JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2004; **47**: 415–427.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.829

Research Article

First no-carrier-added radioselenation of an adenosine-A_1 receptor ligand

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Summary

The precursor synthesis and the no-carrier-added (n.c.a.) radiosynthesis of the adenosine-A_1 receptor ligand 5′-(methyl[^{75}Se]seleno)-N^6-cyclopentyladenosine ([^{75}Se]**1**) are described in this report. A method was developed starting from elemental n.c.a. selenium-75, followed by a three-step polymer-supported radioselenation and deprotection which gave the radioligand with a radiochemical yield of 30%, a radiochemical purity of > 99% and a specific radioactivity of > 300 GBq/mmol (8 Ci/mmol). Preparation time was 40 min. The nonradioactive compound 5′-(methylseleno)-N^6-cyclopentyladenosine (**1**) was pharmacologically evaluated *in vitro* and showed high affinity and selectivity for the adenosine-A_1 receptor. These preliminary results suggest that this compound could be a useful radioligand for the noninvasive imaging of the brain adenosine-A_1 receptors using positron emission tomography (PET) when labelled with the positron emitter selenium-73 (half-life: 7.1 h). Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: adenosine-A_1 receptor ligand; n.c.a. radioselenation; selenium-75; positron emission tomography

Introduction

Purinergic receptors can be subdivided into P_1 and P_2 receptors. So far, four P_1 receptors, adenosine-A_1, -A_{2A}, -A_{2B} and -A_3, have been defined pharmacologically and cloned. All adenosine receptors are G-protein-coupled, activated by adenosine and antagonized by xanthines.[1] Adenosine-A_1 receptors are widely distributed in the central nervous system, and have been extensively characterized in brain where they are expressed in high density (0.5–1 pmol/mg membrane protein). In the periphery, they are found in the heart, in adipose tissue and in the kidneys.

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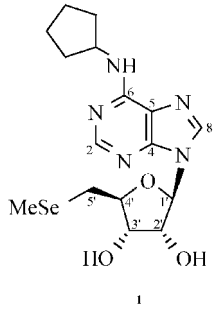
Received 19 June 2003
Revised 20 October 2003
Accepted 4 December 2003

Ligands for the adenosine-A_1 receptor subtype have a broad therapeutic potential because of its wide organ and tissue distribution.[2] Agonists for the adenosine-A_1 receptors could, for example, be useful as sedatives and in the diagnosis of diseases of coronary arteries.[3] However, severe cardiovascular side effects can be expected, caused by the strong hypotensive effects of the adenosine agonists.[4] These side effects are major drawbacks in the therapeutic use of adenosine receptor agonists. Partial agonists might have less pronounced cardiovascular effects and may act more selectively. Another advantage of partial agonists would be that they probably induce less receptor downregulation and desensitization.[5]

Previously, van der Wenden *et al.*[6] have synthesized such partial agonists by substituting the 5′-position of N^6-cyclopentyladenosine. Studies showed that among other 5′-(methylseleno)-N^6-cyclopentyladenosine (**1**) (Scheme 1) proved to be selective for the adenosine-A_1 receptors, displaying an affinity in the nanomolar range.



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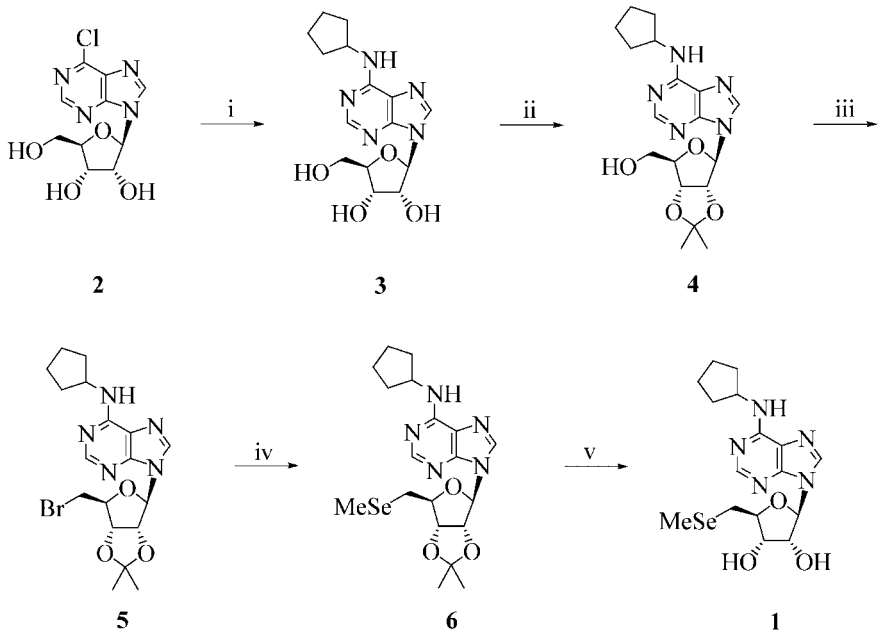
Scheme 1. 5′-(Methylseleno)-N^6-cyclopentyladenosine (1)

Since all adenosine-A_1 receptor ligands, which are under evaluation as PET ligands at present, are labelled with carbon-11[7] and one with fluorine-18,[8,9] the aim of this study was to obtain a much longer-lived radioligand. Therefore, compound **1** was selected for labelling with the positron emitter selenium-73. In order to investigate the general feasibility of an appropriate radioselenation method at the no-carrier-added (n.c.a.) level selenium-75 was used for initial tracer development and *in vitro* evaluation.

Results and discussion

Preparation of the standard and precursor

The standard compound 5′-(methylseleno)-N^6-cyclopentyladenosine (**1**) and the precursor for radioselenation 5′-bromo-2′,3′-isopropylidenedioxy-N^6-cyclopentyladenosine (**5**) were synthesized in five and three steps, respectively, starting from 6-chloropurine riboside (**2**) (Scheme 2).



Scheme 2. Synthesis of 5′-(methylseleno)-N^6-cyclopentyladenosine (1) Reaction conditions: (i) cyclopentylamine, EtOH, reflux; (ii) sulfuric acid (conc.), acetone, 0°C; (iii) triphenylphosphine, carbon tetrabromide, CH_2Cl_2, r.t.; (iv) dimethyldiselenide, sodium borohydride, EtOH, r.t.; (v) acetic acid, reflux

N^6-Cyclopentyladenosine (**3**) was obtained by reacting **2** with cyclopentylamine in ethanol to substitute the chlorine atom.[10] To obtain a selectively reactive 5′-group, the *cis* vicinal 2′- and 3′-hydroxyl groups of N^6-cyclopentyladenosine were selectively protected with an isopropylidene moiety by sulfuric acid-catalyzed reaction in acetone,[11] yielding 2′,3′-isopropylidenedioxy-N^6-cyclopentyladenosine (**4**). Subsequently, by reaction with carbon tetrabromide and triphenylphosphine in CH_2Cl_2, the 5′-hydroxyl group of **4** was replaced by a bromine atom as leaving group,[12] resulting 5′-bromo-2′,3′-isopropylidenedioxy-N^6-cyclopentyladenosine (**5**). This compound was quite unstable and therefore had to be used directly after purification. The reaction between **5** and methyl selenolate, which was obtained by reductive cleavage of dimethyldiselenide with sodium borohydride,[13] yielded 5′-(methylseleno)-2′,3′-isopropylidenedioxy-N^6-cyclopentyladenosine (**6**).

Deprotecting the 2′- and 3′-position of **6** with acetic acid[14] gave the final product 5′-(methylseleno)-N^6-cyclopentyladenosine (**1**) with an overall yield of about 20% (based on **2**).

Radiosynthesis of n.c.a. 5′-(methyl[^{75}Se]seleno)-N^6-cyclopentyladenosine

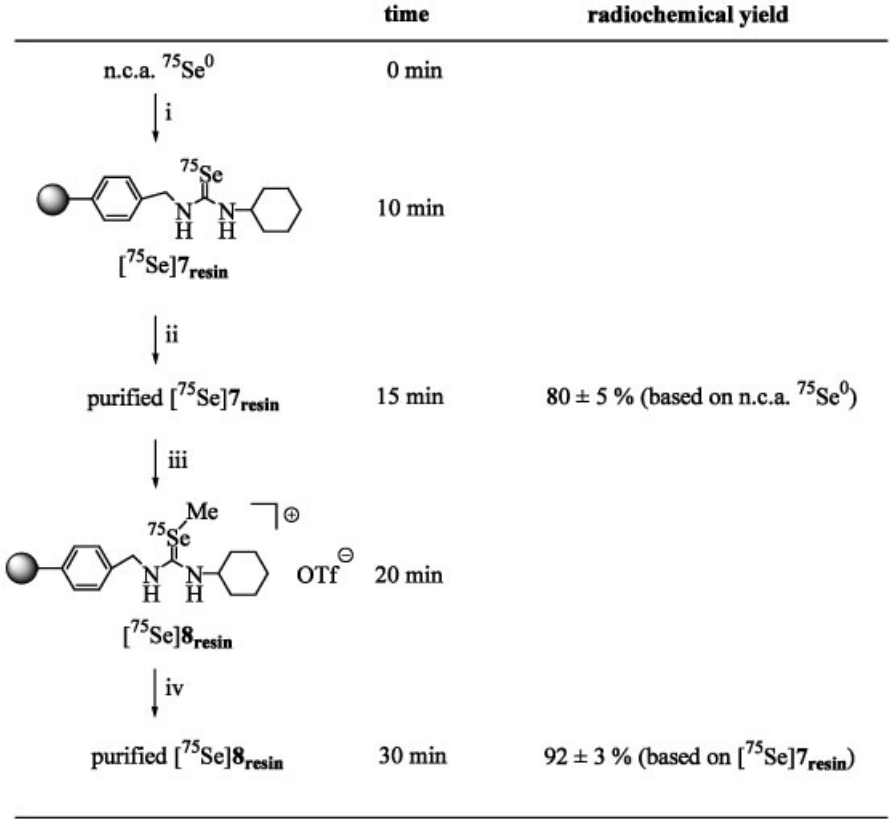
Since the specific activity of a potential radioligand for *in vivo* studies is a crucial factor and should be as high as possible in order to avoid a saturation

of the receptor system by the compound administered, an appropriate n.c.a. labelling method is required.

For convenience, the development and optimization of the radiosynthesis was performed using the longer-lived selenium-75 ($T_{1/2} = 120.4$ days). It has been demonstrated[15] that selenium-75 is a suitable model-nuclide for selenium-73 and that radiosyntheses developed via selenium-75 may serve as well for the preparation of ^{73}Se-labelled compounds without any necessity of modifying the reaction conditions.

A previously developed n.c.a. radioselenation strategy[15] was tested for the preparation of n.c.a. 5′-(methyl[^{75}Se]seleno)-N^6-cyclopentyladenosine ([^{75}Se]**1**).

Starting from n.c.a. elemental selenium-75, cyclohexyl isocyanide and aminomethylated polystyrene, the corresponding polymer-bound [^{75}Se]selenourea ([^{75}Se]**7**$_{resin}$) was synthesized in benzene at 80°C within 10 min with a radiochemical yield (RCY) of 80 ± 5% (based on n.c.a. ^{75}Se^0) (Scheme 3). The



Scheme 3. Polymer-supported radiosynthesis of methyl [^{75}Se]selenouronium salt ([^{75}Se]8_{resin}). Reaction conditions: (i) cyclohexyl isocyanide, aminomethylated polystyrene, benzene, 80°C; (ii) washing with benzene; (iii) methyl triflate, benzene, r.t.; (iv) separation via washing with benzene and THF

intermediate [^{75}Se]**7**$_{resin}$ was purified by washing with benzene. The RCY was determined as activity bound on the resin of purified [^{75}Se]**7**$_{resin}$, since it is likely that ^{75}Se was present on the polymer in no other form than in the corresponding selenourea group.

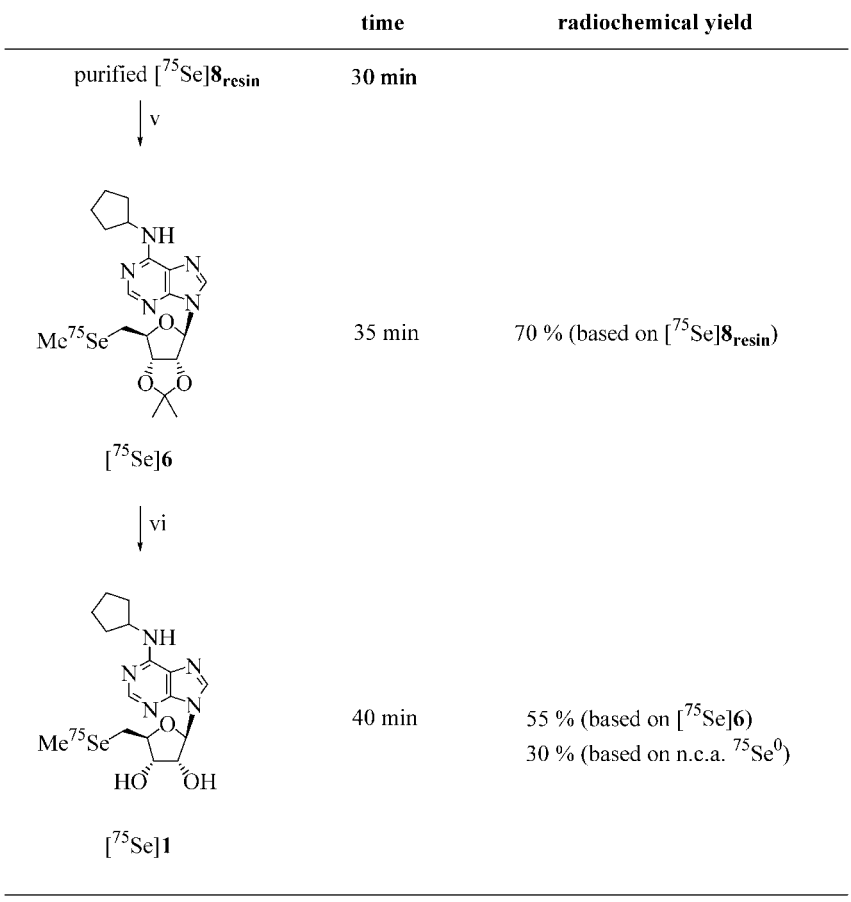
Subsequent alkylation in benzene via methyl triflate yielded the corresponding methyl [^{75}Se]selenouronium salt ([^{75}Se]**8**$_{resin}$) within 5 min with an RCY of 92 $\pm$ 3% (activity bound on the purified resin, based on [^{75}Se]**7**$_{resin}$) (Scheme 3). Purification of [^{75}Se]**8**$_{resin}$ was carried out by repeated washing with benzene and tetrahydrofuran (THF) in order to get rid of surplus alkyl triflate and to determine the RCY of the purified [^{75}Se]**8**$_{resin}$.

Hydrolysis of [^{75}Se]**8**$_{resin}$ by means of tetrabutylammonium hydroxide (TBAH) and alkylation of the resulting methyl [^{75}Se]selenolate with 5′-bromo-2′,3′-isopropylidenedioxy-N^6-cyclopentyladenosine (**5**) gave 5′-(methyl[^{75}Se]seleno)-2′,3′-isopropylidenedioxy-N^6-cyclopentyladenosine ([^{75}Se]**6**) with an RCY of about 70% (based on [^{75}Se]**8**$_{resin}$) under optimized reaction conditions (Scheme 4). Subsequent deprotection of [^{75}Se]**6** using hydrochloric acid at 80°C yielded the final n.c.a. product [^{75}Se]**1** within 5 min and with an RCY of 55% (based on [^{75}Se]**6**). Thus, 5′-(methyl[^{75}Se]seleno)-N^6-cyclopentyladenosine was obtained at the n.c.a. level with an overall RCY of approximately 30% (based on $^{75}Se^0$), with a radiochemical purity of $>$99% and within a total reaction time of 40 min.

With the detection limit of 1 nmol found here and the starting activity used, the specific activity of n.c.a. [^{75}Se]**1** was determined to be more than 300 GBq/mmol (8 Ci/mmol). However, with a theoretical maximal specific activity ($A_{s,max}$) of 37 TBq/mmol (1 Ci/μmol) for carrier-free selenium-75, the actual specific activity will amount to a value in the range between these two limits. Provided production of higher starting activities would not increase the dilution with stable selenium, i.e. the amount of carrier will still remain below 1 nmol, one can suggest that the actual specific activity would increase with the same factor as the starting activity. In case of the positron emitter selenium-73 the theoretical $A_{s,max}$ amounts to 16 TBq/μmol (400 Ci/μmol). Therefore, one can suggest that an even higher specific activity will be obtained if the starting activity is in the GBq-range. Thus, a specific activity appears feasible, which is sufficient to perform *in vivo* receptor studies with ^{73}Se-labelled ligands using PET.

Radioligand binding studies

Results of radioligand binding studies for **1** in the literature[6] are promising regarding selectivity for the adenosine-A_1 receptor, since they showed a 150 fold better affinity for the A_1 vs the A_{2A} receptor, which is probably due to the presence of the N^6-cyclopentyl substituent. Although the K_i value of **1** for the



Scheme 4. Radiosynthesis of 5′-(methyl[^{75}Se]seleno)-N^6-cyclopentyladenosine ([^{75}Se]1). Reaction conditions: (v) TBAH, 5′-bromo-2′,3′-isopropylidenedioxy-N^6-cyclopentyladenosine (5), THF, 70°C; (vi) hydrochloric acid, 80°C

adenosine-A_1 receptor was not very satisfactory with only 76 nM; the K_i value for the A_{2A} receptor amounted to 11 μM. These literature data were obtained using membranes of rat brain cortex with [^{3}H]DPCPX as the radioligand for the A_1 receptor and using rat striatal membranes with [^{3}H]CGS21680 as the radioligand for the A_2 receptor, respectively.

Nevertheless, **1** seems to be a very attractive selective ligand for the A_1 receptor. Therefore, radioligand binding studies were carried out here also under different conditions (cf. Experimental). Its affinity for the adenosine-A_1 receptor was determined by competition with [^{3}H]CPFPX[16] on pig cortical membranes. The K_i value of **1** for the adenosine-A_1 receptor was 0.9 nM (0.8–1.1 nM, 95% confidence interval). On the other hand, **1** had a K_i value of 1.3 μM (0.3–5.5 μM, 95% confidence interval) for the adenosine-A_{2A} receptor

measured by displacement of [^{3}H]CGS21680 from pig striatal membranes. These results suggest, that 5′-(methylseleno)-N^6-cyclopentyladenosine (**1**) is selective for the adenosine-A_1 vs the -A_{2A} receptor with a 1500 fold better affinity. The differences of this study and literature data can be explained by the altered conditions of the radioligand binding studies, i.e. by a more selective radioligand in case of the A_1 receptor study and by tissues of different animals in the studies. Therefore, the results of the biological experiments require further evaluation and make a study on cloned human receptors advisable.

Conclusion

In conclusion, n.c.a. 5′-(methyl[^{75}Se]seleno)-N^6-cyclopentyladenosine ([^{75}Se]**1**) was be synthesized via a polymer-supported radioselenation strategy with an overall radiochemical yield of 30% within 40 min. Furthermore, it was demonstrated, that **1** bound to the pig adenosine-A_1 receptor with a K_i of 0.9 nM. The K_i at the pig adenosine-A_{2A} receptor was 1300 nM, thus giving a selectivity of >1500-fold. These results make the use of this radioligand in its selenium-73 labelled form promising as a PET ligand.

Experimental

Material and methods

All chemicals and solvents were purchased from Aldrich (Germany), Fluka (Switzerland) and Merck (Germany). They were reagent grade or better and used without further purification. [^{3}H]CPFPX was prepared in house, as reported[16] and New England Nuclear provided [^{3}H]CGS 21680. Amino-methylated polystyrene was obtained from Novabiochem (Germany). All selenocompounds were prepared under a slight positive pressure of argon. Flash chromatography was done with Fluka silica gel 60 (220–440 mesh). Mixtures of elution solvents are given as V/V ratios.

Melting point determinations employed a Mettler FP 61 apparatus. Melting points (m.p.) are uncorrected. Analytical thin-layer chromatography (TLC) was performed with precoated silica gel plates (5×7.5 cm plates Type F_{254S}, Merck, Germany). Visualization of TLC slides was by UV. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 200 and 50 MHz, respectively, on a Bruker DPX Avance 200 spectrometer with samples dissolved in d_6-DMSO or $CDCl_3$. ^{13}C NMR were routinely run with broad band decoupling. All chemical shifts are reported in δ ppm using the signals of the solvent as a reference. Mass spectra (MS) were obtained using a Thermoquest Automass Multi mass spectrometer using the electron spray ionization method. Reported values for the mass of selenium containing compounds base on selenium-80.

Analytical radio-HPLC was performed on a system consisting of a Knauer pump 6400 and a Knauer UV/vis photometer 3060 with a detector wavelength of 220 nm. Sample injection was accomplished by a Rheodyne-Injector block 7125. For measurement of radioactivity the outlet of the UV detector was connected to a NaI(Tl) well-type scintillation detector (EG&G ACE Mate^{TM}) and the recorded data were processed by the software system Raytest Winnie (Raytest, Germany). HPLC of aliquots of labelled products and standards was performed using a Lichrosorb RP Select B (250×4 mm) column (CS-Chromatographie Service GmbH, Germany) and a mobile phase consisting of MeOH/H_2O in various concentrations (given as V/V ratios) at a flow rate of 1.0 ml/min. Radio-TLC was performed on Merck silica gel plates with the solvent system CH_2Cl_2/MeOH in various concentrations (given as V/V ratios). The developed TL-chromatograms were measured for radioactivity on an Instant Imager^{TM} (Packard, USA).

*N^6-Cyclopentyladenosine (**3**) [from[10]]*

A mixture of 6-chloropurine ribonucleoside (5.7 g, 20 mmol), cyclopentylamine (11.8 ml, 120 mmol) and EtOH (100 ml) was refluxed for 15 h, and it was evaporated *in vacuo* to dryness. The residue was triturated with a small amount of EtOH, and colorless crystals of N^6-cyclopentyladenosine were obtained. Yield: 4.56 g (68%) (lit.[10]: 75%); m.p.: 78.8°C (lit.: 77–81°C); ^{1}H-NMR (d_6-DMSO): δ 8.36 (s, 1H, H8), 8.21 (s, 1H, H2), 7.80 (d, 1H, N^6H), 5.92–5.86 (d, 1H, H1′), 5.51–5.44 (m, 2H, OH-2′/OH-3′), 5.25–5.19 (m, 1H, OH-5′), 4.72–4.59 (m, 1H, H2′), 4.56 (bs, 1H, HCN^6), 4.19–4.14 (m, 1H, H3′), 4.01–3.96 (m, 1H, H4′), 3.67 (m, 2H, H5′), 1.78 (m, 8H, $H_{cyclopentyl}$); (m, 8 H, $H_{cyclopentyl}$); ^{13}C-NMR: δ 155.3 (C4/C6), 153.1 (C2), 144.9 (C8), 140.4 (C5), 88.8 (C1′), 86.8 (C4′), 74.3 (C2′), 71.5 (C3′), 62.6 (C5′), 56.9 ($C1_{cyclopentyl}$), 33.0 ($C2/5_{cyclopentyl}$), 24.3 ($C3/4_{cyclopentyl}$); MS: *m*/*e* 336 (M + 1, 100%); R_f: 0.68 (CH_2Cl_2/MeOH 1:4).

*2′,3′-Isopropylidenedioxy-N^6-cyclopentyladenosine (**4**) [from[11]]*

N^6-Cyclopentyladenosine (**3**) (4.36 g, 13 mmol) dissolved in acetone (50 ml) was added dropwise to a stirred mixture of acetone (50 ml) and concentrated sulfuric acid (1.3 ml, 26 mmol) at 0°C. After complete addition, the mixture was stirred at r.t. for 90 min. The solution was cooled again to 0°C and neutralized with $NaOH_{aq}$ (20 ml, 20%). The mixture was concentrated and partitioned between H_2O and CH_2Cl_2. The aqueous layer was extracted several times with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), and the solvent was evaporated. The residue was purified via flash chromatography (CH_2Cl_2/MeOH 100:2) to give 2′,3′-isopropylidenedioxy-N^6-cyclopentyladenosine as colorless crystals after evaporation of the eluent.

Yield: 3.79 g (78%); m.p.: 96°C; ^{1}H-NMR (d_6-DMSO): δ 8.36 (s, 1H, H8), 8.23 (s, 1H, H2), 7.81 (d, 1H, N^6H), 6.18–6.14 (d, 1H, H1′), 5.38–5.26 (m, 2H, H2′/OH-5′), 5.01–4.97 (m, 1H, H3′), 4.56 (bs, 1H, HCN^6), 4.14–4.24 (m, 1H, H4′), 3.57–3.53 (m, 2H, H5′), 1.97–1.56 (m + s, 11H, $H_{cyclopentyl}$/$CH_{3,isopropylidene}$), 1.34 (s, 3H, $CH_{3,isopropylidene}$); ^{13}C-NMR: δ 155.8 (C4/C6), 153.4 (C2), 144.9 (C8), 140.2 (C5), 113.9 ($C_{q,isopropylidene}$), 90.6 (C1′), 87.3 (C4′), 84.1 (C2′), 82.3 (C3′), 62.5 (C5′), 57.0 ($C1_{cyclopentyl}$), 33.0 ($C2/5_{cyclopentyl}$), 27.9 ($CH_{3,isopropylidene}$), 26.0 ($CH3,isopropylidene$), 24.3 ($C3/4_{cyclopentyl}$); MS: *m/e* 375 (M, 100%), 204 (35); R_f: 0.35 (CH_2Cl_2/MeOH 25:1).

*5′-Bromo-2′,3′-isopropylidenedioxy-N^6-cyclopentyladenosine (**5**) [from[12]]*

Triphenylphosphine (2.36 g, 9 mmol) in dry CH_2Cl_2 (20 ml) was added dropwise to a stirred solution of **4** (3.38 g, 9 mmol) and CBr_4 (5 g, 15 mmol) in dry CH_2Cl_2 (20 ml) at r.t. After 15 h the solvent was evaporated and the residue was purified via flash chromatography (CH_2Cl_2/MeOH 100:2) to give 5′-bromo-2′,3′-isopropylidenedioxy-N^6-cyclopentyladenosine as an oil after evaporation of the eluent. Yield: 2.09 g (53%); ^{1}H-NMR (d_6-$CDCl_3$): δ 8.36 (s, 1H, H8), 7.86 (s, 1H, H2), 6.11 (d, 1H, H1′), 5.91–5.87 (d, 1H, N^6H), 5.51–5.48 (m, 1H, H2′), 5.20–5.16 (m, 1H, H3′), 4.75–4.35 (bs, 1H, HCN^6), 4.52–4.49 (m, 1H, H4′), 3.72–3.40 (m, 2H, H5′), 2.17–1.50 (m + s, 11H, $H_{cyclopentyl}$/$CH_{3,isopropylidene}$), 1.39 (s, 3H, $CH_{3,isopropylidene}$); ^{13}C-NMR: δ 155.0 (C4/C6), 153.7 (C2), 144.8 (C8), 132.1 (C5), 114.8 ($C_{q,isopropylidene}$), 91.6 (C1′), 87.2 (C4′), 84.6 (C2′), 84.0 (C3′), 54.4 (C5′), 52.8 ($C1_{cyclopentyl}$), 32.3 ($C2/5_{cyclopentyl}$), 27.4 ($CH_{3,isopropylidene}$), 25.7 ($CH_{3,isopropylidene}$), 24.1 ($C3/4_{cyclopentyl}$); MS: *m/e* 440 (M, 100%); R_f: 0.81 (CH_2Cl_2/MeOH 25:1).

*5′-(Methylseleno)-2′,3′-isopropylidenedioxy-N^6-cyclopentyladenosine (**6**) [following[13]]*

5′-Bromo-2′,3′-isopropylidenedioxy-N^6-cyclopentyladenosine (**5**) (1.75 g, 4 mmol), dissolved in EtOH (20 ml), was added dropwise to a stirred mixture of dimethyl diselenide (1.07 ml, 8 mmol) and sodium borohydride (2 g) in EtOH (20 ml). After complete addition, the mixture was stirred at r.t. for 30 min. The solution was concentrated and partitioned between H_2O and CH_2Cl_2. The aqueous layer was extracted several times with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), and the solvent was evaporated. The residue was purified via flash chromatography (CH_2Cl_2/MeOH 100:2) to give 5′-(methylseleno)-2′,3′-isopropylidenedioxy-N^6-cyclopentyladenosine as oil after evaporation of the eluent. Yield: 1.5 g (83%); ^{1}H-NMR (d_6-DMSO): δ 8.35 (s, 1 H, H8), 8.25 (s, 1 H, H2), 7.76 (d, 1 H, N^6H), 6.20 (d, 1 H, H1′), 5.56–5.52 (m, 1 H, H2′), 5.04–5.00 (m, 1 H, H3′), 4.56 (bs, 1 H, HCN^6), 4.37–4.29 (m, 1 H, H4′), 2.87–2.72 (m, 2 H, H5′), 1.74–1.55 (m + 2 s, 14 H,

$H_{cyclopentyl}/SeCH_3/CH_{3,isopropylidene}$), 1.35 (s, 3 H, $CH_{3,isopropylidene}$); ^{13}C-NMR: δ 155.6 (C4/C6), 153.5 (C2), 144.9 (C8), 140.5 (C5), 114.1 ($C_{q,isopropylidene}$), 90.1 (C1′), 87.2 (C4′), 84.7 (C2′), 84.2 (C3′), 58.1 ($C1_{cyclopentyl}$), 33.0 ($C2/5_{cyclopentyl}$), 27.8 (C5′), 27.5 ($CH_{3,isopropylidene}$), 26.0 ($CH_{3,isopropylidene}$), 24.3 ($C3/4_{cyclopentyl}$), 5.0 ($SeCH_3$); MS: *m/e* 454 (M, 100%), 360 (97); *k′*: 4.22 ($MeOH/H_2O$: 7/3); R_f: 0.35 (CH_2Cl_2/MeOH 25:1).

*5′-(Methylseleno)-N^6-cyclopentyladenosine (**1**) [from[14]]*

A solution of **6** (1.36 g, 3 mmol) in acetic acid (60 ml, 65%) was refluxed for 4 h. The solvent was evaporated and the residue was purified via flash chromatography (CH_2Cl_2/MeOH 100:2) to give **5′**-(methylseleno)-N^6-cyclopentyladenosine[6] as a colorless solid. The product recrystallized from EtOH. Yield: 1.08 g (87%); m.p.: 71.3°C (lit.[6]: 66–72°C); ^{1}H-NMR (d_6-DMSO): δ 8.37 (s, 1H, H8), 8.24 (s, 1H, H2), 7.69 (d, 1H, N^6H), 6.18 (d, 1H, H1′), 5.54–5.52 (m, 1H, OH-2′), 5.42–5.36 (m, 1H, OH-3′), 4.80–4.78 (m, 1H, H2′), 4.62–4.39 (bs, 1H, HCN^6), 4.24–4.11 (m, 2H, H3′/H4′), 2.99–2.80 (m, 2H, H5′), 1.96–1.23 (m + s, 11H, $H_{cyclopentyl}/SeCH_3$); ^{13}C-NMR: δ 155.2 (C4/C6), 153.4 (C2), 145.0 (C8), 140.3 (C5), 88.2 (C1′), 85.1 (C4′), 74.1 (C2′), 73.7 (C3′), 56.9 ($C1_{cyclopentyl}$), 33.1 ($C2/5_{cyclopentyl}$), 28.2 (C5′), 24.3 ($C3/4_{cyclopentyl}$), 5.4 ($SeCH_3$); MS: *m/e* 414 (M + 1, 100%); *k′*: 2.07 ($MeOH/H_2O$: 6/4); R_f: 0.22 (CH_2Cl_2/MeOH 25:1).

Production of [^{75}Se]selenium

N.c.a. [^{75}Se]selenium was produced using 20 MeV protons at the compact cyclotron CV-28 of the Forschungszentrum Jülich GmbH via the ^{75}As (p,n)^{75}Se reaction on a solid Cu_3As target. After thermochromatographic separation n.c.a. selenium-75 is available in its oxidized form as [^{75}Se]SeO_3^{2-} in hydrochloric acid. It was then reduced with sulfur dioxide to obtain elemental n.c.a. selenium-75, which was extracted into benzene following procedures earlier described in detail.[17,18]

The radiosynthesis described below was conducted under argon in a conical 5 ml reaction vessel equipped with a magnetic stirring bar and a teflon rubber septum.

N.c.a. 5′-(methyl[^{75}Se]seleno)-N^6-cyclopentyladenosine

The labelled adenosine-derivative was prepared as reported previously[15] for [^{75}Se]selenoethers in general and is briefly described here, starting from elemental n.c.a. selenium-75. The formation of polymer-supported n.c.a. *N*-cyclohexyl [^{75}Se]selenourea-*N′*-methyl polystyrene ([^{75}Se]$\mathbf{7}_{resin}$) was performed at 80°C within 10 min after adding cyclohexyl isocyanide (10 µl, 0.08 mmol) and aminomethylated polystyrene (40 mg) to a solution of n.c.a. $^{75}Se^0$ in

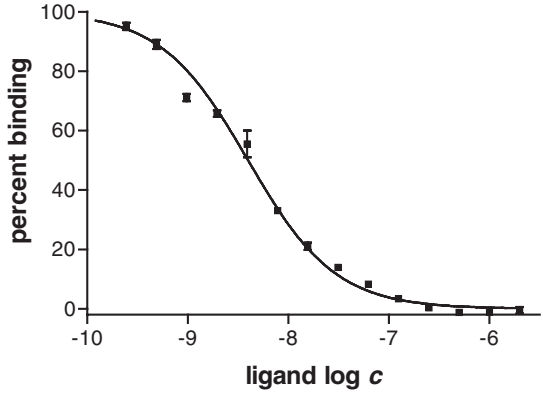
benzene (0.5 ml, typically containing 370 kBq (10 μCi)). [^{75}Se]**7**$_{resin}$ was purified by washing with benzene (2 ml) before use in the following reaction step.

Alkylation of n.c.a. [^{75}Se]**7**$_{resin}$ with trifluoromethanesulfonic acid methyl ester (11 μl, 0.1 mmol) in benzene (0.5 ml) gave the corresponding methyl [^{75}Se]selenouronium salt ([^{75}Se]**8**$_{resin}$) at r.t. within 5 min, which was purified by washing the resin with benzene (2 ml) and THF (2 ml). Hydrolysis with tetrabutylammonium hydroxide (0.06 mmol) in 0.6 ml THF in the presence of 5′-bromo-2′,3′-isopropylidenedioxy-N^6-cyclopentyladenosine (**5**) (44 mg, 0.1 mmol) resulted in 5′-(methyl[^{75}Se]seleno)-2′,3′-isopropylidene-dioxy-N^6-cyclopentyladenosine ([^{75}Se]**6**) at 70°C within 5 min. After deprotection using hydrochloric acid (0.3 ml, 1 N) at 80°C, 5′-(methyl[^{75}Se]seleno)-N^6-cyclopentyladenosine ([^{75}Se]**1**) was obtained within 5 min and analyzed by radio-HPLC and radio-TLC. ([^{75}Se]***1***) was purified and separated by radio-HPLC ($k' = 2.07$; MeOH/H_2O 60:40).

Radioligand binding assays

Corpora striata (for A_{2A}AR assays) and frontal *cortices* (for A_1AR assays) were dissected from pig brain and the tissue was homogenized for 1 min in 20 volumes of ice-cold 50 mM *tris*-HCl buffer, pH 7.4 containing 10 mM $MgCl_2$, soybean trypsin inhibitor (20 μg/ml), Bacitracin (200 μg/ml), and Benzamidine HCl (160 μg/ml) by means of an Ultra Turrax at 20 000 rpm. The homogenate was centrifuged at 48 000 × *g* for 10 min at 4°C (Beckmann Optima L, SW41Ti rotor). The pellet was suspended in 20 volumes of *tris*-HCl, pH 7.4, containing adenosine deaminase (2 U/ml) and trypsin inhibitor (20 μg/ml), and then incubated for 30 min at 37°C. After centrifugation at 48 000 × *g* for 10 min at 4°C the resulting pellet was diluted in 20 volumes of 50 mM *tris*-HCl, pH 7.4, containing 10 mM $MgCl_2$. Aliquots of the homogenate (1 ml) were stored at –80°C. The assays were performed in triplicate by incubating of 20 μl aliquots of the membrane fractions (87 μg protein/assay for A_1-assays, 68 μg protein/assay for A_{2A}-assays) in *tris*-HCl, pH 7.4, containing adenosine deaminase (2 U/ml). Cortical homogenates were used for A_1AR- and striatal homogenates for A_{2A}AR-assays. Incubation was carried out at 20°C for 60 min in a total assay volume of 200 μl. In A_{2A}AR competition experiments [^{3}H]CGS21680 (A_{2A}A receptor radioligand, $K_D = 26$ nM) was used in a concentration of 5 nM. In A_1AR competition experiments [^{3}H]CPFPX (A_1A receptor radioligand, $K_D = 0.62$ nM) was used in a concentration of 2 nM. Centrifugation at 48 000 × *g* for 6 min at 8°C separated bound from free ligand. Supernatants were discarded, the pellets washed with 1 ml ice cold buffer and dissolved by incubating in Solvable™ (500 μl, Canberra-Packard) for 120 min at 50°C. Aliquots of 450 μl were placed in scintillation vials with scintillation cocktail (10 ml, Ultima Gold XR, Canberra-Packard).

Radioactivity was measured in a liquid scintillation analyzer Tri-carb 2300Tr (Canberra-Packard). Protein estimation was performed with a commercial assay (Bio-Rad DC Protein Assay) after solubilization in 15% NH_4OH containing 2% SDS (v,w); human serum albumin served as a standard. K_i was calculated by a computer-assisted curve-fitting program (GraphPad Prism, version 3.0) as exemplified in Scheme 5 for the adenosine A_1 receptor.



Scheme 5. Binding inhibition of [^{3}H]CPFPX by 5′-(methyseleno)-N^6-cyclopentyladenosine (1) in homogenates of pig frontal cortices

Acknowledgements

The authors thank S. Spellerberg for his helpful assistance in preparation of n.c.a. selenium-75 and Dr M. Holschbach and S. Mahbubfar for recording the NMR and mass spectra.

References

1. Williams M, Jarvis MF. Purinergic and pyrimidinergic receptors as potential drug targets. *Biochem Pharmacol* 2000; **59**: 1173–1185.
2. Jacobson KA, van Galen PJM, Williams M. Adenosine receptors: pharmacology, structure-activity relationships, and therapeutic potential. *J Med Chem* 1992; **35**: 407–422.
3. Erion MD. Adenosine Receptors as Pharmacological Tools. *Annu Rep Med Chem* 1993; **28**: 295–304.
4. Dhalla AK, Shryock JC, Shreniwas R, Belardinelli L. Pharmacology and therapeutic applications of adenosine A_1 receptor ligands. *Curr Top Med Chem* 2003; **3**: 369–385.
5. Van der Wenden EM, Von Frijtag Drabbe Künzel JK, Mathot RAA, Danhof M, Ijzerman AP, Soudijn W. Ribose-modified adenosine analogues as potential partial agonists for the adenosine receptor. *J Med Chem* 1995; **38**: 4000–4006.
6. Van der Wenden EM, Carnielli M, Roelen HCPF, Lorenzen A, Von Frijtag Drabbe Künzel JK, Ijzerman AP. 5′-Substituted adenosine analogs as new

high-affinity partial agonists for the adenosine A_1 receptor. *J Med Chem* 1998; **41**: 102–108.

7. Holschbach MH, Olsson RA. Applications of adenosine receptor ligands in medical imaging by positron emission tomography. *Curr Pharm Des* 2002; **8**: 2345–2352.
8. Holschbach MH, Fein T, Krummeich C, Lewis RG, Wutz W, Schwabe U, Unterlugauer D, Olsson RA. A_1 Adenosine receptor antagonists as ligands for positron emission tomography (PET) and single photon emission tomography (SPET). *J Med Chem* 1998; **41**: 555–563.
9. Holschbach MH, Fein T, Wutz W, Boy C, Cremer M, Mühlensiepen H, Hamacher K, Lewis RG, Schwabe U, Müller-Gärtner HW, Coenen HH, Olsson RA. Synthesis and characterization of radiolabelled xanthines: new antagonists for the A_1 adenosine receptor. *Drug Dev Res* 1998; **43**: 71 (Abstr).
10. Kikugawa K, Iizuka K, Ichino M. Platelet aggregation inhibitors. 4. N^6-Substituted adenosines. *J Med Chem* 1973; **16**: 358–364.
11. Schmidt OT. Isopropylidene derivatives. *Methods Carbohydr Chem* 1963; **2**: 318–325.
12. Nair SA, Lee B, Hangauer DG. Synthesis of orthogonally protected L-homocysteine and L-2-amino-4-phosphonobutanoic acid from L-homoserine. *Synthesis* 1995; 810–814.
13. Ahmad R, Saa JM, Cava MP. Regioselective O-demethylation in the aporphine alkaloid series. *J Org Chem* 1977; **42**: 1228–1230.
14. Lewbart ML, Schneider JJ. Preparation and properties of steroidal 17,20- and 20,21-acetonides epimeric at C-20. 1. Derivatives of 5β-pregnan-3α-ol. *J Org Chem* 1969; **34**: 3505–3512.
15. Blum T, Ermert J, Coenen HH. No-carrier-added (n.c.a.) synthesis of asymmetric [^{73,75}Se]selenoethers with isonitriles. *Appl Radiat Isot* 2002; **57**: 51–56.
16. Holschbach M, Wutz W, Schüller M, Bier D, Coenen HH. Tritium-label-led 8-cyclopentyl-3-(3-fluoropropyl)-1-propylxanthine ([^{3}H]CPFPX), a potent and selective antagonist for the A_1 adenosine receptor. *J Label Compd Radiopharm* 2003; **46**: 365–372.
17. Plenevaux A, Guillaume M, Brihaye C, Lemaire C, Cantineau R. Chemical processing for production of no-carrier-added selenium-73 from germanium and arsenic targets and synthesis of L-2-amino-4-([^{73}Se]methylseleno)-butyric acid (L-[^{73}Se]selenomethionine). *Appl Radiat Isot* 1990; **41**: 829–838.
18. Blessing G, Lavi N, Hashimoto K, Qaim SM. Thermochromatographic separation of radioselenium from irradiated Cu3As-target: production of no-carrier added ^{75}Se. *Radiochim Acta* 1994; **65**: 93–98.

