Expedited Articles

Antagonists of the Platelet P_{2T} Receptor: A Novel Approach to Antithrombotic Therapy

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The platelet P_{2T} receptor plays a major role in platelet aggregation, and its antagonists are predicted to have significant therapeutic potential as antithrombotic agents. We have explored analogues of adenosine triphosphate (ATP), which is a weak, nonselective but competitive P_{2T} receptor antagonist. Modification of the polyphosphate side chain to prevent breakdown to the agonist adenosine diphosphate (ADP) and substitution of the adenine moiety to enhance affinity and selectivity for the P_{2T} receptor led to the identification of **10e** (AR-C67085MX), having an IC_{50} of 2.5 nM against ADP-induced aggregation of human platelets. Compound **10e** was the first very potent antagonist of the P_{2T} receptor, with a selectivity for that subtype of the P2 receptor family of >1000-fold. Further modification of the structure produced compound **10l** (AR-C69931MX) having an IC_{50} of 0.4 nM. In vivo, at maximally effective antithrombotic doses, there is little prolongation of bleeding time (1.4-fold), which is in marked contrast to the 5−6-fold found with GPIIb/IIIa antagonists.

Introduction

Platelet adhesion and aggregation are pivotal events in arterial 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. As a component of normal hemostasis, platelet aggregation serves a vital physiological function by arresting bleeding following traumatic transection of blood vessels. However, platelet aggregation at the site of a ruptured atherosclerotic plaque on the surface of, for example, a coronary artery may result in life-threatening partial or complete occlusive arterial thrombosis manifesting as unstable angina or myocardial infarction. Intervention in the process of thrombus formation has long been an attractive therapeutic target for the treatment or prophylaxis of such events, and many approaches, centered on the various components of the process, have been investigated.

Our interest in antithrombotic therapy stemmed from our investigations of P2 receptors (a receptor family whose endogenous agonists are nucleotides) and the working hypothesis that activation of the P_{2T} receptor subtype on platelets by adenosine diphosphate (ADP) is a key event in arterial thrombosis. At the time of our entry into the area, four subtypes of the P2 family were known and, in the absence of structural information and selective antagonists, were then designated P_{2x}, P_{2y}, P_{2z}, and P_{2t}.[1,2] Moreover there was a complete dearth of information on the physiological importance of P2 receptors in man. The application of molecular biological techniques in the last 5 years has greatly expanded the number of variants within the P2 receptor family while simplifying its structure, and the nomenclature has been modified accordingly. Thus the "P_{2y} receptor" comprises a group of transmembrane helix G-protein-coupled receptors (designated $P2Y_1$,etc.), and the "P_{2x} receptor" has been subdivided into eight ion channels ($P2X_{1-8}$), subsuming the P_{2z} type. Many of the members of these subfamilies have been cloned and sequenced.[3]

As yet there is no definitive structural information on the P_{2T} receptor, consequently its true family relationship is unclear, though recent evidence that it is G-protein-coupled[4] suggests it belongs in the P2Y subclass. While other P2 receptors have wide tissue distributions, the P_{2T} subtype has, to date, only been found on platelets. It has recently been shown that ADP stimulates platelets via its action on three distinct P2 receptors ($P2X_1$, $P2Y_1$, and P_{2T}) leading to shape change, exposure of the glycoprotein IIb/IIIa complex (binding sites for cross-linking of platelets by fibrinogen), amplification of the response by release of a range of proaggregatory mediators (including thromboxane A2, 5-HT, and ADP), and sustained aggregation. Studies with the novel selective compounds described herein indicate a pivotal role for the P_{2T} receptor in the aggregatory process, as all of these events, with the exception of shape change, are blocked by P_{2T} receptor antagonists.

The original basis for the chemical program was the observation that ATP is a competitive, albeit weak,

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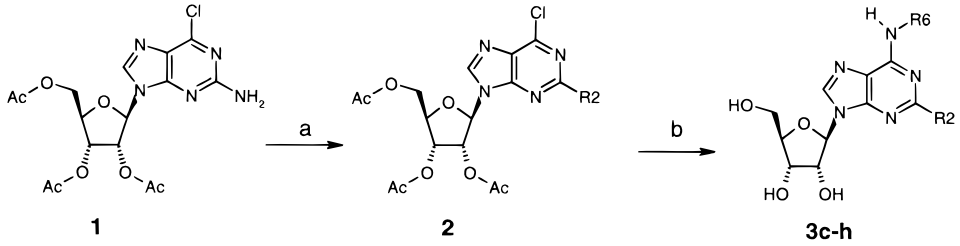
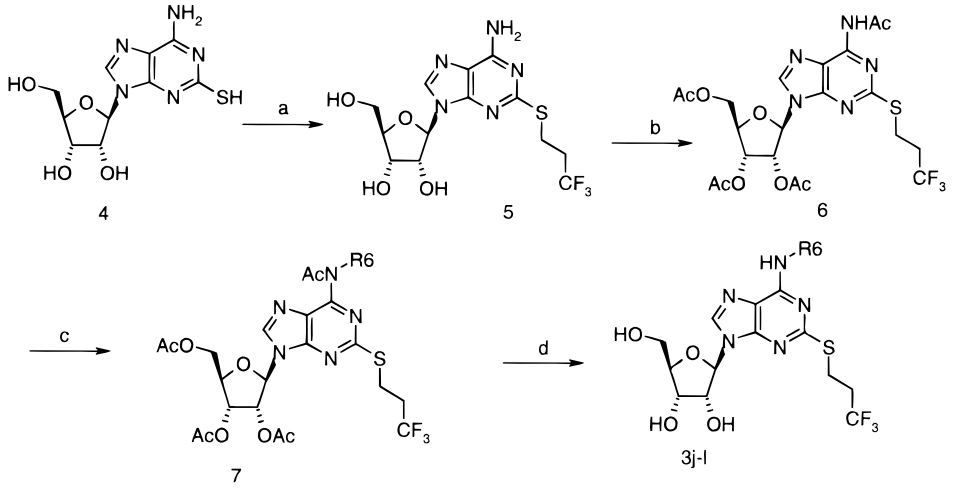
Scheme 1[a]



[a] Reagents: (a) isoamyl nitrite, MeCN, electrophile (e.g., RSSR, CH_2I_2); (b) R^6NH_2.

Scheme 2[a]



[a] Reagents: (a) $CF_3CH_2CH_2I$, NaOH, H_2O; (b) Ac_2O, NaOAc; (c) NaH, DMF, R^6Hal; (d) MeO^-, MeOH, heat.

antagonist of the ADP-induced platelet aggregation. Coupling this information with the exclusive localization of the P_{2T} receptor on the platelet, we concluded that potent and selective ATP analogue antagonists of this receptor would offer great potential as novel antithrombotic agents.

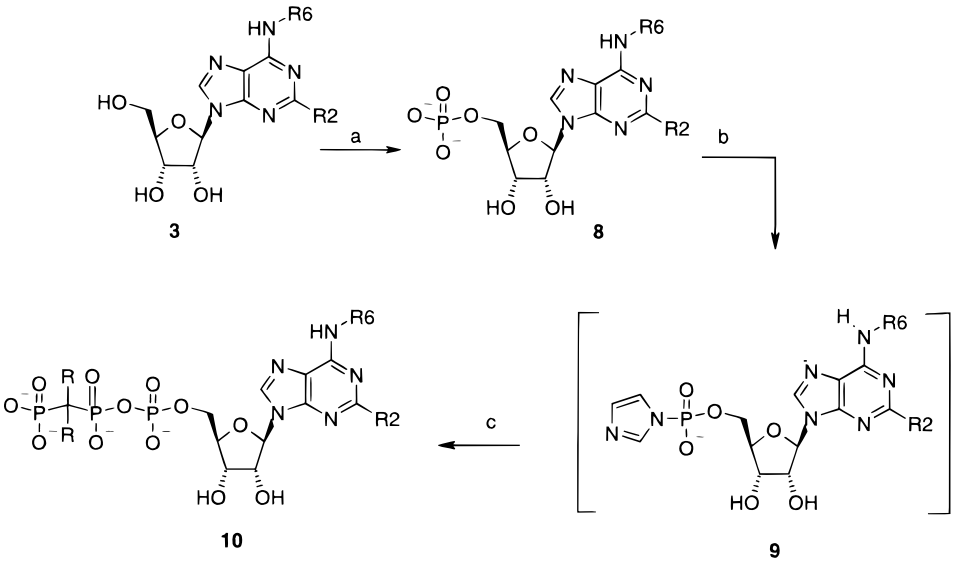
Chemistry

Two general methods were used to prepare the modified adenosine precursors of the target structures, the choice of route being dictated by the accessibility of the necessary reagents for the introduction of the C-2 or N^6 substituent. Method A (Scheme 1) was preferred as offering the broadest possible range of combinations of substituents. Diazotization of the amino group of **1** (prepared from guanosine[5]) with isoamyl nitrite led to the purinyl radical which could be efficiently trapped by disulfides,[6] halogen sources,[7] or other electrophilic agents, giving **2**. Subsequent displacement of the chlorine atom at C-6 by ammonia or amines conveniently also removed the acetyl protecting groups to afford the modified nucleosides **3c**−**h**.

For the preparation of the 3,3,3-trifluoropropylthio-substituted compounds **3i**−**l** (see Table 1) method B (Scheme 2) was used. 2-Mercaptoadenosine[8] (**4**) alkylated readily on the thiol group. Acetylation of the S-alkylated material **5** leading to **6** simultaneously protected the ribose hydroxyls and activated the exocyclic amino group to deprotonation with sodium hydride. This anion could be trapped with a range of electrophiles, affording **7**; subsequent mild treatment with ammonia or methoxide removed the protecting groups to give the adenosine analogues **3i**−**l**.

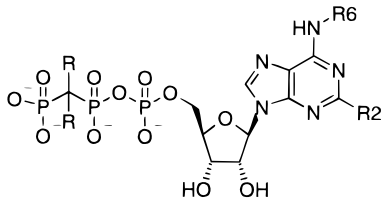
Elaboration of the phosphorus-containing side chain was by the method of Scheme 3. Phosphorylation of the 5′-hydroxyl of the modified adenosine moiety **3** (or **5**) by the method of Yoshikawa[9] afforded the 5′-monophosphates **8** efficiently, which, after removal of the phosphoric acid byproduct by ion-exchange chromatography on a sulfonic acid resin (Dowex 50W (H^+ form)) (using aqueous ammonia as eluant), could be activated by conversion to phosphorimidazolates **9**[10] with carbonyldiimidazole. This unstable intermediate (generated in situ and not isolated) was coupled with the methylenebis(phosphonic acids). Ion-exchange chromatography on weakly basic DEAE Sephadex (using aqueous triethylammonium bicarbonate or ammonium bicarbonate as eluant) was the preferred method of purification of the "stabilized triphosphate products" **10**. Separation of the product and unreacted monophosphate residues was easily monitored by UV detection of the chromophoric components. However separation of the product from the excess of the (non-UV-active) methylenebis(phosphonic acids) could not be determined by the same means and was checked by ^{31}P NMR after the product-containing fractions had been concentrated by freeze-drying. The sensitivity of the ^{31}P nucleus in NMR experiments was sufficiently high as to permit a good determination of "phosphorus purity" of the chromatographic fractions, which were combined as appropriate and freeze-dried again, affording the required products as (alkyl)ammonium salts. These salts were frequently very hygroscopic or even deliquescent; so in the majority of cases stable solid sodium salts were prepared by

Scheme 3[a]



[a] Reagents: (a) $POCl_3$, $(EtO)_3PO$; (b) CDI; (c) $R_2C(PO_3H_2)_2$.

Table 1. P_{2T} Receptor Antagonist Activity (Human Washed Platelets) and Recovery of Aggregation Behavior 20 min Postadministration in Rat



compd	R	R2	R6	pIC_{50}[a]	20 min recovery mean % (spread)[b]
10a[c]	F	H	H	3.5	
10b[c]	Cl	H	H	3.5	
10c	Cl	SEt	H	6.53 ± 0.16	
10d	F	SPr	H	8.16 ± 0.19	
10e	Cl	SPr	H	8.60 ± 0.09	93 (81−100)
10f	Cl	SPr	CH_2CF_3	9.48 ± 0.06	10 (3−16)
10g	Cl	SPr	CH_2CH_2OMe	7.81 ± 0.18	48 (43−56)
10h	Cl	SPr	CH_2CH_2SMe	9.42 ± 0.11	33 (21−43)
10i	Cl	$SCH_2CH_2CF_3$	H	8.87 ± 0.09	
10j	Cl	$SCH_2CH_2CF_3$	CH_2CF_3	9.14 ± 0.11	6 (1−11)
10k	Cl	$SCH_2CH_2CF_3$	CH_2CH_2OMe	8.02 ± 0.15	64 (54−79)
10l	Cl	$SCH_2CH_2CF_3$	CH_2CH_2SMe	9.35 ± 0.21	79 (60−107)

[a] Mean of four determinations except for **10l** (nine determinations). [b] Recovery of ADP-induced platelet aggregation 20 min postdose; mean of four determinations and spread of results. [c] Reference 15.

metathesis with sodium iodide. Even then the salts were heavily hydrated, and they were used as amorphous powders.

Results and Discussion

Human washed platelets were used for the primary screen in vitro. Suspended in a modified Tyrode buffer, they were stimulated to aggregate by the addition of ADP, and the aggregation process was detected using a turbidimetric technique.[11] Aggregation was inhibited by the test compounds in a concentration-dependent manner, from which a pIC_{50} value could be calculated. (Duplicate experiments were conducted in the presence of the A_{2a} adenosine receptor antagonist 8-(sulfophenyl)-theophylline to identify effects mediated via P_1 receptors.) Table 1 reports the pIC_{50} data for a selection of key compounds.

ATP, as a starting point for the discovery of a useful antithrombotic agent, presents a range of intriguing challenges: chemical and biological lability of the polyphosphate moiety, low affinity for the P_{2T} receptor (pIC_{50} 3.6), lack of selectivity between P2 subtypes, and extreme physicochemical properties (log $D_{7.4} \ll -3$). There is an unmet therapeutic need for an *intravenous* antithrombotic agent for use in episodes of acute arterial thrombosis. In such circumstances the highly polar nature of analogues of ATP could be advantageous, and we have identified such a compound that could be used for the treatment of acute thrombotic conditions.

Enzymatic or chemical hydrolysis of ATP is facile, leading to ADP (an endogenous proaggregatory agent),

AMP, and adenosine, which, inter alia, can also inhibit aggregation, via the A_{2A} receptor on platelets. Enhancement of the stability of the polyphosphate side chain to prevent the formation of the proaggregatory diphosphate was the most important preliminary target. The ectonucleotidases that are found on the surface of many cardiovascular tissues generally degrade polyphosphate species by stepwise removal of terminal phosphates. Replacement of the anhydride oxygen between P^{β} and P^{γ} (the central and terminal phosphorus atoms) of ATP by a methylene unit has been shown to afford a degradation-resistant product,[12] though with an important effect upon the ionization behavior of the triphosphate. The pK_a for the final deprotonation of ATP is ~6.6, while for the $\beta\gamma$-methylene compound it is ~8.1. At physiological pH, therefore, ATP may be expected to be predominantly tetra-anionic and the $\beta\gamma$-methylene analogue predominantly a tri-anion. Enhancement of the electronegativity of the $\beta\gamma$-carbon unit by halogenation gives rise to compounds with pK_a values close to those of ATP;[13] this maneuver has been used extensively by Blackburn et al. in investigating the role nucleotides play in various biological processes[14] and in the early general investigation of the P2 receptors by Cusack and Hourani.[15]

We hypothesized that the agonist/antagonist behavior difference of ADP and ATP was the consequence of charge density in the region of space occupied by the terminal phosphate of ATP, which is absent in ADP. The stabilized ATP analogues were all found to be antagonists, but the $\beta\gamma$-dihalomethylene compounds **10a**,**b** (having pK_a values similar to that of ATP) had higher affinities (pIC_{50} 3.5) than the methylene compound. While the dichloro- and difluoro-substituted structures had comparable activities, the dichloro-substituted system was preferred, since dichloromethylenebis(phosphonic acid) (clodronic acid) has been extensively investigated as a pharmaceutical per se and appears to be toxicologically acceptable;[16] moreover its synthesis is simpler and safer than its fluorinated analogue.

Substitutions of C-8 and C-2 of the adenine group were next investigated. The introduction of even small groups at C-8 produced a reduction of affinity (data not shown), while replacement of the hydrogen at C-2 led to important gains in antagonist activity. Taken together these observations suggest the required conformation about the glycosidic bond to be "anti". Although anything other than hydrogen at C-2 conferred enhanced activity, nonpolar moieties were preferred and sulfide-linked chains were particularly advantageous, an effect previously observed by Gough et al.[17] who noted that 2-methylthio-ADP was some 30-fold more potent as a platelet-aggregating agent than ADP. Investigation of the homologous series of alkyl sulfide chains revealed a quite unexpected 100-fold leap of affinity between the *S*-ethyl and *S*-propyl examples (see compounds **10c**,**e**, Table 1); neither further increase in chain length nor substitution found any additional substantial advantage. Selectivity was initially assessed using functional P2X and P2Y assays in rabbit ear artery[18] and guinea pig aorta[19] preparations, respectively, and subsequently in binding assays using cloned receptors. The modifications that enhanced affinity for the P_{2T} receptor had little effect on the potencies at the other P2 receptors (ATP is an agonist in these tissues), and a selectivity of some $>10^4$-fold was found for **10e**.

Being the first example of a highly potent and selective antagonist of any P2 purinoceptor, **10d** (AR-C66096MX, formerly FPL 66096MX) was recognized as an early definitive pharmacological tool for characterizing P_{2T} receptors.[11] The later compound AR-C67085MX (**10e**), having slightly superior receptor affinity and containing the preferred dichloromethylenebis(phosphonic acid) moiety, was taken forward into development for the express purpose of permitting studies of the significance of a P2 receptor in humans.

In vivo studies showed that **10e** had a very short duration of action, in the anesthetized rat, dog, and human ($t_{1/2}$ in human approximately 2 min).[20] In a dog model of arterial thrombosis, a full antithrombotic effect was achieved at a dose (0.1 nmol·kg^{-1}·min^{-1} iv) which produced complete inhibition of platelet aggregation, measured ex vivo, and no detectable hemodynamic effects.[21] Full inhibition of aggregation was established within 15 min of commencement of infusion, and normal baseline aggregation was fully restored within 15 min of cessation of dosing. These onset/offset parameters were maintained even following continuous infusion of the compound for 6 h or more. This highly selective, rapid onset/rapid offset profile was felt to be highly desirable in the acute care setting.

While dialkylation of N^6 of the adenine reduced receptor affinity (the N^6,N^6-diethyl derivative of **10e** was some 1000-fold less potent than **10e**), monoalkylation gave a useful increase. A lipophilic substituent was essential (polar atoms in or upon the chain reduced receptor binding markedly: see, for example, compound **10g** in Table 1), and activity increased with chain length. However, there was an effective upper limit to the length that could be tolerated, as chains of approximately four carbon atoms and longer caused the compound's biological half-life to increase sharply, unacceptably compromising the rapid onset/offset profile shown by **10e** in the dog. Differences in the functional kinetics of compounds were subsequently explored in an anesthetized rat model,[22] in which ADP-induced ex vivo aggregation of blood samples taken at various time points after iv administration of compound was used to follow recovery of aggregatory behavior and hence clearance of compound. In this screen **10e** showed >93% recovery 20 min after dosing. Long substituent chains on N^6 reduced this to <10%, as, surprisingly, did a 2,2,2-trifluoroethyl group (see **10f,j**). A methylthioethyl substituent at this position (**10h**) was found to enhance affinity with somewhat less effect upon half-life. In combination with a 3,3,3-trifluoropropylthio group at C-2, the N^6-methylthioethyl group gave a compound with a half-life similar to that of **10e** (showing 79% recovery of aggregation behavior at the 20 min time point). This compound **10l**, being some 6-fold more potent than **10e**, has been taken forward into development and is currently in phase II of development.

While profound inhibition of platelet function provides the potential for beneficial antithrombotic effects, this may be achieved at the expense of compromised hemostasis, with a subsequent increase in the risk of bleeding, particularly during invasive procedures. To assess

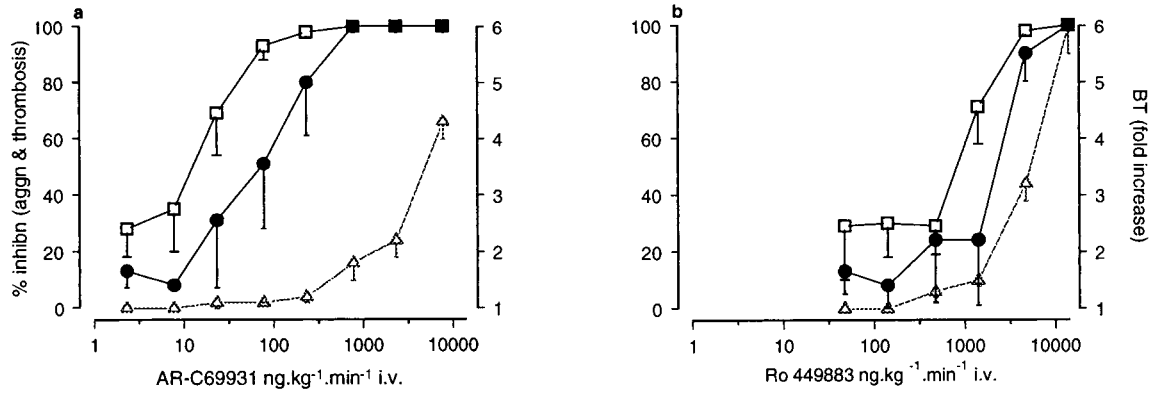


Figure 1. Effects of compound **10l** (AR-C69931MX) (panel a, 5−6 dogs) and the GPIIb/IIIa antagonist Lamifiban (Ro 449883) (panel b, 5 dogs) on femoral artery cyclical flow reduction (●), ADP-induced platelet aggregation ex vivo (□), and bleeding time (tongue) (Δ) of the anesthetized dog. Values are the mean ± SEM. Lamifiban from reference 23.

the potential for undesirable antihemostatic effects, we have examined the relationship between the antithrombotic activity of **10l** and its effect upon bleeding time in the dog. Dose−response relationships show a favorable (98-fold) separation between the desired antithrombotic action and the prolongation of bleeding time. In consequence, the full inhibition of platelet aggregation needed to give an antithrombotic effect is achieved at doses which extend bleeding time by less than 2-fold (see Figure 1a). This substantial separation of the two effects is in marked contrast to the pharmacological behavior of members of the GPIIb/IIIa antagonist class (agents currently attracting considerable interest for antithrombotic therapy). We have examined three examples (of diverse structure and profile), and each has shown a separation of a mere 2−5-fold. Figure 1b shows the data generated with the compound Lamifiban (Ro 449883), which is quite representative of our observations. Full inhibition of aggregation by these GPIIb/IIIa antagonists is achieved at dose levels such that bleeding time is prolonged by some 6−7-fold. Clearly the risk of persistent hemorrhage following administration of a P_{2T} receptor antagonist is likely to be very much less than would be the case with such GPIIb/IIIa antagonists. We believe this substantial separation of the two effects constitutes a major advantage for the P_{2T} antagonist mechanism of action in the modulation of platelet aggregation.

Conclusion

Modification of ATP has led to the identification of **10d** (AR-C66096MX), an important pharmacological tool for the characterization of P_{2T} receptors, and to the discovery of the therapeutically useful analogues **10e** (AR-C67085MX) and **10l** (AR-C69931MX). In a healthy human volunteer study with **10e**, a dose-related inhibition of ex vivo ADP-induced platelet aggregation has been demonstrated, with rapid recovery on termination of the infusion and with little or no prolongation of bleeding time. The same profile has been demonstrated for **10l**, which is in phase II development for the treatment of acute coronary syndromes such as unstable angina and in the setting of percutaneous transluminal coronary artery revascularization. A significant feature of the pharmacological profile of P_{2T} receptor antagonists is the unprecedentedly advantageous separation of antithrombotic activity from effects on bleeding time. On the basis of this clear and consistent advantage, we believe antagonists of the P_{2T} receptor represent a major step forward in the treatment of thrombotic disease.

Experimental Section

Thin layer chromatographic (TLC) analyses were performed using Whatman silica gel F254 plates; visualization used UV light or an iodine chamber. Flash chromatography for routine purification of reaction products used silica gel (230−400 mesh). ^{1}H and ^{31}P NMR spectra were recorded on a Bruker AM360 spectrometer at 360.13 and 145.73 MHz, respectively. ^{1}H chemical shifts are expressed in ppm relative to TMS as an internal standard, while ^{31}P shifts are quoted relative to 85% phosphoric acid (external standard). Mass spectra were obtained on a VG 70-250SEQ spectrometer using fast atom bombardment ionization (FAB). Combustion analyses were performed using a Carlo Erba EA 1108 elemental analyzer. Water content was determined using a Orion AF7LC Karl Fischer titrater.

General Procedures for the Preparation of N^6-Alkyl-2-(alkylthio)adenosines 3. 1. Method A, Scheme 1. (a) 2-(Alkylthio)-6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purines 2. A solution of the guanosine derivative **1** (25 g, 58.5 mmol), isoamyl nitrite (42.51 g, 363 mmol), and the appropriate dialkyl disulfide (585 mmol) in dry acetonitrile (350 mL) was purged thoroughly with nitrogen and then heated at 60 °C for 20 h. The disappearance of starting material was monitored by TLC (EtOAc−60/80 petroleum ether, 1:1). Solvent and other volatiles were removed under reduced pressure, and the residue was purified by column chromatography (EtOAc−60/80 petroleum ether, 1:1 as eluant).

6-Chloro-2-(ethylthio)-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine (2c). Prepared from **1** and diethyl disulfide in 64% yield: MS (FAB) *m*/*z* 473/5 ($M^+ + H^+$), 259 (100%); ^{1}H NMR δ($CDCl_3$) 8.10 (1H, s, H-8), 6.12 (1H, d, J = 5.3 Hz, H-1′), 5.94 (1H, t, J = 5.5 Hz, H-2′), 5.65 (1H, t, J = 5.5 Hz, H-3′), 4.42−4.31 (3H, m, H-4′ + H-5′a + H-5′b), 3.24 (2H, m, SCH_2), 2.15 (3H, s, CH_3CO), 2.12 (3H, s, CH_3CO), 2.11 (3H, s, CH_3CO), 1.12 (3H, t, SCH_2CH_3).

6-Chloro-2-(propylthio)-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine (2d). Prepared from **1** and dipropyl disulfide in 59% yield: MS (FAB) *m*/*z* 487/9 ($M^+ + H^+$), 259 (100%); ^{1}H NMR δ($CDCl_3$) 8.11 (1H, s, H-8), 6.14 (1H, d, J = 5.3 Hz, H-1′), 5.92 (1H, t, J = 5.5 Hz, H-2′), 5.60 (1H, t, J = 5.6 Hz, H-3′), 4.47−4.32 (3H, m, H-4′ + H-5′a + H-5′b), 3.20 (2H, t, J = 7.1 Hz, SCH_2), 2.15 (3H, s, CH_3CO), 2.12 (3H, s, CH_3CO), 2.11 (3H, s, CH_3CO), 1.81 (2H, m, $SCH_2CH_2CH_3$), 1.07 (3H, t, J = 7.3 Hz, $SCH_2CH_2CH_3$).

(b) N^6-Alkyl-2-(alkylthio)adenosines 3f−h. The chloropurine **2** (33 mmol) was suspended in 1:1 aqueous 1,4-dioxane (400 mL) and treated with the appropriate amine (230 mmol) at 95 °C for 24 h. The reaction was monitored by TLC (EtOAc−

MeOH, 15:1). Solvent was evaporated under reduced pressure, and the residue was purified by column chromatography (EtOAc−MeOH, 15:1).

2-(Propylthio)-*N*^{6}-(2,2,2-trifluoroethyl)adenosine (3f). Prepared from **2d** and 2,2,2-trifluoroethylamine in 72% yield: MS (FAB) *m/z* 424 ($M^+ + H^+$), 292 (100%); ^{1}H NMR δ(DMSO-d_6) 8.58 (1H, br s, NH), 8.34 (1H, s, H-8), 5.84 (1H, d, J = 5.7 Hz, H-1′), 5.47 (1H, d, J = 6.2 Hz, OH), 5.21 (1H, d, J = 4.9 Hz, OH), 5.07 (1H, t, J = 5.5 Hz, OH), 4.59 (1H, q, J = 5.7 Hz, H-2′), 4.29 (2H, br, NCH_2), 4.14 (1H, q, J = 4.9 Hz, H-3′), 3.93 (1H, m, H-4′), 3.65 (1H, m, H-5′a), 3.54 (1H, m, H-5′b), 3.06 (2H, m, SCH_2), 1.71 (2H, m, $SCH_2CH_2CH_3$), 0.99 (3H, t, J = 7.5 Hz, $SCH_2CH_2CH_3$).

***N*^{6}-(2-Methoxyethyl)-2-(propylthio)adenosine (3g).** Prepared from **2d** and 2-methoxyethylamine in 87% yield: MS (FAB) *m/z* 400 ($M^+ + H^+$, 100%); ^{1}H NMR δ(DMSO-d_6) 8.23 (1H, s, H-8), 7.89 (1H, br s, NH), 5.81 (1H, d, J = 5.7 Hz, H-1′), 5.45 (1H, d, J = 6.3 Hz, OH), 5.20 (1H, d, J = 4.85 Hz, OH), 5.09 (1H, t, J = 5.4 Hz, OH), 4.60 (1H, m, H-2′), 4.12 (1H, m, H-3′), 3.91 (1H, m, H-4′), 3.90−3.43 (6H, m, H-5′a + H-5′b + NCH_2 + OCH_2), 3.28 (3H, s, OCH_3), 3.07 (2H, m, SCH_2), 1.70 (2H, m, $SCH_2CH_2CH_3$), 0.99 (3H, t, J = 7.3 Hz, $SCH_2CH_2CH_3$).

***N*^{6}-(2-Methylthioethyl)-2-(propylthio)adenosine (3h).** Prepared from **2d** and 2-methylthioethylamine in 76% yield: MS (FAB) *m/z* 416 ($M^+ + H^+$, 100%); ^{1}H NMR δ(DMSO-d_6) 8.28 (1H, s, H-8), 7.81 (1H, br s, NH), 5.82 (1H, d, J = 5.6 Hz, H-1′), 5.47 (1H, d, J = 6.1 Hz, OH), 5.18 (1H, d, J = 4.8 Hz, OH), 5.03 (1H, t, J = 5.5 Hz, OH), 4.65 (1H, m, H-2′), 4.06 (1H, m, H-3′), 3.89 (1H, m, H-4′), 3.64 (2H, m, H-5′a + H-5′b), 3.59 (2H, m, NCH_2), 3.11 (2H, m, SCH_2), 2.52 (2H, m, NCH_2CH_2), 2.05 (3H, s, SCH_3), 1.69 (2H, m, $SCH_2CH_2CH_3$), 0.99 (3H, t, J = 7.4 Hz, $SCH_2CH_2CH_3$).

2. Method B, Scheme 2. 2-(3,3,3-Trifluoropropylthio)-adenosine (5). A suspension of sodium hydride (1.44 g of 60% in mineral oil, 36 mmol) and adenosine-2-thione monohydrate (**4**) (5.32 g, 16.8 mmol) in dry DMF (80 mL) was stirred for 1 h before the addition of 1-chloro-3,3,3-trifluoropropane (8.72 g, 66 mmol), and the reaction was stirred for 36 h. The disappearance of starting material was monitored by TLC (CH_2Cl_2−MeOH, 9:1). The solvent was removed under reduced pressure, and the residue was partitioned between EtOAc and water. Drying of the organic phase and evaporation gave the crude product which was purified by column chromatography (CH_2Cl_2−MeOH, 9:1) to afford a fluffy off-white powder (4.98 g, 76%): MS (thermospray) *m/z* 396($M^+ + H^+$, 100%); UV λ_{max} (EtOH) 207 nm (ϵ 11 200), 234 (ϵ 23 300), 276 (ϵ 15 000); ^{1}H NMR δ(DMSO-d_6) 8.26 (1H, s, H-8), 7.46 (2H, br s, NH_2), 5.82 (1H, d, J = 6.2 Hz, H-1′), 5.43 (1H, d, J = 6.19 Hz, OH), 5.18 (1H, d, J = 4.4 Hz, J = 4.4 Hz, OH), 5.04 (1H, t, J = 5.75 Hz, OH), 4.59 (1H, m, H-2′), 4.11 (1H, m, H-3′), 3.92 (1H, m, H-4′), 3.62−3.54 (2H, m, H-5′a + H-5′b), 3.25 (2H, m, SCH_2), 2.71 (2H, m, $SCH_2CH_2CF_3$).

***N*^{6},*O*,*O*,*O*-Tetraacetyl-2-(3,3,3-trifluoropropylthio)adenosine (6).** The trifluoropropylthio compound **5** (5.15 g, 13 mmol), anhydrous sodium acetate (0.723 g, 8.8 mmol), and acetic anhydride (42 mL, 447 mmol) were heated at 80 °C for 7 h. After cooling the solution was diluted with water (100 mL), stirred at room temperature for 18 h, and then extracted into CH_2Cl_2 (4 × 200 mL). The organic extracts were combined, washed thoroughly with saturated sodium bicarbonate solution (200 mL), dried, and evaporated. The crude product was purified by column chromatography (Et_2O−MeOH, 97:3) to give a colorless foam (5.35 g, 73%): MS (FAB) *m/z* 564 ($M^+ + H^+$), 139 (100%); ^{1}H NMR δ($CDCl_3$) 9.36 (1H, br s, NH), 8.10 (1H, s, H-8), 6.14 (1H, d, J = 5.3 Hz, H-1′), 5.92 (1H, t, J = 5.5 Hz, H-2′), 5.62 (1H, t, J = 5.5 Hz, H-3′), 4.44−4.37 (2H, m, H-4′ + H-5′a), 4.34 (1H, m, H-5′b), 3.18 (2H, m, SCH_2), 2.78 (2H, m, $SCH_2CH_2CF_3$), 2.15 (3H, s, CH_3CO), 2.12 (3H, s, CH_3CO), 2.11 (3H, s, CH_3CO), 2.10 (3H, s, CH_3CO).

***N*^{6}-Alkyl-2-(3,3,3-trifluoropropylthio)adenosines 3j−l.** The tetraacetylated compound **6** (5.067 g, 9 mmol) in dry DMF (100 mL) was added over 3 h to a suspension of sodium hydride (60% in mineral oil, 0.44 g, 11 mmol) in dry DMF (100 mL) containing an appropriate alkyl iodide (27 mmol). The reaction was stirred under a nitrogen atmosphere at 60 °C for 24 h. Disappearance of starting material was monitored by TLC (EtOAc−cyclohexane, 1:1). Solvent was removed under reduced pressure, and the residue was taken in EtOAc (300 mL) and washed with water (3 × 100 mL). Drying and evaporation gave the crude product which was purified by column chromatography (EtOAc−cyclohexane, 1:1).

The alkylated material **7** (7.5 mmol) was dissolved in a solution of 0.1 M sodium hydroxide in methanol (150 mL) and heated under reflux for 30 min. After cooling the solution was acidified with glacial AcOH (0.89 mL, 15 mmol) and evaporated to dryness. The crude product was purified by column chromatography (CH_2Cl_2−MeOH, 95:5).

***N*^{6}-(2,2,2-Trifluoroethyl)-2-(3,3,3-trifluoropropylthio)-adenosine (3j).** Prepared from **6** and 2,2,2-trifluoroethyl iodide in 55% yield: MS (FAB) *m/z* 478 ($M^+ + H^+$, 100%); ^{1}H NMR δ(DMSO-d_6) 8.56 (1H, br s, NH), 8.30 (1H, s, H-8), 5.74 (1H, d, J = 6 Hz, H-1′), 5.41 (1H, d, J = 6.1 Hz, OH), 5.21 (1H, d, J = 5.1 Hz, OH), 5.01 (1H, t, J = 5.5 Hz, OH), 4.57 (1H, m, H-2′), 4.33 (2H, br, NCH_2), 4.19 (1H, m, H-3′), 3.91 (1H, m, H-4′), 3.63−3.58 (2H, m, H-5′a + H-5′b), 3.20 (2H, m, SCH_2), 2.71 (2H, m, $SCH_2CH_2CF_3$).

***N*^{6}-(2-Methoxyethyl)-2-(3,3,3-trifluoropropylthio)adenosine (3k).** Prepared from **6** and 2-methoxyethyl bromide in 51% yield: MS (FAB) *m/z* 454 ($M^+ + H^+$, 100%); ^{1}H NMR δ(DMSO-d_6) 8.19 (1H, s, H-8), 7.80 (1H, br s, NH), 5.84 (1H, d, J = 6.2 Hz, H-1′), 5.40 (1H, d, J = 6.2 Hz, OH), 5.29 (1H, d, J = 4.8 Hz, OH), 5.17 (1H, t, J = 5.7 Hz, OH), 4.59 (1H, m, H-2′), 4.17 (1H, m, H-3′), 3.99 (1H, m, H-4′), 3.60−3.49 (2H, m, H-5′a + H-5′b), 3.45−3.38 (4H, m, NCH_2 + OCH_2), 3.26 (3H, s, OCH_3), 3.18 (2H, m, SCH_2), 2.70 (2H, m, $SCH_2CH_2CF_3$).

***N*^{6}-(2-Methylthioethyl)-2-(3,3,3-trifluoropropylthio)adenosine (3l).** Prepared from **6** and 2-methylthioethyl chloride in 36% yield: MS (FAB) *m/z* 470 ($M^+ + H^+$), 75 (100%); ^{1}H NMR δ(DMSO-d_6) 8.27 (1H, s, H-8), 8.14 (1H, br s, NH), 5.82 (1H, d, J = 6.2 Hz, H-1′), 5.44 (1H, d, J = 6.2 Hz, OH), 5.20 (1H, d, J = 4.9 Hz, OH), 5.08 (1H, t, J = 5.3 Hz, OH), 4.57 (1H, m, H-2′), 4.11 (1H, m, H-3′), 3.92 (1H, m, H-4′), 3.58 (4H, m, NCH_2 + H-5′a + H-5′b), 3.27 (2H, m, SCH_2), 2.72 (4H, m, NCH_2CH_2 + $SCH_2CH_2CF_3$), 2.09 (3H, s, SCH_3).

General Method for the Preparation of $\beta\gamma$-Dihalomethylene-Substituted Triphosphates 10. Scheme 3. Phosphorus oxychloride (0.66 g, 4.3 mmol) was added to a solution of nucleoside analogue **3** (or **5**) (1.1 mmol) in triethyl phosphate (12 mL) cooled to 0 °C. After 5 h the cold solution was poured into ice−water (100 mL) containing sodium bicarbonate (1.45 g, 17.25 mmol), and the mixture was stirred for 45 min. The aqueous solution was washed with Et_2O (2 × 100 mL) and then applied to a column of Dowex 50Wx8 (H^+ form). The column was washed with deionized water until the eluate was at pH 6, and then the product was eluted with 2 M ammonium hydroxide. The product-containing fractions were combined and freeze-dried.

The 5′-monophosphate product **9** (0.85 mmol) and tri-*n*-butylamine (0.157 g, 0.85 mmol) were combined in a small volume of pyridine and evaporated to dryness under reduced pressure. The residue was rendered anhydrous by azeotropic drying with pyridine (3 × 15 mL) followed by anhydrous DMF (2 × 15 mL). It was finally dissolved in anhydrous DMF (10 mL), and carbonyldiimidazole (0.66 g, 4.1 mmol) was added. After 4 h at room temperature methanol (0.209 g, 6.5 mmol) was added, and 30 min later a solution of the mono(tri-*n*-butylammonium) salt of the appropriate (dihalo)methylenebis-(phosphonic acid) (4.85 mmol) in anhydrous DMF (30 mL) was introduced. The mixture was stirred at room temperature for 18 h, then filtered, and evaporated to dryness under reduced pressure. The crude product was purified by ion-exchange chromatography using DEAE-Sephadex and triethylammonium bicarbonate or ammonium bicarbonate solution (gradient 0−0.6 M) as eluant. The product-containing fractions were concentrated by freeze-drying and assayed by ^{31}P NMR. Those found to be pure were combined and freeze-dried again to give the product as an amine salt. If the amine salt was hygroscopic

or deliquescent the sodium salt was prepared: The amine salt was dissolved in methanol (2 mL) and treated with a 1 M solution of sodium iodide in analytical grade acetone (30 mL). The precipitate was collected by centrifugation, washed by repeated suspension in analytical grade acetone (4 × 40 mL), and recentrifugation. Finally the colorless solid product was redissolved in deionized water and freeze-dried.

2-(Ethylthio)-5′-adenylic Acid, Monoanhydride with Dichloromethylenebis(phosphonic acid) (10c). Prepared as the tetrasodium salt in 9% yield from **3c**: ^{1}H NMR δ(D_2O) 8.33 (1H, s, H8), 6.15 (1H, d, J = 5.29 Hz, H1′), 4.84 (1H, m, H2′), 4.62 (1H, m, H3′), 4.42 (1H, m, H4′), 4.36 (2H, m, H5′a and H5′b), 3.29 (2H, m, SCH_2), 1.13 (3H, t, J = 7.3 Hz, SCH_2CH_3); ^{31}P NMR δ(D_2O) 9.23 (d, 1P, J = 18.7 Hz, P$^{\gamma}$), 1.51 (dd, 1P, J^1 = 18.7 Hz, J^2 = 28.6 Hz, P$^{\beta}$), −9.8 (d, 1P, J = 28.7 Hz, P$^{\alpha}$). Anal. ($C_{13}H_{16}Cl_2N_5Na_4O_{12}P_3S\cdot4.5H_2O$) H, N, S; C: calcd, 19.40; found, 20.13.

2-(Propylthio)-5′-adenylic Acid, Monoanhydride with Difluoromethylenebis(phosphonic acid) (10d). Prepared as a triammonium salt in 5% yield from **3d**: ^{1}H NMR δ(D_2O) 8.28 (1H, s, H8), 6.01 (1H, d, J = 4.6 Hz, H1′), 4.80 (1H, m, H2′), 4.46 (1H, m, H3′), 4.28 (1H, m, H4′), 4.15 (2H, m, H5′a and H5′b), 3.05 (2H, t, J = 6.9 Hz, SCH_2), 1.63 (2H, m, $SCH_2CH_2CH_3$), 0.91 (3H, t, J = 7.3 Hz, $SCH_2CH_2CH_3$); ^{31}P NMR δ(D_2O) 4.85 (dt, 1P, J = 53 Hz, P$^{\gamma}$), −1.71 (ddt, 1P, J^1 = 52 Hz, J^2 = 28.9 Hz, P$^{\beta}$), −9.44 (d, 1P, J = 28.9 Hz, P$^{\alpha}$). Anal. ($C_{14}H_{31}F_2N_8O_{12}P_3S\cdot4H_2O$) C, H, N; S: calcd, 4.34; found, 4.89.

2-(Propylthio)-5′-adenylic Acid, Monoanhydride with Dichloromethylenebis(phosphonic acid) (10e). Prepared as the tetrasodium salt in 7% yield from **3d**: ^{1}H NMR δ(D_2O) 8.34 (1H, s, H8), 6.11 (1H, d, J = 5.1 Hz, H1′), 4.81 (1H, m, H2′), 4.66 (1H, m, H3′), 4.44 (1H, m, H4′), 4.32 (2H, m, H5′a and H5′b), 3.15 (2H, t, J = 6.8 Hz, SCH_2), 1.76 (2H, m, $SCH_2CH_2CH_3$), 1.04 (3H, t, J = 7.3 Hz, $SCH_2CH_2CH_3$); ^{31}P δNMR (D_2O) 9.15 (d, 1P, J = 18.6 Hz, P$^{\gamma}$), 1.39 (dd, 1P, J^1 = 18.7 Hz, J^2 = 28.9 Hz, P$^{\beta}$), −9.6 (d, 1P, J = 28.9 Hz, P$^{\alpha}$). Anal. ($C_{14}H_{18}Cl_2N_5Na_4O_{12}P_3S\cdot3H_2O$) C, H, N, S.

***N*^{6}-(2,2,2-Trifluoroethyl)-2-(propylthio)-5′-adenylic Acid, Monoanhydride with Dichloromethylenebis(phosphonic acid) (10f).** Prepared as the tetrasodium salt in 12% yield from **3f**: ^{1}H NMR δ(D_2O) 8.25 (1H, s, H8), 5.95 (1H, d, J = 5.7 Hz, H1′), 4.84 (1H, m, H2′), 4.63 (1H, m, H2′), 4.50 (1H, m, H3′), 4.27−4.12 (5H, m, H4′, H5′a and H5′b, NCH_2), 2.97 (2H, t, J = 6.8 Hz, SCH_2), 1.58 (2H, m, $SCH_2CH_2CH_3$), 0.86 (3H, t, J = 7.35 Hz, $SCH_2CH_2CH_3$); ^{31}P NMR δ(D_2O) 9.70 (d, 1P, J = 18.5 Hz, P$^{\gamma}$), 3.43 (dd, 1P, J^1 = 18.5 Hz, J^2 = 30.38 Hz, P$^{\beta}$), −9.20 (d, 1P, J = 30.41 Hz, P$^{\alpha}$). Anal. ($C_{16}H_{19}F_3Cl_2N_5Na_4O_{12}P_3S\cdot9H_2O$) C, H, N, S, H_2O.

***N*^{6}-(2-Methoxyethyl)-2-(propylthio)-5′-adenylic Acid, Monoanhydride with Dichloromethylenebis(phosphonic acid) (10 g).** Prepared as the tetrasodium salt in 6% yield from **3g**: ^{1}H NMR δ(D_2O) 8.39 (1H, s, H8), 6.14 (1H, d, J = 5.85 Hz, H1′), 4.84 (1H, m, H2′), 4.66 (1H, m, H3′), 4.44 (1H, m, H4′), 4.32 (2H, m, H5′a and H5′b), 3.86 (2H, m, $NHCH_2CH_2OCH_3$), 3.80 (2H, m, $NHCH_2CH_2OCH_3$), 3.46 (3H, s, OCH_3), 3.22 (2H, t, J = 7.1 Hz, SCH_2), 1.80 (2H, m, $SCH_2CH_2CH_3$), 1.07 (3H, t, J = 7.35 Hz, $SCH_2CH_2CH_3$); ^{31}P NMR δ(D_2O) 9.05 (d, 1P, J = 18.7 Hz, P$^{\gamma}$), 1.44 (dd, 1P, J^1 = 18.8 Hz, J^2 = 29.3 Hz, P$^{\beta}$), −9.4 (d, 1P, J = 29.5 Hz, P$^{\alpha}$). Anal. ($C_{17}H_{24}Cl_2N_5Na_4O_{13}P_3S\cdot2H_2O$) C, N, S; H: calcd, 3.16; found, 3.82.

***N*^{6}-(2-Methylthioethyl)-2-(propylthio)-5′-adenylic Acid, Monoanhydride with Dichloromethylenebis(phosphonic acid) (10h).** Prepared as the triammonium salt in 6% yield from **3h**: ^{1}H NMR δ(D_2O) 8.32 (1H, s, H8), 6.05 (1H, d, J = 5.5 Hz, H1′), 4.71 (1H, m, H2′), 4.52 (1H, m, H3′), 4.38 (1H, m, H4′), 4.24 (2H, m, H5′a and H5′b), 3.67 (br s, $NHCH_2$), 3.15 (2H, t, J = 6.9 Hz, SCH_2), 2.58 (2H, m, NCH_2CH_2), 2.04 (3H, s, SCH_3), 1.72 (2H, m, $SCH_2CH_2CH_3$), 1.04 (3H, t, J = 7.2 Hz, $SCH_2CH_2CH_3$); ^{31}P NMR δ(D_2O) 8.83 (d, 1P, J = 18.6 Hz, P$^{\gamma}$), 0.49 (dd, 1P, J^1 = 18.9 Hz, J^2 = 28.9 Hz, P$^{\beta}$), −9.48 (d, 1P, J = 29.0 Hz, P$^{\alpha}$). Anal. ($C_{17}H_{37}Cl_2N_8O_{12}P_3S_2\cdot3H_2O$) C, H, N, S.

2-(3,3,3-Trifluoropropylthio)-5′-adenylic Acid, Monoanhydride with Dichloromethylenebis(phosphonic acid) (10i). Prepared as the tetrasodium salt in 21% yield from **5**: ^{1}H NMR δ(D_2O) 8.38 (1H, s, H8), 6.13 (1H, d, J = 5.7 Hz, H1′), 4.60 (1H, t, J = 4.5 Hz, H3′), 4.39 (1H, m, H4′), 4.28 (2H, m, H5′a and H5′b), 3.34 (2H, t, J = 6.3 Hz, SCH_2), 2.69 (2H, m, $SCH_2CH_2CF_3$); ^{31}P NMR δ(D_2O) 9.22 (d, 1P, J = 19 Hz, P$^{\gamma}$), 1.33 (dd, 1P, J^1 = 19 Hz, J^2 = 29 Hz, P$^{\beta}$), −9.12 (d, 1P, J = 29.0 Hz, P$^{\alpha}$). Anal. ($C_{14}H_{15}Cl_2F_3N_5Na_4O_{12}P_3S\cdot3H_2O$) (C, H, N, S, H_2O.

***N*^{6}-(2,2,2-Trifluoroethyl)-2-(3,3,3-trifluoropropylthio)-5′-adenylic Acid, Monoanhydride with Dichloromethylenebis(phosphonic acid) (10j).** Prepared as the triammonium salt in 4% yield from **3j**: ^{1}H NMR δ(D_2O) 8.32 (1H, s, H8), 6.60 (1H, d, J = 5.7 Hz, H1′), 4.68 (1H, m, H2′), 4.52 (1H, m, H3′), 4.32−4.20 (5H, m, H4′ + H5′a and H5′b + $NHCH_2CF_3$), 3.24 (2H, m, SCH_2), 2.60 (2H, m, $SCH_2CH_2CF_3$); ^{31}P NMR δ(D_2O) 8.82 (d, 1P, J = 18.6 Hz, P$^{\gamma}$), 0.63 (dd, 1P, J^1 = 18.9 Hz, J^2 = 28.9 Hz, P$^{\beta}$), −9.43 (d, 1P, J = 29.0 Hz, P$^{\alpha}$). Anal. ($C_{16}H_{29}Cl_2F_6N_8O_{12}P_3S\cdot4H_2O$) C, H, N, H_2O; S: calcd, 3.53; found, 2.90.

***N*^{6}-(2-Methoxyethyl)-2-(3,3,3-trifluoropropylthio)-5′-adenylic Acid, Monoanhydride with Dichloromethylenebis(phosphonic acid) (10k).** Prepared as the tetrasodium salt in 10% yield from **3k**: ^{1}H NMR δ(D_2O) 8.36 (1H, s, H8), 6.10 (1H, d, J = 5.8 Hz, H1′), 4.88 (1H, m, H2′), 4.60 (1H, m, H3′), 4.41 (1H, m, H4′), 4.38 (2H, m, H5′a and H5′b), 3.81 (2H, m, $NHCH_2CH_2OCH_3$), 3.78 (2H, m, $NHCH_2CH_2OCH_3$), 3.46 (3H, s, OCH3), 3.23 (2H, m, SCH_2), 2.57 (2H, m, $SCH_2CH_2CF_3$); ^{31}P NMR δ(D_2O) 9.0 (d, 1P, J = 18.9 Hz, P$^{\gamma}$), 1.54 (dd, 1P, J^1 = 18.8 Hz, J^2 = 29.3 Hz, P$^{\beta}$), −9.9 (d, 1P, J = 29.7 Hz, P$^{\alpha}$). Anal. ($C_{17}H_{21}Cl_2F_3N_5Na_4O_{13}P_3S\cdot7H_2O$) C, H, N; S: calcd, 3.28; found, 2.81.

***N*^{6}-(2-Methylthioethyl)-2-(3,3,3-trifluoropropylthio)-5′-adenylic Acid, Monoanhydride with Dichloromethylenebis(phosphonic acid) (10l).** Prepared as the triammonium salt in 4% yield from **3l**: ^{1}H NMR δ(D_2O) 8.30 (1H, s, H8), 5.97 (1H, d, J = 5.5 Hz, H1′), 4.65 (1H, m, H2′), 4.47 (1H, m, H3′), 4.28 (1H, m, H4′), 4.17 (2H, m, H5′a and H5′b), 3.67 (br s, $NHCH_2$), 3.21 (2H, t, J = 7.6 Hz, SCH_2), 2.72 (2H, t, J = 6.6 Hz, $SCH_2CH_2CF_3$), 2.58 (2H, m, NCH_2CH_2), 2.04 (3H, s, SCH_3); ^{31}P NMR δ(D_2O) 8.80 (d, 1P, J = 18.6 Hz, P$^{\gamma}$), 0.42 (dd, 1P, J^1 = 18.9 Hz, J^2 = 28.9 Hz, P$^{\beta}$), −9.41 (d, 1P, J = 29.0 Hz, P$^{\alpha}$). Anal. ($C_{17}H_{34}Cl_2F_3N_8O_{12}P_3S_2\cdot3H_2O$) H, N, S; C: calcd, 23.16; found, 23.66.

Biological Assays. The affinity of the test compounds for the P_{2T} receptor was assayed using washed human platelets by the method of Humphries et al.[11] Antithrombotic activity was measured using a cyclic flow reduction model in dog femoral artery.[21] Functional kinetics of the compounds were determined in the anesthetized rat by the method of Humphries et al.[22]

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