An improved radiochemical synthesis of N-succinimidyld 4-^{18}\text{F}-(fluoromethyl)benzoate and its application

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The emerging of $^{18}$F labeled peptide

- Since the 1970s, positron emission tomography (PET) has increased using in the fields of neurology, cardiology and oncology. Small $^{18}$F-labeled peptides are the agents of choice for diagnostic variety of human diseases using positron-emission tomography due to their specific affinity to receptor.

**sketch map**

- Radioligand
- Over expressing receptors
- Tumor Cell
- Imaging
One favourable imaging agent of peptide—vasoactive intestinal peptide (VIP)

VIP is a 28-amino acid peptide of the glucagon-secretin family, initially characterized from porcine duodenum and possessing a wide range of biological actions. VIP receptors are expressed on several human tumor cells: adenocarcinomas of the pancreas, colon, stomach, and liver; gastroenteropancreatic neuroendocrine tumors; and brain tumors. Clinical studies of VIP radiolabeled with $^{123}$I and VIP analog TP3654 radiolabeled with $^{99m}$Tc show promise for imaging a variety of human tumors including pancreatic, gastric, and colorectal carcinoma; and various adenocarcinomas.

The amino acid sequences of VIP

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn
Labelling method

$^{18}$F-labeling of peptide can only be achieved via prosthetic groups using no-carrier-added $^{18}$F-fluorination. Various $^{18}$F-labeled prosthetic groups have been developed to realize the conjugation of $^{18}$F with peptide. Such as: $[^{18}\text{F} ]\text{APF}$, $[^{18}\text{F} ]\text{NPFP}$, $[^{18}\text{F} ]\text{SFB}$, $[^{18}\text{F} ]\text{SFMB}$ and so on.

N-succinimidy1 4-$[^{18}\text{F} ]$fluorobenzoate ($S^{18}\text{FB}$) and N-succinimidy1 4-$[^{18}\text{F} ]$(fluoromethyl) benzoate ($S^{18}\text{FMB}$) are the widely used $^{18}$F-labelled prosthetic groups. They have the similar structure.
The synthesis route of $^{18}$F-SFB

$$\text{TfO}^- + \text{Me}_3\text{N} + \text{COOEt} \xrightarrow{[\text{K2.2.2}]+^{18}\text{F}^-} \text{DMSO} \xrightarrow{\text{OH}^-} \text{[^{18}F]FBA}$$
The synthesis route of $^{18}$FMB
# The compare of $^{18}$FMB and $^{18}$FB

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<th>Advantage</th>
<th>Limitation</th>
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<td><strong>Our choice</strong></td>
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| $^{18}$FMB | ➢ labeling step is simple  
➢ specific activity is higher | ➢ the radiochemical yield is low: about 18%(not decay corrected),  
(Lang and Eckelman)  
➢ the purification step is complicated (HPLC). |
| $^{18}$FB  | ➢ the radiochemical yield is relatively higher : 50-60%,  
(Wester et.al) | ➢ the labeling, isolation and purification steps are complicated.                           |
The Synthesis of $^{18}\text{F}\text{M}B$
The character of SNOB

$^1$H NMR (CDCl$_3$) $\delta$ (ppm) 2.92 (s, 4H), 5.26 (s, 2H), 7.44 (d, 2H), 8.12 (d, 4H), 8.41 (d, 2H)
MS(ESI), (M+H)^+ = 434.15  M = 434.38
SNOB #7

WVL: 254 nm

UV_VIS_1

reverse phase C18 column (10µm 4.6*250mm)
Eluent: CH₃CN  H₂O  acetic acid  70  30  0.1
Flow rate: 0.6ml/min, tᵣ=7.1min
Reaction conditions on the synthesis of $S^{18}\text{FMB}$

Fig. 2 Effect of different temperatures and solvents on labeling yields at the ratio of K2.2.2 : K$_2$CO$_3$ 1:1 in 5 min
Fig. 3  Effect of ratios of K2.2.2 to K₂CO₃ (n) and reaction times on labeling yields in acetonitrile at 80
Our primary results

- The labeling yield is higher—57%.
- The isolation step is greatly simplified with Sep-Pak silica cartridge instead of HPLC.
- Time is shorter: about 20min.
The primary studying of $^{18}$SMB labeling biological molecule

- Labeling of IgG
- Labeling of dipeptide
- Labeling of VIP
The labeling of IgG

IgG (dissolved in potassium phosphate buffer) was added to the solution of S\(^{18}\)FMB in acetonitrile

mixed at room temperature for desired times

Using a Sephadex G-25 column eluted with 0.05 mol/L potassium phosphate buffer (1.5 mL/min).

S\(^{18}\)FMB-IgG was collected

The radiochemical purity was greater than 98%.
The effects of several factors on conjugate yield

Figure 5. Dependence of reaction yields of S^{18}FMB with IgG on concentration of IgG (pH 8.5, 25°C, 1 min, n=3)
Fig. 4  Dependence of IgG labeling with $S^{18}\text{FMB}$ on reaction time 
(0.2 mg/mL IgG, pH 8.5, 25°C)
Fig. 6 pH dependence of IgG labeling with $^{18}$FMB (0.2 mg/mL IgG, 25°C, 15 min)
The optimal conjugate condition of IgG with S18FMB

0.2mg IgG/mL

PH: 7.8-8.5

Reaction time: 15 min

Labeling yield: 80%
The labeling of dipeptide (Acetate gly-phe)

- Acetate gly-phe (HGP) is one of the dipeptides with the simplest structures. It can be very easily available. So it can be a good model compound labeled with fluorine-18 to offer a useful method reference for labeling other bioactive peptides.
The labeling of HGP

$^{18}\text{S} \text{FMB}$ in CH$_3$CN solution mixed with HGP in borate solution, reacted at room temperature

Isolation with Sep-Pak silicon cartridge

$^{18}\text{S} \text{FMB}-\text{HGP}$, the radiochemical purity is greater than 99%
The study of labeling conditions

The effect of HOBT (catalyzer) concentrations on yields: C (peptide) = 1 mg/mL, 5 min, pH = 8.4
The effect of time on labeling yields of HGP with $^{18}$FMB at different pH:C (peptide)=1 mg/mL, 1---pH=9.2, 2---pH=8.4, 3---pH=7.8
The effect of labeling yield on PH
The labeling of VIP

➢ There are three free amino residue of lysine as well as several side chain amino groups available for prosthetic group radiofluorination.

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-
Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn
The labeling of VIP

50µl acetonitriles solution of S\textsuperscript{18}FMB, 50 µg VIP in 10µl of 0.1 M borate buffer (pH7.5)

reacted at room temperature for 10 min.

C\textsubscript{18} HPLC column, using a 25-70% acetonitrile / 0.1 M NaH\textsubscript{2}PO\textsubscript{4} water gradient eluting with flow rate of 1ml/min.

TR=26min, S\textsuperscript{18}FMB-VIP was collected
The HPLC spectrum of UV (280 nm) and Radioactivity

HPLC Column: Vydac C18 2.5×250 mm
Eluent: CH$_3$CN/0.1 M NaH$_2$PO$_4$ 25%-70% (v/v) gradient for 30 min, Flow rate: 1 mL/min
The effect of reaction time (t) on VIP conjugate with $S^{18}$FMB

<table>
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<tr>
<th>Time(min)</th>
<th>1</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>yield(%)</td>
<td>11.3</td>
<td>26.5</td>
<td>34.6</td>
<td>35.0</td>
<td>33.6</td>
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- Reaction time hardly affects the yield when it is 10-30 min.
The effect of pH on VIP conjugate with $S^{18}$FMB

<table>
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<tr>
<th>PH</th>
<th>7.5</th>
<th>8.0</th>
<th>8.5</th>
<th>9.0</th>
</tr>
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<tbody>
<tr>
<td>yield(%)</td>
<td>35.2</td>
<td>26.5</td>
<td>12.5</td>
<td>&lt; 7</td>
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- pH affects the conjugate reaction very greatly. The yield reaches the highest at pH=7.5. The reaction yield is reduced to 12.5% when pH = 8.5.
Conclusion

- $^{18}$FMB is a good bifunctional ligands (BFL) for the labeling of VIP and other biological molecule with $^{18}$F.
Research group

11 staff members
1 post doctor
14 postgraduate students

Research centre of Radiopharmaceuticals, Shanghai Institute of Applied Physics, the Chinese Academy of Sciences.
Thanks for your attention!