Ribose-Modified Nucleosides as Ligands for Adenosine Receptors: Synthesis, Conformational Analysis, and Biological Evaluation of 1′-*C*-Methyl Adenosine Analogues

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1′-*C*-Methyl analogues of adenosine and selective adenosine A_1 receptor agonists, such as N-[(*1R*)-1-methyl-2-phenylethyl]adenosine ((*R*)-PIA) and N^6-cyclopentyladenosine, were synthesized to further investigate the subdomain that binds the ribose moiety. Binding affinities of these new compounds at A_1 and A_{2A} receptors in rat brain membranes and at A_3 in rat testis membranes were determined and compared. It was found that the 1′-*C*-methyl modification in adenosine resulted in a decrease of affinity, particularly at A_1 and A_{2A} receptors. When this modification was combined with N^6 substitutions with groups that induce high potency and selectivity at A_1 receptors, the high affinity was in part restored and the selectivity was increased. The most potent compound proved to be the 1′-*C*-methyl analogue of (*R*)-PIA with a K_i of 23 nM for the displacement of [^{3}H]CHA binding from rat brain A_1 receptors and a >435-fold selectivity over A_{2A} receptors. In functional assays, these compounds inhibited forskolin-stimulated adenylate cyclase with IC_{50} values ranging from 0.065 to 3.4 μM, acting as full agonists. Conformational analysis based on vicinal proton−proton *J*-coupling constants and molecular mechanics calculations using the MM2 force field proved that the methyl group on C1′ in adenosine has a pronounced impact on the furanose conformation by driving its conformational equilibrium toward the north, $\gamma+$, syn form.

Introduction

Adenosine is an endogenous nucleoside with an effect in multiple physiological processes. Its different actions are mediated by binding to extracellular receptors coupled to guanyl nucleotide binding proteins (G-proteins). The potential of adenosine receptors as drug targets was reviewed.[1] Four adenosine receptor subtypes have now been characterized and cloned as follows: A_1, A_{2A}, A_{2B}, and A_3. The activation of A_1 and A_3 receptors causes the inhibition of adenylyl cyclase, activation of phospholipase C, activation of potassium channels, and inhibition of calcium channels influx. The activation of A_{2A} and A_{2B} receptors stimulates adenylyl cyclase via G-protein coupling.[1] The A_{2B} receptors also couple to a phospholipase in human mast cells and may be important in the mediation of allergic reactions.[2] Adenosine receptors are widely distributed throughout the body, and studies on their structure−activity relationships have been recently reviewed.[1,3] Adenosine receptor agonists are being studied for their potential use as various agents: antiarrhythmic,[4] antinociceptive,[5] antilipolytic (A_1 subtype),[6] cerebroprotective and cardioprotective (A_1 and A_3 subtypes),[7] hypotensive,[8] antipsychotic (A_{2A} subtype),[9] antiinflammatory (A_1, A_{2A}, A_{2B}, and A_3 subtypes),[10] anticancer (A_3),[11] and as agents for the treatment of cystic fibrosis (A_{2B}).[12] All known adenosine agonists are closely related to the chemical structure of adenosine itself. Substitution at N^6 or C2 may enhance affinity and may impart A_1/A_2/A_3 selectivity.[1,13] Other modifications of the adenine moiety usually lead to inactive compounds or weakly active compounds.[13]

Structure−activity relationship studies have pointed out that the ribose recognition domain of adenosine and its analogues contributes strongly to affinity at the adenosine receptor subtypes. So, the removal or inversion of 2′- and 3′-hydroxyl groups were generally not well-tolerated by the binding site. However, the amide substitution at the 5′-position as in NECA (5′-*N*-ethylcarboxamidoadenosine) provided increased potency at A_{2A} receptors. Substitution of the hydrogen atoms of the ribose ring by a methyl group in adenosine and adenosine analogues afforded compounds with various affinity and selectivity. Replacement of the 4′-hydrogen by a methyl group in adenosine was poorly tolerated; yet, when combined with other favorable modifications, the potency and selectivity at A_3 receptors may be maintained.[13] The 3′-*C*-methyl adenosine analogue was found to bind weakly with A_1 selectivity.[13]

We have reported that the introduction of a methyl group at the 2′-*C* position in adenosine resulted in a decrease of the affinity, particularly at A_{2A} and A_3 receptors.[14] However, when this modification was combined with N^6 substitutions with groups that induce high potency and selectivity at A_1 receptors, the high affinity was retained and the selectivity was increased. To further investigate the subdomain that binds the ribose moiety, in the present study, we report on the

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Scheme 1[a]

2, 3, 4 R = H, 5 R = Ac, 6, 7, 8 a-d + α-anomers (9a-d), 1a-d, 11, 12

a R = H
b R = cyclopentyl
c R = (*R*)-1-methyl-2-phenylethyl
d R = (*S*)-1-methyl-2-phenylethyl

11a R = Bn, R' = H
11b R = Bn, R' = cyclopentyl
12a R = H, R' = H
12b R = H, R' = cyclopentyl

[a] Reagents: (i) NaH, BnBr, THF; (ii) CH_3Li, Et_2O; (iii) Ac_2O, DMAP, pyridine; (iv) 1.8 M $EtAlCl_2$, MeCN; (v) liquid ammonia or RNH_2, EtOH, Δ; (vi) $HCOONH_4$, 10% Pd/C, MeOH, reflux.

synthesis of a series of 1′-*C*-methyl analogues of adenosine and A_1 selective adenosine receptor agonists (**1a**–**d**).

Chemistry

The synthesis of 1′-*C*-methyladenosine (**1a**, 1′-Me-Ado), N^6-cyclopentyl-1′-*C*-methyladenosine (**1b**, 1′-Me-CPA), N^6-[(1*R*)-1-methyl-2-phenylethyl]-1′-*C*-methyladenosine (**1c**, 1′-Me-(*R*)-PIA), and N^6-[(1*S*)-1-methyl-2-phenylethyl]-1′-*C*-methyladenosine (**1d**, 1′-Me-(*S*)-PIA) was carried out as shown in Scheme 1, using 2,3-*O*-benzylidene-D-ribono-1,4-lactone (**2**)[15] as the starting compound. The reaction of *endo*-5-*O*-benzyl-2,3-*O*-benzylidene-D-ribono-1,4-lactone (**3**), prepared by the benzylation of **2**, with methyllithium in diethyl ether, gave **4** as a mixture of α and *β* anomers. The reaction of **4** with acetic anhydride in pyridine in the presence of 4-(dimethylamino)pyridine afforded the acetoxy derivative 2-*O*-acetyl-6-*O*-benzyl-3,4-*O*-benzylidene-1-deoxy-D-psicofuranose (**5**, anomeric mixture), which was coupled with 6-chloropurine (**6**) in dry acetonitrile in the presence of ethylaluminum dichloride to give 6-chloropurine ribonucleosides **7** (6-chloro-9*H*-(6-*O*-benzyl-3,4-*O*-benzylidene-1-deoxy-D-psicofuranosyl)purine, *β*/α, 9:1 ratio). Amination at the 6-position of derivative **7** with ammonia, cyclopentylamine, *l*-amphetamine, or *d*-amphetamine yielded compounds **8a**–**d** and the α anomers **9a**–**d**. Deprotection of the purified *β* anomer by catalytic transfer hydrogenation with ammonium formate in methanol in the presence of 10% Pd/C gave **1a**–**d**. The assignment of the *β* anomeric structure of compounds **8a**–**d** was performed by nuclear Overhauser enhancement (NOE) experiments. In fact, it was found that when the methyl group hydrogens of **8a**–**d** were irradiated, a NOE effect was observed at the hydrogen atom of the benzylidene protecting group confirming that the 1′-*C*-methyl group and the benzylidene one have a cis conformation. Compounds **1a**–**d** proved to be very unstable in aqueous solutions at pH < 7.

We also attempted another route for the synthesis of title compounds using the isopropylidene protecting group instead of the benzylidene one. However, all attempts of deprotection of isopropylidene derivatives **12a** and **12b**, prepared by the amination of 6-chloro-9*H*-(6-*O*-benzyl-1-deoxy-3,4-*O*-isopropylidene-D-psicofuranosyl)purine (**10**) followed by debenzylation, with several Bronsted and Lewis acid catalysts under different conditions, resulted in base cleavage.

Conformational Analysis

Proton nuclear magnetic resonance (^1H NMR) data and NOE experiments were employed to determine the predominant conformation of the 1′-*C*-methyl derivative of adenosine and adenosine analogues in solution. The relevant H-8 enhancement observed in compounds **1a**–**d** when the methyl group hydrogen atoms of these nucleosides were irradiated supports a spatial arrangment where H-8 and the methyl group are proximate, as would be the case in the syn conformer. However, the observation of a NOE effect also at H-2 when the methyl group hydrogen atoms of compounds **1a**–**d** were irradiated, even though inferior to that observed at H-8, and the appearance of the 5′-hydroxyl resonance as a triplet[16] led us to conclude that a syn conformation in the low range predominates in solutions of these compounds.

Further information concerning the solution conformation was obtained by the coupling constant values. Conformational analysis was based on the vicinal proton–proton *J*-coupling constants $J_{2'3'}$ and $J_{3'4'}$ using D_2O as solvent. $J_{2'3'}$ values of 5.1–5.2 Hz and $J_{3'4'}$ values of 7.0–7.1 Hz were found, very similar to those reported for 1′-methyl-*β*-D-ribofuranosylthymine in the north type puckered conformation.[17] Thus, it can be concluded that

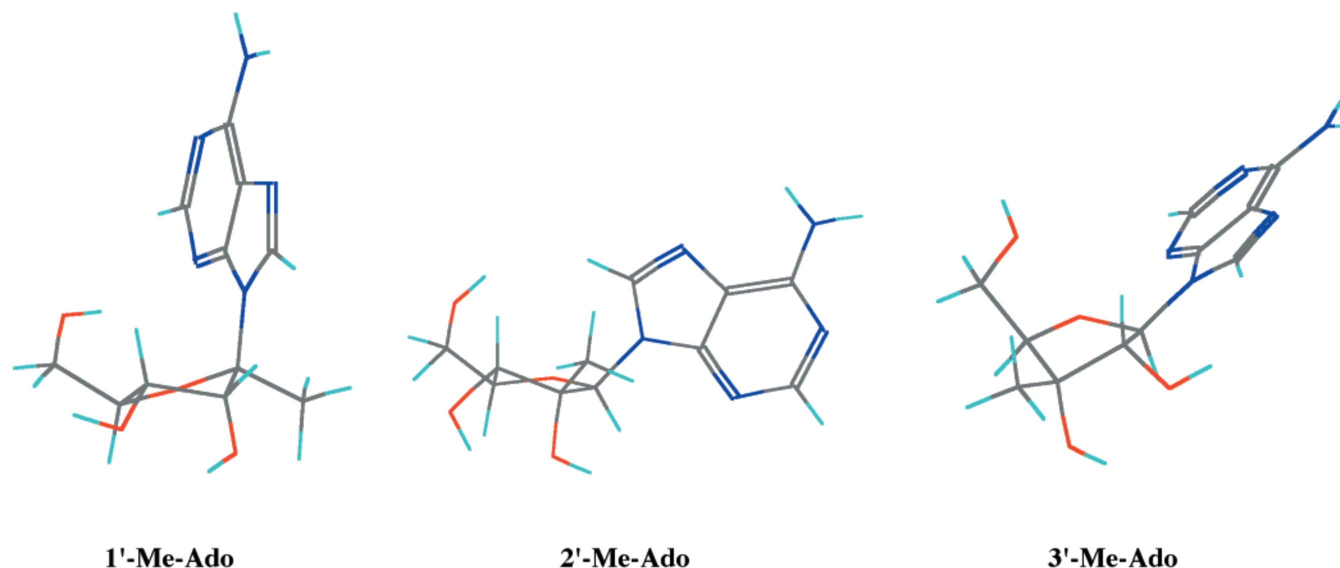

Figure 1. Molecular mechanics (MM2 force field) optimized structures for **1a** (north, $\gamma+$, syn), 2′-*C*-methyladenosine (north, $\gamma+$, anti), and 3′-*C*-methyladenosine (south, $\gamma+$, syn).

Table 1. Affinity of 1′-*C*-Methyl-adenosine Derivatives in Radioligand Assays at Rat Brain A_1 and A_{2A} Receptors and at Rat Testis A_3 Receptors[a–c]

compd	K_i (nM) or % displacement at 10^{-5} M			A_{2A}/A_1	A_3/A_1	A_3/A_{2A}
	A_1[a]	A_{2A}[b]	A_3[c]			
1a (1′-Me-Ado)	7700 ± 500	7%	19%	>1.30	>1.30	
1b (1′-Me-CPA)	100 ± 10	0%	5170 ± 420	>100	52	<0.52
1c (1′-Me-(*R*)-PIA)	23 ± 2	8%	670 ± 30	>435	29	<0.07
1d (1′-Me-(*S*)-PIA)	270 ± 20	0%	6160 ± 480	>37	23	<0.62
2′-Me-Ado	740 ± 60	3400 ± 750	0%	4.6	>13.5	>2.9
3′-Me-Ado	51 000 ± 8300	12%[d]	ND[e]	>202	>202	
4′-Me-Ado	3%[d]	0%[d]	ND[f]			
2′-Me-CPA	9.5 ± 0.8	7200 ± 930	210 ± 40	758	22.1	0.029
2′-Me-(*R*)-PIA	4.7 ± 0.6	7400 ± 110	1080 ± 260	1574	230	0.15
2′-Me-(*S*)-PIA	50 ± 6	17%	10 000 ± 2500	>200	200	<1
CPA	0.3 ± 0.02	380 ± 80	260 ± 7	1266	87	0.07
(*R*)-PIA	0.6 ± 0.02	750 ± 80	53 ± 4[g]	1250	88	0.07
(*S*)-PIA	11 ± 4	1800 ± 570	240 ± 12[g]	164	22	0.13

[a] Displacement of specific [^{3}H]CHA binding in rat brain cortical membranes. [b] Displacement of [^{3}H]CGS21680 binding in rat brain striatal membranes. [c] Displacement of [^{3}H](*R*)-PIA binding in the presence of 150 nM DPCPX in rat testis membranes. [d] Ref 13. [e] Ref 13: percent of displacement of [^{125}I]ABMECA binding in membranes of CHO cells stably transfected with the human A_3-cDNA = 9%. [f] Ref 13: percent of displacement of [^{125}I]ABMECA binding in membranes of CHO cells stably transfected with the human A_3-cDNA = 8%. [g] Ref 19. The values are expressed as K_i ± SEM of three determinations or percent of inhibition of specific radioligand binding at 10 μM compound concentration.

the methyl group on C1′ in **1a**−**d** has a pronounced impact on the furanose conformation by driving the conformational equilibrium toward the north form. The north conformation appears to correspond with the pseudoequatorial location of the Me group, which is sterically favored. Figure 1 shows a molecular mechanics (MM2 force field) optimized structure for north, $\gamma+$, syn **1a** as compared to that of north, $\gamma+$, anti 2′-*C*-methyladenosine and south, $\gamma+$, syn 3′-*C*-methyladenosine.

Biological Evaluation and Discussion

Compound **1a** and its N^6-substituted derivatives were tested in radioligand binding assays to determine their affinities toward adenosine A_1, A_{2A}, and A_3 receptors. Affinities for A_1 and A_{2A} receptors were determined in competition assays in rat brain membranes (A_1) and rat brain striatum (A_{2A}) using, respectively, [^{3}H]CHA (N^6-cyclohexyladenosine) and [^{3}H]CGS21680 (2-[4-(2-carboxyethyl)phenyl]ethyl-amino-5′-*N*-ethylcarboxamidoadenosine) as radioligands.[18] Affinity for A_3 receptors was determined in competition assays for the receptor of rat testis membranes using [^{3}H](*R*)-PIA in the presence of the A_1 selective antagonists DPCPX (8-cyclopentyl-1,3-dipropylxanthine).[19] The data are summarized in Table 1 in comparison with the nonmethylated analogues and 2′-, 3′-, and 4′-*C*-methyladenosine.

1′-Me-Ado showed an affinity for A_1 receptors (K_i = 7.7 μM), 10-fold lower than that of 2′-Me-Ado and 6.6-fold superior to that of 3′-*C*-methyl isomer. At A_{2A} receptors, the 1′-Me-Ado and its analogues (**1a**−**d**) were found to have poor or no affinity, similar to the 3′- and 4′-substituted isomers. At A_3 receptors, 1′-Me-Ado as well as 2′-, 3′-, and 4′-Me-Ado showed no affinity.

As compared with the adenosine affinity for A_1 receptors (10 nM),[20] the substitution of hydrogen atoms of the furanose ring by a methyl group is poorly tolerated at this receptor subtype, in particular at the 3′- and 4′-position, while a similar substitution at the 1′- and 2′-position brings about a less marked reduction

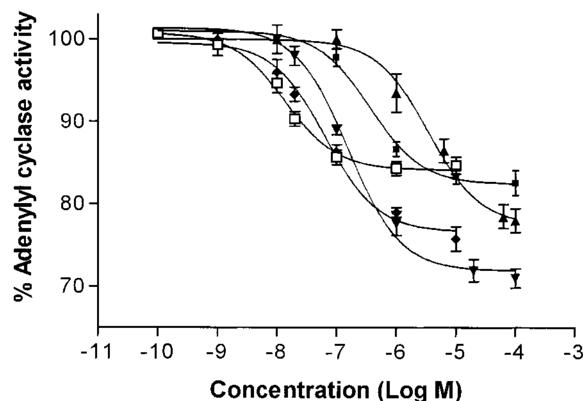

Figure 2. Inhibition of adenylyl cyclase in rat cortical membranes. The assay was carried out as described in Biological Methods in the presence of 100 μM forskolin. Each data point is shown as mean ± SEM of at least three independent experiments. Concentration-dependent effects on adenylyl cyclase by 1′-Me-CPA (■), 1′-Me-(*R*)-PIA (◆), 1′-Me-(*S*)-PIA (▼), 1′-Me-Ado (▲), and CPA (□).

Table 2. Inhibition of Adenylyl Cyclase Activity in Rat Cortical Membranes by 1′-*C*-Methyl-adenosine and Its Derivatives

compd	IC_{50} (nM)[a]	% maximal inhibition
CPA	12 ± 0.7	15.5 ± 2.6
1′-Me-Ado	3440 ± 230	22.0 ± 1.7
1′-Me-CPA	357 ± 23	17.4 ± 0.6
1′-Me-(*R*)-PIA	65 ± 7	23.7 ± 1.8
1′-Me-(*S*)-PIA	140 ± 10	29.0 ± 3.0

[a] IC_{50} values were obtained from nonlinear curve fitting of data using the GraphPad computer program. The maximal inhibitory effects were at 10 μM for CPA and 100 μM for the other compounds. All values are the mean ± SEM of three independent experiments.

of affinity. At A_3, a similar substitution was tolerated only in the case of N6-substituted analogues of 1′-Me-Ado. The order of selectivity of 1′-Me-Ado was A_1 (K_i = 7.7 μM) > A_3 (19%) ≥ A_{2A} (7%); for comparison, the order of selectivity of adenosine was estimated to be A_1 (0.01 μM) > A_{2A} (0.03 μM) > A_3 (1.0 μM).[20] 1′-*C*-Methyl-substituted analogues of the A_1 selective agonists CPA, (*R*)-PIA, and (*S*)-PIA were slightly less potent at A_1 receptors than the 2′-*C*-substituted parent compounds with K_i values in the submicromolar range. The most potent compound proved to be 1′-Me-(*R*)-PIA with an affinity of 23 nM and a selectivity of >435-fold for A_1 vs A_{2A} and 29-fold for A_1 vs A_3. 1′-Me-CPA was 4.3-fold less potent than 1′-Me-(*R*)-PIA and less selective for A_1 vs A_{2A} but more selective for A_1 vs A_3. The stereoselectivity characteristic of the N^6 regions of A_1 receptors, similar to that observed for (*R*)-PIA and (*S*)-PIA and their 2′-*C*-methyl analogues, was maintained.

Compounds **1a**–**d** were also tested in a functional assay at A_1 receptors in rat cortical membranes for the ability to inhibit forskolin-stimulated adenylyl cyclase. The efficacy of these compounds was compared with that obtained for CPA (Figure 2). Compounds **1a**–**d** appeared to be full agonists, with an inhibition of cyclase with IC_{50} values ranging from 0.065 to 3.4 μM (Table 2). Statistical analysis of the results revealed that there were significant differences between the maximal inhibition of adenylyl cyclase activity values obtained for 1′-Me-(*S*)-PIA and CPA ($p < 0.05$).

The affinity of the adenosine receptors for **1a** and its N^6-substituted analogues could depend on the conformations of those molecules. We previously reported that the high affinity and selectivity for A_1 receptors of 2′-*C*-methyl-ribosyl analogues of adenosine may be related to their preferential conformation in solution, which was determined to be anti with a north ($^3T_2$) puckered furanose ring form, while the poor activity of 3′-*C*-methyladenosine might be explained by its marked preference for the south ($^2T_3$) syn conformation, with pseudoequatorial location of the purine ring (Figure 1).[14] We found that 1′-Me-Ado and its N^6-substituted analogues have a marked preference for the N-puckered conformation with the purine ring in the low-range syn conformation with pseudoaxial location. The preference of these compounds for the syn conformation of the purine ring was further confirmed by the fact that 1′-Me-Ado was neither a substrate nor an inhibitor of adenosine deaminase.[21] So, the greater affinity of 1′- and 2′-*C*-methyladenosine for the A_1 receptor, as compared to that of 3′-*C*-methyladenosine, might be explained by their preference for the N-puckered conformation of the modified ribose ring. The anti conformation of the purine ring favors the binding to this receptor subtype, as proved by the greater affinity of N^6-substituted 2′-methyl analogues of adenosine as compared to that of their 1′-*C*-methyl analogues.

In conclusion, the 1′-*C*-methyl modification in adenosine resulted in a decrease of the affinity, which was more marked at A_1 and A_{2A} receptors. When such modification was combined with N^6 substitutions with groups that induce high potency and selectivity at A_1 receptors, the high affinity was in part retained and the selectivity increased. The good affinity and selectivity for A_1 receptors of 1′-*C*-methyl-ribosyl analogues of N^6-substituted adenosine derivatives may be related to the preferential conformation in solution of the modified ribose moiety whose puckered furanose ring form was determined to be north ($^3T_2$), similar to that of their 2′-*C*-methyl analogues. The lower potency of 1′-methyl-N^6-substituted derivatives as compared to that of the 2′-methyl analogues might be explained by their marked preference to adopt a syn conformation about the glycosyl bond, which is not optimal for binding at A_1 receptors.

Experimental Section

Chemistry. Melting points were determined on a Büchi apparatus and are uncorrected. Elemental analyses were determined on an EA 1108 CHNS-O (Fisons Instruments) analyzer. Thin-layer chromatography was run on silica gel 60 F_{254} plates (Merck); silica gel 60 (70–230 and 230–400 mesh, Merck) for column chromatography was used. ^{1}H NMR and ^{13}C NMR spectra were determined with a Varian VXR-300 spectrometer at 300 and 75 MHz, respectively. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. All exchangeable protons were confirmed by the addition of D_2O. Stationary NOE experiments were run on degassed solutions at 25 °C. A presaturation delay of 1 s was used, during which the decoupler low power was set at 20 dB attenuation. Mass spectroscopy was carried out on an HP 1100 series instrument. All measurements were performed in the positive ion mode using an atmospheric pressure electrospray ionization.

5-*O*-Benzyl-2,3-*O*-benzylidene-D-ribono-1,4-lactone (3). To a cooled (0 °C) solution of **2** (4.0 g, 16.93 mmol) in anhydrous tetrahydrofuran (THF, 50 mL), a 60% NaH dispersion (812 mg, 20.29 mmol) was added portionwise over 15 min. Benzyl

bromide (2.41 mL, 20.29 mmol) was then added dropwise at the same temperature. The reaction mixture was allowed to warm slowly to room temperature and then was stirred overnight. Ice was added slowly, and the mixture was then poured into ice-water. After the mixture was extracted with CH_2Cl_2 (3 × 40 mL), the organic layer was washed with 10% brine and water, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography on silica gel eluting with petroleum ether/ethyl acetate (5:1) to give **3** as an oil (2.7 g, 49%). ^{1}H NMR (dimethyl sulfoxide (DMSO)-d_6): δ 3.70 (dd, J = 2.1, 10.8 Hz, 1H, H-5), 3.80 (dd, J = 2.6, 10.8 Hz, 1H, H-5), 4.56 (s, 2H, CH_2C$_6$H$_5$), 4.91 (d, J = 5.7 Hz, 1H, H-2), 4.95 (t, J = 2.3 Hz, 1H, H-4), 5.02 (s, J = 5.7 Hz, 1H, H-3), 6.0 (s, 1H, CH(C$_6$H$_5$)), 7.30–7.45 (m, 10H, arom). Anal. ($C_{19}H_{18}O_5$) C, H, N.

2-*O*-Acetyl-6-*O*-benzyl-3,4-*O*-benzylidene-1-deoxy-D-psicofuranose (5). To a stirred solution of 1.4 M methyllithium in diethyl ether (7.8 mL) at −78 °C under a nitrogen atmosphere was added dropwise a solution of **3** (2.54 g, 7.80 mmol) in anhydrous diethyl ether (70 mL). The mixture was reacted for 45 min and then warmed to 0 °C and treated with 10% aqueous NH_4Cl (70 mL). After the mixture was extracted with diethyl ether (3 × 100 mL), the combined organic layers were washed with ice-cold water (2 × 70 mL) and dried over anhydrous Na_2SO_4. Filtration and concentration under reduced pressure yielded an oil (**4**), which was used for the next step without purification. The oil was dried with anhydrous pyridine (3 × 10 mL) and was dissolved in pyridine (20 mL), and then, acetic anhydride (0.68 mL, 7.20 mmol) and 4-(dimethylamino)pyridine (42 mg, 0.34 mmol) were added. The reaction mixture was stirred at room temperature for 48 h, and then, ice-cold water (60 mL) was added. The aqueous phase was extracted with $CHCl_3$ (3 × 40 mL), and the combined organic layers were washed with a cold, saturated $NaHCO_3$ solution and with ice-cold water (3 × 40 mL) and dried over anhydrous Na_2SO_4. Evaporation to dryness yielded an oily residue that was purified by flash chromatography on silica gel (*n*-hexanes−EtOAc, 90:10) to give **5** as a clear oil (1.40 g, 47%). ^{1}H NMR (DMSO-d_6): δ 1.68 (s, 3H, H-1), 1.92 (s, 3H, OCOCH$_3$), 3.56 (d, J = 6.9 Hz, 2H, H-6), 4.40 (t, J = 7.0 Hz, 1H, H-5), 4.57 (s, 2H, CH_2C$_6$H$_5$), 4.84 (d, J = 6.2 Hz, 1H, H-4), 4.93 (d, J = 6.2 Hz, 1H, H-3), 5.85 (s, 1H, CHC$_6$H$_5$), 7.30–7.50 (2m, 10H, arom). Anal. ($C_{22}H_{24}O_6$) C, H, N.

6-Chloro-9*H*-(6-*O*-benzyl-3,4-*O*-benzylidene-1-deoxy-D-psicofuranosyl)purine (7). To a stirred mixture of **5** (1.35 g, 3.51 mmol) and **6** (1.18 g, 7.65 mmol) in anhydrous acetonitrile (35 mL) was added dropwise a 1.8 M solution of $EtAlCl_2$ in toluene (1.8 mL). The reaction mixture was stirred at room temperature for 2 h and then poured into an ice-cold mixture of saturated $NaHCO_3$ solution (140 mL) and CH_2Cl_2 (240 mL). After the mixture was stirred (10 min), the resulting solution was filtered through a pad of Celite and the separated organic layer was washed with a saturated solution of $NaHCO_3$ (120 mL) and brine (2 × 120 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The oily residue was purified by flash chromatography on silica gel (*n*-hexanes−EtOAc, 75:25) to give **7** as a foam containing the inseparable α,β anomers (85% yield).

9*H*-(6-*O*-Benzyl-3,4-*O*-benzylidene-1-deoxy-β-D-psicofuranosyl)adenine (8a) and 9*H*-(6-*O*-Benzyl-3,4-*O*-benzylidene-1-deoxy-α-D-psicofuranosyl)adenine (9a). A mixture of **7** (285 mg, 0.59 mmol) and liquid ammonia (20 mL) was reacted in a Parr bomb at 60 °C for 5 h. Ammonia was removed, and the residue was purified by flash chromatography on silica gel ($CHCl_3$−MeOH, 97.3) to give **8a** and **9a** in a 9:1 ratio (76% overall yield).

8a: ^{1}H NMR (DMSO-d_6): δ 1.80 (s, 3H, H-1′), 3.50 (m, 2H, H-6′), 4.42 (s, 2H, CH_2C$_6$H$_5$), 4.65 (m, 1H, H-5′), 5.10 (dd, J = 2.5, 5.9 Hz, 1H, H-4′), 5.57 (d, J = 5.9 Hz, 1H, H-3′), 6.15 (s, 1H, CHC$_6$H$_5$), 7.30 (m, 7H, CHC$_6$$H_5$, NH$_2$), 7.40–7.60 (2m, 5H, CH$_2C_6$$H_5$), 8.15 (s, 1H, H-2), 8.22 (s, 1H, H-8). Anal. ($C_{25}H_{25}N_5O_4$) C, H, N.

9a: ^{1}H NMR (DMSO-d_6): δ 2.05 (s, 3H, H-1′), 3.75 (d, J = 4.8 Hz, 2H, H-6′), 4.65 (s, 2H, CH_2C$_6$H$_5$), 4.78 (q, J = 4.4 Hz, 1H, H-5′), 5.0 (d, J = 6.2 Hz, 1H, H-4′), 5.20 (dd, J = 4.6, 6.0 Hz, 1H, H-3′), 5.55 (s, 1H, CHC$_6$H$_5$), 7.15–7.40 (m, 12H, arom., NH$_2$), 8.10 (s, 1H, H-2), 8.28 (s, 1H, H-8). Anal. ($C_{25}H_{25}N_5O_4$) C, H, N.

***N*^{6}-Cyclopentyl-9*H*-(6-*O*-benzyl-3,4-*O*-benzylidene-1-deoxy-β-D-psicofuranosyl)adenine (8b) and *N*^{6}-Cyclopentyl-9*H*-(6-*O*-benzyl-3,4-*O*-benzylidene-1-deoxy-α-D-psicofuranosyl)adenine (9b).** A mixture of **7** (375 mg, 0.78 mmol) in anhydrous EtOH (10 mL) and cyclopentylamine (0.46 mL, 4.68 mmol) under a nitrogen atmosphere was refluxed for 1.5 h. The mixture was concentrated in vacuo, and the residue was purified by flash chromatography on silica gel (*n*-hexanes−EtOAc, 70:30). As the first eluate, **8b** was separated as a foam (313 mg, 76%). ^{1}H NMR (DMSO-d_6): δ 1.53–1.70 (m, 6H, cyclopentyl), 1.80 (s, 3H, H-1′), 1.95 (m, 2H, cyclopentyl), 3.55 (m, 2H, H-6′), 4.40 (s, 2H, CH_2C$_6$H$_5$), 4.60 (m, 1H, NHCH), 4.65 (m, 1H, H-5′), 5.02 (dd, J = 2.5, 5.9 Hz, 1H, H-4′), 5.72 (d, J = 6.5 Hz, 1H, H-3′), 6.0 (s, 1H, CHC$_6$H$_5$), 7.05–7.25 (2m, 5H, CHC$_6$$H_5$), 7.40–7.60 (2m, 5H, CH$_2C_6$$H_5$), 7.70 (d, J = 7.9 Hz, 1H, NH), 8.20 (s, 2H, H-2, H-8). Anal. ($C_{30}H_{33}N_5O_4$) C, H, N.

As the second eluate, **9b** was obtained as a foam (25 mg, 6%). ^{1}H NMR (DMSO-d_6): δ 1.65, 1.90 (2m, 8H, cyclopentyl), 2.05 (s, 3H, H-1′), 3.72 (d, J = 4.8 Hz, 2H, H-6′), 4.22 (t, J = 6.3 Hz, 1H, H-5′), 4.55 (m, 1H, NHCH), 4.65 (s, 2H, CH_2C$_6$H$_5$), 4.80 (m, 1H, H-4′), 5.05 (s, 1H, H-3′), 5.80 (s, 1H, CHC$_6$H$_5$), 7.15–7.45 (m, 10H, CHC$_6$$H_5$, CH$_2C_6$$H_5$), 7.55 (d, J = 8.4 Hz, 1H, NH), 8.10 (s, 1H, H-2), 8.18 (s, 1H, H-8). Anal. ($C_{30}H_{33}N_5O_4$) C, H, N.

***N*^{6}-[(1*R*)-1-Methyl-2-phenylethyl]-9*H*-(6-*O*-benzyl-3,4-*O*-benzylidene-1-deoxy-β-D-psicofuranosyl)adenine (8c) and *N*^{6}-[(1*R*)-1-Methyl-2-phenylethyl]-9*H*-(6-*O*-benzyl-3,4-*O*-benzylidene-1-deoxy-α-D-psicofuranosyl)adenine (9c).** Compound **7** (410 mg, 0.86 mmol) was treated with *l*-amphetamine as described for **8b** (reaction time 6 h). The crude product was chromatographed on a silica gel column ($CHCl_3$−EtOAc, 95:5) to give **8c** as the first eluate (50%) and **9c** as the second eluate (8.0%), both as a foam.

8c: ^{1}H NMR (DMSO-d_6): δ 1.20 (d, J = 6.6 Hz, 3H, CHCH_3), 1.80 (s, 3H, H-1′), 2.75, 3.0 (2m, 2H, CH_2C$_6$H$_5$), 3.47 (dd, J = 7.6, 10.8 Hz, 1H, H-6′), 3.60 (dd, J = 4.8, 10.6 Hz, 1H, H-6′), 4.40 (s, 2H, CH_2C$_6$H$_5$) 4.65 (m, 2H, H-5′, CHCH$_3$), 5.05 (dd, J = 2.5, 5.9 Hz, 1H, H-4′), 5.56 (d, J = 5.9 Hz, 1H, H-3′), 6.15 (s, 1H, CHC$_6$H$_5$), 7.20 (m, 5H, CH$_2$C$_6$$H_5$), 7.40–7.55 (2m, 5H, CHC$_6$$H_5$), 7.70 (d, J = 8.0 Hz, 1H, NH), 8.20 (s, 2H, H-2, H-8). Anal. ($C_{34}H_{35}N_5O_4$) C, H, N.

9c: ^{1}H NMR (DMSO-d_6): δ 1.20 (d, J = 6.8 Hz, 3H, CHCH_3), 2.0 (s, 3H, H-1′), 2.70 (dd, J = 6.8, 13.4 Hz, 1H, CH_2C$_6$H$_5$), 3.0 (dd, J = 7.0, 13.6 Hz, 1H, CH_2C$_6$H$_5$), 3.95 (m, 2H, H-6′), 4.50 (m, 1H, CHCH$_3$), 4.60 (s, 2H, CH_2C$_6$H$_5$) 4.75 (q, J = 4.6 Hz, 2H, H-5′), 5.0 (d, J = 5.8 Hz, 1H, H-4′), 5.20 (dd, J = 4.0, 5.8 Hz, 1H, H-3′), 5.52 (s, 1H, CHC$_6$H$_5$), 7.25 (m, 10H, CH$_2$C$_6$$H_5$, CHC$_6$$H_5$), 7.80 (d, J = 8.0 Hz, 1H, NH), 8.15 (s, 1H, H-2), 8.20 (s, 1H, H-8). Anal. ($C_{34}H_{35}N_5O_4$) C, H, N.

N^6-[(1*S*)-1-Methyl-2-phenylethyl]-9*H*-(6-*O*-benzyl-3,4-*O*-benzylidene-1-deoxy-β-D-psicofuranosyl)adenine (8d) and N^6-[(1*S*)-1-Methyl-2-phenylethyl]-9*H*-(6-*O*-benzyl-3,4-*O*-benzylidene-1-deoxy-α-D-psicofuranosyl)adenine (9d). Compounds **8d** and **9d** were obtained from **7** (360 mg, 0.75 mmol) with *d*-amphetamine as described above (reaction time 15 h). Chromatography on a silica gel column ($CHCl_3$−EtOAc, 95:5) gave **8d** as the first eluate (69%) as a foam. Compound **9d** was also obtained as a foam (5%).

8d: ^{1}H NMR (DMSO-d_6): δ 1.20 (d, J = 6.2 Hz, 3H, CHCH_3),1.80 (s, 3H, H-1′), 2.75 (dd, J = 6.6, 13.5 Hz, 1H, CH_2C$_6$H$_5$), 3.05 (dd, J = 7.5, 13.4 Hz, 1H, CH_2C$_6$H$_5$), 3.50 (m, 2H, H-6′), 4.42 (s, 2H, CH_2C$_6$H$_5$) 4.65 (m, 2H, H-5′, CHCH$_3$), 5.0 (dd, J = 2.6, 5.9 Hz, 1H, H-4′), 5.80 (d, J = 6.0 Hz, 1H, H-3′), 6.15 (s, 1H, CHC$_6$H$_5$), 7.25 (m, 5H, CH$_2$C$_6$$H_5$), 7.40–7.55 (2m, 5H, CHC$_6$$H_5$), 7.70 (d, J = 7.6 Hz, 1H, NH), 8.20 (s, 2H, H-2, H-8). Anal. ($C_{34}H_{35}N_5O_4$) C, H, N.

9d: ^{1}H NMR (DMSO-d_6): δ 1.20 (d, J = 6.6 Hz, 3H, CHCH_3), 2.0 (s, 3H, H-1′), 2.70 (dd, J = 6.6, 13.2 Hz, 1H, CH_2C$_6$H$_5$), 3.05 (dd, J = 7.2, 13.4 Hz, 1H, CH_2C$_6$H$_5$), 3.90 (m,

2H, H-6′), 4.60 (m, 1H, C*H*CH$_3$), 4.62 (s, 2H, C*H*$_2$C$_6$H$_5$), 4.75 (q, 1H, H-5′), 5.0 (d, *J* = 5.9 Hz, 1H, H-4′), 5.20 (dd, *J* = 4.2, 5.8 Hz, 1H, H-3′), 5.55 (s, 1H, C*H*C$_6$H$_5$), 7.10−7.40 (m, 10H, CH$_2$C$_6$*H*$_5$, CHC$_6$*H*$_5$), 7.62 (d, *J* = 7.7 Hz, 1H, NH), 8.15 (s, 1H, H-2), 8.25 (s, 1H, H-8). Anal. (C$_{34}$H$_{35}$N$_5$O$_4$) C, H, N.

General Procedure for the Synthesis of Compounds 1a−d. Compounds **1a−d** were obtained from **8a−d** (0.85 mmol) in MeOH (30 mL) by treatment with 10% Pd/C in the presence of ammonium formate (6.8 mmol) under reflux. After the compounds were cooled at room temperature, the mixture was filtered and the filtrate was evaporated to dryness and purified by chromatography on silica gel to give the desired compounds.

9*H*-(1-Deoxy-*β*-D-psicofuranosyl)adenine (1a). The title compound was synthesized from **8a** (reaction time 1 h) and chromatographed on a silica gel column (CHCl$_3$−MeOH, 85:15) to give a white solid (57%); mp >230 °C dec. ^{1}H NMR (DMSO-*d*$_6$): δ 1.72 (s, 3H, H-1′), 3.50 (m, 2H, H-6′), 3.75−4.0 (m, 2H, H-5′, H-4′), 4.60 (t, *J* = 4.7 Hz, 1H, H-3′), 5.0 (d, *J* = 6.5 Hz, 1H, OH), 5.22 (t, *J* = 5.5 Hz, 1H, OH), 5.70 (d, *J* = 5.0 Hz, 1H, OH), 7.25 (br s, 2H, NH$_2$), 8.12 (s, 1H, H-2), 8.40 (s, 1H, H-8). ^{13}C NMR (DMSO-*d*$_6$): δ 22.6 (C-1′), 60.4 (C-6′), 69.3 (C-4′), 74.6 (C-3′), 83.7 (C-5′), 97.2 (C-2′), 120.4 (C-5), 139.1 (C-8), 148.0 (C-4), 152.1 (C-2), 156.4 (C-6). MS: *m*/*z* 282.1 [MH]$^+$, 304.1 [M + Na$^+$], 320.1 [M + K$^+$]. Anal. (C$_{11}$H$_{15}$N$_5$O$_4$) C, H, N.

N^6-Cyclopentyl-9*H*-(1-deoxy-*β*-D-psicofuranosyl)adenine (1b). Compound **1b** was synthesized from **8b** (reaction time 1.5 h). Purification by chromatography on a silica gel column (CHCl$_3$−MeOH, 95:5) gave **1b** as a white solid (60%); mp 175−177 °C. ^{1}H NMR (DMSO-*d*$_6$): δ 1.60 (m, 6H, cyclopentyl), 1.75 (s, 3H, H-1′), 1.90 (m, 2H, cyclopentyl), 3.55 (m, 2H, H-6′), 3.70−4.0 (m, 2H, H-5′, H-4′), 4.55 (m, 1H, NHC*H*), 4.60 (t, *J* = 4.6 Hz, 1H, H-3′), 4.98 (d, *J* = 6.3 Hz, 1H, OH), 5.20 (t, *J* = 6.3 Hz, 1H, OH), 5.70 (d, *J* = 5.0 Hz, 1H, OH), 7.65 (d, *J* = 8.6 Hz, 1H, NH), 8.20 (s, 1H, H-2), 8.40 (s, 1H, H-8). ^{13}C NMR (DMSO-*d*$_6$): δ 22.6 (C-1′), 23.7 (2C, cyclopentyl), 29.9 (2C, cyclopentyl), 32.7 (cyclopentyl), 60.4 (C-6′), 69.3 (C-4′), 74.6 (C-3′), 83.7 (C-5′), 97.2 (C-2′), 120.5 (C-5), 138.7 (C-8), 148.2 (C-4), 152.0 (C-2), 154.6 (C-6). MS: *m*/*z* 350.3 [MH]$^+$, 372.2 [M + Na$^+$], 388.2 [M + K$^+$]. Anal. (C$_{16}$H$_{23}$N$_5$O$_4$) C, H, N.

N^6-[(1*R*)-1-Methyl-2-phenylethyl]-9*H*-(1-deoxy-*β*-D-psicofuranosyl)adenine (1c). Compound **1c** was synthesized from **8c** (reaction time 2.5 h) and chromatographed on a silica gel column (CHCl$_3$−MeOH, 90:10) (white solid, 52%); mp 128−130 °C. ^{1}H NMR (DMSO-*d*$_6$): δ 1.20 (d, *J* = 6.5 Hz, 3H, CHC*H*$_3$), 1.75 (s, 3H, H-1′), 2.75 (dd, *J* = 6.7, 13.1 Hz, 1H, C*H*$_2$C$_6$H$_5$), 3.0 (dd, *J* = 7.6, 13.4 Hz, 1H, C*H*$_2$C$_6$H$_5$), 3.55 (m, 2H, H-6′), 3.70−4.0 (m, 3H, H-5′, H-4′, C*H*CH$_3$), 4.60 (t, *J* = 4.7 Hz, 1H, H-3′), 5.0 (d, *J* = 6.2 Hz, 1H, OH), 5.20 (t, *J* = 5.5 Hz, 1H, OH), 5.70 (d, *J* = 5.1 Hz, 1H, OH), 7.15, 7.25 (2m, 5H, CH$_2$C$_6$*H*$_5$), 7.65 (d, *J* = 8.3 Hz, 1H, NH), 8.15 (s, 1H, H-2), 8.40 (s, 1H, H-8). ^{13}C NMR (DMSO-*d*$_6$): δ 20.4 (C-1′), 22.6 (CH*C*H$_3$), 41.9 (*C*H$_2$C$_6$H$_5$), 47.2 (*C*HNH), 60.3 (C-6′), 69.2 (C-4′), 74.4 (C-3′), 83.6 (C-5′), 97.2 (C-2′), 120.4 (C-5), 126.2 (C$_6$H$_5$), 128.4 (2C, C$_6$H$_5$), 129.3 (2C, C$_6$H$_5$), 138.8 (C$_6$H$_5$), 139.7 (C-8), 147.1 (C-4), 152.1 (C-2), 154.1 (C-6). MS: *m*/*z* 400.2 [MH]$^+$, 422.2 [M + Na$^+$]. Anal. (C$_{20}$H$_{25}$N$_5$O$_4$) C, H, N.

N^6-[(1*S*)-1-Methyl-2-phenylethyl]-9*H*-(1-deoxy-*β*-D-psicofuranosyl)adenine (1d). The title compound was obtained from **8d** (reaction time 3.5 h) and purified by chromatography on a silica gel column (CHCl$_3$−MeOH, 90:10) (white solid, 50%); mp 128−130 °C. ^{1}H NMR (DMSO-*d*$_6$): δ 1.20 (d, *J* = 6.3 Hz, 3H, CHC*H*$_3$),1.75 (s, 3H, H-1′), 2.75, 3.0 (2m, 2H, C*H*$_2$C$_6$H$_5$), 3.55 (m, 2H, H-6′), 3.70−4.0 (m, 3H, H-5′, H-4′, C*H*CH$_3$), 4.60 (t, *J* = 4.7 Hz, 1H, H-3′), 5.0 (d, *J* = 6.2 Hz, 1H, OH), 5.20 (t, *J* = 5.1 Hz, 1H, OH), 5.70 (d, *J* = 4.8 Hz, 1H, OH), 7.15, 7.25 (2m, 5H, CH$_2$C$_6$*H*$_5$), 7.65 (d, *J* = 8.4 Hz, 1H, NH), 8.15 (s, 1H, H-2), 8.40 (s, 1H, H-8). ^{13}C NMR (DMSO-*d*$_6$): δ 20.4 (C-1′), 22.6 (CH*C*H$_3$), 41.9 (*C*H$_2$C$_6$H$_5$), 47.2 (*C*HNH), 60.3 (C-6′), 69.2 (C-4′), 74.4 (C-3′), 83.6 (C-5′), 97.2 (C-2′), 120.4 (C-5), 126.2 (2C, C$_6$H$_5$), 128.4 (2C, C$_6$H$_5$), 129.3 (C$_6$H$_5$), 138.8 (C$_6$H$_5$), 139.7 (C-8), 147.1 (C-4), 152.1 (C-2), 154.1 (C-6). MS: *m*/*z* 400.2 [MH]$^+$, 422.2 [M + Na$^+$]. Anal. (C$_{20}$H$_{25}$N$_5$O$_4$) C, H, N.

6-Chloro-9*H*-(6-*O*-benzyl-1-deoxy-3,4-*O*-isopropylidene-D-psicofuranosyl)purine (10). The title compound was prepared from 2-*O*-acetyl-6-*O*-benzyl-1-deoxy-3,4-*O*-isopropylidene-D-psicofuranose[22] (1.2 g, 3.4 mmol) as described for **7** (reaction time 1.5 h). After work up, **10** as an inseparable α,*β* mixture was obtained (78% yield).

9*H*-(6-*O*-Benzyl-1-deoxy-3,4-*O*-isopropylidene-*β*-D-psicofuranosyl)adenine (11a). Compound **11a** was obtained from **10** (600 mg, 1.39 mmol) as described for **8a** (reaction time 7 h) and was purified by chromatography on a silica gel column (CHCl$_3$−MeOH, 98:2) (foam , 71%). ^{1}H NMR (DMSO-*d*$_6$): δ 1.35, 1.53 (2s, 6H, C(C*H*$_3$)$_2$), 1.65 (s, 3H, H-1′), 3.42 (dd, *J* = 4.9, 10.4 Hz, 1H, H-6′), 3.52 (dd, *J* = 3.2,10.5 Hz, 1H, H-6′), 4.38 (s, 2H, C*H*$_2$C$_6$H$_5$), 4.51 (t, *J* = 5.1 Hz, 1H, H-5′), 4.80 (dd, *J* = 1.5, 6.0 Hz, 1H, H-4′), 5.51 (d, *J* = 6.0 Hz, 1H, H-3′), 7.10, 7.25 (2m, 7H, CH$_2$C$_6$*H*$_5$, NH$_2$), 8.15 (s, 1H, H-2), 8.20 (s, 1H, H-8). Anal. (C$_{21}$H$_{25}$N$_5$O$_4$) C, H, N.

N^6-Cyclopentyl-9*H*-(6-*O*-benzyl-1-deoxy-3,4-*O*-isopropylidene-*β*-D-psicofuranosyl)adenine (11b). The title compound was obtained from **10** (500 mg, 1.16 mmol) as described for **8b** and was purified by flash chromatography on silica gel (CH$_2$Cl$_2$−EtOH, 99.2:0.8) (foam, 75%). ^{1}H NMR (CDCl$_3$): δ 1.40, 1.60 (2s, 6H, C(C*H*$_3$)$_2$), 1.53−1.70 (m, 6H, cyclopentyl), 1.75 (s, 3H, H-1′), 2.10 (m, 2H, cyclopentyl), 3.45 (dd, *J* = 5.3, 10.5 Hz, 1H, H-6′), 3.55 (dd, *J* = 3.7, 10.4 Hz, 1H, H-6′), 4.35 (q, *J* = 11.9 Hz, 2H, C*H*$_2$C$_6$H$_5$), 4.51 (m, 1H, H-5′), 4.55 (dd, *J* = 1.5, 6.1 Hz, 1H, H-4′), 4.60 (m, 1H, NHC*H*), 5.60 (d, br d, *J* = 6.1 Hz, 2H, H-3′, NH), 7.0, 7.18 (2m, 5H, CH$_2$C$_6$*H*$_5$), 8.0 (s, 1H, H-2), 8.40 (s, 1H, H-8). Anal. (C$_{26}$H$_{33}$N$_5$O$_4$) C, H, N.

9*H*-(1-Deoxy-3,4-*O*-isopropylidene-*β*-D-psicofuranosyl)adenine (12a). The title compound was obtained from **11a** (350 mg, 0.85 mmol) as described for **1a** (reaction time 1 h). Chromatography on a silica gel column (CHCl$_3$−MeOH, 92:8) gave **12a** as a foam (81%).^{1}H NMR (DMSO-*d*$_6$): δ 1.33, 1.53 (2s, 6H, C(C*H*$_3$)$_2$), 1.67 (s, 3H, H-1′), 3.38 (m, 2H, H-6′), 4.28 (t, *J* = 6.6 Hz, 1H, H-5′), 4.76 (dd, *J* = 1.8, 6.2 Hz, 1H, H-4′), 5.08 (t, *J* = 5.1 Hz, 1H, OH), 5.50 (d, *J* = 6.2 Hz, 1H, H-3′), 7.25 (br s, 2H, NH$_2$), 8.15 (s, 1H, H-2), 8.22 (s, 1H, H-8). Anal. (C$_{14}$H$_{19}$N$_5$O$_4$) C, H, N.

N^6-Cyclopentyl-9*H*-(1-deoxy-3,4-*O*-isopropylidene-*β*-D-psicofuranosyl)adenine (12b). The title compound was prepared from **11b** (350 mg, 0.73 mmol), (reaction time 1.5 h) and was purified by chromatography on a silica gel column (CH$_2$Cl$_2$−EtOH, 98:2) (foam, 70%). ^{1}H NMR (DMSO-*d*$_6$): δ 1.33, 1.53 (2s, 6H, C(C*H*$_3$)$_2$), 1.55−1.70 (m, 6H, cyclopentyl), 1.67 (s, 3H, H-1′), 1.90 (m, 2H, cyclopentyl), 3.40 (m, 2H, H-6′), 4.28 (t, *J* = 6.6 Hz, 1H, H-5′), 4.53 (m, 1H, NHC*H*), 4.76 (dd, *J* = 1.8, 6.2 Hz, 1H, H-4′), 5.08 (t, *J* = 5.1 Hz, 1H, OH), 5.50 (d, *J* = 6.2 Hz, 1H, H-3′), 7.65 (d, *J* = 8.6 Hz, 1H, NH), 8.20 (s, 1H, H-2), 8.22 (s, 1H, H-8). Anal. (C$_{19}$H$_{27}$N$_5$O$_4$) C, H, N.

Biological Methods. Materials. [^{3}H]-(*R*)-PIA (37 Ci/mmol), [^{3}H]cAMP (adenosine 3′,5′-cyclic monophosphate, 25 Ci/mmol), and [α-^{32}P]ATP (adenosine 5′-triphosphate, 30−40 Ci/mmol) were from Amersham Corp., while [^{3}H]CHA (sp. act. 32.5 Ci/mmol) and [^{3}H]CGS21680 (37.5 Ci/mmol) were purchased from NEN Life Science Products, Inc. CPA, (*R*)-PIA, NECA, and other agents were purchased from RBI. Forskolin and guanosine 5′-triphosphate were from Sigma-Aldrich Srl. Myokinase, creatine kinase, and adenosine deaminase were obtained from Boehringer-Mannheim (Mannheim, Germany). All other reagents were from standard commercial sources and of the highest grade commercially available.

Receptor Binding Assay. Rat membranes of the cerebral cortex, cerebral striatum, and testis were prepared as previously described.[14] Rat cortical membranes were suspended in 10 volumes of ice-cold buffer A (1 mM ethylenediaminetetraacetic acid (EDTA), 5 mM MgCl$_2$, and 50 mM Tris/HCl, pH 7.7) and homogenized, and binding of [^{3}H]CHA to A$_1$ receptors was measured in triplicate, as previously described.[18a] Rat striatal membranes were suspended in 20 volumes of buffer B (10 mM MgCl$_2$, 50 mM Tris/HCl, pH 7.4) and homogenized,

and binding of [^{3}H]CGS21680 to A_{2a} receptors was performed as previously described.[18b] Rat testis membranes were suspended in 15 volumes of buffer C (1 mM EDTA, 10 mM $MgCl_2$, and 50 mM Tris/HCl, pH 7.4) and homogenized, and binding of [^{3}H](*R*)-PIA to A_3 adenosine receptors was measured in the presence of DPCPX (150 nM) to block A_1 adenosine receptors, as previously described.[19] Compounds were dissolved in DMSO and then diluted with assay buffer, and final DMSO concentrations never exceeded 1%. At least six different concentrations of each compound were used. IC_{50} values, computer-generated using a nonlinear regression formula on a computer program (GraphPad, San Diego, CCA), were converted to K_i values, knowing the K_d values of radioligands in these different tissues and using the Cheng and Prusoff equation.[23]

Adenylyl Cyclase Assay. The cerebral cortex was obtained from male Sprague−Dawley rats sacrificed by cervical dislocation. Fresh tissue was used for membrane preparation, performed as previously described.[14] Adenylyl cyclase activity was measured by monitoring the conversion of [α-^{32}P]ATP to [α-^{32}P]-cAMP, using a previously reported method.[23] The method involved the addition of [α-^{32}P]ATP to membranes in the presence of forskolin to stimulate adenylyl cyclase and papaverine as a phosphodiesterase inhibitor. The assay was performed as previously described.[14] Compounds tested as inhibitors of forskolin-stimulated adenylyl cyclase activity were dissolved in DMSO and then diluted with 50 mM *N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid/NaOH buffer, pH 7.4, so that the final DMSO concentration never exceeded 1%. IC_{50} values were calculated using a nonlinear regression analysis (GraphPad).[24]

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