Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

Supporting information for:

Electron Transfer Dynamics of *Rhodothermus marinus* caa_3 Cytochrome *c* Domain on Biomimetic Films

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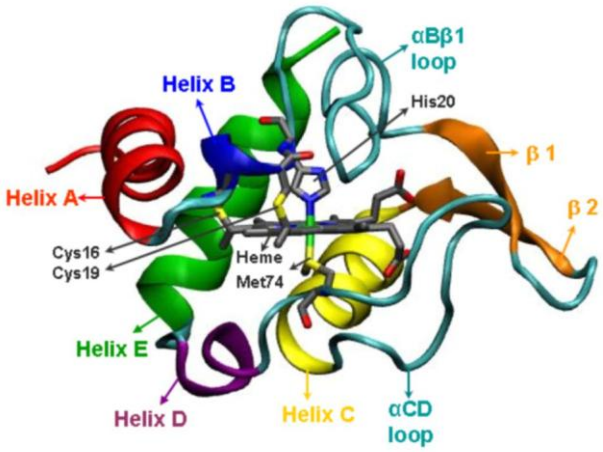

Figure S1. Schematic representation of Cyt-D shown from the partially exposed edge of the heme. Elements of secondary structure, heme group and the axial ligands of the iron are shown.

Table S1. RR and SERR band positions (cm^{-1}) of the ferrous (Fe^{2+}) and ferric (Fe^{3+}) Cyt-D in solution and adsorbed on a C_{11}-OH/C_{10}-CH_3 SAM, respectively.

	Solution		Adsorbed	
Band	Fe^{2+}	Fe^{3+}	Fe^{2+}	Fe^{3+}
ν_{13} (B_{1g})	1234	1234	1233	1234
ν_{21} (A_{2g})	1316	1316	1314	1315
ν_4 (A_{1g})	1364	1373	1362	1374
ν_{20} (A_{2g})	1404	1404	1402	1404
ν_3 (A_{1g})	1495	1500	1495	1500
ν_{11} (B_{1g})	1545	1560	1543	1563
ν_2/ν_{19} (A_{1g}/A_{2g})	1592	1592	1591	1592
ν_{10} (B_{1g})	1625	1640	1623	1640

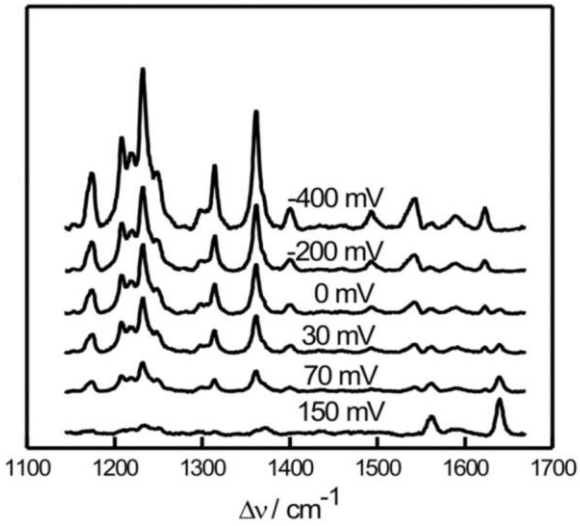

Figure S2. SERR spectra of Cyt-D adsorbed on a 3:1 C_{11}-OH/C_{10}-CH_3 SAM, measured at different potentials.

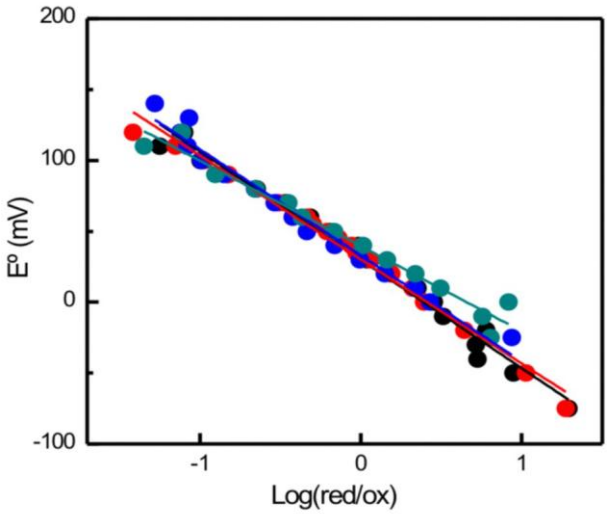

Figure S3. Nernst plots obtained from the quantitative treatment of SERR spectra of Cyt-D adsorbed on a C_n-OH/C_n-CH_3 SAM as a function of the electrode potential. Black: n = 6, E^o = 30 mV; red: n = 11, E^o = 31 mV; blue: n = 16, E^o = 33 mV; green: n = 20, E^o = 39 mV.

Table S2. Structural parameters of the Cyt-D in stable complexes with neutral (n_1 to n_8) and 50% charged (c_1 to c_5) SAMs obtained by MD simulations.

[1]*MD#*	[2]*Zone*	α	φ	[3]μ *(Debye)*	[4]μ *angle*	[5]$d_{Fe\text{-}SAM}$ (Å)	[6]*RMSD*
n_1	I	104	192	352	119	9.63	1.33
n_2	I	106	190	653	111	10.04	1.65
n_3	II	135	221	514	90	10.3	1.15
n_4	II	152	227	447	80	11.18	1.00
n_5	II	160	242	519	84	12.5	1.54
n_6	III	89	245	492	84	13.3	1.47
n_7	IV	169	188	579	96	13.7	0.91
n_8	V	162	81	313	113	15.5	1.21
c_1		72.5	141	814	167	19.7	0.80
c_2		57	167.5	740	161	20.2	1.4
c_3		104	125	690	169	21.4	1.2
c_4		112.5	93	697	147	24.4	1.15
c_5		91.6	120.4	662	170	21.8	0.93

[1]Simulation number. [2]Zone designation as represented in Figure 8. [3]Average dipole moment of Cyt-D in the complex. [4]Angle between the dipole moment vector and the Z axis. [5]Average direct distance between the heme iron to the SAM surface. [6]Root mean square deviation of Cyt-D along the dinamic compared to the crystal structure.

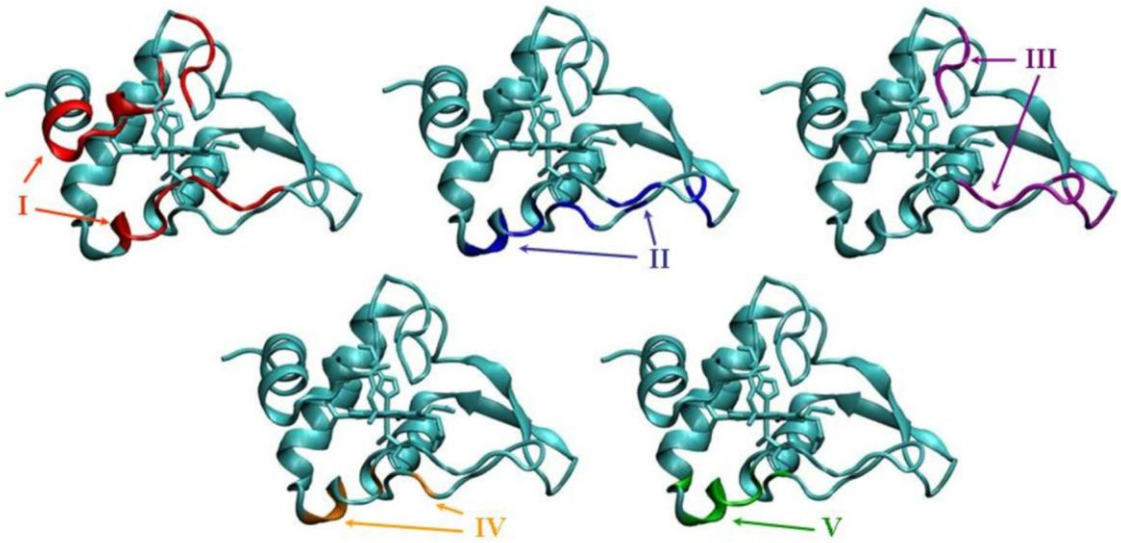

Figure S4. Schematic representation of Cyt-D showing the contact surface residues of each SAM binding zone. Colors from zones I to V are the same as in figure 7.

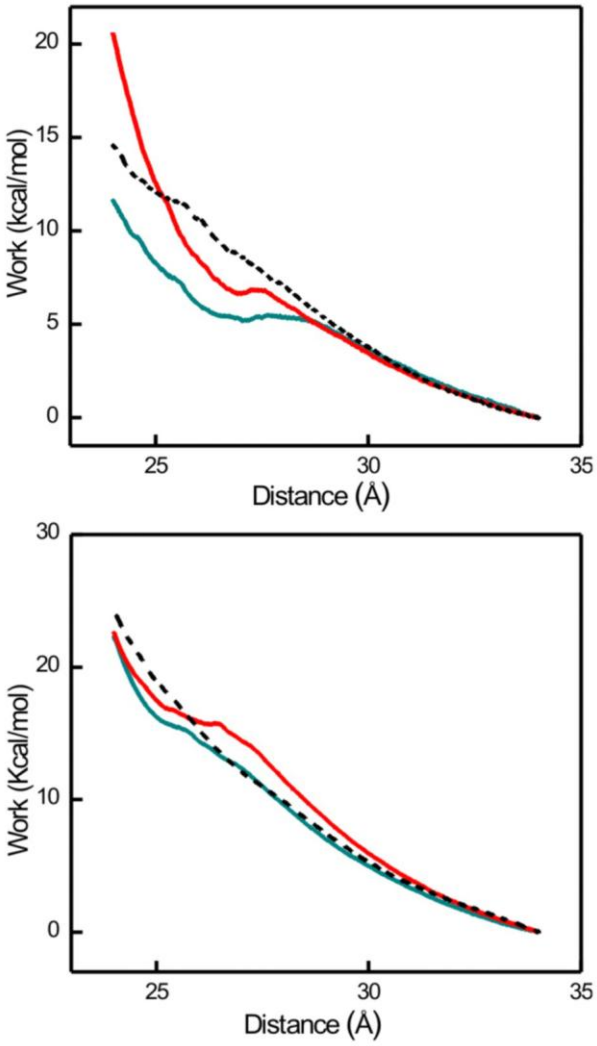

Figure S5. Work profiles obtained by MD simulation for the binding of Cyt-D to a 2:1 C_6-OH/C_5-CH_3 SAM (top) and 2:1 C_5-COOH/C_5-CH_3 SAM (bottom) starting from three different orientations. Red and green lines: binding orientations. Black lines: non-binding orientations.

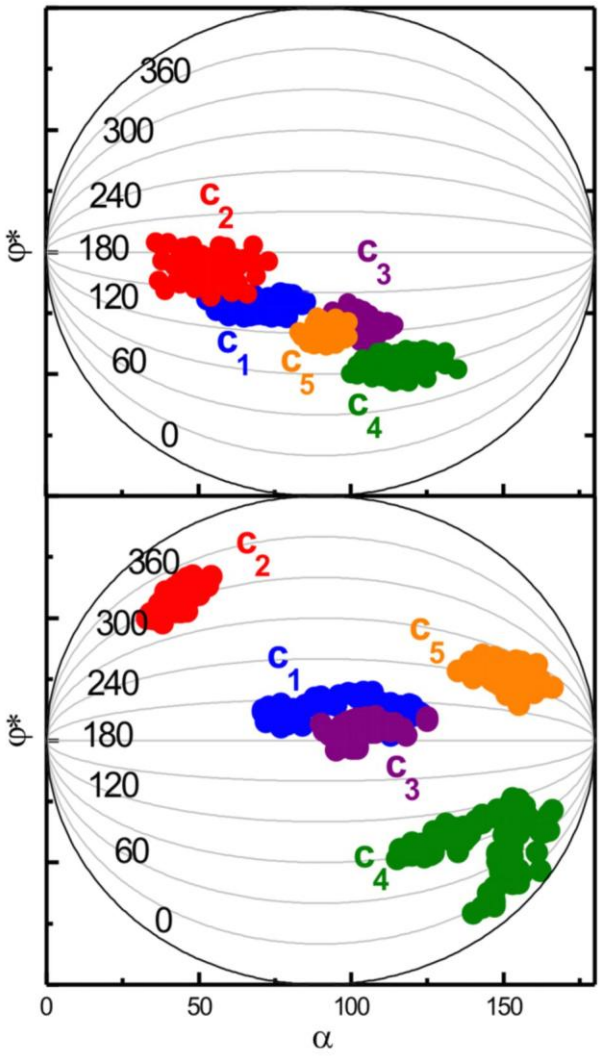

Figure S6. α vs φ* plots for all the Cyt-D/charged C_5-COOH/C_5-CH_3 SAM mixture in 2:1 proportion. Top: assuming 50% deprotonation of the carboxylic acids. Bottom: assuming 10% deprotonation of the carboxylic acids.

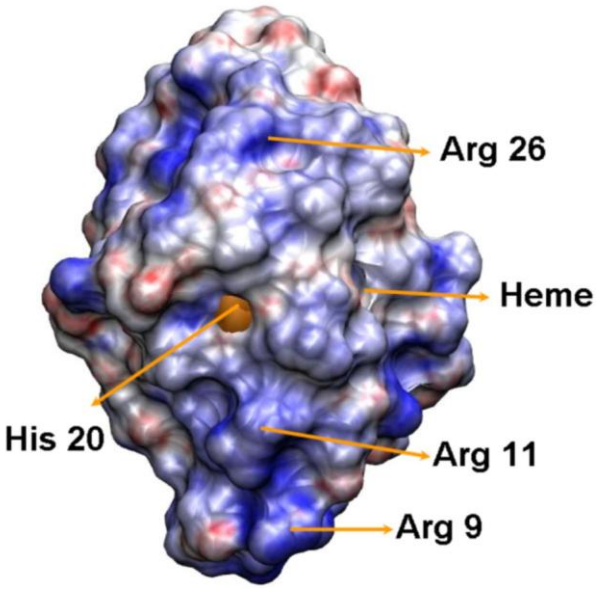

Figure S7. Cyt-D side view with the partially exposed iron axial ligand His 20 in orange. The rest of the protein is colored according to the electrostatic surface potential: the color code indicates negative values in blue and positive values in red.

Synthetic procedures

Eicosane-1-thiol (**3**) and 20-Mercaptoeicosan-1-ol (**9**) were successfully synthesized by modified published procedures.[1–4] Therefore, treatment of *n*-eicosyl bromide under Williamson-type conditions employing potassium thioacetate as nucleophile afforded *S*-Eicosyl ethanethioate (**2**) in 88% yield. On treatment with lithium aluminum hydride at room temperature, this compound was converted into mercaptane **3** in 99% yield. Compound **9** was synthesized via a Wurtz-type coupling as a key step. Then, 1,8-dichlorooctane was reacted with magnesium in the presence of iodine at 65 ºC for 3 hours. The resulting Grignard reagent was added to a solution 1,6-dibromohexane in tetrahydrofuran in the presence of Li_2CuCl_4 and refluxed almost one day to produce 1,20-Dibromoeicosane (**6**) in a low but reproducible yield of 24%. This compound was treated with one equivalent of sodium acetate to afford the monoacetate derivative **7** in 61%, which treated with potassium thioacetate gave rise to 20-(Acetylthio)eicosyl acetate (**8**) in 85% yield. Finally, compound **8** was reacted with lithium aluminum hydride to give the compound **9** in 98% yield.

Experimental

The glassware used in air and/or moisture sensitive reactions was flame-dried and carried out under a dry argon atmosphere. Unless otherwise noted, chemicals were commercially available and used without further purification. Solvents were distilled before use. Tetrahydrofuran was distilled from sodium/benzophenone ketyl. Anhydrous *N*,*N*-dimethylformamide was used as supplied from Aldrich.

Nuclear magnetic resonance spectra were recorded using a Bruker AC-200 MHz or a Bruker AM-500 MHz spectrometers. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane. Coupling constants are reported in Hertz. ^{13}C-NMR spectra were fully decoupled. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet.

High resolution mass spectra were conducted using a Bruker micrOTOF-Q II spectrometer, which is a hybrid quadrupole time of flight mass spectrometer with MS/MS capability.

Melting points were determined using a Fisher-Johns apparatus and are uncorrected.

Column chromatography was performed with E. Merck silica gel (Kieselgel 60, 230-400 mesh). Analytical thin layer chromatography was performed employing 0.2 mm coated commercial silica gel plates (E. Merck, DC-Aluminum sheets, Kieselgel 60 F_{254}).

Elemental analyses were conducted by UMYMFOR using an Exeter CE 440 apparatus.

***S*-Eicosyl ethanethioate (2).** A solution of *n*-eicosyl bromide (compound **1**, 1.043 g, 2.9 mmol) in anhydrous *N*,*N*-dimethylformamide (18 mL) under argon atmosphere was treated with potassium thioacetate (665 mg, 5.82 mmol). The reaction mixture was stirred at 90 ºC for 3 h. The solvent was evaporated and the residue was partitioned between water (50 mL) and methylene chloride (50 mL). The aqueous phase was extracted with methylene chloride (2 × 50 mL). The combined organic layers were dried ($MgSO_4$), and the solvent was evaporated. The product was purified by column chromatography (silica gel) eluting with hexane to afford 849 mg (88% yield) of pure compound **2** as a white solid: R_f = 0.71

(hexane–EtOAc, 19:1); mp 46–47 ºC; ^{1}H NMR (200.13 MHz, CDCl3) δ 0.88 (t, *J* = 6.4 Hz, 3H, H-20), 1.25 (m, 34H, -$(CH_2)_{17}$-), 1.56 (p, *J* = 7.7 Hz, 2H, H-2), 2.32 (s, 3H, SC(O)C*H*$_3$), 2.86 (t, *J* = 7.3 Hz, 2H, H-1); ^{13}C NMR (50.33 MHz, CDCl3) δ 14.13 (C-20), 22.69 (C-19), 28.82 (C-3), 29.12 (C-1), 29.16 (CH_2), 29.36 (CH_2), 29.48 (CH_2), 29.69 (CH_2), 30.64 (SC(O)*C*H$_3$), 31.92 (C-18), 196.08 (S*C*(O)CH$_3$). Anal. Calcd for ($C_{22}H_{44}OS$): C, 74.09; H, 12.44. Found C, 74.18; H, 12.55.

Eicosane-1-thiol (3). To a mixture of lithium aluminum hydride (95 mg, 25 mmol) in anhydrous tetrahydrofuran (7.0 mL) cooled at 0 ºC was added a solution of compound **2** (211.7 mg, 0.59 mmol) in tetrahydrofuran (5.0 mL). The reaction mixture was allowed to reach room temperature and was stirred for 60 min. The reaction was quenched by addition of ethyl acetate (5 mL). The mixture was partitioned between an aqueous saturated solution of sodium potassium tartrate (50 mL) and methylene chloride (50 mL). The aqueous layer was extracted with methylene chloride (2 × 50 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was evaporated. The residue was purified by column chromatography (silica gel) eluting with hexane to give 184.5 mg (99% yield) of pure compound **3** as a white solid: R_f = 0.86 (hexane–EtOAc, 19:1); mp 37–38 ºC; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.88 (t, *J* = 7.0 Hz, 3H, H-20), 1.25 (m, 30H, H-4–H-19), 1.33 (t, *J* = 7.7 Hz, 1H, -S*H*), 1.37 (p, *J* = 7.0 Hz, 2H, H-3), 1.61 (p, J = 7.4 Hz, 2H, H-2), 2.52 (q, *J* = 7.4 Hz, 2H, H-1); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 14.12 (C-20), 22.69 (C-19), 24.66 (C-1), 28.38 (C-3), 29.07 (C-4), 29.36 (C-17), 29.52 (C-5), 29.59 (C-6), 29.69 (C-8 – C16), 31.92 (C-18), 34.06 (C-2). Anal. Calcd for ($C_{22}H_{44}OS$): C, 76.35; H, 13.46. Found C, 76.09; H, 13.46.

1,20-Dibromoeicosane (6). A mixture of 1,8-dichlorooctane (**4**; 1.538 g, 8.4 mmol), iodine (20 mg) and magnesium turnings (408.3 mg 16.8 mmol) in anhydrous tetrahydrofuran (20 mL) was refluxed for 3 h under an argon atmosphere. The reaction mixture was allowed to reach room temperature. Then, the resulting Grignard reagent was slowly cannulated to a solution of 1,6-dibromohexane (**5**; 4.760 g, 19.5 mmol) in tetrahydrofuran (10 mL) in the presence of Li_2CuCl_4 (2 mL, 0.1*M* tetrahydrofuran solution) cooled at 0 ºC. The mixture was refluxed for 22 h. The reaction was monitored by gas chromatography employing an Alltech Heliflex column 60 m × 0.25 mm bonded RSL-150 0.25 μm. Samples were

injected at the initial temperature of 100 ºC at a rate of 10 ºC/min to reach 280 ºC. The retention time for the title compound was 48.09 min. The reaction was quenched by addition of an aqueous saturated solution of ammonium chloride (100 mL). The mixture was extracted with methylene chloride (3 × 50 mL) and the combined organic layers were washed with brine (2 × 100 mL), dried ($MgSO_4$), and the solvent was evaporated. The product was purified by column chromatography (silica gel) eluting with hexane to afford 900 mg (24% yield) of pure compound **6** as a white solid: R_f 0.56 (hexane); mp 63–65 ºC; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.25 (m, 28H, H-4 – H-17), 1.42 (p, *J* = 7.3 Hz, 4H, H-3, H-18), 1.86 (p, *J* = 7.3 Hz, 4H, H-2, H-19), 3.41 (t, *J* = 7.0 Hz, 4H, H-1, H-20); ^{13}C NMR (125.77 MHz, CDCl3) δ 28.17 (C-3, C-18), 28.77 (C-4, C-17), 29.43 (C-5, C-16), 29.54 (C-6, C-15), 29.61 (C-7, C-14), 29.64 (C-8, C-13), 29.67 (C-9, C-10, C-11, C-12), 32.84 (C-2, C-19), 34.08 (C-1, C-20); MS (*m/z*, relative intensity) 361 (1, M^+ – Br), 359 (1, M^+ – Br), 193 (3), 191 (3), 179 (4), 177 (4), 165 (5), 163 (5),151 (11), 149 (11), 137 (48), 135 (53), 55 (100). Anal. Calcd for ($C_{20}H_{40}Br_2$): C, 54.55; H, 9.16. Found C, 55.19; H, 9.19.

20-Bromoeicosyl Acetate (7). A solution of compound **6** (360 mg, 0.82 mmol) in anhydrous *N*,*N*-dimethylformamide (10 mL) under argon atmosphere was treated with anhydrous sodium acetate (70 mg, 0.84 mmol). The reaction mixture was stirred at 80 ºC for 2 h. The reaction was worked up as described for compound **2**. The residue was purified by column chromatography (silica gel) employing a mixture of hexane–EtOAc (99:1) as eluent to afford 210 mg (61% yield) of pure compound **7** as a waxy solid: R_f = 0.52 (hexane); mp 41–43 ºC; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.25 (m, 30H, H-4 – H-18), 1.42 (p, *J* = 7.3 Hz, 2H, H-3), 1.62 (p, *J* = 7.1 Hz, 2H, H-2), 1.85 (p, *J* = 7.2 Hz, 2H, H-19), 2.05 (s 3H, COCH_3), 3.41 (t, *J* = 6.8 Hz, 2H, H-20), 4.05 (t, *J* = 6.8 Hz, 2H, H-1); ^{13}C NMR (125.77 MHz, CDCl3) δ 21.02 (CO*C*H_3), 25.90 (C-3), 28.17 (C-18), 28.59 (C-17), 28.76 (C-2), 32.83 (C-19), 34.08 (C-20), 64.67 (C-1), 171.27 (CO).

20-(Acetylthio)eicosyl acetate (8). A solution of compound **7** (194 mg, 0.46 mmol) in anhydrous *N*,*N*-dimethylformamide (5 mL) under argon atmosphere was treated with potassium thioacetate (106 mg, 0.93 mmol). The reaction mixture was stirred at 90 ºC for 3 h. The reaction was quenched as depicted for compound **2**. The residue was purified by column chromatography employing a mixture of hexane–EtOAc (19:1) to give 162 mg

(85% yield) of pure compound **8** as a white solid. R_f 0.34 (hexane–EtOAc, 9:1); mp 56–57 ºC; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.25 (m, 28H H-4–H-17), 1.34 (m, 4H, H-3, H-18), 1.56 (p, *J* = 7.2 Hz, 2H, H-2), 1.62 (p, *J* = 6.6 Hz, 2H, H-19), 2.05 (s, 3H, OC(O)C*H*$_3$), 2.32 (s, 3H, SC(O)C*H*$_3$), 2.86 (t, *J* = 7.3 Hz, 2H, H-20), 4.05 (t, *J* = 6.9 Hz, 2H, H-1); ^{13}C NMR (125.77 MHz, CDCl3) δ 21.02 (OC(O)*C*H$_3$), 25.89 (C-3), 28.58 (C-18), 28.81 (C-2), 29.11 (C-20), 29.15 (C-16), 29.24 (C-4), 29.47 (CH_2), 29.48 (CH_2), 29.56 (CH_2), 29.67 (CH_2), 29.51 (CH_2), 29.66 (CH_2), 29.67 (CH_2), 30.63 (SC(O)*C*H$_3$), 64.67 (C-1), 171.27 (O*C*(O)CH$_3$), 196.11 (S*C*(O)CH$_3$). HRMS (ESI) Cald for ($C_{24}H_{46}O_3SNa$) $[M+Na]^+$ = 437.3065. Found: 437.3046.

20-Mercaptoeicosan-1-ol (9) To a mixture of lithium aluminum hydride (76 mg, 20 mmol) in anhydrous tetrahydrofuran (4.0 mL) cooled at 0 ºC was added a solution of compound **8** (50 mg, 0.12 mmol) in tetrahydrofuran (4.0 mL) under an argon atmosphere. The reaction mixture was stirred at room temperature for 3 h. The reaction was worked up as depicted for compound **3**. The product was purified by column chromatography (silica gel) eluting with a mixture of hexane–EtOAc (9:1) to afford 39 mg (98% yield) of pure compound **9** as a white solid. R_f 0.28 (hexane–EtOAc, 4:1); mp 62–64 ºC; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.25 (m, H), 1.33 (t, *J* = 7.7 Hz, 1H, S*H*), 1.61 (p, *J* = 7.4 Hz, 2H, H-19), 2.52 (t, *J* = 7.4 Hz, 2H, H-20), 3.64 (t, *J* = 6.5 Hz, 2H, H-1); ^{13}C NMR (125.77 MHz, CDCl3) δ 24.66 (C-20), 25.73 (C-3), 28.38 (C-18), 29.07 (C-17), 29.43 (C-16), 29.51 (C-15), 29.67 (C-4–C-14), 32.80 (C-2), 34.05 (C-19), 63.11 (C-1). HRMS (ESI) Cald for ($C_{20}H_{42}OSNa$) $[M+Na]^+$ = 353.2854. Found: 353.2847.

References

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