Synthesis of the adenosine A_{2A} receptor fluorescent agonist MRS5424

Kenneth A. Jacobson[1] and Francisco Ciruela[2*]

[1]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, USA; [2]Departament de Patologia i Terapèutica Experimental, Universitat de Barcelona-IDIBELL, L'Hospitalet de Llobregat, Spain
*For correspondence: fciruela@ub.edu

[Abstract] MRS5424 is a functional fluorescent agonist for the adenosine A_{2A} receptor ($A_{2A}R$) in which the fluorescent dye Alexa Fluor 532 is covalently attached to the $A_{2A}R$ agonist 2-[[2-[4-[2-(2-aminoethyl)-aminocarbonyl]ethyl]phenyl]ethylamino]-5'-N-ethylcarboxamidoadenosine (APEC). This easy-to-synthesize new $A_{2A}R$ fluorescent ligand was shown to be extremely useful for determining the binding kinetic constants of $A_{2A}R$ in a real-time mode (Fernandez-Duenas *et al.*, 2012). In addition, this fluorescent $A_{2A}R$ ligand is compatible with ligand-receptor interaction studies using fluorescent plate readers. Finally, it is important to mention that even though the sensitivity of this $A_{2A}R$ fluorescent ligand may not be as high as that observed for the marketed $A_{2A}R$ radioactive compounds, the use of such fluorescent derivative may have some advantages over radioactive probes, for example its safe delivery, manipulation and disposal, the short signal acquisition times, the feasibility to automate and to miniaturize, and finally its cost.

Materials and Reagents

1. Alexa Fluor 532 carboxylic acid, *N*-succinimidyl ester (Life Technologies, Invitrogen™)
2. Anhydrous dimethylformamide (DMF; HPLC grade) (Alfa Aesar)
3. Sodium tetraborate labeling buffer (0.1 M, pH 8.5)
4. 2-[[2-[4-[2-(2-aminoethyl)-aminocarbonyl]ethyl]phenyl]ethylamino]-5'-N-ethyl-carboxamidoadenosine (APEC) (NIMH Chemical Synthesis and Drug Supply Program, http://nimh-repository.rti.org/)
5. Triethylammonium acetate (TEAA)-CH_3CN (BioUltra grade) (Sigma-Aldrich)
6. Tetrabutylammonium dihydrogenphosphate-CH_3CN (TBAP) [puriss. ≥99.0% (T)] (Sigma-Aldrich)

Equipment

1. RP-C18(2) semipreparative column (250 x 10.0 mm) (Phenomenex)

2. Hewlett-Packard 1100 HPLC equipped with a Luna 5 µm RP-C18(2) semipreparative column (250 x 10.0 mm) (Figure 1B) (Phenomenex) or a Zorbax SB-Aq 5 µm analytical column (50 x 4.6 mm) (Agilent) (Figure 1A)

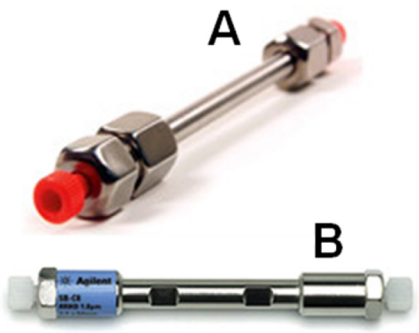

Figure 1. Picture of the Luna 5 µm RP-C18(2) semipreparative column (250 x 10.0 mm) (B) and the Zorbax SB-Aq 5 µm analytical column (50 x 4.6 mm) (A)

3. Diode array detector
4. POLARstar Optima plate-reader (BMG LABTECH)

Procedure

Briefly, MRS5424 (Fernandez-Duenas *et al.*, 2012) was synthesized as follows.

1. Firstly, Alexa Fluor 532 carboxylic acid, *N*-succinimidyl ester (1.0 mg, 1.38 µmol) was dissolved in anhydrous DMF (200 µl).
2. Next, make a 0.1 M sodium tetraborate buffer by dissolving 0.038 g of sodium tetraborate decahydrate for every ml of water. Adjust pH with HCl to 8.5. The labeling buffer should be made just before using it (*i.e.* fresh) since air exposure of this solution will result in carbon dioxide absorption, which will change its pH.
3. Then, 200 l of freshly prepared sodium tetraborate labeling buffer (0.1 M, 1 ml, pH 8.5) containing APEC (1.12 mg, 2.07 µmol) - initially dissolved in anhydrous DMF - was added to the Alexa Fluor 532 solution.
4. The reaction mixture was protected from light and after stirring for 18 h at 4 °C, the mixture was diluted with H_2O (600 µl) and purification was performed by HPLC with a Luna 5 µm RP-C18(2) semipreparative column under the following conditions: flow rate of 2 ml/min; 10 mM triethylammonium acetate (TEAA)-CH_3CN from 100:0 (v/v) to 70:30 (v/v) in 30 min.
5. An homogeneous product corresponding to the MRS5424 was isolated in the triethylammonium salt form with an HPLC retention time of 13.5 min.

6. Analytical purity of this conjugate was checked using a Hewlett-Packard 1100 HPLC equipped with a Zorbax SB-Aq 5 µm analytical column. Mobile phase: linear gradient solvent system: 5 mM TBAP from 80:20 to 40:60 in 13 min; the flow rate was 0.5 ml/min (retention time 9.08 min).
7. Peaks were detected by UV absorption with a diode array detector at 254, 275, and 280 nm, and the yield of MRS5424 was 0.67 mg (31%). ESI-HRMS m/z 1150.4142 $[M + H]^+$, $C_{55}H_{63}N_{11}O_{13}S_2 \cdot H^+$: Calcd. 1150.4127.
8. Finally, in order to check the fluorescence features of the MRS5424 the excitation/emission spectrum was assessed by means of a POLARstar Optima plate-reader.

Acknowledgments

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References

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