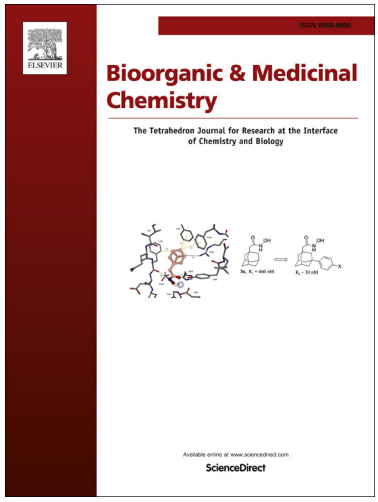

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Synthesis and evaluation of sulfonylethyl-containing phosphotriesters of 3'-azido-3'-deoxythymidine as anticancer prodrugs

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Abstract

A series of bis(sulfonylethyl) and mono(sulfonylethyl) phenyl phosphotriesters of zidovudine (3'-azido-3'-deoxythymidine, AZT) were synthesized as potential anticancer prodrugs that liberate AZT monophosphate via nonenzymatic β-elimination mechanism. Stability studies demonstrated that all the synthesized prodrugs spontaneously liberate AZT monophosphate with half-lives in the range of 0.07-278.8 h under model physiological conditions in 0.1 M phosphate buffer at pH 7.4 and 37 ^{o}C. Analogous to aldophosphamide, the elimination rates were accelerated in the presence of reconstituted human plasma under the same conditions. Among the compounds, **3**, **4**, **8**, and **10** were comparable or superior to AZT against five established human cancerous cell lines in vitro. Moreover, the selected compounds were equally sensitive to both the wild-type osteosarcoma 143B and the thymidine kinase-deficient 143B/TK^{-} cell lines. The findings are consistent with that these compounds deliver AZT monophosphate intracellularly.

Key words:

AZT; AZT monophosphate; sulfonylethyl; phosphotriester; pronucleotide; β-elimination; prodrugs.

1. Introduction

Zidovudine (3'-azido-3'-deoxythymidine, AZT), a nucleoside reverse transcriptase inhibitor, was the first drug approved by FDA for the treatment of HIV/AIDS infections. However, interest in repositioning AZT as an anticancer agent has emerged since 1989.[1] It was reported that AZT could potentiate the anticancer effects of other chemotherapeutic agents such as paclitaxel, 5-fluorouracil, emodin, and cisplatin.[2-5] When used alone, AZT exhibited potent inhibitory activity against human breast cancer, melanoma, and multiple myeloma cell growth.[6-8] The anticancer activity of AZT may be associated with its intracellular conversion to 5'-triphosphate, which functions as a competitive inhibitor of DNA polymerase, and it incorporates itself into the growing DNA strand.[9] It terminates DNA chain replication, which induces apoptosis of the target cells. AZT triphosphate was also found to inhibit human telomerase activity in many cancer cell lines in vitro.[10-12] Approximately 90% of all human cancers exhibit enhanced telomerase activity, and it is proposed that telomerase may be involved in cell immortalization, tumorigenesis and inhibition of apoptosis.[11]

Cellular drug resistance, however, can develop due to prolonged exposure to AZT, which enhances the cellular expression of P-glycoprotein (P-gp), multidrug resistance proteins (MRPs), and breast cancer resistance protein (BCRP), which in turn cause efflux of AZT and its metabolites from the cells.[13-16] In AZT-resistant cells the activity of thymidine kinase is reduced, and prolonged exposure of cells to AZT results in decreased levels of thymidine kinase,[17] the enzyme responsible for the intracellular transformation of AZT into AZT monophosphate. This phosphorylation is essential to the activation of the drug to AZT triphosphate. Moreover, AZT possesses such undesirable pharmacokinetic properties as short half-life and small volume of distribution.[18]

In order to improve the pharmacokinetic profile of AZT and overcome the drawbacks of AZT-based therapy, many pronucleotides of AZT have been developed, and their anticancer activities have been investigated. Iyer et al. evaluated the anticancer activity of a series of AZT phosphoramidate monoesters containing amino acid methyl ester and *N*-alkylamide moieties and found that the synthesized prodrugs were less potent than AZT against MCF-7 and CEM cell lines.[18] When the cytotoxicity of AZT 5'-chloromethylphosphonates against the human cancer cell lines KB and MCF-7 were examined by Celewicz et al., the phosphonates exhibited less anticancer activity than did AZT, and the hydrolysis studies showed that these pronucleotides act as depot forms of AZT rather than prodrugs of AZT 5'-phosphate.[9] More recently, Liu et al. studied a series of AZT phosphorothioamidates and proposed that these pronucleotides were able to release AZT monothiophosphate inside the cell.[19] These prodrugs presented selective toxicity toward cancer cells, as they were more potent than AZT against RKO colon carcinoma, but less cytotoxic than AZT towards 293T embryonic kidney cells.[19]

Substituted sulfonylethyl groups have successfully been employed in our laboratory for the intracellular delivery of phosphate-containing drug molecules in the design of phosphoramide mustard prodrugs[20] and 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP) prodrugs.[21] Now we applied the same approach to AZT, synthesizing and evaluating a series of bis(sulfonylethyl) phosphotriesters of AZT (Figure 1A). The proposed activation mechanism for the these prodrugs involves two successive β-elimination steps to release AZT monophosphate (Scheme 1A). Moreover, phosphodiesterases may have great influence on the activity of the pronucleotides.[22] The phenyl phosphodiester of AZT proved to be a good substrate of type I phosphodiesterase and was hydrolyzed rapidly in the presence of the enzyme.[23] Therefore, mono(sulfonylethyl) phenyl phosphotriesters of AZT (Figure 1B) were also prepared and investigated. The

decomposition of the mono(sulfonylethyl) phenyl prodrugs is expected to follow β-elimination and subsequent phosphodiesterase-hydrolyzing steps (Scheme 1B).

2. Results and Discussion

2.1. Chemistry

All the sulfonylethyl-containing phosphotiesters were synthesized using P(III) (phosphoramidite) chemistry. 2-(phenylthio)ethanol (**1c**) is commercially available. Reaction of 2-chloroethanol with corresponding *p*-substituted thiophenols yielded compounds **2c**-**5c** (Scheme 2). Thiophenol and allyl alcohol were used to prepare 2-(phenylthio)propan-1-ol (**6c**) with sulfur as catalyst (Scheme 3). Other α- and β-substituted thioethanols, compounds **7c**-**10c**, were prepared by adding suitable epoxides to sodium thiophenolate in methanol (Scheme 4). Phosphoramidite intermediates **1b**-**15b** were prepared via two synthetic steps (Scheme 5). In the first step, crystalline bis(diisopropylamino)chlorophosphine was added to the mixture of dry triethylamine and thioethanol derivatives, yielding the bis(diisopropylamino)alkoxyphosphine intermediates. In the second step, the crude products were treated with either thioethanol or phenol derivatives in the presence of the acid catalyst 4,5-dicyanoimidazole (DCI). Intermediates **1b**-**15b** were coupled with AZT using 1*H*-tetrazole in anhydrous acetonitrile, followed by in situ oxidation using *tert*-butyl hydroperoxide (TBHP), resulting in the formation of the phosphotriester derivatives **1a**-**15a**. The thioethyl groups were then oxidized to form sulfonylethyl groups using an excess of *meta*-chloroperoxybenzoic acid (*m*CPBA) to obtain the target compounds **1**-**15** (Scheme 6).

Some compounds were obtained as mixtures of diastereoisomers due to the asymmetrical center formed at the phosphorus atom in the reactions. Moreover, more than two signals were present in ^{31}P NMR spectra of compounds **7**, **8**, and **10** due to the chiral α- and β-carbons of their

sulfonylethyl moieties. Unfortunately, these compounds could not be isolated by preparative TLC or column chromatography and further purification was not attempted. All the synthesized analogues presumably permeate the cell membrane by passive diffusion and release the identical mononucleotide in the cell.

2.2. Stability studies

Stability studies were conducted to determine the half-lives of the synthesized pronucleotides in 0.1 M phosphate buffer at pH 7.4 and 37 ^{o}C and in the presence of human plasma under the same conditions. The results are presented in Table 1.

As anticipated, the phosphotriesters bearing various substituted sulfonylethyl moieties possess different kinetic properties. Compound **2** with a *p*-methyl group was observed to have a slightly longer half-life than compound **1**. The half-life of compound **3** with a *p*-methoxy substitution was twice of that of compound **1**. The electron-donating methyl and methoxy groups make the α-proton of the sulfonylethyl moiety less acidic, resulting in decreased β-elimination rates. Since methoxy is more electron-donating than methyl, compound **3** had a longer half-life than compound **2**. Conversely, the electron-withdrawing *p*-chloro group in **4** and *p*-nitro group in **5** enhance the acidity of α-proton; as a result, compounds **4** and **5** showed higher β-elimination rates than compound **1**. The *p*-substituents confer the β-elimination reactivity in the following order: -NO_2 > -Cl > -Me > -OMe. All the α- and β- substituted analogues provided longer half-lives than the nonsubstituted compound **1**. The α-methyl-substituted prodrug **6** exhibited a much slower elimination rate compared to its β-methyl-substituted counterpart, prodrug **7**. Combination of the steric hindrance, the reduced acidity of α-proton due to the presence of methyl group and the availability of only one α-proton in compound **6** could explain the retarded elimination rate. Compound **8**, with a β-ethyl substitution, showed more chemical stability than

compound **7** because the steric hindrance provided by the ethyl group was more prominent than it was in the methyl group. Both α- and β-methyl substitutions made compound **9** extremely stable: the half-life was approximately 12 days in the phosphate buffer. Unexpectedly, the α-phenyl-substituted prodrug **10** had a much shorter half-life than compound **6**, which has a much smaller substitution at the α position. We had assumed that the electron-inductive effect of the α-phenyl group might increase the acidity of α-proton.

All the mono(sulfonylethyl)phenyl phosphotriesters **11**-**15** exhibited longer half-lives than compound **1**. The half-lives varied slightly, depending on the *p*-substituents on the phenoxy group. The overall trend was that the electron-donating *p*-substitutions decelerated the β-elimination rates. Conversely, electron-withdrawing *p*-substitutions accelerated the elimination rates. Since the half-lives of the analogues were determined by the rate of disappearance of the remaining prodrugs, not the generation of nucleoside monophosphate, it might be argued that the half-lives of these mono(sulfonylethyl) phenyl phosphotriesters could only give the information for the β-elimination rates of the sulfonylethyl group, but the rates of actual release of monophosphate was still not established. Mono(thioethyl) phenyl phosphotriester **12a** which lacks a sulfonyl group displayed much greater stability. It had a half-life 12 h in phosphate buffer, and was stable for 20 h in human plasma, suggesting that the cleavage of the phenoxy group from the phosphate constituted a rate-limiting step for the liberation of AZT monophosphate.

All the synthesized phosphotriesters of AZT displayed a similarly accelerated β-elimination profile in plasma. The extent of enhancement of β-elimination reaction was 2.2-4.2 times in plasma what it was in the phosphate buffer. The results were consistent with the previous findings: plasma proteins, specifically, human serum albumin (HSA), contribute to the faster

elimination of cyclophosphamide, sulfonylethyl analogues of phosphoramide mustard and FdUMP.[20, 21, 24]

2.3. Anticancer activity

The sulfonylethyl prodrugs were investigated by employing MTT assay for their cytotoxic activity against five human cancer cell lines: MCF-7 (breast adenocarcinoma), H460 (lung carcinoma), KB-3-1 (epidermoid carcinoma), SW620 (colorectal adenocarcinoma), and 143B (osteosarcoma). AZT was used as a positive control. The IC_{50} values are presented in Table 2.

Cytotoxicity of AZT and all the synthesized pronucleotides is directly associated with the intracellular level of AZT monophosphate. As all the phosphotriesters were designed to release active metabolite AZT monophosphate inside the cell, their ability to penetrate the cell membrane and rate of intracellular conversion are of great importance in determining their cytotoxic effects.

AZT and all the synthesized compounds displayed the same trend of cytotoxic effects on the tested cell lines. They were most sensitive to H460 cells but displayed highest IC_{50} values in SW620 cells. Compounds **1** and **2** were slightly less toxic than AZT to all the cell lines. Compounds **3** and **4** exhibited higher potency compared to AZT against all the cell lines. Compound **5**, with *p*-nitro substitution, was approximately half as active as AZT. No direct relationship between their stability and cytotoxicity can be deduced based on these results, but a compound with an extremely short half-life may not be a good drug candidate as active AZT monophosphate might be released more in the extracellular medium than in the intracellular compartment. The α-methyl-substituted analogue **6** showed higher IC_{50} value than the β-methyl-substituted analogue **7**, but both were less cytotoxic than AZT. However, analogue **8**, which displayed higher stability than **7**, exhibited slightly better activity than AZT. Compound **9**, which

had an extremely long half-life, was 1/3 to 1/2 as potent against all the cell lines as was AZT. The α-phenyl-substituted analogue **10** was as potent as AZT. Since the intracellular conversion rate might be slow in the prodrugs with extremely high stability, as in the cases of compounds **6** and **9**, the acute toxicity of these prodrugs was reduced due to there being less AZT monophosphate present in the target cells. Moreover, compounds **8** and **10** possess higher lipophilicity due to the presence of β-ethyl and α-phenyl groups, respectively, which may allow for the increased permeation through the cell membrane. Thus lower IC_{50} values were displayed by compounds **8** and **10** than by the other α- and β-substituted sulfonylethyl analogues.

The mono(sulfonylethyl) phenyl phosphotriesters **11**-**15** presented very similar potency, perhaps because of the slow intracellular liberation of AZT monophosphate. Unlike bis(sulfonylethyl) prodrugs which require two consecutive β-elimination steps to liberate active AZT monophosphate, the activation of this series of compounds is a phosphodiesterase-involved process. The level of phosphodiesterases in the target cells might be a determining factor in the intracellular generation of AZT monophosphate.

In order to further probe the mode of action of the phosphotriester prodrugs, we evaluated compounds **3**, **4**, **8**, **10**, **13**, and AZT against both a parental 143B cell line and thymidine kinase-deficient 143B/TK^- cell line. The data are shown in Table 3. The IC_{50} value of AZT for 143B/TK^- cells is more than 12 times as high as that for wild-type 143B cells. However, the IC_{50} values of all the selected analogues were not significantly different for 143B and 143B/TK^- cells. In other words, these compounds could reverse the cellular resistance to AZT. This confirmed that the mechanism of action of these phosphotriester prodrugs involved the intracellular liberation of AZT monophosphate.

3. Conclusion

The phosphotriesters bearing sulfonylethyl pro-moieties were successfully applied to AZT as anticancer prodrugs. Efficient preparation of these compounds were achieved by employing bis(diisopropylamino)chlorophosphine as a phosphitylating agent via P(III) chemistry. Stability studies showed that the half-lives of these compounds vary in a substituent-dependent manner. Higher β-elimination rates were observed for all compounds in model physiological phosphate buffer solution as well as in the presence of human plasma at pH 7.4 and 37 ^{o}C. The in vitro anticancer activities of these compounds were evaluated against MCF-7, H460, KB-3-1, SW620, and 143B cancer cell lines. Compounds **3**, **4**, and **8** were more cytotoxic to five human cancer cell lines than AZT. Compound **3** was most potent among all the synthesized prodrugs. AZT was less than 1/12 as effective against thymidine kinase-deficient 143B/TK^{-} cells as it was against wild-type 143B cells, while all the selected prodrugs were equally potent against both cell lines. These results were consistent with an activation mechanism that involves intracellular delivery of AZT monophosphate, which circumvents the first phosphorylation step.

4. Experimental section

4.1. Chemistry

All water-sensitive experiments were conducted under scrupulously anhydrous conditions using oven-dried glassware. Moisture-sensitive reactions were performed under nitrogen atmosphere. Triethylamine was dried by refluxing over calcium hydride and stored over activated 4Å molecular sieves. Evaporation of solvents was carried out on a rotary evaporator under reduced pressure. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and were uncorrected. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker spectrometer (^{1}H, 400 MHz; ^{13}C, 100 MHz). All ^{13}C and ^{31}P NMR spectra were recorded proton-decoupled. All NMR spectra were run at ambient temperature. Chemical shifts of ^{1}H

NMR spectra are given in δ values relative to tetramethylsilane (TMS) peak used as the internal reference. Chemical shifts of ^{31}P NMR spectra are reported relative to external reference phosphoric acid. Abbreviations for the signal patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quin), sextet (sex), septet (sep), multiplet (m), broad (br), tertiary carbons (C tert), quaternary carbons (C quat). Many ^{1}H NMR signals were split due to the presence of phosphate diastereoisomers in the samples. Elemental analyses were carried out by Atlantic Microlab Inc., Norcross, GA and the observed values were within ±0.4% of the calculated values. The column chromatography refers to flash chromatography carried out using Merck silica gel 60 (40-60 μm) as stationary phase. Preparative thin-layer chromatography was performed on Merck pre-coated glass plates (20 × 20 cm, silica gel GF, 1000 μm thick) purchased from Analtech Inc., Newark, DE, and visualized under 254 nm UV light. AZT was purchased from AK Scientific, Inc., Union City, CA.

4.1.1. Standard procedure 1: preparation of *p*-substituted phenylthioethanol (2c-5c)

Compounds **2c**-**5c** were prepared according to the published procedure.[21]

4.1.1.1. 2-(*p*-Tolylthio)ethanol (2c). Starting with 2-chloroethanol (2.42 g, 30 mmol) and *p*-thiocresol (2.48 g, 20 mmol), product **2c** was obtained as a pale yellow oil (2.68 g, 80%). R_f 0.36 (EtOAc/n-hexane = 1:4); ^{1}H NMR (DMSO) δ 7.26 (2H, m), 7.12 (2H, d, J = 8.4Hz), 3.56 (2H, t, J = 7.2 Hz), 2.99 (2H, t, J = 6.8Hz), 2.26 (3H, s).

4.1.1.2. 2-(*p*-Methoxyphenylthio)ethanol (3c). Starting with 2-chloroethanol (2.42 g, 30 mmol) and 4-methoxythiophenol (2.80 g, 20 mmol), product **3c** was obtained as a colorless solid (2.76 g, 75%). mp 40-41 ºC; R_f 0.28 (EtOAc/n-hexane = 1:4); ^{1}H NMR (DMSO) δ 7.35 (2H, m), 6.91 (2H, m), 3.74 (3H, s), 3.50 (2H, m), 2.91(2H, t, J = 7.0 Hz).

4.1.1.3. 2-(*p*-Chlorophenylthio)ethanol (4c). Starting with 2-chloroethanol (2.42 g, 30 mmol) and 4-chlorothiophenol (2.89 g, 20 mmol), product **4c** was obtained as a pale yellow oil (2.64 g, 70%). R_f 0.24 (EtOAc/n-hexane = 1:4); ^{1}H NMR (DMSO) δ 7.36 (4H, m), 3.58 (2H, q, J = 6.4 Hz), 3.06 (2H, t, J = 6.7 Hz).

4.1.1.4. 2-(*p*-Nitrophenylthio)ethanol (5c). Starting with 2-chloroethanol (2.42 g, 30 mmol) and 4-nitrothiophenol (3.10 g, 20 mmol), product **5c** was obtained as a yellow solid (2.03 g, 51%). mp 61-62 ºC; R_f 0.30 (EtOAc/n-hexane = 1:1); ^{1}H NMR (DMSO) δ 8.12 (2H, m), 7.51 (2H, m), 3.66 (2H, m), 3.21 (2H, t, J = 6.4 Hz).

4.1.2. Preparation of 2-(phenylthio)propan-1-ol (6c)

Compound **6c** was synthesized according to the procedure described previously.[25] Starting with thiophenol (11 g, 0.1 mol) and allyl alcohol (6.96 g, 0.12 mol), product **6c** was obtained as a pale yellow liquid (2.01 g, 12%). R_f 0.50 (EtOAc/n-hexane = 1:3); ^{1}H NMR (DMSO) δ 7.38 (2H, d, J = 7.2 Hz), 7.32 (2H, t, J = 7.6 Hz), 7.23 (1H, t, J = 7.2 Hz), 3.52 (1H, m), 3.31 (2H, m), 1.23 (3H, d, J = 6.1 Hz); ^{13}C NMR (DMSO) δ 134.9 (C quat), 130.4 (C tert), 128.9 (C tert), 126.4 (C tert), 65.1 (C1), 43.9 (C2), 17.6 (CH_3).

4.1.3. Standard procedure 2: preparation of 2-(phenylthio)ethanol derivatives (7c-10c)

Compounds **7c-10c** were synthesized according to the published procedure.[26]

4.1.3.1. 1-(Phenylthio)propan-2-ol (7c). Starting with sodium thiophenolate (1.58 g, 12 mmol) and propylene oxide (0.58 g, 10 mmol), product **7c** was obtained as a pale yellow oil (1.49 g, 89%). R_f 0.50 (EtOAc/n-hexane = 1:3); ^{1}H NMR (DMSO) δ 7.32 (4H, m), 7.16 (1H, t, J = 7.0 Hz), 3.78 (1H, sep, J = 5.9 Hz), 2.96 (2H, m), 1.16 (3H, d, J = 6.3 Hz); ^{13}C NMR (DMSO) δ 136.9 (C quat), 128.9 (C tert), 127.8 (C tert), 125.3 (C tert), 65.2 (C1), 41.0 (C2), 22.5 (CH_3).

4.1.3.2. 1-(Phenylthio)butan-2-ol (8c). Starting with sodium thiophenolate (1.58 g, 12 mmol) and 1,2-butylene oxide (0.72 g, 10 mmol), product **8c** was obtained as a pale yellow oil (1.26 g, 69%). R_f 0.44 (EtOAc/n-hexane = 1:4); ^{1}H NMR (DMSO) δ 7.31 (4H, m), 7.16 (1H, t, J = 7.0 Hz), 3.52 (1H, sex, J = 6.0 Hz), 2.97 (2H, d, J = 6.1 Hz), 1.58 (1H, m), 1.38 (1H, m), 0.88 (3H, t, J = 7.3 Hz).

4.1.3.3. 3-(Phenylthio)butan-2-ol (9c). Starting with sodium thiophenolate (1.58 g, 12 mmol) and 2,3-epoxybutane (0.72 g, 10 mmol), product **9c** was obtained as a pale yellow oil (1.33 g, 73%). R_f 0.42 (EtOAc/n-hexane = 1:4); ^{1}H NMR (DMSO) δ 7.37 (2H, d, J = 7.2 Hz), 7.32 (2H, t, J = 7.6 Hz), 7.21 (1H, t, J = 7.2 Hz), 3.76 (1H, sex, J = 5.5 Hz), 3.35 (1H, m), 1.21 (3H, d, J = 6.8 Hz), 1.12 (3H, d, J = 6.3 Hz).

4.1.3.4. 2-Phenyl-2-(phenylthio)ethanol (10c). Starting with sodium thiophenolate (1.58 g, 12 mmol) and styrene oxide (1.20 g, 10 mmol), product **10c** was obtained as a pale yellow oil (0.92 g, 40%). R_f 0.36 (EtOAc/n-hexane = 1:4); ^{1}H NMR (DMSO) δ 7.30 (10H, m), 4.45 (1H, t, J = 6.6 Hz), 3.77 (2H, m).

4.1.4. Standard procedure 3: preparation of phosphoramidites (1b-15b)

The phosphoramidite intermediates **1b**-**15b** were prepared by a modified procedure previously described.[27] To a stirred solution of thioethanol (4.0 mmol) in anhydrous THF (20 mL) containing triethylamine (6.06 g, 6 mmol) was added bis(diisopropylamino)chlorophosphine (1.28 g, 4.8 mmol) at room temperature under N_2. After 2h, TLC analysis indicated complete conversion of the alcohol into a product with a higher R_f value. After removal of the $(Et_3)N{\cdot}HCl$ salt by filtration and concentration of the filtrate under reduced pressure afforded the product as viscous liquid.

The residue was dissolved in anhydrous CH_2Cl_2 (20 mL) and the corresponding alcohol (3.2 mmol) together with 4,5-dicyanoimidazole (1.89 g, 1.6 mmol) was added at ambient temperature and stirred for 30 min. The resulting mixture was washed with cold saturated $NaHCO_3$ solution (2 × 20 mL) and cold saturated NaCl solution (20 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by preparative TLC with an appropriate solvent containing 1% *N,N*-dimethylethylamine to obtain the products.

4.1.4.1. Bis[2-(phenylthio)ethyl] *N,N*-diisopropylphosphoramidite (1b). Product **1b** was obtained as a yellow viscous liquid (0.81 g, 58%). R_f 0.86 (EtOAc/n-hexane = 1:4 containing 1% *N,N*-dimethylethylamine); ^{1}H NMR ($CDCl_3$) δ 7.36 (4H, d, J = 7.1 Hz), 7.26 (4H, t, J= 7.6 Hz), 7.17 (2H, t, J = 7.3 Hz), 3.85-3.67 (4H, m), 3.61-3.50 (2H, m), 3.18-3.08 (4H, m), 1.12 (12H, d, J = 6.8 Hz); ^{31}P NMR ($CDCl_3$) δ 147.74.

4.1.4.2. Bis[2-(*p*-tolylthio)ethyl] *N,N*-diisopropylphosphoramidite (2b). Product **2b** was obtained as a yellow viscous liquid (0.86 g, 58%). R_f 0.88 (EtOAc/n-hexane = 1:4 containing 1% *N,N*-dimethylethylamine); ^{1}H NMR ($CDCl_3$) δ 7.31 (4H, d, J = 7.2 Hz), 7.11 (4H, d, J = 7.3 Hz), 3.81-3.73 (4H, m), 3.58-3.48 (2H, m), 3.13-3.09 (4H, m), 2.34 (6H, s), 1.16 (12H, d, J = 6.8 Hz); ^{31}P NMR ($CDCl_3$) δ 147.90.

4.1.4.3. Bis[2-(*p*-methoxyphenylthio)ethyl] *N,N*-diisopropylphosphoramidite (3b). Product **3b** was obtained as a yellow viscous liquid (1.04 g, 65%). R_f 0.81 (EtOAc/n-hexane = 1:4 containing 1% *N,N*-dimethylethylamine); ^{1}H NMR ($CDCl_3$) δ 7.36 (4H, d, J = 6.7 Hz), 6.82 (4H, d, J = 6.9 Hz), 3.77 (6H, s), 3.76-3.61 (4H, m), 3.58-3.46 (2H, m), 3.07-2.92 (4H, m), 1.10 (12H, d, J = 6.1 Hz); ^{31}P NMR ($CDCl_3$) δ 147.72.

4.1.4.4. Bis[2-(*p*-chlorophenylthio)ethyl] *N,N*-diisopropylphosphoramidite (4b). Product **4b** was obtained as a yellow viscous liquid (0.81 g, 50%). R_f 0.72 (EtOAc/n-hexane = 1:4 containing 1% *N,N*-dimethylethylamine); ^{1}H NMR (DMSO) δ 7.39-7.32 (8H, m), 3.80-3.63 (4H, m), 3.55-3.43 (2H, m), 3.17 (4H, t, J = 6.5 Hz), 1.06 (12H, d, J = 6.6 Hz); ^{31}P NMR (DMSO) δ 148.56.

4.1.4.5. Bis[2-(*p*-nitrophenylthio)ethyl] *N,N*-diisopropylphosphoramidite (5b). Product **5b** was obtained as a yellow viscous liquid (1.33 g, 79%). R_f 0.72 (EtOAc/n-hexane = 1:1 containing 1% *N,N*-dimethylethylamine); ^{1}H NMR ($CDCl_3$) δ 8.11 (4H, d, J = 8.6 Hz), 7.37 (4H, d, J = 8.9 Hz), 3.96-3.79 (4H, m), 3.64-3.54 (2H, m), 3.39 (4H, t, J = 6.8 Hz), 1.16 (12H, d, J = 6.8 Hz); ^{31}P NMR ($CDCl_3$) δ 147.90.

4.1.4.6. Bis[2-(phenylthio)propyl] *N,N*-diisopropylphosphoramidite (6b). Product **6b** was obtained as a yellow viscous liquid (0.81 g, 54%). R_f 0.88 (EtOAc/n-hexane = 1:4 containing 1% *N,N*-dimethylethylamine); ^{1}H NMR (DMSO) δ 7.40 (4H, d, J = 7.7 Hz), 7.32 (4H, t, J = 7.4 Hz), 7.25 (2H, t, J = 7.3 Hz), 3.68-3.54 (2H, m), 3.53-3.48 (2H, m), 3.47-3.38 (4H, m), 1.27-1.22 (6H, m), 1.05 (12H, t, J = 6.8 Hz); ^{31}P NMR (DMSO) δ 147.65, 147.40.

4.1.4.7. Bis[1-(phenylthio)propan-2-yl] *N,N*-diisopropylphosphoramidite (7b). Product **7b** was obtained as a yellow viscous liquid (1.16 g, 78%). R_f 0.73 (EtOAc/n-hexane = 1:6 containing 1% *N,N*-dimethylethylamine); ^{1}H NMR (DMSO) δ 7.37-7.26 (8H, m), 7.18 (2H, t, J= 7.1 Hz), 3.99-3.86 (2H, m), 3.51 (2H, m), 3.06 (4H, m), 1.25 (6H, m), 1.16 (12H, d, J = 6.2 Hz); ^{31}P NMR (DMSO) δ 146.45, 145.99, 145.48.

4.1.4.8. Bis[1-(phenylthio)butan-2-yl] *N,N*-diisopropylphosphoramidite (8b). Product **8b** was obtained as a yellow viscous liquid (1.04 g, 66%). R_f 0.74 (EtOAc/n-hexane = 1:6 containing 1% *N,N*-dimethylethylamine); ^{1}H NMR (DMSO) δ 7.36-7.25 (8H, m), 7.18 (2H, t, J = 7.3 Hz), 3.95-

3.75 (2H, m), 3.61-3.46 (2H, m), 3.16-3.01 (4H, m), 1.76-1.49 (4H, m), 1.09 (12H, d, J = 6.9 Hz), 0.88-0.81 (6H, m); ^{31}P NMR (DMSO) δ 146.78, 146.21, 145.81.

4.1.4.9. Bis[3-(phenylthio)butan-2-yl] *N,N*-diisopropylphosphoramidite (9b). Product **9b** was obtained as a yellow viscous liquid (0.92 g, 58%). R_f 0.86 (EtOAc/n-hexane = 1:6 containing 1% *N,N*-dimethylethylamine); ^{1}H NMR (DMSO) δ 7.39 (4H, d, J = 7.2 Hz), 7.32 (4H, t, J = 7.4 Hz), 7.24 (2H, t, J = 7.2 Hz), 3.95-3.81 (2H, m), 3.55-3.44 (2H, m), 3.43-3.34 (2H, m), 1.23-1.16 (12H, m), 1.04 (12H, d, J = 6.6 Hz); ^{31}P NMR (DMSO) δ 146.65, 146.16, 145.66.

4.1.4.10. Bis[2-phenyl-2-(phenylthio)ethyl] *N,N*-diisopropylphosphoramidite (10b). Product **11b** was obtained as a yellow viscous liquid (1.39 g, 74%). R_f 0.87 (EtOAc/n-hexane = 1:6 containing 1% *N,N*-dimethylethylamine); ^{1}H NMR (DMSO) δ 7.37-7.20 (20H, m), 4.59-3.50 (2H, m), 3.92-3.62 (4H, m), 3.36-3.25 (2H, m), 0.91 (12H, d, J = 6.9 Hz); ^{31}P NMR (DMSO) δ 147.21, 146.78.

4.1.4.11. Phenyl [2-(phenylthio)ethyl] *N,N*-diisopropylphosphoramidite (11b). Product **11b** was obtained as a yellow viscous liquid (0.64 g, 53%). R_f 0.79 (EtOAc/n-hexane = 1:5 containing 1% *N,N*-dimethylethylamine); ^{1}H NMR (DMSO) δ 7.42-7.24 (6H, m), 7.19 (1H, t, J = 7.2 Hz), 7.06-6.90 (3H, m), 3.90-3.74 (2H, m), 3.72-3.58 (2H, m), 3.21 (2H, t, J = 6.4 Hz), 1.11 (12H, d, J = 7.1 Hz); ^{31}P NMR (DMSO) δ 146.69.

4.1.4.12. 2-(Phenylthio)ethyl *p*-tolyl *N,N*-diisopropylphosphoramidite (12b). Product **12b** was obtained as a yellow viscous liquid (1.12 g, 90%). R_f 0.80 (EtOAc/n-hexane = 1:5 containing 1% *N,N*-dimethylethylamine); ^{1}H NMR (DMSO) δ 7.41-7.23 (4H, m), 7.18 (1H, t, J = 7.1 Hz), 7.06-6.92 (4H, m), 3.88-3.72 (2H, m), 3.70-3.55 (2H, m), 3.20 (2H, t, J = 6.3 Hz), 2.55 (3H, s), 1.11 (12H, d, J = 6.9 Hz); ^{31}P NMR (DMSO) δ 146.52.

4.1.4.13. *p*-Methoxyphenyl [2-(phenylthio)ethyl] *N,N*-diisopropylphosphoramidite (13b). Product **13b** was obtained as a yellow viscous liquid (1.05 g, 81%). R_f 0.79 (EtOAc/n-hexane= 1:5 containing 1% *N,N*-dimethylethylamine); ^{1}H NMR (DMSO) δ 7.42-7.25 (4H, m), 7.20 (1H, t, J = 7.4 Hz), 6.94-6.78 (4H, m), 3.89-3.74 (2H, m), 3.69 (3H, s), 3.67-3.49 (2H, m), 3.20 (2H, t, J = 6.5 Hz), 1.11 (12H, d, J = 6.8 Hz); ^{31}P NMR (DMSO) δ 146.48.

4.1.4.14. *p*-Fluorophenyl [2-(phenylthio)ethyl] *N,N*-diisopropylphosphoramidite (14b). Product **14b** was obtained as a yellow viscous liquid (1.10g, 87%). R_f 0.79 (EtOAc/n-hexane = 1:5 containing 1% *N,N*-dimethylethylamine); ^{1}H NMR (DMSO) δ 7.37 (2H, d, J = 7.5 Hz), 7.31 (2H, t, J = 7.4 Hz), 7.20 (1H, t, J = 7.2 Hz), 7.11 (2H, t, J = 7.6 Hz), 7.01-6.92 (2H, m), 3.89-3.73 (2H, m), 3.72-3.56 (2H, m), 3.19 (2H, t, J = 6.8 Hz), 1.12 (12H, d, J = 6.8 Hz); ^{31}P NMR (DMSO) δ 147.91.

4.1.4.15. *p*-Chlorophenyl [2-(phenylthio)ethyl] *N,N*-diisopropylphosphoramidite (15b). Product **15b** was obtained as a yellow viscous liquid (0.92 g, 70%). R_f 0.80 (EtOAc/n-hexane = 1:5 containing 1% *N,N*-dimethylethylamine); ^{1}H NMR (DMSO) δ 7.42-7.24 (6H, m), 7.20 (1H, t, J = 7.2 Hz), 6.99 (2H, d, J = 8.0 Hz), 3.91-3.72 (2H, m), 3.71-3.58 (2H, m), 3.21 (2H, t, J = 6.5 Hz), 1.11 (12H, d, J = 7.0 Hz); ^{31}P NMR (DMSO) δ 147.72.

4.1.5. Standard procedure 4: preparation of phosphotriester derivatives of AZT (1a-15a)

AZT phosphotriesters **1a**-**15a** were prepared according to the published procedure of with modifications.[28] 1*H*-tetrazole (8.8 mL, 4 mmol, 0.45 M in acetonitrile, 4 equiv.) was added to a stirred solution of 3'-azido-3'-deoxythymidine (267 mg, 1 mmol) and phosphoramidite (1.2 mmol) in anhydrous acetonitrile (8 mL) at room temperature. After 3 h, the reaction mixture was cooled to 0 ºC, and a solution of TBHP (0.44 mL, 2.4 mmol, 5.5 M in nonane, 2.4 equiv.) was added; the reaction mixture was then allowed to warm to room temperature for over 1 h. The solvent

was removed under reduced pressure. The residue was diluted in CH_2Cl_2 (50 mL) and washed with 10% $Na_2S_2O_3$ solution (50 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL) and the combined organic phase was washed with water (100 mL). The organic layer was dried over anhydrous Na_2SO_4, and evaporated to dryness under reduced pressure followed by preparative TLC purification to get the desired compounds.

4.1.5.1. 3'-Azido-3'-deoxythymidin-5'-yl bis[2-(phenylthio)ethyl] phosphate (1a). Product **1a** was obtained as a colorless viscous liquid (0.26 g, 42%). R_f 0.58 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.39 (1H, br s, NH), 7.45 (1H, s), 7.40-7.27 (8H, m), 7.21 (2H, t, J = 7.0 Hz), 6.12 (1H, t, J = 6.5 Hz), 4.43 (1H, q, J = 5.9 Hz), 4.24-4.15 (2H, m), 4.09 (4H, q, J = 6.9 Hz), 4.02-3.93 (1H, m), 3.24 (4H, t, J = 6.5 Hz), 2.43-2.25 (2H, m), 1.75 (3H, s); ^{31}P NMR (DMSO) δ -0.39.

4.1.5.2. 3'-Azido-3'-deoxythymidin-5'-yl bis[2-(*p*-tolylthio)ethyl] phosphate (2a). Product **2a** was obtained as a colorless viscous liquid (0.29 g, 45%). R_f 0.59 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.39 (1H, br s, NH), 7.44 (1H, s), 7.30-7.22 (4H, m), 7.12 (4H, d, J = 7.2 Hz), 6.12 (1H, t, J = 6.8 Hz), 4.42 (1H, q, J = 5.8 Hz), 4.21-4.14 (2H, m), 4.05 (4H, q, J = 6.8 Hz), 3.99-3.92 (1H, m), 3.22-3.11 (4H, m), 2.38-2.32 (2H, m), 2.26 (6H, s), 1.74 (3H, s); ^{31}P NMR (DMSO) δ -0.38.

4.1.5.3. 3'-Azido-3'-deoxythymidin-5'-yl bis[2-(*p*-methoxyphenylthio)ethyl] phosphate (3a). Product **3a** was obtained as a pale yellow viscous liquid (0.27 g, 40%). R_f 0.53 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR ($CDCl_3$) δ 9.03 (1H, br s, NH), 7.42-7.33 (5H, m), 6.88-6.80 (4H, m), 6.21 (1H, t, J = 6.5 Hz), 4.34-4.26 (2H, m), 4.25-4.21 (1H, m), 4.19-4.12 (4H, m), 4.03-3.96 (1H, m), 3.79 (6H, s), 3.08-2.99 (4H, m), 2.46-2.24 (2H, m), 1.90 (3H, s); ^{31}P NMR ($CDCl_3$) δ -0.45.

4.1.5.4. 3'-Azido-3'-deoxythymidin-5'-yl bis[2-(*p*-chlorophenylthio)ethyl] phosphate (4a). Product **4a** was obtained as a pale yellow viscous liquid (0.26 g, 38%). R_f 0.55 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.39 (1H, br s, NH), 7.45 (1H, s), 7.41-7.33 (8H, m), 6.13 (1H, t, J = 6.7 Hz), 4.43 (1H, q, J = 6.1 Hz), 4.23-4.15 (2H, m), 4.10 (4H, q, J = 6.9 Hz), 3.99-3.93 (1H, m), 3.29-3.22 (4H, m), 2.42-2.26 (2H, m), 1.75 (3H, s); ^{31}P NMR (DMSO) δ -0.44.

4.1.5.5. 3'-Azido-3'-deoxythymidin-5'-yl bis[2-(*p*-nitrophenylthio)ethyl] phosphate (5a). Product **5a** was obtained as a pale yellow viscous liquid (191 mg, 27%). R_f 0.48 (CH_2Cl_2/MeOH = 95:5); ^{1}H NMR (DMSO) δ 11.32 (1H, br s, NH), 8.10 (4H, d, J = 8.1 Hz), 7.52 (4H, t, J = 6.3 Hz), 7.43 (1H, s), 6.10 (1H, t, J = 6.3 Hz), 4.42 (1H, q, J = 6.2 Hz), 4.26-4.12 (6H, m), 3.98-3.92 (1H, m), 3.48-3.40 (4H, m), 2.42-2.26 (2H, m), 1.74 (3H, s); ^{31}P NMR (DMSO) δ -0.48.

4.1.5.6. 3'-Azido-3'-deoxythymidin-5'-yl bis[2-(phenylthio)propyl] phosphate (6a). Product **6a** was obtained as a pale yellow viscous liquid (0.2 g, 31%). R_f 0.62 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.37 (1H, br s, NH), 7.45 (1H, s), 7.43-7.37 (4H, m), 7.36-7.30 (4H, m), 7.29-7.23 (2H, m), 6.12 (1H, t, J = 6.4 Hz), 4.42 (1H, q, J = 5.7 Hz), 4.23-4.13 (2H, m), 4.05-3.86 (5H, m), 3.57-3.49 (2H, m), 2.41-2.28 (2H, m), 1.75 (3H, s), 1.23 (6H, t, J = 5.6 Hz); ^{31}P NMR (DMSO) δ -0.50.

4.1.5.7. 3'-Azido-3'-deoxythymidin-5'-yl bis[1-(phenylthio)propan-2-yl] phosphate (7a). Product **7a** was obtained as a pale yellow viscous liquid (0.25 g, 39%). R_f 0.61 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.37 (1H, br s, NH), 7.47 (1H, s), 7.38-7.26 (8H, m), 7.19 (2H, t, J = 7.2 Hz), 6.15-6.08 (1H, m), 4.55-4.45 (2H, m), 4.43-4.36 (1H, m), 4.24-4.08 (2H, m), 3.98-3.91 (1H, m), 3.24-3.13 (4H, m), 2.41-2.26 (2H, m), 1.75 (3H, s), 1.38-1.29 (6H, m); ^{31}P NMR (DMSO) δ -1.80, -1.93, -1.96, -2.17.

4.1.5.8. 3'-Azido-3'-deoxythymidin-5'-yl bis[1-(phenylthio)butan-2-yl] phosphate (8a). Product **8a** was obtained as a pale yellow viscous liquid (0.17 g, 25%). R_f 0.61 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.37 (1H, br s, NH), 7.48 (1H, s), 7.39-7.25 (8H, m), 7.24-7.15 (2H, m), 6.15-6.07 (1H, m), 4.45-4.37 (2H, m), 4.36-4.30 (1H, m), 4.22-4.10 (2H, m), 3.99-3.91 (1H, m), 3.25-3.16 (4H, m), 2.40-2.25 (2H, m), 1.76 (3H, s), 1.74-1.56 (4H, m), 0.87-0.79 (6H, m); ^{31}P NMR (DMSO) δ -1.44, -1.56, -1.59, -1.81.

4.1.5.9. 3'-Azido-3'-deoxythymidin-5'-yl bis[3-(phenylthio)butan-2-yl] phosphate (9a). Product **9a** was obtained as a pale yellow viscous liquid (0.17 g, 25%). R_f 0.61 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.38 (1H, br s, NH), 7.47 (1H, s), 7.43-7.36 (4H, m), 7.34-7.28 (4H, m), 7.27-7.22 (2H, m), 6.16-6.06 (1H, m), 4.52-4.45 (2H, m), 4.43-4.38 (1H, m), 4.20-4.11 (2H, m), 3.98-3.90 (1H, m), 3.59-3.49 (2H, m), 2.36-2.28 (2H, m), 1.76 (3H, s), 1.36-1.26 (6H, m), 1.24-1.18 (6H, m); ^{31}P NMR (DMSO) δ -1.42, -1.48.

4.1.5.10. 3'-Azido-3'-deoxythymidin-5'-yl bis[2-phenyl-2-(phenylthio)ethyl] phosphate (10a). Product **10a** was obtained as a pale yellow viscous liquid (0.28 g, 38%). R_f 0.60 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.39 (1H, br s, NH), 7.39-7.29 (21H, m), 6.15-6.05 (1H, m), 4.70-4.56 (2H, m), 4.33-4.20 (3H, m), 4.18-4.06 (2H, m), 3.98-3.86 (2H, m), 3.85-3.78 (1H, m), 2.33-2.18 (2H, m), 1.69 (3H, s); ^{31}P NMR (DMSO) δ -0.83, -0.86, -0.88.

4.1.5.11. 3'-Azido-3'-deoxythymidin-5'-yl phenyl [2-(phenylthio)ethyl] phosphate (11a). Product **11a** was obtained as a pale yellow viscous liquid (0.24 g, 43%). R_f 0.65 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.38 (1H, br s, NH), 7.45 (1H, s), 7.42-7.28 (6H, m), 7.26-7.15 (4H, m), 6.13 (1H, t, J = 6.6 Hz), 4.45 (1H, q, J = 5.8 Hz), 4.40-4.27 (2H, m), 4.25-4.17 (2H, q, J = 6.6 Hz), 4.03-4.01 (1H, m), 3.31-3.24 (2H, m), 2.42-2.26 (2H, m), 1.71 (3H, s); ^{31}P NMR (DMSO) δ -5.28, -5.38.

4.1.5.12. 3'-Azido-3'-deoxythymidin-5'-yl 2-(phenylthio)ethyl *p*-tolyl phosphate (12a). Product **12a** was obtained as a pale yellow viscous liquid (0.20 g, 35%). R_f 0.65 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.37 (1H, br s, NH), 7.44 (1H, s), 7.40-7.28 (4H, m), 7.22 (1H, t, J = 6.8 Hz), 7.16 (2H, t, J = 7.0 Hz), 7.06 (2H, d, J = 8.1 Hz), 6.14 (1H, t, J = 6.7 Hz), 4.44 (1H, q, J = 5.9 Hz), 4.39-4.25 (2H, m), 4.23-4.16 (2H, m), 4.03-3.96 (1H, m), 3.32-3.20 (2H, m), 2.44-2.30 (2H, m), 2.27 (3H, s), 1.71 (3H, s); ^{31}P NMR (DMSO) δ -5.23, -5.32.

4.1.5.13. 3'-Azido-3'-deoxythymidin-5'-yl *p*-methoxyphenyl [2-(phenylthio)ethyl] phosphate (13a). Product **13a** was obtained as a pale yellow viscous liquid (0.29 g, 49%). R_f 0.65 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.37 (1H, br s, NH), 7.45 (1H, d, J = 10.1 Hz), 7.39-7.29 (4H, m), 7.25-7.19 (1H, m), 7.11 (2H, d, J = 9.1 Hz), 6.95-6.86 (2H, m), 6.13 (1H, t, J = 6.8 Hz), 4.44 (1H, q, J = 5.6 Hz), 4.36-4.26 (2H, m), 4.25-4.16 (2H, m), 4.01-3.97 (1H, m), 3.73(3H, s), 3.30-3.21 (2H, m), 2.43-2.28 (2H, m), 1.71 (3H, s); ^{31}P NMR (DMSO) δ -4.92, -5.03.

4.1.5.14. 3'-Azido-3'-deoxythymidin-5'-yl *p*-fluorophenyl [2-(phenylthio)ethyl] phosphate (14a). Product **14a** was obtained as a pale yellow viscous liquid (0.28 g, 49%). R_f 0.49 (CH_2Cl_2/MeOH = 95:5); ^{1}H NMR (DMSO) δ 11.36 (1H, br s, NH), 7.44 (1H, d, J = 9.2 Hz), 7.39-7.29 (4H, m), 7.25-7.18 (4H, m),6.13 (1H, t, J = 6.7 Hz), 4.45 (1H, q, J = 5.9 Hz), 4.40-4.27 (2H, m), 4.26-4.19 (2H, m), 4.02-3.97 (1H, m), 3.31-3.24 (2H, m), 2.42-2.28 (2H, m), 1.71 (3H, s); ^{31}P NMR (DMSO) δ -5.27, -5.38.

4.1.5.15. 3'-Azido-3'-deoxythymidin-5'-yl *p*-chlorophenyl [2-(phenylthio)ethyl] phosphate (15a). Product **15a** was obtained as a pale yellow viscous liquid (0.22 g, 37%). R_f 0.50 (CH_2Cl_2/MeOH = 95:5); ^{1}H NMR (DMSO) δ 11.37 (1H, br s, NH), 7.43 (3H, t, J = 7.2 Hz), 7.38-7.28 (4H, m), 7.22 (3H, t, J = 8.2 Hz),6.14 (1H, t, J = 5.8 Hz), 4.45 (1H, q, J = 5.9 Hz),

4.41-4.29 (2H, m), 4.24 (2H, q, J = 6.3 Hz), 4.08-3.98 (1H, m), 3.32-3.22 (2H, m), 2.46-2.28 (2H, m), 1.72 (3H, s); ^{31}P NMR (DMSO) δ -5.53, -5.63.

4.1.6. Standard procedure 5: preparation of sulfone products (1-15)

Oxidation of sulfide to sulfone was achieved according to the procedure described previously with some modifications.[29] To a stirring solution of a sulfide-containing phosphate (0.5 mmol) in CH_2Cl_2 (10 mL) was added an excess amount of *m*CPBA (0.56 g, 2.5 mmol, 5 equiv.). The reaction mixture was stirred at ambient temperature for 3 h and then concentrated at reduced pressure. The residue was diluted with CH_2Cl_2 (30 mL) and washed with water (30 mL), and the water layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined extract was washed with saturated NaCl solution (30 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated under reduced pressure and the crude product was purified by preparative TLC to afford the sulfone product.

4.1.6.1. 3'-Azido-3'-deoxythymidin-5'-yl bis[2-(phenylsulfonyl)ethyl] phosphate (1). Product **1** was obtained as a white foam (198 mg, 58%). R_f 0.51 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.39 (1H, br s, NH), 7.94-7.86 (4H, m), 7.75 (2H, t, J = 7.2 Hz), 7.71-7.61 (4H, m), 7.42 (1H, s), 6.12 (1H, t, J = 6.6 Hz), 4.39 (1H, q, J = 5.7 Hz), 4.24-4.12 (4H, m), 4.06-3.96 (2H, m), 3.93-3.87 (1H, m), 3.76-3.66 (4H, m), 2.44-2.26 (2H, m), 1.75 (3H, s); ^{31}P NMR (DMSO) δ -1.44. Anal. Calcd for $C_{26}H_{30}N_5O_{11}PS_2$: C, 45.68; H, 4.42; N, 10.24; S, 9.38. Found: C, 45.50; H, 4.54; N, 9.98; S, 9.15.

4.1.6.2. 3'-Azido-3'-deoxythymidin-5'-yl bis[2-(*p*-tolylsulfonyl)ethyl] phosphate (2). Product **2** was obtained as a white foam (135 mg, 38%). R_f 0.52 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.40 (1H, br s, NH), 7.84-7.72 (4H, m), 7.49-7.43 (4H, m), 7.41 (1H, s), 6.13 (1H, t, J = 6.5 Hz), 4.39 (1H, q, J = 5.7 Hz), 4.22-4.12 (4H, m), 4.06-3.93 (2H, m), 3.92-3.86 (1H, m),

3.72-3.63 (4H, m), 2.40 (6H, s), 2.38-2.29 (2H, m), 1.74 (3H, s); ^{31}P NMR (DMSO) δ -1.38. Anal. Calcd for $C_{28}H_{34}N_5O_{11}PS_2 \cdot 1/4H_2O$: C, 46.96; H, 4.86; N, 9.78; S, 8.95. Found: C, 46.94; H, 4.84; N, 9.41; S, 8.80.

4.1.6.3. 3'-Azido-3'-deoxythymidin-5'-yl bis[2-(*p*-methoxyphenylsulfonyl)ethyl] phosphate (3). Product **3** was obtained as a white foam (141 mg, 38 %). R_f 0.47 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.37 (1H, br s, NH), 7.85-7.77 (4H, m), 7.40 (1H, s), 7.22-7.10 (4H, m), 6.13 (1H, t, J = 6.6 Hz), 4.39 (1H, q, J = 5.7 Hz), 4.25-4.13 (4H, m), 4.07-3.99 (2H, m), 3.94-3.89 (1H, m), 3.85 (6H, s), 3.74-3.60 (4H, m), 2.41-2.26 (2H, m), 1.75 (3H, s); ^{31}P NMR (DMSO) δ -1.30. Anal. Calcd for $C_{28}H_{34}N_5O_{13}PS_2 \cdot 1/5CH_3COOCH_2CH_3$: C, 45.44; H,4.71; N,9.20; S, 8.42. Found: C, 45.37; H, 4.69; N, 8.98; S, 8.10.

4.1.6.4. 3'-Azido-3'-deoxythymidin-5'-yl bis[2-(*p*-chlorophenylsulfonyl)ethyl] phosphate (4). Product **4** was obtained as a white foam (120 mg, 32%). R_f 0.49 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.39 (1H, br s, NH), 7.95-7.88 (4H, m), 7.78-7.70 (4H, m), 7.41 (1H, s), 6.13 (1H, t, J = 6.7 Hz), 4.42-4.38 (1H, m), 4.25-4.17 (4H, m), 4.05-3.99 (2H, m), 3.92-3.89 (1H, m), 3.82-3.75 (4H, m), 2.42-2.25 (2H, m), 1.75 (3H, s); ^{31}P NMR (DMSO) δ -1.43. Anal. Calcd for $C_{26}H_{28}Cl_2N_5O_{11}PS_2 \cdot 1/2CH_3COOCH_2CH_3$: C, 42.22; H, 4.05; N, 8.79; S, 8.05. Found: C, 42.43; H, 4.03; N, 8.62; S, 7.92.

4.1.6.5. 3'-Azido-3'-deoxythymidin-5'-yl bis[2-(*p*-nitrophenylsulfonyl)ethyl] phosphate (5). Product **5** was obtained as a white foam (162 mg, 42%). R_f 0.48 (CH_2Cl_2/MeOH = 19:1); ^{1}H NMR (DMSO) 11.35 (1H, br s, NH), 8.51-8.40 (4H, m), 8.24-8.12 (4H, m), 7.37 (1H, s), 6.09 (1H, t, J = 6.5 Hz), 4.36 (1H, q, J = 5.8 Hz), 4.29-4.17 (4H, m), 4.01-3.94 (2H, m), 3.93-3.79 (5H, m), 2.42-2.26 (2H, m), 1.75 (3H, s); ^{31}P NMR (DMSO) δ -1.57. Anal. Calcd for

$C_{26}H_{28}N_7O_{15}PS_2 \cdot 1/4CH_3COOCH_2CH_3$: C, 40.76; H, 3.80; N, 12.32; S, 8.06. Found: C, 40.80; H, 3.87; N, 12.07; S, 8.00.

4.1.6.6. 3'-Azido-3'-deoxythymidin-5'-yl bis[2-(phenylsulfonyl)propyl] phosphate (6). Product **6** was obtained as a white foam (160 mg, 45%). R_f 0.54 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.37 (1H, br s, NH), 7.87 (4H, t, J = 7.7 Hz), 7.77 (2H, t, J = 7.6 Hz), 7.66 (4H, t, J = 7.4 Hz), 7.43 (1H, s), 6.13 (1H, t, J = 6.8 Hz), 4.47-4.36 (1H, m), 4.22-4.10 (4H, m), 4.09-4.01 (2H, m), 3.98-3.89 (1H, m), 3.78-3.66 (2H, m), 2.42-2.28 (2H, m), 1.76 (3H, s), 1.20 (6H, m); ^{31}P NMR (DMSO) δ -1.16. Anal. Calcd for $C_{28}H_{34}N_5O_{11}PS_2 \cdot 3/5H_2O$: C, 46.55; H, 4.91; N, 9.69; S, 8.88. Found: C, 46.81; H, 4.89; N, 9.31; S, 8.69.

4.1.6.7. 3'-Azido-3'-deoxythymidin-5'-yl bis[1-(phenylsulfonyl)propan-2-yl] phosphate (7). Product **7** was obtained as a white foam (174 mg, 49%). R_f 0.50 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.37 (1H, br s, NH), 7.97-7.88 (4H, m), 7.76 (2H, t, J = 7.5 Hz), 7.69-7.62 (4H, m), 7.45 (1H, s), 6.13 (1H, t, J = 7.0 Hz), 4.82-4.62 (2H, m), 4.48-4.34 (1H, m), 4.19-4.06 (2H, m), 3.97-3.89 (1H, m), 3.75-3.63 (4H, m), 2.41-2.27 (2H, m), 1.75 (3H, s), 1.40-1.27 (6H, m); ^{31}P NMR (DMSO) δ -2.98, -3.03, -3.77. Anal. Calcd for $C_{28}H_{34}N_5O_{11}PS_2 \cdot 1/4H_2O$: C, 46.96; H, 4.86; N, 9.78; S, 8.95. Found: C, 47.35; H, 5.01; N, 9.43; S, 8.58.

4.1.6.8. 3'-Azido-3'-deoxythymidin-5'-yl bis[1-(phenylsulfonyl)butan-2-yl] phosphate (8). Product **8** was obtained as a white foam (236 mg, 64%). R_f 0.53 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.38 (1H, br s, NH), 7.98-7.86 (4H, m), 7.79-7.72 (2H, m), 7.70-7.62 (4H, m), 7.45 (1H, s), 6.13 (1H, t, J = 6.8 Hz), 4.67-4.53 (2H, m), 4.45-4.35 (1H, m), 4.25-4.08 (2H, m), 3.99-3.87 (1H, m), 3.78-3.58 (4H, m), 2.43-2.28 (2H, m), 1.75 (3H, s), 1.73-1.56 (4H, m), 0.80 (6H, t, J = 7.3 Hz); ^{31}P NMR (DMSO) δ -2.32, -2.67, -2.73, -3.37. Anal. Calcd for $C_{30}H_{38}N_5O_{11}PS_2$: C, 48.71; H, 5.18; N, 9.47; S, 8.67. Found: C, 48.50; H, 5.13; N, 9.19; S, 8.39.

4.1.6.9. 3'-Azido-3'-deoxythymidin-5'-yl bis[3-(phenylsulfonyl)butan-2-yl] phosphate (9). Product **9** was obtained as a white foam (207 mg, 56%). R_f 0.52 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.37 (1H, br s, NH), 7.91-7.83 (4H, m), 7.81-7.74 (2H, m), 7.72-7.63 (4H, m), 7.45 (1H, s), 6.13 (1H, t, J = 6.8 Hz), 4.83-4.66 (2H, m), 4.45-4.32 (1H, m), 4.19-4.02 (2H, m), 3.95-3.83 (1H, m), 3.73-3.58 (2H, m), 2.42-2.31 (2H, m), 1.76 (3H, s), 1.37-1.27 (6H, m), 1.15 (6H, t, J = 7.7 Hz); ^{31}P NMR (DMSO) δ -2.31, -2.46. Anal. Calcd for $C_{30}H_{38}N_5O_{11}PS_2$: C, 48.71; H, 5.18; N, 9.47. Found: C, 49.04; H, 5.21; N, 9.23.

4.1.6.10. 3'-Azido-3'-deoxythymidin-5'-yl bis[2-phenyl-2-(phenylsulfonyl)ethyl] phosphate (10). Product **10** was obtained as a white foam (175 mg, 42%). R_f 0.52 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.37 (1H, br s, NH), 7.69 (2H, t, J = 7.7 Hz), 7.65-7.58 (4H, m), 7.57-7.50 (4H, m), 7.38-7.24 (7H, m), 7.22-7.13 (4H, m), 6.09 (1H, t, J = 6.8 Hz), 5.03-4.91 (2H, m), 4.59-4.33 (4H, m), 4.32-4.23 (1H, m), 3.99-3.77 (3H, m), 2.34-2.22 (2H, m), 1.71 (3H, s); ^{31}P NMR (DMSO) δ -1.17, -1.22, -1.28. Anal. Calcd for $C_{38}H_{38}N_5O_{11}PS_2$: C, 54.60; H, 4.58; N, 8.38; S, 7.67. Found: C, 54.64; H, 4.82; N, 8.12; S, 7.43.

4.1.6.11. 3'-Azido-3'-deoxythymidin-5'-yl phenyl [2-(phenylsulfonyl)ethyl] phosphate (11). Product **11** was obtained as a pale yellow foam (154 mg, 52%). R_f 0.50 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.36 (1H, br s, NH), 7.95-7.86 (2H, m), 7.75 (1H, t, J = 7.2 Hz), 7.65 (2H, t, J = 7.3 Hz), 7.46-7.31 (3H, m), 7.28-7.18 (1H, m), 7.13 (2H, d, J = 7.7 Hz), 6.13 (1H, t, J = 6.5 Hz), 4.48-4.32 (3H, m), 4.30-4.16 (2H, m), 4.02-3.92 (1H, m), 3.86-3.72 (2H, m),2.43-2.28 (2H, m), 1.74 (3H, s); ^{31}P NMR (DMSO) δ -5.90, -6.05. Anal. Calcd for $C_{24}H_{26}N_5O_9PS \cdot 1/3CH_3COOCH_2CH_3$: C, 49.00; H, 4.65; N, 11.28. Found: C, 49.29; H, 4.66; N, 11.25.

4.1.6.12. 3'-Azido-3'-deoxythymidin-5'-yl 2-(phenylsulfonyl)ethyl *p*-tolyl phosphate (12). Product **12** was obtained as a white foam (175 mg, 58%). R_f 0.51 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.38 (1H, br s, NH), 7.94-7.85 (2H, m), 7.75 (1H, t, J = 7.4 Hz), 7.65 (2H, t, J = 7.4 Hz), 7.41 (1H, d, J = 11.9 Hz), 7.15 (2H, t, J = 8.0 Hz), 7.01 (2H, d, J = 8.2 Hz), 6.13 (1H, t, J = 6.7 Hz), 4.43-4.30 (3H, m), 4.27-4.15 (2H, m), 4.01-3.92 (1H, m), 3.83-3.71 (2H, m), 2.44-2.30 (2H, m), 2.27 (3H, s), 1.70 (3H, s); ^{31}P NMR (DMSO) δ -5.71, -5.85. Anal. Calcd for $C_{25}H_{28}N_5O_9PS$: C, 49.59; H, 4.66; N, 11.57; S, 5.30. Found: C, 49.64; H, 4.77; N, 11.29; S, 5.13.

4.1.6.13. 3'-Azido-3'-deoxythymidin-5'-yl *p*-methoxyphenyl [2-(phenylsulfonyl)ethyl] phosphate (13). Product **13** was obtained as a white foam (180 mg, 58%). R_f 0.54 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.36 (1H, br s, NH), 7.94-7.87 (2H, m), 7.75 (1H, t, J = 7.5 Hz), 7.65 (2H, t, J = 7.5 Hz), 7.42 (1H, d, J = 12.1 Hz), 7.04 (2H, d, J = 8.1 Hz), 6.89 (2H, t, J = 7.8 Hz), 6.13 (1H, t, J = 6.8 Hz), 4.48-4.41 (1H, m), 4.39-4.29 (2H, m), 4.27-4.13 (2H, m), 3.99-3.92 (1H, m), 3.84-3.75 (2H, m), 3.74-3.66 (3H, s), 2.43-2.28 (2H, m), 1.71 (3H, s); ^{31}P NMR (DMSO) δ -5.40, -5.54. Anal. Calcd for $C_{25}H_{28}N_5O_{10}PS$: C, 48.31; H, 4.54; N, 11.27; S, 5.16. Found: C, 48.09; H, 4.64; N, 11.23; S, 5.14.

4.1.6.14. 3'-Azido-3'-deoxythymidin-5'-yl *p*-fluorophenyl [2-(phenylsulfonyl)ethyl] phosphate (14). Product **14** was obtained as a white foam (140 mg, 46%). R_f 0.49 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.37 (1H, br s, NH), 7.94-7.87 (2H, m), 7.75 (1H, t, J = 7.4 Hz), 7.65 (2H, t, J = 7.3 Hz), 7.42 (1H, d, J = 11.8 Hz), 7.25-7.13 (4H, m), 6.13 (1H, t, J = 6.8 Hz), 4.47-4.32 (3H, m), 4.31-4.17 (2H, m), 4.01-3.93 (1H, m), 3.84-3.73 (2H, m), 2.44-2.28 (2H, m), 1.71 (3H, s); ^{31}P NMR (DMSO) δ -5.77, -5.92. Anal. Calcd for $C_{24}H_{25}FN_5O_9PS$: C, 47.29; H, 4.13; N, 11.49; S, 5.26. Found: C, 47.56; H, 4.35; N, 11.18; S, 5.06.

4.1.6.15. 3'-Azido-3'-deoxythymidin-5'-yl *p*-chlorophenyl [2-(phenylsulfonyl)ethyl] phosphate (15). Product **15** was obtained as a white foam (200 mg, 64%). R_f 0.49 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (DMSO) δ 11.39 (1H, br s, NH), 7.93-7.87 (2H, m), 7.75 (1H, t, J = 7.6 Hz), 7.65 (2H, t, J = 7.7 Hz), 7.43 (3H, t, J = 8.2 Hz), 7.18 (2H, d, J = 8.5 Hz), 6.13 (1H, t, J = 6.5 Hz), 4.48-4.33 (3H, m), 4.31-4.18 (2H, m), 4.01-3.93 (1H, m), 3.85-3.72 (2H, m),2.46-2.29 (2H, m), 1.72 (3H, s); ^{31}P NMR (DMSO) δ -6.05, -6.18. Anal. Calcd for $C_{24}H_{25}ClN_5O_9PS\cdot 1/2H_2O$: C, 45.40; H, 4.13; N, 11.03; S, 5.05. Found: C, 45.79; H, 4.19; N, 10.79; S, 5.04.

4.2. Stability studies

4.2.1. Stability tests of sulfonylethyl nucleotide analogues in 0.1 M phosphate buffer at pH 7.4

Each incubation mixture contained 0.4 mL of pre-incubated 0.1 M phosphate buffer at pH 7.4 and 0.1 mL of the drug solution (5 mg/mL) prepared in acetonitrile. The vials were incubated at 37 °C in a shaking water bath (Dubnoff Metabolic Shaking Incubator, Precision Scientific) for varying time intervals. The incubation was initiated by the addition of the compound and terminated by the addition of 0.1 mL 2.0 M acetate buffer (pH 5) and 0.2 mL ethanol (0.4 mL ethanol for compound **5**) and immediately cooled in crushed ice. An aliquot of the resulting mixture (15 μL) were spotted on 10 × 20 mm silica gel TLC plates and eluted with appropriate solvent system (CH_2Cl_2/MeOH = 95:5). The starting materials from all the samples were visualized as a dark spot under UV light and quantitatively analyzed by using the Uniscan Video Densitometer. The results were expressed as the percentage of starting material remaining after each incubation period relative to the zero time incubation control sample. These results were

plotted against the respective incubation times and the half-lives were calculated from rate constant k of the decay equation ($t_{1/2} = \ln 2/k$).

4.2.2. Stability tests of sulfonylethyl nucleotide analogues in the presence of human plasma

Lyophilized human plasma (Lee Biosolutions, Inc., St. Louis, MO) was used for this study and it was reconstituted immediately prior to the experiment. The protocol used for the study was essentially the same as in **4.2.1** except the following: Each incubation mixture contained 0.4 mL of reconstituted solution of human plasma in 0.1 M phosphate buffer at pH 7.4 and 0.1 mL drug solution. After the termination of the incubation, the vials were cooled in crushed ice and centrifuged at high speed (3000 × g) for 30 min to precipitate the proteins. Subsequently, an aliquot of the resulting supernatant (15 μL) was spotted on TLC plates, developed and analyzed as described above in procedure 4.2.1.

4.3. In vitro cytotoxicity assessment

4.3.1. Chemicals and equipment

Dulbecco's modified Eagle's medium (DMEM), Eagle's minimum essential medium (EMEM), fetal bovine serum (FBS), penicillin/streptomycin and trypsin 0.25% were products of Hyclone, Thermo Scientific, Logan, UT. Phosphate buffered saline (PBS) 20× concentrate (pH 7.5) was purchased from AMRESCO, Solon, OH. 5-Bromo-2'-deoxyuridine (5-BrdU) was purchased from Alfa Aesar, Ward Hill, MA. Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-yl)-2,5-diphenyltetrazolium bromide (MTT), and other chemicals were obtained from Sigma Chemical Co., St. Louis, MO. OPSYS microplate reader was purchased from Dynex Technologies, Chantilly, VA.

4.3.2. Cell lines and cell culture

The cell lines MCF-7, H460, KB-3-1, and SW620 were cultured at 37 °C, 5% CO_2 with DMEM containing 10% FBS and 1% penicillin/streptomycin. The 143B cell line (ATCC No. CRL-1543) was cultured at 37 °C, 5% CO_2 with EMEM containing 10% FBS and 1% penicillin/streptomycin. The cell line 143B/TK$^-$ (ATCC No. CRL-8303) was maintained in the EMEM with 0.015 mg/mL 5-BrdU. All cells were grown as adherent monolayer in drug-free culture media for more than 2 weeks before the assay.

4.3.3. Cytotoxicity determination by MTT assay

A modified MTT colorimetric assay[30] was utilized to detect the sensitivity of cells to AZT and anticancer prodrugs in vitro. In the cytotoxicity assay, stocking solutions of the compounds were prepared at 10 mM using diemthyl sulfoxide (DMSO). Then 0.5 mM PBS (PH 7.4) was used to dilute the stocking solutions of compounds into different concentrations (0.03 μM to 300 μM) right before the MTT assay. Briefly, cells were seeded in 180 μL medium in 96-well plates in triplicate at 5000~6000 cells/well. After incubation at 37 °C, 5% CO_2 for 24 h to allow the cells to attach to the wells, different concentrations of AZT and the synthesized compounds (20 μL/well) were added into designated wells. After 72 h of incubation at 37 °C, 20 μL of MTT solution (4 mg/mL) was added to each well. The plates were further incubated at 37 °C for 4 h, allowing viable cells to change the yellow-colored MTT into dark-blue formazan crystals. Subsequently, the MTT medium was removed from each well without disturbing the cells, and 100 μL of DMSO was added into each well. Plates were placed on a shaking table to thoroughly mix the formazan into the solvent. Finally, the absorbance was determined at 570 nm by OPSYS microplate reader. The percentage of cell survival was expressed as the absorbance of the cells treated with certain drug concentration relative to that of the untreated control cells. IC_{50} values were obtained from the slopes of cell survival rate versus log drug concentration curves.

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References and notes

1. Chow, W. A.; Jiang, C.; Guan, M. *Lancet Oncol.* **2009**, *10*, 61.

2. Johnston, J. S.; Johnson, A.; Gan, Y.; Wientjes, M.G.; Au, J. L. *Pharm. Res.* **2003**, *20*, 957.

3. Brunetti, I.; Falcone, A.; Calabresi, P.; Goulette, F. A.; Darnowski, J. W. *Cancer Res.* **1990**, *50*, 4026.

4. Chen, P.; Liu, Y.; Sun, Y.; Chen, C.; Qi, Y.; Zhang, Y. *Pharm. Biol.* **2013**, *51*, 1586.

5. Mattson, D. M.; Ahmad, I. M.; Dayal, D.; Parsons, A. D.; Aykin-Burns, N.; Li, L.; Orcutt, K. P.; Spitz, D. R.; Dornfeld, K. J.; Simons, A. L. *Free Radic. Biol. Med.* **2009**, *46*, 232.

6. Wagner, C. R.; Ballato, G.; Akanni, A. O.; McIntee, E. J.; Larson, R. S.; Chang, S. L.; Abulhajj, Y. J. *Cancer Res.* **1997**, *57*, 2341.

7. Humer, J.; Ferko, B.; Waltenberger, A.; Rapberger, R.; Pehamberger, H.; Muster, T. *Melanoma Res.* **2008**, *18*, 314.

8. Pereira, J.; Levy, D.; Ruiz, J. L.; Brocardo, G. A.; Ferreira, K. A.; Costa, R. O.; Queiroz, R. G.; Maria, D. A.; Neto, A. E.; Chamone, D. A.; Bydlowski, S. P. *Anticancer Agents Med. Chem.* **2013**, *13*, 186.

9. Celewicz, L.; Jóźwiak, A.; Ruszkowski, P.; Laskowska, H.; Olejnik, A.; Czarnecka, A.; Hoffmann, M.; Hładoń, B. *Bioorg. Med. Chem.* **2011**, *19*, 6375.

10. Jeng, K. S.; Sheen, I. S.; Jeng, W. J. *Hepatogastroenterology.* **2011**, *58*, 2091.

11. Falchetti, A.; Franchi, A.; Bordi, C.; Mavilia, C.; Masi, L.; Cioppi, F.; Recenti, R.; Picariello, L.; Marini, F.; Del Monte, F.; Ghinoi, V.; Martineti, V.; Tanini, A.; Brandi, M. L. *J. Bone Miner*

Res. **2005**, *20*, 410.

12. Melana, S. M.; Holland, J. F.; Pogo, B. G. *Clin. Cancer Res.* **1998**, *4*, 693.

13. Jorajuria, S.; Dereuddre-Bosquet, N.; Becher, F.; Martin, S.; Porcheray, F.; Garrigues, A.; Mabondzo, A.; Benech, H.; Grassi, J.; Orlowski, S.; Dormont, D.; Clayette, P. Antivir. Ther. **2004**, *9*, 519.

14. Wang, X.; Nitanda, T.; Shi, M.; Okamoto, M.; Furukawa, T.; Sugimoto, Y.; Akiyama, S.; Baba, M. *Biochem. Pharmacol.* **2004**, *68*, 1363.

15. Schuetz, J. D.; Connelly, M. C.; Sun, D.; Paibir, S. G.; Flynn, P. M.; Srinivas, R. V.; Kumar, A.; Fridland, A. *Nat. Med.* **1999**, *5*, 1048.

16. Fukuda, Y.; Schuetz, J. D. *Biochem. Pharmacol.* **2012**, *83*, 1073.

17. Di Vito, M.; Bozzi, A.; Ferretti, A.; Cianfriglia, M.; Barca, S.; Signoretti, C.; Lenti, L.; d'Agostino, F.; Strom, R.; Podo, F. *Biochem. Pharmacol.* **1997**, *54*, 979.

18. Iyer, V. V.; Griesgraber, G. W.; Radmer, M. R.; McIntee, E. J.; Wagner, C. R. *J. Med. Chem.***2000**, *43*, 2266.

19. Liu, W.; Zhang, L.; Zhou, H.; Yang, C.; Miao, Z.; Zhao, Y. *Nucleosides Nucleotides Nucleic Acids.* **2013**, *32*, 161.

20. Jain, M.; Fan, J.; Baturay, N. Z.; Kwon, C. H. *J. Med. Chem.* **2004**, *47*, 3843.

21. Sun, Y. W.; Chen, K. M.; Kwon C. H. *Mol. Pharm.* **2006**, *3*, 161.

22.Schlienger, N.; Beltran, T.; Périgaud, C.; Lefebvre, I.; Pompon, A.; Aubertin, A. M.; Gosselin, G.; Imbach, J. L. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3003.

23. Gouy, M. H.; Jordheim, L. P.; Lefebvre, I.; Cros, E.; Dumontet, C.; Peyrottes, S.; Périgaud, C. *Bioorg. Med. Chem.* **2009**, *17*, 6340.

24. Kwon, C. H. *Arch. Pharm. Res.* **1999**, *22*, 533.

25. Fuson, R. C.; Koehneke, J. H. **1949**, *14*, 706.

26. Conte, V.; Di Furia, M.; Licini, G.; Modena, G.; Sbampato, G.; Valle, G. *Tetrahedron: Asymmetry.* **1991**, *2*, 257.

27. Marugg, J. E.; Burik, A.; Tromp, M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1986**, *27*, 2271.

28. Schlienger, N.; Peyrottes, S.; Kassem, T.; Imbach, J. L.; Gosselin, G.; Aubertin, A. M.; Périgaud, C. *J. Med. Chem.* **2000**, *43*, 4570.

29. Griffin, R. J.; Henderson, A.; Curtin, N. J.; Echalier, A.; Endicott, J. A.; Hardcastle, I. R.; Newell, D. R.; Noble, M. E.; Wang, L. Z.; Golding, B. T. *J. Am. Chem. Soc.* **2006**, *128*, 6012.

30. Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936.

Graphical Abstract

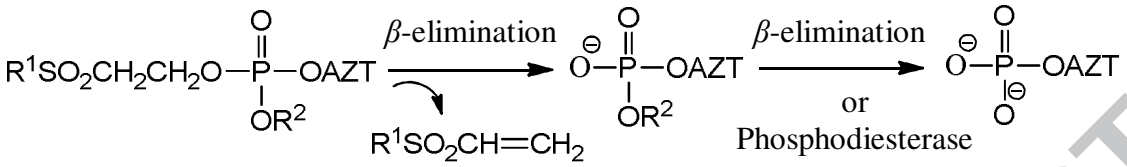
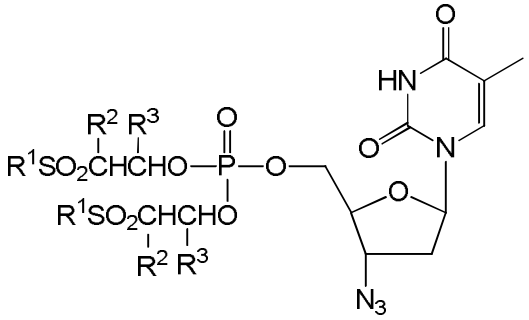


Figure 1. Structures of sulfonylethyl-containing AZT phosphotriesters.

A. Bis(sulfonylethyl) phosphotriesters



1. R^1 = phenyl, R^2 = H, R^3 = H;

2. R^1 = *p*-tolyl, R^2 = H, R^3 = H;

3. R^1 = *p*-methoxyphenyl, R^2 = H, R^3 = H;

4. R^1 = *p*-chlorophenyl, R^2 = H, R^3 = H;

5. R^1 = *p*-nitrophenyl, R^2 = H, R^3 = H;

6. R^1 = phenyl, R^2 = CH_3, R^3 = H;

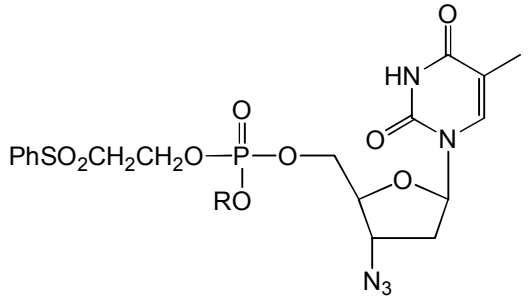
7. R^1 = phenyl, R^2 = H, R^3 = CH_3;

8. R^1 = phenyl, R^2 = H, R^3 = CH_2CH_3;

9. R^1 = phenyl, R^2 = CH_3, R^3 = CH_3;

10. R^1 = phenyl, R^2 = phenyl, R^3 = H.

B. Mono(sulfonylethyl) phenyl phosphotriesters



11. R = phenyl;

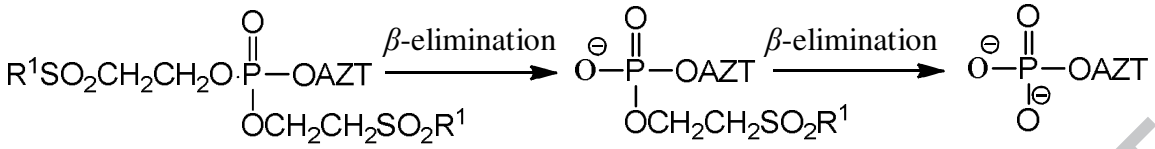
12. R = *p*-tolyl;

13. R = *p*-methoxyphenyl;

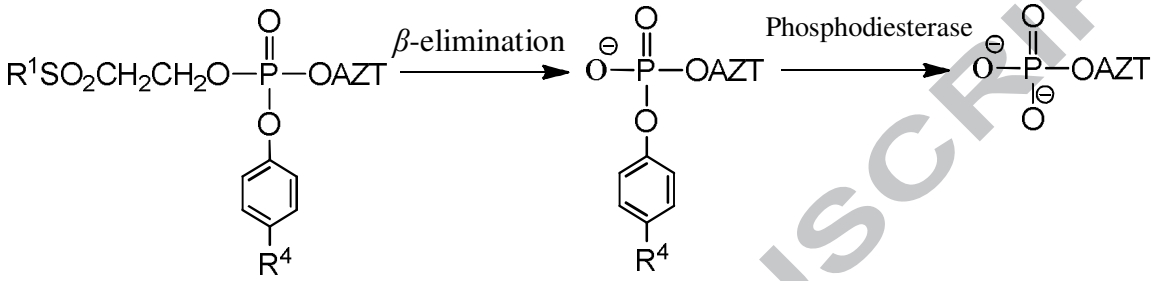
14. R = *p*-fluorophenyl;

15. R = *p*-chlorophenyl.

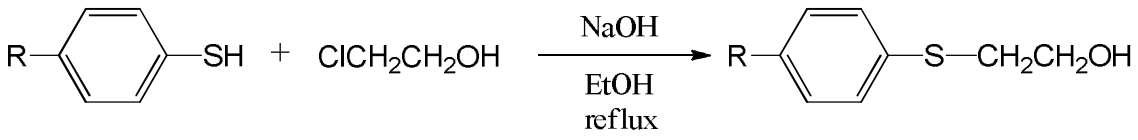
A.



B.



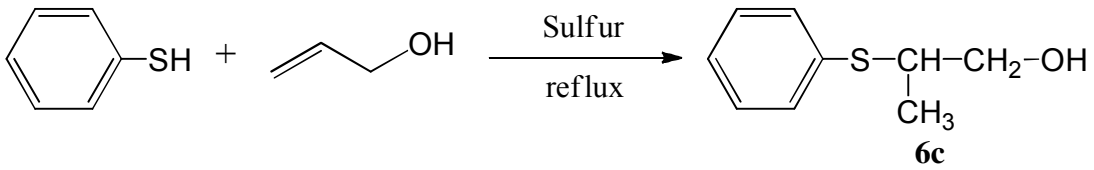
Scheme 1. Proposed activation mechanisms of AZT phosphotriester prodrugs.



2c-**5c**

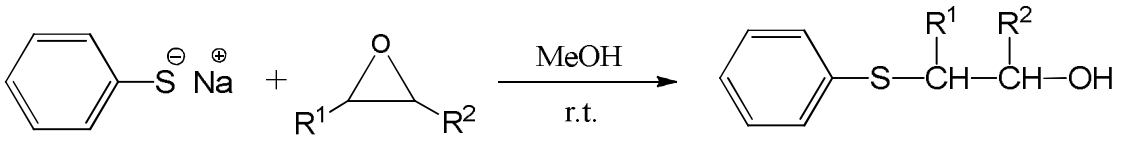
2c: R = CH_3; **3c**: R = CH_3O; **4c**: R = Cl; **5c**: R = NO_2.

Scheme 2. Synthesis of *p*-substituted phenylthioethanol derivatives **2c**-**5c**.



6c

Scheme 3. Synthesis of 2-(phenylthio)propan-1-ol **6c**.

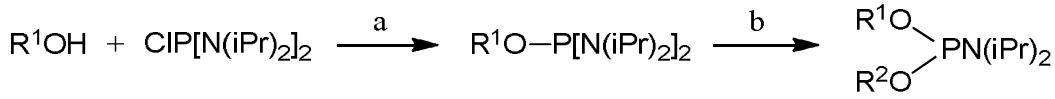


7c-**10c**

7c: R^1= H, R^2 = CH_3; **8c**: R^1= H, R^2 = CH_2CH_3; **9c**: R^1 = CH_3, R^2 = CH_3;

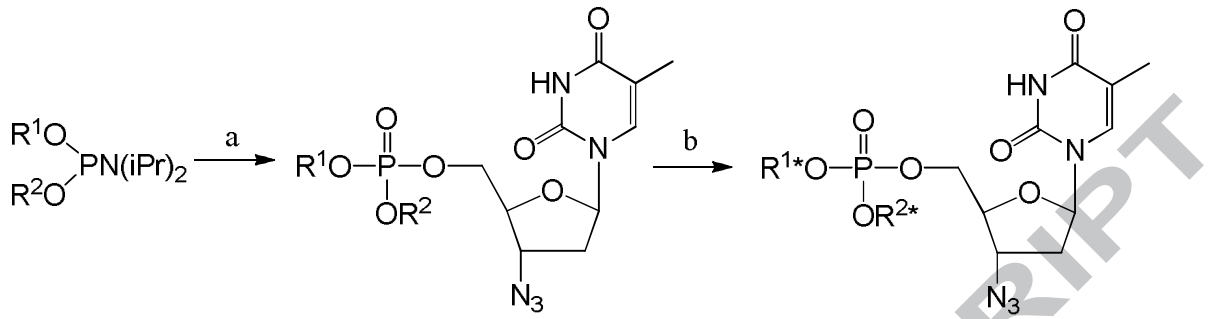
10c: R^1 = Ph, R^2 = H.

Scheme 4. Synthesis of α- and β-substituted 2-(phenylthio)ethanol derivatives **7c**-**10c**.



1c-**10c** **1b**-**15b**

Scheme 5. Synthesis of phosphoramidite intermediates **1b**-**15b**. Reagents and conditions: (a) Et_3N, THF, room temperature, N_2, 2h; (b) R^2OH (**1c**-**10c**, phenol and *p*-substituted phenols), 4,5-dicyanoimidazole, CH_2Cl_2, room temperature, N_2, 15min.



1b-15b **1a-15a** **1-15**

R^1* and R^2* indicate the corresponding sulfone product of thioethyl group.

Scheme 6. Synthesis of sulfonylethyl-containing phosphotriesters **1-15**. Reagents and conditions: (a) AZT, 1*H*-tetrazole, ACN, r.t., followed by TBHP at 0 ºC, then r.t. for 1h; (b) *m*CPBA, CH_2Cl_2, r.t., 3h.

Table 1

Half-lives of sulfonylethyl-containing AZT phophotriesters in model physiological conditions (0.1 M phosphate buffer, pH 7.4, 37 °C) and in the presence of human plasma

	Half-lives (h)[a]	
Compd	Phosphate buffer	Human plasma[b]
1	0.71 ± 0.07	0.23 ± 0.05
2	1.09 ± 0.09	0.40 ± 0.04
3	1.44 ± 0.14	0.55 ± 0.03
4	0.36 ± 0.03	0.13 ± 0.02
5	0.07 ± 0.006	0.02 ± 0.0005
6	13.57 ± 0.91	4.21 ± 0.79
7	3.56 ± 0.63	1.35 ± 0.18
8	5.42 ± 0.45	1.77 ± 0.16
9	278.83 ± 29.26	70.12 ± 4.65
10	2.16 ± 0.32	0.51 ± 0.04
11	1.22 ± 0.05	0.45 ± 0.05
12	1.32 ± 0.22	0.60 ± 0.12
13	1.53 ± 0.17	0.60 ± 0.07
14	1.08 ± 0.08	0.37 ± 0.08
15	0.79 ± 0.09	0.35 ± 0.03

[a] Mean ± SD (standard deviation) of three independent experiments.

[b] Human plasma reconstituted in 0.1 M phosphate buffer, pH 7.4.

Table 2

The cytotoxic effects of AZT and the synthesized phosphotriesters on MCF-7, H460, KB-3-1, SW620 and 143B cell lines

	$IC_{50} \pm SD^a$ (μM)				
Compd	MCF-7	H460	KB-3-1	SW620	143B
1	16.33 ± 1.05	7.59 ± 0.69	10.98 ± 0.33	43.04 ± 1.07	16.32 ± 1.11
2	19.61 ± 1.42	11.28 ± 0.39	14.44 ± 0.77	46.56 ± 0.54	19.63 ± 1.45
3	6.38 ± 0.75	1.71 ± 0.19	4.68 ± 0.54	7.62 ± 0.53	6.36 ± 0.46
4	9.46 ± 0.58	4.47 ± 0.58	7.84 ± 0.61	11.64 ± 0.70	9.68 ± 0.52
5	22.88 ± 0.86	15.22 ± 0.68	20.35 ± 0.65	57.00 ± 1.01	23.09 ± 1.02
6	27.57 ± 0.57	23.70 ± 0.56	25.190 ± 0.85	68.21 ± 1.29	27.44 ± 0.63
7	22.43 ± 1.79	14.59 ± 1.20	18.37 ± 0.41	54.82 ± 1.49	22.54 ± 2.37
8	12.22 ± 1.03	5.13 ± 0.40	8.28 ± 0.16	27.84 ± 1.30	12.41 ± 1.11
9	26.83 ± 0.90	22.17 ± 0.55	24.18 ± 0.48	66.66 ± 1.19	26.43 ± 1.10
10	14.07 ± 0.33	7.13 ± 0.50	9.82 ± 0.65	42.16 ± 0.94	14.17 ± 0.11
11	25.27 ± 0.91	18.22 ± 0.77	21.74 ± 1.61	62.12 ± 2.21	25.43 ± 1.48
12	28.17 ± 0.83	24.70 ± 0.83	27.10 ± 0.91	71.74 ± 2.25	28.33 ± 1.29
13	20.39 ± 0.66	12.33 ± 0.57	15.15 ± 1.68	47.26 ± 3.81	20.24 ± 0.67
14	26.17 ± 0.65	20.78 ± 1.00	23.30 ± 0.64	64.46 ± 1.53	26.55 ± 0.60
15	25.03 ± 1.02	17.85 ± 0.88	21.36 ± 0.70	61.32 ± 2.78	24.42 ± 1.15

AZT	13.74 ± 1.11	6.81 ± 0.74	9.17 ± 0.28	39.11 ± 0.39	13.10 ± 0.97

[a] IC_{50}: concentration that inhibited cell survival by 50% (means ± SD).

Values in the table are representatives of at least three independent experiments performed in triplicate.

Table 3

The cytotoxic effects of AZT and phosphotriesters on 143B and 143B/TK$^-$ cell lines

	$IC_{50} \pm SD^a$ (μM)		
Compd	143B	143B/TK$^-$	CR[b]
3	5.45 ± 0.32	5.16 ± 0.21	0.9
4	7.83 ± 0.44	8.80 ± 0.47	1.1
8	11.46 ± 0.23	13.32 ± 0.77	1.2
10	15.93 ± 0.35	19.74 ± 0.56	1.2
13	20.22 ± 0.36	25.28 ± 0.90	1.3
AZT	12.25 ± 0.60	148.42 ± 3.16	12.1

[a] IC_{50}: concentration that inhibited cell survival by 50% (means ± SD).

[b] CR: cytotoxicity ratio is IC_{50} of 143B/TK$^-$ cells / IC_{50} of 143B cells.

Values in the table are representatives of at least three independent experiments performed in triplicate.

Graphical Abstract