Supplementary figure legends

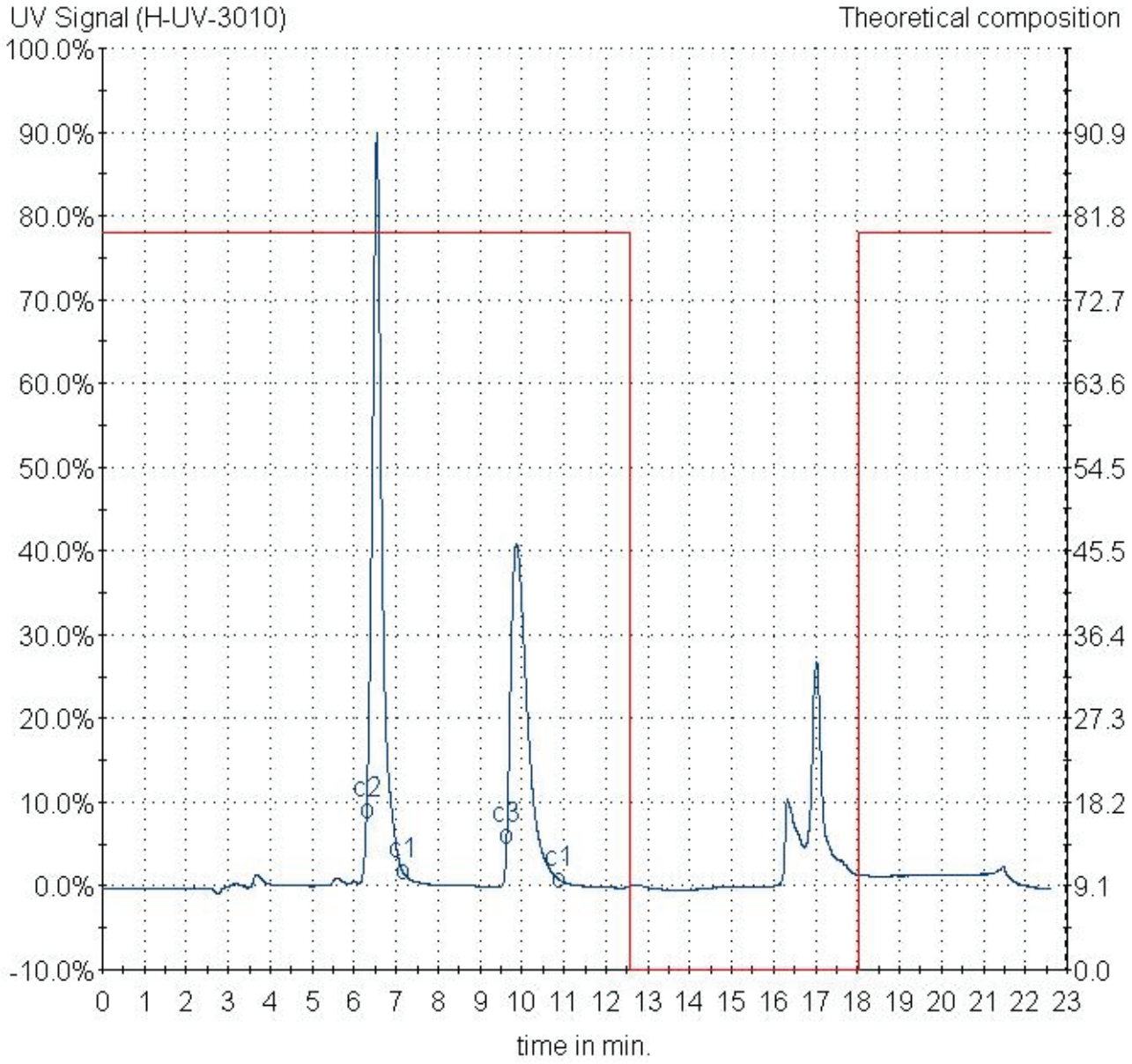
Supplementary figure 1. Representative chromatogram of preparative HPLC runs; fractions containing corresponding peaks at RT 6.2 to 7.3 min (peak 1) and RT 9.6 to 10.8 min (peak 2) were collected and used for further experiments. Red line denotes the theoretical composition of the mobile phase with "pump A" ratio marked on the right y-axis.

Supplementary figure 2. LC-MS analysis of a sample, taken from the reaction mixture of allylmalonyl-SNAC thioester synthesis before preparative HPLC. **A)** UV chromatogram **B)** MS chromatogram represented as total ion count. In both chromatograms two main peaks are visible, »peak 1« at 2.50 min and »peak 2« at 2.94 min. **C)** Fragments of "peak1" with retention time 2.50 min determining allylmalonyl-SNAC monothioester (**10**) with m/z 246 ($[M+H]^+$) and sodium adduct ($[M+Na]^+$) with m/z 268. **D)** Fragments of "peak2" with retention time 2.94 min determining allylmalonyl-diSNAC double thioester (**11**) with m/z 347 ($[M+H]^+$) and sodium adduct ($[M+Na]^+$) with m/z 369.

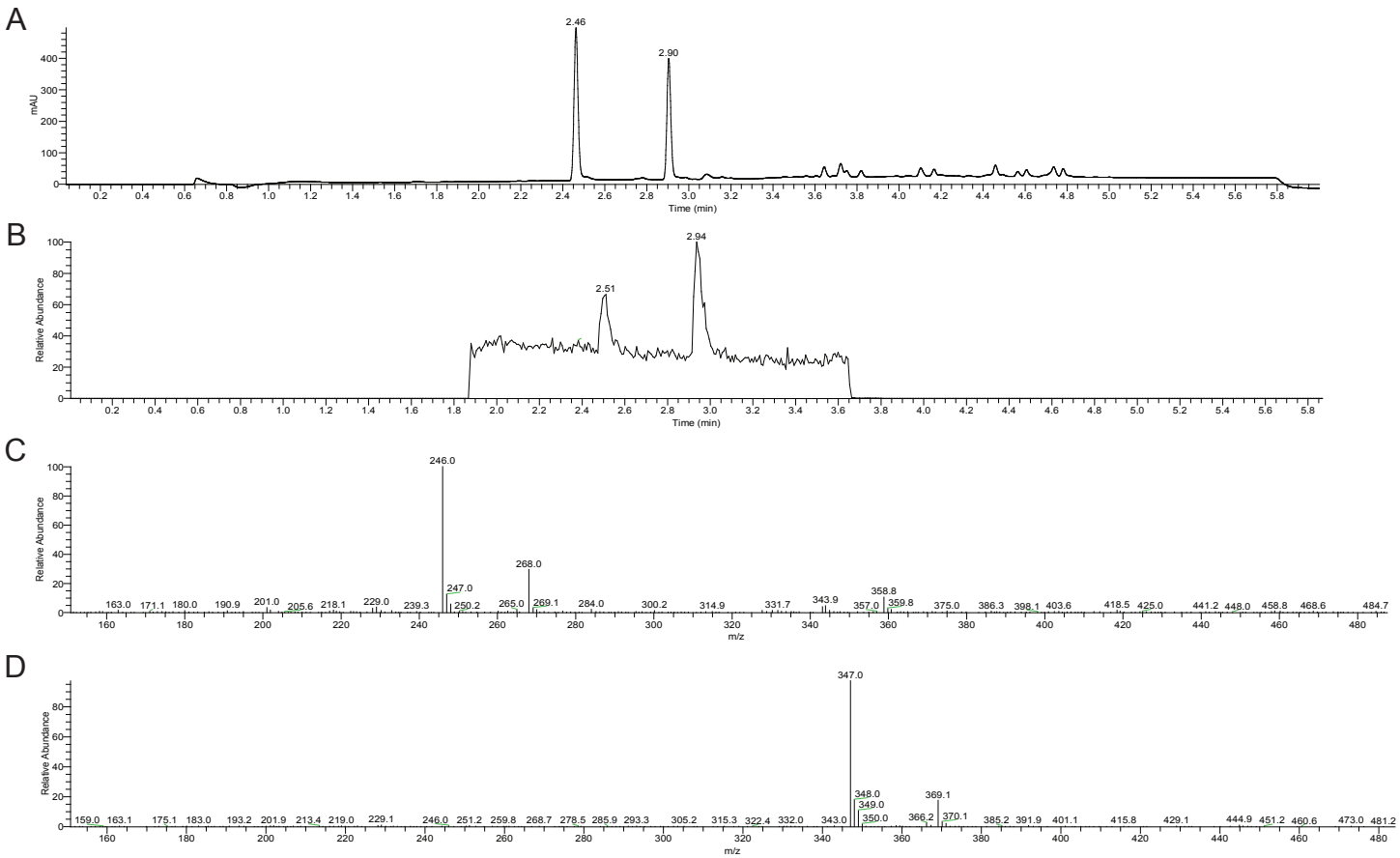
Supplementary figure 3. **A)** 1H NMR-spectrum of isolated material from fractions of the "peak 1" corresponding to the structure of (2-((2-acetamidoethylthio)carbonyl)pent-4-enoic acid. **B)** 1H NMR-spectrum of isolated material from fractions of the "peak 2" corresponding to the structure of (S,S-bis(2-acetamidoethyl)-2-allylpropanebis (thioate).

Supplementary figure 4. Representative multiple reaction monitoring (MRM) mass chromatograms of FK506 in the following samples: **a)** purified FK506 standard, **b)** cultivation broth extract of the wild type strain, **c)** cultivation broth extract of *allK* inactivated strain, **d)** cultivation broth extract of *allK* inactivated strain after feeding with allylmalonyl-SNAC (**10**) and **e)** cultivation broth extract of *allK* inactivated strain after feeding with allylmalonyl-diSNAC (**11**).

Supplementary figure 1

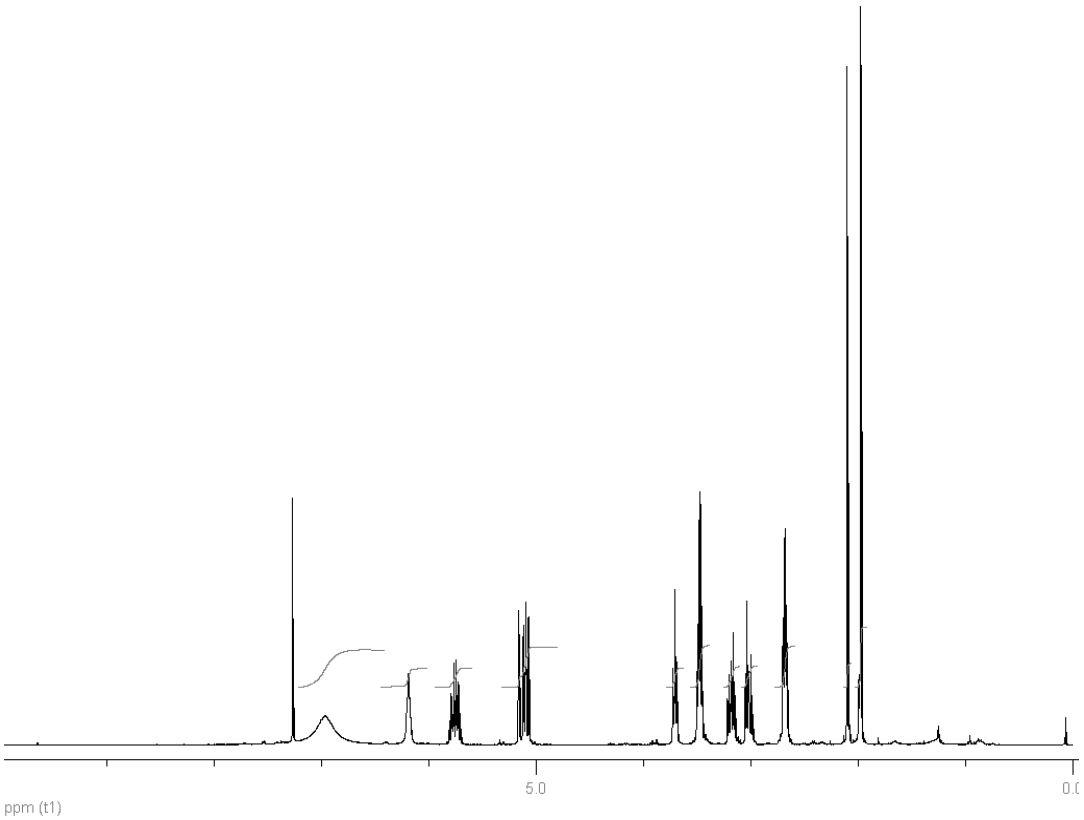


Supplementary figure 2

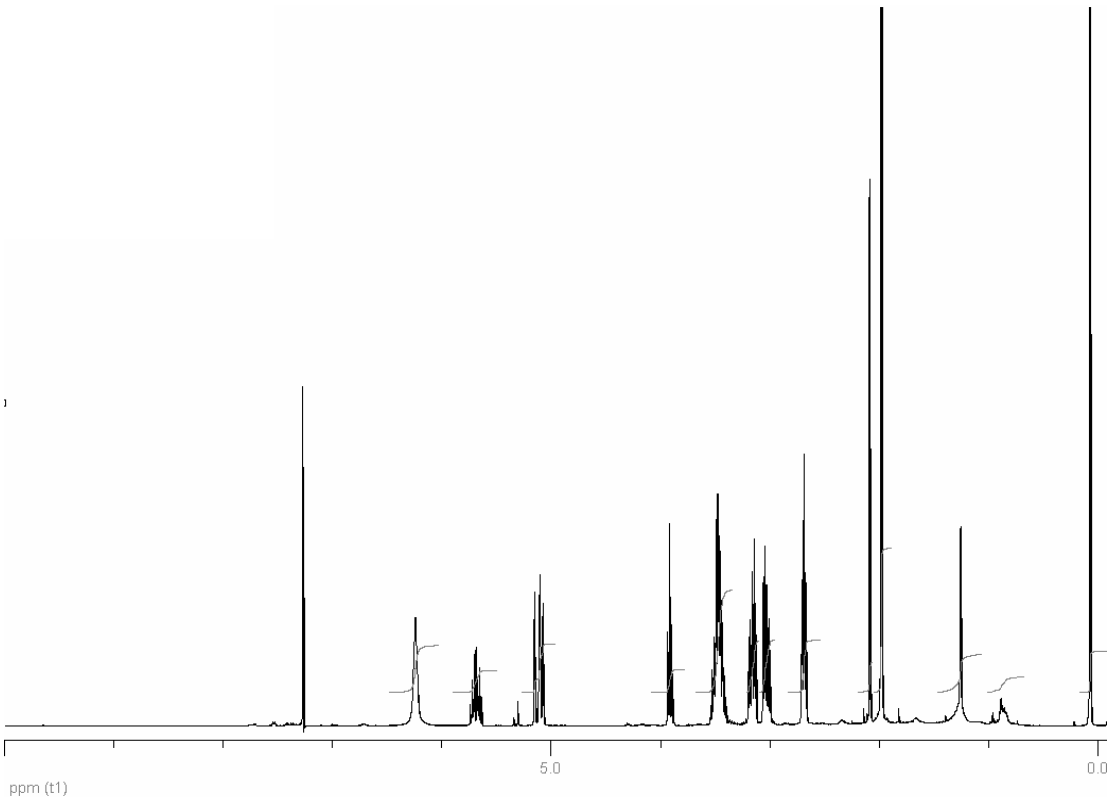


Supplementary figure 3

A



B



Supplementary figure 4