ExciTides: NTP-derived probes for monitoring pyrophosphatase activity based on excimer-to-monomer transitions

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Supporting Information (SI2)

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1a (cpADP-Im)

<table>
<thead>
<tr>
<th>Structure</th>
<th>HPLC of purified product (method A)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="HPLC" /></td>
</tr>
</tbody>
</table>

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$^1$H NMR (500 MHz, D$_2$O)

$^{31}$P NMR (202 MHz, D$_2$O)
### 1b cpGDP-Im

**Structure**

![Structure Diagram](image)

- $2 \text{ Li}^+ + 2'$-O isomer

**Reaction HPLC**

![HPLC Chart](image)

**Purified HPLC**

![HPLC Chart](image)
2'-O isomer:

3'-O isomer:
1H NMR

31P NMR (202 MHz)
1c cpCDP-Im

Structure

HPLC of purified product (Method A)

$^1$H NMR (500 MHz, D$_2$O)

$^3$P NMR (202.5 MHz, D$_2$O)

2a cpATPC$_2$H

Structure of purified product (method B)

+ 2'-O isomer

HPLC of purified product (method B)
$^{1}H$ NMR (400 MHz, D$_2$O)
31P NMR (162 MHz, D2O)

2b cpATPC$_3$H$_3$

Structure

HPLC of purified product (Method B)
HR MS

^1H NMR (400 MHz, D_2O)
$^{31}$P NMR (162 MHz, D$_2$O)

2c cpATPOC$_3$H$_3$

Structure

HPLC of purified product (Method B)
HR MS

$^1$H NMR (400 MHz, D$_2$O)

HDO
3a cpGTPC$_2$H

3 HNEt$_3^+$ + 2'-O isomer

HPLC of purified product (method B)

DAD1 A, S=50,4 Refr=360,100 (KOPCIAL/GTPCPC2000288 D)
H NMR (400 MHz, D<sub>2</sub>O)
3b cpGTP$_3$H$_3$

Structure

HPLC of purified product (method A)
$^1$H NMR (500 MHz, D$_2$O)
$^{31}P$ NMR (202 MHz, D$_2$O)

$3c$ cpGTPOC$_3$H$_3$

Structure

HPLC of purified product (method A)

VWD1 A, Wavelength=254 nm (KOPCIAL/GTPCPOC3-2017-06-30-10-29-20-1496.D)
$^1$H NMR (500 MHz, D$_2$O)
$^3$P NMR (202 MHz, D$_2$O)

$4a \text{2'}-\text{O-cp}^{m7}\text{GTPC}_2\text{H}$ (R1)

Structure

$3 \text{NH}_4^+$
HPLC of purified product (method A)

HR MS

$^1$H NMR (400 MHz, D$_2$O)
3P NMR (162 MHz, D$_2$O)

4b 3'-O-cep$^m$7GTPC$_2$H (R2)

Structure

HPLC of purified product
(method A)
$^3$P NMR (162 MHz, D$_2$O)

4c 2'-O-cp$^{m7}$GTPC$_3$H$_3$ (R1)

HPLC of purified product (method A)
$^3\text{P} NMR (162 MHz, D$_2$O)

$4d$ 3'-O-cp$^{m7}$GTPC$_3$H$_3$ (R2)

Structure

HPLC of purified product (method A)
$\text{HR MS}$

$\text{H NMR (400 MHz, D}_2\text{O)}$
31P NMR (162 MHz, D2O)

4-e 2'-O-cp^m7GTPOC3H3 (R1)

Structure

HPLC of purified product (method A)

VWD1 A, Wavelength=254 nm (K0FCIAL/M7/GTPCPOC3_R1-2017-09-29-15-22-19-1489.D)
$^3$P NMR (162 MHz, D$_2$O)

$4f\ 3'$-O-cp$^{m7}$GTPOC$_3$H$_3$ (R2)

Structure
HPLC of purified product

(method A)

HR MS

$^1$H NMR (400 MHz, D$_2$O)
$^3$P NMR (162 MHz, D$_2$O)

Structure

$3 \text{NH}_4^+ \quad + 2'$-O isomer

5 cpCTPOC$_3$H$_3$
HPLC of purified product (Method A)

HR MS

$^1$H NMR (500 MHz, D$_2$O) $^1$H NMR (500 MHz, D$_2$O)
Cyanovinylene Dye - OH

$^{31}$P NMR (202 MHz, D$_2$O)
### $^{13}$C NMR (101 MHz, DMSO)

<table>
<thead>
<tr>
<th>Chemical Shift (ppm)</th>
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<tbody>
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<td>165.17</td>
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<td>153.66</td>
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<td>152.98</td>
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<td>58.71</td>
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<td>54.32</td>
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### $^1$H NMR (400 MHz, DMSO)

<table>
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<tr>
<th>Chemical Shift (ppm)</th>
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<tbody>
<tr>
<td>8.14</td>
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<tr>
<td>3.11</td>
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</tbody>
</table>

**Note:** The above table and diagram illustrate the chemical shifts for both $^{13}$C NMR and $^1$H NMR spectroscopy. The shifts are measured in parts per million (ppm) and are observed in deuterium dimethyl sulfoxide (DMSO) as the solvent medium.
Cyanovinylene Dye - Ms

Structure

$^1$H NMR (400 MHz, DMSO)
### 1H NMR (400 MHz, DMSO)

**Structure**

6a Cyanovinylene Dye - N₃
$^{13}$C NMR (101 MHz, DMSO)

Structure

7a
Reaction HPLC (Method C)

HPLC of purified product (method C)

HR MS
7b

Structure

HPLC of purified product (method C)

Reaction HPLC (method C)

\[ 3 \text{NH}_4^+ + 2'\text{-O-isomer} \rightarrow \text{purified product} \]
7c

Structure

3 NH4+

= 2’-O-isomer
Reaction HPLC (method C)

$t = 0$

Reaction HPLC (method C)

$t = 3$ h

HR MS

HPLC of purified product (method C)
Structure

HPLC of purified product (method C)

Reaction HPLC (method C)

HPLC of purified product (method C)
m/z = 1:

\[ 7e \]

**Structure**

\[ 3 \text{NH}_4^+ \] + 2'-O-isomer

m/z = 2:

\[ 7e \]
Reaction HPLC (method C)

$t = 0$

HPLC of purified product (method C)

HR MS
Structure

Reaction HPLC (method C)

$t = 0$

HPLC of purified product (method C)
m/z = 1:

Structure

7g

3 NH₄⁺ + 2'O-isomer
Reaction HPLC (Method C)

$t = 0$

HPLC of purified product (method C)

$t = 1$ h
7h

Structure

3 NH₄⁺ + 2'O-isomer
Reaction HPLC (method C)

$t = 0$

DAD1 A, Sig=254,4 Ref=360,100 (PW\SONDAOC3PY32025.D)

mAU

0 1000

200

400

600

800

1000

min

$t = 1\ h$

DAD1 A, Sig=254,4 Ref=360,100 (PW\SONDAOC3PY32026.D)

0 5 10 15

200

400

600

800

1000

min

HPLC of purified product (method C)

DAD1 A, Sig=254,4 Ref=360,100 (PW\SONDAOC3PYR3251.D)

0 5 10 15

200

400

600

800

1000

min
Structure

Reaction HPLC (method C + additional absorbance detection at 362 nm)

HPLC of purified product (method C + additional absorbance detection at 362 nm)
Structure

$t = 0$:

$3 \text{NH}_4^+$

$t = 5.5 \text{h}$:

Reaction HPLC (method C)

HPLC of purified product (method C)
Z=1

Z=2

8b

Structure
Reaction HPLC (method C)

HPLC of purified product (method C)

$t = 0$

$t = 5 h$
Z=1

109966, M+Na=513.22, RT 0.04, 0.56 AV 56 NL 2.0956
T: 7788 p.ESI Mass [08/05/2005/00]

Z=2

109966, M+Na=513.22, RT 0.04, 0.56 AV 56 NL 3.0527
T: 7788 p.ESI Mass [08/05/2005/00]

Structure

![Structural Diagram]

3 NH₄⁺
Reaction HPLC (method C)

HPLC of purified product (method C)
HR MS

Z=1

8d

Structure

NH₄⁺
Reaction HPLC (method C)

HPLC of purified product (method C)

t = 0

t = 0.5 h
Structure

$3 \text{NH}_4^+$

$t = 0$

Reaction HPLC (method C)

$3 \text{NH}_4^+$

$t = 0.5$ h

HPLC of purified product (method C)
Reaction HPLC (method C)

$t = 0$

HPLC of purified product (method C)
Reaction HPLC (method D)

$t = 0$

HPLC of purified product (method D)

$t = 0.75\ h$
Z=1

8h

Structure
Reaction HPLC (method D)

$t = 0$

HPLC of purified product (method D)

$t = 4$ h
**Structure**

- Reaction HPLC (method D)
  - $t = 0$
  - $t = 2$ h

- HPLC of purified product (method D)
HR MS

Structure

3 NH₄⁺

Reaction HPLC (method D)

t = 0

DAD1 A, Sig=254,4 Ref=360,100 (KOPCIAL/SONDAGTPOC3PY01.D)

mAU

2.5  5   7.5 10   12.5 15 17.5

0  250  500  750 1000 1250 1500

0.262 7.003


t = 0.5 h

DAD1 A, Sig=254,4 Ref=360,100 (KOPCIAL/GTP_OC3_PYR2757.D)

mAU

2.5  5  7.5 10  12.5 15 17.5

0  50  100  150  200  250  300

1.188 7.309 10.370 16.966

1.188 7.309 10.370 16.966
HPLC of purified product
(method D)

HR MS
<table>
<thead>
<tr>
<th>Reaction HPLC (method C)</th>
<th>HPLC of purified product (method C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t = 0 )</td>
<td></td>
</tr>
<tr>
<td>( t = 2 \text{~h} )</td>
<td></td>
</tr>
</tbody>
</table>

- Structure

![Structure Image]

![HPLC Chart at t = 0]

![HPLC Chart at t = 2 h]
Structure

Reaction HPLC (method C)

HPLC of purified product (method C)
HR MS

11c

Structure

\[ \text{Reaction HPLC (method C)} \]

\[ t=0 \]

\[ t=2h \]


VWD1 A, Wavelength=254 nm (PW\ATPCTBDA 2016-01-25 12-04-56.D)
HPLC of purified product (method C)

VWD1 A, Wavelength=254 nm (PW\ATPCTBDA2016-06-1512-46-00.D)

HR MS
HPLC of purified product (method D)

Reaction HPLC (method D)

t=0

DAD1 A, Sig=254.4 Ref=360,100 (KOPCIALLATPOC3_PY000176.D)

mAU

0 2 4 6 8 10 12 14 16 18 minute

3 NH4+

3 NH4+

HPLC of purified product (method D)

DAD1 A, Sig=254.4 Ref=360,100 (KOPCIALLATPOC3_PY000291.D)

mAU

0 2 4 6 8 10 12 14 16 minute

3 NH4+

3 NH4+

Structure