Supporting Information

for

Functional Characterization of the recombinant \( N \)-Methyltransferase Domain from the Multienzyme Enniatin Synthetase

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Results

**MALDI-TOF analysis of \( N \)-Me-\( L \)-Val-SNAC formation by ENMT**

The reflector spectra of the sample as well as of the standard substance \( N \)-Me-\( L \)-Val-SNAC contain, beside a number of peaks origination from the matrix DHB, a peak at \( m/z \) 233, which corresponds to the protonated molecular mass of the methylated \( L \)-Val-SNAC variant (spectrum 1 and 2).

The PSD spectrum of the \( N \)-Me-\( L \)-Val-SNAC standard shows, beside a peak at \( m/z \) 72, a characteristic fragment ion at \( m/z \) 86, which was used as the diagnostic marker for the presence of a methylated valine fragment (spectrum 3 and 4). The unmethylated variant \( L \)-Val-SNAC on the other hand only produced a fragment ion at \( m/z \) 72, indicative for the presence of an unmethylated valine fragment (spectrum 5).
Figure S1. Positive ion MALDI-TOF mass spectra of N-Me-L-Val-SNAC and ENMT products.
**Saturation transfer difference (STD) NMR spectroscopy**

STD measurements were carried as described in the Experimental Section.

**Figure S2.** A) $^1$H spectrum of 2 mM AMP in the presence of 10 µM ENMT and assignment of signals. B) Saturation transfer difference (STD) spectra.

**Figure S3.** Structural formula of AMP
Figure S4. A) $^1$H spectrum of 0.1 mM AdoMet in the presence of 5 µM BSA and assignment of signals. B) Saturation transfer difference (STD) spectra. For this experiment we used a 5 mm $^1$H/$^{13}$C micro probehead. The molar ratio of AdoMet/BSA was 20:1.