

# Acyclic and Cyclopropyl Analogues of Adenosine Bisphosphate Antagonists of the P2Y<sub>1</sub> Receptor: Structure–Activity Relationships and Receptor Docking

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The activation of P2Y<sub>1</sub> receptors in platelets contributes to platelet aggregation, and selective antagonists are sought as potential antithrombotic agents. We reported (Kim et al. *J. Med. Chem.* **2000**, *43*, 746–755) that acyclic analogues of adenine nucleotides, containing two phosphate groups on a symmetrically branched aliphatic chain, attached at the 9-position of adenine, are moderately potent P2Y<sub>1</sub> receptor antagonists. In this study we have varied the chain structure, to include asymmetric substitution, olefinic, and cyclopropyl groups. These antagonists inhibited the stimulation of phospholipase C in turkey erythrocyte membranes induced by 30 nM 2-MeS-ADP in the micromolar range. In the series of symmetrically branched aliphatic groups substituted with two phosphate groups, the optimal antagonist potency occurred with the 2-methylpropyl group. A 2-chloro-*N*<sup>6</sup>-methyladenine derivative, 2-[2-(2-chloro-6-methylaminopurin-9-yl)methyl]propane-1,3-bisoxo(diammoniumphosphate) (**7**), was a full antagonist at the P2Y<sub>1</sub> receptor with an IC<sub>50</sub> value of 0.48  $\mu$ M. Esterification of one of the phosphate groups or substitution with *O*-acetyl greatly reduced the antagonist potency at the P2Y<sub>1</sub> receptor. Removal of a methylene group of **7** or inclusion of an olefinic or cyclopropyl group also reduced potency. A pair of enantiomeric glycerol derivatives demonstrated a 5-fold stereoselectivity for the *S*-isomer. Stereoisomerically defined analogues of **7** containing a cyclopropyl group in place of the branched carbon were less potent than **7** as antagonists, with IC<sub>50</sub> values of 2–3  $\mu$ M. No agonist activity was observed for these analogues. A new rhodopsin-based molecular model of the P2Y<sub>1</sub> receptor indicated that the optimal docked orientation of the two monophosphate moieties relative to the adenine N<sup>6</sup> (compared to a rigid, bicyclic analogue) was consistent with the dependence of antagonist potency on chain length. The 3'-phosphate was predicted to occupy a restricted space, deeper in the binding cleft than the 5'-phosphate location. In summary, modification of the flexible spacer chain linking bisphosphate groups to the adenine moiety provided many moderately potent antagonists.

## Introduction

P2 receptors, which are activated by purine and/or pyrimidine nucleotides, consist of two families: G protein-coupled receptors, termed P2Y, and ligand-gated cation channels, termed P2X. Numerous subtypes have been cloned within each family regulating a diverse range of functions in the central and peripheral nervous systems, the cardiovascular system, the endocrine system, lungs, intestines, muscle, and the immune system.<sup>1–3</sup>

The P2Y<sub>1</sub> receptor has been cloned from chick, turkey, human, rat, and mouse.<sup>4–6</sup> ADP induces aggregation of human platelets by acting at both P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors.<sup>7–10</sup> The P2Y<sub>1</sub> receptor antagonist *N*<sup>6</sup>-methyl-2'-deoxyadenosine 3',5'-bisphosphate has been shown to inhibit ADP-induced platelet aggregation.<sup>11,12</sup> A mouse line lacking expression of the P2Y<sub>1</sub> receptor has been

reported,<sup>13,14</sup> and the absence of the P2Y<sub>1</sub> receptors in platelets of these mice interfered with ADP-promoted aggregation.

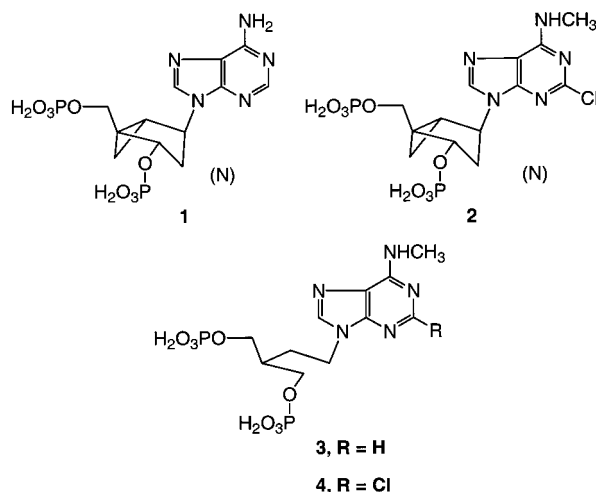
We have developed selective P2Y<sub>1</sub> receptor agonists and antagonists of high affinity for use as pharmacological probes.<sup>15–20</sup> With simplified pharmacophores we are exploring the steric and electronic constraints of the receptor binding site and the structural basis of receptor activation. Various naturally occurring bisphosphates of adenosine (e.g., adenosine 3',5'-bisphosphate) act as competitive antagonists at the P2Y<sub>1</sub> receptor,<sup>15</sup> and *N*<sup>6</sup>-methyl and 2-chloro groups on the adenine moiety have been found to increase the potency and selectivity of the bisphosphate derivatives acting as antagonists.<sup>16</sup> Among ribose modifications, both rigidifying (e.g., bicyclic carbocycles)<sup>19</sup> and adding flexibility (e.g., acyclic modifications)<sup>20</sup> have led to effective P2Y<sub>1</sub> receptor ligands. Rigid rings in the bicyclic "methanocarba" series (Figure 1) have defined a preference at the receptor binding site for the northern (N) conformation. Compound **1** ((1*R*,2*S*,4*S*,5*S*)-1-[(phosphato)methyl]-4-(6-aminopurin-9-yl)bicyclo [3.1.0]-hexane-2-phosphate), an (N)-methanocarba analogue, is a potent agonist (EC<sub>50</sub> = 155 nM), 120-fold more potent than the corresponding

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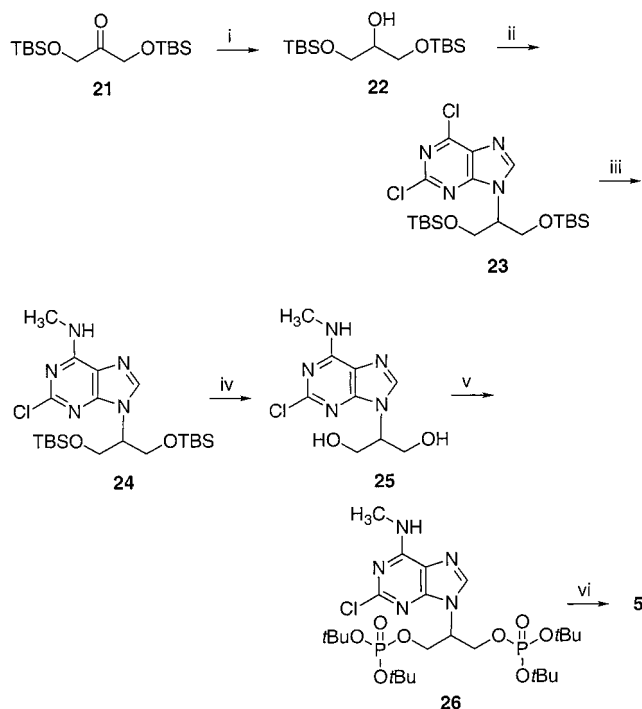


**Figure 1.** Structures of rigid and acyclic nucleotide analogues found to be receptor agonist (**1**) or antagonists (**2–4**).

southern (S) isomer. Compound **2**, the corresponding 2-Cl-*N*<sup>6</sup>-methyl analogue, is a potent P2Y<sub>1</sub> receptor antagonist (IC<sub>50</sub> = 52 nM).

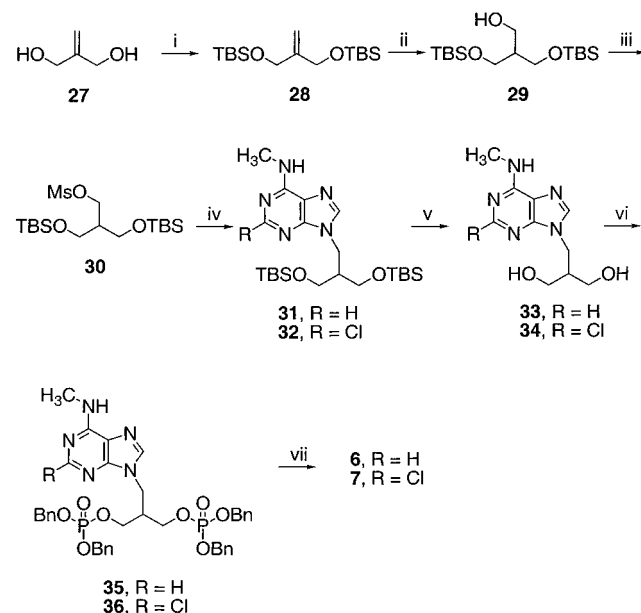
The present study explores the SAR of nucleotide analogues containing acyclic aliphatic chains, such as **3** and **4**, which act as P2Y<sub>1</sub> receptor antagonists.<sup>20</sup> Compound **3** (2-[2-(6-methylaminopurin-9-yl)ethyl]-propane-1,3-bisphosphate) and its 2-chloro- analogue were found to be selective antagonists of the P2Y<sub>1</sub> receptor with EC<sub>50</sub> values of 1.60 and 0.84  $\mu$ M, respectively. Compound **4** was shown to be inactive at the rat P2X<sub>1</sub> receptor.<sup>21</sup> We have introduced shorter chains than in the previous study<sup>20</sup> and also elements of rigidity,

**Scheme 1.** Synthesis of Isopropyl Bisphosphate, Compound **5**<sup>a</sup>



<sup>a</sup> Reaction conditions: (i) NaBH<sub>4</sub>, EtOH, 0 °C, 30 min, 91%; (ii) 2,6-dichloropurine, DEAD, PPh<sub>3</sub>, THF, rt, overnight, 68%; (iii) CH<sub>3</sub>NH<sub>2</sub>, THF, rt, 4 h, 88%; (iv) Dowex 50x-8 200, MeOH, reflux, overnight, 75%; (v) Et<sub>2</sub>NP(OtBu)<sub>2</sub>, tetrazole, THF, rt, 20 min, then MCPBA, 73%; (vi) Dowex 50x-8 200, MeOH:H<sub>2</sub>O = 1:1, 80 °C, 16 h, 63%.

**Scheme 2.** Synthesis of Isobutyl Bisphosphates, Compounds **6** and **7**<sup>a</sup>



<sup>a</sup> Reaction conditions: (i) TBSCl, TEA, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, rt, overnight, 99%; (ii) BH<sub>3</sub>, THF, rt, overnight, then H<sub>2</sub>O<sub>2</sub>, NaOH, 60%; (iii) MsCl, TEA, CH<sub>2</sub>Cl<sub>2</sub>, rt, 1 h; (iv) 2-chloro-*N*<sup>6</sup>-methylaminopurine (for **32**) or *N*<sup>6</sup>-methylaminopurine (for **31**), K<sub>2</sub>CO<sub>3</sub>, 18-crown-6, DMF, 60% two-step yield for **31**, 72% two-step yield for **32**; (v) AcOH:H<sub>2</sub>O:THF = 3:2:2, rt, 2 d, 79% for **33**, 93% for **34**; (vi) tetra-n-butylpyrophosphate, NaH, THF, rt, 1 h, 81% for **35**, 53% for **36**; (vii) BCl<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 5 °C, 2 d, 75% for **6**, 41% for **7**.

such as olefinic and cyclopropyl groups. Molecular modeling of the receptor and its docked ligands, by homology to the recently reported high-resolution structure of rhodopsin,<sup>22</sup> was carried out. In general, modifying the flexible spacer chain linking bisphosphate groups to the adenine moiety, within spatial requirements as defined by the docked positions of the phosphates relative to the adenine N<sup>6</sup>, provided many moderately potent antagonists.

## Results

**Chemical Synthesis.** Acyclic and cyclopropyl analogues **5–20** of adenosine bisphosphates were prepared in the ammonium salt form and tested biologically as antagonists at the P2Y<sub>1</sub> receptor (Table 1). The nucleotide analogues were characterized by high-resolution mass spectrometry and by HPLC in two different solvent systems (Table 2).

The synthesis of these nucleotide analogues and the corresponding nucleoside precursors are shown in Schemes 1–10. Symmetrical (Schemes 1, 2, and 4) and chiral acyclic aliphatic (Scheme 5), olefinic (Scheme 6), and cyclopropyl moieties (Schemes 7–10) were included. In general, introduction of the 2-chloro-*N*<sup>6</sup>-methyl- (or 2-H) adenine moiety was accomplished either by direct alkylation of a mesylate with 2-chloro-*N*<sup>6</sup>-methylaminopurine or by a Mitsunobu reaction utilizing 2,6-dichloropurine, followed by displacement of 6-chloro with methylamine. During the displacement, the diols were protected by silylation (e.g., TBS, *tert*-butyldimethylsilyl) or masked as a precursor olefin (Scheme 4).

Subsequently, phosphorylation of each nucleoside analogue (yields in Table 2) was achieved in two steps. First, benzyl- or *tert*-butyl-protected phosphate groups

**Table 1.** In Vitro Pharmacological Data for Antagonism of Turkey Erythrocyte P2Y<sub>1</sub> Receptors, Indicated by the Inhibition of PLC Stimulation Elicited by 30 nM 2-MeSADP, for at Least Three Separate Determinations. "P" = Phosphate

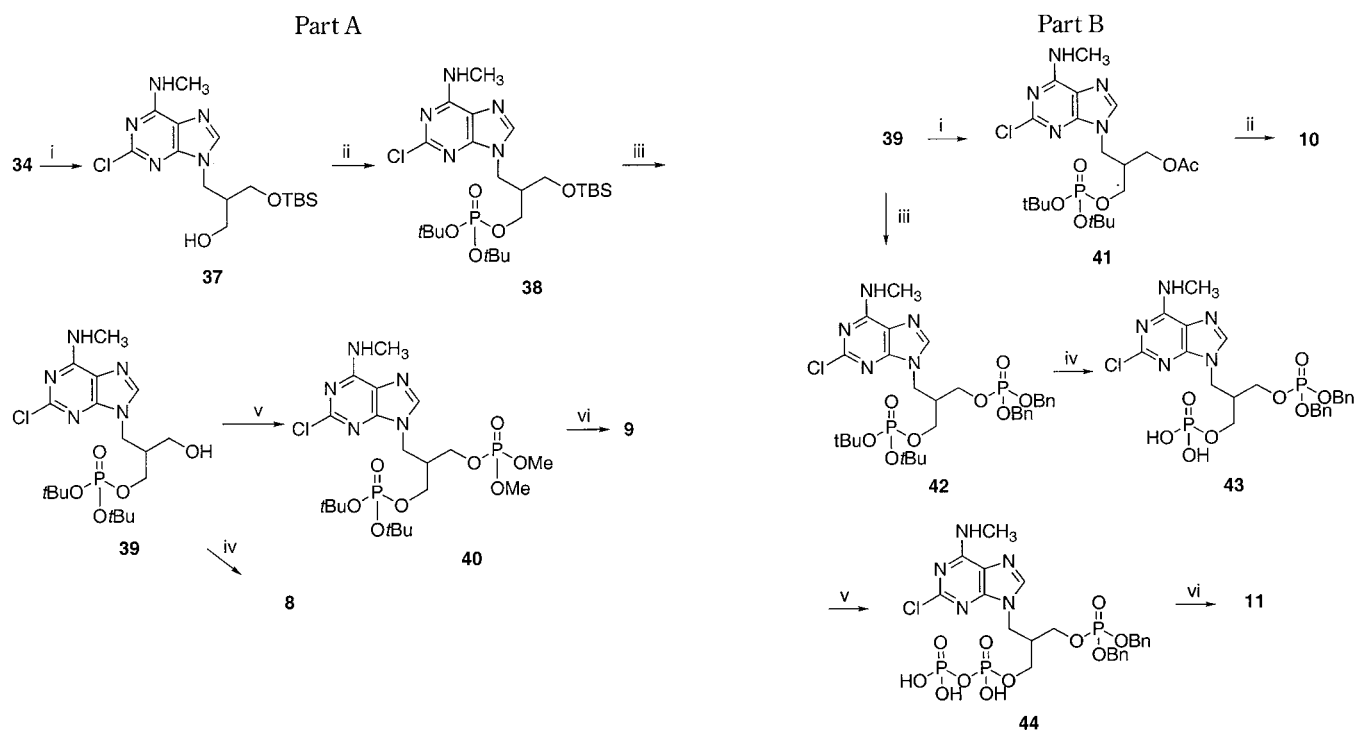
Compound	R =	R' =	Antagonist Effect, <sup>a</sup> % of max.	IC <sub>50</sub> , μM (n = 3, unless noted)
5		Cl	100	4.56 ± 1.38
6		H	100	1.09 ± 0.51
7		Cl	100	0.48 ± 0.13
8		Cl	68 ± 7	22.6 ± 14.6
9		Cl	78 ± 7	31 ± 11
10		Cl	65 ± 8	37.6 ± 12.6
11		Cl	100	0.23 ± 0.10
3		H	100	1.60 ± 0.47
4		Cl	99 ± 1	0.84 ± 0.13
12		Cl	100	2.04 ± 0.94 (4)
13		Cl	100	7.86 ± 3.13
14		Cl	100	1.47 ± 0.43
15		Cl	100	2.10 ± 0.97
16		Cl	100	1.74 ± 0.80
17		Cl	100	2.17 ± 0.85
18		Cl	100	2.70 ± 0.66
19		Cl	100	2.36 ± 0.63
20		Cl	100	1.90 ± 0.24

<sup>a</sup> At 100 μM, none of the acyclic adenosine bisphosphate nucleotide analogues displayed agonist activity at the P2Y<sub>1</sub> receptor in turkey erythrocyte membranes.

**Table 2.** Synthetic Data for Nucleotide Derivatives, Including Structural Verification Using High Resolution Mass Spectroscopy and Purity Verification Using HPLC

no.	formula	FAB (M - H <sup>+</sup> )		HPLC (rt; min) <sup>a</sup>		yield (%) <sup>b</sup>
		calcd	found	system A (purity %)	system B (purity %)	
5	C <sub>9</sub> H <sub>13</sub> O <sub>8</sub> N <sub>5</sub> ClP <sub>2</sub>	415.9928	415.9940	4.981 (97)	8.900 (98)	63
6	C <sub>10</sub> H <sub>16</sub> O <sub>8</sub> N <sub>5</sub> P <sub>2</sub>	396.0474	396.0482	7.779 (99)	6.639 (98)	70
7	C <sub>10</sub> H <sub>15</sub> O <sub>8</sub> N <sub>5</sub> ClP <sub>2</sub>	430.0084	430.0094	6.655 (95)	6.453 (97)	41
8	C <sub>10</sub> H <sub>14</sub> O <sub>5</sub> N <sub>5</sub> ClP	350.0421	350.0423	8.963 (97)	2.942 (99)	79
9	C <sub>12</sub> H <sub>19</sub> O <sub>8</sub> N <sub>5</sub> ClP <sub>2</sub>	458.0397	458.0416	13.579 (95)	5.534 (99)	76
10	C <sub>12</sub> H <sub>16</sub> O <sub>8</sub> N <sub>5</sub> ClP <sub>2</sub>	392.0527	392.0517	7.452 (99)	4.206 (99)	59
11	C <sub>10</sub> H <sub>16</sub> O <sub>11</sub> N <sub>5</sub> ClP <sub>3</sub>	509.9748	509.9751	6.062 (99)	7.970 (96)	72
12	C <sub>12</sub> H <sub>19</sub> O <sub>8</sub> N <sub>5</sub> ClP <sub>2</sub>	458.0397	458.0385	7.865 (99)	6.205 (100)	69
13	C <sub>9</sub> H <sub>13</sub> O <sub>8</sub> N <sub>5</sub> ClP <sub>2</sub>	415.9928	415.9945	5.960 (100)	8.466 (99)	44
14	C <sub>9</sub> H <sub>13</sub> O <sub>8</sub> N <sub>5</sub> ClP <sub>2</sub>	415.9928	415.9938	5.314 (99)	7.610 (100)	55
15	C <sub>11</sub> H <sub>15</sub> O <sub>8</sub> N <sub>5</sub> ClP <sub>2</sub>	442.0084	442.0086	5.279 (99)	8.565 (99)	63
16	C <sub>12</sub> H <sub>19</sub> O <sub>8</sub> N <sub>5</sub> ClP <sub>2</sub> (FAB positive)	458.0397	458.0392	10.648 (96)	8.564 (98)	77
17	C <sub>12</sub> H <sub>19</sub> O <sub>8</sub> N <sub>5</sub> ClP <sub>2</sub> (FAB positive)	458.0397	458.0415	10.391 (99)	8.457 (99)	57
18	C <sub>12</sub> H <sub>17</sub> O <sub>8</sub> N <sub>5</sub> ClP <sub>2</sub>	456.0241	456.0256	7.882 (100)	6.176 (100)	71
19	C <sub>11</sub> H <sub>15</sub> O <sub>8</sub> N <sub>5</sub> ClP <sub>2</sub>	442.0084	442.0089	5.036 (96)	8.592 (98)	61
20	C <sub>12</sub> H <sub>17</sub> O <sub>8</sub> N <sub>5</sub> ClP <sub>2</sub>	456.0241	456.0251	7.211 (98)	6.522 (100)	55

<sup>a</sup> Purity of each derivative was  $\geq 95\%$ , as determined using HPLC with two different mobile phases. System A: gradient of 0.1 M TEAA/CH<sub>3</sub>CN from 95/5 to 40/60. System B: gradient of 5 mM TBAP/CH<sub>3</sub>CN from 80/20 to 40/60. <sup>b</sup> The percent yields refer to yield for deprotection sequence.

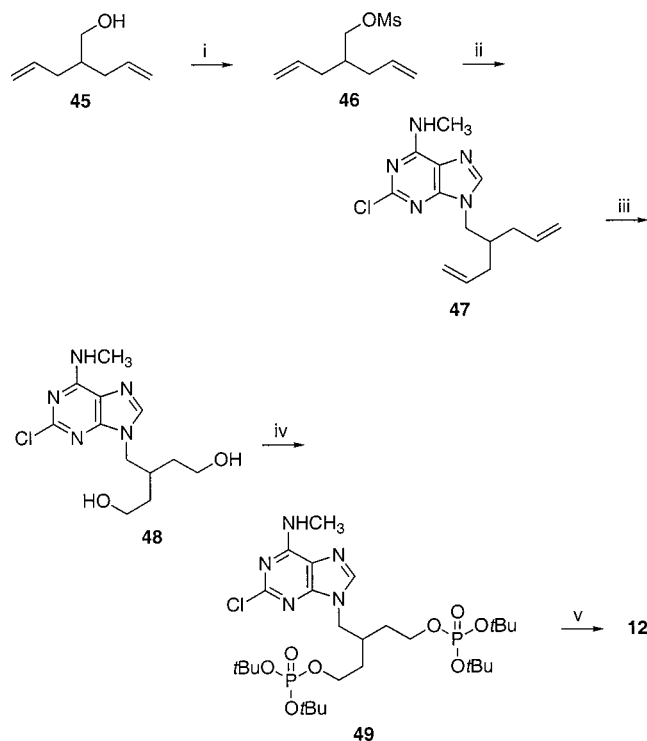
**Scheme 3.** Synthesis of Asymmetrically Phosphorylated Isobutyl Derivatives, **8–11**<sup>a</sup>

<sup>a</sup> Part A, reaction conditions: (i) TBSCl, imidazole, DMF, rt, overnight, 38%; (ii) Et<sub>2</sub>NP(OtBu)<sub>2</sub>, tetrazole, THF, rt, 20 min, then MCPBA, 83%; (iii) TBAF, THF, rt, 10 min, 76%; (iv) 5% TFA in CH<sub>2</sub>Cl<sub>2</sub>, rt, 2 h, 79%; (v) (MeO)<sub>2</sub>P(O)Cl, NaH, THF, rt, 1 h, 31%; (vi) 5% TFA in CH<sub>2</sub>Cl<sub>2</sub>, rt, 2 h, 76%. Part B, reaction conditions: (i) Ac<sub>2</sub>O, TEA, CH<sub>2</sub>Cl<sub>2</sub>, rt, 2 h, 96%; (ii) 5% TFA in CH<sub>2</sub>Cl<sub>2</sub>, rt, 30 min, 59%; (iii) Pr<sub>2</sub>NP(OBn)<sub>2</sub>, tetrazole, THF, rt, 20 min, then MCPBA, 45%; (iv) 5% TFA in CH<sub>2</sub>Cl<sub>2</sub>, rt, 30 min, 89%; (v) C(O)(imi)<sub>2</sub>, NEt<sub>3</sub>·H<sub>3</sub>PO<sub>4</sub>, DMF, rt, 3 d, 74%; (vi) TMSBr, rt, 10 min, 72%.

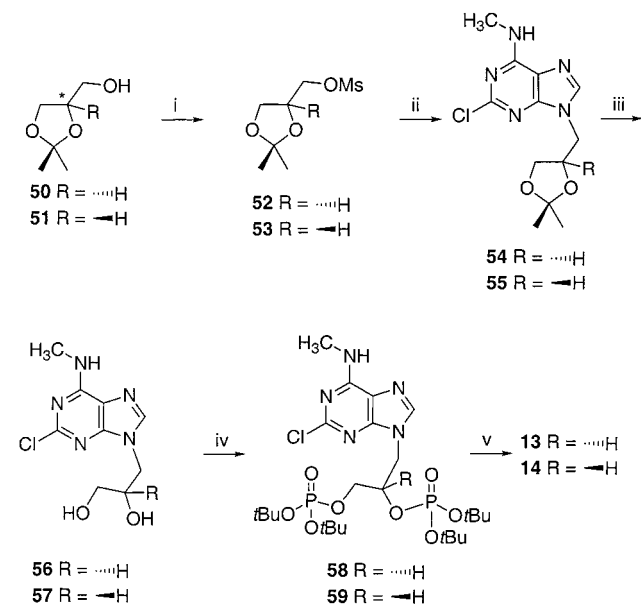
were incorporated, thus facilitating product purification at this intermediate stage. Benzyl phosphates were introduced by the treatment of diols with NaH and tetrabenzylpyrophosphate (Schemes 2, 3B, and 7).<sup>20</sup> The yields for this method of phosphorylation were variable, being limited by the solubility of the diols in THF. In such cases, use of DMF as solvent failed to give the desired product. Alternatively, bisphosphates were made via the *tert*-butyl phosphate esters,<sup>23</sup> which were synthesized by treatment of diols with di-*tert*-butyl diethylphosphoramidite and tetrazole in THF, followed by

oxidation with *m*-chloroperoxybenzoic acid (MCPBA, Schemes 1, 3A, 4–6, and 8–10). The two-step synthesis of *tert*-butyl phosphates had the following advantage over the tetrabenzylpyrophosphate method: limited solubility of the diols in THF could be overcome since these conditions allowed use of excess phosphoramidite and tetrazole reagents with overnight stirring, which either slowly or rapidly resulted in a homogeneous reaction mixture without significant side reactions.

To synthesize compound **11**, containing both mono-phosphate and diphosphate groups, it was necessary to

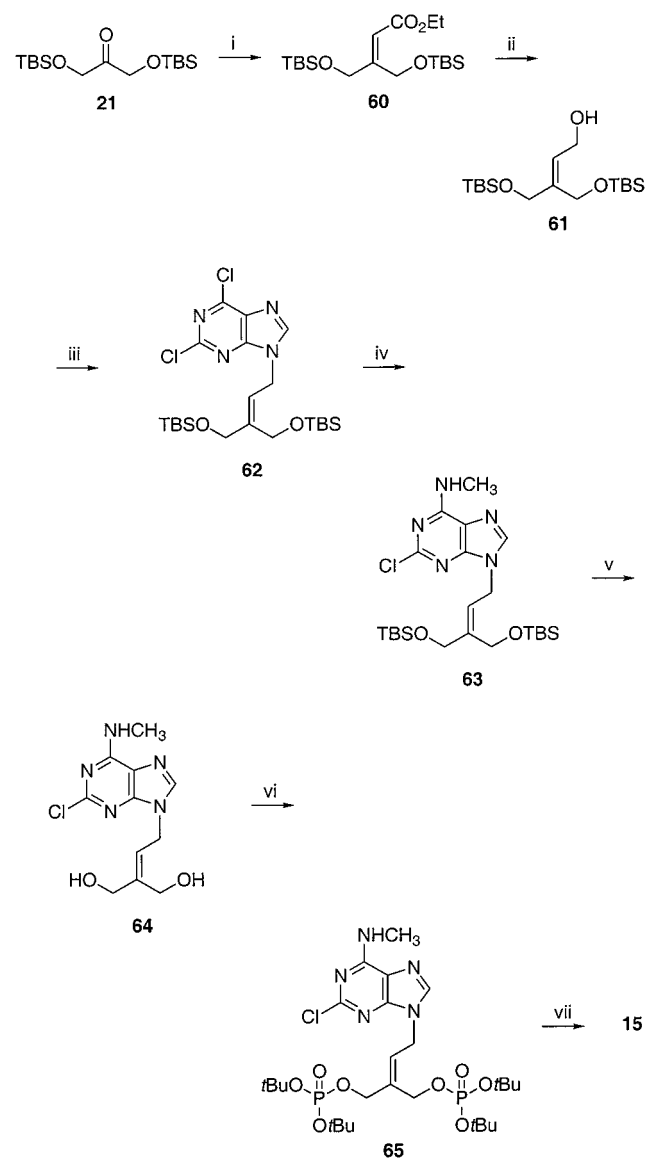
**Scheme 4.** Synthesis of Compound **12**<sup>a</sup>

<sup>a</sup> Reaction conditions: (i) MsCl, TEA, CH<sub>2</sub>Cl<sub>2</sub>, rt, 1 h; (ii) 2-chloro-8-methylaminopurine, K<sub>2</sub>CO<sub>3</sub>, 18-crown-6, DMF, 60–70 °C, overnight, 60% two-step yield; (iii) (a) OsO<sub>4</sub>, NaIO<sub>4</sub>, acetone/H<sub>2</sub>O = 4:1, rt, 4 h, (b) NaBH<sub>4</sub>, EtOH, 0 °C, 2 h, 32% two-step yield; (iv) Et<sub>2</sub>NP(OtBu)<sub>2</sub>, tetrazole, THF, rt, 1 h then MCPBA, 73%; (v) 5% TFA in CH<sub>2</sub>Cl<sub>2</sub>, rt, 30 min, 69%.

**Scheme 5.** Synthesis of *R*- and *S*-Glyceryl Bisphosphate Derivatives, Compounds **13** and **14**<sup>a</sup>

<sup>a</sup> Reaction conditions: (i) MsCl, TEA, CH<sub>2</sub>Cl<sub>2</sub>, rt, 1 h; (ii) 2-chloro-8-methylaminopurine, K<sub>2</sub>CO<sub>3</sub>, 18-crown-6, DMF, 80–90 °C, 12 h, 50% two-steps yield for **54**, 54% two-steps yield for **55**; (iii) Dowex 50x-8 200, MeOH, 40 °C, 4 h, 88% for **56**, 85% for **57**; (iv) Et<sub>2</sub>NP(OtBu)<sub>2</sub>, tetrazole, THF, rt, overnight then MCPBA, 56% for **58**, 69% for **59**; (v) 5% TFA in CH<sub>2</sub>Cl<sub>2</sub>, rt, 1 h, 44% for **13**, 55% for **14**.

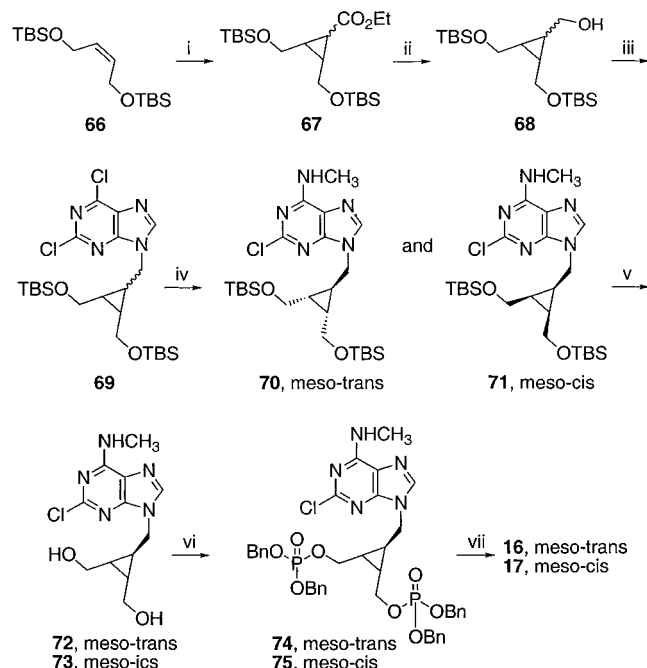
distinguish between the two diols present. This was accomplished through the synthesis of **42**, containing di-*tert*-butyl- and dibenzyl-protected phosphate groups

**Scheme 6.** Synthesis of Isopentenyl Bisphosphate, Compound **10**<sup>a</sup>

<sup>a</sup> Reaction conditions: (i) EtO<sub>2</sub>CCH<sub>2</sub>P(O)(OEt)<sub>2</sub>, NaH, THF, rt, 2 h, 85%; (ii) DIBAL, toluene, –78 °C to rt, 1 h, 74%; (iii) 2,6-chloropurine, DEAD, PPh<sub>3</sub>, THF, rt, 2 h, 57%; (iv) CH<sub>3</sub>NH<sub>2</sub>, THF, rt, 6 h, 85%; (v) AcOH:H<sub>2</sub>O:THF = 1:2:2, 40 °C, 5 h, 86%; (vi) Et<sub>2</sub>NP(OtBu)<sub>2</sub>, tetrazole, THF, rt, 1 h then MCPBA, 35%; (vii) Dowex 50x-8 200, MeOH/H<sub>2</sub>O = 1:1, 50 °C, 3 h, 63%.

(Schemes 3A,B), which was smoothly achieved in four steps from **34**. The *tert*-butyl group was readily deprotected selectively in the presence of a dibenzyl phosphate ester by treatment with 5% trifluoroacetic acid in methylene chloride. The conversion of monophosphate of **43** to the diphosphate in moderate yield utilized a phosphoryl imidazolidine intermediate, which reacted with triethylammonium phosphate.<sup>24</sup> Preparation of analogues **16** and **17** (Scheme 7) required the synthesis of an intermediate, **68**, using a modification of a literature procedure, which also permitted the assignment of the stereochemical identity of each isomer.<sup>25</sup> *meso-cis*- (minor), **71**, and *meso-trans*-cyclopropylmethyl (major), **70**, isomers were separated after condensation of the mesylate with adenine moieties, using silica gel column chromatography. The preparation of a racemic cyclopropylmethyl moiety, leading to **18**, was accom-



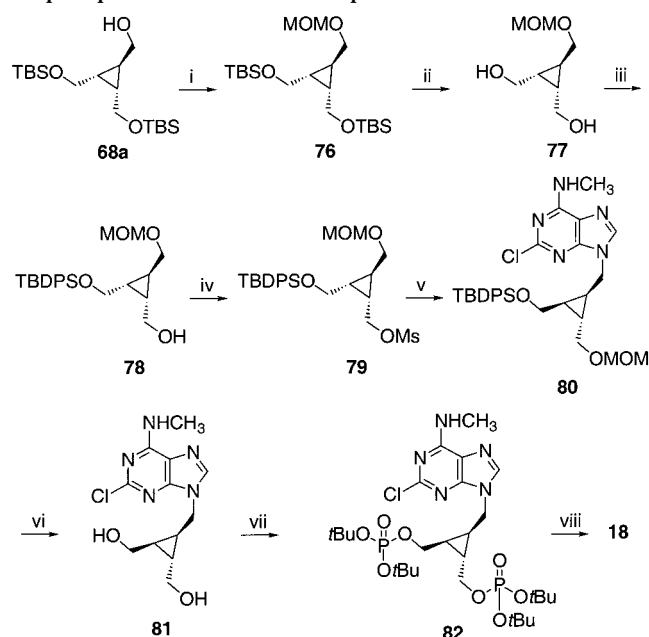
**Scheme 7.** Synthesis of *meso-cis*- and *meso-trans*-Cyclopropyl Bisphosphate Derivatives, Compounds **16** and **17**<sup>a</sup>

<sup>a</sup> Reaction conditions: (i)  $\text{N}_2\text{CH}_2\text{CO}_2\text{Et}$ ,  $\text{Rh}_2(\text{OAc})_4$ ,  $\text{CH}_2\text{Cl}_2$ , rt, 17 h, 61%; (ii)  $\text{LiBH}_4$ , diethyl ether, toluene, 100 °C, 1 h, 93%; (iii) 2,6-chloropurine, DEAD,  $\text{PPh}_3$ , THF, rt, overnight, 59%; (iv)  $\text{CH}_3\text{NH}_2$ , THF, rt, 5 min then separation, total 75%, 70:71 = 2.5:1; (v)  $\text{AcOH}:\text{THF}:\text{H}_2\text{O} = 3:1:1$ , rt, 4 h; (vi)  $(\text{BnO})_2(\text{O})\text{P}_2\text{O}$ , LDA, THF, -78 °C to rt, overnight, 46% two-step yield for **74**, 33% two-step yield for **75**; (vii)  $\text{BCl}_3$ ,  $\text{CH}_2\text{Cl}_2$ , -78 °C for 1 h and 6–7 °C for 2 d, 77% for **16**, 57% for **17**.

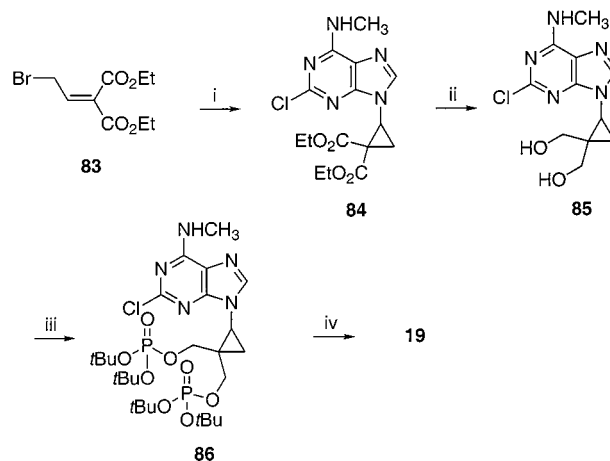
plished through a mesylate displacement (Scheme 8), utilizing TBDPS- (*tert*-butyl-diphenylsilyl) and MOM- (methoxymethyl) protected intermediates. Another cyclopropyl derivative, **19**, was synthesized through displacement of a bromo diester, **83**, with ring closure, followed by diester reduction to generate the diol group (Scheme 9).<sup>26</sup>

**Biological Activity.** Adenine nucleotides activate a  $\text{P2Y}_1$  receptor in turkey erythrocyte membranes to markedly stimulate inositol lipid hydrolysis by phospholipase C.<sup>27–29</sup> The analogues were screened for activation or inhibition of  $\text{P2Y}_1$  receptor promoted-effects on phospholipase C, as indicated by the accumulation of inositol phosphates in membranes isolated from [ $^3\text{H}$ ]inositol-labeled turkey erythrocytes (Table 1). As in our previous study,<sup>20</sup> none of the acyclic adenosine bisphosphate nucleotide analogues displayed agonist activity at the  $\text{P2Y}_1$  receptor in turkey erythrocyte membranes. Concentration–response curves were obtained for each compound for inhibition of the effects of a fixed concentration (30 nM) of the agonist 2-MeSADP.

An acyclic 2-chloro- $N^6$ -methyladenine derivative, **7**, containing a symmetric isobutyl bisphosphate moiety, was a potent  $\text{P2Y}_1$  receptor antagonist, with an  $\text{IC}_{50}$  value of 0.48  $\mu\text{M}$ . The corresponding 2-H derivative, **6**, displayed 2-fold weaker antagonist potency. Thus, antagonists **6** and **7** were roughly twice as potent as the next higher homologues, **3** and **4**, respectively. The lower homologue, the isopropyl derivative, **5**, was nearly an order of magnitude less potent than **7**.

**Scheme 8.** Synthesis of Racemic Cyclopropyl Bisphosphate Derivative, Compound **18**<sup>a</sup>

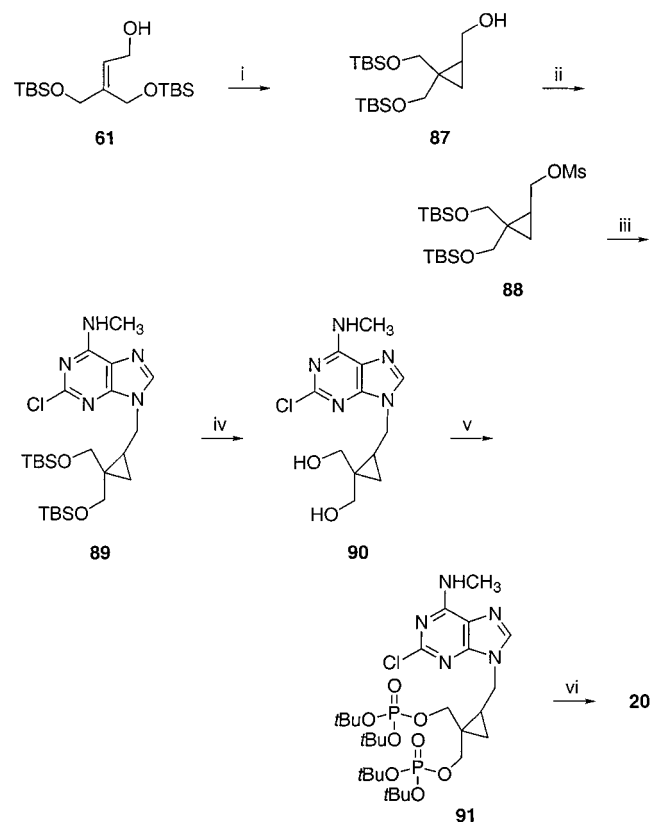
<sup>a</sup> Reaction conditions: (i)  $\text{CH}_3\text{OCH}_2\text{Cl}$ , Hünig base,  $\text{CH}_2\text{Cl}_2$ , rt, 4 h, 93%; (ii) TBAF, THF, rt, 3 h, 95%; (iii) TBDPSCl (1 equiv), DMAP (1 equiv),  $\text{CH}_2\text{Cl}_2$ , rt, 3 d, 70%; (iv)  $\text{MsCl}$ , TEA,  $\text{CH}_2\text{Cl}_2$ , rt, 1 h, 95%; (v) 2-chloro- $N^6$ -methylaminopurine,  $\text{K}_2\text{CO}_3$ , DMF, 70 °C, overnight, 71%; (vi)  $c\text{-HCl}:\text{MeOH} = 1:3$ , 80 °C, 4 h, 65%; (vii)  $\text{Et}_2\text{NP}(\text{O}i\text{Bu})_2$ , tetrazole, THF, rt, 20 min then MCPBA, 62%; (viii) 5% TFA in  $\text{CH}_2\text{Cl}_2$ , rt, 30 min, 71%.

**Scheme 9.** Synthesis of Cyclopropyl Bisphosphate Derivative, Compound **19**<sup>a</sup>

<sup>a</sup> Reaction conditions: (i) 2-chloro- $N^6$ -methylaminopurine,  $\text{K}_2\text{CO}_3$ , DMF, 35 °C, 2 d, 41%; (ii) DIBAL,  $\text{CH}_2\text{Cl}_2$ , -78 °C to rt, 2 h, 70%; (iii)  $\text{Et}_2\text{NP}(\text{O}i\text{Bu})_2$ , tetrazole, THF, rt, 20 min then MCPBA, 85%; (iv) 5% TFA in  $\text{CH}_2\text{Cl}_2$ , rt, 20 min, 61%.

Replacement of one of the phosphate groups of **7** with an uncharged moiety (i.e., racemic alcohol, **8**, phosphotriester, **9**, or *O*-acetyl ester, **10**) resulted in a 500- to 1000-fold loss of potency. Replacement of a phosphate group with diphosphate, **11**, increased potency.

Methylene homologation at the phosphate ester positions of **7**, resulting in compound **12**, decreased potency at the  $\text{P2Y}_1$  receptor 4-fold. Removal of a phosphate-linked methylene group of **7**, resulting in an enantiomeric pair of glycerol derivatives, **13** and **14**, demonstrated a 5-fold stereoselectivity of binding at the  $\text{P2Y}_1$  receptor. The *S*-isomer, **14**, was favored with an  $\text{IC}_{50}$

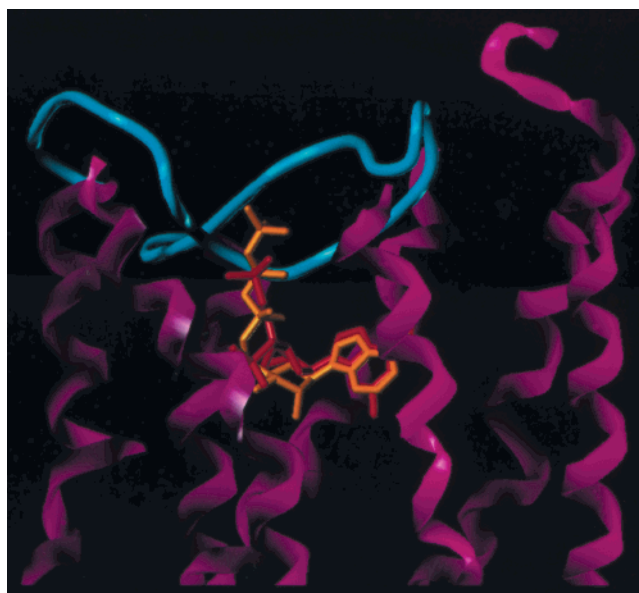
**Scheme 10.** Synthesis of Cyclopropyl Bisphosphate Derivative, Compound **20**<sup>a</sup>

<sup>a</sup> Reaction conditions: (i)  $\text{Et}_2\text{Zn}$ ,  $\text{CH}_2\text{I}_2$ ,  $\text{CH}_2\text{Cl}_2$ , rt, overnight, 96%; (ii)  $\text{MsCl}$ , TEA,  $\text{CH}_2\text{Cl}_2$ , rt, 1 h, 93%; (iii) 2-chloro- $N^6$ -methylaminopurine,  $\text{K}_2\text{CO}_3$ , 18-crown-6, DMF, 60 °C, overnight, 62%; (iv)  $\text{AcOH}:\text{THF}:\text{H}_2\text{O} = 1:2:2$ , 40 °C, overnight, 55%; (v)  $\text{Et}_2\text{NP}(\text{O}t\text{Bu})_2$ , tetrazole, THF, rt, 30 min then MCPBA, 40%; (vi) 5%  $\text{TFA}:\text{CH}_2\text{Cl}_2:\text{THF} = 10:20:1$ , rt, 30 min, 55%.

value of 1.47  $\mu\text{M}$ . Introduction of an olefinic group, i.e., compound **15**, decreased antagonist potency compared to **7** at the  $\text{P2Y}_1$  receptor 4-fold. *meso-trans*-, **16**, and *meso-cis*-, **17**, isomers of a cyclopropylmethyl derivative (two of the four possible stereoisomers) failed to display stereoselectivity of binding, and the  $\text{IC}_{50}$  values were approximately 2  $\mu\text{M}$ . The racemate of the remaining two isomers, **18**, as well as **19** and **20**, had a similar potency. Thus, the antagonistic potency among cyclopropyl derivatives was nearly invariable.

**Molecular Modeling.** To better understand the affinities of the acyclic bisphosphate adenine analogues and, in particular, the correspondence between the receptor bound conformers of these compounds and the rigid, high affinity analogues, we carried out molecular modeling of the ligand–receptor complexes. The human  $\text{P2Y}_1$  receptor model used for this purpose included the seven transmembrane helical domains (TMs) and the second extracellular loop (EL2) and was built in homology to the recently published X-ray structure of bovine rhodopsin.<sup>22</sup> In this X-ray structure EL2, but not EL1 and 3, appears to interact directly with retinal.

Using this construct, docking experiments were carried out to establish a binding site for both prototypical agonists ATP or ADP and an antagonist, **2**.<sup>19</sup> These experiments were guided by our previous modeling experiments,<sup>30,31</sup> in which parts of the binding pocket accommodating the adenine moiety have been identi-

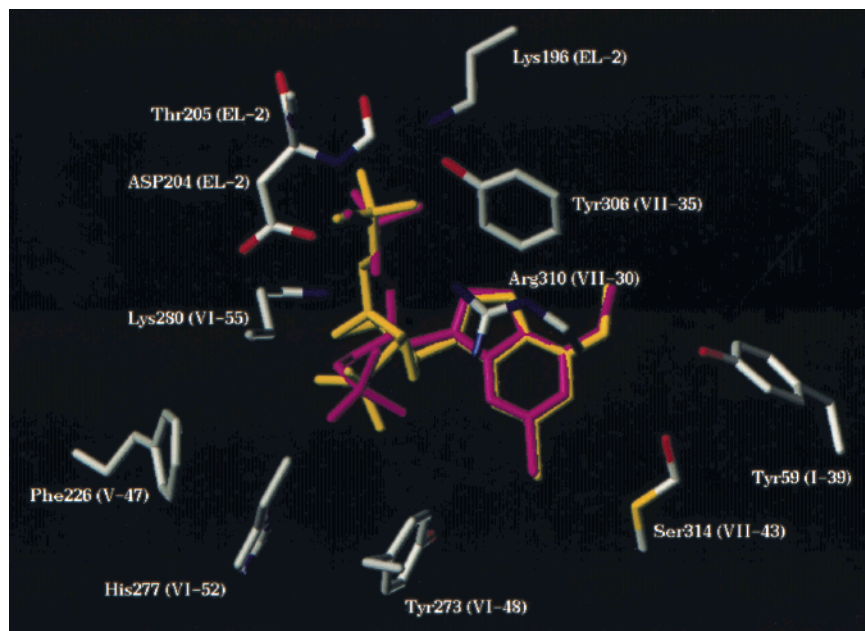


**Figure 2.** Structural correspondence of ATP (orange) and **2** (red), superimposed in the putative receptor binding site. TMs (magenta) and EL2 (cyan), joining TM4 and 5, are shown. The adenine moieties occupy the same binding subsite.

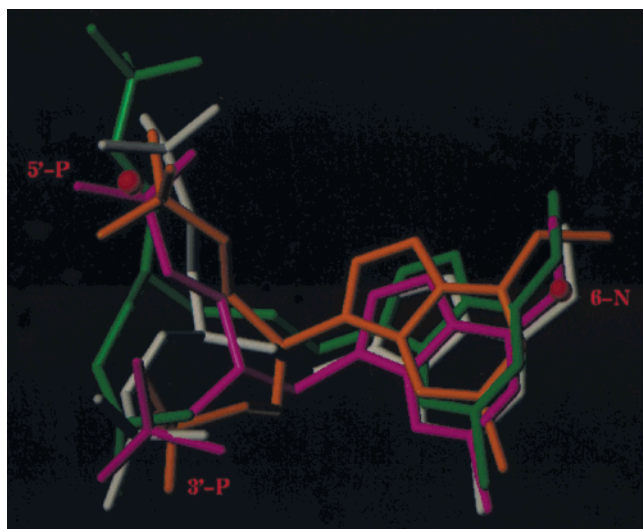
fied, by mutagenesis studies,<sup>30</sup> and by possible modes of superposition of the two ligand structures.

The energy-optimized structures of  $\text{P2Y}_1$  receptor complexes with ATP and **2** were practically superimposable with respect to the protein backbone atoms. The structural correspondence of the two ligands (Figure 2) indicated that in both cases the adenine moieties occupied the same binding subsite. According to this model, the 5'-phosphate of **2** overlapped the  $\beta$ -phosphate moiety of ATP, and the 3'-phosphate of **2** had no structural equivalent in the bound ATP structure. As in the previous model,<sup>30</sup> neither the ribose ring of ATP nor the methanocarba ring of **2** participated in binding interactions with the receptor.

Using the receptor-**2** complex as a template, the various acyclic bisphosphates were docked into the binding site and the models reoptimized. The structural correspondence between the receptor-bound conformer of **4** and that of **2** is shown in Figure 3. This figure also depicts the various residues involved in specific interactions with the ligands, including Lys280 and Arg310, which interacted with both phosphate groups of the ligands,<sup>30</sup> and other residues. The residues Lys196, Tyr306, and Asp204 apparently were involved in polar interactions with the phosphate group corresponding to the 5'-phosphate of **2**. The residues Phe226, Tyr273, and His277 formed a sterically restricted pocket to accommodate the 3'-phosphate of **2**. The residues Tyr59, Tyr273, and Ser314 interacted with the adjacent adenine nitrogen atoms (Figure 3). The similarity in positioning of the binding elements for **2** and **4** with respect to the modeled receptor manifold (see Figure 3) was consistent with the more potent inhibition of the  $\text{P2Y}_1$  receptor by the latter (see Table 1). Furthermore, ligands homologous to **4** (having 0 to 3 methylenes adjacent to adenine N-9), representing a >100-fold span of  $\text{IC}_{50}$  values, displayed varying degrees of correspondence to this pharmacophoric arrangement based on **2**. An overall comparison of the bound conformations of **4** and the three homologues is shown in Figure 4, while specific



**Figure 3.** Structural correspondence between the receptor bound conformer of **4** (magenta) and that of **2** (yellow), depicting the residues involved in specific interactions with the ligands. Lys280 and Arg310 interact with both phosphate groups of the ligands.



**Figure 4.** Comparison of the bound conformations of **4** (white) and three homologous derivatives, **5** (orange), **7** (magenta), and the analogue containing an *n*-propyl spacer **94** (green).

deviations of atoms corresponding to 3'-P, 5'-P, and 6-N of **2** are listed in Table 3.

Table 3 compares the receptor-bound conformations of rigid and acyclic P2Y<sub>1</sub> antagonists. A relatively high affinity was maintained, as long as the position of a given antagonist did not deviate significantly with respect to 6-N and 3'-P of the template. In contrast, the binding pocket, including the relatively flexible EL2, provided significantly more room for the group bearing the 5'-phosphate. To further examine this suggestion, the docked conformations of three potent, cyclic P2Y<sub>1</sub> antagonists,<sup>18,19</sup> **92** (2'-deoxy-2-chloro-*N*<sup>6</sup>-methyladenosine-3',5'-bisphosphate), **2**, and **93** (phosphoric acid mono-[3-(2-chloro-6-methylaminopurin-9-yl)-2-phosphonooxymethylcyclobutyl] ester) (Figure 5). Indeed, the requirements with respect to positioning the 5'-phosphate in the receptor appeared not

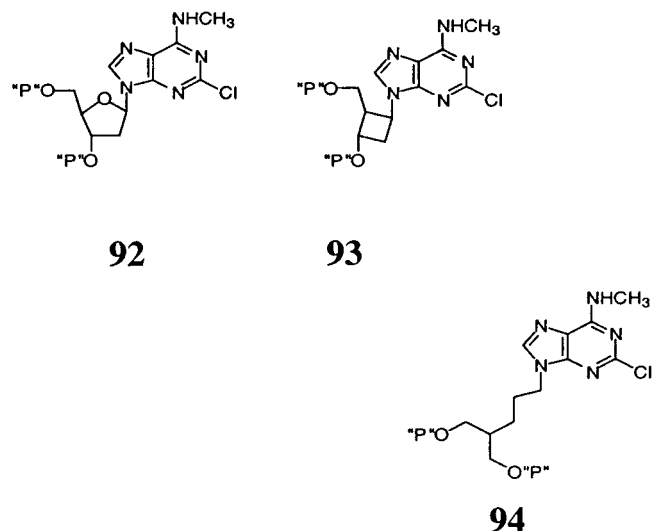
**Table 3.** Distances in Angstroms between Pharmacophoric Points of P2Y<sub>1</sub> Docked Antagonists and Template Compound **2**<sup>a</sup>

compd		3'-P	5'-P	6-N
<b>92</b>		0.35	0.36	0.05
<b>93</b>		0.28	1.88	0.20
<b>5</b>		0.42	0.79	1.27
<b>7</b>		0.38	0.50	0.27
<b>4</b>		0.42	1.08	0.49
<b>94</b>		0.33	2.22	0.7
<b>12</b>		0.44	1.78	0.43
<b>13</b>		0.45	0.83	1.24
<b>14</b>		0.45	1.40	0.46
<b>15</b>		0.16	0.42	0.25
<b>16</b>		0.50	0.86	0.21
<b>17</b>		0.27	1.60	0.30
<b>18</b>	isomer 1	0.78	0.72	0.50
	isomer 2	0.27	1.31	0.27
<b>19</b>	<i>R</i> -config	0.10	0.81	0.15
	<i>S</i> -config	0.15	0.83	0.21
<b>20</b>	<i>R</i> -config	0.26	0.63	0.18
	<i>S</i> -config	0.08	0.54	0.10

<sup>a</sup> Compound **2** (MRS 2279) is (1*R*,2*S*,4*S*,5*S*)-1-[(phosphato)methyl]-4-(2-chloro-6-methylaminopurin-9-yl)bicyclo [3.1.0]-hexane-2-phosphate. Compounds are grouped as cyclic template compounds (**92**, **93**), acyclic bisphosphates of variable chain length (0–3 methylenes) (**5**, **7** (MRS 2298), **4**, **94**), other acyclic compounds (**12**–**15**), and cyclopropyl derivatives (**16**–**20**). Compounds **92** (2'-deoxy-2-chloro-*N*<sup>6</sup>-methyladenosine-3',5'-bisphosphate (MRS 2216), IC<sub>50</sub> 0.206  $\mu$ M), **93** (phosphoric acid mono-[3-(2-chloro-6-methylaminopurin-9-yl)-2-phosphonooxymethylcyclobutyl] ester (MRS 2264), IC<sub>50</sub> 0.805  $\mu$ M), and **94** (2-[3-(6-methylaminopurin-9-yl)-propyl]propane-1,3-bisphosphate, IC<sub>50</sub> >30  $\mu$ M) were reported previously.<sup>19,20</sup>

to be very stringent, since the location of the corresponding phosphate of **93** deviated considerably from that of **2**. Such a model is also consistent with the high activity of **11** (see Table 1), where the adenine and monophosphate binding elements could be expected to occupy receptor subsites analogous to those of **7**. The flexible chain bearing the diphosphate moiety allows for its accommodation in a similar way to that of the  $\alpha$ - and  $\beta$ -phosphates in ADP or ATP. The relative orientation of the three pharmacophoric elements in the





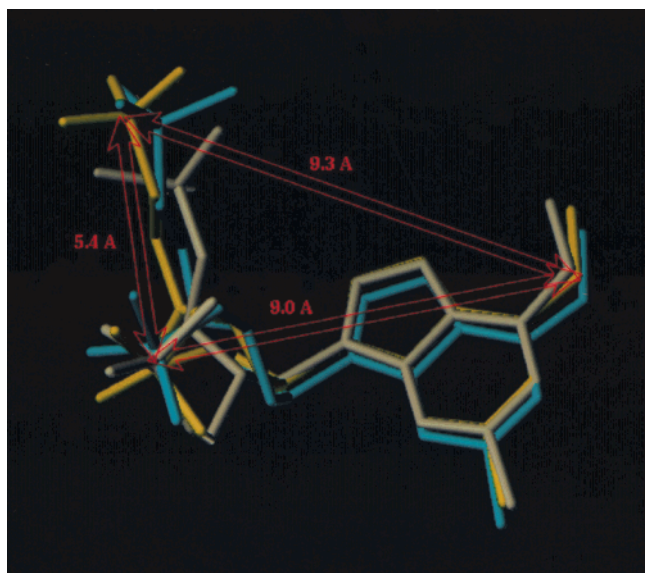
template structure of **2** is presented (Figure 5). The resulting distances differ somewhat from those cited in our previous publication<sup>30</sup> due to the refined receptor model used in the present study.

## Discussion

We have improved upon the affinity of **4** and found the optimal length of the spacer chain to be one methylene, i.e., **7**. Numerous cyclopropyl analogues were designed to add an element of rigidity, by analogy to antagonists of metabotropic glutamate receptors in which selectivity was achieved specifically depending on the stereochemistry.<sup>32</sup> However, all of the cyclopropyl analogues displayed *K<sub>i</sub>* values in the 1–2  $\mu$ M range. Although the lead bisphosphate structure, upon which these analogues were based, i.e., **4**, was selective for the P2Y<sub>1</sub> subtype,<sup>33</sup> it will nevertheless be useful to examine the acyclic compounds as agonists and/or antagonists at other subtypes of P2 receptors.

We have refined our previous modeling of the P2Y<sub>1</sub> receptor<sup>30</sup> utilizing the high-resolution template structure of rhodopsin now available.<sup>22</sup> In general, the two receptor models are similar with respect to the overall juxtaposition of the side chains forming the nucleotide binding pocket (Figure 3), while differing in the length of the individual helices and in their mutual orientation within the helical bundle. In addition, a portion of EL2 bridging TM4 and TM5 is deeply embedded in the TM-manifold, much like the  $\beta$ 4 strand of the antiparallel  $\beta$ -sheet of EL2 in rhodopsin.<sup>22</sup> Thus, while both P2Y<sub>1</sub> receptor models<sup>30</sup> predict proximity of EL2 to the path of approach of the ligand toward the TM binding site, the present model indicates participation of certain residues of this loop in coordination of the ligand bound within the TM domain (Figure 2).

Previous modeling studies<sup>19,30</sup> suggested that agonists and antagonists may share a common binding site within the receptor. In particular cases, substitution of a single *N*<sup>6</sup>-methyl group converted a pure P2Y<sub>1</sub> agonist into an antagonist, apparently without affecting affinity. The first modeling experiment carried out with this P2Y<sub>1</sub> receptor model was the docking of the prototypical agonists ATP and ADP, aimed to rationalize the apparently superior binding of the latter to the receptor.<sup>31</sup> The binding site model satisfied the following binding re-



**Figure 5.** Docked conformations of three relatively rigid potent P2Y<sub>1</sub> antagonists (see Table 3 for chemical names): **92** (cyan, a riboside), **2** (yellow), and **93** (white, a cyclobutyl analogue). Also shown is the relative orientation of the three pharmacophoric elements in the template structure of **2**. The requirements with respect to positioning the 5'-phosphate in the receptor appeared not to be very stringent, since the location of the corresponding phosphate of **93** deviated considerably from that of **2**.

quirements: differentially accommodating (a) the conformationally constrained agonists ATP and ADP; (b) rigid antagonists such as **2** (Figure 2); (c) flexible, acyclic antagonists reported here (Figure 4).

Initially, basic residues needed to accommodate the phosphate moieties of the ligands were identified (Lys128, Lys196, Lys280, Arg310). The model suggested that the  $\beta$ -phosphate moieties of ATP and ADP, as well as the 5'-phosphate substituent of **2**, may occupy a position adjacent to the antiparallel  $\beta$ -sheet of EL2. This interpretation was based on the mutual orientation of these basic side chains, as well as the juxtaposition of the main chain amide nitrogens of residues Asp204 and Thr205. In this position the 5'-phosphate moiety did not seem to be sterically constrained, and its main interactions were with the flexible lysine and arginine side chains. Interestingly, according to this ligand orientation, the  $\gamma$ -phosphate of ATP should not contribute to affinity due to its location above the  $\beta$ 4 strand of the EL2  $\beta$ -sheet.

In contrast to the relaxed steric requirements for accommodation of the 5'-phosphate moiety of **2**, the receptor seemed to present a restricted binding pocket for the 3'-phosphate substituent. Consequently, it appeared that the ligand binding environment of the P2Y<sub>1</sub> receptor consisted of rigidly defined binding subsites for the adenine and the 3'-phosphate moieties (using ligand **2** as structural template) while positioning of the 5'-phosphate was less stringent. Such notion is consistent with the affinities of the different P2Y<sub>1</sub> ligands in which these three pharmacophoric elements are held together by various spacers, such as carbocyclic and heterocyclic rings or carbon chains. In all these cases, the ligand-bound conformation seemed to conform to the proposed model (Table 3). We note that compound **11**, where the monophosphate and the diphosphate substituents mimic

the 3'-substituent of **2** and the 5'-substituent of ADP, respectively, was the most potent of the acyclic P2Y<sub>1</sub> antagonists reported here.

In conclusion, two binding pockets for monophosphate moieties were defined. The flexibility of the spacer chain linking bisphosphate groups to the adenine moiety, in general, allowed a relaxation of spatial requirements, although the more potent antagonists in the acyclic series conformed to a three-point model of the docked positions of the phosphates relative to the adenine N<sup>6</sup>. This was especially true for the series of symmetrically substituted homologues **3–7** and **94** (Table 3). Elongation of the ethylene group of **4** was previously shown to greatly decrease potency at the P2Y<sub>1</sub> receptor.<sup>20</sup> We have identified the optimal chain length for P2Y<sub>1</sub> antagonism to be one methylene group, in **7**. We have now achieved potency in an acyclic derivative comparable to the best antagonists in the ribose series.<sup>16</sup> Thus, both approaches of rigidifying ribose<sup>19</sup> and ring opening<sup>20</sup> have proven successful design approaches for P2Y<sub>1</sub> antagonists.

## Experimental Section

### Chemical Synthesis. Materials and Instrumentation.

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

<sup>1</sup>H NMR spectra were obtained with a Varian Gemini-300 spectrometer using CDCl<sub>3</sub>, DMSO-*d*<sub>6</sub>, or D<sub>2</sub>O 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). <sup>31</sup>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. Low-resolution CI-NH<sub>3</sub> (chemical ionization) mass spectra were carried out with a Finnigan 4600 mass spectrometer and high-resolution EI (electron impact) mass spectrometry with a VG7070F mass spectrometer at 6 kV. High-resolution FAB (fast atom bombardment) mass spectrometry was performed with JEOL SX102 spectrometer using 6-kV Xe atoms following desorption from a glycerol matrix.

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<sub>3</sub>CN 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<sub>3</sub>CN, 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 derivatives showed more than 95% purity as determined using HPLC.

**1,3-Bis-(*tert*-butyldimethylsilanyloxy)propan-2-ol (22).** To a cooled (0 °C) and stirred solution of disilyl ketone **21** (1.20 g, 3.77 mmol) in ethyl alcohol (6 mL) was added a solid sodium borohydride (80 mg, 2.11 mmol) in one pot. After the mixture was stirred at 0 °C for 30 min, water (10 mL) was added, and solid ammonium bicarbonate (5 g) was added at the same temperature to quench excess residual sodium borohydride. The resulting mixture was partitioned between ethyl acetate (50 mL) and brine (20 mL). The organic layer was washed with brine/water (1:1, 10 mL × 2), dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to give a crude disilyl alcohol **22** (1.10 g, 91%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.07 (s, 12H), 0.90 (s, 18H), 3.54–3.72 (m, 5H).

**Method A. Mitsunobu Reaction and Substitution of 6-Chloro Group by Methylamine To Introduce *N*-Methylamino-2-chloro- (or hydro-)purine Moiety.** The alcohol **22** (250 mg, 0.78 mmol), triphenylphosphine (307 mg, 1.17 mmol), and 2,6-dichloropurine (189 mg, 0.858 mmol) were

mixed and dried by high vacuum pump for about 30 min. The mixture was dissolved in anhydrous tetrahydrofuran (5 mL) and cooled to 0 °C. To this solution was dropwise added diethylazodicarboxylate (149 mg, 0.855 mmol) via syringe. After the mixture was stirred for 2–4 h at room temperature (the progress of the reaction was monitored by thin-layer chromatography, and if necessary it was overnight stirred), tetrahydrofuran was removed in vacuo. The residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 5:1) to give a desired 2,6-dichloropurine analogue **23** (260 mg, 68%).

The 2,6-dichloropurine analogue **23** was treated with 2 M methylamine in tetrahydrofuran (5 mL, 10 mmol), stirred for 5 h, filtrated through silica gel (10 g), and washed with hexanes/ethyl acetate (1:2). Evaporation of the filtrate gave a desired 6-amino-2-chloropurine analogue **24** (225 mg, 88%).

**9-[2-(*tert*-Butyldimethylsilyloxy)-1-(*tert*-butyldimethylsilyloxymethyl)ethyl]-2,6-dichloro-9H-purine (23).** <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.008 (s, 6H), 0.014 (s, 6H), 0.84 (s, 18H), 3.99 (dd, 2H, *J* = 5.2, 10.6 Hz), 4.08 (dd, 2H, *J* = 5.4, 10.6 Hz), 4.81 (m, 1H), 8.34 (s, 1H). MS (CI/NH<sub>3</sub>) *m/z* 491 (M + H)<sup>+</sup>.

**{9-[2-(*tert*-Butyldimethylsilyloxy)-1-(*tert*-butyldimethylsilyloxymethyl)ethyl]-2-chloro-9H-purin-6-yl}-methylamine (24).** <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.008 (s, 6H), 0.014 (s, 6H), 0.84 (s, 18H), 3.94 (dd, 2H, *J* = 5.4, 10.3 Hz), 4.01 (dd, 2H, *J* = 5.1, 10.3 Hz), 4.70 (m, 1H), 6.04 (bs, 1H), 7.91 (s, 1H). MS (CI/NH<sub>3</sub>) *m/z* 486 (M + H)<sup>+</sup>.

**Method B. Hydrolysis of TBS Group or Acetonide Group with Acidic Dowex Resin.** A mixture of disilyl ether **24** (215 mg, 0.442 mmol), Dowex 50x-8 200 (500 mg), and methanol (5 mL) was stirred overnight at refluxing temperature. The resin was removed by filtration of the resulting mixture through glass filter (10–20 μm, pore size). The filtrate was evaporated in vacuo to give diol **25** (85 mg, 75%). (This method was also used in deprotection of acetonide group of **54** and **55**).

**2-(2-Chloro-6-methylaminopurin-9-yl)propane-1,3-di-ol (25).** <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 2.91 (bs, 3H), 3.76 (dd, 2H, *J* = 5.4, 11.4 Hz), 3.83 (dd, 2H, *J* = 7.0, 11.4 Hz), 4.46 (m, 1H), 8.15 (s, 1H). MS (CI/NH<sub>3</sub>) *m/z* 258 (M + H)<sup>+</sup>.

**Method C. Preparation of Bis(di-*tert*-butyl phosphate) from Diol.** To a stirred suspension of diol **25** (22 mg, 0.0854 mmol) and diethyl di-*tert*-butylphosphoramidite (53 mg, 0.213 mmol) in anhydrous tetrahydrofuran (5 mL) at room temperature was added solid tetrazole (36 mg, 0.513 mmol) in one pot. The reaction mixture was stirred at room temperature for 20 min (sometimes overnight) and then cooled to –78 °C. To this cooled reaction mixture was added solid MCPBA (57–85%, calculated by 57%, 64 mg), and the resulting mixture was stirred at –78 °C for 30 min and at room temperature for 10 min. After removal of tetrahydrofuran in vacuo, the residue was directly purified by preparative thin-layer chromatography (CHCl<sub>3</sub>/MeOH = 20/1) to give **26** (40 mg, 73%).

**Phosphoric Acid Di-*tert*-butyl Ester 2-(2-Chloro-6-methylaminopurin-9-yl)-3-(di-*tert*-butoxyphosphoryloxy)-propyl Ester (26).** <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.39 (s, 18H), 1.41 (s, 18H), 3.17 (bs, 3H), 4.34 (dd, 2H, *J* = 5.4, 11.0, 11.0 Hz), 4.47 (dd, 2H, *J* = 6.6, 11.0, 11.0 Hz), 5.00 (m, 1H), 6.16 (bs, 1H), 7.88 (s, 1H). MS (CI/NH<sub>3</sub>) *m/z* 642 (M + H)<sup>+</sup>, 659 (M + NH<sub>4</sub>)<sup>+</sup>.

**3-(*tert*-Butyldimethylsilyloxy)-2-(*tert*-butyldimethylsilyloxymethyl)propene (28).** To a stirred solution of propenediol **27** (3.0 g, 34.1 mmol), triethylamine (6.90 g, 68.2 mmol), and 4-(dimethylamino)pyridine (20 mg, 0.264 mmol) in methylene chloride (30 mL) at room temperature was added solid *tert*-butyldimethylsilyl chloride (10.7 g, 71.5 mmol). After being stirred overnight at room temperature, the resulting mixture was diluted with ethyl acetate (100 mL). The organic layer was washed with 1 N hydrochloric acid (20 mL), saturated aqueous sodium bicarbonate (30 mL × 2), brine/water (1:1, 20 mL × 2), dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to dryness to give crude **28** (10.7 g, 99%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.07 (s, 12H), 0.92 (s, 18H),



4.17 (t, 4H,  $J = 1.5$  Hz), 5.09 (t, 2H,  $J = 1.5$  Hz). MS (CI/NH<sub>3</sub>)  $m/z$  317 ( $M + H^+$ )<sup>+</sup>.

**3-(*tert*-Butyldimethylsilyloxy)-2-(*tert*-butyldimethylsilyloxymethyl)propan-1-ol (29).** A 1 M solution of borane (in tetrahydrofuran, 34.2 mL, 34.2 mmol) was added dropwise to a cooled (0 °C), stirred solution of **28** (3.6 g, 11.37 mmol) in anhydrous tetrahydrofuran (10 mL). After being stirred at the same temperature, the reaction mixture was warmed to room temperature and stirred overnight. The resulting mixture was treated with potassium carbonate (10 g, 72.4 mmol) and 30% hydrogen peroxide (20 mL) at 0 °C, stirred at room temperature for 2 h, and partitioned between ethyl acetate (100 mL) and water (50 mL). The organic layer was separated, washed with brine/water (1:1, 20 mL  $\times$  3), dried over anhydrous sodium sulfate, filtered, concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 2:1) to give **29** (2.28 g, 60%). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.07 (s, 12H), 0.90 (s, 18H), 1.90 (m, 1H), 3.73 (d, 2H,  $J = 6.0$  Hz), 3.75 (d, 2H,  $J = 6.0$  Hz), 3.78 (d, 2H,  $J = 5.1$  Hz). MS (CI/NH<sub>3</sub>)  $m/z$  335 ( $M + H^+$ )<sup>+</sup>.

**Method D. Mesylation and N-Alkylation To Introduce N<sup>6</sup>-Methylamino-2-chloro- (or 2-hydro)purine Moiety.** To a cooled (0 °C), stirred solution of alcohol **29** (1.47 g, 4.40 mmol) and triethylamine (1.34 g, 13.2 mmol) in methylene chloride (10 mL) was dropwise and neatly added methanesulfonyl chloride (756 mg, 6.60 mmol). After being stirred at 0 °C for 10 min and at room temperature for 1 h, the resulting mixture was partitioned between ethyl acetate (50 mL) and brine (20 mL). The organic layer was separated and washed with saturated sodium bicarbonate (20 mL) and brine/water (1:1, 10 mL  $\times$  2), dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to give a crude mesylate **30** (1.90 g). The mesylate **30** was used in the following N-alkylation without further purification.

A mixture of crude mesylate **30** (1.9 g), 2-chloro-6-methylaminopurine (808 mg, 4.40 mmol), potassium carbonate (912 mg, 6.60 mmol), 18-crown-6 (10 mg, mmol), and dimethylformamide (10 mL) was stirred overnight at 40–60 °C. The resulting mixture was partitioned between ethyl acetate (150 mL) and brine/water (40 mL:20 mL). The organic layer was separated, washed with brine (20 mL  $\times$  4), and dried over anhydrous sodium sulfate, filtered, concentrated to dryness. The residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 1:1) to give **32** (1.47 g, 72% two steps yield).

**Methanesulfonic Acid 3-(*tert*-Butyldimethylsilyloxy)-2-(*tert*-butyldimethylsilyloxymethyl)propyl Ester (30).** <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.06 (s, 12H), 0.89 (s, 18H), 2.10 (m, 1H), 3.00 (s, 3H), 3.63 (dd, 2H,  $J = 5.2$ , 10.2 Hz), 3.68 (dd, 2H,  $J = 5.8$ , 10.2 Hz), 4.30 (d, 2H,  $J = 5.8$  Hz). MS (CI/NH<sub>3</sub>)  $m/z$  413 ( $M + H^+$ )<sup>+</sup>.

**{9-[3-(*tert*-Butyldimethylsilyloxy)-1-(*tert*-butyldimethylsilyloxymethyl)-propyl]-9H-purin-6-yl]-methylamine (31).** **Method D.** Compound **31** (280 mg, 60% two steps yield) was obtained from **30** (410 mg, 0.993 mmol). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.04 (s, 12H), 0.90 (s, 18H), 2.32 (m, 1H), 3.22 (bs, 3H), 3.54 (dd, 2H,  $J = 5.9$ , 10.2 Hz), 3.65 (dd, 2H,  $J = 4.8$ , 10.2 Hz), 4.22 (d, 2H,  $J = 7.2$  Hz), 5.79 (bs, 1H), 7.75 (s, 1H), 8.41 (s, 1H). MS (CI/NH<sub>3</sub>)  $m/z$  466 ( $M + H^+$ )<sup>+</sup>.

**{9-[3-(*tert*-Butyldimethylsilyloxy)-1-(*tert*-butyldimethylsilyloxymethyl)propyl]-2-chloro-9H-purin-6-yl]-methylamine (32).** **Method D.** <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.04 (s, 12H), 0.90 (s, 18H), 2.29 (m, 1H), 3.20 (bs, 3H), 3.52 (dd, 2H,  $J = 5.9$ , 10.2 Hz), 3.64 (dd, 2H,  $J = 5.0$ , 10.2 Hz), 4.19 (d, 2H,  $J = 7.0$  Hz), 5.93 (bs, 1H), 7.71 (s, 1H). MS (CI/NH<sub>3</sub>)  $m/z$  500 ( $M + H^+$ )<sup>+</sup>.

**Method E. Deprotection of Disilyl Ether and Acetonide Groups with Acetic Acid/THF/Water.** A solution of disilyl ether **32** (300 mg, 0.644 mmol) in acetic acid/tetrahydrofuran/water (3 mL:2 mL:2 mL) was stirred at 50 °C for 1 day. All volatile material was removed in vacuo, and the residue was washed with hexanes/ethyl ether (1:1) to give diol **34** (163 mg, 93%).

**2-(6-Methylaminopurin-9-ylmethyl)propane-1,3-diol (33).** **Method E.** Compound **33** (106 mg, 79%) was obtained from **31** (260 mg, 0.993 mmol). <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  2.23 (m, 1H), 3.12 (bs, 3H), 3.54 (m, 4H), 4.33 (d, 2H,  $J = 6.7$  Hz), 8.05 (s, 1H), 8.26 (s, 1H). MS (CI/NH<sub>3</sub>)  $m/z$  238 ( $M + H^+$ )<sup>+</sup>.

**2-(2-Chloro-6-methylaminopurin-9-ylmethyl)propane-1,3-diol (34).** **Method E.** <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  2.23 (m, 1H), 3.06 (bs, 3H), 3.54 (m, 4H), 4.27 (d, 2H,  $J = 6.7$  Hz), 8.00 (s, 1H). MS (CI/NH<sub>3</sub>)  $m/z$  272 ( $M + H^+$ )<sup>+</sup>.

**Method F. Preparation of Tetrabenzylbisphosphates from Diol.** To a cooled (0 °C), stirred diol **34** (50 mg, 0.184 mmol) and tetrabenzylpyrophosphate (247 mg, 0.423 mmol) in anhydrous tetrahydrofuran (5 mL) was added solid sodium hydride (80 mg, 50% in mineral oil, ~1.67 mmol) in one pot. The reaction mixture was stirred at 0 °C for 10 min and at room temperature for 30 min. The resulting mixture was poured into ice–water (20 mL) solution, and the aqueous layer was extracted with ethyl acetate (20 mL  $\times$  3). The combined organic phase was washed with brine (10 mL), dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate/methanol = 15:1) to afford a desired bis(dibenzyl phosphate) **36** (82 mg, 53%). (In the case of cyclopropanediols **73** and **72**, lithium diethylamide was used as a base as substitute for sodium hydride.)

**Phosphoric Acid Dibenzyl Ester 3-(Bisbenzyloxyphosphoryloxy)-2-(6-methylaminopurin-9-ylmethyl)propyl Ester (35).** **Method F.** Compound **35** (59 mg, 81%) was obtained from **33** (23 mg, 0.097 mmol). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  2.57 (m, 1H), 3.19 (bs, 3H), 3.90 (m, 4H), 4.03 (d, 2H,  $J = 6.9$  Hz), 5.01 (m, 8H), 5.99 (q, 1H,  $J = 5.1$  Hz), 7.22–7.38 (m, 20H), 7.61 (s, 1H), 8.34 (s, 1H). <sup>31</sup>P NMR (D<sub>2</sub>O)  $\delta$  –22.363. MS (positive-ion FAB)  $m/z$  758 ( $M + H^+$ )<sup>+</sup>.

**Phosphoric Acid Dibenzyl Ester 3-(Bisbenzyloxyphosphoryloxy)-2-(2-chloro-6-methylaminopurin-9-ylmethyl)propyl Ester (36).** **Method F.** <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  2.49 (m, 1H), 3.17 (bs, 3H), 3.86 (m, 4H), 3.97 (d, 2H,  $J = 7.0$  Hz), 4.89–5.08 (m, 8H), 5.99 (bs, 1H), 7.15–7.0.38 (m, 20H), 7.57 (s, 1H). MS (CI/NH<sub>3</sub>)  $m/z$  792 ( $M + H^+$ )<sup>+</sup>.

**( $\pm$ )-3-(*tert*-Butyldimethylsilyloxy)-2-(2-chloro-6-methylaminopurin-9-ylmethyl)propan-1-ol (( $\pm$ )-37).** To a stirred solution of diol **34** (370 mg, 1.34 mmol), imidazole (140 mg, 2.06 mmol), in dimethylformamide at room temperature was added solid *tert*-butyldimethylsilyl chloride (226 mg, 1.50 mmol) in one pot. The reaction mixture was overnight stirred at room temperature. Dimethylformamide was removed in vacuo, and the residue was purified by preparative thin-layer chromatography (chloroform/methanol = 10:1) to give monosilyl derivative, **37** (200 mg, 38%). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.05 (s, 6H), 0.89 (s, 9H), 2.22 (m, 1H), 3.15 (bs, 3H), 3.35–3.52 (m, 2H), 3.51 (dd, 1H,  $J = 7.1$ , 10.4 Hz), 3.61 (dd, 1H,  $J = 5.0$ , 10.4 Hz), 4.32 (m, 2H), 6.60 (bs, 1H), 7.71 (s, 1H). MS (positive-ion FAB) 386 ( $M + H^+$ )<sup>+</sup>.

**( $\pm$ )-Phosphoric Acid Di-*tert*-butyl Ester 3-(*tert*-Butyldimethylsilyloxy)-2-(2-chloro-6-methylaminopurin-9-ylmethyl)propyl Ester (38).** **Method C.** Compound **38** (250 mg, 83%) was obtained from **37** (200 mg, 0.52 mmol). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.04 (s, 6H), 0.90 (s, 9H), 1.49 (s, 18H), 2.49 (m, 1H), 3.19 (bs, 3H), 3.56 (dd, 1H,  $J = 5.4$ , 10.5 Hz), 3.61 (dd, 1H,  $J = 4.8$ , 10.5 Hz), 3.88–4.06 (m, 2H), 4.24 (d, 2H,  $J = 6.9$  Hz), 6.05 (bs, 1H), 7.77 (s, 1H). MS (positive-ion FAB) 578 ( $M + H^+$ )<sup>+</sup>.

**( $\pm$ )-Phosphoric Acid Di-*tert*-butyl Ester 2-(2-Chloro-6-methylaminopurin-9-ylmethyl)-3-hydroxy-propyl Ester (39).** A solution of silyl phosphate **38** (250 mg, 0.432 mmol) in tetrahydrofuran (10 mL) at room temperature was treated with tetrabutylammonium fluoride (0.52 mL, 0.52 mmol, 1 M solution in tetrahydrofuran). The reaction mixture was stirred for 10 min and applied directly to preparative thin-layer chromatography (chloroform/methanol = 10:1) to give **39** (190 mg, 76%). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.51 (s, 9H), 1.52 (s, 9H), 2.41 (m, 1H), 3.19 (bs, 3H), 3.40–3.56 (m, 2H), 3.84–4.11 (m, 2H), 4.23–4.40 (m, 2H), 6.34 (bs, 1H), 7.81 (s, 1H). MS (positive-ion FAB) 464 ( $M + H^+$ )<sup>+</sup>.

(±)-Phosphoric Acid Di-*tert*-butyl Ester 2-(2-Chloro-6-methylaminopurin-9-ylmethyl)-3-(dimethoxyphosphoryloxy)propyl Ester (**40**). To a stirred solution of **39** (10 mg, 0.0216 mmol) and dimethylphosphoryl chloride (12 mg, 0.083 mmol) in anhydrous tetrahydrofuran at room temperature was added solid sodium hydride (8.6 mg, 0.215 mmol, 60% in mineral oil) in one pot. The reaction mixture was stirred for 1 h at the same temperature, followed by quenching with water under water-ice bath. The mixture was applied to preparative thin-layer chromatography (chloroform/methanol = 10:1) to give **40** (3.8 mg, 31%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.497 (s, 9H), 1.500 (s, 9H), 2.73 (m, 1H), 3.18 (bs, 3H), 3.80 (d, 3H, *J* = 11.1 Hz), 3.82 (d, 3H, *J* = 11.1 Hz), 3.86–4.19 (m, 4H), 4.21–4.38 (m, 2H), 6.17 (bs, 1H), 7.85 (s, 1H). MS (positive-ion FAB) (*M* + *H*)<sup>+</sup>.

(±)-Acetic Acid 2-(2-Chloro-6-methylaminopurin-9-ylmethyl)-3-(di-*tert*-butoxyphosphoryloxy)propyl Ester (**41**). A mixture of **39** (11.5 mg, 0.0248 mmol), triethylamine (9.4 mg, 0.0933 mmol), and acetic anhydride (3.80 mg, 0.0372 mmol) was stirred for 2 h at room temperature. The resulting mixture was purified by preparative thin-layer chromatography (chloroform/methanol = 15:1) to give **41** (12 mg, 96%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.497 (s, 9H), 1.502 (s, 9H), 2.07 (s, 3H), 2.70 (m, 1H), 3.18 (bs, 3H), 3.86–4.05 (m, 2H), 4.09 (d, 2H, *J* = 5.9 Hz), 4.29 (d, 2H, *J* = 7.0 Hz), 6.08 (bs, 1H), 7.79 (s, 1H). MS (positive-ion FAB) 506 (*M* + *H*)<sup>+</sup>.

(±)-Phosphoric Acid Dibenzyl Ester 2-(2-Chloro-6-methylaminopurin-9-ylmethyl)-3-(di-*tert*-butoxyphosphoryloxy)propyl Ester (**42**). To a stirred suspension of **39** (13 mg, 0.028 mmol) and diisopropyl dibenzylphosphoramidite (29 mg, 0.084 mmol) in anhydrous tetrahydrofuran (2 mL) at room temperature was added solid tetrazole (11.7 mg, 0.167 mmol) in one pot. The reaction mixture was stirred at room temperature for 20 min and cooled to –78 °C. To this cooled reaction mixture was added solid MCPBA (57–85%, calculated by 57%, 25 mg), and the resulting mixture was stirred at –78 °C for 30 min and at room temperature for 10 min. After removal of tetrahydrofuran in vacuo, the residue was directly purified by preparative thin-layer chromatography (CHCl<sub>3</sub>/MeOH = 20/1) to give **42** (9.2 mg, 45%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.46 (s, 18H), 2.61 (m, 1H), 3.18 (bs, 3H), 3.79–4.08 (m, 4H), 4.14 (d, 2H, *J* = 7.0 Hz), 4.97–5.13 (m, 4H), 6.04 (bs, 1H), 7.28–7.42 (m, 10H), 7.74 (s, 1H). MS (positive-ion FAB) 724 (*M* + *H*)<sup>+</sup>.

(±)-Phosphoric Acid Dibenzyl Ester 2-(2-Chloro-6-methylaminopurin-9-ylmethyl)-3-phosphonooxypropyl Ester (**43**). Method I. Compound **43** (8 mg, 89%) was obtained from **42** (9.1 mg, 0.0126 mmol) as a triethylammonium salt. <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 2.65 (m, 1H), 3.07 (bs, 3H), 3.93–4.13 (m, 4H), 4.24 (d, 2H, *J* = 7.0 Hz), 5.03 (d, 4H, *J* = 8.9 Hz), 7.34 (s, 10H), 8.07 (s, 1H). HPLC 16.372 min (purity >98%) in solvent system A, 13.449 min in system B.

(±)-Phosphoric Acid Dibenzyl Ester 2-(2-Chloro-6-methylaminopurin-9-ylmethyl)-3-phosphonophosphonooxypropyl Ester (**44**). Monotriethylamine salt **43** (8 mg, 0.0112 mmol) was dissolved in anhydrous dimethylformamide (1 mL), followed by an addition of carbonyldiimidazole (5.46 mg, 0.0337 mmol). The mixture was stirred for 20 min at room temperature and then triethylammonium phosphate (13.4 mg, 0.067 mmol) in anhydrous dimethylformamide (0.5 mL) was added. The reaction mixture was stirred for 3 days at room temperature. All volatile material was removed by nitrogen stream. The residue was purified with an ion-exchange column chromatography using Sephadex-DEAE-25 resin with a linear gradient (0.01 to 1.0 M) of 1.0 M ammonium bicarbonate as the mobile phase to give phosphate **44** (6 mg, 74%) as ammonium salt. <sup>1</sup>H NMR (D<sub>2</sub>O) δ 2.66 (m, 1H), 2.87 (bs, 3H), 3.91–4.19 (m, 4H), 4.20–4.41 (m, 2H), 4.65–4.90 (m, 2H), 7.06–7.25 (m, 5H), 7.30–7.41 (m, 5H), 8.08 (s, 1H). High-resolution MS (negative-ion FAB) calcd for C<sub>24</sub>H<sub>28</sub>O<sub>11</sub>N<sub>5</sub>ClP<sub>3</sub> [*M* – *H*]<sup>–</sup> 690.0687, found 690.0684.

Methanesulfonic Acid 2-Allylpent-4-enyl Ester (**46**) Method D. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.96 (m, 1H), 2.17 (t, 4H, *J* = 7.0 Hz), 3.15 (s, 3H), 4.14 (d, 2H, *J* = 5.5 Hz), 5.03–5.16 (m, 4H), 5.76 (m, 2H).

[9-(2-Allylpent-4-enyl)-2-chloro-9H-purin-6-yl]methylamine (**47**). Method D. Compound **47** (69 mg, 60% two steps yield) obtained from **45** (50 mg, 0.396 mmol). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.07 (t, 4H, *J* = 6.6 Hz), 2.23 (m, 1H), 3.18 (s, 3H), 4.06 (d, 2H, *J* = 7.2 Hz), 5.01–5.11 (m, 4H), 5.75 (m, 2H). MS (positive-ion FAB) 292 (*M* + *H*)<sup>+</sup>.

3-(2-Chloro-6-methylaminopurin-9-ylmethyl)pentane-1,5-diol (**48**). A mixture of diene **47** (39 mg, 0.134 mmol), osmium tetroxide (0.3 mL of stock solution, 1 g OsO<sub>4</sub>/100 mL H<sub>2</sub>O), sodium metaperiodate (340 mg, 1.59 mmol), and a mixed solvent (5 mL, acetone/water = 4:1) was stirred at room temperature for 4 h. All volatile materials were removed in vacuo to dryness. The residue was stirred in a mixed solvent (ethyl acetate/methanol = 10:1) and filtrated through a short column packed with silica gel (20 g). The filtrate was collected and evaporated, and the residue was dissolved in ethyl alcohol in an ice–water bath. Solid sodium borohydride was added to this solution, and the reaction mixture was stirred for 2 h at room temperature, followed by quenching excess sodium borohydride with 1 N hydrochloric acid. After removal of all volatile materials in vacuo to dryness, the residue was purified by preparative thin-layer chromatography (chloroform/methanol = 10:1) to give **48** (13 mg, 32%). <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 1.43–1.65 (m, 4H), 2.27 (m, 1H), 3.07 (bs, 3H), 3.51–3.71 (m, 4H), 4.18 (d, 2H, *J* = 7.2 Hz), 8.05 (s, 1H). MS (positive-ion FAB) 300 (*M* + *H*)<sup>+</sup>.

Phosphoric Acid Di-*tert*-butyl Ester 3-(2-Chloro-6-methylaminopurin-9-ylmethyl)-5-(di-*tert*-butoxyphosphoryloxy)-pentyl Ester (**49**). Method C. Compound **49** (20 mg, 73%) obtained from **48** (12 mg, 0.040 mmol). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.47 (s, 36H), 1.65–1.75 (m, 4H), 2.29 (m, 1H), 3.18 (bs, 3H), 3.93–4.16 (m, 4H), 4.21 (d, 2H, *J* = 6.7 Hz), 6.12 (bs, 1H), 7.84 (s, 1H). MS (positive-ion FAB) 684 (*M* + *H*)<sup>+</sup>.

(4*R*)-Methanesulfonic Acid 2,2-Dimethyl-[1,3]dioxolan-4-ylmethyl Ester (**52**) (from *R*-Isomer Alcohol **50**). Method D. Crude **52** (700 mg) was obtained from **50** (500 mg, 3.78 mmol). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.36 (s, 3H), 1.44 (s, 3H), 3.06 (s, 3H), 3.82 (dd, 1H, *J* = 5.5, 8.8 Hz), 4.10 (dd, 1H, *J* = 6.5, 8.8 Hz), 4.22 (d, 2H, *J* = 5.2 Hz), 4.37 (m, 1H). MS (CI/NH<sub>3</sub>) *m/z* 211 (*M* + *H*)<sup>+</sup>, 228 (*M* + NH<sub>4</sub>)<sup>+</sup>.

(4*R*)-Methanesulfonic Acid 2,2-Dimethyl-[1,3]dioxolan-4-ylmethyl Ester (**53**) (from *S*-Isomer Alcohol **51**). Method D. Crude **53** (850 mg) was obtained from **51** (500 mg, 3.78 mmol), and it showed the identical proton NMR spectrum to **52**. MS (CI/NH<sub>3</sub>) *m/z* 228 (*M* + NH<sub>4</sub>)<sup>+</sup>.

(*R*)-[2-Chloro-9-(2,2-dimethyl-[1,3]dioxolan-4-ylmethyl)-9H-purin-6-yl]-methylamine (**54**) (Originated from *R*-Isomer **50**). Method D. Compound **54** (567 mg, 50% two steps yield) was obtained from **50** (500 mg, 3.78 mmol). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.33 (s, 3H), 1.39 (s, 3H), 3.19 (bs, 3H), 3.69 (dd, 1H, *J* = 5.8, 8.8 Hz), 4.14 (dd, 1H, *J* = 6.6, 8.8 Hz), 4.20 (dd, 1H, *J* = 6.4, 14.4 Hz), 4.27 (dd, 1H, *J* = 3.2, 14.4 Hz), 4.46 (m, 1H). MS (CI/NH<sub>3</sub>) 298 *m/z* (*M* + *H*)<sup>+</sup>.

(*S*)-[2-Chloro-9-(2,2-dimethyl-[1,3]dioxolan-4-ylmethyl)-9H-purin-6-yl]-methylamine (**55**) (Originated from *S*-Isomer **51**). Method D. Compound **55** (611 mg, 54% two steps yield) was obtained from **51** (500 mg, 3.78 mmol), and it showed the identical proton NMR spectrum and mass spectrum to **54**. MS (CI/NH<sub>3</sub>) 298 *m/z* (*M* + *H*)<sup>+</sup>.

(*R*)-3-(2-Chloro-6-methylaminopurin-9-yl)propane-1,2-diol (**56**) (Originated from *R*-Isomer **50**). Method B. Compound **56** (114 mg, 88%) was obtained from **54** (150 mg, 0.504 mmol). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ (d, 3H, *J* = 3.6 Hz), 3.32 (dd, 1H, *J* = 6.2, 11.1 Hz), 3.40 (dd, 1H, *J* = 5.1, 11.1 Hz), 3.82 (m, 1H), 3.95 (dd, 1H, *J* = 8.5, 13.9 Hz), 4.25 (dd, 1H, *J* = 3.4, 13.9 Hz), 8.04 (s, 1H), 8.16 (m, 1H). MS (CI/NH<sub>3</sub>) *m/z* 258 (*M* + *H*)<sup>+</sup>.

(*S*)-3-(2-Chloro-6-methylaminopurin-9-yl)propane-1,2-diol (**57**) (Originated from *S*-Isomer **51**). Method B. Compound **57** (110 mg, 85%) was obtained from **55** (150 mg,



0.504 mmol) and it showed the identical proton NMR spectrum and mass spectrum to **56**.

**(R)-Phosphoric Acid Di-*tert*-butyl Ester 1-(2-Chloro-6-methylaminopurin-9-ylmethyl)-2-(di-*tert*-butoxyphosphoryloxy)ethyl Ester (58) (Originated from *R*-Isomer 50).** Method C. Compound **58** (42 mg, 56%) was obtained from **56** (30 mg, 0.116 mmol).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.34 (s, 9H), 1.44 (s, 9H), 1.507 (s, 9H), 1.513 (s, 9H), 3.18 (bs, 3H), 4.04–4.27 (m, 2H), 4.34–4.55 (m, 2H), 4.78 (m, 1H), 6.50 (bs, 1H), 7.88 (s, 1H). MS ( $\text{CI}/\text{NH}_3$ )  $m/z$  642 ( $\text{M} + \text{H}^+$ ) $^+$ .

**(S)-Phosphoric Acid Di-*tert*-butyl Ester 1-(2-Chloro-6-methylaminopurin-9-ylmethyl)-2-(di-*tert*-butoxyphosphoryloxy)ethyl Ester (59) (Originated from *R*-Isomer 50).** Method C. Compound **59** (43 mg, 69%) was obtained from **57** (25 mg, 0.097 mmol).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.34 (s, 9H), 1.44 (s, 9H), 1.507 (s, 9H), 1.513 (s, 9H), 3.18 (bs, 3H), 4.04–4.27 (m, 2H), 4.34–4.55 (m, 2H), 4.78 (m, 1H), 6.21 (bs, 1H), 7.85 (s, 1H). MS ( $\text{CI}/\text{NH}_3$ )  $m/z$  642 ( $\text{M} + \text{H}^+$ ) $^+$ .

**4-(*tert*-Butyldimethylsilyloxy)-3-(*tert*-butyldimethylsilyloxy)methylbut-2-enoic Acid Ethyl Ester (60).** Neat ethyl (diethoxy)phosphoacetate (3.10 g, 13.8 mmol) was added dropwise to a cooled (0 °C), stirred suspension of sodium hydride (50%, 662 mg, 13.8 mmol) in anhydrous tetrahydrofuran (60 mL). After being stirred at room temperature for 1 h until the reaction mixture became homogeneous, the reaction mixture was cooled to 0 °C, and to this solution was added a solution of **21** (4.0 g, 12.6 mmol) in anhydrous tetrahydrofuran (10 mL) at the same temperature. The reaction mixture was stirred at room temperature for 2 h and poured into ethyl acetate (100 mL) and water (50 mL). The organic layer was separated, washed with brine/water (1:1, 20 mL  $\times$  3), dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 15:1) to give **60** (4.17 g, 85%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.07 (s, 6H), 0.10 (s, 6H), 0.90 (s, 9H), 0.93 (s, 9H), 1.29 (t, 3H,  $J = 7.2$  Hz), 4.16 (q, 2H,  $J = 7.2$  Hz), 4.44 (m, 2H), 4.87 (m, 2H), 5.98 (m, 1H). MS ( $\text{CI}/\text{NH}_3$ )  $m/z$  389 ( $\text{M} + \text{H}^+$ ) $^+$ .

**4-(*tert*-Butyldimethylsilyloxy)-3-(*tert*-butyldimethylsilyloxy)methylbut-2-en-1-ol (61).** To a cooled (–78 °C), stirred solution of **60** (1.3 g, 3.34 mmol) in toluene (20 mL) was added a 1 M solution of DIBAL (in tetrahydrofuran, 8.0 mL, 8.0 mmol). After being stirred for 2 h at –20 °C, the mixture was warmed to room temperature, stirred for 1 h, and cooled to 0 °C, followed by an addition of methanol (7 mL). The resulting mixture was partitioned between 10% Rochelle salt solution (100 mL) and ethyl acetate (100 mL). This two-phase solution was stirred until the two phases were clearly separated with more addition of Rochelle salt. The organic layer was separated, washed with brine/water (1:1, 20 mL  $\times$  2), dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 1:1) to give **61** (860 mg, 74%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.08 (s, 6H), 0.09 (s, 6H), 0.91 (s, 9H), 0.92 (s, 9H), 4.10–4.28 (m, 6H), 5.82 (m, 1H). MS ( $\text{CI}/\text{NH}_3$ )  $m/z$  347 ( $\text{M} + \text{H}^+$ ) $^+$ .

**9-[4-(*tert*-Butyldimethylsilyloxy)-3-(*tert*-butyldimethylsilyloxy)methylbut-2-enyl]-2,6-dichloro-9H-purine (62).** Method A. Crude **62** (230 mg, 57%) was obtained from **61** (270 mg, 0.779 mmol).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.05 (s, 6H), 0.11 (s, 6H), 0.89 (s, 9H), 0.91 (s, 9H), 4.19 (s, 2H), 4.25 (s, 2H), 5.03 (d, 2H,  $J = 7.5$  Hz), 5.78 (t, 1H,  $J = 7.5$  Hz), 8.17 (s, 1H).

**9-[4-(*tert*-Butyldimethylsilyloxy)-3-(*tert*-butyldimethylsilyloxy)methylbut-2-enyl]-2-chloro-9H-purin-6-yl-methylamine (63).** Method A. Compound **63** (168 mg, 85%) was obtained from crude **62** (200 mg, 0.386 mmol).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.05 (s, 6H), 0.09 (s, 6H), 0.89 (s, 9H), 0.90 (s, 9H), 3.19 (bs, 3H), 4.20 (s, 2H), 4.33 (s, 2H), 4.89 (d, 2H,  $J = 7.4$  Hz), 5.76 (t, 1H,  $J = 7.4$  Hz), 7.74 (s, 1H). MS ( $\text{CI}/\text{NH}_3$ )  $m/z$  512 ( $\text{M} + \text{H}^+$ ) $^+$ .

**2-[2-(2-Chloro-6-methylaminopurin-9-yl)-ethylidene]-propane-1,3-diol (64).** Method E. Compound **64** (100 mg, 86%) was obtained from **63** (210 mg, 0.410 mmol).  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  2.91 (s, 3H), 3.96 (s, 2H), 4.12 (s, 2H), 4.85 (d,

2H,  $J = 7.4$  Hz), 5.60 (t, 1H,  $J = 7.4$  Hz), 8.11 (s, 1H), 8.20 (bs, 1H). MS ( $\text{CI}/\text{NH}_3$ )  $m/z$  284 ( $\text{M} + \text{H}^+$ ) $^+$ .

**Phosphoric Acid Di-*tert*-butyl Ester 4-(2-Chloro-6-methylaminopurin-9-yl)-2-(di-*tert*-butoxyphosphoryloxymethyl)but-2-enyl Ester (65).** Method C. Compound **65** (40 mg, 34%) was obtained from **64** (50 mg, 0.176 mmol).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.45 (s, 18H), 1.49 (s, 18H), 3.18 (bs, 3H), 4.52 (d, 2H,  $J = 6.7$  Hz), 4.70 (d, 2H,  $J = 9.1$  Hz), 4.96 (d, 2H,  $J = 7.1$  Hz), 5.95 (t, 1H,  $J = 7.3$  Hz), 6.17 (bs, 1H), 7.87 (s, 1H). MS ( $\text{CI}/\text{NH}_3$ )  $m/z$  668 ( $\text{M} + \text{H}^+$ ) $^+$ .

**2,3-Bis-(*tert*-butyldimethylsilyloxy)methyl)cyclopropanecarboxylic Acid Ethyl Ester (67).** A solution of ethyl diazoacetate (containing <10% methylene chloride, 9 mL) at room temperature was added dropwise over 18 h, using a syringe pump, to a stirred solution of (*Z*)-1,4-bis[(*tert*-butyldimethylsilyloxy)-2-butene **66** (25 g, 78.95 mmol) and rhodium-(II) acetate dimer (410 mg, 0.928 mmol) in methylene chloride (50 mL). After the mixture was stirred for an additional 6 h, the solvent was removed in vacuo, and the residue was chromatographed on silica gel eluting 0.5–2% ethyl acetate in hexanes to give a mixture **67** of *meso-cis* and *meso-trans* isomers (19.27 g, 61%, *meso-cis:meso-trans* = 7.36 g: 11.91 g). **67**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.01–0.08 (m, 12H), 0.80–0.99 (m, 18H), 1.20–1.35 (m, 3H), 1.52–1.88 (m, 3H), 3.65–4.26 (m, 6H).

**[2,3-Bis-(*tert*-butyldimethylsilyloxy)methyl)cyclopropyl]methanol (68).** To a stirred solution of the mixture **67** (400 mg, 0.99 mmol) in ether (5 mL) and toluene (2.7 mL) at room temperature was added solid lithium borohydride (22 mg, 1.01 mmol), and the reaction mixture was stirred at 100 °C with continuous removal of ethyl ether. More lithium borohydride (40 mg, 2.02 mmol) was added. After the mixture was stirred for 1 h at the same temperature, ice (20 g) was added in one pot with a rigorous stirring to decompose excess lithium borohydride, and the resulting mixture was stirred for 30 min. The aqueous layer was extracted with ethyl acetate (50 mL), and the organic layer was washed with water (30 mL  $\times$  2), dried over anhydrous sodium sulfate, filtered, concentrated in vacuo to give a crude mixture of *meso-cis* and *meso-trans* cyclopropanol isomers **68** (320 mg, 1:1.6, 93%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.02–0.11 (m, 12H), 0.87–0.95 (m, 18H), 1.01–1.54 (m, 3H), 3.46–3.95 (m, 6H). MS ( $\text{CI}/\text{NH}_3$ )  $m/z$  361 ( $\text{M} + \text{H}^+$ ) $^+$ .

**9-[2,3-Bis-(*tert*-butyldimethylsilyloxy)methyl)cyclopropylmethyl]-2,6-dichloro-9H-purine (69).** Method A. A mixture of two isomers **69** (147 mg, 59%) was obtained from a mixture **68** (160 mg, 0.464 mmol).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.01–0.20 (m, 12H), 0.61–0.89 (m, 18H), 1.42–1.55 (m, 3H), 3.54–4.48 (m, 6H). 8.59 and 8.61 (two singlets, 1H).

***meso*-1,2-*trans*-[9-[2,3-Bis-(*tert*-butyldimethylsilyloxy)methyl)cyclopropylmethyl]-2-chloro-9H-purin-6-yl]-methylamine (70) (*meso-trans*).** Method A. Compound **70** (78 mg) and **71** (31 mg) were obtained from **69** (147 mg, 0.276 mmol) and separated by silica gel column chromatography.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.03 (s, 12H), 0.87 (s, 18H), 1.20–1.35 (m, 3H), 3.18 (bs, 3H), 3.62 (dd, 2H,  $J = 5.9, 10.3$  Hz), 3.78 (dd, 2H,  $J = 4.4, 10.3$  Hz), 4.05 (d, 2H,  $J = 7.3$  Hz), 6.11 (bs, 1H), 8.19 (s, 1H). MS ( $\text{CI}/\text{NH}_3$ )  $m/z$  526 ( $\text{M} + \text{H}^+$ ) $^+$ .

***meso*-1,2-*cis*-[9-[2,3-Bis-(*tert*-butyldimethylsilyloxy)methyl)cyclopropylmethyl]-2-chloro-9H-purin-6-yl]-methylamine (71) (*meso-cis*).** Method A. Compound **70** (78 mg) and **71** (31 mg) were obtained from **69** (147 mg, 0.276 mmol) and separated by silica gel column chromatography.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.06 (s, 6H), 0.07 (s, 6H), 0.90 (s, 18H), 1.35–1.55 (m, 3H), 3.19 (bs, 3H), 3.78 (dd, 2H,  $J = 8.8, 11.7$  Hz), 3.92 (dd, 2H,  $J = 5.9, 11.7$  Hz), 4.32 (d, 2H,  $J = 5.9$  Hz), 6.07 (bs, 1H), 8.18 (s, 1H). MS ( $\text{CI}/\text{NH}_3$ )  $m/z$  526 ( $\text{M} + \text{H}^+$ ) $^+$ .

***meso*-1,3-*trans*-[2-(2-Chloro-6-methylaminopurin-9-yl)-methyl]-3-hydroxymethylcyclopropyl]methanol (72) (*meso-trans*).** Method E. Crude **72** (50 mg) was obtained from **70** (78 mg, 0.148 mmol).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.24 (m, 1H), 1.38 (m, 2H), 3.05 (bs, 3H), 3.48–3.71 (m, 2H), 4.08 (d, 2H,  $J = 6.9$  Hz), 8.13 (s, 1H). MS ( $\text{CI}/\text{NH}_3$ )  $m/z$  298 ( $\text{M} + \text{H}^+$ ) $^+$ .

**meso-1,3-*cis*-[2-(2-Chloro-6-methylaminopurin-9-yl-methyl)-3-hydroxymethylcyclopropyl]methanol (73) (meso-*cis*).** Method E. Crude **73** (20 mg) was obtained from **71** (31 mg, 0.059 mmol).  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  1.38–1.63 (m, 3H), 3.07 (bs, 3H), 3.72–3.93 (m, 4H), 4.36 (d, 2H,  $J = 7.1$  Hz), 8.16 (s, 1H). MS ( $\text{CI}/\text{NH}_3$ )  $m/z$  298 ( $\text{M} + \text{H}^+$ ) $^+$ .

**meso-1,3-*trans*-Phosphoric Acid Dibenzy Ester 2-(bis-benzyloxyphosphoryloxymethyl)-3-(2-chloro-6-methylaminopurin-9-ylmethyl)cyclopropylmethyl Ester (74) (meso-*trans*).** Method F. Compound **74** (56 mg, 46% two steps yield from **70**) was obtained from crude **72** (50 mg).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.14–1.43 (m, 3H), 3.10 (bs, 3H), 3.82–3.97 (m, 6H), 4.90–5.06 (m, 8H), 5.99 (bs, 1H), 7.21–7.39 (m, 20H), 7.68 (bs, 1H).  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  –22.458. MS ( $\text{CI}/\text{NH}_3$ )  $m/z$  818 ( $\text{M} + \text{H}^+$ ) $^+$ . High-resolution MS (positive-ion FAB) calcd for  $\text{C}_{40}\text{H}_{42}\text{N}_5\text{O}_8\text{ClP}_2\text{Cs}$  [ $\text{M}^{2+} + \text{H}^+ + \text{Cs}^{2+}$ ] $^+$  950.1252, found 950.1229.

**meso-1,3-*cis*-Phosphoric Acid Dibenzy Ester 2-(bis-benzyloxyphosphoryloxymethyl)-3-(2-chloro-6-methylaminopurin-9-ylmethyl)cyclopropylmethyl Ester (75) (meso-*cis*).** Method F. Compound **75** (17 mg, 33% two steps yield from **71**) was obtained from crude **73** (20 mg).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.46 (m, 2H), 1.61 (m, 2H), 3.15 (bs, 3H), 3.97 (dd, 2H,  $J = 7.8$ , 10.8 Hz), 4.04 (d, 2H,  $J = 6.9$  Hz), 4.18 (dd, 2H,  $J = 6.6$ , 10.8 Hz), 4.90–5.19 (m, 8H), 5.99 (bs, 1H), 7.23–7.40 (m, 20H), 7.70 (bs, 1H).  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  –22.338. MS ( $\text{CI}/\text{NH}_3$ )  $m/z$  818 ( $\text{M} + \text{H}^+$ ) $^+$ . High-resolution MS (positive-ion FAB) calcd for  $\text{C}_{40}\text{H}_{42}\text{N}_5\text{O}_8\text{ClP}_2\text{Cs}$  [ $\text{M}^{2+} + \text{H}^+ + \text{Cs}^{2+}$ ] $^+$  950.1252, found 950.1298.

**1,2-Bis-(*tert*-butyldimethylsilanyloxymethyl)-3-methoxymethoxymethylcyclopropane (76).** Chloromethyl ether (234 mg, 2.90 mmol) was added dropwise by syringe over 2 min to a stirred solution of cyclopropyl alcohol **68a** (1.0 g, 2.90 mmol) and diisopropylethylamine (563 mg, 4.36 mmol) in methylene chloride (15 mL) cooled in an ice-water bath. The reaction mixture was stirred for 1 h at the same temperature, for 4 h at room temperature. Ice (10 g) was added, the mixture was stirred for 10 min, and water (30 mL) was added. The aqueous layer was extracted with ethyl acetate (150 mL), and the organic layer was washed with brine/water (30 mL  $\times$  2, 1/1), dried over sodium sulfate, filtered, and concentrated to give **76** (1.05 g, 93%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.05 (s, 6H), 0.90 (s, 9H), 0.94–1.16 (m, 3H), 3.36 (s, 3H), 3.45 (d, 1H,  $J = 5.9$  Hz), 3.66–3.76 (m, 4H), 4.66 (s, 2H). MS ( $\text{CI}/\text{NH}_3$ )  $m/z$  405 ( $\text{M} + \text{H}^+$ ) $^+$ .

**(2-Hydroxymethyl-3-methoxymethoxymethylcyclopropyl)methanol (77).** A mixture of disilyl ether **76** (640 mg, 1.65 mmol), tetrabutylammonium fluoride (2.47 mL, 2.47 mmol, 1 M solution in tetrahydrofuran), and tetrahydrofuran (5 mL) was stirred for 3 h at room temperature. After removal of volatile materials in vacuo, the residue was purified by column chromatography (ethyl acetate/methanol = 10:1) to give diol **77** (275 mg, 95%). MS ( $\text{CI}/\text{NH}_3$ )  $m/z$  194 ( $\text{M} + \text{NH}_4^+$ ) $^+$ .

**( $\pm$ )-1,2-*cis*-1,3-*trans*-[2-(*tert*-Butyldiphenylsilyloxy-methyl)-3-methoxymethoxymethylcyclopropyl]methanol (( $\pm$ )-**78**).** Neat *tert*-butyldiphenylsilyl chloride (498 mg, 1.56 mmol) was added to a stirred solution of diol **77** (275 mg, 1.56 mmol) and (dimethylamino)pyridine (190 mg, 1.56 mmol) in methylene chloride (7 mL). The reaction mixture was stirred for 3 days at room temperature. Methylene chloride was removed in vacuo, and the residue was purified by column chromatography (hexanes/ethyl acetate = 1:1) to give **78** (479 mg, 70%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.93 (m, 1H), 1.07 (s, 9H), 1.21 (m, 1H), 1.41 (m, 1H), 3.30 (s, 3H), 3.34–3.49 (m, 4H), 4.02 (dd, 1H,  $J = 5.4$ , 12.0 Hz), 4.11 (dd, 1H,  $J = 5.4$ , 11.5 Hz), 4.57 (s, 2H), 7.36–7.50 (m, 6H), 7.65–7.76 (m, 4H). MS ( $\text{CI}/\text{NH}_3$ )  $m/z$  415 ( $\text{M} + \text{H}^+$ ) $^+$ .

**( $\pm$ )-1,2-*cis*-1,3-*trans*-Methanesulfonic Acid 2-(*tert*-Butyldiphenylsilyloxy-methyl)-3-methoxymethoxymethylcyclopropylmethyl Ester (( $\pm$ )-**79**).** Method D. Crude ( $\pm$ )-**79** (538 mg, 95%) was obtained from ( $\pm$ )-**78** (479 mg, 1.15 mmol).

**( $\pm$ )-1,2-*cis*-1,3-*trans*-[9-[2-(*tert*-Butyldiphenylsilyloxy-methyl)-3-methoxymethoxymethyl-cyclopropylmethyl]-2-chloro-9H-purin-6-yl]-methylaniline (( $\pm$ )-**80**).**

**Method D.** Compound ( $\pm$ )-**80** (430 mg, 71%) was obtained from ( $\pm$ )-**79** (520 mg, 1.05 mmol).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.05 (s, 9H), 1.04–1.46 (m, 3H), 3.18 (bs, 3H), 3.28 (s, 3H), 3.24–3.45 (m, 2H), 3.50 (dd, 1H,  $J = 6.0$ , 10.4 Hz), 3.70 (dd, 1H,  $J = 8.0$ , 11.4 Hz), 3.98 (dd, 1H,  $J = 5.1$ , 11.4 Hz), 4.28 (dd, 1H,  $J = 7.0$ , 14.6 Hz), 4.54 (m, 2H), 6.18 (bs, 1H), 7.33–7.49 (m, 6H), 7.61–7.74 (m, 4H), 8.05 (s, 1H). MS (positive-ion FAB) 580 ( $\text{M} + \text{H}^+$ ) $^+$ .

**( $\pm$ )-1,2-*cis*-1,3-*trans*-[2-(2-Chloro-6-methylaminopurin-9-ylmethyl)-3-hydroxymethylcyclopropyl]methanol (( $\pm$ )-**81**).** A mixture of silyl ether ( $\pm$ )-**80** (230 mg, 0.467 mmol), *c*-HCl (2 mL), and methanol (6 mL) was stirred at 80  $^\circ\text{C}$  for 4 h. All volatile materials were removed by nitrogen purging. The residue was dissolved in methanol (20 mL) and treated with potassium carbonate (100 mg). The mixture was stirred at room temperature for 4 h, filtered, and evaporated. The residue was purified by PTLC (chloroform/methanol = 4:1) to give diol ( $\pm$ )-**81** (90 mg, 65%).  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  1.07–1.43 (m, 3H), 3.08 (bs, 3H), 3.32–3.66 (m, 3H), 3.92 (dd, 1H,  $J = 5.8$ , 11.8 Hz), 4.20–4.34 (m, 2H), 8.20 (s, 1H). MS (positive-ion FAB) 298 ( $\text{M} + \text{H}^+$ ) $^+$ .

**( $\pm$ )-1,2-*cis*-1,3-*trans*-Phosphoric Acid Di-*tert*-butyl Ester 2-(2-Chloro-6-methylaminopurin-9-yl)-3-(di-*tert*-butoxyphosphoryloxymethyl)cyclopropylmethyl Ester (( $\pm$ )-**82**).** Method C. Compound ( $\pm$ )-**82** (46 mg, 62%) was obtained from diol ( $\pm$ )-**81** (32 mg, 0.108 mmol).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.43 (s, 9H), 1.45 (s, 9H), 1.49 (s, 18H), 3.18 (s, 3H), 3.73–3.98 (m, 3H), 4.15–4.42 (m, 3H), 6.19 (bs, 1H), 7.93 (s, 1H). MS (positive-ion FAB) 682 ( $\text{M} + \text{H}^+$ ) $^+$ .

**( $\pm$ )-2-(2-Chloro-6-methylaminopurin-9-yl)cyclopropane-1,1-dicarboxylic Acid Diethyl Ester (( $\pm$ )-**84**).** A mixture of allyl bromide **83** (115 mg, 0.435 mmol), potassium carbonate (71 mg, 0.52 mmol), *N* $^6$ -methyl-2-chloropurine (80 mg, 0.435 mmol), and dimethylformamide (3 mL) was stirred for 2 days at 30–35  $^\circ\text{C}$ . After the usual aqueous workup using ethyl acetate (40 mL) and 5% NaCl solution (20 mL  $\times$  3), the organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography (hexanes/ethyl acetate = 1:5) to give ( $\pm$ )-**84** (68 mg, 41%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.01 (t, 3H,  $J = 7.1$  Hz), 1.32 (t, 3H,  $J = 7.1$  Hz), 2.06 (dd, 1H,  $J = 6.6$ , 8.2 Hz), 2.74 (t, 1H,  $J = 6.3$  Hz), 3.15 (bs, 3H), 3.87–4.06 (m, 2H), 4.19–4.39 (m, 3H), 6.19 (bd, 1H,  $J = 3.6$  Hz), 7.68 (s, 1H). MS (positive-ion FAB) 368 ( $\text{M} + \text{H}^+$ ) $^+$ .

**( $\pm$ )-[2-(2-Chloro-6-methylaminopurin-9-yl)-1-hydroxymethylcyclopropyl]methanol (( $\pm$ )-**85**).** To a cooled (–78  $^\circ\text{C}$ ), stirred solution of diester ( $\pm$ )-**84** (33 mg, 0.0860 mmol) in methylene chloride was added 1 M DIBAL (in hexanes, 0.52 mL, 0.52 mmol). The reaction mixture was stirred for 20 min at the same temperature and for 1 h at room temperature. After quenching with methanol (0.5 mL), the resulting solution was directly purified by PTLC (chloroform/methanol = 4:1) to give ( $\pm$ )-**85** (17 mg, 70%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 0.5 mL +  $\text{CD}_3\text{OD}$  30  $\mu\text{L}$ )  $\delta$  1.14 (dd, 1H,  $J = 4.5$ , 6.8 Hz), 1.32 (t, 1H,  $J = 6.3$ , 6.7 Hz), 3.07 (bs, 3H), 3.11 (d, 1H,  $J = 12.4$  Hz), 3.29 (dd, 1H,  $J = 4.3$ , 7.7 Hz), 3.60 (d, 1H,  $J = 12.2$  Hz), 3.70 (ABq, 1H,  $J = 12.0$  Hz), 7.09 (bs, 1H), 7.74 (s, 1H). MS (positive-ion FAB) 284 ( $\text{M} + \text{H}^+$ ) $^+$ .

**( $\pm$ )-Phosphoric Acid Di-*tert*-butyl Ester 2-(2-Chloro-6-methylaminopurin-9-yl)-1-(di-*tert*-butoxyphosphoryloxymethyl)cyclopropylmethyl Ester (( $\pm$ )-**86**).** Method C. ( $\pm$ )-**86** (30 mg, 85%) was obtained from ( $\pm$ )-**85** (15 mg, 0.053 mmol).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.39 (s, 9H), 1.41 (s, 9H), 1.51 (s, 9H), 1.52 (s, 9H), 1.63 (t, 1H,  $J = 7.3$  Hz), 1.78 (dd, 1H,  $J = 4.9$ , 7.0 Hz), 3.17 (bs, 3H), 3.58 (dd, 1H,  $J = 5.0$ , 7.7 Hz), 3.74–3.87 (m, 2H), 4.07–4.21 (m, 2H), 6.10 (bs, 1H), 7.79 (s, 1H). MS (positive-ion FAB) 668 ( $\text{M} + \text{H}^+$ ) $^+$ .

**( $\pm$ )-[2,2-Bis-(*tert*-butyldimethylsilanyloxymethyl)-cyclopropyl]methanol (( $\pm$ )-**87**).** To a stirred, cooled (0  $^\circ\text{C}$ ) solution of allyl alcohol **61** (220 mg, 0.635 mmol) in methylene chloride (8 mL) was added diethylzinc (1 M in hexane, 4.45 mL, 4.45 mmol), followed by an addition of diiodomethane (510 mg, 1.91 mmol). After 20 min at room temperature, the mixture was allowed to reach rt and stirred overnight. Aqueous



saturated ammonium chloride was carefully added, and the resulting mixture was extracted with ether (30 mL), washed successively with saturated sodium bicarbonate (20 mL), brine (20 mL), dried over sodium sulfate, filtered, evaporated. The residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 4:1) to give **87** (220 mg, 96%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.04 (s, 6H), 0.11 (s, 3H), 0.12 (s, 3H), 0.35 (t, 1H, *J* = 4.1 Hz), 0.70 (dd, 1H, *J* = 5.0, 8.4 Hz), 0.90 (s, 9H), 0.92 (s, 9H), 1.19 (m, 1H), 2.87 (d, 1H, *J* = 10.3 Hz), 3.25 (d, 1H, *J* = 11.0 Hz), 3.26 (t, 1H, *J* = 12.1 Hz), 3.96 (dd, 1H, *J* = 5.4, 12.1 Hz), 4.13 (d, 1H, *J* = 10.3 Hz), 4.29 (d, 1H, *J* = 11.0 Hz). MS (positive-ion FAB) 361 (M + H)<sup>+</sup>.

(±)-**Methanesulfonic Acid 2,2-bis-(tert-butyl)dimethylsilyloxymethyl)cyclopropylmethyl Ester ((±)-88)**. **Method D.** Crude (±)-**88** (250 mg, 93%) was obtained from (±)-**87** (220 mg, 0.610 mmol).

(±)-**[9-[2,2-Bis-(tert-butyl)dimethylsilyloxymethyl)cyclopropylmethyl]-2-chloro-9H-purin-6-yl]methylamine ((±)-89)**. **Method D.** Compound (±)-**89** (185 mg, 62%) was obtained from crude (±)-**88** (250 mg, 0.570 mmol). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.02 (s, 6H), 0.04 (s, 3H), 0.06 (s, 3H), 0.054 (t, 1H, *J* = 5.0 Hz), 0.79 (dd, 1H, *J* = 5.0, 8.7 Hz), 0.88 (s, 9H), 0.89 (s, 9H), 1.31 (m, 1H), 3.20 (bs, 3H), 3.42 (d, 1H, *J* = 10.2 Hz), 3.62 (d, 1H, *J* = 11.1 Hz), 3.66 (d, 1H, *J* = 10.3 Hz), 3.97 (d, 1H, *J* = 11.0 Hz), 4.04 (dd, 1H, *J* = 9.1, 14.3 Hz), 4.42 (dd, 1H, *J* = 6.0, 14.4 Hz), 8.08 (s, 1H). MS (positive-ion FAB) 526 (M + H)<sup>+</sup>.

(±)-**[2-(2-Chloro-6-methylaminopurin-9-ylmethyl)-1-hydroxymethylcyclopropyl]methanol ((±)-90)**. **Method E.** Compound (±)-**90** (56 mg, 55%) was obtained from (±)-**89** (180 mg, 0.342 mmol). <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 0.60 (t, 1H, *J* = 5.4 Hz), 0.77 (dd, 1H, *J* = 5.1, 8.7 Hz), 1.37 (m, 1H), 3.08 (bs, 3H), 3.41 (d, 1H, *J* = 11.4 Hz), 3.62 (d, 1H, *J* = 11.1 Hz), 3.65 (d, 1H, *J* = 11.8 Hz), 3.99 (d, 1H, *J* = 12.0 Hz), 4.28 (d, 2H, *J* = 7.3 Hz), 8.18 (s, 1H). MS (positive-ion FAB) 298 (M + H)<sup>+</sup>.

(±)-**Phosphoric Acid Di-tert-butyl Ester 2-(2-chloro-6-methylaminopurin-9-ylmethyl)-1-(di-tert-butoxyphosphoryloxymethyl)cyclopropylmethyl Ester ((±)-91)**. **Method C.** Compound (±)-**91** (23 mg, 40%) was obtained from (±)-**90** (25 mg, 0.0840 mmol). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.77 (t, 1H, *J* = 5.7 Hz), 1.03 (dd, 1H, *J* = 5.7, 8.4 Hz), 1.21–1.41 (m, 1H), 1.44 (s, 9H), 1.45 (s, 9H), 1.49 (s, 18H), 3.17 (bs, 3H), 3.74 (dd, 1H, *J* = 5.9, 10.4 Hz), 3.95 (dd, 1H, *J* = 5.4, 11.5 Hz), 4.04 (dd, 1H, *J* = 6.6, 10.6 Hz), 4.27 (d, 2H, *J* = 6.8 Hz), 4.38 (dd, 1H, *J* = 4.5, 11.4 Hz), 6.66 (bs, 1H), 7.95 (s, 1H). MS (positive-ion FAB) 682 (M + H)<sup>+</sup>.

**Method G. Deprotection of tert-Butylphosphates with Dowex 50 Resin.** A mixture of bis(di-tert-butyl phosphate) **26** (20 mg, mmol) and Dowex 50 resin (100 mg) and methanol/water (1:1, 5 mL) was stirred at 80 °C for 16 h. The resulting mixture was filtered off through glass filter and washed with water (5 mL × 2), the combined filtrate was lyophilized, and 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 bisphosphate **5** (9.0 mg, 63%) as an ammonium salt.

**Phosphoric Acid Mono-[2-(2-chloro-6-methylaminopurin-9-yl)-3-phosphonooxypentyl] Ester, Ammonium Salt (5).** <sup>1</sup>H NMR (D<sub>2</sub>O) δ 3.03 (s, 3H), 4.35 (m, 2H), 4.46 (m, 2H), 5.16 (m, 1H), 8.97 (s, 1H). <sup>31</sup>P NMR (D<sub>2</sub>O) 4.107. MS (negative-ion FAB) 416 (M – H)<sup>–</sup>.

**Method H. Deprotection of Benzyl Phosphates with Boron Trichloride.** Bis(dibenzyl phosphate) **36** (37 mg, 0.0442 mmol) was dissolved in anhydrous methylene chloride (3 mL) and cooled to 0 °C. To this solution was added 1 M boron trichloride (in dichloromethane, 0.442 mL, 0.442 mmol), and the reaction mixture was stirred at 5 °C for 2 d (the progress of the reaction was monitored by HPLC). After the deprotection was done, the resulting mixture was quenched by 1 M triethylammonium acetate (5 mL), followed by addition of water (5 mL). This mixture was lyophilized, and the residue was purified with an ion-exchange column chromatography using Sephadex-DEAE-25 resin with a linear gradient (0.01

to 0.5 M) of 0.5 M ammonium bicarbonate as the mobile phase to give bisphosphate **7** (9.6 mg, 41%).

**Phosphoric Acid Mono-[2-(6-methylaminopurin-9-ylmethyl)-3-phosphonooxypropyl] Ester, Ammonium Salt (6).** **Method H.** Compound **6** (12.0 mg, 70%) was obtained from **35** (28 mg, 0.369 mmol). <sup>1</sup>H NMR (D<sub>2</sub>O) δ 2.57 (m, 1H), 3.11 (bs, 3H), 3.87 (m, 4H), 4.36 (d, 2H, *J* = 7.1 Hz), 8.17 (s, 1H), 8.22 (s, 1H). <sup>31</sup>P NMR (D<sub>2</sub>O) –21.005. MS (negative-ion FAB) 396 (M – H)<sup>–</sup>.

**Phosphoric Acid Mono-[2-(2-chloro-6-methylaminopurin-9-ylmethyl)-3-phosphonooxypropyl] Ester, Ammonium Salt (7).** **Method H.** Compound **7** (9.6 mg, 41%) was obtained from **36** (37 mg, 0.0467 mmol). <sup>1</sup>H NMR (D<sub>2</sub>O) δ 2.56 (m, 1H), 3.04 (bs, 3H), 3.87 (m, 4H), 4.28 (d, 2H, *J* = 7.3 Hz), 8.09 (s, 1H). <sup>31</sup>P NMR (D<sub>2</sub>O) –20.975 (s).

**Method I. Deprotection of tert-Butylphosphates with Trifluoroacetic Acid.** A solution of tert-butylphosphate **39** (5 mg, 0.011 mmol) in 5% trifluoroacetic acid in methylene chloride was stirred for 2 h at room temperature. All volatile material was removed in vacuo. The residue was purified using ion-exchange column chromatography on Sephadex-DEAE-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, 79%) as an ammonium salt.

(±)-**Phosphoric Acid Mono-[2-(2-chloro-6-methylaminopurin-9-ylmethyl)-3-hydroxypropyl] Ester, Ammonium Salt ((±)-8)**. **Method I.** Compound **8** (3.3 mg, 79%) was obtained from (5.0 mg, 0.0107 mmol). <sup>1</sup>H NMR (D<sub>2</sub>O) δ 2.55 (m, 1H), 3.11 (s, 3H), 3.69 (d, 2H, *J* = 5.9 Hz), 3.82–4.02 (m, 2H), 4.44 (d, 2H, *J* = 7.8 Hz), 8.87 (s, 1H). <sup>31</sup>P NMR (D<sub>2</sub>O) 1.292 (s).

(±)-**Phosphoric Acid Mono-[2-(2-chloro-6-methylaminopurin-9-ylmethyl)-3-(dimethoxyphosphoryloxy)propyl] Ester, Ammonium Salt ((±)-9)**. **Method I.** Compound **9** (2.5 mg, 76%) was obtained from **40** (3.8 mg, 0.00664 mmol). <sup>1</sup>H NMR (D<sub>2</sub>O) δ 2.73 (m, 1H), 3.09 (bs, 3H), 3.67 (d, 3H, *J* = 3.2 Hz), 3.71 (d, 3H, *J* = 3.2 Hz), 3.96 (m, 2H), 4.16 (m, 2H), 4.38 (d, 2H, *J* = 6.0 Hz), 8.14 (s, 1H). <sup>31</sup>P NMR (D<sub>2</sub>O) 0.664 (bs), 1.538 (s).

(±)-**Acetic Acid 2-(2-Chloro-6-methylaminopurin-9-ylmethyl)-3-phosphoryloxypentyl Ester, Ammonium Salt ((±)-10)**. **Method I.** Compound **10** (6 mg, 59%) obtained from **41** (12 mg, 0.0237 mmol). <sup>1</sup>H NMR (D<sub>2</sub>O) δ 1.99 (s, 3H), 2.73 (m, 1H), 3.09 (bs, 3H), 3.98 (m, 2H), 4.12 (d, 2H, *J* = 5.1 Hz), 4.44 (d, 2H, *J* = 6.3 Hz), 8.60 (s, 1H). <sup>31</sup>P NMR (D<sub>2</sub>O) 0.352 (s).

(±)-**Diphosphoric Acid Mono-[2-(2-chloro-6-methylaminopurin-9-ylmethyl)-3-phosphonooxypentyl] Ester, Ammonium Salt ((±)-11)**. Compound **44** (6 mg, 0.00808 mmol) was dissolved in methylene chloride (1 mL), and trimethylsilyl bromide (60 μL, 0.455 mmol) was added. The reaction mixture was stirred at room temperature for 10 min, followed by quenching with 0.1 M triethylammonium bicarbonate (5 mL). Methylene chloride was removed in vacuo, and the residue was lyophilized and purified using ion-exchange column chromatography on Sephadex-DEAE-25 resin with a linear gradient (0.01–1.0 M) of 1.0 M ammonium bicarbonate as the mobile phase to give bisphosphate **11** (3.5 mg, 72%) as an ammonium salt. <sup>1</sup>H NMR (D<sub>2</sub>O) δ 2.59 (m, 1H), 3.05 (bs, 3H), 3.74–4.09 (m, 4H), 4.31 (d, 2H, *J* = 7.1 Hz), 8.12 (s, 1H). <sup>31</sup>P NMR (D<sub>2</sub>O) –10.457 (bs), 0.774 (s).

**Phosphoric Acid Mono-[3-(2-chloro-6-methylaminopurin-9-ylmethyl)-5-phosphonooxypentyl] Ester, Ammonium Salt (12).** **Method I.** Compound **12** (8.0 mg, 69%) was obtained from **49** (15 mg, 0.0219 mmol) as an ammonium salt. <sup>1</sup>H NMR (D<sub>2</sub>O) δ 1.61–1.85 (m, 4H), 2.41 (m, 1H), 3.08 (s, 3H), 3.80–4.09 (m, 4H), 4.33 (d, 2H, *J* = 7.3 Hz), 8.95 (s, 1H). <sup>31</sup>P NMR (D<sub>2</sub>O) 0.689 (s).

(*R*)-**Phosphoric Acid Mono-[1-(2-chloro-6-methylaminopurin-9-ylmethyl)-2-phosphonooxyethyl] Ester, Ammonium Salt (13).** **Method I.** Compound **13** (5.0 mg, 44%) was obtained from **58** (15 mg, 0.0234 mmol) as an ammonium salt. <sup>1</sup>H NMR (D<sub>2</sub>O) δ 3.07 (bs, 3H), 3.97 (m, 2H), 4.32–4.60 (m, 3H), 8.10 (s, 1H). <sup>31</sup>P NMR (D<sub>2</sub>O) –0.446, –1.673.

**(S)-Phosphoric Acid Mono-[1-(2-chloro-6-methylaminopurin-9-ylmethyl)-2-phosphonooxyethyl] Ester, Ammonium Salt (14).** Method I. Compound **14** (8.3 mg, 55%) was obtained from **59** (20 mg, 0.0311 mmol) as an ammonium salt.  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  3.06 (bs, 3H), 3.78 (t, 2H,  $J = 4.5$  Hz), 4.30–4.55 (m, 3H), 8.17 (s, 1H).  $^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ ) 3.851, 2.745. MS (negative-ion FAB) 416 ( $\text{M} - \text{H}^+$ ) $^-$ .

**Phosphoric Acid 4-(2-Chloro-6-methylaminopurin-9-yl)-2-phosphonooxymethylbut-2-enyl Ester, Ammonium Salt (15).** Method I. Compound **15** (9.7 mg, 63%) was obtained from **65** (20 mg, 0.0299 mmol) as an ammonium salt.  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  3.03 (bs, 3H), 4.53 (d, 2H,  $J = 7.0$  Hz), 4.67 (d, 2H,  $J = 7.0$  Hz), 5.01 (d, 2H,  $J = 6.6$  Hz), 6.03 (m, 1H), 8.61 (s, 1H).  $^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ ) 0.342.

**meso-1,3-trans-Phosphoric Acid Mono-[2-(2-chloro-6-methylaminopurin-9-ylmethyl)-3-phosphonooxymethylcyclopropylmethyl] Ester, Ammonium Salt (16).** Method H. Compound **16** (28.0 mg, 77%) was obtained from **74** (56 mg, 0.068 mmol) as an ammonium salt.  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  1.42–1.52 (m, 3H), 3.05 (bs, 3H), 3.93 (m, 4H), 4.13 (d, 2H,  $J = 6.6$  Hz), 8.32 (s, 1H).  $^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ ) –21.217. MS (positive-ion FAB) 458 ( $\text{M} + \text{H}^+$ ) $^+$ .

**meso-1,3-cis-Phosphoric Acid Mono-[2-(2-chloro-6-methylaminopurin-9-ylmethyl)-3-phosphonooxymethylcyclopropylmethyl] Ester, Ammonium Salt (17).** Method H. Compound **17** (6.2 mg, 57%) was obtained from **75** (17.0 mg, 0.0208 mmol) as an ammonium salt.  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  1.67 (m, 2H), 1.82 (m, 1H), 3.07 (bs, 3H), 4.05 (m, 2H), 4.22 (m, 2H), 4.40 (d, 2H,  $J = 6.9$  Hz), 8.28 (s, 1H).  $^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ ) –21.287. MS (positive-ion FAB) 458 ( $\text{M} + \text{H}^+$ ) $^+$ .

**( $\pm$ )-1,2-cis-1,3-trans-Phosphoric Acid Mono-[2-(2-chloro-6-methylaminopurin-9-ylmethyl)-3-phosphonooxymethylcyclopropylmethyl] Ester, Ammonium Salt (( $\pm$ )-18).** Method H. Compound (( $\pm$ )-**18**) (11.0 mg, 71%) was obtained from (( $\pm$ )-**82**) (20 mg, 0.0293 mmol) as an ammonium salt.  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  1.41 (m, 1H), 1.49–1.67 (m, 2H), 2.99 (bs, 3H), 3.62–3.97 (m, 4H), 4.15–4.28 (m, 4H), 8.48 (s, 1H).  $^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ ) 0.583.

**( $\pm$ )-Phosphoric Acid Mono-[2-(2-chloro-6-methylaminopurin-9-yl)-1-phosphonooxymethylcyclopropylmethyl] Ester (( $\pm$ )-19).** Method I. Compound (( $\pm$ )-**19**) (7.0 mg, 61%) was obtained from (( $\pm$ )-**86**) (15 mg, 0.0225 mmol) as an ammonium salt.  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  1.69 (t, 1H,  $J = 7.8$  Hz), 1.77 (dd, 1H,  $J = 4.6, 7.2$  Hz), 3.06 (bs, 3H), 3.49 (dd, 1H,  $J = 4.4, 11.3$  Hz), 3.79 (dd, 1H,  $J = 4.6, 7.8$  Hz), 3.91 (dd, 1H,  $J = 6.4, 11.0$  Hz), 4.10 (dd, 1H,  $J = 4.4, 11.3$  Hz), 4.35 (dd, 1H,  $J = 6.7, 11.0$  Hz), 8.67 (s, 1H).  $^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ ) 0.183, 0.644 (s).

**( $\pm$ )-Phosphoric Acid Mono-[2-(2-chloro-6-methylaminopurin-9-ylmethyl)-1-phosphonooxymethylcyclopropylmethyl] Ester (( $\pm$ )-20).** Method I. Compound (( $\pm$ )-**20**) (5.0 mg, 55%) was obtained from (( $\pm$ )-**91**) (12 mg, 0.0176 mmol) as an ammonium salt.  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  0.85 (t, 1H,  $J = 5.5$  Hz), 1.06 (dd, 1H,  $J = 5.8, 8.7$  Hz), 1.63 (m, 1H), 3.08 (bs, 3H), 3.67 (dd, 1H,  $J = 4.8, 10.5$  Hz), 3.86 (dd, 1H,  $J = 3.6, 11.3$  Hz), 4.01 (dd, 1H,  $J = 5.5, 10.6$  Hz), 4.20–4.40 (m, 3H), 8.39 (s, 1H).  $^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ ) 0.548 (s).

**Pharmacological Analyses.** P2Y<sub>1</sub> receptor promoted stimulation of inositol phosphate formation by adenine nucleotide analogues was measured in turkey erythrocyte membranes as previously described.<sup>27,28</sup> 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-[ $^3\text{H}$ ]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<sub>2</sub> at 37 °C. Erythrocyte ghosts were prepared by rapid lysis in hypotonic buffer (5 mM sodium phosphate, pH 7.4, 5 mM MgCl<sub>2</sub>, 1 mM EGTA) as described.<sup>27</sup> Phospholipase C activity was measured in 25  $\mu\text{L}$  of [ $^3\text{H}$ ]inositol-labeled ghosts (approximately 175  $\mu\text{g}$  of protein, 200–500,000 cpm/assay) in a medium containing 424  $\mu\text{M}$  CaCl<sub>2</sub>, 0.91 mM MgSO<sub>4</sub>, 2 mM EGTA, 115 mM KCl, 5 mM KH<sub>2</sub>PO<sub>4</sub>, and 10 mM Hepes, pH 7.0. Assays (200  $\mu\text{L}$  final volume) contained 1  $\mu\text{M}$  GTP $\gamma\text{S}$ , and

the indicated concentrations of nucleotide analogues. Ghosts were incubated at 30 °C for 5 min, and total [ $^3\text{H}$ ]inositol phosphates were quantitated by anion exchange chromatography as previously described.<sup>27,28</sup>

**Data Analysis.** Agonist potencies were calculated using a four-parameter logistic equation and the GraphPad software package (GraphPad, San Diego, CA). EC<sub>50</sub> values (mean  $\pm$  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<sub>50</sub> values (mean  $\pm$  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 replicated in at least three separate experiments, carried out with different membrane preparations using duplicate or triplicate assays.

**Computational Methods.** A molecular model of the human P2Y<sub>1</sub> receptor was built and optimized using Sybyl 6.6<sup>34</sup> modeling package, following the homology modeling approach described in our previous studies<sup>30,35</sup> and using the recently reported X-ray structure of bovine rhodopsin<sup>22</sup> as a structural template. All calculations were performed on a Silicon Graphics Octane R12000 workstation. Briefly, sequences of the P2Y<sub>1</sub> transmembrane domains, identified in our previously published model, were amended by comparison to the corresponding domains of rhodopsin, according to a published sequence alignment.<sup>36</sup> Transmembrane P2Y<sub>1</sub> helices were built from these sequences, in homology to the corresponding helices of rhodopsin, and minimized individually. The minimized helices were then grouped by adding one at a time until a helical bundle (TM region), matching the overall characteristics of the crystallographic structure of rhodopsin, was obtained. The TM region was further modified by adding the amino acid residues 189–213, which comprise EL2. The conformation of EL2 was initially modeled according to the corresponding domain in rhodopsin including the Cys124–Cys202 disulfide bond. At each step the structures were minimized using the Tripos force field with Amber<sup>37</sup> all-atom force parameters until the *rms* value of the conjugate gradient (CG) was <0.1 kcal/mol/Å. A fixed dielectric constant = 4.0 was used throughout these calculations. A model of adenosine 5'-triphosphate (ATP) was constructed from the crystallographic coordinates of ATP-phosphoglycerate kinase.<sup>38</sup> Models of **92** and other nucleotides were constructed from ATP using the "Sketch Molecule" module of Sybyl. The ligands were minimized in Sybyl (using MOPAC calculated partial atomic charges) and were rigidly docked into the helical bundle using graphical manipulation coupled to continuous energy monitoring (Dock module of Sybyl). Whenever a final position was reached, consistent with a local energy minimum, the complexes of receptor and ligand were subjected to an additional CG minimization run of up to 1500 steps.

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## References

- (1) Fredholm, B. B.; Abbracchio, M. P.; Burnstock, G.; Dubyak, G. R.; Harden, T. K.; Jacobson, K. A.; Schwabe, U.; Williams, M. Toward a revised nomenclature for P1 and P2 receptors. *Trends Pharm. Sci.* **1997**, *18*, 79–82.
- (2) North, R. A.; Barnard, E. A. Nucleotide receptors. *Curr. Opin. Neurobiol.* **1997**, *7*, 346–357.
- (3) Burnstock, G.; Williams, M. P2 Purinergic Receptors: Modulation of cell function and therapeutic potential. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 862–869.
- (4) Webb, T. E.; Simon, J.; Krishek, B. J.; Bateson, A. N.; Smart, T. G.; King, B. F.; Burnstock, G.; Barnard, E. A. Cloning and functional expression of a brain G-protein-coupled ATP receptor. *FEBS Lett.* **1993**, *324*, 219–225.



- (5) Schachter, J. B.; Li, Q.; Boyer, J. L.; Nicholas, R. A.; Harden, T. K. Second messenger cascade specificity and pharmacological selectivity of the human P2Y<sub>1</sub>-purinoceptor. *Br. J. Pharmacol.* **1996**, *118*, 167–173.
- (6) King, B. F.; Townsend-Nicholson, A.; Burnstock, G. Metabotropic receptors for ATP and UTP: exploring the correspondence between native and recombinant nucleotide receptors. *Trends Pharmacol. Sci.* **1998**, *19*, 506–514.
- (7) Jin, J.; Daniel, J. L.; Kunapuli, S. P. Molecular basis for ADP-induced platelet activation. II. The P2Y<sub>1</sub> receptor mediates ADP-induced intracellular calcium mobilization and shape change in platelets. *J. Biol. Chem.* **1998**, *273*, 2030–2034.
- (8) Hechler, B.; Leon, C.; Vial, C.; Vigne, P.; Frelin, C.; Cazenave, J. P.; Gachet, C. The P2Y<sub>1</sub> receptor is necessary for adenosine 5'-diphosphate-induced platelet aggregation. *Blood* **1998**, *92*, 152–159.
- (9) Jarvis, G. E.; Humphries, R. G.; Robertson, M. J.; Leff, P. ADP can induce aggregation of human platelets via both P2Y<sub>1</sub> and P2<sub>T</sub> receptors. *Br. J. Pharmacol.* **2000**, *129*, 275–282.
- (10) Hollopeter, G.; Jantzen, H.-M.; Vincent, D.; Li, G.; Englund, L.; Ramakrishnan, V.; Yang, R.-B.; Nurden, P.; Nurden, A.; Julius, D.; Conley, P. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature* **2001**, *409*, 202–207.
- (11) Boyer, J. L.; Adams, M.; Jang, S. Y.; Ravi, R. G.; Jacobson, K. A.; Harden, T. K. Development of P2Y<sub>1</sub> receptor antagonists. *Drug Dev. Res.* (Abstract S14-02) **2000**, *50*, 21.
- (12) Gachet, C. The role of ADP receptors in experimental thrombosis and haemostasis. *Drug Dev. Res.* (Abstract S11-03) **2000**, *50*, 19.
- (13) Leon, C.; Hechler, B.; Freund, M.; Eckly, A.; Vial, C.; Ohlmann, P.; Dierich, A.; LeMeur, M.; Cazenave, J. P.; Gachet, C. Defective platelet aggregation and increased resistance to thrombosis in purinergic P2Y<sub>1</sub> receptor-null mice. *J. Clin. Invest.* **1999**, *104*, 1731–1737.
- (14) Fabre, J. E.; Nguyen, M.; Latour, A.; Keifer, J. A.; Audoly, L. P.; Coffman, T. M.; Koller, B. H. Decreased platelet aggregation, increased bleeding time and resistance to thromboembolism in P2Y<sub>1</sub>-deficient mice. *Nat. Med.* **1999**, *5*, 199–202.
- (15) Boyer, J. L.; Romero-Avila, T.; Schachter, J. B.; Harden, T. K. Identification of competitive antagonists of the P2Y<sub>1</sub> receptor. *Mol. Pharmacol.* **1996**, *50*, 1323–1329.
- (16) Camaioni, E.; Boyer, J. L.; Mohanram, A.; Harden, T. K.; Jacobson, K. A. Deoxyadenosine-bisphosphate derivatives as potent antagonists at P2Y<sub>1</sub> receptors. *J. Med. Chem.* **1998**, *41*, 183–190.
- (17) Boyer, J. L.; Mohanram, A.; Camaioni, E.; Jacobson, K. A.; Harden, T. K. Competitive and selective antagonism of P2Y<sub>1</sub> receptors by N<sup>6</sup>-methyl 2'-deoxyadenosine 3',5'-bisphosphate. *Br. J. Pharmacol.* **1998**, *124*, 1–3.
- (18) Nandanan, E.; Camaioni, E.; Jang, S. Y.; Kim, Y.-C.; Cristalli, G.; Herdewijn, P.; Secrist, J. A.; Tiwari, K. N.; Mohanram, A.; Harden, T. K.; Boyer, J. L.; Jacobson, K. A. Structure activity relationships of bisphosphate nucleotide derivatives as P2Y<sub>1</sub> receptor antagonists and partial agonists. *J. Med. Chem.* **1999**, *42*, 1625–1638.
- (19) Nandanan, E.; Jang, S. Y.; Moro, S.; Kim, H.; Siddiqi, M. A.; Russ, P.; Marquez, V.; Busson, R.; Herdewijn, P.; Harden, T. K.; Boyer, J. L.; Jacobson, K. A. Synthesis, biological activity, and molecular modeling of ribose-modified adenosine bisphosphate analogues as P2Y<sub>1</sub> receptor ligands. *J. Med. Chem.* **2000**, *43*, 829–842.
- (20) Kim, Y. C.; Gallo-Rodriguez, C.; Jang, S. Y.; Nandanan, E.; Adams, M.; Harden, T. K.; Boyer, J. L.; Jacobson, K. A. Acyclic analogues of deoxyadenosine 3',5'-bisphosphates as P2Y<sub>1</sub> receptor antagonists. *J. Med. Chem.* **2000**, *43*, 746–755.
- (21) Brown, S. G.; King, B. F.; Kim, Y.-C.; Burnstock, G.; Jacobson, K. A. Activity of novel adenine nucleotide derivatives as agonists and antagonists at recombinant rat P2X receptors. *Drug Dev. Res.* **2000**, *49*, 253–259.
- (22) Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Trong, I. L.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M. Crystal structure of rhodopsin: a G protein-coupled receptor. *Science* **2000**, *289*, 739–745.
- (23) Perich, J. W.; Johns, R. B. Di-*tert*-butyl *N*, *N*-diethylphosphoramidite. A new phosphitylating agent for the efficient phosphorylation of alcohols. *Synthesis* **1988**, *2*, 142–144.
- (24) Zatorski, A.; Goldstein, B. M.; Colby, T. D.; Jones, J. P.; Pankiewicz, K. W. Potent inhibitors of human inosine monophosphate dehydrogenase Type II. Fluorine-substituted analogues of thiazole-4-carboxamide adenine dinucleotide. *J. Med. Chem.* **1995**, *38*, 1098–1105.
- (25) Sekiyama, T.; Hatsuya, S.; Tanaka, Y.; Uchiyama, M.; Ono, N. Synthesis and antiviral activity of novel acyclic nucleosides: Discovery of a cyclopropyl nucleoside with potent inhibitory activity against Herpes viruses. *J. Med. Chem.* **1998**, *41*, 1284–1298.
- (26) Green, G. R.; Kinney, P. M.; Choudary, B. M. Regiospecific Michael additions with 2-aminopurines. *Tetrahedron Lett.* **1992**, *33*, 4609–4612.
- (27) Boyer, J. L.; Downes, C. P.; Harden, T. K. Kinetics of activation of phospholipase C by P2Y purinergic receptor agonists and guanine nucleotides. *J. Biol. Chem.* **1989**, *264*, 884–890.
- (28) Harden, T. K.; Hawkins, P. T.; Stephens, L.; Boyer, J. L.; Downes, P. Phosphoinositide hydrolysis by guanosine 5'-(gamma-thio)triphosphate-activated phospholipase C of turkey erythrocyte membranes. *Biochem. J.* **1988**, *252*, 583–593.
- (29) Filtz, T. M.; Li, Q.; Boyer, J. L.; Nicholas, R. A.; Harden, T. K. Expression of a cloned P2Y purinergic receptor that couples to phospholipase C. *Mol. Pharmacol.* **1994**, *46*, 8–14.
- (30) Moro, S.; Guo, D.; Camaioni, E.; Boyer, J. L.; Harden, T. K.; Jacobson, K. A. Human P2Y<sub>1</sub> receptor: molecular modeling and site-directed mutagenesis as tools to identify agonist and antagonist recognition sites. *J. Med. Chem.* **1998**, *41*, 1456–1466.
- (31) Moro, S.; Hoffmann, C.; Jacobson, K. A. Role of the extracellular loops of G protein-coupled receptors in ligand recognition: A molecular modeling study of the human P2Y<sub>1</sub> receptor. *Biochemistry* **1999**, *38*, 3498–3507.
- (32) Pellicciari, R.; Marinozzi, M.; Costantino, G.; Natalini, B.; Moroni, F.; Pellegrini-Giampietro, D. (2*R*,1'*S*,2'*R*,3'*S*)-2-(2'-Carboxy-3'-phenylcyclopropyl)glycine (PCCG-13), the first potent and selective competitive antagonist of phospholipase D-coupled metabotropic glutamate receptors: Asymmetric synthesis and preliminary biological properties. *J. Med. Chem.* **1999**, *42*, 2716–2720.
- (33) Palmer, R. K.; Boyer, J. L.; Schachter, J. B.; Nicholas, R. A.; Harden, T. K. Agonist action of adenosine triphosphates at the human P2Y<sub>1</sub> receptor. *Mol. Pharmacol.* **1998**, *54*, 1118–1123.
- (34) The program SYBYL 6.3 is available from TRIPOS Associates, St. Louis, MO, 1993.
- (35) van Rhee, A. M.; Fischer, B.; van Galen, P. J. M.; Jacobson, K. A. Modelling the P2Y purinoceptor using rhodopsin as template. *Drug Des. Discovery* **1995**, *13*, 133–154.
- (36) Horn, F.; Weare, J.; Beukers, M. W.; Hörsch, S.; Bairoch, A.; Chen, W.; Edvardsen, Ø.; Campagne, F.; Vriend, G. GPCRDB: an information system for G protein-coupled receptors. *Nucleic Acids Res.* **1998**, *26*, 277–281.
- (37) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. An all-atom force field for simulation of protein and nucleic acids. *J. Comput. Chem.* **1986**, *7*, 230–252.
- (38) Watson, H. C.; Walker, N. P.; Shaw, P. J.; Bryant, T. N.; Wendell, P. L.; Fothergill, L. A.; Perkins, R. E.; Conroy, S. C.; Dobson, M. J.; Tuite, M. F.; et al. Sequence and structure of yeast phosphoglycerate kinase. *EMBO J.* **1982**, *1*, 1635–1640.

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