**Title:** COMPOSITION AND METHOD FOR INHIBITING PLATELET AGGREGATION

**Abstract:**
This invention is directed to a method of preventing or treating diseases or conditions associated with platelet aggregation. The method is also directed to a method of treating thrombosis. The method comprises administering to a subject a pharmaceutical composition comprising a therapeutic effective amount of P2Y\(_{12}\) receptor antagonist compound, wherein said amount is effective to bind the P2Y\(_{12}\) receptors on platelets and inhibit ADP-induced platelet aggregation. The P2Y\(_{12}\) receptor antagonist compounds useful for this invention include mononucleoside 5'-monophosphates, mononucleoside polyphosphates, and dinucleoside polyphosphates of general Formula I, or salts thereof. The present invention also provides novel compounds of mononucleoside 5'-monophosphates and dinucleoside diphosphates. The present invention further provides pharmaceutical formulations comprising a pharmaceutical carrier and mononucleoside 5'-monophosphates or dinucleoside diphosphates.
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COMPOSITION AND METHOD FOR INHIBITING PLATELET AGGREGATION

TECHNICAL FIELD

This invention relates to mono- and dinucleoside polyphosphate compounds and the method of using such compounds in the prevention or treatment of diseases or conditions associated with platelet aggregation, including thrombosis in humans and other mammals.

BACKGROUND OF THE INVENTION

Hemostasis is the spontaneous process of stopping bleeding from damaged blood vessels. Precapillary vessels contract immediately when cut; within seconds, thrombocytes, or blood platelets, are bound to the exposed matrix of the injured vessel by a process called platelet adhesion. Platelets also stick to each other in a phenomenon known as platelet aggregation to form a platelet plug to stop bleeding quickly.

An intravascular thrombus results from a pathological disturbance of hemostasis. Platelet adhesion and aggregation are critical events in intravascular thrombosis. Activated under conditions of turbulent blood flow in diseased vessels or by the release of mediators from other circulating cells and damaged endothelial cells lining the vessel, platelets accumulate at a site of vessel injury and recruit further platelets into the developing thrombus. The thrombus can grow to sufficient size to block off arterial blood vessels. Thrombi can also form in areas of stasis or slow blood flow in veins. Venous thrombi can easily detach portions of themselves called emboli that travel through the circulatory system and can result in blockade of other vessels, such as pulmonary arteries. Thus, arterial thrombi cause serious disease by local blockade, whereas venous thrombi do so primarily by distant blockade, or embolization. These conditions include venous thrombosis, thrombophlebitis, arterial embolism, coronary and cerebral arterial thrombosis, unstable angina, myocardial infarction, stroke, cerebral embolism, kidney embolisms and pulmonary embolisms.

A number of converging pathways lead to platelet aggregation. Whatever the initial stimulus, the final common event is crosslinking of platelets by binding fibrinogen to a membrane binding site, glycoprotein IIb/IIIa (GPIIb/IIIa). Compounds that are antagonists for GPIIb/IIIa receptor complex have been shown to inhibit platelet aggregation (U.S. Patent Nos. 6,037,343 and 6,040,317). Antibodies against GPIIb/IIIa have also been shown to have high antiplatelet efficacy (The EPIC investigators, New Engl. J. Med. (1994) 330:956-961). However, this class of antiplatelet agents sometimes causes bleeding problems.
Thrombin can produce platelet aggregation largely independently of other pathways but substantial quantities of thrombin are unlikely to be present without prior activation of platelets by other mechanisms. Thrombin inhibitors such as hirudin are highly effective antithrombotic agents. However, functioning as both antiplatelet and anti-coagulant agents, thrombin inhibitors again can produce excessive bleeding. (The TIMI 9a investigators, The GUSTO Iia investigators, Circulation, 90: 1624-1630 (1994); Circulation, 90: 1631-1637 (1994); Neuhaus K. L. et al., Circulation, 90: 1638-1642 (1994))

Various antiplatelet agents have been studied for many years as potential targets for inhibiting thrombus formation. Some agents such as aspirin and dipyridamole have come into use as prophylactic antithrombotic agents, and others have been the subjects of clinical investigations. To date, the powerful agents such as disintegrins, and the theinopyridines ticlopidine and clopidogrel have been shown to have substantial side effects, while agents such as aspirin have useful but limited effectiveness (Hass, et al., N. Engl. J. Med., 321:501-507 (1989); Weber, et al., Am. J. Cardiol. 66:1461-1468 (1990); Lekstrom and Bell, Medicine 70:161-177 (1991)). In particular, use of the theinopyridines in antiplatelet therapy has been shown to increase the incidence of potentially life threatening thrombotic thrombocytopenic purpura (Bennett, C.L. et al. N. Engl. J. Med., (2000) 342: 1771-1777).

Aspirin, which has a beneficial effect on platelet aggregation (Br. Med. J. (1994) 308: 81-106; 159-168), acts by inducing blockade of prostaglandin synthesis. Aspirin has no effect on ADP-induced platelet aggregation, and thus has limited effectiveness on platelet aggregation. Furthermore, its well documented high incidence of gastric side effects limits its usefulness in many patients. Clinical efficacy of some newer drugs, such as ReoPro (7E3), is impressive, but recent trials have found that these approaches are associated with an increased risk of major bleeding, sometimes necessitating blood transfusion (New Engl. J. Med. (1994) 330:956-961). Thus it appears that the ideal “benefit/risk” ratio has not been achieved.


ADP induces platelet aggregation, shape change, secretion, influx and intracellular mobilization of Ca^{2+}, and inhibition of adenyl cyclase. Binding of ADP to platelet receptors is required for elicitation of the ADP-induced platelet responses. There are at least three P2 receptors expressed in human platelets: a cation channel receptor P2X_{1}, a G protein-coupled
receptor P2Y₁, and a G protein-coupled receptor P2Y₁₂ (also referred to as P2Y₅ and P2T). The P2X₁ receptor is responsible for rapid calcium influx and is activated by ATP. The role of P2X₁ receptors in the process of platelet aggregation is not fully understood. However, it has been suggested that the P2X₁ receptor participates in platelet shape change (Rolf, et al., *Thromb Haemost.* 85:303-308, 2001), and in platelet thrombi formation under high shear forces. (Hechler, et al., *J Exp Med.* 198: 661-667, 2003). The P2Y₁ receptor is responsible for calcium mobilization, shape change and the initiation of aggregation. P2Y₁₂ receptor is responsible for inhibition of adenylyl cyclase and is required for full aggregation. (Hourani, et al., The Platelet ADP Receptors Meeting, La Thuile, Italy, March 29-31, 2000)

Ingall et al. (*J. Med. Chem.* 42: 213-220, (1999)) describe a dose-related inhibition of ADP-induced platelet aggregation by analogues of adenosine triphosphate (ATP), which is a weak, nonselective but competitive P2Y₁₂ receptor antagonist. Zamecnik (USPN 5,049,550) discloses a method for inhibiting platelet aggregation in a mammal by administering to said mammal a diadenosine tetraphosphate compound of App(CH₂)ppA or its analogs. Kim et al. (USPN 5,681,823) disclose P¹, P⁴-dithio-P², P³-monochloromethylene 5', 5'' diadenosine P¹, P⁴-tetraphosphate as an antithrombotic agent. The thienopyridines ticlopidine and clopidogrel, which are metabolized to antagonists of the platelet P2Y₁₂ receptor, are shown to inhibit platelet function *in vivo* (Quinn and Fitzgerald, *Circulation* 100:1667-1672 (1999); Geiger, et al., *Arterioscler. Thromb. Vasc. Biol.* 19:2007-2011 (1999)). However, these thienopyridines have a number of therapeutic disadvantages:

- Due to the irreversible nature of these inhibitors on the P2Y₁₂ receptor, subjects treated with thienopyridines are at a high risk of bleeding if a surgical procedure is necessary. For elective surgeries, discontinuation of the drug is necessary for at least five to ten days since production of new platelets is necessary to restore hemostasis, exposing the subject to a high risk of thrombotic events during this period (Kapetanakis, et al., *Eur Heart J.* 26: 576-583, 2005).
- Subjects treated with the standard dose regimen of these compounds present a large inter-individual variability in the pharmacological effect of the drug, with a significant proportion of patients underprotected from the occurrence of ischemic events. (Gurbel, et al., *Circulation* 107: 2908-2913, 2003; Aleil, et al., *J Thromb Haemost.* 3: 85-92, 2005).
There is a need in the area of cardiovascular and cerebrovascular therapeutics for a direct, reversible P2Y\textsubscript{12} receptor inhibitor that does not require metabolism and has a fast onset and offset of action that can be used in the prevention and treatment of thrombosis, with minimal side effects, such as excessive unwanted prolongation of bleeding, while preventing or treating target thrombi.

**SUMMARY OF THE INVENTION**

This invention is directed to a method of preventing or treating diseases or conditions associated with platelet aggregation and/or platelet activation; such diseases include venous thrombosis, thrombophlebitis, arterial embolism, coronary and cerebral arterial thrombosis, unstable angina, myocardial infarction, stroke, cerebral embolism, kidney embolisms and pulmonary embolisms. The method is also directed to a method of preventing, treating or reducing the incidence of: thrombosis, thrombotic events, embolic events or pathological conditions associated with such events, where the thrombosis, thrombotic event or embolic event occurs during or after surgery.

The method comprises administering to a subject a pharmaceutical composition comprising a therapeutic effective amount of P2Y\textsubscript{12} receptor antagonist compound, wherein said amount is effective to bind the P2Y\textsubscript{12} receptors on platelets and inhibit ADP-induced platelet aggregation.

The P2Y\textsubscript{12} receptor antagonist compounds useful for this invention include compounds of general Formula I, or salts thereof:

**Formula I**

![Chemical structure](attachment:image.png)

wherein:

A
X₁, X₂, and X₃ are independently oxygen, methylene, monochloromethylene, dichloromethylene, monofluoromethylene, difluoromethylene, or imido;
T₁, T₂, W, and V are independently oxygen or sulfur;
m = 0, 1 or 2;
n = 0 or 1;
p = 0, 1, or 2;
where the sum of m+n+p is from 0 to 5; (monophosphate to hexaphosphate)
M = H or a pharmaceutically-acceptable inorganic or organic counterion;
D₁ = O or CH₂;
B’ is a purine or a pyrimidine residue according to general Formulae IV and V which is linked to the 1’ position of the furanose or carbocycle via the 9- or 1- position of the base, respectively;
Y’ = H, OH, or OR₁;
Z’ = H, OH, or OR₂;
with the proviso that when A = M, at least one of Y’ and Z’ is equal to OR₁ or OR₂ respectively;
A = M, or
A is a nucleoside residue which is defined as:

```
  B
 / \  
/   \  
Y   Z
```

and which is linked to the phosphate chain via the 5’ position of the furanose or carbocycle;
wherein:
D₂ = O or CH₂;
Z = H, OH, or OR₃;
Y = H, OH, or OR₄;
with the proviso that at least one of Y’, Z’, Y and Z is equal to OR₁, OR₂ OR₃ or OR₄ respectively;
B is a purine or a pyrimidine residue according to general Formulae IV and V which is linked to the 1’- position of the furanose or carbocycle via the 9- or 1- position of the base, respectively;
R₁, R₂, R₃, and/or R₄ are residues which are linked directly to the 2'- and/or 3'-hydroxyls of the respective furanose or carbocycle via a carbon atom according to Formula II, or linked directly to two (2'- and 3')-hydroxyls of the respective furanose or carbocycle via a common carbon atom according to Formula III, such that from one to four independent residues of R₁, R₂, R₃ and R₄ falling within the definition of Formula II are present or from one to two independent residues made up of R₁+R₂ and/or R₃+R₄ are present.

The present invention also provides novel compounds of mononucleoside 5'-monophosphates and dinucleoside 5'-diphosphates. The present invention further provides pharmaceutical formulations comprising compound of Formula I and a pharmaceutically acceptable carrier.

**BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 shows the effect of inhibition of ADP-induced aggregation by different compounds.

Figure 2 shows the kinetics of inhibition of ADP-induced aggregation following intravenous administration of INS50589 in mice.

Figure 3 shows the protection against thromboembolism-induced mortality in mice by the treatment with INS50589.

Figure 4 shows the dose-dependent inhibition of platelet aggregation by continuous IV infusion of INS50589 in dogs.

Figure 5A shows the platelet aggregation at the indicated times during and after administration of INS50589 at 0.3 mg/kg/h in Dogs. Figure 5B shows the plasma levels of INS50589 at the indicated times during and after the infusion of INS50589.

**DETAILED DESCRIPTION OF THE INVENTION**

**Definitions**

When present, unless otherwise specified, the following terms are generally defined as, but are not limited to, the following:

Alkyl groups are from 1 to 12 carbons inclusively, either straight chained or branched, with or without unsaturation and with or without heteroatoms, are more preferably from 2 to 8 carbons inclusively, and most preferably 2 to 6 carbons inclusively.
Cycloalkyl groups from 3 to 12 carbons inclusively, more preferably from 3 to 10 carbons inclusively, and most preferably 3 to 6 carbons inclusively, with or without unsaturation, and with or without heteroatoms.

Aralkyl groups are from 1 to 8 carbons inclusively in the alkyl portion, are more preferably from 1 to 6 carbons inclusively in the alkyl portion, and most preferably are 1 to 4 carbons inclusively in the alkyl portion; as included in the alkyl definition above, the alkyl portion of an aralkyl group can include one or more positions of unsaturation such as a double bonds or a triple bond in the chain when the chain includes two or more carbon atoms; the alkyl portion of an aralkyl group can also include one or more heteroatoms and/or substituents; the aryl portion of an aralkyl group can be a monocyclic or polycyclic moiety from 3 to 8 carbons inclusively per ring in the aryl portion, more preferably from 4 to 6 carbons inclusively per ring, and most preferably 5 to 6 carbons inclusively per ring; the aryl portion of an aralkyl group can also bear one or more substituents and/or heteroatoms.

Aryl groups are either monocyclic or polycyclic, are from 3 to 8 carbons inclusively per ring, are more preferably from 4 to 6 carbons inclusively per ring, and are most preferably 5 to 6 carbons inclusively per ring; aryl groups can also bear substituents and/or heteroatoms.

Heteroaralkyl groups are from 1 to 8 carbons inclusively in the alkyl portion, are more preferably from 1 to 6 carbons inclusively in the alkyl portion, and most preferably are 1 to 4 carbons inclusively in the alkyl portion; as included in the alkyl definition above, the alkyl portion of a heteroaralkyl group can include one or more positions of unsaturation such as a double bonds or a triple bond in the chain when the chain includes two or more carbon atoms; the alkyl portion of a heteroaralkyl group can also include one or more heteroatoms and/or substituents; the heteroaryl portion of a heteroaralkyl group can be a monocyclic or polycyclic moiety from 3 to 8 carbons inclusively per ring in the heteroaryl portion and containing from 1 to 4 heteroatoms inclusively per ring, more preferably from 4 to 6 carbons inclusively per ring, and most preferably 5 to 6 carbons inclusively per ring; the heteroaryl portion of an heteroaralkyl group can also bear one or more substituents and/or heteroatoms.

Heteroaryl groups are either monocyclic or polycyclic, contain from 1 to 4 heteroatoms inclusively per ring, are from 3 to 8 atoms inclusively per ring, are more preferably from 4 to 6 atoms inclusively per ring, and are most preferably 5 to 6 atoms inclusively per ring; heteroaryl groups can also bear substituents and/or heteroatoms.

Substituents on the foregoing groups can be, but are not limited to, hydroxy, nitro, methoxy, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, thioalkyl, alkoxy,
carboxyl, carboxamido, alkylsulfonyl, alkylsulfonylamino, sulfonamido, cyano, amino, substituted amino, trifluoromethyl, trifluoromethoxy, phenyl, pyridyl, imidazolyl, cyclopropyl, cyclopentyl, and cyclohexyl; and preferred heteroatoms are oxygen, nitrogen, and sulfur.

Diastereomers are stereoisomers (isomers of identical constitution but differing three-dimensional architecture), which do not bear a mirror-image relation to each other.

Pharmaceutically acceptable salts are salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects. Pharmaceutically acceptable salt forms include various polymorphs as well as the amorphous form of the different salts derived from acid or base additions. The acid addition salts can be formed with inorganic or organic acids. Illustrative but not restrictive examples of such acids include hydrochloric, hydrobromic, sulfuric, phosphoric, citric, acetic, propionic, benzoic, napthoic, oxalic, succinic, maleic, malic, adipic, lactic, tartaric, salicylic, methanesulfonic, 2-hydroxyethanesulfonic, toluenesulfonic, benzenesulfonic, camphorsulfonic, and ethanesulfonic acids. The pharmaceutically acceptable base addition salts can be formed with metal or organic counterions and include, but are not limited to, alkali metal salts such as sodium or potassium; alkaline earth metal salts such as magnesium or calcium; and ammonium or tetraalkyl ammonium salts, i.e., $\text{NX}_4^+$ (wherein $X$ is $\text{C}_{1-4}$). In the case of mono- or di-phosphates of nucleosides of the present invention, the salt forms are typically to be alkali-earth metals such as sodium, potassium, lithium or basic salts such as ammonium.

Solvates are addition complexes in which a compound is combined with a pharmaceutically acceptable cosolvent in some fixed proportion. Cosolvents include, but are not limited to, water, methanol, ethanol, 1-propanol, isopropanol, 1-butanol, isobutanol, tert-butanol, acetone, methyl ethyl ketone, acetonitrile, ethyl acetate, benzene, toluene, xylene(s), ethylene glycol, dichloromethane, 1,2-dichloroethane, N-methylformamide, N, N-dimethylformamide, N-methylacetamide, pyridine, dioxane, and diethyl ether. Hydrates are solvates in which the cosolvent is water. It is to be understood that the definition of the compound of the present invention encompasses all possible hydrates and solvates, in any proportion, which possess the stated activity.
P2Y_{12} receptor antagonist compounds

The P2Y_{12} receptor antagonist compounds useful for preventing or treating diseases or conditions associated with platelet aggregation and/or platelet activation include compound of general Formula I, or a pharmaceutically acceptable salt, solvate, or hydrate thereof:

\[
\text{Formula I}
\]

wherein:

- X_1, X_2, and X_3 are independently oxygen, methylene, monochloromethylene, dichloromethylene, monofluoromethylene, difluoromethylene, or imido;
- T_1, T_2, W, and V are independently oxygen or sulfur;
- m = 0, 1 or 2;
- n = 0 or 1;
- p = 0, 1, or 2;

where the sum of m + n + p is from 0 to 5; (from monophosphate to hexaphosphate)

- M = H, or a pharmaceutically-acceptable inorganic or organic counterion;
- D_1 = O or CH_2

B' is a purine or a pyrimidine residue according to general Formulae IV and V which is linked to the 1'- position of the furanose or carbocycle via the 9- or 1- position of the base, respectively;

- Y' = H, OH, or OR_1;
- Z' = H, OH, or OR_2; with the proviso that when A = M, at least one of Y' and Z' is OR_1 or OR_2;

A = M, or

A is a nucleoside residue which is defined as:
and which is linked to the phosphate chain via the 5' - position of the furanose or carbocycle; wherein:

\[ \begin{align*}
D_2 &= \text{O or CH}_2; \\
Z &= \text{H, OH, or OR}_3; \\
Y &= \text{H, OH, or OR}_4;
\end{align*} \]

with the proviso that at least one of \( Y', Z', Y \) and \( Z \) is equal to OR\(_1\), OR\(_2\) OR\(_4\) or OR\(_3\) respectively.

B is a purine or a pyrimidine residue according to general Formulae IV and V which is linked to the 1' position of the furanose or carbocycle via the 9- or 1- position of the base, respectively;

\( R_1, R_2, R_3, \) and/or \( R_4 \) are residues which are linked directly to the 2' - and/or 3' - hydroxyls of the respective furanose or carbocycle via a carbon atom according to Formula II, or linked directly to two (2' - and 3' -) hydroxyls of the respective furanose or carbocycle via a common carbon atom according to Formula III, such that from one to four independent residues of \( R_1, R_2, R_3 \) and \( R_4 \) falling within the definition of Formula II are present or from one to two independent residues made up of \( R_1+R_2 \) and/or \( R_3+R_4 \) are present;

**Formula II**

\[ \begin{align*}
\text{R}_5 \quad \text{O} \quad \text{C} \quad \text{R}_6 \\
\text{R}_7
\end{align*} \]

wherein:

- O is the corresponding 2' - and/or 3' - oxygen of the respective furanose or carbocycle;
- C is a carbon atom;

\( R_5, R_6, \) and \( R_7 \) are \( \text{H, alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl, or substituted aryl, such that the moiety defined according to Formula II is an ether; or} \)

\( R_5 \) and \( R_6 \) are \( \text{H, an alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl, or substituted aryl, and R}_7 \) is \( \text{alkoxy, cycloalkoxy, aralkyloxy, aryloxy, substituted aralkyloxy, or substituted aryloxy such that the moiety defined according to Formula II is an acyclic acetal or ketal; or} \)
R₅ and R₆ are taken together as oxygen or sulfur doubly bonded to C, and R₇ is alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl, or substituted aryl, such that the moiety defined according to Formula II is an ester or thioester; or

R₅ and R₆ are taken together as oxygen or sulfur doubly bonded to C, and R₇ is amino or mono- or disubstituted amino, where the substituents are alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl, or substituted aryl, such that the moiety according to Formula II is a carbamate or thiocarbamate; or

R₅ and R₆ are taken together as oxygen or sulfur doubly bonded to C, and R₇ is alkoxy, cycloalkoxy, aralkyloxy, aryloxy, substituted aralkyloxy, or substituted aryloxy, such that the moiety according to Formula II is a carbonate or thiocarbonate; or

R₇ is not present and R₅ and R₆ are taken together as oxygen or sulfur doubly bonded to C and both the 2'- and 3'- oxygens of the respective furanose or carbocycle are directly bound to C to form a cyclical carbonate or thiocarbonate;

Formula III

\[ \begin{array}{c}
\text{O} \\
\text{C} \\
\text{O}
\end{array} \]

wherein:

the O atoms are the 2'- and 3'- oxygens of a furanose or carbocycle; and the 2'- and 3'- oxygens of the furanose or carbocycle are linked by a common carbon atom (C) to form a cyclical acetal, cyclical ketal, or cyclical orthoester;

for cyclical acetals and ketals, R₈ and R₉ are independently hydrogen, alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl, substituted aryl, or can be joined together to form a homocyclic or heterocyclic ring composed of 3 to 8 atoms, preferably 3 to 6 atoms;

for cyclical orthoesters, R₈ is hydrogen, alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl, or substituted aryl, R₉ is alkoxy, cycloalkoxy, aralkyloxy, aryloxy, substituted aralkyloxy, or substituted aryloxy.

When R₅, R₆ and R₇ are not the same, or when R₈ and R₉ are not the same, a compound according to Formula I can exist in several diastereomeric forms. The general structure of Formula I includes all diastereomeric forms of such materials, when not specified.
otherwise. Formula I also includes mixtures of compounds of Formula I, including mixtures of enantiomers, diastereomers and/or other isomers in any proportion.

One embodiment of the invention is that A = M, wherein M = H or a pharmaceutically acceptable inorganic or organic counterion. In such an embodiment, the compound can be a nucleoside monophosphate, nucleoside diphosphate, nucleoside triphosphate, nucleoside tetraphosphate, nucleoside pentaphosphate, or nucleoside hexaphosphate with one or both of the 2’- and/or 3’- positions of the furanose or carbocycle modified. Most preferred are nucleotide monophosphates, nucleotide diphosphates, nucleotide triphosphates, and nucleotide tetraphosphates. When T₂, W, V, or T₁ are sulfur, the preferred position for this atom is on the terminal phosphorous of the polyphosphate chain (i.e. the phosphorous furthest removed from the nucleoside residue).

For monophosphates, where m, n, and p are all equal to zero, preferably R₈ is hydrogen and R₉ is aryl or aralkyl, with 1, 2, 3, or 4 carbons inclusively in the alkyl portion of an aralkyl group, and 6 carbons inclusively in the aryl portion of an aralkyl or aryl group; when the number of carbons in the alkyl portion of an aralkyl group is 2, the carbon atoms are most preferably connected by either a double or triple bond.

Another embodiment of the invention is that A is a nucleoside residue defined as:

```
B   D₂
\   / \
\ /   \ /
Y    Z
```

and linked to the phosphate chain via the 5’- position of the furanose or carbocycle (to give a dinucleoside polyphosphate with at least one of the 2’-, 3’-, 2’’- and 3’’’- positions of the furanose or carbocycle moieties modified to be OR₁, OR₂, OR₄ or OR₃ respectively).

When T₂, W, V, and/or T₁ are sulfur, the preferred positions (for sulfur) are T₁ and T₂.

Further provisions are that when either D₁ or D₂ are oxygen, the corresponding furanose is preferably in the β-configuration; and that the corresponding furanose is preferably in the β-D-configuration.
In one embodiment, compounds of general Formula I are molecules whose structures fall within the definitions of Formula Ia and Formula Ib:

**Formula Ia**

$$
\begin{align*}
B & \quad O \quad T_2 \quad X_3 \quad OM \\
D_1 & = O \text{ or } CH_2; \\
D_2 & = O \text{ or } CH_2; \\
B' & \quad Z' \\
Y & \quad Y' \\
Z & \quad Z' \\
D_1 & \quad D_2 \quad D_3
\end{align*}
$$

wherein:

- $D_1 = O$ or $CH_2$;
- $D_2 = O$ or $CH_2$;
- $B$ and $B'$ are independently purine or pyrimidine residues according to general Formula IV or V;
- $m$ and $p = 0$, $1$ or $2$; $n = 0$ or $1$; such that the sum of $m + n + p$ is from $0$ to $5$,
- preferably $0$ to $4$, and most preferably $0$ to $3$;
- $X_1$, $X_2$, and $X_3$ = are independently $O$, $NH$, $CH_2$, $CHF$, $CHCl$, $CF_2$, $CCl_2$;
- $T_1$, $T_2$, $V$, and $W$ are independently $O$ or $S$;
- $M = H^+$, $NH_4^+$, $Na^+$ or other pharmaceutically-acceptable inorganic or organic counterion;
- $Y' = H$, $OH$, or $OR_1$;
- $Z' = OH$ or $OR_2$;
- $Z = OH$ or $OR_3$;
- $Y = H$, $OH$, or $OR_4$, where $R_1$, $R_2$, $R_3$, and $R_4$ fall under the definition of general Formulae II or III, provided that at least one of $Y'$, $Z'$, $Z$ and $Y$ is $OR_1$, $OR_2$, $OR_3$, or $OR_4$.

Preferred compounds of Formula Ia include:

- $D_1 = O$ or $CH_2$;
- $D_2 = O$ or $CH_2$;
- $X_1$, $X_2$, and $X_3 = O$;
- $T_1$, $T_2$, $V$, and $W = O$; or
\[ \text{Formula Ib} \]

\[
\begin{align*}
D_1 &= O \text{ or CH}_2; \\
D_2 &= O \text{ or CH}_2; \\
X_1 \text{ and } X_3 &= O; \\
X_2 &= \text{methylene, monochloromethylene, dichloromethylene, monofluoromethylene,} \\
& \text{difluoromethylene, or imido;} \\
T, T_1, T_2, V, \text{ and } W &= O; \text{ or} \\
D_1 &= O \text{ or CH}_2; \\
D_2 &= O \text{ or CH}_2; \\
m, n, \text{ and } p &= 1; \text{ or} \\
X_1 \text{ and } X_3 &= O; \\
X_2 &= \text{methylene, monochloromethylene, dichloromethylene, monofluoromethylene,} \\
& \text{difluoromethylene, or imido;} \\
T_1 \text{ and } T_2 &= S; \\
V \text{ and } W &= O.
\end{align*}
\]

\[ D_1 = O \text{ or CH}_2; \]

\[ n \text{ and } p = 0, 1, \text{ or } 2 \text{ such that the sum of } n + p \text{ is from 0 to 3;} \]

\[ A = M; \text{ wherein } M = H^+, \text{NH}_4^+, \text{Na}^+ \text{ or other pharmaceutically-acceptable inorganic or} \]

\[ \text{organic counterion;} \]

B' is a purine or pyrimidine residue according to general Formulae IV and V;

\[ X_1 \text{ and } X_2 \text{ are independently O, NH, CH}_2, \text{ CHF, CHCl, CF}_2, \text{ CCl}_2; \]

\[ T_1, V, \text{ and } W \text{ are independently O or S;} \]

\[ Y' = H, \text{OH, or OR}_1, \]

14
$Z' = H, OH$ or $OR_2$, where $R_1$ and $R_2$ fall under the definitions of general Formulae II or III; with the proviso that at least one of $Y'$ and $Z'$ is $OR_1$ or $OR_2$, respectively.

Preferred compounds of Formula Ib include:

5  
$D_1 = O$ or $CH_2$;
$n$ and $p = 0, 1, or 2$ such that the sum of $n + p$ is from 0 to 3, preferably 1 to 2;
$X_1$ and $X_2 = O$;
$T_1, V,$ and $W = O$; or

10  
$D_1 = O$ or $CH_2$;
$X_1$ and $X_2 = O$;
$T_1$ and $V = O$;
$W = S$; or

15  
$D_1 = O$;
$n$ and $p = 0$ such that the sum of $n + p$ is 0;
$V = O$;
$B'$ is a purine residue of general Formula IV;
$Y'$ and $Z'$ fall under the definition of general Formula III; or

20  
$D_1 = O$ or $CH_2$;
$p = 0, 1, or 2, n = 1,$ such that the sum of $n + p$ is from 1 to 3;
$X_1 = O$;
$X_2 =$ methylene, monochloromethylene, dichloromethylene, monofluoromethylene, difluoromethylene, or imido;
$T_1, V,$ and $W = O$;
$Y' = H, OH,$ or $OR_1$;
$Z' = H, OH$ or $OR_2$, where $R_1$ and $R_2$ fall under the definition of general Formulae II or III; with the proviso that at least one of $Y'$ and $Z'$ is $OR_1$ or $OR_2$, respectively.

25  
Several preferred compounds also are described by Formulae Ia-1 and Ib-2:
For compounds of Formula I, B and B' can independently be a purine residue, as in Formula IV, linked through the 9- position, or a pyrimidine residue, as in Formula V, linked through the 1- position. The ribosyl moieties in Formulae Ia, Ib, Ia-1, and Ib-1 are in the D-configuration as shown, but can also be L-, or D- and L-. The D- configuration is preferred for ribosyl moieties.
Formula V

wherein:

5 \( R_{10} \) and \( R_{14} \) independently are hydroxy, oxo, amino, mercapto, alkylthio, alkoxy, arylox, alkylamino, cycloalkylamino, aralkylamino, arylamino, diaralkylamino, diarylamino, or dialkylamino, where the alkyl groups are optionally linked to form a heterocycle; or

\( R_{10} \) and \( R_{14} \) independently are acylamino, provided that they incorporate an amino residue from the C-6 position of the purine or the C-4 position of the pyrimidine; or

when \( R_{10} \) in a purine or \( R_{14} \) in a pyrimidine has as its first atom nitrogen, \( R_{10} \) and \( R_{11} \) or \( R_{14} \) and \( R_{15} \) can be taken together to form a 5-membered fused imidazole ring (to give an etheno compound), optionally substituted on the etheno ring with one or more alkyl, cycloalkyl, aralkyl, or aryl moieties, as described for \( R_8-R_9 \) above;

\( J \) is carbon or nitrogen, with the provision that when \( J = \text{nitrogen,} \ R_{12} \text{ is not present;} \)

10 \( R_{11} \) is hydrogen, O (adenine 1-oxide derivatives) or is absent (adenine derivatives);

\( R_{15} \) is hydrogen, or acyl (e.g. acetyl, benzoyl, phenylacetyl, with or without substituents);

\( R_{12} \) is hydrogen, alkyl, bromo, azido, alkylamino, arylamino or aralkylamino, alkoxy, arylox or aralkyloxyl, alkylthio, arthio or aralkylthio, or \( \omega\text{-A(C}_{1,6}\text{alkyl})B}\), wherein A and B are independently amino, mercapto, hydroxy or carboxyl;

\( R_{13} \) is hydrogen, chlorine, amino, monosubstituted amino, disubstituted amino, alkylthio, arthio, or aralkylthio, where the substituent on sulfur contains up to a maximum of 20 carbon atoms, with or without unsaturation, and with or without substituents on the chain;

20 \( R_{16} \) is hydrogen, methyl, alkyl, halogen, alkyl, alkenyl, substituted alkenyl, alkynyl, or substituted alkynyl.
Compounds according to Formulae IV and V where R₁₀ or R₁⁴ is acylamino fall within the scope of Formula VI:

\[
\text{Formula VI}
\]

wherein:
- NH is the amino residue at the C-6 position in a purine or the amino residue at the C-4 position in a pyrimidine;
- C is a carbon atom;
- \( W₁ \) is oxygen or sulfur;
- \( R₁₇ \) is amino or mono- or disubstituted amino, with the amino substituent(s) being alkyl, cycloalkyl, aralkyl, or aryl, with or without further substituents, unsaturation, or heteroatoms, such that the moiety according to Formula VI is a urea or thiourea; or
- \( R₁₇ \) is alkoxy, aralkyloxy, aryloxy, substituted aralkyloxy, or substituted aryloxy, such that the moiety according to Formula VI is a carbamate or thiocarbamate; or
- \( R₁₇ \) is alkyl, cycloalkyl, aralkyl, or aryl, with or without substituents or heteroatoms, such that the moiety according to Formula VI is an amide; with definitions of alkyl, cycloalkyl, aralkyl, or aryl groups as previously defined for comparable groups in \( R₅ \) to \( R₉ \).

**Novel Compounds**

Novel compounds of the present invention include compounds of Formula Ia, wherein \( B \) and \( B' \) are independently pyrimidine (pyrimidine/pyrimidine dinucleotide), provided that when \( m + n + p = 1 \), \( R₁₆ = \text{CH}_₃ \), and \( R₅ \) and \( R₆ \) are taken together as oxygen doubly bonded to C, then \( R₇ \) is not equal to \( \text{CH}_₃ \) (\( Z' \) does not equal to acetate); also provided that when \( m + n + p = 3 \), \( B \) and \( B' \) = uridine, and \( R₅ \) and \( R₆ \) are taken together as oxygen doubly bonded to C, then \( R₇ \) is not equal to phenyl for \( Y' = \text{OR}_₁ \) and/or \( Y = \text{OR}_₄ \) (Y and \( Y' \) does not equal to benzoyl); further provided that when \( m + n + p = 1 \), then both \( R₈ \) and \( R₉ \) are not \( \text{CH}_₃ \) (\( Z' \) and \( Y' \) taken together do not equal isopropylidene).
Novel compounds of the present invention also include compounds of Formula Ia, wherein B is a purine or residue according to general formula IV, and B' is a pyrimidine residue according to general formula V, (purine/pyrimidine dinucleotide); provided that Y' is not equal to OCH₃ when Z', Y, or Y' = H or OH; further provided that R₈ is not equal to OCH₂CH₃ when R₉ = H (Z' and Y' or Z and Y taken together do not equal to an orthoethylester).

Novel compounds of the present invention also include compounds of Formula Ia, wherein B and B' are independently a purine residue according to general formula IV, (purine/purine dinonucleotide); provided that (a) Y or Y' is not equal to OCH₃ when R₁₀ = NH₂ or O; (b) R₈ is not equal to OCH₃ or OCH₂CH₃ when R₉ = H; (c) both R₈ and R₉ are not equal to CH₃; (d) when m+n+p = 1, then R₈ and R₉ does not equal OCH₂CH₃; (e) when R₁₀ = NH₂, and when R₅ and R₆ are taken together as oxygen doubly bonded to C, then R₇ is not equal to ortho-methylnaphthoaminophenyl; (f) when m+n+p = 1, and when R₅ and R₆ are taken together as oxygen doubly bonded to C, then R₇ is not equal to CH(CH₂CH₂SCH₃)NHS(o-NO₂-Ph) or CH(CH₂Ph)NHS(o-NO₂-Ph).

Novel compounds of the present invention include compounds of Formula Ib (mononucleotide), provided that when n = 1, both X₁ and X₂ are not O; and when n = 0, X₁ is not O; and provided when Y' = H, that X₂ is independently O, CH₂, CHF, CHCl, CF₂, CCl₂; also provided that when R₁₀ = NH₂ or O, and when R₅ and R₆ are taken together as oxygen doubly bonded to C, then R₇ is not equal to ortho-methylnaphthoaminophenyl; further provided that when n = p = 1, X₂ = CH₂ and B' = adenosine, then R₁ and R₂ are not equal to naphtthylhexmethyl, napthylhexethylmethylene, or phenylmethylene.

Novel dinucleotide 5'-diphosphates compounds include compounds of Formula Ia-1:

**Formula Ia-1**

![Diagram](image)

wherein:

V = O;
M = H or a pharmaceutically-acceptable inorganic or organic counterion;
D₁ and D₂ = O;
Y' = H, OH, or OR₁;
Z' = H, OH or OR₂;
Z = H, OH or OR₃;
Y = H, OH, or OR₄, where R₁, R₂, R₃, and R₄ fall under the definition of general
Formulae II or III, provided that at least one of Y', Z', Z and Y is OR₁, OR₂, OR₃, or OR₄;
R₁, R₂, R₃, and R₄ are residues which are linked directly to the 2' and/or 3' hydroxyls
of the furanose via a carbon atom according to Formula II, or, preferably, comprise a moiety
linked to the 2' and 3' hydroxyls of the furanose or carbocycle via a common carbon atom
according to Formula III,

```
Formula III
     \   /
    O   O
    \ /  /
     R₈ ---- R₉
```

wherein:
the O atoms are the 2'- and 3'- oxygens of the furanose; and
the 2'- and 3'- oxygens of the furanose are linked by a common carbon atom to form a
cyclical acetal; and
R₈ is hydrogen; and
R₉ is selected from the group consisting of aralkyl, aryl, substituted aralkyl, and substituted
aryl;
in which the aralkyl groups are straight chained from 1 to 5 carbons, with or without
unsaturation and without heteroatoms in the alkyl portion, and are monocyclic moieties from
5 to 6 carbons in the aryl portion; and the aryl groups are monocyclic moieties from 4 to 6
carbons, with or without heteroatoms;

B' is a purine residue according to general Formula IV
wherein:
R₁₀ is acylamino, according to Formula VI; and
R₁₇ is amino or mono- or disubstituted amino such that the moiety according to Formula VI is
a urea;

J = carbon;
R₁₁ is absent;
R\textsubscript{12} is hydrogen; and
R\textsubscript{13} is hydrogen.

Novel mononucleoside 5'-monophosphates compounds include compounds of

Formula Ib-1:

![Formula Ib-1]

wherein:

V = O;

M = H or a pharmaceutically-acceptable inorganic or organic counterion;

D\textsubscript{1} = O;

Y' = H, OH, or OR\textsubscript{1};

Z' = H, OH, or OR\textsubscript{2}; with the proviso that at least one of Y' and Z' is OR\textsubscript{1} or OR\textsubscript{2};

R\textsubscript{1} and R\textsubscript{2} are residues which are linked directly to the 2' and/or 3' hydroxyls of the furanose via a carbon atom according to Formula II, or both R\textsubscript{1} and R\textsubscript{2} are linked to the 2' and 3' hydroxyls of the furanose via a common carbon atom according to Formula III,

Formula III

![Formula III]

wherein:

the O atoms are the 2'- and 3'- oxygens of the furanose; and

the 2'- and 3'- oxygens of the furanose are linked by a common carbon atom to form a
cyclical acetal; and

R\textsubscript{8} is hydrogen; and

R\textsubscript{9} is selected from the group consisting of aralkyl, aryl, substituted aralkyl, and substituted aryl;
in which the aralkyl groups are straight chained from 1 to 5 carbons, with or without
unsaturation and without heteroatoms in the alkyl portion, and are monocyclic moieties from
5 to 6 carbons in the aryl portion; and the aryl groups are monocyclic moieties from 4 to 6
carbons, with or without heteroatoms;

B' is a purine residue according to general Formula IV

wherein:
R_{10} is acylamino, according to Formula VI; and
R_{17} is amino or mono- or disubstituted amino such that the moiety according to Formula VI is
a urea;

J = carbon;
R_{11} is absent;
R_{12} is hydrogen; and
R_{13} is hydrogen.

The compounds of the present invention fall under the definition of general Formula I,
which is further divided into general Formulae Ia (dinucleotides), Ib (mononucleotides), Ia-1
(dinucleoside diphosphates) and Ib-1 (mononucleoside monophosphates). While potent and
selective P2Y_{12} antagonists can be found within any of these subdivisions, mononucleotides
have an advantage over dinucleotides in terms of ease of synthesis and cost. In general,
mononucleoside diphosphates and mononucleoside triphosphates falling under general
Formula Ib are more potent antagonists at P2Y_{12} than the corresponding mononucleoside
monophosphates of Formula Ib-1. However, nucleoside 5'-monophosphates and their
analogues are easier to prepare and have greater chemical and biological stability comparing
with mononucleoside diphosphates and mononucleoside triphosphates. Thus, for synthetic
reasons, a nucleoside 5'-monophosphate with appropriate druglike properties is sometimes
more advantageous than other mononucleotides bearing more than one phosphate, or related
dinucleotides. For dinucleotides falling under general formula Ia, those having only two
phosphates as described by formula Ia-1 are most desirable, as they can be prepared under
simple reaction conditions in good yield from the corresponding mononucleoside 5'-
monophosphates falling under formula Ib-1. When attacked by enzymes in the bloodstream,
nucleoside 5'-monophosphate only gives one byproduct from loss of the phosphate.

Dinucleoside diphosphates are the easiest dinucleotides to make; they are stable, and give a
limited number of breakdown products, in contrase to dinucleotides with longer phosphate
chain lengths. For inhibition of platelet aggregation, nucleoside 5'-monophosphate and
dinucleoside diphosphates are preferred compounds.

Two modifications can be made to compounds of general Formulas Ia-1 and Ib-1 to
render them potent antagonists of the platelet P2Y$_{12}$ receptor. In general, a preferred starting
material of nucleoside 5'-monophosphate is adenosine 5'-monophosphate (AMP), or an
AMP derivative, as it contains the appropriate functional groups for the desired modifications
and gives rise to more potent and selective antagonists compared to similar modifications of
other commonly available nucleotide monophosphates. The first modification is to install an
aryl or aralkyl acetal bridging the 2'- and 3'- hydroxyls of the ribose, with the nature of the
aryl or aralkyl group as previously described. Of these described groups, the most preferred
are phenyl, benzyl, and styryl, which are the acetals arising from the reaction between the 2'
and 3' hydroxyl groups of the ribose and benzaldehyde, phenylacetaldehyde, and
cinnamaldehyde, respectively. These moieties provide several important advantages over
many similar modifications. Firstly, they are derived from readily available, low cost
aldehydes or aldehyde equivalents (for example, aldehyde, dialkyl acetals). Secondly, these
moieties allow the synthesis of either of the two possible diastereomers that arise from the
addition of the acetal to the chiral ribose residue, via several synthetic strategies. Finally,
molecules bearing these acetal moieties provide for potent analogues of the present invention.

The second modification is to add an aminocarbonyl or substituted aminocarbonyl
group to the 6-amino position of the adenine base, resulting in a urea moiety at that position.
Substituents on the urea moiety fall under the definition of the amino substituents of R$_{17}$, i.e.,
alkyl, cycloalkyl, aralkyl, or aryl, with or without substituents, unsaturation, or heteroatoms.
These urea substituents can be broadly categorized as either aromatic or aliphatic in nature. A
preferred substituent chosen from aryl groups is phenyl. When the urea group is an aliphatic
urea, a preferred substituent on nitrogen is a linear, branched, or cyclic, C$_1$-C$_6$ alkyl; with or
without unsaturation. Preferred urea moieties are linear alkyl ureas from 2 to 6 carbons
inclusively, or cyclic alkyl ureas having 3 to 6 carbons in the ring. More preferred urea
moieties are linear alkyl ureas containing from 2 to 4 carbons inclusively in the chain or
cycloalkyl ureas having from 3 to 5 carbons inclusively in the ring.

Novel compounds having the above modifications can be shown in general as
formulae Ia-2 and Ib-2.
wherein:
R\textsubscript{18} and R\textsubscript{18}' are independently phenyl, benzyl, or styryl; and
R\textsubscript{19} and R\textsubscript{19}' are independently C\textsubscript{2} to C\textsubscript{6} alkyl; C\textsubscript{3} to C\textsubscript{6} cycloalkyl; alkylcycloalkyl with one to two carbon atoms in the alkyl portion, and three to six carbons in the cycloalkyl portion;

phenyl; substituted or unsubstituted.

For example, R\textsubscript{19} and R\textsubscript{19}' are independently ethyl, propyl, butyl, cyclopropyl, cyclopropylethyl, cyclobutyl, cyclopentyl, or phenyl.

While R\textsubscript{18} and R\textsubscript{18}' and R\textsubscript{19} and R\textsubscript{19}' can be independently a given group, they are preferably identical pairs for practical synthetic reasons.
wherein:

5 R\textsubscript{18} is phenyl (benzaldehyde acetal), benzyl (phenylacetaldehyde acetal) or styryl (cinnamyl acetal);

R\textsubscript{19} is C\textsubscript{2} to C\textsubscript{6} alkyl, C\textsubscript{3} to C\textsubscript{6} cycloalkyl, alkylcycloalkyl with one to two carbon atoms in the alkyl portion, and three to six carbons the cycloalkyl portion, phenyl, substituted or unsubstituted.

10 For example, R\textsubscript{19} is ethyl, propyl, butyl, cyclopropyl, cyclopropylmethyl, cyclobutyl, cyclopentyl, or phenyl.

An aspect of the present invention is that, while any of Formula I compounds is capable of antagonism of ADP-induced platelet aggregation, it is the combination of both 2'3' and 6-N modifications in the same molecule that renders a highly potent and selective P2Y\textsubscript{12} antagonist compound.

Another aspect of the present invention is the effect of compound structure on the resultant potency in washed platelets versus potency in whole blood. In general, the potency of a given compound is lower in whole blood versus washed platelets, ostensibly the result of increased binding of the compound to the higher levels of blood proteins in the former. This property is particularly acute for nucleoside 5'-monophosphates versus the corresponding di-
and triphosphates, since there are fewer ionizable groups available in the former to offset the lipophilic acetal and urea groups, which presumably increase protein binding in whole blood. Unexpectedly, we found that compounds having aliphatic ureas gave-comparable results when tested in whole blood and washed platelet assays. Furthermore, we found that compounds with aliphatic ureas were more potent than their aromatic counterparts in washed platelets.


Novel dinucleoside 5'- diphosphate compounds include P1, P2-di-(2', 3'-cinnamyl acetal-6-N-ethylurea adenosine 5'-) diphosphate (compound 44), P1, P2-di-(2', 3'-phenylpropargyl acetal-6-N-ethylurea adenosine 5'-) diphosphate (compound 45), P1, P2-di-(2', 3'-phenylacetaldehyde acetal-6-N-ethylurea adenosine 5'-) diphosphate (compound 46), and P1, P2-di-(2', 3'-phenyl acetal-6-N-ethylurea adenosine 5'-) diphosphate (compound 47).

Novel mononucleoside 5'- monophosphate compounds include 2', 3'-phenylacetaldehyde acetal-6-N-phenylurea AMP (compound 22), 2', 3'-phenylacetaldehyde acetal-6-N-n-hexylurea AMP (compound 23), 2', 3'-phenylacetaldehyde acetal-6-N-ethylurea AMP (compound 24), 2', 3'-phenylacetaldehyde acetal-6-N-cyclopentylurea AMP (compound 25), 2', 3'-cinnamyl acetal-6-N-n-hexylurea AMP (compound 26), 2', 3'-
cinnamyl acetal-6-N-ethyleurea AMP (compound 27), 2', 3'- cinnamyl acetal-6-N-phenyleurea AMP (compound 28), 2', 3'- cinnamyl acetal-6-N-n-propylurea AMP (compound 29), 2', 3'- cinnamyl acetal-6-N-n-butylurea AMP (compound 30), 2', 3'- phenylpropargyl acetal-6-N-phenyleurea AMP (compound 31), 2', 3'- phenylpropargyl acetal-6-N-n-hexylurea AMP (compound 32), 2', 3'- phenylpropargyl acetal-6-N-n-butylurea AMP (compound 33), 2', 3'- phenylpropargyl acetal-6-N-n-propylurea AMP (compound 34), 2', 3'-phenylpropargyl acetal-6-N-ethylurea AMP (compound 35), 2', 3'- benzaldehyde acetal-6-N-ethylurea AMP (compound 36), 2', 3'- benzaldehyde acetal-6-N-n-propylurea AMP (compound 37), 2', 3'- benzaldehyde acetal-6-N-n-butylurea AMP (compound 38), 2', 3'- benzaldehyde acetal-6-N-n-hexylurea AMP (compound 39), 2', 3'-benzaldehyde acetal-6-N-cyclopentylurea AMP (compound 40), 2'3'-(trans)cinnamyl acetal-6-N-ethylurea AMP(compound 41), 2'3'-(trans)phenyl acetal-6-N-ethylurea AMP(compound 42), and 2'3'-(cis)phenyl acetal-6-N-ethylurea AMP(compound 43). In Compounds 41-43, cis or trans refers to the relative position of hydrogen atoms on the dioxole ring.

The structures of the novel Compounds 1-47 are shown as follows. In the following structures, hydrogens that are understood to be present have been omitted for the sake of simplicity. Tautomers drawn represent all tautomers possible. As diastereomers are generated with the introduction of the acetal group, structures containing this moiety without the stereochemistry explicitly defined (Compounds 1-20 and 23-26) are taken to mean either of the possible diastereomers alone or a mixture of diasteromers in any ratio.
Compound 2

Compound 3

2-(3-trifluoromethylpropyl)thio-6-(2-methylthio) ethylamino-2',3'-(benzyl)methylenedioxy purine riboside 5'-α,β-difluoromethylene diphosphate
Compound 8

Compound 9
Compound 10

Compound 11
Compound 14

P1-[2-(3-trifluoromethylpropyl)thio-6-(2-methylthio)ethylamino 2',3'-[benzyl]methylene dioxy purine riboside]-P4-(2',3'-[benzyl]methylene dioxy uridine) tetraphosphate

Compound 15
Compound 20

Compound 21
Compound 22

Compound 23

Compound 24
Compound 27

Compound 28
Compound 37

Compound 38

Compound 39
Compound 40

Compound 41
**Pharmaceutical Formulations**

The present invention additionally provides novel pharmaceutical formulations comprising compounds of Formula Ia, Ib, Ia-1, Ib-1, Ia-2, or Ib-2 and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers can be selected by those skilled in the art using conventional criteria. Pharmaceutically acceptable carriers include, but are not limited to, saline solution, aqueous electrolyte solutions, isotonicity modifiers, water polyethers such as polyethylene glycol, polyvinyls such as polyvinyl alcohol and povidone, cellulose derivatives such as methylcellulose and hydroxypropyl methylcellulose, polymers of acrylic acid such as carboxypolymethylene gel, polysaccharides such as dextrans, and glycosaminoglycans such as sodium hyaluronate and salts such as sodium chloride and potassium chloride.

The pharmaceutical formulation of the present invention provides an aqueous solution comprising water, suitable ionic or non-ionic tonicity modifiers, suitable buffering agents, chelating agent, pH adjusters, and a compound of Formula Ia, Ib, Ia-1, Ib-1, Ia-2, or Ib-2 at 0.005 to 3% w/v, wherein said aqueous solution has a tonicity of 200-400 mOsm/kG and a pH of 4-9. In a preferred embodiment, the pharmaceutical formulation comprises 0.025-3, 0.05 to 2.5% or 0.01 to 1.5% w/v of a compound of Formula Ia, Ib, Ia-1, Ib-1, Ia-2, or Ib-2. In another preferred embodiment, the pharmaceutical formulation has the tonicity of 220-360 or 250-350 mOsm/kG. In another preferred embodiment, the pharmaceutical formulation has a pH of 4.5-8.5 or 5.5-7.5. In yet another preferred embodiment, the chelating agent in the amount of 0.005-0.01% w/v; preferably 0.008-0.01% w/v.
The pharmaceutical formulation can be sterilized by filtering the formulation through a sterilizing grade filter, preferably of a 0.22-micron nominal pore size. The pharmaceutical formulation can also be sterilized by terminal sterilization using one or more sterilization techniques including but not limited to a thermal process, such as an autoclaving process, or a radiation sterilization process, or using pulsed light to produce a sterile formulation. In one embodiment, the pharmaceutical formulation is a concentrated solution of the active ingredient; the formulation can be serially diluted using appropriate acceptable sterile diluents prior to intravenous administration.

In one embodiment, the tonicity modifier is ionic such as NaCl, for example, in the amount of 0.5-0.9 % w/v, preferably 0.6-0.9 % w/v.

In another embodiment, the tonicity modifier is non-ionic, such as mannitol, dextrose, in the amount of at least 2%, or at least 2.5%, or at least 3%, and no more than 7.5%; for example, in the range of 3-5 %, preferably 3.5-5%, and more preferably 4.2-5% w/v. Mannitol and dextrose are preferred over other possible non-ionic tonicity modifiers such as, propylene glycol and related glycols, polyethylene glycol of varying molecular ranges, sorbitol, polymers such as polyvinyl alcohol, PVP, sorbitol, sucrose, and trehalose, because most of these non-ionic tonicity modifiers are not suitable for long-term administration via the intravenous route. For example, sucrose and trehalose are not metabolized when given via the intravenous route and are excreted unchanged, thus, they are not candidates for an intravenous dosage form. In addition, the formulation containing mannitol has the advantages of being adaptable for freeze-drying or lyophilization.

In one embodiment, the pharmaceutical formulation comprises 0.5-0.9 % ionic tonicity modifier such as sodium chloride; the formulation optionally contains additional buffering agents (such as sodium phosphates and/or sodium citrate) within a range of 0.01-0.2% w/v, a chelating agent in a range of 0.005 – 0.01% w/v, and pH adjusters. Such an aqueous composition has a tonicity of 250-350 mOsm/kg and is formulated at a physiologically acceptable pH. However, depending on the compound type and concentration, this NaCl formulation tends to form a precipitate at higher concentration (>0.1% w/v) of some compounds.

Nucleoside compounds, including mono-, di-, and tri-phosphates, are generally soluble in aqueous solutions. However, depending on the degree and nature of substituent groups as well as the salt forms, the relative aqueous solubility may be altered.
In order to prepare aqueous solution formulations for intravenous administration, it is preferred that the solution be clear (i.e. free of precipitates), isotonic, and at a physiologically acceptable pH. The aqueous solubility of the compounds of the present invention varies as a function of both the substitution and the salt form; with the latter having a greater effect on aqueous solubility. The salt forms of some compounds, even at a low concentration (such as <0.05% w/v), tend to form precipitates or have a limiting solubility in a normal aqueous saline solution (0.9% NaCl), which is a typical vehicle for intravenous administration.

Applicants have discovered that pharmaceutical formulations prepared using the compounds of the present invention in a non-ionic, iso-osmotic based vehicle provided clear, isotonic preparations at physiological pH. The level of non-isotonic toxicity modifiers such as mannitol or dextrose in the pharmaceutical formulations varied as a function of the desired target concentration of the said compounds (since the compounds themselves had an impact on the toxicity and contributed to the overall toxicity of the preparations). The pharmaceutical formulations made in non-ionic vehicles can be adjusted for a target pH over a wide range without compromising the solubility of the parent compound.

In one embodiment, the present invention provides a pharmaceutical formulation comprising a compound of Formula I, Ia, Ib, Ia-1, Ib-1, Ia-2, or a pharmaceutically acceptable salt, solvate, or hydrate thereof, in an amount of 0.0005-0.3% w/v, a buffering agent, a chelating agent (such as EDTA), a non-ionic toxicity modifier, wherein the pharmaceutical formulation has a toxicity of 250-350 mOsm/kg and pH of 4-9. A preferred non-ionic toxicity modifier is dextrose or mannitol, in the amount of at least 2%, or at least 2.5%, or at least 3%, and no more than 7.5%; for example, in the range of 3-5, preferably 3.5-5, and more preferably 4.2-5% w/v. Such pharmaceutical formulation may or may not contain any ionic toxicity modifier such as NaCl. For example, the pharmaceutical formulation contains a compound of 2', 3'- phenylacetaldehyde acetal-6-N-ethyurea AMP (compound 24), 2', 3'-cinnamyl acetal-6-N-ethyurea AMP (compound 27), 2', 3'- benzaldehyde acetal-6-N-ethyurea AMP (compound 36), P₁, P₄-di-(2', 3'-cinnamyl acetal-6-N-ethyurea adenosine 5'-) diphosphate (compound 44), P₁, P₄-di-(2', 3'-phenylacetaldehyde acetal-6-N-ethyurea adenosine 5'-) diphosphate (compound 46), or P₁, P₄-di-(2', 3'-phenyl acetal-6-N-ethyurea adenosine 5'-) diphosphate (compound 47).
Preparation of the compounds

The compounds of the present invention can be conveniently synthesized by those skilled in the art using well-known chemical procedures. Mononucleoside mono-, di- and triphosphates can be obtained from commercial sources or can be synthesized from the nucleoside using a variety of phosphorylation reactions which can be found in the chemical literature. Symmetrical and unsymmetrical dinucleotide polyphosphates can be prepared by activation of a nucleoside mono-, di- or triphosphate with a coupling agent such as, but not limited to, dicyclohexylcarbodiimide or 1,1'-carbonyldiimidazole, followed by condensation with another nucleoside mono-, di-, or triphosphate, which can be the same or different as the activated moiety. Activation of nucleoside triphosphates with dicyclohexylcarbodiimide gives a cyclical trimetaphosphate as the activated species, which can be advantageously reacted with a variety of nucleophiles to install unique substituents on the terminal phosphate of a triphosphate.

The compounds of the present invention can be prepared by derivatization or substitution at the level of the nucleoside, followed by phosphorylation and condensation as previously described, or the reactions can be carried out directly on the preformed mono- or dinucleotides. In the general Formulae Ia and Ib, the substituents at Y', Z', Y, and Z can be esters, carbamates, or carbonates, which are generally described by Formula II. Esters can be readily prepared by reacting a hydroxyl group of the furanose in a nucleoside or nucleotide with an activated form of an appropriate organic acid, such as an acid halide or acid anhydride in the presence of an organic or inorganic base. Alternately, use of a suitable coupling reagent such as dicyclohexylcarbodiimide, 1,1'-carbonyldiimidazole and the like to activate the organic acid can be used to achieve the same result.

Carbamates or thiocarbamates can be most conveniently prepared by reaction of a hydroxyl group of the furanose in a nucleoside or nucleotide with any of a number of commercially available isocyanates or isothiocyanates, respectively, in an inert solvent. Alternately, when a desired isocyanate or isothiocyanate cannot be obtained from commercial sources, it can be prepared from the corresponding amine by the use of phosgene or thiophosgene, respectively, or their chemical equivalents. Carbonates or thiocarbonates can be synthesized by reacting the hydroxyl groups of a furanose in a nucleoside or nucleotide with an appropriate haloformate in the presence of an organic or inorganic base.
In the general Formulae Ia, Ib and Ib-1, the substituents at Y' and Z', and Y and Z, when taken together, can be taken to mean acetals, ketals or orthoesters, as described in Formula III.

Acetals and ketals can be readily prepared by reaction of the neighboring 2'- and 3'- hydroxyl groups of the furanose in an appropriate nucleoside or nucleotide with an aldehyde or ketone, respectively, or their chemical equivalents, in the presence of an acid catalyst. Particularly advantageous is the use of an organic acid, which can effect the transformation without affecting the integrity of the rest of the molecule. Alternately, strong acids such as trichloroacetic, p-toluensulfonic, methanesulfonic and the like can be employed in catalytic amounts, in conjunction with inert solvents. Most preferred is formic acid, which can be removed by evaporation under reduced pressure, and is ideally suited to serve as both solvent and catalyst for these reactions. Alternately, trifluoroacetic acid can be substituted for formic acid in the reaction; provided that the reaction is carried out at low temperatures and the aldehyde or aldehyde equivalent used to prepare the acetal is stable to strong acid conditions.

Either of the two possible diastereomers that arise from the addition of the acetal to the chiral ribose residue can be synthesized by various procedures. In one example for preparing 2', 3'-phenyl acetal-6-N-ethylurea AMP, one of the diastereomers of the acetal substituted with phenyl (cis-isomer, Compound 43) is prepared by the reaction between benzaldehyde and the 2' and 3' hydroxyl groups of the ribose at low temperature such as -10 to 0°C. The other trans isomer (Compound 42) is prepared by first performing the same acetal-forming reaction at room temperature to produce an equilibrium mixture of both cis and trans diastereomers, then followed by selective degradation under aqueous acid conditions of the cis-isomer. In another example for preparing 2', 3'-cinnamyl acetal-6-N-ethylurea AMP (compound 27), a mixture of cis and trans isomers of the acetal substituted with styryl is prepared by the reaction between cinnamaldehyde and the 2' and 3' hydroxyl groups of the ribose at temperature such as 20°C. The trans isomer (Compound 41) is prepared from the mixture by selective degradation under aqueous acid conditions of the cis-isomer.

Cyclical orthoesters can be prepared by reaction of the neighboring 2'- and 3'- hydroxyl groups of a furanose with an acyclic orthoester, in the presence of an acid. When the nucleoside or nucleotide to be derivatized is a purine that contains a 6-amino functionality or is a pyrimidine that contains a 4-amino functionality, it can be converted to the respective urea or thiourea by treatment with an isocyanate or isothiocyanate, respectively, as was
previously described for carbamates or thiocarbamates of the 2'- or 3'-hydroxyls of a furanose. It was found that reactions of such an amino group with isocyanates or isothiocyanates can be carried out in the presence of one or more hydroxyl groups on a furanose, by appropriate manipulation of the stoichiometry of the reaction.

All of the derivitization reactions described herein can be carried out on preformed dinucleotide polyphosphates, which results in multiple products dependent on reaction stoichiometry and on whether multiple reactive groups are present. When multiple products are obtained, these can be conveniently separated by the use of preparative reverse phase high performance liquid chromatography (HPLC). Particularly advantageous is the use of C18 or phenyl reverse phase columns, in conjunction with gradients that start with ammonium acetate buffer and end with methanol. The use of a buffer provides for nucleotide stability and improved peak shape of the eluting products and the use of methanol allows for effective desorption of these lipophilic compounds from the column. Particularly advantageous is the use of ammonium acetate buffer solutions in conjunction with methanol, as these solvents are miscible in all proportions and can be readily removed from the chromatographed products by evaporation, followed by lyophilization.

While separation of multiple products can be done by HPLC, another strategy is to use nucleosides or nucleotides which contain only a single reactive functionality, whether because only one is present, or by the use of protecting groups to block side reactions at other positions in the molecule. This can be done at the level of preformed dinucleotide polyphosphates, or alternately, can be carried out on nucleoside mono-, di-, or triphosphates, leading to novel products in their own right, or can be coupled to other nucleoside mono-, di, or triphosphates by the methods which have already been described.

The above reactions and purification techniques can also be applied to carba-ribose analogues (e.g., D1 = CH2) of nucleosides, nucleotides and their derivatives, and the terms such as “mononucleotide” and “dinucleotide” also apply to the carba-ribose analogues and other derivatives defined by Formulae I-IV.

Those skilled in the art will recognize various synthetic methodologies that can be employed to prepare non-toxic pharmaceutically acceptable salts and acylated prodrugs of the compounds.
Use of P2Y₁₂ Receptor Antagonist Compounds

This invention provides a method of preventing or treating diseases or conditions associated with platelet aggregation and/or platelet activation. The method also provides a method of treating thrombosis. The method comprises administering to a subject a pharmaceutical composition comprising a therapeutic effective amount of P2Y₁₂ receptor antagonist compound, wherein said amount is effective to bind the P2Y₁₂ receptors on platelets and inhibit ADP-induced platelet aggregation.

The compounds of general Formula I are antagonists of the effect of ADP on its platelet membrane receptor, the P2Y₁₂ receptor. The compounds of general Formula I are useful in therapy, in particular in the prevention or treatment of platelet aggregation. The compounds provide efficacy as antithrombotic agents by their ability to block ADP from acting at its platelet receptor site and thus prevent platelet aggregation. The compounds provide a more efficacious antithrombotic effect than aspirin, but with less profound effects on bleeding than antagonists of the fibrinogen receptor.

The P2Y₁₂ receptor antagonists of this invention, in contrast with currently available marketed products clopidogrel (Plavix®) and ticlopidine (Ticlid®), bind to the P2Y₁₂ receptor in a reversible fashion and therefore, the effects of the treatment with compounds described in this invention are reversed by the simple discontinuation of the treatment, restoring the hemostatic functionality of the platelet as necessary. Since platelets are non-nucleated cell particles that lack the ability to synthesize new proteins, treatment of subjects with irreversible P2Y₁₂ antagonists results in the impairment of platelet function that lasts for the lifespan of the platelet (approximately 8 to 10 days). The use of irreversible P2Y₁₂ antagonists such as clopidogrel has been associated with increases in blood loss, transfusion requirements and rate of reoperation after cardiac surgery (Kapetanakis, et al., Eur Heart J. 26: 576-83, 2005). To avoid these complications, subjects undergoing elective surgeries are required to discontinue the treatment with irreversible antagonists for at least five days prior to the surgery, which increases the risk of a thrombotic event during this period. Therefore, the compounds described in this invention represent an advantage over the currently marketed compounds.

The ADP-induced platelet aggregation is mediated by the simultaneous activation of both P2Y₁₂ and P2Y₁ receptors, thus the combined administration of the Formula I compounds with antagonists of platelet P2Y₁ receptors can provide a more efficacious antithrombotic effect at concentrations of each antagonist that are below the effective
concentrations to block each receptor subtype in other systems, resulting in a decrease of the potential manifestation of adverse effects. In addition, these compounds can be used in conjunction with lower doses of other platelet aggregation inhibitors, which work by different mechanisms, to reduce the possible side effects of said agents.

The compounds of the present invention are useful as anti-thrombotic agents, and are thus useful in the treatment or prevention of unstable angina, coronary angioplasty (PTCA) and myocardial infarction.

The compounds of the present invention are useful in the treatment or prevention of primary arterial thrombotic complications of atherosclerosis such as thrombotic stroke, peripheral vascular disease, and myocardial infarction without thrombolysis.

The compounds of the invention are useful for the treatment or prevention of arterial thrombotic complications due to interventions in atherosclerotic disease such as angioplasty, endarterectomy, stent placement, coronary and other vascular graft surgery.

The compounds of the invention are useful for the treatment or prevention of thrombotic complications of surgical or mechanical damage such as tissue salvage following surgical or accidental trauma, reconstructive surgery including skin flaps, and “reductive” surgery such as breast reduction.

The compounds of the present invention are useful for the prevention of mechanically-induced platelet activation in vivo, for example, caused by cardiopulmonary bypass, which results in temporary platelet dysfunction (prevention of microthromboembolism). The compounds of the present invention are useful for prevention of mechanically-induced platelet activation in vitro. For example, the compounds are useful in the preservation of blood products, e.g. platelet concentrates, prevention of shunt occlusion such as renal dialysis and plasmapheresis, and thrombosis secondary to vascular damage/inflammation such as vasculitis, arteritis, glomerulonephritis and organ graft rejection.

The compounds of the present invention are useful in disorders with a diffuse thrombotic/platelet consumption component such as disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, heparin-induced thrombocytopenia and pre-eclampsia/eclampsia.

The compounds of the invention are useful for the treatment or prevention of venous thrombosis such as deep vein thrombosis, veno-occlusive disease, hematological conditions such as thrombocythemia and polycythemia, and migraine.
The compounds of the present invention are useful in treating a mammal to alleviate the pathological effects of atherosclerosis and arteriosclerosis, acute MI, chronic stable angina, unstable angina, transient ischemic attacks and strokes, peripheral vascular disease, arterial thrombosis, preeclampsia, embolism, restenosis or abrupt closure following angioplasty, carotid endarterectomy, and anastomosis of vascular grafts.

The compounds of the present invention are useful in treating chronic or acute states of hyper-aggregability, such as disseminated intravascular coagulation (DIC), sepsis, surgical or infectious shock, post-operative and post-partum trauma, cardiopulmonary bypass surgery, incompatible blood transfusion, abruptio placenta, thrombotic thrombocytopenic purpura (TTP), snake venom and immune diseases, are likely to be responsive to such treatment.

The compounds of the present invention are useful in treating diseases or conditions associated with platelet activation and/or aggregation produced by the contact of blood with an artificial device. In one embodiment, the artificial device is a paracorporeal artificial lung and an extracorporeal membrane oxygenation device. In another embodiment, the artificial device is an internal implantable artificial heart. In another embodiment, the artificial device is an apheresis instrument used to remove or isolate a specific component of the blood, and returning the remaining blood components to the donor. In yet another embodiment, the artificial device is a hemodialysis instrument.

The compounds of the present invention are useful in vitro to inhibit the aggregation of platelets in blood and blood products, e.g. for storage, or for ex vivo manipulations such as in diagnostic or research use. In such applications, the compounds are administered to the blood or blood product.

Finally, if the compounds of the present invention have sufficient binding affinity and bear a fluorescent moiety, they are useful as biochemical probes for the P2Y\textsubscript{12} receptor.

In a preferred embodiment, the compounds are used in the treatment of unstable angina, coronary angioplasty and myocardial infarction.

In another preferred embodiment, the compounds are useful as adjunctive therapy in the prevention or treatment of thrombotic disorders, such as coronary arterial thrombosis during the management of unstable angina, coronary angioplasty and acute myocardial infarction, i.e. perithrombolysis. The compounds are administered in combination with other antiplatelet and/or anticoagulant drugs such as heparin, aspirin, GP IIb/IIIa antagonists, or thrombin inhibitors.
This invention provides a method of inhibiting platelet aggregation and clot formation in a mammal, especially a human, which comprises administering to the subject a compound of Formula (I) and a pharmaceutically acceptable carrier.

This invention further provides a method for inhibiting the reocclusion of an artery or vein following fibrinolytic therapy, which comprises administering to a subject a compound of Formula (I) and a fibrinolytic agent. When used in the context of this invention, the term fibrinolytic agent is intended to mean any compound, whether a natural or synthetic product, which directly or indirectly causes the lysis of a fibrin clot. Plasminogen activators are a well known group of fibrinolytic agents. Useful plasminogen activators include, for example, anistreplase, urokinase (UK), pro-urokinase (pUK), streptokinase (SK), tissue plasminogen activator (tPA) and mutants, or variants thereof, which retain plasminogen activator activity, such as variants which have been chemically modified or in which one or more amino acids have been added, deleted or substituted or in which one or more functional domains have been added, deleted or altered such as by combining the active site of one plasminogen activator or fibrin binding domain of another plasminogen activator or fibrin binding molecule.

Extracorporeal circulation is routinely used for cardiovascular surgery in order to oxygenate blood. Platelets adhere to surfaces of the extracorporeal circuit. Platelets released from artificial surfaces show impaired hemostatic function. Compounds of the invention can be administered to prevent adhesion.

Other applications of these compounds include prevention of platelet thrombosis, thromboembolism and reocclusion during and after thrombolytic therapy and prevention of platelet thrombosis, thromboembolism and reocclusion after angioplasty of coronary and other arteries and after coronary artery bypass procedures.

The active compounds can be administered systemically to target sites in a subject in need such that the extracellular concentration of a P2Y12 agonist is elevated to block the binding of ADP to P2Y12 receptor, thus inhibit the platelet aggregation. The term systemic as used herein includes subcutaneous injection, intravenous, intramuscular, intrasternal injection, intravitreal injection, infusion, inhalation, transdermal administration, oral administration, rectal administration and intra-operative instillation.

For systemic administration such as injection and infusion, the pharmaceutical formulation is prepared in a sterile medium. The active ingredient, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Adjuvants such
as local anesthetics, preservatives and buffering agents can also be dissolved in the vehicle. The sterile indubitable preparation can be a sterile indubitable solution or suspension in a non-toxic acceptable diligent or solvent. Among the acceptable vehicles and solvents that can be employed are sterile water, saline solution, or Ringer's solution.

Another method of systemic administration of the active compound involves oral administration, in which pharmaceutical compositions containing active compounds are in the form of tablets, lozenges, aqueous or oily suspensions, viscous gels, chewable gums, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

For oral use, an aqueous suspension is prepared by addition of water to dispersible powders and granules with a dispersing or wetting agent, suspending agent one or more preservatives, and other excipients. Suspending agents include, for example, sodium carboxymethylcellulose, methylcellulose and sodium alginate. Dispersing or wetting agents include naturally-occurring phosphatides, condensation products of an allylene oxide with fatty acids, condensation products of ethylene oxide with long chain aliphatic alcohols, condensation products of ethylene oxide with partial esters from fatty acids and a hexitol, and condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anydrides. Preservatives include, for example, ethyl, and n-propyl p-hydroxybenzoate. Other excipients include sweetening agents (e.g., sucrose, saccharin), flavoring agents and coloring agents. Those skilled in the art will recognize the many specific excipients and wetting agents encompassed by the general description above.

For oral application, tablets are prepared by mixing the active compound with nontoxic pharmaceutically acceptable excipients suitable for the manufacture of tablets. These excipients can be, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example, starch, gelatin or acacia; and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glycercyl monostearate or glyceryl distearate can be employed. Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin or
olive oil. Formulation for oral use can also be presented as chewable gums by embedding the active ingredient in gums so that the active ingredient is slowly released upon chewing.

Additional means of systemic administration of the active compound to the target platelets of the subject would involve a suppository form of the active compound, such that a therapeutically effective amount of the compound reaches the target sites via systemic absorption and circulation.

For rectal administration, the compositions in the form of suppositories can be prepared by mixing the active ingredient with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the compound. Such excipients include cocoa butter and polyethylene glycols.

The active compounds can also be systemically administered to the platelet aggregation sites through absorption by the skin using transdermal patches or pads. The active compounds are absorbed into the bloodstream through the skin. Plasma concentration of the active compounds can be controlled by using patches containing different concentrations of active compounds.

One systemic method involves an aerosol suspension of respirable particles comprising the active compound, which the subject inhales. The active compound would be absorbed into the bloodstream via the lungs, and subsequently contact the target platelets in a pharmaceutically effective amount. The respirable particles can be liquid or solid, with a particle size sufficiently small to pass through the mouth and larynx upon inhalation; in general, particles ranging from about 1 to 10 microns, but more preferably 1-5 microns, in size are considered respirable.

Another method of systemically administering the active compounds to the platelet aggregation sites of the subject involves administering a liquid/liquid suspension in the form of eye drops or eye wash or nasal drops of a liquid formulation, or a nasal spray of respirable particles that the subject inhales. Liquid pharmaceutical compositions of the active compound for producing a nasal spray or nasal or eye drops can be prepared by combining the active compound with a suitable vehicle, such as sterile pyrogen free water or sterile saline by techniques known to those skilled in the art.

Intravitreal delivery can include single or multiple intravitreal injections, or via an implantable intravitreal device that releases P2Y12 antagonists in a sustained capacity. Intravitreal delivery can also include delivery during surgical manipulations as either an
adjunct to the intraocular irrigation solution or applied directly to the vitreous during the surgical procedure.

For systemic administration, plasma concentrations of active compounds delivered can vary according to compounds; but are generally $1 \times 10^{-10}$-$1 \times 10^{-4}$ moles/liter, and preferably $1 \times 10^{-8}$-$1 \times 10^{-5}$ moles/liter.

The pharmaceutical utility of P2Y$_{12}$ antagonist compounds of this invention is indicated by their inhibition of ADP-induced platelet aggregation. This widely used assay, as described in S.M.O. Hourani et al. Br. J. Pharmacol. 105, 453-457 (1992) relies on the measurement of the aggregation of a platelet suspension upon the addition of an aggregating agent such as ADP.

The invention is illustrated further by the following examples that are not to be construed as limiting the invention in scope to the specific procedures described in them.

**EXAMPLES**

Example 1

2′(3′)-O-((phenylaminocarbonyl) -uridine 5′)-triphosphate

Uridine 5′- triphosphate, ditributylammonium salt (100 mg, 0.176 mmol; prepared from the trisodium salt by treatment with Dowex 50Wx4 H$^+$ in water, followed by mixing the protonated species with an excess of tributylamine, stripping and lyophilization) was dissolved in dry DMF (1 mL) and phenylisocyanate (19 μL, 0.176 mmol) added. The reaction mixture was heated at 45°C for 15 minutes, at which point a further portion of phenylisocyanate (19 μL, 0.176 mmol) was added. The solution was heated at 45°C overnight and the DMF was removed on a rotary evaporator. The residual oil was partitioned between water (2 mL) and ethyl acetate (2 mL) and the layers were separated. The aqueous layer was extracted twice more with ethyl acetate (2 mL each) and the water was removed on a rotary evaporator. The residue was dissolved in water (1.5 mL) and the product isolated by repeated injections onto a preparative HPLC column (Alltech Nucleotide/Nucleoside C18, 7μm, 10 X 250 mm, gradient from 0.1 M ammonium acetate to methanol over 30 minutes, 5 mL/min, monitor at 260 nm). The yield of the carbamate was 26mg (22%, calculated for the tetraammonium salt). 1H NMR showed the product to be a mixture of 2′ and 3′ carbamates.
The product so obtained can be used for the purposes of this invention per se or can be activated with a suitable coupling agent (e.g. a carbodiimide) and reacted with a variety of nucleotides to generate novel dinucleoside polyphosphates.

1H NMR (D2O, 300 MHz): δ 4.10-4.47 (m, 4H), 5.17 (m, 1H), 5.83 (dd, 1H), 5.96 (m, 1H), 7.04 (t, 1H), 7.25 (m, 4H), 7.79 (m, 1H). 31P NMR (D2O, 121.47 MHz): δ -9.54 (m, 1P), -10.20 (m, 1P), -21.87 (m, 1P).

Example 2

2'(3')-O-(phenylaminocarbonyl)-P1,P4-di( uridine 5'-)tetraphosphate

["monophenylcarbamate Up4U"] Di- 2'(3')-O-(phenylaminocarbonyl)-P1,P4-di( uridine 5'-)tetraphosphate ["diphenylcarbamate Up4U"] and Tri- 2'(3')-O-(phenylaminocarbonyl)-P1,P4-di( uridine 5'-)tetraphosphate ["triphenylcarbamate Up4U"]

P1,P4-Di( uridine 5'-) tetraphosphate, ditributylammonium salt (211 mg, 0.182 mmol; prepared from the tetrasodium salt by treatment with Dowex 50Wx4 H⁺ in water, followed by mixing the protonated species with an excess of tributylamine, stripping and lyophilization) was dissolved in dry DMF (2 mL) and phenylisocyanate (40 μL, 3.64 mmol) added in a single portion. The homogeneous reaction mixture was heated overnight at 45°C, whereupon TLC (silica gel, 50% isopropanol / 50% ammonium hydroxide) indicated a substantial conversion to two products. The solvent was removed on a rotary evaporator and the residue was partitioned between water (7 mL) and ethyl acetate (10 mL). The layers were separated, and the aqueous was extracted twice more with ethyl acetate. (10 mL each). The water was removed from the aqueous extract and the residual oil lyophilized overnight. The solid obtained was reconstituted in water (3 mL) and the two products separated by repeated injections onto a semipreparative HPLC column (Alltech Nucleotide/Nucleoside C18, 7μm, 10 X 250 mm, gradient from 0.1 M ammonium acetate to methanol over 30 minutes, 5 mL/min, monitor at 260 nm). Stripping and lyophilization gave the mono-phenylcarbamate (48 mg, 27 % yield), di-phenylcarbamate (16 mg, 8% yield) and a trace amount of the triphenylcarbamate, as the tetraammonium salts. All three products were mixtures of the corresponding 2'/3' regiosomers.
Monophenylcarbamate: $^1$H NMR (D$_2$O, 300 MHz): δ 4.08-4.65 (m, 9H), 5.14 (d, 1H), 5.75-5.94 (m, 4H), 7.01 (t, 1H), 7.22 (m, 4H), 7.76 (m, 2H). $^{31}$P NMR (D$_2$O, 121.47 MHz): δ -10.17 (m, 2P), -21.81 (m, 2P).
Diphenylcarbamate: $^1$H NMR (D$_2$O, 300 MHz): δ 4.13-4.43 (m, 8H), 5.12 (m, 2H), 5.84 (m, 4H), 7.01 (m, 2H), 7.21 (m, 8H), 7.75 (dd, 2H). $^{31}$P NMR (D$_2$O, 121.47 MHz): δ -10.19 (m, 2P), -21.65 (m, 2P).
Triphenylcarbamate: $^1$H NMR (D$_2$O, 300 MHz): δ 4.29 (m, 7H), 4.510 (m, 1H), 5.27 (m, 2H), 5.87 (m, 4H), 7.09 (m, 15H), 7.76 (d, 2H). $^{31}$P NMR (D$_2$O, 121.47 MHz): δ -10.30 (m, 2P), -21.73 (m, 2P).

Example 3

P$^1$P$^4$-Tetra-((2'(3')-O-(phenylaminocarbonyl) di(urate 5')-tetraphosphate
[tetraphenylcarbamate Up4U”]

This derivative was prepared according to the method of example 2. P$^1$P$^4$-Di(urate 5')-tetraphosphate, ditributylammonium salt (200 mg, 0.172 mmol) was treated with 16 eq of phenylisocyanate (300 uL, 2.76 mmol) in DMF and stirred overnight at 35°C. The solvent was evaporated and the excess reagents removed by extraction of an aqueous solution of the product with ethyl acetate. Following preparative HPLC as previously described, 93 mg (30% yield) of the tetraphenylcarbamate was obtained.

Example 4

2',3'-((benzyl)methyleneoxy-P$^1$P$^4$-di(urate 5')-tetraphosphate [“Mono 2'/3'
benzylacetal Up4U”] and P$^1$P$^4$-Di-2',3'-((benzyl)methyleneoxy) di(urate 5'
)tetraphosphate [“Di 2'/3' benzylacet al Up4U”]

P$^1$P$^4$-Di(urate 5')-tetraphosphate, tetrasodium salt (290 mg, 0.332 mmol) was dissolved in 98% formic acid and phenylacetaldehyde, dimethyl acetal (110 uL, 0.662 mmol) added. The reaction was stirred at ambient temperature for 3 days, at which point TLC (silica gel, 50% isopropanol / 50% ammonium hydroxide) and HPLC (C18) showed good conversion to two less polar products. The formic acid was removed on a rotary evaporator, and the residue
partitioned between 0.7 M ammonium bicarbonate (15 mL) and butyl acetate (15 mL). The layers were separated and the aqueous was washed with a further portion of butyl acetate (10 mL). The aqueous layer was stripped and the residue lyophilized overnight. The crude product was dissolved in water (5 mL) and the components separated by preparative HPLC (Waters Novapak C18, 6um, 25 X 100 mm, gradient from 0.1 M ammonium acetate to methanol over 30 minutes, 30 mL/min, monitor at 260 nm). The yield of the monoacetal was 88 mg (28%) and of the diacetal 60mg (17%), both as the tetraammonium salts.

Monoacetal: $^1$H NMR (D$_2$O, 300 MHz): $\delta$ 2.99 (d, 2H), 4.01-4.32 (m, 8H), 4.77 (m, 2H), 5.33 (m, 2H), 5.74 (d, 1H), 5.81 (m, 2H), 7.21 (m, 5H), 7.64 (d, 1H), 7.79 (d, 1H). $^{31}$P NMR (D$_2$O, 121.47 MHz): $\delta$ -10.18 (m, 1P), -10.78 (m, 1P), -22.00 (m, 2P).

Diacetal: $^1$H NMR (D$_2$O, 300 MHz): $\delta$ 2.98 (d, 4H), 3.99 (m, 4H), 4.27 (m, 2H), 5.27 (m, 2H), 5.36 (m, 2H), 5.73 (d, J= 8.1 Hz, 2H), 7.21 (m, 10H), 7.61 (d, J= 8.1 Hz, 2H). $^{31}$P NMR (D$_2$O, 121.47 MHz): $\delta$ -10.57 (m, 2P), -21.81 (m, 2P).

Example 5

$2',3'$-((benzyl)methyleneedioxy) P$^1$,P$^3$- uridine 5'- triphosphate ["2'3' phenylacetaldehyde acetal Up3U"] and P$^1$,P$^3$-Di-(2',3'-((benzyl)methyleneedioxy) uridine 5')-triphosphate ["di 2'3' phenylacetaldehyde acetal Up3U"]

P$^1$,P$^3$-Di(uridine 5') triphosphate, trisodium salt (100 mg, 0.129 mmol) was dissolved in 98% formic acid and phenylacetaldehyde, dimethyl acetal (64 uL, 0.386 mmol) added. After overnight stirring at room temperature, the formic acid was removed, and the residue partitioned between 1 M sodium bicarbonate and ethyl acetate. Following removal of the organic layer, the product was purified on preparative HPLC, as previously described. Following lyophilization, 40 mg (36%) of the monoacetal and 24 mg (19%) of the diacetal were obtained.

Monoacetal: $^1$H NMR (D$_2$O, 300 MHz): $\delta$ 7.7s (d, 2H), 7.54 (d, 2H), 7.16 (s, 5H), 5.70 (m, 3H), 5.31 (s, 1H), 5.23 (s, 1H), 4.66 (m, 2H), 4.10 (m, 8H), 2.93 (d, 2H). $^{31}$P NMR (D$_2$O, 121.47 MHz): $\delta$ -10.30 (m, 1P), 10.81 (m, 1P), -21.99 (m, 1P).

Diacetal: $^1$H NMR (D$_2$O, 300 MHz): $\delta$ 7.51 (d, 2H), 7.15 (m, 10H), 5.65 (d, 2H), 5.31 (d, 2H), 5.20 (t, 2H), 4.63 (m, 2H), 4.13 (m, 2H), 3.88 (m, 4H), 2.90 (d, 4H). $^{31}$P NMR (D$_2$O, 121.47 MHz): $\delta$ -10.75 (m, 2P), -21.97 (m, 1P).
Example 6

P³⁻,2',3'-(benzyl)methyleneoxy) (uridine 5'⁻) P⁴⁻( deoxycytidine 5'⁻) tetraphosphate
["2'3' phenylaceteldehyde acetal Up4dC"]

P³⁻ (uridine 5'⁻) P⁴⁻( deoxycytidine 5'⁻) tetraphosphate, tetrasodium salt (100 mg, 0.16
5 mmol) was dissolved in 98% formic acid (1 mL), and phenylaceteldehyde, dimethyl acetel
(57 uL, 0.384 mmol) added. After overnight stirring, the formic acid was removed and the
residue partitioned between 1 M sodium bicarbonate and ethyl acetel. After separation of
the layers, the product was purified on preparative HPLC, as previously described. Yield 40
mg (36 %). This product was amenable to subsequent modification of the deoxy cytidine
10 base by the procedures described in examples 9-13, giving rise to lipophilic bifunctional
molecules falling within the scope of this invention.

Monoacetal: ¹H NMR (D₂O, 300 MHz): δ 7.98 (d, 1H), 7.62 (d, 1H), 7.21 (m, 5H), 6.11 (m,
2H), 5.74 (d, 1H), 5.39 (d, 1H), 5.31 (t, 1H), 4.77 (m, 2H), 4.45 (m, 1H), 4.32 (m, 1H), 4.03
(m, 5H), 2.99 (d, 2H), 2.29 and 2.21 (m, 2H). ³¹P NMR (D₂O, 121.47 MHz): δ -10.15 (m,
15 1P), -10.68 (m, 1P), -21.98 (m, 2P).

Example 7

3'-O-(phenylaminocarbonyl) -2'-deoxy (uridine 5')- monophosphate

Deoxyuridine 5'- monophosphate, tetrabutylammonium salt (135 mg, 0.274 mmol;
20 prepared from the disodium salt by treatment with Dowex 50Wx4 H⁺, followed by stirring the
resultant neutral species with excess tributylamine, stripping and lyophilization) was
dissolved in dry DMF (1 mL). Phenylisocyanate (60 uL, 0.547 mmol) was added and the
mixture heated overnight at 45°C, at which time TLC (silica gel, 50% isopropanol / 50%
ammonium hydroxide) and HPLC (C18) indicated a substantial conversion to a less polar
product. The DMF was stripped on a rotary evaporator and the oily residue partitioned
between water (10 mL) and ethyl acetel (10 mL). The layers were separated and the aqueous
layer was rewashened with ethyl acetel (2 X 10 mL). The water was removed and the residue
was dissolved in water (2 mL). The product was isolated by repeated injections onto
30 semipreparative HPLC (Alltech Nucleotide/Nucleoside C18, 7um, 10 X 250 mm, gradient
from 0.1 M ammonium acetel to methanol over 30 minutes, 5 mL/min, monitor at 260 nm).
The yield was 67 mg as the dianmonium salt (53 %).

¹H NMR (D₂O, 300 MHz): δ 2.21 (m, 2H), 3.84 (s, 2H), 4.13 (s, 1H), 5.08 (d, 1H), 5.63 (d,
1H), 6.06 (t, 1H), 6.89 (br. t, 1H), 7.10 (m, 4H), 7.72 (d, 1H).
$^{31}$P NMR (D$_2$O, 121.47 MHz): δ -2.31 (s).

**P$_1$-(3'-O-(phenylaminocarbonyl)-2'-deoxyuridine 5')-P$_4$-(uridine 5')-tetraphosphate**

Uridine 5'-triphosphate, ditributylammonium salt (prepared from the trisodium salt by treatment with Dowex 50Wx4 H$^+$, followed by stirring the resultant neutral species with excess tributylamine, stripping and lyophilization) is treated with 1.5 equivalents of dicyclohexylcarbodiimide in DMF for 2 hours at room temperature. The dicyclocexylurea is filtered off, and the resultant uridine 5'-cyclical triphosphate is treated with 3'-O-(phenylaminocarbonyl) -2'-deoxy (uridine 5')- monophosphate, which is in the monotributylammonium salt form. The reaction mixture is stirred for several days at 45°C, and the solvent is removed. The products are separated by preparative HPLC, as has been previously described.

**Example 8**

2'(3')-(2-methylamino)benzoyl-P$_1$,P$_4$-di(uridine 5')-tetraphosphate ("MANT Up4U") and P$_1$,P$_4$-Di-(2'(3')-(2-methylamino)benzoyl uridine 5')-tetraphosphate ("Bis MANT Up4U")

P$_1$,P$_4$-Di(uridine 5')-tetraphosphate, tetrascalate salt (800 mg, 0.93 mmol) was dissolved in water (5 mL) and the pH adjusted to 7.6 by the addition of solid sodium bicarbonate. N,N-dimethylformamide (DMF, 5 mL) was added, followed by N-acetic anhydride (231 mg, 1.3 mmol) and the suspension was heated at 50°C for 2.5 hrs. TLC (silica gel, 50% isopropanol, 50% ammonium hydroxide) indicated that the reaction was not done by this time, so a further portion of N-acetic anhydride (100 mg, 0.56 mmol) was added and the reaction heated for another hour. The DMF was removed on a rotary evaporator and the residue was dissolved in a minimum of water and applied to a DEAE Sephadex A-25 column (3 X 60 cm). The column was eluted with a stepwise gradient from water to 1 M ammonium bicarbonate and the eluent monitored with a UV detector set at 254 nm. The two products that eluted were collected separately and the solvent was removed from each and the residue lyophilized overnight. $^1$H NMR indicated that the first product to elute was the monoacylated compound, while the latter was the diacylated derivative, and that both were mixtures with the acylation at either the 2' or 3' hydroxyls, but without two carbamates on the same sugar. The yield of the monoaminobenzoylated product was 150 mg (16%); the yield of the diaminobenzoylated compound was 91 mg (8.7%).
Monoaminobenzoylated derivative: $^1$H NMR ($D_2O$, 300MHz): $\delta$ 2.70 (s, 3H), 4.09-4.55 (m, 9H), 5.34 (m, 1H), 5.71 (m, 2H), 5.83 (dd, 1H), 6.01 (m, 1H), 6.57 (m, 1H), 6.65 (m, 1H), 7.25 (t, 1H), 7.72 (d, 2H), 7.81 (m, 2H). $^{31}$P NMR ($D_2O$, 121.47 MHz): $\delta$ -10.20 (m, 2P), -21.83 (m, 2P).

Diaminobenzoylated derivative: $^1$H NMR ($D_2O$, 300 MHz): $\delta$ 2.69 (s, 6H), 4.15-4.51 (m, 8H), 5.27 (m, 2H), 5.86 (m, 4H), 6.60 (m, 4H), 7.30 (m, 2H), 7.79 (m, 4H). $^{31}$P NMR ($D_2O$, 121.47 MHz): $\delta$ -10.16 (m, 2P), -21.76 (m, 2P).

Example 9

$P^1$-(4-N-(4-methoxyphenyl)aminocarbonylcytidine 5’-)$-P^4$-(uridine 5’-) tetraphosphate

$P^1$-(cytidine 5’-)$-P^4$-(uridine 5’-) tetraphosphate, ditributylammonium salt (50 mg, 0.043mmol; prepared from the tetraammonium salt by treatment with Dowex 50Wx4 H$^+$ in water, followed by mixing the protonated species with an excess of tributylamine in methanol, stripping and lyophilization) was dissolved in dry DMF (1mL) and tributylamine (10 uL, 0.43 mmol), and p-methoxyphenylisocyanate (8.4 uL, 0.648 mmol) were added in a single portion. The homogeneous reaction mixture was heated overnight at 35°C, whereupon TLC (silica gel, 50% isopropanol / 50% ammonium hydroxide) and HPLC (C18) indicated a substantial conversion to a single product. The solvent was removed on a rotary evaporator and the residue dissolved in water (1mL). The product was isolated by repeated injections onto a semi-preparative HPLC column (Alltech Nucleotide/Nucleoside C18, 7um, 10 X 250 mm, gradient from 0.1 M ammonium acetate to methanol over 30 minutes, 5 mL/min, monitor at 260 nm). Stripping and lyophilization gave the p-methoxyphenylurea (24 mg, 55 % yield), as the tetraammonium salt.

The product so obtained can be derivatized on the 2’ and/or 3’ hydroxyl groups according to the foregoing methods (e.g. Examples 2-6).

$^1$H NMR ($D_2O$, 300 MHz): $\delta$ 3.59 (s, 3H), 4.01-4.20 (m, 10H), 5.68 (m, 3H), 6.19 (d, 1H), 6.71 (d, 2H), 7.18 (d, 2H), 7.67 (d, 1H), 8.06 (d, 1H). $^{31}$P NMR ($D_2O$, 121.47 MHz): $\delta$ -10.13 (m, 2P), -21.76 (m, 2P).
Example 10

P\(^1\)-((4-bromophenyl)ethenocytidine 5'-)-P\(^4\)- (uridine 5'-) tetraphosphate

P\(^1\)-(cytidine 5'-)-P\(^4\)-(uridine 5'-) tetraphosphate, tetrosodium salt (500 mg, 0.57 mmol) was dissolved in water (5 mL) and a solution of 2,4'-dibromoacetophenone (792 mg, 2.85 mmol) in DMF (15 mL) added. The mixture was heated overnight at 40°C, and a further portion of the dibromoketone (400 mg, 1.44 mmol) in DMF (5 mL) added. The reaction was heated a further 5 hrs, and the solvents removed by evaporation. The residue was partitioned between water (20 mL) and ethyl acetate (25 mL) and the layers separated. The aqueous layer was washed with further ethyl acetate (2x15 mL) and the aqueous evaporated to dryness. The residue was dissolved in water (5 mL) and the product was isolated by repeated injections onto a semi-preparative HPLC column (see example 6 for conditions). The yield of the pure etheno compound was 80 mg (13.5%)

\(^1\)H NMR (D\(_2\)O, 300 MHz): \(\delta\) 4.06 (m, 8H), 4.36 (m, 2H), 5.64 (dd, 2H), 6.07 (d, 1H), 6.74 (d, 1H), 7.45 (d, 2H), 7.54 (d, 2H), 7.59 (d, 1H), 7.63 (d, 1H), 7.93 (s, 1H). \(^31\)P NMR (D\(_2\)O, 121.47 MHz): \(\delta\) -10.09 (m, 2P), -21.59 (m, 2P).

Example 11

P\(^1\)-((4-bromophenyl)etheno-2'-deoxycytidine 5'-)-P\(^4\)- (uridine 5'-) tetraphosphate

Example 11 product was prepared from 100 mg P\(^1\)-(2'-deoxycytidine 5')-P\(^4\) (uridine 5') tetraphosphate, tetrosodium salt and 2,4'-dibromoacetophenone, according to the general method of example 10. Yield= 35 mg (30%).

\(^1\)H NMR (D\(_2\)O, 300 MHz): \(\delta\) 2.31 (m, 2H), 4.03 (m, 8H), 5.60 (dd, 2H), 6.41 (t, 1H), 6.73 (d, 1H), 7.53 (m, 5H), 7.65 (d, 1H), 7.93 (s, 1H). \(^31\)P NMR (D\(_2\)O, 121.47 MHz): \(\delta\) -10.11 (m, 2P), -21.58 (m, 2P).

Example 12

P\(^1\), P\(^4\)-Di((4-bromophenyl)ethenocytidine 5'-) - tetraphosphate

Example 12 product was prepared from 50 mg P\(^1\),P\(^4\)-Di(cytidine 5') - tetraphosphate, tetrosodium salt and 2,4'-dibromoacetophenone, according to the general method of example 10. Yield= 20 mg (29%).

\(^1\)H NMR (D\(_2\)O, 300 MHz): \(\delta\) 4.24 (m, 10H), 5.98 (d, 2H), 6.39 (d, 2H), 7.14 (m, 8H), 7.45 (m, 4H). \(^31\)P NMR (D\(_2\)O, 121.47 MHz): \(\delta\) -10.13 (m, 2P), -21.68 (m, 2P).
Example 13

P1-((4-phenylphenyl)ethenocytidine 5')-P4-(cytidine 5')- tetraphosphate

Example 13 product was prepared from 50 mg P1,P4-Di(cytidine 5')- tetraphosphate, tetrasodium salt and 2-bromo-4'-phenylacetophenone, according to the general method of example 10. Yield= 15 mg (13%).

1H NMR (D2O, 300 MHz): δ 4.10 (m, 10H), 5.48 (d, 1H), 5.87 (m, 2H), 6.68 (d, 1H), 7.20 (m, 3H), 7.36 (m, 6H), 7.68 (m, 3H). 31P NMR (D2O, 121.47 MHz): δ -10.08 (m, 2P), -21.78 (m, 2P).

The products of examples 11-13 can be further derivatized according to the methods of Examples 1-8, to give bifunctional molecules that fall within the scope of the invention.

Example 14

2', 3'- phenylacetaldehyde acetal adenosine 5'-monophosphate

Adenosine 5'-monophosphate, free acid (10.0 g, 28.8 mmol) was dissolved in trifluoroacetic acid (50 mL) and phenylacetaldehyde, dimethylacetal (18.50 mL, 121 mmol) added. The reaction was stirred at ambient temperature for 3 hours, after which the trifluoroacetic acid was evaporated and the residue partitioned between 1 M sodium bicarbonate (80 mL) and ethyl acetate (40 mL). The layers were separated, and the product was isolated from the aqueous layer via C18 preparative HPLC. Yield= 7.50 g (59 %). The ammonium salt so obtained was converted to the mono-tributylammonium salt via treatment with a slight excess of tributylamine in aqueous N,N-dimethylformamide, followed by evaporation and drying.

1H NMR (D2O, 300 MHz): δ 3.06 (d, 2H), 3.86 (m, 2H), 4.39 (m, 1H), 4.91 (m, 1H), 5.18 (m, 1H), 5.36 (t, 1H), 5.63 (d, 1H), 7.23 (m, 5H), 8.09 (s, 1H), 8.20 (s, 1H). 31P NMR (D2O, 121.47 MHz): δ 2.17 (s).

Example 15

2', 3'- cinnamyl acetal adenosine 5'-monophosphate

Adenosine 5'-monophosphate, free acid (1.0 g, 2.88 mmol) was dissolved in 98% formic acid (5 mL) and cinnamaldehyde (1.14 g, 8.65 mmol) added. The reaction was stirred at ambient temperature for 3 hours, after which the formic acid was evaporated and the residue partitioned between 1 M sodium bicarbonate (25 mL) and ethyl acetate (20 mL). The
layers were separated, and the product was isolated from the aqueous layer via preparative HPLC. Yield = 0.202 g (15%).

$^1$H NMR (D$_2$O, 300 MHz): $\delta$ 3.97 (m, 2H), 4.50 (m, 1H), 5.04 (m, 1H), 5.29 (m, 1H), 5.65 (d, 0.4H), 5.86 (d, 0.6H), 6.24 (m, 2H), 6.87 (dd, 1H), 7.27 (m, 3H), 7.43 (m, 2H), 8.12 (d, 1H), 8.28 (d, 1H).

$^{31}$P NMR (D$_2$O, 121.47 MHz): $\delta$ 1.42 (d).

Example 16

2', 3'-phenylacetaldehyde acetal-6-N-phenylurea adenosine 5'-monophosphate (compound 22)

2',3'-phenylacetaldehyde acetal adenosine 5'-monophosphate, tributylammonium salt (prepared according to example 14, 1.0 g, 2.15 mmol) was dissolved in N,N-dimethylformamide (10 mL) and phenylisocyanate (1.17 g, 10.72 mmol) added. The reaction was heated at 35°C for 4 hrs, after which the solvent was removed and the residue partitioned between 1 M sodium bicarbonate (30 mL) and ethyl acetate (25 mL). The layers were separated and the product isolated from the aqueous layer via preparative HPLC. Yield = 0.85 g (68%).

$^1$H NMR (D$_2$O, 300 MHz): $\delta$ 2.97 (d, 2H), 3.81 (m, 2H), 4.31 (m, 1H), 4.78 (m, 1H), 4.98 (m, 1H), 5.23 (t, 1H), 5.63 (d, 1H), 6.74 (m, 1H), 6.96 (m, 4H), 7.19 (m, 5), 8.12 (s, 1H), 8.30 (s, 1H).

$^{31}$P NMR (D$_2$O, 121.47 MHz): $\delta$ 1.19 (s).

Example 17

2', 3'-cinnamyl acetal-6N-ethyurea adenosine 5'-monophosphate (Compound 27)

Compound 27 was prepared according to example 16, starting with 2’3’ cinnamyl acetal adenosine 5'-monophosphate (Example 15) and substituting ethyl isocyanate for phenylisocyanate. Yield = 65%.

$^1$H NMR (D$_2$O, 300 MHz): $\delta$ 1.07 (t, 3H), 3.21 (q, 2H), 3.93 (m, 2H), 4.45 (m, 1H), 4.99 (m, 1H), 5.28 (m, 1H), 5.54 (d, 0.3H), 5.70 (d, 0.7H), 5.95 (m, 1H), 6.14 (m, 1H), 6.61 (dd, 1H), 7.14 (m, 5H), 8.29 (m, 2H).

$^{31}$P NMR (D$_2$O, 121.47 MHz): $\delta$ 1.93 (d).
Example 18

Trans-2', 3'-cinnamyl acetal-6N-ethyurea adenosine 5'-monophosphate (compound 41)

Compound 41 (trans-2', 3'-cinnamyl acetal-6N-ethyurea adenosine 5'-monophosphate) was obtained by the separation of the two diastereomers contained within Compound 27 (Example 17) by HPLC. Compound 41 is typically converted to the bis-sodium salt form to enhance its isolation from solution and to increase the stability of the resultant dry powder.

HPLC Method: Column: Phenomenex Synergi Polar RP, 4 μm, 80 angstrom, 150 x 3.0 mm; Mobile Phase: 0.1 M ammonium acetate buffer, pH = 5:acetonitrile (70:30);
Detection: UV, 254 nm; Column temperature: RT; Flow Rate: 1.5 mL/min; Retention time of Compound 41 = 6.4 min.

Example 19

NMR data of trans-2', 3'-cinnamyl acetal-6N-ethyurea adenosine 5'-monophosphate (Compound 41)

Chemical Name: Phosphoric acid mono-{6-[6-(3-ethyl-ureido)-purin-9-yl]-2-styryl-tetrahydro-furo[3,4-d][1,3]dioxol-4-ylmethyl} ester bis sodium salt
Molecular Formula: C_{22}H_{23}N_{6}Na_{2}O_{8}P
Molecular Weight: 576.41

^{1}H NMR (D_{2}O, 300 MHz): δ 1.11 (t, 3H, J = 7.3 Hz), 3.24 (q, 2H, J = 3.27 Hz), 3.90 (d, 2H), 4.49 (m, 1H), 4.99 (m, 1H), 5.07 (dd, 1H, J = 3.3 Hz), 5.32 (dd, 1H, J = 6.4 Hz), 5.79 (d, 1H, J = 6.6 Hz), 6.02 (dd, 1H, J = 6.6 and 16.0 Hz), 6.18 (d, 1H, J = 3.7 Hz), 6.62 (d, 1H, J = 16.0 Hz), 7.14-7.23 (m, 5H), 8.33 (s, 1H), 8.47 (s, 1H).

^{13}C NMR (D_{2}O, 121 MHz):
14.2, 63.9, 80.24, 83.54, 83.9, 87.83, 119.23, 142.11, 149.27, 149.34, 150.87 \(^{31}\)P NMR (D\(_2\)O, 75 MHz): \(\delta\ 5.168\) (s). Optical rotation = -0.201° at a concentration of 5mg/mL in water (specific rotation = -50.4°)

5

**Example 20**

**Compounds 31-40**

2', 3'-phenyl acetal adenosine 5'-monophosphate was prepared from the reaction between adenosine 5'-monophosphate, free acid, and benzaldehyde in trifluoroacetic acid according to Example 15; the yield was 82%.

10

2', 3'-phenyl acetal adenosine 5'-monophosphate was further elaborated into Compounds 36-40. Compound 36 was prepared by acylation of the adenine 6 position with the ethyl isocyanate according to the general method of Example 16. Compounds 37-40 are likewise derived from the reaction between 2', 3'-phenyl acetal adenosine 5'-monophosphate and an appropriate isocyanate (propyl, butyl, hexyl, and cyclopentyl, respectively).

15

Similarly, Compounds 31, 32, 33, and 35 were prepared first by reacting adenosine 5'-monophosphate, free acid and phenylpropargyl aldehyde in formic acid (35% yield), followed by acylation of the adenine 6 position with the appropriate isocyanate (phenyl, hexyl, butyl, or ethyl respectively). Compound 34 is likewise prepared from 2', 3'-phenylpropargyl acetal adenosine 5'-monophosphate and propyl isocyanate.

20

**Example 21**

**Platelet Aggregation Assays**

Blood was collected from healthy volunteers into syringes containing 1/6 final blood volume of anti-coagulant ACD (65mM citric acid, 85mM sodium citrate, 110mM dextrose) for washed platelet (WP) preparation or into a syringe containing a final concentration of 10 units/mL heparin or 300 \(\mu\)M PPACK for whole blood (WB) assays. The blood collected for whole blood assays was maintained at room temperature and immediately tested as described below. The blood collected for WP was centrifuged at 180g for 15 minutes and the supernatant (platelet rich plasma) was removed. The platelet rich plasma was centrifuged and the platelets were pelleted and resuspended in a buffer consisting of (mM): NaCl (137), KCl (2.7), CaCl\(_2\) (2) MgCl\(_2\) (1), NaH\(_2\)PO\(_4\) (3), Glucose (5), HEPES (10), pH 7.4, 0.2% BSA.

30

These centrifugations and washes were repeated twice following by resuspension in the media described above containing 0.25 U apyrase/mL. Platelet aggregation was measured using the
optical mode of a ChronoLog aggregometer (Havertown, PA). Five hundred µl of platelet suspension containing 1 mg/mL Fibrinogen were warmed to 37°C and stirred at 1000 rpm. Indicated concentrations of ADP were added to the sample and aggregation was monitored for 8 minutes. The effect of the compounds described in this invention were studied following the same protocol with the exception that the inhibitor was incubated for 2-5 minutes prior to the addition of a maximally effective concentration of ADP. For whole blood aggregation, blood was diluted 1:1 with saline and then aggregation was performed in the same manner as described above using the impedance mode of the aggregometer. The potency of agonists and inhibitors of platelet aggregation was calculated from both, the rate of aggregation and the maximal extent of aggregation obtained for each determination by fitting the data to a four-parameter logistic equation using the GraphPad software package (GraphPad Corp. San Diego, CA).

The ability of P2Y<sub>12</sub> antagonists to inhibit platelet aggregation is presented in this application as the percent inhibition of the aggregation induced by a maximally effective concentration of ADP. When a broad range of concentrations of P2Y<sub>12</sub> antagonist was tested (usually from 1nM to 100 µM), an IC<sub>50</sub> value was also obtained. IC<sub>50</sub> values represent the concentration of antagonist needed to inhibit by 50% the aggregation elicited by a given concentration of ADP.

Example 22

**Effect of Different Compounds on ADP-Induced Aggregation**

Different compounds were tested for their inhibition of ADP-induced aggregation and their IC<sub>50</sub> according to the protocols in Example 19; the results are shown in Figure 1. The bar graphs in the figure illustrate the effect of 100 µM concentration of the compound on ADP-induced platelet aggregation, and the data are expressed as % inhibition of the ADP response.

Figure 1 shows the structure and abbreviated name of each compound and its activity. Where hydrogens are understood to be present, they have been omitted for the sake of simplicity. For example, for the first structure of the figure, it is implied that there are hydrogens at the 3'-position of the pyrimidine ring, at the 3'-position of the ribose on the oxygen, and on the nitrogen of the carbamate at the 2'-position of the ribose. In addition, as disclosed within the scope of the present invention, it is implied that the oxygens that are not doubly bonded to the phosphorous atoms are either present in the ionized form as salts with a counterion, or are bonded to a hydrogen atom. For simplicity, some of the structures in the
figure are portrayed in the salt form, but this should not be interpreted as excluding the possibility that hydrogens could be present instead.

Several parent compounds, Up4U, Ip4U, Up3U, and Cp4U, without modifications on the furanose hydroxyl groups, have been included at the end of the figure to illustrate the utility of the present invention. However, these unmodified parent compounds do not inhibit the ADP-induced aggregation and are not within the scope of the present invention.

Example 23

**Calcium Mobilization Assay**

For calcium mobilization assays, cells expressing P2Y₁, P2Y₂, and P2Y₆ were seeded in black wall/clear bottom cell culture plates (Corning Inc., Corning, NY), and assays were conducted 48 hours after plating. On the day of the assay, the growth medium was aspirated and replaced with a solution of Fluo-3 AM (2.5 μM final concentration) in an assay buffer consisting of (mM): KCl (10.0), NaCl (118), CaCl₂ (2.5), MgCl₂ (1.0), HEPES (20), glucose (10), pH 7.4. After a 60-minute incubation with Fluo-3 AM at 25°C, cells were washed free of dye. Cells were treated with different concentrations of test compound followed 1-2 minutes later with the addition of a maximally effective concentration of the cognate receptor agonist. Intracellular calcium levels in response to the treatment of cells with the test compound and the receptor agonist were continuously monitored in each well simultaneously by measuring the changes in fluorescence intensity using the a fluorescence imaging plate reader (FLIPR, Molecular Devices Corp., Sunnyvale, CA). The results of this assay are presented in Table I.

Example 24

**Effect of Different Compounds on ADP-Induced Aggregation and on Activation of P2Y Receptors**

Various compounds were tested for their effects on inhibition of ADP-induced platelet aggregation using the washed platelet preparation as described in Example 21. In addition, the effects of these compounds on the activation of P2Y₁, P2Y₂, P2Y₄, and P2Y₆ receptors were estimated according the calcium mobilization described in Example 23. The results are shown in Table 1. All compounds tested showed no response (NR) at P2Y₁, P2Y₂, P2Y₄, and P2Y₆ receptors. Data presented are from the average of at least two separate experiments.
TABLE 1

<table>
<thead>
<tr>
<th>Compound #</th>
<th>Platelet Aggregation IC₅₀ (nM)</th>
<th>Calcium Mobilization</th>
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<td></td>
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<td>P2Y₁</td>
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<tr>
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<td>160 ± 27</td>
<td>NR</td>
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<tr>
<td>23</td>
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<td>NR</td>
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<td>10</td>
<td>NR</td>
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<td>NR</td>
</tr>
<tr>
<td>47</td>
<td>55</td>
<td>NR</td>
</tr>
</tbody>
</table>

Example 25

Effects of Compounds on Platelet Aggregation In Vivo

To evaluate the ability of these compounds to inhibit platelet aggregation in vivo, an experimental protocol similar to the method of R. G. Humphries et al. (Br. J. Pharmacol. 115:1110-1116, 1995) will be performed.

Surgical Preparation and Instrumentation: Male Sprague-Dawley rats are anesthetized. Body temperature is maintained at 37 ± 0.5°C with a heating lamp. Animals breathe spontaneously and a tracheotomy is performed to ensure a patent airway. A cannula containing heparinized saline is introduced into the left femoral artery and connected to a transducer to record blood pressure and heart rate. Cannulae containing non-heparinized
saline are introduced into the left common carotid artery and left jugular vein for withdrawal
of arterial blood samples and i.v. administration of compounds, respectively.

Experimental Protocol: Either compound or vehicle is administered to each animal as an
infusion. Blood samples are taken immediately prior to the first infusion, at the end of each
infusion and 20 min after cessation of the final infusion for measurement of platelet
aggregation ex vivo. Immediately after sampling, ADP-induced platelet aggregation is
measured in duplicate in 0.5 ml blood samples diluted 1:1 with saline and incubated at 37°C
for 4 min. For the final minute of this period, cuvettes are transferred to aggregometer and
the sample stirred at 900 rpm. ADP (3 μM) is added in a volume of 20 μl and the aggregation
response is recorded.

Example 26

Inhibition of Thrombus Formation in Anesthetized Rats

To evaluate the effect of these compounds on thrombus formation in vivo, the
following experimental protocol is performed.

Rats (CD-1; male; approximately 350 grams; Charles River, Raleigh, NC), are
anesthetized with sodium pentobarbital (70 mg/kg i.p.). The abdomens are shaved and a 22
gauge intravenous catheter is inserted into a lateral tail vein. A midline incision is made and
the intestines are wrapped in saline-soaked gauze and positioned so the abdominal aorta is
accessible. The inferior vena cava and abdominal aorta are carefully isolated and a section
(approx. 1 cm) of the abdominal aorta (distal to the renal arteries proximal to the bifurcation)
is dissected. All branches from the aorta in this section are ligated with 4-0 silk suture. A 2.5
mm diameter flow probe connected to a Transonic flow meter is placed on the artery and a
baseline (pre-stenosis) flow is recorded. Two clips are placed around the artery decreasing
the vessel diameter by approximately 80%. A second baseline flow measurement is taken
(post-stenosis) and the hyperemic response is tested. Animals are then treated with either
compound or saline i.v., via tail vein catheter. Thrombosis is induced five minutes after
treatment by repeated external compressions of the vessel with hemostatic forceps. Two
minutes post-injury, the vessel compressions are repeated and a 10-minute period of flow
monitoring is started. Animals are monitored continuously for a minimum of the first ten
minutes post-injury. After twenty minutes (post-injury), a flow measurement is repeated and
the animals are euthanized. The section of the aorta that includes the injured section is
harvested and placed in 10% formalin for possible histologic evaluation.
Example 27

Inhibition of Thrombus Formation in Anesthetized Dogs

To evaluate the effect of these compounds on dynamic thrombus formation *in vivo*, the following experimental protocol similar to the method of J. L. Romson et al. (*Thromb. Res.* 17:841-853, 1980) is performed.

*Surgical Preparation and Instrumentation:* Briefly, purpose-bred dogs are anesthetized, intubated and ventilated with room air. The heart is exposed by a left thoracotomy in the fifth intercostal space and suspended in a pericardial cradle. A 2-3 cm segment of the left circumflex coronary artery (LCCA) is isolated by blunt dissection. The artery is instrumented from proximal to distal with a flow probe, a stimulation electrode, and a Goldblatt clamp. The flow probe monitors the mean and phasic LCCA blood flow velocities. The stimulation electrode and its placement in the LCCA and the methodology to induce an occlusive coronary thrombus have been described previously (J. K. Mickelson et al., *Circulation* 81:617-627, 1990; R. J. Shebuski et al., *Circulation* 82:169-177, 1990; J. F. Tschopp et al., Coron. Artery Dis. 4:809-817, 1993).

*Experimental Protocol:* Dogs are randomized to one of four treatment protocols (n=6 per treatment group) in which the control group receives saline i.v. and the three drug-treated groups are administered compound i.v. Upon stabilization from the surgical interventions, dogs receive either saline or compound. After approximately 30 minutes, an anodal current is applied to the LCCA for 180 min. The number and frequency of cyclic flow variations (CFV) that precede formation of an occlusive thrombus are recorded. These cyclic phenomena are caused by platelet thrombi that form in the narrowed lumen as a result of platelet aggregation (J. D. Folts et al., *Circulation* 54:365-370, 1976; Bush et al., *Circulation* 69:1161-1170, 1984). Zero flow in the LCCA for a minimum of 30 minutes indicates a lack of antithrombotic efficacy (L.G. Frederick *et al.*, *Circulation* 93:129-134, 1996).

Example 28

Dose-Dependent Inhibition of Platelet Aggregation in Mice

In order to evaluate the effects of inhibition of platelet aggregation *in-vivo* by the compounds described in this invention, experimental protocols similar to those described by Leon et al. (*Circulation* 103:718-723, 2001) were performed.
Anesthetized mice were injected intravenously with either saline or with different doses of trans-2', 3'-cinnamyl acetal-6N-ethylurea adenosine 5'-monophosphate, bis sodium salt (Compound 41, also named as INS50589), usually between 1 and 100 µg/kg. Five minutes after the administration of the compound, 700 µL of blood were obtained from each animal. The blood of two animals was combined and immediately processed to obtain platelet rich plasma for the assessment of platelet aggregation stimulated by 1 and 5 µM ADP. Platelet aggregation was measured using optical aggregometry as described in Example 21. The P2Y₁₂ receptor antagonists described here and tested in this ex-vivo model produced a dose-dependent inhibition of platelet aggregation.

Example 29

Reversible Inhibition of Platelet Aggregation by P2Y₁₂ Receptor Antagonists in Mice

A key characteristic of the compounds described in this invention is the reversible nature of the inhibition of platelet aggregation. The model system described in Example 28 was also used to study the kinetics of in-vivo inhibition of platelet aggregation with selected compounds. Briefly, a maximally effective concentration of test compound was administered intravenously, and blood samples were obtained before and at different times (usually 0, 1, 5, 15 and 30 minutes) after the administration of trans-2', 3'-cinnamyl acetal-6N-ethylurea adenosine 5'-monophosphate, bis sodium salt (Compound 41, also named as INS50589).

Blood samples were processed immediately and aggregation induced by 1 µM and 5 µM ADP was estimated as described in Example 28. Complete inhibition of ADP-induced platelet aggregation was observed five minutes after administration of the test compound, and by 30 minutes the aggregation response to ADP returned to values similar to those observed in control animals, suggesting that the effect of the test compound in mice is reversible (Figure 2).

Example 30

Prevention of Thromboembolism-Induced Mortality in Mice by the Treatment with P2Y₁₂ Receptor Antagonists.

Ten anesthetized mice were treated with vehicle (control) or with a single bolus intravenous administration of test compound (usually between 10 and 25 mg/kg). Five minutes after treatment, the animals were injected with a mixture of 0.3 mg/kg collagen and 60 µg/kg epinephrine. The survival time after the administration of collagen and epinephrine in animals pretreated with saline or 25 mg/kg of the test compound (INS50589) are shown
using the Kaplan-Meier plot in Figure 3. Ninety percent of the vehicle-pretreated animals
died within 6 minutes after the IV injection of collagen and epinephrine. In contrast,
mortality resulting from systemic intravascular thromboembolism was observed in only 20%
of the animals pretreated with the test compound (Figure 3). These results demonstrate a
significant antithrombotic effect of the \textit{in vivo} administration of compounds described in this
invention.

Example 31

\textbf{Inhibition of Platelet Aggregation by Administration of P2Y$_{12}$ Antagonists in Dogs}

Two weeks prior to the study, beagle dogs were implanted with jugular catheters and
vascular access ports. Groups of four dogs (two of each sex) were fitted with jackets and
connected to ambulatory infusion pumps for drug administration.

The day of the study, dogs were treated with a continuous infusion of different
concentrations of test compound (usually between 0.1 and 1.5 mg/kg/h) for 90 minutes at a
rate of 3 mL/kg/h. Approximately 30 minutes and 15 minutes prior to the start of infusion
(predose samples), at different times during each infusion period (usually 10, 30, 60, and 90
minutes), and after the termination of the infusion (usually 5, 10, 20, 30, 40, 60, 120, and 240
minutes and 18, 20, 22, and 24 hours), blood was withdrawn from a cephalic vein catheter for
the assessment of \textit{ex-vivo} whole blood platelet aggregation assays and/or for bioanalytical
determination of the plasma levels of the test compound. Platelet aggregation was estimated
using the impedance mode of a CronoLog aggregometer as described in Example 21, and the
plasma levels of the test compound were analyzed on an API 4000 LC/MS/MS system
coupled with an Agilent 1100 series liquid chromatograph.

As illustrated in Figure 4, continuous intravenous administration of Compound 41
(INS50589) produced a dose-dependent inhibition of platelet aggregation. The maximal
inhibitory effect was observed with the administration of 0.3 mg/kg/h.

The kinetics and reversibility of the inhibition of platelet aggregation produced by the
test compound were also studied. As illustrated in Figure 5A, the pharmacodynamic effect of
the test compound reached steady state by 30 minutes and the inhibition of platelet
aggregation was rapidly reversed upon termination of the infusion. These results demonstrate
the ability of the test compound to rapidly inhibit platelet aggregation within minutes of
initiating continuous IV infusion, and to allow for nearly complete restoration of platelet
aggregation within an hour following termination of infusion.
The pharmacological effect of the administration of the test compound correlated closely with the plasma levels of the compound. Total plasma concentrations of the test compound reached steady state between 30 and 60 minutes and decreased rapidly after discontinuation of the administration of the compound. One-compartment model analysis of the plasma levels of the test compound after the end of infusion indicated that the compound was cleared from plasma with an estimated half-life of approximately 7 minutes, as illustrated in Figure 5B.

In conclusion, the pharmacokinetic and pharmacodynamic profile of Compound 41 (INS50589) is consistent with a rapid onset and offset of action that results in a rapid and readily reversible control of platelet function.

Although the invention has been described with reference to the presently preferred embodiments, it should be understood that various modifications could be made without departing from the scope of the invention.
WHAT IS CLAIMED:

1. A compound of Formula Ib-2, or a pharmaceutically acceptable salt, solvate, or hydrate thereof;

```
Formula Ib-2
```

wherein:

- $R_{18}$ is phenyl, benzyl, or styryl;
- $R_{19}$ is $C_2$ to $C_6$ alkyl; $C_3$ to $C_6$ cycloalkyl; alkylcycloalkyl with one to two carbon atoms in the alkyl portion, and three to six carbons in the cycloalkyl portion; phenyl; substituted or unsubstituted.

2. The compound according to Claim 1, wherein $R_{19}$ is ethyl, propyl, butyl, cyclopropyl, cyclopropylmethyl, cyclobutyl, cyclopentyl, or phenyl.

3. The compound according to Claim 1, which is 2'3' phenylacetaldehyde acetal-6-N-phenylurea AMP; 2'3' phenylacetaldehyde acetal-6-N-n-hexylurea AMP; 2'3' phenylacetaldehyde acetal-6-N-ethylurea AMP; 2'3' phenylacetaldehyde acetal-6-N-cyclopentylurea AMP; 2'3' cinnamyl acetal-6-N-n-hexylurea AMP; 2'3' cinnamyl acetal-6-N-ethylurea AMP; 2'3' cinnamyl acetal-6-N-phenylurea AMP; 2'3' cinnamyl acetal-6-N-n-
propylurea AMP; 2′3′ cinnamyl acetal-6-N-n-butylurea AMP; 2′3′ phenylpropargyl acetal-6-N-phenylurea AMP; 2′3′ phenylpropargyl acetal-6-N-n-hexylurea AMP; 2′3′ phenylpropargyl acetal-6-N-n-butylurea AMP; 2′3′ phenylpropargyl acetal-6-N-n-propylurea AMP; 2′3′ phenylpropargyl acetal-6-N-ethylurea AMP; 2′3′ benzaldehyde acetal-6-N-ethylurea AMP; 2′3′ benzaldehyde acetal-6-N-n-propylurea AMP; 2′3′ benzaldehyde acetal-6-N-n-butylurea AMP; 2′3′ benzaldehyde acetal-6-N-n-hexylurea AMP; 2′3′ benzaldehyde acetal-6-N-cyclopentylurea AMP; 2′3′-(trans)cinnamyl acetal-6-N-ethylurea AMP, 2′3′-(trans)phenyl acetal-6-N-ethylurea AMP; 2′3′-(cis)phenyl acetal-6-N-ethylurea AMP.

4. The compound according to Claim 3, which is 2′3′-(trans)cinnamyl acetal-6-N-ethylurea AMP, 2′3′-(trans)phenyl acetal-6-N-ethylurea AMP; or 2′3′-(cis)phenyl acetal-6-N-ethylurea AMP.

5. A compound of Formula Ia-2, or a pharmaceutically acceptable salt, solvate, or hydrate thereof;

**Formula Ia-2**

wherein:

R$_{18}$ and R$_{18′}$ are independently phenyl, benzyl, or styryl; and
R₁₉ and R₁₉' are independently C₂ to C₆ alkyl; C₃ to C₆ cycloalkyl; alkylcycloalkyl with one to two carbon atoms in the alkyl portion, and three to six carbons in the cycloalkyl portion; phenyl; substituted or unsubstituted.

6. The compound according to Claim 5, wherein R₁₉ and R₁₉' are independently ethyl, propyl, butyl, cyclopropyl, cyclopropylmethyl, cyclobutyl, cyclopentyl, or phenyl.

7. The compound according to Claim 5 or 6, wherein R₁₈ and R₁₈' and R₁₉ and R₁₉' are identical pairs.

8. The compound according to Claim 5, which is P¹, P²-di-(2', 3'-cinnamyl acetal-6-N-ethylurea adenosine 5'-) diphosphate; P¹, P²-di-(2', 3'-phenylpropargyl acetal-6-N-ethylurea adenosine 5'-) diphosphate; P¹, P²-di-(2', 3'-phenylacetaldehyde acetal-6-N-ethylurea adenosine 5'-) diphosphate; or P¹, P²-di-(2', 3'-phenyl acetal-6-N-ethylurea adenosine 5'-) diphosphate.

9. A pharmaceutical formulation comprising a pharmaceutically acceptably carrier and the compound according to any one of Claims 1-8, or a pharmaceutically acceptable salt, solvate, or hydrate thereof.

10. The pharmaceutical formulation according to Claim 9, wherein said compound is 2'3'-(trans)cinnamyl acetal-6-N-ethylurea AMP, 2'3'-(trans)phenyl acetal-6-N-ethylurea AMP; or 2'3'-(cis)phenyl acetal-6-N-ethylurea AMP.

11. A method of preventing or treating diseases or conditions associated with platelet aggregation and/or platelet activation comprising:
   administering to a subject the pharmaceutical formulation according to Claim 9 or 10, wherein said compound is effective to bind P₂Y₁₂ receptors on platelets and inhibit ADP-induced platelet aggregation.

12. The method according to Claim 11, wherein said compound reversibly inhibits ADP-induced platelet aggregation.
13. The method according to Claim 11, wherein said pharmaceutical composition is administered in combination with other antiplatelet and/or anticoagulant drugs.

14. The method according to Claim 11, wherein said diseases or conditions associated with platelet aggregation are disorders or procedures characterized by thrombosis, primary arterial thrombotic complications of atherosclerotic disease, thrombotic complications of interventions of atherosclerotic disease, thrombotic complications of surgical or mechanical damage, mechanically-induced platelet activation, shunt occlusion, thrombosis secondary to vascular damage and inflammation, indications with a diffuse thrombotic/platelet consumption component, venous thrombosis, coronary arterial thrombosis, pathological effects of atherosclerosis and arteriosclerosis, platelet aggregation and clot formation in blood and blood products during storage, chronic or acute states of hyper-aggregability, reocclusion of an artery or vein following fibrinolytic therapy, platelet adhesion associated with extracorporeal circulation, thrombotic complications associated with thrombolytic therapy, thrombotic complications associated with coronary and other angioplasty, or thrombotic complications associated with coronary artery bypass procedures.

15. The method according to Claim 14, wherein said disorders or procedures associated with thrombosis are unstable angina, coronary angioplasty, or myocardial infarction; said primary arterial thrombotic complications of atherosclerosis are thrombotic stroke, peripheral vascular disease, or myocardial infarction without thrombolysis; said thrombotic complications of interventions of atherosclerotic disease are angioplasty, endarterectomy, stent placement, coronary or other vascular graft surgery; said thrombotic complications of surgical or mechanical damage are associated with tissue salvage following surgical or accidental trauma, reconstructive surgery including skin flaps, or reductive surgery; said mechanically-induced platelet activation is caused by cardiopulmonary bypass resulting in microthromboembolism and storage of blood products; said shunt occlusion is renal dialysis and plasmapheresis; said thromboses secondary to vascular damage and inflammation are vasculitis, arteritis, glomerulonephritis or organ graft rejection; said indications with a diffuse thrombotic/platelet consumption component are disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, heparin-induced thrombocytopenia, or pre-eclampsia/eclampsia; said venous thrombosis are deep vein thrombosis, veno-occlusive disease, hematological conditions, or migraine; and said coronary
arterial thrombosis is associated with unstable angina, coronary angioplasty or acute myocardial infarction.

16. The method according to Claim 14, wherein said pathological effects of atherosclerosis and arteriosclerosis are arteriosclerosis, acute myocardial infarction, chronic stable angina, unstable angina, transient ischemic attacks, strokes, peripheral vascular disease, arterial thrombosis, preeclampsia, embolism, restenosis or abrupt closure following angioplasty, carotid endarterectomy, or anastomosis of vascular grafts; said chronic or acute states of hyper-aggregability is caused by DIC, septicemia, surgical or infectious shock, post-operative trauma, post-partum trauma, cardiopulmonary bypass surgery, incompatible blood transfusion, abruptio placenta, thrombotic thrombocytopenic purpura, snake venom or immune diseases.

17. The method according to Claim 11, wherein said diseases or conditions associated with platelet aggregation and/or platelet activation are produced by the contact of blood with an artificial device.

18. The method according to Claim 17, wherein said artificial device is a hemodialysis instrument, a paracorporeal artificial lung and an extracorporeal membrane oxigenation device, an internal implantable artificial heart, an apheresis instrument used to remove or isolate a specific component of the blood and returning the remaining blood components to a donor.

19. The method according to Claim 11, wherein said administering is systemic administration of said compound to a subject.

20. The method according to Claim 19, wherein said systemic administration is oral administration.

21. A method for in vitro inhibiting the aggregation of platelets in blood or blood product, comprising the step of administering to the blood or blood product the compound according to any one of Claims 1-8, or a salt, solvate, or hydrate thereof.
22. A pharmaceutical formulation comprising a compound of Formula I, or a pharmaceutically acceptable salt, solvate, or hydrate thereof, in an amount of 0.0005-0.3% w/v, a buffering agent, a chelating agent, a non-ionic tonicity modifier, wherein the pharmaceutical formulation has a tonicity of 250-350 mOsm/kg and pH of 4.9:

![Formula I](image)

wherein:

- $X_1$, $X_2$, and $X_3$ are independently oxygen, methylene, monochloromethylene, dichloromethylene, monofluoromethylene, difluoromethylene, or imido;
- $T_1$, $T_2$, $W$, and $V$ are independently oxygen or sulfur;
- $m = 0, 1$ or $2$;
- $n = 0$ or $1$;
- $p = 0, 1$, or $2$;
- where the sum of $m + n + p$ is from 0 to 5;
- $M = H$, or a pharmaceutically-acceptable inorganic or organic counterion;
- $D_1 = O$ or $CH_2$;
- $B'$ is a purine or a pyrimidine residue according to general Formulae IV and V which is linked to the 1'-position of the furanose or carbocycle via the 9- or 1- position of the base, respectively;
- $Y' = H$, OH, or $OR_1$;
- $Z' = H$, OH, or $OR_2$; with the proviso that when $A = M$, at least one of $Y'$ and $Z'$ is $OR_1$ or $OR_2$;
- $A = M$, or
- $A$ is a nucleoside residue which is defined as:
and which is linked to the phosphate chain via the 5'-position of the furanose or carbocycle; wherein:

\[ D_2 = \text{O or CH}_2; \]

\[ Z = \text{H, OH, or OR}_3; \]

\[ Y = \text{H, OH, or OR}_4; \]

with the proviso that at least one of \( Y', Z', Y \) and \( Z \) is equal to \( \text{OR}_1, \text{OR}_2, \text{OR}_4, \) or \( \text{OR}_3 \) respectively.

\( B \) is a purine or a pyrimidine residue according to general Formulae IV and V which is linked to the 1'-position of the furanose or carbocycle via the 9- or 1-position of the base, respectively;

\( R_1, R_2, R_3, \) and/or \( R_4 \) are residues which are linked directly to the 2'- and/or 3'-hydroxyls of the respective furanose or carbocycle via a carbon atom according to Formula II, or linked directly to two (2'- and 3'-) hydroxyls of the respective furanose or carbocycle via a common carbon atom according to Formula III, such that from one to four independent residues of \( R_1, R_2, R_3 \) and \( R_4 \) falling within the definition of Formula II are present or from one to two independent residues made up of \( R_1+R_2 \) and/or \( R_3+R_4 \) are present;

**Formula II**

\[ \overset{2'}{\text{O}} \overset{3'}{\text{C}} \text{R}_5 \text{R}_6 \text{R}_7 \]

wherein:

\( O \) is the corresponding 2'- and/or 3'-oxygen of the respective furanose or carbocycle;

\( C \) is a carbon atom;
R₅, R₆, and R₇ are H, alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl, or substituted aryl, such that the moiety defined according to Formula II is an ether; or

R₅ and R₆ are H, an alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl, or substituted aryl, and R₇ is alkoxy, cycloalkoxy, aralkyloxy, aryloxy, substituted aralkyloxy, or substituted aryloxy such that the moiety defined according to Formula II is an acyclic acetal or ketal; or

R₅ and R₆ are taken together as oxygen or sulfur doubly bonded to C, and R₇ is alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl, or substituted aryl, such that the moiety defined according to Formula II is an ester or thioester; or

R₅ and R₆ are taken together as oxygen or sulfur doubly bonded to C, and R₇ is amino or mono- or disubstituted amino, where the substituents are alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl, or substituted aryl, such that the moiety according to Formula II is a carbamate or thiocarbamate; or

R₅ and R₆ are taken together as oxygen or sulfur doubly bonded to C, and R₇ is alkoxy, cycloalkoxy, aralkyloxy, aryloxy, substituted aralkyloxy, or substituted aryloxy, such that the moiety according to Formula II is a carbonate or thiocarbonate; or

R₇ is not present and R₅ and R₆ are taken together as oxygen or sulfur doubly bonded to C and both the 2'- and 3'- oxygens of the respective furanose or carbocycle are directly bound to C to form a cyclical carbonate or thiocarbonate;

\[
\text{Formula III}
\]

\[
\begin{array}{c}
\text{C} \\
\text{O} \\
R_8 \\
\text{O} \\
R_9
\end{array}
\]

wherein:

the O atoms are the 2'- and 3'- oxygens of a furanose or carbocycle; and the 2'- and 3'- oxygens of the furanose or carbocycle are linked by a common carbon atom (C) to form a cyclical acetal, cyclical ketal, or cyclical orthoester;

for cyclical acetals and ketals, R₈ and R₉ are independently hydrogen, alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl, substituted aryl, or can be joined together to form a homocyclic or heterocyclic ring composed of 3 to 8 atoms, preferably 3 to 6 atoms;

for cyclical orthoesters, R₈ is hydrogen, alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl, or substituted aryl, R₉ is allyloxy, cycloalkyloxy, aralkyloxy, aryloxy, substituted aralkyloxy, or substituted aryloxy.
23. The pharmaceutical formulation according to Claim 22, wherein said non-isotonic
tonicity modifier is a mannitol or dextrose.

24. The pharmaceutical formulation according to Claim 23, wherein said non-isotonic
tonicity modifier is in the amount of 3-5%.
<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
<th>ADP-induced aggregation (%)</th>
<th>IC_{50} (uM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="structure" /></td>
<td>2' or 3' phenylcarbamate UTP</td>
<td><img src="image2.png" alt="bar_chart" /></td>
<td><img src="image3.png" alt="IC50_chart" /></td>
</tr>
<tr>
<td><img src="image4.png" alt="structure" /></td>
<td>2', 3' di-phenylcarbamate UTP</td>
<td><img src="image5.png" alt="bar_chart" /></td>
<td><img src="image6.png" alt="IC50_chart" /></td>
</tr>
<tr>
<td><img src="image7.png" alt="structure" /></td>
<td>2'3' phenylacetaldehyde acetal ADP</td>
<td><img src="image8.png" alt="bar_chart" /></td>
<td><img src="image9.png" alt="IC50_chart" /></td>
</tr>
<tr>
<td><img src="image10.png" alt="structure" /></td>
<td>dl [3'(phenylcarbamate)dU/p2dU]</td>
<td><img src="image11.png" alt="bar_chart" /></td>
<td><img src="image12.png" alt="IC50_chart" /></td>
</tr>
</tbody>
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**FIG. 1-1**
<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
<th>ADP-induced aggregation (%)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (uM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Chemical Structure]</td>
<td>di 2′3′ phenylacetaldehyde acetal Ap4U</td>
<td><img src="image" alt="Graph" /></td>
<td>0.18</td>
</tr>
<tr>
<td>[Chemical Structure]</td>
<td>2′3′ phenylacetaldehyde acetal Ip4U</td>
<td><img src="image" alt="Graph" /></td>
<td></td>
</tr>
<tr>
<td>[Chemical Structure]</td>
<td>2′3′ phenylacetaldehyde acetal Up4U</td>
<td><img src="image" alt="Graph" /></td>
<td></td>
</tr>
<tr>
<td>[Chemical Structure]</td>
<td>di 2′3′ phenylacetaldehyde acetal Ip4U</td>
<td><img src="image" alt="Graph" /></td>
<td></td>
</tr>
</tbody>
</table>

*FIG. 1-3*
<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>ADP-induced aggregation (%)</th>
</tr>
</thead>
<tbody>
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<td>2-naphthylaldehyde acetol AgO4A</td>
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<td>100 µM</td>
</tr>
<tr>
<td>2-naphthylaldehyde acetol Up4A</td>
<td><img src="image2.png" alt="Structure" /></td>
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<td>100 µM</td>
</tr>
<tr>
<td>Tetr phenyl carbamate Up4U</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>100 µM</td>
<td>100 µM</td>
</tr>
<tr>
<td>di-2,3-benzaldehyde acetol Up4U</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>100 µM</td>
<td>100 µM</td>
</tr>
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</table>

**FIG. 1-4**
<table>
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<tr>
<th><strong>Structure</strong></th>
<th><strong>Name</strong></th>
<th><strong>ADP-Induced aggregation (%)</strong></th>
<th><strong>IC&lt;sub&gt;50&lt;/sub&gt; (uM)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Structure" /> <img src="image2" alt="Structure" /></td>
<td>di 2',3' benzaldehyde acetal Up4U</td>
<td><img src="image3" alt="Graph" /></td>
<td><img src="image4" alt="Graph" /></td>
</tr>
<tr>
<td><img src="image5" alt="Structure" /> <img src="image6" alt="Structure" /></td>
<td>2',3' benzaldehyde acetal Up4U</td>
<td><img src="image7" alt="Graph" /></td>
<td><img src="image8" alt="Graph" /></td>
</tr>
<tr>
<td><img src="image9" alt="Structure" /> <img src="image10" alt="Structure" /></td>
<td>di 2',3' phenylacetaldehyde acetal Cp4U</td>
<td><img src="image11" alt="Graph" /></td>
<td><img src="image12" alt="Graph" /></td>
</tr>
<tr>
<td><img src="image13" alt="Structure" /> <img src="image14" alt="Structure" /></td>
<td>2',3' phenylacetaldehyde acetal Cp4U</td>
<td><img src="image15" alt="Graph" /></td>
<td><img src="image16" alt="Graph" /></td>
</tr>
<tr>
<td>Name</td>
<td>Structure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2',3'-phenylalanine</td>
<td><img src="image1.png" alt="Structure 1" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>acetal Lipid A</td>
<td><img src="image2.png" alt="Structure 2" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2',3'-phenylalanine</td>
<td><img src="image3.png" alt="Structure 3" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>acetal Lipid A</td>
<td><img src="image4.png" alt="Structure 4" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d4 2',3'-benzaldehyde</td>
<td><img src="image5.png" alt="Structure 5" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>acetal CpG</td>
<td><img src="image6.png" alt="Structure 6" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2',3'-benzaldehyde</td>
<td><img src="image7.png" alt="Structure 7" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>acetal Lipid A</td>
<td><img src="image8.png" alt="Structure 8" /></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ADP-induced aggregation (IC<sub>50</sub> (uM))**

<table>
<thead>
<tr>
<th>Concentration (uM)</th>
<th>Control</th>
<th>100 uM</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td><img src="image9.png" alt="Bar 1" /></td>
<td><img src="image10.png" alt="Bar 2" /></td>
</tr>
<tr>
<td>50</td>
<td><img src="image11.png" alt="Bar 3" /></td>
<td><img src="image12.png" alt="Bar 4" /></td>
</tr>
<tr>
<td>0</td>
<td><img src="image13.png" alt="Bar 5" /></td>
<td><img src="image14.png" alt="Bar 6" /></td>
</tr>
</tbody>
</table>

**FIG. 1-6**
<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>IC₅₀ (uM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2',3' benzaldehyde acetate-UpΔ4I</td>
<td><img src="image1" alt="Structure 1" /></td>
<td>100 uM</td>
</tr>
<tr>
<td>2',3' benzaldehyde acetate-UpΔ4C</td>
<td><img src="image2" alt="Structure 2" /></td>
<td>100 uM</td>
</tr>
<tr>
<td>2',3' benzaldehyde acetate-CpΔ4I</td>
<td><img src="image3" alt="Structure 3" /></td>
<td>100 uM</td>
</tr>
<tr>
<td>2',3' benzaldehyde acetate-UpΔ4C</td>
<td><img src="image4" alt="Structure 4" /></td>
<td>100 uM</td>
</tr>
</tbody>
</table>

ADP-induced aggregation (%)

- 100 uM - Control
- 80 uM
- 0 uM
<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
<th>ADP-induced aggregation (%)</th>
<th>IC$_{50}$ (uM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Structure" /></td>
<td>2',3' phenylpropionaldehyde acetal Up4U</td>
<td><img src="image2" alt="Graph" /></td>
<td></td>
</tr>
<tr>
<td><img src="image3" alt="Structure" /></td>
<td>di 2',3' phenylpropionaldehyde acetal Up4U</td>
<td><img src="image4" alt="Graph" /></td>
<td></td>
</tr>
<tr>
<td><img src="image5" alt="Structure" /></td>
<td>2',3' benzaldehyde acetal Cp4C</td>
<td><img src="image6" alt="Graph" /></td>
<td></td>
</tr>
<tr>
<td><img src="image7" alt="Structure" /></td>
<td>Bis MANT Up4U</td>
<td><img src="image8" alt="Graph" /></td>
<td></td>
</tr>
</tbody>
</table>

FIG. 1-8
<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
<th>ADP-induced aggregation (%)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="structure1.png" alt="" /></td>
<td>Mant Up4U</td>
<td><img src="bar_chart1.png" alt="Bar Chart1" /></td>
<td>35</td>
</tr>
<tr>
<td><img src="structure2.png" alt="" /></td>
<td>Di 2/3' benzylacetal Up4U</td>
<td><img src="bar_chart2.png" alt="Bar Chart2" /></td>
<td>2.35</td>
</tr>
<tr>
<td><img src="structure3.png" alt="" /></td>
<td>Mono 2/3' benzylacetal Up4U</td>
<td><img src="bar_chart3.png" alt="Bar Chart3" /></td>
<td>100</td>
</tr>
<tr>
<td><img src="structure4.png" alt="" /></td>
<td>Triphenyl carbamate Up4U</td>
<td><img src="bar_chart4.png" alt="Bar Chart4" /></td>
<td>7.5</td>
</tr>
</tbody>
</table>

FIG. 1-9
<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>UpSU</td>
<td><img src="image1" alt="UpSU Structure" /></td>
</tr>
<tr>
<td>Gp4U</td>
<td><img src="image2" alt="Gp4U Structure" /></td>
</tr>
</tbody>
</table>

**IC<sub>50</sub> (µM)**

- **Control**: 100 µM
- **UpSU**: 50 µM, 100 µM
- **Gp4U**: 50 µM, 100 µM

**ADP-Induced Aggregation (%)**

- 0%, 50%, 100%
FIG. 2
FIG. 3
FIG. 4

ADP-Induced Platelet Aggregation (% predose)

predose 0.03 0.1 0.3 1

INS50589 (mg/kg/h)
FIG. 5A

ADP-induced Platelet Aggregation (% predose)

Time (min)

FIG. 5B

INS50589 Plasma levels (ng/ml)

Time (min)