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RESEARCH ARTICLE

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Derivatives of 3′-Azidothymidine with 6-Cyanopyridone as Base or as Phosphoramidate Ester and their Antiretroviral Activity

Jianyang Han,[a] Jakob Arnold,[a] Christophe Pannecouque,[b] Graciela Andrei,[b] Robert Snoeck,[b] and Clemens Richert*[a]

[a] Institute for Organic Chemistry, University of Stuttgart, Pfaffenwaldring 55, DE-70569 Stuttgart, Germany, e-mail: lehrstuhl-2@oc.uni-stuttgart.de

[b] Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, Herestraat 49, B-3000 Leuven, Belgium

Strongly pairing ethynylpyridone *C*-nucleosides are attractive surrogates for thymidine in oligonucleotides. Exploratory work on the antiviral activity of 3′-azidothymidine (AZT) derivatives with ethynylpyridone as base had identified strong lipophilicity as a limiting factor. Two strategies are being pursued to overcome this issue. In order to make the base more polar, the ethynyl group has been replaced with a cyano group, leading to a cyanopyridone *C*-nucleoside, whose eleven-step synthesis is reported here, together with the synthesis of a 3′-azido-2′,3′-dideoxynucleoside derivative. The base pairing with adenine in a DNA duplex was studied by UV melting analysis of a self-complementary hexamer containing the 6-cyano-2′-deoxynucleoside instead of thymidine. A melting point increase of 2 °C compared to the unmodified control was found. The other strategy employs a phosphoramidate prodrug design with less lipophilic amino acid esters. Here, anti-HIV test of the alaninyl and prolinyl methyl esters of AZT gave promising results in cell culture experiments, increasing the selectivity index up to 5.8-fold for the III_B strain and up to 5-fold for the ROD strain of the virus, as compared to the parent nucleoside. These findings help to design the next generation of pyridone *C*-nucleosides with potential applications as antivirals.

Keywords: antiviral agents, HIV, nucleosides, nucleotides, prodrugs.

Introduction

Viral infections pose a threat to humanity. New viruses and new variants of known viruses can cause pandemics that seriously affect the livelihood and economic well-being of billions of people. One recent example is the COVID-19 pandemic caused by the SARS-CoV-2 virus,[1] leading to extensive human suffering and a severe economic downturn even though the virus is closely related to known viruses.[2] The current pandemic not only causes a significant burden, it also shifts public attention and funding away from other viral diseases, like HIV, which continue to claim millions of lives per year.[3–5] Both new and known viral diseases call for the development of effective and inexpensive treatment options to avoid the effects of the disease itself and the indirect effects caused by social distancing and other physical interventions. The search for new therapeutics should include studies on new antiretrovirals.[6]

One prime target for antiviral compounds are nucleic acid polymerases. The majority of viruses uses own enzymes for the tasks involved in replicating their genomes.[7] Those viral polymerases are therefore promising molecular targets that may be inhibited without interfering with host enzymes unnecessarily. Further, the mode of action of polymerases is well understood, so that inhibitors may be designed based on established principles.[8] If the inhibitor is accepted by the polymerase, resulting in incorporation at the

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3′-terminus of the growing chain, further elongation may be prevented by employing a sugar moiety that lacks the 3′-hydroxy group for formation of the next phosphodiester. Alternatively, a modified nucleoside may cause delayed termination[9] or an increase level of mutations.[10,11]

A number of successful antivirals have been generated from nucleosidic structures.[12,13] Among the successful antiretrovirals is 3′-azido-2′,3′-dideoxythymidine (AZT, zidovudine).[14,15] Zidovudine was the first nucleoside inhibitor showing *in vitro* anti-HIV activity and continues to be used to treat HIV infections, mostly in combination with other antiretrovirals.[5,16] It contains unmodified thymine as nucleobase. Thymine is known to be the nucleobase that gives the weakest pairing at the terminus of primer-template duplexes.[17] Increased base pairing strength may help to outcompete the natural substrate, thus lowering the concentration required to achieve the inhibitory effect. Pyridone *C*-nucleosides with an ethynyl substituent at the 6-position have been shown to pair more strongly with adenine in complementary strands than thymine.[18,19] Both E and W (*Figure 1*) increase UV-melting points of oligonucleotide duplexes significantly,[20,21] and an activated monophosphate of W was found to lead to incorporation preferentially over TMP in enzyme-free primer extension.[22]

Tests on its effect against HSV1 showed activity in the micromolar range in Vero cells in a plaque reduction assay, but both the parent nucleoside and a ProTide prodrug construct suffered from low solubility due to strong lipophilicity.[23] ProTides are among the most successful examples of nucleotide prodrugs.[23–27] They were first developed in the context of HIV.[24,25] They feature a phosphate group masked by an *O*-aryl group and an *N*-linked amino acidyl ester. The free nucleotide is released intracellularly in a series of steps, starting with the enzymatic hydrolysis of the amino acidyl ester and subsequent hydrolysis of the phosphoramidate.[28,29]

In our previous work, we employed the alaninyl ester known from sofosbuvir[30] and remdesivir,[31] and did not test less lipophilic alternatives. Here, we report the synthesis of a more polar derivative of AZW, containing a cyano rather than an ethynyl group, together with exploratory studies on two ProTide constructs of AZT with a less lipophilic amino acid ester moiety. One of these is a new construct with a prolinyl methyl ester as amino acid component, motivated by our observation of facilitated release for such species.[29]

Results and Discussion

The starting point of our study was 3′-azido-2′,3′-deoxythymidine (AZT), an HIV nucleoside reverse transcriptase inhibitor in clinical use against HIV.[32] We sought to improve its activity by replacing the nucleobase with a pyridone derivative. Pyridone *C*-nucleosides with an ethynyl substituent at the 6-position bind more strongly with adenine than their natural counterparts (thymine or uracil).[20,21] However, the ethynyl group renders the base very lipophilic,[23] so that a more polar replacement is desirable. Cyano groups are isoelectronic to ethynyl groups but feature a polarized C≡N bond and a H-bond acceptor that will reside in the minor groove of a duplex with a complementary strand. This led to compound **1** as the target molecule in one branch of our study.

The synthesis of the cyanopyridone *C*-nucleoside **1** is shown in *Scheme 1*. Aminobromopyridine **2** was prepared in three steps following literature protocols[20] in an overall yield of 58%. Next, a cyano group was introduced through a *Rosenmund–von Braun* reaction[33] to obtain cyanopyridine **3**, which was converted to diazonium salt **4**, followed by nucleophilic substitution to give the iodide **5** in 46% yield over three steps. The cyanation was performed at 110 °C for three days with *N*-methyl-2-pyrrolidone as solvent, avoiding an explosive decomposition that was

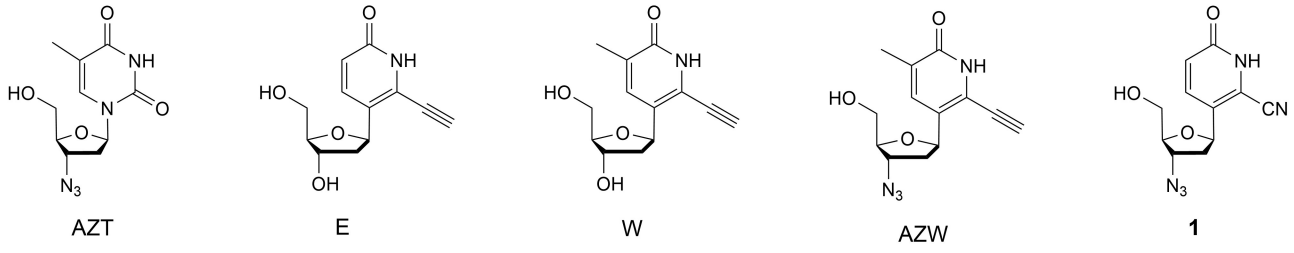
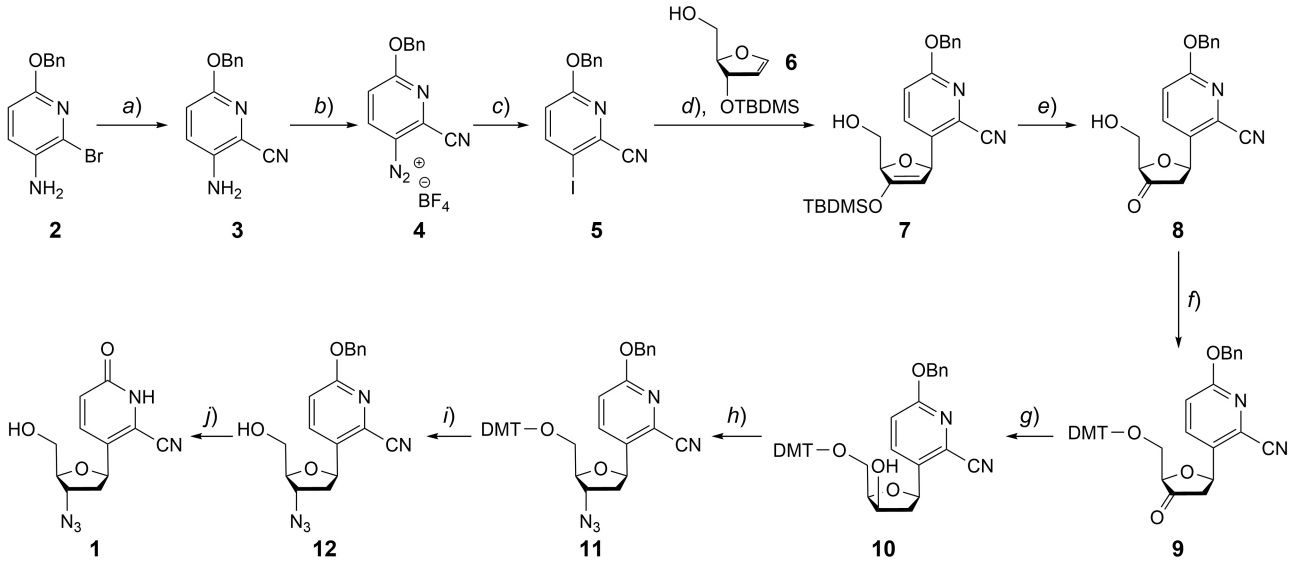


Figure 1. Chemical structure of the azidonucleoside **1** containing a cyanopyridone nucleobase.



Scheme 1. Synthetic route to cyanopyridone azidodeoxynucleoside **1**. *a*) 1. CuI, KI, 1,10-phenanthroline, *N*-methyl-2-pyrrolidone; 2. cyanohydrin $(CH_3)_2C(OH)CN$, NBu_3, *N*-methyl-2-pyrrolidone, 76% over 2 steps. *b*) $BF_3 \cdot OEt_2$, $^tBuNO_2$, THF. *c*) KI, MeCN, 60% over 2 steps. *d*) $Pd(OAc)_2$, $P(PhF_5)_3$, Ag_2CO_3, MeCN. *e*) $3HF \cdot NEt_3$, THF, 49% over 2 steps. *f*) 4,4-dimethoxytrityl chloride, 4-dimethylaminopyridine, pyridine. *g*) $NaBH_4$, THF/EtOH, 51% over 2 steps. *h*) PPh_3, diisopropyl azodicarboxylate, diphenylphosphoryl azide, THF, 73%. *i*) trichloroacetic acid, CH_2Cl_2, 82%. *j*) BCl_3, CH_2Cl_2, 42%.

encountered when using DMSO and microwave irradiation. Glycal **6** was synthesized in four steps in 40% overall yield, following our preferred route.[20] Then, glycal and aglycone were coupled through a *Heck* reaction to yield enol ether **7**, followed by desilylation to ketone **8**. Subsequently, the 5′-hydroxy group of **8** was DMT protected, and the keto group of **9** was diastereoselectively reduced to xylonucleoside **10** as the main isomer in 51% yield. The *ribo*-configured epimer of **10** was removed by column chromatography. The subsequent *Mitsunobu* reaction introduced the 3′-azido group with inversion of configuration to provide **11** in 73% yield. The DMT group of azidonucleoside **11** was removed under acidic condition to obtain **12**, the benzyl group of which was removed with boron trichloride at −40 °C, leading to the target cyanonucleoside **1**.

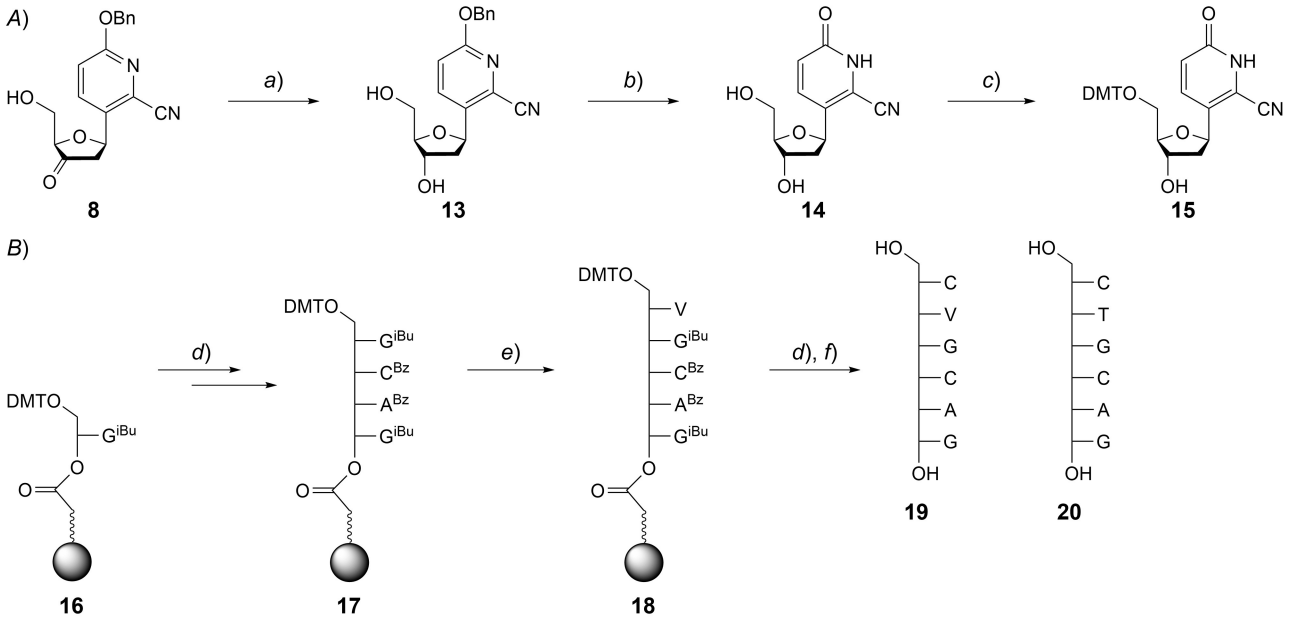
After successful preparation of the azidonucleoside containing the cyanopyridone nucleobase (**1**, abbreviated 'AZC'), its activity against HIV was tested *in vitro*. The results are compiled in *Table 1*. In the assays, AZT was used as positive control, and either compound was tested on two strains (III_B and ROD) of the human immunodeficiency virus. While AZT gave the expected double digit nanomolar activity, a 50% inhibitory concentration of AZC was not reached in the nanomolar concentration range assayed.

Table 1. Results of the anti-HIV tests of azidonucleosides AZT and AZC (**1**) with IC_{50} as 50% inhibitory concentration and CC_{50} as 50% cytotoxic concentration.

	AZT	**1**
IC_{50} of III_B strain	0.026 μM	> 478 μM
CC_{50} of III_B strain	> 7.48 μM	> 478 μM
IC_{50} of ROD strain	0.010 μM	> 478 μM
CC_{50} of ROD strain	> 7.48 μM	> 478 μM

To evaluate whether the cyanonucleoside is able to pair with adenine in a DNA duplex, we decided to synthesize an oligodeoxynucleotide containing the cyanopyridone deoxynucleoside residue, which we abbreviate as 'V' in this context. We chose to prepare this oligonucleotide by the on-support phosphitylation approach reported earlier.[20] This called for the synthesis of a 5′-DMT protected 2′-deoxynucleoside as building block (*Scheme 2,A*). For this, ketone **8** was diastereoselectively reduced using sodium triacetoxyborohydride to yield the *ribo*-configured deoxynucleoside **13**. The benzyl protecting group was removed by TMS−I generated *in situ* to give **14**. In the final step, the 5′-position was again DMT protected to obtain building block **15** for coupling to the phosphitylated chain on solid support.

In the event, chain assembly started with commercially available controlled pore glass (cpg) **16** loaded



Scheme 2. *A*) Synthesis of deoxynucleoside **15**, starting from ketone **8**. *a*) $NaBH(OAc)_3$, MeCN, 88%. *b*) TMS-Cl, NaI, MeCN, 75%. *c*) DMT-Cl, DMAP, pyridine, 46%. *B*) Synthesis of oligonucleotide **19**. *d*) Automated DNA chain extension cycles involving 1. TCA, CH_2Cl_2; 2. phosphoramidite, 4,5-dicyanoimidazole, MeCN; 3. Ac_2O, methylimidazole, pyridine, THF; 4. I_2, pyridine, THF, H_2O; *e*) coupling through on-support phosphitylation: 1. TCA, CH_2Cl_2; 2. diisopropylammonium tetrazolide $(^iPr_2N)_2P(OC_2H_4CN)$; 3. **15**, tetrazole; 4. I_2, pyridine, THF, H_2O. *f*) 1. TCA, CH_2Cl_2; 2. NH_4OH. Bz = benzoyl, ^{i}Bu = isobutyryl.

with the 2′-deoxyguanosine residue that later constituted the 3′-terminus of the sequence 5′-CVGCAG-3′ (*Scheme 2,B*). After three chain extension cycles to **17**, the 5′-position was phosphitylated, followed by coupling of cyanopyridone **15** to obtain protected strand **18**. A subsequent chain extension cycle introduced the 5′-terminal deoxycytidine residue, and deprotection followed by HPLC purification gave oligonucleotide **19**, the structure of which was confirmed by MALDI-TOF mass spectrometry (*Figure S25*, *Supporting Information*).

Unmodified DNA reference strand 5′-CTGCAG-3′ (**20**) was purchased as control compound for hexamer **19** and was also used in the subsequent UV-melting experiments. Changes in UV absorbance at 260 nm were measured in a temperature range of 5–85 °C in 100 mM phosphate buffer, 1 M NaCl and pH 7.0. *Figure 2* shows the melting profiles for the two self-complementary duplexes. It can be discerned that duplex $(\mathbf{19})_2$ with its two cyanopyridone residues facing adenines is thermally more stable than its unmodified DNA counterpart. Numerically, the increase in melting point is 2.0 °C (25 °C *vs.* 27 °C for the duplex of **19**), as determined by the points of inflection of the curves, but the cyanopyridones also change the hyperchromicity due to their longer wavelength absorbance maximum, as compared to those of canonical DNA bases, so that this numerical value is probably the lower limit of the overall stabilizing effect.

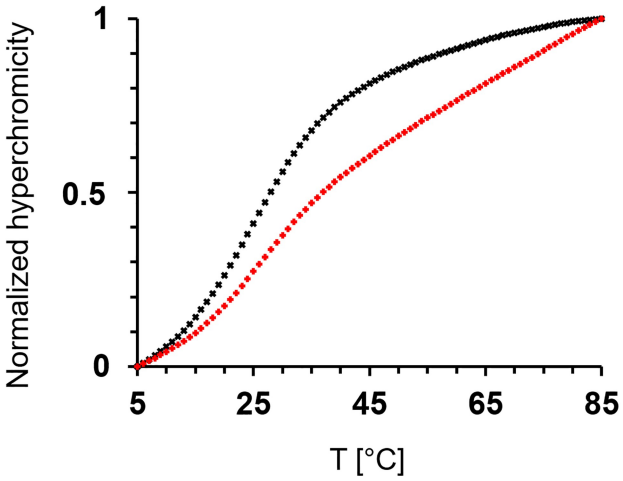


Figure 2. Cyanopyridone *C*-nucleosides have a stabilizing effect on a DNA duplex: UV-melting curves of the duplexes of oligodeoxynucleotides **19** (red) and reference **20** (5′-CTGCAG-3′, black) at 260 nm and a heating rate of 1 °C per minute, as determined in 0.1 M phosphate buffer, pH 7.0, 1 M NaCl.

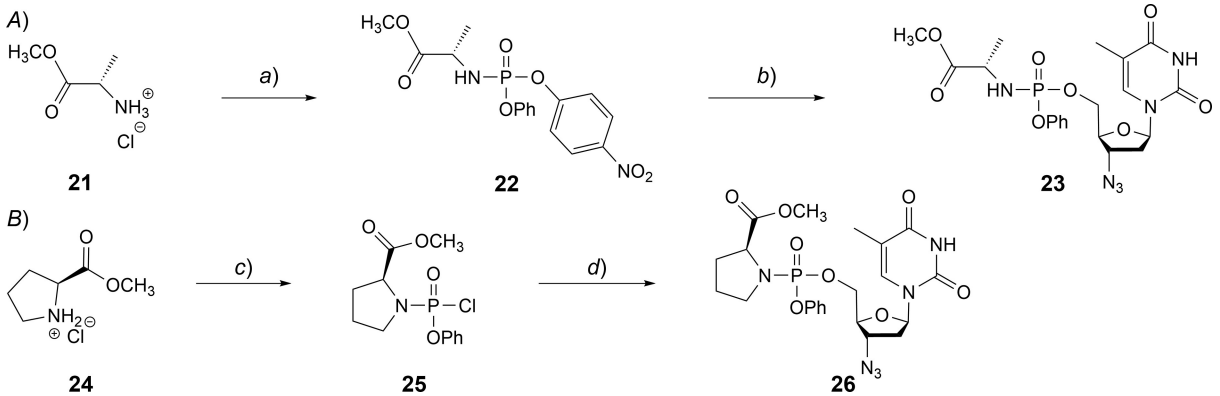
Since the melting curve data suggested favorable base pairing properties, we suspected unsuccessful intracellular phosphorylation as one reason for the low antiviral activity of **1**. Therefore, an exploratory set of experiments was performed on less lipophilic ProTide phosphoramidates, based on azidonucleoside AZT as established antiretroviral API. Besides known alaninyl methyl ester **23**,[24,25] which was prepared to test the synthetic method and to serve as positive control, prolinyl phosphoramidate **26** was selected as target. The latter represents a design that should also allow for non-enzymatic release of the free monophosphate intracellularly.[29]

Our synthesis of alanine methyl ester phosphoramidate **23** involved the formation of nitrophenyl phosphorylating reagent **22** from the amino acid ester and phenyl dichlorophosphate (*Scheme 3,A*). This reagent was coupled with AZT to obtain ProTide **23** in 87% yield, which is significantly higher than the 63% reported by an earlier method.[24,25] The second phosphoramidate with proline methyl ester was synthesized as shown in *Scheme 3,B*. Here, proline methyl ester hydrochloride (**24**) was converted to chlorophosphoramidate **25** as reagent, which was reacted with AZT to the desired prolinyl methyl ester **26** in an overall yield of 32%. Either of the ProTides was then subjected to antiviral testing.

The results of the tests on antiviral activity and the toxicity on uninfected cells are shown in *Figure 3,A* and *3,B*, respectively. From this data, the selectivities were calculated that are compiled in *Table 2*. This activity data indicates high potency for both **23** and **26**, with IC_{50} values well below that of didanosine. At the same time, both phosphoramidates show low toxicity. For methyl ester **23**, the most favorable CC_{50}/IC_{50} ratios were obtained, but the values for **26** are also well above those for both didanosine and nevirapine and not far from those for zidovudine, indicating that either form of prodrug construct is a valid choice for the design of future drug candidates.

Conclusions

Reported here is the 11-step synthesis of a new cyanopyridone *C*-nucleoside that proceeds in an overall yield of 9%. The cyanopyridone was incorporated in an oligonucleotide by solid-phase synthesis and was shown to have a duplex-stabilizing effect when placed opposite deoxyadenosine residues. In unphosphorylated form, the cyanopyridone azidonucleoside derived from the 2′-deoxynucleoside was not found to be active against HIV in our assay system. However, the two phosphoramidate prodrug designs employed



Scheme 3. Synthesis of ProTide phosphoramidates; with *A*) the synthesis of **23** and *B*) the synthesis of **26**. *a*) 1. Phenyl dichlorophosphate, NEt_3, CH_2Cl_2; 2. *p*-nitrophenol, NEt_3, CH_2Cl_2, 52% over 2 steps. *b*) AZT, $MgCl_2$, $(^iPr)_2NEt$, MeCN, 87%. *c*) Phenyl dichlorophosphate, NEt_3, CH_2Cl_2. *d*) AZT, *N*-methylimidazole, CH_2Cl_2, 32%.

Table 2. Selectivity index of the activity of AZT phosphoramidates against the III_B and ROD strains of HIV versus zidovudine, didanosine and nevirapine as positive controls.[a]

	Zidovudine	Didanosine	Nevirapine	Compound **23**	Compound **26**
CC_{50}/IC_{50} of III_B strain	> 284	> 26	> 105	1644	167
CC_{50}/IC_{50} of ROD strain	> 706	> 43	> 1	3529	185

[a] See the *Experimental Section* for further details.

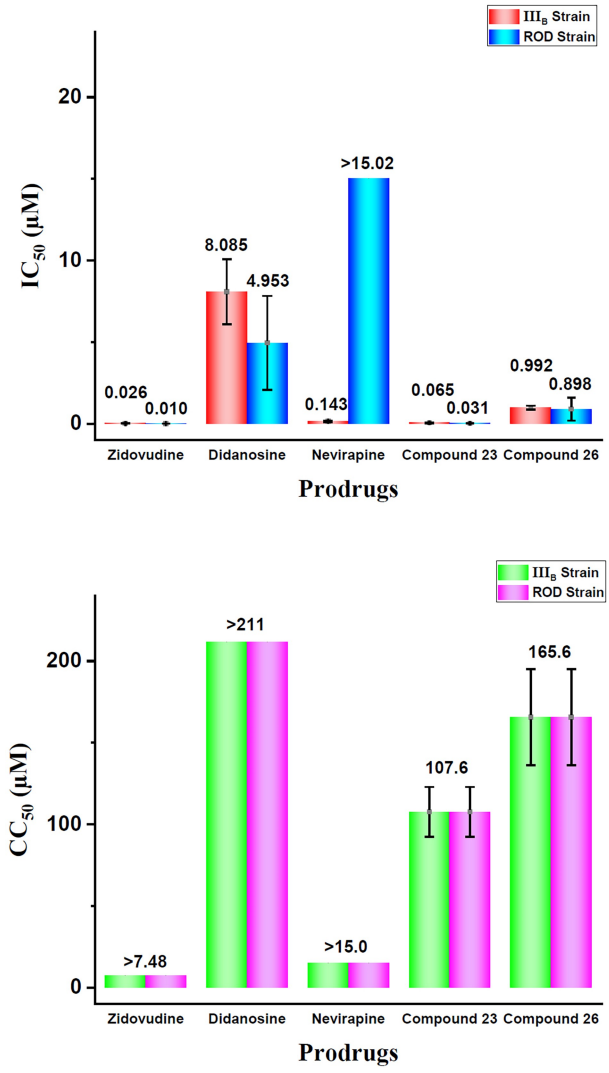


Figure 3. Plots of biological tests: *A*) antiviral activity against HIV in MT-4 cells for two strains of the virus, and *B*) cytotoxicity to exponentially growing MT-4 cells, as determined using the MTT assay.

in the second branch of our project offer an opportunity to test whether a singly phosphorylated species, as more advanced metabolite of the cellular phosphorylated cascade, helps to capitalize on the favorable base pairing properties of the cyanopyridone. Synthetic efforts along these lines are planned.

Experimental Part

General

Chemicals and solvents were purchased from commercial sources, including *Sigma–Aldrich* (Deisenhofen, Germany), *Acros Organics* (Geel, Belgium) and *TCI* (Eschborn, Germany), and used without further purification. The reactions were performed under inert atmosphere unless otherwise noted. Thin layer chromatography (TLC) was performed on pre-coated *ALUGRAM Xtra SIL G/UV254* sheets (*Macherey Nagel*, Düren, Germany). Spots were visualized under UV light (254 nm or 366 nm) and/or staining with Seebach solution (25 g phosphomolybdic acid hydrate, 10 g cerium (IV) sulfate tetrahydrate and 60 mL concentrated sulfuric acid ad 1 L water), followed by heating. For column chromatography, silica 60 M (0.040–0.063 mm, *Macherey Nagel*) was used. Product-containing fractions were visualized by TLC. The NMR spectra were recorded on *Bruker Advance* 300 MHz, 400 MHz, 500 MHz, or 700 MHz spectrometers. Mass spectra (ESI or HR-ESI-MS) were measured on a *Bruker micrOTOF-Q* spectrometer.

5-Amino-2-benzyloxy-6-cyanopyridine (**3**). In a 10 mL flask, bromopyridine **2** (1.00 g, 3.58 mmol), CuI (68.2 mg, 0.36 mmol, 0.1 equiv.), 1,10-phenanthroline (129 mg, 0.72 mmol, 0.2 equiv.) and KI (292 mg, 1.80 mmol, 0.5 equiv.) were suspended in dry *N*-methyl pyrrolidone (3 mL). The flask was sealed and stirred at 110 °C for 6 h under argon. Then acetone cyanohydrin (0.36 mL, 3.94 mmol, 1.1 equiv.) followed by tributylamine (1.00 mL, 4.30 mmol, 1.2 equiv.) were added and the mixture was stirred further at 110 °C for 60 h. The suspension was cooled to room temperature and then filtered through *Celite* eluting with diethyl ether (100 mL). The organic solution was washed twice with brine (10 % NaCl in H_2O, 2×50 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with petroleum ether/AcOEt (5:1 to 1:1, *v/v*). Cyanopyridine **3** (614 mg, 2.73 mmol, 76 %) was obtained as a colorless solid. R_f (petroleum ether/AcOEt 3:1): 0.37. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.45–7.33 (*m*, 5 H); 7.10 (*d*, *J* = 8.6, 1 H); 6.86 (*d*, *J* = 8.7, 1 H); 5.28 (*s*, 2 H); 4.11 (*s*, 2 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 156.6; 142.8; 137.0; 128.6; 128.4; 128.2; 128.1; 118.1; 116.5; 111.5; 68.3. HR-ESI-MS: 248.080 ($C_{13}H_{11}N_3O_5^+$, $[M+Na]^+$; calc. 248.079).

2-Benzyloxy-6-cyanopyridin-5-yl diazonium tetrafluoroborate (**4**). Cyanopyridine **3** (427 mg, 1.90 mmol) was dissolved in dry THF (3 mL) and cooled to −10 °C. Then, $BF_3 \cdot OEt_2$ (0.35 mL, 2.85 mmol, 1.5 equiv.) was added, followed by the addition of *tert*-butyl nitrite (0.27 mL, 2.28 mmol, 1.2 equiv.), and the mixture was stirred for 10 min at −10 °C. A light-yellow precipitate formed, and the solid was collected by vacuum filtration. This solid is labile and was used

directly in the next step without purification after drying *in vacuo* at 20 °C for 1 h. (Caution: Diazonium salts can be explosive and should be handled with care).

2-Benzyloxy-6-cyano-5-iodopyridine (**5**). To a solution of crude diazonium salt **4** (1.90 mmol) in dry acetonitrile (20 mL), potassium iodide solid (380 mg, 2.28 mmol, 1.2 equiv.) was added in one portion. The resulting mixture was stirred at room temperature for 2 h under argon. After TLC indicated full conversion, water (10 mL) was added, and the mixture was extracted twice with AcOEt (2×30 mL). The combined organic solution was dried over Na_2SO_4, and the solvent was removed *in vacuo*. The residue was purified by column chromatography using petroleum ether/AcOEt (9:1, *v/v*) as eluent. Compound **5** (381 mg, 1.13 mmol, 60% over two steps) was obtained as a light-yellow solid. TLC (petroleum ether/AcOEt 8:2, *v/v*): R_f = 0.75. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.97 (*d*, *J* = 8.5, 1 H); 7.46–7.33 (*m*, 5 H); 6.77 (*d*, *J* = 8.6, 1 H); 5.38 (*s*, 2 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 163.1; 148.4; 135.8; 135.7; 128.6; 128.5; 128.4; 117.7; 117.5; 87.5; 68.9. HR-ESI-MS: 358.965 ($C_{13}H_9IN_2O^+$, $[M+Na]^+$; calc. 358.965).

1′-β-(2-Benzyloxy-6-cyanopyridin-5-yl)-3′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-didehydro-1′,2′-dideoxy-D-ribofuranose (**7**). Palladium acetate (36.4 mg, 0.16 mmol, 0.2 equiv.) and tris(pentafluorophenyl)phosphine (172 mg, 0.32 mmol, 0.4 equiv.) were dissolved in dry acetonitrile (2 mL) and stirred at room temperature for 30 min. A suspension of aglycone **5** (381 mg, 1.13 mmol, 1.4 equiv.), Ag_2CO_3 (223 mg, 0.81 mmol, 1.0 equiv.) and glycal **6** (187 mg, 0.81 mmol, 1.0 equiv.) in dry acetonitrile (2 mL) were prepared in a separate flask. The solution of the Pd-catalyst was added to the suspension, and the reaction mixture was stirred for 20 h at room temperature. After TLC indicated full conversion, the slurry was filtered through celite, followed by washing with AcOEt. The solvent was removed *in vacuo*. And the crude product **7** was obtained as a brown waxy solid, that was used directly in the next step.

1′-β-(2-Benzyloxy-6-cyanopyridin-5-yl)-1′,2′-dideoxy-3′-oxo-D-ribofuranose (**8**). In a polypropylene tube, crude **7** (0.81 mmol) was dissolved in dry THF (10 mL), followed by the addition of $3HF \cdot NEt_3$ (264 µL, 1.62 mmol, 2.0 equiv.). The solution was shaken at room temperature for 30 min. The reaction was quenched by the addition of methoxytrimethylsilane (0.6 mL), and the mixture shaken for another 30 min. The suspension was filtered through *Celite* and concentrated under reduced pressure. The residue was purified by column chromatography using petroleum ether/AcOEt (9:1 to 1:1, *v/v*) as eluent. Compound **8** (128 mg, 0.40 mmol, 49% over two steps) was obtained as an orange waxy solid. R_f (petroleum ether/AcOEt 1:1, *v/v*) 0.49. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.95 (*d*, *J* = 8.6, 1 H); 7.48–7.33 (*m*, 5 H); 7.08 (*d*, *J* = 8.6, 1 H); 5.52 (*dd*, *J* = 11, 6.0, 1 H); 5.41 (*s*, 2 H); 4.10 (*t*, *J* = 3.3, 1 H); 4.03–4.01 (*m*, 2 H); 3.03 (*dd*, *J* = 11, 6.0, 1 H); 2.44 (*dd*, *J* = 11, 6.0, 1 H); 1.99 (*t*, *J* = 6.1, 1 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 211.7; 163.5; 137.5; 136.1; 134.4; 128.6; 128.4; 128.3; 128.1; 116.9; 82.4; 77.2; 73.8; 68.8; 61.5; 44.7. HR-ESI-MS: 347.100 ($C_{18}H_{16}N_2O_4^+$, $[M+Na]^+$; calc. 347.100).

1′-β-(2-Benzyloxy-6-cyanopyridin-5-yl)-1′,2′-dideoxy-5′-*O*-(dimethoxytrityl)-3′-oxo-D-ribofuranose (**9**). Ketone **8** (128 mg, 0.40 mmol) was co-evaporated twice from dry pyridine (5 mL) and then dissolved in dry pyridine (3 mL). Then, 4-dimethylaminopyridine (4.9 mg, 0.04 mmol, 0.1 equiv.) was added to the flask, and the resulting solution was stirred for 30 min at room temperature. 4,4′-Dimethoxytriyl chloride (DMT-Cl, 407 mg, 1.20 mmol, 3.0 equiv., freshly recrystallized from petroleum ether) was dissolved in dry pyridine (2 mL), and this solution was added to the reaction flask. The resulting mixture was stirred for 18 h at room temperature, until TLC showed full conversion. The solution was concentrated *in vacuo*, and the residue was taken up in a small volume of AcOEt. The suspension was filtered through silica gel, using petroleum ether/AcOEt (7:3, *v/v*) as eluent. The filtrate was concentrated under reduced pressure, to obtain crude **9** as a light-yellow foam. This crude product was directly used in the next step without further purification.

1′-β-(2-Benzyloxy-6-cyanopyridin-5-yl)-1′,2′-dideoxy-5′-*O*-(dimethoxytrityl)-D-xylofuranose (**10**). Crude **9** (0.40 mmol) was dissolved in dry THF (2 mL). Absolute ethanol (2 mL) was added, and this solution was cooled to −60 °C. Sodium borohydride (22.7 mg, 0.60 mmol, 1.5 equiv.) was ground to a fine powder and added to the flask. The mixture was stirred for 2 h at −60 °C. Then, cold acetone (−20 °C, 2 mL) was added slowly while the flask temperature was kept at −60 °C). The cold mixture was poured into AcOEt (50 mL), followed by the addition of saturated aqueous bicarbonate solution (50 mL). The two phases were

separated, and the organic layer was washed twice with brine (10% NaCl in H_2O, 2×50 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with petroleum ether/AcOEt (9:1 to 7:3, *v/v*). Pure xylonucleoside **10** (128 mg, 0.21 mmol, 51% over two steps) was obtained as an off-white foam. R_f (petroleum ether/AcOEt 7:3, *v/v*): 0.52. ^{1}H-NMR (400 MHz, CD_3CN): 8.00 (*d*, *J*=8.4, 1 H); 7.50–7.22 (*m*, 14 H); 7.06 (*d*, *J*=8.6, 1 H); 6.87 (*d*, *J*=8.8, 4 H); 5.37 (*s*, 2 H); 5.24 (*dd*, *J*=8.9, *J*=5.4, 1 H); 4.40–4.37 (*m*, 1 H); 4.11–4.07 (*m*, 1 H); 3.77 (*s*, 6 H); 3.45–3.30 (*m*, 2 H); 2.96 (*d*, *J*=4.1, 1 H); 2.81–2.73 (*m*, 1 H); 1.80–1.76 (*m*, 1 H). ^{13}C-NMR (176 MHz, CD_3CN): 163.7; 159.6; 146.2; 139.8;139.6; 137.7; 137.1; 137.0; 130.9; 129.4; 129.1; 129.0; 128.8; 127.8; 127.7; 117.4; 117.0; 113.9; 87.0; 83.9; 76.1; 72.8; 69.1; 63.8; 55.8; 43.8. HR-ESI-MS: 651.247 ($C_{39}H_{36}N_2O_6^+$, $[M+Na]^+$; calc. 651.247).

3′-Azido-1′-β-(2-benzyloxy-6-cyanopyridin-5-yl)-5′-*O*-(dimethoxytrityl)-1′,2′,3′-trideoxy-D-ribofuranose (**11**). Triphenylphosphine (82.9 mg, 0.32 mmol, 2.0 equiv.) was dissolved in dry THF (0.3 mL) and cooled to 0 °C in an ice bath. A solution of diisopropyl azodicarboxylate (62.0 µL, 0.32 mmol, 2.0 equiv.) in dry THF (0.2 mL) was prepared and added dropwise to the flask. The mixture was stirred for 15 min at 0 °C until formation of a white precipitate was observed. To this, compound **10** (99.3 mg, 0.16 mmol), dissolved in dry THF (0.5 mL), was added slowly, followed by the addition of diphenylphosphoryl azide (67.9 µL, 0.32 mmol, 2.0 equiv.) at 0 °C. The resulting mixture was allowed to warm to room temperature, and the suspension turned into a clear solution. The light-yellow solution was stirred further for 16 h at room temperature. After TLC indicated full conversion, water (0.5 mL) was added, and the mixture was poured into AcOEt (20 mL). This organic solution was washed twice with brine (10% NaCl in H_2O, 2×10 mL), dried over Na_2SO_4, and evaporated to dryness *in vacuo*. The residue was purified by column chromatography, using petroleum ether/AcOEt (9:1 to 8:2, *v/v*), to yield azide **11** (75.1 mg, 0.12 mmol, 73%) as an off-white foam. R_f (petroleum ether/AcOEt 7:3, *v/v*): 0.75. ^{1}H-NMR (400 MHz, CD_3CN): 7.89 (*d*, *J*=8.4, 1 H); 7.47–7.22 (*m*, 14 H); 7.04 (*d*, *J*=8.6, 1 H); 6.87 (*d*, *J*=8.4, 4 H); 5.37 (*s*, 2 H); 5.27 (*dd*, *J*=8.9, *J*=5.4, 1 H); 4.33–4.30 (*m*, 1 H); 4.05–4.02 (*m*, 1 H); 3.79 (*s*, 6 H); 3.31 (*d*, *J*=4.4, 2 H); 2.47–2.42 (*m*, 1 H); 2.17–2.11 (*m*, 1 H). ^{13}C-NMR (100 MHz, CD_3CN): 164.1; 159.7; 145.9; 139.0;137.7; 136.8; 136.7; 131.0; 129.5; 129.1; 129.0; 128.9; 128.8; 128.5; 127.9; 117.6; 116.7; 114.1; 87.3; 84.8; 76.9; 69.2; 64.6; 63.8; 55.9; 40.3. HR-ESI-MS: 676.252 ($C_{39}H_{35}N_5O_5^+$, $[M+Na]^+$; calc. 676.253).

3′-Azido-1′-β-(2-benzyloxy-6-cyanopyridin-5-yl)-1′,2′,3′-trideoxy-D-ribofuranose (**12**). To DMT-protected azidonucleoside **11** (43.0 mg, 65.8 µmol), deblock solution (3% trichloroacetic acid in CH_2Cl_2, 3.5 mL) was added. The solution turned red immediately, and this solution was stirred for 30 min at room temperature. After TLC-indicated full conversion, the mixture was poured into AcOEt (30 mL). The organic solution was washed twice with aqueous $NaHCO_3$ solution (saturated, 20 mL) and once with brine (10% NaCl in H_2O, 20 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The concentrate was purified by column chromatography using petroleum ether/AcOEt (7:3 to 1:1, *v/v*), yielding compound **12** (19 mg, 54 µmol, 82%) as an off-white solid. R_f (petroleum ether/AcOEt 1:1, *v/v*): 0.53. ^{1}H-NMR (400 MHz, CD_3OD): 8.04 (*d*, *J*=8.4, 1 H); 7.45–7.29 (*m*, 5 H); 7.11 (*d*, *J*=8.6, 1 H); 5.38 (*s*, 2 H); 5.28 (*dd*, *J*=9.9, *J*=5.8, 1 H); 4.33–4.27 (*m*, 1 H); 4.01–3.97 (*m*, 1 H); 3.73 (*d*, *J*=4.4, 2 H); 2.47–2.40 (*m*, 1 H); 2.15–2.09 (*m*, 1 H). ^{13}C-NMR (100 MHz, CD_3OD): 164.6; 139.4; 137.9; 136.9; 129.5; 129.3; 129.1; 128.8; 117.7; 116.7; 86.8; 77.5; 69.5; 64.3; 63.3; 40.8. HR-ESI-MS: 352.140 ($C_{18}H_{17}N_5O_3^+$, $[M+H]^+$; calc. 352.140).

3′-Azido-1′-β-(6-cyano-2-pyridon-5-yl)-1′,2′,3′-trideoxy-D-ribofuranose (**1**). Compound **12** (26.2 mg, 74 µmol) was dissolved in dry CH_2Cl_2 (0.7 mL), and the solution was cooled to −78 °C. Boron trichloride (1.0 M solution in CH_2Cl_2, 0.56 mL, 560 µmol, 7.5 equiv.) was added dropwise to the flask through a syringe. The mixture was allowed to slowly warm to −40 °C and stirred at this temperature for 5 h. Then, the mixture was cooled to −78 °C again and was then quenched by addition of methanol (0.5 mL). The solvents were removed under reduced pressure, and the residue was applied to a silica column. The product was purified by chromatography, eluting with petroleum ether/AcOEt (1:1 to 3:7, *v/v*). The deprotected azidonucleoside **1** (8.1 mg, 31 µmol, 42%) was obtained as colorless crystalline solid. R_f (petroleum ether/AcOEt 1:1, *v/v*): 0.13. ^{1}H-NMR (400 MHz, CD_3OD): 7.93 (*d*, *J*=8.4, 1 H); 6.90 (*d*, *J*=8.5, 1 H); 5.20 (*dd*, *J*=9.9, *J*=5.6, 1 H); 4.33–4.27 (*m*, 1 H); 4.01–3.97 (*m*, 1 H); 3.73 (*d*, *J*=4.4, 2 H); 2.40–2.33 (*m*, 1 H); 2.17–2.10 (*m*, 1 H). ^{13}C-NMR (100 MHz, CD_3OD): 165.0; 140.0; 133.2; 119.9; 115.4; 86.8; 77.4; 64.4; 63.4; 40.6. HR-ESI-MS: 284.076 ($C_{11}H_{11}N_5O_3^+$, $[M+Na]^+$; calc. 284.075); UV/Vis (methanol): λ_{max}=297, ε_{297}=5403 $M^{-1}cm^{-1}$.

1′-β-(2-Benzyloxy-6-cyanopyridin-5-yl)-1′,2′-dideoxy-D-ribofuranose (**13**). Ketone **8** (146 mg, 0.45 mmol) was dissolved in dry acetonitrile (5 mL) under argon and cooled to 0 °C. Then, sodium triacetoxyborohydride (237 mg, 1.12 mmol, 2.5 equiv.) was added, and the mixture stirred for 3 h at 0 °C until TLC monitoring indicated complete conversion. Methanol (7 mL) was added, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (30 g) with a gradient of methanol (0–5%) in CH_2Cl_2 to give *C*-nucleoside **13** (90 mg, 0.28 mmol, 62%) as a faint orange oil. R_f (CH_2Cl_2/methanol 9:1, *v/v*): 0.52. ^{1}H-NMR (700 MHz, $CDCl_3$): 7.79 (*d*, *J* = 8.6, 1 H); 7.47 (*d*, *J* = 7.1, 2 H); 7.41 (*t*, *J* = 7.3, 2 H); 7.37 (*t*, *J* = 7.3, 1 H); 7.03 (*d*, *J* = 8.8, 1 H); 5.44–5.43 (*m*, 1 H); 5.41 (*s*, 2 H); 4.57–4.55 (*m*, 1 H); 4.08–4.07 (*m*, 1 H); 3.94–3.84 (*m*, 2 H); 2.39 (*dd*, *J* = 13.0, 6.2, 1 H); 2.04 (*dd*, *J* = 13.2, 9.2, 1 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 163.0; 137.8; 136.2; 135.6; 128.5; 127.5; 116.5; 87.8; 76.6; 73.5; 68.7; 63.1; 44.0. HR-ESI-MS: 349.115 ($C_{18}H_{18}N_2O_4^+$, $[M+Na]^+$; calc. 349.115).

1′-β-(6-Cyano-2-pyridon-5-yl)-1′,2′-dideoxy-D-ribofuranose (**14**). Benzyl-protected *C*-nucleoside **13** (100 mg, 0.31 mmol) was dissolved in dry acetonitrile (1.5 mL) under argon atmosphere in a *Schlenk* tube over molecular sieves (3 Å). To this mixture was added sodium iodide (46 mg, 0.31 mmol, 1.0 equiv.) and freshly distilled TMS chloride (40 µL, 0.31 mmol, 1.0 equiv.) under argon atmosphere, followed by stirring for 1 h at room temperature. When monitoring by TLC indicated incomplete conversion, additional TMS chloride (40 µL, 0.31 mmol, 1.0 equiv.) and sodium iodide (46 mg, 0.31 mmol, 1.0 equiv.) were added. After stirring for 1 h, the reaction mixture was applied directly to a silica column (15 g), eluting with a gradient of 0–7% methanol in CH_2Cl_2. Ribonucleoside **14** (55 mg, 0.23 mmol, 75%) was obtained as an orange glass. R_f (CH_2Cl_2/methanol = 9:1, *v/v*): 0.23. ^{1}H-NMR (700 MHz, CD_3OD): 7.94 (*d*, *J* = 8.5, 1 H); 6.89 (*d*, *J* = 9.8, 1 H); 5.35–5.30 (*m*, 1 H); 4.38–4.36 (*m*, 1 H); 3.98–3.95 (*m*, 1 H); 3.69–3.68 (*m*, 2 H); 2.24 (*dd*, *J* = 5.9, 7.5, 1 H); 1.99–1.94 (*m*, 1 H). ^{13}C-NMR (125 MHz, CD_3OD): 164.8; 140.2; 133.9; 115.2; 89.6; 77.3; 74.3; 63.7; 43.8: HR-ESI-MS: 259.068 ($C_{11}H_{12}N_2O_4^+$, $[M+Na]^+$; calc. 259.068). UV/Vis (H_2O): λ_{max} = 311, ε_{311} = 2144 $M^{-1}cm^{-1}$, ε_{260} = 1876 $M^{-1}cm^{-1}$.

1′-β-(6-Cyano-2-pyridon-5-yl)-1′,2′-dideoxy-5′-O-(dimethoxytrityl)-D-ribofuranose (**15**). The DMT protection followed the protocol for preparation of compound **9**, as described above. Ribonucleoside **14** (22 mg, 0.09 mmol) was reacted with DMT chloride (46 mg, 0.14 mmol, 1.4 equiv.) and 4-dimethylaminopyridine (1.2 mg, 0.01 mmol, 0.1 equiv.) in dry pyridine (1 mL). The crude was purified by column chromatography (gradient 0–5% methanol in CH_2Cl_2) to yield nucleoside **15** (23 mg, 0.04 mmol, 45%) as a colorless glass. R_f (CH_2Cl_2/methanol 95:5, *v/v*): 0.76. ^{1}H-NMR (700 MHz, CD_3CN): 7.77 (*d*, *J* = 8.7, 1 H); 7.48–7.22 (*m*, 9 H); 6.90 (*d*, *J* = 8.8, 1 H); 6.88 (*d*, *J* = 8.8, 4 H); 5.32–5.29 (*m*, 1 H); 4.36–4.34 (*m*, 1 H); 4.03–4.02 (*m*, 1 H); 3.79 (*s*, 6 H); 3.25–3.22 (*m*, 2 H); 2.28–2.24 (*m*, 1 H); 1.96–1.93 (*m*, 1 H). ^{13}C-NMR (125 MHz, CD_3CN): 163.5; 159.2; 145.7; 138.9; 136.6; 136.5; 134.3; 130.6; 130.5; 128.6; 128.4; 127.4; 125.7; 118.3; 115.5; 113.6; 87.3; 86.5; 76.4; 73.7; 64.8; 55.5; 46.3; 43.1: HR-ESI-MS: 539.211 ($C_{32}H_{30}N_2O_6$, $[M+H]^+$; calc. 539.210).

(*S*)-Methyl 2-(((4-nitrophenoxy)(phenoxy) phosphoryl) amino) propanoate (**22**). Phenyl dichlorophosphate (214 µL, 1.43 mmol) was dissolved in dry CH_2Cl_2 (1 mL) and cooled to 0 °C. A solution of L-alanine methyl ester hydrochloride (0.20 g, 1.43 mmol, 1.0 equiv.) in dry CH_2Cl_2 (1 mL) was prepared and added to the flask. The reaction mixture was cooled to −30 °C and stirred for 30 min in the cold, followed by the dropwise addition of triethylamine (396 µL, 2.86 mmol, 2.0 equiv.). The slurry was allowed to slowly warm to room temperature and stirred overnight. The resulting suspension was cooled to 0 °C, and 4-nitrophenol (200 mg, 1.43 mmol, 1.0 equiv.) was added in one portion. Triethylamine (198 µL, 1.43 mmol, 1.0 equiv.) was added slowly, and the resulting mixture was stirred at room temperature for 12 h. The slurry was filtered, and the filtrate was concentrated *in vacuo*. Then, THF (2 mL) was added, the mixture was stirred for 5 min, then kept at 4 °C for 1 h. The precipitate was filtered off, and the solvent was removed under reduced pressure. The residue was then purified by column chromatography, eluting with petroleum ether/AcOEt (7:3 to 1:1, *v/v*), to afford compound **22** (282 mg, 0.74 mmol, 52%) as a colorless waxy solid (mixture if diastereomers). R_f (petroleum ether/AcOEt = 7:3, *v/v*): 0.21. ^{1}H-NMR (500 MHz, $CDCl_3$): 8.12–8.08 (*m*, 2 H); 7.29–7.07 (*m*, 7 H); 4.08–4.00 (*m*, 1 H); 3.85 (*q*, *J* = 11, 1 H); 3.58 (*s*, 3 H); 1.30–1.25 (*m*, 3 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 173.5; 155.6; 150.3; 144.8; 130.0; 125.7; 125.6; 120.9; 120.2; 52.8; 50.5; 21.1. ^{31}P-NMR (202 MHz, $CDCl_3$): −3.25, −3.30. HR-ESI-MS: 403.067 ($C_{16}H_{17}N_2O_7P^+$, $[M+Na]^+$; calc. 403.067).

Phosphoramidate ProTide with L-Alanine Methyl Ester (**23**). In a *Schlenk* flask, zidovudine (AZT, 40.6 mg, 0.15 mmol), nitrophenyl-phosphoramidate **22** (145 mg, 0.38 mmol, 2.5 equiv.) and anhydrous $MgCl_2$ (13.8 mg, 0.15 mmol, 1.0 equiv.) were suspended in dry MeCN (1.5 mL). The slurry was heated to 50 °C and stirred for 15 min in an argon atmosphere. Then, *N,N*-diisopropylethylamine (65 μL, 0.38 mmol, 2.5 equiv.) was added, and the resulting mixture was stirred at 50 °C for 1 h. After cooling to room temperature, the mixture was poured into AcOEt (20 mL). The organic solution was washed with aqueous citric acid solution (5 %, 10 mL), NH_4Cl/H_2O (saturated, 10 mL), twice with K_2CO_3/H_2O (5 %, 10 mL) and finally with brine (10 mL). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The concentrate was purified by column chromatography, eluting with CH_2Cl_2/methanol (99 : 1 to 95 : 5, *v/v*), to yield compound **23** as a mixture of diastereomers (66.1 mg, 0.13 mmol, 87 %) as a colorless foam. R_f (CH_2Cl_2/methanol = 100 : 5, *v/v*): 0.32. The spectroscopic data were in agreement with the literature.[24]

Phosphoramidate ProTide with *L*-Proline Methyl Ester (**26**). Phenyl dichlorophosphate (90 μL, 0.60 mmol, 2.0 equiv.) was dissolved in dry CH_2Cl_2 (0.5 mL) and cooled to 0 °C. Then, L-proline methyl ester hydrochloride (100 mg, 0.60 mmol, 2.0 equiv.) was added, and the solution was cooled to −30 °C. A solution of triethylamine (166 μL, 1.20 mmol, 4.0 equiv.) in dry CH_2Cl_2 (0.5 mL) was prepared and slowly added. The resulting mixture was allowed to warm to room temperature and stirred for 1 h under argon. After full conversion of the starting material, the solvent was removed *in vacuo*, THF (1 mL) was added, and the slurry was filtered. The filtrate was dried *in vacuo*, and the crude residue was stored in the cold before use. In a separate flask, zidovudine (AZT, 80.2 mg, 0.30 mmol) was suspended in dry CH_2Cl_2 (1 mL), followed by the addition of *N*-methylimidazole (96 μL, 1.20 mmol, 4.0 equiv.). After cooling to −10 °C, a solution of the crude reagent in dry CH_2Cl_2 (0.5 mL) was added dropwise. The resulting mixture was stirred while allowing to warm from −10 °C to room temperature, and then for an additional 12 h at room temperature. The slurry was concentrated, and was then applied to a silica column, followed by chromatography, eluting with CH_2Cl_2/methanol (100 : 1 to 100 : 5, *v/v*). Phosphoramidate **26** (51.3 mg, 96 μmol, 32 %, mixture of diastereomers) was obtained as a colorless foam. R_f (CH_2Cl_2/methanol = 100 : 5, *v/v*): 0.46. ^{1}H-NMR (700 MHz, $CDCl_3$): 8.29 (*s*, 1 H); 7.48–7.46 (*m*, 1 H); 7.36–7.30 (*m*, 2 H); 7.24–7.17 (*m*, 3 H); 6.26 (*t*, *J* = 7.0, 1 H); 4.50–4.45 (*m*, 2 H); 4.42–4.38 (*m*, 1 H); 4.36–4.33 (*m*, 1 H); 4.11–4.07 (*m*, 1 H); 3.71 (*s*, 3 H); 3.36–3.27 (*m*, 2 H); 2.36–2.30 (*m*, 1 H); 2.14–2.07 (*m*, 2 H); 2.01–2.00 (*m*, 1 H); 1.92 (*s*, 3 H); 1.91–1.90 (*m*, 1 H); 1.84–1.82 (*m*, 1 H). ^{13}C-NMR (176 MHz, $CDCl_3$): 174.3; 163.4; 150.6; 150.1; 135.4; 130.1; 125.3; 119.8; 111.3; 84.6; 82.9; 65.9; 61.1; 60.7; 52.5; 46.7; 37.6; 31.1; 25.4; 12.7. ^{31}P-NMR (283 MHz, $CDCl_3$): 1.98. HR-ESI-MS: 557.152 ($C_{22}H_{27}N_6O_8P^+$, $[M+Na]^+$; calc. 557.152).

Oligonucleotide 19. The oligodeoxynucleotide sequence 5′-GCAG-3′ was assembled on long chain alkylamine controlled pore glass (LCAA cpg) by automated solid-phase synthesis using an *H-8* DNA synthesizer from *K&A Laborgeräte* (Schaafheim, Germany) according to the manufacturer's recommendation. For this, the support loaded with the first nucleoside, cpg **16** (1 μmol loading) was placed in the synthesis column, followed by coupling cycles with successive deblocking (3 % trichloroacetic acid in CH_2Cl_2), coupling (0.25 M 4,5-dicyanoimidazole and the respective phosphoramidite in dry acetonitrile), capping (cap A, 9 % acetic anhydride in THF plus cap B, THF/pyridine/methylimidazole, 8 : 1 : 1), and oxidation (3 % iodine in THF/pyridine/water). After three cycles, cpg-bound **17** was obtained. Then, **17** was treated with deblock solution and removed from the DNA synthesizer. After drying *in vacuo*, diisopropylammonium tetrazolide (27 mg, 150 μmol) was added, followed by brief vacuum drying. Then, dry acetonitrile (1 mL) and molecular sieves (3 Å) were added, followed by the addition of 2-cyanoethyl-*N,N,N′,N′*-tetraisopropylphosphordiamidite (100 μL, 315 μmol). The mixture was shaken for 90 min at room temperature. The supernatant was removed, and the solid phase was washed with dry acetonitrile (3 × 800 μL). Subsequently, a solution of nucleoside **15** (10 mg, 20 μmol) in tetrazole-contained acetonitrile (0.45 M, 400 μL) was added. The mixture was shaken for 2 h. The supernatant was removed and a solution (0.02 M iodine in THF/pyridine/water, 750 μL) was added. The mixture was shaken for 10 min, and the supernatant was removed. The remaining solid support was washed with acetonitrile (5 × 800 μL) and dried *in vacuo* to give solid support **18**. The 5′-terminal deoxyguanosine residue was then added on the DNA synthesizer using the chain extension cycle given above, followed by a final deblocking step. Oligonucleotide **19** (35 nmol, 4 %) was obtained after deprotection and cleavage from the solid phase by treating

with 25% aqueous ammonia solution (1 ml) in a polypropylene reaction vessel at 55 °C overnight and HPLC purification (*C18* column, gradient of 0–15% MeCN in H_2O, 0.6 mL/min, 35 min). MALDI-TOF-MS: 1785 ($C_{60}H_{73}N_{22}O_{33}P_5^-$, [M-H]$^-$; calc. 1783).

UV-Melting Curves

Measurements of oligonucleotides solutions were carried out on a *Lambda 25* spectrometer from *Perkin Elmer* (Waltham, USA), using Templab 2.0 software for the gradient. Absorbance was monitored over the temperature range of 5–80 °C, with a gradient of 1 °C/min. Evaporation was suppressed with parafilm sealing. Absorbance was measured at 260 nm using a cuvette of 1 cm path length containing a buffer solution (1 M sodium chloride, 0.1 M phosphate buffer, pH = 7) and an oligonucleotide concentration of 8.8 μM. Four curves were measured, two heating and two cooling curves, with the first measurement preceded by an annealing step consisting of brief heating to 80 °C, followed by cooling to 5 °C. Melting points were determined as the extremum of the first derivative of the curves, using the UV-Winlab software. Melting points are the average of four experiments.

Antiviral Assays

Evaluation of the antiviral activity of the compounds against HIV in MT-4 cells was performed using the MTT assay as described below. Stock solutions (10×final concentration) of test compounds were added in 25 μL volumes to two series of triplicate wells to allow for the simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial five-fold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a *Biomek 3000* robot (*Beckman Instruments*, Fullerton, CA, USA). Untreated HIV- and mock-infected cell samples were included as controls. HIV stock (50 μL) at 100–300 $CCID_{50}$ (50% cell culture infectious doses) or culture medium was added to either the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effects of the test compound on uninfected cells to assess the test compounds' cytotoxicity. Exponentially growing MT-4 cells were centrifuged for 5 min at 220 g, and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells/mL, and 50 μL volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock-and HIV-infected cells was examined spectrophotometrically using the MTT assay. The MTT assay is based on the reduction of yellow colored 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (*Acros Organics*) by mitochondrial dehydrogenase activity in metabolically active cells to a blue-purple formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (*Infinite M1000*, *Tecan*) at two wavelengths (540 and 690 nm). All data were calculated using the median absorbance value of three wells. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the test compound that reduced the absorbance (OD_{540}) of the mock-infected control sample by 50%. The concentration achieving 50% protection against the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration.

Supporting Information

Supporting information for this article (^{1}H- and ^{13}C-NMR spectra, as well as a MALDI-ToF mass spectrum of **19**) is available on the WWW under https://doi.org/10.1002/hlca.202200157.

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Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Author Contribution Statement

J. H. and *J. A.* performed the syntheses and analyzed the data, *C. P.*, *G. A.*, and *R. S.* conceived and performed antiviral assays. *C. R.* designed and led the project. *C. R.*, *J. H.*, *J. A.* and *C. P.* wrote the manuscript.

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