JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
*J Label Compd Radiopharm* 2006; **49**: 1117–1124.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.1131

**Research Article**

# Synthesis of 2-[$^{11}$C]methoxy-3,17β-estradiol to measure the pharmacokinetics of an antitumor drug candidate, 2-methoxy-3,17β-estradiol

Jiyoung Mun, Ronald J. Voll and Mark M. Goodman*
*Department of Radiology, Division of Radiological Sciences, Emory University, Atlanta, GA 30322, USA*

## Summary

2-Methoxy-3,17β-estradiol, an endogenous estrogen metabolite, showed cytotoxicity in various cancer cell lines and also has antiangiogenic and proapoptotic activities. Clinical I and II trials of 2-methoxy-3,17β-estradiol for multiple myeloma, advanced solid tumors, metastatic breast and prostate cancer are underway. We prepared 2-[$^{11}$C]methoxy-3,17β-estradiol to measure the pharmacokinetics and organ distribution of 2-methoxy-3,17β-estradiol in clinical trials. 2-[$^{11}$C]Methoxy-3,17β-estradiol was synthesized from a precursor, 2-hydroxy-3,17β-*O*-bis(methoxymethyl)estradiol, in two steps with over 99% radiochemical purity. The overall reaction time was 45 min and the decay-corrected radiochemical yield was 32.9%. The distribution coefficient ($logP_{7.4}$) of 2-[$^{11}$C]methoxy-3,17β-estradiol at pH 7.4 was measured as 2.95. Copyright © 2006 John Wiley & Sons, Ltd.

Received 18 March 2005; Revised 18 September 2005; Accepted 27 October 2005

**Key Words:** 2-methoxy-3,17β-estradiol (2ME2,2ME); positron emission tomography (PET); carbon-11; pharmacokinetics

## Introduction

2-Methoxy-3,17β-estradiol (2-methoxyestradiol, 2ME2, 2ME, Figure 1) is an endogenous estrogen metabolite, which has been reported to show cytotoxicity in various cancer cell lines.[1,2] The colchicine binding site of tubulin is occupied by 2ME2, which results in destabilization of microtubule.[3–5] 2ME2 reduced HIF-1, hypoxia inducible factor, activity

*Correspondence to: Mark M. Goodman, Department of Radiology, Division of Radiological Sciences, Emory University, 1364 Clifton Road, Atlanta, GA30322, USA. E-mail: mgoodma@emory.edu

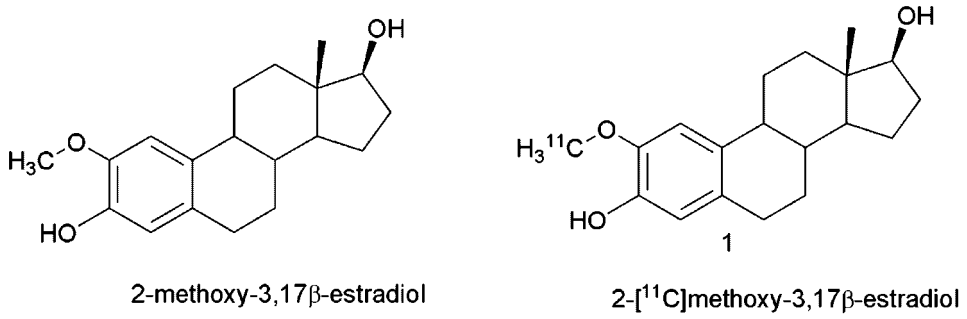



**Figure 1. Structures of 2-methoxy-3,17β-estradiol and 2-[$^{11}$C]methoxy-3,17β-estradiol**

and HIF-1α nuclear accumulation, which is related to antiangiogenic and proapoptotic activity.[6–11] 2ME2 is being evaluated in phase I and II clinical trials for advanced solid tumors, multiple myeloma, prostate and metastatic breast cancer.[12] One hundred and eighty three references were searched by SciFinder relating to anticancer activity of 2ME2 from 2004 to 2006.

The purpose of this paper is to develop a reliable labeling method of 2-methoxyestradiol with carbon-11 for positron emission tomography (PET) study. PET can be used to obtain pharmacokinetics, organ distribution and access to a target organ of a drug candidate in human clinical trials, when a drug candidate can be labeled without change in chemical structure.[13] Generally, blood and plasma pharmacokinetic data can be obtained from conventional methods in human clinical trials, whereas, organ distribution and tumor/tissue pharmacokinetic data are limited to body secretions and biopsy samples. PET of a whole body can provide quantified organ distribution as well as tumor/tissue pharmacokinetic data.[13] A tracer quantity of a drug candidate is used in a PET study (nanomolar to micromolar quantity, depending on a study model) and half life of carbon-11 is only 20.4 min. Thus, chemical toxicity from the drug is within allowable limits.[14] The availability of the drug candidate labeled with a positron emitter itself is usually the limiting factor for a PET study. 2ME2 has an inherent methoxy group, which can be labeled with [$^{11}$C]$CH_3I$ from a phenol precursor, which makes it ideal for PET study.

A synthesis of 2-[$^{11}$C]methoxy-3,17β-estradiol was published by Park *et al.* with 8–11% decay-corrected radiochemical yield during 38 min to 40 min of the total reaction time as a symposium abstract in 1999.[15] We would like to present a modified synthesis to give a better radiochemical yield and to provide a formulation for PET study.



DOI: 10.1002/jlcr

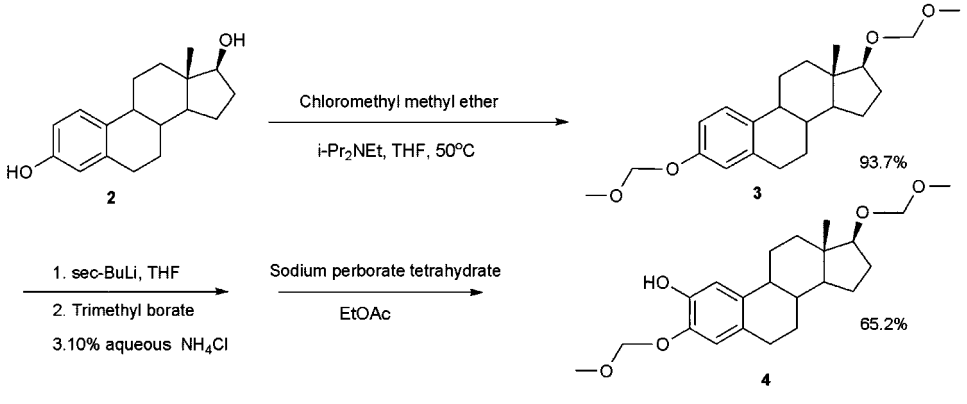
## Results and discussion

### *Chemistry*

2-Hydroxy-3,17β-*O*-bis(methoxymethyl)estradiol was synthesized from 3,17β-estradiol in two steps in 61% yield (Scheme 1). The synthetic method, published by Paaren Herbert *et al.* was slightly modified.[16,17] 3,17β-Estradiol was protected with chloromethyl methyl ether to generate 3,17β-*O*-bis (methoxymethyl)estradiol in 93.7% yield. 2-Hydroxy-3,17β-*O*-bis(methoxymethyl)estradiol was synthesized by lithiation with *sec*-butyllithium at −78°C, subsequent addition of trimethyl borate, followed by oxidation with sodium perborate tetrahydrate at room temperature in 65.2% yield. In the last step, after addition of trimethyl borate, the temperature of the reaction mixture was elevated to 0°C. It was essential to stir the reaction mixture for more than 1 h to get a good yield. Temperature fluctuation between 0°C and room temperature did not affect the yield. Product formation was monitored with TLC ($R_f = 0.1$, eluent 1:6 = EtOAc: Hexane) after taking out small aliquots of the reaction mixture and quenching them with saturated aqueous ammonium chloride solution. Oxidation step was performed with saturated aqueous sodium perborate tetrahydrate solution overnight. NMR spectrometry of 2-hydroxy-3,17β-*O*-bis(methoxymethyl)estradiol was verified by the previous literature values.[16,17] 2-Methoxy-3,17β-estradiol was purchased from Sigma.

### *Radiochemistry*

2-[$^{11}$C]Methoxy-3,17β-estradiol was synthesized from 2-hydroxy-3,17β-*O*-bis (methoxymethyl)estradiol in two steps by modification of the labeling method, published by Park *et al.* (Scheme 2).[15] 2-Hydroxy-3,17β-*O*-bis(methoxymethyl) estradiol in DMF was deprotonated with NaH and then methylated with [$^{11}$C]$CH_3I$ at 80°C for 10 min. The resulting 2-[$^{11}$C]methoxy-3,17β-*O*-bis

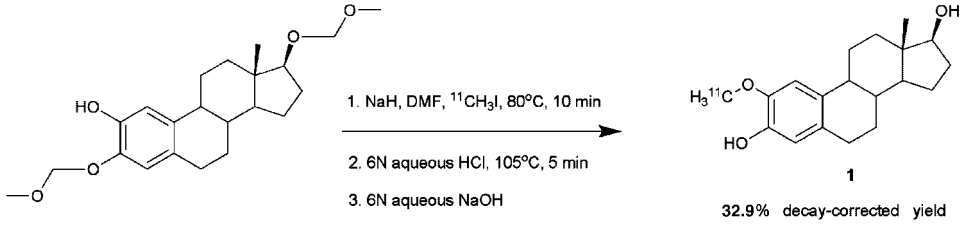



**Scheme 1. Synthesis of 2-hydroxy-3,17β-*O*-bis(methoxymethyl)estradiol**



DOI: 10.1002/jlcr

(methoxymethyl)estradiol was deprotected by 6 N aqueous HCl at 125°C for 5 min. After neutralization with 6 N aqueous NaOH, crude 2-[$^{11}$C] methoxy-3,17β-estradiol, was purified by means of semi-preparative HPLC. 2-[$^{11}$C]Methoxy-3,17β-estradiol was concentrated in 1 ml ethanol *via* a solid phase extraction procedure based upon the method described by Lemaire *et al.* [18] The distribution coefficient ($\log P_{7.4}$) of 2-[$^{11}$C]methoxy-3,17β-estradiol, measured in 1-octanol and 0.02 M phosphate buffer at pH 7.4, was 2.95. 2-[$^{11}$C]Methoxy-3,17$^{11}$-estradiol was formulated to dissolve in 10% ethanolic, 45% aqueous 3-hydoxypropyl γ-cyclodextrin solution. 3-Hydoxypropyl γ-cyclodextrin was used as a delivery vehicle of 2-[$^{11}$C]methoxy-3,17β-estradiol to increase solubility in serum. The overall reaction time was 45 ± 12.5 min ($n = 5$) and the decay-corrected radiochemical yield was 32.9 ± 8.3% ($n = 5$) based on the production of [$^{11}$C]$CH_3I$. The identity of 2-[$^{11}$C]methoxy-3, 17β-estradiol was confirmed by comparing the retention time on an analytical HPLC system with a co-injected authentic sample of 2-methoxy-3, 17β-estradiol. 2-[$^{11}$C]Methoxy-3,17β-estradiol was synthesized with more than 99% radiochemical purity based on the radiometric detection.




**Scheme 2. Synthesis of 2-[$^{11}$C]methoxy-3,17β-estradiol**

## Experimental

### *General*

All chemicals were analytical grade, purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) and used without further purification. 2-Methoxyestradiol was purchased from Sigma. Glassware was flame-dried under argon gas and reactions were performed under argon gas. Two hundred and fifty micrometer layers of F-254 silica gel absorbed on aluminum plates, purchased from Whatman Ltd. (Clifton, NJ, USA), was utilized for thin-layer chromatography (TLC) analyses. Silica gel 60 from EMD was used for column chromatography. $^1$H NMR spectra were recorded on a Varian spectrometer at 400 MHz or 300 MHz and referenced to the NMR solvent (chemical shifts in ppm values, *J*-values in Hz). No-carrier-added [$^{11}$C]$CO_2$ was produced from the bombardment of $^{14}N_2$ gas containing 2% $^{16}O_2$ by a Siemens 11 MeV RDS 112 negative-ion cyclotron at Emory University



DOI: 10.1002/jlcr

Hospital through the $^{14}N[p,\alpha]^{11}C$ reaction. A GE MicroLab methyl iodide system was utilized to convert $[^{11}C]CO_2$ to $[^{11}C]CH_3I$. C-18 SepPaks were purchased from Waters Inc. (Milford, MA, USA).

*Chemistry*

*Synthesis of 3,17β-O-bis(methoxymethyl)estradiol (**3**).* Diisopropylethylamine (14.7 ml, 84.8 mmol) and chloromethyl methyl ether (6 ml, 79.0 mmol) were added to a solution of 3,17β-estradiol (2) (3.82 g, 14.0 mmol) in tetrahydrofuran (24 ml) at room temperature, to form a white suspension. The reaction mixture was stirred between 50°C and 55°C for 24 h to form a yellowish solution with white solid on the wall of the reaction flask. After cooling, 20% aqueous $NH_4Cl$ (10 ml) was added to the reaction mixture, which was extracted with diethyl ether (20 ml × 3). The organic layer was washed with 20% aqueous $NH_4Cl$ (10 ml × 2) and brine (20 ml). Then, the organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by chromatography on silica ($R_f$ = 0.3, eluent 1:5 = EtOAc:Hexane) to give 3,17β-*O*-bis(methoxymethyl)estradiol (3) (4.74 g, 93.7%) as clear highly viscous oil. $^1H$ NMR ($CDCl_3$) δ = 0.82 (s, 1 H); 1.18–2.33 (m, 13 H); 2.84–2.88 (m, 2 H); 3.39 (s, 3 H); 3.48 (s, 3 H); 3.63 (t, 1 H, $J$ = 8); 4.67 (dd, 2 H, $J$ = 6.6, $J$ = 0.95); 5.16 (s, 2 H); 6.78 (d, 1 H, $J$ = 2.8); 6.825 (dd, 1 H, $J$ = 8.5, $J$ = 2.8); 7.22 (d, 1 H, $J$ = 8.5).

*Synthesis of 2-hydroxy-3,17β-O-bis(methoxymethyl)estradiol (**4**).* *sec*-Butyllithium (7.7 ml, 10.8 mmol, 1.4 M in cyclohexane) was added to the solution of 3,17β-*O*-bis(methoxymethyl)estradiol (0.945 g, 2.62 mmol) in tetrahydrofuran (14 ml) at −78°C slowly, so that the temperature of the solution did not exceed −68°C. The reaction mixture was stirred for 1 h at −78°C and trimethylborate (1.19 ml, 10.67 mmol) was added to the reaction mixture at a rate such that the temperature of the solution did not elevate more than 10°C. The reaction mixture was stirred for 30 min at −78°C. The temperature of the reaction mixture was allowed to increase to 0°C and stirred for at least 1 h. Saturated aqueous ammonium chloride (3 ml) was added to the reaction mixture, which was stirred to form a white emulsion. Water (10 ml) and ethyl acetate (20 ml) was added to the reaction mixture, followed by extraction and then the aqueous layer was extracted two more times (10 ml × 2) with ethyl acetate. Saturated aqueous sodium perborate tetrahydrate (50 ml) was added to the combined organic layer (60 ml). The mixture was stirred vigorously overnight and extracted with ethyl acetate (50 ml × 3). The organic layer was washed with brine (70 ml) and dried with $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by chromatography on silica ($R_f$ = 0.3, eluent 1:4 = EtOAc:Hexane) to give 2-hydroxy-3,17β-*O*-bis(methoxymethyl)estradiol (4) (0.643 g, 65.2%) as clear highly viscous oil. $^1H$ NMR ($CDCl_3$) δ = 0.82(s, 3 H);



DOI: 10.1002/jlcr

1.18–2.27 (m, 13 H); 2.76–2.81(m, 2 H); 3.38(s, 3 H); 3.53 (s, 3 H); 3.62 (t, 1 H, $J = 8.5$); 4.67 (dd, 2 H, $J = 6.6$, $J = 0.95$); 5.17 (s, 2 H); 5.75 (bs, 1 H); 6.80 (s, 1 H); 6.90 (s, 1 H).

*Radiochemistry*

*Radiosynthesis of [$^{11}$C]$CH_3I$.* [$^{11}$C]$CH_3I$ was produced from [$^{11}$C]$CO_2$ by a GE MicroLab methyl iodide system. [$^{11}$C]$CO_2$ was reduced to [$^{11}$C]$CH_4$ by $NiH_2$ and then [$^{11}$C]$CH_4$ was iodinated by $I_2$ at 720°C. The preparation time was approximately 15 min.

*Preparation of 2-[$^{11}$C]methoxy-3,17β-estradiol.* 2-Hydroxy-3,17β-*O*-bis(methoxymethyl)estradiol (2.0 mg) was dissolved in anhydrous *N,N*-dimethylformamide (DMF, 200 µl) in a 2 ml septum-sealed v-vial. Slow stream (approximately 30 ml/min) of [$^{11}$C]$CH_3I$ was added to the reaction mixture with a vent at 0°C. The reaction mixture was heated at 80°C for 10 min and cooled down for 1 min in a cold water bath. Aqueous hydrochloric acid (6 N, 100 µl) was added to the reaction vial and then the reaction mixture was heated at 125°C for 5 min. Aqueous sodium hydroxide (6 N, 100 µl) was added to the reaction mixture, after it was cooled down for 1 min in a cold water bath. The reaction mixture was diluted with HPLC eluent (0.5 ml, 50:50:0.1 = $CH_3CN$: water: $NaH_2PO_4$) and injected onto a semi-preparative HPLC column (X-Terra Prep $RP_{18}$, 5 µm, 19 × 100 mm, Waters, Inc.). The HPLC eluate was monitored using a radioactivity probe (SRI Instruments Peak Simple Chromatography Data System Model 202, Torrance, CA, USA; Carrol-Ramsey Model 101-S-DC-P single channel detector with preamplifier and standard probe, Carrol-Ramsey Associates, Berkley, CA, USA) and each 1 min fraction was collected with 9 ml/min elution rate. 2-[$^{11}$C]Methoxy-3,17β-estradiol was eluted in the 8-min fraction.

*Dose formulation of 2-[$^{11}$C]methoxy-3,17β-estradiol.* The fraction, containing 2-[$^{11}$C]methoxy-3,17β-estradiol, was concentrated via a solid phase extraction procedure based upon the method described by Lemaire *et al.* [18] All transfers of liquid were performed using vacuum suction. The fraction of 2-[$^{11}$C]methoxy-3,17β-estradiol (9 ml) was diluted with sterile water (18 ml) and drawn to a C-18 SepPak (pre-activated with 10 ml of ethanol, followed by 10 ml of water), which was then washed with saline (20 ml) and ethanol (0.5 ml). 2-[$^{11}$C]Methoxy-3,17β-estradiol was eluted from the C-18 SepPak with 1 ml of ethanol. The ethanolic solution (1 ml) of 2-[$^{11}$C]methoxy-3,17β-estradiol was diluted with 9 ml of 45% aqueous 3-hydroxypropyl γ-cyclodextrin solution. The reaction time was 45 ± 12.5 min ($n = 5$) and the decay-corrected radiochemical yield was 32.9 ± 8.3% ($n = 5$) based on



DOI: 10.1002/jlcr

[$^{11}$C]$CH_3I$ production. The specific activity was 0.9 Ci/μmol at the end of synthesis.

*Quality control of 2-[$^{11}$C]methoxy-3,17β-estradiol dose*. The identity of 2-[$^{11}$C]methoxy-3,17β-estradiol was examined by comparing retention time with standard 2-methoxy-3,17β-estradiol, purchased from Sigma. 2-[$^{11}$C] Methoxy-3,17β-estradiol and standard 2-methoxy-3,17β-estradiol were co-injected onto analytical HPLC (C18, 5 μm, 3.9 × 150 mm, Waters Inc.). Both UV absorbance (254 nm) and radiometric detection (IN/US Gamma RAM radiometric detector; IN/US Systems Inc., FL, USA) were monitored at the same time. Both of 2-[$^{11}$C]methoxy-3,17β-estradiol and 2-methoxy-3,17β-estradiol were eluted at the same retention time ($R_t$ = 3.5 min, eluent 50:50:0.1 = $CH_3CN$: water: $NaH_2PO_4$, rate = 1 ml/min). The radiochemical purity was determined by the percentage of the peak area of 2-[$^{11}$C]methoxy-3,17β-estradiol with the radiometric detection. 2-[$^{11}$C]Methoxy-3,17β-estradiol in a dose vial showed over 99% radiochemical purity.

*Measurement of distribution coefficient ($logP_{7.4}$)*.[18,19] 1-Octanol (10 ml) and phosphate buffer (5 ml, 0.02 M, pH 7.4) were pre-saturated with each other in a separatory funnel (30 ml) and 2-[$^{11}$C]methoxy-3,17β-estradiol (40 μCi) in ethanol (200 μL) was added to 1-octanol layer. The layers were mixed for 3 min and the bottom layer was discarded. Aliquots (2 ml) of the 1-octanol layer were added to four test tubes containing 2 ml phosphate buffer (0.02 M, pH 7.4). The test tubes were mechanically shaken for 10 min and centrifuged for 5 min at 1000 g. 0.5 ml of the organic layer was transferred to a test tube and the rest of the organic layer was discarded for each test tube. 0.5 ml of the aqueous layer was transferred to a test tube from each test tube. The activity of each layer was measured with a Packard Cobra automated gamma-counter for four samples. The distribution coefficient was calculated by the ratio of activity in 1-octanol layer to aqueous layer. The average distribution coefficient of 2-[$^{11}$C]methoxy-3,17β-estradiol ($\log P_{7.4}$) was 2.95 $\pm$ 0.3 ($n$ = 4).

## Conclusion

2-[$^{11}$C]Methoxy-3,17β-estradiol was synthesized in two steps with 32.9 $\pm$ 8.3% ($n$ = 5) of the decay-corrected radiochemical yield during 45 $\pm$ 12.5 min ($n$ = 5) of the total reaction time, based on [$^{11}$C]$CH_3I$ production. The precursor of the labeling, 2-hydroxy-3, 17β-*O*-bis(methoxymethyl) estradiol, was prepared in two steps from 3,17β-estradiol in 61% of the isolated yield. 2-[$^{11}$C]Methoxy-3,17β-estradiol was formulated in 10% ethanolic, 45% aqueous 3-hydroxypropyl γ-cyclodextrin solution with over 99% radiochemical purity and 0.9 Ci/μmol of specific activity at the end of synthesis.



DOI: 10.1002/jlcr

2-[$^{11}$C]Methoxy-3,17β-estradiol can be used to measure the pharmacokinetics and organ distribution of 2-methoxy-3,17β-estradiol in clinical trials.

**Acknowledgements**

We thank the cyclotron staff at Emory University for their collaboration in this work.

## References

1. Breuer H, Knuppen R. *Naturwissenschaften* 1960; **12**: 280–281.
2. Gelbke HP, Knuppen R. *J Steroid Biochem* 1976; **7**: 457–463.
3. Seegers JC, Aveling ML, van Aswegen CH, Cross M, Koch F, Joubert WS. *J Steroid Biochem* 1989; **32**: 797–809.
4. D'Amato RJ, Lin CM, Flynn E, Folkman J, Hamel E. *Proc Natl Acad Sci USA* 1994; **91**: 3964–3968.
5. Hamel E, Lin CM, Flynn E, D'Amato RJ. *Biochemistry* 1996; **35**: 1304–1310.
6. Fotsis T, Zhang Y, Pepper MS, Adlercreutz H, Montesano R, Nawroth PP, Schweigerer L. *Nature* 1994; **368**: 237–239.
7. Klauber N, Parangi S, Hamel E, Flynn E, D'Amato RJ. *Cancer Res* 1996; **57**: 81–86.
8. Mabjeesh NJ, Escuin D, Lavallee TM, Pribluda VS, Swartz GM, Johnson MS, Willard MT, Zhong H, Simons JW, Giannakakou P. *Cancer Cell* 2003; **3**: 363–375.
9. Beasley NJ, Wykoff CC, Watson PH, Leek R, Turkey H, Gatter K, Pastorek J, Cox GU, Ratcliffe P, Harris AL. *Cancer Res* 2001; **61**: 5262–5267.
10. Aebersold DM, Burri P, Beer KT, Laissue J, Djonov V, Greiner RH, Semenza GL. *Cancer Res* 2001; **61**: 2911–2916.
11. KouKourakis MI, Giatromanolaki A, Sivridis E, Simopoulos C, Turley H, Talks K, Gatter KC, Harris AL. *Int J Radiat Oncol Biol Phys* 2002; **53**: 1192–1202.
12. Ricker JL, Chen Z, Yang XP, Pribluda VS, Swartz GM, Waes CV. *Clin Cancer Res* 2004; **10**: 8665–8673.
13. Fishman AJ, Alpert NM, Rubin RH. *Clin Pharmacokinet* 2002; **41**: 581–602.
14. Bergstrom M, Grahnen A, Langstrom B. *Eur J Clin Pharmacol* 2003; **59**: 357–366.
15. Park BN, Choe YS, Chi DY, Choi Y, Lee KH, Kim BT. *J Label Compd Radiopharm* 1999; **42**: s432–s433.
16. Paaren HE, Duff SR. US Patent 6448419, 2002.
17. Edsall AB, Mohanakrishnan AK, Yang D, Fanwick PE, Hamel E, Hanson AD, Agoston GE, Cushman M. *J Med Chem* 2004; **47**: 5126–5139.
18. Lemaire C, Plenevaux A, Aerts J, Del fiore G, Brihaye C, Le Bars D, Comar D, Luxen A. *J Label Compd Radiopharm* 1999; **42**: 63–75.
19. Wilson AA, Houle JN. *J Label Compd Radiopharm* 1999; **42**: 1277–1288.



DOI: 10.1002/jlcr