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Structure Activity Relationship of (N)-Methanocarba Phosphonate Analogues of 5'-AMP as Cardioprotective Agents Acting Through a Cardiac P2X Receptor

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Abstract

P2X receptor activation protects in heart failure models. MRS2339 **3**, a 2-chloro-AMP derivative containing a (N)-methanocarba (bicyclo[3.1.0]hexane) system, activates this cardioprotective channel. Michaelis–Arbuzov and Wittig reactions provided phosphonate analogues of **3**, expected to be stable *in vivo* due to the C-P bond. After chronic administration via a mini-osmotic pump (Alzet), some analogues significantly increased intact heart contractile function in calsequestrin-overexpressing mice (genetic model of heart failure) compared to vehicle-infused mice (all inactive at the vasodilatory $P2Y_1$ receptor). Two phosphonates, (1'S,2'R,3'S,4'R,5'S)-4'-(6-amino-2-chloropurin-9-yl)-2',3'-(dihydroxy)-1'-(phosphonomethylene)-bicyclo[3.1.0]hexane **4** and its homologue **9**, both 5'-saturated, containing a 2-Cl substitution, improved echocardiography-derived fractional shortening (20.25% and 19.26%, respectively, versus 13.78% in controls), while unsaturated 5'-extended phosphonates, all 2-H analogues, and a CH_3-phosphonate were inactive. Thus, chronic administration of nucleotidase-resistant phosphonates conferred a beneficial effect, likely via cardiac P2X receptor activation. Thus, we have greatly expanded the range of carbocyclic nucleotide analogues that represent potential candidates for the treatment of heart failure.

Introduction

The actions of extracellular nucleotides in cell signaling are mediated by two classes of cell surface purinergic receptors: P2X receptors are ligand-gated ion channels activated by extracellular ATP, and P2Y receptors are G protein-coupled receptors activated by both adenine and uracil nucleotides.[1,2] In the heart, a variety of P2 receptors are expressed.[3]

Cardiac P2X receptors represent a novel and potentially important therapeutic target for the treatment of heart failure. A P2X receptor on the cardiomyocyte mediates cardioprotection and is activated by ATP or its potent analogue 2-MeSATP **1** (Chart 1), as demonstrated using the calsequestrin (CSQ) model of cardiomyopathy. Extracellular ATP can cause an ionic current in murine, rat, and guinea pig cardiac ventricular myocytes.[4–6] The $P2X_4$ receptor is an

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Supporting Information Available: Updated synthetic methods for compound **3** and NMR spectral data and HPLC traces of selected nucleotide derivatives are included. This material is available free of charge via the Internet at http://pubs.acs.org.

important subunit of the native cardiac P2X receptor, which mediates ionic current induced by extracellular ATP.[4] This P2X current was up-regulated in cardiac ventricular myocytes of the CSQ hearts. Furthermore, cardiac myocyte-specific overexpression of the $P2X_4$ receptor can mimic the beneficial effects following chronic infusion of P2X agonist analogues. This analysis suggested that regulation of this cardiac P2X receptor is protective in cardiac hypertrophy or failure.

(1'S,2'R,3'S,4'R,5'S)-4-(6-amino-2-chloro-9H-purin-9-yl)-1-[phosphoryloxymethyl] bicyclo[3.1.0]hexane-2,3-diol, (MRS2339, **3**) is an (N)-methanocarba monophosphate derivative of 2-chloro-AMP **2** that contains a rigid bicyclic ring system (bicyclo[3.1.0]hexane) in place of ribose that we reported to to be effective in an *in vivo* model of cardiomyopathy.[8] This ring system impedes hydrolysis of the 5'-phosphate in a model compound by its nucleotidase.[9] Compound **3** induced a current in the CSQ myocyte similar to that by compound **1**, characteristic of the action of the $P2X_4$ receptor.[8] Chronically administered compound **3** rescued the hypertrophic and heart failure phenotype in the CSQ-overexpressing mouse.[7] When administered via an Alzet mini-osmotic pump, it significantly increased longevity as compared to vehicle-injected mice. The improvement in survival was associated with decreases in heart weight/body weight ratio and in cross-section area of the cardiac myocytes. Compound **3** was devoid of any vasodilator action in aorta ring preparations indicating that its salutary effect in heart failure was not due to any vascular unloading.

Activation of this myocyte P2X receptor leads to the opening of a nonselective cation channel permeable to Na^+, K^+, and Ca^{2+}. The current is inward at negative membrane potentials, reverses near 0 mV, and becomes outward at positive potentials.[4] The continuous activation of this receptor channel under the resting or negative membrane potentials would produce an inward current while its activation during depolarized portions of the action potential should lead to an outward current. These ionic currents represent a possible ionic mechanism by which the cardiomyocyte P2X channel achieves its protective effect.

We have explored the structure activity relationship (SAR) of phosphonate analogues of **3** in a model of cardioprotection. Although a (N)-methanocarba nucleoside 5'-monophosphate was shown to be a poor substrate of 5'-nucleotidase (CD73),[9] replacement of the phosphoester group of **3** with a phosphonate would be expected to further increase the *in vivo* half-life because of the stability of the C-P bond. Phosphonate analogues of nucleotides and other known ligands, in some cases, have been shown to display activity at P2 receptors.[14–17]

Results

Chemical Synthesis

The phosphonate derivatives on varied carbon skeletons (Table 1) were synthesized by the methods shown in Scheme 1 – Scheme 6. In some cases, the phosphorous atom was bonded directly to the 5' carbon atom (Scheme 1, Scheme 2), while in other cases, a carbon atom was added at that position to form either a saturated or unsaturated nucleotide analogue (Scheme 3–Scheme 5). Alternately, a methylphosphonate group was included in compounds **11** and **12**, which were otherwise equivalent to **4** and **5**, respectively (Scheme 6). The 2 position contained either hydrogen (in compounds **5**, **8**, **10**, **12**) or Cl (as in the known active compound **3** and the novel analogues **4**, **7**, **9**, **11**). The reference compound **3** was synthesized by a modification of the reported method,[13] which lead to improved yields (Supporting Information).

Known alcohol **13**[13] was protected as a *O*-*tert*-butyldimethylsilyl ether using TBDPS-Cl, imidazole and DMAP to get compound **14**, followed by the reduction of the ethyl ester using DIBAL-H in anhydrous THF resulting in compound **15** in very good yield (Scheme 1). In order

to introduce an iodo group at the 5' position, a classical two step procedure was implemented. This involved the initial activation of the 5'-alcohol as a mesylate followed by an S_N2 nucleophilic attack of iodide on the activated 5'-position resulting in the 5'-iodo compound **17** in 95% yield. The iodo compound **17** was subjected to classical Michaelis–Arbuzov reaction conditions with excess triethylphosphite and heating up to 110 °C for 17 h to provide a phosphonate diester **18** in excellent 94% yield (Scheme 1). Desilylation using TBAF resulted in alcohol **19**, which was a suitable substrate for Mitsunobu base coupling reactions.

The alcohol **19** was used as a common key intermediate to synthesize phosphonates **4** and **5** (Scheme 2). Synthetic procedures for phosphonates **4** and **5** involved initial Mitsunobu base coupling reaction using triphenylphosphine, diisopropyl azodicarboxylate, and the corresponding purine base followed by amination at the 6 position of the purine ring using 2M NH_3 in isopropanol (Scheme 2). Finally, the simultaneous deprotection of both the phosphonate diester and the acetonide of **21** and **23** was achieved upon treatment with freshly opened iodotrimethylsilane to get target phosphonates **4** and **5**, respectively (Scheme 2). Our efforts to use alternative relatively milder reagents, bromotrimethylsilane and Dowex-50 ion exchange resin, resulted in partial deprotection (results not shown).

The synthetic routes to the elongated saturated and unsaturated phosphonate derivatives **7–10** are shown in Scheme 3–Scheme 5. The saturated phosphonate **10** was synthesized by oxidation of known alcohol **24**[13] to the 5'-aldehyde **25** in 80% yield. It is noteworthy that not even a small amount of decomposition of the aldehyde was observed after storage at room temperature (rt) for several days. The α,β-unsaturated alkyl phosphonate ester **26** was prepared from aldehyde **25** in a Wittig-type reaction using tetraisopropyl methylenediphosphonate and sodium hydride in anhydrous THF.[22,23] The *E*-configuration of the resulting alkene could be inferred from the large coupling constant (^3J = 17.1 Hz). Amination followed by hydrolysis of phophonate diester and acetonide resulted in α,β-unsaturated alkyl phosphonate **7** (Scheme 3).

Catalytic hydrogenation of **27** in the presence of H_2 (3 bar), palladium on carbon and MeOH: 2M aq. NaOH (1:1, v/v)[24,25] resulted in the expected olefin reduction and dechlorination to give the corresponding saturated phosphonate diester **28**. Compound **28** was converted to the long chain saturated alkyl phosphonate **10** using the previously described iodotrimethylsilane deprotection reaction conditions. To our surprise, our various efforts to synthesize **36** (Scheme 5) from **27** by olefin reduction, running the reaction at atmospheric pressure and using less weight percent of catalyst, either resulted in an incomplete reaction or generated an inseparable mixture of dehalogenated and halogenated products (results not shown).

Hence, in order to synthesize phosphonates **8** and **9**, we decided to install the phosphonate diester before the Mitsunobu base coupling reaction, as described in Scheme 4 and Scheme 5. The 5'-alcohol of compound **15** was oxidized using Dess-Martin periodinane reaction to get aldehyde **29**. Similar to the aldehyde **25**, aldehyde **29** also displayed considerable stability at rt. The α,β-unsaturated alkyl phosphonate diester **30** was obtained using the previously described Wittig-type reaction conditions. Desilylation under standard conditions resulted in the formation of alcohol **31**, which served as the key intermediate for the synthesis of long chain unsaturated and saturated alkyl phosphonates **8** and **9**, respectively.

A Mitsunobu base coupling reaction on compound **31** using triphenylphosphine, 6-chloropurine, and diisopropyl azodicarboxylate followed by amination and hydrolysis of the phophonate diester and acetonide resulted in formation of long chain α,β-unsaturated alkyl phosphonate **8**. Sequential catalytic hydrogenation of the resulting vinyl phosphonate diester **31** in the presence of palladium on carbon, a Mitsunobu base coupling reaction, and amination provided the long chain saturated phosphonate diester **36**. Simultaneous deprotection of the phosphonate diester and the acetonide using iodotrimethylsilane resulted in the desired

phosphonate **9** along with the formation of corresponding dehalogenated product phosphonate **10**.

The synthetic approach to methylphosphonates **11** and **12** is shown in Scheme 6. It involved initial 5'-bromination using CBr_4 and treatment with triphenylphosphine and triethylamine to result in 5'-bromosugar **37** in 81% yield. A subsequent Michaelis–Arbuzov reaction using diethyl methylphosphite, followed by desilylation with TBAF gave the 5'-methylphosphonate monoester **38** with 1-alcohol **39** as an inseparable mixture of diastereomers. A further Mitsunobu base coupling reaction with 2,6-dichloropurine, followed by amination and final deprotection gave the desired methylphosphonate **11** along with corresponding dehalogenated methylphosphonate **12**.

Biological Evaluation

Various phosphonate derivatives were infused subcutaneously individually via an Alzet minipump in CSQ mice. After 28 days of infusion, the *in vivo* heart function was assessed using echocardiography-derived fractional shortening (FS), which is the ratio of the change in the diameter of the left ventricle between the contracted and relaxed states. Thus, a lower percentage represents a decrease in function. Two of the phosphonates, **4** and **9**, were able to cause an improved FS as compared to vehicle (Figure 1, Table 1) and in comparison to the reference nucleotide **3**. Other analogues tested in this model (2-H analogues **5**, **8**, and **10**, and 2-Cl analogues **7** and **11**) had lower FS values. Compounds **7**, **8**, and **10** were not protective at this dose, i.e. FS in vehicle control-treated CSQ mice was similar to that from mice treated chronically with these nucleotides. Thus, in the saturated phosphonate series, the orientation of the phosphorous relative to the methanocarba ring was somewhat structurally permissive, although inclusion of an olefin in the spacer prevented the cardioprotective action. In **11**, one OH group of the phosphonate has been replaced with CH_3. This reduces the overall charge on the molecule and is intended to make it more bioavailable. Evidently, the binding site of the receptor requires both oxygens for the most favorable improvement in FS. Therefore, as summarized in Figure 1, the most significant improvement in FS was associated with the saturated homologues containing a 2-Cl substitution and an unmodified phosphonate group, **4** and **9**.

An echocardiographic image of compound **4**- versus normal saline (NS)-infused CSQ hearts is shown in Figure 2. An increased shortening of both the septum and left ventricular (LV) free wall was evident in the heart from mice treated with **4** in comparison to that from vehicle (NS)-infused mice.

We also evaluated the ability of the nucleotide analogues to activate the human $P2Y_1$ receptor (Table 1). This receptor is vasodilatory, and agonist action at this subtype would be expected to be relevant to the observed cardiac effects. Compound **3** was previously reported to activate phospholipase C (PLC) mediated by the human $P2Y_1$ receptor.[9] The phosphonate derivatives were tested in a FLIPR assay of calcium flux induced in 1321N1 astrocytoma cells stably expressing the human $P2Y_1$ receptor. The known $P2Y_1$ receptor agonist 2-MeSADP induced a Ca^{2+} flux with an EC_{50} of 10.3±0.4 nM (n = 3) in the transfected cells (Figure 3), but in control 1321N1 astrocytoma cells there was no change in intracellular Ca^{2+} in response to 10 μM 2-MeSADP. At concentrations up to 10 μM, the phosphonate analogues **4 – 11** produced no effect in the same assay. However, compound **3** was active in this assay as an agonist, with an EC_{50} of 722±55 nM (n = 3). The maximal effect of **3** was ~80–90% of that of the full agonist 2-MeSADP.

Discussion

Retrosynthetic analysis

We set out to synthesize 5'-phosphonate and 5'-methyl phosphonate derivatives of (N)-methanocarba-adenosine **5**, **12** or the corresponding 2-Cl derivatives **4**, **11** using the previously reported intermediate **13**.[13] At least two synthetic pathways leading to these target molecules can be envisioned *viz*. routes A and B (Scheme 7A) based on the installation of phosphonate group. Route A involves phosphonate installation at the nucleoside level to generate key intermediate **42**, which could be used as common intermediate for the generation of both phosphonates and methyl phosphonates **43** using Michaelis–Arbuzov reaction conditions. Route B involves phosphonate installation at the sugar level on halogenated intermediates **17** and **37** to get intermediates **18** and **38**, respectively. Route B does not have a common intermediate, like route A, and, as a result, it involves a longer and more laborious synthetic sequence. Generally, the Michaelis–Arbuzov reaction conditions to generate phosphonate derivatives require long reaction times (24 to 48 h) at elevated temperatures (120 to 180 °C). Moreover, removal of trialkyl phosphite reagent needs high temperatures and high vacuum. Because of these harsh conditions, a Michaelis–Arbuzov reaction at the nucleoside level generally results in very poor yields (less than 25% yield) of the desired phosphonates[29] along with the formation of a dark-colored thermal degraded products of the nucleosides. On the other hand, Michaelis–Arbuzov reactions at the sugar level generally result in very good yields. 29 Hence, although route B is time consuming and contains more synthetic steps than route A, we have decided to obtain these phosphonate derivatives via route B, believing that it would be reliable with good yields.

Long chain saturated and unsaturated phosphonates of (N)-methanocarba adenine or 2-Cl adenine derivatives **7–10** could be achieved from the same starting compound **13** as for the phosphonates **4**, **5**, **11** and **12**. Similar to the synthesis of phosphonates **4**, **5**, **11** and **12**, these phosphonates could possibly be obtained via two synthetic routes *viz*, routes C and D (Scheme 7B), based on the installation of the phosphonate group. The long chain unsaturated phosphonate could be introduced by oxidation of the 5'-alcohol of either nucleoside **24** or compound **15** followed by the Wittig–type reaction using tetraisopropyl methylenediphosphonate and NaH to provide phosphonate diester **26** or **30**, respectively.[22, 23] One could expect this reaction to proceed smoothly at both nucleoside and sugar stages. Since route C has a common intermediate **26** for the synthesis of long chain saturated and unsaturated 5'-phosphonates **7–10**, we have decided to explore the synthesis of these phosphonates by the shorter synthetic route C.

Biological analysis

There is increasing evidence that chronic activation of the native cardiac P2X receptor by nucleotide analogues protects against the progression of heart failure. The $P2X_4$ receptor is an essential subunit of this native receptor, but we do not know what other P2X subtypes are present. The cardiac myoctye receptor is not identical to the vascular $P2X_4$ receptor, which has a key role in the response of endothelial cells to changes in blood flow.[27]

The synthetic nucleotide analogue **3** activates the native cardiac P2X receptor, as indicated in electrophysiological experiments with normal cardiac myocytes and those that overexpress CSQ and based on its *in vivo* ability to improve the heart failure phenotype of these animals. The rigid carbocyclic ring system contained in this derivative stabilizes nucleotides toward the action of nucleotidases. Therefore, compound **3** is expected to be more stable than the corresponding riboside. In the present study, we have synthesized fully hydrolysis-resistant adenosine monophosphate derivatives based on phosphonate linkages. The C-O-P bond of **3** was found to be stable over 24 h in aqueous medium at pH 1.5 to simulate the acidity of the

stomach, however incubation at 37°C in the presence of mammalian cell membranes (1321N1 astrocytoma cells) resulted in considerable hydrolysis of the 5'-phosphate of **3** (data not shown). Therefore, a more stable structural alternate to the 5'-phosphate linkage was sought.

An *in vivo* screen of cardiac function was used to test the novel analogues. Thus, the results of this chronic study likely reflect both pharmacodynamic and pharmacokinetic factors. Several of the novel phosphonate analogues displayed the same agonist activity as **3** in protecting the heart muscle when chronically administered in the CSQ model. The SAR analysis showed that considerable cadioprotection was associated with specific structural features of the phosphonate derivatives. The variation in the chain length and saturation at the 5' carbon provided consistent results in the *in vivo* screen. Two of the phosphonates, **4** and **9**, both saturated homologues containing a 2-Cl substitution, improved FS, while the unsaturated phosphonates and 2-H analogues were inactive. The most favorable FS (20.25%, compared to 13.78% in controls) was observed for (1'S,2'R,3'S,4'R,5'S)-4'-(6-amino-2-chloropurin-9-yl)-2',3'-(dihydroxy)-1'-(phosphonomethylene)-bicyclo[3.1.0]hexane **4**, which is the equivalent of **3** in which the 5'-O has been excised. The higher homologue **9** displayed a FS of 19.26%. Thus, it is possible to extend the SAR around compound **3**, for chronic activation of the cardiac P2X receptor leading to a beneficial effect in heart failure.

The activity measured here does not reflect direct measurements at a P2X receptor, and the likely association with this mechanism is due to the close resemblance to compound **3**, which clearly activates a $P2X_4$ receptor-dependent ion flux that has been shown to protect in this model of cardiomyopathy. It is not feasible to study the analogues at a recombinant homotrimeric $P2X_4$ receptor system,[12] because the endogenous cardiac P2X receptor is thought to be composed of $P2X_4$ receptor subunits in heteromeric association with a yet unidentified P2X subtype. The $P2X_4$ receptor is known to associate with other P2X receptor subtypes, and these heterotrimers, for example heteromers with $P2X_1$ subunits, are pharmacologically distinct from $P2X_4$ homotrimers.[26] More recently, the existence of $P2X_4/P2X_7$ heteromers has also been described.[31]

The $P2X_4$ receptor also is distributed widely through the central and peripheral nervous systems, the epithelial layers of ducted glands and airways, bladder smooth muscle, gastrointestinal tract, uterus, and fat cells.[32] Its activation in spinal microglial cells participates in the pathogenesis of chronic pain.[33] Might the effects of overactivation of $P2X_4$ receptors oustide the myocardium complicate the use of such a phosphonate derivative for treatment of cardiac failure and cardiomyopathy? We have no evidence that these monophosphate analogues activate a $P2X_4$ receptor channel at any other location. This is a topic for further investigation.

Another site of action of adenine nucleotides in cardiac tissue is the metabotropic $P2Y_1$ receptor, which causes a nitric oxide-dependent relaxation of the vascular smooth muscle. Therefore, we tested the nucleotides as $P2Y_1$ receptor agonists to account for the possibility that the observed cadiovascular effects of the phosphonate derivatives were a result of activation of an endothelial $P2Y_1$ receptor. Compound **3** was initially characterized in assays of PLC as a weak $hP2Y_1$ receptor agonist (EC_{50} 1.89 μM),[9] and that conclusion is consistent with the potency obseved here in inducing calcium transients in the same cell line. All of the phosphonate derivatives tested were inactive at the $P2Y_1$ receptor. This suggests the use of these compounds as more selective pharmacological probes of the endogenous cardiac P2X receptor than compound **3**. However, it is worth noting that the cardioprotection provided by **3** was shown to be independent of the $P2Y_1$ receptor by its inability to dilate aortic rings and by use of a $P2Y_1$-selective antagonist MRS2500. This antagonist could not block the membrane current evoked by **3** under voltage clamp in mouse cardiac myocytes.[8]

In conclusion, we have greatly expanded the range of carbocyclic nucleotide analogues that represent potential candidates for the treatment of heart failure. A more chemically and biologically stable linkage than the phosphate group in compound **3** has been introduced in the form of the phosphonate groups,[30] which in several cases preserve heart contractile function in a genetic model of heart failure. Facile routes for the synthesis of phosphonate analogues of **3** in the conformationally constrained (N)-methanocarba series were developed using Michaelis–Arbuzov and Wittig reactions. A further advantage of the phosphonate linkage is that the undesired activity as agonist of the $P2Y_1$ receptor has been eliminated. The beneficial effects of these nucleotidase-resistant agonists can now be explored in additional models of cardiac failure and cardiomyopathy.

Experimental Procedures

General methods

Compound **13** was either synthesized as reported[13] or obtained as a custom synthesis from Natland International Corporation (Research Triangle Park, NC). All other reagents and solvents (regular and anhydrous) were of analytical grade and obtained from commercial suppliers and used without further purification. Reactions were conducted under an atmosphere of argon whenever anhydrous solvents were used. All reactions were monitored by thin-layer chromatography (TLC) using silica gel coated plates with a fluorescence indicator which were visualized: a) under UV light, b) by dipping in 5% conc. H_2SO_4 in absolute ethanol (v/v) followed by heating, or c) by dipping in a solution of anisaldehyde:H_2SO_4 (1:2, v/v) in MeOH followed by heating. Silica gel column chromatography was performed with silica gel (SiO_2, 200–400 mesh, 60Å) using moderate air pressure. Evaporation of solvents was carried out under reduced pressure at a temperature below 50 °C. After column chromatography, appropriate fractions were pooled, evaporated and dried at high vacuum for at least 12 h to give the obtained products in high purity. ^{1}H NMR and ^{31}P NMR ascertained sample purity. No corrections in yields were made for solvent of crystallization. ^{1}H NMR and ^{31}P NMR spectra were recorded at 300 MHz and 121.5 MHz, respectively. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane or deuterated solvent as the internal standard (dH: $CDCl_3$ 7.26 ppm). For compounds **38 – 41**, the integral of the H3'-signal of the least predominant isomer was set to 1.0. Systematic compound names for bicyclic nucleosides are given according to the von Baeyer nomenclature.[21] High resolution mass spectroscopic (HRMS) measurements were performed on a proteomics optimized Q-TOF-2 (Micromass-Waters) using external calibration with polyalanine. Observed mass accuracies are those expected on the basis of known performance of the instrument as well as the trends in masses of standard compounds observed at intervals during the series of measurements. Reported masses are observed masses uncorrected for this time-dependent drift in mass accuracy.

Purification of the nucelotide derivatives for biological testing was performed by HPLC with a Luna 5μ RP-C18(2) semipreparative column (250 × 10.0 mm; Phenomenex, Torrance, CA) under the following conditions: flow rate of 2 mL/min; 10 mM triethylammonium acetate (TEAA)-CH_3CN from 100:0 (v/v) to 70:30 (v/v) in 30 min and isolated in the triethylammonium salt form. Analytical purity of compounds was checked using a Hewlett–Packard 1100 HPLC equipped with Zorbax SB-Aq 5 μm analytical column (50×4.6 mm; Agilent Technologies Inc, Palo Alto, CA). Mobile phase: linear gradient solvent system: 5 mM TBAP (tetrabutylammonium dihydrogenphosphate)-CH_3CN from 80:20 to 40:60 in 13 min; the flow rate was 0.5 mL/min. Peaks were detected by UV absorption with a diode array detector at 254, 275, and 280 nm. All derivatives tested for biological activity showed >99% purity by HPLC analysis (detection at 254 nm).

(1'S,2'R,3'S,4'R,5'S)-4'-(6-Amino-2-chloropurin-9-yl)-2',3'-(dihydroxy)-1'-(phosphonomethylene)-bicyclo[3.1.0]hexane (4)

Nucleoside **21** (30 mg, 0.064 mmol) was coevaporated with anhydrous toluene (3 × 3 mL) and dissolved in anhydrous CH_2Cl_2 (3 mL). To this soution was added iodotrimethylsilane (91 μl, 0.64 mmol). After stirring for 17 h, the reaction mixture was cooled to 0 °C followed by the addition of ice-cold H_2O (25 mL) and CH_2Cl_2 (25 mL). The phases were separated, and the aqueous phase washed with CH_2Cl_2 (1 × 35 mL) and diethyl ether (3 × 35 mL). The resulting aqueous phase evaporated to dryness and purified by HPLC (retention time: 19.1 min) to afford **4** (8.5 mg, 23%) as a white solid material. ESI-HRMS *m/z* 374.0397 $[M - H]^-$, $C_{12}H_{14}ClN_5O_5P^-$: Calcd. 374.0421); ^1H NMR (D_2O) δ 8.21 (s, 1H), 4.71 (s, 1H), 4.57 (d, 1H, *J* = 6.6 Hz), 4.01 (d, 1H, *J* = 6.6 Hz), 3.19 (q, 24H, *J* = 7.2 Hz), 2.23 (t, 1H, 15.5 Hz), 1.63–1.77 (m, 2H), 1.42–1.49 (m, 1H), 1.26 (t, 36H), 0.96–1.04 (m, 1H). ^{31}P NMR (D_2O) δ 23.68. Purity >99% by HPLC (retention time: 4.51 min).

(1'S,2'R,3'S,4'R,5'S)-4'-(6-Aminopurin-9-yl)-2',3'-(dihydroxy)-1'-(phosphonomethylene)-bicyclo[3.1.0]hexane (5)

Nucleoside **23** (25 mg, 0.057 mmol) was coevaporated with anhydrous toluene (3 × 3 mL) and dissolved in anhydrous CH_2Cl_2 (3 mL). Iodotrimethylsilane (83 μl, 0.57 mmol) was added. After stirring for 15 h, the reaction mixture was cooled to 0 °C followed by the addition of ice-cold H_2O (25 mL) and CH_2Cl_2 (25 mL). The phases were separated, and the aqueous phase was washed with CH_2Cl_2 (1 × 35 mL) and diethyl ether (3 × 35 mL). The resulting aqueous phase was evaporated to dryness and purified by HPLC (retention time: 17.5 min) to afford **5** (6.8 mg, 27%) as a white solid material. ESI-HRMS *m/z* 340.0817 $[M - H]^-$, $C_{12}H_{15}N_5O_5P^-$: Calcd. 340.0811); ^1H NMR (D_2O) δ 8.36 (s, 1H), 8.20 (s, 1H) 4.75 (s, 1H), 4.63 (d, 1H, *J* = 6.1 Hz), 4.09 (d, 1H, *J* = 6.1 Hz), 3.19 (q, 6H, *J* = 7.2 Hz), 1.95–2.18 (m,2H), 1.75–1.84 (m, 1H), 1.40–1.46 (m, 1H),1.26 (t, 9H, *J* = 7.2 Hz), 0.92–1.02 (m, 1H). ^{31}P NMR (D_2O) δ 25.36. Purity >99% by HPLC (retention time: 2.9 min)

(1'S,2'R,3'S,4'R,5'S)-4'-(6-Amino-2-chloropurin-9-yl)-2',3'-(dihydroxy)-1'-[(E)-phosphonoethenyl]-bicyclo[3.1.0]-hexane (7)

Nucleoside **27** (12 mg, 0.023 mmol) was coevaporated with anhydrous toluene (3 × 2 mL) and dissolved in anhydrous CH_2Cl_2 (2 mL). Iodotrimethylsilane (35 μl, 0.24 mmol) was added. After stirring for 18 h, the reaction mixture was cooled to 0 °C, followed by the addition of ice-cold H_2O (15 mL) and CH_2Cl_2 (15 mL). The phases were separated, and the aqueous phase was washed with CH_2Cl_2 (1 × 25 mL) and diethyl ether (3 × 35 mL). The resulting aqueous phase was evaporated to dryness and purified by HPLC (retention time: 22.8 min) to afford **7** (2.5 mg, 28%) as a white solid material. ESI-HRMS *m/z* 386.0403 $[M - H]^-$, $C_{13}H_{14}N_5ClO_5P^-$: Calcd. 386.0421); ^1H NMR (D_2O) δ 7.99 (s, 1H), 6.21–6.36 (m, 1H), 6.06 (t, 1H, *J* = 17.5 Hz), 4.84–4.89 (m,1H), 4.06 (d, 1H, *J* = 6.6 Hz), 3.22 (q, 3H, *J* = 7.2 Hz), 1.99–2.06 (m,1H), 1.78–1.87 (m, 1H), 1.29 (t, 6H, *J* = 7.2 Hz), 1.21–1.26 (m, 1H). ^{31}P NMR (D_2O) δ 14.68. Purity >99% by HPLC (retention time: 4.3 min)

(1'S,2'R,3'S,4'R,5'S)-4'-(6-Aminopurin-9-yl)-2',3'-(dihydroxy)-1'-[(E)-phosphonoethenyl]-bicyclo[3.1.0]hexane (8)

Nucleoside **33** (20 mg, 0.042 mmol) was coevaporated with anhydrous toluene (3 × 5 mL) and dissolved in anhydrous CH_2Cl_2 (5 mL). Iodotrimethylsilane (60 μl, 0.42 mmol) was added. After stirring for 17 h, the reaction mixture was cooled to 0 °C, followed by the addition of ice-cold H_2O (25 mL) and CH_2Cl_2 (25 mL). The phases were separated and the aqueous phase was washed with CH_2Cl_2 (1 × 35 mL) and diethyl ether (3 × 35 mL). The resulting aqueous phase was evaporated to dryness and purified by HPLC (retention time: 16.5 min) to afford **8** (11.8 mg, 78%) as a white solid material. ESI-HRMS *m/z* 352.0821 $[M - H]^-$,

$C_{13}H_{15}N_5O_5P^+$: Calcd. 352.0811); ^{1}H NMR (D_2O) δ 8.30 (s, 1H), 8.06 (s, 1H), 6.30–6.44 (m, 1H), 6.07 (t, 1H, *J* =17.5), 4.97 (s, 1H), 4.89 (d, 1H, *J* = 7.2 Hz), 4.09 (d, 1H, *J* = 7.2 Hz), 3.21 (q, 3H, *J* = 7.2 Hz), 2.03–2.10 (m, 2H), 1.84–1.89 (m, 1H), 1.29 (t, 7H, *J* = 7.2 Hz). ^{31}P NMR (D_2O) δ 15.71. Purity >99% by HPLC (retention time: 3.5 min)

(1'S,2'R,3'S,4'R,5'S)-4'-(6-Aminopurin-9-yl)-2',3'-(dihydroxy)-1'-(phosphonoethenyl)-bicyclo [3.1.0]hexane (10)

Nucleoside **28** (15 mg, 0.032 mmol) was coevaporated with anhydrous toluene (3 × 2 mL) and dissolved in anhydrous CH_2Cl_2 (2 mL). Iodotrimethylsilane (45 μl, 0.32 mmol) was added. After stirring for 15 h, the reaction mixture was cooled to 0 °C followed by the addition of ice-cold H_2O (15 mL) and CH_2Cl_2 (15 mL). The phases were separated, and the aqueous phase was washed with CH_2Cl_2 (1 × 25 mL) and diethyl ether (3 × 35 mL). The resulting aqueous phase was evaporated to dryness and purified by HPLC (retention time: 16.6 min) to afford **10** (6.7 mg, 47%) as a white solid material. ESI-HRMS *m/z* 354.0970 $[M - H]^-$, $C_{13}H_{17}N_5O_5P^-$: Calcd. 354.0967); ^{1}H NMR ($CDCl_3$) δ 8.20 (s, 1H), 8.14 (s, 1H), 4.76 (s, 1H), 4.58 (d, 1H, *J* = 6.1 Hz), 4.09 (d, 1H, *J* = 6.1 Hz), 3.21 (q, 3H, *J* = 7.2 Hz), 1.69–2.14 (m, 4H), 1.59–1.69 (m, 1H), 1.39–1.349 (m, 1H), 1.29 (t, 6H, *J* = 7.2 Hz), 0.81–0.92 (m, 1H). ^{31}P NMR (D_2O) δ 27.95. Purity >99% by HPLC (retention time: 2.91 min)

(1'S,2'R,3'S,4'R,5'S)-4'-(6-Amino-2-chloropurin-9-yl)-2',3'-dihydroxy-1'-(methylphosphonicacid)-bicyclo[3.1.0]hexane (11) and (1'S,2'R,3'S,4'R,5'S)-4'-(6-Aminopurin-9-yl)-2',3'-dihydroxy-1'-(methylphosphonicacid)-bicyclo[3.1.0]hexane (12)

Nucleoside **29** (15 mg, 0.034 mmol) was coevaporated with anhydrous toluene (3 × 3 mL) and dissolved in anhydrous CH_2Cl_2 (4 mL). Iodotrimethylsilane (91 μl, 0.33 mmol) was added. After stirring for 19 h, the reaction mixture was cooled to 0 °C, followed by the addition of ice-cold H_2O (25 mL) and CH_2Cl_2 (25 mL). The phases were separated and the aqueous phase was washed with CH_2Cl_2 (1 × 35 mL) and diethyl ether (3 × 35 mL). The resulting aqueous phase was evaporated to dryness and purified by HPLC (retention time: 16.8 min) to afford **11** (1.3 mg, 11%) and **12** (0.8 mg, 20%, combined yield) as a white solid material.

Analytical data of compound 11—ESI-HRMS *m/z* 372.0625 $[M-H]^-$, $C_{13}H_{16}ClN_5O_4P^-$: Calcd. 372.0628); ^{1}H NMR (D_2O) δ 8.23 (s, 1H), 4.76–4.79 (m, 1H), 4.63 (d, 1H, *J* = 6.2 Hz), 4.12 (d, 1H, *J* = 6.2 Hz), 3.21 (q, 2H, *J* = 7.2 Hz), 2.34 (t, 1H, *J* = 15.3 Hz), 1.75–1.86 (m, 1H), 1.68–1.75 (m, 1H), 1.51–1.56 (m, 1H) 1.35 (d, 3H, *J* = 13.2 Hz), 1.26 (t, 1H, *J* = 7.2 Hz) 0.96–1.04 (m, 1H). ^{31}P NMR (D_2O) δ 46.01. Purity >99% by HPLC (retention time: 4.19 min).

Analytical data of compound 12—ESI-HRMS *m/z* 338.1016 $[M-H]^-$, $C_{13}H_{17}N_5O_4P^-$: Calcd. 338.1018); ^{1}H NMR (D_2O) δ 8.26 (s, 1H), 8.25 (s, 1H), 4.76–4.79 (m, 1H), 4.63 (d, 1H, *J* = 6.2 Hz), 4.12 (d, 1H, *J* = 6.2 Hz), 3.21 (q, 2H, *J* = 7.2 Hz), 2.34 (t, 1H, *J* = 15.3 Hz), 1.75–1.86 (m, 1H), 1.68–1.75 (m, 1H), 1.51–1.56 (m, 1H) 1.35 (d, 3H, *J* = 13.2 Hz), 1.26 (t, 1H, *J* = 7.2 Hz) 0.96–1.04 (m, 1H). ^{31}P NMR (D_2O) δ 46.0. Purity >99% by HPLC (retention time: 5.91 min).

Ethyl-(1S,2R,3S,4S,5S)-2,3-*O*-(isopropylidene)-4-*O*-(*tert*-butyldimethylsilyl)-bicyclo[3.1.0] hexanecarboxylate (14)

Known alcohol **13** (0.83 g, 3.40 mmol) was coevaporated with anhydrous toluene (2 × 10 mL) and dissolved in anhydrous CH_2Cl_2 (25 mL). Imidazole (0.69 g, 10.20 mmol), DMAP (0.04 g, 0.34 mmol) and *tert*-butylchlorodiphenylsilane (1.74 mL, 6.81 mmol) were added. After stirring at rt for 16 h, the reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with sat. aq. $NaHCO_3$ (1 × 30 mL). The aqueous phase was back-extracted with CH_2Cl_2 (2 × 50

mL). The combined organic phase was evaporated to dryness, and the resulting crude residue was purified by silica gel column chromatography (0–8% EtOAc in petroleum ether, v/v) to afford compound **14** (1.52 mg, 93%) as a colorless oil. R_f = 0.3 (10% EtOAc in CH_2Cl_2, v/v); ESI-HRMS *m/z* 519.1981 ([M + K]$^+$, $C_{28}H_{36}O_5Si{\cdot}K^+$: Calcd. 519.1969); ^{1}H NMR ($CDCl_3$) δ 7.69–7.80 (m, 4H, Ph), 7.32–7.45 (m, 6H, Ph), 5.11 (d, 1H, *J* = 6.6 Hz), 4.42 (t, 1H, *J* = 6.1 Hz), 3.99–4.18 (m, 3H), 2.17–2.25 (m, 1H), 1.91 (t, 1H, *J* = 5.5 Hz), 1.57 (s, 3H), 1.45–1.53 (m, 1H), 1.18–1.24 (m, 6H), 1.07 (s, 9H).

(1S,2R,3S,4S,5S)-1-Hydroxymethyl-2,3-*O*-(isopropylidene)-4-*O*-(*tert*-butyldimethylsilyl)-bicyclo[3.1.0]hexane (15)

Compound **14** (0.98 g, 2.04 mmol) was coevaporated with anhydrous toluene (2 × 20 mL), dissolved in anhydrous THF (30 mL) and cooled to −70 °C. DIBAL-H (1.5 M in toluene 10.8 mL, 16.32 mmol) was added slowly to this solution over 20 min. After stirring at −70 °C for 3 h, the reaction was quenched with the very careful addition of ice-cold MeOH (20 mL), followed by warming the reaction mixture to rt. 1 M cold H_2SO_4 (20 mL) was added to the mixture and it was stirred for 1 h, followed by addition of CH_2Cl_2 (100 mL). The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 35 mL). The combined organic phase was evaporated to dryness, and the resulting residue was purified by silica gel column chromatography (0–45% EtOAc in petroleum ether, v/v) to afford compound **15** (0.74 g, 82%) as a colorless oil. R_f = 0.4 (50% EtOAc in petroleum ether, v/v); ESI-HRMS *m/z* 461.2112 ([M + Na]$^+$, $C_{26}H_{34}O_4Si{\cdot}Na^+$: Calcd. 461.2124); ^{1}H NMR ($CDCl_3$) δ 7.71–7.78 (m, 4H, Ph), 7.31–7.44 (m, 6H, Ph), 4.73 (d, 1H, *J* = 6.5 Hz), 4.44 (t, 1H, *J* = 6.5 Hz), 4.09 (t, 1H, *J* = 6.5 Hz), 3.59–3.67 (m, 1H), 3.41–3.49 (m, 1H), 1.57–1.59 (m, 4H), 1.33 (t,1H, *J* = 5.5 Hz), 1.20 (s, 3H), 1.07 (s, 9H), 0.56–0.68 (m, 1H).

(1S,2R,3S,4S,5S)-2,3-*O*-(Isopropylidene)-1-methanesulfonyloxymethyl-4-*O*-(*tert*-butyldimethylsilyl)-bicyclo[3.1.0]hexane (16)

Compound **15** (0.59 g, 1.36 mmol) was coevaporated with anhydrous toluene (2 × 20 mL), dissolved in anhydrous CH_2Cl_2 (30 mL) and cooled to 0 °C. Triethylamine (0.95 mL, 6.79 mmol) and methanesulfonyl chloride (0.22 mL, 2.72 mol) were added at 0 °C over 10 min. After warming the reaction mixture to rt, it was stirred for 17 h. Then, ice-cold H_2O (25 mL) was added and the mixture was extracted with EtOAc (2 × 45 mL). The combined organic phase was washed with sat. aq. $NaHCO_3$ (2 × 35 mL) and evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–50% EtOAc in petroleum ether, v/v) to afford compound **16** (0.68 g, 96%) as a colorless oil. R_f = 0.5 (50% EtOAc in petroleum ether, v/v); ESI-HRMS *m/z* 555.1623 ([M + K]$^+$, $C_{27}H_{36}O_6SSi{\cdot}K^+$: Calcd. 555.1639); ^{1}H NMR ($CDCl_3$) δ 7.69–7.77 (m, 4H, Ph), 7.31–7.45 (m, 6H, Ph), 4.69 (d, 1H, *J* = 6.5 Hz), 4.47 (t, 1H, *J* = 6.5 Hz), 4.37–4.43 (dd, 1H, *J* = 10.9 Hz), 4.07 (t, 1H, *J* = 6.5 Hz), 3.90–3.95 (dd, 1H, *J* = 10.9 Hz), 2.99 (s, 3H), 1.67–1.72 (m, 2H), 1.55 (s, 3H), 1.20 (s, 3H), 1.08 (s, 9H), 0.72–0.79 (m, 1H).

(1S,2R,3S,4S,5S)-1-Iodomethyl-2,3-*O*-(isopropylidene)-4-*O*-(*tert*-butyldimethylsilyl)-bicyclo [3.1.0]hexane (17)

Compound **16** (0.68 g, 1.32 mmol) was coevaporated with anhydrous toluene (2 × 20 mL), and the residue dissolved in anhydrous 1,4-dioxane (25 mL). NaI (0.59 g, 3.94 mol) was added to the mixture, and it was heated to 65 °C. After stirring for 17 h, the reaction mixture was cooled to rt and diluted with H_2O (25 mL) and CH_2Cl_2 (75 mL). The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 35 mL). The combined organic phase was evaporated to dryness, and the resulting residue was purified by silica gel column chromatography (0–20% EtOAc in petroleum ether, v/v) to afford compound **17** (0.69 g, 95%) as a colorless oil. R_f = 0.5 (20% EtOAc in petroleum ether, v/v); ESI-HRMS *m/z* 549.1322 ([M

+ H]$^+$, $C_{26}H_{33}IO_3Si{\cdot}H^+$: Calcd. 549.1322); ^{1}H NMR ($CDCl_3$) δ 7.68–7.77 (m, 4H, Ph), 7.32–7.46 (m, 6H, Ph), 4.69 (d, 1H, *J* = 6.5 Hz), 4.43 (t, 1H, *J* = 6.5 Hz), 4.09 (t, 1H, *J* = 6.5 Hz), 3.55–3.60 (dd, 1H, *J* = 10.5 Hz), 3.97–4.02 (dd, 1H, *J* = 10.5 Hz), 2.02 (t, 1H, *J* = 4.9 Hz), 1.54–1.57 (s, 4H), 1.20 (s, 3H), 1.07 (s, 9H), 0.83–0.90 (m, 1H).

Diethyl-(1S,2R,3S,4S,5S)-2,3-*O*-(isopropylidene)-4-*O*-(*tert*-butyldimethylsilyl)-bicyclo[3.1.0] hexane phosphonate (18)

Compound **17** (0.68 g, 1.23 mmol) was dissolved in triethylphosphite (17 mL), and the mixture was heated to 110 °C. After stirring for 17 h, the reaction mixture was cooled to rt and evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–90% EtOAc in petroleum ether, v/v) to afford compound **18** (0.65 g, 94%) as a colorless oil. R_f = 0.3 (EtOAc); ESI-HRMS *m/z* 559.2665 ([M + H]$^+$, $C_{30}H_{43}O_6PSi{\cdot}H^+$: Calcd. 559.2645); ^{1}H NMR ($CDCl_3$) δ 7.71–7.77 (m, 4H, Ph), 7.30–7.43 (m, 6H, Ph), 4.80 (d, 1H, *J* = 6.5 Hz), 4.47 (t, 1H, *J* = 6.5 Hz), 4.10 (t, 1H, *J* = 6.5 Hz), 3.91–4.05 (m, 4H), 2.22 (t, 1H, *J* = 16.5 Hz), 1.63–1.71 (m,1H), 1.57–1.61 (m, 2H), 1.55 (s, 3H), 1.22 (t, 3H, *J* = 7.2 Hz), 1.20 (t, 3H, *J* = 7.2 Hz), 1.19 (s, 3H), 1.07 (s, 9H), 0.53–0.60 (m, 1H). ^{31}P NMR ($CDCl_3$) δ 29.93.

Diethyl-(1S,2R,3S,4S,5S)-4-hydroxy-2,3-*O*-(isopropylidene)-bicyclo[3.1.0]hexane phosphonate (19)

Compound **18** (0.65 g, 1.16 mmol) was dissolved in a mixture of THF (20 mL) and tetrabutylammonium fluoride (1 M in THF, 2.91 mL, 2.91 mmol). After stirring for 17 h, the reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–7% MeOH in EtOAc, v/v) to afford compound **19** (0.33 g, 88%) as a colorless oil. R_f = 0.3 (5% MeOH in EtOAc, v/v); ESI-HRMS *m/z* 321.1466 [M + H]$^+$, $C_{14}H_{25}O_6P{\cdot}H^+$: Calcd. 321.1467); ^{1}H NMR ($CDCl_3$) δ 5.02 (d, 1H, *J* = 6.1 Hz), 4.50–4.58 (m, 2H), 4.02–4.17 (m, 4H), 2.32–2.37 (m, 1H), 2.26 (t, 1H, *J* = 16.5 Hz), 1.88–1.96 (m,1H), 1.61–1.74 (m, 1H), 1.54 (s, 3H), 1.32 (t, 6H, *J* = 7.2 Hz), 1.28 (s, 3H),1.21–1.27 (m, 1H), 0.60–0.67 (m, 1H). ^{31}P NMR ($CDCl_3$) δ 29.01.

Diethyl-(1'S,2'R,3'S,4'R,5'S)-4'-(2,6-dichloropurin-9-yl)-2',3'-*O*-(isopropylidene)-bicyclo [3.1.0]hexane phosphonate (20)

Diisopropyl azodicarboxylate (97 μL, 0.49 mmol) was added at rt to a mixture of triphenylphosphine (128 mg, 0.49 mmol) and 2,6-dichloropurine (92 mg, 0.49 mmol) in anhydrous THF (3 mL). After stirring for 30 min, a solution of compound **19** (78 mg, 0.25 mmol) in THF (3 mL) was added to the mixture. After stirring for 51 h, the reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–4% MeOH in EtOAc, v/v) to afford nucleoside **20** (90 mg, 75%) as a white solid material. R_f = 0.5 (5% MeOH in EtOAc, v/v); ESI-HRMS *m/z* 491.1013 [M + H]$^+$, $C_{19}H_{25}Cl_2N_4O_5P{\cdot}H^+$: Calcd. 491.1018); ^{1}H NMR ($CDCl_3$) δ 8.82 (s, 1H), 5.39 (d, 1H, *J* = 6.5 Hz), 5.10 (s, 1H), 4.61 (d, 1H, *J* = 6.5 Hz), 4.02–4.21 (m, 4H), 2.46 (t, 1H, *J* = 16.5 Hz), 1.91–2.06 (m,1H), 1.74–1.82 (m, 1H), 1.54 (s, 3H), 1.32 (t, 3H, *J* = 7.2 Hz), 1.26 (t, 3H, *J* = 7.2 Hz), 1.24 (s, 3H),1.08–1.21 (m, 1H), 0.97–1.06 (m, 1H).

Diethyl-(1'S,2'R,3'S,4'R,5'S)-4'-(6-amino-2-chloropurin-9-yl)-2',3'-*O*-(isopropylidene)-bicyclo [3.1.0]hexane phosphonate (21)

Nucleoside **20** (90 mg, 0.19 mmol) was treated with 2 M NH_3 in *i*-PrOH (5 mL), and the mixture was heated to 70 °C and stirred for 17 h. The reaction mixture was evaporated to dryness, and the resulting residue was purified by silica gel column chromatography (0–5% MeOH in CH_2Cl_2, v/v) to afford nucleoside **21** (70 mg, 80%) as a white solid material. R_f = 0.5 (5% MeOH in CH_2Cl_2, v/v); ESI-HRMS *m/z* 472.1519 [M + H]$^+$, $C_{19}H_{27}ClN_5O_5P{\cdot}H^+$: Calcd. 472.1517); ^{1}H NMR ($CDCl_3$) δ 8.31 (s, 1H), 5.98 (s, 2H), 5.36 (d, 1H, *J* = 7.1 Hz), 4.97 (s,

1H), 4.61 (d, 1H, *J* = 6.5 Hz), 4.03–4.19 (m, 4H), 2.39 (t, 1H, *J* = 16.5 Hz), 2.03–2.17 (m, 1H), 1.70–1.77 (m, 1H), 1.52 (s, 3H), 1.32 (t, 3H, *J* = 7.2 Hz), 1.25 (t, 3H, *J* = 7.2 Hz), 1.23 (s, 3H), 1.18–1.21 (m, 1H), 0.96–1.04 (m, 1H).

Diethyl-(1'S,2'R,3'S,4'R,5'S)-4'-(6-chloropurin-9-yl)-2',3'-*O*-(isopropylidene)-bicyclo[3.1.0] hexane phosphonate (22)

Diisopropyl azodicarboxylate (100 μL, 0.50 mmol) was added at rt to a mixture of triphenylphosphine (133 mg, 0.50 mmol) and 6-chloropurine (96 mg, 0.50 mmol) in anhydrous THF (3 mL). After stirring the mixture for 30 min, a solution of compound **19** (81 mg, 0.26 mmol) in THF (3 mL) was added. After stirring for 17 h, the reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–4% MeOH in EtOAc, v/v) to afford nucleoside **22** (100 mg, 87%) as a white solid material. R_f = 0.5 (5% MeOH in EtOAc, v/v); ESI-HRMS *m/z* 457.1417 $[M + H]^+$, $C_{19}H_{26}ClN_4O_5P{\cdot}H^+$: Calcd. 457.1408); ^{1}H NMR ($CDCl_3$) δ 8.84 (s, 1H), 8.78 (s, 1H), 5.39 (d, 1H, *J* = 6.5 Hz), 5.15 (s, 1H), 4.62 (d, 1H, *J* = 6.5 Hz), 4.07–4.21 (m, 4H), 2.44 (t, 1H, *J* = 16.5 Hz), 1.94–2.18 (m, 1H), 1.83–1.90 (m, 1H), 1.58 (s, 3H), 1.33 (t, 3H, *J* = 7.2 Hz), 1.24–1.30 (m, 4H), 0.97–1.06 (m, 1H).

Diethyl-(1'S,2'R,3'S,4'R,5'S)-4'-(6-aminopurin-9-yl)-2',3'-*O*-(isopropylidene)-bicyclo[3.1.0] hexane phosphonate (23)

Nucleoside **22** (100 mg, 0.22 mmol) was treated with 2 M NH_3 in *i*-PrOH (5 mL) and heated up to 70 °C. After stirring for 19 h, the reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–6% MeOH in CH_2Cl_2, v/v) to afford nucleoside **23** (75 mg, 79%) as a white solid material. R_f = 0.4 (5% MeOH in CH_2Cl_2, v/v); ESI-HRMS *m/z* 438.1912 $[M + H]^+$, $C_{19}H_{28}N_5O_5P{\cdot}H^+$: Calcd. 438.1906); ^{1}H NMR ($CDCl_3$) δ 8.38 (s, 1H), 8.36 (s, 1H), 5.54 (s, 2H), 5.36 (d, 1H, *J* = 7.2 Hz), 5.03 (s, 1H), 4.63 (d, 1H, *J* = 7.2 Hz), 4.06–4.20 (m, 4H), 2.38 (t, 1H, *J* = 16.5 Hz), 1.97–2.11 (m, 1H), 1.78–1.85 (m,1H), 1.68 (s, 3H), 1.32 (t, 3H, *J* = 7.2 Hz), 1.27 (t, 3H, *J* = 7.2 Hz), 1.23 (s, 3H),1.18–1.21 (m, 1H), 0.95–1.02 (m, 1H).

(1'S,2'R,3'S,4'R,5'S)-4'-(2,6-Dichloropurin-9-yl)-1'-formyl-2,3-*O*-(isopropylidine)-bicyclo [3.1.0]hexane (25)

Known nucleoside[13] **24** (150 mg, 0.41 mmol) was coevaporated with anhydrous toluene (2 × 8 mL) and dissolved in anhydrous CH_2Cl_2 (8 mL). Dess-Martin periodinane (257 mg, 0.61 mmol) was added. After stirring for 1 h, the reaction mixture was diluted with EtOAc (50 mL) and washed with an aqueous mixture of $Na_2S_2O_3$ and $NaHCO_3$ (3 × 35 mL). The aqueous phase was then extracted with EtOAc (2 × 35 mL). The combined organic phase was evaporated to dryness, and the resulting residue was purified by silica gel column chromatography (0–100% EtOAc in petroleum ether, v/v) to afford compound **25** (120 mg, 80%) as a white solid material. R_f = 0.6 (EtOAc); ESI-HRMS *m/z* 369.0527 ($[M + H]^+$, $C_{15}H_{14}Cl_2N_4O_3{\cdot}H^+$: Calcd. 369.0521); ^{1}H NMR ($CDCl_3$) δ 9.62 (s, 1H), 8.05 (s, 1H), 5.94 (d, 1H, *J* = 7.2 Hz), 4.97 (s, 1H), 4.83 (d, 1H, *J* = 7.2 Hz), 2.22–2.29 (m, 1H), 1.73 (t, 1H, *J* = 6.1 Hz), 1.57 (s, 3H), 1.30 (s, 3H).

(1'S,2'R,3'S,4'R,5'S)-4'-(2,6-Dichloropurin-9-yl)-1'-[diisopropyl-(E)-ethenylphosphonate]-2', 3'-*O*-(isopropylidine)-bicyclo[3.1.0]hexane (26)

Tetraisopropyl methylenediphosphonate (165 μL, 0.51 mmol) was added to a suspension of NaH (60% dispersion in mineral oil, 25 mg, 1.02 mmol) in anhydrous THF (2 mL) at 0 °C. After H_2 evolution ceased, a solution of aldehyde **25** (125 mg, 0.34 mmol) in anhydrous THF (3 mL) was added dropwise carefully at 0 °C. After stirring at 0 °C for 1 h, the mixture was warmed to rt. After stirring at rt for 1 h, the reaction mixture was cooled to 0 °C, and ice-cold

H_2O (20 mL) was added. The phases were separated, and the aqueous phase was extracted with EtOAc (3 × 35 mL). The combined organic phase was evaporated to dryness, and the resulting residue was purified by silica gel column chromatography (0–4% MeOH in EtOAc, v/v) to afford nucleoside **26** (150 mg, 83%) as a white solid material. R_f = 0.3 (EtOAc); ESI-HRMS *m/z* 531.1313 ([M + H]$^+$, $C_{22}H_{29}Cl_2N_4O_5P{\cdot}H^+$: Calcd. 531.1331); ^{1}H NMR ($CDCl_3$) δ 8.04 (s, 1H), 6.50–6.65 (m, 1H), 5.97 (t, 1H, *J* = 17.1 Hz), 5.53 (d, 1H, *J* = 7.2 Hz), 4.98 (s, 1H), 4.77 (d, 1H, *J* = 7.2 Hz), 4.60–4.74 (m, 2H), 1.82–1.90 (m, 1H), 1.59 (s, 3H), 1.22–1.38 (m, 16H), 0.83–0.90 (m, 1H). ^{31}P NMR ($CDCl_3$) δ 16.64.

(1'S,2'R,3'S,4'R,5'S)-4'-(6-Amino-2-chloropurin-9-yl)-1'-[diisopropyl-(E)-ethenylphosphonate]-2,3-O-(isopropylidine)-bicyclo-[3.1.0]-hexane (27)

Nucleoside **26** (100 mg, 0.19 mmol) was treated with 2 M NH_3 in *i*-PrOH (5 mL) and heated to 70 °C. After stirring for 16 h, the reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–8% MeOH in CH_2Cl_2, v/v) to afford nucleoside **27** (85 mg, 88%) as a white solid material. R_f = 0.3 (5% MeoH in EtOAc, v/v); ESI-HRMS *m/z* 512.1821 ([M + H]$^+$, $C_{22}H_{31}ClN_5O_5P{\cdot}H^+$: Calcd. 512.1830); ^{1}H NMR ($CDCl_3$) δ 7.69 (s, 1H), 6.52–6.68 (m, 1H), 5.94 (t, 1H, *J* = 17.5 Hz), 5.75 (s, 2H), 5.51 (d, 1H, *J* = 7.2 Hz), 4.91 (s, 1H), 4.76 (d, 1H, *J* = 7.2 Hz), 4.59–4.73 (m, 2H), 1.80–1.89 (m, 1H), 1.61 (s, 3H), 1.21–1.37 (m, 15H), 1.08–1.17 (m, 1H), 0.77–0.95 (m, 1H).

(1'S,2'R,3'S,4'R,5'S)-4'-(6-Aminopurin-9-yl)-1'-(diisopropyl-phosphonoethenyl)-2',3'-*O*-(isopropylidine)-bicyclo[3.1.0]hexane (28)

Nucleoside **27** (20 mg, 0.04 mmol) was dissolved in a mixture of MeOH and aqueous 2 M NaOH (3 mL, 2:1, v/v). 10% Pd/C (20 mg) and H_2 (3 bar) were added to this solution. After stirring the mixture for 19 h, the catalyst was removed by filtration through a Celite pad, which was washed with MeOH (40 mL), and the filtrate was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–10% MeOH in EtOAc, v/v) to afford nucleoside **28** (15 mg, 79%) as white solid material. R_f = 0.5 (15% MeOH in EtOAc, v/v); ESI-HRMS *m/z* 480.2385 ([M + H]$^+$, $C_{22}H_{34}N_5O_5P{\cdot}H^+$: Calcd. 480.2376); ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1H), 7.79 (s, 1H), 5.80 (s, 2H), 5.19 (d, 1H, *J* = 7.2 Hz), 4.83 (s, 1H), 4.74 (d, 1H, *J* = 7.2 Hz), 4.63–4.73 (m, 2H), 1.60–2.35 (m, 4H), 1.52 (s, 3H), 1.44–1.51 (m, 1H), 1.33 (s, 6H), 1.31 (s, 6H), 1.23 (s, 3H), 1.04–1.09 (m, 1H), 0.76–0.83 (m, 1H). ^{31}P NMR ($CDCl_3$) 30.04.

(1S,2R,3S,4S,5S)-1-Formyl-2,3-O-(isopropylidene)-4-*O*-(*tert*-butyldimethylsilyl)-bicyclo [3.1.0]hexane (29)

Compound **15** (0.63 g, 1.43 mmol) was coevaporated with anhydrous toluene (2 × 25 mL) and dissolved in anhydrous CH_2Cl_2 (25 mL). Dess-Martin periodinane (0.91 g, 2.13 mmol) was added to this solution. After stirring for 4 h, the reaction mixture was diluted with EtOAc (50 mL) and washed with an aqueous mixture of $Na_2S_2O_3$ and $NaHCO_3$ (3 × 50 mL). The aqueous phase was extracted with EtOAc (2 × 50 mL). The combined organic phase was evaporated to dryness and the resulting residue was purified by silica gel column chromatography (0–25% EtOAc in petroleum ether, v/v) to afford aldehyde **29** (452 mg, 73%) as a colorless oil. R_f = 0.6 (50% EtOAc in petroleum ether, v/v); ESI-HRMS *m/z* 459.1986 ([M + Na]$^+$, $C_{26}H_{32}O_4Si{\cdot}Na^+$: Calcd. 459.1968); ^{1}H NMR ($CDCl_3$) δ 8.92 (s, 1H), 7.68–7.78 (m, 4H, Ph), 7.31–7.48 (m, 6H, Ph), 5.13 (d, 1H, *J* = 6.5 Hz), 4.41 (t, 1H, *J* = 6.5 Hz), 4.16 (t, 1H, *J* = 6.5 Hz), 2.19–2.28 (m, 1H), 2.10–2.18 (m, 1H), 1.55 (s, 3H), 1.43–1.51 (m, 1H), 1.23 (s, 3H), 1.09 (s, 9H).

(1S,2R,3S,4S,5S)-1-[Diisopropyl-(E)-phosphonoethenyl]-2,3-*O*-(isopropylidene)-4-*O*-(*tert*-butyldimethylsilyl)-bicyclo[3.1.0]hexane (30)

Tetraisopropyl methylenediphosphonate (475 μL, 1.47 mmol) was added to a suspension of sodium hydride (71 mg, 2.95 mmol, 60 % dispersion in mineral oil) in anhydrous THF (6 mL) at 0 °C. After H_2 evolution ceased, a solution of aldehyde **29** (0.43 g, 0.98 mmol) in anhydrous THF (4 mL) was added dropwise carefully at 0 °C. After stirring at 0 °C for 1 h, the mixture was warmed to rt. After stirring at rt for 1h, the mixture was cooled to 0 °C, and ice-cold H_2O (20 mL) was added. The phases were separated, and the aqueous phase was extracted with EtOAc (3 × 35 mL). The combined organic phase was evaporated to dryness and the resulting residue was purified by silica gel column chromatography (0–70% EtOAc in petroleum ether, v/v) to afford nucleoside **30** (0.24 mg, 48%) as a white solid material. R_f = 0.4 (70% EtOAc in petroleum ether, v/v); ESI-HRMS *m/z* 599.2938 ($[M + H]^+$, $C_{33}H_{47}O_6PSi{\cdot}H^+$: Calcd. 599.2958); ^{1}H NMR ($CDCl_3$) δ 7.69–7.77 (m, 4H, Ph), 7.31–7.45 (m, 6H, Ph), 6.24–6.40 (m, 1H), 5.66 (t, 1H, *J* = 17.5 Hz), 4.75 (d, 1H, *J* = 6.5 Hz), 4.52–4.64 (m, 2H) 4.42 (t, 1H, *J* = 6.5 Hz), 4.11 (t, 1H, *J* = 6.5 Hz), 1.93–1.99 (m, 1H), 1.78–1.85 (m, 1H), 1.57 (s, 3H), 1.19–1.32 (m, 15H), 1.07 (s, 9H), 0.93–1.01 (m, 1H).

(1S,2R,3S,4S,5S)-1-[Diisopropyl-(E)-phosphonoethenyl]-4-(hydroxy)-2,3-*O*-(isopropylidene)-bicyclo[3.1.0]hexane (31)

Compound **30** (0.45 g, 0.76 mmol) was dissolved in THF (10 mL) and tetrabutylammonium fluoride (1.0 M in THF, 2.3 mL, 2.3 mmol) was added. After stirring for 13 h, the reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–7% MeOH in EtOAc, v/v) to afford compound **31** (0.26 g, 98%) as a colorless oil. R_f = 0.3 (EtOAc); ESI-HRMS *m/z* 361.1790 ($[M + H]^+$, $C_{17}H_{29}O_6P{\cdot}H^+$: Calcd. 361.1780); ^{1}H NMR ($CDCl_3$) δ 6.34–6.49 (m, 1H), 5.74 (t, 1H, *J* = 17.5 Hz), 4.99 (d, 1H, *J* = 6.5 Hz), 4.47–4.69 (m, 4H), 2.40 (d, 1H, *J* = 9.5 Hz), 2.07–2.15 (M, 1H), 1.59–1.63 (m, 1H), 1.58 (s, 3H), 1.22–1.34 (m, 15H), 0.93–1.07 (m, 1H).

(1'S,2'R,3'S,4'R,5'S)-4'-(6-Chloropurin-9-yl)-1'-[diisopropyl-(E)-phosphonoethenyl] -2',3'-*O*-(isopropylidene)-bicyclo[3.1.0]hexane (32)

Diisopropyl azodicarboxylate (90 μL, 0.45 mmol) was added at rt to a mixture of triphenylphosphine (117 mg, 0.45 mmol) and 6-chloropurine (70 mg, 0.45 mmol) in anhydrous THF (5 mL). After stirring for 30 min, a solution of the compound **31** (80 mg, 0.23 mmol) in THF (5 mL) was added. After stirring for 60 h, the reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–55% acetone in petroleum ether, v/v) to afford nucleoside **32** (92 mg, 85%) as a white solid material. R_f = 0.4 (60% acetone in petroleum ether, v/v); ESI-HRMS *m/z* 519.1532 ($[M + Na]^+$, $C_{22}H_{30}ClN_4O_5P{\cdot}Na^+$: Calcd. 519.1540); ^{1}H NMR ($CDCl_3$) δ 8.71 (s, 1H), 8.07 (s, 1H), 6.49–6.63 (m, 1H), 5.96 (t, 1H, *J* =17.5), 5.53 (d, 1H, *J* = 6.5 Hz), 5.02 (s, 1H), 4.79 (d, 1H, *J* = 6.5 Hz), 4.61–4.73 (m, 2H), 1.86–1.92 (m, 1H), 1.58–1.63 (m, 1H), 1.54 (s, 3H), 1.22–1.38 (m, 16H).

(1'S,2'R,3'S,4'R,5'S)-4'-(6-Aminopurin-9-yl)-1'-[diisopropyl-(E)-phosphonoethenyl]-2',3'-*O*-(isopropylidene)-bicyclo[3.1.0]hexane (33)

Nucleoside **32** (90 mg, 0.19 mmol) was treated with 2 M NH_3 in *i*-PrOH (7 mL) and heated to 70 °C. After stirring for 17 h, the reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–12% MeOH in CH_2Cl_2, v/v) to afford nucleoside **33** (74 mg, 85%) as a white solid material. R_f = 0.2 (10% MeOH in EtOAc, v/v); ESI-HRMS *m/z* 478.2198 ($[M + H]^+$, $C_{22}H_{32}N_5O_5P{\cdot}H^+$: Calcd. 478.2219); ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1H), 7.73 (s, 1H), 6.51–6.66 (m, 1H), 5.96 (t, 1H, *J* =17.5), 5.47–5.54 (m,

3H), 4.95 (s, 1H), 4.78 (d, 1H, *J* = 6.5 Hz), 4.60–4.72 (m, 2H), 1.86–1.94 (m, 1H), 1.53–1.57 (m, 4H), 1.24–1.37 (m, 16H).

(1S,2R,3S,4S,5S)-1-(Diisopropyl-phosphonoethenyl)-4-(hydroxy)-2,3-*O*-(isopropylidene)-bicyclo[3.1.0]hexane (34)

Compound **31** (30 mg, 0.083 mmol) was dissolved in MeOH (3 mL). 10% Pd/C (25 mg) and H_2 (3 bar) was added. After stirring the mixture for 17 h, the catalyst was removed by filtration through a Celite pad, which was washed with MeOH (40 mL), and the filtrate was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–90% acetone in petroleum ether, v/v) to afford nucleoside **34** (22 mg, 72%) as white solid material. R_f = 0.3 (5% MeOH in EtOAc, v/v); ESI-HRMS *m/z* 363.1933 ($[M + H]^+$, $C_{17}H_{31}O_6P{\cdot}H^+$: Calcd. 363.1937); ^{1}H NMR ($CDCl_3$) δ 4.61–4.76 (m, 2H), 4.43–4.54 (m, 2H), 4.17–4.35 (m, 1H), 2.32 (d, *J* = 9.8 Hz, 1H), 1.43–1.93 (m, 8H), 1.22–1.37 (m, 15H), 1.07–1.14 (m,1H), 0.47–0.56 (m, 1H).

(1'S,2'R,3'S,4'R,5'S)-4'-(2,6-Dichloropurin-9-yl)-1'-(diisopropyl-phosphonoethenyl)-2',3'-*O*-(isopropylidene)-bicyclo[3.1.0]hexane (35)

Diisopropyl azodicarboxylate (93 μL, 0.47 mmol) was added at rt to a mixture of triphenylphosphine (123 mg, 0.47 mmol) and 2,6-dichloropurine (89 mg, 0.47 mmol) in anhydrous THF (4 mL). After stirring for 30 min, a solution of the compound **34** (85 mg, 0.24 mmol) in THF (4 mL) was added. After stirring for 65 h, the reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–5% MeOH in EtOAc, v/v) to afford nucleoside **35** (50 mg, 40%) as a white solid material. R_f = 0.4 (5% MeOH in EtOAc, v/v); ESI-HRMS *m/z* 533.1497 ($[M + H]^+$, $C_{22}H_{31}Cl_2N_4O_5P{\cdot}H^+$: Calcd. 533.1487); ^{1}H NMR ($CDCl_3$) δ 8.09 (s, 1H), 5.20 (d, 1H, *J* = 7.2 Hz), 4.86 (s, 1H), 4.73 (d, 1H, *J* = 7.2 Hz), 4.63–4.73 (m, 2H), 2.25–2.44 (m, 1H), 1.79–2.09 (m, 2H), 1.58–1.70 (m, 1H), 1.52 (s, 3H), 1.43–1.52 (m, 1H), 1.28–1.35 (m, 12H), 1.24 (s, 3H), 1.04–1.11 (m,1H), 0.78–0.87 (m, 1H).

(1'S,2'R,3'S,4'R,5'S)-4'-(6-Amino-2-chloropurin-9-yl)-1'-(diisopropyl-phosphonoethenyl)-2',3'-*O*-(isopropylidene)-bicyclo[3.1.0]hexane (36)

Nucleoside **35** (50 mg, 0.094 mmol) was treated with 2 M NH_3 in *i*-PrOH (5 mL) and heated to 70 °C. After stirring for 19 h, the reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–10% MeOH in CH_2Cl_2, v/v) to afford nucleoside **36** (34 mg, 71%) as a white solid material. R_f = 0.4 (8% MeOH in EtOAc, v/v); ESI-HRMS *m/z* 514.1978 ($[M + H]^+$, $C_{22}H_{33}ClN_5O_5P{\cdot}H^+$: Calcd. 514.1986); ^{1}H NMR ($CDCl_3$) δ 7.73 (s, 1H), 5.84 (s, 2H), 5.20 (d, 1H, *J* = 6.5 Hz), 4.76 (s, 1H), 4.72 (d, 1H, *J* = 6.5 Hz), 4.62–4.71 (m, 2H), 2.24–2.40 (m, 1H), 1.75–2.08 (m, 1H), 1.56–1.74 (m, 5H), 1.40–1.47 (m, 1H), 1.28–1.35 (m, 12H), 1.24 (s, 3H), 1.01–1.06 (m,1H), 0.74–0.82 (m, 1H).

(1'S,2'R,3'S,4'R,5'S)-4-(6-Amino-2-chloropurin-9-yl)-2',3'-(dihydroxy)-1'-(phosphonoethenyl)-bicyclo[3.1.0]hexane (9)

Nucleoside **23** (25 mg, 0.049 mmol) was coevaporated with anhydrous toluene (3 × 4 mL) and dissolved in anhydrous CH_2Cl_2 (4 mL). Iodotrimethylsilane (70 μl, 0.49 mmol) was added. After stirring for 19 h, the reaction mixture was cooled to 0 °C followed by the addition of ice-cold H_2O (25 mL) and CH_2Cl_2 (25 mL). The phases were separated, and the aqueous phase was washed with CH_2Cl_2 (1 × 35 mL) and diethyl ether (3 × 35 mL). The resulting aqueous phase was evaporated to dryness and purified by HPLC (retention time: 21.5 min) to afford **9** (8.5 mg) and **10** (1.3 mg, 53%, combined yield) as a white solid materials. ESI-HRMS *m/z* 388.0574 $[M - H]^-$, $C_{13}H_{16}ClN_5O_5P^-$: Calcd. 388.0578); ^{1}H NMR (D_2O) δ 8.15 (s, 1H), 4.74 (s, 1H), 4.61 (d, 1H, *J* = 7.2 Hz), 4.11 (d, 1H, *J* = 7.2 Hz), 3.21 (q, 3H, *J* = 7.2 Hz), 1.70–2.11

(m, 4H), 1.63–1.71 (m, 1H), 1.33–1.38 (m, 1H), 1.29 (t, 3H, *J* = 7.2 Hz), 0.81–0.91 (m, 1H). ^{31}P NMR (D_2O) δ 28.26. Purity >99% by HPLC (retention time: 4.6 min)

(1S,2R,3S,4S,5S)-1-Bromomethyl-2,3-*O*-(isopropylidene)-4-*O*-(*tert*-butyldimethylsilyl)-bicyclo[3.1.0]hexane (37)

Compound **15** (0.30 g, 0.69 mmol) was coevaporated with anhydrous toluene (3 × 10 mL) and dissolved in anhydrous CH_2Cl_2 (8 mL). CBr_4 (0.46 g, 1.36 mmol) triphenylphosphine (0.36 g, 1.36 mmol), and triethylamine (0.3 mL, 2.07 mmol) were added. After stirring for 17 h, the reaction mixture was diluted with CH_2Cl_2 (50 mL) and sat. aqueous NaCl (25 mL). The phases were separated and aqueous phase was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic phase was evaporated to dryness and the resulting residue was purified by silica gel column chromatography (0–20% EtOAc in petroleum ether, v/v) to afford compound **37** (0.28 g, 81%) as a colorless oil. R_f = 0.8 (50% EtOAc in petroleum ether, v/v); ESI-HRMS *m/z* 523.1296 ($[M + Na]^+$, $C_{26}H_{33}BrO_3Si{\cdot}Na^+$: Calcd. 523.1280); ^{1}H NMR ($CDCl_3$) δ 7.67.77 (m, 4H, Ph), 7.30–7.45 (m, 6H, Ph), 4.77 (d, 1H, *J* = 6.5 Hz), 4.44 (t, 1H, *J* = 6.5 Hz), 4.08 (t, 1H, *J* = 6.5 Hz), 3.76 (d, 1H, *J* = 10.5 Hz), 3.13 (d, 1H, *J* = 10.5 Hz), 1.80–1.87 (m, 1H), 1.60–1.70 (m, 1H), 1.55 (s, 3H), 1.21 (s, 3H), 1.05 (s, 9H), 0.71–0.86 (m, 1H).

(1S,2R,3S,4S,5S)-1-*C*-(Ethoxymethylphosphinyl)-2,3-*O*-(isopropylidene)-4-*O*-(*tert*-butyldimethylsilyl)-bicyclo[3.1.0]hexane (38)

Compound **37** (0.28 g, 0.56 mmol) was dissolved in diethylmethylphosphite (4 mL) and heated up to 110 °C. After stirring for 17 h, the reaction mixture was cooled to rt and evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–90% EtOAc in petroleum ether, v/v) to afford inseparable diastereomeric mixture of compound **38** (0.28 g, 95%) as a colorless oil. R_f = 0.6 (5% MeOH in EtOAc, v/v); ESI-HRMS *m/z* 529.2532 ($[M + H]^+$, $C_{29}H_{41}O_5PSi{\cdot}H^+$: Calcd. 529.2539); ^{1}H NMR ($CDCl_3$) δ 7.68–7.77 (m, 6.8H, Ph), 7.29–7.46 (m, 10.2H, Ph), 4.75 (d, 0.7H, *J* = 6.5 Hz), 4.68 (d, 1H, *J* = 6.5 Hz), 4.43–4.49 (m, 1.7H), 3.87–4.14 (m, 5.1 H), 1.73–1.92 (m, 3.4H), 1.62 (s, 5.1H), 1.57–1.60 (m,1.7H), 1.48 (d, 3H, *J* = 3.4 Hz), 1.44 (d, 2.1H, *J* = 3.4 Hz), 1.26 (t, 3H, *J* = 7.2 Hz), 1.22 (t, 2.1H, *J* = 7.2 Hz), 1.19 (s, 2.1H), 1.18 (s, 3H), 1.09–1.13 (m, 1.7H), 1.07 (s, 15.3H), 0.52–0.69 (m, 1H).

(1S,2R,3S,4S,5S)-1-*C*-(Ethoxymethylphosphinyl)-4-hydroxy-2,3-*O*-(isopropylidene)-bicyclo [3.1.0]hexane (39)

Compound **38** (0.30 g, 0.57 mmol) was dissolved in THF (10 mL) and tetrabutylammonium fluoride (1.0 M in THF, 1.70 mL, 1.70 mmol) was added. After stirring for 21 h, the reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–15% MeOH in EtOAc, v/v) to afford an inseparable diastereomeric mixture of compound **39** (0.15 g, 91%) as a colorless oil. R_f = 0.2 (15% MeOH in CH_2Cl_2, v/v); ESI-HRMS *m/z* 291.1366 ($[M + H]^+$, $C_{13}H_{23}O_5P{\cdot}H^+$: Calcd. 291.1361); ^{1}H NMR ($CDCl_3$) δ 4.89–5.03 (m, 2H), 4.50–4.60 (m, 4H), 3.97–4.14 (m, 4H), 2.34–2.40 (m, 2H), 1.95 (t, 2H, *J* = 7.7 Hz), 1.90 (t, 2H, *J* = 7.7 Hz),1.78–1.87 (m, 2H), 1.66 (s, 6H), 1.55 (d, 3H, *J* = 3.9 Hz), 1.50 (d, 3H, *J* = 3.9 Hz), 1.29–1.35 (m, 6H), 1.28 (s, 6H), 1.23–1.26 (m, 2H), 0.60–0.71 (m, 2H).

(1'S,2'R,3'S,4'R,5'S)-1'-*C*-(Ethoxymethylphosphinyl)-4'-(2,6-dichloropurin-9-yl)-2',3'-*O*-(isopropylidene)-bicyclo[3.1.0]hexane (40)

Diisopropyl azodicarboxylate (360 μL, 1.82 mmol) was added at rt to a mixture of triphenylphosphine (0.48 g, 1.82 mmol) and 2,6-dichloropurine (0.35 g, 1.82 mmol) in anhydrous THF (5 mL). After stirring for 30 min, a solution of the compound **39** (0.27 g, 0.91 mmol) in THF (5 mL) was added. After stirring for 60 h, the reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–10%

MeOH in EtOAc, v/v) to afford inseparable diastereomeric mixture of nucleoside **40** (0.25 mg, 60%) as a white solid material. R_f = 0.2 (10% MeOH in EtOAc, v/v); ESI-HRMS *m/z* 461.0899 ([M + H]$^+$, $C_{18}H_{23}Cl_2N_4O_4P{\cdot}H^+$: Calcd. 461.0912); ^{1}H NMR ($CDCl_3$) δ 8.79 (s, 0.5H), 8.51 (s, 1H), 5.45 (d, 0.5H, *J* =7.2 Hz), 5.32 (d, 1H, *J* = 7.2 Hz), 5.06 (s, 0.5H), 4.96 (s, 1H), 4.66 (d, 1.5H, *J* = 7.2 Hz), 3.98–4.21 (m, 3H), 2.40–2.51 (m, 0.5H), 2.15–2.24 (m, 1H), 1.93–2.11 (m, 1.5H), 1.63–1.75 (m, 0.5H), 1.61 (s, 5.5H), 1.59 (d, 3H, *J* = 3.9 Hz), 1.55 (d, 1.5H, *J* = 3.9 Hz), 1.35 (t, 3H, *J* = 7.2 Hz), 1.25 (t, 1.5H, *J* = 7.2Hz), 1.24 (s, 4.5H), 1.18–1.23 (m, 1.5H), 0.95–1.13 (m, 1.5H).

(1'S,2'R,3'S,4'R,5'S)-4'-(6-Amino-2-chloropurin-9-yl)-1'-*C*-(ethoxymethylphosphinyl)-2',3'-*O*-(isopropylidene)-bicyclo[3.1.0]hexane (41)

Nucleoside **40** (0.20 g, 0.44 mmol) was treated with 2M NH_3 in *i*-PrOH (8 mL) and heated to 70 °C. After stirring for 15 h, the reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–7% MeOH in CH_2Cl_2, v/v) to afford nucleoside **41** (150 mg, 79%) as a white solid material. R_f = 0.4 (10% MeOH in CH_2Cl_2, v/v); ESI-HRMS *m/z* 442.1416 ([M + H]$^+$, $C_{18}H_{26}ClN_5O_4P{\cdot}H^+$: Calcd. 442.1411); ^{1}H NMR ($CDCl_3$) δ 8.21 (s, 0.5H), 8.00 (s, 1H), 5.96 (s, 3H), 5.40 (d, 0.5H, *J* = 6.5 Hz), 5.30 (d, 1H, *J* = 6.5 Hz), 4.91 (s, 0.5H), 4.81 (s, 1H), 4.62–4.71 (d, 1.5H, *J* = 7.2 Hz), 4.10–4.22 (m, 2H), 3.98–4.09 (m, 1H), 3.60–3.80 (m, 1H), 2.64 (t, 1H, *J* = 15.3 Hz), 2.35 (t, 0.5H, *J* = 15.3 Hz), 2.01–2.17 (m, 0.5H), 1.87 (t, 1.5H, *J* = 15.8 Hz), 1.75 (s, 4.5H), 1.55–1.69 (m, 4.5H), 1.36 (t, 3H, *J* = 7.2 Hz), 1.25 (t, 1.5H, *J* = 7.2Hz), 1.24 (s, 4.5H), 1.17–1.22 (m, 1.5H), 0.96–1.07 (m, 1.5H).

Biological evaluation

CSQ mice and compound administration

Mice displaying the CSQ model of severe cardiomyopathy and heart failure were bred and maintained according to a previously described method (7). The CSQ transgenic (TG) mice were originally provided by Dr. Larry Jones[10,11] and developed hypertrophy followed by a lethal heart failure phenotype with death near the age of 3 months.

Compound **3** and its analogues were dissolved in phosphate-buffered saline, pH=7.4 at 3.3 μM (200 μL total volume), filtered for sterility for *in vivo* administration at 6 μL per day for 28 days via a mini-osmotic pump (Alzet) in the CSQ mice. Intact heart function in vivo was assessed by echocardiography following infusion of nucleotide or vehicle.

Mouse echocardiography

Transthoracic echocardiography was performed using a linear 30-MHz transducer according to manufacturer's instructions (Vevo 660 High Resolution Imaging System from VisualSonics, Toronto, Canada) similar to previously described methods.[18,19] Two dimensional-targeted M-mode echocardiographic measurements were carried out at mid-papillary muscle level. Mice were anesthetized with 1% isoflurane using a vaporizer as previously described.[20] Left ventricular end-diastolic (LVEDD) and end-systolic (LVESD) diameters, and FS (defined as LVEDD-LVESD/LVEDD) were measured. Parameters were measured digitally on the M-mode tracings and were averaged from more than 3 cardiac cycles.

Activation of human $P2Y_1$ receptors

Activity at the h$P2Y_1$ receptor was quantified in 1321N1 human astrocytoma cells stably expressing this receptor, obtained from Prof. T. K. Harden, University of North Carolina School of Medicine, Chapel Hill, NC. The procedure for measuring intracellular calcium using a FLIPR in response to nucleotide derivatives is similar to that described by Mamedova et al.[28] Cells were grown overnight in 100 ml of medium in 96-well flatbottom plates at 37 °C at

5%CO_2 or until they reached ~80% confluency. The calcium-4 assay kit (Molecular Devices, Sunnyvale, CA) was used as directed with no washing of cells. Cells were loaded with 40 mL dye with probenecid in each well and incubated for 1 h at rt. The compound plate was prepared with dilutions of various compounds in Hank's Buffer at pH 7.2. Samples were run in duplicate with a FLIPR-Tetra (Molecular Devices) at rt. Cell fluorescence (excitation = 485 nm; emission = 525 nm) was monitored following exposure to a compound. Increases in intracellular calcium are reported as the maximum fluorescence value after exposure minus the basal fluorescence value before exposure.

Data analysis

Unless otherwise indicated, data were provided as mean ± standard error of the mean. For analysis of multiple groups, one-way ANOVA and posttest comparison were used. Student's t-test for paired or unpaired samples was used to evaluate the effects of experimental interventions; $P<0.05$ was taken as statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

5'-AMP	adenosine 5'-monophosphate
CSQ	calsequestrin
DIBAL-H	diisobutylaluminium hydride
DMEM	Dulbecco's modified Eagle medium
FS	fractional shortening
HEPES	*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectroscopy
LV	left ventricular
LVEDD	left ventricular end-diastolic diameter
LVESD	left ventricular end-systolic diameter
NS	normal saline
PLC	phospholipase C
SAR	structure activity relationship
TBAF	tetrabutylammonium fluoride
TBAP	tetrabutylmmonium dihydrogen phosphate
TBDPS-Cl	*tert*-butyl(chloro)diphenylsilane
THF	tetrahydrofuran
TEAA	triethylammonium acetate

Acknowledgments

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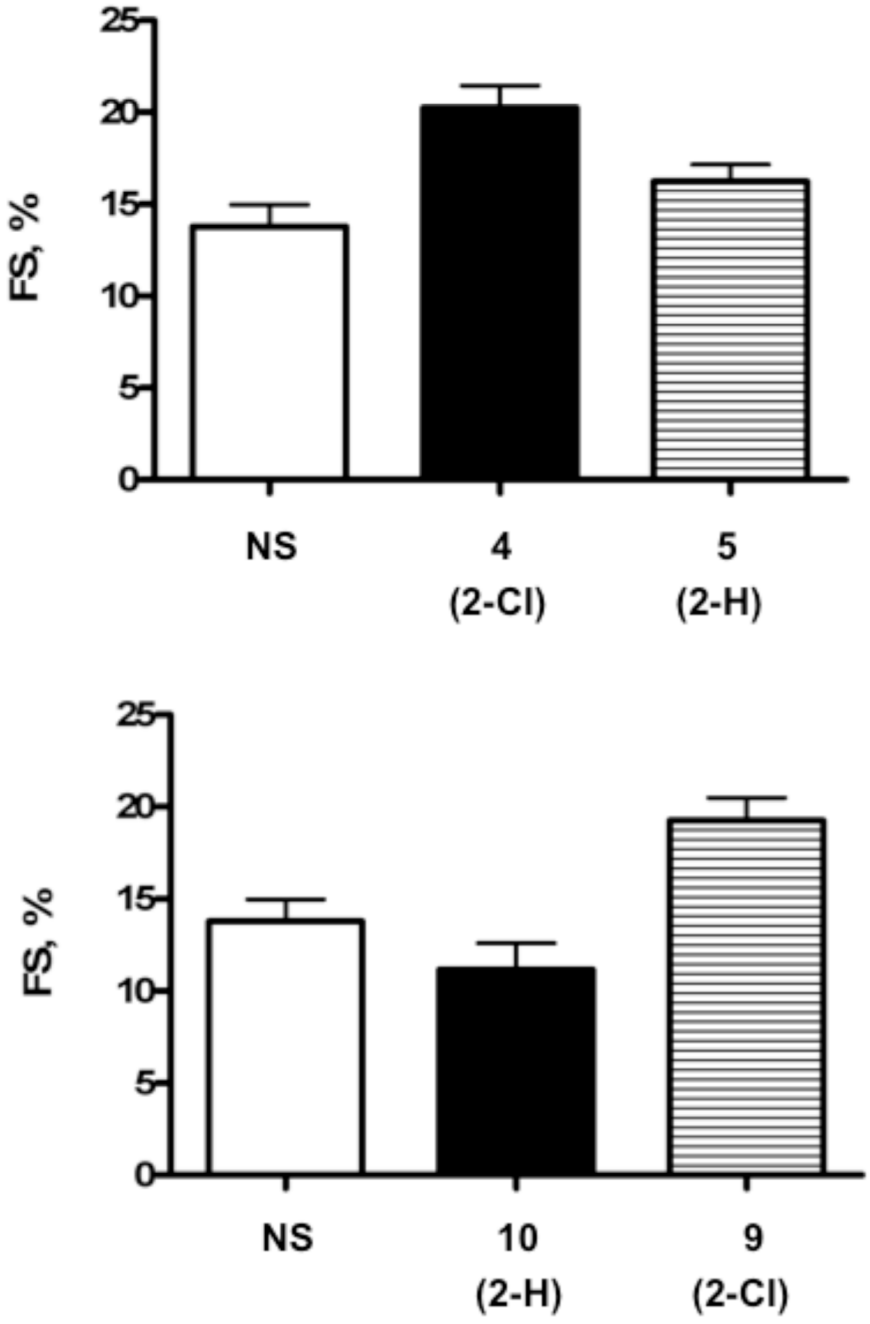


Figure 1.

Beneficial effects of 2-Cl substituted phosphonate derivatives of (N)-methanocarba AMP in heart failure mice. Various derivatives of phosphonates were dissolved in sterile NS at 3.3 μM and were infused subcutaneously individually via an Alzet minipump in CSQ mice as described in Methods. After 28 days of infusion, the *in vivo* heart function was assessed using echocardiography-derived FS. (A) The 2-Cl substituted 5'-phosphonate derivative **4** was able to improve *in vivo* cardiac contractile performance in heart failure mice as compared to normal saline-treated heart failure animals (one-way ANOVA with posttest comparison, $P<0.05$) while the unsubstituted **5** was ineffective ($P>0.05$). (B) Similarly, 2-Cl substituted higher

homologue **9** was able to enhance cardiac contractile function ($P<0.05$), while the parent unsubstituted **10** did not improve the contractile function ($P>0.05$). Data were mean ± SE.

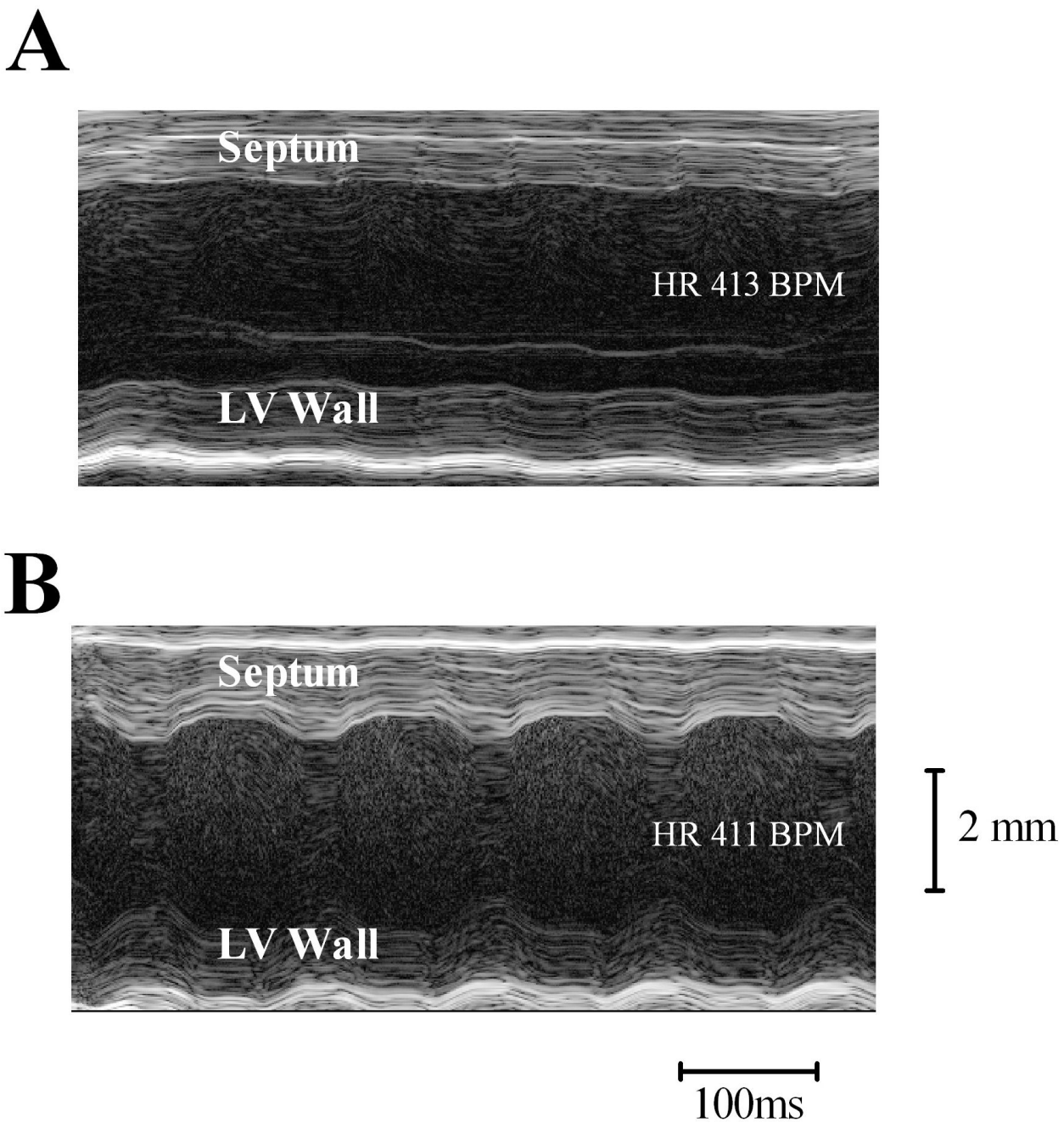


Figure 2.

Chronic infusion of compound **4** resulted in improved echocardiographically derived FS in CSQ heart failure mice. Following chronic subcutaneous infusion of NS or compound **4**, two-dimensional directed M-mode echocardiography was carried out as described in Methods. The heart rate (HR) is indicated on each figure. Representative M-mode echocardiography was shown for a CSQ animal infused with NS (A) and for a CSQ mouse infused with compound **4** (B). A heart from the NS-infused mice showed less shortening of both septum and LV free wall than did compound **4**-infused mice.

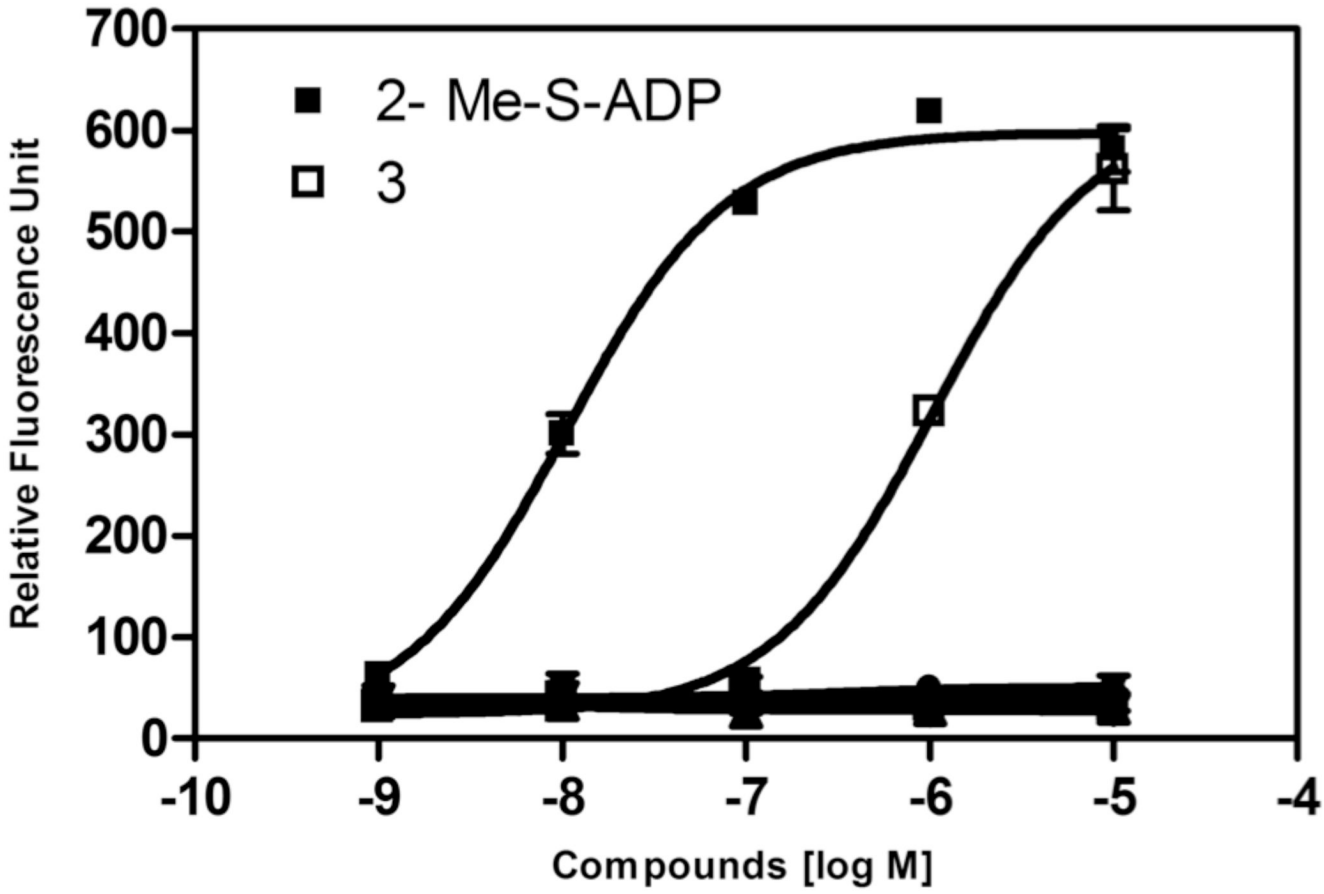
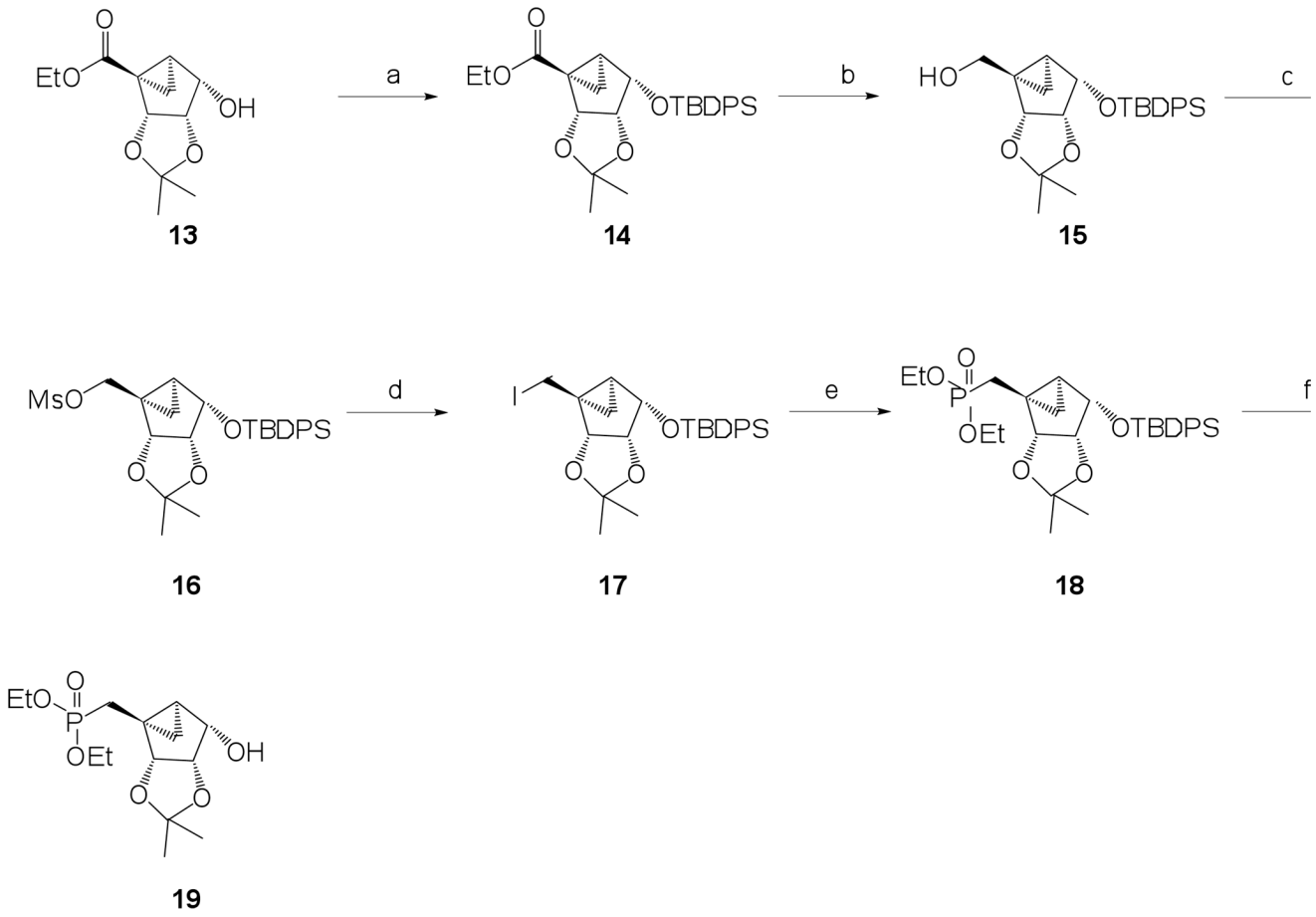


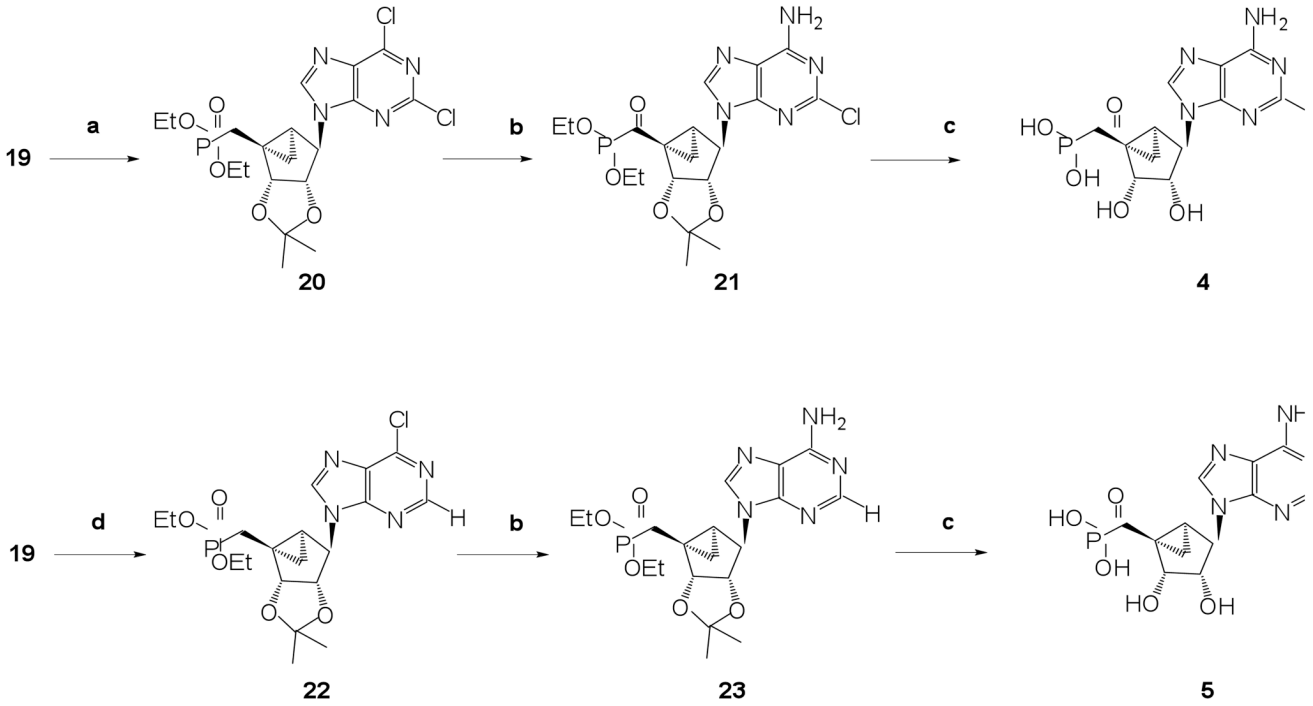
Figure 3.

Changes in intracellular calcium in 1321N1 human astrocytoma cells stably expressing the hP2Y$_1$ receptor. Fluorescence in response to a known hP2Y$_1$ receptor agonist 2-MeSADP (EC$_{50}$ 10.3±0.4 nM), compound **3** (EC$_{50}$ 722±55 nM), or the phosphonate analogues (compounds **4 – 11**, all inactive at 10 μM) was quantified using a FLIPR-Tetra.



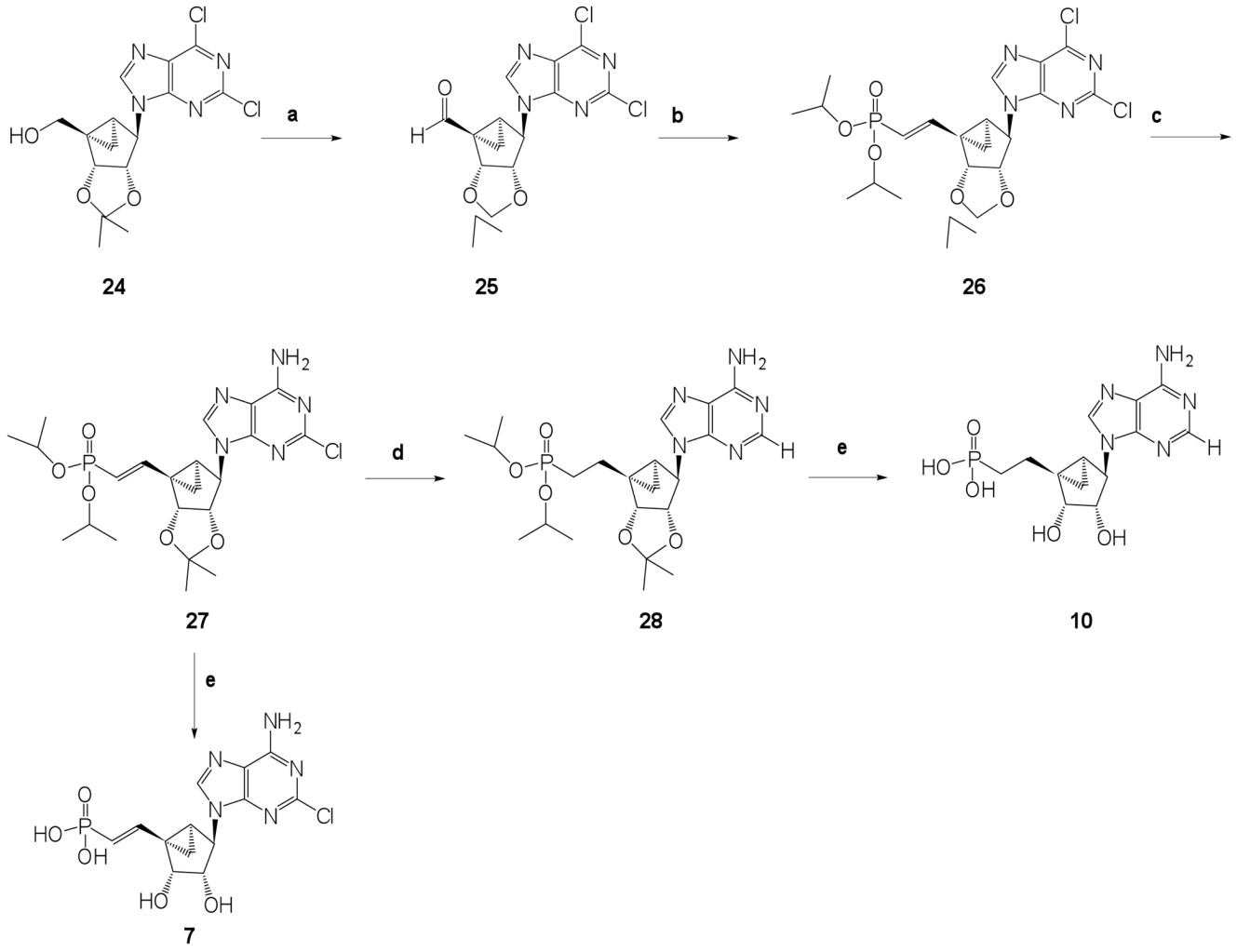
Scheme 1.

Reagents and Conditions. a) *tert*-Butylchlorodiphenylsilane, imidazole, DMAP, an. CH_2Cl_2, 93%; b) DIBAL-H, an. THF, 82 %; c) Methanesulfonyl chloride, triethylamine, an. CH_2Cl_2, 96%; d) NaI, 65 °C, an. 1,4-dioxane, 95%; e) Triethylphosphite, 94%; f) TBAF, THF, 88%.



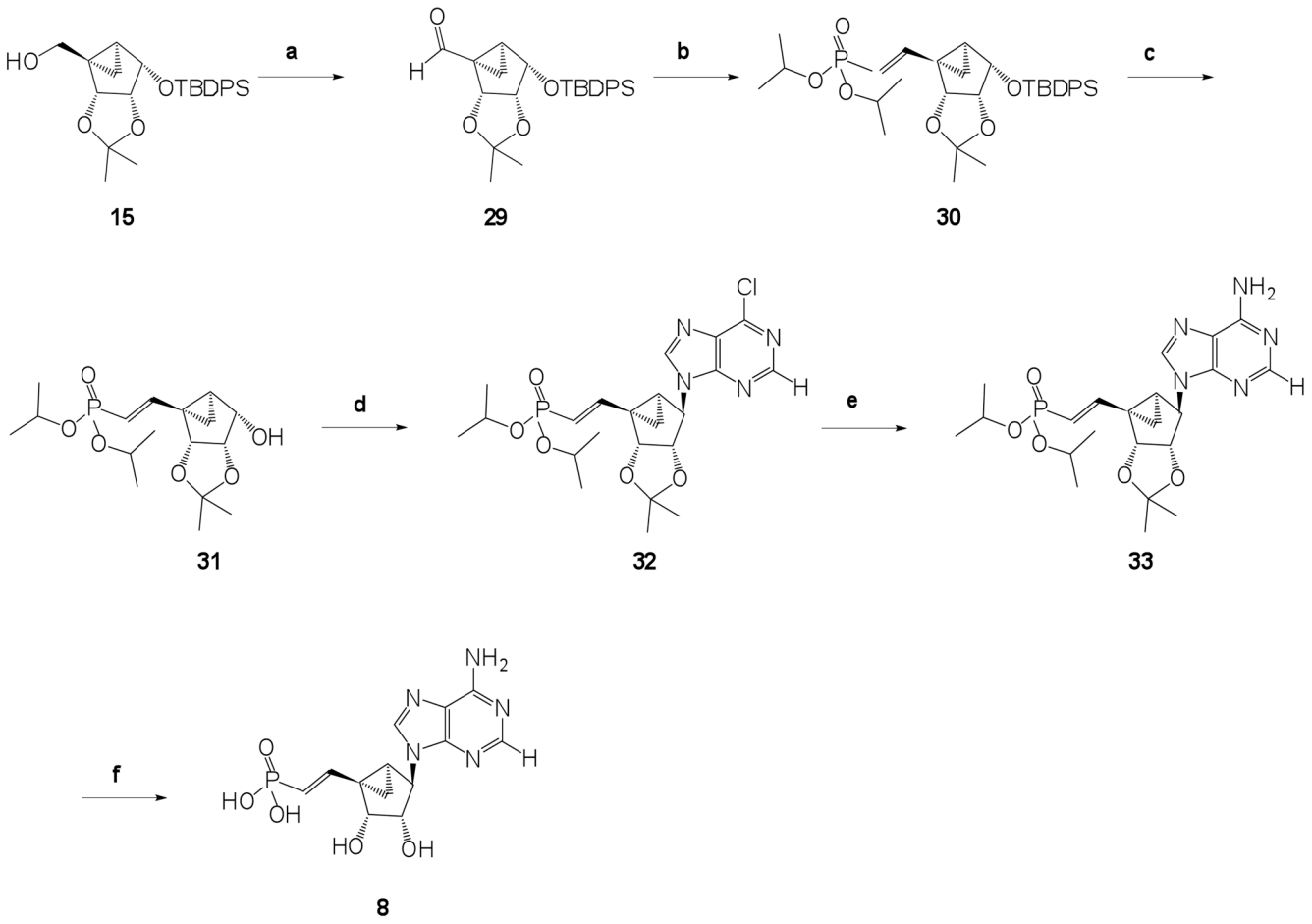
Scheme 2.

Reagents and Conditions. a) Triphenylphosphine, 2,6-dichloropurine, diisopropyl azodicarboxylate, an. THF, 75%; b) 2 M NH_3 in *i*-PrOH, 70 °C, 80% for **9**, 79% for **11**; c) Iodotrimethylsilane, an. CH_2Cl_2, 23% for **4** and 27% for **5**; d) Triphenylphosphine, 6-chloropurine, diisopropyl azodicarboxylate, an. THF, 87%.



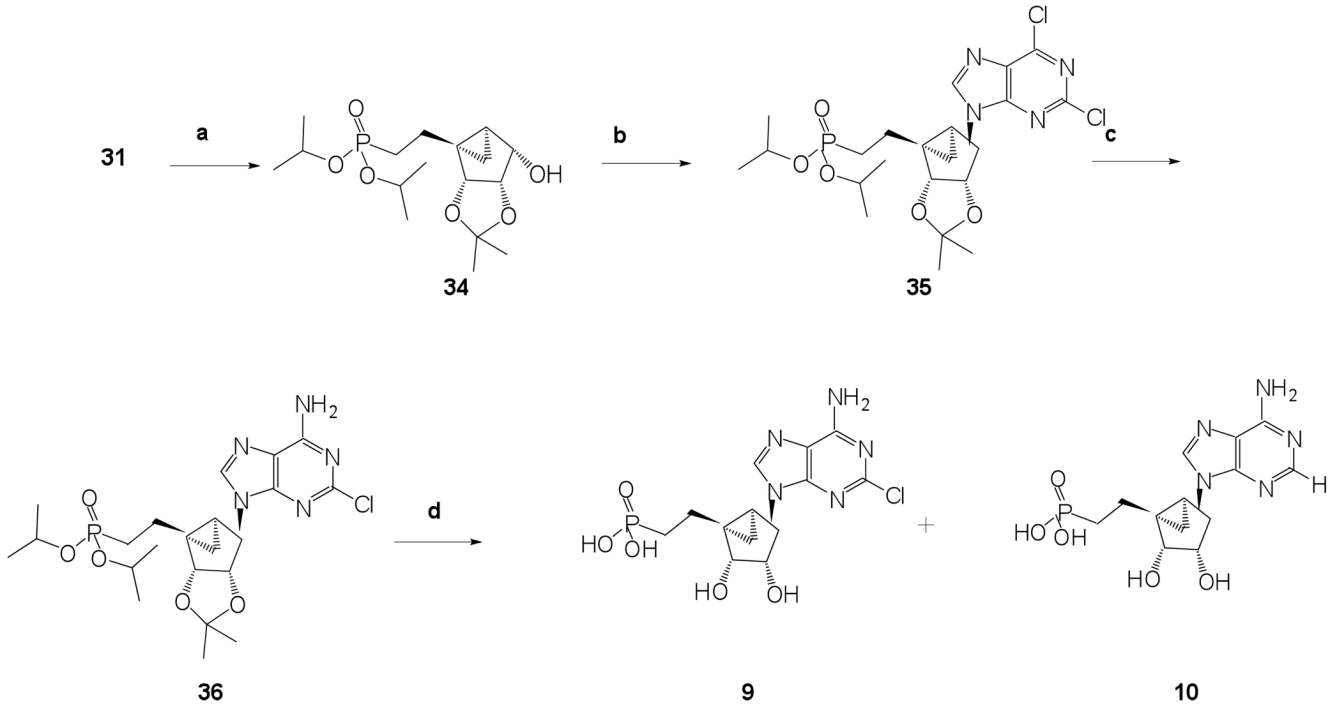
Scheme 3.

Reagents and Conditions. a) Dess-Martin periodinane, an. CH_2Cl_2, 80%; b) Tetraisopropyl methylenediphosphonate, NaH, an. THF, 83%; c) 2 M NH_3 in *i*-PrOH, 70 °C, 80%; d) 10% Pd/C, H_2 (3 bar), MeOH:2M aq. NaOH (1:1, v/v), 79%; e) Iodotrimethylsilane, an. CH_2Cl_2, 47% for **10** and 28% for **7**.



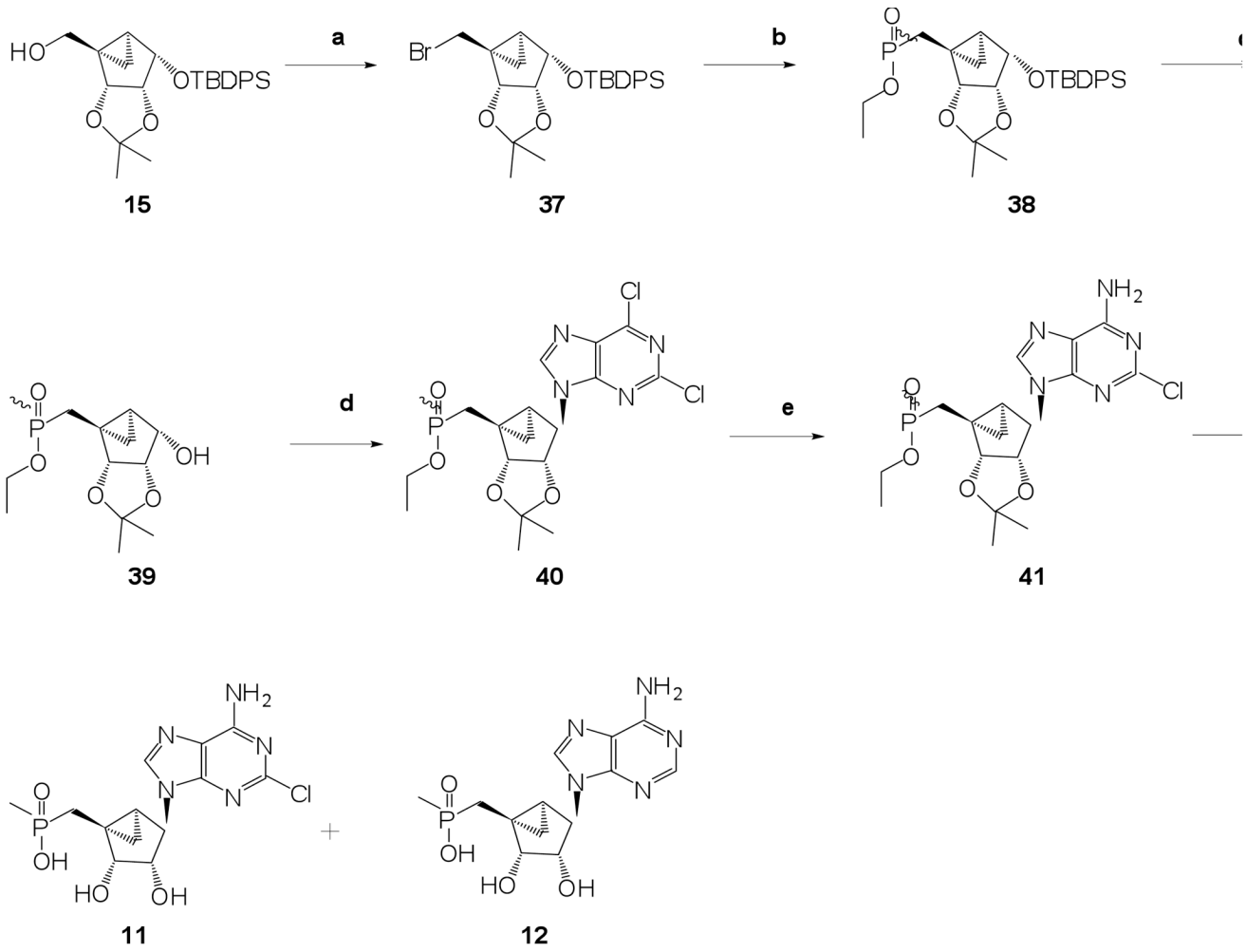
Scheme 4.

Reagents and Conditions. a) Dess-Martin periodinane, an. CH_2Cl_2, 72%; b) Tetraisopropyl methylenediphosphonate, NaH, an. THF, 48%; c) TBAF, THF, 88%; d) Triphenylphosphine, 6-chloropurine, diisopropyl azodicarboxylate, an. THF, 85%; e) 2 M NH_3 in *i*-PrOH, 70 °C, 85%; f) Iodotrimethylsilane, an. CH_2Cl_2, 78%.



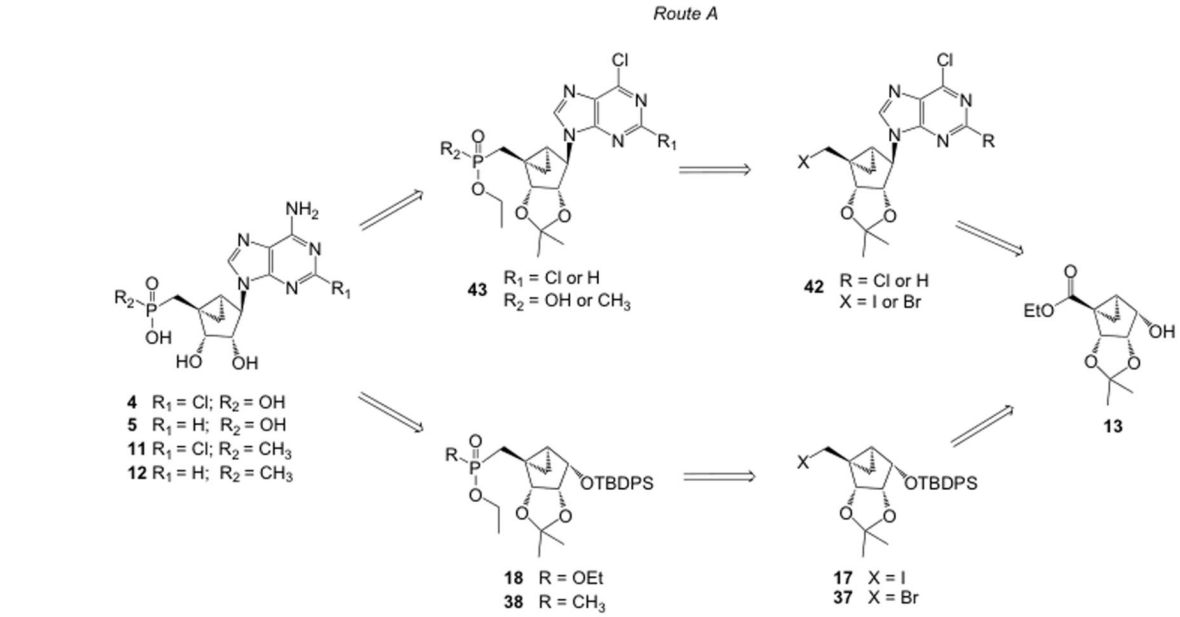
Scheme 5.

Reagents and Conditions. a) 10% Pd/C, H_2 (3 bar), MeOH, 72%; b) Triphenylphosphine, 2,6-dichloropurine, diisopropyl azodicarboxylate, an. THF, 40%; c) 2 M NH_3 in *i*-PrOH, 70 °C, 71%; d) Iodotrimethylsilane, an. CH_2Cl_2, 45% combined yield.

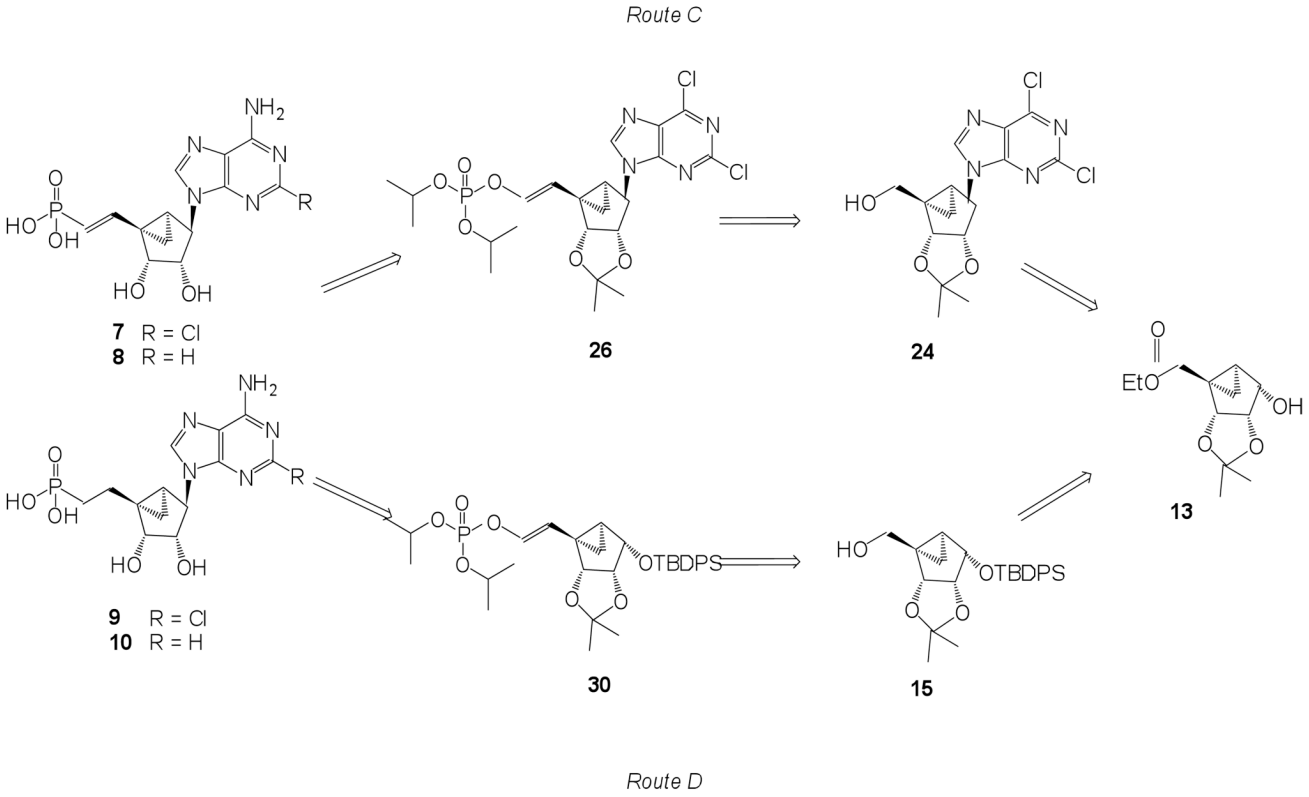


Scheme 6.

Reagents and Conditions. a) CBr_4, triphenylphosphine, triethylamine, 81%; b) Diethylmethylphosphite, 110 °C, 95%; c) TBAF, THF, 91%, d) Triphenylphosphine, 2,6-dichloropurine, diisopropyl azodicarboxylate, an. THF, 75%; e) 2 M NH_3 in *i*-PrOH, 70 °C, 60%; f) Iodotrimethylsilane, an. CH_2Cl_2, 20% combined yield.



Scheme 7B



Scheme 7.

A) Retrosynthetic analysis of 5’-phosphonate and 5’-methyl phosphonates of (N)-methanocarba adenine or 2-Cl adenine derivatives. Use of route B, in which the phosphonate was introduced prior to the nucleobase, proved successful. B) Retrosynthetic analysis of saturated and unsaturated long chain 5’-phosphonates of (N)-methanocarba adenine or 2-Cl adenine derivatives. Use of the shorter synthetic route C, in which the nucleobase was introduced prior to the phosphonate, proved successful.

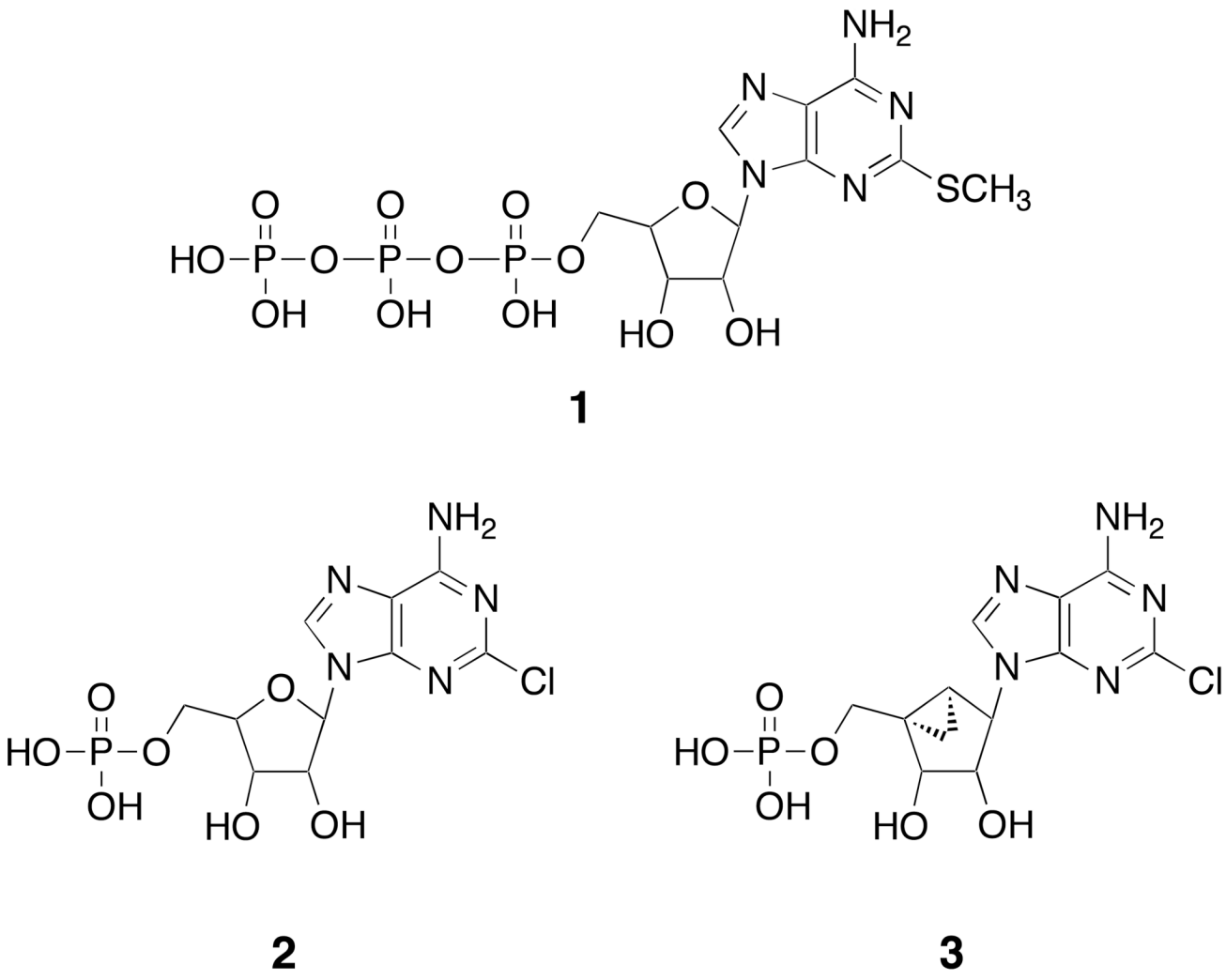
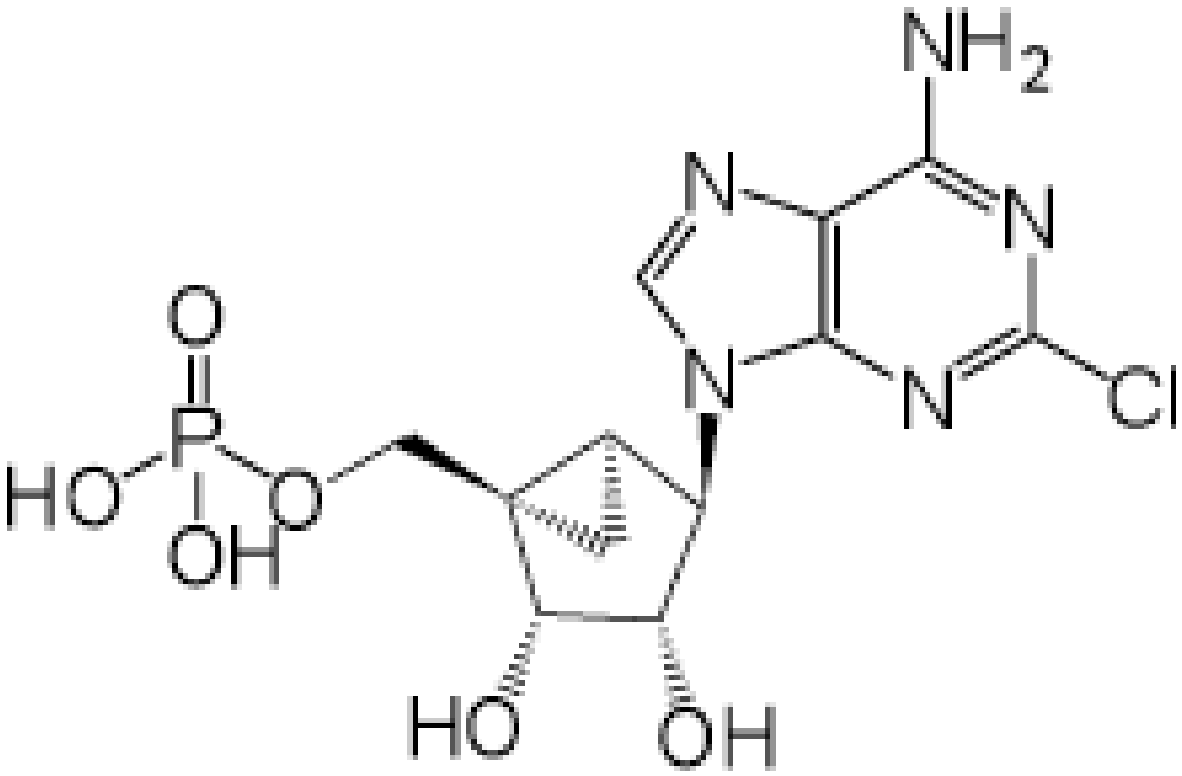
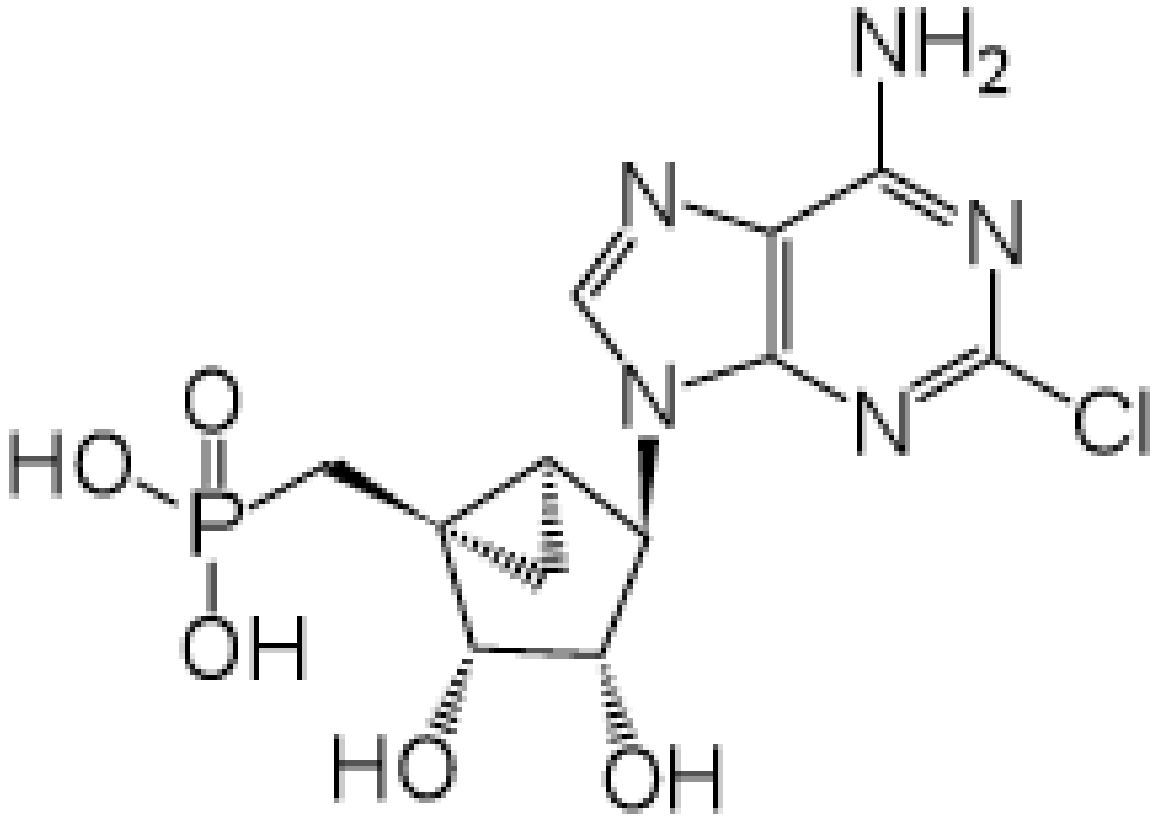
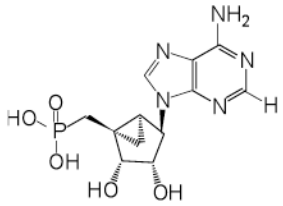


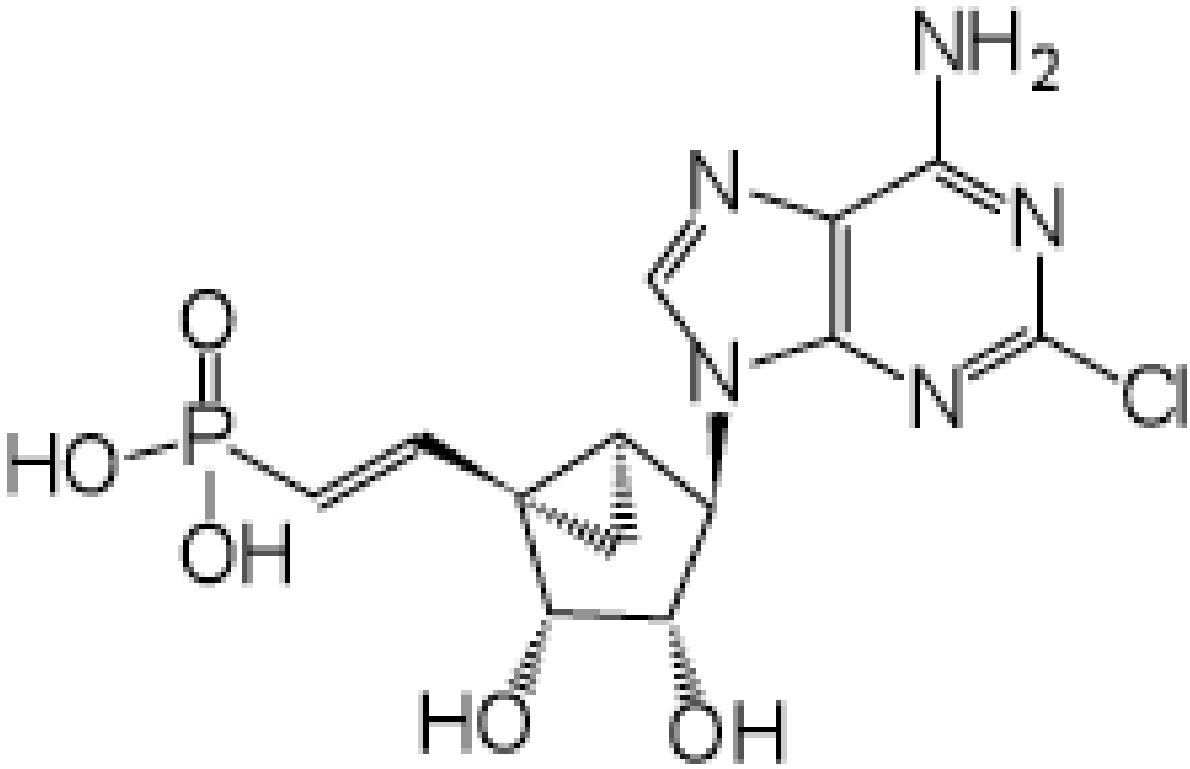
Chart 1.
Representative adenine nucleotide derivatives that display activity at various P2 receptors. Compound **3** served as the lead compound for the present set of phosphonate analogues.

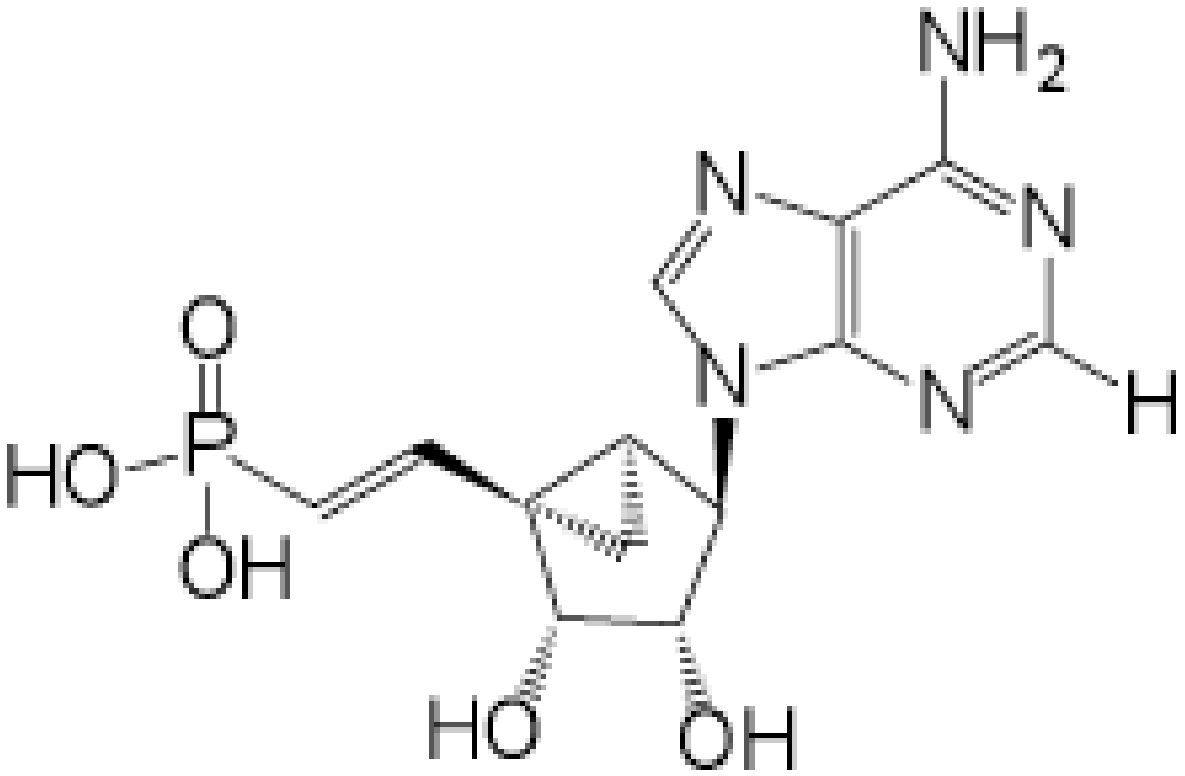
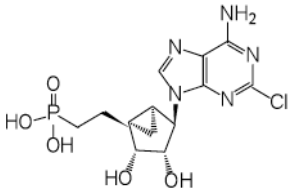
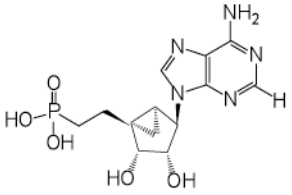
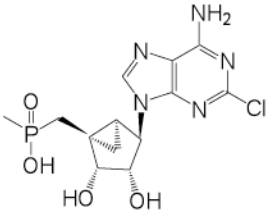
Table 1

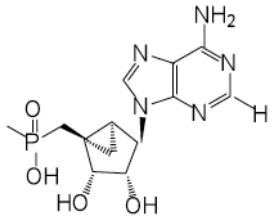
Phosphonate analogues: structure and effects on *in vivo* heart function as determined by echocardiography-derived FS in CSQ heart failure mice.

No	Structure	FS in % in CSQ Mice[a]
3	 MRS 2339	15.47 ± 1.15

No	Structure	FS in % in CSQ Mice[a]
4		20.25 ±1.19
5		16.23 ± 0.93

No	Structure	FS in % in CSQ Mice[a]
7		12.12 ±1.2

No	Structure	FS in % in CSQ Mice[a]
8		13.88 ± 2.12
9		19.26 ± 1.23
10		11.15 ±1.44
11		15.0 ± 1.2

No	Structure	FS in % in CSQ Mice[a]
12		ND

[a] at 3.3 μM. The vehicle control mice displayed a %FS of 13.78 ± 1.19% (n=16).

ND – not determined.