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Synthesis of the Main Metabolite in Human Blood of the A_1 Adenosine Receptor Ligand $[^{18}F]$CPFPX

Marcus H. Holschbach,*,† Dirk Bier,† Walter Wutz,† Sabine Willbold,‡ and Ray A. Olsson†

Institute of Neuroscience and Medicine (INM-5) and Central Division of Analytical Chemistry, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany

m.holschbach@fz-juelich.de

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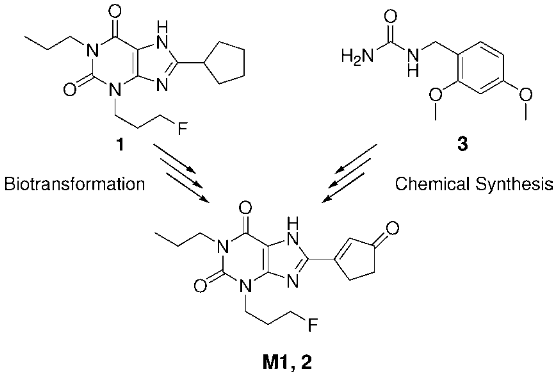
ABSTRACT



In human blood, the PET radiotracer $[^{18}F]$CPFPX (1) is metabolized to numerous metabolites, one (M1) being the most prominent in plasma 30 min p.i. Because the mass of injected tracer is ≤5 nmol, concentrations in plasma are too low to analyze. Human liver microsomes generate main metabolites having HPLC retention times identical to those in plasma. HPLC−MS tentatively identified M1 as 2. Synthesis of 2 and identical HPLC−MS spectra of 2 and M1 confirmed that assignment.

The radiofluorinated isotopologue of the potent and selective A_1 adenosine receptor antagonist (A_1AR antagonist) 8-cyclopentyl-3-(3-fluoropropyl)-1-propylxanthine (CPFPX, **1**), namely $[^{18}F]$8-cyclopentyl-3-(3-fluoropropyl)-1-propylxanthine ($[^{18}F]$CPFPX, **1***),[1] is used as a radioligand to image the A_1AR in human brain by positron emission tomography (PET).[2] Because the brain does not metabolize this ligand and metabolites formed in the periphery do not cross the blood−brain−barrier,[3] specifically the bound ligand accounts for a large fraction of brain tissue radioactivity. However, such is not the case for ligand in the periphery, where it undergoes rapid conversion to a number of more polar metabolites in quantities that obscure specific binding.[3] Using the ligand in humans for clinical and research studies[4−10] and with regard to future research focusing on the develop-

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[†] Institute of Neuroscience and Medicine.

[‡] Central Division of Analytical Chemistry.

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ment of metabolically more stable ligands with improved bioavailability, information about its metabolism is required.

Because the dose of radiotracer administered to humans is so small, typically 0.5−5 nmol, it is extremely difficult to characterize the metabolites in plasma even by LC−MS. However, in vitro metabolism by human liver microsomes (HLM)[12] and recombinant human CYP1A2 (hCYP1A2) seem reasonable alternatives for generating quantities of metabolites sufficient for analyzing their structures.

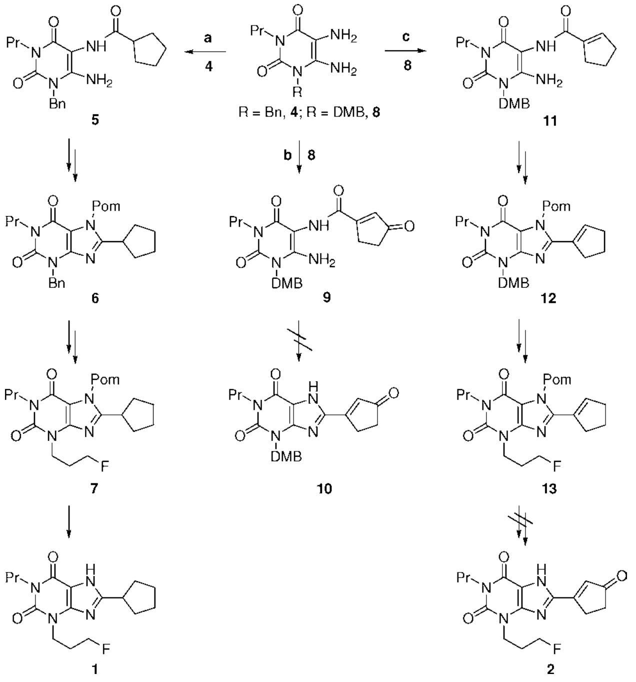
In a recent study,[11] radio-TLC of extracts of plasma from humans given [^{18}F]CPFPX identified nine radiolabeled metabolites. Extracts of reactions catalyzed by HLM contained four of those metabolites and extracts of hCYP1A2 reactions all nine. Thus, like naturally occurring xanthines such as caffeine (1,3,7-trimethylxanthine) and theophylline (1,3-dimethylxanthine), CPFPX undergoes oxidation in liver microsomes mainly by CYP1A2; however, unlike the natural xanthines, dealkylation does not occur. That study[12] used LC−MS to analyze the metabolites generated by HLM; the m/z of the major metabolite, **M1**, was 335 $[M + H]^+$, consistent with an enone species. Cone voltage induced in-source dissociation analysis suggested that **M1** was 3-(3-fluoropropyl)-8-(3-oxocyclopent-1-enyl)-1-propylxanthine (**2**).

It is not surprising that the cyclopentyl moiety should be an important site of metabolism. As pointed out previously,[11] molecular modeling of the interaction of CPFPX with the catalytic site of CYP1A2 places the cyclopentane moiety of CPFPX in close proximity to the enzyme's heme prosthetic group.

It is well known that during metabolism a cyclopentyl moiety can easily be hydroxylated and subsequently oxidized to the respective ketone.[13−15] To the best of our knowledge, a double hydroxylation−dehydration process as postulated for the metabolic transformation of the cyclopentyl moiety of [^{18}F]CPFPX has not yet been described in the literature. The fact that this metabolic step is favored in the case of [^{18}F]CPFPX is most probably due to the generation of a very stable conjugated double bond system resulting from the dehydration of an initially formed ring-hydroxylated species to furnish a cycloalkene which is subsequently hydroxylated at a carbon atom adjacent to the double bond (α-oxidation) followed by dehydrogenation to yield an enone. It is noteworthy that metabolite **M1** is an intensely fluorescent molecule[11] for which a large planar π-electron system is a prerequisite. The conjugation of the double bonds in the imidazole heterocycle with the cyclopentene double bond and an additional carbonyl function in the cyclopentenyl moiety is a structural feature common to a group of well-known fluorescent organic polymethine dyes, the merocyanines.[17,18]

Once LC−MS tentatively identified 8-(3-oxocyclopent-1-enyl)-3-(3-fluoropropyl)-1-propylxanthine (**2**) as the likeliest structure of **M1** it was necessary to synthesize it as a proof of structure. A first synthetic approach was based on the synthesis of the parent ligand CPFPX (**1**)[19] (path **a** in Scheme 1, **4** → **1**) by substituting the original cyclopentyl ring by a cyclopentenyl moiety. In the published synthesis,[19] a set of orthogonal protecting groups consisting of a benzyl (Bn) and a pivaloyloxymethyl (Pom) group was used for the regioselective incorporation of a 3-fluoropropyl substituent at N-3 of the xanthine heterocycle in a late synthetic step. Since unsaturation in the cycloalkenyl building block used in the envisioned

Scheme 1. Key Steps in the Synthesis of CPFPX **1** and Unsuccessful Approaches to **2**[a]



[a] For details, see the text.

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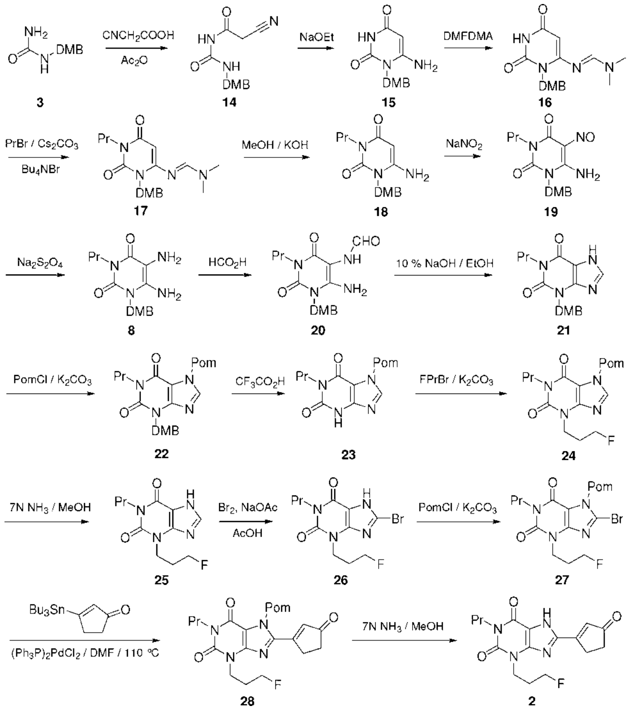
synthesis was not compatible with hydrogenation conditions necessary to remove an amide benzyl protecting group, an alternative protecting group was needed. It is well documented that introduction of methoxy substituents into the aromatic benzylic core leads (poly)methoxy benzyl derivatives with increased acid sensitivity.[20] Thus, a 4-methoxybenzyl (*p*-methoxybenzyl, PMB) amide can be efficiently cleaved under acidic (trifluoroacetic acid, TFA) conditions. Starting the synthesis with 1-PMB-6-aminouracil instead of 1-benzyl-6-aminouracil, it became obvious that during PMB cleavage (TFA, reflux overnight) concomitant hydrolysis of the Pom group on N-7 of the xanthine imidazole ring had occurred. However, protection with a more acid labile 2,4-dimethoxybenzyl (DMB) group[20] allowed the selective cleavage of the latter under much milder acidic conditions (TFA, 50 °C, 5 h) while leaving the Pom group completely unaffected. Having established an efficient combination of protecting groups, various ways to introduce a functionalized cyclopentane ring into CPFPX were examined. Condensation of the key intermediate 5,6-diamino-1-(2,4-dimethoxybenzyl)-3-propyluracil (**8** in Scheme 1) with 3-oxocyclopent-1-enecarboxylic acid[21] (path **b** in Scheme 1, **8** → **10**) under standard coupling reactions (DMF, EDAC, DMAP) gave the desired amide **9** in moderate (35−40%) yield, but subsequent ring closure either induced by aqueous base (LiOH, NaOH, Ca(OH)$_2$) or by heating with condensing agents (HMDS, BTSA) decomposed the amide and furnished only intractable mixtures. The next approach (path **c** in Scheme 1, **8** → **2**) envisioned the condensation of the DMB protected diaminouracil **8** with cyclopent-1-enecarboxylic acid, ring closure to the respective xanthine and subsequent oxidation of the carbon atom α to the double bond of the cyclopentene ring (allylic oxidation). The protected xanthine heterocycle **13** was obtained in a straightforward manner using the classical Traube purine synthesis[22] followed by Pom-protection of N-7 to give **12**, hydrolysis of the DMB moiety, and subsequent fluoropropylation of N-3 to yield **13**. Allylic oxidation of the cyclopentene ring was tried with a variety of oxidants and catalysts according to published methods[23−25] for the allylic oxidation of cyclopentene esters. Among the oxidizing systems investigated were CrO$_3$−acetic anhydride−acetic acid,[23] Pd(OH)$_2$−*tert*-butyl−hydroperoxide,[24] and Rh$_2$(cap)−*tert*-butyl hydroperoxide−K$_2$CO$_3$,[25] but all efforts to isolate even traces of the desired enone were unsuccessful.

Finally, a completely different synthetic strategy to compound **2** was followed (Scheme 2), namely a Pd-catalyzed Stille cross-coupling reaction[26] of a brominated xanthine with tri-*n*-butylstannyl-2-cyclopentene-1-one. This synthesis required several modifications to the approach used to synthesize CPFPX.[19] 1-(2,4-Dimethoxybenzyl)uracil **15** was prepared by condensation of 2,4-dimethoxybenzylurea **3** with cyanoacetic acid[22] followed by sodium ethanolate induced ring closure. The 6-amino-1-(2,4-dimethoxybenzyl)-3-propyluracil **18** was synthesized according to a modification of a known procedure[27] for the regioselective alkylation of the uracil N-3 position via protection of the amino group at the 6-position as the *N*-[(dimethylamino)methylene] derivative. Thus, reaction of **15** with dimethylformamide dimethyl acetal (DMF/DMA) in dimethyl formamide (DMF) at 40 °C yielded the 6-*N*-[(dimethylamino)methylene] derivative **16** in excellent yield. Phase-transfer-catalyzed (PTC) alkylation[28] of **16** with 1-bromopropane, CsCO$_3$, and tetrabutylammonium bromide as a catalyst in a mixed solvent system of DMF and acetonitrile gave the 3-*n*-propyl derivative **17**. Alkaline hydrolysis of the *N*-[(dimethylamino)-methylene] group using KOH in methanol furnished 6-amino-1-(2,4-dimethoxybenzyl)-3-propyluracil **18**.

Scheme 2. Synthesis of Compound **2**



Subsequent nitrosation of **18** at the C-5 position with NaNO$_2$ in aqueous acetic acid afforded the 5-nitroso derivative **19**. Reduction of the nitroso group with Na$_2$S$_2$O$_4$ in dilute aqueous ammonium hydroxide solution[19] gave 5,6-diamino-1-(2,4-dimethoxybenzyl)-3-propyluracil (**8**), and 3-(2,4-dimethoxy)benzyl-1-propylxanthine (**21**) was obtained by

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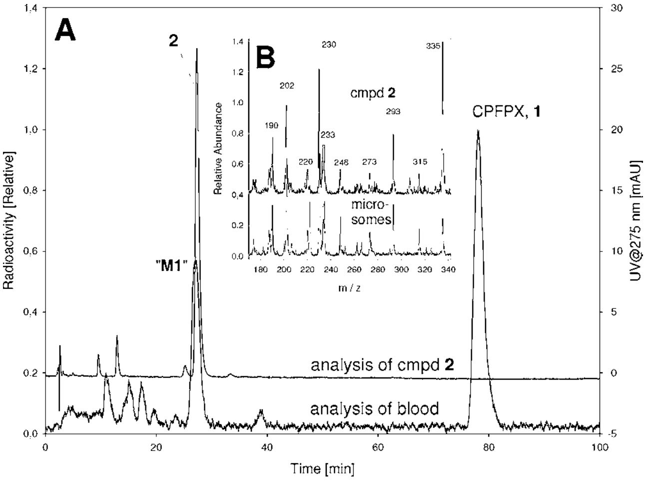

Figure 1. (A) HPLC analysis of the metabolites of [^{18}F]CPFPX in human plasma 30 min postinjection (bottom trace) and of synthetic compound **2** (top trace). (B) ESI-MS "in-source" fragmentation spectra of **M1** generated by HLM (bottom trace) and of synthetic compound **2** (top trace).

condensing the diaminouracil **8** with formic acid[29] to afford carboxamide **20**, which was not isolated but directly subjected to alkaline ring closure. Alkylation with pivaloyloxymethyl chloride (Pom-Cl) protected N-7 of xanthine **21** to give **22**, which then underwent debenzylation by treatment with neat trifluoroacetic acid to form **23**. Subsequent alkylation of N-3 by 1-bromo 3-fluoropropane gave **24**. Attempts to brominate compound **24** at the 8-position under a variety of conditions were unsuccessful, most probably due to the sterically hindering and electron-withdrawing Pom group that prevented electrophilic bromination of C-8. Thus, the Pom group was removed by alkaline hydrolysis yielding 3-(3-fluoropropyl)-1-propylxanthine **25**, which was brominated with bromine and sodium acetate in acetic acid to generate bromoxanthine **26** in excellent yield. All attempts to perform a Pd-catalyzed Stille cross-coupling between compound **26** and tri-*n*-butylstannyl-2-cyclopenten-1-one, prepared from 3-ethoxy-2-cyclopenten-1-one,[30] were unsuccessful. We assumed that this was due to interferences of the organostannane with the free imidazole NH function of the xanthine core. A similar observation has been described[31] during the synthesis of 8-alkynyladenine analogues by the cross-coupling of 8-bromoadenine with alkynes.[31] These authors concluded that preparation of the 8-alkynyladenine analogues was difficult by direct coupling and suggested that efficient coupling requires protecting N-9. Accordingly, N-7 of xanthine **26** was reprotected with a Pom group, giving key intermediate **27**. Finally Pd-catalyzed Stille coupling of **27** with tri-*n*-butylstannyl-2-cyclopenten-1-one gave **28**, which, upon deprotection in methanolic ammonia, supplied compound **2**.

On HPLC the retention times and LC−MS fragmentation spectra of **M1** and **2** coincided exactly (parts A and B of Figure 1, respectively).

Experiments examining the metabolism of **M1** by HLM showed little or no consumption of this substrate after 4 h. This was demonstrated in control experiments ($n = 3$) by comparison of the areas of the respective **M1**-UV signals at $t = 0$ with those obtained at $t = 4$ h (using Student's t test the null hypothesis is significant with $p = 0.01$). Under identical conditions, the consumption of CPFPX used as a control was ∼90%. Thus, **M1** appears to be at best a very poor substrate for further metabolism by HLM. This resistance of **M1** to the most important enzymes of phase I metabolism, together with its relatively high lipophilicity (LogP_{calc} = 1.45, calculated with "Marvin" from http://www.chemaxon.com), which would slow its elimination from plasma more than those of more polar metabolites (e.g., log P_{calc} values for monohydroxylated cyclopentyl derivatives range from 0.4 to 0.6)), might account for why **M1** is the major metabolite in plasma.

Supporting Information Available: Detailed descriptions of experimental procedures and spectroscopic data for compounds **2**, **3**, **8**, and **14**−**28**.This material is available free of charge via the Internet at http://pubs.acs.org.

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