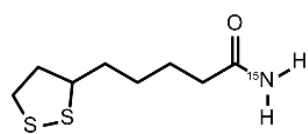


Small-molecule dissolution of stress granules by redox modulation benefits ALS models

In the format provided by the
authors and unedited

Supplementary Note 1

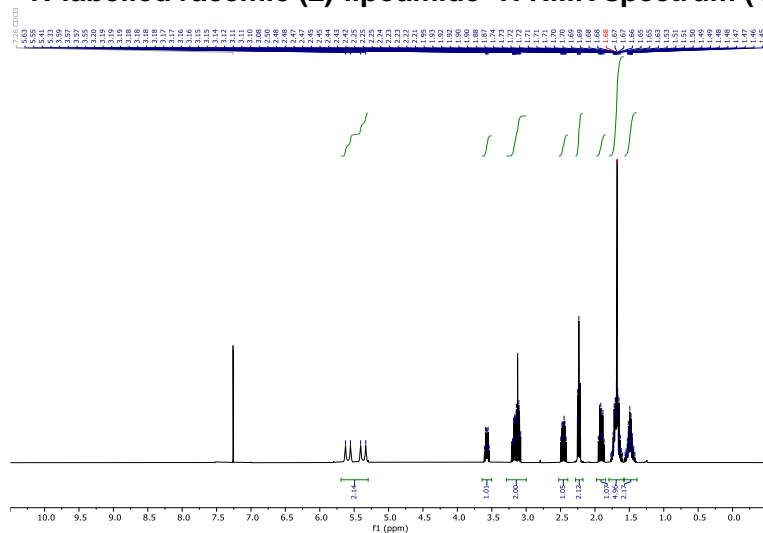
Synthesis of ^{15}N -labelled racemic (\pm)-lipoamide



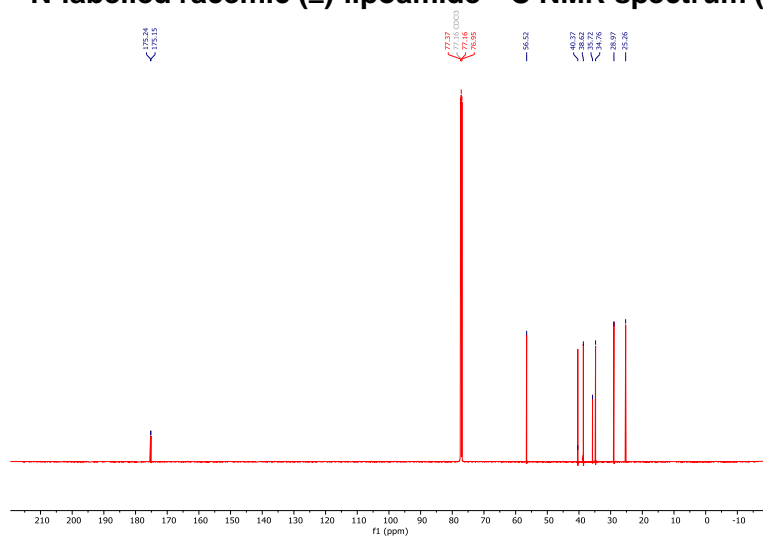
Racemic (\pm)-lipoic acid (1.08 g, 5.23 mmol), *N*-hydroxysuccinimide (660 mg, 5.73 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (1.10 g, 5.74 mmol) were dissolved in dry DMF (20 mL) under argon at 25 °C. Then, the mixture was stirred for 4 h, and it was diluted with ethyl acetate (100 mL). The mixture was washed with water (100 mL), saturated aqueous NaHCO_3 (100 mL) and again with water (2×100 mL). The organic layer was dried over anhydrous MgSO_4 , filtered and the solvent was removed from the filtrate by rotary evaporation to obtain the crude racemic (\pm)-lipoic acid NHS ester. The crude NHS ester obtained from the above process was dissolved in dry DCM (20 mL) at 25 °C. Then, $^{15}\text{NH}_4\text{Cl}$ (500 mg, 9.18 mmol) and triethylamine (1.10 mL, 7.89 mmol) were added to the mixture. The mixture was stirred for 20 h. The solution was diluted with DCM (100 mL), washed with water (100 mL), saturated aqueous NaHCO_3 (100 mL) and again with water (2×100 mL). The organic layer was dried over anhydrous MgSO_4 , filtered and the solvent was removed from the filtrate MgSO_4 by rotary evaporation to give crude ^{15}N -labelled racemic (\pm)-lipoamide, which was further purified by silica gel flash column chromatography (DCM/methanol, 30:1). The solvent was removed by rotary evaporation and [^{15}N](\pm)-lipoamide was obtained as a yellow solid (554 mg, 51% yield).

TLC R_f (DCM/methanol, 20:1) = 0.60; **MP** 130 °C (lit¹ MP 129–131 °C for unlabelled racemic (\pm)-lipoamide); **^1H NMR** (400 MHz, CDCl_3): δ 5.52 (d, J = 88.0 Hz, 1H, NH_{cis}), 5.44 (d, J = 88.4 Hz, 1H, NH_{trans}), 3.57 (m, 1H, SSCH), 3.21 – 3.08 (m, 2H, SSCH_2), 2.50 – 2.42 (m, 1H, $\text{SSCH}_2\text{CH}_{\text{trans}}$), 2.23 (t, J = 8.0 Hz, 2H, CH_2CONH_2), 1.95 – 1.87 (m, 1H, $\text{SSCH}_2\text{CH}_{\text{cis}}$), 1.77 – 1.60 (m, 4H), 1.56 – 1.42 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CONH}_2$); **^{13}C NMR** (151 MHz, CDCl_3) δ 175.2 (d, J = 13.6 Hz, CONH_2), 56.5 (SSCH), 40.4 (SSCH_2CH_2), 38.6 (SSCH_2), 35.7 (CH_2CONH_2), 34.8 ($\text{CH}_2\text{CH}_2\text{CONH}_2$), 29.0 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{CONH}_2$), 25.3 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CONH}_2$); **^{15}N NMR** (61 MHz, CDCl_3) δ = 101.8 (t, J = 88.6 MHz, CONH_2); **HRMS** (ESI^+) m/z [MNa^+] calcd for $\text{C}_8\text{H}_{15}\text{O}^{15}\text{NS}_2\text{Na}$, 229.0458; found 229.0459; **IR** ($\text{neat}/\text{cm}^{-1}$): 3352m, 3176m, 2937m, 1650s, 1629m, 1464m, 1413s.

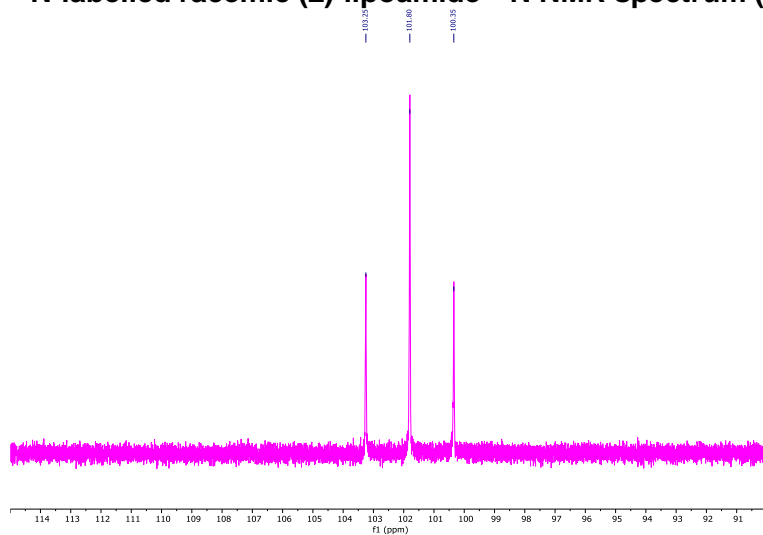
^{15}N -labelled racemic (\pm)-lipoamide ^1H NMR spectrum (400 MHz, CDCl_3)



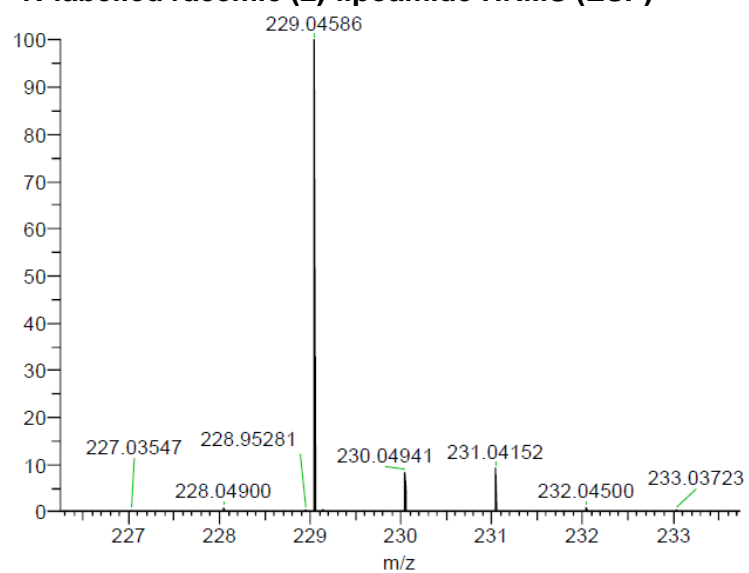
^{15}N -labelled racemic (\pm)-lipoamide ^{13}C NMR spectrum (151 MHz, CDCl_3)



^{15}N -labelled racemic (\pm)-lipoamide ^{15}N NMR spectrum (61MHz, CDCl_3)



¹⁵N-labelled racemic (±)-lipoamide HRMS (ESI⁺)

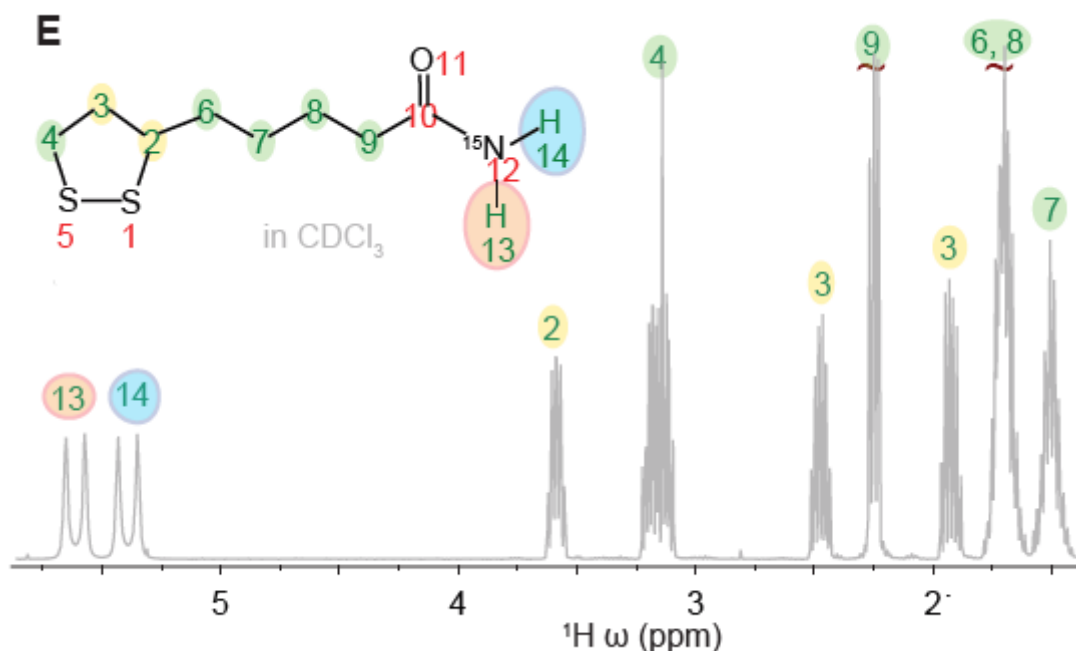


Formula (M)	Ion Formula	m/z	m/z (Calc)	Diff (ppm)
C8 H15 O [15]N S2	C8 H15 O [15]N S2 Na	229.04585	229.04576	0.4

HRMS data suggested that the ¹⁵N labelling efficacy is at ~99%.

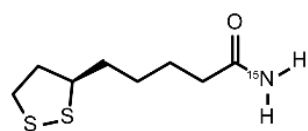
DFT calculations for the assignment of the ^{15}N -labelled racemic (\pm)-lipoamide ^1H NMR

Density functional theory (DFT) calculations were performed to confirm the assignments of proton resonances in the ^1H NMR spectrum obtained from ^{15}N -labelled racemic (\pm)-lipoamide. An optimised structure for the [^{15}N](\pm)-lipoamide was generated using Gaussian098 from which shielding tensors were calculated, enabling isotropic and anisotropic components to be determined. DFT calculations used the B3LYP density functional with the 6-31G(d) basis set.²



DFT calculations suggest that the *cis*-amide proton 13 (*cis* to the alkyl chain) should have a larger chemical shift than the *trans*-amide proton 14 (*trans* to the alkyl chain), and that the proton bound to carbon 3 closest in proximity to the proton bound to carbon 2 should have a larger chemical shift than the other proton on carbon 3.

Synthesis of ^{15}N -labelled (*R*)-(+)-lipoamide



(*R*)-(+)-Lipoic acid (1.08 g, 5.23 mmol), *N*-hydroxysuccinimide (660 mg, 5.73 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.10 g, 5.74 mmol) were dissolved in DMF (20 mL) under argon at 25 °C. Then, the mixture was stirred for 4 h, and it was diluted with ethyl acetate (100 mL). The mixture was washed with water (100 mL), saturated aqueous NaHCO_3 (100 mL) and again with water (2×100 mL). The organic layer was dried over anhydrous MgSO_4 , filtered and the solvent was removed from the filtrate by rotary evaporation to obtain the crude (*R*)-(+)-lipoic acid NHS ester. This crude NHS ester obtained from the above process was dissolved in dry DCM (20 mL) at 25 °C. Then, $^{15}\text{NH}_4\text{Cl}$ (500 mg, 9.18 mmol) and triethylamine (1.10 mL, 7.89 mmol) were added to the mixture. The mixture was stirred for 20 h. The solution was diluted with DCM (100 mL), washed with water (100 mL), saturated aqueous NaHCO_3 (100 mL) and again with water (2×100 mL). The organic layer was dried over anhydrous MgSO_4 , filtered and the solvent was removed from the filtrate by rotary evaporation to give crude ^{15}N -labelled (*R*)-(+)-lipoamide, which was further purified by silica gel flash column chromatography (DCM/methanol, 30:1). The solvent was removed by rotary evaporation and [^{15}N]-(*R*)-(+)-lipoamide was obtained as yellow solid (555 mg, 51% yield).

TLC R_f (DCM/methanol, 20:1) = 0.60; **MP** 131.5 °C; **^1H NMR** (400 MHz, CDCl_3) δ 5.60 (d, J = 88.0 Hz, 1H, NH_{cis}), 5.48 (d, J = 88.8 Hz, 1H, NH_{trans}), 3.57 (m, 1H, SSCH), 3.21 – 3.08 (m, 2H, SSCH_2), 2.49 – 2.42 (m, 1H, $\text{SSCH}_2\text{CH}_{\text{trans}}$), 2.23 (t, J = 8.0 Hz, 2H, CH_2CONH_2), 1.95 – 1.86 (m, 1H, $\text{SSCH}_2\text{CH}_{\text{cis}}$), 1.73 – 1.63 (m, 4H), 1.54 – 1.43 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CONH}_2$); **^{13}C NMR** (151 MHz, CDCl_3) δ = 175.3 (d, J = 13.6 Hz, CONH_2), 56.5 (SSCH), 40.4 (SSCH_2CH_2), 38.6 (SSCH_2), 35.7 (CH_2CONH_2), 34.7 ($\text{CH}_2\text{CH}_2\text{CONH}_2$), 29.0 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{CONH}_2$), 25.3 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CONH}_2$); **^{15}N NMR** (61 MHz, CDCl_3) δ = 102.1 (t, J = 88.7 MHz, CONH_2); **HRMS** (ESI^+) m/z [M^+] calcd for $\text{C}_8\text{H}_{15}\text{O}^{15}\text{NS}_2$, 207.0638; found 207.0637; **IR** (neat/ cm^{-1}): 3343m, 3178m, 2937w, 1653s, 1631s, 1462w, 1413s; **Optical rotation**: $[\alpha]_D^{25} +105.6$ ($c=2.0$, CHCl_3).

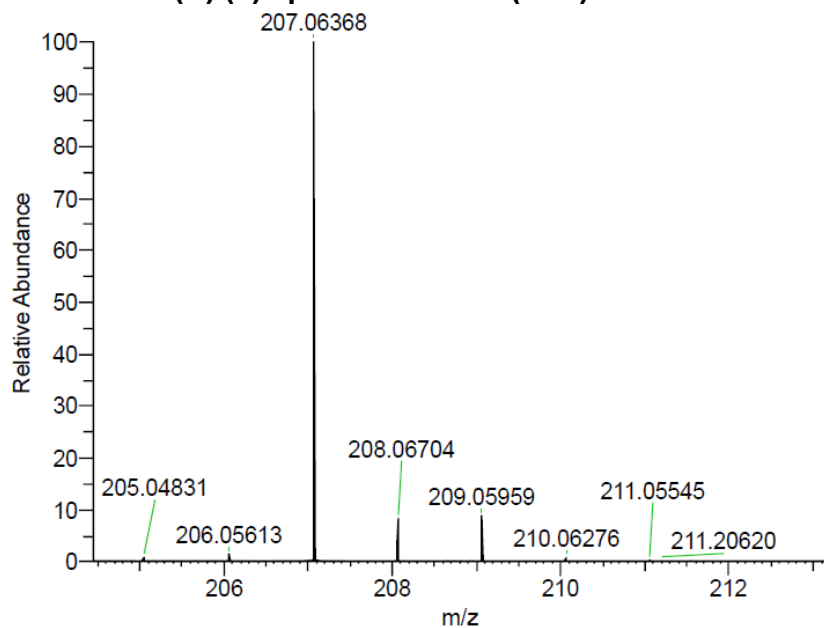
¹H NMR spectrum of compound **10** in CDCl₃. The x-axis represents the chemical shift (δ) in ppm, ranging from 0.0 to 10.0. The spectrum shows several peaks, with integrations provided for the main signals. A chemical structure of compound **10** is shown in the top right corner.

Chemical Shift (ppm)	Integration
~7.2	2.24
~3.5	1.00
~3.2	1.96
~2.3	1.06
~2.0	2.22
~1.8	1.16
~1.5	2.27

¹³C NMR spectrum (CDCl₃) of compound 10. The x-axis represents the chemical shift in ppm, ranging from -10 to 210. The spectrum shows a solvent triplet at 77.16 ppm and several peaks in the aliphatic region. Key peaks are labeled with their chemical shifts: 175.35, 175.26, 77.37, 77.16, 76.95, 56.51, 43.37, 38.60, 35.72, 34.25, 29.95, and 25.26.

13C NMR spectrum of compound 10. The x-axis represents chemical shift in ppm, ranging from 91 to 114. The spectrum shows three distinct peaks: a small peak at 103.59 ppm, a large central peak at 102.13 ppm, and a medium peak at 100.68 ppm. The baseline is relatively flat with some noise.

¹⁵N-labelled (*R*)-(+)-lipoamide HRMS (ESI⁺)



Formula (M)	Ion Formula	m/z	m/z (Calc)	Diff (ppm)
C ₈ H ₁₅ O [15]N S ₂	C ₈ H ₁₅ O [15]N S ₂	207.06368	207.06382	-1.38

HRMS data suggested that the ¹⁵N labelling efficacy is at ~99%.

References

- (1) Reed, L. J.; Koike, M.; Levitch, M. E.; Leach, F. R. Studies on the Nature and Reactions of Protein-Bound Lipoic Acid. *J. Biol. Chem.* **1958**, 232 (1), 143–158. [https://doi.org/10.1016/S0021-9258\(18\)70382-7](https://doi.org/10.1016/S0021-9258(18)70382-7).
- (2) Karunanithy, G.; Cnossen, A.; Müller, H.; Peeks, M. D.; Rees, N. H.; Claridge, T. D. W.; Anderson, H. L.; Baldwin, A. J. Harnessing NMR Relaxation Interference Effects to Characterise Supramolecular Assemblies. *Chem. Commun.* **2016**, 52 (47), 7450–7453. <https://doi.org/10.1039/C6CC02544G>.