Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 14 December 2008, Revised 3 February 2009, Accepted 4 February 2009 Published online 1 April 2009 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1597

Synthesis and *in vitro* evaluation of ^{18}F-labelled di- and tri(ethylene glycol) metomidate esters

Maria Erlandsson,[a] Håkan Hall,[b,c] and Bengt Långström[a]*

By replacing the alkyl chain in a metomidate ester with ^{18}F-labelled di- or tri(ethylene glycol) chains, two ^{18}F-labelled PET tracers, i.e. 2-(2-[^{18}F]fluoroethoxy)ethyl 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylate (1) and 2-[2-(2-[^{18}F]fluoroethoxy)-ethoxy]ethyl 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylate (2), were synthesized. Two precursors, 2-(2-bromoethoxy)ethyl 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylate and 2-[2-(2-chloroethoxy)ethoxy]ethyl 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylate, were prepared and used in one-step nucleophilic [^{18}F]fluorination reactions using conventional and microwave heating. Organ distribution, frozen section autoradiography and metabolite analysis were performed. The decay-corrected radiochemical yields of 1 and 2 were 26 ± 8 and 23 ± 8%, respectively, when they were prepared using conventional heating. By performing microwave heating, the reaction time could be decreased and the yields of analogues 1 and 2 could be increased to 57 ± 12 and 51 ± 18%, respectively. Organ distribution studies in the rat showed considerable uptake in the lungs, adrenals and liver. Both compounds bound with low nonspecific binding (1: approx. 20–30%; 2: 2.9% or lower) to tissue from pig and human normal and pathologic adrenals. Metabolite analyses were performed in rats after 5 and 30 min for tracer 1 (20 ± 6 and 2 ± 1%) and tracer 2 (27 ± 5 and 6 ± 4%). Both compounds are interesting candidates for the detection of different types of adrenal disorders.

Keywords: n.c.a. nucleophilic ^{18}F-fluorination; di- and tri(ethylene glycol); metomidate; analogues

Introduction

1–[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylic acid esters (MTO) are potent inhibitors of 11-β-hydroxylase, a key enzyme in the synthesis of cortisol and aldosterone within the adrenal cortex.[1,2] MTO analogues labelled with β^+-emitting radionuclides such as ^{11}C ($t_{1/2}$ = 20.3 min) and ^{18}F ($t_{1/2}$ = 109.7 min) are thus of interest.

In clinical studies, the radiotracer [^{11}C]methyl 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylate has shown high uptake in lesions of adrenocortical origin, including adenomas, but low uptake in lesions of nonadrenocortical origin.[3–7] Recently, ^{11}C-labelled 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylic acid ester analogues have been synthesized and biologically evaluated.[8] The ^{18}F-labelled tracer analogue 2-[^{18}F]fluoroethyl 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylate ([^{18}F]FETO) has also shown similar pharmacokinetic and pharmacodynamic properties.[9,10]

A crucial factor in the utility of radiolabelling procedures with short-lived radionuclides is the time required for synthesis and purification. There is thus a need to find synthetic strategies that can reduce the synthesis time. Microwave heating has been used for the radiolabelling of different organic molecules with short-lived radionuclides such as ^{11}C and ^{18}F.[11] This technique not only shortens the chemical reaction time but also reduces side reactions, increases the yield and improves reproducibility.[11]

The biological properties of the lead tracer have been shown to be relatively insensitive to the changes in the ester part.[12] This made us interested to explore the one-step synthesis of two ^{18}F-labelled analogues, 2-(2-[^{18}F]fluoroethoxy)ethyl 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylate and 2-[2-(2-[^{18}F]fluoroethoxy) ethoxy]ethyl 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylate. The one-step ^{18}F-labelling syntheses of the two analogues were performed with both conventional and microwave heating.

The two new tracers were evaluated with regard to organ distribution, frozen section autoradiography and metabolism to find out whether the di- and tri(ethylene glycol) modifications confer different biological properties.

Results and discussion

Chemistry

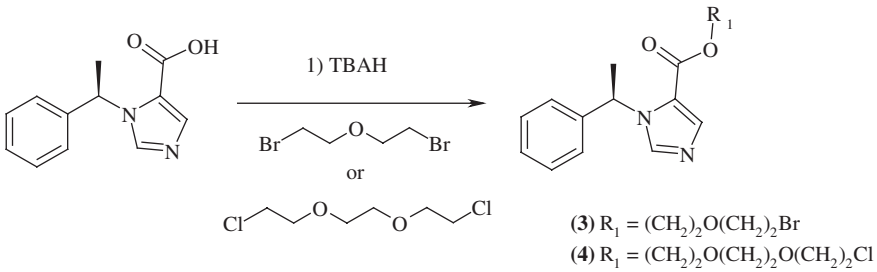
To circumvent the problem of high lipophilicity that can result from the addition of a fluoroalkyl group, we have developed

[a]*Department of Biochemistry and Organic Chemistry, Uppsala University, Box 576, Husargatan 3, BMC, S-751 23 Uppsala, Sweden*

[b]*Uppsala Applied Science Lab, GE Healthcare, Box 967, S-751 09 Uppsala, Sweden*

[c]*Department of Public Health and Caring Sciences, Uppsala University, Uppsala Science Park, S-751 85 Uppsala, Sweden*

**Correspondence to: Bengt Långström, Department of Biochemistry and Organic Chemistry, Uppsala University, Box 576, Husargatan 3, BMC, S-751 23 Uppsala, Sweden.*
E-mail: Bengt.Langstrom@biorg.uu.se



Scheme 1. Synthesis of precursor compounds **3** and **4**.

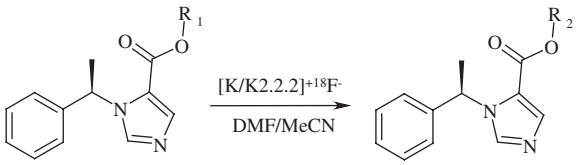
two PET tracers based on the metomidate structure, which contain di- or tri(ethylene glycol) substituents with [^{18}F]fluorine atoms attached at the end of the chains. By varying the chain length of the ethylene glycol group in compounds **1** and **2**, it will be possible to adjust the lipophilicity and to maintain a relatively small size. The calculated Log *P* values for the compounds were 2.47 ± 0.59 (**1**) and 2.11 ± 0.69 (**2**), which are lower compared with the ^{11}C-labelled MTO lead.[8,13] These analogues may be interesting for further biological studies, because of decreased lipophilicity, which is one of the key determinants of pharmacokinetic properties.

Two precursors, compounds **3** and **4**, were synthesized by adding 2-bromoethyl ether or 1,2-*bis*(2-chloroethoxy)ethane to the pre-treated 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylic acid (Scheme 1). Compound **3** could not be synthesized using either conventional or microwave heating due to the formation of byproducts. Instead, the best yield of compound **3**, 53%, was obtained when the reaction temperature was allowed to slowly rise from 0°C to room temperature over 20 h. In the synthesis of precursor **4**, both conventional and microwave heating were evaluated. The disadvantage of conventional heating using an oil bath is that the vessel walls are heated up first, causing a temperature gradient in the solution. However, very fast heating can be obtained using microwave irradiation because the sample is heated from inside, more uniformly at each point.[14] The synthesis of **4** using microwave heating gave a higher yield of the desired product and decreased the formation of the byproducts compared with the synthesis using conventional heating. The yield of compound **4** could be increased from 33 up to 56%.

Compounds **1** and **2** were synthesized from their respective halogen–ether chains by reacting compounds **3** and **4** with [K/K2.2.2]$^{+}$^{18}F^{-} (Scheme 2). Using conventional heating at 150°C for 15 min produced **1** and **2** with specific radioactivities of 20 ± 4 and 175 ± 18 GBq/μmol, respectively, after a 50–60-min synthesis (Table 1). The radiochemical purity of both compounds, as determined by analytical HPLC, was >95% and the decay-corrected (d.c.) radiochemical yields were 26 ± 8% for **1** and 23 ± 8% for **2**.

In the literature, microwave heating has also been used to enhance PET radiolabelling reactions, including one-step nucleophilic [^{18}F]fluorination reactions, so that they become faster, cleaner and higher yielding.[15,16]

Microwave heating was performed on compounds **3** and **4** using different temperatures and solvents to find the optimized reaction condition (Tables 2 and 3). The reactions were heated in the microwave oven for periods of 2–120 s and the samples were collected from the reaction mixtures at different time points and HPLC-analysed (Tables 2 and 3). When the optimized



(**3**) $R_1 = (CH_2)_2O(CH_2)_2Br$ (**1**) $R_2 = (CH_2)_2O(CH_2)_2{}^{18}F$
(**4**) $R_1 = (CH_2)_2O(CH_2)_2O(CH_2)_2Cl$ (**2**) $R_2 = (CH_2)_2O(CH_2)_2O(CH_2)_2{}^{18}F$

Scheme 2. One-step labelling synthesis.

condition was found for each compound, the syntheses were performed again and each reaction was HPLC-analysed and the final d.c. radiochemical yield and SRA were determined (Table 1).

Five different temperatures (60, 100, 150, 170 and 200°C) were tested in the synthesis of analogue **1**, and *N*,*N*-dimethylformamide, acteonitrile and dimethyl sulfoxide were used as solvents. The microwave syntheses of analogue **1** indicated that temperature was more important in determining the yield than the solvent was. Optimally, the reaction temperature should be as high as the substrate and the product tolerate before decomposing, or as high as the reaction solvent allows. In this case 200°C was the temperature limit when *N*,*N*-dimethylformamide/acetonitrile, dimethyl sulfoxide/*N*,*N*-dimethylformamide or pure *N*,*N*-dimethylformamide was used. The low yield of **1** might be a result from a reaction between dimethyl sulfoxide and the precursor **3**. When acetonitrile was used, the best temperature was 150°C; after that point, too much pressure was built up in the vial. Two seconds of microwave heating was enough to obtain the highest d.c. radiochemical yield in the reaction, regardless of the temperatures. A temperature increase to 200°C over 2 s gave the best yields; at longer reaction times, the product started to decompose. Compared with the conventional heating, microwave heating increased the d.c. radiochemical yield of **1** from 26 ± 8 to 57 ± 12%.

The solvent mixture was an important factor in determining the yield of **2**. However, by increasing the temperature from 150 to 200°C, the yield was doubled. To take advantage of the microwave-heating effect, the reaction had to be carried out in a solvent with high dielectric constant.[17] Water was used first because it is the most common polar solvent, but in this case it decomposed the precursor. The precursor was also destroyed when the reaction was performed in *N*,*N*-dimethylformamide/water. *N*,*N*-dimethylformamide and dimethyl sulfoxide were also tested as solvents. A 3:1 mixture of *N*,*N*-dimethylformamide and dimethyl sulfoxide gave the best yield. When more dimethyl

Table 1. Radiochemical yield and specific activity for compounds **1** and **2**

Compound	Radiochemical yield[a] (%) Conventional heating	Specific activity[b] (GBq/μmol) Conventional heating	Radiochemical yield[a,c] (%) Microwave heating	Specific activity[b,c] (GBq/μmol) Microwave heating[c]
1	26 ± 8 (n = 5)	20 ± 4 (n = 2)	57 ± 12 (n = 4)	46 ± 8 (n = 2)
2	23 ± 8 (n = 4)	175 ± 18 (n = 2)	51 ± 18 (n = 6)	19 ± 7 (n = 4)

[a]D.c., based upon [^{18}F]fluoride at start and radioactivity of HPLC-purified product. n = number of experiments.
[b]Ratio of radioactivity to amount of substance, d.c. to time at start of synthesis.
[c]Compound **3** reacted in acetonitrile/*N*,*N*-dimethylformamide at 200°C for 2 s; compound **4** reacted in dimethyl sulfoxide/*N*,*N*-dimethylformamide 3:1 at 200°C for 2 s.

Table 2. Results from the microwave synthesis to find the optimized reaction condition for analogue **1**

Product yield at different reaction conditions[a] (%)	Heating time (s)						
	2	10	15	20	30	60	120
MeCN/DMF 200°C	66 ± 5	60 ± 1	58 ± 4	57 ± 5	55 ± 2	55 ± 4	54 ± 4
MeCN/DMF 170°C	64 ± 4	56 ± 4	58 ± 3	58 ± 3	59 ± 5	58 ± 4	57 ± 4
MeCN/DMF 150°C	55 ± 6	55 ± 6	55 ± 3	56 ± 3	55 ± 4	63 ± 7	57 ± 1
MeCN/DMF 100°C	26 ± 3	28 ± 8	30 ± 6	31 ± 5	31 ± 6	31 ± 7	30 ± 5
MeCN/DMF 60°C	18 ± 10	10 ± 6	10 ± 5	10 ± 4	11 ± 6	11 ± 4	10 ± 2
DMSO/DMF 200°C	15 ± 4	13 ± 1	13 ± 1	18 ± 2	18 ± 2	14 ± 5	15 ± 4
MeCN 150°C	63 ± 1	63 ± 5	62 ± 1	68 ± 7	64 ± 4	63 ± 1	67 ± 3
DMF 200°C	53 ± 21	48 ± 15	48 ± 11	46 ± 13	47 ± 12	39 ± 2	44 ± 11

[a]MeCN, acetonitrile; DMF, *N*,*N*-dimethylformamide. Average values from two experiments. The yields are d.c. based upon [^{18}F]fluoride at start and radioactivity of HPLC-purified product.

Table 3. Results from the microwave synthesis to find the optimized reaction condition for analogue **2**

Product yield at different reaction conditions[a] (%)	Heating time (s)						
	2	10	15	20	30	60	120
MeCN/DMF 200°C	15 ± 1	22 ± 4	22 ± 3	20 ± 2	21 ± 2	22 ± 2	21 ± 4
MeCN/DMF 150°C	9 ± 2	11 ± 4	11 ± 5	10 ± 2	10 ± 3	11 ± 5	11 ± 3
DMSO/DMF 3:1 200°C	35 ± 5	33 ± 5	32 ± 5	30 ± 3	31 ± 2	32 ± 4	32 ± 6
DMSO/DMF 6:1 200°C	21 ± 2	19 ± 2	22 ± 2	25 ± 6	21 ± 2	18 ± 2	21 ± 3
DMF 200°C	19 ± 1	21 ± 1	20 ± 2	20 ± 2	21 ± 2	23 ± 1	23 ± 1
MeCN 200°C	15 ± 1	20 ± 4	22 ± 6	25 ± 6	24 ± 6	24 ± 7	26 ± 6
MeCN/DMF 1:1 200°C	18 ± 12	17 ± 11	20 ± 13	19 ± 11	19 ± 11	19 ± 12	17 ± 12

[a]MeCN, acetonitrile; DMF, *N*,*N*-dimethylformamide. Average values from two experiments. The yields are d.c. based upon [^{18}F]fluoride at start and radioactivity of HPLC-purified product.

sulfoxide was used, the yield decreased due to higher water content. When the reaction using precursor **4** was performed in dimethyl sulfoxide, the yield was higher compared with the reaction with precursor **3**. The reason for this may be that the reaction rate was slower with the chloro derivative then with the bromo derivative. A practical problem with dimethyl sulfoxide is that the workup procedure is complicated by the need to remove the solvent at the end of the reaction. However, when acetonitrile and *N*,*N*-dimethylformamide were used as solvents, the yield decreased to lower levels than were obtained with conventional heating. To find out if solvatization was a problem, the less polar solvent dioxane was added to *N*,*N*-dimethylformamide and *N*,*N*-dimethylformamide/dimethyl sulfoxide, but in these cases no products were obtained; only unreacted starting material and ^{18}F were recovered. Microwave heating at 200°C for 2 s increased the d.c. radiochemical yield for analogue **2** to 51 ± 18%, compared with 23 ± 8% with conventional heating.

For analogue **1**, it was possible to increase the specific activity with microwave heating to 46 ± 8 GBq/μmol, compared with 20 ± 4 GBq/μmol with conventional heating. However, the result was the opposite for analogue **2**; the specific activity decreased from 175 ± 18 GBq/μmol with conventional heating to 19 ± 7 GBq/μmol with microwave heating (Table 1).

Microwave heating should produce an efficient internal heat transfer and result in minimized wall effects compared with conventional heating, in which the temperature of the reaction vessel is higher than that of the reaction mixture.[18] The activity left on the walls of the reaction vial after washing was 13 ± 6% (n = 10) in the microwave vial and 9 ± 4% (n = 6) in the vial used for conventional heating. Hence, our experiments indicate that the

fluoride was attracted to the glass walls of the reaction vial regardless of whether microwave or conventional heating was used.

Biology

Organ distribution

The distribution (relative to blood) of **1** and **2** in various male rat organs is shown in Figure 1. The main distribution was to lungs, adrenals, pancreas, kidney and liver, whereas the accumulation was considerably lower in other organs. The accumulation of **1** was approximately tenfold higher in the lung than that of **2**. The accumulation in the adrenals was time-dependent, and was markedly lower after 60 min with both compounds, whereas it increased over time in the bone. Low radioactivity was found in the brain (cerebrum or cerebellum).

Frozen section autoradiography

Both ligands bound specifically to different tissues from pig and human normal and pathologic adrenals (Figure 2). The nonspecific binding was low for **2** (2.9% of total in the pig adrenals, not visible in the normal and pathologic human tissue) but was approximately 20–30% for **1** (pig adrenals). The two radioligands showed qualitatively similar binding to the different human normal and pathologic tissues, although some quantitative differences could be observed.

Analysis of radiolabelled metabolites in plasma

Metabolite studies were performed on **1** and **2** in rats. Blood samples were collected 5 and 30 min after injection, and the amounts of unchanged tracer and radioactive metabolites they contained were measured. Duplicate experiments were performed for each tracer and time point (Table 4). For tracer **1**, the metabolite analysis revealed that 20 ± 6 and 2 ± 1% of the material were intact 5 and 30 min after injection, respectively. The recovery was 79 ± 34%. One hydrophilic metabolite was observed. For tracer **2**, the analysis revealed that 27 ± 5 and 6 ± 4% were intact 5 and 30 min after injection, respectively. The recovery was 86 ± 16%. One hydrophilic metabolite was observed.

The biological evaluation of the metomidate analogues **1** and **2** was in accordance with previous results.[3,4,9] Hence, strong

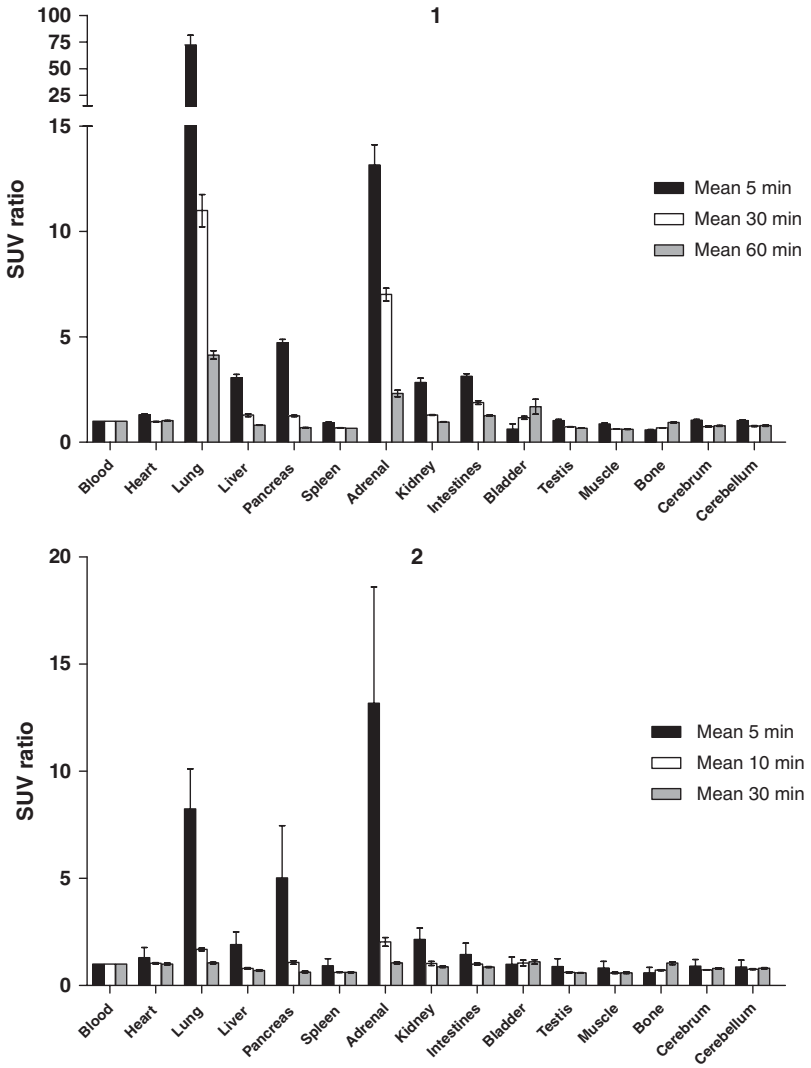


Figure 1. Organ distribution of **1** and **2** in male rats (SUV, relative blood). The rats were injected intravenously with 10 MBq/per animal ($n = 4$ per time point) of one of the two tracers.

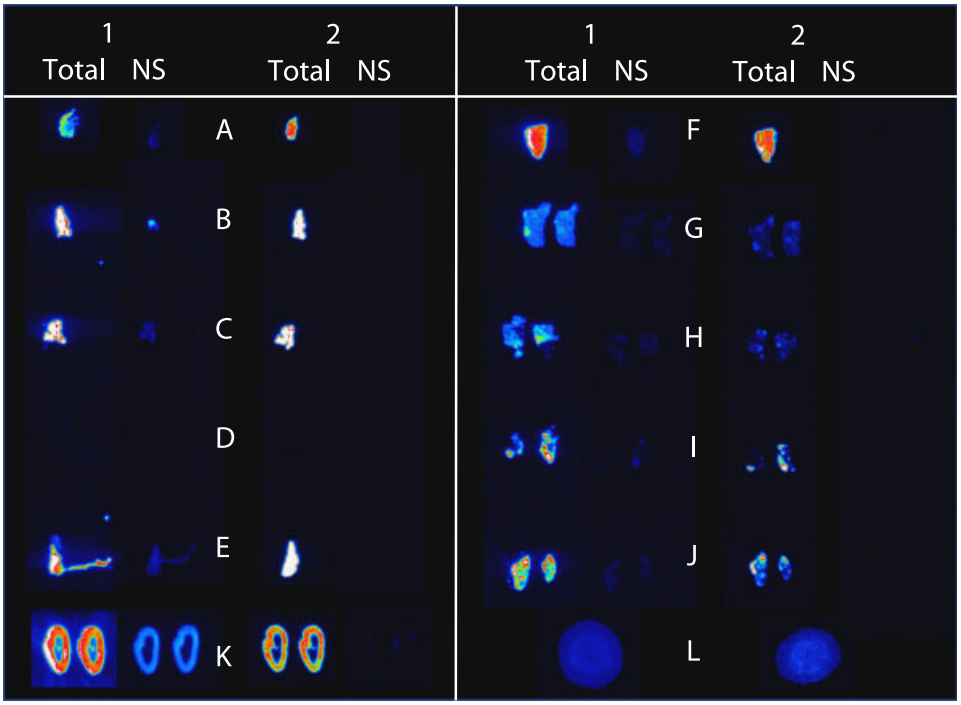


Figure 2. Autoradiography showing total binding of **1** and **2** to various human (A–J) and pig (K) adrenal tissues. A, B: normal adrenals; C, D: adrenal cortex cancer; E, F: aldosterone-producing adenoma; G, H: cortisol-producing adenoma; I, J: adrenal cortex hyperplasia; K: pig adrenal; L: standards on paper. The left tissue shows the tracer binding and the tissue to the right is under blocking conditions. The slides were incubated for 50 min with 0.01–0.1 MBq/mL of one of the two tracers. Ten micromolar etomidate was added to block specific binding.

Table 4. Results from metabolite studies for compounds **1** and **2** performed in rats

Tracer	Intact tracer[a] after 5 min (%)	Intact tracer[a] after 30 min (%)	Recovery[b] (%)
1	20 ± 6	2 ± 1	79 ± 34
2	27 ± 5	6 ± 4	86 ± 16

n = number of experiments.
[a]Intact radiotracer = (radiotracer fraction/all fractions) × 100. *n* = 2.
[b]Recovery = (all fractions/analysed sample) × 100. *n* = 4.

accumulation was seen in normal pig and human adrenal cortex in autoradiography and in rat adrenals in the organ distribution. The activity is slightly increased in bone, which indicates defluorination. The autoradiographic studies also show that both compounds are likely candidates for the detection of different types of adrenal carcinomas. The metabolism, probably due to cleavage of the ester, is rapid for both compounds, which might limit their usefulness as tracers for the *in vivo* visualization of adrenal tumours. Both compounds also accumulate in the lung, pancreas and liver, further indicating rapid metabolism in line with earlier metabolism data.[19]

Experimental

General chemistry

Liquid chromatographic (LC) analysis was performed with a Hitachi LaChromElite, pump L2130, injector L-220 and an L2400 UV detector in series with a β^+-flow detector. The following mobile phases were used: acetonitrile:water (50:7) (A), 25 mM aqueous potassium phosphate (B), 0.05 M aqueous ammonium formate, pH 3.5 (C), and acetonitrile (D). For analytical LC, an ACE 5 C18-HL 250 mm × 4.6 mm column was used at a flow rate of 1.5 mL/min. For semi-preparative LC, an ACE 5 C18-HL 250 mm × 10 mm column was used at a flow rate of 5 mL/min. Synthia, an automated synthesis system, was used for LC injection and fraction collection.[20] Data collection and LC control were performed using a Beckman System Gold chromatography software package (USA). Radioactivity was measured in an ion chamber (Veenstra Instrumenten bv, VDC-202, Holland). A portable dose-rate meter (Långenäs elektriska AB, Sweden) was used to estimate radioactivity during synthesis. Unlabelled reference substrates were synthesized and used for comparison in the LC analysis of the ^{18}F-labelled compounds. Syntheses of precursors and references were conducted under a nitrogen atmosphere using dried glassware and magnetic stirring. The 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylates were synthesized from the corresponding enantiopure phenylethylamine as described in the literature.[21] All other chemicals and anhydrous solvents were bought from Sigma-Aldrich or Acros Organics. Analytical thin layer chromatography (TLC) was

performed using Merck silica gel 60 F_{254}, and spots were visualized using UV light or I_2. Preparative TLC was carried out on 1-mm plates pre-coated with silica gel 60 F_{254}. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ (7.26 ppm ^1H, 77.0 ppm ^{13}C) or $(CD_3)_2SO$ (2.5 ppm ^1H, 39.5 ppm ^{13}C) on a Varian Unity 400 spectrometer (400 MHz for ^1H, 100.6 MHz for ^{13}C and 376.3 MHz for ^{19}F nuclei) or on a Varian Inova spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C nuclei). ^{19}F NMR spectra were recorded in $CDCl_3$ with $CFCl_3$ as the internal standard. Mass spectra was recorded on a Quattro Premier instrument from Waters, which has a triple quadrupole with electrospray ionization and was operated in positive mode, or on a Finnigan AQA using the mobile phases acetonitrile (0.1% formic acid) and water (0.1% formic acid). A Gilson 322 pump and a Phenomenex® Gemeni 5-μm C18 110A 150 mm × 3.00 mm column were also used. Microwave experiments were performed with a SmithCreator™ oven with monomodal radiation or on an Initiator 2.0 (Biotage AB, Uppsala, Sweden).

[^{18}F]fluoride was produced at Uppsala Imanet, GE Healthcare, using the Scanditronix MC17 cyclotron and the $^{18}O(p, n)^{18}F$ nuclear reaction through proton irradiation of ^{18}O-enriched water (95%, Rotem). The product solution of $^{18}F^-$ in water was transferred from the cyclotron target by an HPLC pump and trapped on a QMA filter (ABX, advanced biochemical compounds, pre-conditioned Sep-PAK®, light QMA cartridge with CO_3^{2-} as counter-ions, Radeberg). The column was purged with helium for 1 min. The [^{18}F]fluoride adsorbed on the resin was eluted into a reaction vial with 2 mL of the following solution: 12 mL of a 96:4 (by volume) acetonitrile:water mixture containing Kryptofix 2.2.2 and potassium carbonate (molar ratio 2:1).[22] The solution was then evaporated and co-evaporated with anhydrous acetonitrile (2 × 1 mL) to dryness in a nitrogen stream at 110°C to give the dried $[K/K2.2.2]^+{}^{18}F^-$ complex.

General method for one-step ^{18}F-labelling with conventional heating

The dried $[K/K2.2.2]^+{}^{18}F^-$ complex was dissolved in anhydrous *N*,*N*-dimethylformamide (0.2 mL) followed by the addition of the precursor (3.3 ± 0.3 mg), dissolved in anhydrous acetonitrile (50 μL) and *N*,*N*-dimethylformamide (0.2 mL). The mixture was heated in a closed vessel at 150°C for 15 min. The crude product was dissolved in water (2 mL) and injected onto the semi-preparative LC (**1** and **2**; t_R = 8.88 and 8.59 min, respectively) using the mobile phase C:D (55:45). The organic solvent was evaporated from the collected product fractions and the residue was dissolved in 2 mL of propylene glycol and phosphate buffer (pH = 7.5, 1:16). The product was then collected into a sterile vial. The radiochemical purities determined by analytical LC were >98% (**1** and **2**; t_R = 5.46 and 4.61 min, respectively, mobile phase A:B (50:50)).

General method for one-step ^{18}F-labelling with microwave heating

To a microwave vessel (0.2–0.5 mL) containing the precursor (2.5 ± 0.4 mg of **3** or **4**) dissolved in an appropriate solvent, the dissolved $[K/K2.2.2]^+{}^{18}F^-$ complex was added. A magnetic stir bar was used in all syntheses. The temperature and pressure were monitored during the course of the reaction. The reaction vial was cooled with pressurized air after irradiation. To find out the best reaction condition, the experiments were performed for 2, 10, 15, 20, 30, 60 and 120 s and repeated twice. Samples were taken from the reaction mixture at the different time points and analysed by preparative LC to determine the d.c. radiochemical yield. The recovery for the analogues, calculated from the following formula: recovery = (injected activity on the HPLC column/collected activity) × 100, was 102 ± 14% (n = 21). When the optimized condition was found the experiment was performed again and the crude product was purified using the method described for conventional heating. The d.c. radiochemical yield and the specific radioactivity were finally determined.

Precursor and reference synthesis

*2-(2-Bromoethoxy)ethyl 1-[(1R)-1-phenylethyl]-1H-imidazole-5-carboxylate (**3**)*

A solution of 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylic acid (0.036 g, 0.16 mmol) in dichloromethane was activated by adding tetrabutylammonium hydroxide (1 M in methanol, 0.2 mL). The reaction mixture was evaporated and co-evaporated with dichloromethane, then further dried under vacuum. The dried complex was then dissolved in anhydrous acetonitrile (0.5 mL) and cooled to 0°C. 2-Bromoethyl ether (40 μL, 0.32 mmol) was added and the mixture was brought to room temperature and stirred for 20 h. Dichloromethane was added, and the resulting mixture was extracted with water. The organic phase was concentrated to a small volume. The crude product was purified by preparative TLC (dichloromethane:methanol, 9.5:0.5) to give the title compound **3** as an oil (32 mg, 53%). ^1H NMR ($CDCl_3$): δ 7.84–7.81 (m, 2H), 7.35–7.26 (m, 3H), 7.19–7.17 (m, 2H), 6.34 (q, J = 7.4 Hz, 1H), 4.37 (t, J = 4.9 Hz, 2H), 3.81–3.75 (m, 4H), 3.43 (t, J = 6.1 Hz, 2H), 1.86 (d, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 159.9, 140.9, 137.8, 129.0, 128.7, 128.2, 127.0, 126.3, 71.2, 69.0, 63.55, 55.85, 30.3, 22.3. LC-MS (ESI^+), *m/z* 367 (50.7%), 369 (49.3%) $[M+H]^+$.

*2-(2-Fluoroethoxy)ethyl 1-[(1R)-1-phenylethyl]-1H-imidazole-5-carboxylate (**1**)*

Tetrabutylammonium fluoride (1 M in tetrahydrofuran, 0.33 mL) was added to a solution of **3** (0.1 g, 0.29 mmol) in anhydrous *N*,*N*-dimethylformamide (0.5 mL). The reaction mixture was heated at 130°C for 3 h. Dichloromethane was added and the resulting mixture was extracted with water. The organic phase was dried with magnesium sulfate and concentrated to a small volume. The crude product was purified by preparative TLC (dichloromethane:methanol, 9.9:0.1) to give the title compound **1** as an oil (4.6 mg, 5%). ^1H NMR ($CDCl_3$, 500 MHz): δ 7.83 (s, 1H), 7.74 (s, 1H), 7.35–7.17 (m, 5H), 6.33 (q, J = 7.0 Hz, 1H), 4.60–4.58 (m, 1H), 4.50–4.36 (m, 2H), 4.22–4.19 (m, 1H), 4.06–4.04 (m, 1H), 3.94–3.92 (m, 1H), 3.79–3.70 (m, 2H), 1.85 (d, J = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 167.8, 151.6, 144.5, 129.1, 129.0, 128.2, 126.4, 125.8, 87.5, 69.5, 66.1, 62.7, 55.7, 22.3. ^{19}F NMR δ −223.3 (m, F). LC-MS (ESI^+), *m/z* 307 $[M+H]^+$.

*2-[2-(2-Chloroethoxy)ethoxy]ethyl 1-[(1R)-1-phenylethyl]-1H-imidazole-5-carboxylate (**4**)*

A solution of 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylic acid (0.062 g, 0.29 mmol) in dichloromethane was activated by adding tetrabutylammonium hydroxide (1 M in methanol, 0.35 mL). The reaction mixture was evaporated and

co-evaporated with dichloromethane, then further dried under vacuum. The dried complex was dissolved in anhydrous acetonitrile (0.30 mL) and cooled to 0°C. 1,2-*Bis*(2-chloroethoxy)-ethane (0.45 mL, 2.85 mmol) was added and the reaction mixture was brought to room temperature and stirred for 1 h. The reaction was then microwave heated at 60°C for 15 h. Dichloromethane was added and the resulting mixture was extracted with water and concentrated to a small volume. The crude product was purified by preparative TLC (dichloromethane:methanol, 9.5:0.5) to give the title compound **4** as an oil (58 mg, 56%). ^{1}H NMR ($CDCl_3$): δ 7.85 (s, 1H), 7.83 (s, 1H), 7.36–7.18 (m, 5H), 6.36 (q, $J = 7.1$ Hz, 1H), 4.37 (q, $J = 5.4$ Hz, 2H), 3.77–3.60 (m, 10H), 1.87 (d, $J = 7.1$ Hz, 3H).^{13}C NMR ($CDCl_3$): δ 160.0, 146.2, 140.6, 139.5, 137.0, 129.0, 128.3, 126.6, 126.4, 71.4, 70.7, 69.1, 63.8, 55.9, 42.8, 22.2. LC-MS (ESI^+), *m/z* 367 (75.8%), 369 (24.2%) $[M+H]^+$.

*2-[2-(2-Fluoroethoxy)ethoxy]ethyl 1-[(1R)-1-phenylethyl]-1H-imidazole-5-carboxylate (**2**)*

Tetrabutylammonium fluoride in tetrahydrofuran (45 μL) was added to a solution of **4** (0.012 g, 0.03 mmol) in anhydrous *N*,*N*-dimethylformamide (0.4 mL). The reaction mixture was heated in the microwave at 200°C for 1.4 h. Dichloromethane was added and the resulting mixture was extracted with water. The organic phase was concentrated to a small volume. The crude product was purified by semi-preparative LC (C:D, 50:50) to give the title compound **2** as an oil (8.2 mg, 70%). ^{1}H NMR ($CDCl_3$): δ 7.76 (s, 1H), 7.71 (s, 1H), 7.35–7.17 (m, 5H), 6.36 (q, $J = 7.1$ Hz, 1H), 4.26–4.16 (m, 2H), 1.86 (d, $J = 7.1$ Hz, 4H), 1.68–1.65 (m, 3H) 1.42–1.38 (m, 3H), 0.96–0.93 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 155.8, 138.7, 130.0, 129.0, 128.1, 126.4, 125.0, 104.9, 84.1, 70.9, 69.3, 63.7, 62.2, 59.7, 55.5, 20.0. ^{19}F NMR δ −223.2 (m, F). LC-MS (ESI^+), *m/z* 351 $[M+H]^+$.

General biology

Male Sprague–Dawley rats were used. All animals were handled according to the guidelines set forth by the Swedish Animal Welfare Agency, and the experiments were approved by the local Ethics Committee for Animal Research, permit no: C234/5. Tissues from the adrenals of human subjects were taken during surgery at Uppsala University Hospital in accordance with permission obtained from the local ethical committee. Adrenals from pigs were obtained from the local slaughterhouse. All tissues were frozen immediately after removal and stored at −70°C until cryosectioning.

Data analysis: Calculations were performed using Microsoft Excel (Microsoft, USA) and Prism v.5.01 (GraphPad Software, USA). The images were prepared using ImageQuant 5.1 (Molecular Dynamics, USA) and Microsoft Excel and PowerPoint (Microsoft).

Organ distribution

In order to evaluate uptake in normal tissues, biodistribution studies were performed. The rats were injected intravenously with 10 MBq/animal (**1**: 281 ± 11, range 260–295 g ($n = 12$; $n = 4$ per time point); **2**: 318 ± 12, range 300–340 g ($n = 12$; $n = 4$ per time point)) of the two tracers, respectively. The animals were sacrificed in a high concentration of CO_2 after 5, 10 and 30 min, and selected organs were dissected out. Blood, heart, lung, liver, pancreas, spleen, adrenal, kidney, intestine, urinary bladder, testis, muscle, bone (femur), brain (cerebrum) and cerebellum were collected and weighed, and their radioactivity was determined and corrected for radioactive decay in an automated γ-counter. Organ values were calculated as standardized uptake value (SUV), calculated as follows:

$$\mathrm{SUV} = \frac{\mathrm{ACT(Bq/g)}}{\mathrm{DOSE(Bq)/BW(g)}}$$

where ACT is the measured concentration of radioactivity corrected for physical decay, DOSE is the administered amount of radioactivity (Bq) and BW is the body weight of the subject (g).

Frozen section autoradiography

Sections (25 μm) were prepared in a cryomicrotome and put on SuperFrost glass slides (SuperFrost®Plus, MenzelGläser, Germany). The slides were kept in a freezer (−20°C) for 1 day until used. At the start of the experiment, the slides were pre-incubated for 10 min in Tris–HCl (50 mM, pH 7.4) buffer. The slides were then transferred to containers that held **1** or **2** (0.01–0.1 MBq/mL) in Tris–HCl buffer. In a duplicate set of containers, 10 μM etomidate was added to block specific binding. After incubation for 50 min, the slides were washed 3 × 3 min in cold Tris–HCl buffer. The slides were dried in a heated (37°C) oven and exposed to phosphor imaging plates (Molecular Dynamics) for 2 h. Twenty microlitres of the incubate was pipetted onto a filter paper (standard activity area), which was exposed in parallel to the experimental sections. The plates were then scanned in a Phosphor Imager Model 400S using 100-μm pixel width (Molecular Dynamics).

Metabolite study

The metabolite analysis in plasma was performed in rats at the 5- and 30-min time points. At each time point, one male rat was injected via the tail vein with 50 MBq radioligand. The animals were sacrificed after 5 or 30 min in a high concentration of CO_2. The plasma was obtained by a 4-min centrifugation of the venous blood at 4000 rpm at 4°C. Plasma proteins were precipitated by adding acetonitrile (600 μL) that contained unlabelled reference to the plasma (600 μL). The resulting mixture was centrifuged at 4000 rpm at 4°C for 2 min. The supernatant was filtered through a 0.2-μm pore size membrane (Corning Incorporated, Corning, NY, USA) under centrifugation at 4000 rpm at 4°C for 2 min. The sample was analysed by HPLC to separate the radioligand from its radioactive metabolites. This was performed by injecting the sample (99 μL) on the same column used for semi-preparative LC. The tracer and its metabolite were eluted with C:D (**1**: 50:50 vol/vol and **2**: 45:55 vol/vol) at a flow rate of 5 mL/min. The eluent was collected in two fractions. Both tracers eluted in the second fraction (**1**: $t_R = -2.20$ and 5.29 min and **2**: $t_R = 1.90$ and 5.08 min), whereas their metabolites eluted in the first fraction. Observation of the UV peak from the unlabelled standard indicated that **1** and **2** eluted in the second fraction. Even if no detectable radiopeak co-eluted with the unlabelled standard, the fraction was collected and the radioactivity of the fraction was measured in a γ-counter.

Conclusion

One-step ^{18}F-labelling method for the synthesis of **1** and **2** is presented. These compounds, which are ethoxy-modified at the ester part of the methyl 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylate, are less lipophilic than the alkyl-substituted metomidate tracer, and the biological evaluation indicates that both compounds are likely candidates for the detection of different types of adrenal carcinomas.

The d.c. radiochemical yields of **1** and **2** were 26 ± 8 and 23 ± 8%, respectively, after a 50–60-min synthesis with conventional heating. The reaction became faster and higher yielding when microwave heating was employed. Specifically, the d.c. radiochemical yield for analogues **1** and **2** was increased to 57 ± 12 and 51 ± 18%, respectively.

According to the literature,[23] microwave reactors have been integrated into fully automated radiofluorination modules. The one-step microwave heated ^{18}F-labelling synthesis proposed in this article could also be fully automated by integrating a microwave reactor into, for example, the commercial synthesizer TRACERlab FX $_{FN}$.

Acknowledgement

This work was conducted in collaboration with Uppsala Imanet, GE Healthcare. We are grateful to Elisabeth Bergström-Petterman for her assistance in the biological study.

References

[1] I. Varga, K. Rácz, R. Kiss, L. Fütö, M. Tóth, O. Sergev, E. Gláz, *Steroids* **1993**, *58*, 64–68.
[2] H. Vanden Bossche, G. Willemsens, W. Cools, D. Bellens, *Biochem. Pharmacol.* **1984**, *33*, 3861–3868.
[3] M. Bergström, T. A. Bonasera, L. Lu, E. Bergström, C. Backlin, C. Juhlin, B. Långström, *J. Nucl. Med.* **1998**, *39*, 982–989.
[4] M. Bergström, C. Juhlin, T. A. Bonasera, A. Sundin, B. Långström, *J. Nucl. Med.* **2000**, *41*, 275–282.
[5] T. S. Khan, A. Sundin, C. Juhlin, B. Långström, M. Bergström, B. Eriksson, *Eur. J. Nucl. Med.* **2003**, *30*, 403–410.
[6] G. Zettinig, M. Mitterhauser, W. Wadsak, A. Becherer, C. Pirich, H. Vierhapper, B. Niederle, R. Dudczak, K. Kletter, *Eur. J. Nucl. Med.* **2004**, *31*, 1224–1230.
[7] H. Minn, A. Salonen, J. Friberg, A. Roivainen, T. Viljanen, J. Långsjö, J. Salmi, M. Välimäki, K. Någren, P. Nuutila, *J. Nucl. Med.* **2004**, *45*, 972–979.
[8] F. Karimi, M. Erlandsson, Ö. Lindhe, B. Långström, *J. Labelled Compd. Radiopharm.* **2008**, *51*, 273–276. DOI: 10.1002/jlcr.1517.
[9] W. Wadsak, M. Mitterhauser, *J. Labelled Compd. Radiopharm.* **2003**, *46*, 379–388. DOI: 10.1002/jlcr.680.
[10] W. Wadsak, M. Mitterhauser, G. Rendl, M. Schuetz, L. K. Mien, D. E. Ettlinger, R. Dudczak, K. Kletter, G. Karanikas, *Eur. J. Nucl. Med. Mol. Imaging* **2006**, *33*, 669–672.
[11] N. Elander, J. R. Jones, S. Y. Lu, S. Stone-Elander, *Chem. Soc. Rev.* **2000**, *29*, 239–249.
[12] I. M. Zolle, M. L. Berger, F. Hammerschmidt, S. Hahner, A. Schirbel, B. Peric-Simov, *J. Med. Chem.* **2008**, *51*, 2244–2253.
[13] www.acdlabs.com.
[14] J. Tierney, P. Lindström, *Microwave Assisted Organic Synthesis*, Blackwell Publishing Ltd, Oxford, **2005**.
[15] J. R. Jones, S. Y. Lu, Microwave-enhanced radiochemistry, in *Microwaves in Organic Synthesis* (Ed.: A. Loupy), 1st ed., Wiley-VCH Verlag GmbH & Co., Weinheim, **2002**, pp. 456–459.
[16] S. Stone-Elander, N. Elander, *J. Labelled Compd. Radiopharm.* **2002**, *45*, 715–746.
[17] N. Leadbeater, H. Torenius, *J. Org. Chem.* **2002**, *67*, 3145–3148.
[18] www.personalchemistry.com.
[19] D. E. Ettlinger, W. Wadsak, L. K. Mien, M. Machek, L. Wabnegger, G. Rendl, G. Karanikas, H. Viernstein, K. Kletter, R. Dudczak, M. Mitterhauser, *Eur. J. Nucl. Med. Mol. Imaging* **2006**, *33*, 928–931.
[20] P. Bjurling, R. Reineck, G. Westerberg, A. D. Gee, J. Sutcliffe, B. Långström, *Proceedings of the VIth Workshop on Targetry and Target Chemistry*, Vancouver, Canada, **1995**, pp. 282–284.
[21] E. F. Godefroi, P. A. Janssen, C. A. Van Der Eycken, A. Van Heertum, C. Niemegeers, *J. Med. Chem.* **1965**, *6*, 220–223.
[22] N. A. Gomzina, D. A. Vasil'ev, R. N. Krasikova, *Radiochemistry* **2002**, *44*, 366–409.
[23] N. Lazarova, F. G. Siméon, J. L. Musachio, S. Lu, V. W. Pike, *J. Labelled Compd. Radiopharm.* **2007**, *50*, 463–465.