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DIRECT INCORPORATION OF [^{11}C]CARBON DIOXIDE FOR LABELING BIOACTIVE MOLECULES. AN APPLICATION TO [^{11}C] LABELED TAMOXIFEN+

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SUMMARY

A one-pot synthesis of [^{11}C] labeled tamoxifen has been developed via reductive carboxylation. In this approach, [^{11}C]CO_2 is reacted with the N-trimethylsilyl derivative of desmethyltamoxifen, followed by *in situ* sodium bis (2-methoxyethoxy)aluminum hydride reduction, to afford impure [^{11}C] labeled tamoxifen, which, on purification over a basic alumina-silica gel column, provided pure [^{11}C] tamoxifen in excellent radiochemical yield (65% to 84%) and radiochemical purity (>99%). The specific activity of [^{11}C]tamoxifen was 250-400 Ci/mmol at the end of bombardment.

Key Words: tamoxifen, breast carcinoma, carbon-11 labeling

+A preliminary report of this work was presented at the Society of Nuclear Medicine Meeting, June 14-17, 1988, San Francisco, U.S.A. See Ram S., Coleman R.E. and Spicer L.D., J. Nucl Med. 29, 1325 (1988).

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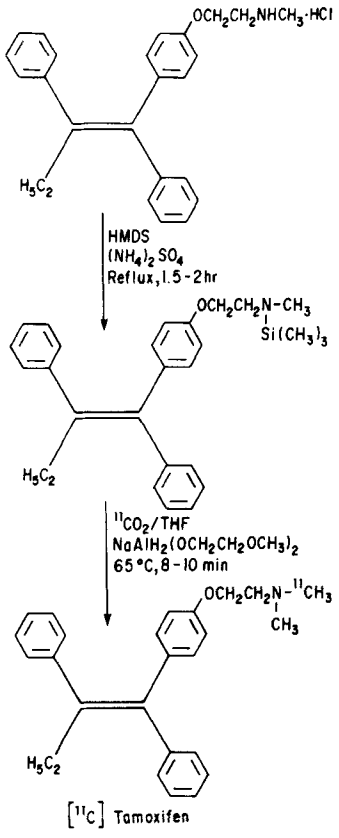
INTRODUCTION

It is well established that estrogens play an important role in the etiology and development of breast carcinoma (1), which is the most prevalent cancer among adult women. The early diagnosis and treatment of this disease will have significant impact in improving the quality of life and/or decreasing the mortality rate in a substantial number of women. Tamoxifen, 1-[4-(2-dimethylaminoethoxy)phenyl]-1,2-diphenyl-1-butene is a non-steroidal, antiestrogenic drug used widely in the treatment of human breast carcinomas. This prototype drug possesses high binding affinity for antiestrogen binding sites (Kd=1nM) but a low binding affinity for estrogen receptors (Kd=80nM) (2). P.E.T. studies with [^{11}C] labeled tamoxifen may be valuable for assessment of estrogen receptors in breast cancer patients.

In our efforts to develop fast and efficient synthetic methods for [^{11}C] labeled radiopharmaceuticals using [^{11}C]CO_2 as a radiolabeled precursor in the presence of a wide variety of labile functionalities such as halogens (Cl, Br), -C=C-, -C≡C-, C=N, OH, etc., we have investigated direct fixation of [^{11}C]CO_2 (3-4) in the synthesis of tamoxifen. The general method we use involves moderate or mild reaction conditions and is easily adaptable for on-line synthesis of [^{11}C] labeled radiopharmaceuticals in high radiochemical yield and specific activity. An alternate method using [^{11}C] methyl iodide has previously been reported (5-7). In our approach to this specific radiopharmaceutical, reaction of [^{11}C]CO_2 with the N-TMS derivative of desmethyltamoxifen at room temperature is used to give the trimethylsilyl-carbamate derivative, which, following <u>in situ</u> reduction with a 35% solution of sodium bis(2-methoxyethoxy)aluminum hydride in toluene at 65°C for 8-10 min, generates no carrier added [^{11}C] labeled tamoxifen without affecting the carbon-carbon double bond. The overall reaction is seen in Scheme 1.



Scheme 1

RESULTS AND DISCUSSION

The radiochemical yields and purity data for labeled tamoxifen prepared on three (N=3) separate occasions using the method described are given in Table 1. It is clear this new reductive carboxylation approach produces good radiochemical yields (65-84%) without particular optimization of reaction conditions and high purity (>99%) [^{11}C] tamoxifen. The time required for the manual synthesis and purification is 36-50 min from the end of bombardment. While this is certainly acceptable, the time for synthesis can be shortened by automation of the reaction, and we are currently developing such a procedure. The final product, [^{11}C] tamoxifen, was characterized on the basis of its chromatographic behavior (HPLC, TLC, GC) by comparing it with samples prepared

TABLE 1. Summary of results for [^{11}C] labeled tamoxifen prepared from [^{11}C] carbon dioxide

Exp.No.	Precursor [^{11}C]CO_2 Activity Trapped[a]	Radiochemical Yield[b] (%)	Radiochemical Purity (%)	Reaction Time (in min)
1	56.8 mCi	65	>99	50
2	58.0 mCi	74	99	43
3	30.2 mCi	84	>99	36

[a]Directly from the cyclotron target.
[b]Radiochemical yields are corrected to EOB.

by reductive carboxylation of desmethyltamoxifen using cold [^{12}C]CO_2 and also with an authentic sample of tamoxifen. On HPLC (Econosphere, 10 μm silica gel column, size 4.6 x 250 mm), tamoxifen and desmethyltamoxifen had elution times of 4.19 min and 7.69 min, respectively, using $CH_3CO_2C_2H_5$:CH_3OH:28% NH_4OH (9:0.9:0.1;V/V) as the mobile phase with a flow rate of 1 ml/min. Separated components were monitored by U. V. absorption at 254 nm. The radioHPLC of [^{11}C] labeled tamoxifen showed only one radioactivity peak at 4.2 min as shown in Figure 1.

The R_f values for cold tamoxifen and desmethyltamoxifen were 0.63 and 0.38, respectively, [silica gel, E. Merck plates, $CH_3CO_2C_2H_5$:CH_3OH:28%NH_4OH (9:0.9:0.1)]. The R_f value of synthesized tamoxifen was the same as with authentic compound and spectral data such as ^{1}H-NMR [^{1}H-NMR ($CDCl_3$), δ (ppm) 0.93 (t, 3H, CH_3), 2.26 [s, 6H, $N(CH_3)_2$], 2.43 (q, 2H, C-CH_2), 2.63 (t, 2H, CH_2-N), 3.90 (t, 2H, OCH_2), 6.5-7.4 (m, 14-H, Ar-H)] were also identical to an authentic sample of tamoxifen. The radiochemical purity of [^{11}C] labeled tamoxifen was determined by HPLC. The specific activity of [^{11}C]tamoxifen was found to be 250-400 Ci/mmol [at the end of bombardment]. This specific activity represents neither maximum [^{11}C]CO_2 production nor optimized synthesis, both of which will determine the attainable specific activity.

This reductive carboxylation approach readily produces no carrier added, high specific activity and high purity [^{11}C] tamoxifen in excellent radiochemical

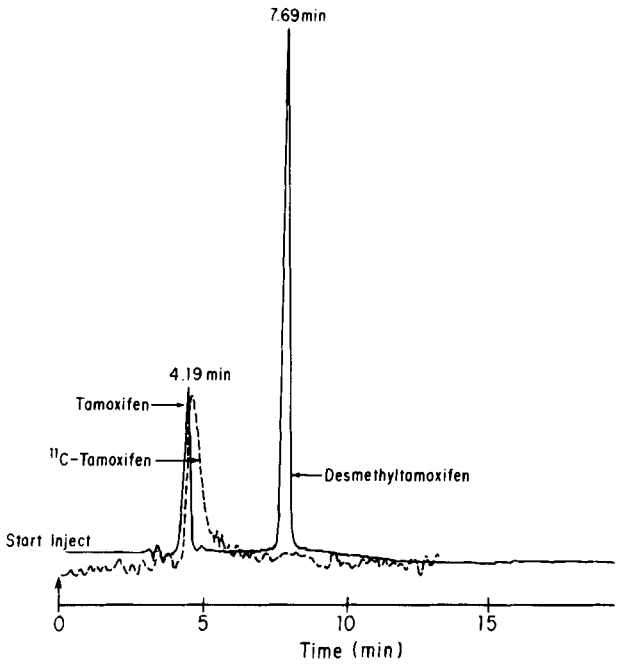


Figure 1: Chromatogram for the preparative separation of [^{11}C] labeled tamoxifen and desmethyltamoxifen. Chromatographic conditions are described in the text. _____ UV absorption curve 254 nm, ... radioactivity curve.

yield from [^{11}C]CO_2, while requiring minimum manipulation. The results obtained demonstrate the unique, direct application of the method in the preparation of [^{11}C] labeled radiopharmaceuticals in the presence of a labile moiety, the carbon-carbon double bond. Reductive carboxylation is a general method for synthesis of a wide variety of [^{11}C] radiopharmaceuticals with labile functionalities (8) and is adaptable for automation. It might be noted also that this new one-pot synthesis does not require HPLC separation, a feature which is advantageous in the synthesis of short-lived isotopes.

EXPERIMENTAL

General

All chemicals were of research grade and were used as obtained from the commercial suppliers. ^{1}H-NMR spectra were obtained using General Electric GN-500 MHz and GN-300 MHz spectrometers. The chemical shift values are

reported in parts per million on the δ scale using TMS as an internal reference. High pressure liquid chromatography and thin layer chromatography were performed on an Econosphere silica gel column and E. Merck silica gel plates, respectively, under conditions described in the preceding section. The starting materials desmethyltamoxifen and tamoxifen were generous gifts from Dr. G. F. Costello, ICI Pharmaceuticals Group, England. $[^{11}C]CO_2$ was produced by the $^{14}N(p,\alpha)^{11}C$ reaction on a nitrogen gas target using the Duke University Medical Center CS-30 cyclotron (Computer Technology and Imaging, Inc.) and was used directly after passing the gas over a Cu/CuO trap, but without further processing (9).

Synthetic Procedures

Preparation of N-trimethylsilyl desmethyltamoxifen (10,11).

A mixture of desmethyltamoxifen hydrochloride salt (4.5 mg, 12.13 umole) and ammonium sulfate (1.5 mg) (a catalytic amount) in HMDS (1.8 ml) was stirred at reflux temperature for 1.5-2.0 hr under an argon atmosphere. The excess of HMDS was removed under reduced pressure on a steam bath using a rotary evaporator. The resulting residue was dissolved in dry THF (0.3 ml) and used as such for the next step. Formation of the N-TMS derivative was confirmed by 500 MHz ^{1}H-NMR in $CDCl_3$ solvent; 0.16 [s, 9H, $Si(CH_3)_3$], 0.93 (t, 3H, $C-CH_3$), 2.42 to 2.50 (m, 5H, $C=C-CH_2$,$N-CH_3$), 2.90 (t, 2H, $-N-CH_2$), 3.92 (t, 2H, $-OCH_2-C$), 6.45 to 7.38 (m, 14H, Ar-H).

Radioalkylation of N-TMS-desmethyltamoxifen with $[^{11}C]$ carbon dioxide.

$[^{11}C]$Carbon dioxide produced by the ^{14}N (p,α) ^{11}C nuclear reaction was bubbled into a 10 ml two-neck, conical-shaped reaction flask fitted with a rubber septum which contained the N-TMS derivative of desmethyltamoxifen in dry THF (12.13 umole, 0.3 ml) at room temperature. After trapping the $[^{11}C]$ carbon dioxide, a 35% solution of sodium bis(2-methoxyethoxy)aluminum hydride in toluene (0.25 ml) was added, and the reaction mixture was heated to 65°C for 8-10 min. Upon cooling, the excess reducing agent was decomposed with saturated $NaHCO_3$ solution (0.3 ml). The product was diluted with ethyl

acetate (1.5 ml x 2) and the organic layer was withdrawn with a syringe. The residue was again washed with an ethyl acetate:methanol:28% NH_4OH (9:0.9:0.1) mixture (2 ml x 2). The combined organic mixture (7 ml) was passed without added solvent through a column (size 1 cm x 15 cm) which contained, in series, 4 gm of Na_2SO_4, 1.5 gm of basic alumina, and 1.5 gm of silica gel. Evaporation of the organic layer on a rotary evaporator afforded pure [^{11}C] tamoxifen which contained trace amounts of unreacted starting material. The purity of [^{11}C] labeled tamoxifen was checked by TLC and radioHPLC.

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