Synthesis and Antiviral Evaluation of (1,4-Disubstituted-1,2,3-Triazol)-(E)-2-Methyl-but-2-Enyl Nucleoside Phosphonate Prodrugs

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Abstract: A series of hitherto unknown (1,4-disubstituted-1,2,3-triazol)-(E)-2-methyl-but-2-enyl nucleosides phosphonate prodrugs bearing 4-substituted-1,2,3-triazoles were prepared in a straight approach through an olefin acyclic cross metathesis as the key synthetic step. All novel compounds were evaluated for their antiviral activities against HBV, HIV and SARS-CoV-2. Among these molecules, only compound 15j, a hexadecloxypropyl (HDP)/((isopropyloxycarbonyl-oxymethyl)-ester (POC) prodrug, showed activity against HBV in Huh7 cell cultures with 62% inhibition at 10 µM, without significant cytotoxicity (IC₅₀ = 66.4 µM in HepG2 cells, IC₅₀ = 43.1 µM in HepG2 cells) at 10 µM.

Keywords: nucleosides; olefin cross metathesis; ultrasound; copper-catalyzed azide-alkyne cycloaddition (CuAAC); antiviral properties; HBV; HIV; SARS-CoV-2

1. Introduction

Acyclic nucleoside phosphonates (ANPs), such as (R)-PMPA [9-9(R)-2-(phosphonomethoxy)propyl]adenine, 1 and PMEA [9-2-(phosphonomethoxy)ethyl] adenine, 2 discovered by A. Holy and E. De Clercq in 1986, led to a new family of nucleotide analogs which has attracted considerable attention, [1–4]. In order to improve the oral absorption of these phosphate analogs, ANPs are delivered as prodrugs [bis(POC)-PMPA (3) or bis(POM)-PMEA (4)]; prodrug moiety [5] has previously focused on acyloxyalkyl-ester (pivaloyloxymethyl, POM) [6,7], or (isopropyloxycarbonyl-oxymethyl)-ester, POC [8], alkoxyalkyl groups (hexadecloxypropyl, HDP) [9], and more recently on phosphonooamides (ProTides) [10,11]. Currently, several ANP prodrugs (alone or in combination) are FDA-approved drugs against DNA and RNA viruses. In our search for antiviral compounds, we have discovered a new class of acyclic nucleoside phosphonates based on a 4-phosphono-but-2-en-1-yl skeleton, with the double bond having trans stereochemistry [12–14]. We have shown that this modification allows for the mimicry of the three-dimensional geometry provided by the backbone of PMEA, PMPA, and CDV ((S)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine) while maintaining an electronic contribution similar to that brought by the oxygen atom [12]. Our group has published a series of bis(POM)-(1,4-disubstituted-1,2,3-triazol)-(E)-but-2′-enyl nucleoside phosphonates [15]. Among them, the
compound 5 exhibits significant potency against human hepatitis C virus (HCV) infections at 10 μM (95% of inhibition) meanwhile our synthesized bis(POC)-(E)-2-methyl-but-2-enylguanine 6 (unpublished data) showed antiviral activity against hepatitis B virus (HBV) with an EC50 of 8.5 μM without significant cytotoxicity (Figure 1).

Figure 1. Structure of selected acyclic nucleoside phosphonates (ANPs) and target derivatives. PMPA: 2-(phosphonomethoxy)propyladenine; PMEA: 9-(2-(phosphonomethoxy)ethyl) adenine; POM: pivaloyloxymethyl; POC: (isopropyloxycarbonyl)-oxymethyl)-ester.

For nucleosides, it appears that chemical alterations (such as a methyl group at 2′ position) at the sugar (or acyclic side-chain) moiety or at the heterocycle such as 1,2,3-triazoles [16] often lead to marked differences in antiviral activity. Based on these findings and on our data for compounds 5 and 6, we were interested in the synthesis of hitherto unknown (1,4-disubstituted-1,2,3-triazol)-(E)-2-methyl-but-2-enyl nucleosides phosphonate prodrugs and we wish to compare the impact of bis(POC) with the mixed HDP/POC biolabile moiety on the antiviral activity. All compounds were evaluated against hepatitis B virus (HBV), human immunodeficiency virus (HIV-1) and the newly detected severe acute respiratory syndrome (SARS-CoV-2).

2. Results and Discussions

2.1. Chemistry

First, we synthetized the bis(POC)allylphosphonate 7 from dimethylallylphosphonate according our previously reported method [14]. Then, optimized olefin cross-metathesis reaction between 7 and 2-methylprop-2-en-1-ol (8) was performed in dry dichloromethane with a Hoveyda–Grubbs catalyst (15 mol%) under ultrasonic irradiation at 55 °C during 24 h [17], (Scheme 1). The desired compound 9 was obtained in an excellent 94% yield with only E-isomer; no trace of Z-isomer was detected (NOESY NMR, see Supplementary Materials). Compound 9 was converted to the corresponding mesylate and the obtained sulfone was used directly in the next step without further purification. The introduction of the azido group on the mesylated compound was realized with sodium azide in DMF at room temperature for 5 h to afford compound 10 in excellent 93% yield.

Scheme 1. Reagents and conditions: (a) Hoveyda–Grubbs catalyst (15 mol%), dry CH2Cl2, 55 °C, 24 h, 94%; (b) (i) MsCl, Et3N, dry CH2Cl2, 0 °C to room temperature (rt), 40 min; (ii) NaN3, DMF, rt, 5 h, 93%.
Compound 10 was engaged in a regioselective copper-catalyzed azide-alkyne 1,3-dipolar cycloaddition (CuAAC) [18,19] with seventeen various terminal alkynes (dipolarophiles) selected to bear bulky, polar and apolar groups. The desired 1,4-disubstituted-1,2,3-triazoles 11a–q were isolated in good yields ranging from 57% to 91% through adequate methods A or B (Table 1). Only compounds 11g and 11i were isolated in poor yields. For 11m, the 2-nitrophenylboronic acid (method B) was added to the solution in order to avoid the decarboxylation of the desired compound [20].

Table 1. Copper-catalyzed azide alkyne cycloaddition to triazolo compounds 11a–q.

| Entry | R | Product | Yield (%) |
|-------|---|---------|-----------|
| 1 a   |   | 11a     | 83        |
| 2 a   |   | 11b     | 88        |
| 3 a   |   | 11c     | 85        |
| 4 a   |   | 11d     | 84        |
| 5 a   |   | 11e     | 91        |
| 6 a   |   | 11f     | 79        |
| 7 a   |   | 11g     | 48        |
| 8 a   | CH$_2$OH | 11h | 71        |
| 9 a   | C(O)H  | 11i | 21        |
| 10 a  | C(O)NH$_2$ | 11j | 78        |
| 11 a  | NH$_2$ | 11k | 94        |
| 12 a  | NO$_2$ | 11l | 69        |
| 13 b  | COOH  | 11m | 57        |
| 14 a  | OMe   | 11n | 63        |
| 15 a  | OEt   | 11o | 64        |
| 16 a  | NHMe  | 11p | 82        |
| 17 a  | NHEt  | 11q | 91        |

*Method A: CuSO$_4$.5H$_2$O, sodium ascorbate, 1BuOH/H$_2$O (2:1), 40 °C, time (followed by thin layer chromatography (TLC)); b Method B: 2-nitrophenylboronic acid, CH$_2$Cl$_2$, rt, 42 h.*
From the antiviral evaluation of 11a–q (see hereafter), it appears that compounds 11a, 11c, 11e, 11j, and 11l have a >40% inhibition of HBV, at 10 μM, with low cytotoxicity. Thus, we focused our attention only to the synthesis of their HDP/POC analogs 15a, 15c, 15e, 15j, 15l (Scheme 2). Starting from HDP/POC allylphosphonate 12, obtained from dimethylallylphosphonate according to our previous method [14], a cross metathesis reaction of 12 and 2-methyl-2-propen-1-ol (8) in dry dichloromethane using the Hoveyda–Grubbs catalyst (15 mol%) under ultrasonic irradiation at 55 °C during 24 h afforded the desired compound 13 in 88% yield as only E-isomer.

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\text{Scheme 2. Reagents and conditions: (a) Hoveyda–Grubbs catalyst (15 mol%), dry CH}_2\text{Cl}_2, 55 \degree \text{C, 24 h, 88%; (b) (i) MsCl, Et}_3\text{N, dry CH}_2\text{Cl}_2, 0 \degree \text{C to rt, 40 min; (ii) NaN}_3, \text{DMF, rt, 5 h, 84%; (c) CuSO}_4.5\text{H}_2\text{O, sodium ascorbate, tBuOH/H}_2\text{O (2:1)}, 40 \degree \text{C, 2~18 h, 68~80%).}
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Compound 13 was then activated by mesylation from 0 °C to room temperature (rt) in dry dichloromethane in the presence of methanesulfonyl chloride during 40 min and the corresponding sulfone was directly engaged in the next step without further purification. Mesylated intermediate was then solubilized in DMF, in the presence of sodium azide at room temperature for 5 h to afford compound 14 in 84% yield. The structure of 14 was confirmed by NOESY NMR experiment (Figure 2).

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\text{Figure 2. NOESY NMR spectra of 14.}
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Finally, the CuAAC reaction of 14 with various substituted phenylacetylenes and non-aromatic terminal alkynes afforded a series of HDP/POC-(1,4-disubstituted-1,2,3-triazol)-2′-methyl-but-2′-eny phosphonate 15a, 15c, 15e, 15j, 15l, respectively, in good yields ranging from 68% to 80%.
2.2. Antiviral Evaluation

All synthesized compounds, the (1,4-disubstituted-1,2,3-triazol)-(E)-2-methyl-but-2-enyl nucleosides phosphonate prodrugs \(11a-q, 15a, 15c, 15e, 15j, 15l\), were tested for their antiviral activities in vitro against HIV, HBV and SARS-CoV-2 viruses; the results are summarized in Table 2 and represent means from triplicate wells.

Table 2. Antiviral activity and cytotoxicity of synthesized compounds in cellular assays at 10 µM.

| Cmpd | Huh7 % Inhibition | HepAD38 % Inhibition | Huh7 % Cell Viability | Vero E6 % Inhibition | PBM % Inhibition | PBM Cytotoxicity (IC50, µM) | HepG2 Cytotoxicity (IC50, µM) |
|------|----------------------|----------------------|-----------------------|----------------------|------------------|-----------------------------|-----------------------------|
| 11a  | 51                   | 19.6                 | 96                    | <1                   | <1               | 50.2                        | 18.3                        |
| 11b  | 25                   | 14.4                 | 88                    | <1                   | <1               | 19.4                        | 16.2                        |
| 11c  | 42                   | <1                   | 89                    | <1                   | 44.4             | 47.8                        | 51.8                        |
| 11d  | 39                   | 38.8                 | 100                   | <1                   | 41.8             | 23.7                        | 59.6                        |
| 11e  | 50                   | 33.5                 | 95                    | <1                   | 29.9             | 9.2                         | 90.8                        |
| 11f  | <1                   | 36.3                 | 100                   | <1                   | 30.7             | 25.5                        | >100                        |
| 11g  | 10                   | 17.3                 | 79                    | <1                   | 12.3             | 28.3                        | >100                        |
| 11h  | 28                   | 15.1                 | 100                   | 12                   | <1               | 76.4                        | >100                        |
| 11i  | 33                   | 6.1                  | 95                    | <1                   | 28.4             | 75.1                        | >100                        |
| 11j  | 40                   | 31.3                 | 100                   | <1                   | <1               | 76.8                        | >100                        |
| 11k  | <1                   | 6.1                  | 100                   | 5                    | 42               | 51.5                        | 57.3                        |
| 11l  | 40                   | 42.6                 | 100                   | 10                   | 38.8             | 23.5                        | 85.3                        |
| 11m  | 12                   | 41.3                 | 57                    | <1                   | 12.9             | >100                        | >100                        |
| 11n  | 28                   | 3.9                  | 60                    | <1                   | <1               | 40.9                        | >100                        |
| 11o  | <1                   | 41.2                 | 60                    | <1                   | 22.4             | 67.5                        | >100                        |
| 11p  | 14                   | <1                   | 54                    | <1                   | 36.4             | 78.3                        | >100                        |
| 11q  | <1                   | <1                   | 90                    | <1                   | 31.2             | 80.6                        | >100                        |
| 15a  | <1                   | ND                   | 95                    | <1                   | ND               | ND                         | ND                          |
| 15c  | 14                   | 28.8                 | 47                    | <1                   | 28.2             | 50.5                        | >100                        |
| 15e  | 8                    | ND                   | 100                   | <1                   | ND               | ND                         | ND                          |
| 15j  | 62                   | <1                   | 74                    | <1                   | 32.2             | 43.1                        | 66.4                        |
| 15l  | 12                   | ND                   | 100                   | <1                   | ND               | ND                         | ND                          |
| Entecavir | 92 - | 96 - - | - | - | - | - | - |
| Remdesivir | - | - | 100 | - | - | - | - |

ND, not determined; PBM, peripheral blood mononuclear cell; Cmpd, compound.

None of the compounds tested displayed anti-HIV activity compared to positive control AZT (EC50 of 0.008 µM (data not shown)). None of the compounds displayed anti-SARS-CoV-2 activity compared to positive control remdesivir. Compounds \(11a, c-e, 11j\) and \(11l\) were found to exhibit a moderate anti-HBV activity compared to entecavir (on Huh7 cells) and lamivudine (on HepAD38).

It is worth noting that the substituent on triazole ring has a dramatic effect on HBV activity. In fact, compounds with hydrophobic aryl groups (electron-donating \(11a-e\) and electron-withdrawing \(11l\)) exhibited good activity (except for \(11b\), whereas triazolyl-ANPs with alkyl \((11f, g)\) or polar substituents such as alcohol \((11h)\), aldehyde \((11i)\), formamide \((11k)\) and amide \((11l)\) and carboxylic derivatives \((11m-q)\) have only low activity. We can speculate that the hydrophobic pocket (formed by A87, F88, P177, L180, and M2) found in the rear of the RT dNTP binding site of HBV could interact with hydrophobic aryl residues through donor atom-π interaction [21].
Compound 15j, a HDP/POC prodrug, a ribavirin analog, is the most active drug against HBV in this serial, with 62% inhibition at 10 µM. However, generally speaking, the bis(POC) ester prodrugs are more active than their HDP/POC counterparts. The cytotoxicity of test compounds was performed in different cell systems. Positive control cycloheximide exhibited expected toxicity in the peripheral blood mononuclear (PBM) and HepG2 cells, with an IC$_{50}$ (µM) of 1.0 and 1.5 µM, respectively (data not shown).

3. Materials and Methods

3.1. Chemistry General Section

Commercially available chemicals were provided as reagent grade and used as received. Some reactions requiring anhydrous conditions were carried out using oven-dried glassware and under an atmosphere of dry argon. All anhydrous solvents were provided from commercial sources as very dry reagents. 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 sulfuric acid (H$_2$SO$_4$ 5% in ethanol) stain followed by charring at average 150 °C. Flash column chromatography was performed on silica gel 60 M (0.040–0.063 mm, E. Merck). The infrared spectra were measured with the Perkin–Elmer Spectrometer. The $^1$H and $^{13}$C NMR spectra were recorded on the BrukerAvance DPX 250 or BrukerAvance 400 Spectrometer (Bruker, Champs sur Marne, France). 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 distortionless enhancement by polarization transfer (DEPT) experiments. $^1$H and $^{13}$C signals were attributed on the basis of H–H and H–C correlations. High resolution mass spectra were performed on a Bruker Q-TOF MaXis spectrometer (Bruker Daltonics, Bremen, Germany) by the “Fédération de Recherche” ICOA/CBM (FR2708) platform. LC-MS data were acquired on a Thermo-Fisher UHPLC-MSQ system equipped with an electron spray ionization source (ESI). The temperature of the source was maintained at 350 °C. Initially, the cone voltage was set at 35 V and after 5 min was increased to 75 V. In full scan mode, data were acquired between 100 and 1000 m/z in the positive mode with a 1.00 s scan time. In addition, a UV detection was performed with a diode array detector at three wavelengths 273, 254 and 290 nm, respectively. A water/methanol (70%/30%) solution mixture with 0.1% formic acid was used as the mobile phase. The composition of the mobile phase was increased to 100% methanol with 0.1% formic acid with a 7% ramp. The flow rate was set at 0.300 mL min$^{-1}$. Samples diluted in the mobile phase were injected (3 µL) on a C18 column (X-terra, Waters), with a 2.1 mm internal diameter, and 100 mm length, and placed into an oven at 40 °C. The electronic extraction of ions was performed and the subsequent areas under the corresponding chromatographic peaks determined. The compounds’ name follows the IUPAC recommendations.

3.2. Antiviral Evaluation

3.2.1. HBV Assay

In Huh7 cells: antiviral activity against HBV in cell culture was measured as previously described [22]. Briefly, Huh7 cells were transfected with plasmid pCI_HBVpg1820 containing 1.1 unit of HBV genome under the control of a human cytomegalovirus (CMV) promoter. The drugs were stored at 1000× in DMSO at −20 °C. For each experiment, the drugs were diluted in DMEM cell culture medium (Thermo Fisher Scientific, Artenay, France) to a final concentration of 10 µM. All drugs were tested in triplicate in two independent experiments. After 4 days of culture, intracapsid viral DNA was quantified by duplex real-time PCR, using primers described elsewhere, on a LightCycler 480 II apparatus (Roche Diagnostics France, Meylan, France) using TaqMan Universal PCR Mastermix II without UNG (Thermo Fisher Scientific, Artenay, France). Cells were quantified using primers by real-time PCR, using primers HPRT1_F (5’-TGCAGACTTTGCTTTCCCTGGTC) and HPRT1_R (5’-CAAGCTTGCACCTTGGACCAC), on a LightCycler 480 II apparatus.
Using a LightCycler® 480 SYBR Green I Master (Roche Diagnostics France, Meylan, France). Cycling reactions were performed with the following parameters: 5 min at 95 °C, followed by 45 cycles of 10 s at 95 °C, 15 s at 60 °C, 10 s at 72 °C. The inhibition of HBV replication (%) and cell culture viability (%) were quantified using the following formulas.

\[
\text{Inhibition of HBV replication (\%) = } 1 - \frac{\text{HBV DNA copy number}_{\text{Drug}}}{\text{HBV DNA copy number}_{\text{Cell culture medium}}}
\]

\[
\text{Cell viability (\%) = } \frac{\text{HPRT1 DNA copy number}_{\text{Drug}}}{\text{HPRT1 DNA copy number}_{\text{Cell culture medium}}}
\]

In HepAD38 cells were seeded at 50,000 cells/well in collagen-coated 96-well plates. Test compounds or 2′,3′-dideoxy-3′-thiacytidin (3TC) (control) were added to HepAD38 cells to a final concentration of 10 µM.

Real-Time PCR for HBV DNA. The experiment lasted 7 days. On day 7, total DNA was purified from the supernatant using a commercially available kit (DNeasy 96 Blood & Tissue kit, Qiagen). The HBV DNA was amplified in a real-time PCR assay using a LightCycler 480 (Roche) as described elsewhere. All samples were tested in duplicate in two to three independent experiments. Analysis: The concentration of compound that inhibited HBV DNA replication by 50% (EC_{50}) was determined by linear regression.

3.2.2. HIV Assay

The assay was performed as described by Schinazi et al. with minor modifications. Briefly, human PBMC cells were stimulated with PHA/IL-2 prior to infection with HIV-1 LAI (MOI 0.1) for 5 days in the presence of test compounds with a final concentration of 10 µM (LA-338-366) or AZT (control). The supernatants were harvested, and the HIV-1 RT was quantified. The median effective concentrations (EC_{50/90}) were determined using the method of Belen’kii and Schinazi.

3.2.3. SARS-CoV-2 Assay

Vero E6 (ATCC CRL-1586) cells were grown in a minimal essential medium (MEM) (Life Technologies, Carlsbad, CA, USA) with 7.5% heat-inactivated fetal calf serum (FCS), at 37 °C with 5% CO₂ with 1% penicillin/streptomycin (PS, 5000 U.mL⁻¹ and 5000 µg.mL⁻¹, respectively; Life Technologies) and supplemented with 1% non-essential amino acids (Life Technologies). SARS-CoV-2 strain BavPat1 was obtained from Pr Drosten through EVA GLOBAL (https://www.european-virus-archive.com, accessed on 23 February 2021). To prepare the virus working stock, a 25 cm² culture flask of confluent Vero E6 cells growing with MEM medium with 2.5% FBS (Life Technologies) was inoculated at MOI 0.001. The cell supernatant medium was harvested at the peak of infection and supplemented with 25 mM HEPES (Sigma, St. Louis, MO, USA) before being stored frozen in small aliquots at -80 °C. All experiments were conducted in a BSL3 laboratory. One day prior to infection, for the antiviral screening 5 × 10^4 Vero E6 cells were seeded in 100 µL of the assay medium (containing 2.5% FCS) in 96 well plates. The next day, four 2-fold serial dilutions of compounds (20 µM to 2.5 µM, in duplicate) were added to the cells (25 µL/well, in the assay medium) as well as two internal well controls of viral inhibition corresponding to the addition of 10 µM of Remdesivir (BLDpharm, Hyderabad, India). Three virus control wells were supplemented with 25 µL of assay medium (positive controls hereafter named vc) and three cell control wells were supplemented with 50 µL of the medium (negative controls, hereafter named nc). After 15 min, 25 µL of a virus mix diluted in 2.5% FCS-containing medium was added to the wells at MOI 0.002. Three days after infection, the cell supernatant media was discarded and CellTiter-Blue® reagent (Promega, Madison, WI, USA) was added following the manufacturer’s instructions. Plates were incubated for 2 h prior to recording the fluorescence (560/590 nm) with a Tecan Infinite 200Pro machine. From the measured OD_{590nm}, the inhibition percentage was calculated as follows: ((OD_{590nm} value- mean OD_{590nm} value of vc)/(mean OD_{590nm} of nc- mean...
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OD$_{590}$nm value of vc)*100. All data obtained were analyzed using GraphPad Prism 7 software (Graph pad software).

3.2.4. Cytotoxicity Assays

Assays were performed in human peripheral blood mononuclear (PBMC) and human liver (HepG2) cells via MTS assay using the CellTiter 96® Non-Radioactive Cell Proliferation (Promega) kit as previously described$^3$. Cytotoxicity was expressed as the concentration of test compounds that inhibited cell proliferation by 50% (IC$_{50}$) and calculated using the Chou and Talalay method$^{[28]}$.

3.3. Experimental Section

(E)-4-Hydroxy-3-methyl-but-2-enyl-bis(POC)phosphonate (9)

To a solution of $E$-bis(POC)allylphosphonate (7) (500 mg, 2.0 equiv., 1.41 mmol) and 2-methyl-2-propen-1-ol (8) (59 $\mu$L, 1.0 eq., 0.71 mmol) in dry CH$_2$Cl$_2$ (5 mL), Hoveyda–Grubbs catalyst (66 mg, 15 mol%, 0.11 mmol) was added. The catalyst addition was performed in five equal portions of 3 mol% (13.20 mg, 0.021 mmol) at t = 0, 3, 6, 9 and 21 h over the course of the reaction. The solution was sonicated at 55 °C under nitrogen atmosphere for 24 h. Volatiles were evaporated and the residue was purified by silica gel column chromatography (EtOAc/PE, 7:3) to give the desired phosphonate derivative 9 (264 mg, 94%) as brown oil.

$^{1}$H NMR (400 MHz, CDCl$_3$) δ 5.65–5.57 (m, 4H, O-CH$_2$-O), 5.41–5.37 (m, 1H, CH=C), 4.87 (sept., J = 6.2 Hz, 2H, CH(CH$_3$)$_2$), 3.98 (d, J = 5.3 Hz, 2H, CH$_2$-OH), 2.69 (dd, J = 22.7, 7.6 Hz, 2H, CH$_2$-P), 2.34 (s, 1H, OH), 1.65 (d, J = 4.5 Hz, 3H, CH$_3$), 1.28 (d, J = 6.3 Hz, 12H, CH(CH$_3$)$_2$).

$^{13}$C NMR (101 MHz, CDCl$_3$) δ 153.2 (C=O), 141.6, 141.5 (CH=C), 111.6, 111.5 (CH=C), 84.1, 84.1 (O-CH$_2$-O), 73.3 (CH(CH$_3$)$_2$), 68.0, 68.0 (CH$_2$-OH), 27.3, 25.9 (CH$_2$-P), 21.6 (CH(CH$_3$)$_2$), 13.9, 13.9 (CH$_3$).

HRMS (ESI): m/z [M+H]$^+$ calcd for C$_{31}$H$_{28}$O$_{10}$P: 399.1416, found: 399.1415.

(E)-4-Azido-3-methyl-but-2-enyl-bis(POC)phosphonate (10)

To a solution of (E)-4-hydroxy-3-methyl-but-2-enyl-bis(POC)phosphonate 9 (264 mg, 1.0 equiv., 0.66 mmol) in anhydrous CH$_2$Cl$_2$ (5 mL) were added dropwise methanesulfonyl chloride (56 $\mu$L, 1.1 equiv., 0.73 mmol) and anhydrous triethylamine (102 $\mu$L, 1.2 equiv., 0.73 mmol) at 0 °C under the argon. After 30 min stirring at room temperature, the mixture was diluted with CH$_2$Cl$_2$ and washed with brine solution (3 × 10 mL). The organic layers were dried over MgSO$_4$, filtrated and concentrated in vacuo. The residue was directly used for the next step without further purification. Thus, it was dissolved in anhydrous DMF (8 mL) and sodium azide (216 mg, 5.0 equiv., 3.32 mmol) was added under nitrogen atmosphere. After 5 h stirring at room temperature, the mixture was diluted with EtOAc, washed with water and then extracted with EtOAc. The combined organic layers were washed with brine solution (5 × 10 mL), dried over MgSO$_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/PE, 4:6) to give 10 (261 mg, 93%) as a colorless oil.

$^{1}$H NMR (400 MHz, CDCl$_3$) δ 5.71–5.61 (m, 4H, O-CH$_2$-O), 5.48–5.42 (m, 1H, CH=C), 4.92 (sept., J = 6.2 Hz, 2H, CH(CH$_3$)$_2$), 3.71 (d, J = 4.0 Hz, 2H, CH$_2$-N$_3$), 2.75 (dd, J = 23.1, 7.9, Hz, 2H, CH$_2$-P), 1.73 (d, J = 4.5 Hz, 3H, CH$_3$), 1.32 (d, J = 6.2 Hz, 12H, CH(CH$_3$)$_2$).

$^{13}$C NMR (101 MHz, CDCl$_3$) δ 153.2 (C=O), 136.2, 136.1 (C=CH), 116.4, 116.3 (CH=C), 84.2, 84.1 (O-CH$_2$-O), 73.3 (CH(CH$_3$)$_2$), 58.6, 58.6 (CH$_2$-N$_3$), 27.6 (CH$_2$-P), 26.2 (CH$_2$-P), 21.6 (CH(CH$_3$)$_2$), 14.9, 14.9 (CH$_3$).

$^{31}$P NMR (162 MHz, CDCl$_3$) δ 27.85. IR ν$_{max}$ 2985.61, 2937.37, 2100.12, 1755.01, 1255.06, 1031.52, 984.34, 949.97, 904.26, 832.67, 788.47 cm$^{-1}$. HRMS (ESI): m/z [M+Na]$^+$ calcd for C$_{35}$H$_{31}$N$_3$O$_{10}$P: 446.12988, found: 446.12990.

General procedures A for CuAAC reaction

To a solution of (E)-4-Azido-3-methyl-but-2-enyl-bis(POC)phosphonate 10 (1.0 equiv.) and alkene (1.3 equiv.) in tBuOH/H$_2$O (2:1) were added sodium ascorbate (0.6 equiv.) and CuSO$_4$5H$_2$O (0.1 equiv.). The resulting suspension was stirred at 40 °C until completion (followed by TLC), then the crude mixture was co-evaporated five times with methanol.
(10 mL). The residue was purified by silica gel column chromatography using elution gradient of petroleum ether/ethyl acetate to give the desired bis(POC) phosphonate derivatives.

\[[E]-4-[4-(4-Propylphenyl)triazol-1-yl]-3-methyl-but-2-enyl\]-isopropoxycarboxyloxymethoxy)phosphoryloxyethyl isopropyl carbonate (11a)

The title compound was prepared from 10 (101 mg, 0.24 mmol) and 1-ethyl-4-propylenobenzene (49 μL, 0.31 mmol) following the general procedure A; the resulting suspension was stirred for 2 h at 40 °C. After purification on a silica gel column chromatography (EtOAc/PE, 4:6), the desired pure compound 11a (112 mg, 83%) was obtained as a colorless oil. 1H NMR (250 MHz, CDCl3) δ 7.76 (s, 1H, H^3), 7.74 (d, J = 5.7 Hz, 2H, H^2a), 7.25–7.17 (d, J = 8.5 Hz, 2H, H^2A), 5.77–5.51 (m, 4H, O-CH2-O; 1H, CH=C), 4.98–4.80 (m, 2H, CH2-N; 2H, CH(CH3)2), 2.78 (dd, J = 23.1, 7.8 Hz, 2H, CH2-P), 2.59 (t, J = 7.6 Hz, 2H, Ar-CH2), 1.70–1.58 (m, 3H, 3H, CH; 2H, Ar-CH2-CH2), 1.27 (d, J = 6.3 Hz, 12H, Ar-CH(CH3)2), 0.93 (t, J = 7.3 Hz, 3H, CH3-CH2-CH2-CH3). 13C NMR (101 MHz, CDCl3) δ 153.2 (C=O), 148.4 (C^2A), 143.0 (C^3Ar), 135.9, 135.8 (CH=C), 128.8 (CHAr), 128.0 (C^4Ar), 125.7 (CHAr), 119.0 (CH^3), 118.1, 118.0 (CH=C), 84.2, 84.1 (O-CH2-O), 73.4 (CH(CH3)2), 57.8, 57.8 (CH2-N), 35.4 (Ar-CH2-CH2-CH2-CH3), 33.5 (Ar-CH2-CH2-CH2-CH3), 27.8 (CH2-P), 26.4 (CH2-P), 23.2 (Ar-CH2-CH2-CH2-CH3), 21.6, 21.6 (CH(CH3)2), 14.2, 14.2 (CH3), 13.9 (Ar-CH2-CH2-CH2-CH3). 31P NMR (162 MHz, CDCl3) δ 27.47. HRMS (ESI): m/z [M+H]^+ calcd for C28H42N2O9P: 658.2418, found: 658.2421.

\[[E]-4-[4-(Butylphenyl)triazol-1-yl]-3-methyl-but-2-enyl\]-isopropoxycarboxyloxymethoxy)phosphoryloxyethyl isopropyl carbonate (11b)

The title compound was prepared from 10 (102 mg, 0.24 mmol) and 1-butyl-4-ethylnylbenzene (55 μL, 0.31 mmol) following the general procedure A; the resulting suspension was stirred for 2 h at 40 °C. After purification on a silica gel column chromatography (EtOAc/PE, 4:6), the desired pure compound 11b (123 mg, 88%) was obtained as a colorless oil. 1H NMR (250 MHz, CDCl3) δ 7.76 (d, J = 7.8 Hz, 2H, H^2A), 7.75 (s, 1H, H^3), 7.22 (d, J = 7.8 Hz, 2H, H^2A), 5.73–5.63 (m, 4H, O-CH2-O), 5.60–5.54 (m, 1H, CH=C), 4.94 (d, J = 5.0 Hz, 2H, CH2-N), 4.90 (sept, J = 6.3 Hz, 2H, CH(CH3)2), 2.80 (dd, J = 23.3, 7.9 Hz, 2H, CH2-P), 2.62 (t, J = 7.8 Hz, 2H, Ar-CH2-CH2-CH2-CH3), 1.64–1.58 (m, 3H, CH3; 2H, Ar-CH2-CH2-CH2-CH3), 1.40–1.34 (m, 2H, Ar-CH2-CH2-CH2-CH3), 1.28 (d, J = 6.3 Hz, 12H, Ar-CH(CH3)2), 0.92 (t, J = 7.3 Hz, 3H, CH3-CH2-CH2-CH2-CH3). 13C NMR (101 MHz, CDCl3) δ 153.2 (C=O), 148.4 (C^2A), 143.0 (C^3Ar), 135.9, 135.8 (CH=C), 128.8 (CHAr), 128.0 (C^4Ar), 125.7 (CHAr), 119.0 (CH^3), 118.1, 118.0 (CH=C), 84.2, 84.1 (O-CH2-O), 73.4 (CH(CH3)2), 57.8, 57.8 (CH2-N), 35.4 (Ar-CH2-CH2-CH2-CH3), 33.5 (Ar-CH2-CH2-CH2-CH3), 27.8 (CH2-P), 26.4 (CH2-P), 23.2 (Ar-CH2-CH2-CH2-CH3), 21.6, 21.6 (CH(CH3)2), 14.2, 14.2 (CH3), 13.9 (Ar-CH2-CH2-CH2-CH3). 31P NMR (162 MHz, CDCl3) δ 27.46. HRMS (ESI): m/z [M+Na]^+ calcd for C28H42N2O9PNa: 604.2394, found: 604.2393.

\[[E]-4-[4-(Pentylphenyl)triazol-1-yl]-3-methyl-but-2-enyl\]-isopropoxycarboxyloxymethoxy)phosphoryloxyethyl isopropyl carbonate (11c)

The title compound was prepared from 10 (96 mg, 0.23 mmol) and 1-ethyl-4-pentylbenzene (57 μL, 0.29 mmol) following the general procedure A; the resulting suspension was stirred for 2 h at 40 °C. After purification on a silica gel column chromatography (EtOAc/PE, 4:6), the desired pure compound 11c (115 mg, 85%) was obtained as a colorless oil. 1H NMR (400 MHz, CDCl3) δ 7.76 (s, 2H, H^2A), 7.74 (s, 1H, H^3), 7.22 (d, J = 7.8 Hz, 2H, H^2A), 5.72–5.64 (m, 4H, O-CH2-O), 5.61–5.56 (m, 1H, CH=C), 4.94 (d, J = 5.1 Hz, 2H, CH2-N), 4.90 (sept, J = 6.2 Hz, 2H, CH(CH3)2), 2.80 (dd, J = 23.1, 7.8 Hz, 2H, CH2-P), 2.62 (t, J = 7.8 Hz, 2H, Ar-CH2(CH2)3CH3), 1.64–1.59 (m, 3H, CH3; 2H, Ar-CH2-CH2-CH2-CH2-CH3), 1.33–1.32 (m, 4H, Ar-CH2-CH2-CH2-CH2-CH3), 1.28 (d, J = 6.3 Hz, 12H, CH(CH3)2), 0.89 (t, J = 7.3 Hz, 3H, Ar-CH(CH2)3CH3). 13C NMR (101 MHz, CDCl3) δ 153.2 (C=O), 148.4 (C^2A), 143.0 (C^3Ar), 135.9, 135.7 (CH=C), 128.8 (CHAr), 128.0 (C^4Ar), 125.7 (CHAr), 119.0 (CH^3), 118.1, 118.0 (CH=C), 84.2, 84.1 (O-CH2-O), 73.4 (CH(CH3)2), 57.8, 57.8 (CH2-N), 35.7 (Ar-CH2-(CH2)3CH3), 31.4, 31.1 (Ar-CH2-CH2-CH2-CH2-CH3), 27.8 (CH2-P), 26.4 (CH2-P), 23.9 (Ar-CH2-CH2-CH2-CH2-CH3), 22.5 (Ar-CH2(CH2)3CH3), 21.6, 21.6 (CH(CH3)2), 14.2, 14.2 (CH3), 14.0 (Ar-CH2(CH2)4-CH3). 31P NMR (162 MHz, CDCl3) δ 27.51. HRMS (ESI): m/z [M+Na]^+ calcd for C28H42N2O9PNa: 618.25508, found: 618.25473.
[[(E)-4-(4-(Hexylphenyl)triazol-1-yl)-3-methyl-but-2-enyl]-isopropoxycarboxyl]methoxyphosphoryl]oxymethyl isopropyl carbonate (11d)

The title compound was prepared from 10 (93 mg, 0.22 mmol) and 1-ethyl-4-hexybenzene (60 µL mg, 0.29 mmol) following the general procedure A; the resulting suspension was stirred for 2 h at 40 °C. After purification on a silica gel column chromatography (EtOAc/PE, 35:65), the desired pure compound 11d (112 mg, 84%) was obtained as a yellow oil. 

1H NMR (400 MHz, CDCl₃) δ 7.75 (d, J = 5.4 Hz, 2H, H Ar), 7.72 (s, 1H, H Ar), 7.20 (d, J = 7.8 Hz, 2H, H Ar), 5.70–5.62 (m, 4H, O-CH₂-O), 5.59–5.53 (m, 1H, CH=C), 4.92 (d, J = 3.8 Hz, 2H, CH₂-CH=), 4.88 (sept., J = 6.2 Hz, 2H, CH(CH₃)₂), 2.78 (dd, J = 23.1, 7.8 Hz, 2H, CH₂-P), 2.60 (t, J = 7.8 Hz, 2H, Ar-CH₂-(CH₂)=CH₃), 1.62–1.56 (m, 3H, CH₃; 2H, Ar-CH₂-CH₂-(CH₂)₂CH₃), 1.33–1.25 (m, 18H, Ar-CH₂-CH₂-(CH₂)₂CH₃, CH(CH₃)₂), 0.86 (t, J = 7.0 Hz, 3H, Ar-(CH₂)₃-CH₃). 13C NMR (101 MHz, CDCl₃) δ 153.2 (C=O), 148.3 (C₆Ar), 143.0 (C₆Ar), 135.9, 135.8 (CH=C), 128.8 (CH₆Ar), 128.0 (C₆Ar), 125.7 (CH₆Ar), 119.1 (CH₆Ar), 118.1, 117.9 (CH=C), 84.2, 84.1 (O-CH₂-O), 73.4 (CH(CH₃)₂), 57.8, 57.7 (CH₂-N), 35.7 (Ar-CH₂-(CH₂)₄-CH₃), 31.7, 31.3 (Ar-CH₂-CH₂-(CH₂)₂CH₃), 28.9 (Ar-CH₂-CH₂-CH₂-CH₂-CH₃), 27.8 (CH₂-P), 26.4 (CH₂-P), 23.9 (Ar-CH₂-CH₂-CH₂-CH₂-CH₂-CH₃), 22.6 (Ar-CH₂-(CH₂)₂-CH₂), 21.6, 21.6 (CH(CH₃)₂), 14.2, 14.21, 14.1 (Ar-(CH₂)₃-CH₃), 31P NMR (162 MHz, CDCl₃) δ 27.50. HRMS (ESI): m/z [M+Na⁺] calcld for C₂₅H₄₄N₅O₇P: 632.27073, found: 632.27074.

[[(E)-4-(4-Heptylphenyl)triazol-1-yl)-3-methyl-but-2-enyl]-isopropoxycarboxyl]methoxyphosphoryl]oxymethyl isopropyl carbonate (11e)

The title compound was prepared from 10 (113 mg, 0.27 mmol) and 1-ethyl-4-hexybenzene (78 µL, 0.35 mmol) following the general procedure A; the resulting suspension was stirred for 2 h at 40 °C. After purification on a silica gel column chromatography (EtOAc/PE, 3:7), the desired pure compound 11e (151 mg, 91%) was obtained as a yellow oil. 

1H NMR (400 MHz, CDCl₃) δ 7.76 (d, J = 2.4 Hz, 2H, H Ar), 7.74 (s, 1H, H Ar), 7.22 (d, J = 7.8 Hz, 2H, H Ar), 5.72–5.64 (m, 4H, O-CH₂-O), 5.62–5.56 (m, 1H, CH=C), 4.94 (d, J = 3.8 Hz, 2H, CH₂-N), 4.89 (sept., J = 6.3 Hz, 2H, CH(CH₃)₂), 2.79 (dd, J = 23.1, 7.8 Hz, 2H, CH₂-P), 2.61 (t, J = 7.8 Hz, 2H, Ar-CH₂-(CH₂)₂CH₃), 1.64–1.58 (m, 3H, CH₃; 2H, Ar-CH₂-CH₂-(CH₂)₂CH₃), 1.33–1.25 (m, 20H, Ar-CH₂-CH₂-CH₂-CH₂-CH₃, CH(CH₃)₂), 0.87 (t, J = 7.1 Hz, 3H, Ar-(CH₂)₃-CH₃). 13C NMR (101 MHz, CDCl₃) δ 153.2 (C=O), 148.4 (C₆Ar), 143.0 (C₆Ar), 135.9, 135.8 (CH=C), 128.8 (CH₆Ar), 128.0 (C₆Ar), 125.6 (CH₆Ar), 119.0 (CH₆Ar), 118.2, 118.1 (CH=C), 84.2, 84.1 (O-CH₂-O), 73.4 (CH(CH₃)₂), 57.9, 57.8 (CH₂-N), 35.7 (Ar-CH₂-(CH₂)₄-CH₃), 31.8, 31.4 (Ar-CH₂-CH₂-(CH₂)₂CH₃), 29.7, 29.2, 29.1 (Ar-CH₂-CH₂-CH₂-CH₂-CH₂-CH₃), 27.8 (CH₂-P), 26.4 (CH₂-P), 22.6 (Ar-(CH₂)₃-CH₃), 21.6, 21.5 (CH(CH₃)₂), 14.2, 14.1 (CH₃), 14.1 (Ar-(CH₂)₆-CH₃). 31P NMR (162 MHz, CDCl₃) δ 27.46. HRMS (ESI): m/z [M+H⁺] calcld for C₃₀H₄₃N₇O₇P: 624.30444, found: 624.30373.

[[(E)-4-(4-Cyclopropyltriazol-1-yl)-3-methyl-but-2-enyl]-isopropoxycarboxyl]methoxyphosphoryl]oxymethyl isopropyl carbonate (11f)

The title compound was prepared from 10 (94 mg, 0.22 mmol) and cyclopropylacetylene (24 µL, 0.29 mmol) following the general procedure A; the resulting suspension was stirred for 2 h at 40 °C. After purification on a silica gel column chromatography (EtOAc/PE, 4:6), the desired pure compound 11f (86 mg, 79%) was obtained as a colorless oil. 

1H NMR (250 MHz, CDCl₃) δ 7.23 (s, 1H, H Ar), 5.72–5.62 (m, 4H, O-CH₂-O), 5.54–5.45 (m, 1H, CH=C), 4.92 (sept., J = 6.2 Hz, 2H, CH(CH₃)₂), 4.83 (d, J = 4.1 Hz, 2H, CH₂-N), 2.76 (dd, J = 23.1, 7.8 Hz, 2H, CH₂-P), 2.00–1.89 (m, 1H, CH₂CH₂CH₃), 1.59 (d, J = 5.5 Hz, 3H, CH₃), 1.31 (dd, J = 6.3, 1.3 Hz, 12H, CH(CH₃)₂), 0.95–0.82 (m, 4H, CH₂CH₂). 13C NMR (101 MHz, CDCl₃) δ 153.2 (C=O), 150.7 (C₆Ar), 136.01, 135.9 (CH=C), 119.4 (CH=H), 117.8, 117.7 (CH=C), 84.1, 84.1 (O-CH₂-O), 73.4 (CH(CH₃)₂), 57.6, 57.6 (CH₂-N), 27.7, 26.4 (CH₂-P), 21.7, 21.6 (CH(CH₃)₂), 14.2, 14.1 (CH₃), 7.8 (CH₂CH₂), 6.7 (CH₂CH₂CH₃). 31P NMR (162 MHz, CDCl₃) δ 27.6. HRMS (ESI): m/z [M+H⁺] calcld for C₂₀H₃₃N₇O₇P: 490.19489, found: 490.19492.

[[(E)-4-(4-tert-Butyltriazol-1-yl)-3-methyl-but-2-enyl]-isopropoxycarboxyl]methoxyphosphoryl]oxymethyl isopropyl carbonate (11g)
The title compound was prepared from 10 (98 mg, 0.23 mmol) and 3,3-dimethyl-1-butyne (37 µL, 0.30 mmol) following the general procedure A; the resulting suspension was stirred for 24 h at 40 °C. After purification on a silica gel column chromatography (EtOAc/PE, 6:4), the desired pure compound 11g (56 mg, 48%) was obtained as a colorless oil. 31H NMR (400 MHz, CDCl3) δ 7.25 (s, 1H, H5), 5.71–5.62 (m, 4H, O-CH2-O), 5.51–5.45 (m, 1H, CH=C), 4.92 (sept, j = 6.2 Hz, 2H, CH(CH3)2), 4.85 (d, j = 3.9 Hz, 2H, CH2-N), 2.76 (dd, j = 23.1, 7.8 Hz, 2H, CH2-P), 1.60 (d, j = 4.5 Hz, 3H, CH3), 1.33 (s, 9H, CH3), 1.31 (d, j = 6.3, Hz, 12H, CH(CH3)2). 13C NMR (101 MHz, CDCl3) δ 158.2 (C(=Ar)), 153.1 (C=O), 136.1, 136.0 (CH=C), 118.4 (CH=Ar), 117.5 (CH=C), 84.2, 84.1 (O-CH2-O), 73.4 (CH(CH3)2), 57.6, 57.5 (CH2-N), 30.8 (C(CH3)3), 30.3 (C(CH3)3), 27.7, 26.3 (CH2-P), 21.6, 21.5 (CH(CH3)2), 14.3, 14.2 (CH3). 31P NMR (162 MHz, CDCl3) δ 27.62. HRMS (ESI): m/z [M+H]+ calcd for C21H27N3O6P: 506.22619, found: 506.22601.

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[(E)-4-[4-(Hydroxymethyl)triazol-1-yl]-3-methyl-but-2-enyl]-[isoproxy carbonyloxy- methoxy]phosphoryl[oxy methyl isopropyl carbonate (11h]
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The title compound was prepared from 10 (105 mg, 0.25 mmol) and propargyl alcohol (19 µL, 0.32 mmol) following the general procedure A; the resulting suspension was stirred for 24 h at 40 °C. After purification on a silica gel column chromatography (MeOH/CH2Cl2, 5/95), the desired pure compound 11h (84 mg, 71%) was obtained as a yellow oil. 31H NMR (400 MHz, CDCl3) δ 7.55 (s, 1H, H5), 5.67–5.59 (m, 4H, O-CH2-O), 5.49–5.44 (m, 1H, CH=C), 4.92–4.86 (m, 4H, CH(CH3)2), 4.75 (s, 2H, CH2OH), 2.74 (dd, j = 23.1, 7.8 Hz, 2H, CH2-P), 1.57 (d, j = 4.6 Hz, 3H, CH3), 1.28 (d, j = 5.6, Hz, 12H, CH(CH3)2). 13C NMR (101 MHz, CDCl3) δ 153.2 (C=O), 135.7, 135.6 (CH=C), 121.5 (CH=Ar), 111.8, 118.0 (CH=C), 84.1, 84.0 (O-CH2-O), 73.5 (CH(CH3)2), 57.7, 57.6 (CH2-N), 56.5 (CH2OH), 27.7, 26.3 (CH2-P), 21.6, 21.5 (CH(CH3)2), 14.3, 14.2 (CH3). 31P NMR (162 MHz, CDCl3) δ 27.40. HRMS (ESI): m/z [M+Na]+ calcd for C18H30N3NaO10P: 502.156102, found: 502.155885.

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[(E)-4-(4-Formyltriazol-1-yl)-3-methyl-but-2-enyl]-[isoproxy carbonyloxy-methoxy]phosphoryl[oxy methyl isopropyl carbonate (11i)
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The title compound was prepared from 10 (82 mg, 0.19 mmol) and 3,3-diethoxy-1-propyne (36 µL, 0.25 mmol) following the general procedure A; the resulting suspension was stirred for 2 h at 40 °C. After purification on a silica gel column chromatography (EtOAc/PE, 6/4), the desired pure compound 11i (19 mg, 21%) was obtained as a colorless oil. 31H NMR (400 MHz, CDCl3) δ 10.14 (s, 1H, CHO), 8.14 (s, 1H, H5), 5.71–5.63 (m, 4H, O-CH2-O), 5.60–5.57 (m, 1H, CH=C), 4.98 (d, j = 3.9 Hz, 2H, CH2-N), 4.92 (sept, j = 6.2 Hz, 2H, CH(CH3)2), 2.78 (dd, j = 23.3, 7.8 Hz, 2H, CH2-P), 1.63 (d, j = 4.8 Hz, 3H, CH3), 1.31 (d, j = 6.2 Hz, 12H, CH(CH3)2). 13C NMR (101 MHz, CDCl3) δ 184.9 (CHO), 153.1 (C=O), 148.0 (CH=Ar), 134.8, 134.6 (CH=C), 125.2 (CH=Ar), 119.5, 119.4 (CH=C), 84.2, 84.1 (O-CH2-O), 73.5 (CH(CH3)2), 58.1, 58.0 (CH2-N), 27.8, 26.4 (CH2-P), 21.6, 21.5 (CH(CH3)2), 14.3, 14.2 (CH3). 31P NMR (162 MHz, CDCl3) δ 27.04. HRMS (ESI): m/z [M+H]+ calcd for C18H29N3O10P: 478.158507, found: 478.158468.

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[(E)-4-(4-Carbamoyletriazol-1-yl)-3-methyl-but-2-enyl]-[isoproxy carbonyloxy-methoxy]phosphoryl[oxy methyl isopropyl carbonate (11j)
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The title compound was prepared from 10 (106 mg, 0.25 mmol) and propiolamide (22 mg, 0.33 mmol) following the general procedure A; the resulting suspension was stirred for 14 h at 40 °C. After purification on a silica gel column chromatography (MeOH/CH2Cl2, 3/97), the desired pure compound 11j (96 mg, 78%) was obtained as a colorless oil. 31H NMR (400 MHz, Acetone-d6) δ 8.31 (s, 1H, H5), 7.38 (s, 1H, NH), 6.78 (s, 1H, NH), 5.70–5.63 (m, 4H, O-CH2-O), 5.59–5.57 (m, 1H, CH=C), 5.08 (d, j = 4.0 Hz, 2H, CH2-N), 4.91 (sept, j = 6.2 Hz, 2H, CH(CH3)2), 2.84 (dd, j = 22.4, 7.9 Hz, 2H, CH2-P), 1.66 (d, j = 3.9 Hz, 3H, CH3), 1.29 (d, j = 6.3 Hz, 12H, CH(CH3)2). 13C NMR (101 MHz, Acetone-d6) δ 161.6 (NH2=O), 153.1 (C=O), 143.4 (CH=Ar), 135.4, 135.3 (CH=C), 126.0 (CH=O), 118.4, 118.3 (CH=C), 84.2, 84.1 (O-CH2-O), 72.8 (CH(CH3)2), 57.2, 57.1 (CH2-N), 27.3, 25.9 (CH2-P), 20.9 (CH(CH3)2), 13.6, 13.5 (CH3). 31P NMR (162 MHz, CDCl3) δ 27.05. HRMS (ESI): m/z [M+H]+ calcd for C18H30N3O10P: 493.169406, found: 493.169337.


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[[(E)-4-(4-Octanoylaminomethyltriazol-1-yl)-3-methyl-but-2-enyl]- (isopropoxycarbonyl氧基methyl)phosphoryl][oxymethyl isopropyl carbonate (11k)]
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The title compound was prepared from 10 (109 mg, 0.26 mmol) and N-N(prop-2-yn-1-yl)octanamide (56 mg, 0.33 mmol) following the general procedure A; the resulting suspension was stirred for 24 h at 40 °C. After purification on a silica gel column chromatography (MeOH/CH₂Cl₂, 3/97), the desired pure compound 11k (147 mg, 94%) was obtained as a yellowish oil. ¹³C NMR (101 MHz, CDCl₃) δ 173.2 (NH-C=O), 153.2 (C=O), 145.1 (CH₂), 135.6, 135.5 (CH=CH), 121.8 (CH₃), 118.2, 118.1 (CH=CH), 84.6, 84.1 (CH₂-CH₂), 73.4 (CH(CH₃)₂), 57.8, 57.7 (CH₂-N), 36.6 (ArCH₂), 27.8, 57.2 (CH₂-CH₂-N), 36.6 (ArCH₂), 35.0 (O=C(CH₂)₂CH₃), 31.7 (O=C(CH₂)₂CH₂CH₃), 29.3 (O=C(CH₂)₂CH₂CH₂CH₂CH₃), 29.0 (O=C(CH₂)₂CH₂CH₂CH₂CH₃), 27.8 (CH₂-CH₂-P), 26.4 (CH₂-P), 25.6 (O=C(CH₂)₂CH₂CH₂CH₃), 22.6 (O=C(CH₂)₂CH₃), 21.6, 21.6 (CH₃CH₂), 14.3, 14.2 (CH₃), 14.1 (O=C(CH₂)₂CH₃). ³¹P NMR (162 MHz, CDCl₃) δ 27.31. HRMS (ESI): m/z [M+Na⁺]⁺ calcd for C₂₆H₄₅N₄NaO₁₀P: 627.27551, found: 627.275980.

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[[*(E)-4-(4-Nitrophenyltriazol-1-yl)-3-methyl-but-2-enyl]-(isopropoxycarbonyl氧基methyl)phosphoryl][oxymethyl isopropyl carbonate (11)]
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The title compound was prepared from 10 (100 mg, 0.24 mmol) and 1-ethyl-4-nitrobenzene (45 mg, 0.31 mmol) following the general procedure A; the resulting suspension was stirred for 2 h at 40 °C. After purification on a silica gel column chromatography (EtOAc/PE, 9/1), the desired pure compound 11 (93 mg, 69%) was obtained as a yellowish oil. ¹³C NMR (101 MHz, CDCl₃) δ 153.2 (C=O), 147.3 (C²⁻), 146.1 (C⁴⁻), 137.0 (C⁷⁻), 135.3, 135.2 (CH=CH), 126.2 (CH²⁻), 124.2 (CH⁴⁻), 121.0 (CH⁷⁻), 119.1, 119.0 (CH=C), 84.2, 84.1 (O=CH₂-CH₂), 73.5 (CH=CH₂), 58.2, 58.1 (CH₂-N), 27.8, (CH₂-P), 26.4 (CH₂-P), 21.6, 21.5 (CH₃CH₂), 14.3, 14.3 (CH₃). ³¹P NMR (162 MHz, CDCl₃) δ 27.26. HRMS (ESI): m/z [M+H⁺]⁺ calcd for C₂₅H₃₂N₄O₁₁P: 571.179971, found: 571.179979.

1,1′-[[(E)-4-Bis(isopropoxycarbonyloxymethoxy)phosphoryl]-2-methyl-but-2-enyl]triazole-4-carboxylic acid (11m)

To a solution of propiolic acid (49 µL, 1.1 equiv., 0.80 mmol) and (E)-4-Azido-3-methyl-but-2-enyl-bis(POC)phosphonate 10 (307 mg, 1.0 equiv., 0.73 mmol) in anhydrous CH₂Cl₂ (10 mL) was added 2-nitrophenylboronic acid (25 mg, 0.21 equiv., 0.15 mmol) at room temperature. The solution was stirred for 42 h at room temperature. After evaporation of the solvent, the residue was purified by silica gel chromatography (MeOH/EtOAc, 1/9) to give compound 11m (203 mg, 57%) as a colorless oil. ¹³C NMR (400 MHz, CD₂Cl₂) δ 8.28 (s, 1H, H²), 5.72–5.64 (m, 4H, O=CH₂-CH₂), 5.57–5.51 (m, 1H, CH=CH), 5.06 (d, J = 4.4 Hz, 2H, CH₂-N), 4.94 (sept., J = 6.4 Hz, 2H, CH₂-CH₂), 2.91 (dd, J = 23.2, 7.9 Hz, 2H, CH₂-P), 1.67 (d, J = 4.8 Hz, 3H, CH₃), 1.33 (d, J = 6.2 Hz, 12H, CH₃CH₂). ¹³C NMR (101 MHz, CD₂Cl₂) δ 153.2 (C=O), 136.0, 135.8 (CH=CH), 126.7 (CH²⁻), 117.5, 117.3 (CH=C), 84.4, 84.3 (O=CH₂-CH₂), 73.1 (CH=CH₂), 57.2, 57.1 (CH₂-N), 26.8, 25.4 (CH₂-P), 20.5 (CH₃CH₂), 13.6, 13.1 (CH₃). ³¹P NMR (162 MHz, CD₂Cl₂) δ 27.94. HRMS (ESI): m/z [M+H⁺]⁺ calcd for C₁₈H₂₀N₅O₁₁P: 494.153422, found: 494.153404.

Methyl-1,1′-[[(E)-4-Bis(isopropoxycarbonyloxymethoxy)phosphoryl]-2-methyl-but-2-enyl]triazole-4-carboxylate (11n)

The title compound was prepared from 10 (124 mg, 0.29 mmol) and methyl propiolate (34 µL, 0.38 mmol) using the general procedure A; the resulting suspension was stirred for 2 h at 40 °C. After purification on a silica gel column chromatography (EtOAc/PE,
6/4), the desired pure compound 11n (92 mg, 63%) was obtained as a colorless oil. 1H NMR (250 MHz, CD2OD) δ 8.57 (s, 1H, H3), 5.79–5.69 (m, 4H, O-CH2-O), 5.65–5.56 (m, 1H, CH=CH2), 5.14 (d, J = 4.2 Hz, 2H, CH2-N), 4.99 (sept., J = 6.4 Hz, 2H, CH(CH3)2), 4.00 (s, 3H, OCH3), 2.97 (dd, J = 23.2, 7.9 Hz, 2H, CH2-P), 1.74 (d, J = 4.7 Hz, 3H, CH3), 1.39 (d, J = 6.2 Hz, 12H, CH(CH3)2). 13C NMR (101 MHz, CDCl3) δ 161.0 (O=CO-OMe), 153.1 (C=O), 140.3 (C=Ar), 135.1, 134.9 (CH=CH2), 127.4 (CH=Ar), 119.1, 118.9 (CH=CH2), 84.2, 84.1 (O-CH2-O), 73.4 (CH(CH3)2), 58.0, 57.9 (CH2-N), 52.1 (OCH3), 27.8, 26.4 (CH2-P), 21.6, 21.5 (CH3(CH2)2), 14.3, 14.2 (CH3). 31P NMR (162 MHz, CDCl3) δ 27.19. HRMS (ESI): m/z [M+Na]+ calc for C19H30N3NaO11P: 530.151016; found: 530.151002.

Ethyl-1-[(E)-4-[bis(isopropoxy carbonyloxymethoxy)phosphoryl]-2-methyl-but-2-enyl] triazole-4-carboxylate (11o)

The title compound was prepared from 10 (120 mg, 0.28 mmol) and methyl propiolate (37 μL, 0.37 mmol) using the general procedure A; the resulting suspension was stirred for 2 h at 40 °C. After purification on a silica gel column chromatography (EtOAc/PE, 5/5), the desired pure compound 11o (94 mg, 64%) was obtained as a colorless oil. 1H NMR (400 MHz, CD2OD) δ 8.53 (s, 1H, H3), 5.74–5.66 (m, 4H, O-CH2-O), 5.59–5.54 (m, 1H, CH=CH2), 5.10 (d, J = 4.2 Hz, 2H, CH2-N), 4.95 (sept., J = 6.3 Hz, 2H, CH2(CH3)2), 4.43 (q, J = 7.1 Hz, 2H, OCH2CH3), 2.93 (dd, J = 23.2, 7.9 Hz, 2H, CH2-P), 1.70 (d, J = 4.8 Hz, 3H, CH3), 1.42 (t, J = 7.1 Hz, 3H, OCH2CH3), 1.34 (d, J = 6.7 Hz, 12H, CH(CH3)2). 13C NMR (101 MHz, CD2OD) δ 161.5 (O=CO-Et), 153.2 (C=O), 139.7 (C=Ar), 135.8, 135.6 (CH=CH2), 128.2 (CH=Ar), 117.9, 117.8 (CH=CH2), 84.3, 84.2 (O-CH2-O), 73.1 (CH2(CH3)2), 60.8 (OCH2CH3), 57.2, 57.1 (CH2-N), 26.8, 25.4 (CH2-P), 20.5 (CH2(CH3)2), 13.2 (OCH2CH3), 13.1, 13.0 (CH3). 31P NMR (162 MHz, CD2OD) δ 27.92. HRMS (ESI): m/z [M+Na]+ calc for C20H32N3NaO11P: 544.16666, found: 544.168837.

[(E)-4-(4-Methylcarbamoyl)triazol-1-yl)-3-methyl-but-2-enyl-(isopropoxy carbonyloxymethoxy)phosphoryl]oxymethyl isopropyl carbonate (11p)

The title compound was prepared from 10 (121 mg, 0.29 mmol) and N-methylprop-2-ynamide (31 mg, 0.37 mmol) following the general procedure A; the resulting suspension was stirred for 2 h at 40 °C. After purification on a silica gel column chromatography (MeOH/CH2Cl2, 1/99), the desired pure compound 11p (118 mg, 82%) was obtained as a colorless oil. 1H NMR (400 MHz, CD2OD) δ 8.34 (s, 1H, H3), 5.74–5.66 (m, 4H, O-CH2-O), 5.58–5.52 (m, 1H, CH=CH2), 5.09 (d, J = 4.0 Hz, 2H, CH2-N), 4.96 (sept., J = 6.2 Hz, 2H, CH2(CH3)2), 2.98 (s, 3H, NH2CH3), 2.92 (dd, J = 23.2, 7.9 Hz, 2H, CH2-P), 1.69 (d, J = 4.6 Hz, 3H, CH3), 1.35 (d, J = 6.3 Hz, 12H, CH(CH3)2). 13C NMR (101 MHz, CD2OD) δ 161.7 (O=CO-CH3), 153.2 (C=O), 142.9 (C=Ar), 136.0, 135.8 (CH=CH2), 125.7 (CH=Ar), 117.5, 117.4 (CH=CH2), 84.4, 84.3 (O-CH2-O), 73.1 (CH2(CH3)2), 57.1, 57.0 (CH2-N), 26.8, 25.4 (CH2-P), 24.7 (NHCH3), 20.5 (CH2(CH3)2), 13.1, 13.1 (CH3). 31P NMR (162 MHz, CD2OD) δ 27.91. HRMS (ESI): m/z [M+Na]+ calc for C21H32N4NaO11P: 529.167001, found: 529.166551.

([(E)-4-(Ethylcarbamoyl)triazol-1-yl]-3-methyl-but-2-enyl-(isopropoxy carbonyloxymethoxy)phosphoryl]oxymethyl isopropyl carbonate (11q)

The title compound was prepared from 10 (115 mg, 0.27 mmol) and N-ethylprop-2-ynamide (34 mg, 0.35 mmol) following the general procedure A; the resulting suspension was stirred for 2 h at 40 °C. After purification on a silica gel column chromatography (MeOH/CH2Cl2, 1/99), the desired pure compound 11q (128 mg, 91%) was obtained as a colorless oil. 1H NMR (400 MHz, CD2OD) δ 8.35 (s, 1H, H3), 5.75–5.67 (m, 4H, O-CH2-O), 5.59–5.53 (m, 1H, CH=CH2), 5.09 (d, J = 4.0 Hz, 2H, CH2-N), 4.96 (sept., J = 6.2 Hz, 2H, CH2(CH3)2), 3.47 (q, J = 7.2 Hz, 2H, NHCH2CH3), 2.93 (dd, J = 23.1, 7.9 Hz, 2H, CH2-P), 1.70 (d, J = 4.7 Hz, 3H, CH3), 1.35 (d, J = 6.2 Hz, 12H, CH(CH3)2), 1.28 (t, J = 7.2 Hz, 3H, NHCH2CH3). 13C NMR (101 MHz, CD2OD) δ 160.8 (O=CO-NH), 153.2 (C=O), 142.99 (C=Ar), 136.0, 135.8 (CH=CH2), 125.7 (CH=Ar), 117.5, 117.4 (CH=CH2), 84.4, 84.3 (O-CH2-O), 73.1 (CH2(CH3)2), 57.1, 57.0 (CH2-N), 33.7 (NHCH2CH3), 26.8, 25.4 (CH2-P), 20.5 (CH2(CH3)2), 13.6 (NHCH2CH3), 13.1, 13.0 (CH3). 31P NMR (162 MHz, CD2OD) δ 27.91. HRMS (ESI): m/z [M+Na]+ calc for C23H35N4NaO11P: 543.182651, found: 543.182603.

(E)-4-Hydroxy-3-methyl-but-2-enyl-(HDP/POC) phosphate (13)
To a solution of HDP/POC allylphosphonate (12) (1.00 g, 2.0 equiv., 1.94 mmol) and 2-methyl-2-propen-1-ol (8) (81 µL, 1.0 equiv., 0.97 mmol) in dry CH₂Cl₂ (10 mL), Hoveyda-Grubbs catalyst (91 mg, 15 mol%, 0.15 mmol) was added. The catalyst addition was performed in five equal portions of 3 mol% (18.20 mg, 0.029 mmol) at t = 0, 3, 6, 9 and 21 h over the course of the reaction. The solution was sonicated at 55 °C under nitrogen atmosphere for 24 h. Volatiles were evaporated and the residue was purified by silica gel column chromatography (EtOAc/PE, 5/5) to give the desired phosphonate derivative 13 (480 mg, 88%) as brown oil. ¹H NMR (400 MHz, CDCl₃) δ 5.67–5.59 (m, 2H, O-CH₂-), 5.47–5.41 (m, 1H, CH=CH₂). 13C NMR (101 MHz, CDCl₃) δ 153.3 (C=O), 140.8, 140.7 (CH=CH₂), 112.6, 112.5 (CH=CH₂), 84.4, 84.3 (O-CH₂-O), 73.1 (CH₂(CH₃)₂), 71.2 (CH₂(CH₂)₄CH₂-O), 68.1, 68.0 (CH₂-OH), 66.5, 66.4 (C₆), 63.3, 63.2 (C₅), 31.9, 30.7, 30.6 (C₄), 29.7, 29.5, 29.4, 27.1 (CH₂-P), 26.2, 25.7 (CH₂-P), 22.7, 21.6 (CH(CH₂)₃O), 14.1 (CH(CH₂)₅O), 13.9, 13.8 (CH₃). ¹³C NMR (162 MHz, CDCl₃) δ 26.71. HRMS (ESI): m/z [M+H]^+ calcd for C₂₉H₆₄O₇P: 565.38368, found: 565.38568.

(E)-4-Azido-3-methyl-but-2-enyl-(HDP/POC) phosphonate (14)

To a solution of (E)-4-hydroxy-3-methyl-but-2-enyl-(HDP/POC)phosphonate 13 (277 mg, 1.0 equiv., 0.49 mmol) in anhydrous CH₂Cl₂ (5 mL) were added dropwise methansulfonyl chloride (42 µL, 1.1 equiv., 0.54 mmol) and anhydrous triethylamine (75 µL, 1.1 equiv., 0.54 mmol) at 0 °C under the argon. After 40 min stirring at room temperature, the mixture was diluted with CH₂Cl₂ and washed with water (3 × 10 mL) and brine. The organic layer was dried over MgSO₄, filtrated and concentrated in vacuo. The residue was directly used for the next step without further purification. To a solution of the above product in anhydrous DMF (8 mL) was added sodium azide (159 mg, 5.0 equiv., 2.45 mmol) under nitrogen atmosphere. After 5 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 water (5 × 10 mL), brine, dried over MgSO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/PE, 4/6) to give (E)-4-Azido-3-methyl-but-2-enyl-(HDP/POC) phosphonate 14 (242 mg, 84%) as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ 5.69–5.58 (m, 2H, O-CH₂-), 5.51–5.41 (m, 1H, CH=CH₂), 4.91 (sept., J = 6.3 Hz, 1H, CH(CH₃)₂), 4.22–4.07 (m, 2H, H²), 3.70 (d, J = 3.9 Hz, 2H, CH₂-N₃), 3.46 (t, J = 6.2 Hz, 2H, H²), 3.37 (t, J = 6.7 Hz, 2H, CH₃(CH₂)₃CH₂-O), 2.68 (dd, J = 22.7, 7.7 Hz, 2H, CH₂-P), 1.90 (quint., J = 6.3 Hz, 2H, H²), 1.73 (d, J = 4.3 Hz, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 153.3 (C=O), 140.8, 140.7 (CH=CH₂), 112.6, 112.5 (CH=CH₂), 84.4, 84.3 (O-CH₂-O), 73.1 (CH₂(CH₃)₂), 71.2 (CH₂(CH₂)₄CH₂-O), 68.1, 68.0 (CH₂-OH), 66.5, 66.4 (C₆), 63.3, 63.2 (C₅), 31.9, 30.7, 30.6 (C₄), 29.7, 29.5, 29.4, 27.1 (CH₂-P), 26.2, 25.7 (CH₂-P), 22.7, 21.6 (CH(CH₂)₃O), 14.1 (CH(CH₂)₅O), 13.9, 13.8 (CH₃). ¹³C NMR (162 MHz, CDCl₃) δ 26.71. HRMS (ESI): m/z [M+H]^+ calcd for C₂₉H₆₄O₇P: 590.39286, found: 590.39341.

General Procedure B for Huisgen 1,3-dipolar cycloaddition

To a solution of terminal alkyne (1.3 equiv.) and (E)-4-Azido-3-methyl-but-2-enyl-(HDP/POC) phosphonate 14 (1.0 equiv.) in tBuOH/H₂O (2:1) were added sodium ascorbate (0.6 equiv.) and CuSO₄·5H₂O (0.1 equiv.). The resulting suspension was stirred at 40 °C until completion (followed by TLC), then the crude mixture was co-evaporated five times with methanol. The residue was purified by silica gel column chromatography using an elution gradient of petroleum ether/ethyl acetate to give the desired HDP/POC phosphonate derivatives.

[3-Hexadecyloxypropoxy]-[(E)-3-methyl-4-[4-(4-propylphenyl)triazol-1-yl]but-2-enyl] phosphoryl]oxymethyl isopropyl carbonate (15a)
The title compound was prepared from 14 (123 mg, 0.21 mmol) and 1-ethyl-4-
propylybenzene (43 µL, 0.27 mmol) following the general procedure B; the resulting
 suspension was stirred for 2 h at 40 °C. After purification on a silica gel column chromatography (EtOAc/PE, 4/6), the desired pure compound 15a (104 mg, 68%) was obtained as a colorless oil. 1H NMR (400 MHz, CDCl₃) δ 7.75 (s, 1H, H²), 7.73 (s, 2H, H¹⁵α), 7.22 (d, J = 8.0 Hz, 2H, H¹⁵β), 5.70 - 5.56 (m, 3H, O-CH₂-O; 1H, CH=C), 4.94 (s, 2H, CH₂-N), 4.89 (sept., J = 6.3 Hz, 1H, CH(CH₃)₂), 4.42 - 4.11 (m, 2H, H²), 3.44 (t, J = 6.1 Hz, 2H, H⁶), 3.35 (t, J = 6.7 Hz, 2H, CH₃(CH₃)₂CH₂-O), 2.73 (dd, J = 22.8, 7.8 Hz, 2H, CH₂-P), 2.60 (t, J = 7.5 Hz, 2H, Ar-CH₂CH₂CH₂CH₃), 1.90 (quint., J = 6.3 Hz, 2H, H²), 1.68 - 1.63 (m, 3H, CH₂), 1.29 - 1.25 (m, 32H, CH₃(CH₃)₁₂CH₂CH₂-O, CH(CH₃)₂), 0.95 (t, J = 7.3 Hz, 3H, CH₂CH₂CH₃), 0.88 (t, J = 7.0 Hz, 3H, CH₂(CH₃)₂O), 13C NMR (101 MHz, CDCl₃) δ 153.3 (C=O), 148.3 (C¹⁵α), 142.8 (C¹⁵β), 128.9 (CH³), 128.0 (CH²), 125.6 (CH⁴), 118.9 (CH=C), 118.8 (CH²), 84.4 (O-CH₂-O), 73.2 (CH(CH₃)₂), 71.2 (CH₃(CH₃)₁₂CH₂-O), 66.4 (C⁶), 63.5 (C⁴), 57.9 (CH₂-
N), 37.8 (Ar-CH₂CH₂CH₃), 31.9, 30.7 (C₆), 29.6 (CH₂-P), 24.5 (Ar-CH₂CH₂CH₃), 22.7, 21.6, 21.5 (CH(CH₃)₂), 14.3 (C=CCH₃), 14.1 (CH₃(CH₃)₁₂O), 31P NMR (162 MHz, CDCl₃) δ 27.46. HRMS (ESI): m/z [M+H]+ calcd for C₄₀H₆₀N₃NaO₃P: 734.486765, found: 734.486600.

[3-Hexadecyloxypropoxy-[(E)-3-methyl-4-(4-pentylphenyl)triazol-1-yl]but-2-enyl]
phosphoryloxymethyl isopropyl carbonate (15c)

The title compound was prepared from 14 (93 mg, 0.16mmol) and 1-ethyl-4-
pentylbenzene (40 µL, 0.21 mmol) following the general procedure B; the resulting
 suspension was stirred for 2 h at 40 °C. After purification on a silica gel column chromatography (EtOAc/PE, 4/6), the desired pure compound 15c (89 mg, 74%) was obtained as a colorless oil. 1H NMR (400 MHz, CDCl₃) δ 7.74 (d, J = 3.8 Hz, 2H, H¹⁵α), 7.73 (s, 1H, H¹⁵β), 7.22 (d, J = 8.0 Hz, 2H, H¹⁵β), 5.70 - 5.56 (m, 3H, O-CH₂-O; 1H, CH=C), 4.94 (s, 2H, CH₂-N), 4.90 (sept., J = 6.2 Hz, 1H, CH(CH₃)₂), 4.42 - 4.11 (m, 2H, H²), 3.44 (t, J = 6.1 Hz, 2H, H⁶), 3.35 (t, J = 6.7 Hz, 2H, CH₃(CH₃)₁₂CH₂-O), 2.73 (dd, J = 22.8, 7.8 Hz, 2H, CH₂-P), 2.62 (t, J = 7.8 Hz, 2H, Ar-CH₂CH₂CH₂CH₃), 1.90 (quint., J = 6.3 Hz, 2H, H²), 1.65 - 1.61 (m, 5H, CH₃, Ar-CH₂CH₂CH₂CH₂CH₃), 1.52 (quint., J = 7.0 Hz, 2H, CH₃(CH₃)₁₂CH₂CH₂-O), 1.29 - 1.25 (m, 36H, CH₃(CH₃)₁₂CH₂CH₂-O, CH(CH₃)₂), Ar-CH₂CH₂CH₂CH₂CH₃), 0.88 (t, J = 6.9 Hz, 6H, Ar-(CH₂)₄CH₃, CH₃(CH₃)₁₅O). ¹³C NMR (101 MHz, CDCl₃) δ 153.2 (C=O), 148.4, 148.3 (C¹⁵α), 143.1 (C¹⁵β), 135.2, 135.0 (C₆), 128.8 (CH²), 127.9 (CH=C), 125.6 (CH⁴), 118.9 (CH=C), 118.8 (CH²), 84.4 (O-CH₂-O), 73.2 (CH(CH₃)₂), 71.2 (CH₃(CH₃)₁₂CH₂-O), 66.4 (C⁶), 63.6 (C⁴), 57.8 (CH₂-N), 35.7 (Ar-CH₂CH₂CH₂CH₃), 31.9, 31.5, 31.1, 30.8, 30.7 (C₆), 29.8, 29.7, 29.6, 29.5, 29.4, 26.2 (CH₂-P), 22.7, 22.5, 21.6, 21.5 (CH(CH₃)₂), 14.3, 14.2 (C=CCH₃), 14.1 (CH₃(CH₃)₁₂O), 14.0 (Ar-CH₂CH₂CH₃). ³¹P NMR (162 MHz, CDCl₃) δ 27.56. HRMS (ESI): m/z [M+Na]+ calcd for C₄₂H₇₂N₃NaO₃P: 784.50009, found: 784.499917.

[3-Hexadecyloxypropoxy-[(E)-3-methyl-4-(4-heptylphenyl)triazol-1-yl]but-2-enyl]
phosphoryloxymethyl isopropyl carbonate (15e)

The title compound was prepared from 14 (113 mg, 0.19 mmol) and 1-ethyl-4-
heptylbenezene (56 µL, 0.25 mmol) following the general procedure B; the resulting
 suspension was stirred for 2 h at 40 °C. After purification on a silica gel column chromatography (EtOAc/PE, 4/6), the desired pure compound 15e (118 mg, 78%) was obtained as a colorless oil. 1H NMR (400 MHz, CDCl₃) δ 7.73 (t, J = 3.9 Hz, 3H, H¹⁵α, H¹⁵β), 7.22 (d, J = 8.0 Hz, 2H, H¹⁵α), 5.70 - 5.60 (m, 3H, O-CH₂-O; 1H, CH=C), 4.94 (s, 2H, CH₂-N), 4.89 (sept., J = 6.3 Hz, 1H, CH(CH₃)₂), 4.20 - 4.11 (m, 2H, H²), 3.43 (t, J = 6.1 Hz, 2H, H⁶), 3.35 (t, J = 6.7 Hz, 2H, CH₃(CH₃)₁₂CH₂-O), 2.73 (dd, J = 22.8, 7.8 Hz, 2H, CH₂-P), 2.63 (t, J = 7.8 Hz, 2H, Ar-CH₂(CH₂)₅CH₃), 1.90 (quint., J = 6.3 Hz, 2H, H²), 1.65 (d, J = 4.1 Hz, 3H, C=CCH₃), 1.60 (quint., J = 7.2, Ar-CH₂CH₂CH₂CH₂CH₃), 1.52 (quint., J = 7.0 Hz, 2H, CH₃(CH₃)₁₂CH₂CH₂-O), 1.29 - 1.25 (m, 40H, CH₃(CH₃)₁₂CH₂CH₂-O, CH(CH₃)₂, Ar-CH₂CH₂CH₂CH₂CH₃), 0.93 (t, J = 7.3 Hz, 3H, Ar-(CH₂)₉CH₃), 0.88 (t, J = 6.7 Hz, 3H, CH₃(CH₃)₁₅O). ¹³C NMR (101 MHz, CDCl₃) δ 153.3 (C=O), 143.5 (C¹⁵α), 135.2 (C¹⁵β), 135.1 (C₆), 129.5, 129.3 (C₆), 128.8 (C₆), 127.9 (CH=C), 125.6 (CH²), 118.9 (CH=C), 84.5, 84.4 (O-CH₂-O), 73.3 (CH(CH₃)₂),
71.2 (CH₃(CH₂)₁₁CH₂-O), 66.4 (C), 63.6, 63.5 (C₈), 57.8 (CH₂-N), 35.4 (Ar-CH₂(CH₂)₅CH₃), 33.5, 32.0, 31.4, 30.8, 30.7 (C₈), 30.2, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 27.6, 26.2 (CH₂-P), 22.7, 22.3, 21.6, 21.6 (CH(CH₃)₂), 14.3 (C=C=CH₃), 14.1 (CH₃(CH₂)₃(O)), 13.9 (Ar-CH₂(CH₂)₅CH₃).

3¹P NMR (162 MHz, CDCl₃) δ 27.49. HRMS (ESI): m/z [M+H]⁺ calcd for C₄₄H₇₇N₃O₅P: 790.549365, found: 790.549322.

([(E)-4-(4-Carbamoyl)triazol-1-yl]-3-methyl-but-2-enyl)-(3-hexadecyloxypropoxy)phosphoryl]oxyethyl isopropyl carbonate (15)

The title compound was prepared from 14 (166 mg, 0.28 mmol) and propiolamide (25 mg, 0.37 mmol) following the general procedure B; the resulting suspension was stirred for 18 h at 40 °C. After purification on a silica gel column chromatography (MeOH/CH₂Cl₂, 3/97), the desired pure compound 15j (130 mg, 70%) was obtained as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ 8.07 (s, 1H, H5), 7.05 (s, 1H, NH₂), 5.76 (s, 1H, NH₂), 5.69–5.58 (m, 2H, O-C₃H₂-O; 1H, CH=C), 4.94 – 4.91 (m, 2H, CH₂-N; 1H, CH(CH₃)₂), 4.22–4.12 (m, 2H, H₆), 3.46 (t, J = 6.1 Hz, 2H, H₆), 3.37 (t, J = 6.7 Hz, 2H, CH(CH₃)₂), 2.71 (dd, J = 22.9, 7.8 Hz, 2H, CH₂-P), 1.90 (quint., J = 6.3 Hz, 2H, H₅), 1.62 (d, J = 4.4 Hz, 3H, C=CCH₃), 1.52 (quint., J = 7.0 Hz, 2H, CH₃(CH₂)₁₁CH₂-O), 1.31–1.24 (m, 32H, CH₃(CH₂)₁₁CH₂-O, CH(CH₃)₂), 0.87 (t, J = 7.0 Hz, 3H, CH(CH₂)₁₁CH₂-O). ¹³C NMR (101 MHz, CDCl₃) δ 161.8 (NH₂C=O), 153.2 (C₂O), 143.1 (C₁₅Ar), 134.2, 134.1 (C=CH₂), 125.6 (CH₄N⁺), 120.1, 120.0 (CH=), 84.5, 84.4 (O-C₃H₂-O), 73.2 (CH(CH₂)₂), 71.3 (CH₃(CH₂)₁₁CH₂-O), 66.4 (C), 63.6, 63.5 (C₈), 58.2, 58.1 (CH₂-N), 31.9, 30.8, 30.7 (C₈), 29.7, 29.6, 29.6, 29.5, 29.3, 27.6, 26.2, 26.1 (CH₂-P), 22.7, 21.6 (CH(CH₃)₂), 14.3, 14.2 (C=CCH₃), 14.1 (CH₃(CH₂)₁₁CH₂-O). ³¹P NMR (162 MHz, CDCl₃) δ 27.11. HRMS (ESI): m/z [M+H]⁺ calcd for C₃₂H₆₆N₄O₃P: 659.414328, found: 659.414346.

[3-Hexadecyloxypropoxy-[(E)-3-methyl-4-[4-(nitrophenyl)triazol-1-yl]but-2-enyl]phosphoryl]oxyethyl isopropyl carbonate (15l)

The title compound was prepared from 14 (115 mg, 0.20 mmol) and 1-ethyl-4-nitrobenzene (37 mg, 0.25 mmol) following the general procedure B; the resulting suspension was stirred for 3 h at 40 °C. After purification on a silica gel column chromatography (EtOAc/PE, 5/5), the desired pure compound 15l (102 mg, 71%) was obtained as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ 8.27 (d, J = 8.8 Hz, 2H, H₄Ar), 8.01 (d, J = 8.9 Hz, 2H, H₅Ar), 7.98 (s, 1H, H₅), 5.70 - 5.58 (m, 3H, O-C₃H₂-O; 1H, CH=), 4.97 (s, 2H, CH₂-N), 4.89 (sept., J = 6.3 Hz, 1H, CH(CH₃)₂), 4.22–4.11 (m, 2H, H₆), 3.44 (t, J = 6.2 Hz, 2H, H₆), 3.35 (t, J = 6.6 Hz, 2H, CH(CH₂)₁₁CH₂-O), 2.73 (dd, J = 22.8, 7.8 Hz, 2H, CH₂-P), 1.90 (quint., J = 6.2 Hz, 2H, H₅), 1.66 (d, J = 4.4 Hz, 3H, C=CCH₃), 1.52 (quint., J = 7.0 Hz, 2H, CH₃(CH₂)₁₁CH₂-O), 1.27–1.23 (m, 32H, CH₃(CH₂)₁₁CH₂-O, CH(CH₃)₂), 0.86 (t, J = 7.0 Hz, 3H, CH₃(CH₂)₁₁CH₂-O). ¹³C NMR (101 MHz, CDCl₃) δ 153.2 (C=O), 147.3 (C₁₅Ar), 146.0 (C₈Ar), 134.9 (C₈Ar), 134.7, 134.5 (CH=), 126.1 (CH₄Ar), 124.3 (CH₁₁Ar), 121.0 (CH₄O⁺), 119.8, 119.7 (CH=), 84.4, 84.3 (O-C₃H₂-O), 73.3, 73.1 (CH(CH₂)₂), 71.2 (CH₃(CH₂)₁₁CH₂-O), 66.5, 66.3 (C₈), 63.6, 63.5 (C₈), 58.1, 58.0 (CH₂-N), 31.9, 30.8, 30.7 (C₈), 29.7, 29.6, 29.6, 29.6, 29.5, 29.5, 27.5, 26.1, 26.1 (CH₂-P), 22.7, 21.6, 21.6 (CH(CH₃)₂), 14.4, 14.3 (C=CCH₃), 14.1 (CH₃(CH₂)₁₁CH₂-O). ³¹P NMR (162 MHz, CDCl₃) δ 27.31. HRMS (ESI): m/z [M+Na]⁺ calcd for C₃₇H₅₁N₄NaO₃P: 759.406837, found: 759.406817.

4. Conclusions

In summary, we have efficiently synthesized various hitherto unknown ANP prodrugs bearing the (E)-2'-methyl-but-2'-enyl aliphatic side-chain with 1,4-substituted-1,2,3-triazoles as nucleobase. The convergent synthesis was based on olefin acyclic cross-metathesis and CuAAC cross-coupling reaction as the key steps. All those compounds were evaluated against HBV, HIV and SARS-CoV-2 viruses for their antiviral properties. Among them, compound 15j, a HDF/POC produrg with R = C(O)NH₂, a ribavirin analog, showed 62% inhibition (at 10 μM) without significant cytotoxicity (IC₅₀ 66.4 μM in HepG2 cells and IC₅₀ = 43.1 μM in HepG2 cells). Further structural optimization of both the (E)-2'-methyl-but-2'-enyl aliphatic side-chain and the heterocycle is underway, alongside more detailed
biological testing of the most active compound, with the aim of improving further its antiviral potency.

Supplementary Materials: The following are available online at https://www.mdpi.com/1420-3046/12/2/549/51-0186-4.

Author Contributions: Conceptualization, V.R. and L.A.A.; chemistry of all compounds, T.A.; biological evaluation on HBV, J.M., C.G.-G., D.B., T.R.M. and R.F.S.; biological evaluation on SARS-CoV-2, C.B., F.T., B.C.; writing—original draft preparation, T.A., V.R., L.A.A.; supervision, L.A.A. and V.R. All authors have read and agreed to the published version of the manuscript.

Funding: T. Abduauains thanks the China Scholarship Council for the PhD fellowship. We thank L. Bassit and O. Russell (from Pr. Raymond F. Schinazi’s laboratory) for the biological evaluations. R.F.S. is supported in part by NIH grant P30AI050409. L.A.A. thanks the LABEX SynOrg (ANR-11-LABX-0029) for support.

Conflicts of Interest: No potential conflict of interest was reported by the authors.

Sample Availability: Samples of the compounds are available on request from the corresponding authors.

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