First Example of Phosphoramidate Approach Applied to a 4′-Substituted Purine Nucleoside (4′-Azidoadenosine): Conversion of an Inactive Nucleoside to a Submicromolar Compound versus Hepatitis C Virus

Plinio Perrone,[†] Felice Daverio,[†] Rocco Valente,[†] Sonal Rajyaguru,[‡] Joseph A. Martin,[‡] Vincent Lévêque,[‡] Sophie Le Pogam,[‡] Isabel Najera,[‡] Klaus Klumpp,[‡] David B. Smith,[‡] and Christopher McGuigan*[,†]

Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff CF10 3XF, UK, and Roche Palo Alto, 3431 Hillview Avenue, Palo Alto, California 94304

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We report on the synthesis of the anti hepatitis C virus (HCV) agent 4′-azidoadenosine (**1**) and the application of the phosphoramidate ProTide technology to this nucleoside. The synthesis of **1** was achieved through an epoxide intermediate followed by regio- and stereoselective ring opening by azidotrimethylsilane in the presence of a Lewis acid. Compound **1** did not inhibit HCV replication in cell culture at concentrations up to 0.1 mM. However, a submicromolar active agent could be derived from **1** by the application of the ProTide technology. All the phosphoramidates prepared were L-alanine derivatives with variations in the aryl moiety and in the ester part of the amino acid. The benzyl ester and the 1-naphthyl phosphate (**18**) had the best activity in replicon assay. Phosphoramidates (**18**−**21**) achieved a significant improvement in antiviral potency over the parent nucleoside (**1**) with no increase in cytotoxicity.

Introduction

The hepatitis C virus (HCV) was identified for the first time in 1989 as a single-stranded positive sense RNA virus of the flaviviridae family.[1] HCV is the most common blood-borne infection and a major cause of chronic liver disease and liver transplantation in industrialized countries. According to the World Health Organization (WHO), more than 170 million people are estimated to be chronically infected by this virus, representing the 3.1% of worldwide population.[2]

Current therapy for HCV treatment is both poorly tolerated and has limited efficacy, with less than 50% response rates among patients infected with the most prevalent virus genotype. Therefore, there is a need for more efficient and better tolerated anti HCV agent.

The HCV genome is a single strand RNA molecule of approximately 9600 nucleotides,[1] containing coding sequences for structural and nonstructural HCV proteins, flanked by 5′- and 3′-nontranslated regions (5′-NTR and 3′-NTR)[a] at the extremities of the HCV genome.[1,3,4]

Modified nucleosides already represent an important class of polymerase inhibitors of other viruses, such as HSV and HIV. It is notable that nucleoside derivatives generally need to be phosphorylated to their corresponding triphosphate by host cell (or in the case of some antiherpetics viral) kinases to be converted to their corresponding pharmacologically active species.

However, in many cases nucleoside analogues are poor substrates for nucleoside kinases. Moreover, the dependence on phosphorylation for activation of a particular nucleoside may be a problem in cells where the nucleoside kinase activity is low or even lacking. The pharmacologically active triphosphate species cannot be considered as a possible drug candidate because of high instability and poor cellular permeation. For many nucleosides the first phosphorylation constitutes the rate-limiting step in the synthetic pathway to triphosphate formation, suggesting that monophosphate analogues might be useful antiviral agents. However, nucleoside monophosphates are also generally unstable in blood and show poor membrane permeation.[9]

In order to bypass this first rate-limiting step required of nucleoside analogues, our group has developed in the past a suitable nucleotide delivery strategy. The aryloxy-phosphoramidate (**2**) allows the bypass of the initial nucleoside kinase dependence, by the intracellular delivery of the monophosphorylated nucleoside analogue in the membrane permeable "ProTide" form.[10,11] This technology increases the lipophilicity of the nucleoside monophosphate analogues and the intracellular availability by passive diffusion. Previously we have reported the success of the ProTide approach applied to various nucleoside analogues including d4A, ddA, L-Cd4A, and d4T.[12−14]

In these examples the corresponding aryloxy-phosphoramidates have shown an enhancement in antiviral activity against target viruses such as HIV and HBV compared to the parent nucleoside analogues *in vitro*. In contrast to the nucleoside analogues, the phosphoramidates retained full antiviral activity in kinase-deficient cell lines.

The putative monophosphate release pathway involves initial enzyme-catalyzed cleavage of the carboxy ester, followed by the internal nucleophilic attack of the acid residue on the phosphorus center, displacing the aryloxy group.[13] The putative transient, cyclic mixed-anhydride is then hydrolyzed to the corresponding amino acid phosphomonoester which has been observed both *in vitro* and *in vivo*.[13]

4′-Azidocytidine has been identified as a potent inhibitor of HCV replication in cell culture.[8] The corresponding 4′-azidocytidine triphosphate is a competitive inhibitor of RNA synthesis by HCV polymerase.[8] As part of an effort to characterize structure−activity relationships in this series of 4′-substituted

* Corresponding author. Tel. +44 29 20874537; fax: +44 29 20874537; e-mail: mcguigan@cardiff.ac.uk.

[†] Cardiff University.

[‡] Roche Palo Alto.

[a] Abbreviations: 5′-NTR, 5′-nontranslated region; 3′-NTR, 3′-nontranslated; HIV, human immunodeficiency virus; HSV, herpes simplex virus; d4A/d4T, 2′,3′-dideoxy-2′,3′-didehydroadenosine/thymidine; ddA, 2′,3′-dideoxyadenosine; L-Cd4A, (1*R*,*cis*)-4-(6-amino-9*H*-purin-9-yl)-2-cyclopentene-1-methanol.

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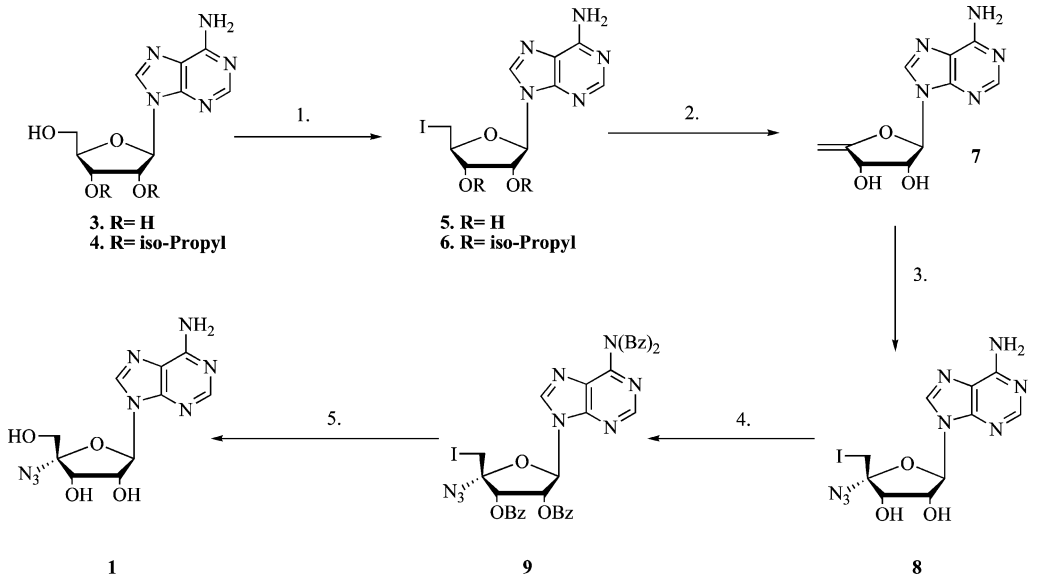
Scheme 1. Initial Pathway for the Synthesis of 4′-Azidoadenosine (**1**)[a]



[a] 1. See Table 1; 2. tBuOK, pyridine; 3. ICl, NaN_3, DMF; 4. BzCl, pyridine; 5. a. MCPBA, CH_2Cl_2/H_2O, b. MeONa, MeOH.

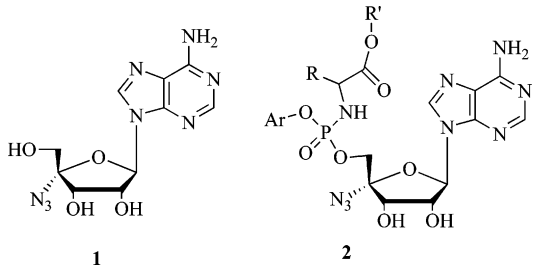


Figure 1. 4′-Azidoadenosine (**1**) and general structure of its corresponding aryloxy-phosphoramidate.

Table 1. Conditions Attempted for the Synthesis of 5′-Deoxy-5′-iodoadenosine

solvent	reagent	source of iodine	conditions
dioxane/pyridine	Ph_3P	I_2	r.t.
NMP	Ph_3P/imidazole	I_2	r.t.
HMPT	Ph_3P	CI_4	r.t.
pyridine	Ph_3P	I_2	r.t.
pyridine	PS-Ph_3P	I_2	50 °C

nucleosides, 4′-azidoadenosine (**1**) was synthesized and evaluated as a potential inhibitor of HCV replication.[16] The ProTide approach was applied to **1** to explore monophosphate delivery and a potential increase of the antiviral activity. In addition it was envisaged that delivering the adenosine analogue as a monophosphate derivative could reduce the extent of intracellular metabolism by deamination.

Results and Discussion

Chemistry. The synthesis of 4′-azidoadenosine (**1**) was initially performed using the same conditions reported in the literature (Scheme 1).[17] The selective 5′-iodination of adenosine was first attempted in the presence of dioxane−pyridine as solvent, but because of the low solubility of the starting material (**3**), the yield was poor. In an attempt to increase the yield, different conditions were attempted (Table 1). The first alternative was to use triphenylphosphine, iodine, and an excess of imidazole in a solution of *N*-methylpyrrolidinone (NMP).[18] This procedure resulted in only a modest 26% yield with suboptimal purity of the desired product 5′-deoxy-5′-iodoadenosine (**5**).[18]

Another attempt was performed using pyridine as solvent. In this case, after purification by column chromatography, ^1H NMR and ^{31}P NMR showed clear evidence of the presence of triphenylphosphine oxide. An extraction in EtOAc/H_2O was explored as a purification method without success.

In order to use a different source of iodine, we tried the iodination reaction using CI_4 and triphenylphosphine in a solution of adenosine and hexamethylphosphoric acid triamide (HMPA).[19] The difficulty of removing the solvent was a limitation of this reaction on large scale.

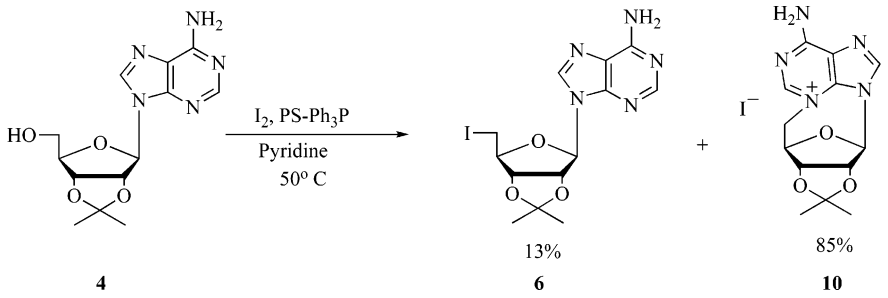
As it was proving difficult to avoid the presence of triphenylphosphine mixed with the desired product, the same reaction was performed using triphenylphosphine supported on resin (polystyrene, PS-triphenylphosphine). In this case the desired product (**5**) was obtained pure and in reasonable yield. Because of the high polarity of **5**, this route required large amounts of EtOAc to achieve extraction.

In an attempt to increase the lipophilicity of the desired 5′-iodo derivative the same reaction was also performed using as starting material the 2′,3′-isopropylidene-protected adenosine (**4**) with PS-triphenylphosphine. In this case, the reaction required an increase in temperature to 50 °C. After purification by extraction, the pure desired product (**6**) was obtained in poor yield (13%). We noted the presence of a high polar compound: it was isolated and fully characterized, and by H NMR we attribute the structure to the cyclic product of intramolecular nucleophilic substitution in the 5′-position from the nitrogen in 3-position (**10**, Scheme 2).[20−22]

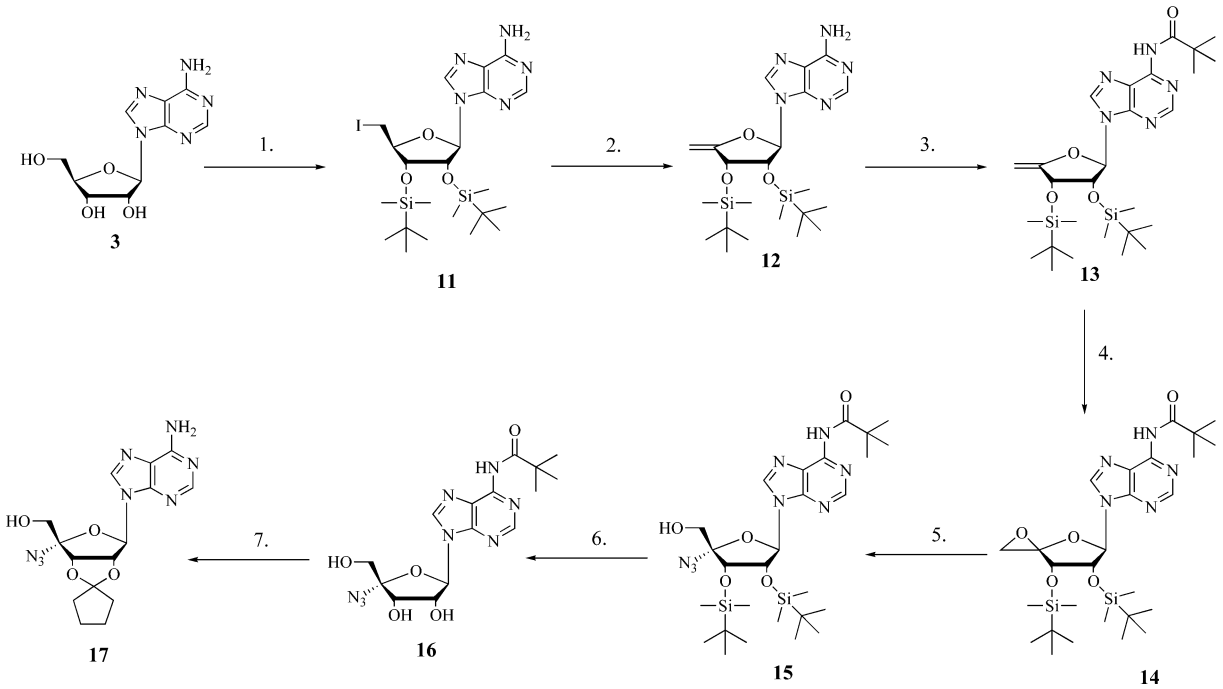
Because of the impossibility to avoid the use of the column chromatography to purify **5**, this first step was repeated on a large scale using column chromatographic purification. The elimination step was performed according the reported procedure (Scheme 1) in the presence of a solution of potassium *tert*-butoxide in pyridine.[17] The regio- and stereoselective addition of iodo-azide resulted successful only in the first attempt. The final three steps involved the full protection of adenosine followed by iodine displacement and deprotection with methanolic ammonia (Scheme 1).

A different synthetic pathway (Scheme 3) was envisioned in which the introduction of the azido group at 4′-position was achieved trough an epoxide intermediate. In the literature this

Scheme 2. Iodination Attempted with PS-Ph_3P and Structure of the Side Product Isolated (**10**)



Scheme 3. Alternative Pathway for the Synthesis of 2′,3′-Cyclopentylidene-4′-azidoadenosine[a]



[a] 1. a. I_2, pyridine, Ph_3P; b. TBDMSCl, imidazole, pyridine; 2. tBuOK, pyridine; 3. pivaloyl chloride, DIEA, DCM; 4. DMDO 1 M in acetone; 5. $TMSN_3$, $SnCl_4$; 6. TBAF, THF;.7. a. dimethoxycyclopentane; b. NH_3/MeOH.

method has never been applied to introduce an azido group to a purine nucleoside.

The modified synthetic pathway started with the selective 5′-iodination followed by protection of 2′ and 3′-OH with *tert*-butyl dimethylsilyl groups. The elimination of HI was performed in the same conditions described above. The key step was the synthesis of the epoxide (**14**) and the subsequent ring opening reaction. In order to avoid N1-oxidation with DMDO, it was necessary to acylate the 6-NH_2 group (**13**). The unsaturated derivative of adenosine (**13**) was converted to the corresponding epoxide (**14**) in the presence of 1 M solution of dimethoxydioxirane, which has been previously reported as stereoselective reagent for such epoxidation reactions.[23] The ring opening reaction was then performed in the presence of a Lewis Acid (tin tetrachloride) and azidotrimethylsilane. The obtained product (**15**) was subsequently deprotected in 2′- and 3′-positions in presence of a solution of tetrabuthylammonium fluoride (TBAF). For the synthesis of the 5′-phosphoramidate the protection with a cyclopentylidine ketal group of 2′ and 3′-OH was performed (**16**). This protection guaranteed a selective 5′-phosphorylation and an increased in the solubility of the nucleoside in THF, which was the solvent used in the synthesis of the phosphoramidate.

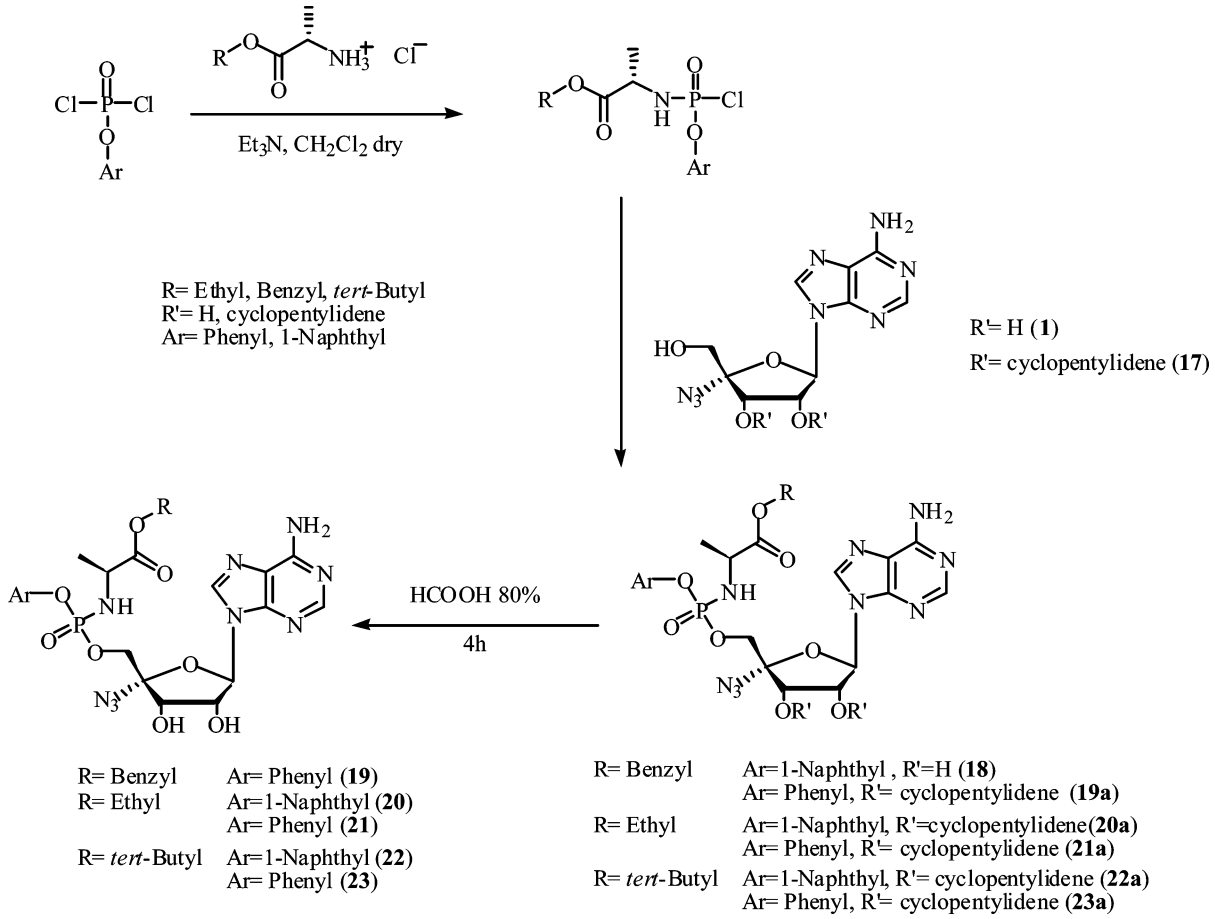
The pivaloyl group was removed in presence of a saturated solution of ammonia in methanol to give the desired product (**17**) in high yield (87%).

The synthesis of the phosphoramidate was performed following the Uchiyama procedure in the presence of *tert*-butyl magnesium chloride (1 M solution in THF).[24] The deprotection step was performed in the presence of a solution of 80% HCOOH in water for 4 h at room temperature (Scheme 4). Because of the stereochemistry at the phosphorus center, the final compounds were isolated as mixtures of two diastereoisomers. The confirmation of the presence of two diastereoisomers was shown by ^{31}P NMR (two peaks) and ^{1}H NMR (splitting of many of the nucleoside signals).

Considering the established SAR previously reported, we decided to focus on L-alanine phosphoramidates with ester and aryl moiety variation. In previous work L-alanine had shown the best activity with 1-naphthyl as aryl moiety and the benzyl group as ester. We choose to have a variation of three ester (*tert*-butyl, ethyl, and benzyl) and 2 aryl moieties (1-naphthyl and phenyl).[14]

Antiviral Activity. The phosphoramidates (**18**−**23**) were characterized *in vitro* as inhibitors of HCV replication in the HCV replicon assay using similar conditions as described.[8] Data are presented in Table 2 as EC_{50} values representing the concentration of compound reducing HCV replication by 50% and CC_{50} values representing the concentration of compound reducing cell viability by 50% as determined using the WST assay. All of the compounds showed CC_{50} values >100 μM. The parent compound (**1**) did not inhibit HCV replication (EC_{50}

Scheme 4. Synthesis of 4'-Azidoadenosine Phosphoramidates[a]



[a] Compound **18** has been synthesized using **1**, and it did not need deprotection. Compound **19**, **20**, **21**, **22**, **23** have been synthesized using 2′,3′-cyclopentylidene-4′-azidoadenosine (**17**).

Table 2. Anti-HCV Activity and Cytotoxicity Data for AZA and Aryl Phosphoramidate Nucleoside Analogues

compd	ester	aryl moiety	EC_{50} (μM)	CC_{50} (μM)
18	benzyl	α-naphthyl	0.22	>100
19	benzyl	phenyl	4.00	>100
20	ethyl	α-naphthyl	0.59	>100
21	ethyl	phenyl	1.50	>100
22	*tert*-butyl	α-naphthyl	>100	>100
23	*tert*-butyl	phenyl	>100	>100
4-azidoadenosine (**1**)			>100	>100

> 100 μM). In contrast, a number of phosphoramidate derivatives (**18**−**21**) showed potent inhibition of HCV replication. Assuming that 4′-azidoadenosine-5′-triphosphate is the active HCV polymerase inhibitor, these results support the notions that (a) the active phosphoramidates successfully delivered 4′-azidoadenosine monophosphate to the replicon cells, that (b) 4′-azidoadenosine (**1**) is a poor substrate for kinases in replicon cells and that (c) 4′-azidoadenosine monophosphate is efficiently phosphorylated to the 5′-triphosphate in replicon cells. Therefore, the phosphoramidate approach could successfully overcome the first phosphorylation of **1** and converted an inactive nucleoside analogue into a potent inhibitor of HCV replication.

Considering previously reported SAR of phosphoramidates, we chose to focus our attention on L-alanine which previously provided the most potent pharmacological activity in a range of antiviral and antiproliferative assays.

Among the three esters tested, the *tert*-butyl esters (**22**, **23**) were found to be inactive as inhibitors of HCV replication, whereas benzyl and ethyl esters were potent inhibitors with either 1-naphthyl or phenyl as aryl moiety (**18**, **19**). The lack of antiviral activity of *tert*-butyl ester phosphoramidates may be related to the relative stability of tertiary esters to enzyme-mediated hydrolysis. Comparing compound **18** and **19**, we noticed an increase of antiviral activity of 20 fold for the 1-naphthyl relative to phenyl as aryl moiety. This difference might be related to the higher lipophilicity and/or the decrease of pK_a of 1-naphthyl relative to phenyl. In the case of ethyl ester, instead the difference observed between 1-naphpthyl (**20**) and phenyl phosphoramidate (**21**) was not significant. There was no significant difference in antiviral potency between the ethyl and benzyl esters, indicating that both of these esters are good substrates for the esterase involved in phosphoramidate conversion.

In conclusion, the most potent compounds in this series of L-alanine ProTides were the 1-naphthyl derivatives with either benzyl (**18**) or ethyl ester moieties (**20**). These compounds inhibited HCV replication in the HCV replication system with EC_{50} values of 0.22 and 0.59 μM, respectively. In contrast the parent nucleoside 4′-azidoadenosine (**1**) was inactive as an antiviral agent in this system (EC_{50} > 100 μM). Therefore, the ethyl and benzyl phosphoramidates provided a more than 3 log improvement in antiviral potency of the ProTide as compared to the parent nucleoside in the cell-based system.

Experimental Procedures

Biology. The HCV replicon assay was performed in the stable replicon cell line 2209−23 derived from Huh-7 cells stably transfected with a bicistronic HCV replicon (genotype 1b) expressing the renilla luciferase reporter gene, as described.[8]

Chemistry. General Procedures. All experiments involving water-sensitive compounds were conducted under scrupulously dry conditions. Anhydrous tetrahydrofuran and dichloromethane were purchased from Aldrich. Proton, carbon and phosphorus nuclear magnetic resonance (^{1}H, ^{13}C, ^{31}P NMR) spectra were recorded on a Bruker Avance spectrometer operating at 500, 125, and 202 MHz, respectively. All ^{13}C and ^{31}P spectra were recorded proton-decoupled. All NMR spectra were recorded in $CDCl_3$ at room temperature (20 °C ± 3 °C). Chemical shifts for ^{1}H and ^{13}C spectra are quoted in parts per million downfield from tetramethylsilane. Coupling constants are referred to as *J* values. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), broad signal (br), doublet of doublet (dd), doublet of triplet (dt), or multiplet (m). Chemical shifts for ^{31}P spectra are quoted in parts per million relative to an external phosphoric acid standard. Many proton and carbon NMR signals were split because of the presence of (phosphate) diastereoisomers in the samples. The mode of ionization for mass spectroscopy was fast atom bombardment (FAB) using MNOBA as matrix. Column chromatography refers to flash column chromatography carried out using Merck silica gel 60 (40−60 μM) as stationary phase.

For convenience, standard procedures have been given, as similar procedures were employed for reactions concerning the synthesis of precursors and derivatives of ProTides. Variations from these procedures and individual purification methods are given in the main text.

Standard Procedure 1: Preparation of 2′,3′-Di-*O*-cyclopentylidene-4′-azidoadenosine Phosphoramidates. ^{t}BuMgCl (2.0 mol equiv) and 2′,3′-di-*O*-cyclopentylidene-4′-azidoadenosine (1.0 mol equiv) were dissolved in dry THF (31 mol equiv) and stirred for 15 min. Then a 1 M solution of the appropriate phosphorochloridate (2.0 mol equiv) in dry THF was added dropwise and then stirred overnight. A saturated solution of NH_4Cl was added, and the solvent was removed under reduced pressure to give a yellow solid, which was subsequently purified by column chromatography.

Standard Procedure 2: Preparation of 4′-**Azidoadenosine Phosphoramidates.** The appropriate 2′,3′-di-*O*-cyclopetylidene-4′azidoadenosine phosphoramidate was added to a solution 80% formic acid in water. The reaction was stirred at room temperature for 4 h. The solvent was removed under reduced pressure, and the obtained yellow oil was subsequently purified by column chromatography.

Standard Procedure 3: Preparation of 4′-Azidoadenosine Phosphoramidates. ^{t}BuMgCl (2.0 mol equiv) and 4′-azidoadenosine (1.0 mol equiv) were dissolved in dry THF (31 mol equiv) and stirred for 15 min. Then a 1 M solution of the appropriate phosphorochloridate (2.0 mol equiv) in dry THF was added dropwise and then stirred overnight. A saturated solution of NH_4Cl was added, and the solvent was removed under reduced pressure to give a yellow solid, which was purified by column chromatography.

Synthesis of 5′-Deoxy-5′-iodoadenosine (5). Iodine (35.43 g, 0.140 mol) and triphenylphosphine (36.80 g, 0.140 mol) were added to a solution of adenosine (**3**, 25 g, 0.093 mol) in pyridine (200 mL). After 2 h a saturated solution of $Na_sS_2O_3$ was added; the solvent was removed under reduced pressure, and the yellow solid was purified by column chromatography using as eluent $CHCl_3$/MeOH 9:1. The obtained product (60 g, 0.159 mol, >100%) was pure enough for the following reaction. δ_H (d_6-$(CH_3)_2SO$): 8.82 (2H, s, $NH_2$6), 8.59 (1H, s, H2), 8.36 (1H, s, H8), 5.95 (1H, d, H1′, J = 5.6 Hz), 4.75 (1H, t, H2′), 4.16 (1H, t, H3′), 4.01 (1H, m, H4′), 3.60 (1H, m, H5′), 3.46 (1H, m, H5′).

Synthesis of 1-(5-Deoxy-β-D-*erythro*-pent-4-enofuranosyl)-adenine (7). tBuOK (47.0 g, 0.418 mol) was added to a solution of 5′-deoxy-5′-iodoadenosine (**5**, 35 g, 0.093 mol) in pyridine (200 mL), and the reaction was stirred for 1 h at 80 °C. The solvent was removed under reduced pressure, and the black solid was purified by column chromatography using as eluent a mixture of $CHCl_3$/MeOH 9:1, and then 8:2 and 7:3. The product was obtained as brown solid (18.54 g, 0.074 mol, 80%). δ_H (d_6-$(CH_3)_2SO$): 8.37 (1H, s, H2), 8.16 (1H, s, H8), 7.32 (2H, s, $NH_2$6), 6.16 (1H, d, H1′, J = 5.3 Hz), 5.73 (1H, s, OH2′), 5.58 (1H, s, OH3′), 4.83 (1H, t, H2′), 4.73 (1H, t, H3′), 4.31 (1H, s, H5′), 4.21 (1H, s, H5′).

Synthesis of 4′-Azido-5′-deoxy-5′-iodoadenosine (8). Sodium azide (11.73 g, 0.180 mol) was added to a solution of ICl (14.65 g, 0.090 mol) in DMF (50 mL) and stirred at 30 °C for 20 min. Then a solution of 1-(5-deoxy-β-D-erythro-pent-4-enofuranosyl)-adenine (**7**, 9 g, 0.036 mol) in DMF (200 mL) was added dropwise in 30 min. After 1 h a saturated solution of $Na_sS_2O_3$ was added, and the solvent was removed under reduced pressure. The solid was dissolved in MeOH, and the precipitate was removed by filtration. The solvent was removed under reduced pressure, and the yellow solid was purified by column chromatography using as eluent $CHCl_3$/MeOH 9:1. The desired product was obtained as a yellow solid (19 g, 0.045 mol, >100%). δ_H (d_4-CH_3OH): 8.86 (1H, s, H2), 8.78 (1H, s, H8), 6.26 (1H, d, H1′, J = 5.1 Hz), 5.28 (1H, m, H2′), 4.73 (1H, t, H3′), 3.71 (1H, s, H5′), 3.68 (1H, s, H5′). For C-13 NMR data see SI.

Synthesis of *N*,*N*-Dibenzoyl-2′,3′-di-*O*-benzoyl-4′-azido-5′-deoxy-5′-iodoadenosine (9). To a solution of 4′-azido-5′-deoxy-5′-iodoadenosine (**8**, 18 g, 0.040 mol) in pyridine, benzoyl chloride (33 mL, 0.320 mol) and dimethylaminopyiridine (4.9 g, 0.040 mol) were added. After 15 h the solvent was removed under reduce pressure, and the dark solid obtained was purified by column chromatography using as eluent EtOAc/hexane 3:7. The desire product was obtained as a yellow solid (8.5 g, 0.010 mol, 35%). δ_H (d_4-CH_3OH): 8.76 (1H, s, H2), 8.67 (1H, s, H8), 8.06−7.86 (10H, m, benzoyl), 7.63−7.40 (10H, m, benzoyl), 6.88 (1H, d, H3′, J = 3.0 Hz), 6.60−6.59 (2H, m, H2′, H1′-adenosine), 3.93 (1H, m, H5′).

Synthesis of 4′-Azidoadenosine (1). To a solution of *N*,*N*-dibenzoyl-2′,3′-di-*O*-dibenzoyl-4′-azido-5′-deoxy-5′-iodoadenosine (**9**, 2.20 g, 2.64 mmol) in CH_2Cl_2 (20 mL, saturated with 1% of water) was added 85% MCPBA (*m*-chloroperbenzoic acid) (3.63 g, 15.84 mmol), and the reaction was stirred at 40 °C for 1 h. EtOAc was added and then washed with a saturated solution of $Na_sS_2O_3$. The organic layer was dried using $MgSO_4$, and then the solvent was removed under reduced pressure. The yellow solid was dissolved in a solution 1 N MeONa in MeOH (6 mL) for 1 h. The product (200 mg, 0.649 mmol, 24%) was obtained after purification by column chromatography using as eluent $CHCl_3$/ MeOH 9:1 with 1% NH_4OH_{concd} δ_H (d_4-CH_3OH): 8.31 (1H, s, H2), 8.19 (1H,s, H8), 6.25 (1H, d, H1′, J = 6.4 Hz), 5.00 (1H, t, H2′), 4.53 (1H, d, H3′), 3.77 (1H, d, H5′, J = 12.2 Hz), 3.60 (1H, d, H5′, J = 12.2 Hz). MS (ES) m/e: 331.1 (MNa^+, 100%); Accurate mass: $C_{10}H_{12}N_8O_4$-Na required 331.0879, found 331.0886.

Synthesis of 2′,3′-Di-*O*-isopropylidene-5′,*N*3-anhydro-5′-deoxyadenosine (10). δ_H (DMSO): 9.41 (1H, s, H2), 9.31 (1H, s, H8), 8.54 (1H, NH6), 5.87 (1H, s, H1′), 5.07−5.01 (3H, m, H2′, H5′, H5′), 4.59−4.51 (1H, m, H3′, H4′), 1.45 (3H, s, 1 CH_3-isopropylidene), 1.21 (3H, s, 1 CH_3-isopropylidene).

Synthesis of 2′,3′-Di-*O*-*tert*-butyldimethylsilyl-5′-deoxy-5′-iodoadenosine (11). Iodine (14.22 g, 0.056 mol) and triphenylphosphine (14.22 g, 0.056 mol) were added to a solution of adenosine (**3**, 10 g, 0.037 mol) in pyridine (45 mL). After 2 h *tert*-butyldimethylsilyl chloride (24.54 g, 0.149 mol) and imidazole (20.36 g, 0.299 mol) were added at 0 °C. After 30 min, the reaction was slowly warmed at room temperature and stirred for 15 h. The solvent was removed under reduced pressure, and the crude was purified by column chromatography using as eluent a mixture of EtOAc/hexane in gradient: 1:9, 8:2, 7:3, 65:35, 50:50, and 6:4. The pure product (10 g, 0.0165 mol, 44%) was a white solid. δ_H ($CDCl_3$): 8.38 (1H, s, H2), 7.92 (1H, s, H8), 5.87 (1H, d, H1′, J = 5.2 Hz), 5.25 (2H, $NH_2$6), 5.23 (1H, t, H2′), 4.42 (1H, m, H3′), 4.16 (1H, m, H4′), 3.73 (1H, m, H5′), 3.41 (1H, m, CH5′), 1.00 (9H, s, 3 CH_3-*tert*-butyl), 0.81 (9H, s, 3 CH_3-*tert*-butyl), 0.21 (3H,

s, CH_3-silyl), 0.16 (3H, s, CH_3-silyl), −0.10 (3H, s, CH_3-silyl), −0.27 (3H, s, CH_3-silyl).

Synthesis of 2′,3′-Di-*O*-*tert*-butyldimethylsilyl-1-(5-deoxy-β-D-*erythro*-pent-4-enofuranosyl)-adenine (12). tBuOK (0.627 g, 4.95 mmol) was added to a solution of 2′,3′-di-*O*-*tert*-butyldimethylsilyl-5′-deoxy-5′-iodoadenosine (**11**, 1.0 g, 1.65 mmol) in pyridine (15 mL), and the reaction was stirred for 3 h at room temperature. The solvent was removed under reduced pressure, and the solid was purified by column chromatography using as eluent a mixture of EtOAc/hexane in gradient: 7:3, 65:35, 50:50, and then 8:2 and 7:3. The pure product was obtained as white solid (0.700 g, 1.46 mmol, 89%). δ_H ($CDCl_3$): 8.22 (1H, s, H2), 7.72 (1H, s, H8), 5.96 (1H, d, H1′, J = 6.2 Hz), 5.52 (2H, s, $NH_2$6), 4.92 (1H, m, H2′), 4.44 (1H, d, H3′, J = 4.2 Hz), 4.37 (1H, d, H5′, J = 2.3 Hz), 4.13 (1H, d, H5′, J = 2.3 Hz), 0.80 (9H, s, 3 CH_3-*tert*-butyl), 0.61 (9H, s, 3 CH_3-*tert*-butyl), 0.00 (3H, s, CH_3-silyl), −0.22 (3H, s, CH_3-silyl), −0.45 (3H, s, CH_3-silyl), −0.27 (3H, s, CH_3-silyl).

Synthesis of 6*N*-Pivaloyl-2′,3′-di-*O*-*tert*-butyldimethylsilyl-1-(5-deoxy-β-D-*erythro*-pent-4-enofuranosyl)-adenine (13). To a solution of 2′,3′-di-*O*-*tert*-butyldimethylsilyl-1-(5-deoxy-β-D-erythro-pent-4-enofuranosyl)-adenine (**12**, 3.6 g, 7.53 mmol) in anhydrous DCM (145 mL) were added tBuOCl (*tert*-butanoyl chloride, 1.86 mL, 15.10 mmol) and diisopropylethylamine (2.63 mL, 15.10 mmol), and the reaction was stirred for 3 h at room temperature. The reaction was washed three times with a saturated solution of $NaHCO_3$. The organic layer was dried with $MgSO_4$, and the solvent was removed under reduced pressure. The crude was purified by column chromatography using as eluent a mixture of EtOAc/hexane in gradient: 1:10, 1:5, 1:1. The pure compound was obtained as white solid (4.0 g, 7.13 mmol 95%). δ_H ($CDCl_3$): 8.62 (1H, s, H2), 8.42 (1H, s, NH6), 7.90 (1H, s, H8), 6.02 (1H, d, H1′, J = 6.2 Hz), 4.91 (1H, m,), 4.42 (1H, d, H3′, J = 4.2 Hz), 4.38 (1H, d, H5′, J = 2.3 Hz), 4.15 (1H, d, H5′, J = 2.3 Hz), 1.26 (9H, s, 3 CH_3-pivaloyl), 0.80 (9H, s, 3 CH_3-*tert*-butyl), 0.60 (9H, s, 3 CH_3-*tert*-butyl), 0.00(3H, s, CH_3-silyl), −0.22 (3H, s, CH_3-silyl), −0.47 (3H, s, CH_3-silyl).

Synthesis of 6*N*-Pivaloyl-2′,3′-di-*O*-*tert*-butyldimethylsilyl-4′,5′-epoxy-adenosine (14). 6*N*-Pivaloyl-2′,3′-di-*O*-*tert*-butyldimethylsilyl-1-(5-deoxy-β-D-erythro-pent-4-enofuranosyl)-adenine (13, 2.0 g, 3.56 mmol) was added to a 0.1 solution of DMDO (dimethoxydioxirane) in acetone (110 mL). The reaction was stirred for 15 min at room temperature, and then the solvent was removed under reduced pressure to give a the pure white product (2.05 g, 3.56 mmol, 100%). δ_H ($CDCl_3$): 8.70 (1H, s, H2), 8.07 (1H, s, NH6), 8.07 (1H, s, H8), 6.11 (1H, d, H1′, J = 5.2 Hz), 4.94 (1H, m, H2′), 4.12 (1H, d, H3′, J = 3.8 Hz), 3.10 (1H, d, H5′, J = 3.5 Hz), 2.94 (1H, d, H5′, J = 3.5 Hz), 1.31 (9H, s, 3 CH_3-pivaloyl), 0.85 (9H, s, 3 CH_3-*tert*-butyl), 0.71 (9H, s, 3 CH_3-*tert*-butyl), 0.03 (3H, s, CH_3-silyl), 0.00 (3H, s, CH_3-silyl), −0.14 (3H, s, CH_3-silyl), −0.33 (3H, s, CH_3-silyl).

Synthesis of 6*N*-Pivaloyl-2′,3′-di-O-*tert*-butyldimethylsilyl-4′-azidoadenosine (15). To a solution of 6*N*-pivaloyl-2′,3′-di-*O*-*tert*-butyldimethylsilyl-4′,5′epoxy-adenosine (**14**, 3.09 g, 5.34 mmol) in anhydrous DCM (200 mL) were added $TBSN_3$ (*tert*-butylazidosilane, 2.10 mL, 16.02 mmol) and $SnCl_4$ (tin chloride, 3.4 mL, 16.02 mmol) at −78 °C. The reaction was stirred for 30 min, and then it was slowly warmed at room temperature and stirred for other 30 min. A saturated solution of $NaHCO_3$ was added until the reaction reached pH 7. The white emulsion formed was filtered on a celite pad, and the solution was washed three times with a saturated solution of $NaHCO_3$. The organic layer was dried with $MgSO_4$. The solvent was removed under reduced pressure, and the crude was purified by column chromatography using as eluent a mixture of EtOAc/hexane in gradient: 50:50 and 70:30. The pure product was obtained as white solid (1.6 g, 52%). δ_H ($CDCl_3$): 8.58 (1H, s, H2), 8.50 (1H, s, NH6), 7.91 (1H, s, H8), 6.26 (1H, m, OH5′), 5.84 (1H, d, H1′, J = 7.9 Hz), 5.08 (1H, m, H2′), 4.22 (1H, d, H3′, J = 4.4 Hz), 3.64 (1H, m, H5′), 3.31 (1H, m, H5′), 1.24 (9H, s, 3 CH_3-pivaloyl), 0.83 (9H, s, 3 CH_3-*tert*-butyl), 0.59 (9H, s, 3 CH_3-*tert*-butyl), 0.03(3H, s, CH_3-silyl), 0.00 (3H, s, CH_3-silyl), −0.32 (3H, s, CH_3-silyl), −0.88 (3H, s, CH_3-silyl).

Synthesis of 6*N*-Pivaloyl-4′-azidoadenosine (16). To a solution of 6*N*-pivaloyl-2′,3′-di-*O*-tert-butyldimethylsilyl-4′-azidoadenosine (**15**, 2.8 g, 4.52 mmol) in THF (30 mL) was added a 1 M solution of TBAF (tetrabutylammonium fluoride, 9.0 mL, 9.0 mmol), and the reaction was stirred at room temperature for 1 h. The solvent was removed under reduced pressure to give a yellow oil that was purified by column chromatography using as eluent a mixture of $CHCl_3$/MeOH 85:15. The pure product was obtained as white solid (1.15 g, 2.93 mmol, 76%). δ_H (d_6-$(CH_3)_2SO$): 10.23 (1H, s, NH6), 8.77 (1H, s, H2), 8.74 (1H, s, H8), 6.33 (1H, d, H1′, J = 6.1 Hz), 5.97 (1H, d, OH2′, J = 4.7 Hz), 5.82 (1H, d, OH3′, J = 5.96 Hz), 5.65 (1H, m, OH5′), 4.94 (1H, m, H2′), 4.48 (1H, m, H3′), 3.67 (1H, m, H5′), 3.51 (1H, m, H5′), 1.33 (9H, s, 3 CH_3-pivaloyl).

Synthesis of 2′,3′-Di-*O*-cyclopentylidene-4′-azidoadenosine (17). 6*N*-Pivaloyl-2′,3′-di-*O*-cyclopentylidene-4′-azidoadenosine (1.12 g, 2.44 mmol) was dissolved in a solution of MeOH saturated with NH_3. The solution was sealed at room temperature overnight. The solvent was removed under reduced pressure, and the crude was purified by column chromatography using as eluent a mixture of $CHCl_3$/MeOH in gradient: 100:0, 98:2, and 90:10. The pure product was obtained as white solid (0.80 g, 2.13 mmol, 87%). δ_H ($CDCl_3$): 8.44 (1H, s, H2), 8.00 (1H, s, H8), 6.23 (1H, d, H1′, J = 5.1 Hz), 6.20 (2H, s, $NH_2$6), 5.46 (1H, m, H2′), 5.21 (1H, d, H3′, J = 5.6 Hz), 3.99 (1H, d, H5′, J = 12.3 Hz), 3.75 (1H, d, H5′, J = 12.3 Hz), 2.39−2.22 (2H, m, CH_2-cyclopentyl), 2.00−1.85 (6H, m, CH_2-cyclopentyl).

Synthesis of 4′-Azidoadenosine 5′-*O*-[α-Naphthyl(benzyloxy-l-alaninyl) Phosphate (18). Prepared according to Standard Procedure 3, from 4′-azidoadenosine (165.6 mg, 0.537 mmol), ^{t}-BuMgCl (1.34 mL 1 M solution of THF, 1.343 mmol), and α-naphthyl(benzyloxy-l-alaninyl) phosphorochloridate (1.34 mL of solution 1 M in THF, 1.343 mmol). The crude was purified by column chromatography, using as eluent $CHCl_3$/MeOH (85:15), and preparative HPLC. The obtained pure product was a white solid (20.2 mg, 0.0306 mmol, 6%). δ_P (d_4-CH_3OH): 3.71, 3.67; δ_H (d_4-CH_3OH): 8.26 (1H, d, H2), 8.17 (1H, s, H8), 8.17 (1H, s, CH-naphthyl), 7.88 (1H, d, CH-naphthyl, J = 7.9 Hz), 7.69 (1H, m, CH-naphthyl), 7.53−7.43 (4H, m, 3 CH-naphthyl, 1 CH-phenyl), 7.38−7.25 (5H, CH-naphthyl, 4 CH-phenyl), 6.28 (1H, d, H1′, J = 5.1 Hz), 5.05 (2H, m, CH_2-benzyl), 4.95 (1H, m, H2′), 4.70 (1H, d, H3′, J = 5.4 Hz), 4.40 (2H, m, H5), 4.05 (1H, m, CHα), 1.28 (3H, m, CH_3-alanine). MS (E/I) 698.1852 (MNa), $C_{30}H_{30}N_9O_8NaP$ requires 698.1853. Anal. ($C_{30}H_{30}N_9O_8NaP$) C, H, N.

Synthesis of 2′,3′-Di-*O*-cyclopentylidene-4′-azidoadenosine 5′-*O*-[Phenyl(benzyloxy-l-alaninyl)] Phosphate (19a). Prepared according to Standard Procedure 1, from 2′,3′-di-*O*-cyclopentylidene-4′-azidoadenosine (**17**, 150 mg, 0.40 mmol), ^{t}BuMgCl (1.00 mL, 1 M solution in THF, 1.00 mmol), and phenyl(benzyloxy-L-alaninyl) phosphorochloridate (1.00 mL of solution 1 M in THF, 1.00 mmol). The crude was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The obtained pure product was a white solid (200 mg, 0.290 mmol, 72%). δ_P ($CDCl_3$): 2.28; δ_H ($CDCl_3$): 8.22 (1H, s, H2), 7.90 (1H, s, H8), 7.27−7.19 (6H, m, 1 CH-phenyl, 5 CH-benzyl), 7.13−7.10 (3H, m, 2 CH-phenyl), 7.97−6.98 (2H, m, 2 CH-phenyl), 6.33 (1H, s, H1′), 6.20 (1H, s, $NH_2$6), 5.12−4.98 (4H, CH_2-benzyl, H2′, H3′), 4.42 (2H, m, NH-alanine), 4.23 (1H, m, H5′), 4.13 (1H, m, H5′), 3.98 (1H, m, CHα), 2.16−2.03 (2H, m, CH_2-cyclopentyl), 1.68−1.62 (6H, m, 3 CH_2-cyclopentyl), 1.25 (3H, d, CH_3-alanine, J = 6.9 Hz).

Synthesis of 4′-Azidoadenosine 5′-*O*-[Phenyl(benzyloxy-l-alaninyl)] Phosphate (19). Prepared according to Standard Procedure 2, from 2′,3′-di-*O*-cyclopentylidene-4′-azidoadenosine 5′-*O*-[phenyl(benzyloxy-l-alaninyl)] phosphate (**19a**, 200 mg, 0.290 mmol) and a solution 80% of HCOOH in water (10 mL). The crude was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The obtained pure product was a white solid (100 mg, 0.159 mmol, 55%). δ_P (d_4-CH_3OH): 3.38. 3.21; δ_H (d_4-CH_3OH): 8.30 (1H, s, H2), 8.21 (1H, s, H8), 7.44−7.33 (1H, m, CH-phenyl), 7.32−7.28 (7H, m, 2 CH-phenyl, 5 CH-benzyl), 7.25−7.15 (2H, m, 2 CH-phenyl), 6.30 (1H, d, H1′, J = 5.1 Hz), 5.12 (2H, m, CH_2-benzyl), 4.96 (1H, m, H2′), 4.68 (1H, d, H3′, J

= 5.4 Hz), 4.32 (1H, m, H5′), 4.23 (1H, m, H5′), 3.92 (1H, m, CHα), 1.28 (3H, m, CH_3-alanine). MS (E/I) 648.1696 (MNa^+), $C_{26}H_{28}N_9O_8NaP$ requires 648.1696. Anal. ($C_{26}H_{28}N_9O_8P$) C, H, N.

Synthesis of 2′,3′-Di-*O*-cyclopentylidene-4′-azidoadenosine 5′-*O*-[α-naphthyl(ethyloxy-l-alaninyl)] Phosphate (20a). Prepared according to Standard Procedure 1, from 2′,3′-di-*O*-cyclopentylidene-4′-azidoadenosine (**17**, 150 mg, 0.40 mmol), ^{t}BuMgCl (1.00 mL, 1 M solution in THF, 1.00 mmol), and α-naphthyl(ethyloxy-l-alaninyl) phosphorochloridate (1.00 mL of solution 1 M in THF, 1.00 mmol). The crude was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The obtained pure product was a white solid (250 mg, 0.369 mmol, 92%). δ_P ($CDCl_3$): 2.74; δ_H ($CDCl_3$): 8.19 (1H, s, H2), 7.91 (1H, m, CH-naphthyl), 7.83 (1H, s, H8), 7.80 (1H, d, CH-naphthyl, J = 4.85 Hz), 7.52 (1H, d, CH-naphthyl, J = 8.2 Hz), 7.44−7.29 (3H, m, CH-naphthyl), 7.24 (1H, m, CH-naphthyl), 7.42−7.21 (1H, m, CH-naphthyl), 6.17 (1H, d, H1′, J = 2.3 Hz), 6.03 (1H, s, $NH_2$6), 5.06 (1H, m, H2′), 4.96 (1H, d, H3′, j= 6.5 Hz), 4.35, 4.25 (2H, m, NH-alanine, H5′), 4.22 (1H, m, H5′), 4.00−3.91 (2H, m, CHα, CH_2-ethyl), 2.18−2.03 (2H, m, CH_2-cyclopentyl), 1.67−1.60 (6H, m, 3 CH_2-cyclopentyl), 1.23 (3H, d, CH_3-alanine), 1.08 (3H, CH_3-ethyl).

Synthesis of 4′-Azidoadenosine 5′-*O*-[α-naphthyl(ethyloxy-l-alaninyl)] Phosphate (20). Prepared according to Standard Procedure 2, from 2′,3′-di-*O*-cyclopentylidene-4′-azidoadenosine 5′-*O*-[α-naphthyl(ethyloxy-l-alaninyl)] phosphate (250 mg, 0.369 mmol), and a solution 80% of HCOOH in water (10 mL). The crude was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The obtained pure product was a white solid (100 mg, 0.169 mmol, 51%). δ_P (d_4-CH_3OH): 3.72, 3.68; δ_H (d_4-CH_3OH): 8.27 (1H, s, H2), 8.13 (1H, s, H8), 8.07 (1H, m, CH-naphthyl), 7.83 (1H, m, CH-naphthyl), 7.66 (1H, m, CH-naphthyl), 7.52−7.43 (3H, m, 3 CH-naphthyl), 7.38−7.32 (1H, m, CH-naphthyl), 6.30 (1H, d, H1′-adenosine, J = 5.1 Hz), 4.97 (1H, m, H2′), 4.75 (1H, d, H3′, J = 5.5 Hz), 4.43 (1H, m, H5′), 4.36 (1H, m, H5′), 4.06−3.89 (2H, m, CH_2-ethyl), 3.73 (1H, m, CHα), 1.27 (3H, m, CH_3-alanine), 1.14 (3H, CH_3-ethyl). MS (E/I) 636.1682 (MNa^+), $C_{25}H_{28}N_9O_8NaP$ requires 636.1696. Anal. ($C_{25}H_{28}N_9O_8P$) C, H, N.

Synthesis of 2′,3′-Di-*O*-cyclopentylidene-4′-azidoadenosine 5′-*O*-[phenyl(ethyloxy-l-alaninyl)] Phosphate (21a). Prepared according to Standard Procedure 1, from 2′,3′-di-*O*-cyclopentylidene-4′-azidoadenosine (**17**, 150 mg, 0.40 mmol), ^{t}BuMgCl (1.00 mL, 1 M solution in THF, 1.00 mmol), and phenyl(ethyloxy-L-alaninyl) phosphorochloridate (1.00 mL of solution 1 M in THF, 1.00 mmol). The crude was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The obtained pure product was a white solid (210 mg, 0.335 mmol, 84%). δ_P ($CDCl_3$): 2.34; δ_H ($CDCl_3$): 8.31 (1H, s, H2), 7.97 (1H, s, H8), 7.23−7.22 (2H, m, 2 CH-phenyl), 7.12−7.09 (3H, m, 3 CH-phenyl), 6.31 (1H, d, H1′, J = 2.2 Hz), 6.23 (1H, s, $NH_2$6), 5.24 (1H, m, H2′), 5.14 (1H, d, H3′, J = 6.3 Hz), 4.35−4.30 (2H, m, NH-alanine, H5′), 4.28−4.22 (1H, m, H5′), 4.15−4.11 (2H, m, CH_2-ethyl), 3.99 (1H, m, CHα), 2.26−2.14 (2H, m, CH_2-cyclopentyl), 1.76−1.69 (6H, m, 3 CH_2-cyclopentyl), 1.33 (3H, CH_3-ethyl), 1.23 (3H, d, CH_3-alanine).

Synthesis of 4′-Azidoadenosine 5′-*O*-[Phenyl(ethyloxy-l-alaninyl)] Phosphate (21). Prepared according to Standard Procedure 2, from 2′,3′-di-*O*-cyclopentylidene-4′-azidoadenosine 5′-*O*-[phenyl-(ethyloxy-l-alaninyl)] phosphate (210 mg, 0.335 mmol) and a solution 80% of HCOOH in water (10 mL). The crude was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The obtained pure product was a white solid (100 mg, 0.159 mmol, 48%). δ_P (d_4-CH_3OH): 3.44. 3.28; δ_H (d_4-CH_3OH): 8.27 (1H, s, H2), 8.18 (1H, s, H8), 7.36−7.28 (2H, m, 2 CH-phenyl), 7.16−7.11 (3H, m, 3 CH-phenyl), 6.38 (1H, d, H1′, J = 5.0 Hz), 4.96 (1H, m, H2′), 4.25 (1H, m, H5′), 4.15 (2H, m, CH_2-ethyl), 3.76 (1H, m, CHα), 1.27 (3H, d, CH_3-ethyl, J = 7.2 Hz), 1.22 (3H, m, CH_3-alanine). MS (E/I) 586.1544 (MNa^+), $C_{21}H_{26}N_9O_8NaP$ requires 586.1540. Anal. ($C_{21}H_{26}N_9O_8P$) C, H, N.

Synthesis of 2′,3′-Di-*O*-cyclopentylidene-4′-azidoadenosine 5′-*O*-[α-Naphthyl(*tert*-butyloxy-l-alaninyl)] Phosphate (22a). Prepared according to Standard Procedure 1, from 2′,3′-*O*-cyclopentylidene-4′-azidoadenosine (**17**, 150 mg, 0.40 mmol), ^{t}BuMgCl (1.00 mL, 1 M solution in THF, 1.00 mmol), and α-naphthyl(*tert*-butyloxy-l-alaninyl) phosphorochloridate (1.00 mL of solution 1 M in THF, 1.00 mmol). The crude was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The obtained pure product was a white solid (220 mg, 0.308 mmol, 77%). δ_P ($CDCl_3$): 2.96; δ_H ($CDCl_3$): 8.17 (1H, s, H2-adenosine), 8.05 (1H, m, CH-naphthyl), 7.82 (1H, s, H8), 7.72−7.65 (2H, m, CH-naphthyl), 7.54−7.44 (2H, 2 CH-naphthyl), 7.42−7.21 (2H, m, 2 CH-naphthyl), 6.47 (1H, s, $NH_2$6), 6.14 (1H, d, H1′), 4.97 (2H, m, H2′, H3′), 4.61 (1H, m, NH-alanine), 4.30 (1H, m, H5′), 4.19 (1H, m, H5′), 3.94 (1H, m, CHα), 2.13−2.01 (2H, m, CH_2-cyclopentyl), 1.65−1.56 (6H, m, 3 CH_2-cyclopentyl), 1.36 (9H, 3 CH_3-*tert*-butyl), 1.23 (3H, m, CH_3-alanine).

Synthesis of 4′-Azidoadenosine 5′-*O*-[α-naphthyl(*tert*-butyloxy-l-alaninyl)] Phosphate (22). Prepared according to Standard Procedure 2, from 2′,3′-di-*O*-cyclopentylidene-4′-azidoadenosine 5′-*O*-[α-naphthyl(*tert*-butyloxy-l-alaninyl)] phosphate (220 mg, 0.308 mmol), and a solution 80% of HCOOH in water (10 mL). The crude was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The obtained pure product was a white solid (100 mg, 0.169 mmol, 51%). δ_P (d_4-CH_3OH): 3.85, 3.73; δ_H (d_4-CH_3OH): 8.25 (1H, s, H2), 8.21 (1H, s, H8), 8.10 (1H, m, CH-naphthyl), 7.84 (1H, m, CH-naphthyl), 7.64 (1H, m, CH-naphthyl), 7.51−7.48 (2H, m, 2 CH-naphthyl), 7.45−7.32 (2H, m, 2 CH-naphthyl), 6.29 (1H, d, H1′, J = 5.1 Hz), 4.96 (1H, m, H2′), 4.72 (1H, d, H3′, J = 5.54 Hz), 4.42 (1H, m, H5′), 4.36 (1H, m, H5′), 3.85 (1H, m, CHα), 1.35 (9H, s, 3 CH_3-*tert*-butyl), 1.25 (3H, d, CH_3-alanine, J = 6.3 Hz). MS (E/I) 664.2015 (MNa^+), $C_{27}H_{32}N_9O_8NaP$ requires 664.2009. Anal. ($C_{27}H_{32}N_9O_8P$) C, H, N.

Synthesis of 2′,3′-Di-*O*-cyclopentylidene-4′-azidoadenosine 5′-*O*-[phenyl(*tert*-butyloxy-l-alaninyl)] Phosphate (23a). Prepared according to Standard Procedure 1, from 2′,3′-di-*O*-cyclopentylidene-4′-azidoadenosine (**17**, 150 mg, 0.40 mmol), ^{t}BuMgCl (1.00 mL, 1 M solution in THF, 1.00 mmol), and phenyl(*tert*-butyloxy-L-alaninyl) phosphorochloridate (1.00 mL of solution 1 M in THF, 1.00 mmol). The crude was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The obtained pure product was a white solid (210 mg, 0.356 mmol, 71%). δ_P ($CDCl_3$): 2.43; δ_H ($CDCl_3$): 8.28 (1H, s, H2), 7.97 (1H, s, H8), 7.27−7.00 (5H, m, CH-phenyl), 6.55 (1H, s, $NH_2$6), 5.77 (1H, m, H1′), 5.17 (1H, m, H2′), 5.16 (1H, d, H3′, J = 2.1 Hz), 3.98 (1H, m, NH-alanine), 4.29 (1H, m, H5′), 4.20 (1H, m, H5′), 3.88 (1H, m, CHα), 2.21−2.09 (2H, m, CH_2-cyclopentyl), 1.76−1.63 (6H, m, 3 CH_2-cyclopentyl), 1.37 (9H, 3 CH_3-*tert*-butyl), 1.26 (3H, d, CH_3-alanine, J = 7.0 Hz).

Synthesis of 4′-Azidoadenosine 5′-*O*-[Phenyl(*tert*-butyloxy-l-alaninyl)] Phosphate (23). Prepared according to Standard Procedure 2, from 2′,3′-di-*O*-cyclopentylidene-4′-azidoadenosine 5′-*O*-[phenyl(*tert*-butyloxy-l-alaninyl)] phosphate (210 mg, 0.356 mmol), and a solution 80% of HCOOH in water (10 mL). The crude was purified by column chromatography, using as eluent $CHCl_3$/MeOH (95:5). The obtained pure product was a white solid (100 mg, 0.169 mmol, 47%). δ_P (d_4-CH_3OH): 3.42; δ_H (d_4-CH_3-OH): 8.29 (1H, s, H2), 8.21 (1H, s, H8), 7.32−7.29 (2H, m, 2 CH-phenyl), 7.19−7.14 (3H, m, 3 CH-phenyl), 6.33 (1H, d, H1′, J = 5.1 Hz), 4.97 (1H, m, H2′), 4.72 (1H, d, H3′, J = 5.45 Hz), 4.36 (1H, m, H5′), 4.27 (1H, m, H5′), 3.77 (1H, m, CHα), 1.42 (9H, s, 3 CH_3-*tert*-butyl), 1.25 (3H, d, CH_3-alanine). MS (E/I) 614.1839 (MNa^+), $C_{23}H_{30}N_9O_8NaP$ requires 614.1853. Anal. ($C_{29}H_{30}N_9O_8P$) C, H, N.

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Supporting Information Available: Microanalytical data of target compounds and ^{13}C NMR data of intermediates and products. This material is available free of charge via the Internet at http://pubs.acs.org.

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