Radioligand for in vivo Imaging of A3 Adenosine Receptors

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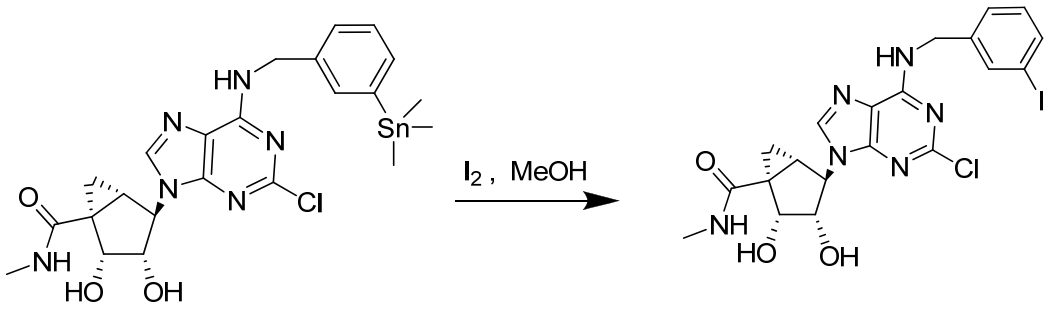
Background

Adenosine regulates many physiological functions through specific cell membrane receptors. The A_3 adenosine receptor (A_3AR) plays an important role in brain ischemia, immunosuppression, and bronchospasm in several animal models.[1] The A_3AR is also elevated in tumors and its expression level correlates to tumor responsiveness to therapy, for instance with the A_3AR agonist IB-MECA.[2, 3] The related compound ^{125}I-AB-MECA which was introduced in 1994 is a radioligand for A_3AR.[2] Radioligands for A_3AR may be promising diagnostic markers for cancer and possibly other diseases while characterization and quantification of the A_3AR *in vivo*, in patients, may also be a useful tool in both research and therapeutic studies. A new nucleoside derivative (MRS1898) is highly potent and selective for the A_3AR compared with other agonists for A_3AR such as IB-MECA,[4] and we synthesized a radioiodine analog for preliminary *in vitro* studies.

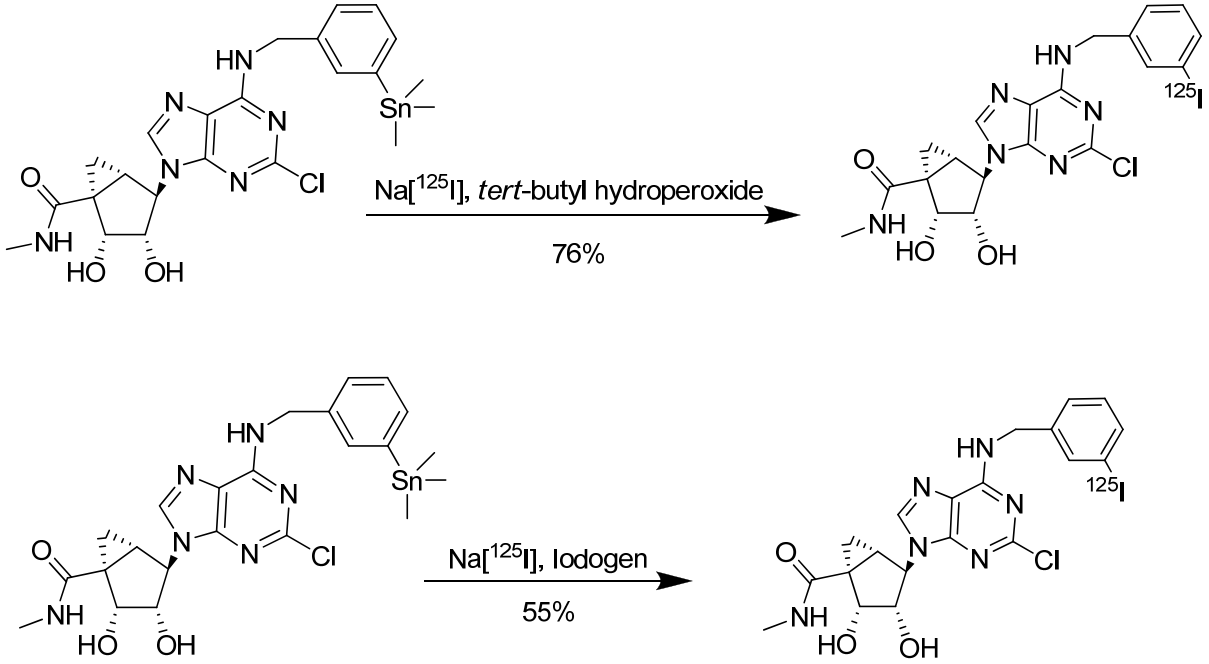
Chemistry

The 'cold' iodination and the radioiodination of MRS1898 were done by iododestannylation from a trimethylstannyl precursor. This new radioligand should permit better analysis of the tissue distribution of the A3AR. Two different radioiodination methods were used, with the 'TBHP' (tertiary-butyl hydroperoxide) method providing the superior yield.

Scheme 1. Non-radioactive Labeling of MRS1898



Scheme 2. Radiosyntheses of MRS1898



Experimental

General. All chemicals were purchased from Aldrich Chemical Company except where noted. Sodium [125I]iodide (17.4 Ci/mg) in NaOH (1.0x10-5 M) was supplied by Perkin–Elmer Life and Analytical Science. Iodogen was purchased from Pierce Biotechnology. High performance liquid chromatography was performed using an Agilent 1200 Series LC-MS system or a Beckman Gold HPLC system equipped with a Model 126 programmable solvent module, a Model 168 variable wavelength detector, a β–Ram Model 4 radioisotope detector, and Beckman System Gold remote interface module SS420X, using 32 Karat®software. These analyses were

performed on Agilent Eclipse XDB-C18 (3.5 µm, 3.0x75 mm) and XDB-C18 (5 µm, 4.6x250 mm) columns.

Iodo-MRS1898. The iodination of MRS1898 was performed using iodine in methanol (Scheme 1). The trimethylstannyl precursor (0.2 mg, 0.34 µmol) was dissolved in 50 µL of methanol in an eppendorf vial and to this solution was added iodine-methanol solution (100µL of 8mg/mL). The vial contents were shaken for 30 minutes, and the crude product analyzed on an Agilent 1200 Series LC-MS. Iodo-MRS1898 was elute at 7.6 minutes with a molecular weight 555.1 that shows $[M+H]^+$.

Radiolabeling of MRS1898. Method 1: The radioiodination of MRS1898 was performed using *tert*-butyl hydroperoxide (TBHP) as an oxidant (Scheme 2).[5] 15 µL of TBHP (10% solution chloroform) was added into a v-vial. To this solution were added acetic acid solution (4µL, 3% in chloroform) and sodium [^{125}I]iodide solution (4 µL, 14.8 MBq, 3.7 GBq/mL), followed by trimethylstannyl precursor (50 µL, 0.2 mg in chloroform). The reaction mixture was mixed under vortex for 30 minutes at room temperature. The final labeled product was separated using a Beckman System Gold HPLC equipped with an Agilent Eclipse XDB-C18 column (5.0 µm, 4.6×250 mm) under the following conditions: Solvent A was 0.1% TFA in water, solvent B was 0.1% TFA in acetonitrile. A linear gradient of solvent B from 20-50% over 25 minutes was used at a flow rate of 1 mL/minute. I-125-MRS1898 eluted at 23 minutes. The fraction containing I-125-MRS1898 was concentrated and loaded onto an Agilent Eclipse XDB-C18 column (3.5 µm, 3.0×75 mm). The mobile phase was the same as above and a linear gradient of solvent B from 20-45% over 12 minutes was used at a flow rate of 1 mL/minute. I-125-MRS1898 eluted at 9.0 minutes. The radiochemical purity was over 98%, the specific activity was 3.94Ci/mg, and the radiochemical yields were 75.6%.

Method 2: The second radioiodination of the trimethylstannyl MRS1898 with Na[^{125}I] iodide was accomplished using iodogen as an oxidant (Scheme 2). Iodogen solution (60 µL, 0.33 mg/mL in chloroform) and sodium [^{125}I]iodide solution (3 µL, 11.1 MBq, 3.7 GBq/mL) were transferred into a v-vial. To this solution was added trimethylstannyl precursor (50 µL, 0.2 mg in

chloroform). The reaction mixture was mixed under vortex for 30 minutes at room temperature and the I-125-MRS1898 was purified using the same conditions as used in method 1. The radiochemical purity was over 98%, the specific activity was 3.94Ci/mg, and the radiochemical yields were 54.5%. The I-125-MRS1898 was stored in solution (ethanol: water=9:1, v/v), containing 1% ascorbic acid for inhibition of radiolytic processes. Its stability over time is reported in Table 1.

Table 1. The radiochemical purities of I-125-MRS1898 solution in different times

Time (days)	2	8	11	20	25	27
Radiochemical purity	98%	98.5%	98.5%	100%	100%	100%

References

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