Synthesis of $^{11}$C-Labelled Haloalkanonitriles and Examples of their Use in Some Alkylation Reactions

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The synthesis of the $^{11}$C-labelled bifunctional precursors 4-iodobutyro-$^{11}$C]nitrile (1), 4-tosloylbutyro-$^{11}$C]nitrile (2), 5-iodoaleryl-$^{11}$C]nitrile (3), 5-tosloylaleryl-$^{11}$C]nitrile (4) and 4-bromopentano-$^{11}$C]nitrile (5) are presented. The nucleophilic substitution reactions of $^{11}$C]cyanide with dibromides, diiodides, ditosylates or mixed iodotosylates producing 1-5 have been carried out in different solvents and the labelled products were obtained in 62-98% radiochemical yields (not isolated), with a total synthesis time of 5 min counted from the end of the hydrogen $^{11}$C]cyanide synthesis. The labelled haloalkanonitriles 1 and 3 have also been used in some alkylation reactions with various carbon and oxygen nucleophiles.

Biomolecules labelled with short-lived positron-emitting radionuclides, such as $^{11}$C, $^{13}$N, $^{15}$O and $^{18}$F ($t_{1/2} = 20.3$, 10.0, 2.0 and 110 min, respectively) can be used in combination with positron emission tomography (PET) to study biochemical and physiological processes in vivo. In the development of radiotracers for use in PET studies it is necessary to increase the number of labelled precursors to open up new methods for the fast and reliable synthesis of biomedically interesting, labelled molecules. The introduction of functional groups in the labelled precursor makes it possible to obtain more differentiated molecular structures in rapid labelling synthesis. Another important aspect is that molecules labelled in more than one position seem to be useful in the understanding of data from PET studies. These facts verify the need to increase the number of available $^{11}$C-labelled precursors.

$^{11}$C-Labelled alkyl iodides have proved useful because of their ease of production and reactivity towards many nucleophiles. To date, non-functionalized primary and secondary $^{11}$C]alkyl iodides and $^{11}$C]phenethyl iodides have been prepared in addition to functionalized alkyl iodides such as $^{11}$C]benzyl iodides and methoxy-$^{11}$C]benzyl iodides. Other useful reactive precursors are $^{11}$C]nitromethane and $^{11}$C]aldehyde$^{7a,b}$. $^{11}$C]Acrylonitrile and $^{11}$C]cinnamonic acid have been synthesized recently $^{8a,b}$.

The synthesis of the $^{11}$C-labelled precursors 4-iodobutyro-$^{11}$C]nitrile (1), 4-tosloylbutyro-$^{11}$C]nitrile (2) and 5-iodoaleryl-$^{11}$C]nitrile (3), 5-tosloylaleryl-$^{11}$C]nitrile (4) are here presented (Scheme 1). The labelled nitriles were prepared in a one-pot procedure from potassium $^{11}$C]cyanide via a nucleophilic substitution reaction with the corresponding diiodide, ditosylate, or mixed iodotosylate compound with the amino polyether 4,7,13,16,21,24-hexaoxa-1,10-diazacyclo[8.8.8]hexacosane (Kryptofix [2.2.2]) as an anion-activating agent. The substitution reactions have been performed in tetrahydrofuran (THF) and the reactions with the diiodides in THF or N,N-dimethylformamide (DMF). The iodoo- and tosloyl-alkanonitriles were used without purification in some alkylation reactions with various carbon and oxygen nucleophiles. To test the usefulness of the labelled iodoalkanonitriles (1, 3) as reactive precursors in labelling syntheses, alkylation reactions were performed on a chiral glycine derivative [α]-2-hydroxypropan-3-ylidene]glycine tert-butyl ester$^{10}$ under anhydrous conditions in THF (Scheme 2), dimethyl malonate in DMF (Scheme 3) and sodium 3-nitrophenolate in DMF (Scheme 4). The use of these functionalized precursors opens up the possibilities for the synthesis of various carbon and oxygen labelled molecules.

Scheme 1.

\[
\begin{align*}
X-(\text{CH}_2)_n-Y + K^{11}\text{CN} & \xrightarrow{\text{K_2CO_3} / \text{K}^+ / \text{base} / 0-70^\circ \text{C} / 5 \text{min}} X-(\text{CH}_2)_n^{11}\text{CN} \\
(a) & X = Y = \text{iodo} \quad X = \text{iodo} \quad n = 3 \quad 1,3 \\
(b) & X = Y = \text{tosloxy} \quad X = \text{tosloxy} \quad 2,4 \\
(c) & X = \text{tosloxy} \quad Y = \text{iodo} \quad X = \text{tosloxy} \quad 2,4 \\
\quad \text{base: KOH} 
\end{align*}
\]

\text{Scheme 2.}

n = 3 or 4

$^{11}$C]nitrile

\[
\begin{align*}
\text{OH} & \xrightarrow{1) \text{TMPD/Py} / \text{THF} / 40^\circ \text{C}} \text{N-C(OH)}_2 \text{COO}^+ \\
\text{OH} & \xrightarrow{2) \text{I-(CH}_2)_n-Y} \text{N-C(OH)}_2 \text{COO}^+ \\
\quad \text{Thermodynamic equilibrium} \\
6a; & n = 3 \\
6b; & n = 4 
\end{align*}
\]

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possibility of preparing, e.g., basic and acidic $^{13}$C-labelled amino acids such as $[6-^{13}$C]lysine and the analogous acid 2-amino[6-$^{13}$C]adipic acid.

The synthesis of 4-bromopentanol[13C]nitrite (5) is also presented, as an example of a branched labelled haloalkanocnitrile. Compound 5 was synthesized via a nucleophilic substitution reaction from the corresponding dibromide compound in four different solvents, THF, DMF, dimethyl sulfoxide (DMSO) and acetonitrile (MeCN).

**Experimental**

General. $^{13}$C was produced at the tandem van der Graaff accelerator at the The Svedberg Laboratory at Uppsala University using 10 MeV protons in the $^{14}$N(p,α)$^{13}$C reaction on a nitrogen target. The $^{13}$C-carbon dioxide obtained was trapped in a lead-shielded oven containing 4 Å molecular sieves and transported to the chemistry laboratory. Hydrogen $^{13}$C-cyanide was then produced by the reaction of $^{13}$C-carbon dioxide with H$_2$/Ni catalyst at 400°C to $^{13}$C-methane followed by NH$_3$/Pt at 1000°C. The production time from $^{13}$C-carbon dioxide to $^{13}$C-cyanide was ca. 4 min.

HPLC analyses were performed on a Hewlett-Packard 1090 liquid chromatograph with a UV-diode array detector in series with β$^+$-flow detector. The column used was 250×4.6 mm (i.d.) C-18 Nucleosil 10 μm (A). The mobile phases used were 0.01 M potassium dihydrogen phosphate, pH 4.6 (B) and methanol (C), flow 2.0 ml min$^{-1}$. For analytical GC, a Hewlett-Packard 5880A gas chromatograph (flame ionization detector) was used with a 2.5% Apiezon Chromosorb W-ÅW DMCS, 100/120 1/8”×2.0 m DMCS-GLAS column in series with a β$^+$-gasflow detector. The GC-MS analyses were performed with a capillary GC (Varian 3400) connected to a mass spectrometer (Finnigan Incos 50 Mass Spectrometer), column: 30 m×0.32 mm, liquid phase DB-5, film thickness 0.25 μm, temperature gradient; 70–250°C, 10°C min$^{-1}$. NMR spectra were run on a Varian XL-300 spectrometer, $^{13}$C NMR spectra (75.4 MHz) with CDCl$_3$ as the solvent. Tetrahydrofuran (THF) was dried by distillation from sodium–benzophenone under a nitrogen atmosphere.

**General procedure for synthesis of iodo- and toslyoxy-alkanoid[13C]nitriles (1–4), Scheme 1, and 4-bromopentano[13C]nitrite (5).** In a septum-equipped reaction vial, 2.5 mg (7.5 μmol) Kryptofix [2.2.2] and 0.5 μl 1.0 M aqueous potassium hydroxide solution (0.5 μmol) were dissolved in

### Table 1. Radiochemical yields under different reaction conditions for the labelled bromo-, iodo- and toslyoxy-alkanocnitriles.

<table>
<thead>
<tr>
<th>Reaction (see Scheme 1)</th>
<th>Product</th>
<th>Radiochemical yield (%)$^a$</th>
<th>Solvent$^b$</th>
<th>Reaction temp./°C</th>
<th>Reaction time/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>4-iodobutyro[13C]nitrite (1)</td>
<td>94</td>
<td>THF</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>(b)</td>
<td>4-toslyoxybutyro[13C]nitrite (2)</td>
<td>92</td>
<td>THF</td>
<td>60</td>
<td>5</td>
</tr>
<tr>
<td>(c)</td>
<td>4-toslyoxybutyro[13C]nitrite (3)</td>
<td>98</td>
<td>THF</td>
<td>70</td>
<td>5</td>
</tr>
<tr>
<td>(b)</td>
<td>5-toslyoxyvalero[13C]nitrite (4)</td>
<td>98</td>
<td>THF</td>
<td>70</td>
<td>4</td>
</tr>
<tr>
<td>(c)</td>
<td>4-bromopentano[13C]nitrite (5)</td>
<td>73</td>
<td>THF</td>
<td>80</td>
<td>5</td>
</tr>
</tbody>
</table>

$^a$Determined by HPLC analysis of the reaction mixture as a percentage of the total amount of radioactivity in the sample, based on the total amount of [13C]cyanide. $^b$0.5 ml reaction volume with (10–20)×10$^{-3}$ mol of substrate.
400 µl solvent. Hydrogen [14C]cyanide, prepared as briefly described above, was trapped on-line at room temperature. After trapping, 10–20 µmol substrate in 200 µl solvent were added. Results and reaction conditions for the synthesis of 1–4 are presented in Table 1. The labelled products (1, 3) were used directly in the alkylation reactions. Identity and radiochemical purity for compounds 1–4 were determined by HPLC using column A (solvents B/C 60/40 v/v linear gradient to 10/90 over 4–7 min, flow 2 ml min⁻¹, column temp. 40°C, wavelength 254 nm) and for compound 5 column A (solvent B/C 80/20 linear gradient to 10/90 over 2–10 min, flow 2 ml min⁻¹, column temp. 40°C, wavelength 254 nm).

**Synthesis of compounds 6a and 6b, Scheme 2.** The ketol imine 6 (30 mg, 0.11 mmol) was dissolved in THF/DMF/U (1,3-dimethyl-2,4,5,6-tetrahydro-2-pyrimidinone) (150/50 µl and 255 µl (0.21 mmol) of a solution of 0.75 ml butyllithium (1.6 M in hexane), 195 µl TMP (2,2,6,6-tetramethylpiperdine) and 525 µl THF were added at −40°C. Base solution (1.9 equiv.) was added, calculated from the molar amount of ketol imine. The solution turned red immediately which indicated anion formation and was then allowed to stand for at least 10 min while the iodoalkano-[14C]nitriles (1, 3) were prepared as described above. Compound 1 or 3 was added, and the reaction mixture kept at −40°C for 10 min. The identity and radiochemical purity were determined by HPLC, using column A (solvents B/C 60/40 v/v linear gradient to 10/90 over 4–7 min, flow 2 ml min⁻¹, column temp. 40°C, wavelength 254 nm). The retention times were 4.2, 6.2, 8.4 and 9.5 min for 4-iodobutyro[14C]nitrile (1), 5-iodovaleral[14C]nitrile (3), the alkylated products 6a and 6b, respectively.

**Synthesis of dimethyl 2-[3-([14C]cyano)propyl]malonate (7a) and dimethyl 2-[4-([14C]cyano)butyl]malonate (7b), Scheme 3.** In a 1 ml septum-equipped glass vial, 10 mg (76 µmol) dimethyl malonate and 3.5 g (73 µmol) sodium hydride (50 % dispersion in oil) were dissolved in 200 µl DMF. The turbid solution was heated at 80°C for 10 min before the labelled 4-iodobutyro[14C]nitrile (1) or 5-iodovaleral[14C]nitrile (3) prepared according to the procedure described above, was transferred to the malonate-anion. The reaction mixture was then heated at 130°C for 10 min. The identity and radiochemical purity of the products were determined by HPLC under the following conditions: column A (solvents B/C 70/20 v/v linear gradient to 10/90 over 2–7 min, flow 2 ml min⁻¹, column temp. 40°C, wavelength 230 nm). The retention times were 6.2, 6.8, 5.5 and 6.0 min for 4-iodobutyro[14C]nitrile (1), 5-iodovaleral-[14C]nitrile (3), dimethyl 2-[3-(14C]cyanopropyl)malonate (7a) and dimethyl 2-[4-(14C]cyanobutyl)malonate (7b), respectively. The corresponding reference compound was mixed with the radioactive sample and the crude product purified by HPLC. The fractions were evaporated to dryness, the residue dissolved in 1 ml diethyl ether and subsequently analysed with GC-MS. The retention times for the alkyl malonates were 10.7 (7a) and 12.2 min (7b) on the gas-chromatogram. For the conditions for GC analysis, see the Experimental section under General.

**Synthesis of 3-nitrophenyl alkano[14C]nitrile ethers (8a,b) Scheme 4.** In a 2 ml septum-equipped glass vial, 16 mg (0.1 mmol) sodium 3-nitrophenolate were dissolved in 400 µl DMF. 4-Iodobutyro[14C]nitrile (1) or 5-iodovaleral[14C]-nitrile (3), prepared according to the one-pot procedure described above in DMF, was added to the reaction vial with a syringe. The reaction mixture was heated at 120°C for 10 min, to yield the labelled products in ca. 70 % (8a) and 98 % (8b) radiochemical yields. The identity and radiochemical purity were determined by HPLC using column A (solvents B/C 60/40 v/v linear gradient to 10/90 over 4–7 min, flow 2 ml min⁻¹, column temp. 40°C, wavelength 254 nm). The retention times were 7.4 and 7.9 min for 3-([14C]cyano)propyl 3-nitrophenyl ether (8a) and 4-([14C]cyano)butyl 3-nitrophenyl ether (8b), respectively.

**Results and discussion**

4-Iodo- and 4-tosloxy-butyro[14C]nitrile (1, 3) and 5-ido- and 5-tosloxy-valeralo[14C]nitrile (2, 4) were prepared as shown in Scheme 1. A nucleophilic substitution reaction between [14C]cyanide and the corresponding diodoate, ditosylate or mixed idotosylate compound gave the labelled nitriles (1–4) in 85–98 % radiochemical yield within 5 min. It was observed that the reaction between the diidoalkanes (n = 3 or 4) and [14C]cyanide was not very sensitive to the reaction temperature. Within 5 min the corresponding nitriles (1, 3) were obtained in 90–98 % radiochemical yield in the temperature range 0–130°C. The reactions gave similarly high yields in DMF as in THF (Table 1). This permits the choice of a suitable solvent for subsequent alkylation reactions. The tosloxy-containing alkane substrates were converted into the expected tosloxyalkanono-nitriles (2, 4) at ca. 70°C within 5 min, in THF (Table 1). The substrates for the tosloxyalkanono[14C]nitriles were synthesized from the corresponding diodoide compounds.

The molar proportions in the substitution reaction between the diidoalkane and silver tosylate was chosen such that the ditosloxy- and iditosloxy-alkanes (n = 3 or 4) were formed in the same reaction, then separated by dry column flash chromatography.

The synthesis of the corresponding 14C-labelled nitrile with n = 2, from diiido-, diitosloxy- or ditosloxy-ethane was not successful. An unidentified product was obtained in > 80 % radiochemical yield within 5 min in the temperature range 0–130°C. The possibility of an elimination reaction taking place producing acrylo[14C]nitrile was ruled out by adding acrylonitrile as a reference compound and comparing the retention times by HPLC. Work is in progress to identify the product. Obviously, other methods are needed to produce 3-idopropiol[14C]nitrile.

The use of 14C-labelled iodoalkanono-nitriles (1, 3) in alkyla-
tion reactions have been investigated with three nucleophiles.

(1) Carbon nucleophile: \((+)-2\)-hydroxyquin-3-yliodeneglycine tert-butyl ester Scheme 2. After having formed the anion, an alkylation reaction was carried out under anhydrous reaction conditions in THF at \(-40^\circ\text{C}\). After 10 min, all the \(^{13}\text{C}\)-labelled iodoalkanitrite \((1,3)\) had been consumed to yield the alkylation products \((6\text{a, b})\) in \(> 80\%\) radiochemical purity and in \(> 90\%\) radiochemical yield. The major impurity was unchanged \(^{13}\text{C}\)-cyanide. The asymmetric alkylation reaction makes possible the formation of \(1-6^{13}\text{C}\)-lysine or 2,7-diamino[7-13C]heptanoic acid after removal of the protecting groups and reduction of the nitrile to an amine. Removal of the protecting groups with strong base produces the analogous acidic amino acids 2-amino[6-13C]adipic acid and 2-amino[7-13C]pimelic acid, the former of which has been synthesized, and will be presented elsewhere.

(2) Carbon nucleophile: dimethyl malonate Scheme 3. The anion was formed by addition of sodium hydride. After the addition of 1 or 3, the reaction mixture was heated for 10 min at \(130^\circ\text{C}\) and the corresponding \(^{13}\text{C}\)-labelled dimethyl (cyanooalkyl)malonates \((7\text{a, b})\) were obtained in \(> 98\%\) radiochemical yield. The high yields were not achieved at lower reaction temperatures with longer reaction times. To verify their identity, the labelled products were trapped after addition of a reference compound on an analytical HPLC-column and analysed by GC-MS; the mass spectra corresponded to the proposed products. To confirm that the labelling position was correct a \(^{13}\text{CN}^{15}\text{CN}\)-synthesis was carried out and the labelled product was purified by HPLC. The \(^{13}\text{C}\) NMR spectrum of the \(^{13}\text{C}\)-labelled product \((7\text{a})\) showed only one peak at 119.7 ppm, the same chemical shift as for the nitrile carbon in dimethyl 2-(3-cyanopropyl)malonate, Fig. 1. The monoalkylated dimethyl malonates \((7\text{a, b})\) open up the possibility of a wide range of reactions and new ways of producing \(^{13}\text{C}\)-labelled compounds, e.g., carboxylic acids with basic and acidic functional side-chain groups.

(3) Oxygen nucleophile: sodium 3-nitrophenoletate Scheme 4. To study the alkylation capacity, the reaction of 3-nitrophenoletate with \(^{13}\text{C}\)-labelled iodoalkanitnitriles \((1,3)\) was used as a model reaction. After 10 min at \(120^\circ\text{C}\) the radiochemical yield of labelled oxygen nucleophile was \(70\%\) \((8\text{a})\) and \(98\%\) \((8\text{b})\).

4-Bromopentanof[13C]nitrile \((5)\) was prepared from 1,3-dibromobutane via a substitution reaction with [13C]cyanide. The reaction was carried out in four different solvents at different reaction temperatures and times. The fact that the radiochemical yields are 62–98% permits the most suitable solvent for a following alkylation reaction to be chosen, anhydrous or not. It was observed the amount of by-products increased with the reaction temperature; elimination products are probably formed. An explanation could be that at higher temperatures elimination becomes the predominant reaction pathway at the expense of substitution.

The identities of the \(^{13}\text{C}\)-labelled precursors and alkylation products were proved by addition of reference substances, characterized by \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectroscopy and GC-MS. The signal from the UV-detector was simultaneous with the radio detector signal, corrected for the time delay between the detectors. To confirm the identity of the two labelled iodoalkanitnitriles \((1,3)\), reference compounds were added to the radioactive sample and analysed by GC with a \(\beta^+\)-flow detector. The radioactive signal
corresponded to the reference signal obtained from the flame ionization detector. The identity of the iodoalkanoneitriles (1, 3) was further verified by TLC. The radioactive sample with added reference was eluted with ether–hexane 90:10 on a TLC plate (SiO₂). The plate was cut into pieces and the radioactivity measured on the different strips. All the radioactivity had moved to the same spot as the reference. To check that all the radioactive material injected into the HPLC analytical column had been eluted, the radioactivity at the outlet of the column was compared with the amount in the injected volume. No discrepancy between the measured radioactivities was found. This indicates that only the HPLC-detected labelled products were formed in the ¹³CN-substitution reactions.

The high yields and rates of production of ¹³C-labelled iodo-, tosylxoy-, and bromo-nitriles (1, 3, 5) of routes (1), (2) and (3) are very interesting for the labelling of biochemical molecules for PET studies.

References


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