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# Preparation of 5-hydroxymethyl-pyrimidine based nucleosides: a reinvestigation

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**Abstract:** 5-Hydroxymethyl-pyrimidine-based nucleobases have attracted attention in the last years due to their important role in gene regulation. It prompted the development of efficient routes to the corresponding nucleoside building blocks for further incorporation into oligonucleotides. Several pathways have been employed in recent years to access properly protected 5-hydroxymethyl-pyrimidine-based nucleosides, including hydroxymethylation of 2'-deoxyuridine, radical bromination of thymidine followed by bromine substitution, or carbonylative coupling through Stille conditions starting from 5-iodo-2'-deoxyuridine. In this study, we review the different approaches currently used to introduce the 5-hydroxymethyl moiety, we reinvestigate some of them, and finally, we propose an alternative route based on the oxidation of the methyl of thymidine followed by its reduction that allows access to protected 5-hydroxymethyl-2'-deoxyuridine in a simple way with good yields. Finally, we present some examples of application including the synthesis of base J and 5-azidomethyl-2'-deoxyuridine.

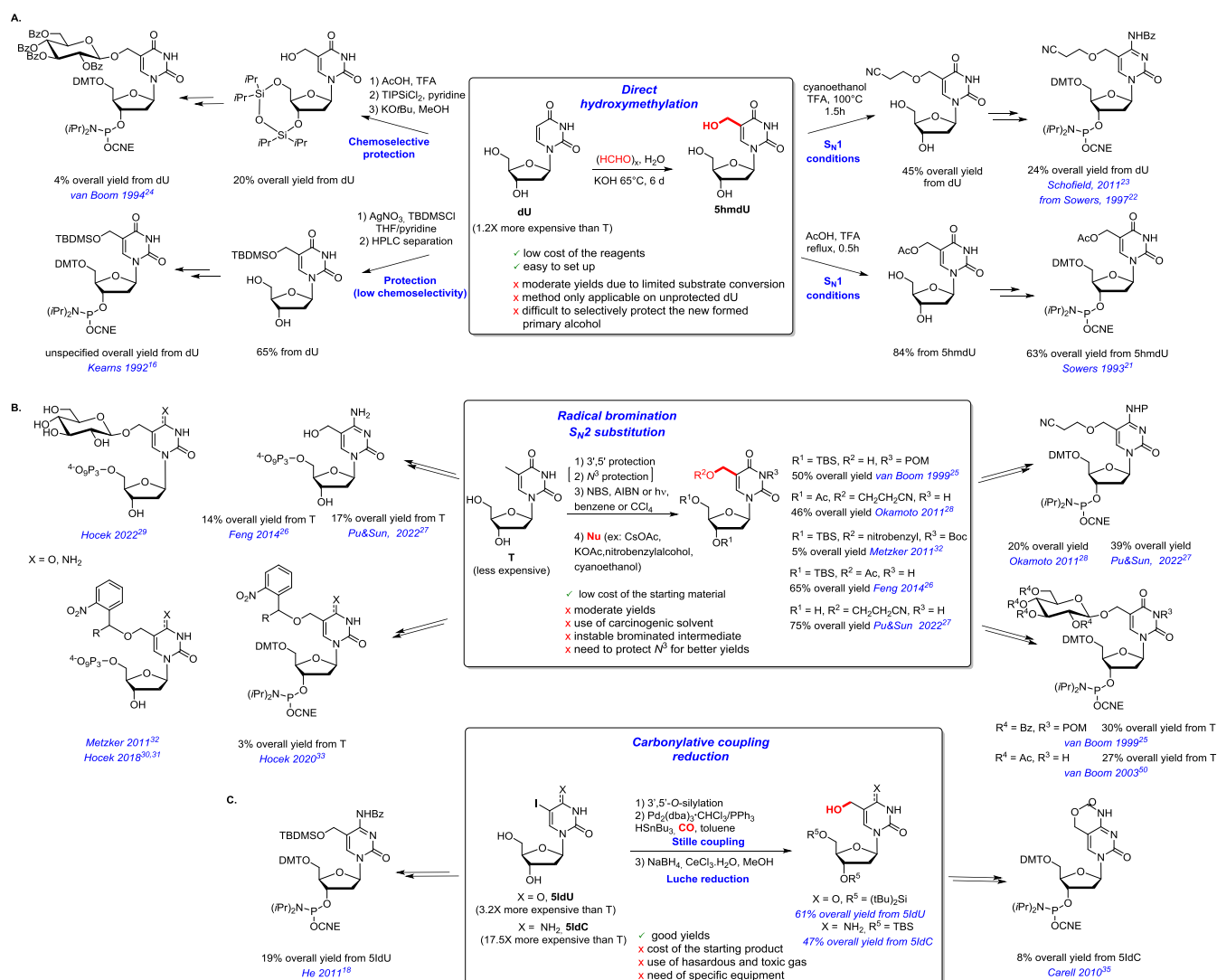
## Introduction

In the last years, the discovery of oxidative lesions or enzymatically-catalyzed oxidation at the C-5 methyl of pyrimidines within DNA<sup>[1],[2],[3]</sup> have prompted the development of efficient routes to the corresponding nucleoside building blocks for further incorporation into oligonucleotides thus enabling the study of their biological role. Hence, several 5-hydroxymethyl-pyrimidine-based nucleobases are reported in different genomes. Among them, 5-hydroxymethylcytosine (5hmC) is produced in eukaryotes by enzymatic oxidation of 5-methylcytosine (5mC) by the TET proteins<sup>[4],[5]</sup> and was found to be involved in gene regulation.<sup>[6]</sup> 5-hydroxymethyluracil (5hmU) was reported as a product of thymidine oxidative DNA damage.<sup>[7],[8]</sup> It was also found in phage genome<sup>[9],[10]</sup>. Interestingly, the presence of 5hmU in the DNA templates was found to enhance transcription by bacterial RNA polymerases.<sup>[11],[12]</sup> Studies also suggest that 5hmU may also arise via thymidine enzymatic oxidation in eukaryotes.<sup>[1]</sup> Derived from 5hmU, the glycosylated nucleobase  $\beta$ -D-glucosyl-5-hydroxymethyluracil (glc-5hmU) or base J, is a rare DNA modification identified in parasite genomes and is proposed to act as an epigenetic mark.<sup>[2],[13]</sup> Base J is introduced within DNA in two steps from a specific thymidine which is first oxidized into 5-hydroxymethyl-2'-deoxyuridine (5hmdU) and then glycosylated.<sup>[14],[15]</sup> So far, several phosphoramidites or deoxynucleosides triphosphate building blocks have been developed to incorporate 5-hydroxymethyl-pyrimidine derivatives in DNA by using either oligonucleotide solid phase synthesis or polymerases, respectively. Currently, there are three major

routes to synthetically obtain 5-hydroxymethyl derivatives of pyrimidine nucleosides as precursors of these building blocks (Figure 1).

First, 5hmdU can be easily prepared from 2'-deoxyuridine (dU) using a single step reaction without protecting groups (Figure 1A). Indeed, hydroxymethylation of dU can be accomplished in either water-formaldehyde or paraformaldehyde solutions, in the presence of potassium hydroxide or triethylamine as bases. Mayol and Kearns group disclosed this reaction by heating dU in the presence of paraformaldehyde and potassium hydroxide<sup>[16]</sup> according to a procedure previously described by Shiao.<sup>[17]</sup> The yield of this reaction is not reported, but various subsequent works using this method report that 5hmdU can be isolated after silica gel column purification in 60-80% yields. To access synthons for their incorporation in DNA, the selective protection of the allylic alcohol remains challenging due to the 5' primary alcohol of the deoxyribose unit. Hence, its protection as a silyl ether in the presence of TBDMSCl and silver nitrate afforded a mixture of mono- and di-O-silylated compounds requiring an HPLC separation to isolate the product of interest in 65% yield (Figure 1A).<sup>[16]</sup> Treatment of the mixture with di-*tert*-butylsilyl bistriflate was shown to facilitate the resolution of the isomers through the formation of a cyclic silyl ring between 3' and 5' for the desired mono-O-silylated product.<sup>[18]</sup> Selective O-5 chemical transformations of 5hmdU were however reported. Hence, the 5-hydroxyl group was differentiated thanks to its pseudobenzylic properties. Following the initial works of Cline and Fink<sup>[19]</sup> on 5-substituted pyrimidines and the one of Scheit<sup>[20]</sup> on uridine derivatives, Sowers accomplished a O-5 selective reaction of 5hmdU in refluxing glacial acetic acid in the presence of trifluoroacetic acid to furnish the acetylated derivative in good yield under S<sub>N</sub>1 conditions,<sup>[21]</sup> which was used to prepare a phosphoramidite derivative (Figure 1A). The 5-cyanoethyl derivative was selectively prepared according to the same procedure allowing the conversion to the corresponding 5-hydroxymethyl-2'-deoxycytidine (5hmdC) phosphoramidite (Figure 1A).<sup>[22],[23]</sup> Van Boom's group described the first synthesis of the suitably protected base J phosphoramidite building unit from the 5-O-acetyl derivative of 5hmdU as intermediate using the Sower's procedure (Figure 1A).<sup>[24]</sup> This method for synthesizing 5-hydroxymethyl derivatives of pyrimidine nucleobases offers several advantages: it requires inexpensive reagents and starting material, and is simple to implement. However, using this pathway, the fully deprotected 5hmdU is obtained, which makes selective protection of the 5-hydroxyl group tricky.

Then, another important method for the preparation of 5hmdU derivatives is the one performed through radical bromination of the 5-methyl position of protected thymidine using



**Figure 1.** Overview of the main synthetic methods for the synthesis of 5-hydroxymethyl-pyrimidine-based nucleobases. A. From 2'-deoxyuridine: hydroxymethylation. B. From thymidine: radical bromination followed by bromide substitution. C. From 5-iodo-2'-deoxyuridine: carbonylative coupling followed by reduction.

*N*-bromosuccinimide in benzene or  $\text{CCl}_4$  and a radical initiator (AIBN or irradiation). Van Boom improved his precedent base J phosphoramidite synthesis by considering thymidine as a starting product through radical bromination of a bis-silylated thymidine derivative and substitution of the resulting crude allylic bromide with cesium acetate, thus affording fully protected 5hmdU (Figure 1B).<sup>[25]</sup> Radical bromination was also used to prepare the 5hmdC triphosphate derivative<sup>[26],[27]</sup>, the cyanoethyl-protected 5hmdC phosphoramidite<sup>[28],[27]</sup>, the glc-5hmdU and glc-5hmdC triphosphate derivatives<sup>[29]</sup> as well as photolabile 2-nitrobenzyl alkylated 5hmdU triphosphates<sup>[30],[31],[32]</sup> and photolabile 2-nitrobenzyl alkylated 5hmdC/U phosphoramidites (Figure 1B).<sup>[33]</sup> This pathway has several drawbacks such as the modest reported yields, the need to engage the bromination product in the following reactions without purification to avoid its degradation, and the use of toxic carcinogenic solvents.

Alternatively, Crouch described the synthesis of 5-formyl derivative of dU (5fdU) through standard Stille coupling conditions starting from 5-iodo derivative of dU (5IdU) and CO in the presence of Pd(0) catalyst and  $\text{HSnBu}_3$ .<sup>[34]</sup> Carell used this carbonylative coupling to prepare 5hmdC phosphoramidite with a cyclic carbamate as a protective group.<sup>[35]</sup> Hence, 5-iodo-3',5'-

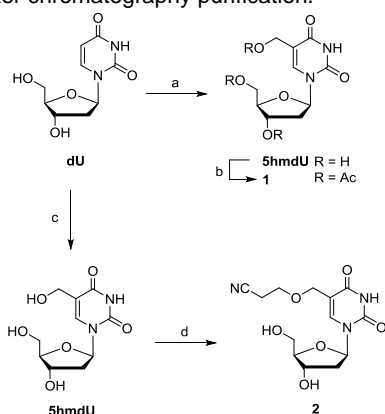
di-*O*-TBDMS-2'-deoxycytidine was used as starting material, thus leading to the corresponding 5-formyl-dC analogue in good yield. This latter has been reduced using Luche<sup>[36]</sup> conditions with  $\text{NaBH}_4$  in the presence of  $\text{CeCl}_3$  to avoid hydride reaction at C-6 position giving the corresponding 5hmdC analogue (Figure 1C). He's group used this method with 5-iodo-3',5'-di-*O*-TBDMS-2'-deoxyuridine as starting material to synthesize a 5hmdC phosphoramidite with TBDMS as the 5-hydroxyl protecting group (Figure 1C).<sup>[18],[37]</sup> This carbonylative coupling approach gives the best yields what counterbalances the fact that it is the method which uses the most expensive starting material. However, it requires toxic CO and specific equipment contrary to the previous methods.

In the course of an ongoing project on the synthesis of 5hmdU derivatives, it seemed that the introduction of the 5-hydroxymethyl moiety on the pyrimidine nucleobase represents a limiting step to obtain an efficient synthesis of building blocks derivatives. The aim of the present study is *i*) to reinvestigate some of the existing methods previously described to access 5hmdU in order to improve yields and *ii*) to investigate an under-explored pathway relying on a biomimetic approach, namely the direct oxidation of thymidine. Hence, we have studied the

oxidation reaction of thymidine to form 5-formyl-deoxyuridine (5fdU) which can then be reduced to 5hmdU under Luche conditions. This oxidation/reduction sequence allows to obtain 3',5'-acetylated or 3',5'-silylated 5hmdU in good yields, which makes it a valuable method to access building blocks of interest for their incorporation into oligonucleotides.

## Results and Discussion

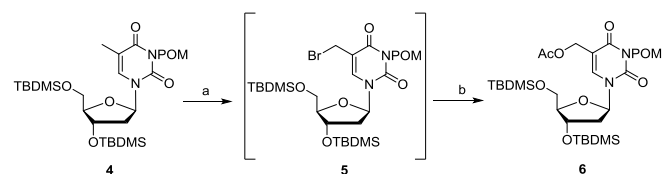
First, we reinvestigated the direct hydroxymethylation of 2'-deoxyuridine (Scheme 1) according to the procedure initially described by Shiau<sup>[17]</sup> and modified by Sowers.<sup>[38]</sup> For this purpose, dU was reacted with formaldehyde in aqueous solution in the presence of triethylamine to obtain 5hmdU after silica gel column purification. In the previously described protocols, a high amount of formaldehyde is used (more than 40 equivalents). According to <sup>1</sup>H NMR spectra, we observed that following the described protocols, the resulting 5hmdU contains significant triethylammonium formate salts, even after purification on silica gel. By reducing formaldehyde by half, dU was entirely converted and the amount of salts was significantly reduced in the product. In an attempt to remove the residual salts, the 5hmdU was reacted in the presence of acetic anhydride and DMAP to give the per-*O*-acetylated 5hmdU **1** without requirement of purification to obtain the pure product (77% yield in two steps). Interestingly, the triethylammonium formate salts, that were present in 5hmdU, disappeared. We assume they were converted through acid-base equilibria in volatile by-products (*i.e.*, acetic acid, trimethylamine, formic anhydride). Alternatively, dU was reacted with paraformaldehyde in 5M aqueous KOH solution for 3 days at 90°C to obtain the 5hmdU in 67% yield after chromatography purification.



**Scheme 1.** Reagents and conditions: a) 37% HCHO (20 eq), Et<sub>3</sub>N (13 eq), H<sub>2</sub>O, 3 days 80°C; b) Ac<sub>2</sub>O, pyridine, DMAP, 16h, r.t., 77% (two steps); c) (HCHO)<sub>n</sub> in 5M KOH aq, 90°C, 3 days, 67% d) Amberlyst 15 (1g/mmol 5hmdU), cyanoethanol (5 eq), MeCN, r.t., 90%.

The unprotected 5hmdU obtained by this procedure was successfully used in chemoselective etherification to prepare several 5-hydroxymethyl derivatives of 2'-deoxyuridine in the presence of alcohol promoted by the acidic Amberlyst 15 resin, as reported in our recent study.<sup>[39]</sup> For example, the cyanoethyl-protected 5hmdU **2** was obtained in an excellent 90% yield compared to previously procedures reporting only 50% yields.<sup>[27],[39],[40]</sup> This two-step procedure therefore provides an easy access to a precursor of phosphoramidite building block for 5hmdU or 5hmdC-containing oligonucleotide synthesis.

Next, we reinvestigated the bromination of TBDMS-protected thymidine **4** in the presence of *N*-bromosuccinimide (NBS) freshly recrystallized and azobisisobutyronitrile (AIBN) as radical initiator followed by direct bromine substitution by acetate to give **6** (Scheme 2). After several attempts to improve the yield of this reaction, we have not been able to obtain more than 55% yield for the two steps, in agreement with the highest yields reported in the literature. Our recommendations and observations are as follows: *i*) the *N*<sup>3</sup> protection of the nucleobase of 5hmdU with a pivaloyloxymethyl (POM) protecting group was shown to be crucial to observe significant yields of bromination; *ii*) a decrease in yield was observed when we tried to scale up (beyond 1g); *iii*) the bromination needs a rigorous control of the reaction to avoid the formation of a secondary product through dibromination. The dibrominated compound leads to the formylated product (5-formyl-deoxyuridine) after treatment of the crude product, which was observed when we add too much reagent in order to consume the starting thymidine. Thus, we tried to find a good compromise between reaction time and quantities of reagents to form the 5-bromomethyl-2'-deoxyuridine (5BrmdU) **5** as the major product, which cannot be isolated and was directly subjected to the bromide substitution step. The conditions described in Scheme 2 are those which, in our hands, have led to the best results.

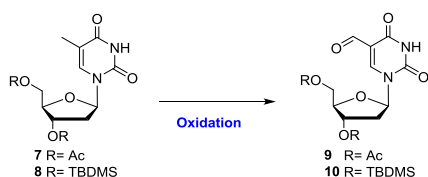


**Scheme 2.** Reagents and conditions: a) NBS (1.6 eq), AIBN (0.2 eq), benzene, 45 min, 95°C. b) CsOAc (1.5 eq), DMF, 1h, r.t., 55%.

In a recent paper, Pu and Sun described a procedure in which they converted the 5BrmdU intermediate to cyanoethyl ether via simultaneous alcoholysis with cyanoethanol and cleavage of TBDMS by HBr generated *in situ* at ambient temperature affording **2** in 78% yield<sup>[27]</sup>.

Finally, we turned to another way to obtain the 5-hydroxymethyl moiety onto the pyrimidine nucleobase. In Nature, the reaction is catalyzed by the 2-oxoglutarate- and Fe(II)-dependent dioxygenases TET or JBP to introduce the 5-hydroxymethyl moiety onto the carbon of the pseudo-benzylic position of thymine or methylcytosine within DNA. As thymidine is the cheapest starting substrate, we thought it would be interesting to re-evaluate previously described biomimetic methods to obtain 5-formyl-2'-deoxyuridine (5fdU), which could then be easily reduced to 5hmdU. A route to 3',5'-*O*-acetyl-5-formyl-2'-deoxyuridine was described in 1993 by Matsuda<sup>[42]</sup> based on oxidation of 3',5'-*O*-acetylthymidine in the presence of potassium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>), copper sulfate (CuSO<sub>4</sub>) and 2,6-lutidine in a water/acetonitrile mixture (52% yield). This method has been further used in other studies and the product is obtained in yields ranging from 20 to 60%.<sup>[41],[43],[44]</sup> The mechanism is presumed to involve the formation of an allylic radical.<sup>[45]</sup> Matsuda also reported the preparation of 3',5'-*O*-bis(*tert*-butyldimethylsilyl)-5-formyl-2'-deoxyuridine following the same procedure in 63% yield.<sup>[44]</sup> First, we reproduced the conditions described by Matsuda starting from either the acetylated or the TBDMS-protected thymidine **7** or **8** and we obtained similar yields as described (Table 1, entries 1 and 2).

The two main reported difficulties of this reaction that we have indeed encountered are: *i*) the low resolution of the starting material and product on TLC which makes it difficult to accurately monitor the reaction; *ii*) after 2h of reaction, a more polar by-product appears, so the reaction should be stopped before the complete disappearance of the starting thymidine. In the initial publication, this by-product was not characterized. We isolated the more polar by-product starting from **7** or **8** and characterized them as the 5-carboxy-2'-deoxyuridine derivatives (see supporting information). When performed under argon in the same conditions, we did not observe this additional oxidation and, interestingly, the *O*-acetylated thymidine **7** was converted in the corresponding acetylated 5-formyl-2'-deoxyuridine in 91% yield (Table 1, entry 3). In the case of the TBDMS-protected thymidine **8**, the yield was lower since it has not been completely consumed, thus making the separation difficult during the purification step. Under these conditions, the compound **9** was obtained in 51% yield (Table 1, entry 4). After 2.5h, the formation of the 5-carboxy-2'-deoxyuridine (5cadU) was observed and we thus decided to take advantage of this secondary reaction to improve the separation of the compound of interest since 5fdU and 5cadU have very different polarity. Hence, by increasing the quantities of reagents, we observed the total consumption of the starting product within 75 min with the concomitant formation of small amounts of 5cadU. The compound **9** was isolated in 65% yield (Table 1, entry 5).



**Table 1.** Optimization of  $K_2S_2O_8$  oxidation of thymidine according to the deoxyribose protecting group.

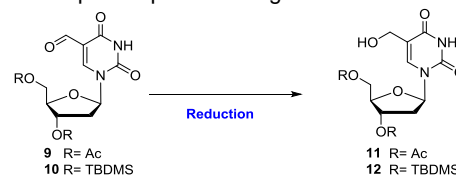
Entry	R	Eq. of $K_2S_2O_8$ , CuSO <sub>4</sub> , 2,6-lutidine	Conditions	Yield <sup>[a]</sup>
1	Ac	2 eq., 0.4 eq., 3.6 eq.	2h	60% <sup>[b]</sup>
2	TBDMS	2 eq., 0.4 eq., 3.6 eq.	2h	54% <sup>[b]</sup>
3	Ac	2 eq., 0.4 eq., 3.6 eq.	2h, under argon	91%
4	TBDMS	2 eq., 0.4 eq., 3.6 eq.	2h, under argon	51%
5	TBDMS	4 eq., 0.8 eq., 7.2 eq.	75min, under argon	65% <sup>[b]</sup>

[a] Isolated yield after flash chromatography (silica gel).

[b] Formation of 5cadU observed.

With 3',5'-protected-5-formyl-2'-deoxyuridine in our hands, we furthermore investigated the conditions for aldehyde reduction. For this purpose, we implemented two reduction conditions. First, we performed the reduction in methanol in the presence of cerium chloride under Luche conditions ( $NaBH_4$ ,  $CeCl_3$ ) as already described by He and Nielsen<sup>[18],[44]</sup> and for reduction of acylpyrimidine nucleosides to homologues of hmU and hmC by Hocek.<sup>[46]</sup> Alternatively, we tried the reduction in water in the presence of borazane according to a procedure described to reduce 3',5'-di-*O*-acetyl-5-formyl-2'-deoxyuridine aldehyde to alcohol.<sup>[46]</sup> This procedure was proved to be compatible with the presence of various base-labile protecting groups, including acetyl group, and acid-labile protecting groups, including TBDMS. We performed the reduction under Luche conditions of 3',5'-di-*O*-acetyl-5-formyl-2'-deoxyuridine **9**. We obtained a modest yield of 58% despite a full conversion observed by TLC

(Table 2, entry 1) probably related to the partial solubility of the product in the aqueous phase during extraction.



**Table 2.** Optimization of the conditions for the 5fdU reduction according to the deoxyribose protecting group.

Entry	R	Conditions	Yield
1	Ac	$CeCl_3 \cdot 7H_2O$ , $NaBH_4$ , MeOH, 45 min, 0°C to r.t.	58% <sup>[a]</sup>
2	Ac	$CeCl_3 \cdot 7H_2O$ , $NaBH_4$ , MeOH, 45 min, 0°C to r.t. <sup>[b]</sup>	92% <sup>[a]</sup>
3	Ac	$BH_3 \cdot NH_3$ (1,1 eq), $H_2O$ , 10 min, r.t.	92%
4	TBDMS	$CeCl_3 \cdot 7H_2O$ , $NaBH_4$ , MeOH, 30 min, 0°C to r.t.	90% <sup>[a]</sup>
5	TBDMS	$BH_3 \cdot NH_3$ (1,1 eq), $H_2O$ , 16h, 80°C	51% <sup>[a]</sup>
6	TBDMS	$BH_3 \cdot NH_3$ (1,1 eq), THF/ $H_2O$ , 1h, r.t.	87% <sup>[a]</sup>
7	TBDMS	$BH_3 \cdot NH_3$ (1,1 eq), MeOH, 1h, r.t.	90% <sup>[a]</sup>

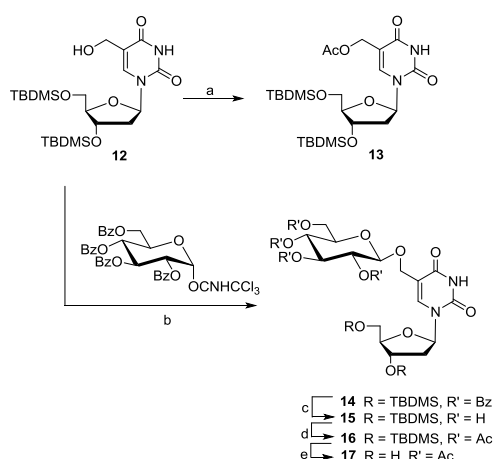
[a] Isolated yield after flash chromatography (silica gel).

[b] Work-up: methanol evaporation before extraction.

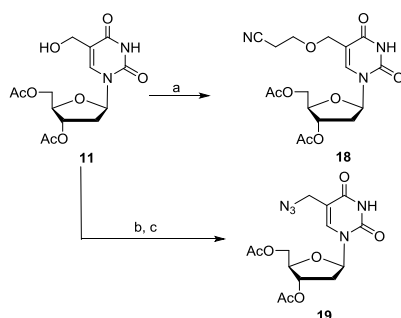
By evaporating methanol before the extraction step, the reduced compound **11** was obtained in 92% yield (Table 2, entry 2). We also performed the reduction of **9** in the presence of borazane. The compound was totally reduced within 10 minutes and **11** was isolated in a good yield of 92% (Table 2, entry 3). We also applied Luche reduction conditions on 3',5'-*O*-bis(*tert*-butyldimethylsilyl)-5-formyl-2'-deoxyuridine **10** and the reduction product **12** was obtained in 90% yield (Table 2, entry 4). The reduction of **10** was also performed in the presence of borazane. While the reaction gives modest yields when performed in water (Table 2, entry 5), it gives good yields when performed either in a mixture of THF/ $H_2O$  or in methanol (Table 2, entries 6, 7).

To demonstrate the suitability of this method to prepare 5hmdU-based building blocks of interest for oligonucleotide synthesis, we designed and synthesized two orthogonal protected 5hmdU, the  $\beta$ -D-glucopyranosyl-5-hydroxymethyl-2'-deoxyuridine (Glc-5hmdU or base J) and an azidomethyl derivative of dU (Schemes 3 and 4). The 3',5'-di-*t*-butyldimethylsilyl-5-acetyloxymethyl-2'-deoxyuridine **13**, a valuable precursor for synthesizing 5hmdC derivatives, was obtained from **12** with an overall yield of 59%. Compared to other described procedures, except a paper by Feng,<sup>[27]</sup> which describes its achievement with 65% yield by the radical bromination pathway, most of the studies mention yields between 24 and 40% for this compound.<sup>[24],[25],[49]</sup> Indeed, as previously mentioned, in our hands, the radical bromination was poorly efficient when the  $N^3$  is unprotected, an observation that was already reported by others. Glc-5hmdU was obtained by glycosylation reaction of acceptor 3',5'-TBDMS-protected 5hmdU **12** using the 2,3,4,6-tetra-*O*-benzoyl- $\alpha$ -D-glucopyranosyl trichloroacetimidate in the presence of trimethylsilyl triflate (TMSOTf) as promoter. The glycosylated compound **14** was then subjected to deprotection/protection steps to obtain Glc-5hmdU **17** with an acetylated glucose moiety suitable for oligonucleotide synthesis according to van Boom procedure. Compound **17** was isolated in similar yields (Scheme 3).<sup>[50]</sup> From

acetylated 5hmdU **11**, the cyanoethyl-protected 5hmdU **18**, useful for solid-phase oligonucleotide synthesis, was obtained in 75% overall yield from thymidine, which is a significant improvement of the yields previously obtained by other methods (46%) (Scheme 4).<sup>[29]</sup> Interestingly, we implemented our previously reported O-5 transformation of 5-(hydroxymethyl)-2'-deoxyuridine through etherification in the presence of 2-cyanoethanol promoted by acidic Amberlyst 15 resin at room temperature, which was found also suitable for the acetylated dU. 5-(Azidomethyl)-2'-deoxy-3,5-di-O-acetyluridine **19** useful for the synthesis of modified nucleosides through click chemistry was obtained from **11** in 84% yield, *i.e.*, 70% overall yield from thymidine, which is also an improvement compared to other methods (31-45%) (Scheme 4).<sup>[51],[17]</sup>



**Scheme 3.** Reagents and conditions: a) Ac<sub>2</sub>O (1.2 eq), pyridine, r.t., 16h, quant.; b) BzGlucosyl trichloroacetimidate (1.1 eq), TMSOTf (0.15 eq), molecular sieves powder 4Å, DCE, 0°C to r.t., 1h, 63%; c) K<sub>2</sub>CO<sub>3</sub> (1.1 eq), MeOH, r.t., 16h, quant.; d) Ac<sub>2</sub>O (8 eq), pyridine, r.t., 16h, 99%; e) HF·NEt<sub>3</sub> (2.1 eq), pyridine, 16h, r.t., 89%.



**Scheme 4.** Reagents and conditions: a) Amberlyst 15 (1g/mmol **11**), 2-cyanoethanol (5 eq), MeCN, r.t., 48h, 89%; b) TMSCl (5 eq), dioxane, 65°C, 16h; c) NaN<sub>3</sub> (3 eq), DMF, 65°C, 1h, 84% (two steps).

## Conclusion

Several 5-hydroxymethyl-pyrimidine based nucleobases were discovered in DNA of different organisms. In order to study their biological role when they are enzymatically introduced or their impact when it is an oxidative lesion, the synthesis of precursors that can be incorporated into oligonucleotides has been actively developed over the last twenty years. An exhaustive review of the different methods used to date shows that biomimetic approaches should be reconsidered as valuable approach to obtain 5hmdU precursors. Indeed, the use of the combination of the oxidation of the pseudo-benzylic carbon followed by the reduction of the resulting aldehyde to access pyrimidine precursors with the 5-hydroxymethyl moiety has been under-

explored to date, probably due to the moderate yields reported for the oxidation step. In fact, each of these steps were used separately in some procedures, *i.e.*, oxidation of thymidine to obtain 5fdU precursors or Luche reduction after a Stille carbonylative coupling, but the combination of these two steps was not optimized to date. After revisiting some of the classical methods used to access 5hmdU precursors, it seems to us, that this route is however easy to implement, inexpensive and allows an easy scale-up since similar yields were obtained at a scale from a few hundred mg to 3 g. Hence, we have defined optimal conditions for obtaining 5hmdU in two steps from two differently protected thymidine. Acetylated and TBDMS protected 5hmdU can be obtained after oxidation of the methyl and then reduction of the aldehyde in an overall yield of 92% and 59%, respectively. These conditions allow to obtain these orthogonally protected 5hmdU precursors and derivatives for oligonucleotide synthesis in good yields by a procedure easy to set up compared to those described.

## Experimental Section

### Essential Experimental Procedures/Data.

**3',5'-Di-O-acetyl-5-formyl-2'-deoxyuridine (9):** According to Table 1 entry 3, 3',5'-di-O-acetylthymidine **7** (2.00 g, 6.1 mmol), K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (3.30 g, 12.3 mmol, 2.0 eq) and CuSO<sub>4</sub>·5H<sub>2</sub>O (0.56 g, 2.3 mmol, 0.4 eq) were successively added in a round bottom flask under argon atmosphere. 100 mL of acetonitrile/water solution (1/1, v/v) and 2,6-lutidine (2.6 mL, 22 mmol, 3.6 eq) were then added. The reaction mixture was stirred for 2 h at 65°C. The progress of the reaction was monitored by TLC (EtOAc/cyclohexane, 2:1). Brine (50 mL) was then added and the resulting mixture was extracted three times with EtOAc (3 x 75 mL). The combined organic phase was dried over Na<sub>2</sub>SO<sub>4</sub> and then concentrated *in vacuo*. The crude product was finally purified by flash silica gel column chromatography (cyclohexane/EtOAc, 4:1) to furnish the product **9** (1.90 g, 91% yield). R<sub>f</sub> = 0.57 (EtOAc). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ = 10.04 (s, 1H, H-7), 9.00 (bs, 1H, NH), 8.50 (s, 1H, H-6), 6.34 (dd, *J* = 5.8 Hz, *J* = 7.8 Hz, 1H, H-1'), 5.26 (dt, *J* = 2.2 Hz, *J* = 6.6 Hz, 1H, H-3'), 4.36 (m, 3H, H-5', H-4'), 2.62 (ddd, *J* = 2.2 Hz, *J* = 5.8 Hz, *J* = 14.2 Hz, 1H, H-2'), 2.30 (m, 1H, H-2'), 2.22 (s, 3H, OAc), 2.14 (s, 3H, OAc) ppm. <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ = 185.7 (C<sub>7</sub>), 170.6 (C<sub>CO</sub>), 170.3 (C<sub>CO</sub>), 161.7 (C<sub>2</sub>), 149.2 (C<sub>4</sub>) 144.3 (C<sub>6</sub>), 111.7 (C<sub>5</sub>), 86.1 (C<sub>1</sub>), 83.1 (C<sub>4'</sub>), 73.9 (C<sub>3</sub>), 63.6 (C<sub>5</sub>), 38.7 (C<sub>2'</sub>), 20.8 (C<sub>OAc</sub>), 20.6 (C<sub>OAc</sub>) ppm. HRMS (ESI): *m/z* calcd. for C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>NaO<sub>8</sub> [M+Na<sup>+</sup>]: 363.0804; found: 363.0792.

**3',5'-Di-O-(tert-butylidimethylsilyl)-5-formyl-2'-deoxyuridine (10):** According to Table 1 entry 5, 3',5'-di-O-tert-butylidimethylsilylthymidine **8** (109 mg, 0.23 mmol), CuSO<sub>4</sub>·5H<sub>2</sub>O (46 mg, 0.2 mmol, 0.8 eq), K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (252 mg, 0.92 mmol, 4.0 eq) and 2,6-lutidine (0.19 mL, 1.61 mmol, 7.2 eq) were successively added to a CH<sub>3</sub>CN/H<sub>2</sub>O (1/1, v/v) solution (5 mL) under an argon atmosphere. The resulting mixture was stirred for 1h15 at 65°C. The reaction mixture was filtered through celite and washed with brine (50 mL). This solution was extracted three times with EtOAc (3 x 30 mL), and the combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated. The crude product was finally purified by flash silica gel column chromatography (cyclohexane/EtOAc, 4:1) to afford the product **10** (73 mg, 65% yield) and the 5caU product (13 mg, 11%). R<sub>f</sub> = 0.7 (cyclohexane/EtOAc, 2:1). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ = 10.01 (s, 1H, H-7), 8.86 (s, 1H, NH), 8.57 (s, 1H, H-6), 6.25 (dd, *J* = 7.8 Hz, *J* = 5.7 Hz, 1H, H-1'), 4.43 (m, 1H, H-3'), 4.09 (ddd, *J* = 4.7 Hz, *J* = 2.7 Hz, *J* = 2.5 Hz, 1H, H-4'), 3.91 (dd, *J* = 11.4 Hz, *J* = 2.7 Hz, 1H, H-5'), 3.80 (dd, *J* = 11.4 Hz, *J* = 2.5 Hz, 1H, H-5'), 2.46 (ddd, *J* = 13.2 Hz, *J* = 5.7 Hz, *J* = 2.0 Hz, 1H, H-2'), 2.07 (ddd, *J* = 13.2 Hz, *J* = 7.8 Hz, *J* = 5.6 Hz, 1H, H-2'), 0.92 (s, 9H, *t*BuSi), 0.91 (s, 9H, *t*Bu-Si), 0.13 (2s, 6H, Me<sub>2</sub>Si), 0.11 (s, 3H, MeSi), 0.10 (s, 3H, MeSi)

ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 185.7 ( $\text{C}_7$ ), 161.8 ( $\text{C}_4$ ), 149.1 ( $\text{C}_2$ ), 145.6 ( $\text{C}_6$ ), 111.0 ( $\text{C}_5$ ), 89.2 ( $\text{C}_4$ ), 87.2 ( $\text{C}_1$ ), 72.9 ( $\text{C}_3$ ), 63.1 ( $\text{C}_5$ ), 42.5 ( $\text{C}_2$ ), 25.9 ( $\text{C}_{\text{Bu}}(\text{TBDMS})$ ), 25.7 ( $\text{C}_{\text{Bu}}(\text{TBDMS})$ ), 18.0 ( $\text{C}_{\text{q}}(\text{TBDMS})$ ), 18.6 ( $\text{C}_{\text{q}}(\text{TBDMS})$ ), -5.6 ( $\text{C}_{\text{MeSi}}$ ), -5.5 ( $\text{C}_{\text{MeSi}}$ ), -4.8 ( $\text{C}_{\text{MeSi}}$ ), -4.6 ( $\text{C}_{\text{MeSi}}$ ) ppm. HRMS (ESI):  $m/z$  calcd. for  $\text{C}_{14}\text{H}_{18}\text{N}_2\text{NaO}_6\text{Si}_2$  [ $\text{M}+\text{Na}^+$ ]: 507.2323; found: 507.2501.

**3',5'-Di-O-acetyl-5-hydroxymethyl-2'-deoxyuridine (11):** According to Table 2 entry 2, 3',5'-di-O-acetyl-5-formyl-2'-deoxyuridine **9** (41 mg, 0.12 mmol) and  $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$  (134 mg, 0.36 mmol, 3.0 eq) were dissolved in methanol (1 mL). The reaction mixture was cooled to  $0^\circ\text{C}$  and  $\text{NaBH}_4$  (5.5 mg, 0.15 mmol, 1.2 eq) was added portionwise. The mixture was stirred at room temperature for 45 min and the reaction was monitored by TLC (EtOAc). The reaction mixture was concentrated *in vacuo* and EtOAc (10 mL) was then added. The organic phase was washed with a saturated aqueous  $\text{NaHCO}_3$  solution (10 mL) and the aqueous phase extracted with EtOAc (3 x 10 mL). The combined organic phases were dried over  $\text{Na}_2\text{SO}_4$ . The crude product was purified by flash silica gel column chromatography (EtOAc). Product **11** was obtained as a yellowish solid (38 mg, 92% yield).

According to Table 2 entry 3, 3',5'-di-O-acetyl-5-formyl-2'-deoxyuridine **9** (50 mg, 0.15 mmol) and  $\text{BH}_3 \cdot \text{NH}_3$  (5 mg, 0.15 mmol, 1.0 eq) were dissolved in water (0.6 mL). The mixture was stirred at room temperature for 10 min and progress of the reaction was monitored by TLC (EtOAc). EtOAc (5 mL) was then added, and the organic phase was washed with an aqueous saturated  $\text{NaHCO}_3$  solution (5 mL). The aqueous phase was extracted with EtOAc (3 x 5 mL), and the combined organic phase was dried over  $\text{Na}_2\text{SO}_4$ . Product **11** was obtained as yellowish solid (46 mg, 92% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 9.31 (bs, 1H, NH), 7.56 (s, 1H, H-6), 6.37 (dd,  $J$  = 5.6 Hz,  $J$  = 8.6 Hz, 1H, H-1'), 5.26 (dt,  $J$  = 1.9 Hz,  $J$  = 6.6 Hz, 1H, H-3'), 4.51 (d,  $J$  = 13.0 Hz, 1H, H-7), 4.42 (m, 2H, H-5', H-7), 4.35 (dd,  $J$  = 3.1 Hz,  $J$  = 12.0 Hz, 1H, H-5'), 4.30 (m, 1H, H-4'), 2.66 (bs, 1H, OH), 2.54 (dd,  $J$  = 5.6 Hz,  $J$  = 14.4 Hz, 1H, H-2'), 2.22 (m, 1H, H-2'), 2.17 (s, 3H, OAc), 2.15 (s, 3H, OAc) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 170.5 ( $\text{C}_{\text{CO}}$ ), 170.4 ( $\text{C}_{\text{CO}}$ ), 162.8 ( $\text{C}_2$ ), 149.8 ( $\text{C}_4$ ), 135.8 ( $\text{C}_6$ ), 114.5 ( $\text{C}_5$ ), 85.2 ( $\text{C}_1$ ), 82.5 ( $\text{C}_4$ ), 74.2 ( $\text{C}_3$ ), 63.8 ( $\text{C}_5$ ), 58.5 ( $\text{C}_7$ ), 37.7 ( $\text{C}_2$ ), 20.9 ( $\text{C}_{\text{OAc}}$ ), 20.8 ( $\text{C}_{\text{OAc}}$ ) ppm. HRMS (ESI):  $m/z$  calcd. for  $\text{C}_{14}\text{H}_{18}\text{N}_2\text{NaO}_8$  [ $\text{M}+\text{Na}^+$ ]: 365.0961; found: 365.0952.

**3',5'-Di-O-(tert-butylidimethylsilyl)-5-hydroxymethyl-2'-deoxyuridine (12):** According to Table 2 entry 4, at  $0^\circ\text{C}$  and under an argon atmosphere,  $\text{NaBH}_4$  (7 mg, 0.18 mmol, 1.2 eq) was added portionwise to a solution of 3',5'-di-O-(tert-butylidimethylsilyl)-5-formyl-2'-deoxyuridine **10** (64 mg, 0.13 mmol) and  $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$  (157 mg, 0.42 mmol, 3.0 eq) in methanol (1.5 mL). The resulting mixture was then stirred for 30 min at room temperature. The reaction mixture was concentrated *in vacuo*, and the product was purified by flash silica gel column chromatography (cyclohexane/EtOAc, from 4:1 to 2:1) to afford **12** (58 mg, 90% yield).

According to Table 2 entry 7, 3',5'-di-O-(tert-butylidimethylsilyl)-5-formyl-2'-deoxyuridine **10** (100 mg, 0.2 mmol) and  $\text{BH}_3 \cdot \text{NH}_3$  (6 mg, 0.2 mmol, 1.0 eq) were dissolved in methanol (2 mL). The mixture was stirred at room temperature for 1h and the progress of the reaction monitored by TLC (cyclohexane/EtOAc, 2:1). EtOAc (10 mL) was added, and the organic phase was washed with a saturated aqueous  $\text{NaHCO}_3$  solution (10 mL). The aqueous phase was extracted with EtOAc (3 x 10 mL) and the combined organic phases were dried over  $\text{Na}_2\text{SO}_4$ . The crude product was purified by flash silica gel column chromatography (cyclohexane/EtOAc, 2:1) and product **12** was obtained as yellowish solid (88 mg, 90% yield).  $R_f$  = 0.13 (cyclohexane/EtOAc, 2:1).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 8.68 (bs, 1H, NH), 7.70 (t,  $J$  = 1.7 Hz, 1H, H-6), 6.29 (dd,  $J$  = 5.7 Hz,  $J$  = 8.5 Hz, 1H, H-1'), 4.40 (m, 3H, H-3', H-7), 3.97 (m, 1H, H-4'), 3.87 (dd,  $J$  = 2.7 Hz,  $J$  = 11.5 Hz, 1H, H-5'), 3.77 (dd,  $J$  = 2.7 Hz,  $J$  = 11.5 Hz, 1H, H-5'), 2.31 (ddd,  $J$  = 2.1 Hz,  $J$  = 5.7 Hz,  $J$  = 13.2 Hz, 1H, H-2'), 2.03 (ddd,  $J$  = 6.9 Hz,  $J$  = 8.5 Hz,  $J$  = 13.2 Hz, 1H, H-2'), 0.94 (s, 9H,  $t\text{BuSi}$ ), 0.92 (s, 9H,  $t\text{BuSi}$ ), 0.13 (2s, 6H,  $\text{Me}_2\text{Si}$ ), 0.11 (s, 3H,  $\text{MeSi}$ ), 0.10 (s, 3H,  $\text{MeSi}$ ) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 163.8 ( $\text{C}_4$ ),

149.9 ( $\text{C}_2$ ), 137.2 ( $\text{C}_6$ ), 113.7 ( $\text{C}_5$ ), 88.0 ( $\text{C}_1$ ), 85.2 ( $\text{C}_4$ ), 72.2 ( $\text{C}_3$ ), 62.9 ( $\text{C}_5$ ), 58.9 ( $\text{C}_7$ ), 41.5 ( $\text{C}_2$ ), 25.9, 25.7 ( $\text{C}_{\text{Bu}}(\text{TBDMS})$ ), 18.4, 17.9 ( $\text{C}_{\text{q}}(\text{TBDMS})$ ), -4.6 ( $\text{C}_{\text{MeSi}}$ ), -4.8 ( $\text{C}_{\text{MeSi}}$ ), -5.4 ( $\text{C}_{\text{MeSi}}$ ), -5.4 ( $\text{C}_{\text{MeSi}}$ ) ppm. HRMS (ESI):  $m/z$  calcd. for  $\text{C}_{22}\text{H}_{42}\text{N}_2\text{NaO}_6\text{Si}_2$  [ $\text{M}+\text{Na}^+$ ]: 509.2479; found: 509.2459.

**3',5'-Di-O-(tert-butylidimethylsilyl)-5-acetoxymethyl-2'-deoxyuridine (13):** Compound **12** (660 mg, 1.37 mmol, 1.0 eq) was dissolved in 10 mL pyridine and acetic anhydride (0.15 mL, 1.64 mmol, 1.2 eq) was added. The reaction mixture was stirred overnight at room temperature. The reaction was monitored by TLC (cyclohexane/EtOAc, 2:1). The mixture was concentrated *in vacuo* and co-evaporated with toluene to obtain **13** (718 mg, quantitative yield) as a white foam.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 8.40 (s, 1H, NH), 7.83 (s, 1H, H-6), 6.29 (dd,  $J$  = 7.6 Hz,  $J$  = 5.8 Hz, 1H, H-1'), 4.85 (d,  $J$  = 12.0 Hz, 1H, H-7), 4.80 (d,  $J$  = 12.0 Hz, 1H, H-7), 4.43 (td,  $J$  = 3.1 Hz,  $J$  = 5.8 Hz, 1H, H-3'), 3.98 (q,  $J$  = 3.1 Hz, 1H, H-4'), 3.85 (dd,  $J$  = 11.4 Hz,  $J$  = 3.1 Hz, 1H, H-5'), 3.80 (dd,  $J$  = 11.4 Hz,  $J$  = 3.1 Hz, 1H, H-5'), 2.33 (ddd,  $J$  = 13.3 Hz,  $J$  = 5.8 Hz,  $J$  = 3.1 Hz, 1H, H-2'), 2.07 (m, 4H, OAc, H-2'), 0.92 (s, 9H,  $t\text{BuSi}$ ), 0.91 (s, 9H,  $t\text{BuSi}$ ), 0.13 (2s, 6H,  $\text{Me}_2\text{Si}$ ), 0.11 (s, 3H,  $\text{MeSi}$ ), 0.10 (s, 3H,  $\text{MeSi}$ ) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 170.8 ( $\text{C}_{\text{CO}}$ ), 162.6 ( $\text{C}_4$ ), 149.9 ( $\text{C}_2$ ), 141.0 ( $\text{C}_6$ ), 109.1 ( $\text{C}_5$ ), 88.1 ( $\text{C}_4$ ), 85.5 ( $\text{C}_1$ ), 72.3 ( $\text{C}_3$ ), 63.0 ( $\text{C}_5$ ), 59.1 ( $\text{C}_6$ ), 41.5 ( $\text{C}_2$ ), 25.9 ( $\text{C}_{\text{Bu}}(\text{TBDMS})$ ), 25.7 ( $\text{C}_{\text{Bu}}(\text{TBDMS})$ ), 18.0 ( $\text{C}_{\text{q}}(\text{TBDMS})$ ), 18.6 ( $\text{C}_{\text{q}}(\text{TBDMS})$ ), -4.6 ( $\text{C}_{\text{MeSi}}$ ), -4.8 ( $\text{C}_{\text{MeSi}}$ ), -5.5 ( $\text{C}_{\text{MeSi}}$ ), -5.6 ( $\text{C}_{\text{MeSi}}$ ) ppm.

**5-(2,3,4,6-Tetra-O-benzoyl- $\beta$ -D-glucopyranosyloxymethyl)-3',5'-di-O-(tert-butylidimethylsilyl)-2'-deoxyuridine (14):** A mixture of the acceptor **12** (400 mg, 0.89 mmol) and 2,3,4,6-tetra-O-benzoyl- $\alpha$ -D-glucopyranosyl trichloroacetimidate (0.720 g, 0.97 mmol, 1.1 eq) as the donor, was dried by two successive co-evaporations with dichloroethane (2 x 8 mL). The mixture was dissolved in dichloroethane (8 mL) and stirred for 25 min with 4 Å powered molecular sieves (410 mg). TMSOTf (24  $\mu\text{L}$ , 0.13 mmol, 0.15 eq) was then added and the resulting solution was stirred at room temperature for 50 min under an argon atmosphere. The reaction was then quenched by addition of triethylamine (0.1 mL) and filtered through celite. The crude solid was purified by flash silica gel column chromatography (cyclohexane/EtOAc, 4:1), affording product **14** (590 mg, 63% yield).  $R_f$  = 0.5 (cyclohexane/EtOAc, 7:3).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 8.09-7.81 (m, 8H, Ar), 7.64 (s, 1H, H-6), 7.60-7.29 (m, 12H, Ar), 6.11 (dd,  $J$  = 7.5 Hz,  $J$  = 6.0 Hz, 1H, H-1'), 5.92 (t,  $J$  = 9.7 Hz, 1H, H-3'), 5.70 (t,  $J$  = 9.7 Hz, H-4'), 5.50 (dd,  $J$  = 9.7 Hz,  $J$  = 8.0 Hz, 1H, H-2'), 5.12 (d,  $J$  = 8.0 Hz, 1H, H-1'), 4.67 (dd,  $J$  = 12.2 Hz, 3.3 Hz, 1H, H-6'), 4.52 (d,  $J$  = 1.2 Hz, 2H, H-7), 4.46 (dd,  $J$  = 12.2 Hz, 4.2 Hz, 1H, H-6'), 4.36 (m, 1H, H-3'), 4.18 (m, 1H, H-5'), 3.93 (m, 1H, H-4'), 3.74 (d,  $J$  = 3.3 Hz, 2H, H-5'), 2.27 (ddd,  $J$  = 13.3 Hz,  $J$  = 5.7 Hz,  $J$  = 2.1 Hz, 1H, H-2'), 1.95 (ddd,  $J$  = 13.3 Hz,  $J$  = 7.5 Hz,  $J$  = 6.0 Hz, 1H, H-2'), 0.91-0.87 (2s, 18H,  $t\text{BuSi}$ ), 0.10, 0.09, 0.09, 0.07 (4s, 12H,  $\text{Me}_2\text{Si}$ ) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 166.1, 165.7, 165.2, 165.1 ( $\text{C}_{\text{CO}}$ ), 162.3 ( $\text{C}_4$ ), 149.5 ( $\text{C}_2$ ), 138.8 ( $\text{C}_6$ ), 133.5 ( $\text{C}_{\text{q}}(\text{Ar})$ ), 133.4, 133.1, 133.1, 129.9, 129.8 ( $\text{C}_{\text{CH, Ar}}$ ), 129.3, 128.9, 128.8 ( $\text{C}_{\text{q}}(\text{Ar})$ ), 128.4, 128.4, 128.3, 128.3 ( $\text{C}_{\text{CH, Ar}}$ ), 110.9 ( $\text{C}_5$ ), 101.6 ( $\text{C}_1$ ), 88.5 ( $\text{C}_4$ ), 85.9 ( $\text{C}_1$ ), 72.7 ( $\text{C}_3$ ), 72.4 ( $\text{C}_3$ ), 72.2 ( $\text{C}_5$ ), 71.5 ( $\text{C}_2$ ), 69.5 ( $\text{C}_4$ ), 64.3 ( $\text{C}_7$ ), 62.8 ( $\text{C}_5$ ), 62.7 ( $\text{C}_6$ ), 41.3 ( $\text{C}_2$ ), 26.0 ( $\text{C}_{\text{Bu}}(\text{TBDMS})$ ), 25.9 ( $\text{C}_{\text{Bu}}(\text{TBDMS})$ ), 18.4 ( $\text{C}_{\text{q}}(\text{TBDMS})$ ), 18.1 ( $\text{C}_{\text{q}}(\text{TBDMS})$ ), -4.4, -4.6, -5.2 ( $\text{C}_{\text{MeSi}}$ ), -5.3 ( $\text{C}_{\text{MeSi}}$ ) ppm. HRMS (ESI):  $m/z$  calcd. for  $\text{C}_{56}\text{H}_{68}\text{N}_2\text{NaO}_{15}\text{Si}_2$  [ $\text{M}+\text{Na}^+$ ]: 1087.4056; found: 1087.4060.

**3',5'-Di-O-(tert-butylidimethylsilyl)-5-( $\beta$ -D-glucopyranosyloxymethyl)-2'-deoxyuridine (15):** Compound **14** (547 mg, 0.51 mmol) was dissolved in MeOH (3 mL) and  $\text{K}_2\text{CO}_3$  (71 mg, 0.51 mmol, 1.1 eq) was added. The reaction mixture was stirred overnight at room temperature. The reaction was quenched by addition of acidic Dowex 50 W resin. The resulting mixture was filtered and the solvent removed under reduced pressure to obtain **15** (328 mg, quantitative yield).  $R_f$  = 0.25 (cyclohexane/EtOAc/MeOH, 5:3:2).  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  = 7.64 (s, 1H, H-6), 6.36 (dd,  $J$  = 5.5 Hz,  $J$  = 8.2 Hz, 1H, H-1'), 4.58 (d,  $J$  = 11.8 Hz, 1H, H-7), 4.47 (m, 1H, H-3'), 4.40 (d,  $J$  = 11.8 Hz, 1H, H-7), 4.37 (d,  $J$  = 7.8 Hz, 1H, H-1'), 3.90 (m, 1H, H-4'), 3.83 (m, 1H, H-5'), 3.75 (m, 2H, H-6'), 3.71 (dd,  $J$  = 4.9 Hz,  $J$  = 11.9 Hz, 1H, H-5'), 3.35 (m, 3H, H-3', H-4', H-5'), 2.25 (m, 1H, H-2'), 2.12 (m, 1H, H-2'), 0.95 (2s, 18H,  $t\text{BuSi}$ ), 0.14

(s, 12H, Me<sub>2</sub>Si) ppm. <sup>13</sup>C NMR (75 MHz, CD<sub>3</sub>OD): δ = 163.9 (C<sub>4</sub>), 150.6 (C<sub>2</sub>), 139.7 (C<sub>6</sub>), 110.8 (C<sub>5</sub>), 102.7 (C<sub>1'</sub>), 87.9 (C<sub>4</sub>), 85.7 (C<sub>1'</sub>), 76.6 (C<sub>2</sub>), 73.6 (C<sub>3'</sub>), 72.7 (C<sub>4'</sub>), 70.1 (C<sub>3</sub>), 63.5 (C<sub>5</sub>), 63.0 (C<sub>5</sub>), 62.2 (C<sub>6</sub>), 61.3 (C<sub>7</sub>), 39.9 (C<sub>2</sub>), 25.1 (C<sub>BU</sub>(TBDMS)), 24.8 (C<sub>BU</sub>(TBDMS)), 17.8 (C<sub>q</sub>(TBDMS)), 17.4 (C<sub>q</sub>(TBDMS)), -5.8 (C<sub>MeSi</sub>), -5.9 (C<sub>MeSi</sub>), -6.4 (C<sub>MeSi</sub>), -6.5 (C<sub>MeSi</sub>) ppm. HRMS (ESI): *m/z* calcd. for C<sub>28</sub>H<sub>52</sub>N<sub>2</sub>NaO<sub>11</sub>Si<sub>2</sub> [M+Na<sup>+</sup>]: 671.3007; found: 671.3001.

**5-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxymethyl)-3',5'-di-O-(tert-butylidimethylsilyl)-2'-deoxyuridine (16):** Compound **15** (330 mg, 0.5 mmol) was dissolved in pyridine (5 mL) and acetic anhydride (380 μL, 4.0 mmol, 8.0 eq) was added to the resulting solution. The mixture was stirred overnight at room temperature and the progress of the reaction was monitored by TLC (EtOAc). The mixture was concentrated *in vacuo* and co-evaporated with toluene (2 x 20 mL) to furnish compound **16** as a pale yellow solid (410 mg, 99% yield). R<sub>f</sub> = 0.13 (cyclohexane/EtOAc, 2:1). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ = 8.42 (bs, 1H, NH), 7.70 (s, 1H, H-6), 6.26 (dd, *J* = 8.0 Hz, *J* = 5.7 Hz, 1H, H-1'), 5.23 (t, 1H, *J* = 9.5 Hz, H-3''), 5.09 (t, *J* = 9.5 Hz, 1H, H-4''), 4.98 (dd, *J* = 9.5 Hz, *J* = 8.0 Hz, 1H, H-2''), 4.70 (d, *J* = 8.0 Hz, 1H, H-1''), 4.50 (d, *J* = 12 Hz, 1H, H-7), 4.44 (d, *J* = 12 Hz, 1H, H-7), 4.40 (m, 1H, H-3'), 4.27 (dd, *J* = 12.3 Hz, *J* = 4.4 Hz, 1H, H-5'), 4.15 (dd, *J* = 12.3 Hz, *J* = 1.9 Hz, 1H, H-5'), 3.99 (dd, *J* = 4.4 Hz, *J* = 1.9 Hz, 1H, H-4'), 3.84 (dd, *J* = 3.5 Hz, *J* = 11.4 Hz, 1H, H-6''), 3.78 (dd, *J* = 3.2 Hz, *J* = 11.4 Hz, 1H, H-6''), 3.72 (ddd, *J* = 9.5 Hz, *J* = 3.5 Hz, *J* = 3.2 Hz, 1H, H-5''), 2.32 (ddd, *J* = 13.2 Hz, *J* = 5.7 Hz, *J* = 2.3 Hz, 1H, H-2'), 2.09 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.01 (m, 1H, H-2'), 0.90 (1s, 18H, tBu-Si), 0.12 (1s, 3H, Me-Si), 0.10 (1s, 3H, Me-Si), 0.09 (1s, 6H, Me<sub>2</sub>-Si) ppm. <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ = 170.4 (C<sub>CO</sub>), 170.1 (C<sub>CO</sub>), 169.5 (C<sub>CO</sub>), 169.4 (C<sub>CO</sub>), 162.8 (C<sub>4</sub>), 149.9 (C<sub>2</sub>), 139.3 (C<sub>6</sub>), 110.6 (C<sub>5</sub>), 101.1 (C<sub>1'</sub>), 88.3 (C<sub>4</sub>), 85.9 (C<sub>1'</sub>), 72.8 (C<sub>3'</sub>), 72.7 (C<sub>3</sub>), 72.0 (C<sub>5'</sub>), 71.1 (C<sub>2'</sub>), 68.3 (C<sub>4'</sub>), 64.5 (C<sub>7</sub>), 63.3 (C<sub>6'</sub>), 61.8 (C<sub>5</sub>), 41.5 (C<sub>2</sub>), 25.9 (C<sub>BU</sub>(TBDMS)), 25.7 (C<sub>BU</sub>(TBDMS)), 20.7 (C<sub>OAc</sub>), 20.6 (C<sub>OAc</sub>), 20.6 (C<sub>OAc</sub>), 20.6 (C<sub>OAc</sub>), 18.4 (C<sub>q</sub>(TBDMS)), 18.0 (C<sub>q</sub>(TBDMS)), -4.4 (C<sub>MeSi</sub>), -4.6 (C<sub>MeSi</sub>), -5.2 (C<sub>MeSi</sub>), -5.3 (C<sub>MeSi</sub>) ppm. HRMS (ESI): *m/z* calcd. for C<sub>36</sub>H<sub>60</sub>N<sub>2</sub>NaO<sub>15</sub>Si<sub>2</sub> [M+Na<sup>+</sup>]: 839.3430; found: 839.3397.

**5-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxymethyl)-2'-deoxyuridine (17):** Compound **16** (25 mg, 0.03 mmol) was dissolved in pyridine (0.5 mL) under an argon atmosphere and Et<sub>3</sub>N·HF complex (11 μL, 0.06 mmol, 2.0 eq) was then added. The reaction mixture was stirred overnight at room temperature. The reaction was then quenched by addition of EtOAc (2 mL) and brine (2 mL). The crude product was extracted three times with EtOAc (3 x 5 mL) and the combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub>. Solvent was removed under reduced pressure and the crude product was purified by flash silica gel chromatography (EtOAc) affording product **17** as white foam (16 mg, 89% yield). R<sub>f</sub> = 0.33 (EtOAc). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ = 8.14 (bs, 1H, NH), 7.88 (bs, 1H, H-6), 6.38 (t, *J* = 6.6 Hz, 1H, H-1'), 5.27 (t, 1H, *J* = 9.7 Hz, H-3''), 5.11 (t, *J* = 9.7 Hz, 1H, H-4''), 5.07 (dd, *J* = 9.7 Hz, *J* = 6.6 Hz, 1H, H-2''), 4.73 (dd, *J* = 1.5 Hz, *J* = 13.3 Hz, 1H, H-7), 4.65 (m, 1H, H-3'), 4.62 (d, *J* = 7.7 Hz, 1H, H-1''), 4.39 (dd, *J* = 13.3 Hz, *J* = 1.5 Hz, 1H, H-7), 4.32 (dd, *J* = 4.9 Hz, *J* = 12.6 Hz, 1H, H-6''), 4.14 (m, 1H, H-6''), 4.01 (m, 1H, H-4'), 4.00 (m, 1H, H-5'), 3.87 (m, 1H, H-5'), 3.75 (ddd, *J* = 2.3 Hz, *J* = 4.9 Hz, *J* = 9.7 Hz, 1H, H-5''), 2.32 (m, 2H, H-2'), 2.11 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.03 (s, 3H, OAc) ppm. <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ = 170.5 (C<sub>CO</sub>), 170.4 (C<sub>CO</sub>), 169.8 (C<sub>CO</sub>), 169.4 (C<sub>CO</sub>), 164.9 (C<sub>2</sub>), 151.3 (C<sub>4</sub>), 136.6 (C<sub>6</sub>), 109.4 (C<sub>5</sub>), 100.4 (C<sub>1'</sub>), 84.9 (C<sub>1'</sub>), 86.9 (C<sub>4</sub>), 72.1 (C<sub>5'</sub>), 72.4 (C<sub>3'</sub>), 72.3 (C<sub>3</sub>), 71.4 (C<sub>2'</sub>), 68.3 (C<sub>4'</sub>), 64.5 (C<sub>7</sub>), 62.2 (C<sub>5</sub>), 61.8 (C<sub>6'</sub>), 41.0 (C<sub>2</sub>), 20.9 (C<sub>OAc</sub>), 20.7 (C<sub>OAc</sub>), 20.5 (C<sub>OAc</sub>), 20.4 (C<sub>OAc</sub>). HRMS (ESI): *m/z* calcd. for C<sub>24</sub>H<sub>32</sub>N<sub>2</sub>NaO<sub>15</sub> [M+Na<sup>+</sup>]: 611.1700; found: 611.1689.

**3',5'-Di-O-acetyl-5-(2-cyanoethoxy)methyl-2'-deoxyuridine (18):** 3',5'-Di-O-acetyl-5-hydroxymethyl-2'-deoxyuridine **11** (100 mg, 0.29 mmol) was dissolved in 1.5 mL of acetonitrile. Acidic Amberlyst 15 (292 mg) and 3-hydroxypropionitrile (0.10 mL, 1.45 mmol, 5 eq) were added. The reaction mixture was stirred at room temperature for 48h, while progress

of the reaction was monitored by TLC (EtOAc/cyclohexane, 7:3). Solvent was removed under reduced pressure and the resulting crude product was purified by flash silica gel chromatography (EtOAc/cyclohexane, 7:3) affording product **18** (103 mg, 89% yield). R<sub>f</sub> = 0.79 (cyclohexane/EtOAc/MeOH, 5:3:2). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ = 8.90 (bs, 1H, NH), 7.64 (t, *J* = 0.9 Hz, 1H, H-6), 6.31 (dd, *J* = 5.6 Hz, *J* = 8.4 Hz, 1H, H-1'), 5.25 (dt, *J* = 1.9 Hz, *J* = 6.5 Hz, 1H, H-3'), 4.36 (m, 5H, H-5', H-7, H-4'), 3.79 (t, *J* = 6.2 Hz, 2H, CH<sub>2</sub>), 2.66 (t, *J* = 6.2 Hz, 2H, CH<sub>2</sub>), 2.53 (ddd, *J* = 1.9 Hz, *J* = 5.6 Hz, *J* = 14.2 Hz, 1H, H-2'), 2.24 (m, 1H, H-2'), 2.14 (s, 3H, OAc), 2.13 (s, 3H, OAc) ppm. <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ = 170.3 (C<sub>CO</sub>), 170.3 (C<sub>CO</sub>), 162.1 (C<sub>2</sub>), 149.8 (C<sub>4</sub>), 137.4 (C<sub>6</sub>), 117.7 (C<sub>CN</sub>), 111.5 (C<sub>5</sub>), 85.6 (C<sub>1'</sub>), 82.6 (C<sub>4'</sub>), 74.3 (C<sub>3</sub>), 65.5 (C<sub>8</sub>), 65.2 (C<sub>5</sub>), 63.8 (C<sub>7</sub>), 37.7 (C<sub>2</sub>), 20.9 (C<sub>OAc</sub>), 20.8 (C<sub>OAc</sub>), 18.8 (C<sub>9</sub>) ppm.

**3',5'-Di-O-acetyl-5-azidomethyl-2'-deoxyuridine (19):** 3',5'-Diacetyl-5-hydroxymethyl-2'-deoxyuridine **11** (480 mg, 1.4 mmol) was dissolved in freshly distilled dioxane (15 mL). Chlorotrimethylsilane (771 mg, 7.1 mmol, 5.0 eq) was then added under argon atmosphere. The reaction mixture was stirred overnight at 65°C and the solvent was removed under reduced pressure. The resulting crude product was dissolved in DMF (5 mL) and NaN<sub>3</sub> (277 mg, 4.3 mmol, 3.0 eq) was added to the solution. The reaction was stirred 1 h at 65°C and then filtered. DMF was removed under reduced pressure and the crude product was purified by flash silica gel column chromatography (EtOAc/cyclohexane, 7:3) to obtain **19** as a yellow oil (0.432 g, 84% yield). R<sub>f</sub> = 0.48 (EtOAc/cyclohexane, 7:3). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ = 8.40 (s, 1H, NH), 7.58 (s, 1H, H-6), 6.33 (dd, *J* = 5.5 Hz, *J* = 8.5 Hz, 1H, H-1'), 5.24 (m, 1H, H-3'), 4.39 (dd, *J* = 4.2 Hz, *J* = 11.9 Hz, 1H, H-5'), 4.34 (dd, *J* = 4.2 Hz, *J* = 11.9 Hz, 1H, H-5'), 4.27 (m, 1H, H-4'), 4.19 (m, 2H, H-7), 2.56 (ddd, *J* = 2.0 Hz, *J* = 5.5 Hz, *J* = 14.0 Hz, 1H, H-2'), 2.14 (m, 1H, H-2'), 2.16 (s, 3H, OAc), 2.14 (s, 3H, OAc) ppm. <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ = 170.3 (C<sub>CO</sub>), 170.3 (C<sub>CO</sub>), 164.9 (C<sub>2</sub>), 151.3 (C<sub>4</sub>), 137.3 (C<sub>6</sub>), 110.3 (C<sub>5</sub>), 85.4 (C<sub>4'</sub>), 82.6 (C<sub>1'</sub>), 74.1 (C<sub>3</sub>), 63.7 (C<sub>5</sub>), 47.1 (C<sub>7</sub>), 37.9 (C<sub>2</sub>), 20.9 (C<sub>OAc</sub>), 20.6 (C<sub>OAc</sub>) ppm. HRMS (ESI): *m/z* calcd. for C<sub>14</sub>H<sub>17</sub>N<sub>5</sub>NaO<sub>7</sub> [M+Na<sup>+</sup>]: 390.1026; found: 390.1015.

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