Photorelease of Tyrosine from Alpha-Carboxy-6-nitroveratryl (αCNV) Derivatives

Supplementary Information

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Details of kinetics and quantum yield measurements S6-S11

NMR spectra:

Allyl 2-(4-(tert-butyl)phenoxy)-2-(4,5-dimethoxy-2-nitrophenyl)acetate (4):

$^1$H NMR (300 MHz, CDCl$_3$) S12

$^{13}$C NMR (75 MHz, CDCl$_3$) S13

(2S)-Methyl 3-(4-(2-(allyloxy)-1-(4,5-dimethoxy-2-nitrophenyl)-2-oxoethoxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate (5):

$^1$H NMR (300 MHz, CDCl$_3$) S14

$^{13}$C NMR (75 MHz, CDCl$_3$) S15

2-(4-(tert-Butyl)phenoxy)-2-(4,5-dimethoxy-2-nitrophenyl)acetic acid (6):

$^1$H NMR (300 MHz, d$_6$-acetone) S16

$^{13}$C NMR (75 MHz, d$_6$-acetone) S17

(1S)-1-Carboxy-2-(4-(carboxy(4,5-dimethoxy-2-nitrophenyl)methoxy)phenyl)ethanaminium 2,2,2-trifluoroacetate (7):

$^1$H NMR (300 MHz, d$_6$-acetone) S18

$^{13}$C NMR (75 MHz, d$_6$-acetone) S19

2-(3,4-Bis(ethoxycarbonylmethoxy)phenyl)acetic acid ethoxycarbonylmethyl ester:

$^1$H NMR (300 MHz, CDCl$_3$) S20

$^{13}$C NMR (100 MHz, CDCl$_3$) S21

2-(4,5-Bis(ethoxycarbonylmethoxy-2-nitro)phenyl)acetic acid ethoxycarbonylmethyl ester (8):

$^1$H NMR (300 MHz, CDCl$_3$) S22

$^{13}$C NMR (75 MHz, CDCl$_3$) S23
Diethyl 2,2′-((4-(1-diazo-2-(2-ethoxy-2-oxoethoxy)-2-oxoethyl)-5-nitro-1,2-phenylene)bis(oxy))diacetate:

$^1$H NMR (300 MHz, CDCl$_3$) S24

$^{13}$C NMR (75 MHz, CDCl$_3$) S25

2-(4,5-Bis(ethoxycarbonylmethoxy)-2-nitrophenyl)-2-bromoacetic acid ethoxycarbonylmethyl ester (9):

$^1$H NMR (300 MHz, CDCl$_3$) S26

$^{13}$C NMR (75 MHz, CDCl$_3$) S27

Diethyl 2,2′-((4-(1-(4-((tert-butyl)phenoxy)(carboxy)methyl)-5-nitro-1,2-phenylene)bis(oxy))diacetate (10):

$^1$H NMR (300 MHz, CDCl$_3$) S28

$^{13}$C NMR (75 MHz, CDCl$_3$) S29

Diethyl 2,2′-((4-(1-(4-(S)-2-((tert-butoxycarbonylamino)-3-methoxy-3-oxopropyl)phenoxy)-2-(2-ethoxy-2-oxoethoxy)-2-oxoethyl)-5-nitro-1,2-phenylene)bis(oxy))diacetate (11):

$^1$H NMR (300 MHz, CDCl$_3$) S30

$^{13}$C NMR (75 MHz, CDCl$_3$) S31

2,2′-((4-(4-(tert-Butyl)phenoxy)(carboxy)methyl)-5-nitro-1,2-phenylene)bis(oxy))diacetic acid (12):

$^1$H NMR (300 MHz, d$_6$-acetone) S32

$^{13}$C NMR (75 MHz, d$_6$-acetone) S33

(1S)-2-(4-((4,5-Bis(carboxymethoxy)-2-nitrophenyl)(carboxy)methoxy)phenyl)-1-carboxyethanaminium 2,2,2-trifluoroacetate (13):

$^1$H NMR (300 MHz, D$_2$O) S34

$^{13}$C NMR (75 MHz, D$_2$O) S35
**tert-Butyl 2-(4,5-dimethoxy-2-nitrophenyl)acetate:**

\[^1\text{H} \text{NMR (300 MHz, CDCl}_3\text{)}\]  
\[^{13}\text{C NMR (75 MHz, CDCl}_3\text{)}\]

**tert-Butyl 2-diazo-2-(4,5-dimethoxy-2-nitrophenyl)acetate**

\[^1\text{H} \text{NMR (300 MHz, CDCl}_3\text{)}\]  
\[^{13}\text{C NMR (75 MHz, CDCl}_3\text{)}\]

**tert-Butyl 2-bromo-2-(4,5-dimethoxy-2-nitrophenyl)acetate (14):**

\[^1\text{H} \text{NMR (300 MHz, CDCl}_3\text{)}\]  
\[^{13}\text{C NMR (125 MHz, CDCl}_3\text{)}\]

**(2S)-tert-Butyl 3-(4-(2-(allyloxy)-1-(4,5-dimethoxy-2-nitrophenyl)-2-oxoethoxy)phenyl)-2-((\text{tert-butoxycarbonyl})amino)propanoate (17):**

\[^1\text{H} \text{NMR (300 MHz, CDCl}_3\text{)}\]  
\[^{13}\text{C NMR (100 MHz, CDCl}_3\text{)}\]

**(2S)-Methyl 3-(4-(2-(\text{tert-butoxy})-1-(4,5-dimethoxy-2-nitrophenyl)-2-oxoethoxy)phenyl)-2-(2,2,2-trifluoroacetamido)propanoate (18):**

\[^1\text{H} \text{NMR (300 MHz, d}_6\text{-acetone)}\]  
\[^{13}\text{C NMR (75 MHz, d}_6\text{-acetone)}\]

**(1S)-2-(4-(2-(Allyloxy)-1-(4,5-dimethoxy-2-nitrophenyl)-2-oxoethoxy)phenyl)-1-carboxyethanaminium 2,2,2-trifluoroacetate**

\[^1\text{H} \text{NMR (300 MHz, d}_6\text{-acetone)}\]

**(2S)-2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-(2-(allyloxy)-1-(4,5-dimethoxy-2-nitrophenyl)-2-oxoethoxy)phenyl)propanoic acid (19)**

\[^1\text{H} \text{NMR (400 MHz, CDCl}_3\text{)}\]  
\[^{13}\text{C NMR (100 MHz, CDCl}_3\text{)}\]
(2S)-2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-(2-(tert-butoxy)-1-(4,5-dimethoxy-2-nitrophenyl)-2-oxoethoxy)phenyl)propanoic acid (20)

$^1$H NMR (300 MHz, CDCl$_3$)  S49

$^{13}$C NMR (75 MHz, CDCl$_3$)  S50

**Mosher’s amide of +/- tyrosine**

$^{19}$F NMR (282 MHz, d$_6$-acetone)  S51

**Mosher’s amide of tyrosine released by photolysis of Mosher’s amide of 7**

$^{19}$F NMR (282 MHz, d$_6$-acetone)  S52
**Kinetic measurements**

The measurements were performed in aerated H$_2$O or EtOH/H$_2$O (4:1) solutions, as indicated, using a pump – probe setup equipped with a frequency - tripled Nd-YAG laser (BMI, 355 nm, 6 ns pulses) as an excitation source. An intensified CCD (Andor Technologies Instaspec V) or a photomultiplier (Hamamatsu R 446 UR connected to a transient digitizer) was used for detection for the spectral and temporal measurements, respectively.

**Kinetic Measurements for Compound 7**

Measurements made in water.

![Graph of transient absorption after 10 μs](image)

Transient absorption after 10 μs

![Graph of decay monitored at 420 nm](image)

Decay monitored at 420 nm ($k = 2.4 \times 10^4$ s$^{-1}$)
Chi squared = 5.2555e-3
Parameters: Standard deviations:
A = 2.9284e-2 ΔA = 1.4412e+4
x0 = -9.9136e-6 Δx0 = 20.1604
t0 = 4.0963e-5 Δt0 = 1.5494e-6
const = 1.4108e-2 Δconst = 2.0486e-4

Kinetic Measurements for Compound 13

Measurements made in water.

Transient absorption after 10 µs

Decay monitored at 420 nm ($k = 2.3 \times 10^4 \text{ s}^{-1}$)
Chi squared = 3.7828e-3

Parameters:
- A = 3.3573e-2
- x0 = -6.2146e-6
- t0 = 4.3683e-5
- const = 1.1171e-2

Standard deviations:
- ΔA = 4436.0902
- Δx0 = 5.7719
- Δt0 = 1.1500e-6
- Δconst = 1.8916e-4

**Kinetic Measurements for Compound 5**

Measurements made in ethanol/water (4:1)

![Graph showing transient absorption after 1 µs]

Decay monitored at 420 nm ($k = 1.3\times10^5$ s$^{-1}$)

Chi squared = 1.3074e-2

Parameters:
- A = 3.7668e-2
- x0 = -2.0372e-6
- t0 = 7.6637e-6
- const = 6.4542e-3

Standard deviations:
- ΔA = 2.9417e+4
- Δx0 = 5.9850
- Δt0 = 4.0326e-7
- Δconst = 1.6965e-4
Kinetic Measurements for Compound 11

Measurements made in ethanol/water (4:1)

Transient absorption after 1 µs

Decay monitored at 420 nm \( (k = 7.0 \times 10^4 \text{ s}^{-1}) \)

Chi squared = 3.1589e-3

Parameters:

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<td>A</td>
<td>2.3738e-2</td>
<td>( \Delta A = 3.7685e+4 )</td>
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<tr>
<td>x0</td>
<td>-1.3965e-5</td>
<td>( \Delta x0 = 22.5644 )</td>
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<tr>
<td>t0</td>
<td>1.4214e-5</td>
<td>( \Delta t0 = 1.0238e-6 )</td>
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<td>const</td>
<td>7.7570e-3</td>
<td>( \Delta \text{const} = 1.1187e-4 )</td>
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Quantum yield determination

Quantum yields were determined using an optical bench consisting of a 0101 Schoeffel lamp housing equipped with a xenon-mercury lamp (150 W) and a 77250 Oriel monochromator. Irradiations were carried out in aerated water at 20 °C at 365 nm (band-width of 10 nm) and followed by HPLC analysis.

The photon flux was determined using potassium ferrioxalate actinometry (Hatchard – Parker actinometer):\(^1\) A 3.0 mL solution of ferrioxalate (0.006 M) in H\(_2\)SO\(_4\) (0.05M) was irradiated and the moles of photoproduct were determined from the change in optical density at 510 nm and the extinction coefficient (11100 M\(^{-1}\) cm\(^{-1}\)) 1 h after addition of 0.1 % of 1,10-phenanthroline to the photolyzed solution. The known quantum yield of photoproduct (1.26)\(^2\) was used to calculate the photon flux (I = 5.48x10\(^{-7}\) E/min).

1. HPLC Analysis of Tyr.

Conditions: 70:30 acetonitrile:water (isocratic), 1 mL/min, detector at 270 nm.

Calibration curve:

![Graph showing calibration curve](image)

\[
\text{Area (270 nm)} = \frac{\text{[Tyr]}}{\text{M}} \times 3.175.5518 + 9674.1127 \\
\text{[Tyr] / M} = 1.4036e+9 \\
\text{Correlation coefficient:} = 0.9944 \\
\text{Significance of correlation:} = 18.3480 \%
\]
2. Quantum yield determination for compound 7

Solution in water at 1.389 mM, irradiated at 365 and analyzed at 10 min intervals.

Quantum yield = 0.19

3. Quantum yield determination for compound 13

Solution in water at 1.095 mM, irradiated at 365 and analyzed at 10 min intervals.

Quantum yield = 0.11

