Organic LETTERS

Letter

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Highly Stereoselective Synthesis of Lamivudine (3TC) and Emtricitabine (FTC) by a Novel *N*-Glycosidation Procedure

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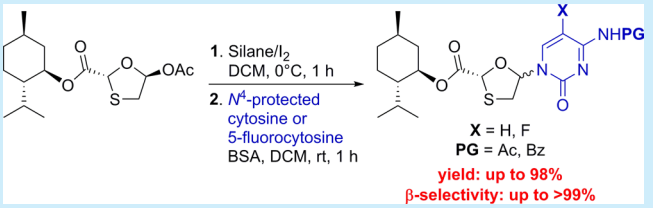
Supporting Information

ABSTRACT: The combined use of silanes (Et_3SiH or PMHS) and I_2 as novel *N*-glycosidation reagents for the synthesis of bioactive oxathiolane nucleosides 3TC and FTC is reported. Both systems (working as anhydrous HI sources) were devised to act as substrate activators and *N*-glycosidation promoters. Excellent results in terms of chemical efficiency and stereoselectivity of the reactions were obtained; surprisingly, the nature of the protective group at the N4 position of (fluoro)cytosine additionally influenced the stereochemical reaction outcome.



In the absence of effective vaccines able to control viral infections, clinical use of chemically modified nucleosides currently represents the core of any chemotherapeutic treatment aiming at a substantial and prolonged suppression of viral replication.[1,2] Because of the structural relationship with their natural counterparts, synthetic nucleosides can deeply interfere with various viral life cycles, mainly at a transcriptional level, by blocking the information flow enclosed in the viral genomes.[1a] Among sugar-modified nucleosides, those having a 1,3-oxathiolane moiety as deoxyribose bioisostere have received considerable attention over the last two decades, owing to their remarkable antiviral properties, especially as reverse transcriptase inhibitors (RTIs).[3−5] As result of the powerful biological activities and favorable toxicological profiles,[3] the two oxathiolane nucleosides Lamivudine (3TC, **1**) and Emtricitabine (FTC, **2**) have been licensed for the treatment of human immunodeficiency viruses (HIV-1 and HIV-2) and hepatitis B virus (HBV) infections, whether administered individually or in combination with other inhibitors[5,6] (Figure 1). In addition, a number of other promising oxathiolane nucleosides including Apricitabine (ATC, **3**) and Racivir (RCV, **4**) (Figure 1) are currently undergoing clinical evaluation as antiretroviral agents.[7,8]

Over the years, the increasing clinical request for oxathiolane nucleosides has justified the development of a wide variety of synthetic approaches allowing their preparation in high purity and on large scale.[4,9,10] Despite their structural simplicity, access to these compounds has represented a major synthetic challenge, because of (a) the need for a chemistry enabling stereochemical control of two potentially epimerizable stereocenters and (b) the need for stereoselective *N*-glycosidation methodologies required in the absence of directing groups adjacent to the glycosidation site.

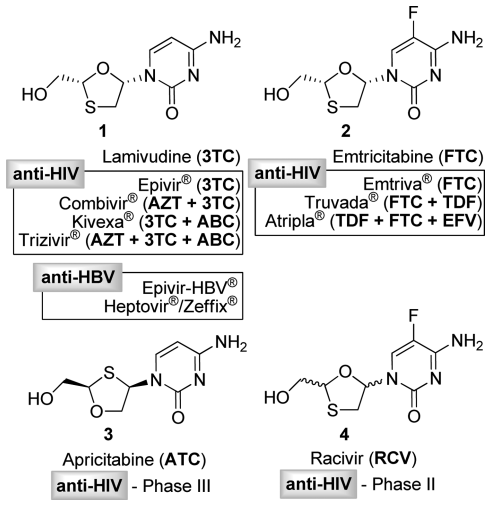


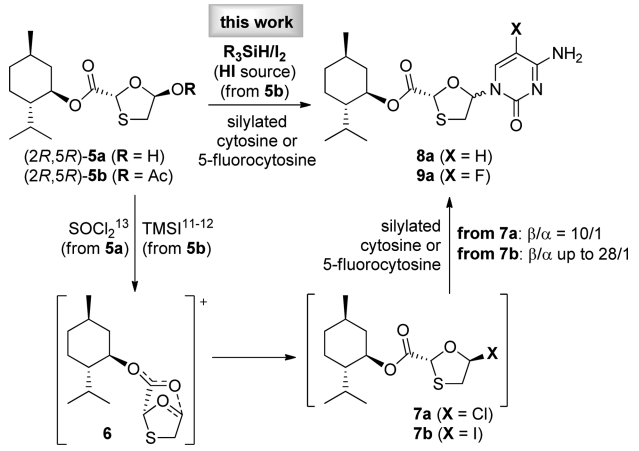
Figure 1. Oxathiolane nucleosides currently approved or undergoing clinical evaluation as antiviral agents.

Some among the most efficient approaches aimed at the stereoselective oxathiolane nucleoside synthesis have been focused on preparation of nucleoside precursors **8** and **9** (Scheme 1).[11−15] One of the methods of choice for the industrial production of 3TC and FTC follows the procedure suggested by Whitehead et al.,[13,15] which involves the reaction between L-menthyl ester-containing (5*S*)-5-chlorooxathiolane **7a** (prepared from 5-hydroxyoxathiolane **5a** with 2*R*,5*R* configuration at the oxathiolane ring by reaction with $SOCl_2$) and presilylated cytosine or 5-fluorocytosine, leading to nucleoside precursors **8a**

Received: April 5, 2015

DOI: 10.1021/acs.orglett.5b00982
Org. Lett. XXXX, XXX, XXX−XXX

Scheme 1. Synthetic Approaches to Oxathiolane Nucleoside Precursors 8 and 9



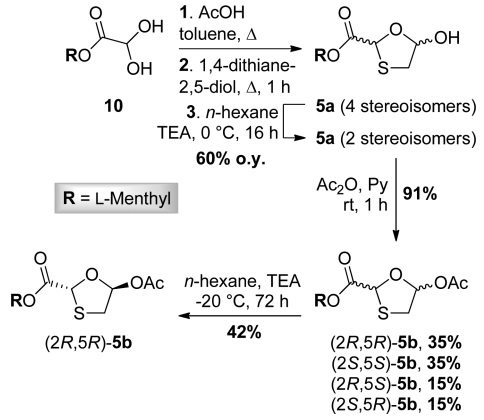
and **9a** in satisfying yields (**8a**: 66%;[13] **9a**: 81%[15a]) and anomeric selectivity (**8a**: α:β = 10:1;[13] **9a**: not given) (Scheme 1). This approach represented a convenient alternative to the previous methodology by Tse et al.,[11,12] who used the more unstable and expensive (although more efficient) TMSI as *N*-glycosidation reagent (**8a**: 75%, β:α = 23:1;[12] **9a**: 91%, β:α = 28:1[11]) starting from acetate (2*R*,5*R*)-**5b** (Scheme 1). As widely reported, in both cases the high β-selectivity is due to formation of oxonium ion **6**, stabilized through anchimeric assistance by the menthyl ester function.[13]

In the framework of our interest into new *N*-glycosidation methods,[16] the search for more efficient, stereoselective, and cost-effective variants of the above approaches caused us to explore the synthetic potential of the *N*-glycosidation reagent based on the combined use of silanes [especially triethylsilane (Et_3SiH) and polymethylhydrosiloxane (PMHS)] and iodine (I_2). Use of these systems in organic synthesis already has numerous precedents in the literature, especially in carbohydrate chemistry.[17] The Et_3SiH/I_2 system is an established reagent devised for a wide variety of transformations, including iodination,[18] regioselective Bn group removal,[19] *O*-glycosylation[20a] (even in stereoselective fashion),[20b] and rearrangement reactions.[21] The PMHS/I_2 system has demonstrated an analogous synthetic potential;[22] the very low cost of PMHS[23] makes the latter an even more convenient option for large-scale applications.

As widely reported, both Et_3SiH/I_2 and PMHS/I_2 systems act as sources of anhydrous HI, which is believed to be the actual reagent in most of the above trasformations.[17] Herein, the latter was conceived to be used both as acetate **5b** activator (enabling its conversion into 5-α-iodooxathiolane **7b**) and as glycosidation promoter (Scheme 1).

Access to enantiopure 5-acetoxyoxathiolane (2*R*,5*R*)-**5b** was explored using the synthetic strategy reported by Whitehead et al.[13] (Scheme 2). The coupling reaction between commercially available L-menthyl glyoxylate monohydrate (**10**) and 1,4-dithiane-2,5-diol, followed by treatment of the crude mixture with a TEA-containing *n*-hexane solution provided hemiacetal **5a** (60% yield). Literature procedure reports an early formation of four stereoisomers **5a** [with the following isomeric distribution: *trans*-(2*R*,5*R*)-**5a**, 35%; *trans*-(2*S*,5*S*)-**5a**, 15%; *cis*-(2*R*,5*S*)-**5a**, 35%; *cis*-(2*S*,5*R*)-**5a**, 15%], while the subsequent addition of *n*-hexane/TEA is claimed to enable both the rapid interconversion among the stereoisomers and the selective precipitation of the

Scheme 2. Synthesis of (2*R*,5*R*)-5b from Glyoxylate 10



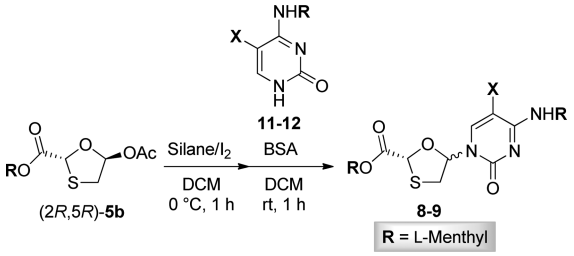
sole (2*R*,5*R*)-**5a**.[13] However, in our hands, the exact repetition of this methodology (as well as that of a more recent variation)[14] on laboratory scale (10 mmol) always gave a roughly equimolar mixture of two hemiacetal stereoisomers **5a** (60% overall yield) containing the (2*R*,5*R*) isomer. In addition, hydroxyl group acetylation of the last ones (Ac_2O/Py) led again to a base-mediated isomerization, providing a mixture of four acetates **5b** (91%). Differently from the aforementioned Whitehead's distribution[13] of hemiacetals **5a**, we identified[24] as the two major acetate stereoisomers **5b** those having the C2 and C5 stereocenters in a *trans* relationship [*trans*-(2*R*,5*R*)-**5b**, 35%; *trans*-(2*S*,5*S*)-**5b**, 35%; *cis*-(2*R*,5*S*)-**5b**, 15%; *cis*-(2*S*,5*R*)-**5b**, 15%] (Scheme 2). Accordingly, an accurate separation of the two main isomers was mandatory to ensure a high optical purity to the target nucleosides: indeed, while the *trans* acetate with the (2*R*,5*R*) configuration represented a suitable building block en route to (−)-oxathiolane nucleosides, conversely the use of the stereoisomer with (2*S*,5*S*) configuration allows the synthesis of the corresponding (+)-enantiomers. Gratifyingly, selective recrystallization of the sole (2*R*,5*R*)-**5b** could be achieved by treatment of the mixture with *n*-hexane containing a catalytic amount of TEA (42% yield). Isomerization at C2 (but not at C5) position, enabling conversion of the minor *cis*-(2*S*,5*R*)-**5b** into the corresponding *trans*-(2*R*,5*R*)-**5b**, was also observed at this stage.

Silane/I_2-mediated *N*-glycosidation was then tested by reaction of (2*R*,5*R*)-**5b** with cytosine (**11a**), 5-fluorocytosine (**12a**), and their N^4-benzoyl and N^4-acetyl derivatives **11b,c** and **12b** (Table 1). The reaction involved the treatment of **5b** with a premixed silane/I_2 solution followed by the *one-pot* addition of presilylated nucleobase solutions. First attempts with unprotected cytosine **11a** using Et_3SiH/I_2 and PMHS/I_2 as *N*-glycosidation reagents provided, already after 1 h at rt, the corresponding nucleoside **8a** in excellent yields (Et_3SiH: 98%; PMHS: 86%) and, somewhat in line with previous methods, with high anomeric selectivities (β:α = 15:1, 88% de) (entries 1 and 2).

Under the same conditions, the reaction of **5b** with 5-fluorocytosine **12a** gave higher selectivities (β:α = 35:1, 94% de) and similar yields (Et_3SiH: 91%; PMHS: 84%) (entries 10 and 11). The reaction of **5b** with protected nucleobases was then considered: surprisingly, we found a dramatic improvement in the β/α ratio when N^4-benzoyl- and N^4-acetyl(5-fluoro)-cytosines **11b,c** and **12b** were used (entries 3−8, 12, and 13). As an example, treatment of **5b** with Et_3SiH/I_2 or PMHS/I_2 and **11c** gave the corresponding nucleoside precursor **8c** with a 80−

DOI: 10.1021/acs.orglett.5b00982
Org. Lett. XXXX, XXX, XXX−XXX

Table 1. Silane/I_2-Mediated *N*-Glycosidation Reaction



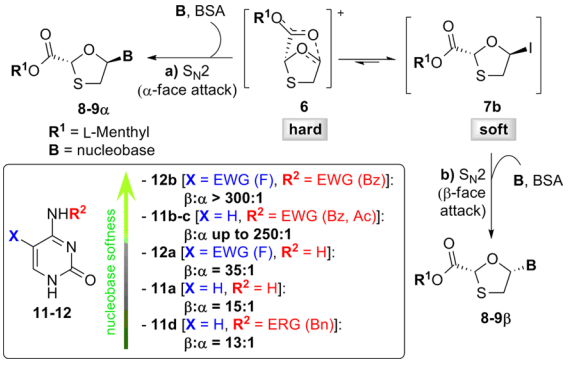
		nucleobase					
entry	silane		R	X	nucleoside	yield (%)	selectivity[a,b] (de, %)
1	Et_3SiH	**11a**	H	H	**8a**	98	88
2	PMHS	**11a**	H	H	**8a**	86	88
3	Et_3SiH[c]	**11b**	Bz	H	**8b**	72	95
4	PMHS[c]	**11b**	Bz	H	**8b**	73	95
5	Et_3SiH[d]	**11b**	Bz	H	**8b**	87	98
6	PMHS[d]	**11b**	Bz	H	**8b**	94	>99
7	Et_3SiH	**11c**	Ac	H	**8c**	95	97
8	PMHS	**11c**	Ac	H	**8c**	80	98
9	Et_3SiH[d]	**11d**	Bn	H	**8d**	62[e]	86
10	Et_3SiH	**12a**	H	F	**9a**	91	94
11	PMHS	**12a**	H	F	**9a**	84	94
12	Et_3SiH	**12b**	Bz	F	**9b**	85	98
13	PMHS	**12b**	Bz	F	**9b**	90	>99

[a]Determined by ^1H NMR analysis (400–600 MHz). [b]The structure of β- and α-nucleosides was determined by reduction of the menthyl ester group of **8a** and **9a** and subsequent NMR comparison of the corresponding reduction products with literature data.[25] [c]Silane/I_2 addition to the reaction mixture immediately after premixing of the two reagents. [d]Silane/I_2 addition to the reaction mixture carried out 15 min after premixing of the two reagents. [e]Mixture of regioisomeric N^1 and N^3 nucleosides (as suggested by NMR analysis).

95% yield and β/α ratios of 68:1–85:1 (97–98% de) (entries 7 and 8). Notably, the stereochemical outcome of the reactions was significantly influenced by the mixing times of silanes with I_2. As an example, when addition of Et_3SiH/I_2 to **5b** was performed immediately after premixing of the two reagents (entry 3), the reaction with N^4-benzoyl cytosine **11b** gave the corresponding nucleoside **8b** with an anomeric ratio of about 36:1 (95% de); in the same reaction, a much higher β-selectivity (β:α = 83:1, 97% de) was instead observed when Et_3SiH and I_2 were mixed for 15 min before addition[26] (entry 5). With PMHS/I_2, the same conditions provide even larger selectivity differences, with the β/α ratios increasing from 35:1 (95% de; entry 4) to 250:1 (>99% de; entry 6). The reaction with N^4-benzoylfluorocytosine **12b** similarly provided exceedingly high β-selectivities using either Et_3SiH/I_2 (98% de; entry 12) and PMHS/I_2 (>99% de; entry 13).

The results reported in Table 1 highlight the superiority of PMHS/I_2 regarding the stereoselectivity of the reactions, while with Et_3SiH/I_2 higher yielding conversions were observed in almost all cases. It is worth noting that, considering the selectivity differences between the two systems, the participation of further reactive species other than HI in the reaction mechanism cannot be excluded. The dramatic stereoselectivity improvement of the reactions with N^4-protected cytosines **11b,c** and **12b** compared to those with **11a** and **12a** also deserve some comments. At first glance, these results even appeared counterintuitive (the bulkier the N^4-amino group in the nucleobase, the higher the *cis*-selectivity). A good agreement between experimental data and

Scheme 3. Silane/I_2-Mediated *N*-Glycosidation and "Protective Group Effect": A Working Hypothesis



the HSAB (hard and soft acids and bases) theory was instead found. Accordingly, starting from the well-established[13] statement that an equilibrium exists between a minor, "hard" β-oxonium ion **6** (leading to α-configured S_N2 products) and a major, "soft" α-glycosyl iodide **7** (leading to β-configured products), we reasoned that the installation of an electron-withdrawing group (EWG) at the N4 position of the nucleobases **11** and **12** (e.g., in **11b,c** and **12b**) would make the corresponding N1 position a "softer" site than in **11a** and **12a**. This would explain the large preference of **11b,c** and **12b** for **7b** (as result of preponderant soft–soft interactions), whereas in the case of **11a** and **12a** a minor β-selectivity would be based on a non-negligible hard–hard interaction with **6** (Scheme 3). It should be also noted that, along the same lines, the higher selectivity in the reaction of **12a** compared with that in the reaction of **11a** can be similarly explained as a consequence of the presence of the fluorine atom at C5 position.

On the basis of these assumptions, the presence of an electron-releasing group (ERG) such as in the N^4-benzylcytosine (**11d**), able to reduce (albeit slightly) the softness of the corresponding persilylated nucleobase, was supposed to worsen the β-selectivity. As a result, treatment of **5b** with Et_3SiH/I_2 and persilylated **11d**[27] gave nucleoside precursor **8d** with an anomeric ratio of ca. 13:1 (86% de), additionally in low yield[28] (Table 1, entry 9).

With nucleoside precursors **8** and **9** in hand, attention was then turned to L-menthyl group reduction to afford target 3TC and FTC (Scheme 4). In line with previous reports,[12] treatment of **8a** (R = H) with $LiAlH_4$ provided, after 1 h at 0 °C, the corresponding free nucleoside **1** in a satisfying 83% yield. Conversely, the same procedure, starting from N^4-Bz or N^4-Ac derivatives **8b,c**, only provided the corresponding ester reduction products in low (58% from **8c**) or even negligible amounts (<10% from **8b**). Alternatively, reduction of **8b** and **9b** (R = Bz) was conceived by two-stages, one-pot procedure involving early N^4-acyl group removal by "superheated" MeOH[29] to give **8a** and **9a**, followed by addition of a reducing agent to the crude reaction mixture (Scheme 4). However, treatment of **8c** with MeOH at 100 °C as described by Robins et al.[29] provided, already after 1 h, large amounts of the epimerization product (2*S*,5*S*)-**8a** along with the expected nucleoside with (2*R*,5*S*) configuration [(2*R*,5*S*)-**8a**: (2*S*,5*S*)-**8a** = 2.2:1]; incidentally, detection in the crude of other nucleoside species containing methyl ester groups also suggested formation of transesterification products **13**. On the other hand, a temperature decrease led to a substantial minimization of the epimerization degree [T = 50 °C, (2*R*,5*S*)-**8a**: (2*S*,5*S*)-**8a** = 24:1; T = 40 °C, (2*R*,5*S*)-**8a**: (2*S*,5*S*)-**8a** =

DOI: 10.1021/acs.orglett.5b00982
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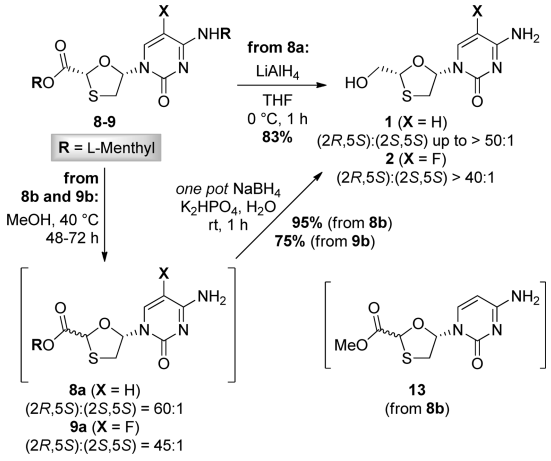
Scheme 4. 3TC and FTC Synthesis by One-Pot Reduction of Nucleoside Precursors 8 and 9



60:1]. Eventually, one-pot addition of $NaBH_4$ and K_2HPO_4 to the crude reaction mixture gave lamivudine (**1**) in an excellent 95% overall yield and with a high anomeric selectivity (β:α > 50:1). The same protocol, repeated on nucleoside **9b**, provided unprotected emtricitabine **2** with similar results (75% overall yield; β:α > 40:1) (Scheme 4).

In summary, we have provided a first look at the synthetic potential of Et_3SiH/I_2 and PMHS/I_2 as novel *N*-glycosidation reagents, as exemplified by the synthesis of the antiviral drugs oxathiolane nucleosides 3TC and FTC. Because of the low cost and high stability of the reagents, the chemical efficiency, and the exceedingly high stereoselectivity of the reactions, this approach can be reasonably considered as an effective alternative to the existing methodologies and reagents devoted to the same end. Crucial in influencing the stereochemical reaction outcome, an unprecedented role played by the N4 protective groups of (fluoro)cytosine has been also observed and rationalized on the basis of their capacity to increase nucleobase softness. Far beyond the scope of this work, this last finding opens up new opportunities in the stereoselective synthesis of β-nucleosides not relying on the presence of stereodirecting groups. A more comprehensive investigation on this topic aimed at studying scope and limitations of silane/I_2 in nucleoside synthesis is ongoing and will be published elsewhere.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and copies of NMR spectra. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b00982.

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Notes

The authors declare no competing financial interest.

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(23) An average cost of ca. 0.2 €/g has been estimated according to the main suppliers of chemicals (Mar 3, 2015).

(24) The structures of (2*R*,5*R*)-**5b** and (2*S*,5*S*)-**5b** were established by 1D and 2D NMR studies (see the Supporting Information) and confirmed by NMR and $[\alpha]_D$ comparison with literature data (ref 11).

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