SUPPORTING INFORMATION

Light-Triggered Conversion of Non-Ionic into Ionic Surfactants: Towards Chameleon Detergents for 2-D Gel Electrophoresis

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General Methods

Solvents and starting materials were used as received. THF was distilled under N_2 atmosphere over sodium/benzophenone, while methylene chloride and acetonitrile were distilled under N_2 atmosphere over calcium hydride prior to use. Column chromatography was carried out with 130-400 mesh silica gel. NMR spectra were recorded on Bruker AB 250 (250.1 and 62.9 MHz for ^{1}H and ^{13}C, respectively) and AC500 as well as Delta JEOL Eclipse 500 (500 and 126 MHz for ^{1}H and ^{13}C, respectively) spectrometers at 23 ± 2 ^{o}C using residual protonated solvent signal as internal standard (^{1}H: $\delta(CHCl_3)$ = 7.24 ppm, and ^{13}C: $\delta(CHCl_3)$ = 77.0 ppm. Mass spectrometry was performed on Perkin-Elmer Varian Type MAT 771 and CH6 (EI), Type CH5DF (FAB), or Bruker Reflex with 337 nm laser excitation (MALDI-TOF) instruments. Elemental analyses were performed on a Perkin-Elmer EA 240. The HPLC system consisted of a 125 mm (∅ = 4 mm) Nucleodur 100-5-C18ec column employing UV-detection at 220 nm with an methanol:water = 80:20 eluent flow of 0.8 mL/min.

Optical Spectroscopy

UV-visible absorption spectra were recorded on a Cary 50 spectrophotometer equipped with a Peltier thermostated cell holder (ΔT ± 0.05 °C). Emission spectra were measured using a Cary Eclipse fluorescence spectrophotometer equipped with a Peltier thermostated cell holder (ΔT ± 0.05 °C). Slit widths were set to 5 nm bandpass for both excitation and emission. Spectra were corrected for variations in photomultiplier response and lamp intensity over wavelength using correction curves generated on the instrument, followed by normalization considering the optical density of the sample at the excitation wavelength. All solvents employed were of spectrophotometric grade. All spectra were recorded at 25 °C on degassed samples under continuous stirring within the cuvette holder.

Determination of Critical Micelle Concentrations (CMCs)

The method developed by Kalyanasundaram and Thomas[1] was adapted. In a typical experiment, 50 μL of a pyrene solution (100 μM in methanol) were placed in a fluorescence cuvette equipped with stirring bar and then the solvent was removed in a stream of nitrogen. After complete evaporation, an aliquot of the aqueous solution of the surfactant was added and the total volume adjusted to 2.5 mL by addition of water. After sonication for 7 min, a stream of nitrogen was bubbled through the solution for 2 min. The thus prepared sample was then placed into fluorescence spectrometer and excited at λ = 339nm.

Irradiation Experiments

Irradiation experiments were performed on degassed solutions (N_2 or Ar for 2 min) of surfactants **7a-d** in water using a LOT-Oriel 1000 W medium-pressure xenon lamp (XBO) equipped with interference filters ($\lambda_{max\ T}$ = 313 nm @ 12% T, FWHM = 11 nm). Irradiation experiments were followed by UV/vis spectroscopy and HPLC analysis (see MS Figure 2a,b). For illustration, both a spectral comparison of the UV/vis spectra of substrate **7d** and the primary nitrosoaldehyde photoproduct as well as the corresponding extinction difference diagram[2] showing non-linear behavior indicative of parallel and/or subsequent reactions are shown in Figure 1a,b.

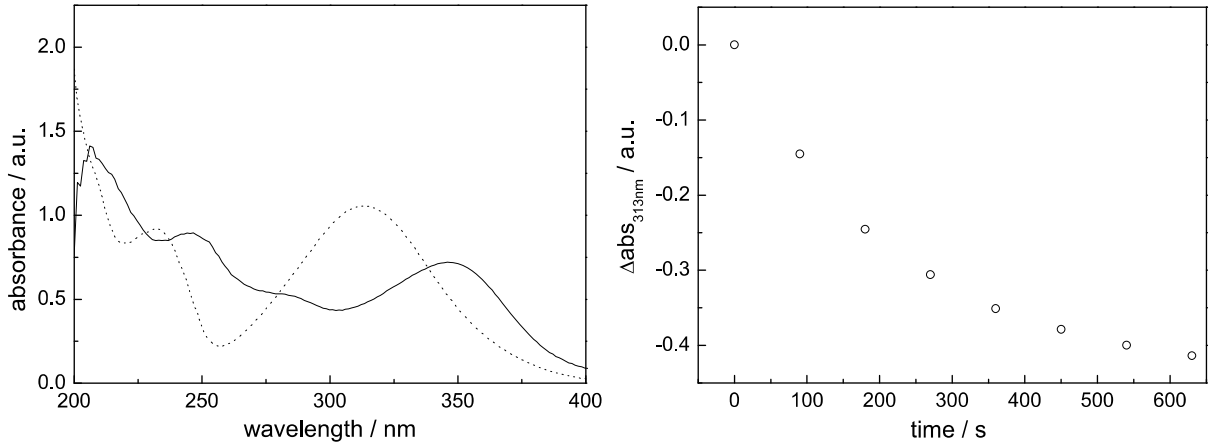


Figure 1. a) UV/vis absorption spectra of **7d** (····· 120 µM in water) and its primary photoproduct (——), acquired during HPLC separation and scaled using isosbestic point at λ = 280 nm. b) Respective extinction difference diagram for irradiation (λ_{irr} = 313 nm) of **7d** (120 µM in water), corresponding to MS Figure 2a.

Synthesis

MeO(PEG)$_{16}$-tosylate **3**.[3] MeO(PEG)$_{16}$-OH **2** (8.00 g, 10.85 mmol) and NaOH (1.00 g, 25.00 mmol) were dissolved in 30 mL of THF and 10 mL H_2O and the solution was colled in an ice bath to 0 °C. *p*-Toluenesulfonyl chloride (4.17g, 21.90 mmol) was dissolved in 10 mL THF and added dropwise over a periode of 5 min and then the reaction mixture was stirred for 24 h at 0 °C. For work-up 50 mL of ice-cold brine were added, the mixture extracted with methylene chloride (5x10 mL), the organic layers combined and dried over $MgSO_4$, and the solvent was removed in vacuo. Column chromatography (first eluting with CH_2Cl_2 to remove excess tosyl chloride, then eluting with 5 vol% MeOH in CH_2Cl_2) provided 5.55g of the product as a white solid (58 %). R_f (5 vol% MeOH in CH_2Cl_2) = 0.35; ^{1}H-NMR (250 MHz, $CDCl_3$): δ (ppm) = 7.76 (d, J = 8.2 Hz, 2 H, Ar-*H*), 7.31 (d, J = 8.2 Hz, 2 H, Ar-*H*), 4.12 (t, J = 4.6 Hz, 2 H, C*H*$_2$-O$_3$S), 3.75-3.45 (m, 63 H, C*H*$_2$), 3.34 (s, 3 H, OC*H*$_3$), 2.41 (s, 3 H, Ar-C*H*$_3$); ^{13}C-NMR (62.5 MHz, $CDCl_3$): δ (ppm) = 144.5, 132.9, 129.6, 127.7, 71.7, 70.5, 70.3,

69.0, 68.4, 58.7, 21.3; MS (MALDI-TOF, α-cyano-4-hydroxycinnamic acid) *m/z* = 869.5 ($[M_{n=15}+Na]^+$), 913.6 ($[M_{n=16}+Na]^+$), 957.6 ($[M_{n=17}+Na]^+$).

5-MeO(PEG)$_{16}$-2-nitrobenzaldehyde **4**. 5-Hydroxy-2-nitrobenzaldehyde **1** (1.57 g, 9.43 mmol), MeO(PEG)$_{16}$-tosylate **3** (6.00 g, 6.74 mmol), K_2CO_3 (1.52 g, 11 mmol), tetra(*n*-butyl)ammonium iodide (0.26 g, 0.7 mmol), and 18-crown-6 (0.09g, 0.34 mmol) were placed in a flask and 75 mL of dry acetonitrile were added. The reaction mixture was stirred at 50 °C under nitrogen for 24 h. Then, the mixture was filtered and the solid residue washed with ethyl acetate. The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. Column chromatography (gradient 1 vol% MeOH in CH_2Cl_2 → 5 vol% MeOH in CH_2Cl_2) provided 5.37 g of the product as a white solid (90 %). R_f (5 vol% MeOH in CH_2Cl_2) = 0.27; ^{1}H-NMR (250 MHz, $CDCl_3$): δ (ppm) = 10.31 (s, 1 H, C*H*O), 8.02 (d, J = 9.1 Hz, 1 H, Ar-*H*), 7.20 (d, J = 2.7 Hz, 1 H, Ar-*H*), 7.06 (dd, J = 9.1 Hz, 2.7 Hz, 1 H, Ar-*H*), 4.16 (t, J = 4.6 Hz, 2 H, Ar-OC*H*$_2$), 3.78 (t, J = 4.6 Hz, 2 H, Ar-OCH$_2$C*H*$_2$), 3.70-3.30 (m, 60 H, C*H*$_2$), 3.24 (s, 3 H, OC*H*$_3$); ^{13}C-NMR (62.5 MHz, $CDCl_3$): δ (ppm) = 188.0, 163.0, 141.8, 133.9, 126.8, 118.7, 113.7, 71.5, 68.9, 68.3, 58.6; MS (MALDI-TOF, α-cyano-4-hydroxycinnamic acid) *m/z* = 864.6 ($[M_{n=15}+Na]^+$), 908.7 ($[M_{n=16}+Na]^+$), 952.7 ($[M_{n=17}+Na]^+$).

5-MeO(PEG)$_{16}$-2-nitrobenzyl alcohol **5**. $NaBH_4$ (11 mg, 0.28 mmol) was placed in a flask, dry ethanol (1.00 mL, 17 mmol) was added, and the flask was placed in a ultrasonic bath for 1 min. To this mixture a solution of 5-MeO(PEG)$_{16}$-2-nitrobenzaldehyde **4** (0.50 g, 0.56 mmol) in 15 mL of dry THF was added slowly. The reaction mixture was stirred at room temperature for 2 h under nitrogen. Then, 50 mL of cold brine were added to the reaction mixture, the aqeous layer was extraction with ethyl acetate (5x10 mL), the combined organic layers were washed with sat. aq. $NaHCO_3$ solution, dried over $MgSO_4$, and the solvent was evaporated. Column chromatography (5 vol% MeOH in CH_2Cl_2) provided 4.37 g of the product as a white solid (88 %) that was immediately used for preparation of **7a-d**. R_f (5 vol% MeOH in CH_2Cl_2) = 0.10; ^{1}H-NMR (250 MHz, $CDCl_3$): δ (ppm) = 8.13 (d, J = 9.6 Hz, 1 H, Ar-*H*), 7.35 (d, J = 2.9 Hz, 1 H, Ar-*H*), 6.87 (dd, J = 9.6 Hz, 2.9 Hz, 1 H, Ar-*H*), 4.99 (s, 2 H, C*H*$_2$-OH), 4.24 (t, J = 5.1 Hz, 2 H, Ar-OC*H*$_2$), 3.87 (t, J = 5.1 Hz, 2 H, Ar-OCH$_2$C*H*$_2$), 3.89-3.44 (m, 56 H, C*H*$_2$), 3.35 (s, 3 H, OC*H*$_3$), 2.84 (broad s, 2 H, O*H*).

General procedure for synthesis of 5-MeO(PEG)$_{16}$-2-nitrobenzyl alkylsulfonates **7a-d**. All operations were performed in the dark under red-light illumination to exclude unwanted

photochemical degradation of the product. 5-MeO(PEG)$_{16}$-2-nitrobenzyl alcohol **5** (1 equiv.) and triethylamine (3 equiv.) were dissolved in methylene chloride (~0.2 M in **5**) and cooled to 0 °C on an ice-bath under a maintained nitrogen atmosphere. Then, a ~0.2 M solution of the respective alkylsulfonyl chloride **6a-d** (2 equiv.) in methylene chloride was added slowly at 0 °C. After stirring at 0 °C for 1 h, brine was added and the aqueous layer was extracted several times with methylene chloride. The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo. Column chromatography (first eluting with CH_2Cl_2 to remove excess alkylsulfonyl chloride, then eluting with 5 vol% MeOH in CH_2Cl_2) yielded the products in good yields as white solids.

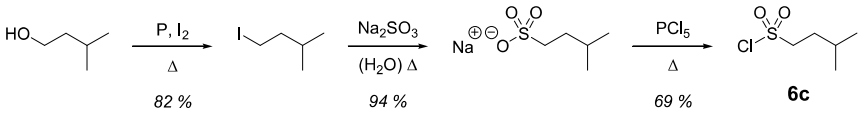
7a (75 %): ^{1}H-NMR (250 MHz, $CDCl_3$): δ (ppm) = 8.21 (d, J = 9.6 Hz, 1 H, Ar-*H*), 7.25 (d, J = 2.9 Hz, 1 H, Ar-*H*), 6.97 (dd, J = 9.6 Hz, 2.9 Hz, 1 H, Ar-*H*), 5.65 (s, 2 H, Ar-C*H*$_2$-O$_3$S), 4.24 (t, J = 4.4 Hz, 2 H, Ar-OC*H*$_2$), 3.88 (t, J = 4.4 Hz, 2 H, Ar-OCH$_2$C*H*$_2$), 3.75-3.50 (m, 51 H, C*H*$_2$), 3.36 (s, 3 H, OC*H*$_3$), 3.21 (t, J = 7.4 Hz, 2 H, O$_3$S-C*H*$_2$), 1.89 (p, J = 7.4 Hz, 2 H, O$_3$S-CH$_2$C*H*$_2$), 1.25 (m, 21 H,C*H*$_2$), 0.87 (t, J = 6.6 Hz, 3 H, C*H*$_3$); MS (FAB, *meta*-nitrobenzyl alcohol/CH_2Cl_2 matrix (*m*NBA)) *m/z* = 1096.8 ([M$_{n=15}$+Na]$^+$); Anal. (%): C: 56.12, H: 8.51, N: 1.12 (calcd for n = 14: C: 55.85, H: 8.69, N: 1.36; calcd for n = 15: C: 55.79, H: 8.71, N: 1.30; calcd for n = 16: C: 55.74, H: 8.73, N: 1.25).

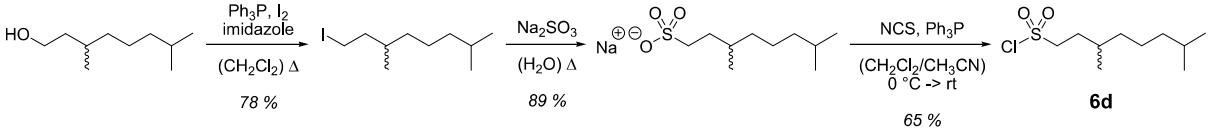
7b (89 %): ^{1}H-NMR (250 MHz, $CDCl_3$): δ (ppm) = 8.17 (d, J = 9.1 Hz, 1 H, Ar-*H*), 7.21 (d, J = 2.7 Hz, 1 H, Ar-*H*), 6.93 (dd, J = 9.6 Hz, 2.9 Hz, 1 H, Ar-*H*), 5.61 (s, 2 H, Ar-C*H*$_2$-O$_3$S), 4.20 (t, J = 4.4 Hz, 2 H, Ar-OC*H*$_2$), 3.84 (t, J = 4.4 Hz, 2 H, Ar-OCH$_2$C*H*$_2$), 3.89-3.44 (m, 58 H, C*H*$_2$), 3.32 (s, 3 H, OC*H*$_3$), 3.20 (t, J = 8.2 Hz, 2 H, O$_3$S-C*H*$_2$), 1.88-1.85 (m, 2 H, O$_3$S-CH$_2$C*H*$_2$), 1.47-1.42 (m, 2 H, C*H*$_2$), 1.23 (m, 8 H, C*H*$_2$), 0.85 (t, J = 5.1 Hz, 3 H, C*H*$_3$); ^{13}C-NMR (62.5 MHz, $CDCl_3$): δ (ppm) = 163.3, 139.5, 133.7, 127.8, 114.3, 114.1, 71.7, 70.5, 70.2, 69.1, 68.2, 58.8, 53.3, 50.7, 31.4, 28.7, 28.6, 27.9, 23.3, 22.3, 13.8; MS (FAB, *m*NBA) *m/z* = 999.3 ([M$_{n=14}$+Na]$^+$), 1043.1 ([M$_{n=15}$+Na]$^+$), 1087.8 ([M$_{n=16}$+Na]$^+$).

7c (86 %): ^{1}H-NMR (250 MHz, $CDCl_3$): δ (ppm) = 8.17 (d, J = 9.1 Hz, 1 H, Ar-*H*), 7.21 (d, J = 2.7 Hz, 1 H, Ar-*H*), 6.93 (dd, J = 9.6 Hz, 2.9 Hz, 1 H, Ar-*H*), 5.61 (s, 2 H, Ar-C*H*$_2$-O$_3$S), 4.20 (t, J = 4.4 Hz, 2 H, Ar-OC*H*$_2$), 3.84 (t, J = 4.4 Hz, 2 H, Ar-OCH$_2$C*H*$_2$), 3.89-3.44 (m, 58 H, C*H*$_2$), 3.32 (s, 3 H, OC*H*$_3$), 3.18 (t, J = 4.1 Hz, 2 H, O$_3$S-C*H*$_2$), 1.77-1.71 (m, 3 H, C*H*$_2$C*H*), 0.91 (d, J = 6.4 Hz, 6 H, CH(C*H*$_3$)$_2$); ^{13}C-NMR (62.5 MHz, $CDCl_3$): δ (ppm) = 163.3, 139.5, 133.7, 127.8, 114.3, 114.1, 71.7, 70.5, 70.2, 69.1, 68.2, 58.8, 53.3, 49.4, 31.7, 26.9, 21.8; MS (FAB, *m*NBA) *m/z* = 956.5 ([M$_{n=14}$+Na]$^+$), 1000.1 ([M$_{n=15}$+Na]$^+$), 1044.2 ([M$_{n=16}$+Na]$^+$).

7d (85 %): ^{1}H-NMR (250 MHz, $CDCl_3$): δ (ppm) = 8.20 (d, J = 10.1 Hz, 1 H, Ar-*H*), 7.21 (d, J = 2.7 Hz, 1 H, Ar-*H*), 6.96 (dd, J = 9.6 Hz, 2.7 Hz, 1 H, Ar-*H*), 5.64 (s, 2 H, Ar-CH_2-O_3S), 4.22 (t, J = 4.5 Hz, 2 H, Ar-OCH_2), 3.87 (t, J = 4.6 Hz, 2 H, Ar-OCH$_2$CH_2), 3.89-3.44 (m, 60 H, CH_2), 3.32 (s, 3 H, OCH_3), 3.20 (t, J = 4.5 Hz, 2 H, O_3S-CH_2), 1.84-1.80 (m, 2 H, C*H*), 1.57-1.47 (m, 2 H, CH_2-CH$_2$-S), 1.26-1.20 (m, 4 H, CH_2), 1.16-1.11 (m, 2 H, CH_2), 0.91 (d, J = 6.4 Hz, 3 H, CH_3), 0.84 (d, J = 6.4 Hz, 6 H, CH(CH_3)$_2$); ^{13}C-NMR (62.5 MHz, $CDCl_3$): δ (ppm) = 163.3, 139.5, 133.7, 127.8, 114.3, 114.1, 71.7, 70.5, 70.2, 69.1, 68.2, 58.8, 53.3, 49.4, 39.8, 36.7, 31.7, 30.5, 26.9, 23.4, 21.8, 20.9; MS (EI, 300 °C) *m/z* = 1049.1 ([$M_{n=15}$+Na]$^+$), 1092.6 ([$M_{n=16}$+Na]$^+$, 1133.6 ([$M_{n=17}$+Na]$^+$).

Synthesis of sulfonyl chlorides **6c,d**:





3-Methylbutanesulfonyl chloride **6c** was prepared following or closely adapting known literature procedures starting from isoamyl alcohol via iodination,[4] Strecker reaction,[5] and chlorination[6] in an overall 53 % yield. ^{1}H-NMR (250 MHz, $CDCl_3$): δ (ppm) = 3.67-3.61 (m, 2 H, S-CH_2), 1.95-1.89 (m, 2 H, CH_2-CH$_2$-S), 1.78-1.70 (m, 1 H, C*H*), 0.96 (d, J = 7.3 Hz, 6 H, CH_3).

3,7-Dimethyloctanesulfonyl chloride **6d** was prepared following or closely adapting known literature procedures starting from 3,7-dimethyloctan-1-ol via iodination,[7] Strecker reaction,[5] and chlorination[8] in an overall 45 % yield. ^{1}H-NMR (250 MHz, $CDCl_3$): δ (ppm) = 3.76 (t, J = 4.5 Hz, 2H, S-CH_2), 2.20-1.93 (m, 2 H, C*H*), 1.74-1.60 (m, 2 H, CH_2-CH$_2$-S), 1.41-1.35 (m, 4 H, CH_2), 1.26 (t, J = 4.5 Hz, 2 H, CH_2), 1.05 (d, J = 6.4 Hz, 3 H, CH_3), 0.96 (d, J = 6.4 Hz, 6 H, CH(CH_3)$_2$).

Dodecylsulfonic acid (laurylsulfonic acid) **8** was prepared by an adapted literature procedure[9] in 92 % yield. ^{1}H-NMR (500 MHz, D_2O): δ (ppm) = 2.82 (t, J = 4.5 Hz, 2 H, CH_2-S), 1.74 (p, J = 4.5 Hz, 2 H, CH_2-CH$_2$-S), 1.4-1.2 (m, 18 H, CH_2), 0.86 (t, J = 4.5 Hz, 3 H, CH_3); ^{13}C-NMR (125 MHz, D_2O): δ (ppm) = 79.4, 60.0, 58.0, 57.9, 57.6, 57.4, 56.8, 52.5, 50.7, 41.8; MS (FAB, glycerin/DMSO): *m/z* = 249.2 ([M+H]$^+$).

Protein Extraction and Western Blot Analysis

Rat L4 dorsal root ganglia (DRG) were transferred into homogenization buffer [50 mM Tris-HCl, pH 7.4 containing 150 mM NaCl, 2 mM EDTA and 2% sodium dodecylsulfate (SDS) or 3,7-di-methyloctylsulfonic acid] supplemented with a complete protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN) and homogenized with an ultrasonic tissue homogenizer. Homogenized DRGs were incubated under shaking at 10 °C overnight and solubilized proteins were extracted by centrifugation at 20.000 g for 15 min at 10 °C. The protein concentration of both samples was determined using the micro BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL) with bovine serum albumin (BSA) as a standard. Proteins were denaturated by boiling in sample buffer (3% SDS, 10 (v/v) glycerol, 5% (v/v) β-mercaptoethanol, 0.025% (w/v) bromphenol blue, 62.5 mM Tris-HCl, pH 6.8) for 10 min and electrophoresed on 4-15% pre-cast polyacrylamide gels (Biorad, Hercules, CA) in 25 mM Tris containing 192 mM glycine and 0.1% SDS. Proteins were electrophoretically transferred to nitrocellulose by using the semi-dry method (transfer time 1h at 10 V with 47.9 mM Tris, 38.9 mM glycine, 0.038% (w/v) SDS and 20% (v/v) methanol). The membrane was stained with Ponceau S (0.1% (w/v) Ponceau S in 5% acetic acid) to visualize the proteins and the lanes were separated from each other by cutting the membrane into strips using a scalpel. The strips were transferred to a stripe box and blocked by shaking overnight in the presence of 5% BSA in Tris buffered saline (TBS; 20 mM Tris, pH 7.4 containing 150 mM NaCl) containing 0.1 % Tween 20 (TBS-T). Strips analyzed for TRPV4 immunoreactivity (ir) were incubated under shaking with an 1:500 dilution of a rabbit anti-TRPV4 antiserum (Alomone Labs, Jerusalem, Israel) in TBS-T containing 5% BSA (antibody dilution buffer) whereas strips analyzed for β1-integrin ir were incubated with an 1:100 dilution of a rabbit anti-β1-integrin antiserum (Santa Cruz Biotechnology, Santa Cruz, CA) in antibody dilution buffer. All stripes were washed with TBS-T (three times, 15 min each) and TRPV4 and β1-integrin ir were revealed with a 1:1000 dilution of an horseradish-peroxidase (HRP) conjugated anti-rabbit antibody (Santa Cruz Biotechnology) in antibody dilution buffer. After washing with TBS-T (3 times, 15 min each) immunoreactivities were visualized by using the enhanced chemiluminescence detection system (ECL, Pierce Biotechnology). To confirm that each lane of the SDS-PAGE was, indeed, loaded with the same amount of proteins the stripes were stripped by a 30 min incubation at 37 °C with 62.5 mM Tris-HCl, pH 6.8 containing 100 mM β-Mercaptoethanol and 2% SDS and washed extensively with TBS-T. The stripes were blocked by a 1 h incubation with TBS-T containing 5% BSA and probed for 2 h at room temperature (RT) with a 1:500 dilution of a mouse anti-β actin antibody (Abcam, Cambridge,

MA) in antibody dilution buffer. After three washing steps with TBS-T (three times, 15 min each) stripes were incubated with an 1:1000 dilution of an respective anti-mouse-HRP conjugated 2nd antibody for 2 h at RT. Stripes were washed again with TBS-T (three times, 15 min each) and anti-β actin ir was visualized using the ECL system.

References

(1) Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1977**, *99*, 2039-2044.

(2) (a) Mauser, H. *Z. Naturforschg.* **1968**, *23b*, 1025-1030. (b) Mauser, H. *Z. Naturforschg.* **1968**, *23b*, 1021-1025. (c) Mauser, H.; Niemann, H. J.; Kretschmer, R. *Z. Naturforschg.* **1972**, *27b*, 1349-1353. (d) Mauser, H.; Starrock, V.; Niemann, H. J. *Z. Naturforschg.* **1972**, *27b*, 1354-1359.

(3) Bahr, J. L.; Yang, J.; Kosynkin, D. V.; Bronikowski, M. J.; Smalley, R. E.; Tour, J. M. *J. Am. Chem. Soc.* **2001**, *123*, 6536-6542.

(4) Adams, R.; Voorhees, V. *J. Am. Chem. Soc.* **1919,** *41*, 789-798.

(5) Reed, R. M.; Tartar, H.V. *J. Am. Chem. Soc.***1935**, *57*, 570-572.

(6) Burlingham, B. T.; Widlanski, T. S. *J. Am. Chem. Soc.* **2001**, *123*, 2937-2945.

(7) Ornstein, P. L.; Bleisch, T. J.; Arnold, M. B.; Wright, R. A.; Johnson, B. G.; Schoepp, D. D. *J. Med. Chem.* **1998**, *41*, 346-357.

(8) Kokotos, G.; Kotsovolou, S.; Constantinou-Kokotou, V.; Wu, G.; Olivecrona, G. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2803-2806.

(9) Noller, C. R.; Gordon, J. J. *J. Am. Chem. Soc.* **1933**, *55*, 1090-1094.