USE OF A MICROWAVE CAVITY TO REDUCE REACTION TIMES IN RADIOLABELLING WITH [^{11}C]CYANIDE

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Summary

Advantages of using a microwave cavity over thermal treatment are demonstrated for radiolabelling reactions with [^{11}C]cyanide. For comparison purposes, two literature syntheses involving typical cyanide chemistry at rather vigorous conditions were investigated: cyano-de-halogenation with subsequent hydrolysis of the nitrile and the Bücher-Strecker synthesis of an aromatic amino acid. Comparable yields were obtained with intensities <100 W in reaction times that were 1/15 to 1/20th those used in thermal methods. Even rates of slower nucleophilic substitutions could be increased by manipulating the polarity of the medium. For the short-lived radionuclide carbon-11, such time gains result in radioactivity gains at the end-of-synthesis on the order of 70-100%.

Key words: [^{11}C]cyanide, microwave cavity, salt effects, cyano-de-halogenation, Bücher-Strecker

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Introduction

Cyanide labelled with carbon-11 is an useful precursor for labelling radiopharmaceuticals with a positron-emitting nuclide. The ^{11}C-nitrile initially formed is usually an intermediate which can be converted to other functionalities such as acids, amides, amines etc. In spite of the fact that multi-step syntheses are often involved, quite a large number of endogenous compounds and drugs have been successfully prepared for *in vivo* studies of their distribution and metabolic pathways in animals or man with positron emission tomography (PET) (for a review see ref 1).

Due to the short half-life of carbon-11 ($t_{\frac{1}{2}}$=20.3 min) it is important that reaction times are kept as short as possible, especially where multi-step syntheses are concerned. Since the mid-1980s microwave ovens have been used to shorten reaction times in organic syntheses (2). They have been used in PET chemistry for quickly evaporating solvents (3) and in radiolabelling with [^{18}F]fluoride (4,5,6). Some studies have also described the production of radiolabelling

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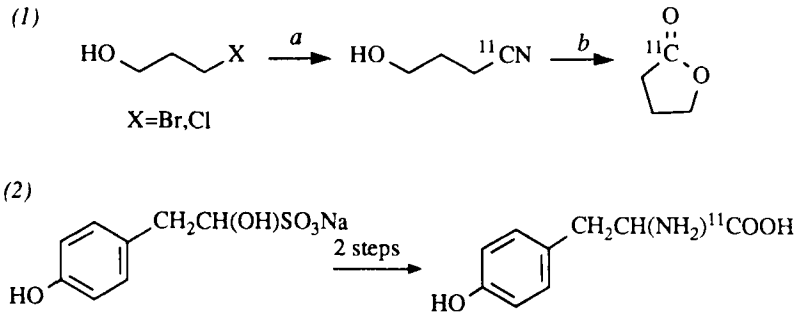
precursors in a microwave discharge: [^{13}N]ammonia (7) and [^{11}C]carbon disulfide and [^{11}C]hydrogen cyanide (8). A coaxial resonance microwave cavity has recently been presented as a variable means of generating high electromagnetic fields in small samples and its use in radiolabelling with ^{18}F has been illustrated (9, 10).

The purpose of this study, which has been preliminarily reported recently (11) was to investigate the use of this microwave resonance cavity to shorten reaction times in typical radiolabelling reactions with [^{11}C]cyanide: *(1)* a cyano-de-halogenation reaction and the subsequent hydrolysis of the nitrile and *(2)* the Bücher-Strecker synthesis of an amino acid (Scheme 1). The examples, chosen for their relative difficulty under thermal conditions, are from the published syntheses of [^{11}C]busulphan (12) and of D/L-[1-^{11}C]tyrosine (13).

Scheme 1



The study was designed to compare the time requirements for obtaining the same yields with microwave or thermal treatment. All reductions in reaction times lead to an end-of-synthesis (E.O.S.) radioactivity gain (due to the loss of less radioactivity from decay) expressed by the following equation:

$$A_G = \left(\frac{Y_M * e^{\{T_M * \ln(0.5)/t_{\frac{1}{2}}\}}}{Y_T * e^{\{T_T * \ln(0.5)/t_{\frac{1}{2}}\}}} - 1 \right) * 100$$

A_G=Radioactivity gain in per cent
Y_M,Y_T=Microwave and thermal yield (per cent of total radioactivity)
T_M,T_T=*Time requirement for microwave* and thermal treatment, respectively
$t_{\frac{1}{2}}$=Half-life of carbon-11, 20.3 min

Experimental

General

3-Bromopropanol (BrPrOH) was obtained from Merck and 3-chloropropanol (ClPrOH) from Aldrich. The salts, solvents, acids and bases were all of analytical grade and commercially available. p-Hydroxyphenylacetaldehyde bisulphite adduct was synthesized by a literature method (14).

^{11}C-Labelled ammonium cyanide ([^{11}C]NH_4CN) was produced in a two-step on-line conversion from [^{11}C]CO_2 (15) and trapped in water (0.5 mL). The radionuclide was obtained in a batch production using the Scanditronix MC 16 cyclotron at the Karolinska Hospital/Institute by irradiation with 17 MeV protons in the $^{14}N(p,\alpha)^{11}C$ reaction.

The microwave equipment (9) consisted of a separate magnetron connected to the coaxial microwave resonance cavity via a coaxial cable. The frequency was fixed at 2450 MHz and the intensity could be varied between 0-200 W. The applied intensities were not corrected for heat losses. The reaction vessel used was a stoppered Pyrex tube (i.d. 4 mm).

Analytical HPLC was performed using an LDC Constametric III pump, a Rheodyne injector (7125 with a 250 μL loop), an Erma ERC 7510 refractometer or an Erma ERC 7210 UV spectrophotometer in series with a Beckman model 170 β-flow radiodetector. The detectors were connected to a Shimadzu C-R2AX integrator. The analytical column used for reaction *(1)* was a Hamilton PRP-1 (305 x 7.0 mm, 10 μm). The mobile phase was CH_3CN:H_2O (5:95) at a flow rate of 3 mL/min. Retention times for [^{11}C]cyanide, [1-^{11}C]-4-hydroxybutyronitrile and [1-^{11}C]-γ-butyrolactone were 4.4 min, 5.1 min and 7.7 min, respectively. For reaction *(2)* a Waters μ-Bondapak NH_2 column (300 x 3.9 mm, 10 μm) was used for the analysis. The mobile phase was CH_3CN:H_2O (85:15) at a flow rate of 3 mL/min. Retention times for [^{11}C]cyanide and [1-^{11}C]tyrosine were 1.5 min and 7.5 min, respectively.

Chemistry

Cyano-de-halogenation (Rxn 1a)

Thermal: The published (12) thermal procedure was: At the end of trapping of [^{11}C]NH_4CN,

BrPrOH (0.3 mL, 3.3 mmol) dissolved in methanol (0.2 mL) or ClPrOH (0.3 mL, 3.6 mmol) was added and the solution was heated at 90 °C for 7 min with stirring. Radiochemical yields with BrPrOH: 60% end-of-trapping (E.O.T.) and with ClPrOH 12% (E.O.T.).

Microwave: BrPrOH (0.1 mL, 1.1 mmol) dissolved in methanol (0.1 mL) or ClPrOH (0.1 mL, 1.2 mmol) was added to the Pyrex tube, containing the aqueous solution (0.3 mL) of [^{11}C]NH_4CN situated in the microwave cavity. The reaction mixture with BrPrOH was treated with an intensity of 50 W for 0.5 min and ClPrOH with 70 W for 0.75 min.

The effect of the addition of salts (up to molar ratios of salt:halide=1:35) on radiochemical yields with thermal or microwave treatment was also investigated. Carrier KCN or NH_4Cl, KCl, KBr, K_3PO_4 or K_2CO_3 was added to the reaction vessel prior to the addition of the substrate. After the treatment with microwaves, the vessel was cooled and the reaction mixture was analyzed by radio-HPLC.

Hydrolysis of [1-^{11}C]-4-hydroxybutyronitrile (Rxn 1b)

Thermal: The thermal conditions (12) were: After the addition of H_2SO_4 (18 M, 0.5 mL), the reaction mixture was heated at 150 °C for 10 min. Radiochemical conversion: > 80% (E.O.T.) (based on formed nitrile).

Microwave: Concentrated H_2SO_4 (18 M, 0.1 mL) was added to the reaction mixture of [1-^{11}C]-4-hydroxybutyronitrile and treated with microwaves at an intensity of 50 W for 1 min. The reaction mixture was cooled in ice-water and immediately analyzed by radio-HPLC.

The Bücher-Strecker synthesis of D/L-[1-^{11}C]tyrosine (Rxn 2)

Thermal: The thermal conditions used (13) were: The [^{11}C]cyanide was trapped in a stainless steel reaction vessel containing NaOH (0.005 M, 1 mL), KCN (0.12 mmol), $(NH_4)_2CO_3$ (0.63 mmol) and p-hydroxyphenylacetaldehyde bisulfite adduct (0.33 mmol). The vessel was closed and heated at 170 °C for 10 min. After cooling, NaOH (10 M, 0.5 mL) was added. The reaction vessel was once more heated at 170 °C for 10 min. Radiochemical yield: 40-60% (E.O.T.).

Microwave: To a Pyrex tube containing p-hydroxyphenylacetaldehyde bisulphite adduct (0.1 mmol), $(NH_4)_2CO_3$ (0.19 mmol) and KCN (0.06 mmol), was added the aqueous solution of

[^{11}C]NH_4CN (0.5 mL). The solution was treated with microwaves at 80 W for 0.5 min. Aqueous NaOH (10 M, 0.1 mL) was subsequently added and the reaction mixture was treated at 80 W for another 0.5 min. The sample was cooled in ice-water and immediately analyzed by radio-HPLC.

Results and Discussion

The synthesis of nitriles from cyanide and organic substrates is a flexible means of increasing a carbon chain by one and generating a variety of functional groups on the terminal carbon. In PET radiochemistry [^{11}C]cyanide has been commonly used as a nucleophilic radiolabelling reagent since the label is often introduced late in the synthesis thus keeping the number of steps with subsequent isolation at a minimum. The incorporation of cyanide is favored by use of a polar medium. Aqueous medium is an advantage for reactions with [^{11}C]NH_4CN. It can be trapped directly at high flow rates and the ammonia added in the on-line production is usually not removed. This is not the case if, for example, aprotic solvents are used.

The labelling reactions chosen for this microwave investigation were all performed in aqueous media thermally. Commercially available microwave magnetrons generally have a set frequency at 2450 MHz which corresponds to a resonance frequency in the microwave spectrum of water. They are therefore well suited for optimizing reactions performed in water, but have also been shown to be useful for reactions in non-aqueous solutions where either the solvent or reagents have appropriate dielectric constants and loss factors (2, 4-6, 9, 10, 16)

Cyano-de-bromination (Rxn 1a)

The nucleophilic displacement of the bromide in BrPrOH with [^{11}C]CN^- was reported to proceed to a radiochemical yield of 60% after 7 min with heating at 90 °C. To find the microwave conditions necessary for obtaining the same yields, the intensity of the field and the time of treatment were varied. It was found that 60 W for 0.5 min was sufficient to produce [1-^{11}C]-4-hydroxybutyronitrile in 60% yield. Addition of carrier KCN did not affect the thermal yields but did increase the microwave yields by a factor 1.23 (Figure 1). The radioactivity gains achieved using the microwave cavity instead of thermal treatment in the nucleophilic reaction of [^{11}C]cyanide and BrPrOH are thus:

Radioactivity gain:	BrPrOH no-carrier-added (NCA)	25 %
	BrPrOH carrier KCN	56 %

Cyano-de-chlorination (Rxn 1a)

ClPrOH reacted more slowly than BrPrOH in the nucleophilic substitution by $[^{11}C]CN^-$ (11), giving radiochemical yields of only 12%. With microwave treatment it was necessary to use more power than with BrPrOH (70 W, 0.75 min) to obtain approximately the same yields (10%). Carrier KCN added to the reaction mixture increased both the thermal yields (by a factor of 1.9), and the microwave yields by a factor of 4.5 (Figure 1).

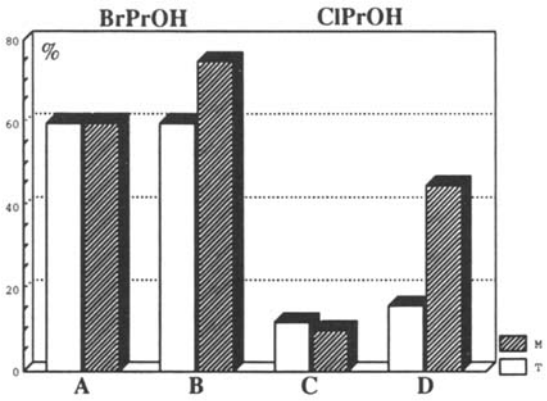


Figure 1: Radiochemical conversion of cyano-de-halogenations with thermal and microwave treatment respectively: (A) BrPrOH, NCA (B) BrPrOH, carrier KCN (C) ClPrOH, NCA and (D) ClPrOH, carrier KCN.

The addition of carrier, adjusting the molar ratio of reagents, is sometimes necessary so that reasonable yields can be obtained in the time constraints imposed by the radionuclide. In the microwave field, carrier may also contribute by increasing the polarity of the medium and thereby it's susceptibility to the electromagnetic field (16). However, the use of carrier may not always be advisable: for example, the additional mass may cause pharmacological reactions or partially block potential binding sites for the radioligand. For this reason, we investigated whether the yields of the slower cyano-de-chlorination could be affected by increasing the polarity with

non-cyanide salts (NH_4Cl, KCl, KBr, K_3PO_4 and K_2CO_3). The microwave results were compared with those obtained from thermal treatment (Figure 2).

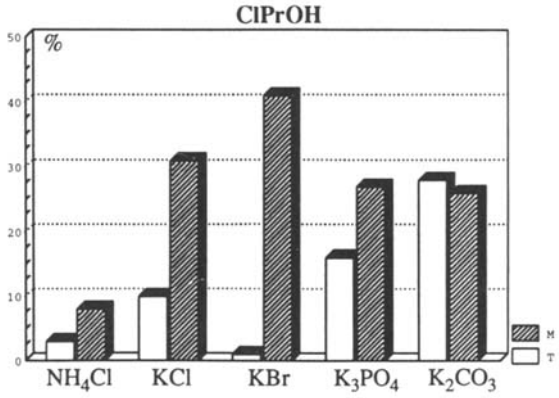


Figure 2: The effects of salts on conversions of [^{11}C]cyanide in cyano-de-chlorinations with thermal and microwave treatment.

NH_4Cl addition decreased radiochemical yields both under thermal and microwave treatment. Since the trapping solution is already saturated with the processing ammonia gas, the only new contribution is the Cl^-. The addition of large amounts of the leaving group may tend to reverse the direction of the reaction (common ion-effect). KCl addition showed the same effect as NH_4Cl on the thermal reaction (same or slightly lower yields), but, with microwave treatment, the yields increased by a factor of 3.1. Here the interaction of the ionic species with the electromagnetic field apparently overrides the dampening effect of the common ion addition.

KBr, K_3PO_4 and K_2CO_3 additions also increased the microwave yields (by factors of 4, 2.8 and 2.7, respectively) in a fashion similar to carrier cyanide. The results are consistent with, but, except for K_2CO_3, are larger than the corresponding yields obtained with thermal treatments. They demonstrate the feasibility of increasing the rates of slow reactions by manipulating the polarity of the samples.

Hydrolysis of [1-^{11}C]-4-hydroxybutyronitrile to [1-^{11}C]-γ-butyrolactone (Rxn 1b)

Hydrolysis of nitriles to acid functionalities may be achieved with either concentrated acid or base at relatively high temperatures. In the literature synthesis of [^{11}C]busulphan, acidic conditions were chosen since the carboxylic acid generated reacted intramolecularly with the

γ-hydroxy group to form [^{11}C]-γ-butyrolactone. The lipophilicity of the cyclized γ-butyrolactone could thus be used to facilitate separation of the product from the aqueous medium.

The ^{11}C-nitrile formed in the cyano-de-bromination above was hydrolyzed with concentrated acid to yield [1-^{11}C]-γ-butyrolactone. Under thermal treatment the ^{11}C-nitrile was converted to ^{11}C-lactone in 80% yield after 10 min at 150 °C. Microwave treatment gave the same conversions after 1 min at 50 W.

Radioactivity gain:	Hydrolysis step	36%

In summary, the total synthesis time for the two-step synthesis (*Rxn 1a+1b*) of [1-^{11}C]-γ-butyrolactone from BrPrOH and [^{11}C]cyanide is, for thermal treatment, 17 min and, for microwave treatment, 1.5 min. The microwave radioactivity gains for the NCA and carrier-added syntheses are thus:

Radioactivity gain:	S_N2 and hydrolysis, NCA	73%
	S_N2 and hydrolysis, carrier KCN	112%

The Bücher-Strecker synthesis of D/L-[1-^{11}C]tyrosine (Rxn 2)

The Bücher-Strecker synthesis is a versatile, widely used method for preparing α-amino acids. The method has been used to label amino acids with carbon-11 in the 1 or 2 position (for a review see ref 17). Heating [^{11}C]cyanide, an aldehyde precursor, $(NH_4)_2CO_3$ and sometimes NH_4Cl in aqueous solution generates an intermediate hydantoin which is hydrolyzed *in situ* with base. Carrier cyanide is almost always included since yields are usually low under non-carrier-conditions. High temperature (>150°C) and relatively long reaction times (ca 10 min per step) are generally necessary for reasonable yields of the D/L-[1-^{11}C]amino acid.

To investigate the utility of the microwave cavity in reducing Bücher-Strecker reaction times in syntheses with [^{11}C]cyanide, the literature synthesis of D/L-[1-^{11}C]tyrosine was used as an example. Under thermal conditions, conversion of cyanide on the order of 40-60% were obtained after 2 x 10 min at 170 °C in a steel bomb. Similar conversions (60%) with microwave treatment were obtained using slightly higher intensity (80 W) than for the nucleophilic substitutions above, but the reaction times for the hydantoin formation and hydrolysis were

radically reduced (2 x 0.5 min). The Pyrex tube was stoppered during the microwave treatment, so that even here pressure build-up in the reaction vessel contributed to the yields obtained. Reduction of the total reaction time from 20 min to 1 min yielded therefore the following:

Radioactivity gain:	2-step Bücher-Strecker	91%

Conclusions

It has been demonstrated here that a microwave cavity can be successfully used to shorten reaction times for typical syntheses involving NCA and carrier added [^{11}C]cyanide. Though the microwave reactions were not optimized, yields comparable to or better than thermal procedures were easily obtained with low microwave intensity (<100 W) in reaction times of 0.5-1 min for cyano-de-halogenations, nitrile hydrolysis and a Bücher-Strecker synthesis. The reduction in reaction times are consistent with increases in reaction rates due to higher temperatures from superheating of the reaction medium and pressure build-up in the reaction vessel during microwave treatment.

Increasing the polarity of the medium by addition of salts also increased the radiochemical conversion of [^{11}C]cyanide by up to a factor of 4 for the slower cyano-de-chlorination reaction. This effect was observed even when the common ion Cl^- was added as KCl, an addition which depressed the reaction rates thermally. A possible explanation for this effect is that ionic species, being even more susceptible to microwave treatment than dipolar compounds, increase the samples absorption of microwaves at any given intensity, thereby accelerating the reaction even more.

The use of microwave technology instead of thermal treatment potentially implies a simplification of the technical apparatus used in some multi-step syntheses. The cavity does not retain heat after treatment of the sample. The same apparatus can therefore be used for different reactions vessels or, alternatively, the same vessel requiring several different temperatures for successive chemical transformations.

The time reductions for the short-lived radionuclide carbon-11 were on the order of one half-life of the radionuclide. Therefore, for comparable yields, nearly 100% more radioactive

product is obtained at E.O.S. with microwave treatment than for the thermal procedure. This has the practical implication that less starting radioactivity is required to produce a given amount of radiotracer or that radiolabelling routes previously inaccessible due to the time constraints imposed by the half-life of the nuclide may now be possible.

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