Supporting information

Streptomyces albus: a New Cell Factory for Non-Canonical Amino Acids Incorporation into Ribosomally Synthetized Natural Products

Mariia Lopatniuk[1], Maksym Myronovskyi[1], and Andriy Luzhetskyy[1,2]*

1. Department of Pharmaceutical Biotechnology, Saarland University, 66123 Saarbrücken, Germany
2. Helmholtz-Institute for Pharmaceutical Research Saarland, Campus, Building C2.3, 66123 Saarbrücken, Germany

Antibiotic conc. µg/ml / Sample name	0	62.5	31.3	15.6	7.81	3.91	1.95	0.98	0.49	0.24	0.12	0
cinnamycin	0.04	0.042	0.038	0.062	0.04	0.052	0.063	0.049	0.053	**1.071**	**1.247**	**1.153**
deoxycinnamycin	0.033	0.044	0.049	**1.305**	**0.994**	**1.037**	**0.876**	**1.318**	**1.131**	**1.323**	**1.253**	**1.349**
deoxycin-10Alk	0.04	0.064	**0.841**	**1.259**	**1.179**	**1.229**	**1.19**	**1.19**	**1.258**	**1.295**	**0.894**	**1.057**
deoxycin-2Alk	0.038	0.068	0.088	0.051	**1.4**	**1.137**	**1.065**	**1.255**	**1.281**	**1.094**	**1.416**	**1.029**

Table S1. MIC analysis of the new cinnamycins compared to cinnamycin and deoxycinnamycin against the test-culture *Bacillus subtilis*. The viability of the strain was detected by optical density of the culture. Values lower 0.1 were considered as not viable, and values higher than 0.8 correspond to a growing culture, indicating resistance.

Primer name	Primers sequences, direction 5´-3´
CinL-Hind-for	AAAAAAGCTTCTTGTAGAAGGCGAGCATCG
CinL-Bgl-rev	ATGGGACCCGACCACGCCCAGATCTGCTCCTCGGGCACGAGCCG
CinR-Bgl-for	CGGCTCGTGCCCGAGGAGCAGATCTGGGCGTGGTCGGGTCCCAT
CinR-Not-rev	AAAAGCGGCCGCCGTCGGAGGTGGTGAAGAG
FTAG-for	GAGCTGCAGCTTCGGCCCGTAGACCTTCGTGTGCGACGGCA
FTAG-rev	TGCCGTCGCACACGAAGGTCTACGGGCCGAAGCTGCAGCTC
RTAG-for	CACGGAAGCCTTCGCCTGCTAGCAGAGCTGCAGCTTCGGCC
RTAG-rev	GGCCGAAGCTGCAGCTCTGCTAGCAGGCGAAGGCTTCCGTG
Cin1-for	TGGCAGAACAACAGCGACA
Cin2-rev	GTGGAACTTGTCGGCCAGT
CinXdel-for	CTCGACCCGGCCCGGTTCGGGCGCGAGATGAAGGCGGTCACGGA GGTACCAATACTTGACATATCACTGT
CinXdel-rev	GGTTGGCGATGCAGTGCCAGAAGAGCTTCTCCGGGTCCTGCTCCA CCTTGGGTACCTCAGGCGCCGGGGGCGGTG
cinXchk-for	TGATCGGCATCTCCGGGTTC
cinXchk-rev	CGGTCCTGCTCCGAACATC
CinCat-for	GGTTCATGTGCAGCTCCATCAGCAAAAGGGGATGATAAGTTTATC ACCACTGTGGTGTGACCGGAACAG
CinCat-rev	CCGACGCCCGTACAACCGTCGGCCTGCCTCGCGGGTCTAACGAAA ATGGAAGTGTAAAGCCTGGGGTGC
CinHyg-for	CGTCCCTTGACTTCTGGACCAAGGACATCGCCGCCACGGAAGCCT TCGCCGTTTAAACAATACTTGACATATCACTGT
CinHyg-rev	CGCGTCATCGCACCGGGCGAGCATTACCGCCTAGAGGCAGCAGC CACTTAGTTTAAACTCAGGCGCCGGGGGCGGTGT

Table S2. Oligonucleotides sequences used for amplification reaction

Figure S.1. Bioassay of two *S. albus* strains, harboring the plasmids pCinCatInt and pCindelX, against *Bacillus subtilis*.

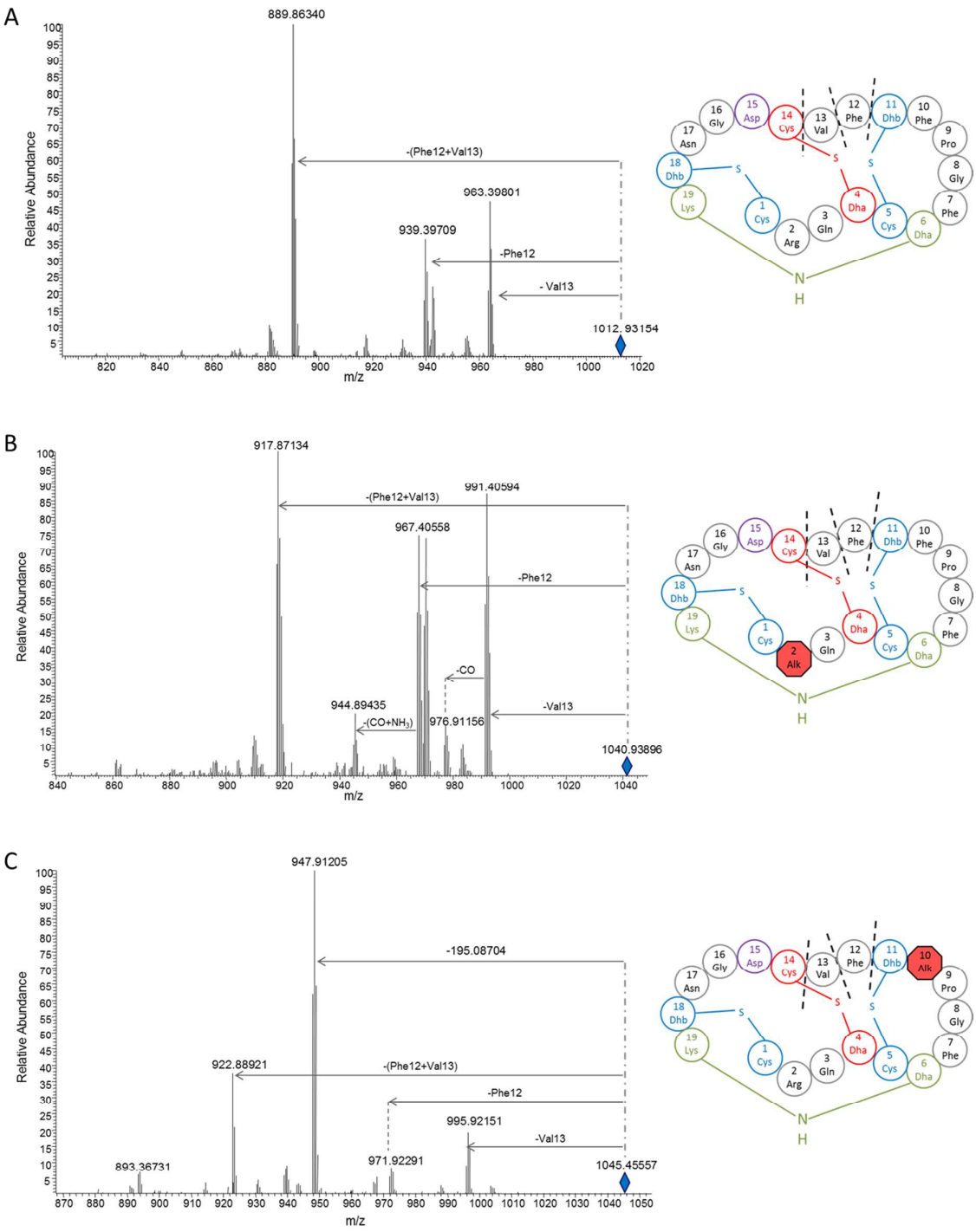

Figure S.2. MS/MS fragmentation analysis of deoxycinnamycin, deoxycin-10Alk and deoxycin-2Alk; positive mode ionization. Fragmentation positions are indicated with dashed lines. (A) Fragmentation of the deoxycinnamycin triple-charged ion with a mass $[2023.848 + 3H]^{3+} = 675.623$ Da. (B) Fragmentation of the deoxycin-10Alk triple-charged ion with a mass $[2088.896 + 3H]^{3+} = 697.306$ Da. (C) Fragmentation of the deoxycin-2Alk triple-charged ion with a mass $[2079.862 + 3H]^{3+} = 694.295$.

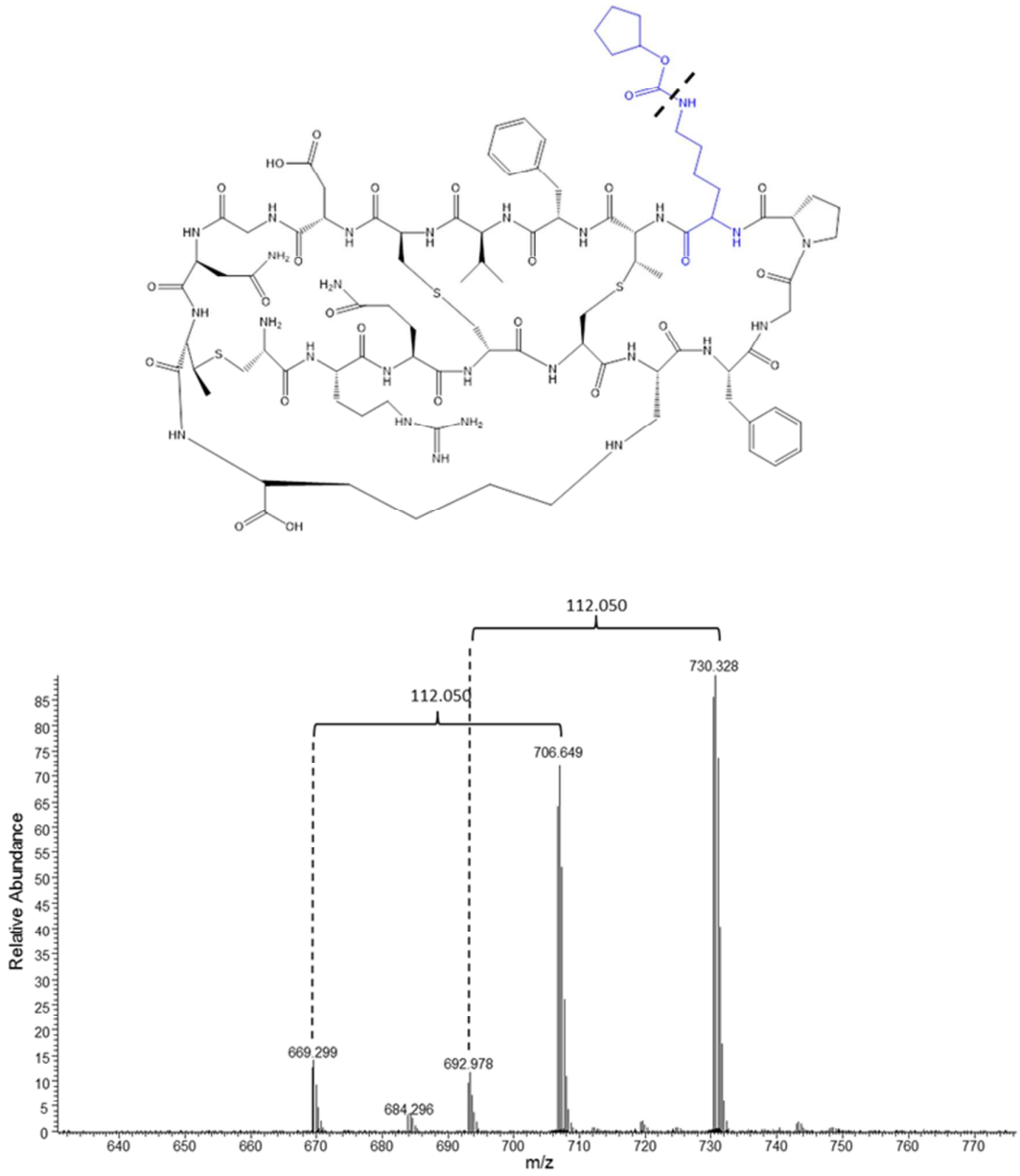

Figure S.3. Non-selective fragmentation of deoxycin-10Cyc and its butanol adduct during ionization. Loss of a mass of 112,050 Da from the triple-charged ion $[2116.925 + 3H]^{3+}$ = 706.649 Da and its butanol adduct $[2116.925 + But + 3H]^{3+}$ = 730.328 Da correspond to the loss of the cyclopentyl formate group of the Cyc residue. The cleavage position is indicated with dashed line.

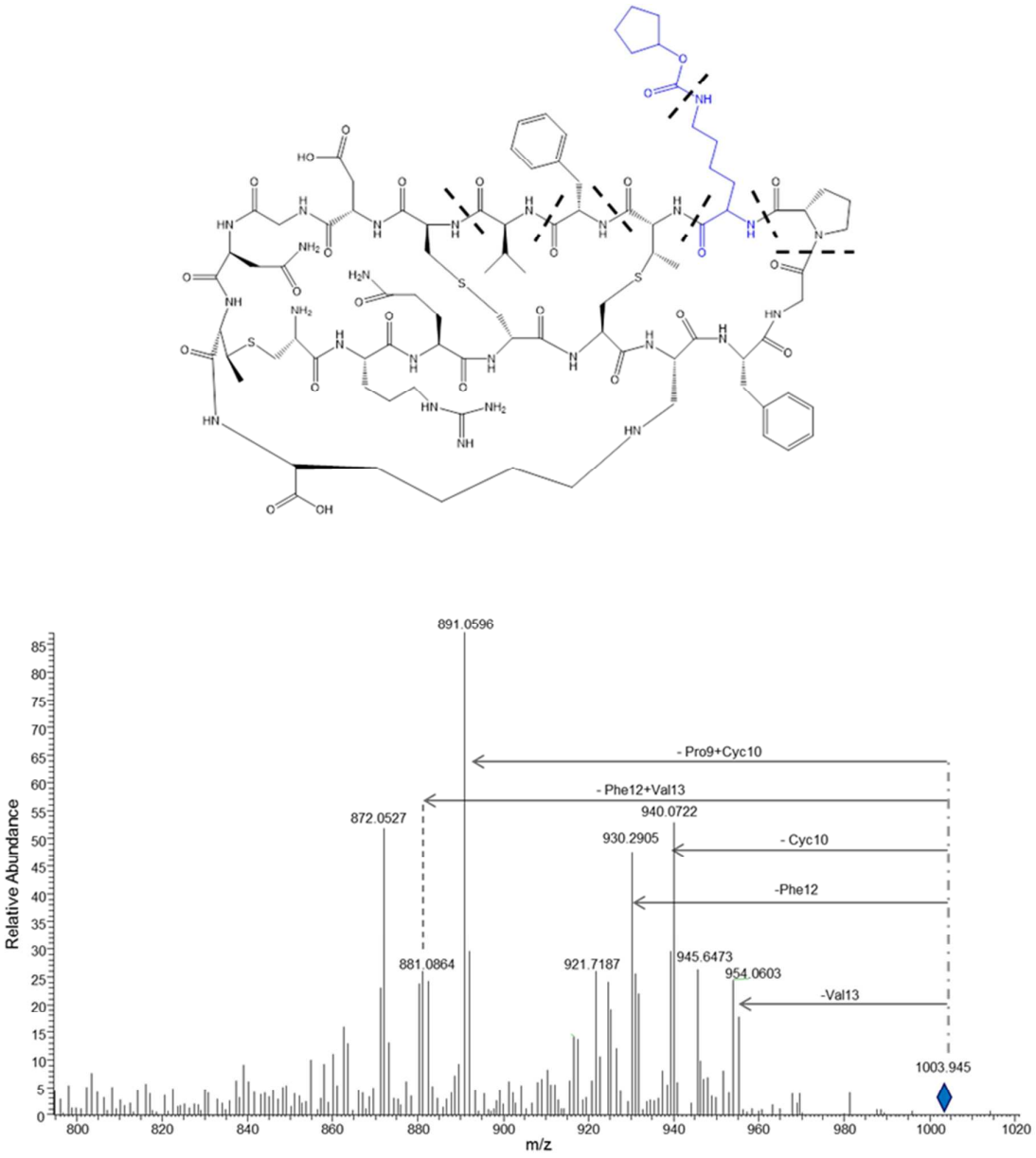

Figure S.4. MS/MS fragmentation analysis of the triple-charged ion of deoxycin-10Cyc, with a mass $[2004.875 + 3H]^{3+} = 669.299$, lacking the cyclopentyl formate group; positive mode ionization. Fragmentation positions are indicated with dashed lines.

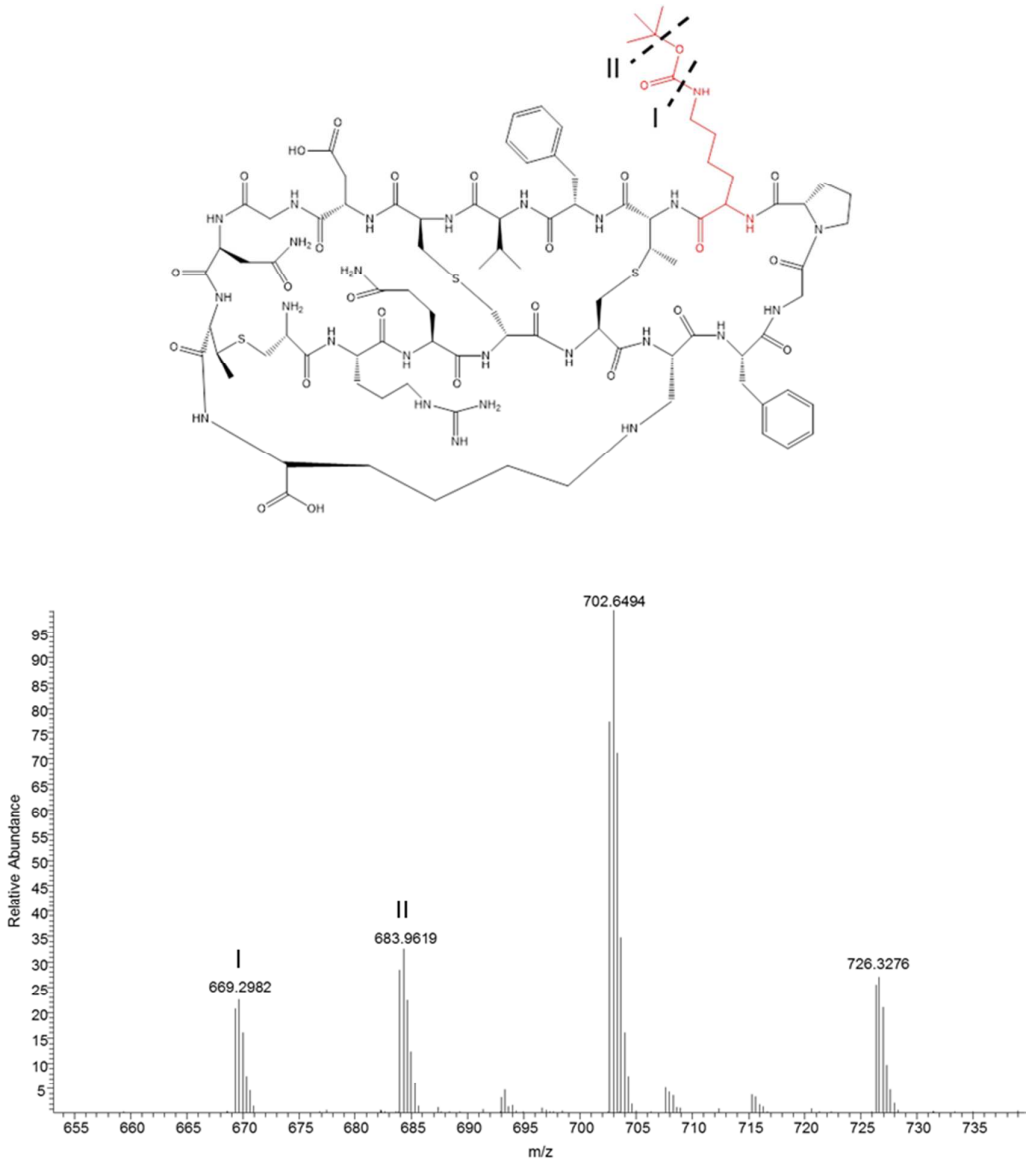

Figure S.5. Non-selective fragmentation of deoxycin-10Boc during ionization. Cleavage positions are indicated with dashed lines.