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Potential application of thymidylate kinase in nucleoside analogue activation in *Plasmodium falciparum*

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ABSTRACT

Plasmodium falciparum thymidylate kinase (*Pf*TMPK) shows a broad range of substrate tolerance when compared to the corresponding human enzyme. Besides 2′-deoxythymidine monophosphate (dTMP), *Pf*TMPK can phosphorylate 3′-azido-2′,3′-dideoxythymidine monophosphate (AZTMP) very efficiently. In contrast, human thymidylate kinase (hTMPK) is 200 times less active towards AZTMP. We were interested to see if we could use *Pf*TMPK to activate 3′-azido-2′,3′-deoxythymidine (AZT) derivatives as a strategy to treat malaria. *P. falciparum* lacks a pyrimidine nucleoside kinase which usually activates nucleoside and nucleoside analogues to the corresponding monophosphates. Therefore, several prodrug analogues of AZT and related nucleoside monophosphates were prepared and analysed for antiparasitic activity. The prodrugs showed an increase in activity over the parent nucleoside analogues, which showed no inhibition of parasite growth at the concentration tested. The evidence here reported provides a strategy that could be exploited for further anti-malarial design.

1. Introduction

Malaria is a major health problem in many areas of the world; due to increased resistance and failure of the current treatments there is a need for new drugs for both treatment and prophylaxis of malaria that are well tolerated.[1] The enzyme *Plasmodium falciparum* thymidylate kinase (*Pf*TMPK) is a potential drug target in *P. falciparum*.[2] The role of this enzyme is the phosphorylation of deoxythymidine monophosphate to deoxythymidine diphosphate. Kinetic data obtained with *Pf*TMPK shows that it is able to tolerate a broad range of substrates, which distinguishes it from other thymidylate kinases reported in the literature, in particular the human enzyme.[2,3] Thus, one novel property is the capacity to phosphorylate AZTMP 200 times more efficiently (k_{cat}/K_M 200 s^{-1} mM^{-1})[3] compared to the corresponding human thymidylate kinase (hTMPK) (k_{cat}/K_M 1 s^{-1} mM^{-1}).[4] Another feature is that *Pf*TMPK can phosphorylate efficiently dGMP (k_{cat}/K_M 370 s^{-1} mM^{-1}),[2,3] which compares very favourably to the value for dTMP (k_{cat}/K_M 460 s^{-1} mM^{-1}).[3]

We were interested to see if we could use *Pf*TMPK to selectively activate AZT as a strategy to treat malaria. AZT is a prodrug used in the treatment of HIV/AIDS; the active species is AZT triphosphate (AZTTP), which exerts its action against the viral enzyme reverse transcriptase, by acting as a chain terminator to DNA polymerization.[5,6] It acts as an alternate substrate and is incorporated into the growing DNA chain, and because it lacks a 3′-hydroxyl, incorporation leads to chain termination.[7,8] AZT is activated firstly to AZTMP by the action of human thymidine kinase, secondly to AZTDP by human thymidylate kinase and then to AZTTP by human nucleoside diphosphate kinase.[8] The rate limiting step in the activation of AZT is actually the phosphorylation of AZTMP to AZTDP, which is catalysed by human thymidylate kinase, when AZT is used as an antiviral.[9] However, as AZTMP is a much better substrate for *Pf*TMPK than for hTMPK, it is probable that AZTMP will be activated much more rapidly in the malaria parasite compared to the human cell. Any subsequent anti-malarial activity would rely on AZTTP being a reasonable substrate/inhibitor of malarial DNA polymerases.

The first step of activation of AZT, when used in the treatment of HIV/AIDS is conversion to AZTMP by human thymidine kinase. However, *P. falciparum* does not encode the enzyme pyrimidine nucleoside kinase[10] required for the formation of the nucleotide monophosphate and so no mechanism for the activation of nucleoside analogues to the nucleoside monophosphate is available in the parasite. To achieve some activity these nucleoside analogues must be phosphorylated to the corresponding nucleotides prior to entering the parasites. On the other hand, it is known that nucleotide analogues have poor bioavailability due to the presence of the phosphate group, which is highly charged at physiological pH, preventing the passive diffusion through cellular membranes and there are also stability issues due to the presence of 5′-nucleotidases.[11] A way to circumvent the lack of a malarial thymidine

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kinase would be to deliver AZTMP in a masked form, or as a prodrug (Fig. 1). A number of phosphate masking strategies have been developed and reported in the literature.[11] One of the most successfully applied technologies is the use of phosphoramidates developed by McGuigan et al.[12,13]

Therefore we decided to make masked phosphate derivatives of several nucleoside analogues. Firstly we selected AZT (**1**) as AZTMP is known to be a good substrate of the *Pf*TMPK.[3] Then we decided to investigate 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T) (**2**); interestingly for this nucleoside analogue, the rate limiting step in its activation as an antiviral is conversion of d4T to d4T-MP,[15] which is a different step to that for AZT. This would provide an interesting comparison to AZT. Another observation from our work is that 2′-deoxyguanosine (dGMP) is a good substrate of *Pf*TMPK.[2] Therefore we decided to investigate a masked phosphate version of 3′-azido-2′,3′-deoxyguanosine (AZG) (**3**). Finally we decided to prepare the masked phosphate of α-3′-azido-2′,3′-dideoxythymidine (α-AZT) (**4**) to see the effect of altered stereochemistry on the activity. Therefore we prepared the phosphoramidate-based prodrugs of AZT, d4T, AZG and α-AZT (Fig. 2). Once the prodrug is inside cells, the masked nucleotide should be enzymatically cleaved and the free phosphate released.

2. Results and discussion

2.1. Chemistry

3′-Azido-2′,3′-dideoxyguanosine (AZG, **3**) was synthesized following key conditions reported in the literature (Scheme 1).[16] The first step was to protect the amine group of guanine base. Initially this was attempted with the BOC group, but this was not successful, although several different conditions[17] were tested. Alternatively the use of isobutyric anhydride in the presence of an excess of trimethylchlorosilane proved to be successful[18] and the protected nucleoside **6** was obtained in good yield. The inversion of the stereochemistry of C-3′ was achieved in two steps after the protection of the 5′-hydroxyl with TBDPS. The 3′-OH was first oxidized to the corresponding ketone, using Dess–Martin reagent and then reduced to alcohol with $NaBH_4$. The reaction produced a diastereomeric mixture of **7** and **8** with a 1:4 ratio. Stereoisomers **7** and **8** could be separated by column chromatography. The 3′ hydroxyl was then converted into the corresponding azide **9** with inversion of configuration using diphenyl-2-pyridylphosphine, DIAD and NaN_3 with a yield of 40%. The final product AZG **3** was obtained after the two de-protections to remove the isobutyl ester and the TBDPS group under standard conditions.

The synthesis of α-AZT is shown in Scheme 2. α-Deoxythymidine **(10)** was prepared in four steps following literature methods.[19,20] α-Deoxythymidine was firstly protected in 5′ position with TBDPS (attempts to use the TBDMS were not successful due to the instability of the TBDMS group under the subsequent Mitsunobu conditions). Mesylation of the 3′-OH was then carried out using Mitsunobu conditions with methanesulfonic acid[21,22] to give the required mesylate **12** in reasonable yield (56%). The Mitsunobu reaction is widely used for the combination of an alcohol and an acidic species HX (methanesulfonic acid in this study), and results in direct replacement of the hydroxyl group

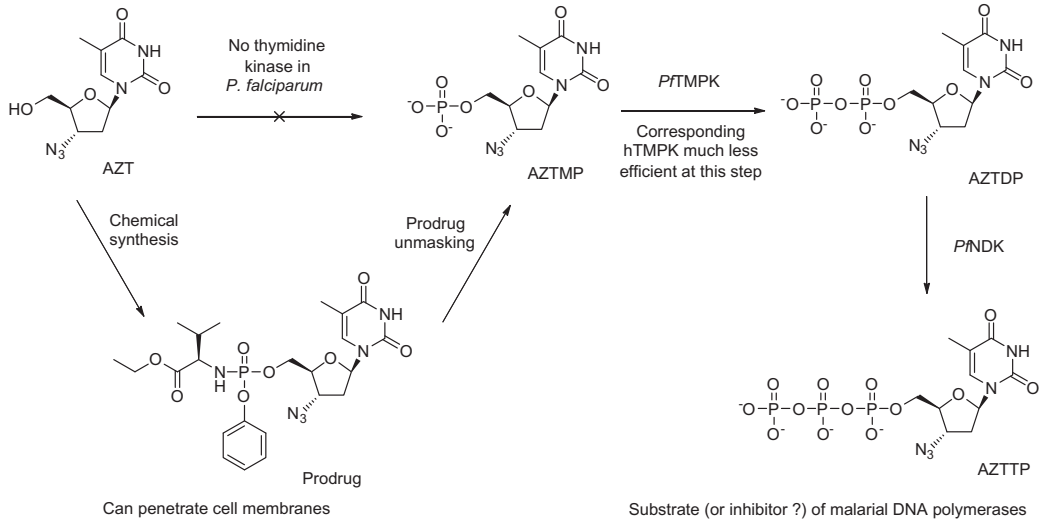


Figure 1. The strategy used in this study. *P. falciparum* lacks the enzyme of thymidine kinase, which is needed to phosphorylate AZT to be AZTMP. In the present strategy, AZT is masked as a prodrug for parasite delivery. The prodrug is released as AZTMP. AZTMP is first phosphorylated to be AZTDP by *Pf*TMPK, a process which is 200 times more efficient than with the corresponding human enzyme. AZTDP would be further phosphorylated by *Plasmodium* nucleoside diphosphate kinase[14] (*Pf*NDK) to AZTTP, which can act as a substrate or inhibitor of *Plasmodium* DNA polymerases and interfere with parasite DNA replication.

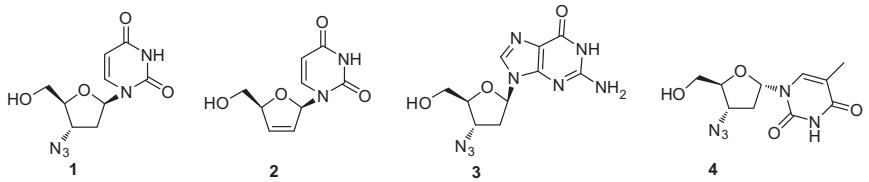
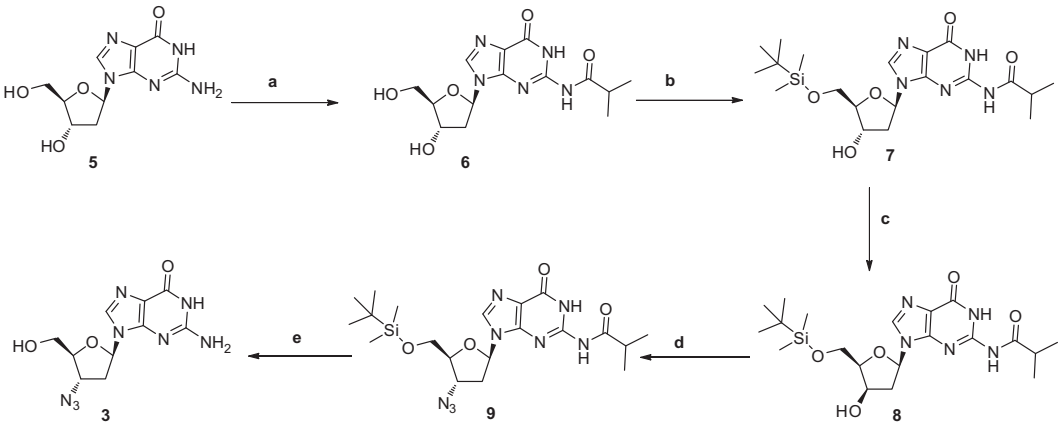
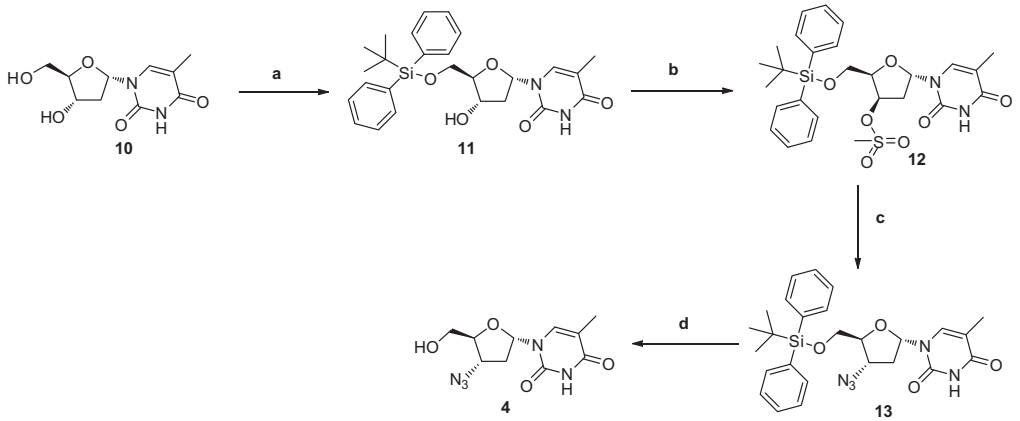


Figure 2. Four selective nucleoside analogues, which are used for the study. 3′-azido-2′,3′-dideoxythymidine AZT (**1**), 2′,3′-didehydro-2′,3′-dideoxythymidine d4T (**2**), 3′-azido-2′,3′-dideoxyguanosine AZG (**3**) and α-3′-azido-2′,3′-dideoxythymidine α-AZT (**4**).



Scheme 1. The synthetic pathway for AZG. Reagents and conditions: (a) Trimethylchlorosilane, isobutyric anhydride, pyridine, 60%; (b) TBDMS–Cl, imidazole, pyridine, 78%; (c) (I) Dess–Martin periodinane, DCM; (II) $NaBH_4$, Acetone, −60 °C, 12 h, 71%; (d) $PyPh_2P$, DIAD, NaN_3, 40%; (e) (I) TBAF, THF; (II) NaOMe, DCM/MeOH, 45 °C, 72%.



Scheme 2. The synthetic pathway for α-AZT. Reagents and conditions: (a) TBDPS–Cl, imidazole, pyridine, 83%; (b) MsOH, Ph_3P, DIAD, THF, 24 h, 56%; (c) NaN_3, DMF, 12 h, 40%; (d) TBAF, THF, 95%.

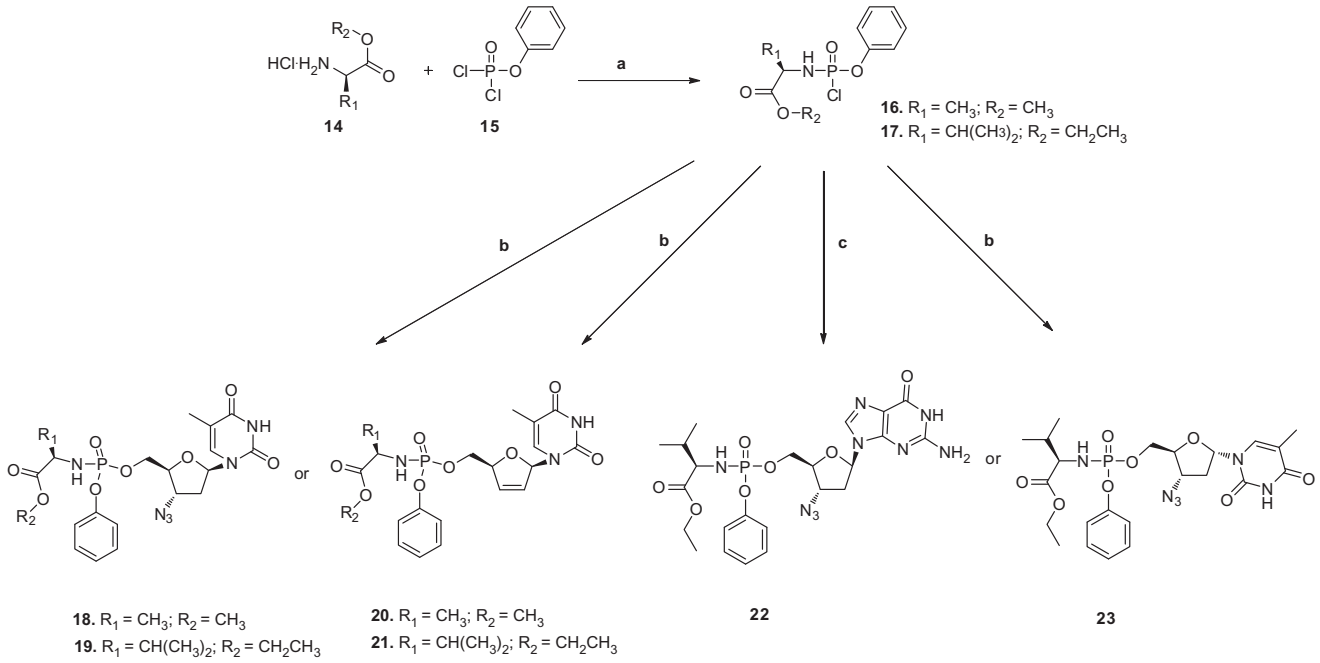
by X with strict inversion of configuration.[21] This procedure allowed us to invert the stereochemistry of the 3′-C, although there was some difficulty in the removal of the triphenylphosphine oxide. Conversion of 3′-methanesulfonate group into azide was performed by S_N2 displacement with NaN_3 in DMF, which gave **13** with the re-inverted stereochemistry of the 3′-C. The de-protection of TBDPS by TBAF in THF produced the final product α-AZT **4**.

The synthesis of phosphoramidates of AZT, d4T and α-AZT was performed according to the method developed by McGuigan et al.[7,13,23] This consists in the reaction of the amino acid ester with phenyl dichlorophosphate to form compounds **16** and **17** and subsequent coupling with the 5′-OH of the nucleosides in the presence of *N*-methyl imidazole (Scheme 3). This procedure afforded: the two phosphoramidates of AZT **18** and **19**; the two d4T phosphoramidates **20** and **21**; and the phosphoramidate of α-AZT **23**. For the synthesis of the AZG phosphoramidate **22**, the phosphochloridate **17** was coupled to the nucleoside following the Uchiyama procedure[24] in the presence of *tert*-butyl magnesium chloride in THF. Again also this method worked well and the desired prodrug **22** was obtained in good yield. The masking group derived from valine was slightly more active against *P. falciparum* than that from the alanine derivative for both AZT and d4T (see later), therefore AZG and α-AZT were only coupled with valine derived phosphoramidate.

2.2. Anti-malarial activity

Compounds were screened for their potency in vitro against *P. falciparum* using the fluorescence-based SYBR-green assay reported in the literature.[25] Table 1 shows the inhibition of *P. falciparum* growth obtained at a compound concentration of 100 μM. EC_{50} values which correspond to the concentration of compounds that reduces *P. falciparum* growth by 50% were further obtained for some of the compounds. As predicted the parent nucleosides **1**–**4** did not inhibit significantly *P. falciparum* growth at a concentration of 100 μM presumably due to the lack of activation by a thymidine kinase. By contrast, the prodrugs of the azides all showed improved anti-malarial activities compared to the parent nucleoside analogues with EC_{50} values in the micromolar range. It is reasonable to assume that the prodrugs are internalised and inside the parasites, are metabolized to yield the nucleoside monophosphates. These are then converted to the diphosphate form by *Pf*TMPK and subsequently to the triphosphates by *Plasmodium* nucleoside diphosphate kinase.[14] We hypothesise that the compounds act at the level of DNA replication, where they are chain terminators of *Plasmodium* DNA polymerisation.

Interestingly the d4T prodrugs had significantly lower activity—it may be that d4T monophosphate is a poor substrate of the *Pf*TMPK. It appeared to be possible to change the stereochemistry from β to α with little effect on activity or to replace the thymine



Scheme 3. Synthesis of phosphoramidates of AZT, d4T, AZG and α-AZT. Reagents and conditions: (a) TEA, DCM, −78 °C, 70%; (b) NMI, THF, −78 °C to rt, 70–80%; (c) ^{t}BuMgCl, THF, 12 h, 46%.

Table 1
Inhibition of growth of *P. falciparum* cultured in vitro

Compound	Nucleoside	(%) Growth inhibition at 100 μM	EC_{50} (μM)
1 (AZT)	AZT	0	>100
18	AZT	35 ± 2%	120
19	AZT	69 ± 1%	80
2 (d4T)	d4T	0	>100
20	d4T	9 ± 1%	>100
21	d4T	18 ± 1%	>100
3 (AZG)	AZG	0	>100
22	AZG	80 ± 1%	66
4 (α-AZT)	α-AZT	0	>100
23	α-AZT	78 ± 2%	67

with a guanine. We can speculate that α-AZTMP and AZG-MP are both substrates for *Pf*TMPK in a similar way to AZTMP. The valine derivative **19** was marginally more active than the alanine derivative **18** for the AZT derivatives and hence we used the valinyl derivatives for the α-AZTMP and AZG-MP prodrugs.

Although the present compounds have insufficient activity for the progressing into antimalarials, all the prodrugs clearly showed improved activity against the parasite compared to the parent nucleoside analogues. It has been described that apart from inhibiting viral reverse transcriptase and incorporating into viral DNA,[26] these kinds of nucleoside analogues also exhibit some affinity for cellular polymerases and can be incorporated into mitochondrial DNA where they arrest DNA chain extension and are able to affect an exonucleolytic repair mechanism.[27] It is therefore possible that the antiplasmodial activity observed is due to direct incorporation into DNA or inhibition of parasite replication.

The lack of potent activity of these compounds may be due to several reasons. First, reduced cell permeability to *P. falciparum* may be an issue. Second, we ignore how efficiently the prodrug is converted to the active species. Finally, the nucleoside triphosphates may be relatively poor substrates or inhibitors of *Plasmodium* DNA polymerase. Further information regarding the uptake and intracellular metabolism of these compounds would aid in the design of compounds with increased antiplasmodial activity.

3. Conclusion

In this paper, we have taken advantage of the unique substrate specificity of *Pf*TMPK compared to that of the human enzyme for designing compounds that would be toxic to *Plasmodium* parasites. In particular we have exploited the observations that both AZTMP and dGMP are good substrates for the *Pf*TMPK but not for human TMPK. Since malaria parasites lack a thymidine kinase activity that would be required for the phosphorylation of nucleoside analogues, a prodrug strategy was devised in order to efficiently deliver the nucleoside monophosphates into cells. Three nucleoside derivatives of AZT, AZG and α-AZT exhibit significant growth inhibition of *Plasmodium* with EC_{50} values in micromolar range, while d4T derivatives were less active. Further optimization of the nucleoside moiety to provide improved cellular uptake, activation and binding to *Plasmodium* DNA polymerases could yield more potent inhibitors of *Plasmodium* growth.

4. Experimental

4.1. In vitro assays

In vitro activity against the erythrocytic stages of *P. falciparum* was determined by using a SYBR-green assay.[25] The parasite *P. falciparum* 3D7 was cultured using standard methods, and synchronized using 5% sorbitol as previously described.[28] Compounds were dissolved in DMSO at 100 mM and added to 48 h post-synchronization parasite cultures incubated in RPMI 1640 medium with hypoxanthine (150 μM), $NaHCO_3$ (0.2%), gentamycin (12.5 μg/mL), Albumax (0.5%), human serum (2%) and washed human red cells O+ at 5% haematocrit (0.3% parasitaemia). Chloroquine was used as standard drug. Experiments were carried out at least twice independently and the different concentrations were tested in duplicate. After 48 h of growth, 100 μl of SYBR-green I (Molecular Probes) in lysis buffer (Tris 20 mM, pH 7.5, EDTA 5 mM, saponin 0.008%, triton X-100 0.08%, 0.2 μl of SYBR-green/ml of lysis buffer) was added to each well, and mixed and after 1 h of incubation in the dark at rt, fluorescence was measured with

excitation and emission wavelength bands centred at 485 and 530 nm. The percent inhibition of each compound at each concentration was determined. EC_{50} values were calculated from hyperbolic or sigmoidal dose–response curves using Sigmaplot 10.0.

4.2. Chemistry

General: Chemical and solvents were purchased from the Sigma-Aldrich Chemical Company, Fluka, VWR, Acros, Fisher Chemicals and Alfa Aesar. ^{1}H NMR, ^{13}C NMR, ^{31}P NMR were recorded on a Bruker Avance DPX 500 spectrometer (^{1}H at 500.1 MHz, ^{13}C at 125.8 MHz and ^{31}P at 202 MHz). Chemical shift (δ) are expressed in ppm. Signal splitting patters are described as singlet (s), broad singlet (br s), double (d), triplet (t), quarter (q), multiplet (m). Low resolution electrospray (ES) mass spectra were recorded either on a Applied Biosystem Mariner API-TOF biospectrometry Workstation spectrometer or on a Bruker MicroTof mass spectrometer, run in a positive ion mode, using either methanol, methanol/water (95:5), or water/acetonitrile (1:1) + 0.2% formic acid as the mobile phase. High resolution electrospray measurements were performed on a Bruker Daltonics MicroTOF mass spectrometer. Column chromatography was carried out using Silica Gel 60 from Fluka. Thin layer chromatography (TLC) was carried out on Merck Silica Gel 60 F254 plates using UV light or PMA for visualization. TLC data are given as the R_f value with the corresponding eluent system specified in brackets.

4.2.1. *N*2-Isobutyryl-2′-deoxyguanosine[18] (6)

2′-Deoxyguanosine (1 g, 3.744 mmol) was co-evaporated with pyridine (20 ml) three times and dried overnight. It was then suspended in dry pyridine (20 ml). Trimethylchlorosilane (2.37 ml, 18.73 mmol) was added. The resulting solution was stirred at rt for 2 h. Isobutyric anhydride (3.11 ml, 18.73 mmol) was added and the mixture was stirred at rt under Ar for 4 h. The reaction was cooled in an ice bath, and H_2O (6 ml) was added. After 15 min 33% aqueous ammonia (6 ml) was added and the reaction was stirred for 15 min. The solution was then evaporated to near dryness and the residue was dissolved in H_2O (60 ml). The aqueous layer was washed with DCM (50 ml) and crystallization occurred quickly in water. The compound was then filtered and dried. The product could further purified by chromatography (MeOH/DCM = 20:80) to yield the product **6** (763 mg, 60%) as a white solid. TLC (10% MeOH/DCM) R_f = 0.09; ^{1}H NMR (500 MHz, DMSO-d$_6$) δ 12.05 (br s, 1H, N*H*), 11.72 (br s, 1H, N*H*), 8.24 (s, 1H, *H*8), 6.21 (q, *J* = 6.5 Hz, 1H, *H*1′), 5.32 (d, *J* = 3.8 Hz, 1H, 3′O*H*), 4.97 (t, *J* = 5.4 Hz, 1H, 5′O*H*), 4.37 (m, 1H, *H*3′), 3.83 (m, 1H, *H*4′), 3.51–3.56 (m, 2H, *H*5′ and *H*5′), 2.76–2.78 (m, 1H, C*H*CH$_3$, *i*-Bu), 2.54–2.56 (m, 1H, *H*2′), 2.27–2.29 (m, 1H, *H*2′), 1.12 (dd, 6H, CHC*H*$_3$ *i*-Bu); ^{13}C NMR (125 MHz, DMSO-d$_6$) δ 180.1 (*C*OCH), 154.8 (*C*6), 148.4, 148.1 (*C*2 and *C*4), 137.4 (*C*8), 120.1 (*C*5), 87.7 (*C*1′), 82.9 (*C*4′), 70.4 (*C*3′), 61.4 (*C*5′), 39.3 (*C*2′), 34.7 (*C*HCH$_3$), 18.8 (CH*C*H$_3$); LC–MS (ES$^+$): *m*/*z* (%) 222 (71) [C$_9$H$_{12}$N$_5$O$_2$]$^+$, 338 (100) [M+H]$^+$; HRMS (ES$^+$): calcd for C$_{14}$H$_{20}$N$_5$O$_5$ [M+H]$^+$ 338.1459 *m*/*z*, found 338.1457 *m*/*z*.

4.2.2. 5′-*O*-*tert*-Butyldimethylsilyl-*N*2-isobutyryl-2′-deoxy-3′-beta-guanosine[16] (7)

A stirred solution of **6** (750 mg, 2.22 mmol) in 5 ml pyridine was treated with TBDMS–Cl (361 mg, 2.4 mmol). After 16 h, the reaction mixture was transferred into ethyl acetate and the solution was washed with H_2O, brine and dried over $MgSO_4$. The reaction mixture was evaporated and purified by chromatography on silica to yield **7** as a white solid (780 mg, 78%). TLC (5% MeOH/DCM) R_f = 0.18; ^{1}H NMR (500 MHz, DMSO-d$_6$) δ 12.05 (s, 1H, N*H*), 11.68 (s, 1H, N*H*), 8.13 (s, 1H, *H*8), 6.17–6.19 (m, 1H, *H*1′), 5.34 (d, *J* = 3.7 Hz, 1H, 3′-O*H*), 4.34 (s, 1H, *H*3′), 3.83 (d, *J* = 3.2 Hz, *H*4′), 3.72–3.75 (m, 1H, *H*5′), 3.65–3.68 (m, 1H, *H*5′), 2.71–2.76 (m, 1H, C*H*CH$_3$, *i*-Bu), 2.52–2.57 (m, 1H, *H*2′), 2.27–2.30 (m, 1H, *H*2′), 1.09 (d, *J* = 6.5 Hz, 6H, CHC*H*$_3$, *i*-Bu), 0.82 (s, 9H, SiCC*H*$_3$), 0.00 (s, 6H, Si*CH*$_3$); ^{13}C NMR (125 MHz, DMSO-d$_6$) δ 180.1 (*C*OCH), 154.8 (*C*6), 148.3, 148.1 (*C*2 and *C*4), 137.1 (*C*8), 120.2 (*C*5), 87.1 (*C*1′), 82.9 (*C*4′), 70.0 (*C*3′), 63.1 (*C*5′), 39.3 (*C*2′), 34.7 (*C*HCH$_3$, *i*-Bu), 25.8 (Si*C*CH$_3$), 18.9, 18.8 (CH*C*H$_3$, *i*-Bu), 18.0 (SiC*C*H$_3$), −5.5 (Si*C*H$_3$); LC–MS (ES$^+$): *m*/*z* (%) 452 (100) [M+H]$^+$; HRMS (ES$^+$): calcd for C$_{20}$H$_{34}$N$_5$O$_5$Si$_1$ [M+H]$^+$ 452.2324 *m*/*z*, found 452.2324 *m*/*z*.

4.2.3. 5′-*O*-*tert*-Butyldimethylsilyl-*N*2-isobutyryl-2′-3′-alpha-deoxyguanosine[16] (8)

To a chilled solution (0 °C) of Dess–Martin periodinane (424 mg, 1 mmol) in DCM (3 ml) was added **7** (320 mg, 0.7 mmol) and the result solution stirred at 0 °C. After 60 min, the cooling bath was removed and stirred continued at rt. After 3 h, the reaction mixture was cooled to −60 °C and anhydrous 2-propanol (3 ml) was added. Stirring was continued for a further 12 h at −60 °C. Acetone (3 ml) was added and the slurry was allowed to come to rt. The resulting solution was washed with $NaHCO_3$, H_2O and Brine. After being dried with $MgSO_4$ and evaporated the solvent, the product was purified by chromatography on silica to give **8** (224 mg, 71%) as a white solid. TLC (5% MeOH/DCM) R_f = 0.20; ^{1}H NMR (500 MHz, DMSO-d$_6$) δ 12.05 (s, 1H, N*H*), 11.70 (s, 1H, N*H*), 8.14 (s, 1H, *H*8), 6.11 (d, 1H, *J* = 4.1 Hz, *H*1′), 5.36 (d, *J* = 3.3 Hz, 1H, 3′-O*H*), 4.34 (d, *J* = 3.7 Hz, 1H, *H*3′), 3.91–3.96 (m, 2H, *H*4′ and *H*5′), 3.73–3.76 (m, 1H, *H*5′), 2.67–2.75 (m, 2H, *H*2′ and C*H*CH$_3$, *i*-Bu), 2.21 (d, *J* = 14.5 Hz, 1H, *H*2′), 1.09 (d, *J* = 6.8 Hz, 6H, CC*H*$_3$, *i*-Bu), 0.82 (s, 9H, SiCC*H*$_3$), 0.00 (s, 6H, Si*CH*$_3$); ^{13}C NMR (125 MHz, DMSO-d$_6$) δ 180.1 (*C*OCH$_3$), 154.8 (*C*6), 148.2 (*C*4 and *C*2), 138.0 (*C*8), 119.8 (*C*5), 85.4 (*C*1′), 82.5 (*C*4′), 68.8 (*C*3′), 62.1 (*C*5′), 41.0 (*C*2′), 34.7 (*C*CH$_3$, *i*-Bu), 25.8 (Si*C*CH$_3$), 18.9, 18.8 (CC*H*$_3$, *i*-Bu), 18.0 (SiC*C*H$_3$), −5.3, −5.4 (Si*C*H$_3$); LC–MS (ES$^+$): *m*/*z* (%) 452 (100) [M+H]$^+$; HRMS (ES$^+$): calcd for C$_{20}$H$_{34}$N$_5$O$_5$Si$_1$ [M+H]$^+$ 452.2324 *m*/*z*, found 452.2321 *m*/*z*.

4.2.4. 3′-Azido-5′-*O*-*tert*-butyldimethylsilyl-*N*2-isobutyryl-2′,3′-dideoxyguanosine[16] (9)

To a stirred slurry of **8** (210 mg, 0.47 mmol) and NaN_3 (91 mg, 1.4 mmol) in DMF (2 ml) was added a solution of diphenyl-2-pyridylphosphine (184 mg, 0.7 mmol) and DIAD (137 μl, 0.7 mmol) in DMF (2 ml). After 8 h, H_2O was added to stop the reaction, and DMF was removed by evaporation. The reaction mixture was purified by column chromatography using ethyl acetate/hexane (9:1) to give **9** as a solid (89 mg, 40%). TLC (10% MeOH/DCM) R_f = 0.49; ^{1}H NMR (500 MHz, $CHCl_3$-d_1) δ 11.90 (s, 1H, *N*–*H*), 8.40 (br s, 1H, N*H*), 7.87 (s, 1H, *H*8), 6.05 (t, *J* = 6.1 Hz, 1H, *H*1′), 4.30 (q, *J* = 5.3 Hz, 1H, *H*3′), 3.94–3.95 (m, 1H, *H*4′), 3.70–3.78 (m, 2H, *H*5′ and *H*5′), 2.50–2.56 (m, 2H, *H*2′ and C*H*CH$_3$), 2.37–2.41 (m, 1H, *H*2′), 1.14–1.22 (m, 6H, CHC*H*$_3$, *i*-Bu), 0.80 (s, 9H, SiCC*H*$_3$), 0.00 (s, 3H, Si*CH*$_3$), 0.00 (s, 3H, Si*CH*$_3$); ^{13}C NMR (125 MHz, $CHCl_3$-d_1) δ 178.2 (*C*O), 155.4 (*C*6), 147.5 (*C*2 and *C*4), 136.5 (*C*8), 121.6 (*C*5), 85.0 (*C*1′), 83.5 (*C*4′), 62.9 (*C*3′), 60.6 (*C*5′), 38.4 (*C*2′), 36.6 (*C*CH$_3$, *i*-Bu), 25.9 (Si*C*CH$_3$), 19.0, 18.9 (CC*H*$_3$), 18.4 (SiC*C*H$_3$), −5.3, −5.5 (Si*C*H$_3$); LC–MS (ES$^+$): *m*/*z* (%) 477 (100) [M+H]$^+$.

4.2.5. 3′-Azido-2′,3′-dideoxyguanosine[29] (3)

Compound **9** (210 mg, 0.44 mmol) was dissolved in 6 ml THF. TBAF (440 μl, 0.44 mmol) was added into the solution. The reaction was stirred at rt for 3 h and TLC was used to observe the disappearance of the starting compound **9**. The solvent of THF was evaporated to remove. Without purification, we further de-protected the isobutyric group by 0.5 M NaOMe in the solution of 3 ml DCM/MeOH (1:1) in 45 °C overnight. After purification by column chromatography using 20% MeOH in DCM as eluent to yield required compound **3** as a white solid (90 mg, 72%). TLC (10% MeOH/DCM) R_f = 0.12; ^{1}H NMR (500 MHz, DMSO-d$_6$) δ 10.78 (br s, 1H, N*H*), 7.93 (s, 1H, *H*8), 6.60 (s, 2H, N*H*$_2$), 6.07 (t, *J* = 6.4 Hz,

1H, *H*1′), 5.16 (t, *J* = 5.4 Hz, 1H, *OH*), 4.57 (m, 1H, *H*3′), 3.87 (q, *J* = 4.6 Hz, 1H, *H*4′), 3.56 (m, 1H, *H*5′), 2.77 (m, 1H, *H*2′), 2.43 (m, 1H, *H*2′); ^{13}C NMR (125 MHz, DMSO-d_6) δ 156.7 (*C*6), 153.8 (*C*2 and *C*4), 150.8 (*C*5), 135.3 (*C*8), 84.5 (*C*1′), 82.1 (*C*4′), 61.3 (*C*3′), 60.9 (*C*5′), 36.2 (*C*2′); LC–MS (ES$^+$): *m*/*z* (%) 152 (100) $[C_5H_6N_5O]^+$, 293 (76) $[M+H]^+$; HRMS (ES$^+$): calcd for $C_{10}H_{13}N_8O_3$ $[M+H]^+$ 293.1105 *m*/*z*, found 293.1094 *m*/*z*.

4.2.6. 5′-*O*-*tert*-Butyldiphenylsilyl-α-thymidine (11)

α-Deoxythymidine (726 mg, 3 mmol) in dry DMF (6 ml) was added to a stirred solution of TBDPS–Cl (780 μl, 3 mmol) and imidazole (450 mg, 6.6 mmol) in dry DMF (6 ml) at rt. After 4 h, water (30 ml) was added and the mixture was extracted with diethyl ether (50 ml × 2). The combined extracts were washed with saturated $NaHCO_3$ (50 ml) and H_2O (50 ml), dried with $MgSO_4$ and reduced in vacuo. Column chromatography with 5% MeOH/DCM as eluent gave the product **11** as a white solid (1.19 g, 83%). TLC (10% MeOH/DCM) R_f = 0.33; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.28 (s, 1H, N*H*), 7.76 (s, 1H, *H*6), 7.44–7.65 (m, 10H, *H*-Ph), 6.20 (q, *J* = 3.5 Hz, 1H, *H*1′), 5.44 (d, *J* = 2.9 Hz, 1H, 3′-O*H*), 4.36 (s, 1H, *H*3′), 4.28 (s, 1H, *H*4′), 3.62–3.65 (m, 2H, *H*5′ and *H*5′), 2.58–2.63 (m, 1H, *H*2′), 1.93–1.96 (m, 1H, *H*2′), 1.78 (s, 3H, CH_3), 1.01 (s, 9H, SiCCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.8 (*C*2), 150.5 (*C*4), 136.9 (*C*6), 135.1, 132.7, 132.6, 130.0, 128.0 (*C*-Ph), 108.7 (*C*5), 88.2 (*C*1′), 85.4 (*C*4′), 70.5 (*C*3′), 64.4 (*C*5′), 39.0 (*C*2′), 26.6 (*C*$(CH_3)_3$), 18.7 (*C*H_3), 12.3 (*C*H_3); LC–MS (ES$^+$): *m*/*z* (%) 481 (100) $[M+H]^+$; HRMS (ES$^+$): calcd for $C_{26}H_{33}N_2O_5Si_1$ $[M+H]^+$ 481.2153 *m*/*z*, found 481.2150 *m*/*z*.

4.2.7. 3′-*O*-Methanesulfonyl-5′-*O*-*tert*-butyldiphenylsilyl-α-thymidine (12)

Methanesulfonic acid (35 μl, 0.53 mmol) was added to a solution of **11** (120 mg, 0.25 mmol) and triphenylphosphine (196 mg, 0.75 mmol) in dry THF (0.8 ml) under Ar. The temperature was raised to 40 °C and DIAD (147 μl, 0.75 mmol) was added dropwise over a period of 12 min with vigorous stirring, and with careful exclusion of moisture. The reaction mixture became a yellow colour, but changed to a white and viscous after 15 min. The mixture was stirred vigorously for 24 h at 40 °C under Ar. The volatiles were then removed under reduced pressure and the residue was re-dissolved in DCM and purified by column chromatography using 5% MeOH in DCM as eluent to give product **12** as a white solid (78 mg, 56%). TLC (10% MeOH/DCM) R_f = 0.22; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.38 (s, 1H, N*H*), 7.60 (s, 1H, *H*6), 7.42–7.67 (m, 10H, *H*-Ph), 6.21 (t, *J* = 6.9 Hz, 1H, *H*1′), 5.50 (q, *J* = 3.8 Hz, 1H, *H*3′), 4.68 (q, *J* = 4.9 Hz, 1H, *H*4′), 3.82–3.86 (m, 1H, *H*5′), 3.73–3.76 (m, 1H, *H*5′), 3.36 (s, 3H, SCH_3), 2.64–2.67 (m, 2H, *H*2′ and *H*2′), 1.81 (s, 3H, CH_3), 1.00 (s, 9H, SiCCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.8 (*C*2), 150.4 (*C*4), 136.7 (*C*6), 135.11, 135.07, 132.51, 132.45, 131.5, 129.95, 127.93, 127.89 (*C*-Ph), 109.7 (*C*5), 84.8 (*C*1′), 81.5 (*C*4′), 80.2 (*C*3′), 62.0 (*C*5′), 37.7, 37.5 (*C*2′ and SCH_3), 26.5 (Si*C*), 18.7 (SiCCH_3), 12.1 (CH_3); LC–MS (ES$^+$): *m*/*z* (%) 559 (100) $[M+H]^+$; HRMS (ES$^+$): calcd for $C_{27}H_{34}N_2O_7S_1Si_1$ $[M+H]^+$ 559.1915 *m*/*z*, found 559.1920 *m*/*z*.

4.2.8. 3′-Azido-5′-*O*-*tert*-butyldiphenylsilyl-3′-deoxy-α-thymidine (13)

A solution of **12** (151 mg, 0.3 mmol) and NaN_3 (39 mg, 0.6 mmol) in DMF (5 ml) was heated to 60 °C overnight. The reaction mixture was evaporated in vacuo with toluene. The solution was cooled and filtered. The filtrate was co-evaporated with toluene to remove DMF. The final azide was purified by column chromatography using 5% MeOH/DCM as eluent to give **13** as a white solid (61 mg, 40%). TLC (10% MeOH/DCM) R_f = 0.28; ^{1}H NMR (500 MHz, $CHCl_3$-d_1) δ 8.94 (s, 1H, N*H*), 7.32–7.58 (m, 10H, *H*-Ph), 7.23 (s, 1H, *H*6), 6.16 (m, 1H, *H1′*), 4.25 (m, 2H, *H*3′ and *H*4′), 3.64 (d, *J* = 3.8 Hz, 2H, *H*5′ and *H*5′), 2.75 (m, 1H, *H*2′), 2.08 (m, 1H, *H*2′), 1.88 (s, 3H, CH_3), 1.00 (s, 9H, SiCCH_3); ^{13}C NMR (125 MHz, $CHCl_3$-d_1) δ 163.9 (*C*4), 150.4 (*C*2), 135.6 (*C*6), 135.5, 135.3, 132.6, 132.4, 130.14, 130.11, 128.0 (*C*-Ph), 110.6 (*C*5), 86.6, 86.5 (*C*1′ and *C*4′), 64.2 (*C*5′), 61.6 (*C*3′), 38.5 (*C*2′), 26.9 (SiCCH_3), 19.2 (*C*H_3), 12.8 (SiCCH_3); LC–MS (ES$^+$): *m*/*z* (%) 506 (100) $[M+H]^+$.

4.2.9. 3′-Azido-3′-deoxy-α-thymidine[30] (4)

Compound **13** (60 mg, 0.12 mmol) was de-protected the TBDPS group by TBAF (120 μl, 0.12 mmol) in 3 ml THF using the procedure of compound **3**, the reaction mixture was then purified by column chromatography using 5% MeOH/DCM as eluent to give the product **4** as a white solid (30 mg, 95%). TLC (10% MeOH/DCM) R_f = 0.17; ^{1}H NMR (500 MHz, acetone-d_6) δ 9.87 (br s, 1H, N*H*), 7.43 (s, 1H, *H*6), 6.06 (q, *J* = 3.8 Hz, 1H, *H*1′), 4.31–4.34 (m, 1H, *H*3′), 4.21 (q, *J* = 4.0 Hz, 1H, *H*4′), 3.52–3.58 (m, 2H, *H*5′ and *H*5′), 2.74–2.75 (m, 1H, *H*2′), 2.05–2.10 (m, 1H, *H*2′), 1.72 (s, 3H, CH_3); ^{13}C NMR (125 MHz, acetone-d_6) δ 164.4 (*C*4), 151.3 (*C*2), 136.6 (*C*6), 110.5 (*C*5), 86.9, 86.6 (*C*1′ and *C*4′), 63.0 (*C*5′), 62.1 (*C*3′), 38.4 (*C*2′), 12.6 (*C*H_3); LC–MS (ES$^+$): *m*/*z* (%) 127 (100) $[C_5H_7N_2O_2]^+$, 268 (38) $[M+H]^+$; HRMS (ES$^+$): calcd for $C_{10}H_{14}N_5O_4$ $[M+H]^+$ 268.1040 *m*/*z*, found 293.1039 *m*/*z*.

4.2.10. Phenyl methoxyalaninyl phosphochloridate (16) and phenyl ethyloxyvalinyl phosphochloridate (17)

The procedure for the preparation of **16** and **17** was carried out according to the literature methods.[23,31]

4.2.11. General procedure: Preparation of 3′-azido-3′-deoxy-nucleoside phosphoramidites

3′-Azido-3′-deoxy-nucleoside (0.3 mmol, 1 equiv) was dissolved in THF (2 ml), and phosphorochloridate (0.9 mmol, 3 equiv) and *N*-methylimidazole (1.8 mmol, 6 equiv) were added dropwise with vigorous stirring in −78 °C. After 12 h, the solvent was removed under vacuum. The residue was dissolved in chloroform (15 ml) and washed with 1 M hydrochloric acid solution (2 × 15 ml), then water (3 × 15 ml). The organic phase was dried with $MgSO_4$ and evaporated under vacuum, and the residue was purified by chromatography on silica by elution with 5% MeOH in DCM to give the final product.

4.2.12. 3′-Azido-5′-phenyl methoxyalaninyl phosphate-3′-deoxy-thymidine (18)

3′-Azido-3′-deoxy-thymidine (80 mg, 0.3 mmol) was reacted with phenyl methoxyalaninyl phosphochloridate **16** (249 mg, 0.9 mmol) to give the final product **18** as a white solid (120 mg, 80%). TLC (10% MeOH/DCM) R_f = 0.52; ^{1}H NMR (500 MHz, $CHCl_3$-d_1) δ 8.73 (d, *J* = 9.1 Hz, 1H, N*H*), 7.09–7.28 (m, 6H, *H*-Ph and *H*6), 6.06–6.13 (m, 1H, *H*1′), 4.17–4.32 (m, 3H, *H*5′, *H*5′ and *H*4′), 3.90–4.03 (m, 2H, *H*3′ and N*H*), 3.75–3.79 (m, 1H, C*H*NH), 3.63–3.70 (m, 3H, CH_3), 2.26–2.36, 2.04–2.16 (m, 2H, *H*2′), 1.78–1.83 (m, 3H, *H*7), 1.28–1.32 (m, 3H, CH_3); ^{13}C NMR (125 MHz, $CHCl_3$-d_1) δ 173.8 (*C*O), 163.5 (*C*4), 150.4, 150.13 (*C*2), 150.10 (*C*-Ph), 135.32, 135.28 (*C*6), 129.9, 125.4, 125.3, 120.10, 120.06, 120.0 (*C*-Ph), 111.44, 111.42 (*C*5), 85.2, 84.9 (*C*1′), 82.43, 82.37, 82.3, 82.2 (C4′), 65.8, 65.72, 65.67 (*C*5′), 60.4, 60.3 (*C*3′), 52.6 (OCH_3), 50.4, 50.2 (*C*HNH), 37.3 (*C*2′), 21.0, 20.94, 20.89 (NHCHCH_3), 12.5, 12.4 (*C*7); ^{31}P NMR (202 MHz, $CHCl_3$-d_1) δ 2.50, 2.80; LC–MS (ES$^+$): *m*/*z* (%) 509 (100) $[M+H]^+$; HRMS (ES$^+$): calcd for $C_{20}H_{26}N_6O_8P_1$ $[M+H]^+$ 509.1544 *m*/*z*, found 509.1563 *m*/*z*.

4.2.13. 3′-Azido-5′-phenyl ethyloxyvalinyl phosphate-3′-deoxy-thymidine (19)

3′-Azido-3′-deoxy-thymidine (80 mg, 0.3 mmol) was reacted with phenyl ethyloxyvalinyl phosphochloridate **17** (287 mg, 0.9 mmol) to give the final product **19** as a white solid (103 mg,

70%). TLC (10% MeOH/DCM) R_f = 0.50; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.34 (s, 1H, N*H*), 7.49 (s, 1H, *H*6), 7.15–7.21, 7.31–7.37 (m, 5H, *H*-Ph), 6.11–6.15 (m, 1H, *H*1′), 5.91–5.97 (m, 1H, N*H*), 4.45 (q, *J* = 1.9 Hz, 1H, *H*3′), 4.20–4.28 (m, 2H, *H*5′), 4.14–4.18 (m, 1H, *H*4′), 3.99–4.15 (m, CH_2), 3.50–3.56 (m, 1H, C*H*NH), 2.29–2.42 (m, 2H, *H*2′and *H*2′), 1.90–1.96 (m, 1H, C*H*), 1.75, 1.77 (s, 3H, *H*7), 1.13–1.18 (m, 3H, CH_3), 0.75–0.88 (m, 6H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.4, 172.2 (*C*O), 163.6 (*C*4), 150.7, 150.6, 150.3 (*C*2 and *C*-Ph), 135.8, 135.7 (*C*6), 129.52, 129.49, 124.59, 124.5, 120.2, 120.1, 120.02, 120.0 (*C*-Ph), 109.91, 109.87 (*C*5), 83.7 (*C*1′), 81.51, 81.45, 81.35 (*C*4′), 65.6, 65.4 (*C*5′), 60.33, 60.29, 60.2, 60.1, 60.0 (*C*HNH and OCH_2CH$_3$), 35.7, 35.6 (*C*2′), 31.4, 31.3, 31.2, 31.1 (CH$_3$*C*HCH$_3$), 18.83, 18.75, 18.03, 17.79 (*C*H$_3$CH*C*H$_3$), 14.0 (OCH$_2$*C*H$_3$), 12.03, 11.99 (*C*7); ^{31}P NMR (202 MHz, DMSO-d_6) δ 4.60, 4.70; LC–MS (ES$^+$): *m*/*z* (%) 551 (100) [M+H]$^+$; HRMS (ES$^+$): calcd for $C_{23}H_{32}N_6O_8P_1$ [M+H]$^+$ 551.2014 *m*/*z*, found 551.2002 *m*/*z*.

4.2.14. 5′-Phenyl methoxyalaninyl phosphate-2′,3′-didehydro-3′-deoxythymidine (20)

2′,3′-Didehydro-3′-deoxythymidine (40 mg, 0.18 mmol) was reacted with phenyl methoxyalaninyl phosphochloridate **16** (149 mg, 0.54 mmol) to give the final product **20** as white solid (63 mg, 75%). TLC (10% MeOH/DCM) R_f = 0.45; ^{1}H NMR (500 MHz, CHCl$_3$-*d*$_1$) δ 8.29 (d, *J* = 16.1 Hz, 1H, N*H*), 7.18–7.37 (m, 6H, *H*-Ph and *H*6), 7.03–7.07 (m, 1H, *H*3′), 6.30–6.31 (m, 1H, *H*1′), 5.92 (q, *J* = 6.45 Hz, 1H, *H*2′), 5.04 (q, *J* = 5.81 Hz, 1H, *H*4′), 4.26–4.35, 4.39–4.43 (m, 2H, *H*5′), 3.96–4.05 (m, 1H, C*H*NH), 3.73 (d, *J* = 5.85 Hz, 1H, OC*H*$_3$), 3.57–3.69 (m, 1H, N*H*), 1.84, 1.89 (d, *J* = 0.8 Hz, 3H, *H*7), 1.36 (q, *J* = 10.0 Hz, 3H, CHC*H*$_3$); ^{13}C NMR (125 MHz, CHCl$_3$-*d*$_1$) δ 173.91, 173.85 (*C*O), 163.40, 163.35 (*C*4), 150.62, 150.57 (*C*2 and *C*-Ph), 135.9, 135.6 (*C*6), 133.4, 133.1 (*C*3′), 129.82, 129.77 (*C*2′), 127.5, 127.3, 125.28, 125.22, 120.19, 120.15, 120.07, 120.03 (*C*-Ph), 111.4, 111.3 (*C*5), 89.9, 89.6 (*C*1′), 84.72, 84.65, 84.57 (*C*4′), 67.2, 67.1, 66.6, 66.5 (*C*5′), 52.62, 52.60 (*C*HNH), 50.2, 50.1 (OCH_3), 21.05, 21.01, 20.98, 20.94 (NHCH*C*H$_3$), 12.4, 12.3 (*C*7); ^{31}P NMR (202 MHz, CHCl$_3$-*d*$_1$) δ 2.46, 3.10; LC–MS (ES$^+$): *m*/*z* (%) 466 (100) [M+H]$^+$; HRMS (ES$^+$): calcd for $C_{20}H_{24}N_3O_8P_1$ [M+H]$^+$ 466.1374 *m*/*z*, found 466.1356 *m*/*z*.

4.3.15. 5′-Phenyl ethyloxyvalinyl phosphate-2′,3′-didehydro-3′-deoxythymidine (21)

2′,3′-Didehydro-3′-deoxythymidine (40 mg, 0.18 mmol) was reacted with phenyl ethyloxyvalinyl phosphochloridate **17** (0.54 mmol) to give the final product **21** as a solid (50 mg, 60%). TLC (10% MeOH/DCM) R_f = 0.45; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.37 (s, 1H, N*H*), 7.30–7.36, 7.15–7.18 (m, 6H, *H*-Ph and *H*6), 6.84 (d, *J* = 1.9 Hz, 1H, *H*3′), 6.38–6.44 (m, 1H, *H*2′), 6.01 (d, *J* = 1.9 Hz, 1H, *H*1′), 5.87–5.93 (m, 1H, N*H*), 4.97, 5.01 (s, 1H, *H*4′), 3.95–4.27 (m, 4H, *H*5′ and OCH_2CH$_3$), 3.42–3.49 (m, 1H, C*H*NH), 1.84–1.92 (m, 1H, CH$_3$CH_2CH$_3$), 1.66, 1.70 (d, *J* = 0.6 Hz, 3H, *H*7), 1.11–1.17 (m, 3H, OCH$_2$CH_3), 0.72–0.82 (m, 6H, CH_3CHCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.5, 172.2 (*C*O), 163.77, 163.75 (*C*4), 150.8, 150.54, 150.50 (*C*2 and OC*P*h), 136.0, 135.9 (*C*6), 133.5, 133.4 (*C*3′), 129.50, 129.48 (*C*2′), 126.93, 126.90, 124.6, 124.5, 120.19, 120.15, 120.0, 119.9 (*C*-Ph), 109.69, 109.66 (*C*5), 89.0, 88.9 (*C*1′), 84.4, 84.34, 84.27 (*C*4′), 66.8, 66.2 (*C*5′), 60.4, 60.24, 60.17, 60.0 (*C*HNH), 54.9 (OCH_2CH$_3$), 31.4, 31.3, 31.23, 32.17 (CH$_3$*C*HCH$_3$), 18.8, 18.7, 18.1, 17.8 (*C*H$_3$CHCH_3), 14.03, 13.98 (OCH$_2$*C*H$_3$), 11.94, 11.91 (*C*7); ^{31}P NMR (202 MHz, DMSO-d_6) δ 4.11, 4.65; LC–MS (ES$^+$): *m*/*z* (%) 508 (100) [M+H]$^+$; HRMS (ES$^+$): calcd for $C_{23}H_{31}N_3O_8P_1$ [M+H]$^+$ 508.1843 *m*/*z*, found 508.1838 *m*/*z*.

4.3.16. 3′-Azido-5′-phenyl ethyloxyvalinyl phosphate-2′,3′-dideoxyguanidine (22)

^{t}BuMgCl (30 μl, 0.3 mmol) and 3′-azido-2′,3′dideoxyguanidine (44 mg, 0.15 mmol) were dissolved in dry THF (3.5 ml) and stirred for 15 min. Then phenyl ethyloxyvalinyl phosphochloridate **17** (95 mg, 0.3 mmol) in dry THF was added dropwise and stirred overnight. A saturated solution of NH_4Cl was added, and the solvent was removed under reduced pressure to give the final **22** as a white solid (40 mg, 46%). TLC (10% MeOH/DCM) R_f = 0.31; ^{1}H NMR (500 MHz, acetone-d_6) δ 11.20 (s, 1H, N*H*); 7.79 (s, 1H, *H*8), 6.97–7.23 (m, 5H, *H*-Ph), 6.54, 6.61 (s, 2H, NH_2), 6.12–6.15 (m, 1H, *H*1′), 4.81–4.86 (m, 1H, N*H*), 4.66–4.69 (m, 1H, *H*3′), 4.41–4.45, 4.53–4.58 (m, 1H, *H*3′), 4.13–4.17, 4.20–4.27 (m, 1H, *H*5′), 4.03–4.06, 4.08–4.11 (m, 1H, *H*4′), 3.90–4.02 (m, 2H, CH_2CH$_3$), 3.57–3.65 (m, 1H, C*H*NH), 2.81–2.88, 2.91–2.97 (m, 1H, *H*2′), 2.39–2.46 (m, 1H, *H*2′), 1.84–1.90 (m, 1H, CH$_3$C*H*CH$_3$), 1.02–1.07 (m, 3H, CH$_2$CH_3), 0.72–0.77 (m, 6H, CH_3CHCH_3); ^{13}C NMR (125 MHz, acetone-d_6) δ 172.48, 172.45, 172.3 (*C*O), 157.7 (*C*6), 153.91, 153.87 (*C*2 and *C*4), 151.22, 151.17, 151.1, 150.9, 150.8 (*C*5 and *C*-Ph), 136.7, 136.4 (*C*8), 129.52, 129.45, 124.6, 120.5, 120.44, 120.39, 120.35, 117.8, 117.7 (*C*-Ph), 84.4, 84.2 (*C*1′), 82.82, 82.81, 82.7 (*C*4′), 66.0, 65.92, 65.89, 65.85 (*C*5′), 61.7 (*C*3′), 60.59, 60.57, 60.40, 60.36 (*C*HNH and OCH_2CH$_3$), 36.4, 36.2 (*C*2′), 32.0, 31.9, 31.8, 31.7 (CH$_3$CHCH$_3$), 18.5, 17.3, 17.1 (*C*H$_3$CHCH_3), 13.64, 13.62 (CH$_2$*C*H$_3$); ^{31}P NMR (202 MHz, acetone-d_6) δ 4.38, 4.63, 4.87; LC–MS (ES$^+$): *m*/*z* (%) 576 (100) [M+H]$^+$; HRMS (ES$^+$): calcd for $C_{23}H_{31}N_9O_7P_1$ [M+H]$^+$ 576.2079 *m*/*z*, found 576.2070 *m*/*z*.

4.3.17. 3′-Azido-5′- phenyl ethyloxyvalinyl phosphate-3′-deoxy-α-thymidine (23)

3′-Azido-3′-deoxy-α-thymidine (38 mg, 0.14 mmol) was reacted with phenyl ethyloxyvalinyl phosphochloridate **17** (134 mg, 0.42 mmol) to give the final product **23** as a white solid (40 mg, 52%). TLC (10% MeOH/DCM) R_f = 0.34; ^{1}H NMR (500 MHz, CHCl$_3$-*d*$_1$) δ 9.21, 9.18 (s, 1H, N*H*), 7.09–7.30 (m, 6H, *H*-Ph and *H*6), 6.02–6.06 (m, 1H, *H*1′), 4.30 (d, *J* = 2.5 Hz, 1H, *H*4′), 4.04–4.18 (m, 5H, *H*3′, *H*5′, *H*5′ CH_2CH$_3$), 3.66–3.79 (m, 2H, C*H*NH, N*H*), 2.64–2.70, 2.38–2.44 (m, 1H, *H*2′), 1.91–2.09 (m, 2H, CH$_3$C*H*CH$_3$, *H*2′), 1.86, 1.87 (d, *J* = 1.1 Hz, 3H, CH_3), 1.16–1.24 (m, 3H, CH_3), 0.80–0.87 (m, 6H, CH_3); ^{13}C NMR (125 MHz, CHCl$_3$-*d*$_1$) δ 172.8, 172.7 (*C*O), 163.89, 163.86 (*C*4), 150.7, 150.6 (*C*2), 150.4, 150.3 (*C*-Ph), 135.2, 135.1 (*C*6), 129.84, 129.80, 125.3, 125.2, 120.34, 120.30, 120.04, 120.00 (*C*-Ph), 111.0, 110.8 (*C*5), 86.5, 86.4 (*C*1′), 84.2, 84.1 (*C*4′), 66.1, 66.0 (*C*5′), 61.5, 61.4 (*C*3′), 61.0 (*C*H$_2$CH$_3$), 60.2, 60.0 (*C*HNH), 37.9 (*C*2′), 32.1, 32.03, 31.98 (CH$_3$CHCH$_3$), 19.0, 17.3 (CH$_3$CHCH_3), 14.3, 14.2 (CH$_2$CH_3), 12.7 (*C*7′); ^{31}P NMR (202 MHz, CHCl$_3$-*d*$_1$) δ 3.66, 4.00; LC–MS (ES$^+$): *m*/*z* (%) 568 (100) [M+NH$_3$]$^+$, 551 (73) [M+H]$^+$; HRMS (ES$^+$): calcd for $C_{23}H_{32}N_6O_8P_1$ [M+H]$^+$ 551.2014 *m*/*z*, found 551.2026 *m*/*z*.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.07.006.

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