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Acyclic Analogues of Deoxyadenosine 3′,5′-Bisphosphates as $P2Y_1$ Receptor Antagonists

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Abstract

$P2Y_1$ receptors are activated by ADP and occur on endothelial cells, smooth muscle, epithelial cells, lungs, pancreas, platelets, and in the central nervous system. With the aid of molecular modeling, we have designed nucleotide analogues that act as selective antagonists at this subtype. The present study has tested the hypothesis that acyclic modifications of the ribose ring, proven highly successful for nucleoside antiviral agents such as gancyclovir, are generalizable to P2Y receptor ligands. Specifically, the binding site of the $P2Y_1$ receptor was found to be sufficiently accommodating to allow the substitution of the ribose group with acyclic aliphatic and aromatic chains attached to the 9-position of adenine. Three groups of adenine derivatives having diverse side-chain structures, each containing two symmetrical phosphate or phosphonate groups, were prepared. Biological activity was demonstrated by the ability of the acyclic derivatives to act as agonists or antagonists in the stimulation of phospholipase C in turkey erythrocyte membranes. An acyclic N^6-methyladenine derivative, 2-[2-(6-methylaminopurin-9-yl)-ethyl]-propane-1,3-bisoxy(diammoniumphosphate) (**10**), containing an isopentyl bisphosphate moiety, was a full antagonist at the $P2Y_1$ receptor with an IC_{50} value of 1.60 μM. The corresponding 2-Cl derivative (**11**) was even more potent with an IC_{50} value of 0.84 μM. Homologation of the ethylene group at the 9-position to 3–5 methylene units or inclusion of

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cis- or *trans*-olefinic groups greatly reduced antagonist potency at the $P2Y_1$ receptor. Analogues containing a diethanolamine amide group and an aryl di(methylphosphonate) were both less potent than **10** as antagonists, with IC_{50} values of 14 and 16 μM, respectively, and no agonist activity was observed for these analogues. Thus, the ribose moiety is clearly not essential for recognition by the turkey $P2Y_1$ receptor, although a cyclic structure appears to be important for receptor activation, and the acyclic approach to the design of P2 receptor antagonists is valid.

Introduction

P2 receptors, which are activated by purine and/or pyrimidine nucleotides, consist of two families: G protein-coupled receptors termed P2Y, of which five mammalian subtypes have been cloned, and ligand-gated cation channels termed P2X, of which seven mammalian subtypes have been cloned.[1,2] The nomenclature of P2 receptors and their various ligand specificities has been reviewed.[2–5] The chick brain $P2Y_1$ receptor was the first P2 subtype to be cloned.[6] Subsequently, $P2Y_1$ receptors have been cloned from human, cow, turkey, rat, and mouse.[7,8] $P2Y_1$ receptors occur on endothelial cells,[9] smooth muscle,[9] epithelial cells,[10] lungs,[10] platelets,[11] pancreas,[12] and in the central nervous system.[13] The role of this receptor in vivo is largely unknown, and few pharmacological probes are available. Thus, the development of selective $P2Y_1$ receptor agonists and antagonists of high affinity is a critical need.

Boyer et al. in 1996[14] reported that various naturally occurring bisphosphates of adenosine (e.g. adenosine 3′,5′-bisphosphate, A3P5P) act as competitive antagonists of this subtype. Synthetic modifications which have increased the potency and selectivity of the bisphosphate derivatives include N^6-methyl (e.g. **3**, **4**, **6**, and **7**), 2-chloro (e.g. **4** and **7**), 2-deoxy (e.g. **2–7**), and the carbocyclic analogue of ribose (e.g. **5–7**) (Figure 1).[15–18] Compound **3** (MRS 2179) has a K_B value at human $P2Y_1$ receptors of 100 nM and does not interact with other P2 receptor subtypes at that concentration.[16] Molecular modeling of nucleotides docked in the putative, principal binding site of the $P2Y_1$ receptor[18,19] suggests that neither the ring oxygen atom nor 2′- and 3′-oxygens of the ribose moiety are involved in specific stabilizing interactions, such as H-bonding, with the receptor. Using this structural insight, we have designed modified ribose analogues including deoxy-,[15,17] carbocyclic,[17] and methanocarba-[18] modifications, which proved to retain receptor affinity. Thus, the structural requirements surrounding the ribose moiety in $P2Y_1$ receptor ligands provide latitude of modification.

The present study introduces a more radical departure from the ribose ring structure than in the previous work,[18] i.e. acyclic aliphatic chains and a pendant aromatic ring. For nucleosides that act as antiviral agents, among the most effective modifications are the acyclic ribose modifications.[20–22] Such aglycosyl antiviral nucleosides include acyclovir and gancyclovir, **1**, in which a 2-methylbutyl group can be considered to overlay the ribose ring. Analogues based on the design concept of gancyclovir, although more flexible, may still adopt the receptor-preferred conformation of the ribose or cyclopentyl rings. We wished to test the hypothesis that this approach, found successful for antiviral nucleosides, is generalizable to P2 receptor ligands. Are the steric requirements of the binding site of the

$P2Y_1$ receptor, in which specific cationic amino acid side chains form putative electrostatic bonds to either triphosphate or bisphosphate groups,[19] sufficiently permissive to allow the substitution of ribose with highly flexible acyclic substitutions?

Results

Chemical Synthesis.

Acyclic analogues similar in concept to gancyclovir[21] were synthesized as shown in Scheme 1.[22] The analogues, **8–11**, contained a branched aliphatic (isopentyl) group, and the only differences present were in the substitution pattern of the adenine moiety. The N^6-methyl and 2-chloro modifications of adenine have been included, due to the anticipated enhancement of affinity (2-Cl versus 2-H) and perturbation of the relative degree of agonist and antagonist character, with the N^6-methyl group expected to minimize agonist effects. In the previous study, methyl substitution of the exocyclic amino group tended to convert partial agonists into pure antagonists. The ethylene group of **10** at the 9-position of adenine was homologated (Scheme 2) to propyl, **12**, butyl, **13**, and pentyl, **14**, and to *cis*- and *trans*-butenyl, **15** and **16**. Furthermore, several amide-containing groups were incorporated through a carboxymethyl group at the 9-position of the adenine (Scheme 3), including a diethanolamine derivative, **17**, and an aniline 3,5-di(methylphosphonate), **18**. All three of these sets of analogues contained symmetrical bisphosphate (or phosphonate) moieties. Characterizations by high-resolution mass spectrometry, HPLC in two different solvent systems, and the yields of phosphorylation of each compound tested are summarized in Table 1.

Biological Activity.

Adenine nucleotides markedly stimulate inositol lipid hydrolysis by phospholipase C in turkey erythrocyte membranes,[23] through activation of a $P2Y_1$ receptor.[24] The 5′-diphosphate standard used in screening these analogues, 2-methylthioadenosine-5′-diphosphate (2-MeSADP), has a higher agonist potency than the corresponding triphosphate[26] for stimulation of inositol phosphate accumulation in membranes isolated from [^{3}H]inositol-labeled turkey erythrocytes.

As in our previous studies,[15–19] the bisphosphate analogues prepared in the present study were tested separately for agonist and antagonist activity in the PLC assay at the $P2Y_1$ receptor in turkey erythrocyte membranes, and the results are reported in Table 2. Concentration–response curves were obtained for each compound alone and in combination with the agonist 2-MeSADP (10 nM). Concentration–response curves for representative compounds, **11**, **17**, and **18**, are shown in Figure 2.

An acyclic N^6-methyladenine derivative, **10**, containing a symmetric isopentyl bisphosphate moiety was a $P2Y_1$ receptor antagonist with an IC_{50} value of 1.60 μM. The unsubstituted adenine derivative, **8**, displayed only weak antagonist properties, and the 2-chloro-substituted derivative, **9**, was somewhat more potent. The corresponding 2-chloro-N^6-methyl derivative, **11**, was even more potent with an IC_{50} value of 0.84 μM. Homologation of the ethylene group of **10** at the 9-position to 3–5 methylene units or inclusion of *cis*- or

trans-olefinic groups, i.e. compounds **12–16**, greatly reduced antagonist potency at the $P2Y_1$ receptor. None of the aliphatic acyclic derivatives, **8–16**, displayed agonist activity.

Analogues containing a diethanolamine amide group, **17**, and an aryl di(methylphosphonate), **18**, were both less potent than **11** as antagonists, with IC_{50} values of 14 and 16 μM, respectively, and no agonist activity was observed for these analogues.

Discussion

The present study has identified new pharmacological probes of $P2Y_1$ receptors that are to be considered adenine derivatives containing a negatively charged aglycosyl side chain, rather than nucleoside analogues. Thus, the ribose moiety is clearly not essential for recognition by the turkey $P2Y_1$ receptor, although a cyclic structure appears to be important for receptor activation. Unlike the corresponding ribose series,[17,18] the two isopentyl analogues, **8** and **9**, which contained an unsubstituted exocyclic amino group displayed no agonist properties. Therefore, the acyclic approach to the design of P2 receptor antagonists is valid, and three highly divergent lead structural classes (gancyclovir-type alkyl chain and homologues, **8–16**, alkyl amide, **17**, and aryl amide, **18**) have been presented. In a recently published study, which also indicates that an intact ribose moiety is not critical for interaction with P2 receptors, various chain-substituted xanthine derivatives were found to interact as antagonists with cardiac P2X receptors.[27] In these derivatives short alkyl groups replaced the ribose moiety of hypothetical xanthosine nucleotide structures. Specifically, for the $P2Y_1$ receptor, we have proposed, based on molecular modeling, that there are no required hydrogen bonds between the receptor and the ribose ring oxygen of a bound nucleotide ligand. This prediction was supported by the synthesis of highly potent carbocyclic bisphosphate analogues.[17,18]

While in the previous study of ribose derivatives the addition of a methyl group to the exocyclic amine tended to convert partial agonists into full antagonists, in the present study only antagonism of $P2Y_1$ receptor-mediated effects was observed. The highest affinity among the three novel structures of the present study occurred with the 2-chloro-N^6-methyl gancyclovir equivalent, i.e. compound **11**. Although further structural refinement would be desirable to optimize affinity, the affinities of these analogues are comparable to recently synthesized, moderately potent antagonists of the $P2Y_1$ receptor, based on ribose.[17,18] Although the lead bisphosphate structures, upon which these analogues are based, i.e. **2–7**, are selective for the $P2Y_1$ subtype, it will nevertheless be useful to examine the acyclic compounds as agonists and/or antagonists at other subtypes of P2 receptors.

The phosphonate group was included in **18** as a potentially more stable alternative to phosphate that would preserve the required negative charges. In the previous study, a 5′-*O*-phosphonylmethyl analogue of ribose was found to interact with $P2Y_1$ receptors,[17] although it was 4-fold less potent than the corresponding bisphosphate but with the same ratio of agonist/antagonist properties. The present structural leads may prove useful in the search for other phosphate group substitutes.

The moderately potent $P2Y_1$ receptor antagonists found in the present study, principally **11**, now require characterization at other P2 receptor subtypes. Compound **11** (10 μm) was recently shown to be inactive as either agonist or antagonist at rat $P2X_1$ receptors expressed in *Xenopus* oocytes.[30] If proven selective for the $P2Y_1$ subtype, these ligands serve as pharmacological probes for P2 receptor function in platelets[11,28,29] and other systems.

Experimental Section

Chemical Synthesis. Materials and Instrumentation.

Nucleosides and synthetic reagents were purchased from Sigma Chemical Co. (St. Louis, MO) and Aldrich (St. Louis, MO).

^{1}H NMR spectra were obtained with a Varian Gemini-300 spectrometer using $CDCl_3$, DMSO-d_6, or D_2O as a solvent. The chemical shifts are expressed as ppm downfield from tetramethylsilane or as relative ppm from DMSO (2.5 ppm) or HOD peaks (4.78 ppm). ^{31}P NMR spectra were recorded at room temperature by use of Varian XL-300 spectrometer (121.42 MHz); orthophosphoric acid (85%) was used as an external standard. High-resolution FAB (fast atom bombardment) mass spectrometry was performed with a JEOL SX102 spectrometer using 6-kV Xe atoms. The phosphate and phosphonate derivatives were previously desorbed from glycerol or magic bullet matrix. Low-resolution CI-NH_3 (chemical ionization) mass spectrometry was carried out with a Finnigan 4600 mass spectrometer and high-resolution EI (electron impact) mass spectrometry with a VG7070F mass spectrometer at 6 kV.

The determinations of purity were performed with a Hewlett-Packard 1090 HPLC system using an SMT OD-5–60 C18 analytical column (250 mm × 4.6 mm, Separation Methods Technologies, Inc., Newark, DE) in two different linear gradient solvent systems. One solvent system (A) was 0.1 M triethylammonium acetate buffer:CH_3CN in ratios of 95:5 to 40:60 for 20 min with flow rate 1 mL/min. The other (B) was 5 mM tetrabutylammonium phosphate buffer:CH_3CN, 80:20 to 40:60, in 20 min with flow rate 1 mL/min. Peaks were detected by UV absorption using a diode array detector. All phosphate and phosphonate derivatives showed more than 95% purity as determined using HPLC.

2-[2-(6-Amino-purin-9-yl)-ethyl]-propane-1,3-bisoxy(diammoniumphosphate) (8).

Compound **28** (35 mg, 0.044 mmol) was dissolved in a mixture of methanol (2 mL) and water (1 mL) and hydrogenated over 10 mg of 10% Pd/C at 25 °C for 24 h under 3 atm H_2. The catalyst was removed by filtration, and the methanol was evaporated. The residue was purified with an ion-exchange column chromatography using Sephadex-DEAE A-25 resin with a linear gradient (0.01 to 0.5 M) of 0.5 M ammonium bicarbonate as the mobile phase to give 18 mg of **8** (88%). ^{1}H NMR (D_2O) 1.96 (3H, m, -CH_2-, -CH-), 3.78 (4H, m, 2×-CH_2O-), 4.36 (2H, t, J = 7.8 Hz, -NCH_2-), 8.20 (1H, s, H-8), 8.23 (1H, s, H-2). ^{31}P NMR (D_2O) −18.24 (s, 1′-P, 3′-P).

2-[2-(6-Amino-2-chloro-purin-9-yl)-ethyl]-propane-1,3-bisoxy(diammoniumphosphate) (9).

To a solution of 90 mg of **28** (0.11 mmol) and 1 mL of anhydrous anisole in 3 mL of anhydrous CH_2Cl_2 was added 1 mL of BCl_3 solution in CH_2Cl_2 at −78 °C under nitrogen atmosphere. After the mixture was stirred for 1 h at −78 °C and for 48 h at 4 °C, 5 mL of 1 M triethylammonium bicarbonate buffer was added to the mixture. The aqueous layer was washed with CH_2Cl_2 two times and evaporated to dryness under reduced pressure. The residue was purified with an ion-exchange column chromatography using Sephadex-DEAE A-25 resin with a linear gradient (0.01 to 0.5 M) of 0.5 M ammonium bicarbonate as the mobile phase to give 28 mg of **9** (62%). ^{1}H NMR (D_2O) 1.93 (3H, m, -CH_2-, -CH-), 3.85 (4H, m, 2×-CH_2O-), 4.27 (2H, t, J = 6.8 Hz, -NCH_2-), 8.14 (1H, s, H-8). ^{31}P NMR (D_2O) −20.07 (s, 1′-P, 3′-P).

2-[2-(6-Methylamino-purin-9-yl)-ethyl]-propane-1,3-bisoxy(diammoniumphosphate) (10).

Starting from 53 mg of **29** (0.066 mmol) with the same procedure shown in the preparation of **8**, 10 mg of **10** was obtained (37%). ^{1}H NMR (D_2O) 1.90–2.00 (3H, m, CH_2, CH), 3.07 (3H, bs, N^6-CH_3), 3.82 (4H, m, 2CH_2-O), 4.32–4.37 (2H, m, CH_2-N), 8.16 (1H, s, H-2), 8.21 (1H, s, H-8). ^{31}P NMR (D_2O) 2.94 (s, 1′-P, 3′-P).

2-[2-(2-Chloro-6-methylamino-purin-9-yl)-ethyl]-propane-1,3-bisoxy(diammoniumphosphate) (11).

Starting from 80 mg of **29** (0.1 mmol) with the same procedure shown in the preparation of **9**, 30 mg of **11** was obtained (54%). ^{1}H NMR (D_2O) 1.94 (3H, m, -CH_2-, -CH-), 3.04 (3H, bs, N^6-CH_3), 3.87 (4H, m, 2×-CH_2O-), 4.26 (2H, t, J = 6.8 Hz, -NCH_2-), 8.08 (1H, s, H-8). ^{31}P NMR (D_2O) −20.47 (s, 1′-P, 3′-P). HRMS (FAB–) Calcd 446.0397; Found: 446.0410. HPLC 5.63 min (purity >98%) in solvent system A, 10.26 min in system B.

2-[3-(6-Methylamino-purin-9-yl)-propyl]-propane-1,3-bisoxy(diammoniumphosphate) (12).

Starting from 9 mg of **34a** (0.0114 mmol) with the same procedure shown in the preparation of **8**, 2.6 mg **12** was obtained (45%). ^{1}H NMR (D_2O) 1.37 (2H, m, -CH_2-), 1.96 (3H, m, -CH_2-, -CH-), 3.12 (3H, bs, N^6-CH_3), 3.84 (4H, dd, J = 5.9 Hz, 4.7 Hz, 2×-CH_2O-), 4.23 (2H, t, J = 6.8 Hz, -NCH_2-), 8.15, 8.26 (2H, 2s, H-2, H-8). ^{31}P NMR (D_2O) −21.06 (s, 1′-P, 3′-P).

2-[4-(6-Methylamino-purin-9-yl)-butyl]-propane-1,3-bisoxy(diammoniumphosphate) (13).

Starting from 18 mg of **34b** (0.0225 mmol) with the same procedure shown in the preparation of **8**, 5.1 mg of **13** was obtained (44.5%).^{1}H NMR (D_2O) 1.37 (4H, m, CHCH_2CH_2), 1.86 (3H, m, -CH_2-, -CH-), 3.01 (3H, bs, N^6-CH_3), 3.81 (4H, m, 2CH_2OP), 4.23 (2H, t, J = 6.6 Hz, NCH_2), 8.09, 8.22 (2H, 2s, H-2, H-8). ^{31}P NMR (D_2O) −20.16 (s, 1′-P, 3′-P).

2-[5-(6-Methylamino-purin-9-yl)-pentyl]-propane-1,3-bisoxy(diammoniumphosphate) (14).

Starting from 18 mg of **34c** (0.0225 mmol) with the same procedure shown in the preparation of **8**, 5.4 mg of **14** was obtained (47%). ^{1}H NMR (D_2O) 1.30 (4H, m, CHC$H_2CH_2CH_2$), 1.85 (3H, m, -CH_2-, -CH-), 3.08 (3H, bs, N^6-CH_3), 3.83 (4H, m,

2CH_2OP), 4.18 (2H, t, J = 6.9 Hz, NCH_2), 8.05, 8.17 (2H, 2s, H-2, H-8). ^{31}P NMR (D_2O) −20.51 (s, 1′-P, 3′-P).

2-[4-(6-Methylamino-purin-9-yl)-*cis*-but-2-enyl]-propane-1,3-bisoxy(diammoniumphosphate) (15).

Starting from 18 mg of **34d** (0.0226 mmol) with the same procedure shown in the preparation of **9**, 5.4 mg of **15** was obtained (47%). ^{1}H NMR (D_2O) 2.10 (1H, m, CH), 2.38 (2H, t, J = 6.9 Hz, CHCH_2CH=), 3.15 (3H, bs, N^6-CH_3), 3.92 (4H, t, J = 6 Hz, 2CH_2OP), 4.93 (2H, d, J = 5.7 Hz, NCH_2), 5.76 (1H, dt, J = 10.8, 6.9 Hz, CH=), 5.87 (1H, dt, J = 10.8, 7.8 Hz, CH=), 8.18, 8.23 (2H, 2s, H-2, H-8). ^{31}P NMR (D_2O) −20.82 (s, 1′-P, 3′-P).

2-[4-(6-Methylamino-purin-9-yl)-*trans*-but-2-enyl]-propane-1,3-bisoxy(diammoniumphosphate) (16).

Starting from 18 mg of **34e** (0.0226 mmol) with the same procedure shown in the preparation of **9**, 4.5 mg of **16** was obtained (39%). ^{1}H NMR (D_2O) 1.99 (1H, m, CH), 2.18 (2H, t, J = 6.8 Hz, CHCH_2CH=), 3.13 (3H, bs, N^6-CH_3), 3.83 (4H, t, J = 5.9 Hz, 2CH_2OP), 5.76 (1H, dt, J = 15.6, 6.8 Hz, CH=), 5.87 (1H, dt, J = 15.6, 5.9 Hz, CH=), 8.13, 8.25 (2H, 2s, H-2, H-8) (NCH_2 signal was overlapped with DHO signal), ^{31}P NMR (D_2O) −20.79 (s, 1′-P, 3′-P).

9-Bis(2-phosphatoethylamino)-acetyl-2-chloro-6-methylamino-purine Triammonium Monotriethylammonium Salt (17).

Starting from 35 mg of **43** (0.041 mmol) using the same procedure shown for the preparation of **9**, 6.5 mg of **17** was obtained (29%). ^{1}H NMR (D_2O) 3.09 (3H, bs, N^6-CH_3), 3.65–3.69 (2H, m, CH_2), 3.85–3.87 (2H, m, CH_2), 3.95–3.98 (2H, m, CH_2), 4.10–4.13 (2H, m, CH_2), 5.36 (2H, s, -NCH_2-CO), 8.06 (1H, s, H-8). ^{31}P NMR (D_2O) 1.65 (bs).

(2-Chloro-6-methylamino-purin-9-yl)-acetaminophenyl-3′,5′-bismethylphosphonate Tetraammonium Salt (18).

To a solution of 35 mg of **44** (0.062 mmol) in 1 mL of anhydrous CH_3CN was added 43 μL of trimethylsilyl bromide (0.31 mmol) at 25 °C under N_2 atmosphere. The mixture was stirred for 12 h, and the solvent was removed by N_2 stream. The residue was partitioned between ether and 0.5 M ammonium bicarbonate solution, and the water fraction was purified by an ion-exchange column chromatography using Sephadex-DEAE A-25 resin with a linear gradient (0.01 to 0.5 M) of 0.5 M ammonium bicarbonate as the mobile phase, and UV and HPLC were used to monitor the elution to give 27 mg of **18** (76%). ^{1}H NMR (D_2O) 2.90 (2H, d, J = 20.5 Hz, -CH_2P-), 2.99 (3H, s, -NHCH_3), 3.16 (2H, d, J = 20.5 Hz, -CH_2P-), 5.06 (2H, s, -NCH_2-), 6.97 (1H, s, Ph), 7.14 (1H, s, Ph), 7.26 (1H, s, Ph), 8.02 (1H, s, H-8) ^{31}P NMR (D_2O) 18.61 (t, J = 20.1 Hz), 19.75 (t, J = 20.5 Hz): with proton decoupling off mode.

Dimethyl 2-[2-(6-Amino-2-chloro-purin-9-yl)ethyl]malonate (24).

A mixture of 0.20 g of 6-amino-2-chloro-purine (1.18 mmol), 0.51 g of **21** (1.5 mmol) and 0.42 g of dry potassium carbonate (3 mmol) in 8 mL of anhydrous DMF was stirred at 60

°C for 24 h. The mixture was filtered and the filtrate evaporated to dryness under reduced pressure to give a crude **22**. The crude **22** was dissolved in 5 mL of methanol, and a solution of 54 mg of sodium methoxide in 1 mL of methanol was added with stirring. Stirring was continued for 3 h, during which time a precipitate was formed. The reaction mixture was kept at 0 °C for 30 min, and the white precipitate was collected by filtration and dried under high vacuum to afford 0.31 g of **24** (80%). ^{1}H NMR ($CDCl_3$) 2.48 (2H, q, J = 6.8 Hz, -CH_2-), 3.36 (1H, t, J = 6.8 Hz, -CH-), 3.75 (6H, s, 2×-OCH_3), 4.29 (2H, t, J = 6.8 Hz, -NCH_2-), 5.96 (2H, bs, -NH_2), 7.79 (1H, s, H-8). MS (EI) (M^+) 327. HRMS (EI) Calcd: 327.0734; Found: 327.0723.

Dimethyl 2-[2-(2-Chloro-6-methylamino-purin-9-yl)ethyl]malonate (25).

Starting from 0.37 g of 2-chloro-6-methylamino-purine (2 mmol) and 0.68 g of **21** (2 mmol) with the same procedure shown in the preparation of **24**, 0.34 g of **25** was obtained (50%). ^{1}H NMR ($CDCl_3$) 2.47 (2H, q, J = 6.8 Hz, -CH_2-), 3.19 (3H, s, N^6-CH_3), 3.34 (1H, t, J = 6.8 Hz, -CH-), 3.75 (6H, s, 2×-OCH_3), 4.26 (2H, t, J = 6.8 Hz, -NCH_2-), 5.97 (1H, bs, -NH-), 7.71 (1H, s, H-8). MS (EI) (M^+) 341. HRMS (EI) Calcd: 341.0890; Found: 341.0893.

2-Chloro-9-[4-hydroxy-3-(hydroxymethyl)butyl]-6-amino-purine (26).

To a suspension of 0.30 g of **24** (0.92 mmol) and 0.10 g of sodium borohydride (2.6 mmol) in 1.5 mL of CH_2Cl_2 was added dropwise 1 mL of MeOH, while maintaining the reaction temperature at 25 °C. The mixture was stirred at 25 °C for 4 h, 1 mL of water was added, and the mixture was neutralized by a dropwise addition of concentrated HCl to pH 7.0 with cooling. The mixture was diluted with MeOH and evaporated three times under vacuum, and the crude residue was purified with a silica gel column chromatography ($CHCl_3$/MeOH = 10/1) to give 0.19 g of **26** as a white powder (74%). ^{1}H NMR (CD_3OD) 1.58–1.68 (1H, m, -CH-), 1.92 (2H, q, J = 6.8 Hz, -CH_2-), 3.52–3.68 (4H, m, 2×-CH_2OH), 4.30 (t, J = 6.8 Hz, -NCH_2-), 8.12 (1H, s, H-8). MS (EI) 271 (M^+). HRMS (EI) Calcd: 271.0836; Found: 271.0828.

2-Chloro-9-[4-hydroxy-3-(hydroxymethyl)butyl]-6-methylamino-purine (27).

Starting from 0.24 g of **25** (0.72 mmol) with the same procedure shown in the preparation of **26**, 0.19 g of **27** was obtained (93%) as a white powder. ^{1}H NMR (DMSO-d_6) 1.39–1.47 (1H, m, -CH-), 1.77 (2H, q, J = 6.8 Hz, -CH_2-), 2.91 (3H, d, J = 4.9 Hz, N^6-CH_3), 3.30–3.47 (4H, m, 2×-CH_2OH), 4.16 (2H, t, J = 6.8 Hz, -NCH_2-), 4.34 (1H, t, J = 4.9 Hz, -NH-), 8.15 (1H, s, H-8). MS (CI) 286 (M + 1), HRMS (EI) Calcd: 285.0992; Found: 285.0985.

2-[2-(6-Amino-2-chloro-purin-9-yl)-ethyl]-propane-1,3-bisoxy(dibenzylphosphate) (28).

To a solution of 0.18 g of **26** (0.66 mmol) and 1.43 g of tetrabenzyl pyrophosphate (2.65 mmol) in 10 mL of anhydrous THF was slowly added 1.33 mL of lithium diisopropylamide solution (2.0 M in THF, 2.65 mmol) at −78 °C. After 1 h of stirring, the reaction mixture was warmed to 25 °C and stirred for additional 12 h. A white precipitate, lithium dibenzyl phosphate, was filtered off, and the filtrate was purified with silica gel column chromatography ($CHCl_3$/MeOH = 80/1) to give 0.13 g of **28** as a colorless oil (24%). ^{1}H

NMR($CDCl_3$) 1.70–1.76 (3H, m, -CH_2-, -CH-), 3.84–3.97 (4H, m, 2×-CH_2O-), 4.04 (2H, t, *J* = 6.8 Hz, -NCH_2-), 4.99 and 5.03 (8H, 2d, *J* = 3.9 Hz, 4×-OCH_2-), 6.42 (2H, s, -NH_2), 7.31 (20H, m, 4×-Ph), 7.68 (1H, s, H-8). ^{31}P NMR ($CDCl_3$) −22.22 (s, 1′-P, 3′-P). MS (FAB+) 792 (M^+ + 1). HRMS (FAB–) (M + H) Calcd: 792.2119; Found: 792.2124.

2-[2-(2-Chloro-6-methylamino-purin-9-yl)-ethyl]-propane-1,3-bisoxy(dibenzylphosphate) (29).

Starting from 0.05 g of **27** (0.17 mmol) with the same procedure shown in the preparation of **28**, 0.11 g of **29** was obtained as a colorless oil (78%). ^{1}H NMR ($CDCl_3$) 1.68–1.75 (3H, m, -CH_2-, -CH-), 3.20 (3H, bs, -N^6-CH_3), 3.83–3.96 (4H, m, 2CH_2-O), 4.04 (2H, t, *J* = 6.8 Hz, -NCH_2-), 4.99 and 5.02 (8H, 2d, *J* = 3.9 Hz, 4×-OCH_2-), 6.23 (1H, bs, -NH-), 7.32 (20H, m, 4×-Ph), 7.62 (1H, s, H-8). ^{31}P NMR ($CDCl_3$) −22.42 (s, 1′-P, 3′-P). MS (FAB) 806 (M^+ + 1). HRMS (FAB–) (M + Cs) Calcd: 938.1252; Found: 938.1227.

Triethyl 4-Iodobutane-1,1,1-tricarboxylate (30a).

To a solution of 2.21 g of sodium triethyl methanetricarboxylate (8.7 mmol) in 1:1 toluene/anhydrous DMF (10 mL) was added 5.15 g of 1,3-diiodopropane (17.4 mmol), and the solution was heated at 60 °C for 5 h, during which time a precipitate was formed. After cooling to room temperature, toluene (50 mL) was added and the mixture was filtered. The filtrate was washed with water (4 × 100 mL), dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. Silica gel column chromatography eluting with petroleum ether for the removal of excess of diiodopropane and then 10:1 petroleum ether-ethyl acetate afforded 2.52 g of **1** (72%) as a colorless oil. ^{1}H NMR ($CDCl_3$) 1.29 (9H, t, *J* = 7.8 Hz, -CH_3), 2.05 (2H, m, -CH_2-), 2.22 (2H, m, -CH_2-), 3.19 (2H, t, *J* = 6.6 Hz, -CH_2I), 4.27 (6H, q, *J* = 6.9 Hz, -OCH_2-). MS (FAB+) 401 (M + 1). HRMS (FAB+) Calcd for $C_{13}H_{22}O_6I$: 401.0461; Found: 401.0450.

Triethyl 5-Bromopentane-1,1,1-tricarboxylate (30b).

Starting from 3.61 g of 1,4-dibromobutane (16.7 mmol) with the same procedure shown in the preparation of **30a**, except for the reaction conditions, 80 °C overnight, 2.66 g of **30b** was obtained as a colorless oil (86%). ^{1}H NMR ($CDCl_3$) 1.29 (9H, t, *J* = 6.9 Hz, C*H*$_3$), 1.66 (2H, m, -CH_2-), 1.90 (2H, m, -CH_2-), 2.12 (2H, m, -C*H*$_2$C(CO_2Et)$_3$), 3.41 (2H, t, *J* = 6.9 Hz, -CH_2Br), 4.26 (6H, q, *J* = 6.9 Hz, -OCH_2). MS (FAB^+) 367 (M + 1). HRMS (FAB+) Calcd for $C_{14}H_{24}O_6{}^{79}Br$: 367.0756; Found: 367.0750.

Triethyl 6-Bromohexane-1,1,1-tricarboxylate (30c).

Starting from 3.34 g of 1,5-dibromopentane (14.7 mmol) with the same procedure shown in the preparation of **30b**, 2.11 g of **30c** was obtained as a colorless oil (73%). ^{1}H NMR ($CDCl_3$) 1.29 (9H, t, *J* = 6.9 Hz, -CH_3), 1.50 (4H, m, -CH_2-), 1.88 (2H, m, -CH_2-), 2.12 (2H, m, -C*H*$_2$C(CO_2Et)$_3$), 3.41 (2H, t, *J* = 6.9 Hz, -CH_2Br), 4.27 (6H, q, *J* = 6.9 Hz, -OCH_2). MS (FAB^+) 381 (M + 1). HRMS (FAB+) Calcd for $C_{15}H_{26}O_6{}^{79}Br$: 381.0913; Found: 381.0919.

Triethyl 5-Chloro-*cis*-pent-3-ene-1,1,1-tricarboxylate (30d).

Starting from 1.75 g of *cis*-1,4-dichloro-2-butene (14.0 mmol) with the same procedure shown in the preparation of **30b**, 2.40 g of **30d** was obtained as a colorless oil (100%). ^{1}H NMR ($CDCl_3$) 1.29 (9H, t, J = 6.9 Hz, -CH_3), 2.95 (2H, d, J = 6.9 Hz, -CH_2C(CO_2Et)$_3$), 4.13 (2H, d, J = 6.9 Hz, -CH_2Cl), 4.27 (6H, q, J = 6.9 Hz, −OCH_2), 5.75, 5.82 (2dt, 2H, J = 10.8, 6.9 Hz, -CH=CH-). MS (FAB$^+$) 321 (M + 1). HRMS (FAB+) Calcd for $C_{14}H_{22}O_6Cl$: 321.1105; Found: 321.1093.

Triethyl 5-Bromo-*trans*-pent-3-ene-1,1,1-tricarboxylate (30e).

Starting from 2.99 g of *trans*-1,4-dibromo-2-butene (14.0 mmol) with the same procedure shown in the preparation of **30b**, 1.87 g of **30e** was obtained as a colorless oil (73%). ^{1}H NMR ($CDCl_3$) 1.29 (9H, t, J = 6.9 Hz, -CH_3), 2.88 (2H, d, J = 7.8 Hz, -CH_2C(CO_2Et)$_3$), 3.91 (2H, d, J = 7.8 Hz, -CH_2Br), 4.26 (6H, q, J = 6.9 Hz, -OCH_2), 5.80, 5.98 (2dt, 2H, J = 14.7, 7.8 Hz, -CH=CH-). MS (FAB+) 365 (M + 1). HRMS (FAB+) Calcd for $C_{14}H_{22}O_6{}^{79}Br$: 365.0600; Found: 365.0586.

Dimethyl 2-[3-(6-Methylamino-purin-9-yl)propyl]malonate (32a).

A mixture of 0.373 g of N^6-methyladenine (2.5 mmol), 1.1 g of **30a** (2.75 mmol), and 0.50 g of dry powdered potassium carbonate (3.6 mmol) in 8 mL of anhydrous DMF was stirred at 60 °C for 24 h. The mixture was filtered while hot, and the filtrate evaporated to dryness under reduced pressure to give a syrupy crude **31a** (R_f 0.52, 10:1 $CHCl_3$: MeOH). The crude **31a** was dissolved in methanol (4 mL), and sodium methoxide (67.5 mg, 1.25 mmol) was added with stirring. After the mixture was stirred for 1 h at room temperature, 10% HCl was added to raise the pH to 7, and methanol was removed under vacuum. Brine was added, and the mixture was extracted with CH_2Cl_2 (3 × 30 mL). The organic fractions were combined, dried (anhydrous Na_2SO_4), filtered, and evaporated. The oily residue was purified by column chromatography (silica gel) eluting with $CHCl_3$ and then 100:1 $CHCl_3$:MeOH to give 0.484 g of pure **32a** as a white crystalline solid (60%). Mp 99–103 °C; R_f 0.47 (10:1 $CHCl_3$: MeOH). ^{1}H NMR ($CDCl_3$) 1.95 (m, 4H, 2×-CH_2-), 3.22 (3H, bs, N^6-CH_3), 3.42 (1H, t, J = 7.2 Hz, -CH-), 3.73 (6H, s, 2×-OCH_3), 4.22 (2H, t, J = 7.5 Hz, -NCH_2-), 5.85 (1H, bs, -NH-), 7.74 (1H, s, H-2), 8.41 (1H, s, H-8). MS (CI NH_3) 322 (M + H). HRMS (EI) Calcd for $C_{14}H_{19}N_5O_6$: 321.1437; Found: 321.1431.

Dimethyl 2-[4-(6-Methylamino-purin-9-yl)butyl]malonate (32b).

Starting from 1.01 g of **30b** (2.75 mmol) with the same procedure shown in the preparation of **32a**, 0.531 g of **32b** was obtained (63%). Mp 102–104 °C; R_f 0.44 (10:1 $CHCl_3$: MeOH). ^{1}H NMR ($CDCl_3$) 1.40 (m, 2H, -CH_2-), 1.96 (m, 4H, 2×-CH_2-), 3.22 (3H, bs, N^6-CH_3), 3.34 (1H, t, J = 6.9 Hz, -CH-), 3.73 (6H, s, 2×-OCH_3), 4.19 (2H, t, J = 6.9 Hz, -NCH_2-), 5.82 (1H, bs, -NH-), 7.73 (1H, s, H-2), 8.42 (1H, s, H-8). MS (CI NH_3) 336 (M + H). HRMS (EI) Calcd for $C_{15}H_{21}N_5O_6$: 335.1594; Found: 335.1601.

Dimethyl 2-[5-(6-Methylamino-purin-9-yl)pentyl]malonate (32c).

Starting from 1.05 g of **30c** (2.75 mmol) with the same procedure shown in the preparation of **32a**, 0.693 g of **32c** was obtained as a crystalline solid (79%). Mp 91–93 °C; R_f 0.44

(10:1 $CHCl_3$:MeOH). ^1H NMR ($CDCl_3$) 1.36 (m, 4H, 2×-CH_2-), 1.89 (m, 4H, 2×-CH_2-), 3.21 (3H, bs, N^6-CH_3), 3.34 (1H, t, *J* = 7.8 Hz, -CH-), 3.73 (6H, s, 2×-OCH_3), 4.17 (2H, t, *J* = 6.6 Hz, -NCH_2-), 5.81 (1H, bs, -NH-), 7.72 (1H, s, H-2), 8.42 (1H, s, H-8). MS (CI NH_3) 350 (M + H). HRMS (EI) Calcd for $C_{16}H_{23}N_5O_6$: 349.1750; Found: 349.1765.

Dimethyl 2-[4-(6-Methylamino-purin-9-yl)-*cis*-but-2-enyl]malonate (32d).

Starting from 0.88 g of **30d** (2.75 mmol) with the same procedure shown in the preparation of **32a**, 0.452 g of **32d** was obtained as a white solid (57%). Mp 111–112 °C; R_f 0.44 (10:1 $CHCl_3$:MeOH). ^1H NMR ($CDCl_3$) 2.85 (dd, 2H, *J* = 7.8, 6.9 Hz -C*H*$_2$CH=), 3.21 (3H, bs, N^6-CH_3), 3.34 (1H, t, *J* = 6.9 Hz, -CH-), 3.76 (6H, s, 2×-OCH_3), 4.90 (2H, d, *J* = 6.9 Hz, -NCH_2-), 5.65 (1H, dt, *J* = 7.8, 10.8 Hz, -CH=), 5.74 (1H, dt, *J* = 6.9, 10.8 Hz, -CH=), 5.85 (1H, bs, -NH-), 7.81 (1H, s, H-2), 8.42 (1H, s, H-8). MS (CI NH_3) 334 (M + H). HRMS (EI) Calcd for $C_{15}H_{19}N_5O_6$: 333.1437; Found: 333.1445.

Dimethyl 2-[4-(6-Methylamino-purin-9-yl)-*trans*-but-2-enyl]malonate (32e).

Starting from 1.00 g of **5** (2.75 mmol) with the same procedure shown in the preparation of **32a**, 0.20 g of **32e** was obtained as a white solid (24%). Mp 114–118 °C; R_f 0.44 (10:1 $CHCl_3$:MeOH). ^1H NMR ($CDCl_3$) 2.67 (t, 2H, *J* = 6.9 Hz -C*H*$_2$CH=), 3.21 (3H, bs, N^6-CH_3), 3.48 (t, 1H, *J* = 7.8 Hz, -CH-), 3.71 (6H, s, 2×-OCH_3), 4.90 (2H, d, *J* = 5.1 Hz, -NCH_2-), 5.71, 5.79 (2dt, 2H, *J* = 6.0, 15.6 Hz, -CH=CH-), 5.89 (1H, bs, -NH-), 7.71 (1H, s, H-2), 8.42 (1H, s, H-8). MS (CI NH_3) 334 (M + H). HRMS (EI) Calcd for $C_{15}H_{19}N_5O_6$: 333.1437; Found: 333.1439.

9-[5-Hydroxy-4-(hydroxymethyl)pentyl]-6-methylaminopurine (33a).

Starting from 0.172 g of **32a** (0.537 mmol) with the same procedure shown in the preparation of **26**, 0.110 g of **33a** was obtained as white crystals (77%). Mp 156–157 °C; R_f 0.22 (10:1 $CHCl_3$:MeOH). ^1H NMR (DMSO-d_6) 1.23 (2H, m, CHC*H*$_2$C*H*$_2$), 1.47 (1H, m, -CH-), 1.83 (2H, m, -CH_2-), 2.96 (3H, bs, N^6-CH_3), [3.27]-3.39 (4H, m, 2×-C*H*$_2$OH), 4.12 (2H, t, *J* = 6.9 Hz, -NCH_2-), 4.33 (2H, t, *J* = 5.1 Hz, 2×-OH) 7.64 (1H, bs, -NH-), 8.12, 8.22 (2H, 2s, H-2, H-8). MS (CI) 266 (M + 1). HRMS (EI) Calcd for $C_{12}H_{19}N_5O_2$: 265.1539; Found: 265.1537.

9-[6-Hydroxy-5-(hydroxymethyl)hexyl]-6-methylaminopurine (33b).

Starting from 0.166 g of **32b** (0.494 mmol) with the same procedure shown in the preparation of **26**, 0.106 g of **33b** was obtained as white crystals (77%). Mp 138–140 °C; R_f 0.22 (10:1 $CHCl_3$:MeOH). ^1H NMR (DMSO-d_6) 1.23 (4H, m, 2×-CH_2-), 1.41 (1H, m, -CH-), 1.78 (2H, m, -CH_2-), 2.96 (3H, bs, N^6-CH_3), [3.27]-3.39 (4H, m, 2×-C*H*$_2$OH), 4.14 (2H, t, *J* = 6.9 Hz, -NCH_2-), 4.29 (2H, t, *J* = 6 Hz, 2×-OH) 7.64 (1H, bs, -NH-), 8.13, 8.22 (2H, 2s, H-2, H-8). MS (CI) 280 (M + 1). HRMS (EI) Calcd for $C_{13}H_{21}N_5O_2$: 279.1695; Found: 279.1696.

9-[7-Hydroxy-6-(hydroxymethyl)heptyl]-6-methylaminopurine (33c).

Starting from 0.186 g of **7** (0.533 mmol) with the same procedure shown in the preparation of **26**, 0.114 g of **33c** was obtained as white crystals (73%). Mp 139–141 °C; R_f 0.22 (10:1

$CHCl_3$:MeOH). ^{1}H NMR (DMSO-d_6) 1.05–1.35 (6H, m, 3x-CH_2-), 1.40 (1H, m, -CH-), 1.79 (2H, m, -CH_2-), 2.96 (3H, bs, N^6-CH_3), [3,27]-3.39 (4H, m, 2×-CH_2OH), 4.13 (2H, t, J = 6.9 Hz, -NCH_2-), 4.26 (2H, t, J = 5.1 Hz, 2×-OH) 7.64 (1H, bs, -NH-), 8.12, 8.21 (2H, 2s, H-2, H-8). MS (CI) 294 (M + 1). HRMS (EI) Calcd for $C_{14}H_{23}N_5O_2$: 293.1852; Found: 293.1863.

9-[6-Hydroxy-5-(hydroxymethyl)-*cis*-hex-2-enyl]-6-methylamino-purine (33d).

Starting from 0.167 g of **32d** (0.50 mmol) with the same procedure shown in the preparation of **26**, 0.101 g of **33d** was obtained as white crystals (73%). Mp 127–129 °C; R_f 0.22 (10:1 CH Cl_3:MeOH). ^{1}H NMR (DMSO-d_6) 1.61 (1H, m, -CH-), 2.44 (2H, t, J = 6.9 Hz, -CH_2CH=), 2.97 (3H, bs, N^6-CH_3), 3.20–3.43 (4H, m, 2×-CH_2OH), 4.50 (2H, t, J = 5.1 Hz, 2×-OH), 4.89 (2H, d, J = 5.7 Hz, -NCH_2-), 5.67 (2H, m, J_{cis} = 10.8 Hz, -CH=CH-) 7.66 (1H, bs, -NH-), 8.10, 8.22 (2H, 2s, H-2, H-8). MS (CI) 278 (M + 1). HRMS (EI) Calcd for $C_{13}H_{19}N_5O_2$: 277.1539; Found: 277.1549.

9-[6-Hydroxy-5-(hydroxymethyl)-*trans*-hex-2-enyl]-6-methylamino-purine (33e).

Starting from 0.104 g of **32e** (0.31 mmol) with the same procedure shown in the preparation of **26**, 0.066 g of **33e** was obtained as white crystals (76%). Mp 115–117 °C; R_f 0.22 (10:1 $CHCl_3$:MeOH). ^{1}H NMR (DMSO-d_6) 1.51 (1H, m, -CH-), 2.00 (2H, t, J = 6.0 Hz, -CH_2CH=), 2.96 (3H, bs, N^6-CH_3), 3.26–3.43 (4H, m, 2×-CH_2OH), 4.33 (2H, t, J = 5.4 Hz, 2×-OH), 4.89 (2H, d, J = 5.2 Hz, -NCH_2-), 5.65 (2H, m, -CH=CH-) 7.64 (1H, bs, -NH-), 8.07, 8.22 (2H, 2s, H-2, H-8). MS (CI) 278 (M + 1). HRMS (EI) Calcd for $C_{13}H_{19}N_5O_2$: 277.1539; Found: 277.1540.

2-[3-(6-Methylamino-purin-9-yl)-propyl]-propane-1,3-bisoxy(dibenzylphosphate) (34a).

Starting from 29.2 mg of **33a** (0.11 mmol) with the same procedure shown in the preparation of **28**, 19 mg of **34a** was obtained as a colorless oil (22%). ^{1}H NMR ($CDCl_3$) 1.26 (2H, m, -CH_2-), 1.87 (3H, m, -CH_2-, -CH-), 3.19 (3H, bs, N^6-CH_3), 3.87 (4H, dd, J = 5.7 Hz, 6 Hz, 2×-CH_2OP-), 4.03 (2H, t, J = 6.8 Hz, -NCH_2-), 4.95 and 5.02 (8H, 2d, J = 11.7 Hz, 4×-OCH_2Ph), 5.87 (1H, s, -NH-), 7.31 (20H, m, 4×-Ph), 7.62 (1H, s, H-8), 8.39 (1H, s, H-2). ^{31}P NMR ($CDCl_3$) −22.27 (s, 1′-P, 3′-P). MS (FAB+, Cs) 918 (M^+ + Cs). HRMS (FAB+) (M + Cs) Calcd for $C_{40}H_{45}O_8N_5P_2Cs$: 918.1798; Found: 918.1798.

2-[4-(6-Methylamino-purin-9-yl)-butyl]-propane-1,3-bisoxy(dibenzylphosphate) (34b).

Starting from 29 mg of **33b** (0.104 mmol) with the same procedure shown in the preparation of **28**, 26 mg of **34b** was obtained as a colorless oil (31%). ^{1}H NMR ($CDCl_3$) 1.24 (4H, m, 2×-CH_2-), 1.76 (3H, m, -CH_2-, -CH-), 3.20 (3H, bs, N^6-CH_3), 3.88 (4H, m, 2×-CH_2OP-), 4.06 (2H, t, J = 6.9 Hz, -NCH_2-), 4.90 (8H, m, 4×-OCH_2Ph), 5.86 (1H, s, -NH-), 7.31 (20H, m, 4×-Ph), 7.67 (1H, s, H-8), 8.41 (1H, s, H-2). ^{31}P NMR ($CDCl_3$) −22.25 (s, 1′-P, 3′-P). MS(FAB+, Cs) 932 (M^++ Cs). HRMS (FAB+) Calcd for $C_{41}H_{47}O_8N_5P_2Cs$: 932.1954; Found: 932.1979.

2-[5-(6-Methylamino-purin-9-yl)-pentyl]-propane-1,3-bisoxy(dibenzylphosphate) (34c).

Starting from 30 mg of **33c** (0.102 mmol) and 275 mg of tetrabenzyl pyrophosphate (0.511 mmol) with the same procedure shown in the preparation of **28**, 20 mg of **34c** was obtained as a colorless oil (24%). ^{1}H NMR ($CDCl_3$) 1.22 (6H, m, 3×-CH_2-), 1.78 (2H, m, -CH_2-), 1.87 (1H, m, -CH-), 3.21 (3H, bs, N^6-CH_3), 3.91 (4H, m, 2×-C*H*$_2$OP -), 4.11 (2H, t, *J* = 6.9 Hz, -NCH_2-), 4.95, 5.02 (4H, 2d, *J* = 11.7 Hz, 2×-OC*H*$_2$Ph), 4.98, 5.00 (4H, 2d, *J* = 12.0 Hz, 2×-OC*H*$_2$Ph), 5.86 (1H, s, -NH-), 7.30 (20H, m, 4×-Ph), 7.70 (1H, s, H-8), 8.42 (1H, s, H-2). ^{31}P NMR ($CDCl_3$) −22.24 (s, 1′-P, 3′-P). MS (FAB+, Cs) 946 (M + Cs). HRMS (FAB+) Calcd for $C_{42}H_{49}O_8N_5P_2Cs$: 946.2111; Found: 946.2139.

2-[4-(6-Methylamino-purin-9-yl)-*cis*-but-2-enyl]-propane-1,3-bisoxy(dibenzylphosphate) (34d).

Starting from 50 mg of **33d** (0.18 mmol) and 679 mg of tetrabenzyl pyrophosphate (1.26 mmol) with the same procedure shown in the preparation of **28**, 23 mg of **34d** was obtained as a colorless oil (16%). ^{1}H NMR ($CDCl_3$) 1.94 (1H, m, -CH-), 2.16 (2H, t, *J* = 7.8 Hz, -C*H*$_2$CH=), 3.21 (3H, bs, N^6-CH_3), 3.94 (4H, t, *J* = 6 Hz, 2×-C*H*$_2$OP-), 4.66 (2H, d, *J* = 6.9 Hz, -NCH_2-), 5.01 (8H, m, 4×-OC*H*$_2$Ph), 5.49 (1H, dt, *J* = 10.8, 7.8 Hz, -CH=), 5.63 (1H, dt, *J* = 10.8, 6.9 Hz, -CH=), 5.84 (1H, bs, -NH-), 7.30 (20H, m, 4×-Ph), 7.70, 8.40 (2H, 2s, H-2, H-8). ^{31}P NMR ($CDCl_3$) −22.21 (s, 1′-P, 3′-P). MS (FAB+, Cs) 930 (M + Cs). HRMS (FAB+) Calcd for $C_{41}H_{45}O_8N_5P_2Cs$: 930.1798; Found: 930.1802.

2-[4-(6-Methylamino-purin-9-yl)-*trans*-but-2-enyl]-propane-1,3-bisoxy(dibenzylphosphate) (34e).

Starting from 44 mg of **33e** (0.159 mmol) and 555 mg of tetrabenzyl pyrophosphate (1.11 mmol) with the same procedure shown in the preparation of **34a**, 22 mg of **34e** was obtained as a colorless oil (17%). ^{1}H NMR (DMSO-d_6) 1.85 (1H, m, -CH-), 1.98 (2H, t, *J* = 7.8 Hz, -C*H*$_2$CH=), 3.20 (3H, bs, N^6-CH_3), 3.88 (4H, m, 2×-C*H*$_2$OP-), 4.62 (2H, d, *J* = 5.7 Hz, -NCH_2-), 4.99 (8H, m, 4×-OC*H*$_2$Ph), 5.48 (1H, dt, *J* = 15.6, 6.6 Hz, -CH=), 5.60 (1H, dt, *J* = 15.6, 5.7 Hz, -CH=), 5.87 (1H, bs, -NH-), 7.30 (20H, m, 4×-Ph), 7.69, 8.42 (2H, 2s, H-2, H-8). ^{31}P NMR ($CDCl_3$) −22.23 (s, 1′-P, 3′-P). MS (FAB+, Cs) 930 (M^++ Cs). HRMS (FAB+) Calcd for $C_{41}H_{45}O_8N_5P_2Cs$: 930.1798; Found: 930.1802.

3,5-Di(bromomethyl)-1-nitro-benzene (36).

A solution of 1.89 g of 3,5-di(hydroxymethyl)-1-nitrobenzene (**35**, 10 mmol), 5.25 g of triphenylphosphine (20 mmol), and 6.62 g of carbon tetrabromide (20 mmol) in 50 mL of anhydrous ether was stirred at 25 °C for 12 h under N_2 atmosphere. After evaporation, the residue was purified with flash silica gel column chromatography (hex/EtOAc = 5/1) to give 1.6 g of **36** as a bright yellow solid (52%). ^{1}H NMR ($CDCl_3$) 4.53 (4H, s, 2×-CH_2Br), 6.83 (1H, s, Ph), 7.76 (1H, s, Ph), 8.20 (1H, s, Ph). MS (EI) (M^+) 309. HRMS (EI) Calcd 306.8843; Found 306.8859.

Tetramethyl 1-Nitro-benzene-3,5-bis(methylphosphonate) (37).

A solution of 1.4 g of **36** (4.53 mmol) in 15 mL of trimethyl phosphite was heated at 80 °C for 6 h. After evaporation, the residue was purified with flash silica gel column

chromatography ($CHCl_3$/MeOH = 30/1) to give 1.57 g of **37** as a white solid (94%). ^{1}H NMR ($CDCl_3$) 3.25 (4H, d, J = 21.5 Hz, 2×-CH_2P-), 3.72 (3H, s, -CH_3), 3.73 (3H, s, -CH_3), 3.75 (3H, s, -CH_3), 3.76 (3H, s, -CH_3), 7.28 (1H, s, Ph), 7.60 (1H, s, Ph), 8.06 (1H, s, Ph). ^{31}P NMR ($CDCl_3$) −27.09 (m), 33.48 (m). MS (EI) (M^+) 367. HRMS (EI) Calcd 367.0586; Found 367.0592.

Tetramethyl 1-Amino-benzene-3,5-bis(methylphosphonate) (38).

A solution of 40 mg of **37** (4.53 mmol) and 10 mg of 10% Pd/C in 3 mL of methanol was stirred at 25 °C for 1 h under H_2 atmosphere (1 atm). The mixture was filtered through a Celite bed and purified with preparative thin-layer chromatography ($CHCl_3$/MeOH = 20/1) to give 37 mg of **38** as a white solid (100%). ^{1}H NMR ($CDCl_3$) 3.06 (4H, d, J = 21.5 Hz, 2×-CH_2P-), 3.66 (6H, s, 2×-CH_3), 3.70 (6H, s, 2×-CH_3), 6.55 (2H, s, Ph), 6.59 (1H, s, Ph). ^{31}P NMR ($CDCl_3$) −27.09 (m), 33.48 (m): with proton decoupling off mode. MS (EI) (M^+) 337. HRMS (EI) Calcd 337.0844; Found 337.0845.

Ethyl 2-Chloro-6-methylamino-purin-9-yl)-acetate (40).

To a suspension of 0.132 g of NaH (3.3 mmol), which was prewashed with 5 mL of hexanes twice, in 2 mL of anhydrous DMF was added 0.55 g of N^6-methyl-2-chloro-purine (3 mmol) in 3 mL of DMF at 25 °C under N_2 atmosphere. After the mixture was stirred for 30 min, 0.4 mL of ethyl bromoacetate was added, and the stirring was continued for 12 h. After evaporation, the residue was purified with preparative thin-layer chromatography ($CHCl_3$/MeOH = 25/1) to give 0.66 g of **40** as a white solid (82%). ^{1}H NMR ($CDCl_3$) 1.31 (3H, t, J = 6.8 Hz, -CH_3), 3.20 (3H, bs, -N^6-CH_3), 4.26 (2H, q, J = 6.8 Hz, -OCH_2-), 4.93 (2H, s, -NCH_2CO-), 6.01 (1H, bs, -NH-), 7.78 (1H, s, H-8). MS (EI) (M^+) 269. HRMS (EI) Calcd 269.0679; Found 269.0667.

2-Chloro-6-methylamino-9-yl)-acetic Acid (41).

Compound **40** (0.33 g, 1.22 mmol) was dissolved in 6 mL of 1 N NaOH with stirring at 25 °C for 1 h. The completion of the hydrolysis was confirmed by HPLC, and the mixture was neutralized to pH 5~7 with 6 N HCl at 0 °C. A white precipitate was collected by filtration, washed with water, and dried under high vacuum to give 0.245 g of **41** as a white solid (83%). ^{1}H NMR (DMSO-d_6) 2.92 (3H, s, -N^6-CH_3), 4.94 (2H, s, -NCH_2CO-), 8.11 (1H, s, H-8). MS (EI) (M^+) 241. HRMS (EI) Calcd 241.0366; Found 241.0358.

2-Chloro-9-[(diethanolamino)carboxymethyl]-6-methylamino-purine (42).

A mixture of 47 mg of **41** (0.193 mmol), 54 mg of 4-nitrophenol (0.386 mmol), 74 mg of EDAC (0.386 mmol), and 5 mg of DMAP in 1 mL of anhydrous DMF and 1 mL of CH_2Cl_2 was stirred at 25 °C for 2 h. After evaporation, a solution of 26 mg of diethanolamine (0.251 mmol) in 1 mL of methanol was added, and the reaction mixture was stirred at 25 °C for 12 h. The mixture was concentrated to dryness, and the residue was purified with preparative thin-layer chromatography ($CHCl_3$/MeOH = 10/1) and crystallized in MeOH:ether (1:2) solution to give 23 mg of **42** as a white solid (36%). ^{1}H NMR (DMSO-d_6) 2.92 (3H, bs, N^6-CH_3), 3.37 (2H, t, J = 5.9 Hz, N-CH_2), 3.48 (2H, t, J = 5.9 Hz, N-CH_2), 3.52–3.56 (2H,

m, CH_2OH), 3.63–3.65 (2H, m, CH_2OH), 5.17 (2H, s, N-CH_2CO), 7.99 (1H, s, H-8), 8.18 (2H, m, OH). MS (EI) (M^+) 328. HRMS (EI) Calcd 328.1050; Found 328.1038.

9-Bis(2-dibenzylphosphatoethylamino)-acetyl-2-chloro-6-methylamino-purine (43).

To a solution of 23 mg of **42** (0.069 mmol) in 2 mL of anhydrous THF was slowly added 0.2 mL of lithium diisopropylamide solution (2.0 M in THF, 0.4 mmol) at −78 °C. After 15 min of stirring, 215 mg of tetrabenzyl pyrophosphate (0.4 mmol) was added and the mixture was stirred for 30–60 min at −78 °C. The reaction mixture was warmed to 0 °C ~ 25 °C and stirred for additional 12 h. The mixture was concentrated to dryness, and the residue was purified with preparative thin-layer chromatography ($CHCl_3$/MeOH = 10/1) to give 55 mg of **43** (94%). ^{1}H NMR ($CDCl_3$) 3.16 (3H, s, N^6-CH_3), 3.37–3.40 (2H, m, CH_2), 3.45–3.49 (2H, m, CH_2), 3.95–4.07 (4H, m, $2CH_2$), 4.78 (2H, s, -NCH_2-CO), 4.97–5.11 (8H, m, O-CH_2), 7.00 (1H, bs, NH), 7.32 (20H, m, C_6H_5), 7.59 (1H, s, H-8). ^{31}P NMR (CD_3Cl) −0.59 (s), −0.72 (s). HRMS (FAB–) (M + Cs) Calcd 981.1310; Found 981.1313.

Tetramethyl (2-Chloro-6-methylamino-purin-9-yl)-acetaminophenyl-3′,5′-bis(methylphosphonate) (44).

A solution of 24 mg of **41** (0.1 mmol), 34 mg of **38** (0.1 mmol), and 29 mg of EDAC (0.15 mmol) in 3 mL of anhydrous DMF/CH_2Cl_2 (1/2) was stirred at 25 °C for 12 h. After evaporation, the residue was purified with preparative thin-layer chromatography ($CHCl_3$/MeOH = 10/1) to give 46 mg of **44** as a white solid (82%). ^{1}H NMR ($CDCl_3$) 2.89 (3H, bs, -N^6-CH_3), 3.03 (4H, d, J = 21.5 Hz, 2×-CH_2P-), 3.71 (6H, s, 2×-CH_3), 3.74 (6H, s, 2×-CH_3), 4.97 (2H, s, -NCH_2CO-), 6.83 (1H, s, Ph), 7.21 (2H, s, Ph), 7.68 (1H, bs, NH), 7.96 (1H, s, H-8), 9.90 (1H, bs, NH). ^{31}P NMR ($CDCl_3$) 28.96 (m), with proton decoupling off mode. MS (FAB) (M + H) 561. HRMS (FAB) Calcd 561.1183; Found 561.1206.

Pharmacological Analyses.

$P2Y_1$ receptor promoted stimulation of inositol phosphate formation by adenine nucleotide analogues was measured in turkey erythrocyte membranes as previously described.[23,25] The $K_{0.5}$ values were averaged from 3 to 8 independently determined concentration–effect curves for each compound. Briefly, 1 mL of washed turkey erythrocytes was incubated in inositol-free medium (DMEM; Gibco, Gaithersburg, MD) with 0.5 mCi of 2-[^{3}H]*myo*-inositol (20 Ci/mmol; American Radiolabeled Chemicals, Inc., St. Louis, MO) for 18–24 h in a humidified atmosphere of 95% air/5% CO_2 at 37 °C. Erythrocyte ghosts were prepared by rapid lysis in hypotonic buffer (5 mM sodium phosphate, pH 7.4, 5 mM $MgCl_2$, 1 mM EGTA) as described.[23] Phospholipase C activity was measured in 25 μL of [^{3}H]inositol-labeled ghosts (approximately 175 μg of protein, 200–500000 cpm/assay) in a medium containing 424 μM $CaCl_2$, 0.91 mM $MgSO_4$, 2 mM EGTA, 115 mM KCl, 5 mM KH_2PO_4, and 10 mM Hepes, pH 7.0. Assays (200 μL final volume) contained 1 μM GTPγS and the indicated concentrations of nucleotide analogues. Ghosts were incubated at 30 °C for 5 min, and total [^{3}H]inositol phosphates were quantitated by anion-exchange chromatography as previously described.[23,25]

Data Analysis.

Agonist potencies were calculated using a four-parameter logistic equation and the GraphPad software package (GraphPad, San Diego, CA). EC_{50} values (mean ± standard error) represent the concentration at which 50% of the maximal effect is achieved. Relative efficacies (%) were determined by comparison with the effect produced by a maximal effective concentration of 2-MeSADP in the same experiment.

Antagonist IC_{50} values (mean ± standard error) represent the concentration needed to inhibit by 50% the effect elicited by 30 nM 2-MeSADP. The percent of maximal inhibition is equal to 100 minus the residual fraction of stimulation at the highest antagonist concentration.

All concentration–effect curves were repeated in at least three separate experiments carried out with different membrane preparations using duplicate or triplicate assays.

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Abbreviations:

ADP	adenosine 5′-diphosphate
DEAE	diethylaminoethyl
DMAP	4-(dimethylamino)pyridine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
EDAC	1-ethyl3-(3-dimethlyaminopropyl)carbodiimide
FAB	fast atom bombardment (mass spectroscopy)
HPLC	high-pressure liquid chromatography
MRS 2179	N^6-methyl-2′deoxyadenosine 3′,5′-bisphosphate
MS	mass spectroscopy
HRMS	high-resolution mass spectroscopy
LDA	lithium diisoproylamide
2-MeSADP	2-methylthioadenosine-5′-diphosphate
TBAP	tetrabutylammonium phosphate
TBPP	tetrabenzylpyrophosphate
THF	tetrahydrofuran

TMS	trimethylsilyl
pTLC	preparative thinlayer chromatography

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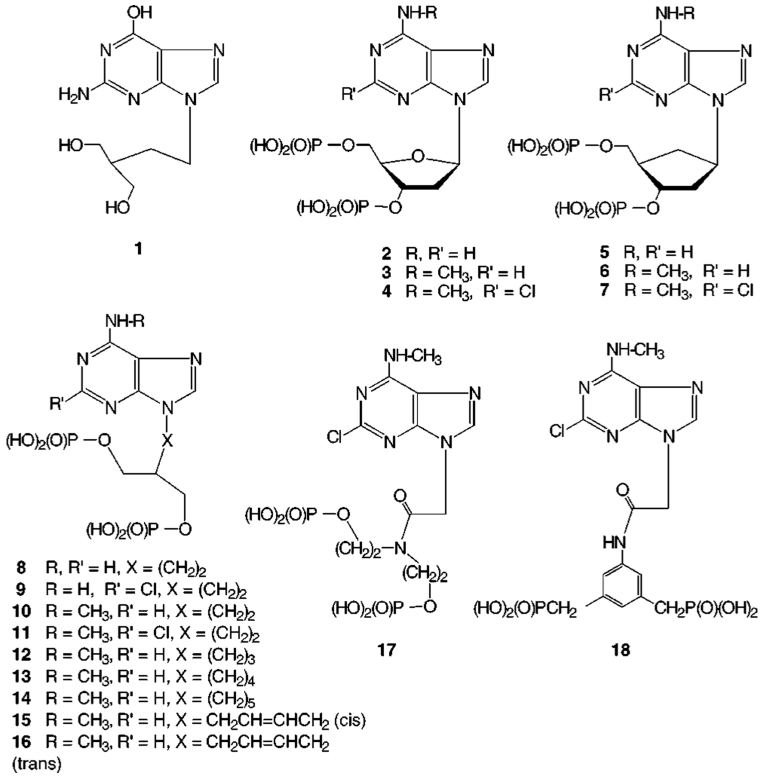


Figure 1.

Structures of acyclic nucleotide analogues synthesized as ligands for the $P2Y_1$ receptor. The corresponding ammonium salts were synthesized and tested for biological activity.

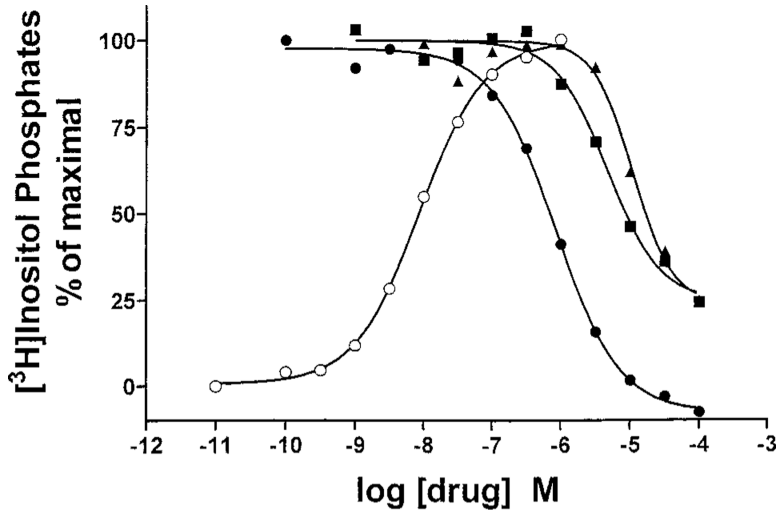
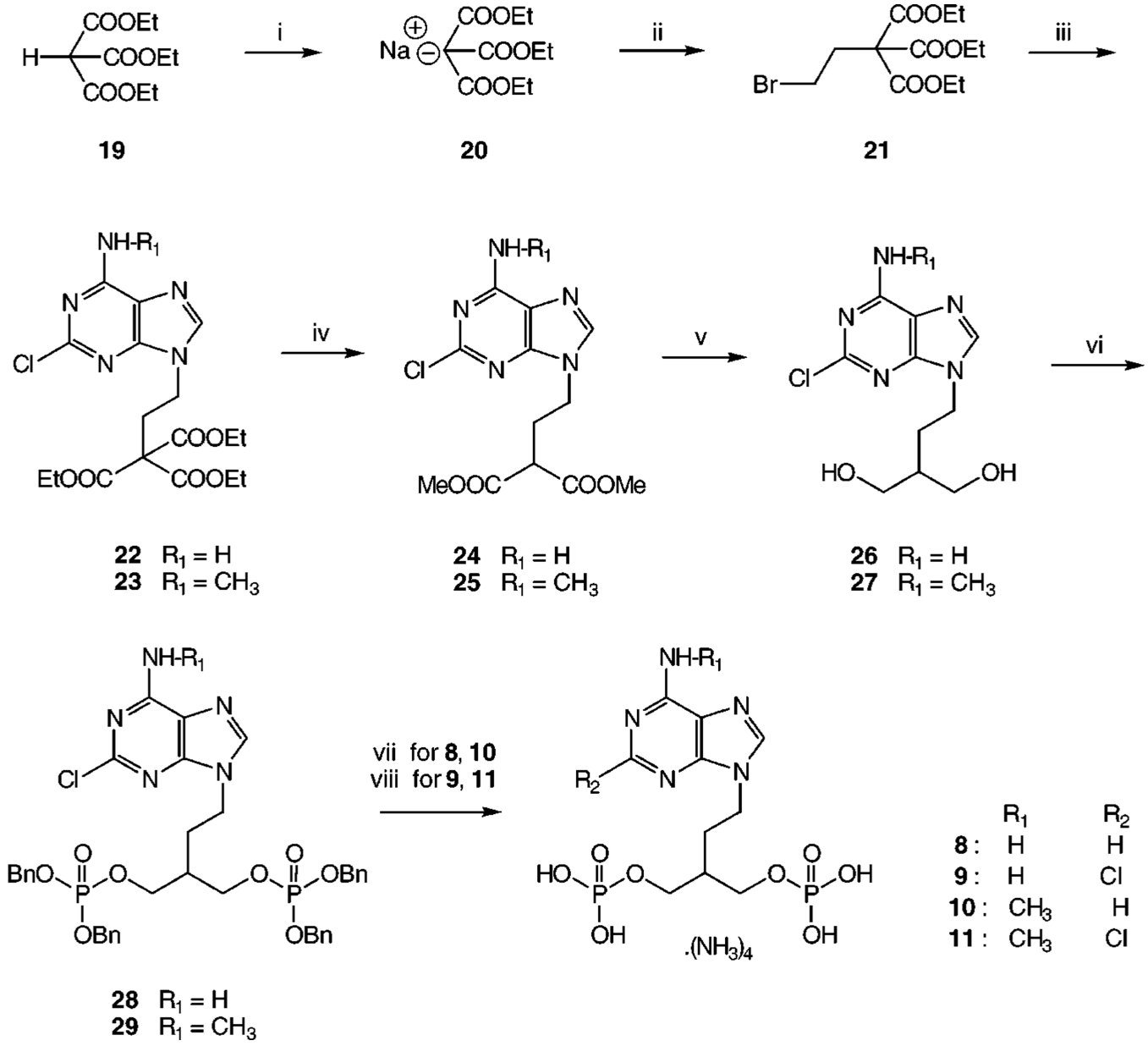


Figure 2.

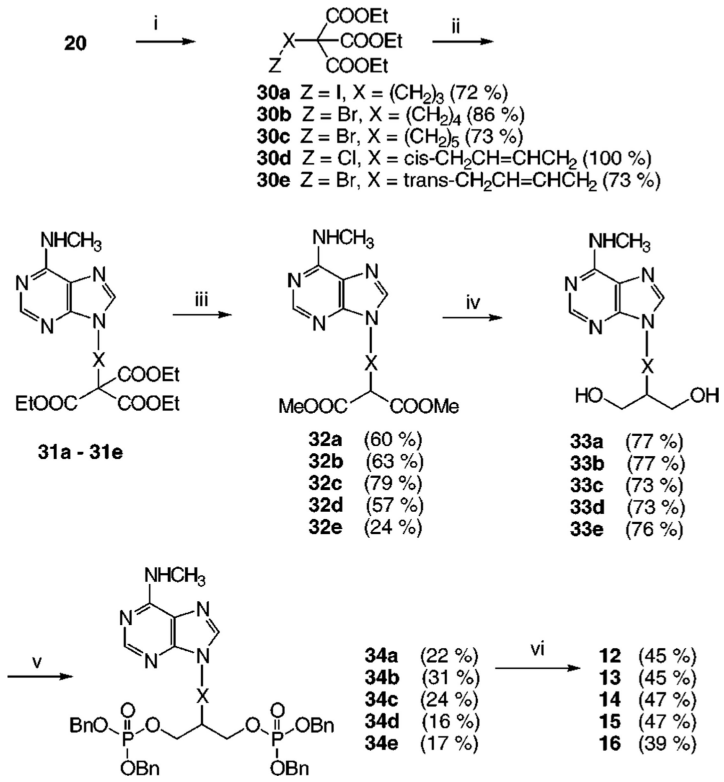
Effects of adenine acyclic bisphosphate derivatives on $P2Y_1$ receptor-activated phospholipase C activity in turkey erythrocyte membranes: both concentration-dependent stimulation of inositol phosphate formation by 2-MeSADP (◯) and its inhibition in the presence of 30 nM 2-MeSADP by compounds **11** (●), **17** (■), and **18** (▲). Membranes from [^{3}H]-inositol-labeled erythrocytes were incubated for 5 min at 30 °C in the presence of the indicated concentrations of 2-MeSADP or of test compound in combination with 30 nM 2-MeSADP. The data shown are typical curves for at least three experiments carried out in duplicate using different membrane preparations.



Scheme 1.

Synthesis of 9-Isopentyladenine Nucleotide Derivatives[a]

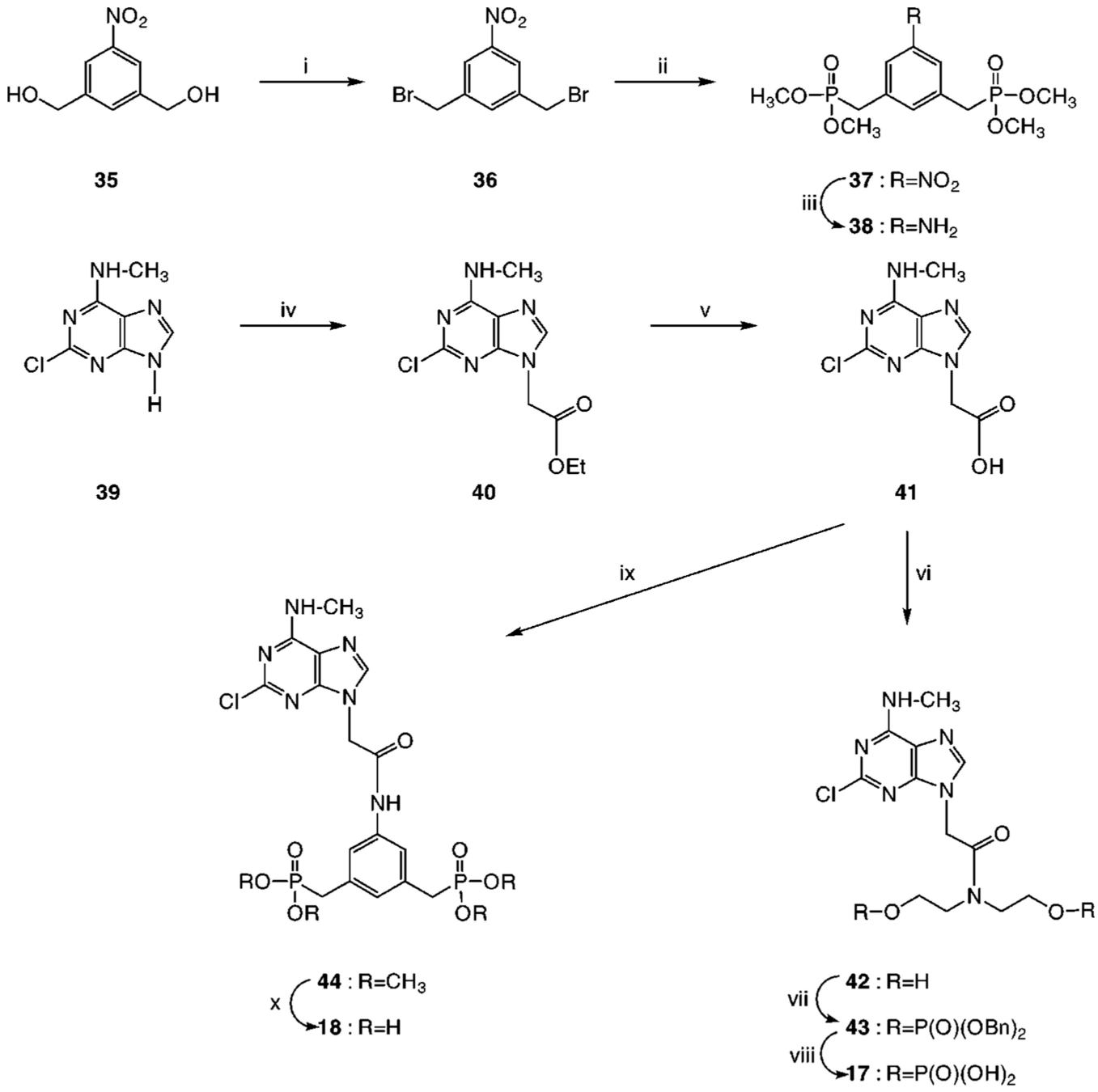
[a] Reagents: (i) NaOEt, ether, 0 °C to RT, 30 min, 85%; (ii) 1,2-dibromoethane, DMF, 90 °C, 10 h, 92%; (iii) adenine analogue, K_2CO_3, DMF, 65 °C, 24 h; (iv) NaOMe, methanol, RT to 15 °C, 50~80% for two steps; (v) $NaBH_4$, CH_2Cl_2:MeOH (3:2), RT, 4–6 h, 74–98%; (vi) DBPP, LDA, THF, −78 °C to RT, 4 h, 24–78%; (vii) H_2/Pd-C, MeOH:H_2O (1:1), RT, 1 atm, 37–88%; (viii) BCl_3, anisole, CH_2Cl_2, −78 °C, 1 h, 0 °C, 48 h, 54−62%.



Scheme 2.

Synthesis of Acyclic Nucleotide Derivatives Containing Groups Larger than Isopentyl at the Adenine 9-Position[a]

[a] Reagents: (i) **30a**: 1,3-diiodopropane; **30b**: 1,4-dibromobutane; **30c**: 1,5-dibromopentane; **30d**: cis-1,4-dichloro-2-butene; **30e**: *trans*-1,4-dibromo-2-butene; 1:1 DMF:toluene, Δ; (ii) N^6-methyladenine, K_2CO_3, DMF, Δ; (iii) NaOMe, methanol, RT; (iv) $NaBH_4$, CH_2Cl_2:MeOH, RT; (v) DBPP, LDA, THF, −78 °C to RT, 16 h; (vi) **12**, **13**, **14**: H_2/10% Pd-C, MeOH, RT, 3 atm; **15**, **16**: BCl_3, CH_2Cl_2, 5 °C.



Scheme 3.

Synthesis of Acyclic Nucleotide Derivatives Containing an Amide Bond[a]

[a] Reagents: (i) $(Ph)_3P$, CBr_4, 25 °C, 12 h, 52%; (ii) $P(OCH_3)_3$, 80 °C, 6 h, 94%; (iii) Pd/C, H_2, 25 °C, 1 h, 100%; (iv) NaH, $BrCH_2COOEt$, 25 °C, 12 h, 82%; (v) 1 N NaOH, 25 °C, 1 h, 83%; (vi) 4-nitrophenol, EDAC, DMAP, 25 °C, 2 h, $HN(CH_2CH_2OH)_2$, 25 °C, 12 h, 36%; (vii) LDA, tetrabenzylpyrophosphate, −78 °C, 0.5 h, 0 °C, 2 h, 94%; (viii) BCl_3, anisole, −78 °C, 1 h, 0 °C, 4 h, 28%; (ix) **38**, EDAC, 25 °C, 12 h, 82%; (x) TMS-Br, 25 °C, 12 h, 45%.

Table 1.

Synthetic Data for Nucleotide Derivatives, Including Structural Verification Using High-Resolution Mass Spectroscopy and Purity Verification Using HPLC

		FAB (M – H$^+$)		HPLC (rt; min)[a]		
no.	formula	calcd	found	system A	system B	yield (%)[b]
8	$C_{10}H_{17}O_8N_5P_2$	398.0631	398.0592	2.68	8.49	21.1
9	$C_{10}H_{16}ClO_8N_5P_2$	432.0241	432.0237	2.70	10.18	14.9
10	$C_{11}H_{19}O_8N_5P_2$	410.0631	410.0630	5.48	5.90	48.4
11	$C_{11}H_{18}ClO_8N_5P_2$	446.0397	446.0410	5.63	10.26	42.1
12	$C_{12}H_{20}O_8N_5P_2$	424.0787	424.0786	4.81	9.82	9.9
13	$C_{13}H_{22}O_8N_5P_2$	438.0944	438.0959	5.26	8.62	14.0
14	$C_{14}H_{24}O_8N_5P_2$	452.1100	452.1101	5.78	8.97	11.3
15	$C_{13}H_{20}O_8N_5P_2$	436.0787	436.0797	4.97	9.03	7.5
16	$C_{13}H_{20}O_8N_5P_2$	436.0787	436.0770	4.55	8.81	6.6
17	$C_{12}H_{19}ClO_9N_6P_2$	487.0290	487.0299	6.80	9.55	27.3
18	$C_{16}H_{19}ClO_7N_6P_2$	503.0401	503.0388	8.28	10.39	

[a] Purity of each derivative was ≥95%, as determined using HPLC with two different mobile phases. System A: gradient of 0.1 M TEAA/CH_3CN from 95/5 to 40/60. System B: gradient of 5 mM TBAP/CH_3CN from 80/20 to 40/60.

[b] The percent yields refer to overall yield for each phosphorylation sequence.

Table 2.

In Vitro Pharmacological Data for Stimulation of PLC at Turkey Erythrocyte $P2Y_1$ Receptors (Agonist Effect) and the Inhibition of PLC Stimulation Elicited by 30 nM 2-MeSADP (Antagonist Effect), for at Least Three Separate Determinations

compd	agonist effect, % of maximal increase[a]	EC_{50}, μM[a]	antagonist effect, % of maximal inhibition[b]	IC_{50}, μM[b] (n)
2 [c]	12 ± 3	6.29 ± 2.54	87 ± 4	5.76 ± 0.68
3 [c]	NE		99 ± 1	0.331 ± 0.059
4 [c]	NE		95 ± 1	0.206 ± 0.053
5 [c]	27 ± 11	7.21 ± 4.40	73 ± 11	2.53 ± 0.57
6[c,e]	NE		99 ± 1	0.331 ± 0.059 (5)
7 [c]	NE		95 ± 1	0.206 ± 0.053
8	NE		63 ± 7[d]	>50
9	NE		94 ± 2	7.57 ± 1.41 (5)
10 [e]	NE		100	1.60 ± 0.47
11 [e]	NE		99 ± 1	0.840 ± 0.130
12	NE		68 ± 4[d]	>30
13	NE		65 ± 6[d]	>50
14	NE		56 ± 2[d]	~100
15	NE		77 ± 3[d]	>50
16	NE		54 ± 6[d]	~100
17	NE		89 ± 3[d]	14.0 ± 6.0 (4)
18	NE		94 ± 2[d]	15.5 ± 4.2

[a] Agonist potencies were calculated using a four-parameter logistic equation and the GraphPad softaware package (GraphPad, San Diego, CA). EC_{50} values (mean ± standard error) represent the concentration at which 50% of the maximal effect is achieved. Relative efficacies (%) were determined by comparison with the effect produced by a maximal effective concentration of 2-MeSADP in the same experiment. Small increase refers to <10% at 100 μM. NE = no effect at 100 μM.

[b] Antagonist IC_{50} values (mean ± standard error) represent the concentration needed to inhibit by 50% the effect elicited by 10 nM 2-MeSADP. The percent of maximal inhibition is equal to 100 minus the residual fraction of stimulation at the highest antagonist concentration. n = 3, unless otherwise indicated in parentheses.

[c] Values are from refs 15 and 17.

[d] For **8** and **12–18**, the percent of maximal antagonist effect indicated was that observed at 100 μM. Higher concentrations were not examined.

[e] **6**, MRS 2179; **10**, MRS 2277; **11**, MRS 2286.