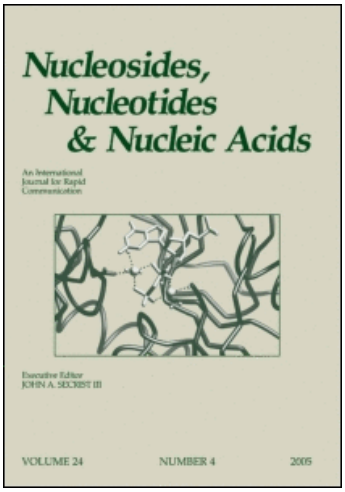

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Synthesis of 2-Azido-(R)-N^6-p-hydroxyphenylisopropyladenosine (R-AHPIA) as Potential Photoaffinity Probe for A_1 Adenosine Receptors

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SYNTHESIS OF 2-AZIDO-(R)-N^6-p-HYDROXYPHENYLISOPROPYLADENOSINE (R-AHPIA) AS POTENTIAL PHOTOAFFINITY PROBE FOR A_1 ADENOSINE RECEPTORS.

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Abstract. The preparation of 2-azido-(R)-N^6-p-hydroxyphenyl-isopropyladenosine (R-AHPIA) (1) starting from 2,6-dichloro--9-(2,3,5 -tri-O-acetyl-β-D-ribofuranosyl)purine (2) is described. This new adenosine analogue exhibits potent agonist activity on A_1 adenosine receptors and could be suitable for photoaffinity labeling studies on the same receptor subtype.

The endogenous nucleoside adenosine has been proposed as a neurotransmitter or neuromodulator acting on several physiological systems by interaction with adenylate cyclase.[1] The molecular components which mediate this interaction have been extensively studied by a number of selected adenosine analogues and as a result two subtypes of membrane associated receptors, A_1(or R_i) and A_2(or R_a), have been identified.[2,3] The A_1 receptors are coupled to an inhibition and the A_2 receptors are coupled to a stimulation of adenylate cyclase activity. Radioligand binding studies

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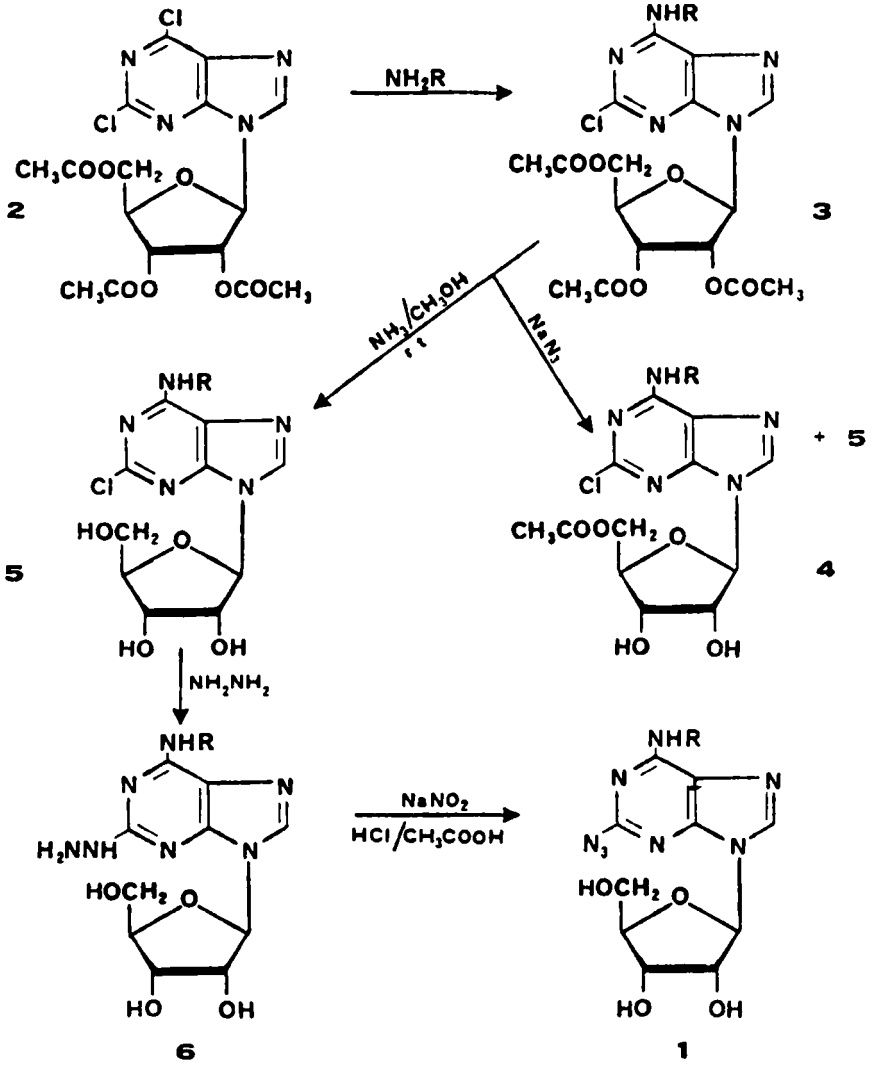
have been performed with tritiated adenosine analogues to identify A_1 receptors in isolated membrane preparations.[4,5] Since the A_1 adenosine receptors in fat cells and in some brain regions are very sensitive to N^6-substituted adenosine derivatives such as (-)N^6-phenylisopropyladenosine (PIA), [^{3}H]PIA has been used as a selective radioligand for A_1 receptors. A derivative of PIA, (-)N^6-p-hydroxyphenylisopropyladenosine [(-)HPIA] has been radioiodinated and the resulting product (-)[^{125}I]HPIA showed a high specific radioactivity which allows identification of the A_1 receptors in tissues with low receptor density.[6-9] On the other hand, covalent labelling of receptors can be obtained with photolabile probes which upon UV-irradiation bind irreversibly to the receptor.[10] These findings prompted us to prepare an adenosine analogue which exhibits potent agonist activity on A_1 receptors and at the same time could be radioiodinated and photolysed.

This paper describes the synthesis of 2-azido-(R)-N^6-p-hydroxyphenylisopropyladenosine (R-AHPIA) (**1**), a new adenosine derivative which possesses the above-required characteristics. Preliminary biological results on binding activity of **1** to both A_1 and A_2 adenosine receptors are also reported.

Chemistry. The synthesis of 2-azido-(R)-N^6-p-hydroxyphenylisopropyladenosine (R-AHPIA) (**1**) was accomplished by the method outlined in Scheme 1. Treatment of 2,6-dichloro-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-purine (**2**)[11] with (-)1-methyl-2-(4-hydroxyphenyl)ethylamine[12,13] in dry acetonitrile effected nucleophilic replacement of halogen at position 6 without removal of the acetyl groups to give compound **3**. An attempt to obtain the title compound **1** by reaction of



SCHEME 1

3 with sodium azide was not successful. The only reactions observed were the selective deacetylation of hydroxylic groups at position 2 and 3 to give compound **4** and the complete deacetylation to give compound **5**. Complete deacetylation of **3** was achieved by treatment with methanolic ammonia to give 2-chloro-(R)-N^6-p-hydroxyphenylisopropyladenosine (**5**). Preparation of the hydrazino derivative **6** was accomplished by treatment of **5** with anhydrous hydrazine at room temperature. This compound was isolated in crystalline form and characterized by NMR and elemental analysis. Treatment of **6** with nitrous acid at 0°C resulted in the isolation of desired compound **1** in good yield.

The infrared spectrum of this product exhibited strong absorption at 2140 cm^{-1} which is characteristic of the azido group. On this basis, in addition to the elemental analysis and the NMR spectrum, the structure of this product has been clearly established. Photolysis of R-AHPIA (**1**) was demonstrated by following changes in the ultraviolet spectrum of an ethanolic solution of the nucleoside ($5x10^{-5}$M) irradiated at 254 nm (figure 1).

BIOLOGICAL EVALUATION

Binding activity of R-AHPIA (**1**) to A_1 adenosine receptors of rat brain membranes was tested in competition studies using [^{3}H]PIA as described.[14] A K_i-value of 1.6 nM was found. The biological activity of the compound was investigated in adenylate cyclase studies with membrane of rat fat cells (A_1 adenosine receptor) and human platelets (A_2 adenosine receptor) as described by Jakobs et al.[15] The IC_{50}-value observed at the A_1 receptor was 35 nM and the EC_{50}-value at the A_2 receptor 2.2 μM. These results show

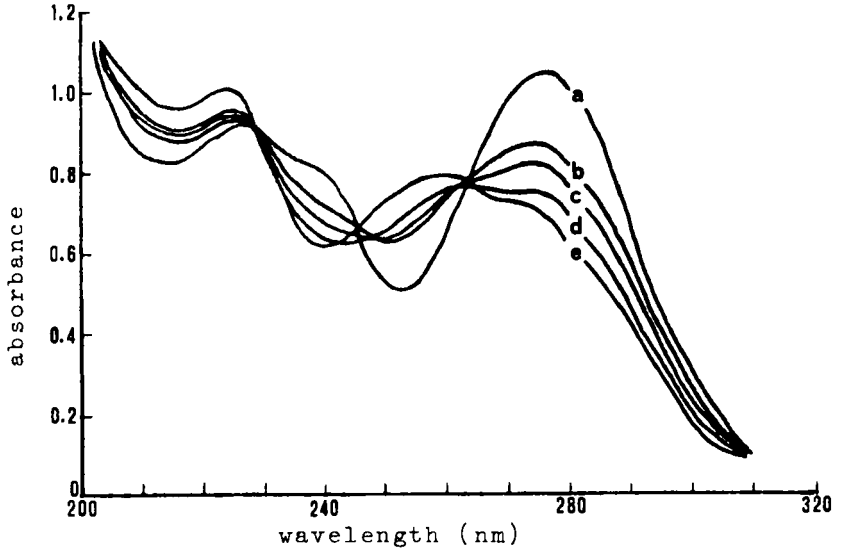


FIGURE 1. Spectral changes during irradiation of R-AHPIA at 254 nm. **a**, Before irradiation; **b**, 2.5 min; **c**, 5 min; **d**, 10 min; **e**, 30 min after irradiation.

that the properties of R-AHPIA at adenosine receptors are comparable to those of the parent compound R-HPIA. More details on these experiments and on photoaffinity labeling studies will be presented separately.[16]

EXPERIMENTAL SECTION

The melting points were determined with a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a Varian FT 80 A spectrometer. All exchangeable protons were confirmed by addition of D_2O. Infrared spectra were recorded on a Perkin-Elmer Model 297 spectrophotometer and ultraviolet on a Perkin-Elmer Model 575 spectrophotometer. TLC was carried out on silica gel 60 F-254 (Merck) precoated TLC plates. Silica gel 60 (Merck) was used for column chromatography.

2-Chloro-(R)-N^6-[1-methyl-2-(4-hydroxyphenyl)ethyl]-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)adenine (3). To a solution of 1,6 g (3.58 mmol) of 2,6-dichloro-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (**2**)[11] in 120 ml of dry acetonitrile was added 1,6 g (10,7 mmol) of (-) 1-methyl-2-(4-hydroxyphenyl)ethylamine,[12,13] and the mixture was stirred at room temperature for 15 h. The solvent was removed in vacuo and the residue was chromatographed on a silica gel column eluting with $CHCl_3$-MeOH (97-3) to give 1 g (50%) of **3** as a viscous pure solid; ^{1}H NMR (Me_2SO-d_6) δ 1.16 (d, 3, J=6,5 Hz, CH_3iPr), 2.03, 2.05, 2.12 (s, 9, three $COCH_3$), 2.43-2.97 (m, 2, CH_2iPr), 4.33 (m, 4, H-5', H-4' and CH iPr), 5.60 (m, 1, H-3'), 5.91 (t, 1, H-2'), 6.15 (d, 1, J=5.4 Hz, H-1'), 6.63 (d, 2, J=8.4 Hz, H-3" and H-5" aromat), 7.05 (d, 2, J=8.2 Hz, H-2" and H-6" aromat), 8.32 (d, 1, J=8.8 Hz, NH), 8.38 (s, 1, H-8), 9.12 (s, 1, OH aromat).

Anal. Calcd. for $C_{25}H_{28}ClN_5O_8$:C, 53.43; H, 5.02; N, 12.46. Found:C, 53.72; H, 5.36; N, 12.11.

2-Chloro-(R)-N^6-[1-methyl-2-(4-hydroxyphenyl)ethyl]-9-(5-O-acetyl-β-D-ribofuranosyl)adenine (4). To a stirred solution of 220 mg (0.39 mmol) of **3** in 20 ml of ethanol was added 127 mg (1.96 mmol) of sodium azide in water. The reaction mixture was refluxed for 24 h and then it was evaporated to dryness in vacuo. Trituration of the residue with $CHCl_3$-MeOH (95:5) gave a mixture from which the precipitated inorganic salts were removed by filtration. Evaporation of the filtrate to dryness in vacuo gave a residue which was chromatographed on a silica gel column. Elution with $CHCl_3$-MeOH (90:10) yielded 75 mg (40%) of **4** and 40 mg (23%) of **5**; ^{1}H NMR (Me_2SO-d_6) δ 1.15 (d, 3, J=6.4 Hz, CH_3 iPr), 2.02 (s, 1, $COCH_3$), 2.35-2.97 (m, 2, CH_2 iPr), 3.96-4.65 (m,

6, H-5', H-4', H-3', H-2' and CH iPr), 5.36 (d, 1, J=5.2 Hz, OH-3'), 5.57 (d, 1, J=5.6 Hz, OH-2'), 5.83 (d, 1, J=4.8 Hz, H-1'), 6.62 (d, 2, J=6.8 Hz, H-3" and H-5" aromat), 7.05 (d, 2, J=8.3 Hz, H-2" and H-6" aromat), 8.22 (d, 1, J=8.7 Hz, NH), 8.33 (s, 1, H-8), 9.1 (s, 1, OH aromat).

Anal. Calcd. for $C_{21}H_{24}ClN_5O_6$:C, 52.77; H, 5.06; N, 14.65. Found: C, 53.09; H, 5.37; N, 14.28.

2-Chloro-(R)-N^6-p-hydroxyphenylisopropyladenosine (5). A solution of **3** (1g, 1.77 mmol) in 25 ml of methanol saturated at 0°C with ammonia was set aside at room temperature for 5 h. The solvent was removed in vacuo and the residue was chromatographed on a silica gel colum eluting with $CHCl_3$-MeOH (87:13) to give 617 mg (80%) of **5** as a pure solid: mp 80-83°C; ^{1}H NMR(Me_2SO-d_6) δ 1.16 (d, 3, J=6.4 Hz, CH_3 iPr), 2.39-3.01 (m, 2, CH_2 iPr), 3.58 (m, 2, H-5'), 3.78-4.65 (m, 4, H-4', H-3', H-2' and CH iPr), 4.98-5.20 (m, 2, OH-5' and OH-3'), 5.45 (d, 1, OH-2'), 5.81 (d, 1, J=5.9 Hz, H-1'), 6.62 (d, 2, J=8.4 Hz, H-3" and H-5" aromat), 7.05 (d, 2, J=8.1 Hz, H-2" and H-6" aromat), 8.22 (d, 1, J=8.6 Hz, NH), 8.37 (s, 1, H-8), 9.1 (s, 1, OH aromat).

Anal. Calcd. for $C_{19}H_{22}ClN_5O_5$: C, 52.37; H, 5.08; N, 16.06. Found: C, 52.61; H, 5.25; N, 15.84.

2-Hydrazino-(R)-N^6-p-hydroxyphenylisopropyladenosine (6). To 6 ml of anhydrous hydrazine, which was cooled in an ice-bath, was added portionwise 590 mg (1.35 mmol) of **5** and the reaction mixture was kept at room temperature for 4 h. Water (15 ml) was added and the solution concentrated in vacuo. This operation was repeated three times to give white crystals which were filtered, washed with water and dried under vacuum: yield of **6**, 410 mg (70%); mp 190-193°C, dec; ^{1}H NMR (Me_2SO-d_6) δ 1.10 (d, 3, J=6.4 Hz, CH_3 iPr), 2.31-3.00

(m, 2, CH_2 iPr) 3.57 (m, 2, H-5') 3.89 (m, 1, H-4'), 4.12 (m, 1, H-3'), 4.27-4.90 (m, 4, H-2', CH iPr and NH_2), 4.97-5.48 (m, 3, OH-5', OH-3' and OH-2'), 5.75 (d, 1, J=6.1 Hz, H-1'), 6.62 (d, 2, J=8.3 Hz, H-3" and H-5" aromat), 6.86-7.49 (m, 4, H-2" and H-6" aromat, two NH), 7.92 (s, 1, H-8), 9.07 (s, 1, aromat).

Anal. Calcd. for $C_{19}H_{25}N_7O_5$: C, 52.89; H, 5.84; N, 22.70. Found: C, 52.98; H, 6.02; N, 22.51.

2-Azido-(R)-N^6-p-hydroxyphenylisopropyladenosine (R-AHPIA) (1). An ice cooled solution of 400 mg (0.92 mmol) of **6** in 6 ml of water was treated with conc hydrochloric acid to obtain pH 2.5. To this mixture was added 0.3 ml of glacial acetic acid and then, dropwise, a cooled solution of 152 mg of sodium nitrite in 10 ml of water. During this addition 7 g of ice was also added to keep the temperature between 0° and 2°C. The reaction mixture was stirred at 0° for 1 h and the crystals that precipitated were collected by filtration. TLC in $CHCl_3$-MeOH (85:15) showed that this solid presented some impurities. The filtrate was neutralized with saturated $NaHCO_3$ solution and then extracted several times with chloroform. The organic layers were dried (Na_2SO_4) and evaporated in vacuo to give a residue which was combined with the filtered solid and chromatographed on a silica gel column. Elution with $CHCl_3$-MeOH (85:15) gave 310 mg (76%) of 2-azido--(R)-N^6-p-hydroxyphenylisopropyladenosine (R-APHIA) (**1**) as white crystals, mp 202-204°C, dec; $[\alpha]_D^{20}$ -83.2 (c=0.5, EtOH); UV (EtOH) before photolysis λ_{max} 228 nm (ε 19040), 277 (ε 21680), after photolysis λ_{max} 224 nm (ε 20880), 262 (ε 16560), 275 (sh, ε 15040); IR ν 2140 cm^{-1} (N≡N); ^1H NMR (Me_2SO-d_6) δ 1.15 (d, 3, J=6.3 Hz, CH_3 iPr), 2.38-3.07 (br m, 2, CH_2 iPr and Me_2SO), 3.37-3.75 (m, 2, H-5'), 3.81-4.19

(m, 2, H-4' and H-3'), 4.26-4.70 (m, 2, H-2' and CH iPr), 4.96-5.27 (m, 2, OH-5' and OH-3'), 5.4 (d, 1, OH-2'), 5.77 (d, 1, J=5.9 Hz, H-1'), 6.61 (d, 2, J=8.3 Hz, H-3" and H-5" aromat), 7.02 (d, 2, J=8.1 Hz, H-2" and H-6" aromat), 8.01 (d, 1, NH), 8.26 (s, 1, H-8), 9.10 (s, 1, OH aromat).

Anal. Calcd. for $C_{19}H_{22}N_8O_5$: C, 51.58; H, 5.01; N, 25.33. Found: C, 51.24; H, 5.27; N, 24.98.

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REFERENCES

1) Berne, R. M.; Rall, T. W. and Rubio, R. Eds; Regulatory function of adenosine, Martinus Nijhoff; Boston 1983.
2) Van Calker, D.; Muller, M.; Hamprecht, B. *Nature*(London) **1978**,276,839.
3) Londos, C.; Cooper, D.M.F.; Wolff, J. *Proc. Natl. Acad. Sci. U.S.A.* **1980**,77,2551.
4) Daly, J. W. *J. Med. Chem.* **1982**,25,197.
5) Schwabe, U. *Trends Pharmacol. Sci.* **1981**,2,299.
6) Munshi, R.; Baer, H. P. *Can. J. Physiol. Pharmacol.* **1982**, 60,1320.
7) Schwabe, U.; Lenschow, V.; Ukena, D.; Ferry, D. R. Glossman, H. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1982**,321, 84.
8) Ukena, D.; Furler, R.; Lohse, M. J.; Engel, G.; Schwabe, U. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1984**,326,233.
9) Linden, J.; Patel A.; Sadek, S. *Circ. Res.* **1985**,56,279.

10) Bayley, H.; Knowles, J. R. *Methods Enzymol.* **1977**, XLVI, 69.

11) Gerster, J. F.; Robins, R. K. *J. Org. Chem.* **1966**, 31, 3258.

12) Mannich, C.; Jacobsohn, W. *Chem. Ber.* **1910**, 43, 189.

13) Van Dijk, J.; Keizer, V. G.; Peelen, J. F.; Moed, H. D. *Rec. Trav. Chim.* **1965**, 84, 521.

14) Lohse, M. J.; Lenschow, V.; Schwabe, U. *Mol. Pharmacol.* **1984**, 26, 1.

15) Jacobs, K. H.; Saur, W.; Schultz, G. *J. Cyclic Nucleotide Res.* **1976**, 2, 381.

16) Klotz, K. N.; Cristalli, G.; Grifantini, M.; Vittori, S.; Lohse, M. J. *J. Biol. Chem.*, in press.

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