

Electronic Supplementary Information

Formation of cationic vesicles by threonine-derived surfactants and gemini surfactants based on conventional or serine-derived headgroups: designing versatile and cytocompatible nanocarriers

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S1. Synthesis of threonine-derived surfactants

A brief description of the synthetic path of the threonine-derived surfactants is presented below.

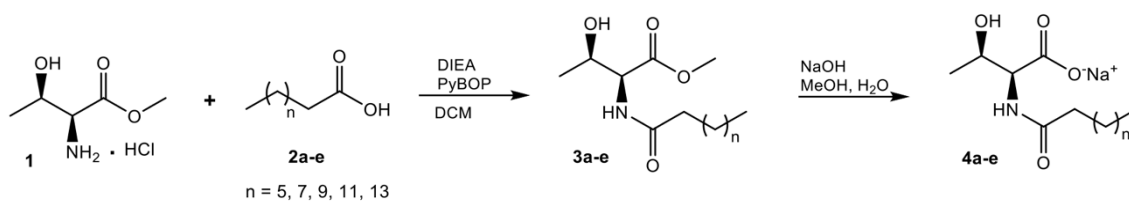


Fig. S.1.1. Synthesis of the threonine-derived surfactants.

Representative procedure for the introduction of the alkyl chain by condensation

N-octanoylthreonine methyl ester (3a): To a solution of **2a** (n=5; 1.3 equiv) in dichloromethane (DCM) at 0 °C and under an Ar atmosphere, 1.5 equiv of the peptide coupling reagent benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) was added, and the mixture was left under stirring for 1 h. Then, a solution of **1** (1 equiv) and N,N-Diisopropylethylamine (DIEA, 3.5 equiv) in DCM was added and the reaction mixture was left with stirring for 48 h. The solvent was removed under reduced pressure and the crude taken in ethylacetate (AcOEt). Washing cycles of HCl (1M), H₂O, NaHCO₃, H₂O were performed until removal of unwanted by-products. The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated. The crude product was submitted to column chromatography on silica gel (hexane/ethyl acetate, 1:2) to afford **3a** as a waxy white compound with an average yield of 75%.

Representative procedure for the hydrolysis of the esters

The sodium salts were readily obtained by saponification of the corresponding esters with sodium hydroxide at room temperature. Thus, the acylated compound (**3a-e**) was dissolved in the minimum volume of methanol and 2/3 drops of water. NaOH was added and the mixture was left to react for 36-48 h. The solvent was evaporated under reduced pressure and the desired product (**4a-e**) isolated by recrystallization from ethyl ether or chloroform with an average yield of 80%.

S2. Spectroscopic data of Thr surfactants

^1H and ^{13}C NMR spectra were recorded with a Bruker Avance III 400 or AMX500 spectrometer. Chemical shifts (δ) are presented in ppm relative to tetramethylsilane (TMS; $\delta = 0$ ppm) as internal standard for CDCl_3 . Coupling constants (J) are in Hertz (Hz). ESI mass spectra were recorded with a Finnigan Surveyor instrument, equipped with a Finnigan LCQ DECA XP MX (Finnigan Corp. San Jose, Calif. USA) mass detector and API (Atmospheric Pressure Ionization) using an electrospray ionization (ESI) interface. The samples were introduced by direct injection and the spectra were obtained in positive mode (m/z range: 50 – 1000).

C8Thr – *N*-octanoylthreonine methyl ester, **3a**

^1H NMR (400 MHz, CDCl_3) δ 6.30 (d, $J = 8.6$ Hz, NH), 4.60 (dd, $J = 8.9, 2.5$ Hz, 1H), 4.37 - 4.30 (m, 1H), 3.76 (s, 3H), 2.54 (brs, -OH), 2.27 (t, $J = 7.6$ Hz, 2H), 1.65 (quint, $J = 7.4$ Hz, 2H), 1.37 – 1.22 (m, 8H), 1.20 (d, $J = 6.4$ Hz, 3H), 0.87 (t, $J = 6.9$ Hz, 3H).

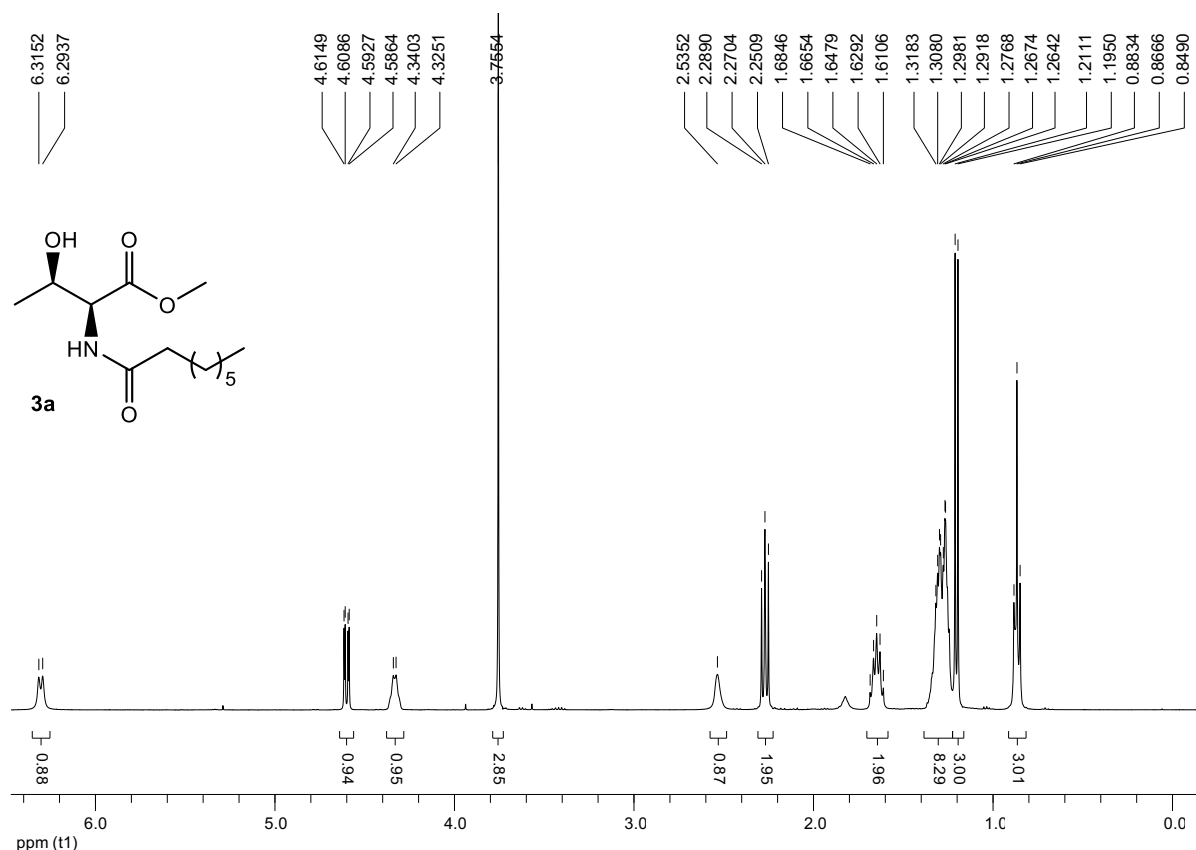


Figure S2.1 – ^1H NMR spectrum of compound **3a**.

C8ThrNa – *N*-octanoylthreonine, sodium salt, 4a

¹H NMR (400 MHz, MeOD) δ 4.28 (d, $J = 3.4$ Hz, 1H), 4.18 (dq, $J = 6.4$, $J = 3.4$ Hz, 1H), 2.29 (dt, $J = 7.6$, $J = 1.6$, 2H), 1.64 (quint, $J = 7.2$ Hz, 2H), 1.40 – 1.26 (m, 8H), 1.13 (d, $J = 6.4$ Hz, 3H), 0.90 (t, $J = 6.9$ Hz, 3H).

¹³C NMR (101 MHz, MeOD) δ 177.4 (C=O), 175.8 (C=O), 69.3 (CH), 60.5 (CH), 37.5 (CH₂), 32.9 (CH₂), 30.4 (CH₂), 30.2 (CH₂), 27.0 (CH₂), 23.7 (CH₂), 20.2 (CH₃), 14.4 (CH₃). HRMS (ESI, MeOH): calcd. 268.15193 [M + H]⁺; found 268.15173.

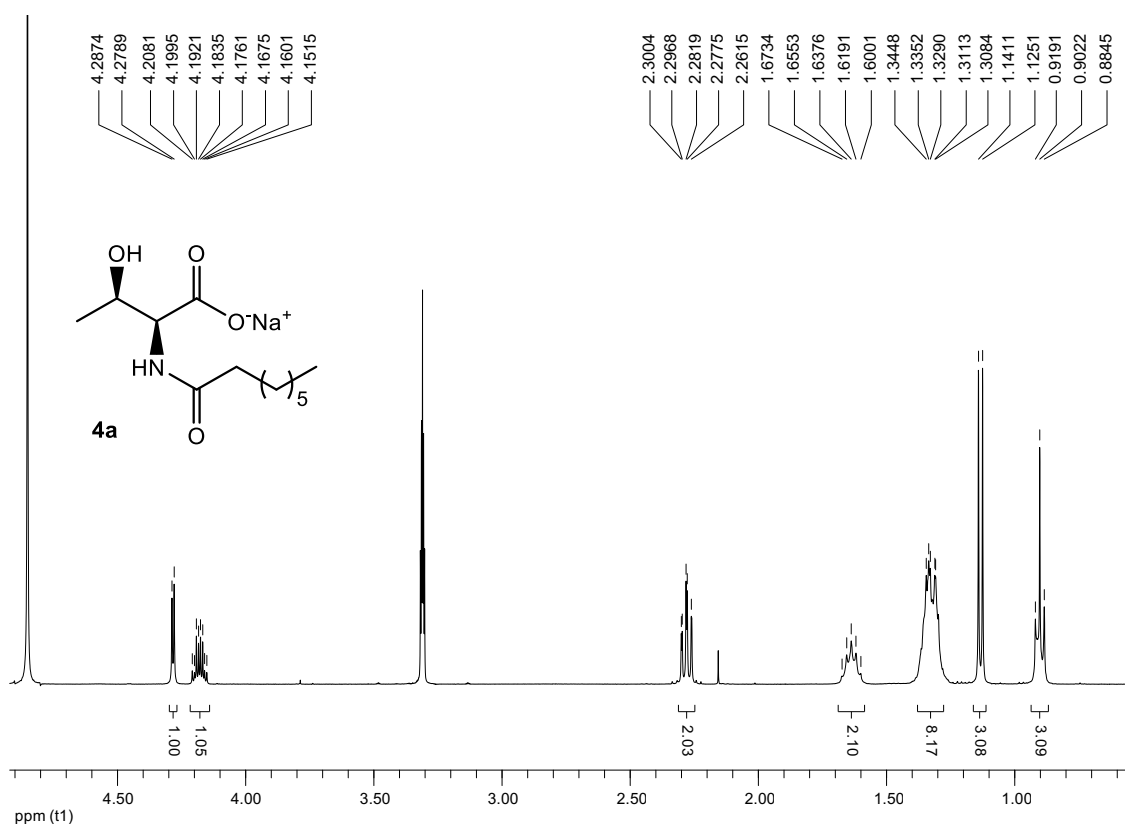


Figure S2.2 – ¹H NMR spectrum of compound 4a.

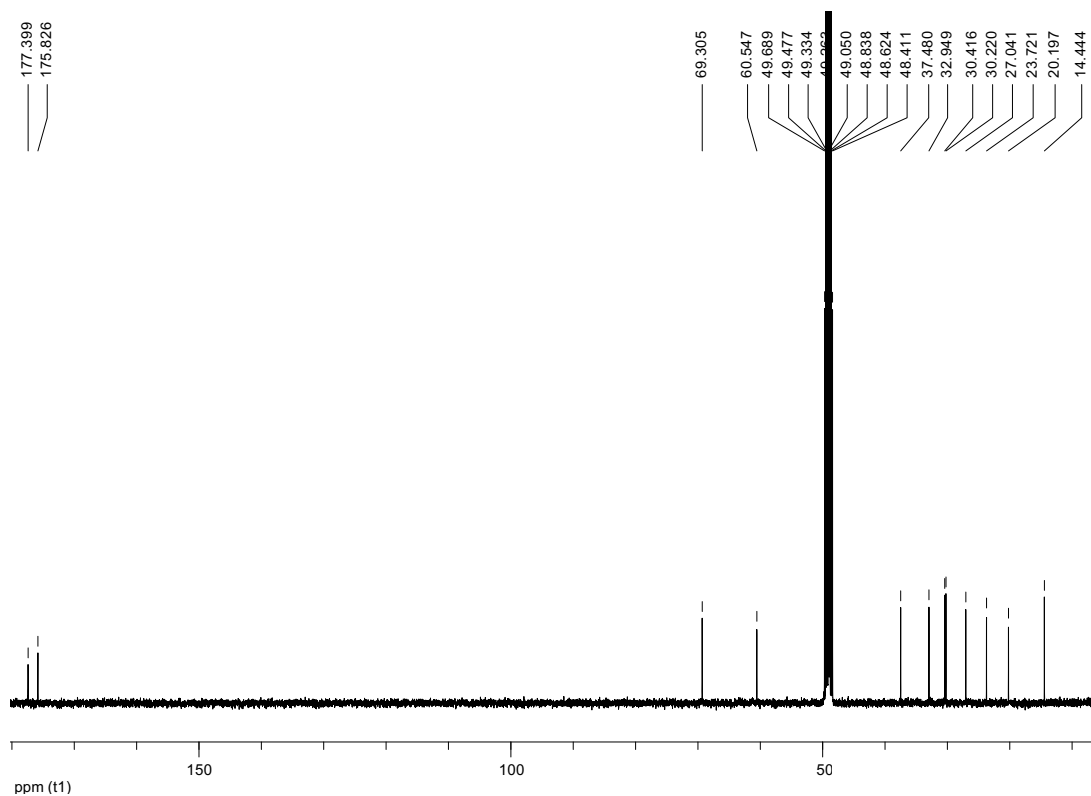


Figure S2.3 – ^{13}C NMR spectrum of compound **4a**.

C10Thr – *N*-decanoylthreonine methyl ester, 3b

^1H NMR (400 MHz, CDCl_3) δ 6.20 (d, $J = 8.6$ Hz, NH), 4.62 (dd, $J = 8.8$, $J = 2.5$ Hz, 1H), 4.38 - 4.31 (m, 1H), 3.77 (s, 3H), 2.28 (t, $J = 7.6$, 2H), 2.18 (brs, -OH), 1.70-1.60 (m, 2H), 1.36 – 1.23 (m, 12H), 1.22 (d, $J = 6.4$ Hz, 3H), 0.87 (t, $J = 6.9$ Hz, 3H).

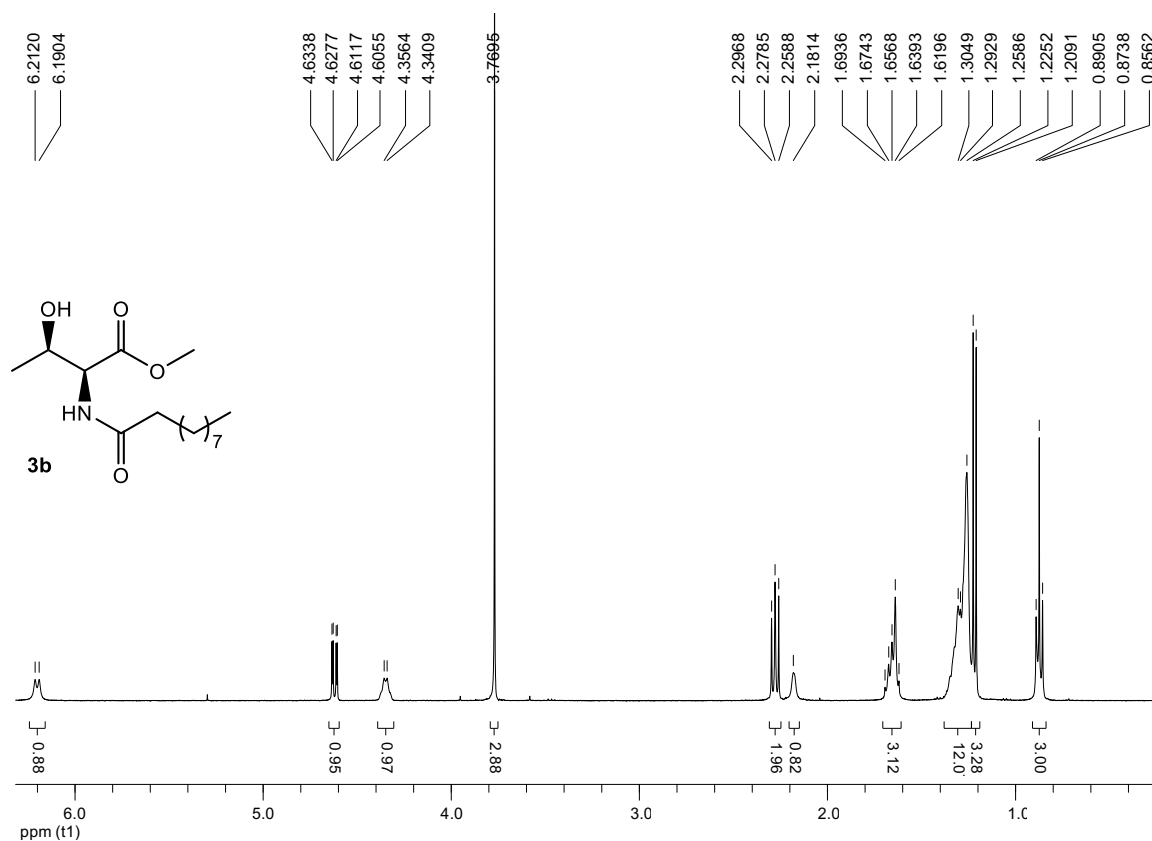


Figure S2.4 – ^1H NMR spectrum of compound **3b**.

C10ThrNa – *N*-decanoylthreonine, sodium salt, **4b**

^1H NMR (400 MHz, MeOD) δ 4.28 (d, $J = 3.4$ Hz, 1H), 4.19 (dq, $J = 6.4$, $J = 3.4$ Hz, 1H), 2.28 (dt, $J = 7.4$, $J = 1.8$ Hz, 2H), 1.63 (quint, $J = 7.1$ Hz, 2H), 1.40 – 1.24 (m, 12H), 1.13 (d, $J = 6.4$ Hz, 3H), 0.90 (t, $J = 6.9$ Hz, 3H).

^{13}C NMR (101 MHz, MeOD) δ 177.4 (C=O), 175.8 (C=O), 69.3 (CH), 60.5 (CH), 37.4 (CH₂), 33.0 (CH₂), 30.6 (CH₂), 30.5 (CH₂), 30.4 (CH₂), 26.9 (CH₂), 23.8 (CH₂), 20.1 (CH₃), 14.4 (CH₃). HRMS (ESI, MeOH): m/z calcd. 296.18323 [M +H]⁺; found 296.18414.

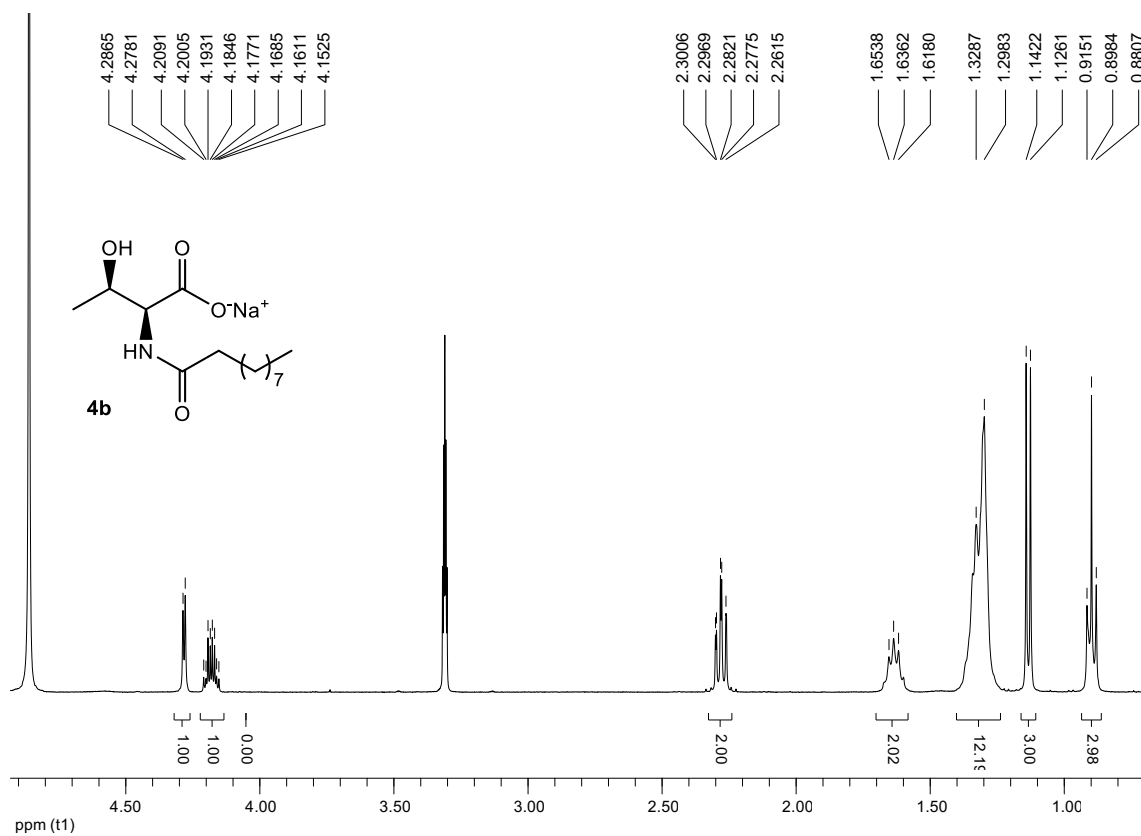


Figure S2.5 – ¹H NMR spectrum of compound **4b**.

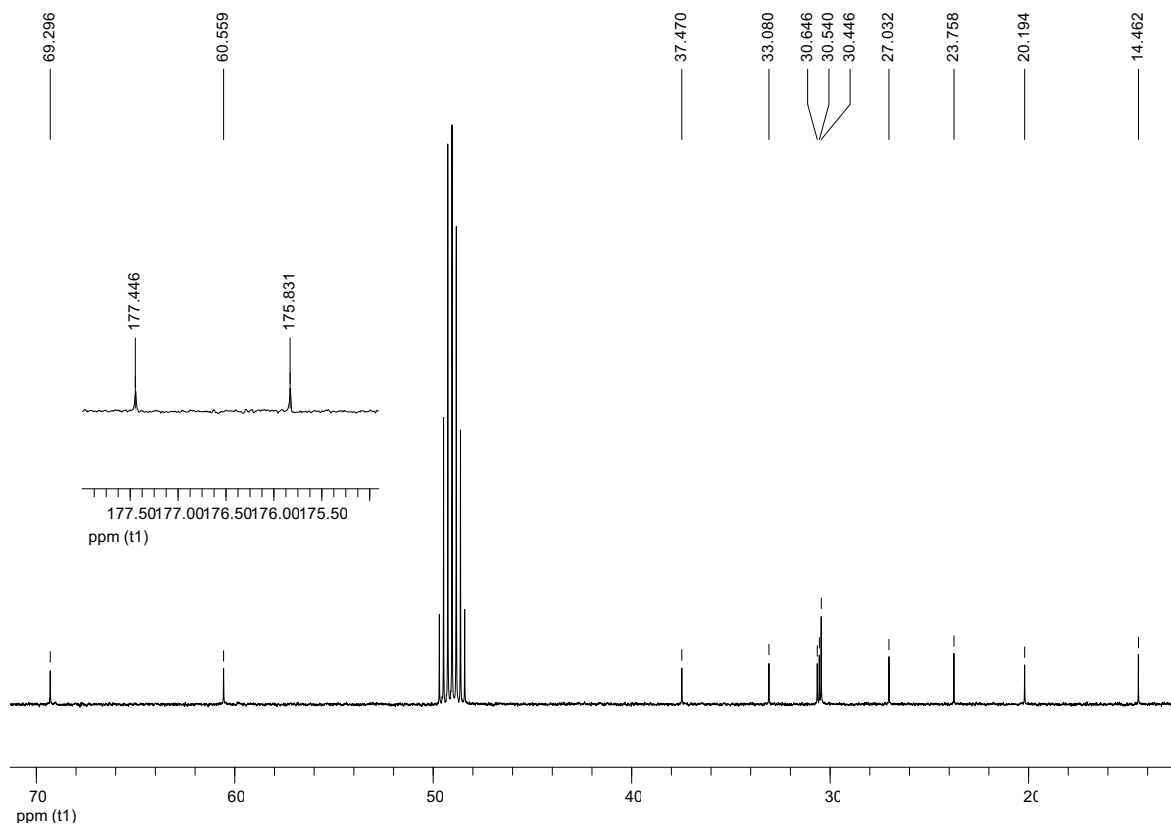


Figure S2.6 – ¹³C NMR spectrum of compound **4b**.

C12Thr – *N*-dodecanoylthreonine methyl ester, **3c**

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 6.17 (t, $J = 8.5$ Hz, NH), 4.62 (dd, $J = 8.8, 2.4$ Hz, 1H), 4.39 - 4.31 (m, 1H), 3.77 (s, 3H), 2.27 (d, $J = 7.6$ Hz, 2H), 2.09 (brs, -OH), 1.66 (quint, $J = 7.4$ Hz, 2H), 1.38 – 1.23 (m, 16H), 1.22 (d, $J = 6.4$ Hz, 3H), 0.88 (t, $J = 6.8$ Hz, 3H).

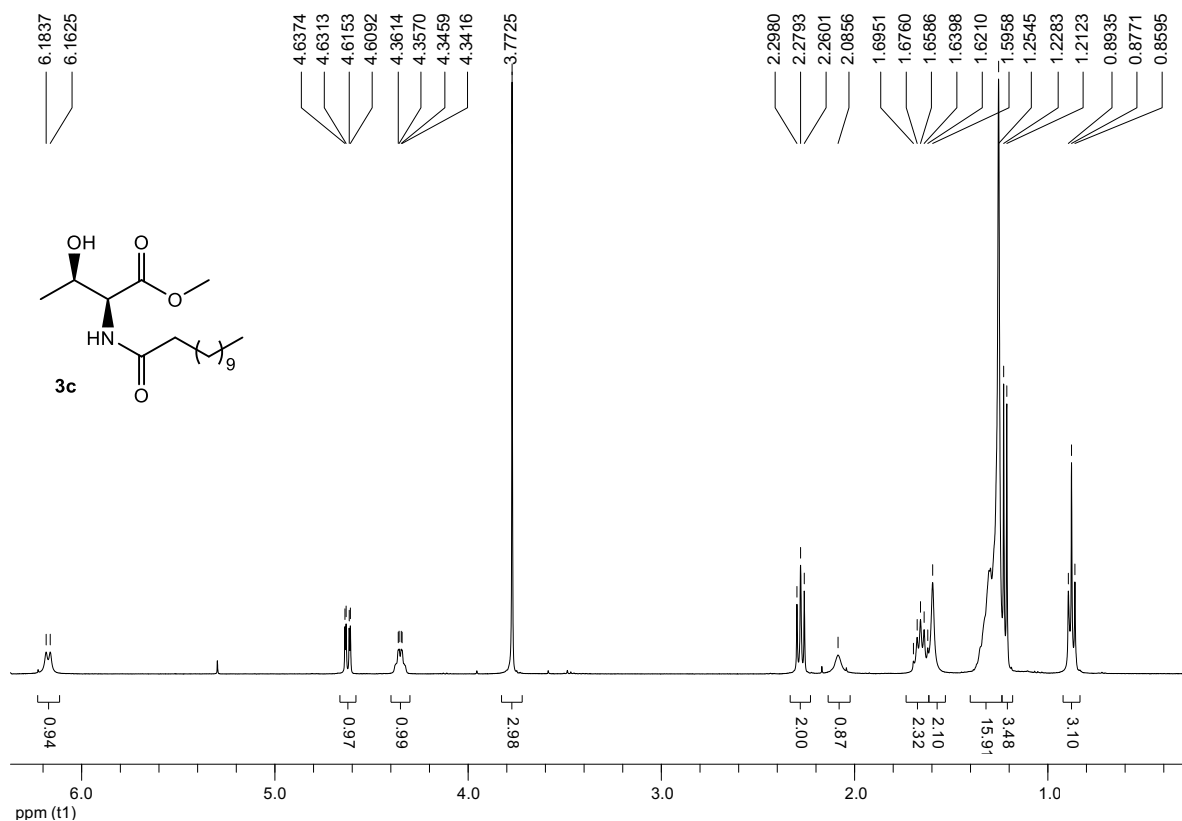
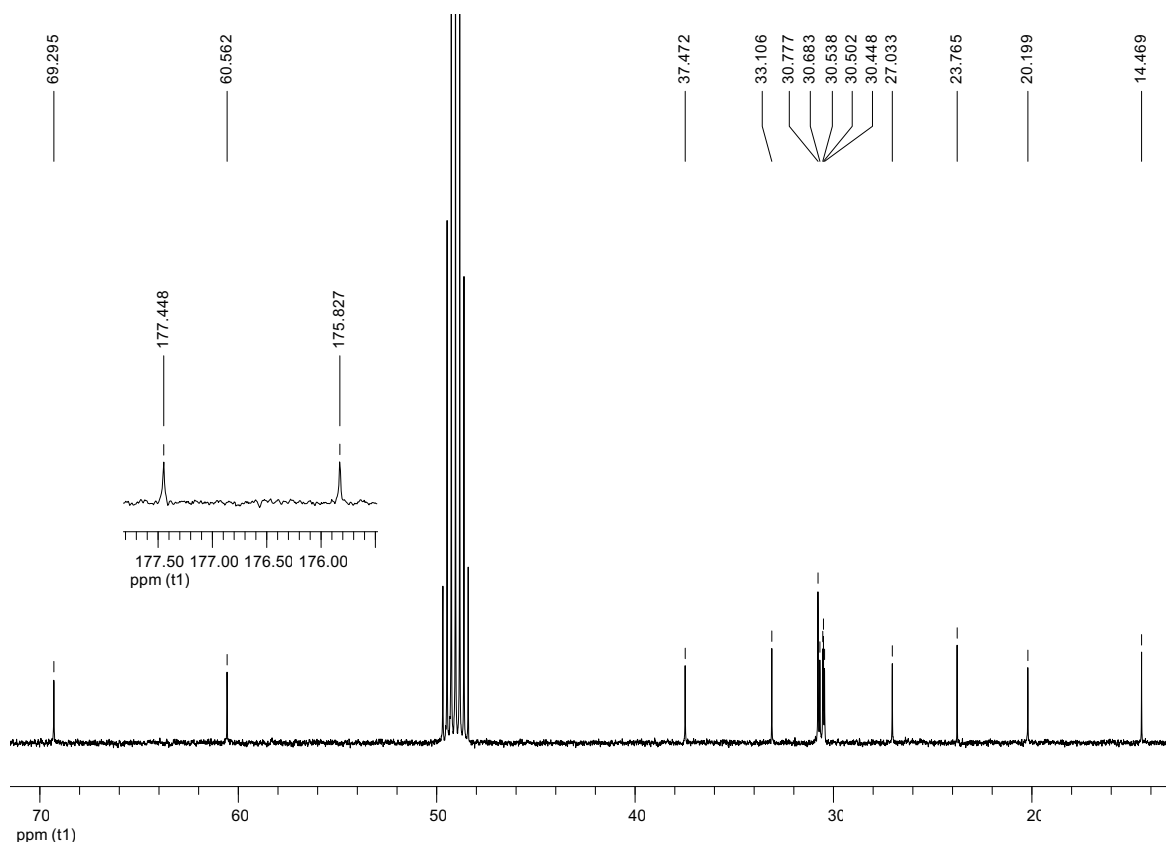
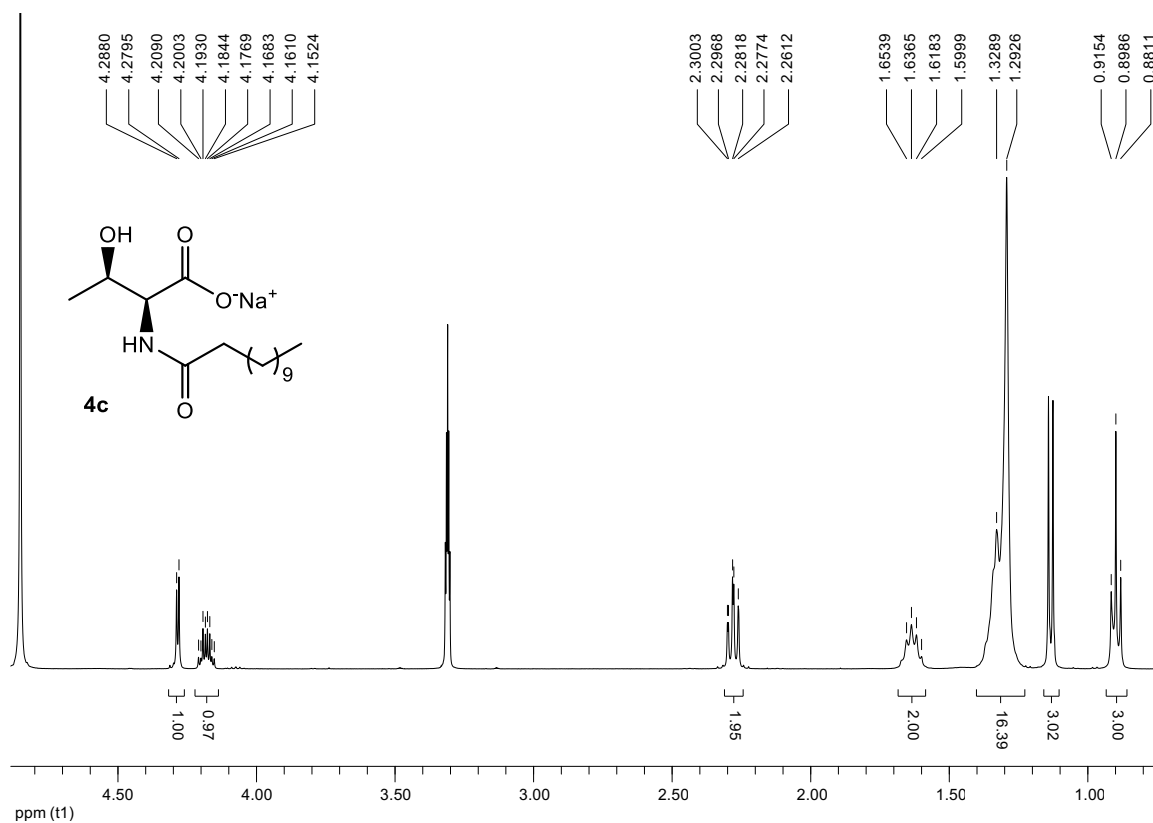


Figure S2.7 – $^1\text{H NMR}$ spectrum of compound **3c**.

C12ThrNa – *N*-dodecanoylthreonine, sodium salt, **4c**

$^1\text{H NMR}$ (400 MHz, MeOD) δ 4.28 (t, $J = 3.4$ Hz, 1H), 4.18 (dq, $J = 6.4, 3.5$ Hz, 1H), 2.28 (dt, $J = 7.6, 1.6$ Hz, 2H), 1.64 (quint, $J = 7.2$ Hz, 2H), 1.40 – 1.23 (m, 16H), 1.13 (d, $J = 6.4$ Hz, 3H), 0.90 (t, $J = 6.9$ Hz, 3H).

$^{13}\text{C NMR}$ (101 MHz, MeOD) δ 177.4 (C=O), 175.8 (C=O), 69.2 (CH), 60.5 (CH), 37.4 (CH₂), 33.1 (CH₂), 30.7 (CH₂), 30.6 (CH₂), 30.5 (3xCH₂), 30.4 (CH₂), 27.0 (CH₂), 23.7 (CH₂), 20.2 (CH₃), 14.4 (CH₃). HRMS (ESI, MeOH): m/z calcd. 324.21453 [$\text{M} + \text{H}$]⁺; found 324.21303.



C14Thr – *N*-tetradecanoylthreonine methyl ester, **3d**

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 6.16 (d, $J = 8.7$ Hz, NH), 4.62 (dd, $J = 8.8, 2.5$ Hz, 1H), 4.01 – 4.31 (m, 1H), 3.77 (s, 3H), 2.28 (dd, $J = 7.8, 7.4$ Hz, 2H), 2.05 (d, $J = 4.7$ Hz, -OH), 1.66 (quint, $J = 7.4$ Hz, 2H), 1.38 – 1.24 (m, 20H), 1.22 (d, $J = 6.4$ Hz, 3H), 0.88 (t, $J = 6.9$ Hz, 3H).

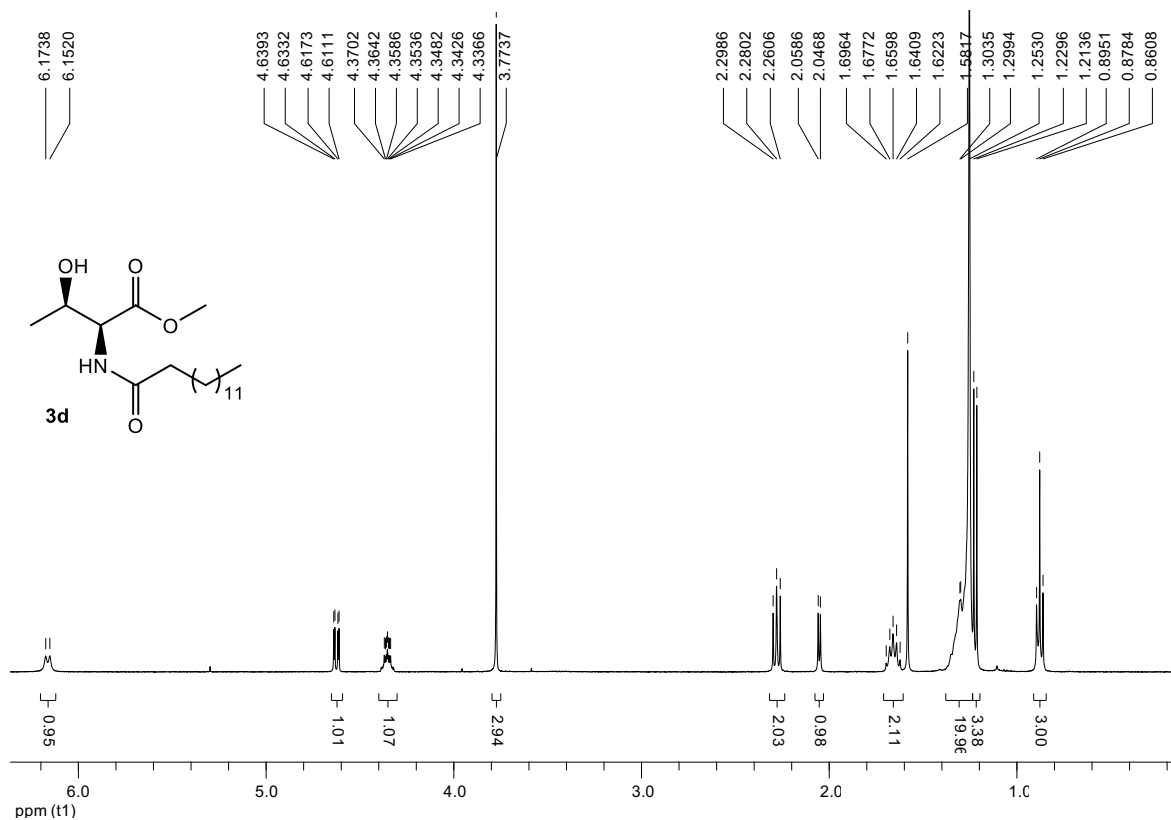


Figure S2.10 – $^1\text{H NMR}$ spectrum of compound **3d**.

C14ThrNa - *N*-tetradecanoylthreonine, sodium salt, **4d**

$^1\text{H NMR}$ (400 MHz, MeOD) δ 4.30 (d, $J = 3.4$ Hz, NH), 4.19 (dq, $J = 6.4, 3.5$ Hz, 1H), 2.32 – 2.26 (m, 2H), 1.71-1.59 (m, 2H), 1.40-1.25 (m, 20H), 1.15 (d, $J = 6.4$ Hz, 3H), 0.91 (t, $J = 6.8$ Hz, 3H).

$^{13}\text{C NMR}$ (101 MHz, MeOD) δ 177.4 (C=O), 175.8 (C=O), 69.2 (CH), 60.5 (CH), 37.4 (CH₂), 33.1 (CH₂), 30.8 (4xCH₂), 30.6 (CH₂), 30.5 (2xCH₂), 30.4 (CH₂), 27.0 (CH₂), 23.7 (CH₂), 20.2 (CH₃), 14.4 (CH₃). HRMS (ESI, MeOH): m/z calcd. 352.24583 [$\text{M} + \text{H}$]⁺; found 352.24589.

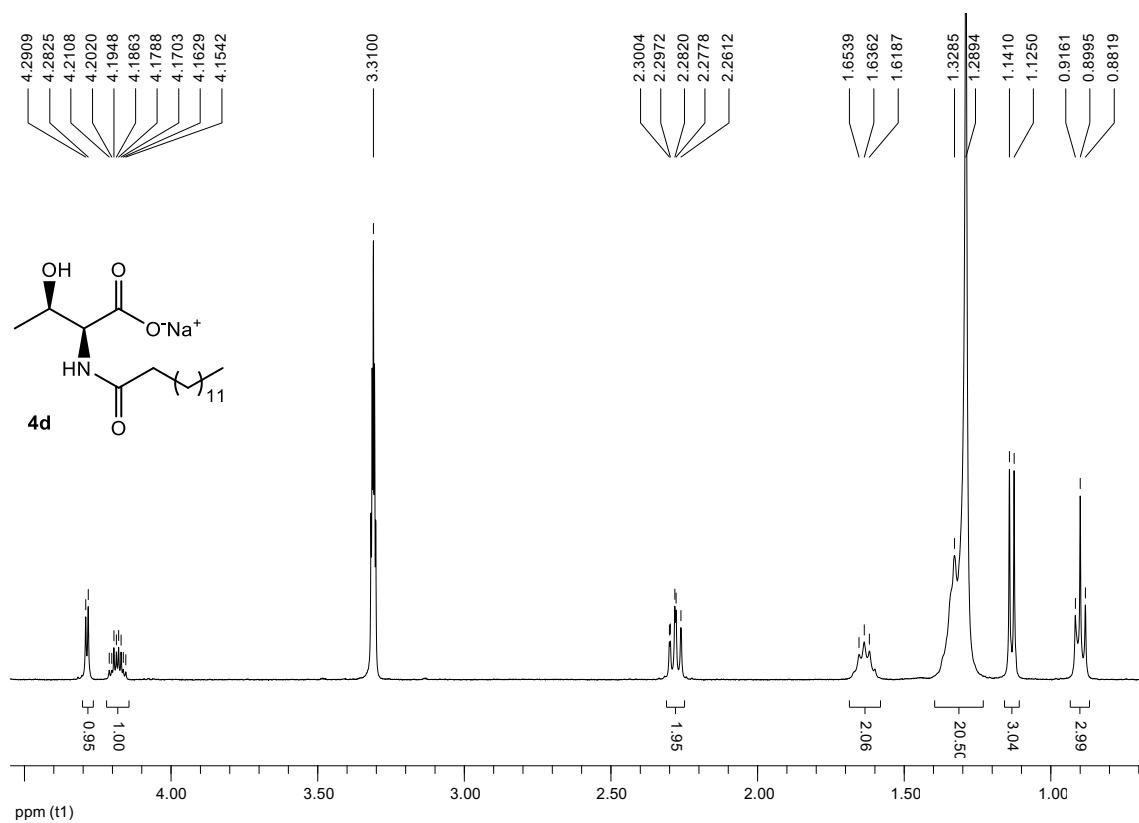


Figure S2.11 – ^1H NMR spectrum of compound **4d**.

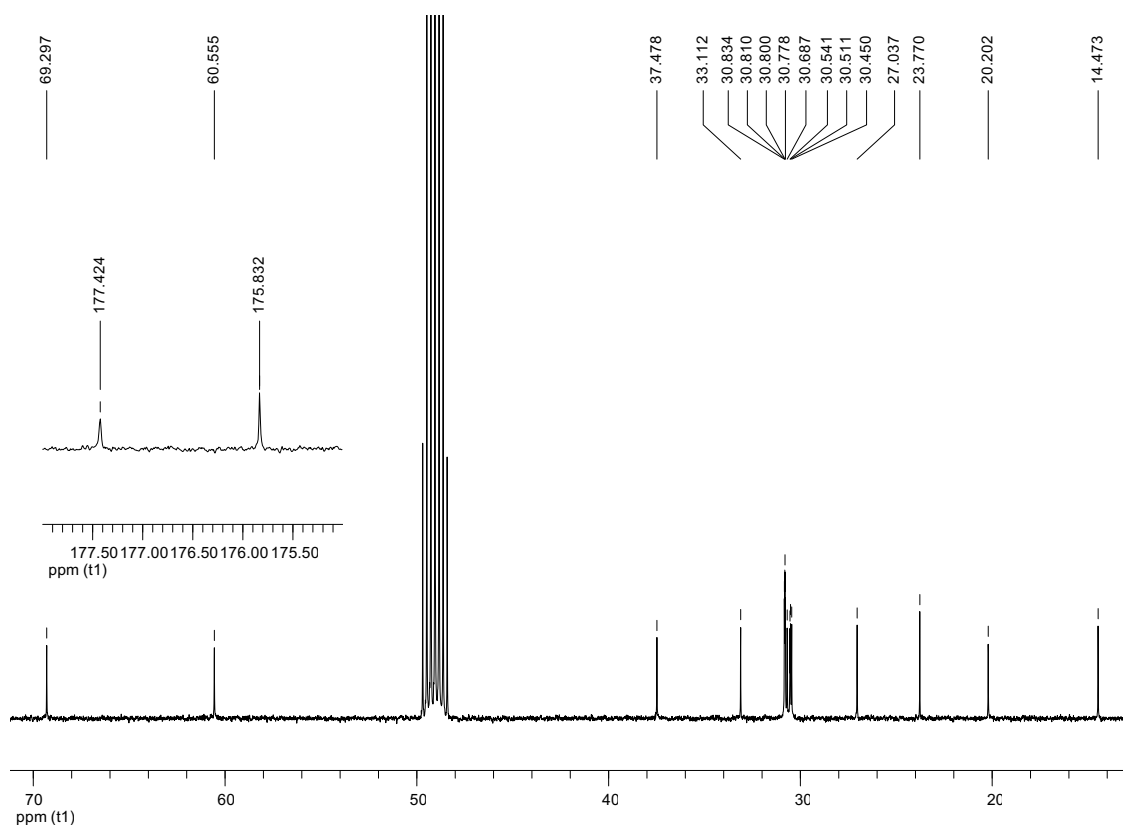


Figure S2.12 – ^{13}C NMR spectrum of compound **4d**.

C16Thr – *N*-hexadecanoylthreonine methyl ester, **3e**

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 6.18 (d, $J = 8.5$ Hz, NH), 4.62 (dd, $J = 8.8, 2.5$ Hz, 1H), 4.39 – 4.31 (m, 1H), 3.77 (s, 3H), 2.28 (dd, $J = 7.8, 7.4$ Hz, 2H), 2.11 (brs, -OH), 1.71 – 1.62 (m, 2H), 1.36 – 1.23 (m, 24H), 1.22 (d, $J = 6.4$ Hz, 3H), 0.88 (t, $J = 6.9$ Hz, 3H).

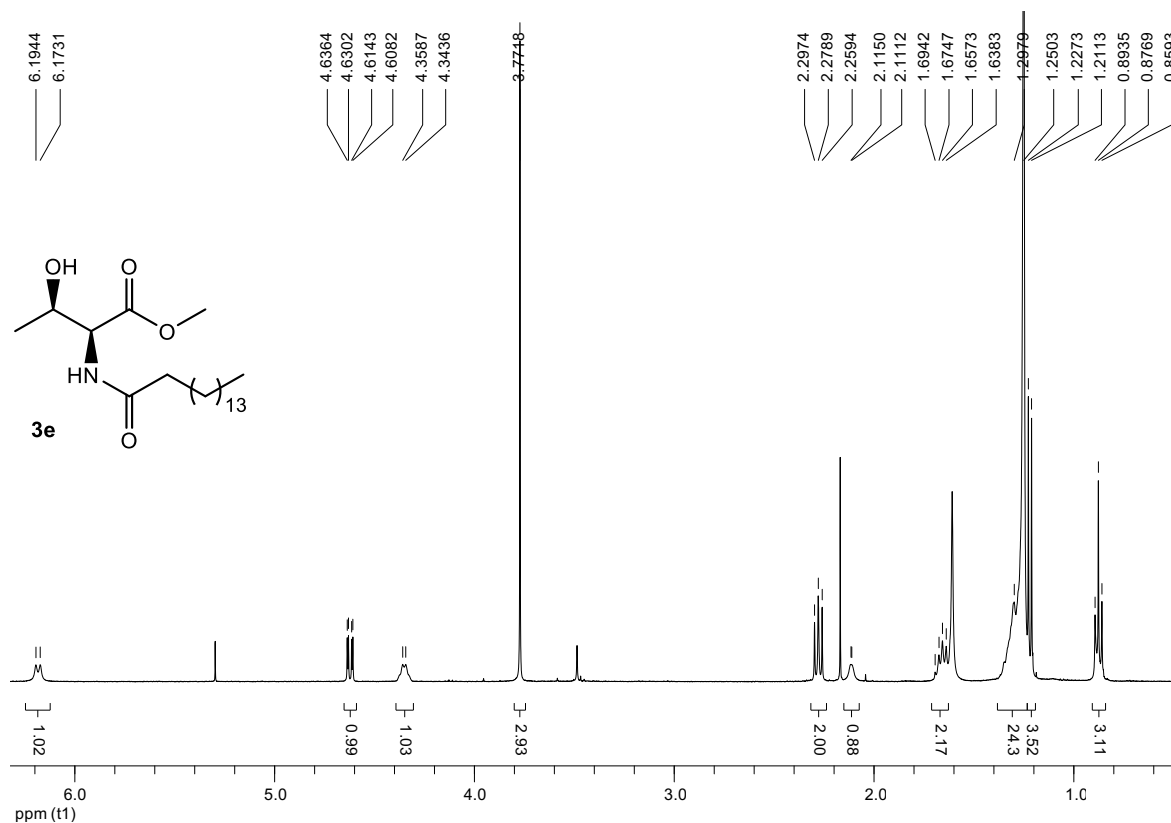


Figure S2.13 – $^1\text{H NMR}$ spectrum of compound **3e**.

N-hexadecanoylthreonine, sodium salt, **4e**

$^1\text{H NMR}$ (400 MHz, MeOD) δ 4.29 (d, $J = 3.4$ Hz, 1H), 4.18 (dq, $J = 6.4, 3.4$ Hz, 1H), 2.28 (dt, $J = 7.6, 1.6$ Hz, 2H), 1.69 – 1.58 (m, 2H), 1.40 – 1.24 (m, 24H), 1.13 (d, $J = 6.4$ Hz, 3H), 0.90 (t, $J = 6.9$ Hz, 3H).

$^{13}\text{C NMR}$ (101 MHz, MeOD) δ 177.4 (C=O), 175.8 (C=O), 69.3 (CH), 60.5 (CH), 37.4 (CH_2), 33.1 (CH_2), 30.8 ($5\times\text{CH}_2$), 30.7 (CH_2), 30.6 (CH_2), 30.5 ($2\times\text{CH}_2$), 30.4 (CH_2), 27.0 (CH_2), 23.7 (CH_2), 20.2 (CH_3), 14.4 (CH_3). HRMS (ESI, MeOH): m/z calcd. 37809.27713 $[\text{M} + \text{H}]^+$; found 380.27740.

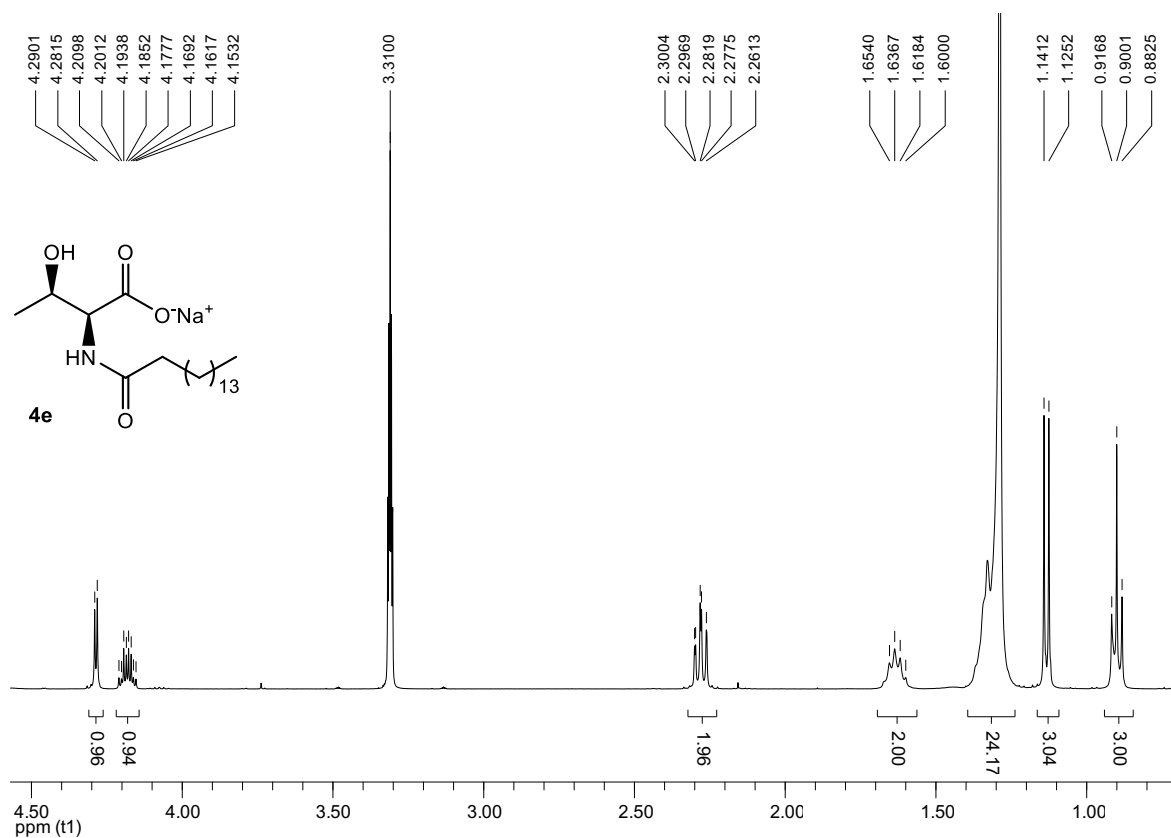


Figure S2.14 – ¹H NMR spectrum of compound **4e**.

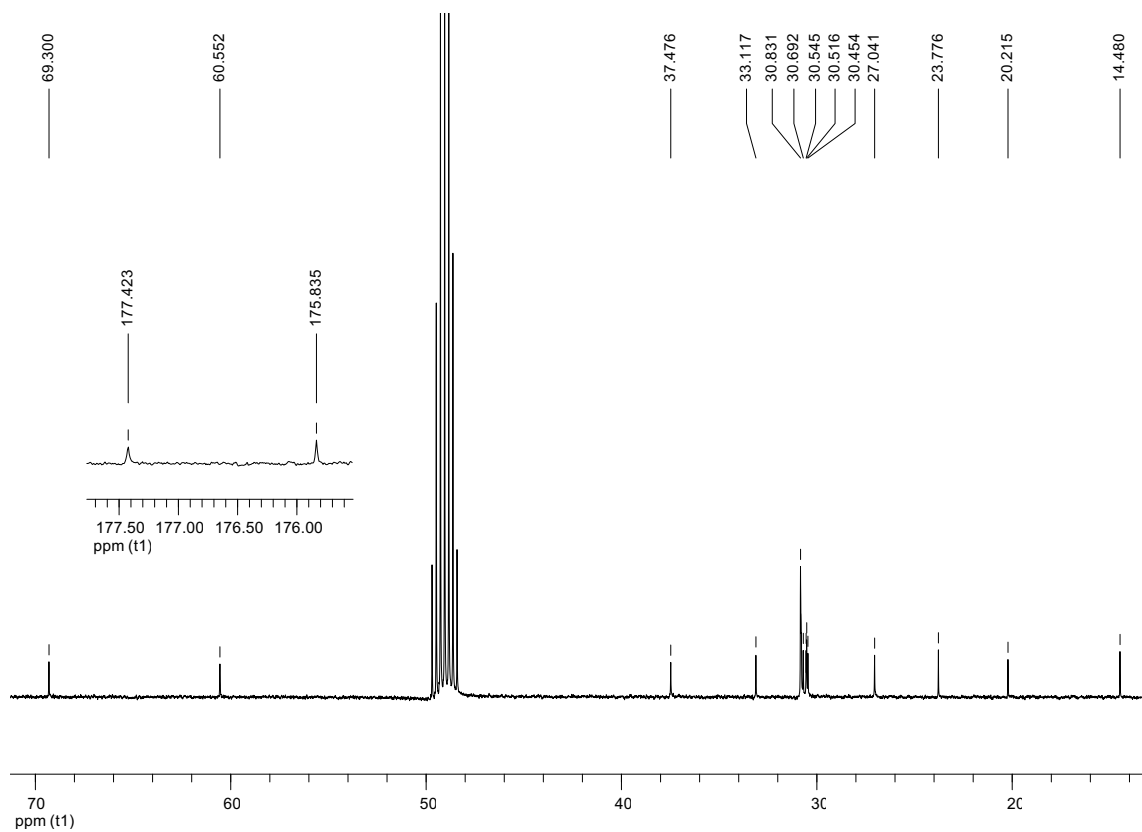


Figure S2.15 – ¹³C NMR spectrum of compound **4e**.

S3. Video-enhanced light microscopy of catanionic mixtures

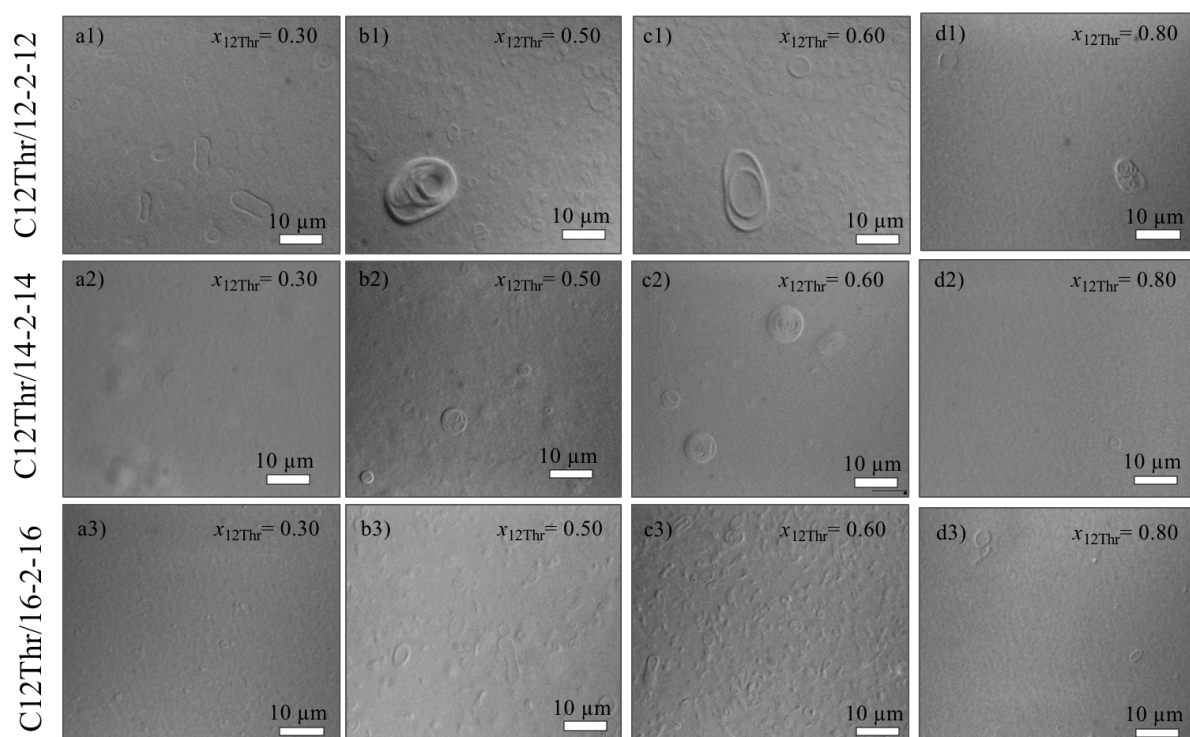


Fig. S3.1 Light micrographs for C12Thr/n-2-n vesicle dispersions, at different $x_{12\text{Thr}}$, at 10 mM and 25 °C.

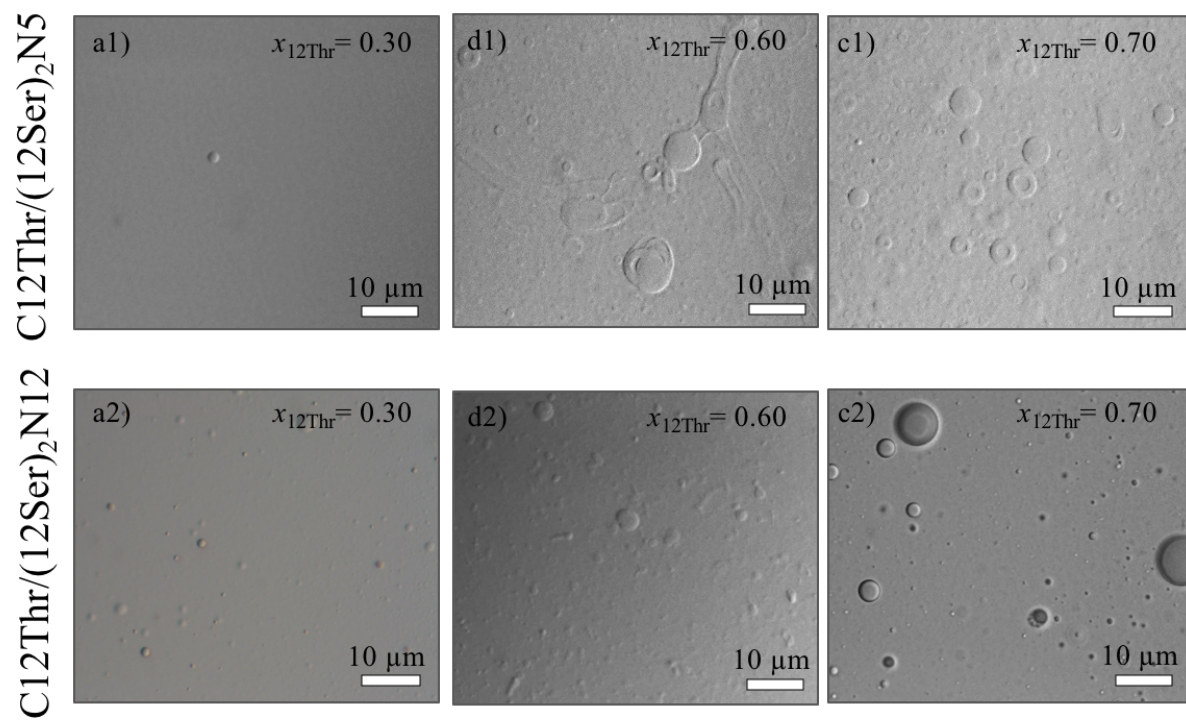


Fig. S3.2 Light micrographs for C12Thr/(12Ser)₂N_x vesicle dispersions, at different $x_{12\text{Thr}}$, at 5 mM and 25 °C.

S4. Cytotoxicity assays

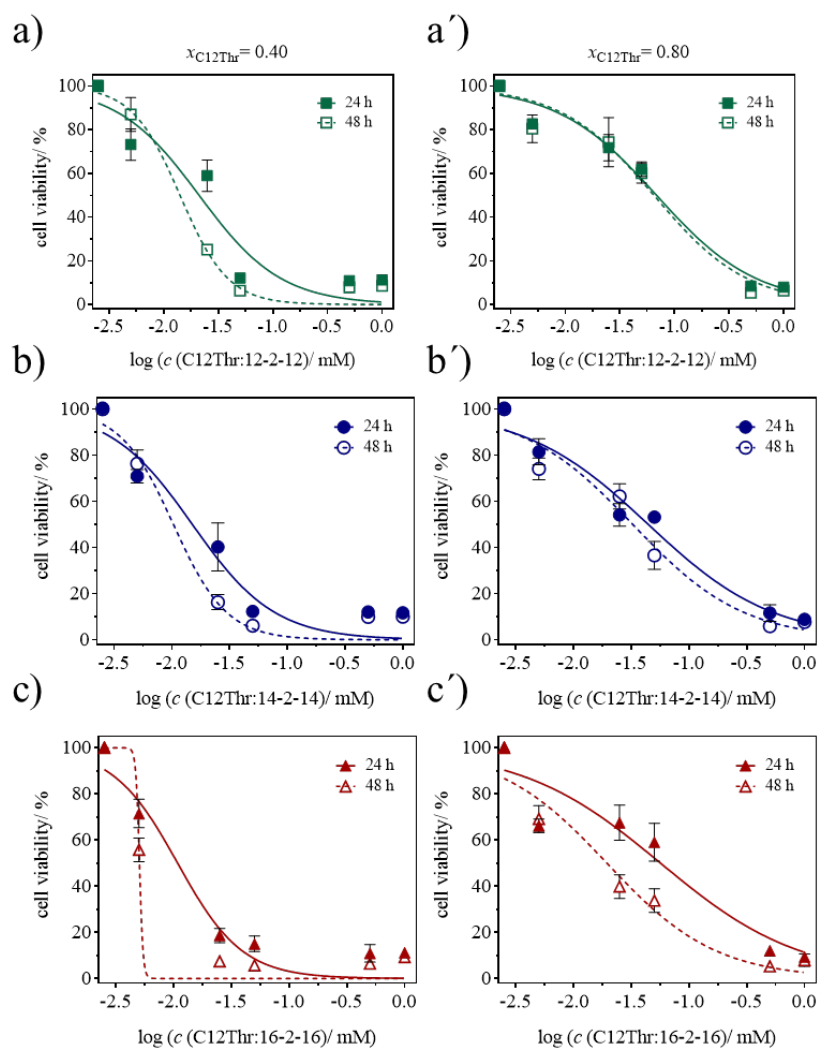


Fig. S4.1 Cytotoxicity induced in L929 cells after 24 (filled symbols) and 48 h (empty symbols) of exposure to C12Thr/gemini n-2-n mixtures: a), b) and c), $x_{C12Thr} = 0.40$; a'), b') and c'), $x_{C12Thr} = 0.80$.

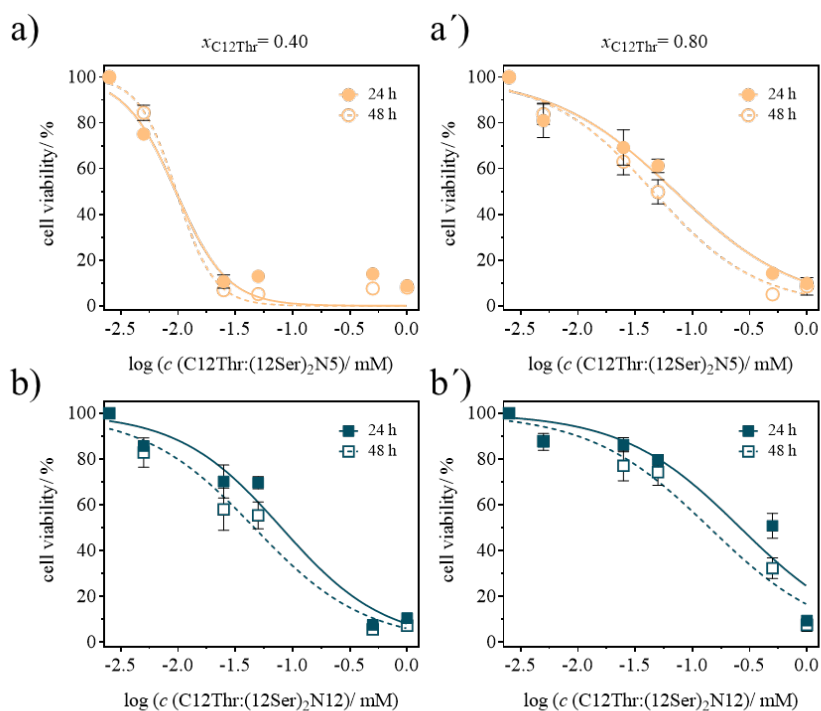


Fig. S4.2 Cytotoxicity induced in L929 cells after 24 (filled symbols) and 48 h (empty symbols) of exposure to C12Thr/(12Ser)₂N_x mixtures: a) and b), $x_{\text{C12Thr}} = 0.40$; a') and b'), $x_{\text{C12Thr}} = 0.80$.

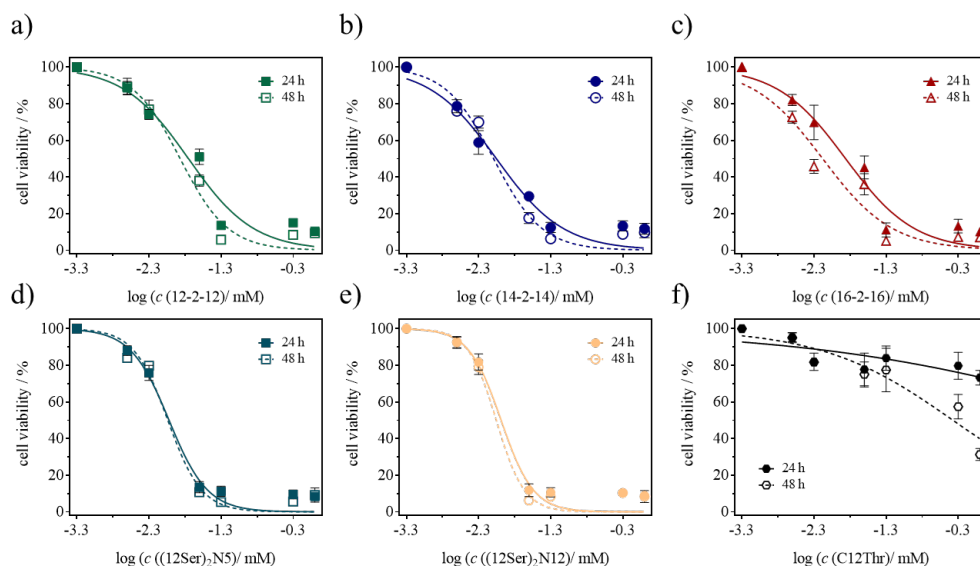


Fig. S4.3. Cytotoxicity induced in L929 cells after 24 (filled symbols) and 48 h (empty symbols) of exposure to neat surfactants: bis-quat gemini surfactants a) 12-2-12, b) 14-2-14 and c) 16-2-16); serine-based surfactants d) (12Ser)₂N₅ and e) (12Ser)₂N₁₂; and f) C12Thr surfactant.