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### **Electronic Supplementary Information**

Accompanying the manuscript

## Fluorophore ATCUN Complexes: Combining Agent and Probe for Oxidative DNA Cleavage

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List of contents:

- **S-1: Experimental section**
- S-2: Determination of peptide yields
- S-3: DNA cleavage experiments
- S-4: UV-vis experiments
- **S-5: Fluorescence experiments**
- S-6: NMR experiment

S-7: Summary properties of fluorophores

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#### **S-1:** Experimental section

#### Synthesis of 2-(2-aminoethyl)-3',6'-bis(diethylamino)spiro[isoindoline-1,9'xanthen]-3-one (4)

Rhodamine B ethylenediamine 4 was prepared as previously described:<sup>1</sup> Ethylenediamine (1.0 mL, 15.08 mmol) was added dropwise to a solution of rhodamine B (1.0 g, 2.01 mmol, *Sigma-Aldrich*) in 25 mL of ethanol. The solution was heated to reflux for 16 h and evaporated to dryness. The resulting residue was dissolved in water and extracted with DCM (2x 15 mL). The combined organic phases were washed with water and dried over Na<sub>2</sub>SO<sub>4</sub>. The solvent was removed by evaporation and the remaining solid was dried *in vacuo*, affording 4 as a pinkish powder (0.96 g, 1.98 mmol, 99%).

<sup>*1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)*:  $\delta$  (ppm) = 1.14 (12 H, t, NCH<sub>2</sub>CH<sub>3</sub>, *J* = 6.8 Hz), 2.38 (2 H, t, NCH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>, *J* = 6.4 Hz), 3.17 (2 H, t, NCH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>, *J* = 6.8 Hz), 3.31 (8 H, q, NCH<sub>2</sub>CH<sub>3</sub>, *J* = 6.9 Hz), 6.24-6.42 (6 H, m, Aryl-*H*), 7.05-7.09 (1 H, m, Aryl-*H*), 7.40-7.45 (2 H, m, Aryl-*H*), 7.86-7.90 (1 H, m, Aryl-*H*).\*\*</sup>



Figure S1.1: Synthesis of rhodamine B ethylenediamine 4.

## Synthesis of *N*-(2-aminoethyl)-5-(dimethylamino)naphthalene-1sulfonamide (5)

Dansyl ethylenediamine **5** was prepared according to a procedure published by Williams *et al.*:<sup>2</sup> Dansyl chloride (0.1 g, 0.37 mmol, *AppliChem*) was dissolved in 10 mL DCM. The solution was added dropwise to a solution of ethylenediamine (3.3 mL, 50.00 mmol) in 2 mL DCM at 0 °C. The mixture was stirred while warming to room temperature for 1 h. The solution was acidified with concentrated HCl and extracted with DCM (2x 20 mL). The aqueous phase was basified with 10 M NaOH (pH 9) and again extracted with DCM (2x 20 mL). The combined organic phase was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness to give **5** as a yellow solid (0.44 g, 0.15 mmol, 41%).

<sup>1</sup>*H* NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) = 2.70 (2 H, t, NHCH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>, J = 6.4 Hz), 2.87 (8 H, m, N(CH<sub>3</sub>)<sub>2</sub> and NHCH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>), 7.17 (1 H, d, 6-CH-dansyl, J = 7.6 Hz), 7.53 (2 H, m, 7-CH- and 3-CH-dansyl), 8.26 (2 H, m, 8-CH- and 4-CH-dansyl), 8.52 (1 H, d, 3-CH-dansyl, J = 8.4 Hz).\*\*



Figure S1.2: Synthesis of dansyl ethylenediamine 5.

## Synthesis of 1-(2-aminoethyl)-3-(3',6'-dihydroxy-3-oxo-3*H*-spiro[isobenzofuran-1,9'-xanthene]-5-yl)thiourea (6)

Fluorescein ethylenediamine **6** was prepared according to a slightly modified literature procedure:<sup>3</sup> A solution of ethylenediamine (0.01 mL, 0.16 mmol) in 5 mL of methanol was added dropwise to a solution of fluorescein isothiocyanate isomer I (0.06 g, 0.16 mmol, *Sigma-Aldrich*) in 3 mL methanol. The reaction mixture was stirred at room temperature for 3 h. The solvent was removed under reduced pressure. The resulting solid was suspended in methanol, filtered and dried in vacuo, affording a bright orange solid (0.06 g, 0.14 mmol, 85 %).

<sup>*1</sup>H NMR (400 MHz, D<sub>2</sub>O):*  $\delta$  (ppm) = 3.16 (2 H, t, NHCH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>, *J* = 5.1 Hz), 3.83 (2 H, t, NHCH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>, *J* = 5.1 Hz), 6.56-6.60 (4 H, m, Aryl-*H*), 7.14-7.24 (3 H, m, Aryl-*H*), 8.26 (2 H, m, 8-CH- and 4-CH-dansyl), 8.52 (1 H, d, 3-CH-dansyl, *J* = 8.4 Hz).\*\*</sup>



Figure S1.3: Synthesis of fluorescein ethylenediamine 6.

# Synthesis of 3-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-2-(*tert*-butoxycarbonylamino)propanoic acid

 $N^2$ -Boc- $N^3$ -Fmoc-2,3-diaminopropanoic acid was prepared according to a procedure of Hopkins *et al.*:<sup>4</sup> To a solution of  $N^2$ -Fmoc-2,3-diaminopropanoic acid (0.40 g, 1.96 mmol, *Sigma-Aldrich*) in 5 mL sodium carbonate solution (10% w/w) was added dropwise a solution of Fmoc-Cl (0.62 g, 2.40 mmol, *Sigma-Aldrich*) in 5 mL dioxane over a period of 30 min at 0 °C. After stirring for 1 h at 0 °C and 1 h at room temperature the reaction was quenched with 60 mL water. The mixture was washed with diethyl ether (2x 25 mL). The aqueous layer was acidified with concentrated HCl (pH 1) and immediately extracted with ethyl acetate (3x 25 mL). The solvent was removed in vacuo to afford  $N^2$ -Boc- $N^3$ -Fmoc-2,3-diaminopropanoic acid as a white solid (0.52 g, 1.23 mmol, 62%).

<sup>*l*</sup>*H NMR* (400 *MHz*, *CDCl*<sub>3</sub>):  $\delta$  (ppm) = 1.44 (9 H, s, <sup>*t*</sup>C<sub>4</sub>*H*<sub>9</sub>), 3.58 (2 H, m, CHC*H*<sub>2</sub>NH), 4.18-4.30 (2 H, m, *α*-*H* and CHCH<sub>2</sub>O), 4.39 (2 H, d, CHC*H*<sub>2</sub>O, *J* = 4.0 Hz), 5.46 (1 H, br s, Fmoc-N*H*), 5.80 (1 H, br s, Boc-N*H*), 7.29-7.74 (8 H, m, Aryl-*H* Fmoc).\*\*



Figure S1.4: Synthesis of N<sup>2</sup>-Boc-N<sup>3</sup>-Fmoc-2,3-diaminopropanoic acid.

### Synthesis of the peptides a, 1a - 3a

Peptide synthesis was performed by using the Fmoc strategy. A rink amide MBHA resin (*Novabiochem*) served as the solid support. As side chain protecting groups Mtt (for histidine), 'Bu (for serine), and Boc (for 2,3-diaminopropanoic acid) were chosen. All amino acids were purchased from *Novabiochem*.

All synthesis steps were performed in dry DMF. The Fmoc deprotection step was performed with 20% piperidine in DMF (20 min) and the coupling with a mixture containing 4 equivalents of the Fmoc protected amino acid, 4 equivalents of activating agent PyBOP (*Novabiochem*), and 100  $\mu$ L DIPEA (2 h) in a syringe reaction vessel. Coupling of rhodamine B was performed in the same manner. For the coupling of dansyl chloride the tertiary base NEt<sub>3</sub> was used instead of DIPEA. Reaction of the peptide with dansyl chloride and fluorescein isothiocyanate was done without addition of an activating agent. The

final cleavage of the peptides from solid support and simultaneous side chain deprotection was performed with TFA (*Carl Roth*) containing 2.5% H<sub>2</sub>O and 2.5% TIS (2 h). The peptides were precipitated by addition of 10 mL of diethyl ether to the TFA solution. The suspensions were centrifuged at 6000 rpm and the residues were washed with 2x 10 mL diethyl ether. The products were dried in vacuo and purified via RP-HPLC (Chromaster 5000, *VWR*, *Hitatchi*).

*RP-HPLC*: C<sub>18</sub> column (10  $\mu$ m, 10 x 250 mm, *Merck*); solvent A: 0.1% TFA in H<sub>2</sub>O (Millipore system), solvent B: 0.1% TFA in acetonitrile (*VWR*); gradient: 5-50% B in 30 min, 50% B for 3 min, back to 5% B in 2 min, 5% B for 5 min; 4 mL/min.

*Retention times:* **a**: 5 min; **1a**: 20 min (non-fluorescent lactam) & 24 min (fluorescent amide); **2a**: 12 min; **3a**: 19 min.

*MS* (*ESI*<sup>+</sup>): **a**:  $m/z = 486.2402 [M+H]^+$  (calc: 486.2419); **1a**:  $m/z = 910.4604 [M]^+$  (calc: 910.4570); **2a**:  $m/z = 719.2885 [M+H]^+$  (calc: 719.2930); **3a**:  $m/z = 875.2891 [M+H]^+$  (calc: 875.2777).

*Yield*: The yields of the peptides were determined by using the extinction coefficients of compounds **5** and **6** and by weighing (for peptides **a** and **1a**) respectively (see S-2).

On a 60  $\mu$ mol scale the yields were **a**: 25.4 mg (52.3  $\mu$ mol, 87%); **1a**: 35.5 mg (34.7  $\mu$ mol, 58%); **2a**: 22.9 mg (31.9  $\mu$ mol, 53%); **3a**: 0.12 mg (0.1  $\mu$ mol, <1%).

### Synthesis of the Cu(II) complexes b, 1b - 3b

The Cu(II) complexes **b** and **1b** - **3b** were prepared by mixing 500  $\mu$ L of a 1 mM solution of the corresponding peptide with 500  $\mu$ L of a 0.8 mM solution of copper(II) chloride at room temperature. The complexes were incubated at room temperature for 24 h and subsequently stored at -20 °C.

*MS* (*ESI*<sup>+</sup>): **b**:  $m/z = 547.1553 [M+H]^+$  (calc: 547.1559); **1b**:  $m/z = 971.3707 [M]^+$  (calc: 971.3710); **2b**:  $m/z = 780.2089 [M+H]^+$  (calc: 780.2069); **3b**:  $m/z = 958.1742 [M+Na]^+$  (calc: 958.1736).

## S-2: Determination of peptide yields

Weighing peptides after synthesis and purification is not always a reliable way to get information about the yield. For example, the yield might be overestimated because of salt contaminants. We thus sought another way for determination. The peptides 1a - 3a do not have side chains with strongly absorbing aromatic residues like Trp and Tyr. Neither do they have a high molecular weight for determination of the yield by standard methods like absorption measurement at 280 nm and the Bradford assay, respectively.<sup>5</sup> For this reason, the calculation of the yield was carried out by using the extinction of the fluorophores. Given that the extinction coefficients of the free fluorophores are different from the ones coupled to the peptide, the fluorophores were functionalized with ethylenediamine in order to mimic the binding motif at the N-terminus as closely as possible (S-1).

Extinction coefficients of compounds 4 - 6 were determined by UV-vis spectroscopy using solutions of known concentration. The results of absorption measurements are shown in Table S2.1.

Compound	Solvent	Absorption maximum (nm)	Extinction coefficient (L mol <sup>-1</sup> cm <sup>-1</sup> )
4	Ethanol + 0.1 M HCl	556	4434
5	Ethanol	336	2582
6	Water + 0.1 M HCl	439	34094

 Table S2.1: Extinction coefficients of compounds 4, 5 and 6.

The carboxylic acid forms of rhodamines and fluoresceins usually are in equilibrium with the corresponding lactons. The reaction of the carboxyl groups with amines leads to amides, which causes an offset of the equilibrium. Rhodamine B shows strong absorption and fluorescence only in the open form, which is present at low pH values (Scheme S2.1, Figure S2.1A).<sup>6</sup> For this reason the extinction coefficient of rhodamine B was determined in hydrochloric acid solution (Table S2.1).



Scheme S2.1: Equilibrium between the two rhodamine B amide forms (amide and spirolactame).

Fluorescein, on the other hand, only shows a shift of the absorption maximum when the pH value changes (Figure S2.1B). The absorption measurements were thus also carried out in acidic solution (Figure S2.1B, orange curve).



**Figure S2.1:** (A) Absorption spectra of **4** in ethanol (orange) and acidic ethanol (blue). (B) Absorption spectra of **6** in water (blue), aq. HCl (orange) and aq. NaOH (green).

The absorption measurements for the determination of the concentration of the solutions of peptides 1a - 3a were carried out by using the calculated extinction coefficients under identical conditions (as listed in Table S2.1). The yields calculated from these measurements were compared with the values obtained by weighing. In case of rhodamine B no correlation between the functionalized dye 4 and peptide 1a was observed. Thus, the yield of 1a could only be determined by weighing. The yields obtained by UV/Vis and weighing, respectively, of peptides 2a and 3a revealed very close values, indicating that the yield obtained by weighing might be also reliable for 1a.

The extensive method for yield determination applied here allowed us to adjust the peptide concentration for following experiments regarding Cu(II) complexation and DNA cleavage precisely, whereas a weighing process might not have been that accurate.

### S-3: DNA cleavage experiments

The cleavage activity of the complexes **b** and **1b** - **3b** towards pBR322 plasmid DNA (Carl Roth) was studied in triplicate using gel electrophoresis. In a typical experiment 1% agarose gels (Lonza) in 0.5X TBE buffer (Fisher Scientific) containing ethidium bromide (0.2 µg/mL, Fisher Scientific) were used. The complexes (different concentrations) in TRIS-HCl buffer (10 mM, pH 7.4, Fisher Scientific) were mixed with plasmid DNA (0.025 µg/µL) and ascorbic acid (0.25 mM, Acros Organics). Deionized water (Millipore system) was added up to a total volume of 8 µL before the samples were incubated for given time at 37 °C. For analysis 1.5 µL of loading buffer (25 mg bromophenol blue and 4 g saccharose added up to a total volume of 10 mL with deionized water) was added and the sample was loaded onto the gel. Electrophoresis was carried out at 40 V for 2 h using an electrophoresis unit (Carl Roth) in 0.5X TBE buffer. Bands were visualised by using fluorescence emission of ethidium bromide and photographed using a gel documentation system (Bio-Rad). The intensity of the bands was measured using the reference DNA (ascorbate added, but no complex) as standard. Taking into account that the supercoiled form I of plasmid DNA has a smaller affinity to bind ethidium bromide, its intensity was multiplied with a correction factor of 1.22.7

Experiments with ROS scavengers were carried out as described above using either 200 mM *tert*-butanol, 200 mM DMSO, 10 mM sodium azide, 2.5 mg/mL catalase (bovine liver, 2000-5000 units/mL, *Sigma-Aldrich*) in 1X phosphate buffered saline (PBS, *Fisher Scientific*) or 313 units/mL superoxide dismutase (bovine liver, 2000-6000 units/mg, suspension in 3.8 M ammonium sulphate, *Sigma-Aldrich*). For the sake of comparison, 214 mM ammonium sulphate and 0.125X PBS were added to all samples, since superoxide dismutase was purchased as a suspension in ammonium sulphate and catalase had to be pre-incubated in PBS at 37 °C for 30 min.

Experiments with  $D_2O$  were conducted similarly to the ones described above except that the solutions were prepared by using  $D_2O$  instead of  $H_2O$  for dilution.

#### **Concentration dependence:**



**Figure S3.1**: Concentration dependent cleavage activity of complexes **1b** - **3b** with respect to pBR322 DNA (0.025  $\mu$ g/ $\mu$ L) in TRIS-HCl buffer (10 mM, pH 7.4) in the presence and absence of ascorbate (0.25 mM) at 37 °C for 1 h.



**Figure S3.2:** Concentration dependent cleavage activity of complex **1b** with respect to pBR322 DNA (0.025  $\mu$ g/ $\mu$ L) in TRIS-HCl buffer (10 mM, pH 7.4) in the presence and absence of ascorbate (0.25 mM) at 37 °C for 1 h.



**Figure S3.3:** Concentration dependent cleavage activity of complex **2b** with respect to pBR322 DNA (0.025  $\mu$ g/ $\mu$ L) in TRIS-HCl buffer (10 mM, pH 7.4) in the presence and absence of ascorbic acid (0.25 mM) at 37 °C for 1 h.



**Figure S3.4:** Concentration dependent cleavage activity of complex **3b** with respect to pBR322 DNA (0.025  $\mu$ g/ $\mu$ L) in TRIS-HCl buffer (10 mM, pH 7.4) in the presence and absence of ascorbate (0.25 mM) at 37 °C for 1 h.

Since a complex concentration of 1b - 3b of only 25  $\mu$ M leads to an obvious cleavage of plasmid DNA, in a further experiment, reduced complex concentrations were tested (Figure S3.5, exemplarily for 1b).



**Figure S3.5:** Concentration dependent cleavage activity of complex **1b** with respect to pBR322 (0.025  $\mu g/\mu L$ ) in TRIS-HCl buffer (10 mM, pH 7.4) in the presence of ascorbate (0.25 mM) at 37 °C for 1 h.

## **Comparative gel electrophoresis:**



**Figure S3.6**: Comparing gel electrophoresis for the cleavage activity of the Cu(II) complexes of peptides **2b**, **b** and Gly-Gly-His (GGH) (each 0.05 mM) of pBR322 (0.025  $\mu$ g/ $\mu$ L) in TRIS-HCl buffer (10 mM, pH 7.4) in the presence and absence of ascorbate (0.25 mM) at 37 °C for 1 h.

## Time dependence:



**Figure S3.7:** Time dependent cleavage activity of complex **1b** (50  $\mu$ M) with respect to pBR322 (0.025  $\mu$ g/ $\mu$ L) in TRIS-HCl buffer (10 mM, pH 7.4) in the presence of ascorbate (0.25 mM) at 37 °C.

## DNA cleavage in D<sub>2</sub>O:



**Figure S3.8:** Cleavage activity of **1b** (50  $\mu$ M) with respect to plasmid DNA pBR322 (0.025  $\mu$ g/ $\mu$ L) in TRIS-HCl buffer (10 mM, pH 7.4) in the presence of ascorbate (0.25 mM) in H<sub>2</sub>O and D<sub>2</sub>O, respectively, at 37 °C for 1 h.



Figure **S3.9**: Cleavage activity of **2b** and **3b** (50 µM) respect to with plasmid DNA pBR322 (0.025  $\mu g/\mu L$ ) in TRIS-HCl buffer (10 mM, pH 7.4) in the presence of ascorbate (0.25 mM) in  $H_2O$  and D<sub>2</sub>O, respectively, at 37 °C for 1 h.





**Figure S3.10:** Cleavage activity of **1b** and **2b** (50  $\mu$ M) with respect to plasmid DNA pBR322 (0.025  $\mu$ g/ $\mu$ L) in TRIS-HCl buffer (10 mM, pH 7.4) in the presence of ascorbate (0.25 mM) and neocuproine (neo) in H<sub>2</sub>O at 37 °C for 1 h.

## S-4: UV-vis experiments:



Figure S4.1: UV-vis spectroscopy of pH dependent binding of Cu(II) to peptide 2a.

1.0 mM peptide 2a was dissolved in Millipore water and the baseline was collected to subtract the absorbance of the peptide. 1.0 equiv. of CuCl<sub>2</sub> was added to the solution and the pH of the resulting solution was lowered to 2. Small portions of diluted KOH were added to the solution, the pH was determined using a pH meter (*Mettler Toledo*), and subsequently the absorption spectra were recorded.<sup>8</sup>

## **S-5: Fluorescence experiments**

Monitoring of the metal ion's redox status during DNA cleavage by fluorescence spectroscopy was carried out with a *Cary Eclipse Fluorescence Spectrophotometer (Varian)*. 0.05 mM solutions of each peptide (1a - 3a) and complex (1b - 3b) were measured. Compounds 2b and 3b were dissolved in TRIS-HCl buffer (10 mM, pH 7.4); 1b was measured in Britton-Robinson buffer<sup>9</sup> (10 mM, pH 5.0) to maintain the fluorescence of rhodamine B.

Complexes 1b - 3b were incubated in the respective buffer in the presence of 0.25 mM ascorbate and 0.2 µg pBR322 plasmid DNA at 37 °C for 1 h to obtain also fluorescence data after DNA cleavage (cf. Fig. 4).

#### **Titration with Cu(II):**



**Figure S5.1**: Fluorescence spectra of the titration of peptide **1a** with  $CuCl_2$  in TRIS-HCl buffer (10 mM, pH 7.4) with an incubation time of 5 min at each addition of Cu(II).

## Titration with ascorbate:



**Figure S5.2:** Fluorescence spectra after addition of ascorbate. (A) **1b** in Britton Robinson buffer (10 mM, pH 5.0). (B) **2b** in TRIS-HCl buffer (10 mM, pH 7.4). (C) **3b** in TRIS-HCl buffer (10 mM, pH 7.4).

## **Fluorescence switching:**



**Figure S5.3:** Fluorescence intensity of peptides **1b** and **2b** after titrating ascorbic acid and hydrogen peroxide alternately in (incubation 1 h at 37 °C after each addition). **1b** in Britton Robinson buffer (10 mM, pH 5.0) @ 484 nm. **2b** in TRIS-HCl buffer (10 mM, pH 7.4) @ 557 nm.

Peptide **3b** was not considered due to the instability of the thiourea bond in the presence of  $H_2O_2$ .

## S-6: NMR experiment



**Figure S6.1:** A) The NMR experiment was performed by mixing 1 mM peptide 2a with 0.8 mM CuCl<sub>2</sub> in 200 mM TRIS-HCl buffer (pH 7.4). All solutions were prepared in D<sub>2</sub>O and degassed with argon for 30 min. B) 5 mM ascorbate in D<sub>2</sub>O was added under argon to the reaction mixture.

## **S-7: Summary properties of fluorophores**

Fluorophore coupled	Rhodamine B	Dansyl	FITC
Coupling on solid support	+	+	+
Yield	+	+	
Fluorescence at pH 7.4		+	+
Stability vs. ascorbate	+	-	+
Stability vs. hydroxyl radicals	-	+	-

\*\* Data consistent with the literature.

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