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Synthesis and biological activity of 2-alkylated deoxyadenosine bisphosphate derivatives as $P2Y_1$ receptor antagonists

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Abstract—A previous study around adenine nucleotides afforded the reference N^6-methyl-2′-deoxyadenosine-3′,5′-bisphosphate (**1a**, MRS 2179) as a selective human $P2Y_1$ receptor antagonist ($pA_2 = 6.55 \pm 0.05$) with antithrombotic properties. In the present paper, we have synthesized and tested in vitro various 2-substituted derivatives with the goal of exploring the 2-position binding region and developing more potent $P2Y_1$ receptor antagonists. Thus, we have adopted a novel and versatile chemical pathway using a palladium-catalyzed cross-coupling reaction with the 2-iodinated derivative **7** as a common intermediate for a very efficient synthesis of the 2-alkyl-N^6-methyl-2′-deoxyadenosine-3′,5′-bisphosphate nucleotides **1e–i**. The biological activity was evaluated through the ability of compounds to inhibit ADP-induced platelet aggregation, intracellular calcium rise and to displace the specific binding of [^{33}P]2-MeSADP. 2-Ethyl and 2-propyl groups appeared to be tolerated, whereas a bulky group or a C_3 linear substituent dramatically decreased potency of antagonists. The 2-ethynyl derivative **1h** ($pA_2 = 7.54 \pm 0.10$) was significantly more potent (10-fold) as an antagonist when compared to the reference **1a**, revealing a potential electronic interaction highly favorable between triple bond orbitals and the $P2Y_1$ receptor at this position.

1. Introduction

P2 receptors, which are activated by extracellular adenine and uracil nucleotides, are widely distributed in many different cell types and regulate a broad range of physiological processes.[1,2] These membrane-bound receptors, collectively called purinergic receptors, are divided into two structurally distinct families: G-protein-coupled receptors (GPCR) termed P2Y, and ligand-gated cation channels termed P2X.

Adenosine diphosphate (**ADP**, Fig. 1) plays a crucial role in hemostasis and thrombosis, and its receptors are potential targets for antithrombotic drugs. Two P2Y receptors are involved in platelet aggregation: the $P2Y_1$ receptor coupled to phospholipase C initiates aggregation through mobilization of calcium stores, whereas the $P2Y_{12}$ receptor coupled to adenylyl cyclase inhibition is essential for a full aggregation response to ADP and the stabilisation of aggregates.[3,4] The latter has been shown to be the target of the antiplatelet thienopyridine compounds ticlopidine and clopidogrel[5,6] and of the ATP analogues, the Astra Zeneca compounds of the AR-C series.[7–9] The $P2Y_1$ and $P2Y_{12}$ receptors are both essential for normal ADP-induced platelet aggregation to occur.[10,11] Recent studies with $P2Y_1$-knockout mice or selective $P2Y_1$ antagonists in experimental thrombosis models have shown that the $P2Y_1$ receptor is a promising potential target for new antithrombotic agents.[12–16] On the other hand, the whole physiological role of this receptor is unknown, and few pharmacological probes are available. Thus, the development of potent and selective $P2Y_1$ receptor antagonists is a critical need.

Boyer et al. reported in 1996 that various endogenous bisphosphates of adenosine (e.g., adenosine-3′,5′-bisphosphate, **A3P5P**) behaved as competitive antagonists of both turkey and human $P2Y_1$ receptors with K_B

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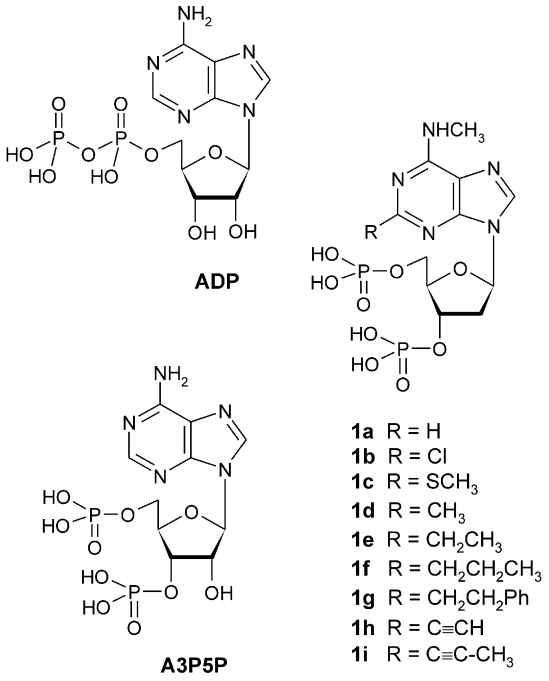


Figure 1. Structures of nucleotides analogues acting on the $P2Y_1$ receptor.

values in the micromolar range.[17] These nucleotides also displayed partial agonist activities at the turkey $P2Y_1$ receptor.[17] Starting from this observation of considerable interest, SAR (structure–activity relationship) studies of derivatives of adenosine bisphosphates have resulted in the reference N^6-methyl-2′-deoxyadenosine-3′,5′-bisphosphate (**1a**, MRS 2179), a competitive antagonist at human and turkey $P2Y_1$ receptors, with a K_B value of 100 nM and an IC_{50} value of 0.33 μM in blocking the effects of 10 nM 2-methylthioadenoine-5′-diphosphate (2-MeSADP).[18,19] The presence of an N^6-methyl group and the absence of a 2′-hydroxyl group both enhanced affinity and turned the agonist character into a pure antagonist at $P2Y_1$ receptor.

Every substitution at the 8-position of the adenine moiety considerably decreased affinity, whereas some substitution at the 2-position was known to be tolerated.[20] The corresponding 2-chloro derivative **1b** was slightly more potent than **1a** with an IC_{50} of 0.20 μM and the 2-methylthio derivative **1c** was an antagonist as potent as the reference **1a**. On the other hand, major synthetic modifications of the ribose moiety including replacement with carbocyclics, smaller and larger rings, acyclics, and conformationally constrained rings have been carried out to increase activity and biological stability.[21]

We have recently reported the synthesis and the pharmacological properties of the 2-methyl derivative **1d**.[22] This compound was a full antagonist at the human $P2Y_1$ receptor with a pA_2 value of 7.11 ± 0.11 and was found to be 4-fold more potent than the reference **1a** ($pA_2 = 6.55 \pm 0.05$). However the effects of longer alkyl groups or aryl groups at 2-position of the adenine moiety have not been described to our knowledge. These interesting results encouraged us to synthesize and test in vitro compounds **1e–i** with the goal of better understand the SAR of 2-substituted derivatives using homology rules and introduction of unsaturation.

The biological activity at human $P2Y_1$ receptor was determined by measuring the ability to inhibit ADP-induced platelet aggregation, shape change, intracellular calcium rise and to displace the specific binding of [^{33}P]2-MeSADP in intact platelets. We also verified that these compounds have no effect on $P2Y_{12}$ receptor by measuring adenylyl cyclase activity.

2. Results and discussion

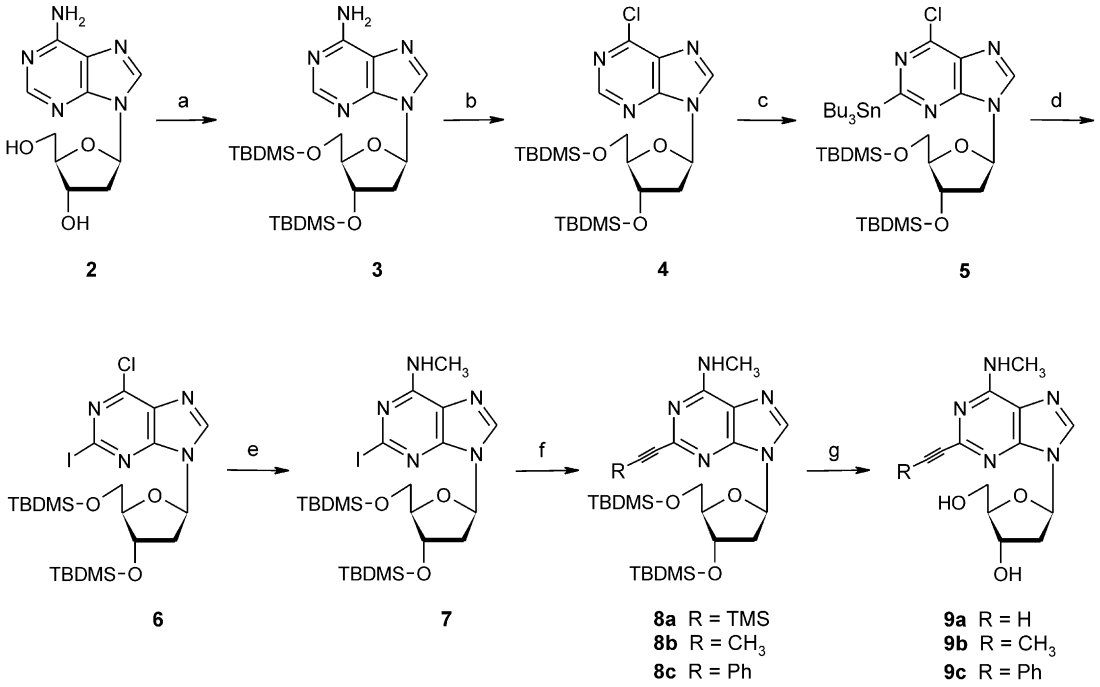
2.1. Chemical synthesis

The recently reported synthesis of the 2-methyl derivative **1d** was long and unsuitable to afford derivatives with different substitutions at 2-position.[22] In this goal, the palladium-catalyzed cross-coupling reaction appeared to be the best tool in C–C bond formation from a 2-halogenated derivative. As shown in Schemes 1 and 2, all N^6-methyl-2′-deoxyadenosine-3′,5′-bisphosphate derivatives **1e–i** were synthesized by Sonogashira reaction of the corresponding alkynes with the iodinated intermediate **7**.

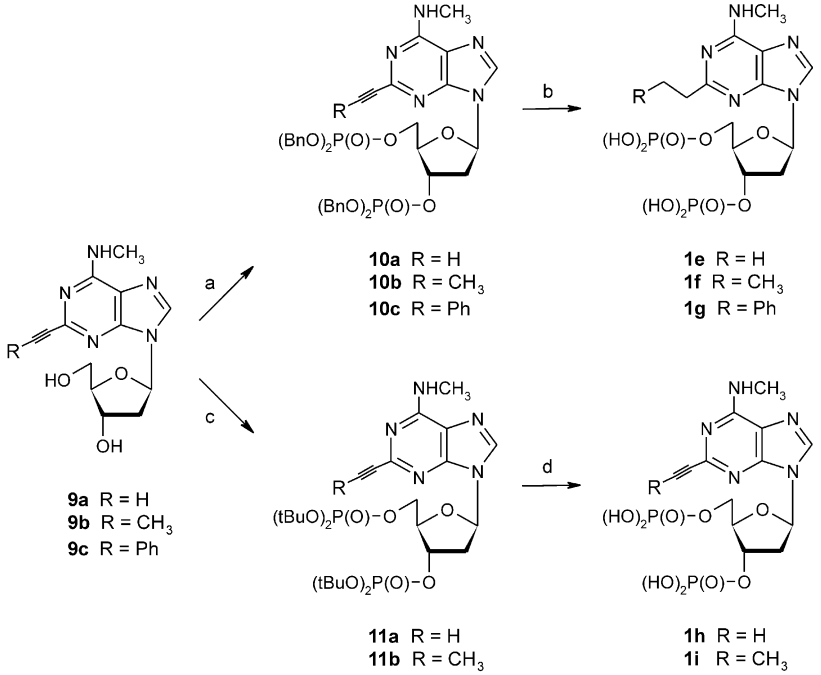
The commercially available 2′-deoxyadenosine (**2**) was first protected as the 3′,5′-disilylated compound **3** in good yield,[23] followed by diazotization-chlorination[24] with isoamyl nitrite in carbon tetrachloride to afford the silylated 6-chloro-2′-deoxynucleoside **4** in 41% yield. Electron-withdrawing effects of the 6-chloro group increased acidity at 2-position of the purine ring according to a previously reported study.[25] Treatment of compound **4** with the bulky base lithium 2,2,6,6-tetramethyl piperidine (LTMP) and tributylstannyl chloride as the electrophile allowed a selective stannylation at 2-position against the 8-position of the purine moiety.[26] To avoid protonolysis of the stannyl group on silica gel during column chromatography, 2-tributylstannyl derivative **5** was not purified and directly treated with iodine to afford the 2-iodo-6-chloro-2′-deoxynucleoside **6** in 55% yield. Nucleophilic displacement of the 6-chloro group with methylamine led to the corresponding 2-iodo-N^6-methyl-2′-deoxyadenosine **7** in good yield. The key intermediate **7** was obtained in five steps and 18% overall yield. This chemical pathway appeared to be more efficient than classical strategies starting from guanosine[23] or 2′-deoxyguanosine.[20]

Then, the intermediate **7** was engaged in a palladium-catalyzed cross-coupling with (trimethylsilyl)acetylene, propyne or phenylacetylene in presence of cuprous iodide to afford 2-substituted derivatives **8a**, **b** or **c** respectively in good yields.[23] Desilylation was achieved with tetrabutylammonium fluoride (TBAF) in acetonitrile to give the desired compounds **9a–c**, which were finally phosphorylated using two different methods (shown in Scheme 2).

The tetrabenzyl pyrophosphate (TBPP) procedure[27] which needs hydrogenolysis to remove the benzyl groups was first displayed with the goal to obtain saturated and flexible alkyl chains at 2-position. Then, transformation of compounds **9a–c** in bis(dibenzyl)phosphates **10a–c** followed by catalytic hydrogenation led to nucleotides **1e–g** in good yields. Alternatively, phosphorylation of nucleosides **9a**,**b** was carried out using a phosphoramidite method[28] to keep unsaturations and rigidity of 2-substituents. The bis(di-*tert*-butyl)phosphate intermediates **11a**,**b** were deprotected under acidic conditions to afford 2-alkynyl derivatives **1h**,**i** in moderate yields.



Scheme 1. Reagents and conditions: (a) TBDMS-Cl, imidazole, DMF, 35 °C, 16 h; (b) isoamyl nitrite, CCl_4, 50 °C, 3 h; (c) LTMP, Bu_3SnCl, THF, −78 °C, 30 min; (d) I_2, THF, rt, 4 h; (e) $MeNH_2$, EtOH, rt, 16 h; (f) $HC{\equiv}C\text{-}R$, $(PPh_3)_2PdCl_2$, CuI, Et_3N, rt, 3 h; (g) TBAF, CH_3CN, rt, 1 h.



Scheme 2. Reagents and conditions: (a) *t*BuOK, TBPP, THF, −40 °C, 30 min; (b) H_2, Pd/C, MeOH, 60 psi, rt, 48 h; (c) $Et_2NP(OtBu)_2$, tetrazole, THF, rt, 2 h, then MCPBA, −78 °C, 30 min; (d) TFA, CH_2Cl_2, rt, 30 min.

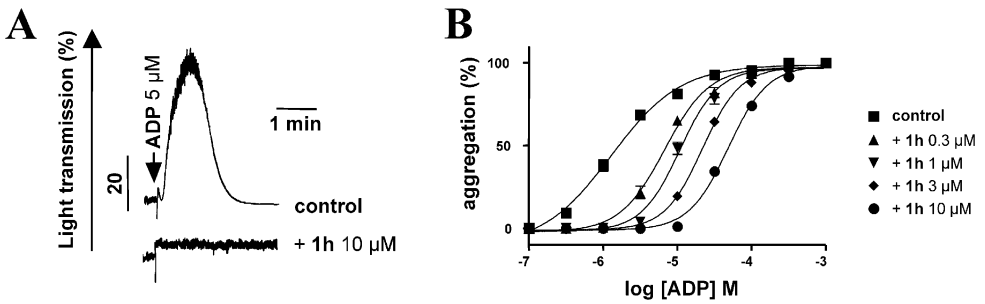


Figure 2. Inhibition of ADP-induced aggregation of washed human platelets by the compound **1h**. (A) Platelet aggregation was evaluated quantitatively using a turbidimetric method across a suspension of washed platelets. Aggregation and shape change induced by 5 μM ADP (control) were inhibited by 10 μM **1h**. Traces are from one representative experiment. (B) The pA_2 value corresponding to the potency of inhibition, was determined by generation a series of concentration-response curves for ADP, in the presence of the indicated increasing concentrations of the compound **1h** added 30 s before ADP. The following Schild regression analysis allows us to determine the pA_2 value. Curves represent the mean of three independent experiments and give a pA_2 of 7.54 ± 0.10.

2.2. Biological activity

2.2.1. Platelet aggregation. The new derivatives **1e–i** prepared in the present study were first tested as antagonists in a platelet aggregation assay induced by ADP using platelet aggregometry. Traces representing the aggregation as a function of time following exposure to 5 μM ADP were shown in Figure 2.

Addition of compounds **1e–i** (100 μM) to washed human platelets 30 s before ADP (5 μM) inhibited platelet aggregation and shape change as shown in the presence of 10 μM **1h** (Fig. 2A), while did not induce shape change or aggregation by themselves even at high concentrations (up to 100 μM) (data not shown). As seen with the reference **1a** no agonist activity was observed.[29] The potency of inhibition was determined by generating a series of concentration-response curves for ADP in the presence of different concentrations of each compound **1e–i**. The compounds caused a parallel shift to the right of the concentration-response curve, but high concentrations of ADP could completely override high concentrations of compounds **1e–i**, as illustrated with the compound **1h** (Fig. 2B). Schild analysis of the inhibition gave pA_2 values summarized in the Table 1 ($n = 3$).

2.2.2. Calcium measurements and inhibition of adenylyl cyclase. The aggregation process involves the activation of two receptors ($P2Y_1$ and $P2Y_{12}$ receptors) and their transduction machinery. In platelets, as previously described, ADP induced simultaneous mobilization of intracellular Ca^{2+} stores and inhibition of adenylyl cyclase, through activation of the $P2Y_1$ and $P2Y_{12}$ receptors, respectively. We have verified that compounds **1e–i** act selectively on the $P2Y_1$ receptor. The intracellular Ca^{2+} rise induced in washed human platelets by ADP could be inhibited by the different derivatives, in the presence or absence of 2 mM external Ca^{2+} (data not shown). As an illustration, the compound **1h** (10 μM) totally inhibited ADP (1 μM)-induced calcium rise (Fig. 3A). Conversely, 100 μM of the compounds **1e–i** had no influence on basal levels of cAMP in human platelets, or on the cAMP levels induced by 10 μM prostaglandin E_1 (PGE_1). The ability of ADP to inhibit PGE_1-stimulated cAMP accumulation was likewise not affected by these molecules in human platelets, whereas AR-C66096,[30] a selective $P2Y_{12}$ receptor antagonist, totally reversed the inhibitory effect of ADP (Fig. 3B). In conclusion, the potential inhibitory effect of **1e–i** on ADP-induced platelet aggregation is entirely due to the antagonistic activity on the $P2Y_1$ receptor.

2.2.3. Binding assays. The affinity of ligands for the $P2Y_1$ receptor could be evaluated quantifying their potency to displace a radioligand. Studies of the binding of ADP to its platelet receptors are currently performed using [^{33}P]2-MeSADP, a radioligand which binds to both $P2Y_1$ and $P2Y_{12}$ receptors.[5,29,31] In order to verify that **1e–i** displaced [^{33}P]2-MeSADP only from $P2Y_1$ receptors, we compared its effects to those of reference **1a**, a well known $P2Y_1$ receptor antagonist.[29] The specific binding of [^{33}P]2-MeSADP to washed human platelets was competitively and partially displaced by **1a** ($K_i = 110$ nM) at approximately 20% of [^{33}P]2-MeSADP sites while the 2-MeSADP totally displaced the radiolabeled 2-MeSADP (Fig. 4, filled square). Similarly, we confirmed that the compounds **1e–i** displaced the radioligand from the $P2Y_1$ binding sites as shown for

Table 1. In vitro pharmacological data for inhibition of ADP-induced aggregation and displacement of the radiolabeled ligand [^{33}P]2-MeSADP to washed human platelets

Compd	R	pA_2[a]	K_i (nM)[b]
1a	H	6.55 ± 0.05	110
1d	CH_3	7.11 ± 0.11	60
1e	CH_2CH_3	6.59 ± 0.14	15
1f	$CH_2CH_2CH_3$	6.75 ± 0.34	140
1g	CH_2CH_2Ph	6.03 ± 0.18	4500
1h	C≡CH	7.54 ± 0.10	10
1i	C≡C–CH_3	4.7	600

[a] The inhibition of ADP-induced platelet aggregation by molecules was measured using aggregometry. The pA_2 value corresponds to the potency of inhibition and was determined by generating a series of concentration-response curves for ADP in the presence of different concentrations of each compound **1a**, **1d–i**. Means of three independent experiments were shown.

[b] The affinity of each compound was evaluated by a competition experiment using the radioligand [^{33}P]2-MeSADP. Displacement experiments were performed using a single concentration of radiolabeled [^{33}P]2-MeSADP in the presence of increasing concentrations of the appropriate unlabeled ligand (**1a**, **1d–i**) and the K_i determined ($n = 1$).

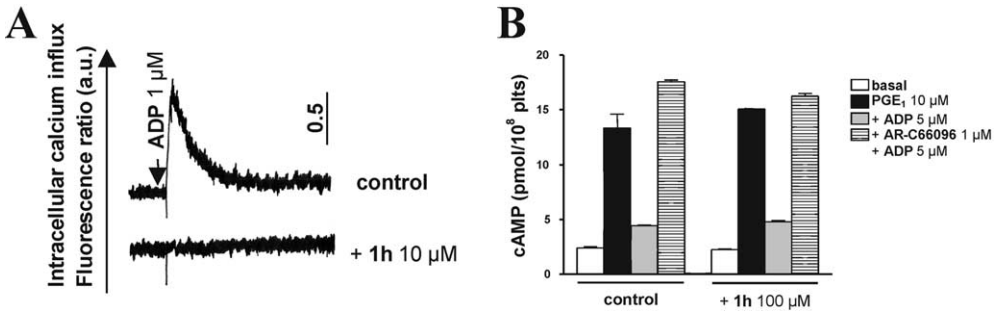


Figure 3. Selective inhibition of the $P2Y_1$ receptor transduction pathway. (A) The antagonistic properties of each compound were evaluated on ADP-induced calcium rise through the $P2Y_1$ receptor in washed human platelets. As an example, the compound **1h** (10 μM) totally abolished the intracellular calcium rise induced by 1 μM ADP in the presence of 2 mM external Ca^{2+}. Graphs are from one experiment representative of three independent experiments. (B) The selectivity of each compound between the platelet $P2Y_1$ and $P2Y_{12}$ receptors was determined by cAMP measurements. The cAMP inhibition induced by ADP through the $P2Y_{12}$ receptor (gray bars) after prestimulation by the prostaglandin PGE_1 (black bars) was not modified by the derivative **1h** as well as each compound. However, a selective $P2Y_{12}$ receptor antagonist (AR-C66096) reversed the inhibition of cAMP induced by ADP (striped bars).

example with the compound **1h** (Fig. 4, open square). The affinity of each compound was evaluated by a competition experiment and gave K_i values summarized in Table 1 ($n = 1$).

The 2-ethyl (**1e**) and 2-propyl (**1f**) derivatives showed pA_2 values similar to the reference **1a** (Table 1). Moreover, the presence of an ethyl group (**1e**) at the 2-position of the adenine moiety enhanced the affinity of the ligand for the $P2Y_1$ receptor ($K_i = 15$ nM) when compared to the reference **1a** (about 8-fold). Short and medium alkyl groups are well tolerated at this position revealing a relatively open area of the $P2Y_1$ receptor around this position. However, the 2-phenylethyl derivative **1g** or the 2-propynyl derivative **1i** displayed low potency as an antagonist, with higher affinity constant ($K_i = 4500$ and 600 nM respectively) than the unsubstituted compound **1a**. A bulky group or a C_3 linear substituent dramatically decreased activity and highlighted some steric hindrance at this position. This topological exploration showed a medium and weakly sterically restricted hydrophobic pocket at the 2-position of the human $P2Y_1$ receptor, which allows various modifications of N^6-methyl-2′-deoxyadenosine-3′,5′-bisphosphate nucleotides to improve

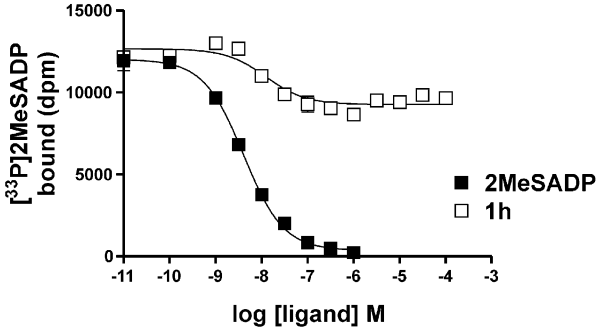


Figure 4. Affinity for the $P2Y_1$ receptor. Competition experiments were performed using a single concentration of the radiolabeled [^{33}P]2-MeSADP on washed human platelets in the presence of increasing concentrations of the each unlabeled compound. The compound **1h** as well as the other compounds (**1e–i**) displaced approximately 20% of [^{33}P]2-MeSADP sites corresponding to the $P2Y_1$ binding sites on platelets, while the remaining binding sites were the $P2Y_{12}$ receptors. Curves are from one experiment.

affinity. On the other hand, compound **1h** ($pA_2 = 7.54 \pm 0.10$) was significantly more potent (10-fold) as an antagonist when compared to the reference **1a** ($pA_2 = 6.55 \pm 0.05$),[29] reflecting the potency-enhancing effect of the 2-ethynyl group. Moreover, this compound exhibited a better affinity ($K_i = 10$ nM) for the human $P2Y_1$ receptor than the reference **1a** (10-fold) and the other derivatives. Thus, an electronic interaction between triple bond orbitals and the receptor at this position seems to be greatly favorable.

3. Conclusion

In summary, we have synthesized and evaluated the 2-alkylated N^6-methyl-2′-deoxyadenosine-3′,5′-bisphosphate derivatives **1e–i**. The chosen chemical pathway allowed us to explore in a very efficient way different substitutions at 2-position by means of the corresponding alkynes and the 2-iodinated derivative as a common intermediate leading to possible topological exploration at this position. The 2-alkylated derivatives **1e–i** were tested as inhibitors of platelet function in comparison to the unsubstituted reference **1a**. 2-Alkyl groups from methyl to propyl appeared to be completely tolerated, whereas a bulky group or a C_3 linear substituent dramatically decreased potency of antagonists. These results reflect a medium hydrophobic pocket at the 2-position of the human $P2Y_1$ receptor. The 2-ethynyl derivative **1h** ($pA_2 = 7.54 \pm 0.10$) was found more potent (10-fold) as an antagonist when compared to the reference **1a** and exhibited high affinity ($K_i = 10$ nM) for its receptor, revealing a potential electronic interaction highly favorable between triple bond orbitals and the human $P2Y_1$ receptor at this position. Furthermore, this 2-ethynyl derivative seems to be the most potent $P2Y_1$ receptor antagonist with a N^6-methyl-2′-deoxyadenosine-3′,5′-bisphosphate scaffold reported to date to our knowledge. In addition, as the 2-ethyl derivative **1e** presented the same potent affinity, the ethynyl derivative **1h** may serve for easy and efficient introduction of tritium in its structure by means of catalytic hydrogenation with tritium gas. This novel radioligand may constitute a new pharmacological tool for further binding experiments.

4. Experimental

4.1. Chemical synthesis

Reagents used for the synthesis were purchased from Sigma-Aldrich (Isle d'Abeau Chesnes, France) and Lancaster (Bischheim-Strasbourg, France). All solvents were obtained from commercial suppliers and used without further purification with the exception of tetrahydrofuran (THF) which was freshly distilled from sodium benzophenone ketyl. Flash chromatography was performed on Geduran silica gel Si 60 (40–63 μm, Merck). Thin-layer chromatography was carried out using plates of silica gel 60 F_{254} (Merck). The spots were visualized under UV light ($\lambda = 254$ nm). All chemical yields are unoptimized and generally represent the result of a single experiment. ^1H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker AC 200 or a Bruker DPX 300 spectrometer at room temperature; orthophosphoric acid (85%) was used as an external standard for ^{31}P NMR calibration. The chemical shifts (δ) are expressed as relative ppm downfield from $CHCl_3$ ($\delta_H = 7.27$, $\delta_C = 77.0$) or DMSO ($\delta_H = 2.52$, $\delta_C = 40.0$), coupling constants (*J*) are given in hertz (Hz), and signals are designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; bs, broad singlet. Electrospray mass spectra (ESMS) and high resolution mass spectra (HRMS) were obtained on a Perseptive-Biosystem Mariner mass spectrometer. Melting points were determined with a Mettler FP62 or a Bibby SMP3 apparatus and are uncorrected (all final nucleotides **1e–i** decomposed between 130–150 °C). The determination of purity was performed with a Waters 600HPLC system using a XTerra MS C_{18} 5 μm analytical column (4.6 mm×250 mm) in the linear gradient solvent system 0.1 M triethylammonium acetate buffer + 50 mM tetrabutylammonium bromide/CH_3CN in ratios of 95/5 to 40/60 for 30 min with flow rate 1 mL/min. Peaks were detected by UV absorption using a Waters 996 photodiode array detector. All final compounds showed more than 95% purity as determined using HPLC.

4.1.1. 3′,5′-bis-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxyadenosine (3). 2′-Deoxyadenosine monohydrate (5.10 g, 18.9 mmol) was suspended in pyridine (20 mL) and evaporated to dryness. This operation was repeated twice, and then, the residue was dissolved in dry DMF (30 mL) under an argon atmosphere. Imidazole (3.87 g, 56.8 mmol) and *tert*-butyldimethylsilyl chloride (6.28 g, 41.7 mmol) were added and the resulting solution was stirred at 35 °C for 16 h. The solvent was removed under reduced pressure, the residue was partitioned between ethyl acetate (100 mL) and water (100 mL) and the aqueous layer was re-extracted with ethyl acetate (100 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (AcOEt/CH_2Cl_2 1/1 then AcOEt/CH_2Cl_2/EtOH 4/5/1) to give **3** (8.77 g, 96%) as a white solid which may be recrystallized from EtOH and Et_2O; mp 131–132 °C. R_f 0.61 (AcOEt/CH_2Cl_2/EtOH 4/5/1). ^1H NMR (300 MHz, $CDCl_3$): δ 0.10, 0.11 (s, 12H, 2×$Si(CH_3)_2$), 0.92 (s, 18H, 2×$SiC(CH_3)_3$), 2.39–2.47 (m, 1H, 2′-H_A), 2.59–2.69 (m, 1H, 2′-H_B), 3.78 (dd, $J = 11.2$, 3.4 Hz, 1H, 5′-H_A), 3.88 (dd, $J = 11.2$, 4.4 Hz, 1H, 5′-H_B), 4.00–4.05 (m, 1H, 4′-H), 4.59–4.65 (m, 1H, 3′-H), 5.69 (bs, 2H, NH_2), 6.46 (t, $J = 6.5$ Hz, 1H, 1′-H), 8.14 (s, 1H, 8-H), 8.36 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.50, −5.40, −4.82, −4,68 (2×$Si(CH_3)_2$), 18.0, 18.4 (2×SiC), 25.9, 26.0 (2×$SiC(CH_3)_3$), 41.3 (2′-C), 62.6 (5′-C), 71.8 (3′-C), 84.3 (1′-C), 87.9 (4′-C), 120.0 (5-C), 140.0 (8-C), 149.6, 153.0 (4-C + 6-C), 155.4 (2-C). MS: *m/z* 480 $(M+H)^+$, 502 $(M+Na)^+$. HRMS for $C_{22}H_{41}N_5O_3Si_2$ $(M+H)^+$ calcd: 480.2826, found: 480.2836.

4.1.2. 9-[3,5-bis-*O*-(*tert*-Butyldimethylsilyl)-2-deoxy-β-D-*erythro*-pentofuranosyl]-6-chloropurine (4). Argon was bubbled through a solution of **3** (8.60 g, 17.9 mmol) in CCl_4 (150 mL) and this solution was cooled to 0 °C. Isoamyl nitrite (7.24 mL, 53.8 mmol) was added and the mixture was stirred at 50 °C for 3 h. The solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (AcOEt/hexane 1/4 then 1/2) to give **4** (3.71 g, 41%) as a yellow syrup. R_f 0.85 (AcOEt/hexane 1/1). ^1H NMR (300 MHz, $CDCl_3$): δ 0.10, 0.12 (s, 12H, 2×$Si(CH_3)_2$), 0.91, 0.93 (s, 18H, 2×$SiC(CH_3)_3$), 2.45–2.53 (m, 1H, 2′-H_A), 2.60–2.68 (m, 1H, 2′-H_B), 3.79 (dd, $J = 11.2$, 2.8 Hz, 1H, 5′-H_A), 3.90 (dd, $J = 11.2$, 3.8 Hz, 1H, 5′-H_B), 4.04–4.08 (m, 1H, 4′-H), 4.61–4.66 (m, 1H, 3′-H), 6.53 (t, $J = 6.5$ Hz, 1H, 1′-H), 8.50 (s, 1H, 8-H), 8.75 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.51, −5.41, −4.84, −4.69 (2×$Si(CH_3)_2$), 18.0, 18.4 (2×SiC), 25.7, 25.9 (2×$SiC(CH_3)_3$), 41.6 (2′-C), 62.7 (5′-C), 71.8 (3′-C), 84.9 (1′-C), 88.2 (4′-C), 132.1 (5-C), 143.8 (8-C), 151.0, 151.1 (4-C + 6-C), 151.8 (2-C). MS: *m/z* 500 (^{35}Cl-$M+H)^+$, 502 (^{37}Cl-$M+H)^+$.

4.1.3. 9-[3,5-bis-*O*-(*tert*-Butyldimethylsilyl)-2-deoxy-β-D-*erythro*-pentofuranosyl]-6-chloro-2-iodopurine (6). 1.6 M BuLi solution in hexane (8.50 mL, 13.6 mmol) was added dropwise to a solution of 2,2,6,6-tetramethylpiperidine (2.87 mL, 17.0 mmol) in anhydrous THF (20 mL) at −78 °C under an argon atmosphere. After stirring for 10 min, a solution of **4** (1.70 g, 3.40 mmol) in anhydrous THF (15 mL) was added dropwise to the mixture at −78 °C. After stirring for 2 min, Bu_3SnCl (2.77 mL, 10.2 mmol) was added to the lithiated mixture and stirring was continued for 30 min at −78 °C. The reaction was quenched by adding saturated aqueous NH_4Cl (10 mL) and the mixture was warmed to room temperature. The mixture was partitioned between ethyl acetate (100 mL) and saturated aqueous $NaHCO_3$ (100 mL) and the aqueous layer was re-extracted with ethyl acetate (100 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was dissolved in THF (25 mL) and iodine (1.30 g, 5.11 mmol) was added to the solution. After stirring for 4 h at room temperature, the reaction mixture was diluted with 10% aqueous $Na_2S_2O_3$ (50 mL) and extracted with ethyl acetate (2×100 mL). The organic layer was washed with brine (20 mL) and evaporated under reduced pressure. The residue was dissolved in ethyl acetate (20 mL) and treated with 15% aqueous KF (10 mL) for 15 min without stirring. The resulting suspension was filtered

and washed with ethyl acetate. The aqueous layer from filtrate was extracted with ethyl acetate (2×50 mL) and the combined organic layer was concentrated under reduced pressure. This KF treatment was carried out once again to remove Bu_3SnCl excess and then, the organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (hexane then AcOEt/hexane gradient from 1/50 to 1/4) to give **6** (1.17 g, 55%) as a yellow syrup. R_f 0.45 (AcOEt/hexane 1/4). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.10, 0.12 (s, 12H, 2×Si(CH_3)$_2$), 0.92, 0.93 (s, 18H, 2×SiC(CH_3)$_3$), 2.43–2.52 (m, 1H, 2′-H_A), 2.59–2.68 (m, 1H, 2′-H_B), 3.79 (dd, J = 11.2, 3.1 Hz, 1H, 5′-H_A), 3.91 (dd, J = 11.2, 3.8 Hz, 1H, 5′-H_B), 4.02–4.06 (m, 1H, 4′-H), 4.61–4.67 (m, 1H, 3′-H), 6.45 (t, J = 6.2 Hz, 1H, 1′-H), 8.40 (s, 1H, 8-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.45, −5.37, −4.80, −4.64 (2×Si(CH_3)$_2$), 18.0, 18.4 (2×SiC), 25.8, 26.0 (2×SiC(CH_3)$_3$), 41.5 (2′-C), 62.6 (5′-C), 71.7 (3′-C), 85.1 (1′-C), 88.3 (4′-C), 116.4 (2-C), 132.2 (5-C), 144.0 (8-C), 150.4, 151.8 (4-C + 6-C). MS: m/z 625 (^{35}Cl-M + H)$^+$, 627 (^{37}Cl-M + H)$^+$, 647 (^{35}Cl-M + Na)$^+$, 649 (^{37}Cl-M + Na)$^+$.

4.1.4. 2-Iodo-N^6-methyl-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (7).

2 M $MeNH_2$ solution in THF (3.0 mL, 6.00 mmol) was added to a solution of **6** (380 mg, 0.608 mmol) in EtOH (10 mL) and the resulting mixture was stirred at room temperature for 16 h. The solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (50 mL) and washed with brine (25 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (AcOEt/hexane 1/3 then 1/2) to give **7** (316 mg, 84%) as a colorless syrup. R_f 0.39 (AcOEt/hexane 1/3). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.09, 0.11 (s, 12H, 2×Si(CH_3)$_2$), 0.91 (s, 18H, 2×SiC(CH_3)$_3$), 2.33–2.43 (m, 1H, 2′-H_A), 2.61–2.70 (m, 1H, 2′-H_B), 3.15 (bs, 3H, NCH_3), 3.76 (dd, J = 11.2, 3.4 Hz, 1H, 5′-H_A), 3.87 (dd, J = 11.2, 4.4 Hz, 1H, 5′-H_B), 3.95–4.00 (m, 1H, 4′-H), 4.60–4.65 (m, 1H, 3′-H), 5.94 (bs, 1H, NH), 6.34 (t, J = 6,5 Hz, 1H, 1′-H), 7.92 (s, 1H, 8-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.47, −5.37, −4.80, −4.66 (2×Si(CH_3)$_2$), 18.0, 18.4 (2×SiC), 25.8, 26.0 (2×SiC(CH_3)$_3$), 40.9 (2′-C), 62.8 (5′-C), 71.9 (3′-C), 84.5 (1′-C), 87.9 (4′-C), 120.1, 120.5 (2-C + 5-C), 138.3 (8-C), 154.9 (4-C), 171.1 (6-C). MS: m/z 620 (M + H)$^+$, 642 (M + Na)$^+$. HRMS for $C_{23}H_{42}IN_5O_3Si_2$ (M + H)$^+$ calcd: 620.1949, found: 620.1971.

4.1.5. N^6-methyl-2-(2-trimethylsilylethynyl)-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy adenosine (8a).

Argon was bubbled through a solution of **7** (280 mg, 0.452 mmol) in Et_3N (6 mL) and then, $(PPh_3)_2PdCl_2$ (32 mg, 0.045 mmol) and CuI (8.6 mg, 0.045 mmol) were added to this solution. (Trimethylsilyl)acetylene (0,12 mL, 0.904 mmol, 2 equiv) was subsequently added dropwise to the reaction mixture, which was then stirred under argon at room temperature for 3 h. The solvent was removed under reduced pressure, the residue was taken up in ethyl acetate (50 mL) and successively washed with 10% aqueous Na_2EDTA (2×25 mL) and brine (25 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (AcOEt/hexane 1/2 then 1/1) to give **8a** (240 mg, 90%) as a yellow syrup. R_f 0.67 (AcOEt/hexane 1/1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.09, 0.10 (s, 12H, 2×Si(CH_3)$_2$), 0.30 (s, 9H, Si(CH_3)$_3$), 0.92 (s, 18H, 2×SiC(CH_3)$_3$), 2.37–2.47 (m, 1H, 2′-H_A), 2.51–2.62 (m, 1H, 2′-H_B), 3.23 (bs, 3H, NCH_3), 3.77 (dd, J = 11.3, 3.4 Hz, 1H, 5′-H_A), 3.86 (dd, J = 11.3, 3.8 Hz, 1H, 5′-H_B), 3.94–3.99 (m, 1H, 4′-H), 4.58–4.65 (m, 1H, 3′-H), 5.80 (bs, 1H, NH), 6.49 (t, J = 6.4 Hz, 1H, 1′-H), 8.11 (s, 1H, 8-H). ^{13}C NMR (50 MHz, $CDCl_3$): δ −5.51, −5.38, −4.82, −4.66 (2×Si(CH_3)$_2$), −0.21 (Si(CH_3)$_3$), 18.0, 18.4 (2×SiC), 25.8, 25.9 (2×SiC(CH_3)$_3$), 41.6 (2′-C), 62.8 (5′-C), 71.8 (3′-C), 83.9 (1′-C), 87.8 (4′-C), 90.1, 103.9 (C≡C-TMS), 119.7 (5-C), 139.1 (8-C), 146.0 (2-C), 155.1 (4-C). MS: m/z 590 (M + H)$^+$, 612 (M + Na)$^+$. HRMS for $C_{28}H_{51}N_5O_3Si_3$ (M + H)$^+$ calcd: 590.3378, found: 590.3379.

4.1.6. N^6-Methyl-2-(prop-1-ynyl)-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (8b).

Propyne (1 mL, 17 mmol) was condensed in a sealed tube at −78 °C and Et_3N (8 mL) was added dropwise. $(PPh_3)_2PdCl_2$ (39 mg, 0.055 mmol), CuI (10 mg, 0.055 mmol) and **7** (340 mg, 0.549 mmol) were added to this solution. The reaction mixture was warmed to room temperature under stirring for 3 h. The solvent was removed under reduced pressure, the residue was taken up in ethyl acetate (50 mL) and successively washed with 10% aqueous Na_2EDTA (2×25 mL) and brine (25 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (AcOEt/hexane 1/2 then 1/1) to give **8b** (230 mg, 79%) as a yellow syrup. R_f 0.34 (AcOEt/hexane 1/2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.09, 0.10 (s, 12H, 2×Si(CH_3)$_2$), 0.91, 0.93 (s, 18H, 2×SiC(CH_3)$_3$), 2.10 (s, 3H, C≡C–CH_3), 2.38–2.47 (m, 1H, 2′-H_A), 2.49–2.58 (m, 1H, 2′-H_B), 3.22 (bs, 3H, NCH_3), 3.77 (dd, J = 11.3, 2.8 Hz, 1H, 5′-H_A), 3.88 (dd, J = 11.3, 3.8 Hz, 1H, 5′-H_B), 3.96–4.02 (m, 1H, 4′-H), 4.57–4.64 (m, 1H, 3′-H), 5.78 (bs, 1H, NH), 6.50 (t, J = 6.4 Hz, 1H, 1′-H), 8.11 (s, 1H, 8-H). ^{13}C NMR (50 MHz, $CDCl_3$): δ −5.53, −5.41, −4.84, −4.66 (2×Si(CH_3)$_2$), 4.57 (C≡C–CH_3), 14.2 (NCH_3), 18.0, 18.4 (2×SiC), 25.8, 26.0 (2×SiC(CH_3)$_3$), 41.7 (2′-C), 62.8 (5′-C), 71.8 (3′-C), 84.0 (1′-C), 87.8 (4′-C), 138.8 (8-C), 146.8 (2-C), 155.2 (4-C), 160.4 (6-C). MS: m/z 532 (M + H)$^+$, 554 (M + Na)$^+$. HRMS for $C_{26}H_{45}N_5O_3Si_2$ (M + H)$^+$ calcd: 532.3139, found: 532.3152.

4.1.7. N^6-Methyl-2-(2-phenylethynyl)-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (8c).

This compound was prepared from **7** (430 mg, 0.694 mmol) and phenylacetylene (0.11 mL, 1.04 mmol) by the procedure described for the preparation of **8a**. Purification by silica gel column chromatography (AcOEt/hexane 1/2 then 1/1) afforded **8c** (350 mg, 85%) as a yellow syrup. R_f 0.44 (AcOEt/hexane 1/1). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.10, 0.11 (s, 12H, 2×Si(CH_3)$_2$), 0.92 (s, 18H, 2×SiC(CH_3)$_3$), 2.40–2.52 (m, 1H, 2′-H_A), 2.55–2.68 (m, 1H, 2′-H_B), 3.27 (bs, 3H, NCH_3), 3.78 (dd, J = 11.2, 3.2 Hz, 1H, 5′-H_A), 3.84 (dd, J = 11.2, 3.9 Hz, 1H, 5′-H_B),

3.97–4.04 (m, 1H, 4′-H), 4.58–4.66 (m, 1H, 3′-H), 5.86 (bs, 1H, NH), 6.52 (t, $J = 6.5$ Hz, 1H, 1′-H), 7.32–7.41, 7.62–7.73 (m, 5H, Ph), 8.13 (s, 1H, 8-H). ^{13}C NMR (50 MHz, $CDCl_3$): δ −5.51, −5.39, −4.82, −4.66 (2×Si$(CH_3)_2$), 14.1 (NCH_3), 18.0, 18.4 (2×SiC), 25.8, 25.9 (2×SiC$(CH_3)_3$), 41.5 (2′-C), 62.8 (5′-C), 71.8 (3′-C), 84.1 (1′-C), 87.8 (4′-C), 122.2 (5-C), 128.2, 139.0, 131.9, 132.5 (Ph), 139.1 (8-C), 155.2 (4-C). MS: m/z 594 $(M+H)^+$, 616 $(M+Na)^+$. HRMS for $C_{31}H_{47}N_5O_3Si_2$ $(M+Na)^+$ calcd: 616.3115, found: 616.3108.

4.1.8. 2-Ethynyl-*N*^{6}-methyl-2′-deoxyadenosine (9a). 1 M TBAF solution in THF (1.12 mL, 1.12 mmol) was added to a solution of **8a** (220 mg, 0.373 mmol) in CH_3CN (10 mL) and the resulting mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH 9/1) to give **9a** (100 mg, 92%) as a white solid which may be recrystallized from EtOH and Et_2O; mp 83–84 °C. R_f 0.39 ($CHCl_3$/MeOH 9/1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.26–2.36 (m, 1H, 2′-H_A), 3.04 (s, 1H, C≡CH), 3.07–3.18 (m, 1H, 2′-H_B), 3.21 (bs, 3H, NCH_3), 3.77–3.88 (m, 1H, 5′-H_A), 3.98–4.07 (m, 1H, 5′-H_B), 4.20–4.25 (m, 1H, 4′-H), 4.81–4.85 (m, 1H, 3′-H), 5.99 (bs, 1H, NH), 6.18 (bs, 2H, 2×OH), 6.31 (dd, $J = 5.4$, 9.6 Hz, 1H, 1′-H), 7.82 (s, 1H, 8-H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 40.8 (2′-C), 63.6 (5′-C), 73.5, 73.8 (3′-C + C≡CH), 87.7 (1′-C), 89.8 (4′-C), 140.3 (8-C). MS: m/z 290 $(M+H)^+$, 312 $(M+Na)^+$. HRMS for $C_{13}H_{15}N_5O_3$ $(M+H)^+$ calcd: 290.1253, found: 290.1247.

4.1.9. *N*^{6}-Methyl-2-(prop-1-ynyl)-2′-deoxyadenosine (9b). This compound was prepared from **8b** (220 mg, 0.414 mmol) and 1 M TBAF solution in THF (0.83 mL, 0.830 mmol) by the procedure described for the preparation of **9a**. Purification by silica gel column chromatography (CH_2Cl_2/MeOH 9/1) afforded **9b** (125 mg, 99%) as a white solid which may be recrystallized from EtOH and Et_2O; mp 91–92 °C. R_f 0.34 (CH_2Cl_2/MeOH 9/1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.10 (s, 3H, C≡C–CH_3), 2.26–2.35 (m, 1H, 2′-H_A), 3.04–3.15 (m, 1H, 2′-H_B), 3.21 (bs, 3H, NCH_3), 3.77–3.88 (m, 1H, 5′-H_A), 4.00 (dd, $J = 12.8$, 1.3 Hz, 1H, 5′-H_B), 4.20–4.24 (m, 1H, 4′-H), 4.78–4.84 (m, 1H, 3′-H), 5.92 (bs, 1H, NH), 6.23 (bs, 2H, 2×OH), 6.31 (dd, $J = 9.4$, 5.3 Hz, 1H, 1′-H), 7.81 (s, 1H, 8-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 4.35 (CH_3), 13.5 (NCH_3), 40.7 (2′-C), 63.0 (5′-C), 72.3 (3′-C), 79.3, 83.6 (C≡C), 86.7 (1′-C), 89.1 (4′-C), 120.0 (5-C), 139.6 (8-C), 146.3 (2-C), 155.2 (4-C), 171.2 (6-C). MS: m/z 304 $(M+H)^+$, 326 $(M+Na)^+$. HRMS for $C_{14}H_{17}N_5O_3$ $(M+H)^+$ calcd: 304.1410, found: 304.1445.

4.1.10. *N*^{6}-Methyl-2-(2-phenylethynyl)-2′-deoxyadenosine (9c). This compound was prepared from **8c** (350 mg, 0.589 mmol) and 1 M TBAF solution in THF (1.18 mL, 1.18 mmol) by the procedure described for the preparation of **9a**. Purification by silica gel column chromatography (CH_2Cl_2/MeOH 9/1) yielded **9c** (203 mg, 94%) as a white solid which may be recrystallized from EtOH and Et_2O; mp 126–128 °C. R_f 0.41 (CH_2Cl_2/MeOH 9/1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.31–2.40 (m, 1H, 2′-H_A), 3.01–3.13 (m, 1H, 2′-H_B), 3.28 (bs, 3H, NCH_3), 3.81–3.91 (m, 1H, 5′-H_A), 4.02–4.09 (m, 1H, 5′-H_B), 4.23–4.26 (m, 1H, 4′-H), 4.80–4.85 (m, 1H, 3′-H), 6.23–6.32 (m, 1H, 1′-H), 6.35 (bs, 3H, NH + 2×OH), 7.37–7.42, 7.68–7.74 (m, 5H, Ph), 7.75 (s, 1H, 8-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 40.9 (2′-C), 63.6 (5′-C), 73.5 (3′-C), 87.7 (1′-C), 89.6 (4′-C), 121.7 (5-C), 128.4, 129.4, 132.7 (Ph), 139.9 (8-C), 155.5 (4-C). MS: m/z 366 $(M+H)^+$, 388 $(M+Na)^+$. HRMS for $C_{19}H_{19}N_5O_3$ $(M+H)^+$ calcd: 366.1566, found: 366.1556.

4.1.11. 2-Ethynyl-*N*^{6}-methyl-2′-deoxyadenosine-3′,5′-bis(dibenzylphosphate) (10a). 1.0 M potassium *tert*-butoxide solution in THF (0.72 mL, 0.720 mmol) was added dropwise to a stirred solution of **9a** (95 mg, 0.328 mmol) in anhydrous THF (10 mL) at −40 °C. After 5 min, tetrabenzyl pyrophosphate (390 mg, 0.720 mmol) was added and the resulting mixture was stirred at −40 °C for 30 min. The reaction was quenched by adding saturated aqueous NH_4Cl (10 mL) and the mixture was allowed to warm to room temperature. The mixture was diluted with water (100 mL) and extracted with ethyl acetate (2×100 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (AcOEt/CH_2Cl_2/EtOH 4/5/1) to give **10a** (200 mg, 75%) as a yellow syrup. R_f 0.59 (AcOEt/CH_2Cl_2/EtOH 4/5/1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.40–2.48 (m, 2H, 2′-H), 2.95 (s, 1H, C≡CH), 3.22 (bs, 3H, NCH_3), 4.02–4.12 (m, 2H, 5′-H), 4.17–4.23 (m, 1H, 4′-H), 5.00, 5.06 (d, 2×4H, 4×CH_2), 5.02–5.10 (m, 1H, 3′-H), 5.88 (bs, 1H, NH), 6.35 (t, $J = 7.2$ Hz, 1H, 1′-H), 7.29, 7.31, 7.36 (s, 20H, 4×Ph), 7.97 (s, 1H, 8-H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 38.6 (2′-C), 66.4 (5′-C), 69.5, 69.6, 69.7, 69.9 (4×CH_2), 72.7 (3′-C), 83.0, 83.7 (1′-C + 4′-C), 128.0, 128.1, 128.2, 128.6, 128.7, 128.8 (4×Ph), 135.4 (C≡CH), 138.7 (8-C), 145.5 (2-C), 155.2 (4-C). MS: m/z 810 $(M+H)^+$, 832 $(M+Na)^+$. HRMS for $C_{41}H_{41}N_5O_9P_2$ $(M+H)^+$ calcd: 810.2458, found: 810.2459.

4.1.12. *N*^{6}-Methyl-2-(prop-1-ynyl)-2′-deoxyadenosine-3′,5′-bis(dibenzylphosphate) (10b). This compound was prepared from **9b** (60 mg, 0.198 mmol) by the procedure described for the preparation of **10a**. Purification by silica gel column chromatography (AcOEt/CH_2Cl_2/EtOH 4/5/1) afforded **10b** (138 mg, 85%) as a colorless syrup. R_f 0.57 (AcOEt/CH_2Cl_2/EtOH 4/5/1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.05 (s, 3H, C≡C–CH_3), 2.34–2.47 (m, 2H, 2′-H), 3.19 (bs, 3H, NCH_3), 4.01–4.11 (m, 2H, 5′-H), 4.16–4.20 (m, 1H, 4′-H), 4.98, 5.06 (d, 2×4H, 4×CH_2), 5.03–5.08 (m, 1H, 3′-H), 6.13 (bs, 1H, NH), 6.39 (dd, $J = 8.1$, 5.9 Hz, 1H, 1′-H), 7.27, 7.29, 7.34 (s, 20H, 4×Ph), 7.95 (s, 1H, 8-H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 4.60 (CH_3), 39.6 (2′-C), 66.4 (5′-C), 69.5, 69.6, 69.7, 69.8 (4×CH_2), 72.7 (3′-C), 82.8, 83.5 (C≡C), 87.6 (1′-C), 90.2 (4′-C), 127.8, 127.9, 128.0, 128.4, 128.5 (4×Ph), 138.8 (8-C), 146.5 (2-C), 155.1 (4-C). MS: m/z 824 $(M+H)^+$, 846 $(M+Na)^+$. HRMS for $C_{42}H_{43}N_5O_9P_2$ $(M+H)^+$ calcd: 824.2614, found: 824.2592.

4.1.13. *N*^{6}-Methyl-2-(2-phenylethynyl)-2′-deoxyadenosine-3′,5′-bis(dibenzylphosphate) (10c). This compound was prepared from **9c** (198 mg, 0.542 mmol) by the proce-

dure described for the preparation of **10a**. Purification by silica gel column chromatography ($AcOEt/CH_2Cl_2$/EtOH 4/5/1) yielded **10c** (456 mg, 95%) as a colorless syrup. R_f 0.69 ($AcOEt/CH_2Cl_2$/EtOH 4/5/1). ^1H NMR (300 MHz, $CDCl_3$): δ 2.42–2.49 (m, 2H, 2′-H), 3.27 (bs, 3H, NCH_3), 4.05–4.13 (m, 2H, 5′-H), 4.18–4.25 (m, 1H, 4′-H), 5.00, 5.06 (d, 2×4H, 4×CH_2), 5.04–5.10 (m, 1H, 3′-H), 5.94 (bs, 1H, NH), 6.40 (t, J = 7.2 Hz, 1H, 1′-H), 7.28-7.39, 7.65–7.68 (m, 25H, 5×Ph), 7.98 (s, 1H, 8-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.7 (2′-C), 66.4 (5′-C), 69.5, 69.6, 69.8 (4×CH_2), 77.9 (3′-C), 83.6 (1′-C), 84.8 (4′-C), 128.0, 128.1, 128.2, 128.6, 128.7, 128.8, 129.0 (5×Ph), 138.4 (8-C), 146.9 (2-C), 155.2 (4-C). MS: m/z 886 $(M+H)^+$, 908 $(M+Na)^+$. HRMS for $C_{47}H_{45}N_5O_9P_2$ $(M+Na)^+$ calcd: 908.2590, found: 908.2606.

4.1.14. 2-Ethyl-*N*^{6}-methyl-2′-deoxyadenosine-3′,5′-bisphosphate (1e). A mixture of **10a** (180 mg, 0.222 mmol) and 10% Pd/C (50 mg) in MeOH (100mL) was shaken in a hydrogenation apparatus under 60 psi pressure at room temperature for 24 h. The catalyst was removed by filtration and washed with MeOH and water. The filtrate was concentrated to dryness and the product was purified by recrystallization from MeOH and Et_2O to furnish **1e** (60 mg, 60%) as a colorless solid; decomp. >130 °C. ^1H NMR (300 MHz, D_2O): δ 1.29 (t, J = 7.2 Hz, 3H, CH_3), 2.68–2.77 (m, 1H, 2′-H_A), 2.82–2.98 (m, 3H, 2′-H_B + CH_2), 3.16 (bs, 3H, NCH_3), 4.03–4.11 (m, 2H, 5′-H), 4.37–4.44 (m, 1H, 4′-H), 4.97–5.05 (m, 1H, 3′-H), 6.51 (t, J = 5.9 Hz, 1H, 1′-H), 8.41 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.1 (CH_3), 31.5 (CH_2), 32.3 (2′-C), 67.8 (5′-C), 76.2 (3′-C), 83.2 (1′-C), 83.8 (4′-C), 139.9 (8-C). ^{31}P NMR (81 MHz, DMSO-d_6): δ-1.37 (3′-OPO_3H_2), 0.20 (5′-OPO_3H_2). MS: m/z no $(M+H)^+$, 178 (adenine fragment). HPLC: R_T = 20.75 min.

4.1.15. *N*^{6}-methyl-2-propyl-2′-deoxyadenosine-3′,5′-bisphosphate (1f). This compound was prepared from **10b** (125 mg, 0.152 mmol) by the procedure described for the preparation of **1e**. Recrystallization from MeOH afforded **1f** (70 mg, 99%) as a white solid; decomp. >130 °C. ^1H NMR (200 MHz, DMSO-d_6): δ 0.94 (t, J = 7.1 Hz, 3H, CH_3), 1.08–1.29 (m, 2H, CH_2), 1.66–1.87 (m, 2H, CH_2), 2.61–2.80 (m, 2H, 2′-H), 2.98 (bs, 3H, NCH_3), 3.93–4.18 (m, 2H, 5′-H), 4.25–4.36 (m, 1H, 4′-H), 4.96-5.12 (m, 1H, 3′-H), 6.38 (t, 1H, 1′-H), 7.71 (bs, 1H, NH), 8.27 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.6 (CH_3), 15.7 (CH_2), 32.1 (CH_2), 32.8 (2′-C), 67.8 (5′-C), 76.4 (3′-C), 83.1 (1′-C), 83.9 (4′-C), 138.9 (8-C), 141.5 (2-C). ^{31}P NMR (81 MHz, DMSO-d_6): δ −1.55 (3′-OPO_3H_2), −0.56 (5′-OPO_3H_2). MS: m/z no $(M+H)^+$, 192 (adenine fragment). HPLC: R_T = 23.13 min.

4.1.16. *N*^{6}-Methyl-2-(2-phenylethyl)-2′-deoxyadenosine-3′,5′-bisphosphate (1g). This compound was prepared from **10c** (455 mg, 0.514 mmol) by the procedure described for the preparation of **1e**. Recrystallization from MeOH yielded **1g** (120 mg, 44%) as a white solid; decomp. >130 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 2.93–3.02 (m, 2H, 2′-H), 3.07 (bs, 3H, NCH_3), 3.98–4.16 (m, 2H, 5′-H), 4.26–4.34 (m, 1H, 4′-H), 4.88 (t, J = 6.8 Hz, 2H, CH_2), 4.99 (t, J = 6.8 Hz, 2H, CH_2), 5.08–5.18 (m, 1H, 3′-H), 6.40 (t, 1H, 1′-H), 7.23–7.35 (m, 5H, Ph), 7.41 (bs, 1H, NH), 8.30 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 34.1 (2′-C), 41.5 (CH_2), 49.3 (CH_2), 67.6 (5′-C), 76.2 (3′-C), 83.4 (1′-C), 83.7 (4′-C), 127.7, 127.8, 128.2, 128.5 (Ph), 137.6 (8-C), 142.0 (2-C). ^{31}P NMR (81 MHz, DMSO-d_6): δ −1.28 (3′-OPO_3H_2), −0.52 (5′-OPO_3H_2). MS: m/z no $(M+H)^+$, 254 (adenine fragment). HPLC: R_T = 27.24 min.

4.1.17. 2-Ethynyl-*N*^{6}-methyl-2′-deoxyadenosine-3′,5′-bis(di-*tert*-butylphosphate) (11a). Di-*tert*-butyl *N*,*N*-diethylphosphoramidite (0.29 mL, 1.04 mmol) and tetrazole (145 mg, 2.07 mmol) were added to a solution of **9a** (100 mg, 0.346 mmol) in anhydrous THF (7 mL). After the solution was stirred at room temperature for 2 h, the reaction mixture was cooled to −78 °C and MCPBA (60–77%, 298 mg, 1.04 mmol) was rapidly added. The reaction mixture was stirred at −78 °C for 30 min and warmed to room temperature for 1 h under stirring. Excess of MCPBA was reduced by 10% aqueous Na_2SO_3 (25 mL) and the mixture was extracted with ethyl acetate (2×50 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography ($AcOEt/CH_2Cl_2$/EtOH 4/5/1) to give **11a** (100 mg, 43%) as a colorless syrup. R_f 0.54 ($AcOEt/CH_2Cl_2$/EtOH 4/5/1). ^1H NMR (200 MHz, $CDCl_3$): δ 1.43, 1.45, 1.48 (s, 36H, 4×$C(CH_3)_3$), 2.67–2.77 (m, 2H, 2′-H), 2.95 (s, 1H, C≡CH), 3.18 (bs, 3H, NCH_3), 4.11–4.19 (m, 2H, 5′-H), 4.37–4.45 (m, 1H, 4′-H), 5.02–5.09 (m, 1H, 3′-H), 6.17 (bs, 1H, NH), 6.52 (t, J = 7.1 Hz, 1H, 1′-H), 8.13 (s, 1H, 8-H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.2 (NCH_3), 29.8, 29.9 ($C(CH_3)_3$), 40.6 (2′-C), 66.1 (5′-C), 71.3 (3′-C), 72.7, 77.3 (C≡CH), 83.8 (1′-C), 85.0 (4′-C), 119.9 (5-C), 139.0 (8-C), 145.4 (2-C), 155.1 (4-C). MS: m/z 674 $(M+H)^+$, 696 $(M+Na)^+$. HRMS for $C_{29}H_{49}N_5O_9P_2$ $(M+H)^+$ calcd: 674.3084, found: 674.3071.

4.1.18. *N*^{6}-Methyl-2-(prop-1-ynyl)-2′-deoxyadenosine-3′,5′-bis(di-*tert*-butylphosphate) (11b). This compound was prepared from **9b** (60 mg, 0.198 mmol) by the procedure described for the preparation of **11a**. Purification by silica gel column chromatography ($AcOEt/CH_2Cl_2$/EtOH 4/5/1) afforded **11b** (97 mg, 71%) as a colorless syrup. R_f 0.49 ($AcOEt/CH_2Cl_2$/EtOH 4/5/1). ^1H NMR (300 MHz, $CDCl_3$): δ 1.48, 1.50, 1.52 (s, 36H, 4×$C(CH_3)_3$), 2.10 (s, 3H, C≡C–CH_3), 2.66–2.75 (m, 2H, 2′-H), 3.22 (bs, 3H, NCH_3), 4.16–4.22 (m, 2H, 5′-H), 4.41–4.47 (m, 1H, 4′-H), 5.03–5.11 (m, 1H, 3′-H), 5.80 (bs, 1H, NH), 6.59 (t, J = 7.2 Hz, 1H, 1′-H), 8.15 (s, 1H, 8-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 4.57 (CH_3), 29.8, 29.9, 30.0 ($C(CH_3)_3$), 39.6 (2′-C), 66.3 (5′-C), 83.2, 83.6 (C≡C), 88.4 (1′-C), 91.7 (4′-C), 119.4 (5-C), 138.6 (8-C), 146.9 (2-C), 155.2 (4-C). MS: m/z 688 $(M+H)^+$, 710 $(M+Na)^+$. HRMS for $C_{30}H_{51}N_5O_9P_2$ $(M+H)^+$ calcd: 688.3240, found: 688.3243.

4.1.19. 2-Ethynyl-*N*^{6}-methyl-2′-deoxyadenosine-3′,5′-bisphosphate (1h). Trifluoroacetic acid (0.15 mL) was added to a solution of **11a** (100 mg, 0.148 mmol) in CH_2Cl_2/MeOH (3 mL/1 mL) and the reaction mixture was stirred at room temperature for 30 min. The solvent

was removed under reduced pressure and the residue was triturated in EtOH. The precipitate was filtered and recrystallized from MeOH and Et_2O to give **1g** (33 mg, 50%) as a white solid; decomp. >130 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.58–2.72 (m, 2H, 2′-H), 2.96 (bs, 3H, NCH_3), 3.27 (s, 1H, C≡CH), 3.94–4.07 (m, 2H, 5′-H), 4.25–4.36 (m, 1H, 4′-H), 4.91–5.02 (m, 1H, 3′-H), 6.38 (t, 1H, 1′-H), 7.82 (bs, 1H, NH), 8.24 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 28.1 (2′-C), 66.2 (5′-C), 75.9 (C≡CH), 76.3 (3′-C), 83.9 (1′-C), 84.7 (4′-C), 140.5 (8-C), 141.3 (2-C). ^{31}P NMR (81 MHz, DMSO-d_6): δ-0.49 (3′-OPO_3H_2), −0.19 (5′-OPO_3H_2). MS: m/z no $(M+H)^+$, 174 (adenine fragment). HPLC: $R_T = 14.12$ min.

4.1.20. N^6-Methyl-2-(prop-1-ynyl)-2′-deoxyadenosine-3′,5′-bisphosphate (1i). This compound was prepared from **11b** (89 mg, 0.129 mmol) by the procedure described for the preparation of **1g**. Recrystallization from MeOH and Et_2O afforded **1h** (35 mg, 58%) as a white solid; decomp. >130 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.06 (s, 3H, C≡C–CH_3), 2.58–2.70 (m, 2H, 2′-H), 2.95 (bs, 3H, NCH_3), 3.96–4.17 (m, 2H, 5′-H), 4.26–4.35 (m, 1H, 4′-H), 4.93–5.15 (m, 1H, 3′-H), 6.37 (t, $J = 7.1$ Hz, 1H, 1′-H), 7.86 (bs, 1H, NH), 8.21 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 4.82 (CH_3), 30.9 (2′-C), 67.1 (5′-C), 76.1 (3′-C), 81.2, 83.4 (C≡C), 84.3 (1′-C), 84.9 (4′-C), 139.7 (8-C), 142.9 (2-C). ^{31}P NMR (81 MHz, DMSO-d_6): δ −0.62 (3′-OPO_3H_2), −0.08 (5′-OPO_3H_2). MS: m/z no $(M+H)^+$, 188 (adenine fragment). HPLC: $R_T = 16.30$ min.

4.2. Biological tests

2-Methylthioadenosine-5′-diphosphate (2-MeSADP), adenosine-5′-diphosphate (ADP), prostaglandin E_1 (PGE_1) were from Sigma (Saint Quentin-Fallavier, France). Human fibrinogen was from Kabi (Stockholm, Sweden), fura-2/acetoxymethyl ester (fura-2/AM) from Calbiochem (Meudon, France) and the cyclic adenosine-3′,5′-monophosphate (cAMP) assay kit from Amersham (Les Ulis, France). Apyrase was purified from potatoes as previously described.[32] [^{33}P]2-MeSADP was provided by Du Pont NEN (Le Blanc Mesnil, France). AR-C66096MX was a generous gift from AstraZeneca (Charnwood, UK).

4.2.1. Washed human platelet aggregation. Washed human platelets were prepared as previously described[32] and resuspended at 3×10^5 platelets/μL in Tyrode's buffer containing 2 mM $CaCl_2$, in the presence of 0.02 U/mL of the ADP scavenger apyrase (adenosine-5′-triphosphate diphosphorylase, EC 3.6.1.5), a concentration sufficient to prevent the desensitization of platelet ADP receptors during storage.[33] Platelets were kept at 37 °C throughout all experiments and aggregation was measured by standard methods.[10,32] Briefly, a 450 μL aliquot of platelet suspension was stirred at 1100 rpm and activated by addition of agonists and for human platelets of human fibrinogen (0.8 mg/mL), in a final volume of 500 μL. The extent of aggregation was estimated quantitatively by measuring the maximum curve height above the baseline.

4.2.2. $[Ca^{2+}]_i$ Measurements. Fura-2/AM-loaded human platelets were prepared as previously described[10] and resuspended in Tyrode's buffer with 2 mM $CaCl_2$. Aliquots of fura-2-loaded platelets were transferred to a 10×10 mm quartz cuvette maintained at 37 °C and fluorescence measurements were performed under continuous stirring, in a PTI Deltascan spectrofluorimeter (Photon Technology International Inc., Princetown, NJ, USA).[10] The excitation wavelength was alternately fixed at 340 or 380 nm, fluorescence emission was determined at 510 nm and results were calculated as the fluorescence ratio (340/380) in arbitrary units.

4.2.3. Measurement of adenylyl cyclase activity. A 450 μL aliquot of washed platelets resuspended in Tyrode's buffer containing 2 mM Ca^{2+} and 1 mM Mg^{2+} was stirred at 1100 rpm in an aggregometer cuvette and the following reagents were added at 30 s intervals: (i) 10 μM PGE_1, (ii) 1 μM AR-C66096MX or different concentrations of compounds **1e–i**, and (iii) 5 μM ADP or vehicle (Tyrode's buffer containing no Ca^{2+} or Mg^{2+}). The reaction was stopped 1 min later by addition of 50 μL of ice-cold 6.6 N perchloric acid. Perchloric acid extracts were centrifuged at 11,000 g for 5 min to eliminate protein precipitate, and cAMP was isolated from the supernatants using a mixture of trioctylamine and Freon (28/22, vol/vol). The upper aqueous phase was lyophilized and the dry residue dissolved in the buffer provided with the commercial radioimmunoassay kit for cAMP measurement.[10]

4.2.4. Binding studies. Competitive binding of [^{33}P]2-MeSADP (850 Ci/mmol) to washed platelets at 37 °C for 5 min was determined as described in earlier work.[31] Displacement experiments were performed using a single concentration of radiolabeled ligand [^{33}P]2-MeSADP (0.2 nM, 200.000 dpm), in the presence of increasing concentrations of the appropriate unlabeled ligand. The reactions were terminated by addition of ice cold Tyrode's buffer and rapid filtration through Whatman GF/C glass fiber filters under vacuum, after which the tubes and filters were rinsed twice. Radioactivity bound to the platelets on the filters was measured by scintillation counting (Wallac 1409 β-counter, count rate (DPM/CPM ± S.E.M.): 1.070 ± 0.0018; Turku, Finland). The inhibition constants for the drugs (K_i) were calculated using the GraphPad software package (GraphPad, San Diego, CA).

References and notes

1. Ralevic, V.; Burnstock, G. *Pharmacol. Rev.* **1998**, *50*, 413.
2. von Kügelgen, I.; Wetter, A. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2000**, *362*, 310.
3. Gachet, C. *Int. J. Hematol.* **2001**, *74*, 375.
4. Storey, R. F.; Newby, L. J.; Heptinstall, S. *Platelets* **2001**, *12*, 443.
5. Hechler, B.; Eckly, A.; Ohlmann, P.; Cazenave, J.-P.; Gachet, C. *Br. J. Haematol.* **1998**, *103*, 858.
6. Geiger, J.; Brich, J.; Hönig-Liedl, P.; Eigenthaler, M.; Schanzenbacher, P.; Herbert, J. M.; Walter, U. *Arterioscler., Thromb., Vasc. Biol.* **1999**, *19*, 2007.

7. Humphries, R. G.; Tomlinson, W.; Ingall, A. H.; Cage, P. A.; Leff, P. *Br. J. Pharmacol.* **1994**, *113*, 1057.
8. Fagura, M. S.; Dainty, I. A.; McKay, G. D.; Kirk, I. P.; Humphries, R. G.; Robertson, M. J.; Dougall, I. G.; Leff, P. *Br. J. Pharmacol.* **1998**, *124*, 157.
9. Daniel, J. L.; Dangelmaier, C.; Jin, J.; Ashby, B.; Smith, J. B.; Kunapuli, S. P. *J. Biol. Chem.* **1998**, *273*, 2024.
10. Hechler, B.; Léon, C.; Vial, C.; Vigne, P.; Frelin, C.; Cazenave, J.-P.; Gachet, C. *Blood* **1998**, *92*, 152.
11. Jin, J.; Kunapuli, S. P. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 8070.
12. Léon, C.; Freund, M.; Ravanat, C.; Baurand, A.; Cazenave, J.-P.; Gachet, C. *Circulation* **2001**, *103*, 718.
13. Léon, C.; Hechler, B.; Freund, M.; Eckly, A.; Vial, C.; Ohlmann, P.; Dierich, A.; LeMeur, M.; Cazenave, J.-P.; Gachet, C. *J. Clin. Invest.* **1999**, *104*, 1731.
14. Fabre, J. E.; Nguyen, M. T.; Latour, A.; Keifer, J. A.; Audoly, L. P.; Coffman, T. M.; Koller, B. H. *Nat. Med.* **1999**, *5*, 1199.
15. Baurand, A.; Gachet, C. *Cardiovasc. Drug Rev.* **2003**, *21*, 67.
16. Lenain, N.; Freund, M.; Léon, C.; Cazenave, J.-P.; Gachet, C. *J. Thromb. Haemost.* **2003**, *1*, 1144.
17. Boyer, J. L.; Romero-Avila, T.; Schachter, J. B.; Harden, T. K. *Mol. Pharmacol.* **1996**, *50*, 1323.
18. Camaioni, E.; Boyer, J. L.; Mohanram, A.; Harden, T. K.; Jacobson, K. A. *J. Med. Chem.* **1998**, *41*, 183.
19. Boyer, J. L.; Mohanram, A.; Camaioni, E.; Jacobson, K. A.; Harden, T. K. *Br. J. Pharmacol.* **1998**, *124*, 1.
20. Nandanan, E.; Camaioni, E.; Jang, S.-Y.; Kim, Y.-C.; Cristalli, G.; Herdewijn, P.; Secrist, J. A.; Tiwari, K. N.; Mohanram, A.; Harden, T. K.; Boyer, J. L.; Jacobson, K. A. *J. Med. Chem.* **1999**, *42*, 1625.
21. Nandanan, E.; Jang, S.-Y.; Moro, S.; Kim, H. O.; Siddiqui, M. A.; Russ, P.; Marquez, V. E.; Busson, R.; Herdewijn, P.; Harden, T. K.; Boyer, J. L.; Jacobson, K. A. *J. Med. Chem.* **2000**, *43*, 829.
22. Raboisson, P.; Baurand, A.; Cazenave, J.-P.; Gachet, C.; Retat, M.; Spiess, B.; Bourguignon, J.-J. *J. Med. Chem.* **2002**, *45*, 962.
23. Nair, V.; Purdy, D. F. *Tetrahedron* **1991**, *47*, 365.
24. Golisade, A.; Van Calenbergh, S.; Link, A. *Tetrahedron* **2000**, *56*, 3167.
25. Kumamoto, H.; Tanaka, H.; Tsukioka, R.; Ishida, Y.; Nakamura, A.; Kimura, S.; Hayakawa, H.; Kato, K.; Miyasaka, T. *J. Org. Chem.* **1999**, *64*, 7773.
26. Kato, K.; Hayakawa, H.; Tanaka, H.; Kumamoto, H.; Shindoh, S.; Shuto, S.; Miyasaka, T. *J. Org. Chem.* **1997**, *62*, 6833.
27. Vacca, J. P.; deSolms, S. J.; Huff, J. R. *J. Am. Chem. Soc.* **1987**, *109*, 3478.
28. Perich, J. W.; Johns, R. B. *Synthesis* **1988**, *2*, 142.
29. Baurand, A.; Raboisson, P.; Freund, M.; Léon, C.; Cazenave, J.-P.; Bourguignon, J.-J.; Gachet, C. *Eur. J. Pharmacol.* **2001**, *412*, 213.
30. Humphries, R. G. *Haematologica* **2000**, *85*, 66 supplement.
31. Gachet, C.; Cattaneo, M.; Ohlmann, P.; Hechler, B.; Lecchi, A.; Chevalier, J.; Mannuccio Mannucci, P.; Cazenave, J.-P. *Br. J. Haematol.* **1995**, *91*, 434.
32. Cazenave, J.-P.; Hemmendinger, S.; Beretz, A.; Sutter-Bay, A.; Launay, J. *Ann. Biol. Clin.* **1983**, *41*, 167.
33. Baurand, A.; Eckly, A.; Bari, N.; Léon, C.; Hechler, B.; Cazenave, J.-P.; Gachet, C. *Thromb. Haemostasis* **2000**, *84*, 484.