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Research Article

Synthesis of [^{11}C-*carbonyl*]hydroxyureas by a rhodium-mediated carbonylation reaction using [^{11}C]carbon monoxide

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Summary

[^{11}C]Hydroxyurea has been successfully labelled using [^{11}C]carbon monoxide at low concentration. The decay-corrected radiochemical yield was 38 ± 3%, and the trapping efficiency of [^{11}C]carbon monoxide in the order of 90 ± 5%. This synthesis was performed by a rhodium-mediated carbonylation reaction starting with azidotrimethylsilane 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). (^{13}C)Hydroxyurea was synthesized using this method and the position of the labelling was confirmed by ^{13}C-NMR. In order to perform accurate LC–MS identification, the derivative 1-hydroxy-3-phenyl[^{11}C]urea was synthesized in a 35 ± 4% decay-corrected radiochemical yield. After 13 μA h bombardment and 21 min synthesis, 1.6 GBq of pure 1-hydroxy-3-phenyl[^{11}C]urea was collected starting from 6.75 GBq of [^{11}C]carbon monoxide and the specific radioactivity of this compound was in the order of 686 GBq/μmol (3.47 nmol total mass). [^{11}C]Hydroxyurea could be used in conjunction with PET to evaluate the uptake of this anticancer agent into tumour tissue in individual patients.

Key Words: [^{11}C]carbon monoxide; [^{11}C]hydroxyurea; rhodium-mediated carbonylation reaction; transporter systems

Introduction

The development of Positron Emission Tomography (PET) for applications in medical diagnosis and drug development[1] has opened up areas for new tracers labelled with β^+-emitting radionuclides such as ^{11}C-carbon ($t_{1/2} = 20.3$ min). Hydroxyurea is a commonly used anti-cancer agent and thus the availability of

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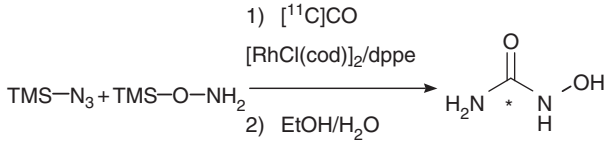
the tracer [^{11}C]hydroxyurea would allow PET studies of the organ distribution and kinetics of hydroxyurea, especially with respect to access to tumours and normal organs, including transport across the blood-brain-barrier.[2] Furthermore, there is interest in evaluation of pharmacokinetic interactions of other pharmacological agents, it has been demonstrated that hydroxyurea is a substrate for cellular efflux systems OATP2 and PgP,[2] and [^{11}C]hydroxyurea is a potential tracer.

[^{11}C]Hydroxyurea/isohydroxyurea have previously been synthesized using ^{11}C-cyanate and hydroxylamine hydrochloride, obtaining a 72% radiochemical yield of crude ^{11}C-labelled compounds.[3] Anyhow, the obtainment of pure [^{11}C]hydroxyurea has never been reported.

The presented synthesis involves the use of the properties of [^{11}C]carbon monoxide to react with a transition metal reagent. A series of transition metal-mediated carbonylation methods were developed in order to introduce the ^{11}C-carbon into the compound. For example selenium was used in the synthesis of ^{11}C-carbamoyl compounds,[4] palladium in the synthesis of [^{11}C]ketones,[5] [^{11}C]amides,[6] [^{11}C]imides,[7] [^{11}C]amines,[8] [^{11}C]hydrazines,[9] [^{11}C]carboxylic acids,[10] [^{11}C]esters[11] and [^{11}C]carbothioates,[11] and recently [^{11}C]isocyanatobenzene was used as a reaction intermediate in a rhodium-mediated synthesis of [^{11}C]*N*,*N*′-diphenylurea and [^{11}C]ethylphenylcarbamate[12] starting from phenyl azide. All these methods permit the synthesis of ^{11}C-labelled compounds in high specific radioactivity. It is known that organic azides readily react with a rhodium complex and undergo a carbonylation reaction at atmospheric pressure, producing ureas and carbamates in good yields in mild conditions.[13] Thus the possibility of synthesizing [^{11}C]hydroxyurea from azidotrimethylsilane via a rhodium-mediated carbonylation reaction was investigated (Scheme 1).

In this report, a two-step synthesis, characterization and purification of [^{11}C]hydroxyurea is described. This procedure was extended in the synthesis of 1-hydroxy-3-phenyl[^{11}C]urea.



Scheme 1. Synthesis of [^{11}C]hydroxyurea. * = ^{11}C/^{13}C. TMS: Trimethylsilyl

Results and discussion

The carbonylation reaction involves the use of a semi-automated synthetic system permitting the efficient production and concentration of [^{11}C]carbon monoxide (estimated to 0.1 mM) and high specific radioactivity.[14] [^{11}C]Carbon

dioxide was produced by cyclotron, transferred to the semi-automated system and there reduced with Zn to [^{11}C]carbon monoxide, concentrated and finally transferred to the 250 µl volume micro-autoclave, a teflon coated stainless steel cavity.[15]

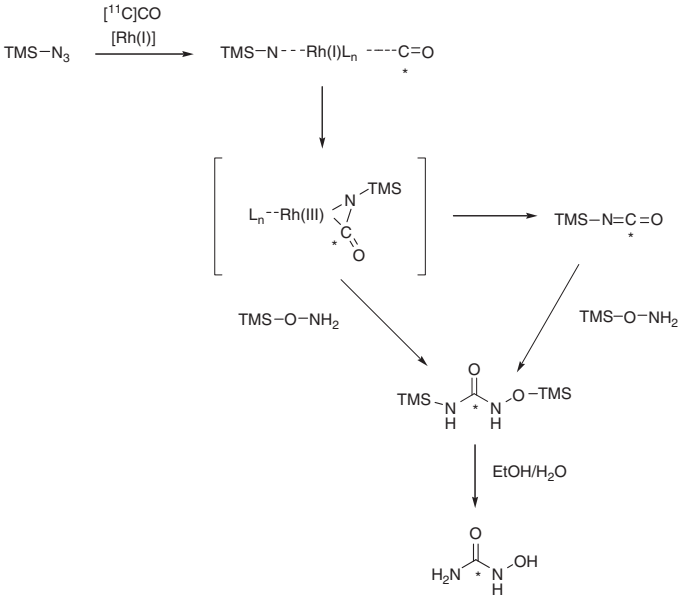
A solution containing azidotrimethylsilane, $[Rh(cod)Cl)]_2$, dppe and *O*-(trimethylsilyl)hydroxylamine in THF was transferred and pressurized to 35 MPa into the micro-autoclave. The reaction proceeded at 120°C for 5 min. After 1 min, a mixture of ethanol–water (70:30) was added to the resulting solution for deprotection of the trimethylsilyl groups. After purification and sterile filtration, the desired [^{11}C]hydroxyurea was obtained in a $38 \pm 3\%$ decay-corrected radiochemical yield in a sterile solution ready for injection. The isolated decay-corrected radiochemical yield was calculated from the initial amount of radioactivity, e.g. [^{11}C]carbon monoxide, and the radioactivity of the semi-preparative LC purified products. The purity of the [^{11}C] hydroxyurea obtained after purification by semi-preparative HPLC was better than 99.5%. The analytical radiochemical yield was based on HPLC analysis of a sample withdrawn from the reaction mixture and decay-corrected from beginning of analysis, and was determined at $78 \pm 2\%$, and the trapping efficiency of [^{11}C]carbon monoxide was $90 \pm 5\%$.

The suggested mechanism of this reaction involves the production of a nitrene compound as a reaction intermediate. The nitrene would react with [^{11}C]carbon monoxide in the presence of the rhodium complex to form an [^{11}C]isocyanate intermediate or a [^{11}C]isocyanate-coordinated rhodium complex intermediate.[16] Both intermediates are expected to have the same reactivity in the presence of *O*-(trimethylsilyl)hydroxylamine to form the TMS-protected [^{11}C]hydroxyurea which can be easily deprotected to [^{11}C] hydroxyurea (Scheme 2). It however remains uncertain if the reaction is concerted or stepwise.

The use of methanol instead of ethanol for the deprotection of the trimethylsilyl groups gave similar results. The observed bi-products were traces of the remaining compound protected by the trimethylsilyl group, and probably some [^{11}C]isocyanate-coordinated rhodium complex.

This procedure could be extended to labelling using (^{13}C)carbon monoxide, containing a stable carbon isotope and at higher concentration. The identity of the labelling position was confirmed by the ^{13}C-NMR analysis of ^{13}C-labelled hydroxyurea. The ^{13}C-NMR signal at 162.5 ppm corresponds to the carbonyl carbon of the authentic cold reference.

Due to the high polarity and low molecular weight of hydroxyurea, it was uncertain whether a clear identification of the labelled compound could be obtained using LC–MS. This problem was solved to some extent by synthesizing a derivative, i.e. 1-hydroxy-3-phenyl[^{11}C]urea (Figure 1) and thus to enable proper LC–MS identification and specific radioactivity measurements.



Scheme 2. Proposed mechanism for the synthesis of [^{11}C]hydroxyurea. * = ^{11}C. TMS: Trimethylsilyl

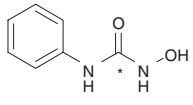


Figure 1. Structure of 1-hydroxy-3-phenyl[^{11}C]urea. * = ^{11}C

1-Hydroxy-3-phenyl[^{11}C]urea was synthesized using a similar procedure starting from phenylazide, and using [Rh(cod)Cl]$_2$/dppe as the rhodium complex and *O*-(trimethylsilyl) hydroxylamine as the nucleophile.

The purified product was obtained in 35 ± 4% radiochemical yield. The analytical radiochemical yield of 1-hydroxy-3-phenyl[^{11}C]urea was 68 ± 2%, and the trapping efficiency of [^{11}C]carbon monoxide was determined to be 90 ± 2%. The product was identified using LC–MS *m*/*z*: 153.

The specific radioactivity of the isolated 1-hydroxy-3-phenyl[^{11}C]urea was determined to be 686 GBq/μmol after 21 min upon bombardment of 13 μA h.

Conclusion

A general methodology for the synthesis of urea derivatives was successfully applied for the synthesis of [^{11}C]hydroxyurea and one analogue, giving the desired purified products in over 35% radiochemical yield (over 70% analytical) in a sterile solution and at over 99.5% purity. The availability of this

efficient and reproducible method for synthesizing [^{11}C]hydroxyurea would allow the study of hydroxyurea in humans.

Considering the chemistry of isocyanates, this method has been applied to one hydroxyurea derivative, and would be applicable to the synthesis of other model compounds, such as [^{11}C]amides[17], [^{11}C]isonitrile[17] and ^{11}C-labelled heterocyclic compounds[18] for the development of novel PET tracers.

This approach will be further explored.

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, Oxygen 4.8), bombarded with 17 MeV protons using a Scanditronix MC-17 cyclotron at Uppsala Imanet. [^{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. The gas flow was passed through a small tube containing zinc at 400°C. The [^{11}C]carbon monoxide produced was trapped again on a short silica column at -196°C. The [^{11}C]carbon monoxide was released by warming the silica column to approximatively 60°C and the radioactivity was transferred into a micro-autoclave.

At the start of the experimental sessions, the stainless steel micro-autoclave was washed with 10–15 ml THF. It was also washed with 1–2 ml THF after each experiment.

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: 0.01 M potassium hydrogen phosphate (A), acetonitrile (B), 9 mg/ml NaCl solution (C), 0.005 M ammonium formiate (pH = 3.5) (D), sterile water (E) and 0.1 M formic acid (F). 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, was used for LC injection and fraction collection.[19] 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 estimations of radioactivity during synthesis, a portable dose-rate meter was used.

In analyses of the ^{11}C-labelled compounds, unlabelled 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 Alto, USA) operating at 400 MHz, and dimethyl sulphoxide-d6 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 i.d.).

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

Synthesis of [^{11}C]hydroxyurea

To a capped 1 ml vial containing a solution of azidotrimethylsilane (7.3 μl, 55 μmol) in dry THF (300 μl), was added the complex prepared *in situ* by mixing $[Rh(cod)Cl]_2$ (0.27 mg, 0.55 μmol) and dppe (0.43 mg, 1.1 μmol). The vial was shaken until the solution was homogeneous, and then left at r.t. for 5 min. After addition of *O*-(trimethylsilyl)hydroxylamine (13.5 μl, 110 μmol), the resulting mixture was transferred to the micro-autoclave, which was pre-charged with [^{11}C]CO. The micro-autoclave was heated to 120°C for 5 min. The crude product was then transferred to a reduced-pressure vial. The reaction mixture was quenched with ethanol:water (70:30), shaken, and left at r.t. for 30 s. After purification, the solution was filtered (Dynagard ME, 22 μm) yielding a solution that was sterile. Radioactivity was measured before and after the vial was flushed with N_2 (the [^{11}C]CO trapping efficiency was determined based on these values). A small amount of crude product was collected and analysed using the reversed phase HPLC. The product was identified using HPLC with an added authentic reference compound.

[^{11}C]Hydroxylurea was analysed using the following HPLC method: solvents A:B (95:5), isocratic for 8 min, linear gradient to 20:80 for 5 min, then 1 min at 80% B; flow, 1.5 ml/min.

[^{11}C]Hydroxyurea was purified using the following HPLC method: solvent C (100%) isocratic for 20 min; flow, 4 ml/min.

Synthesis of 1-hydroxy-3-phenyl[^{11}C]urea. The procedure was the same as described above, except that phenylazide (5.5 μl, 55 μmol) was used.

The product was identified using HPLC with an added authentic reference compound.

1-Hydroxy-3-phenyl[^{11}C]urea was analysed using the following HPLC method: solvent D:B (85:15), isocratic for 15 min; flow, 2 ml/min.

1-Hydroxy-3-phenyl[^{11}C]urea was purified using the following HPLC method: solvent D:B (65:25) isocratic for 20 min; flow, 4 ml/min.

1-Hydroxy-3-phenyl[^{11}C]urea was analysed by LC–MS using the following LC method: solvent F:B (85:15) isocratic for 10 min; flow, 0.3 ml/min; m/z: 153.

Synthesis of (^{13}C)hydroxyurea. The reaction was performed as described before except that the micro-autoclave was pre-charged with [^{11}C]carbon monoxide and (^{13}C)carbon monoxide (450 µl), and then heated to 120°C for 25 min. Azidotrimethylsilane (26.6 µl, 200 µmol), [Rh(cod)Cl]$_2$ (1 mg, 2 µmol), dppe (1.6 mg, 4 µmol), and *O*-(trimethylsilyl)hydroxylamine (49 µl, 400 µmol) were used. The crude product was transferred to a reduced-pressure vial. The reaction mixture was quenched with ethanol:water (70:30), shaken, and left at room temperature for 30 s.

(^{13}C)Hydroxyurea was purified using the following HPLC method: solvent B:E (50:50) isocratic for 20 min; flow, 4 ml/min. ^{13}C-NMR (400 MHz, *d*-DMSO): 162.5 ppm.

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