Distyrylbenzene-aldehydes: identification of proteins in water

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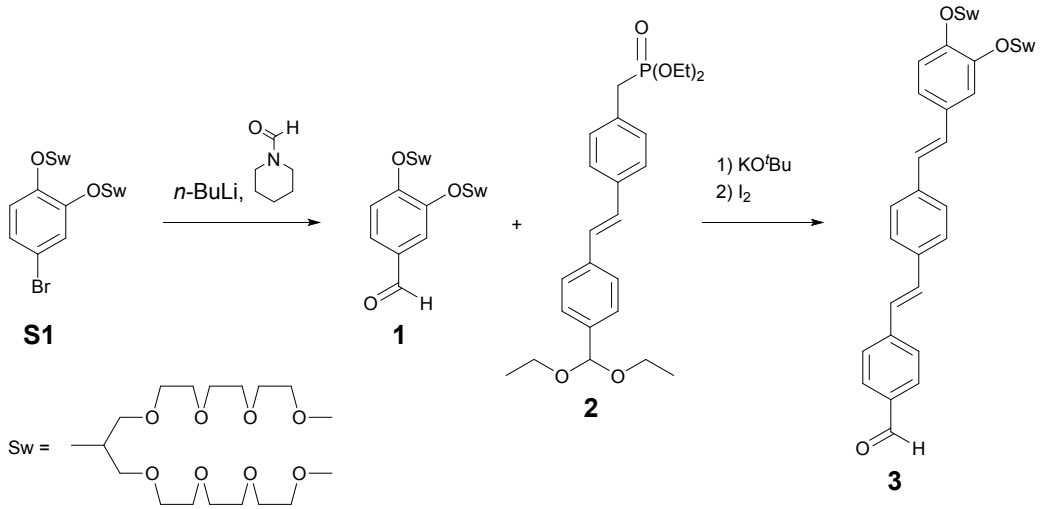
Supporting Information

Contents:

1 Synthesis

General Experimental Methods:

All reagents, solvents and Proteins have been purchased from Sigma Aldrich and were used without further purification unless otherwise specified. Preparation of air- and moisture-sensitive materials was carried out in oven dried flasks under a nitrogen atmosphere using Schlenk techniques. Column chromatography was performed using Standard Grade silica gel 60 Å. Compounds **2**, **4**, and **9** were prepared as reported.[1] ^{1}H NMR spectra were recorded at 298 K on a 300, 400, 500 or 600 MHz spectrometer, and ^{13}C NMR spectra were recorded on a 75, 100, 125 or 150 MHz spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to traces of $CHCl_3$. MS spectra were recorded using fast atom bombardment, electronspray ionization or electron impact detected by magnetic sector and FT-ICR techniques, respectively. Infrared (IR) spectra are reported in wavenumbers (cm^{-1}) and were recorded neat. Absorption spectra were recorded in a rectangular quartz cuvette (light path = 10 mm) on a Jasco UV-VIS V-660 spectrophotometer. Fluorescence spectra were recorded in a conventional quartz cuvette (10 x 10x 40 mm) on a Jasco FP-6500 fluorospectrometer. Photographs were taken with a Canon EOS 7D (objective: EF-S60mm f/2.8 Macro USM) with shutter speed 0.10 s.



Scheme S1 Synthesis of DSB **3**.

13,13'-[(4-Bromobenzene-1,2-diyl)bis(oxy)]bis(2,5,8,11,15,18,21,24-octaoxapentacosane) (S1): In a 100 mL Schlenk flask K_2CO_3 (6.53 g, 47.3 mmol, 7.00 eq) was added to a solution of swallowtail tosylate (8.00 g, 14.9 mmol, 2.20 eq) in 2-butanone (30 mL). The suspension was degassed, 4-bromobenzene-1,2-diol (1.28 g, 6.75 mmol, 1.00 eq) was added and the mixture was refluxed for 4 d (80 °C). The salts were filtered through Celite with dichloromethane as eluent, the solvents were removed by rotary evaporation and the crude product was purified by column chromatography on silica gel (petroleum ether/dichloromethane/ethyl acetate/methanol = 5:3:1:0.6, R_f = 0.09) to yield the desired compound as a pale yellow oil (5.58 g, 6.05 mmol, 90 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.19 (d, *J* = 2.2 Hz, 1H), 6.99 (dd, *J* = 8.6 Hz, 2.2 Hz, 1H), 6.92 (d, *J* = 8.6 Hz, 1H), 4.46-4.36 (m, 2H), 3.70-3.59 (m, 48H), 3.54-3.51 (m, 8H), 3.36 (s, 12H). ^{13}C{^{1}H}-NMR (150 MHz, $CDCl_3$): δ 150.3, 148.5, 125.0, 121.5, 120.2, 78.99, 78.97, 72.0, 71.1, 71.0, 70.7-70.6, 59.1. IR (cm^{-1}): 2868, 1489, 1351, 1255, 1200, 1098, 1040, 943, 849. HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{40}H_{73}BrO_{18}Na$ 943.3873, found 943.3884; *m/z* $[M+K]^+$ calcd for $C_{40}H_{73}BrO_{18}K$ 959.3612, found 959.3618. Elemental analysis: calcd (%) for $C_{40}H_{73}BrO_{18}$: C 52.11, H 7.98, Br 8.67, found: C 51.94, H 7.76, Br 8.53.

3,4-Bis(2,5,8,11,15,18,21,24-octaoxapentacosan-13-yloxy)benzaldehyde (1):
To a solution of **S1** (1.00 g, 1.08 mmol, 1.00 eq) in dry THF (40 mL) *n*-BuLi (2.24 mL of a 1.6 M solution in hexanes, 3.58 mmol, 3.30 eq) was added dropwise at -78 °C and the mixture was stirred for 1.5 h. Then *N*-formylpiperidine (337 µL, 3.04 mmol, 2.80 eq) was added slowly and the reaction mixture was stirred at -78 °C for 4 h before it was quenched with a saturated aqueous NH_4Cl-solution (20 mL) at 0 °C. The layers were separated and the aqueous layer was extracted with dichloromethane (5 x 20 mL). The combined organic layers were dried over $MgSO_4$ and the solvents were evaporated. Purification by column chromatography (silica gel, petroleum ether/dichloromethane/ethyl acetate/methanol = 5:3:1:0.7, R_f = 0.08) afforded the desired compound as a pale yellow oil (720 mg, 827 µmol, 76 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.82 (s, 1H), 7.58 (d, *J* = 1.9 Hz, 1H), 7.45 (dd, *J* = 8.4 Hz, 1.9 Hz, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 4.63 (quin, *J* = 5.1 Hz, 1H), 4.51 (quin, *J* = 5.1 Hz, 1H), 3.74-3.71 (m, 8H), 3.62-3.60 (m, 40H), 3.57-3.51 (m, 8H), 3.36 (s, 12H). ^{13}C{^{1}H}-NMR (100 MHz, $CDCl_3$): δ 190.9, 155.0, 149.3, 130.7, 126.2, 117.7, 116.2, 78.8, 78.4, 72.0, 71.11, 71.09, 70.7-70.6, 59.1. IR (cm^{-1}): 2868, 1687, 1595, 1502, 1454,

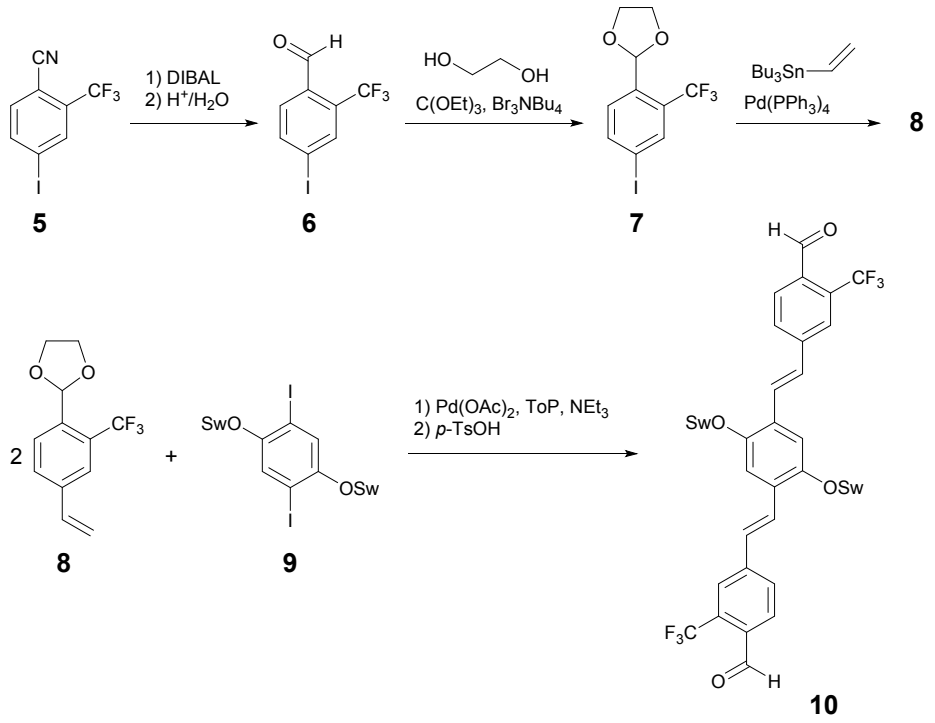
1436, 1351, 1270, 1199, 1099, 1040, 997, 942, 849. HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{41}H_{74}O_{19}Na$ 893.4717, found 893.4717; *m/z* $[M+K]^+$ calcd for $C_{41}H_{74}O_{19}K$ 909.4456, found 909.4456.

4-[(E)-2-(4-{(E)-2-[3,4-Bis(2,5,8,11,15,18,21,24-octaoxapentacosan-13-yloxy)phenyl]ethenyl} phenyl)ethenyl]benzaldehyde (3):

Compound **2** (164 mg, 379 µmol, 1.10 eq) was dissolved in dry THF (4 mL). The mixture was cooled to 0 °C and KO^{t}Bu (50.2 mg, 448 µmol, 1.30 eq) was added carefully. The mixture was stirred at 0 °C for 10 min before aldehyde **1** was added dropwise. The reaction mixture was allowed to warm to rt and stirred overnight. The reaction was quenched by adding water (10 mL) and a saturated aqueous NH_4Cl-solution (10 mL). The layers were separated and the aqueous layer was extracted with DCM (5 x 20 mL). The combined organic extracts were washed with brine and dried over $MgSO_4$ and the solvents were removed by rotary evaporation.

Deprotection:

To a solution of the crude acetal (253 mg, 220 µmol, 1.00 eq) in toluene (4 mL) a catalytic amount of iodine was added. The mixture was refluxed for 4 h and then quenched with a saturated $NaSO_3$-solution. The layers were separated and the aqueous layer was extracted with dichloromethane (4 x 10 mL). The combined organic extracts were dried over $MgSO_4$ and the solvents were evaporated. Column chromatography (silica gel, petroleum ether/dichloromethane/ethyl acetate/methanol = 5:3:1:0.6, R_f = 0.09) afforded the desired compound as a bright yellow oil (214.0 mg, 199 µmol, 64 % over both steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.00 (s, 1H), 7.87 (d, *J* = 8.3 Hz, 2H), 7.66 (d, *J* = 8.3 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.26 (d, *J* = 16.3 Hz, 1H), 7.15 (d, *J* = 16.3 Hz, 1H), 7.08-7.02 (m, 3H), 6.96 (d, *J* = 16.3 Hz, 1H), 4.57-4.46 (m, 2H), 3.76-3.59 (m, 48H), 3.55-3.50 (m, 8H), 3.37 (s, 6H), 3.35 (s, 6H). ^{13}C{^{1}H}-NMR (75 MHz, $CDCl_3$): δ 191.7, 149.3, 143.6, 137.9, 135.7, 135.4, 131.9, 131.8, 130.4, 128.9, 127.4, 127.1, 127.0, 126.9, 126.8, 121.3, 118.4, 116.5, 78.7, 78.6, 72.05, 72.03, 71.1, 70.7-70.6, 59.1. IR (cm^{-1}): 2870, 1503, 1454, 1349, 1268, 1200, 1097, 1051, 962, 847, 539. HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{57}H_{86}O_{19}Na$ 1097.5656, found 1097.5609; *m/z* $[M+K]^+$ calcd for $C_{57}H_{86}O_{19}K$ 1113.5395, found 1113.5419. Elemental analysis: calcd (%) for $C_{57}H_{86}O_{19}$: C 63.67, H 8.06, found: C 63.60, H 8.20.



Scheme S2 Synthesis of DSB **10**.

4-Iodo-2-(trifluoromethyl)benzaldehyde (6):

Under a nitrogen atmosphere a solution of 4-iodo-2-(trifluoromethyl)benzonitrile (1.00 g, 3.37 mmol, 1.00 eq) in dry dichloromethane (10 mL) was treated with DIBAL (4.04 mL of a 1 M solution in DCM, 4.04 mmol, 1.20 eq) at 0 °C. The ice bath was removed and the reaction mixture was stirred at rt for 3 h. The mixture was carefully poured into a mixture of crushed ice (25 g) and 6 N HCl (65 mL) and stirred for 1 h. The layers were separated and the aqueous layer was extracted with dichloromethane (2 x 50 mL). The combined organic extracts were washed with a 10 % aqueous solution of $NaHCO_3$ and brine and dried over $MgSO_4$. The solvents were removed under reduced pressure to yield the desired compound as a slightly yellow solid (1.00 g, 3.33 mmol, 99 %, mp = 74-76 °C). ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.33 (m, 1H), 8.13 (s, 1H), 8.08 (d, *J* = 8.1 Hz, 1H), 7.81 (d, *J* = 8.1 Hz, 1H). ^{13}C{^{1}H}-NMR (125 MHz, $CDCl_3$): δ 188.2 (q, *J* = 2.9 Hz), 141.9, 135.3 (q, *J* = 5.8 Hz), 133.0 (m), 132.1 (q, *J* = 32.9 Hz), 130.3, 122.7 (q, *J* = 275.0 Hz), 101.3. ^{19}F{^{1}H}-NMR (470 MHz, $CDCl_3$): δ -55.81. IR (cm^{-1}): 2359, 1686, 1580, 1559, 1418, 1300, 1271, 1202, 1155, 1115, 1063, 1049, 898, 846, 831, 781, 686, 656, 515, 509, 446. HRMS (EI): *m/z* $[M]^+$calcd for $C_8H_4F_3IO$ 299.9259, found 299.9272.

2-[4-Iodo-2-(trifluoromethyl)phenyl]-1,3-dioxolane (7):

To a suspension of **6** (500 mg, 1.67 mmol, 1.00 eq) and triethyl orthoformate (195 µL, 1.83 mmol, 1.10 eq) in ethylene glycol (1 mL) tetra-*n*-butylammonium tribromide (9.00 mg, 16.7 µmol, 0.01 eq) was added. The reaction mixture was stirred at rt overnight. The reaction mixture was purified directly by column chromatography (silica gel, petroleum ether/ethyl acetate = 20:1, R_f = 0.18) to yield the desired compound as a colorless oil (398 mg, 1.16 mmol, 69 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.99 (m, 1H), 7.94-7.91 (m, 1H), 7.54 (d, *J* = 8.3 Hz, 1H), 6.07 (m, 1H), 4.18-4.03 (m, 4H). ^{13}C{^{1}H}-NMR (125 MHz, $CDCl_3$): δ 141.3 (m), 136.2 (m), 134.8 (q, *J* = 5.8 Hz), 130.4 (q, *J* = 31.8 Hz), 129.8, 123.0 (q, *J* = 275.4 Hz), 99.4 (q, *J* = 2.3 Hz) 94.7, 65.8. ^{19}F{^{1}H}-NMR (280 MHz, $CDCl_3$): δ -57.97. IR (cm^{-1}): 2889, 2359, 1416, 1300, 1271, 1211, 1167, 1122, 1088, 1044, 976, 942, 891, 851, 823, 722, 684, 651, 536, 472. HRMS (EI): *m/z* [M]$^+$calcd for $C_{10}H_8F_3IO_2$ 343.9521, found 343.9524.

2-[4-Ethenyl-2-(trifluoromethyl)phenyl]-1,3-dioxolane (8):

A solution of **7** (690 mg, 2.01 mmol, 1.00 eq) in DMF (20 mL) was degassed. After addition of vinyl tributyltin (642 µL, 2.21 mmol, 1.10 eq) and $Pd(PPh_3)_4$ (116 mg, 100 µmol, 5 mol%) the reaction mixture was stirred at 100 °C overnight. The reaction mixture was allowed to cool to rt, filtered through Celite with dichloromethane as eluent and the solvents were removed under reduced pressure. The residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate = 20:1) two times to yield the desired product as a colorless oil (407 mg, 1.67 mmol, 83 %, R_f = 0.15). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.78 (d, *J* = 8.2 Hz, 1H), 7.68 (m, 1H), 7.62-7.59 (m, 1H), 6.74 (dd, *J* = 17.6 Hz, 10.9 Hz, 1H), 6.11 (m, 1H), 5.84 (d, *J* = 17.6 Hz, 1H), 5.38 (d, *J* = 10.9 Hz, 1H), 4.23-4.02 (m, 4H). ^{13}C{^{1}H}-NMR (125 MHz, $CDCl_3$): δ 138.9, 135.5 (m), 135.4, 129.5 (m), 129.1 (q, *J* = 31.4 Hz), 128.4, 124.1 (q, *J* = 273.6 Hz), 123.7 (q, *J* = 5.8 Hz), 116.5, 99.7 (q, *J* = 2.6 Hz), 65.7. ^{19}F{^{1}H}-NMR (280 MHz, $CDCl_3$): δ -57.87. IR (cm^{-1}): 2890, 1435, 1408, 1316, 1279, 1196, 1162, 1119, 1088, 1049, 987, 958, 943, 917, 902, 846, 821, 739, 723, 667. HRMS (EI): *m/z* [M]$^+$calcd for $C_{12}H_{11}F_3O_2$ 244.0711, found 244.0703.

4,4'-{[2,5-bis(2,5,8,11,15,18,21,24-octaoxapentacosan-13-yloxy)benzene-1,4-diyl]di(*E*)ethene-2,1-diyl}bis[2-(trifluoromethyl)benzaldehyde] (10):

The reaction was performed in a heat-gun dried Schlenk tube under a nitrogen atmosphere. **9** (200 mg, 183 µmol, 1.00 eq) and **8** (98.2 mg, 402 µmol, 2.20 eq) were dissolved in dry DMF (5 mL). $Pd(OAc)_2$ (2 mg, 7.3 µmol, 0.04 eq), tris(*o*-tolyl)phosphine (11.1 mg, 36.5 µmol, 0.20 eq) and triethylamine (0.5 mL) were added. The mixture was stirred at 120 °C for 72 h. After the reaction mixture was cooled to ambient temperature it was poured into 50 mL of water to give a yellow suspension which was extracted with dichloromethane (4 x 50 mL). The combined organic layers were dried over $MgSO_4$ and the solvents were removed under reduced pressure. The residue was purified by column chromatography (silica gel, petroleum ether/dichloro-methane/ethyl acetate/methanol = 5:3:1:0.5, R_f = 0.12) to yield the desired acetal as a yellow oil (138 mg, 104 µmol, 57 %).

Deprotection:

The acetal (114 mg, 85.9 µmol, 1.00 eq) was dissolved in acetone/water = 3:1 (3 mL acetone + 1 mL water) and a catalytic amount of *p*-toluenesulfonic acid was added. The solution was stirred at 40 °C overnight. The reaction was quenched by addition of a saturated aqueous solution of $NaHCO_3$ (10 mL) and dichloromethane (10 mL). The layers were separated and the aqueous layer was extracted with dichloromethane (4 x 10 mL). The combined organic extracts were dried over $MgSO_4$ and the solvents were evaporated. Column chromatography (silica gel, ethyl acetate/methanol = 10:0.6, R_f = 0.28) afforded the desired compound as an orange colored liquid (88 mg, 186 µmol, 92 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.36 (m, 2H), 8.13 (d, *J* = 8.1 Hz, 2H), 7.88-7.84 (m, 4H), 7.70 (d, *J* = 16.5 Hz, 2H), 7.40 (s, 2H), 7.21 (d, *J* = 16.5 Hz, 2H), 4.55 (quin, *J* = 5.0 Hz, 2H), 3.83-3.76 (m, 8H), 3.70-3.57 (m, 40H), 3.50-3.48 (m, 8H), 3.33 (s, 12H). ^{13}C{^{1}H}-NMR (100 MHz, $CDCl_3$): δ 188.4 (m), 151.7, 143.7, 132.1 (m), 131.7 (q, *J* = 32.2 Hz), 129.9, 129.6, 128.8, 128.4, 127.2, 124.3 (q, *J* = 5.7 Hz), 123.9 (q, *J* = 275.2 Hz), 114.8, 80.0, 72.0, 71.2, 70.9, 70.7-70.6 (m), 59.1. ^{19}F{^{1}H}-NMR (280 MHz, $CDCl_3$): δ -55.81. IR (cm^{-1}): 2871, 1692, 1596, 1486, 1456, 1420, 1349, 1320, 1273, 1252, 1199, 1165, 1103, 1050, 966, 926, 850, 806, 667, 535. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{60}H_{85}F_6O_{20}$ 1239.5533, found 1239.5554; *m/z* $[M+Na]^+$ calcd for $C_{60}H_{84}F_6O_{20}Na$ 1261.5352, found 1261.5370. Elemental analysis: calcd (%) for $C_{60}H_{84}F_6O_{20}$: C 58.15, H 6.83, found: C 57.79, H 6.81.

2 Protein Sensing

2.1 Absorption and Emission Spectra

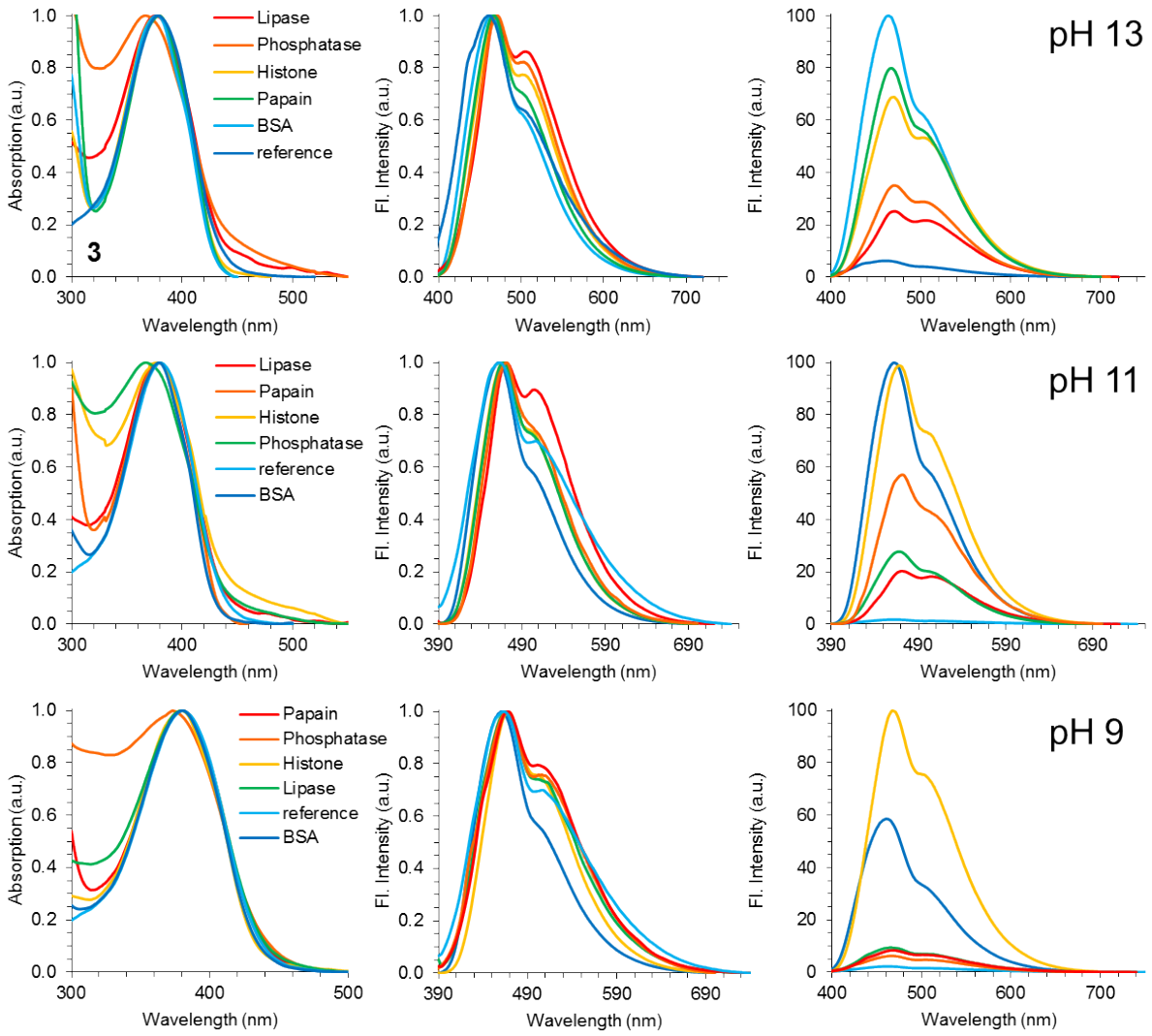


Fig S1 Absorption spectra (left), normalized emission spectra (middle), and non-normalized emission spectra (right) of buffered aqueous solutions (top: pH 13, middle: pH 11, bottom: pH 9) of **3** upon addition of different proteins.

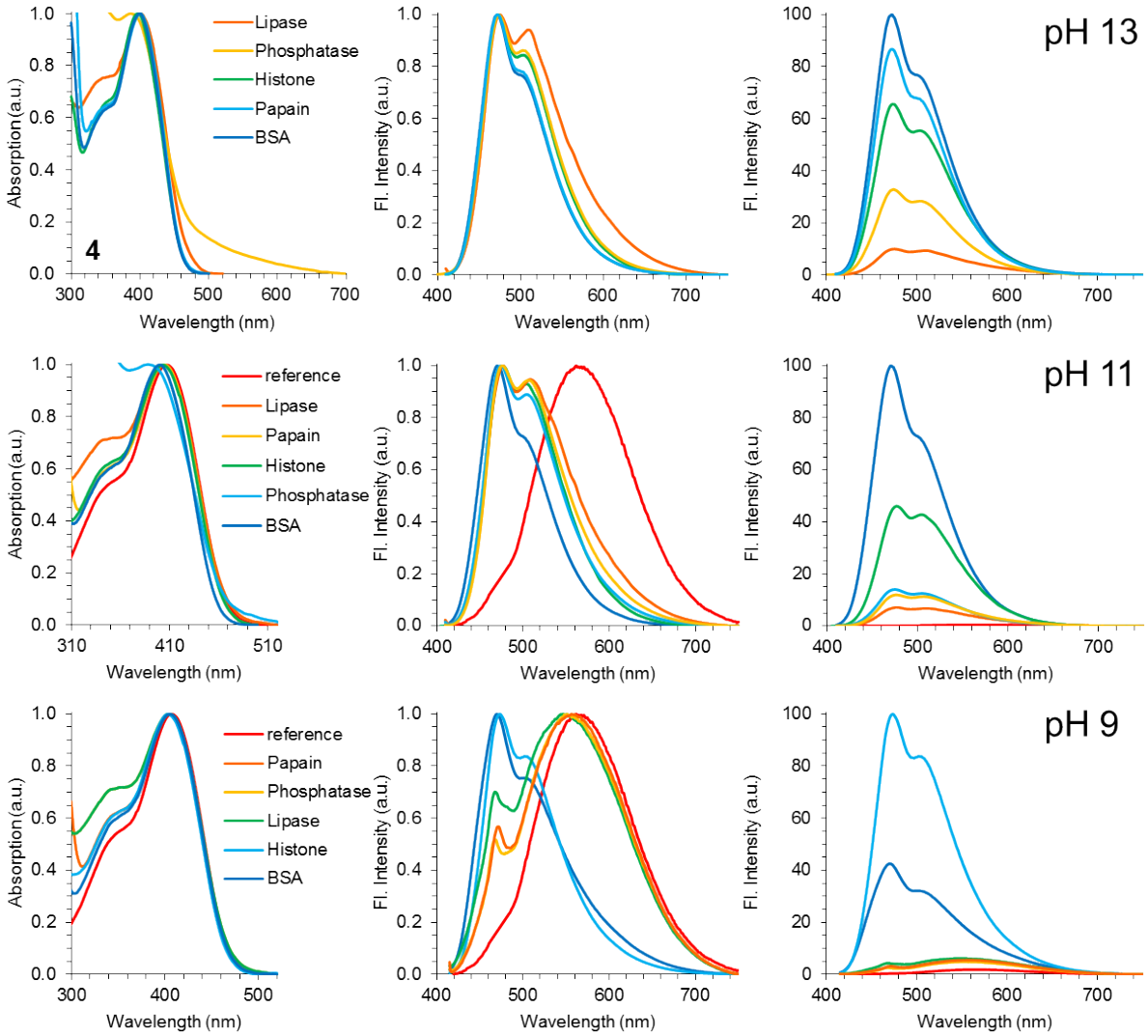


Fig S2 Absorption spectra (left), normalized emission spectra (middle), and non-normalized emission spectra (right) of buffered aqueous solutions (top: pH 13, middle: pH 11, bottom: pH 9) of **4** upon addition of different proteins.

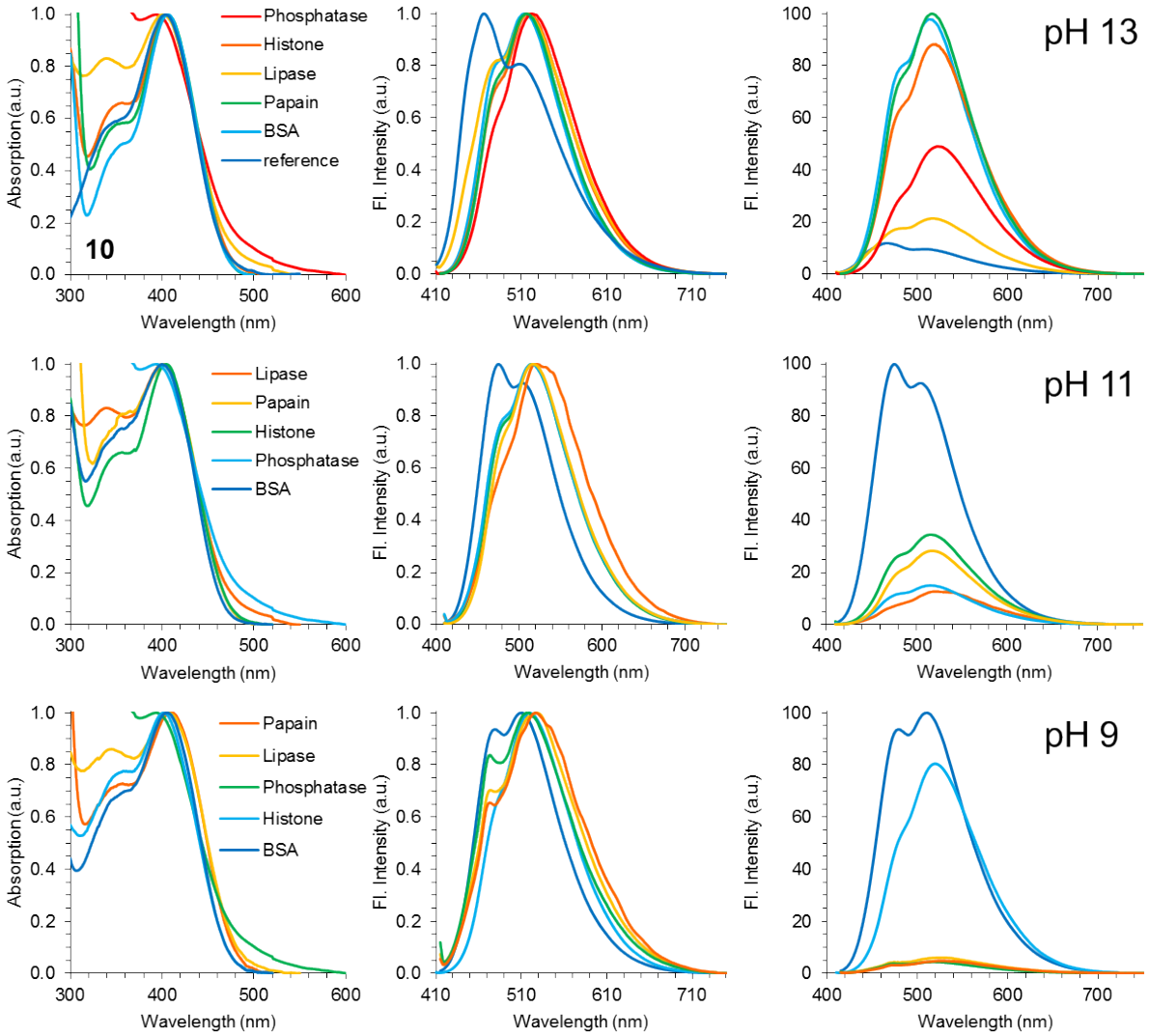


Fig S3 Absorption spectra (left), normalized emission spectra (middle), and non-normalized emission spectra (right) of buffered aqueous solutions (top: pH 13, middle: pH 11, bottom: pH 9) of **10** upon addition of different proteins

2.2 Detection Limit

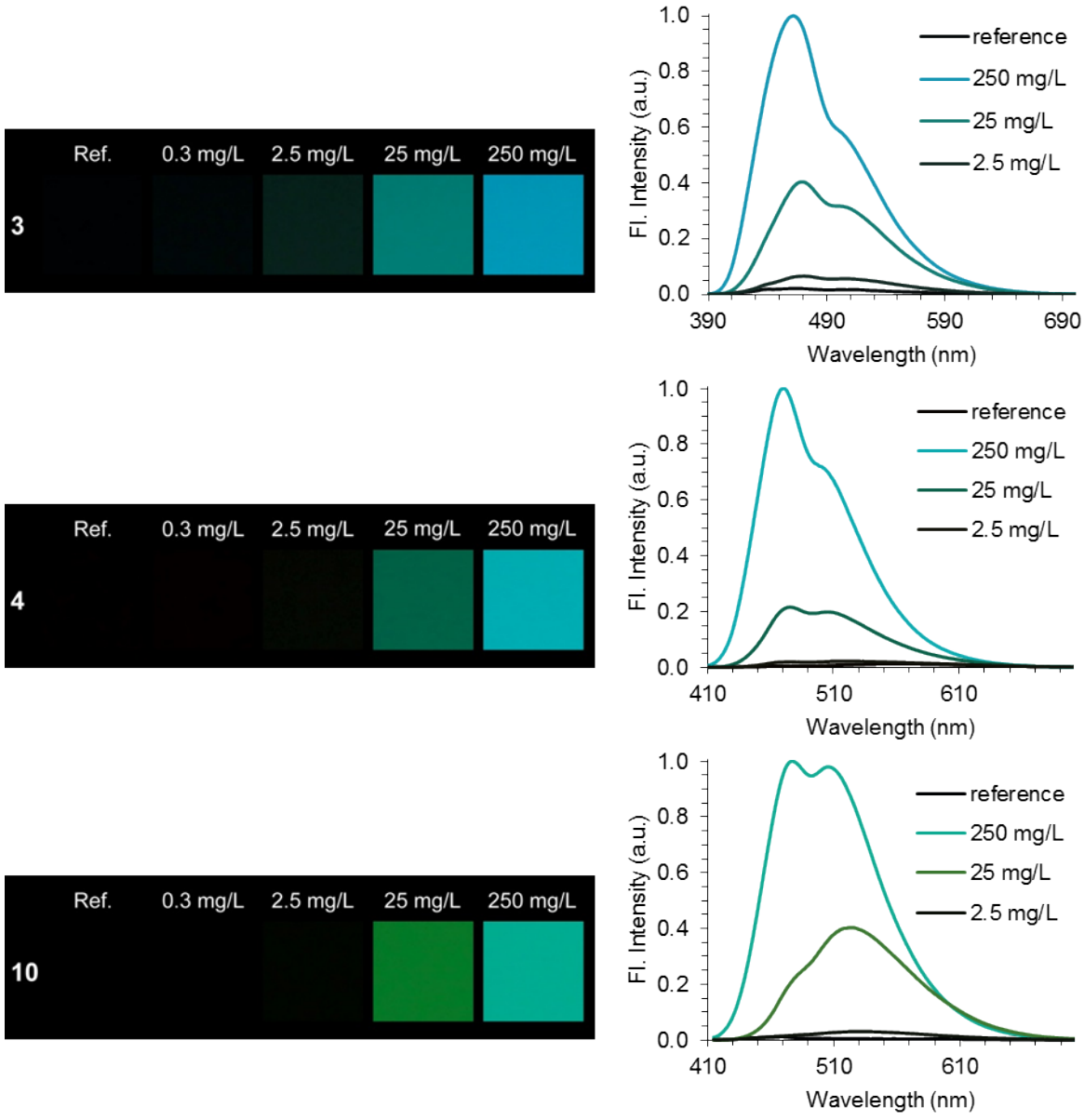


Fig S4 Photographs and fluorescence spectra of buffered aqueous solutions (pH 11, c = 4.4 µM) of **3** (top), **4** (middle) and **10** (bottom) at the concentrations of bovine serum albumin specified in the panel.

2.3 Comparison of Proteins and Amino Acid

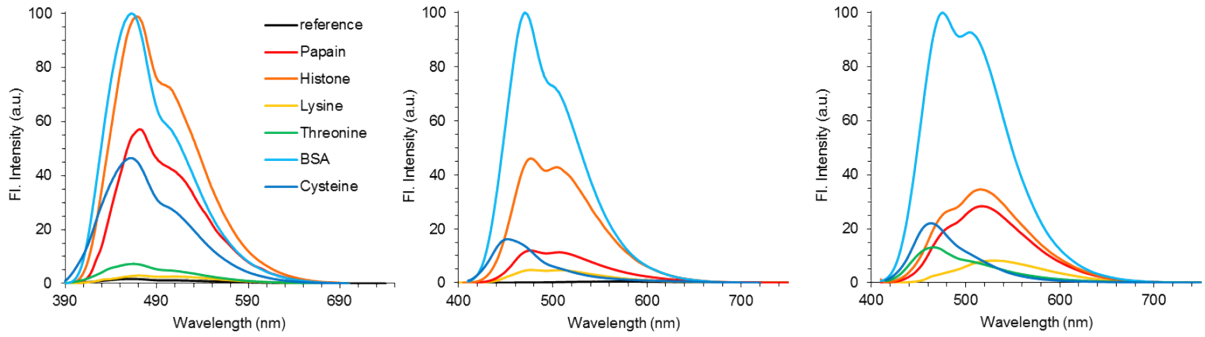


Fig S5 Non-normalized emission spectra of buffered aqueous solutions (pH 11) of **3** (left), **4** (middle) and **10** (right) upon addition of different proteins and amino acids.

2.4 Linear Discriminant Analysis

LDA was performed after 1 h reaction time of buffered aqueous solutions (pH 11, c = 4.4 µM) of **3**, **4** and **10** with albumins or protein shakes (c = 0.25 g/L). The final concentrations for fluorescence measurements were A = 0.038 at 280 nm, which was calibrated using UV/vis spectroscopy. The fluorescence intensity values at 495 nm (albumins) and at 465 nm (protein shakes) were recorded with excitation at 380 nm. This process was repeated for each protein target to generate five replicates of each. Thus, the five albumins (or six protein shakes) were tested against a three fluorophore array (**3**, **4** and **10**) five times to afford a data matrix of 3 fluorophores x 5 albumins (or 6 shakes) x 5 replicates. To obtain a fluorescence reference value the pure buffered fluorophore solution was measured at A_{280} = 0.038 and subtracted from the fluorescence response in presence of analytes. The data matrix was processed using classical linear discriminant analysis (LDA) in SYSTAT (version 13.0). In LDA, all variables were used in the model (complete mode) and the tolerance was set as 0.001. The fluorescence response patterns were transformed to canonical patterns. The Mahalanobis distances of each individual pattern to the centroid of each group in a multidimensional space were calculated and the assignment of the case was based on the shortest Mahalanobis distance. For the blind experiment another 18 unknown albumin samples were subjected to analysis via LDA and treated equally to the training cases. The protein sample preparation, data collection and analysis via LDA were carried out by different persons.

Table S1. Training matrix of fluorescence response patterns of the three DSB array (**3**, **4** and **10**) against five albumin analytes with identical absorption values of A = 0.038 at 280 nm measured with the same excitation wavelength (380 nm). Fluorescence response was recorded at 495 nm and LDA was carried out as described above resulting in the three factors of the canonical scores and group generation.

Analyte	Fluorescence response pattern			Results LDA			
Albumin	**3**	**4**	**10**	Factor 1	Factor 2	Factor 3	Group
BSA	65.868	56.898	58.002	-3.767	-17.536	8.151	1.000
BSA	68.506	56.910	59.042	-2.420	-16.646	9.621	1.000
BSA	65.186	57.243	56.461	-4.019	-17.195	6.540	1.000
BSA	67.414	55.478	59.769	-3.905	-17.198	10.980	1.000
BSA	65.536	55.979	58.968	-4.486	-17.953	9.496	1.000
PSA	95.450	95.930	68.581	40.642	-18.696	-5.892	5.000
PSA	98.055	96.668	69.188	42.467	-17.814	-5.312	5.000
PSA	96.314	95.480	66.632	40.437	-17.053	-6.797	5.000
PSA	98.236	94.965	65.678	40.796	-15.339	-6.621	5.000
PSA	95.561	94.525	65.985	39.286	-16.835	-6.755	5.000
HSA	134.762	77.625	42.771	41.255	22.056	-1.121	2.000
HSA	131.874	78.976	42.485	40.926	20.218	-3.129	2.000
HSA	133.266	76.506	43.210	39.785	21.340	-0.326	2.000
HSA	136.935	77.900	40.537	42.157	24.349	-2.452	2.000
HSA	134.942	75.646	41.398	39.657	23.479	-0.601	2.000
Ovalbumin	75.852	31.723	27.859	-22.271	11.514	7.183	4.000
Ovalbumin	78.225	32.544	29.207	-20.392	11.852	8.199	4.000
Ovalbumin	78.088	32.768	28.590	-20.366	12.037	7.526	4.000
Ovalbumin	78.362	35.013	30.478	-18.292	10.509	7.311	4.000
Ovalbumin	77.688	32.782	29.606	-20.403	11.275	8.179	4.000
Lactalbumin	28.700	17.165	8.368	-57.323	0.404	-8.845	3.000
Lactalbumin	29.159	16.816	8.055	-57.419	0.927	-8.695	3.000
Lactalbumin	28.277	17.073	7.958	-57.639	0.417	-9.197	3.000
Lactalbumin	28.864	16.945	8.222	-57.433	0.638	-8.745	3.000
Lactalbumin	29.570	16.792	7.888	-57.272	1.248	-8.695	3.000

Table S2. Detection and Identification of unknown albumin samples using LDA. All unknown samples could be assigned to the corresponding albumin group defined by the training matrix.

Sample	Fluorescence response pattern			Results LDA				Analyte
#	**3**	**4**	**10**	Factor 1	Factor 2	Factor 3	Group	Albumin
1	98.152	91.164	68.581	38.264	-15.780	-1.540	5	PSA
2	77.560	33.660	27.330	-20.097	12.148	5.750	4	Ovalbumin
3	67.401	55.232	55.055	-4.720	-14.630	7.594	1	BSA
4	70.162	56.818	55.130	-2.253	-13.634	7.165	1	BSA

5	140.874	74.328	39.668	41.129	28.045	0.655	2	HSA
6	27.201	16.347	8.028	-58.669	0.009	-8.872	3	Lactalbumin
7	78.103	31.069	27.669	-21.768	13.045	8.130	4	Ovalbumin
8	102.212	94.533	63.963	42.051	-12.120	-6.546	5	PSA
9	139.328	74.404	41.424	40.715	26.243	1.521	2	HSA
10	29.395	17.235	8.368	-56.953	0.763	-8.716	3	Lactalbumin
11	26.519	15.912	8.245	-59.279	-0.348	-8.554	3	Lactalbumin
12	70.986	55.425	54.882	-2.966	-12.632	8.258	1	BSA
13	75.852	29.317	26.449	-24.279	12.987	7.953	4	Ovalbumin
14	140.777	72.952	39.600	40.034	28.442	1.629	2	HSA
15	73.659	34.206	28.838	-21.259	9.045	5.450	4	Ovalbumin
16	103.089	96.545	67.124	44.391	-13.923	-5.458	5	PSA
17	31.014	17.433	8.129	-56.098	1.718	-8.623	3	Lactalbumin
18	65.868	52.473	56.021	-7.379	-15.151	10.031	1	BSA

Table S3. Training matrix of fluorescence response patterns of the three DSB array (**3**, **4** and **10**) against six protein shake analytes with identical absorption values of A = 0.038 at 280 nm measured with the same excitation wavelength (380 nm). Fluorescence response was recorded at 465 nm and LDA was carried out as described above resulting in the three factors of the canonical scores and group generation

Analyte	Fluorescence response pattern			Results LDA			
Albumin	**3**	**4**	**10**	Factor 1	Factor 2	Factor 3	Group
Whey	74.190	30.763	12.527	-2.710	-6.202	3.028	6.000
Whey	80.538	32.506	12.197	-0.337	-7.150	5.330	6.000
Whey	82.544	33.256	12.079	0.440	-7.263	6.205	6.000
Whey	82.557	34.015	12.446	0.952	-6.442	6.030	6.000
Whey	77.719	33.839	12.799	-0.521	-4.589	4.802	6.000
Egg	114.592	48.660	23.695	26.459	-2.114	-1.352	2.000
Egg	113.047	49.246	24.656	26.930	-0.678	-2.834	2.000
Egg	116.494	50.114	24.652	28.369	-1.220	-1.937	2.000
Egg	115.396	50.199	24.068	27.426	-0.860	-0.977	2.000
Egg	112.833	51.523	24.281	26.962	1.484	-0.804	2.000
Soy	54.833	20.782	11.724	-12.633	-8.269	-3.690	5.000
Soy	58.585	21.193	12.458	-10.471	-9.169	-4.335	5.000
Soy	55.317	21.956	12.465	-11.506	-7.137	-4.217	5.000
Soy	56.756	22.054	12.139	-11.270	-7.704	-3.437	5.000
Soy	54.254	21.959	11.919	-12.417	-6.860	-3.370	5.000
Casein	54.971	33.905	14.475	-7.253	4.982	-0.443	3.000
Casein	59.494	35.602	14.582	-5.141	4.835	0.878	1.000
Casein	58.406	34.709	14.501	-5.803	4.392	0.361	1.000
Casein	58.365	35.259	14.590	-5.619	4.959	0.537	1.000
Casein	58.293	37.073	15.140	-4.746	6.873	0.679	1.000

$Multi_{exp}$	63.907	35.186	14.614	-3.578	2.690	1.023	4.000
$Multi_{exp}$	61.746	36.487	15.530	-3.229	5.044	-0.004	4.000
$Multi_{exp}$	62.603	37.249	14.740	-3.512	5.217	1.934	4.000
$Multi_{exp}$	63.192	37.223	15.104	-2.953	5.057	1.342	4.000
$Multi_{exp}$	61.045	36.436	14.958	-4.042	5.119	0.893	4.000
$Multi_{cheap}$	55.130	33.462	14.076	-7.666	4.387	-0.001	3.000
$Multi_{cheap}$	56.481	34.891	14.017	-6.933	5.200	1.119	1.000
$Multi_{cheap}$	57.214	34.667	15.535	-5.261	5.108	-1.597	3.000
$Multi_{cheap}$	57.183	34.646	16.257	-4.587	5.297	-2.877	3.000
$Multi_{cheap}$	56.629	34.264	15.752	-5.352	5.013	-2.285	3.000

3 NMR spectra

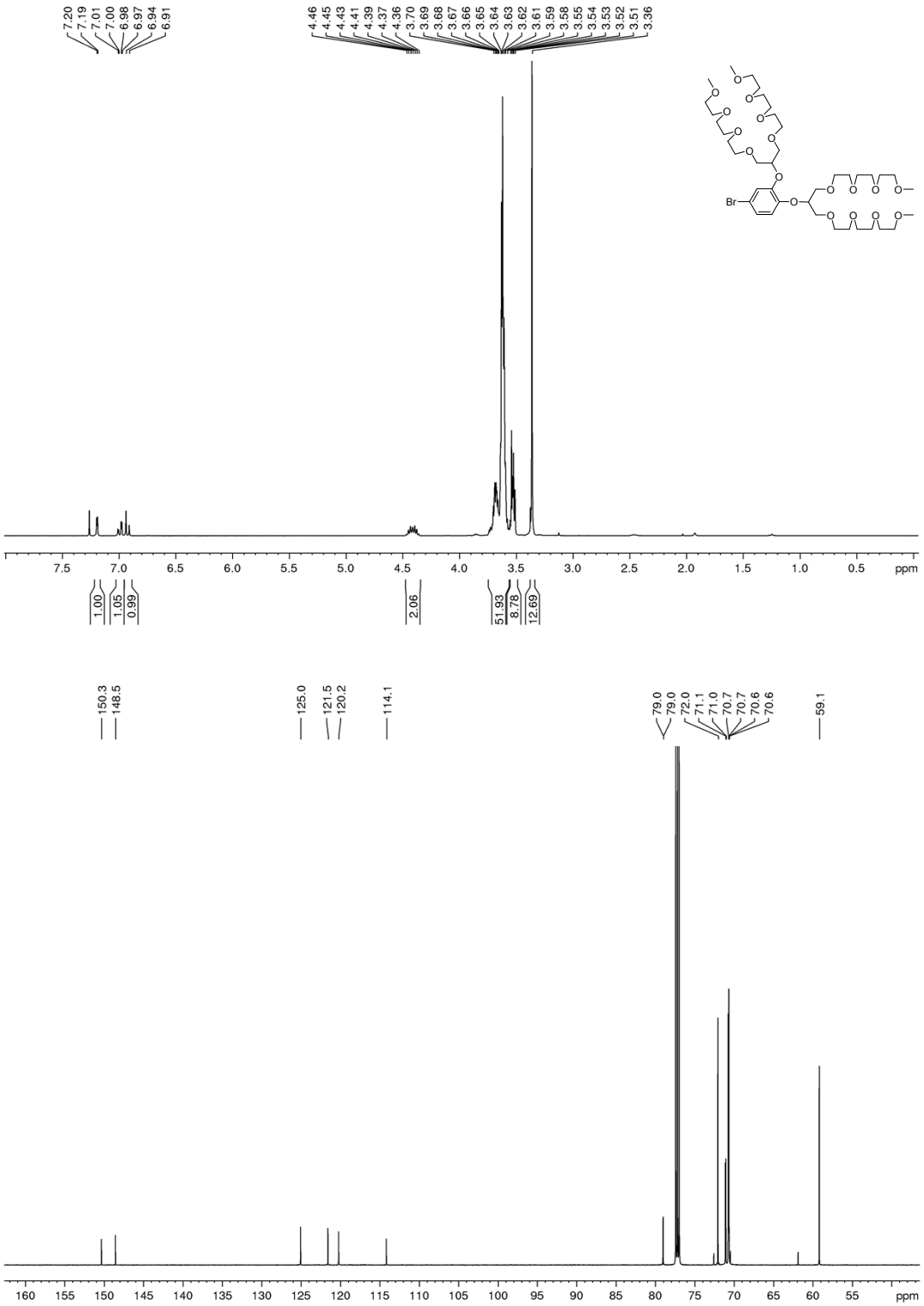


Fig S6 ^{1}H-NMR spectrum (300 MHz, $CDCl_3$, top) and $^{13}C\{^{1}H\}$-NMR spectrum (150 MHz, $CDCl_3$, bottom) of **S1**.

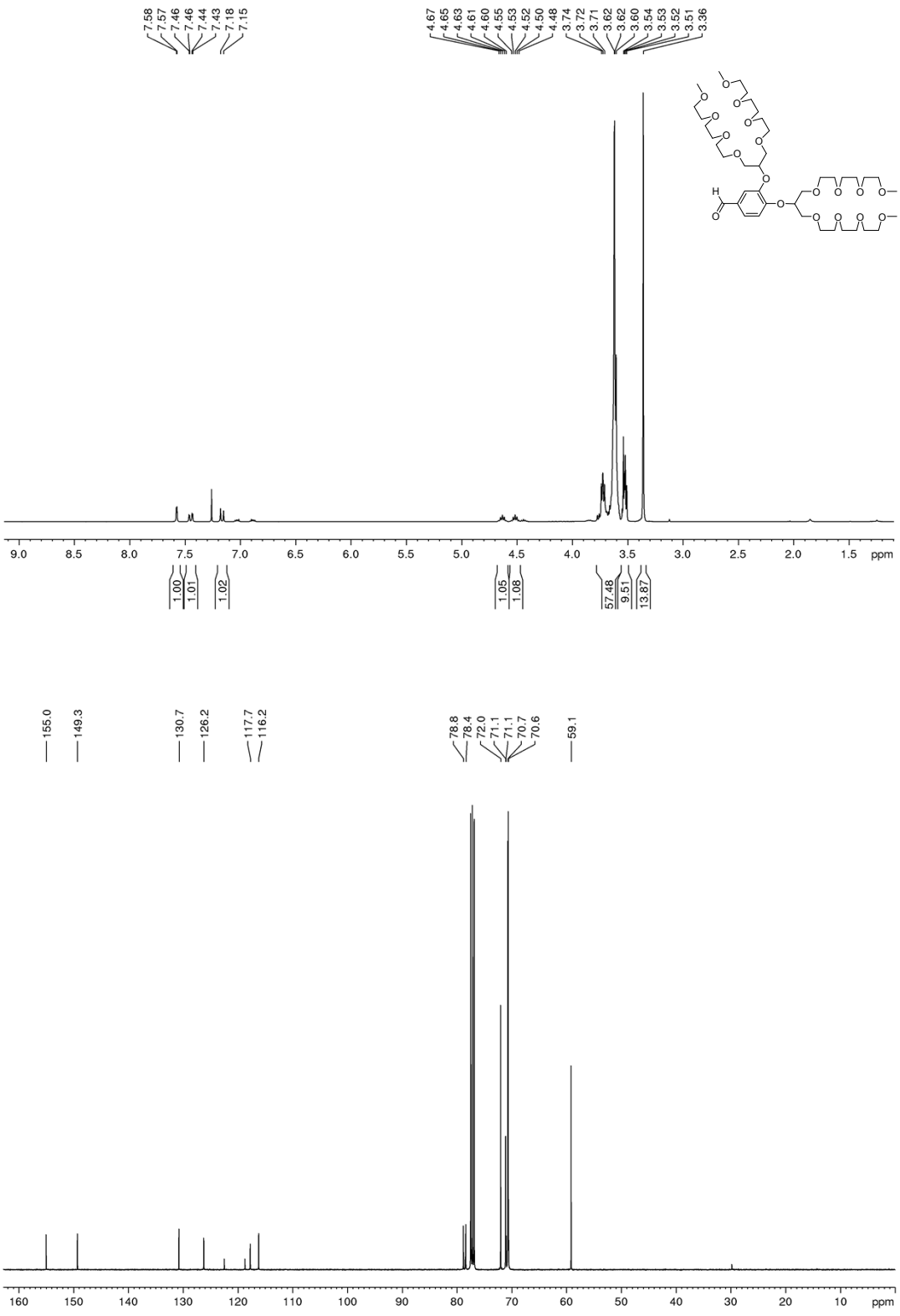


Fig S7 ^{1}H-NMR spectrum (300 MHz, $CDCl_3$, top) and $^{13}C\{^{1}H\}$-NMR spectrum (100 MHz, $CDCl_3$, bottom) of **1**.

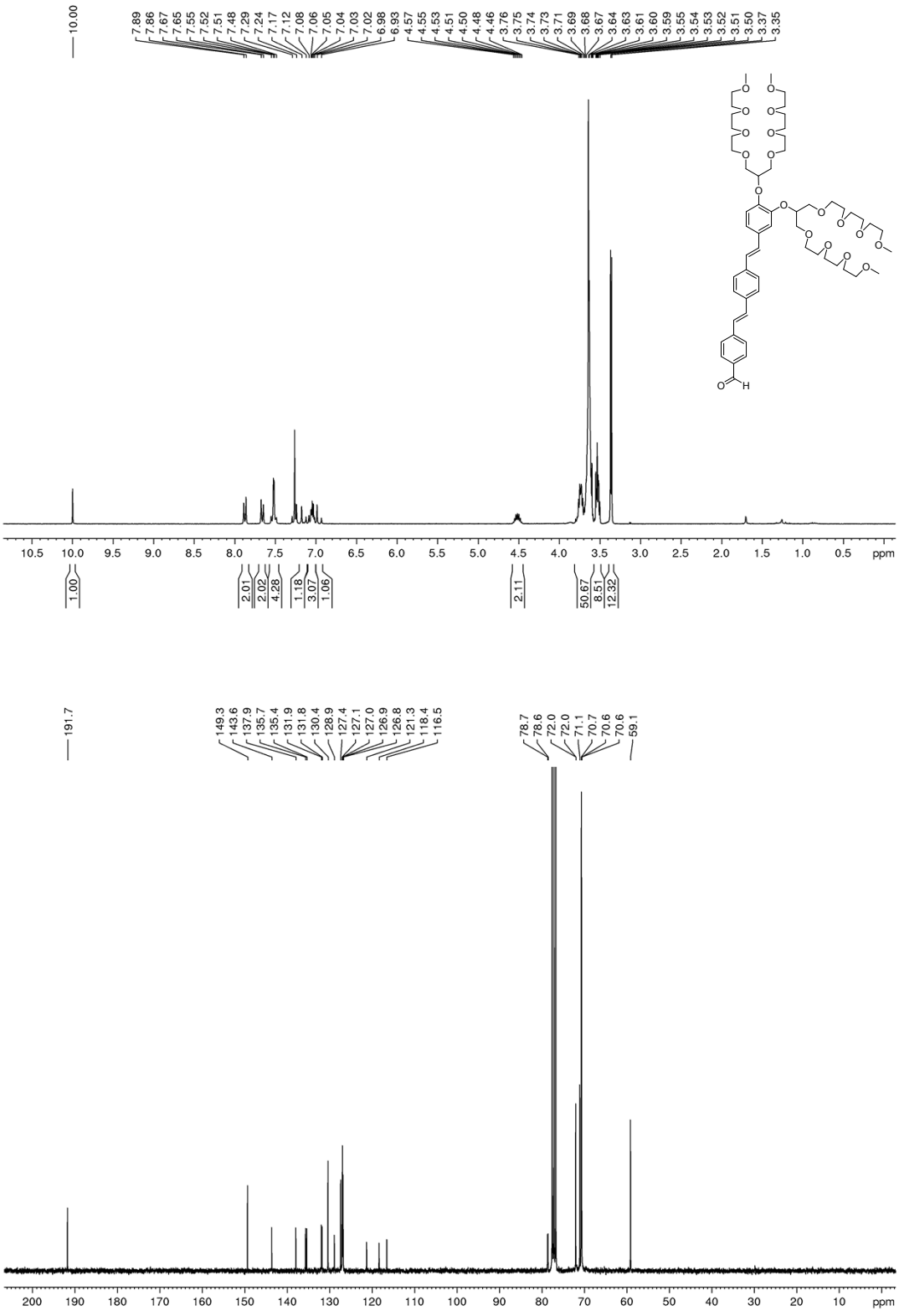


Fig S8 ^{1}H-NMR spectrum (300 MHz, $CDCl_3$, top) and $^{13}C\{^{1}H\}$-NMR spectrum (75 MHz, $CDCl_3$, bottom) of **3**.

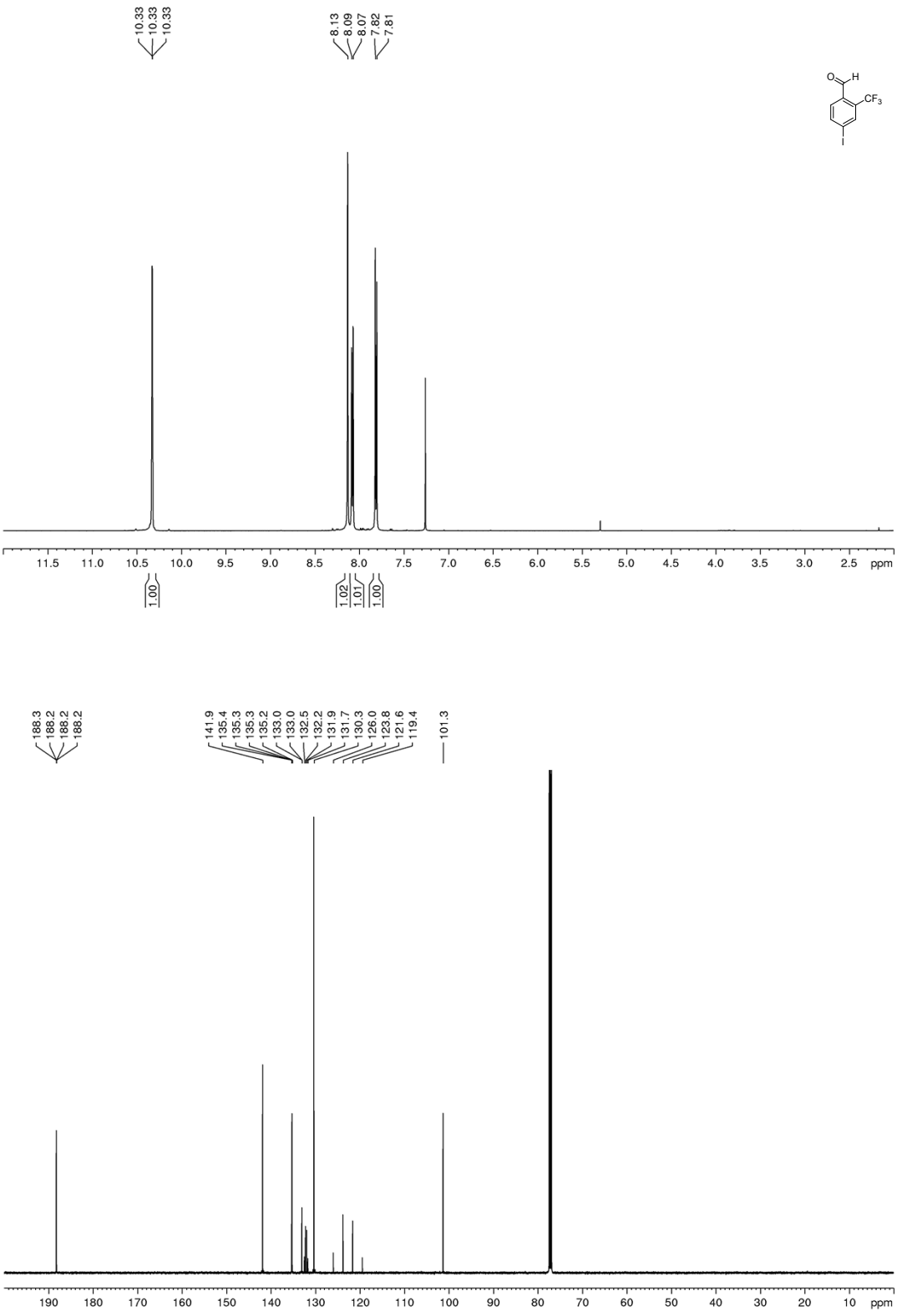


Fig S9 ^{1}H-NMR spectrum (500 MHz, $CDCl_3$, top) and $^{13}C\{^{1}H\}$-NMR spectrum (125 MHz, $CDCl_3$, bottom) of **6**.

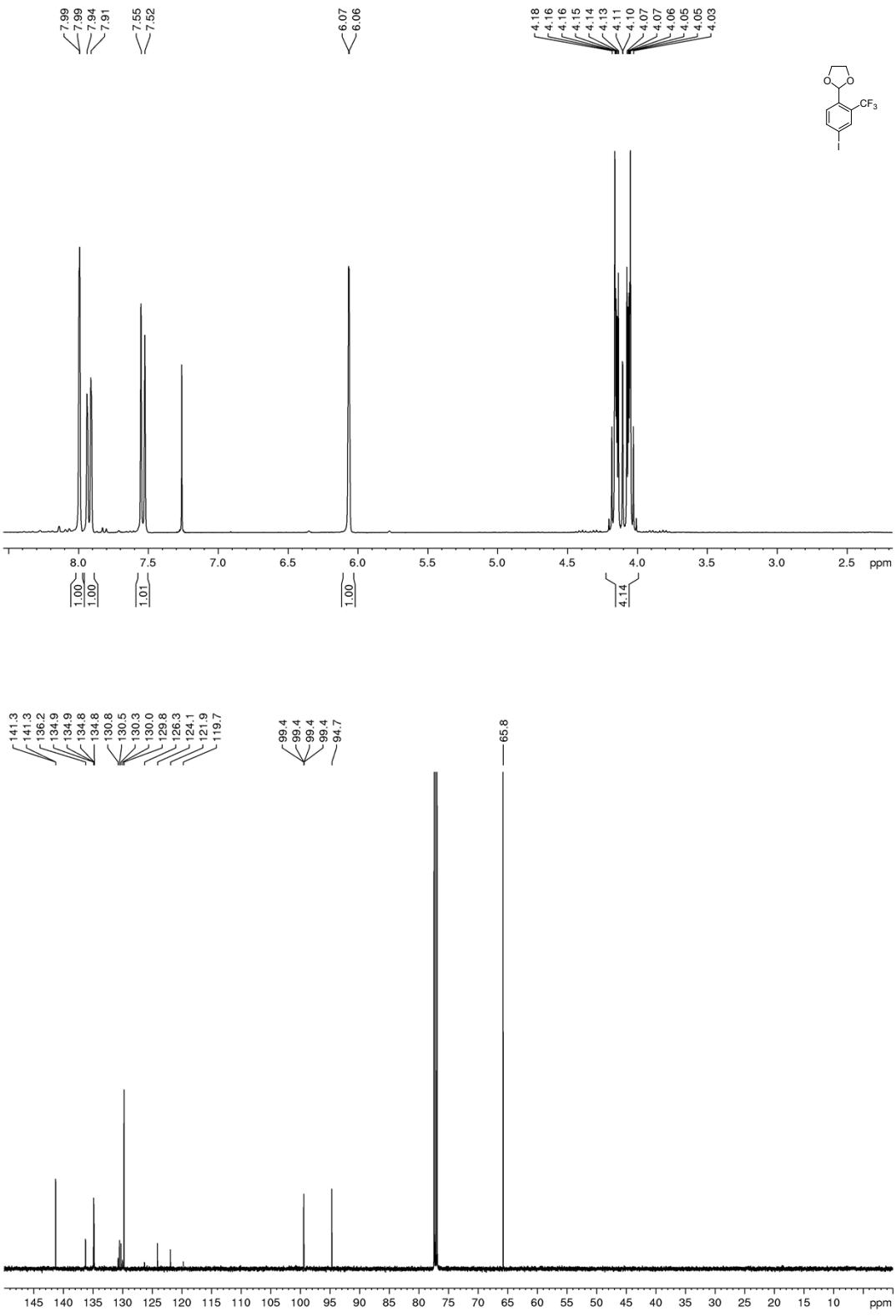


Fig S10 ^{1}H-NMR spectrum (300 MHz, $CDCl_3$, top) and $^{13}C\{^{1}H\}$-NMR spectrum (125 MHz, $CDCl_3$, bottom) of **7**.

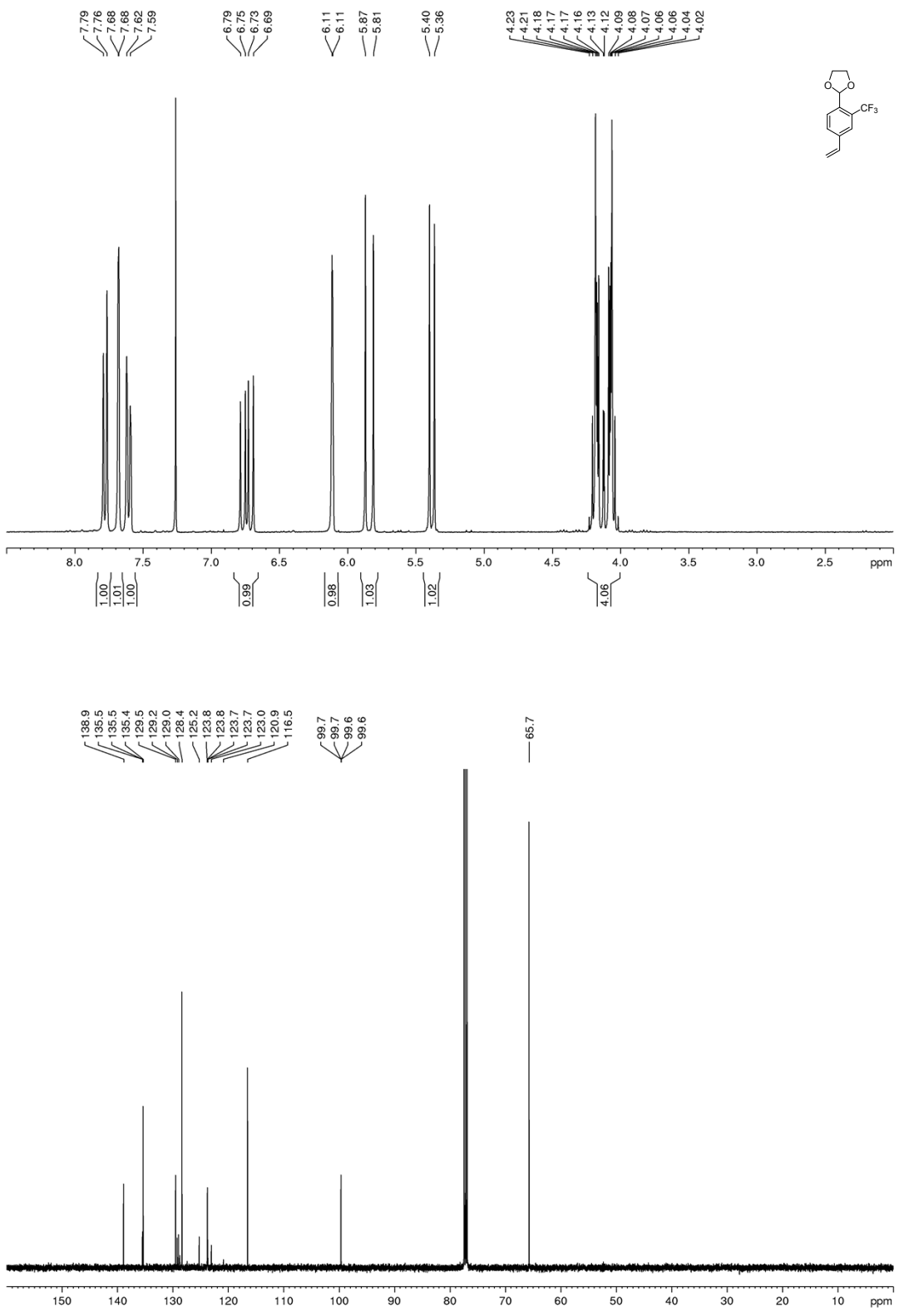


Fig S11 ^{1}H-NMR spectrum (300 MHz, $CDCl_3$, top) and $^{13}C\{^{1}H\}$-NMR spectrum (125 MHz, $CDCl_3$, bottom) of **8**.

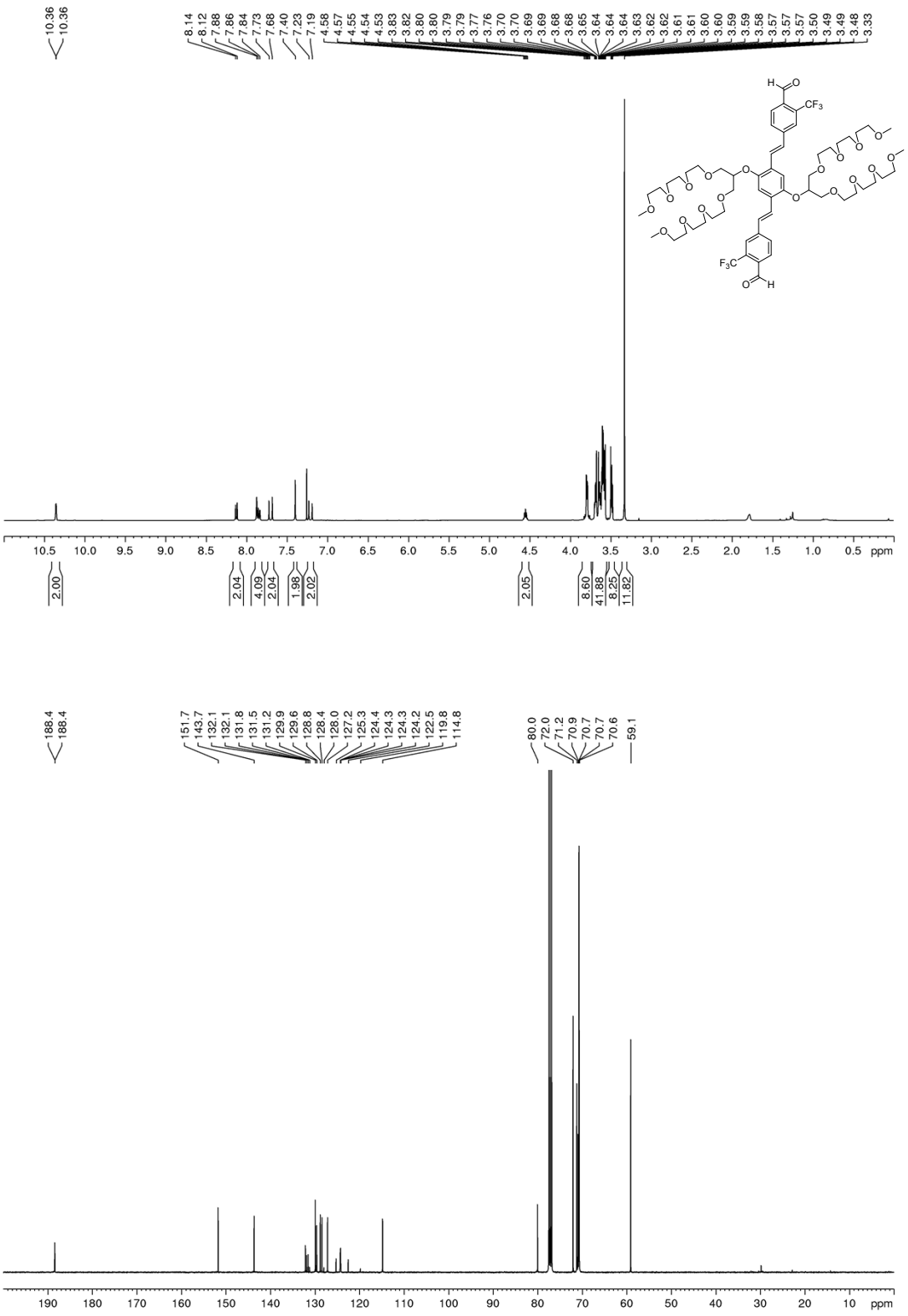


Fig S12 ^{1}H-NMR spectrum (300 MHz, $CDCl_3$, top) and $^{13}C\{^{1}H\}$-NMR spectrum (125 MHz, $CDCl_3$, bottom) of **10**.

Literature:

[1] J. Freudenberg, J. Kumpf, V. Schäfer, E. Sauter, S. J. Wörner, K. Brödner, A. Dreuw, U. H. F. Bunz , *J. Org. Chem.,* 2013, **78**, 4949-4959.