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^{13}C bis-labeled pyrroles: A tool for the identification of the rat metabolism of 3-methyl pyrrole-2,4-dicarboxylic acid 2-propyl ester 4-(1,2,2-trimethyl-propyl) ester

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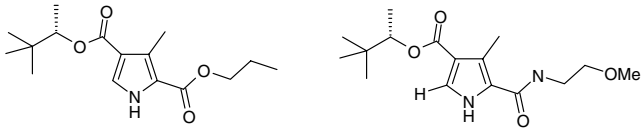
Abstract—Following the recent disclosure of 3-methyl pyrrole-2,4-dicarboxylic acid 2-propyl ester 4-(1,2,2-trimethyl-propyl) ester as a potent and selective mGluR1 non-competitive antagonist, the use of a doubly ^{13}C-labeled analogue to identify, and consequently prevent, metabolically labile positions is reported.

Glutamate, one of the major excitatory neurotransmitters in mammalian brain, exerts its action through ionotropic (specifically NMDA, AMPA, and Kainate)[1–5] and metabotropic receptors (mGluRs).[6,7]

The metabotropic receptors are characterized by a large amino-terminal domain and they belong to the family C of G-protein coupled receptors (GPCR). Through different effectors and second messengers they are able to control crucial functions within the cell;[8–12] moreover, their structures are peculiar and show very little homology with those of other cloned receptors.[13–15] To date, eight mGluR subtypes have been identified and named mGluR1–8 according to the succession of the molecular cloning. These receptors are divided into three main groups on the basis of sequence similarity, pharmacology, and transduction mechanisms: Group I (mGluR1 and mGluR5), Group II (mGluR2 and mGluR3) and Group III (mGluR4, mGluR6, mGluR7, and mGluR8). A number of recent reviews reported the current state of the art of competitive and non-competitive antagonists and modulators of these important receptors.[16–20]

We have recently reported[21–23] that 3-methyl pyrrole-2,4-dicarboxylic acid 2-propyl ester 4-(1,2,2-trimethyl-propyl) ester (**1**, Fig. 1) is a potent and selective non-competitive mGluR1 antagonist endowed with an excellent in vitro and in vivo activity in different animal models of pain. Further derivatives, endowed with a better pharmacokinetic profile in preclinical species (e.g., **2**, Fig. 1), were also recently published.[24,25]

To gain a further understanding of the preclinical profile of this series avoiding the lengthy and expensive synthesis of the [^{14}C]-labeled molecules—generally used in advanced development phase—it was decided to apply some stable isotope [^{13}C] labeling to the pyrrole derivatives.[27–29] This would greatly facilitate the identification of major metabolic pathways in vivo and in vitro, without the need for complex and time-consuming purification processes.



1 2

Figure 1. The recently reported mGluR1 antagonist pyrrole class.

Keywords: mGluR1; Metabotropic; Glutamate; Metabolism.
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Based on the synthetic route described for **1** and **2**,[21–23] the introduction of two ^{13}C atoms (25% of the total amount) into the pyrrole template was decided to generate a well-identifiable 'fingerprint' in the mass and NMR spectra of both the derivatives and their metabolites. Both ^{13}C atoms have been positioned on the core scaffold, thus ensuring that the main metabolic pathways—more likely to affect groups in C2 and C4—did still maintain the stable isotopes useful to their identification. The detailed synthetic route is reported in Scheme 1.

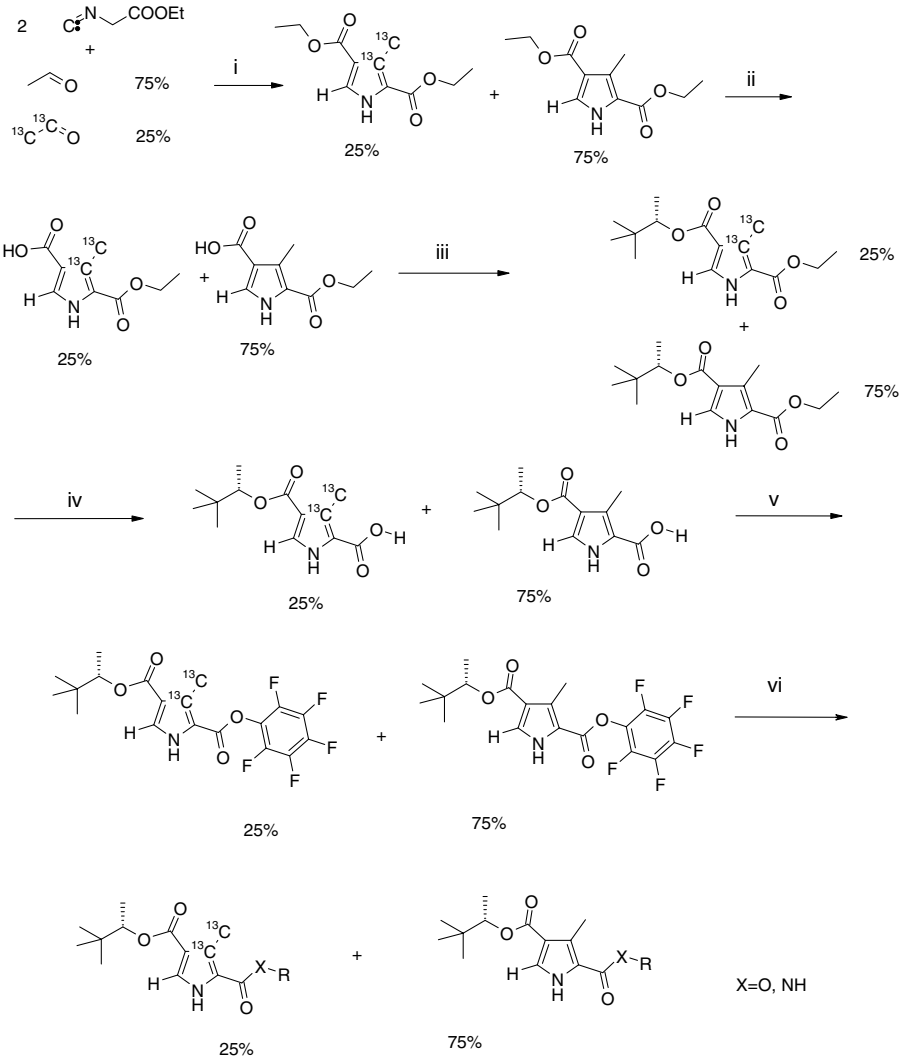
After oral administration[30] of derivatives **1** and **2** to rats, the chromatographic peaks of parent compounds and their major metabolites in biofluid extracts were easily identified by MS and NMR spectroscopy, tracking the dual ^{13}C fingerprints which therefore allowed a quick formulation of some working hypothesis on the degradation pattern of the pyrrole template.

As far as derivative **1** is concerned, the hypothesized metabolites (**3**–**6**) are depicted in Scheme 2. In particular, the mass spectra allowed the identification of two hydroxylated derivatives (**3** and **4**) and two carboxylated ones (**5** and **6**).

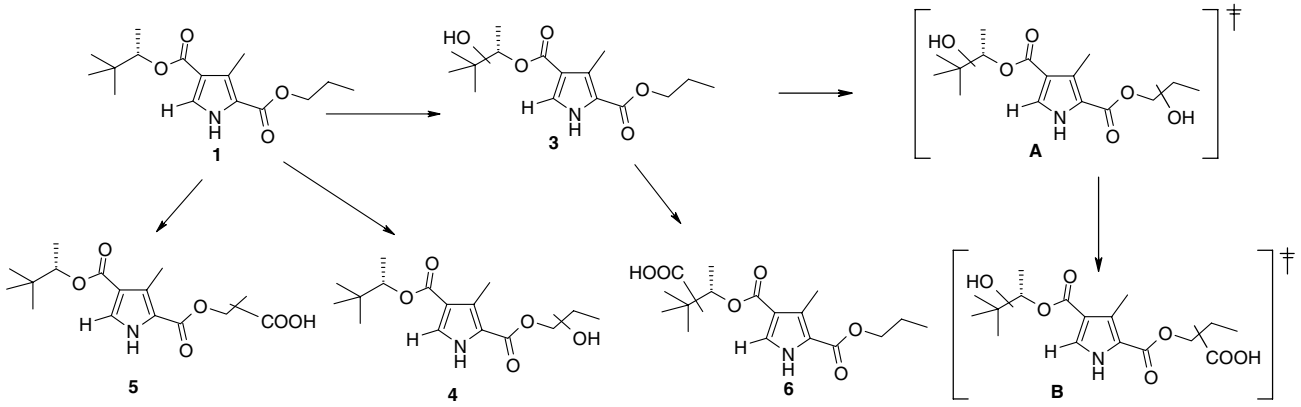
Further MS–MS experiments on the study of the decomposition pattern of carboxylate **5** led to hypothetical metabolic route represented in Scheme 3, allowing the unequivocal attribution of the two derivatives now identified as compounds **7** and **8** through the direct comparison of the spectra available from HPLC–NMR studies of the biological samples and the synthetic ones.

As far as derivative **2** is concerned (Scheme 4), after a rapid cleavage of the methoxy group, the corresponding primary hydroxyl group **9** was readily oxidized to the corresponding carboxylic acid **10**. Also in this case, the working hypothesis was confirmed through the comparison between the spectra coming from the biological samples and the synthetic compounds ad hoc prepared.

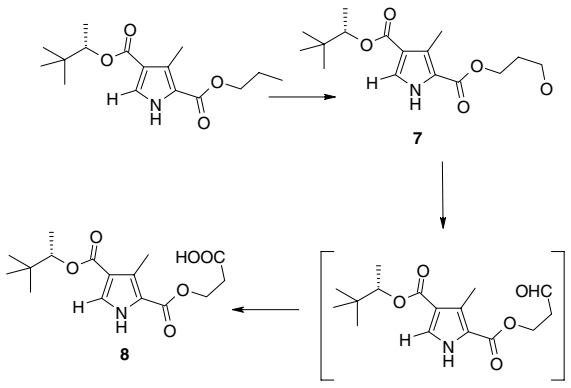
As far as derivative **3** is concerned, the site of hydroxylation could not be unequivocally assigned, due to the



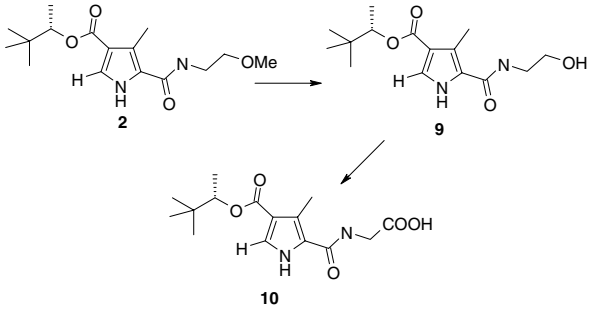
Scheme 1. Reagents and conditions: (i) DBU/THF, 50 °C; (ii) H_2SO_4, 0 °C; (iii) 1—trifluoroacetic anhydride; toluene; 0 °C, 1 h; 2—(*S*) pinacolyl alcohol, rt, 4 h; (iv) LiOH (4 equiv), EtOH; H_2O, 50 °C; (v) $C_6F_5OCOCF_3$ (1.2 equiv), DMF, Py (1.2 equiv), rt, 12 h; (vi) RNH_2 or ROH, 0–60 °C.



Scheme 2. Hypothetical metabolic pathway.



Scheme 3. Identified metabolic pathway.



Scheme 4. Metabolic pathway for amide 2.

competitive biotransformation on the C2-ester,[31] involving the transient intermediates A and B (Scheme 2). Consequently, and to allow enough time to study the metabolic fate of the C4 position, more stable C2[25,26] substituted amides were prepared and LC/NMR/MS studies were performed on freeze-dried rat urines collected after oral administration. The NMR data obtained clearly demonstrated that the *tert*-butyl group of the pinacolyl ester underwent a metabolic hydroxylation. In particular, derivative **11** was rapidly converted to derivative **12** reported in Figure 2.

Two alternative medicinal chemistry strategies to reduce the metabolic clearance were therefore available; the first one was to prevent the *t*-Bu group oxidation; the second one was to identify a suitable replacing group more metabolically stable than the *t*-Bu one.

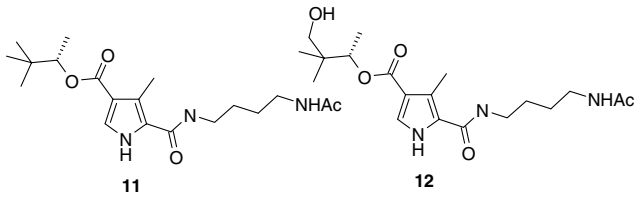


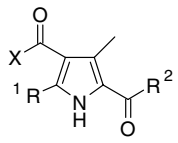
Figure 2. *t*-Bu group was identified as one metabolically labile position within the pinacolyl group.

The second approach is rapidly described below. It was known,[21–26] that appropriate potency could be obtained in the pyrrole template using lipophilic groups in the C4 position; nonetheless, appropriate stereochemistry was also needed. Accordingly, a number of non-bornyl derivatives were prepared via trifluoroacetic mixed anhydride in accordance with previously described methods[21–26] and their in vitro potency on r-mGluR1 receptor was evaluated. Data are reported in Table 1.

While a slight improvement in metabolic stability was achieved through this replacement (data not shown), a clear decrease in potency was also observed with respect to the nanomolar potency of compound **1**,[21–26] confirming once more the need for the particular shape and chirality represented by the pinacolyl substituent in the pyrrole class for the ideal fit within the mGluR1 receptor. Nonetheless, these molecules might constitute the starting point for further exploration.

A double [^{13}C] labeling of the pyrrole template allowed the rapid identification of the metabolic pathway in preclinical species through NMR/LC, HPLC/MS/MS techniques. Some more stable, but less potent derivatives were identified and might represent new potential hits for further exploration.

Table 1. Potency values of the pyrrole derivatives on r-mGluR1a-CHO obtained as previously reported[21–26]



Compound	X	R^1	R^2	pIC_{50}
1		H	$OCH^2CH^2CH^3$	8.4
2		H	$NHCH_2CH_2OMe$	7.5
13		H	OEt	7.7
14		H	$HN(CH_2)_4NHAc$	6.7
15		H	$HN(CH_2)_2OMe$	6.8
16		Me	OEt	7.0
17		Me	$HN(CH_2)_4NHAc$	6.0
18		Me	$HN(CH_2)_2OMe$	6.5
19		H	HN(CH2)2OMe	<5

IC_{50}s were measured from at least six-point inhibition curves and they are the geometric means of at least three independent experiments. No selectivity data were generated at this point on these derivatives.

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30. The research complied with national legislation and with company policy on the Care and Use of Animals and with related codes of practice.
31. The different stability of the C2 esters was previously reported[21,23–25] and is species dependent also in rodents; moreover, it is sensitive to steric hindrance as expected (e.g., the C2 *n*-Pr ester in compound **1** has a $T_{1/2}$ of 2.8 h in mice and 0.2 h in rats, while the corresponding C2 *t*-Bu ester has a $T_{1/2} > 24$ h in mice and >5 h in rats). A detailed comparison of the PK profile of compounds **1** and **2** is reported in Ref. 25.