



Pyrrolidine *N*-alkylphosphonates and related nucleotide analogues: synthesis and stereochemistry

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ABSTRACT

N-Phosphonoalkyl-*trans*-3,4-dihydroxypyrrolidine derivatives were synthesized and exploited as synthons for the preparation of hydroxypyrrolidine nucleoside phosphonic acids, the 3'-deoxynucleoside 5'-phosphate analogues. Simultaneously, an alternative route, the *N*-phosphoalkylation of the preformed pyrrolidine nucleosides employing Mannich- and Michael-type reactions, was investigated to obtain desired nucleotide analogues. In contrast to the latter approach, the former resulted in the formation of two diastereoisomers very likely due to the existence of two possible S_N2 transition states during a nucleophilic displacement. The stereochemistry of the prepared nucleotide analogues was studied by NMR spectroscopy.

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1. Introduction

Over the past few decades, enormous effort has been devoted to the synthesis and biochemical and biological evaluation of analogues of natural nucleosides and nucleotides due to their usefulness as tools in investigations aimed at a more thorough understanding of metabolic processes. In many cases, the form of a triphosphate is the one that effectively starts the active role of such antimetabolites in the *in vivo* processes affecting biosynthesis of DNA and RNA. Based on this knowledge, alternatives to the natural phosphates were sought, and the enzymatically and chemically stable phosphonate moiety was introduced¹ to improve the *in vivo* stability of parent compounds and hence their biological properties.

Systematic biological investigation of the structurally diverse nucleoside phosphonic acids has led to potent antiviral drugs based on both the acyclic phosphonate nucleotides **1**^{2,3} and the cyclic structures **2** and **3** (Fig. 1).⁴ Specifically, acyclic compounds **1** were found to inhibit the replication of DNA viruses and retroviruses, whereas cyclic compounds **2** and **3** exhibited favorable antiviral profiles against HIV strains. The modification of the sugar-

phosphate moiety in nucleotides seems to be the most successful approach in contributing to the pool of potential antivirals. For instance, several types of structurally interesting aza-sugar nucleoside phosphonates, such as the pyrrolidine **4**,^{5,6} isoxazolidine **5**⁷ and **6**,⁸ and aziridine **7**⁹ ring-containing compounds have been reported but none of them, except for analogues **6** (*n*=1), which exerted significant inhibition of reverse transcriptase comparable to AZT in efficiency as well as low cytotoxicity, showed any remarkable antiviral or antimicrobial activities.

Also nitrogen-containing heterocycles bearing phosphonate moiety were reported as biologically active species. Thus, a tetrazole phosphonic acid¹⁰ was shown to inhibit multiplication of HSV II, and imidazole, benzimidazole, and benzotriazole phosphonic acids have been evaluated as plant growth factors.^{11,12} Besides these compounds, many others, such as the triazole,¹³ aziridine,¹⁴ azetidine,¹⁵ pyrazole,¹⁶ and pyrrolidine^{17–23} phosphonates were reported and biologically evaluated. We have recently described prolinol-based nucleoside *N*-methylphosphonates²⁴ as a new class of nucleotide analogues with a conformationally flexible *N*-methylphosphonate moiety. In this paper, we report on similar, flexible-type, 3-hydroxypyrrolidine-based nucleoside *N*-alkylphosphonates **10–13** (Fig. 2) mimicking 3'-deoxynucleoside 5'-phosphates. For the synthesis of these compounds, we considered two routes **A** and **B** (Scheme 1) employing preformed pyrrolidine phosphonate synthons and the recently published²⁵ pyrrolidine nucleosides **8** and **9**, respectively.

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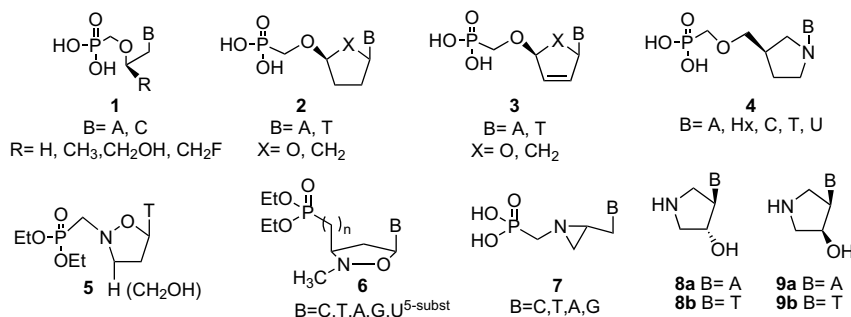


Figure 1.

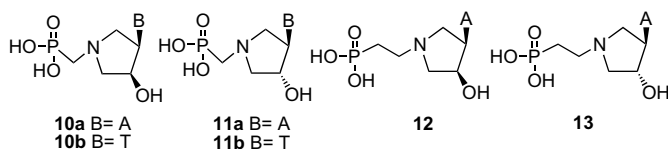
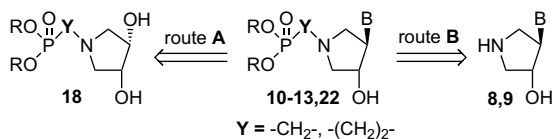


Figure 2.



Scheme 1.

chloride at reflux according to the described procedure.²⁶ Cyclic anhydride **15** was then opened with diisopropyl amino-methylphosphonate²⁷ (**23a**) or 2-aminoethylphosphonate²⁸ (**23b**), and the intermediates **16a** and **16b** were cyclized by heating with acetic anhydride. The formed *O*-acetylated intermediates were deacetylated to **17a** and **17b** with 1 M HCl in methanol (Scheme 2). Reduction of these compounds with borane, generated in situ from sodium borohydride and iodine in THF,^{29,30} afforded pyrrolidine derivatives **18a** and **18b** after treatment of the reaction mixture with hydrochloric acid to decompose the observed pyrrolidine–BH₃ complex.

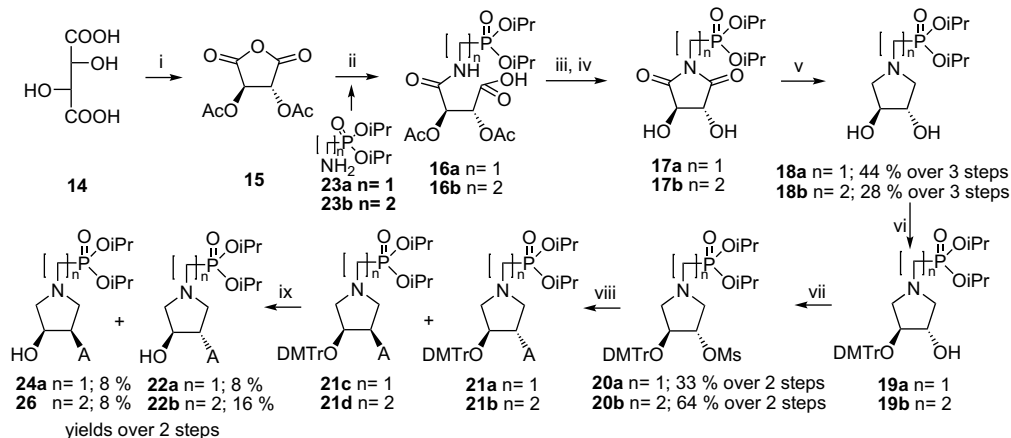
Compounds **18a** and **18b** were dimethoxytritylated in pyridine and subsequently mesylated in DCM in the presence of DMAP. The obtained mesyl derivatives **20a** and **20b** were subjected to the nucleosidation reaction with adenine providing a mixture of two diastereoisomers (**21a/21c**, *trans/cis* ~1:1; **21b/21d**, *trans/cis* ~2:1; determined by ¹H NMR spectroscopy) in a moderate yield. Removal of the dimethoxytrityl protecting groups gave the phosphono diesters **22a/24a** and **22b/26** as diastereomeric mixtures.

The epimerization at the C3 atom in **20a** and **20b** during nucleophilic displacement of the mesyl group could be explained by the participation of the pyrrolidine nitrogen free electron pair. Two hypothetical chiral S_N2 transition state intermediates **A** and **B** (Fig. 3) can be attacked on the C3 atom by adenine either from the *cis* or *trans* position with respect to the dimethoxytrityloxy substituent, providing a mixture of diastereoisomers **21a/21c** and **21b/**

2. Results and discussion

2.1. Chemistry

The synthetic procedure started with L-tartaric acid (**14**), which was transformed into anhydride **15** on treatment with acetyl



Scheme 2. Synthesis of pyrrolidine *N*-alkylphosphonate derivatives. (i) AcCl, reflux; (ii) **23a** or **23b**, DCM; (iii) Ac₂O, 90 °C; (iv) 1 M HCl in MeOH; (v) NaBH₄/I₂/THF, aq HCl; (vi) DMTrCl/pyridine; (vii) MsCl/DMAP/DCM; (viii) Adenine/Cs₂CO₃/DMF; (ix) 80% aq AcOH.

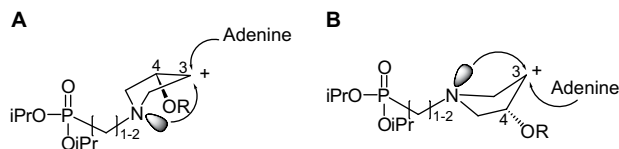


Figure 3. Possible explanation of C3 epimerization during nucleophilic displacement.

21d. Similar epimerization was observed during nucleosidation of 1-*N*-benzyl-3-dimethoxytrityloxy-4-mesyloxyrrolidines.

The observed epimerization provides the possibility to obtain two separable diastereoisomers in one reaction but we did not attempt it in this case.

In order to obtain the desired phosphonates **10–13** in diastereomerically pure forms, we performed *N*-phosphorylation reactions at the level of pyrrolidine nucleosides **8a,b** and **9a,b**. These compounds easily underwent the Mannich-type reaction with formaldehyde and diisopropyl phosphite³¹ to form phosphonates **24a,b** and **25a,b** (Schemes 3 and 4).

Pyrrolidine nucleosides **8a** and **9a** were subjected to the Michael addition on diisopropyl vinylphosphonate³² giving a good yield of diisopropyl phosphonates **26** and **27**. The phosphoester groups of **24a,b** and **25a,b** were removed by treatment with bromotrimethylsilane in acetonitrile to yield the desired phosphonic acids **10a**, **10b**, **11a**, **11b**, **12**, and **13**.

2.2. NMR analysis

¹H, ¹³C, and ³¹P NMR spectra of target nucleotide analogues **10–13** were measured in D₂O and all resonances were unambiguously assigned based on H,H-COSY, H,H-ROESY, H,C-HSQC, and H,C-HMBC experiments.

We have measured NMR spectra of **10–13** at different pD (using D₂O solutions of DCl and NaOD) and found them to be pD dependent with similar behavior as proline. An example of ¹H NMR pD dependence for T-derivative is shown in Figure 4. Whereas at low pD the compound **11b** exists as a free phosphonic acid with

deuterated quaternary pyrrolidine nitrogen, at the high pD it is a salt of phosphonic acid with fast flip-flop movement of the phosphonoalkyl substituent. A special situation occurs at pD 6–8 when phosphonates **10–13** exist as zwitterions and the phosphonoalkyl group occupies an *exo* (trans) arrangement relative to the nucleobase as follows from stereospecific assignment of protons using H,H-ROESY (Fig. 5).

Nothing is known about the conformation of the pyrrolidine ring in the series of novel nucleotide analogues **10–13**. The conformation analysis using vicinal ³J(H,H) coupling constants of pyrrolidine ring protons confronted with a molecular modeling approach is underway and will be published elsewhere.

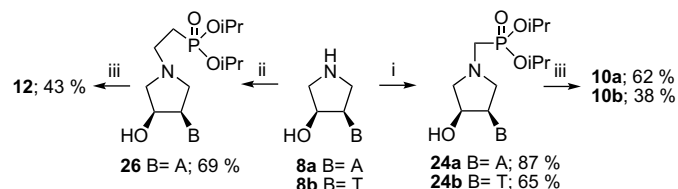
3. Conclusion

Two synthetic routes for the preparation of pyrrolidine nucleoside phosphonates exploiting either nucleosidation of an *N*-phosphonoalkylpyrrolidine synthon with a nucleobase, or *N*-phosphonoalkylation of preformed pyrrolidine nucleoside have been evaluated. In contrast to the latter approach, the former provided a mixture of separable epimers. This experimental arrangement seems to be advantageous in cases when two diastereoisomeric pyrrolidine nucleoside *N*-alkylphosphonates are required, starting from one diastereoisomerically pure *N*-phosphonoalkylpyrrolidine derivative. All synthetic steps in the preparation of *N*-phosphonoalkylpyrrolidine derivatives are straightforward giving good yields of the desired compounds. We can conclude that the described synthetic strategies provide, in several simple steps, new pyrrolidine *N*-alkylphosphonates as well as the appropriate nucleotide analogues whose antiviral, anticancer, and antimicrobial properties are currently underway. NMR-based stereochemical studies of pyrrolidine nucleotide analogues revealed an *exo* (trans) configuration of the *N*-phosphonoalkyl moiety regarding to the pyrrolidine-nucleobase residue. Acquired knowledge will be evaluated at the synthesis of modified oligonucleotides containing pyrrolidine nucleotide units in selected positions.

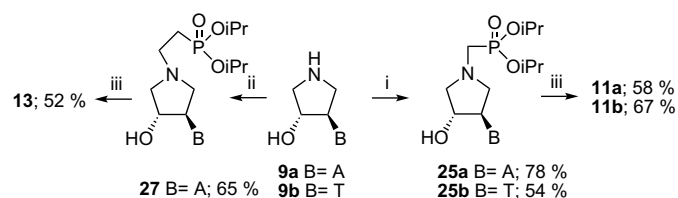
4. Experimental

4.1. General

Unless otherwise stated, all solvents used were anhydrous. Final products were lyophilized from water, and dried over phosphorus pentoxide at rt and 13 Pa. TLC was performed on silica gel pre-coated aluminum plates UV 254 (Fluka), and the compounds were detected by UV light (254 nm), by heating (detection of dimethoxytrityl group; orange color), by spraying with a 1% solution of ninhydrin (to visualize amines), and by spraying with a 1% solution of 4-(4-nitrobenzyl)pyridine in ethanol followed by heating and treating with gaseous ammonia (to expose the blue color of mono- and diesters of phosphonic acids). Preparative column chromatography was carried out on silica gel (40–60 μm; Fluka) neutralized with triethylamine (1 ml/100 g), and the elution was performed at the flow rate of 40 ml/min. The solvent systems used for TLC and preparative chromatography were (v/v) toluene–ethyl acetate 1:1 (T) and chloroform–ethanol 9:1 (C1). Mass spectra were recorded on a ZAB-EQ (VG Analytical) instrument, using FAB (ionization with Xe, accelerating voltage 8 kV, glycerol and thioglycerol were used as matrices) or on LTQ Orbitrap XL (Thermo Fisher Scientific) instrument for ESI. NMR spectra were measured on a Bruker Avance 600 (¹H at 600.1 MHz, ¹³C at 150.9 MHz), Bruker Avance 500, Varian Unity 500 (¹H at 500.0 MHz, ¹³C at 125.7 MHz, ³¹P at 202.3 MHz), and Bruker Avance 400 (¹H at 400.0 MHz, ¹³C at 100.6 MHz, ³¹P at 162.0 MHz) spectrometers in CDCl₃ (referenced to the TMS signal and to the solvent signal—77.0 ppm for ¹³C), DMSO-*d*₆ (referenced to the



Scheme 3. Synthesis of *cis*-nucleotide analogues from pyrrolidine nucleosides. (i) HP(O)(OⁱPr)₂, formaldehyde; (ii) diisopropyl vinylphosphonate, CH₃OH; (iii) Me₃SiBr, DMF (MeCN).



Scheme 4. Synthesis of *trans*-nucleotide analogues from pyrrolidine nucleosides. (i) HP(O)(OⁱPr)₂, formaldehyde; (ii) diisopropyl vinylphosphonate, CH₃OH; (iii) Me₃SiBr, DMF (MeCN).

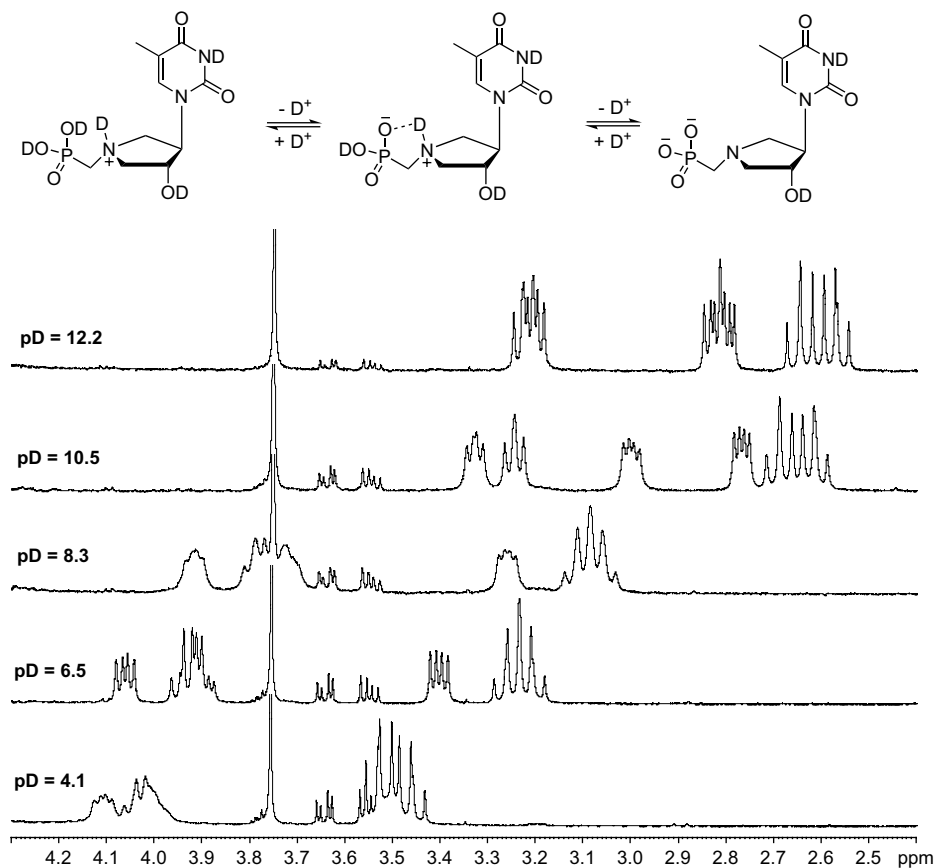


Figure 4. ^1H NMR spectra of **11b** measured at different pD values.

solvent signal—2.5 ppm for ^1H and 39.7 ppm for ^{13}C , or D_2O (referenced to the 1,4-dioxane signal as an internal standard—3.75 ppm for ^1H and 69.3 ppm for ^{13}C) as indicated. $^{31}\text{P}\{^1\text{H}\}$ NMR spectra were referenced to the signal of H_3PO_4 as an internal standard in 2 mm coaxial capillary tube.

4.1.1. (3*S*,4*R*)-(4-(Adenin-9-yl)-3-hydroxypyrrolidin-1-yl)methylphosphonic acid (**10a**)

TMSBr (0.88 ml, 6.7 mmol) was added to a solution of **24a** (0.53 g, 1.3 mmol) in DMF (15 ml), and the mixture was stirred at rt overnight. On evaporation of the reaction mixture in vacuo, the

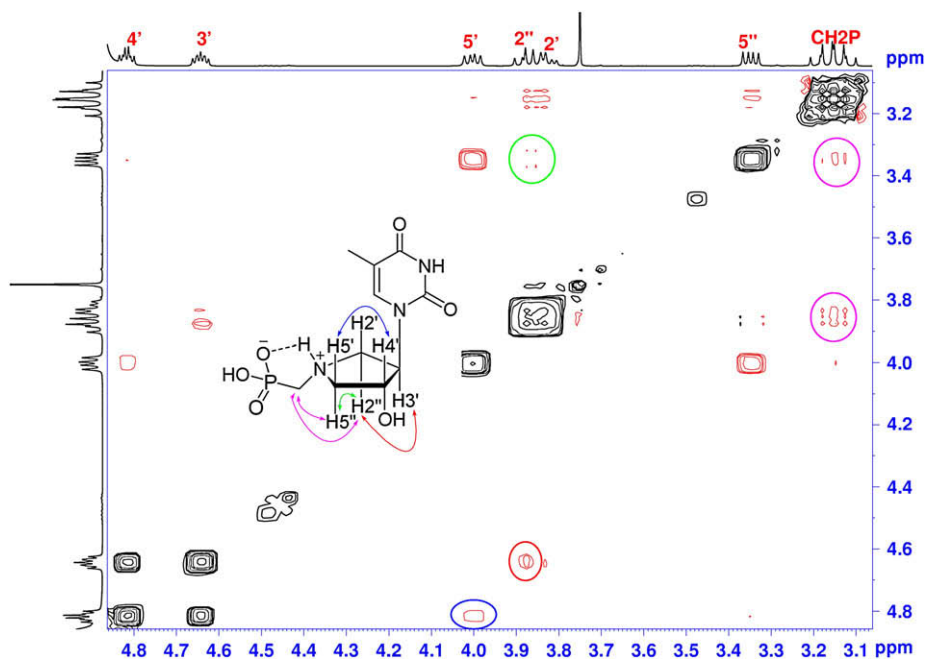


Figure 5. Part of H,H-ROESY spectrum of **11b** at pD ~ 7 showing spatial arrangement in zwitterionic form.

residue was dissolved in 0.5 M TEAB (4 ml) and the volatiles evaporated again. The title compound was obtained by chromatography on DEAE-Sephadex A25 using a linear gradient of 0.2 M TEAB in water, converted to the sodium salt by passing through small column of Dowex 50 in sodium form, and lyophilized from water giving 62% yield (296 mg, 0.8 mmol) of a white amorphous solid.

Mp >350 °C. HRMS (ES⁺) for C₁₀H₁₅N₆O₄PNa (M+H+Na)⁺: calcd 337.0790, found 337.0793. [α]_D²⁰ +26.0 (c- 0.123, H₂O). $\nu_{\max}$ (KBr) 3329 (s), 3163 (vs, br), 1657 (vs), 1606 (s), 1573 (m), 1485 (m), 1422 (m), 1337, 1315 (m), 1216 (m), 1073 (s, br, sh), 909 (w, br), 795 (m), 644 (m). ¹H NMR (500 MHz, D₂O): 3.24 (dd, 1H, $J_{\text{gem}}=14.1$, $J_{\text{H,P}}=11.1$, CH₃H_bP), 3.29 (dd, 1H, $J_{\text{gem}}=14.1$, $J_{\text{H,P}}=11.6$, CH₃H_bP), 3.76 (dd, 1H, $J_{\text{gem}}=12.8$, $J_{2b,3}=5.8$, H-2b-pyrr), 3.80 (dd, 1H, $J_{\text{gem}}=12.8$, $J_{2a,3}=3.7$, H-2a-pyrr), 3.94 (dd, 1H, $J_{\text{gem}}=12.8$, $J_{5b,4}=7.9$, H-5b-pyrr), 4.22 (dd, 1H, $J_{\text{gem}}=12.8$, $J_{5a,4}=5.0$, H-5a-pyrr), 4.88 (ddd, 1H, $J_{3,4}=6.2$, $J_{3,2}=5.8$, 3.7, H-3-pyrr), 5.44 (ddd, 1H, $J_{4,5}=7.9$, 5.0, $J_{4,3}=6.2$, H-4-pyrr), 8.23 (s, 1H, H-8), 8.26 (s, 1H, H-2). ¹³C NMR (125.7 MHz, D₂O): 56.87 (d, $J_{\text{C,P}}=128$, CH₂P), 59.12 (CH-4-pyrr), 59.63 (d, $J_{\text{C,P}}=6$, CH₂-5-pyrr), 63.92 (d, $J_{\text{C,P}}=4$, CH₂-2-pyrr), 71.56 (CH-3-pyrr), 121.07 (C-5), 145.06 (CH-8), 151.81 (C-4), 155.36 (CH-2), 158.39 (C-6). ³¹P NMR (162 MHz, D₂O): 7.92.

4.1.2. (3*S*,4*R*)-(3-Hydroxy-4-(thymine-1-yl)-pyrrolidin-1-yl)methylphosphonic acid (**10b**)

The title compound was prepared via the same procedure as that employed for compound **10a**, using **24b** (0.12 g, 0.31 mmol), TMSBr (0.2 ml, 1.53 mmol), and DMF (5 ml), in a 38% yield (41 mg, 0.12 mmol) as a white amorphous solid.

Mp 289.2 °C. HRMS (FAB⁺) for C₁₀H₁₅N₃O₆P (M+H)⁺: calcd 304.0699, found 304.0689. [α]_D²⁰ +38.1 (c- 0.162, H₂O). $\nu_{\max}$ (KBr) 3595 (m, br, sh), 3525 (m, br, sh), 3320 (s, br, sh), 3268 (s, br), 1402 (m), 1705 (vs), 1669 (vs), 1511 (w, sh), 1485 (m), 1436 (m, br), 1381 (w, sh), 1292 (s), 1145 (s), 1073 (s), 1025 (m), 983 (m), 766 (m). ¹H NMR (500 MHz, D₂O): 1.89 (d, 3H, $J_{\text{CH3,6}}=1.1$, CH₃-5), 3.19 (d, 2H, $J_{\text{H,P}}=11.4$, CH₂P), 3.62 (dd, 1H, $J_{\text{gem}}=12.3$, $J_{2b,3}=5.4$, H-2b-pyrr), 3.75 (dd, 1H, $J_{\text{gem}}=12.3$, $J_{2a,3}=2.0$, H-2a-pyrr), 3.75 (dd, 1H, $J_{\text{gem}}=12.7$, $J_{5a,4}=9.3$, H-5a-pyrr), 4.00 (dd, 1H, $J_{\text{gem}}=12.7$, $J_{5a,4}=6.5$, H-5a-pyrr), 4.70 (ddd, 1H, $J_{3,4}=6.5$, $J_{3,2}=5.4$, 2.0, H-3-pyrr), 5.17 (dt, 1H, $J_{4,5}=9.3$, 6.5, $J_{4,3}=6.5$, H-4), 7.63 (q, 1H, $J_{6,\text{CH3}}=1.1$, H-6). ¹³C NMR (125.7 MHz, D₂O): 14.18 (CH₃-5), 56.65 (d, $J_{\text{C,P}}=130$, CH₂P), 58.46 (d, $J_{\text{C,P}}=5$, CH₂-5-pyrr), 60.36 (CH-4-pyrr), 64.86 (d, $J_{\text{C,P}}=5$, CH₂-2-pyrr), 71.27 (CH-3-pyrr), 112.99 (C-5), 144.53 (CH-6), 155.47 (C-2), 169.37 (C-4). ³¹P NMR (162 MHz, D₂O): 9.16.

4.1.3. (3*R*,4*R*)-(4-(Adenin-9-yl)-3-hydroxypyrrolidin-1-yl)methylphosphonic acid (**11a**)

The title compound was prepared via the same procedure as that employed for compound **10a** from **25a** (0.5 g, 1.26 mmol), TMSBr (0.83 ml, 6.28 mmol), and DMF (15 ml) in a 58% yield (261 mg, 0.73 mmol) as a white amorphous solid.

Mp >350 °C. HRMS (ES⁺) for C₁₀H₁₅N₆O₄PNa (M+H+Na)⁺: calcd 337.0790, found 337.0788. [α]_D²⁰ -7.0 (c- 0.116, H₂O). $\nu_{\max}$ (KBr) 3329 (s), 3163 (vs, br), 1657 (vs), 1606 (s), 1573 (m), 1485 (m), 1422 (m), 1337, 1315 (m), 1216 (m), 1073 (s, br, sh), 909 (w, br), 795 (m), 644 (m). ¹H NMR (500 MHz, D₂O): 3.26 (d, 2H, $J_{\text{H,P}}=11.4$, CH₂P), 3.40 (dd, 1H, $J_{\text{gem}}=12.5$, $J_{2b,3}=5.1$, H-2b-pyrr), 4.01 (dd, 1H, $J_{\text{gem}}=12.8$, $J_{5b,4}=7.1$, H-5b-pyrr), 4.04 (dd, 1H, $J_{\text{gem}}=12.8$, $J_{5a,4}=4.4$, H-5a-pyrr), 4.16 (dd, 1H, $J_{\text{gem}}=12.5$, $J_{2a,3}=6.5$, H-2a-pyrr), 4.76 (ddd, 1H, $J_{3,2}=6.5$, 5.1, $J_{3,4}=3.1$, H-3-pyrr), 5.17 (ddd, 1H, $J_{4,5}=7.1$, 4.4, $J_{4,3}=3.1$, H-4-pyrr), 8.23 (s, 1H, H-8), 8.25 (s, 1H, H-2). ¹³C NMR (125.7 MHz, D₂O): 56.89 (d, $J_{\text{C,P}}=130$, CH₂P), 60.50 (d, $J_{\text{C,P}}=6$, CH₂-5-pyrr), 63.63 (d, $J_{\text{C,P}}=5$, CH₂-2-pyrr), 64.28 (CH-4-pyrr), 77.27 (CH-3-pyrr), 121.55 (C-5), 144.20 (CH-8), 151.10 (C-4), 155.36 (CH-2), 158.39 (C-6). ³¹P NMR (162 MHz, D₂O): 8.57.

4.1.4. (3*R*,4*R*)-(3-Hydroxy-4-(thymine-1-yl)-pyrrolidin-1-yl)methylphosphonic acid (**11b**)

The title compound was prepared, using the procedure reported for compound **10a**, from **25b** (0.15 g, 0.39 mmol) and TMSBr (0.25 ml, 0.39 mmol) in DMF (10 ml) in a 67% yield (90 mg, 0.26 mmol) as a white amorphous solid.

Mp 266.4 °C (dec). [α]_D²⁰ -16.5 (c- 0.121, H₂O). HRMS (FAB⁺) for C₁₀H₁₅N₃O₆P (M-H)⁺: calcd 304.0699, found 304.0689. $\nu_{\max}$ (KBr) 3491 (w, br), 3183 (m, vbr), 1693 (vs), 1656 (s, sh), 1513 (w), 1470 (w), 1444 (w, br), 1384 (w), 1269 (w), 1071 (s, vbr), 909 (w, br), 768 (w). ¹H NMR (500 MHz, D₂O): 1.89 (d, 3H, $J=1.3$, CH₃-5), 3.13 and 3.18 (2×dd, 2×1H, $J_{\text{gem}}=14.1$, $J_{\text{H,P}}=11.4$, CH₂P), 3.35 (dd, 1H, $J_{\text{gem}}=12.0$, $J_{2b,3}=5.8$, H-2b-pyrr), 3.82 (dd, 1H, $J_{\text{gem}}=12.7$, $J_{5b,4}=5.4$, H-5b-pyrr), 3.88 (dd, 1H, $J_{\text{gem}}=12.7$, $J_{5a,4}=9.1$, H-5a-pyrr), 4.00 (dd, 1H, $J_{\text{gem}}=12.0$, $J_{2a,3}=6.9$, H-2a-pyrr), 4.64 (ddd, 1H, $J_{4,5}=9.1$, 5.4, $J_{4,3}=4.0$, H-4-pyrr), 4.82 (ddd, 1H, $J_{3,2}=6.9$, 5.8, $J_{3,4}=4.0$, H-3), 7.52 (q, 1H, $J=1.3$, H-6). ¹³C NMR (125.7 MHz, D₂O): 14.06 (CH₃-5), 56.41 (d, $J_{\text{C,P}}=129$, CH₂P), 59.49 (d, $J_{\text{C,P}}=5$, CH₂-5-pyrr), 63.71 (d, $J_{\text{C,P}}=5$, CH₂-2-pyrr), 69.16 (CH-4-pyrr), 75.31 (CH-3-pyrr), 113.94 (C-5), 145.93 (CH-6), 154.91 (C-2), 169.50 (C-4).

4.1.5. (3*S*,4*R*)-2-(4-(Adenin-9-yl)-3-hydroxypyrrolidin-1-yl)ethylphosphonic acid (**12**)

The title compound was prepared as described for **10a** from **26** (0.904 g, 2.19 mmol) and TMSBr (1.45 ml, 11 mmol) in DMF (20 ml) in a 43% yield (350 mg, 0.94 mmol) as a white amorphous solid.

Mp >350 °C. HRMS (ES⁺) for C₁₁H₁₇N₆NaO₄P (M+H+Na)⁺: calcd 351.0947, found 351.0957. [α]_D²⁰ +31.3 (c- 0.115, H₂O). $\nu_{\max}$ (KBr) 3450 (m, br, sh), 3329 (s), 3264 (s), 3176 (s, br), 3120 (s, br, sh), 1649 (vs), 1604 (s), 1576 (m), 1478 (m), 1418 (m), 1333 (m), 1306 (m), 1220 (m), 1066 (s), 901 (m, br), 798 (m), 647 (m). ¹H NMR (500 MHz, D₂O): 1.95 (m, 2H, CH₂P), 3.44 (m, 2H, CH₂N), 3.56 (dd, 1H, $J_{\text{gem}}=12.4$, $J_{2b,3}=2.4$, H-2b-pyrr), 3.72 (dd, 1H, $J_{\text{gem}}=12.4$, $J_{2a,3}=4.9$, H-2a-pyrr), 3.89 (dd, 1H, $J_{\text{gem}}=11.9$, $J_{5b,4}=8.8$, H-5b-pyrr), 4.01 (dd, 1H, $J_{\text{gem}}=11.9$, $J_{5a,4}=8.2$, H-5a-pyrr), 4.80 (td, 1H, $J_{3,4}=4.9$, $J_{3,2}=4.9$, 2.4, H-3-pyrr), 5.45 (ddd, 1H, $J_{4,5}=8.8$, 8.2, $J_{4,3}=4.9$, H-4-pyrr), 8.29 (s, 1H, H-2), 8.19 (s, 1H, H-8). ¹³C NMR (125.7 MHz, D₂O): 28.62 (d, $J_{\text{C,P}}=125$, CH₂P), 56.78 (CH₂N), 56.322 (CH₂-5-pyrr), 58.14 (CH-4-pyrr), 62.15 (CH₂-2-pyrr), 71.37 (CH-3-pyrr), 121.48 (C-5), 144.46 (CH-8), 151.98 (C-4), 155.33 (CH-2), 158.31 (C-6). ³¹P NMR (162 MHz, D₂O): 17.88.

4.1.6. (3*R*,4*R*)-2-(4-(Adenin-9-yl)-3-hydroxypyrrolidin-1-yl)ethylphosphonic acid (**13**)

The titled compound was prepared according to the procedure for **10a** from **27** (0.144 g, 0.35 mmol) and TMSBr (0.23 ml, 1.75 mmol) in DMF (5 ml) in 52% yield (68 mg, 0.18 mmol) as a white amorphous solid.

Mp >350 °C. HRMS (ES⁺) for C₁₁H₁₇N₆NaO₄P (M+H+Na)⁺: calcd 351.0947, found 351.0950. [α]_D²⁰ -6.8 (c- 0.088, H₂O). $\nu_{\max}$ (KBr) 3465 (w, br, sh), 3319 (s), 3260 (m), 3173 (s, br), 3115 (s, br, sh), 1647 (vs), 1602 (s), 1575 (m), 1476 (m), 1416 (m), 1331 (m), 1301 (m), 1219 (m), 1076 (vs, br), 900 (w, br), 798 (m), 649 (m). ¹H NMR (500 MHz, D₂O): 1.90 (m, 2H, CH₂P), 3.29 (m, 3H, H-2b-pyrr and CH₂N), 3.67 (dd, 1H, $J_{\text{gem}}=11.9$, $J_{5b,4}=6.6$, H-5b-pyrr), 3.75 (dd, 1H, $J_{\text{gem}}=11.6$, $J_{2a,3}=6.5$, H-2a-pyrr), 3.86 (dd, 1H, $J_{\text{gem}}=11.9$, $J_{5a,4}=8.4$, H-5a-pyrr), 4.90 (ddd, 1H, $J_{3,2}=6.5$, 4.9, $J_{3,4}=4.7$, H-3-pyrr), 5.14 (ddd, 1H, $J_{4,5}=8.4$, 6.6, $J_{4,3}=4.7$, H-4-pyrr), 8.14 (s, 1H, H-2), 8.23 (s, 1H, H-8). ¹³C NMR (125.7 MHz, D₂O): 29.05 (d, $J_{\text{C,P}}=126$, CH₂P), 55.19 (CH₂N), 58.10 (CH₂-5-pyrr), 61.32 (CH₂-2-pyrr), 63.76 (CH-4-pyrr), 76.71 (CH-3-pyrr), 121.48 (C-5), 143.56 (CH-8), 151.69 (C-4), 155.28 (CH-2), 158.31 (C-6). ³¹P NMR (162 MHz, D₂O): 18.57.

4.1.7. Diisopropyl ((3*R*,4*R*)-3,4-dihydroxy-2,5-dioxypyrrolidin-1-yl)methylphosphonate (**17a**)

Diisopropyl aminomethylphosphonate (34.73 g, 178.13 mmol) in DCM (300 ml) was added to a solution of 2,3-O-diacetyl-L-

tartaric anhydride (38.5 g, 178.13 mmol) in DCM (500 ml) over 2 h at rt. The mixture was stirred for 2 h more at rt, and then DCM was evaporated. The obtained white foam was dissolved in acetic anhydride (350 ml), and the mixture was stirred at 90 °C overnight. Then, acetic anhydride was evaporated. The diacetyl derivative was purified by flash column chromatography on silica gel using a linear gradient of ethyl acetate in toluene. After evaporation, the product was dissolved in 1 M methanolic HCl (600 ml) and stirred at rt for 8 h. The mixture was concentrated in vacuo at a temperature below 40 °C, and co-evaporated with toluene (2×100 ml) and DCM (2×100 ml). The title compound was obtained by purification on silica gel using a linear gradient of ethanol in chloroform in 87% yield (47.87 g, 154.8 mmol) as a yellowish oil.

HRMS (FAB⁺) for C₁₁H₂₁NO₇P (M+H)⁺: calcd 310.1056, found 310.1049. ¹H NMR (500 MHz, DMSO-*d*₆): 1.23 and 1.24 (2×d, 2×6H, *J*_{vic}=6.2, (CH₃)₂CH), 3.75 (d, 2H, *J*_{H,P}=12.0, CH₂P), 4.32 (br d, 2H, *J*_{vic}=4.9, H-3,4-pyrr), 4.57 (m, 2H, CH(CH₃)₂), 6.41 and 6.42 (2×d, 2×1H, *J*_{vic}=4.9, OH). ¹³C NMR (125.7 MHz, DMSO-*d*₆): 23.86, 24.04, 24.07, and 24.10 (d, *J*_{C,P}=4, (CH₃)₂CH), 34.81 (d, *J*_{C,P}=157, CH₂P), 71.36 and 71.38 (d, *J*_{C,P}=6, CH(CH₃)₂), 74.55 (CH-3,4-pyrr), 173.78 (C-2,5-pyrr). ³¹P NMR (202.3 MHz, DMSO-*d*₆): 17.54.

4.1.8. Diisopropyl ((3*R*,4*R*)-3,4-dihydroxy-2,5-dioxopyrrolidin-1-yl)-ethylphosphonate (**17b**)

A solution of diisopropyl 2-aminoethylphosphonate (4.6 g, 22 mmol) in DCM (40 ml) was added to a solution of 2,3-di-*O*-acetyl-*L*-tartaric anhydride (4.76 g, 22 mmol) in DCM (60 ml) over 2 h at rt. The mixture was stirred for an additional hour at rt. The DCM was evaporated and the resulting white foam was dissolved in acetic anhydride (150 ml). The mixture was stirred at 90 °C for 7 h. The excess of acetic anhydride was evaporated and the mixture was purified on silica gel using a linear gradient of ethyl acetate in toluene. The obtained product was dissolved in 1 M HCl in methanol (200 ml) and stirred at rt for 8 h. The mixture was concentrated in vacuo at a temperature below 40 °C, and co-evaporated with toluene (2×100 ml) and DCM (2×100 ml) to provide diisopropyl ((3*R*,4*R*)-3,4-dihydroxy-2,5-dioxopyrrolidin-1-yl)methylphosphonate (**17b**) in a 95% yield (8.5 g, 21 mmol).

HRMS (ESI⁺) for C₁₂H₂₃NO₇P (M+H)⁺: calcd 324.1207, found 324.1208. ¹H NMR (500 MHz, DMSO-*d*₆): 1.238, 1.240, 1.244, and 1.246 (4×d, 4×3H, *J*_{vic}=6.2, (CH₃)₂CH), 1.91–2.03 (m, 2H, CH₂P), 3.42–3.58 (m, 2H, CH₂N), 4.30 (s, 2H, H-3,4-pyrr), 4.54 and 4.55 (2×dh, 2×1H, *J*_{H,P}=8.0, *J*_{vic}=6.2, CH(CH₃)₂). ¹³C NMR (125.7 MHz, DMSO-*d*₆): 23.87, 23.89, 23.93, and 23.94 (d, *J*_{C,P}=4, (CH₃)₂CH), 24.42 (d, *J*_{C,P}=140, CH₂P), 32.40 (CH₂N), 69.96 and 70.01 (d, *J*_{C,P}=6, CH(CH₃)₂), 74.48 (CH-3,4-pyrr), 174.45 (C-2,5-pyrr). ³¹P NMR (202.3 MHz, DMSO-*d*₆): 25.54.

4.1.9. Diisopropyl ((3*S*,4*S*)-3,4-dihydroxy-2,5-dioxopyrrolidin-1-yl)methylphosphonate (**18a**)

A solution of iodine (43.2 g, 170 mmol) in THF (500 ml) was added dropwise under argon atmosphere to an ice bath cooled suspension of sodium borohydride (18.2 g, 480 mmol) in the solution of diisopropyl ((3*R*,4*R*)-3,4-dihydroxy-2,5-dioxopyrrolidin-1-yl)methylphosphonate (21 g, 68 mmol) **17a** in THF (800 ml) for 2.5 h. The reaction mixture was stirred overnight at rt, then cooled to 0 °C, and aqueous 3 M HCl (100 ml) was added carefully to destroy an excess of sodium borohydride (gas evolving). The mixture was diluted with water (1000 ml) and applied onto a Dowex 50 (H⁺) (2000 ml) column. The column was washed with water (3000 ml) and the product was eluted with 3% aqueous ammonia. After evaporation of solvents, **18a** was obtained in 50% yield (9.6 g, 34 mmol) as a yellowish oil.

HRMS (FAB⁺) for C₁₁H₂₅NO₅P (M+H)⁺: calcd 282.1470, found 282.1473. *ν*_{max} (KBr) 3375 (m, br), 2980 (m), 1469 (m), 1386 (m),

1376 (m), 1255 (m, sh), 1232 (s), 1216 (m, sh), 1179 (m), 1143 (m), 1106 (m), 1074 (m), 1013 (vs), 990 (vs), 890 (m). ¹H NMR (500 MHz, DMSO-*d*₆): 1.23 (d, 12H, *J*_{vic}=6.2, (CH₃)₂CH), 2.48 (dd, 2H, *J*_{gem}=9.6, *J*_{vic}=4.5, H-2b,5b-pyrr), 2.73 and 2.79 (2×dd, 2H, *J*_{gem}=15.0, *J*_{H,P}=11.2, CH₂P), 2.89 (dd, 2H, *J*_{gem}=9.6, *J*_{vic}=5.8, H-2a,5a-pyrr), 3.81 (m, 2H, H-3,4-pyrr), 4.57 (m, 2H, CH(CH₃)₂), 4.83 (d, 2H, *J*_{vic}=4.4, OH). ¹³C NMR (125.7 MHz, DMSO-*d*₆): 23.96 and 24.08 (d, *J*_{C,P}=4, (CH₃)₂CH), 52.41 (d, *J*_{C,P}=163, CH₂P), 62.57 (d, *J*_{C,P}=10, CH₂-2,5-pyrr), 69.77 and 69.79 (d, *J*_{C,P}=7, CH(CH₃)₂), 77.54 (CH-3,4-pyrr). ³¹P NMR (202.3 MHz, DMSO-*d*₆): 23.25.

4.1.10. Diisopropyl 2-((3*S*,4*S*)-3,4-dihydroxy-2,5-dioxopyrrolidin-1-yl)ethylphosphonate (**18b**)

The reduction of this compound **17b** with diborane/THF complex generated in situ was performed according to the procedure described for compound **18a**. Starting from diisopropyl ((3*R*,4*R*)-3,4-dihydroxy-2,5-dioxopyrrolidin-1-yl)methylphosphonate (**17b**) (5.27 g, 16.3 mmol), sodium borohydride (4 g, 106 mmol), and iodine (10.34 g, 40.75 mmol) in THF (200 ml), we obtained **18b** in 30% yield as a yellowish oil (1.44 g, 4.87 mmol).

HRMS (FAB⁺) for C₁₂H₂₇NO₅P (M+H)⁺: calcd 296.1627, found 296.1617. *ν*_{max} (KBr) 3390 (m, br), 2980 (m), 1468 (m), 1386 (m), 1377 (m), 1255 (m, sh), 1220 (s), 1179 (m), 1143 (m), 1106 (s), 1077 (m, sh), 1011 (vs, sh), 990 (vs), 891 (w). ¹H NMR (500 MHz, DMSO-*d*₆): 1.23 (d, 12H, *J*_{vic}=6.2, (CH₃)₂CH), 1.85 (ddd, 2H, *J*_{H,P}=16.3, *J*_{vic}=8.9, 7.6, CH₂P), 2.39 (dd, 2H, *J*_{gem}=9.9, *J*_{vic}=3.8, H-2b,5b-pyrr), 2.59 (m, 2H, CH₂N), 2.82 (dd, 2H, *J*_{gem}=9.9, *J*_{vic}=5.7, H-2a,5a-pyrr), 3.84 (br m, 2H, H-3,4-pyrr), 4.55 (m, 2H, CH(CH₃)₂), 4.94 (br s, 2H, OH). ¹³C NMR (125.7 MHz, DMSO-*d*₆): 23.98 and 24.00 (d, *J*_{C,P}=4, (CH₃)₂CH), 25.46 (d, *J*_{C,P}=138, CH₂P), 49.73 (CH₂N), 60.27 (CH₂-2,5-pyrr), 69.56 (d, *J*_{C,P}=6, CH(CH₃)₂), 77.28 (CH-3,4-pyrr). ³¹P NMR (202.3 MHz, DMSO-*d*₆): 28.15.

4.1.11. Diisopropyl ((3*S*,4*S*)-3-dimethoxytrityloxy-4-hydroxy-2,5-dioxopyrrolidin-1-yl)methylphosphonate (**19a**)

A solution of dimethoxytrityl chloride (8.47 g, 25 mmol) in DCM (50 ml) was added dropwise to the solution of **18a** (5.98 g, 21.26 mmol) and DMAP (3 g, 35 mmol) in DCM (150 ml). The reaction mixture was left aside at rt overnight. The product was obtained by purification on silica gel using a linear gradient of ethyl acetate in toluene in a 40% yield in the form of a yellowish oil (5.0 g, 8.57 mmol).

HRMS (ESI⁺) for C₃₂H₄₃NO₇P (M+H)⁺: calcd 584.2772, found 584.2771. ¹H NMR (500 MHz, DMSO-*d*₆): 1.21, 1.22, 1.23, and 1.24 (4×d, 4×3H, *J*_{vic}=6.2, (CH₃)₂CH), 2.12 (br dd, 1H, *J*_{gem}=10.0, *J*_{5b,4}=3.6, H-5b-pyrr), 2.25 (br m, 1H, H-5a-pyrr), 2.57 (br m, 1H, H-2b-pyrr), 2.70 (br m, 2H, CH₂P), 2.98 (br m, 1H, H-2a-pyrr), 3.75 (s, 6H, CH₃O-DMTr), 3.85 (br m, 1H, H-4-pyrr), 4.04 (br m, 1H, H-3-pyrr), 4.55 (m, 2H, CH(CH₃)₂), 6.87 (m, 4H, H-*m*-C₆H₄-DMTr), 7.22 (m, 1H, H-*p*-C₆H₅-DMTr), 7.29 (m, 6H, H-*o*-C₆H₄-DMTr and H-*m*-C₆H₅-DMTr), 7.43 (m, 2H, H-*o*-C₆H₅-DMTr). ¹³C NMR (125.7 MHz, DMSO-*d*₆): 23.83 and 23.94 (d, *J*_{C,P}=4, (CH₃)₂CH), 51.95 (d, *J*_{C,P}=162, CH₂P), 55.15 (CH₃O-DMTr), 60.67 (d, *J*_{C,P}=10, CH₂-5-pyrr), 61.82 (d, *J*_{C,P}=9, CH₂-2-pyrr), 69.79 (d, *J*_{C,P}=7, CH(CH₃)₂), 76.45 (CH-3), 80.21 (CH-4), 86.08 (C-DMTr), 113.25 and 113.27 (CH-*m*-C₆H₄-DMTr), 126.73 (CH-*p*-C₆H₅-DMTr), 127.82 (CH-*o*-C₆H₅-DMTr), 128.08 (CH-*m*-C₆H₅-DMTr), 130.01 and 130.10 (CH-*o*-C₆H₄-DMTr), 136.47 and 136.76 (C-*i*-C₆H₄-DMTr), 145.83 (C-*i*-C₆H₅-DMTr), 158.24 (C-*p*-C₆H₄-DMTr).

4.1.12. Diisopropyl ((3*S*,4*S*)-4-dimethoxytrityloxy-3-mesyloxy-2,5-dioxopyrrolidin-1-yl)methylphosphonate (**20a**)

Mesyl chloride (3.23 ml, 43 mmol) was added to the solution of **19a** (5 g, 8.57 mmol) and DMAP (5.3 g, 43 mmol) in DCM (50 ml) at 0 °C, and the reaction mixture was stirred at rt for 1 h. The reaction was quenched with water (1 ml), diluted with chloroform (100 ml), and washed with saturated solution of sodium hydrogen carbonate. The organic phase was dried with sodium sulfate and evaporated.

The title product was obtained in an 82% yield (4.6 g, 7 mmol) by purification on silica gel using a linear gradient of ethyl acetate in toluene in the form of a yellowish oil.

HRMS (FAB⁺) for C₃₃H₄₃NO₉PS (M-H)⁻: calcd 660.2396, found 660.2446. ¹H NMR (500 MHz, DMSO-*d*₆): 1.15, 1.16, 1.17, and 1.18 (4×d, 4×3H, *J*_{vic}=6.2, (CH₃)₂CH), 1.82 (dd, 1H, *J*_{gem}=10.0, *J*_{5b,4}=5.2, H-5b-pyrr), 2.07 (dd, 1H, *J*_{gem}=10.0, *J*_{5a,4}=6.6, H-5a-pyrr), 2.64 (d, 2H, *J*_{H,P}=11.5, CH₂P), 2.93 (dd, 1H, *J*_{gem}=11.2, *J*_{2b,3}=5.1, H-2b-pyrr), 2.95 (dd, 1H, *J*_{gem}=11.2, *J*_{2a,3}=3.9, H-2a-pyrr), 3.20 (s, 3H, CH₃S), 3.737 and 3.740 (2×s, 2×3H, CH₃O-DMTr), 3.98 (ddd, 1H, *J*_{4,5}=6.6, 5.2, *J*_{4,3}=2.6, H-4-pyrr), 4.48 (dh, 2H, *J*_{H,P}=7.8, *J*_{vic}=6.2, CH(CH₃)₂), 5.09 (ddd, 1H, *J*_{3,2}=5.1, 3.9, *J*_{3,4}=2.6, H-3-pyrr), 6.89 (m, 4H, H-*m*-C₆H₄-DMTr), 7.23 (m, 1H, H-*p*-C₆H₅-DMTr), 7.26 and 7.28 (2×m, 2×2H, H-*o*-C₆H₄-DMTr), 7.31 (m, 2H, H-*m*-C₆H₅-DMTr), 7.42 (m, 2H, H-*o*-C₆H₅-DMTr).

4.1.13. Diisopropyl 2-((3*S*,4*S*)-4-dimethoxytrityloxy-3-mesyloxypyrrolidin-1-yl)ethylphosphonate (**20b**)

Dimethoxytrityl chloride (1.7 g, 5.0 mmol) was added to the solution of **18b** (1.44 g, 4.9 mmol) in pyridine (20 ml). The reaction mixture was stirred at rt overnight and quenched by the addition of methanol (3 ml). The solvents were evaporated and compound **19b** was obtained by chromatography on silica gel using a linear gradient of ethanol in chloroform in a 64% yield (1.7 g, 2.8 mmol). It was characterized only by MS (HRMS (FAB⁺) for C₃₃H₄₅NO₉P (M+H)⁺: calcd 598.2934, found 598.2921), and used directly for the further step.

Mesyl chloride (1.1 ml, 14.0 mmol) was added to the solution of **19b** (1.7 g, 2.8 mmol) and DMAP (1.7 g, 14.0 mmol) in DCM (40 ml) at 0 °C, and the reaction mixture was stirred at rt for 2 h. The reaction was quenched with water (1 ml), diluted with chloroform (100 ml), and washed with saturated solution of sodium hydrogen carbonate. The organic phase was dried with sodium sulfate and evaporated. The desired product **20b** was obtained by purification on silica gel using a linear gradient of ethanol in chloroform in a 60% yield as a yellowish oil (1.134 g, 1.7 mmol).

HRMS (FAB⁺) for C₃₄H₄₇NO₉PS (M+H)⁺: calcd 676.2709, found 676.2627. ¹H NMR (500 MHz, DMSO-*d*₆): 1.175, 1.18, and 1.19 (3×d, 12H, *J*_{vic}=6.2, (CH₃)₂CH), 1.54 (dd, 2H, *J*_{gem}=10.0, *J*_{5b,4}=5.3, H-5b-pyrr), 1.62 (m, 2H, CH₂P), 1.76 (dd, 1H, *J*_{gem}=10.0, *J*_{5a,4}=6.4, H-5a-pyrr), 2.33 (q, 2H, *J*_{H,P}=*J*_{vic}=8.2, CH₂N), 2.73 (dd, 1H, *J*_{gem}=11.0, *J*_{2b,3}=3.3, H-2b-pyrr), 2.78 (dd, 1H, *J*_{gem}=11.0, *J*_{2a,3}=6.0, H-2a-pyrr), 3.22 (s, 3H, CH₃S), 3.74 (s, 6H, CH₃O-DMTr), 3.96 (ddd, 1H, *J*_{4,5}=6.4, 5.3, *J*_{4,3}=2.8, H-4-pyrr), 4.50 (m, 2H, CH(CH₃)₂), 5.12 (ddd, 1H, *J*_{3,2}=6.0, 3.3, *J*_{3,4}=2.8, H-3-pyrr), 6.89 (m, 4H, H-*m*-C₆H₄-DMTr), 7.25 (m, 1H, H-*p*-C₆H₅-DMTr), 7.30 (m, 4H, H-*o*-C₆H₄-DMTr), 7.31 (m, 2H, H-*m*-C₆H₅-DMTr), 7.45 (m, 2H, H-*o*-C₆H₅-DMTr). ¹³C NMR (125.7 MHz, DMSO-*d*₆): 23.88 and 23.93 (d, *J*_{C,P}=4, (CH₃)₂CH), 25.08 (d, *J*_{C,P}=139, CH₂P), 38.07 (CH₃S), 48.58 (CH₂N), 56.78 and 57.94 (CH₂-2,5-pyrr), 55.26 (CH₃O-DMTr), 69.44 (d, *J*_{C,P}=6, CH(CH₃)₂), 77.99 (CH-3), 85.37 (CH-4), 86.69 (C-DMTr), 113.49 and 113.53 (CH-*m*-C₆H₄-DMTr), 127.04 (CH-*p*-C₆H₅-DMTr), 127.97 (CH-*o*-C₆H₅-DMTr), 128.10 (CH-*m*-C₆H₅-DMTr), 130.04 and 130.13 (CH-*o*-C₆H₄-DMTr), 135.77 and 136.15 (C-*i*-C₆H₄-DMTr), 145.24 (C-*i*-C₆H₅-DMTr), 158.51 and 158.52 (C-*p*-C₆H₄-DMTr).

4.1.14. Diisopropyl (3*S*,4*S*)-2-(4-(adenin-9-yl)-3-dimethoxytrityloxypyrrolidin-1-yl)ethylphosphonate (**21b**) and diisopropyl (3*S*,4*R*)-2-(4-(adenin-9-yl)-3-dimethoxytrityloxypyrrolidin-1-yl)ethylphosphonate (**21d**)

The mixture of **20b** (1.134 g, 1.7 mmol) and adenine (0.27 g, 2 mmol) was co-evaporated with DMF (2×20 ml) and redissolved in DMF (20 ml). Cesium carbonate (0.55 g, 1.7 mmol) was added and the reaction mixture was stirred at 120 °C under a drying tube for two days. A mixture of products **21b** and **21d** (2:1) was obtained by purification on silica gel using a linear gradient of ethanol in

chloroform in a 26% yield as a thick yellowish oil (0.311 g, 0.44 mmol).

HRMS (FAB⁺) for C₃₈H₄₈N₆O₆P (M+H)⁺: calcd 715.3373, found 715.3351. ¹H NMR (500 MHz, DMSO-*d*₆): 1.17–1.22 (m, 24H, (CH₃)₂CH-*cis*+*trans*), 1.72 (m, 4H, CH₂P-*cis*+*trans*), 1.84 (dd, 1H, *J*_{gem}=10.0, *J*_{2b,3}=4.5, H-2b-pyrr-*trans*), 1.86 (dd, 1H, *J*_{gem}=10.5, *J*_{2b,3}=7.2, H-2b-pyrr-*cis*), 2.13 (dd, 1H, *J*_{gem}=10.0, *J*_{2a,3}=7.0, H-2a-pyrr-*trans*), 2.16 (dd, 1H, *J*_{gem}=10.0, *J*_{2a,3}=7.0, H-2a-pyrr-*cis*), 2.40 (m, 4H, CH₂N-*cis*+*trans*), 2.68 (dd, 1H, *J*_{gem}=9.5, *J*_{5b,4}=6.2, H-5b-pyrr-*trans*), 2.72 (dd, 1H, *J*_{gem}=10.0, *J*_{5b,4}=5.4, H-5b-pyrr-*cis*), 2.93 (dd, 1H, *J*_{gem}=10.0, *J*_{5a,4}=3.6, H-5a-pyrr-*cis*), 2.96 (dd, 1H, *J*_{gem}=9.5, *J*_{5a,4}=7.2, H-5a-pyrr-*trans*), 4.34 (br dt, 1H, *J*_{3,5}=7.0, 4.5, *J*_{3,4}=4.5, H-3-pyrr-*trans*), 4.36 (ddd, 1H, *J*_{3,4}=7.6, *J*_{3,5}=7.2, 5.0, H-3-pyrr-*cis*), 4.51 (m, 4H, CH(CH₃)₂-*cis*+*trans*), 5.05 (ddd, 1H, *J*_{4,3}=7.6, *J*_{4,5}=5.4, 3.6, H-4-pyrr-*cis*), 5.09 (ddd, 1H, *J*_{4,5}=7.2, 6.2, *J*_{4,3}=4.5, H-4-pyrr-*trans*), 6.58 and 6.71 (2×m, 2×4H, H-*m*-C₆H₄-DMTr-*trans*), 6.72 and 6.73 (2×m, 2×4H, H-*m*-C₆H₄-DMTr-*cis*), 6.95 (m, 2H, H-*p*-C₆H₅-DMTr-*cis*+*trans*), 7.02–7.17 (m, 12H, H-*o*-C₆H₄-DMTr-*cis*+*trans* and H-*m*-C₆H₅-DMTr-*cis*+*trans*), 7.22 (br s, 2H, NH₂-*cis*), 7.24 (br s, 2H, NH₂-*trans*), 7.45 (m, 2H, H-*o*-C₆H₅-DMTr), 8.145 and 8.148 (2×s, 2×1H, H-2,8-*trans*), 8.17 and 8.46 (2×s, 2×1H, H-2,8-*cis*).

4.1.15. Diisopropyl (3*S*,4*S*)-(4-(adenin-9-yl)-3-hydroxypyrrolidin-1-yl)methylphosphonate (**22a**) and diisopropyl (3*S*,4*R*)-(4-(adenin-9-yl)-3-hydroxypyrrolidin-1-yl)methylphosphonate (**24a**)

Dimethoxytrityl derivatives **21a** and **21c** were prepared from **20a** (4.6 g, 7 mmol), adenine (1.1 g, 8 mmol), and cesium carbonate (2.6 g, 8 mmol) in DMF (40 ml) using the same procedure as described for compound **21b** and **21d**. The mixture of products **21a** and **21c** obtained by purification on silica gel using a linear gradient of H₃ in ethyl acetate was dissolved in 80% aqueous acetic acid (30 ml) and the solution was stirred at rt for 3 h. Acetic acid was removed in vacuo, and the crude residue was co-evaporated with water (2×20 ml), ethanol (2×20 ml), and toluene (2×20 ml). A mixture of products **22a** and **24a** (in the ratio 1:1) was obtained by purification on silica gel using a linear gradient of H₃ in ethyl acetate in a 17% overall yield as a yellowish thick oil (0.48 g, 1.21 mmol).

HRMS (FAB⁺) for C₁₆H₂₈N₆O₄P (M+H)⁺: calcd 399.1910, found 399.1935. For ¹H, ¹³C and ³¹P NMR see compound **24a** (*cis*-isomer) and **25a** (*trans*-isomer).

4.1.16. Diisopropyl (3*S*,4*S*)-2-(4-(adenin-9-yl)-3-hydroxypyrrolidin-1-yl)ethylphosphonate (**22b**) and diisopropyl (3*S*,4*R*)-2-(4-(adenin-9-yl)-3-hydroxypyrrolidin-1-yl)ethylphosphonate (**26**)

Compound **21b** (0.311 g, 0.435 mmol) was dissolved in 80% aqueous acetic acid (30 ml) and the whole was stirred at rt for 3 h. The acetic acid was removed in vacuo, and the crude product was co-evaporated with water (2×20 ml), ethanol (2×20 ml), and toluene (2×20 ml). A mixture of products **22b** and **26** (2:1) was obtained in a 99% yield (0.177 g, 0.43 mmol) by purification on silica gel using a linear gradient of H₃ in ethyl acetate as a thick yellowish oil.

HRMS (FAB⁺) for C₁₇H₃₀N₆O₄P (M+H)⁺: calcd 413.2066, found 413.2084. For ¹H, ¹³C and ³¹P NMR, see compound **26** (*cis*-isomer) and **27** (*trans*-isomer).

4.1.17. Diisopropyl (3*S*,4*R*)-(4-(adenin-9-yl)-3-hydroxypyrrolidin-1-yl)methylphosphonate (**24a**)

Aqueous formaldehyde (14.5 M, 1 ml, 14.5 mmol) was added to the suspension of **8a** (0.34 g, 1.537 mmol) in diisopropyl phosphite (3 ml). The mixture was homogenized using an ultrasonic bath and stirred at 60 °C for 3 h. Aqueous sulfuric acid (0.02 M, 40 ml) was added and the reaction mixture was stirred at rt for two days. The solution was applied onto the column of Dowex 50 (40 ml). The resin was washed with 50% aqueous ethanol (200 ml), and the crude product was eluted with 3% ammonia in an ethanol–water (1:1)

mixture. Pure compound **24a** was obtained by column chromatography on silica gel using a linear gradient of ethanol in chloroform in 87% yield (0.53 g, 1.332 mmol) as a thick yellowish oil.

HRMS (FAB⁺) for C₁₆H₂₈N₆O₄P (M+H)⁺: calcd 399.1910, found 399.1905. ν_{max} (KBr) 3423 (vs, br), 3200 (m, br), 2979 (m), 1641 (s), 1600 (s), 1573 (m, sh), 1469 (m), 1415 (m), 1387 (m), 1376 (m), 1331 (m), 1299 (m), 1245 (m, br, sh), 1222 (m, br), 1179 (m), 1143 (w), 1079 (m), 1062 (m), 1012 (s, sh), 990 (vs), 889 (w), 799 (w), 763 (w, br), 647 (w), 553 (m). ¹H NMR (400 MHz, DMSO-*d*₆): 1.26 and 1.27 (2×d, 12H, *J*_{vic}=6.2, (CH₃)₂CH), 2.85 (dd, 1H, *J*_{gem}=10.2, *J*_{2b,3}=3.7, H-2b-pyrr), 2.91 (dd, 1H, *J*_{gem}=15.0, *J*_{H,P}=11.3, CH_aH_b-P), 2.97 (dd, 1H, *J*_{gem}=15.0, *J*_{H,P}=11.6, CH_aH_b-P), 3.00 (dd, 1H, *J*_{gem}=10.2, *J*_{2a,3}=6.9, H-2a-pyrr), 3.03 (dd, 1H, *J*_{gem}=9.9, *J*_{5b,4}=6.5, H-5b-pyrr), 3.15 (dd, 1H, *J*_{gem}=9.9, *J*_{5a,4}=5.1, H-5a-pyrr), 4.40 (m, 1H, *J*_{3,2}=6.9, 3.7, *J*_{3,4}=6.5, *J*_{3,OH}=5.0, H-3-pyrr), 4.61 and 4.62 (2×dh, 2×1H, *J*_{H,P}=7.8, *J*_{vic}=6.2, CH(CH₃)₂), 5.01 (td, 1H, *J*_{4,3}=6.5, *J*_{4,5}=6.5, 5.1, H-4-pyrr), 5.13 (d, 1H, *J*_{OH,3}=5.0, OH), 7.16 (br s, 2H, NH₂), 8.12 (s, 1H, H-2), 8.16 (s, 1H, H-8). ¹³C NMR (100.6 MHz, DMSO-*d*₆): 24.01 and 24.09 (d, *J*_{C,P}=4, (CH₃)₂CH), 51.14 (d, *J*_{C,P}=163, CH₂P), 55.05 (CH-4-pyrr), 58.87 (d, *J*_{C,P}=11, CH₂-5-pyrr), 62.61 (d, *J*_{C,P}=10, CH₂-2-pyrr), 68.86 (CH-3-pyrr), 69.95 (d, *J*_{C,P}=6, CH(CH₃)₂), 118.20 (C-5), 141.03 (CH-8), 150.19 (C-4), 152.29 (CH-2), 156.02 (C-6). ³¹P NMR (162 MHz, DMSO-*d*₆): 22.49.

4.1.18. Diisopropyl (3*S*,4*R*)-(3-hydroxy-4-(thymine-1-yl)-pyrrolidin-1-yl)methylphosphonate (**24b**)

The title compound was prepared as described for **24a** from **8b** (0.1 g, 0.47 mmol), 14.5 M aqueous formaldehyde (0.5 ml, 7.25 mmol), and diisopropyl phosphite (2 ml) in 65% yield (0.12 g, 0.31 mmol) as a thick yellowish oil.

HRMS (ESI⁺) for C₁₆H₂₈N₃NaO₆P (M+Na)⁺: calcd 412.1608, found 412.1600. ν_{max} (KBr) 3397 (m, vbr), 3190 (m, br), 2980 (m), 1694 (vs, br), 1664 (s, br, sh), 1517 (w, sh), 1472 (m), 1389 (m), 1375 (m), 1280 (s), 1233 (m), 1179 (m), 1143 (m), 1069 (m), 1012 (s, sh), 988 (vs), 888 (m), 764 (w). ¹H NMR (400 MHz, DMSO-*d*₆): 1.26 and 1.27 (2×d, 12H, *J*_{vic}=6.2, (CH₃)₂CH), 1.74 (d, 3H, *J*=1.2, CH₃), 2.85 (dd, 1H, *J*_{gem}=10.0, *J*_{2b,3}=5.9, H-2b-pyrr), 2.65 (dd, 1H, *J*_{gem}=10.8, *J*_{5b,4}=8.0, H-5b-pyrr), 2.76 (dd, 1H, *J*_{gem}=15.0, *J*_{H,P}=10.7, CH_aH_b-P), 2.86 (dd, 1H, *J*_{gem}=10.0, *J*_{2a,3}=2.2, H-2a-pyrr), 2.89 (dd, 1H, *J*_{gem}=15.0, *J*_{H,P}=12.8, CH_aH_b-P), 3.05 (dd, 1H, *J*_{gem}=10.8, *J*_{5a,4}=3.4, H-5a-pyrr), 4.27 (m, 1H, *J*_{3,4}=7.6, *J*_{3,2}=5.9, 2.2, *J*_{3,OH}=4.4, H-3-pyrr), 4.61 (m, 2H, CH(CH₃)₂), 4.92 (ddd, 1H, *J*_{4,5}=8.0, 3.4, *J*_{4,3}=7.6, H-4-pyrr), 5.16 (d, 1H, *J*_{OH,3}=4.4, OH), 7.58 (q, 1H, *J*=1.2, H-6), 11.15 (br s, 1H, NH). ¹³C NMR (100.6 MHz, DMSO-*d*₆): 12.49 (CH₃), 23.98, 24.01, and 24.05 (d, *J*_{C,P}=4, (CH₃)₂CH), 50.88 (d, *J*_{C,P}=165, CH₂P), 55.14 (CH-4-pyrr), 58.04 (d, *J*_{C,P}=10, CH₂-5-pyrr), 63.58 (d, *J*_{C,P}=12, CH₂-2-pyrr), 68.53 (CH-3-pyrr), 69.94 and 69.96 (d, *J*_{C,P}=7, CH(CH₃)₂), 106.86 (C-5), 140.79 (CH-6), 151.54 (C-2), 164.13 (C-4).

4.1.19. Diisopropyl (3*R*,4*R*)-(4-(adenine-9-yl)-3-hydroxypyrrolidin-1-yl)methylphosphonate (**25a**)

The title compound was prepared as described for **24a** from **9a** (0.9 g, 4.09 mmol), 14.5 M aqueous formaldehyde (1.4 ml, 20 mmol), and diisopropyl phosphite (20 ml) in 78% yield (1.27 g, 3.18 mmol) as a thick yellowish oil.

HRMS (FAB⁺) for C₁₆H₂₈N₆O₄P (M+H)⁺: calcd 399.1910, found 399.1907. ¹H NMR (400 MHz, DMSO-*d*₆): 1.24 and 1.25 (2×d, 12H, *J*_{vic}=6.2, (CH₃)₂CH), 2.56 (dd, 1H, *J*_{gem}=10.0, *J*_{2b,3}=4.7, H-2b-pyrr), 2.89 (dd, 1H, *J*_{gem}=15.0, *J*_{H,P}=10.7, CH_aH_b-P), 2.98 (dd, 1H, *J*_{gem}=15.0, *J*_{H,P}=12.4, CH_aH_b-P), 3.09 (d, 2H, *J*_{5,4}=5.8, H-5-pyrr), 3.35 (dd, 1H, *J*_{gem}=10.0, *J*_{2a,3}=6.8, H-2a-pyrr), 4.40 (br m, 1H, *J*_{3,2}=6.8, 4.7, *J*_{3,OH}=4.8, *J*_{3,4}=3.3, H-3-pyrr), 4.60 and 4.61 (2×dh, 2×1H, *J*_{H,P}=7.8, *J*_{vic}=6.2, CH(CH₃)₂), 4.70 (td, 1H, *J*_{4,5}=5.8, *J*_{4,3}=3.3, H-4-pyrr), 5.54 (d, 1H, *J*_{OH,3}=4.8, OH), 7.23 (br s, 2H, NH₂), 8.14 (s, 1H, H-8), 8.18 (s, 1H, H-2). ¹³C NMR (100.6 MHz, DMSO-*d*₆): 24.00 and 24.08 (d, *J*_{C,P}=4, (CH₃)₂CH), 50.94 (d, *J*_{C,P}=163, CH₂P), 58.79 (d, *J*_{C,P}=10, CH₂-5-pyrr), 61.67 (CH-4-pyrr), 62.69 (d, *J*_{C,P}=10, CH₂-2-pyrr), 69.99 (d, *J*_{C,P}=6,

CH(CH₃)₂), 75.06 (CH-3-pyrr), 118.87 (C-5), 139.47 (CH-8), 149.52 (C-4), 152.54 (CH-2), 156.19 (C-6). ³¹P NMR (162 MHz, DMSO-*d*₆): 22.84.

4.1.20. Diisopropyl (3*R*,4*R*)-(3-hydroxy-4-(thymine-1-yl)-pyrrolidin-1-yl)methylphosphonate (**25b**)

The titled compound was prepared as described for **24a** from **9b** (0.15 g, 0.71 mmol), 14.5 M aqueous formaldehyde (0.5 ml, 7.25 mmol), and diisopropyl phosphite (2 ml) in 54% yield (0.15 g, 0.385 mmol) as a thick yellowish oil.

HRMS (ESI⁺) for C₁₆H₂₈N₃NaO₆P (M+Na)⁺: calcd 412.1608, found 412.1611. ¹H NMR (500 MHz, DMSO-*d*₆): 1.21 and 1.22 (2×d, 12H, *J*_{vic}=6.2, (CH₃)₂CH), 1.74 (d, 3H, *J*=1.2, CH₃), 2.34 (dd, 1H, *J*_{gem}=9.7, *J*_{2b,3}=6.0, H-2b-pyrr), 2.79 (dd, 1H, *J*_{gem}=15.2, *J*_{H,P}=10.6, CH_aH_b-P), 2.85 (d, 2H, *J*_{5,4}=5.5, H-5-pyrr), 2.90 (dd, 1H, *J*_{gem}=15.2, *J*_{H,P}=12.6, CH_aH_b-P), 3.26 (dd, 1H, *J*_{gem}=9.7, *J*_{2a,3}=6.9, H-2a-pyrr), 4.12 (br m, 1H, *J*_{3,2}=6.9, 6.0, *J*_{3,OH}=5.1, *J*_{3,4}=3.5, H-3-pyrr), 4.56 (dh, 2H, *J*_{H,P}=7.9, *J*_{vic}=6.2, CH(CH₃)₂), 4.60 (td, 1H, *J*_{4,5}=5.5, *J*_{4,3}=3.5, H-4-pyrr), 5.66 (d, 1H, *J*_{OH,3}=5.1, OH), 7.51 (q, 1H, *J*=1.2, H-6), 11.27 (br s, 1H, NH). ¹³C NMR (125.7 MHz, DMSO-*d*₆): 12.20 (CH₃), 23.86, 23.87, and 23.92 (d, *J*_{C,P}=4, (CH₃)₂CH), 50.95 (d, *J*_{C,P}=163, CH₂P), 58.34 (d, *J*_{C,P}=10, CH₂-5-pyrr), 62.27 (CH-4-pyrr), 62.59 (d, *J*_{C,P}=11, CH₂-2-pyrr), 69.88 and 69.90 (d, *J*_{C,P}=7, CH(CH₃)₂), 74.95 (CH-3-pyrr), 109.19 (C-5), 138.19 (CH-6), 150.98 (C-2), 163.79 (C-4). ³¹P NMR (202.3 MHz, DMSO-*d*₆): 23.42.

4.1.21. Diisopropyl (3*S*,4*R*)-2-(4-(adenine-9-yl)-3-hydroxypyrrolidin-1-yl)ethylphosphonate (**26**)

Diisopropyl vinylphosphonate (1.4 g, 7 mmol) was added to a suspension of compound **8a** (0.7 g, 3.18 mmol) in methanol (30 ml), and the mixture was treated at reflux for five days. The solution was applied onto a column of Dowex 50 (40 ml). The resin was washed with 50% aqueous ethanol (200 ml), and the crude product was obtained by elution with 3% ammonia in an ethanol–water (1:1) mixture. Pure compound **26** was obtained by column chromatography on silica gel using a linear gradient of ethanol in chloroform, providing a 69% yield (0.904 g, 2.192 mmol) of a thick yellowish oil.

HRMS (FAB⁺) for C₁₇H₃₀N₆O₄P (M+H)⁺: calcd 413.2066, found 413.2084. ν_{max} (KBr) 3400 (m, br, sh), 3328 (m, br), 3265 (m, br, sh), 3176 (m, br), 2978 (m), 1662 (s), 1645 (s), 1599 (s), 1573 (m), 1511 (w), 1474 (m), 1416 (m), 1385 (m), 1374 (m), 1334 (m), 1299 (m), 1249 (s, br), 1224 (m, br), 1179 (m), 1143 (m), 1064 (m), 1009 (s, br), 983 (vs, br), 888 (w), 799 (w), 793 (w), 774 (w, br), 647 (w), 529 (m, br). ¹H NMR (600 MHz, DMSO-*d*₆): 1.24 and 1.25 (2×d, 12H, *J*_{vic}=6.2, (CH₃)₂CH), 1.94 (m, 2H, CH₂P), 2.64 (dd, 1H, *J*_{gem}=10.1, *J*_{2b,3}=3.7, H-2b-pyrr), 2.68 (m, 2H, CH₂N), 2.95 (dd, 1H, *J*_{gem}=10.1, *J*_{2a,3}=6.8, H-2a-pyrr), 2.96 (dd, 1H, *J*_{gem}=9.6, *J*_{5b,4}=6.8, H-5b-pyrr), 2.99 (dd, 1H, *J*_{gem}=9.6, *J*_{5a,4}=6.3, H-5a-pyrr), 4.36 (m, 1H, *J*_{3,2}=6.8, 3.7, *J*_{3,4}=6.7, *J*_{3,OH}=5.1, H-3-pyrr), 4.57 and 4.58 (2×dh, 2×1H, *J*_{H,P}=8.0, *J*_{vic}=6.2, CH(CH₃)₂), 4.99 (ddd, 1H, *J*_{4,5}=6.8, 6.3, *J*_{4,3}=6.7, H-4-pyrr), 5.10 (d, 1H, *J*_{OH,3}=5.1, OH), 7.15 (br s, 2H, NH₂), 8.12 (s, 1H, H-2), 8.19 (s, 1H, H-8). ¹³C NMR (100.6 MHz, DMSO-*d*₆): 24.00 (d, *J*_{C,P}=4, (CH₃)₂CH), 25.65 (d, *J*_{C,P}=138, CH₂P), 8.99 (CH₂N), 54.95 (CH-4-pyrr), 56.58 (CH₂-5-pyrr), 60.66 (CH₂-2-pyrr), 68.65 (CH-3-pyrr), 69.40 (d, *J*_{C,P}=6, CH(CH₃)₂), 118.27 (C-5), 141.33 (CH-8), 150.14 (C-4), 152.23 (CH-2), 155.99 (C-6). ³¹P NMR (162 MHz, DMSO-*d*₆): 29.13.

4.1.22. Diisopropyl (3*R*,4*R*)-2-(4-(adenine-9-yl)-3-hydroxypyrrolidin-1-yl)ethylphosphonate (**27**)

The title compound was prepared from **9a** (0.12 g, 0.54 mmol) and diisopropyl vinylphosphonate (0.2 g, 1 mmol) in methanol (10 ml) via the same procedure described for **26**, in a 65% yield (0.144 g, 0.35 mmol) as a thick yellowish oil.

HRMS (FAB⁺) for C₁₇H₃₀N₆O₄P (M+H)⁺: calcd 413.2066, found 413.2074. ¹H NMR (400 MHz, DMSO-*d*₆): 1.234, 1.240, 1.242, and 1.243 (4×d, 4×3H, *J*_{vic}=6.2, (CH₃)₂CH), 1.92 (m, 2H, CH₂P), 2.43 (dd, 1H, *J*_{gem}=9.8, *J*_{2b,3}=4.6, H-2b-pyrr), 2.66 (m, 2H, CH₂N), 2.90 (dd, 1H,

$J_{gem}=9.6$, $J_{5b,4}=5.1$, H-5b-pyrr), 3.00 (dd, 1H, $J_{gem}=9.6$, $J_{5a,4}=7.1$, H-5a-pyrr), 3.18 (dd, 1H, $J_{gem}=9.8$, $J_{2a,3}=7.0$, H-2a-pyrr), 4.41 (br m, 1H, $J_{3,5}=7.0$, 4.6, $J_{3,OH}=5.0$, $J_{3,4}=3.7$, H-3-pyrr), 4.57 and 4.58 (2×dh, 2×1H, $J_{H,P}=7.8$, $J_{vic}=6.2$, CH(CH₃)₂), 4.72 (td, 1H, $J_{4,5}=7.1$, 5.1, $J_{4,3}=3.7$, H-4-pyrr), 5.52 (d, 1H, $J_{OH,3}=5.0$, OH), 7.21 (br s, 2H, NH₂), 8.13 (s, 1H, H-8), 8.24 (s, 1H, H-2). ¹³C NMR (100.6 MHz, DMSO-*d*₆): 23.97 and 23.99 (d, $J_{C,P}=4$, (CH₃)₂CH), 25.60 (d, $J_{C,P}=139$, CH₂P), 48.95 (CH₂N), 57.17 (CH₂-5-pyrr), 60.58 (CH₂-2-pyrr), 61.60 (CH-4-pyrr), 69.41 (d, $J_{C,P}=6$, CH(CH₃)₂), 75.26 (CH-3-pyrr), 118.88 (C-5), 139.77 (CH-8), 149.56 (C-4), 152.48 (CH-2), 156.18 (C-6). ³¹P NMR (162 MHz, DMSO-*d*₆): 28.46.

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