

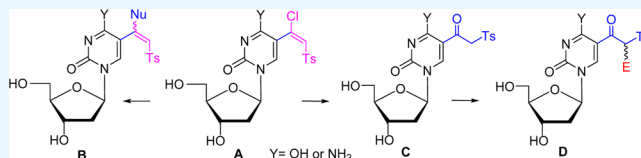
# Pyrimidine Nucleosides with a Reactive ( $\beta$ -Chlorovinyl)sulfone or ( $\beta$ -Keto)sulfone Group at the C5 Position, Their Reactions with Nucleophiles and Electrophiles, and Their Polymerase-Catalyzed Incorporation into DNA

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## Supporting Information

**ABSTRACT:** Transition-metal-catalyzed chlorosulfonylation of 5-ethynylpyrimidine nucleosides provided (*E*)-5-( $\beta$ -chlorovinyl)sulfones **A**, which undergo nucleophilic substitution with amines or thiols affording **B**. The treatment of vinyl sulfones **A** with ammonia followed by acid-catalyzed hydrolysis of the intermediary  $\beta$ -sulfonylvinylamines gave 5-( $\beta$ -keto)sulfones **C**. The latter reacts with electrophiles, yielding  $\alpha$ -carbon-alkylated or -sulfonylated analogues **D**. The 5'-triphosphates of **A** and **C** were incorporated into double-stranded DNA, using open and one-nucleotide gap substrates, by human or *Escherichia coli* DNA-polymerase-catalyzed reactions.



## INTRODUCTION

The 5-modified pyrimidine nucleosides have been extensively studied, and many analogues exhibit a wide range of biological activity.<sup>1,2</sup> For example, (*E*)-5-(2-bromovinyl)-2'-deoxyuridine<sup>3</sup> and bicyclic furanopyrimidine-2-one analogues display antiviral activity against varicella zoster virus (VZV)<sup>4</sup> and 5-(trifluoromethyl)-2'-deoxyuridine is used for the treatment of colorectal cancer.<sup>5</sup> The introduction of reactive groups at the C5 position of pyrimidine nucleobases such as alkyne<sup>6–9</sup> or azide<sup>10–13</sup> for Cu-catalyzed or strain-promoted click chemistry, aldehyde<sup>14</sup> for reductive aminations,<sup>15</sup> ene/diene for cycloaddition reactions,<sup>16–19</sup> vinyl sulfonamide for Michael additions,<sup>20,21</sup> or chloroacetamide for nucleophilic substitutions<sup>22</sup> has been explored<sup>23</sup> for imaging cellular DNA, bioconjugation with DNA-bound proteins, and applications in the fluorescent bioanalysis of DNA and RNA.<sup>24</sup> The 5' triphosphates<sup>8,10,20,25</sup> or 3' phosphoroamidite<sup>26,27</sup> of these probes were incorporated into oligonucleotides by DNA/RNA polymerases or solid-phase synthesis.

The vinyl sulfones are widely used intermediates in organic and medicinal chemistry.<sup>28–30</sup> The ( $\beta$ -halo)vinyl sulfones are gaining attention as a new class of reactive sulfones.<sup>31</sup> Recent synthetic developments include metal-free stereoselective *E*-iodosulfonylation of internal alkynes with sodium phenyl sulfinate and iodine to provide tetrasubstituted olefins,<sup>32</sup> di-*t*-butyl peroxide/I<sub>2</sub>-promoted difunctionalization of alkynes with sodium benzenesulfonates to give (*E*)- $\beta$ -iodovinyl sulfones,<sup>33</sup> and multicomponent reactions with insertion of sulfur dioxide.<sup>34</sup> The application of ( $\alpha$ -halo)vinyl sulfones to organic synthesis as well as nucleoside and medicinal chemistry is also documented.<sup>35–37</sup> The  $\beta$ -keto sulfones are also important

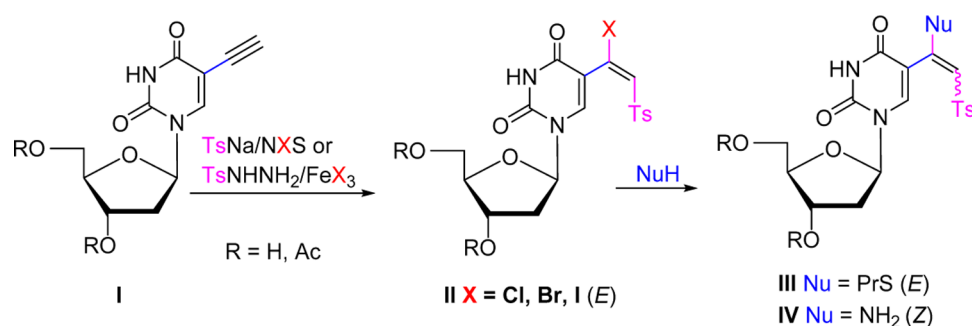
synthetic synthons.<sup>38,39</sup> They have been synthesized from terminal alkynes via pyridine-catalyzed dioxygen-triggered radical reactions<sup>40</sup> or in reactions of aryl/heteroaryl acetylenes with sulfonyl chloride in the presence of catalytic amount of *p*-toluenesulfonic acid.<sup>41</sup> Both ( $\beta$ -halovinyl)sulfones and ( $\beta$ -keto)sulfones exhibit important biological activities. For example, vinyl sulfones act as inhibitors of cysteine proteases<sup>28</sup> and human cathepsin L,<sup>42</sup> whereas ( $\beta$ -keto)sulfones are selective inhibitors of 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1).<sup>43</sup>

We have recently reported a stereoselective synthesis of (*E*)-5-( $\beta$ -halovinyl)sulfone derivatives of uracil nucleosides (e.g., **II**, Figure 1) by transition-metal-catalyzed or radical-mediated halovinylsulfonylation of 5-ethynyluracils **I** with TsNa or TsNHNH<sub>2</sub> in the presence of NXS (X = Br, I) or FeX<sub>3</sub> (X = Cl, Br), respectively, as halogen sources.<sup>44</sup> 5-( $\beta$ -Halovinyl)sulfones **II** underwent efficient stereoselective addition–elimination with nucleophiles (NuH) such as thiols or amines to provide *E*- $\beta$ -thiovinyl or *Z*- $\beta$ -aminovinyl sulfones **III** and **IV**. The 3',5'-di-*O*-acetyl-5-(*E*)-(1-chloro-2-tosylvinyl)-2'-deoxyuridine (**II**; X = Cl, R = Ac; compound **9a** in Table 1) inhibited the growth of L1210 (IC<sub>50</sub> 5.6  $\mu$ M), CEM, and HeLa cancer cells in the lower micrometer range.<sup>45</sup> It also displayed micromolar activity against varicella zoster virus (VZV; EC<sub>50</sub> 4  $\mu$ M).<sup>45</sup> Moreover, the lack of activity of **9a** with the thymidine kinase-deficient VZV strain implies the importance of phosphorylation in the metabolism of these compounds.

Received: March 27, 2018

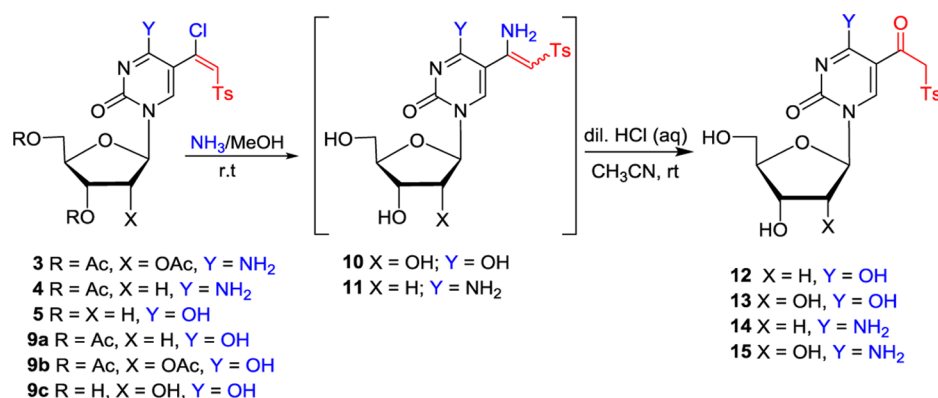
Accepted: April 5, 2018

Published: April 16, 2018



**Figure 1.** Synthesis of uridine 5-( $\beta$ -halovinyl)sulfone analogues and their reactions with nucleophiles.

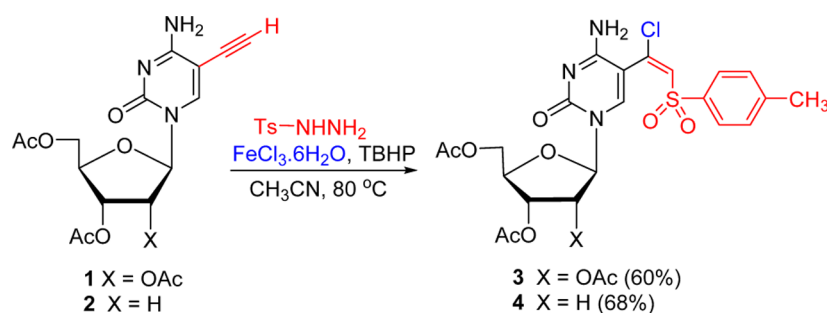
**Table 1.** Conversion of 5-( $\beta$ -Chlorovinyl)sulfones to 5-( $\beta$ -Keto)sulfones<sup>a</sup>



entry	substrate	product	yield (%) <sup>b</sup>
1	5	12	72
2	9a	12	65
3	9b	13	74
4	9c	13	70
5	4	14	59
6	3	15	64

<sup>a</sup>(i) 5-( $\beta$ -Chlorovinyl)sulfones 3–5, 9a–c (0.2 mmol) in NH<sub>3</sub>/MeOH, room temperature (rt), 3–12 h; (ii) 0.1 M HCl in MeCN, rt, 2 h. <sup>b</sup>Isolated yields.

**Scheme 1.** Synthesis of 5-( $\beta$ -Chlorovinyl)sulfone Derivatives of Cytidine and 2'-Deoxycytidine



In this article, we extend the halosulfonylation reaction to cytosine nucleosides and describe the conversion of the uridine and cytosine 5-( $\beta$ -halovinyl)sulfone derivatives to 5-( $\beta$ -keto)-sulfone probes. We report, herein, two classes of 5-modified uracil and cytosine nucleosides, their selective bioconjugation with nucleophiles or electrophiles, and the incorporation of their 5'-phosphates into double-stranded DNA by human or *Escherichia coli* DNA-polymerase-catalyzed reactions. One class contains a ( $\beta$ -chlorovinyl)sulfone probe that efficiently reacts with nucleophiles, including amino acid thiols, via the addition–elimination pathway, whereas the second class bears

a  $\beta$ -keto sulfone probe at the C5 position that reacts with electrophiles and disulfides.

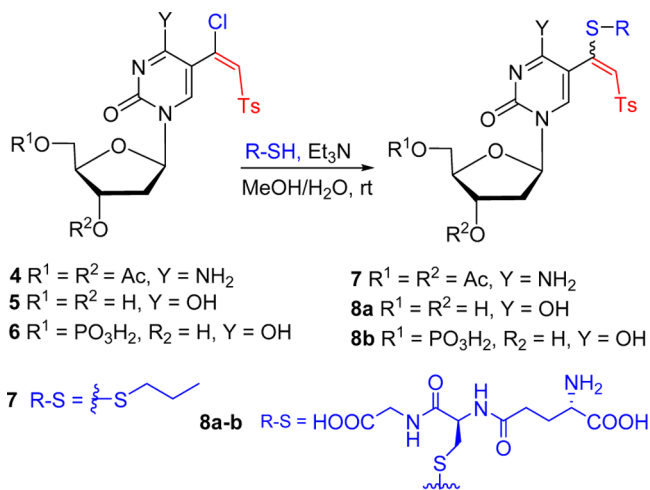
## RESULTS AND DISCUSSION

**Synthesis and Reactivity of ( $\beta$ -Chlorovinyl)sulfone or ( $\beta$ -Keto)sulfone Probes.** The treatment of 2',3',5'-tri-*O*-acetyl-5-ethynylcytosine **1** with tosyl hydrazide (3 equiv) in the presence of FeCl<sub>3</sub> (2 equiv) and *t*-butyl hydroperoxide (TBHP; 4 equiv) gave (*E*)-5-( $\beta$ -chlorovinyl)sulfone **3** (Scheme 1). Analogous chlorosulfonylation of the protected 5-ethynyl-2'-deoxycytidine **2** provided  $\beta$ -chlorovinyl sulfone **4**, which also

demonstrated the stability of the glycosidic bond in the labile 2'-deoxy substrates.

The 5-( $\beta$ -halovinyl)sulfones underwent an efficient addition–elimination reaction with nucleophiles such as amines or thiols,<sup>44</sup> as demonstrated by the reaction of **4** with *n*-PrSH, which provided **7** (80%, Scheme 2). The treatment of 5-(1-

### Scheme 2. Nucleophilic Substitution of 5-( $\beta$ -Chlorovinyl)sulfones with Thiols



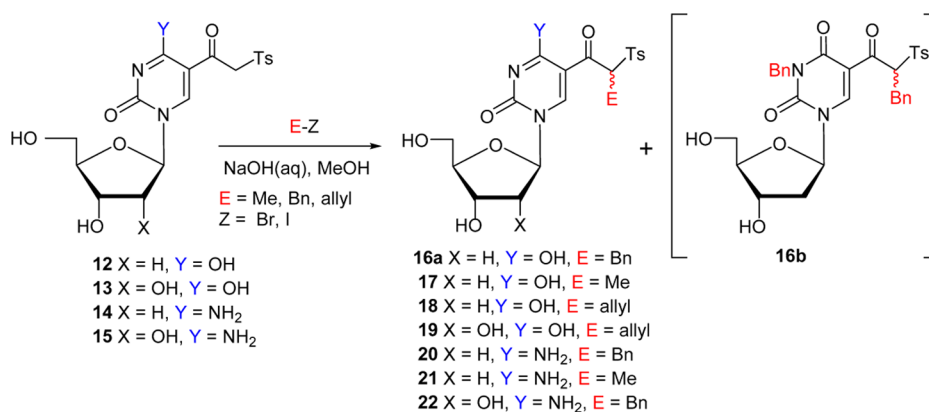
chloro-2-tosylvinyl)-2'-deoxyuridine **5** (56 mM;  $\text{H}_2\text{O}/\text{MeOH}$ , 1:4) with tripeptide L-glutathione (1.5 equiv) in the presence of trimethylamine (TEA, 3 equiv) at room temperature (rt) for 4 h led to nucleophilic substitution of chloride to give **8a** (55%) after high-performance liquid chromatography (HPLC) purification. 5'-*O*-phosphate **6** (47 mM;  $\text{H}_2\text{O}/\text{MeOH}$ , 4:1; see Scheme 4 for the synthesis of **6**) coupled (rt, 4 h) with L-glutathione in aqueous solution in the presence of TEA (3 equiv) affording **8b** (54%) after purification on a Sephadex

column. Phosphate **6** (34 mM) also reacted with glutathione (1.2 equiv) in triethylammonium acetate (TEAA) buffer, under the conditions similar to those for the bioconjugation of glutathione with a chloroacetamide probe attached to the C5 position of dCMP (87 mM),<sup>22</sup> providing **8b** (58%). Because bioconjugation of a chloroacetamide probe attached to short oligodeoxynucleotides with peptides or proteins also occurred efficiently at a lower concentration (0.1  $\mu\text{M}$ ),<sup>22</sup> it can be expected that 5-( $\beta$ -chlorovinyl)sulfone-modified DNA might also serve as a probe for bioconjugation with peptides or proteins.

The treatment of 2',3',5'-tri-*O*-acetyluridine 5-( $\beta$ -chlorovinyl)sulfone **9b** with methanolic ammonia at rt resulted in a concomitant deacetylation and vinylic substitution to give intermediary (*Z*)- $\beta$ -sulfonovinylamine (enamine) **10** as a single isomer.<sup>44,46</sup> We now found that careful acid hydrolysis of **10** with HCl (pH  $\sim$  3–4) in MeCN afforded 5-( $\beta$ -keto)sulfone **13** (74% from **9b**; Table 1, entry 3). Subjection of the unprotected or protected 5-( $\beta$ -chlorovinyl)sulfones of 2'-deoxyuridine (**5** or **9a**) and unprotected uridine (**9c**) to this amination/hydrolysis sequence afforded 5-( $\beta$ -keto)sulfones (**12** or **13**; entries 1–2 and 4). Cytidine and 2'-deoxycytidine sulfones (**3** or **4**) were converted via a similar two-step one-pot protocol into 5-( $\beta$ -keto)sulfones **14** and **15**, illustrating its general character (entries 5 and 6). It is noteworthy that 2'-deoxycytidine substrate **4** provided  $\beta$ -sulfonovinylamine **11** as a mixture of *E/Z* isomers (3:7), which was confirmed by the correlation between H6 (7.84 ppm) and the vinylic proton (4.95 ppm) in the nuclear Overhauser enhancement spectra for the major isomer. The  $\beta$ -keto sulfones attached to the C5 position of pyrimidine nucleobases can be envisioned as mechanistically different probes than 5-( $\beta$ -chlorovinyl)sulfones as they can trap electrophiles rather than nucleophiles because they possess a methylene acidic proton ( $\text{p}K_a = 10\text{--}11$ ).<sup>47</sup>

As anticipated, the treatment of 5-( $\beta$ -keto)sulfone **12** with BnBr in the presence of aqueous NaOH at rt afforded  $\alpha$ -

Table 2.  $\alpha$ -Alkylation of the 5-( $\beta$ -Keto)sulfones of Uracil and Cytosine Nucleosides<sup>a</sup>



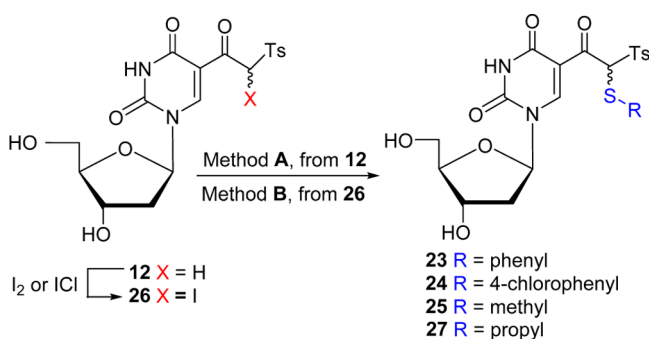
entry	substrate	<i>E-Z</i>	product	yield (%) <sup>b</sup>
1	<b>12</b>	BnBr	<b>16a</b> <sup>c</sup>	50
2	<b>12</b>	MeI	<b>17</b>	49
3	<b>12</b>	AllBr	<b>18</b>	46
4	<b>13</b>	AllBr	<b>19</b>	42
5	<b>14</b>	BnBr	<b>20</b>	68
6	<b>14</b>	MeI	<b>21</b>	80
7	<b>15</b>	BnBr	<b>22</b>	72

<sup>a</sup>5-( $\beta$ -Keto)sulfones **12**–**15** (0.1 mmol), NaOH/ $\text{H}_2\text{O}$  (0.2 mmol)/MeOH, rt, 4–12 h. <sup>b</sup>Isolated yields. <sup>c</sup>Also isolated was **16b** (17%).

monobenzylated product **16a** as a diastereotopic mixture with no  $\alpha$ -dialkylated product observed (Table 2, entry 1). However, the benzylation was also observed at the N3 position and byproduct **16b** (17%) was isolated. The alkylation of **12** with MeI or allyl bromide and of **13** with allyl bromide also provided  $\alpha$ -alkylated products **17–19** (entries 2–4). As expected, 2'-deoxycytosine **14** and cytidine sulfones **15**, which lack an acidic proton at the N3 position, afforded  $\alpha$ -alkylated products **20–22** with higher yields (entries 5–7).

The 5-( $\beta$ -keto)sulfones also react with disulfides in the presence of a TEA-trapping electrophilic phenylsulfenyl<sup>48</sup> ion. Thus, the treatment of 2'-deoxyuridine-derived sulfone **12** with phenyl disulfide (2 equiv) in the presence of TEA in MeCN led to the  $\alpha$ -sulfanylation, affording **23** (36%) as a 1:1 mixture of diastereomers (Scheme 3, method A). Analogous treatment of

**Scheme 3.**  $\alpha$ -Sulfanylation of 5-( $\beta$ -Keto)sulfones of 2'-Deoxyuridine



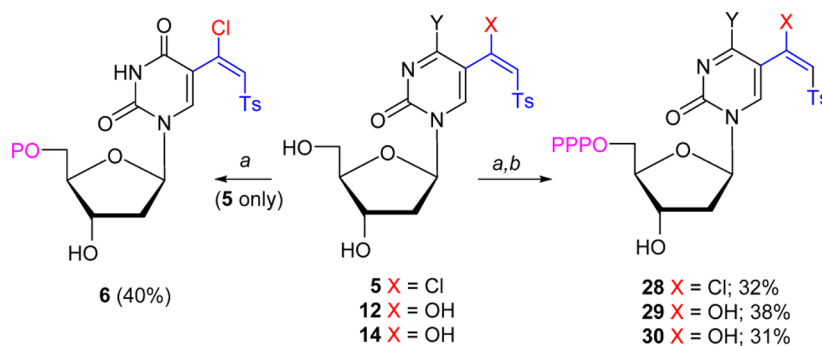
**12** with 4-chlorophenyl disulfide yielded **24** (44%). Sulfanylation of **12** was also successful with alkyl disulfides (e.g., MeSSMe), providing alkylsulfanylated product **25** in 40% yield.

The 5-( $\beta$ -keto)sulfones can also be  $\alpha$ -sulfanylated by a sequence of halogenation and nucleophilic substitution reactions. Thus, the treatment of ( $\beta$ -keto)sulfone **12** with iodine monochloride/NaOH or iodine in the presence of H<sub>2</sub>O<sub>2</sub>/AcOH<sup>49</sup> afforded 5-( $\alpha$ -iodo- $\beta$ -keto)sulfone **26** (35 or 41%) as a 1:1 diastereomeric mixture. Subsequent, displacement of iodide from **26** with PrSH/TEA gave 5-(2-propanethio-2-tosylacetyl)-2'-deoxyuridine **27** (Scheme 3, method B).

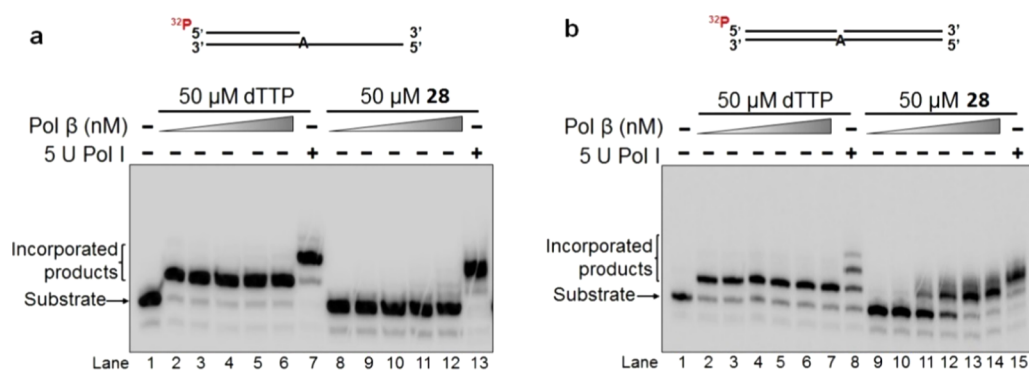
**Phosphorylation and Polymerase-Catalyzed Incorporation into DNA.** The uracil and cytosine nucleosides modified at the C5 position with ( $\beta$ -chlorovinyl)sulfone or ( $\beta$ -keto)sulfone probes were incorporated into DNA fragments via polymerase-catalyzed reactions. Phosphorylation of ( $\beta$ -chlorovinyl)sulfone **5** with POCl<sub>3</sub> (2.5 equiv; 0 °C, 30 min) in the presence of proton sponge (2.5 equiv)<sup>50</sup> followed by quenching of the crude reaction mixture with triethylammonium bicarbonate (TEAB) and purification on a Sephadex column gave 5'-monophosphate **6** (40%; Scheme 4). The reaction of **5** with POCl<sub>3</sub> (2.5 equiv)/proton sponge (2.5 equiv) followed by the treatment with tributylammonium pyrophosphate (TBAPP, 4.2 equiv) and tributylamine (TBA, 2.7 equiv) afforded 5'-triphosphate **28** after Sephadex purification. High-resolution mass spectrometry (HRMS) confirmed the presence of a ( $\beta$ -chlorovinyl) unit in **28**. Subjection of 2'-deoxyuridine 5-( $\beta$ -keto)sulfone **12** to the similar phosphorylation sequence afforded triphosphate **29**. Analogous phosphorylation of 2'-deoxycytidine 5-( $\beta$ -keto)sulfone **14** provided triphosphate **30**.

The 2'-deoxyuridine- and 2'-deoxycytidine-modified nucleotides **28–30** were incorporated into double-stranded DNA using a bacterial replication DNA polymerase, the Klenow fragment of DNA polymerase I (pol I),<sup>51</sup> and a human repair DNA polymerase, DNA polymerase  $\beta$  (pol  $\beta$ ).<sup>52</sup> The incorporation into double-stranded DNA was examined under the conditions that mimic the situations during both DNA leading and lagging strand syntheses using an open template substrate and a one-nucleotide gap substrate, respectively (Figures 2–4). The results showed that pol  $\beta$  was unable to insert **28** into an open template substrate (Figure 2a, lanes 8–12). On the other hand, 5 U pol I was able to incorporate **28** and dTTP into this template (Figure 2a, compare lane 13 with lane 7). Interestingly, both pol  $\beta$  and pol I inserted **28** into the substrate containing one-nucleotide gap (Figure 2b). It is noteworthy that at a high concentration, 5 U pol I is able to incorporate two nucleotides of **28** by the misincorporation of **28** with a template G. The results showed that 5-( $\beta$ -chlorovinyl)sulfone **28** can be incorporated into DNA by replication DNA polymerases during leading strand DNA synthesis, whereas it can be incorporated in double-stranded DNA by both replication and repair DNA polymerases during DNA lagging strand synthesis. The sequences of oligonucleotides for constructing the substrates for testing the incorpo-

**Scheme 4.** Synthesis of 5'-Phosphates of 5-( $\beta$ -Chlorovinyl) and 5-( $\beta$ -Keto)sulfones of 2'-Deoxyuridine and 2'-Deoxycytidine



(a) POCl<sub>3</sub>, PO(OMe)<sub>3</sub>, Proton Sponge, 0 °C, 30 min; (b) TBAPP, TBA, 0 °C, 10 min.



**Figure 2.** Incorporation of 2'-deoxyuridine 5-( $\beta$ -chlorovinyl)sulfone phosphate **28** into a DNA open template substrate (a) and a one-nucleotide gap substrate (b) by DNA polymerases. Lane 1 represents only substrate in both panels. Substrates were incubated with pol  $\beta$  or pol I at 37 °C for 15 min. In panel a, lanes 2–6 represent the reactions containing 1, 5, 10, 25, and 50 nM pol  $\beta$  along with 50  $\mu\text{M}$  dTTP, respectively. Lanes 8–12 indicate the reactions containing various concentrations of pol  $\beta$  (1, 5, 10, 25, and 50 nM) with 50  $\mu\text{M}$  **28**. Lanes 7 and 13 represent the reactions containing 5 U pol I. In panel b, lanes 2–7 represent the reactions containing 0.5, 1, 5, 10, 25, and 50 nM pol  $\beta$  along with 50  $\mu\text{M}$  dTTP, respectively. Lanes 9–14 indicate the reactions containing various concentrations of pol  $\beta$  (0.5, 1, 5, 10, 25, and 50 nM) with 50  $\mu\text{M}$  **28**. Lanes 8 and 15 represent the reactions containing 5 U pol I. Substrates were  $^{32}\text{P}$ -labeled at the 5'-end of the upstream strand and are illustrated above the gels.

**Table 3.** Oligonucleotide Sequences for C5-Modified 2'-Deoxyuridine Triphosphates **28** and **29**<sup>a</sup>

oligonucleotide	nt	sequence (5'–3')
upstream primer	31	GCA GTC CTC TAG TCG TAG TAG CAG ATC ATC A
downstream primer	39	CAA CCG GCA TTA GGT GTA GTA GCT AGA CTT ACT CAT TGC
template strand	71	GCA ATG AGT AAG TCT AGC TAC TAC ACC TAA TGC CGG TTG ATG ATG ATC TGC TAC TAC GAC TAG AGG ACT GC

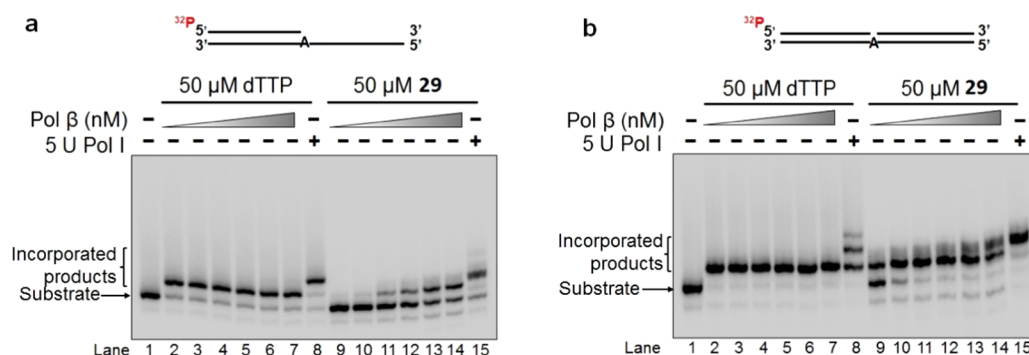
<sup>a</sup>

#### Upstream primer

5'-GCAGTCCTCTAGTCGTAGTAGCAGATCATCA CAACCGGCATTAGGTGTAGTAGCTAGACTTACTCATTGC-3'

3'-CGTCAGGAGATCAGCATCATCGTCTAGTAGTAGTTGGCCGTAATCCACATCATCGATCTGAATGAGTAACG-5'

#### Template strand



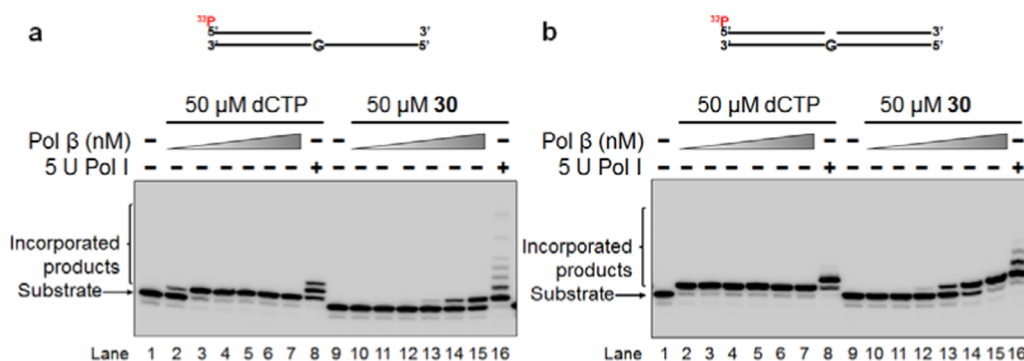
**Figure 3.** Incorporation of 2'-deoxyuridine 5-( $\beta$ -keto)sulfone phosphate **29** into the DNA open template substrate (a) and one-nucleotide gap substrate (b) by DNA polymerases. Substrates were incubated with pol  $\beta$  or pol I at 37 °C for 15 min. In both panels, lane 1 represents only substrate; lanes 2–7 represent the reactions containing 0.5, 1, 5, 10, 25, and 50 nM pol  $\beta$  along with 50  $\mu\text{M}$  dTTP, respectively; lanes 9–14 indicate the reactions containing various concentrations of pol  $\beta$  (0.5, 1, 5, 10, 25, and 50 nM, respectively) with 50  $\mu\text{M}$  **29**; lanes 8 and 15 represent the reactions containing 5 U pol I. Substrates were  $^{32}\text{P}$ -labeled at the 5'-end of the upstream strand and are illustrated above the gels.

ration of **28** are listed in Table 3. The DNA substrate constructed by the oligonucleotides is illustrated below Table 3.

Both pol  $\beta$  and pol I incorporated **29** into DNA open template and one-nucleotide gap substrates (Figure 3). 5 U pol I incorporated same quantity of **29** as dTTP (Figure 3a,b, lane 15). Again, at the high concentrations, 5 U pol I was able to incorporate two nucleotides of **29**. However, the incorporation of **29** by pol  $\beta$  into the open template substrate increased along with the increasing concentrations of the enzyme (0.5–50 nM) (Figure 3a, lanes 9–14). For the one-nucleotide gap substrate, 1.0 nM pol  $\beta$  had already inserted **29** into the DNA (Figure 3b,

lane 10), indicating pol  $\beta$  incorporated **29** into the one-nucleotide gap substrate with a high efficiency. Our results show that 5-( $\beta$ -keto)sulfones can be incorporated into double-stranded DNA more efficiently by repair DNA polymerases than  $\beta$ -(chlorovinyl)sulfones. They also suggest that it is possible that both 5'-triphosphates of 5-( $\beta$ -chlorovinyl)sulfone **28** and 5-( $\beta$ -keto)sulfone **29**, when generated in cells, can be incorporated into genomic DNA by DNA polymerases during DNA replication and repair.

Both pol  $\beta$  and pol I also incorporated 2'-deoxycytidine 5-( $\beta$ -keto)sulfone phosphate **30** into DNA open template and one-



**Figure 4.** Incorporation of 2'-deoxycytidine 5-( $\beta$ -keto)sulfone phosphate **30** into the DNA open template substrate (a) and one-nucleotide gap substrate (b) by DNA polymerases. Substrates were incubated with pol  $\beta$  or pol I at 37 °C for 15 min. In both panels: lanes 1 and 9 represent only substrate; lanes 2–7 represent the reactions containing 0.5, 1, 5, 10, 25, and 50 nM pol  $\beta$  along with 50  $\mu$ M dCTP, respectively; lanes 10–15 indicate the reactions containing various concentrations of pol  $\beta$  (0.5, 1, 5, 10, 25, and 50 nM, respectively) with 50  $\mu$ M **30**; and lanes 8 and 16 represent the reactions containing 5 U pol I. Substrates were  $^{32}$ P-labeled at the 5'-end of the upstream strand and are illustrated above the gels.

**Table 4. Oligonucleotide Sequences for C5-Modified 2'-Deoxycytidine Triphosphate **30**<sup>a</sup>**

oligonucleotide	nt	sequence (5'–3')
upstream primer	30	CCT CTT CCG TCT CTT TCC TTT TAC GTC ATC
downstream primer	18	GGG GGC AGA CTG GGT GGC
template strand	49	GCC ACC CAG TCT GCC CCC GGA TGA CGT AAA AGG AAA GAG ACG GAA GAG G

<sup>a</sup>

#### Upstream primer

5'- CCT CTT CCG TCT CTT TCC TTTT ACGTCATC GGGGGCAGACTGGGTGGC-3'

3'- GGAGAAGGCAGAGAAAGGAAAATGCAGTAGCCCCCGTCTGACCCACCG-5'

#### Template strand

nucleotide gap substrates (Figure 4). 5 U pol I incorporated the same quantity of **30** as dCTP (Figure 4a,b, lane 16). However, the incorporation of **30** by pol  $\beta$  into the open template substrate increased along with the increasing concentrations of the enzyme (0.5–50 nM; Figure 4a, lanes 10–15). For the one-nucleotide gap substrate, 10 nM pol  $\beta$  is required to insert **30** into the DNA (Figure 4b, lane 13). The sequences of oligonucleotides for constructing the substrates for testing the incorporation of **30** are listed in Table 4. The DNA substrate constructed by the oligonucleotides is illustrated below Table 4.

In summary, we have developed two mechanistically different probes attached to the C5 position of uracil and cytosine nucleosides. The first 5-( $\beta$ -chlorovinyl)sulfone probe efficiently reacts with nucleophiles, such as thiols, at rt in aqueous MeOH solution via the conjugated addition–elimination pathway. The second 5-( $\beta$ -keto)sulfone probe reacts with electrophiles such as alkyl halides or aryl/alkyl disulfides. These nucleosides with reactive  $\beta$ -chlorovinyl and  $\beta$ -keto sulfone groups were converted into their 5'-triphosphates and were efficiently incorporated into double-stranded DNA using open and one-nucleotide gap substrates by human or *E. coli* DNA-polymerase-catalyzed reactions. Our results also suggest that if 5-( $\beta$ -chlorovinyl)sulfone and 5-( $\beta$ -keto)sulfone 5'-triphosphates are generated in cells, they could be incorporated into genomic DNA by DNA polymerases during DNA replication and repair.

## EXPERIMENTAL SECTION

$^1$ H (400 MHz),  $^{13}$ C (100.6 MHz), and  $^{31}$ P (161.9 MHz) NMR spectra were recorded at ambient temperature in solutions of DMSO-*d*<sub>6</sub> unless otherwise noted. Reaction progress was monitored by thin-layer chromatography (TLC) on Merck

Kieselgel 60-F<sub>254</sub> sheets with product detection by 254 nm light. HRMS were obtained in time-of-flight (electrospray ionization, ESI) or ESI-Fourier transform ion cyclotron resonance mode. Products were purified by column chromatography using Merck Kieselgel 60 (230–400 mesh) or by automated flash chromatography using a CombiFlash system. Reagent-grade chemicals were used, and solvents were dried by reflux and distillation from CaH<sub>2</sub> under N<sub>2</sub> unless otherwise specified, and an atmosphere of N<sub>2</sub> was used for reactions.

**(E)-2',3',5'-Tri-O-acetyl-5-(1-chloro-2-tosylvinyl)-cytidine (3).** *p*-Toluenesulfonyl hydrazide (90 mg, 0.5 mmol), FeCl<sub>3</sub>·6H<sub>2</sub>O (91 mg, 0.33 mmol), and TBHP (70% in water; 84  $\mu$ L, 0.67 mmol) were added to a stirring solution of 2',3',5'-tri-O-acetyl-5-ethynylcytidine<sup>53</sup> (**1**; 66 mg, 0.167 mmol) in CH<sub>3</sub>CN at rt. The resulting mixture was stirred at 80 °C for 5 h. The volatiles were evaporated, and the residue was purified by column chromatography (MeOH/CHCl<sub>3</sub>; 0  $\rightarrow$  5%) to give **3** (48 mg, 60%):  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  2.10 (s, 3H), 2.13 (s, 3H), 2.15 (s, 3H), 2.39 (s, 3H), 4.38–4.46 (m, 3H), 5.38 (“t”, *J* = 5.6 Hz, 1H), 5.49 (dd, *J* = 5.4, 4.0 Hz, 1H), 6.07 (d, *J* = 4.0 Hz, 1H), 7.10 (s, 1H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.86 (s, 1H);  $^{13}$ C NMR (CDCl<sub>3</sub>)  $\delta$  20.61, 20.63, 20.9, 21.8, 63.1, 70.1, 74.0, 80.1, 89.4, 100.8, 128.1, 130.4, 136.5, 137.8, 139.3, 144.0, 146.0, 153.2, 160.7, 169.5, 169.6, 170.5. HRMS calcd for C<sub>24</sub>H<sub>27</sub><sup>35</sup>ClN<sub>3</sub>O<sub>10</sub>S [M + H]<sup>+</sup> 584.1100, found 584.1132.

**(E)-3',5'-Di-O-acetyl-5-(1-chloro-2-tosylvinyl)-2'-deoxycytidine (4).** The treatment of 3',5'-di-O-acetyl-5-ethynylcytidine<sup>53</sup> (**2** (156 mg, 0.465 mmol) with TsNHNH<sub>2</sub> (261 mg, 1.39 mmol) in the presence of FeCl<sub>3</sub>·6H<sub>2</sub>O (252 mg, 0.93 mmol), as described for **3**, gave **4** (168 mg, 68%):  $^1$ H NMR

(CDCl<sub>3</sub>)  $\delta$  2.10 (s, 3H), 2.12 (s, 3H), 2.20–2.27 (m, 1H), 2.41 (s, 3H), 2.70 (ddd,  $J$  = 14.0, 5.2, 2.0 Hz, 1H), 4.29–4.38 (m, 2H), 4.42 (dd,  $J$  = 12.8, 5.2 Hz, 1H), 5.21–5.27 (m, 1H), 6.33 (dd,  $J$  = 8.0, 5.6 Hz, 1H), 7.01 (s, 1H), 7.32 (d,  $J$  = 8.0 Hz, 2H), 7.61 (d,  $J$  = 8.4 Hz, 2H), 7.91 (s, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  21.02, 21.04, 21.8, 38.8, 63.9, 74.5, 82.9, 86.7, 100.0, 127.9, 130.3, 136.6, 136.7, 140.0, 143.9, 145.9, 154.2, 161.4, 170.5, 170.7. HRMS calcd for C<sub>22</sub>H<sub>25</sub><sup>35</sup>ClN<sub>3</sub>O<sub>8</sub>S [M + H]<sup>+</sup> for 526.1045, found 526.1046.

**5-(1-Chloro-2-tosylvinyl)-2'-deoxyuridine 5'-O-Monophosphate (6).** (MeO)<sub>3</sub>PO (0.85 mL; dried over 3A molecular sieves) was added to the flame-dried flask containing 5-( $\beta$ -chloro)vinyl sulfone **5** (55 mg, 0.124 mmol; dried in vacuum (65 °C over P<sub>2</sub>O<sub>5</sub>)) and proton sponge (67 mg, 0.31 mmol), and the resulting solution was stirred at 0 °C for 5 min under an Ar atmosphere. Freshly distilled POCl<sub>3</sub> (29  $\mu$ L, 47.5 mg, 0.31 mmol) was then added, and stirring was continued for 30 min at 0 °C. The reaction mixture was quenched by adjusting pH to 7.5–7.8 with triethylammonium bicarbonate (TEAB) buffer (2 M, several drops). The residue was dissolved in water (5 mL) and was extracted with EtOAc (3  $\times$  5 mL). The water layer was evaporated and co-evaporated (three times) with a mixture of EtOH/H<sub>2</sub>O (1:1, 5 mL). The residue was chromatographed on a DEAE-Sephadex A-25 column (30  $\times$  1 cm<sup>2</sup>; 3 g of resin) with TEAB (0.05  $\rightarrow$  0.25 M), and the appropriate fractions (TLC, R<sub>f</sub> 0.45; *i*-PrOH/H<sub>2</sub>O/NH<sub>4</sub>OH, 7:2:1) were evaporated in vacuum and co-evaporated five times with a mixture of EtOH/H<sub>2</sub>O (1:1, 10 mL) to remove excess of TEAB salt to give **6** (26 mg, 40%) as a triethylammonium salt: <sup>1</sup>H NMR (D<sub>2</sub>O)  $\delta$  2.26–2.48 (m, 2H), 2.41 (s, 3H), 3.90–4.01 (m, 2H), 4.12–4.20 (m, 1H), 4.46–4.57 (m, 1H), 6.24 (t,  $J$  = 6.0 Hz, 1H), 7.41 (d,  $J$  = 8.0 Hz, 2H), 7.42 (s, 1H), 7.61 (d,  $J$  = 8.0 Hz, 2H), 7.96 (s, 1H); <sup>13</sup>C NMR (D<sub>2</sub>O)  $\delta$  20.8, 38.5, 64.0, 71.0, 85.8, 85.9, 108.5, 127.7, 130.1, 134.6, 135.5, 140.8, 142.7, 146.8, 150.8, 161.7; <sup>31</sup>P NMR (D<sub>2</sub>O)  $\delta$  2.69; HRMS calcd for C<sub>18</sub>H<sub>19</sub><sup>35</sup>ClN<sub>2</sub>O<sub>10</sub>PS [M – H]<sup>–</sup> 521.0192, found 521.0199.

**3',5'-Di-O-acetyl-5-(1-propylthio-2-tosylvinyl)-2'-deoxycytidine (7).** *n*-PrSH (18.1  $\mu$ L, 14.5 mg, 0.19 mmol) and TEA (56  $\mu$ L, 39.5 mg, 0.39 mmol) were sequentially added to a stirred solution of **4** (35 mg, 0.0665 mmol) in MeOH (2 mL) at ambient temperature. After 6 h, the volatiles were evaporated and the residue was purified by silica gel column chromatography (MeOH/CHCl<sub>3</sub>; 0  $\rightarrow$  2%) to give **7** (*E/Z*, 1:1; 30 mg, 80%) as a white solid: <sup>1</sup>H NMR  $\delta$  0.94 (t,  $J$  = 7.2 Hz, 3H), 1.58 (sextet,  $J$  = 7.2 Hz, 2H), 1.87 (s, 1.5H), 1.92 (s, 1.5H), 2.07 (s, 1.5H), 2.08 (s, 1.5H), 2.08–2.22 (m, 1H), 2.37 (s, 1.5H), 2.38 (s, 1.5H), 2.30–2.44 (m, 1H), 2.74–2.93 (m, 2H), 4.09 (dd,  $J$  = 12.0, 3.6 Hz, 0.5H), 4.14–4.21 (m, 2H), 4.24 (“q”,  $J$  = 3.2 Hz, 0.5H), 5.10–5.21 (m, 1H), 6.16 (t,  $J$  = 7.2 Hz, 0.5H), 6.23 (t,  $J$  = 7.0 Hz, 0.5H), 6.53 (s, 0.5H), 6.59 (s, 0.5H), 5.61–6.75 (m, 1H), 7.31 (d,  $J$  = 9.6 Hz, 1H), 7.35 (s, 1H), 7.36 (d,  $J$  = 8.4 Hz, 1H), 7.54 (s, 1H), 7.56 (d,  $J$  = 8.0 Hz, 1H), 7.58 (d,  $J$  = 8.0 Hz, 1H); <sup>13</sup>C NMR  $\delta$  13.3, 13.4, 20.2, 20.41, 20.43, 20.5, 21.1, 34.0, 36.8, 37.4, 63.7, 63.8, 74.0, 74.1, 81.1, 81.4, 84.7, 85.4, 101.1, 101.5, 124.1, 124.6, 127.01, 127.03, 129.7, 129.8, 138.7, 139.0, 143.8, 143.9, 149.0, 153.8, 153.9, 161.8, 162.2, 169.8, 170.0, 170.11, 170.12. HRMS calcd for C<sub>25</sub>H<sub>32</sub>N<sub>3</sub>O<sub>8</sub>S<sub>2</sub> [M + H]<sup>+</sup> 566.1625, found 566.1643.

**S-[(E)-1-(2'-Deoxyuridin-5-yl)-2-tosylvinyl]-L-glutathione (8a).** 5-( $\beta$ -Chloro)vinyl sulfone **5** (25 mg, 0.056 mmol) was dissolved in the mixture of MeOH/H<sub>2</sub>O (1 mL, 4:1). L-Glutathione (25.8 mg, 0.084 mmol) and TEA (23.5  $\mu$ L, 17 mg, 0.17 mmol) were then sequentially added, and the resulting

mixture was stirred at ambient temperature for 4 h. The volatiles were evaporated, and the residue was purified by RP-HPLC (Phenomenex Gemini RP-C18 column (250  $\times$  10 mm<sup>2</sup>, 5  $\mu$ m particle size) using a gradient of MeCN in water (5  $\rightarrow$  10%) for 45 min and a flow rate of 2 mL/min;  $t_R$  = 22 min) to give **8a** (22 mg, 55%) as a triethylammonium salt (white solid): <sup>1</sup>H NMR (D<sub>2</sub>O)  $\delta$  2.14 (q,  $J$  = 7.2 Hz, 2H), 2.27–2.35 (m, 1H), 2.38–2.46 (m, 1H), 2.43 (s, 3H), 2.51 (t,  $J$  = 8.0 Hz, 2H), 3.22–3.42 (m, 2H), 3.72–3.86 (m, 5H), 4.06–4.12 (m, 1H), 4.45 (br, 1H), 4.65–4.69 (m, 1H), 6.24 (t,  $J$  = 6.4 Hz, 1H), 6.94 (br, 1H), 7.42 (d,  $J$  = 8.0 Hz, 2H), 7.62 (d,  $J$  = 8.0 Hz, 2H), 7.94 (s, 1H); <sup>13</sup>C NMR (D<sub>2</sub>O)  $\delta$  20.7, 26.0, 31.4, 39.3, 43.4, 51.8, 54.2, 61.0, 70.2, 85.9, 87.0, 99.6, 107.4, 127.3, 130.2, 132.1, 134.0, 136.2, 142.0, 146.4, 150.7, 161.9, 170.7, 173.9, 174.9, 176.1; HRMS calcd for C<sub>28</sub>H<sub>35</sub>N<sub>3</sub>NaO<sub>13</sub>S<sub>2</sub> [M + Na]<sup>+</sup> 736.1565, found 736.1513.

**S-[(E)-1-(2'-Deoxyuridin-5-yl)-2-tosylvinyl]-L-glutathione 5'-O-Monophosphate (8b).** L-Glutathione (22 mg, 0.07 mmol) and TEA (20  $\mu$ L, 14.3 mg, 0.14 mmol) were sequentially added into the stirred solution of 5'-monophosphate **6** (25 mg, 0.047 mmol) in MeOH/H<sub>2</sub>O (1 mL, 1:4) at ambient temperature. The resulting mixture was stirred for 4 h. The volatiles were evaporated, the residue was purified on a DEAE-Sephadex A-25 column (30  $\times$  1 cm<sup>2</sup>; 3 g of resin) with TEAB (0.1  $\rightarrow$  0.3 M), and the appropriate fractions (TLC, R<sub>f</sub> 0.25; *i*-PrOH/H<sub>2</sub>O/NH<sub>4</sub>OH, 7:2:1) were evaporated in vacuum and co-evaporated five times with a mixture of EtOH/H<sub>2</sub>O (1:1, 10 mL) to remove excess of TEAB salt to give **8b** (20 mg, 54%) as a triethylammonium salt (off-white solid): <sup>1</sup>H NMR (D<sub>2</sub>O)  $\delta$  2.13 (dt,  $J$  = 12.0, 7.8 Hz, 1H, 2H), 2.23–2.38 (m, 2H), 2.41 (s, 3H), 2.47–2.55 (m, 2H), 3.26–3.30 (m, 1H), 3.38 (dd,  $J$  = 12.0, 7.8 Hz, 1H), 3.70–3.76 (m, 3H), 3.92–4.02 (m, 2H), 4.09–4.19 (m, 1H), 4.48 (br, 1H), 4.60–4.71 (m, 1H), 6.22 (t,  $J$  = 6.8 Hz, 1H), 6.85 (s, 1H), 7.40 (d,  $J$  = 8.0 Hz, 2H), 7.60 (d,  $J$  = 8.0 Hz, 2H), 7.77 (s, 1H); <sup>13</sup>C NMR (D<sub>2</sub>O)  $\delta$  20.8, 26.1, 31.4, 33.3, 38.7, 42.2, 48.7, 51.8, 54.2, 58.9, 64.6, 85.8, 85.9, 107.9, 127.3, 129.9, 130.2, 141.9, 146.3, 148.6, 150.7, 161.8, 170.9, 173.9, 174.9, 176.2; <sup>31</sup>P NMR (D<sub>2</sub>O)  $\delta$  3.77; HRMS calculated for C<sub>28</sub>H<sub>35</sub>N<sub>3</sub>O<sub>16</sub>PS<sub>2</sub> [M – H]<sup>–</sup> 792.1264, found 792.1264.

Note: L-Glutathione (6.4 mg, 0.02 mmol) was added to a solution of 5-( $\beta$ -chloro)vinyl sulfone **6** (9 mg, 0.017 mmol) in TEAA buffer (0.5 mL, 0.3 M, pH = 8.3) at rt, and the mixture was stirred for 12 h. Purification as described above gave **8b** (7.8 mg, 58%).

**5-(1-Amino-2-tosylvinyl)-2'-deoxycytidine (11).**  $\beta$ -Chlorovinylsulfone **4** (150 mg, 0.285 mmol) was dissolved in NH<sub>3</sub>/MeOH (5 mL) at 0 °C (ice bath). After stirring for 12 h, the volatiles were evaporated and the residue was column-chromatographed (MeOH/CHCl<sub>3</sub>; 0  $\rightarrow$  10%) to afford **11** (*E/Z*, 30:70; 77 mg, 64%) as a white solid: <sup>1</sup>H NMR  $\delta$  1.98–2.07 (m, 1H), 2.10–2.22 (m, 1H), 2.38 (s, 2.1H), 2.40 (s, 0.9H), 3.46–3.57 (m, 1.4H), 3.64–3.70 (m, 0.6H), 3.78 (q,  $J$  = 3.6 Hz, 0.7H), 3.84 (q,  $J$  = 3.5 Hz, 0.3H), 4.16–4.22 (m, 0.7H), 4.26–4.31 (m, 0.3H), 4.71 (s, 0.3H), 4.95 (s, 0.7H), 4.98 (t,  $J$  = 5.4 Hz, 0.7H), 5.19 (d,  $J$  = 4.4 Hz, 0.7H), 5.22–5.25 (m, 0.3H), 5.27 (d,  $J$  = 4.4 Hz, 0.3H), 6.08 (t,  $J$  = 6.5 Hz, 0.7H), 6.13 (t,  $J$  = 6.4 Hz, 0.3H), 6.32–6.43 (br s, 1H), 6.84–7.01 (br s, 2H), 7.37 (d,  $J$  = 8.4 Hz, 1.4H), 7.45 (d,  $J$  = 8.4 Hz, 0.6H), 7.52–7.65 (br s, 1H), 7.72 (d,  $J$  = 8.0 Hz, 0.6H), 7.80 (d,  $J$  = 8.0 Hz, 1.4H), 7.84 (s, 0.7H), 8.50 (s, 0.3H); <sup>13</sup>C NMR  $\delta$  20.9, 40.6, 61.1, 70.2, 85.5, 87.6, 90.9, 103.6, 125.6, 129.4, 141.3, 141.9,

142.4, 151.4, 153.8, 162.0; HRMS calcd for  $C_{18}H_{23}N_4O_6S$   $[M + H]^+$  423.1333, found 423.1348.

**Conversion of 5-( $\beta$ -Chlorovinyl)sulfones into 5-( $\beta$ -Keto)sulfones: Procedure A.** Acetyl-protected (**3**, **4**, **9a**, or **9b**) or -unprotected (**5** or **9c**) 5-(1-chloro-2-tosylvinyl)-pyrimidine nucleosides were dissolved in methanolic ammonia, and the resulting mixture was stirred at 0 °C  $\rightarrow$  rt for 3–12 h. The volatiles were evaporated, and the residue was dissolved in MeCN (4 mL). The solution was acidified to pH  $\sim$  3–4 with dil. HCl (aq) and stirred for 2 h and then neutralized with dil. NaOH (aq) to pH  $\sim$  6–7. The volatiles were evaporated, and the residue was column-chromatographed to give products **12**–**15**.

**5-(2-Tosylacetyl)-2'-deoxyuridine (12).** The treatment of **5**<sup>44</sup> (300 mg, 0.68 mmol) with methanolic ammonia (10 mL) for 3 h and subsequent acid hydrolysis, as described in procedure A, followed by column chromatography (MeOH/CHCl<sub>3</sub>; 0  $\rightarrow$  5%) gave **12** (207 mg, 72%) as a white solid: <sup>1</sup>H NMR  $\delta$  2.04–2.18 (m, 1H), 2.19–2.28 (m, 1H), 2.40 (s, 3H), 3.50–3.66 (m, 2H), 3.87 (dd,  $J = 6.4, 3.2$  Hz, 1H), 4.13–4.28 (m, 1H), 5.10 (t,  $J = 4.6$  Hz, 1H), 5.15 (s, 2H), 5.29 (d,  $J = 4.4$  Hz, 1H), 6.06 (t,  $J = 6.2$  Hz, 1H), 7.42 (d,  $J = 8.0$  Hz, 2H), 7.74 (d,  $J = 8.0$  Hz, 2H), 8.69 (s, 1H), 11.78 (s, 1H); <sup>13</sup>C NMR  $\delta$  20.9, 40.6, 60.8, 64.3, 70.3, 85.7, 87.9, 111.0, 127.6, 129.5, 136.8, 144.1, 148.0, 148.7, 160.7, 183.5; HRMS calcd for  $C_{18}H_{20}N_2NaO_8S$   $[M + Na]^+$  for 447.0833, found 447.0874.

Analogous treatment of **9a**<sup>44</sup> (90 mg, 0.17 mmol) with methanolic ammonia (3 mL) for 12 h, as described in procedure A, also gave **12** (47 mg, 65%).

**5-(2-Tosylacetyl)uridine (13).** The treatment of **9c**<sup>44</sup> (96 mg, 0.20 mmol) with methanolic ammonia (3 mL) and subsequent acid hydrolysis, as described in procedure A (3 h), followed by column chromatography (MeOH/CHCl<sub>3</sub>; 0  $\rightarrow$  5%) gave **13** (64.5 mg, 70%) as a white solid: <sup>1</sup>H NMR  $\delta$  2.40 (s, 3H), 3.53–3.61 (m, 1H), 3.64–3.73 (m, 1H), 3.88–3.99 (m, 2H), 4.02–4.09 (m, 1H), 5.07–5.24 (m, 4H), 5.50 (d,  $J = 5.2$  Hz, 1H), 5.75 (s, 1H), 7.43 (d,  $J = 8.0$  Hz, 2H), 7.73 (d,  $J = 8.0$  Hz, 2H), 8.78 (s, 1H), 11.79 (s, 1H); <sup>13</sup>C NMR  $\delta$  21.0, 60.1, 64.2, 69.3, 74.3, 84.7, 89.3, 111.2, 127.9, 129.6, 136.8, 144.2, 148.2, 149.4, 161.2, 184.0; HRMS calcd for  $C_{18}H_{21}N_2O_9S$   $[M + H]^+$  for 441.0962, found 441.0954.

Analogous treatment of **9b**<sup>44</sup> (95 mg, 0.16 mmol) with methanolic ammonia (3 mL) for 12 h, as described in procedure A, also gave **13** (53 mg, 74%).

**5-(2-Tosylacetyl)-2'-deoxycytidine (14).** The treatment of **4** (150 mg, 0.28 mmol) with methanolic ammonia (5 mL) for 12 h and subsequent acid hydrolysis, as described in procedure A, followed by column chromatography (MeOH/CHCl<sub>3</sub>; 0  $\rightarrow$  10%) gave **14** (70 mg, 59%): <sup>1</sup>H NMR  $\delta$  2.06–2.18 (m, 1H), 2.26–2.35 (m, 1H), 2.41 (s, 3H), 3.59–3.69 (m, 1H), 3.73 (dt,  $J = 12.0, 4.0$  Hz, 1H), 3.90 (q,  $J = 3.6$  Hz, 1H), 4.28 (quint,  $J = 4.8$  Hz, 1H), 4.86 (d,  $J = 14.4$  Hz, 1H), 4.95 (d,  $J = 14.0$  Hz, 1H), 5.29 (d,  $J = 4.4$  Hz, 1H), 5.33 (t,  $J = 5.2$  Hz, 1H), 6.09 (t,  $J = 6.0$  Hz, 1H), 7.44 (d,  $J = 8.4$  Hz, 2H), 7.75 (d,  $J = 8.0$  Hz, 2H), 8.01–8.15 (m, 2H), 9.00 (s, 1H); <sup>13</sup>C NMR  $\delta$  21.0, 41.1, 60.6, 62.5, 69.2, 86.6, 88.0, 103.0, 128.0, 129.9, 136.0, 144.8, 152.2, 159.0, 162.6, 185.3; HRMS calcd for  $C_{18}H_{22}N_3O_7S$   $[M + H]^+$  424.1173, found 424.1176.

**5-(2-Tosylacetyl)cytidine (15).** The treatment of **3** (146 mg, 0.25 mmol) with methanolic ammonia (4 mL) for 12 h and subsequent acid hydrolysis, as described in procedure A, followed by column chromatography (MeOH/CHCl<sub>3</sub>; 0  $\rightarrow$  8%) gave **15** (70 mg, 64%): <sup>1</sup>H NMR  $\delta$  2.40 (s, 3H), 3.69 (ddd,

$J = 12.4, 4.8, 2.0$  Hz, 1H), 3.86 (ddd,  $J = 12.4, 4.8, 2.0$  Hz, 1H), 3.92–3.97 (m, 1H), 3.98–4.02 (m, 1H), 4.03–4.09 (m, 1H), 4.81 (d,  $J = 14.4$  Hz, 1H), 4.95 (d,  $J = 14.0$  Hz, 1H), 5.04 (d,  $J = 6.4$  Hz, 1H), 5.45 (t,  $J = 5.2$  Hz, 1H), 5.57 (d,  $J = 4.8$  Hz, 1H), 5.71 (d,  $J = 2.0$  Hz, 1H), 7.44 (d,  $J = 8.4$  Hz, 2H), 7.74 (d,  $J = 8.0$  Hz, 2H), 8.00–8.22 (m, 2H), 9.05 (s, 1H); <sup>13</sup>C NMR  $\delta$  21.1, 59.1, 62.4, 67.9, 74.9, 83.9, 91.2, 102.8, 127.9, 129.9, 136.2, 144.6, 152.3, 152.4, 162.6, 185.4; HRMS calcd for  $C_{18}H_{22}N_3O_8S$   $[M + H]^+$  440.1122, found 440.1133.

**Alkylation at the  $\alpha$ -Carbon of 5-( $\beta$ -Keto)sulfones: Procedure B.** The NaOH/H<sub>2</sub>O solution (3 M, 0.2 mmol) was added to a stirred solution of 5-( $\beta$ -keto)sulfones **12**–**15** (0.1 mmol) in MeOH (2 mL) at ambient temperature. After 20 min, the electrophile source (R–X, 0.2 mmol) was added and the resulting solution was stirred for 4–12 h. The reaction mixture was then neutralized with dil. HCl to pH  $\sim$  6.5–7, and the volatiles were evaporated. The residue was column-chromatographed to give products **16**–**22**.

**5-(2-Benzyl-2-tosylacetyl)-2'-deoxyuridine (16a) and 3-N-Benzyl-5-(2-benzyl-2-tosylacetyl)-2'-deoxyuridine (16b).** The treatment of **12** (25 mg, 0.058 mmol) with NaOH (aq) (3.0 M, 39  $\mu$ L) and BnBr (14  $\mu$ L, 20 mg, 0.117 mmol) for 4 h, as described in procedure B, followed by column chromatography (MeOH/CHCl<sub>3</sub>; 0  $\rightarrow$  5%) gave **16b** (6 mg, 17%; 1:1 mixture of diastereomers) followed by **16a** (15 mg, 50%; as a 1:1 mixture of diastereomers). Compound **16a** had: <sup>1</sup>H NMR  $\delta$  2.05–2.21 (m, 1.5H), 2.22–2.30 (m, 0.5H), 2.40 (s, 3H), 3.09–3.26 (m, 2H), 3.55–3.65 (m, 2H), 3.87 (q,  $J = 3.2$  Hz, 1H), 4.17–4.23 (m, 0.5H), 4.24–4.29 (m, 0.5H), 5.10–5.18 (m, 1H), 5.27 (d,  $J = 4.0$  Hz, 0.5H), 5.32 (d,  $J = 4.0$  Hz, 0.5H), 5.97 (t,  $J = 6.2$  Hz, 0.5H), 6.06 (t,  $J = 6.2$  Hz, 0.5H), 6.47–6.61 (m, 1H), 6.99–7.08 (m, 2H), 7.10–7.24 (m, 3H), 7.41 (d,  $J = 8.0$  Hz, 1H), 7.43 (d,  $J = 8.0$  Hz, 1H), 7.67 (d,  $J = 8.4$  Hz, 1H), 7.68 (d,  $J = 8.4$  Hz, 1H), 8.63 (s, 0.5H), 8.69 (s, 0.5H), 11.40 (s, 1H); <sup>13</sup>C NMR  $\delta$  21.1, 31.3, 31.5, 40.5, 40.9, 60.81, 60.83, 69.9, 70.31, 70.33, 85.9, 86.0, 86.4, 88.0, 88.1, 110.8, 111.3, 126.6, 128.5, 128.8, 128.9, 129.7, 134.6, 136.22, 136.24, 145.1, 148.2, 148.3, 148.9, 161.31, 161.32, 186.4, 186.6; HRMS calcd for  $C_{25}H_{27}N_2O_8S$   $[M + H]^+$  515.1483, found 515.1457. Compound **16b** had: <sup>1</sup>H NMR  $\delta$  2.04–2.14 (m, 1H), 2.15–2.29 (m, 1H), 2.30 (s, 1.5H), 2.32 (s, 1.5H), 3.13–3.31 (m, 2H), 3.55–3.68 (m, 2H), 3.86–3.92 (m, 1H), 4.18–4.28 (m, 1H), 4.87–4.97 (m, 2H), 5.21 (q,  $J = 4.0$  Hz, 1H), 5.31 (d,  $J = 4.4$  Hz, 0.5H), 5.36 (d,  $J = 4.4$  Hz, 0.5H), 5.97 (t,  $J = 5.2$  Hz, 0.5H), 6.07 (t,  $J = 6.4$  Hz, 0.5H), 6.46 (dd,  $J = 11.2, 3.6$  Hz, 0.5H), 6.51 (dd,  $J = 12.0, 4.8$  Hz, 0.5H), 7.03–7.09 (m, 2H), 7.13–7.23 (m, 4H), 7.29–7.38 (m, 6H), 7.58 (d,  $J = 8.4$  Hz, 1H), 7.59 (d,  $J = 8.0$  Hz, 1H), 8.65 (s, 0.5H), 8.73 (s, 0.5H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  21.11, 21.12, 31.2, 31.3, 40.7, 41.0, 43.9, 45.7, 60.5, 60.6, 69.6, 69.7, 70.5, 70.6, 87.0, 87.4, 88.2, 88.3, 110.2, 110.8, 126.7, 127.8, 128.4, 128.7, 129.0, 134.4, 136.2, 136.3, 145.0, 145.2, 146.6, 146.8, 149.1, 149.2, 160.3, 160.4, 186.7, 186.9; HRMS calcd for  $C_{32}H_{33}N_2O_8S$   $[M + H]^+$  605.1952, found 605.1976.

**5-(2-Methyl-2-tosylacetyl)-2'-deoxyuridine (17).** The treatment of **12** (40 mg, 0.094 mmol) with NaOH (aq) (3 M, 63  $\mu$ L) and MeI (11.7  $\mu$ L, 26.7 mg, 0.188 mmol) for 4 h, as described in procedure B, followed by column chromatography (MeOH/CHCl<sub>3</sub>; 0  $\rightarrow$  5%) gave **17** (21 mg, 49%) as a 1:1 mixture of diastereomers: <sup>1</sup>H NMR  $\delta$  1.32 (t,  $J = 7.6$  Hz, 3H), 2.11–2.17 (m, 1H), 2.20–2.30 (m, 1H), 2.39 (s, 1.5H), 2.40 (s, 1.5H), 3.53–3.67 (m, 2H), 3.88 (q,  $J = 2.8$  Hz, 1H), 4.21–4.29 (m, 1H), 5.13 (dd,  $J = 9.2, 4.4$  Hz, 1H), 5.30 (d,  $J = 4.3$  Hz,



0.5H), 5.32 (d,  $J = 4.3$  Hz, 0.5H), 5.97 (q,  $J = 6.8$  Hz, 1H), 6.05 (t,  $J = 6.4$  Hz, 0.5H), 6.11 (t,  $J = 6.4$  Hz, 0.5H), 7.42 (d,  $J = 8.0$  Hz, 1H), 7.44 (d,  $J = 8.0$  Hz, 1H), 7.66 (d,  $J = 8.0$  Hz, 1H), 7.67 (d,  $J = 8.0$  Hz, 1H), 8.72 (s, 0.5H), 8.73 (s, 0.5H), 11.78 (s, 1H);  $^{13}\text{C}$  NMR  $\delta$  11.0, 11.2, 21.1, 40.6, 40.8, 60.8, 60.9, 65.4, 70.0, 86.0, 86.1, 88.0, 88.1, 111.0, 111.1, 128.8, 128.9, 129.6, 129.7, 134.5, 144.8, 144.9, 147.8, 148.0, 149.1, 149.2, 161.3, 161.4, 187.6; HRMS calcd for  $\text{C}_{19}\text{H}_{23}\text{N}_2\text{O}_8\text{S}$   $[\text{M} + \text{H}]^+$  439.1170, found 439.1147.

**5-(2-Allyl-2-tosylacetyl)-2'-deoxyuridine (18).** The treatment of **12** (36 mg, 0.084 mmol) with allyl bromide (14.6  $\mu\text{L}$ , 20.3 mg, 0.17 mmol) and NaOH (aq) (3 M, 56  $\mu\text{L}$ ) for 4 h, as described in procedure B, followed by column chromatography (MeOH/CHCl<sub>3</sub>; 0  $\rightarrow$  5%) gave **18** (18 mg, 46%) as a ~1:1 mixture of diastereomers:  $^1\text{H}$  NMR  $\delta$  2.11–2.22 (m, 1.5H), 2.23–2.34 (m, 0.5H), 2.39 (s, 3H), 2.66–2.77 (m, 2H), 3.56–3.67 (m, 2H), 3.89 (q,  $J = 4.0$  Hz, 1H), 4.21–4.29 (m, 1H), 4.92–5.01 (m, 2H), 5.12–5.16 (m, 1H), 5.30 (d,  $J = 4.4$  Hz, 0.5H), 5.34 (d,  $J = 4.0$  Hz, 0.5H), 5.52–5.69 (m, 1H), 6.02 (t,  $J = 6.4$  Hz, 0.5H), 6.09 (t,  $J = 6.4$  Hz, 0.5H), 6.17 (d,  $J = 2.8$  Hz, 0.5H), 6.20 (d,  $J = 2.8$  Hz, 0.5H), 7.42 (d,  $J = 8.2$  Hz, 1H), 7.43 (d,  $J = 8.2$  Hz, 1H), 7.65 (d,  $J = 7.6$  Hz, 2H), 8.70 (s, 0.5H), 8.74 (s, 0.5H), 11.79 (s, 1H);  $^{13}\text{C}$  NMR  $\delta$  21.1, 30.0, 30.1, 40.6, 40.9, 60.8, 60.9, 68.8, 70.01, 70.03, 86.0, 86.4, 88.1, 88.2, 107.7, 111.0, 113.0, 117.7, 117.8, 128.8, 129.0, 129.8, 132.91, 132.93, 134.5, 145.1, 145.2, 148.3, 149.0, 149.1, 161.4, 161.5, 186.7; HRMS calcd for  $\text{C}_{21}\text{H}_{25}\text{N}_2\text{O}_8\text{S}$   $[\text{M} + \text{H}]^+$  465.1326, found 465.1341.

**5-(2-Allyl-2-tosylacetyl)uridine (19).** The treatment of **13** (18 mg, 0.04 mmol) with allyl bromide (7  $\mu\text{L}$ , 9.68 mg, 0.08 mmol) and NaOH (aq) (3 M, 26  $\mu\text{L}$ ) for 4 h, as described in procedure B, followed by column chromatography (MeOH/CHCl<sub>3</sub>; 0  $\rightarrow$  5%) gave **19** (8 mg, 42%; ~1:1 mixture of two diastereomers):  $^1\text{H}$  NMR  $\delta$  2.39 (s, 3H), 2.54–2.57 (m, 1H), 2.68–2.77 (m, 1H), 3.59–3.62 (m, 1H), 3.69–3.77 (m, 1H), 3.92–3.40 (m, 2H), 4.00–4.10 (m, 1H), 4.94–5.00 (m, 2H), 5.13 (d,  $J = 5.2$  Hz, 0.5H), 5.20 (d,  $J = 5.6$  Hz, 0.5H), 5.23–5.29 (m, 1H), 5.49 (d,  $J = 5.2$  Hz, 0.5H), 5.58 (d,  $J = 5.2$  Hz, 0.5H), 5.61–5.66 (m, 1H), 5.71 (d,  $J = 3.6$  Hz, 0.5H), 5.77 (d,  $J = 4.0$  Hz, 0.5H), 6.16 (dd,  $J = 6.0, 3.6$  Hz, 0.5H), 6.18 (dd,  $J = 6.4, 4.0$  Hz, 0.5H), 7.42 (d,  $J = 8.0$  Hz, 1H), 7.43 (d,  $J = 8.4$  Hz, 1H), 7.60–7.68 (m, 2H), 8.81 (s, 0.5H), 8.89 (s, 0.5H), 11.81 (s, 1H);  $^{13}\text{C}$  NMR  $\delta$  21.1, 30.0, 30.1, 59.9, 60.2, 68.8, 68.9, 69.4, 74.4, 74.7, 84.8, 85.0, 89.3, 89.6, 111.3, 111.6, 117.7, 117.8, 128.8, 128.9, 129.7, 132.9, 134.5, 145.22, 145.23, 148.5, 148.6, 149.2, 161.41, 161.43, 186.5, 186.6; HRMS calcd for  $\text{C}_{21}\text{H}_{25}\text{N}_2\text{O}_9\text{S}$   $[\text{M} + \text{H}]^+$  481.1275, found 481.1265.

**5-(2-Benzyl-2-tosylacetyl)-2'-deoxycytidine (20).** The treatment of **14** (36 mg, 0.085 mmol) with BnBr (20.4  $\mu\text{L}$ , 29.4 mg, 0.17 mmol) and NaOH (aq) (3 M, 56  $\mu\text{L}$ ) for 8 h, as described in procedure B, followed by column chromatography (MeOH/CHCl<sub>3</sub>; 0  $\rightarrow$  5%) gave **20** (30 mg, 68%; ~1:1 mixture of two diastereomers):  $^1\text{H}$  NMR  $\delta$  1.95–2.01 (m, 1H), 2.23–2.30 (m, 1H), 2.38 (s, 1.5H), 2.40 (s, 1.5H), 3.20–3.31 (m, 2H), 3.64 (ddd,  $J = 12.0, 5.2, 1.6$  Hz, 0.5H), 3.76 (dd,  $J = 7.2, 4.4$  Hz, 1H), 3.83–3.91 (m, 1H), 3.97 (dd,  $J = 6.8, 3.2$  Hz, 0.5H), 4.20 (quint,  $J = 4.8$  Hz, 0.5H), 4.38 (quint,  $J = 4.6$  Hz, 0.5H), 5.29 (d,  $J = 4.4$  Hz, 0.5H), 5.35 (d,  $J = 4.4$  Hz, 0.5H), 5.48 (dd,  $J = 11.2, 3.2$  Hz, 0.5H), 5.57 (dd,  $J = 10.8, 4.4$  Hz, 0.5H), 5.68 (t,  $J = 5.0$  Hz, 0.5H), 5.75 (t,  $J = 4.6$  Hz, 0.5H), 5.96 (t,  $J = 6.0$  Hz, 0.5H), 6.01 (t,  $J = 5.8$  Hz, 0.5H), 7.04–7.24 (m, 5H), 7.42 (d,  $J = 8.4$  Hz, 1H), 7.46 (d,  $J = 8.4$  Hz, 1H), 7.66–7.71 (m, 2H), 7.98–8.15 (m, 2H), 8.92 (s, 0.5H), 9.05 (s, 0.5H);  $^{13}\text{C}$  NMR  $\delta$

21.0, 21.1, 31.7, 32.1, 41.3, 41.4, 60.4, 60.8, 68.4, 68.9, 69.3, 69.4, 86.1, 86.8, 87.7, 88.0, 104.0, 104.6, 126.7, 126.8, 128.4, 128.5, 128.7, 128.8, 133.1, 133.7, 133.8, 135.7, 145.3, 149.8, 150.9, 151.9, 162.1, 162.4, 187.3, 187.8; HRMS calcd for  $\text{C}_{25}\text{H}_{28}\text{N}_3\text{O}_7\text{S}$   $[\text{M} + \text{H}]^+$  514.1642, found 514.1635.

**5-(2-Methyl-2-tosylacetyl)-2'-deoxycytidine (21).** The treatment of **14** (18 mg, 0.043 mmol) with MeI (5.5  $\mu\text{L}$ , 12.2 mg, 0.086 mmol) and NaOH (aq) (3 M, 29  $\mu\text{L}$ ) for 12 h, as described in procedure B, followed by column chromatography (MeOH/CHCl<sub>3</sub>; 0  $\rightarrow$  5%) gave **21** (15 mg, 80%; ~1:1 mixture of two diastereomers):  $^1\text{H}$  NMR  $\delta$  1.34 (d,  $J = 6.8$  Hz, 1.5H), 1.35 (d,  $J = 6.8$  Hz, 1.5H), 2.03–2.18 (m, 1H), 2.25–2.34 (m, 1H), 2.40 (s, 3H), 3.63 (dt,  $J = 11.6, 4.4$  Hz, 0.5H), 3.69–3.75 (m, 1H), 3.77–3.88 (m, 1H), 3.94 (dd,  $J = 7.6, 3.6$  Hz, 0.5H), 4.20 (quint,  $J = 4.6$  Hz, 0.5H), 4.39 (quint,  $J = 4.8$  Hz, 0.5H), 5.19 (q,  $J = 7.6$  Hz, 0.5H), 5.24–5.31 (m, 1H), 5.33–5.48 (m, 1.5H), 6.01 (t,  $J = 5.6$  Hz, 0.5H), 6.15 (t,  $J = 6.0$  Hz, 0.5H), 7.43 (d,  $J = 7.6$  Hz, 1H), 7.45 (d,  $J = 7.6$  Hz, 1H), 7.63 (d,  $J = 8.4$  Hz, 1H), 7.64 (d,  $J = 8.0$  Hz, 1H), 8.04–8.25 (m, 2H), 9.07 (s, 0.5H), 9.16 (s, 0.5H);  $^{13}\text{C}$  NMR  $\delta$  12.0, 12.02, 21.1, 41.1, 41.2, 60.31, 63.32, 63.3, 68.8, 68.9, 86.0, 86.8, 87.5, 88.0, 103.1, 103.6, 129.0, 129.1, 129.91, 129.93, 133.3, 133.7, 145.1, 145.2, 151.7, 151.9, 152.3, 162.7, 162.9, 188.5, 188.6; HRMS calcd for  $\text{C}_{19}\text{H}_{24}\text{N}_3\text{O}_7\text{S}$   $[\text{M} + \text{H}]^+$  438.1329, found 438.1317.

**5-(2-Benzyl-2-tosylacetyl)cytidine (22).** The treatment of **15** (30 mg, 0.068 mmol) with BnBr (16.2  $\mu\text{L}$ , 23.2 mg, 0.135 mmol) and NaOH (aq) (3 M, 46.0  $\mu\text{L}$ ) for 8 h, as described in procedure B, followed by column chromatography (MeOH/CHCl<sub>3</sub>; 0  $\rightarrow$  5%) gave **22** (26 mg, 72%; ~1:1 mixture of diastereomers):  $^1\text{H}$  NMR  $\delta$  2.38 (s, 1.5H), 2.39 (s, 1.5H), 3.19–3.31 (m, 2H), 3.74–3.90 (m, 2.5H), 3.93–4.05 (m, 2H), 4.14 (dd,  $J = 11.2, 6.0$  Hz, 0.5H), 5.04 (d,  $J = 6.0$  Hz, 0.5H), 5.19 (d,  $J = 6.4$  Hz, 0.5H), 5.49 (dd,  $J = 12.0, 3.6$  Hz, 0.5H), 5.52–5.62 (m, 2.5H), 5.76 (t,  $J = 3.6$  Hz, 0.5H), 5.85 (t,  $J = 4.0$  Hz, 0.5H), 7.10–7.24 (m, 5H), 7.42 (d,  $J = 8.0$  Hz, 1H), 7.45 (d,  $J = 8.0$  Hz, 1H), 7.68 (d,  $J = 8.4$  Hz, 1H), 7.69 (d,  $J = 8.0$  Hz, 1H), 7.99–8.07 (m, 1.5H), 8.15 (d,  $J = 3.6$  Hz, 0.5H), 8.90 (s, 0.5H), 9.06 (s, 0.5H);  $^{13}\text{C}$  NMR  $\delta$  21.1, 31.7, 31.8, 59.2, 59.7, 67.5, 68.1, 68.3, 69.1, 74.7, 75.0, 83.9, 84.0, 90.7, 91.2, 104.1, 104.5, 126.7, 126.8, 128.4, 128.5, 128.8, 128.9, 133.7, 134.0, 135.6, 145.2, 145.3, 151.4, 151.9, 162.1, 162.3, 187.3, 187.9; HRMS calcd for  $\text{C}_{25}\text{H}_{28}\text{N}_3\text{O}_8\text{S}$   $[\text{M} + \text{H}]^+$  530.1592, found 530.1577.

**Incorporation of the Aryl/Alkyl Sulfonyl Group at the  $\alpha$ -Carbon of 5-( $\beta$ -Keto)sulfone of 2'-Deoxyuridine (23–25): Procedure C.** 5-(2-Tosylacetyl)-2'-deoxyuridine **12** (0.1 mmol) was dissolved in 3 mL of MeCN. Then, phenyl disulfide (0.2 mmol) and TEA (0.2–0.5 mmol) were sequentially added, and the resulting mixture was placed in an oil bath and stirred at 70  $^\circ\text{C}$  for 36–72 h. The volatiles were evaporated, and the residue was column-chromatographed to afford products **23–25**.

**5-(2-Phenylthio-2-tosylacetyl)-2'-deoxyuridine (23).** The treatment of **12** (50 mg, 0.115 mmol) with phenyl disulfide (51 mg, 0.23 mmol) and TEA (32  $\mu\text{L}$ , 24 mg, 0.23 mmol), as described in procedure C, for 36 h followed by column chromatography (MeOH/CHCl<sub>3</sub>; 0  $\rightarrow$  5%) afforded **23** (22 mg, 36%) as a 1:1 mixture of diastereomers:  $^1\text{H}$  NMR  $\delta$  2.14–2.34 (m, 2H), 2.38 (s, 3H), 3.57–3.66 (m, 2H), 3.84–3.94 (m, 1H), 4.18–4.30 (m, 1H), 5.14 (t,  $J = 4.4$  Hz, 0.5H), 5.16 (t,  $J = 4.4$  Hz, 0.5H), 5.30 (d,  $J = 4.4$  Hz, 0.5H), 5.33 (d,  $J = 4.4$  Hz, 0.5H), 6.04 (d,  $J = 6.0$  Hz, 0.5H), 6.07 (d,  $J = 6.0$  Hz, 0.5H),

7.20–7.48 (m, 8H), 7.69 (d,  $J = 8.0$  Hz, 2H), 8.84 (s, 0.5H), 8.85 (s, 0.5H), 11.91 (s, 0.5H), 11.93 (s, 0.5H);  $^{13}\text{C}$  NMR  $\delta$  20.1, 39.7, 39.8, 59.7, 68.81, 68.83, 73.0, 73.2, 85.4, 85.5, 87.1, 106.5, 108.2, 127.2, 128.21, 128.22, 128.52, 128.53, 128.6, 130.42, 130.43, 144.31, 144.33, 148.0, 148.1, 148.2, 160.72, 160.73, 183.2, 183.3; HRMS calcd for  $\text{C}_{24}\text{H}_{25}\text{N}_2\text{O}_8\text{S}_2$  [ $\text{M} + \text{H}$ ] $^+$  533.1047, found 533.1069.

**5-(2-(4-Chlorophenylthio)-2-tosylacetyl)-2'-deoxyuridine (24).** The treatment of **12** (50 mg, 0.116 mmol) with 4-chlorophenyl disulfide (67 mg, 0.23 mmol) and TEA (32  $\mu\text{L}$ , 24 mg, 0.23 mmol) for 36 h, as described in procedure C, followed by column chromatography (MeOH/ $\text{CHCl}_3$ ; 0  $\rightarrow$  5%) afforded **24** (29 mg, 44%) as a 1:1 mixture of diastereomers:  $^1\text{H}$  NMR  $\delta$  2.14–2.29 (m, 2H), 2.39 (s, 3H), 3.55–3.60 (m, 1H), 3.62–3.67 (m, 1H), 3.90 (quint,  $J = 2.8$  Hz, 1H), 4.20–4.28 (m, 1H), 5.12–5.17 (m, 1H), 5.30 (d,  $J = 4.0$  Hz, 0.5H), 5.33 (d,  $J = 4.4$  Hz, 0.5H), 6.05 (t,  $J = 6.2$  Hz, 0.5H), 6.07 (t,  $J = 6.0$  Hz, 0.5H), 7.31 (d,  $J = 10.2$  Hz, 1H), 7.37–7.43 (m, 6H), 7.68 (d,  $J = 8.4$  Hz, 2H), 8.84 (s, 1H), 11.93 (s, 0.5H), 11.95 (s, 0.5H);  $^{13}\text{C}$  NMR  $\delta$  21.1, 40.3, 40.4, 60.6, 60.7, 69.82, 69.83, 73.9, 74.0, 86.3, 88.1, 109.1, 129.11, 129.12, 129.51, 129.52, 129.53, 129.6, 130.5, 131.0, 133.3, 145.41, 145.43, 149.00, 149.02, 149.2, 161.7, 161.8, 183.9, 184.0; HRMS calcd for  $\text{C}_{24}\text{H}_{24}\text{ClN}_2\text{O}_8\text{S}_2$  [ $\text{M} + \text{H}$ ] $^+$  567.0657, found 567.0681.

**5-(2-Methylthio-2-tosylacetyl)-2'-deoxyuridine (25).** The treatment of **12** (25 mg, 0.058 mmol) with dimethyl disulfide (26  $\mu\text{L}$ , 27 mg, 0.29 mmol) and TEA (40  $\mu\text{L}$ , 29 mg, 0.29 mmol) for 72 h, as described in procedure C, followed by column chromatography (MeOH/ $\text{CHCl}_3$ ; 0  $\rightarrow$  5%) afforded **25** (11 mg, 40%) as a 1:1 mixture of diastereomers:  $^1\text{H}$  NMR  $\delta$  2.17 (s, 1.5H), 2.18 (s, 1.5H), 2.20–2.33 (m, 2H), 2.42 (s, 3H), 3.53–3.66 (m, 2H), 3.88 (dt,  $J = 8.4$ , 3.2 Hz, 1H), 4.20 (dd,  $J = 9.2$ , 4.4 Hz, 0.5H), 4.25 (dd,  $J = 9.4$ , 4.5 Hz, 0.5H), 5.12 (t,  $J = 4.4$  Hz, 0.5H), 5.13 (t,  $J = 4.5$  Hz, 0.5H), 5.28 (d,  $J = 4.4$  Hz, 0.5H), 5.31 (d,  $J = 4.4$  Hz, 0.5H), 6.01–6.07 (m, 1H), 6.60 (s, 1H), 7.46 (d,  $J = 8.4$  Hz, 2H), 7.76 (d,  $J = 8.4$  Hz, 2H), 8.83 (s, 0.5H), 8.84 (s, 0.5H), 11.88 (s, 1H);  $^{13}\text{C}$  NMR  $\delta$  14.91, 14.92, 21.1, 41.7, 60.6, 60.7, 69.81, 69.83, 70.6, 70.7, 86.3, 86.5, 88.12, 88.13, 108.8, 108.9, 127.0, 128.0, 129.4, 129.6, 134.6, 145.3, 149.0, 149.2, 161.0, 183.4; HRMS calcd for  $\text{C}_{19}\text{H}_{23}\text{N}_2\text{O}_8\text{S}_2$  [ $\text{M} + \text{H}$ ] $^+$  471.0890, found 471.0917.

**5-(2-Iodo-2-tosylacetyl)-2'-deoxyuridine (26).** NaOH (aq) (3 M, 14  $\mu\text{L}$ ) was added into the stirred solution of ( $\beta$ -keto)sulfone **12** (18 mg, 0.042 mmol) in MeOH (1 mL). After 30 min, iodine monochloride (1 M/dichloromethane; 42  $\mu\text{L}$ , 0.042 mmol) was added into the solution and the resulting mixture was continuously stirred for 3 h. The solution was neutralized to pH  $\sim$  6–7 by adding dil. HCl, and volatiles were evaporated. The residue was column-chromatographed (MeOH/ $\text{CHCl}_3$ ; 0  $\rightarrow$  5%) to afford **26** (8 mg, 35%) as a 1:1 mixture of diastereomers:  $^1\text{H}$  NMR  $\delta$  2.10–2.25 (m, 2H), 2.40 (s, 3H), 3.53–3.63 (m, 2H), 3.87 (dt,  $J = 7.6$ , 4.0 Hz, 1H), 4.17–4.25 (m, 1H), 5.13 (t,  $J = 4.4$  Hz, 0.5H), 5.17 (t,  $J = 4.4$  Hz, 0.5H), 5.28 (d,  $J = 4.0$  Hz, 0.5H), 5.31 (d,  $J = 4.4$  Hz, 0.5H), 5.98–6.07 (m, 1H), 7.30 (s, 0.5H), 7.31 (s, 0.5H), 7.44 (d,  $J = 8.0$  Hz, 2H), 7.75 (d,  $J = 8.0$  Hz, 2H), 8.80 (s, 1H), 11.88 (s, 1H);  $^{13}\text{C}$  NMR  $\delta$  21.2, 40.7, 60.6, 60.7, 69.8, 69.9, 86.3, 86.5, 88.11, 88.12, 107.7, 107.8, 128.1, 129.62, 129.63, 129.71, 129.72, 133.31, 133.32, 145.3, 145.4, 149.11, 149.12, 161.5, 184.2, 184.3; HRMS calcd for  $\text{C}_{18}\text{H}_{20}\text{IN}_2\text{O}_8\text{S}$  [ $\text{M} + \text{H}$ ] $^+$  550.9980, found 550.9961.

Alternatively,  $\text{I}_2$  (24 mg, 0.1 mmol) and 50%  $\text{H}_2\text{O}_2$  (aq) (127  $\mu\text{L}$ , 1.88 mmol) were sequentially added into a stirred solution of **12** (80 mg, 0.19 mmol) in AcOH (0.5 mL) at ambient temperature. After 1 h, volatiles were evaporated and the residue was column-chromatographed ( $\text{CHCl}_3$ /MeOH; 100:0  $\rightarrow$  95:5) to give **26** (42 mg, 41%).

**5-(2-Propanethio-2-tosylacetyl)-2'-deoxyuridine (27).** A 1:1 mixture of PrSH (10  $\mu\text{L}$ , 8.2 mg, 0.11 mmol) and TEA (15  $\mu\text{L}$ , 11 mg, 0.11 mmol) was added into a stirring solution of **26** (40 mg, 0.07 mmol) in MeOH (1.5 mL) at rt. After 8 h, the volatiles were evaporated and the residue was column-chromatographed (MeOH/ $\text{CHCl}_3$ ; 0  $\rightarrow$  5%) to give **27** (12.5 mg, 34%) as a 1:1 mixture of diastereomers:  $^1\text{H}$  NMR  $\delta$  0.85 (t,  $J = 7.2$  Hz, 3H), 1.48 (sex,  $J = 7.2$  Hz, 2H), 2.14–2.29 (m, 2H), 2.41 (s, 3H), 2.54–2.61 (m, 1H), 2.68–2.76 (m, 1H), 3.53–3.66 (m, 2H), 3.88 (dt,  $J = 7.2$ , 3.6 Hz, 1H), 4.19–4.27 (m, 1H), 5.11 (t,  $J = 4.4$  Hz, 0.5H), 5.14 (t,  $J = 4.4$  Hz, 0.5H), 5.29 (d,  $J = 4.4$  Hz, 0.5H), 5.32 (d,  $J = 4.0$  Hz, 0.5H), 6.05 (dd,  $J = 10.4$ , 4.4 Hz, 1H), 6.76 (s, 0.5H), 6.78 (s, 0.5H), 7.45 (d,  $J = 8.0$  Hz, 2H), 7.73 (d,  $J = 8.0$  Hz, 2H), 8.82 (s, 1H), 11.91 (s, 1H);  $^{13}\text{C}$  NMR  $\delta$  13.0, 21.2, 22.0, 34.1, 34.2, 40.7, 40.8, 60.7, 69.8, 69.9, 70.2, 70.3, 86.40, 86.42, 88.11, 88.13, 108.9, 109.0, 129.51, 129.52, 129.7, 134.21, 134.23, 145.1, 145.2, 149.10, 149.12, 149.2, 161.6, 161.7, 184.41, 184.42; HRMS calcd for  $\text{C}_{21}\text{H}_{27}\text{N}_2\text{O}_8\text{S}_2$  [ $\text{M} + \text{H}$ ] $^+$  499.1203, found 499.1189.

**(E)-5-(1-Chloro-2-tosylvinyl)-2'-deoxyuridine 5'-O-Triphosphate (28).** ( $\text{MeO}$ ) $_3\text{PO}$  (0.5 mL; dried over 3A molecular sieves) was added to the flame-dried flask containing 5-( $\beta$ -chloro)vinyl sulfone **5** (30 mg, 0.067 mmol; dried in vacuum (65  $^\circ\text{C}$  over  $\text{P}_2\text{O}_5$ )) and proton sponge (37 mg, 0.172 mmol), and the resulting solution was stirred at 0  $^\circ\text{C}$  for 5 min under an Ar atmosphere. Freshly distilled  $\text{POCl}_3$  (16  $\mu\text{L}$ , 26.2 mg, 0.17 mmol) was then added, and stirring was continued for 30 min at 0  $^\circ\text{C}$ . Then, tributylammonium pyrophosphate (TBAPP; 0.5 M/dimethylformamide (DMF); 700  $\mu\text{L}$ , 0.35 mmol) and  $\text{Bu}_3\text{N}$  (50  $\mu\text{L}$ , 39 mg, 0.21 mmol) were added sequentially and stirred for another 10 min at 0  $^\circ\text{C}$ . The reaction mixture was quenched by adjusting pH to 7.5–7.8 with triethylammonium bicarbonate (TEAB) buffer (2 M, several drops). The residue was dissolved in water (5 mL) and was extracted with EtOAc (3  $\times$  5 mL). The water layer was evaporated and co-evaporated (three times) with a mixture of EtOH/ $\text{H}_2\text{O}$  (1:1, 5 mL). The residue was chromatographed on a DEAE-Sephadex A-25 column (30  $\times$  1  $\text{cm}^2$ ; 3 g of resin) with TEAB (0.1  $\rightarrow$  0.5 M), and the appropriate fractions (TLC,  $R_f$  0.30;  $i$ -PrOH/ $\text{H}_2\text{O}$ / $\text{NH}_4\text{OH}$ , 5:2:3) were evaporated in vacuum and co-evaporated five times with a mixture of EtOH/ $\text{H}_2\text{O}$  (1:1, 10 mL) to remove excess of TEAB salt to give **28** (15 mg, 32%) as a triethylammonium salt:  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  2.31–2.38 (m, 1H), 2.41–2.47 (m, 1H), 2.44 (s, 3H), 4.15–4.24 (m, 3H), 4.56–4.67 (m, 1H), 6.27 (t,  $J = 6.7$  Hz, 1H), 7.40–7.47 (m, 3H), 7.65 (d,  $J = 8.0$  Hz, 2H), 8.02 (s, 1H);  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  20.9, 38.7, 65.5, 70.9, 85.9, 86.1, 108.6, 127.8, 130.1, 130.2, 134.8, 140.3, 142.8, 146.9, 150.4, 161.4;  $^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  -23.15, -11.62, -10.83; HRMS calcd for  $\text{C}_{18}\text{H}_{21}^{35}\text{ClN}_2\text{O}_{16}\text{P}_3\text{S}$  [ $\text{M} - \text{H}$ ] $^-$  680.9518, found 680.9517.

**5-(2-Tosylacetyl)-2'-deoxyuridine 5'-O-Triphosphate (29).** The treatment of 5-( $\beta$ -keto)sulfone **12** (40 mg, 0.095 mmol) with  $\text{POCl}_3$  (22.2  $\mu\text{L}$ , 36.4 mg, 0.24 mmol) and TBAPP (0.5 M/DMF; 800  $\mu\text{L}$ , 110 mg, 0.4 mmol), as described for **28**, gave **29** (24 mg, 38%; TLC,  $R_f$  0.30;  $i$ -PrOH/ $\text{H}_2\text{O}$ / $\text{NH}_4\text{OH}$ , 5:2:3) as a triethylammonium salt:  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  2.34–2.40 (m,

1H), 2.45 (s, 3H), 2.46–2.51 (m, 1H), 4.17–4.24 (m, 2H), 4.25–4.31 (m, 1H), 4.59–4.65 (m, 1H), 4.84–4.89 (m, 2H), 6.14 (t,  $J = 6.4$  Hz, 1H), 7.48 (d,  $J = 8.0$  Hz, 2H), 7.76 (d,  $J = 8.0$  Hz, 2H), 8.47 (s, 1H);  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  20.7, 39.3, 58.6, 65.3, 70.9, 86.3, 87.5, 111.0, 128.5, 130.3, 133.9, 147.2, 149.4, 150.0, 161.9, 185.3;  $^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  -23.34, -11.61, -10.97; HRMS calcd for  $\text{C}_{18}\text{H}_{22}\text{N}_2\text{O}_{17}\text{P}_3\text{S}$  [ $\text{M} - \text{H}$ ] $^-$  662.9857, found 662.9859.

**5-(2-Tosylacetyl)-2'-deoxycytidine 5'-O-Triphosphate (30).** The treatment of 5-( $\beta$ -keto)sulfone **14** (9.3 mg, 0.022 mmol) with  $\text{POCl}_3$  (5  $\mu\text{L}$ , 8.1 mg, 0.0528 mmol) and TBAPP (0.5 M/DMF; 330  $\mu\text{L}$ , 0.165 mmol), as described for **28**, gave **30** (4.5 mg, 31%) as a triethylammonium salt after purification on a DEAE-Sephadex A-25 column (30  $\times$  1  $\text{cm}^2$ ; 5 g of resin) with TEAB (0.2  $\rightarrow$  0.7 M) buffer and evaporation and co-evaporation of the appropriate fractions (TLC,  $R_f$  0.25; *i*-PrOH/ $\text{H}_2\text{O}$ / $\text{NH}_4\text{OH}$ , 5:2:3):  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  2.23–2.33 (m, 1H), 2.42 (s, 3H), 2.47–2.56 (m, 1H), 4.19–4.35 (m, 3H), 4.54 (dt,  $J = 10.2, 4.6$  Hz, 1H), 5.91–5.00 (m, 2H), 5.97 (t,  $J = 5.6$  Hz, 1H), 7.48 (d,  $J = 8.4$  Hz, 2H), 7.75 (d,  $J = 7.2$  Hz, 2H), 8.47 (s, 1H);  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  20.8, 40.1, 58.7, 64.6, 69.5, 87.7, 100.0, 104.0, 128.2, 130.5, 133.3, 147.2, 151.1, 154.6, 162.9, 186.5;  $^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  -23.24, -11.82, -10.83; HRMS calcd for  $\text{C}_{18}\text{H}_{23}\text{N}_3\text{O}_{16}\text{P}_3\text{S}$  [ $\text{M} - \text{H}$ ] $^-$  662.0017, found 662.0043.

**Incorporation of 5-Modified Nucleotides into DNA: Materials of Enzymatic Reactions.** All DNA oligonucleotides were synthesized by the Integrated DNA Technologies (IDT; Coralville, IA). The radionucleotide [ $\gamma$ - $^{32}\text{P}$ ]ATP (6000 mCi/mmol) was purchased from MP Biomedicals (Santa Ana, CA). T4 polynucleotide kinase and deoxynucleoside 5'-triphosphates were purchased from Thermo Scientific (Pittsburgh, PA). Micro Bio-Spin TM 6 Columns were purchased from Bio-Rad (Hercules, CA). All other chemicals were purchased from Thermo Scientific (Pittsburgh, PA) and Sigma-Aldrich (St. Louis, MO). Purified human DNA polymerase  $\beta$  (pol  $\beta$ ) was purified following the procedures described previously.<sup>54,55</sup> The Klenow fragment was obtained from New England Biolabs (Ipswich, MA).

**Oligonucleotide Substrates.** Substrates with an upstream primer annealed to the template strand were designated as open template substrates. The substrates were constructed by annealing an upstream primer (31 or 30 nt) with the template strand (71 or 49 nt) at a molar ratio of 1:3. The substrate containing one-nucleotide gap was made by annealing an upstream primer and a downstream primer with the template strand at the molar ratio of 1:3:3. The open template and one-nucleotide gap substrates were employed to mimic the intermediates formed during DNA replication. The sequences of oligonucleotides for constructing the substrates are listed in Tables 3 and 4.

**Enzymatic Activity Assay.** Nucleotide incorporation by DNA polymerases was performed by incubating different concentrations of pol  $\beta$  or 5 U Klenow fragment with 25 nM  $^{32}\text{P}$ -labeled substrates at 37  $^\circ\text{C}$  for 15 min according to a method described previously.<sup>50,51</sup> The enzymatic reactions were assembled in the presence of triphosphates of 5-( $\beta$ -chlorovinyl)sulfone of dU **28** (50  $\mu\text{M}$ ), 5-( $\beta$ -keto)sulfone of dU **29** (50  $\mu\text{M}$ ), or 5-( $\beta$ -keto)sulfone of dC **30**. This allows us to examine if **28**, **29**, or **30** can be directly incorporated into a double-stranded DNA during DNA leading and lagging strand maturation. DNA synthesis was separated in a 15% urea denaturing polyacrylamide gel and the synthesized products

were detected by a Pharos FX Plus PhosphorImager (Bio-Rad Laboratory, CA).

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b00584.

$^1\text{H}$ ,  $^{13}\text{C}$ , and  $^{31}\text{P}$  NMR spectra for all compounds (PDF)

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

H.H. and Z.W. are recipients of the FIU Presidential and Dissertation Year Fellowships, respectively. This work was partially supported by NIH R01 grant ES023569 (Y.L.).

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