residence time (8.4 min) and the temperature (80 °C) was kept constant. As the concentration of silyl product, **8** increases the conversion increased (Figure 10).

Interestingly, when the concentration of pyridinium triflate catalyst decreased from 0.30 M to 0.20 M the conversion was not affected (98%), however, the further reduction in a concentration below 0.20 M did result in lower conversions (Figure 11). In summary, the optimal condition for this step of the flow synthesis was 8.4 min residence time, temperature (80 °C) and concentrations (5-acetoxy oxathiolane **7** 0.30 M, silyl cytosine **8** 0.30 M and pyridinium triflate 0.20 M). As compared to batch process in flow, we reduced the amount of catalyst needed and shortened the reaction time giving a higher yield of product.

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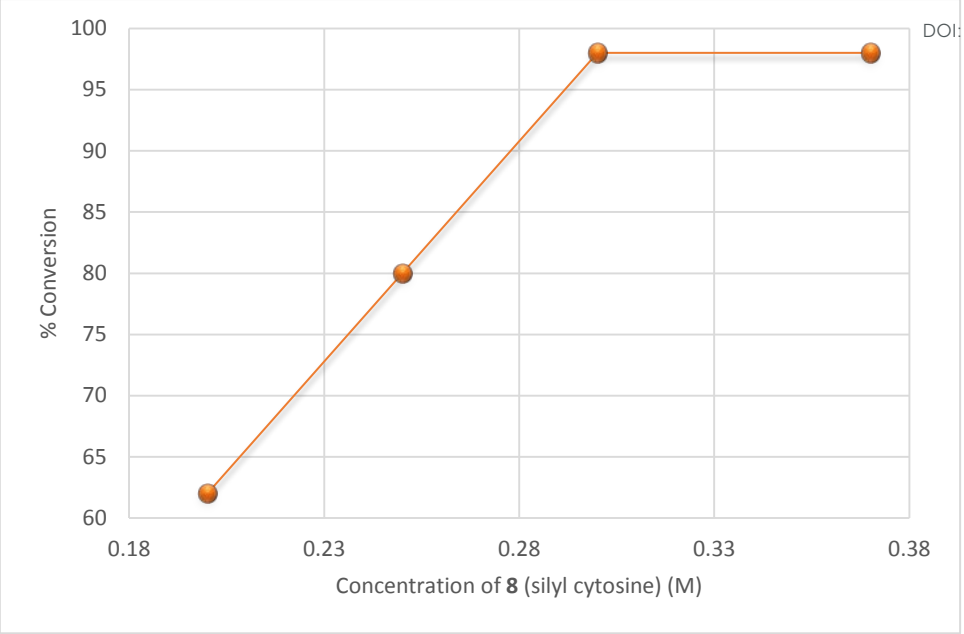

Figure 10 Effect of concentration variations of silyl product **8** on the conversion to **9** at constant residence time 8.4 min temperature (80 °C) at 0.30 M of all reagents.

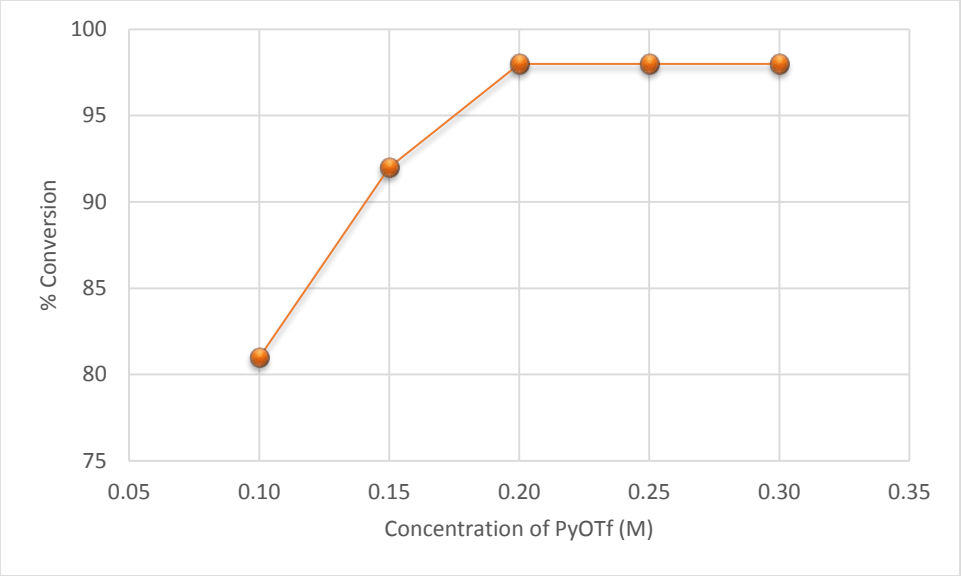

Figure 11 Effect of concentration variations of pyridinium triflate on the conversion to **9** at constant residence time 8.4 min temperature (80 °C) at 0.30 M of other reagents.

Reduction of a nucleoside precursor (9) with $NaBH_4$ in flow:

As the final step in the synthesis, we successfully deprotected the menthyl group using sodium borohydride in the presence of K_2HPO_4 in methanol and water[12] (Figure 12). The preliminary reaction was done with the nucleoside precursor **9** (0.26 M) dissolved in

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methanol and K_2HPO_4 (0.78 M) dissolved in water, and both solutions were pumped into the 0.2 mL LTF-MS microreactor at room temperature and the outlet of this reactor connected to another 0.2 mL LTF-MS, where a solution of $NaBH_4$ (0.52 M) in water and 0.1 mL of 25% NaOH solution was added. This mixture went through the 1.1 mL LTF-VS and the product was collected to afford the crude product, which upon recrystallization yielded the final product **1** (94 %, chiral purity 99.81%).

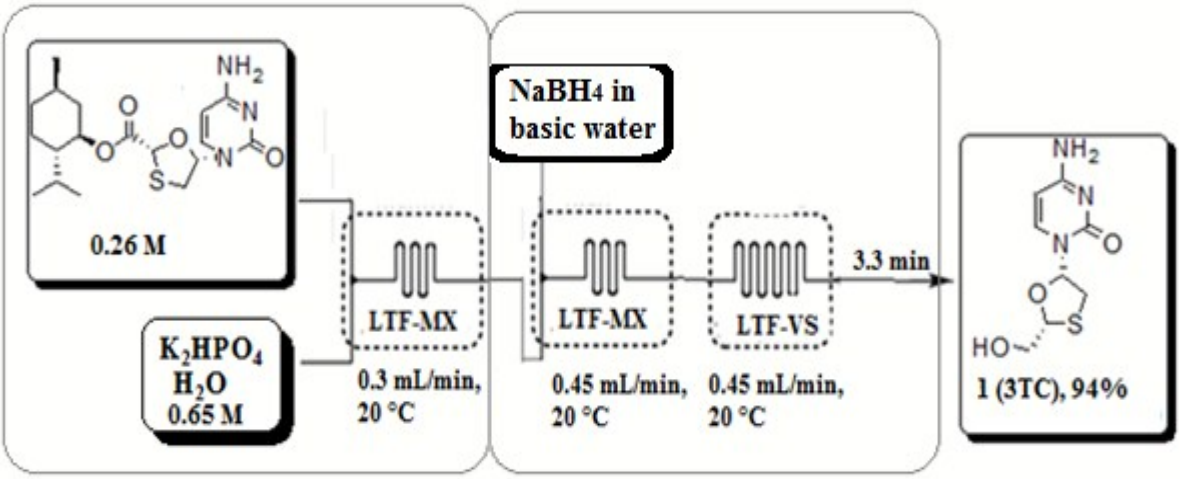

Figure 12 Reduction of nucleosides **9** with $NaBH_4$.

Having developed the preliminary conditions in flow, we then extended the investigation towards the residence time, temperature and optimum concentrations of all reagents. Residence time investigations revealed that the maximum conversion was observed at a residence time of just 3.3 min (100%).

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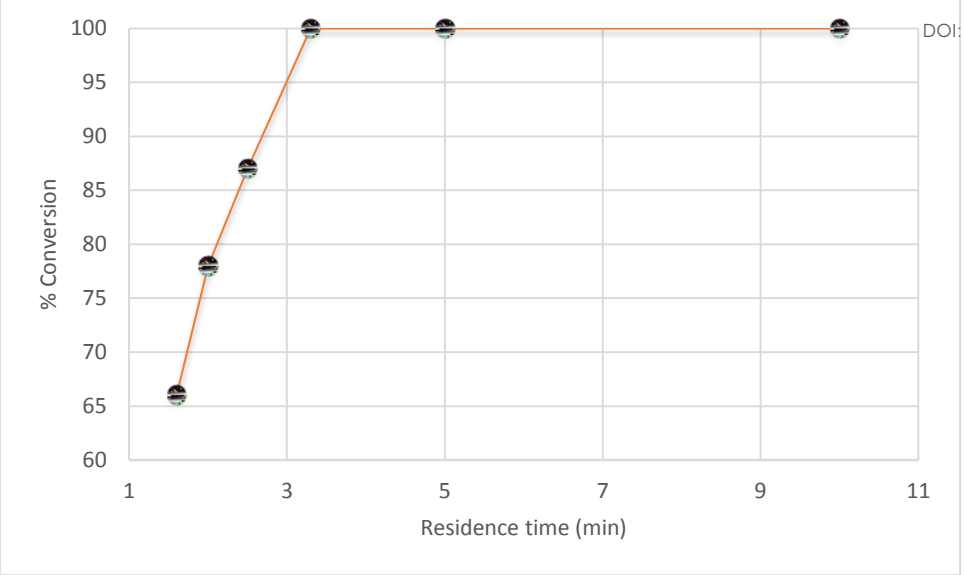


Figure 13 Effect of residence time on conversion to **1** at concentrations of (**9**: 0.26 M, K_2HPO_4: 0.78 M & $NaBH_4$: 0.52 M) and temperature 20 °C.

We then performed a detailed reaction temperature study on this reaction at known residence time. Complete conversion was observed at temperatures of 20 °C and above (Figure 14).

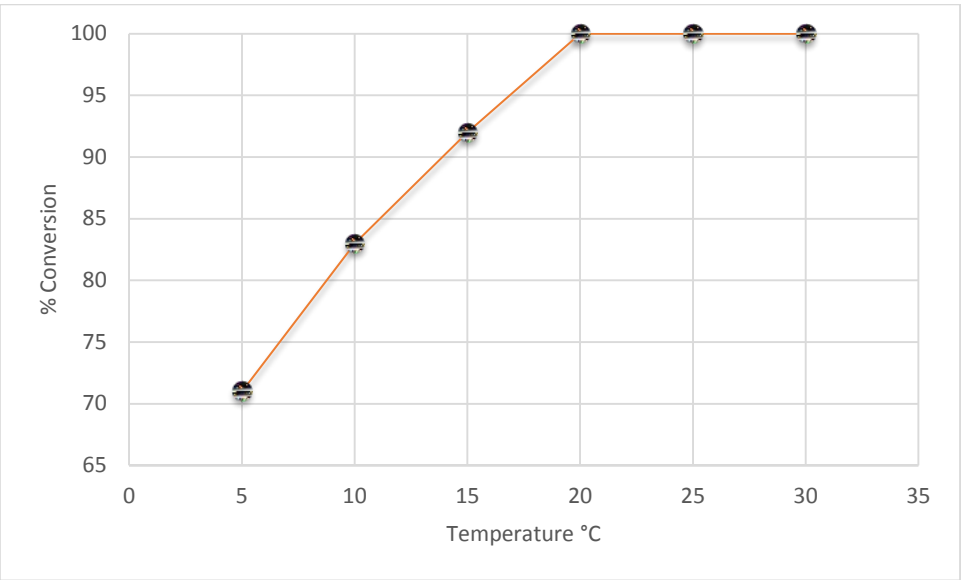


Figure 14 Effect of temperature variations on the conversion to **1** at residence time 3.3 min and concentrations of (**9**: 0.26 M, K_2HPO_4: 0.78 M & $NaBH_4$: 0.52 M).

At final we were keen to investigate the concentration studies on conversion of **1** from compound **9** at a residence time 3.3 min and temperature 20 °C. Firstly, the concentration of K_2HPO_4 from 0.78 M to 0.26 M studied. At 0.78 gives the maximum conversion then it

remains up to 0.65 M, then it slowly dropped (figure 15). After these parameters in hand, we have investigated the concentration range of $NaBH_4$ from 0.52 M to 0.26. The observations are shown in graph (figure 16) and it gives the maximum conversion at 0.52 M and it was sustained until 0.39 M.

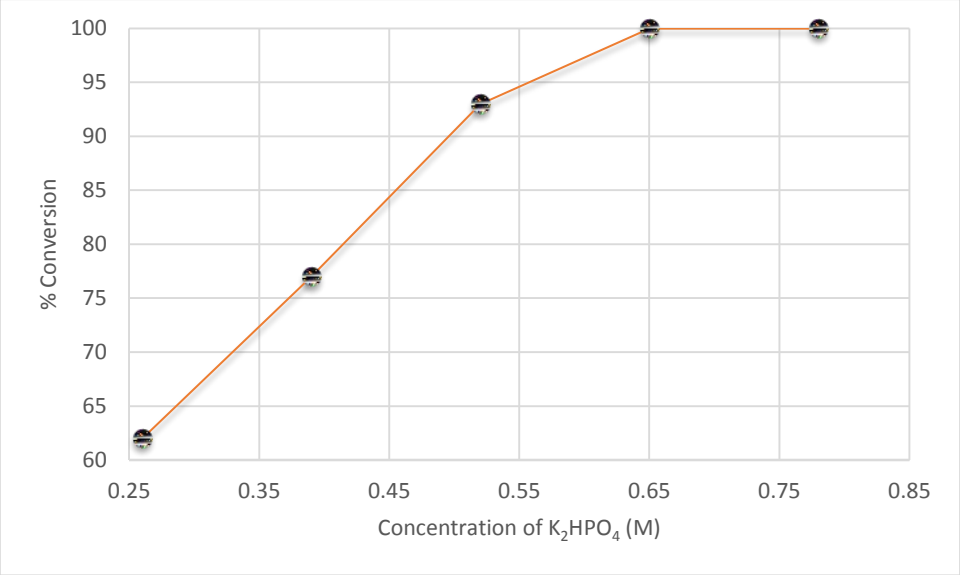


Figure 15 Effect of concentration variations of K_2HPO_4 on the conversion to **1** at residence time 3.3 min temperature (20 °C) and concentrations of (**9**: 0.26 M & $NaBH_4$: 0.52 M).

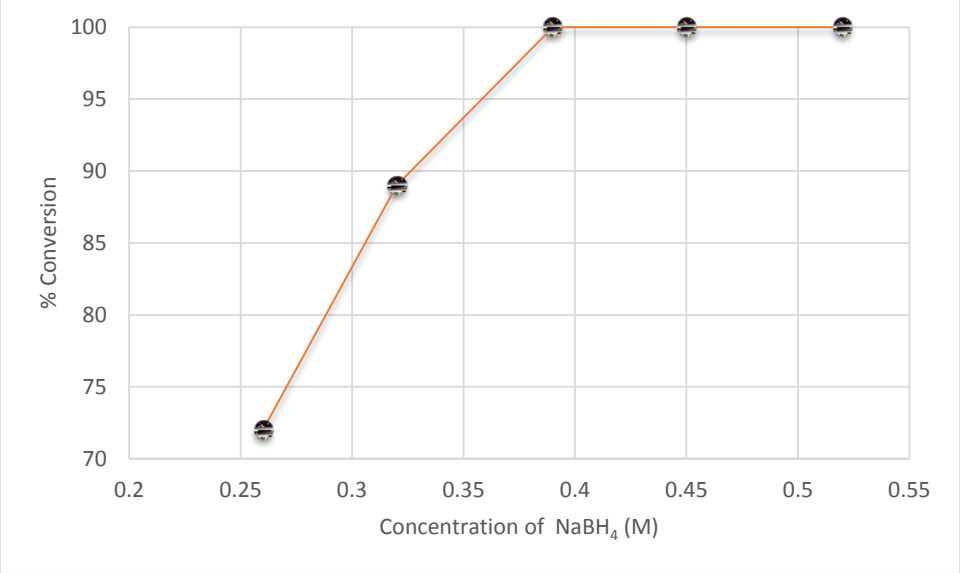
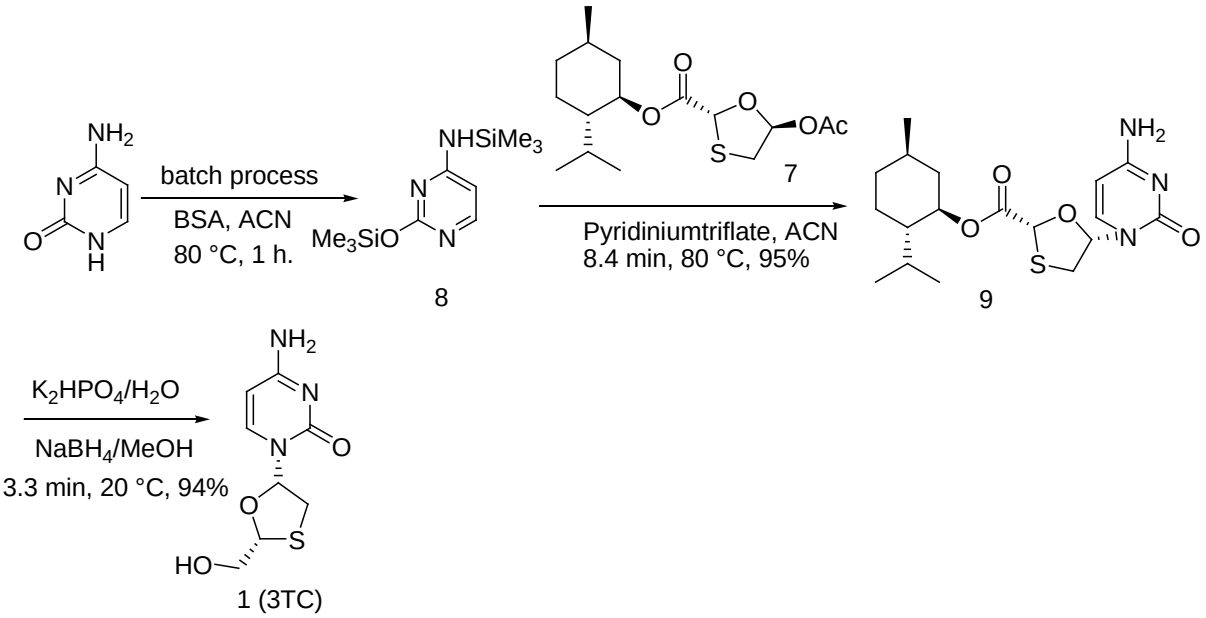


Figure 16 Effect of concentration variations of $NaBH_4$ on the conversion to **1** at residence time 3.3 min temperature (20 °C) and concentrations of (9: 0.26 M & K_2HPO_4: 0.65 M).

In summary, the optimized conditions for the flow synthesis to lamivudine **1** from nucleoside precursor **9** are a residence time 3.3 min, temperature (20 °C) and concentrations (nucleoside precursor **9** 0.26 M, K_2HPO_4 0.65 M and $NaBH_4$ 0.39 M) (Scheme 2).



Scheme 2 Synthesis of lamivudine **1**.

Conclusion

In summary, we have developed the protocol for the synthesis of lamivudine in semi-continuous flow synthesis and also reported optimized conditions for each step. The continuous flow synthesis of 5-acetoxy oxathiolane which is an important tool for synthesis of lamivudine and also achieved using pyridinium triflate as glycosidation reagent with higher yields and desired isomer are highlights of this work. By telescoping the condensation (step 1) and acetylation steps into a single, continuous and uninterrupted reactor network, thereby avoiding the need to isolate and purity of the intermediate product, the 5-acetoxy oxathiolane has been significantly streamlined. High yields, reduced reaction times, temperatures and reduced volumes of reagents enhanced safety and the ease of scalability is improved in the continuous flow synthesis as compared with traditional batch synthesis. A more comprehensive investigation towards the large scale process in continuous flow synthesis of lamivudine is ongoing and will be disseminated when implemented.

Acknowledgements

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Experimental Section

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General: Solvents and starting materials were taken from Aldrich and Alfa Aesar companies, were used as obtained without further purification. The syringe pumps (Chemyx Fusion Classic syringe pumps) and syringes (1 mL, 5 mL, and 10 mL, SGE Luer lock gas tight glass syringes) were purchased from Supelco Company. PTFE tubing refers to Polytetrafluoroethylene tubing. PTFE and Tygon MHLL tubing were purchased from IDEX Health & Sciences. Used different microreactors such as: LTF-MX (Internal volume 0.2 mL regularly used for small volumes 0.1-10 mL/min/channel and mix intensive chemicals), LTF-MS (Internal volume 0.2 mL regularly used for higher volumes 0.5-20 mL/min/channel and insensitive against blocking), LTF-V (Internal volume 1.7 mL used for reaction residence time) and LTF-VS (Internal volume 1.1 mL used for reaction residence time for mix intensive chemicals) were purchased from Little Things Factory-Germany.

The GC- FID analysis was performed using HP-5 MS capillary column (30 mm × 0.25 mm i.d.) and ultra-high purity nitrogen carrier gas. The samples were analyzed by using the following method; injector temperature 250 °C, nitrogen flow rate 0.1 mL/min, oven temperature 80 °C for 3 min and then ramped to 320 °C at 50 °C min^{-1} and final hold-up time 5 min. Chiral HPLC analysis was performed on Cyclobond I 2000 (4.6 x 250 mm) column using 30:70 acetonitrile: buffer (0.025 M ammonium acetate solution).

^{1}H-NMR, ^{13}C NMR, ^{19}F, NOESY and HSQC spectra were recorded on a Bruker AvanceIII-400 MHz spectrometer (400 and 100 MHz, respectively) in $CDCl_3$, DMSO-d_6 and CD_3OD. Chemical shifts in ^{1}H NMR spectra were reported in parts per million (ppm) on the ∂ scale from an internal standard of residual $CHCl_3$ in $CDCl_3$ (7.26 ppm), DMSO in DMSO-d_6 (2.50 ppm) or MeOH in MeOD (3.31 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet and br = broad, dd = doublet of doublet, td = triplet of doublet), coupling constant in hertz (Hz), and integration. Chemical shifts of ^{13}C NMR spectra were reported in ppm from the central peak of $CDCl_3$ (77.16 ppm), DMSO-d_6 (39.52 ppm) or MeOD (49.15 ppm) on the ∂ scale. ^{13}C signals with identical chemical shifts for more than one carbon are specified, and overlapping peaks for multiple carbons are indicated by a shift range. Melting points were recorded using a Mel-Temp melting point apparatus and are uncorrected. Combustion analyses were performed using a CHNS analyzer. Infrared (IR) spectra were recorded on a Perkin-Elmer Model 2000 FT-IR. Optical rotations were measured at 25 °C in the stated

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solvent and matched with standard samples. All data generated from these experiments was plotted using Microsoft Excel to illustrate the trends observed.

(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl) 5-hydroxy-1,3-oxathiolane-2-carboxylate 5

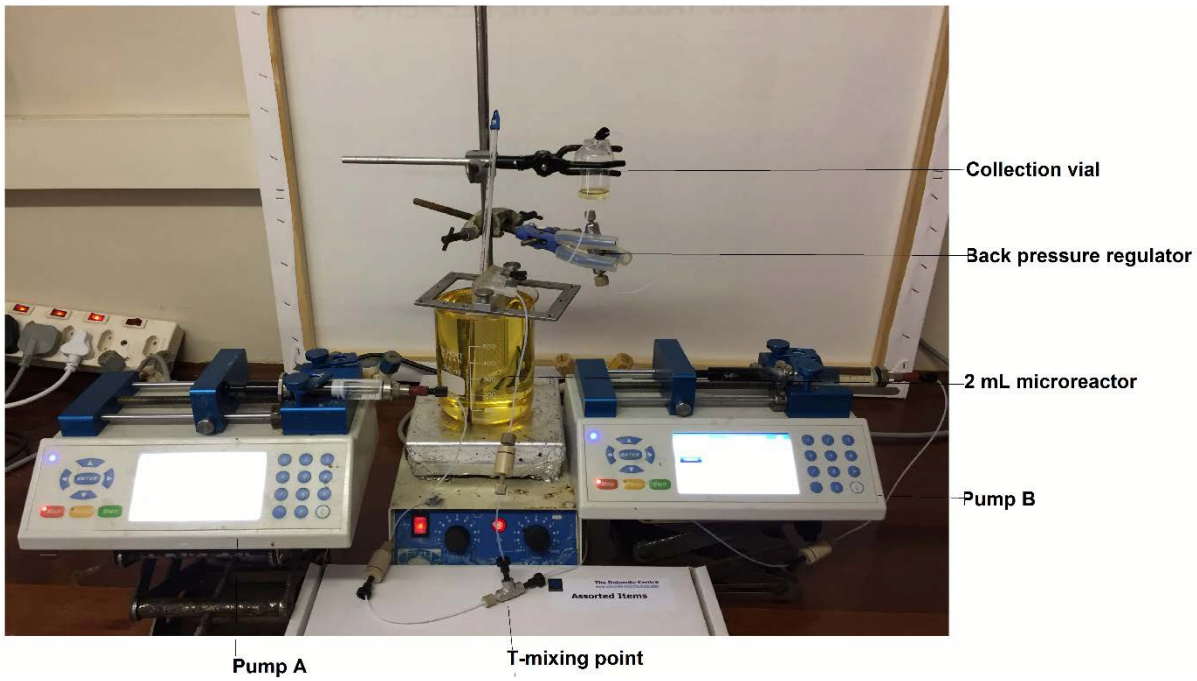

Figure 17: Microreactor setup for the synthesis to 5-hydroxy oxathiolane **5**

A solution of L-menthyl glyoxalate hydrate (0.43 M) in acetonitrile was prepared in reagent bottle **A**. While reagent bottle **B** was with a solution containing 1,4-dithiane 2,5-diol (0.22 M) in acetonitrile, and 0.05 mL of acetic acid. This solution **B** was heated at a temperature between 40-45 °C for 10 min to get the clear solution prior to performing the reaction. Both solutions from reagent bottles **A** and **B** transferred into 10 mL syringes and pumped through a T-piece mixing point to ensure proper mixing and into a 2 mL glass microreactor plate heated at 90 °C at 10 min residence time. A 10 PSI back pressure regulator was fitted onto the fluid line after the reactor plate so as to keep the system pressurized thus enabling the use of acetonitrile above its boiling point (figure 17). The reaction mixture was then collected in a collection vial equipped with a septum (to prevent solvent evaporation). After completion of the reaction, the solvent was evaporated and the reaction mixture was cooled to 0-5 °C. 1% of triethylamine in heptane solution drop wise was thereafter added (The addition of TEA in *n*-heptane/hexane the rapid interconverison among the stereoisomers happened). The mixture was stirred at 0-5 °C for 2-3 h and the formation of a precipitate was observed. The isolated

solid was filtered and washed with cold *n*-heptane to give a (1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl 5-hydroxy-1,3-oxathiolane-2-carboxylate **5**. Yield- 88%, white colour solid, $R_{f\,=}$ 0. 4 (Ethyl acetate : Hexane 1:1), ^{1}H-NMR in $CDCl_3$ (400 MHz): ∂ 0.78 (d, *J* = 6.84, 3H), 0.91(m, 6H), 1.09-1.00 (m, 2H), 1.51-1.42 (m, 2H), 1.70 (d, *J* = 11.84, 1H), 2.02 (bd, *J* = 8.76, 2H), 3.15 (dd, *J* = 4.5, 11.2, 1H), 3.32-3.29 (m, 1H), 4.74 (s, 1H), 5.57 (d, *J* = 8.76, 1H), 5.96 (d, 1H). ^{13}C-NMR in $CDCl_3$ (100 MHz): ∂ 16.27, 20.69, 23.30, 26.16, 31.42, 34.11, 38.46, 40.35, 46.86, 46.07, 80.20, 101.22, 103.20, 172.18. FT-IR (Neat): 3456, 2956, 2864, 1731, 1457, 1387, 1288, 1196, 1041, 986. Anal.calcd for $C_{14}H_{24}O_4S$: C 58.30, H 8.39, S 11.12. Found: C 58.46, H 8.35, S 10.91.

(2*R*,5*R*)-((1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl) 5-acetoxy-1, 3-oxathiolane-2-carboxylate 7:

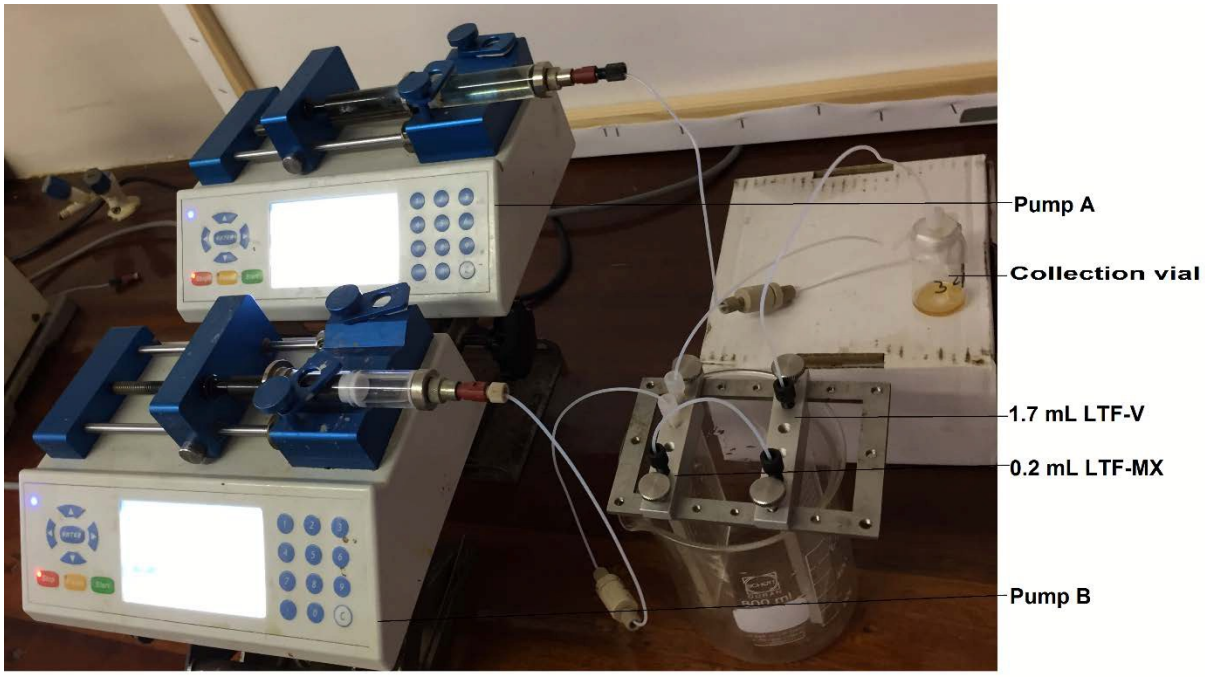

Figure 18: Microreactor setup for the synthesis to 5-acetoxy oxathiolane **7**

Reagent bottle **A** was filled with a solution of (1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl 5-hydroxy-1,3-oxathiolane-2-carboxylate **5** (0.34 M) dissolved in acetonitrile and reagent bottle **B** was filled with a stock solution of acetic anhydride and pyridine (1.73 M) in acetonitrile at room temperature. Both solutions from reagent bottles **A** and **B** transferred into 10 mL syringes and pumped with two separate Chemyx fusion pumps into 0.2 mL LTF-MX microreactor then passed through 1.7 mL LTF-V microreactor to increase the residence time

(4.7 min) at room temperature. The output stream was collected in closed cap vial (figure 18). After the reaction completion was confirmed by TLC (20% ethyl acetate in hexane) & GC, the reaction mixture was evaporated under rotary pressure, quenched with dilute HCl (25 mL). The reaction mixture was extracted with ethyl acetate (3 x 25 mL) washed with water (50 mL), brine solution (50 mL) and dried over anhydrous Na_2SO_4. On concentration of this organic layer under the reduced pressure, a brown colored solid comprising a mixture of diastereomers **6** (95%) was obtained.

The crude product (780 mg) was dissolved in triethylamine (0.1 mL) and hexane (30 mL). The solution was kept at -20 °C for 36 h after which the desired product (2*R*,5*R*)-((1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl) 5-acetoxy-1,3-oxathiolane-2-carboxylate **7** was obtained 375 mg, yield-48%, white colour solid, R_f = 0.52 (Ethyl acetate : Hexane 3:7), M. P. 99-102 °C (Lit 101-102 °C). ^{1}H-NMR in DMSO-d_6(400 MHz): ∂ 0.72 (d, *J* = 6.8, 3H) 0.98-0.81 (m, 7H), 1.03 (t, 2H), 1.47-1.36 (m, 2H), 1.63 (d, *J* = 11.76, 2H), 1.89 (bd, *J* = 12.32, 2H), 2.04 (s, 3H), 3.38-3.23 (m, 2H), 4.63 (td, *J* = 3.88, 1H), 5.76 (s, 1H), 6.78 (d, *J* = 3.8, 1H). ^{13}C-NMR in DMSO-d_6 (100 MHz): ∂ 16.19, 20.71, 21.50, 23.24, 26.15, 31.37, 34.13, 37.33, 40.40, 47.00, 76.10, 79.90, 99.85, 168.58, 169.64. FT-IR (Neat): 3463, 2959, 2932, 2865, 1745, 1730, 1463, 1385, 1287, 1169, 1040, 986, 876. Anal.calcd for $C_{16}H_{26}O_5S$: C 58.16, H 7.93, S 9.70. Found: C 57.90, H 8.20, S 9.83.

Continuous flow synthesis to 5-acetoxy oxathiolane 7 from L- methyl glyoxalate hydrate 3:

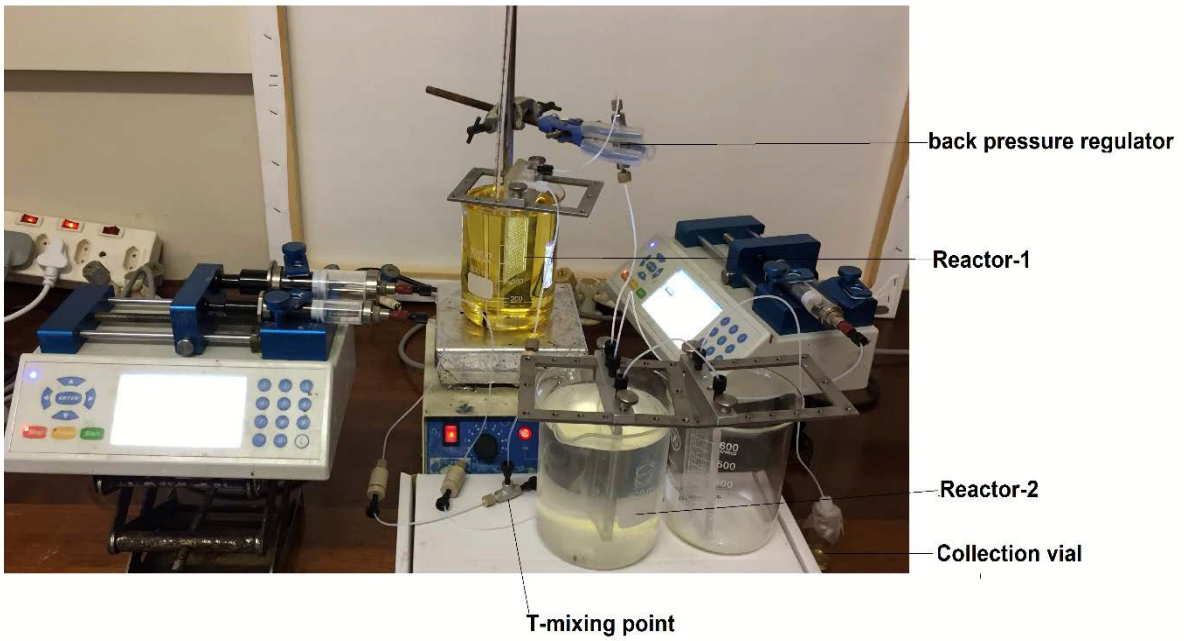

Figure 19: Complete setup for continuous flow synthesis to 5-acetoxy oxathiolane **7**

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Three reagent solutions (stock solutions A, B, and C) were prepared as explained below.

Stock solution **A** was prepared by dissolving L-menthyl glyoxalate hydrate (0.43 M) in acetonitrile.

Stock solution **B** contained 1,4-dithiane 2,5-diol (0.22 M) and acetic acid (0.05 mL) in acetonitrile. This was first heated to 40 °C for 10 min to form a clear solution prior to use.

Stock solution **C** comprised of acetic anhydride and pyridine (1.73 M) in acetonitrile.

All three stock solutions were kept in 100 mL glass reagent bottles.

Stock solutions **A** and **B** were taken into 10 mL syringes and pumped through the Chemyx pump at a flow rate of 0.1 mL/min each, these were mixed at T-piece mixing point and further pumped into a 2 mL glass microreactor plate (reactor-1) which is preheated at 90 °C. The reactor-1 outlet was connected to a 10 PSI back pressure regulator. The output stream of reactor **1** (step 1 product **5**) was connected to 0.2 mL LTF-MX reactor (This LTF-MX microreactor was kept in cooling bath to cool the first step solution) which is already connected to another Chemyx Fusion syringe pump with pyridine and acetic anhydride in acetonitrile (1.73 M). These solutions were passed through another LTF-V (1.7 mL, at RT) reactor (reactor-2) to increase the residence time at room temperature and the product was collected in a closed cap vial (figure 19). After the reaction completion was confirmed by TLC (30% EtOAc in hexane) & GC, the solvent was evaporated. The reaction mixture was quenched with ice-cold water (10 mL) and extracted using ethyl acetate. The organic layer was washed with dilute HCl (10 mL), dried over anhydrous Na_2SO_4 and concentrated under the reduced pressure to give gave 1.25 g (87.4%) compound **6** as brown color solid with the mixture of four diastereomers. A crude sample of compound **6** (1.0 g) was dissolved in a little amount of triethylamine (0.1 mL) and hexane (30 mL). This was kept at -20 °C for 36 h after which the desired product (2*R*,5*R*)-((1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl) 5-acetoxy-1,3-oxathiolane-2-carboxylate **7** was obtained. (450 mg, 45% overall for two steps).

Procedure for the glycosidation reaction in flow:

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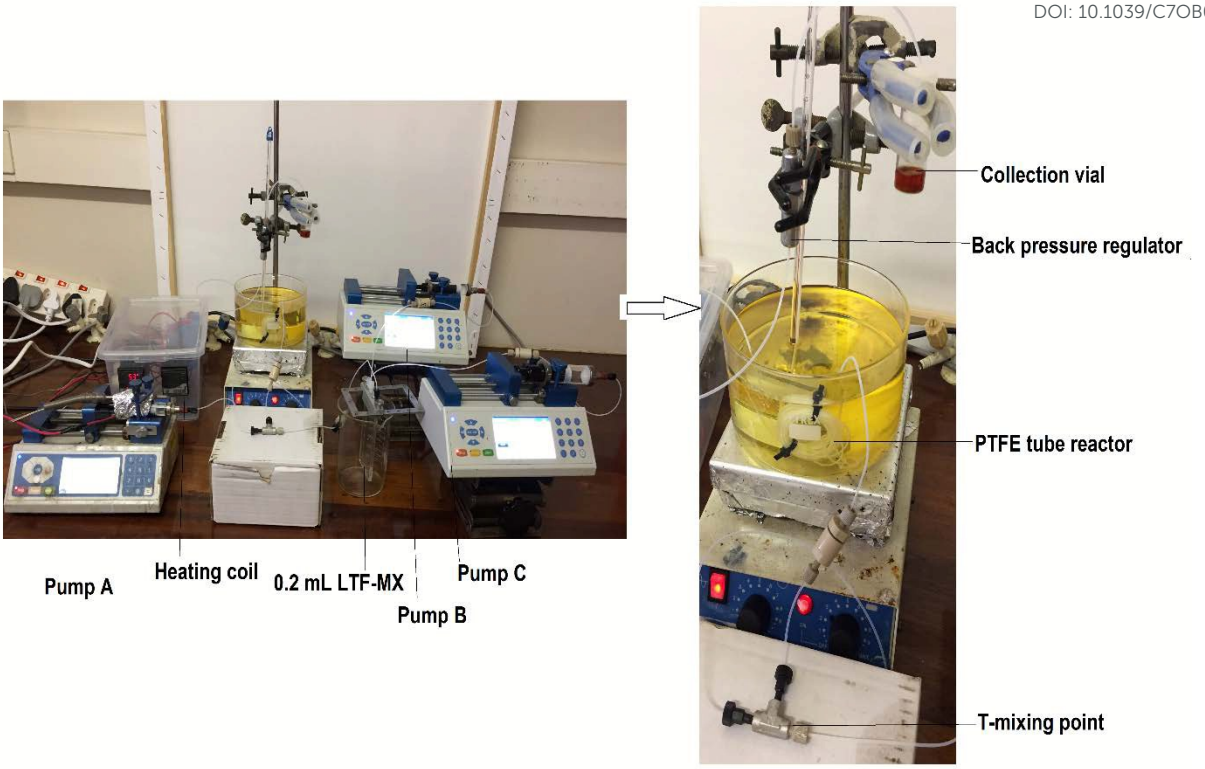

Figure 20: Microreactor setup for glycosidation reaction:

The preparation of silylated cytosine (**8**) was prepared by batch process (cytosine 1.0 eq and BSA 2.25 eq in acetonitrile at 80 °C for 1 hour) and this solution was made up into 0.30 M with acetonitrile in an oven dried, screw-cap volumetric flask.

On the other hand, a solution of (2*R*,5*R*)-((1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl) 5-acetoxy-1,3-oxathiolane-2-carboxylate **7** (0.30 M) and pyridiniumtriflate (0.20 M) in acetonitrile in a separate oven dried, screw-cap volumetric flasks was prepared.

All three prepared solutions were then transferred into 10 mL SGE glass syringes (nucleobase **8** was placed in the syringe wrapped around with the heating tape and maintained at 60 °C during the reaction to prevent the clogging in the syringe). Initially, the two syringes having solutions of compound **7** and pyridinium triflate were pumped into 0.2 mL LTF-MX for mixing then the outlet of this reactor and third syringe pump having nucleobase **8** were connected at T-piece mix joint where they were mixed and further pumped through the PTFE tubing (3.8 mL, 1.6 mm (1/16”) o.d. and 0.8 mm i.d.) at a flow rate of 0.15 mL/min each (residence time 8.4 min). And the PTFE tubing was dipped into an oil bath to keep at 80 °C, the outlet of this tube connected to 10 PSI back pressure regulator. The reaction mixture was then collected into a vial equipped with a septum (to prevent solvent evaporation, figure 20). The solvent in the collected reaction mixture was evaporated under reduced pressure and the

residue obtained was purified by recrystallization in hexane: ethyl acetate: methanol (2:2:1) to get the clear white solid 1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl(2*R*,5*S*)-5-(4-amino-2-oxo-1,2-dihydro-1-pyrimidinyl)-1,3-oxathialane-2-carbaxylate **9**. Yield-95%, white solid, R_f = 0.35 (MeOH: CH_2Cl_2 1:9), M. P. 203-206 °C (Lit 202-203 °C), ^{1}H-NMR in $CDCl_3$ (400 MHz): ∂ 0.78 (d, *J* = 6.84, 3H), 0.95-0.89 (m, 6H), 1.10-1.04 (m, 2H), 1.45 (t, , *J* = 11.82, 2H), 1.72 (bd, *J* = 11.36, 2H), 2.06 (d *J* = 11.76, 1H), 1.96 (t, J = 6.64, 1H), 3.13 (dd, *J* = 6.6, 11.80, 1H), 3.54 (dd, *J* = 4.06, 11.76, 1H), 4.77 (td, *J* = 4.04, 1H), 5. 47 (s, 1H), 5.92 (d, *J* = 7.48, 2H), 6.47 (t, *J* = 5.36, 1H), 8.36 (d, *J* = 6.72, 1H), ^{13}C-NMR in $CDCl_3$ (100 MHz): 16.09, 20.72, 21.93, 23.24, 26.09, 31.46, 34.06, 36.32, 40.75, 47.07, 76.70, 78.49, 90.22, 94.86, 141.87, 155.18, 165.22, 169.75. Anal.calcd for $C_{18}H_{27}N_3O_4S$: C 56.67, H 7.13, N 11.01, S 8.41. Found: C 56.59, H 7.18, N 10.98 S 8.26.

Reduction of nucleoside precursor (9) by flow synthesis:

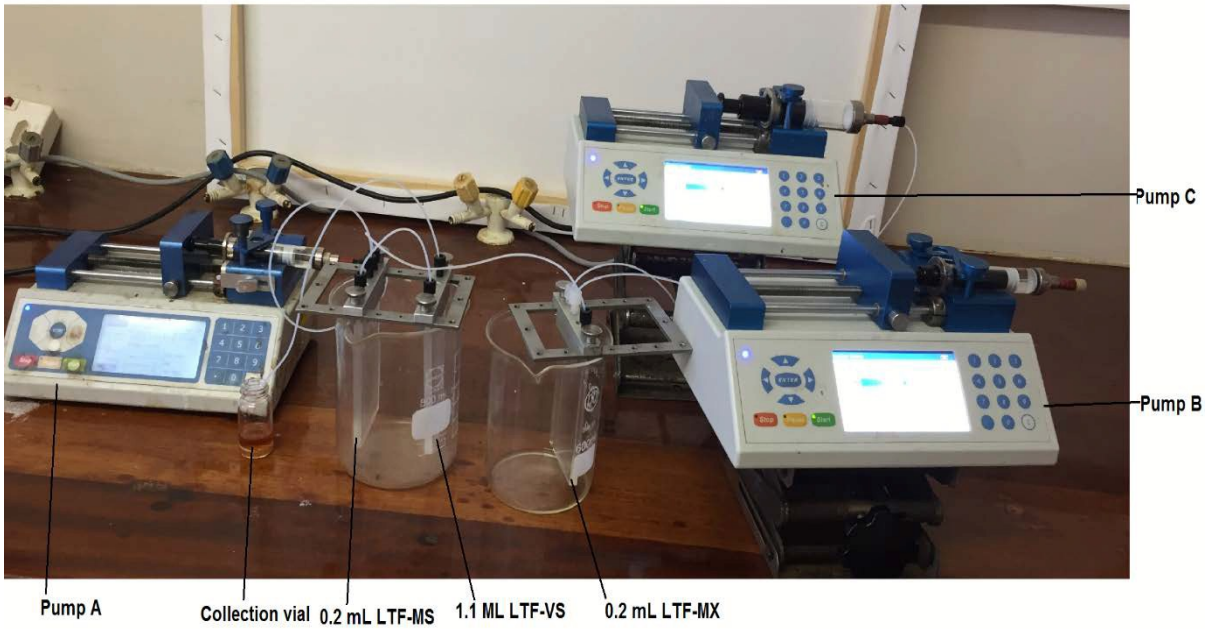

Figure 21: Flow setup for the synthesis to lamivudine

Using three oven dried 10 ml SGE glass syringes, a suspension of nucleoside precursor **9** (0.26 M) in methanol (syringe A), a prepared solution containing potassium hydrogen phosphate (0.65 M) in water (syringe B) and sodium borohydride (0.39 M) in water +0.1 mL 25% w/w sodium hydroxide solution (syringe C) was fed into a microreactor plates with the aid of three Chemyx Fusion syringe pumps. The first two Fluid lines (syringe A and B)

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leading to mixing and passed through the 0.2 mL LTF-MX microreactor. The outlet of this reactor and the third syringe pipe from another Chemyx Fusion syringe pump which contains the $NaBH_4$ solution were added together at 0.2 mL LTF-MS and further into 1.1 mL LTF-VS microreactor at room temperature (3.3 min residence time). The product was collected at the end using closed cap vial (figure 21). The reagent solutions were each fed into the system at a flow rate of 0.15 mL/min residence time 3.3 min. The collected solution was quenched with dil. HCl (pH 4-4.5), extracted with diethyl ether to remove the menthol. The pH of the aqueous layer was adjusted to 7 using saturated $NaHCO_3$ solution. This was then filtered and evaporated under reduced pressure. The crude compound was purified by recrystallization using hexane: EtOAc: methanol (2:2:1) at 65 °C, slowly taken into ambient temperature to give the pure compound of lamivudine **1**. Yield-94%, white colour solid, Chiral purity 99.81%, R_f = 0.30 (MeOH: CH_2Cl_2 1:9), M. P. 162-165 °C (Lit 158-160 °C), ^{1}H-NMR in DMSO-d_6 (400 MHz): ∂ 3.04 (dd, *J* = 4.92, 11.68, 1H), 3.40 (dd, *J* = 5.48, 11.68, 1H), 3.73 (dd, *J* = 5.32, 10.44, 2H), 5.17 (t, *J* = 4.48, 1H), 5.30 (t, *J* = 5.76, 1H), 5.73 (d, *J* = 7.48, 1H), 6.20 (t, *J* = 5.16, 1H), 7.23-7.18 (br, 2H), 7.81 (d, *J* = 7.44, 1H); ^{13}C-NMR in DMSO-d_6 (100 MHz): 36.74, 63.35, 86.28, 87.03, 94.40, 141.43, 155.18, 166.13; Anal.calcd for $C_8H_{11}N_3O_3S$: C 41.91, H 4.84, N 18.33, S 13.99. Found: C 41.88, H 4.71, N 18.19 S 13.

Notes and References:

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Mandala table of contents entry

We report the first semi-continuous flow synthesis of lamivudine, an antiretroviral drug used in the treatment of HIV/AIDS.