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An Alternative and Expedient Synthesis of Radioiodinated 4-Iodophenylalanine

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Abstract

Radiolabeled amino acids have been used extensively in oncology both as diagnostic and therapeutic agents. In our pursuit to develop radiopharmaceuticals to target breast cancer, we were interested in determining the uptake of radioiodinated 4-iodophenylalanine, among other labeled amino acids, in breast cancer cells. In this work, we have developed an alternative method for the synthesis of this agent. The novel tin precursor, (*S*)-*tert*-butyl 2-(*tert*-butoxycarbonylamino)-3-(4-(tributylstannyl)phenyl)propanoate (**3**) was synthesized from the known, corresponding iodo derivative. Initially, the labeled 4-iodophenylalanine was synthesized from the above tin precursor in two steps with radiochemical yields of $91.6 \pm 2.7\%$ and $83.7 \pm 1.7\%$ ($n = 5$), for the radioiodination (first) and deprotection (second) step, respectively. Subsequently, it was synthesized in a single step with an average radiochemical yield of $94.8 \pm 3.4\%$ ($n = 5$). After incubation with MCF-7 breast cancer cells for 60 min, an uptake up to $49.0 \pm 0.7\%$ of the input dose was seen; in comparison, the uptake of [^{14}C]phenylalanine under the same conditions was $55.9 \pm 0.5\%$. Furthermore, the uptake of both tracers was inhibited to a similar degree in a concentration-dependent manner by both unlabeled phenylalanine and 4-iodophenylalanine. With [^{14}C]phenylalanine as the tracer, IC_{50} values of 1.45 mM and 2.50 mM were obtained for Phe and I-Phe, respectively and these values for [^{125}I]I-Phe inhibition were 1.3 and 1.0 mM. In conclusion, an improved and convenient method for the synthesis of no-carrier-added 4-[$^{*}\text{I}$]phenylalanine was developed and the radiotracer prepared by this route demonstrated an amino-acid transporter-mediated uptake in MCF-7 breast cancer cells in vitro that was comparable to that of [^{14}C]phenylalanine.

Keywords

Radiolabeled amino acid; Amino acid transporter; breast cancer

1. Introduction

Compared to normal cells, cancer cells voraciously consume amino acids among other nutrients. Amino acids are transported across the plasma membrane by various amino acid transporters (del Amo et al., 2008; Fuchs et al., 2005). The system L amino acid transporter (LAT1) is up-regulated in a number of cancer cells including breast cancer (Shennan et al.

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2004; Shennan and Thomson 2008). Specific inhibition of this system results in inhibition of cell growth, and has been suggested as a target to inhibit the progression of breast cancer (Shennan and Thomson, 2008). Facilitated by these proteins, amino acids are easily transported across the blood-brain barrier. Given these facts, amino acid transporters are prime targets for radioimaging and targeted radiotherapy of various cancers and a number of radiolabeled amino acid derivatives have been developed (Herrmann et al., 2009; Jager et al., 2001; Langen et al., 2006; McConathy et al., 2008; Yu et al., 2008).

Radioiodinated derivatives of phenylalanine have been investigated as potential tumor imaging and therapeutic agents. A group in Belgium has labeled 2-iodo-phenylalanine (both *D* and *L* forms) with various iodine radioisotopes and evaluated their potential utility for cancer targeting (Bauwens et al., 2007; Bauwens et al., 2010; Kersemans et al., 2006). Samnick and colleagues have published several reports on the synthesis and evaluation of radioiodinated 4-iodophenylalanine (4-I-Phe) including clinical studies. Results from a preliminary study of using 4-iodophenylalanine labeled with the positron emitter ^{124}I in evaluation of the pre- and post-operative status of a single glioma patient by PET imaging have been reported (Farmakis et al., 2008). In another study, the utility of 4- ^{123}I I-Phe was compared with that of 3- ^{123}I iodo- ∇ -methyltyrosine (^{123}I IMT) for the imaging of 11 glioma patients (Hellwig et al., 2008). While the tumor-to-brain ratios for 4- ^{123}I I-Phe were inferior to those of (^{123}I IMT, the authors suggested 4- ^{131}I I-Phe for therapy due to its longer tumor retention. In yet another prospective study involving 67 patients suspected of glioma, SPECT imaging was performed using 4- ^{123}I I-Phe (Hellwig et al., 2010). Although SPECT imaging using 4- ^{123}I I-Phe showed high specificity, false negative findings, especially in low grade glioma, may be a confounding issue. This group of investigators also evaluated the suitability of 4- ^{123}I I-Phe for the diagnosis of pancreatic adenocarcinoma in 7 patients but no tumor uptake was seen (Hellwig et al., 2008). The therapeutic effectiveness of 4- ^{131}I I-Phe has been demonstrated in vitro using glioma cells (Romeike et al., 2004). Moreover, using orthotopic glioma rat models, 4- ^{131}I I-Phe alone or in combination with external beam radiotherapy has been shown to be highly effective in the treatment of glioma (Romeike et al., 2004; Samnick et al., 2009).

We are involved in the development of novel radiolabeled amino acid derivatives primarily to target breast cancers, and as a part of our studies wanted to evaluate the uptake of 4-iodo-phenylalanine in some breast cancer cell lines. Radioiodinated 4-iodo-phenylalanine has been synthesized as early as in 1968 by Counsell et al. using exchange iodination method (Counsell et al., 1968). No-carrier-added (n.c.a.) synthesis of 4- $^{*}\text{I}$ iodophenylalanine by copper-catalyzed iodo-debromination has been reported by several groups including that of Samnick (Farah et al., 1997; Samnick et al., 2001). Halodestannylation is the most facile route for radioiodination—the reaction can be conducted under very mild conditions, results in higher radiochemical yields, and the product can be isolated devoid of any carrier. In addition, in general this reaction is the most practical route for the introduction of the ∇ -particle-emitting heavy halogen ^{211}At into molecules of interest (Vaidyanathan and Zalutsky, 2008). Although astatinated phenylalanine has been obtained in considerably higher radiochemical yields by the copper-catalyzed astato-deiodination than by astato-destannylation (Meyer et al., 2010), separation of the radiohalogenated product from the chemically similar substrate, unlike from the tin precursor, could be challenging. Samnick's group developed an improved n.c.a. synthesis of 4- $^{*}\text{I}$ iodo-*L*-phenylalanine suitable for clinical applications from a tin precursor (Israel et al., 2008). The tin precursor they utilized was a methyl ester of the *N*-Boc-protected phenylalanine derivative. This entailed the use of two steps for the deprotection of the *N*-Boc and the methyl ester groups. In addition, because the methyl ester was hydrolyzed under alkaline conditions, there is the potential for epimerization at the chiral center. In the case of 2-iodophenylalanine, the *L*-isomer has been shown to be better than the *D*-isomer for imaging applications due to its faster blood

clearance and rapid distribution to the peripheral compartment (Kersemans et al., 2006). Although it is not known at this time whether L-isomer of 4-iodophenylalanine will have better biological characteristics over its D-isomer or *vice versa*, to insure chiral homogeneity, and to make the deprotection more facile, we developed a tin precursor wherein the amino group was protected with a Boc group and the acid function was protected with *tert*-butyl ester so that both can be simultaneously removed under acidic conditions.

This paper describes the synthesis of the above tin precursor and its conversion to radioiodinated phenylalanine. We also did some preliminary evaluation of the radiolabeled compound by determining its uptake in a breast cancer cell line *in vitro*.

2. Materials and Methods

2.1. General

All chemicals were purchased from Sigma-Aldrich unless otherwise specified. Sodium [¹²⁵I]iodide (2200 Ci/mmol) as a solution in 0.1N NaOH and [¹⁴C]phenylalanine ([¹⁴C]Phe; 0.1 mCi/ml in 2:98 ethanol:water; 487 mCi/mmol) were procured from Perkin Elmer Life and Analytical Sciences (Boston, MA). Aluminum-backed sheets (Silica gel 60 F254) were used for analytical TLC, and normal-phase column chromatography was performed using silica gel 60 (both obtained from EM Science, Gibbstown, NJ). High-pressure liquid chromatography (HPLC) was performed using Beckman Gold HPLC system equipped with a Model 126 programmable solvent module, a Model 166 NM variable wavelength detector, a Model 170 radioisotope detector and a Beckman System Gold remote interface module SS420X, using 32 Karat® software. For reversed-phase HPLC a 4.6 × 250-mm XTerra RP18 (5 μm) column (Waters) was used. Proton NMR spectra of samples were obtained on a Varian Mercury 400 MHz spectrometer; chemical shifts are reported in δ units using the residual CHCl₃ peak as a reference. Low and high-resolution mass spectra were obtained using DART-MS using an Agilent TOF-MS model 6224 mass spectrometer.

2.2. (S)-*tert*-butyl 2-(*tert*-butoxycarbonylamino)-3-(4-iodophenyl)propanoate (2)

Tert-butyl bromide (27.8 ml, 245 mmol) was added dropwise to a mixture of (S)-2-(*tert*-butoxycarbonylamino)-3-(4-iodophenyl)propanoic acid (2.0 g, 5.1 mmol), *N*-benzyl-*N*,*N*-diethylethanaminium chloride (1.164 g, 5.11 mmol), and anhydrous K₂CO₃ (18.4 g, 133 mmol) in DMA (1.0 ml). The reaction mixture was stirred at 55 °C for 2 h, cooled to 20 °C and 300 ml of cold water was added. The crude product was isolated from this mixture by extraction with ethyl acetate three times. The combined ethyl acetate extract was washed with brine, dried with Na₂SO₄, and concentrated. The crude product was subjected to silica gel chromatography using 0%–20% EtOAc/hexanes to afford (S)-*tert*-butyl 2-(*tert*-butoxycarbonylamino)-3-(4-iodophenyl)propanoate (1.51 g, 3.4 mmol, 66.0% yield): ¹H-NMR (400 MHz, CDCl₃) δ 1.39 (18 H, s), 2.96 (2H, m), 4.40 (1H, d, *J* = 7.2), 4.97 (1H, d, *J* = 8.0), 6.90 (2H, d, *J* = 8.0), 7.58 (2H, d, *J* = 8.0).

2.3. (S)-*tert*-butyl 2-(*tert*-butoxycarbonylamino)-3-(4-(tributylstannyl)phenyl)propanoate (3)

A mixture of **2** (0.10 g, 0.22 mmol), bis(tributyltin) (0.56 ml, 1.12 mmol) and 1,4-dioxane (25 ml) was heated to 100 °C in an oil bath. Bis(triphenylphosphine)palladium(II) dichloride (0.02 g, 0.02 mmol) was added and the mixture stirred at 100 °C for 1.0 h. The reaction mixture was cooled to 0–5 °C in an ice bath and the precipitated metallic palladium was filtered through a bed of Celite. The filtrate was concentrated to dryness to yield a dull golden oil. The crude product was subjected to silica gel column chromatography using 95/5 hexanes/EtOAc to yield (S)-*tert*-butyl 2-(*tert*-butoxycarbonylamino)-3-(4-(tributylstannyl)phenyl)propanoate (0.12 g, 0.19 mmol, 86 %) as a clear oil, which was shown to be more than 99% pure by reversed-phase HPLC (see below for conditions). ¹H-

NMR (400 MHz, CDCl_3) ^1H 0.86 (9H, t, $J = 7.6$), 1.01 (6H, m), 1.31 (6H, m), 1.36 (9H, s), 1.40 (9H, s), 1.50 (6H, m), 3.00 (2H, m), 4.43 (1H, m), 4.99 (1H, d, $J = 8.0$), 7.11 (2H, d, $J = 8.0$), 7.50 (2H, d, $J = 7.6$). MS (DART) m/z : cluster peaks at 612 ($\text{M}+\text{H}$) $^+$, 569, and 500; peaks at higher mass range (900) were seen and are probably due to ion-molecule interactions (Song et al., 2009). HRMS (DART) Calcd for $\text{C}_{30}\text{H}_{54}\text{NO}_4^{120}\text{Sn}$ ($\text{M}+\text{H}$) $^+$: 612.3075. Found: 612.3065 $\pm$ 0.0002 ($n = 4$).

2.4. (S)-2-amino-3-(4-[^{125}I]iodophenyl)propanoic acid, [^{125}I]I-Phe (4)

A) Two-step method: Iodine-125 (2 μl , 0.5–1.5 mCi) was added to a solution of 50 μg of (S)-tert-butyl 2-(tert-butoxycarbonylamino)-3-(4-(tributylstannyl)phenyl)propanoate in 10 μl methanol in a 1-dram vial followed by 10 μl of 3:1 (v/v) mixture of acetic acid and 30% (w/v) hydrogen peroxide. The resultant mixture was sonicated for a period of 15 s and purged with Argon to remove most of the solvent. Any remaining solvent was removed by co-evaporating with 3 H 10 μl of anhydrous methanol using Argon. The residual radioactivity was taken in 500 μl of 10% aqueous acetonitrile and injected onto a reversed-phase HPLC column. The column was eluted at 1 ml/min with a gradient consisting of 0.1% TFA in both water (solvent A) and acetonitrile (solvent B); the proportion of B was kept at 10% for 5 min and then linearly increased to 90% over the next 25 min. The HPLC fractions containing [^{125}I]2 ($t_R = 25.5$ min) were collected and concentrated using a gentle Argon stream. The residual material containing the [^{125}I]2 was diluted with deionized water (5 ml) and loaded onto a C_{18} Sep pak cartridge (Waters). The cartridge was eluted with acetonitrile and 250 μl fractions were collected. The fractions containing majority of the radioactivity were pooled and concentrated using a gentle stream of Argon and the resultant solution was treated with 90% TFA (200 μl) at 65 $^\circ\text{C}$ for 10 min. TFA was evaporated by purging with Argon and taken in 10% aqueous acetonitrile (500 μl) and injected onto a reversed-phase HPLC column as above. The fractions corresponding to [^{125}I]4 ($t_R = 10.5$ min) were collected from which acetonitrile was evaporated with Argon. For use in biological assays, this solution was further diluted with PBS. *B) One step method:* The radioiodination was done essentially as described above and the solvents were evaporated with Argon. The intermediate derivative was deprotected *in situ* by treatment with TFA (90%; 100 μl) at 65 $^\circ\text{C}$ for 10 min. TFA was evaporated and the radioactivity reconstituted in 300 :1 of HPLC mobile phase (90:10 water:acetonitrile) and injected onto the HPLC. The fractions corresponding to [^{125}I]4 ($t_R = 10.5$ min) were collected and processed as above.

2.5. Cell lines and culture conditions

MCF-7 breast adenocarcinoma cells were obtained from the American Type Culture Collection (Manassas, VA) and were routinely cultured in MEM supplemented with fetal bovine serum (10%), penicillin (100 IU/ml) and streptomycin (100 $\mu\text{g}/\text{ml}$). Cells were incubated in a humidified incubator with 5% CO_2 at 37 $^\circ\text{C}$.

2.6. In vitro uptake of [^{125}I]I-Phe and [^{14}C]Phe in breast cancer cell lines

Cells were seeded at a density of 2×10^5 cells/well in 1 ml culture media and incubated overnight at 37 $^\circ\text{C}$. The media was removed and the cells were washed twice with 0.5 ml of PBS. Cells were then incubated with 0.5 ml of Hank's medium containing 100 nCi (0.05 to 0.13 pmol) of [^{125}I]I-Phe in quadruplicate for 5 min and 1 h. At the end of incubation, the cells were washed twice with 0.5 ml PBS and solubilized in 0.5 ml of 0.5N NaOH. The cell lysates were combined with respective distilled water (0.5 ml each) washings and counted in an automated gamma counter (Perkin Elmer Wizard 3 1480) for ^{125}I activity. The assay was repeated using about 100 nCi (0.21 nmol) of [^{14}C]Phe in similar fashion as above except the final solubilized cells were mixed with 14 ml of scintillation cocktail and the activity was counted using a beta counter (LKB Wallac 1214 RACKBETA). Competition assays were performed in the presence of 0–10 mM phenylalanine or iodophenylalanine.

3. Results and Discussion

The system L amino acid transporter (LAT1/CD98hc) is known to be up-regulated in a number of cancer types including breast cancer (Fuchs et al., 2005; Shennan et al., 2008). Being a neutral amino acid, phenylalanine uptake in cancer cells is mediated by LAT1 transport (Boado et al., 2004). Based on this, both ^{18}F -labeled (Kubota et al., 1996) and radioiodinated derivatives of phenylalanine have been developed both as imaging and therapeutic agents. Radioiodinated 4-iodo-L-phenylalanine has been generally evaluated in brain tumor models. In our current study, we developed a new method for the synthesis of 4- ^{125}I -iodo-L-phenylalanine and evaluated the tracer along with ^{14}C -phenylalanine by determining their uptake in MCF-7 breast cancer cells.

The most notable difference in our approach for the synthesis of 4- ^{125}I -iodo-L-phenylalanine from that reported by Samnick's group (Israel et al., 2008) is the utilization of a tin precursor in which both the amino and COOH groups were protected with acid-labile groups—Boc and *tert*-butyl ester, respectively (Figure 1). This precursor (**3**) was synthesized from commercially available *N*-Boc phenylalanine by first converting it to the *tert*-butyl ester (**2**) following a reported procedure (Ousmer M et al., 2006) in 66% yield. The ester **2** was stannylated using Stille reaction to render **3** in 86% yields.

The synthesis of radioiodinated 4-iodo-L-phenylalanine was initially performed in a two-step process. This was done to avoid potential contamination with phenylalanine, a byproduct resulting from the decomposition of excess tin precursor on treatment with acid. It was also hoped that the purified ^{125}I -**2**, after separating it from **3** by HPLC, could be quantitatively converted to the final product thereby avoiding a second HPLC separation. It was possible to generate ^{125}I -**2** from **3** in an average radiochemical yield of $91.6 \pm 2.7\%$ ($n = 5$) in a very short reaction time of 15 seconds. Compound ^{125}I -**2** was purified by HPLC (Figure 2), which separated it from the tin precursor ($t_R = 25.5$ min for ^{125}I -**2** versus 31–32 min for **3**), and after concentrating it from the HPLC fractions, deprotected by treatment with TFA. Contrary to expectations, the deprotection was not complete under these conditions even after 30 min of reaction ($\text{RCY} = 83.7 \pm 1.7\%$; $n = 5$) with a small amount protected product still present. Thus it was necessary to purify the final product by a second HPLC run.

Because the above process was rather lengthy—two HPLC runs and one solid-phase extraction were involved—we evaluated performing the reaction in a single step. Fortunately, the retention time of phenylalanine ($t_R = 3.7$ min) was different from that of 4-iodophenylalanine ($t_R = 10.5$ min) under the HPLC conditions used. In the single step reaction, the tin precursor was subjected to radioiodination under the same set of conditions as above and the resultant radioiodinated intermediate was treated in situ with TFA. The final product was purified by HPLC, and acetonitrile was evaporated from the HPLC fractions. The average radiochemical yield for this single step radio synthesis was $94.8 \pm 3.4\%$ ($n = 5$). The single step synthesis was also performed with ^{131}I (versus ^{125}I used in the above cases) with an average radiochemical yield of $90.0 \pm 3.8\%$ ($n = 3$). However, the difference in the yields that were obtained with ^{131}I was not statistically significant ($p > 0.05$) from that obtained with ^{125}I . Radiochemical purity was greater than 99% and no unlabeled product was present in the final preparation when up to 1.5 mCi of purified ^{125}I -Phe was injected (Figure 3A). Being a no-carrier-added synthesis, the specific activity is expected to be that of the radionuclide. The detection limit for the I-Phe under the HPLC conditions used is 1–2 nmol. Based on this and the fact that no carrier was detected in 1.5 mCi of the product, we estimate a specific activity of greater than 750 Ci/mmol. Overall, the method we present here is more advantageous and convenient than those reported for the synthesis of radiolabeled 4-iodophenylalanine. For example, an average radiochemical yield of $90 \pm 6\%$, compared to $95 \pm 3\%$ that we obtained, has been reported in the latest

publication on its synthesis by Samnick's group (Israel et al., 2008). Their conditions involve 5 min of radioiodination, 15 min at 95 °C for deprotection of Boc group, and 15 min at 100 °C for hydrolysis of methyl ester. We, on the other hand, used 15 seconds for radioiodination and 10 min at 65 °C for the deprotection of both Boc and *tert*-butyl ester groups. It should be pointed out that, in the synthesis of astatinated analogue (Meyer et al., 2010), the ester function in the tin precursor was converted to an acid before subjecting it to radiohalogenation thereby avoiding the hydrolysis step after radiolabeling. However, they have not elaborated on the hydrolysis process and thus it is not clear whether epimerization of the chiral carbon was a possibility. The few attempts that we made to synthesize N-Boc-(4-trialkyltin)phenylalanine by the direct stannylation of **1** were not successful.

To determine the suitability of 4-[¹²⁵I]I-Phe for breast cancer targeting, initially its uptake in MCF-7 cells was determined at two time points and compared it with that of [¹⁴C]phenylalanine. As shown in Figure 4, the uptake at 5 min was similar for both tracers ($18.1 \pm 1.1\%$ vs $17.1 \pm 2.4\%$ for [¹⁴C]Phe and 4-[¹²⁵I]I-Phe, respectively) and the difference was not statistically significant ($p = 0.6$). There was a slight increase in the uptake of [¹⁴C]phenylalanine at 60 min; however, the difference was not statistically significant ($p = 0.4$). On the other hand, the cell-associated radioactivity from 4-[¹²⁵I]I-Phe decreased at 60 min to $13.9 \pm 1.5\%$ ($p = 0.04$) and the difference in uptake between the two tracers at 60 min was statistically significant ($p = 0.02$). Decrease in the uptake of 4-[¹²⁵I]I-Phe in other tumor cells in vitro has been observed by other investigators. For example, its uptake at later time points in both A1207 human glioblastoma and F98 rat glioma cells were significantly less than that seen at the initial time point of 5 min (Israel et al., 2008). On the other hand, in T99 and T3868 human glioblastoma cells, the uptake increased with time reaching a maximum at 30 min and plateaued thereafter (Samnick et al., 2000). It is tempting to speculate that in some cell lines, 4-[¹²⁵I]I-Phe may be susceptible to deiodination and that the decreased cell-associated radioactivity at later time points in certain cases might be due to the washout of the radioiodide. On the other hand, analysis of plasma and urine from glioma patients given 4-[¹²³I]I-Phe intravenously has shown excellent stability of this radiotracer (Samnick et al., 2002). It is also possible that the lower uptake at later time points in some cases might be due to the washout of the tracer mediated by the transporter. Such carrier-mediated efflux of amino acids in MCF-7 cells has been documented (Shennan et al., 2004). With the caveat that a different cell line and a different iodophenylalanine analogue were used, the absolute uptake of 4-[¹²⁵I]I-Phe in MCF-7 cells that we observed in this study was considerably higher than that reported for 2-[¹²⁵I]I-Phe in EF43fgf4 breast cancer cells (14–17% per 0.2 million cells versus ~4% per million cells; see also below) (Kerseman et al., 2006).

MCF-7 cells have been shown to express LAT1 (Shennan et al. 2004; Shennan and Thomson 2008) and phenylalanine has been shown to be a substrate for this transporter (Boado et al., 2004). While 2-iodophenylalanine has been reported to be a substrate for LAT1 (Kerseman et al., 2005), it has been speculated that 4-iodophenylalanine uptake in glioma cells is mediated by system L amino acid transporter (Israel et al., 2008). To investigate whether 4-[¹²⁵I]I-Phe is taken up in MCF-7 cells by LAT1, the uptake of both 4-[¹²⁵I]I-Phe and [¹⁴C]Phe in these cells was determined as a function of the concentration of the unlabeled analogues and the results are presented in Figure 5. The uptake of both tracers after 60 min of incubation in the untreated cells was substantially higher ($55.9 \pm 0.5\%$ and $49.0 \pm 0.7\%$ for [¹⁴C]Phe and [¹²⁵I]I-Phe, respectively) than that we saw in the previous assay (see above). The higher uptake in this assay compared to previous results may be attributed to higher number of cells in each sample for this latter assay (3×10^5 versus 2×10^5). Other factors include differing cell passage number, or biologic variations in cell samples or reagents. Despite these variations, the concordant physiologic behavior of the controls verifies similar uptake properties of [¹⁴C]Phe and 4-[¹²⁵I]I-Phe. With both tracers, the uptake was not significantly affected by both unlabeled compounds up to a concentration

of about 100 :M. However, the uptake was sensitive to competition by both phenylalanine and 4-iodophenylalanine to a similar degree at higher concentrations. With [^{14}C]Phe as the tracer, IC_{50} values 1.45 mM and 2.50 mM were obtained for Phe and I-Phe, respectively and these values for [^{125}I]I-Phe inhibition were 1.3 and 1.0 mM. The uptake of 4-[^{125}I]I-Phe was inhibited to about 31% and 47% of control values by 1 mM phenylalanine and 4-iodophenylalanine, respectively. Samnick's group has shown that the uptake of 4-[^{125}I]I-Phe in glioma cells has been reduced to about 15% control values by the natural amino acid phenylalanine (1mM) (Israel et al., 2008; Samnick et al., 2000). Similarly, the uptake of 2-[^{125}I]I-Phe in EF43fgf4 breast cancer cells was reduced to about 20% of control values by 1 mM concentrations of both phenylalanine and 2-iodophenylalanine. Although the extent of inhibition of uptake of 4-[^{125}I]I-Phe in MCF-7 cells that we observed was somewhat lower than the above values reported in literature, it can be concluded from the above results that 4-[^{125}I]I-Phe is predominantly taken up by MCF-7 cells by LAT1, which mediates the uptake of phenylalanine.

Summary and Conclusions

We have developed a method for the synthesis of radioiodinated 4-iodo-phenylalanine that is more convenient and with higher yields than current literature procedures. This radiolabeled derivative of phenylalanine showed an uptake that was similar to the carbon-14 labeled phenylalanine in MCF-7 breast cancer cells. Furthermore, the uptake of both radiotracers was inhibited by both unlabeled phenylalanine and 4-iodophenylalanine of various concentrations to a similar degree.

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Figure 1.
Scheme for the synthesis of $[^{125}\text{I}]$ iodo-phenylalanine

Figure 2.
HPLC (UV and radioactivity) of reaction mixture from the first step of two-step radiochemical synthesis of 4-[^{*}I]I-Phe.

Figure 3.
HPLC (UV and radioactivity) of (A) purified [^{125}I]I-Phe (1.5 mCi) injected by itself and B) overlaid with the HPLC of a standard of 4-iodo-phenylalanine.

Figure 4. Uptake of [¹⁴C]Phe and 4-[¹²⁵I]I-Phe by MCF-7 cells. MCF-7 breast cancer cells were incubated with 100 nCi [¹⁴C]Phe or 4-[¹²⁵I]I-Phe and percent of input dose of radioactivity that was associated with the cells was measured at 5 and 60 min. Graph shows the mean and standard error of quadruplicate measurements, and is representative of at least 3 independent experiments.

Figure 5.

Competitive inhibition of uptake of [¹⁴C]Phe and 4-[¹²⁵I]I-Phe by Phe and 4-I-Phe. About 100 nCi [¹⁴C]Phe (A) or 4-[¹²⁵I]I-Phe (B) was incubated with MCF-7 cells for 60 min. Unlabeled phenylalanine or 4-iodophenylalanine (0–10000 :M) was used as a competitor as indicated.