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Carbon-11 Labeling of a Potent, Nonpeptide, AT_1-Selective Angiotensin-II Receptor Antagonist: MK-996

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Summary

[α-^{11}C]Benzoyl chloride was synthesized and purified by normal phase HPLC. [^{11}C]MK-996 ([^{11}C]*N*-[[4'[(2-ethyl-5,7-dimethyl-3H-imidazo [4,5-b]pyridin-3-yl)methyl][1,1'-biphenyl]-2-yl]sulfonyl]-benzamide), a potent and selective ligand for the AT_1 receptor, was prepared by *N*-benzoylation of L-159,221 with [α-^{11}C]benzoyl chloride in tetrahydrofuran using lithium bis(trimethylsilyl)amide as a base. The radiotracer was purified by semi-preparative reverse-phase HPLC. The average specific activity was 1162 mCi/μmol calculated at end-of-synthesis (EOS). The average time of synthesis including formulation was 38 minutes.

Key Words: Angiotensin, Benzoyl chloride, Carbon-11, PET, Radiotracer

Introduction

The renin-angiotensin system (RAS) plays a major role in the regulation of blood pressure and in the pathogenesis of hypertension. Two obligatory steps of RAS

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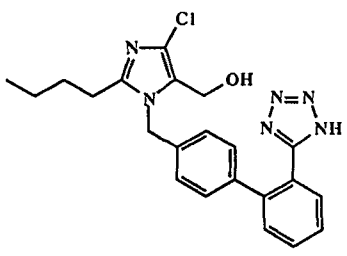
involve the angiotensin-converting enzyme-(ACE)-catalyzed cleavage of the decapeptide angiotensin I (Ang I) to the RAS-active component angiotensin II (Ang II, an octapeptide), and binding of Ang II to specific receptors in various tissues including vascular smooth muscle, adrenal cortex, and kidney (1,2). Interaction of Ang II with these receptors elevates blood pressure by a variety of mechanisms including constriction of vascular smooth muscles and stimulation of the release of aldosterone which leads to sodium retention (3). In the early 1970's, ACE inhibitors were developed that inhibit the conversion of Ang I to Ang II and these drugs (e.g. captopril, enalopril and lisinopril) are now widely used in the treatment of hypertension and heart failure (4,5).

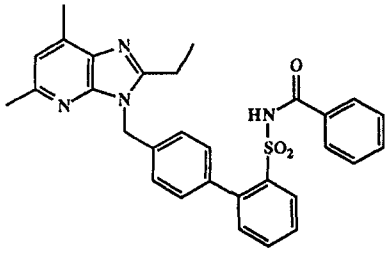
Currently, the focus of research on new antihypertensives that inhibit RAS is on the development of drugs which more specifically inhibit the effects of Ang II by blocking Ang II receptors. Early work with saralasin, a peptidal Ang II antagonist, demonstrated that blockade of Ang II receptors lowers blood pressure in man (6,7). However, development of saralasin (as well as other peptidal Ang II antagonists) as a drug has been hindered by poor oral bioavailability, a property that is common to most peptides. Consequently, there has been substantial effort devoted to the development of orally available, non-peptide, Ang II antagonists (8,9). One such antagonist that has come from this effort is losartan (MK 594 or DuP 753, Compound 1, Figure 1) which is now undergoing clinical trials, preliminary results of which suggest that losartan is a promising new antihypertensive drug (10-12).

Recent *in vitro* binding studies using radiolabeled Ang II antagonists have shown that Ang II receptors exist in at least two subtypes and these have been designated AT_1 and AT_2 (13). Extensive studies have demonstrated that most of the physiological effects of Ang II antagonists are related to blockade of AT_1 receptors while the biological role of AT_2 has not yet been established. Binding studies have demonstrated that losartan binds with high affinity to AT_1 (IC_{50} = 12 nM) and that it is > 10,000-fold selective for AT_1 versus AT_2. Numerous studies have been reported using radiolabeled peptides for the study of Ang II receptors *in vitro* and autoradiographically using post mortem tissue samples. A recent report on the receptor binding properties of [^{125}I][Sar^1,Ile^8]Ang II, a sub-type non-selective Ang II antagonist, suggested that this peptide, if labeled with I-123, may be useful for the study of these receptors non-invasively in living animals and man via SPECT (14). Such studies, and similar studies

using PET, have proven useful in the localization and quantification of receptors in man and offer insight into the role of these receptors in normal and disease states (15-18).



1. Losartan



2. MK-996

Figure 1. Structures of the nonpeptide angiotensin-II antagonists Losartan and MK-996.

As part of our efforts to develop radiotracers for the study of AT_1 receptors in man, we have investigated the development of positron emitting radiotracers that bind with high affinity and selectivity to AT_1 receptors. Although losartan, if labeled with C-11 or F-18 might be useful for this purpose, reports that a similar, tetrazole containing tracer ([^{3}H]L-158,809), in addition to binding to AT_1 receptors, binds to a second, as yet, uncharacterized, biphenyltetrazole binding site in kidney (19). This complication led us to focus our efforts on AT_1 antagonists that do not contain the biphenyltetrazole moiety. MK-996 (L-159,282, Compound 2, Figure 1) is an analog of losartan in which the imidazole ring has been replaced with a bicyclic, imidazopyridine ring, and, more importantly, the tetrazole ring has been replaced by an acidic benzoylsulfonamide moiety (20). *In vitro* binding studies have shown that, MK-996 has a significantly higher affinity for AT_1 than losartan (IC_{50} = 0.15 and 12 nM, respectively) and that binding to the proposed biphenyltetrazole binding site is minimal (21). Like losartan, MK-996 is highly selective for AT_1 (>2000 fold vs. AT_2). We report here on the radiosynthesis, purification, and quality control of [^{11}C]MK-996, a non-peptide AT_1 selective radiotracer which has been shown to be useful for PET studies of angiotensin receptors (22,23).

Materials and Methods

With the exception of L-159,221 and MK-996, all chemicals were available from commercial sources and used as received. L-159,221 (4'-[(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-yl)methyl][1,1'-biphenyl]-2-sulfonamide) and an authentic

sample of MK-996 were both synthesized by chemists at Merck Research Laboratories (20). Radioactivity measurements were made using a Capintec CRC-12 dose calibrator.

Synthesis of [α-^{11}C]Benzoyl chloride

[α-^{11}C]Benzoyl chloride was prepared with minor modifications of a published method (24). Briefly, [^{11}C]carbon dioxide was produced by 16 MeV proton bombardment of a nitrogen gas target using a Scanditronix RNP-16 biomedical cyclotron. At the end-of-bombardment (EOB), the [^{11}C]CO_2 was trapped in a stainless steel coil cooled in liquid nitrogen and evacuated by an oilless vacuum pump. The cooling bath was removed and the [^{11}C]CO_2 transferred to the reaction vessel via a nitrogen sweep gas (~15 to 25 mL/min). The reaction vessel consisted of a teflon septum-sealed 3 mL glass v-vial that was purged with argon and charged with 300 μL of 1.0 M phenylmagnesium chloride (Aldrich) in tetrahydrofuran (THF) and diluted with 300 μL of dry THF. To measure [^{11}C]CO_2 not trapped in the reaction vessel (usually < 20%), an ascarite column (~50 g) connected to the vessel via $^1/_8$ inch teflon tubing was placed in the dose calibrator. After obtaining maximum radioactivity in the v-vial, the sweep gas was turned off and the vessel was warmed at 80°C for 1 minute and then allowed to stand at room temperature for 4 minutes. The mixture was treated with 45 μL of phthaloyl dichloride (Aldrich) and heated at 80°C for 5 minutes.

Purification of [α-^{11}C]Benzoyl chloride by normal phase HPLC

The reaction mixture was concentrated under an argon stream to ~100 μL and injected onto a semi-preparative Waters Associates μPorasil HPLC column (7.8 mm x 30 cm) eluted with hexane at 6 mL/min. The effluent from the column was monitored with a UV detector (254 nm, Waters module 440) and an in-line radioactivity detector (Ortec 449 ratemeter, 575 amplifier, 550 single channel analyzer, with a NaI(Tl) crystal). The radioactive peak corresponding to [α-^{11}C]benzoyl chloride (t_R = 3.2 min, k' = 4.5) was collected in a 10 mL v-vial.

Synthesis and purification of [^{11}C]MK-996

A 0.3 mL reaction vial was flushed with argon and charged with 1 mg (2 μmol) of L-159,221. The precursor was dissolved in 150 μL of dry THF and treated with

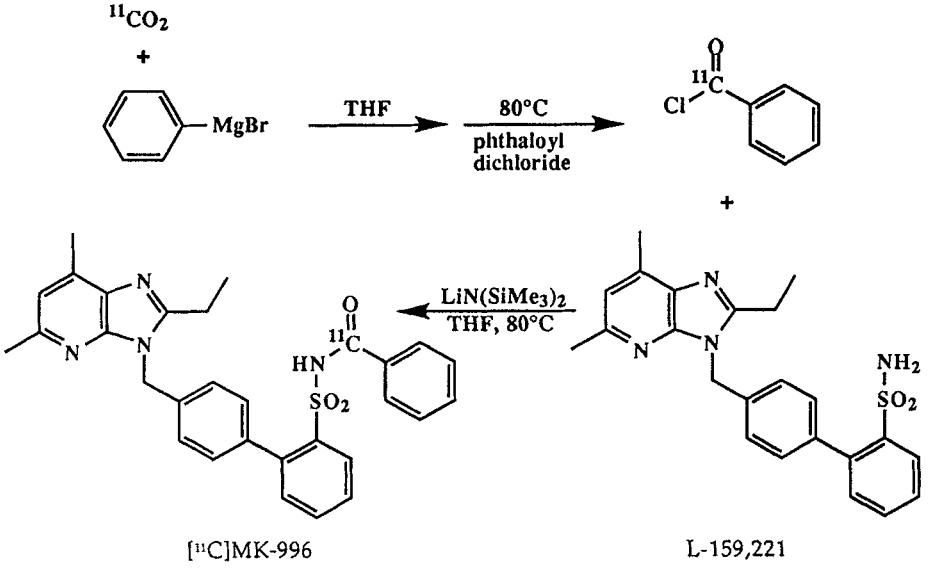
2.6 μL of $LiN(SiMe_3)_2$ (Aldrich, 1.0 M in THF, 2.6 μmol, 1.3 eq.). This solution was stirred for 5 minutes at room temperature and then added to the solution of purified [α-^{11}C]benzoyl chloride in hexane. The reaction mixture was heated at 80°C for 5 minutes and then concentrated under an argon stream. The residue was dissolved in 500 μL of 50:50 acetonitrile:water and injected onto a semi-preparative Waters Associates C_{18} μBondapak HPLC column (7.8 mm x 30 cm) eluted with a mobile phase of 50:50 acetonitrile:water (0.1% trifluoroacetic acid) at 7 mL/min. The radioactive peak corresponding to [^{11}C]MK-996 (t_R = 3.8 min., k' = 2.4) was collected in a rotary evaporator and the solvent evaporated to dryness under reduced pressure. The residue was dissolved in 7 mL of sterile saline and filtered through a sterile 0.22 μm filter (Gelman Acrodisc) into a sterile, pyrogen-free evacuated vial. The solution was diluted with 3 mL of 8.4% sterile aqueous sodium bicarbonate and the radioactivity measured.

Determination of specific activity

A 100 μL aliquot of the final solution of known activity was injected onto an analytical Waters Associates C_{18} μBondapak HPLC column (3.9 mm x 30 cm). A mobile phase of 50:50 acetonitrile:water (0.1% trifluoroacetic acid) at 3 mL/min was used to elute the radioligand (t_R = 2.9 min., k' = 1.5). The area of the UV absorbance peak measured at 254 nm corresponding to carrier product was measured by an automated integrating recorder (Hewlett Packard 3390A) and compared to a standard curve relating mass to UV absorbance.

Results and Discussion

The synthesis of [^{11}C]MK-996 involved the *N*-benzoylation of the benzenesulfonamide precursor L-159,221 with [α-^{11}C]benzoyl chloride (Scheme 1). The radiosynthetic procedure used in the preparation of [α-^{11}C]benzoyl chloride was a modification of one reported previously (24). Briefly, it involved the radiocarboxylation of a solution of phenylmagnesium chloride in THF with [^{11}C]CO_2 with subsequent conversion of the resulting [α-^{11}C]benzoic acid to [α-^{11}C]benzoyl chloride using either phthaloyl dichloride or thionyl chloride. While both reagents accomplished this transformation, phthaloyl dichloride gave consistently better yields (Scheme 1).



Scheme 1. Radiochemical Synthesis of [^{11}C]MK-996.

Purification of [α-^{11}C]benzoyl chloride was achieved by one of four different methods: distillation, solid-phase extraction, liquid-liquid extraction, or normal phase HPLC. Although isolation of [α-^{11}C]benzoyl chloride by distillation was attempted previously (24), distilled [α-^{11}C]benzoyl chloride did not couple with L-159,221 (unpublished results). Both solid-phase extraction (C_{18} Sep-Pak) and liquid-liquid extraction with diethyl ether also proved to be unsuccessful methods when coupling with L-159,221 was attempted (unpublished results). Normal phase semi-preparative HPLC, however, yielded [α-^{11}C]benzoyl chloride 26 minutes after EOB in an average non-decay corrected radiochemical yield of 10% (n=7) based on radioactivity trapped in the Grignard solution. [α-^{11}C]Benzoyl chloride prepared in this manner was of >96% radiochemical purity and reacted at 80°C with L-159,221 in 5 minutes. Pretreatment of the precursor with lithium bis(trimethylsilyl)amide was necessary for the consumption of [α-^{11}C]benzoyl chloride (Scheme 1).

Reverse-phase semi-preparative HPLC (Figure 2) was used to purify [^{11}C] MK-996. With a retention time of 3.8 minutes (k' = 2.4) for [^{11}C]MK-996, a satisfactory separation between the desired product and the precursor L-159,221 (t_R = 2.2 min.) was achieved. The radioactive peak corresponding to the product was collected remotely and the solvent removed by rotary evaporation under high vacuum. The product was

formulated in sterile saline, microfiltered into a sterile, evacuated dose-vial, and diluted with sterile sodium bicarbonate. Using this procedure, the formulated radiotracer proved to be sterile and pyrogen-free using normal methods.

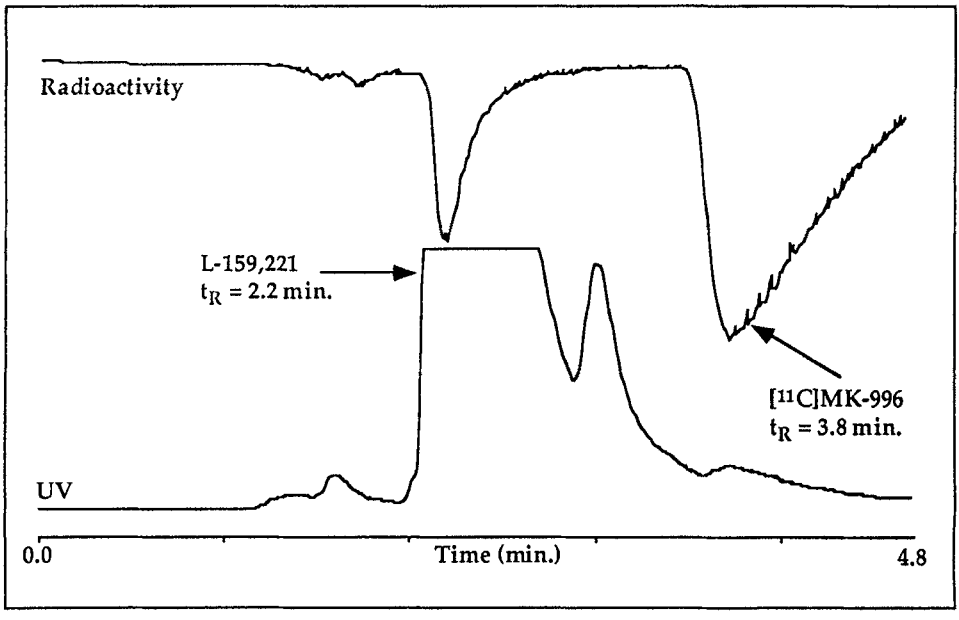


Figure 2. HPLC purification of [^{11}C]MK-996 (t_R = 3.8 min.)

The average time of synthesis of [^{11}C]MK-996 was 38 minutes from EOB with an average non-decay corrected radiochemical yield (based on initial radioactivity trapped in the Grignard) of 3% for three preparations. Both the radiochemical purity and specific activity were determined by analytical reverse-phase HPLC. The resulting chromatogram showed [^{11}C]MK-996 to be of >95% radiochemical purity. The radioactive product co-eluted with an authentic sample of MK-996. The average specific activity calculated at the end of synthesis was 1162 mCi/μmol. Imaging studies (the results of which will be reported elsewhere) have demonstrated that this tracer can be used to image AT_1 receptors in dog kidney (23).

Conclusions

[^{11}C]MK-996 of high specific activity can be synthesized and purified in acceptable radiochemical yields from L-159,221 and [α-^{11}C]benzoyl chloride. A

sufficient amount of the radioligand can be prepared to permit its use for localization and quantitation of angiotensin receptors *in vivo* with PET.

Footnote: The chemical name of MK-996 (a.k.a. L-159,282) is *N*-[[4'[(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-yl)methyl][1,1'-biphenyl]-2-yl]sulfonyl]-benzamide.

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