Preparation of [^{11}C]diethyl oxalate and [^{11}C]oxalic acid and demonstration of their use in the synthesis of [^{11}C]-2,3-dihydroxyquinoxaline

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Summary

A method for the production of two new carbon-11 labelled difunctional radiolabelling precursors, [^{11}C]diethyl oxalate, **2**, and [^{11}C]oxalic acid, **3**, is described. Methyl chloroformate was reacted with no-carrier-added [^{11}C]cyanide to generate the intermediate nitrile, methyl [^{11}C]cyanoformate. Alcoholysis with HCl in ethanol generated **2**, which could subsequently be converted to **3** with aqueous acid. The total time of preparation from end-of-trapping of [^{11}C]cyanide was 6-7 min using combined microwave and thermal treatment or, by exclusively thermal treatment, 15 and 20 min for **2** and **3**, respectively. The radiochemical conversion of [^{11}C]cyanide to **2** and **3** was ~80% and ~70%, respectively. Both **2** and **3** were used in a model reaction with 1,2-phenylenediamine to synthesize the heterocyclic compound, 2,3-dihydroxyquinoxaline, a basic structural unit in antagonists for the excitatory amino acid receptor system.

Key words: Microwaves, PET, [^{11}C]cyanide, [^{11}C]diethyl oxalate, [^{11}C]oxalic acid, [^{11}C]2,3-dihydroxyquinoxaline

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Introduction

A large number of molecules of biological interest have been labelled with short-lived positron-emitting radionuclides for *in vivo* evaluation by positron emission tomography (PET). A critical requirement for the success of these methods has been the ready access to rapidly produced radiolabelling precursors with varying chemical functionalities. One of the most widely used nucleophilic precursors is [^{11}C]cyanide (CN^-) which can be produced on-line from accelerator-produced [^{11}C]carbon dioxide or [^{11}C]methane. The [^{11}C]nitriles generated with this

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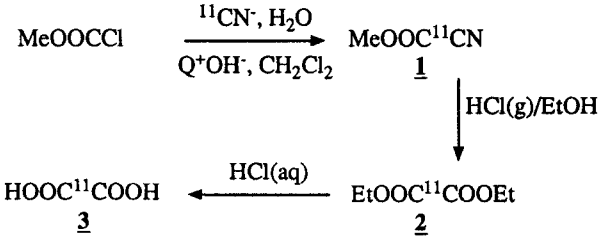
precursor are usually subsequently transformed into other functionalities. [^{11}C]CN^- has also been used to produce a number of labelled compounds that could be potentially used as multifunctional precursors (for example: aliphatic diacids (1,2), diamines (3-6) and diols (7), urea and its derivatives (8,9) as well as various functionalized nitriles (10,11)).

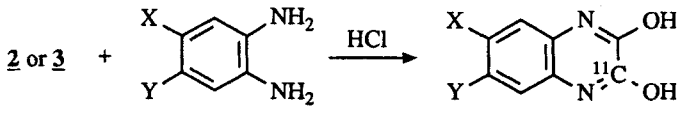
We present here two new difunctional radiolabelling precursors, [^{11}C]diethyl oxalate and [^{11}C]oxalic acid synthesized in 2 and 3 steps, respectively, from no-carrier-added [^{11}C]CN^-. To our knowledge, this is the first synthetic approach for rapidly preparing these ^{11}C-labelled compounds for use as radiolabelling precursors, although [^{11}C]oxalic acid has previously been generated from the oxidation of ^{11}C-labelled propionic acid and α- and β-hydroxypropionic acid (12). The reaction sequence used here is outlined below in Scheme 1. [^{11}C]CN^- was reacted with methyl chloroformate to generate methyl [^{11}C]cyanoformate, **1**. Transformation to [^{11}C]diethyl oxalate, **2**, was performed in one step using HCl(g)/ethanol. After evaporation of the solvent, the diester was hydrolysed to [^{11}C]oxalic acid, **3**, using aqueous acid.

Scheme 1



To demonstrate the potential of **2** and **3** as radiolabelling precursors, a model reaction with 1,2-phenylenediamine (X,Y=H) was performed to generate the 2,3-dihydroxyquinoxaline, [^{11}C]DHQ, as shown below in Scheme 2. This heterocycle is the basic structural unit of a family of compounds that are antagonists for the excitatory amino acid system (13). By varying the substituents on the aromatic ring, a number of ligands (for example, DNQX (X,Y=NO_2), CNQX (X=NO_2, Y=CN) and DCQX (X,Y=Cl)) might be synthesized by similar techniques for *in vivo* evaluation with PET.

Scheme 2



Results and Discussion

The on-line production of [^{11}C]CN$^-$ requires the presence of ammonia in the processing gas (14). As was observed here, a large amount of ammonia in the trapping solution can sometimes disturb the intended radiolabelling reaction. Ammonia has previously been removed in a two-step process by (**a**) trapping the cyanide in water and then distilling as [^{11}C]HCN after the addition of sulfuric acid (15), (**b**) trapping in small volumes of dilute base and evacuating to dryness under high vacuum (14) or by on-line methods using (**c**) powdered boric acid (4) or (**d**) a solution of 50% sulfuric acid (16). As in all clean-up procedures, varying amounts of radioactivity are lost and methods (**a**) and (**b**) require more handling time. Method (**d**) was found to work well for this procedure. At the end-of-trapping (E.O.T.) approximately 15% of the radioactivity remained in the sulfuric acid and the P_2O_5 drying tube. The [^{11}C]HCN generated could be efficiently trapped in water and ethanol (~95%), and well in acetonitrile (~80%).

To make a multi-step precursor synthesis feasible for routine radiolabelling procedures, the combined time of preparation should be as short as possible. Microwave treatment has been increasingly used to speed up reaction times in multi-step procedures thereby increasing the end-of-synthesis yields (17-25). In this study both microwave (26,27) and thermal treatment were used to effect the desired conversions, enabling a comparison of the two methods.

To produce 1, the reaction of methyl chloroformate with [^{11}C]CN$^-$ was first attempted in various polar solvents (28). In water and ethanol no reaction was observed, probably due to the decomposition of the starting material. In acetonitrile conversions of [^{11}C]CN$^-$ to 1 were approximately 40% at 100 °C after 10 min. Microwave treatment with 100 W magnetron input power for 0.5 min under otherwise identical conditions gave nearly the same results. The use of acetonitrile as a solvent in nucleophilic reactions with labelled cyanide has however previously

been reported to result in an exchange of the label with the solvent's nitrile function (29). The risk for lowered specific activity and the moderate yields obtained caused us to abandon this approach.

Methyl cyanoformates have previously been prepared from methyl chloroformate in a slurry with sodium cyanide (30) and with solid-liquid phase catalysis using dichloromethane and a crown-ether (31). Benzoyl nitriles have been synthesized using phase transfer catalysts in the reaction of sodium cyanide with benzoyl chloride (32). The latter method was chosen since the [^{11}C]HCN could be efficiently trapped in the aqueous media and the methyl chloroformate precursor could be subsequently added in an appropriate organic solvent.

Tetrabutylammonium hydroxide or bromide (Q^+OH^- or Q^+Br^-) was dissolved in water and the resulting solution was used to trap [^{11}C]CN^-. At E.O.T. methyl chloroformate dissolved in CH_2Cl_2 was added to the aqueous solution. The two-phase mixture was vigorously stirred at room temperature. When Q^+Br^- was used, a small amount of NaOH was required for reaction. Higher yields were obtained consistently with Q^+OH^-. Analysis by radio-HPLC indicated a radiochemical conversion after 5 min of [^{11}C]CN^- to **1** of ~90% and ~60% for Q^+OH^- and Q^+Br^-, respectively.

Attempts to hydrolyze **1** directly to **3** failed under both acidic and basic conditions. Analytical radio-HPLC indicated that **1** was primarily converted to an unidentified product with the same elution properties as [^{11}C]CN^-, and only a small amount of **3** was detected. Preliminary studies have recently indicated that [^{11}C]nitriles can be converted to [^{11}C]esters in good yields using alcoholic solutions of HCl(g) (Thorell, unpublished observations). This method was used here to produce the diester **2** which could subsequently be hydrolyzed to **3**.

The CH_2Cl_2 solution of **1** (85-90% of the total radioactivity) was transferred to a reaction vessel containing HCl(alc). By microwave treatment with 70 W magnetron input power for 0.5 min or thermal treatment at 60 °C for 10 min, radiochemical conversions on the order of 90% were obtained for **2**, according to analytical HPLC. Use of higher temperatures for the thermal procedure did not appreciably affect the reaction time under otherwise identical conditions. Yields of ~90% could however be obtained in 4-5 min thermally if the CH_2Cl_2 was evaporated

prior to reaction with HCl(alc). This procedure is therefore recommended for the exclusively thermal preparation of 2 in spite of the losses in radioactivity usually associated with evaporation procedures.

Hydrolysis of 2 to 3 required the use of aqueous media. Evaporation of the ethanol/CH_2Cl_2 solvent was performed with both microwave and thermal treatment. After 15 sec with 50 W the evaporation was complete, but approximately half of the activity was lost. Thermal treatment at 60 °C under reduced pressure required 3-4 min with approximately 25% of the total radioactivity lost. Since the product distribution of the residue was the same as prior to the evaporation, these losses are assumed to be due to the difficulty in controlling the evaporation (bumping) and may be reduced by optimizing these procedures technically.

The conversion of 2 to 3 with aqueous HCl was essentially quantitative with magnetron input power of 70 W for 15 sec, according to radio-HPLC analysis. The same conversions were obtained at 150 °C after 2 min under otherwise identical conditions.

The cyclocondensation of phenylenediamine with 2 and 3 was attempted with microwaves at different intensities and different times. At low input magnetron power (50-100 W) no reaction was observed in typical reaction times. At input power >125 W, pressure increased in the vessel which usually resulted in eruption of the septa and vaporization of the reaction mixture. Preliminary tests indicate that the rapid volatilization of the HCl used in the hydrolysis under these conditions is difficultly contained in the vessels used here. [^{11}C]DHQ was detected in the residue, but this method requires modification for safe reliable operation.

Thermal treatment of phenylenediamine and 2 or 3 in aqueous acid at 150 °C gave ~90% conversions to [^{11}C]DHQ after 5 min and 10 min, respectively. Lower temperatures or less substrate gave lower conversions (table 1). These reaction times are however common in carbon-11 chemistry and the results demonstrate the feasibility of using 2 and 3 as radiolabelling precursors to generate ^{11}C-labelled 2,3-dihydroxyquinoxalines. Preliminary experiments indicate that [^{11}C]DCQX (6,7-dichloroquinoxaline-2,3-dione) can be prepared in a similar fashion by the reaction of 2 or 3 with the dichloro phenylenediamine (Thorell, to be reported).

Table 1

		2 → [^{11}C]DHQ			3 → [^{11}C]DHQ		
Substrate	Temp °C	5 min	10 min	20 min	5 min	10 min	20 min
5 mg	120	15%	30%	53%	14%	18%	41%
10 mg	120	29%	40%	71%	23%	37%	88%
5 mg	150	73%	~90%	-	44%	70%	~90%
10 mg	150	~90%	-	-	65%	~90%	-

Conclusions

^{11}C-Labelled diethyl oxalate and oxalic acid were synthesized in a 2- and 3-step procedure, respectively, from [^{11}C]CN^- via methyl [^{11}C]cyanoformate. A combination of microwave and thermal treatment or exclusively thermal treatment could be used to effect the desired transformations. These difunctional radiolabelling precursors were subsequently reacted with 1,2-phenylenediamine to form ^{11}C-labelled 2,3-dihydroxyquinoxaline in a model reaction. The conversions and synthesis times for the individual reactions are summarized in table 2.

Table 2

Reaction	Conversion *		Time *		Total Time **	
	mw	T	mw	T	mw + T	T
^{11}CN→ 1	-	92 n=5	-	5	5	5
1 → 2	87 n=5	89 n=3	0.5	10	5.5	15
2 → 3	99 n=3	99 n=3	0.25	2	6	20
2 → [^{11}C]DHQ	-	87 n=2	-	5	11	23
3 → [^{11}C]DHQ	-	87 n=2	-	10	16	30

* Conversions (%, means of n experiments) and reaction times (min) for the individual step

** Min from E.O.T. Reaction times include the time required to evaporate the solvents in the conversion of 2 to 3

Appreciable time gains were achieved by the use, when possible, of microwave treatment in this synthetic procedure. Under otherwise identical reaction conditions, the total synthesis time from E.O.T. for [^{11}C]DHQ synthesized from 2 or 3 was 10-15 min shorter for the combined technique. This difference in reaction time could be further reduced, however, by modifying the

thermal procedure so that only a single solvent is used in the conversion of **1** to **2**. The conversions and reaction times for either procedure are feasible for radiolabelling procedures with carbon-11. Investigations are therefore continuing on the use of these two new difunctional precursors to synthesize biologically interesting heterocyclic compounds.

Experimental

General

Methyl chloroformate (MeOOCCl), methyl cyanoformate (MeOOCCN), diethyl oxalate (EtOOCCOOEt), 1,2-phenylenediamine (PDA) and 2,3-dihydroxyquinoxaline (DHQ) were obtained from Aldrich, tetrabutylammonium hydroxide (0.8 M in methanol) from Fluka Chemika, tetrabutylammonium bromide from Eastman Kodak Co and HCl gas from Aga Specialgas Sweden. The solvents and reagents were of analytical grade.

Analytical radio-HPLC was performed using a LDC Constametric III pump, a Rheodyne injector (7125 with a 250 μL loop), an Erma ERC-7210 UV-detector (wavelength used 215 or 254 nm) in series with a Beckman 170 β-flow radiodetector. The detectors were connected to a Shimadzu C-R2AX integrator. The analytical columns used were (A) Waters μ-Bondapak C-18 (300 x 3.9 mm, 10 μm), and (B) Biorad Aminex HPX 87-H (300 x 7.8 mm, 9 μm). The mobile phases were, for column A, 1) CH_3CN:H_3PO_4 (0.01 M)=7.5:92.5 at a flow rate of 1.5 mL/min and 2) NH_4OAc (0.01 M) at a flow rate of 1.5 mL/min, and, for column B, H_2SO_4 (0.01 N) at 1.0 mL/min. The retention times for the ^{11}C-labelled compounds on the analytical system used are given in table 3.

Table 3

Compound	Column : Mobile Phase	Retention Time (min)
[^{11}C]CN	A : 2	2.9
1	A : 2	4.9
2	A : 1	6.1
3	B	7.2
[^{11}C]DHQ	A : 1	4.7

The microwave treatment was performed with a prototype microwave cavity whose performance has recently been preliminary reported (26,27). The design of this prototype was based on our previous experiences with the use of a coaxial microwave resonance cavity to speed up radiolabelling reactions with $[^{18}F]F^-$ and $[^{11}C]CN^-$ (24,25). A controllable microwave field with a fixed frequency of 2450 MHz is introduced in a wave-guide type cavity where the reaction sample is placed in a position with maximum electric field strength. Only the cavity unit containing the magnetron is installed in the shielded working area and is connected via cables to a separate control unit outside the hot cell. These investigative studies were performed with <1.85 GBq (~50 mCi) $[^{11}C]CN^-$ and did not address the procedures necessary for handling large amounts of radioactivity. However, the open construction of the wave-guide lends itself well to the insertion and removal of reaction vessels by remote- or automated-control.

Pyrex tubes (10 mL, l=100 mm) equipped with screw caps and silicon/teflon septa were used in the microwave experiments. The reaction volume filled not more than 1/10th of the total volume of the tube, thereby allowing head space for pressure build-up during the treatment.

Radionuclide production

$[^{11}C]$Carbon dioxide (CO_2) was produced batchwise at the Karolinska Hospital/Institute with a Scanditronix MC 16 cyclotron using 17 MeV protons in the $^{14}N(p,\alpha)^{11}C$ reaction and converted on-line to $[^{11}C]$ammonium cyanide (NH_4CN) (14). Immediately after leaving the Pt oven, $[^{11}C]NH_4CN$ was flowed through H_2SO_4 (50%, 2 mL) (16) at 60 °C to generate $[^{11}C]HCN$ which then flowed through a tube containing powdered P_2O_5 (1.5 g).

Methyl $[^{11}C]$cyanoformate, **1**

The $[^{11}C]HCN$ generated as described above was trapped in a pear shaped vessel (5 mL), equipped with rubber stopper and stirring bar, and containing H_2O (0.5 mL) with Q^+OH^- (50 µL, 40 µmol) at 0-4 °C. At E.O.T. MeOOCCl (50 µL, 0.65 mmol) dissolved in CH_2Cl_2 was added to the aqueous solution. The reaction mixture was stirred vigorously for 5 min at room temperature.

[^{11}C]Diethyl oxalate, **2**

The organic phase was transferred to a reaction vessel containing HCl(g) in ethanol (3 M, 0.5 mL). The solution was treated with microwaves with a magnetron input power of 70 W for 0.5 min or heated at 60 °C for 10 min.

Evaporation:

Microwave: The Pyrex tube was vented to the atmosphere by insertion of a needle through the septa. The solvents evaporated rapidly under treatment with a magnetron input power of 50 W for 0.25 min.

Thermal: The reaction vessel was connected to a vacuum line and the solvents evaporated within 3-4 min at 60 °C with stirring.

[^{11}C]Oxalic acid, **3**

Aqueous HCl (4 M, 0.5 mL) was added to the residue remaining after evaporation and treated with a magnetron input power of 70 W for 0.25 min or heated at 150 °C for 2 min.

[^{11}C]2,3-Dihydroxyquinoxaline, [^{11}C]DHQ

The solution of [^{11}C]diethyl oxalate was evaporated and the residue redissolved in aqueous HCl (4 M, 0.5 mL) or the aqueous solution of [^{11}C]oxalic acid was used directly. Reaction with PDA (10 mg, 0.1 mmol) dissolved in H_2O (0.5 mL) was performed in a stoppered vessel with heating at 150 °C for 5 or 10 min, respectively.

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References

1. Allen M.B. and Ruben S. -J. Am. Chem. Soc. 64: 948 (1942)

2. De Spiegeleer B., Goethals P., Slegers G., Gillis E., Van den Bossche W. and De Moerloose P. -J. Nucl. Med. 29: 1107 (1988)

3. Winstead M.B., Dischino D.D., Munder N.A., Walsh C. and Winchell H.S. -Eur. J. Nucl. Med. 5: 165 (1980)

4. Jerabek P.A., Dence C.S., Kilbourn M.R., Welch M.J. and Kadmon D. -Int. J. Nucl. Med. Biol. 12: 349 (1985)

5. McPherson D.W., Fowler J.S., Wolf A.P., Arnett C.D., Brodie J.D. and Volkow N. -J. Nucl. Med. 26: 1186 (1985)

6. Somawardhana C.W., Sajjad M. and Lambrecht R.M. -Appl. Radiat. Isot. 42: 555 (1991)

7. Hassan M., Thorell J.O., Warne N. and Stone-Elander S. -Appl. Radiat. Isot. 42: 1055 (1991)

8. Emran A.M., Boothe T.E., Finn R.D., Vora M.M. and Kothari P.J. -Int. J. Appl. Radiat. Isot. 34: 1013 (1983)

9. Winstead M.B., Chern C.I., Lin T.H., Khentigan A., Lamb J.F. and Winchell H.S. -Int. J. Appl. Radiat. Isot. 29: 443 (1978)

10. Hörnfelt K., Antoni G. and Långström B. -Acta. Chem. Scand. 46: 87 (1992)

11. Antoni G. and Långström B. -Appl. Radiat. Isot. 43: 903 (1992)

12. Nahinsky P., Rice C.N., Ruben S. and Kamen M.D. -J. Am. Chem. Soc. 64: 2299 (1942)

13. Watkins J.C., Krogsgaard-Larsen P. and Honoré T. -Trends Pharmacol. Sci. 11: 25 (1990)

14. Christman D.R., Finn R.D., Karlstrom K.I. and Wolf A.P. -Int. J. Appl. Radiat. Isot. 26: 435 (1975).

15. Van Haver D., Rabi N.A., Vandewalle M., Goethals P. and Vandecasteele C. -J. Label. Cmpds. Radiopharm. 22: 657 (1985)

16. Maeda M., Nishimura S., Fukumura T. and Kojima M. -J. Label. Cmpds. Radiopharm. 25: 233 (1988)

17. Ferrieri R.A., Schlyer D.J., Wieland B.W. and Wolf A.P. -Int. J. Appl. Radiat. Isot. 34: 897 (1983)

18. Niisawa K., Ogawa K., Saito J., Taki K., Karasawa T. and Nozaki T. -Int. J. Appl. Radiat. Isot. 35: 29 (1984)

19. Hwang D.R., Moerlein S.M., Lang L. and Welch M.J. -J. Chem. Soc., Chem. Commun. 1799 (1987)

20. Hwang D.R., Dence C.S., McKinnon Z.A., Mathis C.J. and Welch M.J. -Nucl. Med. Biol. 18: 247 (1991)

21. Hwang D.R., Dence C.S., Gong J. and Welch M.J. -Appl. Radiat. Isot. 42: 1043 (1991)

22. Lemaire C., Cantineau R., Guillaume M., Plenevaux A. and Christiaens L. -J. Nucl. Med. 32: 2266 (1991)

23. Plenevaux A., Lemaire C., Palmer A.J., Damhaut P. and Comar D. -Appl. Radiat. Isot. 43: 1035 (1992)

24. Stone-Elander S. and Elander N. -Appl. Radiat. Isot. 42: 885 (1991)

25. Thorell J.-O., Stone-Elander S. and Elander N. -J. Label. Cmpds. Radiopharm. 31: 207 (1992)

26. Stone-Elander S. and Elander N. -Proceedings, 9:th Int. Symp. Radiopharm. Chem., Paris and J. Label. Cmpds. Radiopharm. (in press) (1992)

27. Stone-Elander S. and Elander N. -J. Nucl. Med. 33: 1026 (1992)

28. Ando T., Kawate T., Yamawaki J. and Hanafusa T. -Synthesis 637 (1983)

29. Jay M., Layton W.J. and Digenis G.A. -Tetrahedron Lett. 21: 2621 (1980)

30. Sheppard W.A. -J. Org. Chem. 27: 3756 (1962)

31. Childs M.E. and Weber W.P. -J. Org. Chem. 41: 3486 (1976)

32. Koenig K.E. and Weber W.P.. -Tetrahedron Lett. 26: 2275 (1974)