Supplementary Information

Mass spectrometry-based ligand binding assays on adenosine A_1 and A_{2A} receptors

Purinergic Signalling

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Synthesis of deuterated internal standards [^{2}H]DPCPX and [^{2}H]ZM-241,385

Results

*Synthesis of [$^2H_4$]DPCPX **2***

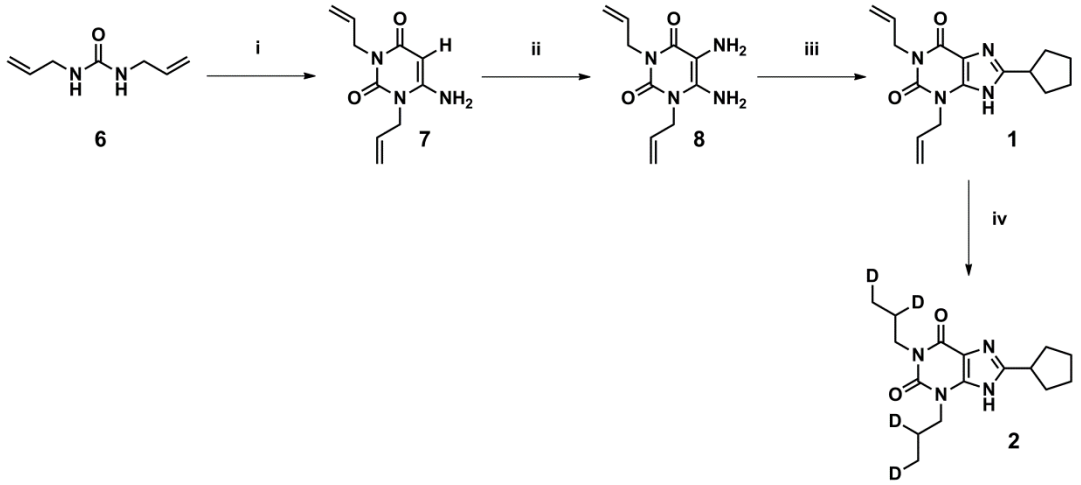
[$^2H_4$]DPCPX **2** was prepared according to the synthetic route shown in Scheme 3 and was adopted from previously described syntheses of non-deuterated DPCPX. Starting from the commercially available diallyl urea **6**, 1,3-diallyl-6-aminouracil **7** was synthesized by condensation with cyanoacetic acid and subsequent base promoted ring closure [1]. **7** was then nitrosylated on the 5-position and subsequently reduced to the diamine **8** [2]. Cyclization of **8** with cyclopentanecarbonyl chloride gave the [$^2H_4$]DPCPX precursor **1** [3]. Finally, the allylic double bonds of compound **1** were reductively deuterated in the presence of Wilkinson's catalyst with $NaBD_4$ as a deuterium source generating deuterium gas *in situ* upon addition of D_2O [7]. The mass spectrum showed a mass range for the ($M+H^+$) species from 305.20 ([$^2H_0$] isotopologue) to 313.27 ([$^2H_8$] isotopologue) in a Gaussian distribution with the desired [$^2H_4$]DPCPX **2** as most abundant isotopologue generating the main mass peak at 309.33**.**

*Synthesis of [$^2H_4$]ZM-241,385 **5***

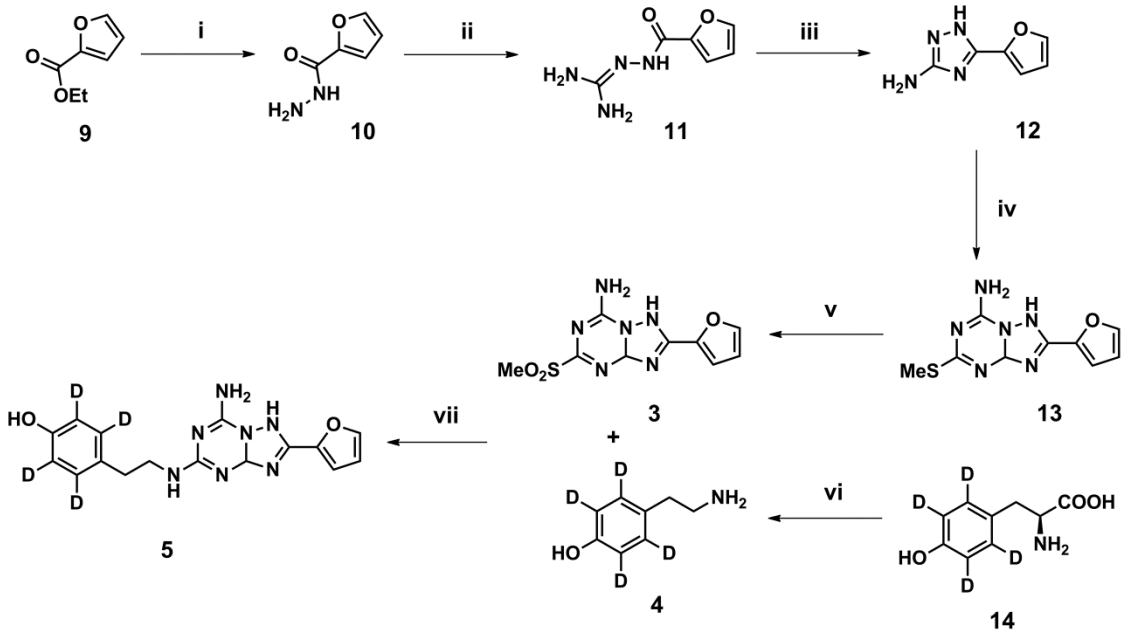
[$^2H_4$]ZM-241,385 **5** was prepared according to the synthetic route shown in Scheme 4 and was adopted from previously described syntheses of non-deuterated ZM-241,385. Commercially available ethyl furan-2-carboxylate **9** was reacted with hydrazine hydrate to give furan-2-carbohydrazide **10.** Through a substitution reaction of **10** with methyl isothiourea hemisulfate diamide **11** was obtained, which underwent a cyclization in water to give the 1,2,4-triazole **12**. In a subsequent cyclization of **12** with dimethyl cyanocarbonimidothioate the triazolotriazine **13** was obtained [4] and the corresponding methylsulfone **3** was prepared by oxidation of the methylthioether with *m*CPBA [5]. For the synthesis of the deuterated building block, [$^2H_4$]tyrosine **14** was enzymatically decarboxylated using tyrosine decarboxylase and pyridoxal-5-phosphate as cofactor to give [$^2H_4$]tyramine **4** [6]. Finally, reaction of the deuterated tyramine **4** with methylsulfone compound **3** yielded the final product [$^2H_4$]ZM-241,385 **5** [4]. MS analysis showed a mass of 342.7 ($M+H^+$) and confirmed the incorporation of 4 deuterium atoms in the final product.

Schemes

Scheme 3 Synthesis of [$^2H_4$]DPCPX (**2**). Reagents and conditions: *i* 1. Cyanoacetic acid, Ac_2O, MW, 80 °C, 4 h, 2. EtOH, NaOH 2M; *ii* 1. $NaNO_2$, 42% AcOH, rt, 1 h, 2. $Na_2S_2O_4$, EtOAc/H_2O = 2/1, rt, 0.5 h, *iii* 1. Cyclopentanecarbonyl chloride, pyridine, dry CH_2Cl_2, N_2-atm, 5 °C to rt, 2 h, 2. NaOH 3M, reflux, 1 h; *iv* $Rh(PPh_3)_3Cl$, $NaBD_4$, D_2O, dry THF, 60 °C, 3.5 h



Scheme 4 Synthesis of [$^2H_4$]ZM-241,385 (**5**). Reagents and conditions: *i* Hydrazine hydrate, EtOH, reflux, 18 h; *ii* Methyl isothiourea hemisulfate, 1% NaOH, rt, 23 h; *iii* H_2O, MW, 160 °C, 1.5 h; *iv* Dimethyl cyanocarbonimidodithioate, 180 °C, 16 h; *v* *m*CPBA, CH_2Cl_2, −5 °C to rt, 18 h; *vi* Tyr-decarboxylase, pyridoxal-5-phosphate, acetate buffer 0.1 M (pH 5.5), H_2O, 37 °C, 17 h; *vii* Et_3N, MeCN, MW, 70 °C, 3 h



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