Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary information

Probing the target-specific inhibition of sensitized protein tyrosine phosphatases with biarsenical probes

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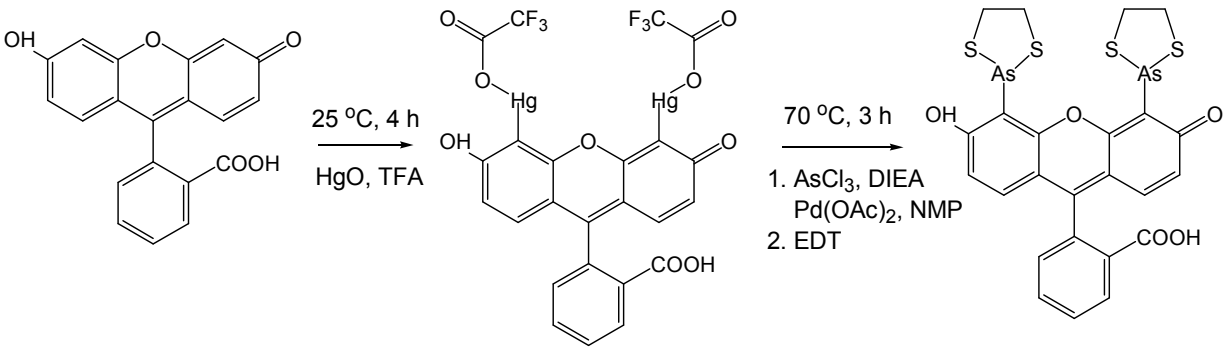
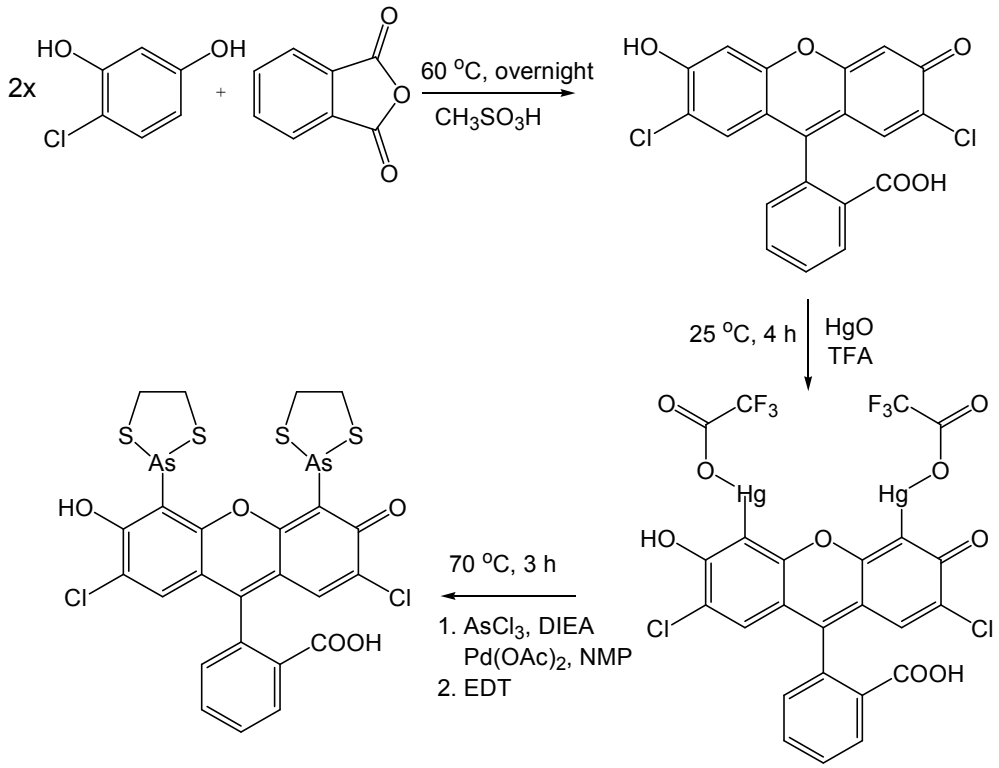
FlAsH-EDT$_2$; *4',5'-bis(1,3,2-dithioarsolan-2-yl)-fluorescein.*

FlAsH-EDT$_2$ was synthesized according to general procedure presented below:



Fluorescein (0.30 g, 0.90 mmol) and mercury(II) oxide (0.43 g, 1.98 mmol) were dissolved in 4.51 ml of trifluoroacetic acid (TFA). The reaction mixture was stirred at room temperature. After 4 h TFA was evaporated and the resulting solid was suspended in 25 ml of water and centrifuged. Solid was washed several times with water and dried in speed-vac. Fluorescein-4',5'-bis(mercuric trifluoroacetate) (100 mg, 0.10 mmol) was suspended in dry NMP (1 ml) and treated with arsenic trichloride (176 µl, 2.09 mmol), DIEA (146 µl, 0.84 mmol) and palladium(II) acetate (0.21 mg) for 3 h at 70 °C. After cooling, the reaction mixture was poured into acetone/0.25 M potassium phosphate buffer pH 7 (10.44 ml, 1:1 v/v), treated with 1,2-ethanedithiol (EDT) (0.42 ml) and extracted with chloroform (3 × 30 ml). Combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in rotary evaporator. The concentrated solution was kept overnight in refrigerator which resulted in precipitation. Next the solid was dissolved in small amount of chloroform and compound was purified by flash chromatography (Gilson PLC2020) on silica gel (PuriFlash SIHP 30µm, 20 g, Interchim) using gradient of ethyl acetate in toluene.

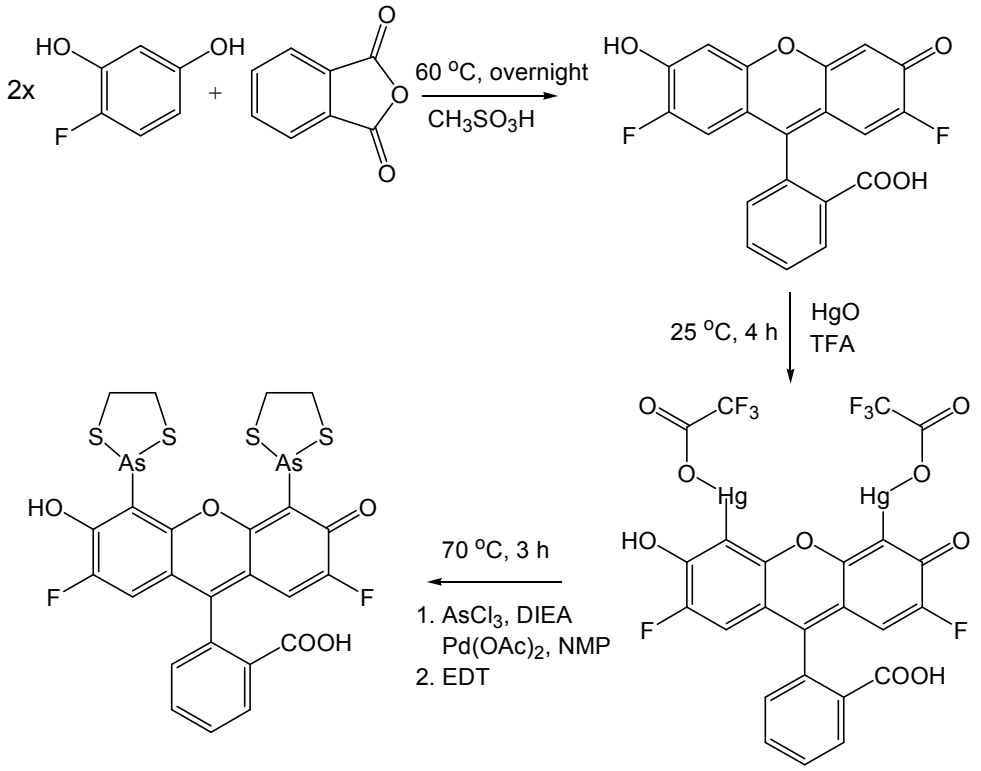
Cl2FlAsH-EDT$_2$; *2',7'-dichloro-4',5'-bis(1,3,2-dithioarsolan-2-yl)-fluorescein.*



4-Chlororesorcinol (1.45 g, 10 mmol) and phthalic anhydride (741 mg, 5 mmol) were dissolved in 25 ml in $MeSO_3H$. Reaction mixture was stirred overnight at 60 ºC. Next 200 ml of ice-cold water was added to the reaction, which precipitated the product. Solid was collected by centrifugation and washed several times with water. Compound was dried overnight in speed-vac. 1.36 g of the product was obtained. Product analyzed using TLC in mixture of methanol and toluene was >90% pure and was therefore used without further purification. 2',7'-dichlorofluorescein (0.30 g, 0.75 mmol) and mercury(II) oxide (0.36 g, 1.66 mmol) were dissolved in 3.74 ml of TFA. The reaction mixture was stirred at room temperature. After 4 h TFA was evaporated and the resulting solid was suspended in 25 ml of water and centrifuged. Solid was washed several times with water and dried in speed-vac. 2',7'-difluoro-fluorescein-4',5'-bis(mercuric trifluoroacetate) (100 mg, 0.10 mmol) was suspended in dry NMP (1 ml) and treated with arsenic trichloride (170 µl, 2.02 mmol), DIEA (140 µl, 0.80 mmol) and palladium(II) acetate (0.20 mg). The reaction mixture was stirred at

70 ºC for 3 h. After cooling, the reaction mixture was poured into acetone/0.25 M potassium phosphate buffer pH 7 (10.44 ml, 1:1 v/v), treated with EDT (0.41 ml) and extracted with chloroform (3 × 30 ml). Combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in rotary evaporator. The concentrated solution was kept overnight in refrigerator which resulted in precipitation. Next the solid was dissolved in small amount of chloroform and compound was purified by flash chromatography (Gilson PLC2020) on silica gel (PuriFlash SIHP 30µm, 20 g, Interchim) using gradient of ethyl acetate in toluene.

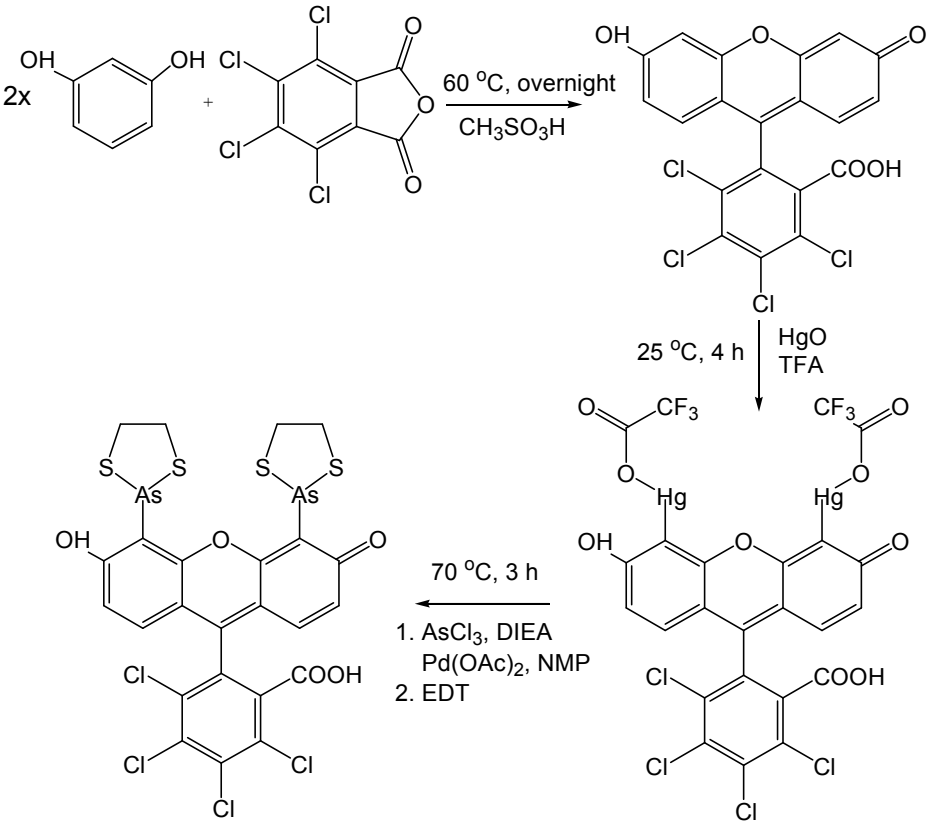
F2FlAsH-EDT$_2$; *2',7'-difluoro-4',5'-bis(1,3,2-dithioarsolan-2-yl)-fluorescein.*



4-Fluororesorcinol (1.28 g, 10 mmol) and phthalic anhydride (741 mg, 5mmol) were dissolved in 25 ml in $MeSO_3H$. Reaction mixture was stirred overnight at 60 ºC. Next 200 ml of ice-cold water was added to the reaction, which precipitated the product. Solid was collected by centrifugation and washed several times with water. 1.31 g of the product was obtained. Product analyzed using TLC in mixture of methanol and toluene was >90% pure

and was therefore used without further purification. 2',7'-difluorofluorescein (0.30 g, 0.82 mmol) and mercury(II) oxide (0.39 g, 1.80 mmol) were dissolved in 3.74 ml of TFA. The reaction mixture was stirred at room temperature. After 4 h TFA was evaporated and the resulting solid was suspended in 25 ml of water and centrifuged. Solid was washed several times with water and dried in speed-vac. 2',7'-dichloro-fluorescein-4',5'-bis(mercuric trifluoroacetate) (105 mg, 0.10 mmol) was suspended in dry NMP (1 ml) and treated with arsenic trichloride (173 µl, 2.05 mmol), DIEA (143 µl, 0.82 mmol) and palladium(II) acetate (0.21 mg). The reaction mixture was stirred at 70 ºC for 3 h. After cooling, the reaction mixture was poured into acetone/0.25M potassium phosphate buffer pH 7 (10.44 ml, 1:1 v/v), treated with EDT (0.41 ml) and extracted with chloroform (3 × 30 ml). Combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in rotary evaporator. The concentrated solution was kept overnight in refrigerator which resulted in precipitation. Next the solid was dissolved in small amount of chloroform and compound was purified by flash chromatography (Gilson PLC2020) on silica gel (PuriFlash SIHP 30µm, 20 g, Interchim) using gradient of ethyl acetate in toluene.

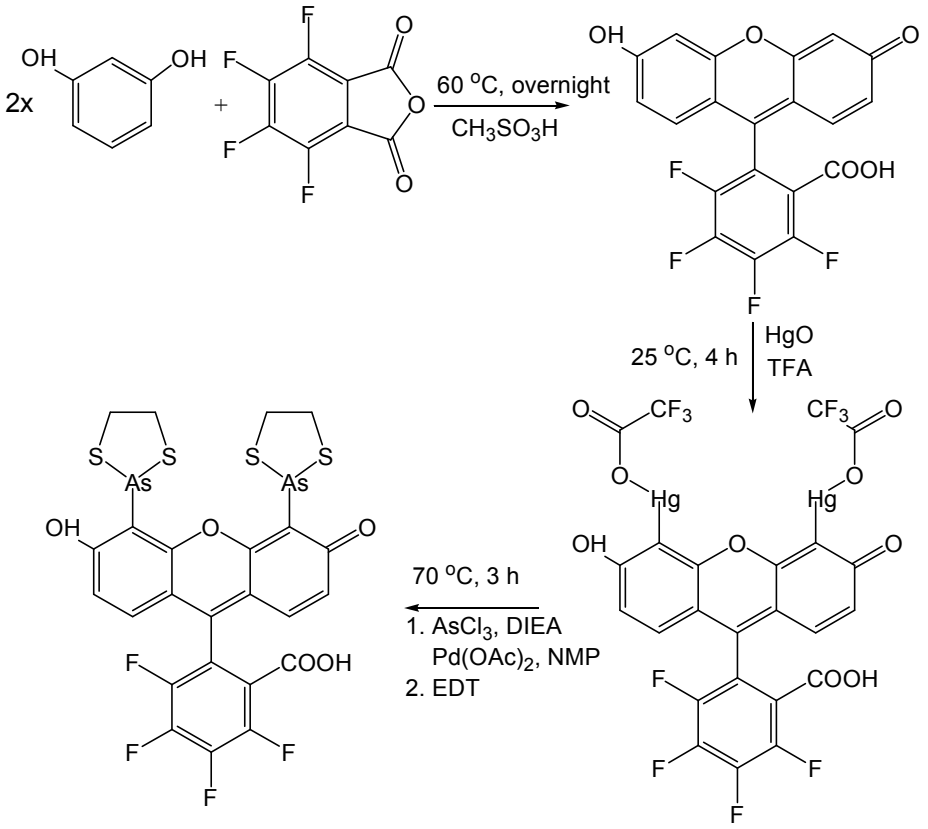
Cl4FlAsH-EDT$_2$; 4*,5,6,7-tetrachloro-4',5'-bis(1,3,2-dithioarsolan-2-yl)-fluorescein*.



Resorcinol (1.10 g, 10 mmol) and 3,4,5,6-tetrachlorophthalic anhydride (1.43 g, 5mmol) were dissolved in 25 ml in $MeSO_3H$. Reaction mixture was stirred overnight at 60 ºC. Next 200 ml of ice-cold water was added to the reaction, which precipitated the product. Solid was collected by centrifugation and washed several times with water. Compound was dried overnight in speed-vac. 1.95 g of the product was obtained. Product analyzed using TLC in mixture of methanol and toluene was >90% pure and was therefore used without further purification. 3,4,5,6-tetrachlorofluorescein (0.30 g, 0.64 mmol), mercury(II) oxide (0.30 g, 1.39 mmol) were dissolved in 3.19 ml of TFA. The reaction mixture was stirred at room temperature. After 4 h TFA was evaporated and the resulting solid was suspended in 25 ml of water and centrifuged. Solid was washed several times with water and dried in speed-vac. 3,4,5,6-tetrachloro-fluorescein-4',5'-bis(mercuric trifluoroacetate) (105 mg, 0.096 mmol) was suspended in dry NMP (1 ml) and treated with arsenic trichloride (162 µl, 1.92 mmol), DIEA (134 µl, 0.77 mmol) and palladium(II) acetate (0.19 mg). The reaction mixture was stirred at

70 ºC for 3 h. After cooling, the reaction mixture was poured into acetone /0.25 M potassium phosphate buffer pH 7 (9.60 ml, 1:1 v/v), treated with EDT (0.37 ml) and extracted with chloroform (3 × 30 ml). Combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in rotary evaporator. The concentrated solution was kept overnight in refrigerator which resulted in precipitation. Next the solid was dissolved in small amount of chloroform and compound was purified by flash chromatography (Gilson PLC2020) on silica gel (PuriFlash SIHP 30µm, 20 g, Interchim) using gradient of ethyl acetate in toluene.

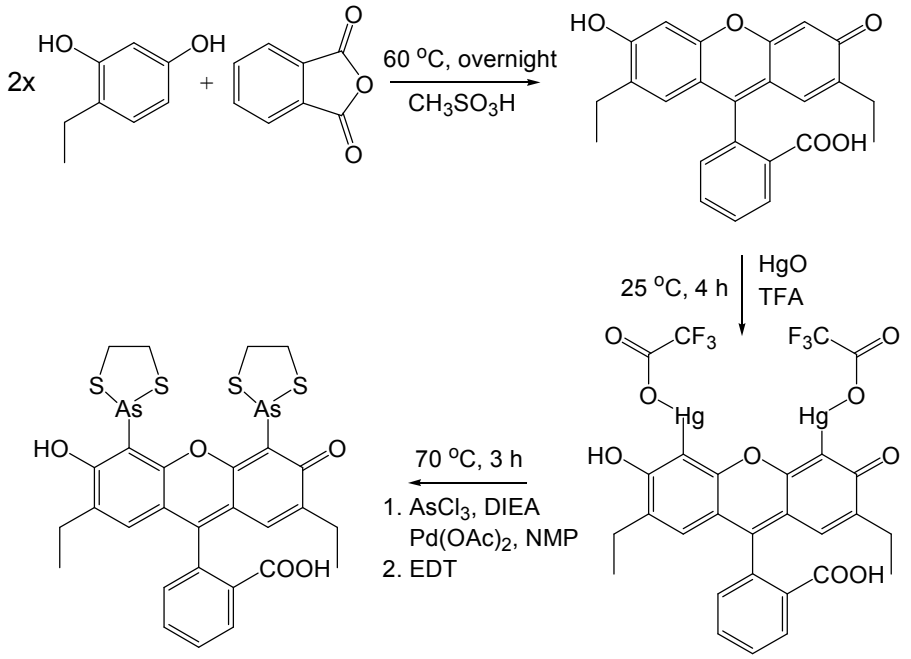
F4FlAsH-EDT$_2$; 4*,5,6,7-tetrafluoro-4',5'-bis(1,3,2-dithioarsolan-2-yl)-fluorescein.*



Resorcinol (1.10 g, 10 mmol) and 3,4,5,6-tetrafluorophthalic anhydride (1.10 g, 5 mmol) were dissolved in 25 ml in $MeSO_3H$. Reaction mixture was stirred overnight at 60 ºC. Next 200 ml of ice-cold water was added to the reaction, which precipitated the product. Solid was collected by centrifugation and washed several times with water. Compound was dried overnight in speed-vac. 1.74 g of the product was obtained. Product analyzed using TLC in

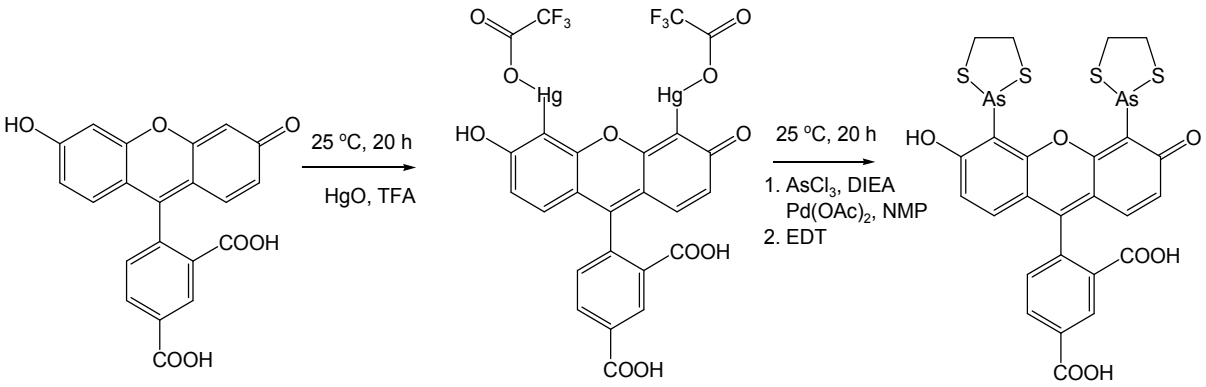
mixture of methanol and toluene was >90% pure and was therefore used without further purification. 3,4,5,6-tetrafluorofluorescein (0.30 g, 0.74 mmol) and mercury(II) oxide (0.35 g, 1.62 mmol) were dissolved in 3.71 ml of TFA. The reaction mixture was stirred at room temperature. After 4 h TFA was evaporated and the resulting solid was suspended in 25 ml of water and centrifuged. Solid was washed several times with water and dried in speed-vac. 3,4,5,6-tetrafluoro-fluorescein-4',5'-bis(mercuric trifluoroacetate) (100 mg, 0.097 mmol) was suspended in dry NMP (1 ml) and treated with arsenic trichloride (164 µl, 1.94 mmol), DIEA (135 µl, 0.78 mmol) and palladium(II) acetate (0.19 mg). The reaction mixture was stirred at 70 ºC for 3 h. After cooling, the reaction mixture was poured into acetone/0.25 M potassium phosphate buffer pH 7 (9.71 ml, 1:1 v/v), treated with EDT (0.39 ml), extracted with chloroform (3 × 30 ml). Combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in rotary evaporator. The concentrated solution was kept overnight in refrigerator which resulted in precipitation. Next the solid was dissolved in small amount of chloroform and compound was purified by flash chromatography (Gilson PLC2020) on silica gel (PuriFlash SIHP 30µm, 20 g, Interchim) using gradient of ethyl acetate in toluene.

Et2FlAsH-EDT$_2$; *2',7'-diethyl-4',5'-bis(1,3,2-dithioarsolan-2-yl)-fluorescein.*



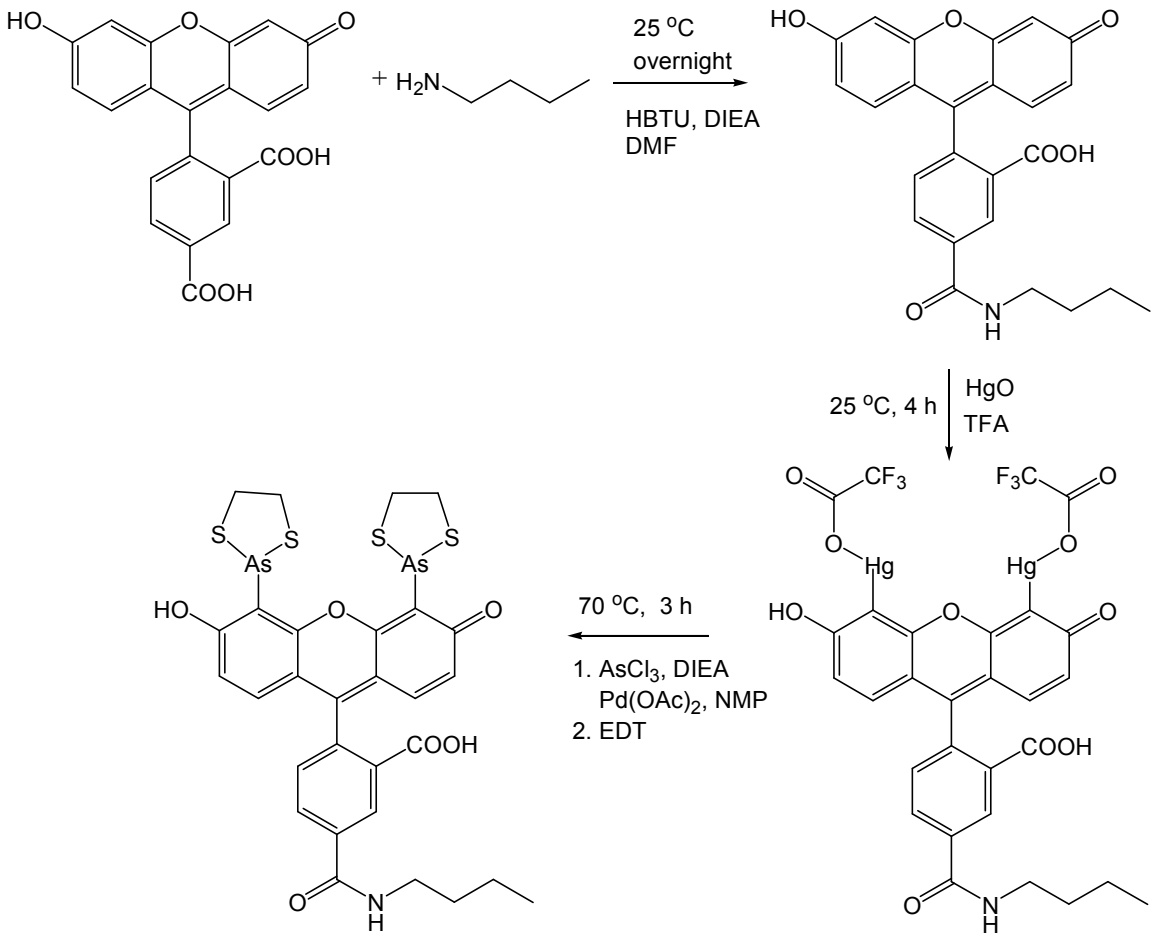
4-Ethylresorcinol (0.5 g, 3.62 mmol) and phthalic anhydride (0.268 g, 1.81 mmol) were dissolved in 9 ml in $MeSO_3H$. Reaction mixture was stirred overnight at 60 ºC. Next 80 ml of ice-cold water was added to the reaction, which precipitated the product. Solid was collected by centrifugation and washed several times with water. Compound was dried overnight in speed-vac. 0.60 g of the product was obtained. Product analyzed using TLC in mixture of methanol and toluene was >90% pure and was therefore used without further purification. 2',7'-diethylfluorescein (0.185 g, 0.48 mmol) and mercuric(II) oxide (0.23 g, 1.06 mmol) were dissolved in 2.38 ml of TFA. The reaction mixture was stirred at room temperature. After 4 h TFA was evaporated and the resulting solid was suspended in 25 ml of water and centrifuged. Solid was washed several times with water and dried in speed-vac. 2',7'-diethylfluorescein-4',5'-bis(mercuric trifluoroacetate) (100 mg, 0.023 mmol) was suspended in dry NMP (0.99 ml) and treated with arsenic trichloride (166 µl, 1.97 mmol), DIEA (137 µl, 0.79 mmol) and palladium(II) acetate (0.20 mg). The reaction mixture was stirred at 70 ºC for 3 h. After cooling, the reaction mixture was poured into acetone/0.25 M potassium phosphate buffer pH 7 (9.87 ml, 1:1 v/v), treated with EDT (0.40 ml), extracted with chloroform (3 × 30 ml). Combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in rotary evaporator. The concentrated solution was kept overnight in refrigerator which resulted in precipitation. Next the solid was dissolved in small amount of chloroform and compound was purified by flash chromatography (Gilson PLC2020) on silica gel (PuriFlash SIHP 30µm, 20 g, Interchim) using gradient of ethyl acetate in toluene.

5-CrAsH-EDT$_2$; *5-Carboxy-4',5'-bis(1,3,2-dithioarsolan-2-yl)-fluorescein.*



5-Carboxyfluorescein (50 mg, 0.13 mmol) and mercury(II) oxide (63.5 mg, 0.29 mmol) were dissolved in 0.665 ml TFA. The reaction mixture was stirred at room temperature. After 4 h TFA was evaporated and the resulting solid was suspended in 10 ml of water and centrifuged. Solid was washed several times with water and dried in speed-vac. 5- Carboxyfluorescein-4',5'-bis(mercuric trifluoroacetate) (109.5 mg, 0.11 mmol) was suspended in dry NMP (1 ml) and treated with arsenic trichloride (184 µl, 2.18 mmol), DIEA (152 µl, 0.87 mmol) and palladium(II) acetate (0.22 mg). After 3 h to the reaction mix was added acetone/0.25 M potassium phosphate buffer pH 7 (9.87 ml, 1:1 v/v) and EDT (0.44 ml). Reaction mixture was extracted with chloroform (3 × 30 ml). Combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in rotary evaporator. The concentrated solution was kept overnight in refrigerator which resulted in precipitation. Next the solid was dissolved in small amount of toluene and compound was purified by flash chromatography (Gilson PLC2020) on silica gel (PuriFlash SIHP 30µm, 20 g, Interchim) using gradient of methanol in toluene.

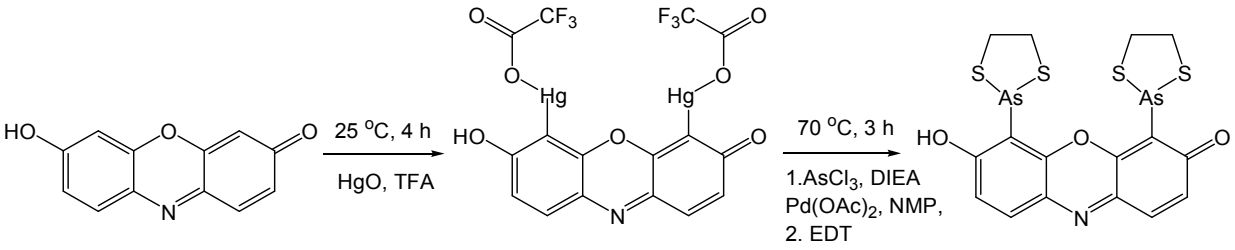
5-BuCrAsH-EDT$_2$; *5-(N-butylamide)-4',5'-bis(1,3,2-dithioarsolan-2-yl)-fluorescein*.



5-Carboxyfluorescein (100 mg, 0.27 mmol), HBTU (102 mg, 0.26 mmol), DIEA (94 µl 5.7 mmol) and *n*-buthylamine (264 µl, 2.68 mmol) were dissolved in 6 ml dimethylformamide (DMF). Reaction mixture was stirred overnight at room temperature. Next the compound was purified by flash chromatography (Gilson PLC2020) on silica gel (PuriFlash SIHP 30µm, 20 g, Interchim) using gradient of methanol in toluene. Fractions containing the pure product, as determined by HPLC, were pooled and evaporated in rotary evaporator. Final yield 92 mg, 80%. 5-(N-buthylamide)fluorescein (50 mg, 0.12 mmol), mercury(II) oxide (50 mg, 0.23 mmol) and TFA (0.58 ml 7.57 mmol) were altogether stirred for 4 h at 25 ºC. After 4 h TFA was evaporated and the resulting solid was suspended in 10 ml of water and centrifuged. Solid was washed several times with water and dried in speed-vac. 5-(N-buthylamide)fluorescein bis(mercuric trifluoroacetate) (40 mg, 37.86 µmol) was suspended in dry NMP (0.38 ml).

Arsenic trichloride (64 µl, 0.76 mmol), DIEA (53 µl, 0.3 mmol) and palladium acetate (0.076 mg) were added and the reaction mixture was stirred at 70 ºC for 3 h. After cooling, the reaction mixture was poured into acetone/0.25M potassium phosphate buffer pH 7 (9.87 ml, 1:1 v/v), treated with EDT (0.40 ml) and extracted with chloroform (3 × 30 ml). Combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in rotary evaporator. The concentrated solution was kept overnight in refrigerator which resulted in precipitation. Next the solid was dissolved in small amount of toluene and compound was purified by flash chromatography (Gilson PLC2020) on silica gel (PuriFlash SIHP 30µm, 20 g, Interchim) using gradient of methanol in toluene.

ReAsH-EDT$_2$; *4,5-Bis(1,3,2-dithiarosolan-2-yl)-resorufin*.



Resorufin (0.30 g, 1.4 mmol) and mercury(II) oxide (0.67 g, 3.1 mmol) were dissolved in 7.04 ml of TFA. The reaction mixture was stirred at room temperature. After 4 h TFA was evaporated and the resulting solid was suspended in 25 ml of water and centrifuged. Solid was washed several times with water and dried in speed-vac. Resorufin 4,5-bis(mercuric trifluoroacetate) (65 mg, 77 µmol) was suspended in dry NMP (0.77 ml). Arsenic trichloride (130 µl, 1.54 mmol), palladium acetate (0.15 mg) and DIEA (108 µl, 0.62 mmol) were added, and the reaction mixture was stirred at 70 ºC for 3 h. After cooling, the reaction mixture was poured into acetone/0.25 M phosphate buffer pH 7 (7.72 ml, 1:1 v/v), treated with EDT (0.31 ml) and extracted with chloroform (3 × 30 ml). Combined organic layers were dried over

anhydrous Na_2SO_4 and evaporated in rotary evaporator. The concentrated solution was kept overnight in refrigerator which resulted in precipitation. Next the solid was dissolved in small amount of toluene and compound was purified by flash chromatography (Gilson PLC2020) on silica gel (PuriFlash SIHP 30μm, 20 g, Interchim) using gradient of ethyl acetate in toluene.

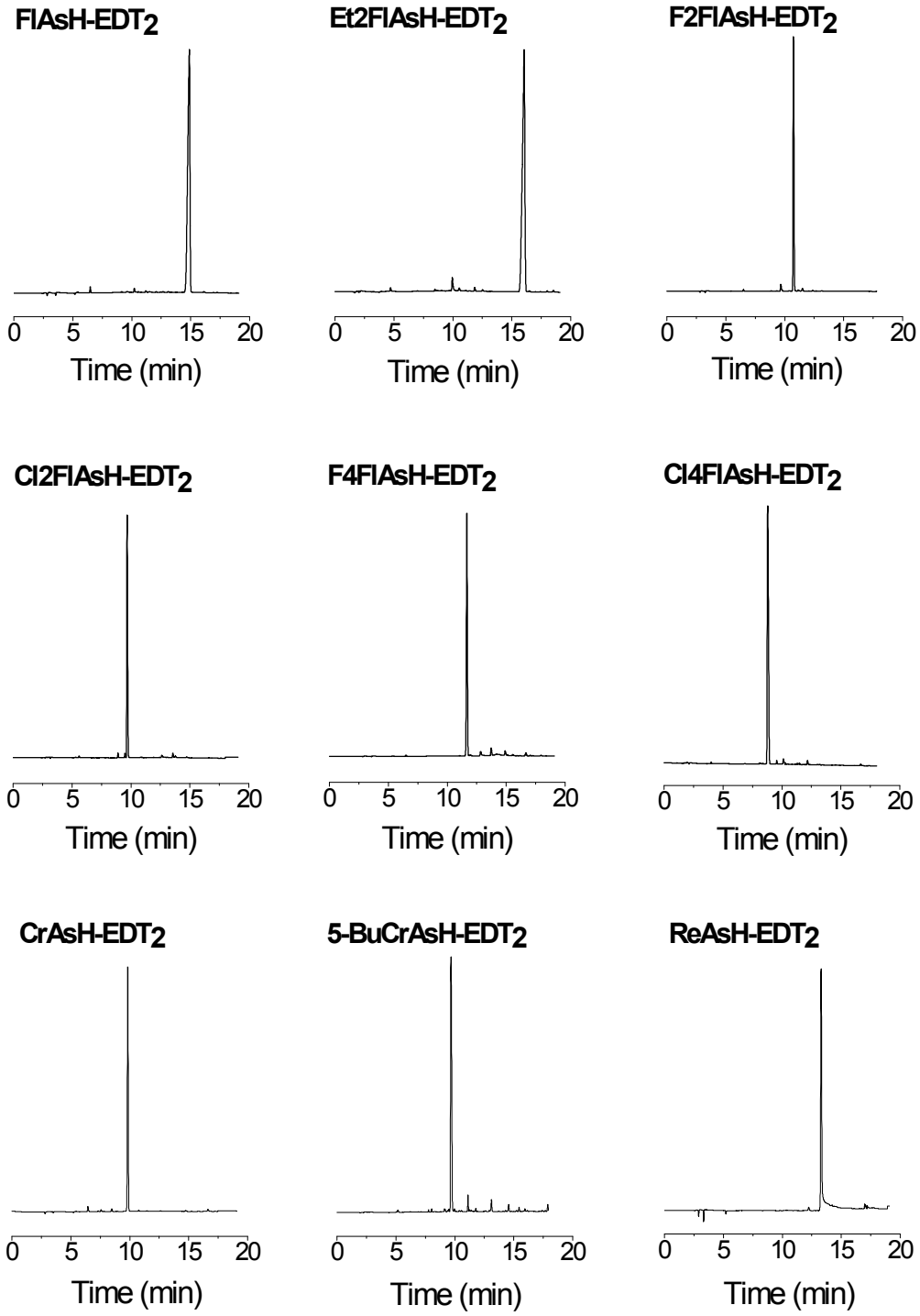


Figure S1. HPLC chromatograms of purified biarsenical probes obtained in this study. The purity was 95% - 99% and was analyzed using HPLC (Dionex Ultimate 3000) equipped with Phenomenex Aeris peptide 3,6 μm C18 column utilizing a gradient of acetonitrile (0-90% in 20 minutes) in 5 mM ammonium carbonate. Diode array detector (DAD) was used for monitoring the absorbance at 280 nm or 500 nm in case of ReAsH.

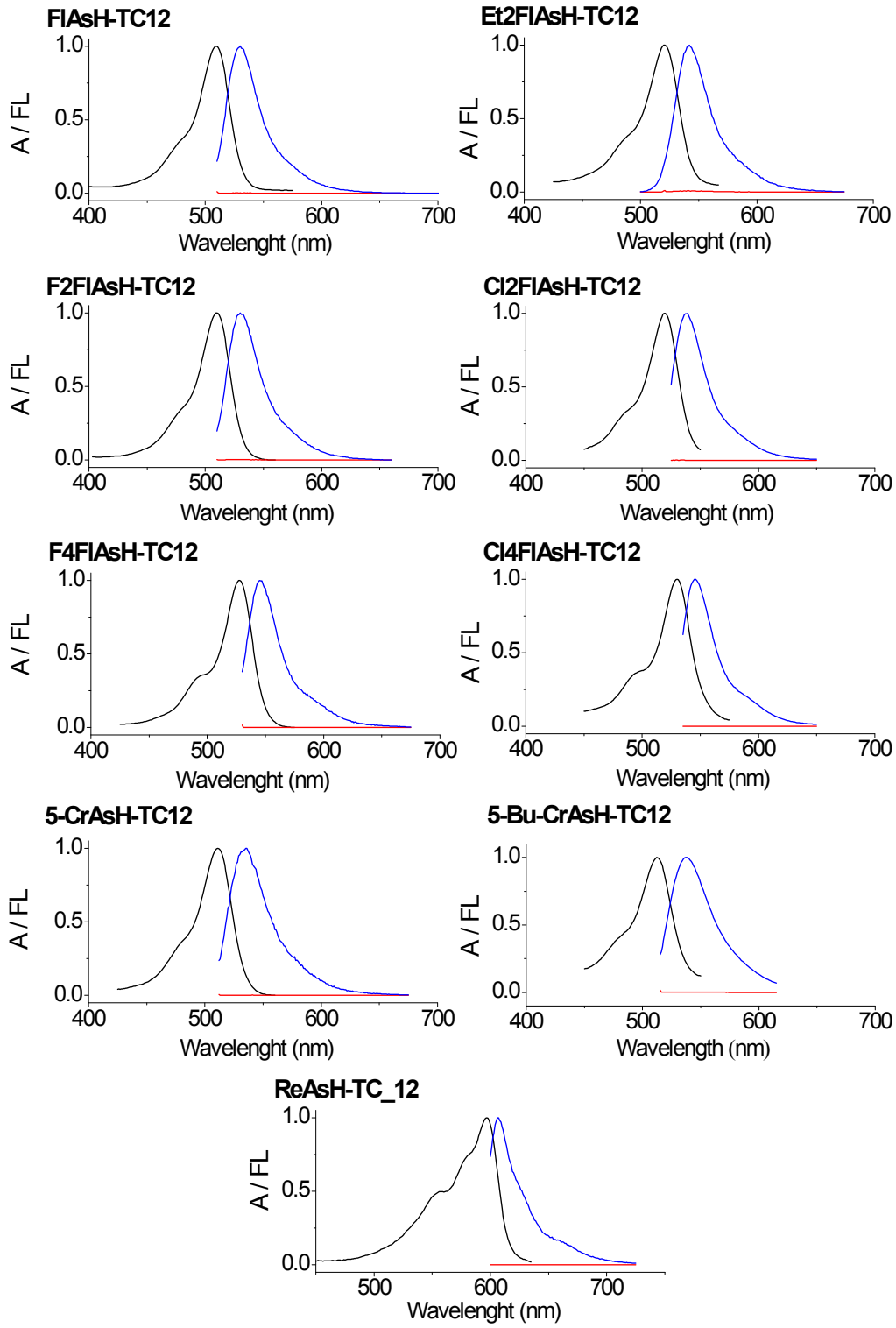


Figure S2. Normalized absorbance and fluorescence spectra of various biarsenical probes. Black denote the absorbance, blue and red the fluorescence of biarsenical probe prior to addition of tetracysteine peptide and fluorescence of biarsenical probe-TC12 (Ac-FLNCCPGCCMEP-amide) complex, respectively. All experiments were performed in 50 mM Na^+-HEPES buffer pH 7.4 with 100 mM NaCl and 120 µM TCEP.

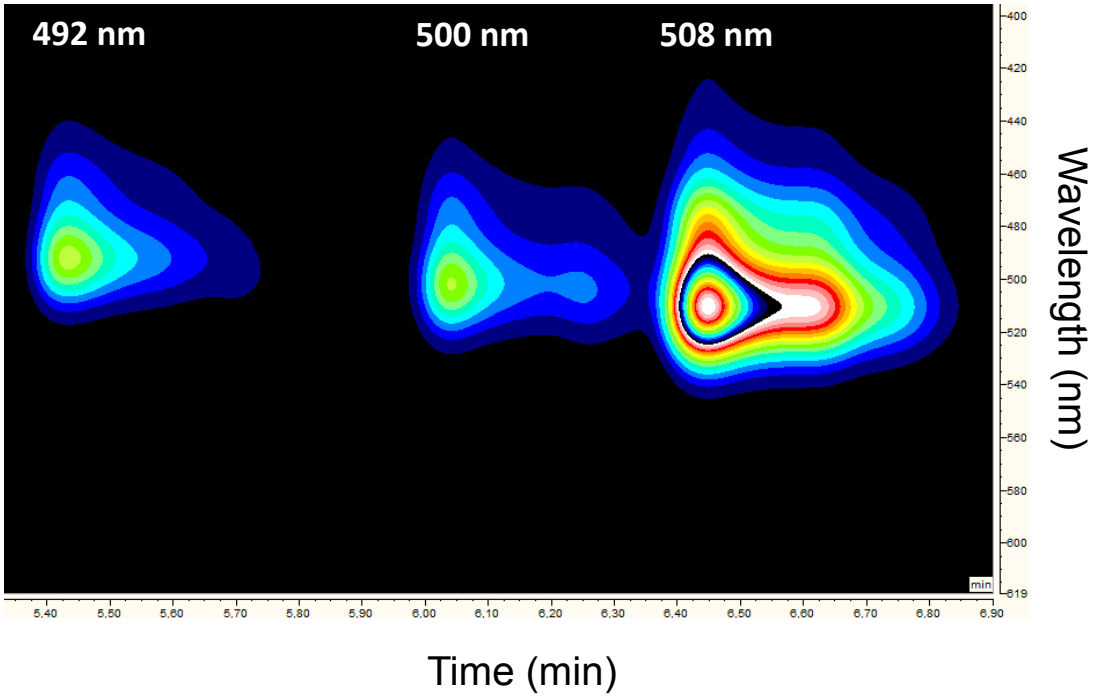


Figure S3. Typical 3D-field chromatogram of synthetic F2FlAsH-EDT_2 before purification. Spots refer to three species present in the crude: parent probe 2',7'-difluorofluorescein (substrate), monoarsenical F2FlAsH-EDT and biarsenical F2FlAsH-EDT_2 species, respectively. Wavelength values denote the absorbance maximum.

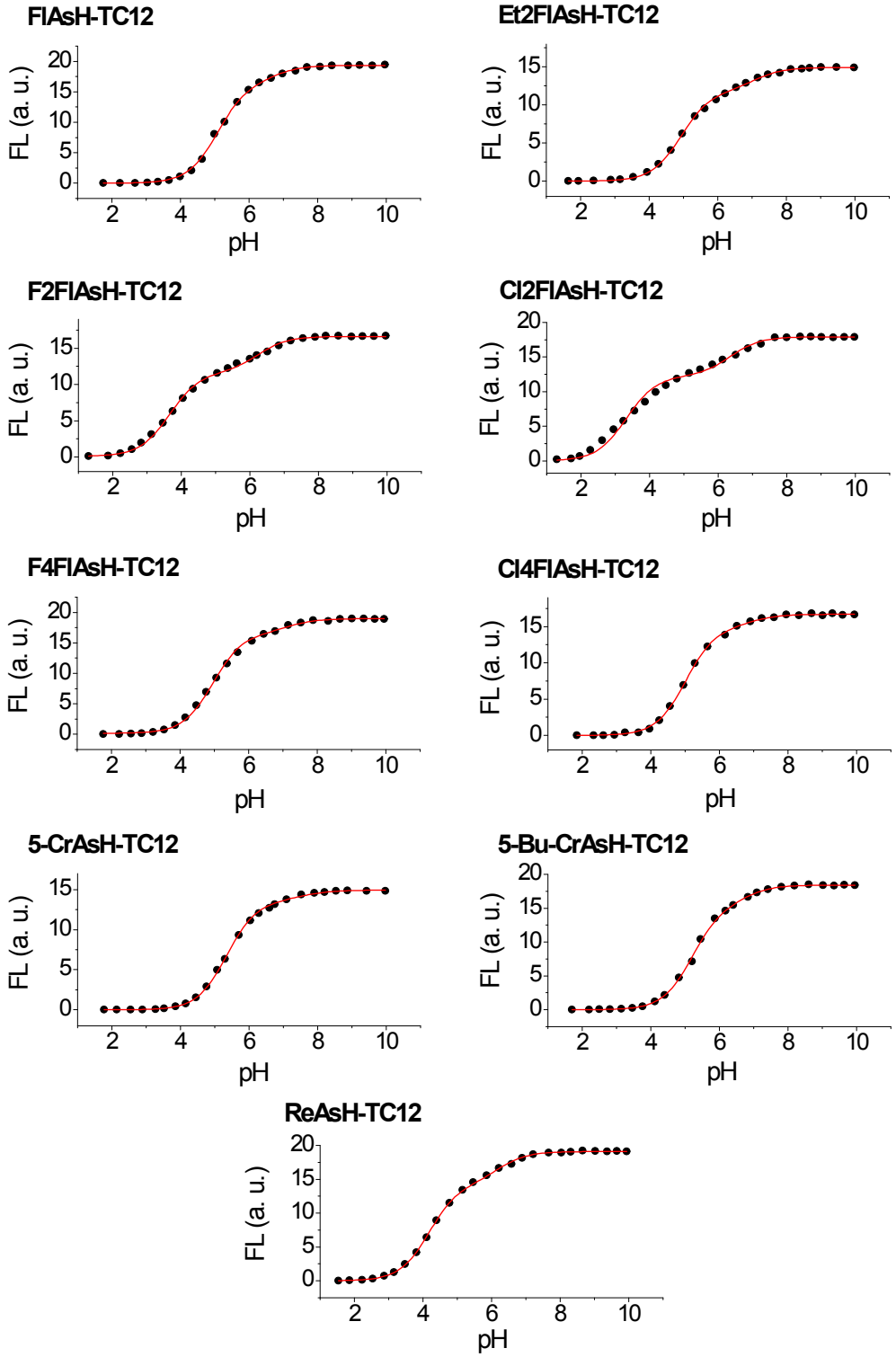


Figure S4. pH titration curves of different biarsenical probes in complex with the tetracysteine peptides TC12 (Ac-FLNCCPGCCMEP-amide). Experimental data were fitted to Equation S1.

Equation S1:

$$y = (d1 * H^2 + dx * K1 * H + d2 * K1 * K2)/(H^2 + K1 * H + K1 * K2)$$

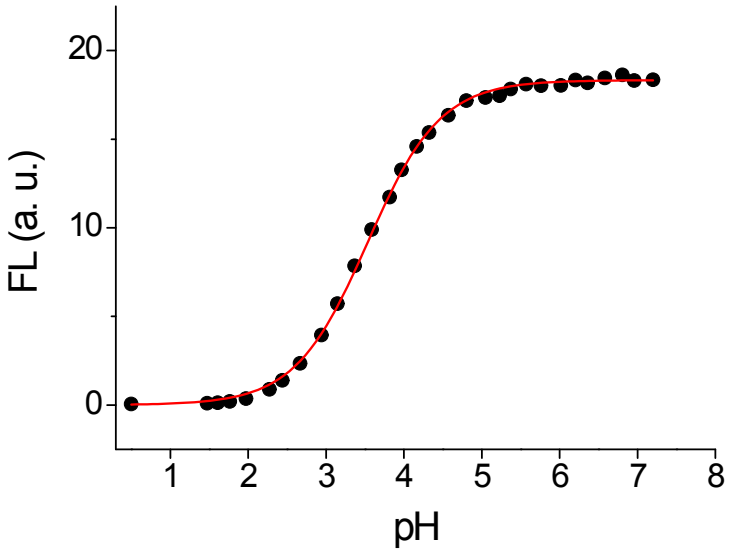


Figure S5. The pH titration of F2FlAsH bound to the peptide Ac-CCPGCC-amide. Data was fitted to Equation S2 (Hill's equation).

Equation S2:

$$y = d1\ (H^n/(H^n + K1^n)) + d2\ (K^n/(H^n + K1^n))$$

where H = 10^(-x), $K1$ = 10^ (-pK_{a1}), $K2$ = 10^ (-pK_{a2}), *d1* and *d2* are the titration end-points and *dx* is the inflexion point; *n* denotes Hill's coefficient.

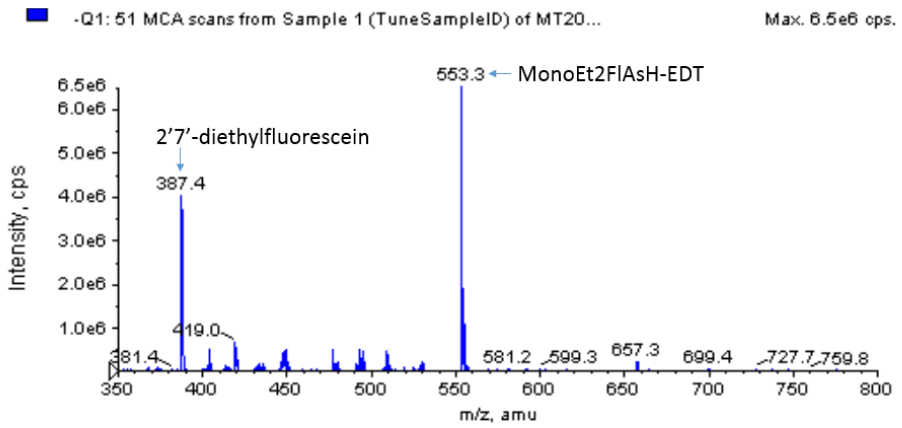


Figure S6. Mass spectra of decomposed Et2FlAsH-EDT_2, showing monoarsenical species and substrate 2'7'-diethylfluoresceine. Et2FlAsH-EDT_2 was added to final concentration of 25 μM into 15 mM NH_4CO_3 buffer and sample was left in darkness for 12 h at room temperature. After that the sample was diluted with solution of 50% H_2O 49% methanol and 1% ethylenediamine and analyzed in mass spectrometer (API 2000, Applied Biosystems). Mass $[M-H]^-$ calculated for 2'7'-diethylfluoresceine and monoarsenical species are 387.1 and 553.0, respectively.

Table S1. Stability of various biarsenical probes in aqueous pH buffered solution. 25 μM biarsenical probe was incubated in the dark in 50 mM Na^+-HEPES buffer, 100 mM NaCl, pH 7.4. Analytical HPLC was performed directly after the addition of the probe (0 h) and 1, 2, 3 and 6 h later. Since monoarsenical and biarsenical species have different maxima of absorption (~10 nm) the percentages of monoarsenical species were estimated from chromatograms collected at 280 nm, with exception of ReAsH-EDT_2 for which absorption at 595 nm was used.

Probe	% of monoarsenical species				
	0 h	1 h	2 h	3 h	6 h
FlAsH-EDT_2	0.14 ± 0.01	0.42 ± 0.02	0.60 ± 0.03	0.72 ± 0.04	1.06 ± 0.05
F2FlAsH-EDT_2	1.86 ± 0.09	1.98 ± 0.09	2.0 ± 0.1	2.3 ± 0.1	2.4 ± 0.1
Cl2FlAsH-EDT_2	0.98 ± 0.05	1.07 ± 0.05	1.28 ± 0.06	1.37 ± 0.07	1.51 ± 0.08
Et2FlAsH-EDT_2	1.72 ± 0.09	3.8 ± 0.2	6.2 ± 0.3	9.2 ± 0.5	15.9 ± 0.8
F4FlAsH-EDT_2	0.16 ± 0.01	0.22 ± 0.01	0.30 ± 0.02	0.41 ± 0.02	0.58 ± 0.03

Cl4FlAsH-EDT$_2$	0.10 ± 0.01	0.31 ± 0.02	0.47 ± 0.02	0.60 ± 0.03	1.18 ± 0.06
5-CrAsH-EDT$_2$	0.97 ± 0.05	1.10 ± 0.06	1.28 ± 0.06	1.53 ± 0.08	1.68 ± 0.08
5-Bu-CrAsH-EDT$_2$	0.65 ± 0.03	1.31 ± 0.07	2.3 ± 0.1	2.7 ± 0.1	3.9 ± 0.2
ReAsH-EDT$_2$	1.19 ± 0.06	1.28 ± 0.06	1.44 ± 0.07	1.64 ± 0.08	2.0 ± 0.1

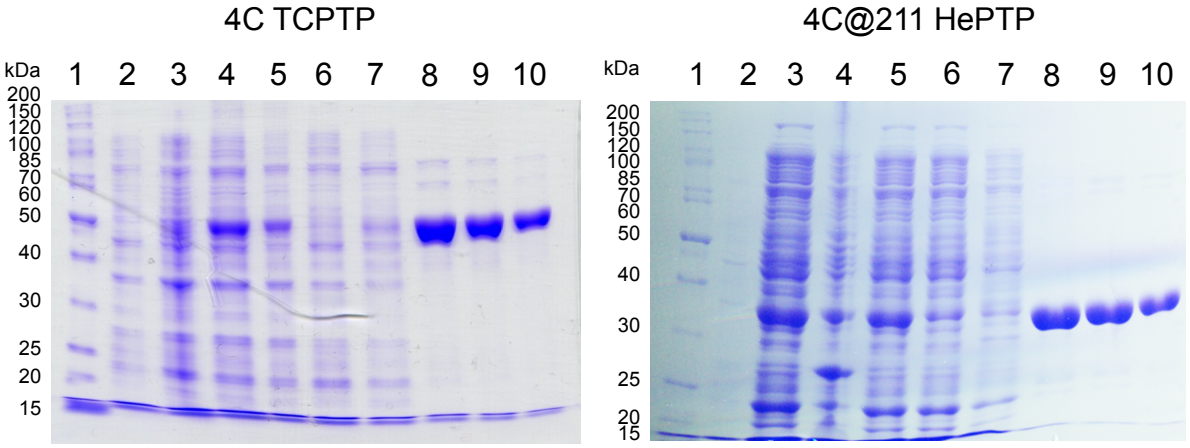


Figure S7. SDS-PAGE gels stained with Coomassie shoing the purification of different protein tyrosine phosphatases TCPTP 4C and HePTP 4C@211. The lanes show: mass marker (Ferementas PageRuler Unstained) (**1**), bacteria before induction of PTP expression (**2**), bacteria after induction with 0.2 mM ITPG (TCPTP) or 0.04% arabinose (HePTP) (**3**), soluble fraction after sonication ((**4**) 4C-3 TCPTP or (**5**) 4C@211 HePTP), unsoluble fraction after sonication ((**5**) 4C-3 TCPTP or (**4**) 4C@211 HePTP), NiNTA flow through fraction (**6**), fraction from washing with 20 mM imidazole (**7**), fraction with protein eluted with 250 mM imidazole (**8-10**).

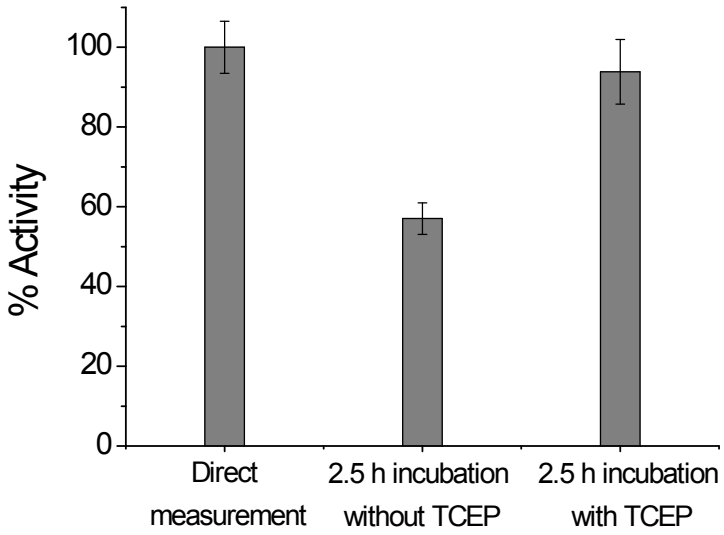


Figure S8. The percentage of 4C TCPTP activity at various conditions. PTPs are stored in buffer containing 1 mM DTT, which ensures the reduced (active) state of the protein. Upon dilution the concentration of DTT is < 1μM, which is too little to keep the protein reduced during 2.5 h of incubation with biarsenical probe at room temperature. The activity was assayed with pNPP as described in *Experimental procedures* using 100 nM TCPTP 4C. The concentration of TCEP was 20 μM. Measurements were performed in triplicates.

Table S2. Values of k_{cat} (s^{-1}) of wild type and sensitized PTP conjugates with various biarsenical probes obtained from Michaelis-Menten equation. Enzyme activity assayed with pNPP as described in *Experimental procedures*.

Biarsenical probe	TCPTP					HePTP	
	WT	P181C/ E187C	P181C+2C	4C	4C@241	WT	4C@211
No probe	10.6 ± 0.2	10.5 ± 0.1	3.81 ± 0.06	14.4 ± 0.3	7.8 ± 0.1	3.7 ± 0.3	0.187 ± 0.003
FlAsH-EDT_2	10.0 ± 0.1	6.58 ± 0.05	2.75 ± 0.03	6.69 ± 0.09	6.70 ± 0.09	3.4 ± 0.2	0.152 ± 0.004
Et2FlAsH-EDT_2	10.3 ± 0.2	5.7 ± 0.1	1.57 ± 0.03	4.75 ± 0.08	7.4 ± 0.1	3.3 ± 0.1	0.093 ± 0.003
F2FlAsH-EDT_2	9.8 ± 0.2	6.60 ± 0.04	2.0 ± 0.03	6.4 ± 0.1	6.4 ± 0.1	3.5 ± 0.2	0.055 ± 0.001
Cl2FlAsH-EDT_2	10.4 ± 0.1	5.79 ± 0.07	2.5 ± 0.03	6.2 ± 0.1	6.2 ± 0.1	3.4 ± 0.2	0.109 ± 0.003
F4FlAsH-EDT_2	10.2 ± 0.1	7.23 ± 0.03	3.02 ± 0.04	6.4 ± 0.1	6.4 ± 0.1	3.6 ± 0.2	0.109 ± 0.003
Cl4FlAsH-EDT_2	12.0 ± 0.2	7.09 ± 0.06	2.77 ± 0.04	6.49 ± 0.09	6.5 ± 0.09	3.5 ± 0.2	0.191 ± 0.004
5-CrAsH-EDT_2	10.4 ± 0.2	7.44 ± 0.07	3.43 ±0.03	6.9 ± 0.1	6.9 ± 0.1	3.4 ± 0.1	0.178 ± 0.003
5-Bu-CrAsH-EDT_2	10.6 ± 0.2	7.15 ± 0.04	2.90 ± 0.03	6.6 ± 0.1	6.6 ± 0.1	3.5 ± 0.2	0.152 ± 0.004
ReAsH-EDT_2	10.69 ± 0.09	6.74 ± 0.04	3.23 ± 0.03	6.45 ± 0.09	6.45 ± 0.09	3.5 ± 0.2	0.152 ± 0.003

Table S3. Values of K_M (mM) of wild type and sensitized PTP conjugates with various biarsenical probes obtained from Michaelis-Menten equation. Enzyme activity assayed with pNPP as described in *Experimental procedures*.

Biarsenical probe	TCPTP					HePTP	
	WT	P181C/ E187C	P181C + 2C	4C	4C@241	WT	4C@211
No probe	1.67 ± 0.08	0.69 ± 0.04	0.99 ± 0.05	1.28 ± 0.08	1.58 ± 0.09	4.1 ± 0.2	3.0 ± 0.2
FlAsH-EDT_2	1.89 ± 0.08	0.60 ± 0.02	0.94 ± 0.04	1.71 ± 0.07	1.71 ± 0.07	4.0 ± 0.3	3.6 ± 0.3
Et2FlAsH-EDT_2	1.56 ± 0.08	0.79 ± 0.09	1.04 ± 0.06	1.47 ± 0.09	1.91 ± 0.08	4.0 ± 0.2	3.7 ± 0.4
F2FlAsH-EDT_2	1.8 ± 0.1	0.61 ± 0.02	0.97 ± 0.05	1.7 ± 0.1	1.7 ± 0.1	4.1 ± 0.3	2.7 ± 0.2
Cl2FlAsH-EDT_2	1.95 ± 0.08	0.67 ± 0.03	0.99 ± 0.04	1.7 ± 0.1	1.7 ± 0.1	4.3 ± 0.3	2.9 ± 0.3
F4FlAsH-EDT_2	1.76 ± 0.07	0.57 ± 0.01	1.09 ± 0.05	1.66 ± 0.09	1.66 ± 0.09	4.4 ± 0.3	3.2 ± 0.3
Cl4FlAsH-EDT_2	1.9 ± 0.1	0.59 ± 0.02	1.05 ± 0.05	1.63 ± 0.07	1.63 ± 0.07	4.3 ± 0.3	3.3 ± 0.2
5-CrAsH-EDT_2	1.7 ± 0.1	0.56 ± 0.02	0.97 ± 0.03	1.62 ± 0.08	1.62 ± 0.08	4.0 ± 0.2	3.6 ± 0.2
5-BuCrAsH-EDT_2	1.61 ± 0.08	0.59 ± 0.01	1.00 ± 0.04	1.71 ± 0.08	1.71 ± 0.08	4.5 ± 0.3	3.6 ± 0.3
ReAsH-EDT_2	1.62 ± 0.04	0.62 ± 0.02	1.03 ± 0.03	1.70 ± 0.07	1.70 ± 0.07	4.5 ± 0.3	3.8 ± 0.3

Table S4. Values of catalytic efficiency (k_{cat}/K_m, s^{-1} mM^{-1}) of wild type and sensitized PTP conjugates with various biarsenical probes assayed with *p*NPP. Individual k_{cat} and K_m values are listed in. Bolded results shows enzyme – probe pairs that have the lowest activity. The experimental error denotes combined experimental errors of the last significant digits of k_{cat}/K_m values as described below.

Biarsenical probe	TCPTP					HePTP	
	WT	P181C/ E187C	P181C + 2C	4C	4C@241	WT	4C@211
No probe	6.4 ± 0.4	15.3 ± 0.9	3.9 ± 0.3	11.3 ± 0.9	4.9 ± 0.4	0.9 ± 0.1	0.062 ± 0.004
FlAsH-EDT_2	5.3 ± 0.3	10.9 ± 0.4	2.9 ± 0.2	3.9 ± 0.2	3.9 ± 0.2	0.9 ± 0.1	0.042 ± 0.005
Et2FlAsH-EDT_2	5.2 ± 0.3	**7.3 ± 0.9**	**1.5 ± 0.1**	**3.2 ± 0.2**	3.8 ± 0.2	0.82 ± 0.07	0.025 ± 0.003
F2FlAsH-EDT_2	5.3 ± 0.4	10.8 ± 0.3	2.0 ± 0.1	3.8 ± 0.3	3.8 ± 0.3	0.8 ± 0.1	**0.020 ± 0.002**
Cl2FlAsH-EDT_2	5.3 ± 0.3	8.7 ± 0.5	2.5 ± 0.1	3.7 ± 0.3	**3.7 ± 0.3**	0.8 ± 0.1	0.037 ± 0.005
F4FlAsH-EDT_2	5.8 ± 0.3	12.6 ± 0.3	2.8 ± 0.2	3.8 ± 0.3	3.8 ± 0.3	0.8 ± 0.1	0.035 ± 0.004
Cl4FlAsH-EDT_2	6.5 ± 0.5	12.1 ± 0.5	2.6 ± 0.2	4.0 ± 0.2	4.0 ± 0.2	0.8 ± 0.1	0.058 ± 0.005
5-CrAsH-EDT_2	6.0 ± 0.4	13.3 ± 0.7	3.5 ± 0.1	4.3 ± 0.3	4.3 ± 0.3	0.83 ± 0.08	0.049 ± 0.004
5-Bu-CrAsH-EDT_2	6.5 ± 0.4	12.1 ± 0.3	2.9 ± 0.1	3.9 ± 0.2	3.9 ± 0.2	0.8 ± 0.1	0.042 ± 0.005
ReAsH-EDT_2	6.6 ± 0.2	11.0 ± 0.4	3.1 ± 0.1	3.8 ±0.2	3.8 ± 0.2	0.8 ± 0.1	0.040 ± 0.004

Estimation of the experimental error in calculation of catalytic efficiency

If the value of y is not directly measured but determined based on measurements of other quantities related x the specified functional dependency is:

$$y = f(x_1, x_2, ..., x_n), \qquad \text{[Equation S3]}$$

To determine the change in the function Δy due to changes in the argument of Δx_1, Δx_2, …, Δx_n calculate the difference in the points $x_i + \Delta x_i$ and x_i, where i = 1, 2, …, n.

$$\Delta y = f(x_1 + \Delta x_1, x_2 + \Delta x_2, ..., x_n + \Delta x_n) - f(x_1, x_2, ..., x_n), \qquad \text{[Equation S4]}$$

In developing the first factor expression in a Taylor's series and retaining the only the words of the first order is obtained following expression:

$$\Delta y = \frac{\partial f}{\partial x_1}\Delta x_1 + \frac{\partial f}{\partial x_2}\Delta x_2 + ... + \frac{\partial f}{\partial x_n}\Delta x_n, \qquad \text{[Equation S5]}$$

which equation that maps the measurement error and the field (exact differential).

Calculation of the dissociation constant from dose-dependent activity measurement using fluorogenic phosphorylated peptide

The relative initial activity of the biarsenical bound enzyme relative to the enzyme without attached probe was plotted against the total concentration of biarsenical probe. Data points were fitted to Equation S6 using Origin 8.6 software

$$y = A_{min}\left(\frac{A_{min} - A_{max}}{2\,cE}\right)\left(\sqrt{cE - x - K_d + (cE + x + K_d)^2 - 4cEx}\right) \qquad [Equation\ S6]$$

where A_{max} and A_{min} are the maximal and minimal values of enzyme activities (activity was normalized in a such a way that maximal enzyme activity was set as 1, while its minimal value depended on experimental activity and was above 0 in all cases); cE is the total concentration of the enzyme and x is the concentration of biarsenical probe and K_d is the dissociation constant.