N^6,C8-Disubstituted Adenosine Derivatives as Partial Agonists for Adenosine A_1 Receptors†

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The synthesis and biological evaluation of N^6,C8-disubstituted derivatives of adenosine as potential partial agonists for adenosine receptors is described. *Via* three routes, two series of compounds were prepared, *viz.*, N^6-cyclopentyladenosine derivatives **3a**−**e** and C8-(cyclopentylamino)adenosine analogs **3e** and **9a**−**d**, respectively. The X-ray structure determination of one of these compounds, N^6-ethyl-8-(cyclopentylamino)adenosine (**9b**), was carried out (orthorhombic, space group $P2_12_12_1$ (No. 19) with $a = 11.039(3)$, $b = 8.708(2)$, and $c = 24.815(12)$ Å, $Z = 4$, $R1 = 0.0974$, $R2_w = 0.2455$). Due to intramolecular hydrogen bonding, the ribose moiety of this compound is in an *anti* conformation. The compounds were tested *in vitro* in radioligand binding studies, yielding their affinities for A_1 and A_{2a} adenosine receptors. All compounds appeared A_1 selective, with affinities in the high nanomolar, low micromolar range. On A_1 receptors the so-called GTP shift was also determined, *i.e.*, the ratio between the affinities measured in the presence and absence of 1 mM GTP. All GTP shifts (values between 1.1 and 3.8) were lower than the GTP shift for CPA (6.0). This GTP shift appeared indicative for partial agonism *in vivo*, since the N^6-cyclopentyladenosine derivatives showed lower intrinsic activities than the prototypic full agonist N^6-cyclopentyladenosine on the decrease in heart rate in conscious, normotensive rats.

Introduction

Extracellular adenosine has significant physiological activity. Through its interaction with adenosine receptors, the compound mediates a large variety of effects, *e.g.*, on the cardiovascular, immune, and central nervous systems. Three subclasses of adenosine receptors have been identified by pharmacological and molecular biological techniques, *viz.*, A_1, A_2, and A_3 receptors. The A_2 receptors have been further divided into A_{2a} and A_{2b} receptors. A large number of both A_1 and A_{2a} selective ligands—both agonists and antagonists—are available (for a recent review and references therein, see ref 1). This is not yet the case for A_{2b} receptors, whereas only A_3 selective agonists have been reported so far.[2]

Due to the ubiquity of adenosine receptor subtypes in the body, the desired activity profile of adenosine receptor ligands is often confounded by serious side effects. In particular, the profound hemodynamic disturbances caused by adenosine receptor agonists have largely precluded their use for other therapeutic targets. In this respect, the design of partial agonists seems worthwhile, since virtually all known agonists behave as full agonists.

Recently, we have reported on the synthesis and biological activity of 8-substituted adenosines.[3] Among the 8-amino-substituted derivatives, several analogs appeared to be partial agonists in a variety of *in vitro* and *in vivo* test systems. However, their affinity toward adenosine receptors was modest. In the present study we have synthesized N^6,C8-disubstituted adenosines. These compounds, while retaining partial agonism, proved to be more potent than the C8-monosubstituted series, with an additional gain in A_1 receptor selectivity.

Chemistry

In our search for the preparation of 8-aminoalkyl-derived N^6-substituted adenosines, we developed and evaluated three possible and different synthetic routes as depicted in Schemes 1−3. The first one is based on the introduction of an 8-alkylamino substituent on the N^6-derivatized 8-halogenide adenosine moiety, whereas the second one concerns the introduction of a 6-alkylamino substituent on the 8-aminoalkyl-derivatized 6-chloropurine riboside as key intermediate. Alternatively, the desired compounds could be obtained by a selective introduction of the alkylamino substituent on the 6,8-dichloropurine riboside, yielding one of the above-mentioned key intermediates, and its subsequent amination.

In the first route (see Scheme 1), our attention was focused on the preparation of key intermediate **2** by direct chlorination of 2′,3′,5′-tri-*O*-acetyl-N^6-cyclopentyladenosine (**1**). Starting compound **1** was easily available by substitution of 6-chloropurine riboside with cyclopentylamine[4] and subsequent acetylation of the formed N^6-cyclopentyladenosine in 78% overall yield. Unfortunately, several attempts to convert **1** into 8-bromo-substituted nucleoside by direct bromination with Br_2/H_2O (pH 4.0), *N*-bromoacetamide, $Br_2/Na_2HPO_4/H_2O$ (pH 7.0), or *N*-bromosuccinimide met with little success. The failure to prepare this intermediate compound was due to cleavage of the glycosidic bond, as

† Dedicated to Professor Ernst Mutschler on the occasion of his 65th birthday.

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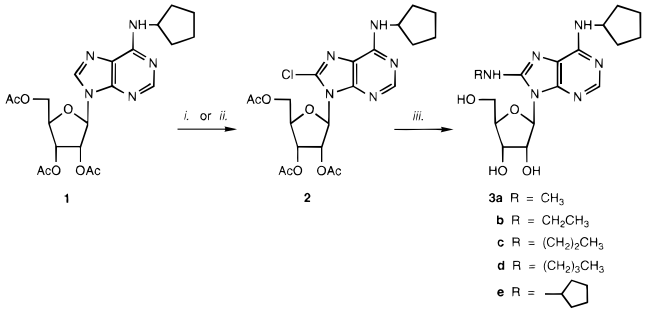
Scheme 1[a]



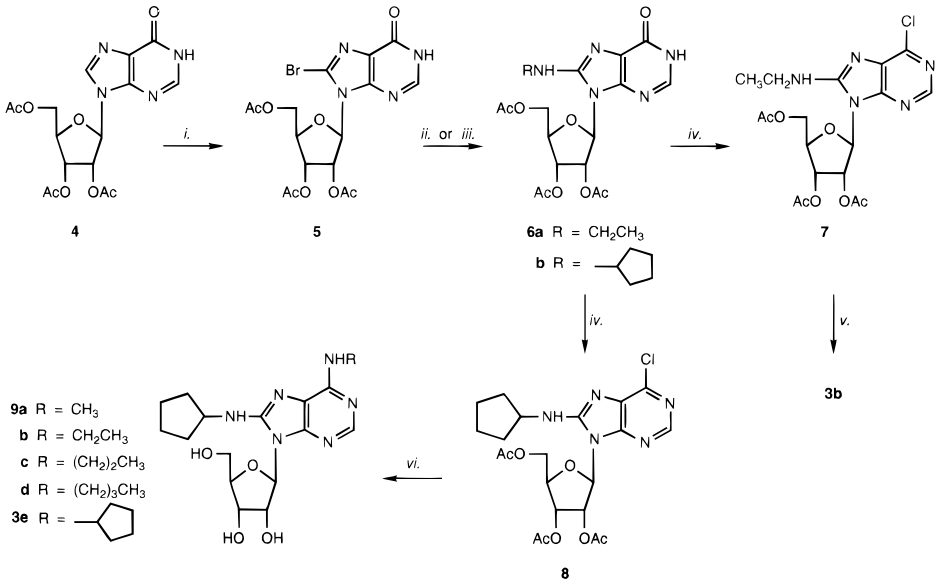
[a] (i) *m*-CPBA, HCl/DMF; (ii) NCS, $ClCH_2CH_2Cl$; (iii) RNH_2/H_2O or dioxane, Δ.

proved by the presence of the acetylated riboside after workup in the first two bromination reactions, or to inertness of the purine ring for electrophilic or radical attack, as proved by the recovery of the starting material after workup in the last two reactions. On the other hand, **2** could be isolated in low yields after chlorination with *m*-chloroperbenzoic acid in an anhydrous hydrochloric acid/DMF solution[5] (29%) or with *N*-chlorosuccinimide in dry dichloroethane[6] (31%). Intended nucleophilic displacement of the chlorine atom in **2** with different monoalkylamines at room temperature resulted only in removal of the acetyl groups, but heating for 1 or more days at temperatures between 50 and 70 °C yielded smooth conversion with simultaneous deacetylation. After isolation and purification, the products **3a**−**e** were obtained in good yields (75−83%). The homogeneity and identity of these products were established by NMR and mass spectroscopy as well as by elemental analysis.

The second route (Scheme 2) started with the fully acetylated inosine **4**, obtained by a known procedure[7,8] that was slightly modified. Bromination of **4** with a saturated $Br_2/Na_2HPO_4/H_2O$ solution (pH 7.0)[9] gave the 8-bromoinosine derivative **5**. Use of this bromination procedure resulted in a substantially higher yield (85%) than bromination with *N*-bromoacetamide (52%).[10] Moreover, it is noteworthy that the present order of reactions has a beneficial effect on the overall yield of **5** (83%) when compared with the reverse order, *i.e.*, bromination[11] and subsequent acetylation[12] (overall 48%). Substitution of **5** with ethylamine or cyclopentylamine and subsequent acetylation of the intermediate products afforded **6a**,**b** in 81% and 82% yields, respectively. Chlorination of **6a**,**b** with an excess of dimethylchloromethyleneammonium chloride (DMCMAC)[13] in chloroform furnished the 6-chloro derivatives **7** and **8** in 56% and 58% yields, respectively. Amination at the 6-position of derivative **7** with cyclopentylamine yielded **3b**, which was in every aspect identical with the product obtained from **2** following the route in Scheme 1. Although this procedure was useful and resulted in products of high quality with acceptable yields (±35%), the preparation of each intermediate in order to obtain the remaining target compounds **3a**,**c**,**d** made this route quite laborious and time-consuming in comparison with the first route described before, and further application was abandoned. We also explored the substitution of the 6-chloro function in **8** with the corresponding amines used above. The formation of the adenosine derivatives **9a**−**d** and **3e**, respectively, could indeed be effected in good yields (76−85%) in a similar way as described above. The reaction time was slightly shorter and the reaction temperature lower during the substitution at the 6-position than with 8-substitution, probably caused by decrease in steric hindrance. Elemental analyses, NMR, and high-resolution mass spectroscopy of compounds **9a**−**d** supported their structures. Moreover, the structure of one of them, namely, **9b**, was corroborated with X-ray single-crystal structure determination (see Figure 1).

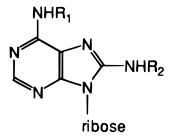
For the preparation of compound **2** or **8** *via* the third method (Scheme 3), 8-bromoinosine derivative **5** was used as starting material. Substitution at the 6-position and simultaneous exchange of the 8-bromo function by treatment with a DMCMAC solution (2 M) in $CHCl_3$[13,14] gave the 6,8-dichloropurine derivative **10**[12,15] in 91% yield after purification by column chromatography. However, nucleophilic attack on **10** with cyclopentyl-

Scheme 2[a]



[a] (i) Br_2, Na_2HPO_4/H_2O; (ii) a. $EtNH_2/H_2O$, Δ, b. Ac_2O/DMAP/pyridine; (iii) a. cyclopentylamine, dioxane, Δ, b. Ac_2O/DMAP/pyridine; (iv) DMCMAC/$CHCl_3$; (v) cyclopentylamine/dioxane, Δ; (vi) RNH_2/H_2O or dioxane, Δ.

Table 1. Adenosine A_1 and A_{2a} Receptor Affinities (Apparent K_i Values for the A_1 Receptor in the Presence and Absence of GTP) and GTP Shifts for the A_1 Receptor of the C8-Amino Monosubstituted and C8-Amino-N^6-disubstituted Adenosine Analogs (Data for the monosubstituted derivatives and CPA are from Van der Wenden *et al.*[3])



			K_i A_1[a] (μM)			
compd	R_1 (N^6)	R_2 (C8)	−GTP	+GTP	GTP shift	K_i A_2,[b] −GTP (μM)
3a	cyclopentyl	-CH_3	0.26 (0.16−0.36)	0.98 (0.88−1.07)	3.8 ± 1.5	20.8 (18.1−23.4)
3b	cyclopentyl	-CH_2-CH_3	0.47 (0.34−0.59)	1.33 (1.10−1.56)	2.8 ± 0.9	10.1 (8.80−11.3)
3c	cyclopentyl	-$(CH_2)_2$-CH_3	0.35 (0.27−0.43)	1.05 (0.89−1.21)	3.0 ± 0.8	7.67 (6.61−8.72)
3d	cyclopentyl	-$(CH_2)_3$-CH_3	0.50 ± 0.08	1.13 ± 0.18	2.3 ± 0.5	12.4 ± 4.0
3e	cyclopentyl	cyclopentyl	1.09 ± 0.08	1.28 ± 0.08	1.2 ± 0.1	45.5 ± 4.0
9a	-CH_3	cyclopentyl	5.61 ± 2.39	15.7 ± 4.0	2.8 ± 1.4	101 (111−92)
9b	-CH_2-CH_3	cyclopentyl	0.31 ± 0.10	1.26 ± 0.23	4.1 ± 1.5	30.2 ± 5.1
9c	-$(CH_2)_2$-CH_3	cyclopentyl	1.59 ± 0.90	1.76 ± 0.29	1.1 ± 0.6	59.4 (44.9−74.0)
9d	-$(CH_2)_3$-CH_3	cyclopentyl	0.76 ± 0.21	0.86 ± 0.16	1.1 ± 0.4	35.3 (19.4−51.1)
	-H	-CH_3	2.42 ± 0.36	18.6 ± 1.9	7.7 ± 1.4	1.85 ± 0.36[c]
	-H	-CH_2-CH_3	6.56 ± 0.71	23.8 ± 1.3	3.6 ± 0.4	3.97 ± 0.64[c]
	-H	-$(CH_2)_2$-CH_3	5.96 ± 0.68	34.7 ± 2.2	5.8 ± 0.8	4.96 ± 1.73[c]
	-H	-$(CH_2)_3$-CH_3	11.4 ± 1.1	45.5 ± 4.2	4.0 ± 0.5	11.5 ± 1.9[c]
	-H	cyclopentyl	na	−	−	na
CPA (N^6-cyclopentyladenosine)			0.0059 (0.0058−0.0060)	0.035 (0.030−0.040)	6.0 ± 0.9	0.58 ± 0.12

[a] Displacement of 0.4 nM [^{3}H]DPCPX (K_D = 0.28 nM) from rat cortical membranes (n = 2−3). [b] Displacement of 5.6 nM [^{3}H]CGS 21680 (K_D = 14.5 nM) from rat striatal membranes, unless stated otherwise (n = 2−3). [c] Displacement of 4 nM [^{3}H]NECA (K_D = 15.3 nM) in the presence of 50 nM CPA from rat striatal membranes[31] (n = 3). −, not determined; na, not active (<50% displacement at 10^{-4} M ligand).

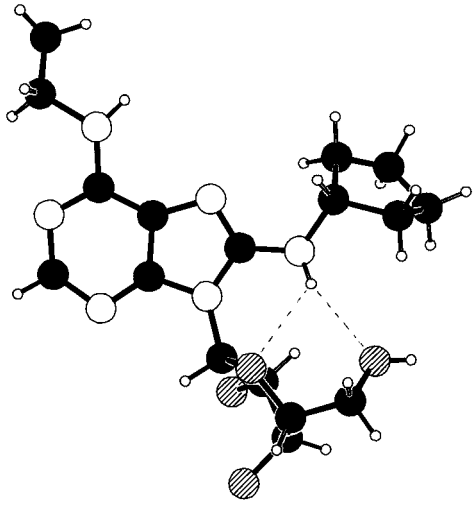
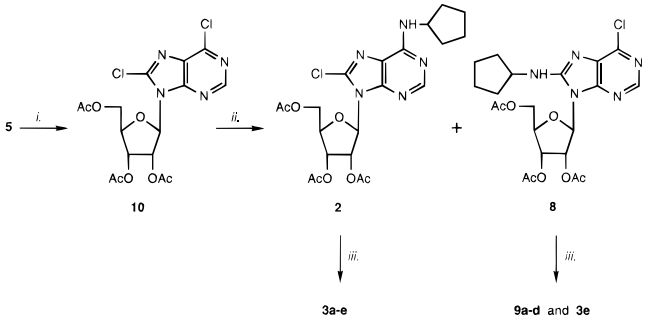


Figure 1. X-ray structure of compound **9b**: small open circles, hydrogen atoms; large filled circles, carbon atoms; open circles, nitrogen atoms; dashed circles, oxygen atoms; dashed lines, possible hydrogen bonds.

amine showed no selectivity in displacement of the 8-chloro function over the 6-chloro function in contrast to the results presented by Sutcliffe and Robins[16] and Szekeres *et al.*[12] when ammonia was used. The two isomeric products (**2** and **8**) formed were obtained in approximately equal amounts according to TLC analysis and could be isolated after separation by column chromatography in 33% and 41% yields, respectively. On the basis of comparison of NMR spectra or retention times of both compounds with that of the intermediate obtained *via* route in Scheme 1 and that one obtained *via* route in Scheme 2, the product with higher R_f value was identified as **2** and the one with lower R_f value as **8**. The chlorinated derivatives could subsequently be used for conversion into **3a**−**e** or **9a**−**d**, as described before.

Scheme 3[a]



[a] (i) DMCMAC/$CHCl_3$; (ii) a. cyclopentylamine/dioxane, 40 °C, b. Ac_2O/pyridine, c. separation by column chromatography; (iii) RNH_2/H_2O or dioxane, Δ (see Scheme 1).

Compound **9b** was studied by single-crystal X-ray analysis. Its structure is shown in Figure 1.

Biological Evaluation

All compounds were tested in radioligand binding assays to determine their affinities toward adenosine A_1 and A_{2a} receptors in rat brain cortex and rat striatum, respectively (Table 1). For A_1 receptors the tritiated antagonist [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine (DPCPX) was used. Displacement experiments were performed in the absence and presence of 1 mM GTP, allowing the determination of the so-called GTP shift (*i.e.*, the ratio of the apparent K_i values in the presence and absence of GTP, respectively). This shift is an *in vitro* parameter often indicative for intrinsic activity.[17] Since no radiolabeled antagonist is available for A_{2a} receptors, the tritiated agonist [^{3}H]CGS21680 was used. This prohibited the determination of a GTP

Table 2. Pharmacodynamics of 8-Alkylamino Derivatives of N^6-Cyclopentyladenosines **3a**−**e** in Normotensive, Conscious Rats[a]

compd	dose (mg/kg)	*n*	heart rate base line (bpm)	max reduction (%)	MAP base line (mmHg)	max reduction (%)
CPA	0.20	6	345 ± 16	54 ± 3	109 ± 5	61 ± 2
8-(methylamino)-CPA (**3a**)	4.8	6	334 ± 16	34 ± 4	105 ± 6	50 ± 5
8-(ethylamino)-CPA (**3b**)	4.8	6	350 ± 15	25 ± 4	100 ± 3	24 ± 3
8-(propylamino)-CPA (**3c**)	4.8	6	344 ± 9	20 ± 5	97 ± 4	15 ± 4
8-(butylamino)-CPA (**3d**)	8.0	6	348 ± 12	8 ± 5	94 ± 3	11 ± 3
8-(cyclopentylamino)-CPA (**3e**)	8.0	5	329 ± 17	2 ± 3	96 ± 1	3 ± 2

[a] The compounds were dissolved in 20% (v/v) DMSO and administered intravenously during 15 min. Data are presented as means ± SE.

shift on A_{2a} receptors, and all experiments on A_{2a} receptors were done in the absence of GTP. Compounds **3a**−**e** were also tested *in vivo*. Heart rate and mean arterial pressure were recorded of conscious, normotensive, and unrestrained rats that received an intravenous infusion of the drugs. The results are presented in Table 2.

Results and Discussion

Three different synthetic routes gave access to the target compounds **3a**−**e** and/or **9a**−**d**. The first one seems useful for straightforward synthesis of **3a**−**e**, although the overall yields (ca. 20%) are rather low. The second route is most suitable for the synthesis of compounds **9a**−**d** or **3e**, despite the again moderate overall yields (ca. 30%). Although the substitution of the dichloropurine derivative **10** in Scheme 3 was not regioselective, this method appeared quite efficient, based on the fact that both intermediates (**2** and **8**) formed could be individually converted after separation into the required target compounds in higher overall yields (ca. 46%).

In Table 1 radioligand binding data are gathered for all synthesized disubstituted end products. For reasons of comparison, data of previously reported C8-monosubstituted adenosines[3] are also incorporated. Substitution of the exocyclic N^6-amino group by cyclopentyl, as in **3a**−**e**, led to compounds that showed higher affinity for the A_1 receptors than the corresponding monosubstituted ones, in both the absence and presence of GTP. On average this increase in affinity was at least 10-fold. In contrast, A_2 receptor affinities were consistently lower throughout the series. This is fully in line with the fact that N^6-cyclopentyl substitution enhances potency and selectivity for adenosine A_1 receptors.[1] Compounds **9a**−**d** were somewhat less potent than the corresponding compounds **3a**−**d**.

Obviously, C8-substitution caused a significant drop in affinity when compared to the reference compound CPA (see also Table 1). A few reports deal with 8-substituted adenosines. Bruns,[18] Jacobson,[19] and Olsson[20] concluded that substituents at this carbon atom often lead to inactive compounds, *e.g.*, for 8-bromoadenosine. It was suggested that such substituents force the ribose ring into the *syn* conformation, whereas the *anti* conformation is thought to be essential for receptor binding. From the crystal structure of **9b** (Figure 1), it is apparent that the *anti* conformation is compatible with 8-substitution. In this particular case a bifurcated hydrogen bond appears to be formed between the -N*H*- element in the 8-position and the 5′-*O*H group in the ribose moiety (distance H···O, 2.3 Å; angle N−H···O, 147°) and the oxygen atom in the ribose ring (distance H···O, 2.4 Å; angle N−H···O, 127°), respectively. Apparently, the bulk tolerance in the "C8-region" is greater than previously thought. The concomitant increases in size and lipophilicity of the C8 substituents in **3a**−**e**, as in the monosubstituted compounds in Table 1, do not influence receptor affinity very much.

Interestingly, 8-substitution also lowered GTP shift values. Full agonists such as CPA and R-PIA (data not shown) consistently have GTP shifts of ca. 6 in our rat membrane preparations. All N^6,C8-disubstituted derivatives have significantly lower values, *viz.*, 1.1−3.8. If this *in vitro* parameter reflected *in vivo* partial agonism, all compounds synthesized would behave as partial agonists. Therefore we considered it worthwhile to further investigate this aspect. We tested the more potent series **3a**−**e** and N^6-cyclopentyladenosine (CPA) in conscious, normotensive rats (Table 2). Upon infusion of the compounds at dosages that caused maximal effects—3-fold higher dosages did not produce higher effects—it was found that CPA, as the reference full agonist, caused a severe bradycardia and hypotension.[21] Heart rate was lowered from 345 bpm (preadministration levels) to ca. 160 bpm at the end of the 15 min infusion, a reduction of 54%. Similarly, mean arterial blood pressure (MAP) was only 43 mmHg after administration of CPA, down by 61% from 109 mmHg. All 8-substituted CPA analogs caused lower maximum reductions in varying degree. With increasing chain length, going from methyl to cyclopentyl substitution, both cardiovascular parameters were less influenced. The maximum reductions in heart rate and MAP were highly correlated ($r = 0.96$). The GTP shifts of CPA and compounds **3a**−**e** appear to be correlated with the maximum reductions possible ($r = 0.98$ for heart rate vs GTP shifts, $r = 0.93$ for MAP vs GTP shifts). Recently, we have shown that the *in vivo* modulation of heart rate is a sensitive pharmacologic end point for A_1 receptor activation,[21] which seems to be corroborated by this high correlation between heart rate reductions and GTP shifts on A_1 receptors.

Conclusion

Our previous efforts in synthesizing partial agonists have led to the identification of theophylline 7-riboside,[22] C8-monosubstituted adenosines,[3] and deoxyribose derivatives of N^6-substituted adenosines[23] as such compounds. The present series of compounds is among the derivatives with the highest A_1 receptor affinity and "controllable" intrinsic activity. It is anticipated that these compounds may be useful tools in pharmacology and biochemistry. Rapid receptor downregulation and desensitization have been demonstrated for full agonists for adenosine receptors. Partial agonists may behave less outspoken in this respect. Second, partial agonism

may induce selectivity of effects due to differences in receptor−effector coupling in various tissues. It would therefore be worthwhile to study the effects of the compounds described on other physiological processes mediated by adenosine receptors, *e.g.*, inhibition of lipolysis and anticonvulsive activity.

Experimental Section

Chemicals and Solvents. Dimethylchloromethyleneammonium chloride (DMCMAC) solution in $CHCl_3$ (2 M) was prepared from dry dimethylformamide and freshly distilled thionyl chloride as described before.[14] All other reagents were of analytical grade.

Chromatography. Thin-layer chromatography (TLC) was carried out using silica F_{254} preformed layers 0.1 mm thick on a plastic backing (Schleicher and Schüll DC Fertigfolien F1500 LS254) in the following mobile phases: A, CH_2Cl_2/CH_3OH, 90/10, v/v; B, ethyl acetate/acetone, 3/1, v/v; C, CH_2Cl_2/CH_3OH, 96/4, v/v; D, diethyl ether/hexane, 4/1, v/v; and E, CH_2Cl_2/CH_3OH, 4/1, v/v. Spots were visualized either under UV (254 or 356 nm) light or by spraying with sulfuric acid/methanol (1:4) or molybdate reagent (H_2O/concentrated H_2SO_4/$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$/$(NH_4)_2Ce(SO_4)_4\cdot 2H_2O$, 90/10/2.5/1, v/v/w/w) and charring at 140 °C for a few minutes. Preparative column chromatography was performed on silica gel (230−400 mesh ASTM), suspended in CH_2Cl_2.

Instruments and Analyses. Elemental analyses (results within ±0.4%) were done for C,H,N (Department of Microanalysis, Groningen University, Groningen, 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 ppm (δ) relative to tetramethylsilane (TMS) as internal standard. All high-resolution mass spectra were measured on a Finnigan MAT TSQ-70 mass spectrometer equipped with an electrospray interface (EI). Experiments were done in positive ionization mode. Samples were dissolved in CH_2Cl_2, diluted with 80/20 methanol/water + 1% acetic acid, and introduced by means of constant infusion at a flow rate of 1 μL/min.

Syntheses. 2′,3′,5′-Tri-*O*-acetyl-N^6-cyclopentyladenosine (1). To a solution of dry 6-chloro-9-β-D-ribofuranosylpurine (1.44 g, 5.00 mmol) in dry EtOH (50 mL) were added Et_3N (0.9 mL, 6.5 mmol) and cyclopentylamine (1.0 mL, 10.1 mmol); the mixture was refluxed for 16 h. After concentration *in vacuo*, the residue was evaporated with toluene (2 × 25 mL) and subsequently dried by evaporation with pyridine (2 × 10 mL). The crude N^6-cyclopentyladenosine was dissolved in pyridine (25 mL), and Ac_2O (2.83 mL, 30.0 mmol), together with a catalytic amount of DMAP, was added. After stirring for 3 h, the reaction was quenched by addition of MeOH (3 mL) and the mixture concentrated under reduced pressure to dryness. The residue was dissolved in CH_2Cl_2 (75 mL) and washed with an aqueous $NaHCO_3$ solution (10%, 50 mL) and H_2O (50 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated. The product was purified by column chromatography eluted with a 0−5% gradient of MeOH in CH_2Cl_2 to give **1** as a white foam: yield 1.81 g (78%); R_f 0.51 (C); ^{1}H NMR ($CDCl_3$) δ 8.38 (s, 1H, H-8), 7.90 (s, 1H, H-2), 6.19 (d, $J_{1',2'} = 5.5$ Hz, 1H, H-1′), 5.93 (t, $J_{1',2'} = J_{2',3'} = 5.5$ Hz, 1H, H-2′), 5.78 (bd, $J = 7.1$ Hz, 1H, 6-NH, exchangeable with CD_3OD), 5.67 (t, $J_{2',3'} = J_{3',4'} = 5.5$ Hz, 1H, H-3′), 4.75−4.52 (m, 1H, N-C*H*, cyclopentyl), 4.46−4.33 (m, 3H, H-4′/H-5′/H-5″), 2.16−1.95 (m, 2H, N-CH-C*H*H, cyclopentyl) coinciding with 2.14, 2.08, 2.00 (3s, 9H, $3CH_3$, Ac), 1.85−1.51 (m, 6H, N-CH-CH*H*, N-CH-CH_2-C*H*$_2$, cyclopentyl); ^{13}C NMR ($CDCl_3$) δ 169.8, 169.0, 168.8 (3C=O, Ac), 154.1 (C-6, C-4), 152.8 (C-2), 137.5 (C-8), 119.5 (C-5), 85.6 (C-1′), 79.7 (C-4′), 72.7 (C-2′), 70.2 (C-3′), 62.7 (C-5′), 51.9 (N-*C*H, cyclopentyl), 32.8, 32.4 (N-CH-*C*H$_2$, cyclopentyl), 23.2 (N-CH-CH_2-*C*H$_2$, cyclopentyl), 20.2, 20.0, 19.9 ($3CH_3$, Ac); MS m/z 462 $(M + 1)^+$.

2′,3′,5′-Tri-*O*-acetyl-8-chloro-N^6-cyclopentyladenosine (2): Method A. To a stirred solution of **1** (461 mg, 1.0 mmol), dried by evaporation with DMF (2 × 5 mL), in HCl/DMF (0.5 M, 3.0 mL, 1.5 mmol) was added a solution of *m*-chloroperbenzoic acid (*m*-CPBA; 50−60%, 1.0 g, 3.0 mmol), dried by evaporation with DMF (2 × 5 mL), in DMF (4.0 mL). After 1 h, an additional amount of *m*-CPBA (500 mg, 1.5 mmol), as solution in DMF (2.0 mL), was added and stirring was continued for another 16 h. The mixture was concentrated to a small volume, and the residue was purified by column chromatography eluted with a 0−4% gradient of acetone in CH_2Cl_2 to give **2** as a white foam: yield 144 mg (29%); R_f 0.55 (C), 0.23 (D); ^{1}H NMR ($CDCl_3$) δ 8.33 (s, 1H, H-2), 6.34 (dd, $J_{1',2'} = 4.4$ Hz, $J_{2',3'} = 6.0$ Hz, 1H, H-2′), 6.10 (d, $J_{1',2'} = 4.5$ Hz, 1H, H-1′), 5.93 (t, $J_{2',3'} = J_{3',4'} = 5.9$ Hz, 1H, H-3′), 5.73 (d, $J = 7.7$ Hz, 1H, NH, exchangeable with CD_3OD), 4.64−4.54 (m, 1H, N-C*H*, cyclopentyl), 4.54−4.50 (m, 1H, H-5″), 4.39−4.36 (m, 2H, H-4′/H-5′), 2.18−2.03 (m, 2H, N-CH-C*H*H, cyclopentyl) coinciding with 2.16, 2.11, 2.05 (3s, 9H, $3CH_3$, Ac), 1.93−1.45 (m, 6H, N-CH-CH*H*, N-CH-CH_2-C*H*$_2$, cyclopentyl); ^{13}C NMR ($CDCl_3$) δ 169.9, 169.4, 169.3 (3C=O, Ac), 152.9 (C-2), 152.8 (C-6, C-4), 136.6 (C-8), 118.1 (C-5), 86.9 (C-1′), 79.8 (C-4′), 71.6 (C-2′), 70.0 (C-3′), 62.5 (C-5′), 52.0 (N-*C*H, cyclopentyl), 32.8, 32.7 (N-CH-*C*H$_2$, cyclopentyl), 23.3 (N-CH-CH_2-*C*H$_2$, cyclopentyl), 20.1, 2 × 20.0 ($3CH_3$, Ac); MS m/z 497 $(M + 1)^+$.

Method B. To a stirred solution of **1** (461 mg, 1.0 mmol) in DCE (5.0 mL) was added NCS (534 mg, 4.0 mmol). After heating at 50 °C for 24 h, precipitated succinimide was filtered and the filtrate evaporated *in vacuo* to dryness. The residue was purified by column chromatography as described before: yield 154 mg (31%); the analytical data (TLC, ^{1}H NMR, ^{13}C NMR, and MS analyses) were in every aspect identical with those described above.

General Procedure for the Amination of Compound 2 into 3a−e. To a solution of **2** (321 mg, 0.65 mmol) in dioxane (10 mL) was added an excess of the corresponding amine, and the mixture was heated at 50−80 °C for 2−5 days. The mixture was concentrated and evaporated with toluene (2 × 50 mL), ethanol (50 mL), and CH_2Cl_2/CH_3OH (50 mL, 1/1, v/v). The crude product was purified by column chromatography with a gradient of CH_3OH in CH_2Cl_2 (98/2 → 90/10, v/v). The appropriate fractions were collected and concentrated to a white solid, which was dried *in vacuo* at 50 °C for 24 h. Recrystallization of small amounts from acetone/ether gave the analytical samples.

***N^6*-Cyclopentyl-8-(methylamino)adenosine (3a).** Reaction was carried out with aqueous methylamine (40%, 25 mL) at 50 °C for 48 h: yield 185 mg (78%); mp 146−148 °C; R_f 0.48 (E); ^{1}H NMR (DMSO-d_6) δ 7.94 (s, 1H, H-2), 6.90 (q, $J = 4.5$ Hz, 1H, 8-NH, exchangeable with D_2O), 6.68 (d, $J = 7.8$ Hz, 1H, 6-NH, exchangeable with D_2O), 5.91 (bt, 1H, 5′-OH, exchangeable with D_2O), 5.85 (d, $J_{1',2'} = 7.3$ Hz, 1H, H-1′), 5.24 (d, $J = 6.7$ Hz, 1H, 2′-OH, exchangeable with D_2O), 5.15 (d, $J = 4.1$ Hz, 1H, 3′-OH, exchangeable with D_2O), 4.64 (AB, which changed by addition of D_2O in dd, $J_{1',2'} = 7.3$ Hz, $J_{2',3'} = 5.5$ Hz, 1H, H-2′), 4.60−4.47 (m, 1H, 6-NH-C*H*, cyclopentyl), 4.11 (m, which changed by addition of D_2O in dd, $J_{2',3'} = 5.3$ Hz, $J_{3',4'} = 1.5$ Hz, 1H, H-3′), 3.96 (m, 1H, H-4′), 3.62 (m, which changed by addition of D_2O in bs, 2H, H-5′/H-5″), 2.87 (d, $J = 4.6$ Hz, which changed by addition of D_2O in s, 3H, N-C*H*$_3$, methyl), 1.96−1.86 (m, 2H, N-CH-C*H*H, cyclopentyl), 1.71−1.48 (m, 6H, N-CH-CH*H*-C*H*$_2$, cyclopentyl); ^{13}C NMR (DMSO-d_6) δ 152.3 (C-6 or C-4), 151.3 (C-6 or C-4), 149.3 (C-8), 149.2 (C-2), 117.1 (C-5), 86.9 (C-1′), 86.0 (C-4′), 71.1 (C-2′, C-3′), 61.8 (C-5′), 52.0 (N-*C*H, cyclopentyl), 33.0, 32.9 (N-CH-*C*H$_2$, cyclopentyl), 29.1 (N-*C*H$_3$, methyl), 23.7 (N-CH-CH_2-*C*H$_2$, cyclopentyl); MS m/z 365 $(M + 1)^+$; Anal. ($C_{16}H_{24}N_6O_4$) C,H,N.

***N^6*-Cyclopentyl-8-(ethylamino)adenosine (3b).** Reaction was carried out with aqueous ethylamine (70%, 10 mL) at 50 °C for 48 h: yield 199 mg (81%); mp 151−153 °C; R_f 0.51 (E); MS m/z 379 $(M + 1)^+$. Anal. ($C_{17}H_{26}N_6O_4$) C,H,N.

***N^6*-Cyclopentyl-8-(*n*-propylamino)adenosine (3c).** Reaction was carried out with *n*-propylamine (5.0 mL) at 70 °C for 72 h: yield 207 mg (80%); mp 138−141 °C; R_f 0.55 (E); MS m/z 393 $(M + 1)^+$. Anal. ($C_{18}H_{28}N_6O_4$) C,H,N.

***N^6*-Cyclopentyl-8-(*n*-butylamino)adenosine (3d).** Reac-

tion was carried out with *n*-butylamine (5.5 mL) at 80 °C for 120 h: yield 198 mg (76%); mp 110−112 °C; R_f 0.60 (E); MS *m*/*z* 407 $(M + 1)^+$. Anal. ($C_{19}H_{30}N_6O_4$) C,H,N.

***N*⁶-Cyclopentyl-8-(cyclopentylamino)adenosine (3e).** Reaction was carried out with cyclopentylamine (5.5 mL) at 80 °C for 120 h: yield 176 mg (82%); mp 121−123 °C; R_f 0.57 (E); MS *m*/*z* 419 $(M + 1)^+$. Anal. ($C_{20}H_{30}N_6O_4$) C,H,N.

2′,3′,5′-Tri-*O*-acetylinosine (4). The preparation of this compound has been described before.[7,8]

2′,3′,5′-Tri-*O*-acetyl-8-bromoinosine (5). To an aqueous Na_2HPO_4 solution (10%, w/v, 75 mL) at room temperature was added Br_2 (2.0 mL), and the mixture was stirred vigorously for 15 min until most of the bromine had dissolved. The decanted bromine solution was added to a solution of dry **4** (1.97 g, 5.0 mmol) in dioxane (75 mL), and the mixture was stirred for 4 days at room temperature. Then again freshly prepared bromine solution (35 mL) was added, and the mixture was stirred for another 3 days at room temperature. After cooling in an ice/water bath, an aqueous $NaHSO_3$ solution (2 N) was added dropwise until the solution became colorless. The water layer was extracted with CH_2Cl_2 (3 × 75 mL). The organic layer was washed with an aqueous $NaHSO_3$ solution (0.2 N, 50 mL) and water (50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The product was purified by column chromatography eluted with a 0−5% gradient of CH_3OH in CH_2Cl_2. The appropriate fractions were collected and concentrated to a white foam: yield 2.04 g (86%); R_f 0.50 (A), 0.65 (B); ^{1}H NMR ($CDCl_3$) δ 13.1 (bs, 1H, 1-NH, exchangeable with CD_3OD), 8.38 (s, 1H, H-2), 6.26 (dd, $J_{1',2'} = 4.8$ Hz, $J_{2',3'} = 5.8$ Hz, 1H, H-2′), 6.13 (d, $J_{1',2'} = 4.8$ Hz, 1H, H-1′), 5.81 (t, $J_{2',3'} = J_{3',4'} = 5.8$ Hz, 1H, H-3′), 4.55−4.29 (m, 3H, H-4′/H-5′/H-5″), 2.17, 2.12, 2.08 (3s, 9H, $3CH_3$, Ac); ^{13}C NMR ($CDCl_3$) δ 169.9, 169.0, 168.9 (C═O, Ac), 156.7 (C-6), 149.3 (C-4), 145.8 (C-2), 125.7 (C-8), 125.1 (C-5), 88.1 (C-1′), 79.6 (C-4′), 71.6 (C-2′), 69.7 (C-3′), 62.4 (C-5′), 20.6, 20.1, 19.9 (CH_3, Ac); MS *m*/*z* 474 $(M + 1)^+$.

2′,3′,5′-Tri-*O*-acetyl-8-(ethylamino)inosine (6a). To a solution of **5** (1.95 g, 4.12 mmol) in dioxane (20 mL) was added an aqueous ethylamine solution (50 mL, 70%, w/v), and the solution was heated at 80 °C in an oil bath. After 48 h the mixture was concentrated under reduced pressure and the residue dried with and dissolved in pyridine (20 mL). To this solution were added Ac_2O (4.50 mL, 47.7 mmol) and a catalytic amount of DMAP, and the mixture was stirred for 3 h at room temperature. The reaction was quenched by addition of MeOH (5 mL), and the mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (75 mL) and washed with an aqueous $NaHCO_3$ solution (10%, w/v, 50 mL) and water (50 mL). The organic layer was dried over $MgSO_4$, filtered, concentrated, and evaporated with toluene (50 mL) and CH_2Cl_2 (25 mL). The product was purified by column chromatography with a 0−6% gradient of CH_3OH in CH_2Cl_2. The appropriate fractions were collected and concentrated to a white foam: yield 1.45 g (81%); R_f 0.35 (A); ^{1}H NMR ($CDCl_3$) δ 12.9 (bs, 1H, 1-NH, exchangeable with CD_3OD), 8.02 (s, 1H, H-2), 6.12 (d, $J_{1',2'} = 6.5$ Hz, 1H, H-1′), 5.82 (t, $J_{1',2'} = J_{2',3'} = 6.3$ Hz, 1H, H-2′), 5.50 (dd, $J_{2',3'} = 6.2$ Hz, $J_{3',4'} = 4.3$ Hz, 1H, H-3′), 5.04 (t, $J = 5.7$ Hz, 1H, 8-NH, exchangeable with CD_3OD), 4.58−4.53 (m, 1H, H-5′), 4.39−4.33 (m, 2H, H-4′/H-5″), 3.65−3.49 (m, 2H, N-C*H*$_2$, ethyl), 2.15, 2.12, 2.05 (3s, 9H, $3CH_3$, Ac), 1.30 (t, $J = 7.1$ Hz, 3H, N-CH_2-C*H*$_3$, ethyl); ^{13}C NMR ($CDCl_3$) δ 169.9, 169.3, 169.2 (3C═O, Ac), 157.8 (C-6), 151.2 (C-4), 148.0 (C-8), 142.2 (C-2), 122.1 (C-5), 84.7 (C-1′), 80.0 (C-4′), 70.7 (C-2′), 69.7 (C-3′), 62.6 (C-5′), 37.8 (N-C*H*$_2$, ethyl), 20.3, 20.1, 20.0 ($3CH_3$, Ac), 14.7 (N-CH_2-C*H*$_3$, ethyl); MS *m*/*z* 438 $(M + 1)^+$.

2′,3′,5′-Tri-*O*-acetyl-8-(cyclopentylamino)inosine (6b). To a solution of **5** (0.71 g, 1.50 mmol) in dioxane (10 mL) was added cyclopentylamine (2.0 mL, 20.2 mmol), and the solution was heated at 80 °C in an oil bath. After 48 h the mixture was concentrated under reduced pressure and the residue dried with and dissolved in pyridine (5.0 mL). To this solution was added Ac_2O (1.0 mL, 10.6 mmol) followed by a catalytic amount of DMAP, and the mixture was stirred for 3 h at room temperature. The reaction was quenched by addition of MeOH (2 mL), and the mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (30 mL) and washed with an aqueous $NaHCO_3$ solution (10%, w/v, 20 mL) and water (20 mL). The organic layer was dried over $MgSO_4$, filtered, concentrated, and evaporated with toluene (20 mL) and CH_2Cl_2 (10 mL). The product was purified by column chromatography with a 0−5% gradient of CH_3OH in CH_2Cl_2. The appropriate fractions were collected and concentrated to a white foam: yield 0.59 g (82%); R_f 0.44 (A); ^{1}H NMR ($CDCl_3$) δ 12.2 (s, 1H, 1-NH, exchangeable with CD_3OD), 7.83 (s, 1H, H-2), 6.63 (d, $J = 6.6$ Hz, 1H, 8-NH, exchangeable with CD_3OD), 6.17 (dd, $J_{1',2'} = 4.7$ Hz, $J_{2',3'} = 6.2$ Hz, 1H, H-2′), 6.08 (d, $J_{1',2'} = 4.7$ Hz, 1H, H-1′), 5.60 (t, $J_{2',3'} = J_{3',4'} = 6.1$ Hz, 1H, H-3′), 4.38−4.34 (m, 1H, H-5′), 4.24−4.06 (m, 2H, H-4′/H-5″), 2.08, 2.07, 2.05 (3s, 9H, $3CH_3$, Ac), 1.78−1.61 (m, 2H, N-CH-C*H*H, cyclopentyl), 1.60−1.48 (m, 6H, N-CH-CH*H*, N-CH-CH_2-C*H*$_2$, cyclopentyl); ^{13}C NMR ($CDCl_3$) δ 170.0, 2 × 169.3 (3C═O, Ac), 157.9 (C-6), 151.1 (C-4), 148.1 (C-8), 142.2 (C-2), 122.3 (C-5), 85.1 (C-1′), 79.8 (C-4′), 71.2 (C-2′), 69.7 (C-3′), 62.7 (C-5′), 54.5 (N-*C*H, cyclopentyl), 33.0, 32.9 (N-CH-*C*H$_2$, cyclopentyl), 23.4 (N-CH-CH_2-*C*H$_2$, cyclopentyl), 20.4, 20.2, 20.1 ($3CH_3$, Ac); MS *m*/*z* 478 $(M + 1)^+$.

2′,3′,5′-Tri-*O*-acetyl-6-chloro-8-(ethylamino)purine riboside (7). To a solution of **6a** (0.44 g, 1.0 mmol), dried by evaporation with dioxane (2 × 5.0 mL), in dry $CHCl_3$ (10.0 mL) was added a DMCMAC solution in $CHCl_3$ (2 M, 1.5 mL, 3.0 mmol). The mixture was heated at 40 °C in an oil bath for 24 h. Thereafter the mixture was allowed to cool with an ice/water bath and added dropwise into a cold aqueous $NaHCO_3$ solution (10%, w/v, 20 mL). The aqueous layer was extracted with two portions of CH_2Cl_2 (50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The product was purified by column chromatography with a 0−8% gradient of acetone in CH_2Cl_2. The appropriate fractions were collected and concentrated to a white foam: yield 0.26 g (56%); R_f 0.49 (C), 0.15 (D); ^{1}H NMR ($CDCl_3$) δ 8.44 (s, 1H, H-2), 6.17 (d, $J_{1',2'} = 6.4$ Hz, 1H, H-1′), 5.84 (t, $J_{1',2'} = J_{2',3'} = 6.2$ Hz, 1H, H-2′), 5.54 (t, $J = 5.6$ Hz, 1H, NH, exchangeable with CD_3OD), 5.51 (dd, $J_{2',3'} = 6.1$ Hz, $J_{3',4'} = 4.2$ Hz, 1H, H-3′), 4.59 (dd, $J_{4',5'} = 4.4$ Hz, $J_{5',5''} = 12.2$ Hz, 1H, H-5′), 4.43−4.40 (m, 1H, H-4′), 4.35 (dd, $J_{4',5''} = 2.6$ Hz, $J_{5',5''} = 12.2$ Hz, 1H, H-5″), 3.72−3.62 (m, 2H, N-C*H*$_2$, ethyl), 2.16, 2.10, 2.04 (3s, 9H, $3CH_3$, Ac), 1.34 (t, $J = 7.3$ Hz, 3H, N-CH_2-C*H*$_3$, ethyl); ^{13}C NMR ($CDCl_3$) δ 170.0, 2 × 169.5 (3C═O, Ac), 154.4 (C-6), 152.6 (C-4), 148.2 (C-2), 143.3 (C-8), 131.6 (C-5), 85.0 (C-1′), 80.6 (C-4′), 71.0 (C-2′), 69.9 (C-3′), 62.8 (C-5′), 38.1 (N-*C*H$_2$, ethyl), 20.6, 20.4, 20.3 ($3CH_3$, Ac), 14.8 (N-CH_2-*C*H$_3$, ethyl); MS *m*/*z* 456 $(M + 1)^+$.

***N*⁶-Cyclopentyl-8-(ethylamino)adenosine (3b) Starting from 7.** To a solution of **7** (228 mg, 0.5 mmol), dried by evaporation with dioxane (2 × 5 mL), in dioxane (10 mL) was added cyclopentylamine (5.0 mL, 51 mmol), and the solution was heated in an oil bath at 50 °C for 48 h. The mixture was concentrated and evaporated with toluene (2 × 50 mL), ethanol (50 mL), and CH_2Cl_2/CH_3OH (50 mL, 1/1, v/v). The crude product was purified by column chromatography with a gradient of CH_3OH in CH_2Cl_2 (98/2 → 90/10, v/v). The appropriate fractions were collected and concentrated to a white solid, which was dried *in vacuo* at 50 °C for 24 h: yield 142 mg (75%); the analytical data (mp, TLC, ^{1}H NMR, ^{13}C NMR, and MS analyses) were in every aspect identical with those described above.

2′,3′,5′-Tri-*O*-acetyl-6-chloro-8-(cyclopentylamino)-purine Riboside (8). To a solution of **6b** (0.25 g, 0.52 mmol), dried by evaporation with dioxane (2 × 5.0 mL), in dry $CHCl_3$ (5.0 mL) was added a DMCMAC solution in $CHCl_3$ (2 M, 1.2 mL, 2.4 mmol). The mixture was warmed at 40 °C in an oil bath for 24 h. Thereafter the mixture was allowed to cool in an ice/water bath and added dropwise into a cold aqueous $NaHCO_3$ solution (10%, w/v, 10 mL). The aqueous layer was extracted with two portions of CH_2Cl_2 (30 mL). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The product was purified by column chromatography with a 0−6% gradient of acetone in CH_2Cl_2. The appropriate fractions were collected and concentrated to a white foam: yield 151 mg (58%); R_f 0.56 (C); ^{1}H NMR ($CDCl_3$) δ 8.42 (s, 1H, H-2), 6.05 (d, $J_{1',2'} = 5.4$ Hz, 1H, H-1′), 5.99 (t,

$J_{1',2'} = J_{2',3'} = 5.5$ Hz, 1H, H-2′), 5.58 (t, $J_{2',3'} = J_{3',4'} = 5.4$ Hz, 1H, H-3′), 5.44 (d, $J = 7.1$ Hz, 1H, NH, exchangeable with CD_3OD), 4.53−4.35 (m, 4H, N-C*H*, cyclopentyl/H-4′/H-5′/H-5″), 2.23−2.15 (m, 2H, N-CH-C*H*H, cyclopentyl), 2.15, 2.07, 2.04 (3s, 9H, $3CH_3$, Ac), 1.77−1.51 (m, 6H, N-CH-CH*H*-C*H*$_2$, cyclopentyl); ^{13}C NMR ($CDCl_3$) δ 170.0, 169.4, 169.3 (3C=O, Ac), 154.2 (C-6), 152.4 (C-4), 148.0 (C-2), 143.3 (C-8), 131.7 (C-5), 85.6 (C-1′), 80.3 (C-4′), 71.6 (C-2′), 70.0 (C-3′), 62.8 (C-5′), 54.7 (N-*C*H, cyclopentyl), 33.1, 33.0 (N-CH-*C*H$_2$, cyclopentyl), 23.3 (N-CH-CH$_2$-*C*H$_2$, cyclopentyl), 20.3, 2 × 20.0 ($3CH_3$, Ac); MS m/z 497 $(M + 1)^+$.

General Procedure for the Amination of 8 into 9a−d. To a solution of **8** (270 mg, 0.5 mmol) in dioxane (10 mL) was added an excess of the corresponding amine, and the mixture was heated at 50−70 °C for 2−3 days. The mixture was concentrated and evaporated with toluene (2 × 50 mL), ethanol (50 mL), and CH_2Cl_2/CH_3OH (50 mL, 1/1, v/v). The crude product was purified by column chromatography with a gradient of CH_3OH in CH_2Cl_2 (98/2 → 90/10, v/v). The appropriate fractions were collected and concentrated to a white solid, which was dried *in vacuo* at 50 °C for 24 h. Recrystallization of small amounts from acetone/ether gave the analytical samples.

8-(Cyclopentylamino)-N^6-methyladenosine (9a). Reaction was carried out with aqueous methylamine (40%, 20 mL) at 50 °C for 24 h: yield 147 mg (81%); mp 128−130 °C; R_f 0.53 (E); ^{1}H NMR (DMSO-d_6) δ 7.96 (s, 1H, H-2), 6.77 (q, $J =$ 4.7 Hz, 1H, 6-NH, exchangeable with D_2O), 6.67 (d, $J = 7.0$ Hz, 1H, 8-NH, exchangeable with D_2O), 5.90 (d, $J_{1',2'} = 7.4$ Hz, 1H, H-1′), 5.81 (t, $J = 4.3$ Hz, 1H, 5′-OH, exchangeable with D_2O), 5.24 (d, $J = 6.5$ Hz, 1H, 2′-OH, exchangeable with D_2O), 5.15 (d, $J = 4.1$ Hz, 1H, 3′-OH, exchangeable with D_2O), 4.60 (AB, which changed by addition of D_2O in dd, $J_{1',2'} = 7.5$ Hz, $J_{2',3'} = 5.4$ Hz, 1H, H-2′), 4.20 (m, 1H, 8-N-C*H*, cyclopentyl), 4.10 (m, which changed by addition of D_2O in dd, $J_{2',3'} = 5.4$ Hz, $J_{3',4'} = 2.0$ Hz, 1H, H-3′), 3.94 (m, 1H, H-4′), 3.62 (m, which changed by addition of D_2O in d, $J = 2.2$ Hz, 2H, H-5′/H-5″), 2.91 (d, $J = 4.7$ Hz, which changed by addition of D_2O in s, 3H, N-C*H*$_3$, methyl), 1.94−1.88 (m, 2H, N-CH-C*H*H, cyclopentyl), 1.67−1.48 (m, 6H, N-CH-CH*H*-C*H*$_2$, cyclopentyl); ^{13}C NMR (DMSO-d_6) δ 152.1 (C-6 or C-4), 151.2 (C-6 or C-4), 149.1 (C-2), 148.9 (C-8), 117.4 (C-5), 86.6 (C-1′), 85.9 (C-4′), 71.0 (C-2′, C-3′), 61.7 (C-5′), 54.3 (N-*C*H, cyclopentyl), 32.6, 32.5 (N-CH-*C*H$_2$, cyclopentyl), 27.5 (N-*C*H$_3$, methyl), 23.8, 23.7 (N-CH-CH$_2$-*C*H$_2$, cyclopentyl); MS m/z 365 $(M + 1)^+$. Anal. ($C_{16}H_{24}N_6O_4$) C,H,N.

8-(Cyclopentylamino)-N^6-ethyladenosine (9b). Reaction was carried out with aqueous ethylamine (70%, 8.0 mL) at 50 °C for 48 h: yield 150 mg (80%); mp 132−133 °C; R_f 0.55 (E); MS m/z 379 $(M + 1)^+$. Anal. ($C_{17}H_{26}N_6O_4$) C,H,N.

8-(Cyclopentylamino)-N^6-*n*-propyladenosine (9c). Reaction was carried out with *n*-propylamine (5.0 mL) at 50 °C for 48 h: yield 168 mg (85%); mp 119−121 °C; R_f 0.61 (E); MS m/z 393 $(M + 1)^+$. Anal. ($C_{18}H_{28}N_6O_4$) C,H,N.

8-(Cyclopentylamino)-N^6-*n*-butyladenosine (9d). Reaction was carried out with *n*-butylamine (5.5 mL) at 50 °C for 72 h: yield 183 mg (76%); mp 100−102 °C; R_f 0.64 (E); MS m/z 407 $(M + 1)^+$. Anal. ($C_{19}H_{30}N_6O_4$) C,H,N.

2′,3′,5′-Tri-*O*-acetyl-6,8-dichloropurine 9-β-D-Ribofuranoside (10). The preparation of this compound has been described before.[12]

2′,3′,5′-Tri-*O*-acetyl-8-chloro-N^6-cyclopentyladenosine (2) and 2′,3′,5′-Tri-*O*-acetyl-8-(cyclopentylamino)-6-chloropurine 9-β-D-Ribofuranoside (8). Compound **10** (1.29 g, 2.88 mmol) was dried with and dissolved in dioxane (15 mL). To this solution was added cyclopentylamine (1.14 mL, 11.5 mmol), and the mixture was warmed in an oil bath at 40 °C for 48 h. The mixture was concentrated under reduced pressure to dryness, and the residue was evaporated with and dissolved in pyridine (5.0 mL). Then, Ac_2O (0.5 mL, 5.3 mmol) was added, and the mixture was stirred for 24 h at room temperature. TLC analysis (C) showed complete conversion of starting material into a mixture of two products. The mixture was concentrated *in vacuo* and evaporated with toluene (2 × 25 mL). The residue was dissolved in CH_2Cl_2 (50 mL) and washed with an aqueous $NaHCO_3$ solution (10%, w/v, 20 mL) and H_2O (20 mL). The organic layer was dried over $MgSO_4$, filtered, concentrated, and evaporated with toluene (50 mL) and CH_2Cl_2 (25 mL). The residue, existing as a mixture of two isomers, was purified by column chromatography with a gradient of diethyl ether/hexane (1/1−95/5, v/v) to give the individual products as white foams.

Faster running isomer 2: yield 0.47 g (33%); the analytical data (TLC, ^{1}H NMR, ^{13}C NMR, and MS analyses) were identical in all aspects with those described above.

Slower running isomer 8: yield 0.59 g (41%); the analytical data (TLC, ^{1}H NMR, ^{13}C NMR, and MS analyses) were identical in all aspects with those described earlier starting from compound **6b**.

Table 3. Crystallographic Data for **9b**

Crystal Data	
formula	$C_{17}H_{26}N_6O_4 \cdot C_4H_{10}O$
molecular weight	452.55
crystal system	orthorhombic
space group	$P2_12_12_1$ (No. 19)
Z	4
a, b, c (Å)	11.039(3), 8.708(2), 24.815(12)
V (Å^3)	2385.4(14)
D_{calcd} (g cm^{-3})	1.260
$F(000)$	976
$\mu_{MoK\alpha}$ (cm^{-1})	0.9
crystal size (mm)	0.03 × 0.50 × 0.63
Data Collection	
T, K	295
θ_{min}, θ_{max} (deg)	1.6, 24.2
radiation (Mo Kα, graphite monochromator) (Å)	0.710 73
scan type	$\omega/2\theta$
$\Delta\omega$ (deg)	$1.69 + 0.35 \tan\theta$
horizontal, vertical aperture (mm)	4.35, 4.00
reference reflections	$\bar{2}05$, $\bar{1}\bar{2}\bar{2}$, $30\bar{2}$
data set	−12:0, −10:0, −27:27
total data	3913
total unique data	3424 ($R_{int} = 0.0815$)
observed data	1450 [$F_o > 4.0\sigma(F_o)$]
Refinement	
no. of refined parameters	298
weighting scheme[a]	$w = 1/[\sigma 2(F_o^2) + (0.1P)^2]$
final $R2_w$, $R1$, S [b]	0.2455, 0.0974, 0.947
$(\Delta/\sigma)_{av}$, $(\Delta/\sigma)_{max}$ in final cycle	0.001, 0.004
min, max residual density (e Å^{-3})	−0.25, 0.23

[a] $P = (\max(F_o^2,0) + 2F_c^2)/3$. [b] $R1 = \sum||F_o| - |F_c||/\sum|F_o|$, $R2_w = [\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]]^{1/2}$.

Crystal Structure Determination and Refinement of 9b. A colorless, plate-shaped crystal of **9b** was glued to the tip of a Lindemann glass capillary and transferred to an Enraf-Nonius CAD4-T diffractometer on rotating anode. Accurate unit-cell parameters and an orientation matrix were determined by least-squares refinement of the setting angles of 25 well-centered reflections (SET4) in the range $9.0° < \theta < 15.3°$. Reduced-cell calculations did not indicate higher lattice symmetry.[24] Crystal data and details on data collection and refinement are given in Table 3. All data were collected in $\omega/2\theta$ scan mode. Data were corrected for Lp effects and the observed linear decay of 2% of the three periodically measured reference reflections. The structure was solved by automated direct methods (SHELXS86[25]). Refinement on F^2 was carried out by full-matrix least-squares technique (SHELXL-93[26]); no observance criterion was applied during refinement. All non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms were refined with a fixed isotropic thermal parameter amounting to 1.5 or 1.2 times the value of the equivalent isotropic thermal parameter of their carrier atoms, for the hydrogen atoms on N7, N8, O12, O13, O15, and the methyl groups, and the other hydrogen atoms, respectively. Weights were introduced in the final refinement cycles. The cyclopentyl moiety was disordered. No attempt was made to model this conformational disorder in view of the limited quality of the data. Final positional parameters are

Table 4. Final Atomic Coordinates and Equivalent Isotropic Thermal Parameters for **9b** ($C_{17}H_{26}N_6O_4 \cdot C_4H_{10}O$) (esd's in parentheses)

atom	*x*	*y*	*z*	*U*(eq) [Ang**2]
O(1)	0.1215(6)	0.5856(6)	0.4866(3)	0.044(3)
O(12)	0.0738(6)	0.7682(8)	0.3613(3)	0.056(3)
O(13)	0.1332(7)	0.9294(6)	0.4531(3)	0.055(3)
O(15)	−0.1367(7)	0.6161(8)	0.5017(3)	0.059(3)
N(1)	0.4140(9)	0.2168(11)	0.3335(4)	0.073(4)
N(3)	0.3473(9)	0.4616(9)	0.3705(4)	0.056(4)
N(6)	0.2978(9)	−0.0011(10)	0.3371(4)	0.071(4)
N(7)	0.1075(8)	0.1959(8)	0.3950(3)	0.042(3)
N(8)	−0.0430(8)	0.3432(8)	0.4368(3)	0.044(3)
N(9)	0.1441(7)	0.4466(7)	0.4082(3)	0.036(3)
C(2)	0.4262(11)	0.3683(13)	0.3464(5)	0.071(5)
C(4)	0.2479(9)	0.3861(10)	0.3846(4)	0.038(4)
C(5)	0.2205(10)	0.2320(10)	0.3744(4)	0.044(4)
C(6)	0.3126(12)	0.1537(12)	0.3481(5)	0.058(4)
C(8)	0.0675(10)	0.3261(9)	0.4137(4)	0.037(3)
C(11)	0.1404(10)	0.6004(9)	0.4306(4)	0.042(4)
C(12)	0.0418(8)	0.7059(9)	0.4107(4)	0.034(3)
C(13)	0.0347(9)	0.8217(8)	0.4584(4)	0.038(3)
C(14)	0.0588(10)	0.7211(9)	0.5063(5)	0.053(4)
C(15)	−0.0502(9)	0.6750(12)	0.5372(5)	0.055(4)
C(61)	0.3920(12)	−0.0924(15)	0.3086(7)	0.105(7)
C(62)	0.3441(17)	−0.207(2)	0.2749(8)	0.176(11)
C(81)	−0.1200(10)	0.2106(10)	0.4463(5)	0.055(4)
C(82)	−0.1727(11)	0.1494(15)	0.3945(5)	0.078(5)
C(83)	−0.2866(15)	0.209(3)	0.3891(7)	0.174(12)
C(84)	−0.3248(14)	0.291(2)	0.4382(7)	0.121(8)
C(85)	−0.2311(11)	0.2557(14)	0.4782(5)	0.071(5)
O(33)	0.1314(9)	0.3346(10)	0.2015(3)	0.085(4)
C(31)	0.2793(14)	0.199(2)	0.1514(7)	0.121(8)
C(32)	0.2522(16)	0.295(2)	0.1984(8)	0.132(10)
C(34)	0.108(2)	0.436(2)	0.2462(6)	0.133(9)
C(35)	−0.011(2)	0.457(3)	0.2562(10)	0.168(11)

[a] $U(\text{eq}) = {}^1/_3$ of the trace of the orthogonalized U.

listed in Table 4. Neutral atom scattering factors and anomalous dispersion corrections were taken from *International Tables for Crystallography*.[27] Geometrical calculations and illustrations were performed with PLATON.[28] All calculations were performed on a DECstation 5000/125.

Radioligand Binding Studies. Adenosine A_1 receptor affinities were determined on rat cortical membranes with [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine (DPCPX) as radioligand according to a protocol published previously.[29] Measurements with [^{3}H]DPCPX were performed in the presence and absence of 1 mM GTP.

Adenosine A_{2a} receptor affinities were determined on rat striatal membranes with [^{3}H]CGS 21680 as radioligand according to Jarvis *et al*.[30]

All data reflect two to three independent experiments, performed in duplicate.

***In Vivo* Pharmacology: Animals and Surgical Preparation.** Adult male normotensive SPF rats of Wistar descent, weighing 200−250 g, were used throughout the study. The animals were housed individually in plastic cages at constant temperature with a normal 12 h light (7:00 a.m.−19:00 p.m.)−dark cycle. Both laboratory chow (Standard Laboratory Rat, Mouse, and Hamster Diets, RMH-TM, Hope Farms, Woerden, The Netherlands) and tap water were available *ad libitum*. Two days before experimentation, cannulas were implanted under light ether anesthesia. For the monitoring of arterial blood pressure, the abdominal aorta was cannulated with 4.5 cm of polythene tubing (i.d. 0.28 mm; Portex, Medica BV, Hertogenbosch, The Netherlands), heat-sealed to 18 cm (i.d. 0.58 mm) of polythene tubing (Portex), by an approach through the left femoral artery. The right jugular vein was cannulated with 12 cm of poly(vinyl chloride) tubing (i.d. ±0.6 mm; Talas, Ommen, The Netherlands) for drug administration. The catheters were guided subcutaneously to the neck where they were exteriorized and anchored in place. To prevent clotting the cannulas were filled with a 25% (g/v) poly(vinylpyrrolidone) (PVP; Brocacef, Maarssen, The Netherlands) solution in physiological saline containing 50 IU/mL heparin (Pharmacy Academic Hospital, Leiden, The Netherlands), which was renewed daily.

Cardiovascular Measurement. Arterial blood pressure was measured from the femoral catheter in the abdominal aorta using a miniature strain gauge P10EZ transducer, equipped with a TA1017 CritiFlo diaphragm dome (both Viggo-Spectramed BV, Bilthoven, The Netherlands). This dome allowed a continuous flushing of the cannula with heparinized saline (20 IU/mL) at a rate of 500 μL/h (Harvard infusion pump 22, Plato, Diemen, The Netherlands). The pressure transducer was placed at the level of the animal heart, when in normal position, and connected to a polygraph amplifier console (RMP6018, Nihon Kohden Corp., Tokyo, Japan). Heart rate was captured from the pressure signal which was used to trigger a tachograph. Signals were recorded on a polygraph and concurrently converted in a CED1401 interface (Cambridge Electronics Design Ltd., Cambridge, England) and fed into a 80387 computer (Philips, Eindhoven, The Netherlands). The data were stored on hard disk for off-line analysis. Data acquisition and reduction were performed with Spike2 computer software (Cambridge Electronics Design Ltd., Cambridge, England).

Pharmacodynamic Experiments. Conscious, free-ranging rats received an intravenous infusion of the compounds, dissolved in 20% (v/v) DMSO/water, in 5 min. Control rats were similarly treated with vehicle only. During the experiments arterial blood pressure and heart rate were continuously monitored. After connection of the arterial catheter to the recording equipment, the rat was allowed to accommodate to the surroundings and the experimental conditions for 30 min. The recording of the hemodynamic parameters was started 15 min prior to the administration, for base-line determination, and was continued for 5.5 h. The administrations took place between 10:00 and 11:00 a.m. to minimize the potential influence of diurnal rhythms. During the experiment the animal had free access to water.

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Supporting Information Available: Further details of the NMR spectra of compounds **3b**−**e** and **9b**−**d**, the structure determination of **9b**, including atomic coordinates for the hydrogen atoms, bond lengths and angles, thermal parameters, and a thermal motion ellipsoid plot for **9b**, and details of the elemental analyses (13 pages); listings of observed and calculated structure factor amplitudes for **9b** (8 pages). Ordering information is given on any current masthead page.

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