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The synthesis of radiolabeled Irbesartan using *N,N*-dimethyl[^{14}C]formamide as a source of carbon-14 isotope

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Irbesartan (Avapro™) is in clinical use as an antihypertensive drug. It is a nonpeptide angiotensin II receptor antagonist. A radiolabeled version of this drug, intended for use in environmental fate studies, was prepared from *N,N*-dimethyl[^{14}C]formamide. Our method of synthesis gave 18% overall yield and a radiochemical purity of 98.22%.

Keywords: angiotensin receptor antagonist; antihypertensive; sartan; Irbesartan

Introduction

Irbesartan is a member of the sartan class of antihypertensive drugs.[1] These nonpeptides interact with the renin–angiotensin system (RAS), a central regulator of blood pressure and homeostasis.[2] Angiotensin I is a decapeptide formed from the action of renin on angiotensinogen. Angiotensin I is further converted by angiotensin converting enzyme (ACE) into angiotensin II, an octapeptide. Binding of angiotensin II to its receptor (AT_1) produces a powerful vasoconstrictor.[3] Since angiotensin II can be formed *in vivo* by enzymes other[4] than ACE, a small molecule-mediated modulation of the RAS system that blocks the action of angiotensin II at receptor level made a better therapeutic target than the inhibition of angiotensin I conversion to angiotensin II. It is the selective antagonism of the biological activity of angiotensin II that underlies the mechanism of action of the sartans, and several compounds in this group including Irbesartan (BMS), Losartan (Merck), Telmisartan (Boehringer Ingelheim), etc., are currently in clinical use.[5] Essential hypertension is a major risk factor in cardiovascular disease and it accounts for a third of global deaths.[6] Irbesartan and the related sartans are useful in the treatment of hypertension and other diseases, such as heart failure, renal insufficiency, glaucoma, and diabetic retinopathy, which are linked with this condition.[7]

Our interest in the synthesis of a radiolabeled analog was prompted by studies of the environmental fate of Irbesartan, the ecological impact of its clinical use as a drug and the derivatives therefrom. An earlier labeled synthesis of Irbesartan (unpublished) utilized [^{14}C]cyclopentanone to install the label. We opted instead for a comparatively less expensive starting labeled reagent and a short synthetic route that takes advantage of intermediates in the known syntheses. Dimethylformamide-(carbonyl-^{14}C), 1,4-dibromobenzene, 5-phenyl-1*H*-tetrazole, and SR48001A met these requirements for a cost effective and expeditious preparation, and the details are as follows.

Experimental

All reactions were carried out under an atmosphere of argon unless otherwise specified. Solvents were of commercial grade and used without purification or drying. Column chromatography was carried out on Merck Kiesegel 60 (230 μ) silica gel. Flash chromatographic separations were performed on a Biotage Flash System using pre-packed silica gel cartridges. TLC visualization reagents included (10% iodine plus 10% AcOH) in 40% aqueous KI and, Cerium sulfate in 10% sulfuric acid. ^{1}H NMR spectra were recorded at 300, 400, or 500 MHz Spectrometer. Chemical shifts are given in ppm relative to tetramethylsilane. Agilent 1100 HPLC System including solvent degasser, pump, automated injector, and a variable UV detector connected to IN/US BetaRam model 4 Flow Detector with a 0.25-mL detector cell was used in the analyses of compounds. The HPLC column was YMC-Park Pro C_{18}, 3 μ, 4.6 × 150 mm. Elution Solvent System A = water:acetonitrile 80:20 (0.1% TFA), and B = water:acetonitrile 20:80 (0.1% TFA) under Gradient Condition of 0–8 min, 0% B; 20 min, 100% B; 21 min, 0% B; 27 min, 0% B, and a flow rate of 1.0 mL/min, with detection by UV at 254 nm. LC/MS analysis was performed on a Finnigan LXQ Mass Spectrometer System, LC/MS method: Generic-B20 (+p ESI full mass). *N,N*-Dimethyl [^{14}C]formamide (Lot ♯ B37, 100 mCi, Sp Act. 56 mCi/mmol) was purchased from GE Healthcare, UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, PH7 9NA, UK.

4-Bromo[^{14}C]benzyl bromide 4

To a stirred solution of 1,4-dibromobenzene (1.18 g, 5 mmol) in dry THF (40 mL) at −78°C was slowly added *n*-BuLi (2.5 M

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solution, 2 mL, 5 mmol) over 45 min. After 1 h, a solution of DMF-^{14}C (purchased as 100 mCi, Sp. Act. 56 mCi/mmol and diluted to 374.5 mg, 5.0 mmol, Sp. Act. 20 mCi/mmol, 100 mCi) in THF (5 mL) was added. It was stirred at −78°C for 1 h, 1 N HCl (15 mL) was added and the reaction mixture was allowed to slowly warm to room temperature. The reaction was extracted with dichloromethane (3 × 50 mL), and the combined organic phases were washed with water (50 mL), brine (50 mL), and dried. Solvent was removed under reduced pressure and the product was dissolved in THF:MeOH (60 mL, 7:3 v/v). It was cooled in ice-water bath, sodium borohydride (274.3 mg, 7.25 mmol) was added, and the reaction was stirred at room temperature for 30 min. Excess borohydride was destroyed by the careful addition of dil. HCl and the reaction was extracted with dichloromethane (3 × 30 mL). The combined organic portion was washed with water, brine, and dried. It was purified by column on silica gel (40% ether in hexane) to afford white solid, 4-bromobenzyl alcohol-^{14}C **3** (690 mg, 3.68 mmol, 73.7%). ^{1}H NMR (400 MHz, $CDCl_3$), δ1.96 (brs, 1H), 4.61 (s, 2H), 7.21 (d, 2H, J = 8.3 Hz), 7.60 (d, 2H, J = 8.3 Hz). ^{13}C-NMR (125.7 MHz, $CDCl_3$), 139.79, 131.62, 128.59, 121.43, 64.52. To a solution of **3** and triethylamine (1.02 mL, 7.36 mmol) in 20 mL dry dichloromethane at 0°C was added methanesulfonyl chloride (401 μL, 5.16 mmol) and the mixture was stirred to warm to room temperature in 1 h. It was diluted with diethyl ether (40 mL) and washed with saturated $NaHCO_3$ (30 mL), water (30 mL), brine (40 mL), and dried over $MgSO_4$. The solution was filtered and evaporated to give the crude mesylate (992 mg). The mesylate and LiBr (800 mg, 9.2 mmol) in dry acetone (120 mL) were refluxed for 2 h and concentrated to a small volume under reduced pressure. It was partitioned between ether and water, the aqueous was further extracted with ether (3 × 30 mL), and the organic portions were combined and dried. Evaporation gave 4-bromo-[^{14}C]benzyl bromide **4** (922 mg, 3.6 mmol, 72%, from DMF). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.32 (s, 2H), 7.17 (d, 2H, J = 8.52 Hz), 7.34 (d, 2H, J = 8.3 Hz).

3-(4-Bromo-[^{14}C]benzyl)-2-butyl-1,3-diazaspiro[4,4]non-1-en-4-one 5

Potassium carbonate (1.53 g, 11.06 mmol) was added to SR48001A (850 mg, 3.68 mmol) in dry DMF (20 mL) and stirred for 45 min at room temperature. 4-Bromobenzyl bromide-^{14}C **4** (922.0 mg, 3.68 mmol) in dry DMF (5 mL) was added and the mixture was stirred at room temperature overnight. The solvent was removed under high vacuum and the residue was partition between water/*n*-heptane/ether (50 mL, 20:20:10 v/v/v). The aqueous was further extracted with heptane/ether (3 × 30 mL, 20:10v/v), and the combined organic was dried and evaporated to give **5** (1.3 g, 3.56 mmol, 96%). ^{1}H-NMR (400 MHz, $CDCl_3$), δ 7.39 (d, J = 8.3 Hz, 2 H), 6.97 (d, J = 8.3 Hz, 2H), 4.55 (s, 2H), 2.21 (overlapping q, J = 7.8 Hz, 2H), 1.9 (brm, 6H), 1.7 (brm, 2H), 1.49 (*m*, 2H), 1.24 (*m*, 2H), 0.80 (tr, 3H).

2-(1-Trityl-1H-tetrazol-5-yl)phenylboronic acid

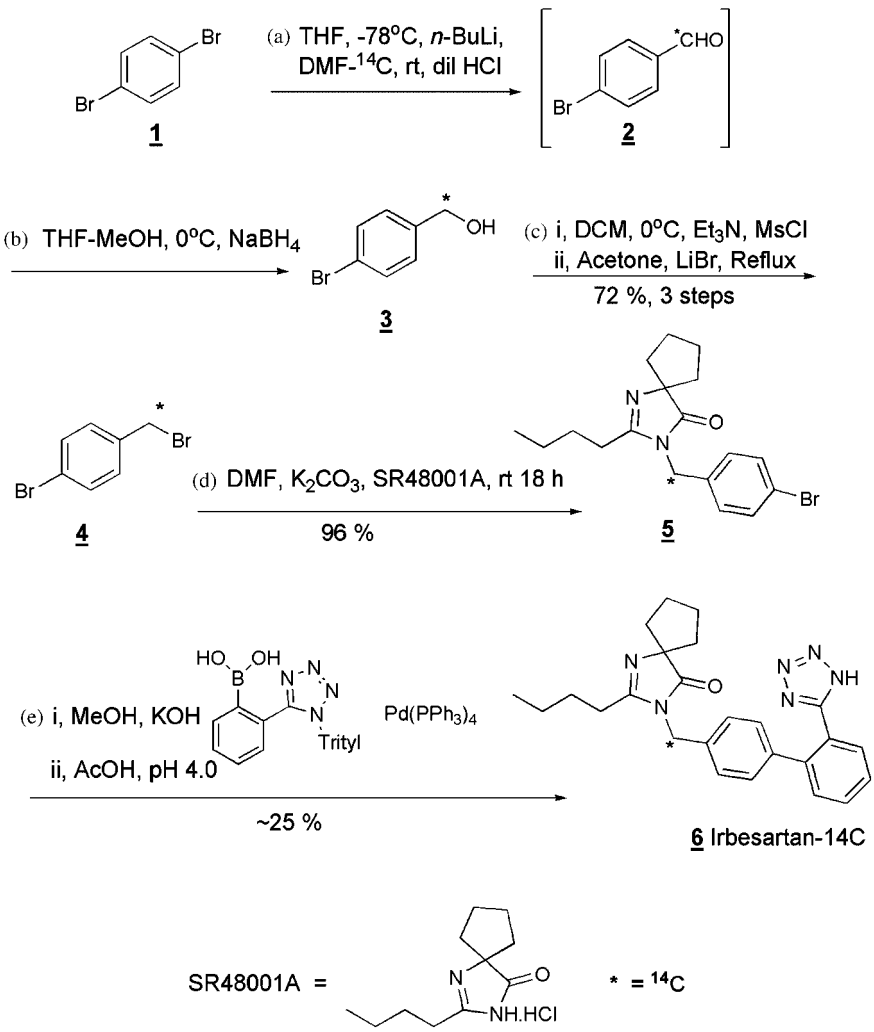
To a stirred solution of 5-phenyltetrazole (14.9 g, 100 mmol) and triethylamine (14.8 mL, 105 mmol) in dry THF (260 mL) at 40°C was added a solution of trityl chloride (29.9 g, 105 mmol) in dry THF (120 mL). After 30 min, the reaction was cooled to 0°C and filtered to remove the salt. The filtrate was placed under argon atmosphere and cooled to −25°C. *n*-BuLi (2.5 M solution in hexanes, 46.16 mL, 115.4 mmol) was added slowly and the reaction was stirred at −20 to −10°C under argon atmosphere for 1 h. The reaction mixture was again cooled to −30°C, triisopropylborate (30.6 mL, 130 mmol) was added and the reaction mixture was stirred and slowly warmed to 10°C over 1 h. After the volatiles were removed by rotary evaporation, 160 mL of THF was added followed by 60 mL of isopropyl alcohol. The mixture was cooled to 0°C and saturated NH_4Cl (40 mL) was added ensuring that temperature stayed below 10°C. The slurry was warmed to room temperature and stirred for 30 min. Water (100 mL) was added over 20 min and the mixture was kept for 2 h during which white solid separated. It was collected by filtration, washed with isopropyl alcohol/water/triethylamine (50:50:2, 100 mL), and dried under high vacuum overnight to give 2-(1-trityl-1H-tetrazol-5-yl)phenylboronic acid (43.2 g, 98 %). ^{1}H-NMR (DMSO-$_{d6}$) δ 7.94 −7.84 (*m*), 7.60–7.39 (*m*), 7.10 −7.08 (*m*), 1.74 (brS).

Irbesartan-^{14}C 6

To a solution of **5** (1.3 g, 3.56 mmol) in methanol (13 mL) was added potassium hydroxide (400 mg, 7.01 mmol). After the solution was flushed with argon for 10 min, it was refluxed for 45 min under argon atmosphere. A solution of 2-(1-trityl-1H-tetrazol-5-yl)phenylboronic acid (2.7 g, 6.23 mmol) and potassium hydroxide (400 mg, 7.01 mmol) in methanol (13 mL) was added. The reaction mixture was heated under reflux for 10 h before it was cooled to room temperature. It was diluted with water (15 mL) and toluene (15 mL); the organic was separated and the aqueous portion was further extracted with toluene (12 mL). The organic portions were discarded; the aqueous was acidified with acetic acid and it was evaporated to a residue. The residue was purified by using flash column chromatography (C_{18} column) using step-gradient Twenty to sixty percent of MeOH in water to give the phenylboronic acid impurity followed by pure fractions of labeled compound. The fractions were combined and while it was being concentrated under reduced pressure a white solid separated. It was collected by filtration and dried under high vacuum to afford Irbesartan-^{14}C **6**, (380 mg, 16.43 mCi, 43.26 μCi/mg or 18.53 mCi/mmol, 25%). Radiochemical purity was determined to be 98.22% at Retention time of 15.1 min (authentic Reference Lot ♯ 6C17210), Flow rate of 1 mL/min and at UV detection wavelength of 254 nm. MS $[M+1]^+$: 429.33 and 431.25. ^{1}H-NMR (300 MHz, $CDCl_3$), 7.90 (d, J = 7.6 Hz, 1H), 7.58 (tr, J = 7.5 Hz, 1H), 7.50 (tr, J = 7.5 Hz, 1H), 7.40 (d, J = 7.58, 1H), 7.14 (d, J = 8.0 Hz, 2H), 7.04 (d, J = 8.0 Hz, 2H), 4.62 (s, 2H), 2.1 (tr, J = 8.5 Hz, 2H), 1.83–1.60 (br *m*, 8H), 1.4 (*m*, 2H), 1.25 (*m*, 2H), 0.79 (tr, 3H).

Results and discussion

In an earlier labeled synthesis, [^{14}C]-2-*n*-butyl-1,3-diazo-spiro-[4,4]-non-1-ene hydrochloride (SR48001A) was generated from [^{14}C]cyclopentanone and elaborated into labeled Irbesartan **6**.[8] Besides that it started from the relatively more expensive [^{14}C]cyclopentanone, we considered the reaction sequence to the -spiro-[4,4]non-1-ene intermediate to be cumbersome. The additional steps required downstream to convert the labeled intermediate SR48001A-^{14}C to Irbesartan-^{14}C were likely to further depress the overall radiochemical yield.



Scheme 1. Synthesis of Irbesartan ^{14}C.

It had been established in earlier work that 4-bromobenzyl bromide-[^{14}C] **4**, easily made from dimethyl[^{14}C]formamide and 1,4-dibromobenzene **1**, may be used to insert the bridging methylene to the heterocycle to make these sartans.[9] With labeled DMF instead of [^{14}C]cyclopentanone, we expected to realize a significant cost saving.

In addition, we were encouraged by the precedence in synthetic literature on Irbesartan indicating that *N*-alkylation of SR48001A with 4-bromobenzyl bromide[10] proceeded in good yield. Therefore, our critical task became the reaction of SR48001A with 4-bromobenzyl bromide to produce **5** for the Suzuki cross-coupling reaction that would follow. Such a cross-coupling reaction with 2-(1-trityl-1*H*-tetrazole-5-yl)phenylboronic acids has been used extensively in making these sartans,[11] and it should provide labeled Irbesartan after the removal of protecting groups. Accordingly in Scheme 1, the labeled 4-bromobenzyl bromide **4** was prepared as follows. A lithium–halogen exchange reaction was conducted on **1** using 1.0 equiv. of *n*-BuLi at −78°C. After 45 min, the reaction mixture was treated with DMF-[1^{14}C-carbony] and stirred for a further 1 h reaction time. Dil HCl was added and the reaction mixture was stirred to warm to room temperature. The resulting 4-bromobenzaldehyde **2** was isolated by extraction method and reduced immediately with sodium borohydride to give the labeled 4-bromobenzyl alcohol **3**. A mesylate prepared from **3** was reacted with LiBr in refluxing acetone to make **4** in 72% yields from labeled DMF. Reaction of SR48001A with **4** gave 3-[4-bromobenzyl]-2-butyl-1,3-diazoSpiro[4,4]-non-1-en-4-one **5** in 96% yield.

A phenylboronic acid derivative for cross-coupling with **5** was prepared from commercially obtained 5-phenyl-1*H*-tetrazole. Our best procedure for making the boronic acid required the protection of the tetrazole as a triphenylmethyl homolog, followed by tetrazole moiety directed ortho-lithiation of the phenyl ring.[11] Triisopropyl borate was the superior reagent for incorporating boron onto the phenyltetrazole after lithiation at −30°C. By this protocol, the requisite boronic acid was made in 98% yield after deprotection with dilute acid. The cross-coupling reaction was carried out with $Pd(PPh_3)_4$ and KOH in methanol and afforded a low yield of 25%. It could be that the low yield was due to the quality of the palladium catalyst. Acidification with acetic acid to a pH 4 provided Irbesartan-^{14}C in 18% overall Radiochemical yield.

Conclusions

From dimethylformamide-(carbonyl-^{14}C), Irbesartan-^{14}C was prepared in 18% overall yield. The synthetic sequence entailed

generating labeled benzaldehyde from dimethylformamide-(carbonyl-^{14}C) and 1,4-dibromobenzene, its reduction to the alcohol, followed by conversion of the latter compound to the benzyl bromide. Thereon in the sequence, known intermediates, other established reaction steps like *N*-alkylation of SR48001A and Suzuki cross-coupling were employed to make Irbesartan-^{14}C. The present sequence is a cost effective and an efficient route to labeled Irbesartan.

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