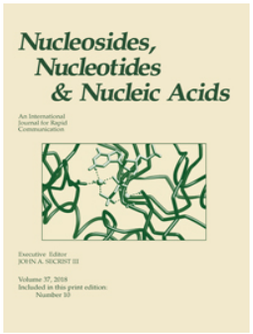

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The development of β-selective glycosylation reactions with benzyl substituted 2-deoxy-1,4-dithio-D-*erythro*-pentofuranosides: enabling practical multi-gram syntheses of 4'-Thio-2'-deoxycytidine (T-dCyd) and 5-aza-4'-thio-2'-deoxycytidine (aza-T-dCyd) to support clinical development

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The development of β-selective glycosylation reactions with benzyl substituted 2-deoxy-1,4-dithio-D-*erythro*-pentofuranosides: enabling practical multi-gram syntheses of 4′-Thio-2′-deoxycytidine (T-dCyd) and 5-aza-4′-thio-2′-deoxycytidine (aza-T-dCyd) to support clinical development

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ABSTRACT

The lack of effective methods to perform direct β-selective glycosylation reactions with 2-deoxy-1,4-dithio-D-*erythro*-pentofuranosides has long been a significant stumbling block for the multi-gram synthesis of 4′-thio-2′-deoxy nucleosides. In addition, previously reported methods for the preparation of appropriately substituted 2-deoxy-1,4-dithio-D-*erythro*-pentofuranosides have proven problematic for large scale synthesis. To address these issues, herein we describe the modification and optimization of previously reported methods to allow for the convenient large scale synthesis of benzyl substituted 2-deoxy-1,4-dithio-D-*erythro*-pentofuranosides. Furthermore, we describe the development of reaction conditions for β-selective glycosylation reactions of benzyl substituted 2-deoxy-1,4-dithio-D-*erythro*-pentofuranosides with both N4-benzoylcytosine and 5-aza-cytosine to enable the practical multi-gram syntheses of the clinical candidates 4′-thio-2′-deoxycytidine (T-dCyd) and 5-aza-4′-thio-2′-deoxycytidine (aza-T-dCyd). Taken together, these new synthetic developments have made possible the preclinical and early clinical development of these important anticancer agents at the National Cancer Institute.

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1. Introduction

Cytidine analogs remain an area of active drug discovery and development having produced four FDA approved drugs for the treatment of cancer. DNA Methyl Transferase I (DNMT1),[1] a maintenance methyltransferase that contributes to the hypermethylation and silencing of tumor suppressor genes, is a major molecular target of two of these drugs, azacytidine and decitabine (Figure 1). The relatively low response rates and adverse effects of these two DNMT1 inhibitors in clinical use has prompted the development of new, modified nucleoside derivatives to address these issues. Indeed, 4′-thio-modified nucleosides such as 4′-thio-2′-deoxycytidine (T-dCyd) **1** (NSC764276) and 5-aza-4′-thio-2′-deoxycytidine (aza-T-dCyd) **2** (NSC777586) potently deplete DNMT1 while inhibiting tumor growth in both *in vitro* and *in vivo* models[2] with less observed toxicity than the corresponding 4-oxo-pentofuranose derived compounds aza-cytidine and decitabine. As a result, both T-dCyd **1** and aza-T-dCyd **2** are currently in Phase I clinical trials at The National Cancer Institute (NCT02423057 and NCT03366116, respectively).

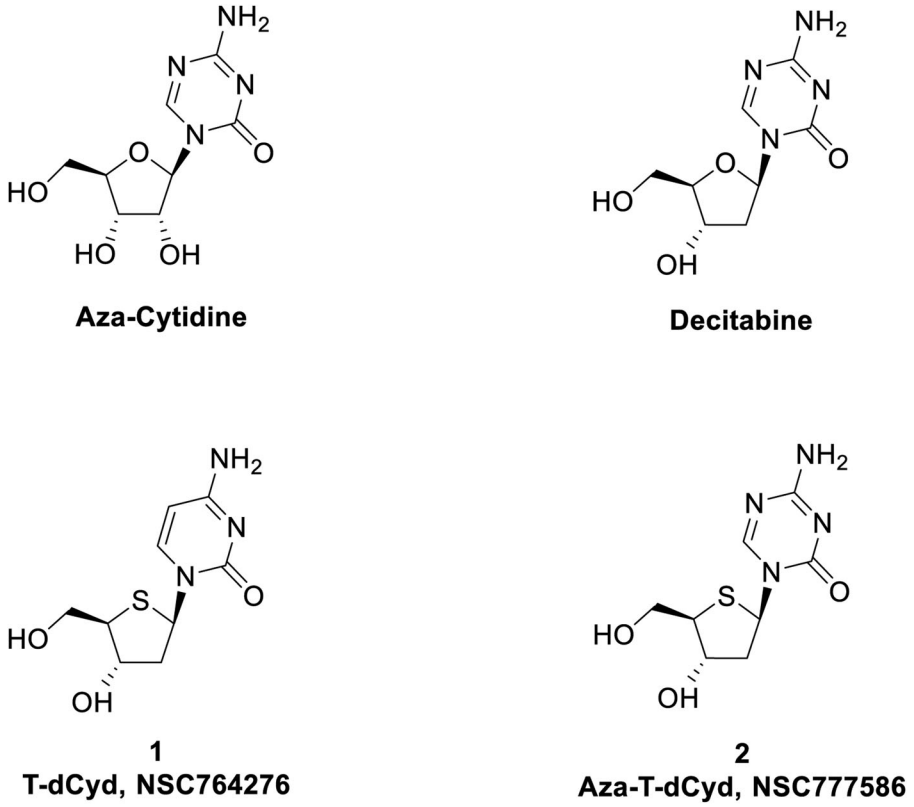


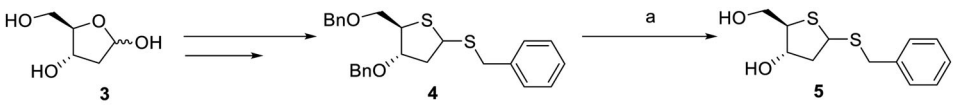
Figure 1. Structures of cytidine analogs.

2. Results and discussion

The syntheses of both T-dCyd (**1**)[3] and aza-T-dCyd (**2**)[4] have been previously reported on small scale. However, due to compounding gross inefficiencies in both the synthesis of the intermediate benzyl 2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside (**5**) and the subsequent glycosylation chemistry, each of the reported approaches proved impractical for the reliable multi-gram production of these compounds required for preclinical and early clinical development. Our objective was to develop practical multi-gram syntheses for both T-dCyd (**1**) and aza-T-dCyd (**2**) that addressed the inadequacies of the earlier methods in order to facilitate the preclinical advancement of these compounds and to establish a robust supply chain to support clinical development.

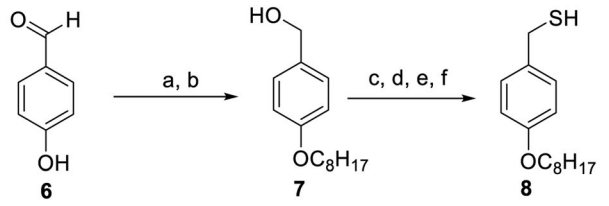
Our initial efforts were directed at addressing the inefficiencies in the synthesis of the intermediate benzyl 2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside (**5**). The synthesis of benzyl 3,5-di-*O*-benzyl-2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside (**4**) was originally described by Walker et al.[5] starting from 2-deoxy-D-*erythro*-ribose (**3**) (Scheme 1). A key step in the syntheses of T-dCyd (**1**) and aza-T-dCyd (**2**) reported by Secrist et al.[2] and Tirwari et al.[3] involved the debenzylation of **4** to provide intermediate **5**. Attempted scale up of this deprotection (BCl_3, DCM, −78 °C) revealed the capricious nature of this reaction as it was found to provide steeply diminishing returns as the scale exceeded 90 grams of substrate. This limitation necessitated serial batchwise processing for this step thereby creating an untenable labor-intensive bottleneck for the synthesis of bulk amounts of diol **5**. In an effort to avoid this bottleneck we initially turned to chemistry first described by Uenishi et al.[6] for the asymmetric synthesis of ethyl 5-acetoxy-3-silyloxy-2-deoxy-4-thio-D-*erythro*-pentofuranoside (**14**). The modifications to this chemistry and the adaptations required to enable the bulk synthesis of the key intermediate (4-octyloxybenzyl) 2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside (**16**) are outlined in Schemes 2 and 3.

The desire to minimize the foul odors associated with the preparation of low molecular weight thioethers led to the selection of the inoffensive 4-octyloxybenzyl mercaptan (**8**)[7] as a key building block for the synthesis of diol **16**. The synthesis of mercaptan **8** is outlined in Scheme 2. Starting with 4-



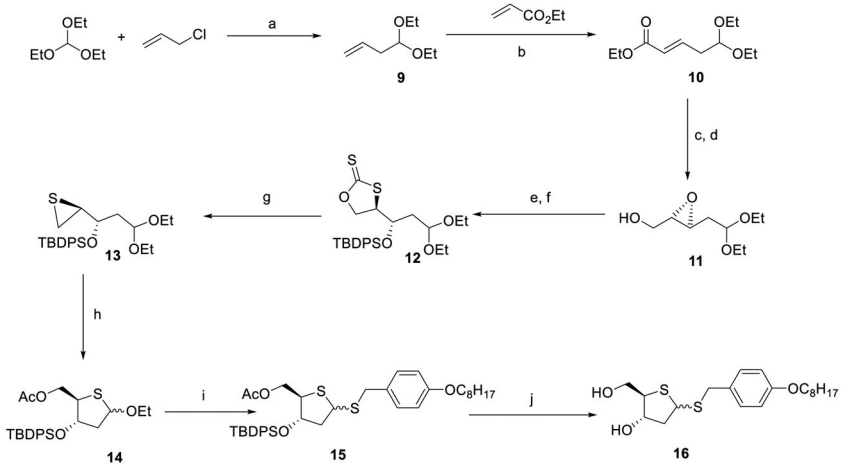
Reagents and Conditions: a) BCl_3, DCM, -78°C

Scheme 1. General approach for the preparation of benzyl 2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside.



Reagents and Conditions: (a) 1-bromooctane, K_2CO_3, MeCN, reflux; (b) $NaBH_4$, MeOH, 23 °C; (c) 12N HCl, MeCN, 23 °C; (d) thiourea, MeCN, reflux; (e) 13N NaOH, reflux; (f) 12N HCl, 15 °C.

Scheme 2. Synthesis of 4-octyoxylbenzyl mercaptan (**8**).



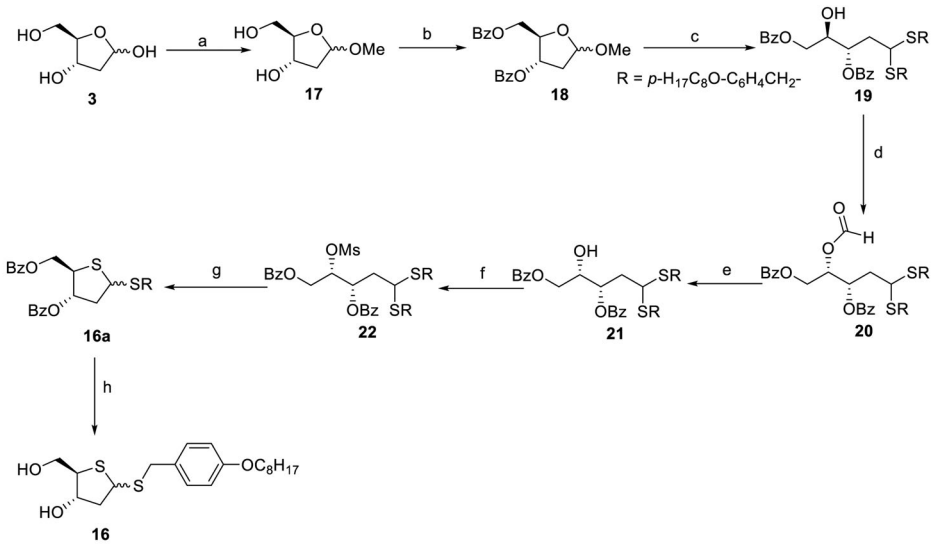
Reagents and conditions: (a) Magnesium, 105°C (86%); (b) 2 mole% Grubbs(II), DCM, reflux (87%); (c) DIBAL-H, DCM, 0°C; (d) Ti(O*i*Pr)$_4$, diethyl (L) tartrate, *t*-Bu-OOH, -22°C (73%); (e) CS_2, LiHMDS, THF, -30°C (92%); (f) TBDPS-Cl, imidazole, DMF, 23°C (86%); (g) K_2CO_3, MeOH, 0°C (92%); (h) HOAC, Ac_2O, KOAc, 120°C (86%); (i) BF_3OEt_2, 4-octyloxyphenylmethanethiol (**8**), DCM, 0°C (75%); (j) LiOH, H_2O, THF, reflux (78%).

Scheme 3. The asymmetric synthesis of (4-octyloxybenzyl) 2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside (**16**).

hydroxy-benzaldehyde (**6**), the synthesis of mercaptan **8** was accomplished on kilogram scale in six linear steps in two reaction pots in 76% overall yield after filtration of crude **8** through a silica pad. This was carried out by alkylation of 4-hydroxy-benzaldehyde (**6**) with 1-bromooctane, which was immediately followed by aldehyde reduction with sodium borohydride in methanol to afford crude 4-octyloxybenzyl alcohol (**7**). Conversion of benzyl alcohol **7** to the corresponding benzyl chloride was achieved by treatment with aqueous 12 N hydrochloric acid and layer separation. Treatment of the crude benzyl chloride with thiourea in refluxing acetonitrile was followed by aqueous base driven hydrolysis of the intermediate thioguanidine in refluxing 13 N sodium hydroxide to give the thiocarbonate sodium salt. Decomposition of this intermediate salt with aqueous 12 N hydrochloric acid cleanly afforded 4-octyloxybenzyl mercaptan (**8**) in 76% overall yield after phase separation and filtration through a silica pad.

The asymmetric synthesis of the key intermediate (4-octyloxybenzyl) 2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside (**16**) generally follows the route described by Uenishi et al.[5] but with some modifications necessary to ultimately enable scale up (Scheme 2). For instance, we greatly streamlined the reported 6 step synthesis of unsaturated ester **10** to a two-step scaleable protocol. In the event, *in situ* generation of allylmagnesium chloride by treatment of allyl chloride with magnesium metal in the presence of triethyl orthoformate afforded 4,4-diethoxybut-1-ene (**9**) in 86% yield. Catalytic cross-metathesis of butene-acetal **9** with an excess of ethyl acrylate in the presence of 2 mol % Grubbs (II) catalyst provided an 87% yield of unsaturated ester **10.** Reduction of ester **10** to the corresponding allylic alcohol with diisobutylaluminum hydride, followed by Sharpless catalytic asymmetric epoxidation gave ((2*S*,3*S*)-3-(2,2-diethoxyethyl)oxiran-2-yl)methanol (**11**) in 73% overall yield. Subsequent reaction of alcohol **11** with carbon disulfide in the presence of lithium hexamethyldisilazane, followed by protection of the intermediate secondary alcohol as the *t*-butyldiphenylsilyl ether, afforded 1,3-oxathiolane-2-thione **12** in 79% overall yield. The replacement of the originally reported TBS ether with the more robust TBDPS ether at this point in the synthesis proved crucial in preserving the yields on scale for the next three steps. The conversion of **12** to the corresponding thiirane **13** was efficiently achieved in excellent yield through treatment with potassium carbonate in methanol. Further treatment of **13** with a mixture of potassium acetate in glacial acetic acid and acetic anhydride yielded the intermediate ethyl 5-acetoxy-3-*t*-butyldiphenylsilyloxy-2-deoxy-4-thio-D-*erythro*-pentofuranoside (**14**). Subsequent reaction of the ethoxy-ether **14** with 4-octyloxybenzyl mercaptan (**8**) in the presence of boron trifluoride diethyletherate resulted in conversion to the corresponding (4-octyloxybenzyl) 2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside **15** in 75% yield. Hydrolysis of **15** with lithium hydroxide resulted in removal of both the acetate and *t*-butyldiphenylsilyl protecting groups to afford diol **16** as a mixture of anomers in 78% yield. Notably, the final deprotection has been performed on greater than 500-gram scale without the requirement for batch-wise processing.

Concurrently we were able to modify and optimize the route originally described by Walker et al.[4] starting from 2-deoxy-D-*erythro*-ribose (**3**) as shown in Scheme 4. In order to eliminate the capricious benzyl deprotection of thioglycoside **4** we proposed to replace the 3,5-dibenzyl protecting scheme from the reported chemistry with more labile benzoyl protecting groups. We were cognizant that the more labile protecting scheme would necessitate some modifications to the downstream chemistry, but we were confident that the benefits would justify the effort. Further, in the interests of odor control, the 4-octyloxybenzyl group was swapped for the benzyl thioglycoside. In the event, 2-deoxy-D-*erythro*-ribose (**3**) was converted to methyl



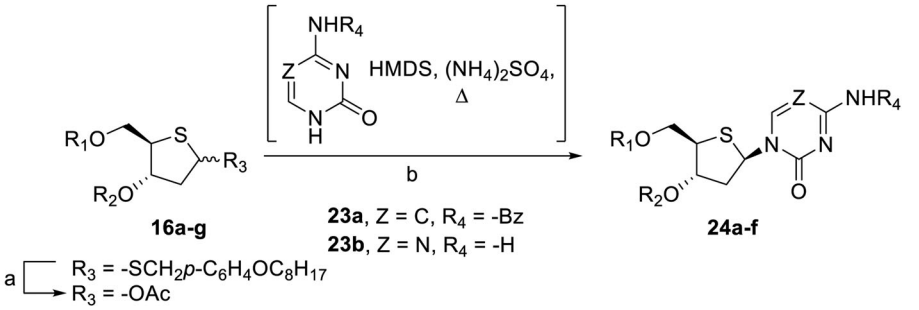
Reagents and Conditions: (a) Dry HCl/MeOH, Ag_2CO_3; (b) BzCl, pyr, 10% DMAP, DCM; (c) 4-octyloxybenzyl mercaptan (**8**), BF_3OEt_2, DCM (70% from 3); (d) HCO_2H, Ph_3P, DIAD, THF (82%); (e) Dry HCl/MeOH THF (95%); (f) MsCl, Et_3N, DCM; (g) $(n\text{Bu})_4NI$, Et_3N, MeCN (69% from **21**); (h) LiOH, H_2O, THF (96%).

Scheme 4. Modification of the chemistry of Walker et al.[4] to enable the bulk synthesis of (4-octyloxybenzyl) 2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside (**16**).

glycoside **17** in the presence of dry HCl in methanol. Benzoyl protection of **17** at the 3 and 5 hydroxy positions was catalyzed by DMAP to efficiently provide diester methyl glycoside **18**. Treatment of **18** with 4-octyloxybenzyl mercaptan (**8**) under catalysis with boron trifluoride diethyl etherate afforded dithioacetal **19** in 70% overall yield from **3**. Mitsunobu inversion of alcohol **19** afforded formate **20** in 82% yield. Cleavage of formate **20** was accomplished with dry hydrochloric acid in methanol/THF to afford a 95% yield of inverted alcohol **21**. Formate cleavage under acidic conditions was determined to be essential to prevent acyl migration. Activation of alcohol **21** as the mesylate **22** was followed by cyclization of **22** in the presence of tetra *n*-butylammonium iodide and triethylamine to provide 3,5-dibenzoyl protected 2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside **16a** in 69% yield from alcohol **21**. The deprotection of **16a** was uneventfully carried out by treatment with aqueous lithium hydroxide to give (4-octyloxybenzyl) 2-deoxy-1,4-dithio-D-*erythro*-penofuranoside **16** as a mixture of anomers in 96% yield. As with the modified chemistry of Uenishi et al.[5] described earlier, this revised route has been successfully executed on large scale to produce bulk **16**.

With robust chemistry in place to efficiently provide bulk quantities of diol **16** we turned our attention to addressing the inefficiencies in the glycosylation reactions of protected **16** with derivatives of cytosine such as *N*4-benzoyl-cytosine (**23a**) and aza-cytosine (**23b**).

There is rich chemical literature[8] describing effective methods for the conversion of 1-thioglycosides or 1-acetates of 2-deoxy-D-*erythro*-



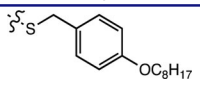
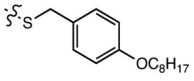
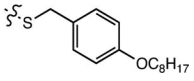
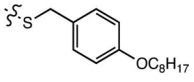
Scheme 5. General approach for the glycosylation of *N*4-benzoyl-cytosine (**23a**) and 5-aza-cytosine (**23 b**).

pentofuranose to the corresponding cytidine or aza-cytidine containing nucleosides in a manner that reliably affords a preponderance of the desired C1-β anomer. Unfortunately, the same wealth of literature methods cannot be found for the direct conversion of the thioglycosides or the corresponding acetates of 2-deoxy-4-thio-D-*erythro*-pentofuranose to the corresponding C1-β anomers of the nucleosides of immediate interest, T-dCyd (**1**) and 5-aza-T-dCyd (**2**). Indeed, the reaction of thioglycosides of 2-deoxy-1,4-thio-D-*erythro*-pentofuranoses with silylated nucleobases is generally reported [2,3] to favor formation of the undesired C1-α anomer or, at best, a 1:1 mixture of anomers. Furthermore, in the course of our studies it became apparent that the lack of chemical equivalence between pentofuranosyl thioglycosides and 4-thiopentofuranosyl thioglycosides meant that application of known β-selective base addition methodology from the former system to the latter system was an unlikely path forward. As a result, we embarked upon an effort to identify conditions that would allow the successful β-selective addition of a silylated-base to an appropriately functionalized (4-octyloxybenzyl) 2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside.

The general approach utilized for reacting an appropriately functionalized 1,4-dithio-D-*erythro*-pentofuranoside with *N*4-benzoyl-cytosine (**23a**) or 5-aza-cytosine (**23b**) after silylation is outlined in Scheme 5. The observations made upon the application of this approach toward the preparation of T-dCyd (**1**) and 5-aza-T-dCyd (**2**) with regard to the interplay between the nature of the diol protection scheme at R_1 and R_2, the leaving group at C1 (R_3) on the 4-thio-D-*erythro*-pentofuranosides (**16a**-**g**), the reaction conditions, and the β/α ratio obtained for the protected nucleoside products (**24a**-**f**) are tabulated in Table 1.

The data in Table 1 points to some general trends that appear to favor increased selectivity for the formation of the desired β anomer of protected nucleosides **24a**-**f**. For instance, it is clear from entries **16a** and **16b**, where R_1 and R_2 are benzoyl, and R_3 is either the 4-octyloxybenzylthio as in **16a**

Table 1. Conditions for the glycosylation of *N*4-benzoyl-cytosine (**23a**) and 5-aza-cytosine (**23 b**).

Entry	R_1, R_2 or R_1 + R_2	R_3	Z	b Conditions	Compound (24)/Observed β/α Ratio	Isolated Yield β Anomer
16a	Bz-, Bz-		C	NIS, MeCN 50-60°C	**24a** 1:1	22-25%
16b	Bz-, Bz-	-OAc	C	TMSOTf, MeCN, 0°C to rt	**24a** 1:2	25%
16b'	Bz-, Bz-	-OAc	N	TMSOTf, MeCN, 0°C to rt	**24b** 1:2	15%
16c	Bn-, Bn-	-OAc	C	TMSOTf, MeCN, 0°C to rt	**24c** 2:3	32%
16d	Ac-, -TBDPS	-OCHO/-OAc	N	TMSOTf, MeCN, 0°C to rt	**24d** 4:5	30%
16e	Ac-, -TBDPS		N	NBS, DCM 0°C to rt	**24d** 5:6	27%
16f	-(*i*Pr)$_2$SiOSi(*i*Pr)$_2$-	-OAc	N	TMSOTf, MeCN, 0°C to rt	**24e** 3:5	25% (SFC)
16g	-(*i*Pr)$_2$SiOSi(*i*Pr)$_2$-		C	NBS, DCM 0°C	**24f** 5:1	54%
16g'	-(*i*Pr)$_2$SiOSi(*i*Pr)$_2$-		N	NBS, DCM 0°C	**24e** 4:1	44%

or acetate as in **16b** that reaction of silylated *N*4-benzoyl-cytosine with the thioglycoside **16a** affords a more favorable β/α ratio (1:1) than reaction with the acetate **16b** (1:2). The comparable amount of isolated β anomer **24a** despite the superior ratio afforded by thioglycoside **16a** is the result of the improved chemical conversion obtained upon reaction with the acetate **16b**. Likewise, entry **16b'** demonstrates that reaction of silylated 5-aza-cytosine with the same acetyl pentofuranoside **16b** provides a 1:2 β/α ratio of the 5-aza-cytidine analog **24b**. The lower isolated yield of β anomer **24b** (15% vs 25% for **24a**) is the result of the consistently poorer reactivity observed for the reaction of silylated 5-aza-cytosine (**23b**) with acetyl 3,5-diacyl substituted 2-deoxy-4-thio-D-*erythro*-pentofuranosides. A further complication observed while using **24b** for the late stage synthesis of 5-aza-T-dCyd (**2**) involves the well-documented[9] hydrolytic instability of the aza-cytosine ring in protic solvents under basic conditions. This property proves to be a serious liability during the final deprotection of **24b** to give 5-aza-T-dCyd (**2**) and leads to significant loss of material. One way we sought to minimize the hydrolytic lability of the aza-cytosine ring during final deprotection was to adopt 3,5-hydroxyl protection schemes for the 2-deoxy-4-thio-D-*erythro*-pentofuranoside derivative that reduced the need to employ base in protic solvents above room temperature during deprotection.

Along these lines, the introduction of a *t*-butyldiphenylsilyloxy group at the 3 position and an acetate at the 5 position of formyl/acetyl 4-

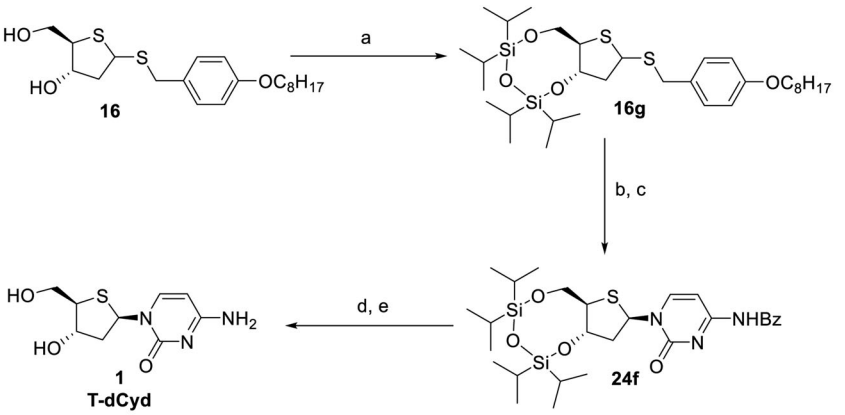
thiopentofuranoside **16d**[10] and (4-octyloxybenzyl) 1,4-dithio-pentofuranoside **16e** while using silylated 5-aza-cytosine (**23b**) as the nucleophile afforded a nearly 1:1 ratio of β and α anomers and an improved isolation of β anomer **24d** for both substrates (27-30% respectively for **16d** & **16e** versus 15% for bis-benzoate protected entry **16b'**). In this case, deprotection of **24d** by sequential treatment with tetra-*n*-butylammonium fluoride and catalytic sodium methoxide (-5 to 0 °C) cleanly precipitated 5-aza-T-dCyd (**2**) from the reaction mixture in 75% yield.[11] These results suggested that silyl protection of the 3 and 5 hydroxy groups might offer the potential for both improved β/α ratios and enhanced reactivity with silylated nucleobases, particularly the more sluggish 5-aza-cytosine, (**23b**), as well as improved recovery on final deprotection of the β anomer to give 5-aza-T-dCyd (**2**).

Initially, as shown in entry **16f**, dual protection the 3 and 5 hydroxy groups of the acetyl 2-deoxy-4-thio-D-*erythro*-pentofuranoside with the tetraisopropyldisiloxane (TIPDS) protecting group afforded the 8-membered silylcycle of the acetate **16f**. Reaction of **16f** with silylated 5-aza-cytosine (**23b**) led to a slightly improved β/α ratio of 3:5 and an isolated yield of 25% for **24e** after chromatography under SFC conditions. In this instance anomer separation through SFC chromatography was required due to the net α selectivity of this acetate coupling along with an unfavorable elution order that made fractionation of the β and α anomers of **24e** over silica gel impractical.

In contrast to these results, dual protection the 3 and 5 hydroxyls of the 1,4-dithio-D-*erythro*-pentofuranoside with the tetraisopropyldisiloxane (TIPDS) protecting group afforded the 8-membered silylcycle of the thioglycoside **16g**. Gratifyingly, reaction of **16g** with silylated *N*4-benzoyl-cytosine (**23a**) gave the precursor to T-dCyd (**1**), **24f**, in a β/α ratio of 5:1 and an isolated yield of 54% of β anomer after silica gel column chromatography (entry **16g**). Similarly, reaction of **16g** with silylated 5-aza-cytosine (**23b**) gave the precursor to 5-aza-T-dCyd (**2**), **24e**, in a β/α ratio of 4:1 and an isolated β anomer yield of 44% after column chromatography over silica gel (entry **16g'**).

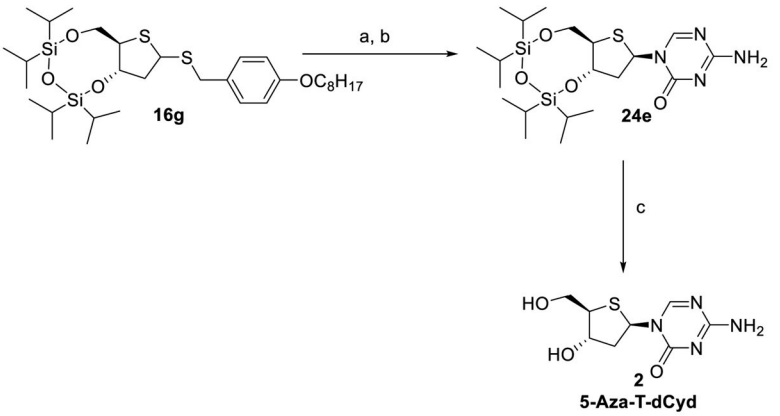
On the basis of these results, our experience indicates that the three essential elements required for the β-diastereoselective addition of nucleobases **23a** and **23b** after silylation to derivatives of 2-deoxy-4-thio-D-*erythro*-pentofuranose **16a-g** are: one, dual protection of the 3 and 5 hydroxyl groups via the 8-membered silylcycle derived from 1,3-dichloro-1,1,3,3-tetraisopropoxydisiloxane; two, a thioglycoside as donor; and three, the use of *N*-bromo-succinimide as the activator[12] in dichloromethane.

The successful application of this methodology to the multi-gram synthesis of T-dCyd (**1**) is outlined in Scheme 6. Protection of diol thioglycoside



Reagents and conditions: (a) Cl-TIPDS-Cl, imidazole, DMF, 23 °C (95%); (b) *N4*-Benzoyl-cytosine (**23a**), HMDS, $(NH_4)_2SO_4$, reflux; (c) NBS, DCM, 0 °C (49%); (d) n-Bu_4NF, THF (93%); (e) 7M Methanolic ammonia (94%).

Scheme 6. The multi-gram synthesis of **T-dCyd** (**1**) (NSC764276).



Reagents and conditions: (a) 5-Aza-cytosine (**23b**), HMDS, $(NH_4)_2SO_4$, reflux; (b) NBS, DCM, 0 °C (44%); (c) H_4NF, MeOH, 60°C (73%).

Scheme 7. The multi-gram synthesis of **5-aza-T-dCyd** (**2**) (NSC777856).

16 as the tetraisopropyldisiloxane gave silylcycle **16g** in excellent yield. Reaction of protected thioglycoside **16g** with silylated *N*4-benzoyl-cytosine **23a** in the presence of *N*-bromosuccinimide, followed by fractionation of the β and α anomers provided protected β anomer **24f** in 49% yield. Treatment of bis silylether **24f** with tetra-*n*-butylammonium fluoride gave a 93% yield of the corresponding diol. Finally, cleavage of the diol/benzamide with methanolic ammonia (7 M, rt) afforded T-dCyd (**1,** NSC764276) in 94% isolated yield.

Similarly, the multi-gram synthesis of 5-aza-T-dCyd (**2**) is outlined in Scheme 7. Starting with protected thioglycoside **16g**, reaction with 5-aza-cytosine **23b** after silylation in the presence of *N*-bromosuccinimide followed by fractionation of the β and α anomers provided protected β

anomer **24e** in 44% yield. Finally, treatment of bis silylether **24e** with ammonium fluoride in refluxing MeOH[13] afforded 5-aza-T-dCyd (**2,** NSC777586) in 73% yield.

Crystallization of T-dCyd (**1**) and aza-T-dCyd (**2**) after deprotection routinely provides API that is remarkably devoid of impurities with enantiomeric excesses and diastereomeric excesses typically greater than 99% and generally approaching the limits of detection. Comparison of the impurity profiles for T-dCyd (**1**) and aza-T-dCyd (**2**) prepared from diol **16** derived from both 2-deoxy-D-ribose (following the methods of Walker et al.[4]) and the modified stereoselective synthesis described by Uenishi et al.[5] revealed no detectable points of difference.

3. Conclusions

Through the modification and optimization of existing chemistry[4,5] we have identified two robust methods for the bulk synthesis of the key intermediate (4-octyloxybenzyl) 2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside (**16**). Furthermore, we have identified conditions for the hitherto unreported β-selective glycosylation reaction between the 3,5-tetraisopropyldisiloxane protected 2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside (**16g**) and the silylated bases of *N*4-benzoyl-cytosine (**23a**) and aza-cytosine (**23b**) to conveniently afford multi-gram quantities of T-dCyd (**1**) and aza-T-dCyd (**2**), respectively. Taken together, these developments represent a significant improvement over reported methods for accessing multi-gram quantities of these important compounds. In addition, the β-selective glycosylation methodology enabled with thioglycoside **16g** may find utility in the synthesis of other 2-deoxy-4-thio-D-*erythro*-pentofuranose derived nucleosides of interest.

Preliminary work to expand the scope of the glycosylation reaction utilizing **16g** beyond *N*4-benzoyl-cytosine (**23a**) and aza-cytosine (**23b**) to other nucleobases including uracil, thymine, and 5-fluoro-cytosine has been initiated. Early efforts in this regard appear promising. The determination of the practical value of this approach will be reported in due course.

4. Experimental

4.1. General methods

Melting points were measured using an Electrothermal Mel-Temp® 3.0 capillary melting point apparatus that was used without correction. Reactions were monitored with TLC using silica gel 60 F254 coated glass plates from Merck KGAa. Purification on laboratory scale via column chromatography was carried utilizing Biotage® or Combiflash® automated

chromatography systems and the commercially available columns specified. Purification on preparative scale was carried out as stated utilizing slurry packed silica gel for flash chromatography (230-400 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded in solution in appropriate deuterated solvents on either a Varian 400 spectrometer at 400 and 100 MHz, respectively or a Bruker 300 Advance spectrometer at 300 and 75 MHz respectively. The chemical shifts are expressed in parts per million (ppm), using the deuterated solvent as internal reference. The multiplicities of the signals are indicated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quadruplet; and m, multiplet, and coupling constants are expressed in Hertz. Combustion analysis data was obtained from either Atantic Microlab, Norcross, GA or Robertson Microlit Laboratories, Ledgewood, NJ.

4.2. Chemical synthesis

4.2.1. Synthesis of 4-octyloxyphenylmethane thiol (8)

4.2.1.1. Preparation of 4-octyloxyphenylmethanol (7). A mixture of 4-hydroxybenzaldehyde (**6**) (430.0 g, 3.52 mol), 1-bromooctane (714.9 g, 3.70 mol) and potassium carbonate (515.7 g, 3.73 mol) in acetonitrile (3.4 L) was refluxed overnight and cooled to room temperature. The solid was filtered off, and the filtrate was concentrated under reduced pressure to give 848.3 g (103%) of crude 4-octyloxybenzaldehyde. The product was dissolved in methanol (2.6 L) and sodium borohydride (44.4 g, 1.17 mol) was added portion-wise to the formed solution, keeping the temperature below 15 °C. The reaction mixture was stirred at room temperature for 1 h. A solution of NaOH (14.33 g, 0.358 mol) in water (200 mL) was added followed by ethyl acetate (1.7 L) and brine (0.5 L). The organic solution was separated, dried over sodium sulfate and evaporated under reduced pressure. Heptane (1 L) was added to the residue and the formed mixture was cooled to 4 °C. The solid was filtered off, washed with ice-cooled heptane, and dried under vacuum to give 765.4 g (92%) of crude compound **7**, which was used in the following step without further purification. ^{1}H NMR spectrum (300 MHz, CDCl3): δ 7.26 (m, 2H), 6.87 (m, 2H), 4.59 (s, 2H), 3.95 (m, 2H), 1.78 m, 3H), 1.47-1.29 (m, 10 H), 0.89 (m, 3H).

*4.2.1.2. Preparation of 4-octyloxyphenylmethanethiol (**8**).* A mixture of 4-octyloxyphenylmethanol (**7**) (765.42 g, 3.24 mol), concentrated HCl (600 mL) and acetonitrile (1.7 L) was stirred overnight at room temperature. Thiourea (296.0 g, 3.89 mol) and acetonitrile (600 mL) were added. The mixture was heated to reflux for 2 h, cooled to room temperature and kept overnight. A solution of sodium hydroxide (518.7 g, 12.97 mol) in water

(1 L) was added. The mixture was heated to reflux temperature for 3 h and cooled to 10 °C. Concentrated HCl (600 mL) was added keeping the temperature below 15 °C. The mixture was extracted with MTBE (3 L) and the extract was dried over magnesium sulfate and concentrated under reduced pressure. Heptane (1 L) was added to the residue, and the mixture was evaporated. Heptane (1.5 L) was added to the residue. The milky solution was kept overnight and filtered through a silica gel pad (500 g). The filtrate was evaporated to give 679.2 g (83%) of compound **8** as a colorless oil. ^{1}H NMR spectrum (300 MHz, CDCl3): δ 7.23 (m, 2H), 6.84 (m, 2H), 3.95 (m, 2H), 3.66 (m, 2H), 1.77 (m, 3H), 1.21-1.59 (m, 10H), 0.88 (m, 3H).

4.2.2. The asymmetric synthesis of (4S,5R)-4-Hydroxy-5-hydroxymethyl-2-((4-(octyloxy)benzyl)thio)-tetrahydrothiophene, (16)

*4.2.2.1. Preparation of 4,4-Diethyoxybut-1-ene (**9**).* The following procedure is an adaptation of the method reported by Cloux, R.; Schlosser, M. *Helv. Chim. Acta*. **1984**, *67*, 1470–1474. To a mixture of magnesium (grit, ≥99.0% (KT) (Aldrich: 63040-250 G-F) (31.8 g, 1.31 mol) and triethoxymethane (123 mL, 0.738 mol) was added 3-chloroprop-1-ene (53.2 mL, 0.653 mol) via addition funnel at a rate of 1 drop every 4 seconds, keeping the temperature under 105 °C. The reaction mixture was vigorously stirred with an overhead stirrer. After addition was complete, the mixture was allowed to cool to room temperature overnight (14 h). The reaction mixture was then chilled to 0 °C, treated with aqueous saturated NH_4Cl solution (200 mL), and stirred vigorously for 4 h. The aqueous layer was then extracted with Et_2O (3 × 150 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated, resulting in a clear and colorless residue. To the clear residue was added a solution of acetic acid (20 mL, 349 mmol), sodium acetate (10 g, 122 mmol), and water (50 mL, 2.78 mol). The mixture was stirred for 2.5 h. Sodium bicarbonate (32.4 g, 386 mmol) and water (about 200 mL) was then slowly added to the mixture. When evolution of CO_2 stopped, the aqueous layer was extracted with Et_2O (2 × 150 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated to afford compound **9** (80.6 g, 559 mmol, 86% yield) as clear and colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.86-5.75 (m, 1H); 5.14-5.06 (m, 1H); 4.54-4.51 (m, 1H); 3.66-3.62 (m, 2H); 3.55-3.49 (m, 2H); 1.23-1.19 (m, 6H).

*4.2.2.2. Preparation of ethyl (E)-5,5-diethoxypent-2-enoate (**10**).* To a solution of 4,4-diethoxybut-1-ene **9** (60.2 g, 417 mmol) and ethyl acrylate (182 mL, 1.67 mol) in DCM (1.39 L) was added [1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(phenylmethylene)(tricyclohexylphosphino)ruthenium (Grubbs II Catalyst) (3.54 g, 4.17 mmol) and the reaction mixture was heated/refluxed at 45 °C for 2 h. A 2nd portion of Grubbs II Catalyst

(350 mg) was added. The reaction mixture was then heated to 45 °C for 14 h. The reaction mixture was allowed to cool to room temperature and the solvent was then removed to afford a black residue. The residue was diluted with DCM and transferred to a 500 mL round-bottom flask. DCM was removed by rotary evaporation. The 500 mL round bottom flask was then attached to a short path distillation head and product was purified by distillation. Ethyl acrylate and residual DCM was first removed by applying heat and vacuum, starting at 60 °C and 200 mbar and gradually increasing to 80 °C and 14 mbar vacuum. Product was then recovered at 98 °C and about 1 torr to afford compound **10** (73.3 g, 338 mmol, 81% yield) as clear and colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 (m, 1H), 5.90 (d, J = 16 Hz, 1H), 4.58 (t, J = 8 Hz, 1H), 4.19 (q, J = 16, 8 Hz, 2H), 3.67 (m, 2H), 3.55 (m, 2H), 2.54 (t, J = 8 Hz, 2H), 1.29 (t, J = 8 Hz, 3H), 1.21 (t, J = 8 Hz, 6H).

4.2.2.3. Preparation of (E)-5,5-Diethoxypent-2-en-1-ol. Ethyl (E)-5,5-diethoxypent-2-enoate (**10**) (63 g, 291 mmol) was dissolved in DCM (1.21 L) in a 5 L three-neck round-bottom flask under nitrogen. The solution was cooled to −78 °C and treated dropwise via addition funnel with DIBAL-H in DCM (699 mL, 699 mmol) over 2.5 h. The reaction was maintained at-78 °C for 2 h. The reaction mixture was then quenched with ethyl acetate (28.5 mL, 291 mmol) and diluted with 1 L saturated sodium potassium tartrate. The mixture was stirred overnight as it warmed to room temperature. The layers were separated, and the aqueous layer was extracted once with DCM (200 mL). The organic layers were combined, dried ($MgSO_4$) and concentrated in vacuo to a colorless oil. The crude material was dissolved in a minimum amount of hexane/DCM, loaded onto a 125 g RediSep Rf filter column and was purified using a 330 g RediSep Rf Gold Silica Gel column, eluting with a 0-30% EtOAc/hexane gradient. The appropriate fractions were collected to afford (E)-5,5-diethoxypent-2-en-1-ol (41 g, 235 mmol, 81% yield) as a clear yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.70 (m, 2H), 4.50 (t, J = 8 Hz, 1H), 4.11 (m, 2H), 3.65 (m, 2H), 3.52 (m, 2H), 2.38 (t, J = 7 Hz, 2H), 1.19 (t, J = 8 Hz, 6H).

*4.2.2.4. Preparation of ((2S,3S)-3-(2,2-Diethoxyethyl)oxiran-2-yl)methanol (**11**).* A 50-L jacked reactor, equipped with overhead stirrer, thermometer, and argon inlet, was charged with a solution of (+)-diethyl L-tartrate (213.4 g, 1.04 mol) in anhydrous DCM (16 L) and activated molecular sieves (1 kg). A solution of titanium tetraisopropoxide (245.17 g, 862.6 mmol) in anhydrous DCM (0.5 L) was added dropwise at −30 °C followed by addition of a 5.5 M solution of *tert*-butyl hydroperoxide (1.57 L, 8.63 mol) while keeping the temperature under −25 °C. The reaction mixture was stirred at this

temperature for 45 min, and a solution of (*E*)-5,5-diethoxypent-2-en-1-ol (742.9 g, 4.31 mol) in anhydrous DCM (1 L) was added at −25 °C. The reaction mixture was stirred at this temperature for 7 h and warmed up to 0 °C. A solution of citric acid (55.24 mol, 287.5 mmol) in water (250 mL) was added. The reaction mixture was stirred at this temperature for 1 h and a solution of triphenylphosphine (1.19 Kg, 4.53 mol) in anhydrous DCM (2 L) was added dropwise while keeping the temperature under 5 °C. Anhydrous magnesium sulfate (500 g) and Celite (300 g) were added and the mixture was stirred at 5 °C for 5 h. The solids were filtered off through a Celite pad and the filtrate was concentrated under reduced pressure. The residue was dissolved in a mixture of ethyl acetate and heptanes (8 L, 1:4). The formed solution was cooled to −9 °C. Additional solids were filtered off, washed with a mixture of ethyl acetate and heptanes (2 L, 1:4), and the filtrate was evaporated. The residue was purified by column chromatography (silica gel, 10 kg; ethyl acetate/heptanes, 1:4, then 2:3) to give 598.4 g (73%) of compound **11** as a pale oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.65 (m, 1H), 3.88 (m, 1H), 3.62 (m, 2H), 3.50 (m, 2H), 3.05 (m, 1H), 2.94 (m, 1H), 1.91 (m, 2H), 1.84 – 1.71 (m, 2H), 1.19 (m, 6H).

4.2.2.5. Preparation of (R)-4-((S)-3,3-diethoxy-1-hydroxypropyl)-1,3-oxathiolane-2-thione. An oven-dried 250 mL two-neck round-bottom flask under nitrogen was charged with carbon disulfide (60.3 mL, 10 mol), dry THF (475 mL) and ((2*S*,3*S*)-3-(2,2-diethoxyethyl)oxiran-2-yl)methanol (**11**) (47.6 g, 250 mmol) and the solution was cooled to −78 °C. The colorless solution was treated dropwise with sodium bis(trimethylsilyl)amide in THF (300 mL, 300 mmol) over 1 h. The deep orange solution was stirred at −78 °C for a total of 2 h and was quenched at −78 °C with acetic acid (17.9 mL, 313 mmol). The reaction then was stirred without the cooling bath until it warmed to −30 °C, was treated with 225 g silica gel (230-400 mesh), and the mixture was concentrated to dryness overnight. The crude plug was placed in a CombiFlash® dry load cartridge, eluted directly onto a 340 g UltraSphere™ SNAP cartridge with a 0-60% EtOAc/hexane gradient into two fractions. First eluting fraction was combined and concentrated to afford 10.15 g (12%) of (*R*)-4-((*S*)-3,3-diethoxy-1-((trimethylsilyl)oxy)propyl)-1,3-oxathiolane-2-thione as a deep yellow oil. Second eluting fraction was combined and concentrated to afford 53.1 g (80%) of (*R*)-4-((*S*)-3,3-diethoxy-1-hydroxypropyl)-1,3-oxathiolane-2-thione as an amber oil. The TMS ether (10.15 g, 30.0 mmol) was dissolved in methanol (110 mL) in a 500 mL one-neck round-bottom flask. The solution was treated with acetic acid (5.4 mL, 94 mmol) and the reaction was stirred for 19 h at room temperature. The reaction was diluted with toluene (200 mL) and concentrated to afford an additional 8.0 g (12%) of the title

compound as a dark yellow oil. Alcohol: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.16 – 5.08 (m, 1H), 4.87 – 4.78 (m, 1H), 4.69 (m, 1H), 4.04 – 3.87 (m, 3H), 3.69 (m, 2H), 3.58 – 3.43 (m, 2H), 1.90 (m, 1H), 1.88 – 1.73 (m, 1H), 1.20 (m, 6H). TMS ether: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.93 (m, 1H), 4.76 (m, 1H), 4.61 (m, 1H), 4.10 (m, 1H), 3.97 (m, 1H), 3.68 – 3.52 (m, 2H), 3.51 – 3.38 (m, 2H), 1.90 (m, 1H), 1.79 (m, 1H), 1.26 (s, 1H), 1.25 – 1.13 (m, 6H), 0.15 (d, J = 0.7 Hz, 9H).

*4.2.2.6. Preparation: (R)-4-((S)-1-((tert-Butyldiphenylsilyl)oxy)-3,3-diethoxypropyl)-1,3-oxathiolane-2-thione (**12**).* (*R*)-4-((*S*)-3,3-Diethoxy-1-hydroxypropyl)-1,3-oxathiolane-2-thione (11.78 g, 44.2 mmol) was dissolved in DMF (44 mL) in a 500 mL one-neck round-bottom flask under nitrogen. The solution was treated with imidazole (7.53 g, 111 mmol) in a single portion followed by *tert*-butylchlorodiphenylsilane (TBDPS-Cl) (23.00 mL, 88 mmol) and the reaction was stirred for 72 h. The mixture was diluted with EtOAc (200 mL), stirred vigorously with 50% saturated 1:1 sodium bicarbonate/sodium chloride (100 mL) for 10 minutes, and the layers were separated. The organic layer was washed with 50% saturated sodium chloride (3 × 50 mL), dried ($MgSO_4$) and concentrated in vacuo to give an amber oil. The crude material was dissolved in hexane and was loaded onto a 50 g UltraSil® SNAP cartridge. The column was eluted over a 100 g UltraSil® SNAP cartridge with a 0-10% EtOAc/hexane gradient while collecting on a Biotage® system. The appropriate fractions were combined and concentrated to afford 19.28 g (86%) of compound **12** as a golden oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 – 7.60 (m, 4H), 7.50 – 7.32 (m, 6H), 4.82 (m, 1H), 4.70 (m, 1H), 4.46 m, 1H), 4.23 (m, 1H), 4.04 (m, 1H), 3.50 – 3.22 (m, 3H), 3.12 (m, 1H), 1.90 (m, 1H), 1.72 (m, 1H), 1.11 (m, 3H), 1.05 (s, 9H), 1.10 – 0.92 (m, 6H).

*4.2.2.7. Preparation of tert-Butyl((S)-3,3-diethoxy-1-((S)-thiiran-2-yl)propoxy)diphenylsilane (**13**).* (*R*)-4-((*S*)-1-((tert-Butyldiphenylsilyl)oxy)-3,3-diethoxypropyl)-1,3-oxathiolane-2-thione (**12**) (11.9 g, 23.57 mmol) was dissolved in anhydrous methanol (140 mL) in a 250 mL one-neck round-bottom flask under nitrogen and the mixture was cooled to 0 °C. The yellow solution was treated with powdered potassium carbonate 325 mesh (3.75 g, 27.1 mmol) and the reaction was stirred 3 h at 0 °C. The mixture was diluted with 1:1 diethyl ether/hexane (500 mL), the insoluble material was removed by filtration through anhydrous potassium carbonate, and the filtrate was concentrated in vacuo to a yellow oil. The crude material was dissolved in a minimum amount of hexane, loaded onto a 50 g UltraSphere® SNAP cartridge and was eluted with a 0-4% EtOAc/hexane gradient on a Biotage® system. The appropriate fractions were combined and concentrated in vacuo to afford 9.67 g (92%) of compound **13** as a colorless oil. ^{1}H NMR (400 MHz,

$CDCl_3$) δ 7.71 (m, 4H), 7.47 – 7.32 (m, 6H), 4.91 (m, 1H), 3.66 – 3.28 (m, 5H), 2.90 (m, 1H), 2.12 – 2.00 (m, 2H), 1.84 (m, 1H), 1.52 (m, 1H), 1.16 (m, 6H), 1.02 (s, 9H).

*4.2.2.8. Preparation of ((2 R,3S)-3-((tert-Butyldiphenylsilyl)oxy)-5-ethoxytetrahydrothiophen-2-yl)methyl acetate (**14**).* *tert*-Butyl((*S*)-3,3-diethoxy-1-((*S*)-thiiran-2-yl)propoxy)diphenylsilane (**13**) (9.67 g, 21.74 mmol) was dissolved in a mixture of acetic acid (25 mL, 437 mmol) and acetic anhydride (30 mL, 318 mmol) in a 250 mL one-neck round-bottom flask under nitrogen. The solution was treated with potassium acetate (10.67 g, 109 mmol) and was placed in an oil bath at 120 °C for 4 h. The mixture was cooled to room temperature, diluted with toluene (500 mL), the solid material was removed by filtration, and the filtrate was concentrated to dryness. The residue was dissolved in a minimum amount of DCM, was loaded onto a 50 g UltraSphere® SNAP cartridge, and was eluted over a 100 g UltraSil® SNAP cartridge with a 0-5% EtOAc/hexane gradient (0-25% of a 20% EtOAc/hexane stock solution) on a CombiFlash® system. The appropriate fractions were combined and concentrated to afford 8.6 g (86%) of compound **14** as 2:3 β/α anomeric mixture as a pale oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 – 7.59 (m, 4H), 7.46 – 7.33 (m, 6H), 5.15 (m, 1H), 4.50 – 4.15 (m, 1H), 4.04 (m, 1H), 3.87 (m, 1H), 3.72 – 3.58 (m, 1H), 3.56 – 3.47 (m, 1H), 3.26 (m, 1H), 2.29 – 2.06 (m, 2H), 1.88 (m, 3H), 1.16 (m, 3H), 1.05 (s, 9H).

*4.2.2.9. Preparation of ((2 R,3S)-3-((tert-Butyldiphenylsilyl)oxy)-5-((4-octyloxybenzyl)thio)- tetrahydrothiophen-2-yl)methyl acetate (**15**).* Boron trifluoride diethyl etherate (278.32 g, 1.96 mol) was added drop wise under argon to a stirred solution of ((2 *R*,3*S*)-3-((*tert*-butyldiphenylsilyl)oxy)-5-ethoxytetrahydrothiophen-2-yl)methyl acetate (**14**) (455.5 g, 980.5 mmol) and 4-octyloxyphenylmethanethiol (**8**) (252.42 g, 1.0 mol) in anhydrous DCM (4.6 L) at 0 °C. The reaction mixture was stirred at this temperature for 2 h. Triethylamine (238.12 g, 2.35 mol) was added dropwise while keeping the temperature under 5 °C, followed by water (2 L). The reaction mixture was stirred for 1 h. The organic solution was separated, dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography (silica gel, ethyl acetate/heptanes, 1:15, then 1:9, and then 1:4) to give 486.9 g (75%) of compound **15** as a pale oil. ^{1}H NMR spectrum (300 MHz, $CDCl_3$): δ 7.79-7.59 (m, 4H), 7.45-7.35 (m, 6H), 7.2 (m, 2H), 6.82 (m, 2H), 4.57 (m, 0.7 H), 4.45 (m, 0.7H), 4.22-4.04 (m, 0.4H), 3.95-3.85 (m, 3H), 3.85-3.65 (m, 2.4H), 3.5 (m, 1H), 2.25-2.00 (m, 1H), 1.87 (m, 3H), 1.75 (m, 2H), 1.50-1.24 (m, 10H), 1.06 (m, 9H), 0.87 (m, 3H).

*4.2.2.10. Preparation of (4S,5R)-4-Hydroxy-5-hydroxymethyl-2-((4-(octyloxy)benzyl)thio)-tetrahydrothiophene, (**16**).* A mixture of a solution of ((2 *R*,3*S*)-3-

((*tert*-butyldiphenylsilyl)oxy)-5-((4-octyloxybenzyl)thio)-tetrahydrothiophen-2-yl)methyl acetate (**15**) (492.1 g, 739.9 mmol) in THF (5.7 L) and a solution of lithium hydroxide monohydrate (310.5 g, 7.4 mol) in water (1.9 L) was heated to reflux for 70 h. The mixture was cooled to room temperature and methyl-*t*-butylether (MTBE) (2 L) was added. The organic solution was separated and the aqueous layer was extracted with MTBE (1 L). The combined organic layers were dried (Na_2SO_4) and evaporated to give 485.5 g of crude product. The crude material was dissolved under heating in heptanes (3 L). The formed solution was cooled to room temperature, and then to 0 °C overnight. The formed solid was filtered off, washed with ice-cold heptanes (0.5 L) and dried under vacuum to give 212.0 g (75%) of compound **16**. The filtrate was evaporated. The residue was purified by column chromatography (silica gel, ethyl acetate/heptanes, 1:9, then 1:4, and then 7:3) to give an additional 10.2 g (3.6%) of compound **16**. The two crops of target compound were combined to afford 222.2 g (78%) of compound **16**. ^{1}H NMR spectrum (300 MHz, $CDCl_3$): δ 7.21 (d, $J = 8.5$ Hz, 2H), 6.84 (d, $J = 8.5$ Hz, 2H), 4.57 (m, 1H), 4.36 (t, $J = 6.3$ Hz, 1H), 3.93, (t, $J = 6.6$ Hz, 2H), 3.83-3.43 (m, 4H), 3.45 (m, 1H), 2.40-2.13 (m, 4H), 1.77 (m, 2H), 1.50-1.20 (m, 10H), 0.89 (m, 3H).

4.2.3. Synthesis of (4S,5R)-4-Hydroxy-5-hydroxymethyl-2-((4-(octyloxy)benzyl)thio)-tetrahydrothiophene, (16) from D-erythro-2-deoxy-ribose

*4.2.3.1. Preparation of (2 R,3S)-2-(hydroxymethyl)-5-methoxytetrahydrofuran-3-ol (**17**).* A 3.0 M solution of hydrogen chloride in methanol (15.0 mL, 45.1 mmol, 0.05 eq) was added drop-wise to a stirred suspension of 2-deoxy-D-ribose (**3**) (120.90 g, 901 mmol) in anhydrous methanol (700 mL) at ambient temperature. The reaction mixture became a brown solution and was stirred for 1.5 hours. Silver carbonate (7.77 g, 28.2 mmol) was added and the reaction mixture was stirred for 1 h (*p*H at 8). The solids were filtered off through a Celite® pad and rinsed with methanol (300 mL). The filtrate was evaporated under reduced pressure. THF (100 mL x 2) was added to the residue and the resulting mixture was evaporated twice to remove traces of methanol. The crude product was dried under high vacuum overnight to give 129.12 g (96.7%) of compound **17** as a brown oil. Product was used in the next step without any further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.15 (m, 1H), 4.45 (m, 0.5H), 4.17 (m, 1H), 4.05 (m, 0.5H), 3.75-3.58 (m, 1H), 3.38 (s, 3H), 2.65 (bs, 2H), 2.30-1.90 (m, 2H).

*4.2.3.2. Preparation of ((2 R,3S)-3-(benzoyloxy)-5-methoxytetrahydrofuran-2-yl)methyl benzoate (**18**).* A solution of benzoyl chloride (269.26 g, 191.55 mmol, 2.2 eq) in anhydrous DCM (60 mL) was added to a stirred solution of (2 R,3S)-2-(Hydroxymethyl)-5-methoxytetrahydrofuran-3-ol (**17**)

(129.00 g, 870.68 mmol) containing 4-N,N-dimethylamino-pyridine (10.61 g, 87 mmole) and pyridine (166.74 g, 2108 mmol, 2.4 eq) in anhydrous DCM (950 mL) cooled at −7 °C through a dropping funnel during a period of one hour while keeping the internal temperature below 0 °C. After the addition was complete, the reaction mixture was spontaneously warmed up to ambient temperature and stirred overnight. After the reaction was completed, the reaction mixture was cooled in an ice bath and quenched with water (600 mL). The cooling bath was removed, and the mixture was stirred at ambient temperature for one hour. The layers were separated. The organic layer was stirred with a solution of sodium bicarbonate (60.00 g, 714 mmol) in water (600 mL) for two hours, followed by stirring with a solution of citric acid monohydrate (36.64 g, 174.1 mmol, 0.2 eq) in water (500 mL) for one hour. The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure to give 325 g of the crude dibenzoyl ester compound **18** as a brown oil. Product was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18-8.10 (m, 4H), 7.62-7.41 (m, 6H), 5.70-5.42 (m, 1H), 5.27-5.18 (m, 1H), 4.69-4.42 (m, 3H), 3.44 (s, 2H), 3.37 (s, 1H), 2.62-2.19 (m, 3H).

*4.2.3.3. Preparation of (2 R,3S)-2-Hydroxy-5,5-bis((4-(octyloxy)benzyl)thio)pentane-1,3-diyldibenzoate (**19**).* 4-Octyloxybenzyl-mercaptan (**8**) (201.01 g, 0.796 mol, 2.5 eq) was added to a stirred solution of ((2 R,3S)-3-(benzoyloxy)-5-methoxytetrahydrofuran-2-yl)methyl benzoate (**18**) (114.16 g, 0.320 mol) in anhydrous DCM (1 L) cooled in a dry-ice/water bath (-1.9 °C), followed by drop-wise addition of boron trifluoride etherate (90.92 g, 0.641 mol, 2.0 eq) while keeping the internal temperature below 0 °C. After the addition was complete, the reaction mixture was stirred in an ice bath (1.0 °C) for 3.5 hours and was added to a stirred mixture of potassium carbonate (265.6 g, 1.92 mol, 6 eq) in water (1 L) and DCM (300 mL) cooled in an ice bath (8-15 °C). After the addition, the ice bath was removed. The mixture was stirred for 50 minutes and then filtered through a Celite® 545 pad. The layers of the filtrate was separated. The organic layer was dried (Na_2SO_4) and evaporated to give a yellowish oil (307.9 g). The crude product was purified by silica gel column chromatography (eluted with ethyl acetate/heptane, 1:9, and then 1:7.5) to give 186.8 g (70%) of compound **19** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17-8.00 (d, $J = 7.9$ Hz, 2H), 7.15-7.03 (d, $J = 7.9$ Hz, 2H), 7.60-7.50 (d, $J = 7.9$ Hz, 2H), 7.55-7.39 (m, 4H), 7.17-7.29 (d, $J = 8.7$ Hz, 2H), 7.15-7.03 (d, $J = 8.7$ Hz, 2H), 6.72-6.69 (d, $J = 8.7$ Hz, 2H), 6.70-6.58 (d, $J = 8.7$ Hz, 2H), 5.42 (m, 1H), 4.60-4.27 (m, 2H), 4.05 (m, 1H), 3.87-3.59 (m, 7H), 2.79 (m, 1H), 2.45 (m, 1H), 2.15 (m, 1H), 1.80-1.65 (m, 4H), 1.50-1.20 (m, 18 H), 0.95-0.79 (m, 6H).

*4.2.3.4. Preparation of (2S,3S)-2-(formyloxy)-5,5-bis((4-(octyloxy)benzyl)thio)pentane-1,3-diyl dibenzoate (**20**).* Triphenylphosphine (117.64 g, 0.449 mol, 2 eq) was added to a stirred solution of (2 R,3S)-2-hydroxy-5,5-bis((4-(octyloxy)-benzyl)thio)pentane-1,3-diyl dibenzoate (**19**) (185.95 g, 0.224 mol) in anhydrous THF (1.4 L). The resulting solution was cooled in an ice bath. Formic acid (20.65 g, 0.448 mol, 2 eq) was added, followed by addition of DIAD (90.7 g, 0.448 mol, 2 eq) dropwise while keeping the internal temperature below 9 °C. After the addition, the reaction mixture was left in the cooling bath and spontaneously warmed up to ambient temperature with stirring overnight. It was diluted with heptane (500 mL), followed by aq $NaHCO_3$ (18.84 g, 0.224 mol in 500 mL of water). The mixture was stirred for 30 minutes. The layers were separated. The aqueous layer was extracted with heptane (200 mL) again. The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue (510 g) was treated with ethyl acetate (500 mL) and heptane (1 L) and stirred for 20 minutes. Solids were filtered off, and the filtrate was concentrated under reduced pressure to give a yellowish oil (316.8 g) which was purified by silica gel column chromatography (eluted with ethyl acetate/heptane 1:9) to give compound **20** (155.96 g, 81.1% yield) as a yellowish oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (s,1H), 7.95 (m, 4H), 7.59 (m, 2H), 7.45 (m, 4H), 7.09 (m, 4H), 6.65 (m, 4H), 5.68 (m, 1H), 5.42 (m, 1H), 4.45 (m, 1H), 4.29 (m, 1H), 3.82-3.63 (m, 8H), 3.55 (m, 1H), 2.31 (m, 1H), 2.05 (m, 1H), 1.75 (m, 4H), 1.50-1.20 (m, 18H), 0.84 (m, 6H).

*4.2.3.5. Preparation of (2S,3S)-2-Hydroxy-5,5-bis((4-(octyloxy)benzyl)thio)pentane-1,3-diyl dibenzoate (**21**).* Hydrogen chloride solution (4.0 M in dioxane, 45.2 mL, 0.181 mol) was added to a stirred solution of (2S,3S)-2-(formyloxy)-5,5-bis((4-(octyloxy)benzyl)thio)pentane-1,3-diyl dibenzoate (**20**) (155.00 g, 0.181 mol) in THF (183 mL) and MeOH (1373 mL). The cloudy solution was stirred at room temperature for 2 hours and concentrated under reduced pressure (bath temperature at 31 °C) and then under high vacuum. The residue was dissolved in DCM (1.2 L). The resulting solution was washed with water (1.2 L), dried ($MgSO_4$), concentrated under reduced pressure and then under high vacuum overnight to give compound **21** (143.6 g, 96%) as a yellowish oil. This product was used in the next step without any further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (d, $J = 7.9$ Hz, 2H), 7.90 (d, $J = 7.9$ Hz, 2H), 7.55 (m, 4H), 7.39 (m, 4H), 7.06 (m, 4H), 6.67 (m, 4H), 5.49 (m, 1H), 4.35 (m, 2H), 3.91-3.64 (m, 8H), 3.57 (m, 1H), 2.39 (m, 1H), 2.24 (m, 1H), 2.15 (m, 1H), 1.80-1.65 (m, 4H), 1.45-1.21 (m, 18 H), 0.84 (m, 6H).

*4.2.3.6. Preparation of (2S,3S)-2-((methylsulfonyl)oxy)-5,5-bis((4-(octyloxy)benzyl)thio)pentane-1,3-diyl dibenzoate (**22**).* A solution of MsCl (23.9 g,

0.209 mol, 1.2 eq) in anhydrous DCM (30 mL) was added dropwise to a stirred solution of (2S,3S)-2-hydroxy-5,5-bis((4-(octyloxy)benzyl)thio)pentane-1,3-diyl dibenzoate (**21**) (144.36 g, 0.174 mol) in anhydrous DCM (950 mL) cooled in an ice bath, followed by a solution of triethylamine (21.1 g, 0.209 mol, 1.2 eq) in anhydrous DCM (20 mL) dropwise while keeping the internal temperature below 5 °C. After the addition, the reaction mixture was left in the ice bath, and was spontaneously warmed up to ambient temperature while stirring overnight. The reaction was quenched with water (800 mL) and the resulting mixture was stirred at room temperature for one hour. The layers were separated. The organic layer was washed with a solution of sodium bicarbonate (17.5 g, 0.209 mol) in water (800 mL) for 40 minutes, and then with a solution of citric acid monohydrate (17.5 g, 0.083 mol) in water (500 mL) for 30 minutes. The organic layer was dried ($MgSO_4$), and concentrated under reduced pressure at 27 °C to give compound **22** (159.54 g, 101.0% yield) as a brown oil. The crude product was used in the next step without any further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (d, $J = 7.9$ Hz, 2H), 7.91 (d, $J = 7.9$ Hz, 2H), 7.77 (m, 2H), 7.47 (m, 4H), 7.15 (d, $J = 8.7$ Hz, 2H), 7.05 (d, $J = 8.7$ Hz, 2H), 6.67 (m, 4H), 5.63 (m, 1H), 5.01 (m, 1H), 4.55 (m, 1H), 4.38 (m, 1H), 4.84-4.65 (m, 8H), 3.58 (m, 1H), 2.98 (s, 3H), 2.36 (m, 1H), 2.18 (m, 1H), 1.75 (m, 4H), 1.45-1.20 (m, 18 H), 0.84 (m, 6H).

*4.2.3.7. Preparation of ((2 R,3S)-3-(benzoyloxy)-5-((4-(octyloxy)benzyl)thio)tetrahydrothiophen-2-yl)methyl benzoate (**16a**).* A solution of (2S,3S)-2-((methylsulfonyl)oxy)-5,5-bis((4-(octyloxy)benzyl)thio)pentane-1,3-diyl dibenzoate (**22**) (159.51 g, 175.81 mmol), triethylamine (35.58 g, 351.6 mmol, 2 eq) and *n*-Bu_4NI (129.88 g, 351.62 mmol, 2 eq) in acetonitrile (2.44 L) was heated at 75-77 °C for 7 days. The reaction mixture was concentrated under reduced pressure. The residue was mixed with ethyl acetate/heptane (1.28 L, 1:3) for 60 minutes. The solids were filtered off, and the filtrate was concentrated under reduced pressure to give a sticky black oil. The residue (203.41 g) was purified by silica gel column chromatography (ethyl acetate/heptane, 1:12) to afford compound **16a** (71.63 g, 69% yield, a mixture of two anomers) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19-7.91 (m, 4H), 7.55 (m, 2H), 7.41 (m, 4H), 7.24 (d, $J = 8.7$ Hz, 2H), 6.83 (d, $J = 8.7$ Hz, 2H), 5.78 (m, 0.75H), 5.65 (m, 0.25H), 4.59-4.38 (m, 3), 4.11 (m, 0.25H), 3.96-3.76 (m, 4.75H), 2.75 (m, 0.25H), 2.59-2.29 (m, 1.75H), 1.76 (m, 2H), 1.45-1.20 (m, 9 H), 0.84 (m, 3H).

*4.2.3.8. Preparation of (2 R,3S)-2-(hydroxymethyl)-5-((4-(octyloxy)benzyl)thio)tetrahydrothiophen-3-ol (**16**).* A mixture of ((2 R,3S)-3-(benzoyloxy)-5-((4-(octyloxy)benzyl)thio)tetrahydrothiophen-2-yl)methyl benzoate (**16a**)

(70.97 g, 119.7 mmol) with THF (710 mL) and lithium hydroxide monohydrate (20.09 g, 478.mmol, 4 eq.) in water (120 mL) was stirred at room temperature for 48 hours. The reaction mixture was diluted with MTBE (700 mL) and brine (700 mL) and stirred for 15 minutes. The layers were separated. The aqueous layer was extracted with MTBE (500 mL) again. The combined organic layers were dried over sodium sulfate, concentrated under reduced pressure to give a light brown oil (53.52 g). The residue was mixed with heptane (500 mL) and then concentrated under reduced pressure to give a white solid (51.73 g). The crude product was stirred with heptane (500 mL) for two hours and then kept at −23 °C overnight. The product was collected by filtration to give a white solid (46.05 g, 95.3% purity by HPLC). This solid was dissolved in DCM (500 mL) and was stirred with a saturated aqueous solution of sodium bicarbonate (500 mL) for one hour. The mixture was filtered through a F-fritted funnel and the layers were separated. The aqueous layer was extracted with DCM (200 mL) again. The combined organic layers were dried (Na_2SO_4), filtered through a Celite® 545 pad, concentrated under reduced pressure and then under high vacuum for 72 hours to give compound **16** (44.2 g, 96.0% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 (d, $J = 8.7$ Hz, 2 H), 6.84 (d, $J = 8.7$ Hz, 2 H), 4.56 (m, 1 H), 4.35 (t, $J = 6.0$ Hz, 1H), 3.93 (t, $J = 6.6$ Hz, 2H), 3.65-3.79 (m, 4 H), 3.44 (m, 1 H), 2.34 (m, 2 H), 2.22 (m, 2 H), 1.77 (m, 2 H), 1.45 (m, 2 H), 1.29 (m, 8 H), 0.89 (t, $J = 6.9$ Hz, 3H); m.p. 69.0-70.5 °C; Combustion Analysis for $C_{20}H_{32}O_3S_2$. Calculated: C, 62.46; H, 8.39; S,16.68, Found: C, 62.52; H, 8.42; S,16.41.

4.2.4. Synthesis of of 4-Amino-1-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)pyrimidin-2(1H)-one (1) (NSC764276, T-dCyd)

*4.2.4.1. Preparation of (6aR,9aS)-2,2,4,4-tetraisopropyl-8-((4-(octyloxy)benzyl)thio)tetrahydro-6H-thieno[3,2-f][1,3,5,2,4]trioxadisilocine (**16 g**).* (2*R*,3*S*)-2-(Hydroxymethyl)-5-((4-(octyloxy)benzyl)thio)tetrahydrothiophen-3-ol (**16**) (598.8 g, 1.56 mol) was combined with imidazole (265 g, 3.89 mol, 2.5 equiv.) in dry DMF (3.1 L) in a 5 three-neck round-bottom flask under argon. The solution was cooled to 2-5 °C, and treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (589.4 g, 1.87 mol, 1.2 equiv.) dropwise over 1.5 h while maintaining the reaction temperature below 5 °C. The reaction was stirred for 21 h as the cooling bath was allowed to expire. The reaction mixture was then poured into cold water (8 L) in a 50 L reactor. The mixture was extracted with EtOAc (3 × 5 L) and the combined organic layer was washed with 50% saturated sodium chloride solution (3 × 3.5 L). The organic layer was dried ($MgSO_4$) and the volatiles were removed in vacuo to provide a crude oil (1132.5 g). The crude material was purified by chromatography over 8 Kg silica gel (230-400 mesh) while eluting with 97%

EtOAc/heptane (57 L). The appropriate fractions were combined and concentrated to afford 927.5 g of compound **16g** in 95% yield as a pale oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (d, $J = 8.7$ Hz, 2H), 6.81 (d, $J = 8.4$ Hz, 2H), 4.59 (m, 0.2H), 4.22 (m, 2H), 4.05 (m, 1), 3.91 (m, 2H), 3.86 – 3.76 (m, 3H), 3.44 (m, 0.8H), 3.33 (m, 0.2), 2.50 (m, 0.8H), 2.40-2.18 (m, 0.4H), 2.00 (m, 0.8H), 1.75 (m, 2H), 1.45-1.20 (m, 10H), 0.92-1.19 (m, 28H), 0.89 (m, 3H).

*4.2.4.2. Preparation of N-(2-oxo-1-((6aR,8R,9aS)-2,2,4,4-tetraisopropyltetrahydro-6H-thieno[3,2-f][1,3,5,2,4]trioxadisilocin-8-yl)-1,2-dihydropyrimidin-4-yl)benzamide (**24f**).* A 3 L three-neck round-bottom flask under argon was charged with *N*4-benzoyl-cytosine (**23a**) (270.2 g, 1.26 mol), ammonium sulfate (4.115 g, 0.031 mol), and hexamethyldisilazane (HMDS) (1.3 L). The suspension was heated to reflux for 18 h. The resulting solution (slight turbidity) was cooled to room temperature and the excess HMDS was removed in vacuo to afford the silylated base as a white solid. The solid was dissolved in anhydrous DCM (2.25 L) and this solution was used directly in the addition step.

(6a*R*,9a*S*)-2,2,4,4-Tetraisopropyl-8-((4-(octyloxy)benzyl)thio)tetrahydro-6H-thieno[3,2-f][1,3,5,2,4]trioxadisilocine (**16g**) (525.2, 0.838 mol) was dissolved in anhydrous DCM (2.26 L) in a 50 L reactor and the solution of silylated *N*4-benzoyl-cytosine (**23a**) in DCM was added. The reaction mixture was cooled to 0 °C while stirring under argon. The solution was treated with *N*-Bromosuccinimide (164 g, 0.92 mol, 1.1 equiv.) in five portions over 30 minutes. The reaction mixture was stirred at 0 °C for 1.5 h. TLC (5% EtOAc/heptane) was used to monitor the consumption of **16g**. After **16g** was consumed completely (1.5 h), the reaction was quenched with sodium thiosulfate (305 g in 2.5 L of water) and stirred for 0.5 h at 0 °C. The mixture was filtered through a pad of Celite 209. The organic layer was separated, and the aqueous layer was extracted with DCM (2 × 2 L). The combined organic layers were dried (MgSO4) and the filtrate was concentrated in vacuo to give 788 g of crude product. Purification by flash chromatography over 12 Kg silica gel (230-400 mesh) eluting with a 20-50% EtOAc/heptane gradient followed by chromatography of mixed fractions ultimately afforded 241.6 g (49%) of compound (**24f**) as an off-white solid. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.82 (d, $J = 7.8$ Hz, 1H), 8.64 (br s, 1H),7.89 (d, $J = 7.8$ Hz, 2H), 7.68-7.45 (m, 4H), 6.11 (d, $J = 6.9$ Hz, 1H), 4.43-4.31 (m, 1H), 4.16 (dd, $J = 12.9$, 3.0 Hz, 1H), 4.00 (d, $J = 12.6$ Hz, 1H), 3.41-3.35 (m, 1H), 2.63-2.50 (m, 1H), 2.37 (dd, $J = 13.2$, 5.1 Hz, 1H), 1.20-0.80 (m, 28 H).

4.2.4.3. Preparation of N-(1-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide. A 3 L three-necked

round-bottom flask under argon was charged with N-(2-oxo-1-((6aR,8R,9aS)-2,2,4,4-tetraisopropyltetrahydro-6H-thieno[3,2-f][1,3,5,2,4]trioxadisilocin-8-yl)-1,2-dihydropyrimidin-4-yl)benzamide (**24f**) (236.4 g, 0.40 mol) and anhydrous THF (1.3 L). The solution was cooled to 0 °C, treated slowly dropwise with a solution of tetra-*n*-butylammonium fluoride in THF (1 M, 1.21 L, 1.21 mol, 3 equiv.) over 1 h, and then it was stirred at that temperature for 2 h. Keeping the bath temperature below 30 °C, the reaction mixture was concentrated to dryness. The residue was dissolved in DCM (2.5 L) and this solution was slowly added to water (2.5 L) with vigorous stirring. After the addition was complete the mixture was stirred an additional 0.5 h. The formed solid was collected by filtration and the filter cake was washed successively with DCM (0.5 L) and water (0.5 L). The solid was dried under vacuum at 40 °C for 18 h to afford 129.5 g (93%) of N-(1-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide as a white solid. ^{1}H-NMR (300 MHz, CD_3OD) δ 8.78 (d, $J = 7.5$ Hz, 1H), 7.97 (d, $J = 7.8$ Hz, 2H), 7.68-7.61 (m, 2H), 5.57-7.50 (m, 2H), 6.40 (t, $J = 6.4$ Hz, 1H), 4.45 (q, $J = 4.5$ Hz, 2H), 3.81 (d, $J = 5.4$ Hz, 2H), 3.46 (q, $J = 5.4$ Hz, 1H), 2.56-2.46 (m, 1H), 2.36-2.26 (m, 1H).

*4.2.4.4. Preparation of 4-Amino-1-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)pyrimidin-2(1H)-one (**1**) (NSC764276, T-dCyd).* A suspension of N-(1-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl) tetrahydrothiophen-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide (129.5 g, 0.373 mol) in anhydrous methanol (4.7 L) was treated with a solution of ammonia in methanol (7 M, 470 mL, 3.29 mol). The suspension was stirred 16 h at room temperature. The clear solution was evaporated to dryness and the residue was stirred with acetonitrile (1 L) containing 15 mL methanol overnight. The solid was collected by filtration, washed with 300 mL acetonitrile, and dried under high vacuum at 40 °C overnight to afford 85.5 g of compound **1** in 94% yield with 99.9% HPLC purity but with 8 mol% methanol by proton NMR. The 85.5 g of solid **1** was dissolved in water (2.5 L) and was dried by lyophilization to remove the residual methanol. The lyophilized solid was dried under vacuum at 45 °C afford 84.1 g (93%) of compound **1** (NSC764276, T-dCyd) as a fine white solid. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.93 (d, $J = 7.5$ Hz, 1H), 7.17 (s, 1H), 7.15 (s, 1H), 6.35 (dd, $J = 8.1$, 6.9 Hz, 1H), 5.77 (d, $J = 7.2$ Hz, 1H), 5.20 (d, $J = 3.9$ Hz, 1H), 5.10 (t, $J = 5.4$ Hz, 1H), 4.33 (t, $J = 3.3$ Hz, 1H), 3.65-3.45 (m, 2H), 3.30-3.22 (m, 1H), 2.20-2.00 (m, 2H). ^{13}C-NMR (75 MHZ, DMSO-d_6) δ 165.3, 155.5, 142.2, 94.8, 73.5, 63.8, 60.6, 58.8, 41.7. Melting Point: 215-216 °C. Combustion analysis: Calculated for $C_9H_{13}N_3O_3S$: C, 44.43; H, 5.39; N, 17.27; S, 13.18. Found: C, 44.51; H, 5.25; N, 17.23; S, 13.36.

4.2.5. Synthesis of 4-Amino-1-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)-1,3,5-triazin-2(1H)-one (2), (NSC777856, 5-Aza-T-dCyd)

*4.2.5.1. Preparation of 4-Amino-1-((6aR,8R,9aS)-2,2,4,4-tetraisopropyltetrahydro-6H-thieno[3,2-f][1,3,5,2,4]trioxadisilocin-8-yl)-1,3,5-triazin-2(1H)-one (**24e**).*

A suspension of 5-azacytosine (**23b**) (150.1 g, 1.34 mol) and ammonium sulfate (4.4 g, 0.033 mol) in hexamethyldisilazane (HMDS, 980 mL) was refluxed in 2-L round-bottomed flask while stirring under argon atmosphere for 21 h. The solution was cooled to room temperature and the excess of HMDS was removed in a rotary evaporator to give a white solid. The residue was dissolved in anhydrous DCM (1 L) and the solution was used directly below.

(6a*R*,9a*S*)-2,2,4,4-Tetraisopropyl-8-((4-(octyloxy)benzyl)thio)tetrahydro-6H-thieno[3,2-f][1,3,5,2,4]trioxadisilocine (**16g**) (280 g, 446.5 mmol) was dissolved in anhydrous DCM (1.3 L) and the solution of the silylated azacytosine was added. The resulting solution was cooled to 0 °C while stirring under argon atmosphere. *N*-Bromosuccinimide (87 g, 491.1 mmol, 1.1 equiv.) was added to the cooled mixture in 5 portions over 30 minutes and the resulting mixture was stirred at 0 °C for 1.5 h. Thin-layer chromatography was used to monitor consumption of starting material **16g** (5% Ethyl acetate/heptane). After **16g** was consumed completely, the reaction mixture was quenched with sodium thiosulfate (160 g in 1.8 L of water) and stirred for 0.5 h at 0 °C. The mixture was filtered through a pad of Celite 209 (200 g). The organic layer was separated, and the aqueous layer was extracted with DCM (1.5 L × 2). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in a 10-L rotary evaporator to give the crude product as a 4:1 anomeric mixture (420 g). Fractionation of the crude material was achieved by column chromatography over 3.5 Kg of silica gel (230-400 mesh) while eluting with a 30-100% ethyl acetate/DCM gradient followed by chromatography of mixed fractions to ultimately afford 90.6 g (42%) of compound **24e** as a white foam. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.99 (s, 1H), 6.22 br s, 1H), 5.95 (d, $J = 6.6$ Hz, 1H), 5.60 (br s, 1H), 4.40-4.26 (m, 1H), 4.13 (dd, $J = 12.8$, 3.0 Hz, 1H), 3.97 (dd, $J = 7.0$, 0.9 Hz, 1H), 3.38-3.30 (m, 1H), 2.57-2.44 (m, 1H), 2.33 (dd, $J = 13.4$, 5.4 Hz, 1H), 1.18-0.80 (m, 28 H).

4.2.5.2. Preparation of 4-Amino-1-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)-1,3,5-triazin-2(1H)-one (2), (NSC777856, 5-Aza-T-dCyd).

A suspension of 4-amino-1-(6a*R*,8*R*,9a*S*)-2,2,4,4-tetraisopropyl-tetrahydro-6H-thieno[3,2-f][1,3,5,2,4] trioxadisilocin-8-yl)-1,3,5-triazin-2(1H)-one (**24e**) (90 g, 184.9 mmol) and ammonium fluoride (34.3 g, 924.4 mmol) in anhydrous methanol (1.3 L) was heated to 60-65 °C for 2.5 h. The mixture was cooled to 15 °C and stirred for 1 h. The precipitated solid was collected

in a filter funnel and washed with anhydrous methanol (2 × 30 mL) to give 28 grams of compound **2** (NSC-777856, 5-Aza-T-dCyd) as white solid after drying. The mother liquor was mixed with Celite 209 (30 g), concentrated to dryness and purified by chromatography on silica gel (150 g) eluting with ethyl acetate/ethanol in a ratio 5:3 (4 L) and then with ethanol (4 L) to give 7 g of crude product. The column isolate was stirred with methanol (70 mL) for 30 min., followed by filtration to provide an additional 3.5 g of **2** as white solid after drying. The two lots of **2** were combined to afford a total of 31.5 g (73%) of compound **2** as a fine white solid. ^{1}H NMR (400 MHz, DMSO- d_6) δ 8.67 (s, 1H), 7.50 (d, J = 5.6 Hz, 2H), 6.06 (t, J = 7.0 Hz, 1H), 5.21 (d, J = 4.0 Hz, 1H), 5.10 (t, J = 5.4 Hz, 1H), 4.30 (q, J = 4.1 Hz, 1H), 3.60 (dt, J = 11.7, 5.9 Hz, 1H), 3.53 (dt, J = 11.2, 5.4 Hz, 1H), 3.25 (td, J = 5.9, 3.5 Hz, 1H), 2.22 (dddd, J = 19.7, 12.8, 9.7, 4.5 Hz, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 165.95, 157.66, 153.80, 73.72, 63.45, 59.99, 59.17, 42.05. Melting Point: 207-209 °C, d. Combustion Analysis. Calculated for $C_8H_{12}N_4O_3S$: C, 39.34; H, 4.95; N, 22.94; S, 13.12. Found: C, 39.28; H, 4.90; N, 22.79; S, 12.93.

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