Advances in the radiolabeling of antibodies with astatine-211: toward simplified procedures and improved radiochemical yields

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Nantes, France
Astatine-211: A promising alpha particle emitter

- Heaviest radiohalogen
- 100% alpha decay
- Half-life ($t_{1/2}$): 7.2 hours
- Product by Arronax Cyclotron

➢ promising candidate for alpha-therapy
Conventional $^{211}$At labeling procedure

Zalutsky 1988:

- **1st step**: Electrophilic destannylation
- **2nd step**: Coupling reaction between radiolabeled precursor and biological vector

Approach used in the **two clinical studies** published to date:

Conventional approach: 2 drawbacks

**Toxic!**

\[ \text{Sn} \rightarrow \begin{array}{c}
\text{\begin{tikzpicture}
\node (a) at (0,0) {\text{At}^{+}};
\node (b) at (1,0) {\text{At}^{-}};
\node (c) at (2,0) {\text{H}_2\text{N-CH}_2-\text{CH}_2-\text{NH}_2};
\node (d) at (3,0) {\text{At}^{+}};
\end{tikzpicture}}
\end{array} \]

**At(+I) form unstable → Reproducibility issues!**

- Difficulties to control precisely the **At**$^+$ form -
  (only picomoles to nanomoles of At are involved)

- Change of **oxidation state** over time due to radiolysis of solvent -
  (Pozzi work (Zalutsky group))

Pourbaix diagram of $^{211}$At

Conventional approach: 2 drawbacks

- Hydrolysis of radiolabeled precursor
- Heterogeneous conjugation!

- Need to use **basic aqueous conditions** to activate lysine (formation of inactive side product)
- Use **high antibody concentration** to reduce hydrolysis reaction ([mAb] = 5mg/mL)
✓ Alternative approach based on nucleophilic astatine (At⁻)?

Arylstannane

At(+I) form unstable

Toxic!

Aryliodonium salt

At(-I) stable in reducing medium

Lower toxicity!
**Precursor Purification**

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Purification method</th>
<th>**[(^{211}\text{At})]\text{SAB RCY (%)*}}</th>
<th>Conjugation yield (%)</th>
<th>Overall RCY (%)*</th>
<th>Procedure duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arylstannane</td>
<td>HPLC</td>
<td>33-58</td>
<td>51-61</td>
<td>20-30</td>
<td>200 ± 10</td>
</tr>
<tr>
<td>Iodonium salt</td>
<td>Silica cartridge</td>
<td>77-84</td>
<td>54-60</td>
<td>53-57</td>
<td>140 ± 10</td>
</tr>
</tbody>
</table>

*Decay corrected

International extention 24 nov 2016.

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**Clinical transfer facilitated...**
Issue II: conjugation

✓ Can bioorthogonal chemistry improve bionconjugation yields?

Can bioorthogonal chemistry improve bionconjugation yields?
Our approach: bioorthogonal click chemistry

3 main advantages:

• **High reaction kinetic**
  - Adaptable to a wide range of radionuclides

• **Great chemoselectivity**
  - Reduction of side reactions and optimum radiolabel efficiencies

• **Good reactivity in water**
  - Applicable to the radiolabeling of antibodies

Bioorthogonal chemistry uses chemical functions that are **stable in biological media and that are inert** to chemical function naturally occurring in biomolecules.
Our approach: bioorthogonal click chemistry

"Five bioorthogonal approaches considered"

<table>
<thead>
<tr>
<th>System</th>
<th>1 azide-BCN</th>
<th>2 azide-DBCO</th>
<th>3 tetrazine-BCN</th>
<th>4 azide-Alkyne</th>
<th>5 tetrazine-TCO</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>$^\text{125}$I or $^\text{211}$At</td>
<td>$^\text{125}$I or $^\text{211}$At</td>
<td>$^\text{125}$I or $^\text{211}$At</td>
<td>$^\text{125}$I or $^\text{211}$At</td>
<td>$^\text{125}$I or $^\text{211}$At</td>
</tr>
<tr>
<td>k (reaction rate constant)</td>
<td>SPAAC (10^{-2} M^{-1} s^{-1})</td>
<td>SPAAC</td>
<td>IEDDA</td>
<td>CuAAC</td>
<td>IEDDA (10^6 M^{-1} s^{-1})</td>
</tr>
</tbody>
</table>

Our approach: bioorthogonal click chemistry

- Proof of concept on model peptides:
  1) Development of radioiodinated and astatinated prosthetic groups
  2) Development of different clickable model peptides
  3) Kinetic study of 5 bioorthogonal systems
Our approach: bioorthogonal click chemistry

• Proof of concept on model peptides:

1) Development of radioiodinated and astatinated prosthetic groups

2) Development of different clickable model peptides

3) Kinetic study of 5 bioorthogonal systems

• Transfer to mAb labeling with $^{211}$At:
1) Development of radioiodinated and astatinated prosthetic groups

• **design of “clickable precursors”**

➢ Synthesis of aryliodonium salts “**azide**” and “**tetrazine**”

![“azide aryliodonium salt”](image1)

and

![“tetrazine aryliodonium salt”](image2)

1) Development of radioiodinated and astatinated prosthetic groups

- design of “clickable precursors”
  - Synthesis of aryliodonium salts “azide” and “tetrazine”
    - “azide aryliodonium salt”
    - “tetrazine aryliodonium salt”

- Radiolabelling procedure with $^{125}$I and $^{211}$At

<table>
<thead>
<tr>
<th>Precursor</th>
<th>X’</th>
<th>t (min)</th>
<th>T(°C)</th>
<th>RCY</th>
<th>Synthon</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{125}$I</td>
<td>60</td>
<td>120°C</td>
<td>84%</td>
<td></td>
<td><img src="image1" alt="Synth" /></td>
</tr>
<tr>
<td>$^{211}$At</td>
<td>30</td>
<td>80°C</td>
<td>86%</td>
<td></td>
<td><img src="image2" alt="Synth" /></td>
</tr>
<tr>
<td>$^{125}$I</td>
<td>60</td>
<td>100°C</td>
<td>95%</td>
<td></td>
<td><img src="image3" alt="Synth" /></td>
</tr>
<tr>
<td>$^{211}$At</td>
<td>30</td>
<td>60°C</td>
<td>85%</td>
<td></td>
<td><img src="image4" alt="Synth" /></td>
</tr>
</tbody>
</table>
2) Synthesis of different clickable model peptides

- **Synthesis of “4 clickable peptides”**

  - Modification with 4 clickable linkers (DBCO, Alkyne, TCO, BCN)

![Chemical structures and reactions](image-url)

3) Kinetic study of 5 bioorthogonal systems

**IEDDDA (1)**

```
H N O
```

![IEDDDA (1) reaction scheme](image1)

**IEDDDA (2)**

```
H N O
```

![IEDDDA (2) reaction scheme](image2)

**CuAAC**

```
H N O
```

![CuAAC reaction scheme](image3)

**SPAAC (1)**

```
H N O
```

![SPAAC (1) reaction scheme](image4)

**SPAAC (2)**

```
H N O
```

![SPAAC (2) reaction scheme](image5)

3) Kinetic study of 5 bioorthogonal systems

3) Kinetic study of 5 bioorthogonal systems

Transfer to mAb labeling

- Preparation of nucleophilic $^{211}\text{At}^{-}$ with Na$_2$SO$_3$ and radiolabeling

$^{211}\text{At}$ (●) in CHCl$_3$

(evaporation)

CHCl$_3$

$^{211}\text{At}_{\text{dry}}$
Transfer to mAb labeling

- Preparation of nucleophilic $\text{At}^-$ with Na$_2$SO$_3$ and radiolabeling

$^{211}\text{At}$ (in CHCl$_3$) in CHCl$_3$

- Evaporation

- Reduction $^{211}\text{At}$

- Radiolabeling precursor

GOOD YIELD

$0 < \text{At}^- < 30$ MBq
Transfer to mAb labeling

- Preparation of nucleophilic $\text{At}^-$ with $\text{Na}_2\text{SO}_3$ and radiolabeling

$^{211}\text{At}$ (●) in $\text{CHCl}_3$

(received from Arronax)

$0 < \text{At}^- < 30$ MBq

$\text{Na}_2\text{SO}_3$ ($\text{H}_2\text{O}$)

GOOD YIELD

$\text{Na}[^{211}\text{At}]\text{At}$

Transfer to mAb labeling

$30$ MBq $< \text{At}^- < 100$ MBq

INCONSITENT YIELD

$\text{Na}[^{211}\text{At}]\text{At}$
Transfer to mAb labeling

- Preparation of nucleophilic At\(^{-}\) with Na\(_2\)SO\(_3\) and radiolabeling

\[ \begin{align*}
\text{Evaporation} & \quad \text{CHCl}_3 \\
\text{reduction} & \quad \text{Na}_2\text{SO}_3 \text{ (H}_2\text{O)} \\
\text{radiolabeling} & \quad \text{precursor} \\
\end{align*} \]

**GOOD YIELD**

- Evaporation
- Reduction 
- Radiolabeling

**GOOD YIELD**

- 0 < At\(^{-}\) < 30 MBq
- Na[\(^{211}\)At]At

**GOOD YIELD**

- 30 MBq < At\(^{-}\) < 100 MBq
- Na[\(^{211}\)At]At

**GOOD YIELD**

- Good reduction with DTT
- whatever the activity involved (>100 MBq)

**GOOD YIELD**

- 0 < At\(^{-}\) < 100 MBq
- Na[\(^{211}\)At]At

**Good reduction with DTT**

- DTT (H\(_2\)O)
- Na[\(^{211}\)At]At

**GOOD YIELD**

- Whatever the activity involved (>100 MBq)
Application of the two bio-orthogonal systems

IEDDA (1)  
system TCO + Tz

---

SPAAC (1)  
system DIBAC + N₃

Immunoreactivity : 82 ± 3%

RCY = 92 ± 4%

Immunoreactivity : 82 ± 5%

RCY = 90%
Conclusions

• New astatinated prosthetic groups were designed with good RCY’s

\[
\begin{align*}
\text{tetrazine-}^{211}\text{At: } & 85 \% \\
\text{azide-}^{211}\text{At: } & 86 \%
\end{align*}
\]

• Scale change: induce lower RCY which needs additional investigations (radiolysis or chemical impurities)
  ➢ High consistency when changing the At reduction method (DTT)

<table>
<thead>
<tr>
<th>Activity</th>
<th>Na\textsubscript{2}SO\textsubscript{3}</th>
<th>DTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>(30 \text{MBq} &lt; ^{211}\text{At} &lt; 100 \text{MBq})</td>
<td>(\times)</td>
<td>(\checkmark)</td>
</tr>
</tbody>
</table>

• Easily transferred to mAb labeling (almost quantitative conjugation) with preservation of the biological vector immunoreactivity

IEDDA (1) system TCO + Tz

- RCY = 92 ± 4%
- Immunoreactivity: 82 ± 3%

SPAAC (1) system DIBAC + N\textsubscript{3}

- RCY = 90%
- Immunoreactivity: 82 ± 5%
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