

Synthesis of Saturated Fatty Acids
 ^{11}C (^{13}C)-labelled in the ω -Methyl Position

Henrik Neu,^{1,2,3} Tor Kihlberg,^{1,2} and Bengt Långström^{1,2,3*}

¹Department of Organic Chemistry, Institute of Chemistry, Uppsala University.

²The Subfemtomole Biorecognition Project, Uppsala University and Japan Science
Technology Corporation.

³Uppsala University PET Centre, UAS,S-751 85 Uppsala, Sweden,

FAX number: +46 18 183390.

E-mail: Bengt.Langstrom@PET.UU.SE

Running Title: ^{11}C -labelled fatty acids

Key Words: ^{11}C -labelled, fatty acids, *tert*-butyl ω -iodocarboxylate, lithium(2-thienyl)iodocuprate

Abstract

A method for the preparation of saturated fatty acids ^{11}C (^{13}C)-labelled in the ω -methyl position is described. A highly reactive zerovalent copper complex was prepared from lithium naphthalenide reduced lithium(2-thienyl)iodocuprate. The labelling precursors were obtained by addition of *tert*-butyl ω -iodocarboxylates to the organocuprate and these were reacted with [^{11}C]methyl iodide to form ^{11}C -labelled,

* Author for correspondence

protected intermediates. The *tert*-butyl ester protecting group was rapidly removed with trifluoroacetic acid, affording fatty acids ^{11}C -labelled in the ω -methyl position. A solid phase extraction method was developed and preceded final HPLC purification. In a typical run starting with 2.75 GBq of [^{11}C]methyl iodide, 375 MBq (66%) [16- ^{11}C]palmitic acid was obtained within 46 min from the end of radionuclide production.

Introduction

Fatty acids ^{11}C -labelled in specific positions have been employed for studies of myocardial metabolism using positron emission tomography (PET).¹ PET has been used as a tool to study the difference in metabolic kinetics between ^{11}C -labelled acetate labelled in the 1- and 2-position, respectively.² While [1- ^{11}C]acetate completes one full turn of the TCA cycle, and is eliminated as [^{11}C]carbon dioxide, [2- ^{11}C]acetate is exponentially eliminated after the second turn. Even long-chain fatty acids labelled in the carboxyl position are indistinguishable from [1- ^{11}C]acetate with respect to the myocardial metabolic pattern. Likewise, long-chain fatty acids labelled in the ω -methyl position behave similarly to [2- ^{11}C]acetate.³ Fatty acids are converted to acetate by β -oxidation and transformed to acetyl-CoA before subsequent oxidation in the TCA cycle.⁴ Thus, the use of position-specific labelling of fatty acids presents a possibility to discriminate between β -oxidation and TCA cycle metabolism in the myocardium using PET.

The aim of this study was to develop a general procedure to prepare ^{11}C -labelled fatty acids, saturated as well as unsaturated, in high yields and short synthesis times. There is also an interest in using the ^{11}C -labelled fatty acids as precursors for enzymatic reactions to produce labelled acyl-CoA, acyl carnitines, phospholipids and

triglycerides. Synthesis of saturated fatty acids labelled with ^{11}C in the carboxyl and ω -methyl position have previously been reported.^{3,5,6} In one method,³ an α,ω -(bis)Grignard reagent was used together with dilithium tetrachlorocuprate, [^{11}C]methyl iodide and carbon dioxide to produce ω -labelled fatty acids. However, this method was found to be unsuitable for ^{11}C -labelling of short-chain fatty acids such as propionic acid and butyric acid. In another method⁵ an ω -Grignard reagent with a furane protected carboxyl group was coupled with [^{11}C]methyl iodide. After oxidative cleavage of the furane ring with ruthenium tetroxide and sodium periodate ω -labelled fatty acids were obtained. Neither of these two methods were applicable for the ^{11}C -labelling of unsymmetrically unsaturated fatty acids (*e.g.* linoleic acid).⁷

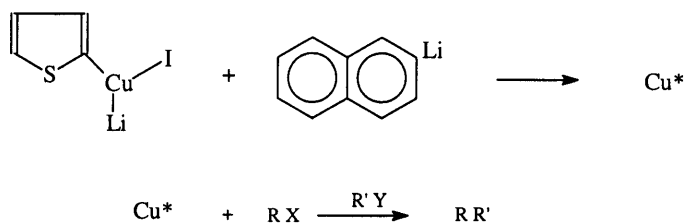
In this paper, a method to synthesize saturated fatty acids ^{11}C -labelled in the ω -methyl position is presented. The ^{11}C -label is incorporated via a coupling reaction between a *tert*-butyl ω -iodocarboxylate and [^{11}C]methyl iodide, using a highly reactive zerovalent copper complex.⁸

Results and discussion

^{11}C -Chemistry

Initial attempts to synthesize saturated fatty acids ^{11}C -labelled in the ω -methyl position utilized an ω -Grignard reagent in which the carboxyl function was masked either as a 1,3-oxazine⁹ or as a magnesium bromo carboxylate.¹⁰ However, when using the ω -Grignard oxazine in coupling reactions with [^{11}C]methyl iodide, no product was formed. The lack of reactivity may be explained by the formation of a stable dimer between the oxazine unit and the Grignard reagent.⁹ Attempts to increase the reactivity using tetramethyldiamine, borontrifluoro-etherate or aluminium trichloride were unsuccessful. The same discouraging result was obtained using a magnesium bromo carboxylate in a similar coupling reaction.

An alternative procedure using a highly reactive copper complex (Cu^*) and various alkyl halides was investigated.¹¹ The copper complex, obtained from the reduction of lithium(2-thienyl)iodocuprate with lithium naphthalenide (Scheme 1), reacted directly with alkylhalides (RBr , RI). The resulting organocuprate could then be reacted with another alkylhalide ($\text{R}'\text{Br}$, $\text{R}'\text{I}$) to produce the coupling product (Scheme 1).⁸ Both substrates may contain functional groups such as esters, ethers, nitriles, ketones and chlorides.



Scheme 1.

The ^{11}C -C bond formation was performed by producing lithium[methyl- ^{11}C](2-thienyl)cuprates from [^{11}C]methyl iodide and Cu^* , followed by reaction with an appropriate alkyl halide¹¹. Using lithium[^{11}C -methyl](2-thienyl)iodocuprate in reaction with heptyliodide, the radiochemical yields of octane were 30-40%. If lithium(2-thienyl)cyanocuprate was used instead of lithium(2-thienyl)iodocuprate the radiochemical yields were less than 5%. Apart from unreacted [^{11}C]methyl iodide, the main labelled side product in these reactions was [^{11}C]methane.¹¹

In the present investigation [^{11}C -methyl]octane was used as a model compound for further development of this ^{11}C -labelling procedure. It was found that the radiochemical yields increased if Cu^* was used to prepare the lithiumalkyl(2-thienyl)cuprate from the unlabelled alkyl iodide prior to the addition of [^{11}C]methyl

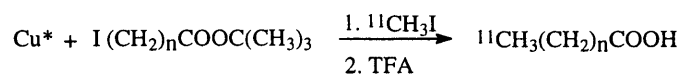
iodide. As illustrated in Table 1, [^{11}C -methyl]octane was obtained in 78% decay-corrected radiochemical yield based on [^{11}C]methyl iodide.

Table 1. Radiochemical yields of fatty acids ^{11}C -labelled in the ω -methyl position.

Compound	Radiochemical yield (n) ^a
[16- ^{11}C]palmitic acid	73 \pm 3% (5)
[12- ^{11}C]laurylic acid	70 \pm 3 % (5)
[8- ^{11}C]octanoic acid	70 \pm 10% (4)
[5- ^{11}C]valeric acid	72 \pm 2% (3)
[4- ^{11}C]butyric acid	64 \pm 7% (3)
[3- ^{11}C]propionic acid	43 \pm 2 % (10)
[1- ^{11}C]octane	78 \pm 3% (5)

^aDecay-corrected radiochemical yields of HPLC purified ^{11}C -labelled fatty acids, counted from [^{11}C]methyl iodide, calculated 35 min from end of radionuclide production. n=number of experiments

Employing this procedure, the *tert*-butyl ω -iodocarboxylate was added to the Cu^* before addition of [^{11}C]methyl iodide. After hydrolysis of the *tert*-butyl ester with trifluoroacetic acid (TFA) (Scheme 2), the fatty acids ^{11}C -labelled in the ω -methyl position were obtained in moderate to high radiochemical yields (64–73%), calculated from trapped [^{11}C]methyl iodide (Table 1). One exception was the [3- ^{11}C]propionic acid which was obtained in 43% radiochemical yield. The total synthesis time was within 45 min from the end of radionuclide production, including sterile formulation of the ^{11}C -labelled fatty acids.



$$n = 1, 2, 3, 6, 10, 14$$

Scheme 2.

This new labelling method is suitable for the synthesis of short-, medium- and long-chain saturated fatty acids as well as unsaturated fatty acids.⁷ The medium- and long-chain fatty acids were obtained in similar radiochemical yields, while [3-¹¹C] propionic acid was obtained in a considerably lower radiochemical yield. An explanation may be that the 2-iodoacetic acid *tert*-butyl ester forms an unstable cuprate because of the short-chain length.

The use of a methylester as the protective group was also investigated. In these experiments lithium hydroxide¹² or trimethylsilyl iodide¹³ were used to hydrolyze the methyl ester. The acids, from the methylester, were never obtained in such good yields as in the reactions using the *tert*-butyl ester as the protective group.

The position of the label was confirmed by analysis of the ¹³C-NMR spectrum of (¹³C-methyl)palmitic acid. The ¹³C-labelled fatty acid was synthesized employing the same method as for the ¹¹C-labelled compound, employing a simultaneous addition of (¹³C)methyl iodide with the [¹¹C]methyl iodide. The ¹³C signal at δ 14.1 ppm corresponded to the same shift of the methyl group in authentic palmitic acid.

Purification of ¹¹C-labelled fatty acids.

In previous reports³ on the synthesis of fatty acids ¹¹C-labelled in the ω -methyl position, wax-like compounds, most likely unlabelled dicarboxylic fatty acids and salts, were formed. These crude product mixtures caused excessively high column pressures and gave irreproducible retention times during the semi-preparative LC purifications. Therefore, a solid phase extraction (SPE) method was developed as a pre-purification step in order to improve the semi-preparative HPLC purification. Using this SPE method, TFA, inorganic salts and high molecular weight compounds were removed. The method was used for fatty acids with chain lengths of 6-14

carbons. By optimizing the water/acetonitrile composition, the ^{11}C -labelled fatty acids were obtained in high radiochemical purities (>95%). Less than 5% of the ^{11}C -labelled fatty acids remained on the SPE column after elution with acetonitrile. The SPE purified labelled fatty acids were obtained within 20 min. With the present SPE method it may be possible to use the pre-purified labelled fatty acids in further chemical transformations such as enzymatic synthesis of acyl-CoA, acyl carnitines, phospholipids and triglycerides, obviating time consuming HPLC purification before the enzymatic synthesis.

Starting materials

The starting materials for the fatty acids were synthesized from the corresponding ω -bromocarboxylic acids. The bromide was substituted with iodide using sodium iodide in acetone.¹⁴ The crude iodo acid was then reacted with isobutene in acidic CH_2Cl_2 .¹⁵ After about 48 hours, when all the acid was consumed, the product was purified and dried. The total chemical yields were 35-80%.

Experimental

General

Radionuclide production was performed on a Scanditronix MC-17 cyclotron at the Uppsala University PET Centre. [^{11}C]Carbon dioxide was prepared by the $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$ reaction using a gas target containing nitrogen and oxygen (AGA, 0.05% oxygen in nitrogen 6.0) bombarded with 17 MeV protons. Synthia, an automated synthesis system, was used for [^{11}C]methyl iodide production, SPE handling, HPLC injection and fraction collection, and formulation.¹⁶

HPLC was performed with a Beckman 126 gradient pump and a Beckman 166 variable wavelength UV absorbance detector at 222 nm in series with a β^+ -flow

detector¹⁷. The following mobile phases were used: 0.05 M ammonium formate, pH 3.5 (A), 0.01 M KH_2PO_4 (B), and acetonitrile/water (50/7, v:v) (C). For analytical HPLC either a Beckman Ultrasphere ODS C_{18} , 5 μm , 250 x 4.6 mm ID (D) or a Spherisorb C6, 5 μm , 250 x 4.6 mm ID (E) column was used at a flow of 2 ml/min. For semi-preparative HPLC either a Beckman Ultrasphere ODS C_{18} , 5 μm , 250 x 10 mm ID (F) column or Beckman Ultrasphere Octyl C_8 , 5 μm , 250 x 10 mm ID (G) column was used at a flow of 5 ml/min. Data collection and HPLC control were performed with Beckman System Gold. In the analysis of the ^{11}C -labelled fatty acids, unlabelled reference substances were used for comparison in the HPLC run using UV absorbance detection at 222 nm.

Flash chromatography was performed using silica gel 60, 230-400 mesh. TLC was performed on Silica 60 plates and developed with 10% phosphomolybdic acid hydrate in ethanol.

NMR spectra were recorded on a Varian XL 300 spectrometer at 300 MHz for ^1H and 75.4 MHz for ^{13}C with chloroform- d_1 as internal standard. All shifts are reported in ppm. THF was distilled under nitrogen from sodium/benzophenone. The synthesis of lithium(2-thienyl)iodocuprate and lithium naphthalenide were performed under an argon atmosphere. The SPE column was pre-conditioned with 5 ml ethanol followed by 5 ml $\text{CH}_3\text{CN}/\text{H}_2\text{O}$. The *tert*-butyl ω -iodocarboxylates were dissolved in dry THF to a concentration of 0.5 M and stored under nitrogen in 250 μl capped vials. 3-Bromopropionic acid, 4-bromobutyric acid, 7-bromoheptanol, 15-bromopentadecanoic acid, 2-bromoacetic acid *tert*-butyl ester, thiophene and copper(I) iodide were purchased from Aldrich. Sodium iodide, acetone, silica gel and TLC plates were purchased from Merck. Butyl lithium was purchased from Lancaster. Isobutene was purchased from AGA. Polysorbatum 80 was purchased from Apoteksbolaget. The C_8 SPE columns were purchased from Applied Separations.

*¹¹C-Labeling**[¹¹C]Methyl iodide¹⁸*

[¹¹C]Carbon dioxide was delivered at a flow of 100 ml/min to a solution of lithium aluminum hydride (50 μmol, 250 μl, 0.2 M in THF) in a stream of nitrogen gas. After evaporation of THF, hydroiodic acid (57%, 0.4 ml) was added and the reaction mixture was heated to 130°C. [¹¹C]Methyl iodide was distilled off and transferred by a stream of nitrogen gas to the reaction vessel.

[16-¹¹C]Palmitic acid

To a THF solution of lithium(2-thienyl)iodocuprate (100 μl, 0.25 M), lithium naphthalenide (100 μl, 0.25 M) was added at -72°C and the resulting mixture kept at -72°C for 10-20 min. Before the transfer of [¹¹C]methyl iodide (2-5 min), 15-iodopentadecanoic acid *tert*-butyl ester (30 μl, 0.5 M in THF) was added. After the transfer of [¹¹C]methyl iodide, the mixture was heated to 70°C for 1 min. The vial was then rapidly cooled to approximately 0°C and TFA (200 μl) added. The vial was heated for another 5 min at 70°C. The crude [16-¹¹C]palmitic acid was diluted to a final volume of 10 ml with CH₃CN/H₂O 50/50 solution and applied to the SPE column. The SPE column was eluted with 3 ml CH₃CN. The eluate was diluted with 1.5 ml CH₃CN/H₂O 50:50 solution and injected onto the HPLC column using column G with mobile phase A/C 10:90 with a linear gradient to 100% C from 1-4 min. For analysis of [16-¹¹C]palmitic acid, column E was used with mobile phase B/C 30:70, *t_R* = 5.3 min.

(16-¹³C)Palmitic acid

Prepared as described for [16-¹¹C]palmitic acid with the exception that (¹³C)methyl iodide (20 μl, 20% w/w in heptane) was added after trapping of [¹¹C]methyl iodide.

The collected fraction was evaporated to dryness, dissolved in CDCl_3 (0.5 ml) and analyzed by ^{13}C -NMR.

[12- ^{11}C]Laurylic acid

Prepared as described for [16- ^{11}C]palmitic acid, starting from 11-iodoundecanoic acid *tert*-butyl ester. $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ 40:60 was used for the solid phase extraction. For semi-preparative HPLC purification column G was used with mobile phase A/C 10:90 with a linear gradient to 100% C from 1-4 min. For analysis of [12- ^{11}C]laurylic acid, HPLC column E was used with mobile phase B/C 40:60, $t_{\text{R}} = 7.4$ min.

[8- ^{11}C]Octanoic acid

Prepared as described for [16- ^{11}C]palmitic acid, starting from 7-iodoheptanoic acid *tert*-butyl ester. $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ 40:60 was used for the solid phase extraction. For semi-preparative HPLC column G was used with mobile phase A/C 10:90 with a linear gradient to 100% C from 1-4 min. For analysis of [8- ^{11}C]octanoic acid, HPLC column E was used with mobile phase B/C 40:60, $t_{\text{R}} = 6.6$ min.

[5- ^{11}C]Valeric acid

Prepared as described for [16- ^{11}C]palmitic acid, starting from 4-iodobutanoic acid *tert*-butyl ester. The crude product was diluted with water (0.5 ml) and sodium hydroxide (5 M, 0.3 ml) and injected onto the semi-preparative HPLC column F eluting with mobile phase A/C 70:30 with a linear gradient to 100% C from 6-9 min. For analysis of [5- ^{11}C]valeric acid, HPLC column E was used with mobile phase B/C 70:30, $t_{\text{R}} = 3.9$ min.

[4- ^{11}C]Butyric acid

Prepared as described for [16- ^{11}C]palmitic acid, starting from 3-iodopropionic acid *tert*-butyl ester. The crude product was diluted with water (0.5 ml) and sodium

hydroxide (5 M, 0.3 ml) and injected onto the semi-preparative HPLC column F eluting with mobile phase A/C 80:20 with a linear gradient to 100% C from 6-9 min. For analysis of [4-¹¹C]butyric acid, HPLC column E was used with mobile phase B/C 85:15, $t_R = 4.0$ min:

[3-¹¹C]Propionic acid

Prepared as described for [16-¹¹C]palmitic acid, starting from 2-iodoacetic acid *tert*-butyl ester. The crude product was diluted with water (0.5 ml) and sodium hydroxide (5 M, 0.3 ml) and injected onto the semi-preparative HPLC column F with mobile phase A/C 95:5 with a linear gradient to 100% C from 6-9 min. For analysis of [3-¹¹C]propionic acid, HPLC column D was used with mobile phase B/C 95:5, $t_R = 4.3$ min.

Formulation of the ¹¹C-fatty acids

Before concentration of the HPLC fraction containing the short chain ¹¹C-labelled fatty acids (3-8 carbons), NaOH (150 μ l, 1 M) was added. After concentration at reduced pressure, 1 ml Polysorbatum 80 in ethanol (10%), was added followed by physiological phosphate buffer (7 ml). For the short-chain fatty acids HCl (0.5 ml, 0.3 M) was added to the phosphate buffer prior to its addition. The resulting solution was passed through a 0.22 μ m sterile filter (Dynagard) into a sterile injection flask.

Starting materials

Lithium(2-thienyl)iodocuprate¹⁹

To a stirred solution of thiophene (0.82 ml, 10.2 mmol) in THF (10 ml) at -72°C, butyllithium (10.0 mmol, 1.6 M, 6.4 ml) was added and the mixture slowly warmed to room temperature. After 30 min, the mixture was slowly transferred by cannula to a

stirred slurry of copper(I) iodide (1.90 g, 10.0 mmol) in THF (10 ml) at -72°C . The mixture was stirred vigorously at -72°C and was allowed to warm to room temperature overnight. THF (13 ml) was added, and the resulting solution was stored in the reaction flask and used as such.

Lithium naphthalenide

Lithium naphthalenide was prepared by reacting lithium (86 mg, 12.5 mmol) and naphthalene (1.86 mg, 14.5 mmol) in THF (50 ml) at room temperature until all lithium had been consumed.

*7-Bromoheptanoic acid*²⁰

7-Bromoheptanol (0.5 g, 2.6 mmol) was added dropwise to a vigorously stirred solution of chromium trioxide (1.25 g, 12.5 mmol) in glacial acetic acid (15 ml) and water (1 ml) cooled to 0°C . After stirring for 20 hours at room temperature, the solution was diluted with water (40 ml) and extracted with ether (3x50 ml), dried and concentrated, affording 0.5 g crude product.

$^1\text{H NMR}$: δ 1.4 (m, 4H), 1.65 (m, 2H), 1.88 (m, 2H), 1.36 (t, 2H), 3.42 (t, 2H)

15-Iodoheptadecanoic acid tert-butyl ester

To a solution of NaI (350 mg, 2.3 mmol) in acetone (20 ml) 15-bromopentadecanoic acid (0.5 g, 1.6 mmol) was added and the mixture stirred for 5 hours at room temperature. The solvent was removed by evaporation, water (25 ml) was added and the mixture was extracted with ether (3x50 ml). The combined organic layers were successively washed with water (25 ml), brine (25 ml), sodium thiosulphate (10%, 25 ml) and dried over Na_2SO_4 . Concentration at reduced pressure afforded the 15-iodoheptadecanoic acid.

The crude 15-iodoheptadecanoic acid was dissolved in freshly distilled CH_2Cl_2 (15 ml), isobutene was added to double the volume followed by 2 drops of concentrated

sulfuric acid. After approximately 48 hours, sodium carbonate (10%, 25 ml) and water (25 ml) were added. The CH_2Cl_2 layer was separated and washed with successively with water (25 ml) and brine (25 ml). The organic phase was dried over Na_2SO_4 and concentrated at reduced pressure. The crude product was purified by flash chromatography (ether/pentane 4:96). The compounds were dried by azeotropic distillation using benzene (3x15 ml). The 15-iodoheptadecanoic acid *tert*-butyl ester (402 mg, 0.94 mmol) was obtained in 59% yield as white crystals.

^1H NMR: δ 1.25 (bs, 20H), 1.44 (s, 9H), 1.55 (m, 2H), 1.82 (m, 2H), 2.20 (t, 2H), 3.18 (t, 2H). ^{13}C NMR: δ 7.4, 25.2, 28.2, 28.6, 29.1, 29.3, 29.5, 29.5, 29.6, 29.6, 29.6, 30.5, 33.6, 35.7, 79.9, 173.4

11-Iodoundecanoic acid tert-butyl ester

The 11-iodoundecanoic *tert*-butyl ester was obtained in 52% yield as a colorless oil.

^1H NMR: δ 1.32 (bs, H), 1.41 (s, 9H), 1.62 (m, 2H), 1.86 (m, 2H), 2.25 (t, 2H), 3.23 (t, 2H). ^{13}C NMR: δ 7.4, 25.1, 28.2, 28.5, 29.1, 29.3, 29.4, 29.4, 30.5, 33.6, 35.6, 79.9, 173.4

7-Iodoheptanoic acid tert-butyl ester

The 7-iodoheptanoic *tert*-butyl ester was obtained in 54% yield as a colorless oil.

^1H NMR: δ 1.35 (m, 2H), 1.45 (s, 9H), 1.58 (m, 2H), 1.82 (m, 2H), 2.20 (t, 2H), 3.18 (t, 2H). ^{13}C NMR: δ 7.0, 24.8, 27.9, 28.1, 30.1, 33.3, 35.4, 80.0.

4-Iodobutanoic acid tert-butyl ester

The 4-iodobutanoic acid *tert*-butyl ester was obtained in 37% yield as a colorless oil.

^1H NMR: δ 1.44 (s, 9H), 2.09 (m, 2H), 2.34 (t, 2H), 3.22 (t, 2H). ^{13}C NMR: δ 5.6, 28.0, 28.6, 35.9, 80.5, 170.8

3-Iodopropionic acid tert-butyl ester

The 3-iodopropionic acid *tert*-butyl ester was obtained in 39% yield as a colorless oil.

^1H NMR: δ 1.45 (s, 9H), 2.86 (t, 2H), 3.28 (t, 2H). ^{13}C NMR: δ -2.8, 28.0, 39.7, 81.4, 170.3

2-Iodoacetic acid tert-butyl ester

To NaI (3.5 g, 25 mmol) in acetone (50 ml) 2-bromoacetic acid *tert*-butyl ester (2.1 g, 10.1 mmol) was added and the resulting mixture stirred for 5 hours at room temperature. The solvent was removed, water (100 ml) added and the resulting mixture was extracted with ether (3x50 ml). The combined organic layers were successively washed with water (50 ml), brine (50 ml) and sodium thiosulphate (10%, 50 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography (pentane/ether 96:4), and afforded 2-iodoacetic acid *tert*-butyl ester (2.0 g, 8.3 mmol) in 82 % yield, as a colorless oil. The 2-iodoacetic acid *tert*-butyl ester was dried by azeotropic distillation using benzene.

^1H NMR: δ 3.5 (s, 2H), 1.35 (s, 9H). ^{13}C NMR: δ -2.5, 27.6, 82.2, 167.8

Acknowledgment

This work was financially supported by the Swedish Natural Science Research Council, grant K-3463

References

-
- ¹ Phelps M., Mazziotta J.C., Schelbert H.R. (eds) -Positron emission tomography and autoradiography: Principles and applications for the brain and heart. Raven Press, New York, 581-661, 1986. Antark M.A. -J. Nucl. Med. Biol. 17: 103 (1990)
- ² Kihlberg, T., Valind, S. and Långström, B. -Int. J. Appl. Radiat. Isot. Part B: J. Nucl. Med. Biol. 21: 1067 (1994)

- ³ Kihlberg, T., Valind, S. and Långström, B. -Int. J. Appl. Radiat. Isot. Part B: J. Nucl. Med. Biol., 21: 1053 (1994)
- ⁴ Stryer L., "Biochemistry", 3rd edn. W. H. Freeman and Company, New York. 1988
- ⁵ Buckman, B.O., VanBrocklin, H.F., Dence, C.S., Bergmann, S.R., Welch, M.J. and Katzenellenbogen J.A. -J. Med. Chem. 37: 2481 (1994)
- ⁶ Jewett D.M., Ehrenkaufner R. L., Ram S., Int. J. Appl. Radiat. Isot. 32: 843. (1985). Walsh M.N., Geltman E.M., Brown M.A., Henes C.G., Weinheimer C.J., Sobel B.E. and Bergman S.R., J. Nucl. Med., 30: 1798 (1989)
- ⁷ Neu H. Kihlberg T and Långström B. -Manuscript in preparation (1997)
- ⁸ Wehmeyer, R.M. and Rieke, R.D. -J. Org. Chem. 52: 5056 (1987). Rieke, R.D. Wehmeyer, R.M., Wu, T.C. and Ebert, G.W. -Tetrahedron, 45: 443 (1989). Rieke, R.D., Wu, T.C., Stinn, D.E. and Wehmeyer, R.M. -Synth. Commun. 19: 1833 (1989)
- ⁹ Meyers A.I., Nabeya A., Adickes H.W., Politzer I.R., Malone G.R., Kovelesky A.C., Nolen R.L. and Portnoy R. C., -J. Org Chem. 38: 36 (1973)
- ¹⁰ Baer T.A. and Carney R.L. -Tetrahedron Lett. 51: 4697 (1976)
- ¹¹ Kihlberg T., Neu H. and Långström B. -Acta Chem. Scand. in press (1997)
- ¹² Viala J. and Labaudinière R. -J.Org Chem. 58: 1280 (1993)
- ¹³ Jung M.E., Lyster M. A., J. Am. Chem. Soc. 99:3: 968 (1977). Olah G.A., Narang S.C., Balaram Gupta GB., Malhotra R. -J. Org. Chem. 44: 1247 (1979)
- ¹⁴ Vogel's: Textbook of Practical Organic Chemistry Fourth edition, John Wiley and Sons New York, p 397, 1978
- ¹⁵ Anderson G.W. and Callahan F.M. -J. Am. Chem. Soc. 82: 3359 (1960)

- ¹⁶ Bjurling P., Reineck R., Westerberg G., Schultz J., Gee A., Sutcliff J. and Långström B. - VI:th Workshop on Targetry and Target Chemistry, Vancouver, B.C., Canada. (1995)
- ¹⁷ Långström B. and Lundquist H. -Radiochem. Radioanal. Lett. 41: 375 (1979)
- ¹⁸ Långström B., Antoni G., Gullberg P., Halldin C., Malmberg P. Någren K., Rimland A., and Svärd H. -J. Nucl. Med. 28: 1037 (1987)
- ¹⁹ Lipschutz B. H., Koerner M., Parker D. A. -Tetrahedron Lett. 28 945 (1987)
- ²⁰ Pattison F.L.M., Stothers J.B., Woolfordd R.G., -J. Chem. Soc. 2255 (1956)