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# Fluorophosphonylated Nucleoside Derivatives as New Series of Thymidine Phosphorylase Multisubstrate Inhibitors

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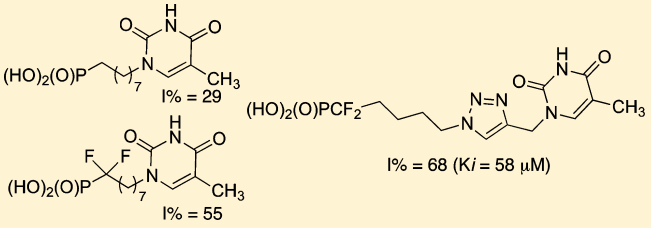
Supporting Information

**ABSTRACT:** The synthesis of new class of potential TPase inhibitors containing a difluoromethylphosphonate function as phosphate mimic is reported. This new series was prepared from a readily available fluorinated building block in few steps. Two series were evaluated as potential inhibitors: a linear series and a conformational constrained series. The activity of these multisubstrate inhibitors depends on the size of the spacer introduced between the pyrimidine ring and the phosphonate function. Best results were observed from triazolyl derivatives, easily obtained from propargylthymine and corresponding azides.




## INTRODUCTION

Platelet-derived endothelial cell growth factor (PD-ECGF) is a protein involved in tumor angiogenesis. This protein at low abundance is overexpressed in many human solid tumors and might be an attractive cancer chemotherapy target for inhibition of tumor angiogenesis, subsequent tumor growth, and metastasis. Because of a structural and genetic similarity between the *Escherichia coli* thymidine phosphorylase (EC 2.4.2.4) and the human recombinant PD-ECGF, it has been suggested that PD-ECGF could be identified as the human thymidine phosphorylase.[1,2] Thymidine phosphorylase (TPase) catalyzes the reversible phosphorolysis of pyrimidine 2′-deoxynucleosides (Figure 1). The

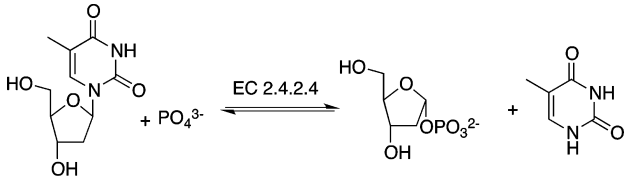



**Figure 1.** Thymidine phosphorylase EC 2.4.2.4.

main product of the conversion, the 2-deoxyribose-1-phosphate, is transformed into 2-deoxyribose (2dR), which stimulates endothelial cell migration and angiogenesis.[3]

An important effect on angiogenesis and apoptosis in tumors has been observed by using thymidine phosphorylase inhibitors.[4,5] The known TPase inhibitors are commonly structurally close to modified nucleosides or acyclonucleosides (Figure 2), and some of them are well-known as antiviral agents.[6] Among these inhibitors, the most efficient on the tumor angiogenesis is a chloropyrrolidinyl uracil derivative called TPI (Figure 2).[4a,5]

Multisubstrate inhibitors, simultaneously bound to the nucleoside and phosphate binding sites, are less efficient than TPI. Their moderate activities could be attributed to the low acidic character of the phosphonate function ($pK_a^2$(phosphonate) = 7.5−8). Indeed, high difference in activities between naturally occurring phosphates and phosphonates has been already reported for purine nucleoside phosphorylase and tyrosine kinase inhibitors.[7] As difluoromethylphosphonates are the best surrogate mimic of naturally occurring phosphates,[8] our work is focused on the synthesis and the evaluation of the inhibitory activities of new fluorinated multisubstrate compounds. Taking into account that the distance between the phosphate oxygen atom and the thymidine $N^1$ atom lies between 4 and 10 Å when the enzymatic substitution occurred,[9] the present study deals with the synthesis of fluorinated phosphonates as multisubstrate inhibitors of TPase containing different spacers such as alkyl chains and heterocycles (Figure 3).

## RESULTS AND DISCUSSION

**Chemistry.** In connection with our previous studies regarding the synthesis of fluorophosphonate derivatives,[10] the preparation of aliphatic series **7**−**10** and **14**−**16** was realized from the readily available tosylate derivatives **1** and **2** (Scheme 1).[11]

We recently reported that 6-chloropurine or $N^3$-benzoylthymine alkylation with fluorophosphonylated tosylates **1** and **2** affording compounds **3**, **4**, and **12** proceeded in good yields and

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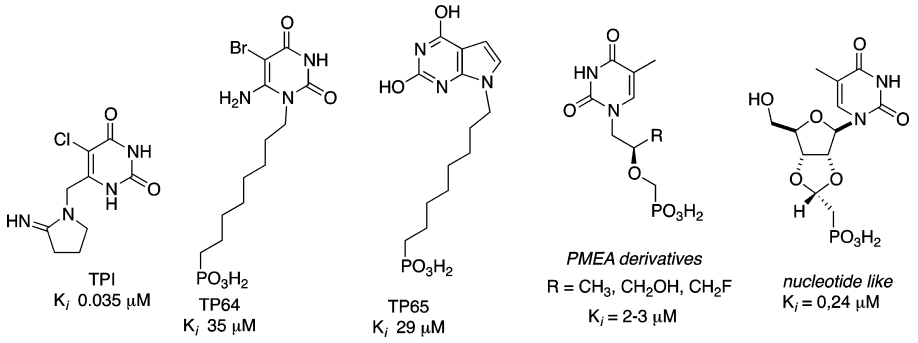



**Figure 2.** Thymidine phosphorylase inhibitors.[6a,b,e,f]

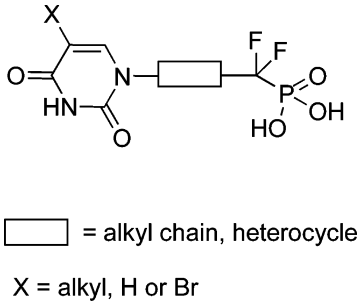
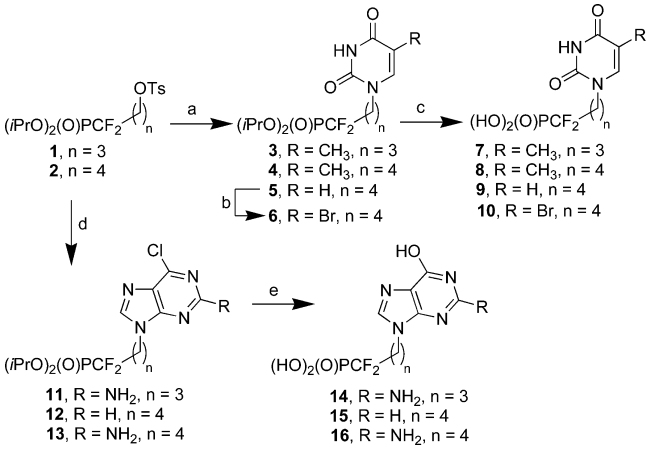



**Figure 3.** Multisubstrate inhibitors.

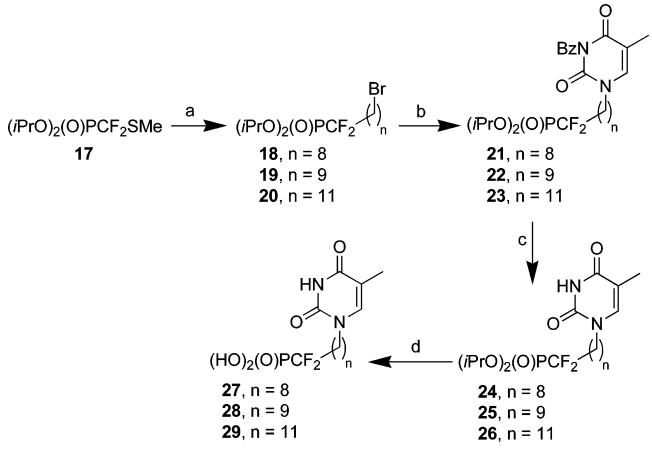
**Scheme 1. Synthesis of Phosphonic Acids 7−10 and 14−16**[a]




[a]Reagents and conditions: (a) (i) $N^3$-benzoylthymine, TMG, DMSO, rt, 15 h; (ii) $MeNH_2$, MeOH, rt, 15 h to give **3** (75%) and **4** (81%); (i) $N^3$-benzoyluracil, TMG, DMSO, rt, 15 h; (ii) $MeNH_2$, MeOH, rt, 15 h to give **5** (78%); (b) NBS, AIBN, THF, 60 °C, 1.5 h (75%); (c) (i) TMSBr, $CH_2Cl_2$, 0 °C to rt, 72 h; (ii) MeOH, rt, 2 h to give **7** (67%), **8** (75%), **9** (70%), and **10** (74%); (d) 6-chloropurine, TMG, DMSO, rt, 15 h to give **12** (67%); 2-amino-6-chloropurine, TMG, DMSO, rt, 15 h to give **11** (60%) and **13** (68%); (e) (i) TMSBr, $CH_2Cl_2$, 0 °C to rt, 72 h; (ii) $H_2O$, rt, 16 h to give **14** (77%), **15** (75%), and **16** (64%).

excellent regioselectivities when the reaction was conducted in the presence of 1,1,3,3-tetramethylguanidine (TMG).[11] These coupling conditions were extended to other nucleic bases. Indeed, tosylates **1** and **2** were treated with 2-amino-6-chloropurine in the presence of TMG. After 15 h of stirring, corresponding alkylation products **11** and **13** were isolated in 60−68% yields. From $N^3$-benzoyluracil and tosylate **2**, the reaction was also very efficient and uracil derivative **5** was obtained in 78% yield after deprotection of the benzoyl group. Bromination of the 5-position of uracil was realized from **5** to afford fluorophosphonylated 5-bromouracil **6** in 75% yield. Cleavage of phosphonic alkyl esters was carried out under standard conditions. Fluorinated phosphonates **3**−**6** were treated with TMSBr (5 equiv) followed by addition of methanol, leading to phosphonic acids **7**−**10** in 67−75% yields after precipitation. From 6-chloropurine derivatives **11**−**13**, an additional step allowing the chlorine atom substitution by a hydroxyl group led to phosphonic acids **14**−**16** in 64−77% yields. Fluorophosphonylated acyclonucleosides **27**−**29**, containing longer alkyl chain spacers (8−11 carbon atoms), were prepared from **17** (Scheme 2).[10c]

**Scheme 2. Synthesis of Phosphonic Acids 27−29**[a]




[a]Reagents and conditions: (a) (i) $^t$BuLi, THF, −78 °C, 10 min; (ii) 1,8-dibromooctane, THF, 30 min at −78 °C, then 1 h at −10 °C to give **18** (52%); 1,9-dibromononane, THF, 30 min at −78 °C, then 1 h at −10 °C to give **19** (51%); 1,11-dibromoundecane, THF, 30 min at −78 °C, then 1 h at −10 °C to give **20** (50%); (b) $N^3$-benzoylthymine, TMG, DMSO, rt, 15 h to give **21** (64%), **22** (77%), and **23** (76%); (c) $MeNH_2$, MeOH, rt, 18 h to give **24** (94%), **25** (76%), and **26** (90%); (d) (i) TMSBr, $CH_2Cl_2$, 0 °C to rt, 72 h; (ii) MeOH, rt, 2 h to give **27** (99%), **28** (88%), and **29** (97%).

Alkylation reactions proceeded smoothly when the carbanion was formed from **17**. In this case, the anion trapped with 1,8-dibromooctane, 1,9-dibromononane, and 1,11-dibromoundecane afforded corresponding difluorophosphonylated bromoalkanes **18**−**20** in 50−52% yields. Products **18**−**20** were reacted with $N^3$-benzoylthymine, and nucleotide analogues **24**−**26** were obtained after nucleobase deprotection. Deprotection of phosphonic esters with TMSBr/MeOH afforded analogues **27**−**29**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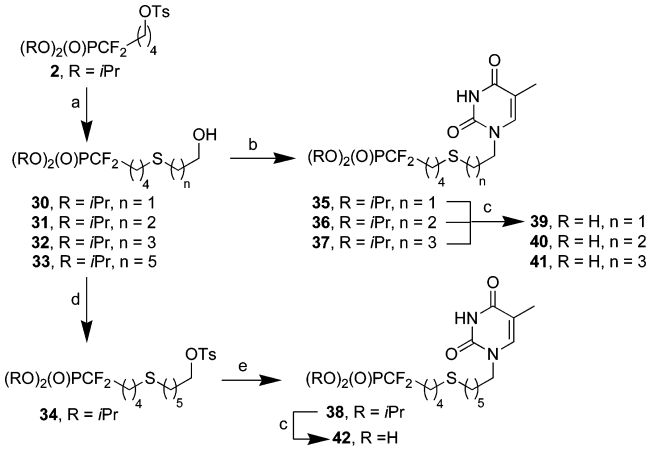
isolated by precipitation in 88−99% yields. In this series, better yields were obtained when intermediates **21**−**23** were isolated prior to debenzoylation reaction. Surprisingly, when the alkylation step was conducted from an anion formed by deprotonation of the diethyl difluoromethylphosphonate by LDA, less than 10% of products were detected in the crude.[12]

In order to modify the conformation of the alkyl chain and the enzymatic activity, the introduction of a sulfur atom was realized (Scheme 3). The presence of a sulfur atom has already been applied with success to improve the activity of PNP inhibitors.[13]

**Scheme 3. Synthesis of Sulfur-Containing Nucleotide Analogues 39−42**[a]

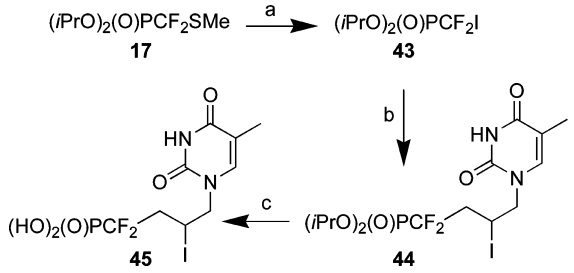



[a]Reagents and conditions. (a) 2-mercaptoethanol, TMG, MeCN, rt, 15 h to give **30** (86%); 3-mercaptopropanol, TMG, MeCN, rt, 15 h to give **31** (77%); 4-mercaptobutanol, TMG, MeCN, rt, 15 h to give **32** (89%); 6-mercaptohexanol, TMG, MeCN, rt, 15 h to give **33** (79%); (b) (i) $N^3$-benzoylthymine, $PPh_3$, DIAD, THF, rt, 15 h; (ii) $MeNH_2$, MeOH, rt, 18 h to give **35** (64%), **36** (55%), and **37** (64%); (c) (i) TMSBr, $CH_2Cl_2$, rt, 72 h; (ii) MeOH, rt, 2 h to give **39** (50%), **40** (86%), **41** (94%), and **42** (83%); (d) TsCl, $NEt_3$, $CH_2Cl_2$, rt, 15 h (79%); (e) (i) $N^3$-benzoylthymine, TMG, rt, 15 h; (ii) $MeNH_2$, MeOH, rt, 15 h (59%).

Sulfur-containing spacers of variable length were prepared by alkylation of thiols using tosylate **2**. This latter was treated with 2 equiv of thiol (2-mercaptoethanol, 3-mercaptopropanol, 4-mercaptobutanol, or 6-mercaptohexanol) in the presence of 1.5 equiv of TMG. After 15 h of stirring in acetonitrile at room temperature, corresponding fluorophosphonylated sulfanyl alcohols **30**−**33** were obtained in 77−89% yields. $N^3$-Benzoylthymine was introduced after activation of alcohols **30**−**33** from the corresponding tosylate or by using the Mitsunobu conditions. From fluorinated hydroxyphosphonate **33**, corresponding tosylate **34** was formed in 79% yield, while tosylation of **30**−**32** was unsuccessful. Alternatively, alcohols **30**−**32** were treated with $N^3$-benzoylthymine in the presence of $PPh_3$ and DIAD to afford corresponding thymine derivatives **35**−**37** in 55−64% yields after deprotection. In this later case, no O-alkylation product was detected in the crude. Indeed, the observed chemical shift was in agreement with the formation of a methylene−nitrogen bond when compared to the starting alcohols **30**−**32** ($^{13}C$ NMR analysis). Furthermore, tosylate **34** was easily transformed into its corresponding alkylated thymine **38**. Finally, deprotection of phosphonate esters **35**−**38** with TMSBr followed by methanolysis and precipitation yielded the targeted phosphonic acids **39**−**42** with good efficiency.

Functionalization of the spacer by introduction of an iodine atom was explored from iododifluoromethylphosphonate **43** and alkenes.[14] Free radical addition onto allylthymine was realized (Scheme 4).

**Scheme 4. Synthesis of Phosphonic Acid 45**[a]




[a]Reagents and conditions. (a) (i) $^t$BuLi, THF, −78 °C, 5 min; (ii) $I_2$, THF, −78 °C, 1 h (81%). (b) Method A: allylthymine, dilauroyl peroxide, $C_2H_4Cl_2$, 80 °C, 3 h (71%). Method B: allylthymine, $Na_2S_2O_4$, $NaHCO_3$, MeCN, $H_2O$, rt, 20 h (17%). Method C: allylthymine, $Et_3B$, $CH_2Cl_2$, rt, 3h (58%). (c) (i) TMSBr, $CH_2Cl_2$, rt, 72 h; (ii) MeOH, rt, 2 h (76%).

Iododifluoromethylphosphonate **43** was reacted with allylthymine in the presence of three different initiators (sodium dithionite, triethylborane, dilauroyl peroxide). Best results were observed in the presence of 0.3 equiv of dilauroyl peroxide. After 3 h of stirring under refluxed dichloroethane, fluorinated iodophosphonate **44** was isolated in 71% yield. It is worthy of note that no protection of the nucleobase is required for the reaction to proceed. After deprotection the corresponding phosphonic acid **45** was obtained in 76% yield.

Finally, phosphonic acids **56**−**62** containing a triazolyl heterocycle were synthesized from fluorophosphonylated azides **46**−**48** and propargyl nucleic bases (Scheme 5).[15] Azido compounds **46**−**48** were easily obtained from their corresponding tosylates and sodium azide, as previously reported.[15] They were reacted with propargyl nucleic bases (1.1 equiv) in the presence of sodium ascorbate (10 mol %) and copper sulfate (5 mol %). From propargylthymine, uracil, 6-chloropurine, and 2-amino-6-chloropurine, corresponding triazolyl derivatives **49**−**55** were isolated in 71−96% yields after 24 h of stirring at room temperature in $^t$BuOH/$H_2O$ (1/1). Phosphonic ester hydrolysis and chlorine substitution by a hydroxyl group, carried out with TMSBr followed by hydrolysis steps with MeOH and/or $H_2O$, afforded pure phosphonic acids **56**−**62** in 34−87% yields after purification by precipitation.

To confirm the effect of the fluorine atoms in the series of targeted acyclic nucleotides, phosphonic acid **65**, as non-fluorinated analogue of **7**, was also prepared. The synthesis was performed in three steps starting from commercially available 1,4-dibromobutane as depicted (Scheme 6).

**Biological Studies.** The magnitude of the size of the spacer was evaluated for compound **49** by X-ray analysis[16] and compared to the value obtained by molecular modeling. In this case, similar values were observed by both methods (Figure 4), and the molecular modeling approach was extended to all the prepared compounds to correlate the enzymatic inhibition percentage with the phosphorus−nitrogen atoms distance. As expected, the different spacers introduced in the series permitted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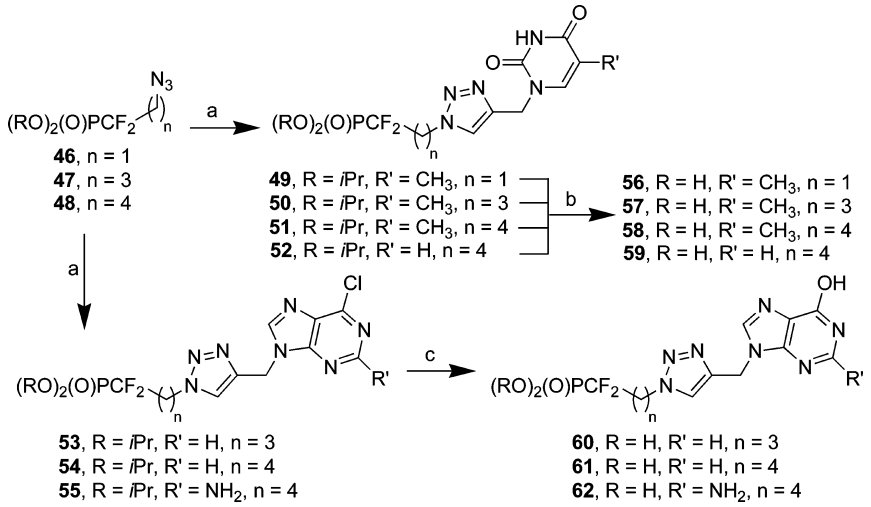
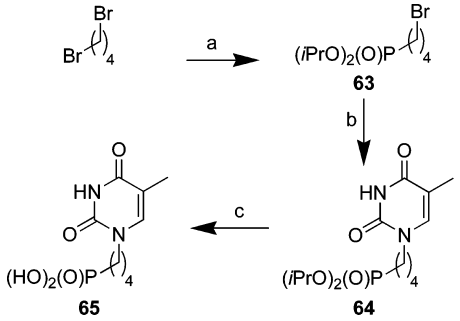
**Scheme 5. Synthesis of Phosphonic Acids 56−62**[a]




[a]Reagents and conditions. (a) propargylthymine, $CuSO_4$, sodium ascorbate, $^t$BuOH, $H_2O$, rt, 24 h to give **49** (71%), **50** (96%), and **51** (93%); propargyluracil, $CuSO_4$, sodium ascorbate, $^t$BuOH, $H_2O$, rt, 24 h to give **52** (85%); propargyl-6-chloropurine, $CuSO_4$, sodium ascorbate, $^t$BuOH, $H_2O$, rt, 24 h to give **53** (85%) and **54** (82%); propargyl-2-amino-6-chloropurine, $CuSO_4$, sodium ascorbate, $^t$BuOH, $H_2O$, rt, 24 h to give **55** (75%); (b) (i) TMSBr, $CH_2Cl_2$, rt, 72 h; (ii) MeOH, rt, 2 h to give **56** (34%), **57** (87%), **58** (73%), and **59** (75%); (c) (i) TMSBr, $CH_2Cl_2$, 0 °C to rt, 72 h; (ii) MeOH, rt, 2 h; (iii) $H_2O$, rt, 16 h to give **60** (56%), **61** (63%), and **62** (81%).

**Scheme 6. Synthesis of Phosphonic Acid 65**[a]




[a]Reagents and conditions: (a) $P(O^iPr)_3$, 130 °C, 24 h (81%); (b) (i) $N^3$-benzoylthymine, TMG, DMSO, rt, 16 h; (ii) $MeNH_2$, MeOH, rt, 16 h (92%); (c) (i) TMSBr, $CH_2Cl_2$, rt, 35 h; (ii) MeOH, rt, 2 h (88%).

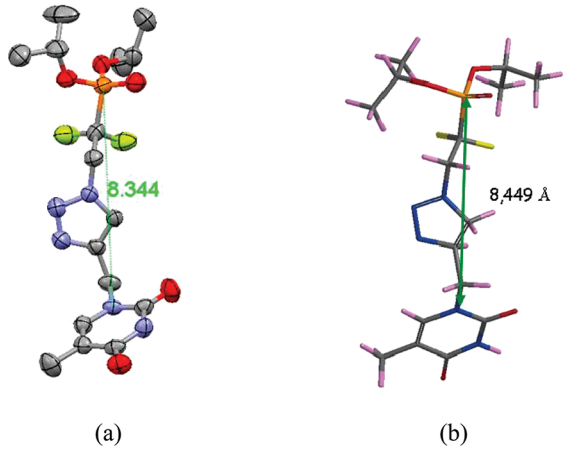



**Figure 4.** (a) X-ray structure of difluorophosphonate **49**. (b) Computational modeling of **49**.

coverage of a large range of distance between 6.5 and 16.3 Å (Table 1). These distances should cover the two possible opened and closed conformations adopted by the enzyme.

**Table 1. TP Inhibition Studies with Phosphonic Acids Containing a Linear Spacer**

| entry | compd | phosphorus−nitrogen distance (Å) | TP inhibition (%) at 1 mM | TP inhibition (%) at 100 μM |
|---|---|---|---|---|
| 1 | 6A5BU | | 100 (100)[6d] | 77 (71)[6d] |
| 2 | POT | | 76 (75)[6d] | 15 (29)[6d] |
| 3 | **7** | 6.60 | 51 | 4 |
| 4 | **8** | 7.38 | 68 | 15 |
| 5 | **9** | 7.38 | 44 | 15 |
| 6 | **10** | 7.38 | 61 | 20 |
| 7 | **14** | 7.81 | 25 | 4 |
| 8 | **15** | 7.82 | 25 | 9 |
| 9 | **16** | 7.82 | 74 | 34 |
| 10 | **27** | 12.57 | 85 | 55 |
| 11 | **28** | 13.84 | 86 | 55 |
| 12 | **29** | 16.33 | 88 | 56 |
| 13 | **39** | 10.23 | 36 | 0 |
| 14 | **40** | 11.72 | 90 | 43 |
| 15 | **41** | 7.82 | 78 | 40 |
| 16 | **42** | 15.22 | 86 | 38 |
| 17 | **45** | 5.36 | 95 | 52 |
| 18 | **65** | 6.56 | 23 | 0 |

Inhibitory activity of phosphonic acids **7**−**10**, **14**−**16**, **27**−**29**, **39**−**42**, **45**, **56**−**62**, and **65** against thymidine phosphorylase from *E. coli* was evaluated using the assay reported by Pérez-Pérez (Tables 1 and 2).[6d] Typically, to a mixture of thymidine, orthophosphate, and TPase in Tris buffer was added phosphonic acid at two concentrations (1 mM, 100 μM). After 20 min of incubation at room temperature followed by 5 min at 90 °C, percentage of inhibition was determined by HPLC after separation of thymidine from thymine. Results were compared to those observed from 6-amino-5-bromouracil (6A5BU), a well-established TPase inhibitor, and 1-phosphonooctylthymine (POT), a moderate inhibitor.[6d] Percentages of inhibition of phosphonic acids containing a linear spacer are listed in Table 1. As expected, introduction of fluorine atoms increased the activity


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**Table 2. TP Inhibition Studies with Conformational Constrained Phosphonic Acids**

| entry | compd | phosphorus−nitrogen distance (Å) | TP inhibition (%) at 1 mM | TP inhibition (%) at 100 μM |
|---|---|---|---|---|
| 1 | 6A5BU | | 100 (100)[6d] | 77 (71)[6d] |
| 2 | POT | | 76 (75)[6d] | 15 (29)[6d] |
| 3 | **56** | 7.82 | 29 | 9 |
| 4 | **57** | 10.86 | 79 | 36 |
| 5 | **58** | 10.86 | 90 | 68 |
| 6 | **59** | 10.86 | 79 | 22 |
| 7 | **60** | 9.95 | 69 | 29 |
| 8 | **61** | 10.83 | 76 | 35 |
| 9 | **62** | 10.83 | 76 | 35 |

as already observed for the inhibition of PNP.[7a] Indeed, compound **7** containing a short spacer was found to be twice more active at 1 mM than its corresponding nonfluorinated analogue **65** (entries 3 and 18). This difference in activity was confirmed when the inhibition percentages of compounds **27** and POT were compared. In addition, the substitution of the pyrimidine ring appeared essential to improve the inhibition of TPase, as observed for TP64 and POT (Figure 5). Unfortunately,

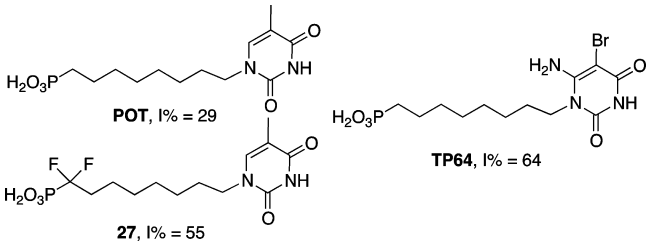


**Figure 5.** Influence of the presence of fluorine atoms and the pyrimidine ring onto the inhibition percentage of TPase at 100 μM.[6d]

the synthesis of the 6A5BU derivatives was attempted but not successful in our hands.

Surprisingly, phosphonic acid **45**, containing an iodine atom in the same size spacer, inhibited TPase at 95% inhibition at 1 mM and 52% at 100 μM (entry 17). The introduction of a supplementary halogen atom into the spacer had a remarkable positive effect on the inhibition compared to **7** (entry 3). This effect is difficult to rationalize; we presume that the iodine atom might occupy the pentose ring pocket of the natural substrate. This later case suggests that TPase would be inhibited in its closed active conformation.[17]

Substitution of thymine by uracil decreased the inhibitory effect, and compound **9** was consequently less active than **8** at 1 mM (entries 5 and 4). However, phosphonic acid **10** in which a bromine atom was introduced onto the 5-position of uracil displayed roughly the same activity as its corresponding thymine derivative **8** (entries 6 and 4), confirming the presence of lipophilic pocket around the nucleic base.[6c] Substitution of thymine by a purine nucleic base such as hypoxanthine and guanine induced a decrease of the activity. Compounds **14** and **15** were less active than their thymine analogues **7** and **8** (entries 3, 4, 7, and 8). In contrast, only phosphonic acid **16** showed a marginal inhibitory effect of 74% at 1 mM (entry 9).

We also noticed that the activity increased with the size of the spacer. Nucleotide analogue **7** exhibited 50% inhibition at 1 mM, while 70% was observed for compound **8** and 85% for compound **27**, both containing, respectively, one and seven additional carbon atoms in the spacer (entries 3, 4, and 10). This pattern was even more pronounced at 100 μM (5% for **7**, 15% for **8**, and 55% for **27**). In this series, results were found to be optimum with **27** and the activity remained unchanged when more than eight carbon atoms were introduced in the spacer. Indeed, compounds **28** and **29** displayed about 85% inhibition at 1 mM and 55% at 100 μM (entries 10, 11, and 12). These results seems to indicate that thymidine phosphorylase is locked in its open, inactive conformation, as already demonstrated by Balzarini and co-workers in a previous study.[6b]

Substitution of a methylene group in the spacer by a sulfur atom did not induce any change in the activity in almost every case. In fact, sulfur-containing phosphonic acids **40**−**42** showed the same activity as their corresponding analogues **27**−**29** (entries 10−12 and 14−16). Surprisingly, the difluorophosphonylated nucleoside **39**, in which the sulfur atom and the thymine are separated by only two carbon atoms, was found to be inactive even at high concentration (entry 13).

PNP inhibition studies realized in the late 1990s revealed that the distance between the nitrogen atom of the nucleic base and the phosphorus atom of the difluoromethylphosphonate function was not the only important factor required for the design of potent inhibitors. In fact, it was also reported that introduction of a cycle (phenyl, cyclopropane, or THF) in the spacer allowed an increase in the activity.[18] In addition, to explore if a click enzymatic reaction could be possible,[19] the tolerance of a triazolyl ring was evaluated. This was confirmed by the difluorophosphonylated nucleoside analogues **56**−**60**, containing a triazolyl ring (Table 2). The presence of a triazolyl ring is tolerated by the enzyme, and phosphonic acids **56**−**60** exhibited moderate to excellent activities at 1 mM (entries 3−9). Compound **56** with only one carbon atom between the difluorophosphonate group and the triazolyl ring displayed a weak inhibition activity (entry 3). This result indicates that the distance between the fluorinated phosphonate and the nucleic base is crucial and reinforces the idea of an inhibition of the enzyme in its opened conformation. Furthermore, we noticed a drastic improvement of the activity when the triazolyl ring was introduced onto a larger spacer. Indeed, nucleotide analogues **57** and **58** were found to be 3 times more active than **56** at 1 mM and up to 7 times at 100 μM (entries 3−5). As a high difference in activity was observed from nonfluorinated (Table 1, POT, **65**) and the fluorinated series (Table 1, **27**, **7**), the synthesis of nonfluorinated triazolyl derivatives was not explored. As previously discussed, substitution of the thymine nucleobase by uracil, hypoxanthine, or guanine had a negative effect. Derivative **58** presented 68% inhibition at 100 μM, while compounds **59**, **61**, and **62** did not exceed 35% at the same concentration (entries 5, 6, 8, 9). The same pattern was observed with **57** and **60** (entries 4 and 7).

Difluorophosphonylated analogue **58** combining the triazolyl ring and a large size spacer appears to be the best inhibitor revealed by this study. Thus, its inhibition constant was evaluated ($K_i$ = 58 μM), and **58** acted as a competitive inhibitor (Figure 6). Note that the other competitive inhibitors TP64 and TP65, evaluated on *E. coli* TPase, presented 142 and 54 μM respectively,[6b] for a similar assay, showing again the superiority of the difluoromethylphosphonates as phosphate surrogates.


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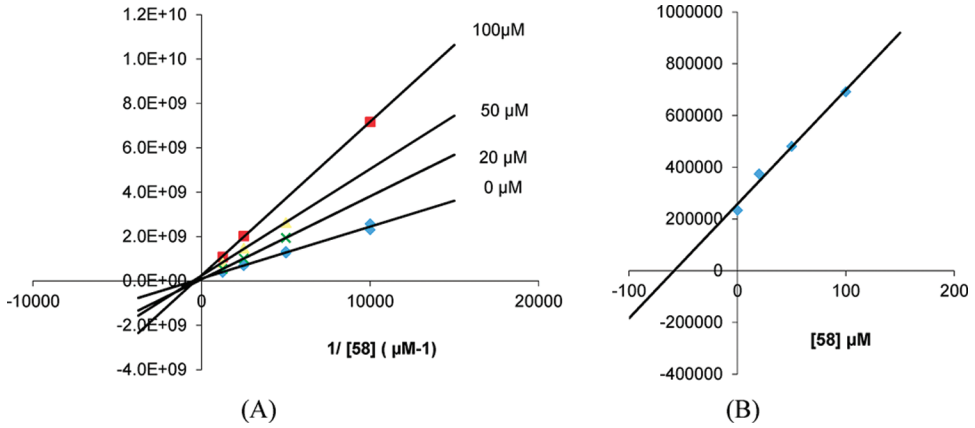


**Figure 6.** (A) Lineweaver–Burk plot and (B) replot of slope versus [**58**]: (■) **58**, 100 $\mu$M; (▲) **58**, 50 $\mu$M; (×) **58**, 20 $\mu$M; (◆) **58**, 0 $\mu$M.

## CONCLUSION

Novel fluorinated acyclic nucleotides have been synthesized to develop new multisubstrate inhibitors of *E. coli* thymidine phosphorylase as potent antiangiogenic factors. The first series contained a difluoromethylphosphonate group and a nucleic base that were separated by linear spacers from different size. A second series of inhibitors functionalized by a triazolyl ring was developed, and their biological activities were evaluated.

For both series, thymine derivatives were much more active than their analogues bearing uracil, guanine, and hypoxanthine nucleic base. It has been confirmed that difluoromethylphosphonate derivatives are more active than their nonfluorinated analogues (**65** vs **7**). However in the fluorinated series, compounds having a short spacer (6–8 Å) are less active except when the iodine atom was present on the alkyl chain (compound **45**). In contrast, a long length spacer up to 16 Å dramatically enhanced the inhibition properties of the compounds. In this series the optimum activity for a chain length of 14–15 Å suggests an inhibition of the enzyme in its opened, inactive conformation, as previously observed in the literature. Substitution of a methylene group in the spacer by a sulfur atom induced no change of the activity. The presence of the triazolyl ring in the spacer is well tolerated by the enzyme, and phosphonic acids **56–60** exhibited moderate to good activities at 1 mM. The best inhibition was obtained with **58** (90% at 1 mM and 68% at 100 $\mu$M) that combined the triazolyl ring and a long spacer. Because of its large size, it is reasonable to assume that **58** inhibits the thymidine phosphorylase in its opened inactive conformation. Finally, these fluorinated acyclic nucleosides are the first examples of multisubstrate TPase inhibitors bearing a difluoromethylphosphonate group as phosphate mimic. These new series will be further exploited in order to improve the activity against thymidine phosphorylase to design new antiangiogenic agents after derivation into their corresponding prodrugs.[6i−k,20] In addition, their activity toward viruses will be evaluated and reported elsewhere.

## EXPERIMENTAL SECTION

**General.** All commercially available reagents were bought from Aldrich and used as received. For anhydrous conditions, the glassware was dried in the oven at 120 °C and cooled to room temperature under a continuous nitrogen flow. THF, $CH_2Cl_2$, $Et_2O$, and $CH_3CN$ were dried in a solvent generator from Innovative Technologies Inc., which uses an activated alumina column to remove water. DMF and $NEt_3$ were distilled under $CaH_2$ or 4 Å molecular sieves. Flash column chromatography was realized on silica gel 60 (40–63 $\mu$m) from Merck with air pressure, and products were detected by thin layer chromatography, in which the spots were visualized by UV irradiation and/or $KMnO_4$ solution. NMR spectra were recorded on a 250 or 400 MHz apparatus in deuterated solvent at 25 °C. $^{31}P$ and $^{19}F$ NMR spectral lines are with respect to the internal references $H_3PO_4$ (capillary) and $CFCl_3$. All chemical shifts are reported in $\delta$ parts per million (ppm), and coupling constants are in hertz (Hz). High-resolution mass data were recorded on a high-resolution mass spectrometer in the EI or ESI mode. IR spectra were recorded on a Perkin-Elmer ATR IR instrument. Analytical HPLC was performed on a Waters systems (model 600 controller, model 717plus autosampler, model 996 photodiode array detector) using reverse phase Phenomenex Gemini C18 110A (5 $\mu$m, 4.6 mm × 250 mm). The mobile phase was 70% methanol, 30% $H_2O$ with 0.05% of TFA, and the flow rate was 1.0 mL/min. UV spectra were recorded using a Waters 996 photodiode array detector. Data were integrated and reported using Waters Empower software. All compounds submitted to enzymatic assays displayed purity of >95% as determined by this method, unless stated otherwise.

**Diisopropyl 1,1-Difluoro-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)pentylphosphonate (5).** *Typical Procedure for the Preparation of Alkyl Spacer from Tosylate Derivatives.* To a stirred solution of tosylate **2** (442.1 mg, 1.0 mmol) in DMSO (5 mL), $N^3$-protected pyrimidine (324 mg, 1.50 mmol) was added followed by dropwise addition of TMG (0.19 mL, 1.50 mmol). The mixture was stirred and monitored by TLC. After completion (15 h), DMSO was evaporated and the residue diluted in $CH_2Cl_2$ (15 mL), washed with water (5 mL) and brine (5 mL). The aqueous layers were extracted with $CH_2Cl_2$ (2 × 10 mL) and the combined organic layers washed with brine (5 mL) and dried over $MgSO_4$. Solvents were evaporated under reduced pressure. To a stirred solution of crude product (382.8 mg, 0.46 mmol) in MeOH (5 mL) was added 40% *N*-methylamine in $H_2O$ (5 mL, 20.0 mmol), and the solution was stirred for 15 h at room temperature. The methanol was evaporated under reduced pressure. The residue was purified by flash column chromatography on silica using EtOAc–pentane (8/2) as eluent to give **5** as a light yellow syrup (298.6 mg, 78%). $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 9.75 (s, 1H), 7.15 (d, $J$ = 8.0 Hz, 1H), 5.66 (d, $J$ = 8.0 Hz, 1H), 4.80 (dsept, $J$ = 6.0 Hz, 2H), 3.71 (t, $J$ = 6.8 Hz, 2H), 1.95–2.15 (m, 2H), 1.68–1.78 (m, 2H), 1.49–1.65 (m, 2H), 1.32 (dd, $J$ = 6.1, 3.1 Hz, 12H); $^{19}F$ NMR ($CDCl_3$, 376 MHz) $\delta$ −112.83 (dt, $J$ = 108.5, 19.8 Hz, 2F); $^{31}P$ NMR ($CDCl_3$, 162 MHz) $\delta$ 5.1 (t, $J$ = 108.5 Hz, 1P); $^{13}C$ NMR ($CDCl_3$, 63 MHz) $\delta$ 164.3, 151.1, 144.7, 121.6 (dt, $J$ = 260.6, 218.1 Hz), 102.4, 73.9 (d, $J$ = 7.1 Hz), 48.8, 33.6 (dt, $J$ = 21.2, 15.1 Hz), 29.9, 24.3 (d, $J$ = 3.4 Hz), 23.9 (d, $J$ = 4.8 Hz), 18.13 (dt, $J$ = 4.9 Hz). LRMS-ESI ($m/z$): $[M + H]^+$ 383 (31), 341 (73), 299 (100). HRMS-ESI ($m/z$): $[M + H]^+$ calcd for $C_{15}H_{26}F_2N_2O_5P$ 383.1547, found 383.1532.

**Diisopropyl 1,1-Difluoro-5-[5-bromo-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]pentylphosphonate (6).** To a suspension of


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**5** (250.0 mg, 0.65 mmol) in dry THF (5 mL) were added *N*-bromosuccunimide (115.7 mg, 0.65 mmol) and AIBN (a pinch). The mixture was heated at 60 °C for 90 min. Then it was diluted with EtOH (10 mL) and filtered through Celite. The filtrate was evaporated and purified by flash column chromatography on silica using $CH_2Cl_2$−MeOH (20/1) as eluent to give **6** (224.8 mg, 75%) as a yellow syrup. $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 10.09 (s, 1H), 7.56 (s, 1H), 4.80 (2H, dsept, $J$ = 6.0 Hz), 3.73 (2H, t, $J$ = 7.2 Hz), 1.99−2.16 (m, 2H), 1.64−1.80 (m, 2H), 1.50−1.62 (m, 2H), 1.31 (dd, $J$ = 6.2, 3.5 Hz, 12H); $^{19}$F NMR ($CDCl_3$, 376 MHz) $\delta$ −112.75 (dt, $J$ = 108.6, 19.6 Hz, 2F); $^{31}$P NMR ($CDCl_3$, 162 MHz) $\delta$ 4.9 (t, $J$ = 108.6 Hz, 1P); $^{13}$C NMR ($CDCl_3$, 101 MHz) $\delta$ 160.1, 150.6, 144.2, 120.4 (dt, $J$ = 260.3, 218.4 Hz), 96.5, 74.0 (d, $J$ = 7.1 Hz), 49.1, 33.5 (dt, $J$ = 21.2, 14.7 Hz), 28.8, 24.1 (d, $J$ = 3.5 Hz), 23.9 (d, $J$ = 4.8 Hz), 18.1 (dt, $J$ = 4.9 Hz). LRMS-ESI ($m/z$): $[M + H]^+$ 461 (58), 419 (100), 377 (75), 229 (2). HRMS-ESI ($m/z$): $[M + H]^+$ calcd for $C_{15}H_{25}BrF_2N_2O_5P$ 461.0653, found 461.0633.

**1,1-Difluoro-5-(6-hydroxy-9*H*-purin-9-yl)pentylphosphonic Acid (9).** *General Procedure for the Hydrolysis of Difluorophosphonylated Pyrimidine Derivatives.* To a cooled solution of **5** (84.1 mg, 0.22 mmol) in anhydrous $CH_2Cl_2$ (5 mL) was added TMSBr (0.174 mL, 1.32 mmol). The mixture was stirred at room temperature for 72 h and then concentrated under vacuum. The residue was diluted in MeOH (2 mL) and stirred for 2 h at room temperature. The solvent was removed, and the crude was diluted in MeOH (0.5 mL). Compound **9** (45.9 mg, 70%) was obtained as a white powder after precipitation in $Et_2O$−$CH_2Cl_2$ (3/1): mp 193 °C; $^1$H NMR (MeOD, 500 MHz) $\delta$ 7.41 (d, $J$ = 9.5 Hz, 1H), 5.62 (d, $J$ = 9.5 Hz, 1H), 3.79 (t, $J$ = 9.0 Hz, 2H), 2.02−2.22 (m, 2H), 1.71−1.83 (m, 2H), 1.57−1.70 (m, 2H); $^{31}$P NMR (MeOD, 202 MHz) $\delta$ 5.7 (t, $J$ = 110.1 Hz, 1P); $^{19}$F NMR (MeOD, 470 MHz) $\delta$ −118.65 (dt, $J$ = 110.1, 23.5 Hz, 2F); $^{13}$C NMR (DMSO, 101 MHz) $\delta$ 163.9, 152.3, 146.8, 121.3 (dt, $J$ = 260.8, 194.2 Hz), 106.5, 43.9, 31.2 (m), 23.5, 17.0 (m). LRMS-ESI ($m/z$): $[M - H]^-$ 297 (90), 277 (6), 254 (100), 234 (8), 185 (32), 111 (36). HRMS-ESI ($m/z$): $[M - H]^-$ calcd for $C_9H_{12}F_2N_2O_5P$ 297.0452, found 297.0443.

**Diisopropyl 4-(2-Amino-6-chloro-9*H*-purin-9-yl)-1,1-difluorobutylphosphonate (11).** *General Procedure for the Introduction of Purine Nucleic Bases.* To a stirred solution of tosylate **1** (200.0 mg, 0.47 mmol) and 2-amino-6-chloropurine (119.5 mg, 0.71 mmol) in DMSO (5 mL) was added dropwise TMG (0.089 mL, 0.71 mmol). The mixture was stirred at 20 °C and monitored by TLC. After completion (15 h), DMSO was evaporated and the residue diluted in $CH_2Cl_2$ (15 mL), washed with water (5 mL), and brine (5 mL). The aqueous layer was extracted with $CH_2Cl_2$ (2 × 10 mL), and the combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, and filtered. Solvents were evaporated under reduced pressure and crude product was purified by flash column chromatography on silica using ethyl acetate−pentane (7/3) as eluent to give **11** (120.1 mg, 60%) as a light yellow syrup. $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 7.78 (s, 1H), 5.28 (sbr, 2H), 4.83 (dsept, $J$ = 6.3 Hz, 2H), 4.14 (t, $J$ = 6.7 Hz, 2H), 2.02−2.40 (m, 4H), 1.33 (dd, $J$ = 5.9, 3.3 Hz, 12H); $^{19}$F NMR ($CDCl_3$, 235 MHz) $\delta$ −112.26 (dt, $J$ = 106.9, 19.0 Hz, 2F); $^{31}$P NMR ($CDCl_3$, 101 MHz) $\delta$ 4.8 (t, $J$ = 106.9 Hz, 1P); $^{13}$C NMR ($CDCl_3$, 101 MHz) $\delta$ 160.1, 154.4, 153.0, 146.7, 131.9, 120.1 (dt, $J$ = 259.8, 216.9 Hz), 73.8 (d, $J$ = 7.0 Hz), 48.1, 32.6 (dt, $J$ = 21.0, 14.9 Hz), 24.4 (d, $J$ = 3.3 Hz), 23.9 (d, $J$ = 4.7 Hz), 22.2 (dt, $J$ = 5.0 Hz). LRMS-ESI ($m/z$): $[M + H]^+$ 427 (62), 385 (100), 342 (23). HRMS-ESI ($m/z$): $[M + H]^+$ calcd for $C_{15}H_{24}ClF_2N_5O_3P$ 426.1273, found 426.1285.

**1,1-Difluoro-4-(2-amino-6-hydroxy-9*H*-purin-9-yl)-pentylphosphonic Acid (14).**[21] *General Procedure for the Hydrolysis of Difluorophosphonylated Purine Derivatives.* To a solution of **11** (93.7 mg, 0.22 mmol) in anhydrous $CH_2Cl_2$ (3 mL) cooled at 0 °C was added TMSBr (0.174 mL, 1.32 mmol). The mixture was stirred at room temperature for 72 h. Volatiles were removed, and the residue was stirred in $H_2O$ (1 mL) for 16 h and dried under reduced pressure. The residue was diluted in MeOH, and compound **14** (54.75 mg, 77%) was obtained as a white solid after precipitation in $Et_2O$−$CH_2Cl_2$: mp 230 °C; $^1$H NMR ($D_2O$, 400 MHz) $\delta$ 8.53 (s, 1H), 4.21 (t, $J$ = 7.5 Hz, 2H), 1.89−2.22 (m, 4H); $^{19}$F NMR ($D_2O$, 376 MHz) $\delta$ −112.43 (dt, $J$ = 112.3, 21.5 Hz, 2F); $^{31}$P NMR ($D_2O$, 162 MHz) $\delta$ 3.6 (t, $J$ = 112.3 Hz 1P); $^{13}$C NMR ($D_2O$, 101 MHz) $\delta$ 166.2, 163.5, 148.6, 141.2, 128.3, 122.4 (m), 46.0, 29.9 (m), 23.9 (m). LRMS-ESI ($m/z$): $[M - H]^-$ 322 (100), 279 (25), 222 (10). HRMS-ESI ($m/z$): $[M - H]^-$ calcd for $C_9H_{11}F_2N_5O_4P$ 322.0517, found 322.0512.

**Diisopropyl 1,1-Difluoro-9-bromononylphosphonate (18).** *General Procedure for the Preparation of Diisopropyl 1,1-Difluorobromoalkylphosphonate.* To a solution of *tert*-butyllithium (2.17 mL 1 M in pentane, 3.47 mmol, 1.3 equiv) in anhydrous THF (40 mL) at −78 °C, difluoromethylphosphonate **17** (700 mg, 2.67 mmol, 1 equiv) was added dropwise. The mixture was stirred for 10 min at −78 °C, and 1,8-dibromooctane (0.98 mL, 5.34 mmol, 2 equiv) was added slowly. The mixture was stirred for 30 min at −78 °C and warmed from −78 to −10 °C over 1 h. The reaction mixture was quenched by addition of saturated $NH_4Cl_{aq}$ (4 mL), and the mixture was extracted with $CH_2Cl_2$ (10 mL), washed with saturated aqueous solution of $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated under reduce pressure. The residue was purified by flash column chromatography using EtOAc/pentane (1/9 and 2/8) as eluent to afford compound **18** (565 mg, 52%) as a colorless oil. $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 4.84 (dsept, $J$ = 6.8 Hz, 2H), 3.40 (t, $J$ = 6.8 Hz, 2H), 2.10−1.95 (m, 2H), 1.85 (quint, $J$ = 6.9 Hz, 2H), 1.60−1.38 (m, 4H), 1.37 (dd, $J$ = 6.1, 4.1 Hz, 12H), 1.35−1.30 (m, 6H); $^{19}$F NMR ($CDCl_3$, 376 MHz) $\delta$ −112.83 (dt, $J$ = 20.0, 109.7 Hz, 2F); $^{31}$P NMR ($CDCl_3$, 161 MHz) $\delta$ 5.7 (t, $J$ = 109.7 Hz, 1P); $^{13}$C NMR ($CDCl_3$, 101 MHz) $\delta$ 120.6 (dt, $J$ = 260.0, 217.1 Hz), 73.3 (d, $J$ = 7.1 Hz), 33.8 (dt, $J$ = 20.9, 14.2 Hz), 33.9, 32.7, 29.1, 29.0, 28.5, 28.0, 24.1 (d, $J$ = 3.5 Hz), 23.7 (d, $J$ = 4.9 Hz), 20.5 (dd, $J$ = 4.7 Hz). LRMS-ESI ($m/z$): $[M + H]^+$ 407 (30), 365 (62), 323 (100). HRMS-ESI ($m/z$): $[M + H]^+$ calcd for $C_{15}H_{31}O_3F_2PBr$ 407.1162, found 407.1161.

**Diisopropyl 1,1-Difluoro-9-(5-methyl-2,4-dihydropyrimidin-1,3(2*H*)-yl)nonylphosphonate (21).** *General Procedure for Thymine Introduction from Bromoalkyl Derivatives.* To a solution of **18** (285 mg, 0.70 mmol) and $N^3$-benzoylthymine (320 mg, 1.39 mmol) in $CH_3CN$ (6 mL) and DMSO (0.5 mL), was added dropwise TMG (0.191 mL, 1.53 mmol). After 15 h of stirring at 20 °C the mixture was poured in $Et_2O$ (10 mL). The organic layer was washed with water (2 × 2 mL), brine (5 mL), dried over $MgSO_4$, filtered, and concentrated under reduce pressure. Compound **21** (249 mg, 64%) was isolated as colorless oil by flash column chromatography using EtOAc/pentane (6/4) as eluent. $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 7.89 (dd, $J$ = 8.0, 0.8 Hz, 2H), 7.62 (dd, $J$ = 7.4 Hz, 1H), 7.47 (dd, $J$ = 7.7 Hz, 2H), 7.09 (s, 1H), 4.82 (dsept, $J$ = 6.4 Hz, 2H), 3.70 (t, $J$ = 7.3 Hz, 2H), 2.20−1.99 (m, 2H), 1.94 (s, 3H), 1.70−1.50 (m, 4H), 1.35 (dd, $J$ = 6.1, 4.5 Hz, 12H), 1.35−1.30 (m, 8H); $^{19}$F NMR ($CDCl_3$, 376 MHz) $\delta$ −112.79 (dt, $J$ = 20.0, 109.6 Hz, 2F); $^{31}$P NMR ($CDCl_3$, 162 MHz) $\delta$ 5.6 (t, $J$ = 109.6 Hz, 1P); $^{13}$C NMR ($CDCl_3$, 101 MHz) $\delta$ 169.2, 163.2, 149.8, 140.2, 134.9, 131.7, 130.4, 129.1, 120.6 (dt, $J$ = 259.0, 217.1 Hz), 110.6, 73.93 (d, $J$ = 7.1 Hz), 48.8, 33.8 (dt, $J$ = 20.9, 14.3 Hz), 29.2, 29.1, 29.0, 28.9, 26.3, 24.1 (d, $J$ = 3.5 Hz), 23.7 (d, $J$ = 4.8 Hz), 20.5 (dd, $J$ = 4.6 Hz, $C_3$), 12.4. LRMS-ESI ($m/z$): $[M + H]^+$ 557 (61), 515 (64), 473 (100), 393 (8), 351 (16). HRMS-ESI ($m/z$): $[M + H]^+$ calcd for $C_{27}H_{40}N_2O_6F_2P$ 557.2592, found 557.2607.

**Diisopropyl 1,1-Difluoro-9-(5-methyl-2,4-dihydropyrimidin-1,3(2*H*)-yl)nonylphosphonate (24).** *General Procedure for the Deprotection of $N^3$-Benzoylthymine.* To a stirred solution of **21** (256 mg, 0.46 mmol) in MeOH (5 mL) was added *N*-methylamine (5 mL, 40% aqueous solution). After 18 h, the mixture was concentrated under reduced pressure. Flash column chromatography using EtOAc/pentane (7/3) afforded **24** (195 mg,

94%) as a colorless oil. $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 9.77 (sbr, 1H), 6.94 (d, $J$ = 1.2 Hz, 1H), 4.77 (dsept, $J$ = 6.2 Hz, 2H), 3.62 (t, $J$ = 7.4 Hz, 2H), 2.05−1.92 (m, 2H), 1.85 (d, $J$ = 1.1 Hz, 3H), 1.65−1.50 (m, 4H), 1.30 (dd, $J$ = 6.2, 4.6 Hz, 12H), 1.27−1.20 (m, 8H); $^{19}$F NMR ($CDCl_3$, 376 Mz) $\delta$ −112.81 (dt, $J$ = 20.0, 109.7 Hz, 2F); $^{31}$P NMR ($CDCl_3$, 162 MHz) $\delta$ 5.6 (t, $J$ = 109.7 Hz, 1P); $^{13}$C NMR ($CDCl_3$, 101 MHz) $\delta$ 164.7, 151.1, 140.4, 120.6 (dt, $J$ = 259.0, 217.0 Hz), 110.5, 73.3 (d, $J$ = 7.1 Hz), 48.4, 33.7 (dt, $J$ = 20.8, 14.2 Hz), 29.1, 29.1, 29.0, 28.9, 26.3, 24.1 (d, $J$ = 3.4 Hz), 23.7 (d, $J$ = 4.9 Hz), 20.5 (dd, $J$ = 4.6 Hz), 12.3. LRMS-ESI ($m/z$): $[M + H]^+$ 453 (92), 411 (100), 369 (51). HRMS-ESI ($m/z$): $[M + H]^+$ calcd for $C_{20}H_{36}N_2O_5F_2P$ 453.2330, found 453.2333.

**1,1-Difluoro-9-(5-methyl-2,4-dihydropyrimidin-1,3(2*H*)-yl)-nonylphosphonic Acid (27).** Following the general procedure used for **9** from difluorophosphonate **24** (99 mg, 0.22 mmol), TMSBr (0.174 mL, 1.32 mmol), and $CH_2Cl_2$ (5 mL), compound **27** was isolated as a white solid (80 mg, 99%): mp 175−180 °C; $^1$H NMR (DMSO, 400 MHz) $\delta$ 11.19 (s, 1H), 7.53 (d, $J$ = 1.2 Hz, 1H), 3.60 (t, $J$ = 7.2 Hz, 2H), 2.00−1.85 (m, 2H), 1.75 (d, $J$ = 1.0 Hz, 3H), 1.60−1.40 (m, 4H), 1.30−1.20 (m, 8H); $^{19}$F NMR (DMSO, 376 Mz) $\delta$ −112.41 (dt, $J$ = 20.1, 101.1 Hz, 2F); $^{31}$P NMR (DMSO, 162 MHz) $\delta$ 4.2 (t, $J$ = 101.1 Hz, 1P); $^{13}$C NMR (DMSO, 101 MHz) $\delta$ 164.4, 151.0, 141.6, 121.5 (dt, $J$ = 257.9, 204.5 Hz), 108.6, 48.7, 47.3, 33.5 (dt, $J$ = 21.0, 14.2 Hz), 28.8, 28.6, 25.9, 20.6 (dt, $J$ = 4.4 Hz), 12.0. LRMS-ESI ($m/z$) $[M - H]^-$ 367 (7), 347 (10), 324 (100), 304 (15). HRMS-ESI ($m/z$): $[M - H]^-$ calcd for $C_{14}H_{22}N_2O_5F_2P$ 367.1234, found 367.1231.

**Diisopropyl 1,1-Difluoro-5-(2-hydroxy-1-ethylsulfanyl)-pentylphosphonate (30).** *General Procedure for the Preparation of Difluorophosphonylated Thioethers.* To a solution of **2** (845 mg, 1.91 mmol) and 2-mercaptoethanol (0.270 $\mu$L, 3.82 mmol) in anhydrous $CH_3CN$ (10 mL) was added TMG (0.350 $\mu$L, 2.86 mmol). The mixture was stirred 15 h at room temperature. The solution was diluted in $Et_2O$ (10 mL) and washed with water (2 × 5 mL). The aqueous layers were combined and extracted with $CH_2Cl_2$ (10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography using EtOAc/pentane (7/3) as eluent to give **30** (572 mg, 86%) as a colorless oil: $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 4.81 (dsept, $J$ = 6.4 Hz, 2H), 3.69 (t, $J$ = 6.0 Hz, 2H), 2.70 (t, $J$ = 6.0 Hz, 2H), 2.52 (t, $J$ = 6.8 Hz, 2H), 2.10−1.95 (m, 2H), 1.70−1.60 (m, 4H), 1.35 (dd, $J$ = 6.0, 3.5 Hz, 12H); $^{19}$F NMR ($CDCl_3$, 376 Mz) $\delta$ −112.70 (dt, $J$ = 19.7, 108.8 Hz, 2F); $^{31}$P NMR ($CDCl_3$, 161 Mz) $\delta$ 5.4 (t, $J$ = 108.8 Hz, 1P); $^{13}$C NMR ($CDCl_3$, 101 MHz) $\delta$ 120.4 (dt, $J$ = 259.3, 217.4 Hz), 73.6 (d, $J$ = 7.1 Hz), 60.4, 35.2, 33.4 (dt, $J$ = 35.6, 14.5 Hz), 31.3, 29.3, 24.1 (d, $J$ = 3.5 Hz), 23.7 (d, $J$ = 4.9 Hz), 20.0 (dd, $J$ = 4.9 Hz). LRMS-ESI ($m/z$): $[M + H]^+$ 349 (15), 331 (100), 289 (38), 271 (2), 247 (10), 229 (2). HRMS-ESI ($m/z$): $[M + H]^+$ calcd for $C_{13}H_{28}O_4F_2PS$ 349.1414, found 349.1412.

**Diisopropyl 1,1-Difluoro-5-(2-(3-benzoyl-5-methyl-2,4-dihydropyrimidin-1(2*H*)-yl)-1-ethanesulfure)pentylphosphonate (35).** *General Procedure for Thymine Introduction from Fluorinated Hydroxyphosphonates.* Alcohol **30** (570 mg, 1.64 mmol), $N^3$-benzoylthymine (453 mg, 1.97 mmol), and $Ph_3P$ (515 mg, 1.97 mmol) in anhydrous THF (12 mL) were cooled at 0 °C. DIAD (0.485 $\mu$L, 2.46 mmol) was introduced dropwise, and the reaction mixture was stirred for 15 h at room temperature. The solvent was evaporated under reduce pressure, and the crude was filtered through a pad of silica gel using EtOAc/pentane (6/4 and 7/3). After concentration, the crude product was diluted with MeOH (17 mL), and *N*-methylamine (17 mL, 40% aqueous solution) was added slowly. After 18 h, the mixture was concentrated under reduced pressure. Flash column chromatography using EtOAc/pentane (8/2) afforded **35** (479.1 mg, 64%) as a colorless oil: $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.80 (s, 1H), 7.03 (d, $J$ = 1.2 Hz, 1H), 4.84 (dsept, $J$ = 6.2 Hz, 2H), 3.85 (t, $J$ = 6.8 Hz, 2H), 2.80 (t, $J$ = 6.8 Hz, 2H), 2.55 (t, $J$ = 6.9 Hz, 2H), 2.10−1.97 (m, 2H), 1.92 (d, $J$ = 1.1 Hz, 3H), 1.67−1.65 (m, 4H), 1.36 (dd, $J$ = 6.2, 4.0 Hz, 12H); $^{19}$F NMR ($CDCl_3$, 376 Mz) $\delta$ −112.81 (dt, $J$ = 19.7, 109.0 Hz, 2F); $^{31}$P NMR ($CDCl_3$, 162 MHz) $\delta$ 5.4 (t, $J$ = 109.0 Hz, 1P); $^{13}$C NMR ($CDCl_3$, 101 MHz) $\delta$ 164.4, 150.9, 140.8, 120.4 (dt, $J$ = 259.5, $J$ = 217.3 Hz), 110.4, 73.6 (d, $J$ = 7.1 Hz), 48.6, 33.4 (dt, $J$ = 20.9, 14.5 Hz), 32.1, 30.8, 29.1, 24.1 (d, $J$ = 3.5 Hz), 23.8 (d, $J$ = 4.9 Hz), 19.9 (dd, $J$ = 4.9 Hz), 12.3. LRMS-ESI ($m/z$): $[M + H]^+$ 457 (75), 415 (100), 373 (70), 331 (7). HRMS-ESI ($m/z$): $[M + H]^+$ calcd for $C_{18}H_{32}N_2O_5F_2PS$ 457.1738, found 457.1752.

**1,1-Difluoro-5-(2-(5-methyl-2,4-dihydropyrimidin-1,3(2*H*)-yl)-1-ethanesulfure)pentylphosphonic Acid (39).** Following the general procedure used for **9** from difluorophosphonate **35** (100 mg, 0.22 mmol), TMSBr (0.174 mL, 1.32 mmol), and $CH_2Cl_2$ (5 mL), compound **39** was isolated as a white solid (41 mg, 50%): mp 155 °C; $^1$H NMR (DMSO, 400 MHz) $\delta$ 11.29 (s, 1H), 7.61 (d, $J$ = 1.2 Hz, 1H), 3.85 (t, $J$ = 6.8 Hz, 2H), 2.79 (t, $J$ = 7.3 Hz, 2H), 2.61 (t, $J$ = 6.7 Hz, 2H), 2.10−1.97 (m, 2H), 1.81 (d, $J$ = 1.0 Hz, 3H), 1.67−1.60 (m, 4H); $^{19}$F NMR (DMSO, 376 MHz) $\delta$ −112.35 (dt, $J$ = 20.0, 100.8 Hz, 2F); $^{31}$P NMR (DMSO, 162 MHz) $\delta$ 4.1 (t, $J$ = 100.8 Hz, 1P); $^{13}$C NMR (MeOD, 101 MHz) $\delta$ 166.9, 152.8, 143.5, 121.9 (dt, $J$ = 257.1, 210.5 Hz), 110.8, 34.4 (dt, $J$ = 21.2, 14.8 Hz), 32.4, 31.2, 30.3 (4C), 21.2 (dd, $J$ = 4.7 Hz), 12.2. LRMS-ESI ($m/z$): $[M + H]^+$ 373 (19), 247 (100), 187 (43). HRMS-ESI ($m/z$): $[M + H]^+$ calcd for $C_{12}H_{20}N_2O_5F_2PS$ 373.0799, found 373.0800.

**Diisopropyl 1,1-Difluoro-3-iodo-4-(5-methyl-2,4-dioxo-3,4-dihydropyridin-1(2*H*)-yl)butylphosphonate (44).** To a refluxed solution of **43** (100.0 mg, 0.29 mmol) and allylthymine (54.0 mg, 0.32 mmol) in 1,2-dichloroethane (2 mL) was added over a period of 1 h via a syringe pump dilauroyl peroxide (35.0 mg, 0.09 mmol) in 1,2-dichloroethane (1 mL). After 3 h, the mixture was cooled, concentrated and the crude product was purified by flash column chromatography using EtOAc/pentane (6/1) as eluent to give **44** (106.0 mg, 71%) as a white solid: mp 110.5 °C; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.87 (s, 1H), 6.98 (d, $J$ = 1.1 Hz, 1H), 4.79 (dsept, $J$ = 6.2 Hz, 2H), 4.59−4.71 (m, 1H), 4.11 (dd, $J$ = 14.5, 10.0 Hz, 1H), 3.80 (dd, $J$ = 14.5, 10.0 Hz, 1H), 2.67−2.96 (m, 2H), 1.88 (d, $J$ = 1.0 Hz, 3H), 1.32 (dd, $J$ = 6.2, 3.0 Hz, 12H); $^{19}$F NMR ($CDCl_3$, 376 MHz) $\delta$ −112.29 (dddd, $J$ = 296.1, 104.0, 25.0, 13.0 Hz, 1F), −109.8 (dddd, $J$ = 296.1, 103.0, 27.1, 11.2 Hz, 1F); $^{31}$P NMR ($CDCl_3$, 162 MHz) $\delta$ 3.5 (dd, $J$ = 104.0, 103.0 Hz, 1P); $^{13}$C NMR ($CDCl_3$, 101 MHz) $\delta$ 164.7, 149.9, 140.1, 119.2 (dt, $J$ = 262.8, 216.8 Hz), 110.4, 74.2 (d, $J$ = 7.0 Hz), 55.3, 41.9 (ddd, $J$ = 20.6, 15.6 Hz), 24.0 (d, $J$ = 3.5 Hz), 23.6 (d, $J$ = 4.6 Hz), 16.6, 12.2. LRMS-ESI ($m/z$): $[M + H]^+$ 509 (28), 466 (87), 425 (100). HRMS-ESI ($m/z$): $[M + H]^+$ calcd for $C_{15}H_{25}F_2IN_2O_5P$ 509.0514, found 509.0519.

**1,1-Difluoro-3-iodo-4-(5-methyl-2,4-dioxo-3,4-dihydropyridin-1(2*H*)-yl)butylphosphonic Acid (45).** Following the general procedure used for **9**, from **44** (112 mg, 0.22 mmol), TMSBr (0.174 mL, 1.32 mmol), and $CH_2Cl_2$ (5 mL), compound **45** was obtained as a white powder (71 mg, 76%): mp 168 °C; $^1$H NMR (DMSO, 400 MHz) $\delta$ 10.51 (s, 1H), 7.19 (d, $J$ = 1.2 Hz, 1H), 4.25−4.73 (m, 1H), 3.85−4.10 (m, 2H), 2.58−2.89 (m, 2H), 1.96 (d, $J$ = 1.0 Hz, 3H); $^{31}$P NMR (DMSO, 162 MHz) $\delta$ 3.6 (dd, $J$ = 104.0, 103.0 Hz, 1P); $^{19}$F NMR (DMSO, 376 MHz) $\delta$ −113.05 (dddd, $J$ = 297.0, 104.0, 25.2, 12.6 Hz, 1F), −111.36 (dddd, $J$ = 297.0, 103.0, 26.7, 11.2 Hz, 1F); $^{13}$C NMR (DMSO, 101 MHz) $\delta$ 163.7, 150.3, 147.5, 121.2 (dt, $J$ = 263.9, 217.6 Hz), 110.9, 57.2, 41.9 (m), 17.1 (m), 14.2. LRMS-ESI ($m/z$): $[M - H]^-$ 422 (64), 295 (100), 126 (2). HRMS-ESI ($m/z$): $[M - H]^-$ calcd for $C_9H_{11}F_2IN_2O_5P$ 422.9418, found 422.9423.

**1-[1-(5-Diisopropoxyphosphono-5,5-difluoropentyl)-1,2,3-triazolo-4-methyl]uracil (52).** *General Procedure for the Preparation of Triazolyl Derivatives.* To a stirred solution of **48** (320.0 mg, 1.02 mmol) in $^t$BuOH (2.5 mL) and $H_2O$ (2.5 mL) were added propargyl uracil (168.1 mg, 1.12 mmol), sodium ascorbate (19.8 mg, 0.10 mmol), and $CuSO_4 \cdot 5H_2O$ (8.00 mg, 0.05 mmol). The mixture was stirred for 24 h at room temperature, then concentrated under reduced pressure. The residue was diluted in $CH_2Cl_2$ (10 mL) and washed with water


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(10 mL). The aqueous layer was extracted with $CH_2Cl_2$ (2 × 10 mL), and combined organic layers were washed with a saturated solution of NaCl (5 mL), dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by flash column chromatography using $CH_2Cl_2/CH_3OH$ (10/1) as eluent to give **52** (401.8 mg, 85%) as a white solid: mp 105 °C; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.69 (s, 1H), 7.59 (s, 1H), 7.23 (d, $J$ = 1.1 Hz, 1H), 5.60 (s, 1H), 4.80 (s, 2H), 4.76 (dsept, $J$ = 6.2 Hz, 2H), 4.16 (t, $J$ = 6.9 Hz, 2H), 1.81−2.06 (m, 4H), 1.52−1.68 (m, 2H), 1.33 (dd, $J$ = 6.1, 3.1 Hz, 12H); $^{19}$F NMR ($CDCl_3$, 376 MHz) $\delta$ −112.90 (dt, $J$ = 106.9, 19.0 Hz, 2F); $^{31}$P NMR ($CDCl_3$, 162 MHz) $\delta$ 5.0 (t, $J$ = 106.9 Hz, 1P); $^{13}$C NMR ($CDCl_3$, 101 MHz) $\delta$ 164.4, 151.3, 142.1, 139.9, 124.0, 119.6 (dt, $J$ = 261.3, 218.0 Hz), 110.9, 74.2 (d, $J$ = 7.1 Hz), 50.1, 43.0, 33.2 (dt, $J$ = 20.1, 14.9 Hz), 29.7, 24.3 (d, $J$ = 3.6 Hz), 23.7 (d, $J$ = 5.1 Hz,), 18.0 (dt, $J$ = 4.7 Hz). LRMS-ESI ($m/z$): $[M + H]^+$ 464 (46), 422 (100), 380 (72). HRMS-ESI ($m/z$): $[M + H]^+$ calcd for $C_{18}H_{29}F_2N_5O_5P$ 464.1874, found 464.1868.

**9-[1-(4-Diisopropoxyphosphono-4,4-difluorobutyl)-1,2,3-triazolo-4-methyl]-6-chloropurine (53).** General procedure used for **52** was followed with **47** (300.0 mg, 1.00 mmol) and propargyl-6-chloropurine (211.9 mg, 1.10 mmol) to afford **53** (418.1 mg, 85%) as a white solid: mp 108 °C; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.66 (s, 1H), 8.31 (s, 1H), 7.72 (s, 1H), 5.51 (s, 2H), 4.73 (dsept, $J$ = 6.0 Hz, 2H), 4.35 (t, $J$ = 7.2 Hz, 2H), 2.05−2.18 (m, 2H), 1.89−2.05 (m, 2H), 1.25 (dd, $J$ = 6.4, 4.0 Hz, 12H); $^{31}$P NMR ($CDCl_3$, 162 MHz) $\delta$ 4.4 (t, $J$ = 106.4 Hz, 1P); $^{19}$F NMR ($CDCl_3$, 376 MHz) $\delta$ −112.34 (dt, $J$ = 106.4, 18.8 Hz, 2F); $^{13}$C NMR ($CDCl_3$, 63 MHz) $\delta$ 156.5, 151.0, 145.4, 131.6, 129.6, 126.2, 123.4, 119.5 (dt, $J$ = 260.8, 218.1 Hz), 74.0 (d, $J$ = 7.2 Hz, 2C), 49.8, 39.1, 31.00 (dt, $J$ = 21.4, 15.1 Hz), 24.1 (d, $J$ = 3.5 Hz, 2C), 23.8 (d, $J$ = 4.7 Hz, 2C), 22.2 (dt, $J$ = 4.8 Hz). LRMS-ESI ($m/z$): $[M + H]^+$ 492 (100), 450 (82), 408 (22), 226 (15), 173 (18). HRMS-ESI ($m/z$): $[M + H]^+$ calcd for $C_{18}H_{26}ClF_2N_7O_3P$ 492.1491, found 492.1470.

**1,1-Difluoro-5-(4-((2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)-1*H*-1,2,3-triazol-1-yl)pentylphosphonic Acid (59).** General procedure used for **9** was followed with **52** (101.9 mg, 0.22 mmol), TMSBr (0.174 mL, 1.32 mmol), and $CH_2Cl_2$ (5 mL) to afford **59** (62.6 mg, 75%) as a white solid: mp 184 °C; $^1$H NMR (DMSO, 400 MHz) $\delta$ 8.15 (s, 1H), 8.07 (s, 1H), 7.87 (s, 1H), 5.80 (s, 2H), 4.54 (t, $J$ = 7.2 Hz, 2H), 1.90−2.15 (m, 2H), 1.70−1.85 (m, 2H), 1.43−1.55 (m, 2H); $^{31}$P NMR (DMSO, 162 MHz) $\delta$ 4.3 (t, $J$ = 97.0 Hz, 1P); $^{19}$F NMR (DMSO, 376 MHz) $\delta$ −112.82 (dt, $J$ = 97.0, 19.9 Hz, 2F); $^{13}$C NMR (DMSO, 101 MHz) $\delta$ 164.9, 151.4, 141.9, 139.3, 129.6, 119.9 (m), 104.1, 55.3, 47.8, 33.5 (m), 28.9, 25.3 (m). LRMS-ESI ($m/z$): $[M - H]^-$ 378 (100), 358 (15), 335 (70), 315 (45). HRMS-ESI ($m/z$): $[M - H]^-$ calcd for $C_{12}H_{15}F_2N_5O_5P$ 378.1235, found 378.1246.

**1,1-Difluoro-4-(4-((6-hydroxy-9*H*-purin-9-yl)methyl)-1*H*-1,2,3-triazol-1-yl)butylphosphonic Acid (60).** General procedure used for **14** was followed with **53** (108.2 mg, 0.22 mmol), TMSBr (202.1 mg, 1.32 mmol), and $CH_2Cl_2$ (5 mL) to afford **60** (47.9 mg, 56%) as a white solid: mp 185 °C; $^1$H NMR ($D_2O$, 400 MHz) $\delta$ 8.45 (s, 1H), 8.23 (s, 1H), 8.12 (s, 1H), 5.62 (s, 2H), 4.49 (t, $J$ = 6.8 Hz, 2H), 2.06−2.25 (m, 2H), 1.85−2.05 (m, 2H); $^{31}$P NMR ($D_2O$, 162 MHz) $\delta$ 4.5 (t, $J$ = 97.2 Hz, 1P); $^{19}$F NMR ($D_2O$, 376 MHz) $\delta$ −112.23 (dt, $J$ = 97.2, 19.9 Hz, 2F); $^{13}$C NMR ($D_2O$, 101 MHz) $\delta$ 155.8, 155.2, 146.0, 144.4, 142.5, 123.3, 121.3, 114.89 (m), 50.1, 42.1, 32.4 (m), 17.3 (m). LRMS-ESI ($m/z$): $[M - H]^-$ 388 (100), 368 (8), 216 (20), 171 (62), 135 (26). HRMS-ESI ($m/z$): $[M - H]^-$ calcd for $C_{12}H_{13}F_2N_7O_4P$ 388.0735, found 388.0718.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Experimental details for the preparation of compounds **7**−**65** not described in the manuscript, HPLC data of all tested compounds (POT, 5BRU, **7**−**10**, **14**−**16**, **27**−**29**, **39**−**42**, **45**, **56**−**61**, **65**), and $^1$H and $^{13}$C NMR spectra for compounds **5**−**65**. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS USED

TPase, thymidine phosphorylase; PD-ECGF, platelet-derived endothelial cell growth factor; 2dR, 2-deoxyribose; TPI, thymidine phosphorylase inhibitor; TMG, tetramethylguanidine; LDA, lithium diisopropylamide; DIAD, diethyl diazodicarboxylate; 6A5BU, 6-amino-5-bromouracyl; POT, 1-phosphonoctylthymine; PNP, purine nucleoside phosphorylase; $K_i$, inhibition constant; TMSBr, trimethylsilyl bromide

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