Development of Spin-Labeled Probes for Adenosine Receptors†

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Functionalized xanthine derivatives bearing a nitroxide moiety at the 3- or 8-position were synthesized as electron paramagnetic resonance (EPR) probes. The 8-cyclopentyl-1-propylxanthine derivative **4**, spin-labeled at N3 by substitution with a nitroxide-bearing dihydropyrrole moiety, was a potent and selective A_1 adenosine receptor antagonist (K_i for A_1 5.5 nM, 1600-fold selectivity vs A_{2A}, >200-fold vs A_{2B}, and 310-fold vs A_3 adenosine receptors). 8-(1-Oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-yl)-1,3-dipropylxanthine **10** (K_i for A_1 8.2 nM) was similarly potent and selective, while 8-(1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)-1,3-dipropylxanthine **11** (K_i for A_1 160 nM) exhibited significantly lower affinity for A_1 adenosine receptors. 8-[4-(((1-Oxyl-2,2,6,6-tetramethylpiperidin-4-yl)amino)-2-oxoethoxy)phenyl]-1-propylxanthine **14**, a 3-unsubstituted xanthine derivative, was found to be a potent A_{2B} adenosine receptor antagonist (K_i for A_{2B} 48 nM) but also exhibited high affinity for A_1 receptors (K_i for A_1 15.7 nM). An X-ray structure of compound **10** was obtained, confirming the proposed structure. The novel spin-labeled A_1-selective or A_1/A_{2B}-nonselective adenosine receptor antagonists may become useful probes for biophysicochemical investigations of adenosine receptors in their membrane environment.

Introduction

Adenosine is a building block of many biologically relevant molecules such as ATP and nucleic acids. In addition, it has important regulatory functions mediated through adenosine receptors (ARs).[1,2] So far four ARs (A_1, A_{2A}, A_{2B}, and A_3) have been characterized and cloned from several mammalian species. A_1 and A_{2A} receptors are so-called "high-affinity" subtypes, activated by adenosine in nanomolar concentrations, while A_{2B} and A_3 receptors ("low-affinity" subtypes) are usually activated by adenosine only at micromolar concentrations.[1−4] ARs are important new drug targets. All ARs are G protein-coupled receptors (GPCR), and being membrane proteins they are not easily amenable to crystallization. Therefore an exact structure elucidation through X-ray diffraction has not been achieved yet. The three-dimensional architecture of only two related membrane proteins, bacteriorhodopsin[5] and (bovine) rhodopsin,[6] has been revealed. These proteins have been used as templates for the modeling of GPCRs. To characterize the adenosine binding site and receptor structure, different indirect approaches have been used, including chemical modification of histidine residues and site-directed mutagenesis.[7,8] However, many characteristics of ARs still have to be revealed, such as the exact process of ligand interaction with the cell membranes and the processes and mechanisms of receptor ligand recognition and signal transduction.

Functionalized receptor ligands have been extensively used for elucidating those processes, including radiolabeled compounds,[9] ligands bearing photoaffinity labels,[10] and fluorescence-labeled probes.[11,12] To the best of our knowledge, spin-labeled ligands as electron paramagnetic resonance (EPR) probes have not been developed for ARs so far.

Stable free radicals of the nitroxide type can be utilized as spin labels and spin probes in electron paramagnetic resonance (EPR) spectroscopy. The fine structure of the resulting spectra provides information about the environment of the radical moiety and allows a description of the dynamical, structural, and redox properties of its surroundings.[13] For this reason spin-labeling can also contribute to the elucidation of the structure, the dynamics, and thus the function of biomolecules such as proteins and nucleic acids. The microgeography of many active sites of enzymes could be described by EPR spectroscopy long before their X-ray structures were obtained.[14−17]

The successful application of spin-labeling methods in enzyme and receptor studies strongly depends on a properly designed spin probe molecule. High biological activity and/or binding affinity of the paramagnetic compound are key requirements.

Nitroxide radicals are paramagnetic compounds.[18] By steric hindrance it is possible to reduce their reactivity. Shielding can be achieved by inserting the nitroxide moiety into a 2,2,5,5- or 2,2,6,6-tetramethylated five- or six-membered nonaromatic ring system (e.g., piperidine, pyrrolidine, or even dihydropyrrole). These stable radicals can be isolated in pure form and stored for up to several years without any decomposition. In addition, they can be functionalized in order to provide bifunc-

† Dedicated to the memory of Dr. Paul Janssen.

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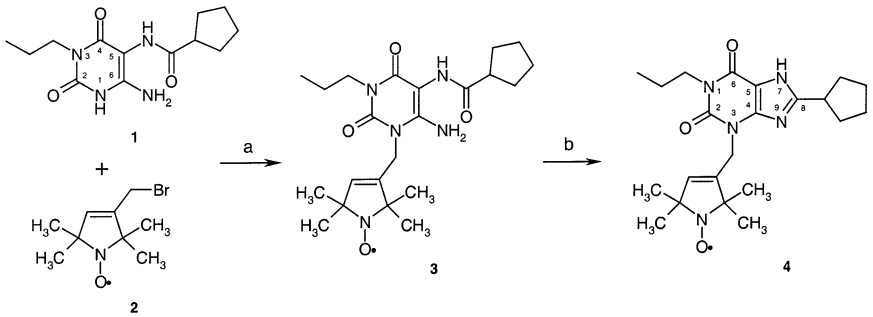
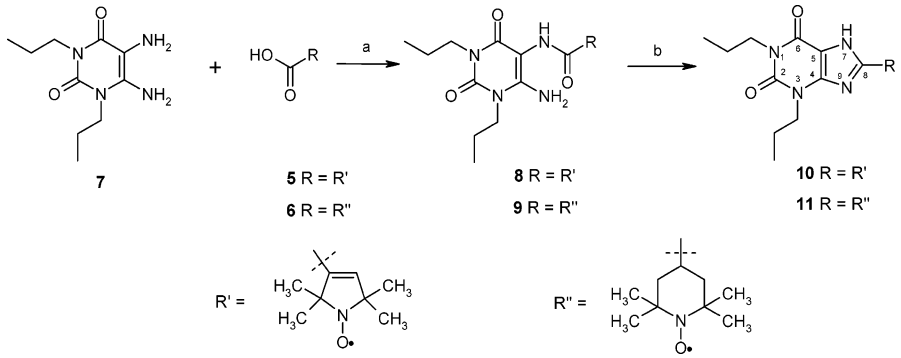
Scheme 1[a]



[a] Reagents, conditions, and yields: (a) K_2CO_3, DMF, rt, 16 h (55% **3**); (b) THF/HMDS, pressure tube, microwave (MW) 100 W, 140 °C, 20 min (28% **4**).

Scheme 2[a]



[a] Reagents, conditions, and yields: (a) EDC, methanol, rt 2 days (67% **8**, 32% **9**); (b) 1 M NaOH/dioxane (1:1), reflux 45 min (75% **10**, 72 % **11**).

tional compounds that can serve to introduce the radical functionality into a diamagnetic compound. The resulting spin-labeled probes can be very powerful molecular tools to study the interaction of ligands with receptors or enzymes by EPR spectroscopy.[13]

Our goal was to develop EPR probes for (a) the A_1 AR, which is the best investigated AR subtype, and (b) for the A_{2B} AR, still the most "enigmatic" AR subtype.[19] Xanthine derivatives are the most intensively investigated class of AR antagonists. Structure−activity relationships are well-known. The A_1 AR receptor tolerates both bulky substituents such as cyclopentyl, phenyl, and 3-noradamantyl in the 8-position and large substituents (e.g., phenethyl) in the 3-position.[4] These positions may therefore be suitable for substitution with bulky nitroxide moieties without significant influence on binding affinity.

On the other hand, A_{2B}-selective AR antagonists have been described in a series of 3-unsubstituted xanthines bearing a para-substituted 8-phenyl residue.[20] To obtain spin-labeled probes for A_{2B} ARs, we therefore planned to attach a nitroxide-bearing moiety to 1-propyl-8-phenylxanthine via a spacer group. The synthesis and physicochemical and biological characterization of four new spin-labeled xanthine derivatives is described, two of which exhibit high affinity and selectivity for A_1 ARs and one of which is a potent but nonselective A_1/A_{2B} receptor ligand.

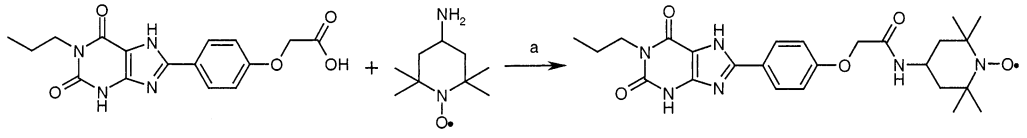
Synthesis

1-Propyl-8-cyclopentylxanthine derivative **4**, labeled in the 3-position, was prepared by condensation of 3-*n*-propyl-5,6-diaminouracil[21] and cyclopentyl carboxylic acid in the presence of (3-dimethylaminopropyl)ethylcarbodiimide hydrochloride (EDC) to yield compound **1**.[22] The N1-atom of the uracil derivative **1** could easily be alkylated in DMF in the presence of potassium carbonate with the spin-labeled alkyl bromide **2**[23] at room temperature, yielding the spin-labeled uracil derivative **3**. Ring closure reactions under elimination of water to yield the final xanthine derivatives are usually performed either in aqueous alkaline solution, or by heating with the nonpolar 1,1,1,3,3,3-hexamethyldisilazane (HMDS) as a solvent and condensing agent. If polar reactants are used, conventional heating with HMDS (bp 126 °C) often requires long reaction times and high reaction temperatures.[24] For the ring closure reaction of **3** to the corresponding spin-labeled xanthine derivative **4**, a mixture of HMDS and tetrahydrofuran (THF) in a microwave-assisted reaction (100 W, 140 °C, 20 min) was found to give the best results, with a yield of 28% (Scheme 1).

Xanthine derivatives **10** and **11**, spin-labeled in the 8-position, were prepared starting from 5,6-diamino-1,3-di-*n*-propyluracil **7**. Coupling of **7** with carboxylic acid derivatives of stable nitroxide radicals **5**[23,25,26] and **6**,[27] respectively, in methanol at room temperature with EDC as a condensing agent led directly to the uracil derivatives **8** and **9**. After a reaction time of 2 days under argon, **8** could be obtained in good and **9** in moderate yields (Scheme 2).

The ring closure reaction of **8** and **9** to their corresponding xanthine derivatives **10** and **11** resulted in better yields when carried out in a mixture of aqueous sodium hydroxide solution and dioxane (1:1) under reflux conditions as compared to the condensation with HMDS. After a reaction time of only 45 min, highly pure **10** and **11** could be obtained in yields of more than 70%

Scheme 3[a]



12 13 14

[a] Reagents, conditions, and yield: (a) EDC, DMAP, DMF/CH_2Cl_2, rt, 6 days, (14% **14**).

(Scheme 2). In the case of **10**, crystallization from dichloromethane/diethyl ether provided sufficiently good crystals for X-ray analysis.

1,8-Disubstituted xanthine derivative **14**, spin-labeled at the 8-residue, was synthesized starting from xanthine derivative **12**.[20] The carboxylic group of xanthine derivative **12** was initially transferred to the corresponding acid chloride, which was subsequently condensed with the spin-labeled amine **13**.[18,28] However, this reaction sequence resulted in only low yields of the desired compound **14** (<5%) associated with the formation of a wide range of side products. Coupling of **12** and **13** in the presence of EDC and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) resulted in better yields of **14** of up to 14% after a reaction time of 6 days with a 2-fold excess of the amine **13** (Scheme 3).[20] Compound **14** was poorly soluble in usual organic solvents, with the exception of dimethylformamide (DMF) and dimethyl sulfoxide (DMSO). Due to its low solubility, **14** was isolated from the reaction mixture by extraction with large quantities of ethyl acetate.

Analytical Characterization of Spin-Labeled Ligands. For the new compounds **4**, **10**, **11**, and **14**, EPR spectra were recorded to prove the presence of the nitroxide group in the molecules and to determine the purity of the compounds. The EPR spectra of freshly prepared 1 mM solutions in dichloromethane, recorded at room temperature, showed the expected three-line spectra with three hyperfine lines for the spin-labeled compounds. ^{14}N Hyperfine coupling constants (a_N) in the range of 14.76−15.93 G could be extracted for **4**, **10**, **11**, and **14**. Further characterization of the final spin-labeled compounds was achieved by thin-layer chromatography, infrared spectroscopy, and mass spectrometry (electrospray ionization mode). Elemental analysis and melting points were also determined. An X-ray structure of compound **10** provided further evidence for the proposed structure (Figure 1).

X-ray Structure Analysis of Compound 10. Since radicals cannot be investigated by standard NMR techniques, structural data gained by X-ray analysis were required. These data will also be valuable for the interpretation of data obtained by biophysicochemical investigations and subsequent molecular modeling studies of receptor−ligand complexes.

The crystal structure of **10** exhibits one molecule with normal intramolecular bond lengths per asymmetric unit. The four molecules per unit cell form two dimers that are held together by van der Waals forces. Each dimer consists of two molecules related by inversion symmetry and bonded via the hydrogen bonds N7−H7···O2 (corresponding to N7−H···O=C6 according to the numbering in Scheme 2: N7−H7 = 0.917(4) Å, H7···O2 = 1.877(9) Å, N7−H7−O2 = 174.40(1)°; see Figure 1). The planar central parts of all dimers are oriented perpendicular to both (100) and alternatingly to [012] and [0−12], respectively. The xanthine ring system is almost planar with an rms value of 0.025 Å for the atom deviations from the least-squares plane defined by N1, C2, N3, C4, C5, C6, N7, C8, N9, O1, O2, and H7. The plane of the pyrroline-1-oxyl ring, defined by C10, C11, C12, N13, C14, O3, and H11 (rms = 0.035 Å), is tilted against the main plane by 14.3(3)°. Detailed crystal data are given in the Supporting Information.

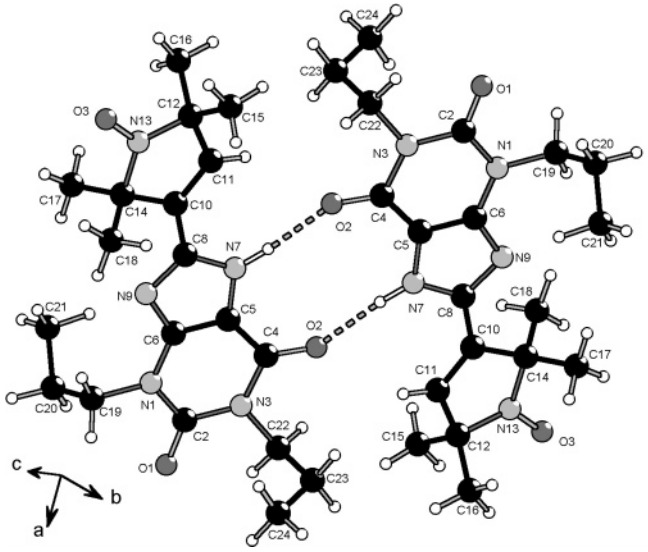


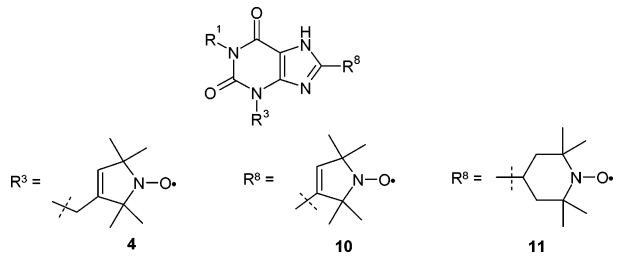
Figure 1. Structure of the spin-labeled xanthine derivative **10** in the crystal. Atoms are drawn by spheres of arbitrary radius.

Biological Activity

The spin-labeled xanthine derivatives were investigated in radioligand binding studies at A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors by use of [^{3}H]-2-chloro-N^6-cyclopentyladenosine ([^{3}H]CCPA), [^{3}H]-(*E*)-3-(3-hydroxypropyl)-8-[2-(3-methoxphenyl)vinyl]-7-methyl-1-prop-2-ynyl-3,7-dihydropurine-2,6-dione ([^{3}H]MSX-2), [^{3}H]-8-[[4-(2-hydroxyethylamino)-2-oxoethoxy]phenyl]-1-propylxanthine ([^{3}H]PSB-298), and [^{3}H]-2-phenyl-8-ethyl-4-methyl-(8*R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-5-one ([^{3}H]PSB-11), respectively, as A_1, A_{2A}, A_{2B}, and A_3 radioligands. Native brain tissues were used for A_1 (cortex) and A_{2A} (striatum) assays. Human recombinant receptors were used for A_{2B} and A_3 receptor assays. It had been shown that species differences for A_1 and A_{2A} receptors between rat and human receptors are moderate; therefore, we assumed that the rat data for these subtypes would be a good estimate for the human receptors as well.[4]

The results of the radioligand binding studies at ARs are presented in Tables1 and 2 and compared with the data of non-spin-labeled analogues. Compound **4** showed high affinity for the A_1 AR and at the same time it was selective for A_1 vs A_{2A} ARs by 1600-fold. Its A_1 affinity

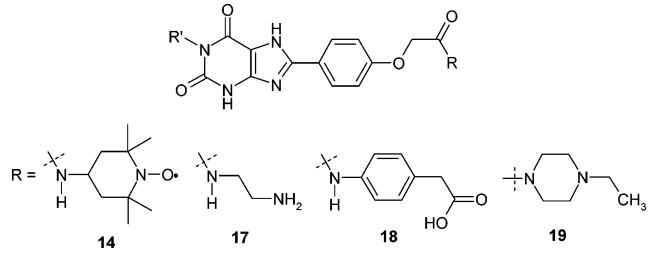
Table 1. Adenosine Receptor Affinities and A_1 Selectivities of 3- and 8-Spin-Labeled Xanthine Derivatives in Comparison with Analogous Standard Compounds



				$K_i \pm$ SEM (nM)			
compd	R^1	R^3	R^8	A_1 affinity, rat vs [^{3}H]CCPA	A_{2A} affinity, rat vs [^{3}H]MSX-2	A_{2B} affinity, human vs [^{3}H]PSB-298	A_3 affinity, human vs [^{3}H]PSB-11
				3- or 8-Spin-Labeled Xanthines			
4	propyl	nitroxide-bearing moiety	cyclopentyl	5.47 ± 0.92	8780 ± 1340	>1000 (23 ± 2% inh[a])	1700 ± 200
10	propyl	propyl	nitroxide-bearing moiety	8.23 ± 1.30	3800 ± 500	3100 ± 1400	~10 000 (47 ± 5% inh[a])
11	propyl	propyl	nitroxide-bearing moiety	160 ± 6	6750 ± 750	>10 000 (25 ± 0% inh.[a])	~10 000 (45 ± 5% inh.[a])
				1,3,8-Trialkylated Xanthines for Comparison			
15	propyl	benzyl	cyclopentyl	8.7[b]	511[c]	nd[d]	54.6
16	propyl	propyl	cyclopentyl	0.9	470	51[e]	795

[a] inh = percent inhibition of radioligand binding at the indicated concentration (1 or 10 μM). [b] Versus [^{3}H]*R*-PIA. [c] Versus [^{3}H]NECA. [d] Not determined. [e] Versus [^{3}H]ZM-241385.

Table 2. Adenosine Receptor Affinities of A_{2B}-Selective Xanthine Derivatives and Their Spin-Labeled Analogue **14**



		$K_i \pm$ SEM (nM)			
compd	R′	A_1 affinity, rat vs [^{3}H]CCPA	A_{2A} affinity, rat vs [^{3}H]MSX-2	A_{2B} affinity, human vs [^{3}H]PSB-298	A_3 affinity, human vs [^{3}H]PSB-11
		Spin-Labeled Xanthine			
14	propyl	15.7 ± 0.7	1270 ± 530	48 ± 0.7	350 ± 100
		Structurally Related Xanthines for Comparison[20]			
17	propyl	24	365	10[a]	nd[b]
18	butyl	41	479	5.3[a]	676
19	butyl	18	290	5.5[a]	nd[b]

[a] Versus [^{3}H]ZM-241380. [b] Not determined.

was similar to that of the related 3-benzyl-5-cyclopentyl-1-propylxanthine (**15**; K_i for A_1 = 8.7 nM),[29] showing that the replacement of the benzyl group by a 1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-ylmethyl group had no significant effect on the affinity. In comparison with DPCPX (**16**),[20] which bears a propyl group in the 3-position, the larger 1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-ylmethyl-substituted derivative **4** was about 6-fold less potent at A_1 ARs, but similarly or even more selective.

1,3-Dipropylxanthines spin-labeled in position 8 with either a 1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-yl group (**10**) or a 1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl group (**11**) were not as potent as their non-spin-labeled analogue DPCPX (**16**). The pyrrolyl nitroxide moiety led to a 9-fold decrease in affinity to A_1 ARs of compound **10** in comparison with xanthine derivative **16**, which is provided with a cyclopentyl group in position 8.

Compound **11** with a larger piperidinyl nitroxide residue was 19-fold less potent than compound **10** (pyrrolyl nitroxide group) and 177-fold less potent than DCPCX. It is evident that the 1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl group is already too bulky and the volume of the piperidine ring in this region of **11** is too large to fit well into the A_1 receptor ligand binding site. The polarity of the nitroxide group may also be unfavorable for binding to the lipophilic pocket of the receptor.

Compound **10** was found to be a potent and highly selective spin-labeled A_1 antagonist (462-fold vs A_{2A}, 377-fold vs A_{2B}, >1000-fold vs A_3), while **11** exhibited only moderate affinity (K_i for A_1 = 160 nM) but was also quite selective (>40-fold) versus all other AR subtypes (Table 1).

Compound **14** was designed as an A_{2B} antagonist. As a matter of fact, **14** showed reasonably high affinity to A_{2B} ARs (K_i = 48 nM); however, it had even somewhat more affinity toward A_1 ARs (K_i = 16 nM) and is therefore a mixed A_1/A_{2B} ligand (Table 2). Compound **14** exhibited good selectivity versus A_{2A} (81-fold) and moderate selectivity versus A_3 ARs (7−22-fold). In comparison with structurally related xanthine derivatives (**17**, **18**, and **19**),[20] spin-labeled compound **14** was somewhat weaker at A_{2B} and more potent at A_1 ARs, resulting in a loss of A_{2B} selectivity (see Table 2).

Conclusions

The synthesis of four spin-labeled xanthine derivatives has been achieved. Two of the compounds (**4** and **10**), bearing spin-labels in different positions exhibit high affinity in the low nanomolar concentration range for A_1 ARs and are highly selective versus all other AR subtypes (≫100-fold). One compound (**14**) was a potent mixed A_1/A_{2B} ligand. The new compounds should be suitable probes for electron paramagnetic resonance studies of A_1 or A_{2B} ARs, respectively. Such probes will allow biophysical studies that may contribute to the understanding of the membrane receptor structures and their interaction with ligands.

Experimental Section

All commercially available reagents were obtained from various producers (Acros, Aldrich, Fluka, Merck, Sigma) and used without further purification. Solvents were used without additional purification or drying, unless otherwise noted. The reactions were monitored by thin-layer chromatography (TLC) on aluminum sheets with silica gel 60 F_{245} (Merck). Column chromatography was carried out with silica gel 0.060−0.200 mm, pore diameter ca. 6 nm. EPR spectra were recorded at room temperature on an EPR spectrometer (Bruker ESP 300) at the Institute Jožef Stefan, Ljubljana, Slovenia. Mass spectra were recorded on an API 2000 (Applied Biosystems, Darmstadt, Germany) mass spectrometer at the Institute of Pharmaceutical Chemistry Poppelsdorf, University of Bonn, Germany, or on a Varian-MAT 311 A mass spectrometer at the Institute Jožef Stefan, Ljubljana, Slovenia. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer at the Institute of Pharmaceutical Chemistry Poppelsdorf, University of Bonn, Germany. Elemental analyses were performed by the Institute of Pharmaceutical Chemistry Endenich, University of Bonn, Germany. The melting points were determined on a Wepa Apotec (Wepa, Höhr-Grenzhausen, Germany) capillary melting point apparatus. The room-temperature X-ray single-crystal analysis was carried out on an AFC6R single-crystal diffractometer (Rigaku) by the Mineralogical−Petrological Institute, University of Bonn, Germany.

Syntheses of Stable Nitroxide Radicals. 1-Oxyl-3-carboxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrole (**5**),[18] 1-oxyl-4-carboxyl-2,2,6,6-tetramethylpiperidine (**6**)[27] and 1-oxyl-4-amino-2,2,6,6-tetramethylpiperidine (**13**)[18,28] were prepared in multistep syntheses from commercially available 2,2,6,6-tetramethylpiperidin-4-one as described.[18,27,28] 1-Oxyl-3-bromomethyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrole (**2**) was prepared from 1-oxyl-3-carboxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrole (**5**) as described.[23,25,26]

6-Amino-1-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-ylmethyl)-5-cyclopentanoylamido-3-propyluracil (3). Compounds **1**[22] (320 mg, 1.15 mmol), **2**[23] (280 mg, 1.20 mmol, 1.05 equiv), and anhydrous K_2CO_3 (240 mg, 1.75 mmol, 1.5 equiv) were suspended in dry DMF (13 mL). The suspension was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was purified on a silica gel column with ethyl acetate/ethyldimethylamine (98:2), yielding 238 mg (55%) of a red oil: IR (KBr) 3320 (NH_2), 2969, 2871, 1700, 1634 cm^{-1}; MS (ESI mode) 434.5 $(M + H)^+$; R_f (ethyl acetate) = 0.35.

8-Cyclopentyl-3-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-ylmethyl)-1-propylxanthine (4). Compound **3** (200 mg, 0.46 mmol) was suspended in 3 mL of THF in a 10 mL pressure tube. Finely ground ammonium sulfate (0.05 g) and 2 mL (10 mmol) of HMDS were added, forming a viscous yellow suspension. After microwaving (100 W, 140 °C, 20 min), 2 mL of methanol was added to the warm (50 °C) mixture. The solvent was removed under reduced pressure and the residue was purified by column chromatography (petrol ether/diethyl ether = 3:1), yielding 54 mg (28%) of a pale-red oil: IR (KBr) 3168 (NH), 2968, 2872, 1712, 1652, 1598 cm^{-1}; MS (ESI mode) 415.3 $(M + H)^+$; a_N = 14.95 G. Anal. ($C_{22}H_{32}N_5O_3$) H; C, calcd 63.75, found, 62.09; N, calcd 16.89, found 15.73. R_f (ethyl acetate) = 0.79.

Reaction of 5,6-Diamino-1,3-dipropyluracil (7) with Spin-Labeled Carboxylic Acids 5 and 6. To a mixture of 0.45 g (2.0 mmol) of 5,6-diamino-1,3-dipropyluracil **7** and 2.05 mmol of **5**[23,25,26] or, respectively, **6** in methanol (10 mL) was added EDC (0.4 g, 2.1 mmol). The mixture was stirred for 2 days under argon and protected from light. The solvent was removed under reduced pressure and the residue was purified by column chromatography (eluent dichloromethane/methanol, 92:8). The product was obtained as a yellow oil **8** or a red oil **9**, respectively.

6-Amino-5-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-yl)carboxamido-3-propyluracil (8). Yellow oil (yield 67%): IR (KBr) 3329 (NH_2), 2972, 2934, 2875, 1702, 1622 cm^{-1}; MS (ESI mode) 393.4 $(M + H)^+$; R_f (eluent dichloromethane/methanol, 9:1) = 0.32.

6-Amino-5-(1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)-carboxamido-3-propyluracil (9). Red oil (yield 32%): IR (KBr) 3210 (NH_2), 2972, 2933, 2877, 1644 cm^{-1}; MS (ESI mode) 409.4 $(M + H)^+$; R_f (eluent dichloromethane/methanol, 9:1) = 0.38.

Preparation of 1,3-Dipropylxanthines with a Spin-Labeled Substituent in the 8-Position (10, 11). Compound **8** or **9**, respectively (0.7 mol), was dissolved in a mixture of 1 M aqueous NaOH solution (8 mL) and dioxane (8 mL) and subsequently heated under reflux for 45 min. The mixture was cooled to room temperature, acidified with ca. 10 mL of a 1 M aqueous HCl solution, and left overnight to complete the precipitation. The product was filtered under reduced pressure, washed with water, and dried at 70 °C.

8-(1-Oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-yl)-1,3-dipropylxanthine (10). Yellow crystals (yield 75%): mp 188 °C; IR (KBr) 3148 (NH), 2969, 2933, 2872, 1702, 1651, 1597 cm^{-1}; MS (ESI mode) 375.4 $(M + H)^+$; a_N = 14.76 G. Anal. ($C_{19}H_{28}N_5O_3$) C; H, calcd 7.54, found 7.48; N, calcd 18.70, found 18.36. R_f (ethyl acetate) = 0.63.

Crystal Structure Data. Yellow single crystals of **10** (FW = 374.46 for $C_{19}H_{28}N_5O_3$) were obtained from a dichloromethane/diethyl ether (1:1) solution.

For a crystal with dimensions of 0.35 × 0.3 × 0.2 mm, the structure was established by X-ray single-crystal diffractometry (Figure 1). Diffraction data were collected with a Rigaku AFC6R four-circle single-crystal diffractometer, equipped with a graphite monochromator, using Mo Kα radiation from a rotating anode generator. Compound **10** crystallizes in the monoclinic space group $P2_1/c$ (no. 14). Unit cell dimensions were determined from 25 reflections ($7.7° < \theta < 11.9°$): a = 13.051(2) Å, b = 17.505(3) Å, c = 9.188(1) Å, β = 103.35(1)°, V = 2042.3(5) Å^3, Z = 4, D_c = 1.218 g/cm^3, $F(000)$ = 804.

Intensities of 7482 reflections with $1.98° < \theta < 20.04°$ were measured by ω scans. The data were corrected for the variations of three standard reflections monitored every 50 reflections. After data reduction, the structure was solved by direct methods with SHELXS-97 and refined on F^2 with SHELXL-97. The non-hydrogen atoms were refined with anisotropic displacement parameters, all hydrogen atoms located by difference Fourier calculations with isotropic displacement parameters. The refinement on a total of 1917 independent reflections varying 357 parameters converged at

$R_1 = 0.0635$ and $wR_2 = 0.0891$ and for 1424 reflections with $I > 2\sigma(I)$ at $R_1 = 0.0364$, $wR_2 = 0.0805$, GoF = 1.047. Final residual electron density features were between −0.12 and +0.11 $e^-/Å^3$.

8-(1-Oxyl-2,2,6,6-tetramethylpiperidin-4-yl)-1,3-dipropylxanthine (11). Red crystals could be obtained by recrystallization from CH_2Cl_2/diethyl ether (ca. 1:1): yield 72%, mp 192 °C; IR (KBr) 3193 (NH), 2972, 2938, 2875, 1703, 1661 cm^{-1}; MS (ESI mode) 391.3 $(M + H)^+$; a_N = 15.93 G; Anal. ($C_{20}H_{32}N_5O_3$) H; C, calcd 61.52, found 61.22; N, calcd 17.93, found 17.66. R_f (ethyl acetate) = 0.57.

8-[4-(((1-Oxyl-2,2,6,6-tetramethylpiperidin-4-yl)amino)-2-oxoethoxy)phenyl]-1-propylxanthine (14). A solution of **12**[20] (0.51 g, 1.48 mmol), **13**[18,28] (0.51 g, 2.98 mmol, 2.0 equiv), EDC (0.63 g, 3.28 mmol, 2.2 equiv), and DMAP (0.1 g, 0.82 mmol, 0.55 equiv) in a mixture of DMF (160 mL) and CH_2Cl_2 (80 mL) was stirred for 6 days at room temperature under argon. The mixture was evaporated to dryness under reduced pressure, and the residue was divided into three portions. Each portion was dissolved in ethyl acetate (350 mL). The solution was washed 5 times with 5% citric acid (70 mL) and then 5 times with a saturated solution of $NaHCO_3$ (70 mL). The ethyl acetate extracts were combined and dried over $MgSO_4$. The solvent was removed under reduced pressure, yielding 105 mg (14%) of a pale red powder, mp > 350 °C; IR (KBr) 2969, 2929, 1716 (C═O), 1640 (C═N), 1055 cm^{-1}; MS (ESI mode) 498.5 $(M + H)^+$; a_N = 15.93 G. Anal. ($C_{25}H_{33}N_6O_5$) C; H, calcd 6.68, found 6.85; N, calcd 16.89, found 16.60. R_f (ethyl acetate) = 0.38.

Biological Studies. Radioligand competition assays were performed as recently described,[24,30] with rat brain cortical membrane preparations as a source of A_1, rat brain striatal membrane preparations as a source for A_{2A} ARs, and membrane preparations containing human A_{2B} or human A_3 adenosine receptors stably expressed in CHO or HEK cells as a source for A_{2B} and A_3 receptors, respectively. Radioligands were obtained from the following sources: [^{3}H]CCPA was from NEN Life Sciences (54.9 Ci/mmol), and [^{3}H]MSX-2 (85 Ci/mmol), [^{3}H]PSB-11 (53 Ci/mmol), and [^{3}H]PSB-298 (124 Ci/mmol) were custom-labeled by Amersham. The precursors were synthesized in our laboratory as previously described.[31−35]

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Supporting Information Available: Details on the X-ray analysis of compound **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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