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Citation for final published version:

Pertusati, Fabrizio , Serafini, Sara, Albadry, Najmiyah, Snoeck, Robert and Andrei, Graciela 2017. Phosphonoamidate prodrugs of C5-substituted pyrimidine acyclic nucleosides for antiviral therapy. *Antiviral Research* 143 , pp. 262-268.
10.1016/j.antiviral.2017.04.013

Publishers page: <http://dx.doi.org/10.1016/j.antiviral.2017.04.013>

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Phosphonoamidate Prodrugs of C5-Substituted Pyrimidine Acyclic Nucleosides for Antiviral Therapy

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Abstract

Acyclic nucleoside phosphonates (ANPs) are nowadays one of the key drugs in the treatment of DNA virus and retrovirus infections. In this work, we report the synthesis and antiviral evaluation of phosphonoamidate and diamidates prodrugs of C5-pyrimidine acyclic nucleosides derivatives functionalized with but-2-enyl-chain. In the phosphonoamidate series, the most active compound **15**, showed sub-micromolar activity against varicella zoster virus (VZV) ($EC_{50} = 0.09-0.5 \mu M$) and μM activity against human cytomegalovirus (HCMV) and herpes simplex virus (HSV). Separation of single diastereoisomers for compound **14**, showed that **14b** had better anti-herpesvirus activity and no cytotoxicity compared to the diastereoisomeric mixture **14**. Very interestingly, phosphonodiamidate **21** showed anti-herpesvirus activity with excellent activity against wild type and thymidine kinase-deficient (TK⁻) VZV strains ($EC_{50} = 0.47$ and $0.2 \mu M$, respectively) and HCMV ($EC_{50} = 3.5-7.2 \mu M$) without any cytotoxicity ($CC_{50} > 100$).

Keywords: Phosphonoamidate; HCMV; C5-pyrimidine acyclic nucleosides; VZV

1. Introduction

Acyclic nucleoside phosphonates (ANPs), nucleotide analogues with a stable P–C bond, are the cornerstone of antiviral therapy (De Clercq and Holy, 2005; De Clercq et al., 1987; Pertusati et al., 2012). Three ANP analogues; cidofovir (**1**, CDV, Vistide)(De Clercq et al., 1987) adefovir dipivoxil (**2**, bis-POM PMEA, Hepsera)(Clercq et al., 1986), tenofovir disoproxil (**3**, TDF, Viread)(Balzarini et al., 1993), were the first ANPs approved for the treatment of severe viral infections (Figure 1). More recently, Tenofovir alafenamide (formerly **GS-7340**), a phosphoroamidate prodrug of Tenofovir, was developed by Gilead Sciences to treat HIV infection and chronic hepatitis B (Callebaut et al., 2015). In November 2015, the FDA approved its use in the form of tenofovir alafenamide fumarate (**4**, TAF) for HIV therapy (<http://www.gilead.com/news/press-releases/2015/11/us-food-and-drug-administration-approves-gileads-single-tablet-regimen-genvoya-elvitegravir-cobicistat-emtricitabine-and-tenofovir-alafenamide-for-treatment-of-hiv1-infection>).

Figure 1. ANPs in the market (1-4) and the novel ANPs reported by Agrofolgio (5-6).

The activities of ANPs are based on the intracellular phosphorylation by two salvage pathway kinases (nucleoside monophosphate [NMP] and nucleoside diphosphate [NDP] kinases) to their diphosphates (ANP-PPs). ANP-PPs mimic the

natural nucleoside triphosphates and are the active form capable of inhibiting the viral DNA polymerases.(De Clercq, 2007; Pertusati et al., 2012)

Since the structure of the ANPs side chain strongly influences their antiviral selectivity, several series of compounds, modified in their acyclic moiety, have been reported. Among them, of particular interest to this project are ANPs analogues with a more rigid acyclic chain. The increased rigidity was achieved by the introduction of a double bond. This structural feature has been found to be important and effective for the antiviral activity of these class of unsaturated ANPs (Stella et al., 2014).

The introduction of an ethenyl unit into the acyclic side chain allows the ANPs to better mimic the conformation of the ribose ring. In addition, because a double bond has an appreciable electron-withdrawing effect, it maintains an electronic contribution similar to the oxygen atom (Hansch and Leo, 1979). This last feature assures that the important second dissociation constant of alkenyl-phosphonic acids can closely mirror those of the natural phosphate.

Surprisingly, almost all biologically active ANP are purine derivative. The only pyrimidine based ANP in the market is Cidofovir. In order to identify novel and more potent ANPs, Agrofoglio's group reported the synthesis of several pyrimidine ANPs in which the oxygen heteroatom has been replaced with a double bond having a *trans* stereochemistry (Oh et al., 2005), (Amblard et al., 2005a), (Amblard et al., 2005b), (Topalis et al., 2011) (McGuigan et al., 2013a).

Among the different ANPs synthesised, compounds **5** and **6** proved to be efficient substrates for the human thymidine monophosphate kinase with their bis-POM prodrugs **7** and **8** (Figure 1) showing remarkable antiviral activity. Compound **7** for example was particularly active against HSV-1 and HSV-2 ($EC_{50} = 3.1$ and 6.1

μM , respectively) and against TK⁺ and TK⁻ VZV strains ($\text{EC}_{50} = 0.41$ and $0.19 \mu\text{M}$, respectively) but not against HCMV (Topalis et al., 2011).

To the best of our knowledge, no other prodrug approach other than bis-POM has been applied to these interesting pyrimidine scaffolds. In search for more potent ANPs, together with a better safety profile, we were interested in study the impact, of the phosphonoamidate and phosphonodiamidate prodrugs on the antiviral activity of ANPs **5** and **6** and compare their antiviral activities with those of the bis-POM derivatives (Topalis et al., 2011). We were also interested in assessing the antiviral activity of the novel compounds against HSV and VZV TK⁺ and TK⁻ strains to prove that our prodrugs are able to bypass the first phosphorylation step.

The phosphoro(no)amidate approach, proved to be extremely successful both on nucleoside (Sofosbuvir for Hepatitis C <https://www.drugs.com/newdrugs/fda-approves-sovaldi-chronic-hepatitis-c-3986.html>) as well as on acyclic nucleosides with the recent approval of the Tenofovir Alafenamide for the treatment of HIV infection and chronic hepatitis (<http://www.gilead.com/news/press-releases/2016/11/us-food-and-drug-administration-approves-gileads-vemlidy-tenofovir-alafenamide-for-the-treatment-of-chronic-hepatitis-b-virus-infection>). Our group also obtained excellent results using the phosphoroamidate approach on adefovir and tenofovir (Pertusati et al., 2014a) as well as on anticancer nucleosides (NUC-1031, phase 3 clinical trial for solid tumours and NUC-3373 among others <http://www.nucanabiomed.com/products.html>).

2. Materials and methods

2.1 Experimental chemistry

All anhydrous solvents were purchased from Sigma–Aldrich, NMR solvents from Cambridge isotope laboratories and reactants from Acros Organics, Sigma Aldrich, Alfa Aesar, Fluka, Carbosynth and Molekula. All reagents commercially available were used without further purification. Analytical Thin Layer Chromatography (TLC): pre-coated aluminium backed plates (60 F254, 0.2 mm thickness, Merck) were visualized under 254 nm UV light or using KMnO₄. Preparative TLC was performed with Analtech blanket 2000, 1000 or 500 m silica gel GF. Flash column chromatography was carried out using silica gel supplied by Fisher (60A, 35–70 mm) or with Biotage Isolera One purification system. Analytical High Performance Liquid Chromatography (HPLC) analysis was performed using both Spectra System SCM (with X-select-C18, 5 μm, 4.8x150 mm column) and Varian Prostar system (LC Workstation-Varian Prostar 335 LC detector). Preparative HPLC was performed with Varian Prostar (with pursuit XRs C18 150 x 21.2 mm column). ¹H NMR (500 MHz), ¹³C NMR (125 MHz), and ³¹P NMR (202 MHz) spectra were recorded on a Bruker Avance 500 MHz spectrometer at 25 °C. Chemical shifts (δ) are quoted in parts per million (ppm) relative to internal MeOD (δ=3.34 ¹H-NMR, δ=49.86 ¹³C-NMR) and CDCl₃ (δ=7.26 ¹H-NMR, δ=77.36 ¹³C-NMR) or external 85% H₃PO₄ (δ=0.00 ³¹P-NMR). Coupling constants (J) are given in Hertz. The following abbreviations are used in the assignment of NMR signals: s (singlet), d (doublet), t (triplet), q (quartet), qn (quintet), m (multiplet), bs (broad singlet), dd (doublet of doublet), dt (doublet of triplet). Low resolution mass spectrometry was performed on a Bruker Daltonics microTOF-LC system, and as a service by the School of Chemistry at Cardiff University. Compounds **5**, **6**, **11** and **12** were

prepared following literature procedures and their ^1H , ^{31}P and ^{13}C NMR were in accordance with the literature.⁹

*2.1.1 General procedure for preparation of phosphoramidates prodrugs (**13-20**)
phosphonodiamidate (**21-23**)*

In a round bottom flask, under an argon atmosphere, the appropriate diester (**11** or **12**) was dissolved in dry acetonitrile (ACN) and treated with an excess of TMSBr (5 equivalents). The mixture was then stirred overnight at room temperature and then the volatiles evaporated without any contact with air. Then, the flask was charged with the appropriate, dry amino acid ester 4- toluene sulfonate salt (1 equivalents with respect to the **11** or **12**), and the solids dissolved in a mixture of dry triethylamine and dry pyridine (1:4 v/v) and heated to 50°C to obtain a homogenous solution. To this, it was added a solution of 2,2-dithiodipyridine (5 equivalents) and triphenylphosphine (5 equivalents) in dry pyridine, prepared in a separate flask, under an argon atmosphere. The resulting mixture was stirred at 50°C for 3-4 hours, to reach the complete conversion as monitored by TLC (DCM/MeOH 95:5 v/v). After evaporating all the volatiles, the residue was treated with a mixture of methanol, water, hexane and toluene (1:1:1:1) and transferred into a separatory funnel. The upper layer (Hexane/toluene) was separated and the aqueous (MeOH/water) extracted 3 times with a mixture of toluene/hexane (1:1). The water/methanol layer was further extracted with DCM (20 mL x 3). The organics were dried over MgSO_4 , filtered and evaporated. The residue was purified with flash column chromatography on silica gel using DCM/MeOH (gradient, usually from 99:1 to

95:5 v/v) as eluting mixture and then by preparative TLC (95:5 v/v) or preparative HPLC (gradient ACN/water 10:90, 100% ACN in 30 minutes, 100% ACN for 5 minutes, then ACN/water 10:90 in 2 minutes).

2.2 *Biological assays*

The compounds were evaluated against the following viruses: herpes simplex virus type 1 (HSV-1) strain KOS, thymidine kinase-deficient (TK⁻) HSV-1 KOS strain resistant to ACV (ACV^r), herpes simplex virus type 2 (HSV-2) strains Lyons and G, varicella-zoster virus (VZV) strain Oka, TK⁻ VZV strain 07-1, human cytomegalovirus (HCMV) strains AD-169 and Davis, vaccinia virus Lederle strain, respiratory syncytial virus (RSV) strain Long, vesicular stomatitis virus (VSV), Coxsackie B4, Parainfluenza 3, Influenza virus A (subtypes H1N1, H3N2), influenza virus B, Reovirus-1, Sindbis, Reovirus-1 and Punta Toro. The antiviral assays were based on inhibition of virus-induced cytopathicity or plaque formation in human embryonic lung (HEL) fibroblasts, African green monkey cells (Vero), human epithelial cells (HeLa) or Madin-Darby canine kidney cells (MDCK). Confluent cell cultures in microtiter 96-well plates were inoculated with 100 CCID₅₀ of virus (1 CCID₅₀ being the virus dose to infect 50% of the cell cultures) or with 20 plaque forming units (PFU) (VZV) in the presence of varying concentrations of the test compounds. Viral cytopathicity or plaque formation was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC₅₀ or compound concentration required to reduce virus-induced cytopathogenicity or viral plaque formation by 50%.

The cytostatic activity measurements were based on the inhibition of cell growth. HEL cells were seeded at a rate of 5×10^3 cells/well into 96-well microtiter plates and allowed to proliferate for 24 h. Then, medium containing different concentrations of the test compounds was added. After 3 days of incubation at 37 °C, the cell number was determined with a Coulter counter. The cytostatic concentration was calculated as the CC_{50} , or the compound concentration required to reduce cell proliferation by 50% relative to the number of cells in the untreated controls. CC_{50} values were estimated from graphic plots of the number of cells (percentage of control) as a function of the concentration of the test compounds. Alternatively, cytotoxicity of the test compounds was expressed as the minimum cytotoxic concentration (MCC) or the compound concentration that caused a microscopically detectable alteration of cell morphology.

3. Results and discussion

3.1 Chemistry

Our research began with the synthesis of the alkenyl-functionalized pyrimidine methyl phosphonates **11** and **12**. The general strategy for the convenient stereocontrolled synthesis of these *trans*-alkene acyclic nucleoside phosphonates from thymine **9** and bromo-uridine **10** (Scheme 1), was based on olefin cross metathesis, developed by Agrofoglio and colleagues. (Topalis et al., 2011) Phosphonic acids **5** and **6** (parent nucleosides) were prepared by treatment of **11** and **12** with an excess of trimethyl bromosilane (TMSBr) followed by aqueous workup (Topalis et al., 2011) (Scheme 1).

Scheme 1: Synthesis of methyl phosphonates **11** and **12** and the parent phosphonic acids **5a** and **6**.

The synthesis of phosphonoamidate prodrugs of these new ANPs was then attempted beginning with the phosphonate esters **11** and **12** using the one-pot procedure reported by Holy (Jansa et al., 2011) and successfully adapted by us for the synthesis of adefovir and tenofovir phosphonoamidate prodrugs (Pertusati et al., 2014b).

Briefly, methylphosphonates **11** and **12** were converted into the correspondent silyl esters by treatment with an excess of trimethylsilyl bromide (Jansa et al., 2011). The silyl derivatives were then treated with an excess of phenol (4 equivalents) and L-alanine-O-neopentyl ester (4 equivalents), in presence of a mixture of triphenylphosphine (5 equivalents) and 2,2-dipyridyl disulphide, (Aldrithiol, 5 equivalents). Under this protocol, only traces of the desired phosphonoamidate **14** were detected (TLC, Mass spectra, and ³¹P-NMR of the crude mixture) with the phosphonodiamidate **21** being the major product. It is clear from these results that the phenol was not nucleophilic enough to compete with the amino acid ester for the replacement of the silyl ester. Indeed, when the more nucleophilic *p*-Methoxy phenol was used as aryl alcohol partner in the above reaction sequence, the desired ProTide **13** was obtained in 18% yield although some diamidate was always isolated. Despite the fact that ProTides bearing para-Methoxy phenol were successfully applied to glucosamine (Serpi et al., 2012), this aryl moiety might be activated too slowly compared to the unsubstituted aryl prodrugs (Derudas et al., 2009). To circumvent this potential problem, we envisaged that increasing the concentration of phenol would allow us to obtain a better yield of the desired ProTide. After several attempts, we found that addition of an excess (6 equivalents) of phenol with respect to the L-alanine neopentyl

ester tosylate (1 equivalent) was sufficient to obtain a reasonable yield of phosphonoamidate (Scheme 2).

Scheme 2: Synthesis of phosphonoamidate and phosphonodiamidate prodrugs from esters 11 and 12;
Reagents and conditions: a) TMSBr, CH₃CN, rt, 8h, Argon atm.; b) Amino acid ester hydrochloride or tosylate (1 equiv.), ArOH (6 equiv.), PPh₃ (6 equiv.), Aldrithiol-2 (6 equiv.) NEt₃/Pyridine (1:4 v/v), 50°C, 4-8 hours; See table 1 for Ar and R definitions and yields

However, the phosphonodiamidates were always obtained alongside with the desired products (ProTide: diamidate roughly 1:1, at best). This fact was not completely unwanted since a one-pot reaction gave us two different prodrugs in a fairly reasonable yield. In few cases, with amino acids having bulky esters (L-Ala-O-hexyl, L-valine-O-pentyl ester, L-isoleucine-Opentyl ester), the yields for both products (mono and diamidate) were very low (1-3 %) probably due to steric reasons. In some circumstances, it was possible to accomplish the separation by column chromatography on silica gel of the two phosphonoamidate diastereoisomers. This was achieved with compounds **14** and **20** that were isolated as single isomers **14a and 14b** and **20a and 20b** (Table 1). Compounds synthesised by this improved procedure are collected in the table 1:

Cpd.	Ar	R	X	Isomers	Yield (%)
5	/	/	CH ₃	Parent Nuc	93
6	/	/	Br	Parent Nuc	12
13	4-OCH ₃ Ph	CH ₂ <i>t</i> Bu	CH ₃	Mix	18
14a	Ph	CH ₂ <i>t</i> Bu	CH ₃	Single diast.	16
14b	Ph	CH ₂ <i>t</i> Bu	CH ₃	Single diast.	10

15	Naph	CH ₂ tBu	CH ₃	Mix	7
16	5,6,7,8-THNaph	CH ₂ Ph	CH ₃	Mix	8
17a	Ph	CH ₂ Ph	Br	Mix	4
17b	Ph	CH ₂ Ph	Br	Single isomer.	3
18	Naph	CH ₂ Ph	Br	Mix	13
19	Ph	CH ₂ tBu	Br	Single diast.	16
20	Ph	CH ₂ Ph	H	Mix	5
20a	Ph	CH ₂ Ph	H	Single diast.	3
20b	Ph	CH ₂ Ph	H	Single diast.	2
21	/	CH ₂ tBu	CH ₃	/	25
22	/	CH ₂ tBu	Br	/	1
23	/	CH ₂ Ph	Br	/	2

Table 1 Compounds synthesised in this study and relative yields.

When, 5-Bromo uracil derivative **12** was subjected to the reaction conditions of Scheme 2, we were able to isolate a third compound (other than phosphonodiamidate, and ProTide). Analysis by mass spectrometry and ¹H-NMR of this new compound, revealed that the bromine in the nucleobase was replaced with a hydrogen. Indeed, uracil derivative **20** was isolated with a 5% overall yield. We attributed the formation of this derivative to the presence of triphenylphosphine in the reaction medium. Indeed, it has been shown that triphenylphosphine is able to debrominate α-bromo-carbonyl compounds (Borowitz and Grossman, 1962; Hoffmann and Diehr, 1962). To investigate if this was the case, we set up a control experiment consisting in the treatment of a solution of 5-Bromo uracil **24** in a 1:4 mixture of triethylamine and pyridine with

triphenylphosphine at 50°C. Indeed, we observed after 3 hours, the formation of the corresponding uracil **25** isolated in 50% yield (Scheme 2).

Scheme 2: Control experiment for the reduction of 5-bromo-uracil 24 to uracil 25 under the reaction conditions of Scheme 2.

The use of TMSBr in the synthetic sequence was considered responsible for the low yield due to the bromination of the alkenyl chain. The low stability of these brominated derivatives, whose formation was observed by mass spectrometry, did not allow their isolation and characterization. The low yield problem was also exacerbated in the case of the 5-bromouracil derivatives. With these substrates, a further reduction in the yield of the desired prodrugs was due to the action of the triphenyl phosphine, which caused the reduction of the C-Br bond in the nucleobase, leading to the formation of the uracil derivatives. The overall result was a lower yield of the desired compounds.

3.2 Antiviral evaluation

With our phosphonoamidates **13-20** and bis-amidates **21-23** in hand, we proceeded to investigate their biological activities against a variety of DNA viruses. In particular, we were interested in assessing if our prodrugs were able to perform better than the bis-POM prodrugs **7** and **8** (Agrofoglio and co-workers (Topalis et al., 2011), against HSV-1, Kos strain, HSV-2 (G strain), (TK⁻) HSV-1 (Kos acyclovir-resistant strain), VZV wild-type OKA strain, TK⁻ VZV (07/1 strain), HCMV strains AD-169 and Davis, vaccinia virus in HEL cell cultures.

As expected from their high polar nature and from literature data¹, phosphonic acids **5** and **6** resulted totally inactive against all viral strains tested in this study, probably due to their inability to permeate inside the cell.

In the phosphonoamidate family (ProTide), all our prodrugs showed good to moderate activity against both TK⁺ and TK⁻ VZV strains in HEL cells except for compound **20** (tested both as 1:1 mixture of diastereoisomers and individual isomers, Table 2, entries 14, 15, 16) which showed no activity against HSV and only a moderate activity against VZV. In this case, the diastereomeric mixture was completely inactive, while one of the two single diastereoisomer **20b**, was slightly more active than the other **20a**.

Compound **15** (the most active in this phosphonoamidate family) proved to have an inhibitory activity against the different HSV-1, HSV-2, VZV and HCMV strains with activity slightly better than the Bis(POM) prodrug **7** in the case of HSV-2 ($EC_{50} = 2.9$ vs $6.1 \mu\text{M}$) and TK⁻ HSV-1 Kos ACV^r, ($EC_{50} = 2.6$ vs $9.2 \mu\text{M}$). **15** was also less toxic than **7** and **8** and in contrast to the Bis(POM) prodrugs proved active against HCMV. Evaluation of single isomers of **14** showed that the slow eluting isomer (HPLC) **14b**, was the most active of the two isomers against HSV, VZV and HCMV (Entry 5 vs. 6) and also the less cytotoxic one (**14b** $EC_{50} > 100$ vs **14a** $EC_{50} = 19.08$). Compound **16** was moderately active against VZV and HSV and inactive against HCMV with a cytotoxicity comparable to **7** (Table 2, Entry 7 vs. 15).

All phosphonoamidate prodrugs having 5-bromouracil as nucleobase were found barely active against HSV, except for **16** that had EC_{50} values comparable to that of the BIS(POM) prodrug **7** (Table 2; Entries 7-12). Although **16** inhibited VZV replication with EC_{50} values in the μM range, it completely lost activity against HCMV.

Compounds **17** to **19** showed anti-HSV and anti-VZV activity like that of the corresponding bis-POM prodrug **8** (Table 2, Entries 8-11 vs 16) but in contrast to the latter prodrug they also displayed some anti-HCMV activity. No striking anti-vaccinia virus was observed with any of the prodrugs having 5-bromouracil as nucleobase. In case of compound **17**, the 1:1 mixture of diastereoisomers showed the same activity as the single isomers **17b**.

Based on our experience (McGuigan et al., 2013b) phosphoro- and phosphonodiamidates are usually less active than the corresponding ProTide, probably due to a slower processing rates considering that an amino acid ester is less easily displaced than an aryl moiety typical of a ProTide. Quite surprisingly, in our study one out of three phosphonodiamidates (**21**) proved active against herpesviruses in HEL cell cultures. Compound **21**, showed interesting activity against HSV-2 G-strain ($EC_{50} = 8.1 \mu\text{M}$), HSV-1 TK⁻ (Kos ACV^r strain) ($EC_{50} = 3.6 \mu\text{M}$) and slightly less active against HSV-1 KOS strain ($EC_{50} = 13.8 \mu\text{M}$), as highlighted in Table 2, Entry 1. This compound had also remarkable activity against TK⁺ and TK⁻ VZV strains ($EC_{50} = 0.47$ and $0.20 \mu\text{M}$, respectively) and proved as active as the reference anti-HCMV drug ganciclovir against human CMV. However, this compound was found inactive against HEL cell infected with Vaccinia virus (Table 2, Entry 1).

Replacement of the methyl group in compound **21** by a bromine group gave compound **22** resulting in detrimental effects for the anti-herpesvirus activity (Table 2 Entry 2). As for compound **21**, also **22** was found totally inactive against Vaccinia virus (Table 2, Entry 2). The diamidate **23** was found totally inactive against HSV, HCMV and vaccinia virus (Table 2, Entry 3) showing only a very weak activity against VZV strains. Collectively, compound **21** showed better activity

than acyclovir against TK⁻ HSV-1 strain (Kos ACV^r), but was found less active than cidofovir, acyclovir and ganciclovir against wild-type HSV-1 and HSV-2 strains. Finally, the diamidate **21** showed the same antiviral activity against TK⁺ and TK⁻ VZV strains than the bis-POM prodrug **7** but lower potency against HSV strains (Table 2, Entries 1 vs Entry 15). Interestingly, its antiviral activity was better than that of the Br-POM prodrug **8** against HSV and VZV except for HSV-1 (Table 2, Entries 1 vs Entry 16). Importantly, while prodrugs **7** and **8** were unable to inhibit HCMV replication at the higher concentration tested (40 μ M), the EC₅₀ values for the phosphonodiamidate **21** against HCMV were comparable to those found for the reference anti-HCMV drug ganciclovir. Although prodrugs **7** and **8** were moderately active against Vaccinia virus, our diamidate **13** was inactive. Finally, all our prodrugs were deprived of activity (EC₅₀ > 100 μ M) against the different RNA viruses tested.

Table 2. Antiviral activity of the synthesized compounds in human embryonic lung (HEL) fibroblasts

Compound	EC ₅₀ ^a (HEL) (μM)							Cytotoxicity (μM)		
	HSV-1 (Kos)	HSV-2 (G)	HSV-1 TK-KOS ACV ^r	VZV TK ⁺ (Oka)	VZV TK ⁻ (07-1)	HCMV (AD-169)	HCMV (Davis)	Vaccinia virus	MCC ^b	CC ₅₀ ^c
5	>100	>100	>100	>100	>100	>100	>100	>100	100	ND
6	>50	>50	>50	>100	>100	>100	>100	>50	>100	ND
7	3.1	6.1	9.2	0.41	0.19	>40	>40	30	≥200	35
8	20	16	19	20	15	>40	>40	33	≥200	34
14a	≥67 ± 47	39 ± 27	25 ± 18	2.13 ± 0.04	0.72 ± 0.27	34.2	13.4	>100	≥100	15 ± 5
14b	8.3 ± 0.9	7.2 ± 0.6	5.8 ± 2.5	0.74 ± 0.32	0.35 ± 0.33	10.1 ± 3.5	5.6 ± 0.5	>100	>100	> 100
15	3.8 ± 3.2	2.9 ± 1.6	2.6 ± 2.0	0.50 ± 0.42	0.09 ± 0.05	9.1 ± 1.3	5.6 ± 0.5	≥73 ± 39	≥100	40 ± 3
16	6.2 ± 3.9	4.4 ± 2.8	4.4 ± 3.4	3.4 ± 3.3	0.8 ± 0.7	>100	>100	>100	≥100	24 ± 15
17	47 ± 18	36 ± 3	25 ± 13	12.9 ± 7.0	10.2 ± 3.4	76	67	≥100	>100	37 ± 19
17b	30 ± 21	36 ± 3	30 ± 21	13.4 ± 9.3	9.7 ± 4.5	63	76	≥79 ± 30	>100	≥86 ± 20
18	35 ± 21	34 ± 0	35 ± 21	15.8 ± 1.8	10.2 ± 3.4	49	55	>100	>100	70 ± 27

19	33.5 ± 23.3	10.5 ± 0.7	27.5 ± 24.7	30.7 ± 2.4	9.3 ± 0.5	45	50	>50	>100	68 ± 27
20	>100	>100	>100	>100	100	>100	>100	>100	>100	ND
20a	>100	>100	>100	72	40	>100	>100	>100	100	ND
20b	>100	>100	>100	39	26	45	41	>100	100	ND
21	13.8 ± 8.8	8.1 ± 3.4	3.6 ± 2.6	0.47 ± 0.16	0.20 ± 0.06	7.2 ± 0.5	3.5 ± 0.8	>100	>100	> 100 ± 0
22	>100	>100	>100	>100	81.8	>100	>100	>100	>100	ND
23	>100	>100	>100	68	42	>100	>100	>100	>100	ND
Brivudin	0.072 ± 0.012	250	>250	0.012 ± 0.010	50.9 ± 16.5	ND	ND	19	>300	>300
Cidofovir	1.81 ± 0.27	1.75 ± 0.36	1.81 ± 0.27	ND	ND	1.26 ± 0.02	0.76 ± 0	17	≥300	>300
Acyclovir	0.42 ± 0.03	0.23 ± 0.02	146	2.9 ± 2.1	96.7 ± 5.8	ND	ND	>250	>440	>440
Ganciclovir	0.047 ± 0.023	0.026 ± 0.006	4	ND	ND	14.2 ± 2.7	7.3 ± 2.9	>100	>350	>350

ND: not done

^aEffective concentration required to reduce virus cytopathic effect (HSV, vaccinia virus and CMV) or plaque formation (VZV) by 50%.

^mminimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology. ^cConcentration required to reduce cell growth by 50%.

Compound **7**: [*E*]-bis(POM)-TbutP – Compound **8**: [*E*]-bis(POM)-5Br-UbutP

4. Conclusions

In conclusion, we have successfully synthesised a series of phosphonoamidate and diamidates prodrugs of C5-pyrimidine acyclic nucleosides derivatives functionalized with but-2-enyl- chain. We were able to demonstrate that the phosphonoamidate approach applied to compound **5** and **6** was capable of *turning* these inactive parent nucleosides to active antiviral agents.

In the phosphonoamidate series, the most active compound **15**, showed sub-micromolar activity against VZV ($EC_{50} = 0.09-0.5 \mu\text{M}$) and μM activity against HCMV and HSV. Importantly, its activity against both wild type and TK⁻ HSV and VZV strains make it a good candidate for the therapy of acyclovir-resistant viruses due to mutations in the viral thymidine kinase gene. In general, our phosphonoamidates have a comparable, and in some cases, better antiviral activity with respect to the bis-POM derivatives. Very interestingly, our prodrugs were able to inhibit HCMV whereas the bis-POM derivatives **7** and **8** were inactive. Other analogues of these structural motifs with more complex amino acid moieties are under investigation in our laboratory.

Acknowledgments

The authors wish also to express their gratitude to Mrs. Ellen De Waegenaere, Mr. Seppe Kelchtermans, Mrs. Leentje Persoons and Mrs. Lies Van Den Heurck for excellent technical assistance. The virological part of this work was supported by the KU Leuven (GOA no. 10/014).

Supplementary Data.

Supplementary data related to this article can be found at <http://>

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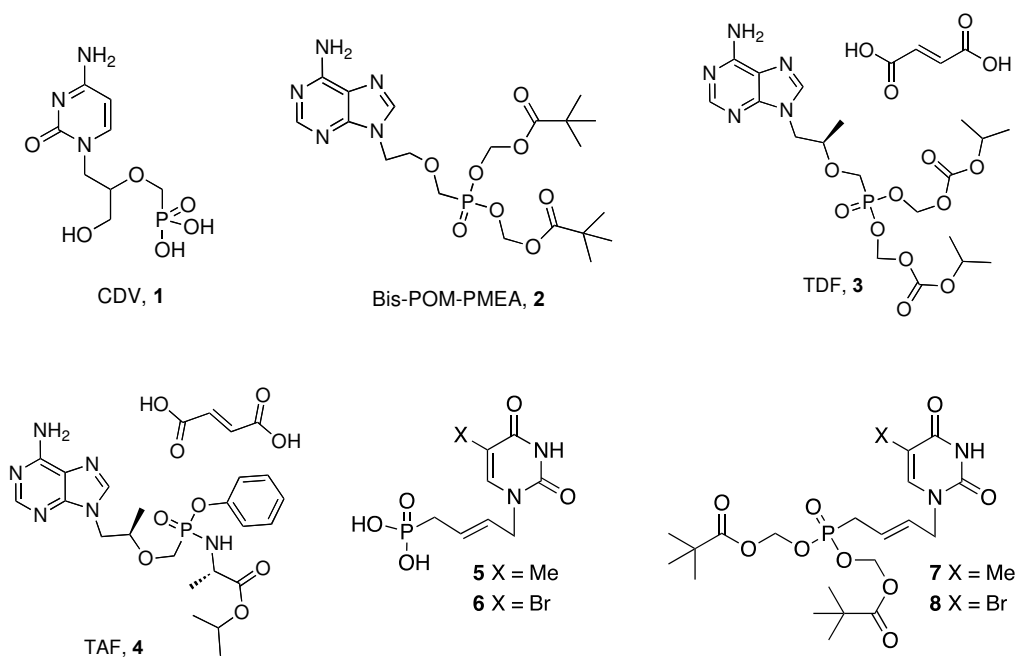
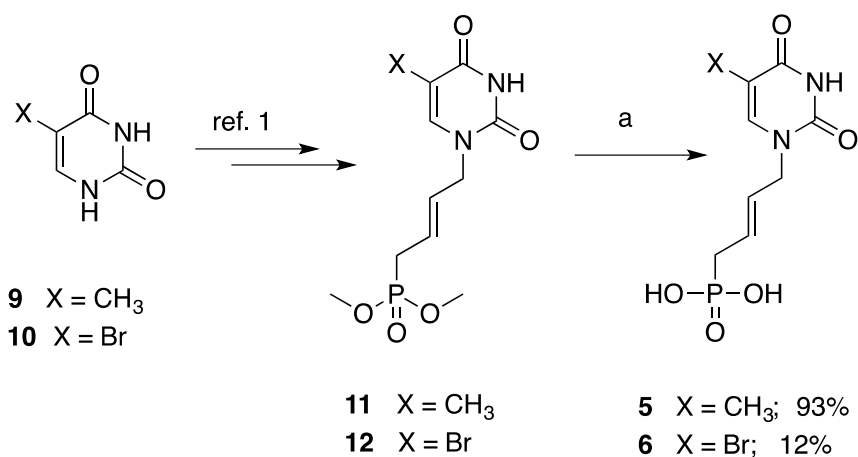
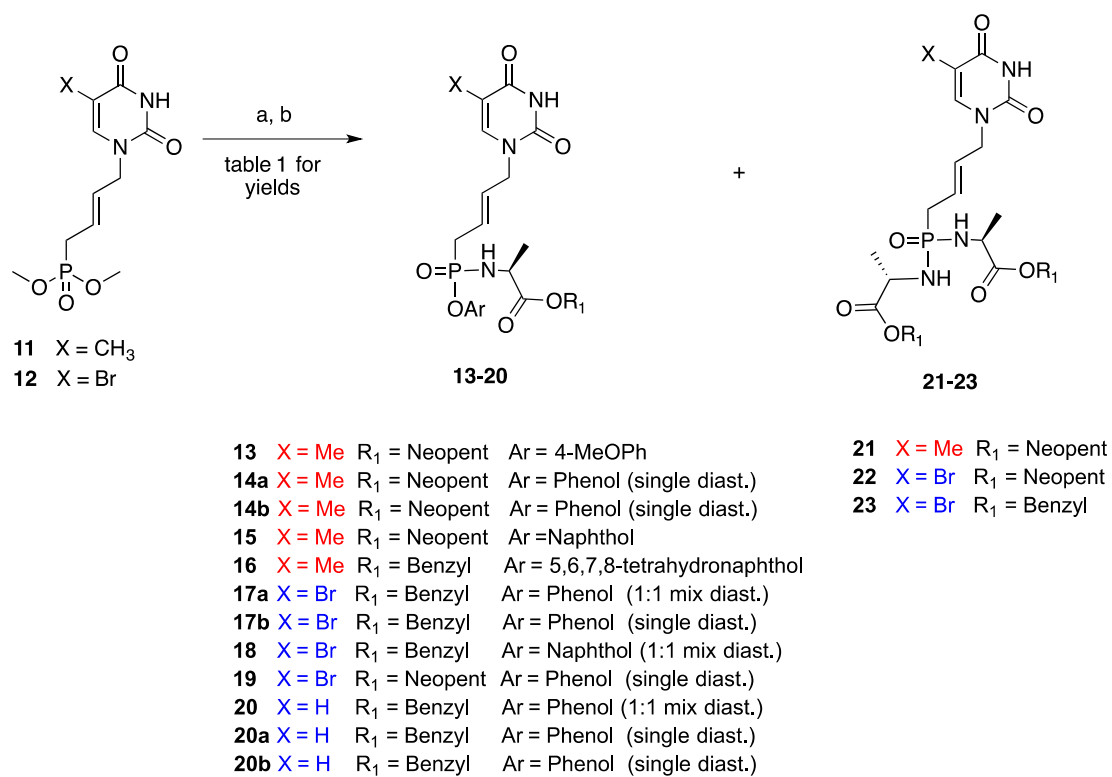


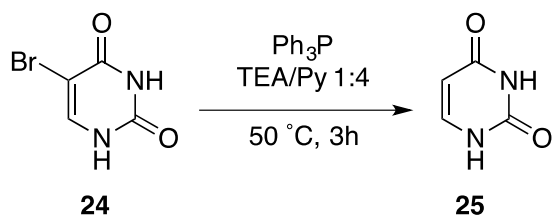
Figure 1



Scheme 1



Scheme 2



Scheme 3

List of captions

Figure 1. ANPs in the market (1-4) and the novel ANPs reported by Agrofoglio (5-6).

Scheme 1: Synthesis of methyl phosphonates 11 and 12 and the parent phosphonic acids 5a and 6.

Scheme 2: Synthesis of phosphonoamidate and phosphonodiamidate prodrugs from esters 11 and 12; Reagents and conditions: a) TMSBr, CH₃CN, rt, 8h, Argon atm.; b) Amino acid ester hydrochloride or tosylate (1 equiv.), ArOH (6 equiv.), PPh₃ (6 equiv.), Aldrithiol-2 (6 equiv.) NEt₃/Pyridine (1:4 v/v), 50°C, 4-8 hours; See table 1 for Ar and R definitions and yields

Scheme 3: Control experiment for the reduction of 5-bromo-uracil 24 to uracil 25 under the reaction conditions of Scheme 2.

Supporting Information

Phosphonate Prodrugs of C5-Substituted Pyrimidine Acyclic Nucleosides for Antiviral Therapy

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Contents:

General procedure and data for target compounds S2-S11

General procedure for the synthesis of phosphoamidates 13-20 and phosphonodiamidates 21-23.

In a round bottom flask, under an argon atmosphere, the desired diester (**11** or **12**) was dissolved in dry acetonitrile (ACN) and treated with an excess of TMSBr (5 equivalents). The mixture was then stirred overnight at room temperature and then the volatiles evaporated without any contact with air. Then the flask was charged with the appropriate, dry amino acid ester *p*-toluene sulfonate salt (1 equivalents with respect to the **11** or **12**), and the solids dissolved in a mixture of dry triethylamine and dry pyridine (1:4 v/v) and heated to 50°C to obtain a homogenous solution. To this, it was added a solution of 2,2-dithiodipyridine (5 equivalents) and triphenylphosphine (5 equivalents) in dry pyridine, prepared in a separate flask, under an argon atmosphere. The resulting mixture was stirred at 50°C for 3-4 hours, to reach the complete conversion as monitored by TLC (DCM/MeOH 95:5 v/v). After evaporating all the volatiles, the residue was treated with a

mixture of MeOH, water, hexane and toluene (1:1:1:1) and transferred into a separatory funnel. The upper layer (hexane/toluene) was separated and the aqueous (MeOH/water) extracted 3 times with a mixture of toluene/hexane (1:1). The water/methanol layer was further extract with DCM (20 mL x 3). The organics were dried over MgSO₄, filtered and evaporated. The residue was purified with flash column chromatography on silica gel using DCM/MeOH (gradient, usually from 99:1 to 95:5 v/v) as eluting mixture and then by preparative TLC (95:5 v/v) and/or preparative HPLC (gradient ACN/water 10:90, 100% ACN in 30 minutes).

Compounds **13**, **15**, **16**, **17a** and **18** were obtained as diastereomeric mixtures. Where possible, the characteristic parameters of the signals of the two isomers are described separately.

(2S)-neopentyl-2-(((4-methoxyphenoxy)((E)-4-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)but-2-en-1-yl) phosphoryl)amino)propanoate (13)

Compound **13** was prepared according to the general procedure from **11** (200 mg 0.632 mmol, 1 eq.), TMSBr (483 mg, 3.16 mmol, 5 eq.) in dry ACN (10 mL), L-alanine-*O*-neopentyl ester tosylate (843 mg, 2.52 mmol, 4 eq.) and *p*MeO-phenol (312 mg, 2.52 mmol, 4 eq.) triphenylphosphine (813 mg, 3.79 mmol, 6 eq.) 2-aldrithiol. (356 mg, 3.79 mmol, 6 eq.). After workup the crude was purified by silica chromatography column using a gradient of DCM/MeOH (99:1 to 95:5 v/v) and providing after preparative TLC (95:5 v/v) **13** (54 mg, 17% yield). ¹H-NMR (500 MHz, CD₃OD) δ_H 7.41(1H, d, *J* = 1.2 Hz, *H*-6), 7.39 (1H, d, *J* = 1.2 Hz, *H*-6), 7.12 (3H, dd, *J* = 9.0 and 2.1 Hz, *H*-Ph), 6.87 (2H, dd, *J* = 9.0 and 2.1 Hz *H*-Ph), 5.82-5.76 (4H, m, NCH₂CH= and =CHCH₂P), 4.36-4.24 (4H, m, CH₂N), 4.03-3.98 (2H, m, CHCH₃), 3.7 (6H, s, OCH₃), 3.34-3.33 (4H, m, CH₂(CH₃)₃), 2.87-2.78 (4H, m, CH₂P), 1.87 (6H, s, CH₃), 1.34 (3H, d, *J* = 7.2 Hz, CHCH₃), 1.26 (3H, d, *J* = 7.3 Hz, CHCH₃), 0.96 (18H, s, C(CH₃)₃). ¹³C-NMR (125 MHz, CD₃OD) δ_C: 175.49 (d, ³*J*_{PC} = 4.9 Hz, COO), 175.13 (d, ³*J*_{PC} = 3.7 Hz, COO), 158.23 (*C*-4), 166.88 (*C*-*ipso* PhOCH₃), 150.45 (*C*-2), 150.38 (d ²*J*_{CP} = 9.1 Hz,

C-ipso PhOP), 142.66 (CH-6), 142.64 (CH-6), 130.91 (d, $^2J_{PC}$ = 14.8 Hz, =CH-CH₂P), 130.90 (d, $^2J_{PC}$ = 14.86 Hz, =CHCH₂P), 122.92 (d, $^3J_{PC}$ = 4.8 Hz, CH-Ph), 122.75 (d, $^3J_{PC}$ = 3.7 Hz, CH-Ph), 115.66 (CH-Ph), 115.64 (CH-Ph), 111.45 (C-5), 111.42 (C-5), 50.12 (CHCH₃), 50.27 (CHCH₃), 56.11 (CH₂N), 56.08 (CH₂N), 32.56 (d, $^1J_{PC}$ = 129.3 Hz, CH₂P), 32.46 (d, $^1J_{PC}$ = 129.5 Hz, CH₂P), 30.90 (C(CH₃)₃), 26.74 (C(CH₃)₃), 26.73 (C(CH₃)₃), 21.45 (d, $^3J_{PC}$ = 5.6 Hz, CHCH₃), 20.85 (d, $^3J_{PC}$ = 4.9 Hz, CHCH₃), 12.29 (CH₃), 12.28 (CH₃) ³¹P-NMR (202 MHz, CD₃OD) δ_P 28.76, 29.35 ppm. HPLC: λ 254 nm, F = 1 mL/min, t_R = 15.14 min. (gradient from ACN/water 10:90 to 100% ACN in 30 minutes. MS(ESI+) *m/z* = 508.2 [M + H⁺].

(2S)-neopentyl2-((((E)-4-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-but-2-en-1-yl)(phenoxy)phosphoryl)amino) propanoate (14a and 14b) and (2S,2'S)-dineopentyl-2,2'-((((E)-4-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-but-2-en-1-yl)phosphoryl)bis(azanediyl))dipropanoate (21)

Compound **14a**, **14b** and **21** were prepared according to the general procedure from **11** (87 mg, 0.275 mmol, 1 eq.), TMSBr (210.6 mg, 1.38 mmol, 5 eq.) in dry ACN (10 mL), L-alanine-*O*-neopentyl ester tosylate (91.1 mg, 0.275 mmol, 1 eq.) and phenol (432.9 mg, 1.65 mmol, 6 eq.) triphenylphosphine (363.6 mg, 1.65 mmol, 6 eq.) 2-aldrithiol. (155.3 mg, 1.65 mmol, 6 eq.). After workup, the crude was purified by silica chromatography column using a gradient of DCM/MeOH (99:1 to 95:5 v/v) and providing preparative TLC (95:5 v/v) **14a** (21.6 mg, 16% yield). R_f(DCM/MeOH 95:5 v/v) = 0.32. ¹H-NMR (500 MHz, CD₃OD) δ_H 7.41 (1H, d, *J* = 1.1 Hz, *H*-6), 7.38-7.34 (2H, m, CH-Ph), 7.21-7.18 (3H, m, CH-Ph), 5.83-5.79 (2H, m, NCH₂CH= and =CHCH₂P), 4.36 (2H, t, *J* = 4.8 Hz, CH₂N), 4.05-3.99 (1H, m, CHCH₃), 3.87, 3.77 (2H, AB, *J*_{AB} = 10.5 Hz, CH₂C(CH₃)₃), 2.87 (2H, ddd, *J* = 6.4 and 4.6 Hz, $^2J_{PH}$ = 20.5 Hz, CH₂P), 1.87 (3H, d, *J* = 1.2 Hz, CH₃), 1.26 (3H, d, *J* = 7.3 Hz, CHCH₃), 0.96 (9H, s, C(CH₃)₃) ppm. ³¹C-NMR (125 MHz, CDCl₃) δ_C 174.05 ((d, $^3J_{PC}$ = 4.9 Hz, COO), 163.9 (C-4), 150.67 (C-2), 150.38 (d $^2J_{CP}$ = 9.1 Hz, *C-ipso* Ph), 139.74 (CH-6), 129.77 (CH-Ph), 129.47 (d, $^2J_{PC}$ = 10.7 Hz, =CHCH₂P), 129.21 (d, $^3J_{PC}$ = 14.7 Hz, NCH₂CH=), 124.64 (CH-Ph),

120.68 (d, $^3J_{PC}=4.5$ Hz, CH-Ph), 111.00 (C-5), 74.71 (CH₂C(CH₃)₃), 49.73 (CHCH₃), 49.49 (CH₂N), 32.44 (d, $^1J_{PC} = 127.2$ Hz, CH₂P), 29.69 (C(CH₃)₃), 26.32 (C(CH₃)₃), 21.53 (d $^3J_{PC} = 6.3$ Hz, CHCH₃), 10.87 (CH₃) ppm. ³¹P-NMR (202 MHz, CD₃OD) δ_P 29.23 ppm. HPLC: $\lambda = 280$ nm, F = 1 mL/min, $t_R = 16.06$ min. (gradient from ACN/water 10:90 to 100% ACN in 30 minutes. MS(ESI+) $m/z = 500.2$ [M + Na⁺] (100%).

Further elution with (DCM/MeOH 95:5 v/v) and preparative TLC (95:5 v/v) followed by preparative HPLC provided **14b** (13.2 mg, 10% yield). R_f (DCM/MeOH 95:5 v/v) = 0.29. ¹H-NMR (500 MHz, CD₃OD) δ_H 7.37 (1H, d, $J = 1.1$ Hz, H-6), 7.35-7.32 (2H, m, CH-Ph), 7.22-7.17 (3H, m, CH-Ph), 5.79-5.76 (2H, m, NCH₂CH= and =CHCH₂P), 4.35 (2H, m, CH₂N), 3.91, 3.82 (2H, AB, $J_{AB} = 10.5$ Hz, CH₂C(CH₃)₃), 3.67-3.60 (1H, m, CHCH₃), 2.86-2.80 (2H, m, CH₂P), 1.87 (3H, s, CH₃), 1.38 (3H, d, $J = 7.2$ Hz, CHCH₃), 0.99 (9H, s, C(CH₃)₃) ppm. ³¹C-NMR (125 MHz, CDCl₃) δ_C 173.79 (d, $^3J_{PC} = 4.9$ Hz, COO), 164.01 (C-4), 150.72 (C-2), 150.43 (d, $^2J_{CP} = 7.84$ Hz, C-*ipso* Ph), 139.66 (CH-6), 129.77 (CH-Ph), 129.24 (d, $^2J_{PC} = 14.7$ Hz, =CHCH₂P), 125.16 (d, $^2J_{PC} = 11.0$ Hz, NCH₂CH=), 124.86 (CH-Ph), 120.47 (d, $^3J_{PC} = 4.9$ Hz, CH-Ph), 111.05 (C-5), 74.78 (CH₂C(CH₃)₃), 49.62 (CHCH₃), 49.23 (CH₂N), 32.79 (d, $^1J_{PC} = 130.8$ Hz, CH₂P), 29.70 (C(CH₃)₃), 29.36 (C(CH₃)₃), 21.79 (d, $^3J_{PC} = 2.5$ Hz, CHCH₃), 12.30 (CH₃) ppm. ³¹P-NMR (202 MHz, CDCl₃) δ_P 28.51 ppm. HPLC: $\lambda = 280$ nm, F = 1 mL/min, $t_R = 16.14$ min (gradient from ACN/water 10:90 to 100% ACN in 30 minutes). MS(ESI+) $m/z = 500.2$ [M + Na⁺] (100%).

Further elution with a gradient DCM/MeOH (95:5 to 93:7 v/v) and preparative TLC (95:5 v/v) afforded **21** (34.9 mg, yield=25%). R_f (DCM/MeOH 95:5 v/v) = 0.20. ¹H-NMR (500 MHz, CD₃OD) δ_H 7.30 (1H, d, $J = 1.2$ Hz, H-6), 5.69-5.58 (2H, m, NCH₂CH= and =CHCH₂P), 4.38-4.28 (2H, m, CH₂N), 4.06-4.96 (2H, m, CHCH₃), 3.78, 3.76 (2H, AB, $J_{AB} = 10.5$ Hz, CH₂C(CH₃)₃), 3.68, 3.65 (2H, AB, $J_{AB} = 10.5$ Hz, CH₂C(CH₃)₃), 2.71-2.58 (2H, m, CH₂P), 1.78 (3H, d, $J = 1.2$ Hz, CH₃), 1.42 (3H, d, $J = 7.0$ Hz, CHCH₃), 1.40 (3H, d, $J = 7.0$ Hz, CHCH₃), 0.86

(9H, s, C(CH₃)₃), 0.85 (9H, s, C(CH₃)₃) ppm. ³¹C-NMR (125 MHz, CD₃OD) δ_c 174.54 (d, ³J_{PC} = 4.9 Hz, COO), 174.43 (d, J = 4.0 Hz, COO), 165.51 (C-4), 151.47 (C-2), 141.31 (CH-6), 128.67 (d, ²J_{PC} = 13.0 Hz, =CHCH₂P), 125.29 (d, ³J_{PC} = 10.2 Hz, NCH₂CH=), 110.03 (C-5), 74.03 (CH₂C(CH₃)₃), 73.96 (CH₂C(CH₃)₃), 48.85 (CH₂N), 48.80 (CHCH₃), 48.50 (CHCH₃), 32.93 (d, ¹J_{PC} = 110.2 Hz, CH₂P), 30.90 (C(CH₃)₃), 25.40 (C(CH₃)₃), 20.13 (d, ³J_{PC} = 6.3 Hz, CHCH₃), 19.92 (d, ³J_{PC} = 5.6 Hz, CHCH₃), 10.94 (CH₃) ppm. ³¹P-NMR (202 MHz, CD₃OD) δ_p 27.35 ppm. HPLC: λ = 280 nm, F = 1 mL/min, t_R = 17.59 min (gradient from ACN/water 10:90 to 100% ACN in 30 minutes). MS(ESI+) m/z = 543.3 [M + H⁺] (50%), m/z = 565.3 [M + Na⁺] (100%).

(2S)-neopentyl-2-(((E)-4-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-but-2-en-1-yl)(naphthalen-1-yloxy)phosphoryl) amino)propanoate (15)

Compound **15** was prepared according to general procedure from **11** (216 mg, 0.752 mmol, 1 eq.) TMSBr (575.99 mg, 0.49 mL, 3.76 mmol, 5 eq.) (in dry ACN (10 mL), 1-naphthol (650.9 mg, 4.51 mmol, 6 eq.), L-alanine-*O*-neopentyl ester tosylate (249.39 mg, 0.752 mmol, 1 eq.), triphenylphosphine (262.3 mg, 4.51 mmol, 6 eq.) and 2-aldrithiol (994.6 mg, 4.51 mmol, 6 eq.). After workup, the crude was purified by silica chromatography column using a gradient of DCM/MeOH (99:1 to 95:5 v/v) and providing after preparative TLC (95:5 v/v) **15** (26 mg, 7% yield). R_f (DCM/MeOH 95:5 v/v) = 0.39. ¹H-NMR (500 MHz, CD₃OD) δ_H 8.02 (2H, d, J = 6.02 Hz, CH-Naph), 7.77 (1H, d, J = 6.02 Hz, CH-Naph), 7.76 (1H, d, J = 6.0 Hz, CH-Naph), 7.58 (2H, dd, J = 8.0, J = 1.0 Hz, CH-Naph), 7.43-7.21 (8H, m, CH-Naph), 7.21 (2H, m, H-6), 5.80-5.65 (4H, m, NCH₂CH= and =CHCH₂P), 4.23-4.17 (4H, m, CH₂N), 4.02-3.92 (2H, m, CHCH₃), 3.72, 3.58 (2H, AB, J_{AB} = 10.5 Hz, CH₂C(CH₃)), 3.60, 3.56 (2H, AB, J_{AB} = 10.5 Hz, CH₂C(CH₃)), 2.94-2.82 (4H, m, CH₂P), 1.65 (3H, d, J = 1.2 Hz, CH₃), 1.18 (3H, d, J = 6.8 Hz, CHCH₃), 1.10 (3H, d, J = 7.2 Hz, CHCH₃), 0.81 (9H, s, C(CH₃)₃), 0.76 (9H, s, C(CH₃)₃) ppm. ³¹C-NMR (125 MHz, CDCl₃) δ_c 174.02 (d, J = 4.2 Hz, COO), 173.57 (d, J = 4.8 Hz, COO), 165.41 (C-4), 165.39 (C-4), 151.44 (C-2), 151.35 (C-2), 146.45 (d, ²J_{PC} = 9.3 Hz, C-*ipso* Naph), 146.31 (d, ²J_{PC} = 9.3 Hz, C-*ipso* Naph), 141.12 (CH-

6), 141.08 (CH-6), 134.91 (C-Naph), 129.68 (d, $^2J_{PC} = 14.9$ Hz, =CHCH₂P), 129.68 (d, $^2J_{PC} = 14.9$ Hz, =CHCH₂P), 127.46, 127.45, (CH-Naph), 126.72 (d, $^3J_{PC} = 5.6$ Hz, C-Naph), 126.59 (d, $^3J_{PC} = 5.6$ Hz C-Naph), 126.36, 126.05 (CH-Naph), 125.13 (d, $^3J_{PC} = 8.4$ Hz, CH-Naph), 124.34 (d, $J = 8.3$ Hz, CH-Naph), 123.91 (d, $^3J_{PC} = 11.0$ Hz, NCH₂CH=), 123.69 (d, $^3J_{PC} = 11.0$ Hz, NCH₂CH=), 121.44 (CH-Naph), 121.31 (CH-Naph), 115.34 (d, $^3J_{PC} = 3.7$ Hz, CH-Naph), 115.11 (d, $^3J_{PC} = 4.2$ Hz, CH-Naph), 110.07 (C-5), 73.88 (CH₂C(CH₃)₃), 73.92 (CH₂C(CH₃)₃), 49.61 (CHCH₃), 49.44 (CHCH₃), 48.78 (CH₂N), 48.67 (CH₂N), 32.22 (d, $^1J_{PC} = 129.6$ Hz, CH₂P), 31.97 (d, $^1J_{PC} = 129.6$ Hz, CH₂P), 30.92 (C(CH₃)₃), 31.97 (C(CH₃)₃), 25.34 (C(CH₃)₃), 25.39 (C(CH₃)₃), 19.94 (d, $^3J_{PC} = 5.1$ Hz, CHCH₃), 19.27 (d, $^3J_{PC} = 5.1$ Hz, CHCH₃), 10.84 (CH₃) ppm. ³¹P-NMR (202 MHz, CDCl₃) δ_P 29.51, 28.86, ppm. HPLC: $\lambda = 280$ nm, F = 1 mL/min, $t_R = 18.23$ min (gradient from ACN/water 10:90 to 100% ACN in 30 minutes,). MS(ESI+) $m/z = 550.2$ [M + Na⁺] (100%).

(2S)-benzyl-2-((((E)-4-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-but-2-en-1-yl))((5,6,7,8-tetrahydronaphthalen-1-yl)oxy)phosphoryl)amino)propanoate (16)

Compound **16** was prepared according to the general procedure, from **11** (116.30 mg, 0.45 mmol, 1 eq.), TMSBr (342.16 mg, 0.30 mL, 2.23 mmol, 5 eq.), dry ACN (5 mL) aldrithiol-2 (598.9 mg, 2.72 mmol, 6 eq.) 5,6,7,8-tetrahydro-1-naphthol (402.9 mg, 2.72 mmol, 6 eq.) L-alanine-*O*-benzyl ester tosylate (159.2 mg, 0.453 mmol, 1 eq.) and triphenylphosphine (713.1 mg, 2.72 mmol, 6 eq.). Then the mixture has been stirred for 5 hours at 50°C. After workup, the crude was purified by silica chromatography column using a gradient of DCM/MeOH (99:1 to 95:5 v/v) and providing after preparative TLC (95:5 v/v) followed by preparative HPLC **16** (21 mg, 8% yield). R_f (DCM/MeOH 95:5 v/v) = 0.25. ¹H-NMR (500 MHz, CDCl₃) δ_H 8.89 (1H, bs, NH), 7.33-7.22 (5H, m, CH-Ar), 7.10 (1H, d, $J = 8.7$ Hz, CH-TetrahydroNaph), 6.91 (1H, d, $J = 1.0$ Hz, H-6), 6.90 (1H, $J = 8.7$ Hz, CH-TetrahydroNaph), 6.76 (1H, $J = 8.1$ Hz, CH-TetrahydroNaph), 5.73-

5.42 (2H, m, NCH₂CH= and =CHCH₂P), 5.05–4.99 (2H, m, CH₂Ph), 4.18 (2H, t, *J* = 5.3 Hz, CH₂N), 4.02–3.94 (1H, m, CHCH₃), 2.69–2.64 (4H, m, CH₂-TetrahydroNaph and CH₂P), 2.56–2.52 (2H, m, CH₂-TetrahydroNaph), 1.80 (3H, s, CH₃), 1.67–1.66 (4H, m, CH₂-TetrahydroNaph), 1.25 (3H, d, *J* = 7.9 Hz, CHCH₃). ³¹C-NMR (125 MHz, CDCl₃) δ_C 173.62 (d, *J* = 5.9 Hz, COO), 164.14 (C-4), 150.80 (C-2), 148.75 (d, ²*J*_{PC} = 8.8 Hz, C-*ipso* TetrahydroNaphOP), 139.59 (CH-6), 139.49 (C-*ipso* CH₂Ph), 135.23 (C-TetrahydroNaph), 129.16 (d, ²*J*_{PC} = 13.7 Hz, =CHCH₂P), 128.65, 128.54, 128.22 (CH-Ar), 128.10 (d, ²*J*_{PC} = 4.8 Hz, C-TetrahydroNaph), 125.92, 125.51 (CH-Ar), 125.26, (d, ²*J*_{PC} = 10.97 Hz, NCH₂CH=) 116.83 (d, ³*J*_{PC} = 3.3 Hz, CH-TetrahydroNaph), 110.02 (C-5), 67.25 (CH₂Ph), 49.69 (d, ²*J*_{PC} = 4.3 Hz, CHCH₃), 49.20 (CH₂N), 32.91 (d, ¹*J*_{PC} = 129.6 Hz, CH₂P), 29.51 (CH₂-TetrahydroNaph), 23.72 (CH₂-TetrahydroNaph), 22.22 (2 x CH₂-TetrahydroNaph), 21.53 (d, ³*J*_{PC} = 4 Hz, CHCH₃), 12.25 (CH₃) ppm. ³¹P-NMR (202 MHz, CDCl₃) δ_P 29.70, 29.19, ppm. HPLC: λ = 280 nm, F = 1 mL/min, t_R = 17.90 min (gradient from ACN/water 10:90 to 100% ACN in 30 minutes). MS (ESI+) *m/z* = 574.2 [M + Na⁺] (100%).

(2S)-benzyl2-(((E)-4-(5-bromo-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-but-2-en-1-yl)(phenoxy) phosphoryl)amino)propanoate (17a and 17b) and (2S)-benzyl-2-(((E)-4-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-but-2-en-1-yl)(phenoxy)phosphoryl)amino)propanoate (20a and 20b)

Compounds **17a**, **17b**, **20a** and **20b** were prepared according to the general procedure, from **12** (309.0 mg, 0.811 mmol, 1 eq.) TMSBr (620.6 mg, 4.05 mmol, 0.53 mL, 5 eq.) dry ACN (10 mL) Aldrithiol-2 (1.07 g, 4.86 mmol, 6 eq.) phenol (457.8 mg, 4.86 mmol, 6 eq.) L-alanine-*O*-benzyl ester tosylate (284.9 mg, 0.811 mmol, 1 eq.) of, triphenylphosphine (1.28 g, 4.86 mmol, 6 eq.) After workup, the crude was purified by silica chromatography column using a gradient of DCM/MeOH (99:1 to 95:5 v/v) and providing after preparative TLC (95:5 v/v) **17a** (17 mg, 4% yield). R_f (DCM/MeOH 95:5 v/v) = 0.30. ¹H-NMR (500 MHz, CD₃OD) δ_H 7.97 (1H, s, *H*-6), 7.96 (1H, s, *H*-6), 7.39–7.29 (14H, m, CH-Ar), 7.20–7.15

(6H, m, CH-Ph), 5.84-5.66 (4H, m, NCH₂CH= and =CHCH₂P), 5.16, 5.12 (2H, d, AB J_{AB} = 10.5 Hz, CH₂Ph), 5.092 (2H, s, CH₂Ph), 4.37-4.29 (4H, m, CH₂N), 4.08-4.00 (2H, m, CHCH₃), 2.87-2.75 (4H, m, CH₂P), 1.30 (3H, d, J = 7.1 Hz, CHCH₃), 1.24 (3H, d, J = 6.9 Hz, CHCH₃) ppm. ³¹C-NMR (125 MHz, CD₃OD) δ_c 173.78 (d, $^3J_{PC}$ = 4.8 Hz, COO), 173.40 (d, $^3J_{PC}$ = 4.8 Hz, COO), 160.71 (C-4), 160.69 (C-4), 150.73 (C-2), 150.63 (C-2), 150.48 (d, $^2J_{PC}$ = 9.2 Hz, C-*ipso* Ph), 150.31 (d, $^2J_{PC}$ = 9.2 Hz, C-*ipso* Ph), 144.85 (C-6), 135.89 (C-*ipso* CH₂Ph), 135.86 (C-*ipso* CH₂Ph), 129.35 (CH-Ar), 129.02 (CH-Ar), 129.10 (d, $^2J_{PC}$ = 13.5 Hz, =CHCH₂P), 128.98 (d, $^3J_{PC}$ = 14.2 Hz, =CHCH₂P), 128.25 (CH-Ar), 128.22 (CH-Ar), 128.05 (CH-Ar), 128.03 (CH-Ar), 128.01 (CH-Ar), 127.97 (CH-Ar), 124.73 (CH-Ar), 124.66 (CH-Ar), 124.50 (d, $^3J_{PC}$ = 9.04 Hz, NCH₂CH=), 124.40 (d, $^3J_{PC}$ = 10.04 Hz, NCH₂CH=), 120.72 (d, $^3J_{PC}$ = 4.3 Hz, CH-Ph), 120.50 (d, $^3J_{PC}$ = 4.5 Hz, CH-Ph), 95.35 (C-5), 66.60 (CH₂Ph), 66.54 (CH₂Ph), 49.58 (CHCH₃), 49.49 (CHCH₃), 49.40 (CH₂N), 49.28 (CH₂N), 31.99 (d, $^1J_{PC}$ = 128 Hz, CH₂P), 31.85 (d, $^1J_{PC}$ = 128 Hz, CH₂P), 19.71 (d, $^3J_{PC}$ = 5.4 Hz, CHCH₃), 19.10 (d, $^3J_{PC}$ = 5.6 Hz, CHCH₃) ppm. ³¹P-NMR (202 MHz, CD₃OD) δ_p 29.13 (0.67P), 28.36 (0.33P). HPLC: λ = 280 nm, F = 1 mL/min, t_R = 16.26 min (gradient from ACN/water 10:90 to 100% ACN in 30 minutes). MS (ESI+) m/z = 584.0 [M + Na⁺] (100%), 586.0 [M + Na⁺] (98%).

Further elution with (DCM/MeOH 95:5 v/v) provided after preparative TLC (95:5 v/v) followed by preparative HPLC **17b** (11.0 mg, 3% yield). R_f (DCM/MeOH 95:5 v/v) = 0.29. ¹H-NMR (500 MHz, CD₃OD) δ_H 7.97 (1H, s, H-6), 7.38-7.30 (7H, m, CH-Ar), 7.21-7.15 (3H, m, CHPh), 5.81-5.69 (2H, m, NCH₂CH= and =CHCH₂P), 5.09 (2H, s, CH₂Ph), 4.38-4.31 (2H, m, CH₂N), 4.06-4.00 (1H, m, CHCH₃), 2.86-2.75 (2H, m, CH₂P), 1.30 (3H, d, J = 6.9 Hz, CHCH₃) ppm. ³¹C-NMR (125 MHz, CD₃OD) δ_c 175.44 (d, $^3J_{PC}$ = 3.7 Hz, COO), 162.82 (C-4), 152.72 (C-2), 152.34 (d, $^2J_{PC}$ = 9.3 Hz, C-*ipso* Ph), 146.81 (CH-6), 137.72 (C-*ipso* CH₂Ph), 131.31 (CH-Ar), 131.01 (d, $^2J_{PC}$ = 14.5 Hz, =CHCH₂P), 130.14 (CH-Ar), 129.93 (CH-Ar), 129.91 (CH-Ar), 126.55 (CH-Ar), 126.34 (d, $^3J_{PC}$ = 11.03 Hz, NCH₂CH=), 122.41 (d, $^3J_{PC}$ = 4.8 Hz, CH-Ph), 97.35 (C-5), 68.51 (CH₂Ph), 51.42 (CHCH₃), 51.24 (CH₂N), 33.73 (d, J_{PC} = 129.0

Hz, CH₂P), 21.61 (d, ³J_{PC} = 5.7 Hz, CHCH₃) ppm. ³¹P-NMR (202 MHz, CD₃OD) δ_P 28.49 ppm. HPLC: λ = 280 nm, F = 1 mL/min, t_R = 16.42 min (gradient from ACN/water 10:90 to 100% ACN in 30 minutes). MS (ESI+) m/z = 584.0 [M + Na⁺] (100%), 586.0 [M + Na⁺] (98%).

Further elution with (DCM/MeOH 94:6 v/v) provided after preparative TLC (95:5 v/v) followed by preparative HPLC **20a** (10.7 mg, 3% yield). R_f (DCM/MeOH 95:5 v/v) = 0.25. ¹H-NMR (500 MHz, CD₃OD) δ_H 7.52 (1H, d, J = 7.9 Hz, H-6), 7.39-7.32 (7H, m, CH-Ar), 7.21-7.16 (3H, m, CH-Ph), 5.82-5.68 (2H, m, NCH₂CH= and =CHCH₂P), 5.66 (1H, d, J = 7.9 Hz, H-5), 5.04, 5.01 (2H, AB J_{AB} = 8 Hz, CH₂Ph), 4.32 (2H, t, J = 4.5 Hz, CH₂N), 4.04 (1H, m, CHCH₃), 2.81 (2H, m, CH₂P), 1.23 (3H, d, J = 7.3 Hz, CHCH₃) ppm. ³¹C-NMR (125 MHz, CD₃OD) δ_C 173.86 (d, ³J_{PC} = 4.0 Hz, COO), 165.34 (C-4), 151.33 (C-2), 150.32 (d, ²J_{PC} = 9.5 Hz, C-*ipso* Ph), 145.44 (CH-6), 135.91 (C-*ipso* CH₂Ph), 129.31 (CH-Ar), 129.22 (d, ²J_{PC} = 14.1 Hz, =CHCH₂P), 128.25 (CH-Ar), 128.02 (CH-Ar), 124.66 (CH-Ar), 124.25 (d, ³J_{PC} = 11.2 Hz, NCH₂CH=), 120.67 (d, ³J_{PC} = 4.4 Hz, CH-Ph), 101.14 (C-5), 66.60 (CH₂Ph), 49.48 (CHCH₃), 49.08 (d, J_{PC} = 2.6 Hz, CH₂N), 33.51 (d, ¹J_{PC} = 129.5 Hz, CH₂P), 19.07 (d, ³J_{PC} = 5.5 Hz, CHCH₃) ppm. ³¹P-NMR (202 MHz, CD₃OD) δ_P 29.58 ppm. HPLC: λ = 280 nm, F = 1 mL/min, t_R = 14.32 minutes (gradient from ACN/water 10:90 to 100% ACN in 30 minutes). MS (ESI+) m/z = 506.1 [M + Na⁺] (100%).

Further elution with (DCM/MeOH 94:6 v/v) provided after preparative TLC (95:5 v/v) followed by preparative HPLC **20b** (5.9 mg, 2% yield). R_f (DCM/MeOH 95:5 v/v) = 0.22. ¹H-NMR (500 MHz, CD₃OD) δ_H 7.41 (1H, d, J = 7.9 Hz, H-6), 7.23-7.18 (7H, m, CH-Ar), 7.08-7.05 (3H, m, CH-Ph), 5.65-5.61 (2H, m, NCH₂CH= and =CHCH₂P), 5.54 (1H, d, J = 7.9 Hz, H-5), 5.09 (2H, s, CH₂Ph), 4.23-4.21 (2H, m, CH₂N), 3.94-3.89 (1H, m, CHCH₃), 2.71-2.65 (2H, m, CH₂P), 1.18 (3H, d, J = 6.9 Hz, CHCH₃) ppm. ³¹C-NMR (125 MHz, CD₃OD) δ_C 173.86 (d, ³J_{PC} = 4.5 Hz, COO), 165.34 (C-4), 151.33 (C-2), 150.32 (d, ²J_{PC} = 10 Hz, C-*ipso* Ph), 145.70 (CH-6), 135.91 (C-*ipso* CH₂Ph), 129.66 (CH-Ar), 129.58 (d, ²J_{PC} = 14.8 Hz, =CHCH₂P), 128.52 (CH-

Ar), 128.29 (CH-Ar), 124.87 (CH-Ar), 124.40 (d, $^3J_{PC} = 10.1$ Hz, NCH₂CH=), 120.74 (d, $^3J_{PC} = 4.6$ Hz, CH-Ph), 101.43 (C-5), 66.79 (CH₂Ph), 49.68 (CHCH₃), 49.28 (d, $^4J_{PC} = 2.1$ Hz, CH₂N), 32.14 (d, $^1J_{PC} = 129.5$ Hz, CH₂P), 19.97 (d, $^3J_{PC} = 5.2$ Hz, CHCH₃) ppm. ³¹P-NMR (202 MHz, CD₃OD) δ_P 28.40 ppm. HPLC: $\lambda = 280$ nm, F = 1 mL/min, $t_R = 14.51$ min (gradient from ACN/water 10:90 to 100% ACN in 30 minutes). MS (ESI+) $m/z = 506.1$ [M + Na⁺] (100%).

(2S)-benzyl-2-((((E)-4-(5-bromo-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-but-2-en-1-yl)(naphthalen-1-yloxy)phosphoryl)amino)propanoate (18) and

(2S,2'S)- dibenzyl 2,2'-((((E)-4-(5-bromo-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-but-2-en-1-yl) phosphoryl)bis(azanediyl))dipropanoate (23)

Compounds **18** and **23** was prepared according to general procedure from **11** (240.0 mg, 0.629 mmol, 1 eq.) of TMSBr (481.9 mg, 3.15 mmol, 0.42 mL, 5 eq.) dry ACN (10 mL) aldrithiol-2 (0.832 g, 3.78 mmol, 6 eq.) naphthol (544.7 mg, 3.78 mmol, 6 eq.) L-alanine-*O*-benzyl ester tosylate (663.9 mg, 1.89 mmol, 3 eq.), triphenylphosphine (0.990 g, 3.78 mmol, 6 eq.) After workup, the crude was purified by silica chromatography column using a gradient of DCM/MeOH (99:1 to 95:5 v/v) and preparative TLC (95:5 v/v) providing **18** (50 mg, 13% yield). R_f (DCM/MeOH 95:5 v/v) = 0.29 – 0.26. ¹H-NMR (500 MHz, CD₃OD) δ_H 8.12 (2H, m, CH-Naph), 7.89-7.87 (4H, m, CH-Naph), 7.67 (2H, d, $J = 8.8$ Hz, CH-Naph), 7.55-7.50 (6H, m, CH-Naph), 7.37-7.26 (12H, m, H-6 and CH-Ph), 5.85-5.74 (2H, m, NCH₂CH=) 5.74-5.70 (2H, m, =CHCH₂P), 5.14-4.95 (4H, m, CH₂Ph), 4.29 (4H, bs, CH₂N), 4.01-4.06 (2H, m, CHCH₃), 2.95-2.91 (4H, m, CH₂P), 1.27 (3H, d, $J = 7.1$ Hz, CHCH₃), 1.19 (3H, d, $J = 7.1$ Hz, CHCH₃) ppm. ³¹C-NMR (125 MHz, CD₃OD) δ_C 173.30 (d, $^3J_{PC} = 3.8$ Hz, COO), 160.66 (C-4), 150.58 (C-2), 146.42 (d, $^2J_{PC} = 9.6$ Hz, C-*ipso* Naph), 144.74 (CH-6), 135.78 (C-*ipso* CH₂Ph), 134.89 (C-Naph), 129.21 (d, $^2J_{PC} = 14.4$ Hz, =CHCH₂P), 128.13, 127.89, 127.50 (CH-Ar) 126.59 (d, $^3J_{PC} = 4.9$ Hz, C-Naph), 126.35 (d, $^3J_{PC} = 10.8$ Hz, NCH₂CH=), 125.15, 124.37, 124.35, 121.30 (CH-Ar), 115.21 (d, $^3J_{PC} = 4.3$ Hz, CH-Naph), 95.39 (C-5), 66.47 (CH₂Ph), 49.51 (CHCH₃), 49.27 (d, $^4J_{PC} = 1.6$ Hz, CH₂N), 32.15 (d, $^1J_{PC} = 129.2$ Hz, CH₂P), 31.95

(d, $^1J_{PC} = 129.2$ Hz, CH_2P), 19.62 (d, $^3J_{PC} = 6.8$ Hz, $CHCH_3$) 19.44 (d, $^3J_{PC} = 6.8$ Hz, $CHCH_3$) ppm. ^{31}P -NMR (202 MHz, CD_3OD) δ_P 26.84, 26.46 ppm. HPLC: $\lambda = 280$ nm, $F = 1$ mL/min, $t_R = 16.50$ (gradient from ACN/water 10:90 to 100% ACN in 30 minutes). MS (ESI+) $m/z = 636.1$ [$M + Na^+$](100%), 638.1 [$M + Na^+$](98%).

Further elution with (DCM/MeOH 95:5 v/v) provided after preparative TLC (95:5 v/v) **23** (7.0 mg, 2% yield). R_f (DCM/MeOH 95:5 v/v) = 0.16. 1H -NMR (500 MHz, CD_3OD) δ_H 7.84 (1H, s, $H-6$), 7.27-7.23 (10H, m, CH -Ph), 5.66-5.52 (2H, m, $NCH_2CH=$ and $=CHCH_2P$), 4.19-4.16 (2H, m, CH_2N), 3.90-3.83 (2H, m, $CHCH_3$), 2.53-2.39 (2H, m, CH_2P), 1.27 (3H, d, $J = 7.2$ Hz, $CHCH_3$), 1.19 (3H, d, $J = 7.2$ Hz, $CHCH_3$) ppm. ^{13}C -NMR (125 MHz, CD_3OD) δ_C 174.23 (d, $^3J_{PC} = 4.7$ Hz, COO), 174.17 (d, $^3J_{PC} = 4.3$ Hz, COO), 160.78 ($C-4$), 150.75 ($C-2$), 144.86 ($CH-6$), 135.96 (C -*ipso* CH_2Ph), 135.93 (C -*ipso* CH_2Ph), 128.24 (CH -Ph), 128.11 (d, $^2J_{PC} = 14.2$ Hz, $=CHCH_2P$), 128.07 (CH -Ph), 128.00, (CH -Ph), 126.03 (d, $^3J_{PC} = 10.4$ Hz, $=CHCH_2N$), 95.40 ($C-5$), 66.63 (CH_2Ph), 66.60 (CH_2Ph), 49.12 (d, $^4J_{PC} = 2.1$ Hz, CH_2N), 48.91 ($CHCH_3$), 48.51 ($CHCH_3$), 33.88 (d, $^1J_{PC} = 110.7$ Hz, CH_2P), 19.73 (d, $^3J_{PC} = 5.9$ Hz, $CHCH_3$), 19.66 (d, $^3J_{PC} = 6.0$ Hz, $CHCH_3$) ppm. ^{31}P -NMR (202 MHz, CD_3OD) δ_P 23.95 ppm. HPLC: $\lambda = 280$ nm, $F = 1$ mL/min, $t_R = 16.53$ minutes (gradient from ACN/water 10:90 to 100% ACN in 30 minutes). MS (ESI+) $m/z = 671.7$ [$M + Na^+$] (98%), 669.1 [$M + Na^+$] (100%).

(2S)-neopentyl-2-((((E)-4-(5-bromo-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-but-2-en-1-yl)(phenoxy)phosphoryl)amino) propanoate (19) and (2S,2'S)-dineopentyl-2,2'-((((E)-4-(5-bromo-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)but-2-en-1-yl)phosphoryl)bis(azanediy))dipropanoate (22)

Compound **19** and **22** were prepared according to the general procedure from **12** (316.0 mg, 0.892 mmol, 1 eq.) TMSBr (682.8 mg, 4.46 mmol, 0.59 mL, 5 eq.) dry ACN (10 mL) aldrithiol-2 (1.18 g, 5.35 mmol, 6 eq.) phenol (503.7 mg, 5.35 mmol, 6 eq.) of, L-alanine-*O*-neopentyl ester tosylate (886.9.9 mg, 2.68 mmol, 3 eq.) and triphenylphosphine (1.40 g, 5.35 mmol, 6 eq.). After workup, the crude was purified by silica chromatography

column using a gradient of DCM/MeOH (99:1 to 95:5 v/v) and providing after preparative TLC (95:5 v/v) followed by preparative HPLC **19** (77 mg, 16% yield). R_f (DCM/MeOH 95:5 v/v) = 0.20. $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ_{H} 7.53 (1H, s, *H*-6), 7.23-7.20 (2H, m, *CH*-Ph), 7.13-7.11 (2H, m, *CH*-Ph), 7.06-7.02 (1H, m, *CHPh*), 5.84-5.76 (1H, m, =*CHCH*₂P), 5.68-5.61 (1H, m, *NCH*₂*CH*=), 4.27 (2H, s, *CH*₂N), 4.11-4.09 (1H, m, *NH*), 4.04-3.97 (1H, m, *CHCH*₃), 3.76, 3.65 (2H, AB, $J_{\text{AB}} = 10.5$ Hz, *CH*₂*C(CH*₃)₃), 2.78-2.75 (2H, m, *CH*₂P), 1.26 (3H, d, $J = 7.4$ Hz, *CHCH*₃), 0.84 (9 H, s, *C(CH*₃)₃) ppm. $^{31}\text{C-NMR}$ (125 MHz, CDCl_3) δ_{C} 173.93 (d, $^3J_{\text{PC}} = 5.2$ Hz, *COO*), 159.76 (*C*-4), 150.42 (d, $^2J_{\text{PC}} = 9.2$ Hz, *C-ips*o Ph), 150.34 (*C*-2), 143.27 (*CH*-6), 129.75 (*CH*-Ph), 128.58 (d, $^2J_{\text{PC}} = 14.2$ Hz, =*CHCH*₂P), 126.16 (d, $^3J_{\text{PC}} = 10.3$ Hz, *NHCH*₂*CH*=), 124.82 (*CH*-Ph), 120.47 (d, $^3J_{\text{PC}} = 4.3$ Hz, *CH*-Ph), 96.65 (*C*-5), 74.75 (*CH*₂*C(CH*₃)₃), 49.68 (*CH*₂N), 49.66 (*CHCH*₃), 32.60 (d, $^1J_{\text{PC}} = 128.5$ Hz, *CH*₂P), 30.90 (*C(CH*₃)₃), 26.34 (*C(CH*₃)₃), 21.60 (d, $^3J_{\text{PC}} = 4$ Hz, *CHCH*₃) ppm. $^{31}\text{P-NMR}$ (202 MHz, CDCl_3) δ_{P} 26.25 ppm. HPLC: $\lambda = 280$ nm $F = 1$ mL/min, $t_{\text{R}} = 17.24$ min (gradient from ACN/water 10:90 to 100% ACN in 30 minutes). MS (ESI+) $m/z = 566.1$ [*M* + *Na*⁺] (98%), 564.1 [*M* + *Na*⁺], (100%)

Further elution with (DCM/MeOH 95:5 v/v) provided after preparative TLC (95:5 v/v) **22** (5.3 mg, 1% yield). R_f (DCM/MeOH 95:5 v/v) = 0.16. $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ_{H} 7.53 (1H, s, *H*-6), 5.66-5.59 (1H, m, *NCH*₂*CH*=), 5.25- 5.11(1H, m, =*CHCH*₂P), 4.29-4.27 (2H, m, *CH*₂N), 4.04-3.99 (2H, m, *CHCH*₃), 3.82, 3.66 (2H, AB, $J_{\text{AB}} = 10.4$ Hz, *CH*₂*C(CH*₃)₃), 3.81, 3.71 (2H, AB, $J_{\text{AB}} = 10.4$ Hz, *CH*₂*C(CH*₃)₃), 3.15-3.07 (2H, m, *NH*), 2.57 (2H, dd, $J = 7.6$, $^2J_{\text{PH}} = 19.6$ Hz, *CH*₂P), 1.37 (3H, d, $J = 7.2$ Hz, *CHCH*₃), 1.34 (3H, d, $J = 7.2$ Hz, *CHCH*₃), 0.87 (9H, s, *C(CH*₃)₃), 0.86 (9H, s, *C(CH*₃)₃) ppm. $^{31}\text{C-NMR}$ (125 MHz, CDCl_3) δ_{C} 174.84 (d, $^3J_{\text{PC}} = 5.1$ Hz, *COO*), 174.75 (d, $^3J_{\text{PC}} = 5.1$ Hz, *COO*), 159.26 (*C*-4), 150.01 (*C*-2), 143.25 (*CH*-6), 128.11 (d, $^2J_{\text{PC}} = 9.9$ Hz, =*CHCH*₂P), 127.58 (d, $^3J_{\text{PC}} = 13.8$ Hz, *NCH*₂*CH*=), 96.73 (*C*-5), 74.82 (*CH*₂(*CH*₃)₃), 74.76 (*CH*₂(*CH*₃)₃), 49.99 (*CH*₂N), 49.00 (*CHCH*₃), 48.55 (*CHCH*₃), 34.26 (d, $^1J_{\text{PC}} = 109.1$ Hz, *CH*₂P), 31.46 (*C(CH*₃)₃), 26.34 (*C(CH*₃)₃), 21.71 (d, $^3J_{\text{PC}} = 4.8$ Hz, *CHCH*₃), 21.51 (d, $^3J_{\text{PC}} = 4.9$ Hz, *CHCH*₃) ppm. $^{31}\text{P-NMR}$ (202 MHz, CD_3OD) δ_{P} 23.68 ppm. HPLC: $\lambda = 280$ nm, $F = 1$

mL/min, $t_R = 18.48$ min (gradient from ACN/water 10:90 to 100% ACN in 30 minutes).

MS (ESI+) $m/z = 629.1$ [M + Na⁺] (100%), 631.1 [M + Na⁺] (98%).