Acyclic Analogues of Adenosine Bisphosphates as P2Y Receptor Antagonists: Phosphate Substitution Leads to Multiple Pathways of Inhibition of Platelet Aggregation

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Activation by ADP of both $P2Y_1$ and $P2Y_{12}$ receptors in platelets contributes to platelet aggregation, and antagonists at these receptor subtypes have antithrombotic properties. In an earlier publication, we have characterized the SAR as $P2Y_1$ receptor antagonists of acyclic analogues of adenine nucleotides, containing two phosphate groups on a symmetrically branched aliphatic chain, attached at the 9-position of adenine. In this study, we have focused on antiaggregatory effects of P2Y antagonists related to a 2-chloro-N^6-methyladenine-9-(2-methylpropyl) scaffold, containing uncharged substitutions of the phosphate groups. For the known nucleotide (cyclic and acyclic) bisphosphate antagonists of $P2Y_1$ receptors, there was a significant correlation between inhibition of aggregation induced by 3.3 μM ADP in rat platelets and inhibition of $P2Y_1$ receptor-induced phospholipase C (PLC) activity previously determined in turkey erythrocytes. Substitution of the phosphate groups with nonhydrolyzable phosphonate groups preserved platelet antiaggregatory activity. Substitution of one of the phosphate groups with O-acyl greatly reduced the inhibitory potency, which tended to increase upon replacement of both phosphate moieties of the acyclic derivatives with uncharged (e.g., ester) groups. In the series of nonsymmetrically substituted monophosphates, the optimal antagonist potency occurred with the phenylcarbamate group. Among symmetrical diester derivatives, the optimal antagonist potency occurred with the di(phenylacetyl) group. A dipivaloyl derivative, a representative uncharged diester, inhibited ADP-induced aggregation in both rat (K_I 3.6 μM) and human platelets. It antagonized the ADP-induced inhibition of the cyclic AMP pathway in rat platelets (IC_{50} 7 μM) but did not affect $hP2Y_1$ receptor-induced PLC activity measured in transfected astrocytoma cells. We propose that the uncharged derivatives are acting as antagonists of a parallel pro-aggregatory receptor present on platelets, that is, the $P2Y_{12}$ receptor. Thus, different substitution of the same nucleoside scaffold can target either of two P2Y receptors in platelets.

Introduction

ATP and other purine and pyrimidine nucleotides act as extracellular signaling molecules through activation of P2 receptors. These receptors are divided into two categories: G protein-coupled receptors, termed P2Y, and ligand-gated cation channels, termed P2X. Seven subtypes have been cloned within each family and function in such systems as the central and peripheral nervous systems, the cardiovascular system, the endocrine system, lungs, intestines, muscle, and the immune system.[1−3] In the cardiovascular system, ADP released from platelets and endothelial cells induces aggregation of platelets by acting at two subtypes of receptors: $P2Y_1$ and $P2Y_{12}$.[4−6] The $P2Y_1$ receptor has been cloned from chick, turkey, human, rat, and mouse,[7−9] and the human $P2Y_{12}$ receptor was cloned recently by several groups. A mouse line lacking expression of the $P2Y_1$ receptor has been constructed,[10,11] and the absence of the $P2Y_1$ receptor in platelets of these mice interfered with ADP-promoted aggregation. A third nucleotide receptor, $P2X_1$, is present on platelets, but its role in aggregation has not yet been determined. The antithrombotic drug clopidogrel acts as a $P2Y_{12}$ receptor antagonist through one of its metabolites.[12] Other $P2Y_{12}$ receptor antagonists based on nucleotide structures related to the potent antagonist **1** are in clinical development.[13] The $P2Y_1$ receptor antagonists N^6-methyl-2′-deoxyadenosine 3′,5′-bisphosphate **2a** and the highly potent, rigid (N)-methanocarba antagonist **3** have been shown to inhibit ADP-induced platelet aggregation.[14−16] Thus, antagonists of either $P2Y_1$ or $P2Y_{12}$ receptor subtypes may display antithrombotic properties.[17] In collaboration with Harden and colleagues, we have

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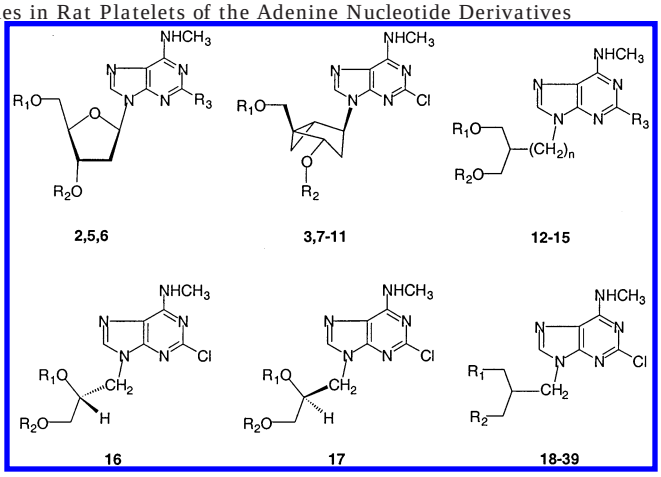
Table 1. Antiaggregatory Activities in Rat Platelets of the Adenine Nucleotide Derivatives



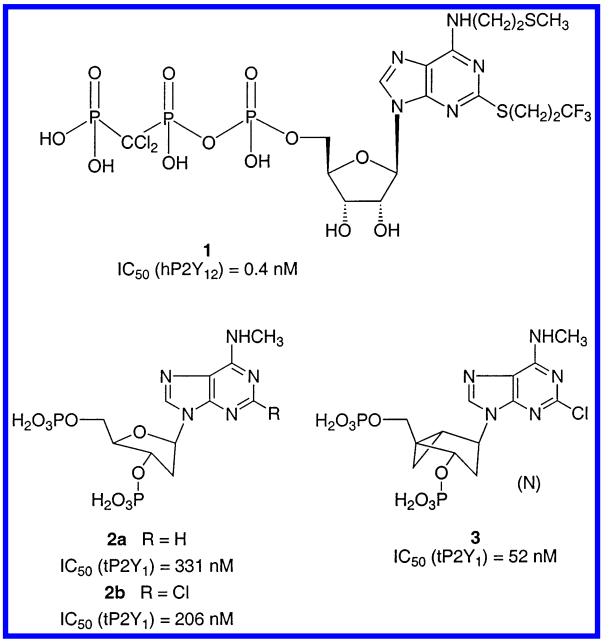
compound	R_1	R_2	R_3	K_i[a] μM (range)
2a	PO_3H_2	PO_3H_2	H	0.58 (0.55−0.61)
2b	PO_3H_2	PO_3H_2	Cl	0.42 (0.33−0.55)
5	PO_3H_2	CH_3CH_2NHCO	Cl	11 (7.9−15)
6	PO_3H_2	$(CH_3)_2CHNHCO$	Cl	6.4 (3.9−11)
3	PO_3H_2	PO_3H_2		0.031 (0.022−0.042)
7	H	PO_3H_2		29 (7.01−20)
8	PO_3H_2	H		33 (11−94)
9	CH_3CO	PO_3H_2		16 (1.0−198)
10	PO_3H_2	CH_3CO		10 (5.0−19)
11	CH_3CO	CH_3CO		29 (25−35)
12 ($n = 2$)	PO_3H_2	PO_3H_2	Cl	0.46 (0.40−0.52)
13 ($n = 1$)	PO_3H_2	PO_3H_2	H	0.51 (0.37−0.70)
14 ($n = 1$)	PO_3H_2	PO_3H_2	Cl	0.30 (0.24−0.37)
15 ($n = 0$)	PO_3H_2	PO_3H_2	Cl	5.5 (4.1−7.3)
16	PO_3H_2	PO_3H_2		2.6 (2.2−3.0)
17	PO_3H_2	PO_3H_2		1.4 (0.87−2.1)
18	OPO_3H_2	CH_3COO		110 (0.002−5700)
19	CH_3COO	CH_3COO		18 (6.2−49)
20	$OPO_3HPO_3H_2$	PO_3H_2		1.1 (0.88−1.3)
21	OPO_3H_2	$(CH_3)_3CCOO$		29 (24−32)
22	OPO_3H_2	C_6H_5COO		18 (14−22)
23	OPO_3H_2	C_2H_5COO		21 (14−31)
24	OPO_3H_2	C_6H_5NHCOO		2.5 (1.8−3.3)
25	C_2H_5COO	C_2H_5COO		23 (17−31)
26	$(CH_3)_3CCOO$	$(CH_3)_3CCOO$		3.6 (2.8−4.5)
27	*trans*-$CH_3CH{=}CHCOO$	*trans*-$CH_3CH{=}CHCOO$		10 (7.4−14)
28	C_6H_5COO	C_6H_5COO		5.1 (3.5−7.5)
29	2-FC_6H_4COO	2-FC_6H_4COO		24 (15−37)

Table 1 (Continued)

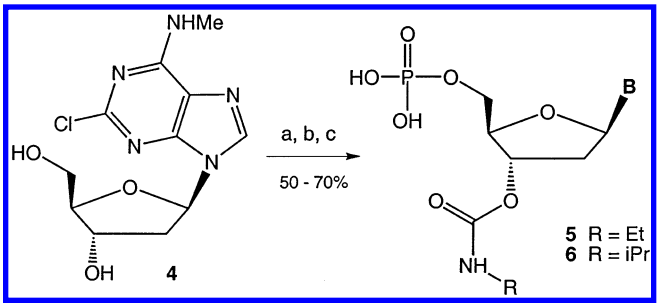
compound	R_1	R_2	R_3	K_I[a] μM (range)
30	2,6-$F_2C_6H_4COO$	2,6-$F_2C_6H_4COO$		33 (19−60)
31	$C_6H_5CH_2COO$	$C_6H_5CH_2COO$		2.0 (0.85−4.7)
32	C_6H_5NHCOO	C_6H_5NHCOO		24 (15−37)
33	C_6H_5OCOO	C_6H_5OCOO		26 (19−37)
34	$HO_2C(CH_2)_2COO$	$HO_2C(CH_2)_2COO$		18 (1.2−278)
35	$C_6H_5CH_2SCSO$	$C_6H_5CH_2SCSO$		87 (54−140)
36	CH_3COO	C_6H_5COO		6.9 (5.2−9.2)
37	CH_3COO	$C_6H_5CH_2COO$		12.3 (7.5−20)
38	PO_3H_2	PO_3H_2		0.68 (0.45−1.0)
39	$(CH_2)_2PO_3H_2$	$(CH_2)_2PO_3H_2$		25 (12−53)

[a] The inhibition of ADP-induced platelet activation by antagonists measured using aggregometry. The antagonist was added and washed with rat platelets, while stirring at 1000 rpm, one min prior to the addition of ADP at 3.3 μM. Percent aggregation was measured 5 min after addition of antagonist. Concentration response curves for antagonists were constructed and the IC_{50} determined. Means for $n = 3$ determinations are shown, unless noted. 95% confidence limits are shown in parentheses. **2a** MRS2179, **2b** MRS2216, **3** MRS2279, **12** MRS2286, **13** MRS2297, **14** MRS2298, **26** MRS2395, **31** MRS2412.

developed selective $P2Y_1$ receptor agonists and antagonists of high affinity for use as pharmacological probes.[14−16,18−20] Various naturally occurring bisphosphates of adenosine (e.g., adenosine 3′,5′-bisphosphate) acted as partial agonists or antagonists at the $P2Y_1$ receptor.[21] The removal of the 2′-hydroxyl group reduced residual agonism of these nucleotide bisphosphates, thus resulting in pure antagonists. Also, acyclic nucleotide analogues, containing mainly symmetrically branched aliphatic chains attached at the 9-position of adenine, were shown to be $P2Y_1$ receptor antagonists.[19,20] The N^6-methyl and 2-chloro substitution of the adenine moiety was found to increase the potency and selectivity of the bisphosphate derivatives acting as competitive $P2Y_1$ antagonists.[14,16,22] Both of these substitutions are included in the present study, in which we have characterized the SAR of acyclic analogues of adenine nucleotides as inhibitors of platelet aggregation.



Scheme 1. Synthesis of Adenine 9-Riboside Derivatives Similar to **2b**, in Which the 3′-Phosphate Group Has Been Replaced with a Urethane Group[a]



[a] Reagents: (a) bis-2-cyanoethyl-*N*,*N*-diisopropylphosporamidite, tetrazole, THF; then *t*-BuOOH; (b) R-NCO, CuBr, Py; (c) bistrimethylsilylacrylamide, DBU, Py. **B** = 2-chloro-6-methylaminopurin-9-yl.

We have focused on uncharged substitutions of the bisphosphate groups among 2-chloro-N^6-methyladenine-9-(2-methylpropyl) derivatives.[20]

Results

Chemical Synthesis. A variety of ribosides and nonriboside analogues of adenine nucleotides (Table 1) were synthesized as shown in Schemes 1−6 as potential inhibitors of platelet aggregation. The focus was on combinations of phosphate groups and phosphate substitutes, an approach that was first tested for feasibility in riboside derivatives (**5**, **6**),[14,15] then extended to rigid methanocarba analogues (**7**−**11**),[16,22] and finally to the acyclic analogues (**12**−**39**).[19,20] Mass spectral and other analytical data for the substances tested biologically are provided in Table 2.

Nonsymmetric substitution of the bisphosphate groups of nucleotide antagonists was accomplished. Adenine-9-riboside 3′-carbamate 5′-phosphate derivatives were synthesized as shown in Scheme 1. The intermediate 2-chloro-N^6-methyl-2′-deoxyadenosine **4** was synthesized as reported.[18] To make the (N)-methanocarba 3′-

Table 2. Synthetic Data for Nucleosides and Nucleotides, Including Structural Verification Using High-Resolution Mass Spectroscopy and Purity Verification Using HPLC

		FAB ($M^+ - H$)		HPLC (rt, min)			
no.	formula	calcd	found	system A[a]	system B[b]	system C[c]	system D[d]
5a	$C_{14}H_{20}ClN_6O_7P$ (for FAB+)	451.0898	451.0917	2.5 (93%)	2.5 (97%)		
6	$C_{15}H_{22}ClN_6O_7P$ (for FAB+)	465.1054	465.1069	2.5 (95%)	2.4 (95%)		
7	$C13_3H_{16}ClN_5O_5P$	388.0578	388.0582	10.3 (99%)	3.4 (99%)		
8	$C_{13}H_{16}ClN_5O_5P$	388.0578	388.0573	10.2 (99%)	10.3 (99%)		
9	$C_{15}H_{18}ClN_5O_5P$	430.0683	430.0687	5.5 (96%)	14.3 (95%)		
10	$C_{15}H_{18}ClN_5O_5P$	430.0683	430.0701	12.1 (97%)	13.7 (96%)		
11	$C_{17}H_{21}ClN_5O_4$ (for FAB+)	394.1282	394.1276			7.3[e] (98%)	6.8[f] (97%)
21	$C_{15}H_{23}ClN_5O_6P$	434.0996	434.0993	13.7 (96%)	15.6 (97%)		
22	$C_{17}H_{19}ClN_5O_6P$	454.0683	454.0701	12.8 (96%)	14.5 (95%)		
23	$C_{13}H_{19}ClN_5O_7P$ (for FAB+)	424.0789	424.0789	11.6 (96%)	13.5 (96%)		
24	$C_{17}H_{20}ClN_6O_6P$	471.0949	471.0960	15.3 (96%)	15.7 (97%)		
25	$C_{16}H_{22}ClN_5O_4$	384.1439	384.1435			3.4 (97%)	3.4 (99%)
27	$C_{18}H_{22}ClN_5O_4$	408.1439	408.1450			3.3 (98%)	3.5 (99%)
28	$C_{24}H_{22}ClN_5O_4$	480.1439	480.1436			4.7 (96%)	4.8 (95%)
30	$C_{24}H_{18}ClF_4N_5O_4$	552.1062	552.1062			3.1 (96%)	3.4 (96%)
31	$C_{26}H_{26}ClN_5O_4$	508.1752	508.1761			3.9 (97%)	4.8 (99%)
32	$C_{24}H_{24}ClN_7O_4$	510.1657	510.1661			3.6 (95%)	3.6 (96%)
33	$C_{24}H_{22}ClN_5O_6$	512.1337	512.1343			3.6 (98%)	4.2 (99%)
34	$C_{18}H_{22}ClN_5O_8$	472.1235	472.1239			2.0 (95%)	1.8 (95%)
35	$C_{26}H_{26}ClN_5O_2S_4$	604.0736	604.0747			8.5 (96%)	22.1[e] (96%)
38	$C_{10}H_{16}ClN_5O_6P_2$	398.0339	398.0196	2.0 (95%)	3.7 (95%)		
39	$C1_4H_{24}ClN_5O_6P_2$ (for FAB^+)	456.0969	456.0982	1.9 (96%)	1.9 (98%)		

[a] System A: 5% CH3CN 95% 0.1 M TEAA to 60% CH_3CN 40% 0.1 M TEAA in 20 min. [b] System B: 20% CH_3CN 80% 5 mM TBAP to 60% CH_3CN 40% 5 mM TBAP in 20 min. [c] System C: 80% CH_3CN 20% MeOH to 20% CH_3CN 80% MeOH in 20 min, unless noted. [d] System D: 80% CH_3CN 20% H_2O to 20% CH_3CN 80% H_2O in 20 min, unless noted. [e] 40% CH_3CN 60% 0.1 M TEAA to 90% CH_3CN 10% 0.1 M TEAA in 20 min. [f] 40% CH_3CN 60% 5 mM TBAP to 90% CH_3CN 10% 5 mM TBAP in 20 min.

monophosphate **7** and 5′-monophosphate **8** analogues (Scheme 2), it was necessary to distinguish the 5′-hydroxy and 3′-hydroxy groups. Regioselective control of acetylation on the 5′-hydroxy group of **40** was accomplished using a catalytic amount of 4-(dimethylamino)pyridine (DMAP). Increasing the amount of DMAP gave a mixture of mono- and diacetate. Phosphorylation of the 3′-hydroxy-5′-acetate **41** was carried out by the phosphoramidite method[23] followed by deprotection, to afford the acetyl phosphate **9** in a moderate yield. Deprotection of the 5′-acetyl group with aqueous ammonia gave the 5′-hydroxy-3′-phosphate **7**.

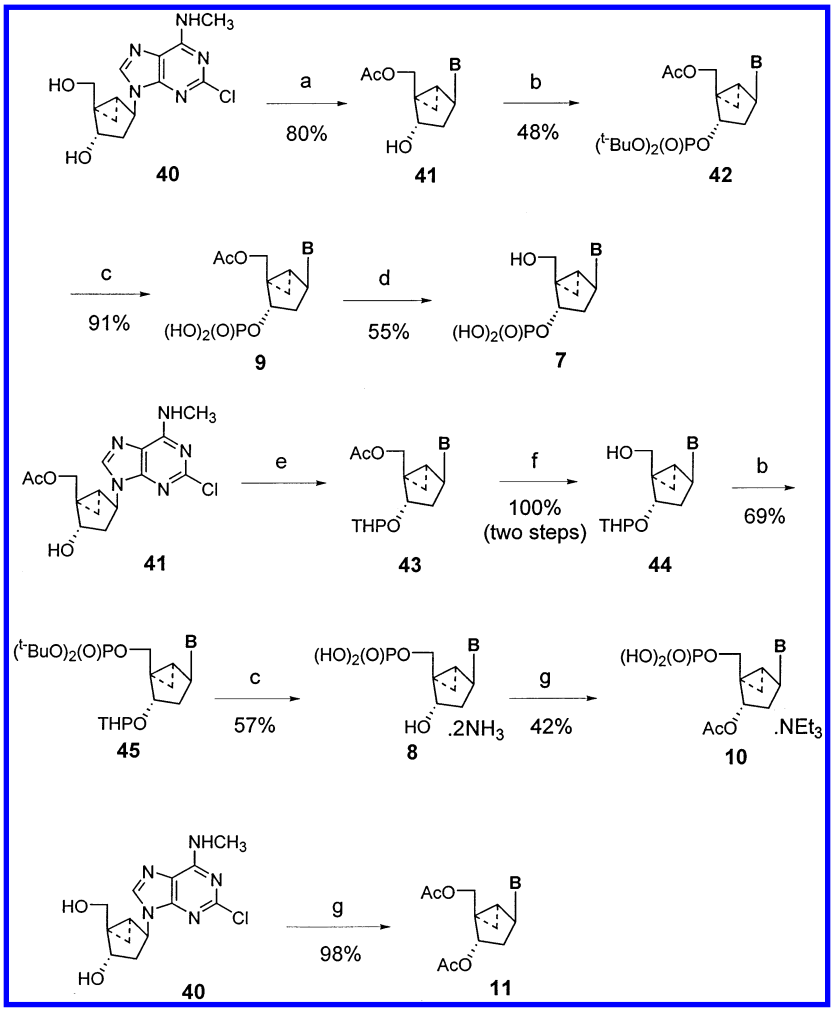
Synthesis of the 5′-phosphate **8** involved masking the 3′-hydroxy group, which was accomplished using a tetrahydropyranyl (THP) protecting group. After deprotection of the acetyl group of **43** and phosphorylation with di-*tert*-butyl *N*,*N*-diethylphosphoramidite followed by oxidation,[23] THP and di-*tert*-butyl groups were removed simultaneously to provide the 3′-hydroxy-5′-phosphate **8**. Acetylation of the free hydroxyl group of **8** upon treatment with acetic anhydride in the presence of DMAP furnished the 3′-acetyl-5′-phosphate **10**.

Acyclic $P2Y_1$ antagonists **12**−**18** and **20** were reported in our previous studies.[19,20] A new, improved method was developed to synthesize related acyclic nucleotide analogues from a commercially available starting material, 2-(hydroxymethyl)-1,3-propanediol. The efficient synthesis of key intermediates **51** and **52** is outlined in Scheme 3. The formation of cyclic ketal **46** from the triol was carried out successfully under acidic condition using a catalytic amount of TsOH.[24] Subsequent mesylation reaction afforded the mesylate[25] **47** which was coupled with 2-chloro-N^6-methylaminopurine to produce the isopropylidene derivative **48** in good yield. Compound **48** was then converted either to a monoacetate **49** or diol **52** selectively using HOAc/THF/H_2O (65/35/10) or 80% acetic acid, respectively. Phosphates **50a** and **50b** were prepared in high yield by the 1*H*-tetrazole promoted phosphitylation of **49** with dibenzyl diisopropylphosphoramidite or di-*tert*-butyl diethylphosphoramidite followed by oxidation of the resulting triester with *m*-CPBA.[23] Then, the acetyl groups of **50a** and **50b** were removed by using K_2CO_3 in MeOH to afford the hydroxy compounds **51a** and **51b**. Typical acetylation conditions (acetic anhydride/DMAP)[26] provided compound **19** from **52**.

Transformation of **51a** or **51b** to the protected target monophosphate compounds **53**−**56** was carried out as shown in Scheme 4. Silane-induced cleavage of the phosphate esters using trimethylsilyl bromide in CH_2Cl_2 at room temperature afforded **21**−**24,** respectively.[27] All of the phosphates were prepared and tested as the ammonium salt form.

The acyclic nonphosphates, that is, esters, carbamates, and carbonates, were synthesized as shown in Scheme 5. The alkyl and aryl acid esters **25**−**31** and **36**−**37** were prepared by acylation of the diol **52** or the monoacetate **49** with the corresponding anhydrides.[28] The use of more reactive acyl chlorides led to undesired substitution at the N^6 position of the purine moiety. The carbamate[29] **32** and carbonate[30] **33** were derived from phenylisocyanate and phenyl chloroformate, respectively. The free acid **34** was prepared from succinic anhydride in pyridine.[31] Compound **52** reacted with CS_2 in the presence of 5 N NaOH and was alkylated in situ with excess benzyl bromide to give the xanthate[32] **35** in 15% yield.

Scheme 2. Synthesis of Adenine 9-(N)-Methanocarba Derivatives Similar to **3**, in Which the 3′- or 5′-Phosphate Ester Groups Have Been Removed or Replaced with Acetyl Groups[a]



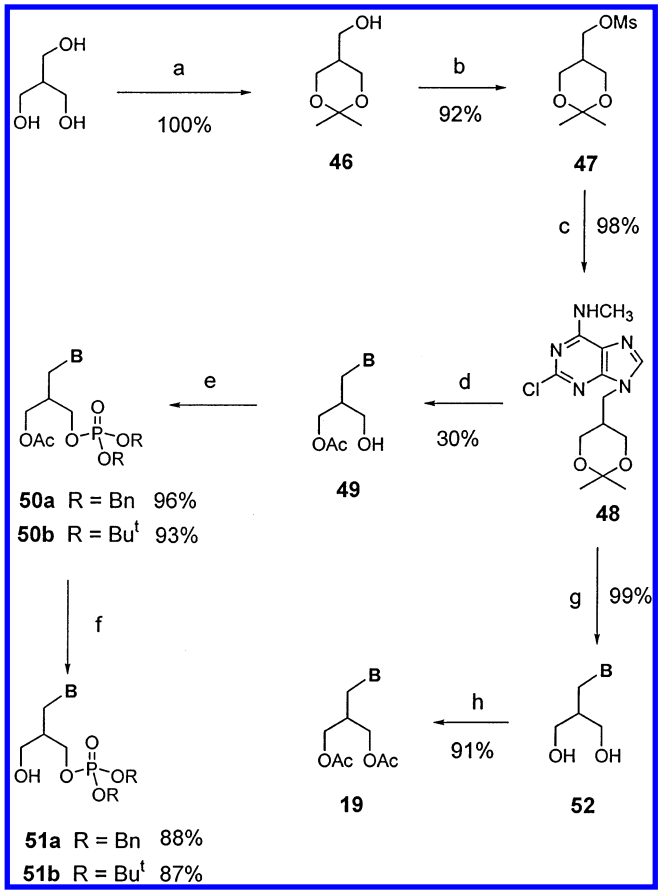
[a] Reagents: (a) Ac_2O, DMAP, Py, CH_2Cl_2; (b) $Et_2NP(OBu^{-t})_2$, 1H-tetrazole, *m*-CPBA, THF/CH_2Cl_2; (c) 10% TFA in CH_2Cl_2; (d) NH_3/H_2O; (e) DHP, TsOH−H_2O; (f) KOH/MeOH; (g) Ac_2O, DMAP, Et_3N, CH_2Cl_2. **B** = 2-chloro-6-methylaminopurin-9-yl.

Two acyclic phosphonate derivatives **38** and **39** were synthesized as shown in Scheme 6. The key step in the synthesis was the Arbuzov reaction for introduction of the phosphonate group by heating a halo derivative (dichloro **63** or dibromo **61**) with triethyl phosphite. The phosphonates differed in the length of the adjacent carbon chains.

Biological Activity. The inhibition of ADP-induced activation of rat platelets by the various nucleotide derivatives as antagonists was measured using platelet aggregometry. Traces representing the percent aggregation as a function of time following exposure to 3.3 μM ADP are shown in Figure 1 for representative compounds. Compounds **2a** and **14** (Figure 1A and 1C) were previously reported as antagonists of turkey $P2Y_1$ receptors,[14,20] and they showed moderately potent inhibition of aggregation in the micromolar range. The monoester **21** and diester **26** (Figure 1B and 1D), analogues of the acyclic derivative **14** in which the number of negative charges was reduced by pivaloyl ester substitution of phosphate, displayed antiaggregatory potencies in the order **14** > **2a** > **26** > **21**.

Concentration−response curves were constructed for inhibition of rat platelet aggregation by all of the compounds tested, and the K_I values are reported in Table 1. Generally the degree of inhibition by various nucleotide antagonists was concentration dependent. Concentration−response curves for selected potent compounds in comparison to known $P2Y_1$ receptor bisphosphate antagonists, the riboside **2b** and the acyclic derivative **14** (Figure 2A and 2B), and two representative derivatives of lesser charge, monophosphate **24** and diester **31** (Figure 2C and 2D), are shown. Acyl substitution of either or both of the phosphate groups of the potent (N)-methanocarba antagonist **3**, that is, **9**−**11**,[16] greatly reduced but did not prevent inhibition of rat platelet aggregation. There was no significant distinction in subsequent biological activity evaluation between acetylation at the 3′- or 5′-position or at both positions simultaneously. In the series of nonsymmetrically substituted acyclic monophosphate derivatives, the optimal antagonist potency occurred with the phenylcarbamate derivative **24**, which had a K_I value of 2.5 μM. Among symmetrical diester derivatives, the optimal antagonist potency occurred with the di(phenylacetyl) derivative **31,** which had a K_I value of 2.0 μM. Other potent inhibitors of platelet aggregation (K_I values ≤ 10 μM) that were neither bisphosphate nor bisphosphonate derivatives were the isopropyl carbamate **6**, a methanocarba derivative **10**, and the dipivaloyl **26**, dibenzoyl **28**, and the mixed benzoyl/acetyl **36** ester derivatives. Substitution of the rings of **28** with fluorine, for ex-

Scheme 3. Synthesis of 2-Chloro-N^6-methyladenine-9-(2-methylpropyl) Derivatives Similar to **14**, in Which Either or Both Phosphate Ester Groups Have Been Removed or Replaced with an Acetyl Group[a]



[a] Reagents: (a) $Me_2C(OMe)_2$, TsOH−H_2O, THF; (b) MsCl, Et_3N, CH_2Cl_2; (c) 2-chloro-6-methylaminopurine, K_2CO_3, DMF, 60 °C; (d) AcOH/H_2O/THF (35:65:10), 70 °C; (e) $^iPr_2NP(OBn)_2$ or $Et_2NP(OBu^{-t})_2$, 1H-tetrazole, *m*-CPBA, THF/CH_2Cl_2; (f) K_2CO_3, CH_3OH; (g) 80% HOAc; (h) Ac_2O, DMAP, Py, CH_2Cl_2. **B** = 2-chloro-6-methylaminopurin-9-yl.

ample, **29** and **30**, reduced potency in inhibition of rat platelet aggregation.

The bisphosphonate derivatives **38** and **39** were inhibitors of rat platelet aggregation. The shorter homologue **38** was the more potent of the two derivatives, with a K_I value of 0.68 μM. Thus, the phosphate group of acyclic inhibitors may be substituted with the nonhydrolyzable phosphonate group.

Substitution of only one of the phosphate groups, for example, **21**, with O-acyl greatly reduced the inhibitory potency, which tended to increase upon replacement of both phosphate moieties of the acyclic derivatives with uncharged (e.g., ester) groups, e.g., **26**. This was also true for the pair of acetyl derivatives **18** (mono) and **19** (di) and similarly for the benzoyl derivatives **22** and **28**. However, the di(phenylcarbamate) derivative **32** was less potent than the corresponding mixed phosphate/carbamate **24.** The di(phenylcarbamate) **32** and di(phenyl carbonate) **33** derivatives were equipotent in inhibition of platelet aggregation.

Effects on the $P2Y_1$ receptor-linked PLC pathway and the $P2Y_{12}$ receptor-linked adenylate cyclase pathway were determined for selected compounds. For the known nucleotide (cyclic and acyclic) bisphosphate antagonists of $P2Y_1$ receptors, ribosides **2**, the potent (N)-methanocarba antagonist **3**, and the acyclics **12**−**17**, there was a significant correlation between inhibition of aggregation and inhibition of turkey $P2Y_1$ receptor-induced PLC activity (Figure 3) determined previously.[19,20] Thus, bisphosphate derivatives in the riboside, (N)-methanocarba, and acyclic series behaved as inhibitors of platelet aggregation in a predictable fashion based on their potency as antagonists of $P2Y_1$ receptor-induced PLC.

However, derivatives in which one or more of the phosphate groups have been replaced with uncharged substitutes did not behave predictably as inhibitors of platelet aggregation on the basis of $P2Y_1$ receptor affinity, which decreased dramatically with the replacement of either or both phosphates with acyl groups.[20] The bisphosphate **14** (Figure 4A), which potently inhibited platelet aggregation, did so without antagonizing the rat platelet $P2Y_{12}$ receptor. However, compound **14** was shown to inhibit PLC stimulated by 30 nM 2-methylthio-ADP in 1321N1 astrocytoma cells expressing the human $P2Y_1$ receptor with an IC_{50} of 50 nM. Conversely, with the uncharged dipivaloyl derivative **26** (Figure 4B), there was no correlation between inhibition of aggregation (moderate) and $P2Y_1$ receptor-induced PLC (essentially absent) activity. However, compound **26** antagonized the inhibition of cAMP induced by ADP in rat platelets in the presence of PGE_1 with an IC_{50} of 7 μM, indicating antagonist activity at the $P2Y_{12}$ receptor.

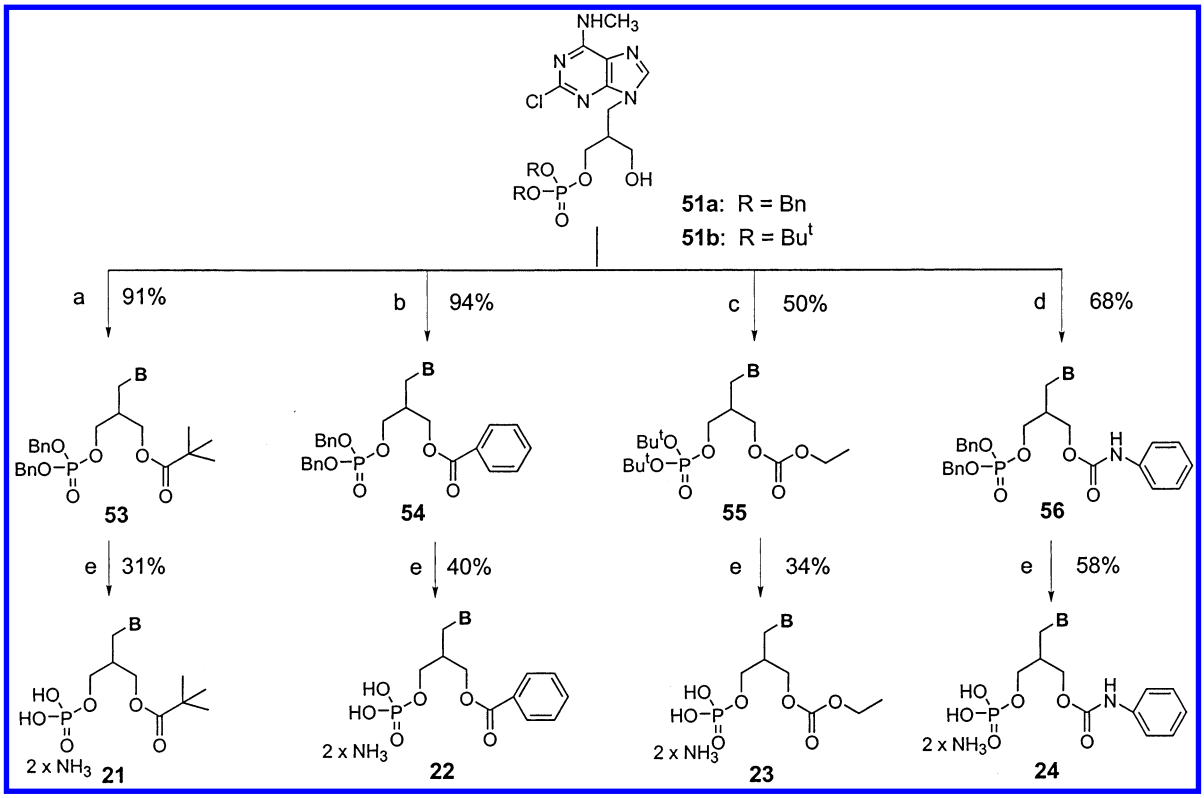
Selected compounds were examined as inhibitors of ADP-induced aggregation of human platelets.[33] Both compound **26**, the dipivaloyl derivative, and compound **31**, the diphenylacetyl derivative, inhibited the aggregation induced by 2 μM ADP in a concentration-dependent manner. In human platelets, **26** and **31** had IC_{50} values of 30 and 45 μM, respectively (Figure 5), and also partially reversed the ADP (5 μM)-induced inhibition of PGE_1-induced cAMP increase (data not shown). **26** or **31** alone had no effect on human platelet cAMP levels.

Discussion

We have demonstrated a correlation between the affinity of moderately potent $P2Y_1$ receptor antagonists (bisphosphate derivatives) and the ability to inhibit platelet aggregation. This quantitative linkage should aid the development of potentially therapeutically interesting inhibitors on the basis of screening in recombinant receptor systems.

The initial objective of this study was to explore the steric and electronic constraints of the $P2Y_1$ receptor binding site and the structural basis of receptor activation and to find uncharged substitutions for the phosphate groups. In the process of looking for less highly charged receptor antagonists, we discovered structurally related antagonists of platelet aggregation that do not act through the PLC-coupled $P2Y_1$ receptor, rather through an ADP-inhibited cyclic AMP pathway in platelets, which is the second messenger of the $P2Y_{12}$ receptor. We therefore propose that uncharged derivatives of known acyclic $P2Y_1$ receptor antagonists of which compound **26** is representative acted as antagonists of this parallel pro-aggregatory receptor present on platelets, that is, the $P2Y_{12}$ receptor. We have

Scheme 4. Synthesis of 2-Chloro-N^6-methyladenine-9-(2-methylpropyl) Derivatives Similar to **14**, in Which One of the Phosphate Ester Groups Has Been Removed or Replaced with an Acyl Group[a]



[a] Reagents: (a) pivalic anhydride, pyridine, CH_2Cl_2; (b) $(PhCO)_2O$, pyridine, DMAP, CH_2Cl_2; (c) ClCOOEt, Et_3N, DMAP, CH_2Cl_2; (d) PhNCO, Et_3N, CH_2Cl_2; (e) (i) TMSBr, CH_2Cl_2; (ii) triethylammonium bicarbonate buffer. **B** = 2-chloro-6-methylaminopurin-9-yl.

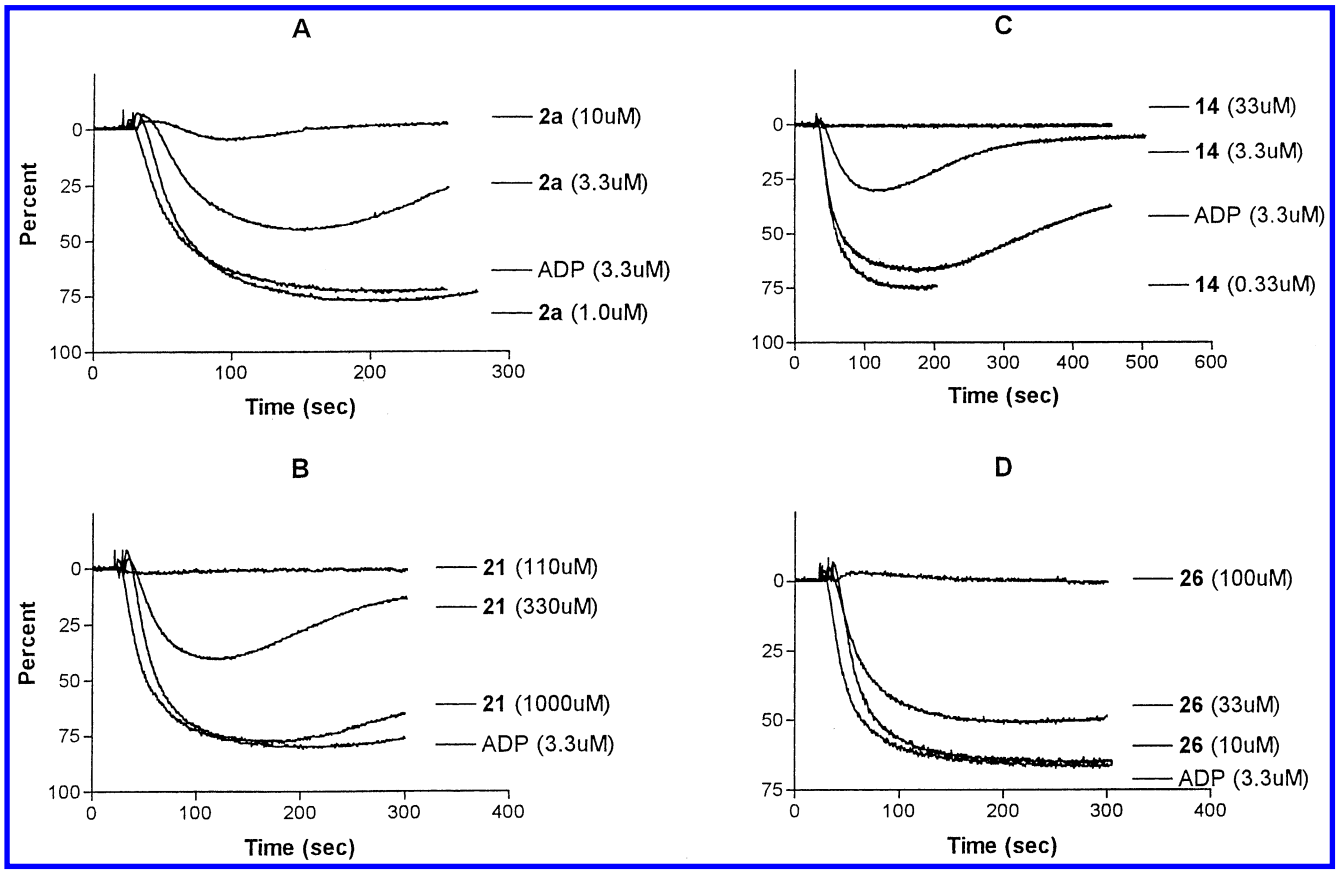
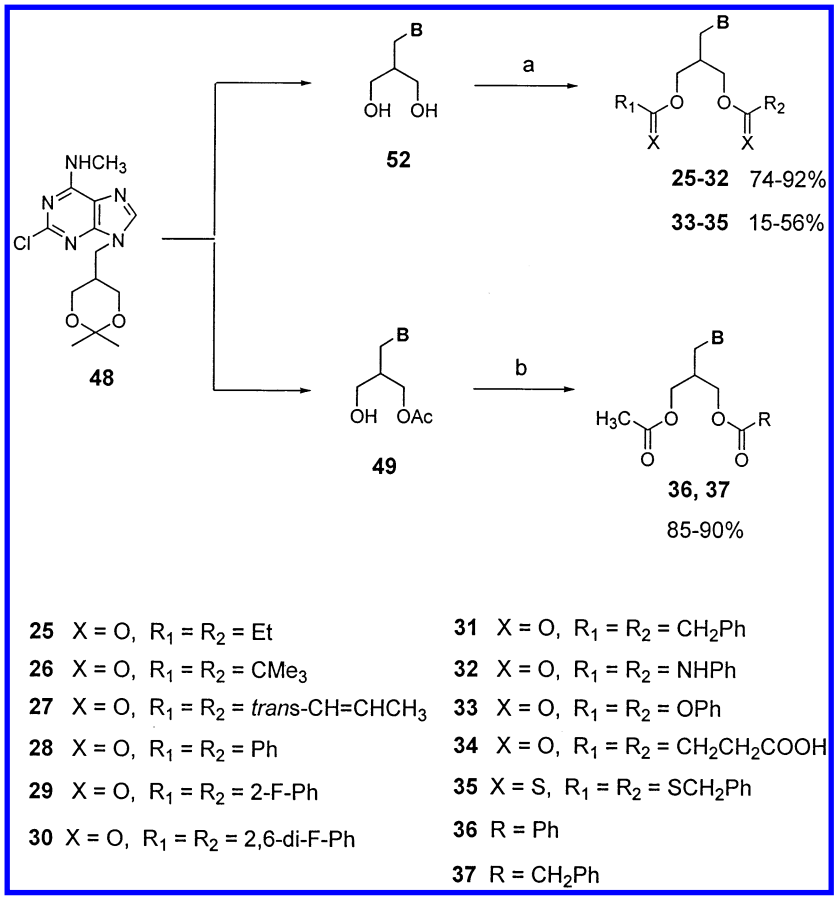


Figure 1. Aggregometry traces for ADP-induced rat platelet aggregation in the presence or absence of various inhibitors: A, riboside **2a**; B, acyclic monophosphate monopivaloyl derivative **21**; C, acyclic bisphosphate **14**; D, acyclic dipivaloyl derivative **26**. In each case 3.3 μM ADP was present.

Scheme 5. Synthesis of 2-Chloro-N^6-methyladenine-9-(2-methylpropyl) Derivatives Similar to **14**, in Which Both of the Phosphate Ester Groups Has Been Replaced Nonsymmetrically with Acyl Groups[a]



[a] Reagents: (a) for **25**, $(CH_3CH_2CO)_2O$, DMAP, pyridine, CH_2Cl_2; for **26**, $(Me_3CCO)_2O$, DMAP, pyridine, CH_2Cl_2; for **27**, crotonic anhydride, DMAP, pyridine, CH_2Cl_2; for **28**, $(PhCO)_2O$, pyridine, DMAP, CH_2Cl_2; for **29**, $(2\text{-F-PhCO})_2O$, pyridine, DMAP, CH_2Cl_2; for **30**, $(2,6\text{-di-F-PhCO})_2O$, pyridine, DMAP, CH_2Cl_2; for **31**, $(PhCH_2CO)_2O$, DMAP, pyridine, CH_2Cl_2; for **32**, PhNCO, Et_3N, CH_2Cl_2; for **33**, ClCOOPh, Et_3N, DMAP, CH_2Cl_2; for **34**, succinic anhydride, pyridine; for **35**, CS_2, NaOH, $PhCH_2Br$, DMSO. (b) For **36**, $(PhCO)_2O$, DMAP, pyridine, CH_2Cl_2; for **37**, $(PhCH_2CO)_2O$, DMAP, pyridine, CH_2Cl_2. **B** = 2-chloro-6-methylaminopurin-9-yl.

demonstrated that this compound failed to interact with the known second messenger coupled to the $P2Y_1$ receptor, that is, phospholipase C; thus, compound **26** appeared to be selective for the $P2Y_{12}$ receptor versus the $P2Y_1$ receptor. The possibility of action through a prostaglandin receptor, activation of which would inhibit platelet aggregation by increasing cAMP, has been ruled out by the demonstration that the antagonists, when added to resting platelet suspensions, did not increase the concentration of platelet cAMP. In contrast, the antagonists prevented the inhibition by ADP of PGE_1-induced increase in human and rat platelet cAMP, indicating that they antagonize the platelet responses to ADP that are mediated by the platelet $P2Y_{12}$ receptor.

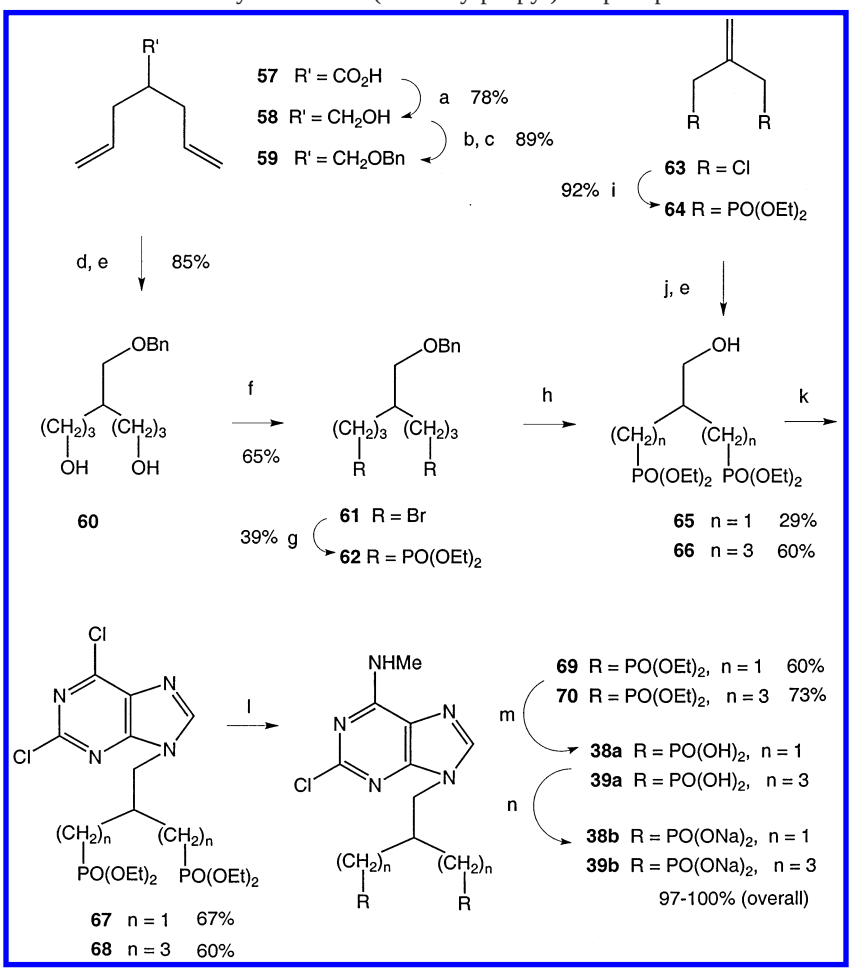
The apparent antagonists of the $P2Y_{12}$ receptor discovered in this study are considerably less potent than the nucleotide/nucleoside antagonists in the series of **1**,[13] some of which have nanomolar potency. Nevertheless, we have shown that different substitution of the same nucleoside scaffold can target either of two P2Y receptors in platelets. The use of a common 9-alkyladenine scaffold promises to aid in the molecular modeling of binding modes of P2Y receptor antagonists[16,19] and in the homology modeling of these receptor subtypes.

In conclusion, this study describes the platelet antiaggreagtory effects of analogues of both cyclic and acyclic nucleotide derivatives. For a subset of these derivatives, that is, those that have bisphosphate groups, there is a correlation between the ability to inhibit platelet aggregation and the potency as antagonists of the phospholipase C-coupled $P2Y_1$ receptor. As the structure was modified to include less highly charged derivatives, substantial antiaggregatory activity remained; however, the mechanism of this blockade of the action of ADP appeared to be through the $P2Y_{12}$ (cyclase-linked) receptor. Both receptor subtypes are present on platelets, and antagonism of each separately has been shown to inhibit aggregation.[4,13,17,22,34] Thus, the interpretation is consistent with known biological pathways. We have identified a new acyclic structural lead for the design of P2Y receptor-based inhibitors of platelet aggregation, which are of low molecular weight and are uncharged.

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). Compounds **2a**, **2b**, **3**, **12**−**18**, and **20** were synthesized as reported.[16,19,20] The final phosphate-containing products were stored in closed vials at 4 °K under nitrogen gas. ^1H 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

Scheme 6. Synthesis of 2-Chloro-N^6-methyladenine-9-(2-methylpropyl) Bisphosphonate Derivatives Similar to **14**[a]



[a] Reagents: (a) $LiAlH_4$, THF; (b) NaH, THF; (c) *n*-Bu_4NI, BnBr; (d) 9-BBN, THF; (e) H_2O_2, NaOH; (f) Br_2, Ph_3P, DMF; (g) $P(OEt)_3$, 155°; (h) H_2 , 5% Pd/carbon, MeOH; (i) $P(OEt)_3$, 155°; (j) 9-BBN, THF, reflux; (k) 2,6-dichloropurine, Ph_3P, DEAD, THF; (l) $MeNH_2$, CH_3CN; (m) Me_3SiI, CH_3CN; (n) Dowex-Na.

or as relative ppm downfield from DMSO (2.5 ppm) or HOD peaks (4.87 ppm). ^{31}P NMR spectra were recorded at room temperature by use of a 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 determination of purity was performed with a Hewlett-Packard 1090 HPLC system using a Luna 5μ C18 analytical column (250 mm × 4.6 mm, Phenomenex) in two different linear gradient solvent systems. For monophosphates: 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 dihydrogen phosphate buffer: CH_3CN, 80:20 to 40:60, in 20 min with flow rate 1 mL/min. For nonphosphates: one solvent system (C) was CH_3CN:CH_3OH in ratio of 80:20 to 20:80 for 20 min with flow rate 1 mL/min; the other system (D) was CH_3CN:H_2O in ratio of 80:20 to 20:80 for 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.

(1*R*,2*S*,4*S*,5*S*) Acetic Acid 4-(2-chloro-6-methylamino-purin-9-yl)-2-hydroxy-bicyclo[3.1.0]hex-1-ylmethyl Ester (41).

To a solution of **40** (18.5 mg, 0.06 mmol) and pyridine (0.05 mL, 0.62 mmol), 4-(dimethylamino)pyridine (2.0 mg, 0.016 mmol) in dry methylene chloride (2 mL) was added acetic anhydride (6 μL, 0.066 mmol) dropwise. After stirring at room temperature for 1 day, the volatile materials were removed in vacuo. The residue was purified by preparative thin-layer chromatography (chloroform:methanol = 5:1) to give a monoacetyl alcohol **41** (16.8 mg, 80%). ^1H NMR (CD_3OD) δ 0.90 (m, 1H), 1.14 (m, 1H), 1.71−2.20 (m, 3H), 2.11 (s, 3H), 3.08 (bs, 3H), 3.99 (d, 1H, J = 12.0 Hz), 4.69 (d, 1H, J = 12.0 Hz), 4.81 (t, 1H, J = 8.4 Hz), 4.96 (d, 1H, J = 6.6 Hz), 8.19 (s, 1H); MS (FAB+) 352 ($M + H^+$).

(1*R*,2*S*,4*S*,5*S*) Acetic Acid 4-(2-chloro-6-methylamino-purin-9-yl)-2-(di-tert-butoxy-phosphoryloxy)-bicyclo[3.1.0]-hex-1-ylmethyl Ester (42). Neat di-*tert*-butyl *N*,*N*-diethylphosphoramidite (47 μL, 0.179 mmol) was added by syringe to a stirred solution of **41** (20 mg, 0.0569 mmol) in anhydrous tetrahydrofuran (1.5 mL) at room temperature, followed by an addition of solid tetrazole (24 mg, 0.343 mmol). After stirring at room temperature for 20 min, the reaction mixture was cooled to −78 °C, and a solution of *m*-CPBA (57 ∼ 85%, 49 mg) in methylene chloride (2 mL) was added rapidly. The reaction mixture was warmed to 0 °C and stirred for 5 min. Triethylamine (0.5 mL, 3.59 mmol) was added to maintain a basic condition to avoid cleavage of *tert*-butyl groups. Purification was accomplished by using preparative thin-layer chromatography (chloroform/methanol = 6:1) to give **42** (15 mg, 48.5%) as an oil. ^1H NMR ($CDCl_3$) δ 0.94 (m, 1H), 1.04−1.22 (m, 1H), 1.46 (s, 18H), 1.48 (s, 18H), 1.70 (m, 1H), 2.15 (s, 3H), 1.94−2.14 (m, 1H), 2.32 (dd, 1H, J = 10.1, 15.2 Hz), 3,18 (bs, 3H), 3.91 (d, 1H, J = 12.1 Hz), 4.79 (d, 1H, J = 12.1 Hz), 5.10

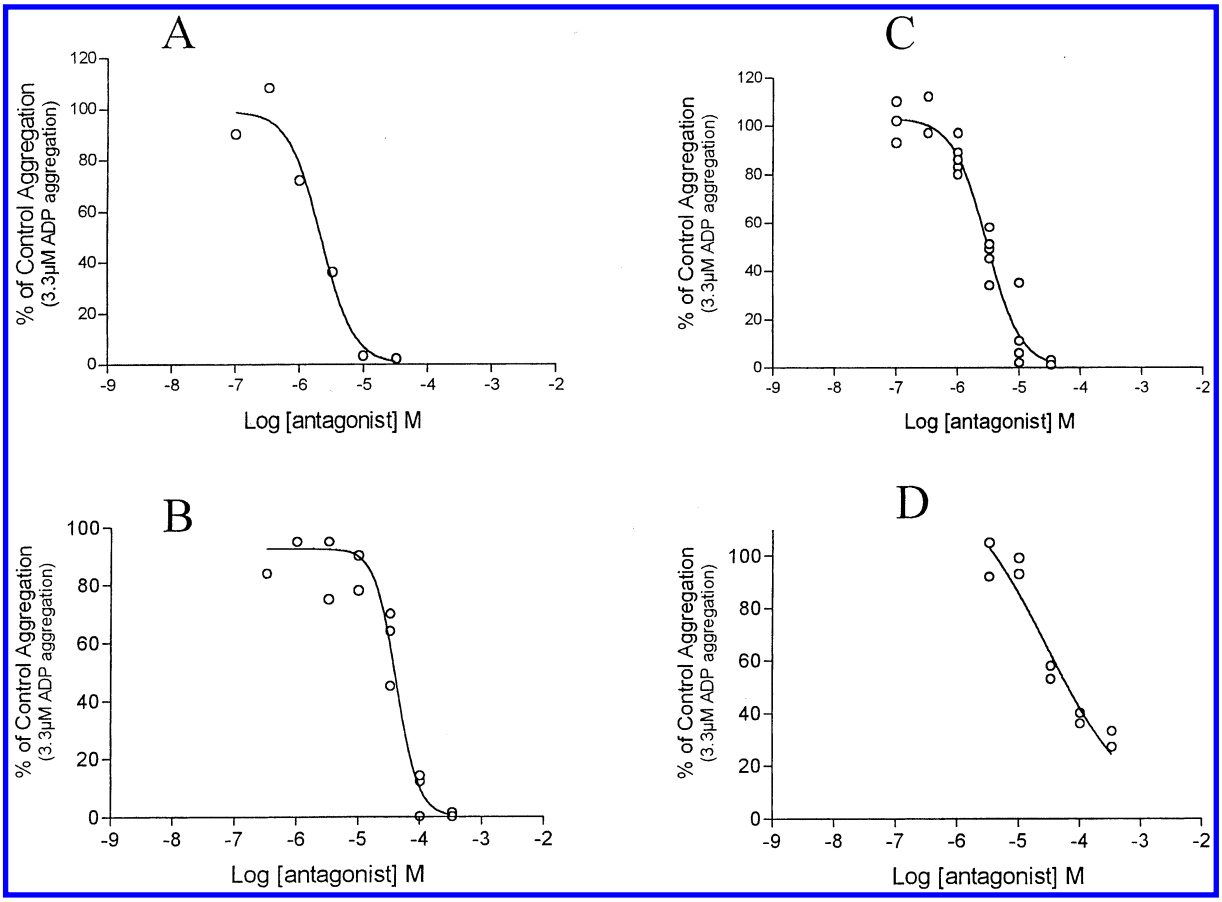


Figure 2. Concentration−response curves for ADP-induced rat platelet aggregation in the presence or absence of various inhibitors: A, riboside **2b**; B, acyclic phenylurethane monophosphate derivative **24**; C, acyclic bisphosphate **14**; D, diphenylacetate derivative **31**. In each case 3.3 μM ADP was present.

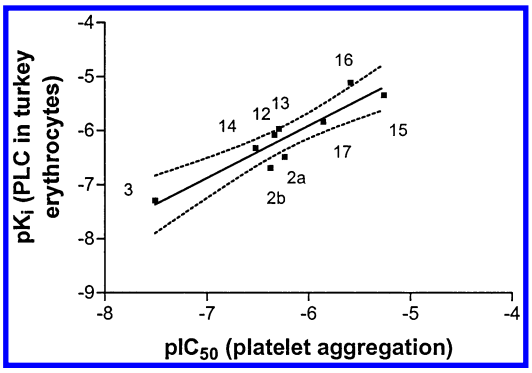


Figure 3. Correlation of inhibition of phospholipase C activity in turkey erythrocytes expressing a native $P2Y_1$ receptor with the potency in the inhibition of rat platelet aggregation of previously reported antagonists **2a**, **2b**, **3**, and **12**−**17**. Values for the PLC inhibition are taken from refs 10, 17, and 18. Dashed curves represent the 95% confidence range ($r^2 = 0.842$).

(d, 1H, J = 6.9 Hz), 5.38 (q, 1H, J = 7.7 Hz), 6.12 (bs, 1H), 8.04 (s, 1H); MS (FAB+) 544 (M + H^+).

(1*R*,2*S*,4*S*,5*S*) Acetic Acid 4-(2-chloro-6-methylamino-purin-9-yl)-2-phosphonooxy-bicyclo[3.1.0]hex-1-ylmethyl Ester (9). A solution of di-*tert*-butyl phosphate **42** (15 mg, 0.0276 mmol) in 10% trifluoroacetic acid in methylene chloride (2 mL) was stirred for 30 min at room temperature. All volatile material was removed in vacuo. The residue was purified using ion-exchange column chromatography on Sephadex-DEAE-A-25 resin and a linear gradient (0.01−0.5 M) of 0.5 M triethylammonium bicarbonate as the mobile phase to give monophosphate **9** (12.1 mg, 91%) as a triethylammonium salt. ^{1}H NMR (D_2O) δ 1.02 (m, 1H), 1.25 (t, 9/4H, J = 7.0 Hz, from triethylamine), 1.27 (t, 9/4H, J = 7.0 Hz, from triethylamine), 1.21−1.33 (m, 1H), 1.89 (dd, 1H, J = 3.9 Hz), 2.02 (m, 1H), 2.12 (s, 3H), 2.38 (dd, 1H, J = 8.0, 15.1 Hz), 3.05 (bs, 3H), 3.19 (q, 3/2H, J = 7.0 Hz), 3.29 (q, 3/2H, J = 7.0 Hz), 3.98 (d, 1H, J = 12.1 Hz), 4.67 (d, 1H, J = 11.8 Hz), 4.93 (d, 1H, J = 6.6 Hz), 5.16 (m, 1H), 8.29 (s, 1H); ^{31}P NMR (D_2O) δ 0.538.

(1*R*,2*S*,4*S*,5*S*) Phosphoric Acid mono-[4-(2-chloro-6-methylamino-purin-9-yl)-1-hydroxymethyl-bicyclo-[3.1.0] hex-2-yl] Ester (7). The acetate **9** (11.4 mg, 0.0237 mmol) was treated with water (10 mL) and 29% ammonia in water (2 mL) and then the reaction mixture was stirred overnight. The ammonia and water were removed in vacuo, and the residue was purified using ion-exchange column chromatography on Sephadex DEAE-A-25 resin and a linear gradient (0.01−0.5 M) of 0.5 M ammonium bicarbonate as the mobile phase to give monophosphate **7** (5.5 mg, 55%) as an ammonium salt.^{1}H NMR (D_2O) δ 0.92 (m, 1H), 1.13 (m, 1H), 1.75 (m, 1H), 1.96 (m, 1H), 2.27 (dd, 1H, J = 7.8, 15.1 Hz), 3.00 (bs, 3H), 3.63 (d, 1H, J = 12.5 Hz), 3.95 (d, 1H, J = 12.5 Hz), 4.67−4.90 (m, 1H), 5.09 (m, 1H), 8.22 (s, 1H); ^{31}P NMR (D_2O) δ 2.368.

(1*R*,2*S*,4*S*,5*S*) [4-(2-Chloro-6-methylamino-purin-9-yl)-2-(tetrahydro-pyran-2-yloxy)-bicyclo[3.1.0]hex-1-yl]methanol (44). Monoacetate **41** (16.8 mg, 0.048 mmol) was dissolved in methylene chloride (2 mL), followed by subsequent addition of *p*-toluenesulfonic acid monohydrate (2 mg, 0.011 mmol) and dihydropyran (5 μL, 0.055 mm0l). The reaction mixture was stirred for 3 h, and the solvent was removed by nitrogen stream to give a crude acetylpyran **43** (23 mg). The residue was used in the next reaction without further purification.

The crude acetylpyran **43** (23 mg) was dissolved in 0.2 M potassium hydroxide (in methanol, 0.36 mL, 0.072 mmol). After stirring for 2 h, the resulting mixture was neutralized with 0.1 M acetic acid (1 mL). All volatile material was removed in vacuo and the residue was purified by preparative

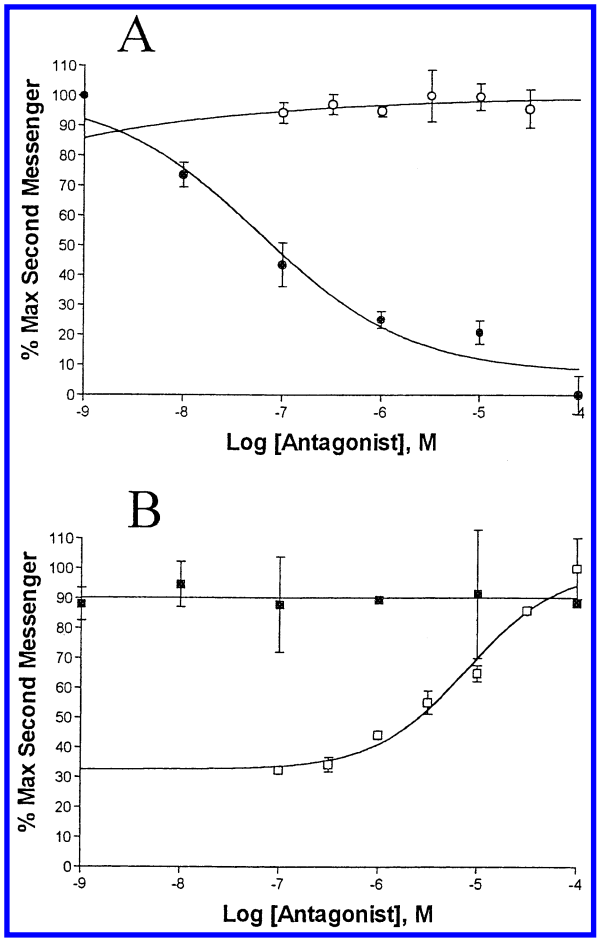


Figure 4. Concentration−response curves for inhibition of P2Y receptor-induced second messengers, either (□) cyclic AMP levels in rat platelets (in the presence of 3.3 μM ADP) or (●) phospholipase C activity in 1321N1 astrocytoma cells expressing the $hP2Y_1$ receptor. Measurements were made in the presence of increasing concentrations of the following inhibitors: A, acyclic bisphosphate **14** and B, acyclic dipivaloyl derivative **26**.

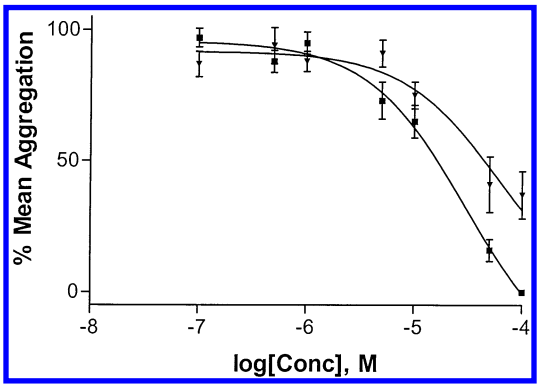


Figure 5. Concentration−response curves for inhibition of ADP-induced aggregation of human platelets by the acyclic dipivaloyl derivative **26** (■) and the di(phenylacetyl) derivative **31** (▼), expressed as percent (mean ± SD) of ADP (5 μM)-induced platelet aggregation in the presence of 1% DMSO (control samples).

thin-layer chromatography (chloroform: methanol = 7:1) to give the hydroxypyran **44** (18.8 mg, 100% two-steps yield) as a mixture of diastereomers. ^{1}H NMR ($CDCl_3$) δ 0.75−2.24 (m, 13H), 3.18 (bs, 3H), 3.20−5.11 (m, 7H), 6.16 (bs, 1H), 7.98, 8.10 (two singlet, 1H).

(1*R*,2*S*,4*S*,5*S*) Phosphoric Acid di-tert-butyl ester 4-(2-chloro-6-methylamino-purin-9-yl)-2-(tetrahydro-puran-2-yloxy)-bicyclo[3.1.0]hex-1-ylmethyl Ester (45). Neat di-*tert*-butyl *N*,*N*-diethylphosphoramidite (52 μL, 0.188 mmol) was added by syringe to a stirred solution of **44** (18.5 mg, 0.047 mmol) in anhydrous tetrahydrofuran (2 mL) at room temperature, followed by an addition of solid tetrazole (20 mg, 0.282 mmol). After stirring at room temperature for 20 min, the reaction mixture was cooled to −78 °C, and a solution of m-CPBA (57 ∼ 65%, 54 mg) in methylene chloride (2 mL) was added rapidly. The reaction mixture was warmed to room temperature and stirred for 5 min. Triethylamine (0.5 mL, 3.59 mmol) was added to maintain a basic condition to avoid cleavage of *tert*-butyl groups. Purification was accomplished by using preparative thin-layer chromatography (chloroform/methanol = 10:1, and then ethyl acetate alone) to give **45** (19 mg, 69%) as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$) δ 0.81−2.24 (m, 13H), 1.51 (s, 18H), 3.11−5.19 (m, 7H), 6.72 (bs, 1H), 8.23, 8.30 (two singlets, 1H).

(1*R*,2*S*,4*S*,5*S*) Phosphoric Acid mono-[4-(2-chloro-6-methylamino-purin-9-yl)-2-hydroxy-bicyclo[3.1.0]hex-1-ylmethyl] Ester (8). A solution of di-*tert*-butyl phosphate **45** (8 mg, 0.014 mmol) in 10% trifluoroacetic acid in a mixed solvent (tetrahydrofuran:water = 2:1, 2 mL) was stirred for 8 h at room temperature. All volatile material was removed in vacuo. The residue was purified using ion-exchange column chromatography on Sephadex DEAE-A-25 resin and a linear gradient (0.01 to 0.5 M) of 0.5 M ammonium bicarbonate as the mobile phase to give monophosphate **8** (3.3 mg, 57%) as an ammonium salt. ^{1}H NMR (D_2O) δ 0.96 (m, 1H), 1.28 (m, 1H), 1.73−1.93 (m, 2H), 2.10 (dd, 1H, J = 8.0, 14.8 Hz), 3.02 (s, 3H), 3.72 (dd, 1H, J = 3.9, 11.3 Hz), 4.45 (dd, 1H, J = 3.1, 11.2 Hz), 4.76−4.88 (m, 2H), 8.38 (bs, 1H); ^{31}P NMR (D_2O) 0.960.

(1*R*,2*S*,4*S*,5*S*) Acetic Acid 4-(2-chloro-6-methylamino-purin-9-yl)-phosphonooxymethyl-bicyclo[3.1.0]hex-2-yl Ester (10). A mixture of hydroxy phosphate **8** (1.5 mg, 3.5 μmol), triethylamine (10 μL, 0.070 mmol), 4-(dimethylamino)-pyridine (0.6 mg, 5 μmol), acetic anhydride (3.3 μL, 0.035 mmol), and methylene chloride (0.5 mL) was stirred for 6 h, followed by an addition of water (0.2 mL). After the resulting mixture was stirred for 1 h, all volatile material was removed in vacuo. The residue was purified using ion-exchange column chromatography on Sephadex DEAE-A-25 resin and a linear gradient (0.01 to 0.5 M) of 0.5 M triethylammonium bicarbonate as the mobile phase to give monophosphate **10** (0.8 mg, 42%) as a triethylammonium salt. ^{1}H NMR (D_2O) δ 1.09 (m, 1H), 1.28 (t, 9H, J = 7.4 Hz), 1.21−1.34 (m, 1H), 1.85−2.01 (m, 2H), 2.36 (dd, 1H, J = 8.0, 14.8 Hz), 3.06 (bs, 3H), 3.19 (q, 6H, J = 7.4 Hz), 3.74 (dd, 1H, J = 5.0, 11.0 Hz), 4.37 (dd, 1H, J = 4.7, 11.0 Hz), 4.96 (d, 1H, J = 6.6 Hz), 5.62 (m, 1H), 8.40 (s, 1H); ^{31}P NMR (D_2O) 0.613.

Acetic Acid 1-acetoxymethyl-4-(2-chloro-6-methylamino-purin-9-yl)-bicyclo[3.1.0]hex-2-yl Ester (11). A mixture of diol **40** (37 mg, 0.12 mmol), pyridine (1 mL), 4-(dimethylamino)pyridine (10 mg, 0.082 mmol), acetic anhydride (58 μL, 0.6 mmol), and methylene chloride (2 mL) was stirred overnight. The resulting mixture was treated with water (0.5 mL), stirred for 1 h, and then all volatile material was removed by nitrogen stream. The residue was purified by preparative thin-layer chromatography (chloroform:methanol = 10:1) to give diacetate **11** (46 mg, 98%) as a white solid. mp 194 °C; ^{1}H NMR ($CDCl_3$) δ 0.98 (m, 1H), 1.14 (dd, 1H, J = 4.1, 6.0 Hz), 1.73 (dd, 1H, J = 3.9, 8.8 Hz), 1.86 (ddd, 1H, J = 7.4, 8.2, 15.4 Hz), 2.09 (s, 3H), 2.17 (s, 3H), 2.39 (dd, 1H, J = 8.0, 15.1 Hz), 3.20 (bs, 3H), 3.91 (d, 1H, J = 12.1 Hz), 4.63 (d, 1H, J = 12.1 Hz), 5.13 (d, 1H, J = 6.9 Hz), 5.69 (dd, 1H, J = 8.3, 8.5 Hz), 5.94 (bs, 1H), 8.05 (s, 1H).

2,2-Dimethyl-[1,3]-dioxan-5-yl)methanol (46). To a solution of 2-(hydroxymethyl)propane-1,3-diol (5.0 g, 45.7 mmol) and 4-toluenesulfonic acid monohydrate (0.27 g, 1.42 mmol) in THF (100 mL) was added 2,2-dimethoxypropane (6.5 mL, 52.9 mmol). The solution was stirred for 2.5 h at room temperature and was then neutralized by the addition of Et_3N (3 mL). The solvent was removed, and the residue was purified by column chromatography on silica gel eluting with CH_3OH/

$CHCl_3$ = 1:10 gave **46** (6.66 g, 99.8%) as a colorless liquid. ^{1}H NMR ($CDCl_3$) δ 4.08 ~ 3.96 (m, 2H), 3.84 ~ 3.7 (m, 4H), 1.92 ~ 1.78 (m, 1H), 1.75 (s, 1H), 1.45 (s, 3H), 1.41 (s, 3H).

Methanesulfonic Acid 2,2-dimethyl-[1,3]-dioxan-5-yl-methyl Ester (47). To approximately 0.2 M solution of alcohol (0.89 g, 6.1 mmol) in CH_2Cl_2 containing a 50% molar excess of Et_3N (1.4 mL) at 0 °C, cooled by ice−water bath, was added a 10% excess of MsCl (0.52 mL) dropwise. Stirred at this constant temperature, the mixture turned yellowish and not clear. It was stirred at 0 °C for 3.5 h and the resulting mixture was diluted with ethyl acetate (30 mL) and stirred with H_2O (10 mL) for 10 min. The organic layer was separated and the upper layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layer was washed with H_2O and brine and dried over Na_2SO_4. The solvent was removed, and the residue was purified by column chromatography on silica gel (MeOH:$CHCl_3$ = 1:20) gave **47** (1.26 g, 92.3%). ^{1}H NMR ($CDCl_3$) δ 4.42 (dd, J = 7.5, 2.4 Hz, 2H), 4.08 (d, J = 12 Hz, 2H), 3.77 (d, J = 12 Hz, 2H), 3.04 (s, 3H), 1.46 (s, 3H), 1.39 (s, 3H).

[2-Chloro-9-(2,2-dimethyl-[1,3]dioxan-5-ylmethyl)-9H-purin-6-yl]-methyl-amine (48). A mixture of 2-chloro-N^6-methyladenine (1.87 g, 10.18 mmol), **47** (2.81 g, 12.54 mmol), and K_2CO_3 (5.23 g, 37.89 mmol) in DMF (50 mL) was stirred at 60 °C overnight. Then the mixture was diluted with EtOAc and filtered. The filtrate was washed with water and brine and dried over Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography on silica gel (MeOH:$CHCl_3$ = 1:15) and gave **48** (3.17 g, 98%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.75 (s, 1H), 6.31 (br, 1H), 4.41 (d, J = 8.1 Hz, 2H), 4.05 (dd, J = 12.6, 2.7 Hz, 2H), 3.56 (dd, J = 11.1, 2.7 Hz, 2H), 3.17 (s, 3H), 2.28 ~ 2.17 (m, 1H), 1.46 (s, 3H), 1.44 (s, 3H).

Acetic Acid 3-(2-chloro-6-methylamino-purin-9-yl)-2-hydroxymethyl-propyl Ester (49). Compound **48** (1.1 g, 3.53 mmol) was dissolved in a solvent of acetic acid (13 mL), THF (2 mL), and H_2O (7 mL), and the mixture was stirred at 65−70 °C overnight. After removal of the solvent, the residue was purified by column chromatography on silica gel (CH_3OH:$CHCl_3$ = 1:20) and gave **49** (0.33 g, 30%) as a thick liquid together with the corresponding diol **9** (0.456 g, 47.6%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.75 (s, 1H), 6.66 (br, 1H), 4.5 ~ 4.2 (m, 3H), 4.2 ~ 4.0 (m, 2H), 3.51 (s, 2H), 3.18 (s, 3H), 2.5 ~ 2.3 (m, 1H), 2.10 (s, 3H).

Acetic Acid 2-(bis-benzyloxy-phosphoryloxymethyl)-3-(2-chloro-6-methylamino-purin-9-yl)-propyl Ester (50a) (Procedure A). 1H-tetrazole (0.4 g, 5.71 mmol) is added in one portion to a stirred solution of the alcohol (0.3 g, 0.958 mmol) and dibenzyl diisopropylphosphoramidite (1 mL, 2.97 mmol) in dry THF (20 mL) and stirred for 20 min at room temperature. The mixture is then cooled to −78 °C (dry ice−acetone) and a solution of *m*-CPBA (0.4 g, 2.32 mmol) in CH_2Cl_2 (2 mL) is rapidly added such that the reaction mixture is kept below 0 °C. After added up, the mixture was allowed to raise to room temperature and stirred for 0.5 h. Then 5% aqueous $NaHSO_3$ was added and the mixture was then transferred to a funnel and extracted with ethyl acetate. The organic layer was washed with $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, and filtered. After removal of the solvent, the residue was purified by column chromatography on silica gel (CH_3OH:$CHCl_3$ = 1:15) to give **50a** (0.526 g, 95.8%). ^{1}H NMR ($CDCl_3$) δ 7.6 (s, 1H), 7.5 ~ 7.28 (m, 10H), 6.22 (br, 1H), 5.16 ~ 4.94 (m, 4H), 4.09 (d, J = 7.9 Hz, 2H), 4.04 ~ 3.82 (m, 4H), 3.16 (s, 3H), 2.7 ~ 2.5 (m, 1H), 2.0 (s, 3H).

Acetic Acid 3-(2-chloro-6-methylamino-purin-9-yl)-2-(di-*tert*-butoxy-phosphoryloxy-methyl)-propyl Ester (50b). Procedure A. Compound **50b** (0.39 g, 92.8%) was obtained from **49** (0.26 g, 0.831 mmol), di-*tert*-butyl diethylphosphoramidite (0.746 mL, 2.49 mmol), 1*H*-tetrazole (0.349 g, 4.98 mmol), *m*-CPBA (0.349 g, 2.02 mmol) in dry THF (10 mL), and CH_2Cl_2 (2 mL). ^{1}H NMR ($CDCl_3$) δ 7.79 (s, 1H), 6.12 (br, 1H), 4.7 ~ 4.4 (m, 2H), 4.3 ~ 3.8 (m, 4H), 3.18 (s, 3H), 2.8 ~ 2.6 (m, 1H), 2.07 (s, 3H), 1.49 (s, 18H).

Phosphoric Acid Dibenzyl Ester 3-(2-chloro-6-methylamino-purin-9-yl)-2-hydroxymethyl-propyl Ester (51a) (Procedure B). A mixture of **50a** (0.5 g, 0.873 mmol) and anhydrous K_2CO_3 (0.121 g, 0.876 mmol) in CH_3OH (15 mL) was stirred at room temperature for 2 h. Then we filtered out the solid, and the given residue was purified with a flash column after evaporation of the solvent (CH_3OH:$CHCl_3$ = 1:15) to give **51a** (0.406 g, 87.6%) as a thick liquid. ^{1}H NMR ($CDCl_3$) δ 7.61 (s, 1H), 7.46 ~ 7.26 (m, 10H), 6.5 (br, 1H), 5.2 ~ 4.9 (m, 4H), 4.5 ~ 4.3 (m, 1H), 4.3 ~ 4.0 (m, 2H), 4.0 ~ 3.7 (m, 2H), 3.5 ~ 3.3 (m, 2H), 3.15 (s, 3H), 2.4 ~ 2.2 (m, 1H).

Phosphoric Acid di-*tert*-butyl Ester 3-(2-chloro-6-methylamino-purin-9-yl)-2-hydroxymethyl-propyl Ester (51b). Procedure B. Compound **51b** (0.255 g, 87%) was obtained from **50b** (0.32 g, 0.632 mmol) and K_2CO_3 (0.133 g, 0.96 mmol) in MeOH (10 mL). ^{1}H NMR ($CDCl_3$) δ 7.80 (s, 1H), 6.08 (br, 1H), 4.5 ~ 4.2 (m, 3H), 4.1 ~ 3.8 (m, 2H), 3.6 ~ 3.3 (m, 2H), 3.18 (s, 3H), 2.5 ~ 2.3 (m, 1H), 1.51 (s, 18H).

2-(2-Chloro-6-methylamino-purin-9-ylmethyl)-propane-1,3-diol (52). The mixture of **48** (0.1 g, 0.32 mmol) in 80% acetic acid (10 mL) was stirred at 70−75 °C for 2.5 h. Then the solvent was removed under reduced pressure by adding toluene. The resulting residue was purified by column chromatography on silica gel (CH_3OH:$CHCl_3$ = 1:6) and afforded **52** (86 mg, 98.6%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 8.2 (br, 1H), 8.05 (s, 1H), 4.61 (t, J = 5.6 Hz, 2H), 4.1 (d, J = 7.3 Hz, 2H), 3.46 ~ 3.24 (m, 4H), 2.91 (d, J = 4.4 Hz, 3H), 2.2 ~ 2.1 (m, 1H).

Acetic Acid 2-acetoxymethyl-3-(2-chloro-6-methylamino-purin-9-yl)-propyl Ester (19) (Procedure C). To the mixture of **52** (0.15 g, 0.552 mmol), acetic anhydride (0.124 g, 1.21 mmol) and pyridine (96 mg, 1.21 mmol) in CH_2Cl_2 (10 mL) was added and DMAP (2 mg) in CH_2Cl_2 (5 mL) at 0 °C. Then the mixture was stirred at room temperature overnight. Then the mixture was quenched by H_2O, neutralized with 0.1 N HCl, and extracted with $CHCl_3$. The organic layer was washed with brine and dried over Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography on silica gel (CH_3OH:$CHCl_3$ = 1:20) and afforded **18** (0.178 g, 91%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.72 (s, 1H), 6.88 (br, 1H), 4.28 (d, J = 8.4 Hz, 2H), 4.2 ~ 4.0 (m, 4H), 3.16 (s, 3H), 2.8 ~ 2.64 (m, 1H), 2.08 (s, 6H). Anal. calc for $C_{14}H_{18}ClN_5O_4$ (355.78): C, 47.26; H, 5.10; N, 19.68. Found: C, 47.53; H, 5.15; N, 19.10.

2,2-Dimethyl-propionic Acid 2-(bis-benzyloxy-phosphoryloxymethyl)-3-(2-chloro-6-methylamino-purin-9-yl)-propyl Ester (53). Procedure C. The mixture of **51a** (20 mg, 0.0376 mmol), pyridine (0.1 mL), and pivalic anhydride (5.6 μL, 0.045 mmol) in CH_2Cl_2 (1 mL) was stirred at room temperature overnight. After workup, **53** was obtained as a pure solid (21 mg, 90.6%). ^{1}H NMR ($CDCl_3$) δ 7.62 (s, 1H), 7.42 ~ 7.3 (m, 10H), 6.73 (br, 1H), 5.14 ~ 4.96 (m, 4H), 4.15 ~ 4.05 (m, 2H), 4.05 ~ 3.85 (m, 4H), 3.17 (s, 3H), 2.66 ~ 2.52 (m, 1H), 1.18 (s, 9H).

2,2-Dimethyl-propionic Acid 3-(2-chloro-6-methylamino-purin-9-yl)-2-phosphono-oxymethyl-propyl Ester, Ammonium Salt (21) (Procedure D). To the solution of **53** (10 mg, 16.3 mmol) in CH_2Cl_2 (1 mL) TMSBr (0.05 mL) in CH_2Cl_2 (1 mL) was added dropwise at room temperature. After added up, the mixture was stirred for an additional 3 h. Then triethylammonium bicarbonate buffer (1M, 5 mL) was added to the mixture and stirred for 30 min. The aqueous layer was washed twice with CH_2Cl_2 and evaporated to dryness under reduced pressure. The residue was purified with ion-exchange column chromatography using Sephadex-DEAE-A-25 resin with a linear gradient (0.01 ~ 0.5 M) of 0.5 M NH_4HCO_3 as the mobile phase. After lyophilization, **21** (2.36 mg, 30.9%) was obtained as a white solid. ^{1}H NMR (D_2O) δ 8.1 (s, 1H), 4.45 ~ 4.24 (m, 2H), 4.22 ~ 4.02 (m, 2H), 4.02 ~ 3.82 (m, 2H), 3.01 (s, 3H), 2.78 ~ 2.54 (m, 1H), 0.89 (s, 9H); ^{31}P NMR (D_2O) 1.016 (s); MS (FAB−): calcd: 436.0987/434.0993; found: 436.0967/434.0996.

Benzoic Acid 2-(bis-benzyloxy-phosphoryloxymethyl)-3-(2-chloro-6-methylamino-purin-9-yl)-propyl Ester (54). Procedure C. Compound **54** (38 mg, 93.5%) was produced from **51a** (34 mg, 0.064 mmol), benzoic anhydride (21.7 mg,

0.096 mmol), pyridine (8 μL, 0.099 mmol), and DMAP (0.5 mg) in CH_2Cl_2 (4 mL). ^1H NMR ($CDCl_3$) δ 8.2 ~ 6.8 (m, 17H), 5.3 ~ 4.8 (m, 4H), 4.4 ~ 3.7 (m, 6H), 3.05 (s, 3H), 2.8 ~ 2.5 (m, 1H).

Benzoic Acid 3-(2-chloro-6-methylamino-purin-9-yl)-2-phosphonooxymethyl-propyl Ester, Ammonium Salt (22). Procedure D. Compound **22** (11.2 mg, 40.4%) was obtained from **54** (36 mg, 56.7 mmol) and TMSBr (0.1 mL) in CH_2Cl_2 (3 mL). ^1H NMR (D_2O) δ 8.05 (s, 1H), 7.6 ~ 7.4 (m, 2H), 7.36 ~ 7.05 (m, 3H), 2.9 ~ 2.7 (m, 1H), 4.55 ~ 4.4 (m, 1H), 4.4 ~ 4.2 (m, 3H), 4.15 ~ 3.95 (m, 2H), 2.61 (s, 3H); ^{31}P NMR (D_2O) 0.814 (s); MS (FAB−): calcd: 456.0666/454.0701; found: 456.0654/454.0683

Carbonic Acid 2-(bis-benzyloxy-phosphoryloxymethyl)-3-(2-chloro-6-methylamino-purin-9-yl)-propyl Ester Ethyl Ester (55). Procedure C. Compound **55** (40 mg, 50%) was produced from **51b** (70 mg, 0.138 mmol), ethyl chloroformate (16 μL, 0.167 mmol), Et_3N (0.1 mL), and DMAP (1 mg) in CH_2Cl_2 (2 mL). The product was purified by column chromatography on silica gel (^{i}PrOH:$CHCl_3$ = 1:15). ^1H NMR ($CDCl_3$) δ 7.61 (s, 1H), 7.46 ~ 7.28 (m, 10H), 5.91 (br, 1H), 5.4 ~ 4.95 (m, 4H), 4.3 ~ 3.8 (m, 8H), 3.16 (s, 3H), 2.8 ~ 2.5 (m, 1H), 1.3 (t, J = 7.14 Hz, 3H).

Carbonic Acid 3-(2-chloro-6-methylamino-purin-9-yl)-2-phosphonooxymethyl-propyl Ester Ethyl Ester, Ammonium Salt (23). Procedure D. Compound **23** (7.3 mg, 34%) was obtained from **55** (35 mg, 0.061 mmol) and TMSBr (0.1 mL) in CH_2Cl_2 (3 mL). ^1H NMR (D_2O) δ 8.09 (s, 1H), 4.4−4.3 (m, 2H), 4.18 (d, J = 5.1 Hz, 2H), 4.02 (q, J = 7.2 Hz, 2H), 3.95−3.85 (m, 2H), 3.03 (s, 3H), 2.79−2.60 (m, 1H), 1.16 (t, J = 7.2 Hz, 3H); ^{31}P NMR (D_2O) 1.106 (s); MS 424 ($M-H^-$).

Phenyl-carbamic Acid 2-(bis-benzyloxy-phosphoryloxymethyl)-3-(2-chloro-6-methylamino-purin-9-yl)-propyl Ester (56) (Procedure E). To a solution of **51a** (60 mg, 0.113 mmol) in dry CH_2Cl_2 (4 mL), Et_3N (5 uL) and phenylisocyanate (15 uL, 0.138 mmol) were added and the mixture was stirred overnight under N_2. The solvent was removed under reduced pressure and the residue was subjected to column chromatography on silica gel (CH_3OH:$CHCl_3$ = 1:10) gave **56** (50 mg, 68%). ^1H NMR ($CDCl_3$) δ 7.63 (s, 1H), 7.45 ~ 7.24 (m, 13H), 7.14 ~ 7.02 (m, 1H), 6.9 ~ 6.78 (m, 1H), 5.95 (br, 1H), 5.3 (s, 1H), 5.15 ~ 4.95 (m, 4H), 4.25 ~ 3.85 (m, 6H), 3.16 (s, 3H), 2.7 ~ 2.55 (m, 1H).

Phenyl-carbamic Acid 3-(2-chloro-6-methylamino-purin-9-yl)-2-phosphonooxy-methyl-propyl Ester, Ammonium Salt (24). Procedure D. Compound **24** (13.5 mg, 58%) was obtained from **56** (30 mg, 0.046 mmol) and TMSBr (0.1 mL) in CH_2Cl_2 (3 mL). ^1H NMR (D_2O) δ 8.08 (s, 1H), 7.45 ~ 7.1 (m, 2H), 7.1 ~ 6.9 (m, 3H), 4.34 (s, 2H), 4.23 (s, 2H), 3.96 (s, 2H), 2.75 (s, 4H). ^{31}P NMR (D_2O) 1.25 (s); MS (FAB−): calcd: 470.806; found: 471.2.

Propionic Acid 3-(2-chloro-6-methylamino-purin-9-yl)-2-propionyloxymethyl-propyl Ester (25). Procedure C. A mixture of **52** (20 mg, 0.074 mmol), pyridine (0.1 mL), propionic anhydride (28.5 mg, 0.221 mmol), and DMAP (0.5 mg) in CH_2Cl_2 (2 mL) was stirred at room temperature overnight. After worked up, it gave compound **25** (25 mg, 88.5%) as a white solid. ^1H NMR ($CDCl_3$) δ 7.71 (s, 1H), 6.08 (br, 1H), 4.26 (d, J = 6.9 Hz, 2H), 4.2 ~ 4.0 (m, 4H), 3.18 (s, 3H), 2.8 ~ 2.65 (m, 1H), 2.36 (q, J = 7.5 Hz, 4H), 1.15 (t, J = 7.5 Hz, 6H). MS (FAB−): calcd: 383.832; found: 384.1

2,2-Dimethyl-propionic Acid 3-(2-chloro-6-methylamino-purin-9-yl)-2-(2,2-dimethyl-propionyloxymethyl)-propyl Ester (26). Procedure C. Compound **26** (0.112 g, 73.6%) was obtained from **52** (94 mg, 0.346 mmol), pyridine (0.1 mL), pivalic anhydride (0.1 mL), and DMAP (1 mg) in CH_2Cl_2:DMF (1:1) (2 mL) as a white solid. ^1H NMR ($CDCl_3$) δ 7.70 (s, 1H), 6.0 ~ 5.8 (br, 1H), 4.23 (d, J = 7.14 Hz, 3H), 4.08 (dd, J = 7.69, 2.19 Hz, 4H), 3.19 (s, 3H), 2.85 ~ 2.65 (m, 1H), 1.22 (s, 18H); MS (FAB−): calcd: 439.94; found: 440.3. Anal. calc for $C_{20}H_{30}ClN_5O_4$ (439.94): C, 54.60; H, 6.87; N, 15.92. Found: C, 54.35; H, 6.97; N, 15.33.

But-2-enoic Acid 2-but-2-enoyloxymethyl-3-(2-chloro-6-methylamino-purin-9-yl)-propyl Ester (27). Procedure C. Compound **27** (58 mg, 76.3%) was obtained from **52** (50 mg, 0.184 mmol), pyridine (0.1 mL), crotonic anhydride (0.1 mL), and DMAP (3 mg) in CH_2Cl_2 (2 mL) as a white solid. ^1H NMR ($CDCl_3$) δ 7.69 (s, 1H), 7.10 ~ 6.9 (m, 2H), 6.1 ~ 5.7 (m, 2H), 4.4 ~ 4.0 (m, 6H), 3.19 (s, 3H), 2.9 ~ 2.7 (m, 1H), 1.91, 1.89 (d, J = 1.37 Hz, 3H).

Benzoic Acid 2-benzoyloxymethyl-3-(2-chloro-6-methylamino-purin-9-yl)-propyl Ester (28). Procedure C. Compound **28** (28.2 mg, 80%) was obtained as a white solid from **52** (20 mg, 0.074 mmol), pyridine (0.1 mL), benzoic anhydride (50 mg, 0.221 mmol), and DMAP (0.5 mg) in CH_2Cl_2 (2 mL). ^1H NMR ($CDCl_3$) δ 8.1 ~ 7.9 (m, 4H), 7.78 (s, 1H), 7.65 ~ 7.5 (m, 2H), 7.5 ~ 7.35 (m, 4H), 6.05 ~ 5.8 (br, 1H), 4.6 ~ 4.35 (m, 6H), 3.14 (s, 3H), 3.15 ~ 2.95 (m, 1H). MS (FAB−): calcd: 479.9; found: 480.3.

2-Fluorobenzoic Acid 2-(2-fluorobenzoyloxymethyl-3-(2-chloro-6-methylamino-purin-9-yl)-propyl Ester (29). Procedure C. Compound **29** (66 mg, 86.8%) was obtained from **52** (40 mg, 0.148 mmol), pyridine (0.1 mL), 2-fluorobenzoic anhydride (116 mg, 0.443 mmol), and DMAP (3 mg) in CH_2Cl_2 (2 mL) as a white solid. ^1H NMR ($CDCl_3$) δ 8.0 ~ 7.9 (t, J = 6.87 Hz, 2H), 7.79 (s, 1H), 7.65 ~ 7.4 (m, 2H), 7.3 ~ 7.0 (m, 4H), 6.19 (br, 1H), 4.7 ~ 4.2 (m, 6H), 3.15 (s, 3H), 3.1 ~ 2.9 (m, 1H). Anal. calc for $C_{24}H_{20}ClF_2N_5O_4$ (515.901): C, 55.87; H, 3.81; N, 13.58; F, 7.37. Found: C, 55.95; H, 4.01; N, 13.53; F, 7.24.

2,6-Difluorobenzoic Acid 2-(2,6-difluorobenzoyloxymethyl-3-(2-chloro-6-methylamino-purin-9-yl)-propyl Ester (30). Procedure C. Compound **30** (37 mg, 91%) was obtained as a white solid from **52** (20 mg, 0.074 mmol), pyridine (0.1 mL), 2,6-difluorobenzoic anhydride (50 mg, 0.168 mmol), and DMAP (1 mg) in CH_2Cl_2 (2 mL). ^1H NMR ($CDCl_3$) δ 7.72 (s, 1H), 7.6 ~ 7.35 (m, 2H), 7.1 ~ 6.9 (m, 4H), 5.92 (br, 1H), 4.7 ~ 4.2 (m, 6H), 3.17 (s, 3H), 3.1 ~ 2.9 (m, 1H).

Phenylacetic Acid 3-(2-chloro-6-methylamino-purin-9-yl)-2-phenylacetoxymethyl-propyl Ester (31). Procedure C. Compound **31** (34.5 mg, 92%) was obtained from **52** (20 mg, 0.074 mmol), pyridine (0.05 mL), phenylacetic anhydride (45 mg, 0.177 mmol), and DMAP (2 mg) in CH_2Cl_2 (2 mL) as a white solid. ^1H NMR ($CDCl_3$) δ 7.5 ~ 7.2 (m, 10H), 7.16 (s, 1H), 6.03 (s, 1H), 4.15 ~ 3.85 (m, 6H), 3.63 (s, 4H), 3.16 (s, 3H), 2.8 ~ 2.55 (m, 1H).

Phenylcarbamic Acid 3-(2-chloro-6-methylamino-purin-9-yl)-2-phenylcarbamoyl-oxymethyl-propyl Ester (32). Procedure E. Compound **32** (75.1 mg, 80%) was obtained as a white solid from **52** (50 mg, 0.184 mmol), phenylisocyanate (48 μL, 0.441 mmol), and Et_3N (20 μL) in dry CH_2Cl_2 (2 mL) under N_2. ^1H NMR ($CDCl_3$) δ 7.83 (s, 1H), 7.50 ~ 7.2 (m, 10H), 7.1 (s, 2H), 6.36 (s, 1H), 4.40 ~ 4.1 (m, 6H), 3.12 (s, 3H), 2.9 ~ 2.7 (m, 1H). MS calcd: 509.950; found: 510.2

Carbonic Acid 3-(2-chloro-6-methylamino-purin-9-yl)-2-phenoxycarbonyloxy-methyl-propyl Ester Phenyl Ester (33). Procedure C. Compound **33** (21.1 mg, 56%) was obtained from **52** (20 mg, 0.074 mmol), Et_3N (41 μL), phenyl chloroformate (20 μL, 0.159 mmol), and DMAP (1 mg) in CH_2Cl_2 (2 mL) as a white solid. The product was purified by column chromatography on silica gel (^{i}PrOH:$CHCl_3$ = 1:15). ^1H NMR ($CDCl_3$) δ 7.77 (s, 1H), 7.5 ~ 7.0 (m, 10H), 6.11 (s, 1H), 4.5 ~ 4.1 (m, 6H), 3.18 (s, 3H), 3.0 ~ 2.9 (m, 1H).

Succinic Acid mono-[2-(3-carboxy-propionyloxymethyl)-3-(2-chloro-6-methylamino-purin-9-yl)-propyl] Ester (34). Compound **52** (50 mg, 0.184 mmol) was dissolved in dry pyridine (2 mL) and succinic anhydride (92 mg, 0.919 mmol) was added under stirring at room temperature. Stirring was continued overnight under exclusion of humidity. The volume was reduced in vacuo and the residue was extracted with $CHCl_3$ (3 × 10 mL) which gave **34** (23 mg, 25%) as a brown solid. ^1H NMR (CD_3OD) δ 8.04 (s, 1H), 7.72 (s, 1H), 4.5 ~ 4.25 (m, 2H), 4.25 ~ 4.0 (m, 4H), 3.08 (s, 3H), 2.8 ~ 2.6 (m, 1H), 2.6 (s, 8H). MS (CI): calcd: 471.9 (M^+); found: 472.0 (M^+H).

Dithiocarbonic Acid S-benzyl Ester O-[2-benzylsulfanylthiocarboxyoxymethyl-3-(2-chloro-6-methylamino-purin-9-yl)-propyl] Ester (35). To a solution of **52** (50 mg, 0.184 mmol) and CS_2 (0.5 mL) in DMSO (1 mL), maintained

at 15 °C, was added dropwise an aqueous 0.1 N NaOH solution (8.2 mL). The mixture was stirred for 20 min and treated dropwise with benzyl bromide (176 mg, 1.029 mmol). The stirring was continued for 1 h, the solvent was removed in vacuo, and the residue was extracted with EtOAc. The organic layer was washed with H_2O, dried over Na_2SO_4, and concentrated. Purification of the residue by column chromatography on silica gel (CH_3OH:$CHCl_3$ = 1:10) gave **35** (17 mg, 15.3%) as white solid. ^{1}H NMR ($CDCl_3$) δ 7.61 (s, 1H), 7.5 ~ 7.1 (m, 10H), 5.87 (s, 1H), 4.7 ~ 4.5 (m, 4H), 4.38 (s, 4H), 4.3 ~ 4.1 (m, 2H), 3.16 (s, 3H), 3.1 ~ 3.0 (m, 1H).

Benzoic Acid 2-acetoxymethyl-3-(2-chloro-6-methylamino-purin-9-yl)-propyl Ester (36). Procedure C. Compound **36** (91 mg, 85%) was obtained from **49** (80 mg, 0.256 mmol), pyridine (0.1 mL), benzoic anhydride (173 mg, 0.764 mmol), and DMAP (2 mg) in CH_2Cl_2 (3 mL) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.98 (d, J = 7.5 Hz, 2H), 7.72 (s, 1H), 7.65 ~ 7.5 (m, 1H), 7.5 ~ 7.3 (m, 2H), 5.94 (s, 1H), 4.5 ~ 4.1 (m, 6H), 3.16 (s, 3H), 3.0 ~ 2.8 (m, 1H). Anal. calc for $C_{19}H_{20}ClN_5O_4$ (417.850): C, 54.61; H, 4.82; N, 16.76. Found: C, 54.47; H, 4.95; N, 16.49.

Phenylacetic Acid 2-acetoxymethyl-3-(2-chloro-6-methylamino-purin-9-yl)-propyl Ester (37). Procedure C. Compound **37** (37 mg, 90%) was obtained from **49** (30 mg, 0.096 mmol), pyridine (0.1 mL), phenylacetic anhydride (73 mg, 0.287 mmol), and DMAP (2 mg) in CH_2Cl_2 (1 mL) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.6 ~ 7.1 (m, 5H), 5.86 (s, 1H), 4.3 ~ 3.8 (m, 6H), 3.66 (s, 2H), 3.18 (s, 3H), 2.9 ~ 2.5 (m, 1H), 2.06 (s, 3H). Anal. calc for $C_{20}H_{22}ClN_5O_4$ (431.876): C, 55.62; H, 5.13; N, 16.22. Found: C, 55.78; H, 5.20; N, 16.09.

Diallylacetic Acetic Acid (57). Diethyl diallylmalonate (10 g, 42 mmol) was dissolved in 25 mL of EtOH and a solution of KOH (5.14 g, 92 mmol) in 15 mL of water was added. The reaction mixture was heated under reflux overnight, concentrated, and the residue dissolved in water. The solution was acidified to pH 2 and extracted with 25 mL of ether three times. The combined organic phases were dried over sodium sulfate and concentrated to give the crude malonic acid. ^{1}H NMR ($CDCl_3$) δ 2.58−2.62 (m, 4H), 5.03−5.12 (m, 4H), 5.63−5.69 (m, 2H), 12.0 (br s, 2H). The crude malonic acid was heated at 155 °C to effect decarboxylation to give 8.18 g (82%) of the desired product. ^{1}H NMR ($CDCl_3$) δ 2.30 (m, 1H), 2.41 (m, 1H), 2.56 (dd, 1H, J = 6, 8 Hz), 5.06 (dd, 1H, J = 2, 10 Hz), 5.10 (dd, 1H, J = 2, 17 Hz), 5.76 (dddd, 1H, J = 3, 4, 10, 17 Hz), 8.5 (br s, 1H).

2-Allyl-4-penten-1-ol (58). A solution of 2-allyl-4-pentenoic acid (**57**) (2.3 g, 16.4 mmol) in 20 mL of dry THF was treated with lithium aluminum hydride (0.76 g, 20 mmol). After the vigorous initial reaction, the mixture was stirred at room temperature overnight. The reaction mixture was treated dopwise with 0.75 mL of water, 0.75 mL of 15% NaOH, and then 3 mL of water. The granular solid was filtered off and rinsed with ether. The combined filtrates were washed with saturated sodium bicarbonate and saturated sodium chloride and then dried over sodium sulfate. Concentration of the filtered solution afforded 1.61 g (78%) of the desired alcohol (**58**). ^{1}H NMR ($CDCl_3$) δ 1.49 (br s, 1H), 1.70−1.74 (m, 1H), 2.10−2.14 (m, 1H), 3.58 (s, 2H, J = 6 Hz), 5.02−5.09 (m, 4H), 5.79−5.85 (m, 2H).

2-Allyl-pent-4-enyloxymethyl)-benzene (59). The alcohol **58** (631 mg, 5 mmol) was dissolved in THF and sodium hydride (144 mg, 6 mmol); tetra-*n*-butylammonium iodide (184 mg, 0.5 mmol) and benzyl bromide (1.03 g, 6 mmol) were added in succession. The reaction mixture was stirred at room temperature for 4 h. Methanol was added followed by water to quench the reaction. The reaction mixture was extracted with ether and dried over sodium sulfate. Concentration gave 1.21 g of a golden liquid which was chromatographed on flash silica gel (10% EtOAc/hexane→EtOAc) to afford 960 mg (89%) of the pure benzyl ether. ^{1}H NMR ($CDCl_3$) δ 1.74−1.76 (m, 1H), 2.02−2.09 (m, 4H), 3.28−3.30 (m, 2H), 4.41 (s, 2H), 4.91−4.97 (m, 4H). 5.67−5.76 (m, 2H), 7.18−7.27 (ArH, 5H).

4-Benzyloxymethyl-heptane-1,7-diol (60). 9-BBN (10.5 mL, 0.5 M in THF) was added to a solution of **59** (1.14 g, 5.27 mmol) in anhydrous THF (10 mL). The solution was refluxed for 1.5 h and cooled to ambient temperature. A 3 M NaOH solution (3.5 mL, 10.5 mmol) and 30% H_2O_2 (3 mL, 10.54 mmol) were added and the reaction was stirred at ambient temperature for 2 h. A second portion of 30% H_2O_2 (1 mL) was added and stirred for an additional 1 h. The solution was concentrated to about half of the volume under vacuum. The reaction solution was treated with EtOAc and brine. The organic layer was dried and concentrated to an oil. The product was purified by column chromatography on silica gel (EtOAc:Hex = 1:9)−(EtOAc:MeOH = 9.8:0.2) to yield **60** (1.13 g, 85%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.42 (m, 5H), 1.69 (m, 2H), 1.89 (m, 2H), 3.37 (d, J = 5.8 Hz, 2H), 4.50 (s, 2H), 7.35 (m, 5H).

[5-Bromo-2-(3-bromo-propyl)-pentyloxymethyl]-benzene (61). In a dried reaction vessel, **60** (1.13 g, 4.48 mmol) and triphenylphosphine (2.58 g, 9.85 mmol) were dissolved in anhydrous DMF (5 mL). Br_2 (0.51 mL, 9.85 mmol) was added dropwise at ambient temperature. The reaction vessel was sealed and heated to 50 °C for 2 h. The reaction was cooled to ambient temperature and extracted with hexane (8 mL × 4). The organic layers were combined and washed with brine (30 mL), dried, and concentrated to a yellow oil. The product was purified by column chromatography on silica gel (EtOAc:Hex = 1:9) to provide a light yellow oil (1.1 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (m, 4H), 1.68 (m, 1H), 1.87 (m, 4H), 3.40 (m, 6H), 4.51 (s, 2H), 7.32 (m, 5H).

[4-Benzyloxymethyl-7-(diethoxy-phosphoryl)-heptyl]-phosphonic Acid Diethyl Ester (62). A sealed reaction vessel containing **61** (1.13 g, 2.99 mmol) dissolved in triethyl phosphite (1.06 mL, 9 mmol) was held at 130 °C for 4 h. The reaction mixture was concentrated and purified by column chromatography on silica gel (CH_2Cl_2:MeOH 1−5%) to yield **62** (570 mg, 39%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.26 (t, 12H, J = 7.08 Hz), 1.35 (m, 2H), 1.40 (m, 2H), 1.58 (m, 9H), 3.30 (d, 2H, J = 5.6 Hz), 4.02 (m, 8H), 4.42 (s, 2H), 7.26 (m, 5H); ^{31}P NMR (162 MHz): δ 35.3.

Diethyl 2-(diethylphosphonomethyl)-allylphosphonate (64). A mixture of 3-chloro-2-chloromethyl-1-propene (**63**, 2.50 g, 20 mmol) and triethyl phosphite (6.65 g, 40 mmol) were heated at 155 °C in a sealed glass pressure vessel overnight. The reaction mixture was distilled to give 6.01 g (92%) of the bisphosphonate (**64**). ^{1}H NMR ($CDCl_3$) δ 1.30−1.34 (m, 12 H), 2.83 (d, 4H, J_{HP}= 24 Hz), 4.09−4.13 (m, 8H), 5.15−5.16 (m, 2H); ^{31}P NMR ($CDCl_3$) δ 26.9.

Tetraethyl 2-hydroxymethyl-1,3-propanebisphosphonate (65). Compound **64** (3.28 g, 10 mmol) was dissolved in 20 mL of THF and 9-BBN (20 mL of 0.5 M in THF, 10 mmol) was added. The reaction mixture was heated under reflux for 1 h and then cooled in an ice bath. Aqueous sodium hydroxide (3 M, 3.3 mL) and 30% hydrogen peroxide (5 mL) were added dropwise and the mixture was stirred for 4 h. Aqueous sodium bisulfite was added and the reaction mixture was concentrated to approximately one-half of its volume. The mixture was treated with ether and saturated sodium chloride. The layers were separated and the organic phase was dried over sodium sulfate and concentrated. The residue was chromatographed on flash silica gel (1:1 EtOAc/hexane to EtOAc) to elute 9-BBN derived byproducts. Elution with 20% MeOH/EtOAc gave 999 mg (29%) of the pure product **65**. ^{1}H NMR ($CDCl_3$) δ 1.24−1.29 (m, 12H), 1.73−2.00 (m, 4H), 2.18−2.36 (m, 1H), 3.63−3.64 (m, 2H), 4.00 (br s, 1H), 4.01−4.06 (m, 8H); ^{31}P NMR ($CDCl_3$) 30.9 ppm.

[7-(Diethoxy-phosphoryl)-4-hydroxymethyl-heptyl]-phosphonic Acid Diethyl Ester (66). A suspension of **62** (570 mg, 1.16 mmol) and 5% Pd/carbon (0.5 g) in MeOH (100 mL) was shaken under 5 psi of H_2 for 10 min. The reaction mixture was filtered through two sheets of filter paper and concentrated to an oil. The product was purified by column chromatography on silica gel (CH_2Cl_2:MeOH, 1−10%) to give **66** (242 mg, 60%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (t, J = 7.04 Hz, 12H), 1.33 (m, 2H), 1.46 (m, 3H), 1.57 (m, 4H), 3.36 (m, 4H), 3.93 (m, 8H); ^{31}P NMR (162 MHz): δ 35.73.

[4-(2,6-Dichloro-purin-9-ylmethyl)-7-(diethoxy-phosphoryl)-heptyl]-phosphonic Acid Diethyl Ester (68). The starting alcohol (**66**) was dried by coevaporation from anhydrous THF (10 mL) two times. Subsequently, a solution of **66** (240 mg, 0.6 mmol) in anhydrous THF (6 mL) was combined with 2,6-dichloropurine (113.4 mg, 0.6 mmol), triphenylphosphine (157.2 mg, 0.6 mmol), and diethylazodicarboxylate (114.8 mg, 0.66 mmol) and stirred at ambient temperature for 2 h. The solution was concentrated and the resulting residue was purified by column chromatography on silica gel (EtOAc:Hex = 1:1, followed by CH_2Cl_2:MeOH = 9:1) to provide **68** (205 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (t, J = 7.04 Hz, 12H), 1.35 (m, 4H), 1.68 (m, 8H), 1.99 (m, 1H), 4.05 (m, 8H), 4.15 (d, J = 6.8 Hz, 2H), 8.13 (s, 1H); ^{31}P NMR (162 MHz): δ 34.5.

[4-(2-Chloro-6-methylamino-purin-9-ylmethyl)-7-(diethoxy-phosphoryl)-heptyl]-phosphonic Acid Diethyl Ester (70). A solution of **68** (206 mg, 0.36 mmol) in acetonitrile (4 mL) and 40% methylamine (78 uL, 0.9 mmol) was stirred at ambient temperature for 40 min. The reaction solution was concentrated and the residue was purified directly by silica gel chromatography (CH_2Cl_2−MeOH, 2−4%) to furnish **70** (151 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (t, 12H, J = 7.04 Hz), 1.32 (m, 2H), 1.58 (m, 8H), 1.97 (m, 1H), 3.12 (s, 3H), 4.02 (m, 10H), 6.60 (s, 1H), 7.75 (s, 1H); ^{31}P NMR (162 MHz) δ 34.7.

[4-(2-Chloro-6-methylamino-purin-9-ylmethyl)-7-phosphono-heptyl]-phosphonic Acid, Sodium Salt (39b). A dried NMR tube equipped with a purge valve was used as the reaction vessel. In d-acetonitrile (0.5 mL), **70** (59 mg, 0.104 mmol) was combined with iodotrimethylsilane (60 uL, 0.41 mmol) and sealed under an inert atmosphere. The reaction was vortexed at ambient temperature for 45 min and analzyed by ^{31}P NMR. A second portion of iodotrimethylsilane (60 μL, 0.41 mmol) was added and after 45 min ^{31}P NMR indicated complete reaction on the basis of a single phosphorus resonance (^{31}P NMR: δ 17.8). The reaction was concentrated to dryness. The residue was combined with 1.6 M triethylammonium carbonate buffer (3 mL), prepared by bubbling CO_2 through an aqueous solution of triethylamine, and the solution was extracted with ethyl ether (3 mL × 5). The aqueous layer was concentrated and passed over the sodium form of DOWEX 50W-8X. The appropriate fractions were combined and lyophilized to yield **39b** in quantitative yield (60 mg) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 1.22 (m, 4H), 1.40 (m, 8H), 1.88 (m, 1H), 2.86 (s, 3H), 3.93 (d, J = 6.8 Hz, 2H), 7.92 (s, 1H); ^{31}P NMR (162 MHz): δ 29.3.

[3-(2,6-Dichloro-purin-9-yl)-2-(diethoxy-phosphoryl-methyl)-propyl]-phosphonic Acid Diethyl Ester (67). The starting alcohol (**65**) was dried by coevaporation from anhydrous THF (80 mL) two times. Subsequently, a solution of **65** (4.4 g, 12.7 mmol) in anhydrous THF (200 mL), was combined with 2,6-dichloropurine (2.65 g, 14 mmol), triphenylphosphine (3.67 g, 14 mmol), and diethylazodicarboxylate (2.2 mL, 14 mmol) and stirred at ambient temperature overnight. The solution was concentrated and the resulting residue was purified by column chromatography on silica gel (EtOAc: Hex=1:1, followed by EtOAc:MeOH = 9.6:0.4) to provide **67** (4.43 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (t, J = 7.1 Hz, 12H), 1.91 (m, 4H), 2.86 (m, 1H), 4.12 (m, 8H), 4.60 (d, J = 6.1 Hz, 2H), 8.46 (s, 1H); ^{31}P NMR (162 MHz, D_2O) δ 31.3.

[3-(2-Chloro-6-methylamino-purin-9-yl)-2-(diethoxy-phosphorylmethyl)-propyl]-phosphonic Acid Diethyl Ester (69). A THF (100 mL) solution of **67** (4.43 g, 8.57 mmol) and 40% methylamine (1.2 mL, 17.14 mmol) was stirred at ambient temperature for 1 h. The reaction solution was concentrated and the residue was purified directly by silica gel chromatography (CH_2Cl_2−MeOH, 1−4%) to furnish **69** (2.64 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (t, 12H, J = 7.1 Hz), 1.77 (m, 2H), 1.91 (m, 2H), 2.68 (m, 1H), 3.01 (s, 3H), 3.98 (m, 8H), 4.33 (d, J = 5.9 Hz, 2H), 7.23 (s, 1H), 7.88 (s, 1H); ^{31}P NMR (162 MHz, $CDCl_3$) δ 33.9.

[3-(2-Chloro-6-methylamino-purin-9-yl)-2-phosphono-methyl-propyl]-phosphonic Acid (38a). In a glovebox, **69** (773 mg, 1.51 mmol) was dissolved into anhydrous acetonitrile (16 mL). Iodotrimethyl-silane (2.0 mL, 13.5 mmol) was added. The reaction was stirred at ambient temperature for 4.5 h and analyzed by ^{31}P NMR. ^{31}P NMR indicated complete reaction on the basis of a single phosphorus resonance (^{31}P NMR: δ 21.2 ppm). The reaction was concentrated to dryness. The residue was combined with water (10 mL) and the solution was extracted with diethyl ether (3 mL × 5). The aqueous layer was concentrated and analyzed by ^{1}H NMR, ^{31}P NMR, and MS. The product was used in the next reaction without further purification. ^{1}H NMR (400 MHz, D_2O) δ 1.85 (m, 2H), 2.04 (m, 2H), 2.74 (m, 1H), 3.06 (s, 3H), 4.41 (d, J = 6.8 Hz, 2H), 8.68 (s, 1H); ^{31}P NMR (162 MHz) δ 31.4; MS (M/e): 398.1.

[3-(2-Chloro-6-methylamino-purin-9-yl)-2-phosphono-methyl-propyl]-phosphonic Acid, Sodium Salt (38b). The phosphonic acid **38a** (603 mg, 1.51 mmol) in water (0.5 mL) was passed over the sodium form of DOWEX 50W-8X. The appropriate fractions were combined and lyophilized to yield **38b** (713 mg, 97%) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 1.72 (m, 2H), 1.92 (m, 2H), 2.64 (m, 1H), 3.03 (s, 3H), 4.32 (d, 2H, J = 6.6 Hz), 8.29 (s, 1H); ^{31}P NMR (162 MHz) δ 30.0; MS (M/e): 398.1.

Biological Activity. Platelet aggregometry: Whole blood was withdrawn from Sprague−Dawley rats (250−300 g: Charles River) from the abdominal aorta of isoflurane anaesthetized animals into sodium citrate, 0.38% [final]. Platelet rich plasma (PRP) was collected by centrifugation at 300 × g for 16 min. Prostaglandin E−1 (Fluka) was added to the PRP to a final concentration of 0.18 μg/mL. Platelets were pelleted by centrifugation at 650 × g for 15 min. Platelets were resuspended in an equal volume of modified phosphate-buffered saline.[35] Platelet concentration was measured on a Coulter counter and adjusted to $3 \times 10^5/\mu$L. Diluted platelets were distributed into polypropylene tubes in 650 μL aliquots and held at 30 °C until ready for use. Washed platelets were activated with ADP at 3.3 μM while stirring at 1000 rpm. ADP was added 1 min after addition of the antagonist being tested. The antagonist was dissolved in either water or a vehicle consisting of DMSO/PEG300 (final concentration during assay 0.05%/0.95%, respectively), which was shown in control experiments not to interfere with the assay. Activation and aggregation was measured by light transmission in a Chrono-Log Model 560 VS aggregometer 5 min after addition of antagonist. K_i values were calculated from IC_{50} values obtained in sigmoidal concentration−response curves using Prizm (GraphPad, San Diego, CA) and EC_{50} values for induction of aggregation by ADP of 420 nM. Studies of human platelet aggregation were carried out as described.[33]

Phospholipase C activity[36] was measured in 1321N1 astrocytoma cells expressing the $hP2Y_1$ receptor. Stimulation of inositol phosphate formation by 2-methylthioadenosine 5-diphosphate (30 nM) was antagonized by various antagonists. Inositol phosphates were measured as described.[36] Membranes from [^{3}H]inositol-labeled membranes were incubated for 5 min at 30 °C in the presence of the indicated concentrations of agonist.

Measurement of cyclic AMP in platelets was carried out as reported.[22]

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