Electronic Supplementary Material (ESI) for MedChemComm.

Supplementary Information

Bioorthogonal metabolic labelling with acyl-CoA reporters: targeting protein acylation

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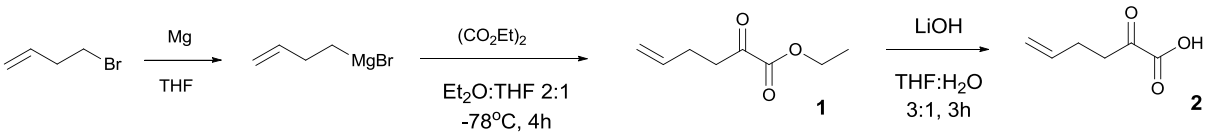
1. Synthesis of 2-oxo-hex-5-enoic acid and its ethyl ester



The synthesis of **1** was performed according to literature procedures.[1] Half of the crude product obtained from the Grignard reaction (40% in **1**) was further purified by flash column chromatography using petroleum ether:EtOAc 19:1 with gradient increase of the percentage of EtOAc to afford **1** as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.77-5.69 (m, 1H), 4.99-4.91 (m, 2H), 4.23 (q, *J* = 7.1 Hz, 2H), 2.86 (t, *J* = 7.2 Hz, 2H), 2.32-2.28 (m, 2H), 1.28 (t, *J* = 7.1 Hz, 3H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 193.7, 160.9, 136.1, 115.7, 62.3, 38.3, 26.9, 13.9 ppm.

The other half was dissolved in THF:H_2O 6:1, 5 eq. of LiOH were added and the solution was stirred for 1h at RT. The mixture was then extracted (2x) with EtOAc and the combined organic phases were dried over Mg_2SO_4 and evaporated, keeping the vacuum higher than 50 mbar at 40°C. The product **2** was obtained as a yellowish oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.83-5.75 (m, 1H), 5.08-5.01 (m, 2H), 3.02 (t, *J* = 7.2 Hz, 2H), 2.42-2.38 (m, 2H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 194.9(x2), 135.8, 116.3, 37.1, 27.0 ppm.

LC-MS: 129 $[M+H]^+$.

2. Metabolic labelling with reporters 1 and 2

CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) kit from Promega was used to test the viability of RAW 264.7 cells after 6h incubation with different concentrations of compounds **1** and **2** at 37 °C and 5% CO_2. 12.5 mM was chosen as the optimum concentration for cell viability.

The incubation of RAW264.7 cells with 12.5 mM of 2-oxo-hex-5-enoate (prepared as a sodium salt in d. H_2O) and ethyl 2-oxohex-5-enoate was performed for 6h at 37 °C and 5% CO_2. Untreated cells were used for negative control experiments. Cell lysis, histone extraction and biotinylation of lysates and extracted histones via the oxidative Heck reaction were performed as described previously.[2]

SDS-PAGE

17 μg of the cell lysates and 10 μg of histones samples were loaded on a SDS PAGE (15%) along with 5.0 μL of the protein ladder. Electrophoresis was performed for 1.5 h at 150 V.

Western blotting and Enhanced Chemiluminescence Assay were performed as described previously.[2] Only endogenous biotinylation of higher molecular weight proteins was observed.[2]

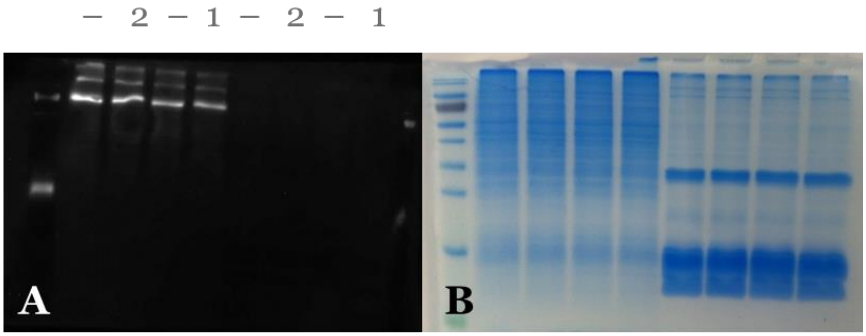


Figure S1. Detection of alkenylated lysates and histone proteins extracted from RAW 264.7 cells via the oxidative Heck reaction using a biotinylated phenylboronic acid. A) Luminescence imaging on PVDF membrane of lysates and histones after 6 h incubation with 12.5 mM of 1) ethyl 2-oxohex-5-enoate and 2) sodium 2-oxo-hex-5-enoate. Blank reactions in absence of the reporters are also shown. B) Coomassie staining of A).

3. NMR spectra

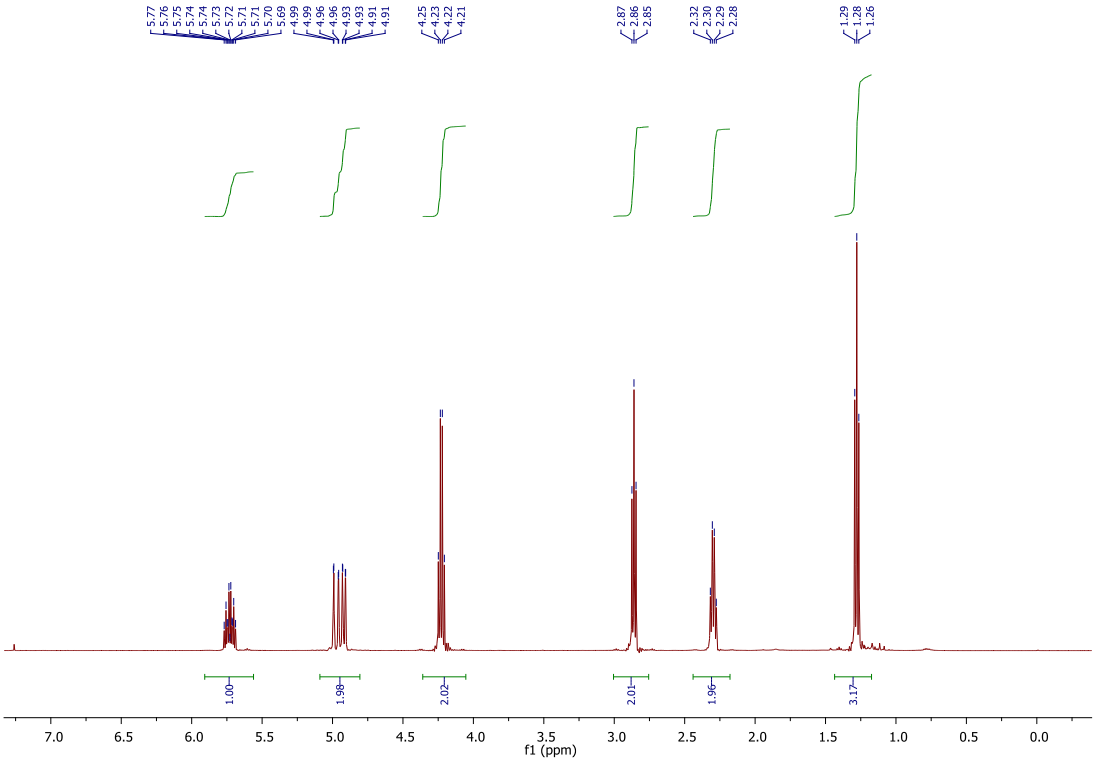


Figure S2. ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound **1**.

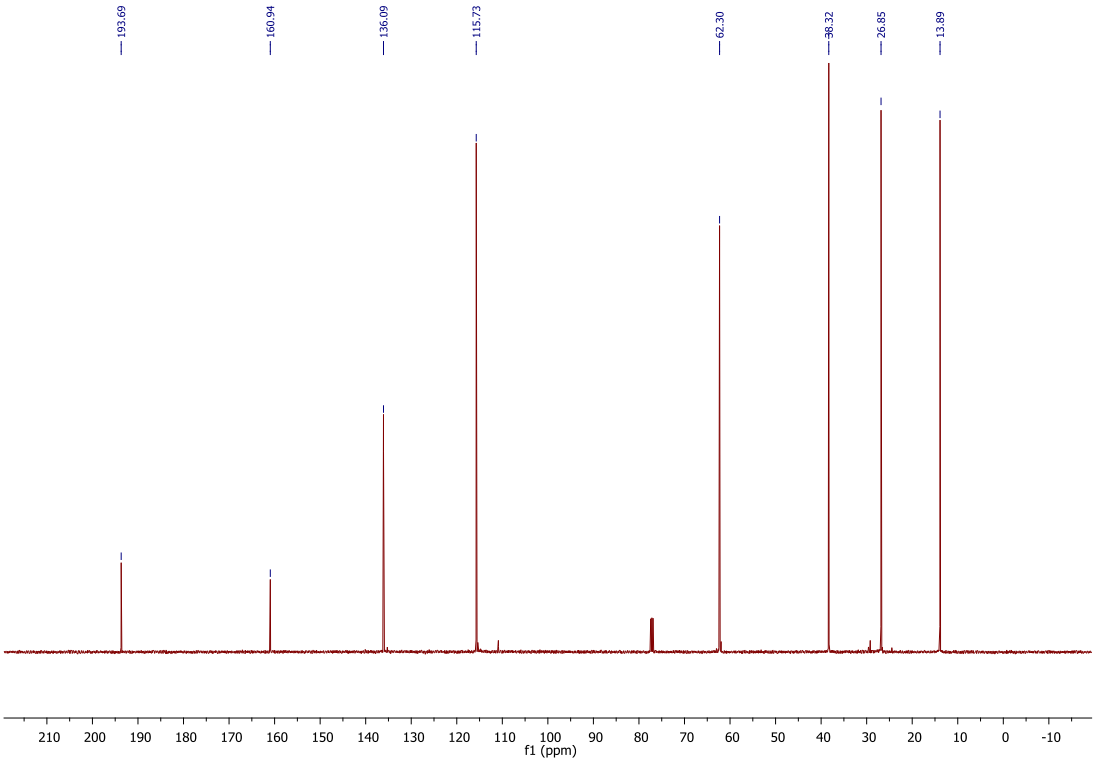


Figure S3. ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of compound **1**.

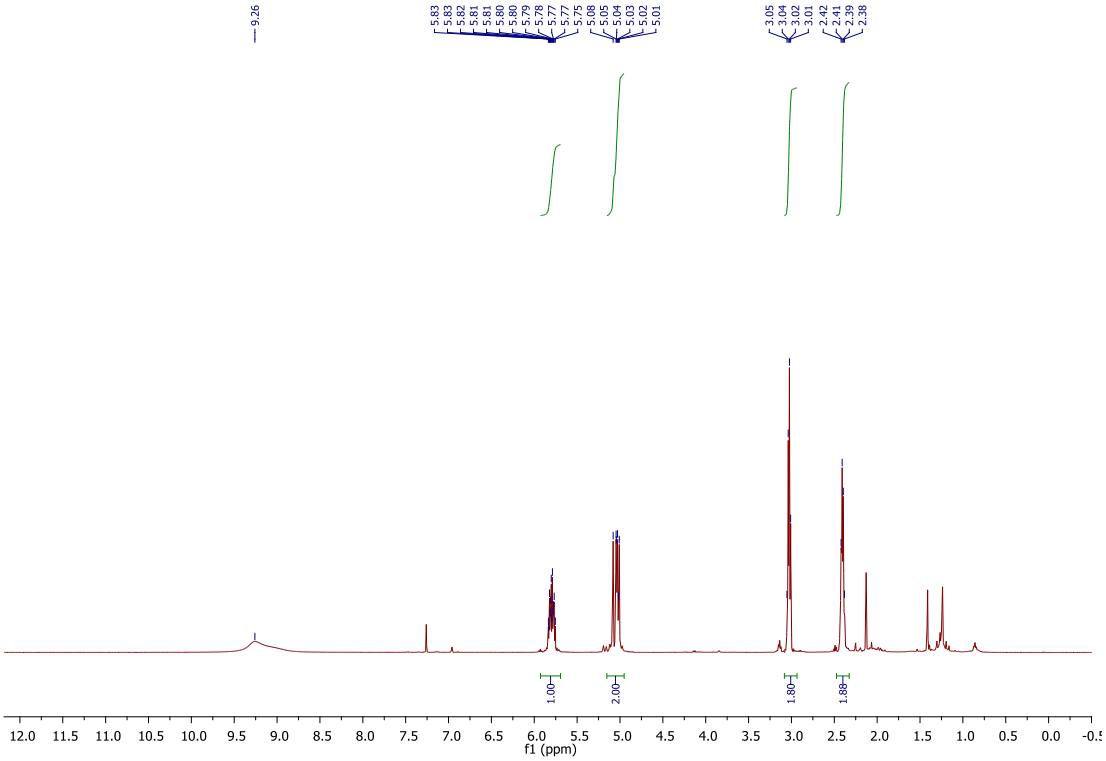


Figure S4. ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound **2**.

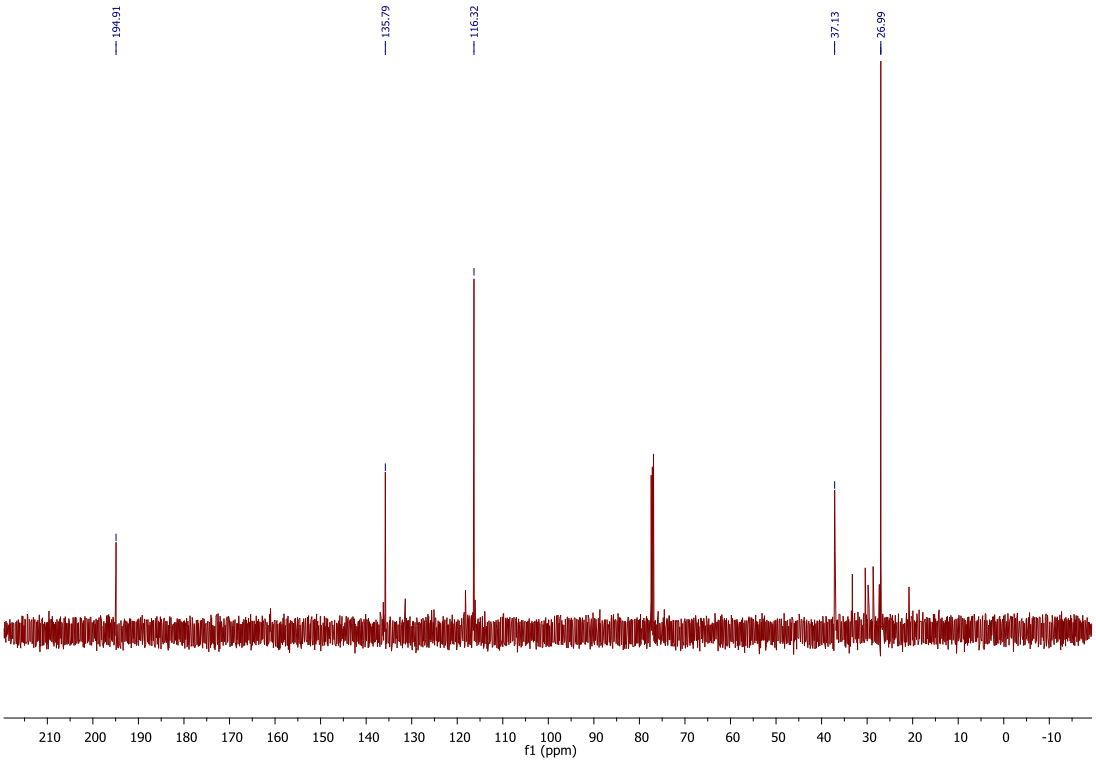


Figure S5. ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of compound **2**.

4. References

1. J. T. Moore, N. V. Hanhan, M. E. Mahoney, S. P. Cramer and J. T. Shaw, *Org. Lett.*, 2013, **15**, 5615–5617.

2. M. E. Ourailidou, P. Dockerty, M. Witte, G. J. Poelarends and F. J. Dekker, *Org. Biomol. Chem.*, 2015, **13**, 3648–3653.