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Asymmetric synthesis of 1,3-oxathiolan-5-one derivatives through dynamic covalent kinetic resolution☆

Yan Zhang, Fredrik Schaufelberger, Morakot Sakulsombat, Chelsea Liu, Olof Ramström *

Department of Chemistry, KTH—Royal Institute of Technology, Teknikringen 30, Stockholm 10044, Sweden

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ABSTRACT

The asymmetric synthesis of 1,3-oxathiolan-5-one derivatives through an enzyme-catalyzed, dynamic covalent kinetic resolution strategy is presented. Dynamic hemithioacetal formation combined with intramolecular, lipase-catalyzed lactonization resulted in good conversions with moderate to good enantiomeric excess (ee) for the final products. The process was evaluated for different lipase preparations, solvents, bases, and reaction temperatures, where lipase B from *Candida antarctica* (CAL-B) proved most efficient. The substrate scope was furthermore explored for a range of aldehyde structures, together with the potential access to nucleoside analog inhibitor core structures.

1. Introduction

Asymmetric synthesis of chiral core structures remains an important field in organic chemistry, particularly for the production of active pharmaceutical ingredients. One such interesting motif is constituted by the 1,3-oxathiolane core. This structure type, together with the related 1,3-oxathiolan-5-ones, are attractive targets, not only for their existence in natural products and broad biological activities, but also due to their importance as intermediates for a range of highly successful and useful pharmaceuticals. For example, they possess inhibitory activities toward human type-II (non-pancreatic) secretory phospholipase A2 (PLA2);[1] the oxathionyl-nucleosides emtricitabine (Coviracil) and lamivudine (3TC) remain two of the most potent antiviral drugs as nucleoside reverse transcriptase inhibitors (NRTIs) for the treatment of diseases, such as human immunodeficiency virus (HIV) or hepatitis B.[2,3] Since the initial discovery of the antiviral activity of this motif, the synthesis of enantiomerically pure 1,3-oxathiolan-5-one derivatives has received significant attention.[4–6] To induce a chiral element into the highly sensitive oxathiolane skeleton, biocatalytic transformations have long been a method of choice owing to its high degree of stereoselectivity, high efficiency, mild reaction conditions, and advantageous environmental properties. For example, classical kinetic resolution protocols were used in the preparation of a lamivudine intermediate through hydrolysis of the undesired enantiomer.[7–11]

Although classical kinetic resolution processes are often very efficient, the maximum theoretical yield is limited to 50% while maintaining the highest possible enantiomeric excess (ee) of the respective transformation. This inherent limitation of the kinetic resolution concept can however be circumvented through the introduction of dynamics in the system. Continuous racemization of the starting material in conjunction with the resolution process, results in dynamic kinetic resolution (DKR), where the maximum theoretical yield can reach 100% without reducing the enantiomeric purity.[12–17] The value of DKR protocols in enzyme-catalyzed asymmetric synthesis has been successfully illustrated by, for example, transition metal-catalyzed racemization of the substrates.[18,19] Another approach involves dynamic covalent reactions, where the racemic substrate is continuously reformed through the reversible nature of participating chemical bonds (Scheme 1).[16,20,21] Both these approaches can be efficiently coupled to enzyme catalysis, leading to kinetic resolution of the optimal product. The dynamic covalent process can furthermore be extended to the resolution of complex systems.[22–27]

Recently, we reported the in situ formation of 1,3-oxathiolan-5-one derivatives through lipase-catalyzed γ-lactonization from a complex dynamic hemithioacetal system.[26] Due to the reversible hemithioacetal transformation, the unselected structures were

* Corresponding author. Fax: +46 8 7912333; e-mail address: ramstrom@kth.se (O. Ramström).

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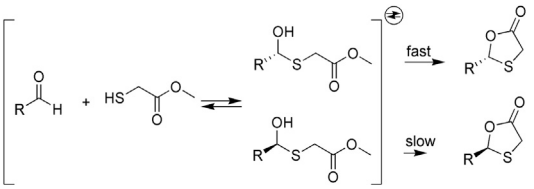


Scheme 1. Asymmetric synthesis of 1,3-oxathiolan-5-ones through dynamic covalent kinetic resolution (DCKR).

instantly recycled back to the starting materials, and the final products after the enzymatic transformation were obtained with both high chemo- and stereoselectivities in a one-pot process. This protocol is particularly useful for enzyme classification and substrate identification, but further exploration of the scope and synthetic utility of this reaction is warranted. Herein we present an optimized asymmetric synthesis of 1,3-oxathiolan-5-one through dynamic covalent kinetic resolution, using hemithioacetal chemistry coupled with a lipase-catalyzed cyclization, as well as its further usage for the construction of oxathiolane nucleoside skeletons.

2. Results and discussion

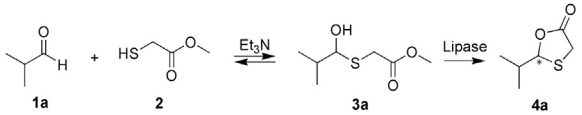
The kinetic resolution function of the overall protocol was based on enzyme-catalyzed transformation of transient, intermediate hemithioacetals. Lipases, which belong to the hydrolase class of enzymes, have in this context proven very efficient and selective for transesterification of secondary alcohols in organic solvents, and together with other advantages, such as commercial availability, absence of cofactor requirements, and broad substrate scope, they were chosen for the resolution process. However, lipases from different sources may behave differently toward the same transformation, and in order to optimize the reaction conditions, screening of a range of lipases for the kinetically controlled lactonization step was initially performed. The lipases from *Pseudomonas fluorescens* (PFL), *Burkholderia* (*Pseudomonas*) *cepacia* (PS, PS-CI, and PS-IM), *Candida antarctica* (CAL-B), and *Candida rugosa* (CRL), were thus probed for the cyclization using a model reaction involving isobutyraldehyde (**1a**) and methyl 2-sulfanylacetate (**2**). The results clearly demonstrated the lipase differentiation (Table 1), and outside of the fact that PS and CRL did not catalyze the reaction under the tested conditions, the use of PS-IM, PS-CI, and PFL led to slow reaction progress and very low enantiomeric excess of the product. The activities are in this case likely challenged by the double role of methyl 2-sulfanylacetate; serving both as substrate precursor and acyl donor. On the other hand, CAL-B provided reasonable conversion and good stereoselectivity, with an enantiomeric ratio (*E*-value) of 12. Thus, CAL-B was chosen for further studies of the reaction.

Lipase catalysis is highly solvent-dependent, and this aspect was next addressed for the current system using the model reaction between compounds **1a** and **2**. Four enzyme-compatible solvents were thus tested: toluene, *tert*-butyl methyl ether (TBME), diethyl ether, and tetrahydrofuran (THF). The cyclized product **4a** was detected in all of the four solvents, of which toluene, the reaction media with lowest polarity, resulted in better enantiomeric excess while the reaction rate was similar to the others. This can be partly ascribed to its low miscibility with water, thus not interrupting the bound water layer on the surface of lipase.

The temperature, another crucial parameter for both the equilibrium distribution and the stereoselectivity of the enzymatic transformation, was subsequently studied. The model reaction was therefore performed at temperatures ranging from 40 °C to −25 °C. In consistency with previous results, the enantioselectivities were improved at lower temperatures, a result that can be explained by a more rigid enzyme structure at lower temperatures, resulting in a better fitting of the optimal enantiomer. The larger activation-energy differences ($\Delta\Delta G^{\ddagger}$) with decreased temperatures can also contribute to the higher enantioselectivity.[28,29] The best ees were obtained at −25 °C, at which point the reaction rate was still reasonably high.

Reversible hemithioacetal formation has proven useful for efficient formation of dynamic systems in both aqueous and organic media.[26,30] In organic solution, base catalysis has been shown to accelerate the reversibility of the reaction, and displace the equilibrium of the system toward the hemithioacetals. Thus, the effect of organic bases on the conversion and stereoselectivity was also investigated in the current study. Besides triethylamine (Et_3N), which was used in the aforementioned dynamic system, other bases, such as morpholine, 4-methylmorpholine (NMM), 4-methylimidazole, and 4-dimethylaminopyridine (DMAP) were tested (Table 2). Among these, the reactions with 4-methylmorpholine resulted in the best enantiomeric excess, but showed slightly lower conversion than using Et_3N within the same timeframe. Incidentally, 4-methylmorpholine was also the weakest base with a pK_a around 7.4. It was also found that increasing the amount of base led to faster reactions, but at the cost of diminished enantioselectivity. This can be explained by the facilitation of the non-enzymatic background cyclization upon higher degree of base, although also resulting in an increase in reversibility rates. In the case of single reactions compared to complex dynamic systems, smaller amounts of base were sufficient for attaining high reversibility rates.

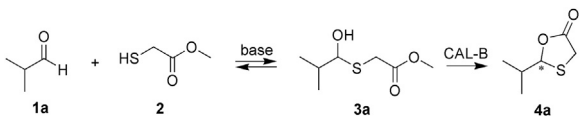
Table 1
Enzyme-catalyzed dynamic covalent kinetic resolution with different lipases[a]



Entry	Enzyme	Conversion[b] (%)	ee[c] (%)
1	CAL-B	35	77
2	PS-IM	11	14
3	PS-CI	8	17
4	PS	0	—
5	CRL	0	—
6	PFL	13	20

[a] Reactions were carried out with **1a** (0.1 mmol), **2** (1.2 equiv), Et_3N (0.5 equiv), 4 Å MS (20 mg), and lipase preparation (50 mg; 5 mg for CAL-B) in toluene (0.6 mL) at −25 °C for 3 days.
[b] Determined by ^{1}H NMR spectroscopy.
[c] The ees of **4a** were determined by HPLC analysis using Daicel Chiralpak OJ column, 99:1 Hex/^{i}PrOH.

Table 2
Enzyme-catalyzed dynamic covalent kinetic resolution with different bases[a]



Entry	Base	Base amount (equiv)	Conversion[b] (%)	ee[c] (%)
1	Triethylamine	0.1	48	80
2	Morpholine	0.1	12	—
3	4-Methylmorpholine	0.1	39	95
4	4-Methylimidazole	0.1	37	80
5	4-Dimethylaminopyridine	0.1	43	86
6	4-Methylmorpholine	0.5	42	83
7	4-Methylmorpholine	1.0	46	83

[a] Reactions were carried out with **1a** (0.1 mmol), **2** (1.2 equiv), base, 4 Å MS (20 mg), and CAL-B (5 mg) in toluene (0.6 mL) at −25 °C for 4 days.
[b] Determined by ^{1}H NMR spectroscopy.
[c] The ees of **4a** were determined by HPLC analysis using Daicel Chiralpak OJ column, 99:1 Hex/^{i}PrOH.

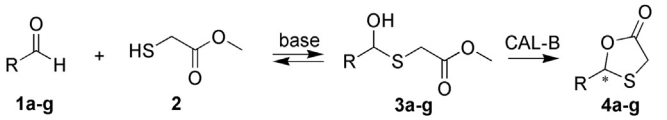
Other efforts to optimize the reaction conditions were also carried out, including lipase loading, substrate concentration, and water activity of the solution. However, it was generally found that the enantioselectivity decreased at higher conversion. At this point it was suspected that methanol, formed as a side product from the cyclization step, could potentially affect the reaction. It is known that short linear alcohols can deactivate lipases through interference with the hydration layer of the enzyme.[31–34] Therefore, 2 equiv of methanol were added to the DKR system at the beginning of the reaction in order to test this effect. A dramatically decreased conversion was indeed observed, supporting the hypothesis of the inhibitory effects of methanol for CAL-B. For the purpose of removing the methanol produced during the reaction, two different methods were adopted for the system. The first one involved addition of anhydrous calcium chloride salt ($CaCl_2$) into the solution. $CaCl_2$ is not only capable of interacting with methanol to form a stable crystalline complex, but also serves as a salt-hydrate pair in the solution to control the water activity.[35,36] After the addition of $CaCl_2$, the conversion increased from 51% to 73%, while the enantiomeric excess remained at a high level of 88%. However, there appeared to be a limitation of methanol binding ability and stability, as the result varied significantly between different aldehydes and also upon changes of reaction conditions. The other method for methanol removal was the addition of a large excess of molecular sieves (MS), which would be able to trap the produced methanol. By addition of 300 mg 4 Å MS per reaction, the reaction went smoothly with a conversion of 73% over a reasonable timeframe, along with a good enantiomeric excess of 81%. More importantly, the methanol trapping effect of MS proved more robust than $CaCl_2$ when applied to other substrates or in other conditions.

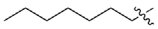
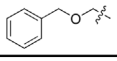
After having identified optimized conditions for the model reaction, a range of other aldehydes (**1b**–**g**) were further evaluated, most of which (**4b**–**g**) gave moderate to good ees with reasonable conversions. For substrates with more branched aliphatic substituents, such as 3-methylbutanal (**1b**), 2-ethylbutanal (**1c**), and cyclohexanecarbaldehyde (**1d**), the enantioselectivities were high, while for the long chain aldehyde octanal (**1e**), much lower enantiomeric excess was obtained. These differences are likely due to how well the structures can be accommodated into the active site of CAL-B. Large substituents without other binding potentials, such as the heptyl group, may have difficulty to fit into the active site of CAL-B.[37,38]

When an aromatic aldehyde was applied to this system (entry 6, Table 3), the reaction was very slow even at the higher temperature of 0 °C, whereas the enantiomeric excess remained at more than 80%. An aldehyde with a protected α-oxygen was also tested (entry 7, Table 3), as this could in principle provide a direct route to interesting chiral NRTI's, such as lamivudine or emtricitabine. Surprisingly, despite the bulky nature of the protecting group, very clean reactions with a high conversion from starting material to the *O*-protected 1,3-oxathiolan-5-one **4g** were achieved, albeit with slightly lower enantioselectivity. This may also be ascribed to the non-optimal fit with the CAL-B stereospecificity pocket.

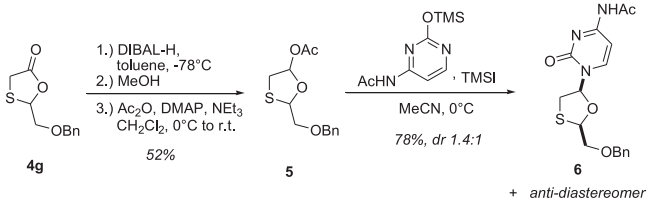
In order to demonstrate that the oxathiolan-5-ones, synthesized with the dynamic covalent kinetic resolution protocol, in principle could be used for the construction of oxathiolane nucleosides, we performed a brief synthesis starting from racemic compound **4g** (Scheme 2). The lactone functionality was first reduced with diisobutyl aluminum hydride to the corresponding lactol, which was subsequently acetylated using standard conditions to obtain oxathiolane product **5** in two steps.[39] This intermediate could then be transformed to the corresponding *O*-protected nucleoside under Vorbrüggen conditions,[2,39] using pre-silylated N^4-acetylcytosine in a trimethylsilyl iodide-catalyzed N-glycosylation procedure. The obtained nucleoside **6** could in principle be further functionalized or deprotected to obtain the pharmaceutical product 3TC.[40]

Table 3
Dynamic covalent kinetic resolution of aldehydes[a]



Entry	R group	Product	Conversion[b] (%)	ee[c] (%)
1		**4a**	73	81
2		**4b**	55	85
3		**4c**	79	90
4		**4d**	67	92
5		**4e**	75	71
6		**4f**	16	83
7		**4g**	93	71

[a] Reactions were carried out with **1a**–**g** (0.1 mmol), **2** (1.2 equiv), NMM (0.1 equiv), 4 Å MS (300 mg), and CAL-B (5 mg) in toluene (0.6 mL) at −25 °C for 4 days.
[b] Determined by ^{1}H NMR spectroscopy.
[c] The ees of **4a** were determined by HPLC analysis using Daicel Chiralpak OJ column.



Scheme 2. Synthesis to nucleoside **6**, an important intermediate for 3TC.

3. Conclusions

In summary, a dynamic covalent kinetic resolution protocol based on reversible hemithioacetal formation was successfully applied to the synthesis of 1,3-oxathiolan-5-one derivatives. Using lipase-catalyzed resolution, selective lactonization of the substrate structures was achieved in a one-pot process. After a series of optimizations of the reaction conditions by use of wild-type CAL-B, good yields and enantioselectivities for a range of substrates were obtained. Furthermore, some of the synthesized 1,3-oxathiolan-5-one derivatives showed potential for a simple access to the core structure of active pharmaceutical nucleoside analogs.

4. Experimental section

4.1. General

Reagents were obtained from commercial suppliers and used as received. Lipase PS 'Amano' IM (EC 3.1.1.3) was purchased from Amano Enzyme Inc. All other lipases: lipases from *Pseudomonas fluorescens* (PFL), *Burkholderia* (*Pseudomonas*) *cepacia* (PS and PS-CI), *Candida antarctica* (CAL-B), and *Candida rugosa* (CRL) were purchased from Sigma–Aldrich. ^{1}H and ^{13}C NMR data were recorded on a Bruker Avance 400 (100) MHz and/or a Bruker Avance 500

(125) MHz, respectively. Chemical shifts are reported as δ values (ppm) with $CDCl_3$ (^{1}H NMR δ 7.26, ^{13}C NMR δ 77.0) or DMSO-d_6 (^{1}H NMR δ 2.50, ^{13}C NMR δ 39.5) as internal standard. *J* values are given in Hertz (Hz). Analytical high performance liquid chromatography (HPLC) with chiral stationary phase was performed on an HP-Agilent 1110 Series controller and a UV detector, using a Daicel Chiralpak OJ column (4.6×250 mm, 10 μm). Solvents for HPLC use were of spectrometric grade. ATR-IR spectroscopy was performed on a Thermo Scientific Nicolet iS10 spectrometer. High resolution mass spectroscopy was performed by the Instrument station of the National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand, and at the Institute of Chemistry at University of Tartu, Estonia. Thin layer chromatography (TLC) was performed on precoated Polygram® SIL G/UV 254 silica plates (0.20 mm, Macherey–Nagel), visualized with UV-detection. Flash column chromatography was performed on silica gel 60, 0.040–0.063 mm (SDS).

4.2. General procedure for dynamic covalent kinetic resolution

Aldehydes **1a**–**g** (0.1 mmol each), methyl 2-sulfanylacetate (0.12 mmol), 4-methylmorpholine (0.01 mmol), and dry toluene (0.75 mL) were added into a 1.5 mL sealed-cap vial containing CAL-B (5 mg) and $CaCl_2$ (20 mg) or 4 Å molecular sieves (300 mg). CAL-B was dried under vacuum for 2 days before use. The vial was kept at −25 °C without stirring, and ^{1}H NMR was used to monitor the reaction progress. After 4 days, the reaction mixture was filtered through a cotton-stoppered pipette. Then, saturated NH_4Cl was added, and the resulting mixture was kept stirring at rt for 30 min. CH_2Cl_2 was subsequently added, and the aqueous layer was extracted three times (3 mL CH_2Cl_2 each). The combined organic layer was dried over $MgSO_4$ and removed in vacuo. The crude products were purified using column chromatography (Hexane/EtOAc=20:1 or 15:1).

4.3. General procedure for the synthesis of racemic compounds

Aldehydes **1a**–**g** (3 mmol), methyl 2-sulfanylacetate (3.6 mmol), and Et_3N (0.3 mmol), were dissolved in toluene (1 mL), to which CAL-B (5 mg), and 4 Å molecular sieves (10 mg) were added. The mixture was stirred at 60 °C for 6 days, and ^{1}H NMR was used to monitor the reaction progress. After completion of the reaction, CAL-B was removed by filtration, and the solution was washed by saturated NH_4Cl, while stirring at rt for 30 min. The mixture was extracted with CH_2Cl_2 (3 mL×3), and the organic layer was dried over $MgSO_4$ and evaporated in vacuo. The crude products were finally purified using column chromatography (Hexane/EtOAc=20:1 or 15:1).

*4.3.1. 2-Isopropyl-1,3-oxathiolan-5-one (**4a**).*[1] Conversion: 73%, enantiomeric excess (ee): 81%, determined by HPLC analysis (Daicel Chiralpak OJ column, 99:1 Hex/^{i}PrOH, 0.5 mL/min; t_R 26.8 min; t_R 30.7 min). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ 1.01 (d, *J*=6.7 Hz, 3H, CH_3), 1.06 (d, *J*=6.7 Hz, 3H, CH_3), 2.06–2.16 (m, 1H, CH), 3.58 (d, *J*=16.3 Hz, 1H, CH_2), 3.68 (d, *J*=16.3 Hz, 1H, CH_2), 5.31 (d, *J*=2.9 Hz, 1H, CH); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 17.2, 18.1, 31.7, 34.5, 87.7, 173.3.

*4.3.2. 2-Isobutyl-1,3-oxathiolan-5-one (**4b**).* Conversion: 55%, enantiomeric excess (ee): 85%, determined by HPLC analysis (Daicel Chiralpak OJ column, 99:1 Hex/^{i}PrOH, 0.5 mL/min; t_R 27.7 min; t_R 30.0 min). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ 0.95 (d, *J*=6.7 Hz, 3H, CH_3), 0.97 (d, *J*=6.7 Hz, 3H, CH_3), 1.61–1.68 (m, H, CH), 1.78–1.88 (m, 1H, CH_2), 1.91–2.00 (m, 1H, CH_2), 3.60 (d, *J*=16.5 Hz, 1H, CH_2), 3.69 (d, *J*=16.5 Hz, 1H, CH_2), 5.54 (t, *J*=6.6 Hz, 1H, CH); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 22.4, 22.5, 25.4, 31.8, 45.6, 81.3, 172.9. HRMS: found 161.0640, calcd for $C_7H_{13}O_2S$ [M+H$^+$] 161.0631.

*4.3.3. 2-(Pentan-3-yl)-1,3-oxathiolan-5-one (**4c**).*[41] Conversion: 79%, enantiomeric excess (ee): 90%, determined by HPLC analysis (Daicel Chiralpak OJ column, 99:1 Hex/^{i}PrOH, 0.5 mL/min; t_R 19.7 min; t_R 22.5 min). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ 0.92 (t, *J*=7.4 Hz, 6H, $(CH_3)_2$), 1.30–1.40 (m, 1H, CH), 1.46–1.56 (m, 3H, $(CH_2)_2$), 1.66–1.74 (m, 1H, $(CH_2)_2$), 3.56 (d, *J*=16.7 Hz, 1H, CH_2), 3.66 (d, *J*=16.7 Hz, 1H, CH_2), 5.50 (d, *J*=6.4 Hz, 1H, CH); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 11.1, 21.5, 21.9, 31.8, 46.4, 85.6, 173.1. HRMS: found 175.0787, calcd for $C_8H_{15}O_2S$ [M+H$^+$] 175.0787.

*4.3.4. 2-Cyclohexyl-1,3-oxathiolan-5-one (**4d**).*[11] Conversion: 67%, enantiomeric excess (ee): 92%, determined by HPLC analysis (Daicel Chiralpak OJ column, 99:1 Hex/^{i}PrOH, 0.5 mL/min; t_R 28.0 min; t_R 32.1 min). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ 1.02–1.33 (m, 6H, $(CH_2)_3$), 1.67–1.83 (m, 4H, $(CH_2)_2$), 1.96 (d, *J*=12.5 Hz, 1H, CH), 3.57 (d, *J*=16.8 Hz, 1H, CH_2), 3.66 (d, *J*=16.8 Hz, 1H, CH_2), 5.29 (s, 1H, CH); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 25.6, 26.2, 27.7, 28.9, 31.5, 43.9, 87.0, 173.1.

*4.3.5. 2-Heptyl-1,3-oxathiolan-5-one (**4e**).*[26] Conversion: 75%, enantiomeric excess (ee): 71%, determined by HPLC analysis (Daicel Chiralpak OJ column, 99:1 Hex/^{i}PrOH, 0.5 mL/min; t_R 42.0 min; t_R 48.5 min). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ 0.88 (t, *J*=6.8 Hz, 3H, CH_3), 1.20–1.37 (m, 10H, $(CH_2)_5$), 1.78–1.86 (m, 1H, CH_2), 1.96–2.06 (m, 1H, CH_2), 3.62 (d, *J*=16.6 Hz, 1H, CH_2), 3.67 (d, *J*=16.6 Hz, 1H, CH_2), 5.48 (t, *J*=6.7 Hz, 2H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 14.2, 22.7, 25.1, 29.1, 31.8, 36.8, 82.6, 173.0.

*4.3.6. 2-(Pyridin-2-yl)-1,3-oxathiolan-5-one (**4f**).*[26] Conversion: 16%, enantiomeric excess (ee): 83%, determined by HPLC analysis (Daicel Chiralpak OJ column, 80:20 Hex/^{i}PrOH, 0.5 mL/min; t_R 51.8 min; t_R 59.2 min). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ 3.79 (d, *J*=16.4 Hz, 1H, CH_2), 3.87 (d, *J*=16.4 Hz, 1H, CH_2), 6.50 (s, 1H, CH), 7.27–7.32 (m, 1H, CH), 7.44 (d, *J*=7.8 Hz, 1H, CH), 7.76 (t, *J*=7.8 Hz, 1H, CH), 8.61 (d, *J*=4.8 Hz, 1H, CH); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 31.7, 81.4, 119.8, 124.1, 137.3, 149.9, 157.0, 173.0.

*4.3.7. 2-(Benzyloxymethyl)-1,3-oxathiolan-5-one (**4g**).*[40] Conversion: 93%, enantiomeric excess (ee): 71%, determined by HPLC analysis (Daicel Chiralpak OD-H column, 95:5 Hex/^{i}PrOH, 0.5 mL/min; t_R 74.3 min; t_R 80.3 min). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ 3.51 (d, *J*=16.3 Hz, 1H, CH_2), 3.66 (dd, J_1=4.3 Hz, J_2=10.9 Hz, 1H, CH_2), 3.68 (d, *J*=16.3 Hz, 1H, CH_2), 3.72 (dd, J_1=4.3 Hz, J_2=10.9 Hz, 1H, CH_2), 4.55 (s, 2H, CH_2), 5.52 (t, *J*=3.9 Hz, 1H, CH), 7.21–7.31 (m, 5H, CH), 7.44 (d, *J*=7.8 Hz, 1H, CH), 7.76 (t, *J*=7.8 Hz, 1H, CH), 8.61 (d, *J*=4.8 Hz, 1H, CH); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 31.1, 73.1, 73.8, 79.9, 127.7, 128.0, 128.6, 137.3, 172.9.

4.4. 5-Acetoxy-2-benzyloxymethyl-1,3-oxathiolane (5)[40]

To a solution of 2-benzyloxymethyl-1,3-oxathiolan-5-one (384 mg, 1.67 mmol) in anhydrous toluene (16 mL) was added a solution of diisobutyl aluminum hydride in hexanes (1 M, 2.5 mL, 2.50 mmol) dropwise over 30 min at −78 °C. The mixture was stirred for 80 min under N_2 atmosphere, keeping the temperature below −70 °C at all times. The reaction was quenched by dropwise addition of cold, anhydrous methanol (5 mL) over 20 min and the reaction mixture was stirred for a further 15 min at −78 °C followed by 2 h at rt. Washing with saturated aqueous Rochelle salt solution (12 mL), distilled H_2O (2×12 mL), and brine (2×12 mL), followed by drying with $MgSO_4$, filtering, and concentration in vacuo yielded a crude oil that was used in the next step without further purification. The crude product mixture was dissolved in

anhydrous CH_2Cl_2 (12 mL) in a round bottom flask and the solution was cooled to 0 °C and evacuated two times with N_2. Et_3N (0.23 mL, 1.67 mmol) was added dropwise under vigorous stirring and the solution was stirred for 10 min, after which acetic anhydride (0.20 mL, 2.12 mmol) and DMAP (65 mg, 0.53 mmol, dissolved in 0.5 mL CH_2Cl_2) were added via syringe. The solution was slowly warmed to rt and stirred under N_2 for 14 h. Quenching by slow addition of saturated aqueous $NaHCO_3$ solution (12 mL) was followed by extraction of the aqueous phase with CH_2Cl_2 (3×12 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated. Purification by column chromatography (19:1 to 15:1 hexanes/EtOAc) yielded an inseparable mixture of the product diastereomers as a slightly yellow, viscous oil (232 mg, 0.87 mmol, 52%). *trans*-**5**: R_f 0.24 (Hexanes/EtOAc 8:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29–7.36 (m, 5H), 6.69 (d, 1H, J=4.3 Hz), 5.53 (dd, 1H, J=4.5, 5.6 Hz), 4.62 (s, 2H), 3.73 (dd, 1H, J=5.9, 10.6 Hz), 3.66 (dd, 1H, J=4.2, 10.6 Hz), 3.31 (dd, 1H, J=4.3, 11.5 Hz), 3.11 (d, 1H, J=11.5 Hz); 2.09 (s, 3H); *cis*-**5**: R_f 0.24 (Hexanes/EtOAc, 8:1); ^{1}H NMR (500 MHz, solvent) δ 7.29–7.36 (m, 5H), 6.59 (d, 1H, J=4.1 Hz), 5.51 (dd, 1H, J=2.3, 4.9 Hz), 4.61 (s, 2H), 3.78 (dd, 1H, J=7.0, 10.5 Hz), 3.73 (dd, 1H, J=5.9, 10.6 Hz), 3.28 (dd, 1H, J=4.2, 11.8 Hz), 3.15 (d, 1H, J=11.8 Hz); 1.99 (s, 3H);^{13} C NMR (125 MHz, $CDCl_3$, mixture of diastereomers) δ 169.8, 169.7, 137.8, 137.7, 128.5, 128.0, 127.82, 127.77, 127.75, 127.68, 99.3, 99.1, 86.1, 84.6, 74.2, 73.6, 73.4, 72.4, 38.0, 37.4, 21.2, 21.1.

4.5. 2-Benzyloxymethyl-1,3-oxathiolan-5-yl-N^4-acetylcytosine (6)

Silylated N^4-acetylcytosine was generated in situ by stirring *N*-acetylcytosine[42] (55.4 mg, 0.362 mmol) with hexamethyldisilazane (1.0 mL, 4.8 mmol) and a crystal of $(NH_4)_2SO_4$ at reflux (135 °C) under argon until all solid had dissolved (approximately 3 h). The solution was slowly cooled to rt, and the hexamethyldisilazane was removed under anhydrous conditions. The system was evacuated five times with argon, and dried under vacuum for 30 min. Meanwhile, 5-acetoxy-2-benzyloxymethyl-1,3-oxathiolane (60.0 mg, 0.224 mmol) was dissolved in anhydrous acetonitrile (6 mL) and stirred with pre-activated 3 Å molecular sieves for 30 min under N_2. The solution was transferred via syringe to the dried silylated N^4-acetylcytosine, and the resulting solution was cooled to 0 °C. TMSI (70.0 μL, 0.504 mmol) was added dropwise over 15 min and the resulting yellow solution was stirred under argon at 0 °C for 1 h. The reaction was quenched by addition of a mixture of ethyl acetate (10 mL) and aqueous $NaHCO_3$ solution (5 wt %, 6 mL) and the resulting two-phase system was stirred at rt for 15 min. The mixture was further diluted with ethyl acetate (10 mL) and the organic phase was washed with saturated aqueous $NaHCO_3$ solution (15 mL), distilled H_2O (2×15 mL), and brine (2×15 mL). Drying with $MgSO_4$, filtration, and concentration in vacuo yielded the crude product mixture as a yellow solid. Purification by column chromatography (EtOAc) allowed for separation of the diastereoisomers to yield the *trans* (28.7 mg, 0.127 mmol, 35%) and *cis* (34.4 mg, 0.157 mmol, 43%) isomers as white solids. *cis*-**6**: R_f 0.09 (EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.43 (s, br, 1H), 8.39 (d, 1H, J=7.5 Hz), 7.35–7.41 (m, 5H), 7.23 (d, 1H, J=7.5 Hz), 6.34 (dd, 1H, J=2.3, 5.2 Hz), 5.36 (t, 1H, J=3.2 Hz), 4.65 (s, 2H), 4.04 (dd, 1H, J=2.8, 11.2 Hz), 3.83 (dd, 1H, J=3.6, 11.2 Hz), 3.58 (dd, 1H, J=5.4, 12.7 Hz), 3.22 (dd, 1H, J=2.2, 12.7 Hz), 2.25 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 162.9, 154.9, 145.6, 137.1, 128.6, 128.2, 127.7, 96.0, 87.5, 73.9, 69.5, 39.5, 29.7, 24.9; 1D-NOE NMR pulse at 6.34 ppm, δ 8.39, 5.36, 3.58, 3.22; 1D-NOE NMR pulse at 5.36 ppm, δ 6.34, 4.04, 3.83, 3.58; *trans*-**6**: R_f 0.12 (EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.15 (s, br, 1H), 7.74 (d, 1H, J=7.5 Hz), 7.41 (d, 1H, J=7.5 Hz), 7.28–7.36 (m, 5H), 7.47 (dd, 1H, J=1.6, 5.1 Hz), 5.68 (t, 1H, J=4.9 Hz), 4.60 (s, 2H), 3.61–3.65 (m, 3H), 3.21 (dd, 1H, J=1.7, 12.5 Hz), 2.25 (s, 3H); HRMS: found 362.1169, calcd for $C_{17}H_{20}N_3O_4S$ [M+H$^+$] 362.1169.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2014.03.059. These data include MOL files and InChIKeys of the most important compounds described in this article.

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