Appl. Radiat. Isot. Vol. 37, No. 5, pp. 391–395, 1986
Int. J. Radiat. Appl. Instrum. Part A
Printed in Great Britain

0883-2889/86 $3.00 + 0.00
Pergamon Journals Ltd

Rapid Reductive-Carboxylation of Secondary Amines, One Pot Synthesis of N′-(4-^{11}C-Methyl)Imipramine

SIYA RAM, RICHARD E. EHRENKAUFER and DOUGLAS M. JEWETT

Cyclotron/PET Facility, Division of Nuclear Medicine, The University of Michigan Medical Center, Ann Arbor, MI 48109, U.S.A.

(*Received* 3 *September* 1985; *in revised form* 20 *November* 1985)

A new rapid high yield synthesis of radiolabeled N′-(4-^{11}C-methyl)imipramine has been developed using a reductive-carboxylation approach, in which $^{11}CO_2$ is reacted with either N′-trimethylsilyldesimipramine or N′-lithium derivative of desimipramine, followed by lithium aluminum hydride reduction, to give *no carrier added* or *carrier added* ^{11}C-labeled imipramine respectively. The final product is characterized by chromatographic and spectroscopic methods.

Introduction

Radioalkylation of secondary amines by direct fixation of [^{11}C]carbon dioxide under moderate reaction conditions is an attractive alternative to the most commonly used alkylating agents such as [^{11}C]methyl iodide ($^{11}CH_3I$) and [^{11}C]formaldehyde ($H^{11}CHO$), which are, in principle, themselves derived from [^{11}C]carbon dioxide. For example, recently, two independent syntheses of N′-(4-^{11}C-methyl)imipramine based on the reductive methylation[1–4] and methylation[5] of desimipramine using $H^{11}CHO/NaBH_4$ and $^{11}CH_3I$ respectively had been reported in the literature. Recently direct fixation of [^{14}C]carbon dioxide into amines has been reported by Pichat *et al.*[6] In our ongoing program, we were interested in direct fixation of $^{11}CO_2$ into various organic molecules, which are potentially of biological interest such as palmitic acid, α-amino acids, etc. for positron emission tomography studies. We therefore developed a new reductive-carboxylation approach, in which secondary amines were carboxylated with [^{11}C]carbon dioxide to their carbamate esters. *In situ* lithium aluminium hydride reduction[7] of these esters, provided N-[^{11}C]methyl derivatives in very good yield.[8] Using this approach, we synthesized a potent antidepressant, N′-(4-^{11}C-methyl)imipramine from desimipramine, which is described in this paper. The procedure is very simple and easily adaptable to automation of the synthesis for human studies.

Experimental

Materials and methods

The starting material, N′-(4-trimethylsilyl)desimipramine (**2**) was prepared by refluxing desimipramine (DMI)-HCl salt (**1**) with 1,1,1,3,3,3-hexamethyldisilazane (HMDS). A standard solution of N′-TMS derivative of DMI was prepared by dissolution in dry THF, which was previously distilled over $LiAlH_4/(C_6H_5)_3C\text{-}Cl$. Chemicals: 1.55 M solution of n-BuLi in n-hexane, 0.5 M solution of $LiAlH_4$ in THF, were purchased from the Aldrich Chemical Company. The synthetic apparatus outlined in Fig. 1, consists of the following parts (a) liquid-nitrogen trap, for the initial trapping of $^{11}CO_2$ in a small stainless steel loop. (b) A 1 mL conical shaped reaction vial, fitted with a Teflon-coated septum and connected to an argon gas cylinder. (c) A 10-mL two-neck conical-shaped reaction flask fitted with rubber septa. (d) A 25-mL Erlenmeyer conical flask containing dry Na_2SO_4. Purity of radiolabeled and cold imipramine was checked by HPLC, two Varian silica gel Si-10 guard columns connected in series [size 4.6 mm × 3 cm, each, which were connected with a small stainless steel tube], with CH_2Cl_2:MeOH:58% NH_4OH (135:15:0.12) as the eluant. Identification of the products was confirmed by TLC (E. Merck, silica gel $60F_{254}$) and Analtech, silica gel (GHLF plates) using mobile phase CH_2Cl_2:MeOH:58% NH_4OH

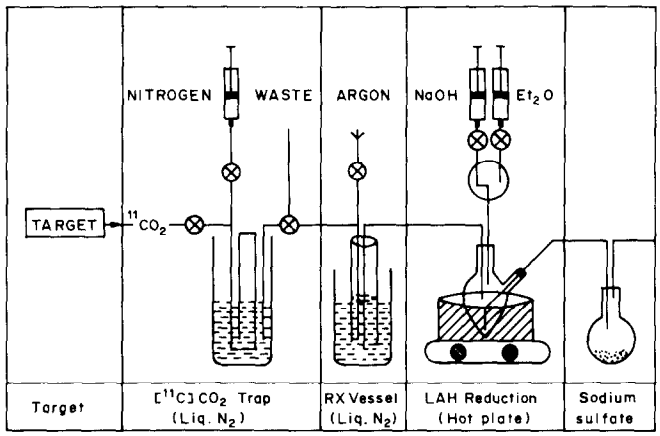

Fig. 1. Apparatus for the synthesis of N′-(4-^{11}C-methyl)imipramine.

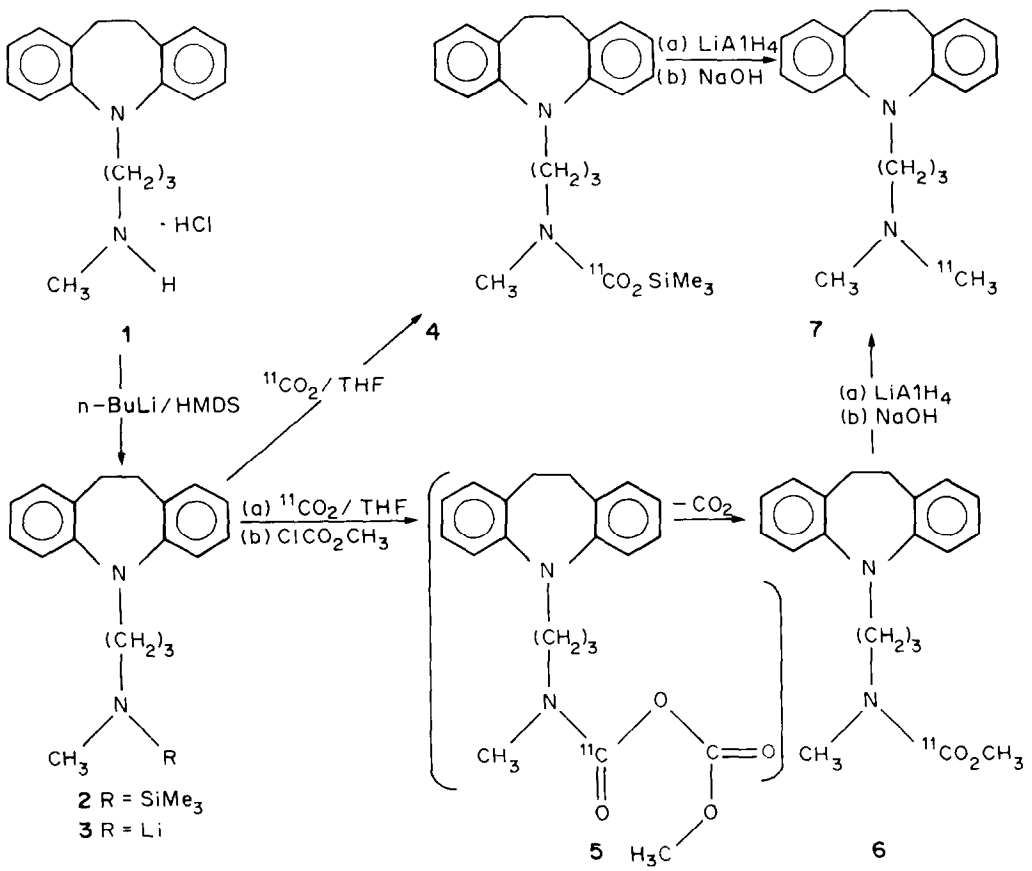

Fig. 2. CA and NCA synthesis of N′-(4-^{11}C-methyl)imipramine.

(9:1:3-drops). The $^{11}CO_2$ was obtained by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction using the University of Michigan CS-30 (The Cyclotron Corporation) cyclotron. The target gas containing mainly $^{11}CO_2$ was used directly without further processing.

Synthetic steps

The ^{11}C-radiolabeled imipramine was synthesized by two independent routes.

(A) No-carrier added synthesis of N′-(4-^{11}C-methyl)-imipramine

The synthetic steps are outlined in Fig. 2.

Synthesis of N′-(4-trimethylsilyl)desimipramine (2). A suspension of DMI-HCl salt (1, 0.5 g) in HMDS (5.0 mL) was stirred at reflux temperature for 12–15 h. The excess HMDS was removed under reduced pressure on a steam bath using a rotary evaporator. The resulting oil was dried under high

vacuum and dissolved in dry THF (20 mL) and stored in a sealed vial under argon pressure. This solution was used as a stock solution for further reactions.

Synthesis of N'-(4-^{11}C-methyl)imipramine (**7**). [^{11}C]carbon dioxide produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction was quantitatively trapped in a stainless steel loop (3.2-mm o.d.) half immersed in liquid nitrogen. After collection, the loop was warmed up to room temperature and [^{11}C]carbon dioxide was purged into the reaction mixture vial, which contained N'-TMS-derivative of DMI (**2**) in dry THF (86 μmol, 0.7 mL). The resulting reaction mixture was heated in a heating block at 55–60°C for 8–10 min. The reaction mixture was then transferred with help of positive pressure of argon, to a 10-mL, two-neck conically-shaped reaction flask, which contained 0.1 M-$LiAlH_4$ solution in dry THF (3.5 mL). The resulting reaction mixture was stirred with heating at 60–65°C for 10 min. After cooling the mixture was decomposed with 6.25 N-NaOH solution (approx. 2 mL). The product was diluted with diethyl ether (2 × 5 mL) and the organic layer was decanted. The combined ether layers were dried over Na_2SO_4 and again decanted to a round-bottom flask. Evaporation of the ether layer on a rotary evaporator, provided ^{11}C-labeled imipramine, which contained some residual unreacted DMI starting material.

Purification of N'-(4-^{11}C-methyl)imipramine. The impure imipramine was dissolved in 1 mL of CH_2Cl_2:MeOH:58% NH_4OH (135:15:0.12) and loaded on to a silica gel column [column size 12 × 1.35 cm, silica gel, E. Merck, 2.0 g, 230–400 mesh] and the compound was eluted with CH_2Cl_2:MeOH:58% NH_4OH system (10 mL) by applying gentle pressure through 50-cm^3 syringe. The fractions 4–6 (1.5 mL, each) which contain almost all the radioactivity were pooled. The maximum activity was found in fraction No. 5. The purity of the thus purified imipramine was checked by radio-HPLC using two guard columns (Si-10, size 4.6 mm × 3 cm, each) connected in series by a small stainless steel tube and CH_2Cl_2:MeOH:58% NH_4OH (135:15:0.12) as an eluant with flow rate of 1 mL/min. Detection of imipramine is performed by u.v. absorption at 254 nm. Imipramine is retained for 2.21 min and desimipramine for 4.75 min. The purity of purified, radiolabeled imipramine was 99.5% as confirmed by TLC.

Identification and quality control. The above pooled radioactivity fractions were evaporated. The u.v. and chromatographic behaviour (HPLC, TLC and GLC) were identical with authentic sample.

(B) Carrier-added synthesis of N'-(4-^{11}C-methyl)-imipramine

Cold synthesis of imipramine. Method I: Synthesis of N'-(4-carbomethoxy)desimipramine (**6**). n-BuLi (9.0 mmol, 5.81 mL of 1.55 M solution of n-BuLi in n-hexane) was added to a stirred suspension of DMI-HCl salt (**1**, 0.906 g, 3.0 mmol) in dry THF (20 mL) at −78°C and stirring was continued for 30 min at −78°C, then methyl chloroformate (0.46 mL, 6.0 mmol) was added. The resulting reaction mixture was stirred 4–5 h at −78°C and 10 h at room temperature. Reaction progress was monitored by TLC [silica gel, CH_2Cl_2:MeOH(9:1)]. When the reaction was complete, the solvent was evaporated under reduced pressure. The residue was redissolved in 5 mL of CH_2Cl_2:MeOH(9:1) and chromatographed over silica gel column [column size, 2 × 35 cm, silica gel, E. Merck, 20 g, 230–400 mesh]. The product was eluted with CH_2Cl_2. Fractions containing the desired product were pooled and evaporated under reduced pressure to give **6** as an oil, yield 0.93 g (95.4%). i.r. (neat) 3018, 2950–20, 2840, 1705, 768, 750 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.47–2.10 (t, 2H, C–CH_2–C), 2.73 (s, 3H, N–CH_3), 2.97–4.45 (m, 11H, N–CH_2–C–C, CO_2CH_3,–CH_2–CH_2–,–CH_2N–CO), 6.64–7.40 (bs, 8H, Ar–H). Anal. Calc'd for: $C_{20}H_{24}N_2O_2$, C, 74.05; H, 7.46; N, 8.63, Found C, 73.94; H, 7.36; N, 8.71. R_f values of product and desimipramine were 0.68 and 0.47 respectively, [silica gel, Analtech plates, CH_2Cl_2:MeOH(98:2)].

Method II: Synthesis of N'-(4-carbomethoxy)-desimipramine (**6**) *using CO_2.* n-BuLi (3.0 mmol, 1.94 mL of 1.55 M solution of n-BuLi in n-hexane) was added to a stirred suspension of desimipramine HCl salt (**1**, 0.302 g, 1.0 mmol) in dry THF (20 mL) and stirring was continued for 30 min at −78°C. Dry CO_2 was then bubbled into the reaction mixture for 5 min at −78°C and 15 min at 55°C, followed by addition of methyl chloroformate (2.0 mmol). The resulting reaction mixture was stirred at reflux temperature for 30 min, the solvent was evaporated under reduced pressure and the resulting residue was purified by column chromatography over silica gel [column size 2 × 35 cm, E. Merck silica gel, 12 g, 230–400 mesh] using CH_2Cl_2 as an eluant. The fractions containing the desired product were pooled and evaporated under reduced pressure to give **6**, an oil; yield 0.31 g (93%). The material was identical in all respects (spectroscopically and analytically) to compound **6** as prepared by Method I.

In situ *reduction of N'-(4-carbomethoxy)desimipramine* (**6**) *to imipramine* (**7**). N'-(4-carbomethoxy)-desimipramine (**6**, 1.0 mmol) in dry THF (20 mL) was prepared as described in Method II. To the above stirred solution, $LiAlH_4$ (0.4 g) was added slowly under argon atmosphere and resulting reaction mixture was refluxed for 30 min. The excess of $LiAlH_4$ was decomposed with 6.25 N-NaOH solution. Diethyl ether (20 mL × 2) was added and the ether layer was decanted. The combined ether layer was dried over Na_2SO_4 and evaporated under reduced pressure to give **7** as an oil in 79% yield, which was identical to the authentic imipramine except having some impurity of n-pentanol, as detected by GLC analysis. The above products were isolated and characterized for identification purposes.

Table 1. Radioanalytical data of the N-(4-^{11}C-methyl)imipramine

Experiment number	Radiochemical yield (%)	Radiochemical purity (%)	Total amount of [^{11}C]carbon dioxide trapped (mCi)
1[a]	67.56	95.83	3.22[b]
2[a]	88.92	97.40	51.23
3[a]	77.65	99.47	65.23
4[c]	63.68	85.15	42.67

[a]No carrier added synthesis.
[b]A tracer experiment was studied.
[c]Carrier added synthesis.

Synthesis of N′-(4-^{11}C-methyl)imipramine (**7**). n-BuLi (0.58 mL, 0.9 mmol, 1.55 M solution of n-BuLi in n-hexane) was added to a 10-mL, two neck conically-shaped reaction flask, fitted with rubber septa, containing a suspension of desimipramine-HCl salt (**1**, 97 mg, 0.3 mmol) in dry THF (6.0 mL). Stirring was continued for 30 min at −78 C. $^{11}CO_2$ was then purged into the reaction mixture. The reaction mixture was heated at 55 C for 5 min, and methyl chloroformate (0.035 mL, 0.45 mmol) was added via a syringe. The resulting reaction mixture was stirred and heated at 50–65 C for 10 min. $LiAlH_4$ (100 mg) was added under an argon atmosphere and heating was continued for additional 15 min. The reaction mixture was cooled and excess of $LiAlH_4$ was decomposed with 6.25 N-NaOH solution (approximately 1–1.5 mL). The organic layer was decanted into a conical flask containing dry Na_2SO_4, the residue was washed with diethyl ether (5 mL × 2) and decanted again to the conical flask. The combined ether layer was decanted into a 25-mL round-bottom flask. Radio TLC analysis results showed only one major radio peak, which corresponds to imipramine. The radiochemical yield was 64% based on radio TLC purity of the crude reaction mixtures. A minor radioacitivty peak (< 10%) identified as n-pentanol (resulting from carboxylation of n-BuLi, followed by LAH reduction) was also observed.

Results and Discussion

In general the reductive-carboxylation procedure has the following possible distinct advantages over current radioalkylation procedures.

(1) Unlike $^{11}CH_3I$ or $H^{11}CHO$, $^{11}CO_2$ is directly available from the cyclotron target for on-line synthesis, without further processing.
(2) The use of $^{11}CO_2$ directly as the radio-precursor can result in a higher specific activity product, since no reduction or oxidation steps are involved in radiolabeled precursor preparation, which may introduce "cold" $^{12}CO_2$.*

The overall manual production of N′-(4-^{11}C-methyl)imipramine required approximately 45 min from the end of bombardment. The trapping of $[^{11}C]CO_2$ from the target gas in the substrate solution required approximately 5 min. The radiochemical yield and purity for *carried added synthesis* were 64% and 85% based on radio TLC of the *crude* reaction mixtures, which were not optimized and are based on the single experiment. In the case of *no carried added synthesis*, the radiochemical yield and purity for *purified* imipramine were 77 and 99.5% respectively, which were also not optimized. The results of four experiments are presented in Table 1. The R_f values of synthesized products checked with authentic compounds and other physicochemical data were also similar to authentic sample. HPLC analysis demonstrated a total of 1 μmol of cold imipramine was produced based on the single experiment, however no attempts were made to purify hydrocarbon traces from the target gas. The radiochemical purity of ^{11}C-labeled imipramine was confirmed by HPLC. Specific activity of the [^{11}C]imipramine was calculated to be 50 mCi/μmol [at the end of cyclotron bombardment (EOB)]. This specific activity represents neither maximum $^{11}CO_2$ production nor optimized synthesis time, both of which will determine the attainable specific activity. The 1 μmol of cold imipramine produced, however, is comparable to or slightly greater than cold amounts reported elsewhere[3,5] and likely represents the amount of $^{12}CO_2$ produced in this system.

Conclusions

High specific activity and high purity N′-(4-^{11}C-methyl)imipramine can be readily synthesized in excellent radiochemical yield from $[^{11}C]CO_2$ by a reductive-carboxylation method. The yield and purity can be optimized and time can be shortened by automation of the reaction. The apparatus is very simple for automation of the synthesis. The reductive-carboxylation approach is easily adaptable to the synthesis of other potentially important radiopharmaceuticals such as promazine, guanifexine, nicotine, etc.

Acknowledgements– Research was supported by NCI training grant No. 5-T32-CA-09015 and by NINCDS grant No. PO1-NS-15655. The authors are thankful to Dr Keith

* LAH may contain traces of $^{12}CO_2$ salts in the reduction of $^{11}CO_2$ to MeOH.
In the oxidation of ^{11}C-MeOH to $^{11}CH_2O$, ^{12}C may be introduced from residual traces of organic solvent (i.e. THF etc.) used in the LAH reduction step.

Mulholland for his valuable suggestions, Roger Lininger for the proton irradiations and Ms Linder Markham for her assistance in the preparation of this manuscipt. We are greatly indebted to Dr T. Pruss, Revlon Health Care for the generous gift of desimipramine.

References

1. Comar D., Maziere M., Marazano C. and Raynaud C. *J. Nucl. Med.* **16,** 521 (1975).
2. Berger G., Maziere M. and Comar D. *J. Labeled Compd. Radiopharm.* **16,** 97 (1979).
3. Berger G., Maziere M., Knipper R., Prenant C. and Comar D. *Int. J. Appl. Radiat. Isot.* **30,** 393 (1979).
4. Maziere M., Berger G. and Comar D. In *Application of Nuclear and Radiochemistry* (Eds Lambrecht R. M. and Morcos N.), pp. 251–270. (Pergamon Press, New York, 1982).
5. Denutte H., Goethals P., Cattoir H., Bogaert M., Vandewalle T., Vandercasteele C., Jonckheere J. and Leenher A. D. *J. Nucl. Med.* **24,** 1185 (1983).
6. (a) Frideling J. P. and Pichat L. *Int. Symp. on the Synthesis and Applications of Isotopically Labelled Compounds.* 6–11 June 1982, Kansas City, MO, U.S.A.; (b) Frideling J. P. Ph.D. Thesis. *Insertion of ^{14}C-carbon dioxide between the N-Si bond of silylated amines.* 4 May 1982. CEN Saclay 9119/Gif/Yvette Cedex, France (In French).
7. Holland H. L. and Johnson G. B. *Tetrahedron Lett.* 3595 (1979).
8. Ram S. and Ehrenkaufer R. E. *Tetrahedron Lett.* **26,** 5367 (1985).