Supplementary Information for

N-Carboxyanhydride-Mediated Fatty Acylation of Amino Acids and Peptides for Functionalization of Protocell Membranes

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General Methods

Anhydrous acetonitrile was purchased from Glen Research (Sterling, VA). Anhydrous dimethylformamide (DMF) and dichloromethane (CH_2Cl_2), amino acids (AAs), oleoyl chloride, and 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid (EPPS) were purchased from Sigma-Aldrich (St. Louis, MO). Oleic acid and octanoic acid were purchased from Nu-Chek Prep, Inc (Elysian, MN). Octanenitrile was purchased from Alfa-Aesar (Ward Hill, MA) and $H_2{}^{18}O$ was purchased from Cambridge Isotope Laboratories (Andover, MA). These reagents were used without further purification unless noted. Purification of the synthesized molecules was performed using a Teledyne Isco combiflash instrument equipped with either a silica gel column or a C18Aq high performance RediSep® column. Nuclear Magnetic Resonance (NMR) spectroscopic analyses were carried out on either a Varian INOVA 400 MHz or a Bruker Ascend 400 MHz spectrometer. Chemical shifts (δ) for ^{1}H NMR spectra were referenced to TMS at δ = 0.00 ppm, or to CHD_2OD at δ = 3.31 ppm. ^{13}C NMR spectra were referenced to $CDCl_3$ at δ = 77.23 ppm, or to CD_3OD at δ = 49.15 ppm. The following abbreviations were used to describe NMR resonances: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), m (multiplet), br (broad), and nfom (non-first order multiplet). Coupling constants (*J*) were reported in Hz. Low-resolution mass spectrometry (LRMS) analyses were performed using a Bruker Daltonics Esquire 6000 mass spectrometer. Liquid chromatography followed by high-resolution mass spectrometry (LC-HRMS) was carried out on an Agilent 6520 Q-TOF or an Agilent 6230 TOF LC/MS-MS instrument (for further details see *Methods* in the main text). UV measurements were conducted on an Amersham Biosciences, Ultraspec 3100-pro UV/VIS spectrophotometer. Membrane growth assays and microscopy of RNA-membrane localization were performed as described in *Methods*.

Table S1. Oligomerization products observed for the reaction of Val-NCA with the specified AA substrates in the presence of either oleic acid vesicles or octanoic acid. Typical reaction conditions: 0.04 mmoles of Val-NCA (suspension) in 2 mL reaction volume, 20 mM AA, 20 mM fatty acid and 300 mM EPPS buffer at an initial pH of 8.5–8.8. The pH of the reaction mixtures drops by up to 0.3 units during the course of the experiments. Aliquots, which were taken at the 24h time point, were diluted 1000 fold before being injected into the LC-HRMS instrument.

Entry	AA	Fatty acid: oleic acid		Fatty acid: octanoic acid	
		2	**Ol-3**	**2**	**Oc-3**
1	Trp (W)	V_nW (n = 1–6)	Ol-V_nW (n = 0–3)	V_nW (n = 1–6)	Oc-V_nW (n = 0–5)
2	Arg (R)	V_nR (n = 1–5)	Ol-V_nR (n = 0–4)	V_nR (n = 1–3)	Oc-V_nR (n = 0–3)
3	Val (V)	V_n (n = 2–8)	Ol-V_n (n = 1–6)	V_n (n = 2–8)	Oc-V_n (n = 1–6)
4	His (H)	V_nH (n = 1–5)	Ol-V_nH (n = 0–5)	V_nH (n = 1–5)	Oc-V_nH (n = 0–5)
5	Lys (K)	V_nK (n = 1–5)	Ol-K	V_nK (n = 1–4)	Oc-V_nK (n = 0–3)
6	Gln (Q)	V_nQ (n = 1–5)	Ol-V_nQ (n = 0–5)*	V_nQ (n = 2–5)	Oc-V_nQ (n = 0–5)
7	Asn (N)	V_nN (n = 2–6)	Ol-V_nN (n = 0–5)	V_nN (n = 2–5)	Oc-V_nN (n = 0–4)

*Ol-V_3Q was not observed

Table S2. HRMS Analysis of the W-containing products outlined in entry 1, Table S1. Overlay of HRMS spectra are presented in the main text.

A. Oleoylated W-containing Products

Molecular Formula	Calc. *m/z*	Obs. *m/z*	Error (ppm)
Ol-W: $C_{29}H_{43}N_2O_3^-$	467.3279	467.3278	-0.2
Ol-VW: $C_{34}H_{52}N_3O_4^-$	566.3963	566.3967	0.7
Ol-V_2W: $C_{39}H_{61}N_4O_5^-$	665.4647	665.4658	1.6
Ol-V_3W: $C_{44}H_{70}N_5O_6^-$	764.5332	764.5335	0.4

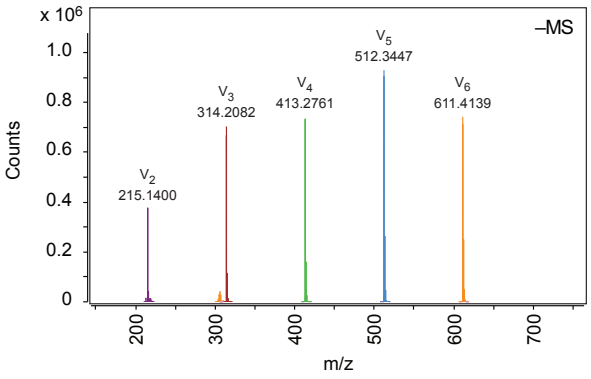
B. Octanoylated W-containing Products

Molecular Formula	Calc. *m/z*	Obs. *m/z*	Error (ppm)
Oc-W: $C_{16}H_{20}N_3O_3^-$	329.1871	329.1873	0.7
Oc-VW: $C_{21}H_{29}N_4O_4^-$	428.2555	428.2555	< 0.1
Oc-V_2W: $C_{26}H_{38}N_5O_5^-$	527.3239	527.3242	0.6
Oc-V_3W: $C_{31}H_{47}N_6O_6^-$	626.3923	626.3917	-1.0
Oc-V_4W: $C_{36}H_{56}N_7O_7^-$	725.4607	725.4607	< 0.1
Oc-V_5W: $C_{41}H_{65}N_8O_8^-$	824.5291	824.5301	1.2

C. V_nW Peptides

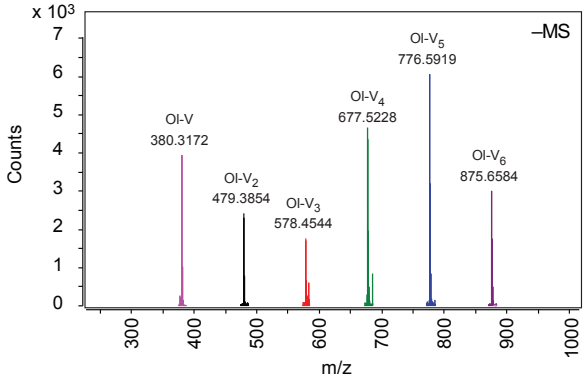
Molecular Formula	Calc. *m/z*	Obs. *m/z*	Error (ppm)
VW: $C_{16}H_{20}N_3O_3^-$	302.1510	302.1503	-2.4
V_2W: $C_{21}H_{29}N_4O_4^-$	401.2194	401.2184	-2.6
V_3W: $C_{26}H_{38}N_5O_5^-$	500.2878	500.2867	-2.3
V_4W: $C_{31}H_{47}N_6O_6^-$	599.3563	599.3556	-1.1
V_5W: $C_{36}H_{56}N_7O_7^-$	698.4247	698.4255	1.2
V_6W: $C_{41}H_{65}N_8O_8^-$	797.4931	797.4931	< 0.1

a



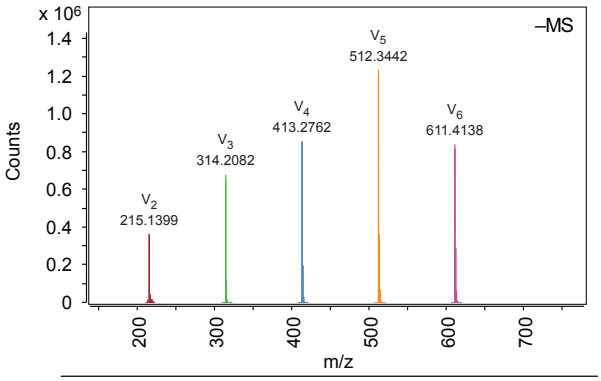
Molecular Formula	Calc. *m/z*	Obs. *m/z*	Error (ppm)
V₂: $C_{10}H_{19}N_2O_3^-$	215.1401	215.1400	-0.5
V₃: $C_{15}H_{28}N_3O_4^-$	314.2085	314.2082	< 0.1
V₄: $C_{20}H_{37}N_4O_5^-$	413.2769	413.2761	-1.9
V₅: $C_{25}H_{46}N_5O_6^-$	512.3454	512.3447	-1.4
V₆: $C_{30}H_{55}N_6O_7^-$	611.4138	611.4139	0.2

b



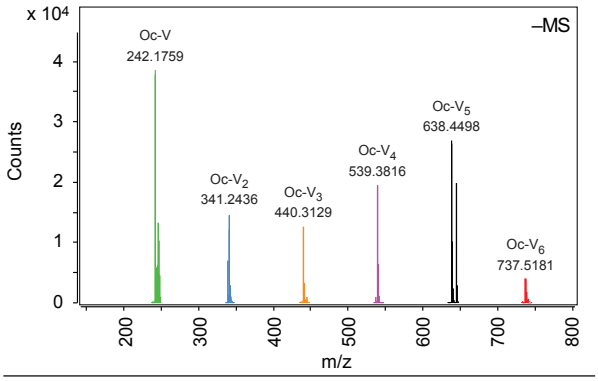
Molecular Formula	Calc. *m/z*	Obs. *m/z*	Error (ppm)
Ol-V: $C_{23}H_{42}NO_3^-$	380.3170	380.3172	0.5
Ol-V₂: $C_{28}H_{51}N_2O_4^-$	479.3854	479.3854	< 0.1
Ol-V₃: $C_{33}H_{60}N_3O_5^-$	578.4538	578.4544	1.0
Ol-V₄: $C_{38}H_{69}N_4O_6^-$	677.5223	677.5228	0.7
Ol-V₅: $C_{43}H_{78}N_5O_7^-$	776.5907	776.5919	1.5
Ol-V₆: $C_{48}H_{87}N_6O_8^-$	875.6591	875.6584	-0.8

c



Molecular Formula	Calc. *m/z*	Obs. *m/z*	Error (ppm)
V₂: $C_{10}H_{19}N_2O_3^-$	215.1400	215.1399	-0.9
V₃: $C_{15}H_{28}N_3O_4^-$	314.2082	314.2082	-1.0
V₄: $C_{20}H_{37}N_4O_5^-$	413.2761	413.2762	-1.7
V₅: $C_{25}H_{46}N_5O_6^-$	512.3447	512.3442	-2.3
V₆: $C_{30}H_{55}N_6O_7^-$	611.4139	611.4138	< -0.1

d



Molecular Formula	Calc. *m/z*	Obs. *m/z*	Error (ppm)
Oc-W: $C_{13}H_{24}NO_3^-$	242.1762	242.1759	-1.2
Oc-VW: $C_{18}H_{33}N_2O_4^-$	341.2446	341.2436	-2.9
Oc-V₂W: $C_{23}H_{42}N_3O_5^-$	440.3130	440.3129	-0.2
Oc-V₃W: $C_{28}H_{51}N_4O_6^-$	539.3814	539.3816	0.4
Oc-V₄W: $C_{33}H_{60}N_5O_7^-$	638.4498	638.4498	< 0.1
Oc-V₅W: $C_{38}H_{69}N_6O_8^-$	737.5182	737.5181	-0.1

Figure S1. Selected ion-HRMS spectra ($[M-H]^{-1}$) of reaction mixtures (24 h) initially consisting of Val-NCA (20 mM), tryptophan (W) (20 mM), EPPS (300 mM) and either oleic acid (20 mM) (**a** and **b**), or octanoic acid (20 mM) (**c** and **d**). HRMS experiments were carried out in negative mode. Panels (**a**) and (**b**) show the oligo-V peptides observed in the presence of oleic acid. Panels (**c**) and (**d**) show the oligo-V peptides observed in the presence of octanoic acid. Ol- is oleoyl and Oc- is octanoyl.

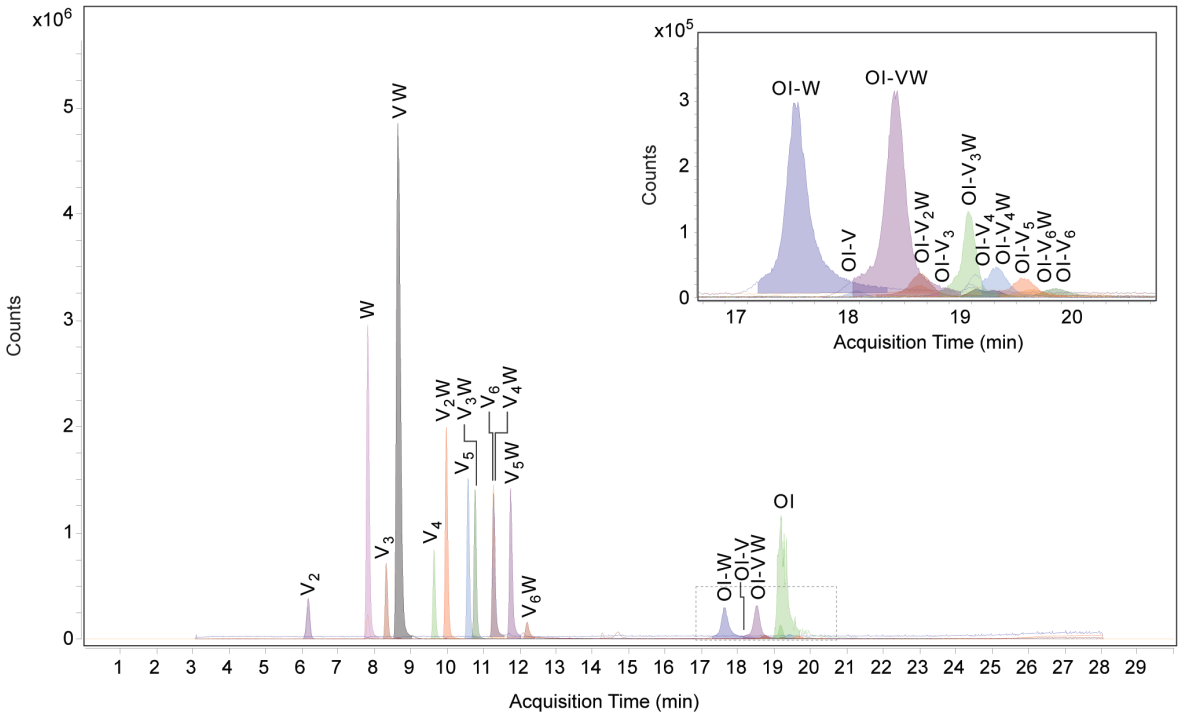
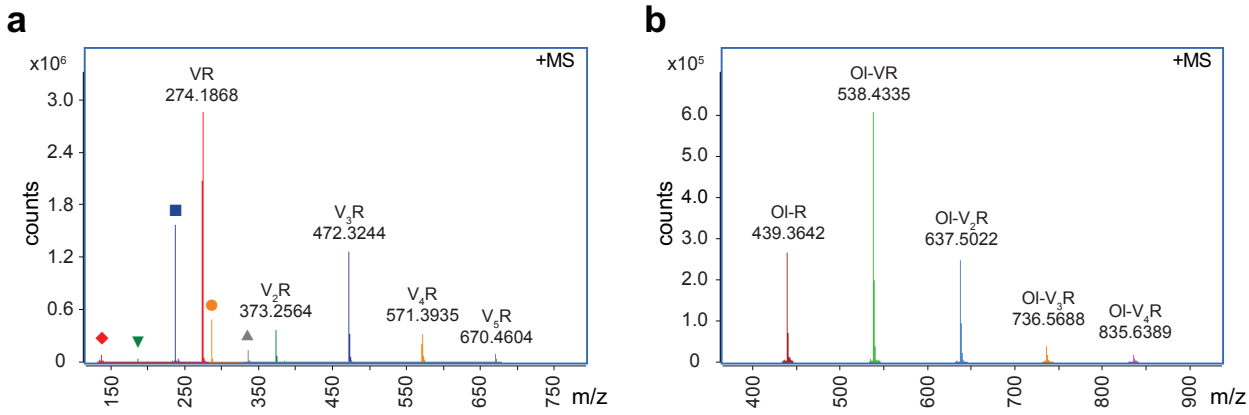


Figure S2. Overlay of HPLC extracted ion chromatogram of a reaction mixture initially containing Val-NCA, oleic acid and Trp, analysis of which is outlined in Fig. 1b-c in the main text.



Molecular Formula	Calc. *m/z*	Obs. *m/z*	Error (ppm)
VR: $C_{11}H_{24}N_5O_3^+$	274.1874	274.1868	-2.2
V$_2$R: $C_{16}H_{33}N_6O_4^+$	373.2558	373.2564	1.6
V$_3$R: $C_{21}H_{42}N_7O_5^+$	472.3242	472.3244	0.4
V$_4$R: $C_{26}H_{51}N_8O_6^+$	571.3926	571.3935	1.6
V$_5$R: $C_{31}H_{60}N_9O_7^+$	670.4610	670.4604	-0.9

Molecular Formula	Calc. *m/z*	Obs. *m/z*	Error (ppm)
Ol-R: $C_{24}H_{47}N_4O_3^+$	439.3643	439.3642	-0.2
Ol-VR: $C_{29}H_{56}N_5O_4^+$	538.4327	538.4335	1.5
Ol-V$_2$R: $C_{34}H_{65}N_6O_5^+$	637.5011	637.5022	1.7
Ol-V$_3$R: $C_{39}H_{74}N_7O_6^+$	736.5695	736.5688	-1.0
Ol-V$_4$R: $C_{44}H_{83}N_8O_7^+$	835.6379	835.6389	1.2

Figure S3. Selected ion-HRMS analyses of reaction mixtures (24 h) initially consisting of Val-NCA (20 mM), arginine (R) (20 mM), EPPS (300 mM) in oleic acid (20 mM) (**a** and **b**). HRMS experiments were carried out in positive mode. Panel (**a**) highlights the observed R-containing unacylated peptides in the presence of oleic acid. Panel (**b**) highlights the observed R-containing oleoylated (Ol-) species. (a) symbols represent the observed second charge state m/z values of the peptide products (◆: VR, ▼: V$_2$R, ■: V$_3$R, ●: V$_4$R, ▲: V$_5$R).

Mechanistic insights into the acylation of amino acids and peptides formed *in situ*

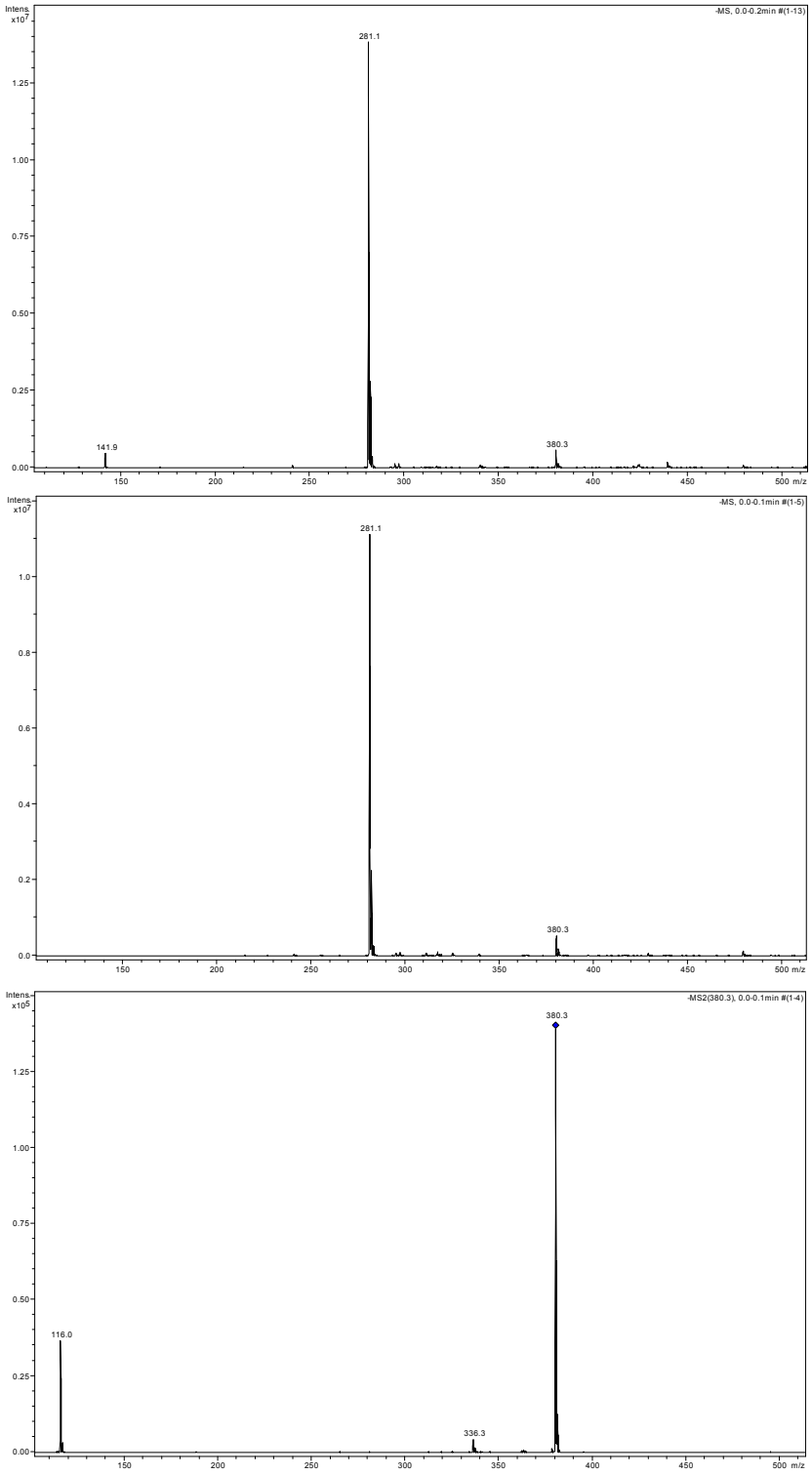


Figure S4. Mass spectroscopic investigation (in negative mode) of the formation of the carboxy-anhydride from Val-NCA, **1** (40 mM) and a mixture of oleic acid (1.5 M) and trimethylamine (1.5 M). ESI spectra of the crude reaction aliquot taken after (top) 15 min and (middle) 6 h of mixing. (Bottom) MS/MS product ion spectrum highlighting the isolated 380 *m*/*z* species and its fragmentation products. $[M - H]^{-1}$ *m*/*z* values; Val-NCA (**1**): 142, oleic acid: 281, N^{α}-oleoylvaline: 380, valine: 116, and decarboxylated N^{α}-oleoylvaline: 336.

Below in Fig. S5 we show MS analyses of lower concentration aqueous reactions in which we used 40 mM ^{18}O -enriched octanoic acid, 40 mM TEA, and ~20 mM Val-NCA at initial pH 8.7. We observed the $[M - H]^{-1}$ = 246 m/z (Fig. S5, top) and $[M - H]^{-1}$ = 246 m/z (bottom) signals in negative ionization mode. The existence of the 246 m/z ($^{18}O_2$-oleylvaline) species, which fragments to yield a y-ion of 118 m/z (mono-^{18}O enriched valine carboxylate) from amide bond dissociation and a decarboxylation product with 200 m/z (mono-^{18}O enriched), suggests that the intramolecular rearrangement (path *i*) occurred. This result is in agreement with Fig. 3c of the main text. Interestingly, when isolating individual peaks within the isotope distribution around this species, we observed that the fragmentation of the 244 m/z signal (^{18}O -oleylvaline) yields y-ions of both 116 and 118 m/z, as well as the decarboxylated ions 200 and 198 m/z, respectively. Partitioning of ^{18}O with the y-ion from fragmentation of ^{18}O-oleylvaline would be expected for an anhydride that arises from ^{18}O-oleate, since the enriched oxygen could be in either the bridging or non-bridging position in the mixed anhydride intermediate. The mono-^{18}O-oleate needed to generate this species could arise from an impurity in the enriched preparation of $^{18}O_2$-oleic acid (Fig. S6, bottom) or from the hydrolysis of the anhydride generated by the reaction of $^{18}O_2$-oleate with Val-NCA. In fact, during

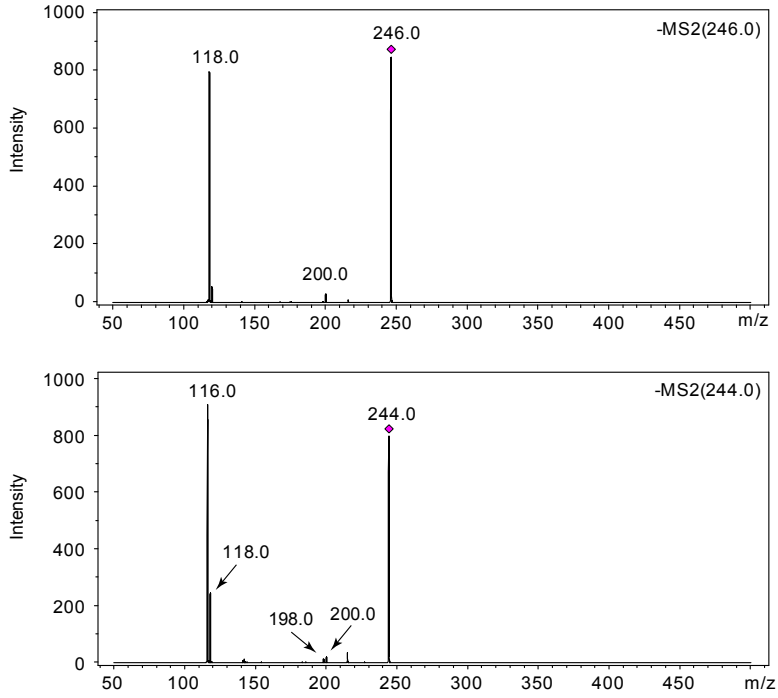


Figure S5. Mechanistic investigation of the formation of a mixed anhydride from Val-NCA and ^{18}O-enriched octanoic acid. (Top and bottom) MS/MS product ion spectra highlighting the isolated ^{18}O-enriched species and their fragmentation products.

the direct MS injection of a crude reaction (Fig. S6), we did observe an increase in the abundance of the 145 m/z peak corresponding to mono-^{18}O-oleate, suggesting that a pathway for hydrolysis of the mixed anhydride exists in a reaction containing Val-NCA.

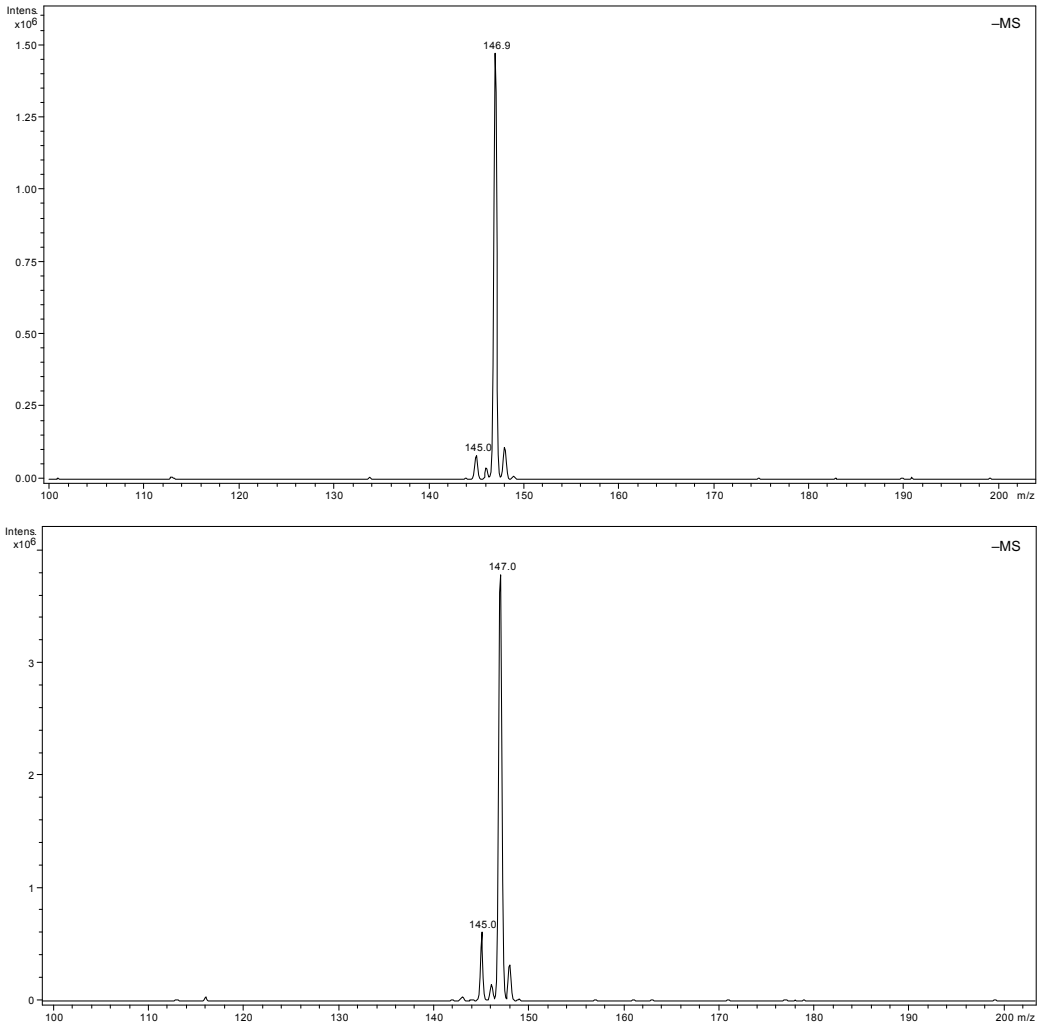


Figure S6. Low-resolution MS spectra of $^{18}O_2$-oleic acid (top) before the reaction was initiated and (bottom) after being mixed (as a triethylammonium salt at ca. pH 8.5 and 20 ºC) with Val-NCA.

Hydrolysis of Val-NCA

Val-NCA (**1**) hydrolyzes rapidly under our reaction conditions. In aqueous buffer at pH 8.5, in the absence of both the fatty acid and free AA, the observed rate constant for hydrolysis, $k_{obs\text{-}h}$, of **1** was measured as ca. 1 min^{-1} by ^1H NMR spectroscopy (Fig. S7).[1] Our hydrolysis study was carried out as follows: At 15 ºC, a 0.14 M d_8-THF solution of **1** (0.6 mL) was added quickly into a vigorously stirred NaEPPS solution (300 mM, pH 8.5, 11.4 mL) to give a 0.007 M solution of **1**, an initial concentration for which the NCA polymerization would be negligible.[1] Reaction aliquots (1 mL) taken over a 2 min period were quenched by addition to 0.05 mL TFA in a 4-mL glass vial, and the unreacted **1** was recovered by $CDCl_3$ (0.6 mL) extraction (vortex for 1 min). For each sample, the organic layer was removed using a gas-tight Hamilton syringe and transferred into a 1-mL vial containing 50 mg Na_2SO_4. After being dried over Na_2SO_4, the $CDCl_3$ solution (ca. 5 mL) was transferred into an NMR tube, and the ^1H NMR spectrum was acquired over 256 scans. The area under the -C*H*$_3$ signals of **1** (two apparent doublets, δ ca. 1.1 ppm) was used to determine the relative mol% of **1**, by normalizing to the peak area of the d_8-THF residual solvent peak (δ ca. 1.7 ppm).

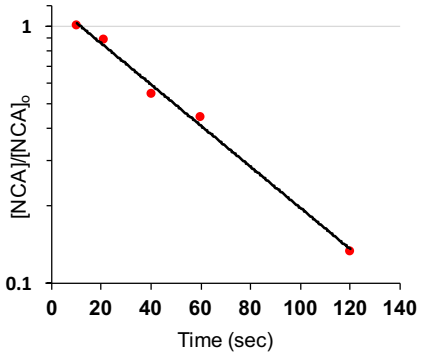


Figure S7. Hydrolysis of Val-NCA (**1**) at 15 ºC and pH 8.5. Observed rate constant for hydrolysis, $k_{obs\text{-}h}$ of **1** is 0.018 sec^{-1}. (R^2 = 0.993).

Standard curves of FRET efficiencies

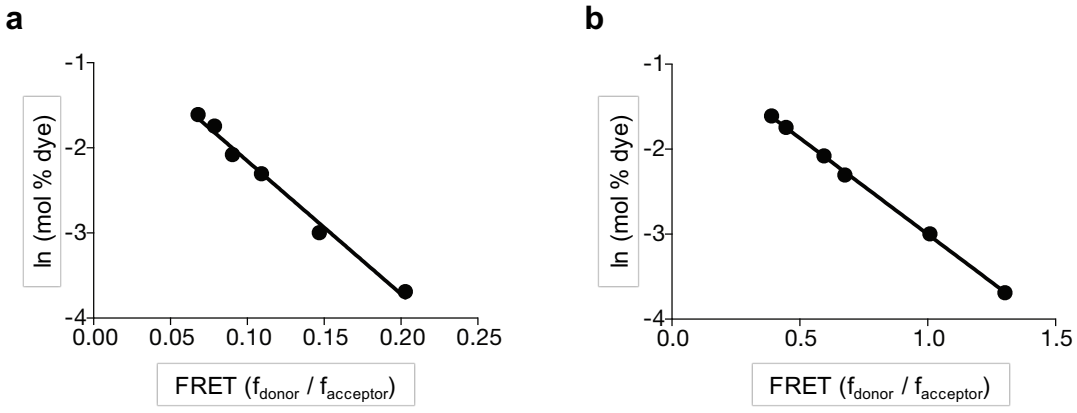
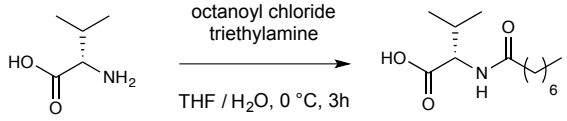


Figure S8. Standard curve of FRET efficiency as a function of mol% FRET dyes in (**a**) oleate and (**b**) POPC vesicles (R^2 > 0.99).

Synthesis of N^{α}-octanoyl-L-valine

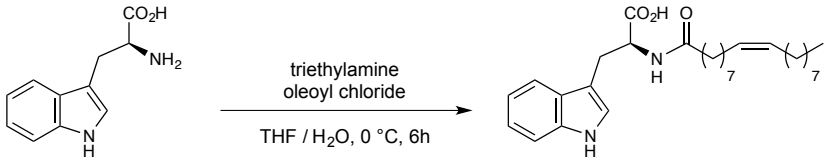


L-valine N^{α}-octanoyl-L-valine

To a stirred suspension of L-valine (175 mg, 1.5 mmol, 1.5 equiv) in a mixture of THF (8 mL) and H_2O (1.5 mL) was added octanoyl chloride (0.17 mL, 1.0 mmol, 1 equiv) and triethylamine (0.6 mL, 4.0 mmol, 4 equiv) at 0 °C. The reaction mixture was stirred for 3 h, then concentrated under reduced pressure. The resulting material was dissolved in CH_2Cl_2 (100 mL) and washed with water. The organic phase was concentrated under reduced pressure, and the resulting crude material was purified by combiflash silica gel chromatography (CH_2Cl_2 / MeOH step gradient). The product fraction was concentrated under reduced pressure to afford N^{α}-octanoyl-L-valine (120 mg, 51% yield, based on octanoyl chloride).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (br s, 1H), 6.15 (d, J = 8.5 Hz, 1H), 4.59 (dd, J = 8.5, J = 4.5 Hz, 1H), 2.27 (t, J = 7.0 Hz, 2H), 2.24 (m, 1H), 1.64 (br quin, J = 7.0 Hz, 2H), 1.36–1.21 (m, 8H), 0.98 (d, J = 7.0 Hz, 3H), 0.95 (d, J = 7.0 Hz, 3H), and 0.88 (br t, J = 7.0 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 175.3, 174.1, 57.0, 36.7, 31.7, 31.0, 29.2, 29.0, 25.7, 22.6, 19.0, 17.7, and 14.0; **LRMS** (ESI): calcd for $[C_{13}H_{25}NO_3 - (H^+)]^-$ 242.1762, found 242.0.

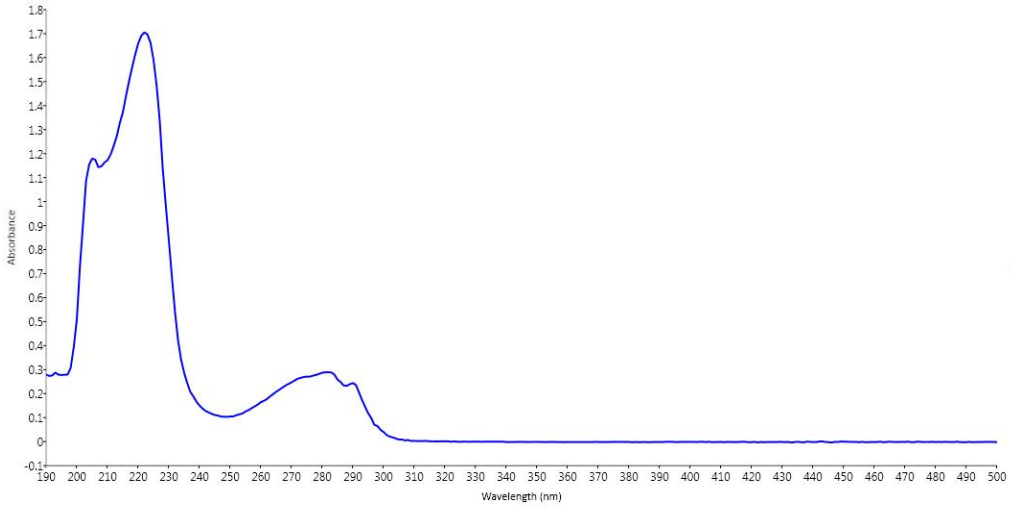
Synthesis of N^{α}-oleoyl-L-tryptophan



L-Tryptophan N^{α}-oleoyl-L-tryptophan

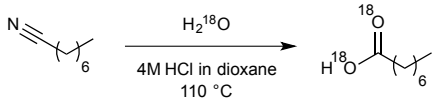
To a stirred suspension of L-tryptophan (200 mg, 1.0 mmol, 1.5 equiv) in a mixture of THF (8.0 mL) and H_2O (1.5 mL) was added oleoyl chloride (0.22 mL, 0.7 mmol, 1 equiv) and triethylamine (0.6 mL, 4.2 mmol, 6 equiv) at 0 °C. The reaction mixture was stirred for 6 h, then concentrated under reduced pressure. The resulting material was dissolved in CH_2Cl_2 (100 mL) and washed with water. The organic phase was concentrated under reduced pressure, and the resulting crude material was purified by combiflash silica gel chromatography (CH_2Cl_2/MeOH

step gradient). The product fraction was concentrated under reduced pressure to afford N^{α}-oleoyl-L-tryptophan (205 mg, 63% yield, based on oleoyl chloride). The ^{1}H-NMR spectrum of the isolated product acquired in $CDCl_3$ matched the reported spectrum.[2] **LRMS** (ESI): calcd for $[C_{29}H_{44}N_2O_3 - (H^+)]^-$ 467.3279, found 467.3. The molar extinction coefficient at 280 nm absorbance (ε_{280}) of N^{α}-oleoyl-L-tryptophan dissolved in MeOH was measured as 5700 $M^{-1}cm^{-1}$ (see the UV spectrum below). In addition, ε_{280} of L-tryptophan dissolved in MeOH/H_2O (1:1) was measured as 5980 $M^{-1}cm^{-1}$.



These ε_{280} values were used to determine the yields of the N^{α}-acylated and unacylated Trp-containing products as described in the main text. Purification was achieved using combiflash reversed-phase C18 chromatography (H_2O/CH_3CN or H_2O/MeOH gradient).

Synthesis of Octanoic-1,1-$^{18}O_2$ acid

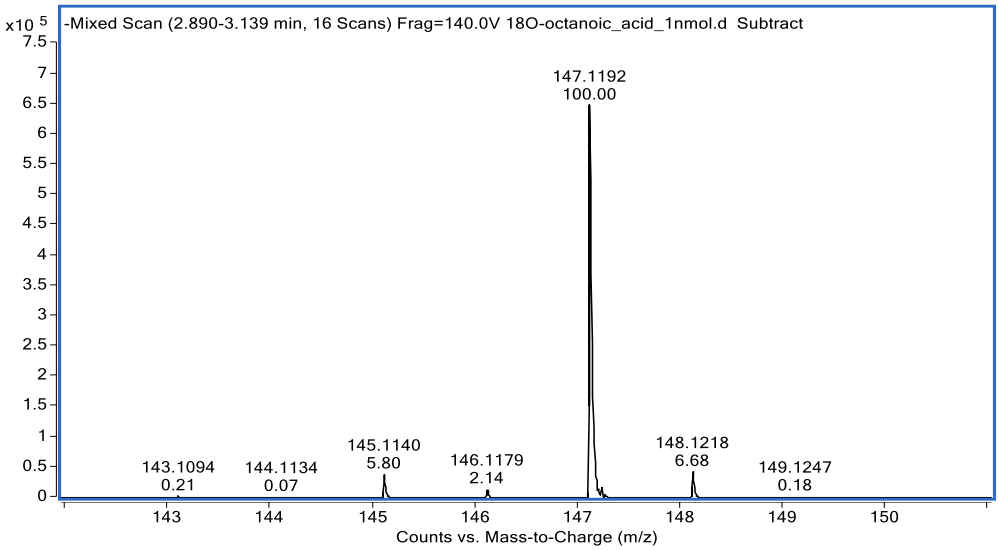


octanenitrile octanoic-1,1-(18)O_2 acid

Inside of a glove box, a 15 mL high pressure reaction vessel equipped with a magnetic stir bar, was charged with octanenitrile (550 μL, 3.6 mmol, 1 equiv), $H_2{}^{18}O$ (97%, 500 μL, 25 mmol, 7 equiv) and 4M HCl in dioxane (3.5 mL). The vessel was sealed tightly with a Teflon cap, then taken out of the glove box and placed in a 110 °C-oil bath. After being stirred for 4 h, the reaction mixture was allowed to cool to room temperature and filtered through a PTFE filter (pore size 0.22

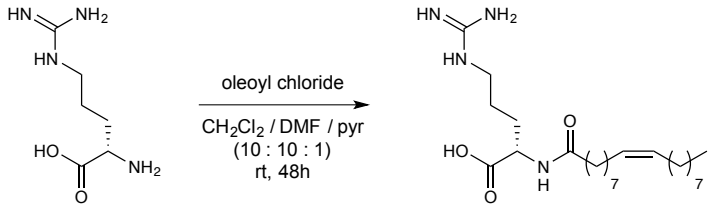
μm). Diethyl ether (8 mL) was added to the filtrate, and the resulting mixture was vortexed in a Falcon tube for ca. 1 min. The organic layer was transferred into another Falcon tube and cooled to 0 °C. Anhydrous triethylamine (1 mL) was added dropwise to remove most of the excess HCl present in the organic liquid as an insoluble triethylammonium chloride salt. The Falcon tube was then vortexed for ca. 1 min and placed in a –30 °C freezer. After 30 min, the tube was centrifuged to pellet the solid material. The supernatant was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting oil was subjected to combiflash silica gel chromatography (with 210–215 nm UV detection) using a hexane/diethyl ether elution mixture (95:5 to 75:25 gradient) to afford octanoic-1,1-$^{18}O_2$ acid (320 mg, 61% yield, 87% enrichment by HRMS).

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.36 (t, J = 7.5 Hz, 2H), 1.64 (br q, J = 7.5, 2H), 1.37–1.24 (m, 8H), and 0.89 (t, J = 7.0 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 179.8, 34.2, 31.9, 29.2, 29.1, 24.9, 22.8 (6x CH_2) and 14.3; **HRMS** (ESI): calcd for $[C_8H_{16}{}^{18}O_2 - (H^+)]^-$ 147.1162, found 147.1191.



m/z calc	m/z obs	natural	enriched	enriched norm.
143.1078	143.1094	91.15	0.18	0.21
144.1112	144.1134	8.11	0.06	0.07
145.1132	145.1140	0.69	5.04	5.80
146.1159	146.1179	0.04	1.86	2.14
147.1184	147.1192	0	86.90	100
148.1209	148.1218	0	5.80	6.68
149.1272	149.1247	0	0.16	0.18

Synthesis of N^{α}-oleoyl-L-arginine



L-arginine N^{α}-oleoyl-L-arginine

To a stirred suspension of L-arginine (180 mg, 1.0 mmol, 2 equiv) in CH_2Cl_2 (3.5 mL), DMF (3.5 mL) and pyridine (0.4 mL) was added oleoyl chloride (0.17 mL, 0.5 mmol, 1 equiv) at room temperature. The reaction mixture was stirred for 48 h, then diluted with 1N HCl (50 mL) and extracted with CH_2Cl_2 (100 mL). The organic phase was concentrated under reduced pressure, and the resulting crude material was purified by combiflash reversed-phase C18 chromatography (solvent A: water, solvent B: 0.1% TFA/acetonitrile, gradient from 40% to 100% solvent B). The aqueous product fraction was transferred into a Falcon tube and freeze-dried to afford N^{α}-oleoyl-L-arginine (54 mg, 26% yield).

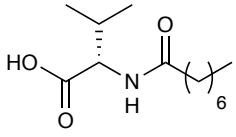
^{1}H NMR (400 MHz, CD_3OD) δ 5.35 (overlapped m, 2H), 4.43 (dd, J = 8.5, 5.5 Hz, 1H), 3.27–3.15 (m, 2H), 2.26 (t, J = 7.5 Hz, 2H), 2.08–2.00 (m, 4H), 1.98–1.89 (m, 1H), 1.77–1.70 (m, 1H), 1.70–1.58 (m, 4H), 1.40–1.24 (overlapped m, 20H), and 0.90 (t, J = 7.0 Hz, 3H) (exchangeable protons were not observed); **^{13}C NMR** (100 MHz, CD_3OD) δ 176.6, 175.4, 158.8, 131.0, 130.9, 53.2, 42.0, 37.0, 33.2, 31.0^+, 31.0^-, 30.8, 30.6, 30.5^+, 30.5^-, 30.4, 31.0, 30.0, 28.3^+, 28.3^-, 27.1, 26.5, 24.0, and 14.6; **HRMS** (ESI): calcd for $C_{24}H_{46}N_4O_3 \bullet H^+$ 439.3643, found 439.3639.

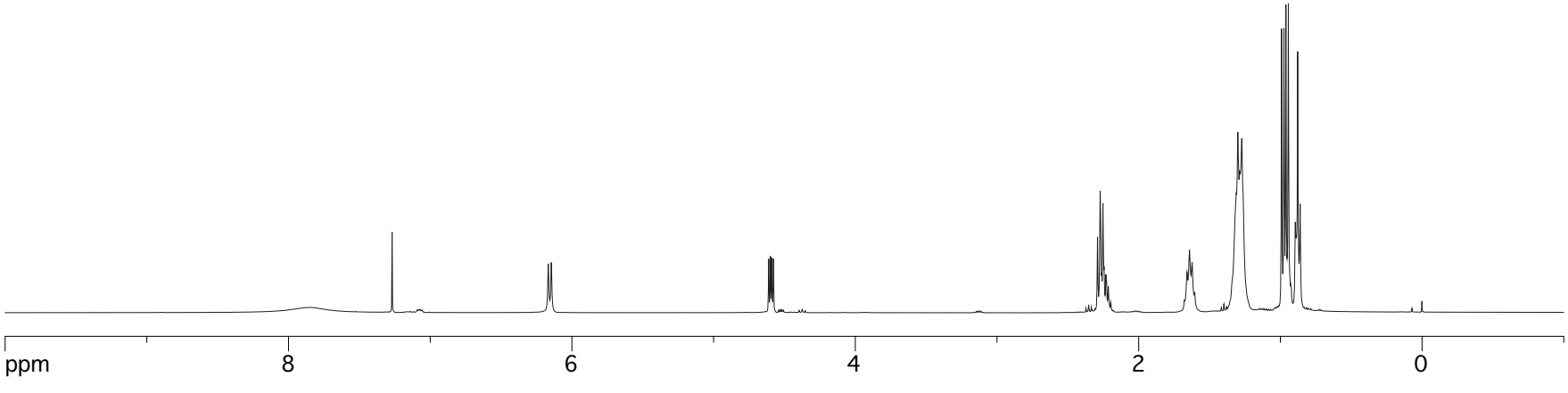
References

1. At pH 6.5, the $k_{obs\text{-}h}$ of **1** has previously been determined to be 0.07 min^{-1} by capillary electrophoresis: Plasson, R.; Biron, J.-P.; Cottet, H.; Commeyras, A.; Taillades, J. *J. Chromatogr., A* **2002**, *952*, 239–248.

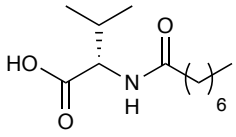
2. Osornio, Y. M., Uebelhart, P., Bosshard, S., Konrad, F., Siegel, J. S. & Landau, E. M., *J. Org. Chem.* **2012**, *77*, 10583–10595.

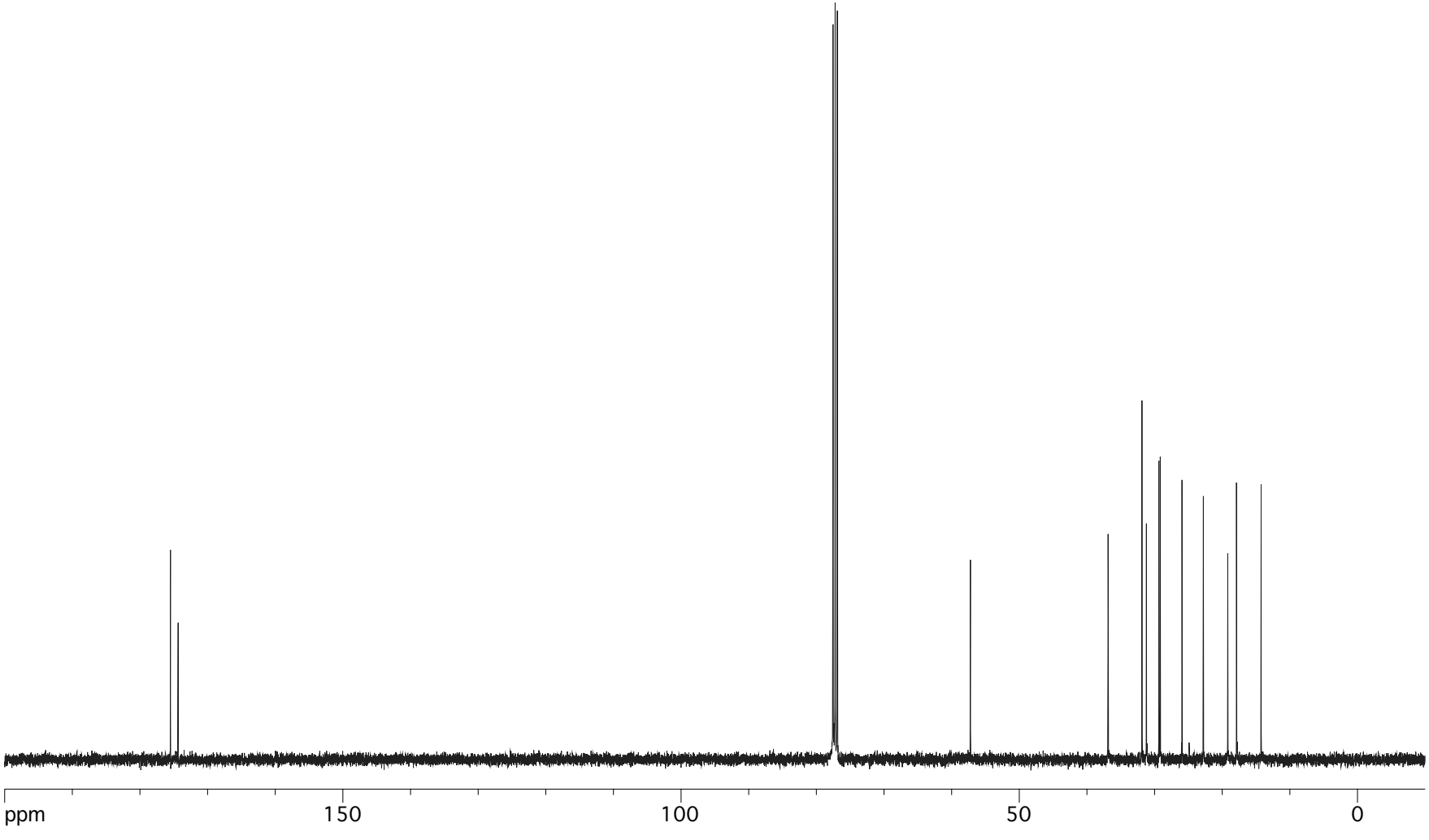
^{1}H NMR Spect. (400 MHz, $CDCl_3$)





^{13}C NMR Spect. (100 MHz, $CDCl_3$)





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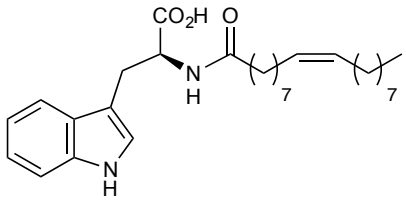

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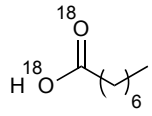

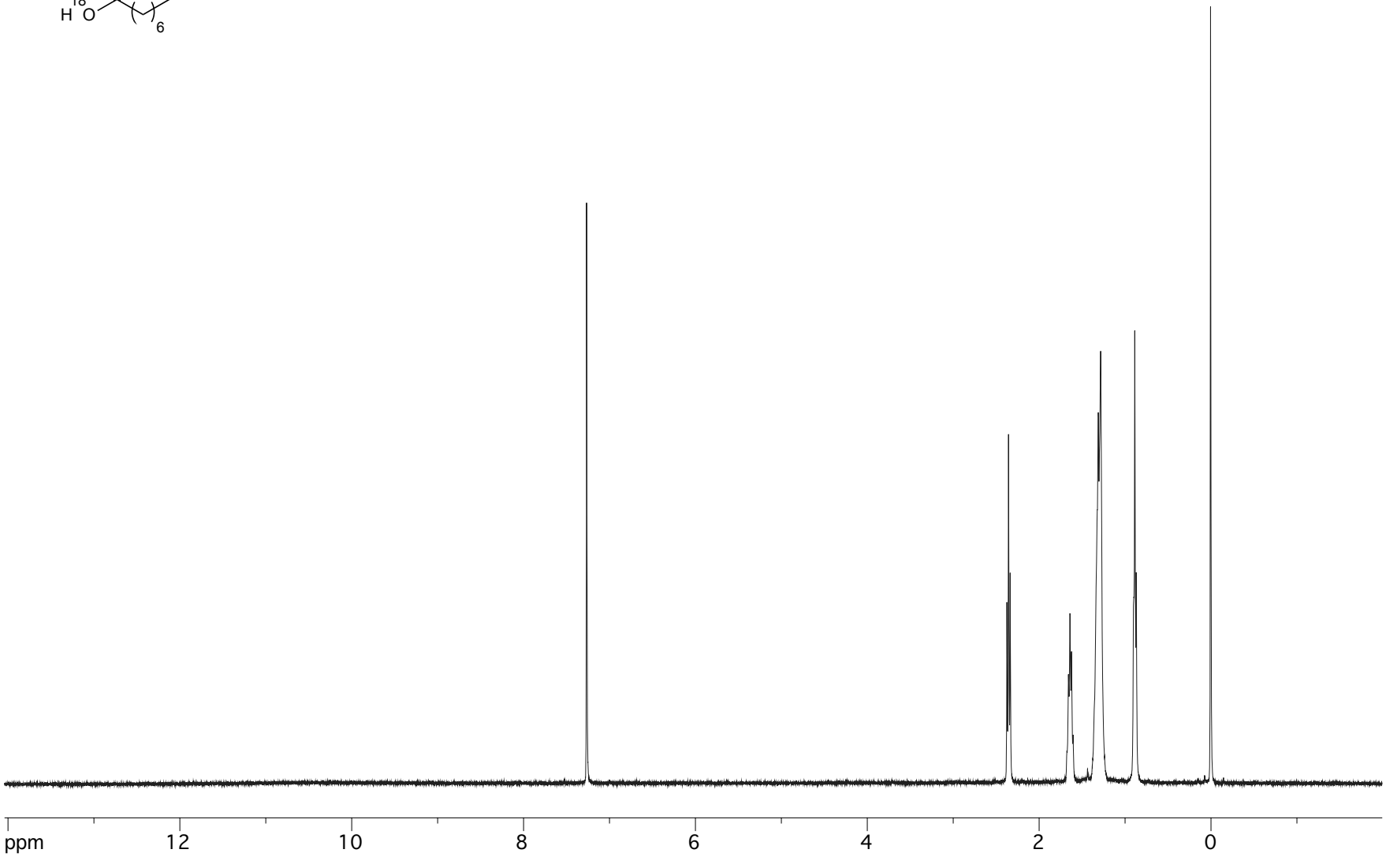

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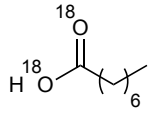

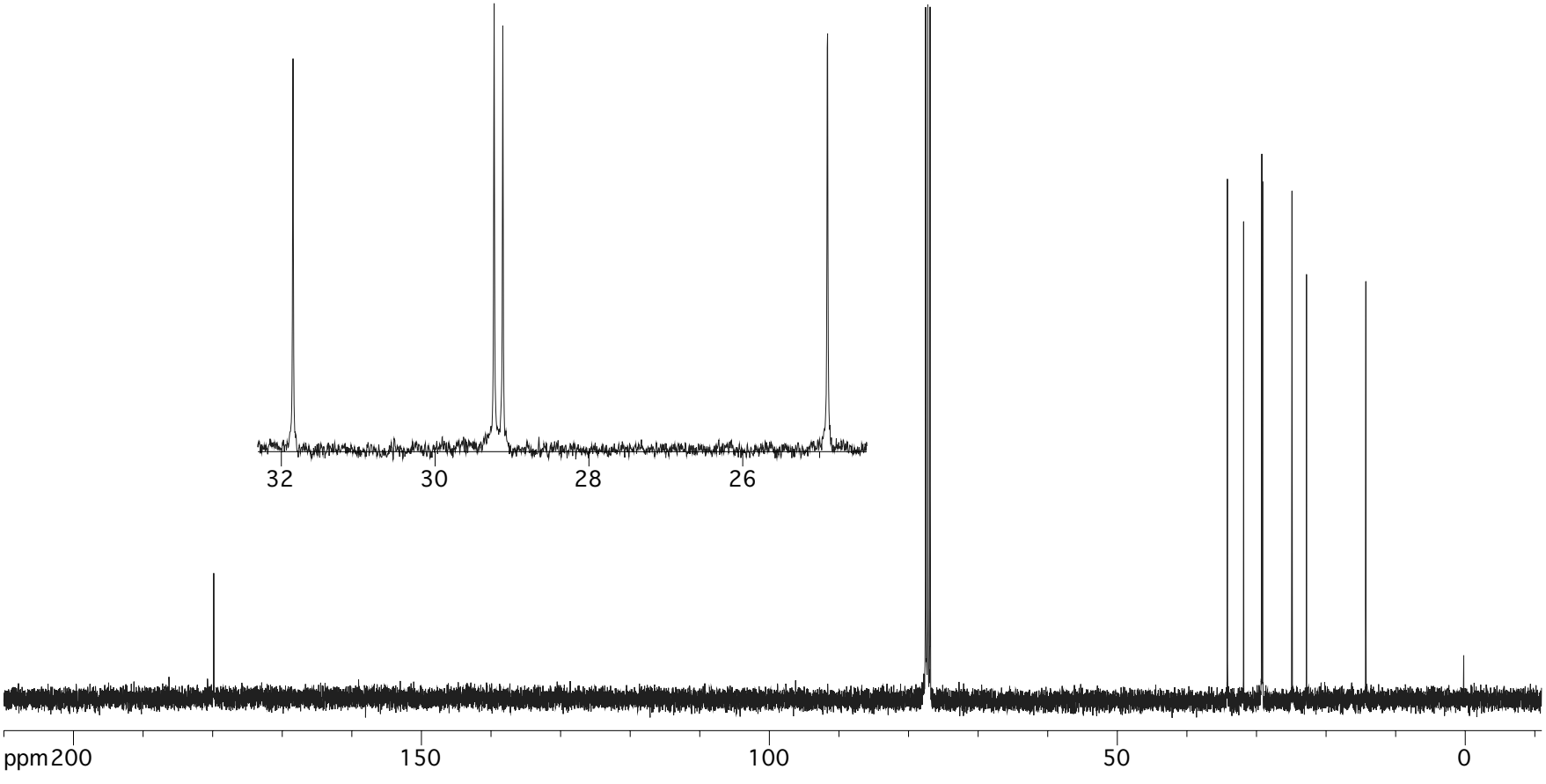

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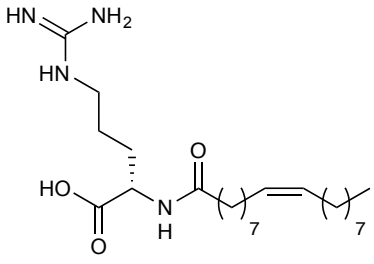

^{13}C NMR Spect. (100 MHz, CD_3OD)