Supplementary Material (ESI) for Organic & Biomolecular Chemistry

SUPPORTING INFORMATION AND SUPLEMENTARY DATA

Efficient Solid Phase Strategy for Preparation of Modified Xanthene Dyes for Biolabelling

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1.-General Information

Unless otherwise noted, all solvents and reagents were used without further purification. HPLC grade methanol (MeOH), peptide grade Dimethylformamide (DMF), Dichloromethane (DCM), Triisopropylsilane (TIS) Diethyl Ether (Et_2O), Trifluoroacetic acid (TFA), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) and N,N'-Diisopropylcarbodiimide (DIC) were obtained from Aldrich and used as received. 2-chlorotrityl chloride resin was purchased from GL Biochem and preactivated prior to use (See details below for the preactivation method). All reactions involving Chlorotrityl chloride resin were performed using disposable filter tubes available from Supelco and agitated using a Blood tube rotator SB1 from Stuart Scientific.

2.-Instrumentation

^{1}H and ^{13}C-NMR spectra were measured on a Bruker DMX 500 spectrometer in the solvents indicated at 298 K. Chemical shifts are reported as δ in units of parts per million (ppm) relative to methanol *d4* (δ 3.30, septet in ^{1}H and 49.15, septet in ^{13}C - NMR). Multiplicities in ^{1}H-NMR are reported as follows: s (singlet), d (doublet), t (triplet), qi (quintuplet), dd (doublet of doublets), m (multiplet), br (broadened). Coupling constants are reported as a *J* value in Hertz (Hz). The number of protons (n) for a given resonance is indicated as nH, and is based on spectral integration values. High resolution mass spectra were recorded by the MS Department of the University of Edinburgh on a Thermo MAT 900 XLP high resolution, double focussing mass spectrometer. Analytical HPLC was conducted on an Agilent 1100 series HPLC system coupled to a Polymer Lab PL-ELS 1000 Evaporative Light Scattering (ELS) detector with UV detection at 220, 254, 260, 282 and 495 nm, Supelco's Discovery® C 18 (50 mm x 2.1 mm x 5μm) was used. Elution was performed with Solvent A (0.1% formic acid in HPLC-grade deionised water) and Solvent B (0.1% formic acid in HPLC-grade methanol) at 1 $mLmn^{-1}$ with a gradient of 5 to 95% B over 3 min, followed by 1 min isocratic at 95% B and ending with a gradient of 95 to 5% B over 1 min, then 1 min isocratic at 95% A. Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected. IR spectra were recorded neat using a Bruker Tensor 27 FT-IR spectrometer. Absorption spectra were recorded

on a Agilent 8453 spectrophotometer using 1cm pathlength fused silica cuvettes using Phosphate buffer as a blank. Fluorescence spectra were recorded on a SPEX Fluoromax, using 1cm pathlength fused silica cuvettes. The excitation and emission slits were 7 nm.

3.-General Procedure for the functionalization of dyes

As said previously 2-chlorotrityl chloride resin was preactivated prior to use. According with the protocol suggested by Harre et al[1] 2-chlorotrityl chloride resin (7.5 mmol) was washed once with DMF (50 mL) and three times with dry DCM (50 mL) and subsequently treated with thionyl chloride (850 μL) in DCM (50 mL). The mixture was gently stirred for 1h at room temperature and filtered. The resin was thereafter washed twice with DMF (50 mL) and three times with DCM (50 mL).

3.1-Dyes thiofunctionalization

A solution of cysteamine (11.75 mmol) and diisopropylethylamine (DIPEA) (11.75 mmol) was dissolved in DMF (15 mL) and added to preactivated 2-chlorotrityl chloride resin (2.25 mmol). The solution mixture was allowed to stir overnight at room temperature. After the coupling a positive ninhydrin test[2] was obtained, confirming that the coupling had been successfully performed. Then, resin **4** was split in three parts in order to perform the dyes functionalization in parallel. For this purpose, to a solution of dye A, B or C in DMF/DCM, Oxyma and DIC were added and the solution was stirred for 20 minutes to allow activation before addition to pre-swell resin **4**. The resultant mixture was allowed to react overnight and after filtration the obtained resin was washed subsequently in DMF, DCM, MeOH and Et_2O to ensure that any unreactive dye has been removed before proceeding to the cleavage. The resin cleavage was performed by adding a cocktail solution (90%TFA/5%TIS/5%DCM) and stirring for 2 hours then filtered off and washed with DCM. The solvent mixture was evaporated under pressure and the obtained product was precipitated in cold Et_2O. Following centrifugation compounds 7, 10 and 13 were obtained. The quantities used for this coupling are summarised in the next table.

	Resin (mmol)	Oxyma (mmol)	DIC (mmol)	DMF/DCM (mL)	Dye (mmol)
Carboxyfluorescein **A**	0.5	1.65	1.65	2.7/0.3	1.65
Carboxytetramethylrhodamine **B**	0.2	0.23	0.23	1.35/0.15	0.23
Carboxynaphthofluorescein **C**	0.2	0.23	0.23	1.35/0.15	0.23

5(6)-(N-thioethyl)fluoresceinamide (10)

Yield: 566 mg (79%).

Mp: 130-135 °C.

IR (cm−1): 2929 (w), 1669 (m), 1114 (s).

ELSD (S50DNEW.M): 3.1 min (79% purity).

HRMS (ES$^-$), $C_{23}H_{16}O_6N_1S_1$ $(M-H)^-$: calcd 434.07038, found 434.07074.

^{1}H-NMR (500 MHz, CD_3OD) δ 8.53 (d, 1H, *J*= 1.00 Hz), 8.31 (dd, 1H, *J*= 1.55, 8.07 Hz), 8.25 (dd, 1H, *J*= 1.32, 8.08 Hz), 8.13 (d, 1H, *J*= 8.01 Hz), 7.71 (s,1H), 7.36 (d, 1H, *J*= 8.10 Hz), 6.72 (dd, 4H, *J*= 2.36, 6.15 Hz), 6.63-6.55 (m, 8H), 3.44 (t, 2H, *J*= 6.72 Hz), 3.19 (t, 2H, *J*= 6.52 Hz). **^{13}C-NMR** (125 MHz, CD_3OD) δ 191.09, 169.82, 164.86, 162.07, 154.28, 143.28, 139.45, 134.91, 130.38, 126.57, 125.27, 119.20, 116.88, 110.79, 103.70, 37.00, 31.69.

5(6)-(N-thioethyl)tetramethylrhodaminamide (13)

Yield: 91 mg (81%).

Mp: 175-180 °C.

IR (cm−1): 2851 (w), 1669 (m), 1173 (s).

ELSD (S50DNEW.M): 3.2 min (97% purity).

HRMS (ES$^-$), $C_{27}H_{26}O_4N_3S_1$ $(M-H)^-$: calcd 488.16495, found 488.16507.

^{1}H-NMR (500 MHz, CD_3OD) δ 8.40-8.35 (m, 1H), 8.33-8.29 (m, 1H), 7.07-7.04 (m, 3H), 6.98 (dd, 2H, *J*= 2.24, 9.52 Hz), 6.88 (d, 2H, *J*= 2.13 Hz), 3.42 (t, 2H, *J*= 6.79 Hz), 3.34 (t, 2H, *J*= 6.82 Hz), 3.22 (s, 12H). **^{13}C-NMR** (125 MHz, CD_3OD) δ 191.21, 166.94, 163.02, 159.88, 159.00, 140.42, 139.200, 137.09, 135.91, 131.84, 119.31, 116.98, 115.69, 114.88, 114.57, 97.55, 41.00, 39.27, 34.93.

5(6)-(N-thioethyl)naphthofluoresceinamide (7)

Yield: 105 mg (86%).

Mp: 240-245°C.

IR (cm−1): 2921 (w), 1679 (m), 1200 (s).

ELSD (S50DNEW.M): 4.1 min (77% purity).

HRMS (ES$^-$), $C_{31}H_{20}O_6N_1S_1$ $(M-H)^-$: calcd 534.10168, found 534.10117.

^{1}H-NMR (500 MHz, CD_3OD) δ: 8.61 (dd, 4H, *J*= 3.62, 8.94 Hz), 8.27-8.17 (m, 3H), 7.65 (s, 1H), 7.34-.7.23 (m, 10H), 7.10 (d, 4H *J*= 2.32 Hz), 6.67 (d, 4H, *J*= 8.73 Hz), 3.40 (t, 2H, *J*= 6.72 Hz), 3.07 (t, 2H, *J*= 6.54 Hz). **^{13}C-NMR** (125 MHz, CD_3OD) δ 170.14, 168.55, 159.0, 155.58, 148.27, 143.66, 139.61, 138.10, 135.27, 131.94, 130.16, 128.75, 126.96, 125.12, 124.912, 120.250, 119.50, 110.67, 110.25, 110.12, 39.23, 34.90.

3.2-Dyes carboxyfunctionalization

A solution of Fmoc-6-aminohexanoic acid (11.75 mmol) and DIPEA (11.75 mmol) was dissolved in DMF (15 mL) and added to preactivated 2-chlorotrityl chloride resin (2.25 mmol). The solution mixture was allowed to stir overnight at room temperature. After the coupling a negative ninhydrin test[2] was obtained, confirming that the coupling had been successfully performed. After Fmoc deprotection (2 x10 minutes in 20% Piperidine in DMF) a positive ninhydrin test was obtained, confirming that the deprotection had been successfully performed. Then, resin **5** was split in three parts in order to perform the dyes functionalization in parallel. For this purpose, to a solution of dye A, B or C in DMF/DCM, Oxyma and DIC were added and the solution was stirred for 20 minutes to allow activation before addition to pre-swell resin **5**. The same protocol described previously for the thiofunctionalization was applied in here, in this case the cleavage cocktail was slightly different (5%TFA/5%TIS/90%DCM). Following this procedure compounds 8, 11 and 14 were obtained. The quantities used for this coupling are summarised in the next table.

	Resin	Oxyma	DIC	DMF/DCM	Dye

	(mmol)	(mmol)	(mmol)	(mL)	(mmol)
Carboxyfluorescein **A**	0.5	1.65	1.65	2.7/0.3	1.65
Carboxytetramethylrhodamine **B**	0.2	0.23	0.23	1.35/0.15	0.23
Carboxynaphthofluorescein **C**	0.2	0.23	0.23	1.35/0.15	0.23

6-[Fluorescein-5(6)-carboxamido]hexanoic acid *(11)*

Yield: 541 mg (67%).

Mp: 205-210 °C.

IR (cm−1): 3376 (w), 1737 (m), 1205 (s).

ELSD (S50DNEW.M): 3.7 min (67% purity).

HRMS (ES$^-$), $C_{27}H_{22}O_8N_1$ $(M-H)^-$: calcd 488.13509, found 488.13575.

^{1}H-NMR (500 MHz, CD_3OD) δ 8.41 (d, 1H, *J*= 1.00 Hz), 8.15 (dd, 1H, *J*= 1.29, 8.01 Hz), 8.07 (m, 1H), 7.58 (s, 1H), 7.27 (d, 1H, *J*= 8.04 Hz), 6.71 (br s, 4H), 6.65-6.55 (m, 8H), 2.29 (t, 2H, *J*= 7.36 Hz), 2.21 (t, 2H, *J*= 7.35 Hz), 1.53 (qi, 2H, *J*= 7.62 Hz), 1.40 (qi, 2H, *J*= 7.43Hz), 1.30 (qi, 2H, *J*= 6.66Hz). **^{13}C-NMR** (125 MHz, CD_3OD) δ 177.57, 170.21, 168.32, 161.48, 155.55, 142.15, 138.12, 135.14, 130.67, 129.13, 125.62, 114.68, 111.77, 103.61, 41.09, 34.83, 30.14, 27.62, 25.79.

6-[Tetramethylcarboxyrhodamine-5(6)-carboxamido]hexanoic acid *(14)*

Yield: 82 mg (65%).

Mp: 180-185 °C.

IR (cm−1): 2993 (w), 1588 (m), 1181 (s).

ELSD (S50DNEW.M): 3.5 min (99% purity).

HRMS (ES$^-$), $C_{31}H_{32}O_6N_3$ $(M-H)^-$: calcd 542.22966, found 542.22981.

^{1}H-NMR (500 MHz, CD_3OD) δ 8.29-8.19 (m, 1H), 8.15-8.09 (m, 1H), 7.09-7.04 (m, 3H), 6.9 (dd, 2H, *J*= 2.32, 9.38Hz), 6.8 (d, 2H, *J*= 2.44 Hz), 3.2 (s, 12H), 2.29-2.21 (m, 4H), 1.65-1.57 (m, 6H). **^{13}C-NMR** (125 MHz, CD_3OD) δ 177.54, 168.25, 163.15, 162.88, 159.06, 139.04, 137.91, 135.32, 132.08, 131.10, 130.24, 115.52, 114.78, 97.48, 40.96, 34.87, 34.510, 30.20, 28.31, 27.65, 26.65, 25.82, 25.44.

6-[naphthofluorescein-5(6)-carboxamido]hexanoic acid *(8)*

Yield: 95 mg (70%).

Mp: 190-195 °C.

IR (cm−1): 2940 (w), 1738 (m), 1203 (s).

ELSD (S50DNEW.M): 4.1 min (98% purity).

HRMS (ES$^-$), $C_{35}H_{26}O_8N_1$ $(M-H)^-$: calcd 588.16639, found 588.16661.

^{1}H-NMR (500 MHz, CD_3OD) δ: 8.57 (d, 4H, *J*= 9.04Hz), 8.13-8.11 (m, 3H), 7.5 (s, 1H) 7.33-7.24 (m, 10H), 7.08 (d, 4H, *J*= 2.22 Hz), 6.68 (dd, 4H, *J*= 1.64, 8.74 Hz), 3.40 (t, 2H, *J*= 7.04 Hz) 2.88 (t, 2H, *J*= 7.61 Hz) 2.29 (t, 4H, *J*= 7.31 Hz) 2.15 (t, 2H, *J*= 7.40 Hz). **^{13}C-NMR** (125 MHz, CD_3OD) δ 177.58, 170.67, 168.35, 159.0, 148.47, 142.58, 138.26, 138.11, 135.67, 130.62, 128.42, 124.94, 124.0, 120.17, 119.43, 110.71, 41.11, 40.58, 34.83, 34.47, 30.137, 29.89, 28.32, 27.62, 25.79, 25.42, 24.25.

3.3-Dyes aminofunctionalization

1,4-Diaminobutane (11.75 mmol) and triethylamine (11.75 mmol) were dissolved in DMF (15 mL) and added to the previously preactivated chlorotrityl resin (2.25 mmol). The solution mixture was allowed to stir overnight at room temperature. After the coupling a positive ninhydrin test was obtained, confirming that the coupling had been successfully performed. Then, resin **6** was split in order to perform the dyes functionalization. The same protocol described previously for the thiofunctionalization was applied in here, in this case the cleavage cocktail was slightly different (20%TFA/5%TIS/75%DCM). Following this procedure compounds 9, 12 and 15 were obtained. The quantities used for this coupling are summarised in the next table.

	Resin (mmol)	Oxyma (mmol)	DIC (mmol)	DMF/DCM (mL)	Dye (mmol)
Carboxyfluorescein **A**	0.5	1.65	1.65	2.7/0.3	1.65
Carboxytetramethylrhodamine **B**	0.2	0.23	0.23	1.35/0.15	0.23
Carboxynaphthofluorescein **C**	0.2	0.23	0.23	1.35/0.15	0.23

5(6)-(N-aminobutyl)fluoresceinamide (12)

Yield: 508 mg (69%).

Mp: 175-180 °C.

IR (cm−1): 3051 (w), 1580 (m), 1169 (s).

ELSD (S50DNEW.M): 3.0 min (76% purity).

HRMS (ES⁻), $C_{25}H_{21}O_6N_2$ $(M-H)^-$: calcd 445.14051, found 445.14005.

^{1}H-NMR (500 MHz, CD_3OD) δ 8.38 (d, 1H, *J*= 1.00 Hz), 8.14 (dd, 1H, *J*= 1.60, 8.02 Hz), 8.08 (dd, 1H, *J*= 1.40, 8.07 Hz), 8.03 (d, 1H, *J*= 7.90 Hz), 7.55 (s,1H), 7.26 (d, 1H, *J*= 8.04 Hz), 6.66 (t, 4H, *J*= 2.26 Hz), 6.56-6.49 (m, 8H), 2.96-2.92 (m, 4H), 1.61-1.55 (m, 4H). **^{13}C-NMR** (125 MHz, CD_3OD) δ 170.46, 168.53, 161.92, 154.32, 142.18, 137.85, 135.37, 130.26, 128.81, 126.47, 126.01, 125.07, 124.11, 114.02, 111.08, 103.66, 40.43, 27.48, 26.00.

5(6)-(N-aminobutyl)tetramethylrhodaminamide (15)

Yield: 105 mg (91%).

Mp: 200-205 °C.

IR (cm−1): 2941 (w), 1673 (m), 1181 (s).

ELSD (S50DNEW.M): 3.1 min (87% purity).

HRMS (ES⁻), $C_{29}H_{31}O_4N_4$ $(M-H)^-$: calcd 499.23508, found 499.23512.

^{1}H-NMR (500 MHz, CD_3OD) δ 8.27-8.15 (m, 1H), 8.14-8.08 (m, 1H), 7.02-6.95 (m, 3H), 6.98 (dd, 2H, *J*= 2.41, 9.43 Hz), 6.73 (d, 2H, *J*= 2.35 Hz), 3.12 (s, 12H), 2.85 (m, 4H), 1.65 (m, 4H). **^{13}C-NMR** (125 MHz, CD_3OD) δ 168.28, 167.41, 162.55, 160.55, 152.92, 139.29, 137.63, 135.50, 131.92, 123.93, 115.55, 114.71, 97.49, 40.92, 40.02, 27.44, 26.00, 25.51.

5(6)-(N-aminobutyl)naphthofluoresceinamide (9)

Yield: 116 mg (93%).

Mp: 190-195 °C.

IR (cm−1): 3346 (w), 1613 (m), 1202 (s).

ELSD (S50DNEW.M): 3.6 min (99% purity).

HRMS (ES⁻), $C_{33}H_{25}O_6N_2$ $(M-H)^-$: calcd 545.17181, found 545.17222.

^{1}H-NMR (500 MHz, CD_3OD) δ: 8.56 (d, 4H, J= 9.05 Hz), 8.15-8.13 (m, 3H), 7.5 (s, 1H), 7.33-7.24 (m, 10H), 7.07 (br s, 4H), 6.66 (t, 4H, J= 8.99 Hz), 2.94-2.93 (m, 4H), 1.53-1.48 (m, 4H). **^{13}C-NMR** (125 MHz, CD_3OD) δ 170.61, 168.51, 159.1, 148.53, 142.38, 138.14, 135.63, 128.47, 124.94, 124.1, 120.22, 119.41, 110.72, 40.0, 27.48, 25.60.

4.-Absorbance Analysis

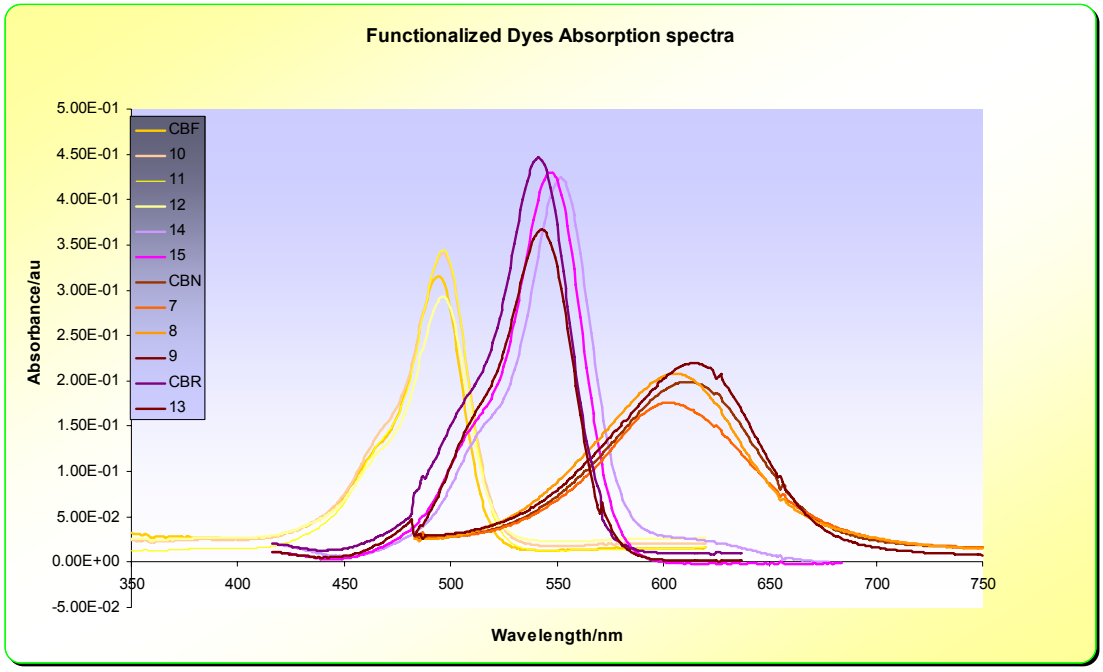


Figure S1. Absorption spectra of commercial dyes, Carboxyfluorescein (CBF), Carboxytetramethylrhodamine (CBR) and Carboxynaphthofluorescein (CBN), thiofunctionalized dyes (**10**, **13** and **7**), carboxyfunctionalized dyes (**11**, **14** and **8**) and aminofunctionalized dyes (**12**, **15** and **9**). Measurements performed in PBS pH 7.4, except the

measurements for the carboxynaphthofluorescein-based derivatives (**7**, **8** and **9**) which were performed in PBS pH 9.1.

5.-Excitation/Emission Analysis

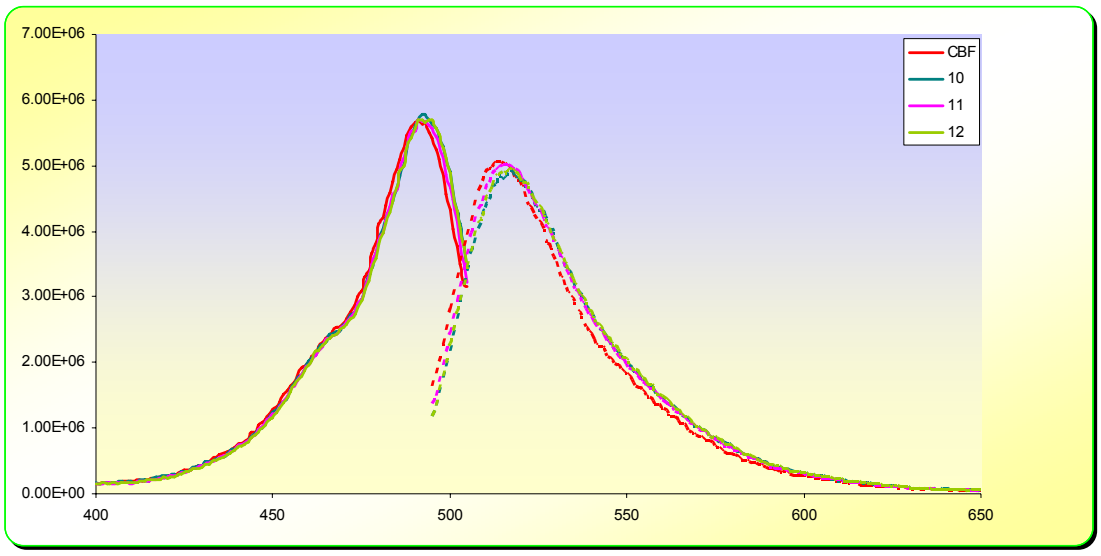


Figure S2. Excitation spectra, on the left, recorded at 513 nm, and emission spectra, on the right hand side, recorded at an excitation wavelength of 491 nm, of CBF and the fluorescein-based derivatives (**10**, **11** and **12**).

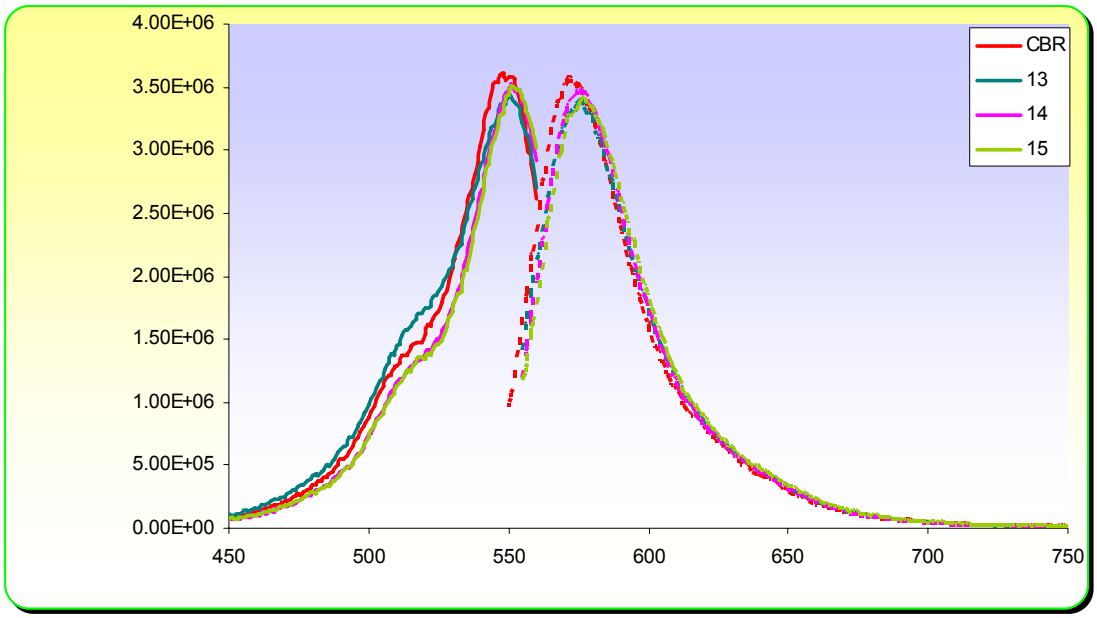


Figure S3. Excitation spectra, on the left, recorded at 572 nm, and emission spectra, on the right hand side, recorded at an excitation wavelength of 543 nm, of CBR and the rhodamine-based derivatives (**13**, **14** and **15**).

6.-Determination of quantum yields

Quantum yields were determined according to the method of Fery-Forgues et al.[4]Compounds to be evaluated were weighed on a microbalance and dissolved in 5 mM phosphate buffer, pH 7.4 (pH 9.1 for napthofluorescein and derivates), such that absorbance values between 0.2-0.5 were obtained at the excitation wavelength. Each sample was then diluted tenfold using additional phosphate buffer, and emission spectra were recorded with a fluorimeter. Carboxyfluorescein derivatives were excited at 491 nm, carboxytetramethylhodamine derivatives at 541 nm and carboxynaphthofluorescein derivatives were excited at 612 nm. Emission spectra were integrated using SpectraAcq software.

Compound	$\lambda_{max(excitation)}$	$\lambda_{max(emission)}$	ε(M cm^{-1})	Φ_r(%)
CBF	492 nm	517 nm	68,000	100%
Compound 10	492 nm	517 nm	83,000	101%
Compound 11	491 nm	495 nm	94,000	101%
Compound 12	492 nm	517 nm	84,000	101%

CBR	543 nm	572 nm	65,000	100%
Compound 13	543 nm	576 nm	137,000	94%
Compound 14	552 nm	575 nm	156,000	94%
Compound 15	547 nm	577 nm	142,000	95%
CBN	598 nm	668 nm	43,000	100%
Compound 7	609 nm	669 nm	58,600	114%
Compound 8	608 nm	667 nm	53,700	85%
Compound 9	614 nm	671 nm	50,100	78%

Table S1. Spectral properties of dyes derivatives reported herein.

7.-Protein labelling

Myoglobine and Cytochorme C from horse heart were purchased from Sigma-aldrich and used as received. To a solution of protein (0.5 mL, 10 mg/mL) in 0.1 M MES buffer, pH 5 (0.65) was added a solution of napthofluorescein derivative **7** (~10 eq.) in 0.1 M MES buffer, pH 5. Freshly prepared solution of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) in distilled water (2 mg/mL) was added (0.1 mL) to the mixture (0.1 mg for each mg of total protein) and incubated for 3 hours with mild agitation (5000 rpm) in a thermomixer at 25 °C. The resulting solution was subjected to gel filtration through a NAP^{TM}-10 Column (GE Healthcare). First of all, the desalting column was equilibrated with the solution phosphate buffered saline (PBS), once all the equilibration buffer was completely inserted into the gel bed the labelled proteins (Cytochrome C or Myoglobin) were add to the column in a volume of 1.0 mL. The sample was allowed to enter into the gel bed completely, next sample was eluted with 1.5mL of buffer, obtaining a solution with the purified sample. The process was repeated twice in order to ensure that any remaining dye stayed retained into the column. The purified sample was checked by absorbance analysis on the UV/Vis. NOTE: GE Healthcare NAP-10 columns are prepacked columns containing Sephadex® G-25 Medium of DNA Grade for rapid and convenient desalting and buffer exchange of nucleic acids, proteins and oligonucleotides (≥10mers).

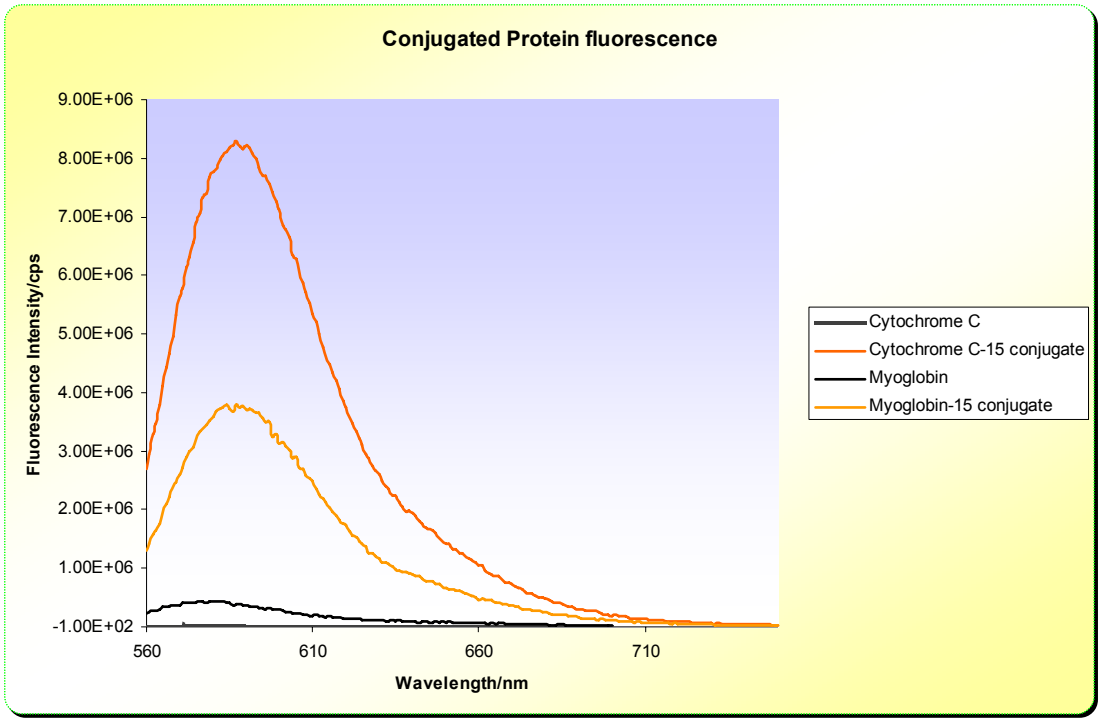


Figure S4. Emission spectra of Proteins (Cytochrome C and Myoglobin) conjugated with **15.** Measurements performed in neutral PBS. Excitation at 545 nm.

Quantitation of protein:dye conjugation (dye:protein or F/P molar ratio) was calculated according with the equation suggested by Thermo Scientific[3].

1. Calculate molarity of the protein:

• ε = protein molar extinction coefficient

• Amax = Absorbance (A) of the dyes solution measured at the wavelength maximum (λmax) for the dyes molecule

• CF = Correction factor; adjusts for the amount of absorbance at 409 nm caused by the dyes.

• Dilution factor = the extent (if any) to which the protein:dye sample was diluted for absorbance measurement

$$\text{Protein concentration } (M) = \frac{A_{280} - (A_{\max} - CF)}{\varepsilon} \times \text{dilution factor}$$

2. Calculate the degrees of labeling:

• ε′ = molar extinction coefficient of the fluorescent dye

$$Moles\ dye\ per\ mole\ protein = \frac{A_{\max}\ of\ the\ labeled\ protein}{\varepsilon' \times protein\ concentration\ (M)} \times dilution\ factor$$

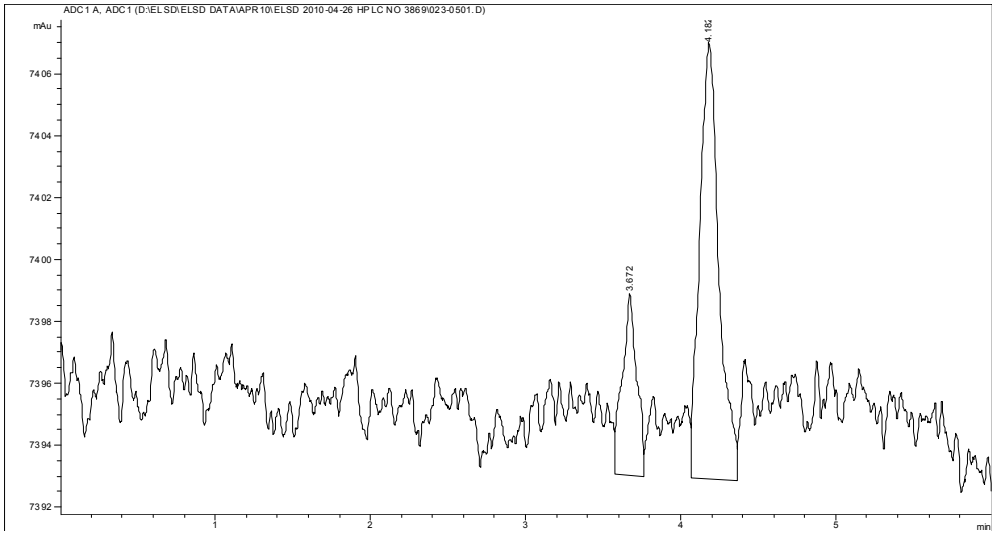
With this equation the following degree of labelling were obtained.

Sample	Degree of labelling*
Cytochrome C-dye9 conjugate	2.82
Myoglobine-dye9 conjugate	3.00
Cytochrome C-dye15conjugate	1.76
Myoglobine-dye15conjugate	1.82

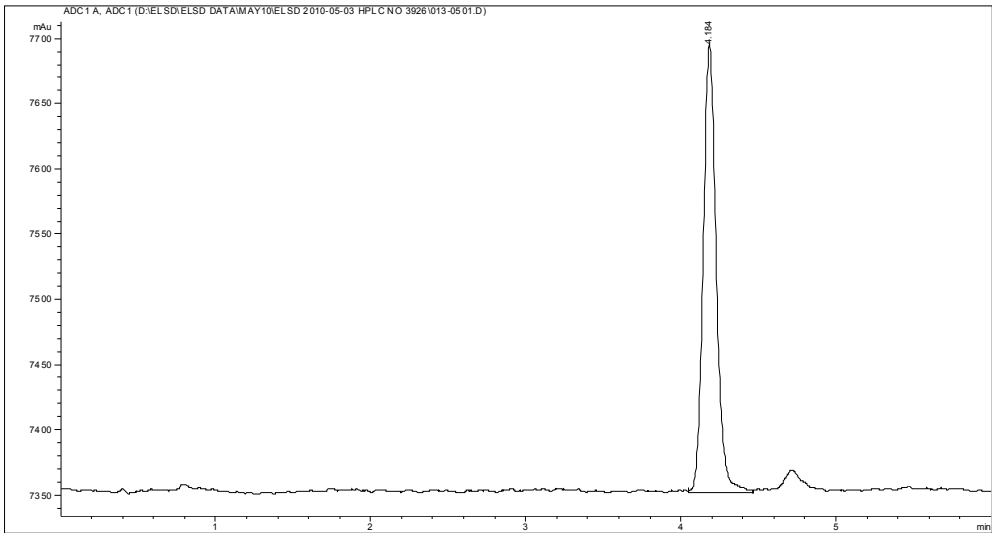
Table S2. Degree of labelling of protein conjugates. * Expressed in mol dye:mol protein ratio (F/P).

8.-ELSD chromatograms

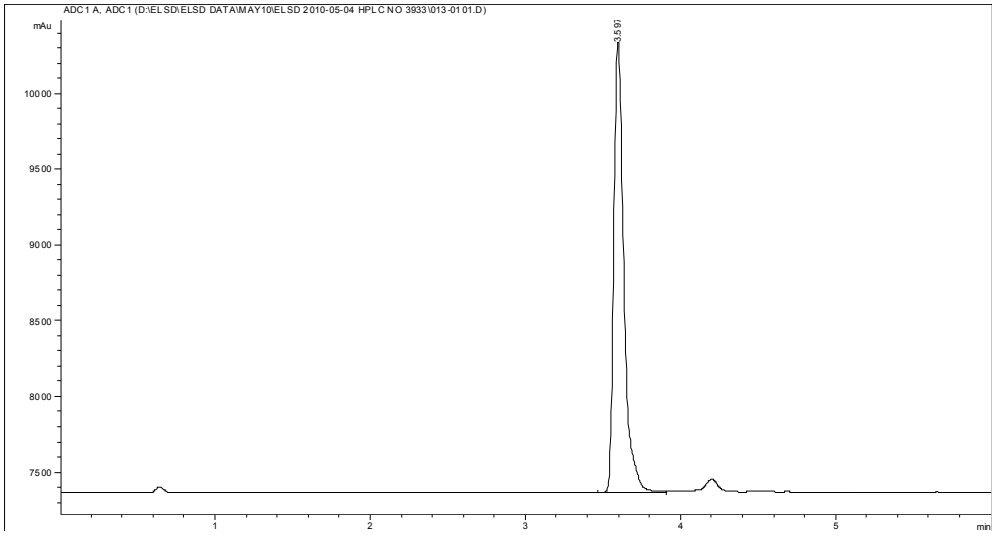
5(6)-(N-thioethyl)naphthofluoresceinamide (7)



6-[naphthofluorescein-5(6)-carboxamido]hexanoic acid *(8)*

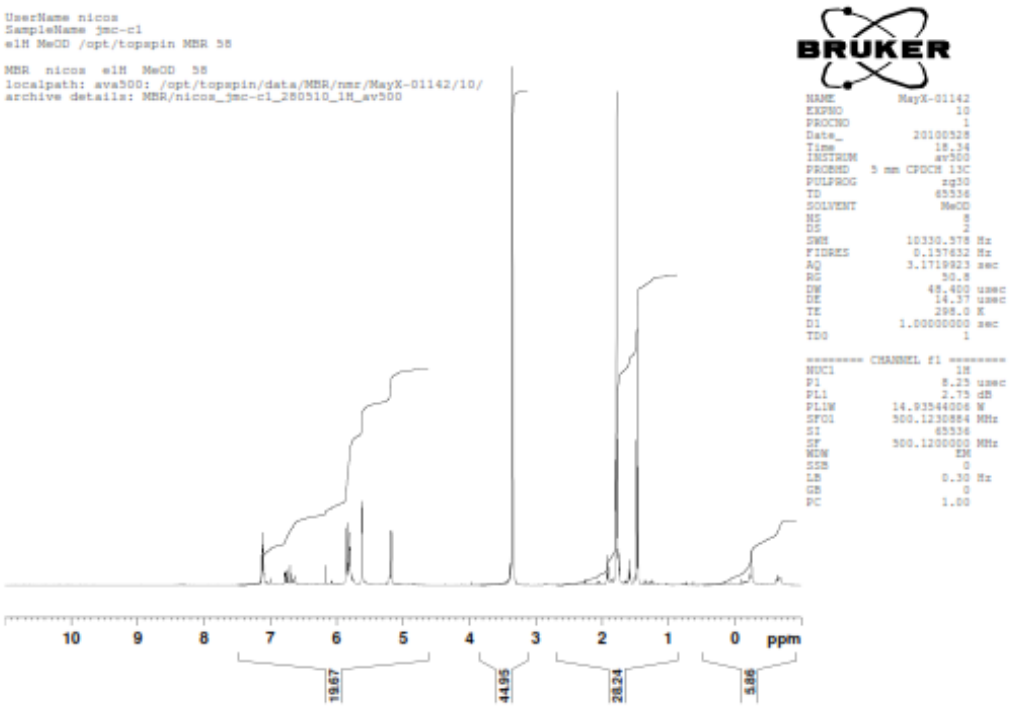


5(6)-(N-aminobutyl)naphthofluoresceinamide (9)

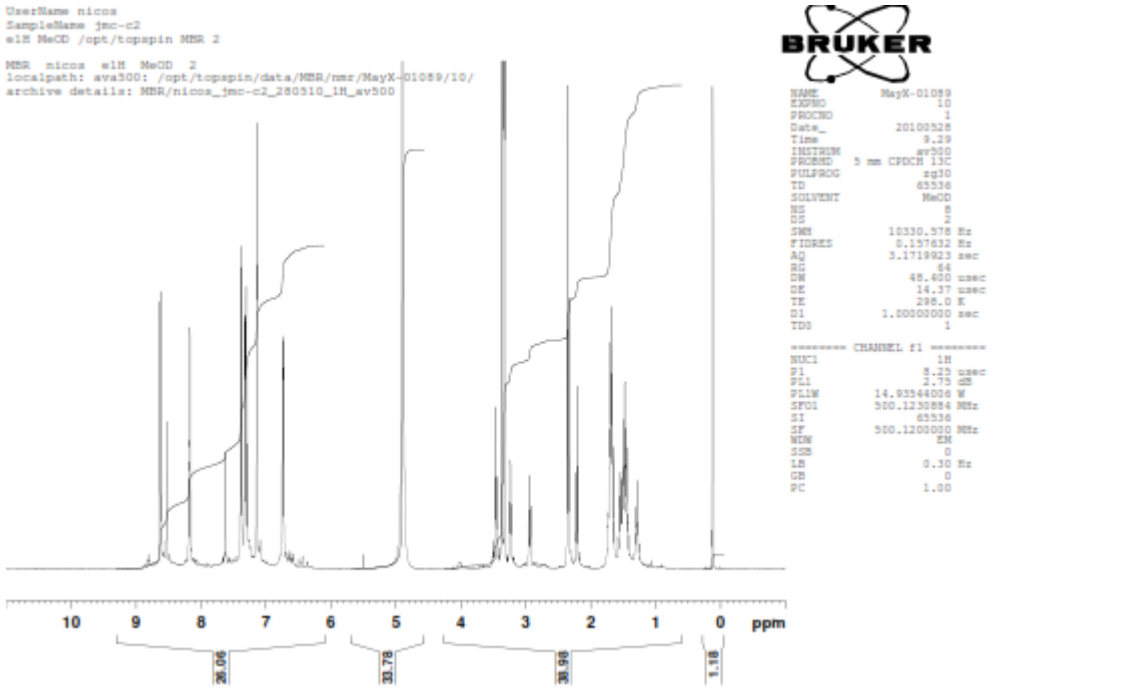


9.-H-NMR spectra

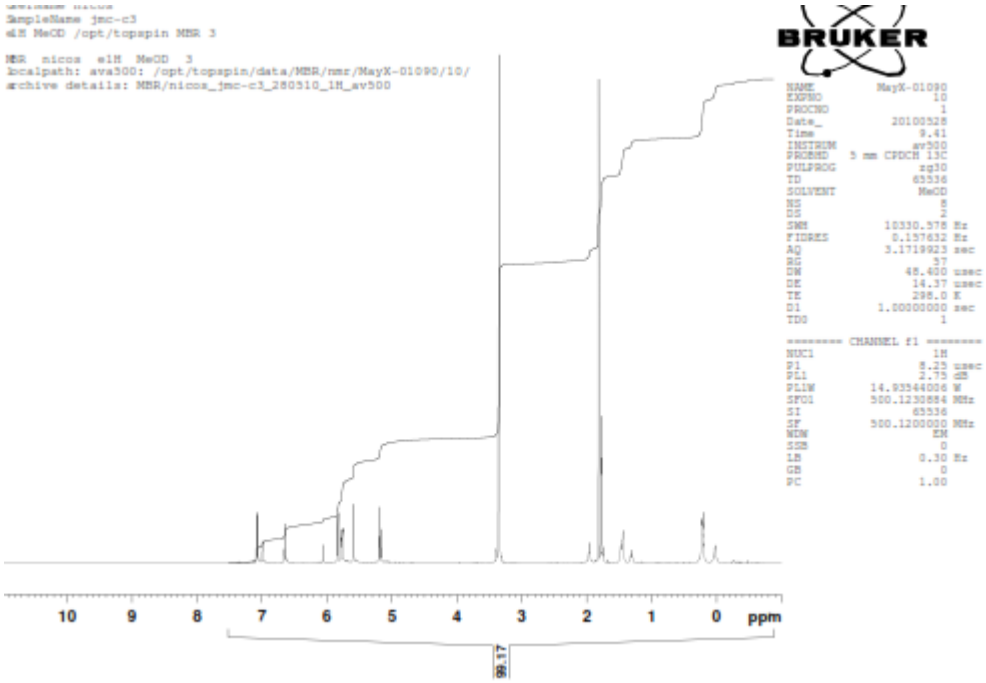
5(6)-(N-thioethyl)naphthofluoresceinamide (7)



6-[naphthofluorescein-5(6)-carboxamido]hexanoic acid *(8)*



5(6)-(N-aminobutyl)naphthofluoresceinamide (9)



9.-References

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