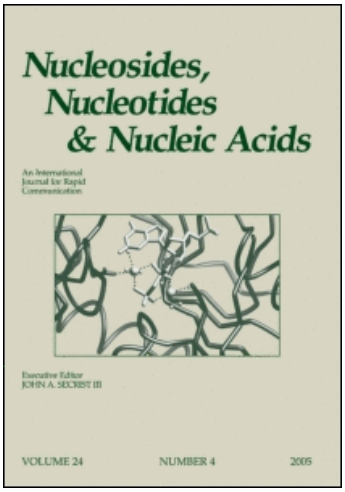

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SYNTHETIC PROCEDURE FOR THE PREPARATION OF NOVEL POTENT AND SELECTIVE A_3 ADENOSINE RECEPTOR RADIOLIGANDS

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ABSTRACT

2-Phenylethynyladenosine and its N^6-methyl derivative were synthesized and evaluated in binding assays at human adenosine receptors stably transfected on CHO cells. Results showed that the N^6-methyl-2-phenylethynyladenosine is endowed with very high affinity and selectivity at A_3 receptor subtype. Hence, an alternative procedure for the synthesis of tritiated N^6-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 neurotrasmission through the activation of four receptor subtypes denominated: A_1, A_{2A}, A_{2B} and A_3 (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_3 receptor subtype.

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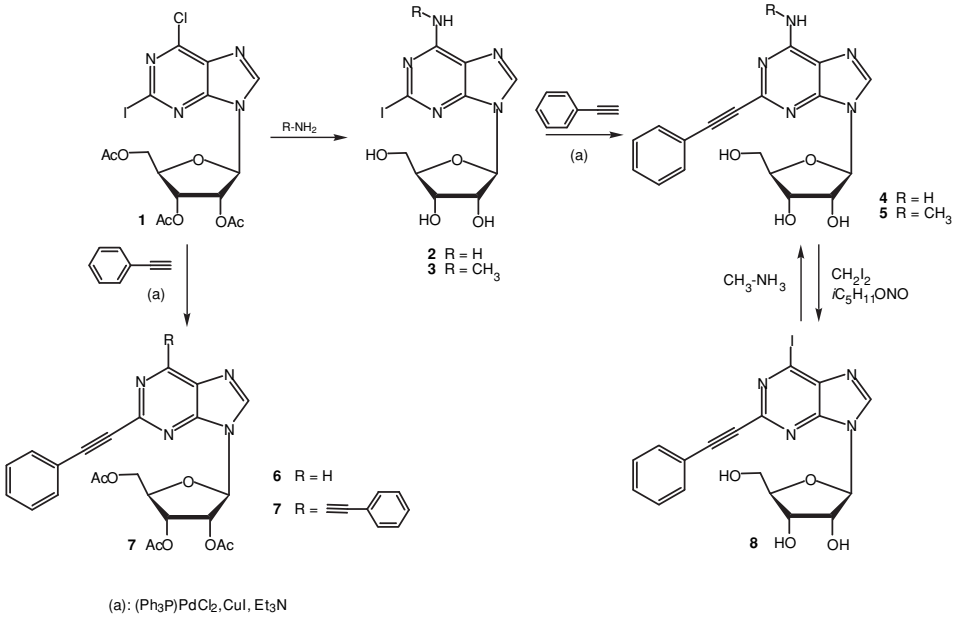
Moreover, the presence of different alkynes modulated the affinity of such derivatives at the other subtypes (A_1, A_{2A}, and A_{2B}). In particular, the 2-phenylethynyl-NECA (PENECA) was found to possess high affinity combined with good selectivity for A_3 receptors (4) ($K_iA_3 = 6.2$ nM; selectivity $A_1/A_3 = 90$, 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 anologue 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 arylcarbamoyl 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. ($K_iA_3 = 3.4$ 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.

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CHEMISTRY

The synthesis of 2-PEAdo (5) (**4**) and N^6-methyl-2-phenylethynyladenosine (**5**) was carried out starting from 6-chloro-2-iodo-9-(2,3,5-tri-*O*-acetyl-β-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^6-methylPEAdo (**5**) (Scheme 1).

Aimed at finding a convenient synthetic procedure for the preparation of radiolabelled N^6-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 isopentylnitrite 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_3 radioligand.

CONCLUSION

The N^6-methylPEAdo is the firstly reported adenosine derivative endowed with very high affinity and selectivity for A_3 adenosine receptor subtype.

The synthetic procedure to prepare the 6-iodo-2-phenylethynylAdo (**8**) could be a general synthetic method to obtain A_3 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. ^{1}H NMR spectra were obtained with Varian VXR 300 MHz spectrometer; δ in ppm, J in Hz. All exchangeable protons were confirmed by addition of D_2O. 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^6-methyl-9-(β-D-ribofuranosyl)adenine (3). To compound **1** (1.86 mmol) methylamine (10 mL) was added and the reaction mixture was

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allowed to stand at −20°C for 1 h. The exceeding amine was evaporated and the residue was chromatographed on a silica gel column eluting with $CHCl_3$-MeOH (93:7) to give **3** (634 mg; 69%) as amorphous solid; ^{1}H NMR (DMSO-d^6) δ 2.92 (d, 3H, $J = 4.1$ Hz, NH-*CH*$_3$), 3.61 (m, 2H, 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. ($C_{11}H_{14}IN_3O_4$) 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 Et_3N (2.3 mL) under an atmosphere of 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 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^6-Methyl-2-(phenylethyn-1-yl)-9-(β-D-ribofuranosyl)adenine (5). The reaction of **3** with phenylethyne for 16 h, followed by chromatography on a silica gel column eluting with $CHCl_3$-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°C for 5 h. After removing the exceeded methylamine the residue was chromatographed on flash silica gel column eluted with $CHCl_3$-cC_6H_{12}:MeOH (87:10:3) to give **5** (42 mg; 73%); mp 229–231°C; ^{1}H NMR (Me_2SO-d_6) δ 2.99 (br s, 3H, NH-*CH*$_3$), 3.64 (m, 2H, 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. ($C_{19}H_{19}N_5O_4$) C, H, N.

2,6-(Diphenylethyn-1-yl)-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (7). The reaction of **1** with phenylethyne for 24 h, followed by chromatography on a silica gel column eluted with $CHCl_3$-cC_6H_{12}-MeCN (52:40:8) gave **7** (293 mg; 51%) as amorphous solid; ^{1}H NMR (DMSO-d_6) δ 2.04 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.15 (s, 3H, $COCH_3$), 4.39 (m, 3H, 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. ($C_{32}H_{26}N_4O_7$) C, H, N.

6-Iodo-2-(phenylethyn-1-yl)-9-(β-D-ribofuranosyl)adenine (8). To a solution of **4** (0.27 mmol) in dry DMF (2 mL) diiodomethane (3.61 mL) and isopentylnitrite (1.12 mL) were added and the mixture was heated at 60°C for 30′. The solvent was evaporated and the residue was chromatographed on a silica gel column eluted with $CHCl_3$-cC_6H_{12}-MeOH (83:10:7) to give **7** (120 mg; 33%) as amorphous solid; ^{1}H NMR (DMSO-d_6) δ 3.68 (m, 2H, 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. ($C_{18}H_{15}IN_4O_4$) C, H, N.

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