Supporting Information

Optimisation of chemical protein cleavage for erythropoietin semi-synthesis using native chemical ligation

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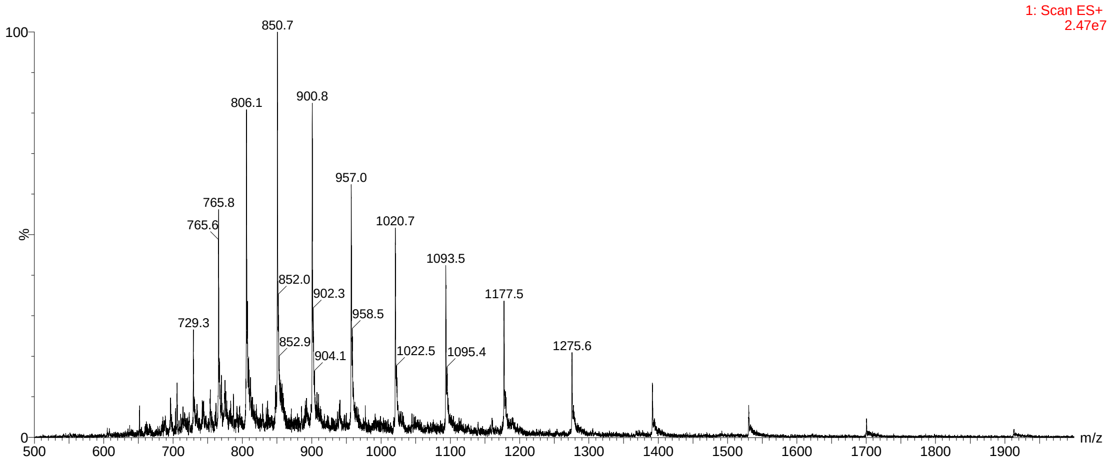
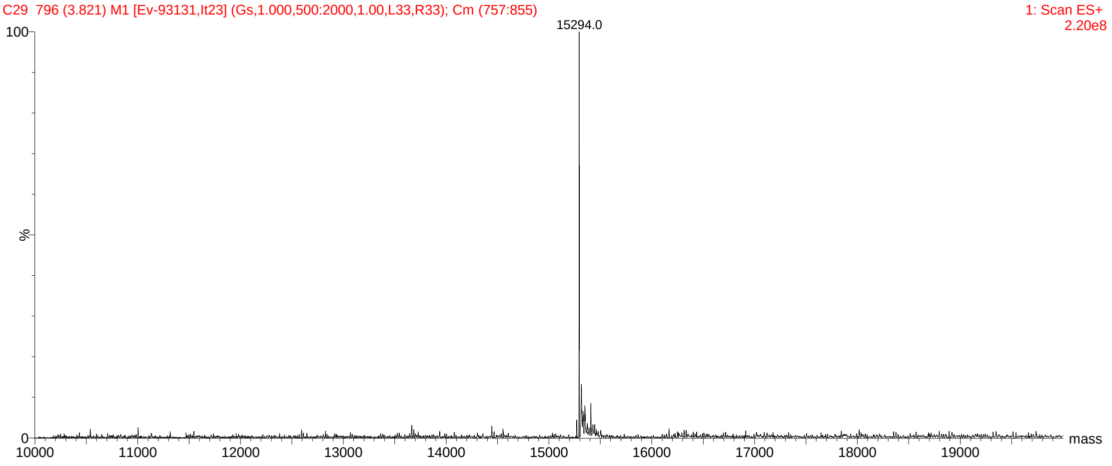
Peptide sequence (EPO 29-166) expressed for NCL studies, calculated average mass after CNBr cleavage = 15292.7. This protein contains 3 mutations M54L, N83K and S131K (the later 2 mutations were introduced to aid solubility)[1]

CAEHC SLNENITVPD TKVNFYAWKR **L**EVGQQAVEV WQGLALLSEA
VLRGQALLV**K** SSQPWEPLQL HVDKAVSGLR SLTTLLRALG AQKEAI**K**PPD
AASAAPLRTI TADTFRKLFR VYSNFLRGKL KLYTGEACRT GDR

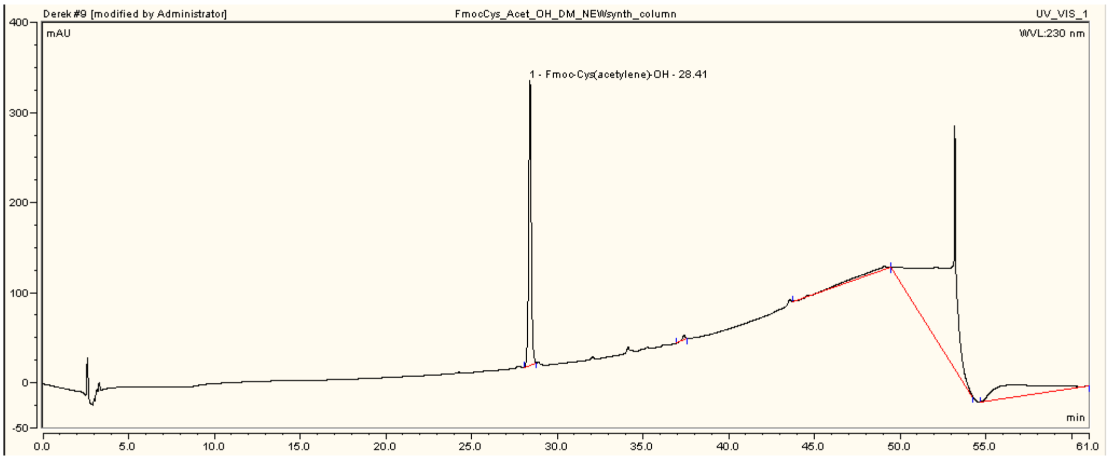
Mass spectrum (raw data) for Bacterial fragment used in CN-Br optimisation studies



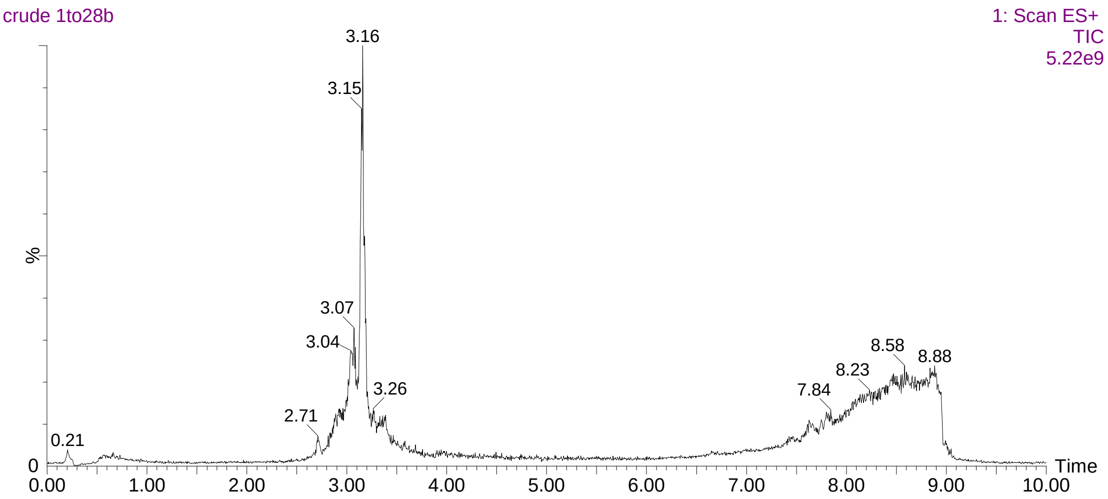
Devonvoluted mass:

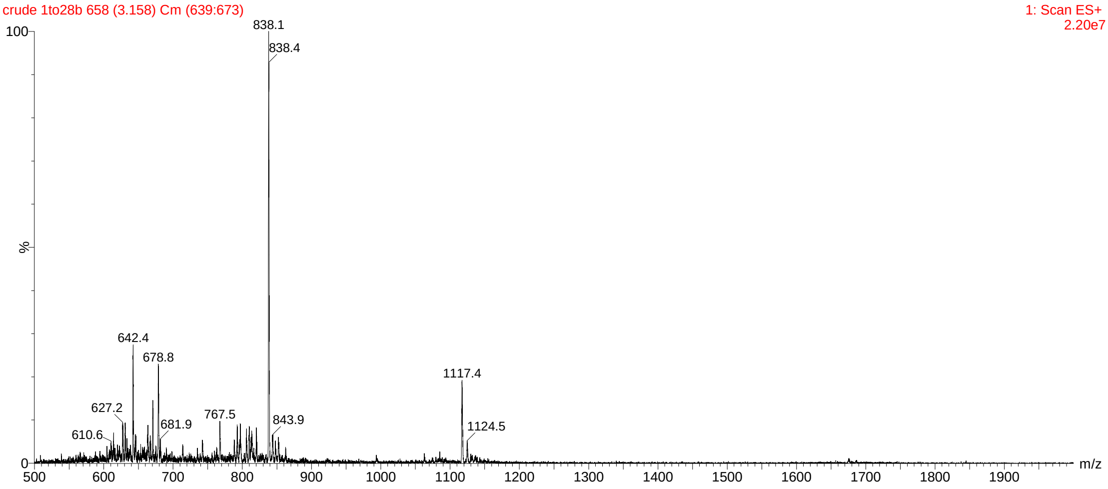


HPLC trace of purified acetylenic amino acid **1**:

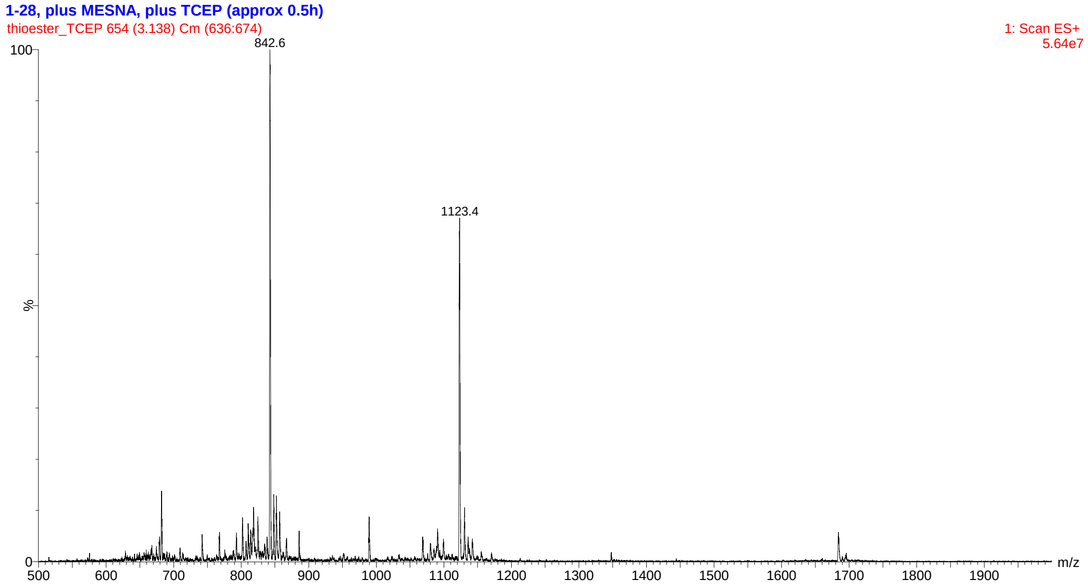


LC-MS of crude EPO residues 1-28 S-benzyl thioester (**2**) prior to HPLC purification.





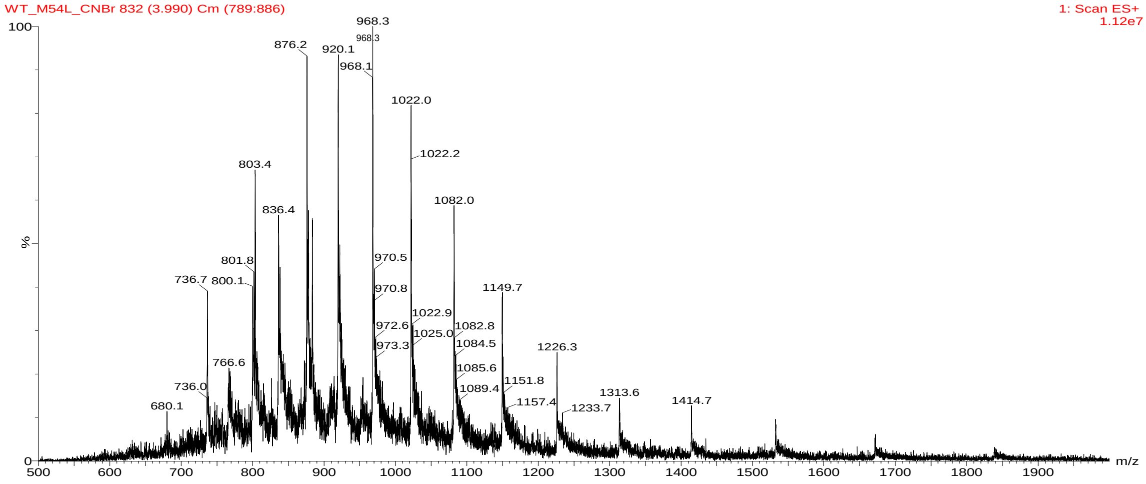
HPLC Purified **2** redissolved in 6 M guanidine hydrochloride containing 300 mM sodium phosphate buffer (pH 8.0), 2 % w.v MESNa and 20 mM TCEP.



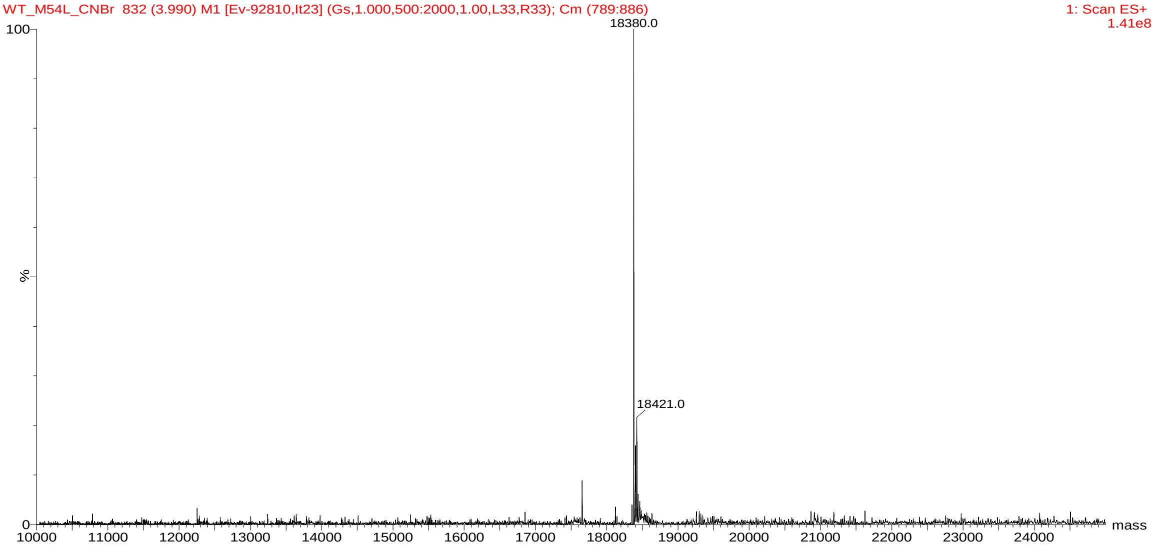
EPO M54L sequence after CNBr cleavage calculated average mass = 18378.1 Da.

APPRLICDSR VLERYLLEAK EAENITTGCA EHCSLNENIT VPDTKVNFYA
WKR**L**EVGQQA VEVWQGLALL SEAVLRGQAL LVNSSQPWEP
LQLHVDKAVS GLRSLTTLLR ALGAQKEAIS PPDAASAAPL RTITADTFRK
LFRVYSNFLR GKLKLYTGEA CRTGDR

Mass spectrum of bacterially produced EPO (M54L) after CNBr treatment and deformylation:



Deconvoluted mass:



1. Cheetham, J. C.; Smith, D. M.; Aoki, K. H.; Stevenson, J. L.; Hoeffel, T. J.; Syed, R. S.; Egrie, J.; Harvey, T. S., NMR structure of human erythropoietin and a comparison with its receptor bound conformation. *Nature Structural Biology* **1998,** 5, (10), 861-866.