Supplementary Information

General: All chemicals were purchased from Sigma-Aldrich, Lancaster or Fluka in analytical grade. The NPE-ATP was purchased from Jena Bioscience. An Agilent ESI-IT-XCT+ spectrometer was used for ESI analysis. ^{1}H NMR and ^{13}C NMR were run at 200 or 300 and 50 or 75 MHz, respectively. Coupling constants (J) are quoted in Hz and chemical shifts (δ) are given in parts per million (ppm) using the residue solvent peaks as reference relative to TMS. An Vydac Analytical C18 column (4.6, 250 mm) or an Acclaim C18 column (4.6, 250 mm) were used for HPLC analysis or a XTerra C18 column (10 x 250 mm) was used for HPLC purification.

Synthesis:

2,4-Dihydroxy-5-chloro-benzaldehyde (1)

2,4-dihydroxybenzaldehyde (8.75 g ; 63 mmol) was dissolved in 200 mL of a sulphuric acid solution (H_2O/H_2SO_4, 50/50, v/v) in a 1 L round bottom flask. N-chloropiperidine (8.3 g ; 70 mmol) was added in 5 min. The mixture was stirred overnight under argon at room temperature. The obtained precipitate was filtered and washed with water. Recrystallization from carbon tetrachloride provided 8.4 g of a white solid. ρ= 77 %

^{1}H NMR (300 MHz, $CDCl_3$) : δ 6.62 (s, 1H, H_3); 7.53 (s, 1H, H_6); 9.70 (s, 1H, COH).

^{13}C NMR (75 MHz, DMSO-D_6) : δ 103.5 (s, C_3); 112.2 (s, C_1); 116.0 (s, C_5); 130.3 (s, C_6); 159.9 (s, C_4); 161.4 (s, C_2) ; 188.8 (s, CHO)

6-chloro-7-hydroxy-coumarin-3-carboxylic acid (2)

Compound **1** (8.4 g, 48.8 mmol) and malonic acid (10.16 g ; 97.7 mmol) were dissolved in 44 mL of anhydrous pyridine in a 250 mL round flask. 4 mL aniline was added dropwisly. The mixture was stirred overnight under argon at room temperature. The obtained precipitate was washed by 70 mL ethanol for 1 h, filtered and washed again with 0.1 M chlorhydric acid, water and ethylic ether. 9.37 g of a yellow solid was obtained. ρ= 80 %

^{1}H NMR (300 MHz, $CDCl_3$) : δ 6.65 (s, 1H, H_8) ; 7.93 (s, 1H, H_5) ; 8.73 (s, 1H, H_4).

^{13}C NMR (75 MHz, DMSO-D_6) : δ 103.7 (s, C_8) ; 112.1 (s , C_4-$\underline{C}$- C_5) ; 114.9 (s, C_3) ; 118.5 (s, C_6) ; 131.6 (s, C_5) ; 149.2 (s, C_4) ; 156.0 (s , C_8-$\underline{C}$-O) ; 157.7 (s, C_7) ; 159.6 (s, C_2) ; 164.9 (s, COOH).

(*tert*-Butoxy)-*N*α,*N*α-bis(2-*tert*-butoxycarbonylmethy)-Nε-benzyloxycarbonyl-L-lysine

To a solution of Nε-benzyloxycarbonyl-L-lysine *tert*-butyl ester (**3a**) (5 g ; 13.4 mmol) in 100 mL of anhydrous DMF were added *tertio*-butyl bromoacetate (8 mL ; 54 mmol) and DIEA (1.5 mL ; 67.5 mmol) dropwisly, The mixture was stirred overnight under argon at 55°C. The reaction mixture was concentrated. The obtained solid was washed 3 times by a cyclohexan/ethyl acetate solution (3/1). The filtrate was concentrated and purified by column chromatography on silica gel, eluting with a cyclohexane/ethyl acetate solution (3/1). 7.5 g of uncoloured oil was obtained. ρ = 98%.

^{1}H NMR (300 MHz, $CDCl_3$) : δ 1.44 (s, 18H, CH_3 *t*Bu) ; 1.47(s, 9H, CH_3 *t*Bu) ; 1.49-1.65 (m, 6H, $CH_{2\beta}$ $CH_{2\chi}$ $CH_{2\delta}$) ; 3.21 (m, 2H, $CH_{2\varepsilon}$) ; 3.31 (t, $^3J_{HH}$ = 7.17 Hz, 1H, CH_α) ; 3.46 (m, 4H, CH_2-CO) ; 5.10 (s, 2H, $CH_{2benzylic}$) ; 7.30 (m, 5H, H_{ar})

(*tert*-Butoxy)-*Nα,Nα*-bis(2-*tert*-butoxycarbonylmethyl)-L-lysine (4a)

(*tert*-Butoxy)-*Nα,Nα*-bis(2-*tert*-butoxycarbonylmethy)-N*ε*-benzyloxycarbonyl-L-lysine (7 g; 12.4 mmol) was dissolved in 280 mL of methanol, followed by addition of 10 % Pd/C (450 mg). The reaction mixture was vigorously stirred for 24 h under H_2 atmosphere at room temperature. Pd/C was removed by filtration over celite and the volatiles from the filtrate were removed under reduced pressure. 5.3 g of uncoloured oil was obtained. ρ = 98%.

^{1}H NMR (200 MHz, CDCl3) : δ 1.45 (s, 18H, CH_3 *t*Bu) ; 1.47(s, 9H, CH_3 *t*Bu) ; 1.49-1.65 (m, 6H, $CH_{2\beta}$ $CH_{2\chi}$ $CH_{2\delta}$) ; 2.69 (t, $^3J_{HH}$ = 6.42 Hz, 2H, $CH_{2\varepsilon}$) ; 3.32 (t, $^3J_{HH}$ = 7.17 Hz, 1H, CH_α) ; 3.48 (m, 4H, CH_2-CO).

(*Methoxy*)-*Nα,Nα*-bis(2-*tert*-butoxycarbonylmethy)-N*ε*-benzyloxycarbonyl-L-lysine

To a solution of N*ε*-benzyloxycarbonyl-L-lysine *tert*-butyl ester (**3b**) (0.5 g ; 1.51 mmol) in 14 mL of anhydrous DMF were added *tertio*-butyl bromoacetate (0.670 mL ; 4.53 mmol) and DIEA (1.05 mL ; 6.05 mmol) dropwise, The mixture was stirred overnight under Argon at 55°C. The reaction mixture was concentrated. The obtained solid was washed 3 times by a cyclohexane/ethyl acetate solution (3/1). The filtrate was concentrated and purified by column chromatography on silica gel, eluting with a cyclohexane/ethyl acetate solution (3/1). 0.632g of yellowish oil was obtained. ρ = 76%.

^{1}H NMR (300 MHz, CDCl3) : δ 1.43 (s, 18H, CH_3 *t*Bu) ; 1.57-1.74 (m, 6H, $CH_{2\beta}$ $CH_{2\chi}$ $CH_{2\delta}$) ; 3.13-3.26 (m, 2H, $CH_{2\varepsilon}$) ; 3.37 (t, $^3J_{HH}$ = 7.50 Hz, 1H, CH_α) ; 3.50 (m, 4H, CH_2-CO) ; 3.66 (s, 3H, OMe) ; 5.08 (s, 2H, $OCH_2\Phi$) ; 7.34 (m, 5H, Har).

(*Methoxy*)-*Nα,Nα*-bis(2-*tert*-butoxycarbonylmethyl)-L-lysine (4b)

(*Methoxy*)-*Nα,Nα*-bis(2-*tert*-butoxycarbonylmethy)-N*ε*-benzyloxycarbonyl-L-lysine (0.63 g; 1.27 mmol) was dissolved in 30 mL of methanol, followed by addition of 10 % Pd/C (50 mg). The reaction mixture was vigorously stirred for 24 h under H_2 atmosphere at room temperature. Pd/C was removed by filtration over celite and the volatiles from the filtrate were removed under reduced pressure. 443.6 mg of uncoloured oil was obtained. ρ = 96%.

^{1}H NMR (300 MHz, CDCl3) : δ 1.44 (s, 18H, CH_3 *t*Bu) ; 1.20-1.79 (m, 6H, $CH_{2\beta}$ $CH_{2\chi}$ $CH_{2\delta}$) ; 2.67 (t, $^3J_{HH}$ = 6.7 Hz, 2H, $CH_{2\varepsilon}$) ; 3.38 (t, $^3J_{HH}$ = 7.50 Hz, 1H, CH_α) ; 3.52-3.55 (m, 4H, CH_2-CO) ; 3.67 (s, 3H, OMe).

(*tert*-Butoxy)-*Nα,Nα*-bis(2-*tert*-butoxycarbonylmethyl)-L-lysine [6-chloro-7-hydroxy-coumarin-3-carboxamide] or *t*-butyl esters-protected NTA-coumarin (5a)

Compound **2** (2.8 g ; 11.9 mmol) was dissolved in 35 mL of anhydrous DMF in a 250 mL flask at 0°C. N-Hydroxysuccinimide (1.5 g ; 13.09 mmol) was added to the reaction mixture and stirred under argon at 0°C for 1 h. DCC (2.7 g ; 13.09 mmol) was added and the reaction mixture was stirred 30 min at 0°C and 2 h at room temperature. A solution of **4a** (3.1 g ; 7.14 mmol) and DIEA (2.28 mL ; 13.09 mmol) dissolved in 15 mL of anhydrous DMF was added to the reaction mixture. After 3 h stirring at room temperature, volatiles were removed under reduced pressure. The product was dissolved in dichloromethane and washed by water. The organic extract was dried over anhydrous sodium sulfate, filtered, concentrated *in vacuo* and purified by column chromatography on silica gel, eluting with a heptane/ethyl acetate solution (1/1). 2.09 g of a yellow solid was obtained. ρ = 45 %.

^{1}H NMR (300 MHz, CDCl3) : δ 1.45 (s, 18H, CH$_3$ *t*Bu) ; 1.46(s, 9H, CH$_3$ *t*Bu) ; 1.63-1.70 (m, 6H, CH$_{2\beta}$ CH$_{2\chi}$ CH$_{2\delta}$) ; 3.33 (t, $^3J_{HH}$ = 7.53 Hz, 1H, CH$_\alpha$) ; 3.40 (m, 2H, CH$_{2\varepsilon}$) ; 3.48 (m, 4H, CH$_2$-CO) ; 7.06 (s, 1H, H$_8$) ; 7,65 (s, 1H, H$_5$) ; 8.75 (s, 1H, H$_4$).

(*Methoxy*)-*Nα,Nα*-bis(2-*tert*-butoxycarbonylmethyl)-L-lysine [6-chloro-7-hydroxy-coumarin-3-carboxamide] or bis-*t*-butyl-mono-methyl esters-protected NTA-coumarin (5b)

Compound **2** (0.58 g ; 2.42 mmol) was dissolved in 7 mL of anhydrous DMF at 0°C. N-Hydroxysuccinimide (0.307 g ; 2.67 mmol) was added to the reaction mixture and stirred under argon at 0°C for 1 h. DCC (0.549 g ; 2.67 mmol) was added and the reaction mixture was stirred 30 min at 0°C and left at room temperature overnight. A solution of **4b** (0.443 g ; 1.21 mmol) and DIEA (0.465 mL ; 2.67 mmol) dissolved in 3 mL of anhydrous DMF was added to the reaction mixture. After 3 h stirring at room temperature, volatiles were removed under reduced pressure. The product was dissolved in dichloromethane and washed by water. The organic extract was dried over anhydrous sodium sulfate, filtered, concentrated *in vacuo* and purified by column chromatography on silica gel, eluting with a heptane/ethyl acetate solution (1/1). 641 mg of a yellow solid was obtained. ρ = 87 %.

^{1}H NMR (200 MHz, CDCl3) : δ 1.44 (s, 18H, CH$_3$ *t*Bu) ; 1.57-1.78 (m, 6H, CH$_{2\beta}$ CH$_{2\chi}$ CH$_{2\delta}$) ; 3.37 (t, $^3J_{HH}$ = 7.2 Hz, 1H, CH$_\alpha$) ; 3.45 (m, 2H, CH$_{2\varepsilon}$) ; 3.52-3.55 (m, 4H, CH$_2$-CO) ; 3.67 (s, 3H, 0Me) ; 7.06 (s, 1H, H$_8$) ; 7.66 (s, 1H, H$_5$) ; 8.76 (s, 1H, H$_4$).

(*tert*-Butoxy)-*Nα,Nα*-bis(2-*tert*-butoxycarbonylmethyl)-L-lysine [6-chloro-7-acetoxy-coumarin-3-carboxamide] 7-acetyl-coumarin-*t*-butyl esters-protected-NTA (6a)

Compound **5a** (44 mg ; 0.067 mmol) was dissolved in 2mL of anhydrous dichloromethane. The solution was stirred overnight under argon atmosphere. Triethylamine (11.3 μL ; 0.081 mmol) and acetyl chloride (5.26 ; 0.074 mmol) were added dropwisly at 0°C. The solution was stirred 2 h at 0°C and 1 h at room temperature under argon atmosphere. The solvent was removed and the crude product was dissolved in water and extracted 3 times by dichloromethane. The combined organic extracts were dried over anhydrous sodium sulfate, filtered, concentrated *in vacuo* and purified by column chromatography on silica gel, eluting with a heptane/ethyl acetate solution (1/1). 37 mg of a yellow solid was obtained. ρ = 87 %.

^{1}H NMR (300 MHz, CDCl$_3$) : δ 1.45 (s, 18H, CH$_3$ *t*Bu) ; 1.46 (s, 9H, CH$_3$ *t*Bu) ; 1.57-1.70 (m, 6H, CH$_{2\beta}$ CH$_{2\chi}$ CH$_{2\delta}$) ; 2.42 (s, 3H, CH$_{3acetyl}$) ; 3.33 (t, $^3J_{HH}$ = 7.53 Hz, 1H, CH$_\alpha$) ; 3.42-3.56 (m, 6H, CH$_{2\varepsilon}$ 2 CH$_2$-CO) ; 7.26 (s, 1H, H$_8$) ; 7.77 (s, 1H, H$_5$) ; 8.82 (s, 1H, H$_4$).

^{13}C NMR (75 MHz, CDCl3) : δ 21.0 (s, CH$_3$acetyl) ; 23.9 (s, CH$_2\chi$) ; 28.5 and 28.6 (2s, CH$_3$ *t*Bu) ; 29.6 (s, CH$_2\delta$) ; 30.8 (s, CH$_2\beta$) ; 40.4 (s, CH$_2\varepsilon$) ; 54.1 (s, CH$_2$CO) ; 65.6 (s, CHα) ; 81.0 and 81.5 (2s, C *t*Bu) ; 112.8 (s, CH$_8$) ; 124.8 (s, C$_4$-$\underline{C}$- C$_5$) ; 127.5 (s, C$_3$) ; 129.3 (s, C$_6$) ; 133.5 (s, CH$_5$) ; 150.4 (s, CH$_4$) ; 153.6 (s, C$_4$-$\underline{C}$-O) ; 155.1 (s,

C_7) ;158.7 (s, C_2) ; 160.9 (s, COONH) ; 168.0 (s, COOacetyl) ; 171.0 and 172.7 (s, COO-*t*Bu).

(Methoxy)-*Nα,Nα*-bis(2-*tert*-butoxycarbonylmethyl)-L-lysine [6-chloro-7-acetoxy-coumarin-3-carboxamide] or 7-acetyl-coumarin-bis-*t*-butyl-mono-methyl esters-protected-NTA (6b)

Compound **5b** (100 mg ; 0.164 mmol) was dissolved in 3 mL of anhydrous dichloromethane. The solution was stirred overnight under argon atmosphere with DIEA (34 µL ; 0.193 mmol) and acetic anhydride (31 µL ; 0.327 mmol). The volatile part was removed under reduced pressure. 86 mg of a yellow solid was obtained. ρ = 80 %.

^{1}H NMR (300 MHz, $CDCl_3$) : δ 1.43 (s, 18H, CH_3 *t*Bu) ; 1.61-1.77 (m, 6H, $CH_{2\beta}$ $CH_{2\chi}$ $CH_{2\delta}$) ; 2.41 (s, 3H, $CH_{3acetyl}$) ; 3.39 (t, $^3J_{HH}$ = 7.6 Hz, 1H, CH_α) ; 3,40-3,48 (m, 2H, $CH_{2\varepsilon}$) ; 3.52-3,54 (m, 4H, CH_2-CO) ; 3.67 (s, 3H, 0Me); 7.25 (s, 1H, H_8) ; 7.76 (s, 1H, H_5) ; 8.81 (s, 1H, H_4).

API-ES-MS : 653.2 $[M+H]^+$

(*tert*-Butoxy)-*Nα,Nα*-bis(2-*tert*-butoxycarbonylmethyl)-L-lysine [-6-chloro-7-(erythro 3'-(4',5'-dimethoxy-2'-nitrophenyl)-2'-butyl)-ethoxy-coumarin-3-carboxamide] 7-DMNPB-coumarin-*t*-butyl esters-protected-NTA (7)

To a solution of **5a** (938 mg ; 1.44 mmol) and the two threo enantiomeres of 3-(4,5-dimethoxy-2-nitrophenyl)butan-2-ol (**11**) (obtained as described by Specht *et al.*[1]) (733 mg ; 2.88 mmol) in anhydrous benzene were added a solution of a 1/1 mixture of triphenylphosphine (1.13 g ; 4.32 mmol)/diisopropyl azodicarboxylate (DIAD) (850 µL ; 4.32 mmol) in anhydrous benzene. The solution was stirred overnight under argon atmosphere. The solvent was removed and the crude product was purified by column chromatography on silica gel, eluting with a heptane/ethyl acetate solution (7/3), followed by semi-preparative reversed-phase HPLC purification using a 10x250 mm Xterra column from waters with isocratic mode, eluting with an acetonitrile/0.1% TFA in water solution (75/25) at 4 mL / min. 832 mg of a yellow solid was obtained. ρ = 65 %.

^{1}H NMR (300 MHz, $CDCl_3$) : δ 1.45 (s, 18H, CH_3 *t*Bu) ; 1.46(s, 9H, CH_3 *t*Bu) ; 1.52-1.70 (m, 12H, $CH_{2\beta}$ $CH_{2\chi}$ $CH_{2\delta}$ CH_{2benz} CH_2) ; 3.33 (t, $^3J_{HH}$ = 7.47 Hz, 1H, CH_α) ; 3.40 (m, 2H, $CH_{2\varepsilon}$) ; 3.48 (m, 4H, CH_2-CO) ; 3.78 (q, $^3J_{HH}$ = 7.47 Hz, 1H, CH_{benz}) ; 3.89 (s, 3H, OCH_3) ; 3.92 (s, 3H, OCH_3) ; 4.84 (q, $^3J_{HH}$ = 6.24 Hz, 1H, CH) ; 6,76 (s, 1H, $H_{6'}$) ; 7,07 (s, 1H, H_8) ; 7.44 (s, 1H, $H_{3'}$) ; 7.65 (s, 1H, H_5) ; 8.74 (s, 1H, H_4).

API-ES-MS : 912,4 $[M+Na]^+$, 890,4 $[M+H]^+$

Nα,Nα-bis(2- carbonylmethyl)-L-lysine [6-chloro-7-(acetoxy -coumarin-3-carboxamide] or 7-acetyl-coumarine-NTA (8a)

Compound **6a** (19.1 mg, 0.027 mmol) was dissolved in 0.5 mL of dry CH_2Cl_2, 0.5 mL of trifluoroacetic acid (TFA) was added dropwisly at room temperature. After 5h at room temperature, the reaction mixture was concentrated *in vacuo*, the product was washed 5 times by cold ethyl ether. 14.8 mg of a yellow solid was obtained. ρ = 99 %.

^{1}H NMR (300 MHz, $CDCl_3$) : 1.57-1.70 (m, 6H, $CH_{2\beta}$ $CH_{2\chi}$ $CH_{2\delta}$) ; 2.42 (s, 3H, $CH_{3acetyl}$) ; 3.33 (t, $^3J_{HH}$ = 7.53 Hz, 1H, CH_α) ; 3.42-3.56 (m, 6H, $CH_{2\varepsilon}$ 2 CH_2-CO) ; 7.26 (s, 1H, H_8) ; 7.77 (s, 1H, H_5) ; 8.82 (s, 1H, H_4).

API-ESI-MS : 525.1 $[M-H]^-$

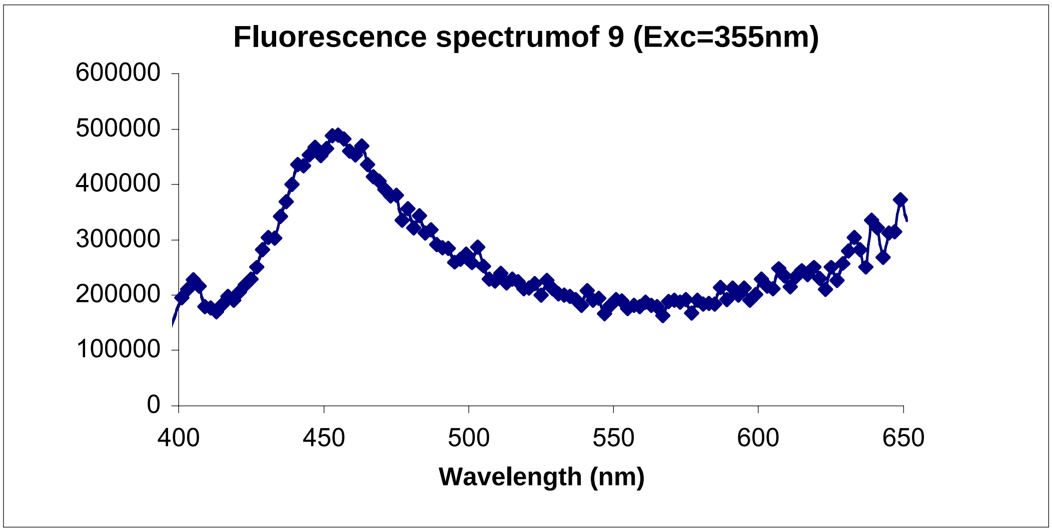
HPLC retention time on a Analytical C18 Acclaim (4.6 x 250 mm) column using a 30 minute linear gradient from 0 to 100% acetonitrile in a 0.1% TFA water solution at 1 mL/min followed by a 10 min isocratic mode of 100% acetonitrile : 32.7 min.
The hydrolysis **8a** to **10** in 0.1M phosphate buffer pH 7.2 was followed by reverse phase HPLC on a Acclaim C18 column (4.6, 250 mm) indicating à $t_{1/2}$ = 4h.

***(Methoxy)-Nα,Nα*-bis(2- carbonylmethyl)-L-lysine [6-chloro-7-(acetoxy - coumarin-3-carboxamide] or 7-acetyl-coumarine-NDA (<u>8b</u>)**

Same procedure as **8a** on **6b** (80 mg, 0.122 mmol) dissolved in 2 mL of dry CH_2Cl_2, using 1 mL of TFA. 65.6 mg of a yellow solid were obtained. ρ = 99 %.
^{1}H NMR (200 MHz, DMSO-d_6) : 1.36-1.70 (m, 6H, $CH_{2\beta}$ $CH_{2\chi}$ $CH_{2\delta}$) ; 2.38 (s, 3H, $CH_{3acetyl}$) ; 3.12-3.34 (m, 7H, $CH_{2\varepsilon}$, CH_α 2xCH_2-CO) ; 3.60 (s, 3H, OMe) ; 7.63 (s, 1H, H_8) ; 8.29 (s, 1H, H_5) ; 8.61 (t, 1H, $^3J_{H-H}$=5,7Hz, NH-CO) ; 8.80 (s, 1H, H_4).

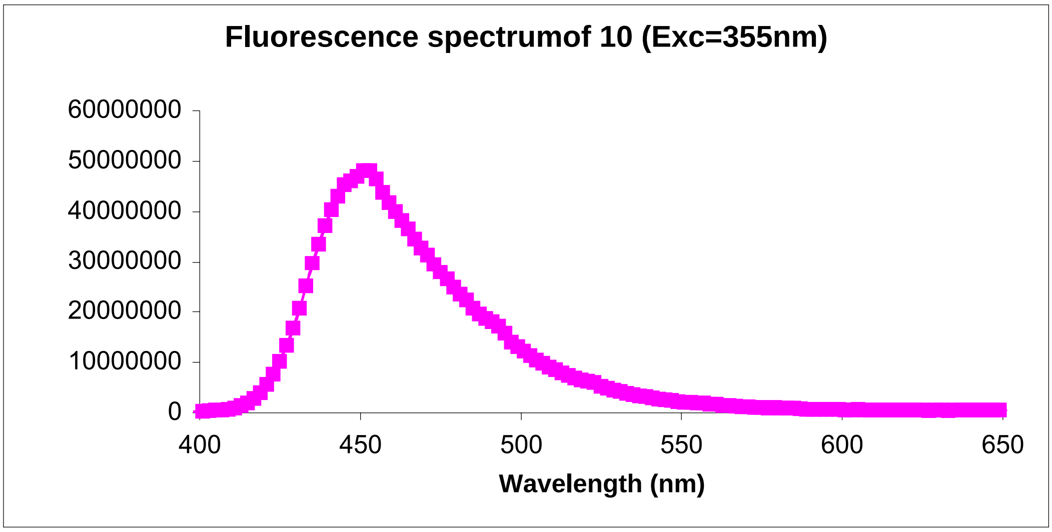
***Nα,Nα*-bis(2- carbonylmethyl)-L-lysine [6-chloro-7-(erythro 3'-(4',5'-dimethoxy-2'-nitrophenyl)-2'-butyl)-ethoxy-coumarin-3-carboxamide] or 7-DMNPB-coumarine-NTA (<u>9</u>)**

Same procedure as **8a** on **7** (65 mg, 0.073 mmol) dissolved in 2 mL of dry CH_2Cl_2, using 2 mL of TFA. 49.1 mg of a yellow solid was obtained. ρ = 93 %.
^{1}H NMR (300 MHz, $CDCl_3$) : δ 1.52-1.70 (m, 12H, $CH_{2\beta}$ $CH_{2\chi}$ $CH_{2\delta}$ CH_{2benz} CH_2) ; 3.33 (t, $^3J_{HH}$ = 7.47 Hz, 1H, CH_α) ; 3.40 (m, 2H, $CH_{2\varepsilon}$) ; 3.48 (m, 4H, CH_2-CO) ; 3.78 (q, $^3J_{HH}$ = 7.47 Hz, 1H, CH_{benz}) ; 3.89 (s, 3H, OCH_3) ; 3.92 (s, 3H, OCH_3) ; 4.84 (q, $^3J_{HH}$ = 6.24 Hz, 1H, CH) ; 6,76 (s, 1H, $H_{6'}$) ; 7,07 (s, 1H, H_8) ; 7.44 (s, 1H, $H_{3'}$) ; 7.65 (s, 1H, H_5) ; 8.74 (s, 1H, H_4).
API-ESI-MS : 720.1 $[M-H]^-$



_Nα,Nα_-bis(2- carbonylmethyl)-L-lysine (6-chloro-7-hydroxy -coumarin-3-carboxamide) or 7-hydroxy-coumarine-NTA (10)

Same procedure as **8a** on **5a** (20 mg, 0.046 mmol) dissolved in 1 mL of dry CH_2Cl_2, using 1 mL TFA. 20.3 mg of a yellow solid was obtained. ρ = 93 %.
^{1}H NMR (300 MHz, DMSO-D_6) : δ 1.30-1.70 (m, 12H, $CH_{2\beta}$ $CH_{2\chi}$ $CH_{2\delta}$ CH_{2benz} CH_2) ; 3.30-3.50 (m, 7H, CH_α $CH_{2\varepsilon}$ CH_2-CO) ; 6.99 (s, 1H, H_8) ; 8.09 (s, 1H, H_5) ; 8.61 (sl, 1H, OH) ; 8.76 (s, 1H, H_4).



One photon photolysis: (Fig. S1)

A solution (4 mL) of 0.04 mM **of 9** in 100 mM phosphate buffer, pH 7.4 was exposed to a 1000W Hg Lamp from Hanovia focused on the entrance slit of a monochromator at 315 nm, 364 nm or 403 nm (± 0.2 nm). The reactions were monitored by UV and aliquots of samples (100 μL) were injected into a Waters 600E HPLC carried out on a Acclaim C18 column (4.6 x 300 nm). Elution was performed at a flow rate of 1 mL/min with a linear gradient of acetonitrile in an aqueous solution of a 50 mM tris buffer pH 6.9 from 0 to 100% (v/v) over 30 min. The compounds were detected by a Waters 2996 PDA detector operating between 200 and 600 nm. Compound **9** and **10** have a retention time of 28.7 and 14.7 min respectively, **10** was quantified during the photolysis of **9** and mumerous photolytic by-products were detected presumably due to a photochemical instability of the 1-propylenyl-2-nitro-4,5-dimethoxybenzene derivatives.

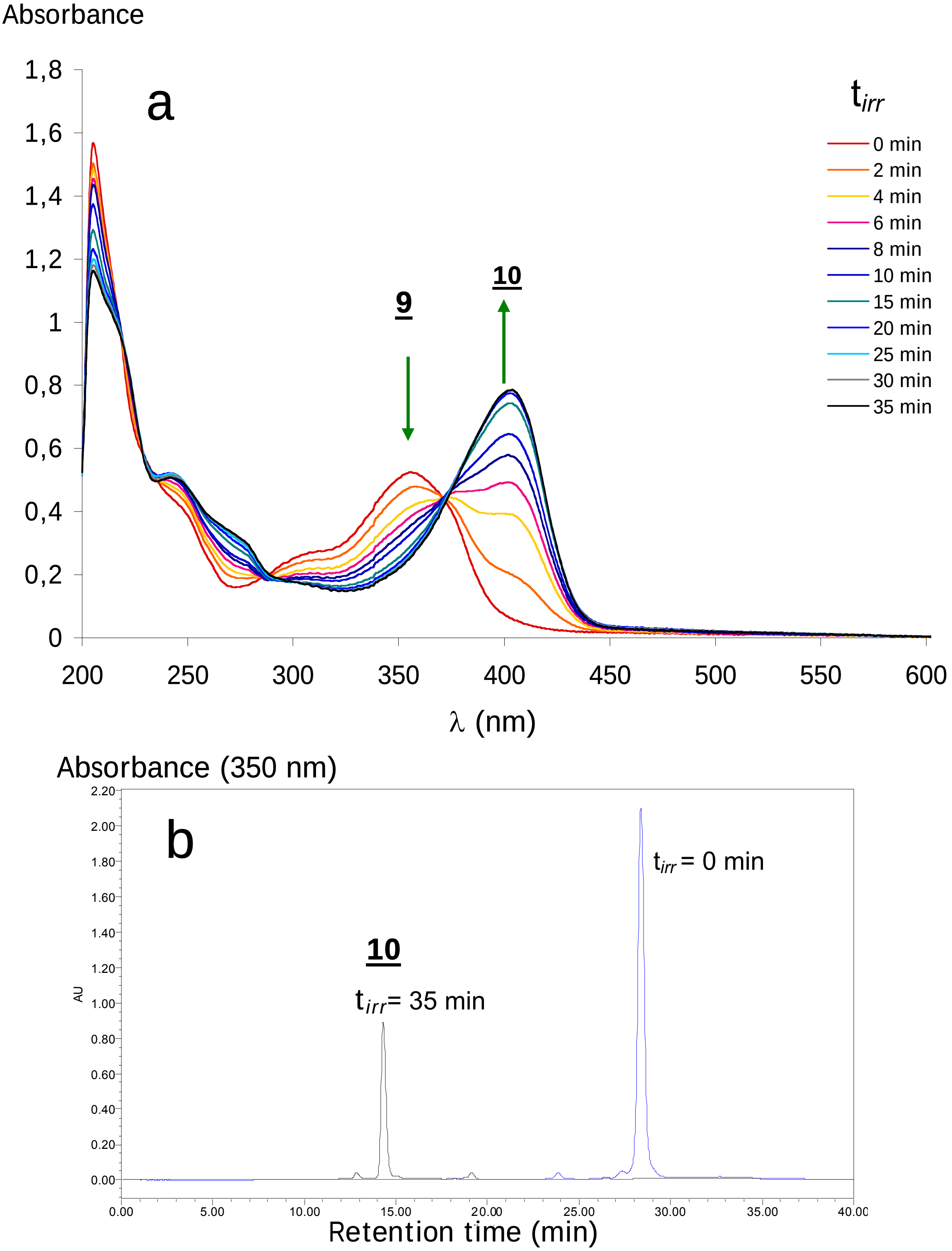


Fig. S1: **9** (0.04 mM in 100 mM phosphate buffer pH 7.4 at 4°C) was exposed to the 364.5-nm line of a Hg-Xe lamp. (a) UV-spectral recording of the photolysis. (b) HPLC analysis.

Two-photon photolysis:
Measurements were carried out in microcuvettes with 10 X 1 X 1 mm illuminated dimensions and 15 µL filling volume (16.10F-Q-10; Starna, Atascadero, CA). Femtosecond near IR pulses from a mode-locked, Ti-sapphire laser (Tsunami pumped by Millenium V; Spectra-Physics) were focused on the center of the sample chamber (10 mm path length) with a 25 mm focal length lens (06LXP003/076; Melles Griot, Irvine, CA) optimized for IR lasers. The two-photon uncaging action cross-section (δ_u) was estimated by referencing to fluorescein, whose fluorescence quantum yield Φ_F (0.9) and two-photon absorption cross-section δ_{aF} (30 GM at 740 nm) were characterized in the literature[2].
The two-photon uncaging action cross-section is given by the equation

$$\delta_u = (N_pK\Phi_F\delta_{aF}C_F)/(<F(t)>C_S)$$

where N_p is the number of product molecules formed per unit time (molecules/s, determined by HPLC analysis); K is the collection efficiency of our experimental setup used to measure the fluorescence of fluorescein emitted at a right angle to the beam and passed through a 520 ± 10 nm bandpass filter (03FIV109, Melles Griot, Irvine, CA); C_F is the concentration of fluorescein (mol/L); $<F(t)>$ is the time averaged fluorescence (photons/s) collected by the detector (silicon photodiode radiometer, SED033 on an IL-1700, International Light, Newburyport, MA); and C_S is the initial concentration of the caged substrate (mol/L).

Quantum yield determination:

The quantum yield for the photoconversion of compound **9**, was determined by comparison with the photolysis of 1-(2-nitrophenyl)ethyl-ATP (NPE-ATP) (Φ=0.63)[3] which was taken as reference in a phosphate buffer (0.1 mM, pH 7.4) at 25°C. These compounds were tested at identical optical densities at the irradiation wavelengths used. Accordingly, a mixture of 0.042 mM of **9** and 0.2 mM of NPE-ATP reference was used. This mixture was photolysed by continuous irradiation at 315 nm, and aliquots were subjected to reversed-phase HPLC to determine the extent of the photolytic conversions. HPLC analysis was carried out on an Vydac Analytical C18 (4.6 x 250 mm) column; elution was performed at a flow rate of 1 mL/min with a linear gradient of acetonitrile in an aqueous solution of 50 mM Tris buffer pH 6.9 from 0 to 100% (v/v) over 30 min. The retention times of **9** and NPE-ATP were 20.4 min and 13.1 min respectively. Quantum yields were calculated by considering conversions up to 30%, to limit as much as possible errors due to undesired light absorption during photolysis.

Laser Flash Photolysis.

An excimer laser was operated at 351 nm (XeF) with a pulse energy of 100 mJ and a pulse width of about 20 ns. A pulsed Xe high-pressure arc was used as the monitoring light. A shutter disposed between the lamp and the sample cell (path-length 4 cm) was opened shortly before the laser flash to avoid photolysis of the starting compound by the Xe arc. The laser flash was perpendicular to the probing light from the pulsed Xe lamp.

Kinetics Records:

The detection system allowed the simultaneous capture of the kinetics at a given wavelength by using a transient digitizer. The analyzing light through the cuvette was gathered with a lens and focused on the entrance slit of a monochromator (410 nm was used to analyze the formation of **10**). The signal was recorded by a photomultiplier and fed into the transient digitizer. Rate constants were calculated by least-squares fitting of decay curves with single exponential function.

Cell membrane permeability studies:

Hela cells were cultured in DMEM medium (Gibco) containing 1 g/L D-glucose, 5% foetal bovine serum and 40 µg/L gentamycin. Cells to be imaged were cultured in an eight-well Lab-Tek II coverglass system (Nunc) at 71 000 cells/cm^2. the cells were incubated 30 min at 37°C with **6a** or **8b** diluted in HBSS buffer (NaCl 8 g/L ; Na_2HPO_4 0,06 g/L ; KCl 0,4 g/L ; KH_2PO_4 0,06 g/L ; $NaHCO_3$ 0,35g/L ; HEPES 100 mM pH 7,35 ; glucose 2 g/L). After three washes with HBSS buffer, confocal microscopy was done on a SP2 AOBS microscope (Leica, Wetzlar, Germany) equipped with an acousto-optical beamsplitter Argon and HeNe lasers were used with a x63/1.4 oil immersion objective.

Fluorescent correlation spectroscopy (FCS):

FCS measurements were performed on a two-photon platform including an Olympus IX70 inverted microscope, as described (Egelé *et al*[4]). Two-photon excitation at 750 nm was provided by a mode-locked Tsunami Ti:sapphire laser pumped by a Millenia V solid-state laser (Spectra Physics).The measurements were carried out in an eight-well Lab-Tek II coverglass system, using a 300-μL volume per well. The focal spot is set about 20 μm above the coverslip. The normalized autocorrelation function, $G(\tau)$ is calculated online by an ALV-5000E correlator (ALV, Germany) from the fluorescence fluctuations, $\delta F(t)$, by $G(\tau)=\langle \delta F(t)\delta F(t+\tau)\rangle/\langle F(t)\rangle^2$, where $\langle F(t)\rangle$ is the mean fluorescence signal and τ is the lag time.

The analysis of $G(\tau)$ can provide information about the underlying mechanisms responsible for the intensity fluctuations such as diffusion of the particles, electronic transition within the molecules and transitions between states of different brightness. For an ideal case of freely diffusing monodisperse fluorescent particles undergoing triplet blinking in a Gaussian excitation volume, the correlation function, $G(\tau)$, calculated from the fluorescence fluctuations can be fitted according to:

$$G(\tau)=\frac{1}{N}\left(1+\frac{\tau}{\tau_D}\right)^{-1}\left(1+\frac{\tau}{S^2\tau_D}\right)^{-1/2} \qquad \text{Eq. 1}$$

where τ_d is the diffusion time (a parameter that is inversely related to the diffusion constant of the molecule), N is the mean number of molecules within the sample volume, s is the ratio between the axial and lateral radii of the sample volume. The excitation volume is about 0.3 μm^3 and s is about 3 to 4. As a compromise between photobleaching and a good signal to noise ratio, we select a power of 5 mW. At this power, the photon counting rate per molecule is about 5 kHz. Typical data recording times are 10 min.

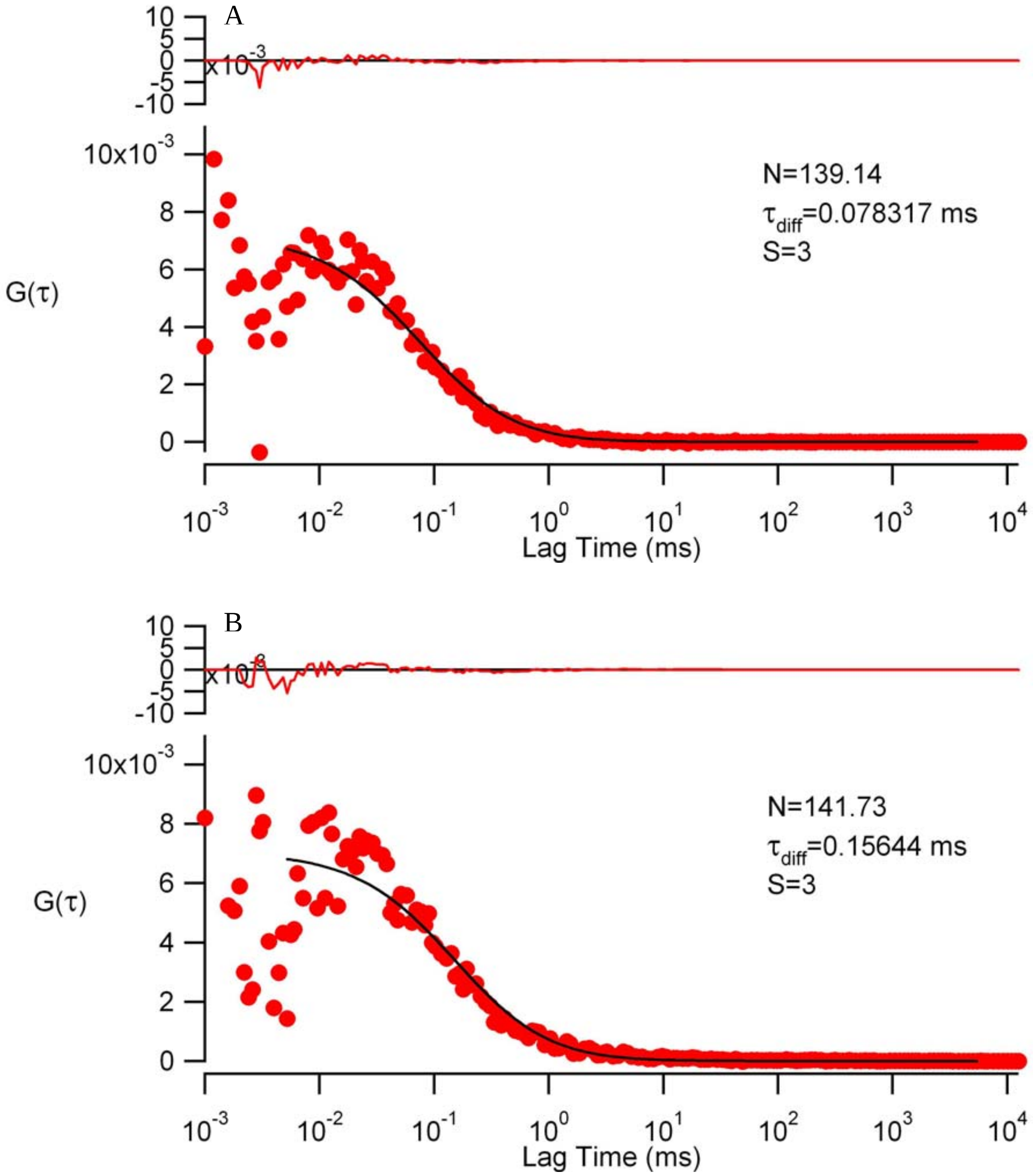


Fig. S2:
Binding of **10(Ni^{2+})** with a His-tagged protein, Bzz1p. Autocorrelation curves of a solution of 1 µM of **10** and 50 µM $NiCl_2$ in HBSS buffer, pH 7.4 (A) and after addition of a His-tagged protein Bzz1p at 5 µM (B). Solid lines correspond to fits of the experimental points with Eq.1.

References:

1. A. Specht, J. S. Thomann, K. Alarcon, W. Wittayanan, D. Ogden, T. Furuta, Y. Kurakawa and M. Goeldner, *Chembiochem*, 2006, **7**, 1690-1695.
2. C. Xu, W. W. Web, *J. Opt. Soc. Am. B* , 1996, **13**, 481 –491.
3. J.W. Walker, G. Reid, J.A. McCray, D.R. Trentham, *J. Am. Chem. Soc.* 1988, ***110****,* 7170-7177.
4. C. Egelé, E. Schaub, E. Piémont, H. de Rocquigny, Y. Mely, *C.R. Biologies*,

2005, **328**, 1041-1051.