Synthesis and Studies of Mononucleoside Glucosyl Phosphotriester Derivatives

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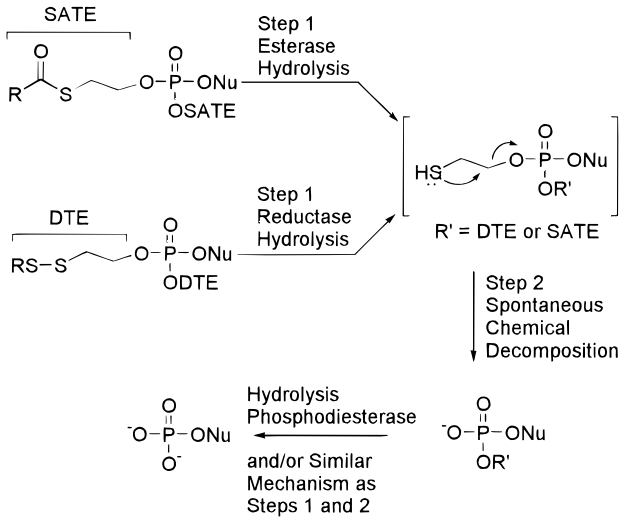
The synthesis and stability studies in several aqueous media of mononucleoside glucosyl phosphotriester derivatives of 3′-azido-3′-deoxythymidine are reported herein. Such neutral mononucleotides, which incorporate *β*-glucopyranosidyl moieties associated to a thioethyl linker as phosphate protecting groups were designed to act as "pronucleotides", giving rise to the corresponding 5′-mononucleotide through a glucosidase-mediated activation mechanism.

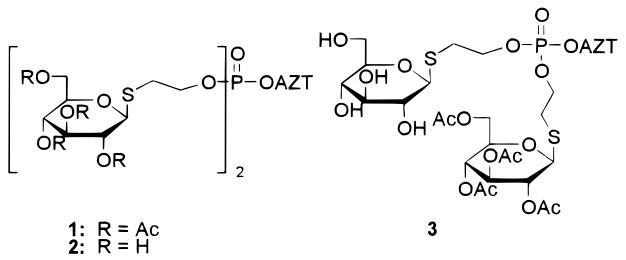
Introduction

In recent years, considerable efforts have been devoted to improve the therapeutic potential of nucleoside analogues by the use of nucleotide prodrugs (pronucleotides).[1−4] Thus, many strategies have been considered to mask or reduce the negative charges of the phosphate function of 5′-mononucleotides. Our work in this topic started with the study of phosphate protections incorporating a thioethyl chain where the thiol is masked either as a disulfide (DTE group)[5] or as a thioester (SATE group).[6] The use of the resulting bis[*S*-((2-hydroxyethyl)sulfidyl)-2-thioethyl] and bis(*S*-acyl-2-thioethyl) mononucleoside phosphotriesters (Scheme 1) allows, respectively, by a reductase- and an esterase-dependent activation process the release of an unstable *O*-2-mercaptoethyl phosphotriester which decomposes spontaneously via intramolecular nucleophilic displacement into the corresponding phosphodiesters and ethylene sulfide. Removal of the remaining phosphate protecting group from the phosphodiester derivatives by either a similar mechanism and/or a phosphodiesterase activity gives rise to the selective delivery of the parent 5′-mononucleotides into the cells.[7−9]

As a part of our anti-HIV drug research program, we decided to evaluate the potentialities of new biolabile phosphate protecting groups involving another enzymatic activation process. In this paper, we report the synthesis, and the stability studies in different media, of mononucleoside phosphotriester derivatives **1**−**3** of 3′-azido-3′-deoxythymidine (AZT), where the thioethyl group is linked to *β*-glucopyranosidyl moieties. Such compounds were designed to be able to liberate the corresponding 5′-mononucleotide through a glucosidase-mediated activation mechanism. Compared to the phosphotriester derivative **2**, the acetyl groups of the pronucleotides **1** and **3**, which are likely to be cleaved by esterases, induce different degrees of lipophilicity and might influence the chemical stability, cellular penetration, and glucosidase activation of the parent phosphotriesters.

Scheme 1





Results and Discussion

Preparation of Pronucleotides 1−3. Because of structural requirements, the synthesis of mononucleoside phosphotriester derivatives **1**−**3** was carried out following two strategies using P^{III} (phosphoramidite) or P^{V} (phosphodiester) intermediates. Thus, the pronucleotides **1**

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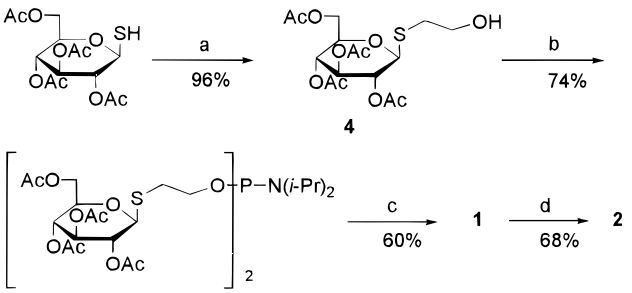
Scheme 2



(a) ICH_2CH_2OH, DBU, toluene. (b) $PCl_2N(i\text{-}Pr)_2$, Et_3N, THF. (c) 1. AZT, 1*H*-tetrazole, THF. 2. *t*BuOOH. (d) Dowex 1X2 (OH^- form), EtOH.

and **2** bearing two identical biolabile phosphate protections can be obtained by coupling AZT with an appropriate phosphoramidite reagent followed by in situ oxidation.[6] This approach involves the use of appropriate protective groups for the carbohydrate hydroxyl functions of the pronucleotide precursor **1** which have to be removed without degradation of the fully deprotected phosphotriester **2**. Classical methods of hydrolysis of base-labile protective groups (e.g. sodium methoxide, methanolic ammonia) cannot be considered, since the phosphotriester function is cleaved by basic and nucleophilic reagents. Furthermore, common protections for the hydroxyl groups of glycosyl derivatives, which could be removed in acidic or neutral media including acetals,[10] silyl derivatives,[11] or ethers were found to be unsuitable for various reasons. For example, the deprotection of 2-hydroxyethyl 2,3,4,6-tetra-*O*-benzyl-1-*β*-D-thioglucoside,[12] used as a model, gave by different methods[13,14] only low yields of 2-hydroxyethyl 1-*β*-D-thioglucopyranoside.

Since enzyme-mediated hydrolysis of ester groups has been reported by using esterases or lipases,[15,16] we decided to prepare the glucosylated phosphotriester **2** from its peracetylated precursor **1** (Scheme 2). The tetraacetylated thioglucoside **4** was obtained by alkylation of the commercially available 2,3,4,6-tetra-*O*-acetyl-1-*β*-D-thioglucopyranose with 2-iodoethanol in presence of DBU. This derivative **4** was coupled with *N*,*N*-diisopropylaminophosphodichloridite[17] yielding the corresponding phosphoramidite **5**, which was stable enough to be purified by flash chromatography. The phosphorylating agent **5** was coupled with AZT, in presence of a large excess of 1*H*-tetrazole, and the phosphotriester derivative **1** was isolated after in situ oxidation of the intermediate phosphite triester with *tert*-butyl hydroperoxide. The treatment of the peracetylated pronucleotide **1** using enzymes such as pig liver esterase (PLE), lipases from porcine pancreas (PPL), or *Candida cylindracea* (CCL) was monitored by HPLC. In all cases, those studies showed a time-dependent accumulation of

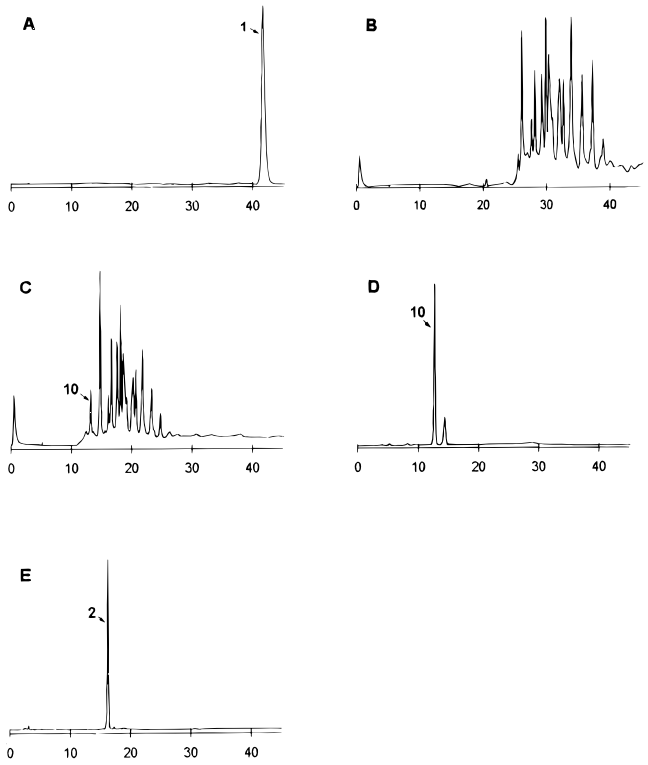


Figure 1. HPLC chromatograms of the treatment of the phosphotriester **1** (A), with PLE (B–D), or Dowex resin (E). Time profiles of the enzymatic reaction at $t = 2$ days (B), 3 days (C), and 10 days (D) in 0.1 M phosphate buffer, pH 7.4, 40 units/μmol, 37 °C. (E) deacetylation by Dowex anion exchange resin (1 × 2, OH^- form), $t = 3$ h at rt.

one major product (Figure 1). Whatever the enzymatic concentrations used (30–50 units/μmol), the resulting product was identified as the corresponding phosphodiester **10** bearing only one deacetylated carbohydrate moiety. The formation of this phosphodiester may be related to the relative instability of thioglycosyl phosphotriester compounds in the aqueous media used in these enzymatic reactions (see below). Finally, the phosphotriester derivative **2** was obtained in satisfactory yield from its phosphotriester precursor **1** by the use of a Dowex anion exchange resin (1 × 2, OH^- form) in ethanol.[18] Only a few examples of this deprotection method have been reported in the literature.[19] Here, the completion of the reaction was monitored by HPLC and the eight acetyl groups of phosphotriester **1** have been cleaved after 3 h at room temperature leading to **2** without appearance of degradation products (Figure 1). Yields were increased by washing the resin with water, ethanol, and ammonium acetate buffer (pH 5.9) in order to minimize partial adsorption of **2** on the resin.

The synthesis of the mixed phosphotriester **3** was carried out by alkylation of the glucosyl phosphodiester **10** with 2-iodoethyl 2,3,4,6-tetra-*O*-acetyl-1-*β*-D-thioglucopyranoside **6**.[20] The alkylating agent **6** was obtained by adapting a published procedure[21] by alkylation of the

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(18) The purity of phosphotriester **2** was checked by reversed-phase HPLC and found to be 98% pure, containing phosphodiester **10** as the only impurity. Higher grades of purity were not achieved due to the lability of the compound in water used for its purification (see stability studies).
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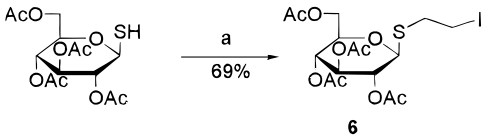
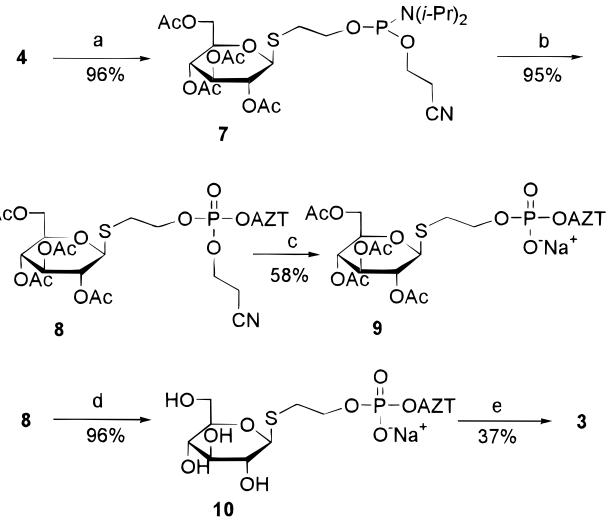
Scheme 3



(a) 1. $BrCH_2CH_2Br$, K_2CO_3, acetone. 2. NaI, acetone.

Scheme 4



(a) $NCCH_2CH_2OP[N(i\text{-}Pr_2)]_2$,1*H*-tetrazole, $HN(i\text{-}Pr)_2$, CH_3CN. (b) 1. AZT, 1*H*-tetrazole, CH_3CN. 2. *t*BuOOH. (c) DBU, THF. (d) NaOMe, MeOH. (e) 1. Dowex 50WX2 [$(n\text{-}C_4H_9)N^+$ form]. 2. **6**, CH_3CN, 80°C.

commercially available 2,3,4,6-tetra-*O*-acetyl-1-β-D-thioglucopyranose with 1,2-dibromoethane and subsequent nucleophilic substitution of the bromine by sodium iodide (Scheme 3). Then, the phosphodiester **10** was synthesized according to known procedures (Scheme 4). Thus, the thioglucoside **4** was phosphitylated with β-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite[22] upon tetrazole activation, in presence of *N*,*N*-diisopropylamine.[23] The resulting phosphoramidite **7** was coupled with AZT, in presence of tetrazole, and oxidized in situ with *tert*-butyl hydroperoxide to yield the phosphotriester **8**. Alkaline deprotection afforded the glucosyl phosphodiester **10** (sodium salt). Subsequent conversion to its tetrabutylammonium salt[24] by a Dowex 50 WX2 exchange resin [$(n\text{-}C_4H_9)N^+$ form], followed by alkylation[25] with the acetylated iodoethyl thioglucoside **6** gave the desired phosphotriester derivative **3**. Alternatively, by DBU treatment the β-cyanoethyl group of phosphotriester **8** was removed selectively to yield the peracetylated phosphodiester **9**, which was used as reference sample in the stability studies.

Structural Analysis. All compounds were fully characterized by ^1H, ^{13}C, and ^{31}P NMR, fast atom bombardment (FAB) mass spectra, and elemental analysis. The β-configuration of the anomeric protons of the thioglucopyranosides in compounds **1**−**10** was deduced from the large ^1H NMR coupling constants ($J_{1,2}$ = 9.6−10.0 Hz) and the chemical shifts (4.5 ppm) of the H_1 doublet. Additionally, proton-coupled ^{13}C NMR experiments showed coupling constants $^1J[^{13}CH(1)]$ of 155 Hz, which are in agreement with reported values for β-thioglucoside derivatives.[26] None of the α-epimers could be detected spectroscopically.

As anticipated, ^{31}P NMR spectral data revealed a single resonance for the compounds **1**,**2** at a chemical shift consistent with the structure of a symmetrical phosphotriester (δ_p −0.98 and −0.36 ppm, respectively). The mixed phosphotriester **3** displayed two closely spaced signals in the ^{31}P NMR spectrum (δ_p −0.41 and −0.47 ppm) corresponding to the presence of diastereoisomers (ratio 1:1), due to the phosphorus stereocenter. Similarly, distinct diastereoisomeric signals and phosphorus coupling were noted on the proton-decoupled ^{13}C NMR spectrum also. Furthermore, the presence of diastereoisomers was apparent from ^1H NMR spectroscopy and analytical HPLC studies. Besides ^{31}P NMR analysis, the phosphotriester structures of **1**−**3** were confirmed by the chemical shifts in ^1H and ^{13}C NMR spectra of the methylene groups bound to the phosphate in comparison with the corresponding NMR signals observed for the phosphodiester derivatives **9**,**10**. For example, ^1H NMR signals for P−O−CH_2 of the glucosidic chain and $H_5/H_{5'}$ of AZT (m, 4.01−4.29 ppm) of **2** are around 0.3 ppm downfield shifted compared to **10**, and the ^{13}C NMR spectrum revealed downfield shifts of 2.8 and 2.4 ppm, respectively. Even if NMR spectral data were very complex, they provided information with regard to structural restraints of such phosphorylated compounds. Thus, the ^1H and ^{13}C NMR spectra of compounds **1** and **5** which incorporate two identical peracetylated glucosyl phosphate protective groups revealed the nonequivalence of the two glucosidic moieties. For example, the ^1H NMR spectrum in $CDCl_3$ of the phosphotriester **1** displayed two distinct doublets for the anomeric protons of the glucosides ($\Delta\delta_H$ = 0.02 ppm). We supposed that these differences are due to diastereotopic protons of the prochiral sugar moities or less likely, to steric interactions of the bulky carbohydrate groups. Mass spectrometry in FAB negative mode showed the molecular ion for the mixed phosphotriester **3** (*m*/*z* 958 $[M - H]^-$), and fragmentations (*m*/*z* 736, 709, 568) consistent with the loss of one out of three different phosphate substituents.

Stability Studies. The hydrolysis kinetics of the compounds **1**−**3** (initial concentration 50 μM) were studied at 37 °C in (i) RPMI 1640 containing 10% heat inactivated fetal calf serum (culture medium, CM), in order to evaluate the stability of these compounds in the extracellular medium used for antiviral evaluation in cell culture systems, (ii) in RPMI 1640 in order to establish the difference between chemical and enzyme-controlled hydrolysis, and (iii) in water, in order to evaluate the influence of nucleophiles present in the RPMI 1640. Crude aliquots of incubates were analyzed by the HPLC "on-line internal-surface reversed-phase (ISRP) cleaning" method previously described.[6] This technique allows the direct analysis of biological samples without pretreatment. Authentic phosphodiesters **9** and **10** were used for calibration and identification of the decomposition products. HPLC/MS coupling experiments (negative electrospray mode) allowed the detection of some analytes presumed to result from deacetylation of the pronucle-

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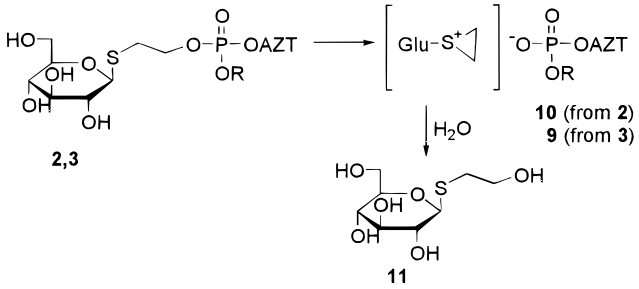
Table 1. Half-lives and Decomposition Products of the Phosphotriesters 1−3 in Water, RPMI 1640, and Culture Medium (CM)

compd	media	$t_{1/2}$, h	resulting products (% of decomposition pathways)
1	water	100	**9**
	RPMI	35	**9** (65%), deacetylated products (35%)
	CM	30	**9** (12%), deacetylated products (88%)
2	water	4.5	**10**
	RPMI	4.5	**10**
	CM	4.5	**10**
3	water	8.8	**9** (92%), **10** (8%)
	RPMI	8.3	**9** (90%), **10** (10%)
	CM	8.3	**9** (90%), **10** (7%), deacetylated products (<3%)

Scheme 5



otides **1** and **3**. The rate constants of the disappearance of the pronucleotides were calculated according to pseudo-first-order kinetic models, which are in accordance with the experimental data.

The half-lives of the pronucleotides **2** and **3** were unexpectedly short and were both similar in all media (Table 1). The phosphotriester **2** decomposed in each medium with a half-life of 4.5 h to give exclusively the corresponding phosphodiester **10**. The formation of this compound as the decomposition product was confirmed by HPLC coinjection with an authentic sample and in D_2O by ^{31}P NMR analysis ($\delta = 0.70$ ppm). In the three media used, the mixed phosphotriester **3** hydrolyzed predominantly into the phosphodiester **9**. In water and RPMI, the unacetylated phosphodiester **10** is the only secondary product observed, while in CM small amounts of deacetylation products are also present. In water, the acetylated phosphotriester **1** decomposed very slowly ($t_{1/2}$ 100 h) to give the acetylated phosphodiester **9** only. In RPMI and CM, deacetylated products predominate and the half-life of the phosphotriester **1** is shorter, but this compound is still more stable than the phosphotriester derivatives **2** and **3**. Deacetylation may be either due to the basicity of the media (pH 7.4) and/or to the remaining esterase activity in the heat-inactivated serum of the CM.

The decomposition of the studied phosphotriester derivatives seems to proceed by chemical mechanisms (apart from deacetylation reactions in CM). In addition to possible direct nucleophilic attacks on the phosphorus atom, the lability in polar aqueous media of the phosphotriesters **2** and **3** (Table 1) might be explained by a similar mechanism to the one reported in the case of 2-chloroethyl 1-β-D-thioglycopyranosides.[20,21] Thus, the mechanism of decomposition of such glycosylated phosphotriesters could involve the participation of the neighboring sulfur atom (Scheme 5), giving rise to the corresponding phosphodiester and to an unstable episulfonium ion[27] which is readily hydrolyzed into the 2-hydroxyethyl thioglucopyranoside **11**. The glucoside **11** has been detected in water (TLC analysis, methanol/acetone 1:9) by comparison with an authentic sample.[20] D-glucose appearance which would result from the hydrolysis of the glucosidic bonds has not been found. Furthermore, it seems that the acetyl protective groups of the sugar moieties may prevent (or at least slow) the rate of this kind of chemical decomposition.

Conclusion

The synthesis of three mononucleoside phosphotriester derivatives of AZT **1**−**3** which incorporate β-glucopyranosidyl moieties associated to a thioethyl linker has been carried out by different phosphorus chemistries. The unacetylated phosphotriester **2** was obtained using a strong anion-exchange resin, and this procedure does not affect the phosphotriester structure. The stability of these new mononucleoside glucosyl phosphotriesters has been investigated in several media including culture medium. Partly or fully deacetylated derivatives exhibited a relatively low stability in all studied media compared to the corresponding SATE or DTE pronucleotides of AZT.[6,7] This may be related to a chemical decomposition mechanism involving the sulfur atom as participating neighboring group. Despite this fact, the half-lives of pronucleotides **1**−**3** seem to be compatible with an in vitro anti-HIV activity. In this respect, the antiretroviral evaluation of the described glucosyl phosphotriester derivatives and the stability studies of these compounds in several biological media, as well as their substrate affinities against purified glucosidases, are currently in progress in our laboratory.

Experimental Section

General Methods. Unless otherwise stated, ^1H NMR spectra were recorded at 400 MHz and ^{13}C NMR spectra at 100 MHz with proton decoupling at ambient temperature. Chemical shifts are given in δ-values referenced to the residual solvent peak chloroform ($CDCl_3$) at 7.26 ppm and 77.0 ppm, dimethyl sulfoxide (DMSO-d_6) at 2.49 ppm and 39.5 ppm) relative to tetramethylsilane (TMS). Deuterium exchange, decoupling, and COSY experiments were performed in order to confirm proton assignments. Coupling constants, J, are reported in Hertz. 2D $^1H-^{13}C$ heteronuclear COSY were recorded for the attribution of ^{13}C signals. Unless otherwise stated, ^{31}P NMR spectra were recorded at ambient temperature at 100 MHz with proton decoupling. Chemical shifts are reported relative to external H_3PO_4. FAB mass spectra were recorded in the positive-ion or negative-ion mode using thioglycerol/glycerol (1:1, v/v, G−T) as matrix. Melting points were determined in open capillary tubes and are uncorrected. Elemental analyses were carried out by the Service de Microanalyses du CNRS, Division de Vernaison (France). Thin-layer chromatography (TLC) was performed on precoated aluminium sheets of silica gel 60 F_{254} (Merck, Art. 9385), visualization of products was accomplished by UV absorbance followed by charring with 5% ethanolic sulfuric acid with heating; phosphorus-containing compounds were detected by spraying with Hanes molybdate reagent.[28] Column chromatography was carried out on silica gel 60 (Merck). Preparative HPLC analyses were performed using a C_{18} reversed-phase column (100 × 40 mm, 6 μM). Stability studies were performed as previously described using an improved "on line ISRP cleaning" method[6] at initial concentrations of 50 μM of substrate. HPLC/MS coupling was performed in the negative electrospray mode as previously reported.[29] Analytical HPLC studies were carried out using a reversed-phase analytical column (Nucleosil, C_{18}, 150 × 4.6 mm, 5 μm) equipped with a

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prefilter, a precolumn (Nucleosil, C_{18}, 5 μm), and a photodiode array detector (detection at 267 nm). The compound to be analyzed was eluted using a linear gradient of 0−50% acetonitrile in 50 mM triethylammonium acetate buffer (pH 7) programmed over a 40-min period with a flow rate of 1 mL/min. All moisture-sensitive reactions were carried out under rigourous anhydrous conditions under an argon atmosphere using oven-dried glassware. Solvents were dried and distilled prior to use and solids were dried over P_2O_5 under reduced pressure at rt. AZT was purchased from Intsel Marsing France; 2,3,4,6-tetra-*O*-acetyl-1-β-D-thioglucopyranose from Sigma; PLE (EC 3.1.1.1., 1340 units/mL) from Fluka and RPMI 1640 from GibcoBRL.

2-Hydroxyethyl 2,3,4,6-Tetra-*O*-acetyl-1-β-D-thioglucopyranoside (4). To a solution of 2,3,4,6-tetra-*O*-acetyl-1-β-D-thioglucopyranose (1.40 g, 3.8 mmol) in toluene (40 mL) at room temperature (rt) was added dropwise 2-iodoethanol (0.45 mL, 5.7 mmol) followed by the addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (1.5-*S*) (DBU, 0.57 mL, 3.8 mmol). The reaction mixture was stirred at rt for 1 h, diluted with water, and extracted four times with CH_2Cl_2. The combined organic layers were washed sequentially with 5% aqueous NaOH, 5% aqueous H_2SO_4, and water, dried over $NaSO_4$, filtered, and evaporated to dryness. The crude oil was chromatographically purified on a silica gel column with a stepwise (0−30%) gradient of EtOAc in CH_2Cl_2. The appropriate fractions were collected, evaporated, and recrystallized in CCl_4 at 4 °C to give pure **4** (1.52 g, 96%) as colorless needles. R_f (EtOAc/CH_2Cl_2 1:4): 0.18. Mp: 74−75 °C; lit.[20] mp 70−73 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 5.23 (t, 1H, J = 9.3), 5.01−5.10 (m, 2H), 4.53 (d, 1H, J = 10.1), 4.18 (m, 2H), 3.72−3.81 (m, 3H), 2.94−3.04 (m, 1H), 2.71−2.81 (m, 2H), 2.10, 2.07, 2.03, 2.01 (4s, 12H). ^{13}C NMR ($CDCl_3$): δ 170.6, 170.1, 169.4, 169.3, 84.1, 75.9, 73.6, 69.5, 68.1, 62.3, 62.0, 35.0, 20.7, 20.6, 20.5. MS FAB$^+$: m/z 409 (M + H)$^+$. $[\alpha]^{25}_D$ = −8 (c 1.0, $CHCl_3$); lit.[20] $[\alpha]_D$ = −14 (c 0.5, $CHCl_3$). Anal. Calcd for $C_{16}H_{24}O_{10}S$: C, 47.05; H, 5.92; S, 7.85. Found: C, 47.30; H, 5.91; S, 7.99.

Bis[*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosidyl)-2-thioethyl] *N*,*N*-Diisopropylphosphoramidite (5). A solution of thioglucoside **4** (2.80 g, 6.86 mmol) and triethylamine (1.53 mL, 10.9 mmol) in dry THF (30 mL) was stirred for 2 h over 4 Å molecular sieve (1g) and then added at −78 °C to a stirred solution of *N*,*N*-diisopropylaminophosphorodichloridite[17] (0.69 g, 3.41 mmol) in dry THF (14 mL). The solution was stirred for 3 h at rt, then filtered to remove triethylamine hydrochloride. Triethylamine (0.47 mL, 3.40 mmol) was added and the filtrate concentrated *in vacuo* to about 5 mL. The product was purified by short column chromatography eluting with EtOAc/cyclohexane (2:3, v/v) containing 1% Et_3N. The appropriate fractions were collected and evaporated to give pure phosphoramidite **5** (2.38 g, 74%) as a colorless foam. R_f (Et_3N/EtOAc/cyclohexane 1:3:6): 0.26. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 5.22 (t, 2H, J = 9.2), 4.97−5.12 (m, 4H), 4.57, 4.56 (2d, 2H, J = 10.0), 4.25 (dd, 2H, J = 4.8, 12.4), 4.12 (dd, 2H, J = 2.2, 12.4), 3.67−3.88 (m, 6H), 3.50−3.65 (m, 2H), 2.75−3.02 (m, 4H), 2.09, 2.05, 2.02, 2.00 (4s, 24H), 1.17 (d, 12H, J = 6.7). ^{13}C NMR ($CDCl_3$): δ 170.6, 170.1, 169.3, 169.2, 83.7, 83.6, 75.8, 73.7, 69.9, 68.2, 63.2 (d, J_{P-C} = 18.1), 63.1 (d, J_{P-C} = 17.5), 62.0, 42.9 (d, J_{P-C} = 12.5), 31.1 (d, J_{P-C} = 7.1), 24.6 (d, J_{P-C} = 7.1), 20.7, 20.6, 20.5. ^{31}P NMR ($CDCl_3$): δ 147.69. MS FAB$^+$: m/z 946 (M + H)$^+$. Anal. Calcd for $C_{38}H_{60}NO_{20}PS_2$: C, 48.24; H, 6.39; N, 1.48. Found: C, 48.30; H, 6.79; N, 1.59.

Bis[*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosidyl)-2-thioethyl] 3′-Azido-3′-deoxythymidin-5′-yl phosphate (1). Sublimed 1*H*-tetrazole (193 mg, 2.75 mmol) was added to a stirred solution of AZT (73 mg, 0.275 mmol) in dry THF (0.74 mL) containing 4 Å molecular sieve (about 500 mg), followed by the dropwise addition at rt of a solution of phosphoramidite **5** (312 mg, 0.330 mmol) in dry THF (0.40 mL). After 45 min, the reaction mixture was cooled to −40 °C and *tert*-butylhydroperoxide (0.22 mL, 0.66 mmol, 3 M in toluene) was added. After further stirring at rt for 90 min, the mixture was diluted with CH_2Cl_2 and washed successively with 10% aqueous $Na_2S_2O_3$ and water, the organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. Column chromatography of the residual syrup on silica gel using a stepwise gradient of 0−3% methanol in CH_2Cl_2 afforded phosphotriester **1** (186 mg, 60%) as a colorless foam. R_f (MeOH/CH_2Cl_2 7:93): 0.50. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 8.41 (br s, 1H), 7.35 (d, 1H, J = 1.2), 6.16 (t, 1H, J = 6.4), 5.24 (m, 2H), 4.98−5.13 (m, 4H), 4.54, 4.52 (2d, 2H, J = 10.0), 4.38−4.45 (m, 1H), 4.13−4.35 (m, 10H), 4.03−4.08 (m, 1H), 3.71−3.76 (m, 2H), 2.82−3.11 (m, 4H), 2.46 (m, 2H), 2.09, 2.06, 2.05, 2.03, 2.01 (5s, 24H), 1.95 (d, 3H, J = 0.8). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 11.36 (s, 1H), 7.47 (d, 1H, J = 1.0), 6.13 (t, 1H, J = 6.6), 5.27 (t, 1H, J = 9.1), 4.80−4.99 (m, 6H), 4.41−4.49 (m, 1H), 3.98−4.19 (m, 12H), 2.77−3.00 (m, 4H), 2.31−2.45 (m, 2H), 2.01, 2.00, 1.99, 1.97, 1.94 (5s, 24H), 1.78 (d, 3H, J = 0.7). ^{13}C NMR ($CDCl_3$): δ 170.6, 170.1, 169.4, 163.3, 150.0, 135,7, 111.4, 85.6, 83.3, 83.2, 82.3 (d, J_{P-C} = 7.6), 76.0, 73.6, 69.6, 68.2, 67.4 (d, J_{P-C} = 6.4), 67.3 (d, J_{P-C} = 6.4), 66.6, 61.9, 60.1, 37.2, 29.8 (d, J_{P-C} = 6.9), 29.7 (d, J_{P-C} = 6.9), 20.7, 20.6, 20.5, 12.5. ^{13}C NMR (DMSO-d_6): δ 170.1, 169.6, 169.3, 169.2, 163.7, 150.4, 135.8, 110.0, 83.7, 82.0, 81.9, 81.3 (d, J_{P-C} = 7.6), 74.5, 72.9, 69.5, 68.1, 66.8, 66.6, 61.9, 59.9, 35.7, 29.6 (d, J_{P-C} = 6.9), 20.5, 20.4, 20.3, 12.9. ^{31}P NMR ($CDCl_3$) δ −0.98. ^{31}P NMR (DMSO-d_6, 81 MHz): δ −0.38. MS FAB$^-$: m/z 1126 (M − H)$^-$. HRMS: calcd for $C_{42}H_{59}N_5O_{25}PS_2$ (M + H$^+$) 1128.2678, found 1128.2618. Anal. Calcd for $C_{42}H_{58}N_5O_{25}PS_2$: C, 44.72; H, 5.18; N, 6.21; S, 5.69. Found: C, 44.78; H, 5.29; N, 6.28; S, 5.74. HPLC: t_R 42.6 min.

Bis(*S*-β-D-glucopyranosidyl-2-thioethyl) 3′-Azido-3′-deoxythymidin-5′-yl Phosphate (2). The phosphotriester **1** (110 mg, 97 μmol) was stirred in ethanol 95 for 3 h with Dowex resin (about 1.1 g, 1 × 2, OH^- form, and washed with ethanol 95). The solution was filtered and the resin was successively washed with water, ethanol 95, and ammonium acetate buffer (0.1M, pH 5.9). The combined fractions were concentrated under reduced pressure and purified by preparative HPLC on a reversed-phase C_{18} column, eluting with CH_3CN/H_2O 9:91. The appropriate fractions were rapidly evaporated and lyophilized to yield the deacetylated phosphotriester **2** (52 mg, 68%) as a white powder. Due to a limited stability in aqueous media, and despite extensive purifications, the title compound was found to be pure at 98% by reversed-phase HPLC, containing the phosphodiester **10** as the only impurity. R_f (MeOH/CH_2Cl_2 3:7): 0.44. ^{1}H NMR (DMSO-d_6): δ 11.3 (br s, 1H), 7.46 (s, 1H), 6.14 (t, 1H, J = 6.6), 5.2, 5.1, 5.0 (3 br s, 6H), 4.47−4.51 (m, 3H), 4.33 (d, 2H, J = 9.6), 4.01−4.29 (m, 6H), 3.90 (m, 1H), 3.67 (m, 2H), 3.35−3.55 (m, 2H), 2.82−3.40 (m, 12H), 2.29−2.45 (m, 2H), 1.80 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 164.1, 150.6, 135.7, 110.0, 85.2, 85.1, 83.8, 81.2 (d, J_{P-C} = 7.9), 81.0, 78.1, 73.0, 69.9, 67.2, 66.6, 61.2, 60.1, 35.7, 29.2, 12.2. ^{31}P NMR (DMSO-d_6): δ −0.36. HRMS: calcd for $C_{26}H_{43}N_5O_{17}PS_2$ (M + H$^+$) 792.1818, found 792.1833. MS FAB$^+$: m/z 792 (M + H)$^+$. HPLC: t_R 16.3 min.

2-Iodoethyl 2,3,4,6-tetra-*O*-acetyl-1-β-D-thioglucopyranoside (6). To a stirred mixture of 2,3,4,6-tetra-*O*-acetyl-1-β-D-thioglucopyranose (970 mg, 2.66 mmol) in acetone (3.5 mL) and potassium carbonate (367 mg, 2.66 mmol) in water (2.5 mL) at rt was added 1,2-dibromoethane (2.3 mL, 26.7 mmol). TLC (EtOAc/toluene 1:4) of the mixture after 1 h at rt showed complete conversion of starting material. Workup as for **5** gave 1.67 g of 2-bromoethyl 2,3,4,6-tetra-*O*-acetyl-1-β-D-thioglucopyranoside,[20] which was pure enough to be used without further purification. This crude white solid was dissolved in acetone (6 mL) and sodium iodide (900 mg, 6.0 mmol) was added. The mixture was stirred under reflux for 2 h in the dark and then filtered and evaporated. The residue was stirred with CH_2Cl_2 and water, and the aqueous layer was extracted three times with CH_2Cl_2. The combined organic layers were washed with 1% aqueous $Na_2S_2O_3$ and water, dried over Na_2SO_4, filtered, and concentrated to a syrup. Purification by column chromatography on silica gel eluting with EtOAc/CH_2Cl_2 (1:19, v/v) and crystallization from CH_2Cl_2/cyclohexane at −20 °C afforded **6** (950 mg, 69%) as colorless needles. R_f (EtOAc/toluene 1:4): 0.42. Mp 124−125 °C; lit.[20] mp 125−126 °C. ^{1}H NMR ($CDCl_3$) in accordance with litera-

(29) Valette, G.; Pompon, A.; Girardet, J.-L.; Cappellacci, L.; Franchetti, P.; Grifantini, M.; La Colla, P.; Loi, A. G.; Périgaud, C.; Gosselin, G.; Imbach, J.-L. *J. Med. Chem.* **1996**, *39*, 1981−1990.

ture.[20] ^{13}C NMR ($CDCl_3$): δ 170.6, 170.0, 169.4, 169.3, 83.8, 76.0, 73.5, 69.7, 68.1, 62.0, 33.4, 20.8, 20.6, 20.5, 3.0. MS FAB$^+$: m/z 519 $(M + H)^+$. $[\alpha]^{25}_D = -35$ (c 1.0, $CHCl_3$); lit.[20] $[\alpha]_D = -35.8$ (c 0.5, $CHCl_3$). Anal. Calcd for $C_{16}H_{23}IO_9S$: C, 37.07; H, 4.47; S, 6.19. Found: C, 37.08; H, 4.47; S, 6.24.

2-Cyanoethyl [*S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosidyl)-2-thioethyl] *N*,*N*-Diisopropylphosphoramidite (7). To a solution of thioglucoside **4** (240 mg, 0.588 mmol) in dry CH_3CN (6 mL) containing 3 Å molecular sieve (500 mg) was added at 0 °C β-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite[22] (0.280 mL, 0.881 mmol), diisopropylamine (0.165 mL, 1.18 mmol) and 1*H*-tetrazole (82 mg, 1.18 mmol). The reaction mixture was stirred for 3 h at rt, and then diluted with EtOAc, washed with brine and water, and dried with Na_2SO_4, filtered, and concentrated *in vacuo*. Purification of the residue by short column chromatography on silica gel eluting with EtOAc/cyclohexane (7:13, v/v) containing 1% of triethylamine afforded a diastereoisomeric mixture (1:1) of **7** (343 mg, 96%) as a colorless foam. R_f (Et_3N/EtOAc/cyclohexane 1:3:6): 0.31. ^{1}H NMR ($CDCl_3$): δ 5.21 (t, 1H, J = 9.4), 5.08, 5.07 (2t, 1H, J = 9.7), 5.01 (t, 1H, J = 9.7), 4.57, 4.50 (2d, 1H, J = 10.0), 4.21−4.26 (m, 1H), 4.12 (dd, 1H, J = 2.0, 12.4), 3.69−3.90 (m, 5H), 3.54−3.64 (m, 2H), 2.78−3.00 (m, 2H), 2.65 (m, 2H), 2.08, 2.05, 2.02, 2.00 (4s, 12H), 1.18, 1.17 (2d, 12H, J = 6.8). ^{13}C NMR ($CDCl_3$): δ 170.6, 170.1, 169.4, 169.3, 117.62, 117.60, 83.6, 83.5, 75.9, 73.8, 69.9, 69.8, 68.3, 68.2, 63.2 (d, J_{P-C} = 17.7), 63.1 (d, J_{P-C} = 17.5), 62.1, 62.0, 58.4 (d, J_{P-C} = 18.6), 58.4 (d, J_{P-C} = 19.1), 43.1 (d, J_{P-C} = 11.1), 31.0 (d, J_{P-C} = 6.8), 24.6 (d, J_{P-C} = 7.1), 20.7, 20.6, 20.5, 20.4, 20.3 (d, J_{P-C} = 6.7). ^{31}P NMR ($CDCl_3$): δ 149.15, 149.04. MS FAB$^+$: m/z 625 (M oxidized + H)$^+$. Anal. Calcd for $C_{25}H_{41}N_2O_{11}PS$: C, 49.33; H, 6.79; N, 4.60. Found: C, 49.28; H, 6.61; N, 4.46.

2-Cyanoethyl [*S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosidyl)-2-thioethyl] 3′-Azido-3′-deoxythymidin-5′-yl Phosphate (8). To a solution of AZT (62 mg, 0.234 mmol) in dry CH_3CN (2 mL) containing 3 Å molecular sieve (0.5 g) was added 1*H*-tetrazole (82 mg, 1.17 mmol) and dropwise a solution of the phosphoramidite **7** (171 mg, 0.280 mmol) in dry CH_3CN (1 mL). The mixture was stirred for 1 h and then cooled to −40 °C. *tert*-Butylhydroperoxide (0.18 mL, 0.56 mmol, 3M in toluene) was added and the solution was stirred again for 1 h. Workup as for **1** and purification by silica gel column chromatography eluting with CH_3OH/CH_2Cl_2 (3:97, v/v) afforded a diastereoisomeric mixture (1:1) of phosphotriester **8** (177 mg, 95%) as a colorless foam. R_f (MeOH/CH_2Cl_2 7:93): 0.38. ^{1}H NMR ($CDCl_3$): δ 8.53 (bs, 1H), 7.46 (s, 1H), 6.15 (t, 1H, J = 6.4), 5.23 (t, 1H, J = 9.4), 4.99−5.11 (m, 2H), 4.53, 4.52 (2d, 1H, J = 10.0), 4.15−4.41 (m, 9H), 4.04 (m, 1H), 3.72 (m, 1H), 3.02−3.08 (m, 2H), 2.77−2.91 (m, 2H), 2.44 (m, 2H), 2.09, 2.06, 2.03, 2.01 (4s, 12H), 1.94 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 170.6, 170.1, 169.5, 169.4, 164.4, 150.0, 135.7, 116.4, 111.5, 85.5, 83.2, 82.0 (d, J_{P-C} = 8.5), 76.0, 73.5, 69.5, 68.1, 68.0, 67.7 (d, J_{P-C} = 6.0), 66.8 (d, J_{P-C} = 6.2), 62.3, 62.2, 61.9, 61.8, 60.0 (d, J_{P-C} = 10.3), 37.1, 29.7, 20.7, 20.6, 20.5, 19.7 (d, J_{P-C} = 7.5), 12.5. ^{31}P NMR ($CDCl_3$): δ −1.29, −1.37. MS FAB$^+$: m/z 791 $(M + H)^+$. Anal. Calcd for $C_{29}H_{39}N_6O_{16}PS$: C, 44.05; H, 4.97; N, 10.63. Found: C, 44.03; H, 4.82; N, 10.57.

[*S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosidyl)-2-thioethyl] 3′-Azido-3′-deoxythymidin-5′-yl Phosphate Sodium Salt (9). To a stirred solution of phosphotriester **8** (52 mg, 66 μmol) in THF (2 mL) was added DBU (15 μL, 100 μmol) at rt. The solution was stirred for 10 min, then evaporated to dryness. The residue was purified by silica gel column chromatography eluting with CH_3OH/CH_2Cl_2 (1:4, v/v), further chromatographed on a column of Sephadex G-10 with water as eluent, converted into its sodium salt by Dowex 50WX2 (Na^+ form) cation exchange resin, and lyophilized to yield phosphodiester **9** (29 mg, 58%) as a white powder. R_f (MeOH/CH_2Cl_2 25:75): 0.40. ^{1}H NMR (DMSO-d_6): δ 11.3 (br s, 1H), 7.81 (s, 1H), 6.15 (t, 1H, J = 6.6), 5.28 (t, 1H, J = 9.4), 4.79−4.96 (m, 3H), 4.48 (m, 1H), 4.14 (dd, 1H, J = 5.4, 12.4), 3.84−4.00 (m, 7H), 2.78−2.90 (m, 2H), 2.29−2.42 (m, 2H), 2.01, 2.00, 1.98, 1.95 (4s, 12H), 1.85 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 170.1, 169.5, 169.3, 169.1, 163.8, 150.5, 136.0, 110.0, 83.5, 82.8, 82.0, 74.4, 73.0, 69.7, 68.1, 64.3−63.7, 61.9, 61.0, 36.1, 30.7, 20.5, 20.4, 20.3, 20.2, 12.2. ^{31}P NMR (DMSO-d_6): δ −1.59. MS FAB$^+$: m/z 760 $(M + H)^+$. Anal. Calcd for $C_{26}H_{35}N_5NaO_{16}PS \cdot 3H_2O$: C, 38.37, N, 8.61. Found: C, 38.13; H, 4.68; N, 8.74. HPLC: t_R 14.4 min.

(*S*-β-D-Glucopyranosidyl-2-thioethyl) 3′-Azido-3′-deoxythymidin-5′-yl Phosphate Sodium Salt (10). Phosphotriester **8** (98 mg, 0.124 mmol) was dissolved in a 1% solution of sodium methoxide in methanol (3 mL) and stirred at rt for 30 min. Evaporation of the solvent, chromatography of the residue by gel permeation (Sephadex G-10, H_2O), passage over a Dowex 50WX2 (Na^+ form) cation exchange resin column, and lyophilization afforded the sodium salt of phosphodiester **10** (70 mg, 96%) as a white powder. R_f (MeOH/CH_2Cl_2 4:6): 0.26. ^{1}H NMR (D_2O): δ 7.64 (s, 1H), 6.02 (t, 1H, J = 6.6), 4.49 (d, 1H, J = 9.9), 4.45 (m, 1H), 4.11 (m, 1H), 3.94−4.09 (m, 4H), 3.78−3.81 (m, 1H), 3.62 (dd, 1H, J = 5.2, 12.4), 3.41 (m, 1H), 3.27−3.36 (m, 2H), 3.21 (m, 1H), 2.81−2.99 (m, 2H), 2.44 (pt, 2H, J = 6.3), 1.86 (s, 3H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 7.78 (s, 1H), 6.13 (t, 1H, J = 6.6), 5.1 (br s, 3H), 4.46−4.53 (m, 1H), 4.26 (d, 1H, J = 9.5), 3.93 (m, 1H), 3.71−3.83 (m, 4H), 3.6 (m, covered partly by H_2O peak), 2.91−3.16 (m, 4H), 2.60−2.88 (m, 2H), 2.15−2.44 (m, 2H), 1.79 (d, 3H, J = 0.7). ^{13}C NMR (D_2O): δ 167.3, 152.3, 137.7, 112.1, 85.7, 85.3, 83.3 (d, J_{P-C} = 8.8), 80.3, 77.5, 72.8, 69.9, 65.7 (d, J_{P-C} = 4.6), 65.4 (d, J_{P-C} = 5.0), 61.2, 60.8, 36.6, 30.5 (d, J_{P-C} = 7.5), 12.1. ^{13}C NMR (DMSO-d_6): δ 166.7, 150.3, 135.0, 109.9, 85.0, 83.9, 82.5, 81.4, 78.1, 73.3, 70.0, 64.4−64.2, 61.5, 61.3, 36.0, 29.7 (d, J_{P-C} = 6.0), 13.2. ^{31}P NMR (D_2O): δ 0.70. ^{31}P NMR (81 MHz, DMSO-d_6): δ 0.01. MS FAB$^+$: m/z 592 $(M + H)^+$. Anal. Calcd for $C_{18}H_{27}N_5NaO_{12}PS \cdot 2H_2O$: C, 34.45; H, 4.98; N, 11.16. Found: C, 34.43; H, 4.83; N, 10.56. HPLC: t_R 12.7 min.

(*S*-β-D-Glucopyranosidyl-2-thioethyl) [*S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosidyl)-2-thioethyl] 3′-Azido-3′-deoxythymidin-5′-yl Phosphate (3). Phosphodiester **10** (20 mg, 34 μmol) was converted into its tetrabutylammonium salt through passage over a Dowex 50 WX2 cation exchange resin column [(n-C_4H_9)N^+ form]. The lyophilized salt was dissolved in dry CH_3CN (10 mL), thioglucoside **6** (250 mg, 0.48 mmol) was added, and the mixture stirred at 80 °C for 8 h. After evaporation of the solvent *in vacuo*, the residue was purified by column chromatography on silica gel using a stepwise gradient of CH_3OH (3−10%) in CH_2Cl_2. Evaporation of the appropriate fractions and lyophilization gave a diastereoisomeric mixture (1:1) of phosphotriester **3** (12 mg, 37%) as a white powder. R_f (MeOH/acetone 1:9): 0.55. ^{1}H NMR (DMSO-d_6): δ 11.35 (br s, 1H), 7.47 (s, 1H), 6.12 (t, 1H, J = 6.6), 5.24−5.29 (m, 1H), 5.18 (d, 1H, J = 5.7), 5.05 (d, 1H, J = 4.6), 4.90−4.98 (m, 3H), 4.84 (t, 1H, J = 9.6), 4.44−4.48 (m, 2H), 4.31, 4.30 (2d, 1H, J = 9.6), 4.09−4.23 (m, 7H), 3.96−4.02 (m, 3H), 3.63−3.67 (m, 1H), 3.38−3.43 (m, 1H), 2.66−3.31 (m, 8H), 2.32−2.43 (m, 2H), 2.10, 2.07, 2.00, 1.97, 1.94 (5s, 12H), 1.79 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 170.0, 169.4, 169.2, 169.1, 163.6, 150.3, 135.65, 109.9, 85.1, 85.0, 83.6, 81.9−81.0, 77.9, 74.3, 72.9, 72.8, 69.8, 69.4, 68.4, 67.9, 67.1, 61.8, 61.1, 59.9, 35.6, 32.0, 20.4, 20.3, 20.2, 20.1, 12.0. ^{31}P NMR (DMSO-d_6): δ −0.41, −0.47. MS FAB$^+$: m/z 960 $(M + H)^+$. Anal. Calcd for $C_{34}H_{50}N_5O_{21}PS_2$: C, 42.54; H, 6.37; N, 8.85. Found: C, 42.82; H, 6.34; N, 9.02. HPLC: t_R 29.7 min.

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Supporting Information Available: A full listing of NMR data, accompanied by subjective peak assignments, of compounds **1**−**10** (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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