5′-*O*-Alkyl Ethers of *N*,2-Substituted Adenosine Derivatives: Partial Agonists for the Adenosine A_1 and A_3 Receptors

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New *N*,5′-di- and *N*,2,5′-trisubstituted adenosine derivatives were synthesized in good overall yields. Appropriate 5-*O*-alkyl-substituted ribose moieties were coupled to 6-chloropurine or 2,6-dichloropurine via Vorbrüggen's glycosylation method. Subsequent amination and deprotection of the intermediates yielded compounds **18**−**35**. Binding affinities were determined for rat adenosine A_1 and A_{2A} receptors and the human A_3 receptor. The ability of compounds **18**−**35** to inhibit forskolin-induced (10 μM) cyclic AMP (cAMP) production and their ability to stimulate guanosine 5′-*O*-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) binding, via either the adenosine A_1 receptor or the adenosine A_3 receptor, were assessed. *N*-Cyclopentyl-substituted adenosine derivatives displayed affinities in the low nanomolar range for the adenosine A_1 receptor, whereas *N*-(3-iodobenzyl)-substituted derivatives had high affinity for the adenosine A_3 receptor. Compound **22** had the highest affinity for the adenosine A_1 receptor (K_i value of 16 nM), and compounds **20** and **26** had the highest affinities for the adenosine A_3 receptor (K_i values of 4 and 3 nM, respectively). A chlorine substituent at the 2-position either did not affect or slightly increased the adenosine A_1 receptor affinity, whereas the A_3 receptor affinity was affected differently, depending on the *N*-substituent. Furthermore, the introduction of chlorine slightly increased the A_3/A_1 selectivity ratio. At the 5′-position, an *O*-methyl substituent induced the highest adenosine A_1 receptor affinity, whereas an *O*-ethyl substituent did so for the A_3 receptor. All compounds showed partial agonistic effects in both the cAMP and [^{35}S]GTPγS assays, although more marked in the latter assay. In general, the 2-chloro derivatives seemed to have lower intrinsic activities compared to the 2-H-substituted compounds on both the adenosine A_1 and the adenosine A_3 receptors. The compounds with an *N*-(3-iodobenzyl) substituent displayed the lowest intrinsic activities. Finally, all compounds also showed partially antagonistic behavior in the [^{35}S]GTPγS assay.

Introduction

Endogenous adenosine exerts its action via specific membrane-bound receptors called P_1-purinoceptors. There are four adenosine receptor subtypes, the adenosine A_1 and A_3 receptors, which inhibit the enzyme adenylate cyclase after activation, and the A_{2A} and A_{2B} receptors, which stimulate adenylate cyclase. The adenosine receptors are widely distributed in the body, and selective ligands for the receptors are required. The potential therapeutic use of adenosine A_1 agonists, for example, in the treatment of type II diabetes[1,2] might be accompanied by a certain extent of cardiotoxicity. Useful cardio- and cerebroprotective actions of chronically administered adenosine A_3 receptor agonists[3,4] often go with unwanted stimulation of the (rat) A_3 receptor in the lungs and immune system, causing bronchoconstriction and the release of allergic mediators.[3] One way to circumvent these side effects is the use of partial agonists,[5] which may exploit the differences in receptor−effector coupling in various tissues and can achieve selectivity of action in vivo.

Partial agonists are compounds that can cause a submaximal stimulation of the receptor at saturating concentrations and block the effect of full agonists to some extent (same binding site at the receptor).[6,7] With the term "intrinsic activity", the ability of a partial agonist to elicit a stimulus from the activation of a single receptor is reflected. In this simplified model,[8] a ligand induces a conformational change in the receptor, thereby conferring affinity to the receptor for the next signaling protein in the transduction cascade (e.g., a G protein). The intensity of the stimulus may be related to the degree of change in conformation, the frequency of change in conformation, and the duration of the induced conformation. In a more complex model,[9] it has been suggested that the active conformation of the receptor (R*) is already one of the variety of conformations that the receptor can form spontaneously, in the absence of an agonist (for a review on this matter see De Ligt et al.[10]). Hence, by selective affinity the agonist (or inverse agonist) either increases or decreases the population of these conformations (R*), respectively, to produce a functional response. This way, a single ligand may have multiple intrinsic efficacies.[9]

Partial agonists selective for the adenosine A_1 receptor that fully inhibit lypolysis in vivo while being devoid of cardiovascular effects have been successfully synthe-

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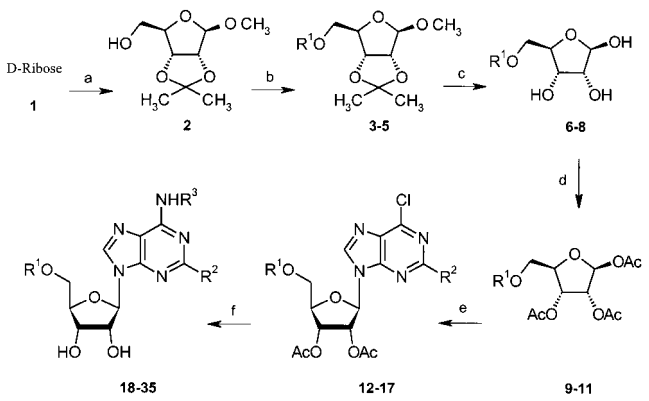
Scheme 1[a]



[a] Reaction conditions: (a) acetone, MeOH, 2,2-dimethoxypropane, HCl(g), (b) NaH, DMF, alkyl halide, $R^1 \simeq CH_3$, C_2H_5, or c-C_3H_5; (c) (i) 0.04 M HCl or (ii) $BaCO_3$; (d) pyridine, DMAP, acetic anhydride; (e) 1,2-dichloroethane, silylated base, TMSOTf, R^2 = H, Cl; (f) (i) EtOH/NH_3 or (ii) absolute EtOH, appropriate amine, R^3 = H, c-C_5H_9, or $CH_2(C_6H_4)$-*m*-I.

sized in our laboratory,[11,12] as have partial agonists for the adenosine A_3 receptor.[13] In these studies, selectivity for the adenosine A_1 or A_3 receptor was obtained by the introduction of either a cyclopentyl or a 3-iodobenzyl substituent at the *N*-position, respectively. Furthermore, alkylthio substituents at the 5′-position induced partial agonism on both the adenosine A_1 and A_3 receptors.[13,14]

In the present study, an *N*-cyclopentyl or an *N*-(3-iodobenzyl) group was selected to introduce either adenosine A_1 or adensosine A_3 receptor selectivity, respectively. Attention has now been focused on the synthetically less accessible 5′-*O*-alkyl ether derivatives to induce partial agonism. The synthesis of some 5′-*O*-methyladenosine derivatives has been published before, although in combination with other *N*-substituents.[15] The effect of the 5′-*O*-alkyl substituents on the intrinsic activity was studied here, in comparison with the 5′-alkyl thioether adenosine derivatives that have shown reduced intrinsic activities,[13,14] as mentioned earlier. When preparing 5′-*O*-alkyl ether adenosine derivatives, one must be careful to prevent alkylation at the other ribose hydroxyl groups,[16] as well as at the nitrogen atoms present in the heterocyclic base. A procedure to prevent these problems may be the glycosylation of appropriate protected 5-*O*-alkyl-substituted riboses to a heterocyclic base, as described by Vorbrüggen et al.[17,18] However, yields have often been reported to be quite low. Finally, in this study, the additional effect of chlorine introduced at the 2-position of the adenosine derivatives on the affinity and intrinsic activity was studied.

The compounds were tested in radioligand binding assays for affinity. Their intrinsic activities were determined in both [^{35}S]GTPγ[S] (guanosine 5′-*O*-(3-[^{35}S]-thio)triphosphate) binding assays[7,19] and cyclic AMP (cAMP) assays.

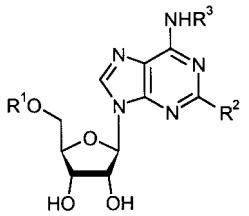
Results and Discussion

Chemistry. The synthesis of the substituted adenosine derivatives **18**−**35** is depicted in Scheme 1. Selective alkylation at the 5′-position of adenosine, or one of its derivatives, is difficult since one must be very careful to prevent alkylation at the more reactive nitrogen atoms present in the base moiety (except for the *N* nitrogen, which is less reactive). Treatment of either 2′,3′-*O*-isopropylideneadenosine or 2′,3′-*O*-isopropylideneinosine with KOH, 18-crown-6, and iodomethane[20,21] yielded the N1-methylated derivative or a mixture of the N1-methylated product together with the N1,5′-dimethylated one, respectively. Refluxing a 5′-chloro-5′-deoxyadenosine derivative in methanolate provided only starting material. Therefore, we chose to alkylate the ribose first, then couple it to the appropriate heterocyclic base, and subsequently perform other reactions.

Protection of the 1-, 2-, and 3-hydroxyl groups of D-ribose (**1**) was performed according to a method described by Leonard et al.[22] with a minor modification. D-Ribose was dissolved in a mixture of acetone, MeOH, and 2,2-dimethoxypropane, and HCl(g) was bubbled directly through the solution, instead of adding MeOH saturated with HCl(g). The protected β-sugar **2** was obtained in good yield (95%). Alkylation of the free hydroxyl group of **2** was performed under standard conditions, i.e., treatment with NaH in DMF with cooling (not in the case of cyclopropyl bromide), followed by the addition of the alkyl halide iodomethane, iodoethane, or cyclopropyl bromide, respectively. Compounds **3**−**5** were obtained in 38−97% yield. Subsequently, the protecting groups of the alkylated compounds **3**−**5** were removed by refluxing the material in aqueous HCl (0.04 M) for 2 h and subsequent neutralization with $BaCO_3$, and compounds **6**−**8** were obtained in good yields.[23] Our first efforts to remove the protecting groups with aqueous sulfuric acid (0.02 M) and EtOH failed. This procedure yielded, besides the desired product, also a significant amount of 1-*O*-ethyl-substituted derivatives.

The hydroxyl groups of **6**−**8** were then protected. In general, benzoyl protecting groups at the 2-, 3-, and 5-positions of the sugar moiety have been used to improve the formation of β-nucleosides over α-nucleosides in the glycosylation reaction, i.e., when the protected sugar is coupled to a heterocyclic base.[17] Treatment of methyl 5-*O*-methyl-D-ribofuranose (**6**) with benzoyl chloride in CH_2Cl_2 and pyridine yielded the desired methyl 2,3-di-*O*-benzoyl-5-*O*-methyl-D-ribofuranoside, although it was difficult to remove the excess benzoyl chloride.[24] The conversion of the 1-*O*-methyl group into a 1-*O*-acetyl group succeeded in reasonable yield by treatment of methyl 2,3-di-*O*-benzoyl-5-*O*-methyl-D-ribofuranoside with acetic acid, acetic anhydride, and sulfuric acid.[24] Since this method is quite laborious, the possibility of using a fully acetyl protected sugar in the Vorbrüggen coupling reaction was explored as well. The hydroxyl groups of **6**−**8** were protected with acetyl groups to obtain compounds **9**−**11**. Then, the peracylated compounds **9**−**11** were coupled to the appropriate heterocyclic base, e.g., 6-chloropurine or 2,6-dichloropurine, according to the Vorbrüggen method[18] to give compounds **12**−**17** in good yields (48−84%). These good yields indicated that peracylated riboses were a good alternative compared to the benzoyl-protected riboses. In fact, recently Vorbrüggen et al. have described that coupling can also be performed with nonprotected riboses, as they can be persilylated prior to reaction with a heterocyclic base.[25]

Table 1. Affinities of *N*,2,5′-trisubstituted Adenosine Analogues at Adenosine A_1, A_{2A}, and A_3 Receptors Expressed as K_i Values (nM ± SEM, $n = 3$) or Percentage of Displacement at 10 μM



				K_i (nM) or % displacement at 10^{-5} M			
compd	R^1	R^2	R^3	A_1[a]	A_{2A}[b]	A_3[c]	A_1:A_3
CPA	H	H	c-C_5H_9	7.14 ± 2.30	45.9%	281 ± 56	0.02
2-Cl-CPA	H	Cl	c-C_5H_9	9.47 ± 0.86	53.1%	204 ± 44	0.05
IBMECA		H	$CH_2(C_6H_4)$-*m*-I	1400 ± 240	39.4%	6.9 ± 0.2	208
2-Cl-IBMECA		Cl	$CH_2(C_6H_4)$-*m*-I	710 ± 41	23.5%	7.2 ± 0.9	98
18	CH_3	H	H	164 ± 37	261 ± 65	40.3 ± 8.1	4.1
19	CH_3	H	c-C_5H_9	33.7 ± 6.9	366 ± 109	79.0 ± 30.0	0.4
20	CH_3	H	$CH_2(C_6H_4)$-*m*-I	179 ± 40	373 ± 80	4.2 ± 0.5	42.6
21	CH_3	Cl	H	169 ± 30	284 ± 55	28.3 ± 13.0	6.0
22	CH_3	Cl	c-C_5H_9	16.3 ± 1.3	1390 ± 140	44.9 ± 4.9	0.4
23	CH_3	Cl	$CH_2(C_6H_4)$-*m*-I	112 ± 10	527 ± 21	7.3 ± 0.2	15.3
24	C_2H_5	H	H	2300 ± 320	415 ± 69	23.3 ± 3.9	98.7
25	C_2H_5	H	c-C_5H_9	27.8 ± 2.2	33.9%	32.3 ± 9.2	0.9
26	C_2H_5	H	$CH_2(C_6H_4)$-*m*-I	198 ± 36	40.4%	3.3 ± 1.0	60.0
27	C_2H_5	Cl	H	327 ± 15	659 ± 168	15.1 ± 6.9	21.7
28	C_2H_5	Cl	c-C_5H_9	27.1 ± 1.8	1730 ± 700	67.0 ± 44.6	0.4
29	C_2H_5	Cl	$CH_2(C_6H_4)$-*m*-I	254 ± 28	848 ± 60	7.4 ± 1.0	34.3
30	c-C_3H_5	H	H	575 ± 52	739 ± 16	45.6 ± 7.4	12.6
31	c-C_3H_5	H	c-C_5H_9	40.5 ± 6.4	24.3%	104 ± 20	0.4
32	c-C_3H_5	H	$CH_2(C_6H_4)$-*m*-I	733 ± 36	43.7%	9.1 ± 1.9	80.5
33	c-C_3H_5	Cl	H	595 ± 84	885 ± 89	26.7 ± 4.3	22.3
34	c-C_3H_5	Cl	c-C_5H_9	80.8 ± 12.0	34.6%	86.0 ± 15.5	0.9
35	c-C_3H_5	Cl	$CH_2(C_6H_4)$-*m*-I	1430 ± 200	38.7%	20.5 ± 8.1	69.8

[a] Displacement of [^{3}H]DPCPX from rat cortical membranes.[32] [b] Displacement of [^{3}H]ZM 241385 from rat striatal membranes.[33] [c] Displacement of [^{125}I]ABMECA from the human A_3 receptor expressed in HEK 293 cells.[35]

From NMR spectroscopy it was concluded that only β-congeners were formed here. The Lewis acid used in the Vorbrüggen method, trimethylsilyl trifluoromethanesulfonate (TMS triflate), belongs to a relatively new family of acids. It was compared in this study with the more traditional Lewis acid stannic chloride ($SnCl_4$), described as high-yielding.[26] Both Lewis acids led to similar yields of the coupled product as a result of the glycosylation of 6-chloropurine and commercially available 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribosfuranose. Amination of compounds **12**−**17** with EtOH/NH_3, cyclopentylamine, or (3-iodobenzyl)amine gave the unprotected substituted adenosine derivatives **18**−**35**.[13,14]

Biological Evaluation. All compounds were tested in radioligand binding assays to determine their affinities for the adenosine A_1 receptor in rat brain cortex, the A_{2A} receptor in rat striatum, and the human A_3 receptor as expressed in HEK 293 cells (Table 1). For the adenosine A_1 receptor, the tritiated antagonist [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX) and, for the adenosine A_{2A} receptor, the tritiated antagonist [^{3}H]ZM 241385(7-amino-2-(2-furyl)-5-[2-(4-hydroxyphenyl)-ethyl]amino[1,2,4]triazolo[1,5-*a*][1,3,5]triazine) were used. Since radiolabeled antagonists are not commercially available for the adenosine A_3 receptor, [^{125}I]ABMECA (*N*-(4-aminobenzyl)-5′-methylcarboxamidoadenosine), an A_3 receptor agonist, was used. Displacement experiments were performed in the absence of GTP (guanosine 5′-triphosphate).

Table 1 displays radioligand binding data for all synthesized di- and trisubstituted final products. Most compounds had lower affinity for the adenosine A_{2A} receptor than for the adenosine A_1 or A_3 receptor. Compounds with an unsubstituted *N* amino group (**18**, **21**, **24**, **27**, **30**, **33**) showed adenosine A_3 receptor selectivity compared to A_1, with A_1:A_3 selectivity ratios ranging from 4.1 for compound **18** to 98.7 for compound **24**. A cyclopentyl group at the *N*-position induced high adenosine A_1 receptor affinities and also the highest selectivity for the A_1 receptor, whereas the *N*-(3-iodobenzyl)adenosine derivatives had the highest adenosine A_3 receptor affinities and were highly selective for this receptor, in line with earlier reports on these *N*-substituents.[13,14,27] For CCPA (2-chloro-*N*-cyclopentyladenosine) it is known that its affinity for the rat adenosine A_1 receptor is about 1.5-fold lower than its affinity for the human A_1 receptor.[28,29] This suggests that the affinity of our compounds for the human A_1 receptor might also be somewhat higher, and therefore the selectivity for the human A_3 receptor might be somewhat less than stated here.

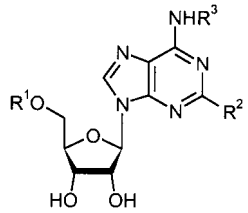
In general, the adenosine A_1 receptor affinities were unchanged or increased (up to 2-fold) when a chlorine was introduced at the 2-position, with a concomitant slight increase in adenosine A_1 receptor selectivity (up to 4-fold). The introduction of chlorine at the 2-position had more variable effects on the affinity and selectivity for the adenosine A_3 receptor. The adenosine A_3 receptor affinities of the *N*-unsubstituted derivatives were slightly increased, whereas for the *N*-(3-iodobenzyl)-substituted compounds they were decreased somewhat. The selectivity for the adenosine A_3 receptor was decreased up

to approximately 4-fold when chlorine was introduced at C2. The effect of chlorine at the 2-position on receptor affinity is difficult to predict in the case of substituted adenosines. It has been shown that for both CPA (*N*-cyclopentyladenosine) and *N*-(3-iodobenzyl)adenosine the affinity and selectivity for the adenosine A_1 receptor are increased when compared to those for the A_{2A} receptor.[30] However, when chlorine was introduced at the 2-position of 5′-*N*-methylcarboxamido-substituted *N*-(3-iodobenzyl)adenosine,[31] the affinity for both the adenosine A_1 and A_{2A} receptors was decreased, while the A_3 receptor affinity increased. On the contrary, in the present study introduction of chlorine at the 2-position of 5′-*O*-alkyl-substituted derivatives slightly decreased the adenosine A_3 receptor selectivity of the compounds compared to that of the A_1 receptor.

The 5′-substituents also influenced the affinity and selectivity of the compounds. For the adenosine A_1 receptor the compounds with a 5′-*O*-methyl group had the highest receptor affinities, whereas the 5′-*O*-cyclopropyl-substituted derivatives displayed the lowest affinities in most cases. Only within the *N*,2-unsubstituted series (**18**, **24**, **30**) 5′-*O*-ethyladenosine had the lowest affinity for the adenosine A_1 receptor. In the case of the adenosine A_3 receptor, the derivatives with a 5′-*O*-ethyl substituent had the highest receptor affinities. The adenosine A_3 receptor affinities of the 5′-*O*-methyl-substituted derivatives were equal or somewhat higher than those of the 5′-*O*-cyclopropyl-substituted ones. Although a 5′-*O*-cyclopropyl group is tolerated fairly well on both the adenosine A_1 and A_3 receptors (K_i values in the low nanomolar range), smaller groups seemed to be preferred, e.g., 5′-*O*-methyl for the adenosine A_1 receptor and 5′-*O*-ethyl for the A_3 receptor. The 5′-*O*-methyl group induced the highest adenosine A_1 receptor selectivity in most cases (within the series containing the same *N*- and 2-substituents), whereas the 5′-*O*-cyclopropyl induced most often the highest adenosine A_3 receptor selectivity compared to that of A_1. The compounds with a 5′-*O*-ethyl substituent had higher affinities than those with a 5′-*O*-methyl for the adenosine A_3 receptor, and had the highest selectivity for this receptor in most cases as well, compared to the A_{2A} receptor. MECA (5′-*N*-methylcarboxamidoadenosine) has been described to have higher affinity (and selectivity) than NECA (5′-*N*-ethylcarboxamidoadenosine) for the adenosine A_3 receptor compared to the A_{2A} receptor. The results in this present study, however, indicate that the adenosine A_3 receptor accommodates the rather large 5′-alkylthio substituents better than the smaller ones (5′-*S*-methyl-5′-thio substituent) and that the adenosine A_3 receptor is well capable of accommodating large 5′-substituents. Finally, compound **26** displayed a higher affinity for the adenosine A_3 receptor (K_i value of 3.3 nM) than both reference compounds IBMECA (*N*-(3-iodobenzyl)-5′-methylcarboxamidoadenosine) and 2-Cl-IBMECA (2-chloro-*N*-(3-iodobenzyl)-5′-methylcarboxamidoadenosine), although its selectivity for the adenosine A_3 receptor compared to the A_1 receptor was somewhat less.

All compounds were also tested in functional assays. First, the ability of the compounds **18**−**35** to inhibit the forskolin (10 μM) induced cAMP production via human adenosine A_1 receptors expressed in CHO cells or by human adenosine A_3 receptors expressed in HEK 293 cells was assessed (Table 2). Note that both the adenosine A_1 and A_3 receptors are coupled to G_i and inhibit the production of cAMP. Second, the modulation of [^{35}S]-GTPγS binding to cell membranes was determined when compounds **18**−**35** were bound to the receptors. Membrane preparations from rat brain cortex (A_1 receptors) and from CHO cells stably transfected with human adenosine A_3 receptors were used (no significant stimulation occurred with membranes prepared from the HEK 293 cells used in the binding studies).

Table 2. Inhibition of Forskolin (10μM) Induced cAMP Production of *N*,5-Di- and *N*,2,5′-Trisubtstituted Adenosine Derivatives at Either the Human Adenosine A_1 or the Human A_3 Receptor



compd	R^1	R^2	R^3	% inhib A_1[a]	% inhib A_3[b]
CPA				69 ± 3	
Cl-IBMECA					83 ± 1
18	CH_3	H	H	75 ± 3	76 ± 2
19	CH_3	H	c-C_5H_9	74 ± 4	58 ± 6
20	CH_3	H	$CH_2(C_6H_4)$-*m*-I	53 ± 10	33 ± 9
21	CH_3	Cl	H	78 ± 4	69 ± 3
22	CH_3	Cl	c-C_5H_9	73 ± 4	42 ± 6
23	CH_3	Cl	$CH_2(C_6H_4)$-*m*-I	37 ± 15	17 ± 7
24	C_2H_5	H	H	57 ± 11	65 ± 7
25	C_2H_5	H	c-C_5H_9	69 ± 6	37 ± 6
26	C_2H_5	H	$CH_2(C_6H_4)$-*m*-I	6 ± 21	11 ± 6
27	C_2H_5	Cl	H	67 ± 7	51 ± 5
28	C_2H_5	Cl	c-C_5H_9	62 ± 6	31 ± 9
29	C_2H_5	Cl	$CH_2(C_6H_4)$-*m*-I	−72 ± 38[c]	7 ± 4
30	c-C_3H_5	H	H	61 ± 2	59 ± 6
31	c-C_3H_5	H	c-C_5H_9	62 ± 4	26 ± 7
32	c-C_3H_5	H	$CH_2(C_6H_4)$-*m*-I	−104 ± 22[c]	10 ± 12
33	c-C_3H_5	Cl	H	49 ± 9	44 ± 8
34	c-C_3H_5	Cl	c-C_5H_9	43 ± 9	19 ± 7
35	c-C_3H_5	Cl	$CH_2(C_6H_4)$-*m*-I	−118 ± 41[c]	−2.6 ± 4

[a] Percentage inhibition (±SEM, $n = 3$). Compared to the reference full agonist CPA (10 μM) in A_1 CHO cells. All compounds were tested at ±100K_i. [b] Percentage inhibition (±SEM, $n = 3$). Compared to the reference full agonist Cl-IBMECA (10 μM). All compounds were tested at ±100K_i. [c] Seemed to behave as inverse (partial) agonists in this assay (in the [^{35}S]GTPγS assay they behaved as "normal" (partial) agonists, Table 3).

Compounds within the three *N*-substituted series showed similar trends in inhibition of the forskolin-induced cAMP production via the adenosine A_1 receptor (Table 2). Compounds with an intact amino group at the 6-position and the *N*-cyclopentyl-substituted derivatives were able to inhibit the cAMP production to a similar extent, almost as well as the reference full agonist CPA, whereas the *N*-(3-iodobenzyl)-substituted derivatives inhibited the forskolin-induced cAMP production somewhat less. Compound **20** had a lower intrinsic activity than compounds **18** and **19**, while only the *N*-substituent is different, implicating that a 3-iodobenzyl group at *N* can induce partial agonism for the adenosine A_1 receptor. Furthermore, within the three *N*-substituted series the 5′-*O*-methyl-substituted derivatives had the highest intrinsic activities compared to the 5′-*O*-ethyl- and 5′-*O*-cyclopropyl-substituted ones. The latter compounds displayed the lowest intrinsic

Table 3. Agonistic Activity of *N*,5′-Di- and *N*,2,5′-Trisubstituted Adenosine Analogues Expressed as the Amount of [^{35}S]GTPγS Bound

				adenosine A_1 receptor		adenosine A_3 receptor	
compd	R^1	R^2	R^3	[^{35}S]GTPγS bound (% CPA)[a]	[^{35}S]GTPγS bound + 1 μM CPA (% CPA)[b]	[^{35}S]GTPγS bound (% NECA)[c]	[^{35}S]GTPγS bound + 1 μM NECA (% NECA)[d]
basal				0	0	0	0
CPA				100	100		
NECA						100	100
18	CH_3	H	H	73 ± 2	91 ± 2	79 ± 3	85 ± 2
19	CH_3	H	c- C_5H_9	68 ± 1	74 ± 1	63 ± 1	72 ± 1
20	CH_3	H	$CH_2(C_6H_4)$-*m*-I	20 ± 0	54 ± 1	27 ± 2	45 ± 3
21	CH_3	Cl	H	64 ± 2	83 ± 1	67 ± 4	69 ± 2
22	CH_3	Cl	c- C_5H_9	53 ± 1	72 ± 1	51 ± 5	74 ± 8
23	CH_3	Cl	$CH_2(C_6H_4)$-*m*-I	14 ± 1	75 ± 2	25 ± 7	43 ± 6
24	C_2H_5	H	H	14 ± 2	101 ± 2	54 ± 4	70 ± 3
25	C_2H_5	H	c-C_5H_9	33 ± 3	53 ± 1	38 ± 5	61 ± 5
26	C_2H_5	H	$CH_2(C_6H_4)$-*m*-I	1 ± 2	70 ± 11	10 ± 4	49 ± 8
27	C_2H_5	Cl	H	31 ± 0	62 ± 0	34 ± 6	51 ± 5
28	C_2H_5	Cl	c-C_5H_9	24 ± 1	47 ± 1	30 ± 6	47 ± 7
29	C_2H_5	Cl	$CH_2(C_6H_4)$-*m*-I	−3 ± 1	55 ± 7	8 ± 4	34 ± 10
30	c-C_3H_5	H	H	22 ± 2	70 ± 1	52 ± 6	70 ± 4
31	c-C_3H_5	H	c-C_5H_9	20 ± 1	53 ± 3	38 ± 7	54 ± 6
32	c-C_3H_5	H	$CH_2(C_6H_4)$-*m*-I	−7 ± 3	44 ± 12	9 ± 5	38 ± 6
33	c-C_3H_5	Cl	H	10 ± 1	55 ± 2	32 ± 5	46 ± 5
34	c−C_3H_5	Cl	c-C_5H_9	8 ± 2	39 ± 7	31 ± 6	57 ± 5
35	c-C_3H_5	Cl	$CH_2(C_6H_4)$-*m*-I	−4 ± 2	94 ± 3	15 ± 7	39 ± 7

[a] Agonistic activity of *N*,2,5′-trisubstituted adenosine analogues expressed as the amount of [^{35}S]GTPγS bound (as a percentage of the amount of [^{35}S]GTPγS bound by 1 μM CPA) via the rat adenosine A_1 receptor (±SEM, $n = 3$). [b] Antagonistic activity of *N*,2,5′-trisubstituted adenosine analogues expressed as the amount of [^{35}S]GTPγS bound in the presence of 1 μM CPA (as a percentage of the amount of [^{35}S]GTPγS bound by 1 μM CPA) via the rat adenosine A_1 receptor (±SEM, $n = 3$). [c] Agonistic activity of *N*,5′-di and *N*,2,5′-trisubstituted adenosine analogues expressed as the amount of [^{35}S]GTPγS bound (as a percentage of the amount of [^{35}S]GTPγS bound by 1 μM NECA) via the human adenosine A_3 receptor (±SEM, $n = 3$). [d] Antagonistic activity of *N*,2,5′-trisubstituted adenosine analogues expressed as the amount of [^{35}S]GTPγS bound in the presence of 1 μM NECA (as a percentage of the amount of [^{35}S]GTPγS bound by 1 μM NECA) via the human adenosine A_3 receptor. (±SEM, $n = 3$).

activities and behaved as partial agonists for the adenosine A_1 receptor in this assay. Here, the effect of the chlorine at the 2-position on the intrinsic activity is ambiguous.

Similar effects at the adenosine A_1 receptor were observed in a [^{35}S]GTPγS assay (Table 3), in the presence of these partial agonists, on the level of the G protein activity. The effect on the intrinsic activity was more pronounced than in the cAMP assay, with in general much lower intrinsic activities of the compounds for the adenosine A_1 receptor (Table 3). It must be noted that in the cAMP assay human adenosine A_1 receptors were used, whereas the studies with [^{35}S]GTPγS were performed with rat A_1 receptors. All compounds behaved as partial agonists for the adenosine A_1 receptor, with the *N*-(3-iodobenzyl)-substituted derivatives having the lowest intrinsic activities. Furthermore, the larger the 5′-substituent, the lower the intrinsic activity, and the introduction of a 2-chloro substituent seemed to reduce the intrinsic activity of the compounds for the adenosine A_1 receptor even further. As for the adenosine A_1 receptor, the intrinsic activities of the compounds for the adenosine A_3 receptor were quite similar in both functional assays (Tables 2 and 3). Here too, the intrinsic activities were lower in the [^{35}S]GTPγS binding assay (Table 3), and all compounds behaved as partial agonists for the adenosine A_3 receptor as well. Again, compounds with an unsubstituted 6-amino group had the highest intrinsic activities, whereas the *N*-(3-iodobenzyl)-substituted derivatives had the lowest. The large 5′-*O*-cyclopropyl substituent and the introduction of chlorine at the 2-position induced low intrinsic activities for the adenosine A_3 receptor, similar to those for the A_1 receptor. Thus, for the adenosine A_3 receptor, the large 5′-*O*-cyclopropyl substituent induced high affinity, similar to the 5′-*O*-methyl substituent, but reduced the intrinsic activity for the adenosine A_3 receptor more than the 5′-*O*-methyl substituent. The compounds (concentration at 100K_i) were also evaluated for their antagonistic effects, an important feature of partial agonists. This evaluation was done by testing the partial agonists in combination with a reference full agonist. All compounds showed partially antagonistic properties for either the adenosine A_1 receptor or the adenosine A_3 receptor in the [^{35}S]GTPγS binding assay in the presence of 1 μM CPA or 1 μM NECA, respectively, by reducing the amount of [^{35}S]GTPγS binding caused by CPA/NECA to a varying extent (Table 3). In almost all cases the amount of [^{35}S]GTPγS bound was less than with CPA/NECA alone, but always higher than that tested alone with the partial agonists. This indicates that partial agonists and full agonists have the same binding site at the receptor.

Conclusions

1,2,3-Tri-*O*-acetyl-5-*O*-alkyl-β-D-ribofuranoses were coupled to an appropriate heterocyclic base, e.g., 6-chloropurine or 2,6-dichloropurine, to yield the corresponding protected nucleosides. Subsequent amination at the 6-position and deprotection of these intermediates gave the novel 5′-*O*-alkylated nucleosides **18**−**35** in good overall yields. The compounds with an unsubstituted 6-amino group displayed higher adenosine A_3 receptor affinities than those for the A_1 receptor. The *N*-cyclopentyl-substituted derivatives had the highest adenosine A_1 receptor affinity (compound **22** with a K_i value of 16 nM), whereas the *N*-(3-iodobenzyl)-substituted derivatives had the highest affinity for the adenosine

A_3 receptor (compound **26** with a K_i value of 3 nM). The adenosine A_1 receptor affinity was either not affected or slightly increased with the introduction of chlorine at the 2-position, while the A_3 receptor affinity was affected by the 2-chlorine, depending on the *N*-substituent. In general, chlorine at the 2-position slightly decreased the A_1:A_3 selectivity ratio. Furthermore, a 5′-*O*-methyl substituent induced the highest adenosine A_1 receptor affinity, whereas a 5′-*O*-ethyl substituent did so for the A_3 receptor.

All compounds were partial agonists in both the cAMP and [^{35}S]GTPγS assays, although more pronounced in the latter. The *N*-(3-iodobenzyl)-substituted derivatives displayed the lowest intrinsic activities for both the adenosine A_1 and A_3 receptors. The introduction of 2-chlorine reduced the intrinsic activity for both receptor subtypes as well. Hence, although the *N*-(3-iodobenzyl) substituent induced the highest affinity for the adenosine A_3 receptor, it also reduced the intrinsic activity. Furthermore, contrary to NECA and MECA, the A_3 receptor appeared to accommodate the larger 5′-*O*-ethyl and even 5′-*O*-cyclopropyl better than the smaller 5′-*O*-methyl group. This is unlike data for the 5′-carboxamido derivatives, in which MECA is more active than NECA. Increasing the size of the 5′-substituent also reduced the intrinsic activity for both the adenosine A_1 and A_3 receptors. Thus, these newly synthesized partial agonists for both the adenosine A_1 and A_3 receptors may be useful tools in further research on adenosine receptors. Compounds **20** and **26** are particularly interesting in view of their higher affinity for the adenosine A_3 receptor than the reference compounds IBMECA and 2-Cl-IBMECA.

Experimental Section

Chemicals and Solvents. Guanosine was obtained from Aldrich (Aldrich Chemie, Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands). All other reagents were from standard commercial sources and of analytical grade. [^{3}H]-DPCPX, [^{3}H]ZM 241385, and [^{125}I]ABMECA were purchased from NEN (Hoofddorp, The Netherlands).

Chromatography. Thin-layer chromatography (TLC) was carried out using aluminum sheets (20 × 20 cm) with silica gel F_{254} from Merck. Spots were visualized under UV light (254 nm). Preparative column chromatography was performed on silica gel (230−400 mesh, ASTM).

Instruments and Analyses. Elemental analyses were performed for C, H, and N (Department of Analytical Chemistry, Leiden University, The Netherlands). ^{13}C NMR spectra were measured at 50.1 MHz with a JEOL JNM-FX 200 spectrometer equipped with a PG 200 computer operating in the Fourier transform mode. ^{1}H NMR spectra were measured at 200 MHz, using the above-mentioned 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 ^{1}H and ^{13}C NMR are given in parts per million (δ) relative to tetramethylsilane (TMS) as internal standard.

All high-resolution mass spectra were measured on a Finnigan MAT900 mass spectrometer equipped with a direct insertion probe for EI experiments (70 eV with resolution 1000) or on a Finnigan MAT TSQ-70 spectrometer equipped with an electrospray interface for ESI experiments. Spectra were collected by constant infusion of the analyte dissolved in 80:20 methanol/H_2O. ESI is a soft ionization technique resulting in protonated, sodiated species in positive ionization mode and deprotonated species in negative ionization mode.

Melting points (not corrected) were determined in a Büchi capillary melting point apparatus.

Synthesis of Methyl 2,3-*O*-*Isopropylidene*-β-D-ribofuranoside (2). A suspension of 88 g (0.59 mol) of dry D-ribose in acetone (400 mL), dimethoxypropane (176 mL), and MeOH (380 mL) was stirred and cooled in an ice bath. Over a period of 4 h, every 30 min HCl(g) was led through the solution, and then the mixture was stirred overnight at room temperature. The mixture was neutralized with pyridine and concentrated in vacuo. The residue was extracted with H_2O and ether. The organic layers were combined, dried ($MgSO_4$), and concentrated: yield 115 g (0.56 mol, 95%); R_f 0.38 (PE40/60−EtOAc, 1:1); ^{1}H NMR ($CDCl_3$) δ 4.97 (s, 1H, H-1), 4.82−4.57 (2 × d, 2H, J = 6.18 Hz, H-2,3), 4.42−4.39 (m, 1H, H-4), 3.71−3.62 (m, 2H, H-5), 3.41 (s, 3H, OCH_3), 1.48, 1.31 (2 × s, 6H, 2 × CCH_3) ppm; ^{13}C NMR ($CDCl_3$) δ 112.02 ($C(CH_3)_2$), 109.8 (C-1), 88.13, 85.64, 81.38 (C-2,3,4), 63.80 (C-5), 55.24 (OCH_3), 26.24, 24.61 (2 × CH_3) ppm.

General Procedure for the Alkylation of Compound 2 to the Methyl 5-*O*-Alkyl-2,3-*O*-isopropylidene-β-D-ribofuranoside Derivatives 3−5. Methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside (**2**; 53.0 g, 0.26 mol) was dissolved in dry dimethylformamide (DMF; 300 mL). This was cooled (0 °C), and NaH (60% in mineral oil, 11.5 g, 0.29 mol) was slowly added. The mixture was allowed to warm to room temperature and cooled again, and the appropriate alkyl halide (0.31 mol) was added very slowly. The mixture was stirred at room temperature overnight. The mixture was treated with MeOH (100 mL) and concentrated in vacuo. It was coevaporated with toluene (2×). The (black) mixture was extracted with water and EtOAc (250 mL each). The water layer was subsequently extracted with CH_2Cl_2. The organic layers were combined, dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography.

Methyl 5-*O*-Methyl-2,3-*O*-isopropylidene-β-D-ribofuranoside (3). The reaction was carried out with **2** (53.0 g, 0.26 mol) and iodomethane (CH_3I; 0.31 mol, 19.4 mL). The mixture was purified by column chromatography (eluent gradient PE40/60, PE40/60−EtOAc, 2:1): yield 55.1 g (0.25 mol, 97%); R_f 0.56 (PE40/60−EtOAc, 1:1); ^{13}C NMR ($CDCl_3$) δ 111.71 ($C(CH_3)_2$), 108.79 (C-1), 84.76, 84.47, 81.70 (C-2,3,4), 73.18 (C-5), 58.38 (OCH_3), 54.03 (CH_2OCH_3), 25.98, 24.49 (2 × $C(CH_3)_2$) ppm.

Methyl 5-*O*-Ethyl-2,3-*O*-isopropylidene-β-D-ribofuranoside (4). The reaction was carried out with **2** (33.4 g, 0.16 mol) and iodoethane (0.20 mol, 15.9 mL). The mixture was purified by column chromatography (eluent gradient PE40/60, PE40/60−EtOAc, 1:1): yield 35.2 g (0.15 mol, 92%); R_f 0.62 (PE40/60−EtOAc, 1:1); ^{13}C NMR ($CDCl_3$) δ 111.41 ($C(CH_3)_2$), 108.55 (C-1), 84.50, 81.55 (C-2,3,4), 70.78 (CH_2CH_3), 65.91 (C-5), 53.85 (OCH_3), 25.77, 24.31 ($C(CH_3)_2$), 14.48 (CH_2CH_3) ppm.

Methyl 5-*O*-Cyclopropyl-2,3-*O*-isopropylidene-β-D-ribofuranoside (5). The reaction was carried out with **2** (43.6 g, 0.21 mol) and cyclopropyl bromide (0.26 mol, 20.6 mL). Prior to treatment with MeOH, the mixture was stirred at room temperature. The mixture was purified by column chromatography (eluent gradient PE40/60, PE40/60−EtOAc, 1:1): yield 19.5 g (79.8 mmol, 38%); R_f 0.70 (PE40/60−EtOAc, 1:1); ^{13}C NMR ($CDCl_3$) δ 111.94 ($C(CH_3)_2$), 108.90 (C-1), 84.88, 84.65, 81.85 (C-2,3,4), 71.19 (C-5), 54.38 (OCH), 53.21 (OCH_3), 26.15, 24.72 ($C(CH_3)_2$), 5.37 (CH_2CH_2) ppm.

General Procedure for the Deprotection of Compounds 3−5 to the 5-*O*-Alkyl-β-D-ribofuranose Derivatives 6−8. The appropriate methyl 5-*O*-alkyl-2,3-*O*-isopropylidene-β-D-ribofuranose (4.48 mmol) was dissolved in 15 mL of HCl (0.04 M) and was refluxed for 2 h. The solution was neutralized with $BaCO_3$, filtered, and concentrated. The mixture was purified by column chromatography.

5-*O*-Methyl-α,β-D-ribofuranose (6). The reaction was carried out with methyl 5-*O*-methyl-2,3-*O*-isopropylidene-β-D-ribofuranoside (**3**; 1 g, 4.58 mmol). The mixture was purified by column chromatography (eluent 10% MeOH in EtOAc): yield 0.44 g (2.68 mmol, 59%); R_f 0.34 (10% MeOH in EtOAc); ^{13}C NMR (MeOD) δ 103.12 (C-1, β), 97.78 (C-1, α), 83.94, 82.36, 77.37, 76.88, 72.61, 72.12 (C-2,3,4, α + β), 73.93 (C-5), 59.51, 59.39 (OCH_3, α + β) ppm.

5-*O*-Ethyl-α,β-D-ribofuranose (7). The reaction was carried out with methyl 5-*O*-ethyl-2,3-*O*-isopropylidene-β-D-ribo-

furanoside (**4**; 1.0 g, 4.85 mmol). The mixture was purified by column chromatography (10% MeOH in EtOAc): yield 0.56 g (3.14 mmol, 65%); R_f 0.40 (10% MeOH in EtOAc); ^{13}C NMR (MeOD) δ 102.88 (C-1, β), 97.51 (C-1, α), 84.09, 82.42, 76.60, 72.53, 72.03 (C-2,3,4, $\alpha + \beta$), 73.40 (C-5), 67.83 (CH_2CH_3), 15.34 (CH_3) ppm.

5-*O*-Cyclopropyl-α,*β*-D-ribofuranose (8). The reaction was carried out with methyl 5-*O*-cyclopropyl-2,3-*O*-isopropylidene-*β*-D-ribofuranoside (**5**; 4.4 g, 18 mmol). The mixture was purified by column chromatography (10% MeOH in EtOAc): yield 2.09 g (11 mmol, 61%); R_f 0.43 (10% MeOH in EtOAc); ^{13}C NMR (MeOD) δ 101.60 (C-1, β), 96.41 (C-1, α), 80.77, 79.42, 74.40, 73.53, 72.03 (C-2,3,4, $\alpha + \beta$), 69.40 (C-5), 56.97 (CH) 7.83 (CH_2CH_2) ppm.

General Procedure for the Acylation of Compounds 6−8 to the 1,2,3-Tri-*O*-acetyl-5-*O*-alkyl-α,*β*-D-ribofuranose Derivatives 9−11. The appropriate 5-*O*-alkyl-α,*β*-D-ribofuranose (2.68 mmol) was dissolved in 25 mL of pyridine. A catalytic amount of (dimethylamino)pyridine (DMAP) and acetic anhydride (8.84 mmol, 843 μL) were added. The mixture was stirred for 2 h at room temperature, concentrated in vacuo and coevaporated with toluene. The oil was extracted with water and EtOAc (25 mL each). The organic layer was dried ($MgSO_4$), concentrated, and purified by column chromatography.

1,2,3-Tri-*O*-Acetyl-5-*O*-methyl-α,*β*-D-ribofuranose (9). The reaction was carried out with 5-*O*-methyl-α,*β*-D-ribofuranose (**6**; 0.44 g, 2.68 mmol). The mixture was purified by column chromatography (eluent 10% MeOH in EtOAc): yield 0.70 g (2.41 mmol, 90%); R_f 0.76 (10% MeOH in EtOAc); ^{13}C NMR ($CDCl_3$) δ 168.98, 168.68, 168.39 (3 × C=O, $\alpha + \beta$), 97.64 (C-1, β), 93.64 (C-1, α), 82.81, 80.30, 73.99, 73.67, 70.20, 69.67 (C-2,3,4, $\alpha + \beta$), 71.75 (C-5), 58.67 (OCH_3), 20.26, 19.70 (3 × $COCH_3$) ppm.

1,2,3-Tri-*O*-Acetyl-5-O-ethyl-α,*β*-D-ribofuranose (10). The reaction was carried out with 5-*O*-ethyl-α,*β*-D-ribofuranose (**7**; 0.56 g, 3.14 mmol). The mixture was purified by column chromatography (eluent 10% MeOH in EtOAc): yield 0.85 g (2.79 mmol, 89%); R_f 0.75 (10% MeOH in EtOAc); ^{13}C NMR ($CDCl_3$) δ 168.68, 168.42, 168.02 (3 × C=O), 97.43 (C-1, β), 93.40 (C-1, α), 82.69, 80.24, 73.50, 70.08, 69.53 (C-2,3,4, $\alpha + \beta$), 69.38, 68.94 (C-5, $\alpha + \beta$), 66.05 (CH_2CH_3), 19.96, 19.41 (3 × $COCH_3$), 14.18 (CH_2CH_3) ppm.

1,2,3-Tri-*O*-Acetyl-5-*O*-cyclopropyl-α,*β*-D-ribofuranose (11). The reaction was carried out with 5-*O*-cyclopropyl-α,*β*-D-ribofuranose (**8**; 3.42 g, 18.0 mmol). The mixture was purified by column chromatography (eluent 10% MeOH in EtOAc): yield 4.74 g (15.0 mmol, 83%); R_f 0.72 (10% MeOH in EtOAc); ^{13}C NMR ($CDCl_3$) δ 169.27, 168.98, 168.74 (3 × C=O), 97.81 (C-1, β), 93.78 (C-1, α), 82.84, 80.36, 73.91, 70.43, 69.88 (C-2,3,4, $\alpha + \beta$), 69.79, 69.38 (C-5, $\alpha + \beta$), 53.50 (CH), 20.55, 20.02 (3 × $COCH_3$), 5.22 (CH_2CH_2) ppm.

General Procedure for the Coupling of Compounds 9−11 to 6-Chloropurine or 2,6-Dichloropurine To Give Compounds 12−17.

Silylation of the Base. The appropriate base (193.8 mg, 1.27 mmol) was treated with 1,1,1,3,3,3-hexamethyldisilazane (HMDS; 5 mL, 23.7 mmol) and 12.5 μL of chlorotrimethylsilane (TMSCl; 0.1 mmol) at 130 °C for 20 h. The silylated compound was concentrated and used without further purification.

Vorbrüggen Coupling. To the appropriate silylated base (12.9 mmol) was added the appropriate ribose (10.3 mmol) in 15 mL of dry 1,2-dichloroethane. The residue was coevaporated twice with dry 1,2-dichloroethane and subsequently dissolved in 75 mL of dry 1,2-dichloroethane. The solution was gently refluxed, and after 5 min TMS triflate (997 μL, 5.16 mmol) was added. The mixture was refluxed for 2 h, cooled to room temperature, and diluted with CH_2Cl_2. It was extracted with 5% $NaHCO_3$ and water. The organic layer was dried ($MgSO_4$), concentrated, and purified by column chromatography.

6-Chloro-9-(2,3-di-*O*-acetyl-5-*O*-methyl-*β*-D-ribofuranosyl)purine (12). The reaction was carried out with silylated 6-chloropurine (12.9 mmol) and 1,2,3-tri-*O*-acetyl-5-*O*-methyl-α,*β*-D-ribofuranose (**9**; 3.0 g, 10.3 mmol). The mixture was purified by column chromatography (eluent 3% acetone in CH_2Cl_2): yield 1.9 g (4.94 mmol, 48%); R_f 0.11 (3% acetone in CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 8.75 (s, 1H, H-8), 8.56 (s, 1H, H-2), 6.41 (d, 1H, J = 6.18 Hz, H-1′), 5.80 (t, 1H, J = 5.49 Hz, H-2′), 5.60−5.56 (m, 1H, H-3′), 4.39 (q, 1H, J = 2.41 Hz, H-4′), 3.69 (dq, 2H, J = 7.21 Hz, J = 2.40 Hz, H-5′), 3.48 (s, 3H, OCH_3), 2.16, 2.03 (2 × s, 6H, 2 × $COCH_3$) ppm.

2,6-Dichloro-9-(2,3-di-*O*-acetyl-5-*O*-methyl-*β*-D-ribofuranosyl)purine (13). The reaction was carried out with silylated 2,6-dichloropurine (10.3 mmol) and **9** (2.39 g, 8.24 mmol). The mixture was purified by column chromatography (eluent gradient 4−6% acetone in CH_2Cl_2): yield 2.91 g (6.94 mmol, 84%); ^{1}H NMR ($CDCl_3$) δ 8.41 (s, 1H, H-8), 6.18 (d, 1H, J = 6.52 Hz, H-1′), 5.60 (dd, 1H, J = 6.18 Hz, J = 5.49 Hz, H-2′), 5.39 (dd, 1H, J = 5.15 Hz, J = 2.06 Hz, H-3′), 4.25−4.23 (m, 1H, H-4′), 3.58−3.48 (m, 2H, H-5′), 3.33 (s, 3H, OCH_3), 2.00, 1.88 (2 × s, 6H, 2 × $COCH_3$) ppm.

6-Chloro-9-(2,3-di-*O*-acetyl-5-*O*-ethyl-*β*-D-ribofuranosyl)-purine (14). The reaction was carried out with silylated 6-chloropurine (11.6 mmol) and 1,2,3-tri-*O*-acetyl-5-*O*-ethyl-α,*β*-D-ribofuranose (**10**; 2.82 g, 9.28 mmol). The mixture was purified by column chromatography (eluent gradient 4−6% acetone in CH_2Cl_2): yield 3.03 g (7.60 mmol, 82%); R_f 0.17 (3% acetone in CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 8.51 (s, 1H, H-8), 8.45 (s, 1H, H-2), 6.20 (d, 1H, J = 6.18 Hz, H-1′), 5.64 (t, 1H, J = 5.15 Hz, H-2′), 5.40−5.36 (m, 1H, H-3′), 4.24−4.18 (m, 1H, H-4′), 3.53 (dq, 2H, J = 13.73 Hz, J = 2.06 Hz, H-5′), 3.42 (q, 2H, J = 6.86 Hz, CH_2CH_3), 1.94, 1.81 (2 × s, 6H, 2 × $COCH_3$), 1.07 (t, 3H, J = 6.86 Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$) δ 169.33, 168.89 (2 × C=O), 151.70, 151.32, 150.56, 143.26, 131.41 (C-2,4,5,6,8), 85.46, 82.78, 74.08, 74.86 (C-1′,2′,3′,4′), 69.35, 66.90 (OCH2, C-5′), 20.28, 19.19 (2 × $COCH_3$), 14.71 (CH_3) ppm.

2,6-Dichloro-9-(2,3-di-*O*-acetyl-5-*O*-ethyl-*β*-D-ribofuranosyl)purine (15). The reaction was carried out with silylated 2,6-dichloropurine (10.3 mmol) and **10** (2.51 g, 8.24 mmol). The mixture was purified by column chromatography (eluent gradient 4−6% acetone in CH_2Cl_2): yield 2.93 g (6.76 mmol, 82%); ^{1}H NMR ($CDCl_3$) δ 8.66 (s, 1H, H-8), 6.39 (d, 1H, J = 6.53 Hz, H-1′), 5.81 (dd, 1H, J = 6.86 Hz, J = 5.49 Hz, H-2′), 5.58 (dd, 1H, J = 7.21 Hz, J = 2.06 Hz, H-3′), 4.43−4.41 (m, 1H, H-4′), 3.75 (dq, 2H, J = 10.64 Hz, J = 2.41 Hz, H-5′), 3.64 (t, 2H, J = 6.87 Hz, CH_2CH_3), 2.19, 2.06 (2 × s, 6H, 2 × $COCH_3$), 1.32 (t, 3H, J = 6.86 Hz, CH_2CH_3) ppm.

6-Chloro-9-(2,3-di-*O*-acetyl-5-*O*-cyclopropyl-*β*-D-ribofuranosyl)purine (16). The reaction was carried out with silylated 6-chloropurine (9.79 mmol) and 1,2,3-tri-*O*-acetyl-5-*O*-cyclopropyl-α,*β*-D-ribofuranose (**11**; 2.48 g, 7.83 mmol). The mixture was purified by column chromatography (eluent gradient 4−6% acetone in CH_2Cl_2): yield 2.09 g (5.10 mmol, 65%); ^{1}H NMR ($CDCl_3$) δ 8.76 (s, 1H, H-8), 8.59 (s, 1H, H-2), 6.42 (d, 1H, J = 6.18 Hz, H-1′), 5.83−5.77 (m, 1H, H-2′), 5.57−5.53 (m, 1H, H-3′), 4.45−4.42 (m, 1H, H-4′), 3.87−3.77 (m, 2H, H-5′), 3.45−3.42 (m, 1H, CH), 2.19, 2.06 (2 × s, 6H, 2 × $COCH_3$), 0.67−0.55 (m, 4H, CH_2CH_2) ppm.

2,6-Dichloro-9-(2,3-di-*O*-acetyl-5-*O*-cyclopropyl-*β*-D-ribofuranosyl)purine (17). The reaction was carried out with silylated 2,6-dichloropurine (8.95 mmol) and **11** (2.26 g, 7.16 mmol). The mixture was purified by column chromatography (eluent gradient 4−6% acetone in CH_2Cl_2): yield 2.55 g (5.73 mmol, 80%); ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1H, H-8), 6.10 (d, 1H, J = 6.18 Hz, H-1′), 5.52−5.46 (m, 1H, H-2′), 5.28−5.25 (m, 1H, H-3′), 4.20−4.17 (m, 1H, H-4′), 3.60 (q, 2H, J = 7.56 Hz, H-5′), 3.24−3.19 (m, 1H, OCH), 1.91, 1.82 (2 × s, 6H, 2 × $COCH_3$), 0.43−0.32 (m, 4H, CH_2CH_2) ppm.

General Procedure for the Amination of Compounds 12−17 to the Substituted Adenosine Derivatives 18−35.

Method A (Compounds 18, 21, 24, 27, 30, and 33). The appropriate 6-chloro-9-(2,3-di-*O*-acetyl-5-*O*-alkyl-*β*-D-ribofuranosyl)purine or the appropriate 2,6-dichloro-9-(2,3-di-*O*-acetyl-5-*O*-alkyl-*β*-D-ribofuranosyl)purine (1.53 mmol) was dissolved in EtOH/NH_3 (30 mL), and the mixture was stirred overnight at room temperature. The mixture was concentrated and purified by column chromatography.

Method B (Compounds 19, 20, 22, 23, 25, 26, 28, 29, 31, 32, 34, and 35). The appropriate 6-chloro-9-(2,3-di-*O*-acetyl-5-*O*-alkyl-*β*-D-ribofuranosyl)purine or the appropriate 2,6-dichloro-9-(2,3-di-*O*-acetyl-5-*O*-alkyl-*β*-D-ribofuranosyl)purine (1.53 mmol) was dissolved in EtOH absolute (10 mL). The

appropriate amine (2.3 mmol) and Et_3N (1.91 mmol) were added, and the mixture was refluxed overnight. The mixture was concentrated, dissolved in EtOH/NH_3 (30 mL), and stirred overnight at room temperature. The mixture was concentrated again and purified by column chromatography.

5′-*O*-Methyladenosine (18). Method A. The reaction was carried out with 6-chloro-9-(2,3-di-*O*-acetyl-5-*O*-methyl-β-**D**-ribofuranosyl)purine (**12**; 682 mg, 1.77 mmol). The mixture was purified by column chromatography (eluent 5% MeOH in CH_2Cl_2): yield 393 mg (1.40 mmol, 79%); mp 112−114 °C; R_f 0.43 (10% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.28 (s, 1H, H-8), 8.14 (s, 1H, H-2), 7.26 (br s, 2H, NH_2), 5.87 (d, 1H, J = 5.49 Hz, OH-2′), 5.25 (d, 1H, J = 5.15 Hz, OH-3′), 4.57 (q, 1H, J = 6.18 Hz, H-2′), 4.13 (q, 1H, J = 4.47 Hz, H-3′), 3.99−3.98 (m, 1H, H-4′), 3.57−3.48 (m, 2H, H-5′), 3.28 (s, 3H, OCH_3) ppm; MS m/z 282 $(M + H)^+$. Anal. ($C_{11}H_{15}N_5O_4 \cdot 0.5H_2O$) C, H, N.

***N*-Cyclopentyl-5′-*O*-methyladenosine (19).** Method B. The reaction was carried out with **12** (589 mg, 1.53 mmol) and cyclopentylamine (2.3 mmol, 227 μL). The mixture was purified by column chromatography (eluent 5% MeOH in CH_2Cl_2): yield 476 mg (1.36 mmol, 89%); mp 164−166 °C; R_f 0.51 (eluent 10% MeOH in CH_2Cl_2). The product was recrystallized from CH_3CN: ^{1}H NMR (DMSO-d_6) δ 8.28 (s, 1H, H-8), 8.18 (s, 1H, H-2), 7.70 (d, 1H, J = 7.55 Hz, NH), 5.89 (d, 1H, J = 4.80 Hz, H-1′), 5.50 (d, 1H, J = 5.15 Hz, OH-2′), 5.26 (d, 1H, J = 5.15 Hz, OH-3′), 4.56 (q, 1H, J = 4.12 Hz, H-2′), 4.13 (q, 1H, J = 4.46 Hz, H-3′), 3.99 (q, 1H, J = 4.12 Hz, H-4′), 3.57−3.42 (m, 2H, H-5′), 3.28 (s, 3H, OCH_3), 2.01−1.83 (m, 2H, cyclopentyl), 1.73−1.52 (m, 4H, cyclopentyl); MS m/z 350 $(M + H)^+$. Anal. ($C_{16}H_{23}N_5O_4 \cdot 0.7CH_3CN$) C, H, N.

***N*-(3-Iodobenzyl)-5′-*O*-methyladenosine (20).** Method B. The reaction was carried out with **12** (363 mg, 0.94 mmol) and (3-iodobenzyl)amine hydrochloride (1.41 mmol, 380 mg). The mixture was purified by column chromatography (eluent 5% MeOH in CH_2Cl_2): yield 397 mg (0.80 mmol, 85%); mp 155−157 °C; R_f 0.54 (eluent 10% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.40 (br s, 1H, NH), 8.33 (s, 1H, H-8), 8.21 (s, 1H, H-2), 7.70 (s, 1H, CC*H*CI), 7.57 (d, 1H, J = 6.52 Hz, CCHCHC*H*), 7.34 (d, 1H, J = 5.15 Hz, CC*H*CH), 7.08 (t, 1H, J = 8.93 Hz, CCHC*H*), 5.89 (d, 1H, J = 4.12 Hz, H-1′), 5.50−5.47 (m, 1H, OH-2′), 5.27−5.25 (m, 1H, OH-3′), 4.68−4.56 (m, 3H, H-2′, NHC*H*$_2$), 4.14−4.13 (m, 1H, H-3′), 4.01−3.98 (m, 1H, H-4′), 3.57−3.53 (m, 2H, H-5′), 3.31 (s, 3H, OCH_3); MS m/z 498 $(M + H)^+$. Anal. ($C_{18}H_{20}N_5O_4$) C, H, N.

2-Chloro-5′-*O*-methyladenosine (21). Method A. The reaction was carried out with 2,6-dichloro-9-(2,3-di-*O*-acetyl-5-*O*-methyl-β-D-ribofuranosyl)purine (**13**; 667 mg, 1.59 mmol). The mixture was purified by column chromatography (eluent 5% MeOH in CH_2Cl_2): yield 382 mg (1.21 mmol, 76%); mp 200−202 °C; R_f 0.49 (10% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.30 (s, 1H, H-8), 7.83 (br s, 2H, NH_2), 5.80 (d, 1H, J = 5.15 Hz, H-1′), 5.53 (d, 1H, J = 6.18 Hz, OH-2′), 5.29 (d, 1H, J = 5.49 Hz, OH-3′), 4.52 (q, 1H, J = 5.84 Hz, H-2′), 4.11−3.99 (m, 2H, H-3′,4′), 3.55−3.46 (m, 2H, H-5′), 3.28 (s, 3H, OCH_3); MS m/z 316 $(M + H)^+$. Anal. ($C_{11}H_{14}ClN_5O_4 \cdot 1.0CH_2Cl_2$) C, H, N.

2-Chloro-*N*-cyclopentyl-5′-*O*-methyladenosine (22). Method B. The reaction was carried out with **13** (505 mg, 1.2 mmol) and cyclopentylamine (1.8 mmol, 178 μL). The mixture was purified by column chromatography (eluent 2% MeOH in CH_2Cl_2): yield 364 mg (0.95 mmol, 79%); mp 124−126 °C; R_f 0.15 (eluent 2% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.33 (br s, 1H, NH), 8.30 (s, 1H, H-8), 5.81 (d, 1H, J = 4.46 Hz, H-1′), 5.51 (d, 1H, J = 3.36 Hz, OH-2′), 5.29 (d, 1H, J = 3.32 Hz, OH-3′), 4.59−4.29 (m, 2H, CH, H-2′), 4.41−3.99 (m, 2H, H-3′,4′), 3.56−3.52 (m, 2H, H-5′), 1.94−1.92 (m, 2H, cyclopentyl), 1.71−1.50 (m, 4H, cyclopentyl); MS m/z 384 $(M + H)^+$. Anal. ($C_{16}H_{22}ClN_5O_4 \cdot 0.1CH_2Cl_2$) C, H, N.

2-Chloro-*N*-(3-iodobenzyl)-5′-*O*-methyladenosine (23). Method B. The reaction was carried out with **13** (372 mg, 0.89 mmol) and 3-iodobenzylamine hydrochloride (1.34 mmol, 360 mg). The mixture was purified by column chromatography (eluent 2% MeOH in CH_2Cl_2): yield 383 mg (0.72 mmol, 81%); mp 84−86 °C; R_f 0.59 (10% MeOH in CH_2Cl_2). The product was recrystallized from CH_3COCH_3: ^{1}H NMR (DMSO-d_6) δ 8.89 (br s, 1H, NH), 8.35 (s, 1H, H-8), 7.73 (s, 1H, CCHCI), 7.60 (d, 1H, J = 5.83 Hz, CCHCHC*H*), 7.33 (d, 1H, J = 6.12 Hz, CC*H*CH), 7.12 (t, 1H, J = 7.55 Hz, CCHC*H*), 5.82 (d, 1H, J = 5.15 Hz, H-1′), 5.54 (d, 1H, J = 5.84 Hz, OH-2′), 5.30 (d, 1H, J = 5.49 Hz, OH-3′), 4.61−4.51 (m, 3H, NHCH_2, H-2′), 4.09−4.00 (m, 2H, H-3′,4′), 3.56−3.52 (m, 2H, H-5′), 3.28 (s, 3H, OCH_3); MS m/z 532 $(M + H)^+$. Anal. ($C_{18}H_{19}ClIN_5O_4 \cdot 0.3CH_3COCH_3$) C, H, N.

5′-*O*-Ethyladenosine (24). Method A. The reaction was carried out with 6-chloro-9-(2,3-di-*O*-acetyl-5-*O*-ethyl-β-D-ribofuranosyl)purine (**14**; 663 mg, 1.70 mmol). The mixture was purified by column chromatography (eluent 5% MeOH in CH_2Cl_2): yield 407 mg (1.38 mmol, 81%); mp 110−112 °C; R_f 0.48 (10% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.32 (s, 1H, H-8), 8.14 (s, 1H, H-2), 7.27 (br s, 2H, NH_2), 5.89 (d, 1H, J = 4.80 Hz, H-1′), 5.49 (d, 1H, J = 5.84 Hz, OH-2′), 5.24 (d, 1H, J = 5.15 Hz, OH-3′), 4.54 (q, 1H, J = 5.14 Hz, H-2′), 4.15 (q, 1H, J = 4.81 Hz, H-3′), 4.00 (q, 1H, J = 4.46 Hz, H-4′), 3.58 (dq, 2H, J = 9.27 Hz, J = 3.78 Hz, H-5′), 3.47 q, 2H, J = 7.21 Hz, CH_2), 1.12 (t, 3H, J = 7.20 Hz, CH_3); MS m/z 296 $(M + H)^+$. Anal. ($C_{12}H_{17}N_5O_4 \cdot 0.2CH_2Cl_2$) C, H, N.

***N*-Cyclopentyl-5′-*O*-ethyladenosine (25).** Method B. The reaction was carried out with **14** (502 mg, 1.26 mmol) and cyclopentylamine (1.89 mmol, 187 μL). The mixture was purified by column chromatography (eluent 5% MeOH in CH_2Cl_2): yield 316 mg (0.87 mmol, 69%); mp 134−136 °C; R_f 0.49 (eluent 10% MeOH in CH_2Cl_2). The product was recrystallized from CH_3CN: ^{1}H NMR (DMSO-d_6) δ 8.31 (s, 1H, H-8), 8.19 (s, 1H, H-2), 7.72 (d, 1H, J = 7.55 Hz, NH), 5.89 (d, 1H, J = 5.15 Hz, H-1′), 5.55−5.25 (m, 2H, OH-2′,3′), 4.53 (t, 1H, J = 4.81 Hz, H-2′), 4.15 (t, 1H, J = 2.40 Hz, H-3′), 4.01−3.96 (m, 1H, H-4′), 3.62−3.48 (m, 2H, H-5′), 3.46 (q, 2H, J = 7.21 Hz, CH_2CH$_3$), 1.99−1.82 (m, 2H, cyclopentyl), 1.78−1.52 (m, 4H, cyclopentyl), 1.11 (t, 3H, J = 7.21 Hz, CH_3); MS m/z 364 $(M + H)^+$. Anal. ($C_{17}H_{25}N_5O_4 \cdot 0.6CH_3CN$) C, H, N.

5′-*O*-Ethyl-*N*-(3-iodobenzyl)adenosine (26). Method B. The reaction was carried out with **14** (367 mg, 0.92 mmol) and (3-iodobenzyl)amine hydrochloride (1.38 mmol, 372 mg). The mixture was purified by column chromatography (eluent 5% MeOH in CH_2Cl_2): yield 339 mg (0.66 mmol, 72%); mp 164−166 °C; R_f 0.40 (10% MeOH in CH_2Cl_2). The product was recrystallized from CH_3CN: ^{1}H NMR (DMSO-d_6) δ 8.45 (br s, 1H, NH), 8.37 (s, 1H, H-8), 8.21 (s, 1H, H-2), 7.71 (s, 1H, CC*H*CI), 7.57 (d, 1H, J = 7.90 Hz, CCHCHC*H*), 7.34 (d, 1H, J = 6.52 Hz, CC*H*CH), 7.09 (t, 1H, J = 6.87 Hz, CCHC*H*), 5.91 (d, 1H, J = 4.46 Hz, H-1′), 5.53 (d, 1H, J = 5.49 Hz, OH-2′), 5.27 (d, 1H, J = 4.80 Hz, OH-3′), 4.67−4.64 (m, 2H, NHCH_2), 4.56 (q, 1H, J = 4.81 Hz, H-2′), 4.17 (q, 1H, J = 4.81 Hz, H-3′), 4.00 (q, 1H, J = 4.12 Hz, H-4′), 3.60 (dq, 2H, J = 9.61 Hz, J = 3.43 Hz, H-5′), 3.47 (q, 2H, J = 6.52 Hz, OCH_2CH$_3$), 1.12 (t, 3H, J = 6.52 Hz, CH_3); MS m/z 512 $(M + H)^+$. Anal. ($C_{19}H_{22}IN_5O_4 \cdot 0.3CH_3CN$) C, H, N.

2-Chloro-5′-*O*-ethyladenosine (27). Method A. The reaction was carried out with 2,6-dichloro-9-(2,3-di-*O*-acetyl-5-*O*-ethyl-β-D-ribofuranosyl)purine (**15**; 656 mg, 1.51 mmol). The mixture was purified by column chromatography (eluent 5% MeOH in CH_2Cl_2): yield 388 mg (1.18 mmol, 78%); mp 117−119 °C; R_f 0.50 (10% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.34 (s, 1H, H-8), 7.83 (br s, 2H, NH_2), 5.81 (d, 1H, J = 5.49 Hz, H-1′), 5.53 (d, 1H, J = 5.83 Hz, OH-2′), 5.28 (d, 1H, J = 5.14 Hz, OH-3′), 4.49 (q, 1H, J = 4.81 Hz, H-2′), 4.13−4.01 (m, 1H, H-3′), 4.01−3.99 (m, 1H, H-4′), 3.62−3.42 (m, 2H, H-5′), 3.47 (q, 2H, J = 6.87 Hz, OCH_2), 1.12 (t, 3H, J = 6.87 Hz, CH_3); MS m/z 330 $(M + H)^+$. Anal. ($C_{12}H_{16}ClN_5O_4 \cdot 0.4CH_2Cl_2$) C, H, N.

2-Chloro-*N*-cyclopentyl-5′-*O*-ethyladenosine (28). Method B. The reaction was carried out with **15** (505 mg, 1.16 mmol) and cyclopentylamine (1.74 mmol, 172 μL). The mixture was purified by column chromatography (eluent 5% MeOH in CH_2Cl_2): yield 323 mg (0.81 mmol, 70%); mp 114−116 °C; R_f 0.55 (10% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.34 (s, 1H, H-8), 8.32 (br s, 1H, NH), 5.82 (d, 1H, J = 5.15 Hz, H-1′), 5.53 (d, 1H, J = 5.18 Hz, OH-2′), 5.29 (d, 1H, J = 5.15 Hz, OH-3′), 4.47−4.37 (m, 2H, CH, H-2′), 4.11−4.10 (m, 1H, H-3′), 4.00 (q, 1H, J = 4.47 Hz, H-4′), 3.62−3.58 (m, 2H, H-5′), 3.47 (q, 2H, J = 7.21 Hz, CH_2CH$_3$), 2.00−1.83 (m, 2H, cyclopentyl),

1.71−1.51 (m, 4H, cyclopentyl), 1.12 (t, 3H, J = 7.21 Hz, CH_3); MS m/z 399 $(M + H)^+$. Anal. ($C_{17}H_{24}ClN_5O_4$) C, H, N.

2-Chloro-5′-*O*-ethyl-*N*-(3-iodobenzyl)adenosine (29). Method B. The reaction was carried out with **15** (375 mg, 0.87 mmol) and (3-iodobenzyl)amine hydrochloride (1.31 mmol, 352 mg). The mixture was purified by column chromatography (eluent 5% MeOH in CH_2Cl_2): yield 366 mg (0.67 mmol, 77%); mp 80−82 °C; R_f 0.53 (10% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.97 (br s, 1H, NH),8.40 (s, 1H, H-8), 7.74 (s, 1H, CCHCI), 7.60 (d, 1H, J = 6.87 Hz, CCHCHC*H*), 7.35 (d, 1H, J = 8.24 Hz, CC*H*CH), 7.12 (t, 1H, J = 7.21 Hz, CCHC*H*), 5.84 (d, 1H, J = 4.46 Hz, H-1′), 5.56 (d, 1H, J = 5.49 Hz, OH-2′), 5.30 (d, 1H, J = 5.15 Hz, OH-3′), 4.60 (d, 2H, J = 4.46 Hz, NHCH_2), 4.51 (d, 1H, J = 5.15 Hz, H-2′), 4.14−4.11 (m, 1H, H-3′), 4.02 (q, 1H, J = 3.43 Hz, H-4′), 3.63−3.58 (m, 2H, H-5′), 3.49 (q, 2H, J = 6.52 Hz, CH_2CH$_3$), 1.13 (t, 3H, J = 6.52 Hz, CH_3); MS m/z 546 $(M + H)^+$. Anal. ($C_{19}H_{21}ClIN_5O_4$·0.8HCON$(CH_3)_2$) C, H, N.

5′-*O*-Cyclopropyladenosine (30). Method A. The reaction was carried out with 6-chloro-9-(2,3-di-*O*-acetyl-5-*O*-cyclopropyl-β-D-ribofuranosyl)purine (**16**; 334 mg, 0.81 mmol). The mixture was purified by column chromatography (eluent 5% MeOH in CH_2Cl_2): yield 172 mg (0.56 mmol, 69%); mp 130−132 °C; R_f 0.49 (10% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.27 (s, 1H, H-8), 8.14 (s, 1H, H-2), 7.28 (br s, 2H, NH_2), 5.87 (d, 1H, J = 4.80 Hz, H-1′), 5.50 (d, 1H, J = 5.84 Hz, OH-2′), 5.26 (d, 1H, J = 5.49 Hz, OH-3′), 4.56 (q, 1H, J = 4.81 Hz, H-2′), 4.10 (q, 1H, J = 4.47 Hz, H-3′), 3.99 (q, 1H, J = 3.78 Hz, H-4′), 3.67−3.59 (m, 2H, H-5′), 0.44−0.40 (m, 4H, OCH_2-CH_2); MS m/z 308 $(M + H)^+$. Anal. ($C_{13}H_{17}N_5O_4$·0.2CH_2Cl_2) C, H, N.

***N*-Cyclopentyl-5′-*O*-cyclopropyladenosine (31).** Method B. The reaction was carried out with **16** (273 mg, 0.66 mmol) and cyclopentylamine (1.00 mmol, 98 μL). The mixture was purified by column chromatography (eluent 5% MeOH in CH_2-Cl_2): yield 173 mg (0.46 mmol, 70%); mp 126−128 °C; R_f 0.53 (10% MeOH in CH_2Cl_2). The product was recrystallized from $(C_2H_5)_2O$: ^{1}H NMR (MeOD) δ 8.17 (s, 1H, H-8), 8.13 (s, 1H, H-2), 5.94 (d, 1H, J = 4.46 Hz, H-1′), 4.44 (t, 1H, J = 4.80 Hz, H-2′), 4.18 (t, 1H, J = 5.15 Hz, H-3′), 4.11−4.06 (m, 1H, H-4′), 3.69 (q, 2H, J = 9.95 Hz, H-5′), 3.24−3.19 (m, 1H, CH), 2.11−1.82 (m, 2H, cyclopentyl), 1.72−1.50 (m, 6H, cyclopentyl), 0.48−0.39 (m, 4H, CH_2CH_2); MS m/z 376 $(M + H)^+$. Anal. ($C_{18}H_{25}N_5O_4$·0.5$(C_2H_5)_2O$) C, H, N.

5′-*O*-Cyclopropyl-*N*-(3-iodobenzyl)adenosine (32). Method B. The reaction was carried out with **16** (196 mg, 0.48 mmol) and (3-iodobenzyl)amine hydrochloride (0.72 mmol, 193 mg). The mixture was purified by column chromatography (eluent 5% MeOH in CH_2Cl_2): yield 186 mg (0.36 mmol, 74%); mp 110−112 °C; R_f 0.49 (10% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.43 (br s, 1H, NH), 8.31 (s, 1H, H-8), 8.21 (s, 1H, H-2), 7.71 (s, 1H, CCHCI), 7.57 (d, 1H, J = 7.55 Hz, CCHCHC*H*), 7.34 (d, 1H, J = 7.89 Hz, CC*H*CH), 7.09 (t, 1H, J = 7.90 Hz, CCHC*H*), 5.89 (d, 1H, J = 5.15 Hz, H-1′), 5.49 (d, 1H, J = 5.83 Hz, OH-2′), 5.27 (d, 1H, J = 5.15 Hz, OH-3′), 4.68−4.55 (m, 2H, NHCH_2), 4.57 (q, 1H, J = 5.49 Hz, H-2′), 4.11 (q, 1H, J = 4.46 Hz, H-3′), 4.00 (q, 1H, J = 4.11 Hz, H-4′), 3.67−3.62 (m, 2H, H-5′), 0.45−0.40 (m, 4H, CH_2CH_2); MS m/z 524 $(M + H)^+$. Anal. ($C_{20}H_{22}IN_5O_4$·0.7CH_3OH) C, H, N.

2-Chloro-5′-*O*-cyclopropyladenosine (33). Method A. The reaction was carried out with 2,6-dichloro-9-(2,3-di-*O*-acetyl-5-*O*-cyclopropyl-β-D-ribofuranosyl)purine (**17**; 649 mg, 1.46 mmol). The mixture was purified by column chromatography (eluent 5% MeOH in CH_2Cl_2): yield 379 mg (1.11 mmol, 76%); mp 122−124 °C; R_f 0.32 (10% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.29 (s, 1H, H-8), 7.83 (br s, 2H, NH_2), 5.79 (d, 1H, J = 5.49 Hz, H-1′), 5.52 (d, 1H, J = 6.18 Hz, OH-2′), 5.29 (d, 1H, J = 5.29 Hz, OH-3′), 4.51 (q, 1H, J = 5.84 Hz, H-2′), 4.08−3.99 (m, 2H, H-3′,4′), 3.68−3.63 (m, 2H, H-5′), 0.45−0.41 (m, 4H, CH_2CH_2); MS m/z 342 $(M + H)^+$. Anal. ($C_{13}H_{16}ClIN_5O_4$·0.8HCON$(CH_3)_2$) C, H, N.

2-Chloro-*N*-cyclopentyl-5′-*O*-cyclopropyladenosine (34). Method B. The reaction was carried out with **17** (543 mg, 1.22 mmol) and cyclopentylamine (1.83 mmol, 180 μL). The mixture was purified by column chromatography (eluent 5% MeOH in CH_2Cl_2): yield 400 mg (0.98 mmol, 80%); mp 104−106 °C; R_f 0.51 (10% MeOH in CH_2Cl_2). The product was recrystallized from $(C_2H_5)_2O$: ^{1}H NMR (DMSO-d_6) δ 8.34 (br s, 1H, NH), 8.28 (s, 1H, H-8), 5.81 (d, 1H, J = 5.15 Hz, H-1′), 5.53 (d, 1H, J = 5.84 Hz, OH-2′), 5.29 (d, 1H, J = 5.15 Hz, OH-3′), 4.50 (q, 1H, J = 5.15 Hz, H-2′), 4.50−4.47 (m, 1H, CH), 4.09−3.99 (m, 2H, H-3′,4′), 3.68−3.63 (m, 2H, H-5′), 1.94−1.89 (m, 2H, cyclopentyl), 1.71−1.50 (m, 4H, cyclopentyl), 0.46−0.41 (m, 4H, $OCHCH_2CH_2$); MS m/z 410 $(M + H)^+$. Anal. ($C_{18}H_{24}ClN_5O_4$·0.2$(C_2H_5)_2O$) C, H, N.

2-Chloro-5′-*O*-cyclopropyl-*N*-(3-iodobenzyl)adenosine (35). Method B. The reaction was carried out with **17** (483 mg, 1.08 mmol) and (3-iodobenzyl)amine hydrochloride (1.63 mmol, 439 mg). The mixture was purified by column chromatography (eluent 5% MeOH in CH_2Cl_2): yield 435 mg (0.78 mmol, 72%); mp 94−96 °C; R_f 0.49 (10% MeOH in CH_2-Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.93 (t, 1H, J = 6.18 Hz, NH), 8.33 (s, 1H, H-8), 7.73 (s, 1H, CC*H*CI), 7.59 (d, 1H, J = 7.90 Hz, CCHCHC*H*), 7.34 (d, 1H, J = 7.55 Hz, CC*H*CH), 7.11 (t, 1H, J = 7.55 Hz, CCHC*H*), 5.82 (d, 1H, J = 5.14 Hz, H-1′), 5.54 (d, 1H, J = 5.84 Hz, OH-2′), 5.30 (d, 1H, J = 5.15 Hz, OH-3′), 4.61−4.48 (m, 3H, H-2′, NHCH_2), 4.09−3.98 (m, 2H, H-3′,4′), 3.68−3.58 (m, 2H, H-5′), 0.45−0.41 (m, 4H, cyclopropyl); MS m/z 559 $(M + H)^+$. Anal. ($C_{20}H_{21}ClIN_5O_4$·0.5H_2O) C, H, N.

Radioligand Binding Studies. Measurements with [^{3}H]-DPCPX in the absence of GTP were performed according to a protocol published previously (rat A_1).[32] Adenosine A_{2A} receptor (rat) affinities were determined according to Gao et al.[33] Adenosine A_3 receptor affinities were determined essentially as described.[34,35] Briefly, assays were performed in 50:10:1 buffer (50 mM Tris/10 mM $MgCl_2$/1 mM ethylenediaminetetraacetic acid (EDTA) and 0.01% 3-([3-cholamidopropyl]dimethylammonio)-1-propanesulfonate (CHAPS)) in glass tubes and contained 50 μL of a HEK 293 cell membrane suspension (10−30 μg), 25 μL of [^{125}I]ABMECA (final concentration 0.15 nM), and 25 μL of ligand. Incubations were carried out for 1 h at 37 °C and were terminated by rapid filtration over Whatman GF/B filters, using a Brandell cell harvester (Brandell, Gaithersburg, MD). Tubes were washed three times with 3 mL of buffer. Radioactivity was determined in a Beckman 5500B γ-counter. Nonspecific binding was determined in the presence of 10^{-5} M (*R*)-PIA.

cAMP Assay for A_1 and A_3. CHO cells expressing either the human adenosine A_1 or the human adenosine A_3 receptor were grown overnight as a monolayer in 24-well tissue culture plates (400 μL/well, 2×10^5 cells/well). cAMP generation was performed in Dulbecco's modified Eagle's medium (DMEM)/*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer (0.60 g of HEPES/50 mL of DMEM, pH 7.4). To each well, washed two times with DMEM/HEPES buffer (250 μL) were added 100 μL of adenosine deaminase (final concentration 5 IU/mL), 100 μL of a mixture of rolipram and cilostamide (final concentration each 50 μM), and 100 μL of agonist (final concentration ±100 times the K_i value). After incubation for 40 min at 37 °C, 100 μL of forskolin (final concentration 10 μM) was added. After 15 min at 37 °C, the reaction was terminated by removing the medium and adding 200 μL of 0.1 M HCl. The wells were stored at −20 °C until assay.

The amounts of cAMP were determined after a protocol with cAMP binding protein[36] with the following minor modifications. As a buffer was used 150 mM K_2HPO_4/10 mM EDTA/0.2% bovine serum albumin (BSA) at pH 7.5. Samples (20 μL + 30 μL of 0.1 M HCl) were incubated for at least 2.5 h at 0 °C before filtration over Whatman GF/B filters. The filters were additionally rinsed with 2 × 2 mL of Tris·HCl buffer (pH 7.4, 4 °C). The filters were counted in Packard emulsifier safe scintillation fluid (3.5 mL) after 24 h of extraction.

GTPγS Binding. The extent of stimulation of [^{35}S]GTPγS binding was determined according to the method of Lorenzen et al.[6,7] with some minor modifications. The final incubation conditions, which allowed the characterization of the synthesized compounds as potential partial agonists of either the adenosine A_1 or A_3 receptor, were as follows.

A_1. The GDP and NaCl concentrations were 10 μM and 100 mM, respectively. Incubations were performed at 25 °C for 90 min with 2 μg of membrane protein (rat adenosine A_1 receptor). CPA was used as a full agonist in this assay, and its

stimulation of [^{35}S]GTPγ[S] binding to the G protein was set to 100% (Table 3).

A_3. The GDP concentration was 1 μM, and NaCl was omitted. Incubations were performed at 25 °C for 90 min with 1−1.5 μg of membrane protein (human adenosine A_3 receptor expressed in CHO cells). NECA was used as a full agonist in this assay, and its stimulation of [^{35}S]GTPγ[S] binding to the G protein was set to 100% (Table 3).

Data Analysis. Apparent K_i and EC_{50} values were computed from the displacement curves by nonlinear regression of the competition curves with the software package Prism (Graph Pad, San Diego, CA).

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