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Synthesis of acyclic nucleoside phosphonates targeting *flavin-dependent thymidylate synthase* in *Mycobacterium tuberculosis*

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ABSTRACT

Flavin-Dependent Thymidylate Synthase (*FDTS*) encoded by ThyX gene was discovered as a new class of *thymidylate synthase* involved in the *de novo* synthesis of dTMP named only in 30 % of human pathogenic bacteria. This target was pursed for the development of new antibacterial agents against multiresistant pathogens. We have developed a new class of ANPs based on the mimic of two natural's cofactors (dUMP and FAD) as inhibitors against *Mycobacterium tuberculosis ThyX*. Several synthetic efforts were performed to optimize regioselective N1-alkylation, cross-coupling metathesis and Sonogashira cross-coupling. Compound **19c** showed a poor 31.8% inhibitory effect on ThyX at 200 μM.

1. Introduction

Since the late 19th century, antibiotics have been used in the treatment of life-threatening infections, however, as a result of antimicrobial resistance (AMR), they are less effective and AMR is becoming a major concern of global public health, especially for *Mycobacterium tuberculosis* (MT).[1] There is an urgent need for new molecules targeting new potential bacterial targets for which the interest arises for their differences between the biochemical pathways in bacteria and eukaryotic cells. The de novo synthesis of 2′-deoxythymidine-5′-monophosphate (dTMP) is crucial to the DNA synthesis. This synthesis can be done in bacteria by either the folate-dependent thymidylate synthase (TS),[2] such as found in *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, or by a flavin-dependent thymidylate synthase (FDTS or *ThyX*; EC 2.1.1.148),[3] such as found in *Helicobacter pylori*, *Borrelia burgdorferi, Chlamydia* species. Other pathogenic bacteria such as *Bacillus anthracis*, *Clostridium botulinum*, and *Mycobacterium tuberculosis* possess both enzymes.[4] FDTS, which is not found in humans (contrary to TS), has no sequence, mechanism or structural similarities with TS.[3,5] Thus, among the new antibacterial targets, FDTS appears to be an excellent target for new antimicrobial drug discovery for deadly microbes.[6]

Based on the commonly approved multistep mechanism reported by *Kohen et al* in 2016,[7] FDTS requires flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide phosphate (NADPH) and CH_2H_4folate to convert dUMP in dTMP. The methylene group was transferred from CH_2H_4folate to the reduced form of FAD ($FADH^-$) and then transferred to C5 of the dUMP, through a bridged methylene intermediate, to afford the dTMP through a last flavin oxidation step. The first specific inhibitor of FDTS, the thiazolidine analog **1** (IC_{50} of 0.057 μM), was reported by Myllykalio *et al.*[8] The same group[9] found that the naphtoquinone derivative **2** exhibited for FTDS a Ki of 28 nM. Herdewijn *et al.*[10] described the monophosphate nucleotide inhibitor **3** (IC_{50} of 0.91 μM) and its acyclic analog **4** (43% inhibition at 50 μM), (Fig. 1). (SEE Fig 2.)

Interestingly, Johar *et al.*[11] and Lescrinier *et al.*[12] have shown that the introduction of a long alkynyl side chains at the C5 position of uridine, such as dodecynyl and tridecynyl, led to potent antimycobacterial activity.

A docking of dUMP and cofactors into the active site of *M. tuberculosis* FDTS (PDB: 3GWC)[26] reveals that cofactors and dUMP were stacked together by π-stacking. They were also maintained into this large and flexible pocket by hydrogen bond interactions at the pyrimidine moiety (Arg 199 and Arg 107) and ribose (*3′*-OH, Gln 103 and Arg 95). The phosphate group acted as an anchor by creation of six hydrogen bonds (Arg 107, Arg 172, Arg 87 and Gln 106). FAD shows only interactions at its pyrimidine moiety (Arg 103 and Arg 95) and its

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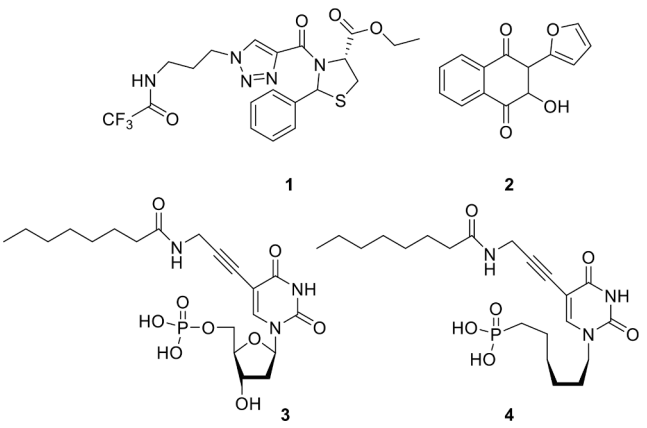


Fig. 1. Structures of *FDTS inhibitors.*

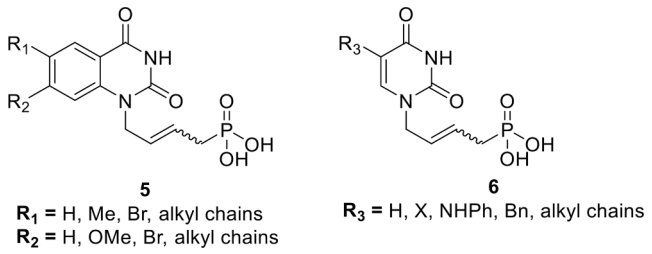


Fig. 2. Structures of synthetized compounds.

diphosphate moiety (Arg 97, Arg 95, Arg 190, His 96 and His 194). No interaction was observed between ribityl and adenine moiety. It appears that the pyrimidine nucleobase and the 5′-phosphate moiety are important in keeping the dUMP into the active site.

Based on FDTS exclusive catalytic mechanism and on the successful development by our group of a new family of unsaturated acyclic nucleoside phosphonates (ANPs), we describe herein new ANP analogs bearing a quinazoline nucleobase (**5**) in order to interact with FAD through π-stacking and hydrophobic pairing interactions. New acyclic dUMP analogs (**6**) substituted at C5 in order to block the methyl transfer were also successfully synthesized (Figure 1).

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis of quinazoline ANPs (12)

Quinazoline derivatives **12a-c** were obtained in five steps starting from anthranilic derivatives **7a-c**, (Scheme 1). Reaction of **7a-c** with urea at 150 °C[13] afforded the corresponding bicycles **8a-c**, respectively. Quinazoline derivatives **8a-c** were regioselectively alkylated at N1 position following a method previously developed by our group. Compounds **8a-c** were first subjected to a silylation step, under microwave irradiation at 80 °C for 5 min in the presence of molecular sieves and BSA. TMSCl, activated NaI and crotyl bromide were then successively added to the reaction mixture which was then subjected to microwave irradiation at 80 °C for 30 min. The corresponding N1-crotylated quinazoline **9a-c** were obtained, respectively, with excellent yields. A N3-Boc protection was realized in the presence of Boc_2O and DMAP in THF under microwave irradiation at 70 °C for 10 min affording **10a-c**. Analogs **10a-c** were directly engaged in a ruthenium-based olefin cross-metathesis with dimethyl allylphosphonate by using Grubbs-II catalyst and DCM under ultrasonication at 55 °C, 80 kHz for 24 h.[14] The crude reaction was not purified and directly engaged in the Boc deprotection with TFA in DCM at rt for 6 h. Compounds **11a-c** were isolated in moderate yields with an inseparable mixture of isomers E/Z (85/15), respectively. Phosphonate derivatives **11a-c** were finally deprotected under McKenna conditions[15] in the presence of TMSBr in ACN under microwave irradiation at 70 °C for 10 min to afford the corresponding phosphonic acid derivatives **12a-c**, in good yield, respectively.

2.1.2. Synthesis of the C7- and C6-substituted quinazoline ANPs

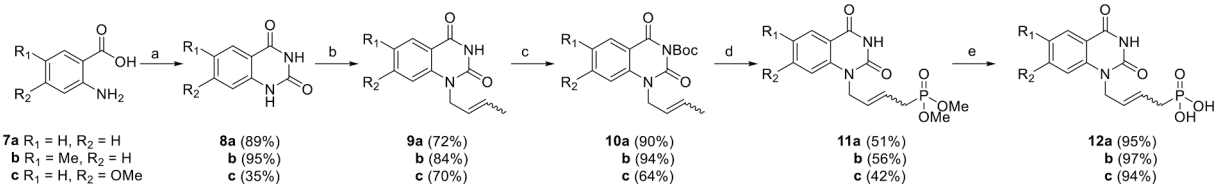
In order to fill the pocket at enzyme active site, we introduced a long alkynyl side chain (**13a-c**) either at the C7-position or at the C6 position of the quinazoline moiety, (Scheme 2 & 3). Firstly, the precursor of the chain, *N*-prop-2-ynyloctanamide **13a** and phenylacetamidoprop-1-ynyl **13b** were isolated by precipitation from propargyl amine **13** in the presence of the corresponding acyl chloride, DIPEA in DCM at 0 °C to rt for 2 h. Meanwhile the 1-hexyl-3-prop-2-ynyl urea **13c** was obtained from **13** and hexylisocyanate in 94% yield.[16]

Thus, for C7 substitution, commercially available 7-bromoquinazoline **14** was regioselectively alkylated to **15**, which was then subjected to the N3-Boc protection to **16**. A ruthenium-based cross-metathesis of **16** with dimethyl allyl phosphonate, (Scheme 2), followed by the N3-Boc removal with TFA in DCM, afforded compound **17** as an inseparable mixture of isomers *E/Z* (85/15). Aryl bromide **17** was then reacted under Pd(0) cross-coupling conditions[17] (CuI, $Pd(PPh_3)_4$ in DMF, reflux, 3 h) with alkynes **13a-c** and Et_3N to afford **18a-c**, respectively, in excellent yields. Subsequent deprotection of C7-dimethylphosphonates **18a-c** with TMSBr in anhydrous acetonitrile afforded the desired phosphonic acids **19a-c** in quantitative yields, (Scheme 2).

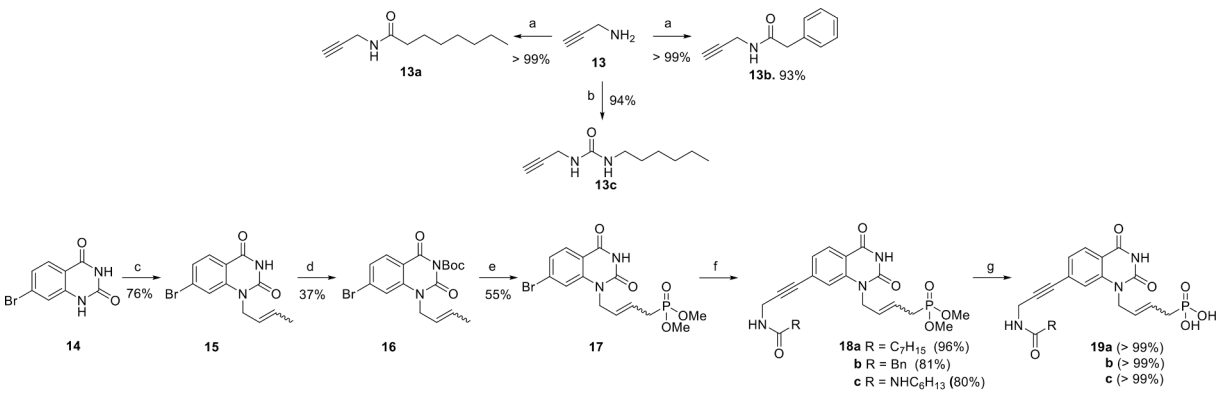
For the synthesis of *C*6-quinazoline, we had to slightly modify the chemical pathway. Side change degradation and non-separable complex mixtures were obtained when the acidic deprotection was carried out due to the hyperconjugation between the N1 and the alkynyl.[18] Thus the 6-bromoquinazoline (**20**) was converted through **21** to the N3-protected crotyl analogue **22**. Ruthenium-catalyzed cross-coupling of **22** with bis (POC)allylphosphonate **23** afforded the ANP **24** (in 58%) which it's directly engaged into the N3-Boc deprotection to **25** in 44% yields (two steps), which was then submitted to a Sonogashira coupling with alkynes **13a-c**, to afford desired compounds **26a-c**, respectively, in moderate yields. The cleavage of POC moiety of **26a-c** was realized using NaOH 0.1 N in deionized water followed by an acidification with Dowex 50WX8 (H^+) to led the phosphonic acid compounds **24a-c**, in moderate to good yields, respectively.

2.1.3. Synthesis of C5-substitued uracil analogues (32a,b and 39)

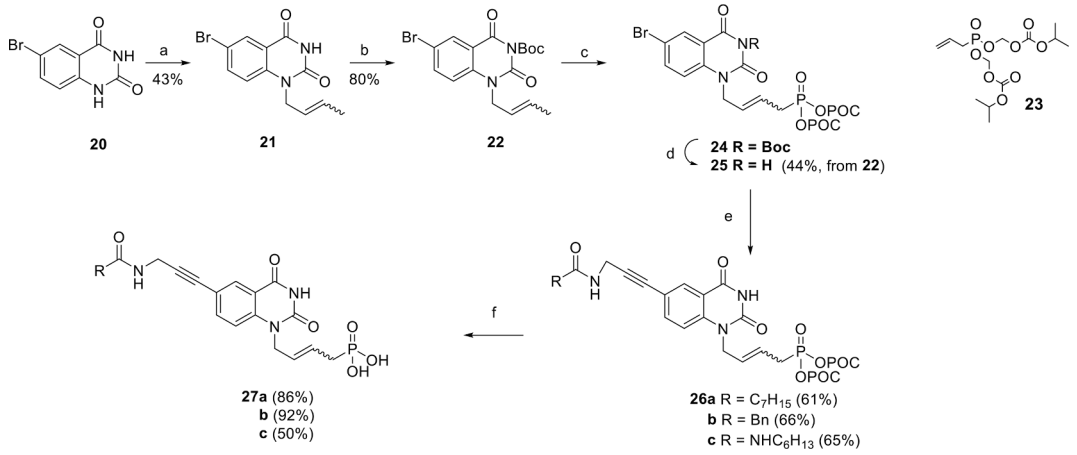
We then turned our attention to the introduction of the uracil acyclic



Scheme 1. *Reagents and conditions*: (a) urea, 150 °C, 12 h.; (b) (i) *bis*(trimethylsilyl)acetamide, CH_3CN, MW, 80 °C, 5 min, (ii) TMSCl, crotyl bromide, NaI_{act}, MW, 80 °C, 30 min; (c) Boc_2O, DMAP, THF, MW, 70 °C, 10 min; (d) (i) dimethyl allylphosphonate, Grubbs-II catalyst, DCM anhyd.,))) 80 kHz, 55 °C, 24 h, (ii) TFA, DCM, rt, 6 h; (e) TMSBr, ACN anhyd., MW, 70 °C, 10 min.



Scheme 2. *Reagents and conditions*: (a) RCOCl, DIPEA, DCM anhyd., 0 °C-rt, 2 h; (b) isocyanate DCM anhyd., 0 °C-rt, 2 h; (c) (i) BSA, CH_3CN, MW, 80 °C, 5 min, (ii) TMSCl, crotyl bromide, NaI_{act}, MW, 80 °C, 30 min; (d) Boc_2O, DMAP, THF, MW 70 °C, 10 min; (e) (i) dimethyl-allylphosphonate, Grubbs-II catalyst, DCM anhyd.,))) 80 KHz, 55 °C, 24 h, (ii) TFA, DCM, rt, 6 h; (f) $Pd(PPh_3)_4$, CuI, Et_3N, alkynes **13a**, **13b** and **13c**, DMF, 70 °C, 3 h; (g) TMSBr, ACN anhyd., rt, 6 h.

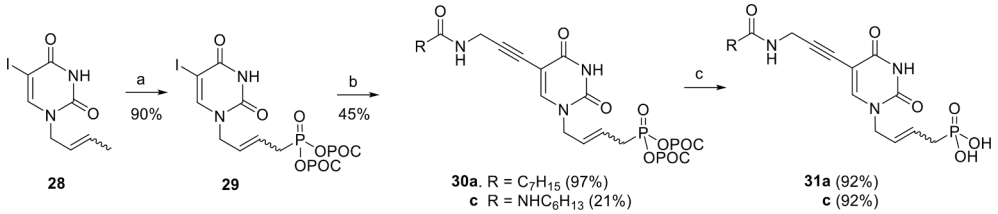


Scheme 3. *Reagents and conditions*: (a) (i) BSA, CH_3CN, MW, 80 °C, 5 min, (ii) TMSCl, crotyl bromide, NaI_{act}, MW, 80 °C, 30 min; (b) Boc_2O, DMAP, THF, MW 70 °C, 10 min; (c) *bis*(POC)-allylphosphonate, G-II, DCM anhyd.,))) 80 KHz, 55 °C, 24 h; (d) TFA, DCM, rt, 6 h; (e) $Pd(PPh_3)_4$, CuI, Et_3N, alkynes **13a**, **13b** and **13c**, DMF, 70 °C, 3 h; (f) NaOH (0.1 N), rt, 4 h, DOWEX 50WX8 (H^+).

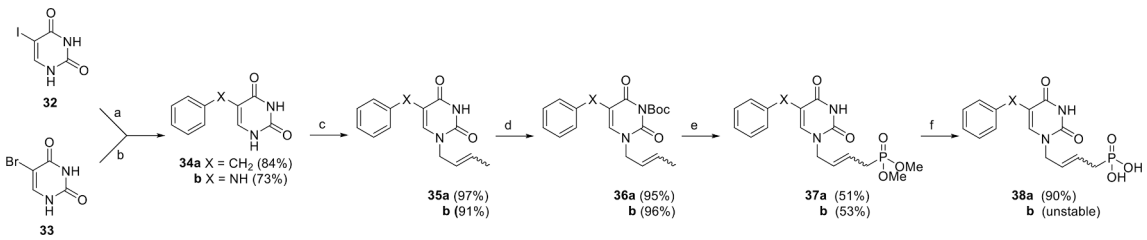
nucleoside phosphonate derivatives at the C5 position of uracil acyclonucleoside phosphonate derivatives (Scheme 4) of various alkynyl chains as reported in literature.[11,12] Thus, the 5-iodo-N1-alkylated uracil **28** obtained as previously described [19] was submitted to a ruthenium-catalyzed cross-metathesis reaction with *bis*(POC) allylphosphonate **23** in the presence of Grubbs-II catalyst in DCM at 55 °C for 24 h, to give **29** in 57% yields, as an inseparable mixture of isomers *E/Z* (85/15). Two alkynes were introduced under Sonogashira cross-coupling conditions (Et_3N, $Pd(PPh_3)_4$, rt, 16 h) to lead to **30a** and **30c**, in 97% and 21% yields, respectively. During this reaction, side products corresponding to the known formation of fluorescent bicyclic furopyrimidine nucleosides were observed (*structures not shown*).[20]

The desired phosphonic acid analogs **31a,c** were obtained from **30a, c**, respectively, by alkaline hydrolysis followed by acidification (Dowex 50WX8 (H^+)), in excellent yields (Scheme 4).

Finally, we developed a third series of ANPs bearing at C5 of uracil either a benzyl or an aniline substituent. Compound **34a** was obtained through a turbo Grignard[21] reaction of 5-iodouracil (**32**) with MeMgCl. LiCl at −20 °C for 30 min in THF, followed by the addition of *i*PrMgCl. LiCl at −20 °C in 30 min and stirred 2 h at room temperature, (Scheme 5). Then CuI.$_2$LiCl in THF was added at −30 °C and stirred at room temperature for 1 h. Finally, addition of benzylbromide at room temperature for 12 h, afforded the desired 5-benzyl-uracil **34a** in 84% yields. Compound **34b**[22] was obtained, through a SN_{Ar} from 5-bromo-uracil **33b** in presence of aniline under thermic condition at 175 °C for 20 min, in good yields. Both **34a** and **34b** were engaged, respectively, in a regioselective N1-alkylation (**35a,b**) followed by a N3-Boc protection to **36a,b** in excellent yields. Ruthenium-catalyzed cross-



Scheme 4. Reagents and conditions: (a) **25**, Grubbs-II catalyst, DCM anhyd., 55 °C, 24 h; (b) $Pd(PPh_3)_4$, CuI, Et_3N, alkynyl chains **13a,b**, DMF, rt, 16 h; (c) NaOH (0.1 N), rt, 4 h, DOWEX 50WX8 (H^+).



Scheme 5. Reagents and conditions: (a) for (**34a**): (i) MeMgCl.LiCl, −20 °C, 30 min, THF, (ii) iPrMgCl.LiCl, −20 °C, 30 min, 2 h, rt. (iii) CuI.2LiCl, −30 °C to rt, 1 h, (iv) BnBr, rt, 12 h.; (b) for (**34b**): aniline, 175 °C, 20 min; (c) (i) BSA, CH_3CN, MW, 80 °C, 5 min, (ii) TMSCl, crotyl bromide, NaI_{act}, MW, 80 °C, 30 min; (d) Boc_2O, DMAP, THF, MW, 10 min, 70 °C; (e) i) allylphosphonate, Grubbs-II catalyst, DCM anhyd.,))) 80 kHz, 55 °C, 24 h, ii) TFA, DCM, rt, 6 h; (f) TMSBr, ACN anhyd., MW, 70 °C, 10 min.

metathesis reaction of **36a,b** with dimethylallylphosphonate afforded **37a,b** as an inseparable mixture of E/Z (85/15), respectively. A final two steps deprotection gave the desired phosphonic acid compound **38a** meanwhile derivative **38b** was not characterized due to its instability.

2.2. Biological evaluation

A NADPH oxidase spectrophotometric assay, adapted from Basta *et al.*,[23] was used to test the *in vitro* inhibitory activities of synthesized compounds on *Mtb* ThyX. As NADPH has a maximum of absorption at 340 nm, ThyX activity was determined by following the decrease of absorbance at λ_{340}. All assays used a kinetic mode of a multilabel microplate reader with an injector, to start the reaction by injecting NADPH (750 μM). In the assay, final concentrations of ThyX and dUMP are 10 μM and 100 μM, respectively. The primary screen was performed with molecules dissolved in DMSO, including DMSO alone as low-activity control. B1-PP146, with a 1,4-benzoxazine moiety and described as tight-binding inhibitors, was used as reference compound.[30] The concentration of screened compounds was 200 μM (Table 1).

Most of the compounds exhibited low inhibition of *Mtb* ThyX at 200 μM; C6-substituted quinazoline AMPs lack of activity compared to their C7-substituted counterparts. Only, quinazoline analog **19c** showed a 31.8% inhibitory effect on ThyX at 200 μM. Docking of **19c** in the active site of *Mtb* ThyX shows that the quinazoline moiety parallels the FAD through π-stacking interactions, moreover the aliphatic chain seems to pass over the FAD (Fig. 3 and SI). This conformation could hinder the approach of different substrates at the N10 position of FAD which seems to be most crucial for flavin substrate binding to enzyme.[31]

Interactions are also formed with residual amino acids involved in the stabilization of natural substrates in the pocket, such as Arg 199 and Arg 95.

Inhibition assays were also performed without addition of FAD or at a lower concentration of dUMP (10 μM) to promote binding of molecules in the active site of *Mtb* ThyX, but no significant inhibitory effect was observed. Since previous work on active ANPs against FDTS, we can hypothesize that the four-carbon-chain linker which connects the nucleobase and the phosphonate moiety is too short. We have thus to optimize the length and flexibility of the linker of **19c** as well as to functionalize it by to adding functional groups in order to mimic the 3′-OH of dUMP.

Table 1
Mtb ThyX inhibition at 200 μM by the NADPH oxidase assay.

Cmpd	% inhibition
12a	15.8
12b	26.2
12c	26.4
19a	14.9
19b	14.7
19c	31.8
27a	0
27b	0
27c	14.9
31a	0
31c	0
38a	23.7
B1-PP146[a]	95.1[b]

[a] B1-PP146, a compound with a 1,4 benzoxazine ring, used as positive control; [b]value at 50 μM. The standard deviations from two independent experiments is between 3 and 9, as a function of the molecules.

3. Conclusion

FDTS of human pathogenic bacteria represent an important target for the development of new antibiotic drugs, even if its mechanism of action is not yet fully elucidated. Twelve new compounds, analogs of acyclic nucleoside phosphoantes, were synthesized and tested for their activity against *M. tuberculosis ThyX*. The synthesized compounds are 5- and 7-substituted quinazoline and 5-substituted uracil linked at N1 to a (E)-but-2-enyl phosphonic acid moiety. Only the quinazoline analog **19b** showed a poor 31.8% inhibitory effect on ThyX at 200 μM. Nevertheless, based on **19b**, future development is needed to develop an optimal acyclic chain and identify more potent compounds.

4. Experimental section

4.1. Chemistry

General information. Commercially available chemicals were of reagent grade and used as received. All reactions requiring anhydrous conditions were carried out using oven-dried glassware and under an atmosphere of dry Ar or N_2. Microwave reactions were carried out in a Biotage Initiator apparatus. The reactions under ultrasound were carried out with Elmasonic P30H apparatus with a frequency of 80 kHz and effective power of 100 W. The reactions were monitored by thin layer chromatography (TLC) analysis using silica gel precoated plates (Kieselgel 60F254, E. Merck). Compounds were visualized by UV irradiation and/or spraying with phosphomolybdic acid (PMA) stain, potassium permanganate solution or ninhydrin stain, followed by charring at around 150 °C. Flash column chromatography was performed on Silica Gel 60 M (0.040–0.063 mm, E. Merck). Melting points were determined with a Kofler Heizbank Reichert Type 7841 apparatus and are uncorrected. The infrared spectra were measured with Perkin–Elmer Spectrometer. The ^1H and ^{13}C NMR spectra were recorded on Bruker Avance DPX 250 or Bruker Avance 400 Spectrometers. Chemical shifts are given in ppm and are referenced to the deuterated solvent signal or to TMS as internal standard and multiplicities are reported as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Carbon multiplicities were assigned by distortion less enhancement by polarization transfer (DEPT) experiments. ^1H and ^{13}C signals were attributed on the basis of H—H and H—C correlations. High Resolution Mass spectra were

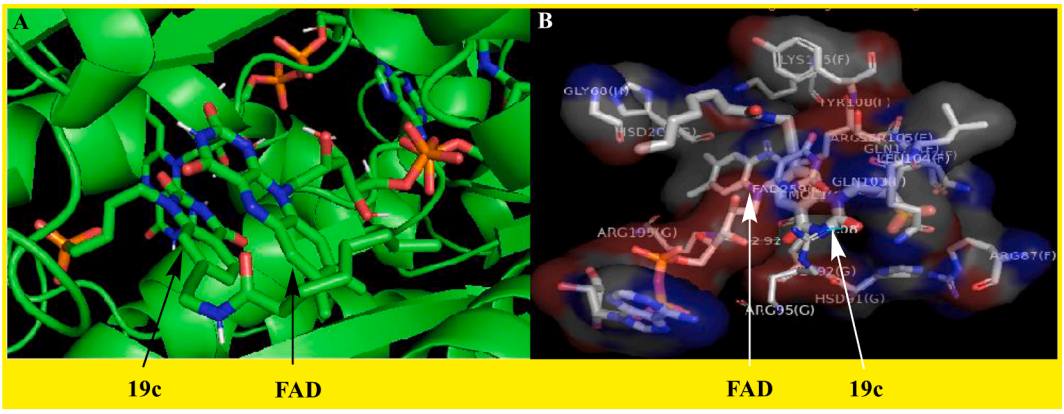


Fig. 3. (A) Modeling of FAD and **19c** in the active site of *Mtb* ThyX. (B) Interactions of **19c** with residual amino acids.

performed on a Bruker Q-TOF MaXis spectrometer by the "Fédération de Recherche" ICOA/CBM (FR2708) platform.

*4.1.1. General synthetic procedure 1 for urea cyclisation for compounds **8a**, **8b** and **8c***

Amino acid compound (10 g, 1 eq.) and urea (87.6 g, 20 eq.) were grinded with a mortar pillar and then heated at 150 °C for 12 h under inert atmosphere in a dry flask. After the mixture was cooled down to 100 °C, 15 ml of water was added and the resultant mixture was stirred for another 15 min. After the reaction mixture was cooled down to room temperature, it was filtered if necessary and aqueous solution of NaOH (1 M, 150 ml) was then added. The mixture was mixed at room temperature another 1 h. After acetic acid was added dropwise until pH 5. Pure compounds were obtained by filtration of precipitates.

*4.1.1.1. Quinazoline-2,4(1H,3H)-dione **(8a)***. Following the general procedure 1, **8a** was prepared from commercially available anthranilic acid **7a** (10 g, 1 eq., 72 mmol) and urea (87.6 g, 20 eq., 1.46 mol). The title compound was obtained after filtration as a white solid (10.4 g, 89%). CAS # 86–96-4.[13] ^{1}H NMR (250 MHz, DMSO-d_6) δ 11.20 (bs, 2H, 2xNH), 7.88 (dd, 1H, J = 8.4, 1.5 Hz, H_5), 7.63 (ddd, 1H, J = 8.4, 7.3, 1.5 Hz, H_6), 7.33–7.04 (m, 2H, $H_{8,7}$).

*4.1.1.2. 6-Methylquinazoline-2,4(1H,3H)-dione **(8b)***. The title compound was prepared from commercially available **7b** to afford after filtration the desired product as a white solid (95%). CAS # 62484–16-6.[13] ^{1}H NMR (250 MHz, DMSO-d_6) δ 11.12 (bs, 2H, 2 × NH), 7.64 (s, 1H, H_5), 7.45 (dd, 1H, J = 8.3, 2.1 Hz, H_8), 7.06 (d, 1H, J = 8.3 Hz, H_7), 2.32 (s, 3H, C-CH_3).

*4.1.1.3. 7-Methoxyquinazoline-2,4(1H,3H)-dione **(8c)***. The title compound was prepared from commercially available **7c** to afford after filtration the desired product as a brown/orange solid (35%). CAS # 62484–12-2.[13] ^{1}H NMR (250 MHz, DMSO-d_6) δ 11.02 (bs, 2H, 2 × NH), 7.75 (d, 1H, J = 8.8 Hz, H_5), 6.72 (dd, 1H, J = 8.8, 2.3 Hz, H_6), 6.58 (d, 1H, J = 2.3 Hz, H_8), 3.78 (s, 3H, O-CH_3).

*4.1.2. General synthetic procedure 2 for crotylation of N1 position for compounds **9a, 9b, 9c, 15, 21, 35a, 35b***

In a microwave tube, a suspension of bicyclic nucleobase (1 eq.) in anhydrous acetonitrile (0.5 M), BSA (2.5 eq.) and 4 Å molecular sieves were sequentially added under inert atmosphere. The tube was then sealed and stirred under microwave irradiation at 80 °C for 5 min. TMSCl (1 eq.), NaI_{act} (1,12 eq.) and crotyl bromide (2 eq.) were successively added. The vial was sealed and heated under microwave irradiation for 30 min at 80 °C. The volatiles were removed under reduced pressure, and the residue diluted in a solution of saturated $NaHCO_3$ and EtOAc. The aqueous layer was extracted with EtOAc (3 × 20 ml). The combined organic layers were washed with $Na_2S_2O_3$ solution and brine, dried over $MgSO_4$ filtered and concentrated under reduced pressure. Pure compounds were obtained after purification on silica gel column chromatography with DCM/MeOH (98:2) as eluent.

*4.1.2.1. 1-(But-2-en-1-yl)quinazoline-2,4(1H,3H)-dione **(9a)***. The title compound was prepared from **8a** to afford after purification the desired product **9a** as a white solid (0.48 g, *E/Z ratio = 85/15*, 72%). CAS # 57397–87-2.[24] ^{1}H NMR (250 MHz, Acetone-d_6) δ 10.23 (bs, 1H, NH), 8.09 (dd, 1H, J = 7.8, 1.7 Hz, H_5), 7.79–7.67 (m, 1H, H_7), 7.40 (d, 1H, J = 8.5 Hz, H_8), 7.29 (dt, 1H, J = 10.0, 7.8 Hz, H_6), 5.84–5.37 (m, 2H, $H_{2',3'}$), 4.83 (d, 1H, J = 6.6 Hz, $H_{1'\text{-minor}}$), 4.70 (d, 2H, J = 5.2 Hz, $H_{1'\text{-major}}$), 1.88–1.82 (m, 1H, $H_{4'\text{-minor}}$), 1.66 (dq, 3H, J = 6.3, 1.4 Hz, $H_{4'\text{-major}}$). Rf : 0.58 (DCM/MeOH 95:5).

*4.1.2.2. 1-(But-2-en-1-yl)-6-methylquinazoline-2,4(1H,3H)-dione **(9b)***. The title compound was prepared from **8b** to afford after purification the desired product **9b** as a white solid (*E/Z ratio = 85/15*, 84%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.15 (bs, 1H, NH), 7.88 (s, 1H, H_5), 7.54 (dd, 1H, J = 8.6, 2.2 Hz, H_7), 7.29 (d, 1H, J = 8.6 Hz, $H_{8\text{-major}}$), 7.21 (d, 1H, J = 8.6 Hz, $H_{8\text{-minor}}$), 5.82–5.47 (m, 2H, $H_{2',3'}$), 4.83–4.77 (m, 2H, $H_{1'\text{-minor}}$), 4.67 (dt, 2H, J = 5.1, 1.4 Hz, $H_{1'\text{-major}}$), 2.39 (s, 3H, C-CH_3), 1.84 (ddt, 3H, J = 7.0, 1.8, 1.1 Hz, $H_{4'\text{-minor}}$), 1.65 (dq, 3H, J = 6.3, 1.5 Hz, $H_{4'\text{-major}}$). ^{13}C NMR (101 MHz, Acetone-d_6) δ 162.4 (C═O), 150.8 (C═O), 140.1 (C_{quat}), 136.8 (C_{arom}), 133.0 (C_{quat}), 129.4 (CH = CH), 129.1 (C_{quat}), 128.4 (C_{arom}), 125.8 (CH = CH), 115.8 (C_{arom}), 44.4 (N-$CH_{2\text{-major}}$), 40.5 (N-$CH_{2\text{-minor}}$), 20.3 (C-CH_3), 17.7 (CH-$CH_{3\text{-major}}$), 13.3 (CH-$CH_{3\text{-minor}}$). HRMS-ESI (*m/z*) $[M + H]^+$ calcd for $C_{13}H_{15}N_2O_2$: 231.1128, found: 231.1127. Rf : 0.63 (DCM/MeOH 95:5).

*4.1.2.3. 1-(But-2-en-1-yl)-7-methoxyquinazoline-2,4(1H,3H)-dione **(9c)***. The title compound was prepared from **8c** to afford after purification the desired product **9c** as a white/brown solid (*E/Z ratio = 85/15*, 70%). ^{1}H NMR (250 MHz, Acetone-d_6) δ 10.03 (bs, 1H, NH), 8.00 (d, 1H, J = 8.5 Hz, H_5), 6.89–6.77 (m, 2H, $H_{6,8}$), 5.88–5.50 (m, 2H, $H_{2',3'}$), 4.83–4.81 (m, 2H, $H_{1'\text{-minor}}$), 4.76 (d, 2H, J = 5.6 Hz, $H_{1'\text{-major}}$), 3.93 (s, 3H, O-CH_3), 1.87 (d, 3H, J = 5.6 Hz, $H_{4'\text{-minor}}$), 1.71–1.63 (m, 3H, $H_{4'\text{-major}}$). ^{13}C NMR (63 MHz, Acetone-d_6) δ 166.0 (C═O), 144.1 (C═O), 130.7 (C_{quat}), 130.6 (CH = CH), 130.1 (C_{quat}), 129.4 (C_{arom}), 125.8 (CH = CH), 122.2 (C_{quat}), 110.3 (C_{arom}), 100.3 (C_{arom}), 56.2 (O-CH_3), 44.68 (N-$CH_{2\text{-major}}$), 40.6 (N-$CH_{2\text{-minor}}$), 17.8 (CH-$CH_{3\text{-major}}$), 13.3 ($CH_{3\text{-minor}}$). HRMS-ESI (*m/z*) $[M + H]^+$ calcd for $C_{13}H_{15}N_2O_2$: 247.1077, found: 247.1077. Rf : 0.67 (DCM/MeOH 95:5).

*4.1.2.4. 7-Bromo-1-(but-2-en-1-yl)quinazoline-2,4(1H,3H)-dione **(15)***. The title compound was prepared from commercially available **14** to afford after purification the desired product *as a beige solid*, in 37 % yields, as a mixture of *E/Z isomers (85/15)*. ^{1}H NMR (400 MHz,

Acetone-d_6) δ 10.22 (bs, 1H, NH), 8.02 (d, 1H, J = 8.4 Hz, H_5), 7.69 (d, J = 1.5 Hz 1H, H_8), 7.54 (dd, 1H, J = 8.4, 1.5 Hz, H_6), 5.89–5.75 (m, 1H, $H_{2'}$), 5.67–5.57 (m, 1H, $H_{3'}$), 4.89 (d, 2H, J = 6.0 Hz, $H_{1'\text{-minor}}$), 4.76 (dt, 2H. J = 5.8, 1.3 Hz, $H_{1'\text{-major}}$), 1.88 (d, 3H, J = 7.0 Hz, $H_{4'\text{-minor}}$), 1.70 (dd, 3H, J = 6.5, 1.5 Hz, $H_{4'\text{-major}}$). ^{13}C NMR (101 MHz, Acetone-d_6) δ 159.6 (C═O), 148.8 (C_{quat}), 148.6 (C═O), 142.4 (C_{quat}), 138.6 (C_{arom}), 130.1 (C_{arom}), 127.2 (CH = CH), 124.9 (CH = CH), 119.1 (C_{arom}), 115.3 (C_{quat}), 45.4 (N-$CH_{2\text{-major}}$), 41.4 (N-$CH_{2\text{-minor}}$), 17.8 (CH-$CH_{3\text{-major}}$), 14.5 (CH-$CH_{3\text{-minor}}$). HRMS-ESI (*m/z*) $[M]^+$ calcd for $C_{12}H_{12}N_2O_2Br$: 295.0075, found: 295.0077. Rf : 0.5 (DCM/MeOH 95:5).

*4.1.2.5. 6-Bromo-1-(but-2-en-1-yl)quinazoline-2,4(1H,3H)-dione (**21**).* The title compound was prepared from commercially available **20** to afford, after purification, the desired product **21** as a beige solid (*E/Z ratio = 85/15*, 43%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.38 (bs, 1H, NH), 8.16 (d, 1H, J = 2.5 Hz, H_5), 7.85 (dd, 1H, J = 9.0, 2.5 Hz, H_7), 7.39 (d, 1H, J = 9.0 Hz, H_8), 5.82–5.72 (m, 1H, $H_{2'}$), 5.61–5.54 (m, 1H, $H_{3'}$), 4.83 (d, 2H, J = 6.4 Hz, $H_{1'\text{-minor}}$), 4.70 (dt, 2H, J = 5.2, 1.4 Hz, $H_{1'\text{-major}}$), 1.85 (dd, 3H, J = 7.0, 1.7 Hz, $H_{4'\text{-minor}}$), 1.67 (dq, 3H, J = 6.4, 1.4 Hz, $H_{4'\text{-major}}$). ^{13}C NMR (101 MHz, Acetone-d_6) δ 161.2 (C═O), 150.5 (C_{quat}), 150.2 (C═O), 141.5 (C_{quat}), 138.3 (C_{arom}), 130.8 (C_{arom}), 129.4 (CH = CH), 125.3 (CH = CH), 118.3 (C_{arom}), 115.4 (C_{quat}), 44.7 (N-$CH_{2\text{-major}}$), 40.7 (N-$CH_{2\text{-minor}}$), 17.8 (CH-$CH_{3\text{-major}}$), 13.3 (CH-$CH_{3\text{-minor}}$). HRMS-ESI (*m/z*) $[M]^+$ calcd for $C_{12}H_{12}N_2O_2Br$: 295.0075, found: 295.0076. Rf : 0.5 (DCM/MeOH 95:5).

*4.1.2.6. 5-Benzyl-1-(but-2-en-1-yl)pyrimidine-2,4(1H,3H)-dione (**35a**).* The title compound was prepared from **34a** to afford after purification the desired product as an oil (*E/Z ratio = 85/15*, 97%). ^{1}H NMR (250 MHz, Acetone-d_6) δ 10.06 (bs, 1H, NH), 7.35 (s, 1H, H_6), 7.31–7.11 (m, 5H, 5 × H_{arom}), 5.82–5.41 (m, 2H, $H_{2',3'}$), 4.39 (d, 2H, J = 7.0 Hz, $H_{1'\text{-minor}}$), 4.26 (dt, 2H, J = 6.0, 1.1 Hz, $H_{1'\text{-major}}$), 3.59 (s, 2H, CH_2), 1.72–1.71 (m, 3H, $H_{4'\text{-minor}}$), 1.69–1.64 (m, 3H, $H_{4'\text{-major}}$). ^{13}C NMR (63 MHz, Acetone-d_6) δ 164.2 (C═O), 151.5 (C═O), 142.3 (C = CH), 140.7 (C_{quat}), 130.8 (CH = CH), 129.5 (2 × C_{arom}), 129.1 (2 × C_{arom}), 126.9 (CH = CH), 125.5 (C_{arom}), 114.3 (C_{quat}), 49.6 (N-$CH_{2\text{-major}}$), 44.5 (N-$CH_{2\text{-minor}}$), 33.0 (C-CH_2), 17.7 (CH-$CH_{3\text{-major}}$), 13.0 (CH-$CH_{3\text{-minor}}$). HRMS-ESI (*m/z*) $[M + H]^+$ calcd for $C_{15}H_{17}N_2O_2$: 257.1284, found: 257.1285. Rf : 0.48 (DCM/MeOH 95:5).

*4.1.2.7. 1-(But-2-en-1-yl)-5-(phenylamino)pyrimidine-2,4 (1H,3H)-dione (**35b**).* The title compound was prepared from **34b** to afford after purification the desired product **35b** as a white solid (*E/Z ratio = 85/15*, 91%). CAS # 4870–31-9. ^{1}H NMR (250 MHz, Acetone-d_6) δ 10.21 (bs, 1H, NH), 7.44 (s, 1H, H_6), 7.18 (dd, 2H, J = 8.6, 7.4 Hz, 2 × H_{arom}), 7.01–6.94 (m, 2H, 2 × H_{arom}), 6.82–6.73 (m, 1H, H_{arom}), 6.32 (bs, 1H, NH), 5.89–5.55 (m, 2H, $H_{2',3'}$), 4.39–4.45 (m, 2H, $H_{1'\text{-minor}}$), 4.32 (ddt, 2H, J = 6.0, 2.2, 1.1 Hz, $H_{1'\text{-major}}$), 1.83–1.81 (m, 3H, $H_{4'\text{-minor}}$), 1.73 – 1.67 (m, 2H, $H_{4'\text{-major}}$). ^{13}C NMR (63 MHz, Acetone-d_6) δ 162.2 (C═O), 145.6 (C═O), 130.9 (C_{quat}), 130.5 (C = CH), 129.9 (2 × C_{arom}), 126.5 (CH = CH), 125.7 (CH = CH), 120.3 (C_{arom}), 118.7 (C_{quat}), 116.5 (2 × C_{arom}), 49.75 (N-$CH_{2\text{-major}}$), 44.7 (N-$CH_{2\text{-minor}}$), 17.8 (CH-$CH_{3\text{-major}}$), 13.1 (CH-$CH_{3\text{-minor}}$). HRMS-ESI (*m/z*) $[M + H]^+$ calcd for $C_{14}H_{16}N_3O_2$: 258.1234, found: 258.1234. Rf : 0.4 (DCM/MeOH 95:5).

*4.1.3. General synthetic procedure 3 for N-Boc protection for compounds **10a, 10b, 10c, 16, 22, 36a, 36b***

Under inert atmosphere to a solution of N1 alkylated compound, respectively, (1 eq.) in dry THF (0.25 M) were successively added dimethylaminopyridine (0.5 eq.) and dicarbonate de di-*tert*-butyle (2 eq.). The sealed tube was heated under microwave irradiation at 70 °C for 10 min. Sealed tube was degas before opened. Volatiles were evaporated under reduced pressure. Pure compounds were obtained after purification on silica gel column chromatography with PE/EtOAc (8:2) as eluent.

*4.1.3.1. Tert-butyl 1-(but-2-en-1-yl)-2,4-dioxo-1,4-dihydroquinazoline-3 (2H)-carboxylate (**10a**).* The title compound was prepared from **9a** to afford after purification the desired product **10a** (*E/Z ratio = 85/15*, 90%) as a white solid . ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.11 (dd, 1H, J = 7.8, 1.6 Hz, H_5), 7.80 (ddd, 1H, J = 8.8, 7.8, 1.6 Hz, H_7), 7.48 (d, 1H, J = 8.8 Hz, H_8), 7.34 (t, 1H, J = 7.8 Hz, H_6), 5.86–5.71 (m, 1H, $H_{2'}$), 5 66–5.41 (m, 1H, $H_{3'}$), 4.86 (d, 2H, J = 5.9 Hz, $H_{1'\text{-minor}}$), 4.73 (d, 2H, J = 4.7 Hz, $H_{1'\text{-major}}$), 1.85 (d, 3H, J = 7.0 Hz, $H_{4'\text{-minor}}$), 1.67 (d, 2H, J = 6.4 Hz, $H_{4'\text{-major}}$), 1.60 (s, 9H. H_{Boc}). ^{13}C NMR (101 MHz, Acetone-d_6) δ 160.1 (C═O), 148.9 (C═O), 141.3 (C_{quat}), 136.7 (C_{arom}), 130.0 (CH = CH), 129.1 (C_{quat}), 128.9 (C_{arom}), 125.2 (CH = CH), 124.0 (C_{arom}), 116.1 (C_{arom}), 116.4 (C_{quat}), 86.5 (O-C_{quat}), 45.3 (N-$CH_{2\text{-major}}$), 41.2 (N-$CH_{2\text{-minor}}$), 27.6 (3 × C-CH_{3Boc}), 17.8 (CH-$CH_{3\text{-major}}$), 13.4 (CH-$CH_{3\text{-minor}}$). HRMS-ESI (*m/z*) $[M + Na]^+$ calcd for $C_{17}H_{20}N_2O_4Na$: 339.1314, found: 339.1314. Rf : 0.81 (PE/ EtOAc 1:1).

*4.1.3.2. Tert-butyl 1-(but-2-en-1-yl)-6-methyl-2,4-dioxo-1,4-dihydroquina zoline-3(2H)-carboxylate (**10b**).* The title compound was prepared from **9b** to afford after purification the desired product **10b** as a white solid (*E/Z ratio = 85/15*, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 1H, H_5), 7.62 (dd, 1H, J = 8.7, 2.1 Hz, H_7), 7.38 (d, 1H, J = 8.7 Hz, H_8), 5.84–5.70 (m, 1H, $H_{2'}$), 5.65–5 40 (m, 1H, $H_{3'}$), 4.84 (d, 2H, J = 6.0 Hz, $H_{1'\text{-minor}}$), 4.71 (d, 2H, J = 4.7 Hz, $H_{1'\text{-major}}$), 2.41 (s, 3H, C-CH_3), 1.85 (d, 3H, J = 7.0 Hz, $H_{4'\text{minor}}$), 1.67 (d, 3H, J = 6.4 Hz, $H_{4'\text{-major}}$), 1.60 (s, 9H, H_{Boc}). ^{13}C NMR (101 MHz, Acetone-d_6) δ 160.2 (C═O), 149.0 (C═O), 137.7 (C_{quat}), 137.6 (C_{arom}), 133.8 (C_{quat}), 129.9 (CH = CH), 128.5 (C_{arom}), 125.5 (C_{quat}), 125.3 (CH = CH), 116.2 (C_{arom}), 115.8 (C_{quat}), 86.4 (O-C_{quat}), 45.2 (N-$CH_{2\text{-major}}$), 41.1 (N-$CH_{2\text{-minor}}$), 27.6 (3 × C-CH_{3Boc}), 20.3 (C-CH_3), 17.8 (CH-$CH_{3\text{-major}}$), 13.4 (CH-$CH_{3\text{-minor}}$). HRMS-ESI (*m/z*) $[M + Na]^+$ calcd for $C_{18}H_{22}N_2O_4Na$: 353.1470, found: 353.1469. Rf : 0.81 (PE / EtOAc 1:1).

*4.1.3.3. Tert-butyl 1-(but-2-en-1-yl)-7-methoxy-2,4-dioxo-1,4-dihydroqui nazoline-3(2H)-carboxylate (**10c**).* The title compound was prepared from **9c** to afford after purification the desired product **10c** as a white solid (*E/Z ratio = 85/15*, 64%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.03 (d, 1H, J = 8.7 Hz, H_5), 6.92 (dd, 1H, J = 8.7, 2.3 Hz, H_6), 6.89 (d, 1H, J = 2.3 Hz, H_8), 5.92–5.73 (m, 1H, $H_{2'}$), 5.67–5.42 (m, 1H, $H_{3'}$), 4.86 (d, 2H, J = 5.8 Hz, $H_{1'\text{-minor}}$), 4.73 (dt, 2H, J = 5.6, 1.3 Hz, $H_{1'\text{-major}}$), 3.96 (s, 3H, O-CH_3), 1.88 (d, 3H, J = 9.7 Hz, $H_{4'\text{-minor}}$), 1.73–1.67 (m, 3H, $H_{4'\text{-major}}$), 1.59 (s, 9H, H_{Boc}). ^{13}C NMR (101 MHz, Acetone-d_6) δ 159.6 (C═O), 149.2 (C═O), 149.1 (C_{quat}), 143.3 (C_{quat}), 130.9 (C_{arom}), 130.1 (CH = CH), 129.0 (C_{quat}), 125.3 (CH = CH), 111.2 (C_{arom}), 109.2 (C_{quat}), 100.6 (C_{arom}), 86.3 (O-C_{quat}), 56.4 (O-CH_3), 45.3 (N-$CH_{2\text{-major}}$), 41.2 (N-$CH_{2\text{-minor}}$), 27.6 (3 × C-CH_{3Boc}), 17.8 (CH-$CH_{3\text{-major}}$), 13.4 (CH-$CH_{3\text{-minor}}$). HRMS-ESI (*m/z*) $[M + H]^+$ calcd for $C_{18}H_{23}N_2O_5$: 347.1600, found: 347.1602. Rf : 0.81 (PE / EtOAc 1:1).

*4.1.3.4. Tert-butyl 7-bromo-1-(but-2-en-1-yl)-2,4-dioxo-1,4-dihydroqui-nazoline-3(2H)-carboxylate (**16**).* The title compound was prepared from **15** to afford after purification the desired product **16** as an oil (*E/Z ratio = 85/15*, 85%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.04 (dd, 1H, J = 8.4, 2.7 Hz, H_5), 7.68 (d, 1H, J = 2.7 Hz, H_6), 7.59–7.50 (m, 1H, H_8), 5.91–5.78 (m, 1H, $H_{2'}$), 5.69–5.45 (m, 1H, $H_{3'}$), 4.90 (d, 2H, J = 6.0 Hz, $H_{1'\text{-minor}}$), 4.78 (dd, 2H, J = 5.4, 1.4 Hz, $H_{1'\text{-major}}$), 1.90 (d, 3H, J = 7.0 Hz, $H_{4'\text{-minor}}$), 1.71 (dd, 3H, J = 6.5, 1.5 Hz, $H_{4'\text{-major}}$), 1.62 (s, 9H, H_{Boc}). ^{13}C NMR (101 MHz, Acetone-d_6) δ 159.6 (C═O), 148.8 (C═O), 148.6 (C_{quat}), 142.4 (C_{quat}), 130.8 (C_{quat}), 130.6 (C_{arom}), 129.8 (CH = CH), 127.2 (C_{arom}), 125.0 (CH = CH), 119.0 (C_{arom}), 115.3 (C_{quat}), 86.8 (O-C_{quat}), 45.4 (N-$CH_{2\text{-major}}$), 41.4 (N-$CH_{2\text{-minor}}$), 27.6 (3 × C-CH_{3Boc}), 17.8 (CH-$CH_{3\text{-major}}$), 14.51 (CH-$CH_{3\text{-minor}}$). HRMS-ESI (*m/z*) $[M + Na]^+$ calcd for $C_{17}H_{19}N_2O_4Na$: 418.0422, found: 418.0422. Rf : 0.80 (PE / EtOAc 1:1).

*4.1.3.5. Tert-butyl 6-bromo-1-(but-2-en-1-yl)-2,4-dioxo-1,4-dihydroquinazoline-3(2H)-carboxylate (**22**).* The title compound was prepared from **21** to afford after purification the desired product 22 as an oil (*E/Z ratio = 85/15*) in 80% yields. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.18 (d, 1H, J = 2.5 Hz, H_5), 7.92 (dd, 1H, J = 9.0, 2.5 Hz, H_7), 7.46 (d, 1H, J = 9.0 Hz, H_8), 5.88–5.71 (m, 1H, $H_{2'}$), 5.65–5.40 (m, 1H, $H_{3'}$), 4.86 (d, 2H, J = 6.1 Hz, $H_{1'\text{-minor}}$), 4.73 (dt, 2H, J = 5.6, 1.3 Hz, $H_{1'\text{-major}}$), 1.84 (d, 3H, J = 8.6 Hz, $H_{4'\text{-minor}}$), 1.67 (dd, 3H, J = 6.5, 1.5 Hz, $H_{4'\text{-major}}$), 1.60 (s, 9H, H_{Boc}). ^{13}C NMR (101 MHz, Acetone-d_6) δ 159.1 (C═O), 148.6 (C═O), 148.5 (C_{quat}), 140.6 (C_{quat}), 139.1 (C_{arom}), 130.9 (C_{arom}), 130.1 (CH = CH), 124.8 (CH = CH), 118.7 (C_{arom}), 118.3 (C_{quat}), 116.1 (C_{quat}), 86.9 (O-C_{quat}), 44.5 (N-$CH_{2\text{-major}}$), 41.2 (N-$CH_{2\text{-minor}}$), 27.6 (3 × C-CH_{3Boc}), 178 (CH-$CH_{3\text{-major}}$), 13.4 (CH-$CH_{3\text{-minor}}$). HRMS-ESI (*m/z*) [M + Na]$^+$ calcd for $C_{17}H_{19}N_2O_4Na$: 418.0420, found: 418.0420. Rf : 0.80 (PE / EtOAc 1:1).

*4.1.3.6. Tert-butyl 5-benzyl-3-(but-2-en-1-yl)-2,6-dioxo-3,6-dihydropyrimidine-1(2H)-carboxylate (**36a**).* The title compound was prepared from **35a** to afford after purification the desired product **36a** as an oil (*E/Z ratio = 85/15*) in 95% yields. ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.47 (s, 1H, H_6), 7.30–7.26 (m, 4H, 4 × H_{arom}), 7.23–7.16 (m, 1H, H_{arom}), 5.83–5.74 (m, 1H, $H_{2'}$), 5.62–5.48 (m, 1H, $H_{3'}$), 4.44 (d, 2H, J = 7.1 Hz, $H_{1'\text{-minor}}$), 4.31 (d, 2H, J = 6.4 Hz, $H_{1'\text{-major}}$), 3.62 (s, 2H, C-CH_2), 1.69 (d, 3H, J = 6.5 Hz, $H_{4'\text{-major}}$), 1.55 (s, 9H, H_{Boc}). ^{13}C NMR (101 MHz, Acetone-d_6) δ 161.6 (C═O), 149.2 (C═O), 142.4 (C = CH), 140.1 (C_{quat}), 131.7 (CH = CH), 130.5 (C_{quat}), 129.6 (2 × C_{arom}), 129.2 (2 × C_{arom}), 127.1 (C_{arom}), 125.8 (CH = CH), 114.0 (C_{quat}), 86.3 (O-C_{quat}), 50.2 (N-$CH_{2\text{-major}}$), 45.2 (N-$CH_{2\text{-minor}}$), 33.1 (C-CH_2), 27.6 (3 × C-CH_{3Boc}), 17.8 (CH-$CH_{3\text{-major}}$), 13.1 (CH-$CH_{3\text{-minor}}$). HRMS-ESI (*m/z*) [M + Na]$^+$ calcd for $C_{20}H_{24}N_2NaO_4$: 379.1628, found: 379.1626. Rf : 0.71 (EDP/AcOEt 1:1).

*4.1.3.7. Tert-butyl 3-(but-2-en-1-yl)-2,6-dioxo-5-(phenylamino)-3,6-dihydropyrimidine-1(2H)-carboxylate (**36b**).* The title compound was prepared from **35b** to afford after purification the desired product as an oil (*E/Z ratio = 85/15*, 96%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.93 (s, 1H, $H_{6\text{-minor}}$), 7.91 (s, 1H, $H_{6\text{-major}}$), 7.39–7.28 (m, 4H, 4 × H_{arom}), 7.18 (t, 1H, J = 7.1 Hz, H_{arom}), 5.86–5.76 (m, 1H, $H_{2'}$), 5.67–5.49 (m, 1H, $H_{3'}$), 4.50 (d, 2H, J = 6.8 Hz, $H_{1'\text{-minor}}$), 4.37 (d, 2H, J = 6.1 Hz, $H_{1'\text{-major}}$), 1.75 (d, 3H, J = 8.0 Hz, $H_{4'\text{-minor}}$), 1.70 (d, 3H, J = 6.5 Hz, $H_{4'\text{-major}}$), 1.57 (s, 9H, H_{Boc}), 1.42 (s, 9H, H_{Boc}). ^{13}C NMR (101 MHz, Acetone-d_6) δ 159.5 (C═O), 154.2 (C_{quat}), 149.0 (C═O), 148.6 (C_{quat}), 143.5 (C = CH), 131.8 (CH = CH), 129.3 (2 × C_{arom}), 126.7 (C_{arom}), 126.6 (2 × C_{arom}), 125.6 (CH = CH), 124.72 (C_{quat}), 118.7 (C_{quat}), 86.8 (O-C_{quat}), 81.6 (O-C_{quat}), 50.7 (N-$CH_{2\text{-major}}$), 45.9 (N-$CH_{2\text{-minor}}$), 28.22 (3 × C-CH_{3Boc}), 27.6 (3 × C-CH_{3Boc}) , 17.8 (CH-$CH_{3\text{-major}}$), 14.5 (CH-$CH_{3\text{-minor}}$). HRMS-ESI (*m/z*) [M + H]$^+$ calcd for $C_{24}H_{32}N_3O_6$: 458.2285, found: 458.2285. Rf : 0.70 (EDP/AcOEt 1:1).

*4.1.4. General synthetic procedure 4 for ruthenium-catalyzed cross-metathesis and subsequent Boc removal for compounds **11a, 11b, 11c, 17, 37a, 37b***

A mixture of protected alkylated nucleobase (1 eq.) and dimethyl allylphosphonate (4 eq.), were stirred at room temperature in freshly distilled DCM (0.1 M), 5 mol% Grubbs 2nd generation catalyst was added. The mixture was sonicated at 55 °C (80 kHz, 100 W) under inert atmosphere. After 2 h, 5 mol% of the Grubbs-II catalyst was added to the solution. The reaction was sonicated for 2 h at 55 °C before the addition of a third portion of 5 mol% of the ruthenium catalyst. The reaction was sonicated 18 h under those conditions. Volatiles were eliminated under reduced pressure. Crude product was applied on short flash chromatography with EtOAc as eluent to afford desired compound which was engaged directly on the Boc removal; thus, a solution of obtained protected phosphonate compound (1 eq.) in freshly distilled DCM (0.05 M) was stirred with trifluoroacetic acid (20 eq.) under inert atmosphere for 6 h. After completion of the reaction, the mixture was diluted in EtOAc and washed twice with saturated $NaHCO_3$ solution, dried over $MgSO_4$ filtered and concentrated under reduced pressure. Pure compounds were purified by column chromatography on silica gel with DCM/MeOH (97:3) as eluent.

*4.1.4.1. Dimethyl (4-(2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphonate (**11a**).* The title compound was prepared from **10a** to afford after purification the desired product as a white/green solid (*E/Z ratio = 85/15*, 51% over two steps). ^{1}H NMR (250 MHz, DMSO-d_6) δ 11.60 (bs, 1H, NH), 8.00 (dd, 1H, J = 7.8, 1.5 Hz, H_5), 7.72 (ddd, 1H, J = 8.7, 7.8, 1.5 Hz, H_7), 7.37 (d, 1H, J = 8.7 Hz, H_8), 7.26 (t, 1H, J = 7.8 Hz, H_6), 5.78–5.51 (m, 2H, $H_{2',3'}$), 4.80–4.77 (m, 2H, $H_{1'\text{-minor}}$), 4.68 (t, 2H, J = 4.7 Hz, $H_{1'\text{-major}}$), 3.70 (s, 3H, $H_{POMe\text{-minor}}$), 3.66 (s, 3H, $H_{POMe\text{-minor}}$), 3.58 (s, 3H, $H_{POMe\text{-major}}$), 3.53 (s, 3H, $H_{POMe\text{-major}}$), 2.93 (dd, 2H, J = 21.7, 6.7 Hz, $H_{4'\text{-minor}}$), 2.66 (dd, 2H, J = 21.7, 6.7 Hz, $H_{4'\text{-major}}$). ^{13}C NMR (63 MHz, DMSO-d_6) δ 161.7 (C═O), 150.0 (C═O), 140.7 (C_{quat}), 135.1 (C_{arom}), 129.2 (d, $J^3_{C\text{-}P}$ = 14.4 Hz, CH = CH), 127.4 (C_{arom}), 122.55 (C_{arom}), 122.5 (d, $J^2_{C\text{-}P}$ = 11.3 Hz, CH = CH), 115.7 (C_{quat}), 115.1 (C_{arom}), 52.2 (O-CH_3), 52.2 (O-CH_3), 43.2 (N-CH_2), 27.9 (d, $J^1_{C\text{-}P}$ = 136.7 Hz, CH-CH_2). ^{31}P NMR (101 MHz, DMSO-d_6) δ 29.58 (P_Z), 29.25 (P_E). HRMS-ESI (*m/z*) [M + H]$^+$ calcd for $C_{14}H_{17}N_2O_5P$: 325.2695, found: 325.2696. Rf : 0.35 (DCM/MeOH 95:5).

*4.1.4.2. Dimethyl (4-(6-methyl-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphonate (**11b**).* The title compound was prepared from **10b** to afford after purification the desired product as a white/green foam (*E/Z ratio = 85/15*, 56% over two steps). ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.25 (bs, 1H, NH), 7.88 (s, 1H, H_5), 7.55 (d, 1H, J = 6.7 Hz, H_7), 7.32 (d, 1H, J = 8.5 Hz, H_8), 5.82–5.62 (m, 2H, $H_{2',3'}$), 4.88 (t, 2H, J = 3.6 Hz, $H_{1'\text{-minor}}$), 4.74 (t, 1H, J = 4.6 Hz, $H_{1'\text{-major}}$), 3.76 (s, 3H, $H_{POMe\text{-minor}}$), 3.74 (s, 3H, $H_{POMe\text{-minor}}$), 3.63 (s, 3H, $H_{POMe\text{-major}}$), 3.61 (s, 3H, $H_{POMe\text{-major}}$), 2.92 (dd, 2H, J = 22.5, 6.1 Hz, $H_{4'\text{-minor}}$), 2.61 (dd, 2H, J = 21.8, 6.9 Hz, $H_{4'\text{-major}}$), 2.38 (s, 3H, C-CH_3). ^{13}C NMR (101 MHz, Acetone-d_6) δ 162.4 (C═O), 150.8 (C═O), 140.0 (C_{quat}), 136.8 (C_{arom}), 133.1 (C_{quat}), 129.2 (d, $J^3_{C\text{-}P}$ = 15.1 Hz, CH = CH), 128.4 (C_{arom}), 123.8 (d, $J^2_{C\text{-}P}$ = 10.8 Hz, CH = CH), 117.0 (C_{quat}), 115.8 (C_{arom}), 52.9 (O-$CH_{3\text{-minor}}$), 52.8 (O-$CH_{3\text{-minor}}$), 52.7 (O-$CH_{3\text{-major}}$), 52.7 (O-$CH_{3\text{-major}}$), 44.4 (N-$CH_{2\text{-major}}$), 40.7 (N-$CH_{2\text{-minor}}$), 29.5 (d, $J^1_{C\text{-}P}$ = 138.58 Hz, CH-$CH_{2\text{-major}}$), 26.5 (d, $J^1_{C\text{-}P}$ = 139.3 Hz, CH-$CH_{2\text{-minor}}$), 20.3 (C-$CH_{3\text{-major}}$). ^{31}P NMR (162 MHz, Acetone-d_6) δ 28.76 (P_Z), 28.28 (P_E). HRMS-ESI (*m/z*) [M + H]$^+$ calcd for $C_{15}H_{19}N_2O_5P$: 339.1104, found: 339.1104. Rf : 0.35 (DCM/MeOH 95:5).

*4.1.4.3. Dimethyl (4-(7-methoxy-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphonate (**11c**).* The title compound was prepared from **10c** to afford after purification the desired product as an oil (*E/Z ratio = 85/15*, 42% over two steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.42 (bs, 1H, NH), 7.90 (d, 1H, J = 8.7 Hz, H_5), 6.83 (dd, 1H, J = 8.7, 2.2 Hz, H_6), 6.77 (d, 1H, J = 2.2 Hz, H_8), 5.64–5.60 (m, 2H, $H_{2',3'}$), 4.79–4.73 (m, 2H, $H_{1'\text{-minor}}$), 4.69 (t, 2H, J = 4.7 Hz, $H_{1'\text{-major}}$), 3.89 (s, 3H, O-CH_3), 3.69 (s, 3H, $H_{POMe\text{-minor}}$), 3.66 (s, 3H, $H_{POMe\text{-minor}}$), 3.55 (s, 3H, $H_{POMe\text{-major}}$), 3.53 (s, 3H, $H_{POMe\text{-major}}$), 2.96 (dd, 2H, J = 22.6, 6.4 Hz, $H_{4'\text{-minor}}$), 2.68 (dd, 2H, J = 21.6, 6.8 Hz, $H_{4'\text{-major}}$). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.6 (C═O), 161.2 (C_{quat}), 150.3 (C═O), 142.6 (C_{quat}), 129.3 (C_{arom}), 128.7 (d, $J^3_{C\text{-}P}$ = 14.5 Hz, CH = CH), 122.6 (d, $J^2_{C\text{-}P}$ = 10.9 Hz, CH = CH), 110.0 (C_{arom}), 108.8 (C_{quat}), 99.3 (C_{arom}), 55.7 (C—O—CH_3), 52.3 (O-$CH_{3\text{-minor}}$), 52.3 (O-$CH_{3\text{-minor}}$), 52.2 (O-$CH_{3\text{-major}}$), 52.1 (O-$CH_{3\text{-major}}$), 43.3 (N-$CH_{2\text{-major}}$), 36.85 (N-$CH_{2\text{-minor}}$), 27.8 (d, $J^1_{C\text{-}P}$ = 136.4 Hz, CH-$CH_{2\text{-major}}$), 24.3 (d, $J^1_{C\text{-}P}$ = 136.7 Hz, CH-$CH_{2\text{-minor}}$). ^{31}P NMR (162 MHz, DMSO-d_6) δ 29.35. HRMS-ESI (*m/z*) [M + H]$^+$ calcd for $C_{15}H_{20}N_2O_6P$: 355.1053, found: 355.1053. Rf : 0.35 (DCM/MeOH 95:5).

*4.1.4.4. Dimethyl (4-(7-bromo-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphonate (**17**).* The title compound was prepared from **16** according to the general procedure 4, affording after purification the desired product **17** as an oil (*E/Z ratio = 85/15*, 55% over two steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (s, 1H, NH), 7.90 (d, 1H, J = 8.4 Hz, H_5), 7.56 (d, 1H, J = 1.6 Hz, H_8), 7.44 (dd, 1H, J = 8.4, 1.6 Hz, H_6), 5.76–5.66 (m, 1H, $H_{2'}$), 5.63–5.51 (m, 1H, $H_{3'}$), 4.80 (t, 2H, J = 3.8 Hz, $H_{1'\text{-minor}}$), 4.68 (t, 2H, J = 4.5 Hz, $H_{1'\text{-major}}$), 3.70 (s, 3H, $H_{POMe\text{-minor}}$), 3.67 (s, 3H, $H_{POMe\text{-minor}}$), 3.58 (s, 3H, $H_{POMe\text{-major}}$), 3.56 (s, 3H, $H_{POMe\text{-major}}$), 2.94 (dd, 2H, J = 22.5, 6.5 Hz, $H_{4'\text{-minor}}$), 2.67 (dd, 2H, J = 21.7, 7.1 Hz, $H_{4'\text{-major}}$). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.2 (C═O), 149.8 (C═O), 141.8 (C_{quat}), 129.2 (C_{arom}), 129.0 (C_{quat}), 128.3 (d, $J^3_{C\text{-}P}$ = 14.5 Hz, CH = CH), 125.6 (C_{arom}), 122.3 (d, $J^2_{C\text{-}P}$ = 10.8 Hz, CH = CH), 117.7 (C_{arom}), 114.9 (C_{quat}), 52.2 (O-CH_3), 52.2 (O-CH_3), 43.2 (N-CH_2), 28.0 (d, $J^1_{C\text{-}P}$ = 136.3 Hz, CH-CH_2). ^{31}P NMR (162 MHz, DMSO-d_6) δ 29.16 (P_E), 28.93 (P_Z). HRMS-ESI (*m/z*) $[M]^+$ calcd for $C_{14}H_{17}BrN_2O_5P$: 403.0052, found: 403.0051. Rf : 0.32 (DCM/MeOH 95:5).

*4.1.4.5. Dimethyl (4-(5-benzyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) but-2-en-1-yl)phosphonate (**37a**).* The title compound was prepared according to the general procedure 4 from **36a** to afford after purification the desired product **37a** as an oil (*E/Z ratio = 85/15*, 51%). ^{1}H NMR (250 MHz, Acetone-d_6) δ 10.43 (bs, 1H, NH), 7.65 (s, 1H, $H_{6\text{-minor}}$), 7.41 (s, 1H, $H_{6\text{-major}}$), 7.31–7.22 (m, 5H, 5 × H_{arom}), 5.79–5.59 (m, 2H, $H_{2',3'}$), 4.47 (t, 2H, J = 4.4 Hz, $H_{1'\text{-minor}}$), 4.32 (t, 2H, J = 4.4 Hz, $H_{1'\text{-major}}$), 3.73 (s, 3H, $H_{POMe\text{-minor}}$), 3.69 (s, 3H, $H_{POMe\text{-major}}$), 3.65 (s, 3H, $H_{POMe\text{-major}}$), 3.59 (s, 2H, C-CH_2), 2.86 (dd, 2H, J = 22.6, 6.6 Hz, $H_{4'\text{-minor}}$), 2.66 (dd, 2H, J = 21.7, 5.8 Hz, $H_{4'\text{-major}}$). ^{13}C NMR (63 MHz, Acetone-d_6) δ 164.4 (C═O), 151.6 (C═O), 142.5 (C = CH), 140.7 (C_{quat}), 130.0 (d, $J^3_{C\text{-}P}$ = 14.4 Hz, CH = CH), 129.4 (2 × C_{arom}), 129.0 (2 × C_{arom}), 126.8 (C_{arom}), 125.2 (d, $J^2_{C\text{-}P}$ = 10.9 Hz, CH = CH), 114.3 (C_{quat}), 52.9 (O-CH_3), 52.8 (O-CH_3), 49.3 (N-$CH_{2\text{-major}}$), 44.8 (N-$CH_{2\text{-minor}}$), 33.0 (C-CH_2), 29.3 (d, $J^1_{C\text{-}P}$ = 138.22 Hz, CH-$CH_{2\text{-major}}$), 25.1 (d, $J^1_{C\text{-}P}$ = 137.8 Hz, CH-$CH_{2\text{-minor}}$). ^{31}P NMR (162 MHz, Acetone-d_6) δ 28.81 (P_Z), 28.58 (P_E). HRMS-ESI (*m/z*) $[M + H]^+$ calcd for $C_{17}H_{22}N_2O_5P$: 365.1258, found: 365.1260. Rf : 0.35 (DCM/MeOH 95:5).

*4.1.4.6. Dimethyl (4-(2,4-dioxo-5-(phenylamino)-3,4-dihydropyrimidin-1 (2H)-yl)but-2-en-1-yl)phosphonate (**37b**).* The title compound was prepared according to the general procedure 4 from **36b** to afford after purification the desired product **37b** as a white/blue foam (*E/Z ratio = 85/15*, 53%). ^{1}H NMR (250 MHz, Acetone-d_6) δ 10.71 (bs, 1H, NH), 7.59 (s, 1H, $H_{6\text{-minor}}$), 7.44 (s, 1H, $H_{6\text{-major}}$), 7.17 (t, 2H, J = 7.4 Hz, 2 × H_{arom}), 6.98 (d, 2H, J = 8.0 Hz, 2 × H_{arom}), 6.75 (t, 1H, J = 7.4 Hz, H_{arom}), 6.47 (bs, 1H, NH), 5.77 (d, 2H, J = 5.1 Hz, $H_{2',3'}$), 4.50 (d, 2H, J = 5.6 Hz, $H_{1'\text{-minor}}$), 4.41–4.35 (m, 2H, $H_{1'\text{-major}}$), 3.70 (s, 3H, $H_{POMe\text{-minor}}$), 3.67 (s, 3H, $H_{POMe\text{-major}}$), 3.63 (s, 3H, $H_{POMe\text{-major}}$), 2.87 (dd, 2H, J = 22.6, 7.6 Hz, $H_{4'\text{-minor}}$), 2.67 (dd, 2H, J = 21.7, 5.6 Hz, $H_{4'\text{-major}}$). ^{13}C NMR (63 MHz, Acetone-d_6) δ 162.5 (C═O), 150.3 (C═O), 145.3 (C_{quat}), 130.2 (d, $J^3_{C\text{-}P}$ = 14.4 Hz, CH = CH), 130.0 (C = CH), 129.9 (2 × C_{arom}), 125.1 (d, $J^2_{C\text{-}P}$ = 10.9 Hz, CH = CH)., 120.2 (C_{arom}), 118.9 (C_{quat}), 116.5 (2 × C_{arom}), 52.9 (O-CH_3), 52.8 (O-CH_3) , 49.5 (N-$CH_{2\text{-major}}$), 45.2 (N-$CH_{2\text{-minor}}$), 29.4 (d, $J^1_{C\text{-}P}$ = 138.38 Hz, CH-$CH_{2\text{-major}}$), 25.1 (d, $J^1_{C\text{-}P}$ = 137.9 Hz, CH-$CH_{2\text{-minor}}$). ^{31}P NMR (162 MHz, Acetone-d_6) δ 28.73. HRMS-ESI (*m/z*) $[M + H]^+$ calcd for $C_{17}H_{21}N_3O_5P$: 366.1213, found: 366.1213. Rf : 0.30 (DCM/MeOH 95:5).

*4.1.5. General synthetic procedure 5 for dimethyl phosphonate deprotection for compounds **12a, 12b, 12c, 19a, 19b, 19c, 38a***

To a dry ACN (15 ml) solution of acyclo nucleosides dialkylphosphonate (1 eq.), TMSBr (15 eq.) was added under inert atmosphere and heated under microwave irradiation at 80 °C for 10 min. MeOH was added and evaporated with heating. MeOH was added again, and this procedure was repeated four times. The residue dissolved in ultrapure H_2O and extracted with DCM. The aqueous layer was lyophilized to yield the pure compound.

*4.1.5.1. (4-(2,4-Dioxo-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl) phosphonic acid (**12a**).* The title compound was prepared according to the general procedure 5 from **11a** to afford after lyophilisation the desired product as a white solid (*E/Z ratio = 85/15*, 95%). ^{1}H NMR (250 MHz, DMSO-d_6) δ 11.58 (bs, 1H, NH), 9.33 (bs, 2H, 2 × H_{POH}), 7.99 (dd, 1H, J = 7.8, 1.3 Hz, H_5), 7.77–7.63 (m, 1H, H_7), 7.38 (d, 1H, J = 8.4 Hz, H_8), 7.25 (t, 1H, J = 7.8 Hz, H_6), 5.77–5.56 (m, 2H, $H_{2',3'}$), 4.78–4.73 (m, 2H, $H_{1'\text{-minor}}$), 4.69–4.59 (m, 2H, $H_{1'\text{-major}}$), 2.67 (dd, 2H, J = 21.2, 5.7 Hz, $H_{4'\text{-minor}}$), 2.38 (dd, 2H, J = 21.3, 5.6 Hz, $H_{4'\text{-major}}$). ^{13}C NMR (63 MHz, DMSO-d_6) δ 161.8 (C═O), 150.0 (C═O), 140.8 (C_{quat}), 135.2 (C_{arom}), 127.4 (C_{arom}), 127.1 (d, $J^3_{C\text{-}P}$ = 14.0 Hz, CH = CH), 125.0 (d, $J^2_{C\text{-}P}$ = 10.2 Hz, CH = CH), 122.5 (C_{arom}), 115.7 (C_{quat}), 115.1 (C_{arom}), 43.4 (N-CH_2), 32.0 (d, $J^1_{C\text{-}P}$ = 134.4 Hz, CH-CH_2). ^{31}P NMR (101 MHz, DMSO-d_6) δ 22.04 (P_Z), 21.72 (P_E). HRMS-ESI (*m/z*) $[M + H]^+$ calcd for $C_{12}H_{13}N_2O_5P$: 296.0635, found: 296.0634.

*4.1.5.2. (4-(6-Methyl-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphonic acid (**12b**).* The title compound was prepared according to the general procedure 5 from **11b** to afford after lyophilisation the desired product as a white solid (*E/Z ratio = 85/15*, 97%). ^{1}H NMR (250 MHz, DMSO-d_6) δ 11.48 (s, 1H, NH), 7.74 (s, 1H, H_5), 7.48 (d, 1H, J = 8.6 Hz, H_7), 7.23 (d, 1H, J = 8.6 Hz, H_8), 6.00–5.59 (bs, 2H, 2 × H_{POH}), 5.61–5.48 (m, 2H, $H_{2',3'}$), 4.71–4.66 (m, 2H, $H_{1'\text{-minor}}$), 4.65–4.52 (m, 2H, $H_{1'\text{-major}}$), 2.61 (dd, 2H J = 22.5, 7.5 Hz, $H_{4'\text{-minor}}$), 2.32 (dd, 2H, J = 22.5, 7.5 Hz, H $H_{4'\text{-major}}$), 2.27 (s, 3H, C-CH_3). ^{13}C NMR (63 MHz, DMSO-d_6) δ 162.2 (C═O), 150.4 (C═O), 139.1 (C_{quat}), 136.5 (C_{arom}), 132.2 (C_{quat}), 127.7 (CH = CH), 127.5 (C_{arom}), 125.2 (d, $J^2_{C\text{-}P}$ = 10.2 Hz, CH = CH), 115.9 (C_{quat}), 115.6 (C_{arom}), 43.8 (N-CH_2), 32.4 (d, $J^1_{C\text{-}P}$ = 134.2 Hz, CH-CH_2), 20.4 (C-CH_3). ^{31}P NMR (101 MHz, DMSO-d_6) δ 21.81 (P_Z), 21.62 (P_E). HRMS-ESI (*m/z*) $[M-H]^-$ calcd for $C_{13}H_{14}N_2O_5P$: 309.0645, found: 309.0645.

*4.1.5.3. (4-(7-Methoxy-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphonic acid (**12c**).* The title compound was prepared according to the general procedure 5 from **11c** to afford after lyophilisation the desired product as a white solid (*E/Z ratio = 85/15*, 94%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.40 (s, 1H, NH), 7.88 (d, 1H, J = 8.8 Hz, H_5), 6.81 (d, 1H, J = 8.8 Hz, H_6), 6.76 (s, 1H, H_8), 6.48 (bs, 2H, 2 × H_{POH}), 5.77–5.53 (m, 2H, $H_{2',3'}$), 4.81–4.72 (m, 2H, $H_{1'\text{-minor}}$), 4.70–4.58 (m, 2H, $H_{1'\text{-major}}$), 3.87 (s, 3H, H_{13}), 2.66 (dd, 2H, J = 20.8, 6.9 $H_{12\text{-minor}}$), 2.40 (dd, 2H, J = 21.4, 6.7 Hz, $H_{12\text{-major}}$). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.7 (C═O), 161.3 (C_{quat}), 150.4 (C═O), 142.8 (C_{quat}), 129.3 (C_{arom}), 127.9 (d, $J^3_{C\text{-}P}$ = 13.1 Hz, CH = CH-$_{minor}$), 125.1 (d, $J^2_{C\text{-}P}$ = 10.3 Hz, CH = CH-$_{major}$), 124.4 (d, J = 10.8 Hz, CH = CH_Z), 110.2 (C_{arom}), 108.8 (C_{quat}), 99.2 (C_{arom}), 55.8 (O-CH_3), 43.6 (N-CH_2), 32.0 (d, J = 134.3 Hz, CH-$CH_{2\text{-major}}$), 28.3 (d, $J^1_{C\text{-}P}$ = 133.1 Hz, CH-$CH_{2\text{-minor}}$). ^{31}P NMR (162 MHz, DMSO-d_6) δ 22.19 (P_Z), 21.80 (P_E). HRMS-ESI (*m/z*) $[M-H]^-$ calcd for $C_{13}H_{14}N_2O_6P$: 325.0594, found: 325.0594.

*4.1.5.4. (4-(7-(3-Octanamidoprop-1-ynyl)-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphonic acid (**19a**).* The title compound was prepared from **18a** to afford after lyophilisation the desired product **19a** as a beige solid (qtf). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (bs, 1H, NH), 8.38 (t, 1H, J = 5.4 Hz, NH), 7.95 (d, 1H, J = 8.0 Hz, H_5), 7.35 (s, 1H, H_8), 7.23 (d, 1H, J = 8.0 Hz, H_6), 5.61 (t, 2H, J = 3.6 Hz, $H_{2',3'}$), 4.65 (d, 2H, J = 4.0 Hz, $H_{1'}$), 4.15 (d, 2H, J = 5.4 Hz, NH-CH_2), 2.37 (dd, 2H, J = 21.9, 4.8 Hz, $H_{4'}$), 2.11 (t, 2H, J = 7.4 Hz, CH_2), 1.49 (p, 2H, J = 7.4 Hz, CH_2), 1.22 (d, 8H, J = 6.3 Hz, 4 × CH_2), 0.83 (t, 3H, J = 6.6 Hz, CH_2-CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.9 (NH-CO), 161.1 (C═O), 149.8 (C═O), 140.7 (C_{quat}), 128.7 (C_{quat}), 127.7 (C_{arom}), 126.7 (d, $J^3_{C\text{-}P}$ = 14.1 Hz, CH = CH), 125.2 (C_{arom}), 124.6 (d, $J^2_{C\text{-}P}$ = 10.1 Hz, CH = CH), 117.3 (C_{arom}), 115.2 (C_{quat}), 91.0 (CH_2-C), 80.6 (C≡C-C), 43.2 (N-CH_2), 34.9 (CO-CH_2), 31.9 (d, $J^1_{C\text{-}P}$ = 135.3 Hz,

CH_2-P), 31.0 (CH_2-CH_2), 28.4 (CH_2-CH_2), 28.4 (NH-CH_2), 28.3 (CH_2-CH_2), 25.0 (CH_2-CH_2), 21.9 (CH_2-CH_2), 13.8 (CH_2-CH_3). ^{31}P NMR (162 MHz, DMSO-d_6) δ 21.37. HRMS-ESI (*m/z*) [M−H]$^-$ calcd for $C_{23}H_{29}N_3O_6P$: 474.1797, found: 474.1799.

*4.1.5.5. (4-(2,4-Dioxo-7-(3-(2-phenylacetamido)prop-1-ynyl)-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphonic acid (**19b**).* The title compound was prepared from **18b** to afford after lyophilisation the desired product **19b** as a beige solid (qtf). ^{1}H NMR (250 MHz, DMSO-d_6) δ 11.64 (bs, 1H, NH), 8.66 (t, 1H, J = 5.4 Hz, NH), 7.96 (d, 1H, J = 8.1 Hz, H_5), 7.36 (s, 1H, H_8), 7.33–7.16 (m, 6H, H_7, 5 × H_{arom}), 5.78–5.52 (m, 2H, $H_{2',3'}$), 4.66 (d, 1H, J = 5.0 Hz, $H_{1'}$), 4.18 (d, 2H, J = 5.3 Hz, NH-CH_2), 3.48 (s, 2H, CH_2), 2.44–2.31 (m, 2H, $H_{4'}$). ^{13}C NMR (63 MHz, DMSO-d_6) δ 170.0 (NH-CO), 161.1 (C═O), 149.8 (C═O), 140.7 (C_{quat}), 135.9 (C_{arom}), 130.5 (C_{quat}), 128.8 (2xC_{arom}), 128.6 (C_{quat}), 128.1 (2xC_{arom}), 127.7 (C_{arom}), 126.3 (CH = CH), 126.3 (C_{arom}), 124.9 (CH = CH), 117.2 (C_{arom}), 115.4 (C_{quat}), 90.6 (CH_2-C), 81.0 (C≡C-C), 43.3 (N-CH_2), 41.9 (NH-CH_2), 31.9 (d, $J^1_{C\text{-}P}$ = 135.3 Hz, CH_2-P), 28.7 (CO-CH_2). ^{31}P NMR (162 MHz, DMSO-d_6) δ 21.33. HRMS-ESI (*m/z*) [M−H]$^-$ calcd for $C_{23}H_{21}N_3O_6P$: 466.1174, found: 466.1178.

*4.1.5.6. (4-(7-(3-(3-Hexylureido)prop-1-ynyl)-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphonic acid (**19c**).* The title compound was prepared from **18c** to afford after lyophilisation the desired product **19c** as a beige solid (qtf). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (bs, 1H, NH), 7.95 (d, 1H, J = 8.0 Hz, H_5), 7.35 (s, 1H, H_8), 7.23 (d, 1H, J = 8.0 Hz, H_7), 5.61 (t, 2H, J = 3.7 Hz, $H_{2',3'}$), 4.66 (d, , 2H, J = 5.0 Hz, $H_{1'}$), 4.10 (s, 2H, NH-CH_2), 2.97 (dt, 2H, J = 12.0, 6.6 Hz, CH_2), 2.38 (dd, 2H, J = 21.7, 4.8 Hz, $H_{4'}$), 1.35 (q, 2H, J = 6.7, 6.3 Hz, NH-CH_2), 1.24 (bs, 6H, 3 × CH_2), 0.85 (t, 3H, J = 6.6 Hz, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.2 (NH-CO-NH), 157.5 (C═O), 149.9 (C═O), 140.9 (C_{quat}), 129.0 (C_{quat}), 127.8 (C_{arom}), 126.9 (d, $J^3_{C\text{-}P}$ = 14.1 Hz, CH = CH), 125.3 (C_{arom}), 124.7 (d, $J^2_{C\text{-}P}$ = 10.1 Hz, CH = CH), 117.3 (C_{arom}), 115.2 (C_{quat}), 92.4 (CH_2-C), 80.5 (C≡C-C), 43.2 (N-CH_2), 31.9 (d, $J^1_{C\text{-}P}$ = 134.3 Hz, CH_2-P), 31.0 (NH-CH_2), 29.8 (CH_2-CH_2), 29.7 (NH-CH_2), 29.6 (CH_2-CH_2), 26.0 (CH_2-CH_2), 22.0 (CH_2-CH_2), 13.9 (CH_2-CH_3). ^{31}P NMR (162 MHz, DMSO-d_6) δ 21.41. HRMS-ESI (*m/z*) [M−H]$^-$ calcd for $C_{22}H_{28}N_4O_6P$: 475.1751, found: 475.1751.

*4.1.5.7. (4-(5-Benzyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)but-2-en-1-yl)phosphonic acid (**38a**).* The title compound was prepared according to the general procedure 5 from **37a** to afford after lyophilisation the desired product **39a** as a white foam (*E/Z ratio = 85/15*) in 90% yields. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.25 (bs 1H, NH), 8.89 (s, 2H, 2 × H_{POH}), 7.60 (s, 1H, $H_{6\text{-}minor}$), 7.57 (s, 1H, $H_{6\text{-}major}$), 7.27–7.17 (m, 4H, 4 × H_{arom}), 7.17–7.09 (m, 1H, H_{arom}), 5.71–5.41 (m, 2H, $H_{2',3'}$), 4.32 (dd, 2H, J = 6.5, 3.2 Hz, $H_{1'\text{-}minor}$), 4.24 (d, 2H, J = 4.7 Hz, $H_{1'\text{-}major}$), 2.62 (dd, 2H, J = 22.1, 7.6 Hz, $H_{4'\text{-}minor}$), 2.48–2.38 (m, 2H, $H_{4'\text{-}major}$). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.7 (C═O), 150.7 (C═O), 142.2 (C = CH), 139.9 (C_{quat}), 128.4 (2 × C_{arom}), 128.3 (2 × C_{arom}), 128.0 (d, $J^3_{C\text{-}P}$ = 14.1 Hz, CH = CH), 126.0 (C_{arom}), 125.9 (d, $J^2_{C\text{-}P}$ = 10.4 Hz, CH = CH), 112.5 (C_{quat}), 48.4 (N-CH_2), 32.8 (d, $J^1_{C\text{-}P}$ = 134.3 Hz, CH-CH_2), 31.9 (C-CH_2). ^{31}P NMR (162 MHz, DMSO-d_6) δ 22.85 (P_Z), 22.25 (P_E). HRMS-ESI (*m/z*) [M−H]$^-$ calcd for $C_{15}H_{16}N_2O_5P$: 305.0799, found: 305.0800.

*4.1.6. N-Prop-2-ynyloctanamide (**13a**)*

Propargylamine (**13**) (1 eq.) was dissolved in anhydrous DCM (0.4 M), DIPEA (1.3 eq.) was added and the solution was cooled to 0 °C. Octanoyl chloride (1.1 eq.) was added dropwise and the reaction mixture was stirred at rt for 2 h. The reaction was quenched with water, then diluted with DCM and washed with $NaHCO_3$ solution and brine. Organic layer was dried over $MgSO_4$ and evaporated. The title compound was obtained without further purification as a solid (qtf). CAS # 422284–34-2.[16] ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.56 (bs, 1H, H_4), 4.06 (dd, 2H, J = 5.2, 2.6 Hz, H_3), 2.25–2.15 (m, 3H), 1.69–1.56 (m, 2H, H_7), 1.38–1.20 (m, 8H), 0.92–0.81 (m, 3H, H_{12}).

*4.1.7. 2-Phenyl-N-prop-2-ynyl-acetamide (**13b**)*

Propargylamine (**13**) (1 eq.) was dissolved in anhydrous DCM (0.41 M), DIPEA (1.3 eq.) was added and the solution was cooled to 0 °C. Phenylacetyl chloride (1.1 eq.) was added dropwise and the reaction mixture was stirred at rt for 2 h. The reaction was quenched with water, then diluted with DCM and washed with $NaHCO_3$ solution and brine. Organic layer was dried over MgSO4 and evaporated. The title compound **13b** was obtained without further purification as a solid (93%). CAS # 174271–37-5.[16] ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.42–7.22 (m, 5H, H_{arom}), 5.54 (bs, 1H, H_4), 4.01 (dd, 2H, J = 5.3, 2.6 Hz, H_3), 3.60 (s, 2H, H_6), 2.18 (t, 1H, J = 2.6 Hz, H_1).

*4.1.8. 1-Hexyl-3-prop-2-ynyl-urea (**13c**)*

Propargylamine (**13**) (1 eq.) was dissolved in anhydrous DCM (0.4 M) under an Ar atmosphere, and the resulting yellow solution was cooled to 0 °C. Hexyl isocyanate (1 eq.) dissolved in anhydrous DCM (0.78 M) was added drop-wise, the ice bath was removed, and the reaction mixture was stirred at rt for 45 min. The solvent was evaporated to obtained title compound **13c** as a solid (94%) without further purification. CAS # 1311414–23-9.[18] ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.40 (bs, 2H, $H_{4,6}$), 3.99 (dd, 2H, J = 5.5, 2.5 Hz, H_3), 3.22–3.12 (m, 2H, H_7), 2.23 (t, 1H, J = 2.5 Hz, H_1), 1.56–1.43 (m, 2H, H_8), 1.38–1.22 (m, 6H, $H_{9,10,11}$), 0.94–0.81 (m, 3H, H_{12}).

*4.1.9. General synthetic procedure 6 for Pd(0) cross-coupling under Sonogashira conditions for compounds **18a, 18b, 18c,***

Under inert atmosphere, to a solution of bromoaryl acyclo nucleoside phosphonate (1 eq.) in dry DMF (0.087 M) were successively added copper iodide (0.2 eq.), triethylamine (3 eq.), alkyne (3 eq.), and Pd(PPh_3)$_4$ (10 mol%). The reaction mixture was heated at 70 °C for 3 h. The reaction was quenched with EtOAc and co-evaporated with heptane. Pure compounds were obtained after purification on silica gel column chromatography using an elution gradient of DCM/MeOH (from 97:3 to 95:5) to give pure product.

4.1.9.1. Dimethyl (4-(7-(3-nonanamidoprop-1-ynyl)-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphonate (18a). The title compound was prepared from **17** to afford after purification the desired product **18a** and alkyne **13a** as a light orange solid (*E/Z ratio = 85/15*, 96%). ^{1}H NMR (250 MHz, Methanol-d_4) δ 8.07 (d, 1H, J = 8.1 Hz, H_5), 7.40 (d, 1H, J = 1.2 Hz, H_8), 7.31 (d, 1H, J = 8.1 Hz, H_6), 5.86 (dt, 1H, J = 14.9, 4.8 Hz, $H_{2'}$), 5.78–5.55 (m, 1H, $H_{3'}$), 4.80 (d, 2H, J = 5.3 Hz, $H_{1'}$), 4.29 (s, 2H, C-CH_2), 3.90 (s, 3H, $H_{POMe\text{-}minor}$), 3.85 (s, 3H, $H_{POMe\text{-}minor}$), 3.77 (s, 3H, $H_{POMe\text{-}major}$), 3.73 (s, 3H, $H_{POMe\text{-}major}$), 2.77 (dd, 2H, J = 21.9, 7.1 Hz, $H_{4'}$), 2.30 (t, 2H, J = 7.5 Hz, C-CH_2), 1.70 (p, 2H, J = 7.3 Hz, CH_2), 1.46–1.28 (m, 8H, 4 × CH_2), 1.01–0.88 (m, 3H, CH_3). ^{13}C NMR (63 MHz, Methanol-d_4) δ 175.9 (CH_2-CO-NH), 163.5 (C═O), 151.9 (C═O), 142.3 (C_{quat}), 131.2 (C_{quat}), 130.2 (d, $J^3_{C\text{-}P}$ = 14.5 Hz, CH = CH), 129.2 (C_{arom}), 127.0 (C_{arom}), 123.0 (d, $J^2_{C\text{-}P}$ = 11.3 Hz, CH = CH), 119.1 (C_{arom}), 116.8 (C_{quat}), 90.8 (CH_2-C), 82.2 (C≡C-C), 53.6 (O-CH_3), 53.5 (O-CH_3), 44.9 (N-CH_2), 36.8 (CO-CH_2), 32.8 (CH_2-CH_2), 30.2 (NH-CH_2), 30.1 (CH_2-CH_2), 30.1 (CH_2-CH_2), 29.3 (d, $J^1_{C\text{-}P}$ = 139.8 Hz, CH-CH_2-P), 26.8 (CH_2-CH_2), 23.6 (CH_2-CH_2), 14.3 (CH_2-CH_3). ^{31}P NMR (101 MHz, Methanol-d_4) δ 30.31 (P_E), 22.90 (P_Z). HRMS-ESI (*m/z*) [M + H]$^+$ calcd for $C_{25}H_{35}N_3O_6P$: 504.2256, found: 504.2257. Rf : 0.21 (DCM/MeOH 95:5).

*4.1.9.2. Dimethyl (4-(2,4-dioxo-7-(3-(2-phenylacetamido)prop-1-ynyl)-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphonate (**18b**).* The title compound was prepared from **17** and alkyne **13b** to afford after purification the desired product **18b** as a light orange solid (81%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.36 (bs, 1H, NH), 8.02 (d, 1H, J = 8.0

Hz, H_5), 7.40 (s, 1H, H_8), 7.38–7.34 (m, 2H, 2 × H_{arom}), 7.33–7.28 (m, 2H, 2 × H_{arom}), 7.27–7.19 (m, 2H, H_7, H_{arom}), 5.77 (td, 2H, J = 5.2, 4.8, 3.5 Hz, $H_{2',3'}$), 4.80 (t, 2H, J = 4.1 Hz, $H_{1'}$), 4.24 (d, 2H, J = 5.2 Hz, C-CH_2), 3.79 (s, 3H, $H_{POMe\text{-}minor}$), 3.76 (s, 3H, $H_{POMe\text{-}minor}$), 3.64 (s, 3H, $H_{POMe\text{-}major}$), 3.62 (s, 3H, $H_{POMe\text{-}major}$), 3.58 (s, 2H, C-CH_2), 2.73–2.63 (m, 2H, $H_{4'}$). ^{13}C NMR (101 MHz, Acetone-d_6) δ 170.8 (CH_2-CO-NH), 161.9 (C═O), 150.8 (C═O), 142.2 (C_{quat}), 137.1 (C_{quat}), 130.2 (C_{quat}), 130.0 (2 × C_{arom}), 129.7 (d, $J^3_{C\text{-}P}$ = 14.4 Hz, CH = CH), 129.1 (2 × C_{arom}), 128.9 (C_{arom}), 127.3 (C_{arom}), 125.8 (C_{arom}), 124.1 (d, $J^2_{C\text{-}P}$ = 10.8 Hz, CH = CH), 119.2 (C_{arom}), 116.7 (C_{quat}), 90.9 (CH_2-C), 82.3 (C≡C-C), 52.9 (O-CH_3), 52.9 (O-CH_3), 44.7 (N-CH_2), 43.3 (CO-CH_2), 29.4 (d, $J^1_{C\text{-}P}$ = 139.3 Hz, CH-CH_2-P). ^{31}P NMR (162 MHz, Acetone-d_6) δ 28.32 (P_E). HRMS-ESI (m/z) [M + H]$^+$ calcd for $C_{25}H_{27}N_3O_6P$: 496.16.31, found: 496.1629. Rf : 0.23 (DCM/MeOH 95:5).

*4.1.9.3. Dimethyl (4-(7-(3-(3-hexylureido)prop-1-ynyl)-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphonate (**18c**).* The title compound was prepared from **17** and alkyne **13c** to afford after purification the desired product **18c** as a light orange solid (*E/Z ratio = 85/15*, 80%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.07 (d, 1H, J = 8.1 Hz, H_5), 7.40 (s, 1H, H_8), 7.30 (d, 1H, J = 8.1 Hz, H_7), 5.84 (dt, 1H, J = 15.1, 5.8 Hz, $H_{2'}$), 5.63 (dq, 1H, J = 15.1, 7.3 Hz, $H_{3'}$), 4.78 (d, 2H, J = 5.8 Hz, $H_{1'}$), 4.20 (s, 2H, C-CH_2), 3.86 (s, 3H, $H_{POMe\text{-}minor}$), 3.83 (s, 3H, $H_{POMe\text{-}minor}$), 3.74 (s, 3H, $H_{POMe\text{-}major}$), 3.71 (s, 3H, $H_{POMe\text{-}major}$), 3.15 (dt, 2H, J = 15.0, 7.1 Hz, NH-CH_2), 2.74 (dd, 2H, J = 21.9, 7.3 Hz, $CH_{4'}$), 1.52 (q, 2H, J = 7.3, 6.8 Hz, CH_2), 1.44–1.30 (m, 6H, 3 × CH_2), 0.94 (q, 3H, J = 5.0, 4.5 Hz, CH_3). ^{13}C NMR (101 MHz, Methanol-d_4) δ 163.5 (C═O), 160.6 (NH-CO-NH), 151.9 (C═O), 142.4 (C_{quat}), 131.5 (C_{quat}), 130.3 (d, $J^3_{C\text{-}P}$ = 14.5 Hz, CH = CH), 129.1 (C_{arom}), 127.0 (C_{arom}), 122.9 (d, $J^2_{C\text{-}P}$ = 11.4 Hz, CH = CH), 119.1 (C_{arom}), 116.7 (C_{quat}), 92.2 (CH_2-C), 81.9 (C≡C-C), 53.6 (O-CH_3), 53.6 (O-CH_3), 44.8 (N-CH_2), 41.1 (NH-CH_2), 32.7 (CH_2-CH_2), 31.2 (NH-CH_2-C), 31.0 (CH_2-CH_2), 29.3 (d, $J^1_{C\text{-}P}$ = 139.3 Hz, CH-CH_2-P), 27.6 (CH_2-CH_2), 27.6 (CH_2-CH_2), 23.6 (CH_2-CH_2), 14.3 (CH_2-CH_3). ^{31}P NMR (162 MHz, Methanol-d_4) δ 30.32 (P_E). HRMS-ESI (m/z) [M + H]$^+$ calcd for $C_{24}H_{34}N_4O_6P$: 505.2210, found: 505.2209. Rf : 0.20 (DCM/MeOH 95:5).

*4.1.9.4. Diisopropyl ((((4-(6-(3-octanamidoprop-1-ynyl)-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphoryl)bis(oxy))bis(methylene)) bis(carbonate) (**26a**).* The title compound was prepared from **25** and alkyne **13a** to affording after purification the desired product **26a** as a light orange solid (61%). ^{1}H NMR (250 MHz, Acetone-d_6) δ 10.29 (bs, 1H, NH), 7.80 (d, 1H, J = 2.1 Hz, H_5), 7.68 (dd, 1H, J = 8.7, 2.1 Hz, H_7), 7.37 (d, 1H, J = 8.7 Hz, H_8), 5.81–5.78 (m, 2H, $H_{2',3'}$), 5.76–5.60 (m, 4H, 4 × O-CH_{2POC}), 4.89 (h, 2H, J = 6.2 Hz, 2 × O-CH_{POC}), 4.80–4.65 (m, 2H, $H_{1'}$), 4.23 (d, 2H, J = 5.3 Hz, NH-CH_2), 2.80–2.60 (m, 2H, $H_{4'}$), 2.22 (t, 2H, J = 7.4 Hz, CH_2), 1.59 (q, 2H, J = 7.2 Hz, CH_2), 1.30–1.04 (m, 20H, 4 × CH_2, 4 × CH_{3POC}), 0.82–0.69 (m, 3H, CH_3). ^{13}C NMR (63 MHz, Acetone-d_6) δ 172.9 (NH-CO), 161.7 (C═O), 153.9 (2 × C═O), 150.7 (C═O), 141.7 (C_{quat}), 138.3 (C_{arom}), 131.5 (C_{arom}), 130.6 (d, $J^3_{C\text{-}P}$ = 15.1 Hz, CH = CH), 122.3 (d, $J^2_{C\text{-}P}$ = 11.8 Hz, CH = CH), 118.0 (C_{quat}), 117.2 (C_{arom}), 116.4 (C_{quat}), 87.8 (CH_2-C), 85.0 (d, J = 6.1 Hz, 2 × O-CH_{2POC}), 81.1 (C≡C-C), 73.6 (2 × O-CH_{POC}), 44.8 (N-CH_2), 36.4 (CO-CH_2), 32.4 (CH_2-CH_2), 31.1 (d, $J^1_{C\text{-}P}$ = 139.2 Hz, CH_2-CH_2), 30.1 (CH_2-CH_2), 29.9 (NH-CH_2), 29.6 (CH_2-CH_2), 26.3 (CH_2-CH_2), 23.2 (CH_2-CH_2), 21.7 (4 × CH_{3POC}), 14.3 (CH_2-CH_3). ^{31}P NMR (101 MHz, Acetone-d_6) δ 26.67. HRMS-ESI (m/z) [M + H]$^+$ calcd for $C_{33}H_{47}N_3O_{12}P$: 708.2889, found: 708.2891. Rf : 0.3 (DCM/MeOH 95:5).

*4.1.9.5. (((4-(2,4-dioxo-6-(3-(2-phenylacetamido)prop-1-ynyl)-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphoryl)bis(oxy))bis(methylene) diisopropyl bis(carbonate) (**26b**).* The title compound was prepared according to the general procedure 6 from **25** and alkyne **13b**, affording after purification the desired product **26b** as a light orange solid (66%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.30 (bs, 1H, NH), 8.04 (d, 1H, J = 2.1 Hz, H_5), 7.70 (dd, 1H, J = 8.7, 2.1 Hz, H_7), 7.39 (d, 1H, J = 8.7 Hz, H_8), 7.37–7.26 (m, 4H, 4 × H_{arom}), 7.26–7.18 (m, 1H, H_{arom}), 5.80 (dt, 1H, J = 15.8, 5.2 Hz, $H_{2'}$), 5.75–5.62 (m, 1H, $H_{3'}$), 5.60 (d, 2H, J = 1.9 Hz, O-CH_{2POC}), 5.59 (d, 2H, J = 1.3 Hz, O-CH_{2POC}), 4.88 (hept, 2H, J = 6.3 Hz, 2 × OCH_{POC}), 4.78 (t, 2H, J = 4.8 Hz, $H_{1'}$), 4.23 (d, 2H, J = 5.4 Hz, C-CH_2), 3.55 (s, 2H, C-H_2), 2.81–2.69 (m, 2H, $H_{4'}$), 1.29 (d, 12H, J = 6.3 Hz, 4 × CH_{3POC}). ^{13}C NMR (101 MHz, Acetone-d_6) δ 170.7 (NH-CO), 163.2 (C═O), 154.0 (2 × C═O), 150.7 (C═O), 138.3 (C_{arom}), 137.0 (C_{quat}), 135.9 (C_{quat}), 131.6 (C_{arom}), 130.6 (d, $J^3_{C\text{-}P}$ = 14.9 Hz, CH = CH), 130.0 (2 × C_{arom}), 129.1 (2 × C_{arom}), 127.4 (C_{arom}), 122.4 (d, $J^2_{C\text{-}P}$ = 11.8 Hz, CH = CH), 118.0 (C_{quat}), 117.4 (C_{quat}), 116.5 (C_{arom}), 87.6 (CH_2-C), 84.9 (d, $J^2_{C\text{-}P}$ = 6.3 Hz, 2 × O-CH_2-O), 81.3 (C≡C-C), 73.7 (2 × O-CH_{POC}), 44.8 (N-CH_2), 43.5 (CO-CH_2), 33.1 (d, $J^1_{C\text{-}P}$ = 139.9 Hz, CH_2-P), 30.4 (NH-CH_2-C), 21.8 (4 × C_{CH3POC}). ^{31}P NMR (162 MHz, Acetone-d_6) δ 26.49. HRMS-ESI (m/z) [M + H]$^+$ calcd for $C_{33}H_{39}N_3O_{12}P$: 700.2253, found: 700.2254. Rf : 0.31 (DCM/MeOH 95:5).

*4.1.9.6. (((4-(6-(3-(3-Hexylureido)prop-1-ynyl)-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphoryl)bis(oxy))bis(methylene) diisopropyl bis(carbonate) (**26c**).*. The title compound was prepared from **25** and alkyne **13c** to afford after purification the desired product **26c** as a light orange solid (65%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.37 (bs, 1H, NH), 8.05–7.98 (m, 1H, H_5), 7.70–7.61 (m, 1H, H_7), 7.37 (d, 1H, J = 8.7 Hz, H_8), 5.90–5.65 (m, 2H, $H_{2'}$, $H_{3'}$), 5.61 (d, 2H, J = 2.0 Hz, O-CH_{2POC}), 5.58 (d, 2H, J = 1.3 Hz, O-CH_{2POC}), 4.88 (hept, 2H, J = 6.3 Hz, 2 × O-CH_{POC}), 4.76 (t, 2H, J = 5.6 Hz, $H_{1'}$), 4.18 (d, 2H, J = 5.5 Hz, C-CH_2), 3.20–3.07 (m, 2H, NH-CH_2), 2.76 (dd, 2H, J = 22.6, 7.2 Hz, $H_{4'}$), 1.47 (t, 2H, J = 7.3 Hz, CH_2), 1.29 (bd, 18H, J = 6.2 Hz, 3 × CH_2, 4 × CH_{3POC}), 0.96–0.75 (m, 3H, CH_3). ^{13}C NMR (101 MHz, Acetone-d_6) δ 161.7 (C═O), 158.5 (NH-CO-NH), 154.0 (2 × C═O), 150.7 (C═O), 141.6 (C_{quat}), 138.3 (C_{arom}), 131.5(C_{arom}), 130.7 (d, $J^3_{C\text{-}P}$ = 15.2 Hz, CH = CH), 122.3 (d, $J^2_{C\text{-}P}$ = 11.8 Hz, CH = CH), 118.3 (C_{quat}), 117.2 (C_{quat}), 116.4 (C_{arom}), 89.1 (CH_2-C), 85.0 (d, J = 6.2 Hz, 2 × O-CH_2-O), 80.9 (C≡C-C), 73.7 (2 × O-CH), 44.8 (N-CH_2), 40.7 (NH-CH_2), 32.3 (CH_2-CH_2), 31.9 (NH-CH_2-C), 31.8 (CH_2-CH_2), 31.0 (d, $J^1_{C\text{-}P}$ = 139.3 Hz, CH-CH_2-P), 27.3 (CH_2-CH_2), 23.3 (CH_2-CH_2), 21.8 (4 × C-CH_{3POC}), 14.3 (CH_2-CH_3). ^{31}P NMR (162 MHz, Acetone-d_6) δ 26.61. HRMS-ESI (m/z) [M + H]$^+$ calcd for $C_{32}H_{46}N_4O_{12}P$: 709.2844, found: 709.2834. Rf : 0.28 (DCM/MeOH 95:5).

*4.1.9.7. Diisopropyl ((((4-(5-(3-octanamidoprop-1-ynyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)but-2-en-1-yl)phosphoryl)bis(oxy))bis(methylene)) bis(carbonate) (**30a**).* The title compound was prepared according to the general procedure 6 but at 70 °C, from **29** and alkyne **13a**, to afford after purification on silica gel column chromatography using an elution gradient of DCM/MeOH (from 97:3 to 95:5) the desired product **30a** as a light orange solid (97%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.99 (bs, 1H, NH), 7.80 (s, 1H, H_6), 7.43 (bs, 1H, NH), 5.90–5.71 (m, 2H, $H_{2',3'}$), 5.66 (dq, 4H, J = 10.7, 5.5 Hz, 2 × O-CH_{2POC}), 4.90 (hept, 2H, J = 6.2 Hz, 2 × O-CH_{POC}), 4.41 (t, 2H, J = 4.9 Hz, $H_{1'}$), 4.14 (d, 2H, J = 5.3 Hz, C-CH_2), 2.81 (dd, 2H, J = 22.6, 6.3 Hz, $H_{4'}$), 2.19 (t, 2H, J = 7.5 Hz, C-CH_2), 1.59 (dd, 2H, J = 9.2, 5.2 Hz, CH_2), 1.30 (bd, 20H, J = 6.3 Hz, 4 × CH_2, 4 × CH_{3POC}), 0.92–0.83 (m, 3H, CH_3). ^{13}C NMR (101 MHz, Acetone-d_6) δ 172.8 (NH-CO), 162.5 (C═O), 154.0 (2 × C═O), 150.7 (C═O), 148.5 (C = CH), 130.7 (d, $J^3_{C\text{-}P}$ = 15.1 Hz, CH = CH), 124.7 (d, $J^2_{C\text{-}P}$ = 11.5 Hz, CH = CH), 99.4 (CH_2-C), 90.2 (C≡C-C), 85.1 (d, J = 6.2 Hz, 2 × O-CH_2-O), 74.8 (C_{quat}), 73.7 (2 × O-CH), 49.9 (N-CH_2), 36.4 (CO-CH_2), 32.4 (CH_2-CH_2), 31.1 (d, $J^1_{C\text{-}P}$ = 139.3 Hz, CH-CH_2-P), 30.4 (CH_2-CH_2), 29.9 (NH-CH_2), 29.7 (CH_2-CH_2), 26.3 (CH_2-CH_2), 23.2 (CH_2-CH_2), 21.8 (4 × C-CH_{3POC}), 14.3 (CH_2-CH_3). ^{31}P NMR (162 MHz, Acetone-d_6) δ 26.64. HRMS-ESI (m/z) [M + H]$^+$ calcd for $C_{29}H_{45}N_3O_{12}P$: 658.2735, found: 658.2723. Rf : 0.29 (DCM/MeOH 95:5).

4.1.9.8. (((4-(5-(3-(3-Hexylureido)prop-1-ynyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)but-2-en-1-yl)phosphoryl)bis(oxy))bis(methylene)

diisopropyl bis(carbonate) ***(30c)***. The title compound was prepared according to the general procedure 6 but at 70 °C, from **29** and alkyne **13b**, to afford after purification on silica gel column chromatography using an elution gradient of DCM/MeOH (from 97:3 to 95:5) the desired product **30b** as a light orange solid (21%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.86 (s, 1H, H_6), 5.90–5.74 (m, 2H, $H_{2',3'}$), 5.72–5.63 (m, 4H, 2 × O-CH_{2POC}), 4.95 (p, 2H, J = 6.3 Hz, 2 × O-CH_{POC}), 4.40 (t, 2H, J = 5.3 Hz, $H_{1'}$), 4.12 (s, 2H, C-CH_2), 3.14 (td, 2H, J = 7.3, 3.1 Hz, NH-CH_2), 2.88 (dd, 2H, J = 22.7, 6.9 Hz, $H_{4'}$), 1.51 (t, 2H, J = 7.0 Hz, CH_2), 1.36 (t, 18H, J = 7.1 Hz, 3 × CH_2, 4 × CH_{3POC}), 0.98–0.91 (m, 3H, CH_3). ^{13}C NMR (101 MHz, Methanol-d_4) δ 164.9 (C═O), 160.5 (NH-CO-NH), 154.6 (2 × C═O), 151.5 (C═O), 149.3 (C = CH), 131.4 (d, $J^3_{C\text{-}P}$ = 15.2 Hz, CH = CH), 124.2 (d, $J^2_{C\text{-}P}$ = 12.0 Hz, CH = CH), 100.1 (CH_2-C), 91.7 (C≡C-C), 85.7 (d, J = 6.6 Hz, 2 × O-CH_2-O), 74.5 (2 × O-CH), 74.4 (C_{quat}), 50.5 (N-CH_2), 41.1 (NH-CH_2), 32.7 (CH_2-CH_2), 31.82 (NH-CH_2-C), 31.2 (CH_2-CH_2), 30.4 (d, $J^1_{C\text{-}P}$ = 139.8 Hz, CH-CH_2-P), 27.6 (CH_2-CH_2), 23.6 (CH_2-CH_2), 21.8 (4xC_{CH3POC}), 14.3 (CH_2-CH_3). ^{31}P NMR (162 MHz, Methanol-d_4) δ 27.51. HRMS-ESI (*m/z*) $[M + H]^+$ calcd for $C_{28}H_{44}N_4O_{12}P$: 659.2676, found: 659.2678. Rf: 0.27 (DCM/MeOH 95:5).

4.1.10. (((4-(6-Bromo-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphoryl)bis(oxy))bis(methylene) diisopropyl bis(carbonate) ***(25)***

A mixture of protected alkylated 6-bromo quinazoline **22** (1 eq.) and *bis*POC allylphosphonate **23** (3 eq.), were stirred at room temperature in freshly distilled DCM (0.1 M). Grubbs 2nd generation catalyst (15 mol%) was added. The mixture was refluxed at 55 °C under inert atmosphere for 24 h. Volatiles were eliminated under reduced pressure. Crude product was applied on short silica gel flash chromatography using an elution gradient of PE/ EtOAc (from 8:2 to 1:1) to afford the desired compound **24** as an oil (*E/Z ratio = 85/15*). HRMS-ESI (*m/z*) $[M]^+$ calcd for $C_{27}H_{37}BrN_2O_{13}P$: 707.1200, found: 707.1211. Rf : 0.3 (EDP/AcOEt 1:1). This product is directly engaged in the deprotection step of Boc group. Then a solution of POC phosphonate mixture (1 eq.) in freshly distilled DCM (0.05 M), was stirred with trifluoroacetic acid (20 eq.) under inert atmosphere for 6 h. After completion of the reaction, the mixture was diluted in EtOAc and washed twice with saturated $NaHCO_3$ solution, dried over $MgSO_4$ filtered and concentrated under reduced pressure. Pure compound **25** was obtained after purification on silica gel column chromatography with DCM/MeOH (97:3) as an oil (44% over two steps). ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.45 (bs, 1H, NH), 8.14 (s, 1H, H_5), 7.85 (d, 1H, J = 9.0 Hz, H_7), 7.38 (d, 1H, J = 9.0 Hz, H_8), 5.85–5.77 (m, 1H, $H_{2'}$), 5.72–5.62 (m, 1H, $H_{3'}$), 5.60 (s, 4H, 2 × H_{CH2POC}), 5.57 (s, 2H, CH_{2POC}), 4.88 (p, 2H, J = 6.2 Hz, 2 × O-CH_{POC}), 4.77 (t, 2H, J = 5.2 Hz, $H_{1'}$), 2.76 (dd, 2H, J = 22.6, 7.2 Hz, $H_{4'}$), 1.29 (d, 12H, J = 6.3 Hz, 4 × CH_{3POC}). ^{13}C NMR (101 MHz, Acetone-d_6) δ 161.3 (C═O), 154.0 (2 × C = O_{POC}), 150.6 (C═O), 141.4 (C_{quat}), 138.4 (C_{arom}), 130.8 (C_{arom}), 130.5 (d, $J^3_{C\text{-}P}$ = 15.1 Hz, CH = CH), 122.4 (d, $J^2_{C\text{-}P}$ = 12.2 Hz, CH = CH), 119.03 (C_{quat}), 118.4 (C_{arom}), 115.6 (C_{quat}), 85.0 (O-CH_{2POC}), 85.0 (O-CH_{2POC}), 73.6 (2 × O-CH_{POC}), 44.8 (N-CH_2), 31.8 (d, $J^1_{C\text{-}P}$ = 139.3 Hz, CH-CH_2), 21.7 (4 × CH-CH_{3POC}). ^{31}P NMR (162 MHz, Acetone-d_6) δ 26.55. HRMS-ESI (*m/z*) $[M]^+$ calcd for $C_{22}H_{29}BrN_2O_{11}P$: 607.0687, found: 607.0688. Rf : 0.49 (DCM/MeOH 95:5).

4.1.11. General synthetic procedure 7 for POC removal for compounds ***27a, 27b, 27c, 31a, 31c***

BisPOC compound were dissolved in an ultrapure water solution of sodium hydroxide (0.1 M). After 4 h stirring at room temperature, the solution was passed acidified to pH 5 with DOWEX 50WX8 (H^+). The solution was filtered and the filtrate was washed two times with DCM. Inorganic layer was lyophilized to give pure compound.

4.1.11.1. (4-(6-(3-Octanamidoprop-1-ynyl)-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphonic acid ***(27a)***. The title compound was prepared according to the general procedure 8 from **26a** to afford after lyophilisation the desired product **27a** as a beige foam (86%). ^{1}H NMR (250 MHz, DMSO-d_6) δ 11.72 (bs, 1H, NH), 8.43–8.25 (m, 1H, NH), 7.91 (s, 1H, H_5), 7.70 (dd, 1H, J = 12.1, 6.0 Hz, H_8), 7.39 (t, 1H, J = 8.6 Hz, H_7), 5.89-0.65 (m, 1H, $H_{2'}$), 5.53 (s, 1H, $H_{3'}$), 4.62 (s, 2H, $H_{1'}$), 4.10 (d, 2H, J = 5.4 Hz, H_{11}), 2.38–2.24 (m, 2H, $H_{4'}$), 2.09 (d, 2H, J = 9.5 Hz, CH_2), 1.51 (q, 2H, J = 7.7, 6.5 Hz, CH_2), 1.23 (bs, 8H, 4 × CH_{21}), 0.84 (q, 3H, J = 6.2 Hz, CH_2-CH_3). ^{13}C NMR (63 MHz, DMSO-d_6) δ 171.9 (NH-CO), 160.9 (C═O), 149.7 (C═O), 140.5, (C_{quat}) 137.3 (C_{arrom}), 130.1 (C_{arrom}), 128.6 (C_{quat}), 127.4 (CH = CH), 126.0 (CH = CH), 116.2 (C_{arrom}), 115.8 (C_{quat}), 87.6 (CH_2-C), 80.0 (C≡C-C), 43.7 (N-CH_2), 33.9 (CO-CH_2), 33.2 (d, $J^1_{C\text{-}P}$ = 139.8 Hz, CH_2-P), 31.6 (CH_2-CH_2), 28.5 (CH_2-CH_2), 28.4 (NH-CH_2), 28.5 (CH_2-CH_2), 25.2 (CH_2-CH_2), 21.9 (CH_2-CH_2), 13.9 (CH_2-CH_3). ^{31}P NMR (162 MHz, DMSO-d_6) δ 18.91. HRMS-ESI (*m/z*) $[M-H]^-$ calcd for $C_{23}H_{29}N_3O_6P$: 474.1799, found: 474.1804.

4.1.11.2. (4-(2,4-Dioxo-6-(3-(2-phenylacetamido)prop-1-ynyl)-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphonic acid ***(27b)***. The title compound was prepared according to the general procedure 8 from **26b** to afford after lyophilisation the desired product **27b** as a beige solid (92%). ^{1}H NMR (250 MHz, DMSO-d_6) δ 11.75 (bs, 1H, NH), 8.62 (d, 1H, J = 5.8 Hz, NH), 7.94 (s, 1H, H_5), 7.77–7.64 (m, 1H, H_9), 7.49–7.06 (m, 6H, H_8, 4 × H_{arom}), 5.63 (dt, 2H, J = 9.9, 6.3 Hz, $H_{2',3'}$), 4.65 (d, 2H, J = 5.0 Hz, $H_{1'}$), 4.13 (d, 2H, J = 5.3 Hz, CH_2), 3.47 (s, 2H, CH_2), 2.39–2.29 (m, 2H, $H_{4'}$). ^{13}C NMR (63 MHz, DMSO-d_6) δ 169.8 (NH-CO), 161.0 (C═O), 149.8 (C═O), 140.5 (C_{quat}), 137.4 (C_{arom}), 136.0 (C_{quat}), 130.1 (C_{arom}), 128.9 (2 × C_{arom}), 128.2 (2 × C_{arom}), 126.6 (d, $J^3_{C\text{-}P}$ = 15.3 Hz, CH = CH), 126.4 (C_{arom}), 125.4 (d, $J^2_{C\text{-}P}$ = 9.3 Hz, CH = CH), 116.2 (C_{arom}), 115.96 (C_{quat}), 87.4 (CH_2-C), 80.2 (C≡C-C), 43.5 (N-CH_2), 42.0 (NH-CH_2), 32.1 (d, $J^1_{C\text{-}P}$ = 131.6 Hz, CH_2-P), 28.9 (CO-CH_2). ^{31}P NMR (162 MHz, DMSO-d_6) δ 21.08. HRMS-ESI (*m/z*) $[M-H]^-$ calcd for $C_{23}H_{21}N_3O_6P$: 466.4178, found: 466.1172.

4.1.11.3. (4-(6-(3-(3-Hexylureido)prop-1-ynyl)-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)but-2-en-1-yl)phosphonic acid ***(27c)***. The title compound was prepared according to the general procedure 8 from **26c** to afford after lyophilisation the desired product **27c** as a beige foam (50%). ^{1}H NMR (250 MHz, DMSO-d_6) δ 11.71 (bs, 1H, NH), 7.90 (s, 1H, H_5), 7.68 (t, 1H, J = 8.1 Hz, H_8), 7.37 (d, 1H, J = 9.0 Hz, H_7), 5.86–5.64 (m, 1H, $H_{2'}$), 5.51 (d, 1H, J = 15.1 Hz, $H_{3'}$), 4.62 (s, 2H, $H_{1'}$), 4.04 (d, 2H, J = 4.6 Hz, H_{11}), 2.99 (q, 2H, J = 6.1, 5.6 Hz, H_{15}), 2.39–2.19 (m, 1H, $H_{4'}$), 1.35 (t, 2H, J = 7.0 Hz, CH_2), 1.23 (bs, 6H, 3 × CH_2), 0.90–0.73 (m, 3H, CH_2-CH_3). ^{13}C NMR (63 MHz, DMSO-d_6) δ 160.9 (NH-CO-NH), 157.5 (C═O), 149.7 (C═O), 140.4 (C_{quat}), 137.3 (C_{arom}), 129.9 (C_{arom}), 128.8 (C_{quat}), 126.1 (d, $J^3_{C\text{-}P}$ = 12.6 Hz, CH = CH), 125.8 (d, $J^2_{C\text{-}P}$ = 10.3H, CH = CH), 116.4 (C_{arom}), 115.9 (C_{quat}), 88.8 (CH_2-C), 79.8 (C≡C-C), 59.3 (N-CH_2), 39.8 (NH-CH_2), 33.2 (d, $J^1_{C\text{-}P}$ = 139.8 Hz, CH_2-P), 31.0 (CH_2-CH_2), 29.9 (CH_2-CH_2), 29.6 (NH-CH_2), 26.0 (CH_2-CH_2), 22.0 (CH_2-CH_2), 13.0 (CH_2-CH_3). ^{31}P NMR (162 MHz, DMSO-d_6) δ 20.95. HRMS-ESI (*m/z*) $[M-H]^-$ calcd for $C_{22}H_{28}N_4O_6P$: 475.11738, found 475.1744.

4.1.11.4. 4-(5-(3-Octanamidoprop-1-ynyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)but-2-en-1-yl)phosphonic acid ***(31a)***. The title compound was prepared according to the general procedure 8 from **30a** to afford after lyophilisation the desired product as a beige foam (92%). ^{1}H NMR (400 MHz, *DMSO-d_6*) δ 11.42 (bs, 1H, NH), 8.32 (s, 1H, NH), 7.96 (s, 1H, H_6), 5.80–5.65 (m, 1H, $H_{2'}$), 5.54–5.43 (m, 1H, $H_{3'}$), 4.23 (s, 2H, $H_{1'}$), 4.04 (d, 2H, J = 5.2 Hz, H_9), 2.27 (dd, 2H, J = 20.7, 8.0 Hz, $H_{4'}$), 2.08 (t, 2H, J = 7.5 Hz, H_{12}), 1.55–1.43 (m, 2H, CH_2), 1.23 (bs, 8H, 4 × CH_2), 0.85 (t, 3H, J = 6.6 Hz, CH_2-CH_3). ^{13}C NMR (63 MHz, DMSO-d_6) δ 171.6 (NH-CO), 161.9 (C═O), 149.6 (C═O), 148.3 (C = CH), 129.2 (CH

= CH), 125.7 (CH = CH), 97.3 (C_{quat}), 89.7 (CH_2-C), 74.0 (C≡C-C), 48.9 (N-CH_2), 35.0 (CO-CH_2), 33.2 (d, $J^1_{C\text{-}P}$ = 131.0 Hz, CH_2-P), 31.1 (CH_2-CH_2), 28.6 (CH_2-CH_2), 28.4 (NH-CH_2), 28.4 (CH_2-CH_2), 25.1 (CH_2-CH_2), 22.1 (CH_2-CH_2), 13.8 (CH_2-CH_3). ^{31}P NMR (162 MHz, DMSO-d_6) δ 19.15. HRMS-ESI (*m/z*) [M−H]$^-$ calcd for $C_{19}H_{27}N_3O_6P$: 424.1173, found: 424.1172.

*4.1.11.5. 4-(5-(3-(hexylcarbamoylamino)prop-1-ynyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)but-2-en-1-yl)phosphonic acid (**31c**).* The title compound was prepared according to the general procedure 8 from **30c** to afford after lyophilisation the desired product **31c** as a beige foam (92%). ^{1}H NMR (250 MHz, DMSO-d_6) δ 11.56 (bs, 1H, NH), 7.93 (s, 1H, H_6), 5.72 (dq, 1H, J = 13.5, 7.8, 6.4 Hz, $H_{2'}$), 5.61–5.43 (m, 1H, $H_{3'}$), 4.24 (s, 2H, $H_{1'}$), 4.05–3.93 (m, 2H, H_9), 2.97 (t, 2H, J = 6.6 Hz, H_{13}), 2.33 (dd, 2H, J = 21.0, 7.2 Hz, $H_{4'}$), 1.41–1.29 (m, 2H, CH_2), 1.24 (bs, 6H, 3 × CH_2), 0.93–0.72 (m, 3H, CH_2-CH_3). ^{13}C NMR (63 MHz, DMSO-d_6) δ 162.1 (C═O), 157.49 (NH-CO-NH), 149.8 (C═O), 148.2 (C = CH), 130.0 (CH = CH), 126.2 (CH = CH), 97.6 (C_{quat}), 91.0 (CH_2-C), 73.8 (C≡C-C), 49.0 (N-CH_2), 41.4 (NH-CH_2), 32.1 (d, $J^1_{C\text{-}P}$ = 134.1 Hz, CH_2-P), 31.2 (NH-CH_2), 29.8 (CH_2-CH_2), 29.5 (CH_2-CH_2), 26.0 (CH_2-CH_2), 22.0 (CH_2-CH_2), 14.0 (CH_2-CH_3). ^{31}P NMR (162 MHz, DMSO-d_6) δ 19.20. HRMS-ESI (*m/z*) [M−H]$^-$ calcd for $C_{19}H_{26}N_3O_6P$: 425.1780, found: 425.1783.

*4.1.11.6. [[4-(5-iodo-2,4-dioxo-pyrimidin-1-yl)but-2-enyl]-(iso-propoxycarbonyloxymethoxy)phosphoryl]oxymethyl isopropyl carbonate (**29**).* A mixture of alkylated 5-iodouracil **28** (1 eq.) and *bis*POC allylphosphonate **23** (3 eq.), were stirred at room temperature in freshly distilled DCM (0.1 M). Grubbs 2nd generation catalyst (15 mol%) was added. The mixture was refluxed at 55 °C under inert atmosphere for 24 h. Volatiles were eliminated under reduced pressure. Crude product was applied on short silica gel flash chromatography using an elution with PE/EtOAc (1:1) as eluent to give pure product as an oil (*E/Z ratio = 95/5*, 45%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.54 (bs, 1H, NH), 8.17 (s, 1H, $H_{6\text{-minor}}$), 8.03 (s, 1H, $H_{6\text{-major}}$), 5.58–5.72 (m, 2H, $H_{2',3}$), 5.71–5.58 (m, 4H, 2 × O-CH_{2POC}), 4.89 (p, 2H, J = 6.2 Hz, 2 × O-CH_{POC}), 4.56–4.52 (m, 2H, $H_{1'\text{-minor}}$), 4.41 (t, 2H, J = 4.9 Hz, $H_{1'\text{-major}}$), 2.91 (dd, 2H, J = 23.2, 7.7 Hz, $H_{4'\text{-minor}}$), 2.81 (dd, 2H, J = 22.6, 6.4 Hz, $H_{4'\text{-major}}$), 1.29 (d, 12H, J = 6.2 Hz, 4 × CH_{3POC}). ^{13}C NMR (101 MHz, Acetone-d_6) δ 160.5 (C═O), 153.1 (2 × C = O_{POC}), 150.6 (C═O), 149.3 (C = CH), 130.1 (d, $J^3_{C\text{-}P}$ = 15.1 Hz, CH = CH), 123.5 (d, $J^2_{C\text{-}P}$ = 11.6 Hz, CH = CH), 84.3 (O-CH_{2POC}), 84.2 (O-CH_{2POC}), 72.9 (2 × O-CH_{POC}), 67.0 (C_{quat}), 49.0 (N-$CH_{2\text{-major}}$), 44.7 (N-$CH_{2\text{-minor}}$), 30.2 (d, J = 138.6 Hz, CH-$CH_{2\text{-major}}$), 26.2 (d, $J^1_{C\text{-}P}$ = 140.3 Hz, CH-$CH_{2\text{-minor}}$), 20.9 (4 × CH-CH_{3POC}). ^{31}P NMR (162 MHz, Acetone-d_6) δ 26.87 (P_E), 25.89 (P_Z). HRMS-ESI (*m/z*) [M + H]$^+$ calcd for $C_{18}H_{27}IN_2O_{11}P$: 605.0383, found: 605.0383. Rf : 0.5 (DCM/MeOH 95:5).

*4.1.12. 5-Benzylpyrimidine-2,4(1H,3H)-dione (**34a**)*

Preparation of LiCl (solution **S1**): A suspension of dry LiCl (2.25 g, 53 mmol) in dry THF (125 ml, 0.5 M) is left stirring at room temperature for 24 h under inert atmosphere until complete dissolution of LiCl. The solution is stored under inert atmosphere upon use. Preparation of CuI.2LiCl (solution **S2**): Under inert atmosphere in a dry flask, dry CuI (0.4 g, 0.1 eq., 2.1 mmol) and LiCl (1.8 g, 2 eq., 42.0 mmol) were mixed in dry THF (21 ml, 0.1 M). 5-Iodouracil (**32**) (5 g, 1 eq., 21.0 mmol) was placed in a dry flask under inert atmosphere. Applying vigorous stirring, the substrate was dried for 15 min under reduced pressure. Solution **S1** (85 ml, 2 eq., 22.0 mmol) was added and stirred 30 min at room temperature. The solution was cooled down to −30 °C and MeMgCl in dry THF (14.3 ml, 2 eq., 22.0 mmol) was added dropwise. After completion of the addition, the resulting solution was stirred at −30 °C for further 30 min, i-PrMgCl·LiCl in dry THF (19.23 ml, 1.2 eq., 25.2 mmol) was then dropwised and the resulting mixture was allowed to warm up to room temperature. After 1 h, the mixture was cooled to –30 °C and solution **S2** (0.21 ml, 0.01 eq., 0.21 mmol) was added dropwise and the resulting mixture was allowed to warm up to room temperature for 30 min. Benzyl bromide (3.21 ml, 1.3 eq., 27.3 mmol) was added at −30 °C dropwise and stirred at 30 min before allowed to warm up at room temperature for 24 h. After the completion of the reaction, it was quenched by addition of MeOH (25 ml). The precipitate was filtered off and dried to afford the pure compound **34a** as a white solid (84%). CAS # 18493–83-9.[22] ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.86 (bs, 2H, 2 × NH), 7.30–7.14 (m, 6H, H_6, 5xH_{Arom}), 3.49 (s, 2H, C-CH_2).

*4.1.13. 5-(Phenylamino)pyrimidine-2,4(1H,3H)-dione (**34b**)*

In a dry flask under inert atmosphere, 5-bromouracil (**33**) (5 g, 1 eq., 26.18 mmol) and aniline derivatives (7.16 ml, 3 eq., 75.54 mmol) were heated at 195 °C for 1 h. After completion of the reaction, a mixture of H_2O/$HCCl_3$ was added to the reaction and stirred during 15 min. The mixture was filtered off and the solid washed with water and ethanol. The solid was dissolved in 5% aqueous NaOH and the residues were filtered off. The filtrate was neutralized with acetic acid and the obtained solid was filtered off and washed with water to afford the desired compound **34b** as a white solid (73%). CAS # 4870–31-9.[21] ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.95 (bs, 2H, 2 × NH), 7.26 (s, 1H, H_6), 7.06 (t, 2H, J = 7.7 Hz, 2xH_{arom}), 6.83 (bs, 1H, H_{arom}), 6.74–6.54 (m, 3H, 2xH_{arom}, NH).

4.2. Biological assays

4.2.1. Mtb ThyX protein expression and purification[25]

The M. *tuberculosis* ThyX enzyme was expressed in *E. coli* BL21(DE3)/pLysS strains containing the recombinant pET24d plasmid carrying the M. tuberculosis H37Rv thyX gene (Rv2754c) as previously described. Before the purification step, 200 μM of flavin-adenine dinucleotide (FAD) cofactor was added to the supernatant after the lysis step to increase the amount of FAD bound to *Mtb* ThyX protein. The solubilized protein extract was loaded on a Hi-Trap Talon 5 ml column (GE Healthcare) previously equilibrated with equilibration buffer containing 30 mM Hepes and 300 mM NaCl at pH 8.0. The His-tagged ThyX protein was eluted with elution buffer (30 mM Hepes pH 8.0, 300 mM NaCl, 500 mM imidazol). The fractions containing Mtb ThyX enzyme were pooled, buffer-exchanged on Econo-Pac PD-10 columns (Bio-rad) with the equilibration buffer, concentrated to a final concentration of 480 μM and stored at −20 °C for further use. The measured absorbance of FAD bound to *Mtb* ThyX at 450 nm showed a ratio FAD to ThyX of 1 to 3 for the purified *Mtb* ThyX chain.

4.2.2. M. Tuberculosis ThyX NADPH oxidase assay

The NADPH oxidation assay for M. tuberculosis ThyX activity in 96-well plates was used to screen the synthesized compounds at a final concentration of 200 μM. All molecules were solubilized in dimethylsulfoxide (DMSO) and used at a 1% final concentration of DMSO during the test. One hundred microlitres of standard reaction mixture contained HEPES 50 mM pH 8, NaCl 30 mM, FAD 50 μM, β-mercaptoethanol 1,43 mM, dUMP 100 μM, NADPH 750 μM, and 10 μM of purified *Mtb*ThyX. Microtitre plates were prepared and transferred to the microplate reader Chameleon II (Hidex). Molecules at 200 μM were incubated with *Mtb*ThyX in the standard reaction mixture for 10 min at 25 °C before starting measurements. The reactions were started by automatically injecting NADPH into individual wells and ThyX activity was determined by following a decrease in absorbance at 340 nm for up to 20 min at 25 °C. The experiment was done in duplicates and samples with added DMSO and enzyme-free reactions were used as positive and negative controls, respectively. % of inhibition was calculated using the following equation: (1-Vi/Vo)*100; Vo and Vi are, respectively, the initial rates of the reaction without or with addition of molecule in the assay.

4.3. Virtual docking

The *Mtb*ThyX protein structure from PDB code 3GWC[26] was used to perform *in silico* molecular docking with the QuickVina 2 software[27]. The A to D chains, water oxygen atoms and UFP cofactor were removed from the structure, keeping only chains F to G and their FAD molecule. Atomic partial charges were assigned and polar hydrogen atoms were added with the Pymol[28] Vina plugin[29]. A cubic search volume of 35 × 35 × 35 Å centered on each active site of the four chains was used. The Arg87, Gln103, Ser105, Arg107, Tyr108 and Arg199 amino acids were chosen to be flexible during the docking attempts. Ten docking poses were generated and the pose with the best score was used to further analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2021.116351.

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