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Research Article

Synthesis of diethyl [*carbonyl*-^{11}C]malonate from [^{11}C]carbon monoxide by rhodium-promoted carbonylation and its application as a reaction intermediate

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Summary

Rhodium-mediated carbonylation reaction was applied to synthesize diethyl [*carbonyl*-^{11}C]malonate using [^{11}C]carbon monoxide at low concentration. The synthesis was performed starting with ethyl diazoacetate, ethanol and the rhodium complex being made *in situ* by chloro(1,5-cyclooctadiene)rhodium(I) dimer ([Rh(cod)Cl]$_2$) and 1,2-*bis*(diphenylphosphino)ethane (dppe), and the reaction is assumed to proceed via a ketene intermediate. The isolated radiochemical yield was 20% (75% analytical radiochemical yield) and the trapping efficiency of [^{11}C]carbon monoxide in the order of 85%. The specific radioactivity of this compound was measured at 127 GBq/μmol (7.28 nmol total mass) after 8 μAh bombardment and 35 min synthesis. The corresponding ^{13}C-labelled compound was synthesized using (^{13}C)carbon monoxide to confirm the position of the carbonyl-labelled atom by ^{13}C-NMR. Diethyl [*carbonyl*-^{11}C]malonate was further used in subsequent alkylation step using ethyl iodide and tetrabutylammonium fluoride to obtain diethyl diethyl [*carbonyl*-^{11}C]malonate in 50% analytical radiochemical yield. Copyright © 2006 John Wiley & Sons, Ltd.

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Introduction

The development of positron emission tomography (PET) as a tracer technology with applications in non-invasive medical diagnosis,[1] biological research and drug development[2] is continuing. Thus, there is a need of new methods for labelling tracers with β^+-emitting radionuclides, and especially ^{11}C ($t_{1/2} = 20$ min) has been in focus.

In our search for new labelling strategies, the malonate has been of an interest[3] since this molecular structure is present in biologically active compounds. It is also known as a versatile precursor for the synthesis of other compounds such as barbiturates, artificial flavorings, vitamin B1 and vitamin B6.

Labelling of malonate structures using various ^{11}C-labelled halides have been previously reported.[3] The synthesized compounds were further used in cyclization step with urea as a labelling method of barbiturates compounds. The radiochemical yields obtained were in the range of 25–30% but this method was restricted to label malonic esters on a 2-alkyl group.

This limitation can be overcome using [^{11}C]carbon monoxide as a precursor using a semi-automated synthetic system permitting an efficient production and concentration of [^{11}C]carbon monoxide in a micro-autoclave[4] and high specific radioactivity.[5] This experimental setup had been successfully used to explore the reactivity of [^{11}C]carbon monoxide in metal-mediated carbonylation reactions and introducing [^{11}C]carbon into a molecule, such as [*carbonyl*-^{11}C]ketones,[6] [*carbonyl*-^{11}C]amides,[7] [*carbonyl*-^{11}C]imides,[8] [^{11}C]amines,[9] [*carbonyl*-^{11}C]hydrazines,[10] [*carboxyl*-^{11}C]acids,[11] [*carbonyl*-^{11}C]esters[12] or [*carbonyl*-^{11}C]carbothioates,[12] and also in radical-mediated reactions.[13] All these methods permit the synthesis of ^{11}C-labelled compounds in high specific radioactivity in a range between 100 and 1500 GBq/μmol.

The use of rhodium-mediated carbonylation with [^{11}C]carbon monoxide and azides for the synthesis of [*carbonyl*-^{11}C]isocyanate compound and its potential use as a versatile intermediate has been previously reported.[14] The presented method deals with the properties of diazo compounds to complex with rhodium complex to form a metallacarbene complex.[15] In the presence of [^{11}C]carbon monoxide,[16] this complex is expected to yield another versatile intermediate, a [*carbonyl*-^{11}C]ketene.[17] The possibility of synthesizing diethyl [*carbonyl*-^{11}C]malonate (**1**) from ethyl diazoacetate via a rhodium-mediated carbonylation reaction was thus explored. In this report, a one-step synthesis,

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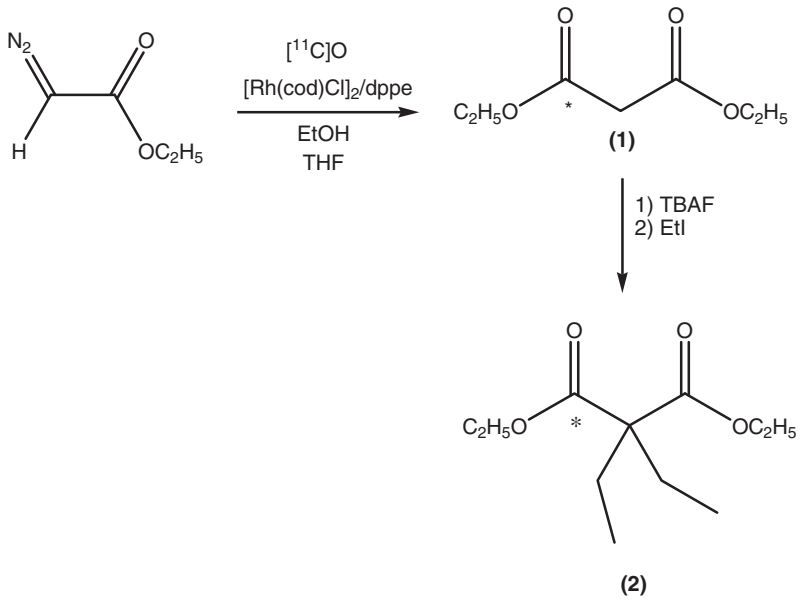

Scheme 1. Synthesis of diethyl [*carbonyl*-^{11}C]malonate and diethyl diethyl [*carbonyl*-^{11}C]malonate. * $=^{11}$C

characterization and purification of diethyl [*carbonyl*-^{11}C]malonate is presented and the availability of diethyl [*carbonyl*-^{11}C]malonate (**1**) as a versatile precursor for further reactions was successfully explored in the synthesis of diethyl diethyl [*carbonyl*-^{11}C]malonate (**2**) (Scheme 1).

Results and discussion

[^{11}C]Carbon dioxide was produced, transferred to the semi-automated system and there reduced to [^{11}C]carbon monoxide by passing it over Zn at 400°C using an apparatus containing concentration traps. The [^{11}C]carbon monoxide is concentrated and finally transferred to a 250 µl stainless steel micro-autoclave.[4] A solution containing ethyl diazoacetate, rhodium complex and ethanol was transferred into the micro-autoclave at a pressure of 35 MPa. The first rhodium complex tested for this reaction was rhodium(II) acetate dimer yielding the desired product in 25% analytical radiochemical yield (Table 1, entry 1). The decay-corrected analytical radiochemical yield was based on LC analysis of a sample withdraw from the reaction mixture, and calculated from the total amount of radioactivity present in the reaction mixture at the start of the synthesis. The results were improved using the complex generated *in situ* by mixing chloro(1,5-cyclooctadiene)-rhodium(I) dimer ([Rh(cod)Cl]$_2$) and 1,2-*bis*(diphenylphosphino)ethane (dppe) (Rh : dppe = 1 : 1) (Table 1, entry 2). However the main improvement

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Table 1. Radiochemical yields of diethyl [*carbonyl*-^{11}C]malonate

Entry	Rhodium complex	Temperature (°C)	Radiochemical yield (%)[a]	Trapping efficiency[b] (%)[c]
1	$Rh(OAc)_2$	100	25	38
2	$[Rh(cod)Cl]_2$/dppe	100	44	78
3	$[Rh(cod)Cl]_2$/dppe[d]	150	75 ± 4 (5)	85 ± 5 (5)

[a] Decay-corrected analytical radiochemical yield. Based on LC analysis of a sample withdraw from the reaction mixture, and calculated from the total amount of radioactivity present in the reaction mixture at the start of the synthesis. [b] Decay-corrected trapping efficiency, the fraction of radioactivity left in the crude product after purge with nitrogen. [c] The figure in brackets is the number of runs. [d] Late addition of EtOH.

occurred with an experimental observation, the late addition of ethanol in the reaction mixture before transfer to the charged micro-autoclave provoked an increase of the analytical radiochemical yield to 75% and of the trapping efficiency of [^{11}C]carbon monoxide to 85%. This observation is probably due to a side reaction of ethanol with ethyl diazoacetate.[18] The reaction proceeds nicely at 100 and 180°C but the best yields were obtained at 150°C when performed for 5 min. After isolation, the diethyl [*carbonyl*-^{11}C]malonate was obtained in 20 ± 7% isolated radiochemical yield. The isolated radiochemical yield was determined by measuring the radioactivity at the start of the synthesis and the radioactivity of the purified product and decay-corrected to the start of the synthesis. The reproducibility of the reaction was highly dependent on the quality of the ethyl diazoacetate.

The reaction occurs by complexation of ethyl diazocetate with the rhodium complex to form a rhodium-carbenoïd complex,[16] releasing nitrogen gas during the process. It was assumed that [^{11}C]carbon monoxide could insert the carbon–rhodium bond, and form a ketene–rhodium complex.[16] However, it remains uncertain if reductive elimination occurs to form the ketene compound[19] or if the ketene–rhodium complex readily reacting with a nucleophile such as ethanol[20] to give diethyl [*carbonyl*-^{11}C]malonate (Scheme 2).

Diethyl [*carbonyl*-^{11}C]malonate was characterized by LC-MS (m/z 160.8) and by comparison of LC-analysis with established reference. The position of the labelling was confirmed by synthesizing the ^{13}C-labelled product using (^{13}C)carbon monoxide at higher concentration. The labelled carbonyl position was identified by the ^{13}C-NMR analysis at 166.7 ppm which corresponds to the carbonyl carbon of the isotopically unmodified authentic reference compound. The specific radioactivity of the isolated diethyl [*carbonyl*-^{11}C]malonate was determined to 127 GBq/μmol after 35 min using a bombardment of 8 μAh.

The utility of diethyl [*carbonyl*-^{11}C]malonate for further synthetic protocols was explored in a model reaction. That proceeded in two steps: the malonate carbanion was generated by adding 2 M tetrabutylammonium fluoride

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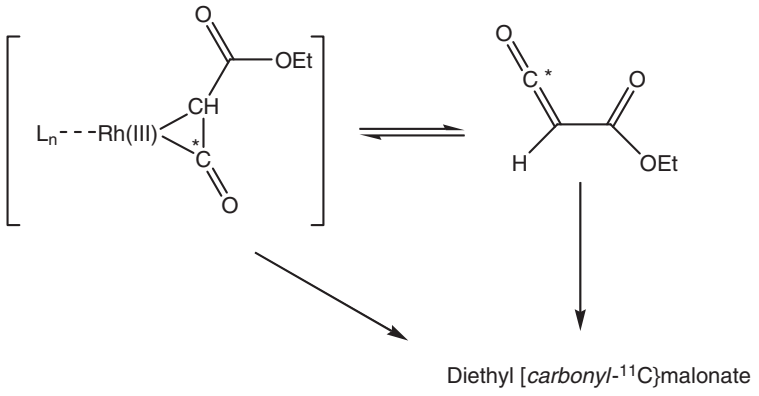

Scheme 2. Potential reaction intermediates in the synthesis of diethyl [*carbonyl*-^{11}C]malonate. * $=^{11}$C

solution in THF (TBAF), and the alkylation occurred using an excess of ethyl iodide. Diethyl diethyl [*carbonyl*-^{11}C]malonate was obtained in 50% analytical radiochemical yield. Strong bases like sodium hydride (NaH) are commonly used for the carbanion formation but milder conditions using tetrabutylammonium fluoride[21] were preferred due to the possible interactions of NaH with a functional group. There are intentions to explore the suitability of [*carbonyl*-^{11}C]ketene as intermediates for more complex labelling protocols.

Conclusion

A synthetic method for labelling of diethyl [*carbonyl*-^{11}C]malonate in the *carbonyl* position using [^{11}C]carbon monoxide in low concentration has successfully been developed. A diazo compound could be successfully used in a rhodium-mediated carbonylation reaction to produce the target compound in $20 \pm 7\%$ isolated radiochemical yield and 127 GBq/μmol specific radioactivity. This reaction was explored as an example of using [*carbonyl*-^{11}C]ketenes as a reaction intermediate to form useful synthetic precursors. As an illustration, this synthesis was extended to a further alkylation step for the production of diethyl diethyl [*carbonyl*-^{11}C]malonate. We are now in the process of utilizing this synthetic pathway towards labelling of series of [*carbonyl*-^{11}C]malonate compounds with the potential in the development of new PET tracers.

Experimental

General

[^{11}C]Carbon dioxide was produced via the ^{14}N(p,α)^{11}C reaction in a gas target containing nitrogen (AGA, Nitrogen 6.0) and 0.1% oxygen (AGA,

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Oxygen 4.8), bombarded with 17 MeV protons using a Scanditronix MC-17 cyclotron at GE Healthcare Imanet Uppsala. [^{11}C]Carbon dioxide was trapped on a Silica column at −196°C. The concentrated gas was released into a slow stream of helium gas (20 ml/min) by heating and passed through a small glass tube containing zinc at 400°C. The [^{11}C]carbon monoxide produced was trapped again on a short silica column at −196°C while unreacted [^{11}C]carbon dioxide is trapped on ascarite material. The [^{11}C]carbon monoxide was then released by warming the silica column to approximately 60°C and the radioactivity was transferred into the 200 µl micro-autoclave.

At the start of the experimental sessions, the stainless steel micro-autoclave had been washed with 10–15 ml THF.

Liquid chromatographic analysis (LC) was performed with a Beckman 126 gradient pump and a Beckman 166 variable wavelength UV-detector (Beckman Coulter, Inc., Fullarton, CA, USA) in series with a β^+-flow detector. The following mobile phases were used: 25 mM potassium dihydrogen phosphate (pH = 3.5) (A), acetonitrile:water, 50:7 (B), 0.005 M ammonium formiate (pH = 3.5) (C), acetonitrile (D) and 0.1 M formic acid (E). For analytical LC, a Beckman Ultrasphere ODS C18 (250 × 4.6 mm i.d.) column was used. For semi-preparative LC, a Beckman Ultrasphere ODS C18 (4 µm, 250 × 10 mm i.d.), column was used. Synthia, an automated synthesis system,[22] was used for LC injection and fraction collection. Data collection and LC control were performed using a Beckman System Gold chromatography software package.

Radioactivity was measured in a VDC-202 ion chamber (Veenstra Instrumenten BV, Joure, Netherlands). For rough measurements of radioactivity during synthesis, a portable dose-rate meter was used.

In analyses of the ^{11}C-labelled compounds, reference substances were used for comparison in all LC runs. Identities of synthesized ^{13}C-labelled compound were determined using NMR. NMR spectra were recorded on a Varian XL 400 NMR spectrometer (Varian Inc., Palo Halto, USA) operating at 400 MHz, and chloroform-d^3 was used as internal standard. LC-MS was performed using a Micromass Quattro Premier Mass Spectrometer (Waters Corp., Milford, MA, USA) with electrospray ionization and a Waters 2695 system for pumping the mobile phase and injecting the samples and an Atlantis column C18 (3 µm, 100 × 2.1 mm id).

All chemicals were purchased from Sigma-Aldrich (Sweden).

Synthesis of diethyl [*carbonyl*-^{11}C]malonate

To a capped vial (1 ml) containing a solution of ethyl diazoacetate (5.8 µl, 55 µmol) in dry THF (300 µl), was added [Rh(cod)Cl]$_2$ (0.27 mg, 0.55 µmol) and dppe (0.43 mg, 1.1 µmol). After addition of excess of desired nucleophile

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(ethanol, 10 μl), the resulting mixture was transferred to the micro-autoclave, which was pre-charged with [^{11}C]carbon monoxide. The micro-autoclave was heated at 150°C for 5 min and the crude product was transferred to a vial with reduced pressure. The radioactivity was measured before and after N_2 was bubbled through the reaction mixture in order to determine the [^{11}C]carbon monoxide trapping efficiency. A small amount of crude product was collected and analyzed by the reversed phase HPLC. The product was identified by LC with an added authentic reference compound.

Diethyl [*carbonyl*-^{11}C]malonate was analyzed using the following HPLC method: solvents A:B (80:20), isocratic for 15 min, flow rate 1.5 ml/min, detection 230 nm, retention time 4.7 min.

Diethyl [*carbonyl*-^{11}C]malonate was purified using the following LC method: solvent A:B (50:50), isocratic for 20 min; flow, 4 ml/min.

Diethyl [*carbonyl*-^{11}C]malonate was analyzed by LC-MS using the following LC method: solvent E:D (70:30), isocratic for 10 min; flow, 0.3 ml/min; *m*/*z*: 160.8.

Synthesis of diethyl (*carbonyl*-^{13}C)malonate

A vial (5 ml) was charged with ethyl diazoacetate (42 μl, 400 μmol), $[Rh(cod)Cl]_2$ (2 mg, 4 μmol), dppe (3.2 mg, 8 μmol) in THF (1 ml) and flushed with nitrogen. The vial was evacuated and charged with (^{13}C)carbon monoxide (10 ml). Ethanol (100 μl) was added. The mixture was heated at 100°C and stirred vigorously for 1 h. Diethyl [^{11}C]malonate was synthesized as described and was added to the reaction mixture. THF was evaporated from the reaction mixture under flow of nitrogen followed by addition of water (500 μl). The products were injected to the semi-preparative LC.

Diethyl (*carbonyl*-^{13}C)malonate was purified using the following LC method: solvent C:D (70:30), isocratic for 20 min; flow, 4 ml/min. ^{13}C-NMR (400 MHz, $CDCl_3$): 166.7 ppm.

Synthesis of diethyl diethyl [*carbonyl*-^{11}C]malonate

Diethyl [*carbonyl*-^{11}C]malonate was synthesized as described. To the crude mixture 300 μl of a 2 M solution of tetrabutyl ammonium fluoride solution in THF was added. The resulting mixture was heated 2 min at 60°C. Ethyl iodide (10 μl) was then added and the mixture was heated 3 min at 60°C. N_2 was bubbled through the reaction mixture in order to remove excess ethyl iodide. A small amount of crude product was collected and analyzed by the reversed phase LC. The product was identified by LC with an added authentic reference compound.

Diethyl diethyl [*carbonyl*-^{11}C]malonate was analyzed using the following LC method: solvents A:B (60:40), isocratic for 15 min, flow rate 1.5 ml/min, detection 230 nm, retention time 7.2 min.

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