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Synthesis and Properties of a Novel Type of Acyclic Nucleoside Phosphonates: 2-(Purin-9-yl)ethoxyphenylphosphonic Acids

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A series of novel acyclic nucleoside phosphonates with a built-in arylphosphonate moiety has been prepared by a microwave-assisted cross-coupling reaction as the key step. Their cytostatic and antiviral activities were tested. The pK_a values of the target *ortho*-, *meta*- and *para*-substituted arylphosphonates were determined by ^{31}P NMR titration studies.

Introduction

Acyclic nucleoside phosphonates (ANPs) represent a group of compounds with remarkable biological activities, especially antiviral effects. The development of these compounds has resulted in several approved drugs (e.g., Adefovir, Figure 1) and is still providing new bioactive compounds.[1,2] Recently, the antimalarial activity of several ANPs (e.g., PEEG, PEEHx, Figure 1) was reported.[3,4]

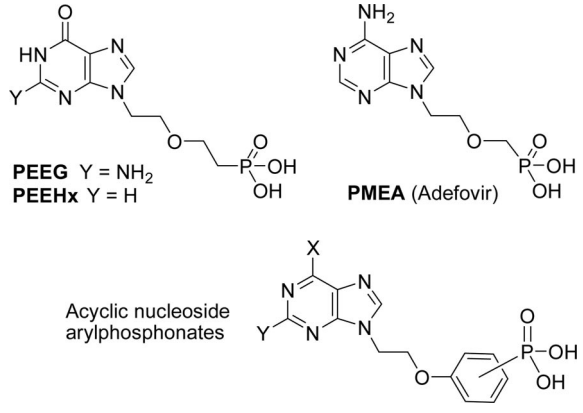

Figure 1. Examples of biologically active acyclic nucleoside phosphonates and proposed structures of their arylphosphonate analogues.

In this type of nucleotide analogue, the heterocyclic base and phosphonate function are linked by various acyclic chains that mimic sugar moieties. The ester oxygen present in the phosphate group of a nucleotide is replaced by a carbon atom. Thus, enzymatic dephosphorylation or chemical hydrolysis is excluded. The absence of a glycosidic bond in the structures of ANPs further increases their resistance to chemical and biological degradation. Another structural advantage is the flexibility of the acyclic chain, which enables the compounds to adopt a conformation suitable for interaction with the active site of the enzyme.

On the basis of the biological activities of several acyclic nucleoside[5] and nucleotide[6] analogues with a built-in aryl moiety in the side-chain, in this work we focused on the synthesis of novel ANPs bearing an arylphosphonate group (Figure 1). This group could improve the binding to the active site of enzymes if hydrophobic or stacking interactions are required. We aimed to evaluate their biological activity and compare their properties with aliphatic parent compounds. To further characterize these new ANPs, the pK_a1 and pK_a2 values of the arylphosphonate group in the *ortho*-, *meta*- and *para*-position were determined by ^{31}P NMR titration studies.

Arylphosphonic acid moieties are key functionalities in many biologically active compounds.[7–9] Owing to the poor reactivity of aryl halides in classic reactions, their accessibility has been limited. However, a general synthetic methodology based on palladium-catalysed cross-coupling has been developed by Hirao et al.[10,11] This method of C–P bond formation has been further improved and modified[12–14] and has also been utilized in nucleoside chemistry.[15] Recently, successful optimization of the microwave-assisted reaction of H-phosphonates with aryl halides was reported.[16]

The above-mentioned procedure together with a well-established methodology for the preparation of ANPs has provided a convenient synthesis of acyclic nucleoside arylphosphonates.

Results and Discussion

Chemistry

The synthesis of the target acyclic nucleoside arylphosphonates was based on the Mitsunobu reaction of purine bases with preformed hydroxy derivatives. The synthesis of

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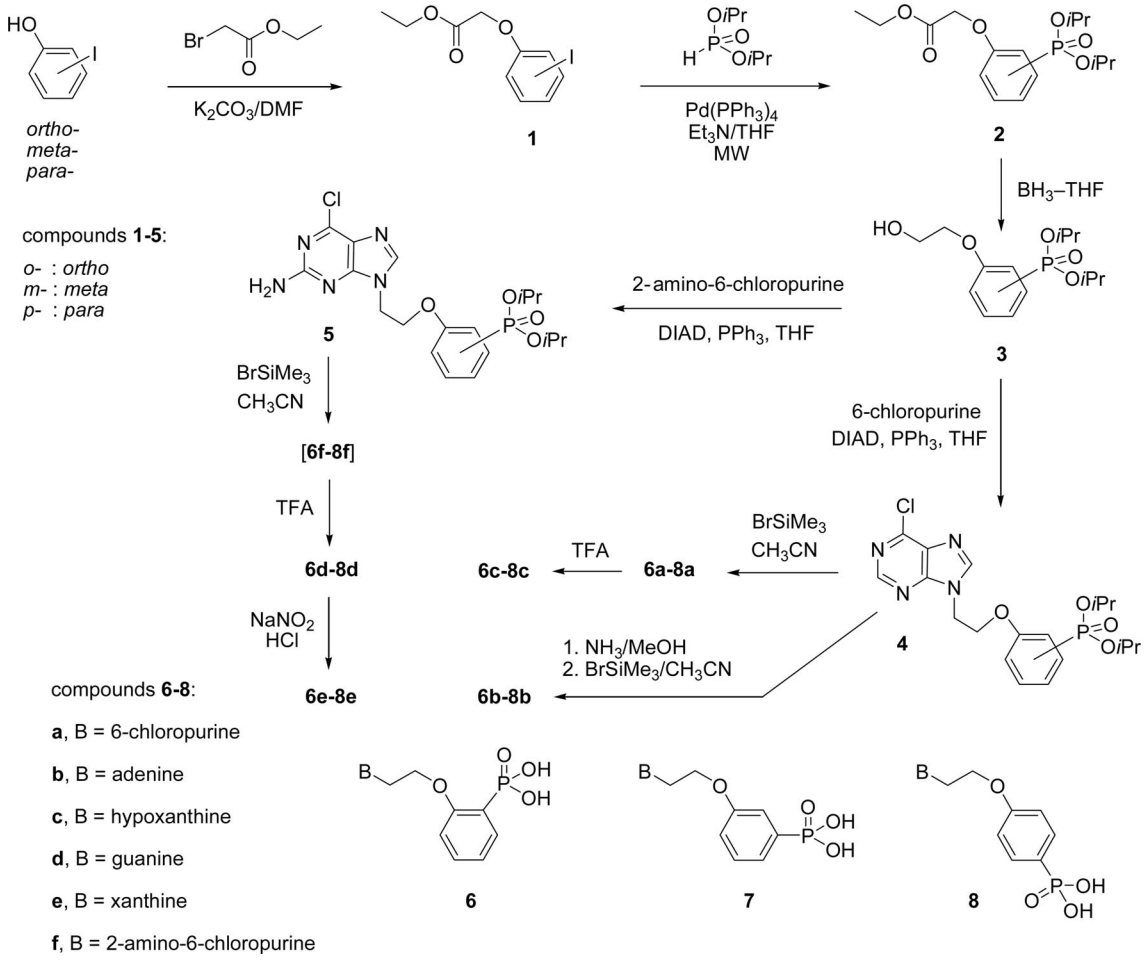


Scheme 1. Synthesis of the acyclic nucleoside phosphonates **6a**–**6f**, **7a**–**7f** and **8a**–**8f**.

these key alcohols **3** with a built-in arylphosphonate diester moiety was carried out in the same manner for *ortho*-, *meta*- and *para*-disubstituted phenyl compounds (Scheme 1). The starting iodophenoxyacetates **1** were prepared according to the literature by the alkylation of iodophenols.[17]

The reaction conditions for successive C–P bond formation by palladium-catalysed cross-coupling had to be optimized. When conventional heating in argon was applied[11] using $[Pd(PPh_3)_4]$ as a catalyst and Et_3N as a base, the coupling of the iodo derivatives **1** with H-phosphonate diisopropyl ester afforded a complex mixture and conversion was low even after 3 d at 90 °C (isolated yield 13%). The microwave-assisted reaction[16] in an argon-flushed septum-closed microwave vial on a 4 mmol scale proceeded reasonably well in tetrahydrofuran with 5% of $[Pd(PPh_3)_4]$ and Et_3N as base. The reaction time was substantially reduced and the yields were approximately 80% for the *meta* and *para* derivatives *m*-**2** and *p*-**2** after 30 min of irradiation at 100 °C and 300 W. The less satisfactory yield of *ortho*-disubstituted arylphosphonate *o*-**2** (45%) was probably caused by side-reactions based on a cyclic mechanism.[18]

The ethyl acetate moiety attached through an oxygen atom to the phenyl served as a latent alcohol. After smooth reduction of derivatives **2** with the BH_3–THF complex, the desired [(2-hydroxyethoxy)aryl]phosphonates **3** were obtained in 90% yields.

To introduce the chain containing the arylphosphonate group at the 9-position of the purine base, the standard alkylation of 6-chloropurine and 2-amino-6-chloropurine was applied under Mitsunobu conditions (THF, DIAD, PPh_3) to form esters **4** and **5**, respectively. Identical procedures were used for the synthesis of all the diisopropyl *o*-, *m*- and *p*-[2-(purin-9-yl)ethoxy]phenylphosphonates **4** and **5** from the corresponding hydroxy derivatives **3** to give predominantly N^9-alkylation products, with only traces of the other regioisomers being detected. The yields were, as expected, approximately 50% for the 2-amino-6-chloropurine derivatives **5**. Higher yields (about 80%) were obtained in the synthesis of 6-chloropurine products *m*- and *p*-**4**.

To prepare ANPs with various purine bases, further transformations of the functional groups at the 6- and/or 2-positions had to be accomplished together with cleavage of the phosphonic acids ester groups. To gain the free phosphonic acids **6a**–**8a** containing the chloropurine base, only treatment of diisopropyl esters **4** with bromotrimethylsilane in dry acetonitrile followed by hydrolysis was needed. Addition of 2,6-lutidine to the reaction mixture prevented the formation of 6-bromopurine compounds.

Adenine ANPs **6b**–**8b** were obtained after ammonolysis of 6-choropurine derivatives *o*-, *m*- and *p*-**4**, respectively, with methanolic ammonia in an autoclave followed by the above-mentioned standard reaction with bromotrimethylsilane and hydrolysis with yields of about 60% over the two steps.

The reverse order of base modification and phosphonate group deprotection was used to prepare the hypoxanthine and guanine derivatives to avoid partial cleavage of the isopropyl esters during hydrolysis of the chloro group. After standard treatment of diisopropyl esters **4** and **5** with bromotrimethylsilane, 75% trifluoroacetic acid was applied in a routine procedure to hydrolyse the halogen at the 6-position of the purine in the free phosphonic acids **6a**–**8a** and crude intermediates **6f**–**8f**. Thus, 6-chloropurine derivatives **6a**–**8a** were converted into hypoxanthine ANPs **6c**–**8c**. 2-Amino-6-chloropurine compounds *o*-, *m*- and *p*-**5** afforded after the two above-mentioned steps guanine ANPs **6d**–**8d**, respectively.

Diazotative oxodeamination[19] at the 2-position of the guanine compounds **6d**–**8d** by $NaNO_2$ in aqueous HCl finally gave xanthine phosphonic acids **6e**–**8e** in satisfactory yields of 71–76%.

Determination of the pK_a Values

The acid–base dissociation constant (pK_a) is a key parameter determining the pharmacokinetic properties of compounds containing ionogenic functional groups. The pH dependences of the ^{31}P chemical shifts of the arylphosphonate group were measured for *o*- (**6c**), *m*- (**7c**) and *p*-hypoxanthine (**8c**) derivatives and, for comparison, also for PEEHx (for details see the Exp. Sect.). The pK_a values were estimated and are summarized in Figure 2.

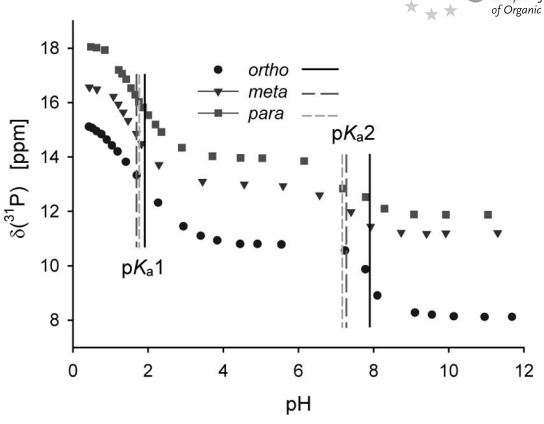
Differences in the pK_a1 values are very small (1.7–1.9) and, in accord with the literature,[20] they increase in the order *meta* < *para* < *ortho*. The values are similar to that of the structurally similar aliphatic phosphonate PEEHx (pK_a1 = 1.8) used as an example of a biologically active ANP.[3]

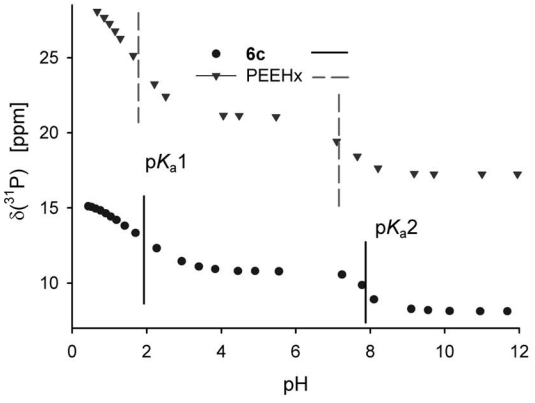
The pK_a2 values are similar for PEEHx and the *meta*- and *para*-disubstituted arylphosphonic acids (7.2, 7.2 and 7.3) and are comparable with literature data for biologically active acyclic nucleoside phosphonates,[21] however, the pK_a2 for the *ortho* derivative **6c** was, as expected,[20] significantly higher (7.9).

The pK_a values of all studied ANPs are slightly higher but comparable with those of the naturally occurring hypoxanthine nucleotide inosine 5′-monophosphate (1.3 and 6.2).[22]

Biological Activity Screening

The title 2-(purin-9-yl)ethoxyphenylphosphonic acids **6a**–**6e**, **7a**–**7e** and **8a**–**8e** were tested for their in vitro inhibition of cell growth in mouse leukaemia L1210 cells, human T-lymphoblastoid CCRF-CEM cell line, human promyelocytic leukaemia HL-60 cells and human cervix carcinoma HeLa S3 cells (Dr. I. Votruba, IOCB, Prague). None of the compounds exhibited considerable cytostatic activity or cytotoxicity. The inhibitor properties of these new ANPs were studied with purine nucleoside phosphorylase (Dr. I. Votruba) and hypoxanthine-guanine-xanthine phosphoribosyltransferase (group of Dr. Luke W. Guddat, Queensland University, Australia), but none of the compounds exhibited significant activity. Because of the probability of these new ANPs exhibiting antiviral activity, all the derivatives are also being subjected to a standard in vitro antiviral activity screening using a wide spectrum of viruses (group of Prof. Jan Balzarini, Rega Institute for Medicinal Research, Leuven, Belgium).





Compounds		pK_a1	pK_a2
6c	*ortho*	1.9	7.9
7c	*meta*	1.7	7.3
8c	*para*	1.8	7.2
PEEHx		1.8	7.2

Figure 2. a) The pH dependence of the ^{31}P NMR chemical shifts of *o*- (**6c**), *m*- (**7c**) and *p*-hypoxantine (**8c**) derivatives. b) Comparison of the pH dependence of the ^{31}P NMR chemical shifts of compound **6c** and PEEHx. c) Table of estimated pK_a values for **6**–**8c** and PEEHx.

Conclusions

An efficient methodology for the synthesis of a novel class of acyclic nucleoside phosphonates with a built-in aryl

moiety in the side-chain has been developed and a series of derivatives containing various purine bases synthesized. The acid–base dissociation constants for the *ortho*-, *meta*- and *para*-substituted arylphosphonic acids were determined as important parameters determining pharmacokinetic properties. Biological activity studies of target ANPs are in progress.

Experimental Section

General: Unless otherwise stated, solvents were evaporated at 40 °C and 2 kPa. NMR spectra were recorded with Bruker Avance 500 (^{1}H at 500 MHz, ^{13}C at 125.8 MHz) and 400 (^{1}H at 400 MHz, ^{13}C at 100.6 MHz, ^{31}P at 202.3 MHz) spectrometers with TMS as the internal standard referenced to the residual solvent signal or H_3PO_4 as the external standard. Mass spectra were measured with a ZAB-EQ (VG Analytical) spectrometer. The chemicals were obtained from commercial sources (Sigma–Aldrich) or prepared according to published procedures.[17] Dimethylformamide and acetonitrile were distilled from P_2O_5 and stored over molecular sieves (4 Å). THF was distilled from sodium/benzophenone under argon. Preparative HPLC purifications were performed on reversed-phase columns packed with 7 μm C18 (Waters Delta 600 chromatograph column, 17 × 250 mm) in batches of around 200 mg of the mixtures using gradient MeOH/H_2O as eluent. The purity of the prepared compounds was determined by combustion elemental analysis (C,H,N); the purity exceeded 95%. Microwave heating was carried out with a single mode cavity Discover® microwave synthesizer (CEM Corp.).

General Procedure for the Synthesis of Ethyl 2-(Diisopropoxyphosphoryl)phenoxyacetates 2: A mixture of iodophenoxyacetate (1.22 g, 4 mmol), [Pd(PPh_3)$_4$] (232 mg, 0.2 mmol), dry triethylamine (0.6 mL), dry tetrahydrofuran (4 mL) and diisopropyl phosphonate (732 mg) in a septum-closed microwave vial was irradiated in the microwave reactor at 100 °C/300 W for 30 min under argon. Then chloroform (50 mL) was added and mixture was subsequently washed with saturated EDTA and NaCl solution. The organic layer was dried with anhydrous magnesium sulfate and the solvent evaporated. The residue was purified by chromatography on silica gel (MeOH in $CHCl_3$).

Ethyl 2-[2-(Diisopropoxyphosphoryl)phenoxy]acetate (*o*-2): Starting from ethyl 2-(2-iodophenoxy)acetate,[17] yield 0.62 g, 45%. ^{1}H NMR ([D$_6$]DMSO): δ = 7.72 (ddd, 1 H, Ar), 7.34 (m, 1 H, Ar), 7.07 (dt, 1 H, Ar), 6.96 (dd, 1 H, Ar), 4.87 (s, 2 H, OCH_2CO), 4.59 (m, 2 H, *i*Pr), 4.18 (q, *J* = 7.1 Hz, 2 H, Et), 1.26 (d, *J* = 6.2 Hz, 6 H), 1.18 (d, *J* = 6.2 Hz, 6 H, *i*Pr), 1.21 (t, *J* = 7.1 Hz, 3 H, Et) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 168.07 (CO), 158.96 [d, *J*(P,C) = 2.1 Hz, O-Ar], 134.34 [d, *J*(P,C) = 6.8 Hz, Ar], 133.87 [d, *J*(P,C) = 1.9 Hz, Ar], 120.66 [d, *J*(P,C) = 13.9 Hz, Ar], 119.74 [d, *J*(P,C) = 184.0 Hz, P-Ar], 112.43 [d, *J*(P,C) = 8.8 Hz, Ar], 69.79 [d, *J*(P,C) = 5.6 Hz, 2 C, *i*Pr], 65.04 (C-1), 60.54 (C-2), 23.55 [dd, *J*(P,C) = 31.9, 4.5 Hz, 4 C, *i*Pr] 13.87 (C-3) ppm. MS (ESI): *m*/*z* = 345 [M + H]$^+$.

Ethyl 2-[3-(Diisopropoxyphosphoryl)phenoxy]acetate (*m*-2): Starting from ethyl 2-(3-iodophenoxy)acetate,[17] yield 1.15 g, 83%. ^{1}H NMR ([D$_6$]DMSO): δ = 7.45 (m, 1 H, Ar), 7.35 (m, 1 H, Ar), 7.16 (m, 2 H, Ar), 4.86 (s, 2 H, OCH_2CO), 4.54 (m, 2 H, *i*Pr), 4.16 (q, *J* = 7.1 Hz, 2 H, Et), 1.27 (d, *J* = 6.2 Hz, 6 H, *i*Pr), 1.16 (d, *J* = 6.2 Hz, 6 H, *i*Pr), 1.20 (t, *J* = 7.1 Hz, 3 H, Et) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 168.39 (CO), 157.24 [d, *J*(P,C) = 18.4 Hz, O-Ar], 131.19 [d, *J*(P,C) = 185.2 Hz, P-Ar], 130.10 [d, *J*(P,C) = 17.0 Hz, Ar], 123.87 [d, *J*(P,C) = 9.1 Hz, Ar], 118.44 [d, *J*(P,C) = 3.0 Hz, Ar], 116.63 [d, *J*(P,C) = 10.9 Hz, Ar], 70.11 [d, *J*(P,C) = 5.5 Hz, 2 C, *i*Pr], 64.66 (C-1), 60.56 (C-2), 23.48 [dd, *J*(P,C) = 18.2, 4.4 Hz, 4 C, *i*Pr), 13.88 (C-3) ppm. MS (ESI): *m*/*z* = 345 [M + H]$^+$.

Ethyl 2-[4-(Diisopropoxyphosphoryl)phenoxy]acetate (*p*-2): Starting from ethyl 2-(4-iodophenoxy)acetate,[17] yield 1.06 g, 77%. ^{1}H NMR ([D$_6$]DMSO): δ = 7.62 (m, 2 H, Ar), 7.06 (m, 2 H, Ar), 4.88 (s, 2 H, OCH_2CO), 4.50 (m, 2 H, *i*Pr), 4.17 (m, 2 H, Et), 1.26 (d, *J* = 6.2 Hz, 6 H, *i*Pr), 1.15 (d, *J* = 6.2 Hz, 6 H, *i*Pr), 1.20 (t, *J* = 7.1 Hz, 3 H, Et) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 168.18 (CO), 160.39 [d, *J*(P,C) = 4.0 Hz, O-Ar], 133.01 (d, *J*(P,C) = 11.3 Hz, 2 C, Ar], 121.76 [d, *J*(P,C) = 192.8 Hz, P-Ar], 114.51 [d, *J*(P,C) = 15.4 Hz, 2 C, Ar], 69.71 [d, *J*(P,C) = 5.6 Hz, 2 C, *i*Pr], 64.46 (C-1), 60.61 (C-2), 23.51 [dd, *J*(P,C) = 16.0, 3.4 Hz, 4 C, *i*Pr], 13.87 (C-3) ppm. MS (ESI): *m*/*z* = 345 [M + H]$^+$.

General Procedure for the Synthesis of Diisopropyl 2-Hydroxyethoxyphenylphosphonates 3: 2-(Diisopropoxyphosphoryl)phenoxyacetate (**2**) (1.1 g, 3.2 mmol) was cooled to –20 °C and a solution of borane in THF (1.0 M, 5 mL) was added under argon. The reaction mixture was stirred at room temperature overnight. Methanol (2 mL) was added at –20 °C and the solution was concentrated after the evolution of hydrogen had stopped. Then ethyl acetate (50 mL) was added and the mixture was washed with saturated NaCl solution. The organic layer was dried with anhydrous magnesium sulfate and the solvent evaporated. The residue was purified by chromatography on silica gel (5% MeOH in $CHCl_3$).

Diisopropyl 2-(2-Hydroxyethoxy)phenylphosphonate (*o*-3): Starting from ethyl 2-[2-(diisopropoxyphosphoryl)phenoxy]acetate (*o*-**2**), yield 0.86 g, 89%. ^{1}H NMR ([D$_6$]DMSO): δ = 7.64 (ddd, 1 H, Ar), 7.56 (m, 1 H, Ar), 7.16 (dt, 1 H, Ar), 7.05 (dd, 1 H, Ar), 4.89 (t, *J* = 5.8 Hz, 1 H, OH), 4.61 (m, 2 H, *i*Pr), 4.11 (t, *J* = 5.2 Hz, 2 H, 1-H), 3.70 (dd, *J* = 10.7, 5.4 Hz, 2 H, 2-H), 1.27 (d, *J* = 6.2 Hz, 6 H, *i*Pr), 1.21 (d, *J* = 6.2 Hz, 6 H, *i*Pr) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 160.37 [d, *J*(P,C) = 2.7 Hz, O-Ar], 134.11 [d, *J*(P,C) = 2.0 Hz, Ar], 133.53 [d, *J*(P,C) = 6.8 Hz, Ar], 120.43 [d, *J*(P,C) = 14.0 Hz, Ar], 118.27 [d, *J*(P,C) = 186.7 Hz, P-Ar], 113.73 [d, *J*(P,C) = 9.0 Hz, Ar], 70.92 (C-1), 69.91 [d, *J*(P,C) = 5.9 Hz, 2 C, *i*Pr], 59.47 (C-2), 23.56 [dd, *J*(P,C) = 26.56, 4.4 Hz, 4 C, *i*Pr] ppm. MS (ESI): *m*/*z* = 303 [M + H]$^+$.

Diisopropyl 3-(2-Hydroxyethoxy)phenylphosphonate (*m*-3): Starting from ethyl 2-[3-(diisopropoxyphosphoryl)phenoxy]acetate (*m*-**2**), yield 0.88 g, 91%. ^{1}H NMR ([D$_6$]DMSO): δ = 7.44 (m, 1 H, Ar), 7.25 (m, 1 H, Ar), 7.15 (m, 2 H, Ar), 4.90 (t, *J* = 5.4 Hz, 1 H, OH), 4.55 (m, 2 H, *i*Pr), 4.02 (t, *J* = 4.9 Hz, 2 H, 1-H), 3.79 (dd, 2 H, *J* = 9.6, 4.8 Hz, 2-H), 1.27 (d, *J* = 6.2 Hz, 6 H, *i*Pr), 1.17 (d, *J* = 6.2 Hz, 6 H, *i*Pr) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 158.11 [d, *J*(P,C) = 18.4 Hz, O-Ar], 130.89 [d, *J*(P,C) = 185.1 Hz, P-Ar], 129.68 [d, *J*(P,C) = 17.1 Hz, Ar], 122.86 [d, *J*(P,C) = 9.1 Hz, Ar], 118.07 [d, *J*(P,C) = 3.1 Hz, Ar], 116.49 [d, *J*(P,C) = 11.0 Hz, Ar], 69.83 [d, *J*(P,C) = 5.6 Hz, 2 C, *i*Pr], 69.44 (C-1), 59.14 (C-2), 23.30 [dd, *J*(P,C) = 16.5, 4.4 Hz, 4 C, *i*Pr] ppm. MS (ESI): *m*/*z* = 303 [M + H]$^+$.

Diisopropyl 4-(2-Hydroxyethoxy)phenylphosphonate (*p*-3): Starting from ethyl 2-[4-(diisopropoxyphosphoryl)phenoxy]acetate (*p*-**2**), yield 0.87 g, 90%. ^{1}H NMR ([D$_6$]DMSO): δ = 7.60 (m, 2 H, Ar), 7.05 (m, 2 H, Ar), 4.89 (t, 1 H, OH), 4.49 (m, 2 H, *i*Pr), 4.04 (t, *J* = 4.9 Hz, 2 H, 1-H), 3.71 (m, 2 H, 2-H), 1.25 (d, *J* = 6.2 Hz, 6 H, *i*Pr), 1.14 (d, *J* = 6.2 Hz, 6 H, *i*Pr) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 161.24 [d, *J*(P,C) = 3.3 Hz, O-Ar], 132.76 [d, *J*(P,C) = 11.1 Hz, 2 C, Ar], 120.49 [d, *J*(P,C) = 192.9 Hz, P-Ar], 114.11 [d, *J*(P,C) = 15.5 Hz, 2 C, Ar], 69.31 [d, 2 C, *J*(P,C) = 5.4 Hz, *i*Pr), 69.26 (C-1), 58.98 (C-2), 23.21 [dd, *J*(P,C) = 16.6, 3.9 Hz, 4 C, *i*Pr] ppm. MS (ESI): *m*/*z* = 303 [M + H]$^+$.

General Procedure for the Synthesis of Diisopropyl 2-(6-Chloropurin-9-yl)ethoxyphenylphosphonates 4 by the Mitsunobu Reaction: Diisopropyl azadicarboxylate (DIAD, 1.6 mL, 8.4 mmol) was added slowly to a solution of triphenyphosphane (2.36 g, 9 mmol) in dry THF (30 mL) cooled to –20 °C under argon. The mixture was stirred for 30 min and this preformed complex was added to the reaction mixture containing 6-chloropurine (1.02 g, 6.6 mmol), dry THF (20 mL) and diisopropyl [(2-hydroxyethoxy)phenyl]phosphonate **3** (3 mmol) at –40 °C under argon. The resulting mixture was slowly warmed to room temperature and stirred overnight. The solvent was evaporated and the crude mixture was purified by chromatography on silica gel (0–5% MeOH in $CHCl_3$).

Diisopropyl 2-[2-(6-Chloropurin-9-yl)ethoxy]phenylphosphonate (*o*-4): Starting from diisopropyl 2-[(2-hydroxyethoxy)phenyl]phosphonate (*o*-**3**), yield 0.64 g, 49%. ^{1}H NMR ([D$_6$]DMSO): δ = 8.93 (s, 1 H, 2-H), 8.81 (s, 1 H, 8-H), 7.69 (m, 1 H, Ar), 7.56 (m, 1 H, Ar), 7.14 (m, 1 H, Ar), 7.06 (m, 1 H, Ar), 4.71 (t, *J* = 4.9 Hz, 2 H, 1′-H), 4.50 (m, 4 H, *i*Pr, 2′-H), 1.21 (d, *J* = 6.2 Hz, 6 H, *i*Pr), 1.06 (d, *J* = 6.2 Hz, 6 H, *i*Pr) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 159.18 [d, *J*(P,C) = 2.3 Hz, O-Ar], 151.82 (C-4), 151.31 (C-2), 148.75 (C-6), 148.01 (C-8), 134.20 (m, 2 C, Ar), 130.55 (C-5), 120.61 [d, *J*(P,C) = 14.0 Hz, Ar], 117.62 [d, *J*(P,C) = 184.2 Hz, P-Ar], 112.43 [d, *J*(P,C) = 8.9 Hz, Ar], 69.74 [d, *J*(P,C) = 5.5 Hz, 2 C, *i*Pr), 65.95 (C-2′), 43.05 (C-1′), 23.35 [dd, *J*(P,C) = 35.1, 4.4 Hz, 4 C, *i*Pr] ppm. MS (ESI): *m*/*z* = 439 [M + H]$^+$.

Diisopropyl 3-[2-(6-Chloropurin-9-yl)ethoxy]phenylphosphonate (*m*-4): Starting from diisopropyl 3-(2-hydroxyethoxy)phenylphosphonate (*m*-**3**), yield 1.16 g, 88%. ^{1}H NMR ([D$_6$]DMSO): δ = 8.80 (s, 1 H, 2-H), 8.79 (s, 1 H, 8-H), 7.40 (m, 1 H, Ar), 7.24 (m, 1 H, Ar), 7.14 (m, 2 H, Ar), 4.72 (t, *J* = 5.1 Hz, 2 H, 1′-H), 4.48 (m, 4 H, *i*Pr, 2′-H), 1.25 (d, *J* = 6.2 Hz, 6 H, *i*Pr), 1.13 (d, *J* = 6.2 Hz, 6 H, *i*Pr) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 157.46 [d, *J*(P,C) = 18.8 Hz, O-Ar], 151.93 (C-4), 151.38 (C-2), 148.84 (C-6), 147.68 (C-8), 131.19 [d, *J*(P,C) = 185.0 Hz, P-Ar], 130.60 (C-5), 129.92 [d, *J*(P,C) = 16.9 Hz, Ar], 123.66 [d, *J*(P,C) = 8.9 Hz, Ar], 118.21 [d, *J*(P,C) = 3.0 Hz, Ar], 116.93 [d, *J*(P,C) = 11.0 Hz, Ar], 70.06 [d, *J*(P,C) = 5.5 Hz, 2 C, *i*Pr], 65.60 (C-2′), 43.16 (C-1′), 23.47 [dd, *J*(P,C) = 17.5, 3.9 Hz, 4 C, *i*Pr] ppm. MS (ESI): *m*/*z* = 439 [M + H]$^+$.

Diisopropyl 4-[2-(6-Chloropurin-9-yl)ethoxy]phenylphosphonate (*p*-4): Starting from diisopropyl 4-(2-hydroxyethoxy)phenylphosphonate (*p*-**3**), yield 1.06 g, 81%. ^{1}H NMR ([D$_6$]DMSO): δ = 8.80 (s, 1 H, 2-H), 8.77 (s, 1 H, 8-H), 7.58 (dd, 2 H, Ar), 7.02 (dd, 2 H, Ar), 4.73 (t, *J* = 5.1 Hz, 2 H, 1′-H), 4.49 (t, *J* = 5.4 Hz, 2 H, 2′-H), 4.46 (m, 2 H, *i*Pr), 1.24 (d, *J* = 6.2 Hz, 6 H, *i*Pr), 1.12 (d, *J* = 6.2 Hz, 6 H, *i*Pr) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 160.61 [d, *J*(P,C) = 3.3 Hz, O-Ar], 151.96 (C-4), 151.44 (C-2), 148.90 (C-6), 147.65 (C-8), 133.08 [d, *J*(P,C) = 11.2 Hz, 2 C, Ar], 130.65 (C-5), 121.61 [d, *J*(P,C) = 192.2 Hz, P-Ar], 114.51 [d, *J*(P,C) = 15.5 Hz, 2 C, Ar], 69.70 [d, *J*(P,C) = 5.5 Hz, 2 C, *i*Pr], 65.46 (C-2′), 43.14 (C-1′), 23.52 [dd, *J*(P,C) = 16.9, 3.9 Hz, 4 C, *i*Pr] ppm. MS (ESI): *m*/*z* = 439 [M + H]$^+$.

General Procedure for the Synthesis of Diisopropyl 2-(2-Amino-6-chloropurin-9-yl)ethoxyphenylphosphonates 5 by the Mitsunobu Reaction: Starting from 2-amino-6-chloropurine, the procedure used was identical to that described above for 6-chloropurine, except that after stirring the reaction mixture overnight, water (20 mL) was added and the mixture was heated at 60 °C for 20 h. The solvent was then evaporated and the crude mixture was purified by chromatography on silica gel (0–7% MeOH in $CHCl_3$).

Diisopropyl 2-[2-(2-Amino-6-chloropurin-9-yl)ethoxy]phenylphosphonate (*o*-5): Starting from diisopropyl 2-(2-hydroxyethoxy)phenylphosphonate (*o*-**3**), yield 0.67 g, 49%. ^{1}H NMR ([D$_6$]DMSO): δ = 8.35 (s, 1 H, 8-H), 7.84 (m, 1 H, Ar), 7.71 (s, 1 H, Ar), 7.13 (s, 1 H, Ar), 7.07 (m, 1 H, Ar), 6.93 (s, 2 H, NH_2), 4.52 (m, 2 H, *i*Pr), 4.43 (t, *J* = 5.3 Hz, 2 H, 1′-H), 4.42 (t, *J* = 5.0 Hz, 2 H, 2′-H), 1.24 (d, *J* = 6.1 Hz, 6 H, *i*Pr), 1.10 (d, *J* = 6.1 Hz, 6 H, *i*Pr) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 159.58 (C-2), 159.27 [d, *J*(P,C) = 2.2 Hz, O-Ar], 153.89 (C-4), 149.13 (C-6), 143.73 (C-8), 132.62 [d, *J*(P,C) = 9.6 Hz, Ar], 128.48 [d, *J*(P,C) = 12.0 Hz, Ar], 123.03 (C-5), 120.54 [d, *J*(P,C) = 14.0 Hz, Ar], 117.68 [d, *J*(P,C) = 184.6 Hz, P-Ar], 112.43 [d, *J*(P,C) = 8.8 Hz, Ar], 69.77 [d, *J*(P,C) = 5.5 Hz, 2 C, *i*Pr], 66.03 (C-2′), 42.27 (C-1′), 23.38 [dd, *J*(P,C) = 32.7, 4.0 Hz, 4 C, *i*Pr] ppm. MS (ESI): *m*/*z* = 454 [M + H]$^+$.

Diisopropyl 3-[2-(2-Amino-6-chloropurin-9-yl)ethoxy]phenylphosphonate (*m*-5): Starting from diisopropyl 3-(2-hydroxyethoxy)phenylphosphonate (*m*-**3**), yield 0.69 g, 51%. ^{1}H NMR ([D$_6$]DMSO): δ = 8.20 (s, 1 H, 8-H), 7.43 (m, 1 H, Ar), 7.23 (s, 1 H, Ar), 7.21 (m, 2 H, Ar), 6.97 (s, 2 H, NH_2), 4.52 (m, 2 H, *i*Pr), 4.45 (t, *J* = 5.3 Hz, 2 H, 1′-H), 4.38 (t, *J* = 5.0 Hz, 2 H, 2′-H), 1.26 (d, *J* = 6.2 Hz, 6 H, *i*Pr), 1.14 (d, *J* = 6.2 Hz, 6 H, *i*Pr) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 159.67 (C-2), 157.53 [d, *J*(P,C) = 1.9 Hz, O-Ar], 154.02 (C-4), 149.21 (C-6), 143.31 (C-8), 131.21 [d, *J*(P,C) = 185.0 Hz, P-Ar], 129.98 [d, *J*(P,C) = 16.8 Hz, Ar], 123.66 [d, *J*(P,C) = 8.9 Hz, Ar], 123.08 (C-5), 118.42 [d, *J*(P,C) = 3.0 Hz, Ar], 116.83 [d, *J*(P,C) = 11.2 Hz, Ar], 70.10 [d, *J*(P,C) = 5.5 Hz, 2 C, *i*Pr], 65.43 (C-2′), 42.23 (C-1′), 23.48 [dd, *J*(P,C) = 17.2, 4.0 Hz, 4 C, *i*Pr] ppm. MS (ESI): *m*/*z* = 454 [M + H]$^+$.

Diisopropyl 4-[2-(2-Amino-6-chloropurin-9-yl)ethoxy]phenylphosphonate (*p*-5): Starting from diisopropyl 4-(2-hydroxyethoxy)phenylphosphonate (*p*-**3**), yield 0.61 g, 45%. ^{1}H NMR ([D$_6$]DMSO): δ = 8.18 (s, 1 H, 8-H), 7.60 (dd, *J* = 12.6, 8.8 Hz, 2 H, Ar), 7.05 (dd, *J* = 8.8, 3.2 Hz, 2 H, Ar), 6.95 (s, 2 H, NH_2), 4.46 (m, 4 H, *i*Pr, 1′-H), 4.40 (t, *J* = 5.0 Hz, 2 H, 2′-H), 1.25 (d, *J* = 6.2 Hz, 6 H, *i*Pr), 1.13 (d, *J* = 6.2 Hz, 6 H, *i*Pr) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 160.64 [d, *J*(P,C) = 3.4 Hz, O-Ar], 159.64 (C-2), 153.99 (C-4), 149.21 (C-6), 143.25 (C-8), 133.08 [d, 2 C, *J*(P,C) = 11.1 Hz, Ar], 123.08 (C-5), 121.54 [d, *J*(P,C) = 192.3 Hz, P-Ar], 114.45 [d, *J*(P,C) = 15.5 Hz, 2 C, Ar], 69.65 [d, *J*(P,C) = 5.5 Hz, 2 C, *i*Pr], 65.31 (C-2′), 42.24 (C-1′), 23.48 [dd, *J*(P,C) = 16.4, 3.9 Hz, 4 C, *i*Pr] ppm. MS (ESI): *m*/*z* = 454 [M + H]$^+$.

General Procedure for the Synthesis of 2-(6-Chloropurin-9-yl)-ethoxyphenylphosphonic Acids 6a–8a: A mixture of diisopropyl ester **4** (440 mg, 1 mmol), acetonitrile (10 mL), $BrSiMe_3$ (0.5 mL) and 2,6-lutidine (0.4 mL) was stirred overnight at room temperature. After evaporation and co-distillation with acetonitrile (5×), the residue was treated with aqueous methanol (2:1, 20 mL) for 0.5 h, evaporated and co-distilled with water. The residue was purified by preparative HPLC (water/methanol) and the product was obtained as a white solid.

2-[2-(6-Chloropurin-9-yl)ethoxy]phenylphosphonic Acid (6a): Starting from diisopropyl 2-[2-(6-chloropurin-9-yl)ethoxy]phenylphosphonate (*o*-**4**), yield 236 mg, 65%. ^{1}H NMR ([D$_6$]DMSO): δ = 9.07 (s, 1 H, 2-H), 8.80 (s, 1 H, 8-H), 7.65 (m, 1 H, Ar), 7.44 (m, 1 H, Ar), 7.01 (m, 2 H, Ar), 4.73 (t, *J* = 4.8 Hz, 2 H, 1′-H), 4.38 (t, *J* = 4.8 Hz, 2 H, 2′-H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 158.93 [d, *J*(P,C) = 2.1 Hz, O-Ar], 151.75 (C-4), 151.19 (C-2), 148.60 (C-6), 148.52 (C-8), 133.02 [d, *J*(P,C) = 6.8 Hz, Ar], 132.71 [d, *J*(P,C) = 1.7 Hz, Ar], 130.46 (C-5), 121.87 [d, *J*(P,C) = 178.8 Hz, P-Ar], 120.22 [d, *J*(P,C) = 13.5 Hz, Ar], 111.96 [d, *J*(P,C) = 8.3 Hz, Ar], 66.23 (C-2′), 42.91 (C-1′) ppm. MS (ESI–): *m*/*z* = 353 [M – H]$^-$. $C_{13}H_{12}ClN_4O_4P\cdot\frac{1}{2}H_2O$ (363.69): calcd. C 42.93, H 3.60, N 15.40; found C 43.04, H 3.63, N 15.32.

3-[2-(6-Chloropurin-9-yl)ethoxy]phenylphosphonic Acid (7a): Starting from diisopropyl 3-[2-(6-chloropurin-9-yl)ethoxy]phenylphosphonate (*m*-**4**), yield 163 mg, 45%. ^{1}H NMR ([D$_6$]DMSO): δ = 8.80 (s, 1 H, 2-H), 8.79 (s, 1 H, 8-H), 7.34 (m, 1 H, Ar), 7.23 (m, 1 H, Ar), 7.13 (m, 1 H, Ar), 7.03 (m, 1 H, Ar), 4.72 (t, *J* = 5.1 Hz, 2 H, 1′-H), 4.43 (t, *J* = 5.1 Hz, 2 H, 2′-H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 157.19 [d, *J*(P,C) = 17.9 Hz, O-Ar], 151.91 (C-4), 151.40 (C-2), 148.86 (C-6), 147.65 (C-8), 135.54 [d, *J*(P,C) = 178.9 Hz, P-Ar], 130.61 (C-5), 129.34 [d, *J*(P,C) = 16.2 Hz, Ar], 123.07 [d, *J*(P,C) = 8.9 Hz, Ar], 116.73 [d, *J*(P,C) = 2.8 Hz, Ar], 116.33 [d, *J*(P,C) = 10.8 Hz, Ar], 65.41 (C-2′), 43.21 (C-1′) ppm. MS (ESI–): *m*/*z* = 353 [M – H]$^-$. $C_{13}H_{12}ClN_4O_4P\cdot{}^2/_5H_2O$ (361.89): calcd. C 43.15, H 3.57, N 15.48; found C 43.45, H 3.59, N 14.95.

4-[2-(6-Chloropurin-9-yl)ethoxy]phenylphosphonic Acid (8a): Starting from diisopropyl 4-[2-(6-chloropurin-9-yl)ethoxy]phenylphosphonate (*p*-**4**), yield 291 mg, 81%. ^{1}H NMR ([D$_6$]DMSO): δ = 8.80 (s, 1 H, 2-H), 8.77 (s, 1 H, 8-H), 7.56 (dd, *J* = 12.5, 8.7 Hz, 2 H, Ar), 6.96 (dd, *J* = 8.7, 2.8 Hz, 2 H, Ar), 4.72 (t, *J* = 5.2 Hz, 2 H, 1′-H), 4.46 (t, *J* = 5.2 Hz, 2 H, 2′-H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 159.53 [d, *J*(P,C) = 3.3 Hz, O-Ar], 151.90 (C-4), 151.41 (C-2), 148.87 (C-6), 147.60 (C-8), 132.18 [d, *J*(P,C) = 11.1 Hz, 2 C, Ar], 130.60 (C-5), 126.26 [d, *J*(P,C) = 186.1 Hz, P-Ar], 113.94 [d, *J*(P,C) = 15.0 Hz, 2 C, Ar], 65.31 (C-2′), 43.10 (C-1′) ppm. MS (ESI–): *m*/*z* = 353 [M – H]$^-$. $C_{13}H_{12}ClN_4O_4P\cdot{}^1/_4H_2O$ (359.03): calcd. C 43.47, H 3.51, N 15.60; found C 43.71, H 3.47, N 15.68.

General Procedure for the Synthesis of 2-(Adenin-9-yl)ethoxyphenylphosphonic Acids 6b–8b: A solution of chloropurine derivative **4** (1 mmol) in methanolic ammonia (60 mL) was stirred in an autoclave at 70 °C for 30 h, the solvent was evaporated and the residue co-distilled with acetonitrile. A mixture of this crude diethyl ester adenine intermediate, acetonitrile (20 mL) and $BrSiMe_3$ (2 mL) was stirred overnight at room temperature. After evaporation and co-distillation with acetonitrile (4×), the residue was treated with aqueous methanol (2:1, 20 mL) for 0.5 h, evaporated and co-distilled with water. The product was obtained as a white solid by filtration.

2-[2-(Adenin-9-yl)ethoxy]phenylphosphonic Acid (6b): Starting from diisopropyl 2-[2-(6-chloropurin-9-yl)ethoxy]phenylphosphonate (*o*-**4**), yield 196 mg, 57% for two steps. ^{1}H NMR ([D$_6$]DMSO): δ = 8.50 (s, 1 H, 2-H), 8.18 (s, 1 H, 8-H), 7.68 (m, 1 H, Ar), 7.44 (m, 1 H, Ar), 7.38 (s, 2 H, NH$_2$), 7.01 (m, 2 H, Ar), 4.60 (m, 2 H, 1′-H), 4.32 (m, 2 H, 2′-H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 159.10 [d, *J*(P,C) = 1.3 Hz, O-Ar], 155.55 (C-6), 151.79 (C-2), 148.99 (C-4), 142.02 (C-8), 133.09 [d, *J*(P,C) = 7.0 Hz, Ar], 132.75 [d, *J*(P,C) = 1.5 Hz, Ar], 121.98 [d, *J*(P,C) = 178.3 Hz, P-Ar], 120.19 [d, *J*(P,C) = 13.6 Hz, Ar], 118.19 (C-5), 112.08 [d, *J*(P,C) = 7.7 Hz, Ar], 67.08 (C-2′), 42.28 (C-1′) ppm. MS (ESI–): *m*/*z* = 334 [M – H]$^-$. $C_{13}H_{14}N_5O_4P\cdot{}^1/_2H_2O$ (344.26): calcd. C 45.35, H 4.39, N 20.31; found C 44.99, H 4.05, N 19.81.

3-[2-(Adenin-9-yl)ethoxy]phenylphosphonic Acid (7b): Starting from diisopropyl 3-[2-(6-chloropurin-9-yl)ethoxy]phenylphosphonate (*m*-**4**), yield 224 mg, 65% for two steps. ^{1}H NMR ([D$_6$]DMSO): δ = 8.21 (s, 1 H, 2-H), 8.16 (s, 1 H, 8-H), 7.32 (s, 2 H, NH$_2$), 7.34 (m, 1 H, Ar), 7.24 (m, 1 H, Ar), 7.15 (m, 1 H, Ar), 7.04 (m, 1 H, Ar), 4.55 (t, *J* = 5.1 Hz, 2 H, 1′-H), 4.37 (t, *J* = 5.1 Hz, 2 H, 2′-H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 157.31 [d, *J*(P,C) = 17.8 Hz, O-Ar], 155.60 (C-6), 152.04 (C-2), 149.39 (C-4), 141.16 (C-8), 135.74 [d, *J*(P,C) = 178.6 Hz, P-Ar], 129.35 [d, *J*(P,C) = 16.2 Hz, Ar], 123.01 [d, *J*(P,C) = 8.9 Hz, Ar], 118.48 (C-5), 116.64 [d, *J*(P,C) = 2.7 Hz, Ar], 116.35 [d, *J*(P,C) = 10.9 Hz, Ar], 65.67 (C-2′), 42.42 (C-1′) ppm. MS (ESI–): *m*/*z* = 334 [M – H]$^-$. $C_{13}H_{14}N_5O_4P\cdot{}^1/_2H_2O$ (344.26): calcd. C 45.35, H 4.39, N 20.31; found C 45.33, H 4.28, N 20.42.

4-[2-(Adenin-9-yl)ethoxy]phenylphosphonic Acid (8b): Starting from diisopropyl 4-[2-(6-chloropurin-9-yl)ethoxy]phenylphosphonate (*p*-**4**), yield 206 mg, 56% for two steps. ^{1}H NMR ([D$_6$]DMSO): δ = 8.21 (s, 1 H, 2-H), 8.20 (s, 1 H, 8-H), 7.57 (m, 2 H, Ar), 7.34 (s, 2 H, NH$_2$), 6.99 (m, 2 H, Ar), 4.55 (m, 2 H, 1′-H), 4.46 (m, 2 H, 2′-H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 159.95 [d, *J*(P,C) = 4.6 Hz, O-Ar], 155.57 (C-6), 151.98 (C-2), 149.43 (C-4), 141.18 (C-8), 132.27 [d, *J*(P,C) = 11.1 Hz, 2 C, Ar], 123.85 [d, *J*(P,C) = 188.6 Hz, P-Ar], 118.51 (C-5), 113.99 [d, *J*(P,C) = 14.9 Hz, 2 C, Ar], 65.65 (C-2′), 42.34 (C-1′) ppm. MS (ESI–): *m*/*z* = 334 [M – H]$^-$. $C_{13}H_{14}N_5O_4P\cdot$MeOH (367.30): calcd. C 45.78, H 4.94, N 19.07; found C 46.42, H 4.97, N 18.67.

General Procedure for the Synthesis of 2-(Hypoxanthin-9-yl)ethoxyphenylphosphonic Acids 6c–8c: A 6-Chloro derivative of type **6a**–**8a** (0.5 mmol) was dissolved in trifluoroacetic acid (75%, 10 mL) and stirred overnight. The solvent was evaporated and the residue co-distilled with water (3×). After preparative HPLC, the pure product was obtained as a white solid.

2-[2-(Hypoxanthin-9-yl)ethoxy]phenylphosphonic Acid (6c): Starting from 2-[2-(6-chloropurin-9-yl)ethoxy]phenylphosphonic acid (**6a**), yield 91 mg, 52%. ^{1}H NMR ([D$_6$]DMSO): δ = 12.29 (br. s, 1 H, NH), 8.45 (s, 1 H, 2-H), 8.06 (s, 1 H, 8-H), 7.66 (m, 1 H, Ar), 7.43 (m, 1 H, Ar), 6.98 (m, 2 H, Ar), 4.56 (t, *J* = 4.8 Hz, 2 H, 1′-H), 4.30 (t, *J* = 4.8 Hz, 2 H, 2′-H) ppm. ^{13}C NMR ([D$_6$]-DMSO): δ = 159.05 [d, *J*(P,C) = 2.0 Hz, O-Ar], 156.48 (C-6), 147.97 (C-4), 145.29 (C-2), 141.39 (C-8), 133.12 [d, *J*(P,C) = 6.8 Hz, Ar], 132.59 (Ar), 123.41 (C-5), 120.17 [d, *J*(P,C) = 13.5 Hz, Ar], 112.05 [d, *J*(P,C) = 8.2 Hz, Ar], 66.82 (C-2′), 42.49 (C-1′) ppm. ^{31}P NMR (D$_2$O): 10.7 ppm. MS (ESI–): *m*/*z* = 335 [M – H]$^-$. $C_{13}H_{13}N_4O_5P\cdot{}^3/_4H_2O$ (349.75): calcd. C 44.64, H 4.18, N 16.02; found C 44.70, H 4.25, N 16.20.

3-[2-(Hypoxanthin-9-yl)ethoxy]phenylphosphonic Acid (7c): Starting from 3-[2-(6-chloropurin-9-yl)ethoxy]phenylphosphonic acid (**7a**), yield 136 mg, 78%. ^{1}H NMR ([D$_6$]DMSO): δ = 12.33 (br. s, 1 H, NH), 8.14 (s, 1 H, 2-H), 8.05 (s, 1 H, 8-H), 7.26 (m, 2 H, Ar), 7.16 (m, 1 H, Ar), 7.00 (m, 1 H, Ar), 4.53 (t, *J* = 5.0 Hz, 2 H, 1′-H), 4.33 (t, *J* = 5.0 Hz, 2 H, 2′-H) ppm. ^{13}C NMR ([D$_6$]-DMSO): δ = 157.12 [d, *J*(P,C) = 1.8 Hz, O-Ar], 156.46 (C-6), 148.30 (C-4), 145.45 (C-2), 140.48 (C-8), 129.00 [d, *J*(P,C) = 16.2 Hz, Ar], 123.74 (C-5), 123.12 [d, *J*(P,C) = 8.8 Hz, Ar], 116.36 [d, *J*(P,C) = 10.6 Hz, Ar], 116.10 [d, *J*(P,C) = 2.2 Hz, Ar], 65.72 (C-2′), 42.78 (C-1′) ppm. ^{31}P NMR (D$_2$O): 10.78 ppm. MS (ESI–): *m*/*z* = 335 [M – H]$^-$. $C_{13}H_{13}N_4O_5P\cdot{}^3/_4H_2O$ (349.75): calcd. C 44.64, H 4.18, N 16.02; found C 44.49, H 3.91, N 15.62.

4-[2-(Hypoxanthin-9-yl)ethoxy]phenylphosphonic Acid (8c): Starting from diisopropyl 4-[2-(6-chloropurin-9-yl)ethoxy]phenylphosphonate (**8a**), yield 107 mg, 61%. ^{1}H NMR ([D$_6$]DMSO): δ = 12.31 (br. s, 1 H, NH), 8.14 (s, 1 H, 2-H), 8.05 (s, 1 H, 8-H), 7.57 (m, 2 H, Ar), 6.97 (dd, *J* = 8.4, 2.2 Hz, 2 H, Ar), 4.55 (t, *J* = 4.9 Hz, 2 H, 1′-H), 4.39 (t, *J* = 4.9 Hz, 2 H, 2′-H) ppm. ^{13}C NMR ([D$_6$]-DMSO): δ = 159.68 [d, *J*(P,C) = 3.3 Hz, O-Ar], 156.489 (C-6), 148.35 (C-4), 145.49 (C-2), 140.49 (C-8), 132.27 [d, *J*(P,C) = 11.0 Hz, 2 C, Ar], 126.28 [d, *J*(P,C) = 186.0 Hz, P-Ar], 123.78 (C-5), 113.99 [d, *J*(P,C) = 15.0 Hz, 2 C, Ar], 65.78 (C-2′), 42.68 (C-1′) ppm. ^{31}P NMR (D$_2$O): 10.78 ppm. MS (ESI–): *m*/*z* = 335 [M – H]$^-$. $C_{13}H_{13}N_4O_5P\cdot{}^4/_5H_2O$ (350.65): calcd. C 44.53, H 4.20, N 15.98; found C 44.12, H 4.03, N 16.39.

General Procedure for the Synthesis of 2-(Guanin-9-yl)ethoxyphenylphosphonic Acids 6d–8d: A mixture of diisopropyl ester **5** (455 mg,

1 mmol), acetonitrile (10 mL), dimethylformamide (2 mL) and $BrSiMe_3$ (1 mL) was stirred overnight at room temperature. After evaporation and co-distillation with toluene (2×) and acetonitrile (5×), the residue was treated with aqueous methanol (2:1, 20 mL) for 0.5 h and the solvents evaporated. The crude intermediate was dissolved in aqueous trifluoroacetic acid (75%, 20 mL) and stirred overnight. The solvent was then evaporated and the residue co-distilled with water (3×). After preparative HPLC, the pure product was obtained as a white solid.

2-[2-(Guanin-9-yl)ethoxy]phenylphosphonic Acid (6d): Starting from diisopropyl 2-[2-(2-amino-6-chloropurin-9-yl)ethoxy]phenylphosphonate (*o*-**5**), yield 159 mg, 43% for two steps. ^{1}H NMR ([D$_6$]-DMSO): δ = 10.58 (br. s, 1 H, NH), 8.041 (s, 1 H, 8-H), 7.67 (m, 1 H, Ar), 7.44 (m, 1 H, Ar), 7.02 (m, 2 H, Ar), 6.48 (s, 2 H, NH_2), 4.35 (t, J = 4.8 Hz, 2 H, 1′-H), 4.24 (t, J = 4.8 Hz, 2 H, 1′-H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 158.92 [d, J(P,C) = 2.1 Hz, O-Ar], 156.36 (C-6), 153.25 (C-2), 150.56 (C-4), 138.22 (C-8), 132.92 [d, J(P,C) = 7.0 Hz, Ar], 132.53 (Ar), 121.76 [d, J(P,C) = 178.9 Hz, P-Ar], 119.93 [d, J(P,C) = 13.6 Hz, Ar], 115.66 (C-5), 111.85 [d, J(P,C) = 8.2 Hz, Ar], 66.50 (C-2′), 41.63 (C-1′) ppm. MS (ESI–): m/z = 350 [M – H]$^-$. $C_{13}H_{14}N_5O_5P{\cdot}H_2O$ (369.08): calcd. C 42.28, H 4.37, N 18.97; found C 42.34, H 4.45, N 18.83.

3-[2-(Guanin-9-yl)ethoxy]phenylphosphonic Acid (7d): Starting from diisopropyl 3-[2-(2-amino-6-chloropurin-9-yl)ethoxy]phenylphosphonate (*m*-**5**), yield 256 mg, 71% for two steps. ^{1}H NMR ([D$_6$]-DMSO): δ = 10.58 (br. s, 1 H, NH), 7.74 (s, 1 H, 8-H), 7.34 (m, 1 H, Ar), 7.27 (m, 2 H, Ar), 7.04 (m, 1 H, Ar), 6.53 (s, 2 H, NH_2), 4.31 (m, 4 H, 1′-H, 2′-H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 157.28 [d, J(P,C) = 17.8 Hz, O-Ar], 156.61 (C-6), 153.56 (C-2), 151.11 (C-4), 137.56 (C-8), 136.11 [d, J(P,C) = 178.0 Hz, P-Ar], 129.29 [d, J(P,C) = 16.1 Hz, Ar], 123.08 [d, J(P,C) = 8.9 Hz, Ar), 116.89 [d, J(P,C) = 7.6 Hz, Ar], 116.29 (C-5), 116.18 [d, J(P,C) = 11.0 Hz, Ar], 65.54 (C-2′), 42.92 (C-1′) ppm. MS (ESI–): m/z = 350 [M – H]$^-$. $C_{13}H_{14}N_5O_5P{\cdot}½H_2O$ (360.26): calcd. C 43.34, H 4.20, N 19.24; found C 43.29, H 4.04, N 19.28.

4-[2-(Guanin-9-yl)ethoxy]phenylphosphonic Acid (8d): Starting from diisopropyl 4-[2-(2-amino-6-chloropurin-9-yl)ethoxy]phenylphosphonate (*p*-**5**), yield 255 mg, 69% for two steps. ^{1}H NMR ([D$_6$]-DMSO): δ = 10.58 (br. s, 1 H, NH), 7.75 (s, 1 H, 8-H), 7.59 (m, 2 H, Ar), 7.00 (m, 2 H, Ar), 6.48 (s, 2 H, NH_2), 4.33 (m, 4 H, 1′-H, 2′-H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 159.72 [d, J(P,C) = 3.3 Hz, O-Ar], 156.57 (C-6), 153.49 (C-2), 151.07 (C-4), 137.54 (C-8), 132.27 [d, J(P,C) = 11.0 Hz, 2 C, Ar], 126.10 [d, J(P,C) = 185.9 Hz, P-Ar], 116.21 (C-5), 113.96 [d, J(P,C) = 15.0 Hz, 2 C, Ar], 65.58 (C-2′), 41.95 (C-1′) ppm. MS (ESI–): m/z = 350 [M – H]$^-$. $C_{13}H_{14}N_5O_5P{\cdot}H_2O$ (369.08): calcd. C 42.28, H 4.37, N 18.97; found C 41.99, H 4.21, N 18.78.

General Procedure for the Synthesis of 2-(Xanthin-9-yl)ethoxyphenylphosphonic Acids 6e–8e: A mixture of a guanine derivative of type **6d**–**8d** (0.5 mmol), aqueous HCl (1 M, 15 mL) and $NaNO_2$ (0.15 g) was stirred for 1 h at room temperature, evaporated and co-distilled with water (2×). The residue was dissolved in hot water and a small amount of MeOH and the pure product was obtained as a white solid by filtration.

2-[2-(Xanthin-9-yl)ethoxy]phenylphosphonic Acid (6e): Starting from 2-[2-(guanin-9-yl)ethoxy]phenylphosphonic acid (**6d**), yield 131 mg, 71%. ^{1}H NMR ([D$_6$]DMSO): δ = 11.84 (br. s, 1 H, NH), 10.77 (s, 1 H, NH), 7.97 (s, 1 H, 8-H), 7.66 (m, 1 H, 7.46 (m, 1 H, Ar), 7.00 (m, 2 H, Ar), 4.45 (t, J = 4.5 Hz, 2 H, 1′-H), 4.25 (t, J = 4.5 Hz, 2 H, 2′-H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 159.12 [d, J(P,C) = 2.1 Hz, O-Ar], 157.78 (C-6), 150.70 (C-2), 140.16 (C-4), 138.02 (C-8), 133.08 [d, J(P,C) = 7.0 Hz, Ar], 132.78 [d, J(P,C) = 1.6 Hz, Ar], 121.99 [d, J(P,C) = 179.0 Hz, P-Ar], 120.34 [d, J(P,C) = 13.5 Hz, Ar], 114.91 (C-5), 112.51 [d, J(P,C) = 8.3 Hz, Ar], 66.93 (C-2′), 43.54 (C-1′) ppm. MS (ESI–): m/z = 351 [M – H]$^-$. $C_{13}H_{13}N_4O_6P{\cdot}H_2O$ (370.26): calcd. C 42.17, H 4.08, N 15.13; found C 42.03, H 3.92, N 15.08.

3-[2-(Xanthin-9-yl)ethoxy]phenylphosphonic Acid (7e): Starting from 3-[2-(guanin-9-yl)ethoxy]phenylphosphonic acid (**7d**), yield 140 mg, 139 mg, 75%. ^{1}H NMR ([D$_6$]DMSO): δ = 11.90 (br. s, 1 H, NH), 10.80 (s, 1 H, NH), 7.74 (s, 1 H, 8-H), 7.36 (m, 1 H, Ar), 7.24 (m, 1 H, Ar), 7.15 (m, 1 H, Ar), 7.03 (m, 1 H, Ar), 4.45 (t, J = 4.5 Hz, 2 H, 1′-H), 4.24 (t, J = 4.5 Hz, 2 H, 2′-H) ppm. ^{13}C NMR ([D$_6$]-DMSO): δ = 157.76 (C-6), 157.27 [d, J(P,C) = 17.8 Hz, O-Ar], 150.66 (C-2), 140.43 (C-4), 137.19 (C-8), 135.64 [d, J(P,C) = 178.8 Hz, P-Ar], 129.37 [d, J(P,C) = 16.2 Hz, Ar], 123.07 [d, J(P,C) = 9.1 Hz, Ar], 116.75 [d, J(P,C) = 2.7 Hz, Ar], 116.30 [d, J(P,C) = 10.9 Hz, Ar], 115.22 (C-5), 66.01 (C-2′), 43.33 (C-1′) ppm. MS (ESI–): m/z = 351 [M – H]$^-$. $C_{13}H_{13}N_4O_6P{\cdot}H_2O$ (370.26): calcd. C 42.17, H 4.08, N 15.13; found C 42.13, H 3.85, N 15.07.

4-[2-(Xanthin-9-yl)ethoxy]phenylphosphonic Acid (8e): Starting from 4-[2-(guanin-9-yl)ethoxy]phenylphosphonic acid (**8d**), yield 141 mg, 76%. ^{1}H NMR ([D$_6$]DMSO): δ = 11.93 (br. s, 1 H, Ar), 10.80 (s, 1 H, NH), 7.72 (s, 1 H, 8-H), 7.59 (m, 2H, Ar), 6.95 (m, 2 H, Ar), 4.49 (t, J = 5.0 Hz, 2 H, 1′-H), 4.29 (t, J = 5.0 Hz, 2 H, 2′-H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 159.65 [d, J(P,C) = 3.3 Hz, O-Ar], 157.39 (C-6), 150.61 (C-2), 140.43 (C-4), 137.18 (C-8), 132.27 [d, J(P,C) = 11.1 Hz, 2 C, Ar], 126.10 [d, J(P,C) = 186.2 Hz, P-Ar], 114.07 (C-5), 113.95 [d, J(P,C) = 5.9 Hz, Ar], 65.80 (C-2′), 43.55 (C-1′) ppm. MS (ESI–): m/z = 351 [M – H]$^-$. $C_{13}H_{13}N_4O_6P{\cdot}H_2O$ (370.26): calcd. C 42.17, H 4.08, N 15.13; found C 42.08, H 3.76, N 15.07.

Determination of the pK_a Values by ^{31}P NMR Titration Studies: Compounds **6c**, **7c** and **8c** (2.5 mg) were dissolved in acetate buffer (0.025 M, 0.5 mL) and deuterium oxide (0.1 mL) and acidified with HCl (2 M), the pH was measured and then the ^{31}P NMR spectrum was acquired. NaOH (0.1 M, one drop) was then added repeatedly, the sample was shaken, and the pH and ^{31}P NMR spectrum were measured. The pH dependence of the ^{31}P chemical shifts was plotted and the pK_a values were estimated to be at the pH at which the phosphorus chemical shift is midway between the chemical shifts of the protonated and unprotonated forms.

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