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Synthesis of Fluorescent Probes for the Detection of Abasic Sites in DNA

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Abstract: The three luminescent molecules **1-3** were prepared as highly sensitive and specific probes for the quantification of a major DNA lesion, the abasic site. These molecules incorporate in their structure the reactive oxyamino function linked to the Dansyl or Lissamine-Rhodamine fluorophores through amido or polyether chains. © 1997 Elsevier Science Ltd.

INTRODUCTION

Abasic sites are probably the most frequent lesions in DNA[1]. They result from cleavage of the N-glycosidic bonds leading to removal of the bases and formation of 2'-deoxyribose residues in DNA. They may spontaneously occur in the cell at physiological pH. The rate of formation is increased as a result of modification of the nucleic bases by alkylating agents or certain therapeutic agents[2]. Abasic sites are also formed as intermediates during the base excision repair process of abnormal or modified bases under the action of highly specialized N-glycosylases[3]. If not repaired, the abasic site may promote misincorporation of nucleotides *in vitro* [4] and can be cytotoxic and mutagenic *in vivo* [5]. It is therefore of great interest to have a simple method for the quantification of this lesion in DNA, to be used both *in vitro* and *in vivo* experiments.

From a chemical point of view the abasic site corresponds to an equilibrium mixture of the cyclic hemiacetals (2'deoxy-D-erythropentafuranose) and the open chain aldehyde which represents less than 1% of the total[6]. Different methods have been reported to detect and titrate abasic lesions in DNA *in vitro*, all of them based upon reaction of nucleophiles, i.e. amino groups or aminooxy functions with the reactive aldehydic form of the deoxyribose moiety. These include the use of radioactive isotopes such as [^{14}C]-labelled methoxyamine[7] or [^{32}P]-postlabelling assay[8] or more recently an ELISA-like assay with a probe bearing a biotin residue[9].

In a general program aimed at designing molecules that specifically recognize abasic sites and that are of interest as cleaving agents for molecular biology experiments [10], or as pharmacological agents to inhibit the repair process in the cell[11], we also engineered molecules that can be used as selective probes to detect and quantitate abasic sites *in vitro*. We made use of a modular approach in which a highly reactive group towards aldehydes is linked to a detection moiety through a chain possessing the required solubility and DNA interaction properties. For the reactive group we chose the aminooxy function which has already been found to be specific for the abasic site[7,9] by forming an oxime ether with the aldehydic function of the ring opened deoxyribose residue. Such oxime ethers are stable in physiological conditions. For the detection the Dansyl and the

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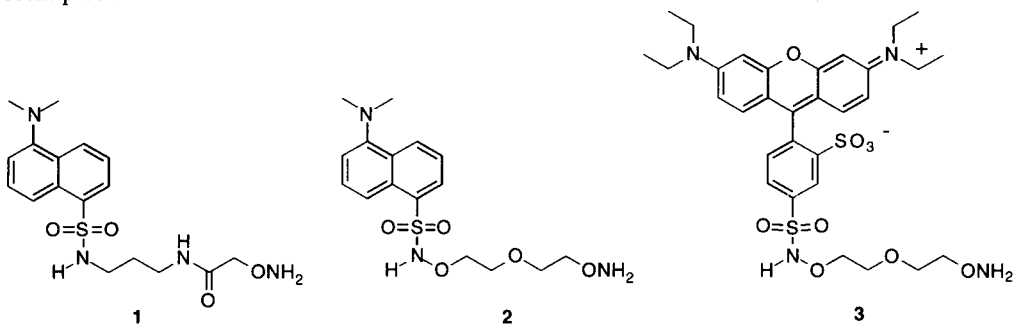
Lissamine-Rhodamine B fluorophores were selected[12]. The label and the reactive aminooxy group were tethered by linkers of two types, one containing a peptide bond, the other ethylether junctions. These two linkers possess comparable lengths but may interact differently with DNA. In this paper we report the synthesis of these fluorescent probes **1-3.**



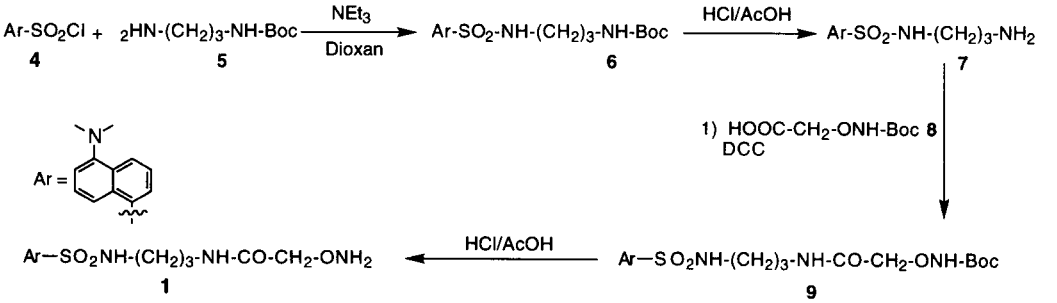
1 **2** **3**

RESULTS AND DISCUSSION

***Synthesis of probe 1*:**

Preparation of probe **1** was accomplished by a straightforward route shown in scheme 1. The luminescent label was introduced by reacting the Dansyl chloride **4** with the monoprotected 1,3-diaminopropane **5** affording compound **6** in 86% yield. The 1,3-diaminopropane was quantitatively monoprotected by a tert-butyloxycarbonyl (t-Boc) group to avoid bis-reaction with the sulfonyl chloride.

The tert-butyloxycarbonyl group on compound **6** was then removed by treatment with 1N HCl in acetic acid at room temperature to furnish the amino derivative **7** in 67 % yield after purification on a chromatography column. Introduction of the reactive aminooxy group was achieved using the commercially available O-(carboxymethyl) hydroxylamine hemihydrochloride. The amino moiety was first protected by a t-Boc group[9b]. Coupling the protected O-(carboxymethyl) hydroxylamine **8** with the amino compound **7** was achieved by applying the DCC method. The t-Boc protection was then removed in mild acidic conditions and the resulting probe **1** was obtained in a 50 % overall yield.



4 **5** **6** **7** **1** **9**

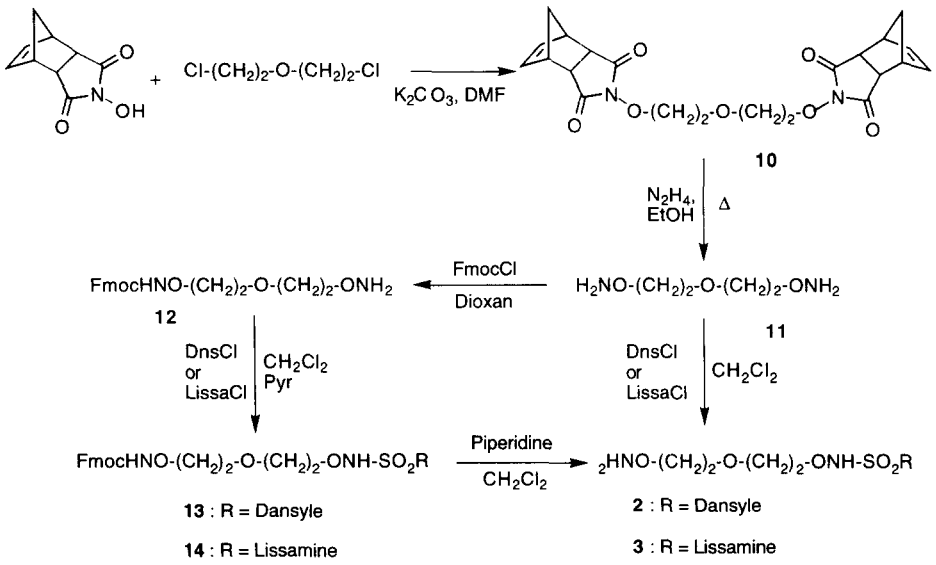
Scheme 1 : Synthesis of compound **1**

We attempted to apply this strategy to fluorescein to maximize detection sensitivity but in the acidic conditions of the deprotection step the fluorophore was found to be unstable. This synthesis has also the

disavantage that the "costly" label is introduced at an early stage of the synthesis. A more versatile synthesis was thus developed in which the fluorophore was introduced at the last stage.

Synthesis of probes 2 and 3:

Preparation of probes **2** and **3** was performed through a "symmetrical" approach as indicated in Scheme 2. The bis-aminooxy chain **11** was prepared in two steps from the commercially available 2,2-dichlorodiethylether. The aminooxy moiety was introduced using N-hydroxy-5-norbornene-2,3-dicarboximide as a precursor. This norbornene derivative was prefered to the usual N-hydroxyphtalimide as the yields of coupling and subsequent deprotection were higher[13]. The protected oxyamine **10** was thus obtained in 92 % yield by heating the two compounds at 50°C in DMF in the presence of potassium carbonate. The aminooxy groups were then deprotected using hydrazine in EtOH. Compound **11** was obtained in high yields and the reaction could be performed on a large scale. We then protected selectively one aminooxy function of **11** with a fluorenylmethoxycarbonyl group (fmoc). This group was chosen as it could be removed in mild alkaline conditions which did not affect the luminescent probe. The fmoc group was introduced by reacting the chloroformate with chain **11** in excess in dioxan to afford the monoprotected compound **12** in 92 % yield after purification by column chromatography (in these conditions, unreacted **11** could be recycled). The luminescent Dansyl and Lissamine-Rhodamine B labels were then introduced by reacting compound **12** with the corresponding sulfonyl chlorides in strictly anhydrous conditions to give **13** and **14** in 54 % and 35 % yield respectively[14]. Other activated forms of the labels like the isothiocyanates could not be used since their reaction products with oxyamines were found to be unstable[15]. Finally, the fmoc protection was removed by basic hydrolysis using piperidine in dichloromethane to afford probe **2** in 40% overall yield from Dansyl chloride and probe **3** in 20% overall yield from Lissamine-Rhodamine B chloride.



Scheme 2: Synthesis of probes **2** and **3**

Alternatively, probes **2** and **3** could be prepared in a one step non selective approach by reacting chain **11** with the Dansyl or Lissamine-Rhodamine B chloride. The crude yields appeared to be better but the separation of the corresponding probes from unreacted **11** used in excess and from the bis-reaction by-products required successive chromatographic separations that led to substantial losses of the desired materials. This method appeared useful for the preparation of the Dansyl probe **2**. It was however rejected, because of the high cost of the Lissamine-Rhodamine B chloride for the synthesis of **3**.

The structure of probes **1**,**2** and **3** were identified using ^1H NMR spectroscopy and accurate mass measurements. The ^1H NMR spectra showed in particular a broad singlet corresponding to the aminooxy protons ($-CH_2ONH_2$) at nearly 5 ppm.

CONCLUSION

We have developed a new series of fluorescent molecules which have been shown to react selectively with aldehydes. For exemple, the Dansyl probe **2** reacted with formaldehyde to afford quantitatively the corresponding oxime ether **15**[16].

Molecules **2** and **3** were used successfully to quantify abasic sites in liver DNA from rats treated by methylating carcinogenic compounds such as nitrosamines. With this method, we are able to detect one abasic site per 100000 nucleotides[16] in DNA.

EXPERIMENTAL PART

General: All commercially available chemical reagents were used without purification. CH_2Cl_2 was distilled over P_2O_5, MeOH over iodine. The protected O-(carboxymethyl) hydroxylamine **8** was prepared as described[9b]. Analytical TLC were performed on 0.2 mm silica 60 coated aluminium foils with F-254 indicator (Merck). Prep. column chromatographies were done using silica gel (Merck 60, 0.063-0.200 mm). Analytical HPLC were performed on a Millipore-Waters equipment (two M-510 pumps, solvent gradient M680) with a UV detector (M490 and diode array 990). Reverse phase µ-Bondapak C-18 Column (Millipore-Waters: 3.9x300 mm) was used with a methanol-water pH2.5 gradient, flow 2 ml/mn for 10 min. Melting points were measured on a Reichert Thermovar apparatus and are uncorrected. ^1H NMR spectra were recorded on Bruker AC200, WM250, AM300 and AM400 spectrometers. Spectra were referenced to the residual proton solvent peaks. Fourier Transform Infrared spectra were performed on an Impact 400 spectrophotometer. Mass spectra were recorded on a Delsi-Nermag R10-10. Elemental analysis and HRMS were performed by "Service Central de Microanalyse du CNRS". In several cases correct elemental analysis could not be obtained due to the polar and hygroscopic character of the compounds.

N-(tert-butoxycarbonyl)-1,3-diaminopropane **5:** To a solution of 1,3-diaminopropane (9.2 mL, 110 mmol) in chloroform (250 mL) was added dropwise at 0°C a solution of di-tertbutyldicarbonate (5 mL, 22 mmol) in chloroform (250 mL). The reaction was stirred overnight and then filtered off. The solution was evaporated and brine was added. The bis-protected by-product was eliminated by filtration and the aqueous phase was extracted with ether. The organic layer was dried (Na_2SO_4) and evaporated to afford compound **5** as a white powder (3.71 g, 95% yield). Mp. 90-92°C. ^1H-NMR ($CDCl_3$, 200 MHz): δppm = 5.10 (1H, br s, N<u>H</u>), 3.00 (2H, q, C<u>H</u>$_2$N), 2.60 (2H, t, C<u>H</u>$_2$N), 1.50 (2H, quint, CH_2-C<u>H</u>$_2$-CH_2), 1.30 (9H, s, C(C<u>H</u>$_3$)$_3$), 1.15 (2H, br s, NH_2). MS (FAB(+), glycerol matrix); m/e = 175 $[M+H]^+$,119 $[M-tBu]^+$.IR (film): 3340, 2440, 1700, 1450, 1300, 1080, 1030, 950, 860, 770 cm^{-1}.

***N-(5-dimethylamino-1-naphthalenesulfonamido)-N'-(tert-butoxycarbonyl)-1,3-diamino-propane* 6:** To a solution of compound **5** (1.95 g, 11.1 mmol) in dioxan (50 mL) was added NEt_3 (1.55 mL, 11.1 mmol) and Dansyl chloride (2 g, 7.4 mmol). The reaction was stirred for 1 hour at r.t. The solvent was then evaporated and the residual oil obtained was diluted in CH_2Cl_2. The organic layer was washed twice with brine, dried (Na_2SO_4) and evaporated to dryness affording compound **6** as a yellow powder (2.605 g, 86%). Mp. 65°C. ^{1}H-NMR ($CDCl_3$, 200 MHz): δppm = 8.50 (1H, d, Dns-H), 8.30 (1H, d, Dns-H), 8.20 (1H, d, Dns-H), 7.50 (2H, m, 2 Dns-H), 7.20 (1H, d, Dns-H), 5.70 (1H, br t, NH), 4.50 (1H, br t, NH), 2.85 (6H, s, $N(CH_3)_2$), 3.05 and 2.90 (4H, 2m, 2 CH_2-NH), 1.50 (2H, m, CH_2-CH_2-CH_2), 1.35 (9H, s, tBu). MS (FAB (-), NBA matrix): m/e = 406 $[M-H]^-$. IR (KBr): 3400, 2940, 1690, 1590, 1520, 1480, 1405, 1365, 1320, 1275, 1250, 1160, 1045, 1090, 1015, 945, 890, 825, 860, 625 cm^{-1}. UV (MeOH): λ_{max} (ε) = 335 (3680), 249 (11070). Anal. Calcd. for $C_{20}H_{29}N_3O_4S$: C 58.95 H 7.17 N 10.31. Found: C 58.67 H 7.54 N 10.28.

***N-(5-dimethylamino-1-naphthalenesulfonamido)-1,3-diaminopropane* 7:** Compound **6** was dissolved in a solution of 1N HCl in AcOH (50mL) and the reaction mixture was stirred for 1hour at r.t. The solvent was then evaporated and the residual oil obtained was diluted with dichloromethane. The organic layer was washed once with brine, dried (Na_2SO_4) and evaporated to dryness. Compound **7** was purified by precipitation in a dichloromethane/hexane solution as a yellow powder (1.01 g, 67%). Mp. 75°C. ^{1}H-NMR: ($CDCl_3$, 200 MHz): δppm = 8.50 (1H, d, Dns-H), 8.30 (1H, d, Dns-H), 8.20 (1H, d, Dns-H), 7.50 (2H, m, 2 Dns-H), 7.20 (1H, d, Dns-H), 3.80 (3H, ech., NH_2 and NH-SO_2), 2.85 (6H, s, $N(CH_3)_2$), 2.95 and 2.60 (4H, 2t, CH_2-NH and CH_2-NH_2), 1.40 (2H, quint, CH_2-CH_2-CH_2). IR (KBr): 3320, 3140, 1730, 1660, 1590, 1510, 1470, 1450, 1420, 1400, 1350, 1250, 1220 cm^{-1}. MS (FAB (+), glycerol matrix): m/e = 308 $[M+H]^+$. UV (MeOH): λ_{max} (ε) = 333 (3787), 249 (11842). Anal. Calcd for $C_{15}H_{21}N_3O_2S$, 2HCl, $1H_2O$: C 45.23 H 6.33 N 10.55. Found: C 45.38 H 5.89 N 10.41.

***Protected Dansyl Probe* 9:** The protected O-(carboxymethyl) hydroxylamine **8** (0.24 g, 1.26 mmol) was dissolved under argon in freshly distilled THF (10 mL) and DCC (0.26 g, 1.26 mmol) was added at 0°C. The reaction mixture was stirred at 0°C for 15 min. Compound **7** (0.25 g, 0.82 mmol) was then added and the solution was stirred for 2 hours. The reaction mixture was then filtered and evaporated to dryness. Compound **9** was purified by silica gel column chromatography (CH_2Cl_2) and was obtained as a yellow powder (0.36 g, 90%). Mp. 80°C. ^{1}H-NMR ($CDCl_3$, 200 MHz): δppm = 8.50 (1H, d, Dns-H), 8.30 (1H, d, Dns-H), 8.20 (1H, d, Dns-H), 8.10 (1H, br t, NH), 7.50 (2H, m, 2 Dns-H), 7.20 (1H, d, Dns-H), 5.70 (1H, ech. t, NH), 4.10 (2H, s, CH_2-O), 3.10 and 2.95 (2H, q, CH_2-NH), 2.85 (6H, s, $N(CH_3)_2$), 1.60 (2H, m, CH_2-CH_2-CH_2), 1.40 (9H, s, $C(CH_3)_3$). MS (FAB (+), NBA matrix): m/e = 480 $[M]^+$, 381 $[M-tBu]^+$. IR (KBr): 3250, 2920, 1700, 1650, 1550, 1450, 1350, 1310, 1250, 1150, 1110, 970, 620 cm^{-1}. UV (MeOH): λ_{max} (ε) = 335 (2724), 250 (8617). Anal. Calcd. for $C_{22}H_{32}N_4O_6S$: C 54.98 H 6.71 N 11.66. Found: C 54.72 H 6.96 N 11.53.

***Dansyl Probe* 1:** Compound **9** (150 mg, 0.31 mmol) was dissolved in a solution of 1N HCl in AcOH (5mL) and the reaction mixture was stirred for 1 hour at r.t. The solvent was then evaporated to dryness. Compound **9** was recristallized from dichloromethane and obtained as a white powder (110 mg, 81%). Mp. 110°C. ^{1}H-NMR (D_2O, 200 MHz): δppm = 8.80 (1H, d, Dns-H), 8.50 (1H, d, Dns-H), 8.40 (1H, d, Dns-H), 8.15 (1H, d, Dns-H), 7.95 (2H, m, 2 Dns-H), 4.50 (2H, s, CH_2ONH_2), 3.50 (6H, s, $N(CH_3)_2$), (2H, q, SO_2NH-CH_2), 3.10 and 3.00 (4H, 2t, 2 CH_2-NH), 1.60 (2H, q, CH_2-CH_2-CH_2). MS (FAB (+), glycerol matrix): m/e = 381 $[M+H]^+$. IR (KBr): 3400, 2650, 1650, 1550, 1430, 1310, 1140, 1090, 1040, 790 cm^{-1}. UV (H_2O): λ_{max} (ε) = 327 (1800), 245 (6180). Anal. Calcd. for $C_{17}H_{24}N_4O_4S$, 2HCl, $1.5H_2O$: C 42.50 H 6.08 N 11.66. Found C 42.44 H 6.38 N 11.38.

***3-oxapentane-1,5-dioxy-endo-2',3'-dicarboxydiimidenorbornene* 10:** To a solution of endo-N-hydroxy-5-norbornene-2,3-dicarboximide (30 g, 0.17 mmol) in DMF (380 mL), K_2CO_3 (24 g, 1 eq) and 2,2-dichlorodiethylether (9.8 g, 68 mmol) were added . The mixture was stirred for 24 h at 50°C. The solvent was then evaporated under vacuum. The solid residue was dissolved in CH_2Cl_2 and the solution was

washed with brine, dried (Na_2SO_4) and evaporated to dryness. Product **10** was obtained as a white powder (27 g, 92 %). Mp. 144-145°C. ^{1}H-NMR ($CDCl_3$, 200 MHz): δ ppm = 6.11 (4H, t, J=2Hz, 2 C<u>H</u>=C<u>H</u>), 4.09 (4H, m, 2 C<u>H</u>$_2$-O-N), 3.70 (4H, m, C<u>H</u>$_2$-O-C<u>H</u>$_2$), 3.38 (4H, m, 4 C<u>H</u>-C=O), 3.18 (4H, m, 4 C<u>H</u>-CH=CH), 1.72 and 1.47 (4H, 2d, J=9Hz, 2 C<u>H</u>$_2$-CH). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ ppm = 171.9 (4 <u>C</u>=O), 134.4 (2 <u>C</u>H=<u>C</u>H), 75.9 (2 <u>C</u>H$_2$-O-N), 68.9 (<u>C</u>H$_2$-O-<u>C</u>H$_2$), 51.2 (2 <u>C</u>H$_2$-CH), 44.5 (4 <u>C</u>H-C=O), 42.4 (4 <u>C</u>H-CH=CH). MS (FAB (+), glycerol matrix): m/e = 429 $[M+H]^+$. UV (EtOH): λ_{max} (ε) = 250 (320). IR (KBr disk): 3000, 2975, 2950, 2890, 1780, 1720 (ν_{CO}), 1450, 1360, 1340, 1290, 1130, 1095, 1050, 1040, 955, 895, 845, 780, 755, 725, 615, 605 cm^{-1}. Anal. Calcd. for $C_{22}H_{24}N_2O_7$: C 61.68; H 5.65; N 6.54. Found: C 61.47; H 5.71; N 6.52.

3-oxapentane-1,5-dioxyamine **11:** Compound **10** (11.9 g, 27.8 mmol) was dissolved in EtOH (160 mL) and then hydrazine (6.2 g, 124 mmol) was added. The solution was refluxed for 2 h then evaporated under vacuum. The residual oil obtained was separated by silica gel column chromatography (CH_2Cl_2/MeOH: 85/15, v/v) to give **11** (3.4 g, 90 %) as an oil. ^{1}H-NMR ($CDCl_3$, 200 MHz): δ ppm = 5.46 (4H, br s, 2 ON<u>H</u>$_2$), 3.82 (4H, m, 2 C<u>H</u>$_2$-ONH$_2$), 3.64 (4H, m, C<u>H</u>$_2$-O-C<u>H</u>$_2$). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ ppm = 74.6 (2 <u>C</u>H$_2$-ONH$_2$), 69.4 (<u>C</u>H$_2$-O-<u>C</u>H$_2$). GC-MS (EI): m/e = 137 ($[M+H]^+$, 3), 104 (9), 102 (12), 88 (10), 78 (19), 60 (100). IR (film): 3310 (ν_{NH}), 3245, 3165, 2915, 2870, 1600, 1465, 1355, 1210, 1130, 1055, 940, 850 cm^{-1}. Anal. Calcd. for $C_4H_{12}N_2O_3$: C 35.29 H 8.88 N 20.58. Found: C 35.06 H 9.03 N 20.28.

N-(9-fluorenylmethoxycarbonyl)-3-oxapentane-1,5-dioxyamine **12:** To a solution of **11** (1.0 g, 7.35 mmol) in dioxan (10 mL), a solution of 9-fluorenylmethylchloroformate (0.3 g, 1.16 mmol) in dioxan (2 mL) was added dropwise under argon. After 30 min. at r.t., the solution was evaporated and the residue was separated on a silica gel chromatography column (CH_2Cl_2/MeOH: 95/5, v/v). Compound **12** was obtained as an oil (0.38 g, 92 %). ^{1}H-NMR ($CDCl_3$, 200 MHz): δ ppm = 8.08 (1H, br s, ON<u>H</u>), 7.75 (2H, m, 2 Ar<u>H</u>), 7.58 (2H, m, 2 Ar<u>H</u>), 7.34 (4H, m, 4 Ar<u>H</u>), 5.42 (2H, br s, ON<u>H</u>$_2$), 4.50 (2H, d, J=7Hz, OC<u>H</u>$_2$-CH), 4.22 (1H, t, J=7Hz, C<u>H</u>-CH$_2$), 3.99 and 3.80 (4H, m, C<u>H</u>$_2$-ONH and C<u>H</u>$_2$-ONH$_2$), 3.66 (4H, m, C<u>H</u>$_2$-O-C<u>H</u>$_2$). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ ppm = 157.2 (<u>C</u>=O), 143.5 (2 <u>C</u>Ar), 141.3 (2 <u>C</u>Ar), 127.8 (2 <u>C</u>HAr), 127.1 (2 <u>C</u>HAr), 125.0 (2 <u>C</u>HAr), 120.0 (2 <u>C</u>HAr), 75.2 and 74.4 (<u>C</u>H$_2$-ONH and <u>C</u>H$_2$ONH$_2$), 69.6 and 69.4 (<u>C</u>H$_2$-O-<u>C</u>H$_2$), 67.1 (<u>C</u>H$_2$-O-C=O), 47.0 (<u>C</u>H-CH$_2$). MS (EI): m/e = 358 (M^+, 2), 179 ($[$fluorenyl-CH$_2]^+$, 96), 178 (100), 165 (39). UV (EtOH): λ_{max} (ε) = 256.1 (9800). IR (film): 3315 (ν_{NH}), 3260, 3080, 3045, 3020, 2940, 2900, 1750(ν_{CO}), 1590, 1450, 1330, 1250, 1120, 1060, 945, 740 cm^{-1}. HRMS: Calcd. for $C_{19}H_{22}N_2O_5$: 358.1529, found: 358.1507.

N-(9-fluorenylmethoxycarbonyl)-N'-(5-dimethylamino-1-naphthalenesulfonamido)-3-oxa-pentane-1,5-dioxyamine **13:** To a solution of compound **12** (137 mg, 0.38 mmol) in CH_2Cl_2 (20 mL), was added pyridine (41.5 mg, 0.53 mmol) and Dansyl chloride (95 mg, 0.35 mmol). The mixture was stirred at r.t. for 2 h, then concentrated under vacuum and purified by silica gel column chromatography (CH_2Cl_2/MeOH: 95/5, v/v). Compound **13** was obtained as pale yellow needles (110 mg, 54 %). Mp. 48-50°C. ^{1}H-NMR ($CDCl_3$, 200 MHz): δ ppm = 8.58 (1H, d, J=6Hz, Dns<u>H</u>), 8.29 (2H, m, 2Dns<u>H</u>), 7.89 (1H, br s, N<u>H</u>), 7.73 (3H, m, N<u>H</u> and 2 Dns<u>H</u>), 7.56-7.22 (8H, m, 8 Fmoc<u>H</u>), 7.10 (1H, d, J=7Hz, Dns<u>H</u>), 4.42 (2H, d, J=7Hz, C<u>H</u>$_2$-CH), 4.17 (1H, t, J=7Hz, C<u>H</u>-CH$_2$), 4.03 and 3.89 (4H, m, C<u>H</u>$_2$-ONHSO$_2$ and C<u>H</u>$_2$-ONHC=O), 3.58 (4H, m, C<u>H</u>$_2$-O-C<u>H</u>$_2$), 2.83 (6H, s, 2 C<u>H</u>$_3$). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ ppm = 157.4 (<u>C</u>=O), 151.9 (<u>C</u>-SO$_2$), 143.4 and 141.2 (4 <u>C</u>Fmoc), 131.9 (<u>C</u>HDns), 131.8 (<u>C</u>-N), 131.5 (<u>C</u>HDns), 130.1 and 129.8 (2 <u>C</u>Dns), 128.6 (<u>C</u>HDns), 127.8, 127.1 and 125.0 (6 <u>C</u>HFmoc), 123.2 (<u>C</u>HDns), 119.9 (2 <u>C</u>HFmoc), 118.6 and 115.2 (2 <u>C</u>HDns), 75.6 and 75.3 (<u>C</u>H$_2$-ONH), 68.6 and 68.2 (<u>C</u>H$_2$-O-<u>C</u>H$_2$), 67.3 (<u>C</u>H$_2$-O-C=O), 46.9 (<u>C</u>H-CH$_2$), 45.3 (2 <u>C</u>H$_3$). MS (FAB (+), NBA matrix): m/e = 592 $[M+H]^+$. UV (EtOH): λ_{max} (ε) = 338.4 (4000), 256.2 (31700). IR (film): 3240 (ν_{NH}), 2940, 2875, 2840, 2790, 1735 (ν_{CO}), 1570, 1455, 1410, 1335, 1250, 1170, 1150, 1060, 945, 800, 760, 745, 625, 570 cm^{-1}. HRMS (FAB (+)) Calcd for $C_{31}H_{34}N_3O_7S$: 592.2117, found 592.2130.

N-(1-sulfonyl-6-(3,6-diethylamino-xanthenyl)-3-sulfonamidophenyl)-N'-(9-fluorenyl-methoxycarbonyl)-3-oxapentane-1,5-dioxyamine **14:** Compound **14** was prepared by the same route as **13** by coupling **12** with Lissamine-Rhodamine sulfonyl chloride. Yield: 35 %. Mp. 135°C. ^{1}H-NMR (DMSO d_6, 250 MHz): δ ppm = 10.87 (1H, br s, ONH), 10.49 (1H, br s, ONH), 8.50 (1H, d, J=1.9Hz, CHLiss), 8.05 (1H, dd, J=8Hz and J=1.5Hz, CHLiss), 7.86 (2H, d, J=7.5Hz, CHFmoc), 7.62 (2H, d, J=7.5Hz, CHFmoc), 7.51 (1H, d, J=8Hz, CHLiss), 7.38 (2H, t, J=7Hz, CHFmoc), 7.28 (2H, t, J=7.5Hz, CHFmoc), 7.04-6.89 (6H, m, CHLiss), 4.34 (2H, d, J=6.5Hz, CH_2-CH), 4.16 (1H, t, J=6.5Hz, CH_2-CH), 4.08 and 3.78 (4H, m, 2 CH_2-ONH-SO_2 and CH_2-ONH-CO), 3.70-3.46 (12H, m, CH_2-O-CH_2 and 4 CH_2-N), 1.18 (12H, t, J=6.5Hz, 4 CH_3). ^{13}C NMR (DMSO d6, 75 MHz): δ ppm = 156.7 (2 CLiss), 156.1(CO), 154.7(2 CLiss), 147.6 (CLiss), 143.3 (2 CFmoc), 140.5 (2 CFmoc), 137.9 (2 CLiss), 134.0 (CLiss), 132.2 (2 CHLiss), 130.3 (CHLiss), 128.3 (CHLiss), 127.4 (2 CHFmoc), 126.9 (CHLiss), 126.8 (2 CHFmoc), 124.8 (2 CHFmoc), 119.9 (2 CHFmoc), 113.4 (2 CHLiss), 113.0 (2 CLiss), 95.1 (2 CHLiss), 75.6 and 74.6 (CH_2-ONH), 67.6 (CH_2-O-CO and CH_2-O-CH_2), 65.5 (CH_2-O-CH_2), 46.3 (CH_2-CH), 45.0 (CH_2-N), 12.5 (CH_3). MS (FAB(+), NBA matrix): m/e = 899 $[M+H]^+$. UV (EtOH): λ_{max} (ε) = 559.1 (96100), 353.4 (7200), 259.3 (46200). IR (KBr disk): 3495 (ν_{NH}), 2980, 2930, 2870, 1735 (ν_{CO}), 1650, 1595, 1470, 1415, 1340, 1275, 1250, 1185, 1135, 1075, 1030, 925, 765, 745, 685, 615 cm^{-1}. HRMS (FAB (+)) Calcd for $C_{46}H_{50}N_4O_{11}S_2$ $[M+H]^+$ 899.2995, found 899.2984.

N-(5-dimethylamino-1-naphthalenesulfonamido)-3-oxapentane-1,5-dioxyamine **2:**

From protected compound **13:** To a solution of **13** (50 mg, 0.14 mmol) in CH_2Cl_2 (5 mL), piperidine (18 mg, 0.21 mmol) was added. After stirring at r.t. for 30 min., the solvent was evaporated to dryness and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH: 95/5, v/v) to give **2** as a pale yellow oil (36.2 mg, 70 %).

From **11:** To a vigourously stirred solution of **11** (504 mg, 3.7 mmol) in CH_2Cl_2 (30 mL), a solution of Dansyl chloride (0.1 g, 0.37 mmol) in CH_2Cl_2 (30 mL) was added dropwise. The mixture was stirred at r.t. for 1 h then evaporated to dryness. Compound **2** was obtained as a yellow oil (94 mg, 69 %) after purification by silica gel column chromatography (CH_2Cl_2/MeOH: 97/3, v/v). ^{1}H-NMR ($CDCl_3$, 200 MHz): δ ppm = 8.56 (1H, d, J=8Hz, ArH), 8.28 (2H, m, 2 ArH), 7.87 (1H, br s, ONH), 7.52 (2H, t, J=8Hz, 2 ArH), 7.15 (1H, d, J=8Hz, ArH), 5.42 (2H, br s, ONH_2), 4.01 (2H, m, CH_2-ONH), 3.72 (2H, m, CH_2-ONH_2), 3.55 (4H, m, CH_2-O-CH_2), 2.85 (6H, s, 2 CH_3). ^{13}C-NMR ($CDCl_3$, 62.5 MHz): δ ppm = 151.6 (C-SO_2), 131.7 (CHAr), 131.6 (CAr-N), 131.3 (CHAr), 130.0 and 129.6 (2 CAr), 128.4, 123.1, 116.5 and 115.1 (4 CHAr), 75.5 and 74.2 (CH_2-ONH and CH_2-ONH_2), 69.1 and 66.1 (CH_2-O-CH_2), 45.3 (2 CH_3). MS (FAB(+), glycerol matrix): m/e = 370 $[M+H]^+$. UV (water): λ_{max} (ε) = 333.0 (3600), 247.1 (13900). IR (film): 3320 (ν_{NH}), 3230, 2945, 2875, 2835, 2785, 1700, 1610, 1585, 1575, 1455, 1410, 1335, 1230, 1200, 1165, 1150, 1060, 945, 835, 790, 710, 620, 570 cm^{-1}. HRMS (FAB (+)): Calcd for $C_{16}H_{24}N_3O_5S$: 370.1437, found 370.1455. Anal. Calcd. for $C_{16}H_{23}N_3O_5S$, 2HCl, 1.5 H_2O: C 41.12 H 5.61 N 8.99. Found: C 41.36 H 5.72 N 9.21.

N-(1-sulfonyl-6-(3,6-diethylamino-xanthenyl)-3-sulfonamidophenyl)-3-oxapentane-1,5-dioxyamine **3:** From **14**: as described for **2**, compound **3** was prepared by piperidine treatment of **14**. Yield: 45 %.

From **11**: to a solution of **11** (1 g, 7.4 mmol) in CH_2Cl_2 (100 mL), Lissamine-Rhodamine B chloride (0.4 g, 0.68 mmol) was added portionwise. The mixture was stirred for 1 h at r.t and the solvent was then evaporated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH: 90/10, v/v) to give probe **3** as a red powder (145 mg, 32 %). Mp. 110°C. ^{1}H-NMR (DMSO d_6, 250 MHz): δ ppm = 8.48 (1H, s, CHAr), 8.02 (1H, dd, J=8.5Hz and J=1.5Hz, CHAr), 7.53 (1H, d, J=8Hz, CHAr), 7.06 (2H, dd, J=9.5Hz and J=1.5Hz, 2 CHAr), 6.93 (4H, m, 4 CHAr), 6.40 (2H, br s, NH_2), 4.05 (2H, m, CH_2-ONH), 3.65-3.50 (14H, m, 3 CH_2-O and 4 CH_2-N), 1.20 (12H, t, 6.5Hz, 4 CH_3). ^{13}C-NMR (DMSO d_6, 75 MHz): δ ppm = 157.2, 155.1, 147.9, 138.2 and 134.3 (8 CAr), 132.6 (2 CHAr), 130.6, 128.4 and 127.2 (3 CHAr), 113.8

(2 CHAr), 113.4 (2 CAr), 95.5 (2 CHAr), 75.9 (CH_2-ONH), 74.2 (CH_2-ONH_2), 68.5 and 67.7 (CH_2-O-CH_2), 45.3 (4 CH_2-N), 12.5 (4 CH_3). MS (FAB(+), glycerol matrix) m/e = 677 $[M+H]^+$, 662 $[M+H-CH_3]^+$. UV (water): λ_{max} (ε) = 569.5 (77700), 257.1 (23900). IR (KBr disk): 3440 (ν_{NH}), 2980, 2925, 2870, 1655, 1590, 1420, 1345, 1275, 1250, 1180, 1125, 1075, 1035, 920, 685, 670 cm^{-1}. HRMS (FAB (+)) Calcd. For $C_{31}H_{40}N_4O_9S_2$ $[M+H]^+$ 677.2315, found 677.2324.

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