

Supporting Information

9-Aminoacridine Peptide Derivatives as Versatile Reporter Systems for Use in Fluorescence Lifetime Assays

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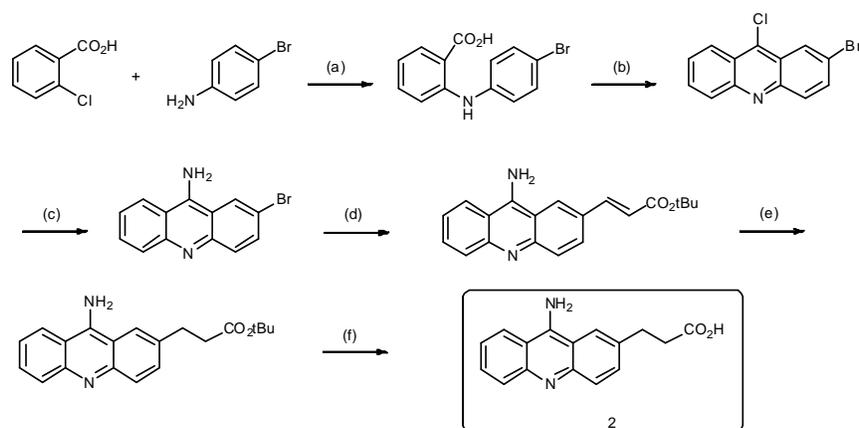
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Experimental

General. ¹H and ¹³C NMR spectra were recorded using a Bruker Advance 500 MHz spectrometer. Chemical shifts (δ) are referenced to the residual solvent peak (DMSO-d₆) and are quoted in parts per million (ppm). HPLC analysis was performed using an Agilent 1200 or Agilent 1100 series system and eluted by a linear gradient of MeCN containing 0.1% TFA and water containing 0.1% TFA. Fluorescence spectra were recorded on an Edinburgh Instruments FLS920 spectrophotometer [Instrument settings: Xe900 lamp source, $\lambda_{\text{ex}} = 405$ nm, step 1, dwell 0.2 s, repeats 1, Ex slit 1 nm, Em slit 1 nm] and fluorescence lifetimes were measured using an Edinburgh Instruments NanoTaurus time-correlated single-photon counting fluorescence lifetime plate reader [Instrument settings: 0.5 s acquisition time, 200 ns time range, 512 channels, excitation by 405 nm laser at 5 MHz with a 450 nm Comar emission filter, data analysis using a single exponential fit]. Absorption spectra were recorded on a Varian Cary 50 UV-Vis spectrophotometer. Mass spectra were recorded using an ABI Mariner electrospray mass time of flight spectrometer. Phosphate buffered saline (PBS) refers to 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4 at 25 °C.

Synthesis of 3-(9-aminoacridin-2-yl) propionic acid **2**

Fig S1. Synthetic scheme for **2**. Experimental protocols are as previously described [R. Ramage, B. Maltman, G. Cotton, S.C.M. Couturier and R.A.S. McMordie, Novel fluorescent dyes and uses thereof PCT/GB2006/004015 2006].



Reagents (a) K_2CO_3 , Cu, CuI, DMF, 23%; (b) POCl_3 , 92%; (c) Phenol, $(\text{NH}_4)_2\text{CO}_3$, 74%; (d) *t*-butyl acrylate, $\text{Pd}(\text{OAc})_2$, $\text{P}(o\text{-tolyl})_3$, DIPEA, DMF, 53%; (e) H_2 , 10% Pd/OH, EtOH, 3 bar, 52%; (f) HCl/dioxane.

Analytical Data for **2**

Extinction coefficient: $\epsilon = 8430 \text{ M}^{-1}\text{cm}^{-1}$ in PBS ($\lambda_{\text{ex}} = 405 \text{ nm}$).

Quantum yield: $\theta = 0.95 \pm 0.02$ in water (reference dimethyl POPOP).

Fluorescence lifetime: $\tau = 17.0 \text{ ns}$ in PBS ($\lambda_{\text{ex}} = 405 \text{ nm}$).

Fig S2. ^1H NMR

Fig S3. ^{13}C NMR

Fig S4. Fluorescence and absorption spectra

Fig S5. HPLC chromatogram

Fig S6. Mass spectrum

Fig S7. X-ray crystal structure

General procedure for the synthesis of 9AA-labelled peptides

Dye coupling: Resin (50 mg) was allowed to swell in DMF (200 μ l). A solution of **2** (3 mg, 0.013 mmol), DIC (25 μ l, 0.013 mmol, 0.5 M in DMF) and HOt (25 μ l, 0.5 M in DMF, 0.013 mmol) in DMF (50 μ l) was added to the resin and sonicated for 3 hours. The resin was then filtered and washed with DMF, DCM and diethyl ether and dried *in vacuo*.

Resin cleavage: The resin was treated with a solution of TFA/TIS/water (95:2.5:2.5, 2 ml) with stirring for 3 hours and then precipitated into cold diethyl ether (10 ml). The precipitated peptide was centrifuged, washed with diethyl ether (10 ml) and lyophilised.

Purification: The crude dye-labelled peptide was purified by semi-preparative reverse phase HPLC using a Luna C18 250 x 10 mm column, flow rate 5 mlmin⁻¹. Gradient: 10-40% B over 40 mins for peptide **3** and 10-50% B over 50 mins for peptide **4**. (buffer A: water cont. 0.1% TFA, buffer B: MeCN containing 0.1% TFA).

Analytical Data for dye labelled peptides

Fig S8. HPLC chromatogram for 9AA-DEVDSK **3**.

Fig S9. Mass spectrum for 9AA-DEVDSK **3**.

Fig S10. HPLC chromatogram for 9AA-DEVDSW **4**

Fig S11. Mass spectrum for 9AA-DEVDSW **4**.

Caspase 3 Assay Procedure

Solutions

Buffer: 20 mM HEPES buffer pH 7.4 containing 10% sucrose, 0.1% CHAPS, 100 mM NaCl, 1 mM EDTA, 10 mM DTT

Substrate solution: 10 μ M 3-(9-aminoacridin-2-yl) propionyl-DEVDSW **4** in buffer.

Product solution: 10 μ M 3-(9-aminoacridin-2-yl) propionyl-DEVDSK **3** in buffer.

Enzyme solution: Caspase 3 (Calbiochem) 10 U/ μ l in buffer

Procedure

To the well of a 96-well plate was added buffer (88 μl), substrate solution (10 μl , 1 μM final conc.) and enzyme (2 μl , 20 U). Reactions were performed in triplicate and also at varying enzyme concentrations. The reaction progress was followed at time intervals using an Edinburgh Instruments NanoTaurus Fluorescence Lifetime Plate Reader against wells containing product and substrate standards.

Fig S2. ^1H NMR spectra for 2

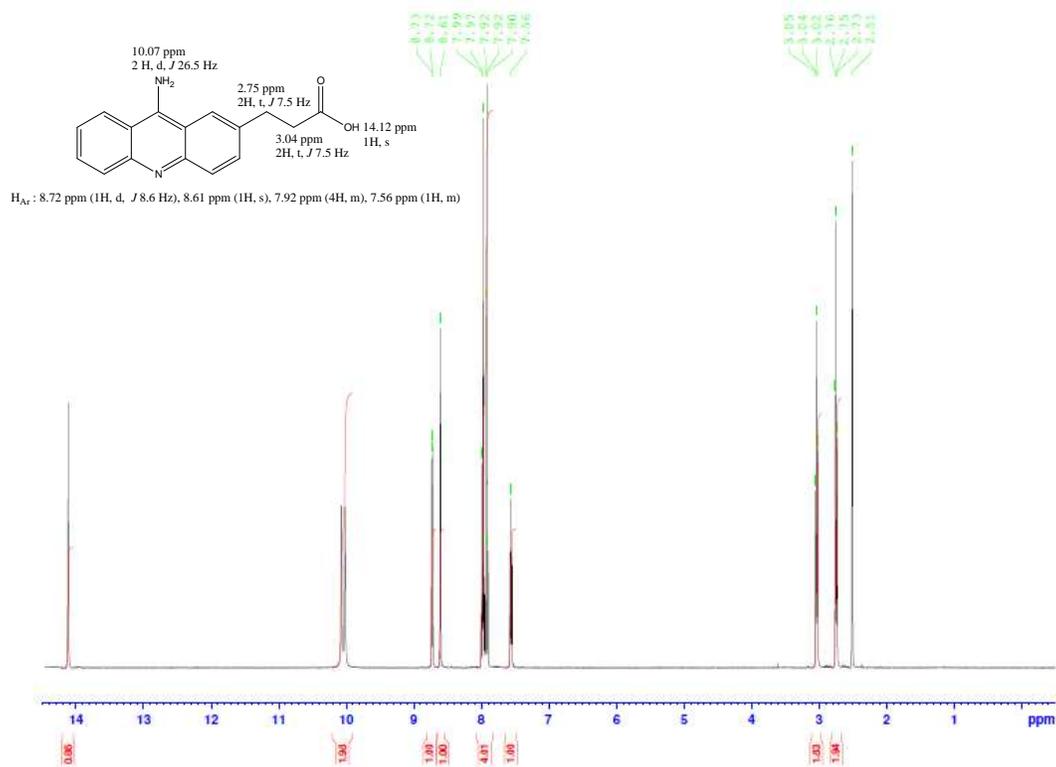


Fig S3. ^{13}C NMR spectra for 2

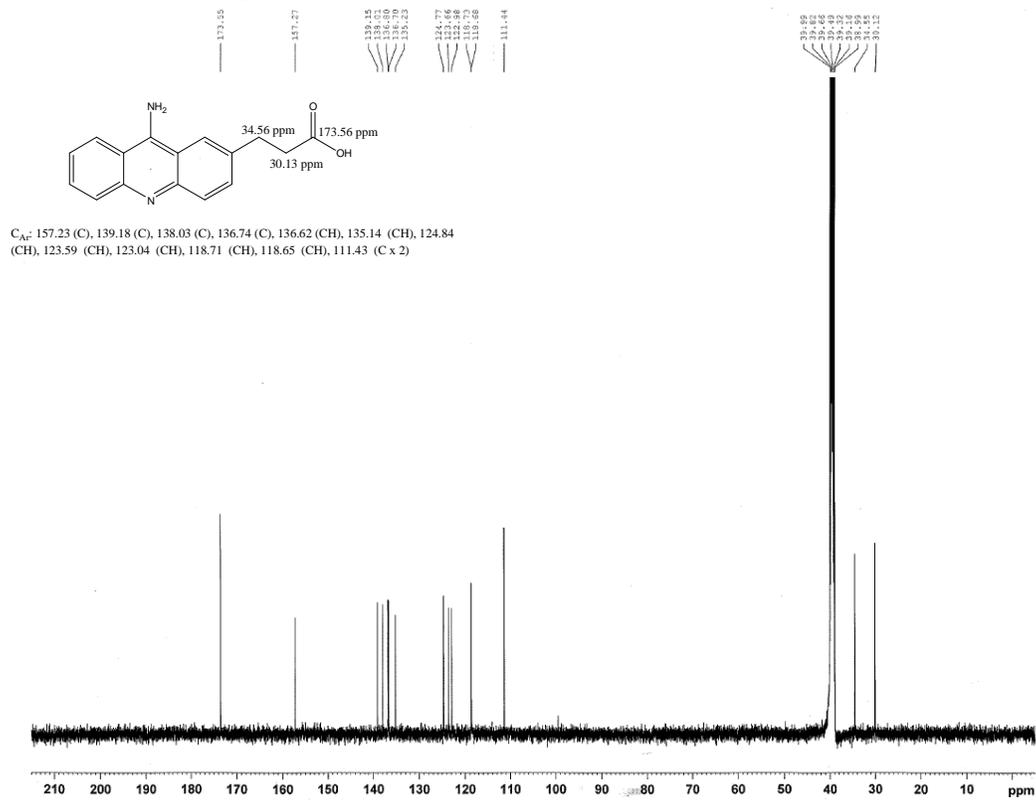


Fig S4. Fluorescence and absorption spectra for **2**. (a) Excitation (solid) and emission (dash) spectra, 500nM in PBS, $\lambda_{\text{ex}} = 405\text{nm}$, $\lambda_{\text{em}} = 450\text{ nm}$. (b) Fluorescence lifetime decay curve, 500nM in PBS, $\lambda_{\text{ex}} = 405\text{nm}$, $\tau = 17.0\text{ ns}$. (c) Absorption spectrum, 20 μM in PBS.

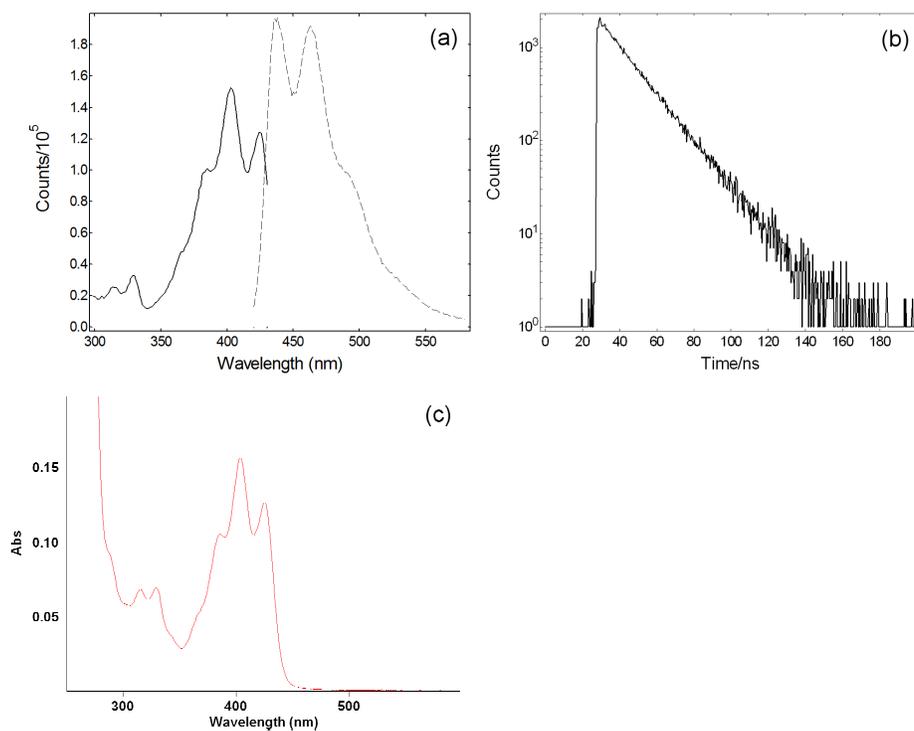


Fig S5. HPLC chromatogram for **2**. Luna C18 250 x 4.6 mm column, 0-73% buffer B 30 mins, flow rate 1 mlmin^{-1} (buffer A: water cont. 0.1% TFA, buffer B: MeCN containing 0.1% TFA). $\lambda_{\text{abs}} = 254\text{ and }405\text{ nm}$.

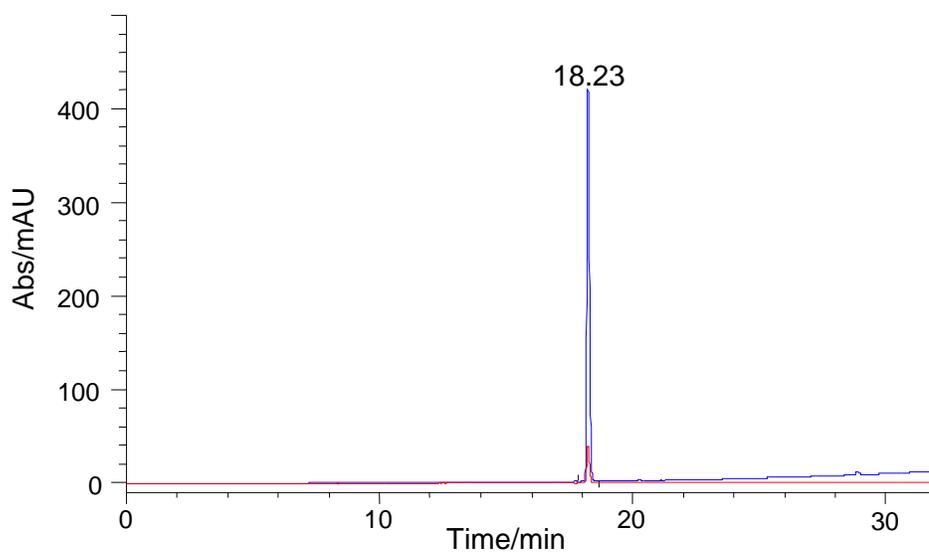
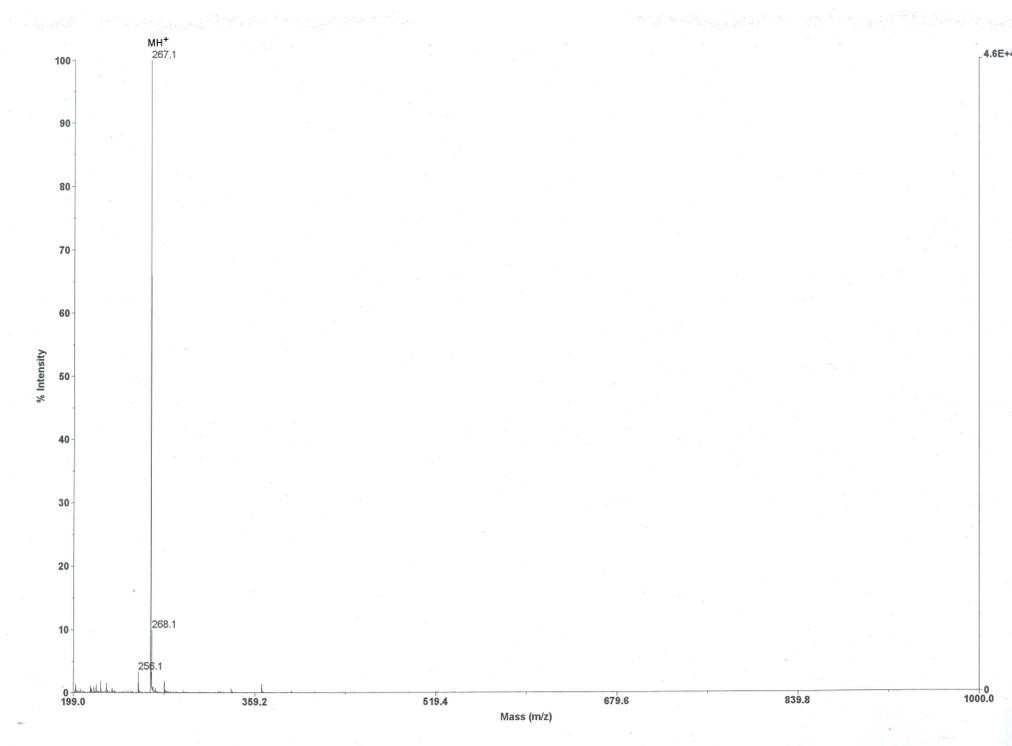


Fig S6. Mass spectrum for **2**. Expected 266.3 amu, obtained 267.1 amu.



S7. X-ray crystal structure of **2**. The structure of **2** was confirmed by X-ray crystallography and shown to be a hydrochloride salt and methanol solvate.

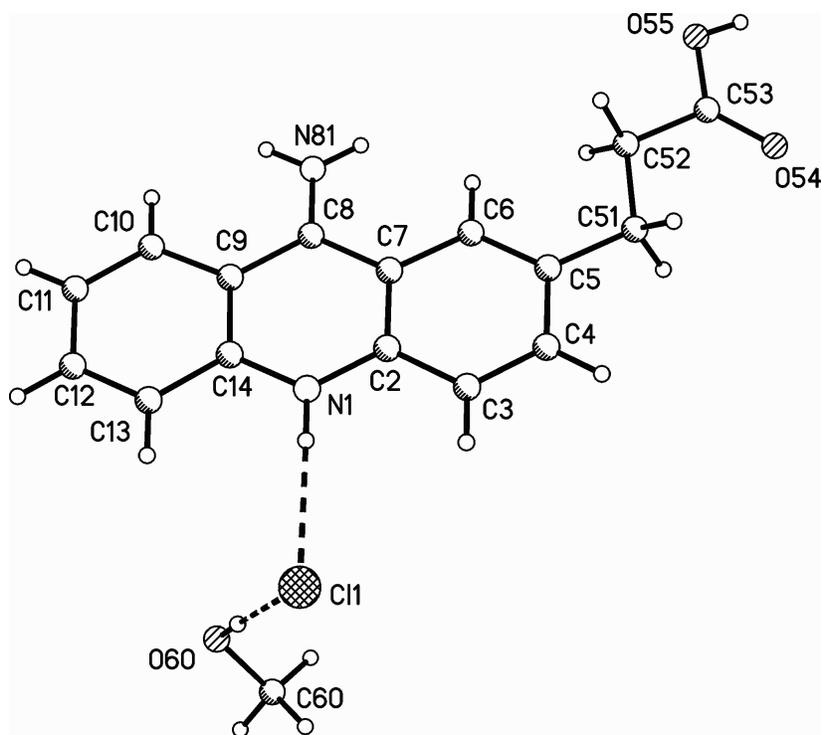


Fig S8. HPLC chromatogram for 9AA-DEVDSK **3**. Luna C18 100 x 4.6 mm column, 0-73% buffer over 30 mins, flow rate 1 mlmin⁻¹ (Buffer A: water cont. 0.1% TFA, Buffer B: MeCN containing 0.1% TFA). $\lambda_{\text{abs}} = 214$ and 405 nm.

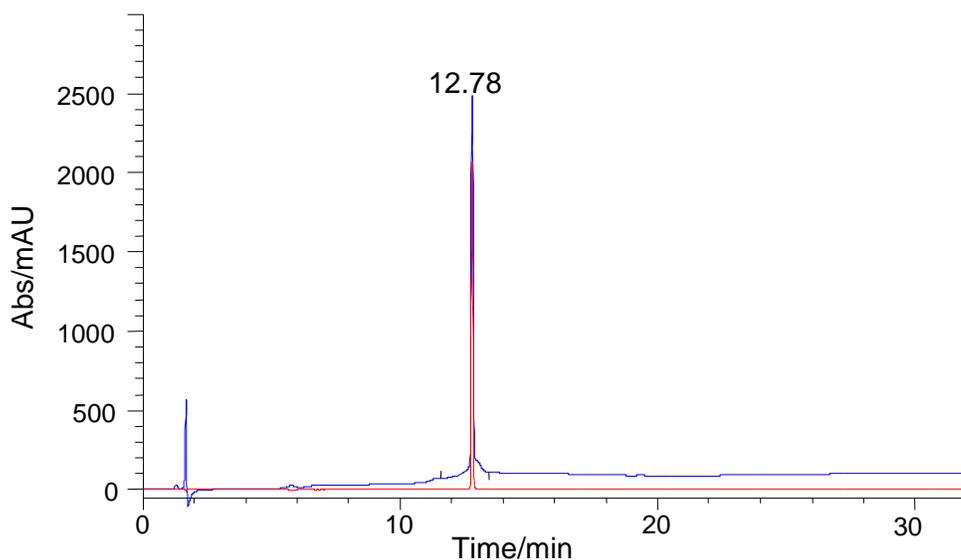


Fig S9. Mass spectrum for 9AA-DEVDSK **3**. Expected 939.0 amu, obtained 938.7 amu.

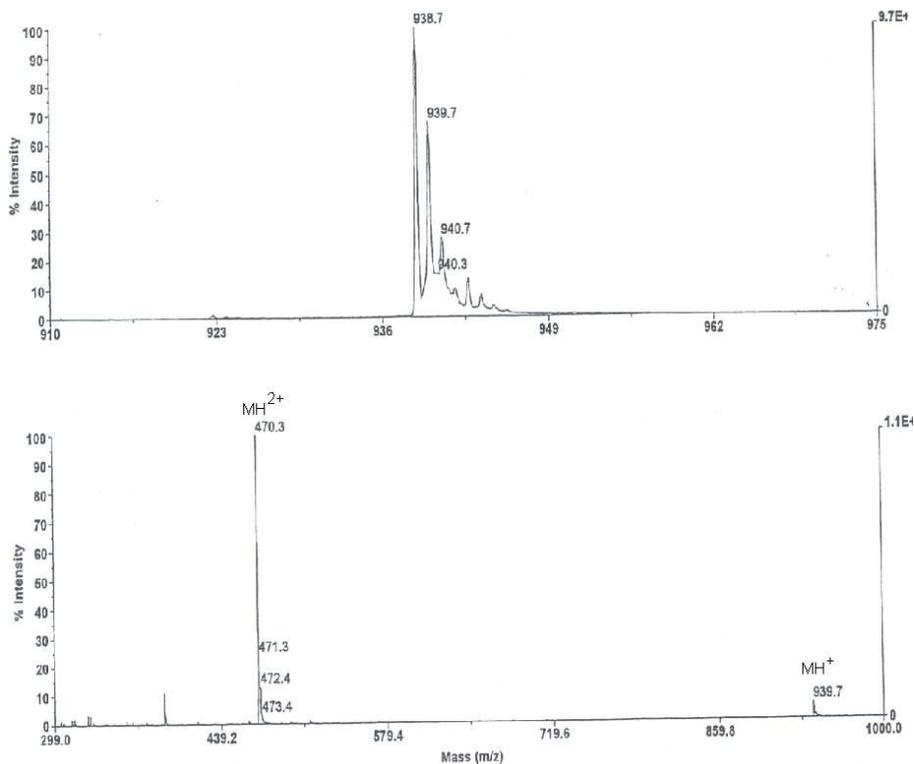


Fig S10. HPLC chromatogram for 9AA-DEVDSW **4**. Luna C18 100 x 4.6 mm column, 0-73% buffer B 30 mins, flow rate 1 mlmin^{-1} (buffer A: water cont. 0.1% TFA, buffer B: MeCN containing 0.1% TFA). $\lambda_{\text{abs}} = 214$ and 405 nm.

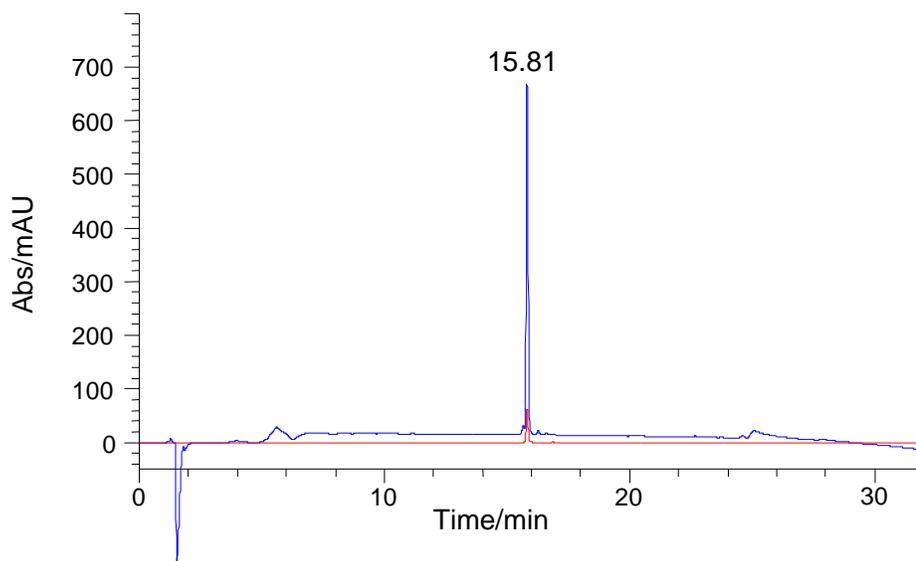
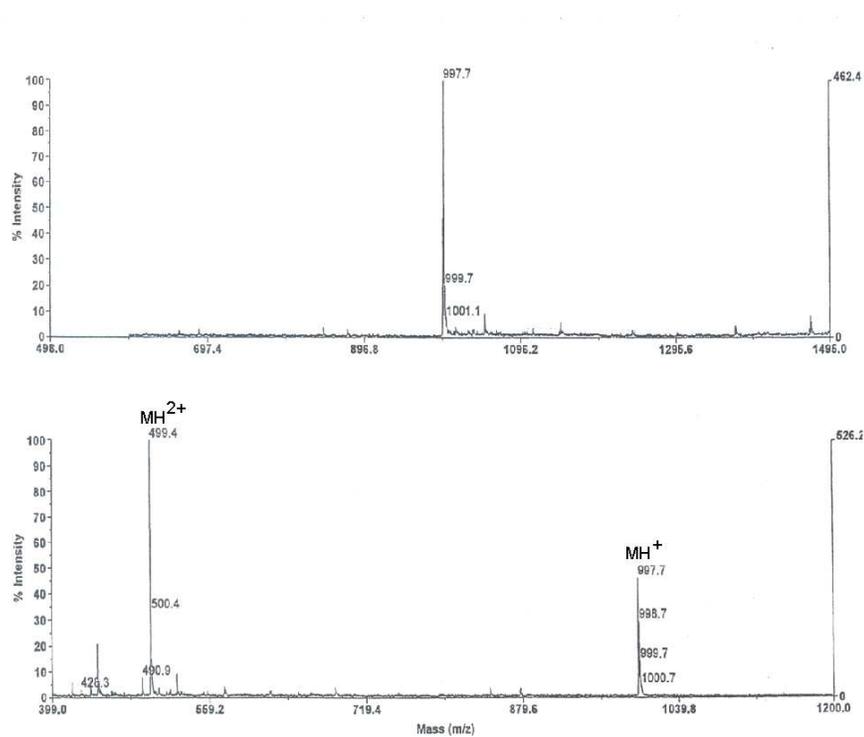


Fig S11. Mass spectrum for 9AA-DEVDSW **4**. Expected 997.0 amu, obtained 997.7 amu.



Data for 9AA 1

Fig S12. Absorption spectrum for **1**, 20 μ M in PBS.

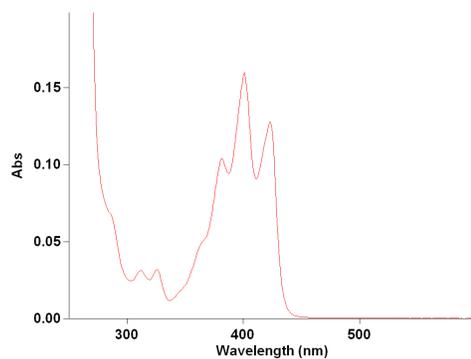


Fig S13. Fluorescence lifetime dependence of **1** (●) and **2** (■) as a function of pH. Measurements were performed at 1 μ M dye concentration in 200 mM sodium phosphate buffer, the pH was adjusted using mixtures of 200 mM monobasic and 200 mM dibasic sodium phosphate buffer and 100 mM sodium hydroxide. Measurements were performed in triplicate and error bars show the standard deviation.

