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Expedient synthesis and biological evaluation of alkenyl acyclic nucleoside phosphonate prodrugs

Elisa Pileggi[a], Michaela Serpi[a], Graciela Andrei[b], Dominique Schols[b], Robert Snoeck[b], Fabrizio Pertusati[a,*]

[a] School of Pharmacy and Pharmaceutical Sciences, Redwood building, King Edwards VII Avenue, CF10 3NB Cardiff, Wales, United Kingdom
[b] Rega Institute for Medical Research, K.U. Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

ABSTRACT

The importance of phosphonoamidate prodrugs (ProTides) of acyclic nucleoside phosphonate (ANPs) is highlighted by the approval of Tenofovir Alafenamide Fumarate for the treatment of HIV and HBV infections. In the present paper we are reporting an expedient, one-pot, two-steps synthesis of allyl phosphonoamidates and diamidates that offers a time saving strategy when compared to literature methods. The use of these substrates in the cross metathesis reactions with alkenyl functionalised thymine and uracil nucleobases is reported. ANPs prodrugs synthesized via this methodology were evaluated for their antiviral activities against DNA and RNA viruses. It is anticipated that the use of 5,6,7,8-tetrahydro-1-napthyl as aryloxy moiety is capable to confer antiviral activity among a series of otherwise inactive uracil ProTides.

1. Introduction

The ProTide approach, pioneered by Chris Mcguigan's group,[1,2] is a powerful technology aimed to optimize intracellular drug delivery and circumvent metabolic bottlenecks in the activation of nucleoside-based antiviral and anticancer drugs. In the last years this technology has displayed a great deal of success in the antiviral field with two compounds in the market: the phosphoramidate Sofosbuvir [3,4] (Sovaldi®) approved in 2013 against HCV infections and the phosphonoamidate tenofovir alafenamide fumarate[5] (TAF, Vemlidy®) approved in 2015 for the treatment of HIV[6,7] and later in 2016 for HBV infections[8,9] (Fig. 1).

Several other ProTides have entered in clinical trials while many others are in preclinical evaluation either as antiviral or anticancer drugs.[2,10,11] Given the tremendous importance of phosphor(n)oamidate prodrugs in the antiviral arena and beyond, after the approval of Sofosbuvir and TAF, the application of the ProTide technology has grown dramatically and it has started to show very promising results in other therapeutic areas as well.[12–14] While there are several efficient procedures to synthesize phosphoroamidate nucleosides, the phosphonoamidate cognate class especially of acyclic nucleoside phosphonates (ANPs) lacks of such plethora of synthetic methodologies.[15]

ANPs play a key role in the treatment of viral infections, and this class of compounds can be regarded as one of the most significant group of drugs in the antiviral field.[16,17] Discovered almost 30 years ago, a great wealth of research has been dedicated to the development of efficient synthetic methodologies that resulted in a great variety of ANPs.[18–22] These new structures offer a potential for the discovery of more effective drugs against a variety of infectious diseases including antiparasitic,[23–29] antimicrobial,[30–33] and antitubercolous[34,35] medicines. Among these synthetic strategies, quite recently, Agrofoglio's group has elaborated a novel, efficient and straightforward synthesis of C5-alkenyl substituted ANPs via olefin cross-metathesis.[36–42] Although structure-activity relationship (SAR) studies on acyclic nucleosides have not clarified their pharmacophore model, the introduction of a rigid structural element such as the double bond has proved to be extremely important for their antiviral activity.[43,44] Precisely, the *trans*-alkene skeleton is able to mimic the three-dimensional geometry of the ribose ring maintaining also an electronic contribution similar to the one provided by the oxygen.[45] There are considerable evidences that the *trans*-alkenyl acyclic nucleotide motif has a strong affinity with recombinant human thymidylate kinase (hTMPK) active site, responsible for the nucleotide phosphorylation and consequently correlated to its antiviral activity.[41]Interestingly, Agrofoglio's group employed the olefin cross-metathesis methodology also for the direct synthesis of a vast array of unsaturated ANPs analogues including *bis*-POM, *bis*-POC, and alkoxyesters prodrugs.[36,38–41,46,47] Although adopting a different procedure, our group extended the range of prodrugs of (*E*)-but-2-enyl-pyrimidine, by synthesising their ProTide and bisamidate derivatives.[48] In this study we showed that the ProTide technology was able to broaden the spectrum of antiviral activity when compared to other

* Corresponding author.
E-mail address: pertusatif1@cf.ac.uk (F. Pertusati).

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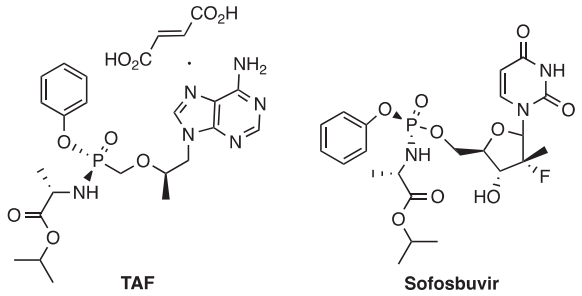


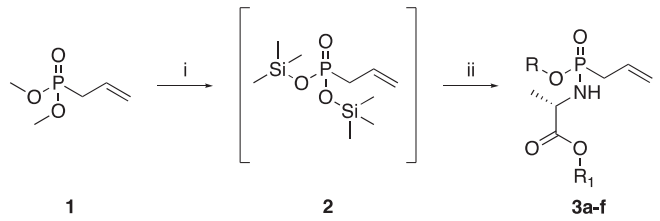
Figure 1. Structures of Sofosbuvir and TAF.

phosphate prodrug approaches. However, we discovered that this methodology suffers from the limitation that only linear olefin must be employed, as with trisubstituted alkenyl derivatives we observed only formation of traces of the desired ProTides. This finding prompted us to investigate the possibility of using the cross-metathesis for the direct synthesis of unsaturated branched ANP phosphonoamidates. At the time we started this investigation, no application of such procedure for the synthesis of ProTides was yet reported. However, during the preparation of this manuscript, a paper reporting the use of the cross metathesis for the synthesis of ProTide derivatives of linear (*E*)-but-2-enyl nucleoside scaffold, was published.[49] The prodrugs described in this work belong to the same family of compounds previously reported by us,[48] and indeed their antiviral profile was in agreement with our published results. In the present article, we would like to report an effective and improved methodology for the synthesis of allyl phosphonoamidate and their further application in olefin cross-metathesis for the synthesis of ANP ProTides. We also anticipate that our two-steps, one-pot methodology can also be applied to the synthesis of symmetrical allyl phosphonodiamidates. Compared with the recently published procedure,[49] our synthetic strategy presents some advantages which we believe, merit consideration.

2. Results and discussion

2.1. Chemistry

Our research began with the synthesis of the aryloxy allylphosphonoamidate synthon **3a**, for which the only literature procedure available is a long and tedious multistep sequence.[50,51] Based on our experience in the application of Holy's one-pot procedure for the direct synthesis of phosphonodiamidates,[52] we envisaged that this protocol could be used to get access to the desired synthon starting from the commercially available dimethyl allylphosphonate **1** (Scheme 1). This methodology was already adapted in our laboratory for the synthesis of adefovir and tenofovir phosphonoamidate prodrugs[53] and more recently for the preparation of (*E*)-but-2-enyl pyrimidine ProTides.[48] Briefly, commercial dimethyl allylphosphonate **1** was converted into the corresponding silyl ester **2**, by reaction with an excess of bromotrimethylsilane (5.0 equivalents). Due to the hydrolytically instability of this ester, **2** was not isolated but immediately dissolved in a mixture



Scheme 1. Synthesis of *O*-Aryl-(L-alanine-ester)-allylphosphonate. *Reagents and conditions*: *i.* TMSBr (5.0 equiv), 2,6-Lutidine (4.0 equiv), CH_3CN, rt, 16 h; *ii.* Amino acid ester hydrochloride (1.0 equiv), aryl-alcohol (6.0 equiv), Et_3N (15.0 equiv), aldrithiol-2 (6.0 equiv), PPh_3 (6.0 equiv), pyridine, 50 °C, 16 h.

Table 1
Substitution pattern and isolated yields of allyl phosphonoamidates **3a–f**.

Entry	Cpds	Aryl	Amino acid	Ester	Yield[a]
1	**3a**	1-Naph	L-Ala	*i*-Pr	79%
2	**3b**	1-Naph	L-Ala	Bz	78%
3	**3c**	Ph	L-Ala	*i*-Pr	65%
4	**3d**	Ph	L-Ala	Bz	42%
5	**3e**	TH-1-Naph	L-Ala	*i*-Pr	55%
6	**3f**	TH-1-Naph	L-Ala	Bz	55%

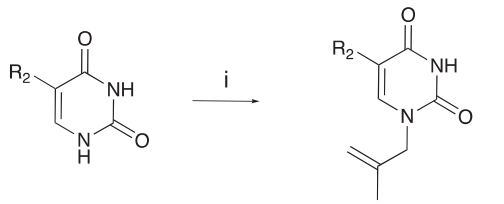
[a] Yield are determined for isolated, purified compounds; see experimental part for details.

of pyridine/Et_3N and treated with the L-alanine isopropyl ester hydrochloride (1.0 equivalents), an excess of 1-naphthol (6.0 equivalents), and a premade solution of PPh_3 (6.0 equivalents) and aldrithiol-2 (6.0 equivalents) in pyridine. After 16 h, the crude mixture did not show the presence of either the desired product or phosphonodiamidate compound (which, based on our experience, is almost invariably formed). We attributed this lack of reactivity to the decomposition of the disilyl ester **2** caused by the release of hydrobromic acid, generated by the hydrolysis of the excess of TMSBr used. Pleasingly, when we attempted the reaction in the presence of 2,6-lutidine (4.0 equivalents) as acid scavenger, the formation of the desired product **3a** was observed (^{31}P NMR and LC-MS analysis of the crude mixture). **3a** was isolated by flash chromatography in excellent yield (79%) (Table 1, Entry 1). Quite surprisingly, no evidence of side reactions[48] (bromination of the double bond and formation of the phosphonodiamidate) have been observed.

With the above methodology, we prepared six different allyl phosphonate analogues **3a-f** in which a variety of aryloxy groups were introduced in combination with two different amino acid esters (L-alanine isopropyl or benzyl esters). From Table 1 it can be appreciated that our method worked well with aryl alcohols with different steric requirements. In particular, we were able to prepare the allyl phosphonoamidates bearing the 5,6,7,8-tetrahydro-1-napthol **3e** and **3f** (Entries 5 and 6, Table 1), which have shown to impart remarkable antiviral activities in compounds of previous series.[48,53]

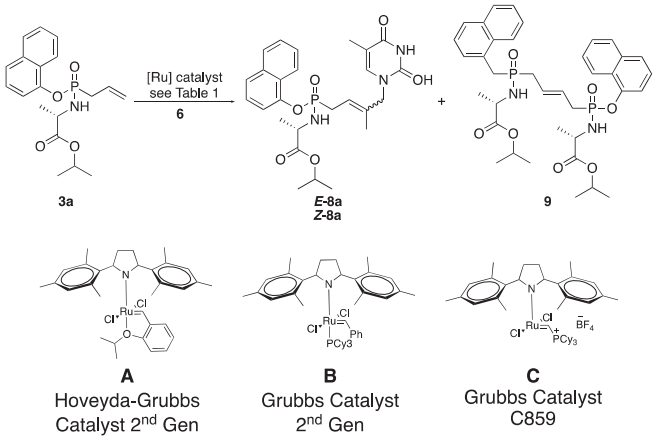
This procedure is short and efficient, representing an improvement of the literature method, which accounts for a 29% overall yield in four steps.[49]

With these allyl phosphonoamidates in hand we began the synthesis of (*E*)-methylbut-2-enyl pyrimidine **6** and **7**, selected as the other partner for the cross-metathesis reaction. These nucleosides and their *bis*-POM prodrugs were originally prepared by Agrofoglio and colleagues,[38] which found the latest to have moderate activities against feline herpes virus (FHV) and feline corona virus (FCoV). Considering that ProTides of alkenyl pyrimidine with "linear" (*E*)-but-2-enyl double bond have shown improved antiviral activities and a broad antiviral spectrum when compared to the corresponding *bis*-POM derivatives, we were now interested in investigating whether ProTide of branched alkenyl pyrimidine might have the same effect. We therefore synthesised a thymine and uracil derivative **6** and **7** as reported in Scheme 2.



Scheme 2. Synthesis of N^1-2′methylallylpyrimidine. *Reagents and conditions*: *i.* 3-Bromo-2-methylpropene (2.0 equiv), BSA (2.5 equivalents), NaI (1.1 equiv), TMSCl (1 equiv), CH_3CN, reflux temperature, 16 h.

Table 2
Screened conditions for CM.[a]



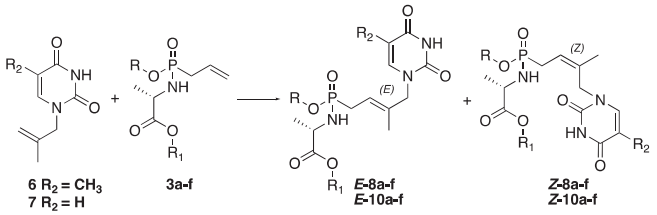
Entry	cat	E-8a/9	E-8a/Z-8a	8a (%)
1[b]	A	1:0.4	1:0.2	24%
2[b]	B	1:1.4	1:0.1	11%
3[b]	C	1:9	1:0.7	3%
4[c]	A	1:0.3	1:0.2	26%
5[c,d]	A	1:0.3	1:0.2	26%

[a] **Reaction conditions:** allyl phosphonoamidate **3a** (1.0 equiv), olefin **6** (2.0 equiv) in CH_2Cl_2 at reflux temperature. Catalyst (5 mol%) added at t = 0, 2, 4 h. Ratio Het/Homo and *E/Z* determined by HPLC.
[b] Reactions sonicated for 24 h.
[c] Reactions sonicated for 36 h.
[d] further addition of the catalyst (5 mol%) after 24 h.

With both alkenyl derivatives in hand we were in the position to investigate the cross-metathesis conditions between the aryloxy allyl-phosphonoamidate synthon **3a** and the olefin **6** as model reaction. First we employed the same CM conditions developed and used by Agrofoglio for the synthesis of the corresponding *bis*-POM alkenyl derivatives[38]. As expected we obtained a mixture of *E/Z* isomers of which the desired compound ***E*-8a** was afforded in 24% yield (Entry 1, Table 2). Both ***E*-8a** and ***Z*-8a** isomers were isolated by preparative reverse phase-HPLC and their configurations were confirmed by NOESY experiments. The homodimer **9** was formed along with the *E/Z* derivatives. Any attempt to improve the reaction outcome using different catalysts (Hoveyda-Grubbs 2nd generation catalyst (**A**), Grubbs 2nd generation catalyst (**B**) and Grubbs catalyst C859 (**C**) failed providing **8a** in similar or lower yield and almost identical *E/Z* ratio (Entries 2–3, Table 2). Since catalyst **A** resulted the best in terms of product/homodimer ratio further screening was conducted keeping **A** as catalyst. Prolonged reaction time (Entry 4, Table 2) resulted in a slightly increased yield that however, was not further improved with addition of more catalyst (Entry 5, Table 2,). These conditions are different from those reported by Agrofoglio in his recent paper,[49] where (*E*)-but-2-enyl pyrimidine ProTides were formed via cross metathesis only when water was used as solvent.

Using these conditions, we prepared different aryloxy phosphonoamidates of both thymine and uracil derivatives. The desired compounds ***E*-8a–f** and ***E*-10a–f** were isolated in moderate yields (Scheme 3, Table 3). In few cases *Z*-isomers (***Z*-8a**, ***Z*-8e**, ***Z*-8f**, ***Z*-10e**) were also isolated in 1 to 7% yield (Scheme 3).

Pleased by the outcome of the above procedure, and to expand the versatility of this methodology, we decided to use the same reaction conditions to prepare the symmetrical phosphonodiamidate **12**. Briefly, the desired *bis*-amidate intermediate **11** was obtained in 52% yield by treating the allyl phosphonate **1** with an excess of TMSBr (in presence of 4.0 equivalents of lutidine) and the resulting silyl diester reacted with an excess (5.0 equivalents) of L-alanine isopropyl hydrochloride (Scheme 4). Compound **11** was then subjected to olefin cross-metathesis reaction with compound **7** under the conditions reported in Scheme 4. Phosphonodiamidate **12** was obtained as a mixture of the *E* and *Z* isomers. The *E*-isomer was isolated in 2% yield, after purification by preparative reverse phase-HPLC.



Scheme 3. ProTide synthesis via cross-metathesis. *Reagents and conditions*: allyl phosphonoamidates **3a–f** (1.0 equiv), olefin **6** or **7** (2.0 equiv) in CH_2Cl_2 at reflux temperature; Hoveyda-Grubbs 2nd generation catalyst (5 mol%) added after 0, 2 and 4 h; reactions sonicated for 24 h.

Since ruthenium catalyst was used during the synthesis, we were interested in measuring its residual amount in the final sample. ICP-MS experiment on compound ***E*-10e** showed ruthenium content of

Table 3
Substitution pattern and isolated yields of phosphonoamidates ***E*-8a–f** and ***E*-10a–f.**

Cpds	R	R_1	R_2	Yield[a]
***E*-8a**	1-Naph	i-Pr	CH_3	36%
***E*-8b**	1-Naph	Bz	CH_3	13%
***E*-8c**	Ph	i-Pr	CH_3	10%
***E*-8d**	Ph	Bz	CH_3	23%
***E*-8e**	TH-1-Naph	i-Pr	CH_3	26%
***E*-8f**	TH-1-Naph	Bz	CH_3	14%
***E*-10a**	1-Naph	i-Pr	H	14%
***E*-10b**	1-Naph	Bz	H	5%
***E*-10c**	Ph	i-Pr	H	10%
***E*-10d**	Ph	Bz	H	18%
***E*-10e**	TH-1-Naph	i-Pr	H	11%
***E*-10f**	TH-1-Naph	Bz	H	5%

[a] Yields were determined for isolated, purified compounds; see experimental part for details.

0.116 mg/g. Further purification[54] will have to be considered if this methodology will be used for preparing compounds progressing to preclinical and clinical evaluation in order to comply the FDA recommended limits for residual metal catalyst in a drug.[55]

2.2. Antiviral activity and serum stability

All the ProTide derivatives synthesised were evaluated against a panel of DNA and RNA viruses as previously described.[48] None of the compounds were active against herpes simplex virus-1 (KOS) (HVS-1), herpes simplex virus-2 (G) (HVS-2), thymidine kinase deficient herpes simplex virus-1 (KOS Acyclovir-resistant strain) (TK^- HSV-1), vaccinia virus (VV), adenovirus-2 (AV-2), human coronavirus (HCoV-229E) in HEL cells, parainfluenza-3 virus (HPIV-3), reovirus-1 (REO-1), vesicular stomatitis virus (VSV), respiratory syncytial virus (RSV) in HeLa cells, influenza A/H1N1, influenza A/H3N2 and influenza B in MDCK cells.

As shown in Table 4, thymine derivatives ***E*-8a–f** showed weak antiviral activity against varicella-zoster virus (VZV TK^+ and TK^-) and human cytomegalovirus (HCMV AD-169 strain and Davis strain) with EC_{50} ranging from 20 to 76 μM, whereas uracil derivatives ***E*-10a-c** were mostly inactive against these viruses with the exception of ***E*-10a** (EC_{50} = 20 μM VZV TK^+) and ***E*-10b** (EC_{50} = 58 μM VZV TK^-). Interestingly uracil derivatives ***E*-10e-f**, bearing the 5,6,7,8-tetrahydro-1-napthol as aryl moiety, resulted slightly active against VZV both TK^+ and TK^- strains, confirming once again the biological potential of this promoiety. No specific information about the 5,6,7,8-tetrahydro-1-naphtol LD_{50} is reported in the literature as for phenol and 1-napthol. However, in previous studies [48,53] we have shown that in an *in vitro* assay the CC_{50} values of ANP ProTides bearing the 5,6,7,8 tehydro-1-napthyl moiety have a comparable CC_{50} values to those bearing phenol and 1-napthol. This is also observed in the presented studies. Remarkably, all the *Z* isomers isolated (***Z*-8a**,**e**,**f** and ***Z*-10e**) showed to some extent antiviral activity against both AD-169 and Davis HCMV strains. Furthermore, compound ***Z*-8e** was found weakly active against Sindbis Virus (SINV), coxsackie virus B4, Punta Toro virus (PTV) and yellow fever virus (YFV) in Vero cells with EC_{50} values in the range of 20–58 μM.

None of the compounds showed significant cytotoxicity. Being able to inhibit VZV, ProTides of allylphosphonate pyrimidine showed a broader antiviral activity than the corresponding *bis*-POM prodrugs, previously reported by Agrofoglio.[41] On the contrary linear alkenyl derivatives showing higher EC_{50} against VZV perform better than those branched, suggesting that a more substituted double bond is detrimental for the antiviral activity.

The metabolic activation of phosphonoamidates follows the same two-enzymatic steps involved in the activation of the phosphoroamidates.[11] Although the use of 5,6,7,8-tetrahydro-1-naphthol as aryloxy group in the ProTides is quite recent we have shown its metabolic activation by carboxypeptidase Y in previous studies.[53] To prove the stability of this class of compound we have performed stability assays of compound ***E*-8e**, in rat and human sera, which indicate a suitable pharmacokinetic profile of the tested phosphonoamidate with a half-life higher than 12 h (Fig. 2).

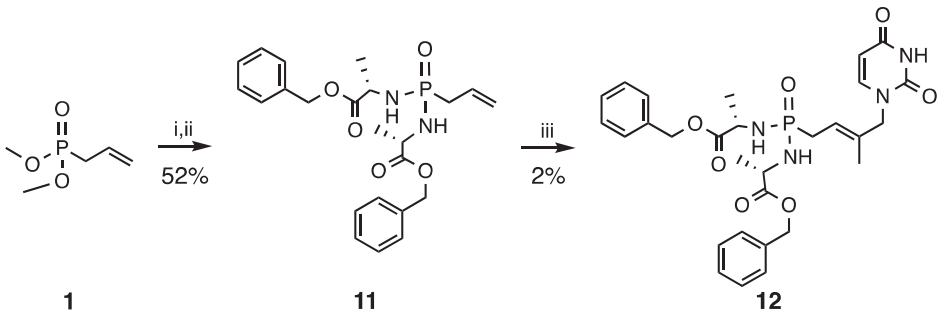
3. Conclusion

In conclusion, we have successfully reported the one pot-two steps synthesis of a family of allyl phosphonoamidates. Our methodology is an important improvement of a recently reported strategy[49] that allows the synthesis of these substrate in a shorter synthetic sequence and with an overall higher yield. We also extended this protocol to the synthesis of hitherto unknown allyl phosphonodiamidate. We also proved that both synthons are capable to undergo alkene cross-metathesis with alkenyl functionalized uracil and thymine nucleobases although the yields need to be further optimized, especially in the case of phosphonodiamidates. These phosphonoamidate prodrugs were evaluated for their biological activity against a panel of DNA and RNA viruses. None of the compounds prepared, showed significant cytotoxicity. ProTides of allylphosphonate pyrimidine showed a broader antiviral activity than the corresponding *bis*-POM prodrugs against VZV infected cells. We have also demonstrated, once again, that the introduction of 5,6,7,8-tetrahydro-1-naphthyl moiety into the ProTide scaffold is capable to increase the antiviral activity of the prodrug. Finally, not only the *E*-isomers showed some biological activity, but also all the *Z* isomers isolated (***Z*-8a**,**e**,**f** and ***Z*-10e**) showed to some extent antiviral activity against both AD-169 and Davis HCMV strains. Further studies directed to the optimization of the cross metathesis procedure especially for the allyl phosphonoamidate, are currently in progress in our laboratory.

4. Experimental section

4.1. Chemistry

All solvents used were anhydrous and used as supplied by Sigma-Aldrich. All commercially available reagents were supplied by either



Scheme 4. Synthesis of symmetrical allyl phosphonodiamidate 12. *Reagents and conditions: i.* TMSBr (5.0 equiv), 2,6-Lutidine (4.0 equiv), CH_3CN, rt, 16 h; *ii.* benzyloxy-L-alanine hydrochloride (5.0 equiv), Et_3N (15.0 equiv), aldrithiol-2 (6.0 equivalents), PPh_3 (6.0 equiv), pyridine, 50 °C, 16 h; iii. N^1-2′-methylallyl-uracil **7** (2 equiv), Hoveyda-Grubbs 2nd generation catalyst (15 mol%), CH_2Cl_2, sonicated for 24 h, at reflux temperature.

Table 4
Antiviral activity of alkenyl ANP ProTides.

Cpds	EC_{50} (HEL cells) (μM)				MCC (HEL cells) (μM)	EC_{50}(Vero cells) (μM)				MCC (Vero cells)(μM)
	VZV		HCMV			SINV	Coxsackie Virus B4	PTV	YFV	
	TK^+	TK^-	AD-169	Davis						
***E*-8a**	44.72	>100	>100	>100	>100	>100	>100	>100	>100	≥20
***E*-8b**	34.2	55.27	>100	>100	>100	>100	>100	>100	>100	>100
***E*-8c**	76.47	>100	>100	>100	>100	>100	>100	>100	>100	≥20
***E*-8d**	55.7	46.66	>100	>100	>100	>100	>100	>100	>100	>100
***E*-8e**	58.48	53.48	>100	>20	>100	>100	>100	>100	>100	≥20
***E*-8f**	50.17	47.19	>100	>100	>100	>100	>100	>100	>100	≥100
***E*-10a**	20	>100	>100	>100	>100	>100	>100	>100	>100	>100
***E*-10b**	100	58.48	>100	>100	>100	>100	>100	>100	>100	>100
***E*-10c**	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
***E*-10d**	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
***E*-10e**	29.91	71.52	>100	>100	>100	>100	>100	>100	>100	>100
***E*-10f**	55.7	52.53	>100	>100	>100	>100	>100	>100	>100	≥100
***Z*-8a**	39.86	41.57	>20	44.72	100	>100	>100	>100	>100	≥20
***Z*-8e**	>20	>20	44.72	>20	100	45	58	45	58	>100
***Z*-8f**	17.03	65.1	76.47	76.47	>100	>100	>100	>100	>100	≥20
***Z*-10e**	58.48	100	>20	54.69	100	>100	>100	>100	>100	>100
Acyclovir	3.55	14.87	–	–	>440	–	–	–	–	–
Brivudin	0.012	0.57	–	–	>300	–	–	–	–	–
Ganciclovir	–	–	11.43	2.29	–	–	–	–	–	–
Cidofovir	–	–	1.24	0.76	–			–	–	–
DS-10.000	–	–	–	–	–	20	7.6	7.6	34	>100
Ribavirin	–	–	–	–	–	>250	>250	126	>250	>250
Mycophenolic acid	–	–	–	–	–	4	>100	6.1	4	>100

EC_{50}: 50% effective concentration or concentration required inhibiting viral induced cytopathic effect (HCMV, SINV, coxsackie virus B4, PTV and YFV) or plaque formation (VZV) by 50%.
MCC: minimal cytotoxic concentration that causes a microscopically alteration of cell morphology.

Sigma-Aldrich or Fisher and used without further purification. All nucleosides and solid reagents were dried for several hours under high vacuum prior to use. For analytical thin-layer chromatography (TLC), precoated aluminium-backed plates (60F-54, 0.2 mm thickness; supplied by E. Merck AG, Darmstadt, Germany) were used and developed by an ascending elution method. For preparative thin-layer chromatography (prep TLC), preparative TLC plates (20 cm × 20 cm, 500–2000 μm) were purchased from Merck. After solvent evaporation, compounds were detected by quenching of the fluorescence, at 254 nm upon irradiation with a UV lamp. Column chromatography purifications were carried out by means of automatic Biotage Isolera One. Fractions containing the product were identified by TLC and pooled,

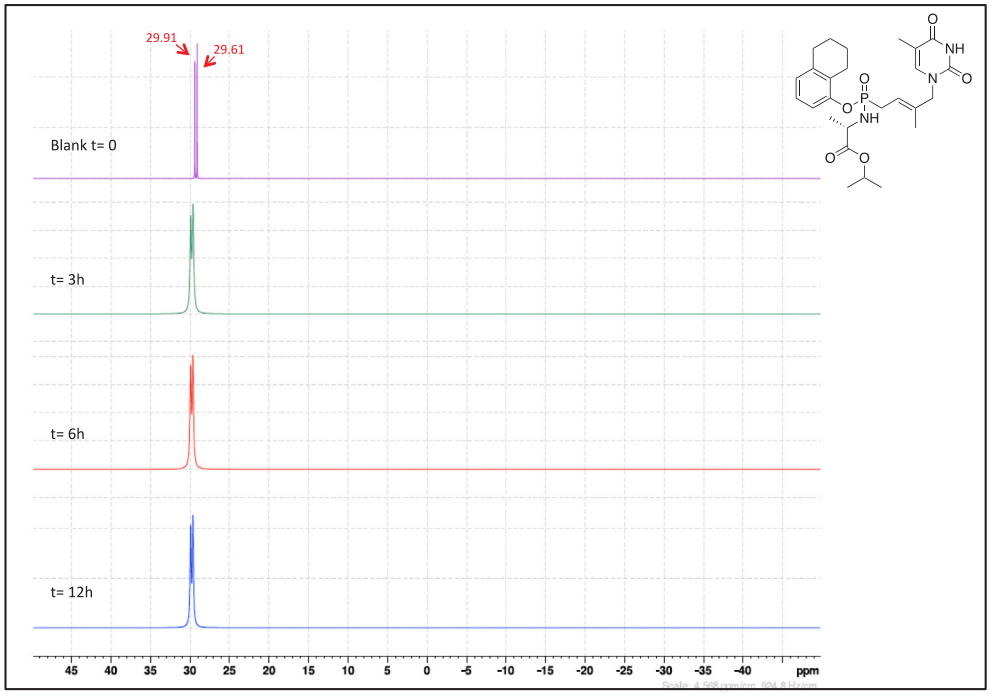


Figure 2. Stability assay of **E-8e** in Human Serum at 37 °C monitored by ^{31}P NMR (202 MHz, DMSO-d_6/H_2O).

and the solvent was removed in vacuo. ^{1}H, ^{31}P and ^{13}C NMR spectra were recorded in a Bruker Avance 500 spectrometer at 500 MHz, 202 MHz and 125 MHz respectively and auto-calibrated to the deuterated solvent reference peak in case of ^{1}H and ^{13}C NMR and 85% H_3PO_4 for ^{31}P NMR experiments. All ^{31}P and ^{13}C NMR spectra were proton-decoupled. Chemical shifts are given in parts per million (ppm) and coupling constants (J) are measured in Hertz (Hz). The following abbreviations are used in the assignment of NMR signals: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (broad singlet), dd (doublet of doublet), ddd (doublet of doublet of doublet), dt (doublet of triplet). The assignment of the signals in ^{1}H NMR and ^{13}C NMR was done based on the analysis of coupling constants and additional two-dimensional experiments (COSY, HSQC). Analytical High-Performance Liquid Chromatography (HPLC) analysis was performed using both Spectra System SCM (with X-select-C18, 5 mm, 4.8 × 150 mm column) and Varian Prostar system (LCWorkstation-Varian Prostar 335 LC detector). Preparative HPLC was performed with Varian Prostar (with pursuit XRs C18 150 × 21.2 mm column). Low and high-resolution mass spectrometry was performed on a Bruker Daltonics MicroTof-LC system (atmospheric pressure ionization, electron spray mass spectroscopy) in positive mode.

The ≥95% purity of the final compounds (***E*-8a–f**, ***E*-10a–f**, ***Z*-8a,e,f** and ***Z*-10e**) was confirmed by HPLC analysis.

4.1.1. General procedure A for the preparation of O-Aryl-(L-alanine-ester)-allylphosphonate (3a–f)

In a round bottom flask, under an argon atmosphere, 2,6-Lutidine (4 eq) and trimethylsilyl bromide (TMSBr, 5 eq) were added to a solution of dimethyl allylphosphonate (1 eq) in anhydrous acetonitrile (8 ml/mmol of allylphosphonate). The mixture was stirred 16 h at room temperature and then the volatiles evaporated without any contact with air. Then the flask was charged with dry aminoacid ester hydrochloride (1 eq), dry aryl-alcohol (6 eq), dry triethylamine (15 eq) and dry pyridine (3 ml/mmol of allylphosphonate) and heated to 50 °C to obtain a homogenous solution. To this mixture was then added a solution of aldrithiol-2 (6 eq) and triphenylphosphine (6 eq) in dry pyridine (3 ml/mmol of allylphosphonate) under argon atmosphere. The resulting mixture was stirred at 50 °C for 16 h. After evaporating all the volatiles, the residue was purified by Biotage Isolera One.

4.1.1.1. O-(1-Naphthyl)-(isopropyloxy-L-alanine)-allylphosphonate (3a).

Prepared according to the standard procedure **A** for the synthesis of allylphosphonoamidate using dimethyl allylphosphonate (500 mg, 3.33 mmol), 2,6-Lutidine (1.55 ml, 13.32 mmol), TMSBr (2.20 ml, 16.65 mmol) in anhydrous acetonitrile (25 ml). For the second step we used dry isopropyloxy-L-alanine hydrochloride (558 mg, 3.33 mmol), dry 1-Naphthol (2.88 g, 19.98 mmol), dry triethylamine (6.9 ml, 49.96 mmol) in dry pyridine (10 ml) and a solution of aldrithiol-2 (4.40 g, 19.98 mmol) and triphenylphosphine (5.24 g, 19.98 mmol) in dry pyridine (10 ml). After evaporation, the mixture was purified by Biotage Isolera One (100 g SNAP cartridge ULTRA, 100 ml/min, gradient eluent system EtOAc/Hexane 10% 1CV, 10–100% 12CV, 100% 2CV), to afford the title compound as a yellow oil (940 mg, 79%). R_f = 0.58 (EtOAc/Hexane – 4:6). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 30.01, 29.43. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 8.19 (d, J = 7.2 Hz, 1H, Ar*H*), 7.89 (d, J = 7.9 Hz 1H, Ar*H*), 7.71–7.69 (m, 1H, Ar*H*), 7.58–7.40 (m, 4H, Ar*H*), 6.07–5.91 (m, 1H, *CH*═), 5.38–5.28 (m, 2H, CH_2═), 5.95–4.82 (m, 1H, *CH*$(CH_3)_2$), 3.99–3.97 (m, 1H, *CH*CH_3 l-Ala), 3.03–2.93 (m, 2H, CH_2P), 1.25 (d, J = 7.8 Hz, 1.5H, CHCH_3 l-Ala), 1.21–1.10 (m, 7.5H, CHCH_3 l-Ala, CH$(CH_3)_2$). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.5 (d, $^{3}J_{C\text{-}P}$ = 4.2 Hz, *C*═O, ester), 173.1 (d, $^{3}J_{C\text{-}P}$ = 4.2 Hz, *C*═O, ester), 146.4 (d, $^{2}J_{C\text{-}P}$ = 8.5 Hz, *C*—O, Ph), 146.3 (d, $^{2}J_{C\text{-}P}$ = 8.5 Hz, *C*—O, Ph), 134.9 (*C*-Ar), 127.4 $^{2}J_{C\text{-}P}$ = 9.3 Hz, *CH*═), 123.3 ($^{2}J_{C\text{-}P}$ = 10.9 Hz, *CH*═), 126.9 (d, $^{3}J_{C\text{-}P}$ = 5.6 Hz *C*-Ar), 126.8 (d, $^{3}J_{C\text{-}P}$ = 4.9 Hz *C*-Ar), 126.3 (*CH*-Ar), 125.95 (*CH*-Ar), 125.90 (*CH*-Ar), 125.1 (*CH*-Ar), 125.0 (*CH*-Ar), 124.3 (*CH*-Ar), 124.2 (*CH*-Ar), 121.6 (*CH*-Ar), 121.4 (*CH*-Ar), 119.7 (d, $^{3}J_{C\text{-}P}$ = 14.2 Hz CH_2═), 119.6 (d, $^{3}J_{C\text{-}P}$ = 13.8 Hz CH_2═), 115.4 (d, $^{3}J_{C\text{-}P}$ = 4.1 Hz *CH*-Ar), 115.2 (d, $^{3}J_{C\text{-}P}$ = 3.4 Hz *CH*-Ar), 68.6 (*CH*$(CH_3)_2$), 68.5 (*CH*$(CH_3)_2$), 49.6 (*CH*CH_3 l-Ala), 49.4 (*CH*CH_3 l-Ala), 33.7 (d, $^{1}J_{C\text{-}P}$ = 129.0 Hz CH_2P), 33.5 (d, $^{1}J_{C\text{-}P}$ = 129.6 Hz CH_2P), 20.5 (CH$(CH_3)_2$), 20.4 (CH$(CH_3)_2$), 20.3 (CH$(CH_3)_2$), 19.7 (d, $^{3}J_{C\text{-}P}$ = 5.4 Hz, CHCH_3 l-Ala), 19.1 (d, $^{3}J_{C\text{-}P}$ = 5.4 Hz, CHCH_3 l-Ala).

4.1.1.2. O-(1-Naphthyl)-(benzyloxy-L-alanine)-allylphosphonate (3b).

Prepared according to the standard procedure **A** for the synthesis of allylphosphonoamidate using dimethyl allylphosphonate (500 mg, 3.33 mmol), 2,6-Lutidine (1.55 ml, 13.32 mmol), TMSBr (2.20 ml, 16.65 mmol) in anhydrous acetonitrile (25 ml). For the second step we used dry benzyloxy-L-alanine hydrochloride (718 mg, 3.33 mmol), dry 1-Naphthol (2.88 g, 19.98 mmol), dry triethylamine (6.9 ml, 49.96 mmol) in dry pyridine (10 ml) and a solution of aldrithiol-2 (4.40 g, 19.98 mmol) and triphenylphosphine (5.24 g, 19.98 mmol) in dry pyridine (10 ml). After evaporation, the mixture was purified by Biotage Isolera One (100 g SNAP cartridge ULTRA, 100 ml/min, gradient eluent system EtOAc/Hexane 10% 1CV, 10–100% 12CV, 100% 2CV), to afford the title compound as a yellow oil (1.1 g, 78%). R_f = 0.58 (EtOAc/Hexane – 4:6). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 30.09, 29.48. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 8.17 (s, 1H, Ar*H*), 7.86 (s, 1H, Ar*H*), 7.69–7.65 (m, 1H, Ar*H*), 7.52–7.22 (m, 9H, Ar*H*), 5.99–5.89 (m, 1H, *CH*═), 5.30–5.24 (m, 2H, CH_2═), 5.09, 5.03 (ABq, J_{AB} = 12.1 Hz, 1H, CH_2Ph), 4.97, 4.93 (ABq, J_{AB} = 12.1 Hz, 1H, CH_2Ph), 4.09–4.07 (m, 1H, *CH*CH_3 l-Ala), 2.95–2.91 (m, 2H, CH_2P), 1.26 (d, J = 6.8 Hz, 1.5H, CHCH_3 l-Ala), 1.16 (d, J = 6.8 Hz, 1.5H, CHCH_3 l-Ala). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.7 (d, $^{3}J_{C\text{-}P}$ = 3.9 Hz, *C*═O, ester), 173.2 (d, $^{3}J_{C\text{-}P}$ = 4.0 Hz, *C*═O, ester), 146.4 (d, $^{2}J_{C\text{-}P}$ = 9.7 Hz, *C*—O, Ph), 146.3 (d, $^{2}J_{C\text{-}P}$ = 10.0 Hz, *C*—O, Ph), 135.8 (*C*-Ar), 135.7 (*C*-Ar), 134.9 (*C*-Ar), 128.17 (*CH*-Ar), 128.12 (*CH*-Ar), 127.9 (*CH*-Ar), 127.8 (*CH*-Ar), 127.48 (*CH*-Ar), 127.42 (*CH*-Ar), 127.3 ($^{2}J_{C\text{-}P}$ = 11.3 Hz, *CH*═), 127.2 ($^{2}J_{C\text{-}P}$ = 11.0 Hz, *CH*═), 126.8 (d, $^{3}J_{C\text{-}P}$ = 5.0 Hz *C*-Ar), 126.7 (d, $^{3}J_{C\text{-}P}$ = 5.3 Hz *C*-Ar), 126.3 (*CH*-Ar), 125.98 (*CH*-Ar), 125.93 (*CH*-Ar), 125.18 (*CH*-Ar), 125.10 (*CH*-Ar), 124.3 (*CH*-Ar), 124.2 (*CH*-Ar), 121.6 (*CH*-Ar), 121.4 (*CH*-Ar), 119.7 (d, $^{3}J_{C\text{-}P}$ = 15.2 Hz CH_2═), 119.6 (d, $^{3}J_{C\text{-}P}$ = 14.9 Hz CH_2═), 115.4 (d, $^{3}J_{C\text{-}P}$ = 3.9 Hz *CH*-Ar), 115.2 (d, $^{3}J_{C\text{-}P}$ = 3.9 Hz *CH*-Ar), 66.5 (CH_2Ph), 66.3 (CH_2Ph), 49.6 (*CH*CH_3 l-Ala), 49.4 (*CH*CH_3 l-Ala), 33.7 (d, $^{1}J_{C\text{-}P}$ = 129.2 Hz CH_2P), 33.5 (d, $^{1}J_{C\text{-}P}$ = 129.7 Hz CH_2P), 19.6 (d, $^{3}J_{C\text{-}P}$ = 5.3 Hz, CHCH_3 l-Ala), 19.0 (d, $^{3}J_{C\text{-}P}$ = 5.8 Hz, CHCH_3 l-Ala).

4.1.1.3. O-Phenyl-(isopropyloxy-L-alanine)-allylphosphonate (3c).

Prepared according to the standard procedure **A** for the synthesis of allylphosphonoamidate using dimethyl allylphosphonate (500 mg, 3.33 mmol), 2,6-Lutidine (1.55 ml, 13.32 mmol), TMSBr (2.20 ml, 16.65 mmol) in anhydrous acetonitrile (25 ml). For the second step we used dry isopropyloxy-L-alanine hydrochloride (558.3 mg, 3.33 mmol), dry phenol (1.88 g, 19.98 mmol), dry triethylamine (6.9 ml, 49.96 mmol) in dry pyridine (10 ml) and a solution of aldrithiol-2 (4.40 g, 19.98 mmol) and triphenylphosphine (5.24 g, 19.98 mmol) in dry pyridine (10 ml). After evaporation, the mixture was purified by Biotage Isolera One (100 g SNAP cartridge ULTRA, 100 ml/min, gradient eluent system EtOAc/Hexane 10% 1CV, 10–100% 12CV, 100% 2CV), to afford the title compound as a yellow oil (670 mg, 65%). R_f = 0.37 (EtOAc/Hexane – 6:4). **^{31}P NMR (202 MHz, $CDCl_3$) δ_P**: 26.77, 26.35. **^{1}H NMR (500 MHz, $CDCl_3$) δ_H**: 7.32–7.28 (m, 2H, Ar*H*), 7.22–7.20 (m, 2H, Ar*H*), 7.14–7.13 (m, 1H, Ar*H*), 5.95–5.82 (m, 1H, *CH*═), 5.32–5.25 (m, 2H, CH_2═), 5.00–4.94 (m, 1H, *CH*$(CH_3)_2$), 4.14–3.96 (m, 1H, *CH*CH_3 l-Ala), 3.51 (dd, $^{2}J_{H\text{-}P}$, $^{3}J_{NH,CH}$ = 10.3 Hz, 0.5H, *NH* l-Ala), 3.41 (dd, $^{2}J_{H\text{-}P}$, $^{3}J_{NH,CH}$ = 10.7 Hz, 0.5H, *NH* l-Ala), 2.81–2.72 (m, 2H, CH_2P), 1.29 (d, J = 7.2 Hz, 1.5H, CHCH_3 l-Ala), 1.23–1.20 (m, 7.5H, CHCH_3 l-Ala, CH$(CH_3)_2$). **^{13}C NMR (125 MHz, $CDCl_3$) δ_C**: 173.5 (d, $^{3}J_{C\text{-}P}$ = 4.7 Hz, *C*═O, ester), 173.1 (d, $^{3}J_{C\text{-}P}$ = 4.7 Hz, *C*═O, ester), 150.6 (d, $^{2}J_{C\text{-}P}$ = 9.1 Hz, *C*—O, Ph), 150.5

(d, $^2J_{C\text{-}P}$ = 9.4 Hz, *C*—O, Ph), 129.4 (*CH*-Ar), 129.3 (*CH*-Ar), 127.5 ($^2J_{C\text{-}P}$ = 11.3 Hz, *CH*═), 127.4 ($^2J_{C\text{-}P}$ = 11.3 Hz, *CH*═), 124.6 (*CH*-Ar), 124.5 (*CH*-Ar), 120.8 (d, $^3J_{C\text{-}P}$ = 4.0 Hz *CH*-Ar), 120.6 (d, $^3J_{C\text{-}P}$ = 4.0 Hz *CH*-Ar), 119.6 (d, $^3J_{C\text{-}P}$ = 14.6 Hz CH_2═), 119.6 (d, $^3J_{C\text{-}P}$ = 14.6 Hz CH_2═), 68.59 (*CH*$(CH_3)_2$), 68.57 (*CH*$(CH_3)_2$), 49.6 (*CH*CH_3 l-Ala), 49.7 (*CH*CH_3 l-Ala), 33.8 (d, $^1J_{C\text{-}P}$ = 129.3 Hz CH_2P), 33.6 (d, $^1J_{C\text{-}P}$ = 129.7 Hz CH_2P), 20.86 (CH(CH_3)$_2$), 20.82 (CH(CH_3)$_2$), 20.81 (CH(CH_3)$_2$), 20.75 (CH(CH_3)$_2$), 20.0 (d, $^3J_{C\text{-}P}$ = 5.3 Hz, CHCH_3 l-Ala), 19.5 (d, $^3J_{C\text{-}P}$ = 5.1 Hz, CHCH_3 l-Ala).

*4.1.1.4. O-Phenyl-(benzyloxy-L-alanine)-allylphosphonate (**3d**)*. Prepared according to the standard procedure **A** for the synthesis of allylphosphonoamidate using dimethyl allylphosphonate (500 mg, 3.33 mmol), 2,6-Lutidine (1.55 ml, 13.32 mmol), TMSBr (2.20 ml, 16.65 mmol) in anhydrous acetonitrile (25 ml). For the second step we used dry benzyloxy-*L*-alanine hydrochloride (718 mg, 3.33 mmol), dry phenol (1.88 g, 19.98 mmol), dry triethylamine (6.9 ml, 49.96 mmol) in dry pyridine (10 ml) and a solution of aldrithiol-2 (4.40 g, 19.98 mmol) and triphenylphosphine (5.24 g, 19.98 mmol) in dry pyridine (10 ml). After evaporation, the mixture was purified by Biotage Isolera One (100 g SNAP cartridge ULTRA, 100 ml/min, gradient eluent system EtOAc/Hexane 10% 1CV, 10–100% 12CV, 100% 2CV), to afford the title compound as a yellow oil (500 mg, 42%). R_f = 0.22 (EtOAc/Hexane – 4:6). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 29.64, 28.99. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 7.35–7.28 (m, 7H, Ar*H*), 7.22–7.14 (m, 3H, Ar*H*), 5.91–5.81 (m, 1H, *CH*═), 5.27–5.18 (m, 2H, CH_2═), 5.14, 5.12 (ABq, J_{AB} = 12.5 Hz, 1H, CH_2Ph), 5.06 (s app, 1H, CH_2Ph), 4.10–4.01 (m, 1H, *CH*CH_3 l-Ala), 2.82–2.75 (m, 2H, CH_2P), 1.31 (d, *J* = 7.2 Hz, 1.5H, CHCH_3 l-Ala), 1.22 (d, *J* = 7.5 Hz, 1.5H, CHCH_3 l-Ala). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 172.3 (d, $^3J_{C\text{-}P}$ = 4.1 Hz, *C*═O, ester), 171.9 (d, $^3J_{C\text{-}P}$ = 3.9 Hz, *C*═O, ester), 149.0 (d, $^2J_{C\text{-}P}$ = 9.5 Hz, *C*—O, Ph), 148.9 (d, $^2J_{C\text{-}P}$ = 9.5 Hz, *C*—O, Ph), 134.37 (*C*-Ar), 134.34 (*C*-Ar), 127.84 (*CH*-Ar), 127.81 (*CH*-Ar), 126.75 (*CH*-Ar), 126.73 (*CH*-Ar), 126.5 (*CH*-Ar), 126.49 (*CH*-Ar), 126.46 (*CH*-Ar), 125.8 ($^2J_{C\text{-}P}$ = 10.1 Hz, *CH*═), 125.7 ($^2J_{C\text{-}P}$ = 10.1 Hz, *CH*═), 123.1 (*CH*-Ar), 123.0 (*CH*-Ar), 119.2 (d, $^3J_{C\text{-}P}$ = 4.3 Hz *CH*-Ar), 119.0 (d, $^3J_{C\text{-}P}$ = 4.3 Hz *CH*-Ar), 118.2 (d, $^3J_{C\text{-}P}$ = 14.5 Hz CH_2═), 118.0 (d, $^3J_{C\text{-}P}$ = 14.6 Hz CH_2═), 65.0 (CH_2Ph), 64.9 (CH_2Ph), 48.09 (*CH*CH_3 l-Ala), 47.9 (*CH*CH_3 l-Ala), 32.1 (d, $^1J_{C\text{-}P}$ = 129.7 Hz CH_2P), 31.9 (d, $^1J_{C\text{-}P}$ = 129.7 Hz CH_2P), 18.2 (d, $^3J_{C\text{-}P}$ = 5.3 Hz, CHCH_3 l-Ala), 17.7 (d, $^3J_{C\text{-}P}$ = 5.3 Hz, CHCH_3 l-Ala).

*4.1.1.5. O-(5,6,7,8-Tetrahydro-1-naphthyl)-(isopropyloxy-L-alanine)-allylphosphonate (**3e**)*. Prepared according to the standard procedure **A** for the synthesis of allylphosphonoamidate using dimethyl allylphosphonate (500 mg, 3.33 mmol), 2,6-Lutidine (1.55 ml, 13.32 mmol), TMSBr (2.20 ml, 16.65 mmol) in anhydrous acetonitrile (25 ml). For the second step we used dry isopropyloxy-*L*-alanine hydrochloride (558 mg, 3.33 mmol), dry 5,6,7,8-tetrahydro-1-naphthol (2.96 g, 19.98 mmol), dry triethylamine (6.9 ml, 49.96 mmol) in dry pyridine (10 ml) and a solution of aldrithiol-2 (4.40 g, 19.98 mmol) and triphenylphosphine (5.24 g, 19.98 mmol) in dry pyridine (10 ml). After evaporation, the mixture was purified by Biotage Isolera One (100 g SNAP cartridge ULTRA, 100 ml/min, gradient eluent system EtOAc/Hexane 10% 1CV, 10–100% 12CV, 100% 2CV), to afford the title compound as a yellow foamy solid (750 mg, 55%). R_f = 0.51 (EtOAc/Hexane – 4:6). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 29.04, 28.43. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 7.17–7.12 (m, 1H, Ar*H*), 7.05–7.00 (m, 1H, Ar*H*), 6.89–6.87 (m, 1H, Ar*H*), 5.99–5.85 (m, 1H, *CH*═), 5.32–5.24 (m, 2H, CH_2═), 5.01–4.88 (m, 1H, *CH*$(CH_3)_2$), 3.98–3.89 (m, 1H, *CH*CH_3 l-Ala), 2.85 (dt, $^2J_{H\text{-}P}$ = $^2J_{H\text{-}H}$ = 20.0 Hz, $^3J_{H\text{-}H}$ = 7.1 Hz, 2H, CH_2P), 2.77–2.74 (m, 4H, Ar*H*), 1.80–1.79 (m, 4H, Ar*H*), 1.30 (d, *J* = 7.1 Hz, 1.5H, CHCH_3 l-Ala), 1.25–1.23 (m, 4.5H, CHCH_3 l-Ala, CH(CH_3)$_2$), 1.19 (d, *J* = 6.05 Hz, 3H, CH(CH_3)$_2$). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.6 (d, $^3J_{C\text{-}P}$ = 4.0 Hz, *C*═O, ester), 173.2 (d, $^3J_{C\text{-}P}$ = 4.0 Hz, *C*═O, ester), 148.7 (d, $^2J_{C\text{-}P}$ = 10.2 Hz, *C*—O, Ph), 148.6 (d, $^2J_{C\text{-}P}$ = 10.6 Hz, *C*—O, Ph), 139.1 (*C*-Ar), 131.3 (*CH*-Ar), 131.2 (*CH*-Ar), 128.6 (d, $^3J_{C\text{-}P}$ = 7.0 Hz *C*-Ar), 128.5 (d, $^3J_{C\text{-}P}$ = 7.5 Hz *C*-Ar), 127.5 ($^2J_{C\text{-}P}$ = 11.0 Hz, *CH*═), 127.4 ($^2J_{C\text{-}P}$ = 11.0 Hz, *CH*═), 125.3 (*CH*-Ar), 125.1 (*CH*-Ar), 119.4 (d, $^3J_{C\text{-}P}$ = 14.6 Hz CH_2═), 119.3 (d, $^3J_{C\text{-}P}$ = 14.6 Hz CH_2═), 116.9 (d, $^3J_{C\text{-}P}$ = 3.1 Hz *CH*-Ar), 116.8 (d, $^3J_{C\text{-}P}$ = 3.5 Hz *CH*-Ar), 68.6 (*CH*$(CH_3)_2$), 68.5 (*CH*$(CH_3)_2$), 49.7 (*CH*CH_3 l-Ala), 49.4 (*CH*CH_3 l-Ala), 33.8 (d, $^1J_{C\text{-}P}$ = 129.5 Hz CH_2P), 33.6 (d, $^1J_{C\text{-}P}$ = 130.1 Hz CH_2P), 29.1 (CH_2-Ar), 23.3 (CH_2-Ar), 22.48 (CH_2-Ar), 22.46 (CH_2-Ar), 22.41 (CH_2-Ar), 20.59 (CH(CH_3)$_2$), 20.56 (CH(CH_3)$_2$), 20.54 (CH(CH_3)$_2$), 20.4 (CH(CH_3)$_2$), 19.9 (d, $^3J_{C\text{-}P}$ = 4.9 Hz, CHCH_3 l-Ala), 19.1 (d, $^3J_{C\text{-}P}$ = 5.4 Hz, CHCH_3 l-Ala).

*4.1.1.6. O-(5,6,7,8-Tetrahydro-1-naphthyl)-(benzyloxy-L-alanine)-allylphosphonate (**3f**)*. Prepared according to the standard procedure **A** for the synthesis of allylphosphonoamidate using dimethyl allylphosphonate (500 mg, 3.33 mmol), 2,6-Lutidine (1.55 ml, 13.32 mmol), TMSBr (2.20 ml, 16.65 mmol) in anhydrous acetonitrile (25 ml). For the second step we used dry benzyloxy-*L*-alanine hydrochloride (718 mg, 3.33 mmol), dry 5,6,7,8-tetrahydro-1-naphthol (2.96 g, 19.98 mmol), dry triethylamine (6.9 ml, 49.96 mmol) in dry pyridine (10 ml) and a solution of aldrithiol-2 (4.40 g, 19.98 mmol) and triphenylphosphine (5.24 g, 19.98 mmol) in dry pyridine (10 ml). After evaporation, the mixture was purified by Biotage Isolera One (100 g SNAP cartridge ULTRA, 100 ml/min, gradient eluent system EtOAc/Hexane 10% 1CV, 10–100% 12CV, 100% 2CV), to afford the title compound as a yellow foamy solid (750 mg, 55%). R_f = 0.51 (EtOAc/Hexane – 4:6). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 28.81, 28.20. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 7.35–7.31 (m, 5H, Ar*H*), 7.17–7.14 (m, 1H, Ar*H*), 7.06–7.94 (m, 1H, Ar*H*), 6.87–6.83 (m, 1H, Ar*H*), 5.94–5.82 (m, 1H, *CH*═), 5.27–5.20 (m, 2H, CH_2═), 5.13, 5.10 (ABq, J_{AB} = 12.2 Hz, 1H, CH_2Ph), 5.04 (AB app t, J_{AB} = 12.8 Hz, 1H, CH_2Ph), 4.12–4.01 (m, 1H, *CH*CH_3 l-Ala), 2.86–2.77 (m, 2H, CH_2P), 2.72–2.67 (m, 4H, Ar*H*), 1.79–1.71 (m, 4H, Ar*H*), 1.32 (d, *J* = 7.1 Hz, 1.5H, CHCH_3 l-Ala), 1.26 (d, *J* = 7.1 Hz, 1.5H, CHCH_3 l-Ala). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.8 (d, $^3J_{C\text{-}P}$ = 3.7 Hz, *C*═O, ester), 173.4 (d, $^3J_{C\text{-}P}$ = 4.1 Hz, *C*═O, ester), 148.8 (d, $^2J_{C\text{-}P}$ = 9.7 Hz, *C*—O, Ph), 148.7 (d, $^2J_{C\text{-}P}$ = 9.4 Hz, *C*—O, Ph), 139.13 (*C*-Ar), 139.11 (*C*-Ar), 135.88 (*C*-Ar), 135.84 (*C*-Ar), 128.6 (d, $^3J_{C\text{-}P}$ = 5.5 Hz *C*-Ar), 128.5 (d, $^3J_{C\text{-}P}$ = 5.8 Hz *C*-Ar), 128.23 (*CH*-Ar), 128.20 (*CH*-Ar), 127.99 (*CH*-Ar), 127.94 (*CH*-Ar), 127.87 (*CH*-Ar), 127.5 ($^2J_{C\text{-}P}$ = 11.3 Hz, *CH*═), 127.4 ($^2J_{C\text{-}P}$ = 11.0 Hz, *CH*═), 125.4 (*CH*-Ar), 125.3 (*CH*-Ar), 125.17 (*CH*-Ar), 125.13 (*CH*-Ar), 119.5 (d, $^3J_{C\text{-}P}$ = 14.6 Hz CH_2═), 119.4 (d, $^3J_{C\text{-}P}$ = 14.8 Hz CH_2═), 117.0 (d, $^3J_{C\text{-}P}$ = 3.4 Hz *CH*-Ar), 116.9 (d, $^3J_{C\text{-}P}$ = 3.1 Hz *CH*-Ar), 66.5 (CH_2Ph), 66.4 (CH_2Ph), 49.7 (*CH*CH_3 l-Ala), 49.4 (*CH*CH_3 l-Ala), 33.8 (d, $^1J_{C\text{-}P}$ = 129.4 Hz CH_2P), 33.7 (d, $^1J_{C\text{-}P}$ = 130.2 Hz CH_2P), 29.18 (CH_2-Ar), 23.38 (CH_2-Ar), 22.5 (CH_2-Ar), 22.48 (CH_2-Ar), 22.43 (CH_2-Ar), 19.8 (d, $^3J_{C\text{-}P}$ = 5.3 Hz, CHCH_3 l-Ala), 19.1 (d, $^3J_{C\text{-}P}$ = 5.3 Hz, CHCH_3 l-Ala).

*4.1.2. General procedure B for the preparation of N^1-2′-methylallylpyrimidine (**6, 7**)*

In a round bottom flask, under an argon atmosphere, to a solution of the nucleobase (1 eq) in anhydrous acetonitrile (2 ml/mmol of nucleobase) was added BSA (2.5 eq). The mixture was refluxed until clear solution was observed (usually 5 min). 3-bromo-2-methylpropene (2.0 eq), NaI (1.1 eq) and TMSCl (1 eq) were then added to the reaction mixture. The solution was refluxed 16 h and then evaporated under reduced pressure. The residue was dissolved in EtOAc, washed with $NaHCO_3$ (aqueous saturated solution), Na_2SO_4 (aqueous saturated solution), H_2O, brine and dried over $MgSO_4$. The resulting mixture was evaporated and the residue was purified by Biotage Isolera One.

*4.1.2.1. N^1-2′-Methylallyl-thymine (**6**)*. Prepared according to the standard procedure **B** for the synthesis of N^1-2′-methylallylpyrimidine using thymine (1.5 g, 11.89 mmol), BSA (7.2 ml, 29.73 mmol), 3-

bromo-2-methylpropene (2.40 ml, 23.79 mmol), NaI (1.96 g, 13.08 mmol) and TMSCl (1.51 ml, 11.89 mmol) in anhydrous acetonitrile (25 ml). After work up and evaporation, the compound was obtained as a pale yellow solid in quantitative yield (2.1 g). R_f = 0.45 (EtOAc/Hexane – 7:3).**^{1}H NMR (500 MHz, CD_3OD) δ_H**: 7.34 (s, 1H, *H*-6), 4.98 (s, 1H, CH_2═), 4.80 (s, 1H, CH_2═), 4.30 (s, 2H, CH_2-N), 1.89 (s, 3H, CH_3, base), 1.76 (s, 3H, CH_3, alkene).

4.1.2.2. N^1-2′-Methylallyl-uracil (7). Prepared according to the standard procedure **B** for the synthesis of N^1-2′-methylallylpyrimidine using uracil (1.5 g, 13.38 mmol), BSA (8.18 ml, 33.46 mmol), 3-bromo-2-methylpropene (2.70 ml, 26.76 mmol), NaI (2.21 g, 14.72 mmol) and TMSCl (1.70 ml, 13.38 mmol) in anhydrous acetonitrile (25 ml). After work up and evaporation, the mixture was purified by Biotage Isolera One (50 g SNAP cartridge ULTRA, 100 ml/min, gradient eluent system EtOAc/Hexane 17% 1CV, 17–100% 10CV, 100% 3CV), to afford the title compound as a pale yellow solid (1.2 g, 51%). R_f = 0.25 (EtOAc/Hexane – 7:3).**^{1}H NMR (500 MHz, CD_3OD) δ_H**: 7.50 (d, *J* = 7.8 Hz, 1H, *H*-6), 5.71 (d, *J* = 7.8 Hz, 1H, *H*-5), 4.98 (s, 1H, CH_2═), 4.81 (s, 1H, CH_2═), 4.33 (s, 2H, CH_2-N), 1.76 (s, 3H, CH_3, alkene).

*4.1.3. General procedure C for the preparation of (E)-N^1-(4′-O-Aryl-(L-alanine-ester)-phosphinyl-2′-methyl-but-2′-enyl)pyrimidine (**E-8a–f**, **E-10a–f**)*

To a solution of *O*-Aryl-(*L*-alanine-ester)-allylphosphonate (1 eq) and N^1-2′-methylallylpyrimidine (2 eq) in dry CH_2Cl_2 (20 ml/mmol allylphosphonate), was added Hoveyda-Grubbs 2nd generation catalyst (15 mol%). The catalyst was added in three equal portion of 5 mol% at t = 0, 2, 4 h over the course of the reaction. The solution was sonicated under argon atmosphere for 24 h. Volatiles were then evaporated, and the residue was purified by Biotage Isolera One. Also a reverse phase chromatography was necessary to gain pure final products.

*4.1.3.1. (E)-N^1-(4′-O-(1-Naphthyl)-(isopropyloxy-L-alanine)-phosphinyl-2′-methyl-but-2′-enyl)thymine (**E-8a**) and (Z)-N^1-(4′-O-(1-naphthyl)-(isopropyloxy-L-alanine)-phosphinyl-2′-methyl-but-2′-enyl)thymine (**Z-8a**)*. Prepared according to the standard procedure **C** for the synthesis of ANP ProTide using *O*-(1-naphthyl)-(isopropyloxy-*L*-alanine)-allylphosphonate **3a** (150 mg, 415 μmol) and N^1-2′-methylallylthymine (150 mg, 830.1 μmol) and Hoveyda-Grubbs 2nd generation catalyst (15 mol%) in dry CH_2Cl_2 (8 ml). After evaporation, the crude was purified by Biotage Isolera One (50 g SNAP cartridge ULTRA, 100 ml/min, gradient eluent system MeOH/CH_2Cl_2 1% 1CV, 1–10% 12CV, 10% 2CV), to afford a mixture of the *E* and *Z* isomers. The two isomers were then separated by reverse Biotage Isolera One (60 g SNAP cartridge KP-C18-HS, 100 ml/min, isocratic eluent system CH_3CN/H_2O 30–60% 12CV) to afford the title compound *E* as pale yellow foamy solid (75 mg, 36%). R_f = 0.23 (CH_2Cl_2/MeOH – 95:5).**^{31}P NMR (202 MHz, CD_3OD) δ_P**: 30.32, 29.54. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 8.13–8.12 (m, 1H, Ar*H*), 7.89–7.87 (m, 1H, Ar*H*), 7.71–7.68 (m, 1H, Ar*H*), 7.57–7.48 (m, 3H, Ar*H*), 7.45–7.39 (m, 1H, Ar*H*), 7.27 (s, 0.5H, *H*-6), 7.26 (s, 0.5H, *H*-6), 5.61–5.56 (m, 1H, *CH*═), 4.93–4.84 (m, 1H, *CH*$(CH_3)_2$), 4.32–4.26 (m, 2H, CH_2-N), 4.01–3.91 (m, 1H, *CH*CH_3 l-Ala), 3.08–2.86 (m, 2H, CH_2P), 1.75 (s, 3H, CH_3, base), 1.67 (s, 3H, CH_3, alkene), 1.27 (d, *J* = 6.9 Hz, 1.5H, CHCH_3 l-Ala), 1.20–1.16 (m, 4.5H, CHCH_3 l-Ala, CH$(CH_3)_2$), 1.13–1.10 (m, 3H, CH$(CH_3)_2$). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.5 (d, $^3J_{C\text{-}P}$ = 3.9 Hz, *C*═O, ester), 173.1 (d, $^3J_{C\text{-}P}$ = 3.5 Hz, *C*═O, ester), 165.34 (*C*-4), 165.32 (*C*-4), 151.69 (*C*-2), 151.61 (*C*-2), 146.5 (d, $^2J_{C\text{-}P}$ = 9.5 Hz, *C*—O, Ph), 146.3 (d, $^2J_{C\text{-}P}$ = 9.5 Hz, *C*—O, Ph), 140.94 (*C*-6), 140.92 (*C*-6), 135.5 (d, $^3J_{C\text{-}P}$ = 14.3 Hz, *C*═), 135.1 (d, $^3J_{C\text{-}P}$ = 14.7 Hz, *C*═), 134.9 (*C*-Ar), 127.48 (*CH*-Ar), 127.46 (*CH*-Ar), 126.7 (d, $^3J_{C\text{-}P}$ = 5.1 Hz *C*-Ar), 126.6 (d, $^3J_{C\text{-}P}$ = 5.1 Hz *C*-Ar), 126.3 (*CH*-Ar), 126.0 (*CH*-Ar), 125.16 (*CH*-Ar), 125.11 (*CH*-Ar), 124.3 (*CH*-Ar), 124.2 (*CH*-Ar), 121.4 (*CH*-Ar), 121.3 (*CH*-Ar), 117.1 ($^2J_{C\text{-}P}$ = 11.1 Hz, *CH*═), 116.6 ($^2J_{C\text{-}P}$ = 10.7 Hz, *CH*═), 115.3 (d, $^3J_{C\text{-}P}$ = 3.5 Hz *CH*-Ar), 115.1 (d, $^3J_{C\text{-}P}$ = 3.9 Hz *CH*-Ar), 110.1 (*C*-5), 68.69 (*CH*$(CH_3)_2$), 68.65 (*CH*$(CH_3)_2$), 53.5 (d, $^4J_{C\text{-}P}$ = 2.7 Hz, CH_2-N), 53.2 (d, $^4J_{C\text{-}P}$ = 2.3 Hz, CH_2-N), 49.7 (*CH*CH_3 l-Ala), 49.5 (*CH*CH_3 l-Ala), 28.3 (d, $^1J_{C\text{-}P}$ = 129.0 Hz CH_2P), 28.1 (d, $^1J_{C\text{-}P}$ = 130.0 Hz CH_2P), 20.55 (CH$(CH_3)_2$), 20.54 (CH$(CH_3)_2$), 20.48 (CH$(CH_3)_2$), 20.40 (CH$(CH_3)_2$), 19.8 (d, $^3J_{C\text{-}P}$ = 5.5 Hz, CHCH_3 l-Ala), 19.1 (d, $^3J_{C\text{-}P}$ = 5.9 Hz, CHCH_3 l-Ala), 13.3 (d, $^4J_{C\text{-}P}$ = 2.3 Hz, CH_3, alkene), 13.2 (d, $^4J_{C\text{-}P}$ = 2.7 Hz, CH_3, alkene), 10.8 (CH_3, base). **HPLC**: Reverse phase HPLC eluting with gradient method CH_3CN/H_2O from 10/90 to 100/0 in 30 min, 1 ml/min, λ = 254 nm and 263 nm, showed one peak with Rt 16.26 min. **HRMS (ESI):** *m/z* $[M+Na]^+$ calcd for $C_{26}H_{32}N_3O_6P$: 536.1926, found: 536.1921.

From PrepHPLC also the *Z* isomer ***Z*-8a** was isolated as pale yellow foamy solid (6 mg, 3%).**^{31}P NMR (202 MHz, CD_3OD) δ_P**: 30.40, 29.66. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 8.19–8.13 (m, 1H, Ar*H*), 7.90–7.85 (m, 1H, Ar*H*), 7.78–7.67 (m, 1H, Ar*H*), 7.57–7.43 (m, 5H, Ar*H*, *H*-6), 5.73–5.65 (m, 1H, *CH*═), 4.97–4.86 (m, 1H, *CH*$(CH_3)_2$), 4.49 (bs, 2H, CH_2-N), 4.04–3.98 (m, 1H, *CH*CH_3 l-Ala), 3.24–3.07 (m, 2H, CH_2P), 1.76–1.70 (m 6H, CH_3, base; CH_3, alkene), 1.27 (d, *J* = 7.0 Hz, 1.5H, CHCH_3 l-Ala), 1.21–1.12 (m, 7.5H, CHCH_3 l-Ala, CH$(CH_3)_2$, CH$(CH_3)_2$). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.5 (d, $^3J_{C\text{-}P}$ = 3.9 Hz, *C*═O, ester), 173.1 (d, $^3J_{C\text{-}P}$ = 3.5 Hz, *C*═O, ester), 165.3 (*C*-4), 151.8 (*C*-2), 151.7 (*C*-2), 146.4 (d, $^2J_{C\text{-}P}$ = 10.2 Hz, *C*—O, Ph), 146.2 (d, $^2J_{C\text{-}P}$ = 10.8 Hz, *C*—O, Ph), 1401.1 (*C*-6), 141.0 (*C*-6), 134.9 (*C*-Ar), 134.8 (d, $^3J_{C\text{-}P}$ = 14.6 Hz, *C*═), 134.5 (d, $^3J_{C\text{-}P}$ = 14.6 Hz, *C*═), 127.4 (*CH*-Ar), 126.9 (d, $^3J_{C\text{-}P}$ = 4.8 Hz *C*-Ar), 126.8 (d, $^3J_{C\text{-}P}$ = 5.3 Hz *C*-Ar), 126.3 (*CH*-Ar), 126.04 (*CH*-Ar), 126.01 (*CH*-Ar), 125.156 (*CH*-Ar), 125.12 (*CH*-Ar), 124.5 (*CH*-Ar), 124.4 (*CH*-Ar), 121.5 (*CH*-Ar), 121.4 (*CH*-Ar), 119.1 ($^2J_{C\text{-}P}$ = 11.1 Hz, *CH*═), 119.0 ($^2J_{C\text{-}P}$ = 10.4 Hz, *CH*═), 115.7 (d, $^3J_{C\text{-}P}$ = 3.4 Hz *CH*-Ar), 115.4 (d, $^3J_{C\text{-}P}$ = 3.4 Hz *CH*-Ar), 110.0 (*C*-5), 68.6 (*CH*$(CH_3)_2$), 49.7 (*CH*CH_3 l-Ala), 49.5 (*CH*CH_3 l-Ala), 47.1 (CH_2-N), 28.2 (d, $^1J_{C\text{-}P}$ = 129.0 Hz CH_2P), 28.0 (d, $^1J_{C\text{-}P}$ = 129.8 Hz CH_2P), 20.51 (CH$(CH_3)_2$), 20.50 (CH$(CH_3)_2$), 20.4 (CH$(CH_3)_2$), 20.3 (CH$(CH_3)_2$), 19.8 (d, $^3J_{C\text{-}P}$ = 5.5 Hz, CHCH_3 l-Ala), 19.0 (d, $^3J_{C\text{-}P}$ = 5.5 Hz, CHCH_3 l-Ala), 10.7 (d, $^4J_{C\text{-}P}$ = 3.0 Hz, CH_3, alkene), 10.6 (CH_3, base). **HPLC**: Reverse phase HPLC eluting with gradient method CH_3CN/H_2O from 10/90 to 100/0 in 30 min, 1 ml/min, λ = 254 nm and 263 nm, showed one peak with Rt 17.90 min.

*4.1.3.2. (E)-N^1-(4′-O-(1-naphthyl)-(benzyloxy-L-alanine)-phosphinyl-2′-methyl-but-2′-enyl)thymine (**E-8b**)*. Prepared according to the standard procedure **C** for the synthesis of ANP |ProTide using *O*-(1-naphthyl)-(benzyloxy-*L*-alanine)-allylphosphonate **3b** (240 mg, 586.1 μmol) and N^1-2′-methylallylthymine (211 mg, 1.17 mmol) and Hoveyda-Grubbs 2nd generation catalyst (15 mol%) in dry CH_2Cl_2 (10 ml). After evaporation, the crude was purified by Biotage Isolera One (120 g ZIP cartridge KP-SIL, 100 ml/min, gradient eluent system MeOH/CH_2Cl_2 1% 1CV, 1–10% 12CV, 10% 2CV), to afford a mixture of the *E* and *Z* isomers. The two isomers were then separated by PrepHPLC (20 ml/min, isocratic eluting system CH_3CN/H_2O – 40/60, 30 min), to afford the title compound as pale yellow foamy solid (43 mg, 13%). R_f = 0.40 (CH_2Cl_2/MeOH – 95:5).**^{31}P NMR (202 MHz, CD_3OD) δ_P**: 30.35, 29.51. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 8.12–8.10 (m, 1H, Ar*H*), 7.88–7.87 (m, 1H, Ar*H*), 7.70–7.66 (m, 1H, Ar*H*), 7.54–7.22 (m, 10*H*, Ar*H*), 5.53–5.45 (m, 1H, *CH*═), 5.12, 5.06 (ABq, J_{AB} = 12.2 Hz, 1H, CH_2Ph), 4.99, 4.95 (ABq, J_{AB} = 12.2 Hz, 1H, CH_2Ph), 4.26–4.20 (m, 2H, CH_2-N), 4.11–4.06 (m, 1H, *CH*CH_3 l-Ala), 3.02–2.86 (m, 2H, CH_2P), 1.74 (s, 3H, CH_3, base), 1.64 (d, *J* = 3.6 Hz 1.5H, CH_3, alkene), 1.61 (d, *J* = 3.5 Hz 1.5H, CH_3, alkene), 1.26 (d, *J* = 6.9 Hz, 1.5H, CHCH_3 l-Ala), 1.18 (d, *J* = 7.2 Hz, 1.5H, CHCH_3 l-Ala). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.7 (d, $^3J_{C\text{-}P}$ = 3.8 Hz, *C*═O, ester), 173.3 (d, $^3J_{C\text{-}P}$ = 3.7 Hz, *C*═O, ester), 165.35 (*C*-4), 165.32 (*C*-4), 151.67 (*C*-2), 151.60 (*C*-2), 146.5 (d, $^2J_{C\text{-}P}$ = 9.7 Hz, *C*—O, Ph), 146.3 (d, $^2J_{C\text{-}P}$ = 9.9 Hz, *C*—O, Ph), 140.9 (*C*-6), 135.8 (*C*-Ar), 135.7 (*C*-Ar), 135.4 (d, $^3J_{C\text{-}P}$ = 14.4 Hz, *C*═), 135.2 (d, $^3J_{C\text{-}P}$ = 14.7 Hz, *C*═), 134.9 (*C*-Ar), 128.19 (*CH*-Ar), 128.12 (*CH*-Ar), 127.9 (*CH*-Ar), 127.8 (*CH*-Ar), 127.5 (*CH*-Ar), 127.4 (*CH*-Ar), 126.7 (d, $^3J_{C\text{-}P}$ = 5.0 Hz *C*-Ar), 126.6 (d, $^3J_{C\text{-}P}$ = 5.3 Hz *C*-Ar), 126.3

(*CH*-Ar), 126.0 (*CH*-Ar), 125.19 (*CH*-Ar), 125.11 (*CH*-Ar), 124.3 (*CH*-Ar), 124.2 (*CH*-Ar), 121.4 (*CH*-Ar), 121.2 (*CH*-Ar), 117.1 ($^2J_{C-P}$ = 10.7 Hz, *CH*═), 116.6 ($^2J_{C-P}$ = 10.7 Hz, *CH*═), 115.4 (d, $^3J_{C-P}$ = 4.0 Hz *CH*-Ar), 115.1 (d, $^3J_{C-P}$ = 3.6 Hz *CH*-Ar), 110.1 (*C*-5), 66.5 (CH_2Ph), 66.4 (CH_2Ph), 53.4 (d, $^4J_{C-P}$ = 2.0 Hz, CH_2-N), 53.2 (d, $^4J_{C-P}$ = 2.2 Hz, CH_2-N), 49.6 (*CH*CH_3 l-Ala), 49.5 (*CH*CH_3 l-Ala), 28.3 (d, $^1J_{C-P}$ = 129.3 Hz CH_2P), 28.1 (d, $^1J_{C-P}$ = 130.2 Hz CH_2P), 19.6 (d, $^3J_{C-P}$ = 5.6 Hz, CHCH_3 l-Ala), 19.0 (d, $^3J_{C-P}$ = 5.3 Hz, CHCH_3 l-Ala), 13.3 (d, $^4J_{C-P}$ = 2.4 Hz, CH_3, alkene), 13.2 (d, $^4J_{C-P}$ = 2.4 Hz, CH_3, alkene), 10.8 (CH_3, base). **HPLC**: Reverse phase HPLC eluting with gradient method CH_3CN/H_2O from 10/90 to 100/0 in 30 min, 1 ml/min, λ = 254 nm and 263 nm, showed one peak with Rt 18.01 min. **HRMS (ESI):** *m/z* $[M+Na]^+$ calcd for $C_{30}H_{32}N_3O_6P$: 584.1926, found: 584.1921.

*4.1.3.3. (E)-N^1-(4′-O-Phenyl-(isopropyloxy-L-alanine)-phosphinyl-2′-methyl-but-2′-enyl)thymine (**E-8c**)*. Prepared according to the standard procedure **C** for the synthesis of ANP ProTide using *O*-phenyl-(isopropyloxy-*L*-alanine)-allylphosphonate **3c** (140 mg, 449.7 μmol) and N^1-2′-methylallylthymine (162 mg, 899.4 μmol) and Hoveyda-Grubbs 2nd generation catalyst (15 mol%) in dry CH_2Cl_2 (8 ml). After evaporation, the crude was purified by Biotage Isolera One (25 g SNAP cartridge ULTRA, 75 ml/min, gradient eluent system MeOH/CH_2Cl_2 1% 1CV, 1–10% 12CV, 10% 2CV), to afford a mixture of the *E* and *Z* isomers. The two isomers were then separated by PrepHPLC (20 ml/min, gradient eluting system CH_3CN/H_2O from 10/90 to 100/0, 30 min), to afford the title compound as pale yellow foamy solid (20.4 mg, 10%). R_f = 0.27 (CH_2Cl_2/MeOH – 94:6). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 29.80, 29.03. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 7.38–7.33 (m, 3H, *H*-6, Ar*H*), 7.22–7.16 (m, 3H, Ar*H*), 5.52 (q, *J* = 6.9 Hz, 0.4H, *CH*═), 5.43 (q, *J* = 6.9 Hz, 0.6H, *CH*═), 4.98 (sept, *J* = 6.2 Hz, 0.4H, *CH*$(CH_3)_2$), 4.92 (sept, *J* = 6.2 Hz, 0.6H, *CH*$(CH_3)_2$), 4.36–4.30 (m, 2H, CH_2-N), 3.97–3.91 (m, 1H, *CH*CH_3 l-Ala), 2.96–2.77 (m, 2H, CH_2P), 1.85 (s, 3H, CH_3, base), 1.72 (s, 1.2H, CH_3, alkene), 1.71 (s, 1.8H, CH_3, alkene), 1.29 (d, *J* = 6.9 Hz, 1.8H, CHCH_3 l-Ala), 1.25 (d, *J* = 6.3 Hz, 1.2H, CH$(CH_3)_2$), 1.23 (d, *J* = 6.2 Hz, 1.2H, CH$(CH_3)_2$), 1.21–1.96 (m, 4.8H, CHCH_3 l-Ala, CH$(CH_3)_2$). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.6 (d, $^3J_{C-P}$ = 4.6 Hz, *C*═O, ester), 173.2 (d, $^3J_{C-P}$ = 4.1 Hz, *C*═O, ester), 165.3 (*C*-4), 151.7 (*C*-2), 151.6 (*C*-2), 150.5 (d, $^2J_{C-P}$ = 9.8 Hz, *C*—O, Ph), 150.3 (d, $^2J_{C-P}$ = 9.5 Hz, *C*—O, Ph), 141.0 (*C*-6), 135.4 (d, $^3J_{C-P}$ = 14.4 Hz, *C*═), 135.0 (d, $^3J_{C-P}$ = 14.4 Hz, *C*═), 129.32 (*CH*-Ar), 129.30 (*CH*-Ar), 124.5 (*CH*-Ar), 124.4 (*CH*-Ar), 120.6 (d, $^3J_{C-P}$ = 4.3 Hz *CH*-Ar), 120.4 (d, $^3J_{C-P}$ = 4.6 Hz *CH*-Ar), 117.2 (d, $^2J_{C-P}$ = 11.0 Hz, *CH*═), 116.6 (d, $^2J_{C-P}$ = 10.8 Hz, *CH*═), 110.1 (*C*-5), 68.67 (*CH*$(CH_3)_2$), 68.63 (*CH*$(CH_3)_2$), 53.5 (d, $^4J_{C-P}$ = 2.4 Hz, CH_2-N), 53.3 (d, $^4J_{C-P}$ = 2.4 Hz, CH_2-N), 49.6 (*CH*CH_3 l-Ala), 49.4 (*CH*CH_3 l-Ala), 28.2 (d, $^1J_{C-P}$ = 129.5 Hz, CH_2P), 28.0 (d, $^1J_{C-P}$ = 130.5 Hz, CH_2P), 20.58 (CH$(CH_3)_2$), 20.53 (CH$(CH_3)_2$), 20.4 (CH$(CH_3)_2$), 19.8 (d, $^3J_{C-P}$ = 5.4 Hz, CHCH_3 l-Ala), 19.1 (d, $^3J_{C-P}$ = 5.4 Hz, CHCH_3 l-Ala), 13.2 (d, $^4J_{C-P}$ = 2.5 Hz, CH_3, alkene), 13.1 (d, $^4J_{C-P}$ = 2.2 Hz, CH_3, alkene), 10.8 (CH_3, base). **HPLC**: Reverse phase HPLC eluting with gradient method CH_3CN/H_2O from 10/90 to 100/0 in 30 min, 1 ml/min, λ = 254 nm and 263 nm, showed one peak with Rt 13.94 min. **HRMS (ESI):** *m/z* $[M+Na]^+$ calcd for $C_{22}H_{30}N_3O_6P$: 486.1770, found: 486.1764.

*4.1.3.4. (E)-N^1-(4′-O-Phenyl-(benzyloxy-L-alanine)-phosphinyl-2′-methyl-but-2′-enyl)thymine (**E-8d**)*. Prepared according to the standard procedure **C** for the synthesis of ANP ProTide using *O*-phenyl-(benzyloxy-*L*-alanine)-allylphosphonate **3d** (200 mg, 556.5 μmol) and N^1-2′-methylallylthymine (200.6 mg, 1.11 mmol) and Hoveyda-Grubbs 2nd generation catalyst (15 mol%) in dry CH_2Cl_2 (8 ml). After evaporation, the crude was purified by Biotage Isolera One (25 g SNAP cartridge ULTRA, 75 ml/min, gradient eluent system 2-propanol/CH_2Cl_2 1% 1CV, 1–10% 12CV, 10% 2CV), to afford a mixture of the *E* and *Z* isomers. The two isomers were then separated by PrepHPLC (20 ml/min, isocratic eluting system CH_3CN/H_2O – 35/65, 30 min), to afford the title compound as pale yellow foamy solid (64 mg, 23%). R_f = 0.42 (CH_2Cl_2/2-propanol – 95:5). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 29.79, 28.99. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 7.36–7.29 (m, 8H, *H*-6, Ar*H*), 7.20–7.14 (m, 3H, Ar*H*), 5.49–5.40 (m, 1H, *CH*═), 5.16, 5.13 (ABq, J_{AB} = 12.3 Hz, 1H, CH_2Ph), 5.08 (s app, 1H, CH_2Ph), 4.28–4.23 (m, 2H, CH_2-N), 4.07–4.01 (m, 1H, *CH*CH_3 l-Ala), 2.89–2.73 (m, 2H, CH_2P), 1.84 (s, 3H, CH_3, base), 1.67–1.64 (m, 3H, CH_3, alkene), 1.30 (d, *J* = 7.0 Hz, 1.5H, CHCH_3 l-Ala), 1.22 (d, *J* = 7.1 Hz, 1.5H, CHCH_3 l-Ala). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.8 (d, $^3J_{C-P}$ = 4.5 Hz, *C*═O, ester), 173.4 (d, $^3J_{C-P}$ = 3.9 Hz, *C*═O, ester), 165.3 (*C*-4), 151.7 (*C*-2), 151.6 (*C*-2), 150.5 (d, $^2J_{C-P}$ = 9.3 Hz, *C*—O, Ph), 150.4 (d, $^2J_{C-P}$ = 9.4 Hz, *C*—O, Ph), 140.98 (*C*-6), 140.97 (*C*-6), 135.9 (*C*-Ar), 135.8 (*C*-Ar), 135.3 (d, $^3J_{C-P}$ = 14.1 Hz, *C*═), 135.0 (d, $^3J_{C-P}$ = 14.0 Hz, *C*═), 129.35 (*CH*-Ar), 129.34 (*CH*-Ar), 128.23 (*CH*-Ar), 128.20 (*CH*-Ar), 128.01 (*CH*-Ar), 128.00 (*CH*-Ar), 127.96 (*CH*-Ar), 127.95 (*CH*-Ar), 124.6 (*CH*-Ar), 124.5 (*CH*-Ar), 120.6 (d, $^3J_{C-P}$ = 4.3 Hz *CH*-Ar), 120.4 (d, $^3J_{C-P}$ = 3.8 Hz *CH*-Ar), 117.2 (d, $^2J_{C-P}$ = 10.7 Hz, *CH*═), 116.6 (d, $^2J_{C-P}$ = 10.7 Hz, *CH*═), 110.13 (*C*-5), 110.11 (*C*-5), 65.5 (CH_2Ph), 66.4 (CH_2Ph), 53.5 (d, $^4J_{C-P}$ = 2.4 Hz, CH_2-N), 53.3 (d, $^4J_{C-P}$ = 2.3 Hz, CH_2-N), 49.6 (*CH*CH_3 l-Ala), 49.4 (*CH*CH_3 l-Ala), 28.2 (d, $^1J_{C-P}$ = 129.7 Hz, CH_2P), 28.0 (d, $^1J_{C-P}$ = 130.3 Hz, CH_2P), 19.7 (d, $^3J_{C-P}$ = 5.3 Hz, CHCH_3 l-Ala), 19.1 (d, $^3J_{C-P}$ = 5.3 Hz, CHCH_3 l-Ala), 13.3 (d, $^4J_{C-P}$ = 1.8 Hz, CH_3, alkene), 13.1 (d, $^4J_{C-P}$ = 2.2 Hz, CH_3, alkene), 10.9 (CH_3, base). **HPLC**: Reverse phase HPLC eluting with gradient method CH_3CN/H_2O from 10/90 to 100/0 in 30 min, 1 ml/min, λ = 254 nm and 263 nm, showed one peak with Rt 15.21 min. **HRMS (ESI):** *m/z* $[M+Na]^+$ calcd for $C_{26}H_{30}N_3O_6P$: 534.1764, found: 534.1764.

*4.1.3.5. (E)-N^1-(4′-O-(5,6,7,8-Tetrahydro-1-naphthyl)-(isopropyloxy-L-alanine)-phosphinyl-2′-methyl-but-2′-enyl)thymine (**E-8e**) and (Z)-N^1-(4′-O-(5,6,7,8-tetrahydro-1-naphthyl)-(isopropyloxy-L-alanine)-phosphinyl-2′-methyl-but-2′-enyl)thymine (**Z-8e**)*. Prepared according to the standard procedure **C** for the synthesis of ANP ProTide using *O*-(5,6,7,8-tetrahydro-1-naphthyl)-(isopropyloxy-*L*-alanine)-allylphosphonate **3e** (200 mg, 547.3 μmol) and N^1-2′-methylallylthymine (197 mg, 1.09 mmol) and Hoveyda-Grubbs 2nd generation catalyst (15 mol%) in dry CH_2Cl_2 (10 ml). After evaporation, the crude was purified by Biotage Isolera One (25 g SNAP cartridge ULTRA, 75 ml/min, gradient eluent system 2-propanol/CH_2Cl_2 1% 1CV, 1–10% 12CV, 10% 2CV), to afford a mixture of the *E* and *Z* isomers. The two isomers were then separated by PrepHPLC (20 ml/min, isocratic eluting system CH_3CN/H_2O – 35/65, 30 min), to afford the title compound *E* as pale yellow foamy solid (72 mg, 26%). R_f = 0.26 (CH_2Cl_2/2-propanol – 95:5). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 29.35, 28.55. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 7.34 (s, 0.5H, *H*-6), 7.33 (s, 0.5H, *H*-6), 7.17–7.12 (m, 1H, Ar*H*), 7.05–7.00 (m, 1H, Ar*H*), 6.89–6.86 (m, 1H, Ar*H*), 5.57–5.52 (m, 0.5H, *CH*═), 5.50–5.44 (m, 0.5H, *CH*═), 5.01–4.88 (m, 1H, *CH*$(CH_3)_2$), 4.36–4.29 (m, 2H, CH_2-N), 3.99–3.91 (m, 1H, *CH*CH_3 l-Ala), 2.94–2.82 (m, 2H, CH_2P), 2.77–2.74 (m, 2H, Ar*H*), 2.69–2.67 (m, 2H, Ar*H*), 1.84 (s, 3H, CH_3, base), 1.80–1.76 (m, 4H, Ar*H*), 1.70 (d, *J* = 2.9 Hz 3H, CH_3, alkene), 1.29 (d, *J* = 7.2 Hz, 1.5H, CHCH_3 l-Ala), 1.25–1.24 (m, 4.5H, CHCH_3 l-Ala, CH$(CH_3)_2$), 1.19 (d, *J* = 6.2 Hz, 3H, CH$(CH_3)_2$). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.7 (d, $^3J_{C-P}$ = 3.8 Hz, *C*═O, ester), 173.2 (d, $^3J_{C-P}$ = 4.1 Hz, *C*═O, ester), 165.3 (*C*-4), 151.7 (*C*-2), 151.6 (*C*-2), 148.8 (d, $^2J_{C-P}$ = 9.4 Hz, *C*—O, Ph), 148.7 (d, $^2J_{C-P}$ = 9.9 Hz, *C*—O, Ph), 141.05 (*C*-6), 141.02 (*C*-6), 139.19 (*C*-Ar), 139.16 (*C*-Ar), 135.1 (d, $^3J_{C-P}$ = 14.1 Hz, *C*═), 134.8 (d, $^3J_{C-P}$ = 14.3 Hz, *C*═), 128.4 (d, $^3J_{C-P}$ = 5.5 Hz *C*-Ar), 128.3 (d, $^3J_{C-P}$ = 5.8 Hz *C*-Ar), 125.4 (*CH*-Ar), 125.3 (*CH*-Ar), 125.1 (*CH*-Ar), 125.0 (*CH*-Ar), 117.4 ($^2J_{C-P}$ = 11.0 Hz, *CH*═), 116.9 ($^2J_{C-P}$ = 10.2 Hz, *CH*═), 116.8 (d, $^3J_{C-P}$ = 4.4 Hz *CH*-Ar), 116.7 (d, $^3J_{C-P}$ = 3.3 Hz *CH*-Ar), 110.1 (*C*-5), 110.1 (*C*-5), 68.66 (*CH*$(CH_3)_2$), 68.62 (*CH*$(CH_3)_2$), 53.6 (d, $^4J_{C-P}$ = 2.4 Hz, CH_2-N), 53.3 (d, $^4J_{C-P}$ = 2.4 Hz, CH_2-N), 49.7 (*CH*CH_3 l-Ala), 49.5 (*CH*CH_3 l-Ala), 29.1 (CH_2-Ar), 28.5 (d, $^1J_{C-P}$ = 129.8 Hz

CH$_2$P), 28.2 (d, $^1J_{C\text{-}P}$ = 131.2 Hz *CH*$_2$P), 23.5 (*CH*$_2$-Ar), 22.47 (*CH*$_2$-Ar), 22.44 (*CH*$_2$-Ar), 22.42 (*CH*$_2$-Ar), 20.6 (CH(*CH*$_3$)$_2$), 20.56 (CH(*CH*$_3$)$_2$), 20.55 (CH(*CH*$_3$)$_2$), 20.4 (CH(*CH*$_3$)$_2$), 19.9 (d, $^3J_{C\text{-}P}$ = 5.2 Hz, CH*CH*$_3$ l-Ala), 19.1 (d, $^3J_{C\text{-}P}$ = 5.8 Hz, CH*CH*$_3$ l-Ala), 13.3 (d, $^4J_{C\text{-}P}$ = 2.4 Hz, *CH*$_3$, alkene), 13.2 (d, $^4J_{C\text{-}P}$ = 2.2 Hz, *CH*$_3$, alkene), 10.8 (*CH*$_3$, base). **HPLC**: Reverse phase HPLC eluting with gradient method CH_3CN/H_2O from 10/90 to 100/0 in 30 min, 1 ml/min, λ = 254 nm and 263 nm, showed one peak with Rt 16.85 min. **HRMS (ESI):** *m/z* $[M+Na]^+$ calcd for $C_{26}H_{36}N_3O_6P$: 540.2239, found: 540.2234.

From PrepHPLC also the *Z* isomer ***Z*-8e** was isolated as pale yellow foamy solid (7 mg, 3%). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 29.41, 28.64. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 7.33 (s, 1H, *H*-6), 7.05–7.00 (m, 1H, Ar*H*), 6.95–6.89 (m, 1H, Ar*H*), 6.80–6.76 (m, 1H, Ar*H*), 5.53–5.48 (m, 1H, *CH*═), 4.88–4.77 (m, 1H, *CH*(CH$_3$)$_2$), 4.38–4.27 (m, 2H, *CH*$_2$-N), 3.86–3.81 (m, 1H, *CH*CH$_3$ l-Ala), 2.98–2.82 (m, 2H, *CH*$_2$P), 2.65–2.62 (m, 4H, Ar*H*), 1.71–1.68 (m, 7H, Ar*H*, CH$_3$, base), 1.61–1.60 (m, 3H, *CH*$_3$, alkene), 1.20–1.07 (m, 9H, CH*CH*$_3$ l-Ala, CH(*CH*$_3$)$_2$). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.7 (d, $^3J_{C\text{-}P}$ = 3.8 Hz, *C*═O, ester), 173.3 (d, $^3J_{C\text{-}P}$ = 3.8 Hz, *C*═O, ester), 165.3 (*C*-4), 151.7 (*C*-2), 151.6 (*C*-2), 148.8 (d, $^2J_{C\text{-}P}$ = 9.5 Hz, *C*—O, Ph), 148.7 (d, $^2J_{C\text{-}P}$ = 9.9 Hz, *C*—O, Ph), 141.05 (*C*-6), 141.02 (*C*-6), 139.19 (*C*-Ar), 139.16 (*C*-Ar), 135.1 (d, $^3J_{C\text{-}P}$ = 14.2 Hz, *C*═), 134.8 (d, $^3J_{C\text{-}P}$ = 14.2 Hz, *C*═), 128.4 (d, $^3J_{C\text{-}P}$ = 5.1 Hz *C*-Ar), 128.3 (d, $^3J_{C\text{-}P}$ = 5.6 Hz *C*-Ar), 125.4 (*CH*-Ar), 125.3 (*CH*-Ar), 125.1 (*CH*-Ar), 125.0 (*CH*-Ar), 119.3 ($^2J_{C\text{-}P}$ = 11.3 Hz, *CH*═), 119.2 ($^2J_{C\text{-}P}$ = 11.0 Hz, *CH*═), 117.2 (d, $^3J_{C\text{-}P}$ = 3.5 Hz *CH*-Ar), 117.0 (d, $^3J_{C\text{-}P}$ = 3.5 Hz *CH*-Ar), 110.0 (*C*-5), 68.66 (*CH*(CH$_3$)$_2$), 68.63 (*CH*(CH$_3$)$_2$), 49.7 (*CH*CH$_3$ l-Ala), 49.5 (*CH*CH$_3$ l-Ala), 47.3 (*CH*$_2$-N), 29.1 (*CH*$_2$-Ar), 28.5 (d, $^1J_{C\text{-}P}$ = 129.8 Hz *CH*$_2$P), 28.2 (d, $^1J_{C\text{-}P}$ = 131.2 Hz *CH*$_2$P), 26.4 (*CH*$_2$-Ar), 26.3 (*CH*$_2$-Ar), 25.8 (*CH*$_2$-Ar), 25.7 (*CH*$_2$-Ar), 20.5 (*CH*$_3$, alkene), 20.4 (*CH*$_3$, alkene), 19.97 (CH(*CH*$_3$)$_2$), 19.93 (CH(*CH*$_3$)$_2$), 19.7 (d, $^3J_{C\text{-}P}$ = 5.2 Hz, CH*CH*$_3$ l-Ala), 19.0 (d, $^3J_{C\text{-}P}$ = 5.8 Hz, CH*CH*$_3$ l-Ala), 10.7 (*CH*$_3$, base). **HPLC**: Reverse phase HPLC eluting with gradient method CH_3CN/H_2O from 10/90 to 100/0 in 30 min, 1 ml/min, λ = 254 nm and 263 nm, showed one peak with Rt 17.90 min.

*4.1.3.6. (E)-N^1-(4′-O-(5,6,7,8-Tetrahydro-1-naphthyl)-(benzyloxy-L-alanine)-phosphinyl-2′-methyl-but-2′-enyl)thymine (**E-8f**) and (Z)-N^1-(4′-O-(5,6,7,8-tetrahydro-1-naphthyl)-(benzyloxy-L-alanine)-phosphinyl-2′-methyl-but-2′-enyl)thymine (**Z-8f**)*. Prepared according to the standard procedure **C** for the synthesis of ANP ProTide using *O*-(5,6,7,8-tetrahydro-1-naphthyl)-(benzyloxy-L-alanine)-allylphosphonate **3f** (200 mg, 483.7 μmol) and *N*^{1}-2′-methylallylthymine (174 mg, 967.4 μmol) and Hoveyda-Grubbs 2nd generation catalyst (15 mol%) in dry CH_2Cl_2 (8 ml). After evaporation, the crude was purified by Biotage Isolera One (25 g SNAP cartridge ULTRA, 75 ml/min, gradient eluent system 2-propanol/CH_2Cl_2 1% 1CV, 1–10% 12CV, 10% 2CV), to afford a mixture of the *E* and *Z* isomers. The two isomers were then separated by reverse Biotage Isolera One (60 g SNAP cartridge KP-C18-HS, 100 ml/min, isocratic eluent system CH_3CN/H_2O 30–60% 12CV) to afford the title compound *E* as pale yellow foamy solid (36 mg, 14%). R_f = 0.23 (CH_2Cl_2/2-propanol – 95:5). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 29.36, 28.51. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 7.36–7.28 (m, 6H, *H*-6, Ar*H*), 7.16–7.12 (m, 1H, Ar*H*), 7.04–6.95 (m, 1H, Ar*H*), 6.89–6.85 (m, 1H, Ar*H*), 5.49–5.42 (m, 1H, *CH*═), 5.15, 5.12 (ABq, J_{AB} = 12.2 Hz, 1H, *CH*$_2$Ph), 5.07, 5.05 (ABq, J_{AB} = 12.6 Hz, 1H, *CH*$_2$Ph), 4.31–4.22 (m, 2H, *CH*$_2$-N), 4.09–4.00 (m, 1H, *CH*CH$_3$ l-Ala), 2.90–2.77 (m, 2H, *CH*$_2$P), 2.74 (bs, 2H, Ar*H*), 2.66 (bs, 2H, Ar*H*), 1.83 (s, 3H, CH$_3$, base), 1.76–1.75 (m, 4H, Ar*H*), 1.66 (d, *J* = 2.9 Hz 1.8H, *CH*$_3$, alkene), 1.64 (d, *J* = 3.1 Hz 1.2H, *CH*$_3$, alkene), 1.31 (d, *J* = 7.0 Hz, 1.5H, CH*CH*$_3$ l-Ala), 1.26 (d, *J* = 7.1 Hz, 1.5H, CH*CH*$_3$ l-Ala). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.8 (d, $^3J_{C\text{-}P}$ = 3.8 Hz, *C*═O, ester), 173.4 (d, $^3J_{C\text{-}P}$ = 3.5 Hz, *C*═O, ester), 165.38 (*C*-4), 165.37 (*C*-4), 151.7 (*C*-2), 151.6 (*C*-2), 148.8 (d, $^2J_{C\text{-}P}$ = 9.8 Hz, *C*—O, Ph), 148.7 (d, $^2J_{C\text{-}P}$ = 9.5 Hz, *C*—O, Ph), 140.9 (*C*-6), 139.2 (*C*-Ar), 139.1 (*C*-Ar), 135.9 (*C*-Ar), 135.8 (*C*-Ar), 135.1 (d, $^3J_{C\text{-}P}$ = 14.5 Hz, *C*═), 134.8 (d, $^3J_{C\text{-}P}$ = 13.9 Hz, *C*═), 128.4 (d, $^3J_{C\text{-}P}$ = 5.4 Hz *C*-Ar), 128.3 (d, $^3J_{C\text{-}P}$ = 5.7 Hz *C*-Ar), 128.2 (*CH*-Ar), 128.1 (*CH*-Ar), 127.96 (*CH*-Ar), 127.92 (*CH*-Ar), 127.8 (*CH*-Ar), 125.4 (*CH*-Ar), 125.3 (*CH*-Ar), 125.1 (*CH*-Ar), 125.0 (*CH*-Ar), 117.4 ($^2J_{C\text{-}P}$ = 10.9 Hz, *CH*═), 116.8 ($^2J_{C\text{-}P}$ = 10.4 Hz, *CH*═), 116.7 (d, $^3J_{C\text{-}P}$ = 3.2 Hz *CH*-Ar), 116.6 (d, $^3J_{C\text{-}P}$ = 3.2 Hz *CH*-Ar), 110.09 (*C*-5), 110.06 (*C*-5), 66.5 (*CH*$_2$Ph), 66.4 (*CH*$_2$Ph), 53.5 (d, $^4J_{C\text{-}P}$ = 2.1 Hz, *CH*$_2$-N), 53.3 (d, $^4J_{C\text{-}P}$ = 2.4 Hz, *CH*$_2$-N), 49.6 (*CH*CH$_3$ l-Ala), 49.5 (*CH*CH$_3$ l-Ala), 29.1 (*CH*$_2$-Ar), 28.2 (d, $^1J_{C\text{-}P}$ = 130.8 Hz *CH*$_2$P), 28.2 (d, $^1J_{C\text{-}P}$ = 130.8 Hz *CH*$_2$P), 23.3 (*CH*$_2$-Ar), 22.45 (*CH*$_2$-Ar), 22.43 (*CH*$_2$-Ar), 22.40 (*CH*$_2$-Ar), 19.7–19.6 (m, CH*CH*$_3$ l-Ala, *CH*$_3$, alkene), 19.0 (d, $^3J_{C\text{-}P}$ = 5.7 Hz, CH*CH*$_3$ l-Ala), 10.8 (*CH*$_3$, base). **HPLC**: Reverse phase HPLC eluting with gradient method CH_3CN/H_2O from 10/90 to 100/0 in 30 min, 1 ml/min, λ = 254 nm and 263 nm, showed one peak with Rt 18.44 min. **HRMS (ESI):** *m/z* $[M+Na]^+$ calcd for $C_{30}H_{36}N_3O_6P$: 588.2239, found: 588.2234.

From PrepHPLC also the *Z* isomer ***Z*-8f** was isolated as pale yellow foamy solid (18 mg, 7%). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 29.38, 28.63. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 7.42–7.33 (m, 6H, *H*-6, Ar*H*), 7.15–7.12 (m, 1H, Ar*H*), 7.07–6.95 (m, 1H, Ar*H*), 6.92–6.86 (m, 1H, Ar*H*), 5.60–5.55 (m, 1H, *CH*═), 5.15 (AB app s, 1H, *CH*$_2$Ph), 5.07 (AB app s, 1H, *CH*$_2$Ph), 4.46–4.26 (m, 2H, *CH*$_2$-N), 4.11–4.03 (m, 1H, *CH*CH$_3$ l-Ala), 3.07–2.90 (m, 2H, *CH*$_2$P), 2.76–2.70 (m, 4H, Ar*H*), 1.83–1.77 (m, 7H, Ar*H*, CH$_3$, base), 1.69 (d, *J* = 5.2 Hz 1.8H, *CH*$_3$, alkene), 1.66 (d, *J* = 5.2 Hz 1.2H, *CH*$_3$, alkene), 1.34 (d, *J* = 6.9 Hz, 1.5H, CH*CH*$_3$ l-Ala), 1.24 (d, *J* = 6.9 Hz, 1.5H, CH*CH*$_3$ l-Ala). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.8 (d, $^3J_{C\text{-}P}$ = 3.8 Hz, *C*═O, ester), 173.4 (d, $^3J_{C\text{-}P}$ = 3.5 Hz, *C*═O, ester), 165.3 (*C*-4), 151.7 (*C*-2), 148.8 (d, $^2J_{C\text{-}P}$ = 9.2 Hz, *C*—O, Ph), 148.7 (d, $^2J_{C\text{-}P}$ = 9.2 Hz, *C*—O, Ph), 141.1 (*C*-6), 141.0 (*C*-6), 139.25 (*C*-Ar), 139.21 (*C*-Ar), 135.9 (*C*-Ar), 135.8 (*C*-Ar), 134.4 (d, $^3J_{C\text{-}P}$ = 14.3 Hz, *C*═), 134.2 (d, $^3J_{C\text{-}P}$ = 13.5 Hz, *C*═), 128.7 (d, $^3J_{C\text{-}P}$ = 5.9 Hz *C*-Ar), 128.6 (d, $^3J_{C\text{-}P}$ = 5.0 Hz *C*-Ar), 128.18 (*CH*-Ar), 128.15 (*CH*-Ar), 127.94 (*CH*-Ar), 127.91 (*CH*-Ar), 127.87 (*CH*-Ar), 127.84 (*CH*-Ar), 125.4 (*CH*-Ar), 125.3 (*CH*-Ar), 125.2 (*CH*-Ar), 125.0 (*CH*-Ar), 119.2 ($^2J_{C\text{-}P}$ = 10.9 Hz, *CH*═), 119.0 ($^2J_{C\text{-}P}$ = 11.8 Hz, *CH*═), 117.2 (d, $^3J_{C\text{-}P}$ = 3.3 Hz *CH*-Ar), 116.9 (d, $^3J_{C\text{-}P}$ = 2.5 Hz *CH*-Ar), 110.05 (*C*-5), 110.02 (*C*-5), 66.5 (*CH*$_2$Ph), 66.4 (*CH*$_2$Ph), 49.7 (*CH*CH$_3$ l-Ala), 49.5 (*CH*CH$_3$ l-Ala), 47.2 (*CH*$_2$-N), 29.1 (*CH*$_2$-Ar), 28.3 (d, $^1J_{C\text{-}P}$ = 129.4 Hz *CH*$_2$P), 28.1 (d, $^1J_{C\text{-}P}$ = 130.2 Hz *CH*$_2$P), 26.4 (*CH*$_2$-Ar), 26.3 (*CH*$_2$-Ar), 25.8 (*CH*$_2$-Ar), 25.7 (*CH*$_2$-Ar), 19.7–19.6 (m, CH*CH*$_3$ l-Ala, *CH*$_3$, alkene), 18.8 (d, $^3J_{C\text{-}P}$ = 5.9 Hz, CH*CH*$_3$ l-Ala), 10.7 (*CH*$_3$, base). **HPLC**: Reverse phase HPLC eluting with gradient method CH_3CN/H_2O from 10/90 to 100/0 in 30 min, 1 ml/min, λ = 254 nm and 263 nm, showed one peak with Rt 19.31 min.

*4.1.3.7. (E)-N^1-(4′-O-(1-Naphthyl)-(isopropyloxy-L-alanine)-phosphinyl-2′-methyl-but-2′-enyl)uracil (**E-10a**)*. Prepared according to the standard procedure **C** for the synthesis of ANP ProTide using *O*-(1-naphthyl)-(isopropyloxy-L-alanine)-allylphosphonate **3a** (150 mg, 415 μmol) and *N*^{1}-2′-methylallyluracil (137 mg, 830.1 μmol) and Hoveyda-Grubbs 2nd generation catalyst (15 mol%) in dry CH_2Cl_2 (8 ml). After evaporation, the crude was purified by Biotage Isolera One (50 g SNAP cartridge ULTRA, 100 ml/min, gradient eluent system MeOH/CH_2Cl_2 1% 1CV, 1–10% 12CV, 10% 2CV), to afford a mixture of the *E* and *Z* isomers. The two isomers were then separated by PrepHPLC (20 ml/min, isocratic eluting system CH_3CN/H_2O – 35/65, 30 min), to afford the title compound as pale yellow foamy solid (28 mg, 14%). R_f = 0.24 (CH_2Cl_2/MeOH – 96:4). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 30.28, 29.49. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 8.14–8.13 (m, 1H, Ar*H*), 7.88–7.84 (m, 1H, Ar*H*), 7.70–7.67 (m, 1H, Ar*H*), 7.58–7.49 (m, 3H, Ar*H*), 7.44–7.38 (m, 2H, *H*-6, Ar*H*), 5.61–5.57 (m, 1.5H, *CH*═, *H*-5), 5.51–5.47 (m, 0.5H, *CH*═), 4.93 (sept, *J* = 6.5 Hz, 0.5H, *CH*(CH$_3$)$_2$), 4.88–4.84 (m, 0.5H, *CH*(CH$_3$)$_2$), 4.33–4.25 (m, 2H, *CH*$_2$-N), 4.04–3.97 (m, 1H, *CH*CH$_3$ l-Ala), 3.08–2.90 (m, 2H, *CH*$_2$P), 1.65 (bs, 3H, *CH*$_3$, alkene), 1.27 (d, *J* = 7.0 Hz, 1.5H, CH*CH*$_3$ l-Ala), 1.20 (d, *J* = 6.2 Hz, 1.5H, CH(*CH*$_3$)$_2$), 1.19 (d, *J* = 6.2 Hz, 1.5H, CH(*CH*$_3$)$_2$), 1.17 (d, *J* = 6.9 Hz, 1.5H, CH*CH*$_3$ l-Ala), 1.12 (d, *J* = 6.2 Hz, 1.5H, CH(*CH*$_3$)$_2$),

1.15 (d, J = 6.2 Hz, 1.5H, CH(CH_3)$_2$). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.6 (d, $^3J_{C\text{-}P}$ = 4.3 Hz, *C*═O, ester), 173.2 (d, $^3J_{C\text{-}P}$ = 4.1 Hz, *C*═O, ester), 165.17 (*C*-4), 165.15 (*C*-4), 151.5 (*C*-2), 151.4 (*C*-2), 146.5 (d, $^2J_{C\text{-}P}$ = 9.7 Hz, *C*—O, Ph), 146.3 (d, $^2J_{C\text{-}P}$ = 9.7 Hz, *C*—O, Ph), 145.2 (*C*-6), 145.1 (*C*-6), 135.2 (d, $^3J_{C\text{-}P}$ = 14.5 Hz, *C*═), 135.4 (d, $^3J_{C\text{-}P}$ = 14.5 Hz, *C*═), 134.9 (*C*-Ar), 127.5 (*CH*-Ar), 127.4 (*CH*-Ar), 126.8 (d, $^3J_{C\text{-}P}$ = 4.9 Hz *C*-Ar), 126.6 (d, $^3J_{C\text{-}P}$ = 5.1 Hz *C*-Ar), 126.3 (*CH*-Ar), 126.1 (*CH*-Ar), 125.2 (*CH*-Ar), 125.1 (*CH*-Ar), 124.3 (*CH*-Ar), 124.2 (*CH*-Ar), 121.5 (*CH*-Ar), 121.3 (*CH*-Ar), 117.4 ($^2J_{C\text{-}P}$ = 11.0 Hz, *CH*═), 116.9 ($^2J_{C\text{-}P}$ = 11.0 Hz, *CH*═), 115.4 (d, $^3J_{C\text{-}P}$ = 3.8 Hz *CH*-Ar), 115.1 (d, $^3J_{C\text{-}P}$ = 3.8 Hz *CH*-Ar), 101.2 (*C*-5), 68.69 (*CH*(CH$_3$)$_2$), 68.66 (*CH*(CH$_3$)$_2$), 53.7 (d, $^4J_{C\text{-}P}$ = 2.3 Hz, CH_2-N), 53.5 (d, $^4J_{C\text{-}P}$ = 2.3 Hz, CH_2-N), 49.7 (*CH*CH$_3$ l-Ala), 49.5 (*CH*CH$_3$ l-Ala), 28.3 (d, $^1J_{C\text{-}P}$ = 128.9 Hz CH_2P), 28.1 (d, $^1J_{C\text{-}P}$ = 129.8 Hz CH_2P), 20.6 (CH(*CH*$_3$)$_2$), 20.56 (CH(*CH*$_3$)$_2$), 20.52 (CH(*CH*$_3$)$_2$), 20.4 (CH(*CH*$_3$)$_2$), 19.8 (d, $^3J_{C\text{-}P}$ = 5.8 Hz, CH*CH*$_3$ l-Ala), 19.1 (d, $^3J_{C\text{-}P}$ = 5.5 Hz, CH*CH*$_3$ l-Ala), 13.3 (d, $^4J_{C\text{-}P}$ = 2.4 Hz, CH_3, alkene), 13.2 (d, $^4J_{C\text{-}P}$ = 2.2 Hz, CH_3, alkene). **HPLC**: Reverse phase HPLC eluting with gradient method CH_3CN/H_2O from 10/90 to 100/0 in 30 min, 1 ml/min, λ = 254 nm and 263 nm, showed one peak with Rt 15.57 min. **HRMS (ESI):** *m/z* [M + Na]$^+$ calcd for $C_{25}H_{30}N_3O_6P$: 522.1770, found: 522.1764.

*4.1.3.8. (E)-N^1-(4′-O-(1-naphthyl)-(benzyloxy-L-alanine)-phosphinyl-2′-methyl-but-2′-enyl)uracil (**E-10b**)*. Prepared according to the standard procedure **C** for the synthesis of ANP ProTide using *O*-(1-naphthyl)-(benzyloxy-*L*-alanine)-allylphosphonate **3b** (240 mg, 586.1 μmol) and *N*^{1}-2′-methylallyluracil (195 mg, 1.17 mmol) and Hoveyda-Grubbs 2nd generation catalyst (15 mol%) in dry CH_2Cl_2 (10 ml). After evaporation, the crude was purified by Biotage Isolera One (120 g ZIP cartridge KP-SIL, 100 ml/min, gradient eluent system MeOH/CH_2Cl_2 1% 1CV, 1–10% 12CV, 10% 2CV), to afford a mixture of the *E* and *Z* isomers. The two isomers were then separated by PrepHPLC (20 ml/min, isocratic eluting system CH_3CN/H_2O – 40/60, 30 min), to afford the title compound as pale yellow foamy solid (13 mg, 5%). R_f = 0.33 (CH_2Cl_2/MeOH – 95:5). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 30.33, 29.48. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 8.01–7.99 (m, 1H, Ar*H*), 7.78–7.64 (m, 1H, Ar*H*), 7.59–7.55 (m, 1H, Ar*H*), 7.44–7.13 (m, 10*H*, *H*-6, Ar*H*), 5.48 (d, J = 7.9 Hz, 1H, *H*-5), 5.42–5.34 (m, 1H, *CH*═), 5.01, 4.96 (ABq, J_{AB} = 12.2 Hz, 1H, CH_2Ph), 4.88, 4.84 (ABq, J_{AB} = 12.2 Hz, 1H, CH_2Ph), 4.16 (bs, 2H, CH_2-N), 4.00–3.94 (m, 1H, *CH*CH$_3$ l-Ala), 2.90–2.75 (m, 2H, CH_2P), 1.51 (d, J = 3.4 Hz 1.5H, CH_3, alkene), 1.49 (d, J = 3.5 Hz 1.5H, CH_3, alkene), 1.15 (d, J = 7.0 Hz, 1.5H, CH*CH*$_3$ l-Ala), 1.06 (d, J = 7.1 Hz, 1.5H, CH*CH*$_3$ l-Ala). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.7 (d, $^3J_{C\text{-}P}$ = 4.3 Hz, *C*═O, ester), 173.3 (d, $^3J_{C\text{-}P}$ = 4.1 Hz, *C*═O, ester), 163.5 (*C*-4), 151.5 (*C*-2), 151.4 (*C*-2), 146.5 (d, $^2J_{C\text{-}P}$ = 9.9 Hz, *C*—O, Ph), 146.3 (d, $^2J_{C\text{-}P}$ = 9.7 Hz, *C*—O, Ph), 145.2 (*C*-6), 145.1 (*C*-6), 135.8 (*C*-Ar), 135.7 (*C*-Ar), 135.3 (d, $^3J_{C\text{-}P}$ = 14.1 Hz, *C*═), 135.2 (d, $^3J_{C\text{-}P}$ = 14.8 Hz, *C*═), 134.9 (*C*-Ar), 128.18 (*CH*-Ar), 128.10 (*CH*-Ar), 127.9 (*CH*-Ar), 127.8 (*CH*-Ar), 126.7 (d, $^3J_{C\text{-}P}$ = 4.9 Hz *C*-Ar), 126.6 (d, $^3J_{C\text{-}P}$ = 4.7 Hz *C*-Ar), 126.3 (*CH*-Ar), 126.08 (*CH*-Ar), 126.06 (*CH*-Ar), 125.17 (*CH*-Ar), 125.10 (*CH*-Ar), 124.3 (*CH*-Ar), 124.2 (*CH*-Ar), 121.4 (*CH*-Ar), 121.3 (*CH*-Ar), 117.3 ($^2J_{C\text{-}P}$ = 11.1 Hz, *CH*═), 116.8 ($^2J_{C\text{-}P}$ = 11.7 Hz, *CH*═), 115.17 (d, $^3J_{C\text{-}P}$ = 3.9 Hz *CH*-Ar), 115.10 (d, $^3J_{C\text{-}P}$ = 3.9 Hz *CH*-Ar), 101.2 (*C*-5), 66.5 (CH_2Ph), 66.4 (CH_2Ph), 53.7 (d, $^4J_{C\text{-}P}$ = 2.6 Hz, CH_2-N), 53.5 (d, $^4J_{C\text{-}P}$ = 2.6 Hz, CH_2-N), 49.6 (*CH*CH$_3$ l-Ala), 49.4 (*CH*CH$_3$ l-Ala), 28.2 (d, $^1J_{C\text{-}P}$ = 129.0 Hz CH_2P), 28.0 (d, $^1J_{C\text{-}P}$ = 129.9 Hz CH_2P), 19.6 (d, $^3J_{C\text{-}P}$ = 5.7 Hz, CH*CH*$_3$ l-Ala), 18.9 (d, $^3J_{C\text{-}P}$ = 5.7 Hz, CH*CH*$_3$ l-Ala), 13.2 (d, $^4J_{C\text{-}P}$ = 2.4 Hz, CH_3, alkene), 13.1 (d, $^4J_{C\text{-}P}$ = 2.4 Hz, CH_3, alkene). **HPLC**: Reverse phase HPLC eluting with gradient method CH_3CN/H_2O from 10/90 to 100/0 in 30 min, 1 ml/min, λ = 254 nm and 263 nm, showed one peak with Rt 15.87 min. **HRMS (ESI):** *m/z* [M + Na]$^+$ calcd for $C_{29}H_{30}N_3O_6P$: 570.1770, found: 570.1764.

*4.1.3.9. (E)-N^1-(4′-O-Phenyl-(isopropyloxy-L-alanine)-phosphinyl-2′-methyl-but-2′-enyl)uracil (**E-10c**)*. Prepared according to the standard procedure **C** for the synthesis of ANP ProTide using *O*-phenyl-(isopropyloxy-*L*-alanine)-allylphosphonate **3c** (140 mg, 449.7 μmol) and *N*^{1}-2′-methylallyluracil (150 mg, 1.11 mmol) and Hoveyda-Grubbs 2nd generation catalyst (15 mol%) in dry CH_2Cl_2 (8 ml). After evaporation, the crude was purified by Biotage Isolera One (25 g SNAP cartridge ULTRA, 75 ml/min, gradient eluent system MeOH/CH_2Cl_2 1% 1CV, 1–10% 12CV, 10% 2CV), to afford a mixture of the *E* and *Z* isomers. The two isomers were then separated by PrepHPLC (20 ml/min, gradient eluting system CH_3CN/H_2O from 10/90 to 100/0, 30 min), to afford the title compound as pale yellow foamy solid (20 mg, 10%). R_f = 0.42 (CH_2Cl_2/MeOH – 95:5). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 29.74, 28.97. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 7.53 (d, J = 7.8 Hz, 0.3H, *H*-6), 7.50 (d, J = 7.8 Hz, 0.7H, *H*-6), 7.38–7.33 (m, 2H, Ar*H*), 7.23–7.16 (m, 3H, Ar*H*), 5.67 (d, J = 7.9 Hz, 1H, *H*-5), 5.54 (q, J = 7.0 Hz, 0.3H, *CH*═), 5.46 (q, J = 7.0 Hz, 0.7H, *CH*═), 5.02–4.89 (m, 1H, *CH*(CH$_3$)$_2$), 4.36–4.35 (m, 2H, CH_2-N), 3.98–3.91 (m, 1H, *CH*CH$_3$ l-Ala), 2.94–2.77 (m, 2H, CH_2P), 1.72–1.71 (m, 3H, CH_3, alkene), 1.29 (d, J = 7.0 Hz, 2.1H, CH*CH*$_3$ l-Ala), 1.25 (d, J = 6.7 Hz, 0.9H, CH(*CH*$_3$)$_2$), 1.23 (d, J = 6.2 Hz, 0.9H, CH(*CH*$_3$)$_2$), 1.21–1.19 (m, 5.1H, CH*CH*$_3$ l-Ala, CH(*CH*$_3$)$_2$). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.5 (d, $^3J_{C\text{-}P}$ = 4.7 Hz, *C*═O, ester), 173.2 (d, $^3J_{C\text{-}P}$ = 4.1 Hz, *C*═O, ester), 165.2 (*C*-4), 151.5 (*C*-2), 151.4 (*C*-2), 150.6 (d, $^2J_{C\text{-}P}$ = 9.6 Hz, *C*—O, Ph), 150.4 (d, $^2J_{C\text{-}P}$ = 9.3 Hz, *C*—O, Ph), 145.32 (*C*-6), 145.30 (*C*-6), 135.2 (d, $^3J_{C\text{-}P}$ = 14.5 Hz, *C*═), 134.8 (d, $^3J_{C\text{-}P}$ = 14.2 Hz, *C*═), 129.3 (*CH*-Ar), 124.6 (*CH*-Ar), 124.4 (*CH*-Ar), 120.6 (d, $^3J_{C\text{-}P}$ = 4.6 Hz *CH*-Ar), 120.4 (d, $^3J_{C\text{-}P}$ = 4.3 Hz *CH*-Ar), 117.6 (d, $^2J_{C\text{-}P}$ = 11.2 Hz, *CH*═), 116.9 (d, $^2J_{C\text{-}P}$ = 10.7 Hz, *CH*═), 101.2 (*C*-5), 68.67 (*CH*(CH$_3$)$_2$), 68.64 (*CH*(CH$_3$)$_2$), 53.8 (d, $^4J_{C\text{-}P}$ = 2.4 Hz, CH_2-N), 53.5 (d, $^4J_{C\text{-}P}$ = 2.1 Hz, CH_2-N), 49.6 (*CH*CH$_3$ l-Ala), 49.4 (*CH*CH$_3$ l-Ala), 28.2 (d, $^1J_{C\text{-}P}$ = 129.7 Hz, CH_2P), 28.0 (d, $^1J_{C\text{-}P}$ = 130.3 Hz, CH_2P), 20.6 (CH(*CH*$_3$)$_2$), 20.5 (CH(*CH*$_3$)$_2$), 20.4 (CH(*CH*$_3$)$_2$), 19.8 (d, $^3J_{C\text{-}P}$ = 5.4 Hz, CH*CH*$_3$ l-Ala), 19.1 (d, $^3J_{C\text{-}P}$ = 5.4 Hz, CH*CH*$_3$ l-Ala), 13.2 (d, $^4J_{C\text{-}P}$ = 2.4 Hz, CH_3, alkene), 13.1 (d, $^4J_{C\text{-}P}$ = 2.4 Hz, CH_3, alkene). **HPLC**: Reverse phase HPLC eluting with gradient method CH_3CN/H_2O from 10/90 to 100/0 in 30 min, 1 ml/min, λ = 254 nm and 263 nm, showed one peak with Rt 13.16 min. **HRMS (ESI):** *m/z* [M + Na]$^+$ calcd for $C_{21}H_{28}N_3O_6P$: 472.1613, found: 472.1608.

*4.1.3.10. (E)-N^1-(4′-O-Phenyl-(benzyloxy-L-alanine)-phosphinyl-2′-methyl-but-2′-enyl)uracil (**E-10d**)*. Prepared according to the standard procedure **C** for the synthesis of ANP ProTide using *O*-phenyl-(benzyloxy-*L*-alanine)-allylphosphonate **3d** (200 mg, 556.5 μmol) and *N*^{1}-2′-methylallyluracil (184.9 mg, 1.11 mmol) and Hoveyda-Grubbs 2nd generation catalyst (15 mol%) in dry CH_2Cl_2 (8 ml). After evaporation, the crude was purified by Biotage Isolera One (25 g SNAP cartridge ULTRA, 75 ml/min, gradient eluent system 2-propanol/CH_2Cl_2 1% 1CV, 1–10% 12CV, 10% 2CV), to afford a mixture of the *E* and *Z* isomers. The two isomers were then separated by PrepHPLC (20 ml/min, isocratic eluting system CH_3CN/H_2O – 35/65, 30 min), to afford the title compound as pale yellow foamy solid (49 mg, 18%). R_f = 0.42 (CH_2Cl_2/2-propanol – 95:5). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 29.75, 28.94. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 7.46 (d, J = 7.8 Hz, 1H, *H*-6), 7.37–7.29 (m, 7H, Ar*H*), 7.20–7.14 (m, 3H, Ar*H*), 5.67 (d, J = 7.8 Hz, 1H, *H*-5), 5.50–5.40 (m, 1H, *CH*═), 5.17, 5.14 (ABq, J_{AB} = 12.3 Hz, 1H, CH_2Ph), 5.08 (s app, 1H, CH_2Ph), 4.31–4.29 (m, 2H, CH_2-N), 4.08–4.04 (m, 1H, *CH*CH$_3$ l-Ala), 2.89–2.74 (m, 2H, CH_2P), 1.67–1.65 (m, 3H, CH_3, alkene), 1.30 (d, J = 6.9 Hz, 1.5H, CH*CH*$_3$ l-Ala), 1.22 (d, J = 7.2 Hz, 1.5H, CH*CH*$_3$ l-Ala). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.8 (d, $^3J_{C\text{-}P}$ = 4.4 Hz, *C*═O, ester), 173.4 (d, $^3J_{C\text{-}P}$ = 3.9 Hz, *C*═O, ester), 165.2 (*C*-4), 151.5 (*C*-2), 150.5 (d, $^2J_{C\text{-}P}$ = 9.2 Hz, *C*—O, Ph), 150.3 (d, $^2J_{C\text{-}P}$ = 10.0 Hz, *C*—O, Ph), 145.2 (*C*-6), 135.8 (*C*-Ar), 135.1 (d, $^3J_{C\text{-}P}$ = 14.4 Hz, *C*═), 134.8 (d, $^3J_{C\text{-}P}$ = 14.4 Hz, *C*═), 129.3 (*CH*-Ar), 128.23 (*CH*-Ar), 128.20 (*CH*-Ar), 128.0 (*CH*-Ar), 127.9 (*CH*-Ar), 124.6 (*CH*-Ar), 124.5 (*CH*-Ar), 120.6 (d, $^3J_{C\text{-}P}$ = 4.0 Hz *CH*-Ar), 120.4 (d, $^3J_{C\text{-}P}$ = 4.4 Hz *CH*-Ar), 117.5 (d, $^2J_{C\text{-}P}$ = 10.6 Hz, *CH*═), 116.9 (d, $^2J_{C\text{-}P}$ = 10.6 Hz, *CH*═), 101.2 (*C*-5), 65.5 (CH_2Ph),

66.4 (*CH*$_2$Ph), 53.8 (d, $^4J_{C\text{-}P}$ = 2.2 Hz, *CH*$_2$-N), 53.5 (d, $^4J_{C\text{-}P}$ = 2.4 Hz, *CH*$_2$-N), 49.5 (*CH*CH$_3$ l-Ala), 49.4 (*CH*CH$_3$ l-Ala), 28.2 (d, $^1J_{C\text{-}P}$ = 129.7 Hz, *CH*$_2$P), 28.0 (d, $^1J_{C\text{-}P}$ = 130.1 Hz, *CH*$_2$P), 19.7 (d, $^3J_{C\text{-}P}$ = 5.4 Hz, CH*CH*$_3$ l-Ala), 19.1 (d, $^3J_{C\text{-}P}$ = 5.2 Hz, CH*CH*$_3$ l-Ala), 13.2 (d, $^4J_{C\text{-}P}$ = 2.2 Hz, *CH*$_3$, alkene), 13.1 (d, $^4J_{C\text{-}P}$ = 2.2 Hz, *CH*$_3$, alkene). **HPLC**: Reverse phase HPLC eluting with gradient method CH_3CN/H_2O from 10/90 to 100/0 in 30 min, 1 ml/min, λ = 254 nm and 263 nm, showed one peak with Rt 14.56 min. **HRMS (ESI):** *m/z* $[M+Na]^+$ calcd for $C_{25}H_{28}N_3O_6P$: 520.1608, found: 520.1608.

*4.1.3.11. (E)-N^1-(4′-O-(5,6,7,8-Tetrahydro-1-naphthyl)-(isopropyloxy-L-alanine)-phosphinyl-2′-methyl-but-2′-enyl)uracil (**E-10e**) and (Z)-N^1-(4′-O-(5,6,7,8-tetrahydro-1-naphthyl)-(isopropyloxy-L-alanine)-phosphinyl-2′-methyl-but-2′-enyl)uracil (**Z-10e**)*. Prepared according to the standard procedure **C** for the synthesis of ANP ProTide using *O*-(5,6,7,8-tetrahydro-1-naphthyl)-(isopropyloxy-*L*-alanine)-allylphosphonate **3e** (200 mg, 547.3 μmol) and *N*^{1}-2′-methylallyluracil (181 mg, 1.09 mmol) and Hoveyda-Grubbs 2nd generation catalyst (15 mol%) in dry CH_2Cl_2 (10 ml). After evaporation, the crude was purified by Biotage Isolera One (25 g SNAP cartridge ULTRA, 75 ml/min, gradient eluent system 2-propanol/CH_2Cl_2 1% 1CV, 1–10% 12CV, 10% 2CV), to afford a mixture of the *E* and *Z* isomers. The two isomers were then separated by PrepHPLC (20 ml/min, isocratic eluting system CH_3CN/H_2O – 35/65, 30 min), to afford the title compound *E* as pale yellow foamy solid (31 mg, 11%). R_f = 0.23 (CH_2Cl_2/2-propanol – 95:5). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 27.84, 27.00. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 7.52–7.49 (m, 1H, *H*-6), 7.17–7.12 (m, 1H, Ar*H*), 7.06–7.00 (m, 1H, Ar*H*), 6.90–6.87 (m, 1H, Ar*H*), 5.67 (d, *J* = 7.9 Hz, 1H, *H*-5), 5.58–5.54 (m, 0.5H, *CH*═), 5.49–5.45 (m, 0.5H, *CH*═), 5.02–4.85 (m, 1H, *CH*(CH$_3$)$_2$), 4.35 (bs, 2H, *CH*$_2$-N), 3.99–3.91 (m, 1H, *CH*CH$_3$ l-Ala), 2.97–2.82 (m, 2H, *CH*$_2$P), 2.76 (bs, 2H, Ar*H*), 2.69 (bs, 2H, Ar*H*), 1.80–1.78 (m, 4H, Ar*H*), 1.71 (d, *J* = 2.9 Hz 3H, *CH*$_3$, alkene), 1.30 (d, *J* = 7.0 Hz, 1.5H, CH*CH*$_3$ l-Ala), 1.25–1.24 (m, 4.5H, CH*CH*$_3$ l-Ala, CH(*CH*$_3$)$_2$), 1.19 (d, *J* = 6.3 Hz, 3H, CH(*CH*$_3$)$_2$). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.7 (d, $^3J_{C\text{-}P}$ = 3.9 Hz, *C*═O, ester), 173.2 (d, $^3J_{C\text{-}P}$ = 4.3 Hz, *C*═O, ester), 165.2 (*C*-4), 151.5 (*C*-2), 151.4 (*C*-2), 148.8 (d, $^2J_{C\text{-}P}$ = 9.5 Hz, *C*—O, Ph), 148.6 (d, $^2J_{C\text{-}P}$ = 9.7 Hz, *C*—O, Ph), 145.35 (*C*-6), 145.31 (*C*-6), 139.2 (*C*-Ar), 139.1 (*C*-Ar), 135.0 (d, $^3J_{C\text{-}P}$ = 14.5 Hz, *C*═), 134.6 (d, $^3J_{C\text{-}P}$ = 14.3 Hz, *C*═), 128.5 (d, $^3J_{C\text{-}P}$ = 5.4 Hz *C*-Ar), 128.3 (d, $^3J_{C\text{-}P}$ = 5.4 Hz *C*-Ar), 125.4 (*CH*-Ar), 125.3 (*CH*-Ar), 125.15 (*CH*-Ar), 125.10 (*CH*-Ar), 117.6 ($^2J_{C\text{-}P}$ = 11.0 Hz, *CH*═), 117.0 ($^2J_{C\text{-}P}$ = 10.9 Hz, *CH*═), 116.8 (d, $^3J_{C\text{-}P}$ = 3.3 Hz *CH*-Ar), 116.7 (d, $^3J_{C\text{-}P}$ = 3.3 Hz *CH*-Ar), 101.2 (*C*-5), 101.1 (*C*-5), 68.67 (*CH*(CH$_3$)$_2$), 68.63 (*CH*(CH$_3$)$_2$), 53.8 (d, $^4J_{C\text{-}P}$ = 2.4 Hz, *CH*$_2$-N), 53.5 (d, $^4J_{C\text{-}P}$ = 2.4 Hz, *CH*$_2$-N), 49.7 (*CH*CH$_3$ l-Ala), 49.5 (*CH*CH$_3$ l-Ala), 29.1 (*CH*$_2$-Ar), 28.5 (d, $^1J_{C\text{-}P}$ = 129.9 Hz *CH*$_2$P), 28.3 (d, $^1J_{C\text{-}P}$ = 130.9 Hz *CH*$_2$P), 23.3 (*CH*$_2$-Ar), 22.47 (*CH*$_2$-Ar), 22.44 (*CH*$_2$-Ar), 22.42 (*CH*$_2$-Ar), 20.6 (CH(*CH*$_3$)$_2$), 20.5 (CH(*CH*$_3$)$_2$), 20.4 (CH(*CH*$_3$)$_2$), 19.9 (d, $^3J_{C\text{-}P}$ = 5.0 Hz, CH*CH*$_3$ l-Ala), 19.1 (d, $^3J_{C\text{-}P}$ = 5.5 Hz, CH*CH*$_3$ l-Ala), 13.3 (d, $^4J_{C\text{-}P}$ = 2.3 Hz, *CH*$_3$, alkene), 13.2 (d, $^4J_{C\text{-}P}$ = 2.0 Hz, *CH*$_3$, alkene). **HPLC**: Reverse phase HPLC eluting with gradient method CH_3CN/H_2O from 10/90 to 100/0 in 30 min, 1 ml/min, λ = 254 nm and 263 nm, showed one peak with Rt 16.14 min. **HRMS (ESI):** *m/z* $[M+Na]^+$ calcd for $C_{25}H_{34}N_3O_6P$: 526.2083, found: 526.2077.

From PrepHPLC also the *Z* isomer ***Z*-10e** was isolated as pale yellow foamy solid (2.5 mg, 1%). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 29.39, 28.62. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 7.50 (d, *J* = 7.6 Hz, 1H, *H*-6), 7.10–7.00 (m, 1H, Ar*H*), 6.95–6.88 (m, 1H, Ar*H*), 6.80–6.75 (m, 1H, Ar*H*), 5.54–5.38 (m 2H, *CH*═, *H*-5), 4.88–4.78 (m, 1H, *CH*(CH$_3$)$_2$), 4.38–4.29 (m, 2H, *CH*$_2$-N), 3.86–3.80 (m, 1H, *CH*CH$_3$ l-Ala), 2.97–2.84 (m, 2H, *CH*$_2$P), 2.66–2.58 (m, 4H, Ar*H*), 1.71–1.65 (m, 4H, Ar*H*), 1.61–1.54 (m, 3H, *CH*$_3$, alkene), 1.20–1.17 (m, 1.5H, CH*CH*$_3$ l-Ala), 1.13–1.07 (m, 7.5H, CH*CH*$_3$ l-Ala, CH(*CH*$_3$)$_2$), **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.7 (d, $^3J_{C\text{-}P}$ = 3.9 Hz, *C*═O, ester), 173.2 (d, $^3J_{C\text{-}P}$ = 4.3 Hz, *C*═O, ester), 165.2 (*C*-4), 151.5 (*C*-2), 151.4 (*C*-2), 148.8 (d, $^2J_{C\text{-}P}$ = 9.5 Hz, *C*—O, Ph), 148.6 (d, $^2J_{C\text{-}P}$ = 9.7 Hz, *C*—O, Ph), 145.5 (*C*-6), 145.4 (*C*-6), 139.2 (*C*-Ar), 135.0 (d, $^3J_{C\text{-}P}$ = 14.5 Hz, *C*═), 134.6 (d, $^3J_{C\text{-}P}$ = 14.3 Hz, *C*═), 128.5 (d, $^3J_{C\text{-}P}$ = 5.4 Hz *C*-Ar), 128.3 (d, $^3J_{C\text{-}P}$ = 5.4 Hz *C*-Ar), 125.3 (*CH*-Ar), 125.2 (*CH*-Ar), 125.1 (*CH*-Ar), 125.0 (*CH*-Ar), 119.5 ($^2J_{C\text{-}P}$ = 10.1 Hz, *CH*═), 119.4 ($^2J_{C\text{-}P}$ = 10.8 Hz, *CH*═), 117.1 (d, $^3J_{C\text{-}P}$ = 3.3 Hz *CH*-Ar), 116.8 (d, $^3J_{C\text{-}P}$ = 3.3 Hz *CH*-Ar), 101.1 (*C*-5), 101.0 (*C*-5), 68.6 (*CH*(CH$_3$)$_2$), 49.7 (*CH*CH$_3$ l-Ala), 49.5 (*CH*CH$_3$ l-Ala), 47.0 (*CH*$_2$-N), 29.1 (*CH*$_2$-Ar), 28.2 (d, $^1J_{C\text{-}P}$ = 128.2 Hz *CH*$_2$P), 28.0 (d, $^1J_{C\text{-}P}$ = 130.5 Hz *CH*$_2$P), 23.4 (*CH*$_2$-Ar), 23.3 (*CH*$_2$-Ar), 22.47 (*CH*$_2$-Ar), 22.43 (*CH*$_2$-Ar), 20.57 (CH(*CH*$_3$)$_2$), 20.53 (CH(*CH*$_3$)$_2$), 20.4 (CH(*CH*$_3$)$_2$), 19.7 (d, $^3J_{C\text{-}P}$ = 4.7 Hz, CH*CH*$_3$ l-Ala), 19.0 (d, $^3J_{C\text{-}P}$ = 5.4 Hz, CH*CH*$_3$ l-Ala), 13.3 (d, $^4J_{C\text{-}P}$ = 2.7 Hz, *CH*$_3$, alkene). **HPLC**: Reverse phase HPLC eluting with gradient method CH_3CN/H_2O from 10/90 to 100/0 in 30 min, 1 ml/min, λ = 254 nm and 263 nm, showed one peak with Rt 16.82 min.

*4.1.3.12. (E)-N^1-(4′-O-(5,6,7,8-Tetrahydro-1-naphthyl)-(benzyloxy-L-alanine)-phosphinyl-2′-methyl-but-2′-enyl)uracil (**E-10f**)*. Prepared according to the standard procedure **C** for the synthesis of ANP ProTide using *O*-(5,6,7,8-tetrahydro-1-naphthyl)-(benzyloxy-*L*-alanine)-allylphosphonate **3f** (200 mg, 483.7 μmol) and *N*^{1}-2′-methylallyluracil (160 mg, 967.4 μmol) and Hoveyda-Grubbs 2nd generation catalyst (15 mol%) in dry CH_2Cl_2 (8 ml). After evaporation, the crude was purified by Biotage Isolera One (25 g SNAP cartridge ULTRA, 75 ml/min, gradient eluent system 2-propanol/CH_2Cl_2 1% 1CV, 1–10% 12CV, 10% 2CV), to afford a mixture of the *E* and *Z* isomers. The two isomers were then separated by PrepHPLC (20 ml/min, isocratic eluting system CH_3CN/H_2O – 40/60, 30 min), to afford the title compound as pale yellow foamy solid (14 mg, 5%). R_f = 0.25 (CH_2Cl_2/2-propanol – 95:5). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 29.33, 28.46. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 7.34 (d, *J* = 7.8 Hz, 1H, *H*-6), 7.26–7.18 (m, 5H, Ar*H*), 7.03–6.99 (m, 1H, Ar*H*), 6.93–6.83 (m, 1H, Ar*H*), 6.77–6.73 (m, 1H, Ar*H*), 5.54 (d, *J* = 7.8 Hz, 0.6H, *H*-5), 5.53 (d, *J* = 7.9 Hz, 0.4H, *H*-5), 5.39–5.29 (m, 1H, *CH*═), 5.04, 5.01 (ABq, J_{AB} = 12.2 Hz, 1H, *CH*$_2$Ph), 4.95, 4.94 (ABq, J_{AB} = 12.2 Hz, 1H, *CH*$_2$Ph), 4.19–4.17 (m, 2H, *CH*$_2$-N), 3.97–3.88 (m, 1H, *CH*CH$_3$ l-Ala), 2.78–2.765 (m, 2H, *CH*$_2$P), 2.63 (bs, 2H, Ar*H*), 2.56 (bs, 2H, Ar*H*), 1.67–1.62 (m, 4H, Ar*H*), 1.54 (d, *J* = 3.8 Hz 1.8H, *CH*$_3$, alkene), 1.52 (d, *J* = 3.9 Hz 1.2H, *CH*$_3$, alkene), 1.20 (d, *J* = 6.9 Hz, 1.8H, CH*CH*$_3$ l-Ala), 1.14 (d, *J* = 7.0 Hz, 1.2H, CH*CH*$_3$ l-Ala). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 173.9 (d, $^3J_{C\text{-}P}$ = 4.0 Hz, *C*═O, ester), 173.4 (d, $^3J_{C\text{-}P}$ = 4.0 Hz, *C*═O, ester), 165.2 (*C*-4), 151.5 (*C*-2), 151.4 (*C*-2), 148.8 (d, $^2J_{C\text{-}P}$ = 9.1 Hz, *C*—O, Ph), 148.7 (d, $^2J_{C\text{-}P}$ = 9.7 Hz, *C*—O, Ph), 145.3 (*C*-6), 145.2 (*C*-6), 139.2 (*C*-Ar), 139.1 (*C*-Ar), 135.9 (*C*-Ar), 135.8 (*C*-Ar), 134.9 (d, $^3J_{C\text{-}P}$ = 14.7 Hz, *C*═), 134.7 (d, $^3J_{C\text{-}P}$ = 14.7 Hz, *C*═), 128.4 (d, $^3J_{C\text{-}P}$ = 4.7 Hz *C*-Ar), 128.3 (d, $^3J_{C\text{-}P}$ = 4.7 Hz *C*-Ar), 128.2 (*CH*-Ar), 128.1 (*CH*-Ar), 127.9 (*CH*-Ar), 127.8 (*CH*-Ar), 125.4 (*CH*-Ar), 125.3 (*CH*-Ar), 125.15 (*CH*-Ar), 125.08 (*CH*-Ar), 117.5 ($^2J_{C\text{-}P}$ = 10.9 Hz, *CH*═), 117.0 ($^2J_{C\text{-}P}$ = 10.9 Hz, *CH*═), 116.8 (d, $^3J_{C\text{-}P}$ = 3.2 Hz *CH*-Ar), 116.6 (d, $^3J_{C\text{-}P}$ = 3.2 Hz *CH*-Ar), 101.17 (*C*-5), 66.5 (*CH*$_2$Ph), 66.4 (*CH*$_2$Ph), 53.8 (d, $^4J_{C\text{-}P}$ = 2.5 Hz, *CH*$_2$-N), 53.5 (d, $^4J_{C\text{-}P}$ = 2.5 Hz, *CH*$_2$-N), 49.6 (*CH*CH$_3$ l-Ala), 49.5 (*CH*CH$_3$ l-Ala), 29.1 (*CH*$_2$-Ar), 28.4 (d, $^1J_{C\text{-}P}$ = 130.0 Hz *CH*$_2$P), 28.2 (d, $^1J_{C\text{-}P}$ = 130.8 Hz *CH*$_2$P), 23.3 (*CH*$_2$-Ar), 22.44 (*CH*$_2$-Ar), 22.42 (*CH*$_2$-Ar), 22.39 (*CH*$_2$-Ar), 19.7 (d, $^3J_{C\text{-}P}$ = 5.4 Hz, CH*CH*$_3$ l-Ala), 19.0 (d, $^3J_{C\text{-}P}$ = 5.6 Hz, CH*CH*$_3$ l-Ala), 13.2 (d, $^4J_{C\text{-}P}$ = 2.3 Hz, *CH*$_3$, alkene), 13.1 (d, $^4J_{C\text{-}P}$ = 2.4 Hz, *CH*$_3$, alkene). **HPLC**: Reverse phase HPLC eluting with gradient method CH_3CN/H_2O from 10/90 to 100/0 in 30 min, 1 ml/min, λ = 254 nm and 263 nm, showed one peak with Rt 17.66 min. **HRMS (ESI):** *m/z* $[M+Na]^+$ calcd for $C_{29}H_{34}N_3O_6P$: 574.2083, found: 574.2077.

*4.1.4. bis(Benzyloxy-L-alanine)-allylphosphonate (**11**)*

In a round bottom flask, under an argon atmosphere, 2,6-Lutidine (1.55 ml. 13.22 mmol) and TMSBr, (2.20 ml, 16.65 mmol) were added to a solution of dimethyl allylphosphonate (500 mg, 3.33 mmol), in anhydrous acetonitrile (25 ml). The mixture was stirred 16 h at room temperature and then the volatiles evaporated without any contact with air. Then the flask was charged with dry aminoacid ester hydrochloride

(3.6 g, 16.65 mmol), dry triethylamine (6.9 ml, 49.96 mmol) and dry pyridine (10 ml) and heated to 50 °C to obtain a homogenous solution. To this mixture was then added a solution of aldrithiol-2 (4.40 g, 19.98 mmol) and triphenylphosphine (5.24 g, 19.98 mmol) in dry pyridine (10 ml) under argon atmosphere. The resulting mixture was stirred at 50 °C for 16 h. After evaporating all the volatiles, the residue was purified by Biotage Isolera One (100 g SNAP cartridge ULTRA, 100 ml/min, gradient eluent system EtOAc/Hexane 10% 1CV, 10–100% 12CV, 100% 2CV and 50 g SNAP cartridge ULTRA, 100 ml/min, gradient eluent system MeOH/EtOAc 0% 1CV, 0–20% 15CV, 20% 3CV), to afford the title compound as a yellow oil (770 mg, 52%). R_f = 0.44 (EtOAc/MeOH – 98:2). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 27.47. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 7.39–7.29 (m, 10*H*, Ar*H*), 5.88–5.79 (m, 1H, *CH*═), 5.19–5.09 (m, 6H, CH_2═, 2xCH_2Ph), 4.07–4.01 (m, 2H, 2x*CH*CH_3 l-Ala), 21.36 (dd, J_G = 19.5 Hz, J = 7.2 Hz, 2H, CH_2P), 1.41 (d, J = 7.0 Hz, 3H, CHCH_3 l-Ala), 1.31 (d, J = 7.2 Hz, 3H, CHCH_3 l-Ala). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 174.28 (d, $^3J_{C\text{-}P}$ = 4.3 Hz, *C*═O, ester), 174.23 (d, $^3J_{C\text{-}P}$ = 4.3 Hz, *C*═O, ester), 135.95 (*C*-Ar), 135.91 (*C*-Ar), 128.5 ($^2J_{C\text{-}P}$ = 10.9 Hz, *CH*═), 128.36 (*CH*-Ar), 128.33 (*CH*-Ar), 128.1 (*CH*-Ar), 128.0 (*CH*-Ar), 119.0 (d, $^3J_{C\text{-}P}$ = 13.0 Hz CH_2═), 66.6 (CH_2Ph), 66.5 (CH_2Ph), 48.9 (*CH*CH_3 l-Ala), 48.5 (*CH*CH_3 l-Ala), 34.7 (d, $^1J_{C\text{-}P}$ = 111.4 Hz CH_2P), 19.9 (d, $^3J_{C\text{-}P}$ = 5.4 Hz, CHCH_3 l-Ala), 19.8 (d, $^3J_{C\text{-}P}$ = 4.3 Hz, CHCH_3 l-Ala).

4.1.5. (*E*)-N^1-(bis(Benzyloxy-L-alanine)-phosphinyl-2′-methyl-but-2′-enyl) uracil (12)

Prepared according to the standard procedure **C** using *bis*(benzyloxy-L-alanine)-allylphosphonate **11** (200 mg, 854.9 μmol) and N^1-2′-methylallyluracil (150 mg, 1.71 mmol) and Hoveyda-Grubbs 2nd generation catalyst (15 mol%) in dry CH_2Cl_2 (10 ml). Volatiles were then evaporated and the residue was purified by Biotage Isolera One (25 g SNAP cartridge ULTRA, 75 ml/min, gradient eluent system 2-propanol/CH_2Cl_2 1% 1CV, 1–10% 12CV, 10% 2CV), to afford a mixture of the *E* and *Z* isomers. The two isomers were then separated by Preparative HPLC (20 ml/min, gradient eluting system CH_3CN/H_2O from 5/95 to 100/0, 30 min), to afford the title compound as pale yellow foamy solid (5 mg, 2%). R_f = 0.30 (CH_2Cl_2/2-propanol – 95:5). **^{31}P NMR (202 MHz, CD_3OD) δ_P**: 27.88. **^{1}H NMR (500 MHz, CD_3OD) δ_H**: 7.36 (d, J = 7.9 Hz, 1H, *H*-6), 7.28–7.17 (m, 10*H*, Ar*H*), 5.56 (d, J = 7.9 Hz, 1H, *H*-5), 5.32–5.28 (m, 1H, *CH*═), 5.06, 4.99 (m, 4H, 2xCH_2Ph), 4.14 (s, 2H, CH_2-N), 3.91–3.84 (m, 2H, 2x*CH*CH_3 l-Ala), 2.55–2.41 (m, 2H, CH_2P), 1.50 (d, J = 3.1 Hz, 3H, CH_3, alkene), 1.26 (d, J = 7.1 Hz, 3H, CHCH_3 l-Ala), 1.18 (d, J = 7.1 Hz, 3H, CHCH_3 l-Ala). **^{13}C NMR (125 MHz, CD_3OD) δ_C**: 174.3 (d, $^3J_{C\text{-}P}$ = 4.6 Hz, *C*═O, ester), 174.1 (d, $^3J_{C\text{-}P}$ = 3.7 Hz, *C*═O, ester), 165.2 (*C*-4), 151.5 (*C*-2), 145.2 (*C*-6), 135.95 (*C*-Ar), 135.91 (*C*-Ar), 133.9 (d, $^3J_{C\text{-}P}$ = 13.8 Hz, *C*═), 128.22 (*CH*-Ar), 128.21 (*CH*-Ar), 128.04 (*CH*-Ar), 128.01 (*CH*-Ar), 127.98 (*CH*-Ar), 127.96 (*CH*-Ar), 118.7 (d, $^2J_{C\text{-}P}$ = 9.7 Hz, *CH*═), 101.2 (*C*-5), 66.58 (CH_2Ph), 66.53 (CH_2Ph), 53.7 (d, $^4J_{C\text{-}P}$ = 2.4 Hz, CH_2-N), 48.8 (*CH*CH_3 l-Ala), 48.5 (*CH*CH_3 l-Ala), 29.0 (d, $^1J_{C\text{-}P}$ = 112.5 Hz, CH_2P), 19.8 (d, $^3J_{C\text{-}P}$ = 5.4 Hz, CHCH_3 l-Ala), 19.6 (d, $^3J_{C\text{-}P}$ = 4.8 Hz, CHCH_3 l-Ala), 13.1 (d, $^4J_{C\text{-}P}$ = 2.0 Hz, CH_3, alkene). **HPLC**: Reverse phase HPLC eluting with gradient method CH_3CN/H_2O from 10/90 to 100/0 in 30 min, 1 ml/min, λ = 254 nm and 263 nm, showed one peak with Rt 15.79 min. **HRMS (ESI):** *m/z* $[M+Na]^+$ calcd for $C_{29}H_{35}N_4O_7P$: 605.2141, found: 605.2136.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2018.05.034.

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