Ribose-Modified Adenosine Analogues as Potential Partial Agonists for the Adenosine Receptor

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We have adopted a practical three-step route for the synthesis of 2′- and 3′-deoxy analogues of N^6-substituted adenosines: protection of the hydroxyl groups, replacement of the N^6-amino by a better leaving group, and combined deprotection and N^6-amination in the last step. This route was used to synthesize deoxy analogues of CPA, CHA, and *R*- and *S*-PIA. The compounds were tested on the adenosine A_1 and A_{2a} receptors in our search for partial agonists for these receptors. The GTP shift was used as an *in vitro* measure for the intrinsic activity of these compounds; the *in vivo* intrinsic activities of the deoxy analogues of CPA and *R*-PIA were determined in the rat cardiovascular system. Thus, it was shown that the hydroxyl groups are determinants for the affinity and intrinsic activity of these analogues. Removal of the 2′- and 3′-hydroxyl groups affects affinity and intrinsic activity, whereas removal of the 5′-hydroxyl group decreases only affinity.

Introduction

Adenosine receptors are widely distributed through organs, and ligands for the adenosine receptors have, therefore, a broad therapeutic potential.[1-3] Agonists for the adenosine receptors could for example be useful as sedatives, and as platelet aggregation inhibitors and can be used in diagnosis of diseases of coronary arteries.[4] However, cardiovascular side effects can be expected to be caused by the strong hypotensive effects of adenosine agonists.[5,6] This is a major drawback in the therapeutic use of adenosine receptor agonists. Thus, partial agonists for the adenosine receptors could be useful, because they may have less pronounced cardiovascular effects.[7,8] Another advantage would be that they probably induce less receptor downregulation and desensitisation and perhaps act more selectively. Agonists for the adenosine receptors are derivatives of adenosine itself (Figure 1): they consist of a purine ring system and an (more-or-less) intact ribose moiety. The ribose moiety is necessary for both high affinity and intrinsic activity. Removal of the ribose moiety was described to abolish the intrinsic activity of adenosine analogues and results, therefore, in antagonists for the adenosine receptor.[9-11] The 2′-hydroxyl group was shown to be essential for high affinity of adenosine and its analogues;[12-14] 2′-deoxy-*R*-PIA (2′-deoxy-N^6(*R*)-(1-phenyl-2-propyl)adenosine), for example, had only micromolar affinity for the A_1 receptor.[12] Removal of the 3′-hydroxyl group led also to a decrease in potency, and it was suggested that this group is essential for activity and affinity.[12,13] The 3′-deoxy analogue of *R*-PIA had approximately 30-fold lower affinity than *R*-PIA itself.[12] The importance of these hydroxyl functions, also for intrinsic activity, was confirmed by the finding that removal of both these groups from CHA (N^6-cyclohexyladenosine) yielded a moderately potent antagonist for the adenosine receptors.[15] The 5′-hydroxyl group, however, can be modified to some extent without a decrease in affinity,[12-14,16] but removal of this group resulted in a decrease in potency. The affinity of 5′-deoxy-*R*-PIA was approximately 25-fold lower than that of *R*-PIA.[12]

Obviously, removal of the ribose hydroxyl groups affects both affinity and intrinsic activity of agonists for the adenosine receptors. Therefore, we synthesized new compounds to explore the contribution of the hydroxyl functions to affinity and intrinsic activity in further detail, *i.e. in vitro* and *in vivo*, because it seemed feasible that partial agonists could be obtained in this way. We synthesized N^6-substituted analogues of 2′- and 3′-deoxyadenosine to compensate for the expected loss in affinity by removal of the hydroxyl groups; a suitable N^6-substituent induces an increase in the affinity for the A_1 receptor. With a practical three-step synthesis we obtained the N^6-cyclopentyl, cyclohexyl, (*R*)-phenylisopropyl, and (*S*)-phenylisopropyl analogues of 2′- and 3′-deoxyadenosine (Figure 2). These compounds and the 5′-deoxy analogues of CPA (N^6-cyclopentyladenosine) and *R*-PIA were tested *in vitro* for their affinity for the adenosine A_1 and A_{2a} receptors and their GTP (guanosine-5′-triphosphate) shift on the A_1 receptors. The GTP shift, *i.e.* the difference in affinity of a ligand in the presence and absence of GTP, was determined in the receptor binding assay and used as an indication for (partial) agonism. The *in vivo* intrinsic activity of some compounds was determined in the cardiovascular system of rats. With the results of this study it was possible to assess the relative contribution of hydroxyl groups to the affinity and intrinsic activity of adenosine analogues and to relate their *in vivo* intrinsic activity to the GTP shift.

Results and Discussion

Chemistry. The route described in the literature for the synthesis of deoxy analogues of *R*-PIA is not very convenient: it involves a multistep deoxygenation of

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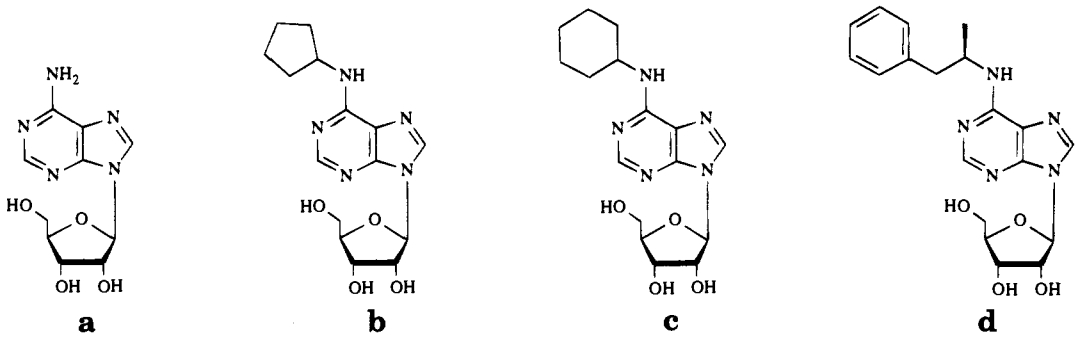


Figure 1. Adenosine (a) and three N^6-substituted analogues: CPA (b), CHA (c), and *R*-PIA (d).

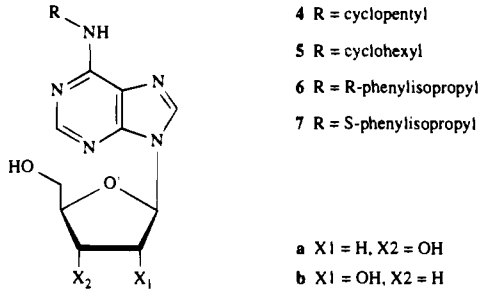
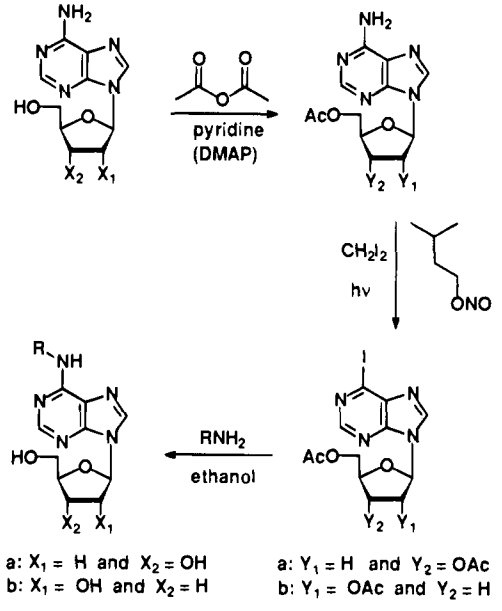


Figure 2. N^6-Substituted deoxyadenosine analogues synthesized.

Scheme 1. Synthesis of N^6-Substituted Deoxyadenosine Analogues



R-PIA, which leads to a mixture of the 2′- and 3′-deoxy analogues.[12] We used a practical short route to convert 2′- and 3′-deoxyadenosine into their N^6-substituted analogues. The route of synthesis, summarized in Scheme 1, included a three-step reaction: protection of the hydroxyl groups, reductive deamination, and combined amination and deprotection. The nonoptimized overall yield of the products was approximately 30%.

The second step was used to replace the N^6-amino group by a better leaving group by treating it with isopentyl nitrite to give a diazo group and substituting it with diiodomethane by an iodo substituent. We tried this reaction first with acetonitrile as solvent and a small excess of the reactants, as described by Nair *et al.* for 2-amino-substituted purines,[17] but this gave very low yields. We found that the yield is much higher if the reaction is carried out with the two reactants, isopentylnitrite and diiodomethane, in excess as solvent, similar to the reaction described by Nair *et al.* with *n*-pentylnitrite.[18] The use of tungsten lamps with this reaction, also described by Nair *et al.*,[18] led to an approximately doubled yield of our product after 2 h. However, we are not certain whether the improved yield is due to the light itself by increasing the rate of the proposed radical reaction or by an additional heating of the mixture by the 150 W tungsten lamps close to the flask.

The last step in the synthesis of the N^6-substituted deoxy analogues, the combined deprotection and amination, had to be carried out at ambient temperature for approximately 2 weeks. Refluxing was not possible for the deoxypurines, due to decomposition of the products at higher temperatures. (For example the low yield of 2′-deoxy-CPA was caused by a 2 h refluxing.) For adenosine itself, however, this step in the reaction could easily be carried out by refluxing the mixture for several hours. The decomposition of the deoxy analogues is probably due to a greater lability of the N9–C1′ glycosidic linkage. An increased rate of breakdown has also been described for deoxyadenosines in the case of acid hydrolysis. The hydrolysis rate was described to increase 15 times by removal of the 3′-hydroxyl and 1200 times by removal of the 2′-hydroxyl group.[19–21] Hydroxyl groups induce electron-withdrawing effects to destabilize a glycosyl carbonium ion, and ribonucleosides are therefore more stabilized than their deoxy analogues. Similarly, the removal of hydroxyl groups could influence the stability of the adenosine analogues during the deacetylation/amination step of the reaction.

Pharmacology: Affinity. The deoxyadenosines, *i.e.* not substituted at the N^6-position, were substrates for the enzyme adenosine deaminase (ADA) that is usually present in receptor binding studies to remove the endogenous adenosine. Thus, we carried out the receptor binding studies with these ligands in the presence of deoxycoformycin, a potent ADA inhibitor with low affinity for the adenosine A_1 receptors, to prevent degradation of these compounds. Therefore, the K_i values of 2′- (**1a**), 3′- (**1b**), and 5′-deoxyadenosine are approximate only. It was not possible to determine the A_{2a} affinities of the deoxyadenosines this way, because the addition of deoxycoformycin disturbed the A_{2a} binding assay.

The K_i values of the deoxy analogues of adenosine, CPA, CHA, and *R*- and *S*-PIA are summarized in Table 1, together with the results for representative full agonists, CPA and *R*-PIA, and an antagonist, 9-methyladenine. Removal of the 2′-hydroxyl group led to a 300-

Table 1. Affinities (K_i Values in the Presence and Absence of GTP) and GTP Shifts of the N^6-Substituted Deoxy Analogues

ligand	K_i (μM) A_1 − GTP	K_i (μM) A_1 + GTP	GTP shift	K_i A_{2a} (μM)
CPA	0.00590 (*0.00578*–0.00602)	0.0352 (*0.0297*–*0.0407*)	6.0 ± 1.5	0.58 ± 0.12
R-PIA	0.011 ± 0.005	0.062 ± 0.004	5.5 ± 2.5	0.14 ± 0.01
9-methyladenine	12.5 ± 2.9	14.7 ± 2.8	1.2 ± 0.4	8.60 ± 0.56
2′-deoxyAdo (1a)	23.3 ± 6.6	–[a]	–[a]	–[a]
2′-deoxy-CPA (**4a**)	1.87 ± 0.52	7.60 (*7.18*–8.01)	4.1 ± 1.2	(*88%*)[b]
2′-deoxy-CHA (**5a**)	8.94 ± 2.77	22.8 (*22.5*–23.1)	2.6 ± 0.8	(*90%*)[b]
2′-deoxy-*R*-PIA (**6a**)	8.45 ± 2.26	14.4 (*14.0*–*14.7*)	1.7 ± 0.6	(*86%*)[b]
2′-deoxy-*S*-PIA (**7a**)	(*91%*)[b]	–[a]	–[a]	(*91%*)[b]
3′-deoxyAdo (**1b**)	7.12 ± 3.62	15.2 ± 2.02	2.1 ± 1.4	–[a]
3′-deoxy-CPA (**4b**)	0.11 ± 0.03	0.47 ± 0.04	4.3 ± 1.2	18.6 ± 6.8
3′-deoxy-CHA (**5b**)	0.31 ± 0.11	1.26 ± 0.16	4.1 ± 1.5	22.1 ± 6.4
3′-deoxy-*R*-PIA (**6b**)	0.35 ± 0.06	1.01 ± 0.12	2.9 ± 0.6	7.36 ± 0.53
3′-deoxy-*S*-PIA (**7b**)	8.26 ± 2.25	17.5 ± 2.8	2.1 ± 0.7	57.2 ± 9.7
5′-deoxyAdo	1.82 ± 0.51	12.1 ± 2.4	6.6 ± 2.3	–[a]
5′-deoxy-CPA	0.07 ± 0.02	0.45 ± 0.02	6.4 ± 1.9	10.1 ± 0.4
5′-deoxy-*R*-PIA	0.30 ± 0.08	1.07 ± 0.09	3.6 ± 1.0	3.67 ± 0.08

[a] –, not determined. [b] Percentage radiolabeled ligand bound to the receptor in the presence of 10^{-5} M ligand (n = 1).

fold decrease in A_1 affinity for CPA (**4a**) and an approximately 800-fold decrease for *R*-PIA (**6a**). The interaction of the 3′- and 5′-hydroxyl groups with the receptor is less substantial, because removal of those groups led to a 10–30-fold drop in affinity. All three hydroxyl groups could be involved in interactions, for example hydrogen bonds, with residues in the receptor, because removal of each of the hydroxyl groups leads to a decrease in affinity. The drop in A_1 affinity by removal of the 2′-hydroxyl moiety was larger than the decrease caused by removal of the 3′- or 5′-hydroxyl group, indicating that the 2′-hydroxyl group is involved in a stronger interaction with the receptor than the two other hydroxyl groups. However, analogues of adenosine in which the 2′- or 3′-hydroxyl group was replaced by another group capable of hydrogen bond formation, *i.e.* an amino group, displayed almost no receptor binding (results not shown). This may be due to a different directionality of amino groups in hydrogen bond formation, or by the different electrostatic properties of this group.

The affinity of the N^6-substituted 2′-deoxy analogues for the A_{2a} adenosine receptor was low: only 10% displacement of [^{3}H]CGS 21680 [2-[[4-(2-carboxyethyl)-phenethyl]amino]-5′-(*N*-ethylcarboxamido)adenosine] binding, at 10 μM ligand concentration. The 3′- and 5′-analogues have micromolar affinities for the A_{2a} receptor.

The deoxy analogues of CPA and *R*-PIA are selective for A_1 receptors versus A_{2a} receptors. The A_1 selectivity of the 3′-deoxy analogues ranges from 7 to 170 times, and 5′-deoxy-CPA and 5′-deoxy-*R*-PIA are 140- and 12-fold A_1 selective, respectively.

Intrinsic Activity. The GTP shifts of the deoxy analogues, listed in Table 1, range from the value obtained for full agonists (5.5–6.0) to almost that of the antagonist 9-methyladenine (1.2), with values between 1.7 and 6.6. The trend that was found for the effect of the hydroxyl groups on affinity was also observed for the GTP shifts. The GTP shifts tend to be low for the 2′-deoxy analogues, and somewhat higher for the 3′-deoxy analogues, and the GTP shifts of the 5′-deoxy analogues reflect the values found for full agonists.

The *in vivo* effects of the 2′- and 3′-deoxy analogues of *R*-PIA were studied in normotensive, conscious rats for two parameters: heart rate, an A_1 effect, and mean arterial pressure, which is reduced by a decrease in cardiac output (A_1 mediated), and by vasodilation (A_2,[22,23] and probably also A_3[6,24] mediated).

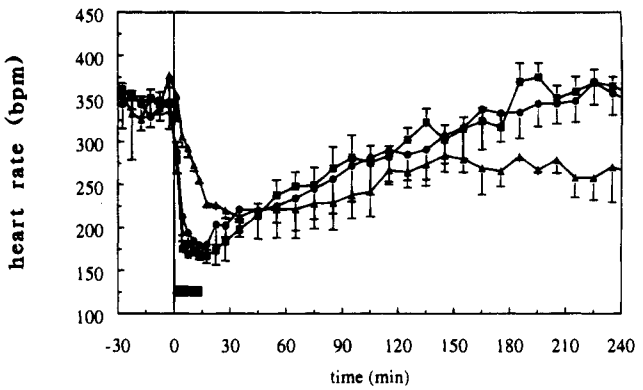


Figure 3. Time course of the averaged heart rate for rats which received 176 μg/kg R-PIA (■, n = 6), 12 mg/kg 3′-deoxy-*R*-PIA (●, n = 3), and 80 mg/kg 2′-deoxy-*R*-PIA (▲, n = 3). The black horizontal bar represents the time of infusion (15 min).

The results of a pilot study on the effects of the *R*-PIA analogues on the heart rate are represented in Figure 3. The compounds were administered in doses that, based on their affinities, assure (near) maximal receptor occupancy, with solubility as a limiting factor. The heart rate before administration (E_0) was approximately 350 bpm, and the decrease in heart rate during administration of the full agonist *R*-PIA was approximately 180 bpm, similar to the E_{max} values described by Mathôt *et al.* for CPA (E_0 369 ± 8 bpm, E_{max} −209 ± 10 bpm).[25] The decrease in heart rate was only slightly less for 3′-deoxy-*R*-PIA, whereas the E_{max} of 2′-deoxy-*R*-PIA was lower than that of the two other compounds. The duration of the maximal effect was longer for the latter compound. The heart rate reached the baseline value much slower in the case of 2′-deoxy-*R*-PIA administration than for the other two compounds, probably due to a difference in disposition of this compound.

The effects of these analogues on the mean arterial blood pressure are displayed in Figure 4. The effect of 3′-deoxy-*R*-PIA was smaller than that of *R*-PIA itself. 2′-Deoxy-*R*-PIA, however, had no significant effect on the arterial blood pressure. Thus, 2′-deoxy-*R*-PIA seems to be a compound that lowers the heart rate (via the A_1

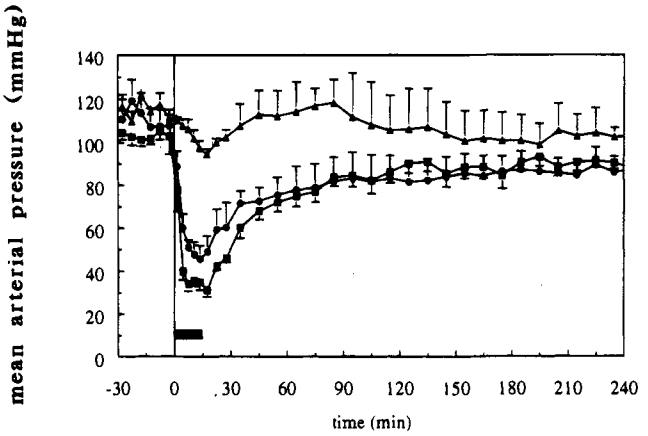


Figure 4. Time course of the averaged mean arterial blood pressure for rats which received 176 μg/kg *R*-PIA (■, $n = 6$), 12 mg/kg 3′-deoxy-*R*-PIA (●, $n = 3$), and 80 mg/kg 2′-deoxy-*R*-PIA (▲, $n = 3$). The black horizontal bar represents the time of infusion (15 min).

receptors) without significantly affecting the mean arterial pressure. The activity of the deoxy analogues of CPA on the cardiovascular system will be published as a full pharmacokinetic–pharmacodynamic modeling study by Mathôt *et al.* In these studies 2′- and 3′-CPA were found to be partial agonists for the cardiovascular A_1 and A_2 adenosine receptors, whereas 5′-deoxy-CPA behaved as a full agonist.

It has been shown for other types of G protein-coupled receptors that the GTP shift has a predictive value for the intrinsic activity of ligands.[26-30] The GTP shift is correlated with the difference in affinity of ligands for the high-affinity, G protein-coupled, state of the receptor and the low-affinity, uncoupled, state. The activation of the G protein by an agonist-bound receptor in the presence of GTP is accompanied by the association of GTP and uncoupling of the G protein from the receptor. A high correlation was shown to exist between the GTP shift of agonists and adenylate cyclase inhibition,[29] adenylate cyclase activation,[30] and *in vivo* intrinsic activity.[28] For the adenosine receptor the GTP shift is indicative for the intrinsic activity in the rat cardiovascular system, because compounds that behaved as partial agonists in the receptor binding studies turned out to be partial agonists *in vivo*. The trends within the groups of N^6-substituted analogues (GTP shift 2′-deoxy < 3′-deoxy < 5′-deoxy ≤ intact ribose) were also found for the intrinsic activity *in vivo*. It can therefore be concluded that the 2′- and 3′-hydroxyl groups are both involved in the mechanism activating the receptor, because removal of one of these groups results in partial agonism, whereas removal of both groups was described to result in antagonism.[15]

In our study we have shown that the monodeoxy analogues are (partial) agonists for the A_1 and A_{2a} receptor. Although it is known that deoxyadenosines bind to the A_3 receptor, it is not yet apparent whether they activate or block this receptor.[14] Nevertheless, agonistic behavior is expected, but testing of these compounds on the A_3 receptors is warranted to verify this supposition.

It has been hypothesized that partial agonists may be of pharmacological use because of their less outspoken cardiovascular effects.[7,8] We found a lower maximal decrease in heart rate and mean arterial blood pressure in rats for most of the compounds that displayed partial agonistic behavior *in vitro*. Moreover, a selectivity of effects was shown for 2′-deoxy-*R*-PIA. Such a compound could be a useful antiarrhytmic substance.

Conclusions

We have found a practical route for the synthesis of 2′- and 3′-deoxy analogues of N^6-substituted adenosines. The hydroxyl groups are determinants for the intrinsic activity of these analogues, and partial agonists with effects intermediate between those for full agonists and antagonists can be obtained by removal of the 2′-hydroxyl or 3′-hydroxyl moiety. Removal of the 3′-hydroxyl group, however, affects intrinsic activity to a lesser extent. Analogously, affinity is more affected by the 2′-hydroxyl group than by the 3′-hydroxyl group. In contrast, removal of the 5′-hydroxyl moiety affects only affinity and not intrinsic activity. We showed that GTP shifts can be used as an easy test system to discriminate between antagonists, agonists and even partial agonists for the adenosine receptors.

Experimental Section

GTP, cyclopentylamine, and 2′-deoxyadenosine were obtained from Aldrich (Brussels, Belgium). 3′-Deoxyadenosine was purchased from Prof. Pfleiderer (Konstanz, Germany). Diiodomethane was obtained from Janssen Chimica (Beerse, Belgium), bovine serum albumin (BSA) and cyclohexylamine from Sigma (St. Louis, MO), and adenosine deaminase (ADA) and *R*-PIA from Boehringer Mannheim (Mannheim, Germany). [^{3}H]DPCPX (1,3-dipropyl-8-cyclopentylxanthine, specific activity 108 Ci/mmol) and [^{3}H]CGS 21680 (specific activity 38.3 Ci/mmol) were purchased from NEN, Du Pont Nemours ('s-Hertogenbosch, The Netherlands). CPA was purchased from RBI (Natick, MA), and BCA and BCA protein assay reagent were purchased from Pierce Chemical Co. (Rockford, IL). All other chemicals were from standard commercial sources and of analytical grade.

The sulfuric acid salts of (*S*)- and (*R*)-1-phenyl-2-aminopropane (D-, and L-amphetamine sulfate, respectively) were kindly provided by Solvay-Duphar (Weesp, The Netherlands). Deoxycoformycin (=pentostatin) and the 5′-deoxy analogues 5′-deoxy-CPA and 5′-deoxy-*R*-PIA were a gift from Parke Davis (Ann Arbor, MI). Professor Olsson (Tampa, FL) provided us with several sugar-modified adenosine analogues, some of which are included in this paper. All gifts are gratefully acknowledged.

Synthesis. ^{1}H-NMR spectra of the intermediates were measured in $CDCl_3$ on a JEOL JNM-FX 200 spectrometer at ambient temperature; the spectra of the end products were determined with a Brucker MSL 400 spectrometer, and the peaks were assigned by selective decoupling. All signals assigned to amino and hydroxyl groups were exchangeable with CD_3OD. The purity of the amphetamine stereoisomers was determined with a Perkin–Elmer 241 polarimeter, by measuring the optical rotation of a 2% (w/w) solution in distilled water at 589 nm and 20 °C. The value found in the literature for such a solution of the sulfuric acid salt of (*S*)-1-phenyl-2-aminopropane (D-amphetamine, sulfate) was $[\alpha]^{20}_D = 21.8$.[31] Melting points were determined in a Büchi capillary melting point apparatus and are uncorrected. Mass spectra were recorded at Solvay-Duphar (Weesp, The Netherlands), by electron impact (EI), chemical ionisation (CI), or fast atom bombardment (FAB) as ionization methods. The elemental analyses of the analogues were determined at the division of microanalysis (University of Groningen, The Netherlands).

2′-Deoxy-3′,5′-di-*O*-acetyladenosine (2*a*). Acetic anhydride (2.2 mL, 23 mmol) was added to a mixture of 2′-deoxyadenosine monohydrate (**1a**, 1.0 g 3.7 mmol), dry pyridine (15 mL), and a catalytic amount of 4-(dimethylamino)pyridine.[32] The reaction mixture was stirred at room

temperature until thin layer chromatography (TLC) analysis (methanol–dichloromethane, 12:88 (v/v)) showed the reaction to be complete (4 h). Subsequently, iced water was added, and the mixture was concentrated and evaporated twice with toluene. The product **2a**, obtained as a yellow oil (1.2 g, 3.7 mmol, quantitative yield), was sufficiently pure for further processing.

^{1}H NMR ($CDCl_3$): δ 2.1 and 2.2 (2 × s, 6H, CH_3, Ac), 2.6 (m, 1H, H2′), 2.9 (m, 1H, H2″), 4.3 (m, 3H, H4′, H5′, H5″), 5.4 (m, 1H, H3′), 6.3 (m (dd), 1H, H1′), 7.3 (s, 2H, N^6H_2), 8.0 (s, 1H, H2), 8.3 (s, 1H, H8).

2′-Deoxy-3′,5′-di-*O*-acetyl-6-iodopurine-9-β-D-riboside (3a). A solution of **2a** (0.68 g, 2.0 mmol) in isopentyl nitrite (5.5 mL, 41 mmol) and diiodomethane (5.0 mL, 62 mmol) was stirred at 60 °C under N_2 and illuminated by two unfrosted 150 W tungsten lamps supported at 0.07 m from the flask.[18] After 2 h the red-brown reaction mixture was cooled and diluted with dichloromethane. The mixture was concentrated on silica gel, and the product was purified by silica gel flash chromatography, first using hexane to remove iodine and subsequently a gradient of methanol in dichloromethane (0–10%).[17] The product **3a** was obtained as a yellow powder (0.60 g, 1.35 mmol, 68%) by crystallization from a small volume of methanol-diisopropyl ether, 1:6 (v/v).

^{1}H NMR ($CDCl_3$): δ 2.1 and 2.2 (2 × s, 6H, CH_3, Ac), 2.7 (m, 1H, H2′), 3.0 (m, 1H, H2″), 4.3 (m, 3H, H4′, H5′, H5″), 5.5 (m, 1H, H3′), 6.5 (m, 1H, H1′), 8.3 (s, 1H, H2), 8.6 (s, 1H, H8).

2′-Deoxy-N^6-cyclopentyladenosine (4a). A mixture of **3a** (0.50 g, 1.12 mmol), cyclopentylamine (0.67 mL, 6.8 mmol), and ethanol (7 mL)[33–35] was refluxed for 6 h and subsequently stirred for 5 days at room temperature. Evaporation of the reaction mixture afforded a brown oil (0.3 g). Crystallization of the product from diisopropyl ether yielded white crystals of **4a** (0.075 g, 0.24 mmol, 21%).

Mass: EI *m/z* 319 (M^+). Mp: 151 °C. ^{1}H NMR ($CDCl_3$): δ 1.53–1.60 (m, 2H, cyclopentyl), 1.65–1.81 (m, 4H, cyclopentyl), 2.08–2.16 (m, 2H, cyclopentyl), 2.30 (dd, $J_{2',2''}$ = 13.4 Hz, $J_{1',2'}$ = 5.5 Hz, 1H, H2′), 2.54 (bs, 1H, 3′-OH), 3.11 (m, 1H, H2″), 3.79 (m, 1H, H5″), 3.98 (dd, $J_{5',5''}$ = 12.9 Hz, $J_{4',5'}$ = 1.6 Hz, 1H, H5′), 4.23 (t, $J_{4',5'}$ = 2.0 Hz, 1H, H4′), 4.60 (bs, 1H, CH, cyclopentyl), 4.80 (d, $J_{2',3'}$ = 4.9 Hz, 1H, H3′), 5.94 (bs, 1H, N^6H), 6.31 (dd, $J_{1',2'}$ = 5.5 Hz, $J_{1',2''}$ = 9.5 Hz, 1H, H1′), 6.83 (bd, $J_{5',5'\text{-OH}}$ = 9.4 Hz, 1H, 5′-OH), 7.77 (s, 1H, H2), 8.32 (s, 1H, H8). Anal. ($C_{15}H_{21}N_5O_3$) C, H, N (±0.5% instead of ±0.4%).

2′-Deoxy-N^6-cyclohexyladenosine (5a). 2′-Deoxy-3′,5′-diacetyl-6-iodopurineriboside, **3a** (0.26 g, 0.58 mmol), was added to a mixture of cyclohexylamine (0.28 mL, 2.5 mmol) and ethanol (3 mL),[33–35] and stirred for 10 days at 40 °C. Purification of the product by silica gel flash chromatography, with a gradient of methanol in dichloromethane (0–5%) and crystallization from diethyl ether, resulted in **5a** as a white powder (0.04 g, 0.12 mmol, 21%).

Mass: EI *m/z* 333 (M^+). Mp: 158–159 °C. ^{1}H NMR ($CDCl_3$): δ 1.19–1.35 (m, 3H, cyclohexyl), 1.41–1.52 (m, 2H, cyclohexyl), 1.66–1.81 (m, 3H, cyclohexyl), 2.09 (dd, *J* = 12.3 Hz, *J* = 3.3 Hz, 2H, cyclohexyl), 2.26 (m, 1H, 3′-OH), 2.29 (dd, $J_{2',2''}$ = 13.5 Hz, $J_{1',2'}$ = 5.5 Hz, 1H, H2′), 3.08–3.15 (m, 1H, H2′), 3.78 (m, 1H, H5′), 3.98 (dd, $J_{5',5''}$ = 12.9 Hz, $J_{4',5'}$ = 1.4 Hz, 1H, H5′), 4.18 (bs, 1H, CH, cyclohexyl), 4.22 (s, 1H, H4′), 4.80 (d, $J_{2',3'}$ = 4.7 Hz, 1H, H3′), 5.77 (bm, 1H, N^6H), 6.31 (dd, $J_{1',2'}$ = 5.5 Hz, $J_{1',2''}$ = 9.6 Hz, 1H, H1′), 6.84 (bs, 1H, 5′-OH), 7.76 (s, 1H, H2), 8.31 (s, 1H, H8). Anal. ($C_{16}H_{23}N_5O_3.^1/_4H_2O$) C, H, N.

1-Phenyl-2-aminopropane. The sulfuric acid salts of (*S*)-, and (*R*)-1-phenyl-2-aminopropane (D- and L-amphetamine, respectively) were converted to the free base by flash chromatography over an aluminium oxide column, with dichloromethane–methanol–ammonium hydroxide, 90:10:1 (v/v). The optical rotation was $[\alpha]^{20}_D$ = 22.4 for (*S*)-1-phenyl-2-aminopropane (D-amphetamine) and $[\alpha]^{20}_D$ = −24.4 for (*R*)-1-phenyl-2-aminopropane (L-amphetamine).

2′-Deoxy-N^6(*R*)-(phenylisopropyl)adenosine (6a). A mixture of **3a** (0.40 g, 0.90 mmol), (*R*)-1-phenyl-2-aminopropane (0.61 g, 4.5 mmol), and ethanol (6 mL)[33–35] was stirred for 18 days at 40 °C. The mixture was concentrated and purified by silica gel flash chromatography, with a gradient of methanol in dichloromethane (0–10%), to yield product **6a** as a white powder (0.21 g, 0.57 mmol, 63%).

Mass: CI *m/z* 370 (MH^+). ^{1}H NMR ($CDCl_3$): δ 1.26 (d, *J* = 6.5 Hz, 3H, CH_3), 2.29 (dd, $J_{2',2''}$ = 13.4 Hz, $J_{1',2'}$ = 5.5 Hz, 1H, H2′), 2.59 (bm, 1H, 3′-OH), 2.84 (dd, *J* = 13.4 Hz, *J* = 7.2 Hz, 1H, CH_2), 3.03 (dd, *J* = 5.7 Hz, *J* = 5.4 Hz, 1H, CH_2), 3.10 (m, 1H, H2′), 3.79 (m, 1H, H5′), 3.98 (d, $J_{5',5''}$ = 12.8 Hz, 1H, H5′), 4.22 (s, 1H, H4′), 4.71 (bm, 1H, CH), 4.78 (d, $J_{2',3'}$ = 4.8 Hz, 1H, H3′), 5.75 (bm, 1H, N^6H), 6.31 (dd, $J_{1',2'}$ = 5.5 Hz, $J_{1',2''}$ = 9.7 Hz, 1H, H1′), 6.84 (bd, $J_{5',\,5'\text{-OH}}$ = 11.1 Hz, 1H, 5′-OH), 7.17–7.32 (m, 5H, Ph), 7.77 (s, 1H, H2), 8.32 (s, 1H, H8).

2′-Deoxy-N^6(*S*)-(phenylisopropyl)adenosine (7a). A mixture of **3a** (0.40 g, 0.90 mmol), (*S*)-1-phenyl-2-aminopropane (0.62 g, 4.6 mmol), and ethanol (6 mL)[33–35] was stirred for 15 days at 40 °C and subsequently concentrated. The product **7a** was obtained as a white powder (0.15 g, 0.41 mmol, 46%) after silica gel flash chromatography with a gradient of methanol in dichloromethane (0–10%) and evaporation of the solvent.

Mass: CI *m/z* 370 (MH^+). Mp: 174–175 °C. ^{1}H NMR ($CDCl_3$): δ 1.26 (d, *J* = 6.6 Hz, 3H, CH_3), 2.29 (dd, $J_{2',2''}$ = 13.4 Hz, $J_{1',2'}$ = 5.5 Hz, 1H, H2′), 2.60 (bm, 1H, 3′-OH), 2.85 (dd, *J* = 13.4 Hz, *J* = 7.2 Hz, 1H, $CH_{2'}$), 3.02 (dd, *J* = 13.4 Hz, *J* = 5.5 Hz, CH_2), 3.10 (m, 1H, H2′), 3.79 (m, 1H, H5′), 3.98 (d, $J_{5',5''}$ = 12.7 Hz, 1H, H5′), 4.23 (s, 1H, H4′), 4.71 (bm, 1H, CH), 4.78 (d, $J_{3',4'}$ = 4.8 Hz, 1H, H3′), 5.70 (bm, 1H, N^6H), 6.31 (dd, $J_{1',2'}$ = 5.5 Hz, $J_{1',2''}$ = 9.7 Hz, 1H, H1′), 6.84 (bd, $J_{5',5'\text{-OH}}$ = 12.0 Hz, 1H, 5′-OH), 7.18–7.31 (m, 5H, Ph), 7.77 (s, 1H, H2), 8.33 (s, 1H, H8). Anal. ($C_{19}H_{23}N_5O_3.^1/_4H_2O$) C,H; N: calcd, 18.73; found, 17.03.

3′-Deoxy-2′,5′-di-*O*-acetyladenosine (2b). Acetic anhydride (1.8 mL, 19 mmol) was added to a mixture of 3′-deoxyadenosine (**1b**, 0.75 g, 3.0 mmol), dry pyridine (10 mL), and a catalytic amount of 4-(dimethylamino)pyridine.[32] The reaction mixture was stirred at room temperature until TLC analysis (methanol–dichloromethane, 12:88 (v/v)) showed the reaction to be complete (4 h). Subsequently, iced water was added and the mixture was concentrated and evaporated twice with toluene. The product **2b**, obtained as a white powder (1.0 g, 3.0 mmol, quantitative yield), was sufficiently pure for further processing.

^{1}H NMR ($CDCl_3$): δ 2.1 and 2.2 (2 × s, 6H, CH_3, Ac), 2.2 (m, 1H, H3′), 2.7 (m, 1H, H3″), 4.2–4.3 (dd, $J_{5',5''}$ = 12.3 Hz, $J_{4',5'}$ = 5.7 Hz, 1H, H5′), 4.4–4.5 (dd, $J_{5',5''}$ = 12.0 Hz, $J_{4',5'}$ = 2.8 Hz, 1H, H5′), 4.6 (m, 1H, H4′), 5.7 (d, $J_{2',3'}$ = 5.7 Hz, 1H, H2′), 5.8 (bs, 2H, N^6H_2), 6.1 (s, 1H, H1′), 8.0 (s, 1H, H2), 8.3 (s, 1H, H8).

3′-Deoxy-2′,5′-di-*O*-acetyl-6-iodo-β-D-purineriboside (3b). A solution of **2b** (1.0 g, 3.0 mmol) in isopentyl nitrite, (8.5 mL, 63 mmol) and diiodomethane (7.5 mL, 93 mmol) was stirred at 60 °C under N_2, and illuminated by two unfrosted 150 W tungsten lamps supported 0.07 m from the flask.[18] After 2 h the red-brown reaction mixture was cooled and diluted with dichloromethane. The product was concentrated on silica gel and purified by silica gel flash chromatography, first using hexane to remove iodine, and subsequently a gradient of methanol in dichloromethane (0–10%).[17] The product **3b** was obtained by evaporation of the solvent as a yellow oil (0.85 g, 1.9 mmol, 63%).

^{1}H NMR ($CDCl_3$): δ 2.1 and 2.2 (2 × s, 6H, Ac), 2.6 (m, 2H, H3′), 4.2–4.3 (dd, $J_{5',5''}$ = 12.3 Hz, $J_{4',5'}$ = 2.6 Hz, 1H, H5′), 4.4–4.5 (dd, $J_{5',5''}$ = 12.3 Hz, $J_{4',5''}$ = 5.1 Hz, 1H, H5″), 4.6 (m, 1H, H4′), 5.7 (d, $J_{1',2'}$ = 5.6 Hz, 1H, H2′), 6.1 (s, 1H, H1′), 8.3 (s, 1H, H2), 8.6 (s, 1H, H8).

3′-Deoxy-N^6-cyclopentyladenosine (4b). A mixture of **3b** (0.42 g, 0.94 mmol), cyclopentylamine (0.52 mL, 5.3 mmol), and ethanol (5 mL)[33–35] was stirred for 15 days at 40 °C and subsequently concentrated. Purification by silica gel flash chromatography with a gradient of methanol in dichloromethane (0–10%) furnished **4b** as a white foam (0.18 g, 0.56 mmol, 60%).

Mass: FAB (glycerol or glycerol/Na^+/K^+ as matrix) *m/z* 320 (MH^+). Mp: 164 °C. ^{1}H NMR ($CDCl_3$): δ 1.49–1.82 (m, 6H, cyclopentyl), 2.06–2.18 (m, 2H, cyclopentyl), 2.29 (m, 1H, H3′), 2.53 (m, 1H, H3″), 3.57 (m, 1H, H5′), 3.96 (dd, $J_{5',5''}$ = 12.5 Hz, $J_{4',5'}$ = 1.0 Hz, 1H, H5′), 4.52 (m, 2H, H4′ and CH, cyclopentyl),

5.04 (dd, $J_{2',3'}$ = 7.8 Hz, $J_{1',2'}$ = 5.9 Hz, 1H, H2'), 5.30 (bs, 1H, 2'-OH), 5.64 (d, $J_{1',2'}$ = 5.7 Hz, 1H, H1'), 5.95 (bm, 1H, N^6H), 6.13 (d, $J_{5',5'\text{-OH}}$ = 5.7 Hz, 1H, 5'-OH), 7.27 (s, 1H, H2), 7.72 (s, 1H, H8). Anal. ($C_{19}H_{23}N_5O_3$) C, H, N.

3'-Deoxy-N^6-cyclohexyladenosine (5b). A mixture of **3b** (0.42 g, 0.94 mmol) and cyclohexylamine (0.44 mL, 3.9 mmol) in ethanol (5 mL)[33-35] was stirred for 14 days at 40 °C. After concentration, the product **5b** was obtained by silica gel flash chromatography with a gradient of methanol in dichloromethane (0–10%) as a white foam (0.17 g, 0.51 mmol, 54%).

Mass: EI *m/z* 333 (M^+), CI *m/z* 334 (MH^+). Mp: 166–167 °C. ^{1}H NMR ($CDCl_3$): δ 1.19–1.70 (m, 6H, cyclohexyl), 1.78 (m, 2H, cyclohexyl), 2.04–2.13 (m, 2H, cyclohexyl), 2.22–2.32 (m, 1H, H3''), 2.57 (m, 1H, H3'), 3.57 (m, 1H, H5'), 3.98 (dt, $J_{5',5''}$ = 12.7 Hz, $J_{4',5'}$ = 1.8 Hz, 1H, H5'), 4.14 (m, 1H, CH, cyclohexyl), 4.31 (bs, 1H, 2'-OH), 4.53 (m, 1H, H4'), 5.04 (m, 1H, H2'), 5.65 (d, $J_{1',2'}$ = 5.9 Hz, 1H, H1'), 5.78 (bm, 1H, N^6H), 6.01 (bd, $J_{5',5'\text{-OH}}$ = 9.3 Hz, 1H, 5'-OH), 7.76 (s, 1H, H2), 8.26 (s, 1H, H8). Anal. ($C_{16}H_{23}N_5O_3$) C, H, N.

3'-Deoxy-N^6(*R*)-(phenylisopropyl)adenosine (6b). A mixture of **3b** (0.40 g, 0.90 mmol), (*R*)-1-phenyl-2-aminopropane (0.56 g, 4.2 mmol), and ethanol (4 mL)[33-35] was stirred for 15 days at 40 °C. The product **6b** was concentrated, purified by silica gel flash chromatography with a gradient of methanol in dichloromethane (0–10%), and crystallized from a small volume of dichloromethane and petroleum ether, yielding a white powder (0.11 g, 0.30 mmol, 33%).

Mass: EI *m/z* 369 (M^+), CI *m/z* 370 (MH^+). Mp: 139–140 °C. ^{1}H NMR ($CDCl_3$): δ 1.28 (d, *J* = 6.6 Hz, 3H, CH_3), 2.27 (dt, $J_{3',3''}$ = 12.6 Hz, $J_{2',3'}$ = $J_{3',4'}$ = 8.6 Hz, 1H, H3'), 2.55–2.63 (m, 1H, H3''), 2.83 (dd, *J* = 13.5 Hz, *J* = 7.3 Hz, 1H, CH_2), 3.01 (dd, *J* = 13.5 Hz, *J* = 5.6 Hz, 1H, CH_2), 3.57 (m, 1H, H5'), 3.97 (dt, $J_{5',5''}$ = 12.7 Hz, $J_{4',5'}$ = $J_{5',5'\text{-OH}}$ = 1.9 Hz, 1H, H5'), 4.28 (bs, 1H, 2'-OH), 4.53 (m, 1H, H4'), 4.66 (bs, 1H, CH), 5.05 (m, 1H, H2'), 5.64 (d, $J_{1',2'}$ = 5.8 Hz, 1H, H1'), 5.73 (bm, 1H, N^6H), 5.98 (bd, $J_{5',5'\text{-OH}}$ = 9.5 Hz, 1H, 5'-OH), 7.17–7.31 (m, 5H, Ph), 7.75 (s, 1H, H2), 8.28 (s, 1H, H8).

3'-Deoxy-N^6(*S*)-(phenylisopropyl)adenosine (7b). A mixture of **3b** (0.40 g, 0.90 mmol), (*S*)-1-phenyl-2-aminopropane (10.56 g, 4.1 mmol), and ethanol (4 mL)[33-35] was stirred for 14 days at 40 °C. The solution was concentrated, and product was purified by silica gel flash chromatography with a gradient of methanol in dichloromethane (0–10%). Crystallization of **7b** from dichloromethane yielded a white powder (0.12 g, 0.33 mmol, 37%).

Mass: EI *m/z* 369 (M^+), CI *m/z* 370 (MH^+). Mp: 184 °C. ^{1}H NMR ($CDCl_3$): δ 1.26 (d, *J* = 6.6 Hz, 3H, CH_3), 2.26 (dt, $J_{3',3''}$ = 12.6 Hz, $J_{2',3'}$ = $J_{3',4'}$ = 8.6 Hz, 1H, H3'), 2.57 (m, 1H, H3'), 2.83 (dd, *J* = 13.5 Hz, *J* = 7.2 Hz, 1H, CH_2), 3.03 (dd, *J* = 13.5 Hz, *J* = 5.8 Hz, 1H, CH_2), 3.57 (m, 1H, H5'), 3.98 (dt, $J_{5',5''}$1H, H5'), 4.07 (bs, 1H, 2'-OH), 4.52 (m, 1H, H4'), 4.55 (bs, 1H, CH), 5.02 (m, 1H, H2'), 5.66 (d, $J_{1',2'}$ = 5.8 Hz, 1H, H1'), 5.78 (bm, 1H, N^6H), 5.95 (bd, $J_{5',5'\text{-OH}}$ = 9.5, 1H, 5'-OH), 7.18–7.32 (m, 5H, Ph), 7.78 (s, 1H, H2), 8.30 (s, 1H, H8). Anal. ($C_{19}H_{23}N_5O_3$) C, H, N.

Receptor Binding Studies. The adenosine A_1 binding assay was carried out on membranes of rat cortical brains. Membranes were prepared according to the method of Lohse *et al.*,[36] except that the membranes were incubated with 2 units/mL ADA at 37 °C before storage, as described by Pirovano *et al.*[8] Protein concentrations were measured with the BCA method.

The adenosine A_1 binding assays were performed with 0.4 nM [^{3}H]DPCPX as the radioligand (K_d 0.28 nM[8]). The assays were performed as originally described by Lohse *et al.*[37] Nonspecific binding was determined in the presence of 10 μM *R*-PIA. The adenosine A_1 displacement studies were carried out both in the absence and in the presence of 1 mM GTP. The displacement by the deoxy analogues of adenosine was determined in the presence of 0.1 μM deoxycoformycin to inhibit the degradation by adenosine deaminase of those ligands.

The adenosine A_{2a} assay was carried out on rat striatal membranes. Striata were dissected from rat brain tissue according to the method described by Glowinsky and Iversen[38] and striatal membranes were prepared according to Bruns *et al.*,[39] except that the membranes were incubated with 2 units/mL ADA at 37 °C before storage.[8] The binding of 6 nM [^{3}H]-CGS 21680 (K_d 14.5 nM, results not shown) to the A_{2a} receptors of rat striatal membranes was determined as originally described by Johansson *et al.*,[40] but CPA was used in a 50 μM concentration to determine the nonspecific binding of [^{3}H]CGS 21680 and 75 μg of striatal protein.

The IC_{50} values are means of three independent experiments performed in duplicate, unless indicated otherwise.

Data Analysis. Apparent K_i values were computed from the displacement curves by nonlinear regression of the competition curves with Inplot (Graph Pad, San Diego, CA). GTP shifts were determined by dividing the apparent K_i value of the ligand in the presence of GTP by the apparent K_i value in the absence of GTP.

***In Vivo* Pharmacology.** The pharmacological studies were carried out as described by Mathôt *et al.*[25] Two days before the experiment, canulas were implanted in adult male normotensive Wistar rats (200–250 g). The rats received an intravenous infusion of the ligands during 15 min. The heart rate was captured from the arterial blood pressure signal, and both were monitored during the experiments. The recording of heart rate and blood pressure was started 30 min prior to the administration, for baseline determination. The amount of drug administered was related to the dose of CPA that was shown to cause a maximal effect (200 μg/kg)[25] and to the affinity of the compound. However, 2'-deoxy-*R*-PIA was administered in a lower dose, due to the limited solubility of this compound. Approximately equipotent doses, based on the receptor affinity and molecular weight of the ligand, were administered, resulting in doses of 176 μg/kg for R-PIA, 12 mg/kg for 3'-deoxy-R-PIA, and 80 mg/kg for 2'-deoxy-R-PIA (42 x K_i, 93 x K_i, and 26 x K_i, respectively).

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