JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2003; **46**: 333–342.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.674

Research Article

A new approach for ^{11}C–C bond formation: synthesis of 17α-(3′-[^{11}C]prop-1-yn-1-yl)-3-methoxy-3,17β-estradiol

F. Wüst*, J. Zessin and B. Johannsen
Institut für Bioanorganische und Radiopharmazeutische Chemie, FZ-Rossendorf e.V., Dresden, Germany

Summary

A new approach for ^{11}C–C bond formation via a Sonogashira-like cross-coupling reaction of terminal alkynes with [^{11}C]methyl iodide was exemplified by the synthesis of 17α-(3′-[^{11}C]prop-1-yn-1-yl)-3-methoxy-3,17β-estradiol. The LC-purified title compound was obtained in decay-corrected radiochemical yields of 27–47% ($n = 8$) based on [^{11}C]methyl iodide within 21–27 min after EOB. In a typical synthesis starting from 9.6 GBq [^{11}C]methyl iodide, 1.87 GBq of 17α-(3′-[^{11}C]prop-1-yn-1-yl)-3-methoxy-3,17β-estradiol was synthesized in radiochemical purity > 99%. The specific radioactivity ranged between 10 and 19 GBq/μmol, and the labeling position was verified by ^{13}C-NMR analysis of the corresponding ^{13}C-labeled compound. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: [^{11}C]methyl iodide; ^{11}C–C bond formation; Sonogashira-like cross-coupling; ^{11}C-labeled steroid

Introduction

The progress of positron emission tomography (PET) as a powerful imaging technique in nuclear medicine and drug research and

*Correspondence to: F. Wüst, Institute of Bioinorganic and Radiopharmaceutical Chemistry, FZ-Rossendorf, PF 51 01 19, 01314 Dresden, Germany. E-mail: f.wuest@fz-rossendorf.de.

Received 28 June 2002
Revised 3 September 2002
Accepted 3 October 2002

development is accompanied by an increasing demand for new radiolabeling methods. This task is especially challenging for the short-lived radionuclide ^{11}C ($t_{1/2}$ = 20.33 min). The short half life imposes major constraints on the synthesis time, and methods for the incorporation of this isotope tend to be limited to those based on readily available labeling precursors.

The most frequently employed reactions in ^{11}C chemistry represent *N*-, *S*- and *O*-methylations using the readily available labeling precursor [^{11}C]methyl iodide. [1–4] However, to further expand the number of ^{11}C-labeled compounds especially the development of novel [^{11}C]carbon–carbon bond forming reactions attracts attention. Furthermore, new methods for [^{11}C]carbon–carbon bond-formation also provide the possibility to place the ^{11}C label at different positions of a given molecule.

Several routes for [^{11}C]carbon–carbon bond-forming reactions have been developed and successfully been applied in ^{11}C chemistry.[5] Commonly applied synthetic methods include the carbonation of organometallic reagents with [^{11}C]CO_2,[6,7] the alkylation of stabilized carbanions with ^{11}C-labeled alkyl halides, [7,8] and nucleophilic reactions employing [^{11}C]cyanide, [9] [^{11}C]methyllithium, [10] [^{11}C]nitromethan, [11,12] triphenylphosphonium [^{11}C]methylide[13] or triphenylarsonium [^{11}C]-methylide. [14] However, the aforementioned methods often require difficult synthetic sequences and they are incompatible with many functional groups. In order to overcome these obstacles, novel technically simple, high-yielding and functional group tolerating synthetic methods for [^{11}C]carbon–carbon bond formations are of particular interest.

In recent years, several transition-metal-mediated cross-coupling reactions have been shown to be effective approaches in the development of [^{11}C]carbon–carbon bond formations. In addition to synthetic methods using cuprate- and copper-zinc-mediated coupling reactions, [15,16] several palladium-mediated [^{11}C]carbon–carbon bond-forming reactions have been found to be effective. As a result palladium-mediated aromatic cyanation,[9] Stille[17,18] and Suzuki cross-coupling reactions[19] as well as carbonylative coupling reactions with [^{11}C]CO [20] are frequently used in ^{11}C chemistry.

The copper–palladium catalyzed coupling of terminal alkynes with aromatic and vinylic halides, also known as the Sonogashira reaction, is an other example for an effective and widely used method to form carbon–carbon bonds.[21] To the best of our knowledge, the Sonoga-

shira-reaction has not yet been employed in ^{11}C chemistry. Although the Sonogashira reaction preferentially utilizes organohalogens containing a sp^2 carbon, we want to expand the scope of the reaction to [^{11}C]methyl iodide as readily available labeling precursor.

In this paper we describe a modified Sonogashira-like reaction for the labeling of terminal alkyne groups with [^{11}C]methyl iodide. The reaction was exemplified by labeling the alkynyl group of the potent contraceptive steroid mestranol **3** with [^{11}C]methyl iodide.

Results and discussion

The synthesis of the reference compound **2** was accomplished by the 1,2-addition of 1-propynylmagnesium bromide to estrone 3-methyl ether **1** in 52% yield (Figure 1).

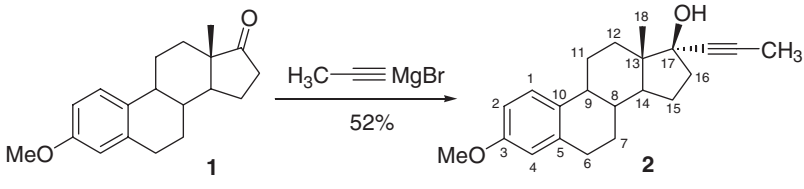

Figure 1. Synthesis of 17α-(3′-prop-1-yn-1-yl)-3-methoxy-3,17β-estradiol 2

The α-selective addition of the Grignard reagent to the 17-keto group in **1** was verified by means of ^{13}C-NMR spectroscopy. Carbon atoms C12 to C18 in compound **2** are affected by the 17α-substitution and the observed shielding(−) and deshielding(+) effects for the carbon atoms C12(−4.9), C13(+3.0), C14(−1.6), C15(−1.0), C16(+8.4), C17(−1.8) and C18(+1.1) in **2** prove the 17α-substitution pattern in comparison with 3,17β-estradiol. [22]

In a series of experiments we investigated several reaction conditions for a sufficient palladium-catalyzed cross-coupling of mestranol **3** with [^{11}C]methyl iodide to form the desired 17α-(3′-[^{11}C]prop-1-yn-1-yl)-3-methoxy-3,17β-estradiol (Figure 2).

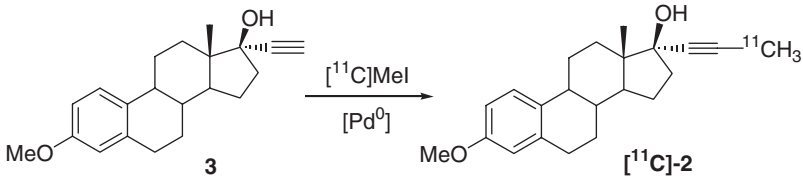

Figure 2. Pd-catalyzed cross-coupling of mestranol 3 with [^{11}C]methyl iodide

The classical conditions of the Sonogashira reaction could not be applied for the cross-coupling with [^{11}C]methyl iodide since it was immediately consumed by the used amine base (e.g. triethylamine). In order to circumvent this problem we made use of 1,8-bis(dimethylamino)-naphthalene as an alternative non-nucleophilic base which is known to withstand alkylation even under harsh reaction conditions. [23] However, only 2–6% of the ^{11}C-labeled cross-coupled product **[^{11}C]-2** could be obtained when the system $Pd[PPh_3]_4$/CuI/1,8-bis(dimethylamino)-naphthalene was subjected to a Sonogashira cross-coupling protocol of alkyne **3** with [^{11}C]methyl iodide. The analysis of the reaction mixture by radio-HPLC revealed only little consumption of [^{11}C]methyl iodide which is indicative of a slow reaction rate under these conditions. The influence of solvent and reaction temperature on the radiochemical yield is summarized in Table 1.

Table 1. Cross-coupling of 3 with [^{11}C]MeI using $Pd[PPh_3]_4$/CuI/1,8-bis (dimethylamino)-naphthalene system

Catalyst / base system	Solvent	Reaction time / temperature	Radiochemical yield[a]
$Pd[PPh_3]_4$ / CuI /1,8-bis(dimethylamino)-naphthalene	CH_3CN	10 min / 60°C	2% ($n = 2$)
$Pd[PPh_3]_4$ / CuI /1,8-bis(dimethylamino)-naphthalene	CH_3CN	10 min / 80°C	4–6% ($n = 3$)
$Pd[PPh_3]_4$ / CuI /1,8-bis(dimethylamino)-naphthalene	THF	10 min / 80°C	2% ($n = 2$)

[a] Determined by radio-HPLC.

Recently a modified procedure for the palladium-catalyzed cross-coupling of terminal acetylens with organohalide electrophiles was reported. [24] In this report several aryl and alkenyl halides were successfully cross-coupled in good to excellent yields with terminal alkynes in the presence of silver(I) oxide, tetrabutylammonium fluoride (TBAF) or tetrabutylammonium hydroxide (TBAOH) as activators. Intrigued by this finding we set up several palladium-catalyzed cross-coupling attempts with [^{11}C]methyl iodide as the coupling partner. In a first series of reactions, $Pd[PPh_3]_4$ was used as catalyst, and silver(I) oxide or TBAF as the activator. The results are summarized in Table 2.

The desired cross-coupled product **[^{11}C]-2** was formed in very low yields of 0.4–4% as shown by radio-HPLC analysis of the reaction

Table 2. Pd-mediated cross-coupling using $Pd[PPh_3]_4/Ag_2O$ or $Pd[PPh_3]_4$/TBAF

Catalyst / activator	Solvent	Reaction time / temperature	Radiochemical yield[a]
$Pd[PPh_3]_4$ / Ag_2O	THF	10 min / 60°C	0.4% ($n = 2$)
$Pd[PPh_3]_4$ / Ag_2O	THF	10 min / 80°C	4% ($n = 2$)
$Pd[PPh_3]_4$ / TBAF	THF	10 min / 80°C	0.4% ($n = 2$)

[a] Determined by radio-HPLC.

mixture. Again, only a low consumption of [^{11}C]methyl iodide was observed which is indicative of a slow reaction rate. However, an increase of the reaction temperature from 60 to 80°C resulted in a higher yield of 4% vs 0.4% of the cross-coupled product **[^{11}C]-2**. This finding prompted us to use a more reactive Pd^0/co-ligand system which should accelerate the reaction rate significantly. It is known that $AsPh_3$ leads to a large rate enhancement in the Stille cross-coupling reaction. [25] In the catalytic cycle of the Stille reaction $AsPh_3$ dissociates more readily from the intermediate Pd(II) species compared to PPh_3 which results in faster coupling rates. Thus, we set up a reaction protocol using $Pd_2(dba)_3$ as the Pd^0-source and $AsPh_3$ as a more readily dissociating co-ligand. In this context we studied the influence of the reaction time and reaction temperature on the yield of the **[^{11}C]-2**. The results are summarized in Table 3.

Table 3. Cross-coupling of 3 with [^{11}C]MeI using $Pd_2(dba)_3/AsPh_3$/TBAF system

Catalyst / co-ligand / Activator	Solvent	Reaction time / temperature	Radiochemical yield[a]
$Pd_2(dba)_3/AsPh_3$/ TBAF	THF	3 min / 40°C	15–33% ($n = 3$)
$Pd_2(dba)_3/AsPh_3$/ TBAF	THF	3 min / 60°C	49–64% ($n = 8$)
$Pd_2(dba)_3/AsPh_3$/ TBAF	THF	5 min / 80°C	14–26% ($n = 3$)
$Pd_2(dba)_3/AsPh_3$/ TBAF	THF	15 min / 60°C	42% ($n = 1$)

[a] Determined by radio-HPLC.

The results clearly show the beneficial effect of the more reactive catalyst/co-ligand system on the reaction rate and therefore on the yield of the cross-coupled product. The reaction was performed in THF and TBAF was used as the base. Without TBAF as an activator the reaction failed completely. In a first run at 40°C, 15–33% of the initially used [^{11}C]methyl iodide was converted into the desired product **[^{11}C]-2** after 3 min. An increase of the reaction temperature to 80°C and an extension

of the reaction time to 5 min gave no improved yield (14–26%) of the desired cross-coupled product.

However, the reaction at 60°C for 3 min yielded 49–64% of steroid **[^{11}C]-2** as shown by the HPLC analysis of the reaction mixture. An extension of the reaction time to 15 min did not improve the yield. After HPLC purification of steroid **[^{11}C]-2** the decay-corrected radiochemical yield was 27–47% ($n = 8$) based on [^{11}C]methyl iodide. Thus, in a typical synthesis starting from 9.6 GBq [^{11}C]methyl iodide, 1.87 GBq of 17α-(3′-[^{11}C]prop-1-yn-1-yl)-3-methoxy-3,17β-estradiol **[^{11}C]-2** could be synthesized within 21–27 min after EOB in radiochemical purity > 99%. The specific radioactivity ranged between 10 and 19 GBq/μmol. It is noteworthy to mention that for a successful reaction a THF-solution of steroid precursor **3** and TBAF has to be added to a preformed solution (40°–60°C for 2 min) of Pd-catalyst-$AsPh_3$-[^{11}C]methyl iodide in THF.

^{13}C-NMR spectroscopy was used for assessing the position of the label. This was done by employing the similiar reaction conditions ($Pd_2(dba)_3$/$AsPh_3$/TBAF) for the labeling of mestranol **3** with (^{13}C)methyl iodide (5 μl) instead of [^{11}C]methyl iodide and subsequent ^{13}C-NMR analysis of the reaction mixture. Figure 3 shows the

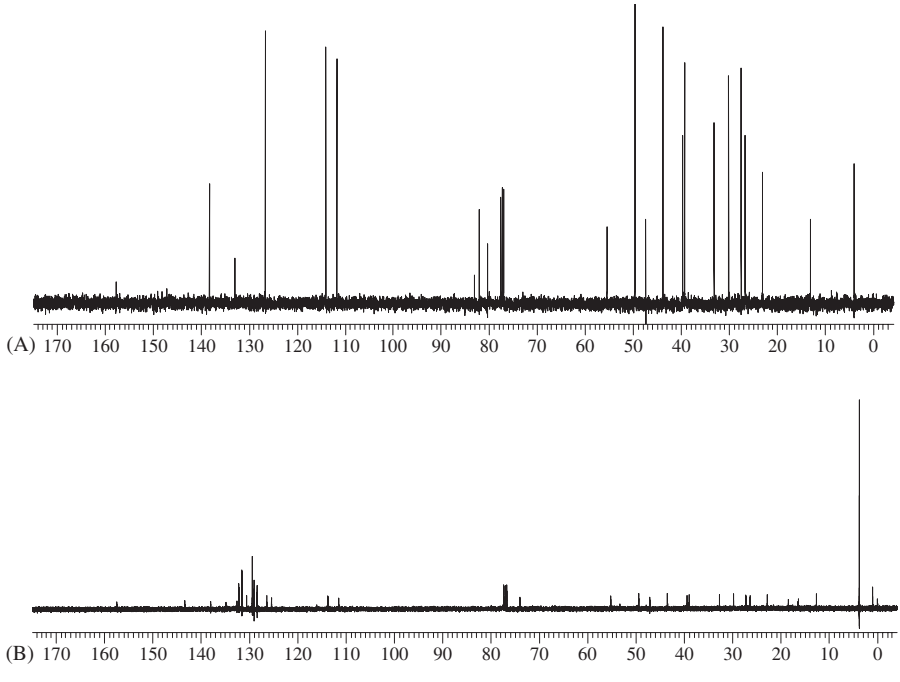

Figure 3. ^{13}C-NMR spectra of steroid 2 (A) and ^{13}C-labeled compound (B)

^{13}C-NMR spectra of reference compound **2** and the corresponding ^{13}C-substituted compound.

The ^{13}C signal at 3.74 ppm of the ^{13}C-substituted compound corresponds with the chemical shift of the methyl group of the 17α-propynyl group in the authentic sample of compound **2**.

In summary, we have developed a novel approach for a [^{11}C]carbon–carbon bond formation via a modified Sonogashira cross-coupling procedure of terminal alkynes with [^{11}C]methyl iodide. The reaction was exemplified by the synthesis of 17α-(3′-[^{11}C]prop-1-yn-1-yl)-3-methoxy-3,17β-estradiol and it allows the convenient coupling of readily available [^{11}C]methyl iodide with terminal alkynes. The reaction proceeds in sufficient radiochemical yields and in short reaction times. The compatibility to functional groups (e.g. OH) makes this procedure a valuable tool for the further expansion of the arsenal of ^{11}C labeled compounds.

Experimental

General

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian Inova-400 at 400 and 100 MHz, respectively. Chemical shifts (δ) are determined relative to the solvent and converted to the TMS scale. Elemental analysis were obtained on a LECO CHNS 932 elemental analyzer. Flash chromatography was conducted according to Still *et al.* [26] Analytical HPLC was conducted using LaChrom systems from Merck-Hitachi.

Thin-layer chromatography (TLC) was performed on Merck silica gel F-254 plastic plates, with visualization under UV (254 nm). Estrone 3-methyl ether was purchased from Sigma, 1-propynylmagnesium bromide (0.5 M in THF) from Aldrich.

Chemical synthesis

17α-(prop-1-yn-1-yl)-3,17β-estradiol-3 methyl ether **2**: To a solution of 1-propynylmagnesium bromide (7 ml, 3.9 mmol) was added estrone 3-methyl ether (110 mg, 0.39 mmol) in small portions. The resulting solution was stirred at room temperature over night under a nitrogen atmosphere. After the addition of saturated NH_4Cl solution (50 ml), extraction with CH_2Cl_2 and drying (Na_2SO_4) the solvent was

evaporated under reduced pressure. The residue was re-dissolved in MeOH (10 ml) and $NaBH_4$ (5 mg) was added to reduce remaining estrone 3-methyl ether. After stirring for 2 h at room temperature the mixture was poured into water (50 ml) followed by extraction (CH_2Cl_2), drying (Na_2SO_4) and evaporation of the solvent. Subsequent purification of the residue by flash chromatography (20% ethyl acetate-hexane) afforded 65 mg (52%) of **2** as a white solid. Melting point 190–191°C. ^{1}H-NMR ($CDCl_3$): δ 0.86 (s, 3 H, 18-CH_3), 1.89 (s, 3 H, C≡C*CH*$_3$), 2.83–2.85 (m, 2 H, 6α/6β-H), 3.78 (s, 3 H, 3-O*CH*$_3$), 6.64 (d, 1 H, J = 2.5 Hz, 4-H), 6.72 (dd, 1 H, J = 8.3 Hz, 2.5 Hz, 2-H), 7.22 (d, 1 H, J = 8.3 Hz, 1-H). ^{13}C-NMR ($CDCl_3$): δ 157.34(3), 137.97(5), 132.64(10), 126.33(1), 113.72(2), 111.42(4), 82.76(-C≡C-CH_3), 81.76(-C≡C-CH_3), 80.06(17), 55.16(3-OCH_3), 49.35(14), 47.12(13), 43.53(9), 39.43(8), 39.00(16), 32.86(12), 29.84(6), 27.22(7), 26.42(11), 22.79(15), 12.77(18), 3.73(-C≡C-CH_3).

Radiochemical syntheses

[^{11}C]CO_2 was produced by the ^{14}N(p,α)^{11}C reaction on a IBA CYCLONE 18/9 cyclotron. [^{11}C]CO_2 was trapped in a loop containing Carbosphere molecular sieve at room temperature and released under a stream of nitrogen at 110°C. [^{11}C]Methyl iodide was prepared according to Crouzel *et al.* [27] in a home built remotely controlled module. [^{11}C]Methyl iodide was transferred in a stream of nitrogen into the reaction vessel at room temperature. After completion of the transfer, the reaction vessel was heated and aliquots were taken for radio-HPLC analysis after the indicated time. The chemical and radiochemical purity was determined with a Purosphere PR-18 column (Merck, 125 mm × 3.0 mm, 5 µm). The mobile phase was water/acetonitrile (50/50) containing 0.1 M ammonium formate (A) and acetonitrile (B). The gradient was the following: HPLC time 0–8 min, (A/B) 100/0–30/70, 8–12 min (A/B) 30/70 isocratic, 12–13 min (A/B) 30/70–100/0, 13–18 min isocratic (A/B) 100/0. t_R **[^{11}C]-2** = 11.5 min.

Semi-preparative HPLC was performed with a Kromasil RP-18 column (300 × 8 mm, 7 µm) isocratically eluted with water/acetonitrile (30/70) containing 0.1 M ammonium formate at a flow rate of 4.5 ml. t_R **[^{11}C]-2** = 11–12 min.The fractions containing the product were collected and diluted with 30 ml of water. The mixture was passed through a Chromafix RP18 cartridge (200 mg, pre-conditioned with 5 ml ethanol

and 10 ml water). The cartridge was washed with water (5 ml) and the product was eluted with ethanol (1 ml).

Cross-coupling with $Pd[PPh_3]_4$/CuI/1,8-bis(dimethylamino)naphthalene

$Pd[PPh_3]_4$ (1 mg (0.86 μmol), CuI (1 mg, 5.2 μmol), 1,8-bis(dimethylamino)-naphthalene (3 mg, 14 μmol) and mestranol **3** (3 mg, 9.7 μmol) were dissolved in 500 μl acetonitrile or THF. After [^{11}C]methyl iodide transfer and subsequent heating (10 min at 60°C or 80°C), aliquots were taken for radio-HPLC analysis.

Cross-coupling with $Pd[PPh_3]_4/Ag_2O$ or TBAF

$Pd[PPh_3]_4$ (1 mg, 0.86 μmol), Ag_2O (1.5 mg, 6.5 μmol) or TBAF(50 μl, 50 μmol, 1 M in THF) and mestranol **3** (3 mg, 9.7 μmol) were dissolved in 500 μl THF. After [^{11}C]methyl iodide transfer, the reaction vessel was heated at 60°C or 80°C for 10 min and aliquots were taken for radio-HPLC analysis.

Cross-coupling with $Pd_2(dba)_3/AsPh_3$/TBAF

1 mg (1.1 μmol) $Pd_2(dba)_3$ and 1.5 mg (4.9 μmol) $AsPh_3$ were dissolved in 300 μl THF and [^{11}C]methyl iodide was transferred into this solution. After completion of the transfer, the reaction vessel was heated for 2–3 min at 60°C. Then TBAF (5 μl, 5 μmol, 1 M in THF) and mestranol **3** (3 mg, 9.7 μmol) in 200 μl of THF were added. The temperature was adjusted at 40°–80°C and at selected time points (3, 5 min or 15 min) aliquots were taken for radio-HPLC analysis.

For the preparative isolation of **[^{11}C]-2**, 2 ml of HPLC eluent (water/acetonitrile (30/70) was added before semi-preparative HPLC purification.

References

1. Halldin C, Stone-Elander S, Thorell JO, Persson A, Sedvall G. *Int J Appl Radiat Isot* 1988; **39**: 993–997.
2. Pike VW, Halldin C, Crouzel C, *et al*. *Nucl Med Biol* 1993; **20**: 503–525.
3. Mazière B, Coenen HH, Halldin C, Någren K, Pike VW. *Int J Radiat Appl Instrum B* 1992; **19**: 497–512.

4. Zessin Z, Gucker P, Ametamey SM, *et al*. *J Label Compd Radiopharm* 1999; **42**: 1301–1312.
5. Långström B, Kihlberg T, Bergström M, *et al*. *Acta Chim Scand* 1999; **53**: 651–669.
6. Winstead MB, Winchell HS, Fawwaz R. *Int J Appl Radiat Isot* 1969; **20**: 859–863.
7. Kilbourn M, Dischino D, Welch MJ. *Int J Appl Radiat Isot* 1984; **35**: 603–605.
8. Antoni G, Långström B. *J Label Compd Radiopharm* 1987; **24**: 125–143.
9. Andersson Y, Bergström M, Långström B. *Appl Radiat Isot* 1994; **45**: 707–714.
10. Dence CS, Napolitano E, Katzenellenbogen JA, Welch MJ. *Nucl Med Biol* 1996; **23**: 491–496.
11. Mäding P, Steinbach J, Johannsen B. *J Label Compd Radiopharm* 2000; **43**: 565–583.
12. Mäding P, Steinbach J, Johannsen B. *J Label Compd Radiopharm* 1997; **39**: 585–599.
13. Kihlberg T, Gullberg P, Långström B. *J Label Compd Radiopharm* 1990; **28**: 1115–1120.
14. Zessin J, Steinbach J, Johannsen B. *J Label Compd Radiopharm* 1999; **42**: 725–736.
15. Neu H, Bonasera TA, Långström B. *J Label Compd Radiopharm* 1997; **41**: 227–234.
16. Wüst F, Dence CS, McCarthy TJ, Welch MJ. *J Label Compd Radiopharm* 2000; **43**: 1289–1300.
17. Björkman M, Doi H, Resul B, Suzuki M, Noyori R, Watanabe Y, Långström B. *J Label Compd Radiopharm* 2000; **43**: 1327–1334.
18. Karimi F, Långström B. *J Label Compd Radiopharm* 2002; **45**: 423–434.
19. Hostetler ED, Fallis S, McCarthy TJ, Welch MJ, Katzenellenbogen JA. *J Org Chem* 1998; **63**: 1348–1351.
20. Kihlberg T, Långström B. *J Org Chem* 1999; **64**: 9201–9205.
21. Sonogashira K, Tohda Y, Hagihara N. *Tetrahedron Lett* 1975; 4467–4470.
22. Dionne P, Poirier D. *Steroids* 1995; **60**: 830–836.
23. Quast H, Risler W, Döllscher G. *Synthesis* 1972; **10**: 558–563.
24. Mori A, Kawashima J, Shimada T, Suguro M, Hirabayashi K, Nishihara Y. *Organic Lett* 2000; **2**: 2935–2937.
25. Farina V, Krishnan B. *J Am Chem Soc* 1991; **113**: 9585–9595.
26. Still WC, Kahn M, Mitra A. *J Org Chem* 1978; **43**: 2923–2925.
27. Crouzel C, Långström B, Pike VW, Coenen HH. *Appl Radiat Isot* 1987; **38**: 601–603.

