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Laboratory note

Radiosynthesis of [tetrazoyl-^{11}C]irbesartan, a non-peptidic angiotensin II antagonist

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Summary — With the aim of visualizing myocardial angiotensin II receptors (AT1 subtypes), [tetrazoyl-^{11}C]2-*n*-butyl-1-[(2'-(1*H*-tetrazol-5-yl)-1,1'-biphenyl-4-yl)methyl]-4-spirocyclo-pentane-2-imidazoline-5-one ([tetrazoyl-^{11}C]irbesartan (SR47436/BMS-186295)) **11** was synthesized in one pot in four steps from [^{11}C]hydrogen cyanide. The labelling process which yielded [tetrazoyl-^{11}C]irbesartan is described in detail and could be applied to the labelling of other ligands which possess the (1*H*-tetrazol-5-yl) moiety. Positron emission tomography (PET) studies were performed in dogs. Heart, lung and blood time–activity curves did not change. Therefore this new radioligand is not suitable for studying myocardial angiotensin II receptors with PET.

angiotensin II / non-peptidic Ang II antagonist / irbesartan / [tetrazoyl-^{11}C] (SR47436/BMS-186295) / positron emission tomography

Introduction

Over the past decade, inhibition of the renin–angiotensin system has emerged as a promising therapeutic approach. The endogenous peptide angiotensin II (Ang II) exerts potent inotropic and chronotropic effects on mammalian cardiac tissue but also has many other biological effects in a wide array of cell, tissue and organ preparations [1]. In addition to its direct effects on myocardial contractility, Ang II has a substantial effect on cardiac function through the sympathetic cardiac nerves [2, 3]. Following the development of the clinical use of angiotensin-converting enzyme inhibitors, antagonists of angiotensin receptors (AT_1 and AT_2) have emerged as new therapeutic agents. High-affinity low-capacity membrane Ang II receptor binding sites have been identified in the myocardium [4]. Characterization of these myocardial AT_1–AT_2 receptors is essential to understanding the mechanism of cardiac effects of Ang II in several diseases. Most of the effects of angiotensin are mediated through the AT_1 receptor, while the role of the AT_2 receptor remains unclear. Following losartan (DuP753 [5]; fig 1), many specific non-peptidic Ang II antagonists have been developed by different companies [6, 7]. Among several compounds, the binding properties of irbesartan (SR47436/BMS-186295) (fig 1) at cardiac AT_1 receptors have been characterized [8]. This compound binds with high affinity (K_d = 0.24 nM) to a single class of myocardial sites (AT_1 receptors). The long-lasting (> 28 h in monkeys) hypotensive action of irbesartan suggested an entero-hepatic cycle or a slow metabolism of this compound [9]. Because of such properties, we selected irbesartan for labelling with carbon-11 to study myocardial Ang II binding sites. As the carbon of the tetrazole moiety has already been labelled with ^{14}C [10], we attempted to label irbesartan with ^{11}C on the tetrazole moiety. An

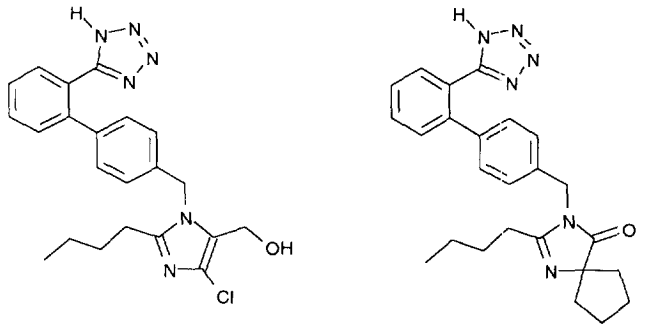

Fig 1. Structures of losartan and irbesartan.

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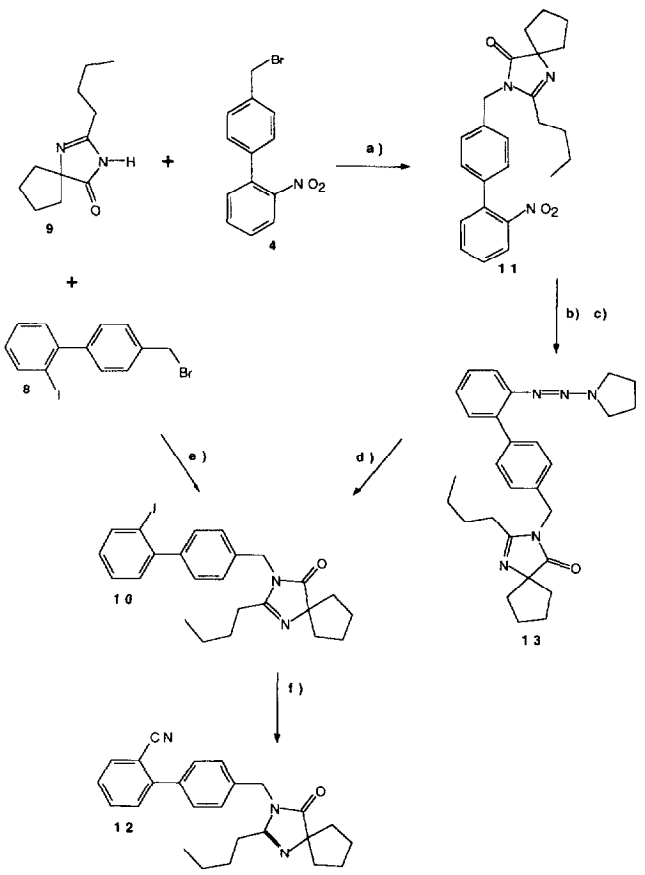
[^{11}C]copper(I)cyanide synthesis was developed to allow the synthesis of [cyano-^{11}C]cyanobiphenyl [11] and a one-pot synthesis in four steps was optimized for [tetrazoyl-^{11}C]irbesartan synthesis.

Chemistry

We have developed a new technique using halogenoaryl compounds and [^{11}C]copper(I)cyanide [11] for labelling a tetrazole moiety with carbon-11. Satisfactory radiochemical yields were obtained for iodoaryl compounds [11]. Our purpose in the present study was to synthesize the iodo precursor (iodo-irbesartan **10**) and to convert it to [tetrazoyl-^{11}C]irbesartan **16**. Two pathways were studied for the synthesis of **10** (fig 2).

First we synthesized the triazene-irbesartan **13** from 2-nitro-4'-bromomethyl-1,1'-biphenyl **4** (fig 2; path A). The synthesis was achieved as depicted in scheme 1. The 2-nitro-4'-methyl-1,1'-biphenyl **3** was obtained by the Ullman biaryl synthesis [13] from 1-iodo-2-nitrobenzene **1** and 4-iodotoluene **2** refluxing in toluene in the presence of copper (scheme 2). Bromination of compound **3** was performed with *N*-bromosuccinimide by refluxing overnight in carbon tetrachloride in the presence of dibenzoylperoxide to give 2-nitro-4'-bromomethyl-1,1'-biphenyl **4** in 92% yield. The *N*-alkylation of 2-*n*-butyl-4-spirocyclopentane-2-imidazoline-5-one **9** was performed using sodium hydride in the presence of 2-nitro-4'-bromomethyl-1,1'-biphenyl **4** giving the nitro-irbesartan **11** in 37% yield (scheme 1). Reduction of the nitro compound **11** by tin(II)chloride in methanol gave the amino derivative in 71% yield. Triazene-irbesartan **13** was obtained according to Foster [12] in 75% yield from the amino derivative. Iodination of **13** was performed according to Foster [12] and gave iodo compound **10** in 32% yield with 23% yield of diiodo compound. Iodination was not reproducible and purification of crude iodo compound was difficult, so we alternatively synthesized iodo-irbesartan **10** from 2-iodo-4'-bromomethyl-1,1'-biphenyl **8** (fig 2; path B). The precursor **10** was synthesized as depicted in scheme 3. Reduction of the nitro compound **3** by tin(II)chloride in methanol led to the amino derivative **5** in 76% yield. 2-Iodo-4'-methyl-1,1'-biphenyl **7** was synthesized from compound **5** via the triazene pathway [12]. The diazo compound **6** was synthesized from amino compound **5** by reaction with hydrochloric acid and sodium nitrite followed by addition of pyrrolidine and potassium hydroxide. After workup and purification, into a methanolic solution of compound **6** was added potassium iodide and trifluoroacetic acid to give 2-iodo-4'-



Scheme 1. Chemical pathway for iodo- and cyano-irbesartan syntheses. Reagents: (a) NaH, DMF, rt, 20 h; (b) $SnCl_2$, MeOH, reflux 20 h; (c) HCl, $NaNO_2$, pyrrolidine, KOH, rt, 2 h; (d) KI, trifluoroacetic acid, rt, 20 h; (e) NaH, DMF, rt, 20 h; (f) CuCN, DMF, rt, 20 h.

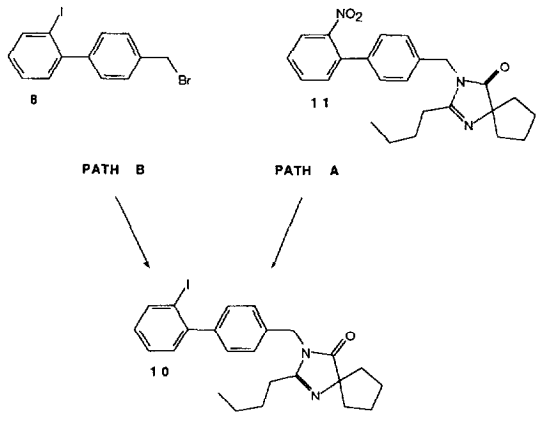
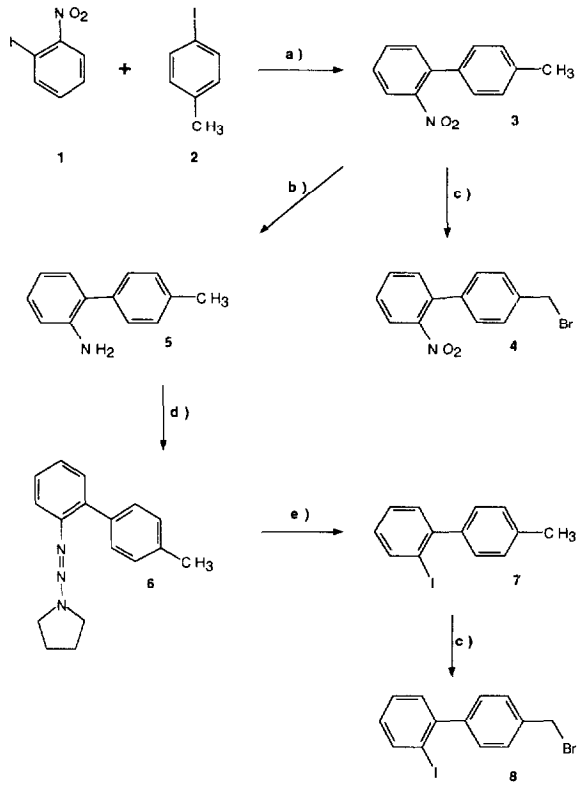


Fig 2. Chemical pathway for iodo-irbesartan **10**.



Scheme 2. Chemical pathway for biphenyl compound syntheses. Reagents: (a) Cu; (b) $SnCl_2$, MeOH, reflux 20 h; (c) NBS, dibenzoylperoxide, CCl_4, reflux 20 h; (d) HCl, $NaNO_2$; pyrrolidine, KOH, rt, 2 h; (e) KI, trifluoroacetic acid, rt, 20 h.

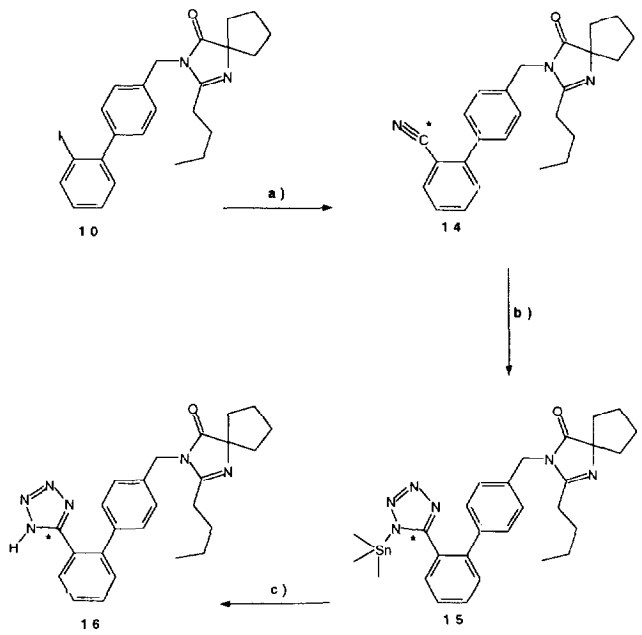
methyl-1,1'-biphenyl **7** in 33% global yield from amino compound **5**. Bromination of 2-iodo-4'-methyl-1,1'-biphenyl **7** was performed with *N*-bromosuccinimide in the catalytic presence of dibenzoylperoxide in carbon tetrachloride to give 2-iodo-4'-bromomethyl-1,1'-biphenyl **8** in 69% yield (scheme 2). Iodo precursor **10** (scheme 1) was obtained in 68% yield by the *N*-alkylation of 2-*n*-butyl-4-spirocyclopentane-2-imidazoline-5-one **9** with 2-iodo-4'-bromomethyl-1,1'-biphenyl **8** as in path B. Cyanodehalogenation of iodo-irbesartan **10** was performed in refluxing *N*,*N*-dimethylformamide for 20 h in the presence of copper(I)cyanide (scheme 1), and gave cyano-irbesartan **12** in 65% yield. This last compound was used as reference for the radiosynthesis.

The radiosynthesis of [tetrazoyl-^{11}C]irbesartan **16** was performed in a single conical vial in four steps, as depicted in scheme 3. The first step was the production of [^{11}C]hydrogen cyanide from [^{11}C]methane [15] and the trapping of [^{11}C]hydrogen cyanide [9]. The second step was the radiosynthesis of [cyano-^{11}C]irbesartan copper complex **14** obtained by the reaction of iodo compound **10** with [^{11}C]hydrogen cyanide in the presence of copper(II)sulfate pentahydrate and sodium metabisulfite at 180 °C in *N*,*N*-dimethylformamide. Then, after cooling the vial, reagents of the third step (trimethyltin chloride in *N*,*N*-dimethylformamide and sodium azide in water) were added to the [cyano-^{11}C]irbesartan copper complex solution. The reaction mixture was heated for 5 min at 180 °C to give **15**. After cooling, the last decomplexation step was performed using ferric chloride and hydrochloric acid. The crude radioactive material **16** was washed on a silica Sep Pak to eliminate most of the salts, and purified by reversed phase HPLC to give pure [tetrazoyl-^{11}C]irbesartan **16** in 3% radiochemical yield.

Results and discussion

[Tetrazoyl-^{11}C]irbesartan **16** could be synthesized easily but in a low radiochemical yield. In order to increase the radiochemical yield attempts are now being made by testing different reagents for synthesis of the tetrazole ring. Therefore the quantities of [tetrazoyl-^{11}C]irbesartan **16** were sufficient to perform myocardial positron emission tomography (PET) studies in dogs.

The shape of the time–activity curves and the level of radioactivity were similar in the myocardium,



Scheme 3. Radiosynthesis of [tetrazoyl-^{11}C]irbesartan **16**. Reagents: (a) Cu^{11}CN, DMF, 180 °C, 2 min; (b) Me_3SnCl, NaN_3, 180 °C, 5 min; (c) HCl, 6 N, $FeCl_3$, 180 °C, 2 min.

blood and lung. Therefore there was no detectable preferential myocardial uptake of [tetrazoyl-^{11}C]irbesartan **16**. These disappointing results could partly be explained by the rapid association rate constant of irbesartan **16** (0.038 ± 0.0006 min^{-1} nM^{-1}) and a slightly less rapid dissociation rate constant (0.013 ± 0.0001 min^{-1}) [8]. In spite of a high affinity for myocardial AT_1 receptors and a prolonged antihypertensive effect, [tetrazoyl-^{11}C]irbesartan **16** is not suitable for imaging myocardial AT_1 receptors with PET.

Experimental protocols

Chemistry

N,N-Dimethylformamide (DMF) was distilled and stored under argon. All reagents were commercially obtained and used without any purification. 1-Iodo-2-nitrobenzene and 4-iodotoluene were purchased from Aldrich. 2-*n*-Butyl-1-spirocyclopentane-2-imidazoline-5-one was a gift from Sanofi Recherche and Bristol Myers Squibb laboratories. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Brucker Ac 300. Mass spectra were obtained using a Nermag R10-10 instrument at 70eV. Flash chromatography was performed on silica (Merck, 40–100 µm).

HPLC analyses were performed using a Waters system (Waters 510 pump) with UV detection at 254 nm (Waters 441 detector). Compound **3** was analyzed on a Hibar Si 60 column (25 cm, 10 mm od, 7 µm). The others were analyzed on a µ-Bondapak column (30 cm, 7.8 mm od, 10 µm). The mobile phases were methanol/water for compounds **4** and **5**, methanol/sodium phosphate solution (1 M NaH_2PO_4/H_3PO_4) for compounds **7**, **8** and **13**, acetonitile/triethylamine phosphate (0.08 M H_3PO_4/triethylamine) for compounds **10** and **12**. Compound **16** was purified on a µ-Bondapak column (30 cm, 7.8 mm od, 10 µm) with acetonitile/triethylamine phosphate (0.08 M H_3PO_4/triethylamine) as mobile phase.

*2-Nitro-4'-methyl-1,1'-biphenyl **3***
To a stirred solution of 1-iodo-2-nitrobenzene **1** (4.96 g, 19.9 mmol) and 4-iodotoluene **2** (4.38 g, 20 mmol) at 180 °C was added copper powder (5.08 g, 80 mmol, 4 eq) in portions over 1 h. After cooling to room temperature the mixture was taken up with toluene and filtered. The filtrate was concentrated under vacuum to remove the toluene. The crude material was purified by flash chromatography on silica using hexane/ethyl acetate (80:20; v/v) as solvent; 0.6 g of pure compound **3** was obtained in 14% yield. HPLC: retention time: 6 min (4 mL/min, normal phase: 95:5, v/v, hexane/methylene chloride). MS (DCI/NH_3): $[M]^+$ = 213, $[M + 18]^+$ = 231. ^{1}H-NMR: (CD_2Cl_2 + TMS), δ: 2.36 (s, 3H), 7.15–7.23 (m, 4H), 7.35–7.45 (m, 2H), 7.52–7.6 (m, 1H), 7.75–7.8 (d, H). ^{13}C-NMR: (CD_2Cl_2 + TMS), δ: 21.7(1C), 124.7(1C), 128.5(2C), 128.8(1C), 130.1(2C), 132.7(1C), 133(1C), 135.3(1C), 136.8(1C), 139(1C), 150.1(1C).

*2-Nitro-4'-bromomethyl-1,1'-biphenyl **4***
A solution of **3** (1.36 g, 6.38 mmol), *N*-bromosuccinimide (1.13 g, 6.35 mmol, 0.99 eq) and dibenzoylperoxide (108 mg, 0.45 mmol, 0.07 eq) in 30 mL carbon tetrachloride was refluxed overnight, cooled to room temperature, filtered and concentrated. The crude material was then purified by flash chromatography on silica gel using heptane as solvent to give 1.7 g of **4** in 92% yield. HPLC: retention time: 4.5 min (5 mL/min, reversed phase: 80:20, v/v, methanol/water). MS (DCI/NH_3): $[M + 18]^+$ = 309–311, $[M + 35]^+$ = 326–328. ^{1}H-NMR: (CD_2Cl_2 + TMS), δ: 4.52 (s, 2H), 7.2–7.29 (m, 2H), 7.32–7.49 (m, 4H), 7.57–7.63 (m, 1H), 7.77–7.85 (m, 1H). ^{13}C-NMR: (CD_2Cl_2 + TMS), δ: 33.8(1C), 124.9(1C), 129(2C), 129.2(1C), 130.1(2C), 132.6(1C), 133.3(1C), 136.2(1C), 138.4(1C), 138.7(1C), 149.8(1C).

*2-Amino-4'-methyl-1,1'-biphenyl, hydrochloride **5***
A solution of 2-nitro-4'-methyl-1,1'-biphenyl **4** (3 g, 14.08 mmol) and tin(II)chloride (27.9 g, 147 mmol) in 50 mL methanol was refluxed overnight under an argon atmosphere. The mixture was then concentrated under vacuum to remove the solvent. The crude material was taken up in water and the solution was adjusted to pH = 9 with sodium bicarbonate. The precipitate of tin hydroxide was filtered off on celite and the solution was extracted with methylene chloride, the extract dried over magnesium sulfate, filtered and concentrated under vacuum to give 3.2 g of crude material. The residue was dissolved in diethyl ether and hydrochloric acid in diethyl ether was added. The precipitate was recovered by filtration, washed with dry diethyl ether and dried under vacuum to provide 2.7 g (10.8 mmol) of **5** in 76% yield. HPLC: retention time: 7 min (5 mL/min, reversed phase: 70:30, v/v, methanol/water) MS (DCI/NH_3): $[M + 1]^+$ = 184, $[M + 18]^+$ = 201. ^{1}H-NMR: (CD_2Cl_2 + TMS), δ: 2.35 (s, 3H), 3.71 (s, 2H), 6.6–6.8 (m, 2H), 7–7.1 (m, 2H). ^{13}C-NMR: (CD_2Cl_2 + TMS), δ: 21.3($1CH_3$), 115.8(1C), 118.7(1C), 128.6(2CH), 129.2(2CH), 129.8(2CH), 130.7(2CH), 137.3(1C), 144.2(1C).

*2-Iodo-4'-methyl-1,1'-biphenyl **7***
To 2.7 g of **5** (10.8 mmol) dissolved in 20 mL water and cooled with an ice bath, was added 6.6 mL 2 N hydrochloric acid (1.2 eq, 13.2 mmol). Then a cold solution of sodium nitrite (0.92 g, 10.8 mmol) was added and the mixture was maintained at 0 to 5 °C while a solution of pyrrolidine (1.01 g, 14.2 mmol, 1.3 eq) and potassium hydroxide (8.07 g, 0.14 mmol, 13 eq) in 20 mL water was added slowly. The reaction mixture was stirred at 25 °C for 2 h. The mixture was extracted with diethyl ether, the extract dried with magnesium sulfate and concentrated under vacuum. The crude material was purified by flash chromatography on silica gel using ethyl acetate/hexane (8:92, v/v) as eluent to give 2.4 g 2-(*N*-pyrrolidinoazo)-4'-methyl-1,1'-biphenyl **6**. This compound (2.4 g, 9 mmol) was dissolved in 20 mL methanol and 5 mL aqueous solution of potassium iodide (1.5 g, 9 mmol) added. To this mixture, maintained at 5 °C, 2 mL trifluoroacetic acid (25.9 mmol, 3.1 eq) was added in dropwise fashion. The mixture was then stirred at room temperature overnight. After evaporation to dryness the residue was dissolved in water, neutralized with sodium bicarbonate and extracted with methylene chloride. The organic phases were washed with water, dried over magnesium sulfate and concentrated. The crude material was purified by flash chromatography on silica gel using ethyl acetate/hexane (3:97, v/v) as solvent to give 1.05 g of **7** in 33% yield from 2-amino-4'-methyl-1,1'-biphenyl **5**. HPLC: retention time: 7.5 min (7 mL/min, reversed phase: 80:20, v/v, methanol/salt solution at pH = 2.3). MS (DCI/NH_3): $[M]^+$ = 294, $[M + 18]^+$ = 312. ^{1}H-NMR: (CD_2Cl_2 + TMS), δ: 2.26 (s, 3H), 6.83–6.87 (dd, 1H), 7–7.25 (m, 6H), 7.78–7.81 (d, 1H). ^{13}C-NMR: (CD_2Cl_2 + TMS), δ: 21.5($1CH_3$), 99.3(1C), 128.6(2CH), 129(2CH), 129.5(2CH), 130.6(1CH), 137.8(1C), 139.9(1CH), 141.7(1C), 146.9(1C).

*2-Iodo-4'-bromomethyl-1,1'-biphenyl **8***
The title compound **8** was prepared from **7** by the procedure described for the preparation of **4** in 69% yield. HPLC:

retention time: 6.1 min (7 mL/min, reversed phase: 80:20, v/v, methanol/salt solution at pH = 2.3). MS (DCI/NH_3): $[M + 1]^+$ = 372–374, $[M + 18]^+$ = 389–391, $[M + 35]^+$ = 407–409. ^{1}H-NMR: (CD_2Cl_2 + TMS), δ: 4.55 (s, 2H), 7–7.06 (m, 1H), 7.26–7.44 (m, 6H), 7.93–7.96 (d, 1H). ^{13}C-NMR: (CD_2Cl_2 + TMS), δ: 34.25($1CH_2$), 98.9(1C), 129(1CH), 129.5(2CH), 129.8(1CH), 130.5(2CH), 130.9(1CH), 138.1(1C), 140.3(1CH), 145(1CH), 146.6(1CH).

2-n-Butyl-1-[(2'-(N-pyrrolidinoazo)-1,1'-biphenyl-4-yl)-methyl]-4-spirocyclo-pentane-2-imidazoline-5-one (triazene-irbesartan) ***13***

To a suspension of oil-free sodium methoxide (1.41 g, 35.3 mmol, 4.7 eq) in 20 mL dry *N,N*-dimethylformamide at room temperature was added a solution of 2-*n*-butyl-4-spirocyclopentane-2-imidazoline-5-one **9** (1.46 g, 7.52 mmol, 1 eq) in 10 mL dry *N,N*-dimethylformamide. The mixture was stirred for 10 min and then a solution of 2-nitro-4'-bromomethyl-1,1'-biphenyl **8** (3.29 g, 11.28 mmol, 1.5 eq) in 20 mL dry *N,N*-dimethylformamide was added dropwise. The reaction mixture was stirred overnight at room temperature and then co-evaporated with toluene under vacuum. The crude material was purified by flash chromatography on silica gel using methylene chloride/ethyl acetate (95:5, v/v) to give 1.14 g of 2-*n*-butyl-1-[(2'-nitro-1,1'-biphenyl-4-yl)methyl]-4-spirocyclopentane-2-imidazoline-5-one (nitro-irbesartan) **12** in 37% yield. 2-*n*-Butyl-1-[(2'-amino-1,1'-biphenyl-4-yl)methyl]-4-spirocyclopentane-2-imidazoline-5-one was prepared from **12** by the procedure described for the preparation of **5** in 71% yield; 770 mg (2.05 mmol) of this compound was immediately dissolved in 20 mL water and cooled in an ice bath. 1.25 mL of 2 N hydrochloric acid (1.2 eq, 2.5 mmol) was added, followed by 5 mL of a cold solution of sodium nitrite (170 mg, 2.46 mmol) and the mixture was maintened at 0 to 5 °C while a solution of pyrrolidine (189 mg, 2.66 mmol, 1.3 eq) and potassium hydroxide (1.49 g, 26.5 mmol, 12.9 eq) in 20 mL water was added slowly. The reaction mixture was stirred at 25 °C for 2 h. The mixture was extracted with diethyl ether, the extract dried with magnesium sulfate and concentrated under vacuum. The crude material was purified by flash chromatography on silica gel using ethyl acetate/hexane (8:92, v/v) as eluent to give 709 mg of 2-*n*-butyl-1-[(2'-(*N*-pyrrolidinoazo)-1,1'-biphenyl-4-yl)methyl]-4-spiro-cyclopentane-2-imidazoline-5-one (triazene-irbesartan) **13** in 75% yield. The global yield of triazene-irbesartan **13** with respect to compounds **8** and **9** was 19%. MS (DCI/NH_3): $[M + 1]^+$ = 458. EI *m/z* (%) = 457(5), 360(40), 318(72), 165(100). ^{1}H-NMR: (CD_2Cl_2), δ: 0.88–0.93 (t, 3H), 1.33–1.40 (m, 2H), 1.57–1.67 (m, 2H), 1.75–2.1 (m, 12H), 2.32–2.04 (t, 2H), 3.25–4.09 (d, 4H), 4.71 (s, 2H), 7.1–7.25 (m, 3H), 7.25–7.45 (m, 2H), 7.45–7.65 (m, 3H). ^{13}C-NMR: (CD_2Cl_2), δ: 14.3($1CH_3$), 22.9($1CH_2$), 24.4($2CH_2$), 26.7($2CH_2$), 28(1CH), 29.2(1CH), 38($2CH_2$), 43.9($1CH_2$), 50($2CH_2$), 77.2($1CH_2$), 118.1(1C), 125.9(1C), 126.5(2CH), 128.7(1C), 131(1C), 131.5(1C), 131.6(2C), 135.8(1C), 136(1C), 140.4(1C), 149(1C), 162(1C), 187(1C).

2-n-Butyl-1-[(2'-iodo-1,1'-biphenyl-4-yl)methyl]-4-spirocyclo-pentane-2-imidazoline-5-one (iodo-irbesartan) ***10***

Path A. To a suspension of oil-free sodium methoxide (322 mg, 8 mmol, 4.3 eq) in 20 mL dry *N,N*-dimethylformamide at room temperature was added a solution of 2-*n*-butyl-4-spirocyclopentane-2-imidazoline-5-one **9** (728 mg, 3.7 mmol, 2 eq) in 5 mL *N,N*-dimethylformamide. The mixture was stirred for 10 min and then a solution of 2-iodo-4'-bromomethyl-1,1'-biphenyl **8** (700 mg, 1.87 mmol, 1 eq) in 5 mL dry *N,N*-dimethylformamide was added dropwise. The reactional mixture was stirred overnight at room temperature and then co-evaporated with toluene under vacuum. The crude material was purified by flash chromatography on silica gel using heptane/ethyl acetate (65:35, v/v) to give 620 mg of iodo-irbesartan **10** in 68% yield.

Path B. Compound **13** (0.49 g, 1.1 mmol) was dissolved in 40 mL acetonitrile and 2 mL aqueous solution of potassium iodide (188 mg, 1.1 mmol) was added. To this mixture, maintained at 5 °C, 2 mL of an aqueous solution of trifluoroacetic acid (0.15 mL, 2 mmol, 2 eq) was added dropwise. The mixture was stirred at room temperature overnight. After evaporation to dryness the residue was dissolved in water, neutralized with sodium bicarbonate and extracted with methylene chloride. The organic phases were washed with water, dried over magnesium sulfate and concentrated. The crude material was purified by flash chromatography on silica gel using ethyl acetate/pentane (30:70, v/v) as solvent to give 168 mg of pure iodo-irbesartan **10** in 32% yield. HPLC: retention time: 6.7 min (5 mL/min, reversed phase: 80:20, v/v, acetonitrile/salt solution at pH 3.1). MS (DCI/NH_3): $[M + 1]^+$ = 487. MS EI *m/z* (%) = 486(12), 444(90), 333(32), 317(30), 293(36), 207(24), 165(100), 152(20), 82(32), 67(54), 55(25), 41(42), 28(30). ^{1}H-NMR: (CD_2Cl_2 + TMS), δ: 0.83–0.88 (t, 3H), 1.2–1.4 (m, 2H), 1.4–1.65 (m, 2H), 1.65–2.1 (m, 8H), 2.2–2.4 (t, 2H), 4.71 (s, 2H), 6.9–7.05 (t, 1H), 7.15–7.5 (m, 6H), 7.9–8 (d, 1H). ^{13}C-NMR: (CD_2Cl_2 + TMS), δ: 14.3($1CH_3$), 23($1CH_2$), 26.7($2CH_2$), 28.1(1CH), 29.3(1CH), 38.1($2CH_2$), 43.9($1CH_2$), 77.2(1C), 99(1C), 126.9(2CH), 129(1CH), 129.7(1CH), 130.5(2CH), 130.8(1CH), 137.1(1C), 140.3(1CH), 144.2(1C), 146.7(1C), 161.9(1C), 187.2(1C).

2-n-Butyl-1-[(2'-cyano-1,1'-biphenyl-4-yl)methyl]-4-spirocyclopentane-2-imidazoline-5-one (cyano-irbesartan) ***12***

To a solution of iodo-irbesartan **10** (210 mg, 0.43 mmol) in 20 mL dry *N,N*-dimethylformamide was added copper(I) cyanide (66.3 mg, 0.74 mmol, 1.7 eq). The resulting mixture was refluxed overnight and then cooled at room temperature, diluted with toluene, filtered on celite and evaporated under vacuum. The crude material was purified by flash chromatography on silica gel using heptane/ethyl acetate (60:40, v/v) then (50:50, v/v) as eluent to give 108 mg of cyano-irbesartan **12** in 65% yield. HPLC: retention time: 4.6 min (5 mL/min, reversed phase: 80:20, v/v, acetonitrile/salt solution at pH 3.1). MS (DCI/NH_3): $[M + 1]^+$ = 386. MS EI *m/z* (%) = 385(5), 356(10), 343(70), 232(40), 192(100). ^{1}H-NMR: (CD_2Cl_2 + TMS), δ: 0.84–0.89 (t, 3H), 1.31–1.39 (m, 2H), 1.60–1.65 (m, 2H), 1.85–2.20 (m, 8H), 2.44–2.50 (m, 2H), 4.77 (s, 2H), 7.28–7.8 (m, 8H). ^{13}C-NMR: (CD_2Cl_2 + TMS), δ: 13.8($1CH_3$), 22.6($1CH_2$), 26.5($2CH_2$), 28.2($1CH_2$), 29.2($1CH_2$), 37.9($2CH_2$), 43.6($1CH_2$), 76.9($1CN_2$), 111.5(1C), 118.8(2C), 127.3(2C), 128.1(1C), 129.7(2C), 130.3(1C), 133.2(1C), 134(1C), 137.4(1C), 138.2(1C), 144.9(1C), 163.3(1C), 185.9(1C).

Radiochemistry

[^{11}C]Methane was produced with a cyclotron by the ^{14}N(*p*, α)^{11}C nuclear reaction [14]. All experiments were performed after 45 min irradiation of a nitrogen/hydrogen (95:5, v/v) target with a 25 μA beam of 20 MeV protons. The irradiation produced 1 Ci (37 GBq) of [^{11}C]methane on average (end of bombardment). [^{11}C]Hydrogen cyanide was produced as usual [14], and trapped with 95% efficiency in an empty conical vial (2 mL) cooled in an ethanol–ice bath. The radiochemical yield was determined from trapped [^{11}C]Hydrogen cyanide.

[Tetrazoyl-^{11}C]2-n-butyl-1-[(2'-(1H-tetrazol-5-yl)-1,1'-biphenyl-4-yl)-methyl]-4-spirocyclopentane-2-imidazoline-5-one: ([tetrazoyl-^{11}C] irbesartan) ***16***

[^{11}C]Hydrogen cyanide was trapped without solvent in a 2-mL conical vial cooled in an ice–ethanol bath. Then 50 µL aqueous solution of copper sulfate (10 µmol) and 50 µL aqueous solution of sodium metabisulfite (10 µmol) were added to the vial followed by an addition of 30 µmol iodo-irbesartan **10** in 200 µL *N,N*-dimethylformamide. The sealed vial was heated at 180 °C for 2 min, and then cooled in an ethanol–ice bath. Then 100 µL *N,N*-dimethylformamide solution of trimethyltin chloride (15 µmol) and 100 µL aqueous solution of sodium azide (20 µmol) were added to the same vial. The sealed vial was heated at 180 °C for 5 min and cooled for 1 min. After evaporation of solvents, decomplexation was performed at 180 °C for 2 min in the presence of hydrochloric acid (50 µL of 1.25 N) and ferric chloride (10 µmol in 50 µL water) in the same vial. After cooling, the crude radioactive material was injected on a silica Sep–Pak™ and washed with heptane, eliminating most of the salts. The [tetrazoyl-^{11}C]irbesartan **16** was eluted with acetonitrile. After evaporation of the solvent, the labelled compound was taken up in mobile phase solvent (acetonitrile/salt solution at pH 3.1) and purified by HPLC on a reversed phase column. 10 mCi (377 MBq) on average of pure [tetrazoyl-^{11}C]irbesartan **16** were obtained at 39 min with a specific radioactivity of 679 mCi/µmol (25 GBq/µmol) on average.

Biology

Animals used in this study were kept in accordance with the guidelines of the committee on care and use of laboratory animals at the Institute of Laboratory Animal Resources, National Research Council. Beagle dogs ($n = 2$) were anaesthetized with pentobarbital, intubated and were imaged in a human brain-scanner (CTI 953B/31; CTI PET systems, Knoxville, TN). Dogs were injected with 2–5 mCi (74–185 MBq) of [tetrazoyl-^{11}C]irbesartan **16** with a specific radioactivity of 679 mCi/µmol (25 GBq/µmol). The PET data acquisition lasted 60 min. Blood samples (femoral artery) were withdrawn at designated times. Myocardial time–concentration curves were measured from a region of interest encompassing the left ventricular myocardium.

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