An Introduction to Positron Emission Tomography (PET)

LECTURE 2

$^{18}$F and $^{11}$C Radiochemistry

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Format & scope of lecture 2

• Recap on general considerations for radiosynthesis
• $^{[18]}\text{F}$ tracer synthesis
  – Electrophilic fluorination
  – Nucleophilic fluorination
• $^{[11]}\text{C}$ tracer synthesis
  – Methylation
  – Carbonylation
Dealing with radioactivity

How do you carry out a chemical reaction if your reactants and products are radioactive?

• **Rule 1.** don’t use your hands!

• **Rule 2.** use a robot – or automated system

• **Rule 3.** do it behind a lead shield to minimise radiation exposure

• **Rule 4.** work fast! Short-lived isotopes don’t hang around for long, so introduce PET isotope in the last synthetic step
Dealing with radioactivity

**GE Fastlab automated synthesiser:**

Characteristics of commonly used positron-emitters

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Half-life</th>
<th>Chemical Form</th>
<th>Nuclear Reaction(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{11}$C</td>
<td>20 min</td>
<td>$^{11}$CO$_2$ / $^{11}$CH$_4$</td>
<td>$^{14}$N(p,$\alpha$)$^{11}$C</td>
</tr>
<tr>
<td>$^{13}$N</td>
<td>10 min</td>
<td>$^{13}$NH$_4^+$ / $^{13}$NO$_x$</td>
<td>$^{16}$O(p,$\alpha$)$^{13}$N</td>
</tr>
<tr>
<td>$^{15}$O</td>
<td>2 min</td>
<td>$^{15}$O$_2$</td>
<td>$^{15}$N(p,n)$^{15}$O , $^{14}$N(d,n)$^{15}$O</td>
</tr>
<tr>
<td>$^{18}$F</td>
<td>110 min</td>
<td>$^{18}$F$^-$ or $^{18}$F$_2$</td>
<td>$^{18}$O(p,n)$^{18}$F , $^{20}$Ne(d,$\alpha$)$^{18}$F</td>
</tr>
</tbody>
</table>
The synthesis of PET tracers

- Hundreds of molecular imaging probes have been developed for PET
- Labelled compounds include ligands, hormones, antibodies, peptides, proteins, oligonucleotides and drugs (medical and illicit!)
- $^{18}F\text{-fluoro-2-deoxy-D-glucose, } [^{18}F]\text{FDG, is the most commonly used PET tracer}$
Fluorine-18 labelled tracer synthesis
Why use fluorine-18?

- **Fluorine-containing natural products are rare**
- **But what about drug molecules?**
  - 5-10% of new drugs contain a fluorine atom
  - Fluorine can enhance lipophilicity – beneficial for drug delivery
  - It is a small atom. VdW radius 1.47 Å (cf. H: 1.20 Å, O: 1.52 Å)
  - Can accept a hydrogen bond
  - C-F bond is very strong (~485 kJ/mol)

- {}^{18}\text{F has a relatively long half-life (110 min)}

- {}^{18}\text{F has a relatively low positron energy (0.69 MeV) (c.f. }^{11}\text{C)}
  - This leads to higher resolution PET images, 2.4 mm range (c.f. }^{11}\text{C)}
Fluorine-18 production

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Product</th>
<th>Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{20}\text{Ne}(d,\alpha)^{18}\text{F}$</td>
<td>$[^{18}\text{F}]\text{F}_2$</td>
<td>electrophilic</td>
</tr>
<tr>
<td>$[^{18}\text{O}]\text{O}_2(p,n)^{18}\text{F}$</td>
<td>$[^{18}\text{F}]\text{F}_2$</td>
<td>electrophilic</td>
</tr>
<tr>
<td>$[^{18}\text{O}]\text{H}_2\text{O}(p,n)^{18}\text{F}$</td>
<td>$^{18}\text{F}^-_{(aq)}$</td>
<td>nucleophilic</td>
</tr>
</tbody>
</table>

**Two types$^{18}\text{F}$ labelling:**

- **Electrophilic substitution reactions**
  - Versatile reactivity BUT low specific activity ($\times$)
  - Typically $\sim$1 GBq/µmol

- **Nucleophilic substitution reactions**
  - Versatile reactivity AND high specific activity ($\checkmark$)
  - Typically $\sim$100 GBq/µmol

Hooker, Harvard Chem 156 course [URL]
Electrophilic $^{18}$F labelling

- $[^{18}\text{F}] F_2$ was the 1st $^{18}$F fluorinating agent
  - Historical significance, used e.g. to make $[^{18}\text{F}]$ FDG
  - Highly reactive, destructive, poor solubility and unselective
  - The reactivity of $F_2$ gas can be partially controlled when converted to a less reactive reagent
    - Acetyl hypofluorite $[^{18}\text{F}]$ AcOF
    - Xenon difluoride $[^{18}\text{F}]$ XeF$_2$
    - Perchloryl fluoride $[^{18}\text{F}]$ ClO$_3$F
    - Selectfluor®

- Low specific activity (carrier added)
- Reduced radiochemical yield (max. RCY is 50%)
- Poor regioselectivity ($F_2$ highly reactive)

- It is still useful for certain applications:
  - e.g. $[^{18}\text{F}]$ fluoro-L-DOPA synthesis

Synthesis of $^{18}$F FDOPA using electrophilic fluorination

- **Commercial route to F-DOPA:**
  - Developmental route to F-DOPA:

![Chemical reaction diagram](attachment:chemical_diagram.png)

Model system for PEG-grafted PS resin

![PET scans](attachment:pet_scans.png)

http://nawrot.psych.ndsu.nodak.edu/courses/465Projects05/parkinsons/symptoms1.htm
Nucleophilic $^{18}$F labelling

- **No carrier added method**
  - Can obtain high RCY
  - Can obtain high specific radioactivity

- **Easier to handle aqueous solution (c.f. $[^{18}$F] $F_2$ gas)**
- **Wide range of possible nucleophilic substitution reactions**

- **Nucleophilic substitution reactions:**
  - Aliphatic
  - Aromatic
Production of nucleophilic $[^{18}\text{F}]$ fluoride

$[^{18}\text{O}]{\text{H}}_{2}{\text{O}}(p,n)$ in cyclotron

$^{18}\text{F}^{-}({\text{H}}_{2}{^{18}\text{O}})_{n}$ Aqueous $[^{18}\text{F}]$fluoride

Addition of kryptofix ($K_{222}$)
Removal of water

Aqueous $[^{18}\text{F}]$fluoride

Anion exchange resin
$K_{2}{\text{CO}}_{3}$ added
$[^{18}\text{O}]{{\text{H}}_{2}}{\text{O}}$ recycled

$[^{18}\text{F}]\text{KF}$ (CH$_3$CN/H$_2$O)
Aqueous $[^{18}\text{F}]$fluoride

Reactive ‘naked’ fluoride
Practicalities of nucleophilic $^{18}\text{F}$ fluoride production

How do you get rid of water?

1) Concentrate fluoride with an anion exchange column.

2) Azeotrope with organic solvent

<table>
<thead>
<tr>
<th>Cosolvent</th>
<th>BP °C</th>
<th>AZ °C</th>
<th>% Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>ethanol</td>
<td>78.4</td>
<td>78.1</td>
<td>4.5</td>
</tr>
<tr>
<td>methanol</td>
<td>64.7</td>
<td></td>
<td>no azeotrope</td>
</tr>
<tr>
<td>n-propanol</td>
<td>97.2</td>
<td>87.7</td>
<td>28.3</td>
</tr>
<tr>
<td>isopropanol</td>
<td>82.5</td>
<td>80.4</td>
<td>12.1</td>
</tr>
<tr>
<td>acetone</td>
<td>56.5</td>
<td></td>
<td>no azeotrope</td>
</tr>
</tbody>
</table>

How do you make fluoride more nucleophilic?

Kryptofix 2.2.2 ($K_{222}$)
Aliphatic nucleophilic substitution

- **Direct introduction of $^{18}$F into target molecule in one step**
- **Quick and simple, but working against time**
- **e.g.**

![Chemical reaction diagram]

- **Choice of leaving group (LG) is important:**
  - Generally, $I^-$ > $Br^-$ > $Cl^-$ >> $F^-$
  - Sulfonate LGs are best: triflate ($CF_3SO_3^-$), mesylate ($MeSO_3^-$), tosylate ($p$-$MeC_6H_4SO_3^-$)

- **Choice of LG may depend not only on the yield and rate of reaction but also on:**
  - Availability and/or stability of precursors
  - Ease of separation of radiolabelled product from precursors
  - Formation of side products
[\textsuperscript{18}F] FDG synthesis

Fully automated [\textsuperscript{18}F] FDG synthesiser

Solid-phase synthesis:

Indirect $^{18}$F labelling

- **Difficulties with direct labelling methods**
  - Often require harsh reactions conditions
  - can destroy target molecule and/or cause unwanted side reactions
  - Unsuitable starting precursor molecules

- **We then need alternative ‘indirect’ methods**
  - Use of ‘prosthetic groups’; i.e. pre-functionalised reactive units containing $^{18}$F
  - Drawback is that multi-step reactions can be time consuming…
  - A compromise

![Chemical structures and reactions](attachment:image.png)
Indirect $^{18}$F labelling – prosthetic groups

- **Prosthetic groups used for indirect labelling:**
  - $[^{18}F]$4-Fluorobenzoic acid
  - $[^{18}F]$4-Fluorobenzaldehyde
  - $[^{18}F]$2-Fluoroethylamine
  - $[^{18}F]$N-Succinimidyl 4-[^{18}F]$fluorobenzoate
  - $[^{18}F]$2-Fluoroethylazide
  - $[^{18}F]$Eph-$[^{18}F]$fluorohydrin

- **Frequently used for labelling peptides e.g.**:
Indirect $^{18}$F labelling – prosthetic groups

• ‘Click’ chemistry can also be used to achieve labelling:
  – 1,3-dipolar cycloaddition of alkynes to azides to form a triazole ring
  – Either partner (azide or alkyne) can be $^{18}$F labelled:

$$R - \text{Cu(II)} \rightarrow R \quad \text{N}_2\text{N}^+\text{N}_2\text{R}$$

• **Strain-promoted, copper-free’ click:**

![Diagram of click chemistry](image)

![PET images](image)
Nucleophilic Aromatic Substitution

- **Some drugs contain an aryl fluoride unit in their structure (e.g. flumazenil)**
- **Preparation by $S_N$Ar is then possible**
  - we need a good leaving group *e.g.* $\text{ArCl} < \text{ArF} < \text{ArNO}_2 ~ \text{ArN}^+\text{Me}_3 < \text{ArI}^+\text{Ar}'$ (aryl iodonium salt)
  - AND we need activating substituent(s) *ortho* and/or *para*
  - Not so simple! – we need more than a good LG
  - Good activating groups: nitro, aldehyde, ketone, nitrile, ester *etc.*

\[ \begin{align*}
\text{EWG} & = \text{electron withdrawing group} \\
X & = \text{leaving group}
\end{align*} \]

- **Aryl iodonium salts are even effective in the absence of additional activation:**

[Diagram of chemical structures and reactions]
Predicting efficiency in nucleophilic $^{18}$F labelling

Hooker, Harvard Chem 156 course [URL]
Carbon-11 labelled tracer synthesis
11C labelling for PET

Advantages of 11C:
• Carbon present in all natural products and almost all drugs
• We can make identical compounds using 11C
• Chemical and biological properties will be identical to the unlabelled compound
• Half-life of 20 min – short, but long enough for multi-step reactions

Disadvantages:
• Half-life of 20 min – short, chemistry needs to be very fast
• Higher positron energy (0.96 MeV) (c.f. 18F)
• Lower resolution PET images, 4.1 mm range (c.f. 18F)
Commonly utilised $^{11}$C-labelling precursors from $^{11}$CO$_2$

$^{11}$CO

- Zn
- R-Mg-X

$^{11}$CO$_2$

- LiAlH$_4$, Ni, 450°C

$^{11}$CH$_3$O$^-$

- HI, $700^\circ$C

$^{11}$CH$_3$I

$^{11}$CH$_4$

- NH$_3$, Pt, 1000°C

$^{11}$CN

$R^{11}$CH$_2$I
**[\[^{11}\text{C}\]]^{-}\text{methylation reactions}**

- **Introduction of a methyl group in a target molecule**
- ‘One-step’ N and O \[^{11}\text{C}\] methylation are the most commonly used \[^{11}\text{C}\] labelling methods
  - $\text{S}_{\text{N}}2$ mechanism
- **For ‘normal’ N-methylation reactions of amines, methylation cannot be controlled**
  - Mixtures of the methylated products are obtained ($2^\circ$, $3^\circ$ and quarternary amines)
  - \[^{11}\text{C}\] methylation: only one product is obtained, due to a stoichiometric excess of the amine

\[
\begin{align*}
\text{R-N} & \text{H} \quad \text{H}_3\text{C-I} \quad \text{base} \\
\text{R-N-CH}_3 & \quad \text{H}_3\text{C-I} \quad \text{base} \\
\text{R-N-CH}_3 & \quad \text{H}_3\text{C-I} \quad \text{base}\cdot\text{HI}
\end{align*}
\]

quarternary amine salt

For \[^{11}\text{C}]\text{CH}_3\text{I}
Methylation stops here
$[^{11}\text{C}]-N$ and $O$-methylation reactions

- Examples of amide, amine and alcohol methylation:

  - **Amine:** $[^{11}\text{C}]$ SCH 23390

  ![Amide reaction][1]

  - **Amide:** $[^{11}\text{C}]$ Flumazenil

  ![Amide reaction][2]

  - **Ether:** $[^{11}\text{C}]$ Raclopride

  ![Ether reaction][3]
\[^{11}\text{C}\]-C-methylation reactions

- \(^{11}\text{C}\)CH\(_3\)-C bonds can be formed using transition metal catalysts (mostly Pd)
  - Stille coupling – organotin reagents
  - Suzuki coupling – organoboron reagents

\[^{11}\text{C}\]\(\text{CH}_3\)l
\text{Pd}(0)\text{ catalyst}
\[\text{Suzuki coupling}\]

\[^{11}\text{C}\]\(\text{CH}_3\)
\text{Pd}(0)\text{ catalyst}
\[\text{Stille coupling}\]

For example:

Hamill \textit{et al.}, \textit{Synapse}, \textbf{2005}, 56, 205
$[^{11}\text{C}]-\text{carbonylations}$

- $[^{11}\text{C}]\text{CO}$ is an available precursor for labelling
- $^{11}\text{C}$ Aldehydes, ketones, esters, amides may be synthesised
\[ ^{11}\text{C} \]-carbonylation - \textit{practicalities} \\

- **Difficulties of using \[^{11}\text{C}\text{CO} as a reagent**
  - low reactivity and low solubility
- **New developments in recent years have permitted the use of \[^{11}\text{C}\text{CO} as a labelling agent**
  - High pressure micro-autoclave system
  - Langstrom, \textit{J. Org. Chem.}, 1999, 64, 9201
  - Formation of BH\(_3\)\[^{11}\text{C}\text{CO} complexes
  - Gee \textit{Chem. Commun.}, 2004, 558
  - Use of supported Pd catalysts and microfluidics
[¹¹C]-carbonylation - examples

- [¹¹C] CO can be inserted into a molecule via a Pd catalysed reaction
- The CO is inserted in a convergent fragment-joining step, e.g.:

\[
\begin{align*}
\text{[¹¹C] CO} & \quad \text{Pd catalyst} \\
\text{[¹¹C] CO} & \quad \text{Pd catalyst}
\end{align*}
\]

• Examples:
  - Raclopride: dopamine D2-antagonist
  - Spiperone: CAD (cationic amphiphilic drug)
  - Cocaine

Mechanism:
Summary

- **Need and demand for new radioligands and methods for their development**
- **Chemists play a vital role in PET**
- **Precursor synthesis**
  - $^{18}\text{F}$ – *tracers*
    - $\text{F}^+$ for electrophilic reactions
    - $\text{F}^-$ for nucleophilic reactions
  - $^{11}\text{C}$ – *tracers*
    - $[^{11}\text{C}] \text{Methyliodide}$ for methylation of amines & cross coupling reactions (Stille and Suzuki)
    - $[^{11}\text{C}] \text{CO}$ for carbonylation reactions