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Synthesis and Pharmacological Evaluation of Identified and Putative Metabolites of the A1 Adenosine Receptor Antagonist Cyclopentyl-3-(3-fluoropropyl)-1-propylxanthine (CPFPX)

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Dedicated to Professor Dr. Ray A. Olsson, a good friend and excellent teacher, in acknowledgement of his contributions to purine chemistry

Abstract

The A_1 adenosine receptor (A_1AR) antagonist [^{18}F]cyclopentyl-3-(3-fluoropropyl)-1-propylxanthine ([^{18}F]CPFPX), used in imaging human brain A_1ARs by PET, is stable in the brain but rapidly undergoes transformation into one major (M1) and several minor metabolites in blood. This report describes the synthesis of putative metabolites of CPFPX as standards for the identification of those metabolites.

Analysis by (radio)HPLC revealed that extracts of human liver microsomes incubated with n.c.a.[^{18}F]CPFPX contained the major metabolite, M1, as well as radioactive metabolites corresponding to derivatives functionalized at the cyclopentyl moiety, but no N^1-despropyl species or metabolites resulting from functionalizations of the N^3-fluoropropyl chain.

The putative metabolites displaced the binding of [^{3}H]CPFPX to the A_1AR in pig brain cortex at K_is between 1.9 and 380 nM and the binding of [^{3}H]ZM 241385 to the A_{2A}AR in pig striatum at K_is greater than 180 nM. One metabolite, a derivative functionalized at the ω-position of the N^1-propyl chain, showed high affinity (K_i 2 nM) to and very good selectivity (> 9000) for the A_1AR.

Keywords: A_1 adenosine receptor ligand, [^{18}F]CPFPX, *in-vitro* metabolism, binding properties, ligand design

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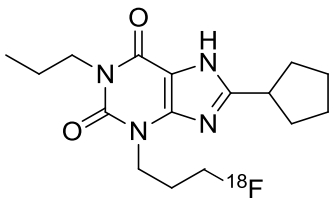
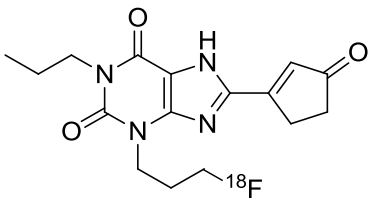
1. Introduction

The radiofluorinated A_1 adenosine receptor (A_1AR) antagonist [^{18}F]8-cyclopentyl-3-(3-fluoropropyl)-1-propylxanthine ([^{18}F]CPFPX) (chart) is the lead diagnostic agent to image that receptor in human brain by means of positron emission tomography (PET).[1,2] Although [^{18}F]CPFPX is not metabolized in the brain, it rapidly undergoes metabolism in the periphery to one main metabolite, **M1** (chart), and a number of more polar metabolites.[3,4]

Chart Structures of radiofluorinated CPFPX and the main metabolite **M1**.



[^{18}F]CPFPX



M1

The rate of metabolism (metabolic stability) of a drug indicates the need for structural modifications to reduce metabolism. Metabolite identification followed by detection of soft spots provides information on the sites to be modified or blocked and thus indicates possible chemical modifications or even new scaffolds. Overall, the information obtained serves as a basis for taking key decisions in the developmental phase of a ligand.

The aim of the present work was to synthesize and evaluate identified and putative CPFPX-metabolites with regard to the development of metabolically stabilized second generation (follow up) ligands. Thus, in the present study putative oxidative (phase I) metabolites of CPFPX were synthesized to provide reference standards for the studies directed toward elucidating the structures of the authentic drug metabolites found in human liver microsome preparations. Pharmacological evaluation of this putative metabolite family was also performed to determine receptor binding affinity and subtype selectivity in comparison to the parent compound.

Since [^{18}F]CPFPX for human applications is prepared under no-carrier-added (n.c.a.) conditions, the amounts of [^{19}F]CPFPX administered are in the low-nanogram range, thus making direct identification of the radioactive metabolites by LC-MS from human blood samples impossible. In recent work it has been shown that human liver microsomes are a convenient and reliable model for the Phase I metabolism of CPFPX.[4]

A comparison by HPLC of extracts from human plasma obtained from blood samples drawn 30 min post tracer injection with extracts from human liver microsomes incubated with unlabelled CPFPX for 30 min showed that the microsome extracts contained compounds having chromatographic behavior similar to all of the radioactive metabolites found in human plasma. LC-MS showed that they had m / z ratios of hydroxy-, oxo-, and unsaturated derivatives but did not allow unambiguous structure determination of the metabolites.[4] The compounds described in the present work should allow to bridge that gap. In liver microsomes, CPFPX was shown to undergo monohydroxylations on three positions of the cyclopentane ring. The synthesized reference compounds should give detailed information about the alcohol regioisomers formed *in vitro*.

Cytochrome P450-mediated oxidation in the liver is the main route of alkylxanthine metabolism,[5] and, presumably, that of CPFPX as well. Cytochrome P450-mediated oxidations of sp^3 carbons such as those in the propyl and cyclopentyl groups of CPFPX have been described in detail.[6-8] Those consist of hydroxylations and oxidations of methyl and methylene groups except those adjacent (alpha) to heteroatoms such as *N*, *O* or *S*. In the latter instance the initially formed hydroxylation product is unstable and the alkyl group is eliminated by hydrolytic cleavage to form the desalkyl xanthine. According to these rules, there are a number of plausible metabolites of CPFPX. Table 1 shows some possible functionalization positions and -products. Besides the already identified metabolites many of these are the targets of the syntheses described below.

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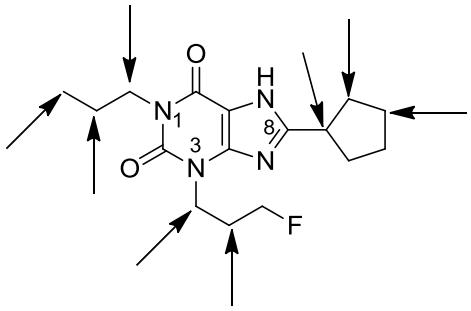
Table 1. Possible CYP 450 functionalization products of CPFPX



m / z $[M+H]^+$	Species	Represents
species found in human blood		
323	CPFPX	intact ligand
339	CPFPX-OH	sp^3-carbon hydroxylation
337	CPFPX=O	oxygenation
323 – 2 x n	unsaturated species	dehydrogenation
species not found human blood		
354	CPFPX-COOH	oxidation of $-CH_3$
281	CPFPX-despropyl	dealkylation of N^1-propyl
262	CPFPX-desfluoropropyl	dealkylation of N^3-fluoropropyl
323 + 16 x n	poly-OH species	multiple hydroxylation
323 + 14 x n	poly=O species	multiple oxygenation

Since all the metabolites found in human blood contain radiofluorine and because there is no significant radiofluorine deposition in bone, it appears that the N^3-fluoropropyl moiety of [^{18}F]CPFPX resists cleavage and further metabolism. The only carbon in the fluoropropyl chain prone to functionalization is carbon C^2 (beta to fluorine) to give the respective β-hydroxy / oxo derivative, which LC-MS did not detect. Accordingly, any stable metabolites must contain alterations of the N^1-propyl and / or C^8-cyclopentyl substituents. Analysis by LC-MS indicated that these metabolites are exclusively Phase I but not Phase II metabolites, that is, they reflect hydroxylation and oxidation but not conjugation.

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With regard to the development of new potent A_1AR selective radioligands and with respect to possible fine tuning of their lipophilicities the synthesized target compounds should be useful for further development.

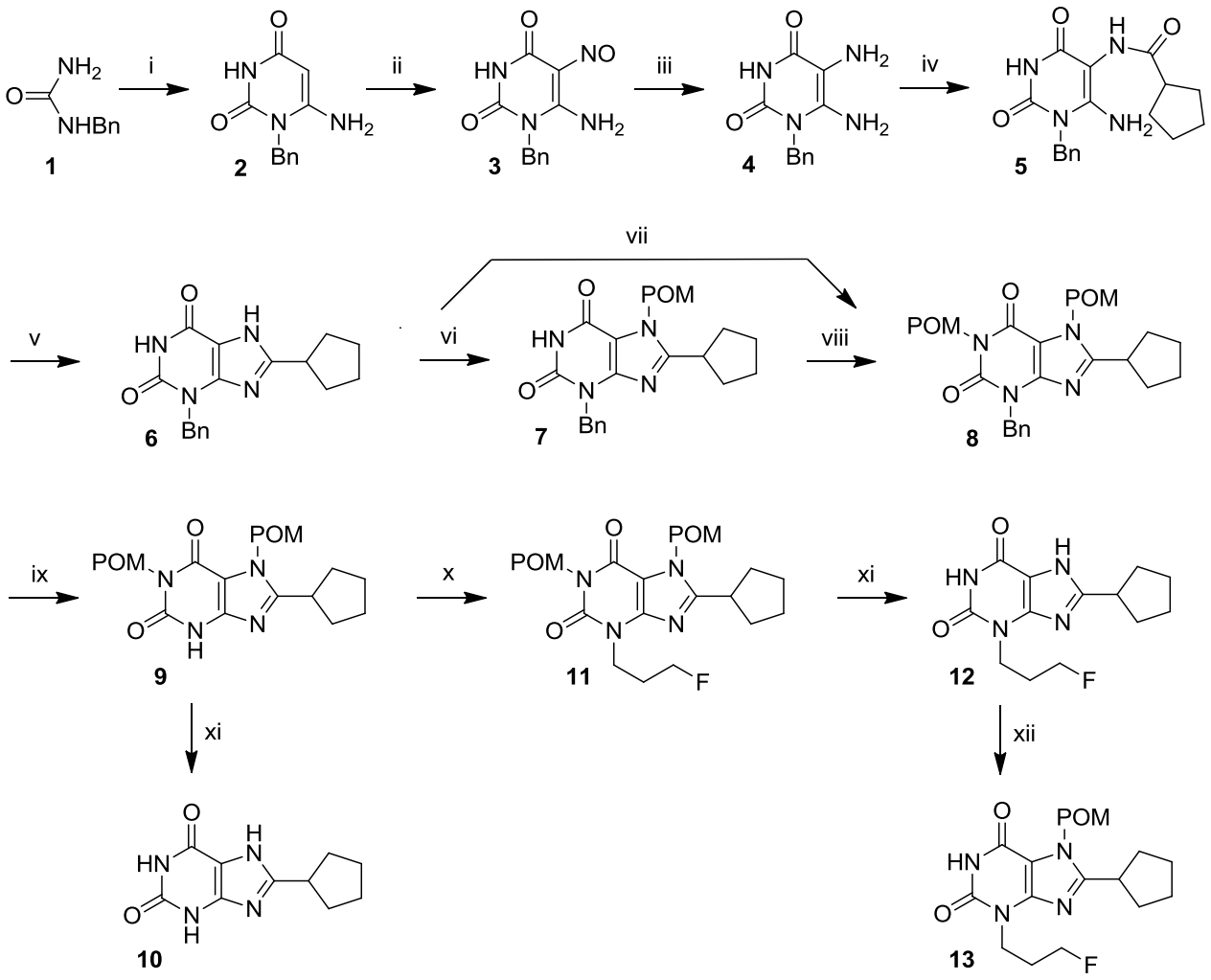
2. Results and discussion

2.1. Chemistry

Earlier work using extracts from human liver microsome preparations incubated with unlabelled CPFPX had already identified CPFPX metabolites by HPLC and LC-MS analysis.[4] Scheme 1 outlines the synthesis of 8-cyclopentyl-3-(3-fluoropropyl)-7-pivaloyloxymethylxanthine, **13**, the key intermediate for the synthesis of 1-substituted 8-cyclopentyl-3-(3-fluoropropyl)xanthines. This synthesis exploited the different reactivities of xanthine N^1, N^3, and N^7 toward alkylation, which is $3 \approx 7 >> 1$. The first stages of the synthesis prepared 3-benzyl-8-cyclopentylxanthine, **6**, in several steps from benzylurea, **1**, and a cyanoacetic ester.[9-11] The synthesis of **6** differed in some details from the approach used earlier.[12] The condensation of benzylurea with methyl cyanoacetate in sodium methoxide rather than with cyanoacetic acid in acetic anhydride cyclized the cyanoacetylurea in one step rather than requiring a separate cyclization in alkali. Nitrosation of 6-amino-1-benzyluracil **2** used isoamyl nitrite rather than $NaNO_2$ and the acylation of 5,6-diaminouracil **4** employed cyclopentane carboxylic acid, a water soluble carbodiimide (EDCI) and 4-dimethylaminopyridine (DMAP) as a catalyst rather than cyclopentane carbonyl chloride and Et_3N. Collectively, these modifications improved the overall yield of **6** to 40 %. Alkylation of **6** with one equivalent of chloromethyl pivalate (POM-Cl) for 3 hours protected N^7 by regioselectively forming the 7-pivaloyloxymethyl (POM) derivative, **7**. Since this reaction is apparently without literature precedent, it was carried out primarily to confirm the preferential alkylation of N^7 over N^1. As expected, using a 3-fold excess of POM-Cl and prolonging the alkylation of **7** for additional 5 hours produced the 1,7-bis-POM derivative, **8**. Alternatively, alkylation of **6** overnight with 3.5 equivalents of POM-Cl gave **8** in one step. Debenzylation by catalytic transfer hydrogenation (CTH) over Pd-C using ammonium formate as the hydrogen donor generated the 1,7-bis-POM, 3-unsubstituted xanthine **9**.[13] Cleavage of both POM groups gave 8-cyclopentylxanthine, **10**. The regioselective introduction of a 3-fluoropropyl group through alkylation of **9** with 1-bromo-3-fluoropropane formed the protected 3-(3-fluoropropyl)xanthine, **11**. Alkaline hydrolysis removed the POM groups, giving one of the putative metabolites, 8-

cyclopentyl-3-(3-fluoropropyl)xanthine, **12**. A short-term alkylation with POM-Cl protected N^7 of **12** to give key intermediate **13**.

Scheme 1 Synthesis of compound **10** and key intermediate **13**



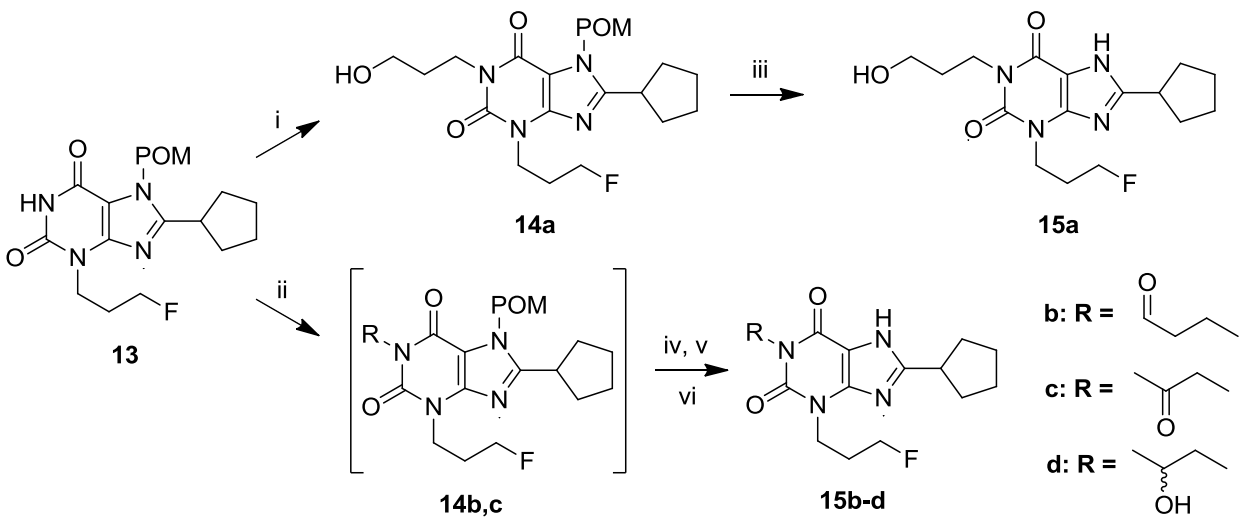
Abbreviations: Bn, benzyl; POM, pivaloyloxymethyl

i: NaOMe, $NCCH_2CO_2Me$, AcOH; ii: isoamyl nitrite; iii: $Na_2S_2O_4$, NH_4OH; iv: $cPCO_2H$, EDCI, DMAP, DMF; v: 2N NaOH, reflux; vi: 1eq POMCl, K_2CO_3, DMF; vii, viii: 3.5 eq POMCl, K_2CO_3, DMF; ix: NH_4HCO_2, Pd / C, MeOH, reflux; x: 1-bromo-3-fluoropropane, K_2CO_3, DMF; xi: NaOH / EtOH; xii: 1eq POMCl, K_2CO_3, DMF.

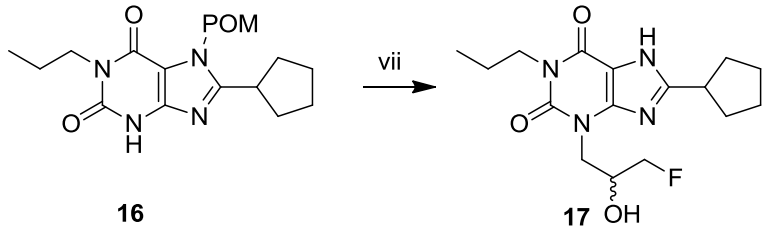
Scheme 2 summarizes the syntheses of the target xanthines. Alkylation of **13** with 3-bromo-1-propanol gave the 1-(3-hydroxypropyl)xanthine **14a** and, after deprotection, the 7-H xanthine, **15a**. Alkylation of **13** with 2-(2-bromoethyl)-1,3-dioxolane and double deprotection gave the 1-

(3-oxopropyl)xanthine **15b**. Alkylation of **13** with chloroacetone and deprotection of the product afforded 1-(2-oxopropyl)xanthine **15c**. Alkylation with chloroacetone followed by reduction with $NaBH_4$ and deprotection without isolation of intermediates yielded the 1-(2-hydroxypropyl)-7H-xanthine, **15d**, as a mixture of the *R* and *S* enantiomers. Alkylation of 1-propyl-8-cyclopentyl-7-pivaloylmethylxanthine **16** with epifluorohydrin in the presence of a catalytic amount of base gave, after deprotection, racemic 3-(3-fluoro-2-hydroxypropyl)-1-propylxanthine, **17**.[14,15]

Scheme 2 Synthesis of putative metabolites functionalized at the *N*-1 and *N*-3 propyl chains



i: K_2CO_3, DMF, 3-bromopropanol, ii: 2-(2-bromoethyl)-1,3-dioxolane (**14b**), chloroacetone (**14c**); iii: NH_3 / MeOH; iv: NaOH / EtOH (**14c** to **15c**); v: 1) NaOH / EtOH, 2) HCl, reflux (**14b** to **15b**); vi: 1) $NaBH_4$ / MeOH, 2) NaOH / EtOH (**14c** to **15d**)



vii: 1) cat K_2CO_3, DMF, epifluorohydrin, 2) NaOH / EtOH;

Scheme 3 depicts the synthesis of the xanthines modified at the cyclopentyl ring. Since the presence of double bonds in the cyclopentenyl moieties and an *O*-benzyl protecting group in the cyclopentyl building block used in the synthesis of compounds **24a-c** was incompatible with the

hydrogenation conditions necessary to remove an amide benzyl protecting group, the acid labile 2,4-dimethoxybenzyl (DMB) protecting group was alternatively installed.[16] Thus, carbodiimide / DMAP induced condensation of the key intermediate 5,6-diamino-1-(2,4-dimethoxybenzyl)-3-propyluracil **18** with with either cyclopent-2-enecarboxylic acid or the isomeric cyclopent-3-enecarboxylic acid followed by alkali induced ring closure of amides **19a** and **19b** as described for the synthesis of **6** furnished xanthines **20a** and **20b**.[16] Xanthine **20c** was obtained by the reaction of diamine **18** with *cis*-2-benzyloxycyclopentane-1-carboxylic acid using the mixed anhydride method followed by alkali induced cyclization of the intermediate amide **19c**. *Cis*-2-benzyloxycyclopentane-1-carboxylic acid was synthesized by benzylation of commercially available *cis*-ethyl 2-hydroxyoxycyclopentane-1-carboxylate with benzyl-2,2,2-trichloroacetimidate followed by ester hydrolysis.[17]

POM protection of N^7 in xanthines **20a-c** yielded compounds **21a-c** which were subsequently deprotected at N^3 by hydrolysis of the DMB groups with TFA to provide **22a-c**. Alkylation with 1-bromo-3-fluoropropane furnished derivatives **23a-c** which upon alkaline hydrolysis of the POM protecting groups at N^7 gave the unsaturated target compounds **24a** and **24b** and the benzyl ether **24c**. Alcohol **25** was synthesized by hydroboration of cyclopentene derivative **24a** with 9-BBN,[18] followed by oxidation with hydrogen peroxide, as a mixture of the *cis* and *trans* isomers. The regioisomeric *cis*-alcohol **26** was obtained by methanesulfonic acid induced debenzylation of **24c**.

Scheme 3 Synthesis of putative metabolites functionalized at the cyclopentyl ring

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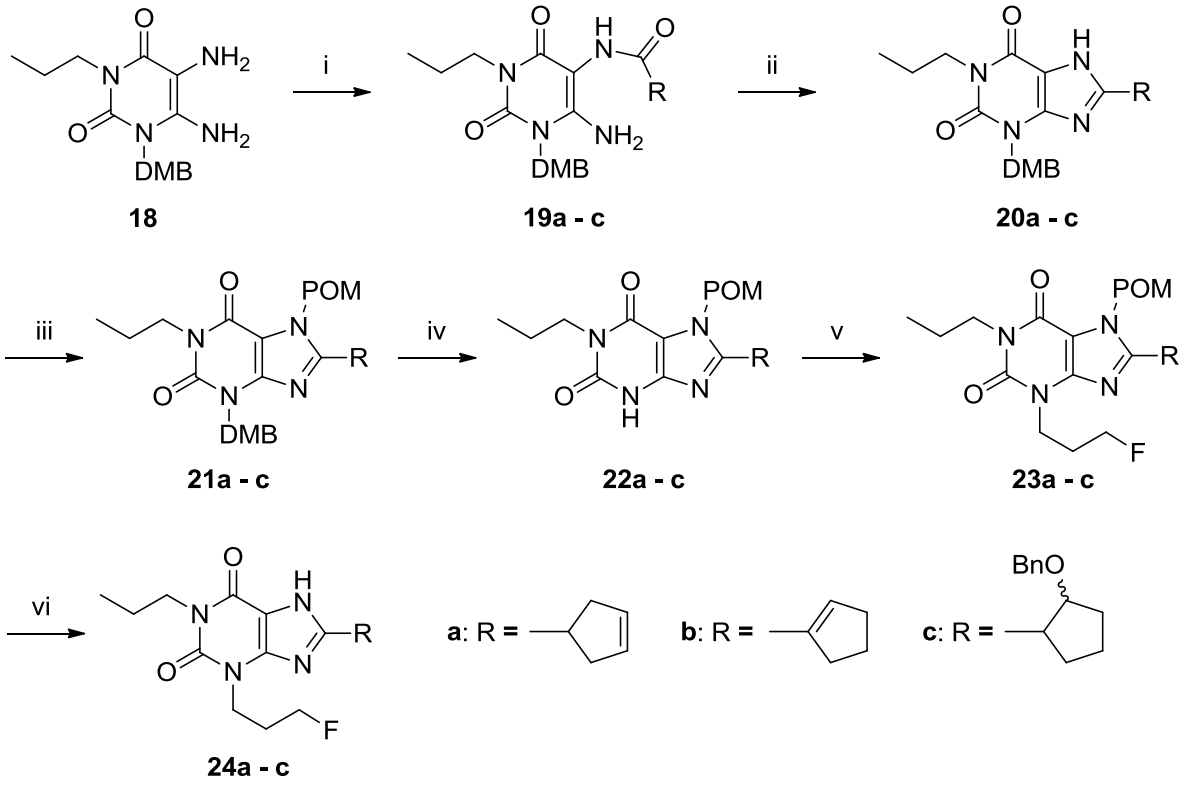
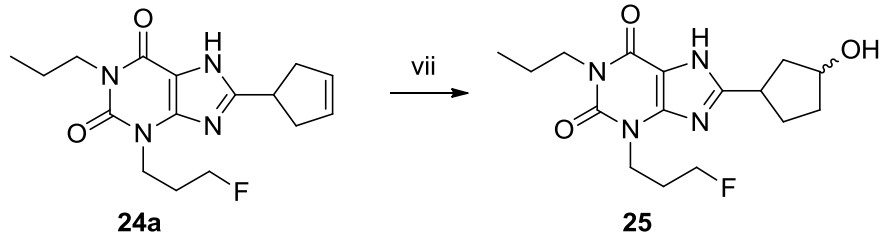


18 **19a - c** **20a - c**

21a - c **22a - c** **23a - c**

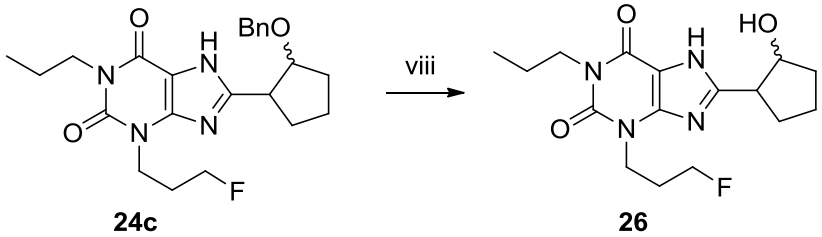
24a - c

i: RCO_2H, EDCI, DMAP (R = a, b), isobutyl chloroformate/NMM (R = c); ii: 2N NaOH, dioxane, reflux; iii: POMCl, K_2CO_3, DMF; iv: TFA, 70°C; v: 1-bromo-3-fluoropropane, K_2CO_3, DMF; vi: NH_3 / MeOH.



24a **25**

vii: 1) 4 eq 9-BBN, 2) H_2O_2, NaOH.



24c **26**

viii: $MeSO_3H$, DCM, 25°C.

Abbreviations: Bn, benzyl; DMB, 2,4-dimethoxybenzyl; POM, pivaloyloxymethyl

Analysis of the xanthines by electrospray ionization-mass spectroscopy detected, in addition to the $[M + H]^+$ species, a second species of *m / z* 60 units greater. This appears to be an adduct of the $[M + H]^+$ species with acetic acid, added to the solvent to increase sensitivity. All the xanthines but not their precursors formed these adducts, whether or not N^1 and N^7 were protected.

2.2. Human liver microsomes

Human liver microsome stability is meanwhile a standard indicator for predicting *in vivo* pharmacokinetic parameters of a compound.[19,20] To generate a chromatographic profile of [^{18}F]CPFPX metabolites formed *in vitro*, human liver microsomes were incubated with n.c.a.[^{18}F]CPFPX and analyzed by (radio)HPLC. The figure shows superimposed chromatograms from the incubation of n.c.a.[^{18}F]CPFPX with human liver microsomes (red radioactive trace) and the same sample spiked with the potential metabolites (blue UV trace). Due to the minute amounts of non radioactive CPFPX present in [^{18}F]CPFPX preparations of low activity (± 500 KBq) the radiolabeled isotopomer and its metabolites are not visible with UV detection at 275 nm. Thus, simultaneous chromatography of the non radioactive and radioactive components allows the direct and unambigous comparison between the synthesized compounds and the *in vitro* formed metabolites in a single HPLC run.

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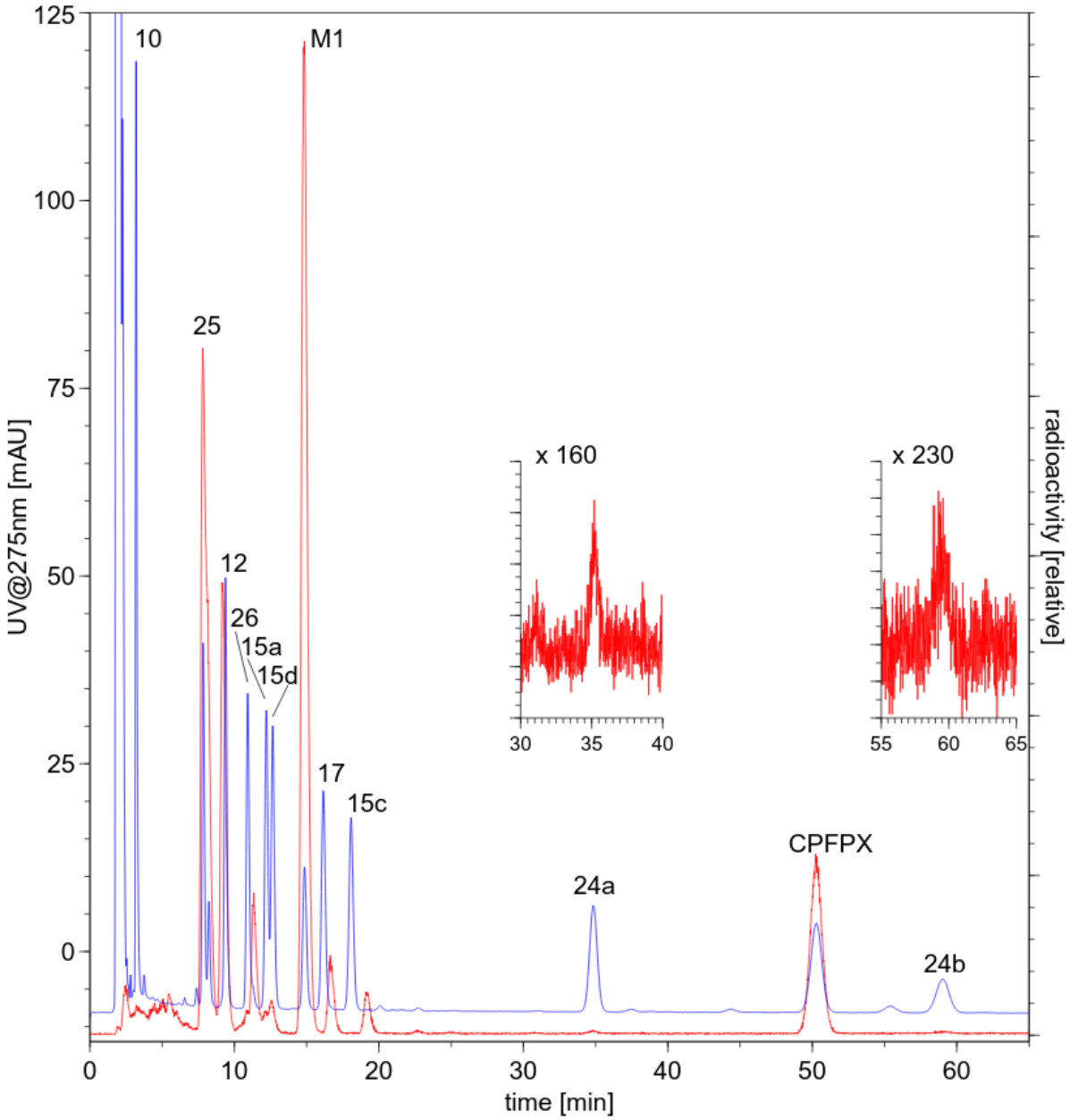
Figure (Radio)HPLC chromatogram of a human liver microsome preparation extract after metabolization of [^{18}F]CPFPX. The extract was subsequently spiked with a mixture of the non-radioactive putative metabolites **12**, **15a-d**, **17**, **24a-b**, **25** and **26** and analyzed in a single run. Blue: UV trace, red: radioactive trace.



2.2.1. Derivatives formed through multiple functionalizations

The most prominent metabolite belonging to this class has already been described earlier.[21] It is the main metabolite of [^{18}F]CPFPX in human blood, **M1**, most likely arising from dehydrogenation, double allylic oxygenation and loss of water from the intermediately formed geminal diol (ketone hydrate) resulting in a highly conjugated double bond system.

2.2.2. Unsaturated derivatives

Representatives of unsaturated metabolites containing double bonds in the alicyclic cyclopentane ring are compounds **24a** and **24b**. Earlier MS investigations [4] had identified these metabolites with an exact mass of 322 $[M+H]^+$, which is 2 Da lower than CPFPX. A decrease of two mass units corresponds to a dehydrogenation reaction.[22] The inserts in the figure indicate the presence of **24a** and **24b**, albeit to a small extent.

2.2.3. Dealkylated derivatives

Compounds **10** and **12** belong to this group of metabolites. In contrast to the naturally occurring methylxanthines where N-demethylation is the main metabolic pathway,[5] no metabolites originating from *N*-dealkylation of [^{18}F]CPFPX could be found. Moreover, since compound **10** does not contain fluorine-18, it would not be detectable in the radioactive channel. The absence of compound **12** makes the existence of compound **10** as well as that of the N^3-dealkylated congener most unlikely.

2.2.4. Oxidative derivatives

2.2.4.1. Alkyl chains

Metabolites functionalized at the N-1-propyl and/or at the N-3-fluoropropyl side chains of the xanthine core are compounds **15a-d** and **17**, respectively. Whereas the N-1-propyl derived metabolites **15a** and **15d** are formed, the putative metabolites **17**, originating from hydroxylation

of C^2 in the N^3-fluoropropylchain, and ketone **15c** are completely absent. These results are in agreement with earlier findings.[4] The observation that compound **17** is not detected might be exploited as a potential strategy in drug design to mask alkyl chain metabolic hotspots through incorporation of a fluorine atom at the terminal carbon (ω-fluorination).[23,24]

2.2.4.2. Cyclopentyl ring

The *in vitro* formation of oxidative metabolites corresponding to the functionalization of cyclopentyl groups has scarcely been described in the literature.[25,26]

In principle there are three chemically different carbon atoms in the cyclopentyl ring of CPFPX prone to monohydroxylation, namely C^1, $C^{2(5)}$, and $C^{3(4)}$. MS studies using extracts of microsome preparations incubated with unlabeled CPFPX have revealed the existence of three hydroxylated regioisomers.[4] Consequently ring-hydroxylated metabolites **25** and **26** have been synthesized. In the first instance one would expect a chromatographic match of the UV-HPLC peaks of **25** and **26** with those of the radioactive trace. However, only compound **25** matched with a radioactivity signal in the chromatogram in the figure. It is well known that stereoisomers are observed in certain disubstituted (and higher substituted) cyclic compounds. Thus, substituted cycloalkanes must be viewed as three-dimensional configurations in order to consider the spatial orientations of the substituents. In non planar ring systems like cyclopentane substituents can adapt equatorial and/or axial orientations resulting in configurational isomers (*cis*-*trans* isomers) which sometimes can be separated by chromatographic methods. The reaction sequence for the synthesis of compound **25** from the cyclopent-3-en precursor **24a** (hydroboration-oxidation) yields a mixture of *cis* and *trans* stereoisomers with a preference for the *trans* isomer (*cis*:*trans* ~ 20:80),[27-29] whereas the *cis* configuration of compound **26** is predetermined by the starting material *cis*-2-hydroxycyclopentanecarboxylic acid. *Cis*-*trans* isomerism might explain the marginal difference in the HPLC retention times of **26** (*cis*) and the putative radioactive metabolite (*trans*). This leads to the assumption that the hydroxyl functions on the cyclopentyl moiety in metabolites **25** and **26** assume *trans* orientation.

These findings are in good agreement with the results of a study on the *in vitro* metabolism of the cyclic nucleotide phosphodiesterase type IV inhibitor RP 73401.[25] The authors clearly showed

that the predominant ring hydroxylated metabolite formed by human liver microsomes was the *trans* alcohol, with the *cis* isomer constituting less than 3% of the amount of total hydroxycyclopentyl metabolite formed.

2.2.5. Binding studies

In binding studies (table 2) using pig brain membranes all examined compounds had lower affinities (higher K_i values) for the A_1 and A_{2A} adenosine receptors than the parent compound CPFPX (K_i 0.47 nM). Differences in K_i values for the A_1AR ranged from 1.9 to 380 nM. The most efficient deactivation was found for metabolite **M1**, the main metabolite in human blood, showing a 800 fold reduction in affinity compared to CPFPX, but four compounds, **12**, **15a**, **15d**, and **17** showed higher subtype selectivity (A_{2A}/A_1) for the A_1AR. The four mentioned compounds are exclusively functionalized at the N-alkyl chains of the xanthine heterocycle. Functionalization at the cyclopentyl ring invariably led to a distinct reduction in subtype selectivity, with the main metabolite **M1** being the most unselective compound. Interestingly compound **15a** has both a high affinity for the A_1AR (K_i 2 nM) and a tenfold higher subtype selectivity (> 9000) compared to CPFPX (800), making **15a** a useful hydrophilic A_1AR ligand for the examination of this receptor outside the brain. Moreover, because of its potentially low unspecific binding, **15a** might be a good candidate for *in vitro* experiments.

Table 2. Affinity of CPFPX metabolites **10**, **12**, **15a-d**, **17**, **24a-b**, **25** and **26** at pig brain membranes.

Cpd	A_1AR (cortex) [^{3}H]CPFPX, K_i [nM][a]	A_{2A}R (striatum) [^{3}H]ZM241385, K_i [nM][a]	selectivity, A_{2A}/A_1
CPFPX	0.47 (0.36 to 0.61)	375 (279 to 504)	798
M1	378 (273 to 525)	7842 (2538 to 24229)	21
10	147	>100 000	>700

	(81 to 265)		
12	34 (24 to 47)	>100 000	>3000
15a	1.12 (0.97 to 1.28)	>10 000	>9000
15b	16.6 (13.6 to 20.1)	>10 000	>600
15c	15 (11 to 21)	>10 000	>650
(±)15d	6.5 (4.8 to 8.8)	>10 000	>1500
(±)17	1.9 (1.5 to 2.4)	1939 (1021 to 3684)	1020
24a	2.8 (1.1 to 2.9)	847 (550 to 1305)	303
24b	6.9 (2.8 to 17)	185 (133 to 258)	27
(±)25	6.5 (3.7 to 12)	189 (117 to 306)	29
(±)26	17.6 (13.4 to 22)	453 (381 to 507)	26

[a] Mean and 95% confidence limits of at least three separate assays.

3. Conclusion

This report describes the *in vitro* metabolic functionalization of the A_1AR antagonist [^{18}F]CPFPX in human liver microsome preparations. Ten putative metabolites were synthesized by the classical Traube synthesis followed by a series of derivatization steps and evaluated as A_1- and A_{2A} adenosine receptor ligands. Metabolic profiling by co-chromatography of the synthesized standards and extracts from human liver microsome incubations revealed, additionally to the known main metabolite M1, the formation of one metabolite resulting from ω–hydroxylation of the N^1-propyl chain and two metabolites arising from oxygenation of the cyclopentyl substituent. The present data suggest that monohydroxylations of the cyclopentyl moiety are stereoselective to give the respective *trans* alcohols. Furthermore two minor metabolites, unsaturated in the cyclopentyl ring, were formed.

The affinity data of the metabolites for the A_1- and A_{2A} adenosine receptor indicate that enzymatic functionalization of either the pendent N^1-side chain or the cyclopentane ring results in the formation of phase I metabolites with lower affinities to both receptor subtypes than the parent compound CPFPX.

Two points can be made about the potential future significance of this study:

- the metabolites are biologically active via a broad affinity range although less affine than the parent compound CPFPX
- the biologically highly active and subtype selective hydroxylated metabolite **15a** provides a convenient starting point for further drug development and the altered lipophilicity of **15a** may offer advantages in terms of unspecific binding and its use as a radioligand.

4. Experimental Section

Melting points were measured on a Büchi Melting Point B 545 apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. Elemental analyses were performed by the Central Division of Analytical Chemistry at the Forschungszentrum Jülich. Analyses indicated by the symbols of the elements are within ± 0.4 % of the theoretical values (table 3). Low resolution mass spectra (MS) were obtained in the ElectroSpray Ionisation (ESI+) mode on a Thermo Finnigan Surveyor MSQ mass spectrometer (Thermo Fisher Scientific GmbH, Dreieich, Germany). Thin layer chromatography (TLC) employed 0.2 mm precoated silica sheets (4 x 8 cm, Alugram SIL G/UV$_{254}$, Macherey-Nagel, Düren, Germany) developed with ethyl acetate / hexane 50 / 50 (v / v). Visualization was by quenching of UV fluorescence at 254 nm and iodine staining. For flash chromatography a Grace Reveleris® iES flash chromatography system equipped with RevealX™ detection, allowing for multisignal (UV/ELSD) collection, and Reveleris® flash silica cartridges (size 40 µm) as stationary phase was employed. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded at 200.13, 50.32, and 188.31 MHz, respectively, by means of a Bruker DPX-200 Avance instrument (Bruker Bio Spin GmbH, Rheinstetten, Germany) in 5% solution at 25°C. Chemical shifts are reported in ppm relative to the residual solvent signal (^{1}H NMR: δ 7.26; ^{13}C NMR: 77.0 for $CDCl_3$, ^{1}H NMR: δ 2.52; ^{13}C NMR: 50.32 for DMSO-d_6). Multiplicities are reported by using the following abbreviations: s; singlet, d; doublet, t; triplet, q; quartet, m; multiplet, br; broad. Coupling constants J (in Hertz) for protons are given in the form $^{n}J_{HX}$, those for carbons as $^{n}J_{CF}$. The protons were assigned with the aid of COSY, and the carbons with the aid of DEPT and HMQC experiments. All solvents and reagents in the highest state of purity available were obtained commercially (Sigma-Aldrich, Taufkirchen, Germany; Alfa Aesar, Karlsruhe, Germany) and used as supplied by the vendor. Methanol for CTH was dried by refluxing over Mg turnings and distillation. Ammonium formate was dried in a dessiccator over P_4O_{10} at room temperature and ambient pressure. For CTH equal weights of substrate and 10% Pd-C and a 10-fold molar excess of ammonium formate was used. Times and temperatures are indicated in individual experiments. Purification of dimethylformamide (DMF) consisted of distillation from calcium hydride and storage in a lightproof container over 4Å molecular sieves. Dichloromethane and toluene were dried by storage over 4Å molecular sieves. Other solvents, chemicals and biochemical reagents were obtained from and used as supplied by Sigma-Aldrich (Taufkirchen,

Germany). Pig brains for membrane preparations were from a local abattoir. The syntheses of [^{3}H]CPFPX and [^{18}F]CPFPX were by the methods cited.[21,30] Reactions were carried out at ambient temperature (± 23°C) under an atmosphere of argon unless otherwise stated.

Table 3. Elemental Analyses of New Compounds

Cpd	Formula		Carbon	Hydrogen	Nitrogen	Oxygen
7	$C_{23}H_{28}N_4O_4$	Calcd	65.08	6.65	13.20	15.08
		Found	65.11	6.61	13.14	15.16
8	$C_{29}H_{38}N_4O_6$	Calcd	64.67	7.11	10.40	17.62
		Found	64.71	7.04	10.48	17.77
9	$C_{22}H_{32}N_4O_6$	Calcd	58.91	7.19	12.49	21.40
		Found	58.88	7.24	12.53	21.32
11	$C_{25}H_{37}FN_4O_6$	Calcd	59.04	7.33	11.02	18.88
		Found	58.97	7.29	10.94	19.01
12	$C_{13}H_{17}FN_4O_2$	Calcd	55.70	6.11	19.99	11.42
		Found	55.75	6.20	19.88	11.52
13	$C_{19}H_{27}FN_4O_4$	Calcd	57.85	6.90	14.20	16.22
		Found	57.87	6.92	14.16	16.27
15a	$C_{16}H_{23}FN_4O_3$	Calcd	56.79	6.85	16.56	14.18
		Found	56.74	6.91	16.38	14.21
15b	$C_{16}H_{21}FN_4O_3$	Calcd	57.13	6.29	16.66	14.32
		Found	56.98	6.14	16.62	14.24
15c	$C_{16}H_{21}FN_4O_3$	Calcd	57.13	6.29	16.66	14.32
		Found	57.21	6.23	16.72	14.24
15d	$C_{16}H_{23}FN_4O_3$	Calcd	56.79	6.85	16.56	14.18
		Found	56.81	6.79	16.51	14.24
16	$C_{16}H_{23}FN_4O_3$	Calcd	56.79	6.85	16.56	14.18
		Found	56.81	6.87	16.48	14.22
17	$C_{16}H_{23}FN_4O_3$	Calcd	56.79	6.85	16.56	14.18
		Found	56.86	6.81	16.52	14.24

19a	$C_{22}H_{28}N_4O_5$	Calcd	61.67	6.59	13.08	18.67
		Found	61.73	6.51	12.99	18.74
19b	$C_{22}H_{28}N_4O_5$	Calcd	61.67	6.59	13.08	18.67
		Found	61.69	6.56	13.14	18.65
20a	$C_{22}H_{26}N_4O_4$	Calcd	64.37	6.38	13.65	15.59
		Found	64.43	6.44	13.52	15.44
20b	$C_{22}H_{26}N_4O_4$	Calcd	64.37	6.38	13.65	15.59
		Found	64.40	6.38	13.66	15.53
20c	$C_{29}H_{34}N_4O_5$	Calcd	67.16	6.61	10.80	15.43
		Found	67.03	6.57	10.74	15.48
21a	$C_{28}H_{36}N_4O_6$	Calcd	64.10	6.92	10.68	18.30
		Found	63.99	6.81	10.52	18.19
21b	$C_{28}H_{36}N_4O_6$	Calcd	64.10	6.92	10.68	18.30
		Found	64.04	6.88	10.63	18.24
21c	$C_{35}H_{44}N_4O_7$	Calcd	66.44	7.01	8.85	17.70
		Found	66.34	6.98	8.83	17.54
22a	$C_{19}H_{26}N_4O_4$	Calcd	60.95	7.00	14.96	17.09
		Found	61.03	6.90	14.72	17.01
22b	$C_{19}H_{26}N_4O_4$	Calcd	60.95	7.00	14.96	17.09
		Found	60.99	7.05	14.88	17.07
22c	$C_{26}H_{34}N_4O_5$	Calcd	64.71	7.10	11.61	16.58
		Found	64.64	7.14	11.52	16.66
23a	$C_{22}H_{31}FN_4O_4$	Calcd	60.81	7.19	12.89	14.73
		Found	61.00	7.11	12.83	14.65
23b	$C_{22}H_{31}FN_4O_4$	Calcd	60.81	7.19	12.89	14.73
		Found	60.77	7.16	12.96	14.81
23c	$C_{29}H_{39}FN_4O_5$	Calcd	64.19	7.24	10.32	14.74
		Found	64.07	7.19	10.33	14.69
24a	$C_{16}H_{21}FN_4O_2$	Calcd	59.99	6.61	17.49	9.99
		Found	60.02	6.81	17.42	10.14

24b	$C_{16}H_{21}FN_4O_2$	Calcd	59.99	6.61	17.49	9.99
		Found	59.99	6.57	17.53	10.06
24c	$C_{23}H_{29}FN_4O_3$	Calcd	64.47	6.82	13.08	11.20
		Found	64.37	6.69	12.93	11.19
25	$C_{16}H_{23}FN_4O_3$	Calcd	56.79	6.85	16.56	14.18
		Found	56.72	6.95	16.55	14.11
26	$C_{16}H_{23}FN_4O_3$	Calcd	56.79	6.85	16.56	14.18
		Found	56.87	6.89	16.53	14.26

6-Amino-1-benzyluracil (2).[31]

A solution of sodium methanolate in methanol (30%, 357 mL, 2 mol) was diluted with methanol to a final volume of 600 mL and heated to reflux. Under efficient stirring N-benzylurea (**1**, 100 g, 0.66 mol) was added, followed by cyanoacetic acid methyl ester (66 g, 0.66 mol). The reaction mixture was stirred at reflux for 6 h, cooled to room temperature and evaporated. Stirring in boiling water (1400 mL) dissolved the syrupy yellow residue and the hot solution was carefully acidified with acetic acid *(CAUTION! VIGOROUS FOAMING!).* After cooling the voluminous precipitate was filtered off, washed with water and dried at 120°C. Recrystallization from 50% aqueous ethanol gave an analytical sample. Yield: 89 g (62%), mp 286°C (50% EtOH).
^{1}H-NMR (DMSO-d_6): δ 4.68 (s, 1H, C-5-*H*), 5.08 (s, 2H, Ph-CH_2), 6.89 (s, 2H, NH_2), 7.27 (m, 5H, C_6H_5), 10.69 (s_{br}, 1H, N*H*). ^{13}C-NMR (DMSO-d_6): δ 44.4 (Ph-*C*H$_2$), 76.4 (*C*-5), 127.2, 128, 129.3 (Ph-*C*-4, *C*-2, *C*-3), 137.5 (Ph-*C*-1), 152.3 (*C*-2), 156.7 (*C*-6), 163.3 (*C*-4). MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{11}H_{11}N_3O_2$ 218.22; Found 218.23.

6-Amino-5-nitroso-1-benzyluracil (3).[32]

Crude **2** (4.34 g, 20 mmol) was suspended by stirring in ethanol (100 mL) and isoamyl nitrite (5 mL, 36 mmole) was added dropwise. Acidification with ethanolic HCl (2.5 N, 1 mL) changed the color from yellow to violet. The reaction mixture was stirred for 0.5 h and cooled in the refrigerator for 2 h. The microcrystalline solid collected by filtration was air-dried. Yield: 4.5 g (92%), mp > 300°C (violet crystals).
^{1}H-NMR (DMSO-d_6): δ 5.17 (s, 2H, Ph-CH_2), 6.89 (s, 2H, NH_2), 7.31 (m, 5H, C_6H_5), 11.28 (s_{br}, 1H, N^3-*H*). ^{13}C-NMR (DMSO-d_6): δ 44.2 (Ph-*C*H$_2$), 127.4, 128.3, 129.4 (Ph-*C*^{4}, *C*^{2}, *C*^{3}), 135.5

(Ph-C^1), 139.5 (C^5), 147.7 (C^2), 149.8 (C^6), 161.3 (C^4). MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{11}H_{10}N_4O_3$ 247.22; Found 247.23.

1-Benzyl-5,6-diaminouracil (4).[15]

Compound **3** (7.4 g 30 mmol) was dissolved in NH_4OH (10%, ca. 70 ml) at 50-60°C to give a brownish solution. The addition of solid $Na_2S_2O_4$ (10 g, 90 mmol) in small portions over 0.3 h discharged the color. Stirring for 2 h longer at 50 - 60°C, followed by cooling, precipitated greenish-grey product, which was filtered off by suction, washed with a small amount of ice-cold water and dichloromethane, and used immediately in the next step. Yield 6.3 g (90%). MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{11}H_{12}N_4O_2$ 232.24; Found 233.24.

N-(6-Amino-1-benzyl-2,4-dioxo-3-propyl-1,2,3,4-tetrahydropyrimidin-5-yl)-cyclopentanecarboxamide (5).[15]

Crude **4** (2.4 g, 10 mmol) was suspended in DMF (50 mL) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC, 1.92 g, 10 mmol) and a catalytic amount (~ 150 mg) of 4-dimethylaminopyridine (DMAP) was added. Under efficient stirring cyclopentanecarboxylic acid (1.08 mL, 10 mmol) was added, and the mixture was stirred for 20 h at ambient temperature. During this time the greenish suspension became a clear brown solution, and sometimes a yellow precipitate formed. The mixture was poured onto ice-water (150 mL), the yellow precipitate was collected by filtration, washed with water (50 mL), and oven-dried at 120°C. Yield 2.82 g (86%). ^{1}H-NMR (DMSO-d_6): δ 1.46 – 1.84 (m, 8H, Cp-CH_2), 2.77 (m, 1H, Cp-C*H*), 5.13 (s, 2H, Ph-CH_2), 6.44 (s, 2H, NH_2), 7.34 (m, 5H, C_6H_5), 8.22 (s, 1H, N*H*CO), 10.74 (s, 1H, N^3-*H*). ^{13}C-NMR (DMSO-d_6): δ 26.6 (Cp-*C*H_2), 30.8 (Cp-*C*H_2), 44.9 (Cp-*C*H), 53.6 (Ph-*C*H_2), 89.2 (C^5), 127.2, 128, 129.3 (Ph-C^4, C^2, C^3), 137.5 (Ph-C^1), 151.2 (C^2), 153.5 (C^6), 160.7 (C^4), 176.9 (*C*=O). MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{17}H_{20}N_4O_3$ 329.37; Found 329.39.

3-Benzyl-8-cyclopentyl-1*H*-purine-2,6(3*H*,7*H*)-dione (6).[15]

Compound **5** was refluxed for 4 h in aqueous 2N NaOH (oil bath 115°C) and cooled to room temperature. Bringing the clear brownish solution to pH 4 - 5 with concentrated HCl precipitated a solid that was filtered off, washed with water and triturated with boiling ethanol to remove

coloured impurities. Filtration and oven drying gave almost colourless product. The xanthine was recrystallized from acetic acid (± 25 mL / g), filtered, washed with ethanol and oven-dried. Yield 2.43 g (91%), mp > 300°C. TLC: Rf 0.21.

^{1}H-NMR (DMSO-*d*$_6$): δ 1.61 – 2.01 (m, 8H, Cp-C*H*$_2$), 3.12 (m, 1H, Cp-C*H*), 5.09 (s, 2H, Ph-C*H*$_2$), 7.33 (m, 5H, C$_6$*H*$_5$), 11.07 (s, 1H, N^1-*H*) 13.18 (s, 1H, N^7-*H*). ^{13}C-NMR (DMSO-*d*$_6$): δ 25.9 (Cp-*C*H$_2$), 32.8 (Cp-*C*H$_2$), 39.6 (Cp-*C*H), 45.8 (Ph-*C*H$_2$), 107.4 (*C*^{5}), 128.2, 128.5, 129.3 (Ph-*C*^{4}, *C*^{2}, *C*^{3}), 138.1 (Ph-*C*^{1}), 150.1 (*C*^{8}), 151.7 (*C*^{4}), 155.1 (*C*^{2}), 158.7 (*C*^{6}). MS (ESI+) m/z: [M+H]$^+$ Calcd for $C_{17}H_{18}N_4O_2$ 311.35; Found 311.32.

3-Benzyl-8-cyclopentyl-7-pivaloyloxymethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (7).

A slurry of **6** (3.1 g, 10 mmol), K_2CO_3 (1.38 g, 10 mmol) and chloromethyl pivalate (POM-Cl, 1.45 mL, 10 mmol) in dry DMF (40 mL) was stirred at 60°C for 3 h. The reaction mixture was poured into ice water and neutralized with 2N HCl. Precipitated product was filtered off and washed with water. The solid was treated with boiling EtOH (50 mL), the mixture was filtered, and the solvent was evaporated *in vacuo* to give an oily yellow residue. Adding MeOH (± 5 mL), scratching with a glass rod and cooling crystallized the product. The off-white solid was filtered off, washed with a small volume of ice cold MeOH and air-dried. Yield: 3.7 g (92 %), mp: 174-175°C (MeOH).

In some preparations traces of the 1,7-diPOM compound formed, hence the crude product mixture was purified by flash cromatography eluting with 50% ethyl acetate in hexane. TLC: Rf 0.65.

^{1}H-NMR (DMSO-*d*$_6$): δ 1.13 (s, 9H, N^7-POM-C*H*$_3$), 1.64 – 2.11 (m, 8H, Cp-C*H*$_2$), 3.40 (m, 1H, Cp-C*H*), 5.09 (s, 2H, Ph-C*H*$_2$), 6.20 (s, 2H, N^7-POM-C*H*$_2$), 7.30 (m, 5H, C$_6$*H*$_5$), 11.26 (s, 1H, N^1-*H*). ^{13}C-NMR (DMSO-*d*$_6$): δ 25.9 (Cp-*C*H$_2$), 27.4 (N^7-POM-*C*H$_3$)), 33 (Cp-*C*H$_2$), 36.4 (Cp-*C*H), 39.1 (N^7-POM-*C*(CH$_3$)$_3$), 45.7 (Ph-C*H*$_2$), 68.1 (N^7-POM-C*H*$_2$), 106.9 (*C*^{5}), 128.5, 129, 129.2 (Ph-*C*^{4}, *C*^{2}, *C*^{3}), 137.8 (Ph-*C*^{1}), 150.3 (*C*^{8}), 155.5 (*C*^{4}), 155 (*C*^{2}), 160.1 (*C*^{6}), 177.4 (*C*=O). MS (ESI+) m/z: [M+H]$^+$ Calcd for $C_{23}H_{28}N_4O_4$ 425.49; Found 425.51.

3-Benzyl-8-cyclopentyl-1,7-(dipivaloyloxymethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (8).

A slurry of **7** (424 mg, 1 mmol), K_2CO_3 (152 mg, 1.1 mmol) and POM-Cl (216 µL, 1.5 mmol) in dry DMF (4 mL) was stirred at 60°C for 5 h. The reaction mixture was poured into ice water and

neutralized with 2N aqueous HCl. The turbid supernatant was decanted from the gummy precipitate and the gum was dissolved in hot MeOH. Addition of water precipitated the product as pale yellowish crystals. The product was recrystallized from 70% aqueous MeOH. Yield: 450 mg (83%), mp: 128-129°C (70% MeOH). TLC: Rf 0.87.
^{1}H-NMR (DMSO-d_6): δ 1.09 (s, 9H, N^{1}-POM-CH_3), 1.13 (s, 9H, N^{7}-POM-CH_3), 1.66 – 2.14 (m, 8H, Cp-CH_2), 3.40 (m, 1H, Cp-C*H*), 5.17 (s, 2H, Ph-CH_2), 5.88 (s, 2H, N^{1}-POM-CH_2), 6.22 (s, 2H, N^{7}-POM-CH_2), 7.33 (m, 5H, C_6H_5), ^{13}C-NMR (DMSO-d_6): δ 26.2 (Cp-*C*H$_2$), 27.4 (POM-*C*H$_3$), 27.5 (POM-*C*H$_3$), 33 (Cp-*C*H$_2$), 36.4 (Cp-*C*H), 39.1 (POM-*C*(CH$_3$)$_3$), 39.2 (POM-*C*(CH$_3$)$_3$), 45.8 (Ph-*C*H$_2$), 65.7 (POM-*C*H$_2$), 68.2 (POM-*C*H$_2$), 106.2 (*C*^{5}), 128.5, 129, 129.30 (Ph-*C*^{4}, *C*^{2}, *C*^{3}), 137.31 (Ph-*C*^{1}), 149.1 (*C*^{8}), 151 (*C*^{4}), 153.5 (*C*^{2}), 161.2 (*C*^{6}), 177.1 (*C*=O), 177.4 (*C*=O). MS (ESI+) m/z: [M+H]$^{+}$ Calcd for $C_{29}H_{38}N_4O_6$ 539.64; Found 539.66.

NOTE: Compound **8** can also be prepared in one step in a similar yield by the reaction of **6** (1 eq) with POM-Cl (3.5 eq) and K_2CO_3 (3.5 eq) in DMF (4 mL / mmol xanthine) for 24 h at 60°C, dilution with water, extraction with DCM, drying of the organic layer over Na_2SO_4, evaporation and crystallizing the clear oil from 70% aqueous MeOH.

8-Cyclopentyl-1,7-(dipivaloyloxymethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (9).
A 50 mL round-bottomed flask was charged with **8** (538 mg, 1 mmol), dry 10% Pd/C (538 mg) and dry ammonium formate (631 mg 10 mmol). Dry MeOH (25 mL) was added and under efficient stirring the flask was immersed in an oil bath preheated to 140°C. After 2h TLC indicated the absence of starting material. The catalyst was removed by filtration over Celite™, the filter cake was washed with DCM (3 x 20 mL) and acetone (3 x 20 mL) and the combined filtrate and washings were evaporated *in vacuo* to give an analytically pure white solid in quantitative yield, mp 138-139°C (MeOH). TLC: Rf 0.73.
^{1}H-NMR (DMSO-d_6): δ 1.01 (s, 9H, N^{1}-POM-CH_3), 1.06 (s, 9H, N^{7}-POM-CH_3), 1.59 – 2.01 (m, 8H, Cp-CH_2), 3.43 (m, 1H, Cp-C*H*), 5.84 (s, 2H, N^{1}-POM-CH_2), 6.18 (s, 2H, N^{7}-POM-CH_2) N^{3}-*H* appears as a broad signal between 3 and 5 ppm. ^{13}C-NMR (DMSO-d_6): δ 26.3 (Cp-*C*H$_2$), 27.4 (POM-*C*H$_3$), 27.6 (POM-*C*H$_3$), 32.9 (Cp-*C*H$_2$), 36.30 (Cp-*C*H), 39.1 (POM-*C*(CH$_3$)$_3$), 39.2 (POM-*C*(CH$_3$)$_3$), 65.6 (POM-*C*H$_2$), 68 (POM-*C*H$_2$), 105.9 (*C*^{5}), 153.9 (*C*^{8}), 155 (*C*^{4}), 160.9 (*C*^{2}),

177.2 (*C*^{6}), 177.7 (*C*=O), 177.8 (*C*=O). MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{22}H_{32}N_4O_6$ 449.51; Found 449.52

8-Cyclopentyl-1*H*-purine-2,6(3*H*,7*H*)-dione (10).

A solution of **9** (448 mg, 1 mmol) in 7N methanolic NH_3 (15 mL) was stirred for 48 h at ambient temperature. Evaporation *in vacuo* gave a solid residue that was recrystallized from MeOH. Yield 215 mg (95%) colourless crystals, m.p. > 300°C (MeOH). TLC: Rf 0.00.

^{1}H-NMR (DMSO-d_6): δ 1.61 – 2.03 (m, 8H, Cp-CH_2), 3.09 (m, 1H, Cp-C*H*),3.60 (s_{br}, 1H, N*H*), 11.38 (s_{br}, 2H, 2 x NH). ^{13}C-NMR (DMSO-d_6): δ 25.9 (Cp-*C*H_2), 32.7 (Cp-*C*H_2), 49.5 (Cp-*C*H), 107.2 (*C*^{5}), 149.9 (*C*^{8}), 152.3 (*C*^{4}), 156.1 (*C*^{2}), 158.6 (*C*^{6}). MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{10}H_{12}N_4O_2$ 221.23; Found 221.22

8-Cyclopentyl-1,7-(dipivaloyloxymethyl)-3-(3-fluoropropyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (11).

A slurry of **9** (448 mg, 1 mmol), K_2CO_3 (152 mg, 1.1 mmol) and 1-bromo-3-fluoropropane (137 µL, 1.5 mmol) in dry DMF (4 mL) was stirred for 24 h at 60°C. Pouring the reaction mixture into ice water and neutralizing with 2N aqueous HCl precipitated the product as a gum that solidified on standing. The solid was recrystallized from MeOH. Yield: 482 mg (95%), mp: 116-117°C (MeOH). TLC: Rf 0.69.

^{1}H-NMR (DMSO-d_6): δ 1.11 (s, 9H, N^{1}-POM-CH_3), 1.12 (s, 9H, N^{7}-POM-CH_3), 1.67 – 2.21 (m, 10H, Cp-CH_2 + N^{3}-CH_2-CH_2), 3.43 (m, 1H, Cp-C*H*), 4.13 (t, 2H, N^{3}-CH_2), 4.51 (dt, *J* = 47.2, 5,7 Hz, 2H, CH_2-F), 5.88 (s, 2H, N^{1}-POM-CH_2), 6.23 (s, 2H, N^{7}-POM-CH_2). ^{13}C-NMR (DMSO-d_6): δ 26.2 (Cp-*C*H_2), 27.4 (POM-*C*H_3), 27.5 (POM-*C*H_3), 29.5 (^{2}J = 19.2 Hz, CH_2-CH_2F), 32.9 (Cp-CH_2), 36.4 (Cp-C*H*), 39.1 (POM-*C*$(CH_3)_3$), 39.1 (^{3}J = 5.5 Hz, N^{3}-*C*H_2), 39.2 (POM-*C*$(CH_3)_3$), 65.7 (POM-*C*H_2), 68.1 (POM-*C*H_2), 82.6 (^{1}J = 162.0 Hz, *C*H_2-F), 106.2 (*C*^{5}), 149.2 (*C*^{8}), 151.1 (*C*^{4}), 159.6 (*C*^{2}), 161.1 (*C*^{6}), 177.1 (*C*=O), 177.5 (*C*=O). ^{19}F-NMR (DMSO-d_6): δ – 219.16. MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{25}H_{37}FN_4O_6$ 509.58; Found 509.60.

8-Cyclopentyl-3-(3-fluoropropyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (12).

A solution of **11** (508 mg, 1 mmol) in a mixture of EtOH (10 mL) and aqueous NaOH (2N, 2 mL, 4 mmol) was stirred at room temperature for 8 h. Diluting the reaction mixture with water and neutralization with acetic acid precipitated the deprotected purine. Collecting the product by filtration, washing with water and drying furnished colourless crystals of **12**. Yield 275 mg (98%), mp 263-264°C (50% MeOH). TLC: Rf 0.23.

^{1}H-NMR (DMSO-d_6): δ 1.63 – 2.02 (m, 10H, Cp-CH_2+ N^3-CH$_2$-CH_2), 3.14 (m, 1H, Cp-CH), 4.04 (t, 2H, N^3-CH_2), 4.48 (dt, J = 47.2, 5,7 Hz, 2H, CH_2-F), 10.98 (s, 1H, N^1H), 13.09 (s, 1H, N^7H). ^{13}C-NMR (DMSO-d_6): δ 25.9 (Cp-CH$_2$), 29.7 (^2J = 19.2 Hz, CH_2-CH$_2$F), 32.8 (Cp-CH$_2$), 39.6 (Cp-CH), 39.7 (^3J = 5.5 Hz, N^3-CH$_2$), 82.7 (^1J = 162.0 Hz, CH$_2$-F), 107.4 (C^5), 150.1 (C^8), 151.8 (C^4), 155.2 (C^2), 158.6 (C^6). ^{19}F-NMR (DMSO-d_6): δ – 218.83. MS (ESI+) m/z: [M+H]$^+$ Calcd for $C_{13}H_{17}FN_4O_2$ 281.30; Found 281.32.

8-Cyclopentyl-7-pivaloyloxymethyl-3-(3-fluoropropyl)-1H-purine-2,6(3H,7H)-dione (13).
A slurry of **12** (2.8 g, 10 mmol) and K_2CO_3 (1.38 g, 10 mmol) in dry DMF (40 mL) was stirred for 0.5 h at ambient temperature. POM-Cl (1.53 mL, 10.5 mmol) was added dropwise and the mixture was stirred at 60°C for 1 h. The reaction mixture was poured into ice water, neutralized with 2N HCl and extracted with diethyl ether (2 x 100 mL). Evaporation of the solvent gave semisolid material that was purified by flash chromatography eluting with 50% ethyl acetate in hexane. The 1,7-diPOM compound (100 mg) eluted first, followed by product **13**. Evaporation of the solvent gave colourless crystals, 2.9 g (74%). An analytical sample was recrystallized from aqueous 80% MeOH, mp: 146-147°C (80% MeOH). TLC: Rf 0.35.

^{1}H-NMR (DMSO-d_6): δ 1.12 (s, 9H, N^7-POM-CH_3), 1.64 – 2.12 (m, 10H, Cp-CH_2 + N^3-CH$_2$-CH_2), 3.38 (m, 1H, Cp-CH), 4.04 (t, 2H, N^3-CH_2), 4.51 (dt, J = 47.2, 5,7 Hz, 2H, CH_2-F), 6.19 (s, 2H, N^7-POM-CH_2), 11.16 (s, 1H, N^1-H). ^{13}C-NMR (DMSO-d_6): δ 26.1 (Cp-CH$_2$), 27.4 (POM-CH$_3$)), 29.6 (^2J = 19.2 Hz, CH$_2$-CH$_2$F), 33 (Cp-CH$_2$), 36.4 (Cp-CH), 39 (N^7-POM-C(CH$_3$)$_3$), 39.5 (^3J = 5.5 Hz, N^3-CH$_2$), 68 (POM-CH$_2$), 82.7 (^1J = 162.0 Hz, CH$_2$-F), 106.9 (C^5), 149.85 (C^8), 151.4 (C^4), 155.1 (C^2), 160.1 (C^6), 177.2 (C=O). ^{19}F-NMR (DMSO-d_6): δ – 218.96. MS (ESI+) m/z: [M+H]$^+$ Calcd for $C_{19}H_{27}FN_4O_4$ 395.44; Found 395.45.

8-Cyclopentyl-7-pivaloyloxymethyl-3-(3-fluoropropyl)-1-(3-hydroxypropyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (14a).

A mixture of K_2CO_3 (166 mg, 1.2 mmol) and **13** (394 mg, 1 mmol) in dry DMF (5 mL) was stirred for 0.5 h, 1-bromo-3-hydroxypropane (134 µL, 1.5 mmol) was added and the suspension was stirred at 70°C for 2 h. After cooling to room temperature the mixture was poured into ice water (100 mL) and the aqueous solution was extracted with diethyl ether (2 x 75 mL). The pooled organic extracts were washed with water (100 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to give a clear, colourless oil. The addition of 40% aqueous methanol (1 mL) followed by scratching with a glass rod gave a semi-solid material which crystallized upon standing overnight in an open vial. Filtration and drying in air gave **14a** that was pure by TLC as colourless, waxy crystals 450 mg, 98%, mp. 80-81°C. TLC: Rf 0.25.

^{1}H-NMR ($CDCl_3$): δ 1.20 (s, 9H, N^7-POM-C*H*$_3$), 1.58 – 2.31 (m, 12H, Cp-C*H*$_2$ + N^1-CH$_2$-C*H*$_2$ + N^3-CH$_2$-C*H*$_2$), 3.25 (m, 1H, Cp-C*H*), 3.53 (br s, 3H, C*H*$_2$OH + O*H*), 4.19 + 4.29 (2t, 4H, N^1-C*H*$_2$ + N^3-C*H*$_2$), 4.53 (dt, *J* = 47.2, 5,7 Hz, 2H, C*H*$_2$-F), 6.24 (s, 2H, N^7-POM-C*H*$_2$). ^{13}C-NMR ($CDCl_3$): δ 26.2 (Cp-*C*H$_2$), 27.3 (POM-*C*H$_3$), 29.6 (^2J = 19.2 Hz, C*H*$_2$-CH$_2$F), 31.2 (N^1-CH$_2$-*C*H$_2$), 33.1 (Cp-*C*H$_2$), 36.7 (Cp-*C*H), 38.1 (POM-*C*(CH$_3$)$_3$), 40.7 (^3J = 5.5 Hz, N^3-*C*H$_2$), 40.8 (N^1-*C*H$_2$), 58.9 (*C*H$_2$-OH), 66.9 (POM-*C*H$_2$), 82.3 (^1J = 162.0 Hz, *C*H$_2$-F), 106.7 (*C*^{5}), 148.75 (*C*^{8}), 152 (*C*^{4}), 155.5 (*C*^{2}), 160.62 (*C*^{6}), 177.7 (*C*=O). ^{19}F-NMR ($CDCl_3$): δ – 220.48. MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{22}H_{33}FN_4O_5$ 453.52; Found 453.54.

8-Cyclopentyl-3-(3-fluoropropyl)-1-(3-hydroxypropyl)-1H-purine-2,6(3H,7H)-dione (15a).

A solution of **14a** (452 mg, 1 mmol) in methanolic ammonia (7N, 20 mL) was stirred for 72 h at room temperature. Evaporation and recrystallization of the residue from 80% aqueous MeOH yielded **15a** as colorless crystals. Yield 285 mg (84%), m.p. 213-214°C (80% MeOH). TLC: Rf 0.06.

^{1}H-NMR (DMSO-d_6): δ 1.55 – 2.21 (m, 12H, Cp-C*H*$_2$ + N^1-CH$_2$-C*H*$_2$ + N^3-CH$_2$-C*H*$_2$), 3.14 (m, 1H, Cp-C*H*), 3.46 (m, 2H, C*H*$_2$OH), 3.93 + 4.11 (2t, 4H, N^1-C*H*$_2$ + N^3-C*H*$_2$), 4.58 (m + t, J = 47.2, 5,7 Hz, 3H, C*H*$_2$-F + O*H*), 13.13 (s, 1H, N^7-*H*). ^{13}C-NMR (DMSO-d_6): δ 25.9 (Cp-*C*H$_2$), 29.6 (^2J = 19.2 Hz, *C*H$_2$-CH$_2$F), 31.9 (N^1-CH$_2$-*C*H$_2$), 32.8 (Cp-*C*H$_2$), 39.2 (N^1-*C*H$_2$), 39.6 (Cp-*C*H), 59.7 (*C*H$_2$-OH), 82.8 (^1J = 162.0 Hz, *C*H$_2$-F), 107.1 (*C*^{5}), 148.4 (*C*^{8}), 151.6 (*C*^{4}), 154.8 (*C*^{2}), 158.7

(C^6). ^{19}F-NMR (DMSO-d_6): δ – 218.84. MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{16}H_{23}FN_4O_3$ 339.38; Found 339.39.

8-Cyclopentyl-3-(3-fluoropropyl)-1-(3-oxopropyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (15b).
A mixture of 8-cyclopentyl-3-(3-fluoropropyl)-7-pivaloyloxymethylxanthine **13** (197 mg, 0.5 mmol) and K_2CO_3 (83 mg, 0.6 mmol) in dry DMF (5 mL) was stirred for 0.5 h, then 2-bromoethyl-1,3-dioxolane (88 µL, 0.75 mmol) and a catalytic amount of KI (25 mg) were added, and the suspension was stirred at room temperature for 24 h. The mixture was poured onto ice-water (100 mL) and the aqueous solution was extracted with diethyl ether (2 x 75 mL). The pooled organic extracts were washed with water (100 mL), dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo to give a clear yellow oil. The oil was dissolved in EtOH (10 mL) and 2N aqueous NaOH (1 mL, 2 mmol) was added. After stirring for 1 h at room temperature the mixture was poured into ice-water (100 mL), acidified to pH 4 by the addition of 2N aqueous HCl, and extracted with diethyl ether (2 x 100 mL). The pooled organic phases were dried over anhydrous Na_2SO_4, filtered and the solvent evaporated in vacuo to give a colourless powder. This was taken up in 0.5N aqueous HCl (10 mL) and the turbid mixture was stirred 2 h at reflux. After cooling the solution was adjusted to pH 5-6 with 1N aqueous NaOH and precipitated **15b** was extracted into dichloromethane (2 x 25 mL). The pooled organic extracts were washed with water (50 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to leave a solid residue. Recrystallization from 50% aqueous MeOH gave off-white crystals. Yield 148 mg (88%), m.p. 223-224°C (50% MeOH. ^{1}H-NMR ($CDCl_3$): δ 1.51 - 2.19 (m, 10H, Cp-CH_2 + N^3-CH$_2$-CH_2), 2.83 (m, 3H, N^1-CH$_2$-CH_2), 3.24 (m, 1H, Cp-CH), 4.32 (t, 2H, N^3-CH_2), 4.57 (dm, J = 47.2, 5,7 Hz, 4H, CH_2-F + N^1-CH_2), 9.87 (s, 1H, CHO), 12.28 (s_{br}, 1H, N^7-H). ^{13}C-NMR ($CDCl_3$): δ 25.8 (Cp-CH$_2$), 29.5 (^2J = 19.2 Hz, CH$_2$-CH$_2$F), 32.8 (Cp-CH$_2$), 36.1 (N^1-CH$_2$), 40.2 (Cp-CH), 40.9 (^3J = 5.5 Hz, N^3-CH$_2$), 43 (N^1-CH$_2$-CH$_2$), 82.3 (^1J = 162.0 Hz, CH$_2$-F),106.9 (C^5), 149.6 (C^8), 151.3 (C^4), 155.6 (C^2), 160.3 (C^6), 200.4 (C=O). ^{19}F-NMR ($CDCl_3$): δ - 220.50. MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{16}H_{21}FN_4O_3$ 337.36; Found 337.38.

8-Cyclopentyl-3-(3-fluoropropyl)-1-(2-oxopropyl)-1H-purine-2,6(3H,7H)-dione (15c).

A mixture of **13** (197 mg, 0.5 mmol) in dry DMF (5 mL) and K_2CO_3 (83 mg, 0.6 mmol) was stirred for 0.5 h, chloroacetone (60 µL, 0.75 mmol) was added, and the suspension was stirred at ambient temperature for 24 h. The mixture was poured onto ice water (100 mL) and the water was extracted with diethyl ether (2 x 75 mL). The pooled organic extracts were washed with water (100 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to a clear yellow oil. The oil was dissolved in EtOH (10 mL) and 2N aqueous NaOH (1 mL, 2 mmol) was added. After stirring for 4 h at ambient temperature the mixture was poured into ice water (100 mL), acidified to pH 4 by the addition of 2N aqueous HCl, and extracted with ethyl acetate (3 x 100 mL). The pooled organic phases were dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated to a colorless powder that was extracted with ethyl acetate (5 mL). Filtration and air-drying provided **15c** as colorless crystals. Yield 132 mg (78%), m.p. 216-218°C. TLC: Rf 0.21. ^{1}H-NMR (DMSO-*d*$_6$): δ 1.03 (d, 3H, C*H*$_3$), 1.61 – 2.20 (m, 10H, Cp-C*H*$_2$ + N^3-CH$_2$-C*H*$_2$), 2.20 (s, 3H, C*H*$_3$), 3.16 (m, 1H, Cp-C*H*), 4.07 (t, 2H, N^3-C*H*$_2$), 4.26 (dt, *J* = 47.2, 5,7 Hz, 2H, C*H*$_2$-F), 4.75 (s, 2H, N^1-C*H*$_2$), 13.21 (s$_{br}$, 1H, N^7-*H*). ^{13}C-NMR (DMSO-*d*$_6$): δ 25.9 (Cp-*C*H$_2$), 27.9 (*C*H$_3$), 29.6 (^{2}*J* = 19.2 Hz, C*H*$_2$-CH$_2$F), 32.8 (Cp-*C*H$_2$), 39.7 (Cp-*C*H), 40.5 (^{3}*J* = 5.5 Hz, N^3-*C*H$_2$), 50.7 (N^1-*C*H$_2$), 82.6 (^{1}*J* = 162 Hz, *C*H$_2$-F), 106.8 (*C*^{5}), 149.5 (*C*^{8}), 151.4 (*C*^{4}), 154.2 (*C*^{2}), 159.3 (*C*^{6}), 203.1 (*C*=O). ^{19}F-NMR (DMSO-*d*$_6$): δ -218.94. MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{16}H_{21}FN_4O_3$ 337.36; Found 337.36.

(±)-8-Cyclopentyl-3-(3-fluoropropyl)-1-(2-hydroxypropyl)-1H-purine-2,6(3H,7H)-dione (15d).

K_2CO_3 (166 mg, 1.2 mmol) was added to a solution of **13** (394 mg, 1 mmol) in dry DMF (5 mL) and the mixture was stirred for 0.5 h. Chloroacetone (120 µL, 1.5 mmol) was added, and the suspension was stirred at ambient temperature for 24 h. The mixture was poured onto ice water (100 mL) and the aqueous solution was extracted with diethyl ether (2 x 75 mL). The pooled organic extracts were washed with water (100 mL), dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo to give a clear yellow oil. The oil was dissolved in dry MeOH (10 mL) and under stirring $NaBH_4$ (45 mg, 1.2 mmol) was added in three portions over 15 min. The mixture was stirred for 0.5 h longer, methanolic ammonia (7N, 30 mL) was added and the mixture was stirred for 48 h at ambient temperature. The solvent was evaporated, the residue was taken up in

ethyl acetate, the organic layer washed with water, dried over anhydrous Na_2SO_4, filtered, and evaporated. Recrystallization from 80% aqueous MeOH gave **15d** as colorless crystals. Yield 300 mg (88%), m.p. 209-210°C (80% MeOH). TLC: Rf 0.11.

^{1}H-NMR (DMSO-d_6): δ 1.03 (d, 3H, CH_3), 1.58 – 2.31 (m, 10H, Cp-CH_2 + N^3-CH$_2$-CH_2), 3.15 (m, 1H, Cp-C*H*), 3.72 (m, 1H, C*H*OH), 3.95 + 4.09 (2t, 4H, N^1-CH_2 + N^3-CH_2), 4.52 (dm, *J* = 47.2, 5,7 Hz, 3H, CH_2-F + O*H*), 12.82 (s_{br}, 1H, N^7-*H*). ^{13}C-NMR (DMSO-d_6): δ 21.8 (*C*H$_3$), 25.9 (Cp-*C*H$_2$), 29.6 (^2J = 19.2 Hz, *C*H$_2$-CH$_2$F),32.8 (Cp-*C*H$_2$), 39.7 (Cp-*C*H), 40.4 (^{3}J = 5.5 Hz, N^3-*C*H$_2$), 48.3 (N^1-*C*H$_2$), 64.4 (*C*H-OH), 82.5 (^1J = 162.0 Hz, *C*H$_2$-F),107.2 (*C*^{5}), 148.4 (*C*^{8}), 151.9 (*C*^{4}), 155.1 (*C*^{2}), 158.6 (*C*^{6}). ^{19}F-NMR (DMSO-d$_6$): δ – 218.80 and – 218.93 (5%). MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{16}H_{23}FN_4O_3$ 339.38; Found 339.38.

(±)-8-Cyclopentyl-3-(3-fluoro-2-hydroxypropyl)1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (17). A mixture of 8-cyclopentyl-1-propyl-7-pivaloyloxymethylxanthine **16** (197 mg, 0.5 mmol),[15] K_2CO_3 (4 mg, 0.005 mmol, 5 mol%) and epifluorohydrin (53 µL, 0.75 mmol) in dry DMF (10 mL) was stirred at 75°C for 6 h,[14] cooled to ambient temperature, poured onto ice-water (150 mL), and extracted with diethylether (2 x 50 mL). The oil obtained by vacuum evaporation and azeotroping with acetonitrile (2 x 20 mL) was dissolved in EtOH (10 mL) containing 2N aqueous NaOH (1 mL, 2 mmol). The mixture was stirred at ambient temperature for 2 h, poured onto ice-water (150 mL), acidified to pH 5-6 by the addition of 0.5N aqueous HCl, and extracted with dichloromethane (2 x 50 mL). Drying of the combined organic reaper dawlayers over anhydrous Na_2SO_4, filtration and rotary evaporation of the solvent *in vacuo* gave **17** as a solid residue that was recrystallized 2 times from ethyl acetate. Yield 152 mg (89%), colourless crystals, m.p. 212-213°C (ethyl acetate). TLC: Rf 0.51.

^{1}H-NMR (DMSO-d_6): δ 0.86 (t, *J* = 7.4 Hz, 3H, CH_3), 1.54 – 2.17 (m, 10H, Cp-CH_2 + N^1-CH$_2$-CH_2), 3.14 (m, *J* = 7.9 Hz, 1H, Cp-C*H*), 3.72 (m, 1H, C*H*-OH), 3.69 - 5.58 (m, 7H, N^1-CH_2 + N^3-CH_2 + CH_2-F + CH-O*H*), 5.37 (s_{br}, 1H, C*H*-OH), 12.82 (s_{br}, 1H, N^7-*H*). ^{13}C-NMR (DMSO-d_6): δ 12.1 (*C*H$_3$), 21.7 (N^1-CH$_2$-*C*H$_2$), 25.9 (Cp-*C*H$_2$), 32.8 (Cp-*C*H$_2$), 39.7 (Cp-*C*H), 42.9 (N^1-*C*H$_2$), 45.9 (N^3-*C*H$_2$), 67.3 (*C*H-OH), 86.1 (^1J = 162.0 Hz, *C*H$_2$-F), 107.1 (*C*^{5}), 148.7 (*C*^{8}), 151.8 (*C*^{4}), 154.8 (*C*^{2}), 158.5 (*C*^{6}). ^{19}F-NMR (DMSO-d_6): δ -227.92. MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{16}H_{23}FN_4O_3$ 339.38; Found 339.39.

N-(6-Amino-1-(2,4-dimethoxybenzyl)-2,4-dioxo-3-propyl-1,2,3,4-tetrahydro-pyrimidin-5-yl)-cyclopent-3-enecarboxamide (19a).

At ambient temperature the DMB protected diamine **18** (3.34 g, 10 mmol) was suspended in a mixture of dioxane (100 mL) and water (40 mL).[16] Under efficient stirring 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC, 1.92 g, 10 mmol) and a catalytic amount (~ 150 mg) of 4-dimethylaminopyridine (DMAP) was added. After the addition of 3-cyclopentene-1-carboxylic acid (1.12 g, 10 mmol) the mixture was stirred for 24 h and poured onto ice-water (150 mL). The formed yellow precipitate was collected by filtration, washed with water (50 mL), and recrystallized from MeOH. Yield 2.9 g (65%), m.p. 189°C (MeOH).

^{1}H-NMR ($CDCl_3$): δ 0.98 (t, *J* = 7.4 Hz, 3H, C*H*$_3$), 1.71 (m, *J* = 7.4 Hz, 2H, CH_3-C*H*$_2$),2.72 (d, *J* = 7.9 Hz , 4H, Cp-C*H*$_2$), 3.22 (m, *J* = 7.9 Hz, 1H, Cp-*H*), 3.83 (s, 3H, 4-OC*H*$_3$), 3.94 (s, 3H, 2-OC*H*$_3$), 3.86 (m, 2H, N^1-C*H*$_2$), 5.14 (s, 2H, Ph-C*H*$_2$), 5.72 (s, 2H, Cpen-*H*), 6.14 (s, 2H, N*H*$_2$), 6.50 (dd, *J* = 8.4, 2.4 Hz, 1H, Ph-*H*-5), 6.57 (d, *J* = 2.3 Hz, 1H, Ph-*H*-3), 7.39 (s, 1H, -N*H*-), 7.57 (d, *J* = 8.4 Hz, 1H, Ph-*H*-6). ^{13}C-NMR ($CDCl_3$): δ 11.4 (*C*H_3), 21.2 (CH_3-*C*H_2), 37.1 (Cp-*C*H), 37.4 (Cp-*C*H_2), 39.8 (Ph-*C*H_2), 43.5 (N^1-*C*H_2), 55.5 (4-O*C*H_3), 55.8 (2-O*C*H_3), 92.1 (*C*^{5}), 98.5 (Ph-*C*^{3}), 106.1 (Ph-*C*^{5}), 115.8 (Ph-*C*^{1}), 129.2 (Cpen-*C*), 132.2 (Ph-*C*^{6}), 147.8 (C^2), 151.2 (*C*^{6}),156.7 (*C*^{4}), 159.8 (Ph-*C*^{2}), 161.2 (Ph-*C*^{4}) 176.1 (*C*=O). MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{22}H_{28}N_4O_5$ 429.21; Found 429.23.

N-(6-Amino-1-(2,4-dimethoxybenzyl)-2,4-dioxo-3-propyl-1,2,3,4-tetrahydro-pyrimidin-5-yl)-cyclopent-1-enecarboxamide (19b).

At ambient temperature the DMB protected diamine **18** (3.34 g, 10 mmol) was suspended in a mixture of dioxane (100 mL) and water (40 mL).[16] Under efficient stirring 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC, 1.92 g, 10 mmol) and a catalytic amount (150 mg) of 4-dimethylaminopyridine (DMAP) was added. After the addition of 3-cyclopentene-1-carboxylic acid (1.12 g, 10 mmol) the mixture was stirred for 24 h and poured onto ice-water (150 mL). The formed yellow precipitate was collected by centrifugation, washed

with water (50 mL), and dried. Yield 2.9 g (65%), m.p. 183-184°C. Very insoluble material. MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{22}H_{28}N_4O_5$ 429.21; Found 429.21.

8-(Cyclopent-3-enyl)-3-(2,4-dimethoxybenzyl)-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (20a). A mixture of carboxamide **19a** (4.3 g, 10 mmol) in 2N NaOH (50 mL, 100 mmol) and dioxane (40 mL) was stirred at 85°C for 12 h. Acidification to pH 4 with concentrated aqueous HCl precipitated a solid that was collected by filtration, washed with water, and recrystallized from ethanol to furnish the title xanthine as yellow crystals. Yield 3.44 g (84%), m.p. 250-251°C (EtOH).

^{1}H-NMR ($CDCl_3$): δ 0.87 (t, *J* = 7.4 Hz, 3H, C*H*$_3$), 1.58 (m, *J* = 7.4 Hz, 2H, CH_3-C*H*$_2$), 2.68 (m, *J* = 7.9 Hz , 4H, Cp-C*H*$_2$), 3.51 (m, *J* = 7.9 Hz, 1H, Cp-*H*), 3.71 (s, 3H, 4-OC*H*$_3$), 3.81 (s, 3H, 2-OC*H*$_3$), 3.86 (m, 2H, N^1-C*H*$_2$), 5.09 (s, 2H, Ph-C*H*$_2$), 5.67 (s, 2H, Cpen-*H*), 6.32 (dd, *J* = 8.4, 2.4 Hz, 1H, Ph-*H*-5), 6.46 (d, *J* = 2.3 Hz, 1H, Ph-*H*-3), 6.72 (d, *J* = 8.4 Hz, 1H, Ph-*H*-6), 12.97 (s_{br}, 1H, N^7-*H*). ^{13}C-NMR ($CDCl_3$): δ 11.5 (*C*H$_3$), 21.3 (CH_3-*C*H$_2$), 37.2 (Cp-*C*H), 38.6 (Cp-*C*H$_2$), 41.5 (Ph-*C*H$_2$), 42.5 (N^1-*C*H$_2$), 55.4 (4-OCH$_3$), 55.6 (2-O*C*H$_3$), 98.4 (Ph-*C*^{3}), 104.4 (Ph-*C*^{5}), 106.9 (*C*^{5}), 117.3 (Ph-*C*^{1}), 127.3 (Ph-*C*^{6}), 129.4 (Cpen-*C*), 148.4 (*C*^{8}), 151.2 (C^4),154. 5 (*C*^{2}), 157.9 (*C*^{6}), 158.3 (Ph-*C*^{2}), 160.3 (Ph-*C*^{4}). MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{22}H_{26}N_4O_4$ 411.20; Found 411.21.

8-(Cyclopent-1-enyl)-3-(2,4-dimethoxybenzyl)-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (20b). A mixture of carboxamide **19b** (4.3 g, 10 mmol) in 2N NaOH (50 mL, 100 mmol) and dioxane (40 mL) was stirred at 85°C for 12 h. Acidification to pH 4 with concentrated aqueous HCl precipitated a solid that was collected by filtration, washed with water, and dried to furnish the title xanthine as orange-red crystals. Yield 2.95 g (72%), m.p. 280-281°C. Very insoluble material. MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{22}H_{26}N_4O_4$ 411.20; Found 411.19.

***cis*-2-Benzyloxycyclopentane-1-carboxylic acid.**

a) cis-Ethyl 2-benzyloxycyclopentane-1-carboxylate.[17]

Under argon benzyl-2,2,2-trichloroacetimidate (4.45 g, 3.34 mL, 18 mmol) was added to a magnetically stirred solution of of *cis*-ethyl 2-hydroxycyclopentane-1-carboxylate (2.37 g, 2.21

mL,15 mmol) in a solvent mixture of dichloromethane (10 mL) and hexane (20 mL). After the addition of neat trifluoromethanesulfonic acid (0.2 mL) the clear yellow solution became turbid (slightly exothermic) and after 5 min the colour changed to light orange. The mixture was stirred at ambient temperature for 3 h (TLC Si 60, hexane / ethyl acetate, 80 + 20, R_f 0.75), the formed solid was removed by filtration, and the filter cake was washed with a mixture of DCM and hexane (10 + 20, 2 x 25 mL). The combined filtrates were washed with sat. aqueous $NaHCO_3$ (50 mL) and water (50 mL), the organic layer was dried over anhydrous Na_2SO_4, filtered and rota-evaporated *in vacuo* furnishing the title compound as a yellow oil (3.72 g , quant.) that was used without further purification for the next step. An aliquot of the benzyl ether was purified by column chromatography on silica gel using 15% ethyl acetate in hexane as an eluent.
^{1}H NMR ($CDCl_3$): δ 1.27 (t, *J* = 7 Hz, 3H, C*H*$_3$), 1.54 – 2.07 (m, 6H, cyclopentane C*H*$_2$), 2.93 (ddd, *J* = 5.9, 5.9, 5.8 Hz, 1H, Cp-C-1-*H*), 4.07 – 4.31 (m, 3H, C*H*$_2$CH$_3$ + Cp-C-2-*H*), 4.50 and 4.61 (AB system, *J* = 12.2 Hz, 2H, C*H*$_2$C$_6$H$_5$), 7.21 – 7.47 (m, 5H, C$_6$*H*$_5$); ^{13}C NMR ($CDCl_3$): δ 14.3, 22.1, 25.5, 31.1, 49.6, 60.3, 71.1, 81.7, 127.4, 128.2, 128.4, 138.7, 172.8.

b) *cis*-2-Benzyloxycyclopentane-1-carboxylic acid.
A solution of crude *cis*-ethyl 2-benzyloxycyclopentane-1-carboxylate (4.96 g, 20 mmol) and 4M NaOH (20 mL, 80 mmol) in ethanol (50 mL) was stirred at ambient temperature for 48 h. The reaction mixture was concentrated *in vacuo* (40°C), water (50 mL) was added to the aqueous residue, and the resultant basic solution was extracted with diethyl ether (2 x 50 mL). The aqueous layer was acidified to pH1 with aqueous HCl (10N, 8.2 mL, 82 mmol), saturated with solid NaCl, and extracted with DCM (2 x 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and rota-evaporated *in vacuo* providing the title compound (2.42 g, 67%) as a yellow oil (TLC Si 60, hexane / ethyl acetate / acetic acid, 80 + 20 + 0.2).
^{1}H NMR ($CDCl_3$): δ 1.58 – 2.36 (m, 6H, cyclopentane C*H*$_2$), 2.92 – 3.03 (m, 1H, Cp-C-1-*H*), 4.25 – 4.36 (m, 1H, Cp-C-2-*H*), 4.50 – 4.71 (m, 2H, C*H*$_2$C$_6$H$_5$), 7.24 – 7.48 (m, 5H, C$_6$*H*$_5$), 10.92 (s$_{br}$, 1H, COO*H*); ^{13}C NMR ($CDCl_3$): δ 23.6, 28.6, 31.1, 50.7, 71.5, 83.5, 127.4, 128.2, 128.4, 138.2, 181.7.

cis-8-(2-Benzyloxycyclopentyl)-3-(2,4-dimethoxybenzyl)-1-propyl-1_*H*_-purine-2,6(3_*H*_,7_*H*_)-dione (20c).

Under an argon atmosphere neat isobutyl chloroformate (10 mmol, 1.3 mL) and 4-methylmorpholine (10 mmol, 1.1 mL) were added to a well stirred ice cold solution of the DMB protected diamine **18** (3.34 g, 10 mmol) in dry DMF (30 mL).[16] At this temperature a solution of 2-benzyloxycyclopentanecarboxylic acid (2.2 g, 10 mmol) in dry DMF (5 mL) was added dropwise. The mixture was stirred for 12 h and allowed to warm to ambient temperature during this time. The reaction mixture was poured onto ice-water (150 mL), the formed yellow precipitate was collected by filtration, washed with water (50 mL), and dried to give the intermediate carboxamide in 79% yield. The amide (3.4 g, 6.5 mmol) was stirred for 12 h at 85°C in a mixture of 2N NaOH (32.5 mL, 65 mmol) and dioxane (22 mL). After cooling to ambient temperature acidification to pH 4 with concentrated aqueous HCl precipitated a solid that was collected by filtration, washed with water, and recrystallized from ethanol to furnish the title xanthine as slightly red crystals. Yield 2.69 g (74%), m.p. 94-96°C (EtOH).
^{1}H-NMR (DMSO-d_6): δ 0.88 (t, J = 7.4 Hz, 3H, CH_3), 1.46-2.17 (m, 8H, CH_3-CH_2 + Cp-CH_2), 3.01-3.26 (m, 1H, Cp-C-1-*H*), 3.71 (s, 3H, 4-OCH_3), 3.82 (s, 3H, 2-OCH_3), 3.87 (m, 2H, N^1-CH_2), 4.08-4.23 (m, 1H, Cp-C-2-*H*), 4.38 (s, 2H, Ph-CH_2-O), 5.07 (s, 2H, Ph-CH_2-N), 6.37 (dd, J = 8.4, 2.4 Hz, 1H, N-CH_2-Ph-*H*-5), 6.59 (d, J = 2.3 Hz, 1H, N-CH_2-Ph-*H*-3), 6.72 (d, J = 8.4 Hz, 1H, N-CH_2-Ph-*H*-6), 7.05-7.38 (m, 5H, O-CH_2-Ph), 13.30 (s_{br}, 1H, N^7-*H*). ^{13}C-NMR (DMSO-d_6): δ 11.6 (*C*H_3), 21.4 (CH_3-*C*H_2), 23.0 (Cp-*C*^{4}), 30.8 (Cp-*C*^{5}), 31.9 (Cp-*C*^{3}), 41.6 (N-*C*H_2-Ph), 42.6 (N-*C*H_2-CH_2-), 45.9 (Cp-*C*^{1}), 55.6 (4-O*C*H_3), 55.9 (2-O*C*H_3), 70.8 (O-*C*H_2-Ph), 85.1 (Cp-*C*^{2}), 98.8 (N-CH_2-Ph-*C*^{3}), 104.9 (N-CH_2-Ph-*C*^{5}), 106.7 (*C*5), 117.3 (N-CH_2-Ph-*C*^{1}), 127.3 (N-CH_2-Ph-*C*^{6}), 127.7 (O-CH_2-Ph), 128.5 (O-CH_2-Ph), 139.1 (O-CH_2-Ph), 148.5 (*C*^{8}), 151.2 (*C*^{4}), 154.4 (*C*^{2}), 156.7 (*C*^{6}), 157.9 (N-CH_2-Ph-*C*^{2}), 160.0 (N-CH_2-Ph-*C*^{4}). MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{29}H_{34}N_4O_5$ 519.25; Found 519.27.

3-(2,4-Dimethoxybenzyl)-8-(cyclopent-3-enyl)-7-pivaloyloxymethyl-1-propyl-1_*H*_-purine-2,6(3_*H*_,7_*H*_)-dione (21a).

At ambient temperature and under the exclusion of moisture K_2CO_3 (1.06 g, 7.7 mmol) was added to a well stirred suspension of **20a** (2.87 g, 7 mmol) in dry DMF (30 mL). The mixture was heated

to 60°C and stirred at that temperature for 0.5 h. PomCl (1.42 mL, 9.8 mmol) was added and stirring was continued for 5 h. The mixture was cooled to ambient temperature, poured onto ice-water (200 mL), and extracted with diethyl ether (2 x 100 mL). The combined organic extracts were washed with water (2 x 100 mL), brine (100 mL), and dried over anhydrous sodium sulfate. Filtration and evaporation *in vacuo* left a yellow solid that was recrystallized from ethanol to provide **21a** as orange crystals. Yield 3.37 g (92%), m.p. 122-123°C (EtOH).
^{1}H-NMR ($CDCl_3$): δ 0.96 (t, *J* = 7.4 Hz, 3H, C*H*$_3$), 1.22 (s, 9H, N^7-POM-C*H*$_3$), 1.69 (m, *J* = 7.4 Hz, 2H, CH_3-C*H*$_2$), 2.82 (d, *J* = 7.9 Hz , 4H, Cp-C*H*$_2$), 3.71 (m, *J* = 7.9 Hz, 1H, Cp-*H*), 3.80 (s, 3H, 4-OC*H*$_3$), 3.82 (s, 3H, 2-OC*H*$_3$), 3.99 (m, 2H, N^1-C*H*$_2$), 5.28 (s, 2H, Ph-C*H*$_2$), 5.76 (s, 2H, Cpen-*H*), 6.28 (s, 2H, N^7-POM-C*H*$_2$), 6.43 (dd, *J* = 8.4, 2.4 Hz, 1H, Ph-*H*-5), 6.46 (d, *J* = 2.3 Hz, 1H, Ph-*H*-3), 7.11 (d, *J* = 8.4 Hz, 1H, Ph-*H*-6). ^{13}C-NMR ($CDCl_3$): δ 11.3 (*C*H_3), 21.3 (CH_3-*C*H_2), 26.9 (N^7-POM-*C*H_3), 34.4 (Cp-*C*H), 38.7 (N^7-POM-*C*$(CH_3)_3$), 38.9 (Cp-*C*H_2), 41.6 (Ph-*C*H_2), 42.8 (N^1-*C*H_2), 55.3 (4-O*C*H_3), 55.4 (2-O*C*H_3), 66.8 (N^7-POM-*C*H_2), 98.5 (Ph-*C*^{3}), 103.9 (Ph-*C*^{5}), 106.6 (*C*^{5}), 117.3 (Ph-*C*^{1}), 128.9 (Ph-*C*^{6}), 129.4 (Cpen-*C*), 148.4 (*C*^{8}), 151.2 (*C*^{4}), 154.7 (*C*^{2}), 158.3 (*C*^{6}) 158.7 (Ph-*C*^{2}), 158.8 (*C*^{4}), 160.3 (Ph-*C*^{4}), 177.3 (*C*=O). MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{28}H_{36}N_4O_6$ 525.26; Found 525.26.

3-(2,4-Dimethoxybenzyl)-8-(cyclopent-1-enyl)-7-pivaloyloxymethyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (21b).

At ambient temperature and under the exclusion of moisture K_2CO_3 (1.06 g, 7.7 mmol) was added to a well stirred suspension of **20b** (2.87 g, 7 mmol) in dry DMF (30 mL). The mixture was heated to 60°C and stirred at that temperature for 0.5 h. PomCl (1.42 mL, 9.8 mmol) was added and stirring was continued for 5 h at 60°C. The mixture was cooled to ambient temperature, poured onto ice-water (200 mL), and the precipitated solid product was collected by filtration. Recrystallization from ethanol furnished **21b** as brick-red crystals. Yield 3.48 g (95%), m.p. 92-94°C (EtOH).
^{1}H-NMR (DMSO-d_6): δ 0.87 (t, *J* = 7.4 Hz, 3H, C*H*$_3$), 1.15 (s, 9H, N^7-POM-C*H*$_3$), 1.58 (m, *J* = 7.4 Hz, 2H, CH_3-C*H*$_2$), 1.93 (m, *J* = 7.4 Hz, 2H, Cp-C^4-C*H*$_2$), 2.51-2.83 (2 m, 4H, Cp-C^3-C*H*$_2$ + Cp-C^5-C*H*$_2$), 3.80 (s, 3H, 4-OC*H*$_3$), 3.83 (s, 3H, 2-OC*H*$_3$), 3.88 (m, 2H, N^1-C*H*$_2$), 5.09 (s, 2H, Ph-C*H*$_2$), 6.26 (s, 2H, N^7-POM-C*H*$_2$), 6.42 (dd, *J* = 8.4, 2.4 Hz, 2H, Ph-*H*-5 + Cp-C^2-C*H*), 6.59 (d, *J*

= 2.3 Hz, 1H, Ph-*H*-3), 6.77 (d, *J* = 8.4 Hz, 1H, Ph-*H*-6). ^{13}C-NMR (DMSO-d_6): δ 11.56 (*C*H$_3$), 21.3 (CH$_3$-*C*H$_2$), 22.8 (Cp-*C*4), 27.0 (N^7-POM-*C*H$_3$), 34.4 (Cp-*C*^{3}), 34.7 (Cp-*C*^{5}), 38.8 (N^7-POM-*C*(CH$_3$)$_3$), 41.5 (Ph-*C*H$_2$), 42.6 (N^1-*C*H$_2$), 55.7 (4-O*C*H$_3$), 56 (2-O*C*H$_3$), 69.1 (N^7-POM-*C*H$_2$), 98.9 (Ph-*C*^{3}), 104.9 (Ph-*C*^{5}), 107.1 (*C*^{5}), 116.8 (Ph-*C*^{1}), 127.6 (Ph-*C*^{6}), 131.5 (Cp-*C*^{2}), 137.9 (Cp-*C*^{1}), 148.2 (*C*^{8}), 150.2 (*C*^{4}), 150.9 (*C*^{2}), 154.4 (*C*^{6}) 157.9 (Ph-*C*^{2}), 160.3 (Ph-*C*^{4}), 176.7 (*C*=O). MS (ESI+) m/z: [M+H]$^+$ Calcd for $C_{28}H_{36}N_4O_6$ 525.26; Found 525.26.

cis-8-(2-Benzyloxycyclopentyl)-3-(2,4-dimethoxybenzyl)-7-pivaloyloxymethyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (21c).

At ambient temperature and under the exclusion of moisture K_2CO_3 (760 mg, 5.5 mmol) was added to a well stirred suspension of **20c** (2.59 g, 5 mmol) in dry DMF (30 mL) and the mixture was stirred at that temperature for 0.5 h. PomCl (0.9 mL, 6.25 mmol) was added and stirring was continued for 48 h. The mixture was poured onto ice-water (200 mL), and the precipitated solid product was collected by filtration. Recrystallization from aqueous methanol furnished **21c** as fawn crystals. Yield 3.02 g (88%), m.p. 118-120°C ($MeOH_{aq}$).

^{1}H-NMR (DMSO-d_6): δ 0.98 (t, *J* = 7.4 Hz, 3H, C*H*$_3$), 1.20 (s, 9H, N^7-POM-C*H*$_3$), 1.59-2.23 (m, 8H, CH$_3$-C*H*$_2$ + Cp-C*H*$_2$), 3.25-3.48 (m, 1H, Cp-C^1-*H*), 3.79 (s, 3H, 4-OC*H*$_3$), 3.82 (s, 3H, 2-OC*H*$_3$), 3.94 (m, 2H, N^1-C*H*$_2$), 4.11-4.25 (m, 1H, Cp-C^2-*H*), 4.41 (s, 2H, Ph-C*H*$_2$-O), 5.06 (s, 2H, Ph-C*H*$_2$-N), 6.23 (s, 2H, N^7-POM-C*H*$_2$), 6.39 (dd, *J* = 8.4, 2.4 Hz, 1H, N-CH$_2$-Ph-*H*^{5}), 6.56 (d, *J* = 2.3 Hz, 1H, N-CH$_2$-Ph-*H*^{3}), 6.70 (d, *J* = 8.4 Hz, 1H, N-CH$_2$-Ph-*H*^{6}), 7.01-7.34 (m, 5H, O-CH$_2$-Ph). ^{13}C-NMR (DMSO-d_6): δ 11.3 (*C*H$_3$), 21.3 (CH$_3$-*C*H$_2$), 22.7 (Cp-*C*^{4}), 26.9 (N^7-POM-*C*H$_3$), 30.8 (Cp-*C*^{5}), 31.9 (Cp-*C*^{3}), 38.8 (N^7-POM-*C*(CH$_3$)$_3$), 41.6 (N-*C*H$_2$-Ph), 43.3 (N-*C*H$_2$-CH$_2$), 45.9 (Cp-*C*^{1}), 55.3 (4-O*C*H$_3$), 55.4 (2-O*C*H$_3$), 66.7 (N^7-POM-*C*H$_2$), 72.0 (O-*C*H$_2$-Ph), 85.6 (Cp-*C*^{2}), 98.5 (N-CH$_2$-Ph-*C*^{3}), 103.9 (N-CH$_2$-Ph-*C*^{5}), 106.6 (*C*^{5}), 117.2 (N-CH$_2$-Ph-*C*^{1}), 127.5 (N-CH$_2$-Ph-*C*^{6}), 127.6 (O-CH$_2$-Ph), 128.3 (O-CH$_2$-Ph), 138.3 (O-CH$_2$-Ph), 148.4 (*C*^{8}), 151.1(*C*^{4}), 154.4 (*C*^{2}), 156.6 (*C*^{6}), 158.3 (N-CH$_2$-Ph-*C*^{2}), 160.3 (N-CH$_2$-Ph-*C*^{4}), 177.2 (*C*=O). MS (ESI+) m/z: [M+H]$^+$ Calcd for $C_{35}H_{44}N_4O_7$ 633.32; Found 633.31.

8-(Cyclopent-3-enyl)-7-pivaloyloxymethyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (22a).

Under stirring xanthine **21a** (2.62 g, 5 mmol) was dissolved in trifluoroacetic acid (TFA, 20 mL) and the solution was heated to 50°C. After 7 h TFA was evaporated *in vacuo* and the solid residue recrystallized from aqueous MeOH to yield **22a** as fawn crystals. Yield 1.5 g (80%), m.p. 141-143°C ($MeOH_{aq}$).

^{1}H-NMR (DMSO-d_6): δ 0.86 (t, J = 7.4 Hz, 3H, CH_3), 1.25 (s, 9H, N^7-POM-CH_3), 1.51 (m, J = 7.4 Hz, 2H, CH_3-CH_2), 2.72 (m, J = 7.9 Hz , 4H, Cp-CH_2), 3.83 (m, 3H, Cp-H + N^1-CH_2), 5.75 (s, 2H, Cpen-H), 6.22 (s, 2H, N^7-POM-CH_2), 11.94 (s_{br}, 1H, N^3-H). ^{13}C-NMR (DMSO-d_6): δ 11.6 (CH_3), 21.3 (CH_3-CH_2), 27.1 (N^7-POM-CH_3), 33.7 (Cp-CH), 38.8 (N^7-POM-C$(CH_3)_3$), 38.9 (Cp-CH_2), 41.6 (N^1-CH_2), 67.5 (N^7-POM-CH_2), 106.1 (C^5), 129.4 (Cpen-C), 147.5 (C^8), 151.2 (C^4), 154.9 (C^2), 159.8 (C^6), 176.7 (C=O). MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{19}H_{26}N_4O_4$ 375.20; Found 375.20.

8-(Cyclopent-1-enyl)-7-pivaloyloxymethyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (22b).

Under stirring xanthin **21b** (2.62 g, 5 mmol) was dissolved in trifluoroacetic acid (TFA, 20 mL) and the solution was heated to 50°C. After 7 h TFA was evaporated *in vacuo* and the solid residue recrystallized from aqueous MeOH to yield **22b** as off-white crystals. Yield 0.98 g (53%), m.p. 193°C ($MeOH_{aq}$).

^{1}H-NMR (DMSO-d_6): δ 0.87 (t, J = 7.4 Hz, 3H, CH_3), 1.14 (s, 9H, N^7-POM-CH_3),1.55 (m, J = 7.4 Hz, 2H, CH_3-CH_2), 1.96 (m, J = 7.4 Hz, 2H, Cp-C-4-CH_2), 2.54-2.87 (2 m, 4H, Cp-C-3-CH_2 + Cp-C-5-CH_2), 3.84 (m, 2H, N^1-CH_2), 6.24 (s, 2H, N^7-POM-CH_2), 6.49 (m, 1H, Cp-C-2-CH), 12.01 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6): δ 11.6 (CH_3), 21.3 (CH_3-CH_2), 22.7 (Cp-C^4), 27.1 (N^7-POM-CH_3), 34.4 (Cp-C^3), 34.8 (Cp-C^5), 38.8 (N^7-POM-C$(CH_3)_3$), 41.7 (N^1-CH_2), 68.9 (N^7-POM-CH_2), 106.8 (C^5), 131.6 (Cp-C^2), 137.4 (Cp-C^1), 147.5 (C^8), 150.2 (C^4), 155.1 (C^2), 159.1 (C^6), 176.6 (C=O). MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{19}H_{26}N_4O_4$ 375.20; Found 375.21.

***cis*-8-(2-Benzyloxycyclopentyl)-7-pivaloyloxymethyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (22c).**

Under stirring xanthine **21c** (2.53 g, 4 mmol) was dissolved in trifluoroacetic acid (TFA, 20 mL) and the solution was heated to 50°C. After 7 h TFA was evaporated *in vacuo* and the oily residue

was purified by chromatography on silica gel (eluent 10% acetonitrile in ethyl acetate) to provide the deprotected xanthine **22c** as a dark-brown viscous oil. Yield 1.64 g (85%).
^{1}H-NMR (DMSO-d_6): δ 0.87 (t, J = 7.4 Hz, 3H, CH_3), 1.09 (s, 9H, N^7-POM-CH_3), 1.57-2.15 (m, 8H, CH_3-CH_2 + Cp-CH_2), 3.20-3.43 (m, 1H, Cp-1-H), 3.84 (m, 2H, N^1-CH_2), 4.04-4.21 (m, 1H, Cp-C-2-*H*), 4.43 (s, 2H, Ph-CH_2-O), 6.26 (s, 2H, N^7-POM-CH_2), 7.25 (m, 5H, O-CH_2-Ph), 11.94 (s_{br}, 1H, N^3-*H*). ^{13}C-NMR (DMSO-d_6): δ 11.6 (*C*H_3), 21.2 (CH_3-*C*H_2), 21.5 (Cp-*C*^{4}), 26.9 (N^7-POM-*C*H_3), 30.9 (Cp-*C*^{5}), 33.1 (Cp-*C*^{3}), 38.7 (N^7-POM-*C*$(CH_3)_3$), 42.7 (N^1-*C*H_2), 45.9 (Cp-*C*^{1}), 66.7 (N^7-POM-*C*H_2), 71.1 (O-*C*H_2-Ph), 85.3 (Cp-*C*^{2}), 106.6 (*C*^{5}), 127.8 (O-CH_2-Ph), 128.5 (O-CH_2-Ph), 138.9 (O-CH_2-Ph), 147.6 (*C*^{8}), 151.2 (C^4), 154.9 (*C*^{2}), 157.9 (*C*^{6}), 176.8 (*C*=O). MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{26}H_{34}N_4O_5$ 483.25; Found 483.24.

8-(Cyclopent-3-enyl)-3-(3-fluoropropyl)-7-pivaloyloxymethyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (23a).

At ambient temperature and under the exclusion of moisture K_2CO_3 (730 mg, 5.3 mmol) was added to a well stirred solution of **22a** (1.65 g, 4.4 mmol) in dry DMF (20 mL). The mixture was heated to 60°C and stirred at that temperature for 0.5 h. 1-Bromo-3-fluoropropane (565 µL, 6.16 mmol) was added and stirring was continued for 18 h. The mixture was cooled to ambient temperature, poured onto ice-water (150 mL), and extracted with diethyl ether (2 x 100 mL). The combined organic extracts were washed with water (2 x 100 mL), brine (100 mL), and dried over anhydrous sodium sulfate. Filtration and evaporation *in vacuo* left a solid that was recrystallized from 50% aqueous MeOH to give **23a** as orange-brown crystals. Yield 1.82 g (95%), m.p. 62-64°C (50% MeOH).
^{1}H-NMR ($CDCl_3$): δ 0.95 (t, J = 7.4 Hz, 3H, CH_3), 1.20 (s, 9H, N^7-POM-CH_3), 1.67 (m, J = 7.4 Hz, 2H, CH_3-CH_2), 2.19 (dt, J = 27.3, 6.1 Hz, 2H, CH_2-CH_2F), 2.83 (d, J = 7.9 Hz , 4H, Cp-CH_2), 3.70 (m, J = 7.9 Hz, 1H, Cp-*H*), 3.97 (m, J = 7.1 Hz, 2H, N^1-CH_2), 4.26 (t, J = 6.7 Hz, 2H, N^3-CH_2), 4.55 (dt, J = 47.2, 5.7 Hz, 2H, CH_2F), 5.76 (s, 2H, Cpen-*H*), 6.25 (s, 2H, N^7-POM-CH_2).
^{13}C-NMR ($CDCl_3$): δ 11.3 (*C*H_3), 21.3 (CH_3-*C*H_2), 26.9 (N^7-POM-*C*H_3), 29.3 (^2J = 19.2 Hz, *C*H_2-CH_2F), 34.4 (Cp-*C*H), 38.9 (Cp-*C*H_2), 39.1 (N^7-POM-*C*$(CH_3)_3$), 40.1 (^3J = 5.5 Hz, N^3-*C*H_2), 42.8 (N^1-*C*H_2), 66.7 (N^7-POM-*C*H_2), 80.4 (^1J = 162.0 Hz , *C*H_2F), 106.6 (*C*^{5}), 128.9 (Cpen-*C*), 147.8

(C^8), 151.2 (C^4), 154.5 (C^2), 158.9 (C^6), 177.2 (*C*=O). ^{19}F-NMR ($CDCl_3$): δ - 219.96. MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{22}H_{31}FN_4O_4$ 435.23; Found 435.23.

8-(Cyclopent-1-enyl)-3-(3-fluoropropyl)-7-pivaloyloxymethyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (23b).

At ambient temperature and under the exclusion of moisture K_2CO_3 (730 mg, 5.3 mmol) was added to a well stirred solution of **22b** (1.65 g, 4.4 mmol) in dry DMF (20 mL). The mixture was heated to 60°C and stirred at that temperature for 0.5 h. 1-Bromo-3-fluoropropane (565 µL, 6.16 mmol) was added and stirring was continued for 18 h at 60°C. The mixture was cooled to ambient temperature and poured onto ice-water (150 mL). After standing overnight at 4°C the solid was collected by filtration recrystallized from 50% aqueous MeOH to give **23b** as yellow crystals. Yield 1.89 g (98%), m.p. 195°C (50% MeOH).

^{1}H-NMR ($CDCl_3$): δ 0.98 (t, *J* = 7.4 Hz, 3H, C*H*$_3$), 1.24 (s, 9H, N^7-POM-C*H*$_3$), 1.68 (m, *J* = 7.4 Hz, 2H, CH$_3$-C*H*$_2$), 1.97-2.39 (m, 4H, Cp-C-4-C*H*$_2$ + C*H*$_2$-CH$_2$F), 2.59-2.75 (m, *2*H, Cp-C*H*$_2$), 2.82-2.98 (m, 2H, Cp-C*H*$_2$), 4.00 (m, *J* = 7.1 Hz, 2H, N^1-C*H*$_2$), 4.30 (t, *J* = 6.7 Hz, 2H, N^3-C*H*$_2$), 4.57 (dt, *J* = 47.2, 5.7 Hz, 2H, C*H*$_2$F), 6.31 (s, 2H, N^7-POM-C*H*$_2$), 6.41 (m, 1H, Cp-C*H*). ^{13}C-NMR ($CDCl_3$): δ 11.3 (*C*H$_3$), 21.3 (CH$_3$-*C*H$_2$), 26.9 (N^7-POM-*C*H$_3$), 29.2 (^2J = 19.2 Hz, *C*H$_2$-CH$_2$F), 34.4 (Cp-*C*3), 34.8 (Cp-*C*5), 38.9 (N^7-POM-*C*(CH$_3$)$_3$), 40.1 (^3J = 5.5 Hz, N^3-*C*H$_2$), 42.9 (N^1-*C*H$_2$), 68.4 (N^7-POM-*C*H$_2$), 80.4 (^1J = 162.0 Hz , *C*H$_2$F), 106.3 (C^5), 131.8 (Cp-C^2), 137.5 (Cp-C^1), 147.9 (C^8), 150.4 (C^4), 151.1 (C^2), 154.6 (C^6) 177.1 (*C*=O). ^{19}F-NMR ($CDCl_3$): δ - 219.11. MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{22}H_{31}FN_4O_4$ 435.23; Found 435.22.

cis-8-(2-Benzyloxycyclopentyl)-3-(3-fluoropropyl)-)-7-pivaloyloxymethyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (23c).

At ambient temperature and under the exclusion of moisture K_2CO_3 (456 mg, 3.3 mmol) was added to a well stirred solution of **23b** (1.45 g, 3 mmol) in dry DMF (20 mL). The mixture was heated to 60°C and stirred at that temperature for 0.5 h. 1-Bromo-3-fluoropropane (303 µL, 3.3 mmol) was added and stirring was continued for 24 h at 60°C. The mixture was cooled to ambient temperature and poured onto ice-water (150 mL). The aqueous mixture was extracted with diethyl ether (2 x 100 mL), the combined organic extracts were washed with water (2 x 100 mL) and

brine (100 mL), and dried over anhydrous sodium sulfate. Filtration and rota-evaporation *in vacuo* gave 706 mg (43%) of a chromatographically pure light brown oil.

^{1}H-NMR ($CDCl_3$): δ 0.97 (t, *J* = 7.4 Hz, 3H, C*H*$_3$), 1.19 (s, 9H, N^7-POM-C*H*$_3$), 1.59-2.38 (m, 10H, C*H*$_2$-CH$_3$+ Cp-C*H*$_2$ + C*H*$_2$-CH$_2$F)), 3.16-3.41 (m, 1H, Cp-*H*^{1}), 3.84 (m, 2H, N^1-C*H*$_2$), 4.11-4.23 (m, 1H, Cp-*H*^{2}), 4.41 (m, 2H, Ph-C*H*$_2$-O), 3.97 (m, 2H, N^1-C*H*$_2$), 4.26 (m, 2H, N^3-C*H*$_2$), 4.55 (dt, *J* = 47.1, 5.6 Hz, 2H, C*H*$_2$F), 6.25 (s, 2H, N^7-POM-C*H*$_2$), 7.18-7.30 (m, 5H, O-CH$_2$-Ph). ^{13}C-NMR ($CDCl_3$): δ 11.4 (*C*H$_3$), 21.3(CH$_3$-*C*H$_2$), 21.5 (Cp-*C*^{4}), 26.9 (N^7-POM-*C*H$_3$), 29.3 (^{2}*J* = 19.2 Hz, *C*H$_2$-CH$_2$F), 30.9 (Cp-*C*^{5}), 33.1 (Cp-*C*^{3}), 38.9 (N^7-POM-*C*(CH$_3$)$_3$), 40.0 (^{3}*J* = 5.5 Hz, N^3-*C*H$_2$), 42.7 (N^1-*C*H$_2$), 45.8 (Cp-*C*^{1}), 66.7 (N^7-POM-*C*H$_2$), 70.9 (O-*C*H$_2$-Ph), 80.3 (^{1}*J* = 162.0 Hz , *C*H$_2$F), 85.3 (Cp-*C*^{2}), 106.6 (*C*^{5}), 127.7 (O-CH$_2$-Ph), 128.9 (O-CH$_2$-Ph), 139.0 (O-CH$_2$-Ph), 147.7 (*C*^{8}), 151.1 (*C*^{4}), 154.7 (*C*^{2}), 158.2 (*C*^{6}), 177.1 (*C*=O). ^{19}F-NMR ($CDCl_3$): δ - 217.90. MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{29}H_{39}FN_4O_5$ 543.29; Found 543.30.

8-(Cyclopent-3-enyl)-3-(3-fluoropropyl)-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (24a).

At ambient temperature compound **23a** (1.3 g, 3 mmol) was stirred overnight in 7N methanolic ammonia (25 mL). After evaporation of the solvent the solid residue was recrystallized from methanol to furnish the deprotected xanthine **24a** as fawn crystals. Yield 750 mg (78%), m.p. 198-200°C (MeOH).

^{1}H-NMR (DMSO-*d*$_6$): δ 0.87 (t, *J* = 7.4 Hz, 3H, C*H*$_3$), 1.57 (m, *J* = 7.4 Hz, 2H, CH$_3$-C*H*$_2$), 2.07 (dt, *J* = 27.3, 6.1 Hz, 2H, C*H*$_2$-CH$_2$F), 2.71 (m, *J* = 7.9 Hz , 4H, Cp-C*H*$_2$), 3.58 (m, *J* = 7.9 Hz, 1H, Cp-*H*), 3.85 (m, *J* = 7.1 Hz, 2H, N^1-C*H*$_2$), 4.11 (t, *J* = 6.7 Hz, 2H, N^3-C*H*$_2$), 4.51 (dt, *J* = 47.2, 5.7 Hz, 2H, C*H*$_2$F), 5.76 (s, 2H, Cpen-*H*), 13.21 (s$_{br}$, 1H, N^7-*H*). ^{13}C-NMR (DMSO-*d*$_6$): δ 11.6 (*C*H$_3$), 21.3 (CH$_3$-*C*H$_2$), 27.9 (N^7-POM-*C*H$_3$), 29.2 (*J* = 19.2 Hz, *C*H$_2$-CH$_2$F), 37.1 (Cp-*C*H), 38.7 (Cp-*C*H$_2$), 41.1 (*J* = 5.5 Hz, N^3-*C*H$_2$), 42.5 (N^1-*C*H$_2$), 74.3 (N^7-POM-*C*H$_2$), 80.9 (*J* = 162.0 Hz , *C*H$_2$F), 106.8 (*C*-5), 129.7 (Cpen-*C*), 148.0 (*C*-8), 151.2 (*C*-4), 154.3 (*C*-2), 158.1 (*C*-6). ^{19}F-NMR (DMSO-*d*$_6$): δ - 218.41. MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{16}H_{21}FN_4O_2$ 321.16; Found 321.15.

8-(Cyclopent-1-enyl)-3-(3-fluoropropyl)-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (24b).

At ambient temperature compound **23b** (1.3 g, 3 mmol) was stirred overnight in 7N methanolic ammonia (25 mL). After evaporation of the solvent the solid residue was recrystallized from methanol to provide the deprotected xanthine **24b** as fawn crystals. Yield 720 mg (75%), m.p. 278°C (MeOH).

^{1}H-NMR (DMSO-d_6): δ 0.88 (t, J = 7.4 Hz, 3H, CH_3), 1.57 (m, J = 7.4 Hz, 2H, CH_3-CH_2), 1.85-2.25 (m, 4H, Cp-C-4-CH_2 + CH_2-CH_2F), 2.41-2.58 (m, *2*H, Cp-CH_2), 2.63-2.80 (m, 2H, Cp-CH_2), 3.85 (m, J = 7.1 Hz, 2H, N^1-CH_2), 4.24 (t, J = 6.7 Hz, 2H, N^3-CH_2), 4.52 (dt, J = 47.2, 5.7 Hz, 2H, CH_2F), 6.46 (m, 1H, Cp-C-2-C*H*), 13.38 (s_{br}, 1H, N^7-*H*). ^{13}C-NMR (DMSO-d_6): δ 11.7 (*C*H_3), 21.5 (CH_3-*C*H_2), 29.2 (^2J = 19.2 Hz, *C*H_2-CH_2F), 33.1 (Cp-*C*^{3}), 33.4 (Cp-*C*^{5}), 40.0 (^3J = 5.5 Hz, N^3-*C*H_2), 42.2 (N^1-*C*H_2), 81.0 (^1J = 162.0 Hz , *C*H_2F), 107.3 (*C*^{5}), 131.1 (Cp-*C*^{2}), 137.1 (Cp-*C*^{1}), 147.9 (*C*^{8}), 149.3 (*C*^{4}), 151.5 (*C*^{2}), 156.1 (*C*^{6}). ^{19}F-NMR (DMSO-d_6): δ - 218.88. MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{16}H_{21}FN_4O_2$ 321.16; Found 321.16.

cis-8-(2-Benzyloxycyclopentyl)-3-(3-fluoropropyl)-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (24c).

At ambient temperature compound **23c** (543 mg, 1 mmol) was stirred in 7N methanolic ammonia (25 mL) for 24 h. After evaporation of the solvent the oily residue was purified by chromatography on silica gel (eluent 5% acetonitrile in ethyl acetate) to give the deprotected xanthine **24c** as a light yellow oil. Yield 348 mg (81%).

^{1}H-NMR (DMSO-d_6): δ 1.01 (t, J = 7.4 Hz, 3H, CH_3), 1.60-2.41 (mm, 10H, CH_3-CH_2 + Cp-CH_2 + FCH_2-CH_2)), 3.29-3.50 (m, 1H, Cp-1-H), 4.04 (m, 2H, N^1-CH_2), 4.13-4.26 (m, 1H, Cp-C-2-*H*), 4.36 (m, 2H, Ph-CH_2-O), 4.21 (m, 2H, N^3-CH_2), 4.60 (dt, J = 47.2, 5.7 Hz, 2H, CH_2F), 7.18-7.31 (m, 5H, O-CH_2-Ph), 12.50 (s_{br}, 1H, N^7-*H*). ^{13}C-NMR (DMSO-d_6): δ 11.4 (*C*H_3), 21.4 (CH_3-*C*H_2), 22.9 (Cp-*C*^{4}), 29.1 (Cp-*C*^{5}), 29.2 (^2J = 19.2 Hz, *C*H_2-CH_2F), 31.1 (Cp-*C*^{3}), 40.1 (^3J = 5.5 Hz, N^3-*C*H_2), 42.7 (N^1-*C*H_2), 46.3 (Cp-*C*^{1}), 71.6 (O-*C*H_2-Ph), 81.5 (^1J = 162.0 Hz , *C*H_2F), 84.8 (Cp-*C*^{2}), 106.8 (*C*^{5}), 127.5 (O-CH_2-Ph), 128.2 (O-CH_2-Ph), 138.4 (O-CH_2-Ph), 148.9 (*C*^{8}), 151.1 (C^4), 155.6 (*C*^{2}), 157.5 (*C*^{6}). ^{19}F-NMR (DMSO-d_6): δ 219.87. MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{23}H_{29}FN_4O_3$ 429.22; Found 429.22.

3-(3-Fluoropropyl)-8-(3-hydroxycyclopentyl)-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (25).

Under an inertgas atmosphere and external cooling with ice a solution of 9-BBN in THF (0.5 M, 8 mL, 4 mmol) was added to a well stirred solution of cyclopentenylxanthine **24a** (320 mg, 1 mmol) in dry THF (10 mL). The ice bath was removed and the mixture was stirred at ambient temperature for 16 h. Water (2 mL) was added cautiously to destroy excess borane. Sodium hydroxide (1N, 1.1 mL, 1.1 mmol) and then 30% hydrogen peroxide (380 µL, 3.7 mmol) were added, and the solution was stirred at ambient temperature for 0.5 h. Brine (75 mL) was added, the phases were separated and the aqueous phase was extracted with THF (2 x 25 mL). The pooled organic phases were washed with brine and dried dried over anhydrous sodium sulfate. Filtration and evaporation *in vacuo* left a residue that was purified by flash chromatography eluting with 5% ethyl acetate in methanol. The product fractions were pooled and the solvents evaporated *in vacuo*. The solid material was triturated with diethyl ether (5 mL) treated with ultrasound (20 s) and collected by fitration to give the title compound **25** (mixture of *cis* and *trans* isomers) as colourless crystals. Yield 220 mg (65%), m.p. 205-206°C.
^{1}H-NMR (DMSO-d_6): δ 0.88 (t, J = 7.4 Hz, 3H, CH_3), 1.35 – 2.33 (m, 10H, 3 Cp-CH_2 + CH_3-CH_2 + CH_2-CH_2F), 3.39 (m, J = 7.9 Hz, 1H, Cp-H), 3.85 (m, J = 7.1 Hz, 2H, N^1-CH_2), 4.11 (t, J = 6.7 Hz, 2H, N^3-CH_2), 4.29 (s_{br}, 1H, Cp-OH), 4.51 (dt, J = 47.2, 5.7 Hz, 2H, CH_2F), 4.69 (s_{br}, 1H, OH), 13.14 (s_{br}, 1H, N^7-H). ^{13}C-NMR (DMSO-d_6): δ 11.6 (CH$_3$), 21.3 (CH_3-CH$_2$), 29.2 (^2J = 19.2 Hz, CH$_2$-CH_2F), 30.6 (Cp-CH$_2$), 35.1 (Cp-CH$_2$), 35.3 (Cp-CH$_2$), 36.9 (Cp-CH), 41.4 (N^1-CH$_2$), 42.2 (^3J = 5.5 Hz, N^3-CH$_2$), 71.9 (Cp-CHOH), 82.4 (^1J = 162.0 Hz , CH$_2$F), 106.6 (C^5), 147.9 (C^8), 151.2 (C^4), 154.3 (C^2), 158.47 (C^6). ^{19}F-NMR (DMSO-d_6): δ - 218.39. MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{16}H_{23}FN_4O_3$ 339.18; Found 339.18.

***cis*-3-(3-Fluoropropyl)-8-(2-hydroxycyclopentyl)-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (26).** At ambient temperature and under the exclusion of moisture methanesulfonic acid (1.5 mL) was added to a well stirred solution of **24c** (215 mg, 0.5 mmol) in dry DCM (3.5 mL). The mixture was stirred at ambient temperature for 12 h and poured onto ice-water (30 mL). Extraction with ethyl acetate (2 x 25 mL), washing of the pooled organic extracts with sat. aqueous Na_2CO_3 (2 x 50 mL) and brine (50 mL), drying over anhydrous sodium sulphate, filtration and rota-evaporation *in vacuo* yielded a solid residue. The residue was purified by chromatography on

silica gel (eluent 30% acetonitrile in ethyl acetate) to provide the deprotected xanthine **26** as a low melting yellow solid. Yield 154 mg (91%).

^{1}H-NMR (DMSO-d_6): δ 0.88 (t, J = 7.4 Hz, 3H, CH_3), 1.44-2.27 (m, 10H, CH_3-CH_2 + Cp-CH_2 + CH_2-CH_2F), 2.81-3.08 (m, 1H, Cp-H^1), 3.63-3.95 (m, 2H, N^1-CH_2), 4.03-4.19 (m, 2H, N^3-CH_2), 4.15-4.29 (m, 1H, Cp-H^2), 4.53 (dt, J = 47.2, 5.7 Hz, 2H, CH_2F), 4.89 (s_{br}, 1H, OH), 13.17 (s_{br}, 1H, N^7-H). ^{13}C-NMR (DMSO-d_6): δ 11.6 (CH$_3$), 21.3 (CH_3-CH$_2$), 22.3 (Cp-C^4), 29.2 (^2J = 19.2 Hz, CH$_2$-CH_2F), 29.4 (Cp-C^5), 30.3 (Cp-C^3), 40.2 (^3J = 5.5 Hz, N^3-CH$_2$), 42.5 (N^1-CH$_2$), 48.1 (Cp-C^1), 77.6 (Cp-C^2), 82.1 (^1J = 162.0 Hz , CH$_2$F), 106.7 (C^5), 148.2 (C^8), 151.2 (C^4), 154.3 (C^2), 157.1 (C^6). ^{19}F-NMR (DMSO-d_6): δ - 218.35. MS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{16}H_{23}FN_4O_3$ 339.18; Found 339.18.

Competitive Radioligand Binding Assays

Frontal cortices (for A_1AR assays) and corpora striata (for A_{2A}AR assays) from pig brains were homogenized for 1 min in 10 volumes of ice-cold 320 mM sucrose, containing soybean trypsin inhibitor (20 µg / mL), bacitracin (200 µg / mL), and benzamidine HCl (160 µg / mL) by means of a Potter at 20,000 rpm under external ice cooling. The homogenate was centrifuged at 1,000 X g for 10 min at 4°C (Beckmann Optima L, SW41Ti rotor). The pellet was discarded and the supernatant was centrifuged at 50,000 X g for 15 min at 4 °C. The resulting pellet was washed once with buffer (50 mM Tris - HCl, pH 7.4, containing 2 U / mL adenosine deaminase) and resuspended in 10 volumes of buffer (same volume as for sucrose, see above), thereafter stored in aliquots (600 µL) at - 80°C.

The assays were performed in triplicate by incubating aliquots of the membrane fractions (65 - 120 µg protein per sample) in Tris HCl, pH 7.4, containing adenosine deaminase (2 U / mL). Cortical homogenates containing A_1ARs were additionally incubated with [^{3}H]CPFPX (2 nM), striatal homogenates containing A_{2A}AR were additionally incubated with [^{3}H]ZM241385 (1.8 nM), all with different competitor concentrations in a total assay volume of 200 µL at 20°C for 90 min. Filtration over GF/C-sheets presoaked in 0.3% polyethylenimine separated bound from free ligand using a sample harvester (Brandel, USA). Filtrates were discarded. The filters with the adhering pellets were washed with ice cold buffer (4 x 2 mL), punched-out into scintillation vials and incubated overnight with scintillation cocktail (8 mL, Ready Save, Beckman Coulter, Krefeld,

Germany). Radioactivity was measured in a liquid scintillation counter. Protein estimation was performed with a commercial assay (Bio-Rad DC Protein Assay) after solubilization in 15% NH_4OH containing 2% SDS (w / v); human serum albumin served as a standard. A computer-assisted curve-fitting program (Graph Pad Prism, version 4.0) calculated IC_{50}- and K_i-values.

Incubation of Human Liver Microsomes with n.c.a.[^{18}F]CPFPX

Human liver microsomes (0.8 mg protein) were dispersed in 0.1 M phosphate buffer (pH 7.4) containing 3.3 mM $MgCl_2$ and an NADPH-generating system consisting of 1.3 mM NADP, 3.3 mM glucose 6-phosphate and 0.4 U glucose 6-phosphate dehydrogenase in a final volume of 1 mL. Incubation was at 37°C. The addition of n.c.a.[^{18}F]CPFPX (500 kBq) initiated the reaction, which was stopped after 30 min by addition of acetonitrile (2 mL). After 2 min of vortex mixing and centrifugation (20.800 X g, 4°C, 1 min.) the supernatant was vacuum evaporated to dryness at ambient temperature. The residue was taken up in 55 µL of a solution prepared by mixing 5 µL of a DMSO solution containing compounds **10**, **12**, **15a,c,d**, **17**, **24a,b, 25**, **26**, **M1** and **CPFPX** (0.2 µg each) and HPLC eluent (50 µL). The resulting suspension was centrifuged (20,800 X g, 4°C, 1 min) to remove sediment.

High-performance liquid chromatography

Analysis of standards, putative metabolites, and liver microsome extracts employed a Kromasil 100-5 C18 column (250 x 4.6 mm) (CS-Chromatographie Service GmbH, Langerwehe, Germany) in a HPLC system consisting of a WellChrom K – 1001 pump (Knauer, Berlin, Germany), a K - 2001 UV detector (Knauer, Berlin, Germany), and a manual sample injector (Type 7125, Rheodyne, Bensheim, Germany) fitted with a 250 µl sample loop. Isocratic elution with water / acetonitrile/ acetic acid (70 / 30 / 0.2, v / v / v) was at a flow rate of 1 mL / min. UV monitoring at 275 nm detected CPFPX and its putative metabolites. For measurement of radioactivity the outflow of the UV detector was connected in series to an on-line NaI(Tl) well-type scintillation detector with a 250 µL detection loop. Chromatograms were corrected for the transit time between the detectors.

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