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Chirality Control in Enzyme-Catalyzed Dynamic Kinetic Resolution of 1,3-Oxathiolanes

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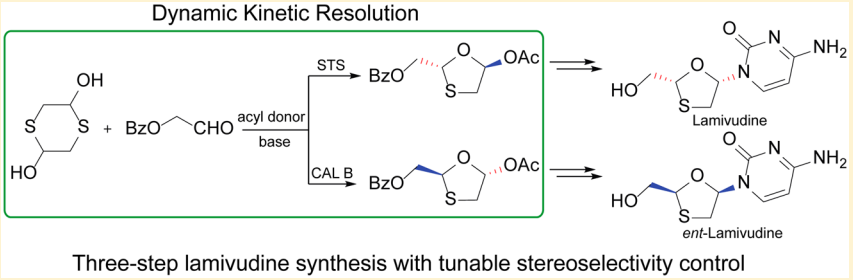
Supporting Information



ABSTRACT: The origin of enantioenrichment in enzyme-catalyzed dynamic kinetic resolution of 1,3-oxathiolane derivatives, key intermediates for asymmetric lamivudine synthesis, was elucidated. The chirality control could be determined by chiral HPLC and NOE NMR spectroscopy using a modified 1,3-oxathiolane compound obtained through enzyme-catalyzed selective hydrolysis. Solvent-dependent stereoselectivity was observed under biphasic conditions using different organic solvents with phosphate buffer.

The human immunodeficiency virus (HIV) has been one of the major threats to human health since its discovery in 1981, and the development of a permanent cure for HIV infection remains one of the greatest challenges to the scientific community. In the past decade, nucleoside analog reverse transcriptase inhibitors, such as lamivudine and emtricitabine (Figure 1), have proven to constitute a successful drug class for the treatment of infections of HIV as well as chronic hepatitis B.[1]

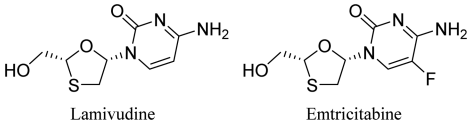
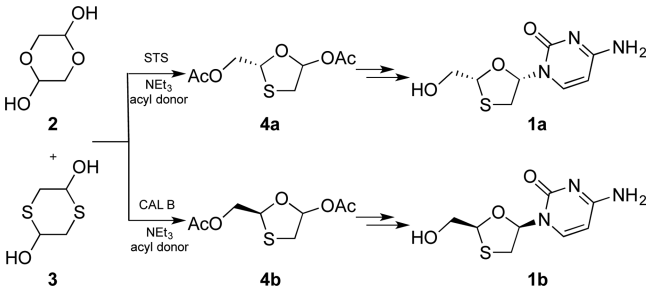


Figure 1. Representative nucleoside analogue reverse transcriptase inhibitors.

By taking advantage of the dynamic formation of hemithioacetals,[2−5] we previously reported an efficient, three-step synthesis of lamivudine through a surfactant-treated subtilisin Carlsberg (STS)-catalyzed dynamic kinetic resolution (DKR) protocol (Scheme 1).[6] The enantiomer of lamivudine was also accessible when STS was replaced by *Candida antarctica* lipase B (CAL B), thus representing a flexible stereocontrol in the asymmetric formation of five-membered oxathiolane derivatives.

The origin of the enantioenrichment effect in these transformations, with respect to which chiral center governs the outcome, is important for further development. Herein, we report a mechanistic study of enzyme-catalyzed asymmetric 1,3-oxathiolane formation where the enzyme selectivities toward the formation of 1,3-oxathiolane intermediates were identified by chiral HPLC and NOE−NMR experiments of a modified 1,3-oxathiolane derivative, obtained through selective enzymatic hydrolysis. An unusual and intriguing solvent-dependence effect regarding the stereoselectivity was also observed in the enzymatic hydrolysis procedure.

Scheme 1. Enzyme-Catalyzed Asymmetric Synthesis of Lamivudine and Its Enantiomer



As shown in Scheme 2, two different hydroxyl groups are generated during the formation of intermediate **5** from compounds **2** and **3**. In principle, enzyme-catalyzed acetylation of either the primary OH group adjacent to ring carbon C-2 (pathway I) or the secondary hemiacetal OH group at carbon C-5 (pathway II) could control the stereochemistry of compound **4**. In general, enzymatic DKR protocols are most

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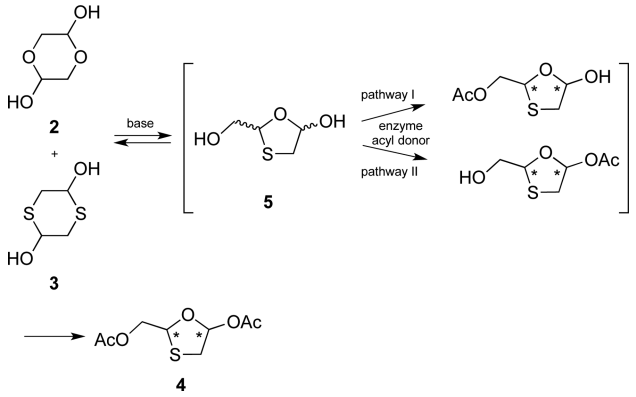
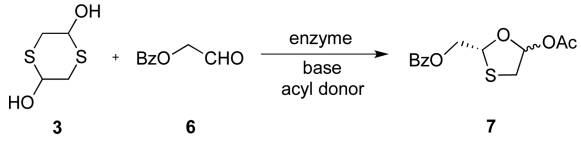
Scheme 2. Two Enzyme-Catalyzed Pathways for the Asymmetric Formation of Compound 4



commonly used for the resolution of secondary alcohols,[7,8] while primary alcohols are considered less suitable due to the lack of chirality at the hydroxyl-carrying carbon. An exception to this notion is the existence of an adjacent chiral center, which provides a complementary fit in the enzyme active site.[9] Intermediate **5** fulfills this requirement owing to the dynamic formation of the 1,3-oxathiolane ring, and enzyme-catalyzed acetylation of the primary OH group could therefore constitute the determining step for the resulting enantioenrichment.

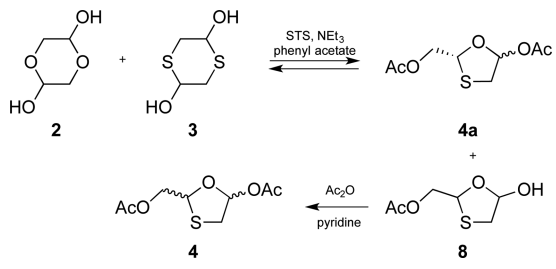
Support for the major importance of the hemiacetal OH came from the synthesis of compound **7** using benzoylated aldehyde **6** (Scheme 3), where high ee's were obtained with

Scheme 3. Asymmetric Synthesis of Compound 7 Using Benzoyl Protected Aldehyde 6



both CAL B and STS.[6] These results indicate that acetylation of the hemiacetal hydroxyl group governs the overall stereoselectivity of the process. Additional support was obtained from a quenching experiment in which the STS-catalyzed DKR process was halted prior to reaching completion. Besides the diacetylated product **4a**, the intermediate, monoacetylated compound **8**, was then obtained in small amounts (Scheme 4). The C-5 hemiacetal OH group of compound **8** was subsequently acetylated for enantiopurity determination, where chiral HPLC analyses revealed the almost racemic structure of compound **4**. Meanwhile, control experiments using the same reagents under the same reaction conditions without enzyme

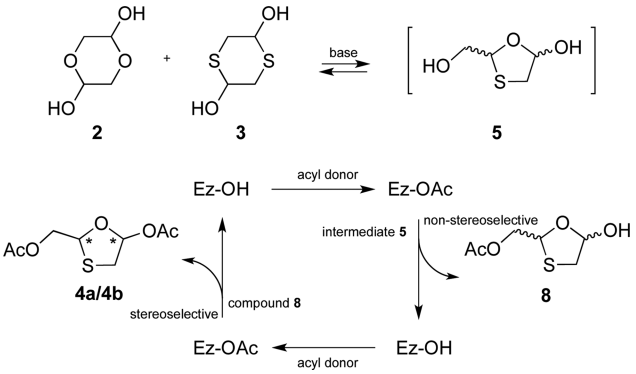
Scheme 4. Synthesis of Monoacetylated Compound 8 and Subsequent Acetylation for Chiral HPLC Analysis



resulted in no product formation, indicating that the enzyme-catalyzed acetylation of the primary OH group is not stereoselective. Thus, these experiments excluded the role of the primary OH group as providing major control of the product stereochemistry.

On the basis of the above observations, an enzyme-mediated reaction pathway could be proposed (Scheme 5). At the outset,

Scheme 5. Proposed Pathway of the Lipase-Catalyzed Asymmetric 1,3-Oxatiolane Derivative formation



the enzyme reacts with the acyl donor, forming the acylated enzyme, which subsequently catalyzes the transesterification of intermediate **5** to yield compound **8** in a nonstereoselective fashion. The liberated enzyme is again acylated in the presence of acyl donor, and the acylated enzyme catalyzes the asymmetric acylation of compound **8**, resulting in the formation of compound **4** and the free enzyme.

The absolute C-2 configuration could be determined for both the CAL B- and STS-catalyzed products by comparing the final product with lamivudine using chiral HPLC. The C-5 configuration, on the other hand, which was directly governing the product stereochemistry, remained unclear due to the racemization of the stereogenic center in the following Vorbrüggen coupling reaction. Literature surveys revealed, in addition, no definite information, and although enzyme selectivities on secondary alcohols have been extensively studied, similar studies with hemiacetal substrates proved absent.

To identify the absolute configuration of the 1,3-oxathiolane derivatives, NOE−NMR spectroscopy was chosen as a convenient tool for the analysis of both compounds **4** and **7**. However, no clear cross-couplings were detected as a consequence of the high flexibility of five-membered oxygen-containing heterocycles.[10−14] Suitable structural modification of the ring substituents would, however, increase the conformational rigidity, in principle resulting in better NOE−NMR signals, and this alternative was thus investigated. On the basis of the structure of compound **4**, the primary ester group was deemed available as a potential modification site, and selective acetyl removal and acylation with a sterically more demanding structure was thus devised. However, regioselective ester hydrolysis is a synthetically challenging task, often resulting in mixtures. In the present case, since the STS system demonstrated faster acylation of the primary alcohol than the hemiacetal group (Scheme 4), an enzyme-catalyzed hydrolysis procedure was consequently designed (Scheme 6). Racemic compound **4** was first synthesized and subsequently subjected to CAL B-catalyzed hydrolytic conditions in a phosphate-buffered saline (PBS)/toluene biphasic system. This resulted in

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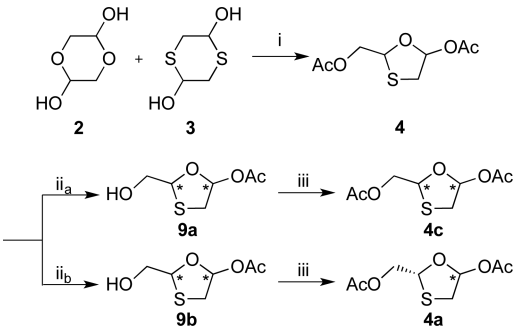
Scheme 6. CAL B-Catalyzed Enantioselective Hydrolysis[a]



[a]Reagents and conditions: (i) pyridine, Ac_2O, 84%; (ii_a) PBS/toluene, CAL B, 77%[a]; (ii_b) PBS/THF, CAL B, 86%[a]; (iii) Ac_2O, triethylamine, DCM, 91% (a, calculated as the yield for the single isomer).

selective hydrolysis of the primary ester functionality, and alcohol **9a** was successfully obtained. To evaluate the enzyme selectivity, subsequent acetylation to compound **4c** was then carried out, after which the chiral HPLC traces were compared with compounds **4a**, **4b**. Compound **4c** was thus identified as an isomer with 68% ee, emanating from the opposite diastereomer leading to isomers **4a** and **4b**. Interestingly, when toluene was replaced by THF, different enzyme diastereoselectivity was observed as the acetylated product of compound **9b** corresponded to the STS-catalyzed DKR product **4b** with very high diastereo- and enantiopurity (Table 1). Solvent dependence in enzyme-catalyzed hydrolyses

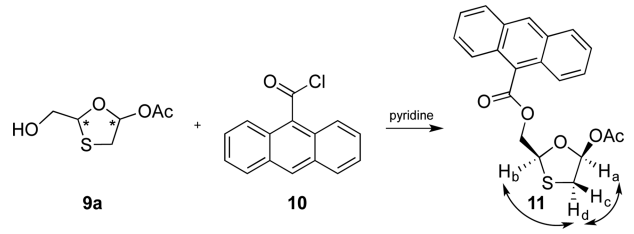
Table 1. Solvent Effects on Hydrolysis of Racemate 4

solvent	dr	ee (%)
PBS/toluene	14.9:1	68
PBS/TBME	11.5:1	59
PBS/THF	<1:99	>99

has been observed in several cases,[15−18] although a detailed rationale for the change in enzyme stereoselectivity remains unclear. A likely explanation is that the conformation of the enzyme active site changes when exposed to different solvent mixtures, resulting in the formation of different enantiomers or diastereomers. Solvents of different polarities, including DMF, DMSO, and TBME, were further evaluated. Of these, the TBME system resulted in similar selectivities as in toluene, albeit with both lower dr and ee. No products were obtained with the more polar solvents DMF and DMSO, likely due to their denaturing effect from dissolving the essential water shell required for enzyme activity.

Following investigation of the selective hydrolysis process, compound **9a** was acylated with anthracene-9-carbonyl chloride **10** to provide compound **11** (Scheme 7), assuming that the bulky anthracene plane would significantly increase the space crowding of the oxathiolane ring, and especially enhance the repulsion with the C-5 acetyl group in case compound **9a** had a *cis* configuration. However, only a very weak NOE−NMR signal was detected between protons H_a and H_b. To further deduce the configuration, protons H_c and H_d were selectively saturated, showing cross peaks between H_d and both H_a and H_b, while only H_a correlated to H_c (the same method used for compound **4** and compound **7** resulted in inconclusive results). These observations lead to the conclusion that compound **11**, as well as compound **9a** (*cf.* Supporting Information), were of

Scheme 7. Synthesis of Compound 9 for NOE−NMR Analysis



cis configuration. Vorbrüggen coupling and hydrolysis reactions of compound **4c** were subsequently conducted to identify the C-2-configuration. Chiral HPLC analysis thus showed that the corresponding nucleoside product was *ent*-lamivudine **1b**, indicating that compound **9a** had a (2*S*,5*R*)-configuration.

Based on NOE−NMR and chiral HPLC analyses, the selectivities of both CAL B and STS in the DKR process for the synthesis of acetylated 1,3-oxathiolane derivatives, as well as the selectivity of CAL B in the reverse hydrolysis, were thus identified (Figure 2). In the DKR process, both CAL B and

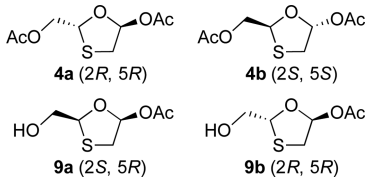


Figure 2. Top: STS and lipase-mediated DKR products; Bottom: CAL B-catalyzed hydrolytic products.

STS favor the formation of the *trans*-1,3-oxathiolane heterocycle. STS shows an *R*-selectivity on the cyclic C-5 hemiacetal (compound **4a**) compared with the *S*-selectivity of the lipase (compound **4b**). In the hydrolytic procedure, the *cis*-isomer **9a** was obtained in the PBS/toluene solvent system while *trans*-isomer **9b** was the exclusive product in the PBS/THF mixture.

CONCLUSIONS

Following our initial study of enzyme-catalyzed asymmetric lamivudine synthesis, the chirality control during the ring-closing process was subsequently established. The enzyme selectivities toward the formation of 1,3-oxathiolane heterocycles were identified by absolute configuration analysis of a modified 1,3-oxathiolane derivative using chiral HPLC and NOE−NMR spectroscopy. A CAL B-catalyzed regio- and stereoselective hydrolysis was applied for the synthesis of the modified compound, where a solvent dependence was also observed. Thus, this study offers a valuable fundamental methodology for the enzyme-catalyzed asymmetric nucleoside synthesis, which can be further utilized in 1,3-oxathiolane-based drug development.

EXPERIMENTAL SECTION

General Experimental Procedures. All commercially available starting materials were of reagent grade and used as received. ^{1}H NMR and ^{13}C NMR data were recorded at 500 (125) MHz. Chemical shifts are reported as δ values (ppm) with $CDCl_3$ (^{1}H NMR δ 7.26, ^{13}C NMR δ 77.16) as internal standard. *J* values are given in hertz (Hz). Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F_{254}, visualized with UV detection. Flash column chromatography was performed on silica gel 60, 0.040−0.063 mm. Analytical

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high-performance liquid chromatography (HPLC) was performed with using a chiral stationary phase. Solvents for HPLC use were of spectrometric grade.

(2*S*,5*R*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-ol (9a). To a solution of 1:1 toluene/PBS (pH = 8.0, 5 mL) with CAL B (20 mg) was added compound **4** (460 mg, 2.1 mmol). The mixture was stirred overnight, at which time the mixture was diluted with 20 mL of EtOAc and washed with satd $NaHCO_3$. The aqueous layer was extracted with EtOAc (20 mL) twice. The combined organic layer was washed with brine and dried over $MgSO_4$. The solvent was concentrated, and the crude product was purified by column chromatography (hexane/EtOAc = 3:1), yielding compound **9a** (53 mg, 77% calculated as a single isomer) as a clear oil, 68% ee, determined in the form of compound **8c** by chiral HPLC analysis (Chiral OJ, λ= 210 nm, Hex/PrOH = 9:1, 0.5 mL/min: ^{1}H NMR (500 Hz, $CDCl_3$) δ = 6.59 (1H, d, J = 4.09 Hz), 5.49 (1H, dd, J = 5.97, 4.38 Hz), 3.78 (2H, m), 3.35 (1H, dd, J = 11.90, 4.31 Hz), 3.21 (1H, d, J = 11.70 Hz), 2.11 (3H, s); ^{13}C NMR (125 Hz, $CDCl_3$) δ = 169.8, 99.1, 87.8, 66.0, 38.2, 21.1; HRMS (ESI-TOF) 201.0197 ([M + Na]$^+$ $C_6H_{10}O_4SNa$, calcd 201.0192); $[\alpha]^{25}_D$ −14.4 (c = 0.5%, MeOH).

(2*R*,5*R*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-ol (9b). Following the same procedure as compound **9a**, toluene was replaced by THF. Compound **9b** was obtained (101 mg, 86% calculated as a single isomer) as a clear oil, 99% ee, determined in the form of compound **4a** by chiral HPLC analysis (Chiral OJ, λ = 210 nm, Hex/*i*PrOH = 9:1, 0.5 mL/min: ^{1}H NMR (500 Hz, $CDCl_3$) δ = 6.62 (1H, d, J = 3.57 Hz), 5.44 (1H, t, J = 3.80 Hz), 3.79 (1H, dd, J = 12.54, 3.08 Hz), 3.68 (1H, dd, J = 12.42, 4.78 Hz), 3.26 (1H, dd, J = 11.46, 4.37 Hz), 3.09 (1H, d, J = 11.87 Hz), 2.04 (3H, s); ^{13}C NMR (125 Hz, $CDCl_3$) δ = 169.4, 98.5, 85.7, 63.9, 37.2, 20.6. HRMS (ESI-TOF): 179.0373 ([M + 1]$^+$, $C_6H_{11}O_4S$, calcd 179.0378); $[\alpha]^{25}_D$ −65.3 (c = 0.3%, $CHCl_3$).

((2*S*,5*R*)-5-Acetoxy-1,3-oxathiolan-2-yl)methyl Acetate (4c). To a solution of compound **9a** (9 mg, 0.05 mmol) in DCM (1 mL) were added triethylamine (8.4 μL, 0.06 mmol) and acetic anhydride. The mixture was stirred at rt for 2 h, diluted with DCM (10 mL), washed with water (2 × 5 mL) and brine, and dried over $MgSO_4$. Solvent was evaporated and the crude product was purified by column chromatography (hexane/EtOAc = 4:1), yielding compound **4c** (9.8 mg, 89%) as a clear oil: ^{1}H NMR (500 Hz, $CDCl_3$) δ = 6.62 (d, J = 4.3 Hz, 1H), 5.52 (dd, J = 7.9, 4.1 Hz, 1H), 4.38 (dd, J = 11.7, 8.0 Hz, 1H), 4.14 (dd, J = 11.7, 4.2 Hz, 1H), 3.21 (d, J = 11.7 Hz, 2H), 2.10 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (125 Hz, $CDCl_3$) δ = 170.5, 169.9, 99.5, 84.7, 67.7, 38.2, 21.4, 20.9.

((2*R*,5*R*)-5-Acetoxy-1,3-oxathiolan-2-yl)methyl Acetate (4a). Following the same procedure as for compound **4c**, compound **4a** was obtained (9.9 mg, 90%) as a clear oil: ^{1}H NMR (500 Hz, $CDCl_3$) δ = 6.68 (d, J = 4.1 Hz, 1H), 5.54 (dd, J = 6.1, 4.0 Hz, 1H), 4.33−4.21 (m, 2H), 3.32 (dd, J = 11.5, 4.2 Hz, 1H), 3.14 (d, J = 11.5 Hz, 1H), 2.10 (s, 6H); ^{13}C NMR (125 Hz, $CDCl_3$) δ = 170.6, 169.9, 99.3, 83.3, 66.0, 37.7, 21.3, 20.9.

(2*S*,5*R*)-2-(2-(Anthracen-9-yl)-2-oxoethyl)-1,3-oxathiolan-5-yl Acetate (11). To a solution of compound **9a** (36 mg, 0.2 mmol) in pyridine (1 mL) was added anthracene-9-carbonyl chloride **10** (96 mg, 0.4 mmol) portion by portion at 0 °C. The reaction was then warmed to rt and stirred overnight. The mixture was diluted with EtOAc (10 mL), washed with satd $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was concentrated and purified by column chromatography (hexane/EtOAc = 5:1), yielding compound **11** (59 mg, 79%) as a pale yellow solid: ^{1}H NMR (500 Hz, $CDCl_3$) δ = 8.47 (1H, s), 8.04 (2H, d, J = 8.77 Hz), 7.95 (2H, d, J = 8.60 Hz), 7.47−7.52 (2H, m), 7.40−7.45 (2H, m), 6.68 (1H, d, J = 4.19 Hz), 5.70 (1H, dd, J = 8.38, 4.19 Hz), 5.02 (1H, dd, J = 11.84, 7.95 Hz), 4.45 (1H, dd, J = 11.84, 3.71 Hz), 3.33 (1H, dd, J = 11.52, 4.34 Hz), 3.23 (2H, d, J = 11.60 Hz), 1.99 (3H, s); ^{13}C NMR (125 Hz, $CDCl_3$) δ = 169.9, 169.0, 130.9, 129.6, 128.6, 127.2, 125.7, 125.1, 99.2, 84.3, 68.3, 38.2, 21.2; HRMS (ESI-TOF) 405.0767 ([M + Na]$^+$, $C_{21}H_{18}O_5SNa$, calcd 405.0773).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b01585.

HPLC chromatograms, NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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