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Synthesis and anticancer activity of some 5-fluoro-2'-deoxyuridine phosphoramidates

Marta Lewandowska[a], Piotr Ruszkowski[b], Kinga Chojnacka[a], Natalia Kleczewska[a], Marcin Hoffmann[a], Karol Kacprzak[a], Lech Celewicz[a,*]

[a]*Faculty of Chemistry, Adam Mickiewicz University, Umultowska St 89 b, 61-614 Poznań, Poland*

[b]*Department of Pharmacology, Poznań University of Medical Sciences, Rokietnicka St 5 a, 60-806 Poznań, Poland*

* Corresponding author. Tel.: +48 61 829 1690; fax: +48 61 829 1555. *E-mail address:* celewicz@amu.edu.pl (L. Celewicz).

Abstract

Two series of novel 4-chlorophenyl *N*-alkyl phosphoramidates of 3'-*O*-(*t*-butoxycarbonyl)-5-fluoro-2'-deoxyuridine (3'-BOC-FdU) (**9a**–**9j**) and 5-fluoro-2'-deoxyuridine (FdU) (**10a**–**10j**) were synthesized by means of phosphorylation of 3'-BOC-FdU (**4**) with 4-chlorophenyl phosphoroditriazolide (**7**), followed by a reaction with the appropriate amine. Phosphoramidates **9a**–**9j** were converted to the corresponding **10a**–**10j** by removal of the 3'-*t*-butoxycarbonyl protecting group (BOC) under acidic conditions. The synthesized phosphoramidates **9a**–**9j** and **10a**–**10j** were evaluated for their cytotoxic activity in five human cancer cell lines: cervical (HeLa), nasopharyngeal (KB), breast (MCF-7), liver (HepG2), osteosarcoma (143B) and normal human dermal fibroblast cell line (HDF) using the sulforhodamine B (SRB) assay. Two phosphoramidates **9b** and **9j** with the *N*-ethyl and *N*-(methoxy-(*S*)-alaninyl) substituents, respectively, displayed remarkable activity in all the investigated cancer cells, and the activity was considerably higher than that of the parent nucleoside **4** and FdU. Among phosphoramidates **10a**–**10j** compound **10c** with the *N*-(2,2,2-trifluoroethyl) substituent showed the highest activity. Phosphoramidate **10c** was more active than the FdU in all the cancer cell lines tested.

Keywords: 5-Fluoro-2'-deoxyuridine phosphoramidates, phosphorylation, cytotoxic activity, human cancer cell lines: HeLa, KB, MCF-7, HepG2, 143B, normal human cell line: HDF

1. Introduction

Several pyrimidine and purine derivatives, especially nucleoside analogues, have found important use as anticancer therapeutics.[1,2] In particular, 5-fluoropyrimidine derivatives including 5-fluorouracil (FUra), 5-fluoro-2'-deoxyuridine (floxuridine, FdU) and 5'-deoxy-5-fluoro-*N*^{4}-pentyloxycarbonyl-cytidine (capecitabine, CAP) play a pivotal role among anticancer drugs (Fig. 1).[3,4] These agents are mostly used in the treatment of colon, breast, gastrointestinal and ovary tumors.[5,6] It was established that intracellularly, each of these agents, i.e. FUra, FdU and CAP, is metabolized to 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP), which acts as a potent, irreversible inhibitor of thymidylate synthase (TS), a key enzyme in DNA synthesis.[7]

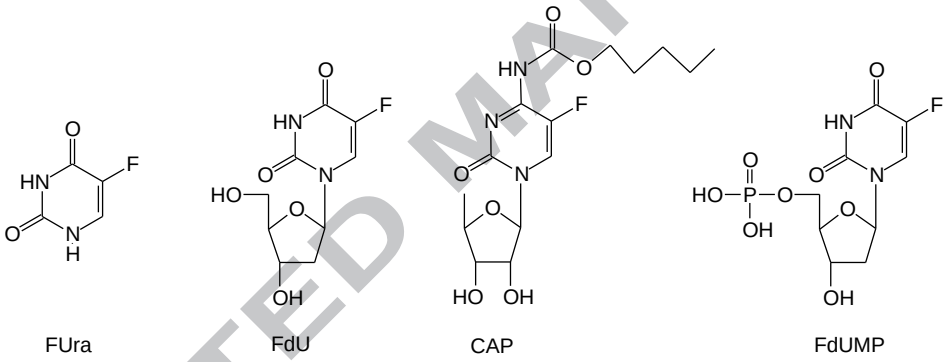


Figure 1. 5-Fluoropyrimidine-based drugs and their active metabolite – FdUMP.

Alternatively, the above mentioned drugs are metabolized to 5-fluoro-2'-deoxyuridine 5'-triphosphate (FdUTP), which can be incorporated into DNA or converted to 5-fluorouridine 5'-triphosphate (FUTP), which in turn can be incorporated into RNA.[7] It has been shown that nucleic acids (DNA and RNA) having thymine or uracil residues, respectively, replaced with a 5-fluorouracil residue are less stable and their function is altered, which leads to the inhibition of cell division.[7] The efficacy of 5-fluoropyrimidine-based drugs is impaired by the frequent development of resistance in tumor cells.[8] One of the main reasons for the occurrence of resistance is decreased levels of thymidine kinase (TK), the enzyme required for the first phosphorylation step of FdU.[8] The direct introduction of FdUMP into cells could therefore circumvent the first phosphorylation step of FdU and possibly the problem of resistance. However, phosphate residues are negatively charged at physiological

pH and FdUMP is too polar to cross the cell membrane.[9,10] In addition, plasma and cell surface phosphohydrolases rapidly convert nucleoside 5'-phosphates into their parent nucleosides.[10] In order to obviate these problems, a considerable effort has been directed into the synthesis of prodrugs (pronucleotides) of FdUMP with a protected 5'-phosphate group.[3,4] These prodrugs are designed to easily penetrate the cell membrane and to release FdUMP inside the cell as a result of chemical or enzymatic hydrolysis.[9,11,12] Moreover, the liberated FdUMP would require only a second and a third phosphorylation for the conversion to FdUTP, the next active metabolite, which can be incorporated into DNA. Several FdUMP prodrugs were synthesized and evaluated for their anticancer activity.[12] Thus, Jones et al. reported the synthesis of a series of cyclic phosphates of FdU and their cytotoxic activity against murine leukemia L1210 cells.[13] Farquhar et al. published synthesis of 5'-(1,3,2-dioxaphosphorinan-2-yl) and 5'-[4-(pivaloyloxy)-1,3,2-dioxaphosphorinan-2-yl] derivatives of FdU.[14,15] Borch et al. described synthesis of series of *N*-haloalkyl phosphoramidate diesters of FdU, which are able to release FdUMP intracellularly.[16-19] Meier et al. reported synthesis of series of cyclosaligenyl FdUMP derivatives as prodrugs of FdUMP.[20] Wagner et al. reported synthesis of *N*-amino acid phosphoramidate monoesters of FdU.[21] Kwon et al. published the synthesis of some sulfonylethyl phosphotriesters of FdU, which release FdUMP by β-elimination reaction.[22] Recently, McGuigan and Balzarini reported synthesis of aryl *N*-amino acid phosphoramidate diesters of FdU.[3,4] Also recently, Jain and Kalman described synthesis of *N*-amino acid 3',5'-cyclic-phosphoramidates of FdU.[23]

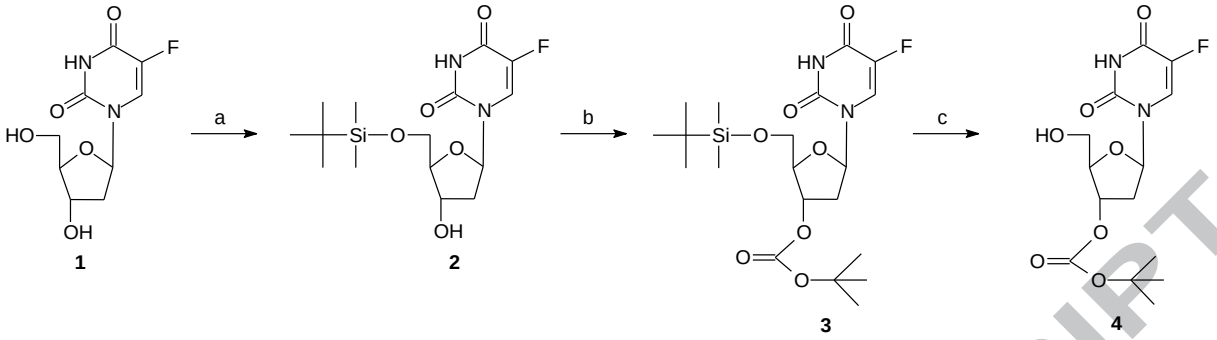
The aim of our study was to synthesize novel phosphoramidate prodrugs of FdU with potential anticancer properties. In this paper, we report the synthesis of 4-chlorophenyl *N*-alkyl phosphoramidate diesters of 3'-*O*-(*t*-butoxycarbonyl)-5-fluoro-2'-deoxyuridine (3'-BOC-FdU) (**9a**–**9j**) and 5-fluoro-2'-deoxyuridine (FdU) (**10a**–**10j**) as well as the evaluation of their cytotoxic activity in five human cancer cell lines: cervical (HeLa), nasopharyngeal (KB), breast (MCF-7) liver (HepG2), osteosarcoma (143B) and normal human dermal fibroblast cell line (HDF).

2. Results and discussion

2.1. Chemistry

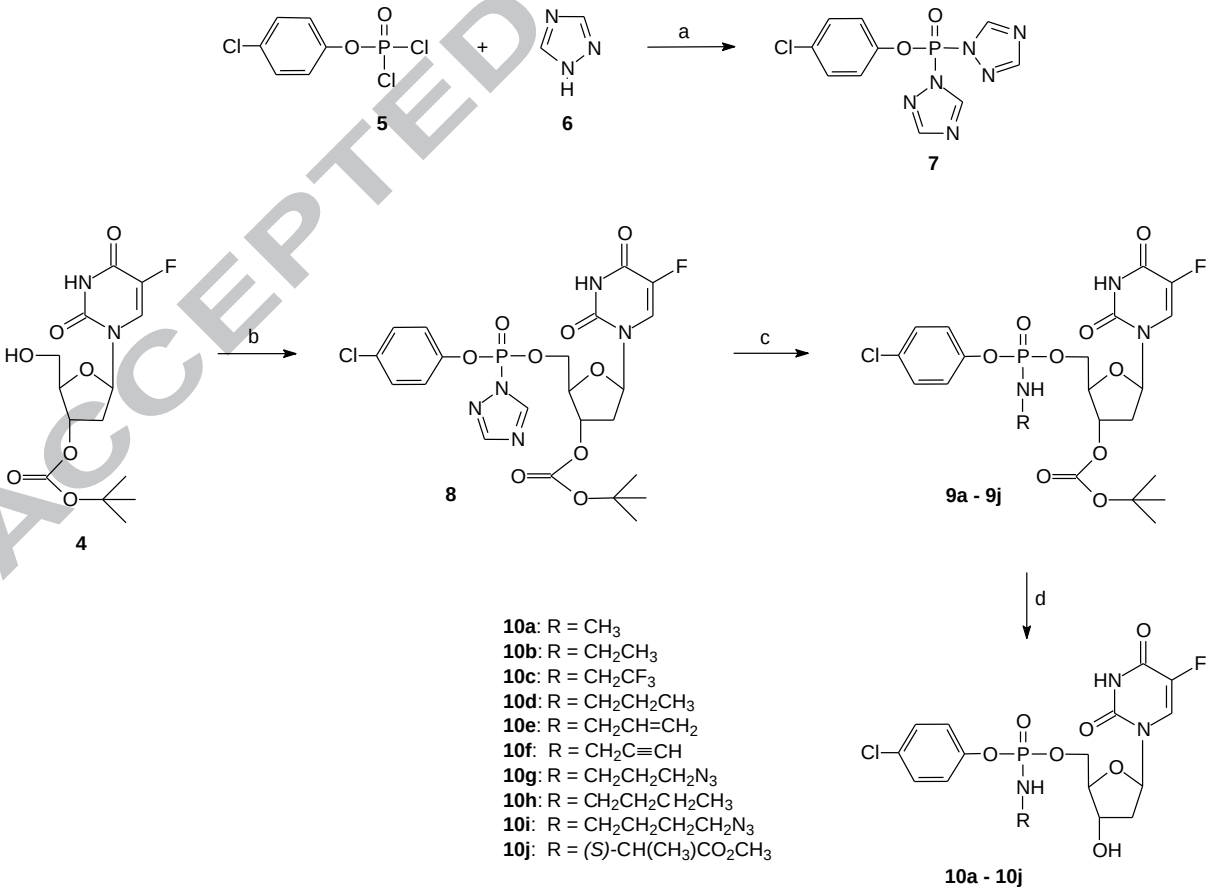
At the beginning of our studies on the synthesis of the 5-fluoro-2'-deoxyuridine phosphoramidates we tried to use for the phosphorylation unprotected at the 3' position FdU but the reaction led to a complex mixture of products. McGuigan and coworkers in their proposition of the synthesis of 5-fluoro-2'-deoxyuridine phosphoramidates also did not protect the 3'-hydroxyl of FdU as a consequence the yield of the reaction was very low in the range of 1-8%.[3] Therefore, we turned our attention on the synthesis of phosphoramidates using as a starting material 5-fluoro-2'-deoxyuridine with the protective group at the 3' position. In our first approach we tried to protect the 3'-hydroxyl of FdU with an acetyl group, but 3'-acetyl-5-fluoro-2'-deoxyuridine was unstable and decomposed during purification by column chromatography on silica gel despite the presence of small amounts of triethylamine in the eluent. Next, we applied the *t*-butyldimethylsilyl protecting group but its removal from the protected phosphoramidate diester of FdU, using tetra-*n*-butylammonium fluoride (TBAF) proceeded with the concomitant cleavage of 4-chlorophenyl group.[24] It seems that the group of choice for the protection of the 3'-hydroxyl of FdU is *t*-butoxycarbonyl group (BOC)[25,26] since introduction of tetrahydropyran-1-yl group results in the formation of a new chiral centre (which causes the appearance of double spots on a TLC plate and broad bands at silica gel column chromatography) and the 5,6-dihydro-4-methoxy-2*H*-pyran reagent to introduce 4-methoxytetrahydropyran-1-yl group is quite expensive.

3'-*O*-(*t*-Butoxycarbonyl)-5-fluoro-2'-deoxyuridine (3'-BOC-FdU, **4**) was synthesized starting from 5-fluoro-2'-deoxyuridine (FdU, **1**) according to the procedure outlined in Scheme 1. In the first step of the synthesis, the 5'-hydroxyl of compound **1** was silylated with *t*-butyldimethylsilyl chloride (TBDMSCl) in the presence of imidazole and 4-*N*,*N*-dimethylaminopyridine (DMAP) in DMF to give intermediate **2**.[27] Then, *t*-butoxycarbonyl protecting group was introduced at the 3'-hydroxyl of compound **2** by means of di-*t*-butyl dicarbonate (DBDC) in the presence of DMAP and triethylamine in dioxane.[26] In the last step, 5'-*O*-*t*-butyldimethylsilyl group was removed from the compound **3** with tetra-*n*-butylammonium fluoride (TBAF) to afford compound **4**.[16]



Scheme 1. Synthesis of 3'-*O*-(*t*-butoxycarbonyl)-5-fluoro-2'-deoxyuridine (3'-BOC-FdU). Reagents and conditions: (a) TBDMSCl, imidazole, DMAP, DMF, rt, 1 h; (b) DBDC, DMAP, Et_3N, dioxane, rt, 1 h; (c) TBAF, THF, rt, 3 h.

A series of novel 4-chlorophenyl *N*-alkyl phosphoramidate diesters of 5-fluoro-2'-deoxyuridine (**10a**–**10j**) was synthesized by phosphorylation of 3'-*O*-(*t*-butoxycarbonyl)-5-fluoro-2'-deoxyuridine (3'-BOC-FdU, **4**) with 4-chlorophenyl phosphoroditriazolide (**7**) according to the synthetic route shown in Scheme 2.



10a: R = CH_3
10b: R = CH_2CH_3
10c: R = CH_2CF_3
10d: R = $CH_2CH_2CH_3$
10e: R = $CH_2CH{=}CH_2$
10f: R = $CH_2C{\equiv}CH$
10g: R = $CH_2CH_2CH_2N_3$
10h: R = $CH_2CH_2CH_2CH_3$
10i: R = $CH_2CH_2CH_2CH_2N_3$
10j: R = *(S)*-$CH(CH_3)CO_2CH_3$

Scheme 2. Synthesis of the 4-chlorophenyloxy *N*-alkyl phosphoramidates of 5-fluoro-2'-deoxyuridine. Reagents and conditions: (a) Et_3N, CH_3CN, rt, 30 min; (b) **7**, pyridine, rt, 1 h; (c) R-NH_2, rt, 1h; (d) TFA, CH_2Cl_2, 0^oC, 40 min.

4-Chlorophenyl phosphoroditriazolide (**7**) was prepared by reaction of 4-chlorophenyl phosphorodichloridate (**5**) with 1,2,4-triazole (**6**) in the presence of triethylamine in acetonitrile. Reaction of compound **7** with 3'-*O*-(*t*-butoxycarbonyl)-5-fluoro-2'-deoxyuridine (**4**) in the presence of pyridine afforded the reactive intermediate **8**, which was treated *in situ* with the appropriate amine (or amine hydrochloride in the presence of triethylamine) to give the 3'-protected products **9a**–**9j** in 65–86% yield. The 3'-*t*-butoxycarbonyl protecting group was removed from compounds **9a**–**9j** with trifluoroacetic acid in dichloromethane at 0 ^oC to afford the desired products **10a**–**10j** in 78–89% yield.

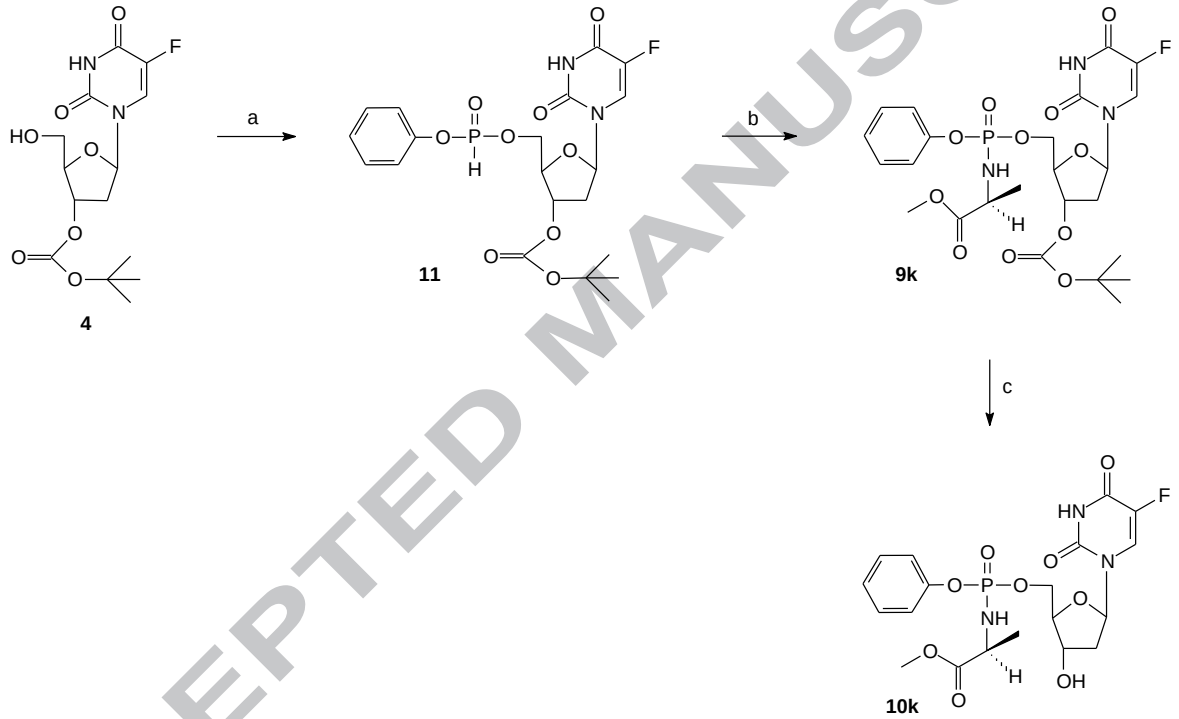
^{31}P NMR spectra of products **9a**–**9j** and **10a**–**10j** revealed the existence of two diastereoisomers due to a chiral center being formed at the phosphorus atom. There were two close signals, in the ratio of approximately 1:1, in each ^{31}P NMR spectrum. Thin layer chromatography of these compounds also disclosed the presence of two diastereoisomers showing two overlapping spots but we were unable to resolve them by silica gel column chromatography. However, it was possible to resolve the two diastereoisomers by HPLC on a reversed-phase column (see experimental data for compound **10a** and **10b**).

It is worth emphasizing that the use of 4-chlorophenyl phosphorodichloride (**5**), rather than its triazolide counterpart **7**, resulted in the formation of a considerable amount of the symmetrical (5'-5')dinucleoside phosphate. The 2- and 4-chlorophenyl phosphoroditriazolides[28-31] have been previously used for the phosphorylation of 5'-protected nucleosides in the phosphotriester synthesis of oligonucleotides. Their successful application in our research resulted in the development of an efficient method for the preparation of 2',3'-didehydro-2',3'-dideoxyinosine phosphoramidates published by us recently.[32]

It should be mentioned that 1-amino-3-azidopropane and its 1-amino-4-azidobutane homologue required as intermediates in the synthesis of phosphoramidates **10g** and **10i** were prepared from 1,3-dibromopropane and 1,4-dibromobutane, respectively, *via* the appropriate diazidoalkanes by a published method.[33]

Phenyloxy *N*-(methoxy-(*S*)-alaninyl) phosphoramidate of 5-fluoro-2'-deoxyuridine (**10k**)[3] was synthesized according to the route shown in Scheme 3 using 3'-*O*-(*t*-

butoxycarbonyl)-5-fluoro-2'-deoxyuridine (**4**) as the starting material.[34] Compound **4** was first reacted with diphenyl phosphite in pyridine to give phenyl H-phosphonate of 5-fluoro-2'-deoxyuridine (**11**). Oxidation of the crude compound **11** with carbon tetrachloride in the presence of triethylamine (Atherton-Todd reaction[35]) generated phenyl chlorophosphate of 5-fluoro-2'-deoxyuridine which was treated *in situ* with L-alanine methyl ester to produce the 3'-*t*-butoxycarbonyl protected phosphoramidate **9k** in 66% yield. The 3'-*t*-butoxycarbonyl protecting group was removed from **9k** with trifluoroacetic acid in dichloromethane at 0 ^{o}C to afford the desired product **10k** in 82% yield.



Scheme 3. Synthesis of the phenyloxy *N*-(methoxy-(*S*)-alaninyl) phosphoramidate of 5-fluoro-2'-deoxyuridine. Reagents and conditions: (a) diphenyl phosphite, pyridine, rt, 1 h; (b) L-alanine methyl ester hydrochloride, CH_3CN, Et_3N, CCl_4, rt, 2 h; (c) TFA, CH_2Cl_2, 0^{o}C, 40 min.

2.2. Biological activity

The synthesized phosphoramidates **9a**–**9j** and **10a**–**10j** were evaluated for their cytotoxic activity in five human cancer cell lines: cervical (HeLa), nasopharyngeal (KB),

breast (MCF-7), liver (HepG2), osteosarcoma (143B) and normal human dermal fibroblast cell line (HDF) employing the sulforhodamine B (SRB) assay.[36] The resulting cytotoxic activity data of the obtained phosphoramidates and the reference compounds are presented in Table 1. Regarding the series of phosphoramidates **9a**–**9j** with 3'-*O*-*t*-butoxycarbonyl substituent, the highest activity was displayed by phosphoramidate **9b** with the *N*-ethyl substituent (IC_{50} = 1.42 μM) in MCF-7 cancer cells. The activity of **9b** was 45 times higher than that of the parent compound **4** and almost nine times higher than that of FdU. A similar order of activity was observed for phosphoramidate **9j** with the *N*-(methoxy-(*S*)-alaninyl) substituent in KB and HeLa cancer cells (IC_{50} = 1.54 and 1.65 μM, respectively). It should be noted that phosphoramidates **9b** and **9j** also showed high activity (IC_{50} in the range of 2.56–4.26 μM) in the other studied cancer cell lines. Interestingly, phosphoramidate **9f** with the *N*-propargyl substituent had good activity (IC_{50} in the range of 3.66–4.71 μM) in all the examined cancer cell lines whereas phosphoramidate **9c** with the *N*-(2,2,2-trifluoroethyl) substituent was found potent (IC_{50} = 4.05 μM) only in MCF-7 cancer cell line. Phosphoramidate **9a** with the *N*-methyl substituent also exhibited somewhat higher activity than FdU in KB, MCF-7 and HepG2 cancer cells (IC_{50} in the range of 6.55–7.28 μM).

The most active phosphoramidates **9b**, **9f** and **9j** have partition coefficient (log *P*) values ranging from 2.15 to 2.37 (Table 1) and can easily penetrate the cell membranes. Once inside the cells, the phosphoramidate 3'-*O*-*t*-butoxycarbonyl group could be hydrolyzed by esterase-type enzyme.

As for the series of phosphoramidates **10a**–**10j** with a free 3'-hydroxyl group, the highest activity was displayed by phosphoramidate **10c** with the *N*-(2,2,2-trifluoroethyl) substituent in all the cancer cells (IC_{50} in the range of 1.82–3.48 μM). It should be noted that this phosphoramidate was significantly more active than the parent nucleoside **1** (FdU) in each cancer cell line tested. Moreover, considerable activity, higher than that of FdU, was shown by phosphoramidate **10a** with the *N*-methyl substituent in HeLa, KB and MCF-7 cancer cells (IC_{50} in the range of 4.44–5.78 μM), and by phosphoramidate **10b** with the *N*-ethyl substituent in KB and HepG2 cancer cells (IC_{50} was respectively 8.21 and 5.39 μM). Disappointingly, phosphoramidate **10j** with the *N*-(methoxy-(*S*)-alaninyl) substituent was less active than its 3'-protected counterpart **9j** in all the cancer cells (IC_{50} in the range of 4.11–6.65 μM). Phosphoramidates with longer *N*-alkyl chain substituents, e.g. in the first series **9d** and **9h** and in the second series **10d** and **10h**, were less potent in all the cancer cells. These phosphoramidates could become hydrophobic to the extent that they are poorly soluble in the

aqueous phase. In addition, hydrophobic compounds are usually more susceptible to metabolism and subsequent elimination.[37]

We noticed that phosphoramidates synthesized by McGuigan[3] exhibited very high cytotoxic activity, for example phenyloxy *N*-(methoxy-(*S*)-alaninyl) phosphoramidate of 5-fluoro-2'-deoxyuridine was shown to have an IC_{50} value of 0.28 μM in HeLa cells. Also compare with the literature data,[38] cytotoxic activity of standards 5-fluorouracil (IC_{50} = 0.54 μM) and FdU (IC_{50} = 0.05 μM) determined in HeLa cells by McGuigan and coworkers are relatively high. Unfortunately, in the description of cytotoxicity assay the authors did not indicate exact concentrations of tested compounds, so experiment of IC_{50} determination can not be followed. Moreover, it is not clear how the amount of viable cells was determined (which dye was used, i.e. SRB or MTT method). In order to clarify this we have synthesized phenyloxy *N*-(methoxy-(*S*)-alaninyl) phosphoramidate of 5-fluoro-2'-deoxyuridine (**10k**) and determined its cytotoxic activity in HeLa cells, IC_{50} was 23.5 μM (Table 1). Thus, the phosphoramidate with a phenyl substituent **10k** was almost six times less active than phosphoramidate with the 4-chlorophenyl substituent **10j** prepared by us.

We anticipated that the 4-chlorophenyl substituted phosphoramidates used in our study would be more susceptible to the intracellular cleavage than the corresponding phenyl counterparts. The 4-chlorophenol anion is a much better leaving group in the S_N2 reaction at the phosphorus than a phenol anion, owing to a strong electron-withdrawing effect of the chlorine atom. Indeed, the phosphoramidates with 4-chlorophenyl substituent were considerably more active than their counterparts with a phenyl substituent (compounds **9k** and **10k**, Table 1). Although the released 4-chlorophenol is toxic, its cytotoxic activity in all the cell lines was observed in the milimolar range whereas the phosphoramidates demonstrated activity in the micromolar range (Table 1 and literature data[39]). Thus, 4-chlorophenol did not contribute to the overall cytotoxic effect.

Table 1

In vitro cytotoxic activity of compounds **9a**–**9k** and **10a**–**10k** in four human cancer cell lines: cervical (HeLa), nasopharyngeal (KB), breast (MCF-7), liver (HepG2) and normal human dermal fibroblast cell line (HDF)

Compound	Cytotoxicity (IC_{50}, μM)[a] ± SD[b]					log *P*[c]
	HeLa	KB	MCF-7	HepG2	HDF	
9a	7.27 ± 0.23	7.28 ± 0.25	8.18 ± 0.26	6.55 ± 0.21	12.37 ± 1.13	2.00

9b	2.66 ± 0.11	3.37 ± 0.12	1.42 ± 0.09	4.26 ± 0.16	6.02 ± 0.02	2.37
9c	14.57 ± 0.27	14.59 ± 0.28	4.05 ± 0.12	18.77 ± 0.54	37.11 ± 0.14	2.92
9d	49.31 ± 0.98	27.17 ± 0.32	20.76 ± 0.28	25.96 ± 0.31	61.08 ± 0.11	2.87
9e	19.10 ± 0.44	16.50 ± 0.36	12.85 ± 0.31	17.02 ± 0.37	27.19 ± 3.02	2.64
9f	4.36 ± 0.17	4.37 ± 0.13	4.71 ± 0.18	3.66 ± 0.14	7.21 ± 0.09	2.15
9g	52.51 ± 0.58	54.93 ± 0.57	58.49 ± 0.59	46.85 ± 0.56	57.22 ± 6.71	2.97
9h	76.02 ± 0.60	66.39 ± 0.57	80.07 ± 0.67	64.19 ± 0.52	86.99 ± 1.16	3.43
9i	36.02 ± 0.23	35.55 ± 0.31	35.39 ± 0.23	58.93 ± 0.47	48.28 ± 0.44	3.24
9j	1.65 ± 0.09	1.54 ± 0.08	2.56 ± 0.11	3.47 ± 0.15	6.21 ± 1.09	2.19
9k	8.34 ± 0.27	8.34 ± 0.26	6.29 ± 0.22	9.28 ± 0.31	17.52 ± 1.02	1.51
10a	5.78 ± 0.20	4.45 ± 0.18	4.44 ± 0.17	9.34 ± 0.38	11.83 ± 0.31	0.34
10b	8.19 ± 0.25	8.21 ± 0.26	19.41 ± 0.54	5.39 ± 0.17	16.33 ± 1.38	0.72
10c	3.09 ± 0.12	1.93 ± 0.09	1.82 ± 0.08	3.48 ± 0.13	4.19 ± 0.95	1.27
10d	50.23 ± 0.84	50.41 ± 0.89	27.21 ± 0.31	41.86 ± 0.42	61.02 ± 0.06	1.22
10e	89.32 ± 0.91	38.67 ± 0.45	94.53 ± 0.97	96.68 ± 0.95	92.14 ± 0.18	0.99
10f	10.55 ± 0.19	25.33 ± 0.28	12.03 ± 0.18	24.48 ± 0.30	31.02 ± 0.28	0.50
10g	22.17 ± 0.24	22.55 ± 0.23	26.98 ± 0.27	22.56 ± 0.24	41.03 ± 0.26	1.32
10h	> 100	> 100	> 100	> 100	> 100	1.78
10i	72.07 ± 0.86	71.31 ± 0.85	67.56 ± 0.81	> 100	> 100	1.59
10j	4.11 ± 0.15	4.22 ± 0.16	4.54 ± 0.17	6.65 ± 0.21	18.44 ± 0.08	0.54
10k	23.50 ± 0.76	18.00 ± 0.61	29.10 ± 0.59	25.93 ± 0.72	43.07 ± 0.67	-0.14
4-chlorophenol	1678 ± 1	1449 ± 1	1876 ± 1	1700 ± 5	1850 ± 4	2.14
FUra	6.23 ± 0.46	4.84 ± 0.15	6.53 ± 0.82	6.60 ± 0.18	7.02 ± 0.20	-0.59
1 (FdU)	6.50 ± 0.24	8.69 ± 1.18	12.19 ± 1.34	8.91 ± 0.34	13.05 ± 0.74	-1.72
4 (3'-BOC-FdU)	63.53 ± 0.74	75.94 ± 0.81	64.21 ± 0.75	80.42 ± 0.85	> 100	-0.07
Cytarabine (standard)	3.54 ± 0.16	4.07 ± 0.08	3.82 ± 0.25	2.86 ± 0.09	4.99 ± 0.84	-2.32[40]

[a] IC_{50} is the compound concentration required to inhibit cell growth by 50%.

[b] SD (standard deviation) of three independent experiments.

[c] log *P* (logarithm of partition coefficient) was calculated using "log $P_{Knowwin}$" method.[41]

The cytotoxic effect was also studied in the normal human dermal fibroblasts (HDF) to assess the toxicity of the prepared phosphoramidates (Table 1). The selectivity index (SI) was calculated as the ratio of the IC_{50} for the normal cell line (HDF) to the IC_{50} for a respective cancerous cell line (Table 2). Higher values of SI indicate greater anticancer specificity and compounds displaying SI's values higher than 3 are considered to be highly

selective.[42] Some of the synthesized phosphoramidates had not only high cytotoxic activity but also displayed low toxicity against normal fibroblast cells and their selectivity index (SI) was higher than 4 (Table 2).

Table 2

The calculated values of the selectivity index (SI) of the compounds more active than FdU

Compound	SI[a]				
	HeLa	KB	MCF-7	HepG2	143B
9b	2.26	1.79	4.24	1.41	1.37
9f	1.65	1.65	1.53	1.97	2.39
9j	3.76	4.03	2.39	1.66	4.11
10a	2.05	2.66	2.66	1.27	5.58
10c	1.36	2.17	2.30	1.20	2.33
10j	4.49	4.37	4.06	2.77	4.58
FUra	1.13	1.45	1.08	1.06	0.54
1 (FdU)	2.01	1.50	1.07	1.47	2.17
Cytarabine (standard)	1.41	1.23	1.31	1.75	2.42

[a] The selectivity index (SI) was calculated for each compound using the formula: SI = IC_{50} for normal cell line HDF/IC_{50} for respective cancerous cell line. A beneficial SI > 1.0 indicates a compound with efficacy against tumor cells greater than the toxicity against normal cells.

Partition coefficient (log *P*) values of phosphoramidates **9a**–**9j** and **10a**–**10j** were calculated[41] to determine a possible correlation between their cytotoxicity data and lipophilicity (Table 1). Phosphoramidates **9a**–**9j** were all more lipophilic than the parent nucleoside **4** (log *P* = -0.07), with log *P* values ranging from 2.00 to 3.43. Similarly, phosphoramidates **10a**–**10j** were all more lipophilic than the parent FdU (log *P* = -1.72), with log *P* values ranging from 0.34 to 1.78. Linear regression analysis revealed some correlations between log *P* values and the cytotoxicity data in the case of compounds **9a**–**9j** (Fig. 2).

(a) (b)

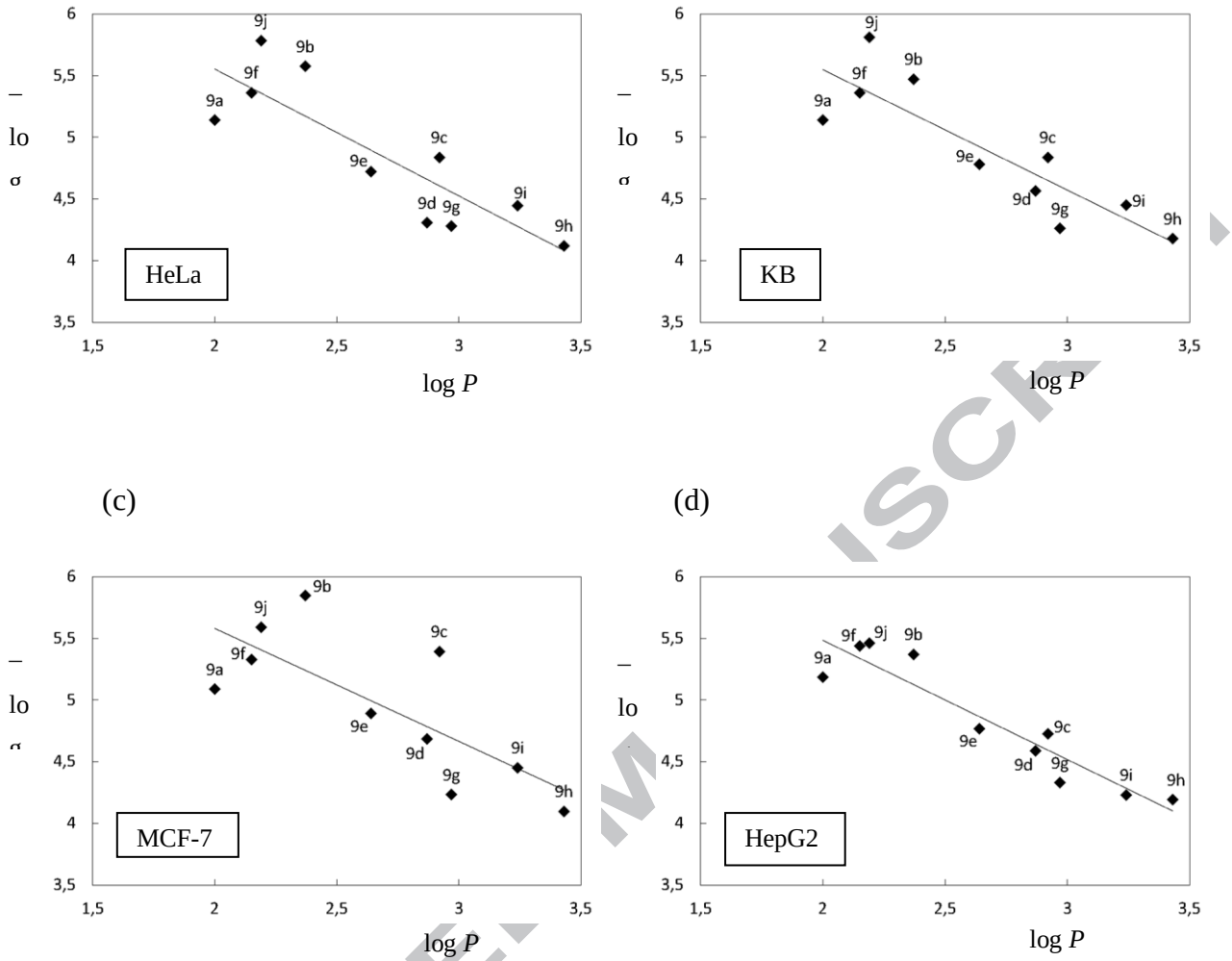


Figure 2. Correlation between log *P* and –log IC_{50} for compounds **9a**–**9j** for four cancer cell lines: (a) HeLa, (b) KB, (c) MCF-7 and (d) HepG2. IC_{50} is expressed in M (mol/L) units.

Interestingly, phosphoramidates **9g**, **9i**, **10g** and **10i** with the *N*-(3-azidopropyl) and *N*-(4-azidobutyl) substituents showed a considerable increase of cytotoxic activity after treatment with Supermix^{TM} which is a mixture of insect cell-expressed human cytochrome P450 isoforms (Table 3).[43] This increase in activity may be explained by the enzymatic reduction of the azido group to an amino function which as a nucleophile intramolecularlly attacks the phosphorus atom or the α-carbon of the *N*-(ω-aminoalkyl) substituent causing the release of FdUMP. These data seem to confirm some prior reports on the reduction of the azido group in 3'-azido-3'-deoxythymidine (AZT)[44] and 9-(β-D-arabinofuranosyl)-6-azidopurine[45] to an amino function by the cytochrome P-450 system. As the data in Table 2

indicate, the increase in cytotoxic activity of phosphoramidates **9d**, **9h**, **10d** and **10h** with the *N*-propyl and *N*-butyl substituents is relatively small after treatment with Supermix™.

Table 3

In vitro cytotoxic activity of compounds **9d**, **9g**, **9h**, **9i** and **10d**, **10g**, **10h**, **10i** in HeLa cancer cell line before and after treatment with Supermix™ (a mixture of cytochrome P450 isoforms)

Compound	Cytotoxicity (IC_{50}, μM)[a] ± SD[b]	
	HeLa	HeLa/Supermix™
9d	49.31 ± 0.98	45.22 ± 0.87
9g	52.51 ± 0.58	24.07 ± 0.22
9h	76.02 ± 0.60	71.35 ± 0.58
9i	36.02 ± 0.23	17.69 ± 0.21
10d	50.23 ± 0.84	45.86 ± 0.59
10g	22.17 ± 0.24	13.49 ± 0.16
10h	> 100	> 100
10i	72.07 ± 0.86	20.64 ± 0.20

[a] IC_{50} is the compound concentration required to inhibit cell growth by 50%.

[b] SD (standard deviation) of three independent experiments.

Finally, phosphoramidates **9a**–**9c**, **9f**, **9j** and **10a**–**10c**, **10f**, **10j** were examined for their cytotoxic activity in human osteosarcoma cells (143B) and thymidine kinase deficient human osteosarcoma cells (143B/TK-), as presented in Table 4. Compounds **1**, **4** and FUra were included as positive controls. It should be noted that phosphoramidate **9c** exhibited the highest activity (IC_{50} = 0.28 μM) among the tested compounds in 143B cancer cells, however, in 143B thymidine kinase deficient cancer cells its activity decreased more than 7-fold. In contrast, phosphoramidates **9f**, **10c** and **10f** showed high activity not only in 143B cancer cells but also in 143B/TK- cancer cells, indicating that these phosphoramidates efficiently bypass the dependence on thymidine kinase. It is interesting that FUra largely retained activity in 143B/TK- cancer cells. This data suggest that FUra has to be directly converted to FdUMP by phosphoribosylation catalyzed by the enzyme orotate phosphoribosyl transferase.[3]

Table 4

In vitro cytotoxic activity of selected compounds in human osteosarcoma cell line (143B) and thymidine kinase deficient human osteosarcoma cell line (143B/TK-)

Compound	Cytotoxicity (IC_{50}, μM)[a] ± SD[b]	
	143B	143B/TK-
9a	6.72 ± 0.38	13.10 ± 0.46
9b	4.40 ± 0.24	9.11 ± 0.12
9c	0.28 ± 0.04	2.01 ± 0.11
9f	3.02 ± 0.13	3.62 ± 0.05
9j	1.51 ± 0.12	3.07 ± 0.14
10a	2.12 ± 0.09	12.72 ± 0.25
10b	2.41 ± 0.04	16.01 ± 0.31
10c	1.80 ± 0.08	2.51 ± 0.12
10f	3.61 ± 0.16	3.90 ± 0.15
10j	4.03 ± 0.26	8.67 ± 0.36
FUra	13.01 ± 0.40	16.31 ± 0.44
1 (FdU)	6.02 ± 0.25	14.10 ± 0.32
4 (3'-BOC-FdU)	18.03 ± 0.60	47.00 ± 0.84
Cytarabine (standard)	2.06 ± 0.18	16.88 ± 0.39

[a] IC_{50} is the compound concentration required to inhibit cell growth by 50%.

[b] SD (standard deviation) of three independent experiments.

3. Conclusion

In conclusion, we have developed an efficient procedure for the synthesis of 4-chlorophenyl *N*-alkyl phosphoramidates of 5-fluoro-2'-deoxyuridine employing 4-chlorophenyl phosphoroditriazolide as a phosphorylating agent and 3'-*O*-(*t*-butoxycarbonyl)-5-fluoro-2'-deoxyuridine as a 3'-protected nucleoside component. The obtained phosphoramidates **9a**–**9j** bearing the 3'-hydroxyl function protected with the 3'-*O*-*t*-butoxycarbonyl group and the corresponding compounds **10a**–**10j** with a free 3'-hydroxyl function were examined for their cytotoxic activity in five human cancer cell lines: cervical (HeLa), nasopharyngeal (KB), breast (MCF-7), liver (HepG2), osteosarcoma (143B) and normal human dermal fibroblast cell line (HDF) using the sulforhodamine B (SRB) assay. In the 3'-hydroxyl protected series **9a**–**9j** two phosphoramidates **9b** and **9j** with the *N*-ethyl and *N*-(methoxy-(*S*)-alaninyl) substituents, respectively, showed the best activity in all the examined cancer cell lines, and their activity was considerably higher than that of the parent

nucleoside **4** and FdU. Phosphoramidate **9f** with the *N*-propargyl substituent displayed also fairly high activity. In the series of phosphoramidates **10a**–**10j**, the highest activity was demonstrated by the compound **10c** with the *N*-(2,2,2-trifluoroethyl) substituent that was considerably more active than the parent nucleoside **1** (FdU) in all the cancer cell lines tested. Phosphoramidate **10j** with the *N*-(methoxy-(*S*)-alaninyl) substituent exhibited also significant activity. Phosphoramidates **9b**, **9j** and **10j** demonstrated not only high cytotoxic activity but also low toxicity against normal fibroblast cells (in certain cases the selectivity index (SI) was higher than 4). Interestingly, phosphoramidates **9g**, **9i**, **10g** and **10i** with the *N*-(3-azidopropyl) or *N*-(4-azidobutyl) substituents, showed a considerable increase of cytotoxic activity after treatment with Supermix^{TM}. Phosphoramidates **9f**, **10c** and **10f** showed high activity in 143B cancer cells and unlike phosphoramidate **9c**, their activity was retained in 143B/TK- cancer cells, suggesting that these phosphoramidates efficiently bypass the dependence on thymidine kinase.

4. Experimental section

4.1. Chemistry

NMR spectra were recorded on a Varian-Gemini 400 MHz spectrometer. Chemical shifts (δ) are reported in ppm relative to the tetramethylsilane (TMS) peak. For ^{31}P NMR spectra 85% phosphoric(V) acid in D_2O was used as an external standard (coaxial inner tube). Mass spectra were measured on a Waters Micromass ZQ electrospray (ES) mass spectrometer. Elemental analyses were performed on EL III elemental analyzer (Elementar Analysensysteme GmbH, Germany). Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} precoated (0.2 mm) plates and vacuum flash column chromatography on silica gel 60 H (5-40 μm) purchased from Merck. High performance liquid chromatography (HPLC) was performed on a Waters chromatograph equipped with a Waters 996 UV-Vis photodiode array detector. Analytical HPLC was carried out on Waters XBridge C18 reversed-phase column (4.6 x 150 mm, 5μm) using phosphate buffer (20 mM Na_2HPO_4, pH was adjusted to 7.1 with H_3PO_4)–methanol (40:60) as an eluting system. The flow rate was 1 mL/min and detection at 266 nm. Chemical reagents were purchased from Sigma-Aldrich.

4.1.1. 5'-*O*-(t-Butyldimethylsilyl)-5-fluoro-2'-deoxyuridine (2)[16]

To a stirred solution of 5-fluoro-2'-deoxyuridine (**1**) (3.69 g, 15 mmol), imidazole (3.06 g, 45 mmol) and DMAP (183 mg, 1.5 mmol) in DMF (75 mL) was added *t*-butyldimethyl silyl chloride (2.94 g, 19.5 mmol) at 0 ^{o}C and the mixture was allowed to warm to room temperature and stirring was continued for 1 h. Then saturated aqueous solution of ammonium chloride (150 mL) was added and the mixture was extracted with ethyl acetate (3 x 75 mL). The combined organic extracts were washed with water (20 mL), dried over anhydrous magnesium sulphate, filtered and evaporated to dryness. The residue was purified by silica gel column chromatography using chloroform–methanol (80:1, v/v) as eluent to afford pure **2** (yield: 3.89 g, 72%). ^{1}H NMR (DMSO-d_6) δ: 0.10 (s, 3H, $SiCH_3$), 0.11 (s, 3H, $SiCH_3$), 0.90 (s, 9H, *t*-Bu), 2.03–2.48 (m, 2H, H-2', H-2''), 3.80–3.98 (m, 2H, H-5', H-5''), 4.08–4.12 (m, 1H, H-4'), 4.43–4.58 (m, 1H, H-3'), 6.37 (pseudo t, 1H, J = 6.5 Hz, H-1'), 7.82 (d, 1H, $J_{H\text{-}F}$ = 6.2 Hz, H-6), 8.10 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: –5.60 ($Si(CH_3)_2$), 18.35 (*t*-C), 25.86 ($C(\underline{C}H_3)_3$), 41.32 (C-2'), 63.48 (C-5'), 72.03 (C-3'), 85.73 (C-1'), 87.87 (C-4'), 124.43 (d, $J_{C\text{-}F}$ = 39.3 Hz, C-6), 140.53 (d, $J_{C\text{-}F}$ = 236.1 Hz, C-5), 149.34 (C-2), 157.47 (d, $J_{C\text{-}F}$ = 26.3 Hz, C-4). ^{19}F NMR (DMSO-d_6) δ: –166.28 (d, 1F, $J_{H\text{-}F}$ = 6.2 Hz). MS-ESI *m/z*: 361 $[M + H]^+$; 383 $[M + Na]^+$; 399 $[M + K]^+$; 359 $[M – H]^–$; 395, 397 $[M + Cl]^–$. Anal. Calcd for $C_{15}H_{25}FN_2O_5Si$: C, 49.98; H, 6.99; N, 7.77. Found: C, 50.03; H, 7.00; N, 7.78.

4.1.2. 3'-*O*-(t-Butoxycarbonyl)-5'-*O*-(t-butyldimethylsilyl)-5-fluoro-2'-deoxyuridine (3)

To a stirred solution of **2** (3.61 g, 10 mmol) and DMAP (159 mg, 1.30 mmol) in dioxane (60 mL) and triethylamine (40 mL) was added di-*t*-butyl dicarbonate (2.84 g, 13 mmol) and stirring was continued at room temperature for 1 h. Then the reaction mixture was evaporated under reduced pressure. The residue was treated with ethyl acetate (100 mL), washed with 5% aqueous solution of sodium bicarbonate (2 x 20 mL) and brine (20 mL), dried over anhydrous magnesium sulphate, filtered and evaporated to dryness. The crude product was purified by silica gel column chromatography using chloroform–methanol (100:1, v/v) as eluent to afford pure **3** (yield: 4.06 g, 88%). ^{1}H NMR (DMSO-d_6) δ: 0.15 (s, 3H, $SiCH_3$), 0.18 (s, 3H, $SiCH_3$), 0.93 (s, 9H, Si-*t*-Bu), 1.51 (s, 9H, O-*t*-Bu), 2.09–2.60 (m, 2H, H-2', H-2''), 3.87–3.99 (m, 2H, H-5', H-5''), 4.22 (m, 1H, H-4'), 5.11 (m, 1H, H-3'), 6.38 (pseudo t, 1H, J = 6.5 Hz, H-1'), 8.05 (d, 1H, $J_{H\text{-}F}$ = 6.0 Hz, H-6), 9.40 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: –5.50 [$Si(CH_3)_2$], 18.40 (Si-*t*-C), 25.91 (Si-$C(\underline{C}H_3)_3$), 27.28 (O-

C($\underline{C}H_3)_3$), 38.51 (C-2'), 63.58 (C-5'), 77.71 (C-3'), 83.24 (*t*-C, BOC), 85.51 (C-1'), 85.57 (C-4'), 123.97 (d, J_{C-F} = 33.8 Hz, C-6), 140.54 (d, J_{C-F} = 235.5 Hz, C-5), 148.75 (C-2), 152.62 (CO, BOC), 156.81 (d, J_{C-F} = 26.9 Hz, C-4). ^{19}F NMR (DMSO-d_6) δ: –166.48 (d, 1F, J_{H-F} = 6.0 Hz). MS-ESI *m/z*: 461 $[M + H]^+$; 483 $[M + Na]^+$; 499 $[M + K]^+$; 459 $[M – H]^–$; 495, 497 $[M + Cl]^–$. Anal. Calcd for $C_{20}H_{33}FN_2O_7Si$: C, 52.16; H, 7.22; N, 6.08. Found: C, 52.21; H, 7.23; N, 6.09.

4.1.3. 3'-*O*-(t-Butoxycarbonyl)-5-fluoro-2'-deoxyuridine (4)

To a stirred solution of **3** (3.90 g, 11.26 mmol) in THF (200 mL) was added 1 M tetra-*n*-butylammonium fluoride in THF (13.51 mL) and stirring was continued at room temperature for 3 h. Then the mixture was evaporated to dryness and the residue was purified by silica gel column chromatography using chloroform–methanol (80:1, v/v) as eluent to afford pure **4** (yield: 2.41 g, 82%). ^{1}H NMR (DMSO-d_6) δ: 1.44 (s, 9H, *t*-Bu), 2.28–2.31 (m, 2H, H-2', H-2''), 3.58–3.68 (m, 2H, H-5', H-5''), 4.08 (m, 1H, H-4'), 5.32 (m, 1H, H-3'), 6.38 (pseudo t, 1H, *J* = 6.8 Hz, H-1'), 8.20 (d, 1H, J_{H-F} = 7.2 Hz, H-6), 11.88 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 27.81 (C($\underline{C}H_3)_3$), 38.95 (C-2'), 63.87 (C-5'), 78.41 (C-3'), 82.11 (*t*-C), 84.81 (C-1'), 85.61 (C-4'), 123.96 (d, J_{C-F} = 33.7 Hz, C-6), 141.24 (d, J_{C-F} = 235.6 Hz, C-5), 148.61 (C-2), 151.97 (CO, BOC), 156.89 (d, J_{C-F} = 26.8 Hz, C-4). ^{19}F NMR (DMSO-d_6) δ: –166.44 (d, 1F, J_{H-F} = 7.2 Hz). MS-ESI *m/z*: 347 $[M + H]^+$; 369 $[M + Na]^+$; 385 $[M + K]^+$; 345 $[M – H]^–$; 381, 383 $[M + Cl]^–$. Anal. Calcd for $C_{14}H_{19}FN_2O_7$: C, 48.55; H, 5.53; N, 8.09. Found: C, 48.61; H, 5.54; N, 8.10.

4.1.4. General procedure for the synthesis of compounds 9a–9j

To a solution of 4-chlorophenyl phosphorodichloridate (**5**) (355 mg, 1.445 mmol) in acetonitrile (3.1 mL) was added 1,2,4-triazole (**6**) (260 mg, 3.757 mmol) followed by triethylamine (300 mg, 2.962 mmol) and the reactants were stirred for 30 min at room temperature. Then to the mixture 3'-*O*-(*t*-butoxycarbonyl)-5-fluoro-2'-deoxyuridine (**4**) (200 mg, 0.578 mmol) and pyridine (3.6 mL) were added. The reaction mixture was stirred at room temperature for a further 1 h and the appropriate amine (2.89 mmol) was added. In the case of synthesis of compounds **9a**–**9c** amine hydrochloride (2.89 mmol) and triethylamine (439 mg, 4.335 mmol) were added. When compound **9j** was synthesized L-alanine methyl ester hydrochloride (2.89 mmol) and triethylamine (439 mg, 4.335 mmol) were added. After 1 h, the reaction mixture was evaporated under reduced pressure. To the residue was added

saturated aqueous sodium bicarbonate (20 mL) and the mixture was extracted with chloroform (3 x 20 mL). The combined chloroform extracts were washed with water (20 mL), dried over anhydrous magnesium sulfate, filtered and evaporated to dryness. The residue was purified by silica gel column chromatography using chloroform – methanol (from 180 : 1 to 80 : 1, v/v) as eluent to afford products **9a**–**9j** (yield 65–86%).

4.1.4.1. 3'-*O*-(t-Butoxycarbonyl)-5-fluoro-2'-deoxyuridine 5'-*O*-(4-chlorophenyl *N*-methylphosphate) (9a)

^{1}H NMR (DMSO-d_6) δ: 1.44 (s, 9H, *t*-Bu), 2.25–2.37 (m, 2H, H-2', H-2''), 2.43 (t, 3H, J = 5.6 Hz, N-CH_3), 4.20–4.30 (m, 2H, H-5', H-5''), 4.67 (m, 1H, H-4'), 5.06–5.15 (m, 1H, H-3'), 5.47 (m, 1H, P-NH), 6.13 (pseudo t, 1H, J = 7.1 Hz, H-1'), 7.18, 7.23 (d, 2H, J = 8.8 Hz, 2H, 4-ClPh), 7.39, 7.44 (d, 2H, J =8.8 Hz, 4-ClPh), 7.95, 7.97 (d, 1H, J_{H-F} =6.9 Hz, H-6), 11.92 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 26.68, 27.29, 35.94, 65.54, 76.53, 81.97, 82.53, 84.58, 122.29, 124.50 (d, J_{C-F} = 34.3 Hz), 129.58, 138.95, 141.29 (d, J_{C-F} = 231.6 Hz), 148.98, 150.45, 152.15, 157.08 (J_{C-F} = 26.1 Hz). ^{19}F NMR (DMSO-d_6) δ: –166.00 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 7.38, 7.53. MS-ESI *m/z*: 550, 552 $[M + H]^+$; 572, 574 $[M + Na]^+$; 588, 590 $[M + K]^+$; 548, 550 $[M - H]^-$; 584, 586, 588 $[M + Cl]^-$. Anal. Calcd for $C_{21}H_{26}ClFN_3O_9P$: C, 45.87; H, 4.77; N, 7.64. Found: C, 45.90; H, 4.78; N, 7.65.

4.1.4.2. 3'-*O*-(t-Butoxycarbonyl)-5-fluoro-2'-deoxyuridine 5'-*O*-(4-chlorophenyl *N*-ethylphosphate) (9b)

^{1}H NMR (DMSO-d_6) δ: 1.02 (t, 3H, J = 7.4 Hz, N-C-CH_3), 1.44 (s, 9H, *t*-Bu), 2.25–2.37 (m, 2H, H-2', H-2''), 2.78–2.93 (m, 2H, N-CH_2-C), 4.19–4.30 (m, 2H, H-5', H-5''), 4.75 (m, 1H, H-4'), 5.06–5.14 (m, 1H, H-3'), 5.59 (m, 1H, P-NH); 6.13 (pseudo t, 1H, J = 7.2 Hz, H-1'), 7.19, 7.23 (d, 2H J = 8.9 Hz, 4-ClPh), 7.38, 7.43 (d, 2H, J = 8.9 Hz, 4-ClPh), 7.94, 7.97 (d, 1H, J_{H-F} = 6.9 Hz, H-6); 11.92 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 17.17, 27.29, 35.51, 36.67, 65.56, 76.21, 81.99, 82.53, 84.67, 122.26, 124.58, 129.16 (d, J_{C-F} = 34.1 Hz), 138.90, 141.30, 149.63 (d, J_{C-F} = 231.3 Hz), 150.60, 152.06, 157.17 (J_{C-F} = 26.1 Hz). ^{19}F NMR (DMSO-d_6) δ: –165.96 (pseudo t, J = 8.0, 1F). ^{31}P NMR (DMSO-d_6) δ: 6.34, 6.47. MS-ESI m/z: 564, 566 $[M + H]^+$; 586, 588 $[M + Na]^+$; 602, 604 $[M + K]^+$; 562, 564 $[M - H]^-$; 598, 600, 602 $[M + Cl]^-$. Anal. Calcd for $C_{22}H_{28}ClFN_3O_9P$: C, 46.86; H, 5.00; N, 7.45. Found: C, 46.90; H, 5.01; N, 7.46.

4.1.4.3. 3'-*O*-(t-Butoxycarbonyl)-5-fluoro-2'-deoxyuridine 5'-*O*-[4-chlorophenyl *N*-(2,2,2-trifluoroethyl)phosphate] (9c)

^{1}H NMR (DMSO-d_6) δ: 1.44 (s, 9H, *t*-Bu), 2.25–2.35 (m, 2H, H-2', H-2''), 3.48–3.72 (m, 2H, N-CH_2), 4.18–4.24 (m, 2H, H-5, H-5''), 4.26–4.31 (m, 1H, H-4'), 5.08 (m, 1H, H-3'), 6.00 (m, 1H, P-NH), 6.13 (pseudo t, 1H, J = 7.2 Hz, H-1'), 7.19, 7.23 (d, 2H, J = 8.9 Hz, 4-ClPh), 7.41, 7.44 (d, 2H, J = 8.9 Hz, 4-ClPh), 7.84, 7.86 (d, 1H, J_{H-F} = 4.3 Hz, H-6), 11.81 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 27.72, 36.55, 42.31, 66.09, 76.45, 81.63, 82.66, 84.54, 121.97, 122.23, 123.68, 125.41 (m), 129.66, 139.00 (d, J_{C-F} = 231.2 Hz), 142.98, 149.95, 152.98, 156.98 (d, J_{C-F} = 26.1 Hz). ^{19}F NMR (DMSO-d_6) δ: –166.49 (m, 1F), –71.53 (t, J = 9.6 Hz, 3F). ^{31}P NMR (DMSO-d_6) δ: 5.44, 5.61. MS-ESI *m/z*: 618, 620 $[M + H]^+$; 640, 642 $[M + Na]^+$; 656, 658 $[M + K]^+$; 616, 618 $[M - H]^-$; 652, 654, 656 $[M + Cl]^-$. Anal. Calcd for $C_{22}H_{25}ClF_4N_3O_9P$: C, 42.77; H, 4.08; N, 6.80. Found: C, 42.82; H, 4.09; N, 6.81.

4.1.4.4. 3'-*O*-(t-Butoxycarbonyl)-5-fluoro-2'-deoxyuridine 5'-*O*-(4-chlorophenyl *N*-*n*-propylphosphate) (9d)

^{1}H NMR (DMSO-d_6) δ: 0.84–0.95 (m, 3H, N-C-C-CH_3), 1.38 (m, 2H, N-C-CH_2-C), 1.44 (s, 9H, *t*-Bu), 2.40–2.56 (m, 2H, H-2', H-2''), 2.81–3.02 (m, 2H, N-CH_2-C-C), 4.06 (m, 1H, H-4'), 3.97–4.14 (m, 2H, H-5', H-5''), 4.28–4.46 (m, 1H, H-3'), 5.21 (m, 1H, P-NH), 6.27 (pseudo t, 1H, J = 8.0 Hz, H-1'), 7.31 (d, 2H, J = 11.4 Hz, 4-ClPh), 7.46 (d, 2H, J = 11.4 Hz, 4-ClPh), 7.98, 8.00 (d, 1H, J_{H-F} = 9.8 Hz, H-6), 11.15 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 11.82, 24.37, 27.51, 37.55, 42.90, 65.86, 76.52, 82.93, 83.34, 84.95, 121.71, 123.19 (d, J_{C-F} = 34.30 Hz), 129.60, 130.07, 140.59 (d, J_{C-F} = 231.40 Hz), 148.87, 149.56, 152.48, 157.95(d, J_{C-F} = 26.20 Hz). ^{19}F NMR (DMSO-d_6) δ: –166.64 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 5.24, 5.45. MS-ESI *m/z*: 578, 580 $[M + H]^+$; 600, 602 $[M + Na]^+$; 616, 618 $[M + K]^+$; 576, 578 $[M - H]^-$; 612, 614, 616 $[M + Cl]^-$. Anal. Calcd for $C_{23}H_{30}ClFN_3O_9P$: C, 47.80; H, 5.23; N, 7.27. Found: C, 47.82; H, 5.24; N, 7.25.

4.1.4.5. 3'-*O*-(t-Butoxycarbonyl)-5-fluoro-2'-deoxyuridine 5'-*O*-(4-chlorophenyl *N*-allylphosphate) (9e)

^{1}H NMR (DMSO-d_6) δ: 1.44 (s, 9H, *t*-Bu), 2.25–2.37 (m, 2H, H-2', H-2''), 3.46 (m, 2H, N-CH_2-C=C), 4.19–4.29 (m, 2H, H-5', H-5''), 5.00, 5.03 (m, 1H, H-4'), 5.07–5.12 (m, 2H, N-C-C=CH_2); 5.14, 5.18 (m, 1H, H-3'), 5.71–5.88 (m, 2H, P-NH, C-CH=C), 6.11 (m, 1H, H-1'), 7.19, 7.23 (d, 2H, *J* = 9.0 Hz, 4-ClPh), 7.39, 7.43 (d, 2H, *J* = 9.0 Hz, 4-ClPh), 7.93, 7.95 (d, 1H, $J_{H\text{-}F}$ = 8.1 Hz, H-6), 11.94 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 27.28, 35.88, 42.94, 65.46, 75.35, 81.91, 82.51, 84.38, 121.96, 122.35, 124.45 (d, $J_{C\text{-}F}$ = 34.30 Hz), 129.51, 136.47, 138.96, 140.08 (d, $J_{C\text{-}F}$ = 231.60 Hz), 148.96, 149.51, 152.04, 156.93(d, $J_{C\text{-}F}$ = 26.10 Hz). ^{19}F NMR (DMSO-d_6) δ: –165.94 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 6.26, 6.41. MS-ESI *m/z*: 576, 578 $[M + H]^+$; 598, 600 $[M + Na]^+$; 614, 616 $[M + K]^+$; 574, 576 $[M - H]^-$; 610, 612, 614 $[M + Cl]^-$. Anal. Calcd for $C_{23}H_{28}ClFN_3O_9P$: C, 47.97; H, 4.90; N, 7.30. Found: C, 48.02; H, 4.91; N, 7.32.

4.1.4.6. 3'-*O*-(t-Butoxycarbonyl)-5-fluoro-2'-deoxyuridine 5'-*O*-(4-chlorophenyl *N*-propargylphosphate) (9f)

^{1}H NMR (DMSO-d_6) δ: 1.44 (s, 9H, *t*-Bu), 2.01 (s, 1H, N-C-C-CH), 2.25–2.40 (m, 2H, H-2', H-2''), 3.14 (m, 2H, N-CH_2-C-C), 3.64–3.71 (m, 2H, H-5', H-5''), 4.20–4.31 (m, 1H, H-4'), 5.05–5.17 (m, 2H, P-NH, H-3') 6.12 (pseudo t, 1H, *J* = 6.1 Hz, H-1'), 7.24 (d, 2H, *J*= 8.4 Hz, 4-ClPh), 7.43 (d, 2H, *J* = 8.4 Hz, 4-ClPh), 7.95 (m, 1H, H-6), 11.95 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 27.30, 30.19, 35.83, 42.54, 65.72, 65.89, 73.51, 76.39, 82.57, 84.27, 121.88, 124.17 (d, $J_{C\text{-}F}$ = 34.20 Hz), 129.52, 139.04, 141.36 (d, $J_{C\text{-}F}$ = 231.50 Hz), 149.44, 149.88, 152.03, 157.09 (d, $J_{C\text{-}F}$ = 26.20 Hz). ^{19}F NMR (DMSO-d_6) δ: –165.82 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 5.56, 5.74. MS-ESI *m/z*: 574, 576 $[M + H]^+$; 596, 598 $[M + Na]^+$; 612, 614 $[M + K]^+$; 572, 574 $[M - H]^-$; 608, 610, 612 $[M + Cl]^-$. Anal. Calcd for $C_{23}H_{26}ClFN_3O_9P$: C, 48.14; H, 4.57; N, 7.32. Found: C, 48.19; H, 4.58; N, 7.33.

4.1.4.7. 3'-*O*-(t-Butoxycarbonyl)-5-fluoro-2'-deoxyuridine 5'-*O*-[4-chlorophenyl *N*-(3-azidopropyl)phosphate] (9g)

^{1}H NMR (DMSO-d_6) δ: 1.44 (s, 9H, *t*-Bu), 1.62 (m, 2H, N-C-CH_2-C-N_3), 2.27–2.40 (m, 2H, H-2', H-2''), 2.82–2.95 (m, 2H, P-N-CH_2), 3.30, 3.39 (t, 2H, *J* = 6.8 Hz, CH_2-N_3), 4.16–4.30 (m, 2H, H-5', H-5''), 4.94 (m, 1H, H-4'), 5.09 (m, 1H, H-3'), 5.69 (m, 1H, P-NH), 6.13 (pseudo t, 1H, *J* = 6.1 Hz, H-1'), 7.20, 7.24 (d, 2H, *J* = 8.9 Hz, 4-ClPh), 7.40, 7.44 (d, 2H, *J* =

8.9 Hz, 4-ClPh), 7.94, 7.95 (d, 2H, J_{H-F} = 6.9 Hz, H-6), 11.94 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 27.27, 30.42, 35.78, 37.74, 48.16, 65.74, 76.29, 81.90, 82.51, 84.55, 121.82, 124.56 (d, J_{C-F} = 34.40 Hz), 129.56, 139.03, 141.36 (d, J_{C-F} = 231.70 Hz), 149.60, 150.66, 152.12, 157.06 (d, J_{C-F} = 26.10 Hz). ^{19}F NMR (DMSO-d_6) δ: –165.96 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 6.30, 6.47. MS-ESI *m/z*: 619, 621 $[M + H]^+$; 641, 643 $[M + Na]^+$; 657, 659 $[M + K]^+$; 617, 619 $[M – H]^–$; 653, 655, 657 $[M + Cl]^–$. Anal. Calcd for $C_{23}H_{29}ClFN_6O_9P$: C, 44.63; H, 4.72; N, 13.58. Found: C, 44.65; H, 4.73; N, 13.60.

4.1.4.8. 3'-*O*-(t-Butoxycarbonyl)-5-fluoro-2'-deoxyuridine 5'-*O*-(4-chlorophenyl *N*-*n*-butylphosphate) (9h)

^{1}H NMR (DMSO-d_6) δ: 0.82 (t, 3H, *J* = 7.3 Hz, N-C-C-C-CH_3), 1.17–1.29 (m, 2H, N-C-C-CH_2C), 1.30–1.40 (m, 2H, N-C-CH_2-C-C), 1.44 (s, 9H, *t*-Bu), 2.25–2.38 (m, 2H, H-2', H-2''), 2.72–2.88 (m, 2H, N-CH_2-C-C-C), 4.19–4.29 (m, 2H, H-5', H-5''), 4.72 (m, 1H, H-4'), 5.07–5.13 (m, 1H, H-3'), 5.57 (m, 1H, P-NH), 6.13 (m, 1H, H-1'), 7.19, 7.23 (d, 2H, *J* = 9.0 Hz, 4-ClPh), 7.38, 7.43 (d, 2H, *J* = 9.0 Hz, 4-ClPh), 7.94, 7.97 (d, 1H, J_{H-F} = 6.9 Hz, H-6), 11.89 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 13.53, 13.71, 19.17, 27.28, 33.15, 35.45, 65.57, 76.37, 82.00, 82.51, 84.71, 121.81, 124.45 (d, J_{C-F} = 34.30 Hz), 129.51, 138.98, 140.13 (d, J_{C-F} = 231.70 Hz), 148.59, 149.60, 152.06, 156.94 (d, *J* = 26.1 Hz). ^{19}F NMR (DMSO-d_6) δ: –166.11 (d, 1F, *J* = 6.9 Hz). ^{31}P NMR (DMSO-d_6) δ: 6.51, 6.65. MS-ESI *m/z*: 592, 594 $[M + H]^+$; 614, 616 $[M + Na]^+$; 630, 632 $[M + K]^+$; 590, 592 $[M – H]^–$; 626, 628, 630 $[M + Cl]^–$. Anal. Calcd for $C_{24}H_{32}ClFN_3O_9P$: C, 48.70; H, 5.45; N, 7.10. Found: C, 48.75; H, 5.46; N, 7.12.

4.1.4.9. 3'-*O*-(t-Butoxycarbonyl)-5-fluoro-2'-deoxyuridine 5'-*O*-[4-chlorophenyl *N*-(4-azidobutyl)phosphate] (9i)

^{1}H NMR (DMSO-d_6) δ: 1.26–1.65 (m, 13H, N-C-CH_2-CH_2-C-N_3, *t*-Bu), 2.31–2.42 (m, 2H, H-2', H-2''), 2.81–2.95 (m, 2H, P-N-CH_2), 3.31 (t, 2H, *J* = 6.4 Hz, CH_2-N_3), 4.20–4.34 (m, 2H, H-5', H-5''), 4.89 (m, 1H, H-4'), 5.07–5.20 (m, 1H, H-3'), 5.69 (m, 1H, P-NH), 6.16 (m, 1H, H-1'), 7.27 (d, 2H, *J* = 8.8 Hz, 4-ClPh), 7.47 (d, 2H, *J* = 8.8 Hz, 4-ClPh), 7.98, 8.00 (d, 1H, J_{H-F} = 6.9 Hz, H-6), 11.98 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 25.53, 27.28, 28.55, 36.26, 40.11, 50.26, 65.87, 76.36, 82.00, 82.58, 84.58, 122.09, 124.60 (d, J_{C-F} = 34.30 Hz),

129.53, 138.88, 141.29 (d, J_{C-F} = 231.70 Hz), 148.79, 149.53, 151.95, 156.58 (d, J_{C-F} = 26.10 Hz). ^{19}F NMR (DMSO-d_6) δ: –165.93 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 6.43, 6.59. MS-ESI *m/z*: 633, 635 $[M + H]^+$; 655, 657 $[M + Na]^+$; 671, 673 $[M + K]^+$; 631, 633 $[M – H]^–$; 667, 669, 671 $[M + Cl]^–$. Anal. Calcd for $C_{24}H_{31}ClFN_6O_9P$: C, 45.54; H, 4.94; N, 13.28. Found: C, 45.60; H, 4.95; N, 13.31.

4.1.4.10. 3'-*O*-(t-Butoxycarbonyl)-5-fluoro-2'-deoxyuridine 5'-*O*-[4-chlorophenyl *N*-(methoxy-(*S*)-alaninyl)phosphate] (9j)

^{1}H NMR ($CDCl_3$) δ: 1.32–1.40 (m, 3H, N-C(CH_3)CO_2C), 1.50 (s, 9H, *t*-Bu), 1.94–2.09 (m, 1H, H-2'), 2.43–2.56 (m, 1H, H-2''), 3.71, 3.74 (2 x s, 3H, O-CH_3), 3.92–4.11 (m, 1H, N-CH(C)CO_2C), 4.21–4.28 (m, 1H, H-4'), 4.37–4.56 (m, 2H, H-5', H-5''), 5.14–5.19 (m, 1H, H-3'), 5.42 (m, 1H, P-NH), 6.25–6.36 (m, 1H, H-1'), 7.12, 7.22 (d, 2H, *J* = 9.1 Hz, 4-ClPh), 7.25, 7.35 (d, 2H, *J* = 9.1 Hz, 4-ClPh), 7.74, 7.79 (d, 1H, J_{H-F} = 6.7 Hz, H-6), 9.65 (br s, 1H, H-3). ^{13}C NMR ($CDCl_3$) δ: 20.75, 20.94 (2 x d, J_{C-P} = 6.7 Hz, N-C($\underline{C}H_3$)CO_2C), 27.64 (3 x CH_3, *t*-Bu), 37.30, 37.53 (C-2'), 50.21, 50.34 (N-$\underline{C}$H(C)CO_2C), 52.44, 52,68 (O-CH_3), 66.34, 66.51 (2 x d, J_{C-P} = 5.1 Hz, C-5'), 76.28 (C-3'), 82.96, 83.06 (*t*-C, BOC), 83.56 (C-1'), 85.02, 85.19 (2 x d, J_{C-P} = 8.2 Hz, C-4'), 121.31, 121.56 (2 x d, J_{C-P} = 4.7 Hz, C-2, C-6 of Ph), 123.68 (d, J_{C-F} = 34.1 Hz, C-6), 129.54, 129.79 (C-3, C-5 of Ph), 130.58, 130.66 (C-4 of Ph), 140.59, 140.63 (2 x d, J_{C-F} = 237.4 Hz, C-5), 148.69, 148.82 (2 x d, J_{C-P} = 6.8 Hz, C-1 of Ph), 148.87, 148.95 (2 x d, J_{C-F} = 3.5 Hz, C-2), 152.57, 152.60 (C=O, BOC), 156.79, 156.82 (2 x d, J_{C-F} = 26.9 Hz, C-4), 173.67, 174.06 (2 x d, J_{C-P} = 3.7 Hz, C=O, Ala). ^{19}F NMR ($CDCl_3$) δ: –166.13 (m, 1F). ^{31}P NMR ($CDCl_3$) δ: 3.36, 3.93. MS-ESI *m/z*: 622, 624 $[M + H]^+$; 644, 646 $[M + Na]^+$; 660, 662 $[M + K]^+$; 620, 622 $[M – H]^–$; 656, 658, 660 $[M + Cl]^–$. Anal. Calcd for $C_{24}H_{30}ClFN_3O_{11}P$: C, 46.35; H, 4.86; N, 6.76. Found: C, 46.46; H, 4.84; N, 6.71.

4.1.5. 3'-*O*-(t-Butoxycarbonyl)-5-fluoro-2'-deoxyuridine 5'-*O*-[phenyl *N*-(methoxy-(*S*)-alaninyl)phosphate] (9k)

To a stirred solution of 3'-*O*-(*t*-butoxycarbonyl)-5-fluoro-2'-deoxyuridine (**4**) (252 mg, 0.728 mmol) in dry pyridine (4 mL) was added dropwise diphenyl phosphite (341 mg, 1.456 mmol) and the stirring was continued at room temperature for 1 h. Then to the mixture was added L-alanine methyl ester hydrochloride (203 mg, 1.456 mmol) followed by acetonitrile (3 mL), triethylamine (810 mg, 8 mmol) and carbon tetrachloride (3 mL). The reaction mixture was stirred at room temperature for 2 h. After this time, the mixture was

evaporated under reduced pressure. To the residue was added saturated aqueous sodium bicarbonate (20 mL) and the mixture was extracted with chloroform (3 x 20 mL). The combined chloroform extracts were washed with water (20 mL), dried over anhydrous magnesium sulfate, filtered and evaporated to dryness. The residue was purified by silica gel column chromatography using chloroform – methanol (from 150 : 1 to 80 : 1, v/v) as eluent to afford pure **9k** (yield: 282 mg, 66%). ^{1}H NMR (DMSO-d_6) δ: 1.28–1.37 (m, 3H, N-C(CH_3)CO_2C), 1.44 (s, 9H, *t*-Bu), 2.21–2.35 (m, 2H, H-2', H-2''), 3.61, 3.68 (2 x s, 3H, O-CH_3), 3.78–3.99 (m, 1H, N-CH(C)CO_2C), 4.12–4.35 (m, 1H, H-4'), 4.81–4.94 (m, 2H, H-5', H-5''), 5.12–5.29 (m, 1H, H-3'), 5.18 (m, 1H, P-NH), 6.12 (m, 1H, H-1'), 7.10-7.28 (m, 3H, Ph), 7.35-7.42 (m, 2H, Ph), 7.92, 7.94 (d, 1H, J_{H-F} = 6.8 Hz, H-6), 11.92 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 20.75, 20.93 (2 x d, J_{C-P} = 6.7 Hz, N-C(<u>C</u>H_3)CO_2C), 27.28 (3 x CH_3, *t*-Bu), 37.30, 37.52 (C-2'), 50.21, 50.33 (N-<u>C</u>H(C)CO_2C), 52.45, 52,68 (O-CH_3), 66.37, 66.51 (2 x d, J_{C-P} = 5.1 Hz, C-5'), 76.28 (C-3'), 82.96, 83.04 (*t*-C, BOC), 83.56 (C-1'), 85.05, 85.19 (2 x d, J_{C-P} = 8.2 Hz, C-4'), 121.34, 121.56 (2 x d, J_{C-P} = 4.7 Hz, Ph), 123.68 (d, J_{C-F} = 34.1 Hz, C-6), 129.54, 129.79 (Ph), 130.58, 130.66 (Ph), 140.59, 140.63 (2 x d, J_{C-F} = 236.8 Hz, C-5), 148.69, 148.83 (2 x d, J_{C-P} = 6.8 Hz, Ph), 148.87, 148.95 (2 x d, J_{C-F} = 3.5 Hz, C-2), 152.57, 152.62 (C=O, BOC), 156.89, 157.12 (2 x d, J_{C-F} = 26.9 Hz, C-4), 173.67, 174.28 (2 x d, J_{C-P} = 3.7 Hz, C=O, Ala). ^{19}F NMR (DMSO-d_6) δ: –165.90 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 4.30, 4.47. MS-ESI *m/z*: 588 $[M + H]^+$; 610 $[M + Na]^+$; 626 $[M + K]^+$; 586 $[M – H]^–$; 622, 624 $[M + Cl]^–$. Anal. Calcd for $C_{24}H_{31}FN_3O_{11}P$: C, 49.07; H, 5.32; N, 7.15. Found: C, 49.16; H, 5.28; N, 7.11.

4.1.6. General procedure for the synthesis of target compounds 10a–10k

To a stirred solution of compound **9** (0.20 mmol) in dichloromethane (8 mL) at 0 °C was added pre-cooled trifluoroacetic acid (8 mL) and stirring was continued at 0 °C for 40 min. Then the mixture was evaporated to dryness. The residue was treated with 5% aqueous solution of sodium bicarbonate (10 mL) and it was extracted with ethyl acetate (3 x 15 mL). The combined organic extracts were washed with water (10 mL), dried over anhydrous magnesium sulphate, filtered and evaporated to dryness. The residue was purified by silica gel column chromatography using chloroform–methanol (gradient from 100:1 to 20:1, v/v) as eluent to afford pure product **10** (yield: 78–89%).

4.1.6.1. 5-Fluoro-2'-deoxyuridine 5'-*O*-(4-chlorophenyl *N*-methylphosphate) (10a)

^{1}H NMR (DMSO-d_6) δ: 2.35–2.42 (m, 2H, H-2', H-2''), 3.10 (m, 3H, N-CH_3), 3.46–3.58 (m, 1H, H-4'), 3.85–4.03 (m, 2H, H-5', H-5''), 4.12–4.31 (m, 1H, H-3'), 5.45 (m, 1H, P-NH), 6.16 (m, 1H, H-1'), 7.21, 7.30 (d, 2H, J = 8.9 Hz, 2H, 4-ClPh), 7.39, 7.44 (d, 2H, J = 8.9 Hz, 4-ClPh), 7.94, 7.96 (d, 1H, J_{H-F} = 4.5 Hz, H-6), 11.99 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 24.32, 38.87, 65.56, 70.00, 70.61, 84.72, 121.70, 124.63 (d, J_{C-F} = 34.1 Hz), 126.72, 129.61, 140.10 (d, J_{C-F} = 231.1 Hz), 149.04, 151.80, 157.05 (d, J_{C-F} = 26.1 Hz). ^{19}F NMR (DMSO-d_6) δ: –166.09 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 7.39, 7.53. MS-ESI m/z: 450, 552 $[M + H]^+$; 472, 474 $[M + Na]^+$; 488, 490 $[M + K]^+$; 448, 450 $[M – H]^–$; 484, 486, 488 $[M + Cl]^{–·}$. Anal. Calcd for $C_{16}H_{18}ClFN_3O_7P$: C, 42.73; H, 4.03; N, 9.34. Found: C, 42.78; H, 4.04; N, 9.36. HPLC: retention time (t_R) of 3.82 and 4.35 min in the ratio 1 : 1.

4.1.6.2. 5-Fluoro-2'-deoxyuridine 5'-*O*-(4-chlorophenyl *N*-ethylphosphate) (10b)

^{1}H NMR (DMSO-d_6) δ: 1.00 (t, 3H, J = 7.2 Hz, CH_3), 2.06–2.18 (m, 2H, H-2', H-2''), 2.86 (m, 2H, N-CH_2), 3.10 (m, 1H, H-4'), 3.90–4.04 (m, 2H, H-5', H-5''), 4.10 - 4.30 (m, 1H, H-3'), 5.55 (m, 1H, P-NH), 6.16 (pseudo t, 1H, J = 6.6 Hz, H-1'), 7.23 (d, 2H, J = 8.9 Hz, 4-ClPh), 7.43 (d, 2H, J = 8.9 Hz, 4-ClPh), 7.91, 7.93 (d, 1H, J_{H-F} = 7.0 Hz, H-6), 11.89 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 16.86, 35.15, 39.10, 65.71, 69.97, 70.55, 84.61, 121.89, 124.51 (d, J_{C-F} = 34.1 Hz), 128.46, 129.49, 140.04 (d, J_{C-F} = 231.3 Hz), 148.96, 149.65, 156.95 (d, J_{C-F} = 26.1 Hz). ^{19}F NMR (DMSO-d_6) δ: –166.27 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 6.34, 6.47. MS-ESI m/z: 464, 466 $[M + H]^+$; 486, 488 $[M + Na]^+$; 502, 504 $[M + K]^+$; 462, 464 $[M – H]^–$; 498, 500, 502 $[M + Cl]^–$. Anal. Calcd for $C_{17}H_{20}ClFN_3O_7P$: C, 44.03; H, 4.35; N, 9.06. Found: C, 44.07; H, 4.36; N, 9.08. HPLC: retention time (t_R) of 4.19 and 4.68 min in the ratio 1 : 1.

4.1.6.3. 5-Fluoro-2'-deoxyuridine 5'-*O*-[4-chlorophenyl *N*-(2,2,2-trifluoroethyl)phosphate] (10c)

^{1}H NMR (DMSO-d_6) δ: 2.15–2.25 (m, 2H, H-2', H-2''), 3.38–3.68 (m, 2H, N-CH_2), 3.18–3.33 (m, 1H, H-4'), 3.89–4.02 (m, 2H, H-5', H-5''), 4.11–4.24 (m, 1H, H-3'), 5.54 (m, 1H, P-NH), 6.15 (pseudo t, 1H, J = 6.4 Hz, H-1'), 7.18, 7.23 (d, 2H, J = 8.9 Hz, 4-ClPh), 7.38, 7.44 (d, 2H, J = 8.9 Hz, 4-ClPh), 7.88, 7.92 (d, 1H, J_{H-F} = 7.3 Hz, H-6), 11.88 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 35.55, 41.34, 65.98, 69.99, 74.84, 84.64, 121.87, 122.54, 124.68 (d, J_{C-F} = 34.2 Hz), 128.84, 129.47 (m), 141.01 (d, J_{C-F} = 231.1 Hz), 148.94, 149.12, 156.94 (d, J_{C-F} = 26.2 Hz). ^{19}F NMR (DMSO-d_6) δ: –166.56 (m, 1F), –71.84 (t, 3F, J = 8.9 Hz). ^{31}P NMR (DMSO-d_6) δ: 5.48; 5.69. MS-ESI m/z: 518, 520 $[M + H]^+$; 540, 542 $[M + Na]^+$; 556, 558 [M

+ K]$^+$; 516, 518 [M – H]$^-$; 552, 554, 556 [M + Cl]$^-$. Anal. Calcd for $C_{17}H_{17}ClF_4N_3O_7P$: C, 39.44; H, 3.31; N, 8.12. Found: C, 39.59; H, 3.32; N, 8.14.

4.1.6.4. 5-Fluoro-2'-deoxyuridine 5'-*O*-(4-chlorophenyl *N*-*n*-propylphosphate) (10d)

^{1}H NMR (DMSO-d_6) δ: 0.81 (t, 3H, J = 8.0 Hz, CH_3), 1.37 (m, 2H, N-C-CH_2-C), 2.08–2.15 (m, 2H, H-2', H-2''), 2.72–2.83 (m, 2H, N-CH_2-C-C), 3.94–3.97 (m, 1H, H-4'), 4.11–4.30 (m, 2H, H-5', H-5''), 4.58–4.64 (m, 1H, H-3'), 5.72 (m, 1H, P-NH), 6.15 (pseudo t, 1H, J = 6.5 Hz, H-1'), 7.22 (d, 2H, J = 11.4 Hz, 4-ClPh), 7.48 (d, 2H, J = 11.4 Hz, 4-ClPh), 7.88, 7.94 (2 x d, 1H, J_{H-F} = 9.4 Hz, H-6), 11.88 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 11.09 (CH_3), 24.27, 24.32 (2 x d, J_{C-P} = 1.6 Hz, N-C-C-C), 38.76, 38.83 (C-2'), 42.66 (P-N-C), 65.56, 65.75 (2 x d, J_{C-P} = 4.6 Hz, C-5'), 69.89, 69.93 (C-3'), 84.50 (C-1'), 84.64, 84.75 (C-4'), 121.66, 121.77 (2 x d, J_{C-P} = 4.9 Hz, C-2, C-6 of Ph), 124.31, 124,45 (2 x d, J_{C-F} = 33.9 Hz, H-6), 128.29 (C-4 of Ph), 129.35 (C-3, C-5 of Ph), 139.86 (d, J_{C-F} = 231.0 Hz, C-5), 148.77 (C-2), 149.46, 149.49 (2 x d, J_{C-P} = 6.0 Hz, C-1 of Ph), 156.76 (d, J_{C-F} = 26.2 Hz, C-4). ^{19}F NMR (DMSO-d_6) δ: –166.34 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 6.48, 6.53. MS-ESI *m/z*: 478, 480 [M + H]$^+$; 500, 502 [M + Na]$^+$; 516, 518 [M + K]$^+$; 476, 478 [M – H]$^-$; 512, 514, 516 [M + Cl]$^-$. Anal. Calcd for $C_{18}H_{22}ClFN_3O_7P$: C, 45.25; H, 4.64; N, 8.79. Found: C, 45.17; H, 4.62; N, 8.81.

4.1.6.5. 5-Fluoro-2'-deoxyuridine 5'-*O*-(4-chlorophenyl *N*-allylphosphate) (10e)

^{1}H NMR (DMSO-d_6) δ: 2.02–2.18 (m, 2H, H-2', H-2''), 3.47 (m, 2H, N-CH_2-C=C), 3.73–4.32 (m, 3H, H-4', H-5', H-5''), 5.01 (m, 1H, H-3'), 5.09–5.29 (m, 2H, N-C-C=CH_2), 5.69–5.85 (m, 2H, P-NH, N-C-CH=C), 6.15 (pseudo t, 1H, J = 7.5 Hz, H-1'), 7.22 (d, 2H, J = 8.9 Hz, 4-ClPh), 7.42 (d, 2H, J = 8.9 Hz, 4-ClPh), 7.90 (d, 1H, J_{H-F} = 7.0 Hz, H-6), 11.89 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 38.66, 43.21, 65.95, 70.07, 84.78, 115.14, 122.05, 124.62 (d, J_{C-F} = 34.3 Hz), 128.63, 129.26, 136.53, 138.87, 140.12 (d, J_{C-F} = 231.3 Hz), 149.05, 149.65, 157.05 (d, J_{C-F} = 26.1 Hz). ^{19}F NMR (DMSO-d_6) δ: –166.18 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 6.24, 6.41. MS-ESI *m/z*: 476, 478 [M + H]$^+$; 498, 500 [M + Na]$^+$; 514, 516 [M + K]$^+$; 474, 476 [M – H]$^-$; 510, 512, 514 [M + Cl]$^-$. Anal. Calcd for $C_{18}H_{20}ClFN_3O_7P$: C, 45.44; H, 4.24; N, 8.83. Found: C, 45.49; H, 4.25; N, 8.85.

4.1.6.6. 5-Fluoro-2'-deoxyuridine 5'-*O*-(4-chlorophenyl *N*-propargylphosphate) (10f)

^{1}H NMR (DMSO-d_6) δ: 2.09 (s, 1H, N-C-C-CH), 2.11–2.22 (m, 2H, H-2', H-2''), 3.11 (m, 2H, N-CH_2-C-C), 3.66–3.75 (m, 2H, H-5', H-5''), 4.18–4.36 (m, 1H, H-4'), 5.09–5.15 (m, 2H, P-NH, H-3'), 6.18 (pseudo t, 1H, J = 6.4 Hz, H-1'), 7.28 (d, 2H, J = 8.7 Hz, 4-ClPh), 7.46 (d, 2H, J = 8.7 Hz, 4-ClPh), 7.89 (d, 1H, J_{H-F} = 6.8 Hz, H-6), 11.95 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 30.37, 48.51, 66.56, 70.53, 74.03, 76.39, 82.38, 85.18, 122.58, 125.09 (d, J_{C-F} = 34.0 Hz), 129.47, 139.04, 141.34 (d, J_{C-F} = 231.7 Hz), 149.52, 149.72, 157.70 (d, J_{C-F} = 26.0 Hz). ^{19}F NMR (DMSO-d_6) δ: –165.79 (d, 1F, J = 6.4 Hz). ^{31}P NMR (DMSO-d_6) δ: 5.55, 5.73. MS-ESI *m/z*: 474, 476 $[M + H]^+$; 496, 498 $[M + Na]^+$; 512, 514 $[M + K]^+$; 472, 474 $[M – H]^–$; 508, 510, 512 $[M + Cl]^–$. Anal. Calcd for $C_{18}H_{18}ClFN_3O_7P$: C, 45.63; H, 3.83; N, 8.87. Found: C, 45.69; H, 3.84; N, 8.89.

4.1.6.7. 5-Fluoro-2'-deoxyuridine 5'-*O*-[4-chlorophenyl *N*-(3-azidopropyl)phosphate] (10g)

^{1}H NMR (DMSO-d_6) δ: 1.60 (m, 2H, N-C-CH_2-C-N_3), 2.07–2.16 (m, 2H, H-2', H-2''), 2.83–2.94 (m, 2H, N-CH_2-C-C-N_3), 3.32 (m, 2H, N-C-C-CH_2-N_3), 3.69 (m, 1H, H-4'), 4.10–4.29 (m, 2H, H-5', H-5''), 5.51 (m, 1H, H-3'), 5.65 (m, 1H, P-NH), 6.16 (pseudo t, 1H, J = 6.8 Hz, H-1'), 7.23 (d, 2H, J = 8.7 Hz, 4-ClPh), 7.44 (d, 2H, J = 8.7 Hz, 4-ClPh), 7.90, 7.93 (2 x d, 2H, J_{H-F} = 6.9 Hz, H-6), 11.89 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 30.22, 35.78, 38.10, 48.32, 65.96, 69.99, 81.90, 84.73, 121.92, 124.62 (d, J_{C-F} = 34.0 Hz), 128.67, 129.63, 140.13 (d, J_{C-F} = 231.3 Hz), 149.05, 149.61, 157.06 (d, J_{C-F} = 26.1 Hz). ^{19}F NMR (DMSO-d_6) δ: –166.20 (d, 1F, J = 5.7 Hz). ^{31}P NMR (DMSO-d_6) δ: 6.31, 6.48. MS-ESI *m/z*: 519, 521 $[M + H]^+$; 541, 543 $[M + Na]^+$; 557, 559 $[M + K]^+$; 517, 519 $[M – H]^–$; 553, 555, 557 $[M + Cl]^–$. Anal. Calcd for $C_{18}H_{21}ClFN_6O_7P$: C, 41.67; H, 4.08; N, 16.20. Found: C, 41.71; H, 4.09; N, 16.23.

4.1.6.8. 5-Fluoro-2'-deoxyuridine 5'-*O*-(4-chlorophenyl *N*-*n*-butylphosphate) (10h)

^{1}H NMR (DMSO-d_6) δ: 0.84 (t, 3H, J = 7.3 Hz, CH_3), 1.20–1.31 (m, 2H, N-C-C-CH_2-C), 1.33–1.42 (m, 2H, N-C-CH_2-C-C), 2.09–2.21 (m, 2H, H-2', H-2''), 2.76–2.94 (m, 2H, N-CH_2-C-C-C), 3.14 (m, 1H, H-4'), 4.00 (m, 2H, H-5', H-5''), 4.13–4.35 (m, 1H, H-3'), 5.58 (m, 1H, P-NH), 6.20 (pseudo t, 1H, J = 6.7 Hz, H-1'), 7.27 (d, 2H, J = 8.9 Hz, 4-ClPh), 7.47 (d, 2H, J = 8.9 Hz, 4-ClPh), 7.94, 7.97 (2 x d, 1H, J_{H-F} = 6.9 Hz, H-6), 11.94 (br s,1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 13.62, 13.81, 19.26, 32.22, 44.66, 65.64, 70.02, 84.29, 121.97, 124.63 (d, J_{C-F} = 34.0 Hz), 127.73, 128.45, 129.59, 140.14 (d, J_{C-F} = 231.3 Hz), 149.06, 149.75, 157.08 (d, J_{C-F} = 26.1 Hz). ^{19}F NMR (DMSO-d_6) δ: –166.20 (pseudo t, 1F, J = 5.3

Hz). ^{31}P NMR (DMSO-d_6) δ: 6.50, 6.66. MS-ESI *m/z*: 492, 494 $[M + H]^+$; 514, 516 $[M + Na]^+$; 530, 532 $[M + K]^+$; 490, 492 $[M - H]^-$; 526, 528, 530 $[M + Cl]^-$. Anal. Calcd for $C_{19}H_{24}ClFN_3O_7P$: C, 46.40; H, 4.92; N, 8.54. Found: C, 46.45; H, 4.93; N, 8.55.

4.1.6.9. 5-Fluoro-2'-deoxyuridine 5'-*O*-[4-chlorophenyl *N*-(4-azidobutyl)phosphate] (10i)

^{1}H NMR (DMSO-d_6) δ: 1.35–1.53 (m, 4H, N-C-CH_2-CH_2-C-N_3), 2.03–2.18 (m, 2H, H-2', H-2''), 2.79–2.90 (m, 2H, N-CH_2-C-C-C-N_3), 3.26 (t, 2H, *J* = 6.7 Hz, N-C-C-C-CH_2-N_3), 3.95 (m, 1H, H-4'), 4.10–4.21 (m, 2H, H-5', H-5''); 4.22–4.28 (m, 1H, H-3'), 5.61 (m, 1H, P-NH), 6.16 (pseudo t, 1H, *J* = 7.1 Hz, H-1'), 7.23 (d, 2H, *J* = 8.8 Hz, 4-ClPh), 7.43 (d, 2H, *J* = 8.8 Hz, 4-ClPh), 7.90, 7.93 (2 x d, 1H, J_{H-F} = 7.0 Hz, H-6); 11.89 (br s,1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 25.41, 28.23, 36.24, 40.12, 50.30, 65.38, 68.82, 84.74, 121.53, 124.57 (d, J_{C-F} = 34.0 Hz), 127.73, 129.06, 140.09 (d, J_{C-F} = 231.2 Hz), 146.84, 149.00, 149.65, 157.00 (d, J_{C-F} = 26.1 Hz). ^{19}F NMR (DMSO-d_6) δ: –166.22 (pseudo t, 1F, *J* = 5.3 Hz). ^{31}P NMR (DMSO-d_6) δ: 6.44, 6.60. MS-ESI *m/z*: 533, 535 $[M + H]^+$; 555, 557 $[M + Na]^+$; 571, 573 $[M + K]^+$; 531, 533 $[M - H]^-$; 567, 569, 571 $[M + Cl]^-$. Anal. Calcd for $C_{19}H_{23}ClFN_6O_7P$: C, 42.83; H, 4.35; N, 6.50. Found: C, 42.89; H, 4.36; N, 6.51.

4.1.6.10. 5-Fluoro-2'-deoxyuridine 5'-*O*-[4-chlorophenyl *N*-(methoxy-(*S*)-alaninyl)phosphate] (10j)

^{1}H NMR (DMSO-d_6) δ: 1.20–1.25 (m, 3H, N-C(CH_3)CO_2C), 2.10–2.14 (m, 2H, H-2', H-2''), 3.58, 3.59 (2 x s, 3H, O-CH_3), 3.69–3.81 (m, 1H, N-CH(C)CO_2C), 4.01–4.12 (m, 1H, H-4'), 4.36–4.54 (m, 2H, H-5', H-5"), 5.08–5.17 (m, 1H, H-3'), 5.32 (m, 1H, P-NH), 6.12–6.26 (m, 1H, H-1'), 7.18, 7.22 (2 x d, 2H, *J* = 9.0 Hz, 4-ClPh), 7.27, 7.38 (2 x d, 2H, *J* = 9.0 Hz, 4-ClPh), 7.84, 7.91 (2 x d, 1H, J_{H-F} = 6.7 Hz, H-6), 11.89 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 19.52, 19.75 (2 x d, J_{C-P} = 6.6 Hz, N-C($\underline{C}H_3$)CO_2C), 38.78 (C-2'), 49.58, 49.76 (N-$\underline{C}$H(C)CO_2C), 51.93 (O-CH_3), 65.88, 66.06 (2 x d, J_{C-P} = 5.2 Hz, C-5'), 69.95, 70.02 (C-3'), 84.59 (C-1'), 84.64, 84.73 (2 x d, J_{C-P} = 8.1 Hz, C-4'), 121.95, 122.08 (2 x d, J_{C-P} = 4.7 Hz, C-2, C-6 of Ph), 124.55, 124.62 (2 x d, J_{C-F} = 33.7 Hz, C-6), 128.66, 128.69 (C-3, C-5 of Ph), 129.52, 130.11 (C-4 of Ph), 140.08, 140.23 (2 x d, J_{C-F} = 230.6 Hz, C-5), 148.92, 149.04 (2 x d, J_{C-P} = 6.7 Hz, C-1 of Ph), 149.44, 149.52 (2 x d, J_{C-F} = 3.5 Hz, C-2), 157.03, 157.22 (2 x d, J_{C-F} = 26.2 Hz, C-4), 173.51, 173.70 (2 x d, J_{C-P} = 3.7 Hz, C=O, Ala). ^{19}F NMR (DMSO-d_6) δ: –166.21 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 4.92, 5.02. MS-ESI *m/z*: 522, 524 $[M + H]^+$; 544,

546 $[M + Na]^+$; 560, 562 $[M + K]^+$; 520, 522 $[M - H]^-$; 556, 558, 560 $[M + Cl]^-$. Anal. Calcd for $C_{19}H_{22}ClFN_3O_9P$: C, 43.73; H, 4.25; N, 8.05. Found: C, 43.64; H, 4.26; N, 8.07.

4.1.6.11. 5-Fluoro-2'-deoxyuridine 5'-*O*-[phenyl *N*-(methoxy-(*S*)-alaninyl)phosphate] (10k)[3]

^{1}H NMR (DMSO-d_6) δ: 1.18–1.27 (m, 3H, N-C(CH_3)CO_2C), 2.25–2.42 (m, 2H, H-2', H-2''), 3.46, 3.55 (2 x s, 3H, O-CH_3), 3.82–3.99 (m, 1H, N-CH(C)CO_2C), 4.08–4.21 (m, 1H, H-4'), 4.30–4.44 (m, 2H, H-5', H-5"), 4.57–4.68 (m, 1H, H-3'), 5.13 (m, 1H, P-NH), 6.20 (m, 1H, H-1'), 7.02-7.20 (m, 3H, Ph), 7.38-7.49 (m, 2H, Ph), 7.88, 7.99 (d, 1H, J_{H-F} = 6.8 Hz, H-6), 11.94 (br s, 1H, H-3). ^{13}C NMR (DMSO-d_6) δ: 19.75, 20.02 (2 x d, J_{C-P} = 6.7 Hz, N-C(<u>C</u>H_3)CO_2C), 38.78, 39.02 (C-2'), 49.77, 49.98 (N-<u>C</u>H(C)CO_2C), 51.93, 52,07 (O-CH_3), 65.87, 65.98 (2 x d, J_{C-P} = 5.1 Hz, C-5'), 69.95, 70,08 (C-3'), 84.35 (C-1'), 85.03, 85.18 (2 x d, J_{C-P} = 8.2 Hz, C-4'), 121.74, 121.96 (2 x d, J_{C-P} = 4.7 Hz, Ph), 124.62 (d, J_{C-F} = 34.0 Hz, C-6), 129.52, 129.78 (Ph), 130.12, 130.26 (Ph), 140.12, 140.23 (2 x d, J_{C-F} = 233.8 Hz, C-5), 148.94, 149.05 (2 x d, J_{C-P} = 6.8 Hz, Ph), 150.84, 150.95 (2 x d, J_{C-F} = 3.5 Hz, C-2), 157.03, 157.15 (2 x d, J_{C-F} = 26.3 Hz, C-4), 173.72, 173.91 (2 x d, J_{C-P} = 3.7 Hz, C=O, Ala). ^{19}F NMR (DMSO-d_6) δ: –166.72 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 4.38, 4.50. MS-ESI *m/z*: 488 $[M + H]^+$; 510 $[M + Na]^+$; 526 $[M + K]^+$; 486 $[M - H]^-$; 522, 524 $[M + Cl]^-$. Anal. Calcd for $C_{19}H_{23}FN_3O_9P$: C, 46.82; H, 4.76; N, 8.62. Found: C, 46.67; H, 4.69; N, 8.51.

4.2. Biological evaluation

4.2.1. Cell cultures

Human cancer cells KB (*carcinoma nasopharynx*) were cultured in RPMI 1640 medium. Human cancer cells MCF-7 (breast cancer cell line) were cultured in DMEM medium. Human cancer cells HeLa (cervical cancer cell line), HepG2 (liver cancer cell line), 143B (osteosarcoma cell line) and 143B/TK- (thymidine kinase deficient osteosarcoma cell line) were cultured in MEM medium. Each medium was supplemented with 10% fetal bovine serum, 1% L-glutamine and 1% penicillin/streptomycin solution. The cell lines were kept in the incubator at 37 °C in a humidified atmosphere (90% RH) containing 5% CO_2. The optimal plating density of cell lines was determined to be 5 x 10^4. The cell lines HeLa, KB, MCF-7, HepG2 and HDF (fetal) were obtained from The European Collection of Cell Cultures (ECACC) supplied by Sigma-Aldrich. The cell lines 143B and 143B/TK- (osteosarcoma)

were purchased from LGC Standards (ATCC). Supermix^{TM} was obtained from Gentest Corporation (Woburn, MA, USA).

4.2.2. In vitro cytotoxicity assay

The protein-staining sulforhodamine B (SRB, Sigma-Aldrich) microculture colorimetric assay, developed by the National Cancer Institute (USA) for in vitro antitumor screening was used in this study, to estimate the cell number by providing a sensitive index of total cellular protein content, being linear to cell density.[36] The monolayer cell culture was trypsinized and the cell count was adjusted to 5 x 10^4 cells. To each well of the 96 well microtiter plate, 100 µL of the cell suspension in a growth medium (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was washed out and 100 µL of six different compound concentrations (0.1, 0.2, 1, 2, 10 and 20 µM) were added to the cells in microtitre plates. The tested compounds were dissolved in DMSO (containing 10% of water) (50 µL) and the content of DMSO did not exceed 0.1%; this concentration was found to be nontoxic to the cell lines. The cells were exposed to compounds for 72 h at 37 °C in a humidified atmosphere (90% RH) containing 5% CO_2. After that, 25 µL of 50% trichloroacetic acid was added to the wells and the plates were incubated for 1 h at 4°C. The plates were then washed out with the distilled water to remove traces of medium and next dried by the air. The air-dried plates were stained with 100 µL of 0.4% sulforhodamine B (prepared in 1% acetic acid) and kept for 30 minutes at room temperature. The unbound dye was removed by washing five times with 1% acetic acid and then the plates were air dried overnight. The protein-bound dye was dissolved in 100 µL of 10 mM unbuffered Tris base (pH 10.5) for optical density determination at 490 nm. All cytotoxicity experiments were performed three times. Cell survival was measured as the percentage absorbance compared to the control (non-treated cells). Cytarabine (Sigma-Aldrich) was used as the internal standard.

4.2.3. Growth of HeLa cancer cells in the presence of recombinant P450 enzymes

The incubation mixture (300 µL) consisted of HeLa cells (approximately 10,000 cells, see previous procedure) suspended in 100 µL of MEM growth medium, 100 µL of solution of investigated compound at six different concentrations (0.1, 0.2, 1, 2, 10 and 20 µM) and human recombinant P450 microsomes suspended in 100 µL of 100 mM phosphate buffer (pH

7.4). Microsomal protein concentration in the final mixture was about 200 µg/mL. The mixture was preincubated at 37 °C for 3 min, and reactions were initiated by addition of 1 mL of 100 mM phosphate buffer (pH 7.4) containing: $MgCl_2$ x $6H_2O$ (2.1 mg), disodium glucose-6-phosphate (10 mg), sodium NADPH (1.2 mg) and glucose-6-phosphate dehydrogenase (0.5 units in 5 µL).[46] Samples were mixed for 5 min on the rotational mixer and then incubated at 37°C for 1 h. Subsequently, the medium was discarded and the test tubes were washed with 100 mM phosphate buffer. Next, 4 mL of the MEM growth medium was added to each test tube and they were incubated at 37 °C for 72 h. The remaining steps were carried out according to the procedure described above for the determination of cellular protein concentration (the SRB assay).

4.2.4. Linear regression analysis

The equations describing the dependence of $-\log IC_{50}$ on log *P* for compounds **9a**–**9j** were as follows:

$-\log IC_{50} = -1.02 \log P + 7.60$, $R^2 = 0.73$, for HeLa cell line,

$-\log IC_{50} = -0.98 \log P + 7.49$, $R^2 = 0.76$, for KB cell line,

$-\log IC_{50} = -0.92 \log P + 7.41$, $R^2 = 0.57$, for MCF-7 cell line,

$-\log IC_{50} = -0.97 \log P + 7.42$, $R^2 = 0.88$, for HepG2 cell line.

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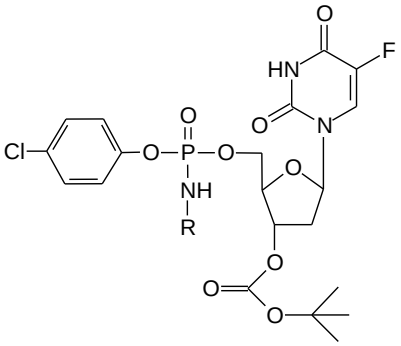
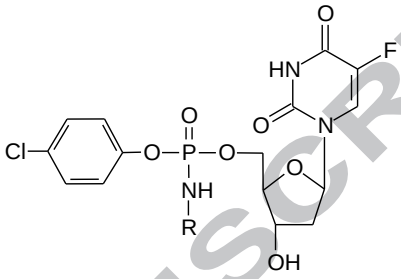
Synthesis and anticancer activity of some 5-fluoro-2'-deoxyuridine phosphoramidates

Marta Lewandowska, Piotr Ruszkowski, Kinga Chojnacka, Natalia Kleczewska, Marcin Hoffmann, Karol Kacprzak and Lech Celewicz*



9b: R = CH_2CH_3
IC_{50} = 1.42 μM (MCF-7)



10c: R = CH_2CF_3
IC_{50} = 1.82 μM (MCF-7)