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Synthesis and biological evaluation of [*carboxyl*-^{11}C]eprosartan

Ola Åberg,[a] Örjan Lindhe,[b] Håkan Hall,[c] Per Hellman,[d] Tor Kihlberg,[b] and Bengt Långström[a,b]*

Essential hypertension occurs in approximately 25% of the adult population and one cause of hypertension is primary aldosteronism. Targeting the angiotensin II AT_1 receptor using PET and an appropriate tracer may offer a diagnostic method for adrenocortical tissue. This report describes the synthesis of the selective AT_1 receptor antagonist [*carboxyl*-^{11}C]eprosartan 10, 4-[2-butyl-5-((*E*)-2-carboxy-3-thiophen-2-yl-propenyl)-imidazol-1-ylmethyl]-[*carboxyl*-^{11}C]benzoic acid, and its precursor (*E*)-3-[2-butyl-3-(4-iodo-benzyl)-3*H*-imidazol-4-yl]-2-thiophen-2-ylmethyl-acrylic acid 9. ^{11}C-carboxylation of the iodobenzyl moiety was performed using a palladium-mediated reaction with [^{11}C]carbon monoxide in the presence of tetra-*n*-butyl-ammonium hydroxide in a micro-autoclave using a temperature gradient from 25 to 140°C over 5 min. After purification by semipreparative HPLC, [*carboxyl*-^{11}C]eprosartan 10 was obtained in 37–54% decay-corrected radiochemical yield (from [^{11}C]carbon monoxide) with a radiochemical purity >95% within 35 min of the end of bombardment (EOB). A 5-μAh bombardment gave 2.04 GBq of 10 (50% rcy from [^{11}C]carbon monoxide) with a specific activity of 160 GBq μmol^{-1} at 34 min after EOB. Frozen-section autoradiography shows specific binding in kidney, lung and adrenal cortex. *In vivo* experiments in rats demonstrate a high accumulation in kidney, liver and intestinal wall.

Keywords: angiotensin II; AT_1; eprosartan; [^{11}C]carbon monoxide; carboxylation

Introduction

Hypertension occurs in approximately 25% of the adult population and predispose for cardiac diseases and arteriosclerosis. Hypertension may be caused by a chronic excessive secretion of aldosterone from the glomerulosa cells of the adrenal cortex; this is known as primary aldosteronism.[1] Primary aldosteronism is the cause of hypertension in 10–15% of all patients with essential hypertension.[2,3] When caused by an aldosterone producing adenoma; primary aldosteronism can be treated by surgical removal of the hypersecreting gland. Since nodular hyperplasia of the adrenal glands may mimic an adenoma; it may be a challenge to determine which of the adrenals to remove.[4]

Another clinical problem is adrenal incidentalomas that are frequently found (1–5%) in CT examinations.[5–7] Though the majority of incidentalomas are benign masses, characterization is difficult and time-consuming since sensitive methods to exclude benign masses, which do not need further attention, are lacking. For this purpose, PET scanning with ^{11}C-metomidate and ^{11}C-hydroxyephederine have been studied.[8,9] As a potential complementary method we are investigating the use of the angiotensin II type 1 receptor (AT_1-R) as target for PET. Pathological adrenal cortex may express different amounts of AT_1-R, adenoma classically been angiotensin-unresponsive while the tissue in idiopathic hyperplasia is angiotensin-responsive. This receptor is present mainly in the zona glomeruosa in human,[10] and is known to also be present in colon, kidney, aorta, CNS and the heart.[10–14] AT_1 up-regulation in the atria of patients with end-stage heart failure has also been observed. Human levels of AT_1 mRNA were altered in hearts with dilated cardiomyopathy as well as old myocardial infarctions.[13–17]

One class of compounds that is targeting the AT_1-receptor is the sartans. Eprosartan is a drug that selectively inhibits the action of angiotensin II on the AT_1 receptor and is prescribed for hypertension. The structure has two carboxylic acid moieties that potentially could be labelled with ^{11}C by carboxylation of the corresponding aryl-halide or vinyl-halide using [^{11}C]carbon monoxide. [^{11}C]Carbon monoxide is a versatile labelled precursor that has been used to synthesize a whole array of different carbonyl compounds by transition metal mediated reactions with high specific activity.[18] Aromatic carboxylations can be performed using [^{11}C]carbon dioxide and a corresponding Grignard reagent, but the reaction is moisture sensitive and is not compatible with acidic functional groups. Another drawback is the laborious preparation and handling of the Grignard reagent to avoid isotopic dilution with atmospheric carbon dioxide. Carbon monoxide on the other hand is less

[a]*Department of Biochemistry and Organic Chemistry, Box 599, Husargatan 3, BMC, S-751 24 Uppsala, Sweden*

[b]*Uppsala Imanet, GE Healthcare, Box 967, S-751 09 Uppsala, Sweden*

[c]*Uppsala ASL, GE Healthcare, Box 967, S-751 09 Uppsala, Sweden*

[d]*Department of Surgery, University Hospital, SE-75185 Uppsala, Sweden*

**Correspondence to: Bengt Långström, Department of Biochemistry and Organic Chemistry, Box 599, Husargatan 3, BMC, S-751 24 Uppsala, Sweden. E-mail: bengt.langstrom@biorg.uu.se*

abundant in the atmosphere, thus [^{11}C]carbon monoxide is less prone to isotopic dilution. Transition-metal mediated/catalyzed carboxylation is a very general reaction and tolerates a wide variety of functional groups.

The metabolic profile of a PET-tracer may influence the decision where to incorporate the radiolabel. However, eprosartan is reported to be very metabolically stable in human and no active metabolites are reported, thus the radiolabel may in this case be incorporated in any of the two carboxylic acid moieties. Following an intravenous administration of [^{14}C]eprosartan, about 61% of the radioactivity was recovered in the feces and about 37% in the urine, indicating that both the biliary and renal routes are important. Elimination occurs via the liver and kidneys, primarily as unchanged drug, with 7% of an intravenous dose excreted in urine as the corresponding acyl glucuronide. Eprosartan is not metabolized by cytochrome P450 enzymes.[19,20]

Previously, the following compounds targeting the AT_1 receptor have been labelled with ^{11}C; [^{11}C-tetrazoyl]irbesartan,[21] [^{11}C]MK-996,[22] [^{11}C]L-159,884[23–27] and [^{11}C]KR31173.[28,29] Here we report the synthesis of [*carboxyl*-^{11}C]eprosartan **10** (Scheme 3). Eprosartan was selected as a tracer candidate as it (a) is extensively investigated in the human, including toxicity studies, (b) is metabolically stable in the human, and (c) can be obtained in high specific activity using a one-step synthesis.

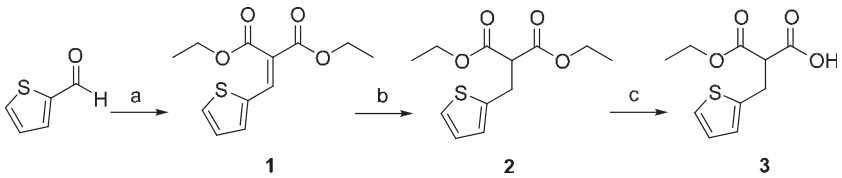
Results and discussion

Precursor synthesis

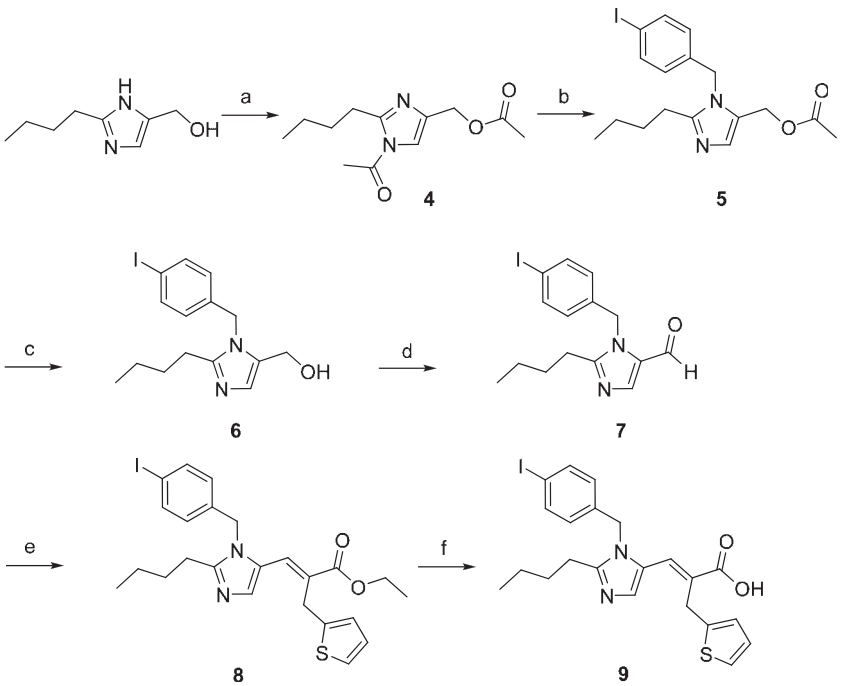
The precursor was synthesized as outlined by Keenan *et al.* (Schemes 1 and 2).[30] A Knoevenagel reaction between 2-thiophenecarboxaldehyde and diethyl malonate formed the condensation product **1**, which was subsequently reduced using sodium borohydride in ethanol to give the diester **2**. The diester was selectively hydrolyzed using potassium hydroxide to give the ester/carboxylic acid **3** in good yields.

2-Butyl-4(5)-(hydroxymethyl)-imidazole was protected by stirring with acetic anhydride to give **4** in good yield. In the next step **4** was combined with trifluoromethanesulfonic acid 4-iodobenzyl ester (prepared from (4-iodophenyl)methanol, DIPEA and triflic anhydride at −78°C), in a one-pot reaction to give the crude acetate **5**. The crude acetate was hydrolyzed using potassium carbonate to give the alcohol **6**. The position of the 4-iodobenzyl group was confirmed by NOE NMR by selective irradiation of the CH_2 groups. Oxidation of the alcohol using technically activated manganese oxide gave the desired aldehyde **7**.

A Knoevenagel-like condensation between the acid **3** and the aldehyde **7** and subsequent elimination of CO_2 formed the intermediate ester **8**.[31] The acid **3** was added in excess due to a competing decarboxylation of the acid. The *E*-geometry of the



Scheme 1. (a) Diethyl malonate, piperidine, benzoic acid in cyclohexane, reflux 20 h. (b) $NaBH_4$ in EtOH, 40 min. (c) KOH 1.0 eq in EtOH, r.t. 48 h.



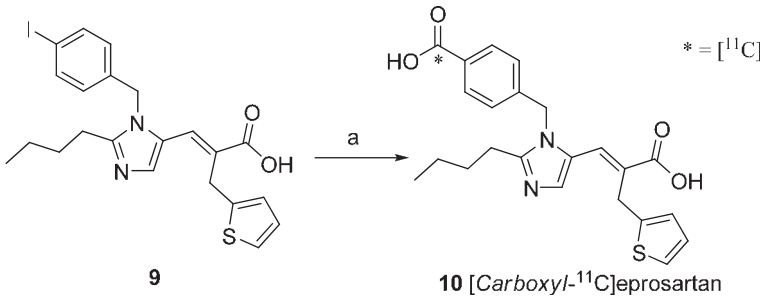
Scheme 2. (a) Acetic anhydride. (b) (4-iodophenyl)methanol, triflic anhydride, DIPEA in dichloromethane. (c) K_2CO_3 in MeOH. (d) Activated MnO_2 in dichloromethane. (e) **3**, piperidine and benzoic acid in toluene. (f) NaOH, EtOH.

double bond *E*-form was confirmed by measuring the $^{3}J_{H\text{-}C}$ coupling constants of the vinyl proton by means of an HMBC experiment. The *cis* coupling to the carbonyl carbon was 7.4 Hz and the *trans* coupling to the CH_2-carbon was 11.2 Hz. The precursor **9** was finally obtained by basic hydrolysis of the ester.

Labelling reaction

The ^{11}C-carboxylation of **9** was performed according to a reported method using a palladium-mediated carboxylation of the aryl iodide with [^{11}C]carbon monoxide in the presence of tetra-*n*-butyl-ammonium hydroxide in a micro-autoclave for 5 min (Scheme 3 and Table 1).[32] Different temperatures were tested using constant reagent concentrations. Temperatures below 120°C gave high conversion of [^{11}C]carbon monoxide, but 61–77% resulted in [^{11}C]carbonate and only 11–31% radiochemical yield of **10** (entries 1–4). The side reaction that trapped the radioactivity as [^{11}C]carbonate occurs probably via the water–gas shift reaction (Scheme 4) and subsequent reaction of [^{11}C]carbon dioxide with OH^-.

The conversion to [^{11}C]carbonate was determined by acidifying the purged crude sample with ammonium formate buffer (pH 3.5) and then purging once more to remove [^{11}C]O_2. Both [^{11}C]O conversion and the amount of [^{11}C]carbonate formed in the reaction rapidly dropped at elevated temperatures. Above 150°C, [^{11}C]carbonate could no longer be detected; instead unidentified lipophilic radioproducts were formed (entries 1–9). The temperature range 65–160°C gave low product yields ($22 \pm 11\%$) and no clear correlation between radiochemical yield and temperature was found. When the amounts of base, water and DMSO were decreased and the temperature was raised from r.t. to around 140°C over 5 min, the reaction was cleaner and formed less [^{11}C]carbonate (entry 10). However, 51% conversion of [^{11}C]carbon monoxide was not satisfactory, so the amount of Pd complex was increased in order to favor the [^{11}C]O insertion that forms the Pd-[^{11}C]acyl complex. This gave 90% [^{11}C]O conversion in a very clean reaction that produced radiochemically pure product (entry 11). The presence of DMSO as a co-solvent had no effect on the radiochemical yields but helped to prevent precipitation while diluting the



Scheme 3. (a) [^{11}C]carbon monoxide, $Pd(PPh_3)_4$, tetra-*n*-butylammonium hydroxide 30 hydrate, water, DMSO, THF, 5 min.

Table 1. Optimization of the ^{11}C-carboxylation reaction

Entry	$Pd(PPh_3)_4$ (mM)	Q-OH[a] (mM)	H_2O[b] (M)	DMSO (M)	Temp (°C)	Conversion of [^{11}C]O (%)[c,d]	Analytical RCY (%)[d,e]
1	5	25	3.5	0.7	65	100	23
2	5	25	3.5	0.7	90	99	31
3	5	25	3.5	0.7	110	98	19
4	5	25	3.5	0.7	120	83	11
5	5	25	3.5	0.7	130	57	28
6	5	25	3.5	0.7	140	36	20
7	5	25	3.5	0.7	150	40	33
8	5	25	3.5	0.7	160	24	16
9	5	25	3.5	0.7	180	30	<6
10	5	25	0.75	–	25–140	$51 \pm 6(2)$	$41 \pm 2(2)$
11	14	6.8	0.90	0.35	25–140	91	91
12	14	6.8	0.20	0.35	25–140	86	82
13	14	6.8	0.20	–	25–140	89	79
14	14	–	–	–	25–140	81	33

Reaction conditions were [^{11}C]carbon monoxide (10–100 nmol), and 200 μL solution of **9** (17 mM), $Pd(PPh_3)_4$, tetra-*n*-butylammonium hydroxide 30 hydrate. Additives were H_2O and DMSO.
[a]Q-OH: tetra-*n*-butylammonium hydroxide 30 hydrate.
[b]The total amount of added water including crystal water from Q−OH.
[c]Decay corrected conversion yield of [^{11}C]carbon monoxide to non-volatile products remaining in the reaction mixture after evaporation of solvent by purging with nitrogen.
[d]The numbers in brackets indicate the number of experiments.
[e]Analytical radiochemical yield as the sum of the decay corrected analytical radiochromatogram and the conversion yield.

$$[^{11}C]O + H_2O \rightleftharpoons [^{11}C]O_2 + H_2$$

Scheme 4. The water–gas shift reaction.

nitrogen-purged crude product for HPLC injection (entry 12 and 13). When base, water and DMSO were omitted the reaction resulted in an unidentified compound (47%) along with the product (33%, entry 14).

For biological deliveries of **10**, the conditions were similar as for entry 13 except that the amount of Pd complex was decreased to 10 mM for practical reasons to avoid precipitation upon dilution of the crude product. Those conditions gave a slightly less clean reaction than entry 11 (as estimated from the preparative UV chromatogram), but at the same time better chromatography, since DMSO was no longer needed to keep crude product mixture in solution. The crude product was purified using semipreparative HPLC and formulated in a phosphate buffer solution with a pH of 7.2. In this way the decay-corrected radiochemical yield of the ^{11}C-labelled and formulated [*carboxyl*-^{11}C]eprosartan **10** was 37–54% ($n = 5$), calculated from [^{11}C]carbon monoxide, within 35 min from EOB and with a radiochemical purity $>95\%$.

Specific activity

High specific activity is important to avoid saturating or perturbating the biological system under study, especially when the binding site density (B_{max}) is low. The concept of using [^{11}C]carbon monoxide as the labelled precursor allows very easy handling and preparation of the reagent solutions because the aryl halide and palladium complex used are not particularly reactive toward carbon monoxide. Additionally, carbon monoxide has low solubility in most organic solvents at normal pressures and temperatures and has low abundance in the atmosphere.[33] This is an advantage over the laborious handling of reactive Grignard reagents used for carboxylation with [^{11}C]carbon dioxide, due to the more abundant carbon dioxide that is prone to cause isotopic dilution and lower specific activity.[34] It should be noted that the basic quaternary ammonium hydroxide salt used in this report is prone to react with carbon dioxide to form carbonate and should be stored under inert atmosphere. Addition of cold carbonate could, at least in theory, lead to isotopic dilution of [^{11}C]carbon monoxide via the water–gas shift reaction. However, in this work we have not noticed tendencies of isotopic dilution from the base. The specific activity of [*carboxyl*-^{11}C]eprosartan **10** was determined following a 5-μAh bombardment giving 10.3 GBq [^{11}C]carbon monoxide at 7.5 min from EOB. At 34 min, 2.04 GBq of **10** (50% from [^{11}C]carbon monoxide) was isolated with a specific activity of 160 GBq μmol^{-1} (520 GBq μmol^{-1} at EOB). A 12-μAh bombardment gave a specific activity of 360 GBq μmol^{-1} at 38 min from EOB (1250 GBq μmol^{-1} at EOB) and is in the same order as previously reported.

[*Carboxyl*-^{11}C]eprosartan *in vitro* autoradiography

Using *in vitro* autoradiography on frozen organ section, specific [*carboxyl*-^{11}C]eprosartan binding (affected by excess of unlabelled eprosartan) was observed in rat lung and kidney as well as pig adrenal, and to some degree also in rat spleen and liver. No specific binding was seen in striatum and cerebellum of rat brain (Figure 1). Studies of [*carboxyl*-^{11}C]eprosartan binding in pig adrenal showed most intense binding in the outer layer of the adrenal cortex (presumably zona glomerulosa) and was also affected by the addition of excess unlabelled eprosartan (Figure 2). However, the binding density (B_{max}) was very low. Quantitation of the binding in whole pig adrenals followed by saturation analysis showed that the binding was saturable, with a K_d-value of approximately 10 nM (Figure 2). The specific binding in pig adrenals was approximately 60% at K_d (10 nM) and 70% at half K_d (5 nM). Specific [*carboxyl*-^{11}C]eprosartan binding was also found in human adrenals, and especially with those affected with aldosterone producing adenomas (Figure 3). It should be noted that no binding at all was found in one case of cortisol producing adenoma and in two cases of adrenocortical hyperplasia (images not shown).

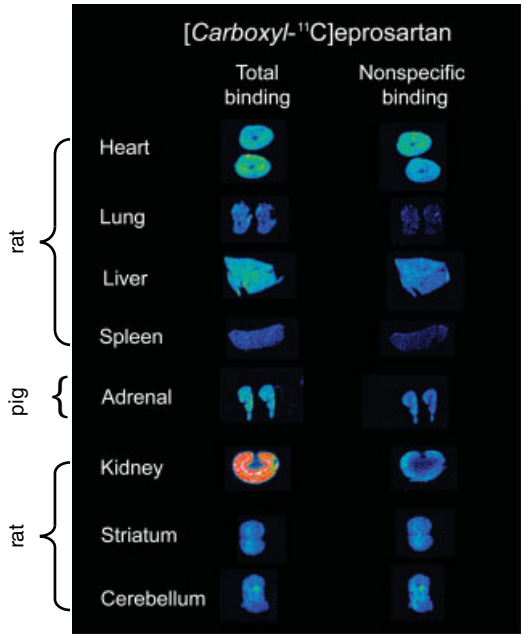


Figure 1. Frozen section autoradiography of [*carboxyl*-^{11}C]eprosartan left, in the right column binding was blocked with 10 mM unlabelled eprosartan.

Several research groups have studied the receptor expression of AT_1 at the mRNA or receptor level in diseased adrenal glands. While the reported levels of AT_1 compared to controls sometimes are contradictory, aldosteron producing adenoma seems in most cases to have a higher degree of variability.[35–46] No studies have yet clearly described differences in AT_1-R expression between adenomas and hyperplasia.

[*Carboxyl*-^{11}C]eprosartan *in vivo* organ distribution

A high accumulation in male rat kidney, liver and intestinal wall was observed as well as retention of uptake after 40 min in lung, testis and fat (Table 2). In an earlier distribution study using pharmacological doses of [^{14}C]eprosartan (3 and 10 mg kg^{-1}) the highest concentrations were found in the intestines, kidney and liver.[47] In the present investigation using tracer amounts of [^{11}C]eprosartan (<0.1 μg kg^{-1}), the highest concentrations were found in the corresponding organs.

Experimental

General

All chemicals were purchased from Aldrich/Fluka and used without further purification. Dry tetrahydrofuran was distilled

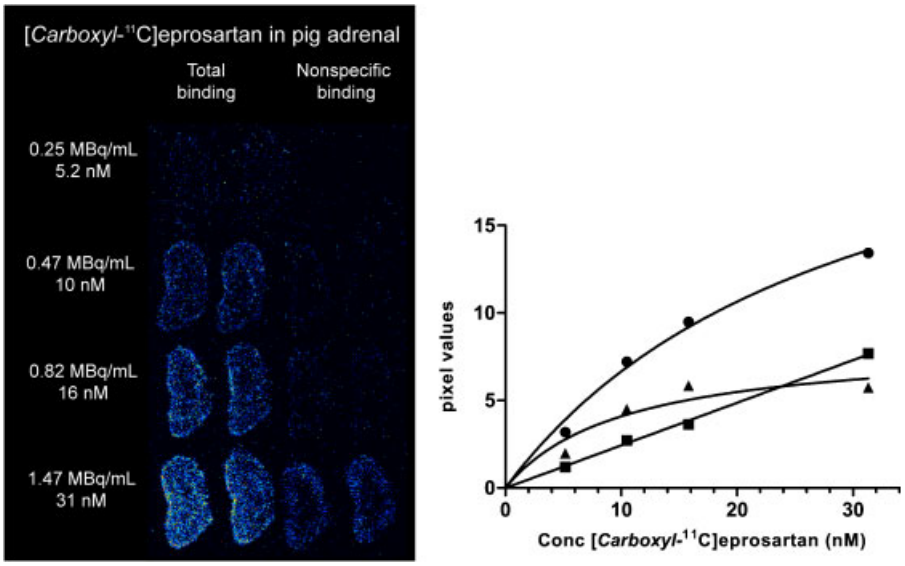


Figure 2. The left panel shows the total and non-specific binding of [*carboxyl*-^{11}C]eprosartan to pig adrenal glands at four different concentrations. Quantitative analysis of the binding was used for a saturation analysis, revealing saturable binding with an estimated of K_d 10 nM and B_{max} of 8.3 pixels (corresponding to approximately 1.1 fmol mg^{-1} tissue). Lower panel: (●) Total binding, (■) non-specific binding, (▲) specifically bound tracer.

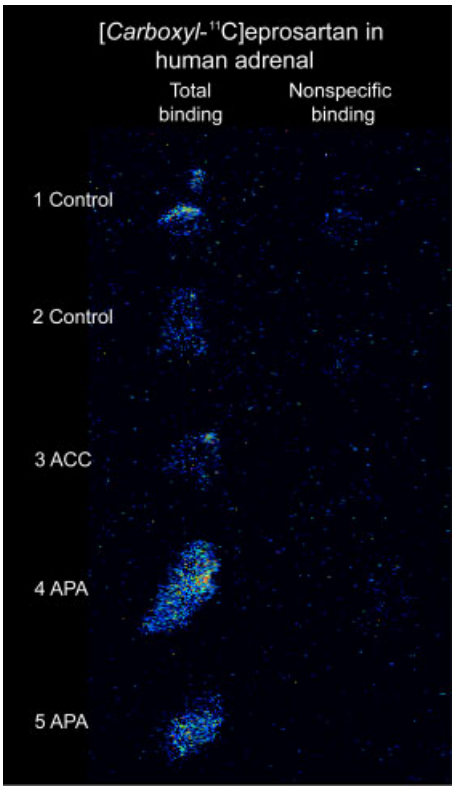


Figure 3. 1 and 2: Parts of two adrenal glands extirpated for non-cortical reasons, but expressing normal cortex 3: from a patient with unspecified adrenocortical cancer (ACC), 4 and 5 from patients with aldosterone producing adenomas (APA).

Table 2. Organ distribution of [*carboxyl*-^{11}C]eprosartan **10** in adult male Sprague Dawley rats 15 and 40 min post injection (SUV, average ± S.E.M.)

Organ distribution of [carboxyl-^{11}C]eprosartan **10** in rats SUV[a] in rat		
	15 min ($n = 9$)	40 min ($n = 8$)
Blood	0.269 ± 0.057	0.130 ± 0.032
Heart	0.116 ± 0.022	0.050 ± 0.008
Lung	0.491 ± 0.094	0.434 ± 0.127
Liver	5.28 ± 0.583	2.52 ± 0.387
Pancreas	0.194 ± 0.025	0.064 ± 0.017
Spleen	0.263 ± 0.046	0.093 ± 0.017
Adrenal	0.875 ± 0.113	0.318 ± 0.054
Kidney	7.00 ± 1.01	2.30 ± 0.358
Intestines	15.41 ± 2.55	3.58 ± 0.570
Urinary bladder	0.417 ± 0.065	0.714 ± 0.283
Testis	0.048 ± 0.004	0.054 ± 0.009
Muscle	0.100 ± 0.018	0.049 ± 0.009
Fat	0.052 ± 0.007	0.059 ± 0.016
Brain	0.010 ± 0.002	0.005 ± 0.001

[a]See experimental section for details.

over sodium and benzophenone. Thin-layer chromatography analyses were performed on pre-coated Merck silica gel plates ($60F_{254}$) and visualized with UV light. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 300, 400 or 500 MHz spectrometer and chemical shifts are given in ppm (δ) using $CHCl_3$ (7.26 ppm for ^{1}H, and 77.16 for ^{13}C) as internal standard. GC-MS analyses were performed on a Finnigan MAT Thermoquest GCQ mass spectrometer equipped with non-polar column SE-54. LC-MS analyses were performed on a Gilson reverse phase HPLC equipped with a Finnigan mass spectrometer (MeCN/H_2O and 0.1% formic acid). [^{11}C]Carbon was produced by a Scandtronix MC-17 cyclotron at the Uppsala Imanet, GE Healthcare by the ^{14}N(p, α)^{11}C nuclear reaction using a gas target containing nitrogen (AGA, Nitrogen 6.0) and 0.05% oxygen (AGA, Oxygen 4.8) that was bombarded with 17 MeV protons. [^{11}C]Carbon was obtained as [^{11}C]carbon dioxide in the target and was reduced to [^{11}C]carbon monoxide in a zinc furnace at 400°C, and transferred to the micro-autoclave using a remote-controlled workstation.[48,49] Analytical HPLC was performed on a Beckman system, equipped with a Beckman 126 pump, a Beckman 166 UV

detector in series with a Bioscan β^+-flow count detector and a Beckman Ultrasphere ODS dp 5 μ column (250 × 4.6 mm). A Gilson 231 XL was used as auto injector. Mobile phase: (A) 50 mM ammonium formate pH 3.5, (B) acetonitrile; 30% B for 10 min then 95% B for 10 min, 1 mL min^{-1}. Purification with semipreparative HPLC was performed on a similar Beckman system equipped with a Genesis C18 120 4 μ (250 × 10 mm). Mobile phase: 65%A, 35% B, 5 mL min^{-1}.

^{11}C-labelling of eprosartan in the carboxyl position

*4-[2-Butyl-5-((E)-2-carboxy-3-thiophen-2-yl-propenyl)-imidazol-1-ylmethyl]-[carboxyl-^{11}C]benzoic acid (**10**)*: The following text describes the procedure used for biological deliveries. To a (0.8 mL) vial was added *tetrakis*(triphenylphosphine)-palladium(0) (4.8 mg, 4.1 μmol) and precursor **9** (3.5 mg, 6.8 μmol). The mixture was purged with argon before dissolving in THF (400 μL). The resulting solution was added to an argon-purged vial containing tetra-*n*-butylammonium hydroxide 30 hydrate (2.2 mg, 2.8 μmol) and then injected into the 200 μL-injection loop of the synthesis system. [^{11}C]Carbon monoxide was transferred to the micro-autoclave (200 μL), which was then pressurized to 35 MPa (5 kpsi) with the reaction mixture from the injection loop. The micro-autoclave was immersed and heated in a heating block set at 150°C for 5 min. The crude product was transferred to an evacuated vial (3 mL) and the radioactivity was measured. The vial was heated in a heating block (70°C) and purged with nitrogen gas to remove unreacted [^{11}C]carbon monoxide and solvent, and the radioactivity was measured again to determine the conversion yield of [^{11}C]carbon monoxide. The crude product was diluted first with MeCN (500 μL) and then water (200 μL), and was purified using semipreparative HPLC (R_t = 5.5–7.5 min). The product fraction was concentrated under reduced pressure or by purging with nitrogen at 100°C and then diluted with phosphate buffer (pH = 7.2) before the radioactivity was measured (dc rcy 37–54%, n = 5). The purity of the final product was analyzed with analytical UV-radio-HPLC (R_t = 6.9 min), and characterization was made by co-eluting with an isotopically unmodified sample. LC-MS, ESI+ for $C_{23}H_{24}N_2O_4S$ *m/z* [M+H]: 425. Specific activity was determined by measuring the concentration, volume and radioactivity of the purified product solution at a specified time point from EOB. An aliquot was withdrawn and 50 μL injections were analyzed by analytical UV-radio-HPLC, λ = 254 nm, (three replicates) and compared to a calibration curve made from three standard solutions (1, 5 and 10 μM) of eprosartan.

Eprosartan precursor synthesis

*Diethyl (thien-2-ylmethylene)malonate (**1**)*: A mixture of 2-thiophenealdehyde (20.15 g, 180 mmol), diethylmalonate (29.63 g, 185 mmol), piperidine (2.4 mL, 24 mmol) and benzoic acid (50 mg, 41 mmol) in cyclohexane (120 mL) was heated to reflux for 22 h under a Dean-Stark water trap. Removal of the solvent gave a brown oil, which was dissolved in diethyl ether (150 mL) and washed with 2 M HCl (3 × 30 mL), sat. aqueous $NaHCO_3$ (3 × 30 mL) and brine (30 mL) then dried over $MgSO_4$. The solvent was removed under reduced pressure to give **1** (39.76 g, 87%) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C) δ: 7.84 (unresolved dd, J = 0.6 Hz, 1H), 7.51 (ddd, J = 0.9, 1.2, 5.1 Hz, 1H), 7.36 (ddd, J = 0.6, 1.2, 3.7 Hz, 1H), 7.07 (dd, J = 3.7, 5.1 Hz, 1H), 4.39 (q, J = 7.1 Hz, 2H, CH_2), 4.28 (q, J = 7.1 Hz, 2H, CH_2), 1.35 (t, J = 7.1 Hz, 3H, CH_3), 1.30 (t, J = 7.1 Hz, 3H, CH_3) ppm. GC-MS for $C_{12}H_{14}O_4S$: *m/z* (rel. intensity) 254 (M^+, 44), 209 (85), 164 (86), 136 (79), 108 (100).

*Diethyl (thien-2-ylmethyl)malonate (**2**)*: $NaBH_4$ (2.94 g, 77.7 mmol) was added portion wise to a solution of **1** (39.54 g, 155.5 mmol) in ethanol (250 mL) at 0°C. The temperature was allowed to rise to r.t. and stirred for 40 min. Glacial acetic acid (~4 mL) was added to pH = 6 (indicated by Merck pH paper), whereupon a white precipitate was formed and removed by filtration. The solvent was removed under reduced pressure to give a brown residue, which was taken up between water (75 mL) and diethyl ether (175 mL). The organic layer was washed with water (50 mL) and brine (40 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to give **2** (35.47 g, 89%) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C) δ: 7.12 (dd, J = 1.2, 5.1 Hz, 1H, Ar-H), 6.88 (dd, J = 3.5, 5.1 Hz, 1H, Ar-H), 6.83 (m, 1H, Ar-H), 4.17 (dq, J = 0.9, 7.2 Hz, 4H, 2 × CH_2), 3.65 (dd, J = 7.2, 8.1 Hz, 1H, CH), 3.42 (m, 2H, CH_2), 1.22 (t, J = 7.2, 6H, 2 × CH_3). GC-MS for $C_{12}H_{16}O_4S$: *m/z* (rel. intensity) 256 (M^+, 84), 182 (64), 154 (43), 137 (99), 110 (100), 97 (55).

*3-Ethoxy-3-oxo-2-(thien-3-ylmethyl)propanoic acid (**3**)*: A solution of KOH (5.10 g, 77.25 mmol) dissolved in ethanol (150 mL) was added drop wise to a solution of **2** (20.0 g, 78.0 mmol) in ethanol (75 mL). The reaction mixture was stirred at r.t. for 48 h and then concentrated. The residue was diluted with water and washed with ether. The aqueous phase was acidified with 2 M HCl to pH = 1 and extracted with ether (3 × 60 mL). The combined extracts were washed with water and brine and then dried over $MgSO_4$. The solvent was removed under reduced pressure to give **3** (15.6 g, 89%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C) δ: 10.66 (brs, 1H, COOH), 7.16 (dd, J = 1.3, 5.1 Hz, 1H, Ar-H), 6.91 (dd, J = 3.5, 5.1 Hz, 1H, Ar-H), 6.86 (dm, J = 3.5 Hz, 1H, Ar-H), 4.22 (q, J = 7.1 Hz, 2H, CH_2CH_3), 3.73 (t, J = 7.4 Hz, 1H, CH), 3.46 (m, 2H, CH_2), 1.25 (t, J = 7.1 Hz, 3H, CH_3) ppm. The compound decompose upon vaporization in GC-MS to the decarboxylated product, 3-thiophen-2-yl-propionic acid ethyl ester, GC-MS for $C_{12}H_{16}O_4S$: *m/z* (rel. intensity) 184 (M^+,100), 120 (88), 97 (66).

*Acetic acid 1-acetyl-2-butyl-1H-imidazol-4-ylmethyl ester (**4**)*: 2-Butyl-4(5)-(hydroxymethyl)imidazole (5.00 g, 32.4 mmol) was added portion wise to a flask containing stirred ice cold acetic anhydride (15.1 mL, 162 mmol). When the reaction mixture occasionally solidified it was warmed a little to dissolve again. The reaction mixture was allowed to slowly rise to r.t. and then stirred for 36 h. The excess acetic anhydride was removed under reduced pressure and the residue was dissolved in dichloromethane and washed with sat. aqueous $NaHCO_3$ (3 × 25 mL) and brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to give **4** (6.99 g, 90%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, 25°C) δ: 7.19 (m, 1H, Ar-H), 4.96 (d, J = 0.8 Hz, 2H), 2.98 (m, 2H), 2.52 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 1.67 (m, 2H, CH_2), 1.39 (m, 2H, CH_2), 0.90 (t, J = 7.4 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ: 170.8, 167.6, 152.6, 136.2, 116.8, 59.7, 30.4, 29.4, 24.5, 22.5, 20.9, 13.7 ppm.

*Acetic acid 2-butyl-3-(4-iodo-benzyl)-3H-imidazol-4-ylmethyl ester (**5**)*: To a solution of triflic anhydride (4.91 g, 17.4 mmol) in dry dichloromethane (30 mL) was added drop wise a solution of diisopropylethylamine (2.30 g, 17.8 mmol) and (4-iodophenyl)-methanol in dichloromethane (20 mL) over 15 min at −78°C. The reaction mixture was stirred at the same temperature for 30 min. A solution of **4** (4.00 g, 16.8 mmol) in dichloromethane was then added drop wise over 15 min at the same temperature.

The reaction mixture was allowed to slowly warm to r.t. and then stirred for 36 h. The solvent was removed under reduced pressure and the residue was taken up in ethyl acetate, washed with sat. aqueous $NaHCO_3$, 2 M HCl and brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to give the crude acetate **5** as a pale solid that was used in the next step without further purification.

*[2-Butyl-3-(4-iodo-benzyl)-3H-imidazol-4-yl]-methanol (**6**)*: The crude acetate **5** was dissolved in methanol (100 mL) and water (10 mL) and treated with K_2CO_3 (4.60 g, 33.0 mmol). The reaction mixture was stirred at r.t. for 90 min. The reaction mixture was filtered and concentrated under reduced pressure and the residue was taken up in ethyl acetate and washed with water and brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to give a white crystalline material, which was purified by flash chromatography ($\emptyset = 7$ cm, $h = 7$ cm) using 10% methanol in dichloromethane as the eluent to give the alcohol **6** (4.24 g, 68%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, 25°C) δ: 7.62 (AA′BB′, 2H, Ar-H, ortho to the iodo substituent), 6.82 (s, 1H, Ar-H on imidazole), 6.70 (AA′BB′, 2H, Ar-H, meta to the iodo subsituent), 5.17 (s, 2H, -CH_2-N), 4.44 (m, 2H, -CH_2-O), 3.94 (brs, 1H, -OH), 2.51 (m, 2H, -CH_2-imidazole), 1.60 (m, 2H, CH_2), 1.30 (m, 2H, CH_2), 0.85 (t, $J = 7.3$ Hz, 3H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ: 150.0, 137.9, 136.4, 131.5, 127.9, 126.0, 93.0, 54.3, 46.4, 29.7, 26.6, 22.4, 13.7 ppm. LC-MS, ESI+ for $C_{15}H_{19}IN_2O$ *m/z* [M+H]: 371.

*2-Butyl-1-(4-iodobenzyl)-1H-imidazole-5-carbaldehyde (**7**)*: To a solution of the alcohol **6** (4.02 g, 10.9 mmol) in dichloromethane (100 mL) was added technically activated MnO_2 (9.44 g, 109 mmol). The heterogeneous mixture was stirred for 90 min at r.t. then filtered through a thin layer of Celite, which was then rinsed with several portions of dichloromethane. The solvent was removed under reduced pressure to give the aldehyde **7** (3.73 g, 93%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C) δ: 9.64 (s, 1H, COH), 7.77 (s, 1H, Ar-H), 7.61 (AA′BB′, 2H, Ar-H), 6.74 (AA′BB′, 2H, Ar-H), 5.49 (s, 2H, CH_2), 2.63 (m, 2H, CH_2), 1.67 (m, 2H, CH_2), 1.34 (m, 2H, CH_2), 0.88 (t, $J = 7.3$ Hz, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25°C) δ: 178.6, 156.6, 143.5, 137.9, 135.8, 131.2, 128.1, 93.2, 47.6, 29.3, 26.4, 22.4, 13.6 ppm.

*(E)-3-[2-Butyl-3-(4-iodo-benzyl)-3H-imidazol-4-yl]-2-thiophen-2-ylmethyl-acrylic acid ethyl ester (**8**)*: A solution of aldehyde **7** (1.46 g, 3.97 mmol), piperidine (0.270 g, 3.18 mmol) and acid **3** (0.905 g, 3.97 mmol), in cyclohexane (25 mL) and 2 mL of toluene was heated in an oil bath at 100°C under a gentle stream of nitrogen. A Dean-Stark trap was used to remove water. Additional portions of acid **3** (in total 0.453 g, 1.99 mmol) in cyclohexane were added at at 2, 5 and 7 h. The reaction mixture was heated for a total of 8 h. The reaction mixture was concentrated under reduced pressure and the residue purified by flash chromatography eluting with 0–3% MeOH in dichloromethane, to give the product (1.392 g, 73%) as pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, 25°C) δ: 7.61 (AA′BB′, 2H, Ar-H) 7.43 (d, $J = 0.8$ Hz, 2H), 7.41 (s, $J = 0.8$ Hz, 1H) 7.06 (dd, $J = 1.2$, 5.2 Hz, 1H), 6.85 (dd, $J = 3.5$, 5.2 Hz, 1H), 6.77 (dm, $J = 3.5$ Hz, 1H), 6.69 (AA′BB′, 2H, Ar-H), 5.08 (s, 2H, CH_2), 4.15 (q, $J = 7.2$ Hz, 2H, CH_2), 4.06 (m, 2H, CH_2), 2.62 (m, 2H, CH_2), 1.66 (m, 2H, CH_2), 1.35 (m, 2H, CH_2), 1.21 (t, $J = 7.2$ Hz, 3H, CH_3), 0.87 (t, $J = 7.2$ Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ: 167.1, 151.3, 141.0, 138.0, 135.5, 131.7, 128.4, 127.6, 127.0, 126.6, 125.2, 124.4, 123.4, 93.3, 60.9, 46.2, 29.5, 28.7, 26.9, 22.3, 14.1, 13.6. LC-MS, ESI+ for $C_{24}H_{27}IN_2O_2S$ *m/z* [M+H]: 535. Decarboxylation of **3** resulted in 3-thiophen-2-yl-propionic acid ethyl ester, a yellow oil that eluted ahead of the desired product **8** during chromatography. ^{1}H NMR (400 MHz, $CDCl_3$, 25°C) δ: 7.22 (dd, $J = 1.2$, 5.1 Hz, 1H), 6.91 (dd, $J = 3.4$, 5.1 Hz, 1H), 6.82 (dd, $J = 1.2$, 3.4 Hz, 1H), 4.15 (q, $J = 7.1$ Hz, 2H), 3.17 (m, 2H), 2.68 (m, 2H), 1.26 (t, $J = 7.1$ Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ: 172.4, 143.2, 126.9, 124.7, 123.6, 60.7, 36.3, 25.3, 14.3 ppm.

*(E)-3-[2-Butyl-3-(4-iodo-benzyl)-3H-imidazol-4-yl]-2-thiophen-2-ylmethyl-acrylic acid (**9**)*: The ester **8** (0.883 g, 1.65 mmol) was hydrolyzed in 10 wt% NaOH in EtOH at r.t. overnight. The reaction was concentrated under reduced pressure and the residue was dissolved in water. The solution was acidified with 2 M HCl and extracted with dichloromethane to give a semicrystalline material that was further purified using flash chromatography, eluting with 0–5% MeOH in dichloromethane to give the acid **9** (0.678 g, 81%) as beige solid. ^{1}H NMR (400 MHz, $CDCl_3$, 25°C) δ: 7.64 (AA′BB′, 2H, Ar-H), 7.51 (s, 1H), 7.47 (s, 1H), 7.11 (dd, $J = 1.3$, 5.3 Hz, 1H), 6.88 (dd, $J = 3.5$, 5.2 Hz, 1H), 6.79 (dm, $J = 3.5$ Hz, 1H), 6.69 (AA′BB′, 2H, Ar-H), 5.12 (s, 2H, CH_2), 4.06 (s, 2H, CH_2), 2.69 (m, 2H), 1.62 (m, 2H, CH_2), 1.31 (m, 2H, CH_2), 0.86 (t, $J = 7.4$ Hz, 3H) ppm. LC-MS, ESI+ for $C_{22}H_{23}IN_2O_2S$ *m/z* [M+H]: 507.

Preparation of eprosartan reference

4-[2-Butyl-5-((E)-2-carboxy-3-thiophen-2-yl-propenyl)-imidazol-1-ylmethyl]-benzoic acid: A sample of the commercially available Teveten®, manufactured by Solvay Pharmaceuticals, was finely powdered in a mortar. The powder (1.99 g) was slurried in water (20 mL) and the pH was adjusted to >12 with 6 M NaOH before the solution was filtered. 6 M HCl was added drop wise to the filtrate until pH was <2. The mixture was filtered and dried to obtain the practically pure organic compound as an off-white crystalline material. ^{1}H NMR (400 MHz, DMSO-d_6, 25°C) δ: 12.90 (br s, 1H, COOH), 7.91 (AA′BB′, 2H, Ar-H), 7.68 (s, 1H, Ar-H), 7.37 (s, 1H, Ar-H), 7.30 (dd, $J = 1.2$, 5.1 Hz, 1H, Ar-H), 7.17 (AA′BB′, 2H, Ar-H), 6.90 (dd, $J = 3.5$, 5.1 Hz, 1H), 6.71 (dm, $J = 3.4$ Hz, 1H), 5.60 (s, 2H, CH_2), 4.00 (s, 2H, CH_2), 2.92 (m, 2H, CH_2), 1.58 (m, 2H, CH_2), 1.28 (m, 2H, CH_2), 0.80 (t, $J = 7.3$ Hz, 3H, CH_3) ppm. LC-MS, ESI+ for $C_{23}H_{24}N_2O_4S$ *m/z* [M+H]: 425.

Frozen section autoradiography[50,51]

Frozen sections (20 μm) were prepared in a cryomicrotome and put on superfrost glass slides. Organs used were: liver, lung, spleen, heart, kidney, striatum and cerebellum from rat as well as pig adrenal. The slides were kept in a freezer (−20°C) until used. At the start of the experiment the slides were pre-incubated for 10 min in TRIS+ (50 mM TRIS+120 mM NaCl+ 5 mM KCl+1 mM $MgCl_2$+2.5 mM $CaCl_2$, pH 7.4) buffer. The slides were then transferred to containers containing [*carboxyl*-^{11}C]-eprosartan in TRIS+buffer. In a duplicate set of containers 10 μM of unlabelled eprosartan was added to block-specific binding. After incubation for 30 min the slides were washed 3 × 3 min in buffer. The slides were dried in a heated (37°C) oven and the exposed to phosphor imaging plates (Molecular Dynamics, USA) for 40 min and scanned in a Phosphor Imager Model 400S (Molecular Dynamics, USA, purchased from Amersham Bioscience, Uppsala, Sweden).

Organ distribution[52,53]

The *in vivo* studies were carried out in adult male Sprague Dawley rats (315–550 g) (Scanbur B&K, Sweden). All animals

were handled according to the guidelines by the Swedish Animal Welfare Agency, and the experiments were approved by the local Ethics Committee for Animal Research, permit no: C234/5.

In order to evaluate uptake in normal tissues, a biodistribution study was performed. Rats were injected intravenously in the tail vein with 10 MBq/kg [*carboxyl*-^{11}C]eprosartan solution. At 15 and 40 min post injection, nine or eight animals/time point were sacrificed and their organ were dissected out, weighed and radioactivity was measured in a well 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 animal (g).

Conclusions

In an effort to find a new tracer for the imaging of aldosterone producing adenomas we have synthesized [*carboxyl*-^{11}C]eprosartan **10**, specifically labelled with ^{11}C in the carboxyl position using a palladium-mediated carboxylation reaction with [^{11}C]carbon monoxide. The labelling reaction gave sufficient radiochemical yields to be used for biological experiments, and with high specific activity.

Biological screening revealed expected binding/accumulation in tissues reported to express the AT_1 receptor, such as kidney, lung and adrenal. The adrenal binding warrants further examination of human adrenal tissues, especially those of pathological origin. ^{11}C-labelled eprosartan is thus a possible tracer for imaging AT_1 receptors *in vivo*.

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