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Original article

Synthesis and broad spectrum antiviral evaluation of *bis*(POM) prodrugs of novel acyclic nucleosides

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ABSTRACT

A series of seventeen *hitherto* unknown ANP analogs bearing the (*E*)-but-2-enyl aliphatic side chain and modified heterocyclic base such as cytosine and 5-fluorocytosine, 2-pyrazinecarboxamide, 1,2,4-triazole-3-carboxamide or 4-substituted-1,2,3-triazoles were prepared in a straight approach through an olefin acyclic cross metathesis as key synthetic step.

All novel compounds were evaluated for their antiviral activities against a large number of DNA and RNA viruses including herpes simplex virus type 1 and 2, varicella zoster virus, feline herpes virus, human cytomegalovirus, hepatitis C virus (HCV), HIV-1 and HIV-2. Among these molecules, only compound **31** showed activity against human cytomegalovirus in HEL cell cultures with at EC_{50} of ~10 μM. Compounds **8a**, **13**, **14**, and **24** demonstrated pronounced anti-HCV activity without significant cytotoxicity at 100 μM.

1. Introduction

The discovery by A. Holý and E. De Clercq in 1986 of broad-spectrum antiviral activity of (*S*)-HPMPA [9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine] and PMEA [9-[2-(phosphonomethoxy)ethyl]adenine] led to a new family of nucleotides designated as acyclic nucleoside phosphonates (ANP) [1–4],. ANPs are nucleotide analogs that are characterized by the presence of a phosphonate group linked to a pyrimidine or purine base through an aliphatic linker. Three of these are approved drugs for the treatment of severe/fatal infectious diseases and represent three different types of ANPs: (i) HPMP derivatives such as (*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (HPMPC, cidofovir (Vistide®)] which is approved for the treatment of cytomegalovirus (CMV) retinitis in AIDS patients [5]; (ii) PME derivatives such as PMEA [adefovir (in its oral prodrug form, adefovir dipivoxil (Hepsera®)] for the treatment of hepatitis B virus infections [6], and (iii) PMP derivatives such as PMPA [tenofovir (in its oral prodrug form, tenofovir disoproxil fumarate (Viread®)] is used for the treatment of HIV infections (AIDS) and hepatitis B virus [7]. From these data, it appears that small chemical alterations in the acyclic side-chain lead to marked differences in antiviral activity and the spectrum of activity of acyclic nucleoside phosphonates against various classes of viral agents [1].

Thus, the synthesis and biological evaluation of a large panel of ANPs were systematically investigated as potential antiviral compounds [1]. In our search for antiviral compounds, we synthesized a new class of acyclic nucleoside phosphonates based on a 4-phosphono-but-2-en-1-yl base motif in which the oxygen heteroatom has been replaced with a double bond having *trans* stereochemistry [8]. We have shown that this modification allows

Abbreviations: VZV, varicella zoster virus; VV, vaccinia virus; HSV, herpes simplex virus; VSV, vesicular stomatitis virus; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; CC_{50}, compound concentration affording 50% inhibition of cell growth; EC_{50}, compound concentration affording 50% inhibition of the viral cytopathicity; MCC, minimum cytotoxic concentration required to afford a microscopically detectable alteration of cell morphology; MDCK, Madin–Darby canine kidney.

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mimicry of the three-dimensional geometry provided by the backbone of PMEA, PMPA, and CDV while maintaining an electronic contribution similar to that brought by the oxygen atom [8]. Several new derivatives are efficiently activated by human thymidylate kinase (hTMPK), and the best substrates were converted to *bis*-(pivaloyloxymethyl)ester phosphonate prodrugs and found to be active against several herpes viruses in cell culture.

On the basis of these findings, it was interesting to design and synthesize *hitherto* unknown ANP analogs bearing the biolabile phosphonate (*E*)-but-2-enyl aliphatic side-chain and a series of modified heterobases selected from the literature as lead nucleobases with antiviral properties, such as cytosine and 5-fluoro cytosine, 2-pyrazinecarboxamide, 1,2,4-triazole-3-carboxamide or 4-substituted-1,2,3-triazoles (Fig. 1). From a chemical synthesis point of view, the strategy based on olefin cross metathesis we have developed to obtain a large library of (*E*)-4-phosphono-but-2′-en-1′-yl pyrimidine nucleosides [9–11].

2. Results and discussion

2.1. Chemistry

First, we turned our attention to the synthesis of cytosine derivatives as cytosine modified nucleosides form a prolific family of antitumor and antiviral agents [12]. As it could be excepted, even if the ruthenium carbene complex **3** (Grubbs–Nolan catalyst 2nd generation) is less affected by free amine and nitrogen-containing groups than the Grubbs's 1st generation catalyst [13], the cross-metathesis reaction between unprotected N^1-crotylated cytosine **2** and *bis*-(POM) allylphosphonate **1** failed (Scheme 1).

The successful cross-metathesis occurred with protected N^1-crotylated cytosines **6a**, **b**. Thus, cytosine **4a** and 5-fluorocytosine **4b** were converted to their N^4-*bis*-Boc cytosine derivatives **5a**, **b**, respectively, through a *N*-peracylation followed by subsequent and regioselective N^1 deprotection by a saturated solution of $NaHCO_3$ in methanol [14,15]. Crotylation of the N^1 position of **5a**, **b** using Cs_2CO_3 and crotyl bromide afforded the desired compounds **6a** (85%) and **6b** (81%). Compound **6a**, **b** were then engaged in the olefin cross metathesis reaction with *bis*(POM)-allylphosphonate **1** using 5 mol% of the (NHC)Ru=CHR Nolan's catalyst, $Cl_2(PCy_3)$(IMes)Ru

(CHPh) (**3**), in dry CH_2Cl_2 (0.1 M) at reflux to afford (*E*)-N^1-(4′-*bis*(POM)-phosphinyl-2′-butenyl)-*bis*-Boc-cytosine **7a**, **b** in moderate yields (43% (for R = H) and 26% (for R = F)). The removal of the Boc group requires oftentimes harsh conditions (e.g. trifluoroacetic acid, trimethylsilyl iodide, hydrochloric acid in ethyl acetate, potassium carbonate, etc.) that are not compatible with the POM moiety. However, Hwu et al. [16] have reported an efficient and milder selective Boc deprotection under neutral conditions using ceric ammonium nitrate (CAN) that is consistent with the stability of the phosphonate biolabile group. Thus, protected ANP **7a**, **b** were reacted with a catalytic amount of ceric ammonium nitrate (CAN) (20 mmol%) in CH_3CN–MeOH (1:1) to give the expected compounds **8a**, **b**, respectively, in moderate yield, with no observed removal of the POM moiety (Scheme 2).

We turned then our attention to the synthesis of the pyrazinecarboxamide derivative **14** since, among the modified nucleobases, a series of pyrazinecarboxamide derivatives (including T-705, favipiravir) developed by Furuta et al. [17,18] have demonstrated good activity in various RNA viral infections. In a first attempt, we struggled to introduce the pyrazine moiety through direct *N*-alkylation of **10** with the corresponding (*E*)-4-phosphono-but-2′-en-1′-yl bromide (**9**), in the presence of K_2CO_3 in anhydrous DMF, (Pathway A). Unfortunately only the *bis*(POM)-but-1,3-dienyl phosphonate **9′** resulting from undesired bromine elimination was obtained, (Scheme 3). Thus, we decided to reach the pyrazine phosphonate analogs **13**, **14** following the same strategy developed for the cytosine derivatives, (Pathway B). Starting from the 3-hydroxypyrazine-2-carboxamide **10**, the N^1-Crotyl-3-oxo-pyrazine-N^3-*bis*-Boc-carboxamide **11** was obtained in 49% yield, via the two step crotylation/*N*-Boc-protection. Next, N^1-crotyl-3-oxo-pyrazine-N^3-*bis*-(Boc)-carboxamide **11** in CH_2Cl_2 was treated in the presence of *bis*(POM)-allylphosphonate **1** and (NHC)Ru=CHR Nolan's catalyst **3** to give the desired product **12** in 22% yield. To obtain the carboxamide **14**, we first applied our previous described methods, using ceric ammonium nitrate and CH_3CN–MeOH (1:1). Surprisingly, the methylester **13** was isolated as the major compound in 47% yield. Thinking that the presence of the undesired product **13** was due to the use of methanol as co-solvent, the preparation of the desired amide **14**, was achieved in CH_3CN using ceric ammonium nitrate in 25% yield.

Based on the above results, we extended this approach to the formation of a ribavirin analog bearing the 1,2,4-triazole derivative [19,20]. Starting from **15**, the protection of both nitrogens provided compound **16**, in quantitative yield, which is directly used in the next step. An attempt to selectively Boc deprotect at the N^1 position

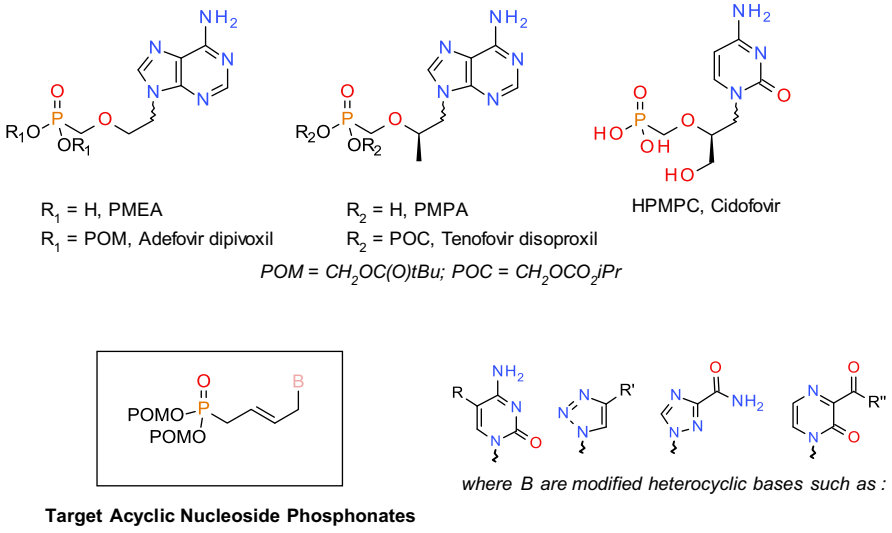
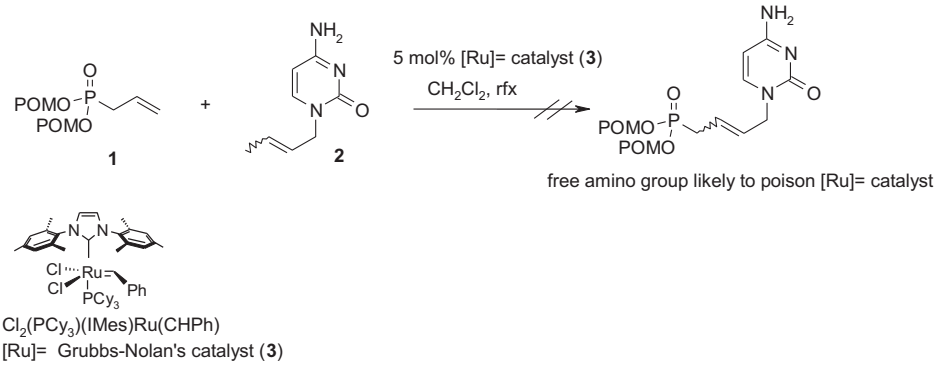


Fig. 1. Structure of selected ANPs and target derivatives.



Scheme 1. Attempted cross-metathesis reaction with *bis*-(POM)-allylphosphonate **1** with unprotected cytosine derivatives.

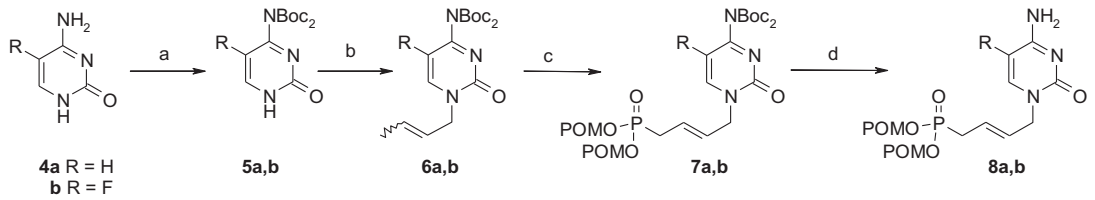
of **16** to **17** failed and gave the unexpected free amide derivative **18**. Alternatively, we decided to successively alkylate the N^1 position of the 1,2,4-triazole-3-carboxamide in the presence of crotyl bromide and Cs_2CO_3, followed by the protection of the free nitrogen in dry THF in the presence of Boc_2O and DMAP at room temperature to give the desired triazole **19** in 34% in two steps (Scheme 4). N^1-crotyl-1,2,4-triazole-3-*bis*-Boc-carboxamide **19** was then subjected to the olefin cross metathesis reaction, with *bis*(POM)-allylphosphonate in CH_2Cl_2 to obtain the desired compound **20** in 16% yield. The *bis*-Boc groups were cleaved according to the previous procedure, by treatment with CAN in a mixture of CH_3CN–MeOH (1:1) to give the free ANP analog **21** in 42% yield (Scheme 4).

To complete our investigation, we elaborated a small library of ANPs in their prodrug form bearing the substituted 1,2,3-triazolyl moiety **24**–**33**. The triazole derivatives were obtained using Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) using selected alkynes and phosphonate azide **22** [21–24],. The introduction of the azide group could be easily performed from the key intermediate previously described. The olefin cross metathesis of *bis*(POM)-allylphosphonate with (*E*)-1,4-dibromobut-2-ene catalyzed by catalyst **3** afforded (*E*)-4-bromo-*bis*(POM)-allylphosphonate (**9**) in 88% yield. Then, the introduction of azido on **9** with sodium azide in DMSO–THF–H_2O (5:2:1) afforded (*E*)-4-azide-*bis*(POM)-allylphosphonate **22** in 69% yield (Scheme 5). Among the modifications on the base moiety, carboxylic acid is an unavoidable function. In a first attempt, following our previously described method C, in the presence of propiolic acid under microwave irradiation, we observed the formation and isolation of the unexpected decarboxylated product **23** in 16% yield [25,26]. To circumvent this decarboxylation, Hall et al. reported boronic acid catalysis (BAC) for the activation of carboxylic acids, to lead to a classic dipolar [3 + 2] cycloaddition with several azides [27]. To obtain the desired (*E*)-4-(4-carboxylic acid-[1,2,3]-triazol-1-yl)methyl-*bis*(POM)-but-2-enylphosphonate derivative **24**, we selected the BAC of the azide–alkyne cycloaddition, which was converted in 58% yield in the presence of 2-nitrophenylboronic acid at room temperature.

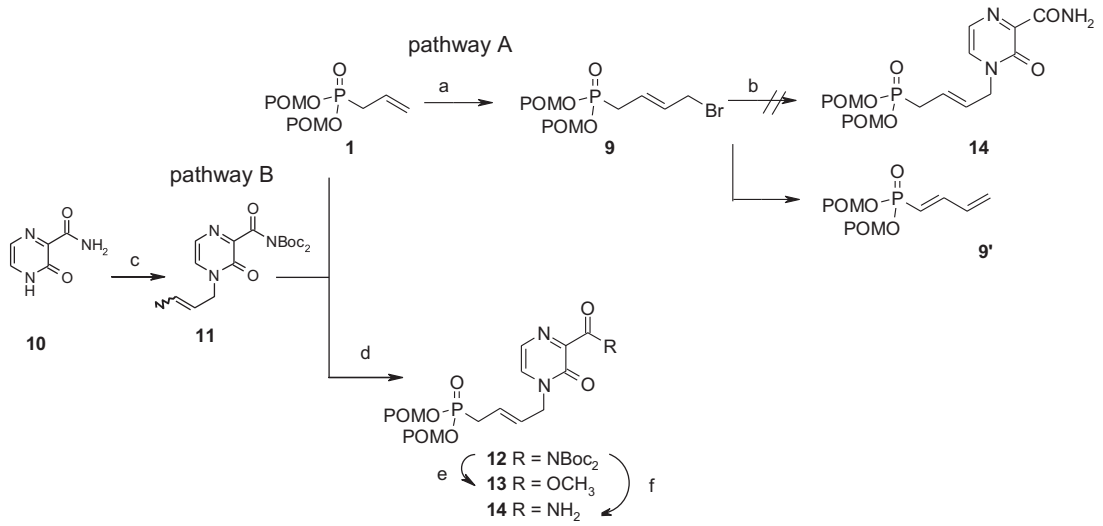
Finally, the copper-catalyzed azide-alkynes 1,3-dipolar cycloaddition (CuAAC) affording chemo-selectively and complete regioselectively the (1,4) substituted-1,2,3-triazoles [22], that permitted the synthesis of a number of ANPs analogs, (Table 1). A first series of *bis*(POM)-(1,4-disubstituted-1,2,3-triazol)-but-2-enyl-phosphonate congeners bearing a substituted 1,2,3-triazole by phenylacetylene moieties (chosen from apolar to bulky substituents) was obtained by CuAAC reaction with Cu(0)/$CuSO_4 \cdot 5H_2O$ as catalyst in the presence of substituted phenylacetylenes, ethynylthiophene and non-aromatic alkynes, in moderate to good yields ranging from 35 to 93% (Table 1, entries 1–6) at room temperature (method A. The reaction between our synthon **22** and 2-ethynylthiophene or prop-2-ynol at room temperature only led to trace amounts of the expected products **31** and **32**. These were, however, isolated. The cycloaddition was then carried out at 60 °C Table 1, entries 7 and 8, method B) to afford compounds **31** and **32** in 45 and 95% yield, respectively. However, the alkyne listed in Table 1, entry 9 did not allow the formation of the triazole product **33** under thermal conditions. Microwave heating is known as a powerful tool that can produce a variety of nucleoside products [23]. Following our previous work [24], the microwave irradiation allowed to obtain in moderate yields the desired ANPs **33** and **34** in 44% and 34% yield respectively (Table 1, entry 9 and 10, method C).

2.2. Antiviral evaluation

The title *bis*(POM) (*E*)-4-phosphono-but-2-en-1-yl acyclic nucleosides were subjected to an *in vitro* antiviral screening using a wide spectrum of viruses, in MDKC cell cultures for anti-influenza virus activity, in Vero cell cultures for an antiviral activity against Para-influenza-3 virus, Reovirus-1, Sindbis virus, Coxsackie B4 virus, Punta Toro virus, in CRFK cell cultures for their anti-geline corona virus and anti-feline herpes virus activity, in HEL cell cultures for vaccinia virus, herpes simplex virus-1, herpes simplex virus-2, vesicular stomatitis virus, varicella-zoster virus (VZV TK^+ and TK^-), human cytomegalovirus (AD-169 strain and Davis strain) inhibitory



Scheme 2. Reagents and conditions: (a) i) Boc_2O, DMAP, dry THF then ii) saturated $NaHCO_3$ aq. solution, methanol, 60 °C, 37% (for R = H) and 24% (for R = F) (yields for two steps); (b) crotyl bromide, Cs_2CO_3, dry DMF, 85% (for R = H) and 81% (for R = F); (c) *bis*-(POM)-allylphosphonate (**1**), 10 mol% [Ru] = catalyst **3**, dry CH_2Cl_2, 45 °C, 43% (for R = H) and 26% (for R = F); (d) ceric ammonium nitrate, CH_3CN:MeOH (1:1), 55% (for R = H) and 35% (for R = F).



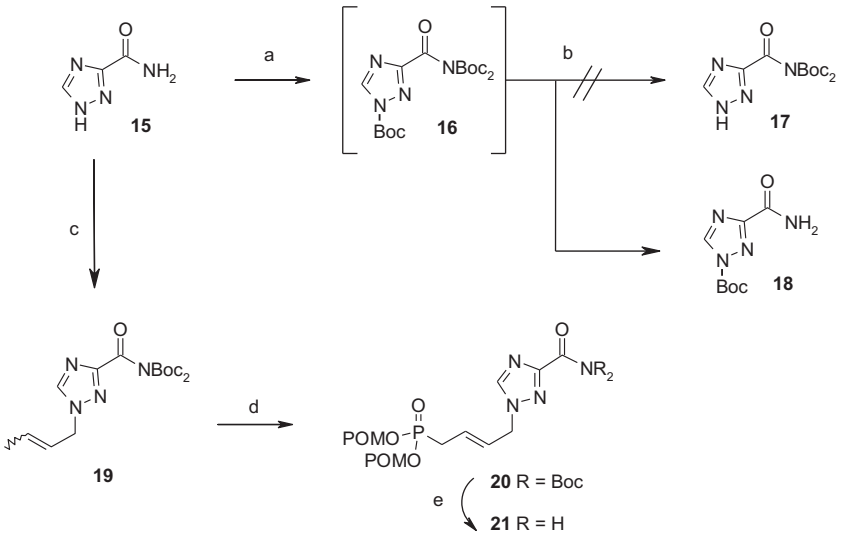
Scheme 3. Reagents and conditions: (a) (*E*)-1,4-dibromobut-2-ene, 10 mol% [Ru] = catalyst **3**, dry CH_2Cl_2, 45 °C, 88%; (b) heterocycle **10**, K_2CO_3, dried DMF; (c) 1) crotyl bromide, Cs_2CO_3, dry DMF then ii) Boc_2O, DMAP, dry THF, 49% (for two steps); (d) 10 mol% [Ru] = catalyst **3**, dry CH_2Cl_2, 45 °C, 22%; (e) ceric ammonium nitrate, CH_3CN–MeOH (1:1), 47%; (f) ceric ammonium nitrate, CH_3CN, 25%.

activity and in HeLa cell cultures for Coxsackie B4 and respiratory syncytial virus inhibitory activity. Among the tested final molecules against human cytomegalovirus in Hel cell cultures (data not shown) only compound **31** showed activity at an EC_{50} of ~10 μM (AD-169 strain) with no observed cytotoxicity at 100 μM (Table 2).

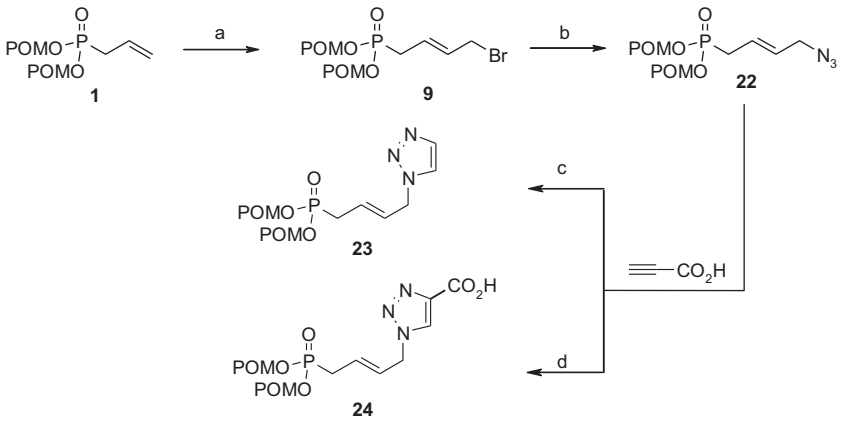
The compounds were also evaluated for inhibition of hepatitis C virus (HCV) in the subgenomic HCV replicon system in Huh 7 cells [28], and cytotoxicity testing was performed in PBMC, human lymphoblastoid CEM and Vero cells [29,30]. Data for anti-HCV activity and anti-HIV activity, and the cytoxicity are shown in Table 3. Many of the compounds were found to be moderately cytotoxic, which must be considered when interpreting the anti-HCV data. Compounds **8a**, **13**, **14**, and **24** demonstrated anti-HCV activity without significant cytotoxicity at 100 μM. The other molecules showed pronounced inhibition at 10 μM, but at compound concentrations that were close to their cytostatic activity.

3. Conclusion

We have efficiently synthesized a series of seventeen *hitherto* unknown ANP analogs bearing the *E*-but-2-enyl aliphatic side chain and a series of modified heterocyclic bases such as cytosine, 5-fluorocytosine, 2-pyrazinecarboxamide, 1,2,4-triazole-3-carboxam ide and 4-substituted-1,2,3-triazoles and evaluated their antiviral activities. Among the tested molecules only compound **31** showed activity against human cytomegalovirus at an EC_{50} of ~10 μM (AD-169 strain) at subtoxic concentrations with no observed cytotoxicity up to 100 μM. Compounds **8a**, **13**, **14**, and **24** demonstrated pronounced anti-HCV activity at 10 μM without significant cytotoxicity at 100 μM. Further structural optimization of both the (*E*)-but-2-enyl aliphatic side chain and the heterocycle is well under way, alongside more detailed biological testing of the most active compounds, with the aim of improving their antiviral potency.



Scheme 4. Reagents and conditions: (a) Boc_2O, DMAP, dry THF then (b) saturated $NaHCO_3$ aq. solution, methanol, 60 °C, 40% (for two steps); (c) i) crotyl bromide, Cs_2CO_3, dry DMF then ii) Boc_2O, DMAP, dry THF, r.t., 34% (for two steps); (d) 10 mol% [Ru] = catalyst **3**, dry CH_2Cl_2, 45 °C, 16%; (e) ceric ammonium nitrate, CH_3CN-MeOH (1:1), 42%.



Scheme 5. Reagents and conditions: (a) (*E*)-1,4-dibromobut-2-ene, 10 mol% [Ru] = catalyst **3**, dry CH_2Cl_2, 45 °C, 88%; (b) sodium azide, DMSO–THF–H_2O (5:2:1), r.t., 69%; (c) (a) Cu(0), $CuSO_4 \cdot 5H_2O$, *t*-BuOH/H_2O (1:1), MW (125 °C), 16%; (d) 2-nitrophenylboronic acid, DCE, r.t., 69%.

4. Experimental section

4.1. Chemistry

Commercially available chemicals were of reagent grade and used as received. Solvents were dried following standard procedures. The reactions were monitored by thin layer chromatography (TLC) analysis using silica gel plates (Kieselgel $60F_{254}$, E. Merck). Column chromatography was performed on Silica Gel 60M (0.040–0.063 mm, E. Merck). The ^1H, ^{31}P and ^{13}C NMR spectra were recorded on a Varian $Inova_{Unity}$ 400 spectrometer (400 MHz) in (d_4) methanol, $CDCl_3$, shift values in parts per million relative to $SiMe_4$ as internal reference. High Resolution Mass spectra (HRMS) were performed on a Bruker maXis mass spectrometer by the "Fédération de Recherche ICOA/CBM (FR 2708) platform".

4.1.1. Bis(POM)-allyl phosphonate (**1**)

Physico-chemical data are in agreement with reported information [9]. CAS number: 1258789-63-7.

4.1.2. N^1-Crotyl cytosine (**2**)

^1H NMR (400 MHz, CD_3OD) δ 7.56 (d, $J = 7.2$ Hz, $1H_{minor}$), 7.54 (d, $J = 7.2$ Hz, $1H_{major}$), 5.88 (d, $J = 7.2$ Hz, 1H), 5.82–5.72 (m, 1H), 5.63–5.47 (m, 1H), 4.47 (d, $J = 7.2$ Hz, 2H, CH_{2minor}), 4.32 (d, $J = 6.0$ Hz, 2H, CH_{2major}), 1.79 (dd, $J = 6.4$, 1.2 Hz, CH_{3minor}), 1.69 (dd, $J = 6.4$, 1.3 Hz, 2H, CH_{3major}). ^{13}C NMR (100 MHz, CD_3OD) δ 158.7, 146.9, 131.4, 130.2, 126.5, 125.6, 95.9, 51.8, 46.7, 17.8, 13.1. HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_8H_{12}N_3O$: 166.09746, found: 166.09748.

4.1.3. N^3,N^3-Bis-Boc-cytosine (**5a**)

To the stirred suspension of cytosine in an argon atmosphere (444.0 mg, 4.0 mmol, 1.0 equiv.) in dry THF (13 mL), DMAP (44.0 mg, 0.4 mmol, 0.1 equiv.) Boc_2O (3.60 g, 16 mmol, 4.0 equiv.) were added. After 20 h stirring at room temperature, the mixture was diluted with EtOAc and then extracted with (2 × 30 mL) EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The resulting *tri*-Boc-cytosine was used for the next step without further purification. To a solution of *tri*-Boc-cytosine in methanol (40 mL) was added saturated $NaHCO_3$ aq. solution (18 mL) at room temperature. After stirring 2 h at 60 °C, confirming the complete consumption of substrate by examining a TLC developed with petroleum ether–EtOAc (1:1). After removal of methanol *in vacuo*, the mixture was diluted with EtOAc (30 mL), quenched with water (20 mL) and finally extracted with EtOAc (2 × 20 mL). The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with petroleum ether–EtOAc (1:1 to 1:20) to give **5a** (465 mg, 37%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 12.88 (s, 1H), 7.66 (d, $J = 7.2$ Hz, 1H), 7.10 (d, $J = 7.1$ Hz, 1H), 1.54 (s, 17H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.9, 158.6, 149.7, 145.7, 96.9, 85.2, 27.9. CAS number: 1108637-28-0.

4.1.4. N^4,N^4-Bis-Boc-5-fluoro-cytosine (**5b**)

In an analogous manner to the preparation of **5a**, **5b** was prepared from 5-fluoro-cytosine (158.0 mg, 24%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, $J = 3.5$ Hz, 1H), 1.46 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.7, 156.8, 149.0, 143.6, 141.2, 134.0, 133.7, 85.2, 27.9. ^{19}F NMR (376 MHz, $CDCl_3$) δ −154.8. HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{14}H_{21}FN_3O_5$: 330.1463, found: 330.1459.

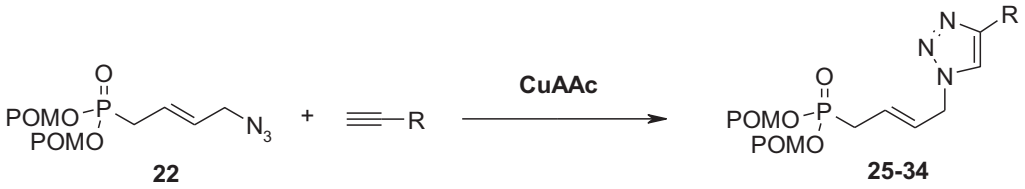
4.1.5. N^4,N^4-Bis-Boc-N^1-crotyl-cytosine (**6a**)

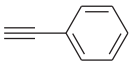
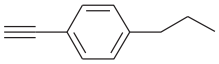
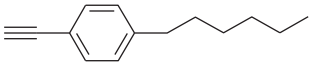
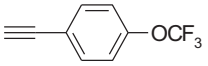
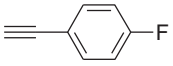
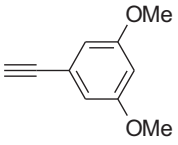
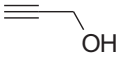
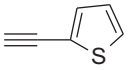
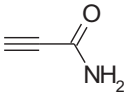
To a solution of **5a** (90.0 mg, 0.29 mmol, 1.0 equiv.) in dry DMF (1 mL) was added Cs_2CO_3 (104.3 mg, 0.32 mmol, 1.1 equiv.) and crotyl bromide (43.0 mg, 0.32 mmol, 1.1 equiv.) at room temperature and stirred under an argon atmosphere for 2 h. The resulting mixture was then diluted with EtOAc (2 × 20 mL), quenched with water and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with petroleum ether–EtOAc (1:1) to give a mixture of *Z* (*minor*)/*E* (*major*) N^4,N^4-*bis*-Boc-N^1-crotyl-cytosine **6a** (90.0 mg, 85%) as a yellow oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, $J = 7.3$ Hz, 1H), 6.95 (d, $J = 7.3$ Hz, 1H), 5.86–5.68 (m, 1H), 5.58–5.43 (m, 1H), 4.48 (d, $J = 7.1$ Hz, 2H, CH_{2minor}), 4.37 (d, $J = 6.4$ Hz, 2H, CH_{2major}), 1.73 (dd, $J = 7.0$, 3.0 Hz, 3H, CH_{3minor}), 1.69 (d, $J = 6.4$ Hz, 3H, CH_{3major}), 1.52 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.4, 162.3, 155.2, 149.8, 147.2, 146.9, 132.1, 131.2, 124.6, 123.5, 96.5, 85.0, 51.8, 46.2, 27.9, 24.0, 18.0, 13.3. HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{18}H_{28}N_3O_5$: 366.2026, found: 366.2023.

4.1.6. N^4,N^4-Bis-Boc-N^1-crotyl-5-fluoro-cytosine (**6b**)

In a similar manner as described for **6a**, a solution of *bis*-Boc-5-fluoro-cytosine **5b** (139.0 mg, 0.42 mmol) in dry DMF (1.6 mL) was treated with crotyl bromide (61.8 mg, 0.46 mmol) and Cs_2CO_3 (150.0 mg, 0.46 mmol), to give a mixture of *Z* (*minor*)/*E* (*major*) N^4,N^4-*bis*-Boc-N^1-crotyl-5-fluoro-cytosine **6b** (130.1 mg, 81%) as a yellow oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, $J = 4.5$ Hz, $1H_{major}$), 7.59 (d, $J = 3.1$ Hz, $1H_{minor}$), 6.02–5.74 (m, 1H), 5.62–5.46 (m, 1H), 4.53 (d, $J = 7.3$ Hz, 2H, CH_{2minor}), 4.42 (d, $J = 6.6$ Hz, 2H, CH_{2major}), 1.76–1.73 (m, 3H), 1.45 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.0,

Table 1
Cycloaddition of CuAAC reaction.[a]



Entry	R	Product	Method	Yield (%)[b]
1		**25**	A	70
2		**26**	A	88
3		**27**	A	93
4		**28**	A	52
5		**29**	A	65
6		**30**	A	35
7		**31**	B	44
8		**32**	B	95
9		**33**	C	44
10		**34**	C	34

Method A: room temperature during 8 h.
Method B: 60 °C during 16 h.
Method C: 125 °C during 1 h under microwave irradiation (MW).
[a] All reactions were performed with 1.0 equiv of (*E*)-4-azide-*bis*(POM)-but-2-enylphosphonate, 1.3 equiv of alkyne, and 5.0 equiv of Cu (0), and 0.25 equiv of $CuSO_4 \cdot 5H_2O$, *t*-BuOH/H_2O (1:1).
[b] Isolated yield.

149.1, 134.0, 133.1, 132.7, 123.6, 122.3, 84.9, 52.2, 46.5, 27.9, 24.1, 18.1, 13.4. ^{19}F NMR (376 MHz, $CDCl_3$) δ −156.54 (d, J = 4.5 Hz), −156.66 (d, J = 4.5 Hz). HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{18}H_{27}FN_3O_5$: 384.1932, found: 384.1929.

4.1.7. (E)-N^4,N^4-Bis-Boc-N^1-(4′-bis(POM)phosphinyl-2′-butenyl) cytosine (**7a**)

To a solution of *bis*(POM)-allylphosphonate **1** (77.0 mg, 0.22 mmol, 1.0 equiv.) and compound **6a** (90.0 mg, 0.25 mmol, 1.1 equiv.) in dry CH_2Cl_2 (2 mL, 0.1 M) was added (NHC)Ru=CHR Nolan's catalyst **3** (17.0 mg, 0.02 mmol, 0.1 equiv.). The reaction mixture was stirred at 45 °C under an argon atmosphere for 16 h. After evaporation of all volatiles, the crude was purified by silica gel chromatography with petroleum ether–EtOAc (1:2 to 1:4) to give **7a** (63.0 mg, 43%) as a yellow oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, J = 7.4 Hz, 1H), 7.01 (d, J = 7.4 Hz, 1H), 5.79–5.70 (m, 1H), 5.69–5.61 (m, 5H), 4.23 (t, J = 4.8 Hz, 2H), 2.68 (dd, J = 22.6, 6.9, 2H), 1.53 (s, 18H), 1.20 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8, 162.3, 154.9, 149.5, 146.9, 129.8, 129.7, 124.1, 123.9, 96.6, 84.9, 81.6, 81.5, 51.2, 38.7, 31.6, 30.2, 27.7, 26.8. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.6. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{30}H_{49}N_3O_{12}P$: 674.3050, found: 674.3048.

4.1.8. (E)-N^4,N^4-Bis-Boc-N^1-(4′-bis(POM)phosphinyl-2′-butenyl)-5-fluoro-cytosine (**7b**)

In a similar manner as described for **7a**, a solution of *bis*(POM)-allylphosphonate (77.0 mg, 0.20 mmol) and **6b** (85.0 mg, 0.22 mmol)

Table 2
Anti-cytomegalovirus activity and cytotoxicity of compound **31** in human embryonic lung (Hel) cells.

Compound	EC_{50}[a] (μM) AD-169 strain	CC_{50}[b] (μM)
31	9.9 ± 1.0	100
Ganciclovir	8.2 ± 0.3	>350
Cidofovir	1.0 ± 0.14	>350

[a] 50% Effective concentration or compound concentration required to inhibit virus-induced cytopathicity by 50%.
[b] 50% Cytotoxic concentration or compound concentration required to reduce the viability of Hel cells by 50%.

in dry CH_2Cl_2 (2 mL, 0.1 M) were treated with (NHC)Ru=CHR Nolan's catalyst **3** (17.0 mg, 0.02 mmol), to give **7b** (35.5 mg, 26%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, J = 4.4 Hz, 1H), 5.82–5.69 (m, 2H), 5.67 (dd, J = 13.8, 5.2 Hz, 2H), 5.63 (dd, J = 13.8, 5.2 Hz, 2H), 4.47 (t, J = 4.4 Hz, 2H), 2.71 (dd, J = 22.9, 5.3 Hz, 2H), 1.45 (s, 18H), 1.20 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 155.9, 153.8, 149.1, 142.0, 139.6, 133.1, 132.8, 128.9, 128.7, 126.2, 126.1, 85.0, 81.9, 81.8, 51.8, 38.9, 31.8, 30.4, 27.9, 27.0. ^{19}F NMR (376 MHz, $CDCl_3$) δ −155.90 (d, J = 4.3 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.2. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{30}H_{48}FN_3O_{12}P$: 692.29539, found: 692.29541.

*4.1.9. (E)-N^1-(4′-Bis(POM)phosphinyl-2′-butenyl)-cytosine (**8a**)*

To a solution of **7a** (44.0 mg, 0.07 mmol, 1.0 equiv.) in 0.7 mL of CH_3CN-MeOH (1:1) was added ceric ammonium nitrate (7.2 mg, 0.013 mmol, 0.2 equiv.) and the reaction solution was stirred at 60 °C for 16 h. After evaporation of all volatiles, the residue was purified by silica gel chromatography with EtOAc to give (*E*)-N^1-(4′-*bis*(POM)phosphinyl-2′-butenyl)-cytosine **8a** (18.2 mg, 55%) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, J = 7.2 Hz, 1H), 5.91 (d, J = 7.2 Hz, 1H), 5.89–5.76 (m, 2H), 5.66 (m, 4H), 4.39 (t, J = 5.2 Hz, 2H), 2.81 (dd, J = 22.5, 7.2 Hz, 2H), 1.23 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.3, 147.1, 132.4, 132.3, 123.6, 123.5, 83.3, 83.2, 51.6, 39.9, 32.0, 30.6, 27.4. ^{31}P NMR (162 MHz, $CDCl_3$) δ 29.8. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{20}H_{33}N_3O_8P$: 474.20045, found: 474.19997.

*4.1.10. (E)-N^1-(4′-Bis(POM)phosphinyl-2′-butenyl)-5-fluoro-cytosine (**8b**)*

In a similar manner as described for **8a**, a solution of **7b** (35.5 mg, 0.05 mmol) in 0.8 mL of CH_3CN–MeOH (1:1) was treated with ceric ammonium nitrate (5.5 mg, 0.01 mmol), to give (*E*)-N^1-(4′-*bis*(POM)phosphinyl-2′-butenyl)-5-fluoro-cytosine **8b** (8.2 mg, 35%) as a colorless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 7.72 (d, J = 6.2 Hz, 1H), 5.89–5.77 (m, 1H), 5.66 (m, 5H), 4.35 (t, J = 5.2 Hz, 2H), 2.82 (dd, J = 22.5, 7.2 Hz, 2H), 1.23 (s, 18H). ^{13}C NMR (100 MHz, MeOD) δ 178.3, 132.1, 132.0, 131.2, 130.9, 124.0, 83.3, 83.3, 51.8, 39.9, 32.0, 30.6, 27.4. ^{19}F NMR (376 MHz, CD_3OD) δ −170.68 (d, J = 5.4 Hz). ^{31}P NMR (162 MHz, CD_3OD) δ 25.7. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{20}H_{32}FN_3O_8P$: 492.19057, found: 492.19055.

*4.1.11. (E)-4-Bromo-bis(POM)-but-2-enylphosphonate (**9**)*

To a solution of *bis*(POM)-allylphosphonate **1** (560 mg, 1.60 mmol, 1.0 equiv.) in 24 mL of dry CH_2Cl_2 was added (*E*)-1,4-dibromobut-2-ene (1.37 g, 6.40 mmol, 4.0 equiv.) and (NHC)Ru=CHR Nolan's catalyst **3** (68.6 mg, 0.08 mmol, 0.05 equiv.). After 16 h of stirring at 45 °C under an argon atmosphere, all volatiles were evaporated and the residue was purified by silica gel chromatography eluting with petroleum ether–EtOAc (4:1) to give (*E*)-4-bromo-*bis*(POM)-but-2-enylphosphonate **9** (623 mg, 88%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.93–5.83 (m, 1H), 5.74–5.62 (m, 5H), 3.92 (dd, J = 7.5, 3.5 Hz, 2H), 2.70 (dd, J = 22.7, 7.3 Hz, 2H), 1.23 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.3, 132.1, 131.9, 122.7, 122.6, 81.2, 38.3, 31.2, 30.9, 29.5, 26.5. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.8. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{16}H_{28}O_7NaBrP$: 465.0654, found: 465.0671. CAS: 1365350-40-8.

*4.1.12. 4-Bis(POM)-but-1,3-diene phosphonate (**9′**)*

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (ddd, J = 22.2, 16.9, 10.6 Hz, 1H), 6.38 (tdd, J = 16.9, 10.6, 1.8 Hz, 1H), 5.81–5.50 (m, 7H), 1.52 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8, 149.7, 149.6, 135.4, 135.1, 126.2, 117.9, 116.0, 81.5, 38.7, 26.8. ^{31}P NMR (164 MHz, $CDCl_3$) δ 18.86.

*4.1.13. N^1-Crotyl-3-oxo-pyrazine-N,N-bis-Boc-carboxamide (**11**)*

In a similar manner as described for **6a**, a solution of 3-hydroxypyrazine-2-carboxamide **10** (139.1 mg, 1.0 mmol) in dry DMF (3 mL) was treated with Cs_2CO_3 (358.4 mg, 1.1 mmol) and crotyl bromide (148.5 mg, 1.1 mmol) for 4 h at 70 °C, to give a crude mixture of (*E*/*Z*) N^1-crotyl-3-oxo-pyrazine-2-carboxamide. After evaporation of all volatiles and rapid silica gel chromatography, the residue (134.0 mg) was treated with DMAP (7.7 mg, 0.07 mmol) and Boc_2O (602.4 mg, 2.76 mmol) in dry THF (3 mL), to give N^1-crotyl-3-oxo-pyrazine-2-*bis*-Boc-carboxamide **11** (134.0 mg, 49%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, J = 4.2 Hz, 1H), 7.22 (d, J = 4.2 Hz, 1H), 5.87–5.70 (m, 1H), 5.53–5.38 (m, 1H), 4.52 (t, J = 7.3 Hz, 2H, CH_{2minor}), 4.40 (t, J = 7.5 Hz, 2H, CH_{2major}), 1.70 (dd, J = 7.0, 1.7 Hz, 3H, CH_{3minor}), 1.65 (dd, J = 7.0, 1.7 Hz, 3H, CH_{3major}), 1.41 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 164.4, 153.4, 150.1, 149.4, 133.2, 132.1, 130.6, 130.4, 123.4, 122.8, 122.4, 85.1, 60.5, 50.7, 45.0, 27.7, 21.1, 17.9, 14.3, 13.3. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{19}H_{28}N_3O_6$: 394.19724, found: 394.19726.

*4.1.14. (E)-N^1-(4′-Bis(POM)-phosphinylbut-2′-enyl)-3-oxo-pyrazine-N,N-bis-Boc-carboxamide (**12**)*

In a similar manner as described for **7a**, a solution of *bis*(POM)-allylphosphonate **1** (273.5 mg, 0.78 mmol) and N^1-crotyl-3-oxo-pyrazine-*N,N-bis*-Boc-carboxamide **11** (338.2 mg, 0.86 mmol) in dry CH_2Cl_2 (8 mL) were treated with (NHC)Ru=CHR Nolan's catalyst **3** (66.2 mg, 0.078 mmol), to give **12** (122.8 mg, 22%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, J = 4.3 Hz, 1H), 7.22 (d, J = 4.3 Hz, 1H), 5.80–5.69 (m, 2H), 5.64 (m, 4H), 4.49 (t, J = 4.8 Hz, 2H), 2.69 (dd, J = 22.2, 5.2 Hz, 2H), 1.47 (s, 18H), 1.21 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 153.4, 149.9, 149.4, 130.1, 128.8, 128.6, 125.8, 125.7, 123.1, 85.3, 82.2, 81.9, 81.8, 50.2, 39.0, 31.9, 28.3, 27.8, 27.1. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.3. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{31}H_{49}N_3O_{13}P$: 702.29871, found: 702.29975.

Table 3
Anti-HCV activity, anti-HIV activity and cytotoxicity of synthesized compounds in cellular assays.

Compound	Anti-HCV activity: % inhibition 10 μM in Huh7 cells	Anti-HIV activity (μM)		Cytotoxicity in CC_{50} (μM)		
	HCV	EC_{50}	EC_{90}	PBM	CEM	Vero
8a	80.4	70.4	>100	>100	>100	>100
8b	73.6	nd	nd	nd	nd	nd
13	76.9	>100	>100	>100	>100	>100
14	60.0	>100	>100	>100	>100	>100
21	73.9	>100	>100	58.3	41.8	86.8
23	85.9	>100	>100	93.1	81.8	87.9
24	53.8	>100	>100	>100	>100	>100
25	83.1	35.8	>100	16.0	31.6	15.6
26	95.6	31.6	>100	35.9	55.3	41.0
27	95.0	43.9	>100	5.9	16.1	10.4
28	60.6	55.2	>100	19.6	37.6	12.7
29	84.3	>100	>100	61.9	31.6	47.0
30	20.8	>100	>100	>100	68.2	86.8
31	78.1	>100	>100	73.2	57.4	87.1
32	0.2	62.3	>100	32.9	31.6	17.1
33	88.7	>100	>100	19.8	16.8	10.3
34	86.5	70.5	>100	62.4	52.9	80.6

*4.1.15. (E)-N^1-(4′-Bis(POM)- phosphinylbut-2′-enyl)-3-oxo-pyrazine-2-methylester (**13**)*

In a similar manner as described for **8a**, a solution of **12** (84.1 mg, 0.12 mmol) in 2 mL of CH_3CN–MeOH (1:1) was treated with ceric ammonium nitrate (13.1 mg, 0.024 mmol), to give **13** (38.6 mg, 47%) as a colorless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 7.80 (d, J = 4.1 Hz, 1H), 7.49 (d, J = 4.1 Hz, 1H), 5.94–5.83 (m, 1H), 5.81–5.73 (m, 1H), 5.71–5.61 (m, 4H), 4.66–4.61 (m, 2H), 3.91 (s, 3H), 2.83 (dd, J = 22.7, 7.1 Hz, 2H), 1.22 (s, 18H). ^{13}C NMR (100 MHz, CD_3OD) δ 178.3, 155.4, 135.1, 130.4, 130.3, 126.1, 126.0, 124.7, 83.4, 83.3, 53.3, 52.0, 39.9, 32.1, 30.7, 27.4. ^{31}P NMR (162 MHz, CD_3OD) δ 26.9. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{22}H_{34}N_2O_{10}P$: 517.19480, found: 517.19455.

*4.1.16. (E)-N^1-(4′-Bis(POM)- phosphinylbut-2′-enyl)-3-oxo-pyrazine-2-carboxamide (**14**)*

In a similar manner as described for **8a**, a solution of **12** (54.3 mg, 0.08 mmol) in CH_3CN (1 mL) was treated with ceric ammonium nitrate (11.0 mg, 0.02 mmol), to give **14** (10.0 mg, 25%) as a colorless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 8.01 (d, J = 4.0 Hz, 1H), 7.74 (d, J = 4.0 Hz, 1H), 5.96–5.76 (m, 2H), 5.69–5.62 (m, 4H), 4.73 (t, J = 5.2 Hz, 2H), 2.85 (dd, J = 22.7, 6.9 Hz, 2H), 1.22 (s, 18H). ^{13}C NMR (100 MHz, CD_3OD) δ 178.3, 154.1, 136.6, 130.1, 130.0, 126.6, 126.1, 125.2, 83.4, 83.3, 53.4, 39.9, 32.0, 30.6, 27.4. ^{31}P NMR (162 MHz, CD_3OD) δ 26.9. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{21}H_{33}N_3O_9P$: 502.19498, found: 502.19489.

*4.1.17. N^1-Crotyl-1,2,4-triazole-N,N-bis-Boc-carboxamide (**15**)*

To a solution of 1,2,4-triazole-3-carboxamide **15** (224.5 mg, 2.0 mmol, 1.0 equiv.) in dry DMF (6 mL) was added Cs_2CO_3 (716.8 mg, 2.2 mmol, 1.1 equiv.) and crotyl bromide (270.0 mg, 2.2 mmol, 1.1 equiv.) and the reaction solution was stirred at room temperature under an argon atmosphere for 2 h and then warmed at 70 °C for 12 h. After concentration to dryness *in vacuo*, the residue was subjected to silica gel chromatography with CH_2Cl_2–MeOH (5:1) and employed in the next step without further purification. The residue (330 mg) was suspended in THF (10 mL), DMAP (44 mg, 0.2 mmol, 0.1 equiv.) and Boc_2O (1.31 g, 6.0 mmol, 3.0 equiv.) were added under an argon atmosphere. The solution was stirred for 20 h at room temperature and then the mixture was diluted with EtOAc and then extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluting with petroleum ether–EtOAc (4:1) to give a mixture of *Z* (*minor*)/*E* (*major*) N^1-crotyl-1,2,4-triazole-3-*bis*-Boc-carboxamide **19** (248.0 mg, 34%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 5.88–5.74 (m, 1H), 5.72–5.63 (m, 1H), 4.82 (d, J = 7.1 Hz, 2H, $CH_{2\text{minor}}$), 4.71 (d, J = 6.6 Hz, 2H, $CH_{2\text{major}}$), 1.71 (dd, J = 6.5, 1.3 Hz, 3H, $CH_{3\text{minor}}$), 1.67 (dd, J = 6.5, 1.3 Hz, 3H, $CH_{3\text{major}}$), 1.36 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.6, 157.1, 149.7, 144.0, 133.4, 131.8, 123.3, 122.0, 84.5, 52.6, 47.1, 27.7, 17.8, 13.2. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{17}H_{27}N_4O_5$: 367.19775, found: 367.19759.

*4.1.18. (E)-N^1-(4′-Bis(POM)-phosphinylbut-2′-enyl)-1,2,4-triazole-N,N-bis-Boc-carboxamide (**20**)*

In a similar manner as described for **7a**, a solution of *bis*(POM)-allylphosphonate (173.1 mg, 0.45 mmol) and N^1-crotyl-1,2,4-triazole-3-*bis*-Boc-carboxamide (185.0 mg, 0.50 mmol) in dry CH_2Cl_2 (4.5 mL, 0.1 M) was treated with (NHC)Ru=CHR Nolan's catalyst **3** (38.1 mg, 0.045 mmol), to give (*E*)-N^1-(4′-*bis*(POM) phosphinyl-2′-butenyl)-1,2,4-triazole-3-*bis*-Boc-carboxamide **20** (48.8 mg, 16%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 5.92–5.72 (m, 2H), 5.67 (dd, J = 13.6, 5.2 Hz, 4H), 5.63 (dd, J = 13.6, 5.2 Hz, 2H), 4.81 (t, J = 4.8 Hz, 2H), 2.71 (dd, J = 22.6, 6.6 Hz, 2H), 1.42 (s, 18H), 1.20 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 160.6, 157.4, 149.8, 144.0, 128.3, 128.2, 126.1, 125.9, 84.7, 81.9, 81.8, 52.3, 38.9, 31.7, 30.3, 28.2, 27.8, 27.0. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.0. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{29}H_{48}N_4O_{12}P$: 675.29986, found: 675.30008.

*4.1.19. (E)-N^1-(4′-Bis(POM)-phosphinylbut-2′-enyl)-1,2,4-triazole-3-carboxamide (**21**)*

In a similar manner as described for **8a**, a solution of (*E*)-N^1-(4′-*bis*(POM)-phosphinyl-2′-butenyl)-1,2,4-triazole-3-*bis*-Boc-carboxamide **20** (44.3 mg, 0.065 mmol) in 1 mL of CH_3CN–MeOH (1:1) was treated with ceric ammonium nitrate (7.2 mg, 0.013 mmol), to give (*E*)-N^1-(4′-*bis*(POM)phosphinyl-2′-butenyl)-1,2,4-triazole-3-carboxamide **21** (14.0 mg, 42%) as a colorless oil.

^{1}H NMR (400 MHz, CD_3OD) δ 8.47 (s, 1H), 6.02–5.92 (m, 1H), 5.80–5.71 (m, 1H), 5.66 (m, 4H), 4.91 (t, J = 4.8 Hz, 2H), 2.85 (dd, J = 22.7, 7.3 Hz, 2H), 1.22 (s, 18H). ^{13}C NMR (100 MHz, CD_3OD) δ 178.3, 163.5, 158.1, 146.4, 131.1, 130.9, 125.4, 125.3, 83.3, 83.3, 52.6, 39.9, 32.0, 30.6, 27.4. ^{31}P NMR (162 MHz, CD_3OD) δ 27.0. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{19}H_{32}N_4O_8P$: 475.19513, found: 475.19522.

*4.1.20. (E)-4-Azido-bis(POM)-but-2-enylphosphonate (**22**)*

To a solution of (*E*)-4-bromo-*bis*(POM)-but-2-enylphosphonate **9** (222.0 mg, 0.50 mmol, 1.0 equiv.) in mixture of DMSO (5 mL), THF (2 mL) and H_2O (1 mL) was added sodium azide (163.0 mg, 2.50 mmol, 5.0 equiv.). After 24 h stirring at room temperature, the mixture was diluted with EtOAc and water and then extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography, eluting with petroleum ether–EtOAc (4:1) to give (*E*)-4-azide-*bis*(POM)-but-2-enylphosphonate **22** (140.0 mg, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.70–5.61 (m, 6H), 3.74 (dd, J = 8.8, 3.5 Hz, 2H), 2.71 (dd, J = 22.4, 6.0 Hz, 2H), 1.21 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 129.8, 123.4, 81.8, 81.7, 38.9, 31.7, 30.3, 27.1. ^{31}P NMR (162 MHz, $CDCl_3$) δ 27.7. IR ν_{max} 3456.4, 2974.2, 2098.6, 1747.5, 1263.4, 1136.1, 960.6 cm^{-1}. HRMS (ESI): m/z [M + Na]$^+$ calcd for $C_{16}H_{28}N_3NaO_7P$: 428.15549, found: 428.15571.

4.2. General procedures for Huisgen 1,3-dipolar cycloaddition

Procedure A: To a solution of alkyne (1.3 equiv.) and (*E*)-4-azido-*bis*(POM)-but-2-enylphosphonate **22** (0.11 mmol, 1.0 equiv.) in *t*-BuOH/H_2O (1:1 ratio, 400 μL) were added Cu powder (11.6 mg, 0.40 mmol, 5.0 equiv.) and $CuSO_4$ (5.0 mg, 0.020 mmol, 0.25 equiv.). The resulting suspension was stirred 8 h at room temperature, then the crude mixture was diluted in EtOAc (1 mL), and directly transferred on a preparative thin layer silica plate to give (*E*)-4′-(1,2,3-triazol-1-yl)-*bis*(POM)-but-2′-enylphosphonate.

Procedure B: Following the procedure described above, the resulting suspension was stirred 16 h at 60 °C.

Procedure C: In a similar procedure, the mixture was stirred 1 h under microwave conditions at 125 °C.

*4.2.1. (E)-1-(4′-Bis(POM)-phosphinylbut-2′-enyl)-1,2,3-triazole (**23**)*

The title compound was prepared from **22** with procedure C to give **23** (16%) as a colorless oil. ^{1}H NMR (250 MHz, CD_3OD) δ 7.95 (d, J = 0.9 Hz, 1H), 7.74 (d, J = 0.9 Hz, 1H), 6.04–5.87 (m, 1H), 5.83–5.58 (m, 5H), 5.06 (t, J = 5.0 Hz, 2H), 2.85 (dd, J = 22.7, 7.2 Hz, 2H), 1.23 (s, 18H). ^{13}C NMR (100 MHz, CD_3OD) δ 178.3, 134.7, 131.3, 131.2, 125.8, 125.2, 125.1, 83.3, 83.2, 52.6, 39.9, 31.9, 30.5, 27.4. ^{31}P NMR (162 MHz, CD_3OD) δ 27.1. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{19}H_{31}N_3O_9P$: 476.17894, found: 476.17924.

*4.2.2. (E)-1-(4′-Bis(POM)- phosphinylbut-2′-enyl)-4-(carboxylic acid)-1,2,3-triazole (**24**)*

To a solution of propiolic acid (14.5 mg, 0.21 mmol, 1.1 equiv.) and (*E*)-4-azido-*bis*(POM)-but-2-enylphosphonate **22** (79.1 mg, 0.19 mmoL, 1.0 equiv.) in 1,2-dichloroethane (500 μL) was added 2-nitrophenylboronic acid (6.7 mg, 0.04 mmol, 0.21 equiv.) at room temperature. The solution was stirred for 30 h. After evaporation of solvent, the residue was purified by silica gel chromatography with EtOAc–MeOH (6:1) to give (*E*)-4-(4-carboxylic acid-[1,2,3]-triazol-1-yl)methyl-*bis*(POM)-but-2′-enylphosphonate **24** (52.7 mg, 58%) as a colorless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 8.10 (s, 1H), 5.93–6.01 (m, 1H), 5.80–5.73 (m, 1H), 5.68 (dd, J = 13.2, 5.2 Hz, 4H), 5.64 (dd, J = 13.2, 5.2 Hz, 4H), 5.03 (t, J = 5.3 Hz, 2H), 2.85 (dd, J = 22.7, 7.3 Hz, 2H), 1.23 (s, 18H). ^{13}C NMR (100 MHz, CD_3OD) δ 178.3, 131.2, 127.1, 125.1, 83.3, 83.3, 52.7, 39.9, 31.9, 30.6, 27.4. ^{31}P NMR (162 MHz, CD_3OD) δ 27.1. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{19}H_{31}N_3O_9P$: 476.17897, found: 476.17924.

*4.2.3. (E)-1-(4′-Bis(POM)-phosphinylbut-2′-enyl)-4-phenyl-1,2,3-triazole (**25**)*

The title compound was prepared from **22** with procedure A to give **25** (70%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, J = 7.6 Hz, 2H), 7.80 (s, 1H), 7.41 (t, J = 7.6 Hz, 2H), 7.32 (t, J = 7.6 Hz, 1H) 5.97–5.88 (m, 1H), 5.84–5.75 (m, 1H), 5.71–5.64 (m, 4H), 5.00 (dd, J = 10.4, 5.2 Hz, 2H), 2.75 (dd, J = 22.7, 7.2 Hz, 2H), 1.21 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0, 148.2, 130.7, 129.4, 129.3, 128.9, 128.3, 125.9, 124.8, 124.7, 119.5, 81.8, 81.7, 51.9, 38.9, 31.7, 30.3, 27.0. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.3. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{24}H_{35}N_3O_7P$: 508.22099, found: 508.22071.

*4.2.4. (E)-1-(4′-Bis(POM)-phosphinylbut-2′-enyl)-4-(4-npropylphenyl)-1,2,3-triazole (**26**)*

The title compound was prepared from **22** using procedure A to give **26** (88%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.72 (d, J = 8.1 Hz, 2H), 7.20 (d, J = 8.1 Hz, 2H), 5.97–5.88 (m, 1H), 5.84–5.70 (m, 1H), 5.71–5.64 (m, 4H), 4.97 (t, J = 5.2 Hz, 2H), 2.73 (dd, J = 22.7, 7.2 Hz, 2H), 2.58 (t, J = 7.5 Hz, 2H), 1.63 (m, 2H), 1.19 (s, 18H), 0.92 (t, J = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0, 148.4, 143.0, 129.5, 129.4, 129.0, 128.1, 125.8, 124.7, 124.6, 119.1, 81.8, 81.7, 51.9, 38.9, 38.0, 31.7, 30.3, 26.9, 24.6, 13.9. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.3. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{27}H_{41}N_3O_7P$: 550.26727, found: 550.26766.

*4.2.5. (E)-1-(4′-Bis(POM)-phosphinylbut-2′-enyl)-4-(4-nhexylphenyl)-1,2,3-triazole (**27**)*

The title compound was prepared from **22** using procedure A to give **27** (93%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.72 (d, J = 8.1 Hz, 2H), 7.20 (d, J = 8.1 Hz, 2H), 5.96–5.88 (m, 1H), 5.82–5.75 (m, 1H), 5.64–5.61 (m, 4H), 4.99 (t, J = 5.2 Hz, 2H), 2.74 (dd, J = 22.7, 7.2 Hz, 2H), 2.62 (t, J = 7.2 Hz, 2H), 1.59 (m, 2H), 1.37–1.19 (m, 8H), 1.20 (s, 18H), 0.84 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0, 148.3, 143.2, 129.5, 129.3, 128.9, 128.0, 125.7, 124.6, 124.5, 119.1, 81.7, 81.6, 51.9, 38.8, 35.8, 31.8, 31.6, 31.4, 30.2, 29.0, 26.9, 22.7, 14.2. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.3. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{30}H_{47}N_3O_7P$: 592.31497, found: 592.31461.

*4.2.6. (E)-1-(4′-Bis(POM)- phosphinylbut-2′-enyl)-4-(4-trifluoromethoxyphenyl)-1,2,3-triazole (**28**)*

The title compound was prepared from **22** using procedure A to give **28** (52%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, J = 8.6 Hz, 2H), 7.82 (s, 1H), 7.26 (d, J = 8.6 Hz, 2H), 5.94–5.89 (m, 1H), 5.82–5.77 (m, 1H), 5.71–5.64 (m, 4H), 5.01 (t, J = 5.2 Hz 2H), 2.75 (dd, J = 22.7, 7.2, 2H), 1.18 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0, 171.3, 147.0, 129.5, 129.2, 129.0, 127.3, 125.1, 125.0, 121.4, 119.7, 81.8, 81.7, 51.9, 38.9, 31.7, 30.3, 27.0, 14.3. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.2. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{25}H_{34}F_3N_3O_8P$: 592.20335, found: 592.20301.

*4.2.7. (E)-1-(4′-Bis(POM)- phosphinylbut-2′-enyl)-4-(4-fluorophenyl)-1,2,3-triazole (**29**)*

Prepared from azide **22** using procedure A, compound **29** was isolated in 65% as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (dd, J = 8.6, 5.4 Hz, 2H), 7.77 (s, 1H), 7.09 (t, J = 8.6 Hz, 2H), 5.96–5.88 (m, 1H), 5.84–5.75 (m, 1H), 5.71–5.64 (m, 4H), 4.99 (t, J = 5.2 Hz, 2H), 2.75 (dd, J = 22.7, 7.2 Hz, 2H), 1.20 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0, 164.0, 161.5, 147.4, 129.3, 129.1, 127.6, 127.5, 126.9, 126.8, 124.9, 124.8, 119.2, 116.0, 115.8, 81.7, 81.6, 51.9, 38.8, 31.7, 30.3, 26.9. ^{19}F NMR (376 MHz, $CDCl_3$) δ −113.68 (ddd, J = 10.5, 6.8, 4.3 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.3. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{24}H_{34}FN_3O_7P$: 526.21159, found: 526.21129. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{24}H_{33}FN_3NaO_7P$: 548.19361, found: 548.19324.

*4.2.8. (E)-1-(4′-Bis(POM)- phosphinylbut-2′-enyl)-4-(3,5-dimethoxyphenyl)-1,2,3-triazole (**30**)*

Prepared from azide **22** following procedure A, compound **30** was isolated in 35% as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.01 (d, J = 2.2 Hz, 2H), 6.44 (t, J = 2.2 Hz, 1H), 5.96–5.88 (m, 1H), 5.83–5.76 (m, 1H), 5.71–5.64 (m, 4H), 5.00 (t, J = 5.2 Hz, 2H), 3.84 (s, 6H), 2.75 (dd, J = 22.7, 7.2 Hz, 2H), 1.21 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0, 161.3, 148.2, 132.5, 129.4, 129.3, 124.8, 124.7, 119.8, 103.8, 100.8, 81.8, 81.7, 55.6, 51.9, 38.9, 31.7, 30.3, 29.8, 27.0. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.3. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{26}H_{39}N_3O_7P$: 568.24210, found: 568.24184.

*4.2.9. (E)-1-(4′-Bis(POM)-phosphinylbut-2′-enyl)-4-(hydroxymethyl)-1,2,3-triazole (**31**)*

The title compound was prepared from **22** using procedure B to give **31** (44%) as a colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.54 (s, 1H), 5.87–5.62 (m, 6H), 4.93 (m, 2H), 4.77 (br s, 2H), 2.71 (dd, J = 22.8, 7.0 Hz, 2H), 2.46 (br s, 1H), 1.20 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.3, 148.5, 129.4, 129.2, 124.9, 124.8, 121.6, 81.8, 81.7, 56.9, 51.9, 39.0, 31.8, 30.4, 27.0. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.4. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{19}H_{33}N_3O_8P$: 462.20003, found: 462.19998.

*4.2.10. (E)-1-(4′-Bis(POM)-phosphinylbut-2′-enyl)-4-(thiophenyl)-1,2,3-triazole (**32**)*

The title compound was prepared from **22** with typical procedure B to give **32** (95%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (s, 1H), 7.36 (dd, J = 3.6, 1.1 Hz, 1H), 7.25 (dd, J = 5.0, 1.1 Hz, 1H), 7.03 (dd, J = 5.0, 3.6 Hz, 1H), 5.94–5.69 (m, 2H), 5.71–5.64 (m, 4H), 4.99 (dd, J = 5.2, 4.8 Hz, 2H), 2.72 (dd, J = 22.7, 7.1 Hz, 2H), 1.18 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 143.4, 133.0, 129.3, 129.1, 127.8, 125.2, 125.0, 124.9, 124.4, 119.0, 81.8, 81.8, 52.0, 52.0, 38.9, 31.7, 30.3, 27.0. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.3. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{22}H_{33}N_3O_7PS$: 514.17714, found: 514.17713.

*4.2.11. (E)-1-(4′-Bis(POM)-phosphinylbut-2′-enyl)-4-(octyl)-1,2,3-triazole (**33**)*

The title compound was prepared from **22** with typical procedure C to give **33** (44%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 5.90–5.72 (m, 2H), 5.71–5.59 (m, 4H), 4.99 (m, 2H), 3.08 (t, J = 7.5 Hz, 2H), 2.72 (dd, J = 22.7, 6.8 Hz, 2H), 1.75–1.68 (m, 2H), 1.39–1.10 (m, 28H), 0.85 (t, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 148.4, 125.9, 125.3, 100.2, 81.9, 81.8, 52.2, 39.8, 39.0, 31.9, 31.9, 30.4, 29.5, 29.3, 27.1, 24.2, 22.8, 14.3. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.0. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{26}H_{47}N_3O_7P$: 544.31454, found: 544.31461.

4.2.12. (E)-1-(4′-Bis(POM)-phosphinylbut-2′-enyl)-4-(carboxamide)-1,2,3-triazole (**34**)

The title compound was prepared from **22** with typical procedure C to give **34** (34%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 6.02–5.91 (m, 1H), 5.79–5.72 (m, 1H), 5.69–5.64(m, 4H), 5.08 (t, $J = 5.3$ Hz, 2H), 2.85 (dd, $J = 22.7$, 7.3, 2H), 1.22 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.3, 131.0, 130.9, 127.5, 125.6, 125.5, 83.4, 83.3, 52.9, 39.9, 31.9, 30.5, 27.4. ^{31}P NMR (162 MHz, $CDCl_3$) δ 27.0. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{19}H_{32}N_4O_8P$: 475.19537, found: 475.19522.

4.3. Antiviral activity assays

The antiviral assays, other than the anti-HIV assays, were based on inhibition of virus-induced cytopathicity or plaque formation in HEL [herpes simplex virus type 1 (HSV-1) (KOS), HSV-2 (G), vaccinia virus, vesicular stomatitis virus, cytomegalovirus (HCMV), and varicella-zoster virus (VZV)], Vero (parainfluenza-3, reovirus-1, Sindbis virus and Coxsackie B4), HeLa (vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus) or MDCK [influenza A (H1N1; H3N2) and influenza B] cell cultures. Confluent cell cultures (or nearly confluent for MDCK cells) in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) or with 20 plaque forming units (PFU) (for VZV) in the presence of varying concentrations (100, 20, … μM) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC_{50} or compound concentration required reducing virus-induced cytopathogenicity or viral plaque (VZV) formation by 50%. The minimal cytotoxic concentration (MCC) of the compounds was defined as the compound concentration that caused a microscopically visible alteration of cell morphology. Alternatively, the cytostatic activity of the test compounds was measured based on inhibition of cell growth. HEL cells were seeded at a rate of 5×10^3 cells/well into 96-well microtiter plates and allowed to proliferate for 24 h. Then, medium containing different concentrations of the test compounds was added. After 3 days of incubation at 37 °C, the cell number was determined with a Coulter counter. The cytostatic concentration was calculated as the CC_{50}, or the compound concentration required to reduce cell proliferation by 50% relative to the number of cells in the untreated controls. The methodology of the anti-HIV assays was as follows: human CEM ($\sim 3 \times 10^5$ cells/cm^3) cells were infected with 100 $CCID_{50}$ of HIV(III_B) or HIV-2(ROD)/ml and seeded in 200 μL wells of a microtiter plate containing appropriate dilutions of the test compounds. After 4 days of incubation at 37 °C, HIV-induced CEM giant cell formation was examined microscopically.

Hepatitis C antiviral activity was evaluated as previously described [28]. Huh 7 Clone B cells containing HCV Replicon RNA were seeded in a 96-well plate at 3000 cells/well, and the compounds were tested at 10 μM in triplicate immediately after seeding. Following five days incubation (37 °C, 5% CO_2), total cellular RNA was isolated using the RNeasy96 well extraction kit from Qiagen. Replicon RNA and an internal control (TaqMan rRNA control reagents, Applied Biosystems) were amplified in a single step multiplex Real Time RT-PCR Assay. The antiviral effectiveness of the compounds was calculated by subtracting the threshold RT-PCR cycle of the test compound from the threshold RT-PCR cycle of the no-drug control (ΔCtHCV). A ΔCt of 3.3 equals a 1-log reduction (equal to 90% less starting material) in Replicon RNA levels. The cytotoxicity of the compounds was also calculated by using the ΔCt rRNA values. RS-446 (2′-C-Me-C) was used as the control and tested at 10 μM. To determine EC50 and EC90 values [31], ΔCt values were first converted into fraction of starting material and then were used to calculate the % inhibition.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.06.053.

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