

## Methanocarba Modification of Uracil and Adenine Nucleotides: High Potency of Northern Ring Conformation at P2Y<sub>1</sub>, P2Y<sub>2</sub>, P2Y<sub>4</sub>, and P2Y<sub>11</sub> but Not P2Y<sub>6</sub> Receptors

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Received August 6, 2001

The potency of nucleotide antagonists at P2Y<sub>1</sub> receptors was enhanced by replacing the ribose moiety with a constrained carbocyclic ring (Nandan, et al. *J. Med. Chem.* **2000**, *43*, 829–842). We have now synthesized ring-constrained methanocarba analogues (in which a fused cyclopropane moiety constrains the pseudosugar ring) of adenine and uracil nucleotides, the endogenous activators of P2Y receptors. Methanocarba-adenosine 5'-triphosphate (ATP) was fixed in either a Northern (N) or a Southern (S) conformation, as defined in the pseudorotational cycle. (N)-Methanocarba-uridine was prepared from the 1-amino-pseudosugar ring by treatment with  $\beta$ -ethoxyacryloyl cyanate and cyclization to form the uracil ring. Phosphorylation was carried out at the 5'-hydroxyl group through a multistep process: Reaction with phosphoramidite followed by oxidation provided the 5'-monophosphates, which then were treated with 1,1'-carbonyldiimidazole for condensation with additional phosphate groups. The ability of the analogues to stimulate phospholipase C through activation of turkey P2Y<sub>1</sub> or human P2Y<sub>1</sub>, P2Y<sub>2</sub>, P2Y<sub>4</sub>, P2Y<sub>6</sub>, and P2Y<sub>11</sub> receptors stably expressed in astrocytoma cells was measured. At recombinant human P2Y<sub>1</sub> and P2Y<sub>2</sub> receptors, (N)-methanocarba-ATP was 138- and 41-fold, respectively, more potent than racemic (S)-methanocarba-ATP as an agonist. (N)-methanocarba-ATP activated P2Y<sub>11</sub> receptors with a potency similar to ATP. (N)-Methanocarba-uridine 5'-triphosphate (UTP) was equipotent to UTP as an agonist at human P2Y<sub>2</sub> receptors and also activated P2Y<sub>4</sub> receptors with an EC<sub>50</sub> of 85 nM. (N)-Methanocarba-uridine 5'-diphosphate (UDP) was inactive at the hP2Y<sub>6</sub> receptor. The vascular effects of (N)-methanocarba-UTP and (N)-methanocarba-UDP were studied in a model of the rat mesenteric artery. The triphosphate was more potent than UTP in inducing a dilatory P2Y<sub>4</sub> response (pEC<sub>50</sub> = 6.1  $\pm$  0.2), while the diphosphate was inactive as either an agonist or antagonist in a P2Y<sub>6</sub> receptor-mediated contractile response. Our results suggest that new nucleotide agonists may be designed on the basis of the (N) conformation that favors selectivity for P2Y<sub>1</sub>, P2Y<sub>2</sub>, P2Y<sub>4</sub>, and P2Y<sub>11</sub> receptors.

### Introduction

P2 receptors, which are activated by purine and/or pyrimidine nucleotides, consist of two families: G protein-coupled receptors termed P2Y, of which six mammalian subtypes have been cloned, and ligand-gated cation channels termed P2X, of which seven mammalian subtypes have been cloned.<sup>1–3</sup> The nomenclature of P2 receptors and their various ligand specificities has been reviewed.<sup>4–7</sup> Uracil nucleotides activate P2Y<sub>2</sub>, P2Y<sub>4</sub>, and P2Y<sub>6</sub> subtypes. Adenine nucleotides are required for the activation of P2Y<sub>1</sub> and P2Y<sub>11</sub> subtypes. Activation of P2Y receptors generally results in the stimulation of phospholipase C (PLC), which generates inositol phosphates and diacylglycerol from phosphatidyl inositol(4,5)bisphosphate,<sup>8</sup> leading to a rise in intra-

cellular calcium and activation of protein kinase C. The P2Y<sub>11</sub> receptor activates adenylyl cyclase in addition to the activation of PLC,<sup>9</sup> and G protein-coupled P2Y receptors in C6 glioma cells<sup>10</sup> and the recently cloned P2Y<sub>12</sub> receptor in platelets<sup>11</sup> inhibit adenylyl cyclase.

The P2Y<sub>1</sub> receptor is present in the heart, skeletal, and various smooth muscles, prostate, ovary, and brain.<sup>12</sup> A selective P2Y<sub>1</sub> receptor agonist may have potential as an antihypertensive or antidiabetic agent.<sup>13,14</sup> The P2Y<sub>2</sub> receptor is associated with the ion secretory actions of uridine 5'-triphosphate (UTP) and adenosine 5'-triphosphate (ATP) in the mucosa of the nasal and tracheal epithelia and is a target in therapeutics of pulmonary diseases, e.g., cystic fibrosis<sup>15,16</sup> and ophthalmic diseases.<sup>17</sup> The P2Y<sub>4</sub> and P2Y<sub>6</sub> receptors may have a role in regulation of blood flow<sup>18</sup> and have been implicated in the gastrointestinal responses to UTP and uridine 5'-diphosphate (UDP).<sup>16</sup> The P2Y<sub>11</sub> receptor subtype is present in the immune system, and the P2Y<sub>12</sub> receptor subtype plays a key role in ADP action in

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**Table 1.** Potencies for Methanocarba Analogues and the Corresponding Naturally Occurring Nucleotides in the Activation of Phospholipase C

compd	analogue	subtype	Northern	
			a, methanocarba	
			EC <sub>50</sub> (μM) of (N)-methanocarba analogue <sup>a</sup>	b, ribose
<b>3a</b>	ATP	tP2Y <sub>1</sub>	0.014 ± 0.003	2.8 ± 0.7
		hP2Y <sub>1</sub>	0.052 ± 0.022	1.5 ± 0.2
		hP2Y <sub>2</sub>	0.091 ± 0.005	0.085 ± 0.012
		hP2Y <sub>4</sub>	32% at 10 μM	<sup>c</sup>
		hP2Y <sub>11</sub>	34.5 ± 4.7% at 10 μM	17.25 ± 2.80
<b>4a</b>	AMP	tP2Y <sub>1</sub>	NE <sup>d</sup>	NE
<b>5a</b>	UTP	hP2Y <sub>2</sub>	0.0159 ± 0.007	0.008 ± 0.002
		hP2Y <sub>4</sub>	0.085 ± 0.005	0.049 ± 0.010
		hP2Y <sub>6</sub>	NE	
		hP2Y <sub>11</sub>	NE	
<b>6a</b>	UDP	hP2Y <sub>6</sub>	NE	0.015 ± 0.004
<b>7a</b>	UMP	hP2Y <sub>6</sub>	NE	NE
compd	analogue	subtype	Southern	
			EC <sub>50</sub> (μM) of (S)-methanocarba analogue <sup>a</sup>	EC <sub>50</sub> (μM) of corresponding ribose nucleotide <sup>a,b</sup>
<b>8a</b>	ATP	tP2Y <sub>1</sub>	3.5 ± 0.66	2.8 ± 0.7
		hP2Y <sub>1</sub>	7.2 ± 1.0	1.5 ± 0.2
		hP2Y <sub>2</sub>	3.7 ± 0.5	0.085 ± 0.012
		hP2Y <sub>4</sub>	NE	<sup>c</sup>
		hP2Y <sub>11</sub>	NE	17.25 ± 2.80
<b>9a</b>	AMP	tP2Y <sub>1</sub>	NE	NE

<sup>a</sup> Average ± SEM. <sup>b</sup> Values from refs 32, 33, 38, and 39. <sup>c</sup> Antagonist effect.<sup>37</sup> <sup>d</sup> NE means "no effect at 100 μM".

platelets. Currently, there are few selective agonists or antagonists at these subtypes.<sup>7</sup> While numerous adenine nucleotide ligands have been explored at the P2Y<sub>1</sub> receptor, few uracil nucleotides have been reported as P2Y receptor agonists.<sup>15,18</sup>

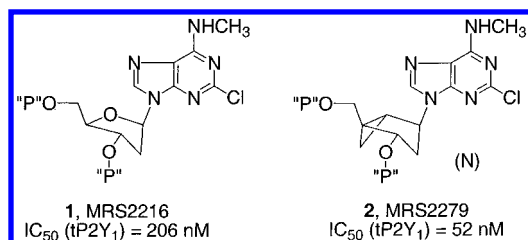
Conformational considerations of both the receptors and their ligands are important in structure-based drug design. The ribose rings of purine and pyrimidine nucleotides can adopt a range of conformations, although the P2 receptors, which they activate, likely prefer specific conformations. Therefore, constraining the riboselike ring of nucleotide analogues would help establish these parameters, and we hypothesize that an analogue in which the ribose has been replaced with a carbocyclic ring locked in a preferred conformation may display unique potency and selectivity. Thus, we have adopted the methanocarba approach,<sup>19</sup> in which fused cyclopropane and cyclopentane rings replace the ribose moiety. Two structural variations, depending on the position of the cyclopropane ring, restrict the ring pucker, i.e., hold the riboselike ring (pseudosugar) in either a Northern (N, 2'-exo) or a Southern (S, 2'-endo) envelope conformation, as defined in the pseudorotational cycle. For two classes of purine ligands, i.e., adenosine bisphosphate derivatives binding at P2Y<sub>1</sub> receptors<sup>20</sup> and adenosine derivatives binding at A<sub>1</sub> and A<sub>3</sub> receptors,<sup>21</sup> a preference for the (N)-methanocarba isomer already has been demonstrated. At P2Y<sub>1</sub> recep-

tors, the ribose-containing antagonist 2-chloro-2'-deoxy-N<sup>6</sup>-methyladenosine-3',5'-bisphosphate, **1**, has been substituted with the (N)-methanocarba modification to yield (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, **2**.<sup>20</sup> Both **1** and **2** are selective antagonists of the turkey erythrocyte P2Y<sub>1</sub> receptor, with **2** (IC<sub>50</sub> 52 nM) approximately 4-fold more potent than **1**.

We wished to determine if the preference for the (N) conformation applies to subtypes other than P2Y<sub>1</sub> receptors and also whether this preference seen for bisphosphate antagonist derivatives is generalizable to 5'-triphosphate agonists and to pyrimidine as well as purine nucleotides. Here, we describe the synthesis and biological characterization in several species of the methanocarba analogues of adenine and uracil nucleotides. For direct comparison, the adenine nucleotide analogues have been locked in both the (N) ring and the (S) ring conformations. In general, the constrained (N) conformation of the pseudoribose ring increased agonist potencies at P2Y<sub>1</sub> receptors and preserved potencies at P2Y<sub>2</sub>, P2Y<sub>4</sub>, and P2Y<sub>11</sub> receptors but not at the UDP-activated P2Y<sub>6</sub> receptor. The corresponding (S) isomer of methanocarba-ATP displayed greatly reduced potency at P2Y<sub>1</sub>, P2Y<sub>2</sub>, and P2Y<sub>11</sub> receptors.

## Results

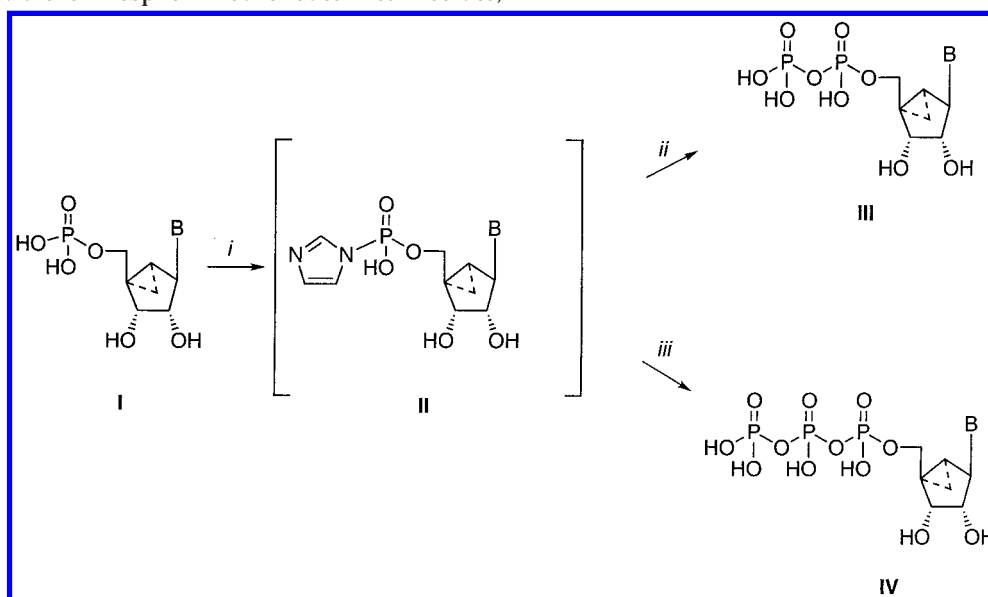
**Chemical Synthesis.** Methanocarba analogues of naturally occurring adenine and uracil nucleotides (Table 1) were synthesized containing fused cyclopropane and cyclopentane rings that fixed the pseudoribose moiety in a rigid (N) or (S) envelope conformation. Nucleotide analogues having either the (N)-methanocarba modification,<sup>19,20</sup> **3a–7a**, or the (S)-methanocarba modification,<sup>22</sup> **8a** and **9a**, were prepared in the ammonium salt form according to the methods shown in Schemes 1–4 and tested biologically as agonists at



**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>e</sup>
		calcd	found	system 1 (purity) (%)	system 2 (purity) (%)	
<b>3a</b>	C <sub>12</sub> H <sub>18</sub> N <sub>5</sub> O <sub>12</sub> P <sub>3</sub>	516.0087	516.0085	4.289 (98)	17.009 <sup>d</sup> (98)	14
<b>4a</b>	C <sub>12</sub> H <sub>16</sub> N <sub>5</sub> O <sub>6</sub> P	356.0760	356.0765	4.178 (99)	8.145 <sup>d</sup> (98)	65
<b>5a</b>	C <sub>11</sub> H <sub>17</sub> N <sub>2</sub> O <sub>14</sub> P <sub>3</sub>	492.9814	492.9832	1.737 (99)	6.764 <sup>b</sup> (96)	22
<b>6a</b>	C <sub>11</sub> H <sub>16</sub> N <sub>2</sub> O <sub>11</sub> P <sub>2</sub>	413.0151	413.0151	2.130 (98)	4.531 <sup>c</sup> (97)	24
<b>7a</b>	C <sub>11</sub> H <sub>15</sub> N <sub>2</sub> O <sub>8</sub> P	333.0488	333.0506	3.235 (99)	3.466 <sup>c</sup> (98)	58
<b>8a</b>	C <sub>12</sub> H <sub>16</sub> N <sub>5</sub> O <sub>6</sub> P	356.0760	356.0765	5.057 (97)	11.091 <sup>d</sup> (99)	60
<b>9a</b>	C <sub>12</sub> H <sub>18</sub> N <sub>5</sub> O <sub>12</sub> P <sub>3</sub>	516.0087	516.0085	5.709 (97)	17.517 <sup>d</sup> (97)	30

<sup>a</sup> Purity of each derivative was  $\geq 95\%$ , as determined using HPLC with two different mobile phases. Purity of all compounds in solvent 1 and compounds **5a**, **6a**, and **7a** in solvent 2 was checked using an SMT OD-5-60 RP-C18 analytical column (250  $\times$  4.6 mm; Separation Methods Technologies, Inc., Newark, DE) in two solvent systems. Compounds **3a**, **4a**, **8a**, and **9a** were checked in solvent 2 using a Phenomenex Luna 5 $\mu$  C18(2) analytical column (250  $\times$  4.6 mm). All gradients were completed in 20 min. System 1: gradient of 0.1 M TEAA/CH<sub>3</sub>CN from 95/5 to 40/60. System 2: as specified below. <sup>b</sup> Gradient of 5 mM TBAP/CH<sub>3</sub>CN from 80/20 to 40/60. <sup>c</sup> Gradient of 0.1 M TEAB/CH<sub>3</sub>CN from 100/0 to 90/10. <sup>d</sup> Gradient of 5 mM TBAP/CH<sub>3</sub>CN from 95/5 to 40/60. <sup>e</sup> The percent yields refer to overall yield for the entire phosphorylation sequence. <sup>f</sup> **3a**, MRS2340; **5a**, MRS 2341.

**Scheme 1.** General Method for Stepwise Phosphorylation of (N)-Methanocarpa Nucleosides: Formation of Di- and Triphosphates via the Phosphorimidazolide Intermediate, II<sup>a</sup>

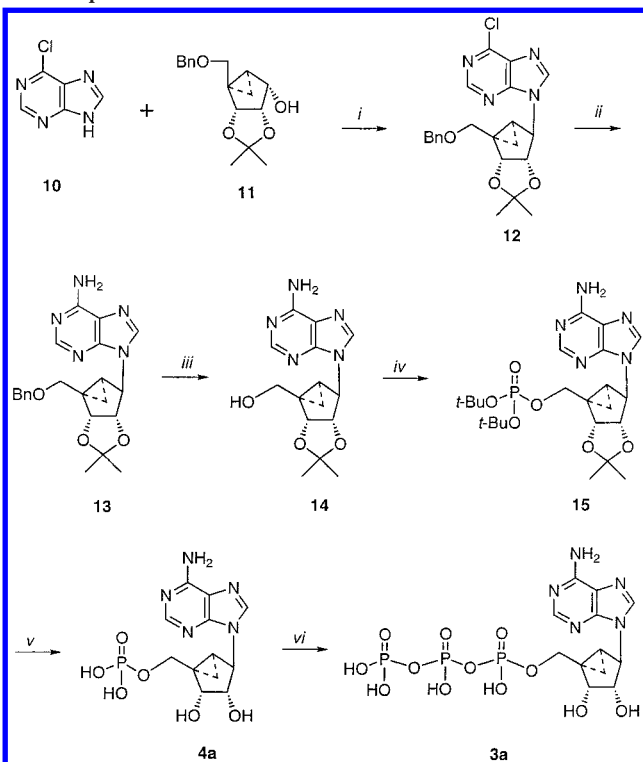
<sup>a</sup> Reagents: (i) 1,1'-Carbonyldiimidazole, DMF, room temperature, 6 h and then 5% aqueous triethylamine, room temperature, 2 h. (ii) Tributylammonium phosphate, DMF, room temperature, 2 days, 43%. (iii) Tributylammonium pyrophosphate, DMF, 3 days, 6–40%.

various P2Y receptors (Table 1). Identity was confirmed using nuclear magnetic resonance (NMR, <sup>1</sup>H and <sup>31</sup>P) and high-resolution mass spectrometry, and purity was demonstrated using high-pressure liquid chromatography (HPLC) in two different solvent systems (Table 2).

Several methods of 5'-phosphorylation of the (N)-methanocarpa nucleoside were compared. A standard method using sequential reaction with phosphorus oxychloride and pyrophosphate to provide nucleoside 5'-triphosphate analogues<sup>23</sup> proved problematic. Several attempts to obtain the (N)-methanocarpa-UTP by variations of this one-pot method were unsuccessful. We have evidence that the one-pot method in the (N) series leads to the formation of cyclic-3',5'-nucleotides (unpublished, V. Marquez). This is due to the close proximity between the 3'-OH and the 5'-OH that can be achieved only in the fixed (N) envelope conformation. On the other hand, these two hydroxyl groups never come close to one another in the antipodal (S) conformers. Therefore, the multistep elongation of a 5'-monophosphate group to di- and triphosphates was pursued. The general method adopted (Scheme 1) consisted of reaction of 1,1'-carbon-

ylidiimidazole<sup>24,25</sup> with a nucleoside 5'-monophosphate, **I**, followed by the addition of either phosphate or pyrophosphate. By this method, the monophosphate analogues were cleanly converted to either di- or triphosphate, **III** or **IV**, respectively, via a phosphorimidazolide intermediate, **II**. Typically, the monophosphate, **I**, was treated with an excess of carbonyldiimidazole in *N,N*-dimethylformamide (DMF) and stirred for 4–6 h. To hydrolyze the cyclic carbonate<sup>24</sup> that formed at the 2',3'-dihydroxy groups, a 5% triethylamine solution in MeOH:water (1/1) was added to the reaction mixture, and stirring was continued for 2 h. HPLC monitoring of this reaction step indicated that the phosphorimidazolide, **II**, was stable to mild base treatment. This intermediate, **II**, was treated with phosphoric acid tributylammonium salt or with pyrophosphoric acid tributylammonium salt to give the diphosphate, **III**, or triphosphate, **IV**, respectively.

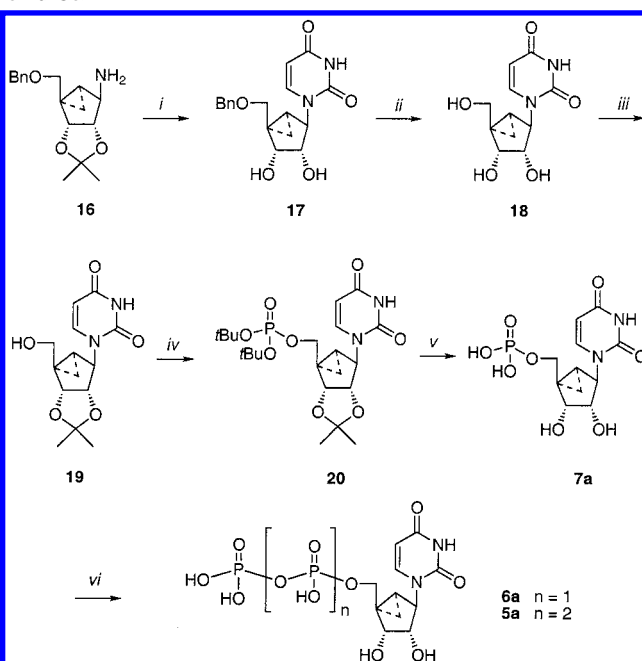
The (N)-methanocarpa analogues of ATP and adenosine 5'-monophosphate (AMP) were prepared by this approach (Scheme 2). The (N)-methanocarpa-adenosine precursors were prepared by the general approaches of

**Scheme 2.** Synthesis of (N)-Methanocarba Adenosine 5'-Phosphate Derivatives<sup>a</sup>

<sup>a</sup> Reagents: (i) DEAD, Ph<sub>3</sub>P, THF (ref 40). (ii) NH<sub>3</sub>/*i*-PrOH, 95%. (iii) Palladium black, HCO<sub>2</sub>H, methanol, 76%. (iv) Di-*t*-butyl *N,N*-diethylphosphoramidite, tetrazole, THF, room temperature, 6 h and then *m*-CPBA, -78 °C, room temperature, 78%. (v) DOWEX 50 × 8–200, methanol, 60–70 °C, 69%. (vi) (1) 1,1'-Carbonyldiimidazole, (2) triethylamine/MeOH, (3) tributylammonium pyrophosphate, 20%.

Marquez and co-workers<sup>19,26,27</sup> or Lee et al.<sup>28</sup> 6-Chloropurine, **10**, was condensed with the protected bicyclo[3.1.0]hexane derivative, **11**,<sup>28</sup> using Mitsunobu conditions to give **12**, which was then treated with a saturated solution of ammonia in THF to give the corresponding adenosine analogue, **13**. Deprotection of a 5'-benzyl group of **13** was carried out smoothly using Pd black/formic acid to give **14** and was followed by phosphorylation using a phosphoramidite method<sup>29</sup> to give the di-*tert*-butyl-protected monophosphate, **15**. The monophosphate, **15**, was deprotected and either tested biologically or carried further to the di- and triphosphates by the method shown in Scheme 1.

The (N)-methanocarba analogues of UTP (**5a**), UDP (**6a**), and UMP (**7a**), were also prepared. A synthetic route to (N)-methanocarba-uridine as a precursor for uracil nucleotide analogues was devised (Scheme 3). Several attempts to condense uracil and the pseudosugar moiety by the Mitsunobu reaction gave only a di-O-alkylated byproduct, likely due to the low solubility of uracil in tetrahydrofuran (THF). A protected uracil derivative having greater solubility in THF, 3-benzoyl-uracil,<sup>30</sup> was used as a reactant in the Mitsunobu reaction; however, an O-alkylated byproduct resulted. Eventually, formation of the uracil moiety was achieved in two sequential steps (Scheme 3).<sup>31</sup> The aminocyclopentane derivative, **16**, was converted to an acryloylureido compound (isolated but not shown in Scheme 3) by treatment with  $\beta$ -ethoxyacryloyl cyanate.<sup>31</sup> Cyclization upon refluxing in 2 N sulfuric acid and

**Scheme 3.** Synthesis of (N)-Methanocarba-uridine 5'-Monophosphate, **7a**, and 5'-Di- and Triphosphates, **6a** and **5a**<sup>a</sup>

<sup>a</sup> Reagents: (i) (a) EtOCH=CHC(=O)NCO, benzene, room temperature, 10 min, 89%; (b) 2 N H<sub>2</sub>SO<sub>4</sub>, MeOH, reflux, 30 min, 67%. (ii) Pd black, HCO<sub>2</sub>H, methanol, 74%. (iii) *p*-Toluene sulfonic acid, acetone, room temperature, 4 h, 86%. (iv) Di-*t*-butyl *N,N*-diethylphosphoramidite, tetrazole, THF, room temperature, 20 min and then *m*-CPBA, -78 °C, room temperature, 79%. (v) DOWEX 50 × 8–200, methanol, 60–70 °C, 86%. (vi) (1) 1,1'-Carbonyldiimidazole, (2) triethylamine/MeOH, (3) tributylammonium phosphate or pyrophosphate, DMF, 2–3 days, 41% (**6a**), 38% (**5a**).

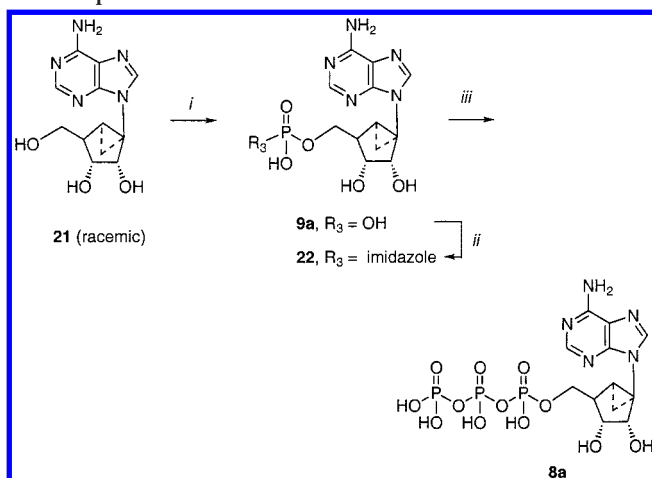
methanol gave the 5'-protected (N)-methanocarba-uracil, **17**, in good yield. Removal of the O-benzyl group with palladium black and formic acid in methanol afforded the triol, **18**. The acetonide of the 2,3-diol, **19**, was prepared, and the primary alcohol was converted to the di-*tert*-butyl phosphate, **20**.<sup>29</sup> Both di-*tert*-butyl and acetonide groups were deprotected simultaneously under acidic conditions, leading to the successful synthesis of monophosphate, **7a**.

The (S)-methanocarba analogues of ATP and AMP, **8a** and **9a** (Scheme 4), respectively, were prepared using a sequential phosphorus oxychloride method from the unprotected, racemic (S)-methanocarba-adenosine, **21**.<sup>22</sup> The monophosphate, **9a**, was isolated and converted to the triphosphate, **8a**, using carbonyldiimidazole.

**Biological Activity.** Methanocarba analogues were tested for agonist activity by measuring P2Y<sub>1</sub> receptor-promoted PLC activity in turkey erythrocyte membranes and by measuring inositol phosphate accumulation in 1321N1 human astrocytoma cells stably expressing the human P2Y<sub>1</sub>, P2Y<sub>2</sub>, P2Y<sub>4</sub>, P2Y<sub>6</sub>, or P2Y<sub>11</sub> receptors. In each case, full concentration effect curves were generated, and EC<sub>50</sub> values from at least three separate experiments were determined (Table 1). The agonist potencies of the corresponding ribose-containing nucleotides (denoted "b") were simultaneously determined for comparison purposes.

The (N)-methanocarba-ATP analogue, **3a**, was 250-fold more potent than the racemic (S)-methanocarba-ATP analogue, **8a**, and 200-fold more potent than ATP, **3b**, at the turkey P2Y<sub>1</sub> receptor (Table 1). The (N)-



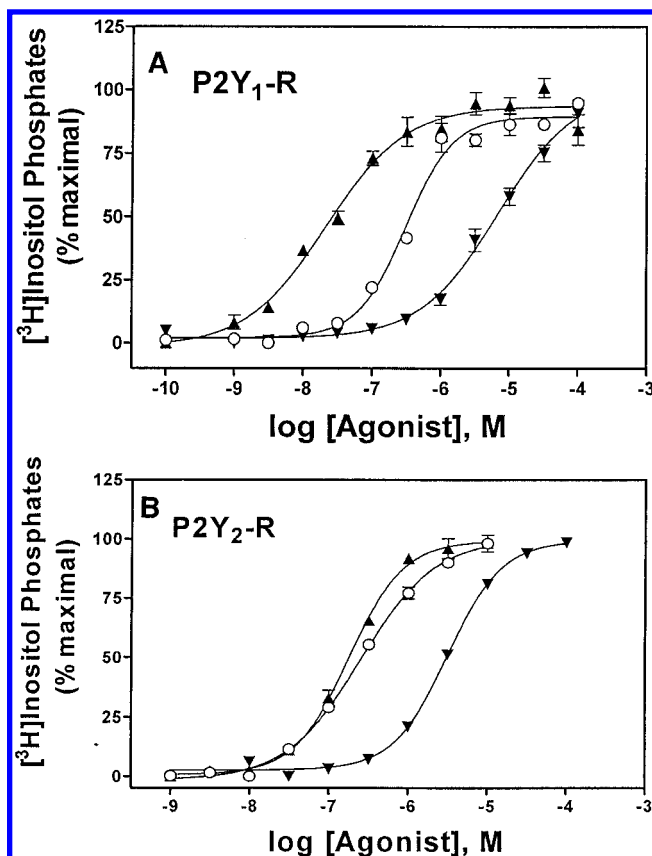
**Scheme 4.** Synthesis of (S)-Methanocarba Adenosine 5'-Phosphate Derivatives<sup>a</sup>

<sup>a</sup> Reagents: (i) POCl<sub>3</sub>, trimethyl phosphate, 60%. (ii) 1,1'-Carbonyldiimidazole, DMF, room temperature, 6 h and then 5% aqueous triethylamine, room temperature, 2 h. (iii) Tributylammonium pyrophosphate, DMF, 3 days, 30%.

methanocarba-ATP analogue, **3a**, was also more potent than the corresponding (S)-methanocarba analogue, **8a**, at the recombinant human P2Y<sub>1</sub> and P2Y<sub>2</sub> receptors, by factors of 138- and 41-fold, respectively (Figure 1 and Table 1). The (N) and (S) isomers of methanocarba-ATP were full agonists at both the P2Y<sub>1</sub> and the P2Y<sub>2</sub> receptors. At the human P2Y<sub>1</sub> receptor (N)-methanocarba-ATP, **3a**, displaying an EC<sub>50</sub> value of 52 nM, was 29-fold more potent than ATP, **3b**. In contrast, the (N)-methanocarba-ATP analogue was equipotent with ATP at the human P2Y<sub>2</sub> receptor and also exhibited a similar potency to ATP at the P2Y<sub>11</sub> receptor. Note that concentration-effect curves for **3a** at the human P2Y<sub>11</sub> receptor were only carried out to 10  $\mu$ M concentration. Nonetheless, these curves predicted an EC<sub>50</sub> for **3a** essentially equivalent to that of ATP ( $\sim$ 20  $\mu$ M). As is the case with ATP, (N)-methanocarba-ATP was not an agonist at the human P2Y<sub>4</sub> receptor. The (N)-methanocarba-AMP analogue, **4a**, was not active at the turkey P2Y<sub>1</sub> receptor.

(N)-Methanocarba analogues of uridine nucleotides also were synthesized as potential agonists at the uracil-activated, P2Y<sub>2</sub>, or uracil selective, P2Y<sub>4</sub> and P2Y<sub>6</sub>, receptors. The high potency in the (N)-methanocarba series was maintained or increased at the human P2Y<sub>2</sub> and P2Y<sub>4</sub> receptor subtypes, where the (N)-methanocarba-UTP analogue, **5a**, was only slightly less potent than UTP (Table 1 and Figure 2). Like UTP, the (N)-methanocarba-UTP analogue was inactive at the human P2Y<sub>6</sub> and P2Y<sub>11</sub> receptors. Surprisingly, the (N)-methanocarba-UDP analogue, **6a**, was inactive at the UDP-selective human P2Y<sub>6</sub> receptor (Figure 3). (N)-Methanocarba-UMP also was without effect at the P2Y<sub>6</sub> receptor.

The vascular effects of (N)-methanocarba-UTP, **5a**, and (N)-methanocarba-UDP, **6a**, were studied in a model of the rat mesenteric artery, in which uracil nucleotides elicit both dilatory and contractile responses (Figures 4–6).<sup>18</sup> The dilatory response is dependent on the activation of endothelial P2Y<sub>2,4</sub> receptors by UTP, and the contractile response is mainly dependent on

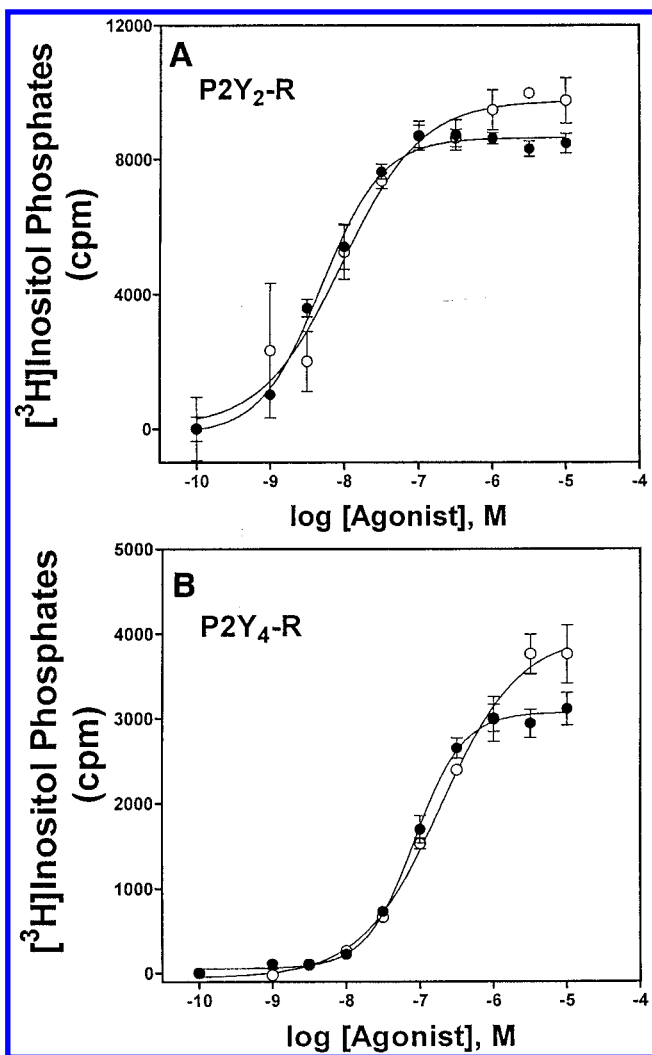


**Figure 1.** Effects of (N)-methanocarba or (S)-methanocarba modification of ATP on PLC activity in 1321N1 astrocytoma cells expressing either hP2Y<sub>1</sub> (A) or hP2Y<sub>2</sub> (B) receptors. Concentration-dependent stimulation of inositol phosphate formation by adenine nucleotides (triphosphates), compounds **3a** ( $\blacktriangle$ ), **3b** ( $\circ$ ), and **8a** ( $\blacktriangledown$ ). Membranes from [<sup>3</sup>H]inositol-labeled membranes were incubated for 5 min at 30 °C in the presence of the indicated concentrations of agonist. The data shown are typical curves for at least three experiments carried out in duplicate using different membrane preparations.

activation of vascular smooth muscle P2Y<sub>6</sub> receptors by UDP with a small P2Y<sub>2,4</sub> receptor-dependent component.

Compound **5a** was significantly more potent than UTP in inducing a dilatory response ( $pEC_{50} = 6.1 \pm 0.2$  for **5a** and  $5.5 \pm 0.2$  for **5b**, Figure 4). The maximal dilatory effects ( $R_{max}$ , as a percentage of the corresponding precontraction with 1  $\mu$ M norepinephrine) observed were  $70.6 \pm 9.5$  and  $50.0 \pm 14.7\%$  for **5a** and **5b**, respectively. In the contractile response (Figure 5), although a response could be observed for UDP, (N)-methanocarba-UDP, **6a**, had no agonist effect at a concentration of 300  $\mu$ M. Compound **5a** induced contractions but much more weakly than the ribose equivalent, UTP. Contraction induced at an agonist concentration of 100  $\mu$ M, expressed as percent of the response to 60 mM potassium, was  $17.2 \pm 6.6\%$  ( $n = 4$ ) for **5a** and  $52.6 \pm 18.6\%$  ( $n = 5$ ) for **5b**. A complete dose-response curve could not be constructed for **5a**, due to limited amounts of the compound. These results are consistent with selectivities observed at the human P2Y receptors, in which the (N)-methanocarba modification precluded activation of P2Y<sub>6</sub> receptors.

Because (N)-methanocarba-UDP, **6a**, did not have any agonistic (contractile) effects on the P2Y<sub>6</sub> receptors in this preparation, we wanted to examine a possible

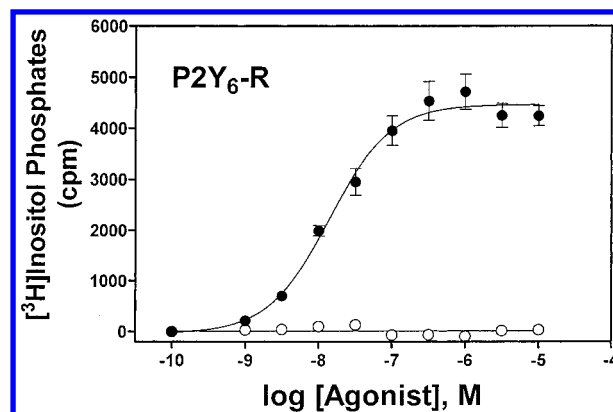


**Figure 2.** Effects of the (N)-methanocarba modification in uracil nucleotides (triphosphates) on activation of PLC in 1321N1 astrocytoma cells expressing either hP2Y<sub>2</sub> (A) or hP2Y<sub>4</sub> (B) receptors, showing concentration-dependent stimulation of inositol phosphate formation by compounds **5a** (○) and **5b** (●). Membranes from [<sup>3</sup>H]inositol-labeled membranes were incubated for 5 min at 30 °C in the presence of the indicated concentrations of agonist. The data shown are typical curves for at least three experiments carried out in duplicate using different membrane preparations.

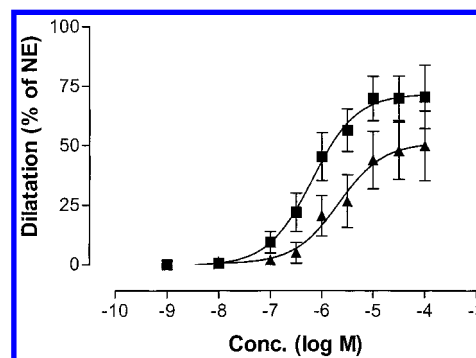
antagonistic effect. (N)-Methanocarba-UDP, **6a**, was demonstrated to lack any antagonistic effects at the UDP-mediated contraction. In this preparation, UDP at 100  $\mu$ M produced a  $33.9 \pm 7.1\%$  contraction ( $n = 4$ ) in the absence of **6a** and a  $34.0 \pm 2.4\%$  contraction ( $n = 4$ ) following pretreatment with **6a** (Figure 6).

## Discussion

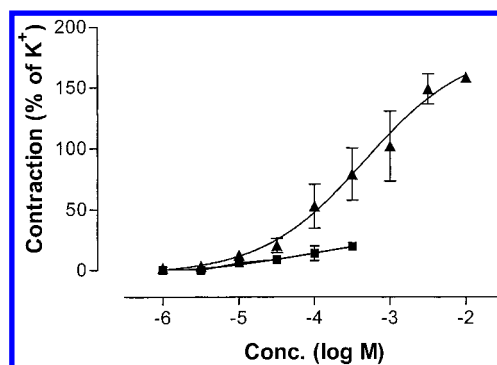
Our previous work with bisphosphate adenosine derivatives as P2Y<sub>1</sub> receptor antagonists revealed that high binding affinity at the P2Y<sub>1</sub> receptor was retained in nonnucleotide bisphosphate analogues.<sup>20</sup> Indeed, replacement of the ribose moiety with a constrained carbocyclic ring, e.g., **2**, enhanced P2Y<sub>1</sub> receptor affinity. Here, we have synthesized ring-constrained methanocarba analogues of ATP and UTP and illustrated that these derivatives exhibit as high or even higher potency for activation of certain P2Y receptors than the corresponding natural nucleotide. As was observed with



**Figure 3.** Effects of the (N)-methanocarba modification in uracil nucleotides (diphosphates) on activation of PLC in 1321N1 astrocytoma cells expressing hP2Y<sub>6</sub> receptors, showing concentration-dependent stimulation of inositol phosphate formation by compound **6b** (●) but not by compound **6a** (○). Membranes from [<sup>3</sup>H]inositol-labeled membranes were incubated for 5 min at 30 °C in the presence of the indicated concentrations of nucleotide. The data shown are typical curves for at least three experiments carried out in duplicate using different membrane preparations.

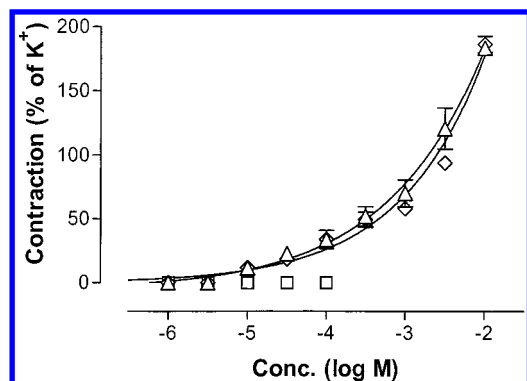


**Figure 4.** Vasodilatory responses to UTP (▲) and **5a** (■) in the rat mesenteric artery. The uracil nucleotides were added after P2X receptor desensitization with 10  $\mu$ M  $\alpha, \beta$ -meATP. Relaxation is expressed as a percentage of an initial contraction induced by 1  $\mu$ M norepinephrine. Data are shown as means  $\pm$  sem of 5–7 vessel segments.



**Figure 5.** Vasocontractile responses to uracil nucleotides UTP (▲) and **5a** (■) in the rat mesenteric artery. The uracil nucleotides were added after P2X receptor desensitization with 10  $\mu$ M  $\alpha, \beta$ -meATP. L-NAME (0.1 mM) was present to inhibit the release of NO. Data are shown as means  $\pm$  sem of 2–4 vessel segments, except for the highest concentration of **5a**, where  $n = 1$ .

bisphosphate antagonists of the P2Y<sub>1</sub> receptor, the presence of a fused cyclopropane moiety constraining a cyclopentane in a pseudorotational (N) conformation produced an agonist at adenine and uridine nucleotide-



**Figure 6.** Vasocontractile responses to UDP ( $\Delta$ ), **6a** ( $\square$ ), and UDP in the presence of  $10\ \mu\text{M}$  **6a** ( $\diamond$ ) in the rat mesenteric artery. The uracil nucleotides were added after P2X receptor desensitization with  $10\ \mu\text{M}$   $\alpha,\beta$ -meATP. L-NAME ( $0.1\ \text{mM}$ ) was present to inhibit the release of NO. Data are shown as means  $\pm$  sem of 2–4 vessel segments.

activated P2Y receptors that was much more potent than the corresponding molecule constrained in the (S) conformation.

The methanocarba modification, originally applied by Marquez and co-workers to antiviral nucleosides, fixes the ring pucker conformation in carbocyclic analogues by virtue of a bridging cyclopropane ring.<sup>26,27,31</sup> The (N) conformation of the pseudorotational cycle was clearly favored in binding of the methanocarba-ATP analogue in the turkey and human P2Y<sub>1</sub> receptors and the hP2Y<sub>2</sub> receptor. The difference in fit in the binding pocket observed with the (N) and (S) conformers of ATP was  $\sim 300$ -fold at the P2Y<sub>1</sub> receptor (turkey) and 41-fold at the hP2Y<sub>2</sub> receptor. The potency enhancement in (N)-methanocarba-ATP vs its ribose equivalent was 350-fold at the P2Y<sub>1</sub> receptor (turkey) and was insignificant at the hP2Y<sub>2</sub> receptor. In another study (in preparation), we have extended this finding of preference for the (N) conformers at the P2Y<sub>1</sub> receptor to molecules that also possess other functional groups, e.g., 2-MeS or N<sup>6</sup>-CH<sub>3</sub> substitution, known to increase binding affinity and selectivity for P2Y receptor agonists.

The observation that the (N)-methanocarba analogue of UDP was inactive at the UDP-activated hP2Y<sub>6</sub> receptor was very surprising given the activity of (N)-methanocarba analogues at the P2Y<sub>1</sub> and P2Y<sub>2</sub> receptors and the fact that (N)-methanocarba-UTP was essentially equipotent with UTP at the hP2Y<sub>4</sub> receptor. Therefore, the binding pocket of the P2Y<sub>6</sub> receptor must markedly differ from that of at least three of its family members. As such, it will be important to extend analyses of activities of potential agonists in the (N) vs (S) conformations to studies of all members of the human P2Y receptor family.

The relative activities observed with the methanocarba analogues of UTP and UDP apparently are retained at rat uracil nucleotide-activated P2Y receptors. (N)-Methanocarba-UTP, **5a**, was more potent than UTP as a vasodilator in the rat mesenteric artery containing intact endothelium, which may be due to greater resistance of (N)-methanocarba-UTP, **5a**, to ectonucleotidase activity or to higher P2Y<sub>2,4</sub> receptor potency. However, (N)-methanocarba-UTP, **5a**, was less potent than UTP for inducing vascular contraction in endothelium-denuded rat mesenteric artery, and (N)-

methanocarba-UDP, **6a**, did not exhibit any contractile effect involving both P2Y<sub>2,4</sub> and P2Y<sub>6</sub> receptor-mediated components.<sup>18</sup> A likely explanation for the inactivity of (N)-methanocarba-UDP, **6a**, is that it does not possess any agonist activity at the rat P2Y<sub>6</sub> receptor.

In previous experiments,<sup>18</sup> we found that the stable UDP analogue,  $\beta$ -thio-UDP, was more potent in the contractile response than a stable UTP analogue, although the reverse was true for the natural pyrimidines (UTP was more potent than UDP). Our interpretation was that the P2Y<sub>6</sub> receptor is a more important vasoconstrictor than P2Y<sub>2,4</sub> and that UTP mediates its contraction mainly after degradation to UDP.

In the present study, (N)-methanocarba-UTP, **5a**, may be degraded to (N)-methanocarba-UDP, **6a**, although it may be more stable than UTP due to the methanocarba substitution. The lack of contractile effect of (N)-methanocarba-UDP, **6a**, can explain the low potency for (N)-methanocarba-UTP, **5a**, as a vasoconstrictor, as compared to its activity as a vasodilator through P2Y<sub>2,4</sub> receptors. Differences in the expression of P2Y<sub>2</sub> and P2Y<sub>4</sub> receptors at endothelium vs smooth muscle could also contribute to this phenomenon.

Thus, it appears that (N)-methanocarba-UDP, **6a**, was neither an agonist nor an antagonist at the rat P2Y<sub>6</sub> receptor, and the weak contractile effect of (N)-methanocarba-UTP, **5a**, was from action at P2Y<sub>2,4</sub> receptors alone. While UDP and its more stable analogue  $\beta$ -thio-UDP can be used to define action at P2Y<sub>6</sub> receptors,<sup>18</sup> it will be useful to have a molecule such as (N)-methanocarba-UTP, **5a**, that exhibits the opposite selectivity, i.e., active at P2Y<sub>2,4</sub> receptors but inactive, itself or through a metabolite, at P2Y<sub>6</sub> receptors.

In conclusion, we have established that constraining the riboselike ring as methanocarba analogues is an important principle for producing P2Y receptor subtype selectivity, similar to our previous demonstration of this phenomenon for P2Y<sub>1</sub> receptor antagonists. These findings promise to be useful in both defining the microscopic determinants of the binding sites on these receptors and in the design of novel pharmacological probes and/or therapeutic agents. We observed that the (N) ring conformation was associated with increased agonist potency at P2Y<sub>1</sub> receptors and preserved agonist potency at P2Y<sub>2</sub>, P2Y<sub>4</sub>, and P2Y<sub>11</sub> but not P2Y<sub>6</sub> receptors. This suggests that new, nucleotide agonists may be designed on the basis of the (N) conformation that favors P2Y receptor subtype selectivity.

## Experimental Section

**Chemical Synthesis.** Nucleosides and synthetic reagents were purchased from Sigma Chemical Co. (St. Louis, MO) and Aldrich (Milwaukee, WI). 2,6-Dichloropurine was obtained from Sigma. Compound **12** was synthesized in our laboratory as described.<sup>28,32</sup>

<sup>1</sup>H NMR spectra were obtained with a Varian Gemini-300 spectrometer (300 MHz) using D<sub>2</sub>O and CDCl<sub>3</sub>, CD<sub>3</sub>OD as a solvent. <sup>31</sup>P NMR spectra were recorded at room temperature by use of a Varian XL-300 spectrometer (121.42 MHz); orthophosphoric acid (85%) was used as an external standard.

Purity of all compounds submitted to biological testing was checked using a Hewlett-Packard 1090 HPLC apparatus equipped with a C18 reverse phase analytical column (250  $\times$  4.6 mm, as specified in Table 2) in two solvent systems. Peaks were detected by UV absorption using a diode array detector. All derivatives showed more than 95% purity in the HPLC systems.



Low-resolution CI-NH<sub>3</sub> (chemical ionization) mass spectra were carried out with 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 a JEOL SX102 spectrometer using 6 kV Xe atoms following desorption from a glycerol matrix. Purification of the nucleotide analogues for biological testing was carried out on (diethylamino)ethyl (DEAE)-A25 Sephadex columns as described above.

**General Phosphorylation Procedure. Synthesis of (N)-Methanocarba-adenosine Derivative (1'S,2'R,3'S,4'R,5'S)-4-(6-Amino-9H-purin-9-yl)-1-[(phenylmethoxy)methyl]bicyclo[3.1.0]hexane-2,3-(O-isopropylidene) (13).** To a solution of **12** (0.2 g, 0.47 mmol) in *i*-PrOH (3 mL) was added NH<sub>3</sub> (2 M solution in *i*-PrOH, 5 mL, 10 mmol), and the reaction mixture was heated at 90 °C in a closed tube for 15 h for complete reaction. The resulting mixture was concentrated under reduced pressure, and the residue obtained was purified by flash chromatography using 9/1 CHCl<sub>3</sub>/MeOH to furnish 0.182 g of **13** (95%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.35 (s, 1H), 8.30 (s, 1H), 7.37 (s, 5H), 5.87 (bs, 2H), 5.31 (d, 1H, *J* = 6.8 Hz), 5.13 (s, 1H), 4.64 (qAB, 2H, *J* = 11.7, 20.5 Hz), 4.51 (d, 1H, *J* = 6.8 Hz), 3.97 (d, 1H, *J* = 10.7 Hz), 3.35 (d, 1H, *J* = 10.7 Hz), 1.72–1.62 (m, 1H), 1.55 (s, 3H), 1.32–1.26 (m, 1H), 1.23 (s, 3H), 1.00–0.93 (m, 1H).

**(1'S,2'R,3'S,4'R,5'S)-4-(6-Amino-9H-purin-9-yl)-1-[hydroxymethyl]bicyclo[3.1.0]hexane-2,3-(O-isopropylidene) (14).** To a solution of **13** (0.182 g, 0.45 mmol) in 5% formic acid in MeOH (15 mL) at room temperature was added 0.15 g of palladium black and stirred at the same temperature for 24 h. The resulting mixture was filtered through a plug of Celite and concentrated under reduced pressure. The crude product obtained was purified by flash chromatography using 9/1 CHCl<sub>3</sub>/MeOH to furnish 0.12 g of **14** (76%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.35 (s, 1H), 7.83 (s, 1H), 5.94 (bs, 2H), 5.61 (d, 1H, *J* = 7.2 Hz), 4.79 (s, 1H), 4.66 (d, 1H, *J* = 7.2 Hz), 4.31 (d, 1H, *J* = 11.5 Hz), 3.26 (d, 1H, *J* = 11.5 Hz), 1.9–1.7 (m, 1H), 1.55 (s, 3H), 1.25 (s, 3H), 1.22–1.10 (m, 1H), 1.10–0.94 (m, 1H).

**Synthesis of Protected (N)-Methanocarba-AMP Derivative. (1'S,2'R,3'S,4'R,5'S)-4-(6-Amino-9H-purin-9-yl)-1-[(di-*tert*-butylphosphato)methyl]bicyclo[3.1.0]hexane-2,3-(O-isopropylidene) (15).** To a stirred solution of di-*tert*-butyl diethylphosphoramidite and **14** (0.040 g, 0.138 mmol) in 4 mL of anhydrous THF, tetrazole (0.029 g, 0.42 mmol) was added, and the mixture was stirred for 6 h at room temperature. The reaction mixture was cooled to –78 °C, and a solution of MCPBA (70% max, 0.04 g) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) was added. The resulting mixture was warmed to room temperature, and the solvent was removed under reduced pressure. The residue obtained was purified by preparative thin-layer chromatography (9/1 CHCl<sub>3</sub>/MeOH), which furnished 0.055 g (78%) of the di-*tert*-phosphate ester **15**. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.37 (s, 1H), 8.19 (s, 1H), 6.46 (bs, 2H), 5.31 (d, 1H, *J* = 6.6 Hz), 5.12 (s, 1H), 4.6–4.42 (m, 2H), 3.92–3.76 (m, 1H), 1.84–1.72 (m, 1H), 1.55 (s, 3H), 1.51 (s, 9H), 1.50 (s, 9H), 1.34–1.26 (m, 1H), 1.23 (s, 3H), 1.12–1.00 (m, 1H).

**(1'S,2'R,3'S,4'R,5'S)-4-(6-Amino-9H-purin-9-yl)-1-[phosphoryloxymethyl]bicyclo[3.1.0]hexane-2,3-diol (4a).** Compound **15** (0.05 g, 0.097 mmol) was heated at 80 °C with DOWEX-50-80-200 resin (0.200 g) in 1/1 MeOH/H<sub>2</sub>O (10 mL) for 1.5 h. The resin was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was purified by a Sephadex column using 200 mL of H<sub>2</sub>O and 200 mL of 0.5 M NH<sub>4</sub>HCO<sub>3</sub> solution, which furnished 0.05 g of **4a** as triethylammonium salt (69%). <sup>1</sup>H NMR (D<sub>2</sub>O): δ 8.54 (s, 1H), 8.17 (s, 1H), 4.31 (dd, 1H, *J* = 4.8, 11.3 Hz), 4.01 (d, 1H, *J* = 6.5 Hz), 3.62 (dd, 1H, *J* = 4.3, 11.3 Hz), 1.86–1.80 (m, 1H), 1.52–1.44 (m, 1H), 1.00–0.92 (m, 1H). <sup>31</sup>P NMR (D<sub>2</sub>O): δ 0.98.

**(±)-5-(6-Amino-9H-purin-9-yl)-1-[phosphoryloxymethyl]bicyclo[3.1.0]hexane-2,3-diol (9a).** To a solution of compound **21** (0.015 g, 0.05 mmol) and Proton Sponge (0.053 g,

0.25 mmol) in trimethyl phosphate was added POCl<sub>3</sub> at 0 °C, and the mixture was stirred for 40 min. Triethylammonium bicarbonate buffer (1.0 M, pH 8.5, 2 mL) was added to the reaction mixture followed by the addition of 2 mL of water. The reaction mixture was lyophilized, and the residue obtained was purified on a Sephadex column eluting with 0.5 M ammonium bicarbonate:water (100 mL:100 mL) to obtain 0.015 g (60%) of the monophosphate, **9a**. <sup>1</sup>H NMR (D<sub>2</sub>O): δ 8.33 (s, 1H), 8.23 (s, 1H), 4.16–4.08 (m, 3H), 3.57 (d, *J* = 10.44 Hz, 1H), 2.40–2.42 (m, 1H), 1.98–1.94 (m, 1H), 1.88–1.82 (m, 1H), 1.46–1.40 (m, 1H). <sup>31</sup>P NMR (D<sub>2</sub>O): δ 0.80.

**Synthesis of Methanocarba ATP Analogues. (1'S,2'R,3'S,4'R,5'S)-4-(6-Amino-9H-purin-9-yl)-1-[triphosphoryloxymethyl]bicyclo[3.1.0]hexane-2,3-diol (3a).** A mixture of **4a** (0.05 g, 0.089 mmol) and 1,1'-carbonyldiimidazole (0.086 g, 0.534 mmol) in 2 mL of anhydrous DMF was stirred at room temperature for 8 h. The reaction mixture was treated with 5% Et<sub>3</sub>N in MeOH and stirred for 2 h. Solvent was removed under reduced pressure, and the residue was dried in high vacuum. To this residue was added tributylammonium pyrophosphate (0.4 g, 0.89 mmol) suspended in 2 mL of anhydrous DMF. To this mixture was added 0.1 mL of tributylamine and stirred at room temperature for 3 days. Triethylammonium bicarbonate (3 mL) was added, and the solvent was removed. The residue obtained was purified by a Sephadex column using 200 mL of H<sub>2</sub>O and 200 mL of 0.5 M NH<sub>4</sub>HCO<sub>3</sub> solution, which furnished 0.010 g of triphosphate **3a** (20%). <sup>1</sup>H NMR (D<sub>2</sub>O): δ 8.50 (s, 1H), 4.73 (s, 1H), 4.58 (dd, 1H, *J* = 4.7, 11.0 Hz), 4.02 (d, 1H, *J* = 6.6 Hz), 3.87 (dd, 1H, *J* = 4.7, 11.0 Hz), 1.96–1.91 (m, 1H), 1.58–1.52 (m, 1H), 1.06–0.98 (m, 1H). <sup>31</sup>P NMR (D<sub>2</sub>O): δ d (–10.27, –10.43), d (–10.67, –10.84), bs –22.61.

**(±)-5-(6-Amino-9H-purin-9-yl)-1-[triphosphoryloxymethyl]bicyclo[3.1.0]hexane-2,3-diol (8a).** Synthesis of **8a** from **9a** (0.007 g, 0.02 mmol), 1,1'-carbonyldiimidazole (0.016 g, 0.1 mmol) and tributylammonium pyrophosphate (0.055 g, 0.12 mmol), was carried out by the same general procedure as for **3a** to furnish 0.003 mg (30%). <sup>1</sup>H NMR (D<sub>2</sub>O): δ 8.33 (s, 1H), 8.23 (s, 1H), 4.42–4.02 (m, 3H), 3.20 (d, *J* = 7.41 Hz, 1H), 2.50–2.64 (m, 1H), 2.02–1.75 (m, 1H), 1.58–1.35 (m, 1H), 1.30–1.22 (m, 1H). <sup>31</sup>P NMR (D<sub>2</sub>O): δ –5.70, –10.47, –21.16.

**(1'S,2'R,3'S,4'R,5'S)-4-(2,4(H,3H)-Dioxypyrimidin-1-yl)-1-[(phenylmethoxy)methyl]bicyclo[3.1.0]hexane-2,3-diol (17).** A solution of β-ethoxyacryloyl cyanate (0.7 mmol) in anhydrous benzene (2 mL) was added rapidly to a stirred solution of amine **16** (105 mg, 0.363 mmol) in anhydrous benzene (1 mL) at room temperature. The reaction mixture was stirred at the same temperature for 10 min, diluted with ethyl acetate (30 mL), and treated with saturated aqueous sodium bicarbonate (10 mL). After the solution was stirred for 20 min at room temperature, the organic layer was separated, washed with brine (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was purified by flash chromatography using 1/2 hexane/ethyl acetate to furnish 126 mg of an intermediate (89%) as a syrup. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.97 (s, 1H), 8.89 (d, 1H, *J* = 7.2 Hz), 7.64 (d, 1H, *J* = 12.2 Hz), 7.20–7.41 (m, 5H), 5.30 (d, 1H, *J* = 12.2 Hz), 5.05 (dd, 1H, *J* = 1.0, 7.2 Hz), 4.58 (s, 2H), 4.55 (dd, 1H, *J* = 1.0, 7.3 Hz), 4.19 (d, 1H, *J* = 7.3 Hz), 3.97 (q, 2H, *J* = 7.1 Hz), 3.63 (d, 1H, *J* = 10.4 Hz), 3.40 (d, 1H, *J* = 10.4 Hz), 1.49 (s, 3H), 1.45 (dddd, 1H, *J* = 1.2, 5.5, 8.0 Hz), 1.35 (t, 3H, *J* = 7.1 Hz), 1.24 (s, 3H), 1.05 (t, 1H, *J* = 5.1 Hz), 0.78 (ddd, 1H, *J* = 1.2, 5.5, 9.2 Hz).

The acryloylureidoheptane (130 mg, 0.302 mmol) was refluxed in 2 N H<sub>2</sub>SO<sub>4</sub> (8 mL). After it was refluxed for 30 min, methanol (5 mL) was added to completely dissolve the substrate and form a homogeneous solution. After the solution was stirred for 10 min at reflux temperature, the methanol was removed by purging with an N<sub>2</sub> stream with heating at 120 °C. The residue (a clear aqueous solution) was refluxed for an additional 30 min. The resulting mixture was cooled to room temperature and neutralized with solid sodium bicarbonate until no further gas evolution was detected. The mixture was lyophilized, and the residue was extracted with a mixed solvent (chloroform/methanol = 1:1, 20 mL × 3) and filtered



through a plug of silica gel to remove all inorganic salts. The filtrate was concentrated in vacuo and purified by flash chromatography using 6/1 CHCl<sub>3</sub>/MeOH to furnish 70 mg of **17** (67.3%) as a syrup. <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 8.18 (d, 1H, *J* = 8.1 Hz), 7.28–7.45 (m, 5H), 5.22 (d, 1H, *J* = 8.1 Hz), 4.77 (s, 1H), 4.60 (d, 1H, *J* = 11.1 Hz), 4.59 (dd, 1H, *J* = 1.5, 6.9 Hz), 4.53 (d, 1H, *J* = 11.1 Hz), 4.22 (d, 1H, *J* = 10.2 Hz), 3.73 (dd, 1H, *J* = 1.4, 6.9 Hz), 3.21 (d, 1H, *J* = 10.0 Hz), 1.49 (dd, 1H, *J* = 4.0, 5.2 Hz), 1.37 (ddd, 1H, *J* = 1.6, 4.0, 8.8 Hz), 0.73 (ddd, 1H, *J* = 1.8, 5.5, 8.8 Hz). MS (*m/e*): 344 (M<sup>+</sup>). CIMS (ammonia) *m/e*: 345 (M<sup>+</sup> + 1).

**(1'*S*,2'*R*,3'*S*,4'*R*,5'*S*)-4-(2,4(*H*,3*H*)-Dioxopyrimidin-1-yl)-1-(hydroxymethyl)bicyclo[3.1.0]hexane-2,3-diol (18)**. Solid palladium black (60 mg) was added to a stirred solution of (1'*S*,2'*R*,3'*S*,4'*R*,5'*S*)-4-(2,4(*H*,3*H*)-dioxopyrimidin-1-yl)-1-[(phenylmethoxy)methyl]bicyclo[3.1.0]hexane-2,3-diol (**17**, 23 mg, 0.0668 mmol) in methanol (4 mL), and 95% formic acid (1.3 mL) was added. The mixture was stirred at 50 °C for 4 h, additional formic acid (1.2 mL) was added, and stirring was continued at 60 °C for 3 h. The resulting mixture was cooled to room temperature, filtered through a plug of Celite, and washed with methanol. The filtrate was evaporated, and the product was purified by silica gel column chromatography (chloroform/methanol = 2:1) to give **18** (12.6 mg, 74%) as a syrup. <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 8.04 (d, 1H, *J* = 8.0 Hz), 5.66 (d, 1H, *J* = 8.0 Hz), 4.72 (s, 1H), 4.58 (dd, 1H, *J* = 1.4, 6.9 Hz), 4.20 (d, 1H, *J* = 11.8 Hz), 3.81 (d, 1H, *J* = 6.9 Hz), 3.24 (d, 1H, *J* = 11.8 Hz), 1.30–1.41 (m, 2H), 0.68 (ddd, 1H, *J* = 1.7, 4.4, 8.0 Hz). CIMS (ammonia) *m/e*: 255 (M<sup>+</sup> + 1), 272 (M<sup>+</sup> + NH<sub>4</sub>).

**(1'*S*,2'*R*,3'*S*,4'*R*,5'*S*)-4-(2,4(*H*,3*H*)-Dioxopyrimidin-1-yl)-1-(hydroxymethyl)bicyclo[3.1.0]hexane-2,3-(*O*-isopropylidene) (19)**. A mixture of triol **18** (20 mg, 0.0787 mmol) and *p*-toluene sulfonic acid monohydrate (50 mg, 0.263 mmol) and acetone (7 mL) was stirred at ambient temperature for 4 h. Triethylamine (0.5 mL) was added to protect the acetonide group. All volatile materials were removed in vacuo, and the residue was purified by preparative thin-layer chromatography using 4/1 chloroform/methanol to give 20 mg of **19** as an oil (86%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.39 (d, 1H, *J* = 8.0 Hz), 5.74 (d, 1H, *J* = 8.0 Hz), 5.32 (dd, 1H, *J* = 1.0, 7.3 Hz), 4.75 (d, 1H, *J* = 7.3 Hz), 4.31 (s, 1H), 4.18 (d, 1H, *J* = 11.4 Hz), 3.31 (d, 1H, *J* = 11.4 Hz), 1.53 (m, 1H), 1.51 (s, 3H), 1.27 (s, 3H), 1.04 (dd, 1H, *J* = 4.7, 5.8 Hz), 0.91 (ddd, 1H, *J* = 1.2, 5.8, 9.2 Hz). CIMS (ammonia) *m/e*: 295 (M<sup>+</sup> + 1), 312 (M<sup>+</sup> + NH<sub>4</sub>).

**(1'*S*,2'*R*,3'*S*,4'*R*,5'*S*)-4-(2,4(*H*,3*H*)-Dioxopyrimidin-1-yl)-1-[(di-*tert*-butylphosphato)methyl]bicyclo[3.1.0]hexane-2,3-(*O*-isopropylidene) (20)**. Neat di-*tert*-butyl *N,N*-diethylphosphoramidite (57 μL, 0.205 mmol) was added by syringe to a stirred solution of **19** (20 mg, 0.0680 mmol) in anhydrous THF (1 mL) at room temperature, followed by an addition of solid tetrazole (43 mg, 0.614 mmol). After the solution was stirred at room temperature for 20 min, the reaction mixture was cooled to –78 °C, and a solution of MCPBA (57 ~ 85%, 58 mg) in methylene chloride (2 mL) was added rapidly. The reaction mixture was warmed to 0 °C and stirred for 5 min. Triethylamine (0.5 mL, 3.59 mmol) was added to maintain a basic condition in order to avoid cleavage of *tert*-butyl groups. Purification was accomplished using preparative thin-layer chromatography 6/1 chloroform/methanol to give 26 mg of **20** (79%) as an oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.04 (d, 1H, *J* = 8.0 Hz), 5.77 (d, 1H, *J* = 8.0 Hz), 5.15 (d, 1H, *J* = 7.3 Hz), 4.95 (s, 1H), 4.46–4.58 (m, 2H), 3.66 (dd, 1H, *J* = 6.7, 11.4 Hz), 1.54 (m, 1H), 1.51 (s, 3H), 1.49 (s, 9H), 1.25 (s, 3H), 0.95 (m, 1H). CIMS (ammonia) *m/e*: 487 (M<sup>+</sup> + 1).

**(1'*S*,2'*R*,3'*S*,4'*R*,5'*S*)-4-(2,4(*H*,3*H*)-Dioxopyrimidin-1-yl)-1-(phosphoryloxymethyl)bicyclo[3.1.0]hexane-2,3-diol, Triethylammonium Salt (7a)**. A mixture of **20** (13 mg, 0.0267 mmol) and DOWEX 50 × 8-200 ion-exchange resin (300 mg) and methanol (4 mL)/water (2 mL) was stirred at 70–80 °C for 12 h. The resulting mixture was filtered and washed with water (~5 mL). The filtrate was lyophilized with 0.1 M triethylammonium bicarbonate to give 10 mg of a triethylammonium salt, **7a** (86%), as a white solid. As a triethylammo-

nium salt: <sup>1</sup>H NMR (D<sub>2</sub>O): δ 8.04 (d, 1H, *J* = 6.0 Hz), 5.93 (d, 1H, *J* = 6.0 Hz), 4.75 (s, 1H), 4.68 (m, 1H), 4.38 (m, 1H), 3.98 (d, 1H, *J* = 4.7 Hz), 3.62 (m, 1H), 3.20 (q, 2H, *J* = 7.3 Hz), 1.58 (m, 1H), 1.37 (m, 1H), 1.28 (t, 3H, *J* = 7.3 Hz), 0.92 (m, 1H). CIMS (ammonia) *m/e*: 487 (M<sup>+</sup> + 1), 272 (M<sup>+</sup> + NH<sub>4</sub>). As a free acid: <sup>1</sup>H NMR (D<sub>2</sub>O): δ 8.04 (d, 1H, *J* = 8.0 Hz), 5.92 (d, 1H, *J* = 8.0 Hz), 4.75 (s, 1H), 4.66 (d, 1H, *J* = 6.8 Hz), 4.42 (m, 1H), 3.98 (d, 1H, *J* = 6.5 Hz), 3.62 (m, 1H), 1.60 (m, 1H), 1.38 (m, 1H), 0.92 (m, 1H). High-resolution MS (negative-ion FAB) calcd for C<sub>11</sub>H<sub>14</sub>N<sub>2</sub>O<sub>8</sub>P [M<sup>2-</sup> + H<sup>+</sup>]<sup>-</sup>, 333.0488; found, 333.0506. HPLC: 3.235 min (99%) in solvent system A, 3.466 min (98%) in system C.

**(1'*S*,2'*R*,3'*S*,4'*R*,5'*S*)-4-(2,4(*H*,3*H*)-Dioxopyrimidin-1-yl)-1-(diphosphoryloxymethyl)bicyclo[3.1.0]hexane-2,3-diol, Ammonium Salt (6a)**. Solid 1,1'-carbonyldiimidazole (6.9 mg, 42.5 μmol) was added to a stirred solution of **7a** (3.0 mg, 6.89 μmol) in anhydrous DMF (0.5 mL) at room temperature. After the solution was stirred at room temperature for 6 h, the reaction mixture was treated with 5% triethylamine solution (2.5 mL, in 1/1 water/methanol) and stirred for 2 h. All volatile materials were removed by purging with nitrogen stream, and the residue dried in vacuo at room temperature and dissolved in anhydrous DMF (0.5 mL). To this solution was successively added tributylamine (30 μL, 126 μmol) and a solution of bis(tributylammonium) salt of phosphoric acid (0.1 mL, in DMF). This salt was prepared by mixing tributylamine (128 mg, 0.69 mmol) and phosphoric acid (33.8 mg, 0.345 mmol) in DMF (1 mL). After this solution was stirred at room temperature for 2 days, 0.2 M triethylammonium bicarbonate (0.2 mL) was added, and the resulting mixture was lyophilized. The residue was purified by ion-exchange column chromatography using a Sephadex-DEAE A-25 resin with a linear gradient (0.01–1.0 M) of 1.0 M ammonium bicarbonate as the mobile phase to give 1.3 mg of **6a** (40.5%) as a white solid. <sup>1</sup>H NMR (D<sub>2</sub>O): δ 8.03 (d, 1H, *J* = 8.0 Hz), 5.96 (d, 1H, *J* = 8.0 Hz), 4.52 (m, 1H), 4.69–4.91 (m, 2H), 3.99 (d, 1H, *J* = 6.8 Hz), 3.77 (m, 1H), 1.64 (m, 1H), 1.39 (m, 1H), 0.95 (t, 1H, *J* = 7.2 Hz). <sup>31</sup>P NMR (D<sub>2</sub>O): δ –10.81, 9.98. MS (negative-ion FAB): 414 [M<sup>2-</sup> + H<sup>+</sup>]<sup>-</sup>. High-resolution MS (negative-ion FAB) calcd for C<sub>11</sub>H<sub>15</sub>N<sub>2</sub>O<sub>11</sub>P<sub>2</sub> [M<sup>2-</sup> + H<sup>+</sup>]<sup>-</sup>, 413.0151; found, 413.0143. HPLC: 2.130 min (98%) in solvent system A, 4.531 (97%) min in system C.

**(1'*S*,2'*R*,3'*S*,4'*R*,5'*S*)-4-(2,4(*H*,3*H*)-Dioxopyrimidin-1-yl)-1-(triphenylphosphoryloxymethyl)bicyclo[3.1.0]hexane-2,3-diol, Triethylamine Salt (5a)**. Solid carbonyldiimidazole (26 mg, 0.16 mmol) was added to a stirred solution of **7a** and free acid (9.0 mg, 26.9 μmol) in anhydrous DMF (0.5 mL) at room temperature. After the solution was stirred at room temperature for 6 h, the reaction mixture was treated with 5% triethylamine solution (2.5 mL, in water/methanol = 1:1) and stirred for 2 h. All volatile materials were removed by purging with nitrogen, and the residue was dried in vacuo at room temperature. The product was purified by ion-exchange column chromatography using a Sephadex-DEAE A-25 resin with a linear gradient (0.01–0.5 M) of 0.5 M triethylammonium bicarbonate as the mobile phase, to give 15 mg of the phosphorimidazolidate monotriethylamine salt, as a white solid. <sup>1</sup>H NMR (D<sub>2</sub>O): δ 7.96 (bs, 1H), 7.81 (d, 1H, *J* = 7.9 Hz), 7.33 (bs, 1H), 7.15 (bs, 1H), 5.85 (d, 1H, *J* = 7.9 Hz), 4.70 (s, 1H), 4.51 (d, 1H, *J* = 6.3 Hz), 4.38 (dd, 1H, *J* = 5.7, 11.3 Hz), 3.94 (d, 1H, *J* = 6.7 Hz), 3.61 (dd, 1H, *J* = 5.3, 11.3 Hz), 3.02–3.38 (m, 6H), 1.47 (m, 1H), 1.13–1.44 (m, 10H), 0.85 (m, 1H).

The phosphorimidazolidate was dissolved in anhydrous DMF (0.5 mL). To this solution was added solid tributylammonium pyrophosphate (from Sigma, 36 mg, 0.0790 mmol) and tributylamine (30 μg, 161 μmol), successively. After the solution was stirred at room temperature for 3 days, 0.2 M triethylammonium bicarbonate (0.2 mL) was added, and the resulting mixture was lyophilized. The residue was purified by ion-exchange column chromatography using a Sephadex-DEAE A-25 resin with a linear gradient (0.01–1.0 M) of 1.0 M ammonium bicarbonate as the mobile phase to give 5.7 mg of **5a** (37.7%) as a white solid. <sup>1</sup>H NMR (D<sub>2</sub>O): δ 8.01 (d, 1H, *J*

= 8.0 Hz), 5.97 (d, 1H,  $J$  = 8.0 Hz), 4.75 (s, 1H), 4.72 (d, 1H,  $J$  = 6.8 Hz), 4.54 (m, 1H), 3.99 (d, 1H,  $J$  = 6.7 Hz), 3.80 (m, 1H), 1.20–1.69 (m, 2H), 0.95 (m, 1H).  $^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ ):  $\delta$  –22.482, d (–10.914, –10.759), –8.979. MS (negative-ion FAB): 515 ( $\text{MHNa}^-$ ), 537 ( $\text{MHN}_2^-$ ). High-resolution MS (negative-ion FAB) calcd for  $\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_{14}\text{P}_3$  [ $\text{M}^{2-} + \text{H}^+$ ] $^-$ , 492.9814; found, 492.9832. HPLC: 1.737 min (99%) in solvent system A, 6.764 min (96%) in system B.

## Pharmacological Analyses

**PLC Activity.** Agonist-promoted stimulation of inositol phosphate formation was measured at human  $\text{P2Y}_1$ ,  $\text{P2Y}_2$ ,  $\text{P2Y}_4$ ,  $\text{P2Y}_6$ , and  $\text{P2Y}_{11}$  receptors stably expressed in 1321N1 astrocytoma cells as previously described.<sup>32,33</sup> Briefly, cells plated in 24 well dishes were labeled with 1  $\mu\text{Ci}$  of [ $^3\text{H}$ ]myo-inositol/well (20 Ci/mmol; American Radiolabeled Chemicals, Inc., St. Louis, MO) in inositol-free Dulbecco's modified Eagle's medium (DMEM). [ $^3\text{H}$ ]Inositol phosphate formation was quantitated the following day during a 10 min incubation at 37 °C in the presence of 10 mM LiCl. The  $\text{EC}_{50}$  values were averaged from 3 to 8 independently determined concentration–effect curves for each compound.

$\text{P2Y}_1$ -receptor-promoted activation of PLC was studied in turkey erythrocyte membranes as we have described.<sup>8,34</sup> Briefly, erythrocytes were 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%  $\text{CO}_2$  at 37 °C. Membranes were prepared and PLC activity was measured in 25  $\mu\text{L}$  of [ $^3\text{H}$ ]inositol-labeled membranes (approximately 175  $\mu\text{g}$  of protein, 200–500 000 cpm/assay) in a medium containing 424  $\mu\text{M}$   $\text{CaCl}_2$ , 0.91 mM  $\text{MgSO}_4$ , 2 mM EGTA, 115 mM KCl, 5 mM  $\text{KH}_2\text{PO}_4$ , and 10 mM HEPES, pH 7.0. Assays (200  $\mu\text{L}$  final volume) contained 1  $\mu\text{M}$   $\text{GTP}\gamma\text{S}$  and the indicated concentrations of nucleotide analogues. Membranes were incubated at 30 °C for 5 min, and total [ $^3\text{H}$ ]inositol phosphates were quantified by anion-exchange chromatography as previously described.<sup>8,34</sup>

Agonist potencies were calculated using a four parameter logistic equation and the GraphPad software package (GraphPad, San Diego, CA).  $\text{EC}_{50}$  values (mean  $\pm$  standard error (SE)) 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 full agonist in the same experiment. All concentration–effect curves were repeated in at least three separate experiments carried out with different membrane preparations using duplicate or triplicate assays.

**Vascular Effects at the Rat Mesenteric Artery.** UTP, UDP,  $\text{N}^G$ -nitro-L-arginine methyl ester hydrochloride (L-NAME),  $\alpha,\beta$ -methylene-ATP ( $\alpha,\beta$ -meATP), and norepinephrine were purchased from Sigma. All substances were dissolved in saline 0.9%.

Vasodilatory responses to cumulatively added UTP and (N)-methanocarba-UTP, **5a**, were examined in segments of the rat mesenteric artery using a myograph by methods previously described.<sup>18</sup> Vessel segments were precontracted with 0.1  $\mu\text{M}$  norepinephrine. The dilatory responses to both UTP and **5a** were studied on the same vessel segment. The dilatory effects of (N)-

methanocarba-UDP, **6a**, were not examined as there are no endothelial  $\text{P2Y}_6$  receptors in the rat mesenteric artery.<sup>18</sup>

Vasocontractile responses of UDP, UTP, (N)-methanocarba-UDP, and (N)-methanocarba-UTP were studied in the presence of 0.1 mM L-NAME to inhibit the effect of endothelial  $\text{P2}$  receptor-stimulated NO release. The antagonistic effect of (N)-methanocarba-UTP on UDP contraction was also assessed.

The negative logarithm of the drug concentration that elicited 50% dilation ( $\text{pEC}_{50}$ ) was determined by fitting the data to the Hill equation, using GraphPad Prism version 3.02.  $R_{\text{max}}$  refers to maximum relaxation calculated as a percentage of the corresponding precontraction with 1  $\mu\text{M}$  norepinephrine. In the contraction experiments, a plateau phase of the maximum contractile response was not reached within the agonist concentrations interval. Experiments were repeated on 2–7 vessel segments for each concentration of the substances. Statistical significance was accepted when  $P < 0.05$ , using Student's t-test for paired data. Values are presented in the graphs as mean  $\pm$  SEM.

**Acknowledgment.** This work was supported by USPHS grants GM38213 and HL54889. R.G.R. thanks Gilead Sciences (Foster City, CA) for financial support.

## 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  $\text{P1}$  and  $\text{P2}$  receptors. *Trends Pharmacol. Sci.* **1997**, *18*, 79–82.
- (2) North, R. A.; Barnard, E. A. Nucleotide receptors. *Curr. Opin. Neurobiol.* **1997**, *7*, 346–357.
- (3) King, B. F.; Townsend-Nicholson, A. Recombinant  $\text{P2Y}$  receptors: the UCL experience. *J. Auton. Nerv. Syst.* **2000**, *81*, 164–170.
- (4) Jacobson, K. A.; Kim, Y.-C.; Camaioni, E.; van Rhee, A. M. Structure activity relationships of  $\text{P2}$  receptor agonists and antagonists. In *The  $\text{P2}$  Nucleotide Receptors*; Turner, J. T., Weisman, G., Fedan, J., Eds.; The Receptors; Humana Press: Clifton, NJ, 1997; Chapter 4, pp 81–107.
- (5) Bhagwat, S. S.; Williams, M.  $\text{P2}$  Purine and Pyrimidine Receptors: Emerging superfamilies of G protein and ligand-gated ion channel receptors. *Eur. J. Med. Chem.* **1997**, *32*, 183–193.
- (6) Fischer, B. Therapeutic applications of ATP-( $\text{P2}$ ) receptors agonists and antagonists. *Expert Opin. Ther. Pat.* **1999**, *9*, 385–399.
- (7) Jacobson, K. A.; Knutsen, L. J. S.  $\text{P1}$  and  $\text{P2}$  purine and pyrimidine receptors. In *Handbook of Experimental Pharmacology, Volume 151/I: Purinergic and Pyrimidinergic Signaling I*; Abbracchio, M. P., Williams, M., Eds.; Springer-Verlag: Berlin, Germany, 2001; pp 129–175.
- (8) 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.
- (9) Communi, D.; Robaye, B.; Boeynaems, J. M. Pharmacological characterization of the human  $\text{P2Y}_{11}$  receptor. *Br. J. Pharmacol.* **1999**, *128*, 1199–1206.
- (10) Boyer, J. L.; Lazarowski, E.; Chen, X.-H.; Harden, T. K. Identification of a  $\text{P2Y}$ -purinergic receptor that inhibits adenylyl cyclase but does not activate phospholipase C. *J. Pharmacol. Exp. Ther.* **1993**, *267*, 1140–1146.
- (11) Hollopeter, G.; Jantzen, H. M.; Vincent, D.; Li, G.; England, L.; Ramakrishnan, V.; Yang, R. B.; Nurden, P.; Nurden, A.; Julius, D.; Conley, P. B. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature* **2001**, *409*, 202–207.
- (12) Janssens, R.; Communi, D.; Piroton, S.; Samson, M.; Parmentier, M.; Boeynaems, J. M. Cloning and tissue distribution of the human  $\text{P2Y}_1$  receptor. *Biochem. Biophys. Res. Commun.* **1996**, *221*, 588–593.
- (13) Crowley, M. R. Oxygen-induced pulmonary vasodilation is mediated by adenosine triphosphate in newborn lambs. *J. Cardiovasc. Pharmacol.* **1997**, *30*, 102–109.



- (14) Loubatières-Mariani, M.-M.; Hillaire-Buys, D.; Chapal, J.; Bertrand, G.; Petit, P. P2 purinoceptor agonists: New insulin secretagogues potentially useful in the treatment of noninsulin-dependent diabetes mellitus. In *Purinergic Approaches in Experimental Therapeutics*; Jacobson, K. A., Jarvis, M. F., Eds.; Wiley: New York, 1997; Chapter 13, pp 253–260.
- (15) Pendergast, W.; Yerxa, B. R.; Douglass, J. G. III; Shaver, S. R.; Dougherty, R. W.; Redick, C. C.; Sims, I. F.; Rideout, J. L. Synthesis and P2Y receptor activity of a series of uridine dinucleoside 5'-polyphosphates. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 157–160.
- (16) Lazarowski, E. R.; Rochelle, L. G.; O'Neal, W. K.; Ribeiro, C. M.; Grubb, B. R.; Zhang, V.; Harden, T. K.; Boucher, R. C. Cloning and functional characterization of two murine uridine nucleotide receptors reveal a potential target for correcting ion transport deficiency in cystic fibrosis gallbladder. *J. Pharmacol. Exp. Ther.* **2001**, *297*, 43–49.
- (17) Fujihara, T.; Murakami, T.; Fujita, H.; Nakamura, M.; Nakata, K. Improvement of corneal barrier function by the P2Y<sub>2</sub> agonist INS365 in a rat dry eye model. *Invest. Ophthalmol. Visual Sci.* **2001**, *42*, 96–100.
- (18) Malmjö, M.; Adner, M.; Harden, T. K.; Pendergast, W.; Edvinsson, L.; Erlinge, D. The stable pyrimidines UDP/βS and UTP/γS discriminate between the P2 receptors that mediate vascular contraction and relaxation of the rat mesenteric artery. *Br. J. Pharmacol.* **2000**, *131*, 51–56.
- (19) Marquez, V. E.; Siddiqui, M. A.; Ezzitouni, A.; Russ, P.; Wang, J.; Wagner, R. W.; Matteucci, M. D. Nucleosides with a twist. Can fixed forms of sugar ring pucker influence biological activity in nucleosides and oligonucleotides? *J. Med. Chem.* **1996**, *39*, 3739–3747.
- (20) Nandanan, E.; Jang, S. Y.; Moro, S.; Kim, H.; Siddiqui, M. A.; Russ, P.; Marquez, V. E.; 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.
- (21) Jacobson, K. A.; Ji, X.-D.; Li, A. H.; Melman, N.; Siddiqui, M. A.; Shin, K. J.; Marquez, V. E.; Ravi, R. G. Methanocarba analogues of purine nucleosides as potent and selective adenosine receptor agonists. *J. Med. Chem.* **2000**, *43*, 2196–2203.
- (22) Ezzitouni, A.; Marquez, V. E. Conformationally locked carbocyclic nucleosides built on a bicyclo[3.1.0]hexane template with a fixed southern conformation. Synthesis and antiviral activity. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1073–1078.
- (23) Fischer, B.; Boyer, J. L.; Hoyle, C. H. V.; Ziganshin, A. U.; Brizzolara, A. L.; Knight, G. E.; Zimmet, J.; Burnstock, G.; Harden, T. K.; Jacobson, K. A. Identification of potent, selective P2Y-purinoceptor agonists—structure–activity–relationships for 2-thioether derivatives of adenosine 5'-triphosphate. *J. Med. Chem.* **1993**, *36*, 3937–3946.
- (24) Hampton, A.; Patel, A. D.; Maeda, M.; Hai, T. T.; Chang, C.-D.; Kang, J. B.; Kappler, F.; Abo, M.; Preston, R. K. Use of adenine nucleotide derivatives to assess the potential of exo-active-site-directed reagents as species- or isozyme-specific enzyme inactivators. 3. Synthesis of adenosine 5'-triphosphate derivatives with N6- or 8-substituents bearing iodoacetyl groups. *J. Med. Chem.* **1982**, *25*, 373–381.
- (25) 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.
- (26) Ezzitouni, A.; Russ, P.; Marquez, V. E. (1S,2R)-[(Benzyloxy)-methyl]cyclopent-3-enol. A versatile synthon for the preparation of 4',1'-a-methano and 1',1'-a-methano carbocyclic nucleosides. *J. Org. Chem.* **1997**, *62*, 4870–4873.
- (27) Siddiqui, M. A.; Ford, H., Jr.; George, C.; Marquez, V. E. Synthesis, conformational analysis, and biological activity of a rigid carbocyclic analogue of 2'-deoxyaristeromycin built on a bicyclo[3.1.0]hexane template. *Nucleosides Nucleotides* **1996**, *15*, 235–250.
- (28) Lee, K.; Cass, C.; Jacobson, K. A. Synthesis using ring closure metathesis and effect on nucleoside transport of a (N)-methanocarba S-(4-nitrobenzyl)thioinosine derivative. *Org. Lett.* **2001**, *3*, 597–599.
- (29) Perich, J. W.; Johns, R. B. Di-tert-butyl N,N-diethylphosphoramidite. A new phosphorylating agent for the efficient phosphorylation of alcohols. *Synthesis* **1988**, *2*, 142–144.
- (30) Csuk, R.; Eversmann, L. Synthesis of difluorocyclopropyl carbocyclic homo-nucleosides. *Tetrahedron* **1998**, *54*, 6445–6456.
- (31) Jeong, L. S.; Buenger, G.; McCormack, J. J.; Cooney, D. A.; Hao, Z.; Marquez, V. E. Carbocyclic analogues of the potent cytidine deaminase inhibitor 1-(β-D-ribofuranosyl)-1,2-dihydropyrimidin-2-one (Zebularine). *J. Med. Chem.* **1998**, *41*, 2572–2578.
- (32) 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.
- (33) Lazarowski, E. R.; Watt, W. C.; Stutts, M. J.; Boucher, R. C.; Harden, T. K. Pharmacological selectivity of the cloned human P2U-purinoceptor: potent activation by diadenosine tetraphosphate. *Br. J. Pharmacol.* **1995**, *116*, 1619–1627.
- (34) 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.
- (35) 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.
- (36) Garrad, R. C.; Otero, M. A.; Erb, L.; Theiss, P. M.; Clarke, L. L.; Gonzalez, F. A.; Turner, J. T.; Weisman, G. A. Structural Basis of Agonist-induced Desensitization and Sequestration of the P2Y<sub>2</sub> Nucleotide Receptor. Consequences of truncation of the C terminus. *J. Biol. Chem.* **1998**, *273*, 29437–29444.
- (37) Kennedy, C.; Qi, A. D.; Herold, C. L.; Harden, T. K.; Nicholas, R. A. ATP, an agonist at the rat P2Y<sub>4</sub> receptor, is an antagonist at the human P2Y<sub>4</sub> receptor. *Mol. Pharmacol.* **2000**, *57*, 926–931.
- (38) Communi, D.; Robaye, B.; Boeynaems, J.-M. Pharmacological characterization of the human P2Y<sub>11</sub> receptor. *Br. J. Pharmacol.* **1999**, *128*, 1199–1206.
- (39) Janssens, R.; Paindavoine, P.; Parmentier, M.; Boeynaems, J.-M. Human P2Y<sub>2</sub> receptor polymorphism: identification and pharmacological characterization of two allelic variants. *Br. J. Pharmacol.* **1999**, *127*, 709–716.
- (40) Lee, K.; Ravi, R. G.; Ji, X.-D.; Marquez, V. E.; Jacobson, K. A. Ring-constrained (N)-methanocarba-nucleosides as adenosine receptor agonists: Independent 5'-uronamide and 2'-deoxy modifications. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1333–1337.

JM010369E