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Base-modified thymidines capable of terminating DNA synthesis are novel bioactive compounds with activity in cancer cells

Kayla M. Borland, Safnas F. AbdulSalam, Morwena J. Solivio, Matthew P. Burke, Patrick R. Wolfkiel, Sean M. Lawson, Courtney A. Stockman, Joel M. Andersen, Skyler Smith, Julia N. Tolstolutskaya, Purujit N. Gurjar, Aron P. Bercz, Edward J. Merino, Vladislav A. Litosh *

Department of Chemistry, University of Cincinnati, 301 Clifton Ct. ML 0172, Cincinnati, OH 45221-0172, USA

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ABSTRACT

Current FDA-approved chemotherapeutic antimetabolites elicit severe side effects that warrant their improvement; therefore, we designed compounds with mechanisms of action focusing on inhibiting DNA replication rather than targeting multiple pathways. We previously discovered that 5-(α-substituted-2-nitrobenzyloxy)methyluridine-5′-triphosphates were exquisite DNA synthesis terminators; therefore, we synthesized a library of 35 thymidine analogs and evaluated their activity using an MTT cell viability assay of MCF7 breast cancer cells chosen for their vulnerability to these nucleoside derivatives. Compound **3a**, having an α-*tert*-butyl-2-nitro-4-(phenyl)alkynylbenzyloxy group, showed an IC_{50} of 9 ± 1 μM. The compound is more selective for cancer cells than for fibroblast cells compared with 5-fluorouracil. Treatment of MCF7 cells with **3a** elicits the DNA damage response as indicated by phosphorylation of γ-H2A. A primer extension assay of the 5′-triphosphate of **3a** revealed that **3aTP** is more likely to inhibit DNA polymerase than to lead to termination events upon incorporation into the DNA replication fork.

1. Introduction

Cancer is one of the most sinister diseases known to mankind, claiming over half a million lives in the US alone during 2014, with more than one and a half million new diagnoses.[1] In contrast to normal cells, cancer cells undergo rapid, abnormal, and uncontrolled division, resulting in a constant requirement for DNA production. Therefore, tampering with this process preferentially affects them and represents a plausible approach to cancer chemotherapy,[2] a major component of cancer treatment, particularly if the tumor is inoperable or has metastasized.

In the past 50 years, nucleoside analogs having general antimetabolite mechanisms of action have substantially impacted cancer treatment. Their structural similarity to physiological nucleosides allows their passage into cells by nucleoside transporters,[3] where they are metabolized into 5′-triphosphates,[4] the active species that interfere with a large variety of intracellular targets. In particular, they inhibit enzymes involved in the synthesis of nucleic acids[5] and nucleotides,[6] signal DNA damage upon their incorporation,[7] obstruct DNA repair,[8] and trigger apoptosis by directly affecting mitochondria.[9] Problematically, the most active clinically approved antimetabolite drugs elicit deadly side effects[10,11] as they also affect rapidly proliferating normal human cells, lymphocytes,[12] and sometimes even non-dividing cells, such as neurons,[13] which substantially narrows their therapeutic windows. Additionally, their efficiency is limited to a relatively short list of malignancies that are predominantly hematological,[14] although gemcitabine[15] has proven to be effective against several solid tumors. Consequently, there is a critical need to discover novel anti-cancer chemotherapeutics with higher selectivity towards cancer cells.

The therapeutic potential of nucleotide species that are routinely used for termination of polymer chain reaction in DNA sequencing by synthesis[16] remains unexplored, primarily due to their poor incorporation by natural polymerases, meaning that their use would require unacceptably high dosage. In the course of developing terminators for a cyclic reversible termination protocol commonly used in DNA sequencing, we recently discovered that N6-(2-nitro)benzyl-2′-deoxyadenosine-,[17] 5-(2-nitro)benzyloxymethylpyrimidine-,[18] and 7-deaza-7-benzyloxymethylpurine-[19,17] 2′-deoxy-5′-triphosphates are incorporated into partial double helix DNA primers by natural polymerases more efficiently than the corresponding natural nucleotides, and then terminate further DNA synthesis by obstructing subsequent nucleotide incorporation. It was also evident that termination of DNA synthesis occurs only in the presence

* Corresponding author. Tel.: +1 513 556 9273; fax: +1 513 556 9239.
E-mail address: Vladislav.Litosh@uc.edu (V.A. Litosh).

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of a bulky group such as a branched alkyl (e.g., isopropyl or *tert*-butyl) linked to the α-benzylic carbon. Considering the high recognition of the novel thymidine 5′-triphosphate DNA terminators by polymerases, we presumed that they would compete with natural nucleotides for incorporation into DNA within cells.

Nucleotides are charged at physiological pH; therefore, they have difficulty penetrating cellular membranes.[20] Additionally, they are susceptible to enzymatic degradation, particularly dephosphorylation.[21] Consequently, nucleoside pro-drugs are commonly used in therapy rather than the corresponding nucleotides, as the pro-drugs are readily translocated into cells by nucleoside transporters[3] and intracellularly phosphorylated by kinases to eventually produce the active species. In particular, thymidine nucleoside analogs are successfully converted into 5′-triphosphates[22] by nucleotide kinases;[23] hence, we hypothesized that base-modified thymidine derivatives would be metabolized within cells to become terminators of DNA synthesis that would be incorporated into the DNA replication fork, thereby obstructing the subsequent addition of natural nucleotides. Consequently, cell division will arrest at the restriction checkpoint, preventing the completion of division, and eventually resulting in apoptosis.[24]

In this paper, we report the synthesis of 35 thymidine analogs bearing a 2-nitrobenzyloxymethyl moiety attached at the C-5 of the uracil nucleobase and evaluate their cytotoxic and cytostatic activities. The lead compound identified from these structure–activity relationship studies was further tested for toxicity to normal cells, DNA damage signaling, and PCR termination by its corresponding 5′-triphosphate. Our contribution to the field of anti-cancer drug discovery is significant as it facilitates the exploration of the therapeutic potential of novel base-modified nucleoside species. These compounds are unlikely to affect other targets than replicating DNA, and show promise to have wider therapeutic windows than present antimetabolites.

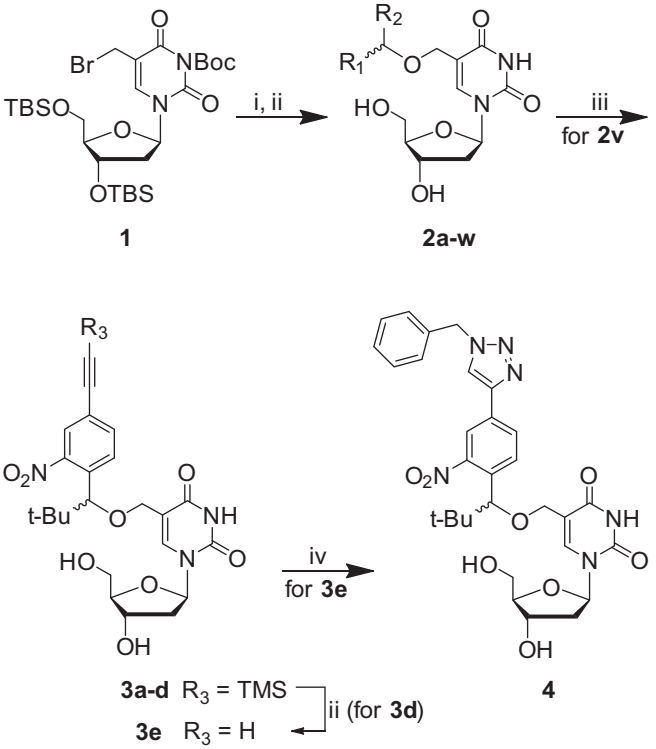
2. Results

2.1. Synthesis of base-modified nucleosides

The bioactive compounds were obtained by heating 5-bromomethyl-3-*N*-(*tert*-butyloxy)carbobyl-3′,5′-bis-(*tert*-butyl)-dimethylsilyl-*O*-2′-deoxyuridine **1**[18] with an appropriate alcohol under neat, anhydrous conditions (see Supporting information for details). Formation of HBr as a byproduct resulted in the in situ elimination of the N3-Boc group, but also in side reactions that made the purification challenging, which at times resulted in low yields. Removal of the residual TBS groups using tetra-*n*-butyl-ammonium fluoride trihydrate yielded derivatives **2a**–**w** (Scheme 1). The di-*tert*-butylcarbinol-oxy-T analog **2x** ($R_1 = R_2 = t$-Bu) could not be synthesized by thermal coupling and was therefore made using mechanochemical conditions.[25] From our previous studies,[19] the attachment of a large group to the *para*-position of the benzyl ring was known to further improve the DNA synthesis termination properties of the base-modified nucleotides. Consequently, the 4-iodo-2-nitrobenzyl derivative **2v** was used for Sonogashira reactions with various terminal alkynes to form derivatives **3a**–**d**. The coupling product (trimethylsilyl)acetylene (**3d**) was exposed to tetra-*n*-butylammonium fluoride trihydrate to generate the acetylenyl derivative **3e**, which was then 'clicked' to benzyl azide to yield compound **4**.

2.2. Cytotoxicity in MCF7 breast cancer cells: elucidation of structure–activity relationship

The MTT bioassay results[26] for the derivatives are summarized in Table 1, and they reveal four important trends in the SAR. First, the presence of at least one phenyl group as either R_1 or R_2 is required,



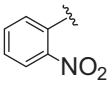
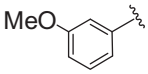
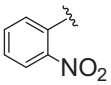
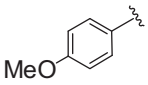
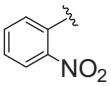
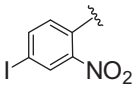
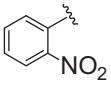
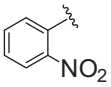
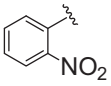
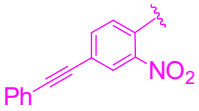
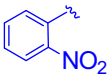
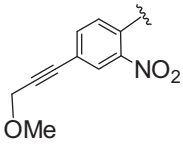
Scheme 1. Synthesis of T-nucleoside analogs. Reagents and conditions: (i) appropriate alcohol, 110–124 °C, 1–3 h; (ii) *n*-Bu4NF, THF, 0 °C to rt, 2–6 h; (iii) $Pd(PPh_3)_4$, appropriate terminal alkyne, CuI, Et_3N, DMF, rt, 6–18 h; (iv) benzyl azide, CuI, Et_3N, MeCN, rt, 4 h.

as neither the bis-isopropyl (**2w**) nor the bis-*tert*-butyl (**2x**) showed significant activity. Second, the presence of *one* nitro group on the phenyl ring is critical for anti-cancer activity, similarly to the bulkiness of the substituent attached to the benzylic α-carbon. Consequently, the IC_{50} values for the non-substituted phenyl derivatives **2d**–**g** are generally much higher compared with the 2-nitro substituted analogs **2p**–**s**. At the same time, the presence of a 2-nitro group in **2m**–**o** does not increase the activities of the smaller α-substituents (H, Me, *i*-Pr) compared to the non-substituted analogs **2a**–**c**. An activity decrease was observed when the α-*tert*-butyl group of the first generation lead compound ***o*-2s** was replaced with a less bulky substituent (**2p**, ***o*-2q**) or moved away from the benzylic α-carbon by just one CH_2 unit (**2r**), which is consistent with DNA synthesis terminating properties of their 5′-triphosphates.[18] Furthermore, neither the replacement of the 2-nitro group with different substituents such as methyl (**2h**), cyano (**2i**), halo (**2j**–**l**), or methoxy (***o*-2u**), nor the introduction of another *ortho*-nitro group (**2t**) improved activity. Third, the electronic characters of the aromatic substituents and their positions on the aromatic ring appear to be related. Therefore, for electron-donating groups in the benzene ring, for example, the methoxy at the *ortho*- (***o*-2u**) or *para*- (***p*-2u**) positions, the activities are lower compared with that of the *meta*-isomer (***m*-2u**). Conversely, the *ortho*- (***o*-2s**, ***o*-2q**) and *para*- (***p*-2q**) nitro-substituted derivatives are substantially more active than their *meta*- counterparts (***m*-2q**, ***m*-2s**). Fourth, derivatization of the initial lead compound ***o*-2s** by the attachment of a large group at the benzyl ring *para*-position via an acetylene linker substantially improved the IC_{50} values, and the (phenyl)alkynyl derivative **3a** was identified as the second generation lead compound.

2.3. Evaluation of selectivity for novel bioactive compound 3a

To evaluate selectivity of the lead compound **3a**, we assessed its toxicity using normal fibroblast cells and compared it to that in

Table 1
IC_{50} values determined by MTT assays using base-modified T-nucleoside analogs and MCF7 breast cancer cells

R_1	R_2	Compd	IC_{50} (μM)	R_1	R_2	Compd	IC_{50} (μM)	R_1	R_2	Compd	IC_{50} (μM)
	H	**2a**	>170		H	**2m**	>200			***m*-2u**	66 ± 10
	Me	**2b**	>200		Me	**2n**	>150			***p*-2u**	150 ± 20
		**2c**	>150			**2o**	>150			**2v**	56 ± 4
		**2d**	88 ± 6			**2p**	74 ± 5			**2w**	>200
		**2e**	>150			***o*-2q**	50 ± 5			**2x**	>110
		**2f**	123 ± 9			**2r**	62 ± 6			**3a**	9 ± 1
		**2g**	188 ± 11			***o*-2s**	42 ± 6			**3b**	74 ± 7
		**2h**	121 ± 3			***m*-2s**	>180			**3c**	16 ± 3
		2i	132 ± 23			***m*-2q**	>180	5-Fluoro-2′-deoxyuridine			38 ± 5
		2j	80 ± 12			***p*-2q**	57 ± 6			**3d**	29 ± 3
		2k	72 ± 7			**2t**	96 ± 7			**3e**	90 ± 10
		2l	60 ± 7			***o*-2u**	88 ± 6			**4**	19 ± 1

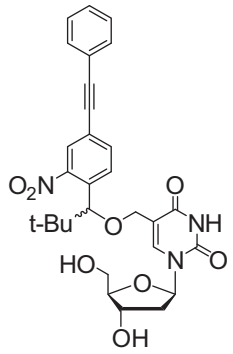
MCF7 breast cancer cells (Fig. 1). The IC_{50} value of **3a** for fibroblast cells was 55 ± 8 μM, showing a selectivity ratio of 6.3 ± 1.6, whereas the selectivity of 5-fluorouracil, the FDA-approved drug used for treating breast cancer, was 1.8 ± 0.5. Therefore, our second-generation lead compound **3a** could potentially have a significantly wider therapeutic window than the current chemotherapeutic drug.

2.4. Evaluation of activity for novel bioactive compound 3a in other cancer cells

The NCI-60 human tumor cell line screen based on the SRB assay gave a growth inhibition of 58% for compound **3a** at 10 μM in MCF7 cells, which is consistent with the IC_{50} value of 9 ± 1 μM

Figure 1. Selectivity of **3a**. Viability of MCF7 cancer cells (black) and fibroblasts (grey) in the presence of **3a**. Selectivity is greater than 5-fold.

Table 2
SRB cellular protein content assay of **3a**



Cancer cell line	10 μM GI (%)
Leukemia **K-562**	38
Leukemia **MOLT-4**	30
Leukemia **RPMI-8226**	51
Leukemia **RPMI-8226**	34
Non-small cell lung **A549/ATCC**	31
Non-small cell lung **EKVX**	36
Non-small cell lung **H460**	34
Non-small cell lung **NCI-H522**	31
Central nervous system **U251**	27
Melanoma **UACC-62**	30
Renal **A489**	26
Renal **UO-31**	30
Prostate **PC-3**	42
Breast **MCF-7**	58

obtained from the MTT assay. Additionally, the lead compound also showed significant activity against leukemia, prostate, renal, melanoma, central nervous system, and non-small lung cancer cell lines (Table 2).

2.5. Activation of DNA damage response

We next confirmed that **3a** elicits a DNA damage response. Most polymerization fork halting events lead to the activation of double strand break repair, a key marker for which is phosphorylation of the histone protein H2A.[27] MCF7 cells were treated with 10 μM **3a**, after 20 h of incubation their nuclei were isolated and used for a quantitative western blot analysis[28] to determine gamma-H2AX levels (Fig. 2). Total protein contents were normalized using the Bradford assay, and the nuclear envelope protein Lamin A served as a loading control. Treatment with **3a** led to a 1.78 ± 0.22 increase in the level of γ-H2AX after twenty-four hours, indicating that the addition of **3a** leads to the termination of DNA synthesis.

2.6. DNA synthesis termination by 5′-triphosphate of 3a

To gain insight into the mechanism of action of **3a**, we synthesized its 5′-triphosphate (**3aTP**) as described in our previous work[18] and examined its incorporation into DNA under conditions approximating the intracellular environment.[29] We performed a primer extension assay using *exo*-Vent polymerase, a random 51-mer template, a fluorescently labeled primer, a 100 μM cocktail of all four natural dNTPs, and various concentrations of acyclo-NTPs as positive control or **3aTP**. Following extension (Fig. 3), it is obvious that **3aTP** inhibits DNA polymerase rather than terminates DNA synthesis as evidenced by the fact that acyclo-TTP showed termination at nucleotide positions 26, 30, 31, 37, and 41, while termination was not observed for **3aTP**. Furthermore, primer extension was halted entirely when the concentration of **3aTP** was increased from 1 to 2 mM, but this was not observed for any of the acyclo nucleotide triphosphates at these concentrations.

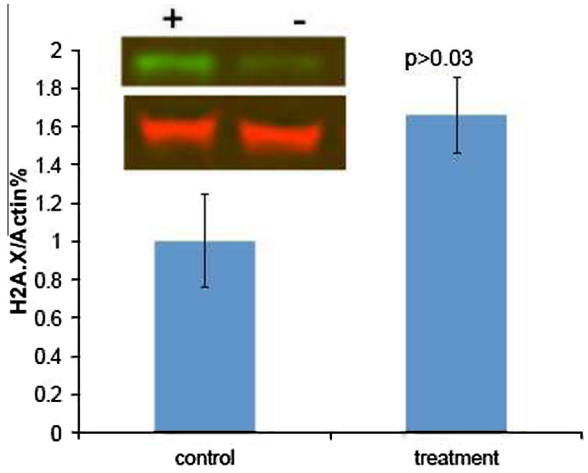


Figure 2. Addition of compound **3a** leads to the activation of the DNA damage response. (inset) Representative Western blot showing a 1.78 ± 0.22 increase after 20 h (p >0.03).

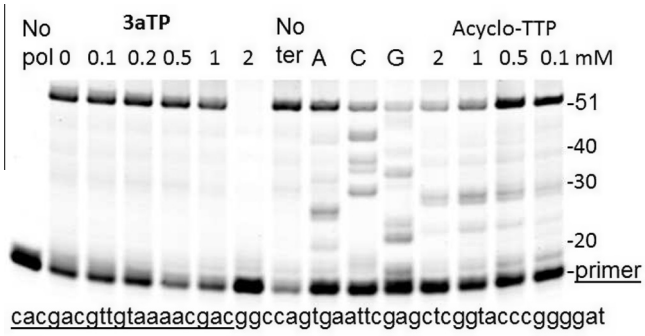


Figure 3. Primer extension assay studies of **3a** triphosphate. Left to right: no polymerase, **3aTP** at 0, 0.125, 0.25, 0.5, 1, 2 mM, no terminating nucleotide, acyclo A, C, G (2 mM each), acylco T at 2, 1, 0.5, 0.125 mM.

3. Conclusions

We have synthesized a library of thymidine derivatives bearing a modified moiety attached to the 5-methyl group. Studies of the structure–activity relationship regarding the cytotoxicity of these base-modified T-nucleosides in MCF7 cancer cells have revealed a lead compound. The DNA damage signaling elicited by the active nucleoside **3a** is consistent with its cellular uptake and 5′-triphosphorylation into the active species **3aTP** that interferes with DNA synthesis. PCR studies using **3aTP** support a mechanism of action that inhibits DNA polymerase rather than being incorporated into the DNA replication fork and blocking nucleotide addition as initially hypothesized, which warrants future studies of polymerase activity affected by these species.

Importantly, this novel anti-cancer bioactive compound is less toxic to normal cells compared with the FDA-approved T-nucleobase analog 5-fluorouracil, which is currently used against breast cancer. Furthermore, we have produced an analog (**3e**) that can undergo click reactions with various azides, facilitating the synthesis of large libraries of diverse triazole compounds that are analogous to **4**. This opportunity offers: (a) the potential for further improvement of cytotoxic activity, particularly in the light of the positive influence of a large substituent when attached at the *para*-position of the benzene ring, and (b) the attachment of imaging modalities for further investigation of intracellular metabolism.

4. Materials and methods

4.1. Synthesis

All chemicals, reagents, and solvents were purchased from Sigma–Aldrich Inc., TCI, and Fisher Scientific, Inc., and used as received unless stated otherwise. All reactions were carried out under an atmosphere of dry argon in oven-dried glassware. Indicated reaction temperatures refer to those of the reaction bath, while room temperature is noted as 25 °C. Pure reaction products were typically dried under high vacuum in the presence of phosphorus pentoxide. Analytical thin layer chromatography (TLC) was performed with glass backed silica plates (5 × 20 cm, 60 Å, 250 μm). Visualization was accomplished using a 254 nm UV lamp. ^{1}H and ^{13}C NMR spectra were recorded on either a Bruker Avance 400 MHz spectrometer or Bruker DPX 500 MHz spectrophotometer using solutions of samples in either of the deturated solvents: chloroform, methanol, acetonitrile, or water. Chemical shifts are reported in ppm with tetramethylsilane as a standard. Data are reported as follows: chemical shift, number of protons, multiplicity (s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, br = broad, m = multiplet, ab = AB pattern), and coupling constants. High resolution mass spectral data were collected on a Shimadzu Q-TOF 6500. All novel compounds were characterized by ^{1}H, ^{13}C, DEPT ^{13}C, ^{31}P (**3aTP**) NMR spectroscopy and high resolution mass spectrometry. The identity of previously made nucleoside derivatives was confirmed comparison of their ^{1}H NMR to the published data (reference provided). HPLC analysis of final products was performed on an Agilent 1200 HPLC with UV detection. Compounds biologically tested were at least 95% pure as judged by ^{1}H NMR and HPLC.

4.1.1. General procedure for preparation of base-modified nucleosides 2a–w

N3-*tert*-butyloxycarbonyl-5-bromomethyl-3′,5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxyuridine (**1**)[18] and appropriate alcohol (4–20 equiv) were heated neat at 110–120 °C for 0.5–3 h under argon atmosphere. The mixture was cooled down to room temperature, dissolved in ethyl acetate (ca. 5 mL), and silica (0.5–1.0 g) was added. The mixture was evaporated, and the solid was applied onto a silica gel chromatography column (hexane/ethyl acetate = 15:1 to 2:1, then dichloromethane/methanol = 0:1 to 10:1). Fractions that were not the starting alcohol were collected, evaporated under reduced pressure, dissolved in tetrahydrofuran (ca. 5 mL), and to this solution chilled at 0 °C tetra-*n*-butylammonium fluoride trihydrate (TBAF) was added (2.5 equiv). The reaction mixture was stirred for 2–3 h while gradually warming up to room temperature. The solvent was removed under reduced pressure and the residue was purified by silica gel (ethyl acetate/methanol = 1:0 to 20:1) to afford product as waxy solid.

4.1.1.1. 5-(Benzyl)oxymethyl-2′-deoxyuridine (2a)[30]. NOTE no TBAF treatment was necessary. Heating **1** (86 mg, 0.132 mmol) with benzyl alcohol (286 mg, 2.346 mmol) for 1.5 h at 118 °C after column chromatography afforded 25 mg (54%) of product. ^{1}H NMR (400 MHz, CD_3OD): δ 8.05 7.33 (m, 5H), 6.27 (t, 1H, J = 6.7 Hz), 4.57 (s, 2H), 4.39 (m, 1H), 4.30 (ab d, 1H, J = 12.6 Hz), 4.24 (ab d, 1H, J = 12.6 Hz), 3.93 (q, 1H, J = 3.5 Hz), 3.78 (ab dd, 1H, J = 12.0, 3.4 Hz), 3.78 (ab dd, 1H, J = 12.0, 3.6 Hz), 2.27 (m, 2H), 2.19 (m, 2H), 1.42 (d, 3H, J = 6.5 Hz).

4.1.1.2. 5-[1-(Phenyl)ethoxymethyl]-2′-deoxyuridine (2b). Heating **1** (121 mg, 0.186 mmol) with α-methylbenzyl alcohol (1-phenyl-1-ethanol) (0.228 g, 1.862 mmol) for 1 h at 114 °C followed by treatment with TBAF (0.303 g, 0.930 mmol) afforded after purification 20 mg (30%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 7.96 and 7.95 (s, 1H), 7.33 (br m, 5H), 6.27 (m, 1H), 4.54 (m, 1H), 4.38 (m, 1H), 4.10 (m, 2H), 3.92 (m, 1H), 3.75 (m, 2H) 2.26 (m, 1H), 2.19 (m, 2H), 1.42 (m, 3H). ^{13}C NMR (400 MHz, CD_3OD) for diastereomers: δ 165.06 and 165.03 (C), 152.09 (C), 144.91 and 144.82 (C), 140.55 and 140.52 (CH), 129.53 (CH), 128.58 and 128.56 (CH), 127.32 and 127.28 (CH), 112.75 and 112.71 (C), 88.93 (CH), 86.50 (CH), 79.42 and 79.34 (CH), 72.22 and 72.18 (CH), 64.41 and 64.22 (CH_2), 62.84 and 62.81 (CH_2), 41.37 and 41.32 (CH_2), 24.53 and 24.42 (CH_3). HRMS (ESI) for $[MH]^+$ $C_{18}H_{23}N_2O_6$ calculated: 363.15506, observed: 363.15516; for $[MNa]^+$ $C_{18}H_{22}N_2O_6Na$ calculated: 385.13701, observed: 385.13712.

4.1.1.3. 5-[1-(Phenyl)-2-(methyl)-1-propoxymethyl]-2′-deoxyuridine (2c). Heating **1** (250 mg, 0.385 mmol) with α-isopropylbenzyl alcohol (2-methyl-1-phenyl-1-propanol) (1.16 g, 7.70 mmol) for 2 h at 124 °C followed by treatment with TBAF (303 mg, 0.963 mmol) afforded after purification 92 mg (61%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (500 MHz, CD_3OD) for diastereomers: δ 7.91 (s, 1H), 7.29 (m, 5H), 6.27 (t, 1H, J = 6.7 Hz), 4.39 (m, 1H), 4.05 (m, 3H), 3.93 (m, 1H), 3.75 (m, 2H), 2.27 (m, 1H), 2.18 (m, 1H), 1.91 (m, 1H), 0.99 (m, 3H), 0.72 (m, 3H). ^{13}C NMR (125 MHz, CD_3OD) for diastereomers: δ 163.63 (C), 150.72 (C), 141.10 and 141.02 (C), 138.94 (CH), 127.80 (CH), 127.23 (CH), 127.14 (CH), 111.56 (C), 87.69 (CH), 87.54 (CH), 85.14 and 85.06 (CH), 70.94 (CH) and 70.87 (CH), 63.39 and 63.20 (CH_2), 61.51 (CH_2), 39.93 (CH_2), 34.67 and 34.61 (CH), 18.09 (CH_3), 18.00 (CH_3). HRMS (ESI) for $[MH]^+$ $C_{20}H_{27}N_2O_6$ calculated: 391.18636, observed: 391.18644; for $[MNa]^+$ $C_{20}H_{26}N_2O_6Na$ calculated: 413.16831, observed: 413.16836.

4.1.1.4. 5-[1-(Phenyl)-1-(cyclohexyl)methoxymethyl]-2′-deoxyuridine (2d). Heating **1** (97 mg, 0.149 mmol) with α-cyclohexylbenzyl alcohol (550 mg, 2.890 mmol) for 2.5 h at 132 °C followed by purification of bis- and mono-TBS products with subsequent treatment with TBAF (103 mg, 0.326 mmol) afforded after purification 22 mg (34%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 7.90 (s, 1H), 7.28 (m, 5H), 6.26 (m, 1H), 4.39 (m, 1H), 4.06 (m, 3H), 3.93 (m, 1H), 3.75 (m, 2H), 2.28 (m, 1H), 2.17 (m, 1H), 2.04 (m, 1H), 1.73 (m, 1H), 1.60 (m, 3H), 1.05 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers δ 165.00 and 164.98 (C), 154.08 (C), 142.40 (C), 140.29 and 140.26 (CH), 129.15 (CH), 128.64 and 128.57 (CH), 128.48 and 128.46 (CH), 112.92 (C), 88.94 and 88.90 (CH), 88.14 (CH), 86.54 and 86.47 (CH), 72.36 and 72.27 (CH), 64.66 and 64.45 (CH_2), 62.94 and 62.90 (CH_2), 45.71 and 45.70 (CH), 41.32 (CH_2), 30.62 and 30.41 (CH_2), 27.65 (CH_2), 27.17 and 27.12 (CH_2). HRMS (ESI) for $[MH]^+$ $C_{23}H_{31}N_2O_6$ calculated: 431.21766, observed: 431.21781; $[MNa]^+$ $C_{23}H_{30}N_2O_6Na$ calculated: 453.19961, observed: 453.19977.

4.1.1.5. 5-[(Diphenyl)methoxymethyl]-2′-deoxyuridine (2e). Heating **1** (250 mg, 0.385 mmol) with diphenylmethanol (1.42 g, 7.70 mmol) for 2.5 h at 120 °C followed by treatment with TBAF (607 mg, 1.925 mmol) afforded after purification 6 mg (3%) of product. ^{1}H NMR (400 MHz, CD_3OD) δ 7.97 (s, 1H), 7.36 (d, 4H, J = 7.9 Hz), 7.29 (m, 4H), 7.21 (m, 2H), 6.26 (t, 1H, J = 6.7 Hz), 5.51 (s, 1H), 4.37 (q, 1H, J = 3.5 Hz), 4.29 (ab d, 1H, J = 12.1 Hz), 4.24 (ab d, 1H, J = 12.1 Hz), 3.93 (m, 1H), 3.75 (ab dd, 1H, J = 12.0, 3.5), 3.70 (ab dd, 1H, J = 12.0, 3.9), 2.27 (m, 1H), 2.17 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 165.04 (C), 152.06 (C), 143.52 (C), 140.53 (CH), 129.34 (CH), 128.47 (CH), 128.09 (CH), 112.64 (C), 88.91 (CH), 86.56 (CH), 84.76 (CH), 72.22 (CH), 64.84 (CH_2), 62.91 (CH_2), 41.28 (CH_2). HRMS (ESI) for $[MH]^+$ $C_{23}H_{25}N_2O_6$ calculated: 425.17071, observed: 425.17082; $[MNa]^+$ $C_{23}H_{24}N_2O_6Na$ calculated: 447.15266, observed: 447.15276.

4.1.1.6. 5-[1-(Phenyl)-3,3-(dimethyl)-1-butoxymethyl]-2′-deoxyuridine (2f). Heating **1** (44 mg, 0.068 mmol) with α-*neo*-pentylbenzyl alcohol (3,3-dimethyl-1-phenyl-1-butanol) (41 mg, 0.239 mmol) for 2.5 h at 110 °C followed by treatment with TBAF (53 mg, 0.170 mmol) afforded after purification 12 mg (43%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3-OD) for diastereomers: δ 7.97 and 7.95 (s, 1H), 7.34 (m, 5H), 6.29 (m, 1H), 4.51 (m, 1H), 4.41 (m, 1H), 4.05 (m, 2H), 3.94 (m, 1H), 3.78 (m, 2H), 2.28 (m, 1H), 2.21 (m, 1H), 1.82 (m, 1H), 1.44 (m, 1H), 0.99 and 0.98 (2s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers δ 163.60 (C), 150.63 (C), 143.72 (C), 139.23 and 139.06 (CH), 128.13 (CH), 127.00 (CH), 126.28 and 126.21 (CH), 111.47 (C), 86.51 (CH), 85.09 and 84.93 (CH), 80.08 and 79.97 (CH), 70.85 (CH), 62.71 and 62.52 (CH_2), 61.52 and 61.49 (CH_2), 51.94 and 51.82 (CH_2), 40.01 (CH_2), 30.01 (C), 29.32 (CH_3). HRMS (ESI) for $[MH]^+$ $C_{22}H_{31}N_2O_6$ calculated: 419.21766, observed: 419.21780; $[MNa]^+$ $C_{22}H_{30}N_2O_6Na$ calculated: 441.19961, observed: 441.19974.

4.1.1.7. 5-[1-(Phenyl)-2,2-(dimethyl)-1-propoxymethyl]-2′-deoxyuridine (2g). Heating **1** (250 mg, 0.385 mmol) with α-*tert*-butylbenzyl alcohol (2,2-dimethyl-1-phenyl-1-propanol) (1.26 g, 7.70 mmol) for 2 h at 120 °C followed by treatment with TBAF (607 mg, 1.925 mmol) afforded after purification 22 mg (21%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3-OD) for diastereomers: δ 7.93 and 7.92 (2s, 1H), 7.32 (m, 5H), 6.28 (m, 1H), 4.42 (m, 1H), 4.06 (m, 3H), 3.95 (m, 1H), 3.77 (m, 2H), 2.30 (m, 1H), 2.19 (m, 1H), 0.91 (s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers: δ 163.61 (C), 150.68 (C), 139.42 (C), 138.73 and 138.62 (CH), 128.24 and 128.20 (CH), 127.18 (CH), 126.95 (CH), 111.61 (C), 87.66 and 87.56 (CH), 87.58 and 87.51 (CH), 85.14 and 84.99 (CH), 70.99 and 70.89 (CH), 63.79 and 63.53 (CH_2), 61.59 (CH_2), 39.94 and 39.87 (CH_2), 35.11 (C), 25.38 (CH_3). HRMS (ESI) for $[MH]^+$ $C_{21}H_{29}N_2O_6$ calculated: 405.20201, observed: 405.20210; for $[MNa]^+$ $C_{21}H_{28}N_2O_6Na$ calculated: 427.18396, observed: 427.18409.

4.1.1.8. 5-[1-(2-Methyl)phenyl-2,2-(dimethyl)propoxymethyl]-2′-deoxyuridine (2h). NOTE: no TBAF treatment was necessary. Heating **1** (125 mg, 0.195 mmol) with α-*tert*-butyl-2-methybenzyl alcohol (3,3-dimethyl-1-(2-methyl)phenyl-1-propanol) (174 mg, 0.776 mmol) for 1 h at 112 °C followed by purification afforded 8 mg (10%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 7.89 and 7.88 (2s, 1H), 7.41 (m, 1H), 7.16 (m, 3H), 6.28 (m, 1H), 4.46 (s, 1H), 4.41 (m, 1H), 4.03 and 4.01 (2s, 2H), 3.95 (m, 1H), 3.76 (m, 2H), 2.38 and 2.37 (2s, 3H), 2.30 (m, 1H), 2.19 (m, 1H), 0.95 (s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers δ 163.57 (C), 150.67 (C), 138.45 and 138.39 (CH), 136.84 (C), 136.74 (C), 129.76 (CH), 127.76 and 127.72 (CH), 126.71 and 126.66 (CH), 124.97 and 124.93 (CH), 111.82 (C), 87.54 and 87.52 (CH), 85.10 and 85.03 (CH), 83.83 and 83.79 (CH), 71.02 (CH), 63.41 and 63.31 (CH_2), 61.59 (CH_2), 39.88 and 39.85 (CH_2), 36.41 and 36.37 (C), 25.43 (CH_3), 19.29 and 19.23 (CH_3). HRMS (ES^+ TOF) for $[MNa]^+$ $C_{22}H_{30}N_2O_6Na$ calculated: 441.20020 observed: 441.19960.

4.1.1.9. 5-[1-(2-Cyanophenyl)-2,2-(dimethyl)propoxymethyl]-2′-deoxyuridine (2i). Heating **1** (114 mg, 0.176 mmol) with α-*tert*-butyl-2-cyanobenzyl alcohol (2,2-dimethyl-1-(2-cyano)-phenyl-1-propanol) (1.61 g, 7.70 mmol) for 2 h at 120 °C followed by treatment with TBAF (607 mg, 1.925 mmol) afforded after purification 14 mg (18%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 7.99 and 7.98 (2s, 1H), 7.89 (d, J = 7.7 Hz, 1H), 7.54 (m, 3H), 6.32 (m, 1H), 5.33 (s, 1H), 4.38 (m, 3H), 3.91 (m, 1H), 3.68 (m, 2H), 2.24 (m, 2H), 1.01 (s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers: δ 164.07 (C), 150.86 (C), 145.06 (C), 137.80 (CH), 131.33 (CH), 130.46 (C), 128.57 (CH), 123.06 and 122.93 (CH), 115.25 (CH), 112.98 (C), 91.70 (CH), 87.56 and 87.52 (CH), 85.11 and 85.02 (CH), 71.00 and 70.92 (CH), 61.55 (CH_2), 58.09 (CH_2), 39.83 (CH_2), 35.58 (C), 24.29 (CH_3). HRMS (ES^+ TOF) for $[MH]^+$ $C_{22}H_{28}N_3O_6$ calculated: 430.19870, observed: 430.19700; for $[MNa]^+$ $C_{22}H_{27}N_3O_6$-Na calculated: 452.17970, observed: 452.18040.

4.1.1.10. 5-[1-(2-Chlorophenyl)-2,2-(dimethyl)propoxymethyl]-2′-deoxyuridine (2j). Heating **1** (250 mg, 0.385 mmol) with α-*tert*-butyl-2-chlorobenzyl alcohol (3,3-dimethyl-1-(2-chloro)phenyl-1-propanol) (540 mg, 2.718 mmol) for 3 h at 118 °C followed by treatment with TBAF (43 mg, 0.136 mmol) afforded after purification 19 mg (11%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 7.91 and 7.89 (2s, 1H), 7.53 (d, 1H, J = 7.6 Hz), 7.31 (m, 3H), 6.27 (m, 1H), 4.67 (s, 1H), 4.41 (m, 1H), 4.10 (m, 1H), 4.00 (m, 1H), 3.95 (m, 1H), 3.76 (m, 2H), 2.27 (m, 2H), 0.97 and 0.96 (2s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers δ 163.50 and 163.47 (C), 150.68 (C), 139.10 and 138.68 (CH), 137.28 (C), 134.38 (C), 129.76 and 129.70 (CH), 128.89 and 128.80 (CH), 128.40 and 128.36 (CH), 111.36 and 111.10 (C), 109.90 (CH), 87.57 and 87.52 (CH), 85.24 and 85.07 (CH), 83.89 and 83.36 (CH), 70.97 (CH), 64.20 and 63.72 (CH_2), 61.54 (CH_2), 39.89 and 39.83 (CH_2), 36.37 and 36.33 (C), 25.15 (CH_3). HRMS (ESI^+) for $[MNa]^+$ $C_{21}H_{27}{}^{35}ClN_2O_6Na$ calculated: 461.14499 observed: 461.14504; for $[MNa]^+$ $C_{21}H_{27}{}^{37}ClN_2$-O_6Na calculated: 463.14213 observed: 463.14210. HRMS (ESI^-) for $[M-H]^-$ $C_{21}H_{26}{}^{35}ClN_2O_6$ calculated: 437.14849 observed: 437.14851; $C_{21}H_{26}{}^{37}ClN_2O_6$ calculated: 439.14561 observed: 439.14656.

4.1.1.11. 5-[1-(2-Bromomethyl)phenyl-2,2-(dimethyl)propoxymethyl]-2′-deoxyuridine (2k). Heating **1** (208 mg, 0.320 mmol) with α-*tert*-butyl-2-bromobenzyl alcohol (3,3-dimethyl-1-(2-bromo)phenyl-1-propanol) (389 mg, 1.607 mmol) for 1 h at 112 °C. Purification afforded 3 mg (2%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 7.89 and 7.86 (2s, 1H), 7.54 (m, 2H), 7.36 (m, 1H), 7.18 (m, 1H), 6.25 (m, 1H), 4.64 and 4.63 (2s, 1H), 4.40 (m, 1H), 4.04 (m, 2H), 3.92 (m, 1H), 3.75 (m, 2H), 2.24 (m, 2H), 0.97 and 0.96 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD) for diastereomers δ 165.03 and 164.88 (C), 152.12 (C), 140.55 and 140.08 (CH), 140.36 (C), 140.26 (C), 133.72 and 133.66 (CH), 131.35 and 131.32 (CH), 130.18 and 130.15 (CH), 128.20 and 128.18 (CH), 112.77 and 112.52 (C), 88.97 and 88.94 (CH), 87.64 and 86.54 (CH), 87.10 and 86.71 (CH), 72.41 and 72.39 (CH), 65.50 and 65.10 (CH_2), 62.99 and 62.97 (CH_2), 41.30 and 41.27 (CH_2), 37.92 and 37.88 (C), 26.68 (CH_3). HRMS (ESI) for $[MH]^+$ $C_{21}H_{28}{}^{79}BrN_2O_6$ calculated: 483.11307 observed: 483.11264, $C_{21}H_{28}{}^{81}BrN_2O_6$ calculated: 485.11103 observed: 485.11055; for $[MNa]^+$ $C_{21}H_{27}{}^{79}BrN_2O_6Na$ calculated: 505.09502 observed: 505.09452, $C_{21}H_{27}{}^{81}BrN_2O_6Na$ calculated: 507.09297 observed: 507.09212.

4.1.1.12. 5-[1-(2,6-Dichlorophenyl)-2,2-(dimethyl)propoxymethyl]-2′-deoxyuridine (2l). Heating **1** (158 mg, 0.243 mmol) with α-*tert*-butyl-2,6-dichlorobenzyl alcohol (3,3-dimethyl-1-(2,6-dichloro)phenyl-1-propanol) (113 mg, 0.486 mmol) for 3.5 h at 102–104 °C followed by treatment with TBAF (77 mg, 0.244 mmol) afforded after purification 8 mg (7%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers (NOTE: due to the presence of two *ortho*-substituents, there is, apparently, restricted rotation of the 2,6-dichlorophenyl group around its 1-C–4-C axis, which thereby makes 3-H non-equivalent to 5-H): δ 7.93 and 7.87 (2s, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.41 (d,

J = 8.5 Hz, 1H), 7.26 (t, J = 8.0 Hz, 1H), 6.30 (m, 1H), 5.08 and 5.06 (2s, 1H), 4.40 (m, 1H), 4.10 (m, 2H), 3.94 (m, 1H), 3.72 (m, 2H), 2.30 (m, 1H), 2.18 (m, 1H), 1.06 (s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers (NOTE: due to the presence of two *ortho*-substituents, there is, apparently, restricted rotation of the 2,6-dichlorophenyl group around its 1-C–4-C axis, which thereby makes 2-CCl non-equivalent to 6-CCl, and accordingly, 3-CH non-equivalent to 5-CH) δ 163.51 and 163.44 (C), 150.72 (C), 139.16 and 139.11 (CH), 137.26 and 137.19 (C), 134.38 (C), 133.43 (C), 131.06 (CH), 128.96 and 128.93 (CH), 128.62 and 128.57 (CH), 110.94 (C), 87.53 and 87.43 (CH), 85.73 and 85.33 (CH), 85.12 and 84.93 (CH), 71.09 and 71.01 (CH), 63.75 and 63.62 (CH_2), 61.67 and 61.64 (CH_2), 39.84 and 39.74 (CH_2), 38.35 (C), 26.78 (CH_3). HRMS (ESI) $[MH]^+$ $C_{21}H_{27}{}^{35}Cl_2N_2O_6$ calculated: 473.12462 observed: 473.12412; for $[MNa]^+$ $C_{21}H_{26}{}^{35}Cl_2N_2O_6Na$ calculated: 495.10656 observed: 495.10611.

4.1.1.13. 5-[1-(2-Nitrobenzyl)oxymethyl]-2′-deoxyuridine (2m)[18]. NOTE: no TBAF treatment was necessary. Heating **1** (220 mg, 0.339 mmol) with 2-nitrobenzyl alcohol (233 mg, 1.524 mmol) for 20 min at 100–105 °C afforded after purification 21 mg (16%) of product. ^{1}H NMR (400 MHz, CD_3OD): δ 8.12 (s, 1H), 8.04 (dd, 1H, J = 8.2, 1.1 Hz), 7.84 (d, 1H, J = 7.0 Hz), 7.71 (dt, 1H, J = 7.6, 1.1 Hz), 7.52 (m, 1H), 6.30 (t, 1H, J = 6.7 Hz), 4.93 (s, 2H), 4.43 (m, 1H), 4.39 (ab d, 1H, J = 11.8 Hz), 4.34 (ab d, 1H, J = 11.8 Hz), 3.95 (q, 1H, J = 3.4 Hz), 3.82 (ab d, 1H, J = 12.0, 3.8 Hz), 3.75 (ab d, 1H, J = 12.0, 3.3 Hz), 2.29 (m, 2H).

4.1.1.14. 5-[1-(2-Nitrophenyl)ethoxymethyl]-2′-deoxyuridine (2n)[18]. Heating **1** (152 mg, 0.234 mmol) with α-methyl-2-nitrobenzyl alcohol (1-(2-nitro)phenyl-1-ethanol) (176 mg, 1.053 mmol) for 1 h at 104 °C afforded after purification 14 mg (15%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 8.01 and 8.00 (2s, 1H), 7.93 (m, 1H), 7.85 (m, 1H), 7.72 (t, 1H, J = 7.5 Hz), 6.27 (m, 1H), 5.09 (m, 1H), 4.42 (m, 1H), 4.11 (m, 2H), 3.94 (m, 1H), 3.78 (m, 2H), 2.26 (m, 2H), 1.52 and 1.52 (2 d, 3H, J = 6.3 Hz). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers δ 163.63 (C), 150.65 (C), 148.48 (C), 139.71 and 139.68 (CH), 138.99 (C), 133.27 and 133.24 (CH), 128.02 (CH), 127.81 and 127.78 (CH), 123.74 and 123.70 (CH), 110.92 and 110.84 (C), 87.58 and 87.56 (CH), 85.13 and 85.12 (CH), 73.19 and 73.01 (CH), 70.81 and 70.78 (CH), 63.60 and 63.46 (CH_2), 61.40 and 61.38 (CH_2), 39.99 (CH_2), 22.47 (CH_3).

4.1.1.15. 5-[1-(2-Nitrophenyl)ethoxymethyl]-2′-deoxyuridine (2o)[18]. Heating **1** (175 mg, 0.270 mmol) with α-isopropyl-2-nitrobenzyl alcohol (1-(2-nitro)phenyl-2-methyl-1-propanol) (400 mg, 2.050 mmol) for 1 h at 105–114 °C afforded after purification 16 mg (14%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 8.01 and 7.98 (2s, 1H), 7.90 (d, 1H, J = 8.5 Hz), 7.77 (m, 1H), 7.51 (m, 1H), 6.27 (m, 1H)H), 4.78 (m, 1H), 4.41 (m, 1H), 4.13 (m, 2H), 3.94 (m, 1H), 3.77 (m, 2H), 2.25 (m, 2H), 1.96 (m, 1H), 0.97 and 0.96 (2d, 3H, J = 6.7 Hz), 0.88 and 0.86 (2d, 3H, J = 7.0 Hz). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers δ 163.63 and 163.56 (C), 150.66 and 150.64 (C), 149.52 (C), 139.66 and 139.50 (CH), 136.52 and 136.51 (C), 132.56 and 132.54 (CH), 129.01 and 128.95 (CH), 128.05 (CH), 123.71 and 123.65 (CH), 111.05 and 110.86 (C), 87.57 (CH), 85.08 and 85.07 (CH), 81.08 and 80.82 (CH), 70.90 (CH), 64.23 and 63.96 (CH_2), 61.48 and 61.45 (CH_2), 39.95 and 39.90 (CH_2), 34.67 (CH), 18.31 and 18.26 (CH_3), 16.64 and 16.57 (CH_3). HRMS (ESI^+) for $[MH]^+$ $C_{20}H_{26}N_3O_8$ calculated: 436.17144, observed: 436.17149; for $[MNa]^+$ $C_{20}H_{25}N_3O_8Na$ calculated: 458.15339, observed: 458.15342. HRMS (ESI^-) for $[M-H]^-$ $C_{20}H_{24}N_3O_8$ calculated: 434.15689, observed: 434.15669.

4.1.1.16. 5-[1-(2-Nitro)phenyl-1-(cyclohexyl)methoxymethyl]-2′-deoxyuridine (2p). Heating **1** (150 mg, 0.231 mmol) with α-cyclohexyl-2-nitrobenzyl alcohol (440 mg, 1.880 mmol) for 2.5 h at 116 °C followed by purification of bis- and mono-TBS products with subsequent treatment with TBAF (73 mg, 0.231 mmol) afforded after purification 28 mg (25%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 7.99 and 7.96 (2s, 1H), 7.89 (d, J = 8.1 Hz, 1H), 7.71 (m, 2H), 7.50 (d, J = 7.6 Hz, 1H), 6.27 (t, J = 6.6 Hz, 1H), 4.78 (m, 1H), 4.42 (m, 1H), 4.12 (m, 3H), 3.94 (m, 1H), 3.77 (m, 2H), 2.28 (m, 1H), 2.22 (m, 1H), 1.87 (m, 1H), 1.70 (m, 2H), 1.30 (m, 4H), 1.17 (m, 4H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers δ 163.57 (C), 150.65 (C), 149.62 and 149.53 (C), 139.61 and 139.51 (CH), 136.21 (C), 132.48 (CH), 129.11 and 129.05 (CH), 128.02 (CH), 123.65 and 123.59 (CH), 111.02 and 110.86 (C), 87.58 (CH), 85.07 (CH), 80.64 and 80.23 (CH), 70.94 and 70.91 (CH), 64.15 and 63.96 (CH_2), 61.50 (CH_2), 44.50 and 44.47 (CH), 39.92 and 39.89 (CH_2), 29.22 and 29.15 (CH_2), 28.05 and 27.98 (CH_2), 26.09 (CH_2), 25.96 and 25.94 (CH_2), 25.78 (CH_2). HRMS (ESI) for $[MH]^+$ $C_{23}H_{30}N_3O_8$ calculated: 476.20274, observed: 476.20292; for $[MNa]^+$ $C_{23}H_{29}N_3O_8Na$ calculated: 498.18469, observed: 498.18486.

4.1.1.17. 5-[{(2-Nitrophenyl)phenyl}methoxymethyl]-2′-deoxyuridine (*o*-2q). Heating **1** (210 mg, 0.323 mmol) with α-phenyl-2-nitrobenzyl alcohol (phenyl(2-nitrophenyl)methanol) (361 mg, 1.576 mmol) for 2.5 h at 110–117 °C followed by purification of bis- and mono-TBS products with subsequent treatment with TBAF (73 mg, 0.231 mmol) afforded after purification 12 mg (7%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 8.04 and 8.00 (2s, 1H), 7.88 (m, 1H), 7.68 (m, 2H), 7.52 (m, 1H), 7.34 (m, 5H), 6.28 (m, 1H), 6.18 and 6.17 (2s, 1H), 4.42 (m, 1H), 4.30 (m, 3H), 3.94 (m, 1H), 3.78 (m, 2H), 2.28 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers δ 163.71 (C), 159.43 (C), 158.87 (C), 150.64 (C), 140.03 and 139.74 (CH), 136.21 and 136.18 (C), 132.60 (CH), 128.13 (CH), 128.11 (CH), 128.09 (CH), 127.58 (CH), 123.92 (CH), 123.87 (CH), 110.71 (C), 87.59 (CH), 85.13 (CH), 78.29 and 78.16 (CH), 70.82 (CH), 64.01 and 63.91 (CH_2), 61.48 (CH_2), 39.95 (CH_2). HRMS (ES^+ TOF) for $[MNa]^+$ $C_{23}H_{29}N_3O_8Na$ calculated: 492.13820, observed: 492.13830.

4.1.1.18. 5-[{(3-Nitrophenyl)phenyl}methoxymethyl]-2′-deoxyuridine (*m*-2q). Heating **1** (200 mg, 0.308 mmol) with α-phenyl-3-nitrobenzyl alcohol (phenyl(3-nitrophenyl)methanol) (350 mg, 1.673 mmol) for 1 h at 120 °C followed by purification of bis- and mono-TBS products with subsequent treatment with TBAF (49 mg, 0.155 mmol) afforded after purification 16 mg (12%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 8.25 and 8.25 (2s, 1H), 8.11 (m, 1H), 8.07 (m, 1H), 7.79 (m, 1H), 7.55 (dt, 1H, J = 8.0, 1.3 Hz), 7.42 (m, 2H), 7.35 (m, 2H), 7.28 (m, 1H), 6.26 (m, 1H), 5.68 (s, 1H), 4.39 (m, 1H), 4.32 (m, 2H), 3.94 (q, 1H, J = 3.4 Hz), 3.74 (m, 2H), 2.29 (m, 1H), 2.20 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers δ 163.69 (C), 150.66 (C), 148.27 (C), 144.96 (C), 141.11 (C), 139.65 (CH), 132.81 and 132.80 (CH), 129.19 (CH), 128.36 (CH), 127.68 (CH), 126.83 and 126.80 (CH), 121.85 (CH), 121.12 and 121.09 (CH), 110.91 (C), 87.65 (CH), 85.21 and 85.19 (CH), 82.08 and 82.03 (CH), 70.87 (CH), 63.79 and 63.68 (CH_2), 61.45 (CH_2), 40.04 (CH_2). HRMS (ESI^+) for $[MH]^+$ $C_{23}H_{24}N_3O_8$ calculated: 470.15634, observed: 470.15581; for $[MNa]^+$ $C_{23}H_{23}N_3O_8Na$ calculated: 492.13828, observed: 492.13777. HRMS (ESI^-) for $[M-H]^-$ $C_{23}H_{22}N_3O_8$ calculated: 468.14124, observed: 468.14101.

4.1.1.19. 5-[{(4-Nitrophenyl)phenyl}methoxymethyl]-2′-deoxyuridine (*p*-2q). Heating **1** (150 mg, 0.231 mmol) with α-phenyl-3-nitrobenzyl alcohol (phenyl(4-nitrophenyl)methanol) (211 mg,

0.923 mmol) for 20 min at 115 °C followed by treatment with TBAF (113 mg, 0.358 mmol) afforded after purification 7 mg (6%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 8.14 (d, 2H, J = 8.7 Hz), 8.03 (s, 1H), 7.61 (d, 2H, J = 8.7 Hz), 7.37 (m, 2H), 7.30 (m, 2H), 7.23 (m, 1H), 6.23 (m, 1H), 5.62 (s, 1H), 4.36 (m, 1H), 4.26 (m, 2H), 3.90 (m, 1H), 3.71 (m, 2H), 2.25 (m, 1H), 2.15 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers δ 166.94 and 165.11 (C), 152.09 and 151.34 (C), 148.53 and 148.00 (C), 142.41 (C), 140.96 (CH), 141.11 (C), 129.77 (CH), 129.64 and 129.54 (C), 129.13 (CH), 128.81 (CH), 128.32 and 128.29 (CH), 124.44 (CH), 112.35 (C), 89.06 (CH), 86.61 (CH), 83.61 (CH), 72.23 (CH), 65.11 and 65.05 (CH_2), 62.84 (CH_2), 41.47 (CH_2). HRMS (ESI$^+$) for $[MH]^+$ $C_{23}H_{24}N_3O_8$ calculated: 470.15634, observed: 470.15582; for $[MNa]^+$ $C_{23}H_{23}N_3O_8Na$ calculated: 492.13828, observed: 492.13780. HRMS (ESI$^-$) for $[M-H]^-$ $C_{23}H_{22}N_3O_8$ calculated: 468.14124, observed: 468.14112.

4.1.1.20. 5-[1-(2-Nitrophenyl)-3,3-(dimethyl)butoxymethyl]-2′-deoxyuridine (2r). Heating **1** (210 mg, 0.323 mmol) with α-*neo*-pentyl-2-nitrobenzyl alcohol (3,3-dimethyl-1-(2-nitro)phenyl-1-butanol) (480 mg, 2.152 mmol) for 2 h at 120 °C followed by treatment with TBAF (607 mg, 1.925 mmol) afforded after purification 18 mg (12%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 8.02 and 8.00 (2s, 1H), 7.90 (d, 1H, J = 8.2 Hz), 7.82 (m, 1H) 7.70 (t, 1H, J = 7.6 Hz), 7.48 (m, 1H), 6.27 (t, 1H, J = 6.9 Hz), 5.14 (m, 1H), 4.42 (m, 1H), 4.05 (m, 2H), 3.94 (m, 1H), 3.78 (m, 2H), 2.28 (m, 1H), 2.19 (m, 1H), 1.71 (m, 1H), 1.53 (m, 1H), 1.05 and 1.04 (2s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers: δ 163.55 and 163.51 (C), 150.64 and 150.60 (C), 148.26 and 148.22 (C), 139.90 and 139.66 (CH), 139.16 (C), 137.73 (CH), 133.16 and 133.11 (CH), 128.29 and 127.82 (CH), 123.73 and 123.67 (CH), 111.10 and 110.88 (C), 87.67 and 87.58 (CH), 85.07 and 84.97 (CH), 75.02 and 74.74 (CH), 70.89 (CH), 63.25 and 63.13 (CH_2), 61.46 (CH_2), 51.05 and 51.01 (CH_2), 40.05 and 39.93 (CH_2), 30.42 and 30.39 (C), 29.40 and 29.38 (CH_3). HRMS (ESI) for $[MNa]^+$ $C_{22}H_{29}N_3O_8Na$ calculated: 486.18430, observed: 486.18520.

4.1.1.21. 5-[1-(2-Nitrophenyl)-2,2-(dimethyl)propoxymethyl]-2′-deoxyuridine (*o*-2s)[18]. Heating **1** (250 mg, 0.385 mmol) with α-*tert*-butyl-2-nitrobenzyl alcohol (2,2-dimethyl-1-(2-nitro)phenyl-1-propanol) (1.61 g, 7.70 mmol) for 2 h at 120 °C followed by treatment with TBAF (607 mg, 1.925 mmol) afforded after purification 33 mg (19%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 8.01 and 7.99 (2s, 1H), 7.81 (m, 2H), 7.68 (m, 1H), 7.51 (m, 1H), 6.28 (t, 1H, J = 6.9 Hz), 4.98 (s, 1H), 4.42 (m, 1H), 4.20 (m, 2H), 3.94 (m, 1H), 3.76 (m, 2H), 2.26 (m, 2H), 0.85 and 0.84 (2s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers: δ 163.60 and 163.55 (C), 150.89 and 150.75 (C), 150.70 (C), 139.80 and 139.41 (CH), 133.81 (C), 131.76 and 131.74 (CH), 129.91 and 129.82 (CH), 128.14 (CH), 123.56 and 123.43 (CH), 111.01 and 110.74 (C), 87.55 (CH), 85.13 and 85.04 (CH), 81.76 and 81.04 (CH), 70.98 and 70.95 (CH), 64.49 and 64.18 (CH_2), 61.52 and 61.46 (CH_2), 39.86 and 39.78 (CH_2), 36.12 and 36.02 (C), 24.84 and 24.82 (CH_3). HRMS (ESI$^+$) for $[MH]^+$ $C_{21}H_{28}N_3O_8$ calculated: 450.18709, observed: 450.18708; for $[MH]^+$ $C_{21}H_{28}N_3O_8$ calculated: 472.16904, observed: 472.16918. HRMS (ESI$^-$) for $[M-H]^-$ $C_{21}H_{26}N_3O_8$ calculated: 448.17254, observed: 448.17258.

4.1.1.22. 5-[1-(3-Nitrophenyl)-2,2-(dimethyl)propoxymethyl]-2′-deoxyuridine (*m*-2s). Heating **1** (223 mg, 0.343 mmol) with α-*tert*-butyl-2-nitrobenzyl alcohol (2,2-dimethyl-1-(3-nitro)phenyl-1-propanol) (0.575 g, 2.748 mmol) for 45 min at 108–112 °C followed by treatment with TBAF (87 mg, 1.925 mmol) afforded after purification 19 mg (12%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 8.15 (m, 2H), 8.00 (s, 1H), 7.70 (m, 1H), 7.57 (m, 1H), 6.24 (m, 1H), 4.41 (m, 1H), 4.21 and 4.20 (2s, 1H), 4.12 (m, 2H), 3.93 (m, 1H), 3.77 (m, 2H), 2.23 (m, 2H), 0.90 and 0.90 (2s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers: δ 165.00 (C), 152.02 (C), 149.20 (C), 143.71 (C), 140.95 and 140.92 (CH), 135.94 and 135.89 (CH), 129.81 and 129.79 (CH), 124.13 (CH), 123.28 and 123.25 (CH) 112.54 (C), 89.74 and 89.61 (CH), 89.06 and 89.02 (CH), 86.54 and 86.50 (CH), 72.36 and 72.32 (CH), 65.58 (CH_2), 62.86 (CH_2), 41.49 and 41.41 (CH_2), 36.56 (C), 26.46 (CH_3). HRMS (ESI) for $[MH]^+$ $C_{21}H_{28}N_3O_8$ calculated: 450.18709, observed: 450.18711; for $[MNa]^+$ $C_{21}H_{27}N_3O_8Na$ calculated: 472.16958, observed: 472.16903; for $[M-H]^-$ $C_{21}H_{26}N_3O_8$ calculated: 448.17254, observed: 448.17236.

4.1.1.23. 5-[1-(2,6-Dinitrophenyl)-2,2-(dimethyl)propoxymethyl]-2′-deoxyuridine (2t). Heating **1** (259 mg, 0.399 mmol) with α-*tert*-butyl-2,6-dinitrobenzyl alcohol (2,2-dimethyl-1-(2,6-dinitro)phenyl-1-propanol) (342 g, 1.345 mmol) for 10 min at 105 °C followed by treatment with TBAF (314 mg, 0.997 mmol) afforded after purification 23 mg (12%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 8.05 (m, 1H), 8.01 and 7.86 (2s, 1H), 7.74 (m, 2H), 6.39 and 6.34 (2t, J = 6.7 Hz, 1H), 5.20 and 5.19 (2s, 1H), 4.44 (m, 1H), 4.25 (m, 2H), 3.94 (m, 1H), 3.74 (m, 2H), 2.33 (m, 2H), 0.87 (s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers (NOTE: due to the presence of two *ortho*-substituents, there is, apparently, restricted rotation of the 2,6-dinitrophenyl group around its 1-C–4-C axis, which thereby makes 2-CNO_2 non-equivalent to 6-CNO_2, and accordingly, 3-CH non-equivalent to 5-CH): δ 163.62 and 163.55 (C), 150.89 and 150.74 (C), 151.25 (C), 151.13 (C), 140.62 and 139.45 (CH), 130.13 and 130.04 (CH), 128.06 and 128.01 (CH), 126.26 and 126.16 (CH), 125.47 and 125.32 (C), 109.97 and 109.84 (C), 87.55 and 87.35 (CH), 85.05 and 84.56 (CH), 82.85 and 82.05 (CH), 71.07 and 70.96 (CH), 66.23 and 65.85 (CH_2), 61.69 (CH_2), 39.82 and 39.50 (CH_2), 37.88 and 37.83 (C), 25.72 (CH_3). HRMS (ESI) for $[MH]^+$ $C_{21}H_{27}N_4O_{10}$ calculated: 495.17217, observed: 495.17218; for $[M-H]^-$ $C_{21}H_{26}N_4O_{10}$ calculated: 493.15762, observed: 493.15754.

4.1.1.24. 5-[1-(2-Methoxyphenyl)-2,2-(dimethyl)propoxymethyl]-2′-deoxyuridine (*o*-2u). Heating **1** (250 mg, 0.385 mmol) with α-*tert*-butyl-2-methoxybenzyl alcohol (3,3-dimethyl-1-(2-methoxy)phenyl-1-propanol) (625 mg, 3.460 mmol) for 2.5 h at 114-128 °C followed by treatment with TBAF (303 mg, 0.963 mmol) afforded after purification 94 mg (56%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 7.82 and 7.81 (2s, 1H), 7.33 (d, 1H, J = 7.8 Hz), 7.21 (m, 1H), 6.90 (m, 2H), 6.25 (m, 1H), 4.62 and 4.61 (2s, 1H), 4.40 (m, 1H), 4.01 (m, 3H), 3.79 (s, 3H), 3.73 (m, 2H), 2.28 (m, 1H), 2.17 (m, 1H), 0.89 and 0.88 (2s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers δ 163.53 (C), 157.92 (C), 150.71 (C), 138.62 and 138.27 (CH), 128.33 (CH), 127.93 (CH), 127.78 (C), 119.65 (CH), 111.82 and 111.56 (C), 109.90 (CH), 87.51 (CH), 85.10 (CH), 81.13 and 80.05 (CH), 71.02 (CH), 63.69 and 63.46 (CH_2), 61.62 (CH_2), 54.33 (CH_3), 39.86 and 39.78 (CH_2), 35.78 and 35.74 (C), 25.21 (CH_3). HRMS (ESI) for $[MH]^+$ $C_{22}H_{31}N_2O_7$ calculated: 435.21258 observed: 435.21261; for $[MNa]^+$ $C_{22}H_{30}N_2O_7Na$ calculated: 457.19452 observed: 457.19451.

4.1.1.25. 5-[1-(3-Methoxyphenyl)-2,2-(dimethyl)propoxymethyl]-2′-deoxyuridine (*m*-2u). Heating **1** (150 mg, 0.231 mmol) with α-*tert*-butyl-3-methoxybenzyl alcohol (3,3-dimethyl-1-(3-methoxy)phenyl-1-propanol) (200 mg, 1.030 mmol) for 2 h at 120 °C followed by treatment with TBAF (182 mg, 0.578 mmol) afforded after purification 5 mg (5%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ

7.91 and 7.91 (2s, 1H), 7.22 (m, 1H), 6.85 (m, 3H), 6.27 (m, 1H), 4.42 (2s, 1H), 4.09 (m, 3H), 3.95 (m, 1H), 3.81 and 3.81 (2s, 3H), 3.77 (m, 2H), 2.27 (m, 2H), 0.92 (s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers δ 159.22 and 159.19 (C), 150.73 and 150.68 (C), 141.36 and 141.17 (C), 138.86 and 138.72 (CH), 128.12 and 128.10 (CH), 120.77 and 120.67 (CH), 115.38 and 115.31 (C), 113.72 and 113.64 (CH), 112.31 (CH), 111.82 and 111.56 (C), 89.60 and 89.45 (CH), 87.59 and 87.52 (CH), 85.15 and 84.98 (CH), 71.00 and 70.90 (CH), 64.07 and 63.66 (CH_2), 61.58 and 61.51 (CH_2), 54.20 (CH_3), 39.94 and 39.89 (CH_2), 35.10 and 35.08 (C), 25.45 and 25.43 (CH_3). HRMS (ESI$^+$) for $[MH]^+$ $C_{22}H_{31}N_2O_7$ calculated: 435.21258 observed: 435.21259; for $[MNa]^+$ $C_{22}H_{30}N_2O_7Na$ calculated: 457.19452 observed: 457.19450. HRMS (ESI$^-$) for $[M-H]^-$ $C_{22}H_{29}N_2O_7$ calculated: 433.19802 observed: 433.19809.

4.1.1.26. 5-[1-(4-Methoxyphenyl)-2,2-(dimethyl)propoxymethyl]-2′-deoxyuridine (*p*-2u). Heating **1** (346 mg, 0.539 mmol) with α-*tert*-butyl-4-methoxybenzyl alcohol (3,3-dimethyl-1-(4-methoxy)phenyl-1-propanol) (620 mg, 2.150 mmol) for 2.5 h at 120 °C followed by treatment with TBAF (870 mg, 2.762 mmol) afforded after purification 11 mg (5%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 7.89 and 7.87 (s, 1H), 7.19 (d, J = 8.6 Hz, 2H), 6.87 (m, 2H), 6.27 (m, 1H), 4.40 (m, 1H), 4.05 (m, 3H), 3.94 (m, 1H), 3.78 and 3.78 (2s, 3H), 3.76 (m, 2H), 2.28 (m, 1H), 2.18 (m, 1H), 0.88 (s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers δ 163.75 (C), 159.05 (C), 150.80 (C), 138.62 and 138.51 (CH), 131.39 and 131.32 (C), 129.24 and 129.22 (CH), 112.62 (CH), 111.78 and 111.72 (C), 89.28 and 89.16 (CH), 87.57 and 87.49 (CH), 85.15 and 85.00 (CH), 71.01 and 70.91 (CH), 63.62 and 63.38 (CH_2), 61.61 and 61.57 (CH_2), 54.28 (CH_3), 39.93 and 39.83 (CH_2), 35.20 (C), 25.38 (CH_3). HRMS (ES$^+$ TOF) $[MNa]^+$ $C_{22}H_{30}N_2O_7Na$ calculated: 457.19510 observed: 457.19490.

4.1.1.27. 5-[1-(4-Iodo-2-nitrophenyl)-2,2-(dimethyl)propoxymethyl]-2′-deoxyuridine (2v). Heating **1** (400 mg, 0.616 mmol) with α-*tert*-butyl-4-iodo-2-nitrobenzyl alcohol (2,2-dimethyl-1-(4-iodo-2-nitro)phenyl-1-propanol) (717 mg, 2.140 mmol) for 2 h at 120 °C followed by treatment with TBAF (607 mg, 1.925 mmol) afforded after purification 163 mg (28%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 8.14 (m, 1H), 8.00 (m, 2H), 7.51 (d, J = 8.4 Hz, 1H), 6.26 (m, 1H), 4.42 (m, 1H), 4.19 (m, 2H), 3.94 (m, 1H), 3.76 (m, 2H), 2.25 (m, 2H), 0.82 and 0.80 (2s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers δ 164.98 and 164.92 (C), 152.34 and 152.24 (C), 152.05 (C), 142.25 and 142.21 (CH), 141.38 and 141.11 (CH), 135.01 and 134.97 (C), 133.45 and 133.35 (CH), 133.05 and 132.96 (CH), 112.17 and 111.94 (C), 92.91 (C), 88.95 and 88.94 (CH), 86.62 and 86.48 (CH), 82.93 and 82.36 (CH), 72.34 and 72.27 (CH), 65.91 and 65.66 (CH_2), 62.85 and 62.80 (CH_2), 41.32 and 41.24 (CH_2), 37.45 and 37.36 (C), 26.15 (CH_3). HRMS (ESI) for $[MH]^+$ $C_{21}H_{27}IN_3O_8$ calculated: 576.08428, observed: 576.08383; for $[MNa]^+$ $C_{21}H_{26}IN_3O_8Na$ calculated: 598.06623, observed: 598.06581.

4.1.1.28. 5-[1-(Isopropyl)-2-(methyl)-1-propoxymethyl]-2′-deoxyuridine (2w). Heating **1** (250 mg, 0.385 mmol) with 2, 4-dimethyl-3-pentanol (0.894 g, 7.70 mmol) for 2 h at 124 °C followed by treatment with TBAF (303 mg, 0.963 mmol) afforded after purification 33 mg (24%) of product. ^{1}H NMR (400 MHz, CD_3OD) δ 8.03 (s, 1H), 6.30 (t, 1H, J = 6.7 Hz), 4.40 (m, 1H), 4.34 (ab d, 1H, J = 11.7), 4.29 (ab d, 1H, J = 11.7), 3.93 (q, 1H, J = 3.5), 3.76 (ab dd, 1H, J = 11.9, 3.6), 3.72 (ab dd, 2H, J = 11.9, 3.7), 2.87 (t, 1H, J = 5.7), 2.29 (m, 1H), 2.23 (m, 1H), 1.84 (m, 2H), 0.94 (m, 12H). ^{13}C NMR (100 MHz, CD_3OD) δ 163.64 (C), 150.76 (C), 139.02 (CH), 109.98 (C), 90.32 (CH), 87.57 (CH), 85.10 (CH), 70.93 (CH), 67.12 (CH_2), 61.54 (CH_2), 39.93 (CH_2), 30.57 (CH), 19.30 (CH_3), 16.73 (CH_3). HRMS (ESI) for $[MH]^+$ $C_{17}H_{29}N_2O_6$ calculated: 357.20201, observed: 357.20210; for $[MNa]^+$ $C_{17}H_{28}N_2O_6Na$ calculated: 379.18396, observed: 379.18400.

4.1.2. N^3-*tert*-Butyloxycarbonyl-5-(di-*tert*-butylcarbinol)oxymethyl-3′,5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxyuridine

Compound **1** (255 mg, 0.392 mmol) and di-*tert*-butylcarbinol (453 mg, 1.140 mmol) were placed in an iron screw-top vial equipped with a ball followed by vigorous shaking at room temperature for 20 h under argon atmosphere. The contents of the vial were dissolved in ethyl acetate (1 mL) and mixed with silica (ca. 500 mg). The solvent was evaporated, and the powder was applied onto a chromatography column (SiO_2, hexane/ethyl acetate = 15:1 to 6:1) to afford 50 mg (18%) of crude product. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (s, 1H), 6.27 (t, 1H, J = 6.7 Hz), 4.49 (m, 1H), 4.44 (m, 2H), 3.95 (q, 2H, J = 3.4 Hz), 3.92 (ab d, 1H, J = 11.0 Hz), 3.75 (ab d, 1H, J = 11.0 Hz), 2.82 (s, 1H), 2.33 (m, 2H), 1.62 (s, 9H), 1.05 (s, 18H), 0.91 (s, 18H), 0.10 (2s, 6H), 0.09 (s, 6H). The product was not further characterized but introduced into the subsequent transformation as is.

4.1.3. 5-(Di-*tert*-butylcarbinol)oxymethyl-3′,5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxyuridine

Intermediate from previous reaction (50 mg, 0.070 mmol) was placed into a round bottom flask and purged with argon for 10 min. Anhydrous acetonitrile (10 mL) and magnesium perchlorate (2 mg, 0.009 mmol) were added, and the reaction mixture was stirred at reflux for 2.5 h under argon atmosphere. The solvent was removed under reduced pressure; the crude product was dissolved in ethyl acetate (1 mL) and mixed with silica (ca. 500 mg). The solvent was evaporated, and the powder was applied onto a chromatography column (SiO_2, hexane/ethyl acetate = 8:1 to 4:1) to afford 22 mg (51%) of product. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.50 (s, 1H), 6.31 (dd, 1H, J = 7.7, 5.9 Hz), 4.41 (s, 2H), 4.38 (m, 1H), 3.95 (m, 1H), 3.77 (ab dd, 1H, J = 10.6, 4.7 Hz), 3.56 (ab dd, 1H, J = 10.6, 7.0 Hz), 2.80 (s, 1H), 2.32 (m, 1H), 1.90 (m, 1H), 1.03 (s, 18H), 0.90 and 0.89 (2s, 18H), 0.09 and 0.07 (2s, 12H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.43 (C), 149.85 (C), 135.41 (CH), 107.73 (C), 96.13 (CH), 87.64 (CH), 85.31 (CH), 72.69 (CH), 68.70 (CH_2), 63.58 (CH_2), 40.04 (CH_2), 38.75 (C), 29.45 (CH_3), 29.20 (CH_3), 25.92 (CH_3), 17.98 (C), −4.70 (CH_3), −5.39 (CH_3).

4.1.4. 5-(Di-*tert*-butylcarbinol)oxymethyl-2′-deoxyuridine (2x)

Intermediate from previous reaction (22 mg, 0.036 mmol) was dissolved in tetrahydrofuran (2.5 mL) chilled at 0 °C by means of ice-water bath. Tetra-*n*-butylammonium fluoride trihydrate (28 mg, 0.090 mmol) was added, and the reaction mixture was stirred for 24 h while gradually warming up to room temperature. The solvent was removed under reduced pressure; the crude product was dissolved in dichloromethane/methanol = 10:1 (1 mL) and was mixed with silica (ca. 200 mg). The solvent was evaporated, and the powder was applied onto a chromatography column (SiO_2, dichloromethane/methanol = 1:0 to 20:1) to afford 6 mg (43%) of product. ^{1}H NMR (500 MHz, CD_3OD) δ 7.93 (s, 1H), 6.32 (t, 1H, J = 6.7 Hz), 4.39 (m, 3H), 3.93 (m, 1H), 3.72 (d, 2H, J = 4.0 Hz), 2.84 (s, 1H), 2.31 (m, 1H), 2.19 (m, 1H), 1.06 (s, 18H). ^{13}C NMR (125 MHz, CD_3OD) δ 163.49 (C), 150.77 (C), 137.59 (CH), 112.33 (C), 94.70 (CH), 87.49 (CH), 85.03 (CH), 71.13 (CH), 68.51 (CH_2), 61.77 (CH_2), 39.71 (CH_2), 38.28 (C), 28.35 (CH_3). HRMS (TOF ES$^+$) for $[MNa]^+$ $C_{19}H_{32}N_2O_6Na^+$ calculated: 407.21580, observed: 407.21600.

4.1.5. Sonogashira reactions

4.1.5.1. 5-[1-(4-{2-Phenylacetylenyl}-2-nitrophenyl)-2,2-(dimethyl)-1-propoxymethyl]-2′-deoxyuridine (3a). Treatment of

38 mg (0.067 mmol) of **2v** with phenylacetylene (21 mg, 0.201 mmol), diisopropylethylamine (148 mg, 1.145 mmol), copper(I) iodide (2.5 mg, 0.013 mmol), and tetrakis(triphenylphosphine)palladium(0) (8 mg, 0.007 mmol) in anhydrous DMF (1 mL) under argon atmosphere for 24 hours followed by solvent removal *in vacuo* and purification using ethyl acetate/methanol = 20:1 system yielded 12 mg (33%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 8.02 and 8.00 (2s, 1H), 7.93 (m, 1H), 7.77 (m, 2H), 7.55 (m, 2H), 7.39 (m, 2H), 6.27 (m, 1H), 4.95 and 4.94 (2s, 1H), 4.42 (m, 1H), 4.19 (m, 2H), 3.93 (m, 1H), 3.76 (m, 2H), 2.25 (m, 2H), 0.84 and 0.83 (2s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers: δ 164.97 and 164.91 (C), 152.07 (C), 151.95 (C), 141.44 and 141.02 (CH), 135.58 and 135.55 (CH), 135.34 and 135.31 (C), 132.75 (CH), 131.71 and 131.62 (CH), 130.14 (CH), 129.64 (CH), 127.52 and 127.42 (CH), 125.00 (C), 123.58 (C), 112.27 and 112.02 (C), 92.61 (C), 88.96 (CH), 87.63 (C), 86.61 and 86.50 (CH), 83.06 and 82.47 (CH), 72.35 and 72.29 (CH), 65.97 and 65.70 (CH_2), 62.87 and 62.83 (CH_2), 41.31 and 41.23 (CH_2), 37.66 and 37.57 (C), 26.24 (CH_3). HRMS (ESI$^+$) for $[MH]^+$ $C_{29}H_{32}N_3O_8$ calculated: 550.21856, observed: 550.21839; for $[MNa]^+$ $C_{29}H_{31}N_3O_8Na$ calculated: 572.20034, observed: 572.20050. HRMS (ESI$^-$) for $[M-H]^-$ $C_{29}H_{30}N_3O_8$ calculated: 548.20384, observed: 548.20335.

4.1.5.2. 5-[1-(4-{3-Methoxy}proyn-1yl-2-nitrophenyl)-2,2-(dimethyl)-1-propoxymethyl]-2′-deoxyuridine (3b). Treatment of 24 mg (0.042 mmol) of **2v** with methyl propargyl ether (15 mg, 0.209 mmol), diisopropylethylamine (38 mg, 0.294 mmol), copper(I) iodide (1.5 mg, 0.008 mmol), and tetrakis(triphenylphosphine)palladium(0) (5 mg, 0.004 mmol) in anhydrous DMF (1 mL) under argon atmosphere for 72 hours followed by solvent removal *in vacuo* and purification using dichloromethane/methanol = 1:0 to 30:1 system yielded 10 mg (64%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 8.02 and 8.00 (2s, 1H), 7.87 (m, 1H), 7.76 (ab d, J = 8.2 Hz, 1H), 7.70 (m, 1H), 6.25 (m, 1H), 4.94 (s, 1H, note: overlapped with HDO), 4.40 (m, 1H), 4.35 (s, 2H), 4.17 (m, 2H), 3.92 (m, 1H), 3.75 (m, 2H), 3.44 (s, 3H), 2.25 (m, 2H), 0.84 and 0.82 (2s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers: δ 163.62 and 163.56 (C), 150.68 (C), 140.02 and 139.71 (CH), 137.15 and 138.83 (C), 134.37 and 134.33 (CH), 134.26 and 134.21 (C), 130.35 and 130.25 (CH), 126.22 and 126.22 (CH), 126.03 (CH), 110.81 and 110.56 (C), 87.60 and 87.34 (CH), 85.18 and 85.07 (CH), 83.21 (C), 81.61 and 81.03 (CH), 80.99 (C), 70.97 and 70.90 (CH), 64.58 and 64.30 (CH_2), 61.47 and 61.43 (CH_2), 59.42 (CH_2), 56.60 (CH_3), 39.92 and 39.84 (CH_2), 36.24 and 36.14 (C), 24.80 (CH_3). HRMS (ESI) for $[MH]^+$ $C_{25}H_{32}N_3O_9$ calculated: 518.21385, observed: 518.21342; for $[MNH_4]^+$ $C_{25}H_{35}N_4O_9$ calculated: 535.24040, observed: 535.23993; $[MNa]^+$ $C_{25}H_{31}N_3O_9Na$ calculated: 540.19580, observed: 540.19529.

4.1.5.3. 5-[1-(4-{3-(7-Coumarin)oxy}proyn-1-yl-2-nitrophenyl)-2,2-(dimethyl)propoxymethyl]-2′-deoxyuridine (3c). Treatment of 68 mg of **2v** with 7-(propargyl)oxycoumarin (48 mg, 0.239 mmol), triethylamine (62 mg, 0.617 mmol), copper(I) iodide (3 mg, 0.016 mmol), and tetrakis(triphenylphosphine)palladium(0) (9 mg, 0.008 mmol) in anhydrous DMF (2 mL) for 4.5 hours under argon atmosphere followed by removal of the solvent *in vacuo* and purification using dichloromethane/methanol = 1:0 to 10:1 system yielded 33 mg (64%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3CN) for diastereomers: δ 9.07 (br s, 1H), 7.88 (s, 1H), 7.84 and 7.81 (2 d, J = 9.5 Hz, 1H), 7.70 (m, 2H), 7.58 (d, J = 8.4 Hz, 1H), 7.04 (m, 1H), 7.01 and 6.89 (2 d, J = 2.6 Hz, 1H), 6.26 (d, J = 9.5 Hz, 1H), 6.17 (m, 1H), 5.10 (s, 2H), 4.90 and 4.89 (2s, 1H), 4.35 (m, 1H), 4.07 (m, 3H), 3.86 (m, 1H), 3.66 (m, 2H), 3.45 (br s, 1H), 3.16 (br s, 1H), 2.20 (m, 2H, overlapped with H_2O), 0.82 and 0.81 (2s, 9H). ^{13}C NMR (100 MHz, CD_3CN) for diastereomers: δ 162.34 and 162.32 (C), 160.66 and 160.51 (C), 155.69 (C), 150.56 (C), 150.34 (C), 150.23 (C), 143.69 (CH), 139.44 and 139.28 (CH), 134.82 and 134.79 (CH), 130.59 and 130.53 (CH), 129.41 (CH), 126.74 and 126.69 (CH), 122.09 (C), 115.80 (C), 115.62 and 115.51 (C), 113.39 and 113.36 (CH), 112.65 (CH), 110.68 and 110.52 (C), 101.99 (CH), 87.34 (CH), 85.77 (C), 85.10 and 84.99 (CH), 84.70 (C), 81.53 and 81.14 (CH), 70.89 and 70.83 (CH), 64.54 and 64.40 (CH_2), 61.59 (CH_2), 56.69 (CH_2), 39.80 (CH_2), 36.34 and 36.29 (C), 25.01 (CH_3). HRMS (ESI) for $[MNa]^+$ $C_{33}H_{33}N_3O_{11}Na$ calculated: 670.20130, observed: 670.20310.

4.1.5.4. 5-[1-(4-{Trimethylsilyl}acetylenyl-2-nitrophenyl)-2,2-(dimethyl)-1-propoxymethyl]-2′-deoxyuridine (3d). Treatment of 87 mg (0.152 mmol) of **2v** with (trimethylsilyl)acetylene (60 mg, 0.607 mmol), diisopropylethylamine (157 mg, 1.124 mmol), copper(I) iodide (5.6 mg, 0.030 mmol), and tetrakis(triphenylphosphine)palladium(0) (17 mg, 0.015 mmol) in anhydrous DMF (3 mL) for 4.5 hours under argon atmosphere followed by removal of the solvent *in vacuo* and purification using ethyl acetate/methanol = 1:0 to 100:1 system yielded 32 mg (38%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 8.01 and 8.00 (2s, 1H), 7.84 (m, 1H), 7.74 (ab d, J = 8.1 Hz, 1H), 7.68 (m, 1H), 6.26 (m, 1H), 4.93 and 4.92 (2s, 1H), 4.40 (m, 1H), 4.18 (m, 2H), 3.92 (m, 1H), 3.74 (m, 2H), 2.24 (m, 2H), 0.83 and 0.82 (2s, 9H), 0.26 (s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers: δ 165.00 and 164.95 (C), 152.10 and 152.00 (C), 151.86 (C), 141.14 and 141.14 (CH), 135.86 and 135.75 (CH), 131.73 and 131.63 (CH), 129.91 (C), 127.84 and 127.74 (CH), 124.83 (C), 112.22 and 111.98 (C), 103.33 (C), 97.79 (C), 89.01 (CH), 86.61 and 86.51 (CH), 83.02 and 82.42 (CH), 72.38 and 72.31 (CH), 66.00 and 65.70 (CH_2), 62.88 and 62.84 (CH_2), 41.33 and 41.25 (CH_2), 37.66 and 37.56 (C), 26.20 (CH_3), -0.25 (CH_3). HRMS (ESI) for $[MH]^+$ $C_{26}H_{36}N_3O_8Si$ calculated: 546.22717, observed: 546.22670; $[MNa]^+$ $C_{26}H_{35}N_3O_8SiNa$ calculated: 568.20911, observed: 568.20911.

4.1.6. 5-[1-(4-Acetylenyl-2-nitrophenyl)-2,2-(dimethyl)-1-propoxymethyl]-2′-deoxyuridine (3e)

Compound **3d** (114 mg, 0.209 mmol) was dissolved in tetrahydrofuran (4 mL) followed by addition of tetra-*n*-butylammonium fluoride trihydrate (90 mg, 0.314 mmol). The reaction mixture was stirred for 6 h, then concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel using ethyl acetate/methanol = 100:1 to afford 73 mg (74%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 8.02 and 8.00 (2s, 1H), 7.90 (m, 1H), 7.76 (ab d, J = 8.2 Hz, 1H), 7.65 (m, 1H), 6.26 (m, 1H), 4.94 and 4.93 (2s, 1H), 4.40 (m, 1H), 4.18 (m, 2H), 3.92 (m, 1H), 3.74 (m, 3H), 2.24 (m, 2H), 0.84 and 0.82 (2s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers: δ 163.60 and 163.55 (C), 150.69 and 150.61 (C), 150.48 (C), 140.01 and 139.91 (CH), 134.73 and 134.52 (CH), 130.94 and 130.26 (CH), 128.46 (C), 126.71 and 126.61 (CH), 122.79 (C), 110.82 and 110.57 (C), 87.61 (CH), 85.20 and 85.10 (CH), 81.61 and 81.01 (CH), 80.48 (C), 80.17 (CH), 70.97 and 70.90 (CH), 64.57 and 64.30 (CH_2), 61.47 and 61.43 (CH_2), 39.93 and 39.86 (CH_2), 36.93 and 36.14 (C), 24.78 (CH_3). HRMS (ESI) for $[MH]^+$ $C_{23}H_{28}N_3O_8$ calculated: 474.18764, observed: 474.18715; $[MNa]^+$ $C_{23}H_{27}N_3O_8Na$ calculated: 496.16958, observed: 496.16915.

4.1.7. 5-[1-{4-(1-Benzyl-1,2,3-triazo-4-yl)-2-nitrophenyl}-2,2-(dimethyl)propoxymethyl]-2′-deoxyuridine (4)

Compound **3e** (3.8 mg, 0.008 mmol) was dissolved in acetonitrile (2 mL) followed by addition of benzyl azide (2 mg, 0.014 mmol), diisopropylethylamine (10 mg, 0.08 mmol) and copper(I) iodide

(0.1 mg, 0.0008 mmol). The reaction mixture was stirred for 2 h under argon atmosphere at room temperature. The reaction mixture was then concentrated under reduced pressure and the residue was purified by column chromatography on silica gel using dichloromethane/methanol = 1:0 to 30:1 to afford 3.7 mg (76%) of product as 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, CD_3OD) for diastereomers: δ 8.49 (s, 1H), 8.27 (m, 1H), 8.07 (m, 1H), 8.01 and 7.99 (2s, 1H), 7.82 (d, 1H, J = 8.3 Hz), 7.37 (m, 5H), 6.24 (m, 1H), 5.66 (s, 2H), 4.96 and 4.95 (2s, 1H), 4.39 (m, 1H), 4.20 (m, 2H), 3.91 (m, 1H), 3.73 (m, 2H), 2.24 (m, 2H), 0.85 and 0.84 (2s, 9H). ^{13}C NMR (100 MHz, CD_3OD) for diastereomers: δ 165.03 and 164.96 (C), 152.63 and 152.48 (C), 152.09 (C), 146.82 (C), 141.34 and 141.05 (CH), 136.62 (C), 134.85 (C), 134.42 and 134.40 (C), 132.16 and 132.06 (CH), 133.10 (CH), 129.75 (CH), 129.69 (CH), 129.19 (CH), 123.32 (CH), 121.67 and 121.55 (CH), 112.32 and 112.06 (C), 88.97 (CH), 86.48 and 86.36 (CH), 83.11 and 82.47 (CH), 72.36 and 72.32 (CH), 65.98 and 65.67 (CH_2), 62.90 and 62.84 (CH_2), 55.18 (CH_2), 41.29 and 41.20 (CH_2), 37.63 and 37.53 (CH), 26.26 (CH_3). HRMS (ESI) for $[MH]^+$ $C_{30}H_{35}N_6O_8$ calculated: 607.25164, observed: 607.25119; for $[MNa]^+$ $C_{30}H_{34}N_6O_8Na$ calculated: 629.23358, observed: 629.23311.

4.1.8. Tetra-triethylammonium 5-[(*R*/*S*)-1-(4-{2-phenylacetylenyl}-2-nitrophenyl)-2,2-(dimethyl)-1-propoxymethyl]-2′-deoxyuridine-5′-triphosphate (3aTP)

Standard procedure was used as previously described.[18] $POCl_3$ (15 μL, 0.163 mmol) was added to a solution of **3a** (19 mg, 0.035 mmol) and proton sponge (30 mg, 0.140 mmol) in trimethylphosphate (0.7 mL) at 0 °C and stirred for two hours under argon atmosphere. Reaction progress was monitored by HPLC and reverse-phase TLC (C18). Additional $POCl_3$ (10 μL, 0.111 mmol) was added, and the mixture was stirred for another one hour. A solution of tri-*n*-butylammonium pyrophosphate (200 mg, 0.366 mmol) and tri-*n*-butylamine (95 μL) in anhydrous DMF (1.0 mL) was added. After five minutes of stirring, triethylammonium bicarbonate buffer (1 M, pH 7.5; 8 mL) was added and then stirred at room temperature for one hour. The reaction was lyophilized to dryness, and the residue was dissolved in water (5 mL), filtered, and purified by reverse phase chromatography using triethylammonium bicarbonate buffer as eluent. Fractions containing the triphosphate (identified by HRMS) were combined and lyophilized to give product in ca 4 mg (10%) as a solid. ^{1}H NMR (400 MHz, D_2O) for diastereomers: δ 7.90 and 7.89 (2s, 1H), 7.82 (m, 1H), 7.73 (m, 2H), 7.52 (m, 2H), 7.40 (m, 2H), 6.21 (m, 1H), 4.88 and 4.88 (2s, 1H), 4.38 (m, 1H), 4.29 (m, 2H), 3.92 (m, 1H), 3.72 (m, 2H), 2.53 (m, 24H), 1.44 (m, 24H), 1.27 (m, 24H), 0.89 (t, 36H, J = 7.3 Hz), 0.79 and 0.77 (2s, 9H). ^{31}P NMR (162 MHz, D2O): δ −3.53 (d, J = 17.8 Hz), −14.63 (d, J = 17.8 Hz), −25.32 (m). HRMS (ESI-TOF): For $[M-H]^-$ $C_{29}H_{33}N_3O_{17}P_3$ calculated: 788.10283, observed: 788.10272.

4.2. Cell cytotoxicity assay (MTT)

MCF7 cells were grown in RPMI 1640 media supplemented with 10% fetal bovine serum, 1% penicillin, 10 nM estrogen and 1 mM insulin. Cells were trypsinized and resuspended at a density of 2.2×10^4 cells per mL. 500 μL of this suspension was added to each well in a 24 well plate. The plates were incubated at 37 °C and 5% CO_2 atmosphere overnight. The media was changed, and plates were dosed in triplicate with compound dissolved in DMSO (DMSO concentration not exceeding 0.5%). Cells were dosed to a final concentration of 200, 100, 50, 25, and 6.25 μM of compound. 5-Fluorouracil was used as a positive control[31] and dosed in the same manner. Plates were incubated for 65 h prior to the addition of MTT solution. 500 μL of a 193 μg/mL MTT and media solution was added to each well. Plates were incubated for 3 h. The MTT solution was removed and 500 μL of DMSO was added to each well. Cells were imaged using GS 800 Bio Rad scanner with Quality One Software or BioTek plate reader with Gene5 software. IC_{50} curves were determined by plotting viability verses compound concentration. Kaleidagraph software was used to calculate the R value for each logarithmic curve fitting. The results are outlined in Table 1, and the IC_{50} curves are in the Supplementary material.

4.3. Detecting double-strand break marker γ-H2AX in MCF7 cells treated with 3a

4.3.1. Protein extraction

Confluent MCF7 cells were treated with **3a** at a concentration equal to the IC_{50} value for 24 h and nuclear protein extracted using a NE-PER Nuclear and Cytoplasmic Extraction Kit (Thermo Scientific) using three independent replicates. Vehicle controls were simultaneously prepared by treatment with DMSO. In detail, cells were harvested by trypsin–EDTA and centrifuged at 500×*g* for 5 min. The cells were washed to remove traces of trypsin by suspending in 1× PBS, followed by centrifugation at 500×*g* for 2–3 min. The supernatant was removed leaving the cell pellet as dry as possible. A 200 μL volume of ice-cold cytoplasmic extraction reagent I (CER-I), treated Halt™ Protease & Phosphatase Inhibitor Cocktail (Thermo Scientific), was added to the pellet. The cell pellet was suspended by vortexing vigorously for 15 s and then incubated on ice for 10 min. The mixture is then treated with 11 μL of ice-cold cytoplasmic extraction reagent II (CER-II) and mixed by vortexing on the highest setting for 5 s followed by incubation on ice of one minute to allow complete release of cytoplasmic contents. The mixture is then vortexed for 5 s followed by centrifugation for 5 min at maximum speed (~16,000×*g*) in a microcentrifuge. The supernatant, containing the cytoplasmic extract is immediately transferred to a pre-chilled tube and placed on ice until storage. The insoluble pellet, containing the nuclei is suspended in ice-cold nuclear extraction reagent (NER), similarly treated with Halt™ Protease & Phosphatase Inhibitor Cocktail (Thermo Scientific). The mixture is vortexed for 15 s at the highest setting and placed on ice for 10 min, with the process repeated every 10 min for a total of 40 min. It is then centrifuged at maximum speed in a microcentrifuge for 10 min. The supernatant, containing the nuclear extract is immediately transferred to a pre-chilled microcentrifuge tube and placed on ice until storage at −80 °C. All centrifugation steps were performed at 4 °C and all cell samples and extracts were kept on ice. Protein concentration in each sample and control was determined by Bradford Assay using Coomassie PlusTM (Bradford) Assay Kit (Thermo Scientific), applying the manufacturer's instruction. In detail, 1000–25 μg/mL concentrations of Albumin Standard by diluting a 2.0 mg/mL stock solution in deionized water, accordingly. The samples were diluted 2× prior to the assay. A volume of 10 μL standard or unknown sample were pipetted into the appropriate wells in a 96-well plate. A volume of 250 μL of the Coomassie Plus Reagent was added to each well and mixed by shaking in a plate shaker for 30 s followed by incubation for 10 min at rt. The absorbance is then measured at 595 nm using a Synergy 4 plate reader (Biotek). The 595 nm measurement for the blank (0 μg/mL protein) was subtracted from the measurements of all other individual standards and unknown sample measurements. A standard curve is prepared by plotting the Blank-corrected measurement for each BSA standard versus its concentration in μg/mL. The standard curve is used to determine the protein concentration of each unknown sample.

4.3.2. Western blot analysis

For Western blot analysis, 20 μg protein from the nuclear extract of each sample and control is loaded in a 10% ExpressPlus™

SDS–PAGE mini-gel (GenScript) and electrophoresed for 1 h to separate the component proteins. The proteins are then transferred into a PVDF membrane using the iBlot Dry Blotting system, conducted at 20 V and 7 min run time. Following transfer, the gel is treated with Coomassie Blue to ensure complete transfer of proteins. The PVDF membrane was wetted in PBS for several minutes. The blocking step was conducted by immersing the membrane in 10 mL Odyssey Blocking Buffer (Licor), with continuous shaking for 1 h. A 1:1000 dilution of the primary antibodies, Rabbit Anti-Histone H2A.X Ab (Cell Signalling Technology) and Mouse Anti -Actin Ab (GenScript), are prepared in 7 mL Odyssey Blocking Buffer. β-Actin, which is present in cells in high levels is used as the loading control, the signal of which is used to normalize the signal of the protein of interest. The membrane is incubated in the diluted primary antibody solution for one hour, with shaking. Following incubation, the membrane is washed 4× for 5 min each at rt in 15 mL PBS + 0.1% Tween 20 (Fisher Scientific) with gentle shaking. The fluorescently labeled secondary antibodies, IRDye 800CW Goat anti-Rabbit antibody (1:15,000 dilution) and IRDye 680RD Goat anti-Mouse Antibody (1:20,000 dilution) are prepared in 10 mL Odyssey Blocking buffer with 0.1% Tween 20 and 0.01% SDS (Fisher Scientific), ensuring minimal exposure to light. After washing with PBS, the membrane was incubated in the secondary antibody solution for 30 min at rt with gentle shaking. The membrane is then washed 4× for 5 min each with 15 mL PBS + 0.1% Tween 20, with gentle shaking and protected from light. To remove residual Tween 20, the membrane is washed with PBS prior to imaging. The membrane is scanned using the Odyssey Infrared Imager (Licor) using the 700 nm channel to detect for β-Actin and the 800 nm channel to detect for γ-H2AX. The intensity of each band was measured using the ImageQuant 5.0 software. All experiments are conducted at $n = 2$.

4.4. DNA synthesis termination studies

Primer extension experiments were performed using a 51-mer nucleotide dsDNA, synthesized from pUC19 plasmid vector (New England Biolabs). A 19-nucleotide forward primer, 5′CACGACGTT GTAAAACGAC3′ was used as a template 5′CACGACGTTGTAAAAC GACGGCCAGTGAATTCGAGCTCGGTACCCGGGGAT3′ (representing 370-420 of pUC 19). The primer was fluorescently-labeled with IRDye700. The oligonucleotide was purified using a Cycle Pure Kit (Omega BioTek). Master mix was made at 2× with a concentration of final in PCR tube reaction of 0.5 μM template, 1× ThermoPol reaction buffer, 0.05 U/μL Vent (*exo*-), and 0.75 μM primer. 100 μM dNTP with varying concentration of Acylco TTP and **3aTP** were added with the master mix. Sequencing reactions were conducted using 10 μL of a solutions containing acyclo-dNTP and dNTP having a final PCR reaction concentration of 2 mM and 100 μM, respectively, followed by the addition of 10 μl master mix. For sequencing the labels on the gel correspond to the sequence produced by the 51-mer template. 10 μL PCR product was mixed with 17 μL PAGE denaturing load dye. After heating at 95 °C for 5 min, 5 μL of the resulting solution was loaded into each well containing a 12% denaturing PAGE gel, which was subsequently run at constant 18 Watts for 35 min. The gel was visualized using the Odyssey Infrared Imaging System (LiCor) with 169 μm resolution and the 700-channel laser source which has a solid-state laser diode at 680 nm and ImageQuant 5.0 software was used to determine density measurements. Experiments were performed in triplicate, and standard errors were calculated.

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Supplementary data

Detailed experimental procedures for synthesis of novel α-substituted benzyl alcohols, NCI-60 human tumor cell line screen full report, DNA synthesis termination data, spectral characterization of molecules (^{1}H, ^{13}C, DEPT ^{13}C, HRMS, HPLC) and the results of MTT assays. This material is available free of charge. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.01.057.

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