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Synthesis and in vitro evaluation of S-acyl-3-thiopropyl prodrugs of Foscarnet

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Abstract—A new enzyme-labile group called S-acyl-3-thiopropyl group (SATP) has been synthesized from allylic esters of phosphonate. After demonstration of the enzyme-labile character of the SATP in cellular extracts, it has been introduced onto the phosphonate moiety of PFA (Foscarnet) to obtain potential lipophilic prodrugs. To ponder the lipophilicity of the triesters of PFA, esters of monomethylether of polyethyleneglycols and of thioglycerol were introduced on the PFA carboxylate moiety. The SATP groups were introduced in an attempt to deliver PFA after bioactivation inside the cells. The PFA prodrugs were evaluated in vitro for their activity against human immunodeficiency viruses (HIV-1 and HIV-2).
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1. Introduction

The AIDS epidemic has resulted in continuous and intensive efforts to find effective chemotherapeutic agents against the human immunodeficiency virus (HIV), the causative agent of the disease. Reverse transcriptase and protease are the targets of choice in the replicative cycle of HIV.[1,2]

Phosphonoformate (PFA, foscarnet) is an effective antiviral agent that has been approved for clinical use in the treatment of cytomegalovirus retinitis in AIDS patients under the name of Foscavir.[3] PFA inhibits the DNA polymerases of herpes viruses, as well as the reverse transcriptase of HIV by blocking the pyrophosphate site in these enzymes. The efficacy of PFA is hampered by poor membrane permeability due to its polyanionic structure at physiological pH. Prodrug approaches can be used to overcome many problems associated with oral bioavailability and cellular permeability of PFA.[4]

The prodrug approaches consist in masking the anionic charges temporarily by a lipophilic ester protective group to enhance cellular permeation. This protective group will be selectively cleaved inside the cell.

For instance, *S*-acylthioalkyl phosphonic esters can be hydrolyzed enzymatically by carboxyesterases which may be more prevalent intracellularly. *S*-acyl-2-thioethyl (SATE)[5–8] and S-acyl-4-thiobutyl (SATB)[9] have been utilized in a prodrug approach to deliver intracellularly the monophosphates of azidothymidine (AZT), 2′,3′-dideoxyuridine (d4T) and stavudine (ddU), and other nucleotides with antiviral activities.[8]

The SATE and SATB behavior was very different; the enzymatic decomposition in cell extracts of the SATE group was instantaneous,[5] whereas the SATB group decomposition was not observed. [9]

It appeared interesting to us to synthesize a new potentially cleavable group by esterase with half-live intermediate between SATE and SATB groups. The *S*-acyl-

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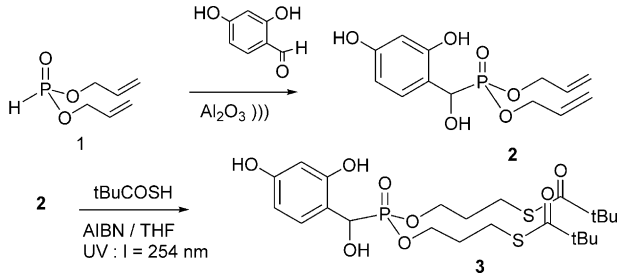


Figure 1. Reaction scheme for model compound.

3-thiopropyl protective group (SATP) could be a good candidate to fulfill the proposed criterion.

Before applying the SATP groups to PFA, we had to verify the enzyme-labile character of SATP group on simple structure model.

In a preliminary work we synthesized a hydroxyresorcylmethyl phosphonate **2** as model for the metabolism studies. The presence of three hydroxyl functions confers the necessary hydrophilicity for water solubilisation [The calculated log P is 2.8 (software ACD logP-Chem CAD)]. Compound **2** was obtained by a Pudovik reaction between the 2,4-resorcylaldehyde and the diallyl phosphonate with basic alumina[10,11] under sonication conditions, followed by addition of thiopivaloic acid on the double bond of allyl groups under UV irradiation ($\lambda = 254$ nm) (Fig. 1).

The decomposition of the model compound **3**, achieved in cellular extracts, mimics the bioconversion of the model in the intracellular compartment. The kinetics was followed over 5 days to 37 °C, with a substrate concentration of 5.10^{-5} M in the cellular extracts in the presence of 2.5% of DMSO. The analysis was performed by 'on-line cleaning HPLC-UV-MS'.[12] The decomposition process of compound **3** is indicated in Figure 2.

The decomposition of the monoester **3″** continues without metabolites detection. Indeed the metabolites of **3″** are very hydrophilic and they are eliminated during the 'cleaning on line' process.

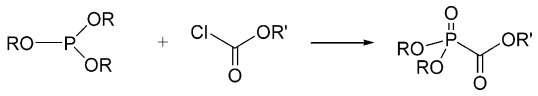


Figure 3. Reaction scheme of Arbusov's reaction between an alkyl chloroformate and a trialkyl phosphate.

The interpretation of the results permitted to show the enzyme-labile character of the SATP group. As expected, the SATP group had a half-life ($T_{1/2}$) of 41 min which is intermediate between those of the ethyl (SATE) and the butyl (SATB) derivatives.

Moreover, new prodrugs of PALA bearing *S*-acyl-3-thio-propyl groups (SATP) have shown cytotoxic activities against human cells lines (SW 1573 lung carcinoma cells). A number of prodrugs displayed a cytotoxic activity in the same order of magnitude of PALA. This biological activities of prodrugs of PALA can be explain by the enzyme-labile character of the SATP group.[13]

2. Synthesis

The prodrug strategy was applied to PFA after the corroboration of the enzyme-labile character of the SATP group. Generally the synthesis of the foscarnet derivatives is achieved according to an Arbusov's reaction between an alkyl chloroformate and a trialkyl phosphite[14] this necessitates temperature conditions incompatible with the thermal stability of allylic esters (Fig. 3).

We opted for a milder method[15] that takes place at room temperature, and in which was used the diallylphosphonate **1** (Fig. 4). The diallyl phosphonate **1**, not commercially available, was prepared [16] from phosphorous trichloride and allylic alcohol in the presence of two equivalents of pyridine. After distillation under reduced pressure, the diallyl phosphonate, was obtained in 75% yield, and kept under argon to avoid its hydrolysis.

Compound **1** was characterized by ^{31}P NMR (decoupled proton), by a signal at 8.9 ppm. In ^{1}H NMR the proton directly bound to the phosphorus appeared at

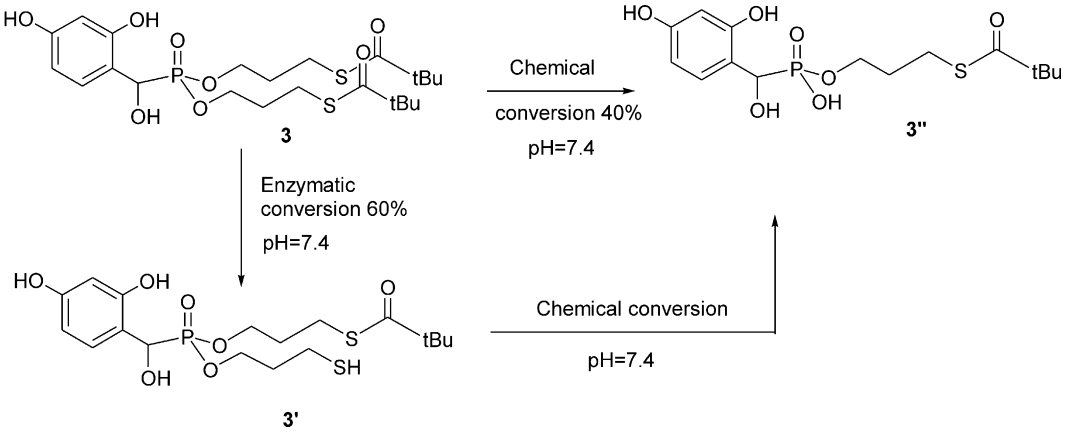


Figure 2. Decomposition process of model compound **3**.

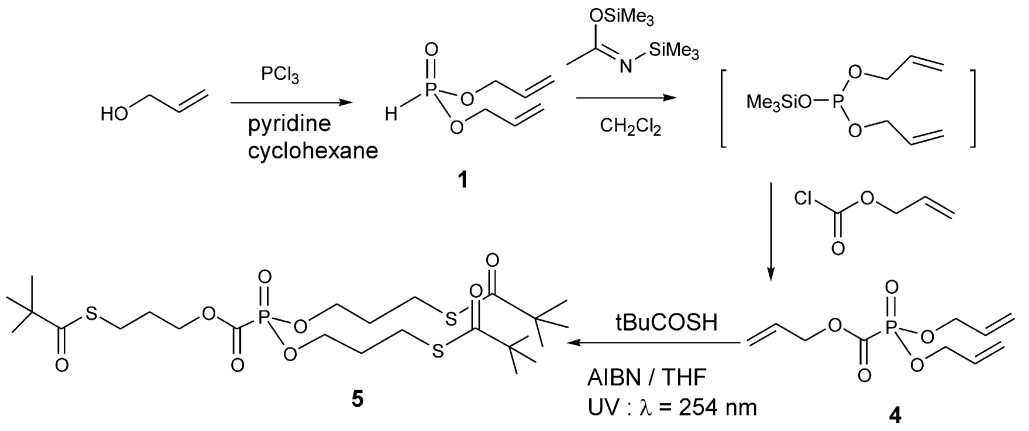


Figure 4. Synthesis of tris (*S*-pivaloyl-3-thiopropyl)phosphonoformate **5**.

6.88 ppm, as a doublet with a coupling constant of $^{1}J_{HP} = 703.1$ Hz.

The synthesis of the triester **4** was then achieved in a 'one pot' procedure (Fig. 4).

The diallyl phosphonate **1** was treated by BSA (*N*,*O*-bis(trimethylsilyl)acetamide), giving the diallyltrimethylsilylphosphite intermediate containing a nucleophilic phosphorus atom. This phosphite was allowed to react with allyl chloroformate leading to triallyl phosphonoformate **4** obtained in 98% yield after neutral alumina column chromatography to avoid the partial cleavage of the allylic esters.

The triallyl phosphonoformate **4** was allowed to react with thiopivaloic acid in THF, the radical addition being initiated with catalytic amounts of AIBN, under UV irradiation ($\lambda = 254$ nm). This step led to the tris-(SATP) phosphonoformate in 64% yield. The structure of the phosphonoformates **4** and **5** was proved by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy and mass spectrometry.

Unfortunately the enzyme-labile triester **5** was characterized by a weak aqueous solubility, a consequence of the high log P value ($\log P_{calc} > 5$). It is worth pointing out that the phosphonoformates must be sufficiently lipophilic to cross the biological membranes but also to dissolve in physiological liquids. The values of log P generally admitted to give good bioavailibility should be within the 2–3.5 range.

Thus, we prepared PFA derivatives (suiting with calculated logP included within the limit values given above) by the introduction of hydrophilic groups derived from polyethylene-glycol monomethylether and glycerol. The S-acyl groups of SATP were pivaloyl or acetyl groups (Table 1).

In these compounds the SATP enzyme-labile groups were introduced on the phosphonic acid moiety and the hydrophilic groups on the carboxylic acid of PFA.

The introduction of polyethyleneglycol monomethylether as carboxylic esters requires the synthesis of the chloroformates of the monomethylether in order to achieve a reaction with diallyl phosphonate as it is described in Figure 4. The chloroformates were prepared in high yields by reaction of polyethyleneglycol monomethylether on phosgene in toluene (Fig. 5).

It is of note that the use of triphosgene, a safe derivative of phosgene, did not lead to the chloroformate derivative but to the chlorinated product via a decarboxylation step. This has been previously observed in the Literature with activated alcohol.[17]

Then several attempts to obtain phosphonoformates of polyethyleneglycol were made. The first strategy concerned the coupling reaction between the diallyl phosphonate **1** and the chloroformiate **7** in the presence of BSA (Fig. 6) following the same procedure described in Figure 3. By this way the phosphonoformate **9** was obtained in 67% yield. The next step was the addition of the thioacid onto the allylic functions. However, this addition of the thiopivaloïc acid on the diallylphosphonoformate of polyethyleneglycol, did not lead to the expected product, but to the degradation of the reagents (Fig. 6).

In fact, the bis enzyme-labile phosphonates **12**, **13** were prepared directly from the phosphorous trichloride by addition of the corresponding alcohol **10**, **11** in presence of pyridine in an analogous manner to the diallyl phos-

Table 1. Bis (RSATP) polyethyleneglycol monomethylether phosphonoformates

Compd	R acyl	*n*	Yield%	$\log P_{calc} \pm 0.6$	$\delta\ ^{31}P$
14	^{t}Bu	1	54	3.7	−3.5
15	Me	1	75	1.3	−3.5
16	^{t}Bu	2	66	3.4	−3.5
17	Me	2	76	0.9	−3.5
18	^{t}Bu	3	62	3.0	−3.5
19	Me	3	75	0.6	−3.5

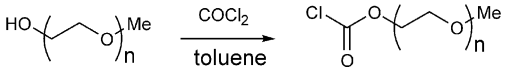


Figure 5. Synthesis of polyethyleneglycol monomethylether chloroformates.

phonate **1**, with 91% yield for R = ^{t}Bu and 81% yield for R = Me (Fig. 7).

The structures of the phosphonates **12** and **13** were proved by ^{31}P NMR spectrometry with the presence of the signal at 8.4 ppm consistent with a H-phosphonate and by ^{1}H NMR spectrometry by a doublet at 6.8 ppm corresponding in the H-P proton ($^1J_{HP}$ = 700 Hz), as well as by the presence of the characteristic signals of the RSATP groups. Phosphonoformates were then obtained by condensation of the bis SATP phosphonate **12**, **13** on the chloroformates of monomethylether of polyethyleneglycol **6**, **7** and **8** (Fig. 8) with yields ranging from 54% to 76% after chromatography, depending on the nature of the acyl group R and the length of the polyethyleneglycol unit (Table 1).

We also considered the use of the thioglycerol and its incorporation on the carboxylate moiety of the PFA. The starting materials, for the preparation of the thioglycerol esters, were the bis SATP phosphonates **12** and **13**. In the first step of the synthesis were prepared the allylic esters **20** and **21** by addition of the allyloxycarbonyl chloride onto the bis SATP phosphonate **12**

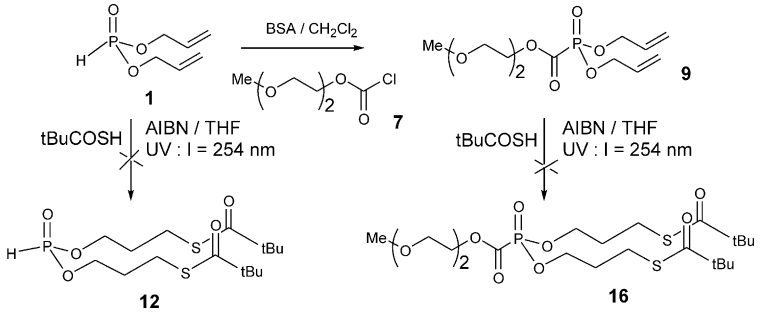


Figure 6. The direct syntheses of Bis SATP phosphonate fail.

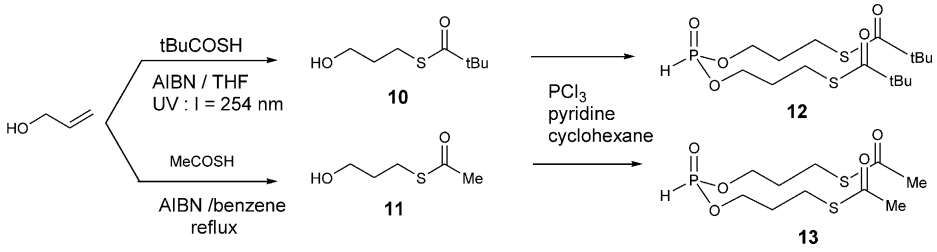


Figure 7. Reaction scheme for the bis enzyme-labile phosphonates.

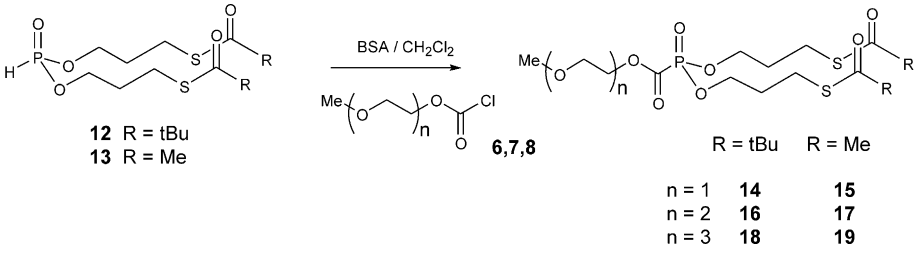


Figure 8. Reaction scheme for Bis (RSATP) polyethyleneglycol monomethylether phosphonoformates.

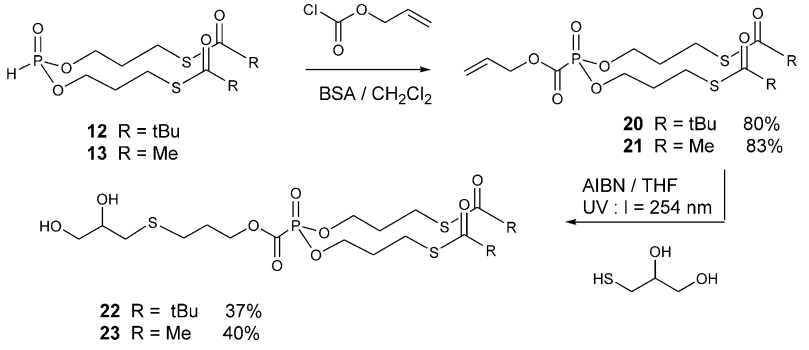


Figure 9. Synthesis of thioglycerol derivatives.

and **13,** respectively. The addition of the thioglycerol onto the allylic esters **19** and **20** was then accomplished under radical conditions, using AIBN as radical initiator and UV irradiation ($\lambda = 254$ nm), leading to **22** in 37% yield and **23** in 40% yield, respectively (Fig. 9).

The structures of the phosphonoformates **14–23** were proved by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy and mass spectrometry.

3. Biological results

The phosphonoformate derivatives **5**, **14–19**, **22** and **23** bearing the enzyme-labile S-acyl-3-thiopropyl group were evaluated for their inhibitory effects on the replication of human immunodeficiency virus (HIV) type 1 (strain III_B) and type 2 (strain ROD) in MT-4 cells (Table 2).

From the present work, it is obvious that none of the synthesized phosphonate derivatives **5–23** surpassed PFA in an in vitro antiviral activity. On the contrary, they proved inactive against HIV at the highest concentration tested (125 μg/mL).

It would seem that the prodrugs of the PFA does not have an antiviral activity in relation with a pondered lipophilicity and only the more lipophilic derivative **5** ($\log P_{calc} = 5.6$) would be capable to penetrate inside the cells to exercise its activity.

This inactivity could be explained by a lack of cellular permeation and/or by a weak esterase activity of the MT- 4 cells. The esterase activity may be different from one cell type to another, and may be most pronounced in cells of the monocyte/macrophage lineage. It may be worth examining whether the SATP-PFA prodrugs would show any anti-HIV activity in these cells.

Table 2. Anti-HIV Activity of phosphonoformate derivatives bearing enzyme labile SATP groups

Compd	Strain	IC_{50} (μg/mL)	CC_{50} (μg/mL)	SI	$\log P_{calc}$
5	III_B	>91	76.85	<1	5.6
	ROD	>83.9	76.85	<1	
14	III_B	>125	>125	>< 1	3.7
	ROD	>125	>125	>< 1	
15	III_B	>125	>125	>< 1	1.3
	ROD	>125	>125	>< 1	
16	III_B	>125	>125	>< 1	3.4
	ROD	>125	>125	>< 1	
17	III_B	>125	>125	>< 1	0.9
	ROD	>125	>125	>< 1	
18	III_B	>125	>125	>< 1	3.0
	ROD	>125	>125	>< 1	
19	III_B	>125	>125	>< 1	0.6
	ROD	>125	>125	>< 1	
22	III_B	>125	>125	>< 1	3.4
	ROD	>125	>125	>< 1	
23	III_B	>125	>125	>< 1	1.0
	ROD	>125	>125	>< 1	
Reference	III_B	14.35	—	>8	<0
Foscarnet	ROD	10.1	—	>12	

4. Experimental

4.1. Chemistry

All chemicals were purchased from Aldrich and used as received. Tetrahydrofuran (THF) was distilled over lithium aluminum hydride under nitrogen immediately prior to use. Diallyphosphite was not commercial and it was prepared in large quantities following the Kennedy procedure.[16]

Merck silica gel 60 F_{254} (0.25 mm) plates were employed for analytical TLC. Compounds were revealed by UV (254 nm), 1% potassium permanganate in water (allylic compounds), 5% phosphomolybdic acid in ethanol or molybdene blue for phosphorous compounds. Merck silica gel 60H, Aldrich alumina (activated neutral, brockmann I, 150 mesh) and Fluka florisil were used for column chromatography. Reactions under ultrasound were performed on a Bransonic 220 ultrasonic cleaning bath. ^{1}H and ^{13}C NMR were recorded on a Brucker apparatus DPX200, AC250 and DRX400. ^{31}P NMR spectra were recorded on a Brucker DPX200. For ^{1}H and ^{13}C we used a numbering system as presented in Chart 1. Chemical shifts are expressed in ppm (δ). Mass spectra were recorded on a JEOL JMS-DX300. The matrixes used were m-nitrobenzylic alcohol (NBA) or glycerol-thioglycerol 1/1. Log P was estimated by means of the software ACD/LogP calculator developed by ACD (Advanced Chemistry Development Inc.) and distributed by the company ChemCAD.

Microanalyses were performed in the analytical department of the CNRS (Vernaison, Rhône, France). C, H and S elemental analyses were done for most of the compounds.

4.1.1. Diallyl(2,4-dihydroxyphenyl)hydroxymethylphosphonate (2). 2,4-Dihydroxybenzaldehyde (2.8 g, 20 mmol, 1 equiv) was dissolved in 3 mL of diallylphosphite **1** (20 mmol, 1 equiv) under vigorous stirring. Then the mixture was completely absorbed on basic alumina (10 g) and put in an ultrasonic cleaning bath for 10 days. The products were extracted from alumina with dichloromethane (3×25 mL). The solvent was evaporated under reduced pressure, the crude product was purified by a florisil column chromatography (ethyl acetate/petroleum ether 50/50), and 0.63 g of compound **2** was obtained as colourless oil in 10% yield. $R_f = 0.25$ (ethyl acetate/petroleum ether 20/80).

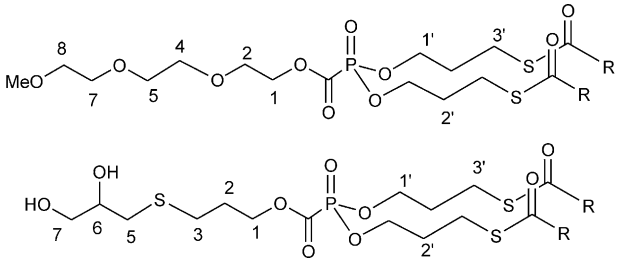


Chart 1.

^{1}H NMR $CDCl_3$/200 MHz: δ (ppm): 4.48 (m, 4H, $POCH_2$); 5.31 (m, 4H, $CH_{(3,3')}$); 5.90 (m, 2H, $CH_{(2)}$); 5.10 (d, $^2J_{HP}$ = 11.4 Hz, 1H, CHOH); 6.13 (d, $^3J_{HH}$ = 8.5 Hz, 1H, H_5); 7.28 (s, 1H, H_3); 7.42 (d, $^3J_{HH}$ = 8.5 Hz, 1H, H_6).^{31}P NMR $CDCl_3$/200 MHz: δ (ppm): 24.4

4.1.2. Bis(S-pivaloyl-3-thiopropyl)-(2,4-dihydroxyphenyl)-hydroxymethylphosphonate (3). In a quartz reactor, a solution of diallyl(2,4-dihydroxyphenyl) hydroxymethylphosphonate **2** (0.63 g, 2.1 mmol, 1 equiv) and thiopivaloic acid (0.85 mL, 7.5 mmol, 3.6 equiv) in the presence of a catalytic amount of AIBN (25 mg) in 18 mL of THF was degassed for 15 min by argon bubbling. The mixture was submitted to UV irradiation (λ = 254 nm) for 45 min, under argon atmosphere at room temperature. Then, the solution was diluted with 80 mL of ethyl acetate and washed twice with a saturated solution of $NaHCO_3$. The organic layer was dried with sodium sulphate and concentrated under reduced pressure. The residual oil was purified by silica gel column chromatography (petroleum ether/ethyl acetate 50/50), and 0.41 g of compound **3** was isolated as colourless oil in 36% yield. R_f = 0.37 (ethyl acetate/petroleum ether 20/80). ^{1}H NMR $CDCl_3$/200 MHz: δ (ppm): 1.20 (s, 18H, CH_3); 1.89 (m, 4H, CH_2); 2.91 (m, 4H, CH_2S); 4.14 (td, $^3J_{HH}$ = 7.1 Hz, $^3J_{HP}$ = 7.2 Hz, 4H, $POCH_2$); 4.44 (s, 1H, CHOH); 5.10 (d, $^2J_{HP}$ = 11.1 Hz, 1H, CHOH); 6.38 (d, $^3J_{HH}$ = 7.7 Hz, 1H, H_5); 6.48 (s, 1H, H_3); 7.03 (d, $^3J_{HH}$ = 7.7 Hz, 1H, H_6); 8.77 (s, OH).

^{13}C NMR $CDCl_3$/100 MHz: δ (ppm): 25.0 (s, CH_2S); 27.8 (s, CH_3); 30.8 (2d, $^3J_{PC}$ = 5.7 Hz, CH_2); 46.9 (s, $C_{(^tBu)}$); 66.2 and 66.7 (2d, $^2J_{PC}$ = 7.3 and 7.1 Hz, $POCH_2$); 69.9 (d, $^1J_{PC}$ = 104.0 Hz, CHOH); 105.3 (s, C_3); 108.5 (s, C_5); 113.4 (s, C_1); 130.2 (d, $^3J_{CP}$ = 7.4 Hz, C_6); 156.7 (d, $^3J_{CP}$ = 4.6 Hz, C_2); 158.4 (s, C_4); 208.0 and 208.1 (2s, CO).

^{31}P NMR $CDCl_3$/200 MHz: δ (ppm): 23.9

MS (Fab +/GT): m/z = 535 $[M+H]^+$; 57 $[^tBu]^+$.

4.2. Decomposition and stability studies of model 3

Far-ultraviolet (UV) HPLC-grade acetonitrile was purchased from Fisons (Loughborough, UK). Triethyl ammonium acetate buffer was purchased from Distribio. The purified water was obtained from a Millipore purifier (Bedford, Massachusetts, USA). The compound **3** was incubated (initial concentration 5.10^{-5} M) at 37 °C either in a full cell extract (pH = 7.4). Incubates were removed at appropriate times and injected as 80 μL samples in a system comprising the cleaning device, the chromatograph and UV detector, and the mass spectrometer.

The analysis by mass spectrometry of the chromatogram identified without ambiguity the various metabolites which were formed in the cellular extract. Spectra were recorded after 2 h of incubation, the retention times observed reporting differences of polarity of the metabolites.

Both first ones metabolites, the thiol (tr = 16.58 min) and the monoester (tr = 13.51 min) quickly form, they were later already observed 15 min of incubation, however the diester (tr = 18.94 min) represents more than 80% of the detected species, it disappears totally only at the end of 4 h. Mainly, the cleavage of the thioester by enzymatic way was observed followed by the rearrangement that leads to the phosphonate monoester. The hydrolysis by chemical way of the phosphonic ester is also observed but it concerns only 40% of the compound.

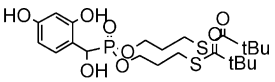
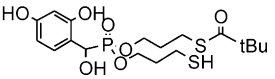
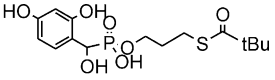
The experiment did not allow detecting the formation of last metabolites because of their too high hydrophilicity (log P < 0) (Table 3).

4.2.1. Triallylphosphonoformate (4). To a solution of diallylphosphonate **1** (2 mL, 12 mmol, 1 equiv) in 20 mL of dichloromethane was added *N*,*O*-bis(trimethylsilyl)-acetamide BSA (4.1 mL, 14 mmol, 1.2 equiv) under nitrogen atmosphere at room temperature. After 3 h, a solution of allylchloroformate (2.55 mL, 24 mmol, 2 equiv) in 20 mL of dichloromethane was added dropwise. Then the reaction was stirred overnight at room temperature. The solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (dichloromethane) to give 2.9 g of compound **4** as colourless oil in 98% yield. R_f = 0.48 (diethyl ether/petroleum ether 2/1). ^{1}H NMR $CDCl_3$/200 MHz: δ (ppm): 4.67 (m, 6H, $POCH_2$ and CO_2CH_2); 5.23 (d, $^3J_{HH}$ = 9.9 Hz, 2H, $CH_{(3p)}$); 5.25 (d, $^3J_{HH}$ = 9.3 Hz, 1H, $CH_{(3c)}$); 5.32 (dd, $^3J_{HH}$ = 17.1 Hz, $^2J_{HH}$ = 1.1 Hz, 1H, $CH_{(3'c)}$); 5.34 (dd, $^3J_{HH}$ = 17.1 Hz, $^2J_{HH}$ = 1.1 Hz, 2H, $CH_{(3'p)}$); 5.89 (m, 3H, $CH_{(2)}$). ^{13}C NMR $CDCl_3$/100 MHz: δ (ppm): 66.9 (d, $^3J_{PC}$ = 5.2 Hz, CO_2CH_2); 68.9 and 69.0 (2d, $^2J_{PC}$ = 6.4 Hz, $POCH_2$); 119.5 (s, $CH_{2\ (3p)}$); 120.4 (s, $CH_{2(3c)}$); 131.0 (s, $CH_{(2c)}$); 132.3 (d, $^3J_{PC}$ = 6.0 Hz, $CH_{(2p)}$); 166.6 (d, $^1J_{PC}$ = 269.5 Hz, CO_2).

^{31}P NMR $CDCl_3$/200 MHz: δ (ppm): −3.4. MS (Fab +/NBA): m/z = 493 $[2M+H]^+$; 247 $[M+H]^+$; 41 $[CH_2=CH\text{-}CH_2]^+$.

4.2.2. Tris(S-pivaloyl-3-thiopropyl)phosphonoformate (5). In a quartz reactor, a solution of triallylphosphonoformate **4** (1.03 g, 4.2 mmol, 1 equiv), and thiopivaloic

Table 3. The half-lives of the various metabolites

Metabolites	logP(±0.6)	Hl[a]	RT[b]
	2.8	27	18.94
	1.5	173	16.58
	0.5		13.51

[a] Hl: Half-lives in min.
[b] RT: retention times in min.

acid (2.5 mL, 22.7 mmol, 5.4 equiv) in the presence of a catalytic amount of AIBN (50 mg) in 35 mL of THF was degassed for 15 min by argon bubbling. The solution was submitted to UV irradiation at $\lambda = 254$ nm for 3 h, under inert atmosphere at room temperature. Then the mixture was diluted with 60 mL of ethyl acetate and washed twice with a saturated solution of $NaHCO_3$. The organic layer was dried with sodium sulphate and concentrated under reduced pressure. The residual mixture was purified by silica gel column chromatography (petroleum ether/ethyl acetate 80/20) to give 1.6 g of compound **5** as pale yellow oil in 64% yield. $R_f = 0.48$ (diethyl ether/petroleum ether 2/1). ^{1}H NMR $CDCl_3$/200 MHz: δ (ppm): 1.19 (s, 27H, CH_3); 1.96 (q^t, $^3J_{HH} = 6.6$ Hz, 6H, CH_2); 2.88 (t, $^3J_{HH} = 7.1$ Hz, 2H, $CH_2S_{(c)}$); 2.91 (t, $^3J_{HH} = 7.1$ Hz, 4H, $CH_2S_{(p)}$); 4.27 (m, 6H, $POCH_2$ and CO_2CH_2). ^{13}C NMR $CDCl_3$/100 MHz: δ (ppm) : 24.8 and 25.1 (2 s, CH_2S); 27.7 and 27.8 (2 s, CH_3); 29.0 (s, $CH_{2\ (c)}$); 30.8 (d, $^3J_{PC} = 6.1$ Hz, $CH_{2\ (p)}$); 46.8 (s, $C_{(^tBu)}$); 64.8 (d, $^3J_{PC} = 4.7$ Hz, CO_2CH_2); 67.3 (d, $^2J_{PC} = 6.5$ Hz, $POCH_2$); 166.8 (d, $^1J_{PC} = 270.2$ Hz, CO_2); 206.6 (2 s, COS). ^{31}P NMR $CDCl_3$/200 MHz: δ (ppm): −3.5. MS (Fab+/GT): $m/z = 601$ $[M+H]^+$; 57 $[^tBu]^+$. Anal. calcd for C_{25} $H_{45}O_8PS_3$ (600.79): C, 49.98; H, 7.55; S, 16.01; found: C, 49.79; H, 7.60; S, 15.88.

4.2.3. 5-methoxy-3-oxapentylchloroformate (7). Diethyleneglycol monomethylether (0.70 mL, 6.0 mmol, 1 equiv) was added drop wise under inert atmosphere to 4.0 mL of phosgene at 20% in toluene (8.1 mmol, 1.35 equiv) cooled 10 min in an ice bath. The mixture was stirred 4 h at room temperature and then the solvents were removed under reduced pressure. The compound **7** was obtained as colourless oil in quantitative yield. ^{1}H NMR $CDCl_3$/400 MHz: δ (ppm): 3.32 (s, 3H, CH_3O); 3.49 (m, 2H, $CH_2O_{(5)}$); 3.59 (m, 2H, $CH_2O_{(4)}$); 3.70 (m, 2H, $CH_2O_{(2)}$); 4.40 (m, 2H, CO_2CH_2). ^{13}C NMR $CDCl_3$/100 MHz: δ (ppm): 59.5 (s, CH_3O); 68.7 (s, $CH_2O_{(2)}$); 71.1 (s, $CH_2O_{(4)}$ and CO_2CH_2); 72.3 (s, $CH_2O_{(5)}$); 151.2 (s, CO_2). The chloroformate of monomethylether of monoethyleneglycol (**6**) and of triethyleneglycol (**8**) were obtained according to the aforementioned procedure in quantitative yield and used in the next step without purification.

4.2.4. Diallyl-(5-methoxy-3-oxapentyloxycarbonyl)-phosphonate (9). *N*,*O*-bis(trimethylsilyl) acetamide BSA (0.88 mL, 3.6 mmol, 1.2 equiv) was added to a solution of diallylphosphonate **1** (3.0 mmol, 1 equiv) in 5 mL of anhydrous dichloromethane, under nitrogen atmosphere at room temperature. After 3 h the chloroformate of monomethylether of diethyleneglycol **7** (9.0 mmol, 3 equiv) in 5 mL of dichloromethane was added dropwise. The reaction was stirred overnight at room temperature. The solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (ethyl acetate/petroleum ether 50/50). A total amount of 0.615 g of compound **9** was isolated as colourless oil. Yield 67%. $R_f = 0.51$(ethyl acetate). ^{1}H NMR $CDCl_3$/400 MHz: (ppm): 3.30 (s, 3H, CH_3O); 3.47 (m, 2H, $CH_2O_{(5')}$); 3.57 (m, 2H, $CH_2O_{(4')}$); 3.69 (m, 2H, $CH_2O_{(2')}$); 4.35 (m, 2H, CO_2CH_2); 4.66 (q, $^3J_{HH} = {}^3J_{HP} = 6.4$ Hz, 4H, $POCH_2$); 5.22 (dd, $^3J_{HH} = 10.4$ Hz, $^2J_{HH} = 1.1$ Hz, 2H, $CH_{(3)}$); 5.34 (qd, $^4J_{HH} = {}^2J_{HH} = 1.4$ Hz, $^3J_{HH} = 17.1$ Hz, 2H, $CH_{(3')}$); 5.91 (m, 2H, $CH_{(2)}$). ^{13}C NMR $CDCl_3$/100 MHz: δ (ppm): 59.5 (s, CH_3O); 65.1 (d, $^3J_{PC} = 5.0$ Hz, CO_2CH_2); 66.8 (d, $^2J_{PC} = 6.1$ Hz, $POCH_2$); 68.9 (s, $CH_2O_{(2')}$); 71.1 (s, $CH_2O_{(4')}$); 72.3 (s, $CH_2O_{(5')}$); 119.1 (s, $CH_{2\ (3)}$); 132.5 (d, $^3J_{PC} = 6.0$ Hz, $CH_{(2)}$); 166.8 (d, $^1J_{PC} = 269.9$ Hz, CO_2). ^{31}P NMR $CDCl_3$/200 MHz: δ (ppm): −4.2. MS (Fab +/NBA): $m/z = 309$ $[M+H]^+$, 41 $[CH_2=CH\text{-}CH_2]^+$.

4.2.5. *S*-pivaloyl-3-thiopropyl alcohol (10). In a quartz reactor, a solution of allyl alcohol (1.4 mL, 37.8 mmol, 1.8 equiv), and thiopivaloic acid (4.2 mL, 37.8 mmol, 1.8 equiv) in the presence of a catalytic amount of AIBN (50 mg) in 150 mL of THF was degassed for 15 min by argon bubbling. The solution was submitted to UV irradiation at $\lambda = 254$ nm for 3 h under inert atmosphere at room temperature. Then the mixture was diluted with 200 mL of ethyl acetate, washed twice with a saturated solution of $NaHCO_3$, then twice with brine. The organic layer was dried with sodium sulphate and concentrated under reduced pressure. The residual oil was purified by silica gel column chromatography (petroleum ether/ethyl acetate 70/30) to give 2.9 g of compound **10** as colourless oil in 80% yield. $R_f = 0.57$. ^{1}H NMR $CDCl_3$/200 MHz: δ (ppm): 1.22 (s, 9H, CH_3); 1.79 (tt, $^3J_{HH} = 6.8$ Hz, $^3J_{HH} = 5.9$ Hz, 2H, CH_2); 2.68 (s, 1H, OH); 2.96 (t, $^3J_{HH} = 6.8$ Hz, 2H, CH_2S); 3.61 (t, $^3J_{HH} = 5.9$ Hz, 2H, CH_2OH). ^{13}C NMR $CDCl_3$/100 MHz: δ (ppm): 25.2 (s, CH_2S); 27.8 (s, CH_3); 32.9 (s, CH_2); 46.9 (s, $C_{(^tBu)}$); 60.8 (s, CH_2OH); 208.9 (s, CO). MS (Fab +/NBA): $m/z = 199$ $[M+Na]^+$; 177 $[M+H]^+$; 85 $[^tBuCO]^+$; 57 $[^tBu]^+$.

4.2.6. *S*-acyl-3-thiopropyl alcohol (11). A solution of allyl alcohol (1.0 mL, 14.7 mmol, 1 equiv), thioacetic acid (2.1 mL, 26.9 mmol, 2 equiv) and AIBN (2.41 g, 14.7 mmol, 1 equiv) in 20 mL of benzene was degassed for 15 min by argon bubbling and then heated to reflux for 4 h. The mixture was cooled to room temperature, washed twice with a saturated solution of $NaHCO_3$, then twice with brine. The organic layer was dried with sodium sulphate and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate 70/30), 1.1 g of compound **11** was obtained as colourless oil in 56% yield. $R_f = 0.28$ (ethyl acetate/petroleum ether 50/50). ^{1}H NMR $CDCl_3$/250 MHz: δ (ppm): 1.75 (q^t, $^3J_{HH} = 6.4$ Hz, 2H, CH_2); 2.28 (s, 3H, CH_3); 2.38 (s, 1H, OH); 2.93 (t, $^3J_{HH} = 6.8$ Hz, 2H, CH_2S); 3.57 (t, $^3J_{HH} = 5.7$ Hz, 2H, CH_2OH).^{13}C NMR $CDCl_3$/100 MHz: δ (ppm): 25.9 (s, CH_2S); 30.9 (s, CH_3); 32.8 (s, CH_2); 60.8 (s, CH_2OH); 197.8 (s, CO).

MS (Fab +/NBA): $m/z = 117$ $[CH_3COSCH_2CH_2CH_2]^+$; 43 $[CH_3CO]^+$.

4.2.7. Bis(*S*-pivaloyl-3-thiopropyl)phosphonate (12). A mixture of S-pivaloyl-3-thiopropyl alcohol **10** (0.83 g, 4.7 mmol, 2 equiv) and pyridine (0.375 mL) was added dropwise to a solution of phosphorus trichloride (0.2 mL, 2.33 mmol, 1 equiv) in 2 mL of cyclohexane at 0 °C.

After 15 min at room temperature, S-pivaloyl-3-thiopropyl alcohol (0.4g, 2.3 mmol, 1 equiv) in a minimum of cyclohexane was added. The mixture was stirred during 4 h 30 min, the formed precipitate was removed by filtration and washed with cyclohexane. The filtrate was concentrated, the residue was diluted in dichloromethane and washed twice with brine. The organic layer was dried with sodium sulphate and the solvent was removed under reduced pressure. The purification of the crude product by silica gel column chromatography (ethyl acetate/petroleum ether 50/50) gave 850 mg of compound **12** as colourless oil in 91% yield. $R_f = 0.43$. ^{1}H NMR $CDCl_3$/250 MHz: (ppm): 1.16 (s, 18H, CH_3); 1.89 (q^t, $^3J_{HH} = 6.6$ Hz, 4H, CH_2); 2.87 (t, $^3J_{HH} = 7.1$ Hz, 4H, CH_2S); 4.07 (td, $^3J_{HH} = 6.2$ Hz, $^3J_{HP} = 8.1$ Hz, 4H, $POCH_2$); 6.77 (d, $^1J_{HP} = 699.6$ Hz, 1H, PH). ^{13}C NMR $CDCl_3$/100 MHz: (ppm): 24.9 (s, CH_2S); 27.7 (s, CH_3); 30.8 (d, $^3J_{PC} = 6.6$ Hz, CH_2); 46.8 (s, $C_{(^tBu)}$); 64.4 (d, $^2J_{PC} = 5.6$ Hz, $POCH_2$); 206.9 (s, COS).^{31}P NMR $CDCl_3$/250 MHz: δ (ppm): 8.4. MS (Fab +/NBA): $m/z = 399$ $[M + H]^+$, 159 $[^tBuCOSCH_2CH_2CH_2]^+$, 57 $[^tBu]^+$.

4.2.8. Bis(*S*-acyl-3-thiopropyl)phosphonate (13). Compound **13** was prepared according to the above procedure (compound **12**) from 0.48 mL of phosphorus trichloride (5.47 mmol, 1 equiv) in 10 mL of cyclohexane, 2.20 g of *S*-acyl-3-thiopropyl alcohol **11** (16.4 mmol, 3 equiv) and 0.88 mL of pyridine (10.9 mmol, 2 equiv). After purification by silica gel column chromatography (eluent: ethyl acetate/petroleum ether 50/50). 1.41 g of compound **13** was isolated as colourless oil in 81% yield. $R_f = 0.28$. ^{1}H NMR $CDCl_3$/400 MHz: δ (ppm): 1.91 (q^t, $^3J_{HH} = 6.6$ Hz, 4H, CH_2); 2.28 (s, 6H, CH_3); 2.90 (t, $^3J_{HH} = 7.0$ Hz, 4H, CH_2S); 4.07 (td, $^3J_{HH} = 6.1$ Hz, $^3J_{HP} = 8.2$ Hz, 4H, $POCH_2$); 6.77 (d, $^1J_{HP} = 701.4$ Hz, 1H, PH). ^{13}C NMR $CDCl_3$/100 MHz: δ (ppm): 25.6 (s, CH_2S); 30.7 (d, $^3J_{PC} = 6.6$ Hz, CH_2); 31.1 (s, CH_3); 64.5 (d, $^2J_{PC} = 5.7$ Hz, $POCH_2$); 195.9 (s, COS). ^{31}P NMR $CDCl_3$/200 MHz: δ (ppm): 9.1.MS (Fab +/NBA): $m/z = 315$ $[M + H]^+$.

4.3. Bis(RSATP)polyethyleneglycol monomethylether phosphonoformates 14–19

4.3.1. General procedure. *N*,*O*-bis(trimethylsilyl)acetamide BSA (0.15 mL, 0.6 mmol, 1.2 equiv) was added to a solution of bis(*S*-acyl-3-thiopropyl)phosphonate (0.5 mmol, 1 equiv) in 2 mL of dichloromethane, under nitrogen atmosphere at room temperature. After 3 h the chloroformate of monomethylether of polyethyleneglycol (1.5 mmol, 3 equiv) in 2 mL of dichloromethane was added dropwise. The reaction was stirred overnight at room temperature. The solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography with ethyl acetate—petroleum ether as eluent. The product was isolated as colourless oil.

4.3.2. [bis(S-pivaloyl-3-thiopropyl)](2-methoxyethyloxycarbonyl)phosphonate (14). The yield was 54%. Chromatographic conditions: ethyl acetate/petroleum ether 50/50.$R_f = 0.40$. ^{1}H NMR $CDCl_3$/400 MHz: δ (ppm): 1.08 (s, 18H, $CH_{3\ (^tBu)}$); 1.84 (q^t, $^3J_{HH} = 6.6$ Hz, 4H, CH_2); 2.80 (t, $^3J_{HH} = 7.1$ Hz, 4H, CH_2S); 3.23 (s, 3H, CH_3O); 3.51 (m, 2H, $CH_2O_{(2)}$); 4.15 (q, $^3J_{HH} = {}^3J_{HP} = 6.5$ Hz, 4H, $POCH_2$); 4.26 (m, 2H, CO_2CH_2). ^{13}C NMR $CDCl_3$/100 MHz: δ (ppm): 24.9 (s, CH_2S); 27.8 (s, $CH_{3\ (^tBu)}$); 30.8 (d, $^3J_{PC} = 6.1$ Hz, CH_2); 46.9 (s, $C_{(^tBu)}$); 59.3 (s, CH_3O); 65.0 (d, $^3J_{PC} = 5.1$ Hz, CO_2CH_2); 67.4 (d, $^2J_{PC} = 6.3$ Hz, $POCH_2$); 70.1 (s, $CH_2O_{(2)}$); 166.9 (d, $^1J_{PC} = 268.0$ Hz, CO_2); 206.9 (s, COS). ^{31}P NMR $CDCl_3$/250 MHz: δ (ppm): −3.5. MS (Fab +/NBA): $m/z = 501$ $[M + H]^+$. Anal. calcd for $C_{20}H_{37}O_8PS_2$ (500.61): C, 47.98; H, 7.45; S, 12.81; found: C, 47.81; H, 7.50; S, 12.68.

4.3.3. [bis(S-acyl-3-thiopropyl)](2-methoxyethyloxycarbonyl) phosphonate (15). The yield was 75%. Chromatographic conditions: ethyl acetate/petroleum ether 50/50.$R_f = 0.40$. ^{1}H NMR $CDCl_3$/100 MHz: δ (ppm): 1.78 (q^t, $^3J_{HH} = 6.5$ Hz, 4H, CH_2); 2.10 (s, 6H, CH_3CO); 2.75 (t, $^3J_{HH} = 7.1$ Hz, 4H, CH_2S); 3.14 (s, 3H, CH_3O); 3.41 (m, 2H, $CH_2O_{(2)}$); 4.06 (q, $^3J_{HH} = {}^3J_{HP} = 6.4$ Hz, 4H, $POCH_2$); 4.18 (m, 2H, CO_2CH_2). ^{13}C NMR $CDCl_3$/100 MHz: δ (ppm): 36.2 (s, CH_2S); 40.3 (d, $^3J_{PC} = 4.9$ Hz, CH_2); 40.6 (s, CH_3CO); 62.9 (s, CH_3O); 67.4 (d, $^3J_{PC} = 4.0$ Hz, CO_2CH_2); 69.1 (d, $^2J_{PC} = 4.8$ Hz, $POCH_2$); 71.4 (s, $CH_2O_{(2)}$); 147.7 (d, $^1J_{PC} = 213.7$ Hz, CO_2); 170.6 (s, COS). ^{31}P NMR $CDCl_3$/250 MHz: δ (ppm): −3.5. MS (Fab +/NBA): $m/z = 417$ $[M + H]^+$. Anal. calcd for $C_{14}H_{25}O_8PS_2$ (416.45): C, 40.38; H, 6.05; S, 15.40; found: C, 40.28; H, 6.10; S, 15.28.

4.3.4. [bis(S-pivaloyl-3-thiopropyl)](5-methoxy-3-oxapentyloxycarbonyl)phosphonate (16). The yield was 66%. Chromatographic conditions: ethyl acetate/petroleum ether 50/50.$R_f = 0.43$ (ethyl acetate). ^{1}H NMR $CDCl_3$/400 MHz: δ (ppm): 1.16 (s, 18H, $CH_{3\ (^tBu)}$); 1.92 (q^t, $^3J_{HH} = 6.6$ Hz, 4H, CH_2); 2.88 (t, $^3J_{HH} = 7.1$ Hz, 4H, CH_2S); 3.31 (s, 3H, CH_3O); 3.47 (m, 2H, $CH_2O_{(5)}$); 3.58 (m, 2H, $CH_2O_{(4)}$); 3.70 (m, 2H, $CH_2O_{(2)}$); 4.22 (q, $^3J_{HH} = {}^3J_{HP} = 6.5$ Hz, 4H, $POCH_2$); 4.35 (m, 2H, CO_2CH_2). ^{13}C NMR $CDCl_3$/100 MHz: δ (ppm): 24.9 (s, CH_2S); 27.8 (s, $CH_{3\ (^tBu)}$); 30.8 (d, $^3J_{PC} = 6.2$ Hz, CH_2); 46.9 (s, $C_{(^tBu)}$); 59.5 (s, CH_3O); 65.2 (d, $^3J_{PC} = 4.9$ Hz, CO_2CH_2); 67.4 (d, $^2J_{PC} = 6.0$ Hz, $POCH_2$); 68.9 (s, $CH_2O_{(2)}$); 71.0 (s, $CH_2O_{(4)}$); 72.3 (s, $CH_2O_{(5)}$); 166.9 (d, $^1J_{PC} = 270.0$ Hz, CO_2); 206.9 (s, COS). ^{31}P NMR $CDCl_3$/250 MHz: δ (ppm): −3.5. MS (Fab +/NBA): $m/z = 545$ $[M + H]^+$. Anal. calcd for $C_{22}H_{41}O_9PS_2$ (544.66): C, 48.51; H, 7.59; S, 11.77; found: C, 48.39; H, 7.63; S, 11.65.

4.3.5. [bis(S-acyl-3-thiopropyl)](5-methoxy-3-oxapentyloxycarbonyl) phosphonate (17). The yield was 76%. Chromatographic conditions: ethyl acetate/petroleum ether 50/50 then 80/20. $R_f = 0.57$ (ethyl acetate). ^{1}H NMR $CDCl_3$/400 MHz: δ (ppm): 1.78 (q^t, $^3J_{HH} = 6.4$ Hz, 4H, CH_2); 2.11 (s, 6H, CH_3CO); 2.76 (t, $^3J_{HH} = 7.1$ Hz, 4H, CH_2S); 3.15 (s, 3H, CH_3O); 3.31 (m, 2H, $CH_2O_{(5)}$); 3.42 (m, 2H, $CH_2O_{(4)}$); 3.53 (m, 2H, $CH_2O_{(2)}$); 4.23 (q, $^3J_{HH} = {}^3J_{HP} = 6.4$ Hz, 4H, $POCH_2$); 4.34 (m, 2H, CO_2CH_2). ^{13}C NMR $CDCl_3$/100 MHz: δ (ppm): 36.2 (s, CH_2S); 40.3 (d, $^3J_{PC} = 5.0$ Hz, CH_2); 40.6 (s, CH_3CO); 63.0 (s, CH_3O); 67.5 (d, $^3J_{PC} = 3.9$ Hz, CO_2CH_2); 69.2 (d, $^2J_{PC} = 4.9$ Hz, $POCH_2$); 70.4 (s,

$CH_2O_{(2)}$); 72.1 (s, $CH_2O_{(4)}$); 73.1 (s, $CH_2O_{(5)}$); 147.7 (d, $^1J_{PC}=213.0$ Hz, CO_2); 170.6 (s, COS). ^{31}P NMR $CDCl_3$/250 MHz: δ (ppm): −3.5. MS (Fab +/NBA): $m/z=461$ $[M+H]^+$. Anal. calcd for $C_{16}H_{29}O_9PS_2$ (460.50): C, 41.73; H, 6.35; S, 13.93; found: C, 41.58; H, 6.40; S, 13.82.

4.3.6. [bis(S-pivaloyl-3-thiopropyl)](8-methoxy-3,6-dioxaoctyloxycarbonyl)phosphonate (18). The yield was 62%. Chromatographic conditions: ethyl acetate/petroleum ether 60/40. $R_f=0.70$ (ethyl acetate). ^1H NMR $CDCl_3$/400 MHz: (ppm): 1.16 (s, 18H, $CH_{3(^tBu)}$); 1.92 (q^t, $^3J_{HH}=6.6$ Hz, 4H, CH_2); 2.88 (t, $^3J_{HH}=7.1$ Hz, 4H, CH_2S); 3.31 (s, 3H, CH_3O); 3.48 (m, 2H, $CH_2O_{(8)}$); 3.58 (m, 6H, $CH_2O_{(4,5,7)}$); 3.70 (m, 2H, $CH_2O_{(2)}$); 4.23 (q, $^3J_{HH}=^3J_{HP}=6.5$ Hz, 4H, $POCH_2$); 4.34 (m, 2H, CO_2CH_2). ^{13}C NMR $CDCl_3$/100 MHz: δ (ppm): 24.9 (s, CH_2S); 27.8 (s, $CH_{3(^tBu)}$); 30.8 (d, $^3J_{PC}=6.4$ Hz, CH_2); 46.8 (s, $C_{(^tBu)}$); 59.4 (s, CH_3O); 65.2 (d, $^3J_{PC}=4.9$ Hz, CO_2CH_2); 67.4 (d, $^2J_{PC}=6.5$ Hz, $POCH_2$); 68.8 (s, $CH_2O_{(2)}$); 71.0 (m, $CH_2O_{(4,5,7)}$); 72.3 (s, $CH_2O_{(8)}$); 166.8 (d, $^1J_{PC}=270.3$ Hz, CO_2); 206.8 (s, COS). ^{31}P NMR $CDCl_3$/250 MHz: δ (ppm): −3.5. MS (Fab +/NBA): $m/z=589$ $[M+H]^+$, 159 $[^tBuCOSCH_2CH_2CH_2]^+$. Anal. calcd for $C_{24}H_{45}O_{10}PS_2$ (588.71): C, 48.96; H, 7.70; S, 10.89; found: C, 48.85; H, 7.74; S, 10.78.

4.3.7. [bis(S-acyl-3-thiopropyl)](8-methoxy-3,6-dioxaoctyloxycarbonyl)phosphonate (19). The yield was 75%. Chromatographic conditions: ethyl acetate/petroleum ether 70/30. $R_f=0.40$ (ethyl acetate). ^1H NMR $CDCl_3$/400 MHz: δ (ppm): 1.94 (q^t, $^3J_{HH}=6.5$ Hz, 4H, CH_2); 2.27 (s, 6H, CH_3CO); 2.92 (t, $^3J_{HH}=7.1$ Hz, 4H, CH_2S); 3.31 (s, 3H, CH_3O); 3.48 (m, 2H, $CH_2O_{(8)}$); 3.58 (m, 6H, $CH_2O_{(4,5,7)}$); 3.69 (m, 2H, $CH_2O_{(2)}$); 4.23 (q, $^3J_{HH}=^3J_{HP}=6.4$ Hz, 4H, $POCH_2$); 4.34 (m, 2H, CO_2CH_2). ^{13}C NMR $CDCl_3$/100 MHz: δ (ppm): 36.2 (s, CH_2S); 40.3 (d, $^3J_{PC}=5.1$ Hz, CH_2); 40.6 (s, CH_3CO); 62.9 (s, CH_3O); 67.5 (d, $^3J_{PC}=3.9$ Hz, CO_2CH_2); 69.1 (d, $^2J_{PC}=5.1$ Hz, $POCH_2$); 70.4 (s, $CH_2O_{(2)}$); 72.1 (m, $CH_2O_{(4,5,7)}$); 73.1 (s, $CH_2O_{(8)}$); 147.7 (d, $^1J_{PC}=213.0$ Hz, CO_2); 170.6 (s, COS). ^{31}P NMR $CDCl_3$/250 MHz: (ppm): −3.5. MS (Fab +/NBA): $m/z=505$ $[M+H]^+$. Anal. calcd for $C_{18}H_{33}O_{10}PS_2$ (504.55): C, 42.85; H, 6.59; S, 12.71; found: C, 42.76; H, 6.62; S, 12.61.

4.3.8. [bis(S - pivaloyl - 3 - thiopropyl)](allyloxycarbonyl)-phosphonate (20). Compound **20** was prepared according to the above procedure (compounds **14**–**19**) with allyl chloroformate. The yield was 80%. Chromatographic conditions: ethyl acetate/petroleum ether 70/30. $R_f=0.75$. ^1H NMR $CDCl_3$/250 MHz: δ (ppm): 1.25 (s, 18H, $CH_{3\ (^tBu)}$); 2.01 (q^t, $^3J_{HH}=6.7$ Hz, 4H, CH_2); 2.95 (t, $^3J_{HH}=7.1$ Hz, 4H, CH_2S); 4.31 (q, $^3J_{HH}=^3J_{HP}=6.5$ Hz, 4H, $POCH_2$); 4.77 (qd, $^4J_{HH}=^4J_{HP}=1.3$ Hz, $^3J_{HH}=5.9$ Hz, CO_2CH_2); 5.38 (m, 2H, $CH_{(3,3')}$); 5.93 (m, 1H, $CH_{(2)}$). ^{13}C NMR $CDCl_3$/100 MHz: δ (ppm): 25.1 (s, CH_2S); 27.8 (s, CH_3); 30.7 (d, $^3J_{PC}=6.1$ Hz, CH_2); 66.8 (d, $^3J_{PC}=5.0$ Hz, CO_2CH_2); 67.4 (d, $^2J_{PC}=6.4$ Hz, $POCH_2$); 120.4 (s, $CH_{2\ (3)}$); 131.0 (s, $CH_{(2)}$); 166.6 (d, $^1J_{PC}=269.8$ Hz, CO_2); 206.9 (s, COS). ^{31}P NMR $CDCl_3$/250 MHz: δ (ppm): −3.5. MS (Fab +/NBA): $m/z=483$ $[M+H]^+$, 159 $[^tBuCOSCH_2CH_2CH_2]^+$, 57 $[^tBu]^+$, 41$[CH_2=CH\text{-}CH_2]^+$.

4.3.9. [bis(S-acyl-3-thiopropyl)](allyloxycarbonyl)phosphonate (21). The same procedure was followed as for compound **20**. The yield was 83%. Chromatographic conditions: ethyl acetate/petroleum ether 50/50. $R_f=0.60$. ^1H NMR $CDCl_3$/400 MHz: δ (ppm): 2.01 (q^t, $^3J_{HH}=6,6$ Hz, 4H, CH_2); 2,27 (s, 6H, CH_3); 2,92 (t, $^3J_{HH}=7,1$ Hz, 4H, CH_2S); 4,22 (q, $^3J_{HH}=^3J_{HP}=6,5$ Hz, 4H, $POCH_2$); 4,70 (dd, $^3J_{HH}=6,0$ Hz, $^4J_{HP}=1,2$ Hz, 2H, CO_2CH_2); 5,26 (dd, $^3J_{HH}=10,5$ Hz, $^2J_{HH}=1,1$ Hz, 1H, $CH_{(3)}$); 5,32 (dd, $^3J_{HH}=17,2$ Hz, $^2J_{HH}=1,1$ Hz 1H, $CH_{(3')}$); 5,88 (m, 1H, $CH_{(2)}$). ^{13}C NMR $CDCl_3$/100 MHz: δ (ppm): 25,5 (s, CH_2S); 30,7 (d, $^3J_{PC}=6,1$ Hz, CH_2); 31,1 (s, CH_3); 66,9 (d, $^3J_{PC}=5,2$ Hz, CO_2CH_2); 67,2 (d, $^2J_{PC}=6,1$ Hz, $POCH_2$); 120,6 (s, $CH_{2\ (3)}$); 131,0 (s, $CH_{(2)}$); 166,6 (d, $^1J_{PC}=270,3$ Hz, CO_2); 195,9 (s, COS). ^{31}P NMR $CDCl_3$/250 MHz: δ (ppm): −3.5. MS (Fab +/NBA): $m/z=399$ $[M+H]^+$, 117 $[MeCOSCH_2CH_2CH_2]^+$, 41 $[CH_2=CH\text{-}CH_2]^+$.

4.3.10. [bis(S-pivaloyl-3-thiopropyl)](6,7-dihydroxy-4-thioheptyloxycarbonyl)phosphonate (22). In a quartz reactor, a solution of [bis(S-pivaloyl-3-thiopropyl)](allyloxycarbonyl) phosphonate **19** (0.388 g, 0.81 mmol, 1 equiv) and 3-mercapto-1,2-propanediol (0.119 mL, 1.45 mmol, 1.8 equiv) in the presence of a catalytic amount of AIBN (8 mg, 0.048 mmol; 0.06 equiv) in 20 mL of THF was degassed for 15 min by argon bubbling. The solution was submitted to UV irradiation at $\lambda=254$ nm during 2 h, under inert atmosphere at room temperature. The solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (ethyl acetate). 0.178 g of compound **22** was isolated as colourless oil. Yield 37%. $R_f=0.38$ (ethyl acetate). ^1H NMR $CDCl_3$/400 MHz: δ (ppm): 1.16 (s, 18H, $CH_{3\ (^tBu)}$); 1.94 (m, 6H, $CH_{2\ (2,2')}$); 2.52–2.66 (m, 6H, $CH_2S_{(3,5)}$ and OH); 2.88 (t, $^3J_{HH}=7.1$ Hz, 4H, $CH_2S_{(3')}$); 3.58 (AB part of an ABX system, $\delta_A=3.66$, $\delta_B=3.51$, $^1J_{AB}=11.3$ Hz, $^3J_{AX}=3.7$ Hz, $^3J_{BX}=6.1$ Hz, 2H, CH_2OH); 3.74 (m, 1H, CHOH); 4.22 (q, $^3J_{HH}=^3J_{HP}=6.5$ Hz, 4H, $POCH_2$); 4.33 (t, $^3J_{HH}=5.9$ Hz, 2H, CO_2CH_2).

^{13}C NMR $CDCl_3$/100 MHz: δ (ppm): 24.9 (s, $CH_2S_{(3')}$); 27.8 (s, $CH_{3\ (^tBu)}$); 28.8 et 28.9 (2s, $CH_2S_{(3,5)}$); 30.8 (d, $^3J_{PC}=6.3$ Hz, $CH_{2\ (2')}$); 35.9 (s, $CH_{2\ (2)}$); 46.9 (s, $C_{(^tBu)}$); 64.9 (d, $^3J_{PC}=4.6$ Hz, CO_2CH_2); 65.7 (s, CH_2OH); 67.5 (d, $^2J_{PC}=6.5$ Hz, $POCH_2$); 70.9 (s, CHOH); 166.7 (d, $^1J_{PC}=269.9$ Hz, CO_2); 207.0 (s, COS). ^{31}P NMR $CDCl_3$/250 MHz: δ (ppm): −3.6. MS (Fab+/NBA): $m/z=591$ $[M+H]^+$, 159 $[^tBuCOSCH_2CH_2CH_2]^+$. Anal. calcd for $C_{23}H_{43}O_9PS_3$ (590.75): C, 46.76; H, 7.34; S, 16.28; found: C, 46.69; H, 7.39; S, 16.19.

4.3.11. [bis(S-acyl-3-thiopropyl)](6,7-dihydroxy-4-thioheptyloxycarbonyl)phosphonate (23). Compound **23** was prepared according to the above procedure (compound **22**) from 0.309 g of [bis(*S*-acyl-3-thiopropyl)](allyloxycarbonyl)phosphonate **21** (0.78 mmol, 1 equiv) and 0.12 mL of 3-mercapto-1,2-propanediol (1.40 mmol, 1.8 equiv) in the presence of 7.6 mg of AIBN (0.047 mmol; 0.06 equiv) in 20 mL of THF . After purification by silica gel column chromatography (eluent: ethyl acetate), 0.156 g of compound **22** was obtained as colourless oil in 40%

yield. $R_f = 0.33$ (ethyl acetate/ethanol 98/2). ^{1}H NMR $CDCl_3$/400 MHz: δ (ppm): 1.95 (m, 6H, $CH_{2\ (2,2')}$); 2.26 (s, 6H, CH_3); 2.53 – 2.66 (m, 6H, $CH_2S_{\ (3,5)}$ and OH); 2.88 (t, $^3J_{HH} = 7.1$ Hz, 4H, $CH_2S_{\ (3')}$); 3.58 (AB part of an ABX system, $\delta_A = 3.66$, $\delta_B = 3.51$, $^1J_{AB} = 11.3$ Hz, $^3J_{AX} = 3.7$ Hz, $^3J_{BX} = 6.1$ Hz, 2H, CH_2OH); 3.73 (m, 1H, CHOH); 4.21 (q, $^3J_{HH} = {}^3J_{HP} = 6.5$ Hz, 4H, $POCH_2$); 4.33 (t, $^3J_{HH} = 5.9$ Hz, 2H, CO_2CH_2). ^{13}C NMR $CDCl_3$/ 100 MHz: δ (ppm): 25.5 (s, $CH_2S_{\ (3')}$); 28.8 and 28.9 (2s, $CH_2S_{\ (3,5)}$); 30.7 (d, $^3J_{PC} = 6.2$ Hz, $CH_{2\ (2')}$); 31.0 (s, CH_3); 35.9 (s, $CH_{2\ (2)}$); 64.8 (d, $^3J_{PC} = 4.5$ Hz, CO_2CH_2); 65.7 (s, CH_2OH); 67.3 (d, $^2J_{PC} = 6.5$ Hz, $POCH_2$); 70.9 (s, CHOH); 166.7 (d, $^1J_{PC} = 270.0$ Hz, CO_2); 195.8 (s, COS). ^{31}P NMR $CDCl_3$/250 MHz: δ (ppm): -−3.6. MS (Fab +/NBA): $m/z = 507$ $[M+H]^+$, 117 $[CH_3COSCH_2CH_2CH_2]^+$, 43 $[CH_3CO]^+$. Anal. calcd for C_{17} $H_{31}O_9PS_3$ (506.59): C, 40.30; H, 6.17; S, 18.99; found: C, 40.19; H, 6.20; S, 18.78.

4.4. Biological studies

Anti-HIV assays were conducted following the procedure described previously.[18]

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