5′-*N*-Substituted Carboxamidoadenosines as Agonists for Adenosine Receptors

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Received August 26, 1998

Novel as well as known 5′-*N*-substituted carboxamidoadenosines were prepared via new routes that provided shorter reaction times and good yields. Binding affinities were determined for rat A_1 and A_{2A} receptors and human A_3 receptors. EC_{50} values were determined for cyclic AMP production in CHO cells expressing human A_{2B} receptors. On all receptor subtypes relatively small substituents on the carboxamido moiety were optimal. Selectivity for the A_3 receptor was found for several analogues (**1a**, **1d**, **1h**, and **1k**). On A_1 receptors a number of compounds, but not 5′-*N*-ethylcarboxamidoadenosine (NECA, **1b**), showed small GTP shifts, which could be indicative of lower intrinsic activities at the A_1 receptor. At the A_{2B} receptor, derivatives **1i**−**k** with modified ethyl substituents had reduced activities compared to the A_{2B} reference agonist NECA (**1b**). Thiocarboxamido derivatives (**8b** and **8c**) displayed considerable although decreased A_{2B} receptor activity. The X-ray structure determination of compound **8b** was carried out. Due to intramolecular hydrogen bonding between the carboxamido NH and the purine N3 in the crystal structure, the ribose moiety of this compound is in a syn conformation. However, theoretical calculations support that NECA (**1b**), and less so **8b**, can readily adopt both the syn and the anti conformation, therefore not excluding the proposed anti mode of binding to the receptor.

Introduction

Adenosine receptors are divided into four subtypes, A_1, A_{2A}, A_{2B}, and A_3. All four receptor subtypes are coupled via a G protein to the cyclic AMP producing enzyme adenylate cyclase, either in an inhibitory (A_1 and A_3 subtypes) or stimulatory manner (A_{2A} and A_{2B} subtypes). Through A_1 and A_{2A} receptors adenosine mediates both cardiovascular, resulting in bradycardia and a lowering of the blood pressure,[1] and renal effects.[2] However, nowadays adenosine receptors are in the scope of interest not only because of their cardiovascular and renal actions but also for their effects on the immune system (A_2 and A_3 subtypes)[3−6] and on the central nervous system (A_1 and A_{2A}).[7] All receptor subtypes have been recently cloned, thus providing tools for studying each receptor subtype solely by using artificially constructed test systems.[8] For the A_1 and A_{2A} receptor, selective agonists, antagonists, and radioligands are now available.[9] For the A_3 receptor there are selective agonists[10] and radioligands,[11] while selective high-affinity antagonists have only recently been reported.[12−14] For the A_{2B} receptor, however, although potent antagonists have recently been demonstrated,[15] no selective compounds have been reported so far.

5′-Carboxamidoadenosines are known as nonselective adenosine receptor agonists. 5′-*N*-Ethylcarboxamidoadenosine (NECA, **1b**, Chart 1) binds to rat and human A_1, A_{2A}, and A_3 receptors with K_i values in the nanomolar range.[16−18] However, a combination of modification of the 5′-position and C2 substitution has led to the potent and selective A_{2A} receptor agonist 2-[*p*-(2-carboxyethyl)-phenylethylamino]-NECA (CGS21680, **2**). Moreover, in addition to an appropriate N^6-substituent, adenosine A_3 receptor selective agonists benefit from a methyl-substituted carboxamido moiety (IBMECA, **3**).[17] As we reported recently, carboxamidoadenosines are the most active agonists on the human A_{2B} receptor.[19] Although far from selective with respect to the other subtypes, NECA was the most potent carboxamide on the A_{2B} receptor with an EC_{50} value in the low micromolar range.

In 1980, several carboxamidoadenosines were evaluated for their cardiovascular effects by Prasad et al.[20] Derivatives with a small 5′-*N*-substituent on the carboxamido function, such as ethyl in NECA, were found to strongly induce coronary vasodilation in dogs. Later, Olsson et al. studied N^6-substituted 5′-carboxamidoadenosine derivatives on their ability to inhibit or stimulate adenylate cyclase via A_1 and A_{2A} receptors, respectively.[21] They also observed that analogues with small substituents showed the highest activities.

Since two more adenosine receptor subtypes have become known, i.e., the A_{2B} and the A_3 receptor, we decided to study the effects of 5′-*N*-carboxamido substitution on all four subtypes of adenosine receptors. Moreover, as 5′-carboxamidoadenosines without modifications on other positions are the most active A_{2B} agonists,[19] we reasoned that substituting carboxamidoadenosines with known or novel 5′-*N*-substituents could add to existing SAR for the A_{2B} receptor. Therefore

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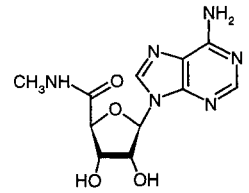
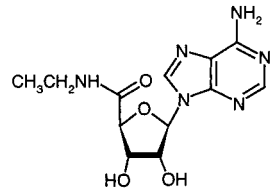
10.1021/jm9804984 CCC: $18.00
Published on Web 03/31/1999

Chart 1. Structures of Reference 5′-Carboxamidoadenosines[a]



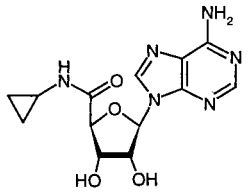
1a (MECA)

	rat
A_1	83.6
A_{2A}	66.8
A_3	72



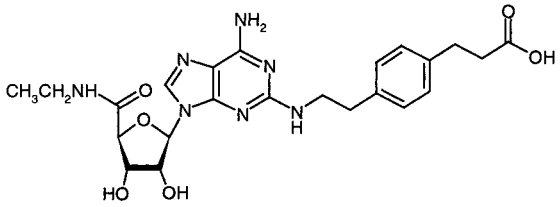
1b (NECA)

	rat	human
A_1	6.3	13.6
A_{2A}	10	
A_{2B}	460	692; 1230; 3100
A_3	113	



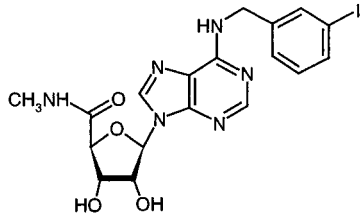
1c (NCPCA)

	rat	human
A_1	6.4	
A_{2A}	13.6	
A_{2B}		5300
A_3	1600	



2 (CGS21680)

	rat	human
A_1	2600	289
A_{2A}	15	27.1
A_{2B}		213000;>1mM
A_3	584	67.1



3 (IBMECA)

	rat	human
A_1	54	3.73
A_{2A}	56	2520
A_{2B}		11000; 200000
A_3	1.1	1.20

[a] K_i values for A_1, A_{2A}, and A_3 receptor subtypes and EC_{50} values on cAMP production for the A_{2B} subtype given in nM.[16−19,39,44−47]

we synthesized carboxamidoadenosines and thiocarboxamidoadenosines with various 5′-*N*-alkyl groups. It was found that, besides 5′-*N*-methylcarboxamidoadenosine (MECA, **1a**), several analogues displayed selectivity toward the A_3 receptor, while a number of derivatives appeared to have nonmaximal intrinsic activities for the A_1 receptor. Furthermore, to study conformation−activity relationships, we evaluated X-ray crystallographic data as well as theoretical calculations on the conformation of one of the novel derivatives (**8b**).

Chemistry

The synthesis of the carboxamidoadenosine derivatives **1a**,**d**−**k** and **8b**,**c** is depicted in Scheme 1. 2′,3′-*O*-Isopropylideneadenosine (**4**) was used as the starting compound for all derivatives. Oxidation of **4** with $KMnO_4$ in 80% acetic acid[21] yielded 2′,3′-*O*-isopropylideneadenosine-5′-uronic acid (**5**) in 67%. Two procedures were followed for the synthesis of the carboxamidoadenosines **6a**−**k**. In method A, the carboxylic acid **5** was first converted to the carboxylate with triethylamine, then reacted with the coupling agent isopropenylchloroformate at 0 °C, immediately followed by the primary amine to obtain the protected carboxamidoadenosines. In method B, 1-methyl-2-chloropyridinium iodide (Mukaiyama's reagent)[22] was used as condensing agent. Thus, the carboxylic acid **5**, the primary amine, Mukaiyama's reagent, and triethylamine were brought together resulting in the formation of the protected carboxamides in high yields.

Deprotection from the 2′,3′-*O*-isopropylidene group was accomplished by stirring in trifluoroacetic acid/water for less than 30 min at room temperature.

Thiocarboxamides **8b** and **8c** were made from the protected carboxamides **7b** and **7c**. The sulfurizing agent used in these reactions was Lawesson's reagent [2,4-bis(4-methoxyphenyl)-1,3-diathia-2,4-diphosphetane-2,4-disulfide]. Deprotection was carried out in the same manner as for the carboxamides in trifluoroacetic acid/water.

Compound **8b** was studied by single X-ray analysis; its structure is shown in Figure 1.

Biological Evaluation

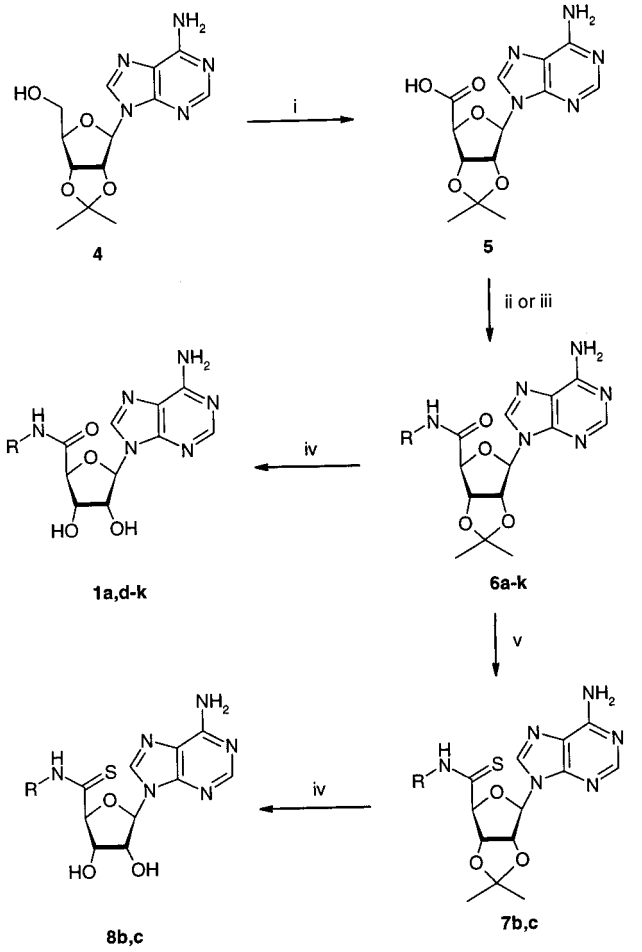
Compounds were tested for their potencies on A_1, A_{2A}, and A_3 adenosine receptors in radioligand binding assays. A_1 and A_{2A} receptor affinities were determined using rat brain cortex membranes and rat brain striatum membranes, respectively. A_3 receptor affinities were measured on membranes prepared from HEK 293 cells stably transfected with human A_3 receptor cDNA. For A_1 receptor affinity the tritiated antagonist [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine (DPCPX) was used. Experiments were performed in the presence and absence of 1 mM GTP, allowing the determination of the so-called GTP shift. The GTP shift is an in vitro parameter often indicative of intrinsic activity. As there were no radiolabeled antagonists commercially available for either the A_{2A} or the A_3 receptor, the tritiated agonist [^{3}H]CGS21680 and the iodinated agonist N^6-(4-amino-3-[^{125}I]iodobenzyl)-5′-*N*-methylcarboxamidoadenosine ([^{125}I]-ABMECA) were used, respectively.

Since there was no binding assay available yet for the A_{2B} receptor, we determined activity by measuring the amount of the second messenger cyclic AMP formed after stimulation of the receptors with the test compounds. For this purpose Chinese hamster ovary (CHO)-K1 cells stably transfected with human A_{2B} receptor cDNA were used.[23] EC_{50} values on cyclic AMP production were thus determined and are presented in Table 1. Activities are shown in micromolar concentrations, whereas binding affinities (K_i values) are expressed in nanomolar concentrations.

Results and Discussion

According to literature procedures, 5′-carboxamidoadenosines have been synthesized with 2′,3′-*O*-isopropy-

Scheme 1. Synthesis of 5′-*N*-Alkyl(thio)carboxamidoadenosines[a]



[a] Reagents and conditions: (i) $KMnO_4$/80% acetic acid, 16 h; (ii) Et_3N/isopropenyl chloroformate/RNH_2 in DMF, 0−5 °C, 5 min (method A); (iii) Mukaiyama's reagent/RNH_2/Et_3N in DMF, 20 °C, 5 min (method B); (iv) trifluoroacetic acid/water, 9/1, v/v, 30 min; (v) Lawesson's reagent, dioxane, 80 °C, 1 h.

lideneadenosine-5′-uronic acid (**5**) as the starting compound. In these procedures the carboxylic acid group was made reactive toward amines by converting it into the acid chloride—and in some cases subsequently to an alkyl ester.[20,21,24] Another method, i.e. the use of a coupling reagent, has been reported for the synthesis of 2′,3′-*O*-isopropylidene-protected carboxamidoinosines.[17] In all cases deprotection from the isopropylidene group was accomplished in hydrochloric acid at elevated temperatures.

For the preparation of the carboxamidoadenosines tested in this study we used coupling reagents to make the uronic acid willing to react. Coupling reagents are preferred to chlorinations, providing a one-step reaction with shorter reaction times. Two coupling reagents were tried for the condensation of the uronic acid **5** with several amines. In method A, isopropenylchloroformate was the condensing agent; in method B, Mukaiyama's reagent[22] served as such. Both procedures gave the predicted carboxamides, but with Mukaiyama's reagent reactions generally proceeded in higher yields (up to 95%). For this reason we used the latter reagent in most cases. Deprotection from the isopropylidene group was performed in trifluoroacetic acid/water at ambient temperature, which proved to be a quick method (<30 min) giving good yields (41−96%).

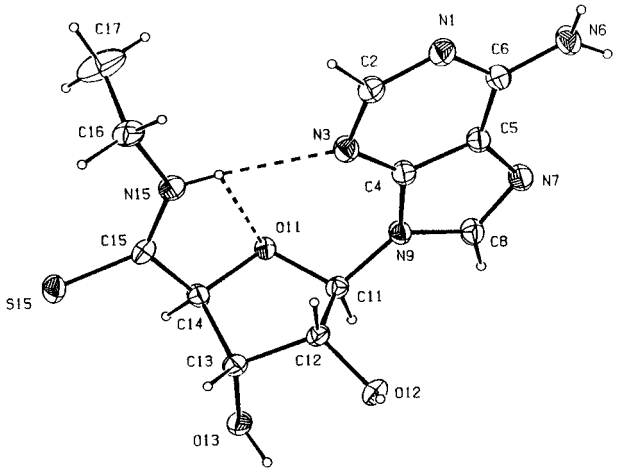
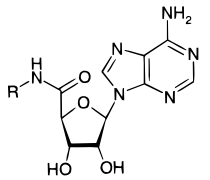


Figure 1. Atomic displacement ellipsoid plot[48] of **8b**, drawn at 50% probability level. Water molecules have been omitted for clarity. Hydrogen atoms are drawn as spheres of arbitrary radius. Intramolecular hydrogen bonds are indicated with dashed lines.

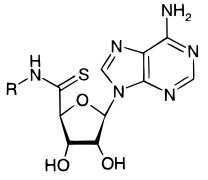
Results on all receptor subtypes are shown in Table 1. Binding affinities are given in nanomolar concentrations and EC_{50} values for cyclic AMP production in micromolar concentrations. The ethyl-substituted analogue **1b** (NECA) and the cyclopropyl-substituted derivative **1c** (NCPCA) were among the most potent analogues on the A_1, the A_{2A}, and also on the A_{2B} subtype. On the human A_3 receptor, the methyl-substituted analogue **1a** (MECA) displayed the highest affinity, with a much higher selectivity than has been observed on the rat A_3 receptor (Chart 1).[17] Larger alkyl substituents resulted in lower activities on all receptor subtypes, generally a decrease of 1−2 orders of magnitude with respect to the most potent derivative; a *tert*-butyl substituent (in **1g**) is apparently too bulky for adenosine receptor activity. Among the cycloalkyl-substituted analogues, the cyclopropyl derivative **1c** displayed a considerably reduced A_3 receptor binding, as had been observed previously by Gallo-Rodriguez et al. at rat A_3 receptors,[17] whereas it retained A_1 and A_{2A} receptor affinity. A low A_3 affinity was also found for the cyclopropyl-substituted thiocarboxamide **8c**. In contrast, the cyclobutyl-substituted analogue **1f** showed, next to high affinities for A_1 and A_{2A}, also a high A_3 receptor binding. Similarly, the cyclopentyl derivative **1h** had a high affinity for the A_3 receptor, while also displaying selectivity for this receptor subtype. Other selective derivatives for the A_3 receptor, besides MECA (**1a**), were the *n*-propyl derivative **1d** and the methoxyl derivative **1k**.

At the A_1 receptor four compounds, **1a**−**c** and **1h**, exhibited large GTP shifts (>6), suggesting them to be full agonists. Among these was NECA (**1b**), which has also been shown to act as a full agonist in a GTP-γS assay at bovine A_1 receptors[25] and is classically considered as such.[26] Other derivatives displayed smaller shifts, which could be indicative of lower intrinsic activities.[27] Recently, it was reported that 5′-substituted N^6-cyclopentyladenosines are partial agonists for the A_1 receptor.[28]

Table 1. Binding Affinities of 5′-*N*-Substituted (Thio)Carboxamidoadenosines in Radioligand Binding Assays at Rat A_1 (in the Absence and Presence of GTP), Rat A_{2A}, and Human A_3 Receptors and Activities for cAMP Production at Human A_{2B} Receptors



1a-k



8b,c

analogue	R	K_i A_1[a,f] −GTP (nM)	K_i A_1[a,f] +GTP (nM)	GTP shift	K_i A_{2A}[b,f] (nM)	K_i A_3[c,f] (nM)	EC_{50} on A_{2B} (cAMP production)[d,e,f] (μM)
1a (MECA)	methyl	1084 ± 104	6840 ± 893	6.3	330 ± 210	6.4 ± 1.1	45 ± 15 (62 ± 14)
1b (NECA)	ethyl	63 ± 16	530 ± 65	8.4	16 ± 10	10.6 ± 0.7	3.1 ± 2.3 (100)[g]
1c (NCPCA)	cyclopropyl	63 ± 4	585 ± 87	9.3	12 ± 7	108 ± 19	5.3 ± 1.6 (100)[g]
1d	*n*-propyl	1240 ± 690	3960 ± 200	3.2	550 ± 360	44 ± 11	29[h] (74)
1e	isopropyl	257 ± 221	804 ± 391	3.1	160 ± 100	211 ± 37	37 ± 13 (83 ± 4)
1f	cyclobutyl	43 ± 19	172 ± 53	4.0	49 ± 14	23 ± 10	33 ± 7 (100)
1g	*tert*-butyl	2726 ± 930	6946 ± 1479	2.6	1340 ± 130	8500 ± 1900	(10% at 100 μM)
1h	cyclopentyl	770 ± 200	4900 ± 1630	6.4	610 ± 290	32 ± 7	54 ± 16 (18 ± 4)
1i	2-chloroethyl	248 ± 7	890 ± 471	3.6	410 ± 320	322 ± 37	32 ± 9 (43 ± 9)
1j	2,2,2-trifluoroethyl	1186 ± 310	2349 ± 400	2.0	405 ± 198	719 ± 257	30 ± 2 (47 ± 13)
1k	methoxyl	1620 ± 430	3960 ± 1250	2.4	623 ± 210	85.3 ± 18.8	45 ± 9 (72 ± 9)
8b	ethyl	141 ± 90	478 ± 154	3.4	130 ± 40	569 ± 129	16 ± 5 (100)
8c	cyclopropyl	137 ± 94	459 ± 190	3.4	130 ± 10	2280 ± 880	13 ± 5 (100)

[a] Displacement of [^{3}H]DPCPX from rat cortical membranes. [b] Displacement of [^{3}H]CGS 21680 from rat striatal membranes. [c] Displacement of [^{125}I]-ABMECA from membranes of HEK 293 cells stably transfected with human A_3 receptor cDNA. [d] Production of cAMP in CHO−K1 cells stably transfected with human adenosine A_{2B} receptor cDNA. [e] E_{max} values in percentages of the response of 100 μM NECA are given in parentheses. [f] Values are taken from three experiments, expressed as means ± SEM, except *h*. [g] EC_{50} values taken from de Zwart et al.[19] [h] $n = 1$.

Concerning the thiocarboxamides **8b** and **8c**, modest to significant decreases in affinity were observed when compared to their carboxamide analogues **1b** and **1c**. At the A_1 receptor a 2- to 3-fold decrease was seen for each pair, at the A_{2A} receptor the difference was 10-fold, at the A_{2B} receptor 2- to 5-fold, and at the A_3 receptor a 50-fold decrease was observed between the ethyl derivatives and 20-fold between the cyclopropyl derivatives.

At the A_{2B} receptor subtype, NECA (**1b**) was the most potent agonist with an EC_{50} value of 3.1 μM and a maximal intrinsic activity. As ethyl is the optimal alkyl substituent, the modified ethyl-substituted analogues **1i** and **1j** are of interest, as well as derivative **1k**, bearing a methoxyl group.[20] EC_{50} values of these compounds were 10-fold higher than that of NECA, and their E_{max} values were significantly lower, indicating decreased intrinsic activities. Maximal intrinsic activities, on the other hand, were observed for NCPCA (**1c**) and for the cyclobutyl derivative **1f**. Modifying the carboxamido functional group into a thiocarboxamido function (compounds **8b** and **8c**) led to ligands with maximal intrinsic activities, but with 2- to 5-fold higher EC_{50} values.

In 1989 the X-ray crystal structure of NECA (**1b**) was reported.[29] NECA was found to be crystallized in a syn conformation. To study structure−activity relationships of (thio)carboxamides we decided to determine the X-ray crystal structure of the active novel derivative 5′-*N*-ethylthiocarboxamidoadenosine (**8b**) (Figure 1) and to compare it with the NECA structure. Similar to NECA, the molecules of **8b** were found to be in the syn conformation due to an intramolecular bifurcated hydrogen bond between the N*H* element of the carboxamido moiety and the purine *N*3 nitrogen atom (distance H···N, 2.2 Å; angle N−H···N, 155°) and the oxygen atom in the ribose ring (distance H···O, 2.3 Å; angle N−H···O, 108°), even though the crystal packing is different as a result of the presence of an extra water solvent molecule, donating to C═S, in the crystals of **8b**. Presumably, the diminished activity of **8b** is therefore not due to an alteration of the agonist conformation in the crystal structure. The sulfur atom in the carboxamide may either be too large for fitting properly in the receptor, or it may diminish the interaction between the carboxamide moiety and the receptor. In this light it is interesting to note that whereas in NECA the C═O and one of the hydroxyl moieties accept hydrogen bonds from the purine 6-NH_2 of an adjacent molecule, the corresponding C═S and OH groups in **8b** accept hydrogen bonds from H_2O. However, caution must be employed when correlating activity with crystal structure data. Conformational preferences of a molecule are well known to be decisively influenced by its surroundings; a different conformation may be found in the solid state (X-ray crystal structure), in solution (NMR experiments), in vacuo (theoretical calculations), or upon binding to a protein.[30] In fact, the syn crystal structure conformation of this nucleoside is just one of the many possible ones, due to the flexibility of the glycosidic bond. From NMR experimental data, Prasad et al.[20] have suggested that in solution a considerable percentage of the NECA molecules is in the syn conformation, proposing also that the syn conformation favors increased biological activity. However, adenosine has been suggested to bind to the receptor in the anti conformation, based on the inability of analogues restricted to the syn conformation to activate adenosine receptors.[31] Moreover, NMR data on H(2′) give information on the conformation of the molecule in solution. Chemical shifts measured in DMSO for this hydrogen atom of derivatives restricted to the syn conformation are approximately 5.2 ppm while analogues that exist exclusively in the anti conformation show values around 4.2 ppm.[32]

For adenosine, an intermediate value of 4.62 has been reported, suggesting that it rapidly changes its conformation with a slight preference for the anti conformation.[33] For **8b** and NECA,[20] H(2′) chemical shifts were found to be close to that of adenosine (4.62 and 4.55, respectively), suggesting that these molecules adopt both conformations in solution, similar to adenosine.

The latter hypothesis was tested by theoretical calculations on the syn and anti conformations of adenosine, NECA, and **8b**. For these calculations, all molecules were allowed to relax fully. Optimization procedures retained the respective puckering of the ribose ring observed in the anti and syn conformations.[34] The glycosidic torsion angle [dihedral (C8N9C1′O1′)] for optimized anti conformations was 7.3, 16.2, and 16.5° and for syn 240.2, 242.3, and 242.5° for adenosine, NECA, and **8b**, respectively, in good agreement with typical values reported elsewhere.[35] Ab initio (HF) energies of syn and anti adenosine presented a negligible difference of 0.22 kcal/mol (favoring the anti as the low-energy conformation), providing therefore further evidence that an equilibrium exists between the syn and anti conformations of this molecule.[33,36] Accordingly, for NECA the difference between syn and anti was 2.1 kcal/mol (favoring the syn conformation) while for **8b** this difference increased to 6.4 kcal/mol. The latter observations, in agreement with both the crystallographic data and the proposed anti modes of binding for such molecules to their receptor,[31] indicate that an equilibrium for NECA and for **8b** may also be present, although somewhat shifted, especially in the case of **8b**, to the syn side. Interestingly, this might as well account for the reduced activity of **8b**.

Conclusion

In this study, we report for the first time activities on all four adenosine receptor subtypes of a series of 5′-*N*-substituted carboxamidoadenosines. In general, derivatives with relatively small 5′-substituents were most potent on all subtypes. Thiocarboxamides were less active than carboxamides. At the A_1 receptor, GTP shifts indicated that a number of derivatives may have a reduced intrinsic activity and may thus be partial agonists. At the A_3 subtype, several analogues were selective, MECA (**1a**) being the most selective in this series. At the A_{2B} receptor NECA (**1b**) is still the most active agonist reported yet, the ethyl substituent appearing optimal for this receptor subtype. Finally, although NECA and its thiocarboxamido analogue **8b** crystallize in the syn conformation, our theoretical calculations support that, similar to adenosine, NECA and less so **8b** can readily adopt both the syn and the anti conformation.

Experimental Section

Chemicals and Solvents. 2′,3′-*O*-Isopropylideneadenosine (**4**) was purchased from ACROS Chimica (Geel, Belgium). All reagents were of analytical grade. Amines were purchased from Aldrich. NECA (**8b**) was purchased from Sigma; NCPCA (**8c**) was a gift of Dr. R. A. Olsson.

Chromatography. Thin-layer chromatography (TLC) was performed using silica F254 preformed layers 0.1 mm thick on a plastic backing (Schleicher and Schuell DC Fertigfolien F 1500 LS 254). Dichloromethane/methanol, 9/1,v/v, was used as the mobile system. Spots were visualized either under UV (254 nm) light or by spraying with sulfuric acid/methanol (1/4, v/v) or molybdate reagent (water/concentrated sulfuric acid/$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$/$(NH_4)_2Ce(SO_4)_4\cdot 2H_2O$, 90/10/2.5/1, v/v/w/w) and heating at 140 °C. Preparative column chromatography was performed on MERCK silicagel (230−400 mesh ASTM).

Instrumental Analyses. ^{13}C NMR spectra were recorded at 50.1 MHz with a JEOL JNM-FX 200 spectrometer equipped with a PG 200 computer operating in the Fourier transform mode. ^1H NMR spectra were measured at 200 MHz, using the abovementioned spectrometer, or at 300 MHz, using a Bruker WM-300 spectrometer equipped with an ASPECT-2000 computer operating in the Fourier transform mode. Chemical shifts for ^1H and ^{13}C NMR are given in ppm (δ) relative to tetramethylsilane as an internal standard. All high-resolution mass spectra (ESI) were measured on a Finnigan MAT TSQ-70 mass spectrometer equipped with an electrospray interface. Experiments were done in positive ionization mode. Samples were dissolved in dichloromethane diluted with 4/1 methanol/water with 1% acetic acid and introduced by means of constant infusion at a flow rate of 1 μL/min. Elemental analysis was done at the Department of Microanalysis, Groningen University, Groningen, The Netherlands. Melting points (not corrected) were determined in a Büchi capillary melting point apparatus.

Syntheses. 2′,3′-*O*-Isopropylideneadenosine-5′-uronic acid (**5**) was synthesized according to literature procedures:[21] yield 67%; ^1H NMR (DMSO-d_6) δ 8.24 (s, 1H, H-8), 8.07 (s, 1H, H-2), 7.27 (s, 2H, NH_2), 6.32 (s, 1H, H-1′), 5.53 (dd, 1H, $J_{3',2'} = 5.5$ Hz, $J_{3',4'} = 2.0$ Hz, H-3′), 5.44 (d, 1H, $J_{2',3'} = 5.5$ Hz, H-2′), 4.68 (d, 1H, $J_{4',3'} = 2.0$ Hz, H-4′), 1.51, 1.34 (2 × s, 2 × 3H, 2 × CH_3, isopropylidene); ^{13}C NMR (DMSO-d_6): δ 170.6 (C-5′), 155.9 (C-6), 152.3 (C-2), 149.1 (C-4), 140.5 (C-8), 118.7 (C-5), 112.6 (Cq, isopropylidene), 89.4 (C-1′), 85.4 (C-4′), 83.7, 83.4 (C-3′, C-2′), 26.5, 24.9 (2 × CH_3, isopropylidene).

Syntheses of Carboxamides 6a−k. Method A. The uronic acid **5** (321 mg, 1.0 mmol) was coevaporated with DMF (3 × 3 mL) and taken up in DMF (5 mL) with triethylamine (204 μL, 1.5 mmol) under a nitrogen atmosphere. After the solution cooled to 0 °C, the coupling reagent isopropenylchloroformate (132 μL, 1.8 mmol) and the appropriate amine (2.3 mmol) were added subsequently. The reaction mixture was stirred for less than 5 min at 0 °C when TLC analysis showed the reactions to be complete. The mixtures were concentated under reduced pressure, redissolved in dichloromethane, washed with an aqueous $NaHCO_3$ solution (10%, 10 mL) and water (2 × 10 mL), dried on $MgSO_4$, and concentrated. The remaining oils were purified by column chromatography (eluent: gradients of methanol in dichloromethane, 0% to 15%).

Method B. Compound **5** (321 mg, 1.0 mmol) was coevaporated with DMF (3 × 3 mL), dissolved in DMF (5 mL), and stirred under a nitrogen atmosphere. Mukaiyama's reagent (2-chloro-1-methylpyridinium iodide, 323 mg, 1.2 mmol), the appropriate amine (1.5 mmol), and triethylamine (346 μL, 2.5 mmol) were subsequently added. The mixtures were stirred at room temperature until completion (TLC analysis, 5 min). The mixtures were concentrated under reduced pressure, redissolved in dichloromethane, washed with an aqueous $NaHCO_3$ solution (10%, 10 mL) and water (2 × 10 mL), dried on $MgSO_4$, and concentrated. The remaining oils were purified by column chromatography (eluent: gradients of methanol in dichloromethane, 0% to 15%).

Deprotection from the 2′,3′-*O*-Isopropylidene group (1a,d−k and 8a,b). Compounds **6a**, **d**−**k** and **7a**,**b** were stirred for 30 min in trifluoroacetic acid/water (9/1, v/v) at room temperature. The solutions were subsequently concentrated under reduced pressure and coevaporated with dry dioxane (3 × 10 mL). The remaining oils were purified by column chromatography (eluent: gradients of methanol in dichloromethane up to 15%). Recrystallization of a small amount from water gave analytical samples.

5′-*N*-Methyl-2′,3′-*O*-isopropylidenecarboxamidoadenosine (6a). Compound **6a** was synthesized according to method A. Five equivalents of methylamine (a solution of methylamine in water (40 wt %), 430 μL) was added: yield 160 mg (41%); R_f 0.40; ^1H NMR ($CDCl_3/CD_3OD$): δ 8.26 (s,

1H, H-8), 8.05 (s, 1H, H-2), 7.44 (s(b), 1H, N*H*), 6.18 (d, 1H, J = 2 Hz, H-1′), 5.39, 5.37 (m + s, 2H, H-2′, H-3′), 4.72 (s, 1H, H-4′), 2.57 (m, 3H, CH_3), 1.63, 1.41 (2 × s, 2 × 3H, 2 × CH_3, isopropylidene); ^{13}C NMR ($CDCl_3$/CD_3OD) 23.44 (*C*H_3).

5′-*N*-Ethyl-2′,3′-*O*-isopropylidenecarboxamidoadenosine (6b). Compound **6b** was synthesized according to method A, starting with 2.00 g (6.2 mmol) of compound **5** and accommodating the amounts of reagents. A solution of ethylamine in water (70 wt %) was used. The compound was isolated as an oil: yield 1.55 g (72%); R_f 0.50; ^{1}H NMR ($CDCl_3$) δ 8.31 (s, 1H, H-8), 7.87 (s, 1H, H-2), 6.92 (s, 2H, NH_2), 5.98 (s, 1H, H-1′), 5.40 (m, 2H, H-2′, H-3′), 4.71 (s, 1H, H-4′), 3.06 (m, 2H, CH_2CH$_3$), 1.63, 1.41 (2 × s, 2 × 3H, 2 × CH_3, isopropylidene), 0.88 (t, 3H, CH$_2$CH_3); ^{13}C NMR ($CDCl_3$) 31.85 (*C*H$_2$CH$_3$), 12.00 (CH$_2$*C*H$_3$).

5′-*N*-Cyclopropyl-2′,3′-*O*-isopropylidenecarboxamidoadenosine (6c). Compound **6c** was synthesized according to method A. Five equivalents of cyclopropylamine was added: yield 200 mg (58%); R_f 0.55; ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1H, H-8), 7.89 (s, 1H, H-2), 7.25 (s, 1H, N*H*), 6.40 (s(b), 2H, NH_2), 6.08 (d, 1H, J = 1.7 Hz, H-1′), 5.38 (m, 2H, H-2′, H-3′), 4.71 (s, 1H, H-4′), 2.56 (m, 1H, C*H*, cyclopropyl), 1.63, 1.39 (2 × s, 2 × 3H, 2 × CH_3, isopropylidene), 0.64, 0.20 (2 × m, 2 × 2H, 2 × CH_2 cyclopropyl); ^{13}C NMR ($CDCl_3$) δ 21.95 (*C*H, cyclopropyl), 5.98, 5.75 (2 × *C*H$_2$, cyclopropyl).

5′-*N*-(*n*-Propyl)-2′,3′-*O*-isopropylidenecarboxamidoadenosine (6d). Method B: yield 66 mg (17%); R_f 0.60; ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1H, H-8), 7.92 (s, 1H, H-2), 7.03 (t, 1H, N*H*), 6.50 (s(b), 2H, NH_2), 6.11 (s, 1H, H-1′), 5.40 (m, 2H, H-2′, H-3′), 4.73 (s, 1H, H-4′), 3.03 (m, 2H, CH_2CH$_2$CH$_3$), 1.63, 1.39 (2 × s, 2 × 3H, 2 × CH_3, isopropylidene), 1.25 (m, 2H, CH$_2$CH_2CH$_3$), 0.75 (t, 3H, CH$_2$CH$_2$CH_3).

5′-*N*-Isopropyl-2′,3′-*O*-isopropylidenecarboxamidoadenosine (6e). Method B: yield 311 mg (85%); R_f 0.46; ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1H, H-8), 8.01 (s, 1H, H-2), 7.63 (s(b), 2H, NH_2), 6.71 (d, 1H, $J_{NH,CH}$ = 7.0 Hz, N*H*), 6.20 (d, 1H, J = 1.4 Hz, H-1′), 5.47 (m, 2H, H-2′, H-3′), 4.72 (s, 1H, H-4′), 3.89 (m, 1H, $J_{CH,CH3} = J_{CH,NH}$ = 7.0 Hz, C*H*(CH$_3$)$_2$), 1.63, 1.40 (2 × s, 2 × 3H, 2 × CH_3, isopropylidene), 1.00, 0.79 (2 × d, 2 × 3H, CH(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 38.97 (*C*H(CH$_3$)$_2$), 20.46, 19.88 (2 × CH(*C*H$_3$)$_2$).

5′-*N*-Cyclobutyl-2′,3′-*O*-isopropylidenecarboxamidoadenosine (6f). Method B: yield 243 mg (64%); R_f 0.50; ^{1}H NMR ($CDCl_3$) δ 8.29 (s, 1H, H-8), 8.13 (s, 1H, H-2), 7.21 (d, 1H, $J_{NH,CH}$ = 8.0 Hz, N*H*), 6.25 (s, 1H, H-1′), 5.48 (m, 2H, H-2′, H-3′), 4.70 (s, 1H, H-4′), 4.17 (m, 1H, C*H*, cyclobutyl), 2.17, 1.95, 1.70, 1.36 (4 × m, 6H, 3 × CH$_2$, cyclobutyl), 1.63, 1.41 (2 × s, 2 × 3H, 2 × CH_3, isopropylidene); ^{13}C NMR ($CDCl_3$) δ 43.5 (*C*H, cyclobutyl), 30.0, 29.6, 14.4 (3 × *C*H$_2$, cyclobutyl).

5′-*N*-(*tert*-Butyl)-2′,3′-*O*-isopropylidenecarboxamidoadenosine (6g). Method B: yield 341 mg (95%); R_f 0.50; ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1H, H-8), 8.04 (s, 1H, H-2), 7.63 (s(b), 2H, NH_2), 7.11 (s, 1H, N*H*), 6.25 (d, 1H, J = 3 Hz, H-1′), 5.55 (m, 2H, H-2′, H-3′), 4.62 (s, 1H, H-4′), 1.61, 1.41 (2 × s, 2 × 3H, 2 × CH_3, isopropylidene), 1.06 (s, 9H, 3 × CH_3, *tert*-butyl); ^{13}C NMR ($CDCl_3$) δ 50.4 (Cq, *tert*-butyl), 27.9 (s, 3 × *C*H$_3$, *tert*-butyl).

5′-*N*-Cyclopentyl-2′,3′-*O*-isopropylidenecarboxamidoadenosine (6h). Method B: yield 383 mg (97%); R_f 0.45; ^{1}H NMR ($CDCl_3$) δ 8.31 (s, 1H, H-8), 8.13 (s, 1H, H-2), 7.30 (s(b), 2H, NH_2), 6.28 (d, 1H, J = 1.7 Hz, H-1′), 5.51 (m, 2H, H-2′, H-3′), 4.73 (s, 1H, H-4′), 4.00 (m, 1H, C*H*, cyclopentyl), 1.62, 1.41 (2 × s, 2 × 3H, 2 × CH_3, isopropylidene), 1.81, 1.65, 1.22, 0.85 (4 × m, 8H, 4 × CH_2, cyclopentyl); ^{13}C NMR ($CDCl_3$) δ 50.0 (*C*H, cyclopentyl), 32.3, 31.6 (2 × CH_2, cyclopentyl).

5′-*N*-(2-Chloroethyl)-2′,3′-*O*-isopropylidenecarboxamidoadenosine (6i). Method A. A solution of 2-chloroethylamine hydrochloride (500 mg, 4.3 mmol) in water (200 μL) was added together with an additional amount of triethylamine (4.3 mmol, 600 μL). TLC analysis indicated the formation of one main product, among four other products with R_f values near that of the main product. This was not a problem for separation: yield 140 mg (38%) as a white powder; ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1H, H-8), 7.91 (s, 1H, H-2), 7.32 (m, 1H, N*H*), 6.68 (s(b), 2H, NH_2), 6.15 (d, 1H, J = 2.5 Hz, H-1′), 5.45 (m, 2H, H-2′, H-3′), 4.76 (s, 1H, H-4′), 3.40 (m, 4H, 2 × CH_2, chloroethyl), 1.63, 1.40 (2 × s, 2 × 3H, 2 × CH_3, isopropylidene); ^{13}C NMR δ 43.1, 40.7 (2 × CH$_2$, chloroethyl).

5′-*N*-(2,2,2-Trifluoroethyl)-2′,3′-*O*-isopropylidenecarboxamidoadenosine (6j). Method B: yield 324 mg (81%); R_f 0.45; ^{1}H NMR data ($CDCl_3$) δ 8.30 (s, 1H, H-8), 7.86 (s, 1H, H-2), 6.37 (s(b), 2H, NH_2), 6.04 (s, 1H, H-1′), 5.25 (m, 2H, H-2′, H-3′), 4.80 (s, 1H, H-4′), 3.80 (m, 1H, CH_2, trifluoroethyl), 1.64, 1.38 (2 × s, 2 × 3H, 2 × CH_3, isopropylidene); ^{13}C NMR δ (q, $J_{C,F}$ = 280 Hz, CH$_2$*C*F$_3$), 40.2 (q, $J_{C,F}$ = 35 Hz, *C*H$_2$CF$_3$).

5′-*N*-Methoxyl-2′,3′-*O*-isopropylidenecarboxamidoadenosine (6k). Method B: yield 170 mg (49%); R_f 0.25; ^{1}H NMR (CD_3OD) δ 8.20 (s, 1H, H-8), 8.15 (s, 1H, H-2), 6.34 (s, 1H, H-1′), 5.64, 5.45 (2 × m, 2H, H-2′, H-3′), 4.70 (d, 1H, J = 1.7 Hz, H-4′), 3.21 (s, 3H, OCH_3), 1.56, 1.39 (2 × s, 2 × 3H, 2 × CH_3, isopropylidene).

5′-*N*-Methylcarboxamidoadenosine (1a): Yield 60 mg (43%); R_f 0.06; ^{1}H NMR data (DMSO-*d*6): δ 8.95 (s, 1H, N*H*), 8.39 (s, 1H, H-8), 8.23 (s, 1H, H-2), 7.45 (s, 2H, NH_2), 5.95 (d, 1H, $J_{1′,2′}$ = 7.7 Hz, H-1′), 4.60 (m, 1H, H-2′), 4.30 (d, 1H, $J_{4′,3′}$ = 0.7 Hz, H-4′), 4.15 (m, 1H, H-3′), 2.71 (d, 3H, CH_3); mp 241 °C (lit. 240−241 °C[20]); MS *m*/*z* 295 [MH]$^+$.

5′-*N*-(*n*-Propyl)carboxamidoadenosine (1d): yield 50 mg (85%); R_f 0.15; ^{1}H NMR data (DMSO-d_6): δ 8.99 (t, 1H, N*H*), 8.37 (s, 1H, H-8), 8.19 (s, 1H, H-2), 7.44 (s, 2H, NH_2), 5.95 (d, 1H, $J_{1′,2′}$ = 7.7 Hz, H-1′), 5.75, 5.55 (2 × d, 2 × 1H, 2′-O*H*, 3′-O*H*), 4.60 (m, 1H, H-2′), 4.31 (d, 1H, $J_{4′,3′}$ = 0.9 Hz, H-4′), 4.12 (t, 1H, H-3′), 3.43 (m, 2H, CH_2CH$_2$CH$_3$), 1.47 (m, 2H, CH$_2$CH_2CH$_3$), 1.05 (t, 3H, CH$_2$CH$_2$CH_3); ^{13}C NMR (DMSO-d_6) δ 172.7 (C-5′), 157.7 (C-6), 153.5 (C-2), 150.6 (C-4), 143.3 (C-8), 121.7 (C-5), 91.2 (C-1′), 86.8 (C-4′), 75.6, 74.2 (C-3′, C-2′), 42.5 (*C*H$_2$CH$_2$CH$_3$), 24.3 (CH$_2$*C*H$_2$CH$_3$), 12.4 (CH$_2$CH$_2$*C*H$_3$); mp 218 °C (lit.: 220−222 °C[20]); MS *m*/*z* 323 [MH]$^+$.

5′-*N*-Isopropylcarboxamidoadenosine (1e): yield 241 mg (87%); R_f 0.12; ^{1}H NMR (DMSO-d_6) δ 8.48 (d, 1H, N*H*), 8.40 (s, 1H, H-8), 8.19 (s, 1H, H-2), 7.50 (s, 2H, NH_2), 5.94 (d, 1H, $J_{1′,2′}$ = 7.5 Hz, H-1′), 5.69, 5.58 (2 × s(b), 2 × 1H, 2′-O*H*, 3′-O*H*), 4.60 (dd, 1H, $J_{2′,1′}$ = 7.5 Hz, $J_{2′,3′}$ = 5.0 Hz, H-2′), 4.29 (d, 1H, $J_{4′,3′}$ = 1.5 Hz, H-4′), 4.11 (dd, 1H, $J_{3′,2′}$ = 4.5 Hz, $J_{3′,4′}$ = 1.5 Hz, H-3′), 3.98 (m, 1H, C*H*(CH$_3$)$_2$, 1.12, 1.07 (2 × d, 2 × 3H, CH(CH_3)$_2$; ^{13}C NMR (DMSO-d_6) δ 168.8 (C-5′), 155.8 (C-6), 152.1 (C-2), 149.1 (C-4), 88.1 (C-1′), 84.7 (C-4′), 73.3, 72.3 (C-3′, C-2′), 40.8 (*C*H(CH$_3$)$_2$), 22.5 (2 × CH(*C*H$_3$)$_2$); mp 145 °C (lit.: 137−141 °C[20]); MS *m*/*z* 323 [MH]$^+$.

5′-*N*-Cyclobutylcarboxamidoadenosine (1f): yield 173 mg (75%); R_f 0.15; ^{1}H NMR (DMSO-d_6) δ 8.86 (d, 1H, N*H*), 8.43 (s, 1H, H-8), 8.22 (s, 1H, H-2), 7.59 (s, 2H, NH_2), 5.95 (d, 1H, $J_{1′,2′}$ = 7.4 Hz, H-1′), 5.60, (s(b), 2H, 2′-O*H*, 3′-O*H*), 4.60 (dd, 1H, $J_{2′,1′}$ = 7.4 Hz, $J_{2′,3′}$ = 4.6 Hz, H-2′), 4.28 (d, 1H, $J_{4′,3′}$ = 1.5 Hz, H-4′), 4.28 (m, 1H, C*H*, cyclobutyl), 4.11 (dd, 1H, $J_{3′,2′}$ = 4.6 Hz, $J_{3′,4′}$ = 1.6 Hz, H-3′), 2.21, 1.95, 1.66 (3 × m, 3 × 2H, 3 × CH_2, cyclobutyl); ^{13}C NMR (DMSO-d_6) δ 168.8 (C-5′), 155.7 (C-6), 151.8 (C-2), 149.2 (C-4), 141.5 (C-8), 119.7 (C-5), 88.3 (C-1′), 84.7 (C-4′), 73.3, 72.5 (C-3′, C-2′), 44.1 (*C*H$_2$, cyclobutyl), 30.5 (2 × *C*H$_2$, cyclobutyl), 15.2 (*C*H$_2$, cyclobutyl); mp 235 °C (lit.: 234−235 °C[20]); MS *m*/*z* 335 [MH]$^+$.

5′-*N*-(*tert*-Butyl)carboxamidoadenosine (1g): yield 146 mg (96%); R_f 0.10; ^{1}H NMR (DMSO-d_6) δ 8.44 (s, 1H, H-8), 8.16 (s, 1H, H-2), 7.72 (s, 1H, N*H*), 7.56 (s, 2H, NH_2), 5.91 (d, 1H, $J_{1′,2′}$ = 7.0 Hz, H-1′), 4.64 (dd, 1H, $J_{2′,1′}$ = 7.0 Hz, $J_{2′,3′}$ = 4.5 Hz, H-2′), 4.23 (d, 1H, $J_{4′,3′}$ = 1.5 Hz, H-4′), 4.09 (dd, 1H, $J_{3′,4′}$ = 1.5 Hz, $J_{3′,2′}$ = 4.5 Hz, H-3′), 1.27 (s, 9H, 3 × CH_3, *tert*-butyl); ^{13}C NMR (DMSO-d_6) δ 52.4 (Cq, *tert*-butyl), 28.9 (s, 3 × *C*H$_3$, *tert*-butyl); mp 140 °C; MS *m*/*z* 337 [MH]$^+$, 359 [MNa]$^+$. Anal. ($C_{14}H_{20}N_6O_4$·1.1H_2O) C, H, N.

5′-*N*-Cyclopentylcarboxamidoadenosine (1h): yield 140 mg (41%); R_f 0.15; ^{1}H NMR (DMSO-d_6) δ 8.43 (s, 1H, H-8), 8.14 (s, 1H, H-2), 7.50 (s, 2H, NH_2), 5.94 (d, 1H, $J_{1′,2′}$ = 7.0 Hz, H-1′), 5.65, 5.52 (2 × s(b), 2 × 1H, 2′-O*H*, 3′-O*H*), 4.60 (dd, 1H, $J_{2′,1′}$ = 7.0 Hz, $J_{2′,3′}$ = 5.0 Hz, H-2′), 4.30 (d, 1H, $J_{4′,3′}$ = 1.5 Hz, H-4′), 4.13 (dd, 1H, $J_{3′,4′}$ = 1.5 Hz, $J_{3′,2′}$ = 5.0 Hz, H-3′), 4.10 (m, 1H, C*H*, cyclopentyl), 1.85, 1.64, 1.51, 1.40 (4 × m, 8H, 4 × CH_2 cyclopentyl); ^{13}C NMR (DMSO-d_6) δ 50.3 (*C*H,

cyclopentyl), 32.3 (2 × CH_2, cyclopentyl), 23.7 (2 × CH_2, cyclopentyl); mp 165 °C (lit.: 165−170 °C[20]); MS *m*/*z* 349 $[MH]^+$.

5′-*N*-(2-Chloroethyl)carboxamidoadenosine (1i): yield 90 mg (72%); R_f 0.22; ^{1}H NMR (DMSO-d_6) δ 8.46 (s, 1H, H-8), 8.18 (s, 1H, H-2), 6.09 (d, 1H, $J_{1',2'}$ = 7.4 Hz, H-1′), 4.74 (dd, 1H, $J_{2',1'}$ = 7.4 Hz, $J_{2',3'}$ = 4.7 Hz, H-2′), 4.51 (d, 1H, $J_{4',3'}$ = 1.7 Hz, H-4′), 4.36 (dd, 1H, $J_{3',4'}$ = 1.7 Hz, $J_{3',2'}$ = 4.7 Hz, H-3′), 3.70 (m, 4H, 2 × CH_2, chloroethyl); ^{13}C NMR (DMSO-d_6) δ 44.7, 43.0 (2 × CH_2, chloroethyl); mp softening at 120 °C, decomposition at 195 °C; MS *m*/*z* 306 $[M - Cl]^+$. Anal. ($C_{12}H_{15}N_6O_4Cl\cdot0.7H_2O\cdot0.3CF_3COOH$) C, H, N.

5′-*N*-(2,2,2-Trifluoroethyl)carboxamidoadenosine (1j): yield 130 mg (45%); R_f 0.15; ^{1}H NMR (DMSO-d_6) δ 10.0 (t, 1H, N*H*), 8.32 (s, 1H, H-8), 8.22 (s, 1H, H-2), 7.47 (s, 2H, NH_2), 5.97 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.87, 5.60 (2 × s(b), 2 × 1H, 2′-O*H*, 3′-O*H*), 4.53 (dd, 1H, $J_{2',1'}$ = 8.0 Hz, $J_{2',3'}$ = 5.0 Hz, H-2′), 4.42 (s, 1H, H-4′), 4.12 (m, 3H, H-3′ + CH_2CF_3); ^{13}C NMR (DMSO-d_6) δ 124.5 (q, $J_{C,F}$ = 280 Hz, CF_3); mp 230 °C; MS *m*/*z* 363 $[MH]^+$. Anal. ($C_{12}H_{13}N_6O_4F_3\cdot1.6H_2O$) C, H, N.

5′-*N*-Methoxylcarboxamidoadenosine (1k): yield 64 mg (43%); R_f 0.05; ^{1}H NMR (DMSO-d_6) δ 8.40 (s, 1H, H-8), 8.17 (s, 1H, H-2), 7.48 (s, 1H, NH), 5.96 (d, 1H, $J_{1',2'}$ = 7.0 Hz, H-1′), 5.75, 5.60 (2 × s(b), 2 × 1H, 2′-O*H*, 3′-O*H*), 4.56 (1H, H-2′), 4.28 (d, 1H, $J_{4',3'}$ = 2.0 Hz, H-4′), 4.21 (d, 1H, $J_{3',4'}$ = 2.0 Hz, H-3′), 3.65 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 64.7 (OCH_3); mp 125 °C (lit. 113 °C, decomp.[20]); MS *m*/*z* 311 $[MH]^+$.

5′-*N*-Ethyl-2′,3′-*O*-isopropylidenethiocarboxamidoadenosine (7b). Compound **6b** (230 mg, 0.66 mmol) and 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (Lawesson's reagent, 50 mg) were dissolved in dioxane (2 mL) and stirred at 80 °C for 1 h. TLC analysis indicated the reaction to be complete (R_f product 0.65). The reaction mixture was diluted with water (10 mL) and extracted with dichloromethane (4 × 20 mL). The combined organic layers were washed with water (2 × 20 mL), dried on $MgSO_4$, and concentrated. The remaining oil was purified by silica gel column chromatography (eluent: dichloromethane/methanol 100/0 to 9/1) to yield 170 mg (71%) of **7b**: ^{1}H NMR ($CDCl_3$) δ 9.33 (s(b), 1H, N*H*), 8.27 (s, 1H, H-8), 8.02 (s, 1H, H-2), 6.80 (s, 2H, NH_2), 6.16 (d, 1H, *J* = 3 Hz, H-1′), 5.30 (m, 2H, H-2′, H-3′), 5.10 (s, 1H, H-4′), 3.63 (m, 2H, CH_2CH_3), 1.67, 1.41 (2 × s, 2 × 3H, 2 × CH_3, isopropylidene), 1.09 (t, 3H, CH_2CH_3); ^{13}C NMR ($CDCl_3$): δ 196.0 (C-5′(C=S)), 39.5 (CH_2CH_3), 12.3 (CH_2CH_3).

5′-*N*-Cyclopropyl-2′,3′-*O*-isopropylidenethiocarboxamidoadenosine (7c). Compound **7c** was synthesized as described for **7b**, starting with compound **6c** (90 mg, 0.25 mmol): yield 65 mg (69%); R_f 0.60; ^{1}H NMR ($CDCl_3$) δ 9.37 (s(b), 1H, N*H*), 8.31 (s, 1H, H-8), 7.87 (s, 1H, H-2), 6.16 (s, 2H, NH_2), 6.01 (d, 1H, *J* = 2.8 Hz, H-1′), 5.20 (m, 2H, H-2′, H-3′), 5.12 (s, 1H, H-4′), 3.26 (m, 2H, CH_2CH_3), 1.66, 1.38 (2 × s, 2 × 3H, 2 × CH_3, isopropylidene), 1.25, 0.51 (2 × m, 2 × CH_2, cyclopropyl); ^{13}C NMR ($CDCl_3$) δ 197.8 (C-5′(C=S)), 26.7 (*C*H, cyclopropyl), 6.2, 5.0 (2 × CH_2).

5′-*N*-Ethylthiocarboxamidoadenosine (8b): yield 136 mg (90%); R_f 0.25; ^{1}H NMR (DMSO-d_6) δ 11.05 (s, 1H, NH), 8.31 (s, 1H, H-8), 8.23 (s, 1H, H-2), 7.51 (s, 2H, NH_2), 5.98 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1′), 5.88, 5.52 (2 × s(b), 2 × 1H, 2′-O*H*, 3′-O*H*), 4.73 (s, 1H, H-4′), 4.55 (t, 1H, H-2′), 4.23 (d, 1H, $J_{2'3'}$ = 4.5 Hz, H-3′), 3.73 (m, 2H, CH_2CH_3), 1.19 (t, 3H, CH_2CH_3); ^{13}C NMR (DMSO-d_6) δ 196.7 (C-5′(C=S)), 156.2 (C-6), 152.8 (C-2), 148.4 (C-4), 141.0 (C-8), 119.8 (C-5), 90.8 (C-1′), 88.4 (C-4′), 75.3, 70.6 (C-3′, C-2′), 12.8 (CH_2CH_3); mp 130 °C; MS *m*/*z* 325 $[MH]^+$, 347 $[MNa]^+$. Anal. ($C_{12}H_{16}N_6O_3S\cdot2.4H_2O$) C, H, N, S.

5′-*N*-Cyclopropylthiocarboxamidoadenosine (8c): yield 45 mg (77%); R_f 0.20; ^{1}H NMR (DMSO-d_6) δ 10.66 (d, 1H, $J_{CH,NH}$ = 4.9 Hz, N*H*), 8.32 (s, 1H, H-8), 8.16 (s, 1H, H-2), 7.48 (s, 2H, NH_2), 5.95 (d, 1H, $J_{1',2'}$ = 9.0 Hz, H-1′), 5.86, 5.51 (2 × d, 2 × 1H, 2′-O*H*, 3′-O*H*), 4.72 (d, 1H, $J_{4',3'}$ = 0.6 Hz, H-4′), 4.55 (m, 1H, H-2′), 4.23 (t, 1H, $J_{3',2'} = J_{3',OH}$ = 4.2 Hz, H-3′), 3.30 (m, H, C*H*, cyclopropyl), 0.92, 0.76, 0.65 (3 × m, 2H + 2 × 1H, 2 × CH_2, cyclopropyl); ^{13}C NMR (DMSO-d_6) δ 197.8 (C-5′(C=S)), 90.6 (C-1′), 88.3 (C-4′), 75.3, 70.7 (C-3′, C-2′), 28.1 (*C*H, cyclopropyl), 6.4, 6.1 (2 × CH_2, cyclopropyl); mp 120 °C; MS *m*/*z* 337 $[MH]^+$. Anal. ($C_{13}H_{16}N_6O_3S\cdot1.7H_2O$) C, H, N.

Radioligand Binding Studies. Adenosine A_1 receptor affinities were determined on rat cortical membranes with [^{3}H]-DPCPX as radioligand according to a protocol published previously.[37] Measurements with [^{3}H]DPCPX were performed in the presence and absence of 1 mM GTP. Adenosine A_{2A} receptor affinities were determined on rat striatal membranes with [^{3}H]CGS21680 as radioligand according to Jarvis et al.[38] Adenosine A_3 receptor affinities were determined on membranes prepared from HEK 293 cells stably transfected with human A_3 receptor cDNA, with [^{125}I]-ABMECA as radioligand.[11,13,39]

Cyclic AMP Generation and Determination. CHO-K1 cells stably transfected with human adenosine A_{2B} receptor cDNA originated from the Garvan Institute, Sydney, Australia. Cyclic AMP generation and determination were performed according to a protocol published previously.[19]

Crystal Structure Determination and Refinement of 8b. $C_{12}H_{16}N_6O_3S\cdot2H_2O$, Mr = 360.39, colorless, plate-shaped crystal (0.05 × 0.4 × 0.6 mm^3), monoclinic, space group *C*2 (no. 5) with *a* = 16.411(7) Å, *b* = 7.814(7) Å, *c* = 13.647(7) Å, β = 111.56(6)°, *V* = 1627.6(19) Å^3, *Z* = 4, D_c = 1.471 g cm^{-3}, 7973 reflections measured, 3730 independent, (1.6° < θ < 27.5°, ω scan, *T* = 150 K, Mo Kα radiation, graphite monochromator, λ = 0.71073 Å) on Enraf-Nonius CAD4 Turbo diffractometer on rotating anode. The structure was solved by automated direct methods (SHELXS97[40]). Refinement on F^2 was carried out by full-matrix least-squares techniques (SHELXL-97-2[41]) for 277 parameters; no observance criterion was applied during refinement. The absolute structure was assigned in accordance with the known configuration of the adenosine moiety. Refinement converged at a final w*R*2 value of 0.0866, *R*1 = 0.0416 (for 3730 reflections with $I > 2\sigma(I)$), *S* = 1.055. A final difference Fourier showed no residual density outside −0.29 and 0.29 e Å^{-3}.

Computational Methods. Studies were performed on a Silicon Graphics O_2 workstation. Manipulation of structures, minimum energy conformations, and calculations were performed with the molecular modeling package Spartan 5.0 (Wavefunction Inc., Irvine, California). Crystallographic data for adenosine (REFCODE=ADENOS01)[42] and NECA (REFCODE=KEMYEG)[29] were imported in Spartan from the CSD[43] while the crystal structure of **8b** was imported from our own X-ray data files. For the anti conformation of NECA and **8b**, adenosine's crystal structure was used as a model after substitution on screen of the respective 5′ side chain (the same method was applied, using the crystal structure of NECA, for deriving the syn conformation of adenosine). Geometry optimization with the Merck molecular mechanics force field and single-point energy calculations with ab initio Hartree−Fock (6-31G* basis set) were performed on these structures to derive the corresponding energies of heat of formation.

Abbreviations

cAMP, cyclic adenosine-5′-monophosphate; CGS21680, 2-[4-(2-carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine; CHO cells, Chinese hamster ovary cells; DMF, dimethyl formamide; DMSO, dimethyl sulfoxide; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; ESI, electrospray ionization; HEK cells, human embryonic kidney cells; HF, Hartree−Fock; IABMECA, N^6-(4-amino-3-iodo-benzyl)-5′-*N*-methylcarboxamidoadenosine; MECA, 5′-*N*-methylcarboxamidoadenosine; MS, mass spectrum; NCPCA, 5′-*N*-cyclopropylcarboxamidoadenosine; NECA, 5′-*N*-ethylcarboxamidoadenosine; NMR, nuclear magnetic resonance; ppm, parts per million; SAR, structure−activity relationship; TLC, thin-layer chromatography.

Acknowledgment. CHO-K1 cells expressing A_{2B} were kindly provided by Dr. Andrea Townsend-Nichol-

son, University College London, London, U.K. Crystallographic studies were supported in part by The Netherlands Foundation of Chemical Research (SON) with financial aid from The Netherlands Organization for Scientific Research (NWO).

Supporting Information Available: The structure determination of compound **8b**, including tables of atomic coordinates, bond lengths and angles, and thermal parameters; details of the elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM9804984