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Large-Scale Stereoselective Synthesis of 1, 3-Oxathiolane Nucleoside, Lamivudine via $ZrCl_4$-Mediated N-Glycosylation

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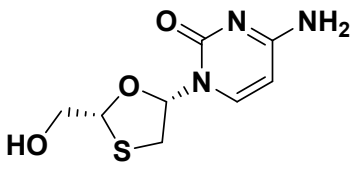
KEYWORDS: Lamivudine (3TC), N-glycosylation, Stereoselectivity, $ZrCl_4$

ABSTRACT: A stereoselective large-scale synthetic process is described to produce 1, 3 – oxathiolane nucleoside, lamivudine (3TC). A mild, inexpensive and readily available zirconium (IV) chloride ($ZrCl_4$) catalyst acts as a substrate activator for the key N-glycosylation step at room temperature. An optimum 0.5 equiv of $ZrCl_4$ is required which gives encouraging results with respect to chemical efficiency and stereoselectivity. The focus of this work was to develop a new Lewis acid (LA) catalyst for N-glycosylation reaction that permits mild and selective synthesis of lamivudine at large scale. It allowed preferential formation of single isomer of nucleoside out of

four possible stereoisomers starting from the corresponding 1, 3-oxathiolane acetate substrate (racemic and/or diastereomeric mixture of isomers). The thermal behavior for the critical N-glycosylation step was also studied by differential scanning calorimetry (DSC) and reaction calorimetry (RC1) techniques.

INTRODUCTION: Lamivudine (3TC™, **1**) (Figure 1.) is a first-generation FDA certified nucleoside reverse transcriptase inhibitor (NRTI) drug class used to treat HIV-1 infection as well as chronic hepatitis B virus (HBV) in the 1990s.[1]



1

Lamivudine (3TC)

Proprietary names where Lamivudine is used as Active pharmaceutical ingradient[1]
Epivir®
Combivir®
Trizivir®
Epzicom®
Triumeq®
Temixys®

Figure 1. 1, 3-oxathiolane nucleoside, lamivudine (3TC) is a currently approved antiviral agent.

In spite of the fact that, lamivudine was endorsed over two decades back, it still remains as a key segment of first-line treatment for HIV on account of its virological efficacy and capacity to be combined with further antiretroviral agents in customary and new combination therapies.[2] It has four stereoisomers however, investigators first reported racemic mixture of lamivudine. It is also known as (±)-BCH-189 to treat HIV-1.[3] BCH-189 enantiomers were further separated out by a method of chiral high pressure liquid chromatography and found to be resistant to both types 1 and 2 of human immunodeficiency viruses. However, the (-)-enantiomer (3TC) has been discovered substantially less cytotoxic than the (+)-enantiomer.[4a, b, c, d] The increasing demand for lamivudine (3TC), **1** has one of the reasons for the progress in a wide variety of synthetic approaches that

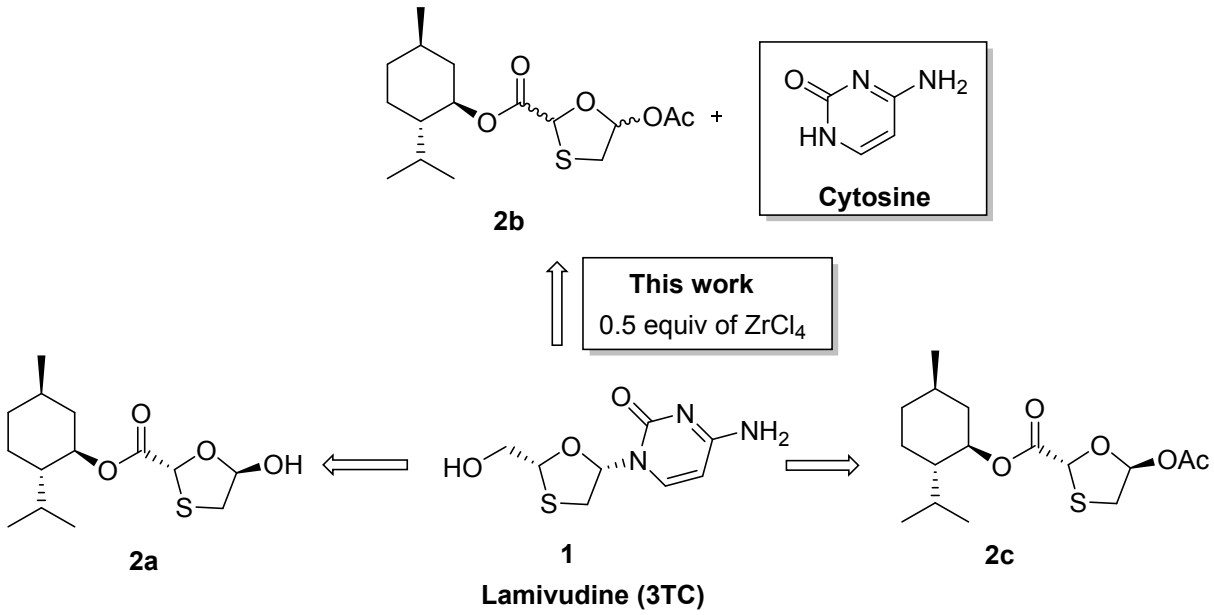
enable its production on a large scale with high enantiomeric purity. Several methodologies for the manufacture of **1** have been described, however, identification as well as implementation of a scalable synthetic sequence becomes necessary and the same outlined here in this work.

The (-)-enantiomer (3TC) holding unnatural L-β-configuration is the first FDA-approved nucleoside analogue.[4d] The construction of many such nucleoside analogues requires the selective glycosylation reaction that is desirable to unite appropriate furanose sugar derivative and nucleobase by forming of C–N glycosidic bonds while maintaining selectivity. Generally, stereo control and/or activation aided by a suitable catalyst or chiral auxiliary portion are obligatory features of any stereoselective glycosylation. Catalytic forms of this reaction are preferable because they require significantly smaller quantities of costly chirality sources. Highly β-selective N-glycosylation reactions has been also reported in literature for the synthesis of lamivudine. The key glycosylation step in lamivudine synthesis where, the enantiomerically pure oxathiolane acetate coupled with silylated cytosine using several Lewis acids (LAs) such as $SnCl_4$, $TiCl_4$, TMS triflate (TMSOTf), silanes [triethylsilane (Et_3SiH) or polymethylhydrosiloxane (PMHS)] and iodine (I_2), pyridinium triflate and trimethylsilyl iodide (TMSI) is described.[5a,b,c] However, some of these methods are still associated with shortcomings based on their toxicity, flammable nature, being expensive and uneasy while handling reagents that limited their practical application in large scale in pharmaceutical manufacturing. Therefore, there is a need for the development of a safe, efficient, inexpensive and industrially viable catalyst for glycosylation method. In the present study, we have established an efficient method for glycosylation through activation of oxathiolane acetate utilizing 0.5 equiv of $ZrCl_4$. The reaction completes within short time for large scale production with high yield at room temperature. The versatileness of this approach is evident from the fact that even the facile glycosylation takes place without isolation of enantiomerically pure

oxathiolane substrate that is superior over previous methods.[5a, b, c.] The oxathiolane acetate taken in situ in solution gives stereo selective single nucleoside isomer out of four possible stereoisomers.

RESULTS AND DISCUSSION: The crucial disconnection common to stereoselective synthetic methods for synthesis of lamivudine **1** is illustrated in Scheme 1 and relies upon a glycosylation approach to link the two suitably substituted 1, 3-oxathiolane derivatives **(2a** or **2b** or **2c)** and cytosine.

Scheme 1. Retrosynthetic approaches for synthesis of **1**



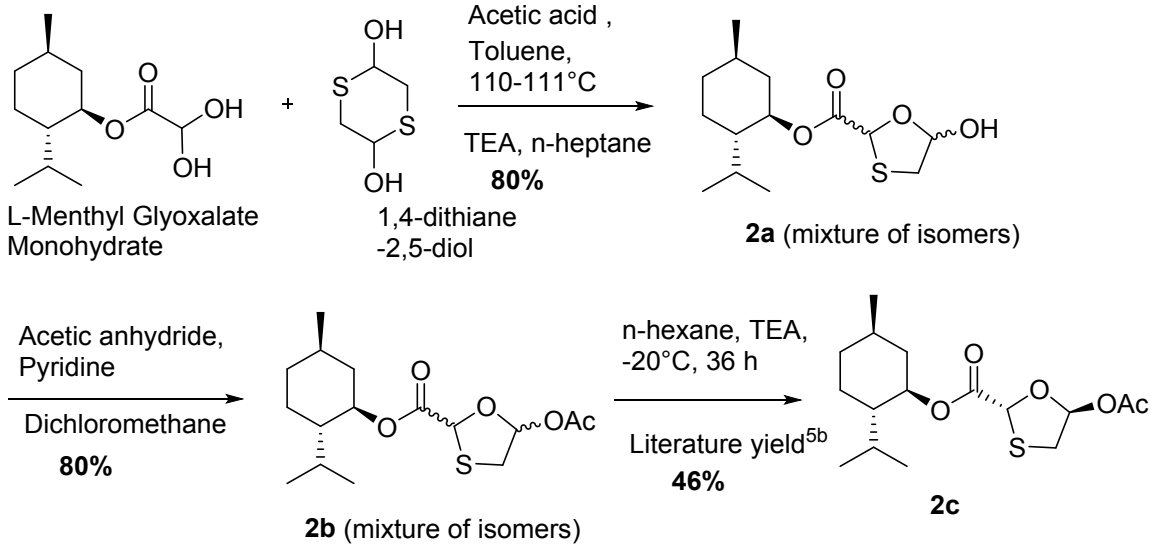
Among the methods available for the industrial manufacturing of lamivudine, the procedure reported by Whitehead and co-workers is the mostly chosen till date.[6] This procedure involves the chiral auxiliary approach where l-menthyl bearing chirally pure oxathiolane lactol **2a** undergoes in situ chlorination reaction with thionyl chloride in presence of catalytic N, N-dimethylformamide (DMF) in dichloromethane solvent (-Cl replaces –OH). Then, silylated cytosine replaces –Cl without any efforts in an S_N2-like nucleophilic substitution to produce β-isomer predominantly. It

is extensively reported that, the β-selectivity could be because of intermediate oxonium ion formation which gets stabilized by neighboring l-menthyl ester group via anchimeric assistance.[6] The method requires extremely efficient crystallization procedure with dynamic kinetic resolution for achieving synthesis of chirally pure oxathiolane lactol **2a**, which in sequence could be used, to produce lamivudine **1**. This approach represents a convenient alternative to previous methodologies[5c, d] for lamivudine synthesis. Previous methodologies uses highly unstable and expensive reagents and requires in a significant quantity and also mandatory requirement of corresponding chirally pure oxathiolane lactol **2a** or its corresponding acetate **2c**. Isolation of oxathiolane acetate (**2c**) (single stereoisomer) from a mixture of isomers (racemic or diastereomeric) by chemical resolution or crystallization or any enzymatic techniques goes in hand with the leaving of 50 per cent (racemic) or extra (diastereomeric mixtures) of the undesired isomers of substrate. Thus, it is somewhat difficult to synthesize oxathiolane acetate **2c** in pure form with quantitative yields. Access to an enantiopure **2c** was explored based on the approach suggested by Whitehead and co-workers.[6] The method involves the reaction of commercially available l-menthyl glyoxylate monohydrate with 1, 4-dithiane 2, 5-diol in toluene and further treatment of crude mixture with triethylamine in n-hexane solvent. Literature procedure reported[6] early formation of four stereoisomers while, the subsequent addition of catalytic amount of triethylamine in n-hexane is preferred to induce rapid interconversion between the stereoisomers. Exact repetition of this methodology on large scale is difficult and not robust. Furthermore, acetylation of pure oxathiolane lactol **2a** with Ac_2O in pyridine as a base could lead to interconversion to form all the four isomers again. Recently developed strategy by Mandala et al.[5b] for preparation of this key intermediate used sodium bicarbonate as a base and acetic anhydride in acetonitrile afforded mixture of diastereomers (93%), crystallization with solution containing

small amount of triethylamine at - 20°C for 36 h required to get desired pure oxathiolane acetate **2c** (46%) (Scheme 2) which is time consuming and critical to maintain such low temperature at large scale.

As a part of efforts focused on developing large-scale manufacturing route to lamivudine, it was decided first to synthesize of oxathiolane lactol **2a** as per literature procedure (Scheme 2).[6] Further, this lactol was converted into an acetate **2b** (racemic and/or diastereomeric mixture of isomers) which was achieved in one step using the reported procedure for a related compound.[5d, 7] The approach for the preparation of **2b**, shown in Scheme 2. Acetylation of oxathiolane lactol **2a** with acetic anhydride and pyridine as a base afforded a mixture of isomers for oxathiolane acetate **2b**.

Scheme 2. Synthesis of oxathiolane acetate **2a, 2b** and **2c**



Attempts to isolate chirally pure isomer of oxathiolane acetate **2c** were unsuccessful as there was a possibility of formation of four lactol isomers of **2a** in solution which were readily acetylated in situ to form four corresponding racemic and/or diastereomeric mixture of isomers. Further, it was difficult to isolate and analyze this pure oxathiolane acetate by chiral HPLC. A specific HPLC

method[7] is also known to determine its isomeric purity. It is known from the literature that, the reaction is very much base sensitive and to avoid base mediated lactol interconversion, as a precautionary measure, the reaction sequence was kept as **2a** to be added in the mixture of dichloromethane and acetic anhydride (3.0 equiv) at cooled condition (acidic media) and thereafter minimum mole of pyridine (0.9 equiv) was added to complete the reaction. Further, the reaction mixture was simply worked up by quenching with water and extracted in dichloromethane followed by distillation afforded the residue. The residue was further treated with n-heptane solvent by heating at 60-70°C for 1 h and cooled to room temperature. The solid precipitate filtered at 5-10°C afforded 80% yield and was of HPLC purity showing 92% purity with 8% amounting to the formation of undesired diastereomer for **2b**. Later, the oxathiolane acetate **2b** was taken in solution form as such without isolation for further glycosylation step at large scale.

Further, we started our investigation on the glycosylation step which is quite crucial to the overall synthetic approach using starting material oxathiolane acetate **2b** as such without any further crystallization or classical resolution. Also, preliminary requirement for glycosylation was a silylation of cytosine completely, which usually described in acetonitrile or chlorinated solvents. In this case, a mixture of bis (trimethylsilyl) acetamide (BSA) as a robust silylating agent and acetonitrile solvent in appeared crucial due to lesser reactivity of cytosine. A screening of solvent was also simultaneously performed, and it was found that BSA was suitable for silylation of cytosine in presence of acetonitrile. Acetonitrile further allowed for better reactivity and solubility that permits the silylation at room temperature in a few minutes. However, use of expensive BSA and acetonitrile would increase the overall process cost at large scale. Silylation with hexamethyl disilazane (HMDS) is also most commonly used method with acid catalysis.[8] The addition of

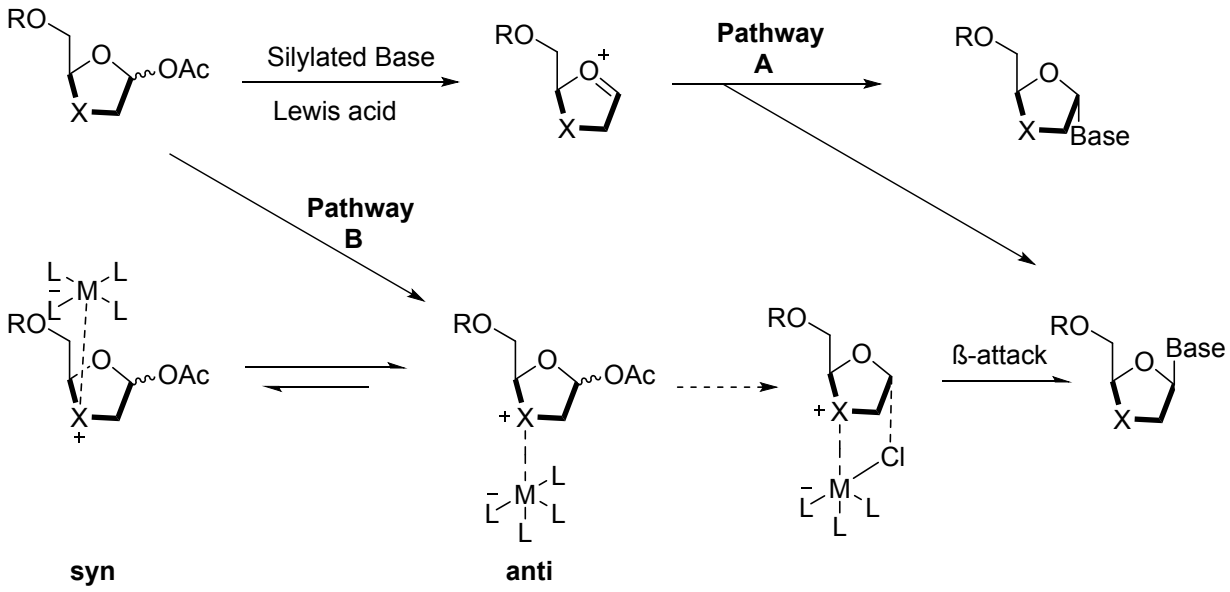
catalytic amount of trimethylsilyl chloride (TMSCl) has been known for silylation of cytosine, hence, this reagent (HMDS with catalytic TMSCl) was used in our method as shown in Scheme 4.

The primary step of each glycosylation reaction is to activate the leaving group, which usually occurs through its interaction with an activator/promoter or, a catalyst more rarely. Reactions of anomeric mixtures of oxathiolane acetate **2b** with silylated cytosine and practically any ordinary LA caused to the formation of inseparable mixture of N-glycosylated anomers. Since only β-isomer demonstrates the advantageous biological activity, any efficient methodologies required the use of a stereoselective nitrogen glycosylation. Tse et al.[4a] reported a practical and efficient preparation of a therapeutically essential nucleoside (3TC), based on a novel diastereoselective N-glycosylation procedure. It is noteworthy to mention that, the high stereo selectivity of the N-glycosylation reaction involving chirally pure oxathiolane acetate **2c** was noticed only when TMSI was applied as the promotor. They used highly expensive and non-stable (even though more effective) TMSI reagent for N-glycosylation reaction starting with oxathiolane acetate **2c** having (2R, 5R)-configuration (Scheme 1) that gave β-isomer in the ratio β: α = 23:1. This β-selectivity could be because of stabilization of oxonium ion by neighboring l-menthyl ester group by means of anchimeric assistance. More recently, Caso et al.[5a] reported the use of silanes (PMHS or Et_3SiH) in combination with iodine (I_2) as novel N-glycosylation reagents for the synthesis of 1, 3-oxathiolane nucleosides lamivudine. These systems (acts as sources of anhydrous HI) were invented to behave as substrate activators and promoters for N-glycosylation reactions. Also, the type of the cytosine protective group at the N^4 position further linked to the outcome of the stereo chemical reaction. But in this case nucleobase protection was an additional requirement for the improvement in the α/β ratio of a nucleoside product. Mandala et al.[5b] also described the use of pyridinium triflate as a novel N-glycosylation reagent first time at high temperature in acetonitrile

solvent. However, in all the cases (TMSI, Silane/I_2 and pyridinium triflate-Mediated N-glycosylation reaction) the particular separation of the isomers of **2b** was mentioned as compulsory for ensuring the target nucleosides with high optical purity.

In 1991, W. B Choi et al.[5c] reported a highly stereoselective base-linking reaction that operated through in situ formation of a complex between an appropriate cyclic precursor and a suitable LA (Scheme 3). The use of stannic (IV) chloride (2 equiv) and dichloromethane as a solvent at ambient temperature condition resulted in the exclusive formation of the β-nucleoside product for lamivudine. This recommends a potentially general approach to the regulation of the stereochemistry in N-glycosylation. The N-glycosylation reaction stereoselectivity could be rationalized based on a preferential interaction between heteroatom and LA.

Scheme 3. Concept of in situ complexation of LAs for controlling nucleoside stereochemistry[5c]



Where R = tert-butyldiphenylsilyl, X = S or O, M = Sn, L= Cl

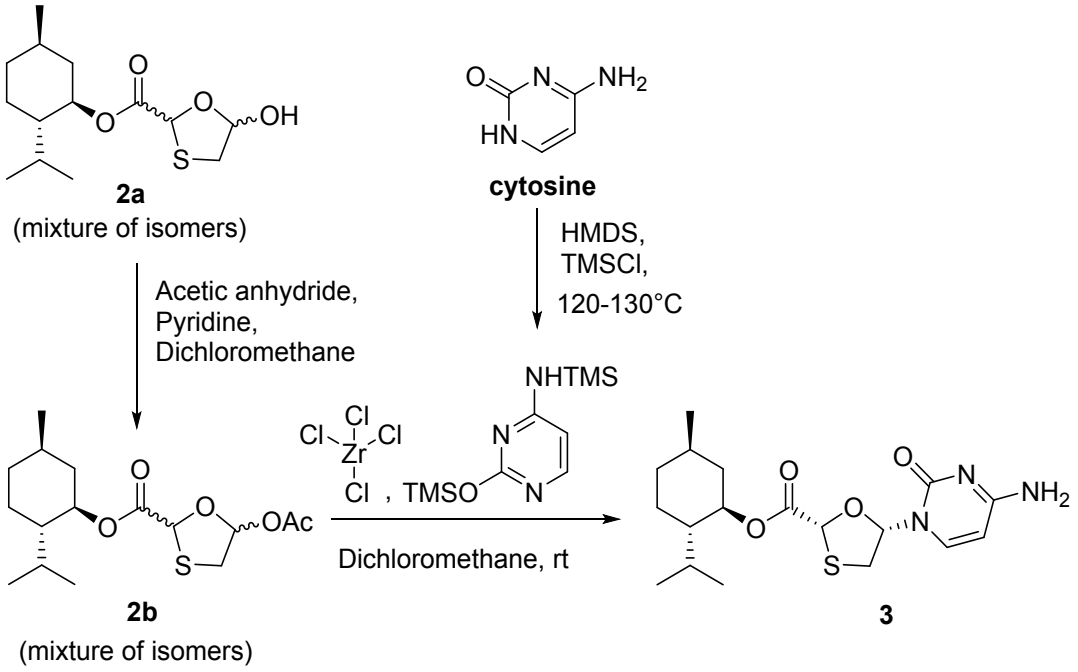
The use of TMSOTf and TMSI, the exclusive purpose of which was to produce an oxonium ion, should adhere to pathway A and results in no stereo controls. However, in examples where LA

could form precomplex to a heteroatom of a ring (i.e., pathway B), selectivity in diastereofacial manner could be accomplished by minimizing destabilization of 1, 2-steric interactions with the aid of complexation anti to the protected hydroxy methyl substituent. At any event, this complexation ought to considerably restrict the methodology of the silylated base moiety to the α-face. The possibility of the associated metal that delivers one chloride ligand to the α-face of carbonium ion could then undergo S_N2 attack and resulted the formation of the β-N-nucleoside.

In the context of our interest in new procedures of N-glycosylation, the investigation for more competent, stereoselective, and cost-effective alternatives to the above mentioned strategies for large scale synthesis of lamivudine caused a great concern to scrutinize the synthetic ability of the N-glycosylation reagents on the basis of above in situ complexation concept of any non-explored LA. Hence, our investigation have been directed towards the search of an appropriate catalyst including hitherto non-explored LA for the said glycosylation which not only gives high chemical yields but also high stereo specificity. Ability of LA to complex with a lone pair of electrons is different for different acids. Hence, one could expect different chemical prospective. Although, in the literature number of LAs like $SnCl_4$, $TiCl_4$, Cu-Triflate, $TiCl_3$ (OiPr), TMSI has been used, they are not effective and none of them gave desirable results either in specificity or chemical yield. Each LA unlike Brønsted acid gives entirely different course of reaction[9a, b]. Contrary to Brønsted acid, where pKa is the essential factor for its efficacy, amongst the LA, the ability of complexing with a host molecule and extent of positive charge generation in the acceptor atom (e.g. complexing with carbonyl oxygen) determines their reaction feature however, it is not predictable. Hence, identifying an appropriate and ideal LA for a specific set of reaction itself becomes a complex phenomenon involving skill and requires extensive experimentation. This would be evident from the facts observed during our experiments using a number of LAs such as

$SnCl_4$, $TiCl_4$, Cu-Triflate, TMSI, $Sc(SO_3CF_3)_3$, $BF_3.OEt_2$ for the said glycosylation reaction with more than 1 mole, and surprisingly none of them gave any useful conversion. Moreover, these LAs suffer from one or more shortcomings such as toxicity, cost and managing complexity or hazards, non-feasibility towards different substituted or functionalized acylation reactants and the process complications, primarily reaction mixture extraction difficulty. Surprisingly, use of tetravalent zirconium salt such as zirconium (IV) chloride satisfies most of the requirements. Zirconium (IV) chloride ($ZrCl_4$) explored as a catalyst at a temperature, preferably between 25-30°C, to obtain a nucleoside product with cis-(-)-configuration stereoselectively almost in quantitative yield (Scheme 4). Thus, in the course of our investigation in the development of stereoselective glycosylation reaction for the efficient synthesis of lamivudine, it was discovered that the zirconium (IV) chloride, among various transition metal chlorides, is the reagent of choice for the glycosylation reaction.

Scheme 4. $ZrCl_4$-Mediated N-Glycosylation reaction



It is evident from the Table 1 (provided in entry 6), that shows that, $ZrCl_4$ gives the correct specificity and optimum chemical yield.

Table 1. Experimental conditions for optimization of different activators for N-glycosylation

entry	activators	equiv	solvent (5 volumes)	temp (°C)	time (h)	Yield (%)	β / α[a] ratio
1	Cu-triflate	1.0	dichloromethane	rt	24	*	*
2	TMSI	1.5	dichloromethane	35-40	18	51	94 / 6
3	$TiCl_4$	1.1	acetonitrile	rt	12	*	*
4	$TiCl_4$	1.1	dichloromethane	35-40	12	30	95.69/4.31
5	$SnCl_4$	1.1	dichloromethane	rt	12	*	*
6	$ZrCl_4$	0.5	dichloromethane	rt	12	60%	98.15/1.85
7	$ZrCl_4$	0.5	acetonitrile	rt	12	*	*
8	$ZrCl_4$	0.5	dichloromethane	rt	12	**	**
9	$BF_3.OEt_2$	1.0	dichloromethane	rt	12	*	*
10	Sc-triflate	1.0	dichloromethane	rt	12	*	*

All these reactions were performed with **2b**, with silylated cytosine using different activators.

[a] β / α ratio analyzed by HPLC method.

*product conversion is less than 5% hence could not isolated them and could not be analyzed.

No reaction where, premixing of donor (oxathiolane acetate, **2b) and acceptor (silylated cytosine) followed by $ZrCl_4$ addition was performed.

Zirconium (IV) chloride, otherwise referred as zirconium tetrachloride ($ZrCl_4$) is a high melting solid and used as a (weak) LA in organic synthesis.[9a, b] In contrast with molecular $TiCl_4$, powdered $ZrCl_4$ comprises a polymeric structure in which every Zr element is octahedrally coordinated. This discrepancy in structure is liable for the striking differences in their properties. $TiCl_4$ is distillable, but zirconium (IV) chloride is a solid powder with a high liquifying point. Most zirconium compounds are relatively less toxic, easily operated, cost effective and can withstand small amount

of moisture. Zirconium (IV) compounds have high coordinating ability that offers them with an adequate LA behavior. Zr4+ has a high value of charge to size ratio (Z2/r = 22.22 e2/m10), relative to the majority of main group elements and lighter and heavier transition metal ions like Li+ (Z2/r = 1.35 e2/m10), Bi3+ (Z2/r = 8.82 e2/m10), In3+ (Z2/r = 11.39 e2/m10), Sc3+ (Z2/r = 12.33 e2/m10), Fe3+ (Z2/r = 13.85 e2/m10), V3+ (Z2/r = 14.06 e2/m10) and Al3+ (Z2/r = 16.98 e2/m10) and is comparatively softer hard acid[10]. This fundamental chemical characteristic of zirconium (IV) chloride has made it an encouraging catalyst with strong acid nature, easy to handle, and lower scale of toxicity. In addition, its relatively high-level abundance and therefore lower cost offer advantageous use in glycosylation.

$ZrCl_4$-catalyzed efficient Ferrier glycosylation procedure stated the mild reaction conditions and shorter reaction time in case of pseudoglycals synthesis and higher stereoselectivity and yields for synthesizing the 2,3-unsaturated glycosylated compounds.[11a,b] The complete stereocontrol approach at anomeric center of 1-thio-D-glucopyranose and higher yields of corresponding condensed compounds were also noticed for the preparation of O-glycosyl disaccharides using $ZrCl_4$ as a LA.[12] Zirconium (IV) chloride is an ideal LA since it is stable, an efficient, low-cost, and an appropriate catalyst. Most of the glycosylation reactions are achieved by pre-mixing donor and acceptor at the same time and further by adding the promoter for donor activation.[13] This strategy did not work in case when, $ZrCl_4$ was used as promotor. Thus, in the present work, we have used alternate strategy which preactivates glycosyl donor initially in the absence of acceptor i.e. preactivation of oxathiolane acetate **2b** with $ZrCl_4$ and then treated with silylated cytosine for glycosylation reaction.

Investigations were undertaken with different moles of $ZrCl_4$ per mole of substrate, e.g. 0.1 equiv, 0.3 equiv, 0.5 equiv and 1.0 equiv for glycosylation reaction, but best yield was obtained

with 0.5 equiv which is less than the mole equivalent of other catalyst used so far for the glycosylation reaction. The mole wise experimental studies of $ZrCl_4$ are comparatively shown in Table 2. Nucleobase variations also gave positive results in case of 5-Fluorocytosine (Table 2. entry -5).

Table 2. Optimization of mole ratio zirconium (IV) chloride as an activator for N-glycosylation

entry	$ZrCl_4$ (equiv)	solvent (5 volumes)	temperature (°C)	time (h)	yield[a] (%)	β / α[b]
1	0.1	dichloromethane	rt	12	29	98.53/1.47
2	0.3	dichloromethane	rt	12	47	97.50/2.50
3	0.5	dichloromethane	rt	12	60	98.15/1.85
4	1.0	dichloromethane	rt	12	58	98.45/1.55
5[c]	0.5	dichloromethane	rt	12	65	98.26/1.76

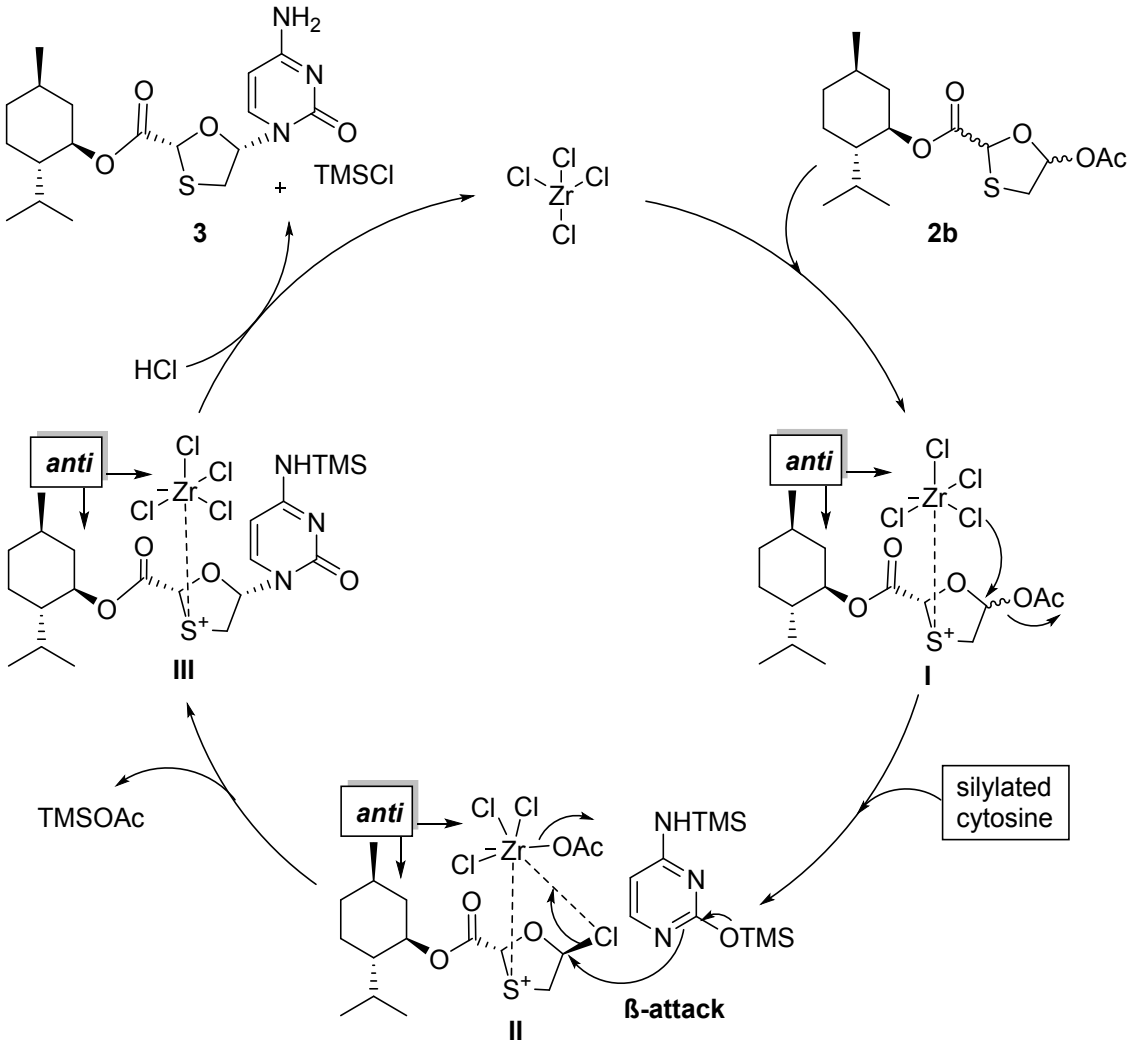
[a] Isolated yield, [b] β / α ratio analyzed by HPLC method, [c] silylated 5-fluorocytosine as a nucleobase is used instead of silylated cytosine.

The notable characteristics of this catalytic glycosylation procedure are associated with lesser reaction times, mild conditions, quantitative yields, better conversions, clean reaction profiles, tolerability of different functional groups, easy isolation of product and operational simplicity.

On the basis of earlier studies in the literature[5c], a reasonable mechanism for the stereoselective N-glycosylation using $ZrCl_4$ is proposed, as shown in Scheme 5. $ZrCl_4$ could form a precomplex to oxathiolane ring heteroatom sulphur (intermediate **I**). The stereofacial selectivity could be accomplished by minimizing the destabilization of 1, 2-steric interactions with the aid of complexing $ZrCl_4$ anti to the l-menthyl ester functionality. Additionally, intermediate **II** could be generated where the Zr metal gives one of its ligand (chloride) to the α-face while removing acyl group as shown in intermediate **I**. The subsequent α-chloro derivative **II** could then follows S_N2-like reaction path with silylated cytosine, constructing the glycosidic C-N bond and results the

formation of β-selective nucleoside intermediate **III**. Finally, deprotection of trimethylsilyl (TMS) group with HCl and removing $ZrCl_4$ catalyst from intermediate **III** produces **3**. This precomplex formation should significantly inhibit the approach of the silylated cytosine to the α-face, at the very least.

Scheme 5. Plausible reaction mechanism for stereoselective N-glycosylation using $ZrCl_4$



Surprisingly, a chiral auxiliary l-menthyl group is needed to be used in executing the process however, the use of optically pure intermediate **2c** and high temperature condition in the

glycosylation step is not a necessary limitation of the present work. The present process is demonstrated for preparing lamivudine at an industrial scale.

Reaction calorimetry evaluation for N-glycosylation step. When considering the migration of a reaction procedure from lab scale to large scale, it is necessary to identify how much heat gets released/dissipated. At a low scale level, the heat produced may not trigger a major concern, however when scaling up, that heat could slowly get build up and be extremely unsafe in turn could result runaway reactions. Since the use of zirconium (IV) chloride as a LA to be performed at large scale, thorough assessment of that heat exchange, stability of the N-glycosylation reaction, its quenching and work up procedure also must be carried out. Safety investigations to determine the onset of degradation points were performed using differential scanning calorimetry (DSC) and reaction calorimetry. The thermal behavior during the preparation of compound **3** from **2b** solution was studied in a reaction calorimeter.

As per protocol of a 50 gm batch input of oxathiolane acetate (**2b**), dichloromethane (250 ml) and 20.2 g of $ZrCl_4$ was charged in single portion while temperature of the reaction mass was maintained at 25°C and stirred for 15 mins. Then, the reaction mass temperature was raised to 30°C. To this 234 g of silylated cytosine reaction mass was charged at 30°C. Sample was removed to assess the decomposition of reaction mixture by DSC. The reaction mass was maintained at 30°C for ½ h. Further, the temperature was raised to 35°C and maintained for 4 h. Then, 200 ml of methanol was added to the reaction mass at 35°C and continued maintaining for ½ h and the decomposition of reaction mixture was checked by DSC by removing samples at different operating parameters.

The above-mentioned protocol was subjected to the reaction calorimeter evaluation utilizing Mettler make SV 01 RC1 reaction calorimeter. The heat changing trends indicated by reaction

calorimetry (RC1) during charging of $ZrCl_4$ followed by silylated cytosine reaction mass and quenching with methanol are shown in Figure 2. The evolution of heat of reaction was calculated using Q flow (exchange from jacket), Q accumulation (of reaction mass), Q dosing (heat inputs by dosed reactants) and Q loss (to surrounding from reactor). Initialization parameters and plots of various parameters, specific heat determination and heat of reaction are given in Table 3.

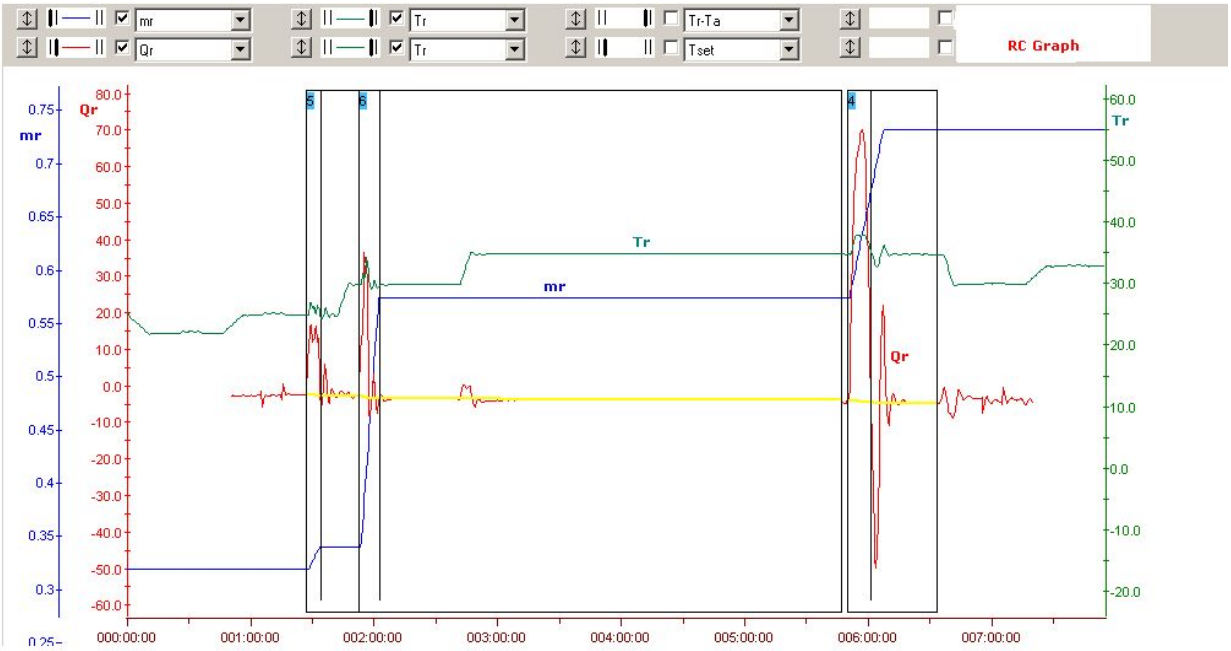


Figure 2. Reaction calorimetric measurement of the N-glycosylation reaction. Tr: reaction temperature (green peak), mr: mass of reaction (blue peak), Qr: heat of reaction (red peak)

Table 3. Reaction calorimetric data for the N-nlycosylation reaction (addition of $ZrCl_4$ and cytosine reaction mass and quenching the reaction with methanol)

unit operation reagents addition	time (min)	reaction mass (kg)	process temperature (°C)	reaction enthalpy ΔH (kJ)	heat of reaction per kg reaction mass (kJ/kg)	specific heat capacity Cpr at start kJ/ (kg. K)	ΔT_{ad} (°C)	MTSR (°C)
$ZrCl_4$	26	0.319	25	6.415	20.1	1.82002	11.05	36.05

cytosine mass	235	0.339	30	8.045	23.6	1.82009	13.03	33.03
methanol	43	0.573	35	28.922	50.4	1.82082	27.71	62.71

Heat of reaction: As shown in Table 3. the heat evolved for zirconium (IV) chloride addition was 6.415 kJ (exotherm) for 0.319 kg of initial reaction mass i.e. 20.1 kJ/kg of initial reaction mass. This could lead to adiabatic temperature rise (ΔT_{ad}) of 11.05°C. The overall heat evolved for cytosine reaction mixture addition was 8.045 kJ (exotherm) for 0.339 kg of initial reaction mass i.e. 23.6 kJ/kg of initial reaction mass. This could lead to ΔT_{ad} of 13.03°C. The heat evolved for methanol addition was 28.922 kJ (exotherm) for 0.573 kg of initial reaction mass i.e. 50.4 kJ/kg of initial reaction mass. This could lead to ΔT_{ad} of 27.71°C.

DSC findings for the reaction mixtures tested at various reaction stages are shown in Table 4. DSC of the reaction mixture samples taken after $ZrCl_4$ addition, cytosine reaction mass addition and quenching reaction mass with methanol (before and after) indicated that their exothermic decomposition points were not much differ from the exothermic decomposition points of starting materials and product formed. It was in the range 215 to 274°C.

Table 4. DSC results at different operating parameters for the reaction mixtures.

Sample	endotherm onset (°C)	exotherm onset[a] (°C)	heat of evolution J/g
2b in dichloromethane	-	231.9	32.0
2b in dichloromethane + $ZrCl_4$	-	215.5	33.1
cytosine reaction mass	234.8	257.8	107.9
cytosine solution addition	-	274.4	33.5
before quenching reaction mass	216	262.0	130.6
after quenching reaction mass	-	255.0	89.6

[a] In the Supporting Information, plots illustrating the DSC results are provided

As depicted in Table 3, the maximum temperature of synthetic reaction (MTSR) during addition of $ZrCl_4$, cytosine reaction mass and methanol charging, calculated as summation of the process temperature and adiabatic temperature rise (ΔT_{ad}), were 36.05°C, 33.03°C and 62.7°C respectively. The onset of decomposition temperature recorded above 215°C as calculated by DSC data of reaction mixtures at various stages, shows that the reaction is safe even though cooling system fails, since the MTSR values are much lower than the onset decomposition temperature. Secondary reactions, such as decomposition with exotherm, can only occur when the MTSR value is greater than their onset temperature. Based on heat evolution data maximum heat flow calculated as depicted in below table 5.

Table 5. Maximum heat flow results

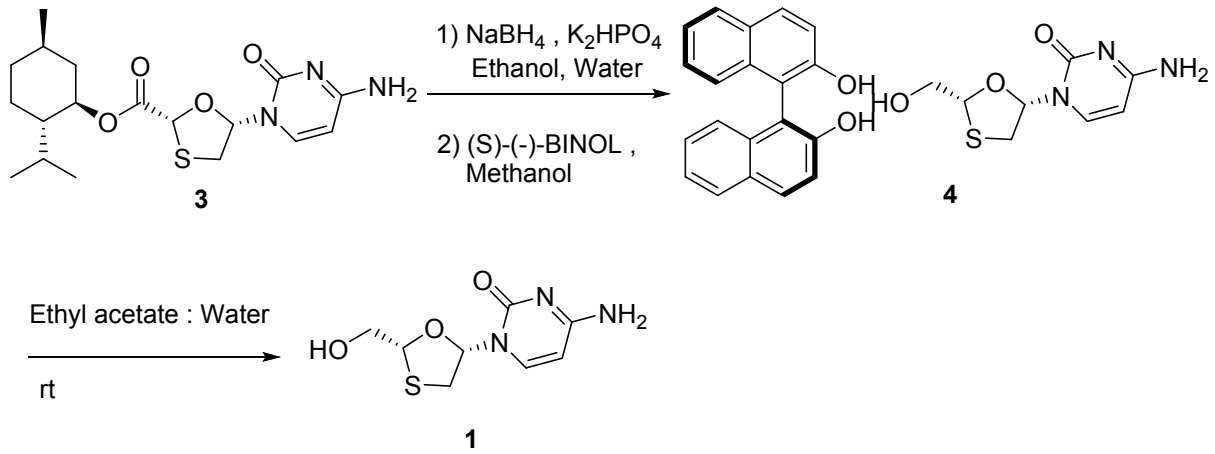
unit operation	reaction mass	maximun heat flow (Q_{max})
$ZrCl_4$ (half addition)	0.323 kg	21W/0.323 kg
Cytosine mass addition (initial phase)	0.379 kg	42W/0.379 kg
Methanol addition (1/3rd portion)	0.632 kg	74W/0.632 kg

Based on the heat evolution data, reaction during methanol addition was instantaneous. Maximum heat flow (Q_{max}) observed during methanol addition was 74 W/0.632 kg of reaction mass. This was observed when almost 1/3rd of methanol was added.

The equipment setup available for a common-purpose reactor could be used in the manufacturing process as per the RC1 trend data. The reaction was scaled up to 174 kg of input in a 3000 L glass line reactor with an adequate jacketed cooling system and outlet connected to the standard scrubber. The addition of zirconium (IV) chloride started at 25.5°C and temperature raised up to 32.0° (time taken for addition was 35 min) recorded by means of a digital temperature indicator, verifying the reflection as in the RC1 experiment and addition of silylated cytosine reaction mass

showed temperature rise from 25.0° to 31.0°C (time taken for addition was 60 min) and addition of methanol to the reaction mass after completion of reaction recorded the temperature rise as 27.2°C to 40.1°C.

Scheme 6. Synthesis of lamivudine



The nucleoside analogue of cis configuration **3** obtained from the glycosylation reaction of the compound **2b** with cytosine thereof was reduced to give a specific stereoisomer lamivudine **1**. Hydride reducing agents like lithium aluminium hydride, lithium borohydride or sodium borohydride are found suitable for such reduction reactions. According to previous reports, lithium aluminium hydride [4a] provides the subsequent nucleoside **1** after stirring 1 h at 0°C, however, gave low yield owing to the degradation during the reaction. It is also reported that stereo integrity is retained utilizing sodium borohydride[13] as the reducing agent in the presence of a buffer like dipotassium hydrogen phosphate. Therefore, this method was used for reduction of l-menthyl ester group to provide the target lamivudine **1** (Scheme 6). After reduction using sodium borohydride and neutralization with aqueous HCl, a practical problem cropped up as lamivudine exhibits high solubility in aqueous media, making it difficult to isolate effectively. The side products salts such as NaCl, KCl and boric acid were in dissolved form along with lamivudine in the aqueous layer. In polar solvents, lamivudine is well isolated from solution by forming a salt with poor solubility.

If required, the water-insoluble salt could then be converted to the free base, or to a various salt thereof by usual methods. As per report salicylate salt[14] is particularly suitable for this purpose, but salicylate salts do not allow to enrich the purity. In the present work, we used our earlier reported[15] (S)-BINOL Co-crystal strategy as lamivudine obtained according to the process was having purity about 98%, i.e. there is 98% ee and 2% of its cis- (+)-isomer. Therefore, enantiomeric purity was further enriched using (S)-BINOL which gave 99.8 % ee. Lamivudine-(S)-BINOL complex compound, **4** obtained was found water insoluble at lower temperature therefore the inorganic salts were removed by simple water slurry to the **4.** It was further easily got separated from (S)-BINOL with extraction procedure in water and ethyl acetate at room temperature. (S)-BINOL reagent was later completely recovered and recycled from ethyl acetate. Lamivudine thus obtained in aqueous layer was isolated by distillation and recrystallized from denatured spirit and acetone mixture in highly pure form.

This stereoselective method for the synthesis of 1, 3-oxathiolane nucleoside analogue using zirconium (IV) chloride ($ZrCl_4$) as an activator in N-glycosylation step[16] was found to be very effective.

CONCLUSION:

The present work aimed in developing a large scale, operationally convenient and scalable synthetic process for preparation of lamivudine **1** reliably reproducible on, at least 33.3 kg scale. The key N-glycosylation step resulted in a stereoselective β-anomer of lamivudine using $ZrCl_4$ starting even with chirally impure substrate where, previous methods fails to attain this. A plausible mechanism was also proposed and discussed for this stereoselective N-glycosylation reaction. The safety of process for the N-glycosylation step was evaluated in detail using DSC and RC1 studies. Furthermore, a problematic isolation of water soluble lamivudine free from inorganic

salts was achieved. The synthesis, carried out at kilogram scale, provided a material that satisfied all regulatory requirements.

EXPERIMENTAL SECTION:

General Remarks: All reagents and solvents purchased commercially and used as received without further purification. Melting points were recorded using an automated melting point apparatus. Specific rotations were measured using a JASCO P-1030. Metrohm instrument is used to determine moisture content by the Karl Fischer titration method. RC1 and DSC studies were carried out at Intertek India Pvt. Ltd, Mumbai: Mettler make SV 01 800 ml capacity all glass reactor equipped with a flow agitator type propeller, jacketed with controlled temperature circulator, condenser, addition funnel and probes.

HPLC analysis was performed on SHIMADZU LC-2010 or UltiMate 3000. HPLC system was equipped with a chiral column (Chiracel OJH, 4.6mm × 250 mm, 5µm). ^{1}H and ^{13}C NMR spectra were measured in CD_3OD or $CDCl_3$ on a Bruker spectrometer (AVANCE-III HD) at frequencies of 500 and 125 MHz, respectively. Proton chemical shifts (δ) are measured in ppm with respect to tetramethylsilane (TMS) (δ) 0 ppm or to residual protons in the solvent as internal standard. Mass spectra were recorded with the ACQUITY UPLC PDA detector utilizing electron spray ionization (ESI).

(1R, 2S, 5R)-2-isopropyl-5-methylcyclohexyl 5-hydroxy-1, 3-oxathiolane-2-carboxylate (2a) : A mixture of toluene 500 mL, l-menthyl glyoxylate monohydrate (100 g, 0.434 mol) and acetic acid (10.5 g, 0.174 mol) was heated to reflux at about 110-111°C. The reaction heating maintained for 3 h to remove water azeotropically. After observing the complete removal of water, cooled the reaction mixture up to 90°C. Distilled off the solvent under vacuum not more than 90°C till about 300 mL solvent remained in the flask. Further cooled the reaction mass to 40-50°C and charged 1, 4-dithiane 2, 5-diol (33.8 g, 0.222 mol). The reaction mixture was heated further for 2

h at 85-90°C. The reaction progress monitored by HPLC method. After completion of the reaction, mixture was filtered through celite bed to remove any traces of undissolved particles. The filtrate was then cooled to 0 to 5°C. To this filtrate, a solution of trimethylamine (6 mL) and n-heptane (600 mL) was added slowly at 0 to 5°C. The resulting slurry was stirred for 6 h at 0 to 5°C. The solid suspension then filtered through Buchner and sucked dried under vacuum. The wet cake was rinsed with precooled n-heptane (200 mL). The solid material was dried in a vaccum oven for 16 h at 45°C under vacuum to provide 100 g of a white solid **2a**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.02 (d, J=4.5 Hz, 1H), 5.92 (bs, 1H), 5.56-5.58 (d, J=5.5 Hz, 2H,), 4.70-4.77 (td, J=5.5 Hz, 3H) , 3.29-3.33 (m, 3H), 3.14-3.17 (dd, J_1= 14, J_2=3, 1H), 3.05-3.15 (d, J=13.5, 1H), 1.94-2.05 (m, 4H), 1.68-1.72 (bd, J=2.5Hz, 4H), 1.41-1.52 (m, 4H), 1.02-1.08 (m, 4H), 0.91-0.93 (m, 14H), 0.75-0.80 (m, 6H) ppm. ^{13}C NMR (125 MHz,$CDCl_3$): δ 172.4, 172.2, 169.2, 103.2, 103.1, 101.2, 101.1, 80.2 , 80.1, 78.0, 77.8, 76.9, 76.7, 75.9, 75.8, 47.0, 46.8, 46.7, 40.6, 40.3, 40.1, 39.9, 38.4, 34.1, 34.0, 31.4, 31.3, 26.2, 26.1, 26.0, 23.3, 23.2, 21.9, 20.7, 16.2, 16.1 ppm. ESI-MS m/z: calcd for: $C_{14}H_{24}O_4S$: 288.1, found [M + NH_4]: 306.0; Loss on drying at 60°C under vacuum was found to be 0.3% w/w. Specific optical rotation -57° (10 mg/mL in methanol at 20°C).

(1R, 2S, 5R)-2-isopropyl-5-methylcyclohexyl 5-acetoxy-1, 3-oxathiolane-2-carboxylate (2b): To a cooled solution of the dichloromethane 250 mL and acetic anhydride (50 mL, 0.52 mol), **2a** (50 g, 0.17 mol) was added at 5-10 °C. The solution was further cooled to 0°C and pyridine (12.5 mL, 0.15 mol) was added dropwise within 60 min and stirred for 1 h. The progress of reaction monitored by thin layer chromatography. The reaction mixture was quenched into water (250 mL and phases were separated. The organic phase washed with water (250 mL). The desired product is in the organic layer. The aqueous layer was extracted again one more time. The combined organic phase was distilled under vacuum to get oily residue. To the oily residue n-heptane (500

mL) was added. The mixture was heated at temperature 65-70°C for 1 h. The reaction mixture further cooled to room temperature and then at 0-5°C. The solid suspension was held for 30 min at same temperature. The solid suspension then filtered through Buchner and sucked dried under vacuum. The wet cake was rinsed with cold n-heptane (75 ml). The solid material was dried for 16 h in a vacuum oven at 40 °C under vacuum to give 45 g of a white solid **2b**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.57-6.72 (d, J=5 Hz, 1H), 5.55-5.56 (s, 1H), 4.63-4.70 (td, J_1= 13.5, J_2=5.5Hz, 1H), 3.36-3.40 (dd, J_1=14.5Hz, J_2=5.1Hz, 1H), 3.09-3.21(d, J=15, 1H), 2.04 (s, 3H),1.86-1.95 (m, 2H), 1.61-1.64 (bd, J=14 Hz, 2H),1.43 (bd, J=4.5Hz, 1H), 1.32-1.38 (bt, J=14.5Hz,1H), 0.91-1.03 (m, 2H), 0.79-0.88 (m, 7H), 0.69-70 (d, J=8.5Hz, 3H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.9, 169.4,168.58, 168.41, 99.55, 98.97, 80.09, 79.7, 79.5, 75.8,75.73,75.6, 46.8, 46.7, 46.6, 40.4, 39.9, 37.4, 37.0, 36.8, 33.9, 31.1, 25.9, 25.8, 25.6, 23.0, 22.9, 21.8, 20.9, 20.6, 20.5, 16.0 ppm. ESI-MS : m/z calcd for: $C_{16}H_{26}O_5S$:330.2 Found: ES^- [M + NH_4^+]: 348.2, and ES^+ [Dimer M +Na^+]:683.2, Specific optical rotation was –62° (c, 0.51, $CHCl_3$) and mp was found to be 105.2°C

(1R, 2S, 5R)-2-isopropyl-3-methylcyclohexyl (2R, 5S)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-1, 3-oxathiolane-2-carboxylate (3): Part a) :Dichloromethane (870L) having water content not more than 0.07% w/w was charged into the glass line reactor under N_2 atmosphere. Acetic anhydride (174 L, 1823.35 mol) was added to the reactor at room temperature. The reaction mixture was further cooled to 0 to 10°C and **2a** (174 kg, 603.2 mol) was charged and stirred for 10 mins. The reaction mixture shows temperature decrease after addition of **2a** (i.e. endothermic reaction). Pyridine (43.5 L, 561.15 mol) was slowly added to the above reaction mixture at 0 to 10°C. The temperature of reaction was allowed to raise up to 10 to 15°C and maintained for 2 h. The reaction progress was monitored by HPLC method to control the content of **2a** below 2.0 % limit. After completion of reaction, the reaction mixture was quenched with water (870 L) and

stirred at 10 to 25°C for 3-4 h. Further, settled the reaction mass and phases were separated. The required product is in the organic phase. The organic phase was then charged into the reactor and added 10% aq. acetic acid solution (870 L). The reaction mixture was stirred, settled and phases were separated. The organic phase was again charged into the reactor and added 10% sodium chloride solution (870 L). The reaction mixture was stirred and settled for 30 min and phases were again separated. The water content of organic phase was analyzed and should be not more than 0.2% w/w, if it was more then solvent had to be distilled out azeotropically and controlled the water content well within the limit not more than 0.2% w/w. This organic layer containing product oxathiolane acetate **2b**.

Part b): Silylation of Cytosine procedure: Meanwhile, hexamethyldisilazane (402 L), cytosine (100.67 kg, 906.12 mol) and trimethylchlorosilane (50 L) were charged into the separate stainless steel reactor under nitrogen atmosphere. This reaction mixture was heated and maintained to 120-130°C till a clear solution obtained. Distilled off the excess solvent under vacuum below 120°C to get white residue. Vacuum was released carefully with nitrogen gas to avoid any moisture contact to this reaction mass. Dichloromethane (348 L) was added to the reaction mass and stirred for 30 min. This silylated cytosine mass was kept under nitrogen atmosphere for further use.

Zirconium (IV) chloride (70.3 kg, 301.7 mol) was added into the organic phase obtained in Part a), and stirred under N_2 atmosphere for 15-30 mins at 20-30°C. After the stirring was completed, the silylated cytosine mass obtained in part b) was charged within 30 to 40 mins at 20 to 40°C by applying continuous cooling to the reactor to avoid exothermic reaction. After completion of addition, the reaction mixture was stirred and maintained for 12 h at room temperature. The progress of reaction was monitored by HPLC method to control the content of **2b** below 4.0 % limit. The reaction mass was then treated with methanol (690 L) by applying cooling and

temperature control (between 20° to 40°C) to avoid exothermic reaction. The reaction solvent was then distilled out under vacuum below temperature 60°C till stirrable volume remains in the reactor. Methanol (174 L) was again charged to the reaction mass and co-distilled to remove traces dichloromethane. Further, after cooling the reaction mass, cyclohexane (348 L) and 10% aq. HCl (1218 L) was added. The resulting solid was stirred for 30 to 60 min at 20 to 30°C. The solid suspension was then centrifuged and kept under spinning till complete expulsion of traces mother liquor was achieved. The wet cake was then spray washed with cyclohexane (174 L) in centrifuge under spinning till expulsion of mother liquor took place. The wet cake was again spray washed with methanol and water (1:1) mixture (870 L) in centrifuge under spinning till expulsion of mother liquor. The solid wet cake was then removed from centrifuge and charged into the reactor containing water (1740 L). The reaction mixture was further treated with trimethylamine (52.2L) and pH was recorded not less than 8. The slurry mass stirred for 30 min and then centrifuged. Centrifuge was kept under spinning till complete expulsion of traces mother liquor. The wet cake again spray washed with water (174 L) in centrifuge under spinning till completed expulsion of mother liquor. The solid wet cake was then removed from centrifuge and charged into the reactor containing water (1740 L). The slurry mass stirred for 30 min and then centrifuged. Centrifuge was kept under spinning till the removal of traces mother liquor. The wet cake again spray washed with water (174 L) in centrifuge under spinning till completed expulsion of mother liquor. The wet cake was finally removed from the centrifuge and analyzed for cytosine content and related substances by HPLC method. The solid wet cake was dried for 16 to 24 h in a vacuum oven at 60°C under vacuum to provide 100.5 Kg of **3**. ^{1}H NMR (500 MHz, CD_3OD): δ 8.32-8.34 (d, J=7.5 Hz, 1H), 6.42-6.44 (dd, J_1=6Hz, J_2=5 Hz, 1H), 5.93-5.94 (d, J=7.5Hz, 1H), 5.63 (s, 1H,),4.78-4.84 (td, J_1=10.8 Hz, J_2=4.1Hz, 1H), 3.56-3.59 (dd, J_1=12.2Hz, J_2=4.7Hz, 1H,), 3.18-3.21 (dd, J_1=12

Hz, J_2=6Hz, 1H), 1.97-2.07 (m, 2H,), 1.73-1.77 (bd, J=12 Hz, 2H), 1.45-1.56 (m, 2H), 1.06-1.17 (m, 2H) ppm; 0.92-0.97(q, J=7 Hz, 7H) , 0.80-0.81(d, J=7 Hz, 3H) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ 171.1, 167.7, 157.9, 142.6, 95.9, 91.2, 79.8, 77.5, 49.4, 41.8, 36.9, 35.2, 32.7, 27.2, 24.2, 22.3, 21.0, 16.4 ppm. ESI-MS : m/z calcd for: $C_{18}H_{27}N_3O_4S$:381.17 Found: ES^-[M -1]: 380.2, and ES^+ [Dimer M +1]:763.2; HPLC purity: 98.4% with major by-product is sum of impurities at RRT about 1.01 & 1.02 observed 1.6%; chiral HPLC purity: 97.4% ; Loss on drying content: 2.6%.

4-amino-1-[(2R, 5S)-(2-hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one (S)-BINOL co-crystal (4) : To the stainless steel reactor, water (225 L) was charged and cooled to 15-20°C followed by di-potassium hydrogen phosphate (205.5 kg, 1162.45 mol) and stirred for 15-20 min at 15-30°C. **3** (150 kg, 393.19 mol) and denatured spirit (1125 L) were added to the reactor and stirred for 15-30 mins. The reaction mixture was further cooled to 10-15°C. A solution of $NaBH_4$ was prepared by dissolving 45 kg of $NaBH_4$ in the precooled (20-25°C) water (450 L) containing 4.5 L of 25% w/w aq. NaOH solution. $NaBH_4$ solution was then added to the reaction mass slowly over the period of 2 h at the temperature at 10 to 15°C. Reaction progress monitored by HPLC to control the content of **3** below 0.5 % limit. After completion of the reaction, the mass of reaction was settled, and phases were separated. The lower aqueous phase was discarded, and upper phase was treated with 50% aq. HCl solution (approx. 48 L) and pH was achieved about 4 to 5. Furthermore, pH was adjusted 6.8 to 7.2 using 10% aq. NaOH solution (35 L). The reaction solvent was then distilled out under vacuum below temperature 50°C till a stirrable volume remains in the reactor. Further, water (375 L) was added to the reaction mixture and reaction mass heated up to 55 to 60°C for 30 mins. The aqueous reaction mass further cooled to 20-30°C and washed with dichloromethane 3 times with 300 L each (By product l-menthol was extracted in this layer). The aqueous reaction mass was finally distilled out under vacuum completely and degassed

for 1 h at the temperature below 50°C. The reaction mixture co-distilled with denatured spirit (300 L) for two times and further denatured spirit (300 L) was charged into the reactor. The water content of this reaction mass was controlled below 2.0% limit. Further quantity of denatured spirit (1500 L) was charged to the reaction mass and heated to achieve temperature level at 70 to 75°C and maintained for 60 mins. The undissolved inorganic solid was hot filtered through sparkler filter at temp 55-60°C and the bed was washed with denatured spirit (75 L). The filtrate was charged into the reactor and the solvent was distilled out under vacuum at temperature below 50°C up to stirrable volume. The reaction mass was further cooled at 20 to 30°C. Methanol (1200 L) and (S)-BINOL (112.5 kg, 392.9 mol) were added to the reaction mass. The reaction mass then heated to get a clear solution at 60-65°C. The reaction mass further treated with activated charcoal (7.5 kg) and hot filtered through celite bed with sparkler filter followed by bed wash with methanol (150 L). The filtrate was charged into the reactor and the solvent was distilled out under vacuum at temperature below 50°C till 300 L solvent remained in the reactor. The reaction mass cooled at 20 to 30°C. The reaction further cooled at 0 to 5°C and stirred for 60 to 120 mins. The solid suspension was filtered through centrifuge. Centrifuge was kept under spinning till the removal of traces mother liquor. The wet cake again spray washed with precooled methanol (75 L) in centrifuge under spinning till completed expulsion of mother liquor. The wet cake was removed from the centrifuge and analyzed for enantiomer and diastereomer content by HPLC method with enantiomer content limit not more than 0.25% and diastereomer content limit not more than 0.5%. The solid wet cake was dried for 4 to 8 h in a vacuum oven at 45°C under vacuum to provide 143.06 Kg of **4**. ^{1}H NMR (500 MHz, CD_3OD):δ 8.06-8.08 (d, J=7.5 Hz, 1H), 7.83-7.89(dd, J_1=21 Hz, J_2=8.5 Hz, 4H),7.30-7.31(d, J=8.5Hz, 2H), 7.24-7.27 (t, J=1Hz, 2H,), 7.16-7.19 (t, J=8.5Hz, 2H), 7.02-7.04 (d, J=9Hz, 2H), 6.29-6.31 (t, J=5.5Hz,1H,), 5.88-5.90 (d, J=7.5 Hz, 1H,), 5.27-

5.29 (t, J=4.0 Hz, 1H), 3.93-3.94 (dd, J_1 =12.5Hz, J_2=3.5Hz, 1H), 3.88-3.89(dd, J_1 =12.5Hz, J_2=4.5Hz, 1H), 3.51-3.54 (dd, J_1 =12Hz, J_2=5.5Hz, 1H), 3.12-3.15 (dd, J_1 =11.7Hz, J_2=4.2Hz, 1H) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ 167.7, 157.9, 154.1, 142.7, 135.8, 130.5, 130.4, 129.0, 127.1, 125.8, 123.8, 119.2, 116.1,95.6, 88.8, 87.9, 64.03, 38.4 ppm. ESI-MS : m/z calcd for $C_{28}H_{25}N_3O_5S$: Found: ES^+ [Dimer M +1]:459.0 and for (S)-BINOL ; ES^- [Dimer M -1] :285.0, Chiral HPLC purity: 99.60%, enantiomer content 0.03%, diastereomer content by HPLC method was 0.42%, Loss on drying content: 0.4% w/w.

4-amino-1-((2R, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl) pyrimidin-2(1H)-one (1) : To the stainless steel reactor, water (525 L) was charged and cooled to 2 to 8°C followed by **4** (105 kg, 203.86 mol) and stirred for 60 to 120 mins. The solid suspension was filtered through centrifuge. Centrifuge was kept under spinning till the removal of traces mother liquor. The wet cake again spray washed with precooled water (100 L) in centrifuge under spinning till the expulsion of mother liquor was completed. The wet cake was removed from the centrifuge and charged into the reactor containing water (525L). Ethyl acetate (525 L) was then charged and heated with the reaction mixture at 40-45°C. The reaction mixture was then stirred to get a clear solution. The reaction mixture was further cooled and settled at 25 to 30°C. The phases were separated, and aqueous layer washed again with ethyl acetate (525 L). (S)-BINOL was recovered from ethyl acetate layer by distillation solvent (97%). Aqueous layer analyzed for (S)-BINOL content by HPLC to control its limit below 50 ppm. Aqueous layer further transferred to reactor through micron filter and the solvent was distilled out under vacuum at below 50°C till a thick mass was obtained. Denatured spirit (92 L) was added to the reactor and stirred for 15-30 min. Further, water content of the reaction mass was checked which found between 10 to 18% w/w. The reaction mass was then heated the reaction mass at 55 to 75°C to get a clear solution.

Simultaneously acetone (462 L) was charged into another reactor and cooled to -10 to -5°C. The hot reaction solution was added to this precooled acetone at -1 to 5°C. The reaction mass was again stirred for 45 to 60 min at 0 to 5°C. The solid suspension was filtered through centrifuge. Centrifuge was kept under spinning till the removal of traces mother liquor. The wet cake again spray washed with precooled mixture of acetone: water: denatured spirit (10:06:2) (52 L) in centrifuge under spinning till the expulsion of mother liquor was completed. The wet cake was removed from the centrifuge and analyzed for enantiomer content and related substances by HPLC method. Enantiomer content found not detected. The solid wet cake was dried for 4 to 8 h in a vacuum oven at 45°C under vacuum to provide 33.3 Kg of **1**. ^{1}H NMR (500 MHz, CD_3OD): δ 8.07-8.08 (d, J=7.5Hz,1H), 6.29-6.31 (t, J=4.1Hz,1H), 5.89-5.91 (d, J=7.5Hz, 1H), 5.28-5.30 (t, J=3.7Hz, 1H), 3.87-3.97 (qd, J1=12.5Hz, J2=4.1Hz, 2H), 3.50-3.54 (dd, J_1 =12Hz, J_2=5.5Hz, 1H), 3.13-3.16 (dd, J_1=12 Hz ,J_2=4 Hz, 1H) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ 167.7, 157.9, 142.7, 95.6, 88.8, 87.9, 64.03, 38.4 ppm. ESI-MS: m/z calcd for: $C_8H_{11}N_3O_3S$: 229.05 Found: ES^+ [Dimer M +1]:459.0; HPLC purity: 99.71%, enantiomer content found not detected, diastereomer content by HPLC method was 0.19%, water content: 1.56 %.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at.

^{1}H NMR, ^{13}C NMR, HPLC data, DSC data (Fig. S1 to S12) (PDF)

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Author Contributions

All authors contributed to the conceptual development of this work. All authors have given approval to the final version of the manuscript.

Notes

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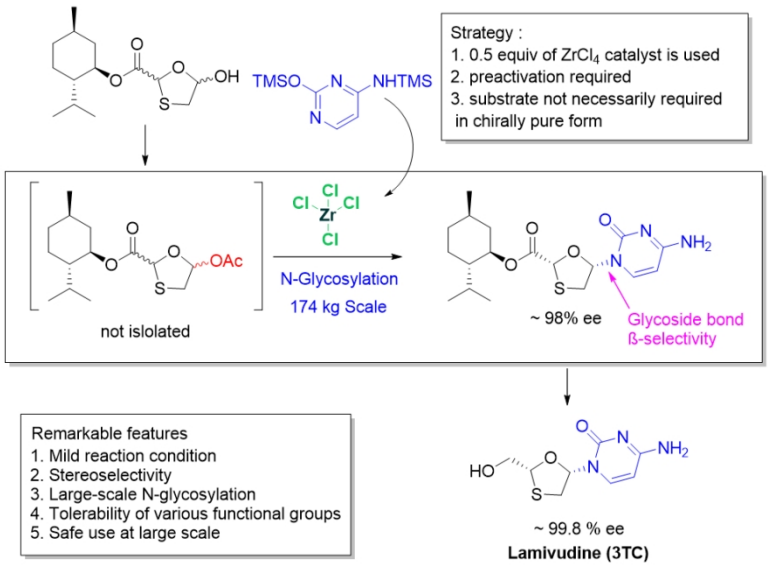

Table of Contents Graphic

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