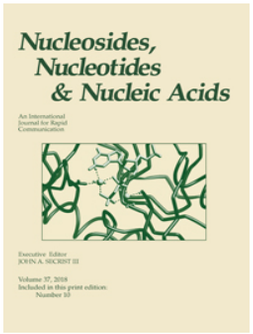

Nucleosides, Nucleotides and Nucleic Acids

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Synthesis and anticancer activity of 3′-[4-fluoroaryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidine analogs and their phosphoramidates

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ABSTRACT

A series of novel 4-chlorophenyl *N*-alkyl phosphoramidates of 3′-[4-fluoroaryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidines (**20**–**49**) was synthesized by means of phosphorylation of 3′-[4-aryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidines (**7**–**11**) with 4-chlorophenyl phosphoroditriazolide (**14**), followed by a reaction with the appropriate amine. The synthesized compounds **7**–**11** and **20**–**49** were evaluated along with four known anticancer compounds for their cytotoxic activity in human cancer cell lines: cervical (HeLa), nasopharyngeal (KB), breast (MCF-7), osteosarcoma (143B) (only selected compounds **20**, **24**, **28**, **32**–**36**, **38**, **40**, **46**) and normal human dermal fibroblast cell line (HDF) using the sulforhodamine B (SRB) assay. Among 3′-[4-aryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidines (**7**–**11**) the highest activity in all the investigated cancer cells was displayed by 3′-[4-(3-fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine (**9**) (IC_{50} in the range of 2.58–3.61 μM) and its activity was higher than that of cytarabine. Among phosphoramidates **20**–**49** the highest activity was demonstrated by *N*-*n*-propyl phosphoramidate of 3′-[4-(3-fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine (**35**) in all the cancer cells (IC_{50} in the range of 0.97–1.94 μM). Also *N*-ethyl phosphoramidate of 3′-[4-(3-fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine (**33**) exhibited good activity in all the used cell lines (IC_{50} in the range of 4.79–4.96 μM).

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Introduction

A number of derivatives of pyrimidine and purine bases, especially nucleoside analogs, has gained wide application as antiviral[1] and anticancer[2] agents. Particularly, 3′-azido-3′-deoxythymidine (AZT, zidovudine), the first approved anti-HIV drug, played an important role in the fight against AIDS in the initial outbreak of its pandemic.[1] In addition, there are also some reports that indicate the use of AZT as an antitumor agent in

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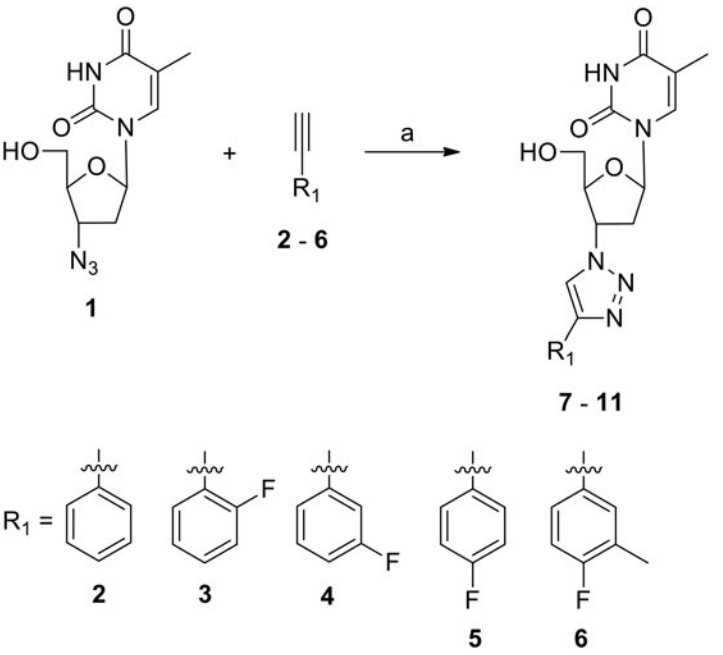
combination with either 5-fluorouracil or methotrexate in the therapy of advanced colon cancer.[3–5] Furthermore, Wagner reported the potent inhibitory activity of AZT in cultured human breast cancer cells.[6] Employing 'click' chemistry approach[7] 3′-azido-3′-deoxythymidine has been converted into a number of 3′-(1,2,3-triazol-1-yl)-3′-deoxythymidine derivatives, which were tested primarily for antiviral activity.[8] Wang et al. reported synthesis of two series of 3′-(1,2,3-triazol-1-yl)-3′-deoxythymidines and their anti-HIV-1 activities.[9] The compounds of the first series containing aryloxymethyl or aryl substituent at position 4 of the triazole moiety were prepared by the copper(I) catalyzed reaction of AZT with the appropriate propargyl aryl ethers or aromatic alkynes and the compounds of the second series with aryloxymethyl or aryl substituent at position 5 of the triazole were obtained by ruthenium(II) catalyzed reaction. The highly active were 3′-(1,2,3-triazol-1-yl)-3′-deoxythymidines with bulky aromatic substituent at the 5 position of the triazole ring, especially compound with 5-(1-naphthyl) substituent (EC_{50} = 0.067 μM). More recently, Wang et al. showed that 3′-(1,2,3-triazol-1-yl)-3′-deoxythymidines having 5′-*O*-*t*-butyldimethylsilyl protecting group and aryl bulky substituent at the 5 position of the triazole ring selectively inhibit West Nile virus and Dengue virus at low micromolar concentrations without inhibiting HIV-1.[10] It is suggested that the observed antiviral activity is likely due to binding to flavivirus methyltransferase (MTase). Van Calenbergh et al. reported the synthesis of some 3′-(1,2,3-triazol-1-yl)-3′-deoxythymidines 4- and 5-substituted at the triazole ring.[11] Two of them bearing either 4-chlorophenyl or 3,4-dichlorophenyl substituent at position 4 of the triazole moiety proved to be potent inhibitors of human mitochondrial thymidine kinase (TK-2), IC_{50} was determined respectively 0.046 and 0.042 μM. Agrofoglio and Eriksson et al. described the synthesis of several 3′-(1,2,3-triazol-1-yl)-3′-deoxythymidines 4-substituted at the triazole ring with alkyl or aryl substituents.[12] These compounds were tested as substrates for human thymidine kinase (hTK-1) and *Ureaplasma parvum* thymidine kinase (*Up*TK). Agrofoglio et al. published the synthesis of several 3′-(1,2,3-triazol-1-yl)-3′-deoxythymidines possessing ω-azidoalkyl substituent at either 4 or 5 position of the triazole ring.[13] The synthesized compounds were evaluated in human PBM cells infected by HIV-1 and none of them exhibited significant activity. Torrence et al. reported the synthesis of several 3′-(1,2,3-triazol-1-yl)-3′-deoxythymidines owning alkyl functionalized substituents at the 4 position of the triazole ring.[14] The obtained compounds were evaluated against broad range of viruses including HIV-1 and HIV-2 but none of them showed considerable activity. Also, before the advent of 'click' chemistry method, efforts were made to synthesize 3′-(1,2,3-triazol-1-yl)-3′-deoxynucleosides mostly using the azide-alkyne 1,3-dipolar cycloaddition (uncatalyzed Huisgen reaction).[15–17]

It is assumed that the mechanism of antiviral as well as anticancer action of many nucleoside analogs, including 3′-azido-3′-deoxythymidine, cytarabine and gemcitabine primarily involves their intracellular conversion to the 5′-triphosphates via the corresponding mono- and diphosphates.[1,2] In the case of AZT, the 5′-triphospate acts as a competitive inhibitor of HIV reverse transcriptase (antiretroviral action[1]) or DNA polymerases (anticancer action[18]) and when incorporated into DNA as 5′-monophospate unit functions as a chain terminator of a growing DNA strand due to the lack of a 3′-hydroxyl group.[1,18] However, 5′-phosphates of nucleoside analogs cannot be considered as useful drugs since they are negatively charged at physiological pH and therefore too polar to cross the cell membranes.[18,19] Besides, the blood and cell surface phosphohydrolases efficiently convert nucleoside 5′-phosphates to the parent nucleosides.[18] In order to obviate these problems, a considerable effort has been directed into the synthesis of nucleoside 5′-monophosphate prodrugs (pronucleotides) with a protected phosphate group.[20] These prodrugs are designed to easily penetrate the cell membrane and to release nucleoside 5′-monophosphate inside the cell as a result of chemical or enzymatic hydrolysis.[18–21] Moreover, the liberated nucleoside 5′-monophosphate would require only a second and a third phosphorylation for the conversion to nucleoside 5′-triphosphate, which can be incorporated into DNA.

The aim of our study was to synthesize novel 3′-(1,2,3-triazol-1-yl)-3′-deoxythymidine derivatives having fluorophenyl substituents at the 4 position of the triazole ring and their phosphoramidates with potential anticancer properties. Why did we focus our attention on this type of 3′-(1,2,3-triazol-1-yl)-3′-deoxythymidines? As is apparent from the above literature data, 3′-(1,2,3-triazol-1-yl)-3′-deoxythymidines with aryl substituents at the 4 or 5 position of the triazole ring exhibited better biological activity than those with alkyl substituents. Introduction of fluorine atom into drug candidate usually enhance biological activity due to fluorine′s special properties: high electronegativity, small size and possibility of forming weak hydrogen bonds.[22] We assumed that 3′-[4-aryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidines are transformed into 5′-triphosphates and incorporated by human polymerases into DNA, hence we synthesized pronucleotides what would release 5′-monophosphates inside the cells which require only the second and the third phosphorylation. We directed our attention to 4-chlorophenyl *N*-alkyl phosphoramidates as pronucleotides with which we have previously had good experience.[23,24]

In this paper, we report the synthesis of 3′-[4-aryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidines (**7**–**11**) and their 4-chlorophenyl *N*-alkyl phosphoramidate diesters (**20**–**49**) as well as evaluation of their cytotoxic activity in four



Scheme 1. Synthesis of 3′-azido-3′-deoxythymidine derived triazoles **7**–**11** via the copper(I) catalyzed azide-alkyne cycloaddition. Reagents and conditions: (a) $CuSO_4$, sodium ascorbate, THF/H_2O (3:1, v/v), rt, 12 h.

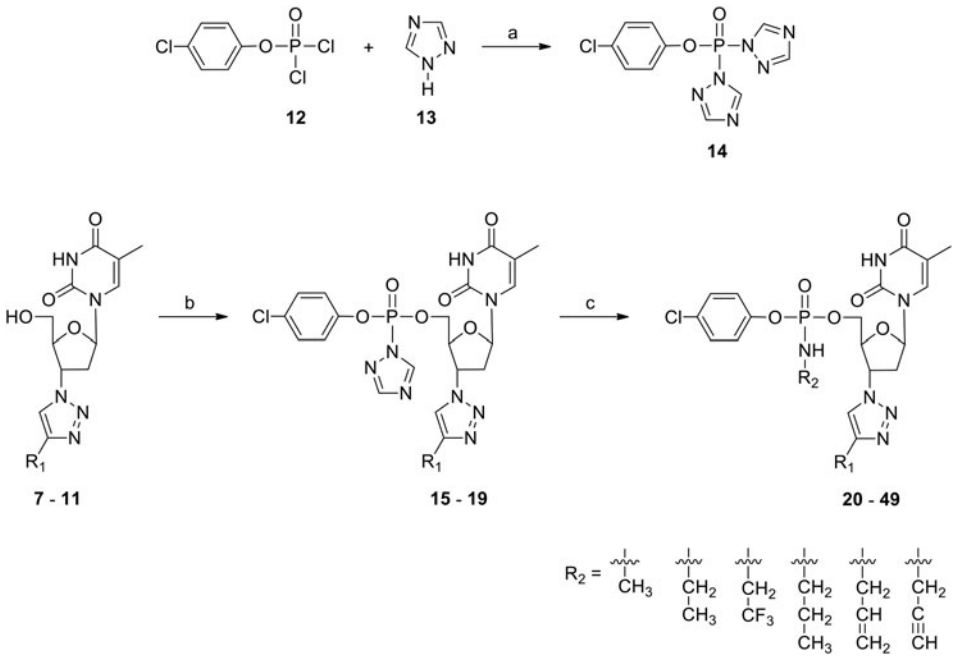
human cancer cell lines: cervical (HeLa), nasopharyngeal (KB), breast (MCF-7) and osteosarcoma (143B).

Results and discussion

Chemistry

A series of 3′-[4-aryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidines (**7**–**11**) was synthesized from 3′-azido-3′-deoxythymidine (**1**) and appropriate ethynylbenzenes (**2**–**6**) employing copper(I)-catalyzed Sharpless click chemistry approach according to the procedure outlined in Scheme 1.[25] Necessary copper(I) cations were generated in situ from copper(II) sulfate and sodium ascorbate in THF-water medium. This reaction proceeded cleanly giving the corresponding products **7**–**11** in 82–90% yield after column chromatography. It should be mentioned that compounds **7** and **10** were previously synthesized using the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC).[11,12]

A series of novel 4-chlorophenyl *N*-alkyl phosphoramidate diesters of 3′-[4-aryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidines (**20**–**49**) was synthesized by phosphorylation of 3′-[4-aryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidines (**7**–**11**) with 4-chlorophenyl phosphoroditriazolide (**14**) according to the synthetic route shown in Scheme 2.[23,24]



Scheme 2. Synthesis of the 4-chlorophenyloxy *N*-alkyl phosphoramidates of 3′-[4-aryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidines **20**–**49**. Reagents and conditions: (a) NEt_3, CH_3CN, rt, 30 min; (b) **14**, pyridine, rt, 1 h; (c) $R–NH_2$, rt, 1 h.

We have tried to synthesize the 4-chlorophenyl *N*-amino acid methyl ester phosphoramidates of 3′-[4-aryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidines, introduced by McGuigan,[26] which are generally characterized by the high biological activity, but the yields of their synthesis were very low (below 5%), that is why we turned our attention to phosphoramidates with *N*-alkyl substituents with which we have previously had good experience.[23,24]

4-Chlorophenyl phosphoroditriazolide (**14**) was prepared by reaction of 4-chlorophenyl phosphorodichloridate (**12**) with 1,2,4-triazole (**13**) in the presence of triethylamine in acetonitrile. Reaction of compound **14** with appropriate 3′-[4-aryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidine (**7**–**11**) in the presence of pyridine afforded the reactive intermediate (**15**–**19**), which was treated in situ with the proper amine (or amine hydrochloride in the presence of triethylamine) to give the desired products **20**–**49** in 52–69% yield.

^{31}P NMR spectra of products **20**–**49** 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 **20** and 2**1**).

It is worth emphasizing that the use of 4-chlorophenyl phosphorodichloridate (**12**), rather than its triazolide counterpart **14**, resulted in the formation of a considerable amount of the symmetrical (5′-5′)dinucleoside phosphate. The 2- and 4-chlorophenyl phosphoroditriazolides[27,28] 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.[29]

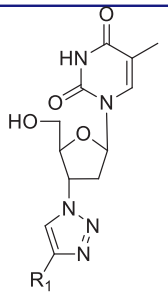
Biological activity

The synthesized 3′-[4-aryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidines (**7**–**11**) were evaluated for their cytotoxic activity in three human cancer cell lines: cervical (HeLa), nasopharyngeal (KB), breast (MCF-7) and normal human dermal fibroblast cell line (HDF) employing the sulforhodamine B (SRB) assay.[30] The resulting cytotoxic activity data of the obtained compounds **7**–**11** as well as the reference compounds: 5-fluorouracil (FUra), 5-fluoro-2′-deoxyuridine (FdU), 3′-azido-3′-deoxythymidine (AZT) and cytarabine (ara-C) are presented in Table 1. The highest activity was displayed by compound **9** with the 3-fluorophenyl substituent (IC_{50} = 2.58 μM) in HeLa and KB cancer cells. The activity of compound **9** was higher than the standards in all cancer cells. Also compound **8** with the 2-fluorophenyl substituent showed fairly high activity (IC_{50} in the range of 7.75–8.26 μM), comparable with that of the reference compounds, in the all studied cancer cell lines. On the other hand, compounds **7** and **10** exhibited only moderate activity, and compound **11** was not active.

The cytotoxic effect was also studied in the normal human dermal fibroblasts (HDF) to assess the toxicity of the prepared 3′-[4-aryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidines (**7**–**11**) (Table 1). The selectivity index (SI) is calculated as the ratio of the IC_{50} for the normal cell line (HDF) to the IC_{50} for a respective cancerous cell line. Higher values of SI indicate greater anticancer specificity. Compounds **8** and **9** had not only high cytotoxic activity but also displayed low toxicity against normal fibroblast cells and their SI was higher than 3, with one exception, compound **9** had SI = 2.5 for the MCF-7 cell line. Whereas, the SI for the reference compounds was in the range of 1.07–2.01.

The synthesized phosphoramidates **20**–**49** were evaluated for their cytotoxic activity in three human cancer cell lines: cervical (HeLa), nasopharyngeal (KB), breast (MCF-7) and normal human dermal fibroblast cell line (HDF) employing the sulforhodamine B (SRB) assay.[30] The resulting

Table 1. *In vitro* cytotoxic activity of compounds **7–11** in three human cancer cell lines: cervical (HeLa), nasopharyngeal (KB), breast (MCF-7) and normal human dermal fibroblast cell line (HDF).



7–11

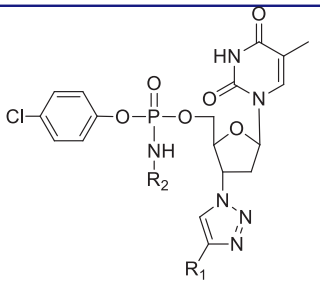
		Cytotoxicity (IC_{50}, μM)[a] ± SD[b]				
Ciompd	R_1	HeLa	KB	MCF-7	HDF	log *P*[c]
7	Ph	35.47 ± 0.42	30.59 ± 0.34	34.65 ± 0.39	63.84 ± 1.21	0.41
8	2-F-Ph	8.26 ± 0.03	8.26 ± 0.05	7.75 ± 0.03	25.61 ± 0.38	0.53
9	3-F-Ph	2.58 ± 0.03	2.58 ± 0.08	3.61 ± 0.05	9.03 ± 0.07	0.55
10	4-F-Ph	44.40 ± 0.53	59.12 ± 0.61	38.72 ± 0.48	66.54 ± 1.30	0.58
11	4-F-3-Me-Ph	>100	>100	>100	>100	0.95
FUra[d]	–	6.23 ± 0.46	4.84 ± 0.15	6.53 ± 0.82	7.02 ± 0.20	−1.31[31]
FdU[d]	–	6.50 ± 0.24	8.69 ± 1.18	12.19 ± 1.34	13.05 ± 0.74	−1.72
AZT[d]	–	10.77 ± 0.66	9.77 ± 0.57	7.67 ± 0.09	14.41 ± 0.58	−0.10
ara-C[d]	–	3.54 ± 0.16	4.07 ± 0.08	3.82 ± 0.25	4.99 ± 0.84	−2.23[31]

[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.[32]
[d]Standards: 5-fluorouracil (FUra), 5-fluoro-2′-deoxyuridine (FdU), 3′-azido-3′-deoxythymidine (AZT) and cytarabine (ara-C).

cytotoxic activity data of the obtained phosphoramidates and the reference compounds are presented in Table 2.

Regarding the phosphoramidates **20–25** with phenyl substituent, the highest activity was displayed by phosphoramidate **24** with *N*-allyl substituent (IC_{50} in the range of 10.18–13.69 μM) in all the examined cancer cell lines. Phosphoramidate **24** was about three times more active than the parent nucleoside **7**. Also more active than the parent nucleoside **7** was phosphoramidate **20** with *N*-methyl substituent (IC_{50} in the range of 19.20–21.47 μM). None of phosphoramidates **26–31** with 2-fluorophenyl substituent showed higher activity than the parent nucleoside **8**. Among phosphoramidates **32–37** with 3-fluorophenyl substituent, the highest activity was demonstrated by phosphoramidate **35** with the *N*-*n*-propyl substituent in all the cancer cells (IC_{50} in the range of 0.97–1.94 μM) and its activity was significantly higher than the parent nucleoside **9**. It should be noted that phosphoramidate **35** was the most potent compound among all tested phosphoramidates **20–49**. Moreover, its activity exceeded the activity of standard compounds. As for phosphoramidates **38–43** with 4-fluorophenyl substituent, the highest activity was exhibited by phosphoramidate

Table 2. *In vitro* cytotoxic activity of phosphoramidates **20–49** in three human cancer cell lines: cervical (HeLa), nasopharyngeal (KB), breast (MCF-7) and normal human dermal fibroblast cell line (HDF).



20–49

			Cytotoxicity (IC_{50}, μM)[a] ± SD[b]				
Compd	R_1	R_2	HeLa	KB	MCF-7	HDF	log P[c]
20	Ph	Me	21.47 ± 0.09	19.20 ± 0.02	19.94 ± 0.08	47.88 ± 1.02	2.47
21	Ph	Et	47.70 ± 0.51	45.15 ± 0.17	46.13 ± 0.10	85.86 ± 0.89	2.85
22	Ph	CF_3CH_2	45.25 ± 0.34	35.26 ± 0.72	36.12 ± 0.19	76.92 ± 1.03	3.40
23	Ph	*n*-Pr	29.95 ± 0.20	30.62 ± 0.50	28.09 ± 0.26	59.91 ± 0.93	3.35
24	Ph	allyl	13.69 ± 0.03	10.18 ± 0.07	11.03 ± 0.12	47.92 ± 0.35	3.12
25	Ph	propargyl	30.82 ± 0.07	30.15 ± 0.08	31.23 ± 0.22	64.72 ± 1.23	2.63
26	2-F-Ph	Me	46.37 ± 0.17	37.23 ± 0.14	35.21 ± 0.24	83.47 ± 0.78	2.59
27	2-F-Ph	Et	81.00 ± 0.17	72.40 ± 0.15	71.54 ± 0.12	>100	2.96
28	2-F-Ph	CF_3CH_2	31.87 ± 0.46	31.87 ± 0.46	30.82 ± 0.19	66.93 ± 1.01	3.52
29	2-F-Ph	*n*-Pr	>100	>100	>100	>100	3.47
30	2-F-Ph	allyl	82.66 ± 1.46	94.01 ± 0.65	91.45 ± 0.90	>100	3.23
31	2-F-Ph	propargyl	84.52 ± 1.88	78.54 ± 1.63	82.12 ± 0.18	>100	2.75
32	3-F-Ph	Me	20.98 ± 0.17	23.02 ± 0.34	19.80 ± 0.17	65.04 ± 1.23	2.61
33	3-F-Ph	Et	4.79 ± 0.02	4.96 ± 0.33	4.96 ± 0.17	15.23 ± 0.09	2.99
34	3-F-Ph	CF_3CH_2	14.11 ± 0.30	13.66 ± 0.46	13.66 ± 0.15	49.38 ± 0.87	3.54
35	3-F-Ph	*n*-Pr	1.78 ± 0.02	1.94 ± 0.02	0.97 ± 0.01	6.12 ± 0.05	3.49
36	3-F-Ph	allyl	79.26 ± 0.65	74.23 ± 0.16	68.08 ± 0.32	>100	3.26
37	3-F-Ph	propargyl	>100	>100	>100	>100	2.77
38	4-F-Ph	Me	23.52 ± 0.02	22.00 ± 0.34	21.03 ± 0.71	75.26 ± 1.21	2.64
39	4-F-Ph	Et	>100	>100	>100	>100	3.01
40	4-F-Ph	CF_3CH_2	16.69 ± 0.61	13.81 ± 0.15	14.16 ± 0.19	58.42 ± 1.07	3.56
41	4-F-Ph	*n*-Pr	>100	>100	>100	>100	3.52
42	4-F-Ph	allyl	42.47 ± 0.13	42.14 ± 0.08	43.11 ± 0.45	89.24 ± 1.28	3.28
43	4-F-Ph	propargyl	92.69 ± 0.03	96.92 ± 0.03	95.21 ± 0.31	>100	2.80
44	4-F-3-Me-Ph	Me	28.76 ± 0.97	27.28 ± 0.47	26.31 ± 0.53	74.78 ± 1.25	3.01
45	4-F-3-Me-Ph	Et	27.66 ± 0.49	28.96 ± 0.81	25.19 ± 0.44	69.15 ± 1.02	3.39
46	4-F-3-Me-Ph	CF_3CH_2	13.82 ± 0.74	8.92 ± 0.59	8.98 ± 0.32	38.37 ± 0.89	3.94
47	4-F-3-Me-Ph	*n*-Pr	30.33 ± 0.79	31.12 ± 0.32	29.38 ± 0.62	78.86 ± 1.13	3.89
48	4-F-3-Me-Ph	allyl	53.88 ± 0.16	49.92 ± 0.16	48.21 ± 0.31	>100	3.66
49	4-F-3-Me-Ph	propargyl	28.94 ± 0.14	28.62 ± 0.08	27.13 ± 0.21	60.77 ± 1.07	3.17
FUra[d]	–	–	6.23 ± 0.46	4.84 ± 0.15	6.53 ± 0.82	7.02 ± 0.20	−1.31[31]
FdU[d]	–	–	6.50 ± 0.24	8.69 ± 1.18	12.19 ± 1.34	13.05 ± 0.74	−1.72
AZT[d]	–	–	10.77 ± 0.66	9.77 ± 0.57	7.67 ± 0.09	14.41 ± 0.58	−0.10
ara-C[d]	–	–	3.54 ± 0.16	4.07 ± 0.08	3.82 ± 0.25	4.99 ± 0.84	−2.32[31]

[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.[32]
[d]Standards: 5-fluorouracil (FUra), 5-fluoro-2′-deoxyuridine (FdU), 3′-azido-3′-deoxythymidine (AZT) and cytarabine (ara-C).

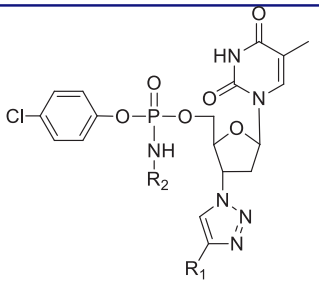
40 with the *N*-(2,2,2-trifluoroethyl) substituent in all the cancer cells (IC_{50} in the range of 13.81–16.69 μM) and it was higher than the parent nucleoside **10**. Regarding the phosphoramidates **44–49** with 4-fluoro-3-methylphenyl substituent, the highest activity was displayed by phosphoramidate **46** with *N*-(2,2,2-trifluoroethyl) substituent (IC_{50} in the range of 8.92–13.82 μM) in all the examined cancer cell lines and it was much higher than the parent nucleoside **11**.

The cytotoxic activity of the most potent phosphoramidate **35** is considerably higher than the parent nucleoside **9** in all the examined cancer cells which means that **35** readily decomposes to 5′-monophosphate and probably undergoes further phosphorylation to 5′-triphosphate, which can be substrate for human DNA polymerases. We have shown the presence of 3′-[4-(3-fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-monophosphate (5′-monophosphate of **9**) in the lysate of HeLa cells previously incubated with phosphoramidate **35** (see experimental section). Similarly, phosphoramidates **20**, **24**, **40** and **46** show higher cytotoxic activity than their parent nucleosides. However, most of the obtained phosphoramidates have comparable or lower cytotoxic activity than the parent nucleosides, which may indicate that they are not efficiently hydrolyzed inside the cells to 5′-monophosphates or further phosphorylation to the 5′-triphosphates proceeds inefficiently. In addition, the cytotoxic activity of 3′-[4-aryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidines (**7–10**) might be due to their inhibition of human thymidine kinases (hTKs) as demonstrated for compounds **7** and **10**.[12]

Finally, phosphoramidates **20**, **24**, **28**, **32–36**, **38**, **40** and **46** were examined for their cytotoxic activity in human osteosarcoma cells (143B) and thymidine kinase deficient human osteosarcoma cells (143B/TK-), as presented in Table 3. It should be noted that phosphoramidate **35** exhibited the highest activity ($IC_{50} = 2.08$ μM) among the tested compounds in 143B cancer cells, however, in 143B thymidine kinase deficient cancer cells its activity decreased more than 13-fold. In contrast, phosphoramidates **24**, **40** and **46** 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 is probably directly converted to FdUMP by phosphoribosylation catalyzed by the enzyme orotate phosphoribosyl transferase.[26]

The cytotoxic effect was also studied in the normal human dermal fibroblasts (HDF) to assess the toxicity of the prepared phosphoramidates **20–49** (Table 2). The calculated values of the selectivity index (SI) of the most active phosphoramidates **20**, **24**, **32–35**, **38**, **40** and **46** are shown in Table 4. Some of the synthesized phosphoramidates had not only high cytotoxic activity but also displayed low toxicity against normal fibroblast

Table 3. *In vitro* cytotoxic activity of selected phosphoramidates in human osteosarcoma cell line (143B) and thymidine kinase deficient human osteosarcoma cell line (143B/TK-).



20, **24**, **28**, **32**–**36**, **38**, **40**, **46**

			Cytotoxicity (IC_{50}, μM)[a] ± SD[b]	
Compounds	R_1	R_2	143B	143B/TK-
20	Ph	Me	18.67 ± 0.19	38.53 ± 0.85
24	Ph	allyl	9.17 ± 0.31	13.10 ± 0.36
28	2-F-Ph	CF_3CH_2	29.86 ± 0.12	75.08 ± 0.42
32	3-F-Ph	Me	28.77 ± 0.02	48.23 ± 0.34
33	3-F-Ph	Et	3.47 ± 0.03	26.45 ± 0.02
34	3-F-Ph	CF_3CH_2	13.06 ± 0.30	27.62 ± 0.15
35	3-F-Ph	*n*-Pr	2.08 ± 0.02	27.98 ± 0.20
36	3-F-Ph	allyl	76.83 ± 0.16	79.42 ± 0.32
38	4-F-Ph	Me	20.01 ± 0.08	65.04 ± 0.41
40	4-F-Ph	CF_3CH_2	12.80 ± 0.08	19.51 ± 0.12
46	4-F-3-Me-Ph	CF_3CH_2	7.61 ± 0.06	15.90 ± 0.15
FUra[c]	–	–	13.01 ± 0.40	16.31 ± 0.44
FdU[c]	–	–	6.02 ± 0.25	14.10 ± 0.32
AZT[c]	–	–	3.37 ± 0.08	64.36 ± 1.50
ara-C[c]	–	–	2.06 ± 0.18	16.88 ± 0.39

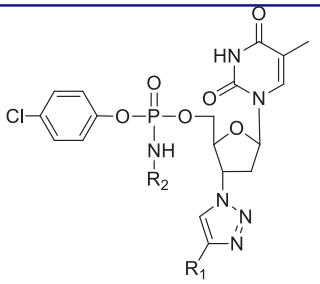
[a]IC_{50} is the compound concentration required to inhibit cell growth by 50%.
[b]SD (standard deviation) of three independent experiments.
[c]Standards: 5-fluorouracil (FUra), 5-fluoro-2′-deoxyuridine (FdU), 3′-azido-3′-deoxythymidine (AZT) and cytarabine (ara-C).

cells and accordingly their selectivity index was usually higher than 3 and in certain cases even higher than 5. The highest selectivity index (SI = 6.31) was displayed by phosphoramidate **35** in MCF-7 cancer cell line.

Conclusion

In conclusion, we have developed an efficient procedure for the synthesis of 4-chlorophenyl *N*-alkyl phosphoramidates of 3′-[4-fluoroaryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidines (**20**–**49**) employing 4-chlorophenyl phosphoroditriazolide as a phosphorylating agent. The obtained phosphoramidates **20**–**49** were examined for their cytotoxic activity in four human cancer cell lines: cervical (HeLa), nasopharyngeal (KB), breast (MCF-7), osteosarcoma (143B) and normal human dermal fibroblast cell line (HDF) using the sulforhodamine B (SRB) assay. The highest activity was demonstrated by *N*-*n*-propyl phosphoramidate of 3′-[4-(3-fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine (**35**) in all the investigated cancer cells and its activity

Table 4. The calculated values of the selectivity index (SI) of the most active phosphoramidates.



20, **24**, **32–35**, **38**, **40**, **46**

			SI[a]			
Compounds	R_1	R_2	HeLa	KB	MCF-7	143B
20	Ph	Me	2.23	2.49	2.40	2.57
24	Ph	allyl	3.50	4.71	4.35	5.23
32	3-F-Ph	Me	3.10	2.83	3.29	2.26
33	3-F-Ph	Et	3.18	3.07	3.07	4.39
34	3-F-Ph	CF_3CH_2	3.50	3.62	3.62	3.78
35	3-F-Ph	*n*-Pr	3.44	3.16	6.31	2.94
38	4-F-Ph	Me	3.20	3.42	3.58	3.76
40	4-F-Ph	CF_3CH_2	3.50	4.23	4.13	4.56
46	4-F-3-Me-Ph	CF_3CH_2	2.78	4.30	4.27	5.04
FUra[b]	–	–	1.13	1.45	1.08	0.54
FdU[b]	–	–	2.01	1.50	1.07	2.17
AZT[b]	–	–	1.34	1.48	1.88	4.28
ara-C[b]	–	–	1.41	1.23	1.31	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.

[b]Standards: 5-fluorouracil (FUra), 5-fluoro-2′-deoxyuridine (FdU), 3′-azido-3′-deoxythymidine (AZT) and cytarabine (ara-C).

was higher than that of the parent nucleoside **9** as well as reference compounds. Also *N*-ethyl phosphoramidate of 3′-[4-(3-fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine (**33**) showed good activity in all the used cell lines. Phosphoramidates **33** and **35** had not only high cytotoxic activity but also displayed low toxicity against normal fibroblast cells (HDF) and consequently their selectivity index was higher than 3. Phosphoramidates **33** and **35** also exhibited high activity in 143B cancer cells, however, in 143B thymidine kinase deficient cancer cells its activity considerably decreased. In contrast, phosphoramidates **24**, **40** and **46** showed significant 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.

Experimental

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 × 150 mm, 5 µm) 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 (now Merck).

General procedure for the synthesis of 3′-[4-aryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidines (7–11)

To a stirred solution of 3′-azido-3′-deoxythymidine (**1**) (1.00 g, 3.742 mmol) and the appropriate ethynylbenzene (compounds **2**–**6**, respectively, 3.742 mmol) in a 1:3 mixture of water and THF (57 mL) was added sodium ascorbate (300 mg, 1.514 mmol) followed by copper(II) sulfate pentahydrate (93 mg, 0.373 mmol, in 1.43 mL of water). The reaction mixture was stirred at room temperature for 12 h, at which time TLC indicated the reaction to be complete. Then the mixture was evaporated under reduced pressure and the residue was purified by flash chromatography on silica gel column using as an eluent the mixture chloroform–methanol (from 80:1 to 10:1, v/v) to afford products **7**–**11** (82–90% yield).

3′-[4-Phenyl-(1,2,3-triazol-1-yl)]-3′-deoxythymidine (7)[11,12]

^1H NMR (DMSO-d_6) δ: 1.83 (d, 3 H, $J = 1.1$ Hz, 5-CH_3), 2.67–2.74 (m, 1H, H-2”), 2.78–2.84 (m, 1H, H-2′), 3.65–3.70 (m, 1H, H-5”), 3.72–3.77 (m, 1H, H-5′), 4.28–4.31 (m, 1H, H-4′), 5.41 (dt, 1H, $J = 5.4$ Hz, $J = 8.7$ Hz, H-3′), 6.46 (pseudo t, 1H, $J = 6.6$ Hz, H-1′), 7.33–7.38 (m, 1H, Ph), 7.44–7.50 (m, 2 H, Ph), 7.85–7.88 (m, 3 H, Ph, H-6), 8.79 (s, 1H, H-5 triazole), 11.38 (br s, 1H, 3-NH). ^{13}C NMR (DMSO-d_6) δ: 12.32 (5-CH_3), 37.18 (C-2′), 59.39 (C-3′), 60.78 (C-5′), 83.89 (C-4′), 84.45 (C-1′), 109.68 (C-5), 121.05 (C-5 triazole), 125.18 (Ph), 128.03 (Ph), 128.99 (Ph), 130.58 (Ph), 136.29 (C-6), 146.58 (C-4 triazole), 150.48 (C-2), 163.77 (C-4). MS-ESI *m/z*: 370 $[M + H]^+$; 392 $[M + Na]^+$; 408 $[M + K]^+$; 368 $[M - H]^-$; 404, 406

$[M + Cl]^-$. Anal. Calcd for $C_{18}H_{19}N_5O_4$: C, 58.53; H, 5.18; N, 18.96. Found: C, 58.42; H, 5.12; N, 18.79.

3′-[4-(2-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine (8)

^{1}H NMR (DMSO-d_6) δ: 1.83 (d, 3 H, $J = 1.1$ Hz, 5-CH_3), 2.66–2.73 (m, 1H, H-2”), 2.80–2.86 (m, 1H, H-2′), 3.64–3.69 (m, 1H, H-5”), 3.71–3.76 (m, 1H, H-5′), 4.30–4.33 (m, 1H, H-4′), 5.49 (dt, 1H, $J = 5.5$ Hz, $J = 8.6$ Hz, H-3′), 6.49 (pseudo t, 1H, $J = 6.7$ Hz, H-1′), 7.32–7.46 (m, 3 H, 2-F-Ph), 7.85 (d, 1H, $J = 1.1$ Hz, H-6), 8.13–8.17 (m, 1H, 2-F-Ph), 8.66 (d, 1H, $J = 3.6$ Hz, H-5 triazole), 11.37 (br s, 1H, 3-NH). ^{13}C NMR (DMSO-d_6) δ: 12.32 (5-CH_3), 37.24 (C-2′), 59.46 (C-3′), 60.77 (C-5′), 83.89 (C-4′), 84.38 (C-1′), 109.69 (C-5), 116.09 (d, $J_{C\text{-}F}$ = 21.2 Hz, 2-F-Ph), 118.25 (d, $J_{C\text{-}F}$ = 13.1 Hz, 2-F-Ph), 123.59 (d, $J_{C\text{-}F}$ = 11.2 Hz, 2-F-Ph), 125.04 (d, $J_{C\text{-}F}$ = 3.1 Hz, C-5 triazole), 127.39 (d, $J_{C\text{-}F}$ = 3.4 Hz, 2-F-Ph), 129.84 (d, $J_{C\text{-}F}$ = 8.4 Hz, 2-F-Ph), 136.33 (C-6), 139.94 (d, $J_{C\text{-}F}$ = 2.4 Hz, C-4 triazole), 150.50 (C-2), 158.49 (d, $J_{C\text{-}F}$ = 247.3 Hz, 2-F-Ph), 163.78 (C-4). ^{19}F NMR (DMSO-d_6) δ: -114.06 (m, 1F). MS-ESI *m/z*: 388 $[M + H]^+$; 410 $[M + Na]^+$; 426 $[M + K]^+$; 386 $[M - H]^-$; 422, 424 $[M + Cl]^-$. Anal. Calcd for $C_{18}H_{18}FN_5O_4$: C, 55.81; H, 4.68; N, 18.08. Found: C, 55.72; H, 4.52; N, 18.01.

3′-[4-(3-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine (9)

^{1}H NMR (DMSO-d_6) δ: 1.83 (d, 3 H, $J = 1.2$ Hz, 5-CH_3), 2.68–2.75 (m, 1H, H-2”), 2.77–2.84 (m, 1H, H-2′), 3.66–3.69 (m, 1H, H-5”), 3.73–3.76 (m, 1H, H-5′), 4.27–4.30 (m, 1H, H-4′), 5.42 (dt, 1H, $J = 5.4$ Hz, $J = 8.7$ Hz, H-3′), 6.46 (pseudo t, 1H, $J = 6.7$ Hz, H-1′), 7.17–7.22 (m, 1H, 3-F-Ph), 7.50–7.55 (m, 1H, 3-F-Ph), 7.65–7.69 (m, 1H, 3-F-Ph), 7.71–7.73 (m, 1H, 3-F-Ph), 7.84 (d, 1H, $J = 1.2$ Hz, H-6), 8.87 (s, 1H, H-5 triazole), 11.38 (br s, 1H, 3-NH). ^{13}C NMR (DMSO-d_6) δ: 12.33 (5-CH_3), 37.16 (C-2′), 59.53 (C-3′), 60.79 (C-5′), 83.90 (C-4′), 84.45 (C-1′), 109.70 (C-5), 111.76 (d, $J_{C\text{-}F}$ = 23.0 Hz, 3-F-Ph), 114.75 (d, $J_{C\text{-}F}$ = 21.0 Hz, 3-F-Ph), 121.23 (d, $J_{C\text{-}F}$ = 2.8 Hz, 3-F-Ph), 121.79 (C-5 triazole), 131.16 (d, $J_{C\text{-}F}$ = 8.6 Hz, 3-F-Ph), 132.96 (d, $J_{C\text{-}F}$ = 8.5 Hz, 3-F-Ph), 136.28 (C-6), 145.50 (d, $J_{C\text{-}F}$ = 2.8 Hz, C-4 triazole), 150.48 (C-2), 163.77 (C-4), 163.79 (d, $J_{C\text{-}F}$ = 243.2 Hz, 3-F-Ph). ^{19}F NMR (DMSO-d_6) δ: -112.26 (td, 1F). MS-ESI *m/z*: 388 $[M + H]^+$; 410 $[M + Na]^+$; 426 $[M + K]^+$; 386 $[M - H]^-$; 422, 424 $[M + Cl]^-$. Anal. Calcd for $C_{18}H_{18}FN_5O_4$: C, 55.81; H, 4.68; N, 18.08. Found: C, 55.61; H, 4.53; N, 18.00.

3′-[4-(4-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine (10)[12]

^{1}H NMR (DMSO-d_6) δ: 1.83 (d, 3 H, $J = 1.2$ Hz, 5-CH_3), 2.67–2.74 (m, 1H, H-2”), 2.77–2.84 (m, 1H, H-2′), 3.64–3.70 (m, 1H, H-5”), 3.71–3.77 (m,

1H, H-5′), 4.27–4.30 (m, 1H, H-4′), 5.41 (dt, 1H, $J = 5.4$ Hz, $J = 8.7$ Hz, H-3′), 6.46 (pseudo t, 1H, $J = 6.7$ Hz, H-1′), 7.31 (m, 2 H, 4-F-Ph), 7.85 (d, 1H, $J = 1.2$ Hz, H-6), 7.90 (m, 2 H, 4-F-Ph), 8.78 (s, 1H, H-5 triazole), 11.38 (br s, 1H, 3-NH). ^{13}C NMR (DMSO-d_6) δ: 12.33 (5-CH_3), 37.17 (C-2′), 59.44 (C-3′), 60.79 (C-5′), 83.90 (C-4′), 84.46 (C-1′), 109.69 (C-5), 115.95 (d, $J_{C\text{-}F}$ = 21.7 Hz, 4-F-Ph), 120.98 (C-5 triazole), 127.15 (d, $J_{C\text{-}F}$ = 3.0 Hz, 4-F-Ph), 127.22 (d, $J_{C\text{-}F}$ = 8.3 Hz, 4-F-Ph), 136.29 (C-6), 145.73 (C-4 triazole), 150.48 (C-2), 161.86 (d, $J_{C\text{-}F}$ = 244.5 Hz, 4-F-Ph), 163.78 (C-4). ^{19}F NMR (DMSO-d_6) δ: -113.43 (m, 1F). MS-ESI *m/z*: 388 $[M + H]^+$; 410 $[M + Na]^+$; 426 $[M + K]^+$; 386 $[M - H]^-$; 422, 424 $[M + Cl]^-$. Anal. Calcd for $C_{18}H_{18}FN_5O_4$: C, 55.81; H, 4.68; N, 18.08. Found: C, 55.59; H, 4.52; N, 18.01.

3′-[4-(4-Fluoro-3-methylphenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine (11)

^{1}H NMR (DMSO-d_6) δ: 1.82 (d, 3 H, $J = 1.3$ Hz, 5-CH_3), 2.30 (d, 3 H, $J = 1.9$ Hz, CH_3 4-F-3-Me-Ph), 2.67–2.74 (m, 1H, H-2”), 2.77–2.83 (m, 1H, H-2′), 3.66–3.71 (m, 1H, H-5”), 3.71–3.76 (m, 1H, H-5′), 4.28 (dt, 1H, $J = 3.6$ Hz, $J = 5.5$ Hz, H-4′), 5.40 (dt, 1H, $J = 5.4$ Hz, $J = 8.7$ Hz, H-3′), 6.45 (pseudo t, 1H, $J = 6.6$ Hz, H-1′), 7.24 (dd, 1H, $J = 8.5$ Hz, $J = 9.7$ Hz, 4-F-3-Me-Ph), 7.70 (ddd, 1H, $J = 2.3$ Hz, $J = 5.0$ Hz, $J = 7.8$ Hz, 4-F-3-Me-Ph), 7.79 (dd, 1H, $J = 2.2$ Hz, $J = 7.6$ Hz, 4-F-3-Me-Ph), 7.84 (d, 1H, $J = 1.3$ Hz, H-6), 8.75 (s, 1H, H-5 triazole), 11.37 (br s, 1H, 3-NH). ^{13}C NMR (DMSO-d_6) δ: 12.32 (5-CH_3), 14.28 (CH_3 4-F-3-Me-Ph), 37.13 (C-2′), 59.41 (C-3′), 60.78 (C-5′), 83.88 (C-4′), 84.46 (C-1′), 109.69 (C-5), 115.56 (d, $J_{C\text{-}F}$ = 22.6 Hz, 4-F-3-Me-Ph), 120.84 (C-5 triazole), 124.55 (d, $J_{C\text{-}F}$ = 8.2 Hz, 4-F-3-Me-Ph), 124.86 (d, $J_{C\text{-}F}$ = 17.6 Hz, 4-F-3-Me-Ph), 126.84 (d, $J_{C\text{-}F}$ = 3.4 Hz, 4-F-3-Me-Ph), 128.41 (d, $J_{C\text{-}F}$ = 5.2 Hz, 4-F-3-Me-Ph), 136.29 (C-6), 145.88 (C-4 triazole), 150.48 (C-2), 160.41 (d, $J_{C\text{-}F}$ = 243.8 Hz, 4-F-3-Me-Ph), 163.77 (C-4). ^{19}F NMR (DMSO-d_6) δ: -117.89 (m, 1F). MS-ESI *m/z*: 402 $[M + H]^+$; 424 $[M + Na]^+$; 440 $[M + K]^+$; 400 $[M - H]^-$; 436, 438 $[M + Cl]^-$. Anal. Calcd for $C_{19}H_{20}FN_5O_4$: C, 56.85; H, 5.02; N, 17.45. Found: C, 56.64; H, 4.95; N, 17.21.

General procedure for the synthesis of target compounds 20–49

To a solution of 4-chlorophenyl phosphorodichloridate (**12**) (355 mg, 1.445 mmol) in acetonitrile (3.1 mL) was added 1,2,4-triazole (**13**) (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′-[4-aryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidine (**7**–**11**) (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 **20**–**22**, **26**–**28**, **20**–**22**, **32**–**34**, **38**–**40** and **44**–**46** amine 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 as an eluent the mixture chloroform – methanol (from 100:1 to 40:1, v/v) to afford products **20**–**49** (yield 52–69%).

3′-[4-Phenyl-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-(4-chlorophenyl N-methylphosphate) (20)

^{1}H NMR ($CDCl_3$) δ: 1.91, 1.92 (d, 3 H, $J = 1.4$ Hz, 5-CH_3), 2.64–2.71 (m, 3 H, N-CH_3), 2.72–2.78 (m, 1H, H-2”), 2.79–2.88 (m, 1H, H-2′), 3.44–3.52 (m, 1H, H-5”), 3.75–3.84 (m, 1H, H-5′), 4.37–4.52 (m, 1H, H-4′), 5.48–5.60 (m, 2 H, P-NH, H-3′), 6.28, 6.40 (pseudo t, 1H, $J = 6.4$ Hz, H-1′), 7.11 (dd, 2 H, $J = 1$. 1H z, $J = 9.1$ Hz, 4-Cl-Ph), 7.17 (dd, 2 H, $J = 1.1$ Hz, $J = 9.1$ Hz, 4-Cl-Ph), 7.31–7.36 (m, 1H, Ph), 7.39–7.46 (m, 2 H, Ph), 7.77–7.82 (m, 3 H, Ph, H-6), 8.20 (s, 1H, H-5 triazole), 9.72 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.58, 12.60 (5-CH_3), 27.77, 27.80 (N-CH_3), 37.62, 37.90 (C-2′), 59.40, 59.42 (C-3′), 61.58 (C-5′), 82.77, 82.94 (C-4′), 85.37 (C-1′), 111.45, 111.55 (C-5), 121.50 (C-5 triazole), 125.80 (Ph), 125.86 (4-Cl-Ph), 128.60 (4-Cl-Ph), 128.64 (Ph), 129.08 (Ph), 130.01 (Ph), 136.39 (4-Cl-Ph), 136.75 (C-6), 148.06 (C-4 triazole), 148.23 (4-Cl-Ph), 150.77 (C-2), 164.10 (C-4). ^{31}P NMR ($CDCl_3$) δ: 7.13, 7.18. MS-ESI *m/z*: 573, 575 $[M + H]^+$; 595, 597 $[M + Na]^+$; 611, 613 $[M + K]^+$; 571, 573 [M – H]$^-$; 607, 609, 611 $[M + Cl]^-$. Anal. Calcd for $C_{25}H_{26}ClN_6O_6P$: C, 52.41; H, 4.57; N, 14.67. Found: C, 52.22; H, 4.42; N, 14.79. HPLC: retention time (t_R) of 8.42 and 8.93 min in the ratio 1:1.

3′-[4-Phenyl-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-(4-chlorophenyl N-ethylphosphate) (21)

^{1}H NMR ($CDCl_3$) δ: 1.10, 1.16 (t, 3 H, $J = 7.2$ Hz, CH_3 Et), 1.91, 1.92 (d, 3 H, $J = 0.9$ Hz, 5-CH_3), 2.63–2.86 (m, 4 H, H-2′, H-2”, N-CH_2), 3.40–3.48 (m, 1H, H-5”), 3.65–3.74 (m, 1H, H-5′), 4.34–4.48 (m, 1H, H-4′), 5.46–5.59 (m, 2 H, P-NH, H-3′), 6.29, 6.41 (pseudo t, 1H, $J = 6.6$ Hz, H-1′), 7.11 (dd, 2 H, $J = 1.1$ Hz, $J = 8.6$ Hz, 4-Cl-Ph), 7.18 (dd, 2 H, $J = 1.1$ Hz, $J = 8.6$ Hz, 4-Cl-Ph), 7.33–7.36 (m, 1H, Ph), 7.39–7.45 (m, 2 H, Ph), 7.78–7.83 (m, 3 H,

Ph, H-6), 8.17 (s, 1H, H-5 triazole), 9.72 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.63, 12.66 (5-CH_3), 17.51 (d, $J_{C\text{-}P}$ = 6.9 Hz, CH_3 Et), 36.28, 36.72 (C-2′), 37.91 (d, $J_{C\text{-}P}$ = 7.0 Hz, N-CH_2), 59.27, 59.35 (C-3′), 65.08, 65.21 (d, $J_{C\text{-}P}$ = 5.2 Hz, C-5′), 82.81, 82.88 (d, $J_{C\text{-}P}$ = 6.6 Hz, C-4′), 86.43, 87.19 (C-1′), 111.24, 111.51 (C-5), 121.44, 121.54 (d, $J_{C\text{-}P}$ = 5.0 Hz, C-5 triazole), 125.86 (Ph), 128.56, 128.58 (4-Cl-Ph), 129.04 (Ph), 129.67 (Ph), 129.93, 129.97 (4-Cl-Ph), 130.16 (Ph), 136.25, 136.58 (4-Cl-Ph), 136.77 (C-6), 148.09, 148.17 (4-Cl-Ph), 149.10, 149.19 (d, $J_{C\text{-}P}$ = 1.5 Hz, C-4 triazole), 150.36, 150.40 (C-2), 163.95, 164.09 (C-4). ^{31}P NMR ($CDCl_3$) δ: 5.88, 6.03. MS-ESI *m/z*: 587, 589 $[M+H]^+$; 609, 611 $[M+Na]^+$; 625, 627 $[M+K]^+$; 585, 587 $[M-H]^-$; 621, 623, 625 $[M+Cl]^-$. Anal. Calcd for $C_{26}H_{28}ClN_6O_6P$: C, 53.20; H, 4.81; N, 14.32. Found: C, 53.02; H, 4.79; N, 14.15. HPLC: retention time (t_R) of 9.31 and 9.83 min in the ratio 1:1.

3′-[4-Phenyl-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-(2,2,2-trifluoroethyl)phosphate] (22)

^{1}H NMR ($CDCl_3$) δ: 1.92 (s, 3 H, 5-CH_3), 2.80–2.98 (m, 1H, H-2”), 3.07–3.18 (m, 1H, H-2′), 3.54–3.68 (m, 4 H, N-CH_2, H-5′, H-5”), 4.46–4.54 (m, 1H, H-4′), 5.43–5.54 (m, 2 H, P-NH, H-3′), 6.21, 6.27 (dd, 1H, *J* = 2.9 Hz, *J* = 5.6 Hz, H-1′), 7.11 (dd, 2 H, *J* = 1.1 Hz, *J* = 9.1 Hz, 4-Cl-Ph), 7.19 (dd, 2 H, *J* = 1.1 Hz, *J* = 9.1 Hz, 4-Cl-Ph), 7.29–7.37 (m, 1H, Ph), 7.38–7.47 (m, 2 H, Ph), 7.78–7.81 (m, 2 H, Ph), 7.91 (s, 1H, H-6), 8.21 (s, 1H, H-5 triazole), 9.29 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.49, 12.55 (5-CH_3), 37.27, 37.42 (C-2′), 43.54, 43.99 (N-CH_2), 59.29, 59.58 (C-3′), 65.51, 65.69 (d, $J_{C\text{-}P}$ = 4.6 Hz, C-5′), 82.55, 82.76 (d, $J_{C\text{-}P}$ = 7.0 Hz, C-4′), 87.73, 88.50 (C-1′), 111.62, 111.70 (C-5), 121.46, 121.58 (d, $J_{C\text{-}P}$ = 4.9 Hz, C-5 triazole), 125.90 (Ph), 128.72, 128.94 (4-Cl-Ph), 129.11 (Ph), 129.59, 129.93 (4-Cl-Ph), 129.66 (Ph), 130.07 (m, CF_3), 130.15 (Ph), 136.78 (C-6), 137.07, 137.41 (4-Cl-Ph), 148.09, 148.17 (4-Cl-Ph), 148.35, 148.79 (d, $J_{C\text{-}P}$ = 5.5 Hz, C-4 triazole), 150.13, 150.26 (C-2), 163.66, 163.76 (C-4). ^{19}F NMR ($CDCl_3$) δ: -74.22, -74.30 (t, 3 F, $J_{H\text{-}F}$ = 8.8 Hz). ^{31}P NMR ($CDCl_3$) δ: 4.01, 4.15. MS-ESI *m/z*: 641, 643 $[M+H]^+$; 663, 665 $[M+Na]^+$; 679, 681 $[M+K]^+$; 639, 641 $[M-H]^-$; 675, 677, 679 $[M+Cl]^-$. Anal. Calcd for $C_{26}H_{25}ClF_3N_6O_6P$: C, 48.72; H, 3.93; N, 13.11. Found: C, 48.61; H, 3.87; N, 13.15.

3′-[4-Phenyl-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-n-propylphosphate] (23)

^{1}H NMR ($CDCl_3$) δ: 0.84, 0.87 (t, 3 H, *J* = 7.3 Hz, CH_3 *n*-Pr), 1.26, 1.47 (sex, 2 H, *J* = 3.6 Hz, CH_2 *n*-Pr), 1.91 (s, 3 H, 5-CH_3), 2.65–2.99 (m, 2 H, H-

2′, H-2”), 3.12 (sex, 2 H, $J = 6.7$ Hz, N-CH_2 *n*-Pr), 3.44–3.83 (m, 2 H, H-5′, H-5”), 4.34–4.52 (m, 1H, H-4′), 5.47–5.58 (m, 2 H, P-NH, H-3′), 6.30, 6.41 (pseudo t, 1H, $J = 6.0$ Hz, H-1′), 7.11 (dd, 2 H, $J = 1.1$ Hz, $J = 8.0$ Hz, 4-Cl-Ph), 7.25–7.29 (m, 1H, Ph), 7.35 (dd, 2 H, $J = 1.1$ Hz, $J = 8.0$ Hz, 4-Cl-Ph), 7.39–7.44 (m, 2 H, Ph), 7.77–7.82 (m, 3 H, Ph, H-6), 8.20 (s, 1H, H-5 triazole), 9.70 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 11.17, 11.20 (CH_3 *n*-Pr), 12.61, 12.65 (5-CH_3), 24.81, 24.87 (CH_2 *n*-Pr), 37.86 (C-2′), 43.55 (d, $J_{C\text{-}P} = 3.4$ Hz, N-CH_2 *n*-Pr), 59.46 (d, $J_{C\text{-}P} = 3.3$ Hz, C-3′), 65.13, 65.37 (d, $J_{C\text{-}P} = 5.1$ Hz, C-5′), 82.79 (C-4′), 86.52, 87.30 (C-1′), 111.32, 111.53 (C-5), 121.41, 121.50 (d, $J_{C\text{-}P} = 5.1$ Hz, C-5 triazole), 125.84 (Ph), 129.05, 129.66 (d, $J_{C\text{-}P} = 4.3$ Hz, 4-Cl-Ph), 129.69 (Ph), 129.93, 129.97 (4-Cl-Ph), 130.08 (Ph), 130.16 (Ph), 136.35, 136.71 (4-Cl-Ph), 136.77 (C-6), 148.09, 148.17 (4-Cl-Ph), 149.10, 149.19 (d, $J_{C\text{-}P} = 3.4$ Hz, C-4 triazole), 150.40, 150.45 (C-2), 164.09, 164.17 (C-4). ^{31}P NMR ($CDCl_3$) δ: 6.02, 6.22. MS-ESI *m/z*: 601, 603 $[M + H]^+$; 623, 625 $[M + Na]^+$; 639, 641 $[M + K]^+$; 599, 601 $[M - H]^-$; 635, 637, 639 $[M + Cl]^-$. Anal. Calcd for $C_{27}H_{30}ClN_6O_6P$: C, 53.96; H, 5.03; N, 13.98. Found: C, 53.89; H, 4.99; N, 13.75.

3′-[4-Phenyl-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-allylphosphate] (24)

^{1}H NMR ($CDCl_3$) δ: 1.91, 1.92 (d, 3 H, $J = 1.3$ Hz, 5-CH_3), 2.69–2.76 (m, 1H, H-2”), 2.79–2.86 (m, 1H, H-2′), 3.13 (sex, 2 H, $J = 7.1$ Hz, N-CH_2-C = C), 3.54–3.64 (m, 2 H, H-5′, H-5”), 4.35–4.51 (m, 1H, H-4′), 5.07–5.25 (m, 2 H, P-NH, H-3′), 5.46–5.57 (m, 2 H, N-C-C = CH_2), 5.72–5.84 (m, 1H, N-C-CH = C), 6.28, 6.38 (dd, 1H, $J = 2.8$ Hz, $J = 5.9$ Hz, H-1′), 7.11 (dd, 2 H, $J_{C\text{-}P} = 1.2$ Hz, $J = 9.0$ Hz, 4-Cl-Ph), 7.17 (dd, 2 H, $J = 1.2$ Hz, $J = 9.0$ Hz, 4-Cl-Ph), 7.22–7.25 (m, 1H, Ph), 7.39–7.44 (m, 2 H, Ph), 7.77–7.81 (m, 3 H, Ph, H-6), 8.18 (s, 1H, H-5 triazole), 9.57 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.51, 12.52 (5-CH_3), 37.66, 37.71 (C-2′), 43.89, 43.92 (N-CH_2), 59.21, 59.29 (d, $J_{C\text{-}P} = 3.3$ Hz, C-3′), 65.07, 65.29 (d, $J_{C\text{-}P} = 4.9$ Hz, C-5′), 82.61, 82.73 (d, $J_{C\text{-}P} = 4.7$ Hz, C-4′), 86.52, 87.33 (C-1′), 111.19, 111.41 (C-5), 120.49 (d, $J_{C\text{-}P} = 4.7$ Hz, CH_2 allyl), 121.40, 121.49 (d, $J_{C\text{-}P} = 4.9$ Hz, C-5 triazole), 125.73 (Ph), 128.60 (pseudo t, $J_{C\text{-}P} = 3.0$ Hz, CH allyl), 129.81, 129.85 (d, $J_{C\text{-}P} = 4.3$ Hz, 4-Cl-Ph), 130.00 (Ph), 130.52, 130.63 (4-Cl-Ph), 134.88 (Ph), 134.93 (Ph), 136.26, 136.62 (4-Cl-Ph), 136.77 (C-6), 148.89, 148.95 (4-Cl-Ph), 150.24 (d, $J_{C\text{-}P} = 3.4$ Hz, C-4 triazole), 150.45 (C-2), 163.85 (C-4). ^{31}P NMR ($CDCl_3$) δ: 5.58, 5.67. MS-ESI *m/z*: 599, 600 $[M + H]^+$; 621, 623 $[M + Na]^+$; 637, 639 $[M + K]^+$; 597, 599 $[M - H]^-$; 633, 635, 637 $[M + Cl]^-$. Anal. Calcd for $C_{27}H_{28}ClN_6O_6P$: C, 54.14; H, 4.71; N, 14.03. Found: C, 53.99; H, 4.69; N, 13.95.

3′-[4-Phenyl-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-propargylphosphate] (25)

^{1}H NMR ($CDCl_3$) δ: 1.90, 1.91 (d, 3 H, $J = 1.4$ Hz, 5-CH_3), 2.01 (s, 1H, CH propargyl), 2.22–2.29 (m, 2 H, H-2′, H-2”), 3.10 (sex, 2 H, $J = 6.8$ Hz, N-CH_2), 3.74–3.86 (m, 2 H, H-5′, H-5”), 4.37–4.56 (m, 1H, H-4′), 5.47–5.59 (m, 2 H, P-NH, H-3′), 6.28, 6.36 (dd, 1H, $J = 5.8$ Hz, $J = 7.0$ Hz, H-1′), 7.13 (dd, 2 H, $J = 1.2$ Hz, $J = 9.1$ Hz, 4-Cl-Ph), 7.17 (dd, 2 H, $J = 1.2$ Hz, $J = 9.1$ Hz, 4-Cl-Ph), 7.22–7.25 (m, 1H, Ph), 7.28–7.29 (m, 2 H, Ph), 7.77–7.82 (m, 3 H, Ph, H-6), 8.18 (s, 1H, H-5 triazole), 9.75 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.64 (5-CH_3), 30.98, 31.25 (N-CH_2), 37.74 (C-2′), 59.42, 59.60 (C-3′), 65.42, 65.61 (d, $J_{C\text{-}P} = 4.7$ Hz, C-5′), 72.04, 72,49 (C propargyl), 77.37, 77.82 (CH propargyl), 82.74, 82.85 (d, $J_{C\text{-}P} = 4.3$ Hz, C-4′), 86.88 (C-1′), 111.42, 111.57 (C-5), 121.60, 121.97 (d, $J_{C\text{-}P} = 4.8$ Hz, C-5 triazole), 125.88 (Ph), 129.08, 129.46 (d, $J_{C\text{-}P} = 4.3$ Hz, 4-Cl-Ph), 129.78 (Ph), 129.99, 130.13 (4-Cl-Ph), 134.87 (Ph), 134.93 (Ph), 136.37, 136.41 (4-Cl-Ph), 136.77 (C-6), 148.23, 148.89 (4-Cl-Ph), 149.25 (d, $J_{C\text{-}P} = 3.4$ Hz, C-4 triazole), 150.41 (C-2), 164.05 (C-4). ^{31}P NMR ($CDCl_3$) δ: 4.86, 4.87. MS-ESI *m/z*: 597, 599 $[M + H]^+$; 619, 621 $[M + Na]^+$; 635, 637 $[M + K]^+$; 595, 597 $[M - H]^-$; 631, 633, 635 $[M + Cl]^-$. Anal. Calcd for $C_{27}H_{26}ClN_6O_6P$: C, 54.32; H, 4.39; N, 14.08. Found: C, 54.19; H, 4.43; N, 13.97.

3′-[4-(2-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-methylphosphate] (26)

^{1}H NMR (DMSO-d_6) δ: 1.80, 1.81 (d, 3 H, $J = 1.1$ Hz, 5-CH_3), 2.43–2.47 (m, 1H, H-2”), 2.49 (m, 3 H, N-CH_3), 2.66–2.79 (sex, 1H, H-2′), 2.83–2.93 (m, 2 H, H-5′, H-5”), 4.31 (m, 1H, H-4′), 5.42–5.50 (m, 1H, H-3′), 5.55–5.61 (m, 1H, P-NH), 6.53, 6.54 (dd, 1H, $J = 2.8$ Hz, $J = 5.6$ Hz, H-1′), 7.11 (dd, 2 H, $J = 1.2$ Hz, $J = 9.1$ Hz, 4-Cl-Ph), 7.17 (dd, 2 H, $J = 1.2$ Hz, $J = 9.1$ Hz, 4-Cl-Ph), 7.19–7.22 (m, 1H, 2-F-Ph), 7.32–7.48 (m, 2 H, 2-F-Ph), 7.68, 7.69 (d, 1H, $J = 1.1$ Hz, H-6), 8.16 (dd, 1H, $J = 1.9$ Hz, $J_{H\text{-}F} = 7.5$ Hz, 2-F-Ph), 8.71 (d, 1H, $J_{H\text{-}F} = 3.6$ Hz, H-5 triazole), 9.68 (br s, 1H, 3-NH). ^{13}C NMR (DMSO-d_6) δ: 12.09, 12.14 (5-CH_3), 26.87 (N-CH_3), 36.68, 36.70 (C-2′), 59.42, 59.44 (C-3′), 65.36 (C-5′), 81.73, 81.83 (C-4′), 84.25, 84.30 (C-1′), 110.03, 110.06 (C-5), 116.09 (d, $J_{C\text{-}F} = 21.1$ Hz, 2-F-Ph), 118.18 (d, $J_{C\text{-}F} = 13.0$ Hz, 2-F-Ph), 121.92, 121.95 (d, $J_{C\text{-}P} = 4.7$ Hz, 4-Cl-Ph), 123.49 (d, $J_{C\text{-}F} = 11.2$ Hz, 2-F-Ph), 123.62, 123.78 (d, $J_{C\text{-}P} = 1.9$ Hz, 4-Cl-Ph), 125.04 (d, $J_{C\text{-}F} = 3.4$ Hz, C-5 triazole), 127.35 (d, $J_{C\text{-}F} = 3.5$ Hz, 2-F-Ph), 129.87 (d, $J_{C\text{-}F} = 8.5$ Hz, 2-F-Ph), 136.14 (C-6), 136.35, 136.39 (4-Cl-Ph), 140.00 (C-4 triazole), 148.03, 148.69 (4-Cl-Ph), 150.46 (C-2), 158.50 (d, $J_{C\text{-}F} = 247.3$ Hz, 2-F-Ph), 163.70 (C-4). ^{19}F NMR (DMSO-d_6) δ: -114.00

(m, 1F). ^{31}P NMR (DMSO-d_6) δ: 7.33, 7.52. MS-ESI *m/z*: 591, 593 $[M+H]^+$; 613, 615 $[M+Na]^+$; 629, 631 $[M+K]^+$; 589, 591 $[M-H]^-$; 625, 627, 629 $[M+Cl]^-$. Anal. Calcd for $C_{25}H_{25}ClFN_6O_6P$: C, 50.81; H, 4.26; N, 14.22. Found: C, 50.62; H, 4.20; N, 14.11.

3′-[4-(2-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-ethylphosphate] (27)

^{1}H NMR (DMSO-d_6) δ: 0.96 (t, 3 H, $J = 7.2$ Hz, CH_3 Et), 1.80, 1.81 (d, 3 H, $J = 1.1$ Hz, 5-CH_3), 2.66–2.78 (m, 2 H, H-2′, H-2”), 2.80–2.89 (m, 2 H, N-CH_2), 4.26–4.32 (m, 2 H, H-5′, H-5”), 4.52–4.56 (m, 1H, H-4′), 5.52–5.63 (m, 2 H, P-NH, H-3′), 6.51, 6.53 (dd, 1H, $J = 3.2$ Hz, $J = 6.7$ Hz, H-1′), 7.19 (dd, 2 H, $J = 1.1$ Hz, $J = 9.0$ Hz, 4-Cl-Ph), 7.20 (dd, 2 H, $J = 1.1$ Hz, $J = 9.0$ Hz, 4-Cl-Ph), 7.32–7.35 (m, 1H, 2-F-Ph), 7.36–7.38 (m, 1H, 2-F-Ph), 7.39–7.41 (m, 1H, 2-F-Ph), 7.67, 7.69 (d, 1H, $J = 1.1$ Hz, H-6), 8.15, 8.18 (dd, 1H, $J = 1.7$ Hz, $J_{H\text{-}F} = 7.6$ Hz, 2-F-Ph), 8.72 (d, 1H, $J_{H\text{-}F} = 3.6$ Hz, H-5 triazole), 11.43 (br s, 1H, 3-NH). ^{13}C NMR (DMSO-d_6) δ: 12.11, 12.17 (5-CH_3), 16.90 (d, $J_{C\text{-}P} = 6.1$ Hz, CH_3 Et), 35.67 (C-2′), 36.69 (N-CH_2), 59.43, 59.44 (C-3′), 65.36, 65.42 (d, $J_{C\text{-}P} = 5.3$ Hz, C-5′), 81.74, 81.81 (d, $J_{C\text{-}P} = 2.7$ Hz, C-4′), 84.23, 84.27 (C-1′), 110.04, 110.06 (C-5), 116.08 (d, $J_{C\text{-}F} = 21.2$ Hz, 2-F-Ph), 118.19 (d, $J_{C\text{-}F} = 12.9$ Hz, 2-F-Ph), 121.93, 121.97 (d, $J_{C\text{-}P} = 4.5$ Hz, 4-Cl-Ph), 123.63 (d, $J_{C\text{-}F} = 11.2$ Hz, 2-F-Ph), 123.62, 123.78 (d, $J_{C\text{-}P} = 1.9$ Hz, 4-Cl-Ph), 125.06 (d, $J_{C\text{-}F} = 3.0$ Hz, C-5 triazole), 127.35 (d, $J_{C\text{-}F} = 3.5$ Hz, 2-F-Ph), 129.89 (d, $J_{C\text{-}F} = 8.3$ Hz, 2-F-Ph), 136.01 (C-6), 136.13, 136.26 (4-Cl-Ph), 140.02 (C-4 triazole), 149.53, 149.59 (4-Cl-Ph), 150.46 (C-2), 158.50 (d, $J_{C\text{-}F} = 247.4$ Hz, 2-F-Ph), 163.72 (C-4). ^{19}F NMR (DMSO-d_6) δ: -114.11 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 6.28, 6.43. MS-ESI *m/z*: 605, 607 $[M+H]^+$; 627, 629 $[M+Na]^+$; 643, 645 $[M+K]^+$; 603, 605 $[M-H]^-$; 639, 641, 643 $[M+Cl]^-$. Anal. Calcd for $C_{26}H_{27}ClFN_6O_6P$: C, 51.62; H, 4.50; N, 13.89. Found: C, 51.40; H, 4.43; N, 13.95.

3′-[4-(2-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-(2,2,2-trifluoroethyl)phosphate] (28)

^{1}H NMR (DMSO-d_6) δ: 1.79, 1.80 (d, 3 H, $J = 1.2$ Hz, 5-CH_3), 2.74 (sex, 1H, H-2″), 2.87 (sex, 1H, H-2′), 3.55-3.68 (m, 2 H, H-5′, H-5″), 4.28-4.38 (m, 2 H, N-CH_2), 4.52-4.56 (m, 1H, H-4′), 5.54-5.59 (m, 2 H, P-NH, H-3′), 6.49, 6.51 (dd, 1H, $J = 6.7$ Hz, H-1′), 7.19 (dd, 2 H, $J_{C\text{-}P} = 1.1$ Hz, $J = 8.9$ Hz, 4-Cl-Ph), 7.20 (dd, 2 H, $J = 1.1$ Hz, $J = 8.9$ Hz, 4-Cl-Ph), 7.33 (d, 1H, $J_{H\text{-}F} = 1.4$ Hz, 2-F-Ph),7.40-7.43 (m, 1H, 2-F-Ph), 7.46-7.47 (m, 1H, 2-F-Ph), 7.65, 7.66 (2 x d, 1H, $J = 1.2$ Hz, H-6), 8.16, 8.17 (2 x dd, $J = 1.7$ Hz,

$J_{H\text{-}F}$ = 7.5 Hz, 2-F-Ph), 8.69 (dd, 1H, J = 2.8 Hz, J = 3.6 Hz, H-5 triazole), 11.41 (br s, 1H, 3-NH). ^{13}C NMR (DMSO-d_6) δ: 12.06, 12.09 (5-CH_3), 36.55, 36.59 (C-2′), 42.21, 42.54 (N-CH_2), 59.32 (C-3′), 65.89, 65.92 (C-5′), 81.59, 81.66 (2 x d, J = 7.2 Hz, C-4′), 84.27 (C-1′), 110.04, 110.08 (C-5), 115.98, 116.19 (2-F-Ph), 123.58, 123.66 (2-F-Ph), 125.03 (d, $J_{C\text{-}F}$ = 3.2 Hz, 2-F-Ph), 126.20 (d, $J_{C\text{-}F}$ = 4.7 Hz, 2-F-Ph), 127.34 (d, $J_{C\text{-}P}$ = 3.6 Hz, 4-Cl-Ph), 128.93 (m, 2-F-Ph), 129.13 (CF_3), 129.56 (C-5 triazole), 129.87 (d, $J_{C\text{-}P}$ = 8.2 Hz, 4-Cl-Ph), 136.18, 136.25 (C-6), 140.01, 140.04 (C-4 triazole), 149.13, 149.19 ($J_{C\text{-}P}$ = 2.9 Hz, 4-Cl-Ph), 150.44 (C-2), 158.50 (d, $J_{C\text{-}F}$ = 247.3 Hz, 2-F-Ph), 163.70 (C-4). ^{19}F NMR (DMSO-d_6) δ: -114.01, 71.80, 71.81 (2 x t, 3 F, J_{HF} = 8.8 Hz). ^{31}P NMR (DMSO-d_6) δ: 5.35, 5.55. MS-ESI *m/z*: 659, 661 $[M + H]^+$; 681, 683 $[M + Na]^+$; 697, 699 $[M + K]^+$; 657, 659 $[M - H]^-$; 693, 695, 697 $[M + Cl]^-$. Anal. Calcd for $C_{26}H_{24}ClF_4N_6O_6P$: C, 47.39; H, 3.67; N, 12.75. Found: C, 47.30; H, 3.33; N, 12.90.

3′-[4-(2-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-n-propylphosphate] (29)

^{1}H NMR (DMSO-d_6) δ: 0.82 (t, 3 H, J = 7.4 Hz, CH_3 *n*-Pr), 1.41 (sex, 2 H, J = 7.3 Hz, CH_2 *n*-Pr), 1.81, 1.85 (2 x d, 3 H, J = 0.8 Hz, 5-CH_3), 2.70-2.80 (m, 2 H, H-2′, H-2″), 2.82-2.96 (m, 2 H, N-CH_2), 3.94-4.06 (m, 2 H, H-5′, H-5″), 4.29-4.35 (m, 1H, H-4′), 4.72-4.80 (m, 2 H, P-NH, H-3′), 6.54 (dd, J = 2.6 Hz, J = 10.8 Hz, H-1′), 7.13 (dd, 2 H, J = 1.0 Hz, J = 9.0 Hz, 4-Cl-Ph), 7.19 (dd, 2 H, J = 1.0 Hz, J = 9.0 Hz, 4-Cl-Ph), 7.35 (s, 1H, H-6), 7.37 (s, 1H, 2-F-Ph), 7.37 (s, 1H, 2-F-Ph), 7.39 (s, 1H, 2-F-Ph), 7.68 (d, 1H, $J_{H\text{-}F}$ = 1.0 Hz, 2-F-Ph), 8.71-8.73 (m, 1H, 2-F-Ph), 8.17 (td, 1H, J = 1.5 Hz, J = 7.6 Hz, H-5-triazole), 11.44 (br s, 1H, 3-NH). ^{13}C NMR (DMSO-d_6) δ: 11.29 (CH_3 *n*-Pr), 12.11, 12.16 (5-CH_3), 24.48, 24.56 (CH_2 *n*-Pr), 36.67 (C-2′), 42.53 (N-CH_2), 59.44 (C-3′), 65.38 (C-5′), 82.33 (C-4′), 84.06 (C-1′), 110.02, 110.05 (C-5), 122.20, (d, $J_{C\text{-}F}$ = 4.8 Hz, 2-F-Ph), 125.01 (d, $J_{C\text{-}F}$ = 3.0 Hz, 2-F-Ph), 127.41 (2-F-Ph), 128.52 (2-F-Ph), 129.13 (4-Cl-Ph), 129.49 (2-F-Ph), 129.71 (C-5 triazole), 129.78 (2-F-Ph), 129.89 (4-Cl-Ph), 136.23 (d, J = 12.9 Hz, C-6), 140.03 (C-4 triazole), 150.44 (C-2), 150.71 (d, $J_{C\text{-}P}$ = 6.4 Hz, 4-Cl-Ph), 158.50 (d, $J_{C\text{-}F}$ = 247.4 Hz, 2-F-Ph), 163.69 (C-3). ^{19}F NMR (DMSO-d_6) δ: -114.01 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 6.46, 6.58. 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 $C_{27}H_{29}ClFN_6O_6P$: C, 52.39; H, 4.72; N, 13.58. Found: C, 52.01; H, 4.90; N, 13.06.

3′-[4-(2-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-allylphosphate] (30)

^{1}H NMR (DMSO-d_6) δ: 1.80, 1.81 (2 x d, 3 H, J = 1.2 Hz, 5-CH_3), 2.72 (sex, 1H, J = 6.8 Hz, H-2″), 2.86 (sex, 1H, J = 6.8 Hz, H-2′), 3.45 (sex, 2 H, J = 6.2 Hz, N-CH_2-C = C), 4.30-4.33 (m, 2 H, H-5′, H-5″), 4.53-4.55 (m, 1H, H-4′), 4.94-5.00 (m, 1H, H-3′), 5.08, 5.14 (2 x sex, 1H, J = 3.5 Hz, P-NH), 5.54-5.61 (m, 2 H, N-C-C = CH_2), 5.66-5.86 (m, 1H, N-C-CH = C), 6.52, 6.53 (2 x dd, J = 3.1 Hz, J = 7.4 Hz, H-1′), 7.21 (dd, 2 H, J = 1.1. Hz, J = 9.0 Hz, 4-Cl-Ph), 7.22 (dd, 2 H, J = 1.1. Hz, J = 9.0 Hz, 4-Cl-Ph), 7.34-7.35 (m, 1H, 2-F-Ph), 7.36-7.38 (m, 1H, 2-F-Ph), 7.42-7.44 (m, 1H, 2-F-Ph), 7.68 (dd, J = 1.4 Hz, J = 2.8 Hz, H-6), 8.15, 8.17 (2 x dd, J = 1.7 Hz, $J_{H\text{-}F}$ = 7.4 Hz, 2-F-Ph), 8.71, 8.72 (2 x d, 1H, J = 2.3 Hz, H-5 triazole), 11.44 (br s, 1H, 3-NH). ^{13}C NMR (DMSO-d_6) δ: 12.14, 12.18 (5-CH_3), 36.67 (C-2′), 43.13, 43.15 (N-CH_2), 59.41, 59.44 (C-3′), 65.49, 65.53 (2 x d, J = 4.7 Hz, C-5′), 81.68, 81.79 (2 x d, J = 7.1 Hz, C-4′), 84.26 (C-1′), 110.04, 110.07 (C-5), 115.10, 115.12 ($J_{C\text{-}F}$ = 1.9 Hz, 2-F-Ph), 116.00, 116.20 (CH_2 allyl), 118.12, 118.25 (2-F-Ph), 121.97, 122.04 (2 x d, $J_{C\text{-}F}$ = 3.4 Hz, 2-F-Ph), 125.06 (d, $J_{C\text{-}P}$ = 3.2 Hz, 4-Cl-Ph), 127.35 (d, $J_{C\text{-}P}$ = 3.5 Hz, 4-Cl-Ph), 129.11 (2-F-Ph), 129.53 (C-5 triazole), 129.86 (d, $J_{C\text{-}F}$ = 8.4 Hz, 2-F-Ph), 136.20 (d, $J_{C\text{-}P}$ = 11.4 Hz, 4-Cl-Ph), 136.39, 136.45 (2 x d, $J_{C\text{-}P}$ = 2.4 Hz, CH allyl), 140.02 (C-6), 145.50 (C-4 triazole), 149.47, 149.53 (2 x d, $J_{C\text{-}P}$ = 2.3 Hz, 4-Cl-Ph), 150.46 (C-2), 158.50 (d, $J_{C\text{-}F}$ = 247.4 Hz, 2-F-Ph), 163.72 (C-4). ^{19}F NMR (DMSO-d_6) δ: -113.98 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 6.18, 6.35. MS-ESI *m/z*: 617, 619 $[M + H]^+$; 639, 641 $[M + Na]^+$; 655, 657 $[M + K]^+$; 615, 617 $[M - H]^-$; 651, 653, 655 $[M + Cl]^-$. Anal. Calcd for $C_{27}H_{27}ClFN_6O_6P$: C, 52.56; H, 4.41; N, 13.62. Found: C, 52.50; H, 4.89; N, 13.22.

3′-[4-(2-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-propargylphosphate] (31)

^{1}H NMR (DMSO-d_6) δ: 1.80, 1.81 (2 x d, 3 H, J = 1.1 Hz, 5-CH_3), 2.14-2.21 (m, 1H, CH propargyl), 2.72 (sex, 1H, J = 6.9 Hz, H-2″), 2.85 (sex, 1H, J = 6.9 Hz, H-2′), 3.11 (dd, 2 H, J = 3.4 Hz, N-CH_2 propargyl), 3.58-3.71 (m, 2 H, H-5″, H-5″), 4.33-4.34 (m, 1H, H-4′), 5.45, 5.49 (2 x t, 1H, J = 7.0 Hz, P-NH), 5.55-5.61 (m, 1H, H-3′), 6.52, 6.54 (2 x pseudo t, 1H, J = 6.7 Hz, H-1′), 7.21 (dd, 2 H, J = 1.2 Hz, J = 9. 1H z, 4-Cl-Ph), 7.22 (dd, 2 H, J = 1.2 Hz, J = 9.1 Hz, 4-Cl-Ph), 7.35 (s, 1H, H-6), 7.37-7.39 (m, 1H, 2-F-Ph), 7.40-7.41 (m, 1H, 2-F-Ph), 7.45-7.46 (m, 1H, 2-F-Ph), 8.15, 8.18 (2 x dd, J = 1.6 Hz, $J_{H\text{-}F}$ = 7.4 Hz, 2-F-Ph), 8.70, 8.72 (2 x d, 1H, J = 3.6 Hz, H-5 triazole), 11.42 (br s, 1H, 3-NH). ^{13}C NMR (DMSO-d_6) δ: 12.15, 12.21 (5-CH_3), 29.90, 30.13 (N-CH_2 propargyl), 36.72 (C-2′), 59.46 (C-3′), 65.66 (d,

$J = 5.0$ Hz, C-5′), 72.64 (CH propargyl), 73.64, 73.71 (C-4′), 82.39 (C propargyl), 84.03 (C-1′), 110.06, 110.12 (C-5), 116.10 (d, $J_{C\text{-}F} = 21.3$ Hz, 2-F-Ph), 122.05, 122.11 (2 x d, $J_{C\text{-}F} = 4.0$ Hz, 2-F-Ph), 122.50 (d, $J_{C\text{-}F} = 4.6$ Hz, 2-F-Ph), 123.67, 123.78 (2 x d, $J_{C\text{-}F} = 3.9$ Hz, 2-F-Ph), 125.05 (d, $J_{C\text{-}P} = 3.3$ Hz, 4-Cl-Ph), 127.36 (d, $J_{C\text{-}P} = 3.3$ Hz, 4-Cl-Ph), 129.20 (C-5-triazole), 129.51 (2-F-Ph), 129.84 (d, $J_{C\text{-}P} = 8.5$ Hz, 4-Cl-Ph), 136.12 (d, $J = 9.8$ Hz, C-6), 149.31, 149.37 (C-4 triazole), 150.15 (d, $J_{C\text{-}P} = 6.5$ Hz, 4-Cl-Ph), 150.45 (C-2), 158.45 (d, $J_{C\text{-}F} = 247.3$ Hz, 2-F-Ph), 163.72 (C-4). ^{19}F NMR (DMSO-d_6) δ: -113.98 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 5,44, 5.67. MS-ESI *m/z*: 615, 617 $[M + H]^+$; 637, 639 $[M + Na]^+$; 653, 655 $[M + K]^+$; 613, 615 $[M - H]^-$; 649, 651, 653 $[M + Cl]^-$. Anal. Calcd for $C_{27}H_{25}ClFN_6O_6P$: C, 52.73; H, 4.10; N, 13.67. Found: C, 52.40; H, 4.31; N, 13.21.

3′-[4-(3-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-methylphosphate] (32)

^{1}H NMR ($CDCl_3$) δ: 1.91 (s, 3 H, 5-CH_3), 2.63-2.64 (m, 1H, H-2″), 2.68 (d, 3 H, $J = 1.2$ Hz, N-CH_3), 2.71-2.72 (m, 1H, H-2′), 2.88 (m, 1H, H-4′), 4.21, 4.23 (2 x d, 1H, $J = 3.8$ Hz, H-5′), 4.60-4.74 (m, 1H, H-5″), 5.49-5.56 (m, 1H, H-3′), 5.58-5.68 (m, 1H, P-NH), 6.23, 6.41 (pseudo t, $J = 5.5$ Hz, H-1′), 7.03 (dd, 2 H, $J = 2.3$ Hz, $J = 8.4$ Hz, 4-Cl-Ph), 7.04 (dd, 2 H, $J_{C\text{-}P} = 2.3$ Hz, $J = 8.4$ Hz, 4-Cl-Ph), 7.20 (s, 1H, 3-F-Ph), 7.22 (s, 1H, 3-F-Ph), 7.31 (s, 1H, 3-F-Ph), 7.44 (s, 1H, H-6), 7.69-7.73 (m, 1H, 3-F-Ph), 8.12 (s, 1H, H-5 triazole), 9.76 (br s, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.51 (5-CH_3), 27.65 (N-CH_3), 36.60 (C-2′), 58.96, 59.38 (C-3′), 64.93 (C-5′), 82.60, 82.68 (C-4′), 82.75, 82.81 (C-1′), 110.91, 111.43 (C-5), 112.70 (d, $J_{C\text{-}F} = 23.2$ Hz, 3-F-Ph), 115.36 (d, $J_{C\text{-}F} = 22.5$ Hz, 3-F-Ph), 116.70 (4-Cl-Ph), 121.27 (d, $J_{C\text{-}P} = 4.8$ Hz, 4-Cl-Ph), 121.42 (d, $J_{C\text{-}P} = 4.3$ Hz, 4-Cl-Ph), 129.37 (3-F-Ph), 129.89 (C-5 triazole), 130.52 (4-Cl-Ph), 130.68 (3-F-Ph), 136.20 (3-F-Ph), 136.57 (C-6) 148.86 (C-4 triazole), 150.22 (C-2), 163.12 (d, $J_{C\text{-}F} = 246.0$ Hz, 3-F-Ph), 163.73 (C-4). ^{19}F NMR ($CDCl_3$) δ: -112.82 (m, 1F). ^{31}P NMR ($CDCl_3$) δ: 7.22, 7.23. MS-ESI *m/z*: 591, 593 $[M + H]^+$; 613, 615 $[M + Na]^+$; 629, 631 $[M + K]^+$; 589, 591 $[M - H]^-$; 625, 627, 629 $[M + Cl]^-$. Anal. Calcd for $C_{25}H_{25}ClFN_6O_6P$: C, 50.81; H, 4.26; N, 14.22. Found: C, 50.40; H, 4.56; N, 14.88.

3′-[4-(3-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-ethylphosphate] (33)

^{1}H NMR ($CDCl_3$) δ: 1.10, 1.13 (td, 3 H, $J = 1.1$ Hz, $J = 7.3$ Hz, CH_3 Et), 1.90 (s, 3 H, 5-CH_3), 2.68-2.75 (m, 2 H, H-2′, H-2”), 2.78-2.89 (m, 2 H, N-CH_2-C), 2.95-3.19 (m, 1H, H-5”), 3.66, 3.89 (quin, 1H, $J = 6.6$ Hz,

$J = 13.2$ Hz, H-5′), 4.35–4.49 (m, 1H, H-4′), 4.62-4.69 (m, 1H, H-3′), 5.49–5.62 (m, 1H, P-NH), 6.28, 6.31 (dd, 1H, $J = 5.1$ Hz, $J = 6.9$ Hz, H-1′), 7.02 (dd, 2 H, $J = 1.2$ Hz, $J = 8.9$ Hz, 4-Cl-Ph), 7.12 (dd, 2 H, $J = 1.2$ Hz, $J = 8.9$ Hz, 4-Cl-Ph), 7.21-7.29 (m, 1H, 3-F-Ph), 7.33-7.40 (m, 1H, 3-F-Ph), 7.51-7.59 (m, 2 H, 3-F-Ph), 8.12, 8.13 (s, 1H, H-6), 8.29 (s, 1H, H-5 triazole), 9.98, 10.10 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.60, 12.64 (5-CH_3), 17.12, 17.19 (d, $J_{C\text{-}P} = 6.5$ Hz, CH_3 Et), 36.68 (C-2′), 37.89 (d, $J_{C\text{-}P} = 8.0$ Hz, N-CH_2), 59.41, 59.50 (C-3′), 65.09, 65.29 (d, $J_{C\text{-}P} = 5.0$ Hz, C-5′), 82.77, 82.89 (d, $J_{C\text{-}P} = 6.3$ Hz, C-4′), 86.44, 87.21 (C-1′), 111.40, 112.74 (C-5), 115.34 (d, $J_{C\text{-}F} = 21.2$ Hz, 3-F-Ph), 116.87 (3-F-Ph), 121.05, 121.29 (C-5 triazole), 121.44, 121.51 (d, $J_{C\text{-}P} = 5.1$ Hz, 4-Cl-Ph), 129.44, 129.69 (d, $J_{C\text{-}P} = 2.1$ Hz, 4-Cl-Ph), 129.95 (d, $J_{C\text{-}F} = 3.7$ Hz, 3-F-Ph), 130.59, 130.70 (d, $J_{C\text{-}P} = 8.5$ Hz, 4-Cl-Ph), 132.33 (d, $J_{C\text{-}F} = 8.4$ Hz, 3-F-Ph), 136.30, 136.66 (C-6), 146.74 (3-F-Ph), 146.99, 147.03 (C-4 triazole), 149.09 (d, $J_{C\text{-}P} = 6.2$ Hz, 4-Cl-Ph), 150.47, 150.50 (C-2), 163.23 (d, $J_{C\text{-}F} = 245.7$ Hz, 3-F-Ph), 164.19, 164.21 (C-4). ^{19}F NMR ($CDCl_3$)δ: -112.91 (m, 1F). ^{31}P NMR ($CDCl_3$) δ: 5.97, 6.19. MS-ESI *m/z*: 605, 607 $[M + H]^+$; 627, 629 $[M + Na]^+$; 643, 645 $[M + K]^+$; 603, 605 $[M - H]^-$; 639, 641, 643 $[M + Cl]^-$. Anal. Calcd for $C_{26}H_{27}ClFN_6O_6P$: C, 51.62; H, 4.50; N, 13.89. Found: C, 51.14; H, 4.31; N, 13.77.

3′-[4-(3-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-(2,2,2-trifluoroethyl)phosphate] (34)

^{1}H NMR ($CDCl_3$) δ: 1.93, 1.94 (s, 3 H, 5-CH_3), 2.31–2.37 (m, 1H, H-2”), 2.48-2.53 (m, 1H, H-2′), 3.08–3.18 (m, 4 H, N-CH_2, H-5′, H-5”), 4.40 (m, 1H, H-4′), 5.42–5.47 (m, 1H, P-NH), 5.48-5.53 (m, 1H, H-3′), 6.22, 6.35 (pseudo t, 1H, $J = 6.8$ Hz, H-1′), 7.14 (dd, 2 H, $J = 2.2$ Hz, $J = 9.1$ Hz, 4-Cl-Ph), 7.18 (dd, 2 H, $J = 2.2$ Hz, $J = 9.1$ Hz, 4-Cl-Ph), 7.20-7.29 (m, 1H, 3-F-Ph), 7.30-7.34 (m, 1H, 3-F-Ph), 7.52-7.59 (m, 1H, 3-F-Ph), 7.69-7.73 (m, 1H, 3-F-Ph), 7.93-7.97 (s, 1H, H-6), 8.24 (s, 1H, H-5 triazole), 9.50 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.57 (5-CH_3), 38.84 (C-2′), 42.33 (N-CH_2), 59.31 (C-3′), 65.78 (C-5′), 82.51 (C-4′), 86.91 (C-1′), 112.72, 112.95 (C-5), 116.84 (3-F-Ph), 121.49-122.42 (C-5 triazole), 123.45 (d, $J_{C\text{-}P} = 4.9$ Hz, 4-Cl-Ph), 128.95 (d, $J_{C\text{-}P} = 1.9$ Hz, 4-Cl-Ph), 129.58 (d, $J_{C\text{-}F} = 2.6$ Hz, 3-F-Ph), 130.17 (m, CF_3), 130.73 (d, $J_{C\text{-}P} = 7.8$ Hz, 4-Cl-Ph), 131.06 (d, $J_{C\text{-}F} = 7.4$ Hz, 3-F-Ph), 133.45 (3-F-Ph), 136.38 (C-6), 144.87 (3-F-Ph), 145.47 (C-4 triazole), 146.96 (d, $J_{C\text{-}P} = 5.8$ Hz, 4-Cl-Ph), 154.74 (C-2), 158.95 (d, $J_{C\text{-}F} = 261.1$ Hz, 3-F-Ph), 162.09 (C-4). ^{19}F NMR ($CDCl_3$) δ: -112.82 (m, 1F), -74.31, -74.23 (t, 3 F, $J_{H\text{-}F} = 9.0$ Hz). ^{31}P NMR ($CDCl_3$) δ: 4.03, 4.12. MS-ESI *m/z*: 659, 661 $[M + H]^+$; 681, 683 $[M + Na]^+$; 697, 699 $[M + K]^+$; 657, 659 $[M - H]^-$; 693, 695, 697 $[M + Cl]^-$. Anal. Calcd for

$C_{26}H_{24}ClF_4N_6O_6P$: C, 47.39; H, 3.67; N, 12.75. Found: C, 47.28; H, 3.57; N, 12.54.

3′-[4-(3-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-n-propylphosphate] (35)

^{1}H NMR (DMSO-d_6) δ: 0.74, 0.75 (t, 3 H, J = 7.4 Hz, CH_3 *n*-Pr), 1.33 (sex, 2 H, J = 7.0 Hz, CH_2 *n*-Pr), 1.80, 1.81 (s, 3 H, 5-CH_3), 2.70–2.80 (m, 1 H, H-2”), 2.83-2.89 (m, 1H, H-2′), 4.31-4.33 (m, 2 H, H-5′, H-5”), 4.51–4.54 (m, 1H, H-4′), 5.48–5.53 (m, 2 H, P-NH, H-3′), 5.58 (sex, 2 H, J = 6.5 Hz, N-CH_2 *n*-Pr), 6.48, 6.49 (pseudo t, 1H, J = 6.7 Hz, H-1′), 7.20 (dd, 2 H, J = 1.0 Hz, J = 9.0 Hz, 4-Cl-Ph), 7.21 (dd, 2 H, J = 1.0 Hz, J = 9.0 Hz, 4-Cl-Ph), 7.39-7.41 (m, 1H, 3-F-Ph), 7.52, 7.54 (t, 1H, $J_{H\text{-}F}$ = 8.0 Hz, 3-F-Ph), 7.64-7.69 (m, 2 H, 3-F-Ph), 7.70, 7.72 (s, 1H, H-6), 8.29 (s, 1H, H-5 triazole), 11.43 (br s, 1H, 3-NH). ^{13}C NMR (DMSO-d_6) δ: 11.06 (CH_3 *n*-Pr), 12.14, 12.19 (5-CH_3), 24.28, 24.34 (d, $J_{C\text{-}P}$ = 1.6 Hz, CH_2 *n*-Pr), 36.63 (C-2′), 42.64, 42.66 (N-CH_2 *n*-Pr), 59.49 (C-3′), 65.37, 65.48 (d, $J_{C\text{-}P}$ = 4.6 Hz, C-5′), 81.76, 81.84 (d, $J_{C\text{-}P}$ = 5.9 Hz, C-4′), 110.04 (C-1′), 111.64, 111.87 (C-5), 114.85 (d, $J_{C\text{-}F}$ = 20.1 Hz, 3-F-Ph), 121.22, 121.24 (C-5 triazole), 121.87 (d, $J_{C\text{-}P}$ = 3.4 Hz, 4-Cl-Ph), 121.91, 121.96 (d, $J_{C\text{-}F}$ = 2.4 Hz, 3-F-Ph), 128.55 (d, $J_{C\text{-}P}$ = 2.2 Hz, 4-Cl-Ph), 129.55 (3-F-Ph), 132.74 (d, $J_{C\text{-}P}$ = 7.4 Hz, 4-Cl-Ph), 132.89 (d, $J_{C\text{-}F}$ = 8.6 Hz, 3-F-Ph), 136.15, 136.31 (C-6), 142.46 (3-F-Ph), 145.34, 145.57 (C-4 triazole), 149.59 (d, $J_{C\text{-}P}$ = 5.3 Hz, 4-Cl-Ph), 150.45 (C-2), 162.61 (d, $J_{C\text{-}F}$ = 243.1 Hz, 3-F-Ph), 163.72, 163.74 (C-4). ^{19}F NMR (DMSO-d_6) δ: -112.14 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 6.50, 6.59. 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 $C_{27}H_{29}ClFN_6O_6P$: C, 52.39; H, 4.72; N, 13.58. Found: C, 52.07; H, 4.44; N, 13.26.

3′-[4-(3-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-allylphosphate] (36)

^{1}H NMR ($CDCl_3$) δ: 1.90 (s, 3 H, 5-CH_3), 2.68–2.76 (m, 1H, H-2”), 2.80–2.87 (m, 1H, H-2′), 3.13 (sex, 2 H, J = 7.0 Hz, N-CH_2-C = C), 3.55–3.65 (m, 2 H, H-5′, H-5”), 4.35–4.52 (m, 1H, H-4′), 4.60-4.64 (m, 1H, 3′), 4.95-5.02, 5.06-5.22 (m, 2 H, N-C-C = CH_2), 5.48–5.61 (m, 1H, P-NH), 5.78 (m, 1H, N-C-CH = C), 6.27, 6.39 (pseudo t, 1H, J = 6.2 Hz, H-1′), 7.01 (dd, 2 H, J = 1.1 Hz, J = 8.4 Hz, 4-Cl-Ph), 7.04 (dd, 2 H, J = 1.1 Hz, J = 8.4 Hz, 4-Cl-Ph), 7.10 (d, 1H, $J_{H\text{-}F}$ = 8.7 Hz, 3-F-Ph), 7.18 (d, 1H, $J_{H\text{-}F}$ = 8.6 Hz, 3-F-Ph), 7.23 (d, 1H, $J_{H\text{-}F}$ = 8.8 Hz, 3-F-Ph), 7.28 (d, 1H, $J_{H\text{-}F}$ = 8.2 Hz, 3-F-Ph), 7.70, 7.73 (s, 1H, H-6), 8.09, 8.11 (s, 1H, H-5 triazole), 9.70, 9.85 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.64 (5-CH_3), 37.79, 37.90 (C-2′), 43.93, 44.05 (N-CH_2), 59.46, 59.49 (C-3′), 65.21, 65.41 (d, $J_{C\text{-}P}$

= 4.7 Hz, C-5′), 82.78, 82.79 (d, $J_{C\text{-}P}$ = 7.8 Hz, C-4′), 86.62, 87.47 (C-1′), 111.31, 111.57 (C-5), 112.77 (d, $J_{C\text{-}F}$ = 23.1 Hz, 3-F-Ph), 115.35 (d, $J_{C\text{-}F}$ = 21.1 Hz, 3-F-Ph), 116.37 (CH_2 allyl), 121.04, 121.26 (C-5 triazole), 121.53, 121.59 (d, $J_{C\text{-}P}$ = 4.6 Hz, 4-Cl-Ph), 128.95, 129.70 (4-Cl-Ph), 129.96 (d, $J_{C\text{-}F}$ = 5.6 Hz, 3-F-Ph), 130.61, 130.66 (d, $J_{C\text{-}P}$ = 9.3 Hz, 4-Cl-Ph), 132.19 (d, $J_{C\text{-}F}$ = 8.4 Hz, 3-F-Ph), 132.32, 132.40 (d, $J_{C\text{-}F}$ = 2.2 Hz, 3-F-Ph), 134.85 (dd, $J_{C\text{-}P}$ = 2.7 Hz, J = 5.7 Hz, CH allyl), 136.38, 136.78 (C-6), 146.94, 147.03 (C-4 triazole), 149.03 (d, $J_{C\text{-}P}$ = 4.9 Hz, 4-Cl-Ph), 150.44 (C-2), 163.25 (d, $J_{C\text{-}F}$ = 245.6 Hz, 3-F-Ph) 164.01, 164.11 (C-4). ^{19}F NMR ($CDCl_3$) δ: -112.93 (m, 1F). ^{31}P NMR ($CDCl_3$) δ: 5.71, 5.83. MS-ESI *m/z*: 617, 619 $[M+H]^+$; 639, 641 $[M+Na]^+$; 655, 657 $[M+K]^+$; 615, 617 $[M-H]^-$; 651, 653, 655 $[M+Cl]^-$. Anal. Calcd for $C_{27}H_{27}ClFN_6O_6P$: C, 52.56; H, 4.41; N, 13.62. Found: C, 52.45; H, 4.35; N, 13.57.

3′-[4-(3-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-propargylphosphate] (37)

^{1}H NMR ($CDCl_3$) δ: 1.91 (s, 3 H, 5-CH_3), 2.25-2.27 (pseudo t, 1H, J = 2.5 Hz, CH propargyl), 2.73–2.80 (m, 1H, H-2”), 2.85-2.92 (m, 1H, H-2′), 3.10-3.17 (sex, 2 H, J = 7.1 Hz, N-CH_2), 3.75–3.82, 3.89-3.95 (m, 2 H, H-5′, H-5”), 4.38–4.45, 4.48-4.55 (m, 1H, H-4′), 4.62-4.68 (m, 1H, P-NH), 5.50-5.61 (m, 1H, H-3′), 6.24, 6.35 (dd, 1H, J = 5.4 Hz, J = 8.9 Hz, H-1′), 6.77 (d, 1H, $J_{H\text{-}F}$ = 9.0 Hz, 3-F-Ph), 7.03 (dd, 1H, $J_{H\text{-}F}$ = 2.6 Hz, J = 8.3 Hz, 3-F-Ph), 7.12 (dd, 2 H, J = 1.0 Hz, J = 8.9 Hz, 4-Cl-Ph), 7.18 (dd, 2 H, J = 1.0 Hz, J = 8.9 Hz, 4-Cl-Ph), 7.36-7.39 (m, 1H, 3-F-Ph), 7.52-7.56 (m, 1H, 3-F-Ph), 7.57, 7.59 (s, 1H, H-6), 8.05, 8.06 (s, 1H, H-5 triazole), 9.35, 9.52 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.66 (5-CH_3), 31.26 (N-CH_2), 37.74 (C-2′), 59.45, 59.61 (C-3′), 65.34, 65.56 (d, $J_{C\text{-}P}$ = 4.9 Hz, C-5′), 72.55 (C propargyl), 80.41 (t, $J_{C\text{-}P}$ = 5.1 Hz, CH propargyl), 82.76, 82.87 (d, $J_{C\text{-}P}$ = 6.8 Hz, C-4′), 87.00, 87.91 (C-1′), 111.43, 111.63 (C-5), 112.64 (d, $J_{C\text{-}F}$ = 23.2 Hz, 3-F-Ph), 115.27 (d, $J_{C\text{-}F}$ = 20.7 Hz, 3-F-Ph), 120.78, 120.94 (C-5 triazole), 121.40 (4-Cl-Ph), 129.36 (4-Cl-Ph), 129.83 (d, $J_{C\text{-}F}$ = 4.8 Hz, 3-F-Ph), 130.52, 130.76 (d, $J_{C\text{-}P}$ = 9.4 Hz, 4-Cl-Ph), 132.11 (d, $J_{C\text{-}F}$ = 8.5 Hz, 3-F-Ph), 132.24, 132.32 (d, $J_{C\text{-}F}$ = 2.1 Hz, 3-F-Ph), 136.63, 137.08 (C-6), 147.04, 147.11 (C-4 triazole), 148.85 (d, $J_{C\text{-}P}$ = 2.3 Hz, 4-Cl-Ph), 150.37 (C-2), 163.27 (d, $J_{C\text{-}F}$ = 245.8 Hz, 3-F-Ph) 163.86, 164.00 (C-4). ^{19}F NMR ($CDCl_3$) δ: -112.87 (m, 1F). ^{31}P NMR ($CDCl_3$) δ: 4.72, 4.73. MS-ESI *m/z*: 615, 617 $[M+H]^+$; 637, 639 $[M+Na]^+$; 653, 655 $[M+K]^+$; 613, 615 $[M-H]^-$; 649, 651, 653 $[M+Cl]^-$. Anal. Calcd for $C_{27}H_{25}ClFN_6O_6P$: C, 52.73; H, 4.10; N, 13.67. Found: C, 52.81; H, 4.23; N, 13.84.

3′-[4-(4-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-(4-chlorophenyl N-methylphosphate) (38)

^{1}H NMR ($CDCl_3$) δ: 1.91, (s, 3 H, 5-CH_3), 2.66–2.70 (m, 3 H, N-CH_3), 2.75–2.89 (m, 1H, H-2”), 2.95–3.19 (m, 1H, H-2′), 3.66, 3.93 (sex, 2 H, J = 7.6 Hz, H-5′, H-5”), 4.21 (dd, 1H, J = 3.9 Hz, J = 5.7 Hz, H-4′), 4.46, 4.67 (m, 1H, H-3′), 5.48-5.62 (m, 1H, P-NH), 6.29, 6.42 (pseudo t, 1H, J = 7.0 Hz, H-1′), 7.12 (dd, 2 H, J = 1.1 Hz, J = 9.1 Hz, 4-Cl-Ph), 7.17 (dd, 2 H, J = 1.1 Hz, J = 9.1 Hz, 4-Cl-Ph), 7.46 (dd, 2 H, $J_{H\text{-}F}$ = 1.3 Hz, J = 4.3 Hz, 4-F-Ph), 7.73-7.81 (m, 2 H, 4-F-Ph), 8.01, 8.05 (s, 1H, H-6), 8.22 (s, 1H, H-5 triazole), 10.2 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.61, 12.64 (5-CH_3), 27.73, 27.77 (N-CH_3), 37.88, 37.99 (C-2′), 59.30, 59.43 (C-3′), 65.12, 65.31 (d, $J_{C\text{-}P}$ = 4.7 Hz, C-5′), 82.75, 82.91 (d, $J_{C\text{-}P}$ = 6.5 Hz, C-4′), 86.60, 87.41 (C-1′), 111.23, 111.57 (C-5), 116.09 (d, $J_{C\text{-}F}$ = 21.9 Hz, 4-F-Ph), 120.24, 120.57 (C-5 triazole), 121.41, 121.52 (d, $J_{C\text{-}P}$ = 5.0 Hz, 4-Cl-Ph), 126.31, 126.33 (d, $J_{C\text{-}P}$ = 3.0 Hz, 4-Cl-Ph), 127.63 (d, $J_{C\text{-}F}$ = 8.2 Hz, 4-F-Ph), 130.02 (d, $J_{C\text{-}F}$ = 3.2 Hz, 4-F-Ph), 130.81 (d, $J_{C\text{-}P}$ = 7.7 Hz, 4-Cl-Ph), 136.43, 136.80 (C-6), 147.24, 147.36 (C-4 triazole), 149.01 (d, $J_{C\text{-}P}$ = 2.1 Hz, 4-Cl-Ph), 150.31, 150.47 (C-2), 162.94 (d, $J_{C\text{-}F}$ = 248.0 Hz, 4-F-Ph), 164.17, 164.25 (C-4). ^{19}F NMR ($CDCl_3$)δ: -113.32 (m, 1F). ^{31}P NMR ($CDCl_3$) δ: 7.14, 7.29. MS-ESI *m/z*: 591, 593 $[M+H]^+$; 613, 615 $[M+Na]^+$; 629, 631 $[M+K]^+$; 589, 591 $[M-H]^-$; 625, 627, 629 $[M+Cl]^-$. Anal. Calcd for $C_{25}H_{25}ClFN_6O_6P$: C, 50.81; H, 4.26; N, 14.22. Found: C, 50.74; H, 4.23; N, 14.25.

3′-[4-(4-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-(4-chlorophenyl N-ethylphosphate) (39)

^{1}H NMR ($CDCl_3$) δ: 1.11, 1.14 (td, 3 H, J = 1.1 Hz, J = 7.2 Hz, CH_3 Et), 1.91 (d, 3 H, J = 1.0 Hz, 5-CH_3), 2.67-2.74 (m, 2 H, H-2′, H-2”), 2.79-2.86 (m, 2 H, N-CH_2-C), 2.97-3.06 (m, 1H, H-5”), 3.43-3.76 (m, 1H, H-5′), 4.35–4.45 (m, 1H, H-4′), 4.62-4.68 (m, 1H, H-3′), 5.47–5.59 (m, 1H, P-NH), 6.29, 6.42 (dd, 1H, J = 5.2 Hz, J = 7.1 Hz, H-1′), 7.11 (dd, 2 H, J = 1.1 Hz, J = 9.0 Hz, 4-Cl-Ph), 7.18 (dd, 2 H, J = 1.1 Hz, J = 9.0 Hz, 4-Cl-Ph), 7.43 (dd, 2 H, $J_{H\text{-}F}$ = 1.3 Hz, J = 2.3 Hz, 4-F-Ph), 7.74-7.81 (m, 2 H, 4-F-Ph), 8.01, 8.02 (s, 1H, H-6), 8.19 (s, 1H, H-5 triazole), 9.85 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.64, 12.67 (5-CH_3), 17.20, 17.26 (d, $J_{C\text{-}P}$ = 3.4 Hz, CH_3 Et), 36.70, 36.72 (C-2′), 37.90 (d, $J_{C\text{-}P}$ = 10.7 Hz, N-CH_2), 59.30, 59.35 (C-3′), 65.01, 65.25 (d, $J_{C\text{-}P}$ = 5.1 Hz, C-5′), 82.77, 82.88 (d, $J_{C\text{-}P}$ = 6.4 Hz, C-4′), 86.42, 87.27 (C-1′), 111.26, 111.55 (C-5), 115.88 (d, $J_{C\text{-}F}$ = 21.8 Hz, 4-F-Ph), 120.29, 120.55 (C-5 triazole), 121.41, 121.53 (d, $J_{C\text{-}P}$ = 5.0 Hz, 4-Cl-Ph), 126.35, 126.38 (d, $J_{C\text{-}P}$ = 2.3 Hz, 4-Cl-Ph), 127.57,127.65 (d, $J_{C\text{-}F}$ = 8.2 Hz, 4-F-Ph), 129.97 (d, $J_{C\text{-}F}$ = 5.2 Hz, 4-F-Ph),

130.60, 130.73 (d, $J_{C\text{-}P}$ = 5.2 Hz, 4-Cl-Ph), 136.27, 136.64 (C-6), 147.04, 147.16 (C-4 triazole), 149.07, 149.13 (d, $J_{C\text{-}P}$ = 4.0 Hz, 4-Cl-Ph), 150.23, 150.28 (C-2), 161.53, 161.67 (C-4), 162.86 (d, $J_{C\text{-}F}$ = 240.2 Hz, 4-F-Ph). ^{19}F NMR ($CDCl_3$)δ: -113.47 (m, 1F). ^{31}P NMR ($CDCl_3$) δ: 5.90, 6.10. MS-ESI *m/z*: 605, 607 $[M+H]^+$; 627, 629 $[M+Na]^+$; 643, 645 $[M+K]^+$; 603, 605 $[M-H]^-$; 639, 641, 643 $[M+Cl]^-$. Anal. Calcd for $C_{26}H_{27}ClFN_6O_6P$: C, 51.62; H, 4.50; N, 13.89. Found: C, 51.27; H, 4.46; N, 13.91.

3′-[4-(4-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-(2,2,2-trifluoroethyl)phosphate] (40)

^{1}H NMR ($CDCl_3$) δ: 1.90, 1.91 (s, 3 H, 5-CH_3), 2.84–2.94 (m, 1H, H-2”), 3.05–3.14 (m, 1H, H-2′), 3.54–3.66 (m, 2 H, H-5′, H-5”), 4.19-4.42 (m, 2 H, N-CH_2), 4.46–4.53 (m, 1H, H-4′), 4.60-4.66 (m, 1H, H-3′), 5.43–5.54 (m, 1H, P-NH), 6.20, 6.26 (dd, 1H, J = 5.6 Hz, J = 7.3 Hz, H-1′), 7.09 (dd, 2 H, J = 1.2 Hz, J = 8.8 Hz, 4-Cl-Ph), 7.24 (dd, 2 H, J = 1.2 Hz, J = 8.8 Hz, 4-Cl-Ph), 7.42 (dd, 2 H, $J_{H\text{-}F}$ = 1.1 Hz, J = 5.4 Hz, 4-F-Ph), 7.73-7.80 (m, 2 H, 4-F-Ph), 7.90, 7.93 (s, 1H, H-6), 8.20 (s, 1H, H-5 triazole), 9.72 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.46, 12.52 (5-CH_3), 37.19, 37.38 (C-2′), 43.50, 43.84 (N-CH_2), 59.49, 59.77 (C-3′), 65.59, 65.78 (d, $J_{C\text{-}P}$ = 5.1 Hz, C-5′), 82.60, 82.82 (d, $J_{C\text{-}P}$ = 7.2 Hz, C-4′), 87.79, 88.74 (C-1′), 111.58, 111.70 (C-5), 115.85 (d, $J_{C\text{-}F}$ = 21.9 Hz, 4-F-Ph), 120.08, 120.31 (C-5 triazole), 121.45, 121.56 (d, $J_{C\text{-}P}$ = 4.8 Hz, 4-Cl-Ph), 121.84 (d, $J_{C\text{-}P}$ = 4.8 Hz, 4-Cl-Ph), 127.63 (d, $J_{C\text{-}F}$ = 8.2 Hz, 4-F-Ph), 129.53 (d, $J_{C\text{-}F}$ = 3.1 Hz, 4-F-Ph), 130.05 (m, CF_3), 131.04, 131.14 (d, $J_{C\text{-}P}$ = 4.2 Hz, 4-Cl-Ph), 136.20, 136.66 (C-6), 147.14, 147.40 (C-4 triazole), 148.71, 148.78 (d, $J_{C\text{-}P}$ = 2.1 Hz, 4-Cl-Ph), 150.35, 150.39 (C-2), 162.97 (d, $J_{C\text{-}F}$ = 247.4 Hz, 4-F-Ph), 163.93, 164.02 (C-4). ^{19}F NMR ($CDCl_3$) δ: -113.26 (m, 1F), -74.24, -74.15 (t, 3 F, $J_{H\text{-}F}$ = 8.9 Hz). ^{31}P NMR ($CDCl_3$) δ: 4.24, 4.37. MS-ESI *m/z*: 659, 661 $[M+H]^+$; 681, 683 $[M+Na]^+$; 697, 699 $[M+K]^+$; 657, 659 $[M-H]^-$; 693, 695, 697 $[M+Cl]^-$. Anal. Calcd for $C_{26}H_{24}ClF_4N_6O_6P$: C, 47.39; H, 3.67; N, 12.75. Found: C, 47.21; H, 3.46; N, 12.29.

3′-[4-(4-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-n-propylphosphate] (41)

^{1}H NMR ($CDCl_3$) δ: 0.85, 0.88 (t, 3 H, J = 7.4 Hz, CH_3 *n*-Pr), 1.25, 1.46 (sex, 2 H, J = 4.1 Hz, CH_2 *n*-Pr), 1.90, 1.91 (s, 3 H, 5-CH_3), 2.85–3.03 (m, 2 H, H-2′, H-2”), 3.13 (sex, 2 H, J = 6.6 Hz, N-CH_2 *n*-Pr), 3.64–3.85 (m, 2 H, H-5′, H-5”), 4.33–4.53 (m, 1H, H-4′), 5.47–5.60 (m, 2 H, P-NH, H-3′), 6.28, 6.41 (dd, 1H, J = 5.8 Hz, J = 7.0 Hz, H-1′), 7.12 (dd, 2 H, J = 1.3 Hz, J = 9.1 Hz, 4-Cl-Ph), 7.18 (dd, 2 H, J = 1.3 Hz, J = 9.1 Hz, 4-Cl-Ph), 7.42 (d,

2 H, $J_{H\text{-}F}$ = 1.3 Hz, J = 5.5 Hz, 4-F-Ph), 7.74-7.81 (m, 2 H, 4-F-Ph), 8.01,8.02 (s, 1H, H-6), 8.20 (s, 1H, H-5 triazole), 9.70 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 11.04, 11.06 (CH_3 *n*-Pr), 12.34, 12.36 (5-CH_3), 25.17, 25.20 (CH_2 *n*-Pr), 36.06 (C-2′), 40.44 (d, $J_{C\text{-}P}$ = 2.0 Hz, N-CH_2 *n*-Pr), 60.37 (C-3′), 66.85 (C-5′), 74.33, 74.63 (C-4′), 95.54 (C-1′), 99.80 (C-5), 116.03 (d, $J_{C\text{-}F}$ = 20.4 Hz, 4-F-Ph), 120.21, 120.48 (d, $J_{C\text{-}P}$ = 4.1 Hz, 4-Cl-Ph), 121.16 (d, $J_{C\text{-}P}$ = 4.1 Hz, 4-Cl-Ph), 124.36, 124.59 (C-5 triazole), 127.97 (d, $J_{C\text{-}F}$ = 8.3 Hz, 4-F-Ph), 128.03 (d, $J_{C\text{-}P}$ = 2.7 Hz, 4-Cl-Ph), 128.45, 128.81 (C-6), 130.02 (d, $J_{C\text{-}F}$ = 2.9 Hz, 4-F-Ph), 131.04, 131.19 (C-4 triazole), 136.83 (d, $J_{C\text{-}P}$ = 3.6 Hz, 4-Cl-Ph), 142.70 (C-2), 162.66 (d, $J_{C\text{-}F}$ = 246.4 Hz, 4-F-Ph), 163.67, 163.78 (C-4). ^{19}F NMR ($CDCl_3$) δ: -113.43 (m, 1F). ^{31}P NMR ($CDCl_3$) δ: 6.05, 6.25. 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 $C_{27}H_{29}ClFN_6O_6P$: C, 52.39; H, 4.72; N, 13.58. Found: C, 52.14; H, 4.97; N, 13.54.

3′-[4-(4-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-allylphosphate] (42)

^{1}H NMR ($CDCl_3$) δ: 1.90, 1.91 (s, 3 H, 5-CH_3), 2.69–2.75 (m, 1H, H-2”), 2.79–2.86 (m, 1H, H-2′), 3.12 (sex, 2 H, J = 7.0 Hz, N-CH_2-C = C), 3.55–3.65 (m, 2 H, H-5′, H-5”), 4.34–4.52 (m, 1H, H-4′), 4.60-4.67 (m, 1H, 3′), 5.07–5.12, 5.14-5.23 (m, 2 H, N-C-C = CH_2), 5.46–5.58 (m, 1H, P-NH), 5.78 (m, 1H, N-C-CH = C), 6.26, 6.38 (dd, 1H, J = 5.3 Hz, J = 7.1 Hz, H-1′), 7.11 (dd, 2 H, J = 1.2 Hz, J = 9.0 Hz, 4-Cl-Ph), 7.18 (dd, 2 H, J = 1.2 Hz, J = 9.0 Hz, 4-Cl-Ph), 7.40 (dd, 2 H, $J_{H\text{-}F}$ = 1.3 Hz, J = 5.2 Hz, 4-F-Ph), 7.75-7.80 (m, 2 H, 4-F-Ph), 7.99, 8.01 (s, 1H, H-6), 8.18 (s, 1H, H-5 triazole), 9.51, 9.68 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.65 (5-CH_3), 37.79, 37.90 (C-2′), 44.03, 44.07 (N-CH_2), 59.33, 59.39 (C-3′), 65.15, 65.39 (d, $J_{C\text{-}P}$ = 5.2 Hz, C-5′), 82.76, 82.88 (d, $J_{C\text{-}P}$ = 6.4 Hz, C-4′), 86.58, 87.48 (C-1′), 111.30, 111.58 (C-5), 116.04 (d, $J_{C\text{-}F}$ = 21.9 Hz, 4-F-Ph), 120.25, 120.49 (C-5 triazole), 121.51 (d, $J_{C\text{-}P}$ = 4.9 Hz, 4-Cl-Ph), 121.62 (d, $J_{C\text{-}P}$ = 4.7 Hz, CH_2 allyl), 126.44 (pseudo t, $J_{C\text{-}P}$ = 3.0 Hz, CH allyl), 127.59, 127.67 (d, $J_{C\text{-}P}$ = 2.1 Hz, 4-Cl-Ph), 129.96 (d, $J_{C\text{-}F}$ = 5.6 Hz, 4-F-Ph), 130.73 (d, $J_{C\text{-}F}$ = 12.1 Hz, 4-F-Ph), 135.00, 135.06 (d, $J_{C\text{-}P}$ = 2.3 Hz, 4-Cl-Ph), 136.32, 136.71 (C-6), 147.21, 147.31 (C-4 triazole), 149.03, 149.10 (d, $J_{C\text{-}P}$ = 2.6 Hz, 4-Cl-Ph), 150.24, 150.41 (C-2), 162.91 (d, $J_{C\text{-}F}$ = 247.8 Hz, 4-F-Ph) 163.79, 163.97 (C-4). ^{19}F NMR ($CDCl_3$) δ: -113.52 (m, 1F). ^{31}P NMR ($CDCl_3$) δ: 5.64, 5.76. MS-ESI *m/z*: 617, 619 $[M+H]^+$; 639, 641 $[M+Na]^+$; 655, 657 $[M+K]^+$; 615, 617 $[M-H]^-$; 651, 653, 655 $[M+Cl]^-$. Anal. Calcd for $C_{27}H_{27}ClFN_6O_6P$: C, 52.56; H, 4.41; N, 13.62. Found: C, 52.94; H, 4.87; N, 13.69.

3′-[4-(4-Fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-propargylphosphate] (43)

^{1}H NMR ($CDCl_3$) δ: 1.88, 1.89 (s, 3 H, 5-CH_3), 2.25-2.29 (m, 1H, CH propargyl), 2.72–2.80 (m, 1H, H-2”), 2.83-2.90 (m, 1H, H-2′), 3.08-3.15 (m, 2 H, N-CH_2), 3.74–3.85 (m, 2 H, H-5′, H-5”), 4.38–4.45, 4.48-4.55 (m, 1H, H-4′), 4.61-4.66 (m, 1H, P-NH), 5.48-5.58 (m, 1H, H-3′), 6.28, 6.36 (dd, 1H, J = 5.8 Hz, J = 7.0 Hz, H-1′), 7.11 (dd, 2 H, J = 1.8 Hz, J = 8.5 Hz, 4-Cl-Ph), 7.16 (dd, 2 H, J = 1.8 Hz, J = 8.5 Hz, 4-Cl-Ph), 7.40 (dd, 2 H, $J_{H\text{-}F}$ = 1.3 Hz, J = 15.9 Hz, 4-F-Ph), 7.74-7.79 (m, 2 H, 4-F-Ph), 7.97, 7.98 (s, 1H, H-6), 8.20 (s, 1H, H-5 triazole), 9.82 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.51 (5-CH_3), 30.35, 31.12 (N-CH_2), 37.69, 37.75 (C-2′), 59.30, 59.55 (C-3′), 65.25, 65.50 (d, $J_{C\text{-}P}$ = 4.8 Hz, C-5′), 72.53 (C propargyl), 80.43 (d, $J_{C\text{-}P}$ = 5.5 Hz, CH propargyl), 82.61, 82.73 (d, $J_{C\text{-}P}$ = 7.1 Hz, C-4′), 86.83 (C-1′), 111.34, 111.48 (C-5), 115.98 (d, $J_{C\text{-}F}$ = 21.8 Hz, 4-F-Ph), 120.04, 120.410 (C-5 triazole), 121.47, 121.89 (d, $J_{C\text{-}P}$ = 4.9 Hz, 4-Cl-Ph), 126.15 (d, $J_{C\text{-}P}$ = 3.2 Hz, 4-Cl-Ph), 127.52 (d, $J_{C\text{-}F}$ = 8.2 Hz, 4-F-Ph), 129.37, 129.88 (d, $J_{C\text{-}F}$ = 4.1 Hz, 4-F-Ph), 130.78, 130.88 (d, $J_{C\text{-}P}$ = 1.3 Hz, 4-Cl-Ph), 136.63, 137.04 (C-6), 146.91, 147.25 (C-4 triazole), 148.69, 148.75 (d, $J_{C\text{-}P}$ = 3.3 Hz, 4-Cl-Ph), 150.41, 150.45 (C-2), 162.84 (d, $J_{C\text{-}F}$ = 247.8 Hz, 4-F-Ph) 167.86 (C-4). ^{19}F NMR ($CDCl_3$) δ: -113.28 (m, 1F). ^{31}P NMR ($CDCl_3$) δ: 4.80, 4.86. MS-ESI *m/z*: 615, 617 $[M+H]^+$; 637, 639 $[M+Na]^+$; 653, 655 $[M+K]^+$; 613, 615 $[M-H]^-$; 649, 651, 653 $[M+Cl]^-$. Anal. Calcd for $C_{27}H_{25}ClFN_6O_6P$: C, 52.73; H, 4.10; N, 13.67. Found: C, 52.43; H, 4.17; N, 13.96.

3′-[4-(4-Fluoro-3-methylphenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-(4-chlorophenyl N-methylphosphate) (44)

^{1}H NMR ($CDCl_3$) δ: 1.86 (d, 3 H, J = 1.2 Hz, 5-CH_3), 2.31 (d, 3 H, J = 1.8 Hz, CH_3 4-F-3-Me-Ph), 2.59-2.67 (m, 1H, H-2′′), 2.68 (d, 3 H, J = 12.4 Hz, N-CH_3), 2.68-2.72 (m, 1H, H-2′), 3.07-3.20 (m, 1H, H-5′′), 3.38-5.42 (m, 1H, H-5′), 4.33-4.53 (m, 1H, H-4′), 4.60-4.70 (m, 1H, P-NH), 5.42-5.61 (m, 1H, H-3′), 6.40 (pseudo t, 1H, J = 7.0 Hz, H-1′), 7.11 (dd, 2 H, J = 1.2 Hz, J = 9.1 Hz, 4-Cl-Ph), 7.16 (dd, 2 H, J = 1.2 Hz, J = 9.1 Hz, 4-Cl-Ph), 7.44 (d, 1H, J = 1.2 Hz, 4-F-3-Me-Ph), 7.47 (d, 2 H, J = 1.2 Hz, 4-F-Me-Ph), 7.80 (s, 1H, 4-F-3-Me-Ph), 7.95 (s, 1H, H-6), 8.02 (s, 1H, H-5 triazole), 9.41 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.65 (5-CH_3), 14.72 (d, $J_{C\text{-}F}$ = 3.4 Hz, CH_3 4-F-Me-Ph), 27.45 (N-CH_3), 37.88, 38.09 (C-2′), 59.02, 59.24 (C-3′), 64.94, 65.12 (2 x d, $J_{C\text{-}P}$ = 4.8 Hz, C-5′), 82.75, 82.92 (2 x d, $J_{C\text{-}P}$ = 6.3 Hz, C-4′), 86.42, 87.20 (C-1′), 111.13, 111.52 (C-5), 115.63 (d, $J_{C\text{-}F}$ = 22.8 Hz, 4-F-3-Me-Ph), 120.00, 120. 46 (C-5 triazole), 121.44, 121.57 (2 x d, $J_{C\text{-}P}$ = 5.0 Hz, 4-Cl-Ph), 124. 92 (d, $J_{C\text{-}F}$ = 8.2 Hz 4-F-3-Me-Ph), 125.51, 125.75 (2 x d, $J_{C\text{-}P}$ = 2.0 Hz, 4-Cl-Ph), 129.00, 129.07 (2 x d,

$J_{C\text{-}P}$ = 1.5 Hz, 4-Cl-Ph), 129.71 (4-F-3-Me-Ph), 130.17 (d, $J_{C\text{-}P}$ = 4.7 Hz, 4-Cl-Ph), 136.18 (4-F-3-Me-Ph), 136.52 (C-6), 147.48(C-4 triazole), 147.56 (4-Cl-Ph), 149.06 (C-2), 161.52 (d, $J_{C\text{-}F}$ = 247.9 Hz, 4-F-3-Me-Ph), 163.89 (C-4). ^{19}F NMR ($CDCl_3$) δ: -117.83 (m, 1F).[31] P NMR ($CDCl_3$) δ: 7.12, 7.18. MS-ESI *m/z*: 605, 607 $[M+H]^+$; 627, 629 $[M+Na]^+$; 643, 645 $[M+K]^+$; 603, 605 $[M-H]^-$; 639, 641, 643 $[M+Cl]^-$. Anal. Calcd for $C_{26}H_{27}ClFN_6O_6P$: C, 51.62; H, 4.50; N, 13.89. Found: C, 51.33; H, 4.70; N, 13.58.

3′-[4-(4-Fluoro-3-methylphenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-(4-chlorophenyl N-ethylphosphate) (45)

^{1}H NMR ($CDCl_3$) δ: 1.10 (2 x t, 3 H, J = 8.2 Hz, CH_3 Et), 1.92 (d, 3 H, J = 0.7 Hz, 5-CH_3), 2.31 (s, 1H, 4-F-3-Me-Ph), 2.65-2.74 (m, 2 H, H-2′, H-2″), 2.76-2.85 (m, 2 H, N-CH_2), 2.98-3.16 (m, 1H, H-5″), 3.54-3.64 (m, 1H, H-5′), 4.32-4.53 (m, 1H, 4′), 5.44-5.59 (m, 2 H, H-3′, P-NH), 6.26, 6.40 (2 x pseudo t, 1 H, J = 6.9 Hz, H-1′), 7.10 (dd, 2 H, J = 1.0 Hz, J = 9.0 Hz, 4-Cl-Ph), 7.18 (dd, 2 H, J = 1.0 Hz, J = 9.0 Hz, 4-Cl-Ph), 7.43 (d, 1 H, J = 1.2 Hz, 4-F-3-Me-Ph), 7.63 (d, 1 H, J = 1.9 Hz, 4-F-3-Me-Ph), 7.67 (d, 1 H, J = 1.8 Hz, 4-F-3-Me-Ph), 7.94, 7.98 (s, 1 H, H-6), 8.19 (s, 1 H, H-5 triazole), 9.26, 9.48 (br s, 1 H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.64, 12.67 (5-CH_3), 14.73 (d, $J_{C\text{-}F}$ = 3.5 Hz, CH_3 4-F-3-Me-Ph), 17.23, 17.31 (2 x d, $J_{C\text{-}P}$ = 3.1 Hz, CH_3 Et), 36.75 (N-CH_2), 37.87, 38.01 (C-2′), 59.24 (d, $J_{C\text{-}P}$ = 5.5 Hz, C-5′), 64.98, 65.22 (2 x d, $J_{C\text{-}P}$ = 5.15 Hz, C-3′), 82.79, 82.90 (2 x d, $J_{C\text{-}P}$ = 6.5 Hz, C-4′), 86.45, 87.25 (C-1′), 111.26, 111.57 (C-5), 115.49, 115.80 (4-F-3-Me-Ph), 120.05, 120.37 (4-F-3-Me-Ph), 121.43, 121.56 (2 x d, $J_{C\text{-}F}$ = 5.0 Hz, 4-F-3-Me-Ph), 124.87, 124.98 (4-Cl-Ph), 125.53, 125.76 (2 x d, $J_{C\text{-}P}$ = 1.7 Hz, 4-Cl-Ph), 129.01, 129.08 (2 x d, $J_{C\text{-}F}$ = 1.8 Hz, 4-F-3-Me-Ph), 129.96, 130.01 (C-4 triazole), 130.63, 130.76 (2 x d, $J_{C\text{-}F}$ = 1.5 Hz, 4-F-Me-Ph), 136.24, 136.59 (C-6), 147.44, 147.56 (4-Cl-Ph), 149.09, 149.18 (2 x d, $J_{C\text{-}P}$ = 2.0 Hz, 4-Cl-Ph), 150.29 (C-5 triazole), 161.53 (d, $J_{C\text{-}F}$ = 247.3 Hz, 4-F-3-Me-Ph), 163.61 (C-2), 163.79 (C-4). ^{19}F NMR ($CDCl_3$) δ: -117.83 (m, 1F). ^{31}P NMR ($CDCl_3$) δ: 5.83, 5.97. MS-ESI *m/z*: 619, 621 $[M+H]^+$; 641, 643 $[M+Na]^+$; 657, 659 $[M+K]^+$; 617, 619 $[M-H]^-$; 653, 657, 659 $[M+Cl]^-$. Anal. Calcd for $C_{27}H_{29}ClFN_6O_6P$: C, 52.39; H, 4.72; N, 13.58. Found: C, 52.20; H, 4.99; N, 13.33.

3′-[4-(4-Fluoro-3-methylphenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-[4-chlorophenyl N-(2,2,2-trifluoroethyl)phosphate] (46)

^{1}H NMR ($CDCl_3$) δ: 1.90 (s, 3 H, 5-CH_3), 2.30 (d, 3 H, J = 1.8 Hz, CH_3 4-F-3-Me-Ph), 2.72–2.99 (m, 1 H, H-2”), 3.30–3.42 (m, 1 H, H-2′), 3.54–3.66

(m, 4 H, N-CH_2, H-5′, H-5”), 4.46–4.51 (m, 1 H, H-4′), 5.55–5.80 (m, 2 H, P-NH, H-3′), 6.20, 6.25 (dd, 1 H, $J = 3.0$ Hz, $J = 6.6$ Hz, H-1′), 7.12 (dd, 2 H, $J = 1.1$ Hz, $J = 9.1$ Hz, 4-Cl-Ph), 7.21 (dd, 2 H, $J = 1.1$ Hz, $J = 9.1$ Hz, 4-Cl-Ph), 7.29–7.35 (m, 1 H, 4-F-3-Me-Ph), 7.40–7.58 (m, 2 H, 4-F-3-Me-Ph), 7.88 (s, 1 H, H-6), 8.29 (s, 1 H, H-5 triazole), 9.29, 9.40 (br s, 1 H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.12, 12.18 (5-CH_3), 37.17, 37.22 (C-2′), 43.80, 43.99 (N-CH_2), 59.30, 59.46 (C-3′), 65.49, 65.65 (d, $J_{C\text{-}P} = 4.6$ Hz, C-5′), 82.20, 82.49 (2 x d, $J_{C\text{-}P} = 4.1$ Hz, C-4′), 87.70, 88.53 (C-1′), 111.20, 111.33 (C-5),115.50, 115.50 (4-F-3-Me-Ph), 121.46, 121.58 (C-5 triazole), 125.90 (4-F-3-Me-Ph), 128.77, 128.91 (4-Cl-Ph), 129.15 (4-F-3-Me-Ph), 129.54, 129.89 (4-Cl-Ph), 129.60 (4-F-3-Me-Ph), 130.10 (m, CF_3), 130.20 (4-F-3-Me-Ph), 135.70 (C-6), 137.20, 137.38 (4-Cl-Ph), 148.09, 148.13 (4-Cl-Ph), 148.31, 148.52 (C-4 triazole), 150.20, 150.45 (C-2), 160.50 (d, $J_{C\text{-}F} = 244.5$ Hz, 4-F-3-Me-Ph), 163.66, 163.76 (C-4). ^{19}F NMR ($CDCl_3$) δ: -74.30, -74.58 (t, 3 F, $J_{H\text{-}F} = 8.2$ Hz). ^{31}P NMR ($CDCl_3$) δ: 4.80, 4.99. MS-ESI *m/z*: 673, 675 $[M + H]^+$; 695, 697 $[M + Na]^+$; 711, 713 $[M + K]^+$; 671, 673 $[M - H]^-$; 707, 709, 711 $[M + Cl]^-$. Anal. Calcd for $C_{27}H_{26}ClF_4N_6O_6P$: C, 48.19; H, 3.89; N, 12.49. Found: C, 48.33; H, 3.75; N, 12.31.

3′-[4-(4-Fluoro-3-methylphenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-(4-chlorophenyl N-n-propylphosphate) (47)

^{1}H NMR ($CDCl_3$) δ: 0.86, 0.88 (2 x t, 3 H, $J = 7.4$ Hz, CH_3 *n*-Pr), 1.48 (sex, 2 H, $J = 8.3$ Hz, CH_2 *n*-Pr), 1.92, 1.93 (2 x d, 3 H, $J = 1.2$ Hz, 5-CH_3), 2.31 (s, 3 H, CH_3 4-F-3-Me-Ph), 2.62-2.87 (m, 1H, H-2′′), 2.98-3.00 (m, 1H, H-2′), 3.12 (sex, 2 H, $J = 7.1$ Hz, N-CH_2 *n*-Pr), 3.33-3.42 (m, 1H, H-5”), 3.64-3.74 (m, 1H, H-5′), 4.32-4.52 (m, 1H, H-4′), 4.61-4.68 (m, 1H, H-3′), 5.45-5.59 (m, 1H, P-NH), 6.27, 6.41 (dd, 1H, $J = 5.4$ Hz, $J = 7.1$ Hz, H-1′), 7.11 (dd, 2 H, $J = 1.2$ Hz, $J = 9.1$ Hz, 4-Cl-Ph), 7.18 (dd, 2 H, $J = 1.2$ Hz, $J = 9.1$ Hz, 4-Cl-Ph), 7.25 (s, 1H, H-6), 7.42 (t, 1H, $J_{H\text{-}F} = 1.4$ Hz, 4-F-3-Me-Ph), 7.52 -7.60 (m, 1H, 4-F-3-Me-Ph), 7.98 (d, $J_{H\text{-}F} = 10.1$ Hz, 4-F-3-Me-Ph), 8.17 (s, 1H, H-5 triazole), 9.5 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 11.20 (d, $J_{C\text{-}P} = 3.5$ Hz, CH_3 *n*-Pr), 12.63, 12.67 (5-CH_3), 14.71 (d, $J_{C\text{-}F} = 3.5$ Hz, CH_3 4-F-Me-Ph), 24.82, 24.91 (CH_2 *n*-Pr), 37.84, 37.99 (C-2′), 43.58 (d, $J_{C\text{-}P} = 2.6$ Hz, N-CH_2), 59.30 (C-3′), 64.97, 65.26 (2 x d, $J_{C\text{-}P} = 5.3$ Hz, C-5′), 82.85, 82.89 (2 x d, $J_{C\text{-}P} = 6.7$ Hz, C-4′), 86.40, 87.26 (C-1′), 111.26, 111.56 (C-5), 115.62 (d, $J_{C\text{-}F} = 22.8$ Hz, 4-F-Me-Ph), 121.41, 121.54 (2 x d, $J_{C\text{-}P} = 5.1$ Hz, 4-Cl-Ph), 124.91 (d, $J_{C\text{-}F} = 8.2$ Hz, 4-F-Me-Ph), 125.62 (d, $J_{C\text{-}P} = 17.7$ Hz, 4-Cl-Ph), 126.01, 126.07 (2 x d, $J_{C\text{-}P} = 3.0$ Hz, 4-Cl-Ph), 129.00 (4-F-Me-Ph), 126.99 (4-F-Me-Ph), 129.95 (d, $J_{C\text{-}F} = 4.2$ Hz, 4-F-Me-Ph), 130.64 (d, $J_{C\text{-}F} = 8.8$ Hz, C-5 triazole), 136.19, 136.58 (C-6), 147.47 (d, $J_{C\text{-}F} =$ C-4 triazole), 149.16 (d, $J_{C\text{-}P} = 2.2$ Hz, 4-Cl-Ph), 150.34

(d, $J_{C\text{-}P}$ = 3.2 Hz, C-2), 161.51 (d, $J_{C\text{-}F}$ = 246.5 Hz, 4-F-Me-Ph), 163.93 (d, $J_{C\text{-}P}$ = 12.0 Hz, C-4). ^{19}F NMR ($CDCl_3$) δ: -117.84 (m, 1F). ^{31}P NMR ($CDCl_3$) δ: 6.01, 6.18. 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 $C_{28}H_{31}ClFN_6O_6P$: C, 53.13; H, 4.94; N, 13.28. Found: C, 53.06; H, 4.99; N, 13.37.

3′-[4-(4-Fluoro-3-methylphenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-(4-chlorophenyl N-allylphosphate) (48)

^{1}H NMR (DMSO-d_6) δ: 1.78, 1.83 (s, 3 H, 5-CH_3), 2.30 (s, 3 H, CH_3 4-F-Me-Ph), 2.67-2.76 (m, 1H, H-2″), 2.77-2.88 (m, 1H, H-2′), 3.44-3.51 (m, 2 H, N-CH_2-C = C), 3.66-3.76 (m, 1H, P-NH), 4.26-4.33 (m, 2 H, H-5′, H-5″), 4.54 (m, 1H, H-4′), 5. 31 (d, 1H, *J* = 1.2 Hz, N-C-C = CH_2), 5.37 (d, 2 H, *J* = 1.4 Hz, N-C-CH = C), 5.42 (m, 1H, H-3′), 6.45, 6.48 (pseudo t, 1H, *J* = 6.7 Hz, H-1′), 7.23 (dd, 2 H, *J* = 3.5 Hz, *J* = 9.4 Hz, 4-Cl-Ph), 7.26 (dd, 2 H, *J* = 3.5 Hz, *J* = 9.4 Hz, 4-Cl-Ph), 7.75 (d, 1H, *J* = 1.7 Hz, 4-F-3-Me-Ph), 7.79 (d, 1H, *J* = 1.8 Hz, 4-F-3-Me-Ph), 7.84 (d, *J* = 0.8 Hz, 4-F-3-Me-Ph), 7.61 (s, 1H, H-5 triazole), 8.78 (s, 1H, H-6), 10.60 (br s, 1H, 3-NH). ^{13}C NMR (DMSO-d_6) δ: 12.15, 12.35 (5-CH_3), 14.32 (d, $J_{C\text{-}F}$ = 3.1 Hz, CH_3 4-F-Me-Ph), 36.65 (C-2′), 37.15 (N-CH_2), 59.40 (d, $J_{C\text{-}P}$ = 13.3 Hz, C-5′), 66.30 (d, $J_{C\text{-}P}$ = 5.6 Hz, C-3′), 81.94 (d, $J_{C\text{-}P}$ = 8. 1H z, C-4′), 84.51 (C-1′), 109.73, 110.11 (C-5), 115.49, 115.71 (2 x d, $J_{C\text{-}F}$ = 3.4 Hz, 4-F-3-Me-Ph), 120.99 (d, $J_{C\text{-}P}$ = 10.4 Hz, CH_2 allyl), 124.59 (d, $J_{C\text{-}F}$ = 6.1 Hz, 4-F-3-Me-Ph), 124.82 (d, $J_{C\text{-}P}$ = 3.2 Hz, 4-Cl-Ph), 125.00 (d, $J_{C\text{-}P}$ = 3.2 Hz, 4-Cl-Ph), 127.27 (d, $J_{C\text{-}F}$ = 8.6 Hz, 4-F-3-Me-Ph), 127.94 (d, $J_{C\text{-}P}$ = 1.1 Hz, 4-Cl-Ph), 129.53 (C-5 triazole), 130.88 (4-F-3-Me-Ph), 136.16 (CH allyl), 136.34 (C-6), 149.85 (C-4 triazole), 149.92 (4-Cl-Ph), 150.49 (d, $J_{C\text{-}P}$ = 3.4 Hz, C-2), 160.43 (dd, $J_{C\text{-}F}$ = 3.1 Hz, $J_{C\text{-}F}$ = 243.8 Hz, 4-F-3-Me-Ph), 163.79 (d, $J_{C\text{-}P}$ = C-4). ^{19}F NMR (DMSO-d_6) δ: -117.79 (m, 1F). ^{31}P NMR (DMSO-d_6) δ: 5.84, 6.29. MS-ESI *m/z*: 631, 633 $[M+H]^+$; 653, 655 $[M+Na]^+$; 669, 671 $[M+K]^+$; 629, 631 $[M-H]^-$; 665, 667, 669 $[M+Cl]^-$. Anal. Calcd for $C_{28}H_{29}ClFN_6O_6P$: C, 53.30; H, 4.63; N, 13.32. Found: C, 53.12; H, 4.74; N, 13.01.

3′-[4-(4-Fluoro-3-methylphenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-O-(4-chlorophenyl N-propargylphosphate) (49)

^{1}H NMR ($CDCl_3$) δ: 1.86 (d, 3 H, *J* = 1.2 Hz, 5-CH_3), 1.92 (t, 1H, *J* = 1.2 Hz, P-NH), 2.24-2.28 (m, 1H, CH propargyl), 2.31 (d, 3 H, *J* = 1.5 Hz, CH_3 4-F-3-Me-Ph), 2.62-2.79 (m, 1H, H-2″), 2.82-2.89 (m, 1H, H-2′), 3.08-3.16 (m, 2 H, N-CH_2 propargyl), 3.75-3.86 (m, 1H, H-5″), 3.98-

4.11 (m, 1H, H-5′), 4.37-4.44 (m, 1H, H-4′), 5.47-5.58 (m, 1H, H-3′), 6.25, 6.31 (2 x pseudo t, 1H, $J = 7.1$ Hz, H-1′), 7.13 (dd, 2 H, $J = 1.2$ Hz, $J = 9.0$ Hz, 4-Cl-Ph), 7.18 (dd, 2 H, $J = 1.2$ Hz, $J = 9.0$ Hz, 4-Cl-Ph), 7.38, 7.41 (2 x d, 1H, $J = 1.2$ Hz, 4-F-3-Me-Ph), 7.64, 7.67 (2 x d, 1H, $J = 1.6$ Hz, 4-F-3-Me-Ph), 7.80 (s, 1H, 4-F-3-Me-Ph), 7.93, 7.95 (s, 1H, H-6), 7.99 (s, 1H, H-5 triazole), 9.27, 9.42 (br s, 1H, 3-NH). ^{13}C NMR ($CDCl_3$) δ: 12.66 (5-CH_3), 14.73 (CH_3 4-F-3-Me-Ph), 29.84 (N-CH_2), 37.76 (d, $J = 2.1$ Hz, C-2′), 59.34, 59.50 (C-5′), 72.03 (C propargyl), 72.51 (C-3′), 80.44, 80.49 (2 x d, $J_{C\text{-}P} = 4.4$ Hz, C-4′), 82.81 (CH propargyl), 86.87, 87.75 (C-1′), 111.39, 111.61 (C-5), 115.64, 115.77 (4-F-3-Me-Ph), 116.86 (4-F-3-Me-Ph), 120.02, 120.20 (4-Cl-Ph), 121.58, 121.66 (2 x d, $J_{C\text{-}P} = 4.9$ Hz, 4-Cl-Ph), 122.02, 122.07 (4-F-3-Me-Ph), 124.88, 124.96 (4-F-3-Me-Ph), 125.57, 125.75 (C-5 triazole), 129.04 (d, $J_{C\text{-}P} = 5.3$ Hz, 4-Cl-Ph), 129.50, 129.77 (4-F-3-Me-Ph), 129.99 (C-4 triazole), 130.85, 130.95 (2 x d, $J_{C\text{-}F} = 1.3$ Hz, C-6), 147.49, 147.59 (C-2), 150.34 (d, $J_{C\text{-}P} = 3.5$ Hz, 4-Cl-Ph), 161.53 (d, $J_{C\text{-}F} = 246.7$ Hz, 4-F-3-Me-Ph), 163.84, 163.97 (C-4). ^{19}F NMR ($CDCl_3$) δ: -117.77 (m, 1F). ^{31}P NMR ($CDCl_3$) δ: 4.72, 5.33. MS-ESI *m/z*: 629, 631 $[M + H]^+$; 651. 653 $[M + Na]^+$; 667, 669 $[M + K]^+$; 627, 629 $[M - H]^-$; 663, 665, 667 $[M + Cl]^-$. Anal. Calcd for $C_{28}H_{27}ClFN_6O_6P$: C, 53.47; H, 4.33; N, 13.36. Found: C, 53.30; H, 4.63; N, 13.32.

Biological evaluation

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), 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 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).

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.[30] 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.

Determination of the presence of 3′-[4-(3-fluorophenyl)-(1,2,3-triazol-1-yl)]-3′-deoxythymidine 5′-monophosphate (5′-monophosphate of 9) in the lysate of HeLa cells incubated with phosphoramidate 35

The HeLa cells were incubated with phosphoramidate **35** for 24 h at 37 °C in a humidified atmosphere (90% RH) containing 5% CO_2 (see previous procedure). The supernatant was removed, and the cells were treated with 60% methanol in water and left to lyse overnight.[33] The cell lysate suspension was centrifuged and the supernatant was filtered through a Millipore HA (0.45 µm) filter and the filtrate was lyophilized. The reside was dissolved in water and analyzed by HPLC/MS. An authentic sample of 5′-monophosphate of **9** was obtained by phosphorylation of compound **9** with phosphoryl chloride in the presence of water and pyridine in acetonitrile by the method published by Sowa and Ouchi.[34] HPLC analysis was carried out on Waters X-Terra MS C18 reversed-phase column (2.1 x 100 mm, 3.5 µm) using mixture water-acetonitrile (9:1, v/v) as the eluent at a flow rate 0.1 mL/min. Synthesized 5′-monophosphate of **9** (the reference compound) eluted as a peak with a retention time of 8.15 min, whereas a peak

with a similar retention time of 8.20 min appeared on the lysate chromatogram, both peaks on the ESI mass spectra in the negative ion mode showed the expected m/z value for $[M - H]^-$ of 466.

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