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A New Process for Synthesis of Apricitabine, 2-(*R*)-Hydroxymethyl-4-(*R*)-(cytosin-1′-yl)-1,3-oxathiolane, an Anti-HIV NRTI

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Supporting Information

ABSTRACT: Apricitabine is a novel inhibitor of the HIV virus reverse transcriptase polymerase which is currently in clinical development for the treatment of AIDS. A new process for the preparation of apricitabine is presented which requires only three steps from 2-(*R*)-benzoyloxymethyl-1,3-oxathiolane. The new process produces the *cis*-(2*R*,4*R*) isomer in greater than 99% diastereomeric excess by preferential crystallisation of the conglomerate form of the novel 2-(*R*)-benzoyloxymethyl-4-(*R*)-(*N*-benzoylcytosin-1-yl)-1,3-oxathiolane intermediate without requiring chromatography. Deprotection of the intermediate in 88% yield then gives chiral apricitabine, in 30% overall yield. The new method avoids a lengthy salt formation/-break stage, does not require toluene sulphonic acid, and introduces no new byproduct to the manufacturing process.

INTRODUCTION

Apricitabine (ATC) 2-(*R*)-hydroxymethyl-4-(*R*)-(cytosin-1′-yl)-1,3-oxathiolane is the (−)-enantiomer[3] which as the free base exists as a single polymorph. ATC when administered orally in a simple dosage form achieves bioavailability of >80% with no food effect. ATC is classed as a deoxycytidine analogue nucleoside reverse transcriptase inhibitor (NRTI) and is in phase III clinical development for the treatment of human immunodeficiency virus (HIV) infection. ATC shows good antiviral activity in vitro, against wild-type HIV-1 and against HIV-1 laboratory strains and clinical isolates with the M184 V mutation in the viral RT enzyme, a mutation which makes the virus resistant to the approved cytidine-based nucleotides, 3TC (2-(*R*)-hydroxymethyl-5-(*S*)-(cytosin-1′-yl)-1,3-oxathiolane, **1**) and FTC (**2**)[1a−c] (Figure 1); thus, there is an unmet clinical need for ATC.[2a−f] ATC has shown exceptional efficacy and safety in clinical trials of both treatment-naïve and treatment-experienced HIV-1-infected patients, including those with virus harbouring the M184 V mutation, with or without thymidine analogue mutations (TAMs). The chronic nature of the HIV infection requires that the drug components of the current standard of care of highly active antiretroviral therapy (HAART) regimens are safe and well-tolerated. ATC has a suitable safety profile to be incorporated in a HAART regime since in clinical trials to date at more than 148 weeks of BID dosing there have been no adverse events attributed to ATC.

ATC was first synthesised by Belleau and Nguyen-Ba[3] as an approximately equal percentage of the two cis and two trans forms after separation by flash chromatography. The synthesis used a sila-Pummerer rearrangement of the racemic sulphoxide with tetrabutyl ammonium acetate and Ac_2O to give the 4(*R*)- and 4(*S*)-acetate intermediate. Optimisation of the sila-Pummerer reaction by Belleau[4] involved maintaining a high concentration of acetate ions in the acetic anhydride, whereby tetra-*n*-butyl ammonium was found to be superior as a counterion to sodium. In the subsequent Vorbruggen step the coupling gave 60% yield of the *cis*- and *trans*-isomers, in a ratio of 7:3.

The current synthesis of ATC at 100-kg plant scale (Scheme 1) is the result of two further rounds of process development.[5] The method proceeds by peroxide oxidation of the 2-(*R*)-benzoyloxymethyl-1,3-oxathiolane (**4**), (2-(*R*)-oxathiolane) to give the oxathiolane-*S*-oxide (**5**) as a mixture of isomers at the sulphur. The next step proceeds via a planar carbocation and therefore is independent of the ratio of isomers at the sulphur. For a representative batch of 2-*R*-oxathiolane and 2-*S*-oxathiolane of 64:36 the sulphoxidation gave a ratio of *E*:*Z* isomers of 2.8:1, based upon peak areas from chiral HPLC. Step 2 of the process (Scheme 1) is a combined sila-Pummerer rearrangement and Vorbruggen coupling of the oxathiolane-*S*-oxide (**5**) with *N*-acetylcytosine in the presence of trimethylsilyliodide, triethylamine and catalytic amounts of copper(II)chloride in dichloromethane to give the intermediate, 2-benzoyloxymethyl-4-(*N*-acetylcytosin-1-yl)-1,3-oxathiolane (**6**). The intermediate (**6**) at step 2 has less than 70% chiral purity because it contains 30% of the *trans*-(2*R*,4*S*)-isomer formed during the coupling step and a small percentage of the *cis*-(2*S*,4*S*) and *trans*-(2*S*,4*R*)-isomers, formed from 2-(*S*)-oxathiolane that is present in the starting material. Step 3 is the deprotection to give (±)2-hydroxymethyl-(±)4-(cytosin-1′-yl)-1,3-oxathiolane. It was shown that the isomeric mixture could be resolved by formation of diastereomeric salts with chiral acids,[6] thereby avoiding chromatography. In particular the inexpensive, achiral *p*-toluene sulphonic acid was observed[7] to give a conglomerate salt form (**7**), allowing the isomers to be separated by preferential crystallisation. This use of toluene sulphonic acid does, however, require a sensitive analytical assay to ensure the absence of alkyl toluene sulphonic esters which are potential genotoxins.[8]

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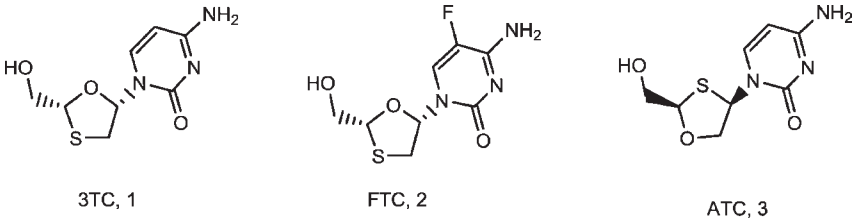
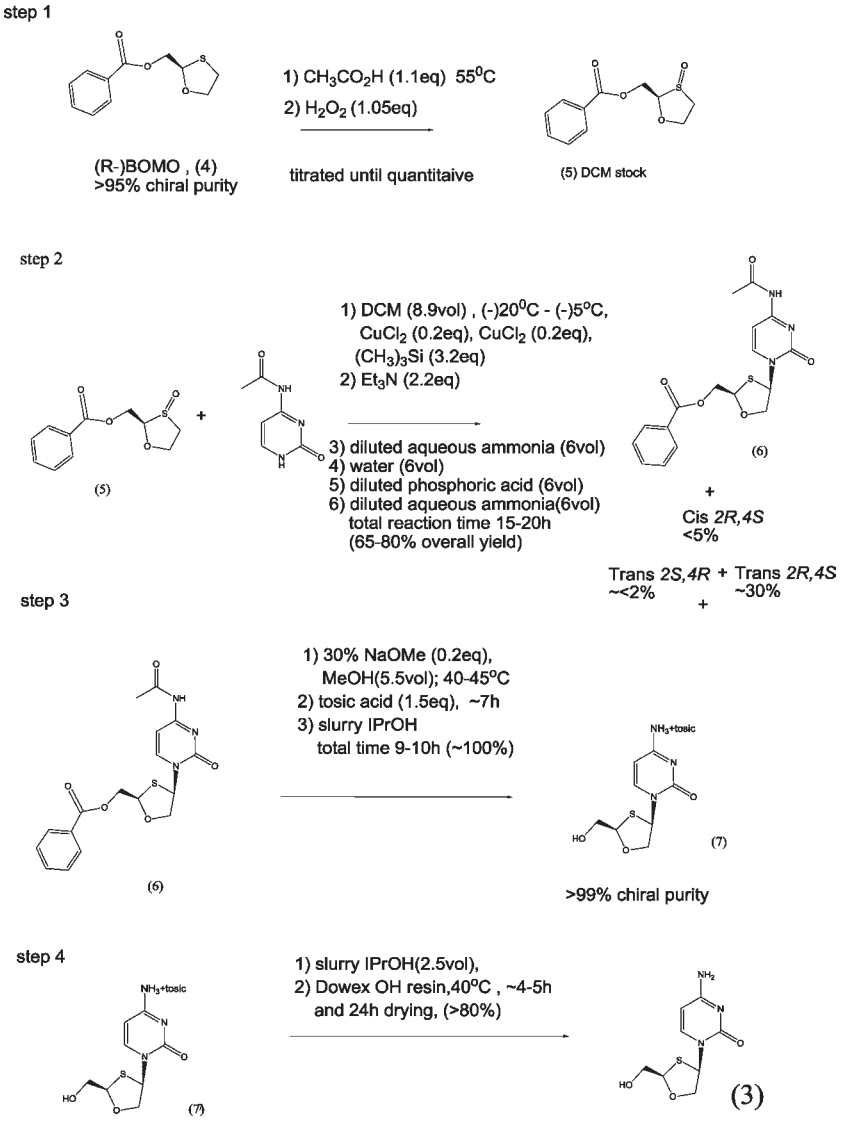


Figure 1.

Scheme 1. Existing process for manufacture of ATC



Step 4 is then removal of the acid counterion by an ion-exchange resin, such as Dowex 550A-OH (140% w/w) to give ATC (**3**). The limited solubility of the free base form of ATC (**3**) requires this process to be run as a continuous cycle, separating the product as it crystallises from the solution. In summary the current industrial-scale process is carried out in four stages from 2-(*R*)-oxathiolane (**4**), giving ATC (**3**) in greater than 99% chiral purity (Scheme 1). Unfavorable aspects of this process include the use of the tosic acid and the lengthy salt formation−salt break process in stages 3 and 4.

This paper presents a new route of synthesis of ATC (**3**) in three steps from chiral 2-(*R*)-oxathiolane (**4**). This method utilises the observation[9] that 2-benzoyloxymethyl-4-(*N*-benzoyl-cytosin-1′-yl)-1,3-oxathiolane (**10**) forms a conglomerate. The method is significantly different from the existing method described above since it does not involve the salt formation step

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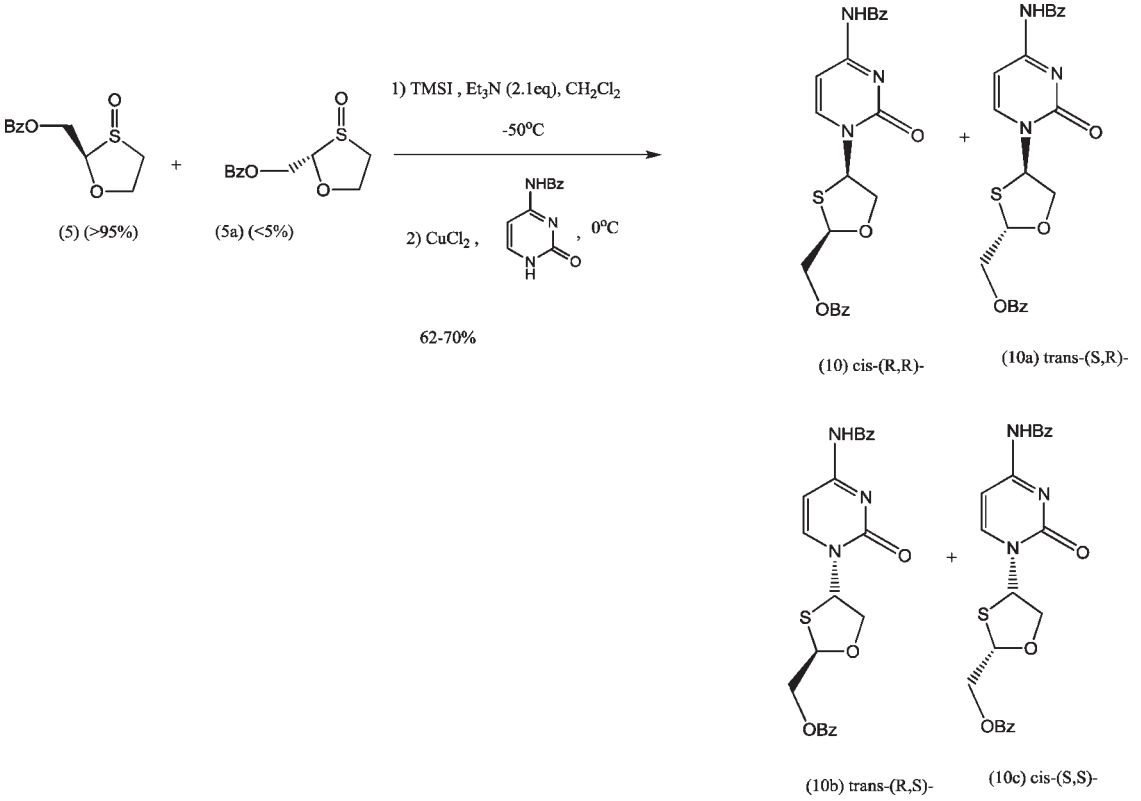
Scheme 2



and so avoids the use of *p*-toluene sulphonic acid. Consequently the new method is also only three stages in length, not needing the salt-break step to give the free base form of ATC (**3**). Importantly, for a new method to supply clinical material, which already has a defined specification, the new method does not introduce any new impurities to the process. We have investigated the coupling conditions, the byproduct formed, and the optimal conditions for the recrystallisation, including the influence of selection of the amino and hydroxyl protecting groups.

RESULTS AND DISCUSSION

2-(*R*)-Benzoyloxymethyl-1,3-oxathiolane-*S*-oxide (**5**) is prepared from 2-(*R*)-benzoyloxymethyl-1,3-oxathiolane (**4**) as in the previous process and used as a mixture of isomers at the sulphur centre.

Step 2 of the new process now involves coupling of *N*-benzoylcytosine with the oxathiolane-*S*-oxide (**5**) (Scheme 2), in a change from the use of *N*-acetylcytosine in the former process (Scheme 1).

The reaction proceeds through an initial activation of (**5**) undergoing a sila-Pummerer rearrangement.

Although the chemical yield of all the coupled isomers is high at 86%, the content of the desired isomer is only 62%, and so the byproducts were studied in an effort to understand the mechanism of the reaction. Two byproducts, the olefin (**8**) and hydroxyoxathiolane (**9**) were identified. (Figure 2).

The olefin (**8**) is an easily identified byproduct in the reaction, formed in an average reaction at about 10% (with respect to the total coupled product), possibly via an elimination reaction. The

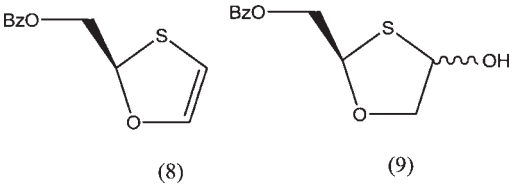


Figure 2

yield of olefin (**8**) decreases to about 7% (with respect to the total coupled product) in more efficient reactions and can be seen in increased amounts in less efficient reactions.

The likely route to hydroxy oxathiolane (**9**) is from an attack of water on an activated C-4 in the oxathiolane intermediate, which has a precedent in the work of Nishizono on sila-Pummerer reactions.[10] It is likely that these byproduct are common to the old and new methods, although they have not previously been described.

Numerous Lewis acids and catalysts have been investigated in the literature for the coupling of acetylcytosine to sulphoxides such as (**5**) (Scheme 2).[11] Catalysts such as $CuCl_2$ are believed to play a role by inducing a β-attack of the silylated-*N*-benzoyl cytosine, resulting in an increase in cis-selectivity during the coupling. For the next sections where we examined various variables of the reaction, a standard set of reaction conditions was followed (see footnotes of Table 1), similar to those used in the old process (Scheme 1), however substituting *N*-acetyl cytosine with *N*-benzoylcytosine (Table 1, entry 6). The reactions were conducted at 1-g scales with respect to the oxathiolane-*S*-oxide (**5**). The first variable that was studied was the

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Table 1. Influence of varying the equivalents of TMSI on stage 2 yield and chiral purity

expt #[a]	TMSI (equiv)	crude mass recovery %	% coupled products (**10**−**10c**)[b]	% olefin (**8**) byproduct[b]	% *cis*-(2*R*,4*R*)-**10**
1	2.1	72	no coupling product detected	–	–
2	2.5	74	no coupling product detected	–	–
3	2.9	82	60	6.9	75
4[c]	3.1	94	60	12	73
5	3.1	88	52	5.7	75
6[d]	3.1	84−89	62−70	6.2−7	74

[a] For this set of experiments a standard method was followed: to a solution of 1 equiv of oxathiolane-*S*-oxide (**5**) in dichloromethane at −50 °C, 2.1 equiv of triethylamine was added, followed by the addition of 3.1 equiv of TMSI, while keeping the temperature below −40 °C . Allowing 1 h for the sila-Pummerrer rearrangement, 0.2 equiv of copper chloride was added, followed by 1 equiv of benzoyl cytosine. Next the reaction was stirred at 0 °C, overnight. After overnight stirring, the reaction was brought up to room temperature for 30 min, before quenching with water. Workup involved sequential washing with 5% ammonia, 2% phosphoric acid, and 1 M sodium thiosulphate. [b] Percent estimated by comparing NMR integrations of relevant protons. For the coupled products (*cis*- and *trans*-) the anomeric proton (C1−H) (which shows a chemical shift of 6.6 ppm in $CDCl_3$ in both *cis*- and *trans*-isomers) was used for comparison. For the olefin (**8**), C-2 olefin proton (chemical shift: 6.04 in $CDCl_3$) or/and tertiary C-4 proton (chemical shift: 6.02 ppm in $CDCl_3$) was used. [c] TMSI was added last. [d] "Standard conditions", using *N*-benzoyl cytidine.

Table 2. Influence of varying the amount of triethylamine on stage 2 yield and chiral purity

expt #	triethylamine (equiv)	% coupled products (**10**−**10c**)[a]	% olefin (**8**)[a]	% *cis*-(2*R*,4*R*)-**10**
1	1.05	no coupled product[b]	not seen	–
std conditions[c]	2.1	62−70	6.2−7	74
2	3.15	50	8.5	73
3	4.2	30	15	difficult to assess due to interfering peaks

[a] Percentage estimated from NMR integrations see footnote *a* in Table 1. [b] Reduced oxathiolane was the major product (57%). [c] "Standard conditions", using *N*-benzoyl cytidine.

Table 3. Influence of varying the amount of $CuCl_2$ on stage 2 yield and chiral purity

expt #	$CuCl_2$ (equiv)	% crude mass recovery	% coupled products (**10**−**10c**)[a]	% olefin (**8**)[a]	% *cis*-(2*R*,4*R*)-**10**
1	0	87	63	12.6	64
2	0.05	85	69	6.9	70
3	0.1	77	69	6.9	71
4	0.3	81	60	6.6	76
5[b]	0.2	84−89	62−70	6.2−7.0	74

[a] Percentage estimated from NMR integrations. See footnote (a) of Table 1. [b] "Standard conditions", using *N*-benzoyl cytidine.

stoichiometry and order of addition of the TMSI while keeping constant the quantities of the other reagents (Table 1).

The results in Table 1 show that 3 equiv of TMSI are needed for the reaction. Two equiv of TMSI would be needed for the sila-Pummerer reaction and at least 1 equiv needed for the silylation of benzoyl cytosine. In entry 4 (Table 1) the TMSI was added last; however, the reaction was less efficient compared to the results achieved under the standard conditions (entry 6, Table 1) as shown by the increased amount of olefin (**8**) at 20%. In entry 5 (Table 1) the *N*-benzoylcytosine was first treated with TMSI in presence of triethylamine, and then finally the oxathiolane-*S*-oxide (**5**) dissolved in dichloromethane was added. Again the reaction was of low efficiency as evidenced by lower extent of reaction. Next the quantity of triethylamine required was also investigated as shown in Table 2.

When 1 equiv of triethylamine was used (entry 1, Table 2), no coupling was seen. Of interest is the observation that no olefin byproduct (**8**) had formed. This possibly shows that negligible sila-Pummerer rearrangement had taken place. When there was excess base (entries 2 and 3, Table 2), the reaction becomes less efficient, and the amount of byproduct increases, arising from competing eliminations from the sila-Pummerer reaction. Next we looked at the effects of varying $CuCl_2$, compared to the 0.2 equiv used in the standard conditions, while keeping the other reagents constant (Table 3). Reducing the amount of $CuCl_2$ used was investigated with the objectives being to use less copper and to reduce the concern of copper contamination in the final product. However, this was expected to be at the cost of a slight decrease in the cis bias of the product stereochemistry.

The above data (entry 1, Table 3) shows that even without $CuCl_2$ there is a bias in the reaction towards formation of the desired *cis*-(2*R*,4*R*)-isomer (**10**). The presence of some $CuCl_2$, (0.05 equiv, entry 2 Table 3) increases the cis-selectivity albeit not by much. Using 0.1 equiv of $CuCl_2$ and averaging the results over many reactions, the *cis*-/*trans*- ratio is actually within the range 2.3−2.6:1 (entry 3, Table 1), as compared to the standard conditions using 0.2 equiv of $CuCl_2$ which gives a ratio of 2.5−2.9:1 (entry 5, Table 1). It is notable that the standard conditions using *N*-acetyl cytosine tend to give a *cis*-/*trans*- ratio of 2.3:1. A further increase in the amount of $CuCl_2$ used to 0.3 equiv, also increases the ratio of the desired *cis*-(2*R*,4*R*)-isomer (**10**), but not by much (entry 4, Table 3). Therefore, the results

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show that we can reduce the amount of $CuCl_2$ used without affecting the outcome of the reaction too much.

For the coupling reaction, the $CuCl_2$ used has to be anhydrous, while formation of the hydrate happens rapidly, over 2−3 h of exposure to air at room temperature, and so we investigated the effect of hydrated $CuCl_2$ on the reaction. In fact using the standard conditions of 0.2 equiv of $CuCl_2$, but now in its hydrated form, gave similar mass recovery but only 34% yield of the coupled products **10**−**10c** and 28.6% of the olefin (**8**), compared to the results of the standard conditions of 62−70% yield and 6.2−7.0% of olefin (**8**). These results show that hydration of the copper chloride gives a poor yield and an increased level of byproduct.

To summarise, the use of 0.1 equiv of $CuCl_2$ reduces the *cis*-/*trans*- ratio in comparison to that of the standard conditions when using *N*-benzoylcytosine, but not significantly, compared to results from the old process (Scheme 1).

It was found that recrystallisation of the crude product mixture in methanol gives predominantly the desired *cis*-(2*R*,4*R*)-isomer **10**. Optical purity can be further improved by sequential recrystallisations or entrainment. This observation is particularly useful when starting with less optically pure starting material and is a particularly important finding of the present study. Recrystallising in 14−15× volumes of methanol (with respect to the mass of the crude) gave almost exclusively the *cis*-(*R*,*R*)-isomer. Evidence that **3** was recrystallising as a conglomerate form was confirmed by the X-ray analysis of the crystals.

The structure of **3** presented in Figure 3 consists of two molecules of methanol which form a H-bonded bridge between N3−H and O4, which is further stabilised by a weak hydrogen bond to N2.

Recrystallisation of the methanol solvate of **3** from toluene gave superior quality crystals of **3** as a nonsolvate, the structure of which is presented in Figure 4 of Supporting Information. Clearly, the structures of **3** from methanol and **3** from toluene exist in different conformations. The difference in the conformations is indicated by the O(1)−C(3)−(C4)−O(2) dihedral angle; for the methanol solvate this is gauche (65.1(9)°), whereas in the structure from toluene this is essentially anti, 179.1(3)°. These results suggest a basis for the preferential crystallisation from methanol.

Next we looked at oxathiolane protected with acetyl and *p*-toluoyl groups and coupling to benzoyl cytosine. Thus, (*R*)-oxathiolane (**4**) was cleanly debenzoylated with sodium methoxide in methanol to yield the hydroxymethyl-1,3-oxathiolane (**11**). The free hydroxy group was protected with acetyl and toluoyl groups, to yield **12** and **15**, respectively, and these were oxidised with H_2O_2/AcOH to the corresponding sulphoxides **13** and **16** (Scheme 3). The sulphoxides were next subjected to TMSI-induced coupling with *N*-benzoylcytosine (Scheme 3), to give the *O*-acetoxybenzoylcytosine coupled product **14** in 58% chemical yield and 68% chiral purity based on the desired *cis*-(2*R*,4*R*)-isomer.

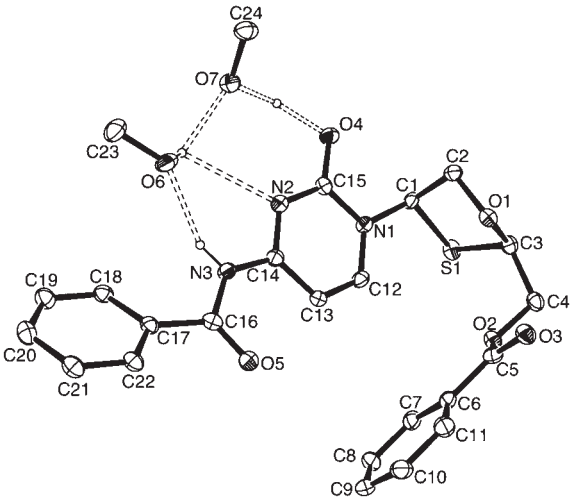

Figure 3. Thermal ellipsoid plot of (3), crystals from methanol. Ellipsoids are at the 20% probability level.

Scheme 3

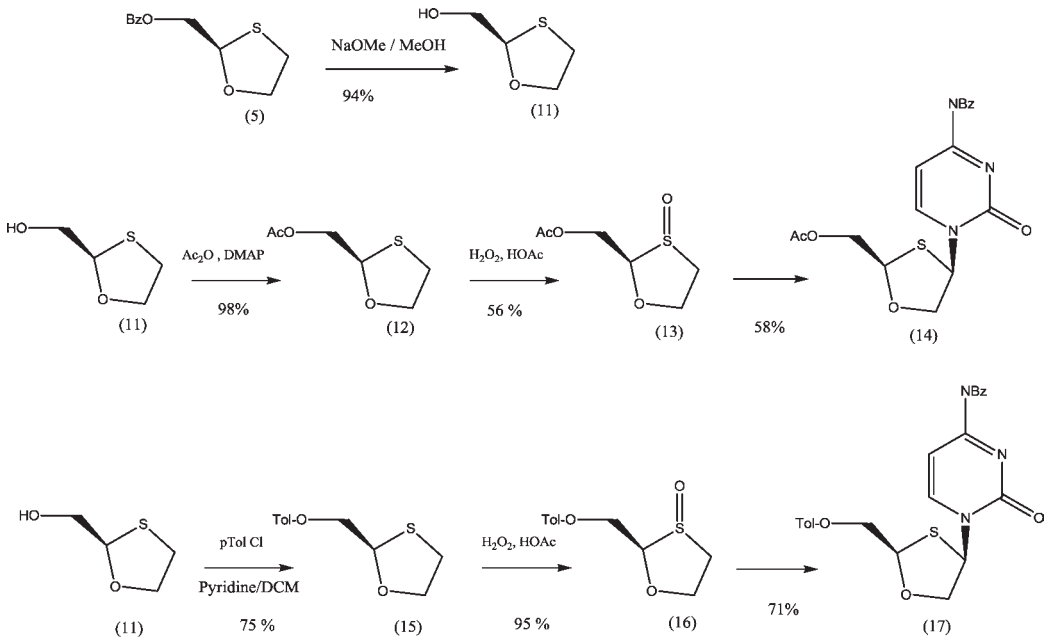

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Table 4. Selection of *N*-cytidine protecting group

expt #	*O*-acyl group	% coupled products	% *cis*-(2*R*,4*R*)-**10**	yield of *cis*-(2*R*,4*R*)-**10**	*cis*-(**10** + **10c**)/ *trans*-(**10a** + **10b**) ratio after recrystallisation
1−4[a]	benzoyl	62−70	74	40−45	>99:1
5	acetyl	66	69	46	1:4
6	isobutyryl	64	73	47	no recrystallisation from MeOH
7	pivalyl	64	74	47	no recrystallisation from MeOH

[a] Average of four experiments.

Table 5. Influence of chiral purity of the feed oxathiolane on the chiral recrystallisation, with or without entrainment

expt #	2-(*R*)-/2-(*S*)- oxathiolane (**4**) ratio[b]	*cis*-(2*R*,4*R*)-**10**/*cis*-(2*S*,4*S*)-**10c** ratio of the crude coupled product[c]	presence of *trans*-isomer after recrystallisation[d] (%)	*cis*-(2*R*,4*R*)-**10**/*cis*-(2*S*,4*S*)-**10c** ratio without entrainment	*cis*-(2*R*,4*R*)-**10**/ *cis*-(2*S*,4*S*)-**10c** ratio with entrainment
1	98.:1.7	–	<1	99.0 − 99.4:1.0−0.6	99.5−99.8:0.5−0.2
2	94.2:5.8	96:4	<1	98.14:1.86	99.33:0.67
3	90:10	94.2:5.8	<1	97.67:2.33	99.23:0.77
4	81.7:18.3	89.5:10.5	2−4	95.1:4.9	98.2:1.8

[a] Mixtures of 2-(*R*)- and 2-(*S*)-oxathiolane were oxidised to the corresponding *S*-oxide under the standard conditions with H_2O_2/HOAc (Scheme 1), and then the reaction was worked up as before; the crude was recrystallised with and without entrainment. Crystallisation was achieved by removing solvent from the crude material and then refluxing in 14 volumes of methanol until all the material was in solution. Next, this was allowed to cool with stirring while the temperature was monitored. At the temperature of approximately 55 °C, the stirred mixture was seeded with pure *cis*-(*R*,*R*)-**10**, allowed to come to room temperature, and stirred overnight. The crystallised mixture was then filtered, washed with methanol, and analyzed via chiral HPLC. As a control, an identical sample was refluxed in methanol to dissolve the mixture and then allowed to cool without any stirring or seeding. [b] Calculation based on purities of starting ratio of 2-(*R*)-oxathiolane/2-(*S*)-oxathiolane estimated from HPLC peak areas. [c] Percentage estimated from HPLC chromatogram peak areas. [d] Percent estimated by comparing NMR integrations of the tertiary C-4 proton of *cis*- and *trans*-isomers. For the *cis*-isomers, C-4 proton shows a chemical shift 5.54 ppm in $CDCl_3$, while for the *trans*-isomer, C-4 proton shows a chemical shift 5.95 ppm in $CDCl_3$.

The *O*-toluoyloxy-oxathiolane coupled product **17** was formed in 71% chemical yield and 74% chiral purity based on the desired *cis*-(2*R*,4*R*)-isomer.

We found that the *O*-acetoxyoxathiolane-*N*-benzoylcytosine coupled product **14** did not crystallise at all from methanol, and the *O*-toluoyloxy-oxathiolane-*N*-benzoylcytosine coupled product **17** was less soluble in methanol, when compared to *O*-benzoyloxy-oxathiolane-benzoylcytosine such that it could not be used in a recrystallisation. We then investigated *O*-benzoyl oxathiolanes (**4**) with a range of different *N*-acyl cytosines (Table 5).

As shown in Table 4 in combination with the *O*-benzoyl-oxathiolane (**4**) only the *N*-benzoylcytosine (entries 1−4 Table 4) gave the recrystallisation to the desired chirally pure *cis*-(2*R*,4*R*)-**10**), while acetyl (entry 5, Table 4) recrystallised to predominantly the *trans*-isomer, and isobutyryl (entry 6, Table 4) or pivalyl (entry 7, Table 4) failed to selectively recrystallise the desired compound.

A mixture of enantiomers can crystallise either by the formation of a racemic compound or by the formation of a conglomerate. In a racemic compound, the crystals are in a lattice with a regular arrangement of both enantiomers in equal amounts. However, in a conglomerate, molecules of one enantiomer are preferentially attracted to the same enantiomer, thus crystallising as a physical mixture of crystals belonging to one enantiomer or the other.[12] That the *O*,*N*-benzoyl compound (**10**) formed a conglomerate is a highly fruitful observation since less than 10% of all racemate salts exhibit conglomerate behaviour.[13] If the material of interest crystallises as a conglomerate, then a process known as entrainment (essentially by using the pure crystals as seeds for crystallisation of the enantiomer of interest) could be used to preferentially crystallise the desired enantiomer, enhancing the optical purity.

Entrainment could be affected by many variables such as the amount of seed crystals and the duration of stirring.

Next we oxidised varied proportions of 2-(*R*)- and 2(*S*)-oxathiolanes under the standard conditions and gave the results as in Table 5.

In summary, Table 5 shows that there is a substantial optical purity enhancement during the crystallisation, even without entrainment. Even starting with an oxathiolane of low 2-(*R*)-/2-(*S*)- ratio of 80:20, the (2*R*,4*R*)-/(2*S*,4*S*)- ratio after recrystallisation, (entry 4, column 5, Table 5), has increased to 95:5, even without entrainment. However, with entrainment, the enhancement is even higher at 98:2 (entry 4, column 6, Table 5). Starting with oxathiolane of an 2-(*R*)-/2-(*S*)- ratio of 90:10 gave a final purity over 99:1. (entry 3, Table 5, column 6). This information shows that starting oxathiolane need not be highly chirally pure to get a high product purity.

However it is also interesting to note that as the optical purity of the starting material goes down, the quality of the separation of *cis*-**10** and -**10c** from *trans*-**10** and -**10b** also decreases. Generally below a 2-(*R*)-/2-(*S*)- ratio of 90/10, trace presence of the *trans*-isomer could be seen. (see entries 4 and 5, Table 5). However, a second recrystallisation should remove this *trans*-isomer (**10a** and **10b**).

With a true conglomerate, entrainment induced optical purity enhancement could occur with either enantiomer. To test this, we did an analogous reaction with a oxathiolane-*S*-oxide derived from feed enriched in the *S*-isomer oxathiolane (**4a**) of a 2-(*R*)-/2-(*S*)- ratio 12.1:87.9. The crude reaction mixture derived from the coupling of *S*-configuration oxathiolane-*S*-oxide with *N*-benzoyl-cytosine was recrystallised with and without entrainment. Indeed, the results with entrainment giving crystals of 98.5% (2*S*,4*S*)-isomer were comparable to the analogous experiment where the oxathiolane was predominantly the 2-(*R*)-isomer (entry 3, Table 5). We also showed that a nucleoside containing fluorocytidine was amenable to the same procedure (unpublished results).

We investigated the effect of the time of crystallisation.

Standing overnight was found to be optimum and convenient. At 1.5 or 3 h intervals it appeared the crystallisation was not

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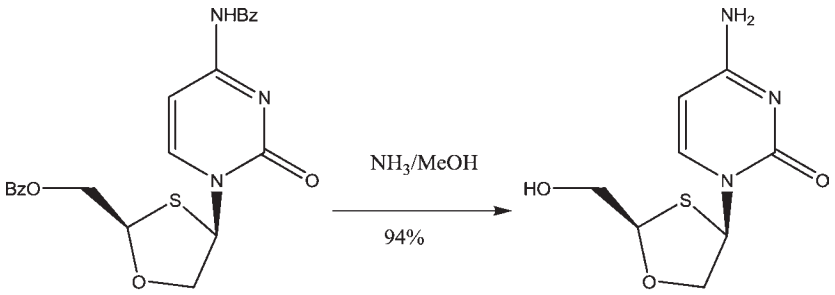
Table 6. Influence of the duration of recrystallisation on chiral purity

		optical purity	
experiment[a]	time allowed for crystallisation	recrystallisation without seeding or stirring[b]	recrystallisation with seeding (14 mg/g crude)[b]
1	1.5 h	98.4/1.6	99.3/0.7
2	3 h	98.4/1.6	99.4/0.6
3	3 days	98.6/1.4	99.5/0.5

[a] Starting with oxathiolane (**4**) of a 2-(*R*)-/2-(*S*)- ratio of 97/3, reaction under standard conditions (see table 1) and recrystallation of the crude coupled product (**10**−**10c**) from 14 volumes of methanol. [b] Ratio of *cis*-(2*R*,4*R*)- (**10**)/**10a** + **10b** + **10c**

Scheme 4



(10) (3)

complete (entries 1 and 2 respectively, table 6), and thus we generally used overnight standing. Keeping more than 1 day improved the purity by, on average, only 0.1%, which is not justified by the extra time (entry 3, Table 6).

Then we tested the effects of the variables: seeding and stirring.[17] In the optimal conditions with stirring and seeding the chiral purity for the desired *cis*-(2*R*,4*R*) compared to the *cis*-(2*S*,4*S*)-isomer was 99.17 to 0.83%, whereas without stirring the yields were 98.55 and 1.45%, respectively. Results show that stirring is always better than no stirring and that even stirring without adding seeds gave higher optical purity products than in recrystallisations where seeds had been added, but no stirring is done. Crystallisation is essentially complete by 1 day, but purity improves slightly with a longer duration

For recrystallisations with entrainment we found that using methanol combined with small amounts of other alcohols (ethanol, *n*-propanol, isopropanol, *n*-butanol) and water gave similar results to using pure methanol. We found that while 1% water in the methanol gave acceptable enhancements, 5% water started to reduce the optical purity noticeably. Other solvents and solvent mixtures including ethanol or ethyl acetate/petroleum spirits did not give the desired enhancements in optical purities.

To summarise for steps 1 and 2 so far, coupling and recrystallisation as an average of optimised runs, the desired fully protected *cis*-(2*R*,4*R*)-nucleoside is obtained in 37% yield based on the input sulphoxide and greater than 99% diastereomeric excess.

Having coupled *N*-benzoyl cytosine to 2-(*R*)-oxathiolane (**4**), the third step of the synthesis is the removal of the benzoyl protecting groups to obtain ATC (**3**) (scheme 2). We investigated deprotection using three methods: catalytic NaOMe/MeOH, NH_3/MeOH, or NH_4OH/MeOH.

We first looked at hydrolysis by NaOMe (0.1 equiv). This deprotects quantitatively; however, it requires an aqueous workup to get rid of the salts nonchromatographically, and at the 8-g scale the yield was only 61% of ATC (**3**) due to partial water solubility of the ATC (**3**). Alternatively, we looked at NH_3/MeOH. From 15 g of bis-benzoyl protected ATC (**10**) stirring overnight with NH_3/MeOH cleanly deprotected the molecule; after removal of the solvent, slurrying in acetone then removed the more lipophilic byproduct and yielded the product very cleanly in 94% yield of the desired ATC (**3**). The reaction was equally successful whether the starting material was predominantly (2*R*,4*R*)-**10** or (2*S*,4*S*)-isomers.

Using NH_4OH/MeOH the deprotection is slower, but it is clean and at the 3 g scale gave the desired ATC (**3**) in 86% yield. The use of NH_4OH/MeOH, however, involves the removal of water and thus is less convenient in larger scale than NH_3/MeOH. In comparsion to the original route to ATC (scheme 1) in the new method *N*-benzoylcytidine is used (schemes 2 and 4), and this deprotects to give methylbenzoate, which was already produced from deprotection of the oxathiolane group during the original method. Therefore, overall this new method does not introduce any new byproduct compared to the old process, which is an advantage that avoids lengthy toxicological profiling of related substances or degradants.

CONCLUSIONS

We have developed a new three step synthesis of ATC (**3**) that on average gives 30% yield from 2-(*R*)-oxathiolane (**4**), compared to the current method which at the laboratory (50-g scale) gave 25−30% overall yield. In addition the new synthesis avoids a lengthy salt formation/break stage, does not require toluene sulphonic acid, and introduces no new byproduct to the process. The new synthesis was facilitated by the recrystallisation of the bis-benzoyl intermediate as a conglomerate form.

EXPERIMENTAL SECTION

All reactions were conducted under a nitrogen atmosphere using oven-dried glassware. *N*-Benzoylcytosine was purchased from Shanghai PI Chemicals. TMSI was synthesised in-house and was distilled after the synthesis. Triethylamine was distilled over KOH. The solvents were used without distillation. Low temperatures for reactions were maintained by using a Thermo-Neslab cryostat. The NMR spectra were run on a Varian high-field NMR spectrometer running at 400 MHz. Thin layer chromatography was performed on Machery-Nagel precoated plastic silica gel plates (0.22 mm). HPLC analyses were done on a Waters 510 HPLC system, with a Chiralpak AD, 250 mm × 4.6 mm (ID) column, detecting at 254 nm. The solvent system was 20% MeOH in acetonitrile, running isochratically, at a flow rate of 2 mL/min. The free base form of the final products (**3**) was insufficiently soluble to be run in the normal phase chiral HPLC system. Reverse phase HPLC for the free base (**3**) was performed on a Cyclobond column, using 0.05% TFA at a flow rate of 0.27 mL/min at 254 nM. In review it has been noted that the methods described below may still be adapted further to plant scale, by options such as carry-over of the solution of intermediate **5** and avoiding evaporations to dryness; however, the underlying process will remain as described herein.

X-RAY CRYSTALLOGRAPHY

Crystals of **3** from methanol or from toluene respectively were mounted in low-temperature oil and then flash cooled to 130 K using an Oxford low-temperature device. Intensity data were collected at 130 K with an Oxford XCalibur X-ray diffractometer with Saphire CCD detector using Cu Kα radiation (graphite crystal monochromator λ = 1.54184 Å). Data were reduced and corrected for absorption.[14] The structures were solved by direct methods and difference Fourier synthesis using the SHELX suite of programs[15] as implemented within the WINGX[16] software. Thermal ellipsoid plots were generated using the program ORTEP-3 integrated within the WINGX suite of programs. **Crystal data for 3 (Methanol modification)** $[C_{22}\ H_{19}\ N_3\ O_5\ S].\ (CH_3OH)_2$, M = 501.55, T = 130.0(1) K, λ = 1.54178 Å, monoclinic, space group P2(1), a = 10.844(2) Å, b = 5.4572(7) Å, c = 20.500(3) Å, α = 90.424(12)°, V = 1213.1(3) Å^3, Z = 2, D_c = 1.373 mg M^{-3}, μ(Cu Kα) 1.614 mm^{-1}, F(000) = 528, crystal size 0.2 mm × 0.01 mm × 0.01 mm. 4596 reflections measured, 2953 independent reflections (R_{int} = 0.074); the final R was 0.0841 [$I > 2\sigma(I)$], and wR(F^2) was 0.2369 (all data). **Crystal data for 3 (Toluene modification)** $C_{22}\ H_{19}\ N_3\ O_5S$, M = 437.46, T = 130.0(1) K, λ = 1.54178 Å, monoclinic, space group P2(1), a = 13.0343(5) Å, b = 5.4468(2) Å, c = 14.8865(4) Å, β = 104.761(3)°, V = 1021.99(6) Å^3, Z = 2, D_c = 1.422 mg M^{-3}, μ(Cu Kα) 1.761 mm^{-1}, F(000) = 456, crystal size 0.2 × 0.05 × 0.05 mm. 5637 reflections measured, 3150 independent reflections (R_{int} = 0.037); the final R was 0.0417 [$I > 2\sigma(I)$], and wR(F^2) was 0.0905 (all data).

2-(*R*)-Benzoyloxymethyl-1,3-oxathiolane-*S*-oxide (5, 5a). To a stirred mixture of 2-(*R*)-benzoyloxymethyl-1,3-oxathiolane (**4**) (118 g, 0.526 mol) and glacial acetic acid (47 g, 0.790 mol) in a 500-mL round-bottomed flask fitted with an air/water condenser, at 40 °C, was added hydrogen peroxide (35% in water) (65 mL, 0.736 mol) in four portions at approximately 10-min intervals. The initial additions are very exothermic. This was stirred at this temperature for 1 h and at room temperature for 1 h. The mixture was then transferred to a 1-L beaker and was diluted with dichloromethane (500 mL). While stirring, a solution of 10% sodium sulphite in water (500 mL) was added in small portions (initially vigorous reaction). The organic layer was separated, and this was next stirred with an aqueous saturated sodium carbonate solution (500 mL) until there was no bubbling. The organic layer was separated, washed with brine, and dried with magnesium sulphate. The resulting hazy, colorless solution was filtered through Celite to obtain a clear solution. This was evaporated to give the product as colorless viscous oil, which solidified to a white, low-melting solid, melting point 65−70 °C. This material was used for the next step in the synthesis without further purification. Yield: 114 g (90%) ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.05 (d), 7.9 (d), 7.5 (m), 7.4 (m), 4.6−4.8 (m), 4.4 (m), 4.1 (m), 3.2 (m), 3.1 (m), 2.7 (m). (The NMR spectrum is complex due to the presence of (*E*)- and (*Z*)-*S*-oxide diastereomers). This material is a mixture of (*E*)- and (*Z*)-diastereomers of the ratio 2.8:1 for a representative sample of 2-(*R*)-oxathiolane and 2-(*S*)-oxathiolane ratio 64:36 respectively, oxidation gave the four diastereomers, *R*,*E* (R_t 6.4 min), *S*,*E* (R_t 5.3 min), *R*,*Z* (R_t 5.0 min), and *S*,*Z* (R_t 4.7 min) of relative peak areas by chiral HPLC of 47.6, 26.3, 16.6, and 9.5% respectively. This equates to an *E*/*Z* ratio of 2.8:1. Chiral HPLC was with the standard conditions but with 25% MeOH in MeCN at a flow rate of 1 mL/min.

2-(*R*)-Benzoyloxymethyl-4-(*R*)-(*N*-benzoylcytosin-1-yl)-1,3-oxathiolane (10−10c). 2-(*R*,*S*)-Benzoyloxymethyl-1,3-oxathiolane-*S*-oxide (**5**, **5a**) (12.0 g, 0.050 mol) was dissolved in dichloromethane in a 500-mL three-neck flask and this was cooled to −50 °C. To this was added triethylamine (15.3 mL, 0.110 mol), followed by iodotrimethylsilane (21.4 mL, 0.150 mol) via a dropping funnel, dropwise, at a rate so that the internal temperature was between −30 °C and −50 °C. The resulting light-yellow solution was stirred for 45 min while maintaining the temperature between −40 and −50 °C. To the reaction mixture was added copper(II) chloride (1.3 g, 0.010 mol); after 5 more minutes was added *N*-benzoylcytosine (**1**) (10.1 g, 0.047 mol). The resulting mixture was stirred at −50 °C for 15 min and then was allowed to warm to 0 °C over 1 h. The reaction mixture was stirred at this temperature overnight. After overnight stirring, the reaction was stirred at room temperature for 1 h, cooled again in ice, and quenched with the addition of water (100 mL) followed by 5% ammonia (100 mL). This was stirred for 5 min, diluted with dichloromethane (50 mL), and filtered through a Celite plug. The plug was washed with additional dichloromethane (2 × 50 mL), and the combined filtrates were poured into a separating funnel. The organic layer was separated, washed with 2% phosphoric acid (2 × 60 mL) and again with 2.5% ammonia (2 × 100 mL). The combined aqueous layers were re-extracted with dichloromethane (100 mL). The combined organic layers were dried with magnesium sulphate, filtered, and evaporated to give a light-yellow/brown thick oil 17.8 g (86% recovery). This crude mixture consisted of *cis*-**3** and *trans*-**4** combined coupled product at 62% purity (NMR) and with a *cis*-/*trans*-isomer ratio 2.86:1 (NMR)

Recrystallisation of the Crude 2-(*R*,*S*)-Benzoyloxymethyl-4-(*R*,*S*)-(*N*-benzoylcytosin-1-yl)-1,3-oxathiolane (10, 10a, 10b, 10c) To Give Pure 2-(*R*)-Benzoyloxymethyl-4-(*R*)-(*N*-benzoylcytosin-1-yl)-1,3-oxathiolane (10). To the crude (*R*,*S*)-benzoyloxymethyl-4-(*R*,*S*)-(*N*-benzoylcytosin-1-yl)-1,3-oxathiolane (**10**, **10a**, **10b**, **10c** (17.8 g) in a 500-mL round-bottom flask was added 14.5 times (by volume) methanol (258 mL), and the mixture was refluxed until a clear solution could be seen.

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This was then allowed to cool to room temperature gradually and was left standing overnight. The resulting crystallised product was filtered, followed by washing with methanol (2 × 100 mL) and drying under vacuum. The resulting slightly coloured, feathery, crystalline solid on analysing with NMR showed that it is 99.1% the *cis*-(2R,4R)-isomer **10**, with 0.9% of the *cis*-(2*S*,4*S*)-isomer 10c, Isolated yield, 7.2 g (35% yield for the isomer). ^{1}H NMR($CDCl_3$): δ 8.5 (br s, 1H), 8.25 (d, 1H), 8.0 (d, 2H), 7.8 (d, 2H), 7.6 (m, 2H), 7.45 (m, 4H) 7.3 (poorly resolved d, 1H), 6.6 (d, 1H) 5.5(t, 1H), 4.8 (m, 2H), 4.5 (d, 1H), 4.05 (dd, 1H). Chiral HPLC for *cis*-(2*R*,4*R*) isomer (**10**) R_t 12.14 min. Melting point 163−165 °C.

Synthesis of 2-(*R*)-Hydroxymethyl-4-(*R*)-(cytosin-1-yl)-1, 3-oxathiolane (3) Using Sodium Methoxide Deprotection. 2-(*R*)-Benzoyloxymethyl-4-(*R*)-(*N*-benzoylcytosin-1-yl)-1,3-oxathiolane (3.3 g, 0.007 mol) was dissolved in a mixture of dichloromethane (8 mL) and methanol (10 mL) with heating. To this was added sodium methoxide (0.043 g, 0.0008 mol) in methanol (2 mL), and the mixture was stirred overnight. After overnight stirring, the mixture was evaporated and was chromatographed on a silica gel column (4 cm × 18 cm), eluting using a gradient of 20−50% methanol in ethyl acetate. Combination and evaporation of the appropriate fractions yielded 1.5 g (88% yield) of the product (**3**) as an off-white powder. ^{1}H NMR (DMSO): δ 7.8 (d, 1H), 7.0−7.2 (broad d, 2H), 6.3 (d, 1H), 5.7 (d, 1H), 5.1 (t, 1H), 4.4 (d, 1H), 3.9 (m, 1H), 3.7(m, 2H), OH peak not resolved. Melting point 210−211 °C. R_t (RP HPLC) 16.7 min, achiral purity 99.2%.

Recrystallisation with Entrainment. To the crude 2-(*R*)-benzoyloxymethyl-4-(*R*,*S*)-(*N*-benzoylcytosin-1-yl)-1,3-oxathiolane (**10** + **10c**) (10.3 g) in a 100-mL round-bottom flask, was added 14.0 times (by volume) methanol (144.2 mL), and the mixture was refluxed until a clear solution could be seen. This was then allowed to cool with stirring while the temperature was monitored with a thermometer. When the temperature reached 53 °C, the solution was seeded with 144 mg of pure 2-(*R*)-benzoyloxymethyl-4-(*R*)-(*N*-benzoylcytosin-1-yl)-1,3-oxathiolane (**10**) while vigorously stirring. Following the seeding, the rapidly crystallising mixture was stirred vigorously overnight. The resulting crystallised product was then filtered, followed by washing with methanol (50 mL). Once all the mother liquor and the subsequent washing had passed through, the resulting crystallised white product was rewashed slowly with methanol (2 × 100 mL) and dried under vacuum. The resulting white crystalline solid on analyzing with NMR showed that it is >99% the *cis*-isomer. Isolated yield, 4.4 g (44% yield for the recrystallisation, based on the crude). Optical purity: *cis*-(2*R*,4*R*-)(**10**)/*cis*-(2*S*,4*S*-) (**10c**), 99.3:0.7.

Recrystallisation without Entrainment. To the crude 2-(*R*)-benzoyloxymethyl-4-(*R*,*S*)-(*N*-benzoylcytosin-1-yl)-1,3-oxathiolane (**10** + **10c**) (8.0 g) in a 100-mL round-bottom flask, was added approximately 14.0 times (by volume) methanol (112 mL), and the mixture was refluxed until a clear solution could be seen. This was then allowed to cool without stirring overnight. The resulting crystallised product was then filtered, followed by washing with methanol (50 mL). Once all the mother liquor and the subsequent washing had passed through, the resulting crystallised white product was rewashed slowly with methanol (2 × 100 mL) and dried under vacuum. The resulting white crystalline solid on analyzing with NMR showed that it is >99% the *cis*-isomer. Isolated yield, 3.36 g (42% yield for the recrystallisation, based on the crude). Optical purity: *cis*-(2*R*,4*R*)-(**10**)/*cis*-(2*S*,4*S*) (**10c**), 98.1:1.9.

2-(*S*)-Benzoyloxymethyl-4-(*R* and *S*)-(*N*-benzoylcytosin-1-yl)-1,3-oxathiolane. 2-(*S*)-Benzoyloxymethyl-1,3-oxathiolane-*S*-oxide (**5a**) (2.0 g, 0.0083 mol) (optical purity **5**/**5a** = *R*/*S* = 12.1:87.9) was dissolved in dichloromethane (40 mL) in a 100-mL three-neck flask under nitrogen, and this was cooled to −50 °C by means of a cryostat. To this was added triethylamine (1.27 mL, 0.009 mol, and 1.1 equiv). This was followed by the dropwise addition of iodotrimethylsilane (2.5 mL, 0.017 mol, 2.1 equiv) via a dropping funnel, at a rate such that the internal temperature remained below −40 °C. The resulting light-yellow solution was stirred for 30 min, while also maintaining the temperature at −50 °C. Next was added to the reaction mixture triethylamine (1.15 mL, 0.0083 mol, 1.0 equiv), again followed by the repeat dropwise addition of iodotrimethylsilane (1.2 mL, 0.0083 mol, and 1.0 equiv). Next, oven-dried anhydrous copper(II) chloride (0.11 g, 0.0008 mol) was added, and after 5 min *N*-benzoylcytosine (1.79 g, 0.0083 mol) was added. The resulting mixture was allowed to warm to 0 °C and was stirred at this temperature overnight. After overnight stirring, the reaction was allowed to warm to room temperature and was stirred at room temperature for 90 min. The reaction mixture was next quenched with the addition of water (25 mL). This was stirred for 5 min and was filtered through a Celite plug. The plug was washed with additional dichloromethane (3 × 25 mL), and the combined filtrates were poured into a separating funnel. The organic layer was separated and washed sequentially with 5% ammonia in water (2 × 25 mL), 2% phosphoric acid (2 × 25 mL), and again with 5% ammonia (25 mL). The combined aqueous layers were re-extracted with dichloromethane (25 mL). The combined organic layers were then washed with 1 M sodium thiosulphate (25.0 mL). The resulting light-yellow solution was dried with magnesium sulphate, filtered, and evaporated to give a light-yellow/brown thick oil 2.4 g (71% recovery). This crude mixture consisted of *cis* (**10** and **10c**) and *trans* (**10a** and **10b**) combined coupled product at 69% purity (NMR) and with a *cis*/*trans* isomer ratio 2.4:1 (NMR).

Recrystallisation of the Crude 2-(*S*)-Benzoyloxymethyl-4-(*R* and *S*)-(*N*-benzoylcytosin-1-yl)-1,3-oxathiolane To Give Pure 2-(*S*)-Benzoyloxymethyl-4-(*S*)-(*N*-benzoylcytosin-1-yl)-1,3-oxathiolane (10c). *Recrystallisation with Entrainment.* To the crude 2-(*S*)-benzoyloxymethyl-4-(*R*,*S*)-(*N*-benzoylcytosin-1-yl)-1,3-oxathiolane (2.4 g) in a 100-mL round-bottom flask was added approximately 14.0 times (by volume) methanol (34 mL), and the mixture was refluxed until a clear solution could be seen. This was then allowed to cool with stirring while monitoring the temperature with a thermometer. When the temperature reached 53 °C, the solution was seeded with 34 mg of previously recrystallised 2-(*S*)-benzoyloxymethyl-4-(*S*)-(*N*-benzoylcytosin-1-yl)-1,3-oxathiolane (**10**) while vigorously stirring. Following the seeding, the rapidly crystallising mixture was stirred vigorously overnight. The resulting crystallised product was then filtered, followed by washing with methanol (10 mL). Once all the mother liquor and the subsequent washing had passed through, the resulting crystallised white product was rewashed slowly with methanol (2 × 10 mL) and dried under vacuum. The resulting white crystalline solid on analyzing with chiral HPLC showed that it is 97.8% of the *cis*-(2*S*,4*S*)-isomer (**10**), R_t 8.4 min, with *cis*-(2*R*,4*R*) at 2.2%. Isolated yield, 0.96 g (40% yields for the recrystallisation, based

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on the crude). Optical purity: *cis*-(2*R*,4*R*) (**10**)/*cis*-(2*S*,4*S*) (**10c**) = 1.5:98.5. Melting point 142−144 °C.

Recrystallisation of 10c without Entrainment. To the crude 2-(*S*)-Benzoyloxymethyl-4-(*R*,*S*)-(*N*-benzoylcytosin-1-yl)-1,3-oxathiolane (13.95 g) in a 250-mL round-bottom flask, was added approximately 14.0 times (by volume) methanol (194.6 mL). The mixture was refluxed until a clear solution could be seen. This was then allowed to cool without stirring overnight. The resulting crystallised product was then filtered, followed by washing with methanol (50 mL). Once all the mother liquor and the subsequent washing had passed through, the resulting crystallised white product was rewashed slowly with methanol (50 mL) and dried under vacuum. The resulting white crystalline solid on analyzing with NMR showed that it is >99% the *cis*-isomer. Isolated yield, 4.8 g (34% yield for the recrystallisation, based on the crude). Optical purity: *cis*-(2*R*,4*R*) (**10**)/*cis*-(2*S*,4*S*) (**10c**) = 2.2:97.8.

2-(*R*)-Hydroxymethyl-4-(*R*)-(cytosin-1-yl)-1,3-oxathiolane (3) Using Methanolic Ammonia. 2-(*R*)-Benzoyloxymethyl-4-(*R*)-(*N*-benzoylcytosin-1-yl)-1,3-oxathiolane (**10**) (15 g, 0.028 mol) was dissolved in a methanolic ammonia (approximately 2M) solution (250 mL). The initial slurry was stirred overnight. After overnight stirring, the resulting clear solution was filtered through Celite, evaporated to dryness, and slurried in acetone (100 mL). This yielded an off-white powdery solid which was filtered and washed with acetone (2 × 25 mL) and dried to yield the product (**3**); 6.5 g (94%)

^{1}H NMR (DMSO): δ 7.8 (d, 1H), 7.0−7.2 (broad d, 2H), 6.3 (d, 1H), 5.7 (d, 1H), 5.3 (t, 1H) (OH peak - not always resolved), 5.1 (t, 1H), 4.4 (d, 1H), 3.9 (m, 1H), 3.7(m, 2H), OH peak not resolved. Melting point 210−211 °C.

2-(*S*)-Hydroxymethyl-4-(*S*)-(cytosin-1-yl)-1,3-oxathiolane. 2-(*S*)-Benzoyloxymethyl-4-(*S*)-(*N*-benzoylcytosin-1-yl)-1,3-oxathiolane (1.0 g, 0.0022 mol) was dissolved in a methanolic ammonia (approximately 2 M) solution (20 mL). The initial slurry was stirred overnight. After overnight stirring, the resulting clear solution was evaporated to dryness and slurried in acetone (20 mL). This yielded an off-white powdery solid which was filtered and washed with acetone (2 × 10 mL) and dried to yield the title product: 0.47 g (88%) ^{1}H NMR (DMSO): δ 7.8 (d, 1H), 7.0−7.2 (broad d, 2H), 6.3 (d, 1H), 5.7 (d, 1H), 5.3 (t, 1H) (OH peak - not always resolved), 5.1 (t, 1H), 4.4 (d, 1H), 3.9 (m, 1H), 3.7(m, 2H), OH peak not resolved. Melting point 218−220 °C. R_t (RP HPLC) 48.1 min, achiral purity 98.9%.

■ ASSOCIATED CONTENT

Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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