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### SYNTHETIC PROCEDURE FOR THE PREPARATION OF NOVEL POTENT AND SELECTIVE A<sub>3</sub> ADENOSINE RECEPTOR RADIOLIGANDS

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## SYNTHETIC PROCEDURE FOR THE PREPARATION OF NOVEL POTENT AND SELECTIVE A<sub>3</sub> ADENOSINE RECEPTOR RADIOLIGANDS

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### ABSTRACT

2-Phenylethynyladenosine and its N<sup>6</sup>-methyl derivative were synthesized and evaluated in binding assays at human adenosine receptors stably transfected on CHO cells. Results showed that the N<sup>6</sup>-methyl-2-phenylethynyladenosine is endowed with very high affinity and selectivity at A<sub>3</sub> receptor subtype. Hence, an alternative procedure for the synthesis of tritiated N<sup>6</sup>-methyl-2-phenylethynyladenosine was set up to introduce tritiated methylamine in the final step.

### INTRODUCTION

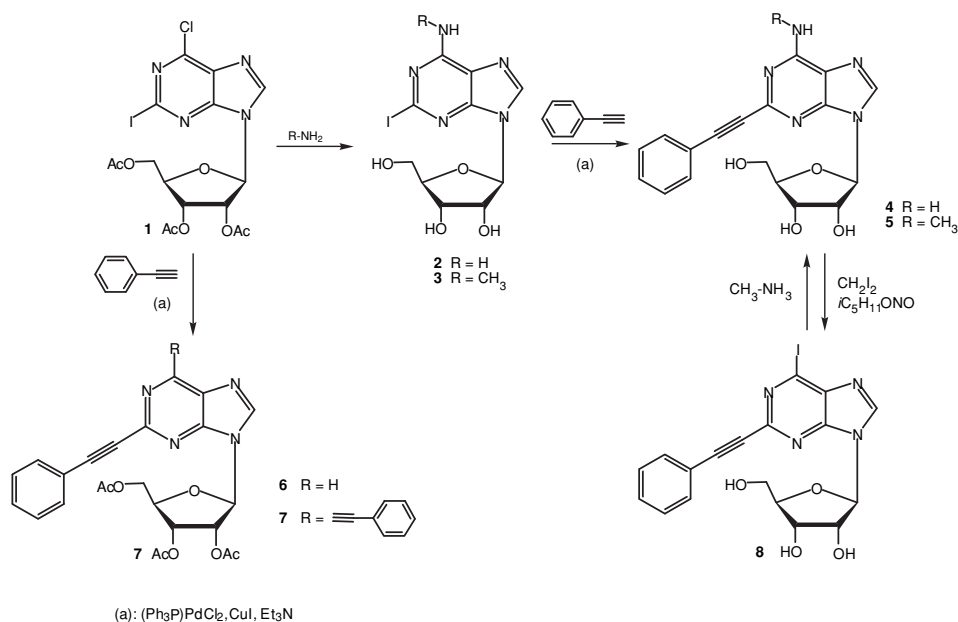
There is evidence that the purine nucleoside adenosine (Ado) specifically modulates neurotransmission through the activation of four receptor subtypes denominated: A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub> (1). Over the last few years many efforts have been directed toward discovery of potent and selective adenosine agonists. To this purpose we synthesized a number of adenosine-5'-N-ethyluronamide (NECA) derivatives substituted at the C-2-position with various (ar)alkynyl chains (2,3). Binding studies at human recombinant adenosine receptors (AdoRs) showed that the 2-alkynyl derivatives of NECA possess high affinity at the A<sub>3</sub> receptor subtype.

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Moreover, the presence of different alkynes modulated the affinity of such derivatives at the other subtypes ( $A_1$ ,  $A_{2A}$ , and  $A_{2B}$ ). In particular, the 2-phenylethynyl-NECA (PENECA) was found to possess high affinity combined with good selectivity for  $A_3$  receptors (4) ( $K_i A_3 = 6.2$  nM; selectivity  $A_1/A_3 = 90$ , and  $A_{2A}/A_3 = 100$ ). Aimed at investigating the role of the ethylcarboxamido group in 5' position and to simplify the structure of this molecule we synthesized the corresponding adenosine analogue 2-phenylethynyladenosine (PEAdo, **4** (**5**), Scheme 1). Furthermore, a methyl group was introduced on the amine in 6 position of PEAdo to obtain **5**, since the presence of a substituent on the 6-amino group of adenosine and NECA drives the agonist affinity toward  $A_1$  and  $A_3$  receptors. The choice of a small substituent was due to the fact that the presence of a sterically hindered substituent, like an arylcarbonyl group, decreases the  $A_3$  receptor affinity of 2-alkynylNECA derivatives (6,7).

Preliminary binding studies at human AdoRs, stably transfected on CHO cells (8), showed that the 2-PEAdo (**4**) possesses good and comparable affinity at AdoRs in comparison to PENECA. The most exciting results were obtained with PEAdo (**5**), a compound endowed with high affinity and very high selectivity for  $A_3$  receptors. ( $K_i A_3 = 3.4$  nM; selectivity  $A_1/A_3 = 500$  and  $A_1/A_{2A} = 2500$ ). These findings prompted us to set up a different synthetic procedure for the synthesis of a new  $A_3$  radioligand.



Scheme 1.



## CHEMISTRY

The synthesis of 2-PEAdo (**5**) (**4**) and N<sup>6</sup>-methyl-2-phenylethynyladenosine (**5**) was carried out starting from 6-chloro-2-iodo-9-(2,3,5-tri-*O*-acetyl- $\beta$ -D-ribofuranosyl)-9H-purine (**1**) (**9**). Treatment of **1** with liquid ammonia or methylamine gave the derivatives **2** (**9**) or **3**, respectively. The alkyne in 2-position was introduced by reacting **2** or **3** with phenylethyne using a modification of the palladium catalyzed cross-coupling reaction (**10**) to give PEAdo (**4**) and N<sup>6</sup>-methylPEAdo (**5**) (Scheme 1).

Aimed at finding a convenient synthetic procedure for the preparation of radiolabelled N<sup>6</sup>-methyl-2-phenylethynyladenosine we coupled **1** with phenylethyne, using the cross-coupling reaction conditions, to obtain **6**. The 6-chlorine atom of **6** could be easily substituted by tritiated methylamine to give the corresponding radioligand of **5**. Unfortunately, reaction of **1** with phenylethyne did not give **6** but the disubstituted derivative **7**. An alternative synthetic route was designed to avoid the undesired disubstitution. Hence, 2-PEAdo (**4**) was used as the starting material. Reaction of this compound with diiodomethane and isopentyl nitrite gave the versatile synthon 2-phenylethynyl-6-iodoadenosine (**8**) from which the desired compound (**5**) was obtained by treatment with methylamine (Scheme 1). This procedure allows the introduction in the final step and with high yield of a tritiated methylamine, leading to potent and selective A<sub>3</sub> radioligand.

## CONCLUSION

The N<sup>6</sup>-methylPEAdo is the firstly reported adenosine derivative endowed with very high affinity and selectivity for A<sub>3</sub> adenosine receptor subtype.

The synthetic procedure to prepare the 6-iodo-2-phenylethynylAdo (**8**) could be a general synthetic method to obtain A<sub>3</sub> adenosine receptor radioligands, since the presence of an iodine atom at the C-6-position of 2-alkynyladenosines allows the introduction of tritiated amines in the final step.

## EXPERIMENTAL

Melting points were determined with a Büchi apparatus and are uncorrected. <sup>1</sup>H NMR spectra were obtained with Varian VXR 300 MHz spectrometer;  $\delta$  in ppm, *J* in Hz. All exchangeable protons were confirmed by addition of D<sub>2</sub>O. TLC were carried out on pre-coated TLC plates with silica gel 60 F-254 (Merck). For column chromatography, silica gel 60 (Merck) was used. Elemental analyses were determined on Carlo Erba model 1106 analyser and are within  $\pm 0.4\%$  of theoretical values.

**2-Iodo-N<sup>6</sup>-methyl-9-( $\beta$ -D-ribofuranosyl)adenine (**3**).** To compound **1** (1.86 mmol) methylamine (10 mL) was added and the reaction mixture was



allowed to stand at  $-20^{\circ}\text{C}$  for 1 h. The exceeding amine was evaporated and the residue was chromatographed on a silica gel column eluting with  $\text{CHCl}_3$ -MeOH (93:7) to give **3** (634 mg; 69%) as amorphous solid;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  2.92 (d, 3H,  $J = 4.1$  Hz,  $\text{NH}-\text{CH}_3$ ), 3.61 (m, 2H,  $\text{CH}_2$ -5'), 3.95 (m, 1H, H-4'), 4.14 (m, 1H, H-3'), 4.53 (m, 1H, H-2'), 5.82 (d, 1H,  $J = 6.1$  Hz, H-1'), 8.14 (d, 1H, NH), 8.31 (s, 1H, H-8). Anal. ( $\text{C}_{11}\text{H}_{14}\text{IN}_3\text{O}_4$ ) C, H, N.

**Cross-coupling reaction for the synthesis of 5 and 7.** To a solution of **3** or **1** (0.51 mmol) in dry DMF (15 mL), and  $\text{Et}_3\text{N}$  (2.3 mL) under an atmosphere of  $\text{N}_2$  were added bis(triphenylphosphine)palladium dichloride (8.1 mg, 0.012 mmol) and CuI (0.51 mg, 0.003 mmol). The phenylethyne (3.1 mmol) was added and the reaction mixture was stirred under an atmosphere of  $\text{N}_2$  at room temperature for the time reported for each compound. The solvent was removed *in vacuo* and the residue was chromatographed on a silica gel column eluting with a suitable mixture of solvents to give **5** or **7** as amorphous solids:

**N<sup>6</sup>-Methyl-2-(phenylethyn-1-yl)-9-( $\beta$ -D-ribofuranosyl)adenine (5).** The reaction of **3** with phenylethyne for 16 h, followed by chromatography on a silica gel column eluting with  $\text{CHCl}_3$ -MeOH (92:8), gave **5** (120 mg; 84%), after crystallization from EtOH.

The same compound was also obtained by reacting **8** (0.15 mmol) with methylamine (2 mL) at  $5^{\circ}\text{C}$  for 5 h. After removing the exceeded methylamine the residue was chromatographed on flash silica gel column eluted with  $\text{CHCl}_3$ - $\text{cC}_6\text{H}_{12}$ :MeOH (87:10:3) to give **5** (42 mg; 73%); mp  $229\text{--}231^{\circ}\text{C}$ ;  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$  2.99 (br s, 3H,  $\text{NH}-\text{CH}_3$ ), 3.64 (m, 2H,  $\text{CH}_2$ -5'), 3.98 (m, 1H, H-4'), 4.15 (m, 1H, H-3'), 4.57 (m, 1H, H-2'), 5.93 (d, 1H,  $J = 6.2$  Hz, H-1'), 7.47 (m, 3H, H-Ph), 7.65 (m, 2H, H-Ph), 8.07 (m, 1H, NH), 8.47 (s, 1H, H-8). Anal. ( $\text{C}_{19}\text{H}_{19}\text{N}_5\text{O}_4$ ) C, H, N.

**2,6-(Diphenylethyn-1-yl)-9-(2,3,5-tri-O-acetyl- $\beta$ -D-ribofuranosyl)purine (7).** The reaction of **1** with phenylethyne for 24 h, followed by chromatography on a silica gel column eluted with  $\text{CHCl}_3$ - $\text{cC}_6\text{H}_{12}$ -MeCN (52:40:8) gave **7** (293 mg; 51%) as amorphous solid;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  2.04 (s, 3H,  $\text{COCH}_3$ ), 2.08 (s, 3H,  $\text{COCH}_3$ ), 2.15 (s, 3H,  $\text{COCH}_3$ ), 4.39 (m, 3H,  $\text{CH}_2$ -5' and H-4'), 5.74 (m, 1H, H-3'), 6.00 (m, 1H, H-2'), 6.40 (d, 1H,  $J = 5.3$  Hz, H-1'), 7.57 (m, 6H, H-Ph), 7.74 (m, 6H, H-Ph), 8.99 (s, 1H, H-2). Anal. ( $\text{C}_{32}\text{H}_{26}\text{N}_4\text{O}_7$ ) C, H, N.

**6-Iodo-2-(phenylethyn-1-yl)-9-( $\beta$ -D-ribofuranosyl)adenine (8).** To a solution of **4** (0.27 mmol) in dry DMF (2 mL) diiodomethane (3.61 mL) and isopentyl-nitrite (1.12 mL) were added and the mixture was heated at  $60^{\circ}\text{C}$  for 30'. The solvent was evaporated and the residue was chromatographed on a silica gel column eluted with  $\text{CHCl}_3$ - $\text{cC}_6\text{H}_{12}$ -MeOH (83:10:7) to give **7** (120 mg; 33%) as amorphous solid;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  3.68 (m, 2H,  $\text{CH}_2$ -5'), 4.01 (m, 2H, H-4'), 4.20 (m, 1H, H-3'), 4.55 (m, 1H, H-2'), 6.03 (d, 1H,  $J = 5.2$  Hz, H-1'), 7.53 (m, 3H, H-Ph), 7.73 (m, 2H, H-Ph), 9.00 (s, 1H, H-8). Anal. ( $\text{C}_{18}\text{H}_{15}\text{IN}_4\text{O}_4$ ) C, H, N.



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