Are the pKa values of free fatty acids in aqueous solution abnormally high? An NMR and computational perspective

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ELECTRONIC SUPPLEMENTARY MATERIAL (ESI)

Methods

Materials

Caproleic acid, purity ≥ 96% and oleic acid, purity ≥ 99% (GC) were purchased from Sigma-Aldrich Chemie Gmbh (Germany) and were used without further purification. D_2O (99.95%) and 3-(trimethylsilyl) propionic-2,2,3,3,-d_4 acid sodium salt (TMSP-d_4) (99%) were obtained from Deutero GMBH (Kastellaum, Germany).

Samples preparation for NMR experiments

To avoid the overwhelming H_2O resonance, D_2O (99.95%) was used as a solvent. Commercial glass electrodes function properly in D_2O when calibrated with standard aqueous buffers at pH 4 and 7. A correction of 0.40 has been recommended to be added to pH meter readings to get pD values,[12] however, recent studies showed that for pKa (H, D) < 8, no correction is needed for any D_2O content.[13,14] In the present work, therefore, the pH-meter readings (METTLER TOLEDO S400) were used without correction for pKa isotope effects. The pD was adjusted by addition of either DCl or NaOD solution. The pD values were measured before and after each NMR experiment.

NMR experiments of caproleic acid at various pD values were performed at c = 2 mM (well below the CMC) and c = 150 mM (well above the CMC). At c = 2 mM the solution remained unchanged over a period of several weeks at pD values 1.90 to 9.45. At c = 150 mM the solution remained constant for several weeks for pD values 10.0 to 6.0. For pD < 6.0 a second aggregation species was observed. On standing the solution for approximately two weeks at pD = 1.9, the major aggregate was transformed to the second one.

NMR experiments for oleic acid were performed at c = 2 mM which is well above the CMC of ~ 6μM.[15] Investigation of oleic acid in the non-aggregated state would require concentrations below 1 μM which are beyond the sensitivity limits of NMR spectroscopy.

NMR experiments

NMR experiments were performed at 298 K on a Bruker Avance Neo 500 spectrometer (University of Ioannina, Ioannina, Greece) equipped with a broadband inverse probe and a Bruker Avance Neo 800 equipped with a cryogenically cooled probe (Slovenian NMR Centre, National Institute of

Chemistry, Ljubljana, Slovenia). The NMR spectrometers were controlled by the software TopSpin 4.0.9 (Bruker BioSpin, Rheinstetten, Germany). The 2D $^{1}H–^{13}C$ HSQC and HMBC, and the 2D DOSY experiments were carried out using the above software. The experimental time required for the various NMR experiments is reported in the captions of the respective Figures.

In the NMR titration curve, the observed chemical shift, δ_{obs}, is the weighted average of the respective concentrations of the non-ionic δ_{AH} and ionic δ_{A^-} forms as follows:[16]

$$\delta_{obs} = \frac{\delta_{AH}[AH] + \delta_{A^-}[A^-]}{[AH] + [A^-]}$$

which substituted into the Henderson-Hasselbalch equation results in the following equation:

$$\delta_{obs} = \frac{\delta_{AH}10^{-pH} + \delta_{A^-}10^{-pk_a}}{10^{-pH} + 10^{-pk_a}}$$

Plotting the experimental chemical shift against pD values yields a sigmoidal curve, with the infection point representing the pKa value. Non-linear regression analysis of the experimental δ_{obs} vs. pD values, results in the pKa, δ_{AH} and δ_{A^-} values of caproleic acid and oleic acid in Table S1.

DFT calculations

The DFT calculations were performed using the aug-cc-pVDZ basis set, consisting of a primitive set of 10 *s* functions, 5 *p* functions, and 2 *d* functions, contracted to 4*s*, 3*p*, and 2*d* functions, offering balanced accuracy for valence and noncovalent interactions. Solvent effects were included through the IEFPCM polarizable continuum model[11] with water as the dielectric medium. Tight convergence criteria were used in all optimizations, and frequency calculations were performed to confirm the nature of the stationary points and to obtain thermochemical corrections. In implicit solvent models, the counterpoise correction cannot be applied because solvation energy depends on the total electron density and the cavity of the entire system; ghost basis functions do not correspond to a physically meaningful solvated state, making the corrected monomer energies unphysical.[17a-d]

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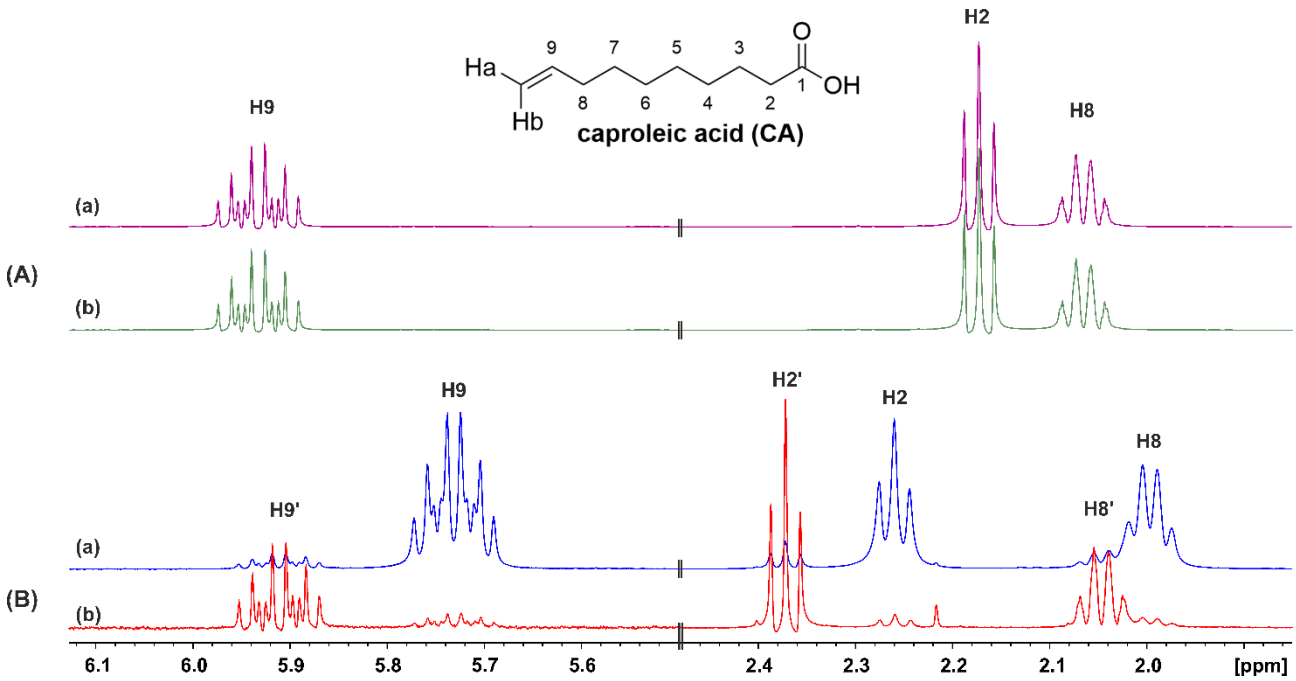

Figure S1. ^{1}H NMR spectra of 150 mM caproleic acid at: (A) pD = 9.3; (b) was recorded after 10 days. (B) pD = 1.9; (b) was recorded after 2 weeks. Experimental time for each NMR spectrum 2 min.

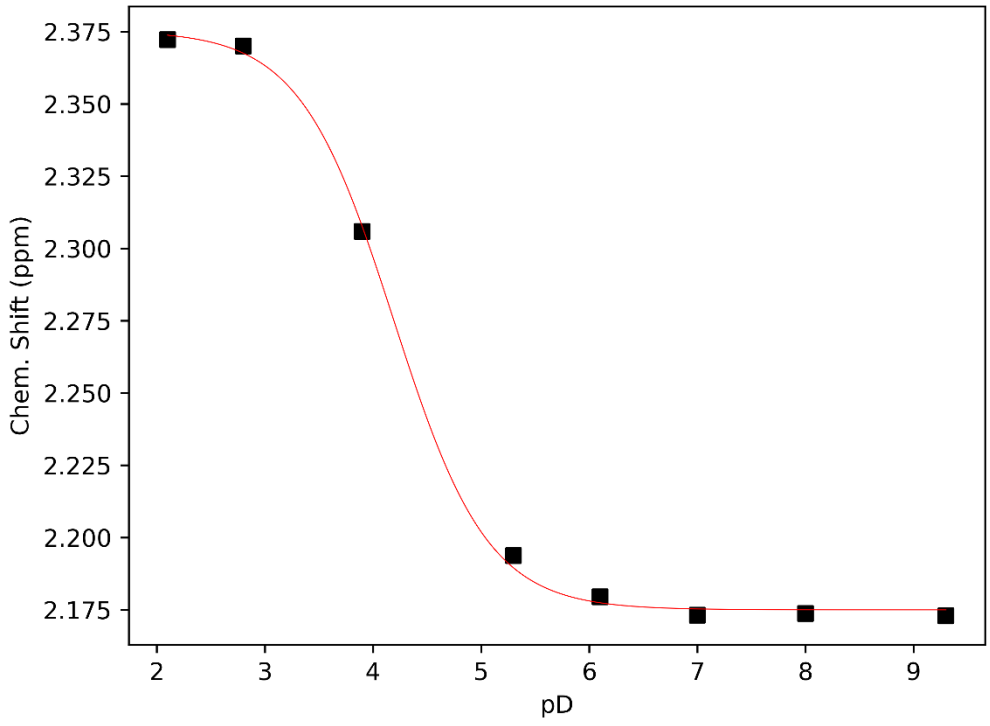

Figure S2. pD dependence of the C$\underline{H_2}$COO protons of caproleic acid (150 mM). Experimental time for each NMR spectrum 2 min.

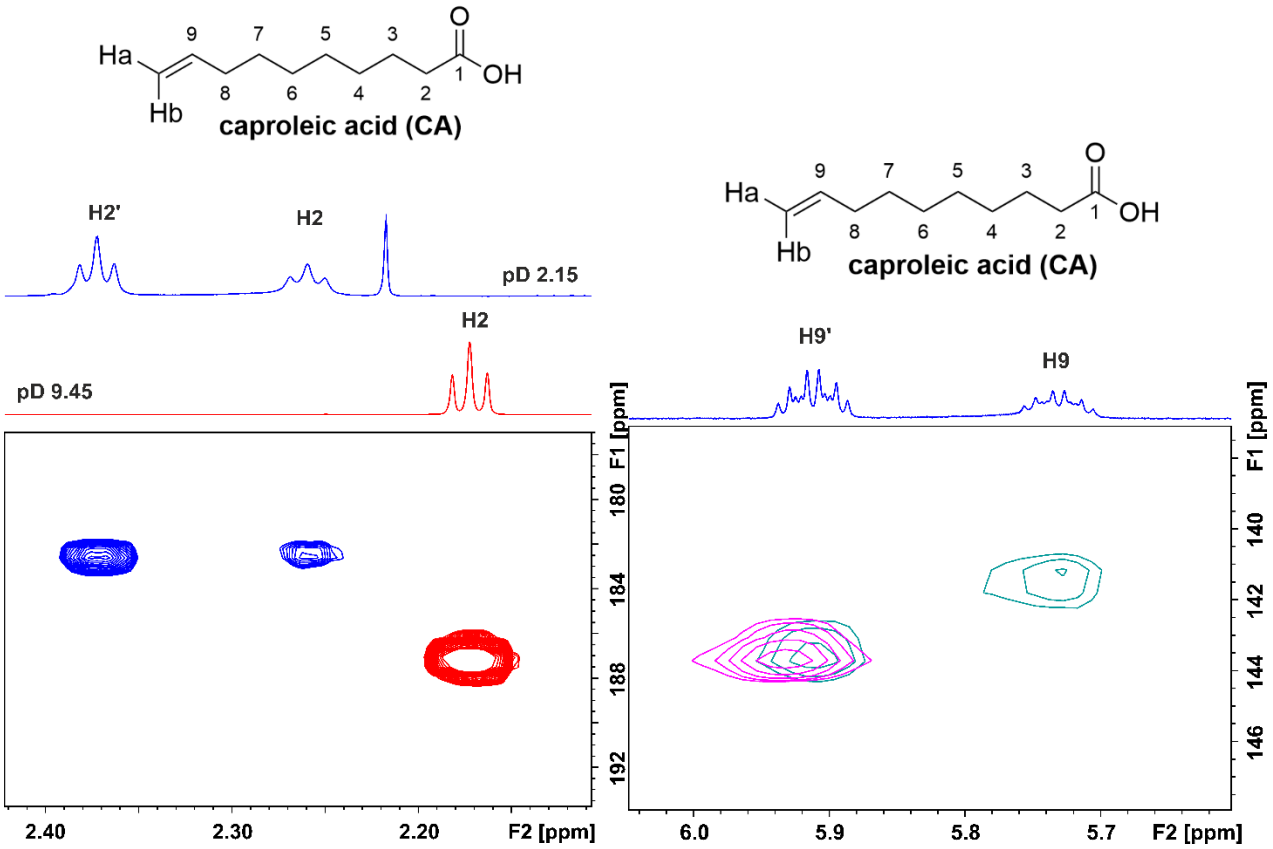

Figure S3. 800 MHz ^{1}H-^{13}C HSQC NMR spectra of 150 mM caproleic acid in D_2O at pD 9.45 (purple) and pD 2.15 (blue) (number of scans = 4, number of increments = 256, T = 298K). Experimental time 29 min.

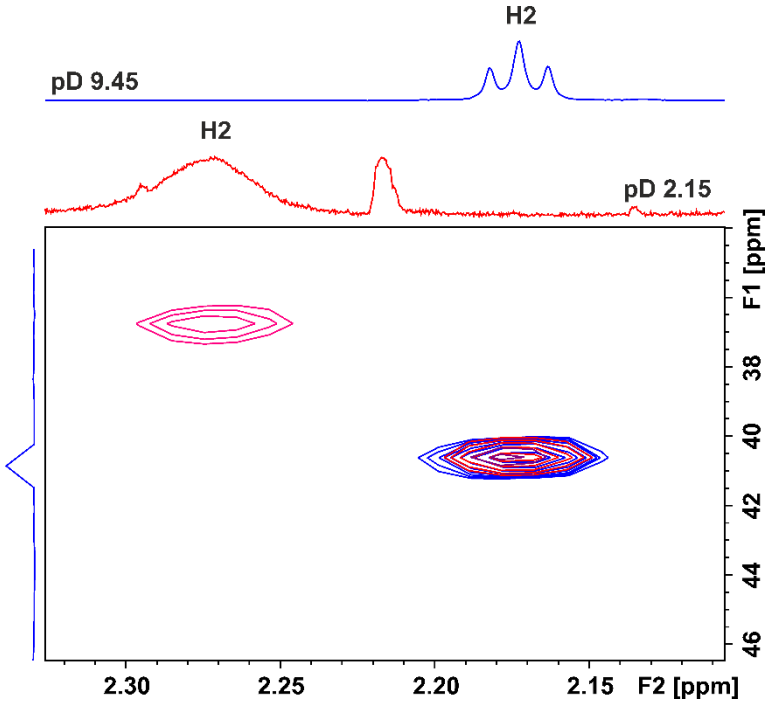

Fig. S4 800 MHz ^{1}H-^{13}C HSQC NMR spectra of 2 mM oleic acid in D_2O at pD 2.1 (red) and pD 9.45 (blue) (number of scans = 16, number of increments = 256, T = 298K). ⇦ denotes an impurity. Experimental time 1 h, 50 min.

Table S1. pKa[a] values and δ_{AH} and δ_{A^-} of caproleic acid (CA) and oleic acid (OA)

Compound (concen.)	**Conc.**	**pKa**	**δ_{AH} (ppm)**	**δ_{A^-} (ppm)**	**$\Delta\delta(\delta_{AH} - \delta_{A^-})$ (ppm)**
caproleic acid	2 mM	3.79±0.05	2.36	2.17	0.19
caproleic acid	150 mM	4.30±0.05	2.37	2.18	0.19
oleic acid	2 mM	3.95±0.08	2.37	2.18	0.19

[a] Average values of three independently prepared solutions.

DFT-ωB97X-D level of theory and aug-cc-pVDZ basis set
Implicit solvation model: IEFPCM polarizable continuum model

Optimized structures

1. Dimer of the oleate anion consistent with the NOE results

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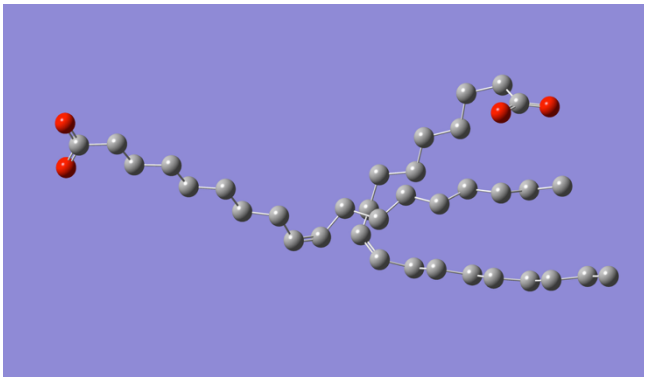
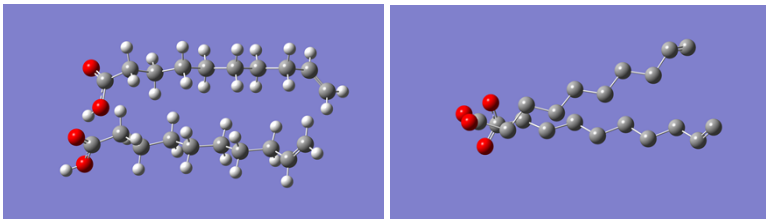
C	12.09400	2.19500	-0.23600
C	10.82400	1.79000	-1.00600
C	9.91500	0.76400	-0.33900
C	8.69400	0.41700	-1.19000
C	7.77200	-0.60900	-0.53500
C	6.56300	-0.97200	-1.39300
C	5.64000	-1.99300	-0.73400
C	4.42800	-2.35500	-1.60200
C	3.56200	-3.40800	-0.96600
C	2.40600	-3.20600	-0.32200
C	1.70200	-1.89300	-0.12500
C	0.46200	-1.75800	-1.01500
C	-0.42000	-0.58000	-0.61700
C	-1.55300	-0.30800	-1.60400
C	-2.61700	0.63700	-1.05500
C	-3.61700	1.10100	-2.11200
C	-4.83300	1.80800	-1.51900
C	-5.73300	2.43500	-2.58000
O	12.83000	3.05100	-0.80300
O	12.31900	1.66300	0.88300
H	10.25900	2.71400	-1.21100
H	11.14700	1.41900	-1.99100
H	10.48700	-0.15300	-0.13300
H	9.58300	1.14500	0.63800
H	8.12100	1.33500	-1.40100
H	9.02800	0.03100	-2.16700
H	8.34600	-1.52300	-0.31300
H	7.42500	-0.21800	0.43600
H	5.98900	-0.05800	-1.62000
H	6.90900	-1.36900	-2.36100
H	6.20900	-2.90800	-0.50400
H	5.28100	-1.59900	0.23000
H	3.84800	-1.44600	-1.80900
H	4.78800	-2.72700	-2.57400
H	3.94400	-4.43200	-1.02000
H	1.88900	-4.07900	0.08900
H	1.38400	-1.81700	0.92400
H	2.37700	-1.04700	-0.31000
H	0.78100	-1.65900	-2.06500
H	-0.13400	-2.68200	-0.95400
H	-0.83700	-0.78500	0.37900
H	0.19300	0.32800	-0.51100
H	-1.13200	0.10600	-2.53400
H	-2.03300	-1.26000	-1.88600
H	-3.15300	0.13500	-0.23400
H	-2.13500	1.51800	-0.60300
H	-3.10500	1.77500	-2.81700
H	-3.95200	0.23600	-2.71000
H	-5.40900	1.08500	-0.92400
H	-4.49700	2.59000	-0.82500
H	-6.63500	2.87300	-2.13300
H	-5.20100	3.23300	-3.11800
H	-6.05300	1.69100	-3.32400
C	-4.30300	4.55900	0.96200
C	-3.75100	4.40300	2.39000
C	-2.51200	3.52000	2.53100
C	-2.80300	2.01900	2.47800
C	-1.53200	1.19100	2.67300
C	-1.75200	-0.29900	2.93400
C	-0.48100	-0.97100	3.45800
C	-0.61800	-2.48200	3.72400
C	-0.38800	-3.33200	2.50200
C	-1.31100	-3.83200	1.67300
C	-2.79400	-3.61600	1.73900
C	-3.32100	-3.00300	0.43800
C	-4.76700	-2.53000	0.53600
C	-5.32800	-2.07300	-0.80600
C	-6.69300	-1.40100	-0.70400
C	-7.23400	-0.94500	-2.05800
C	-8.44400	-0.02200	-1.94600
C	-9.01300	0.38500	-3.30300
O	-3.46800	4.70200	0.02900
O	-5.55900	4.58200	0.83200
H	-3.50600	5.42400	2.72700
H	-4.55400	4.04100	3.04700
H	-1.79100	3.78600	1.74400
H	-2.02500	3.74000	3.49400
H	-3.52200	1.76800	3.27400
H	-3.28800	1.76000	1.52500
H	-0.87500	1.31400	1.79800
H	-0.98000	1.61100	3.53000
H	-2.55000	-0.42500	3.68300
H	-2.10800	-0.79800	2.02100
H	0.34600	-0.79400	2.75300
H	-0.19800	-0.47000	4.39600
H	0.12200	-2.77400	4.48200
H	-1.60600	-2.68100	4.16300
H	0.66000	-3.54700	2.27600
H	-0.96200	-4.42700	0.82300
H	-3.30900	-4.57400	1.91400
H	-3.05300	-2.95800	2.58000



H	-2.68500	-2.15100	0.16100
H	-3.22000	-3.73600	-0.37700
H	-5.39700	-3.33500	0.94700
H	-4.81900	-1.69700	1.25600
H	-4.62100	-1.36800	-1.26800
H	-5.39000	-2.93600	-1.48900
H	-7.41300	-2.08700	-0.23000
H	-6.61200	-0.53200	-0.03200
H	-6.43400	-0.42400	-2.60700
H	-7.49500	-1.82800	-2.66300
H	-9.22700	-0.51800	-1.35100
H	-8.15000	0.87900	-1.38500
H	-9.87400	1.05800	-3.19000
H	-8.25500	0.90600	-3.90500
H	-9.34500	-0.49600	-3.87000

2. Dimer of the caproleic acid



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C	-5.07600	1.52700	0.72700
C	-3.68700	1.23600	1.22100
C	-2.63100	1.65600	0.18400
C	-1.21000	1.58200	0.74300
C	-0.15700	1.67700	-0.36000
C	1.27100	1.84300	0.15400
C	2.29400	1.90200	-0.97800
C	3.74200	2.02300	-0.48700
C	4.69300	2.30100	-1.61500
C	5.63700	1.47100	-2.06500
O	-5.68700	0.86700	-0.10200
O	-5.60600	2.62700	1.26000
H	-3.52600	1.77000	2.16400
H	-3.61300	0.15800	1.41000
H	-2.72200	1.00900	-0.69900
H	-2.83000	2.68300	-0.15400
H	-1.06400	2.39400	1.47100
H	-1.07500	0.64300	1.30200
H	-0.21400	0.77400	-0.99000
H	-0.39900	2.52500	-1.01900
H	1.33800	2.76500	0.75200
H	1.52200	1.01700	0.83600
H	2.20400	1.00300	-1.60600
H	2.05900	2.76000	-1.62800
H	3.80000	2.84600	0.24200
H	4.03500	1.10500	0.04100
H	4.56500	3.26700	-2.11300
H	6.27600	1.74100	-2.90600
H	5.80400	0.49200	-1.61300
H	-6.48000	2.77000	0.86700
C	-3.91300	-2.04700	-0.79000
C	-2.58900	-2.64200	-1.18500
C	-1.53000	-2.10600	-0.20800
C	-0.09500	-2.39700	-0.63400
C	0.90200	-1.96300	0.43800
C	2.35700	-1.99900	-0.01500
C	3.32800	-1.58900	1.08800
C	4.79300	-1.61900	0.63900
C	5.72600	-1.13100	1.70700
C	6.49100	-0.04000	1.62300
O	-4.63400	-2.48600	0.08800
O	-4.17500	-0.91300	-1.44200
H	-2.64600	-3.73400	-1.12600
H	-2.34200	-2.34500	-2.21100
H	-1.65000	-1.01700	-0.11600
H	-1.71700	-2.52900	0.78900
H	0.02700	-3.47000	-0.84400
H	0.11700	-1.86500	-1.57500
H	0.65400	-0.94100	0.76300
H	0.77800	-2.60200	1.32600
H	2.61000	-3.01200	-0.36900
H	2.48600	-1.32700	-0.87900
H	3.07900	-0.57400	1.43500
H	3.20000	-2.25200	1.95800
H	5.05600	-2.65200	0.36300
H	4.91100	-1.00900	-0.26900
H	5.74700	-1.72000	2.63000
H	7.13600	0.26700	2.44700
H	6.49900	0.58500	0.72700
H	-4.88100	-0.40800	-0.97100

3. Dimer of the caprolate anion

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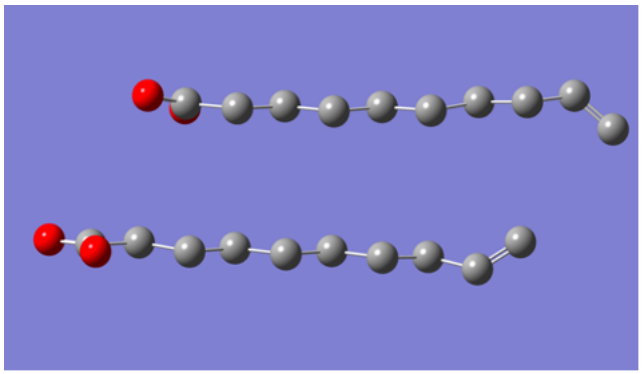

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C	-2.40300	1.41200	0.55900
C	-1.14600	1.81900	-0.20500
C	0.11600	1.82200	0.65200
C	1.36400	2.24400	-0.11800
C	2.61800	2.30900	0.75800
C	3.80800	2.86400	0.02900
C	5.03100	2.32900	0.01900
O	-7.23800	0.55300	0.30400
O	-6.19500	1.18900	-1.57600
H	-5.06200	1.55900	1.32900
H	-4.71300	-0.08600	0.81800
H	-3.49300	0.77300	-1.18900
H	-3.83000	2.42900	-0.70100
H	-2.54600	2.09600	1.41200
H	-2.26100	0.40900	0.98900
H	-1.00600	1.13800	-1.06000
H	-1.29200	2.82300	-0.63600
H	-0.02800	2.50600	1.50500
H	0.27000	0.82100	1.08300
H	1.53500	1.54900	-0.95600
H	1.19000	3.23400	-0.56800
H	2.39900	2.95900	1.62200
H	2.85400	1.31400	1.16100
H	3.63400	3.79100	-0.52700
H	5.85100	2.79700	-0.52700
H	5.25600	1.41000	0.56200
C	-3.17500	-2.41700	0.31200
C	-1.90200	-2.04100	-0.46600
C	-0.60400	-1.99700	0.33100
C	0.60200	-1.61400	-0.52500
C	1.91900	-1.62200	0.24600
C	3.12700	-1.27600	-0.62000
C	4.45500	-1.38800	0.12200
C	5.66400	-1.13200	-0.78500
C	6.96500	-1.19200	-0.04000
C	7.85900	-0.20300	0.03300
O	-3.13400	-2.41600	1.57100
O	-4.18900	-2.69300	-0.38900
H	-1.80400	-2.74500	-1.30500
H	-2.09000	-1.05800	-0.92600
H	-0.70600	-1.28900	1.16500
H	-0.42200	-2.98200	0.78800
H	0.68100	-2.31100	-1.37400
H	0.44000	-0.61700	-0.96300
H	1.85800	-0.91700	1.09000
H	2.06900	-2.62000	0.68900
H	3.14700	-1.94600	-1.49500
H	3.02000	-0.25300	-1.01600
H	4.46800	-0.67900	0.96400
H	4.54400	-2.39300	0.56500
H	5.66700	-1.89400	-1.58100
H	5.56100	-0.15400	-1.27800
H	7.17100	-2.12900	0.48800
H	8.78400	-0.31200	0.59900
H	7.69400	0.75100	-0.47400