Electronic Supporting Information to:

New insights into ergot alkaloid biosynthesis in *Claviceps purpurea*: An agroclavine synthase EasG catalyses, *via* a non-enzymatic adduct with reduced glutathione, the conversion of chanoclavine-I aldehyde to agroclavine

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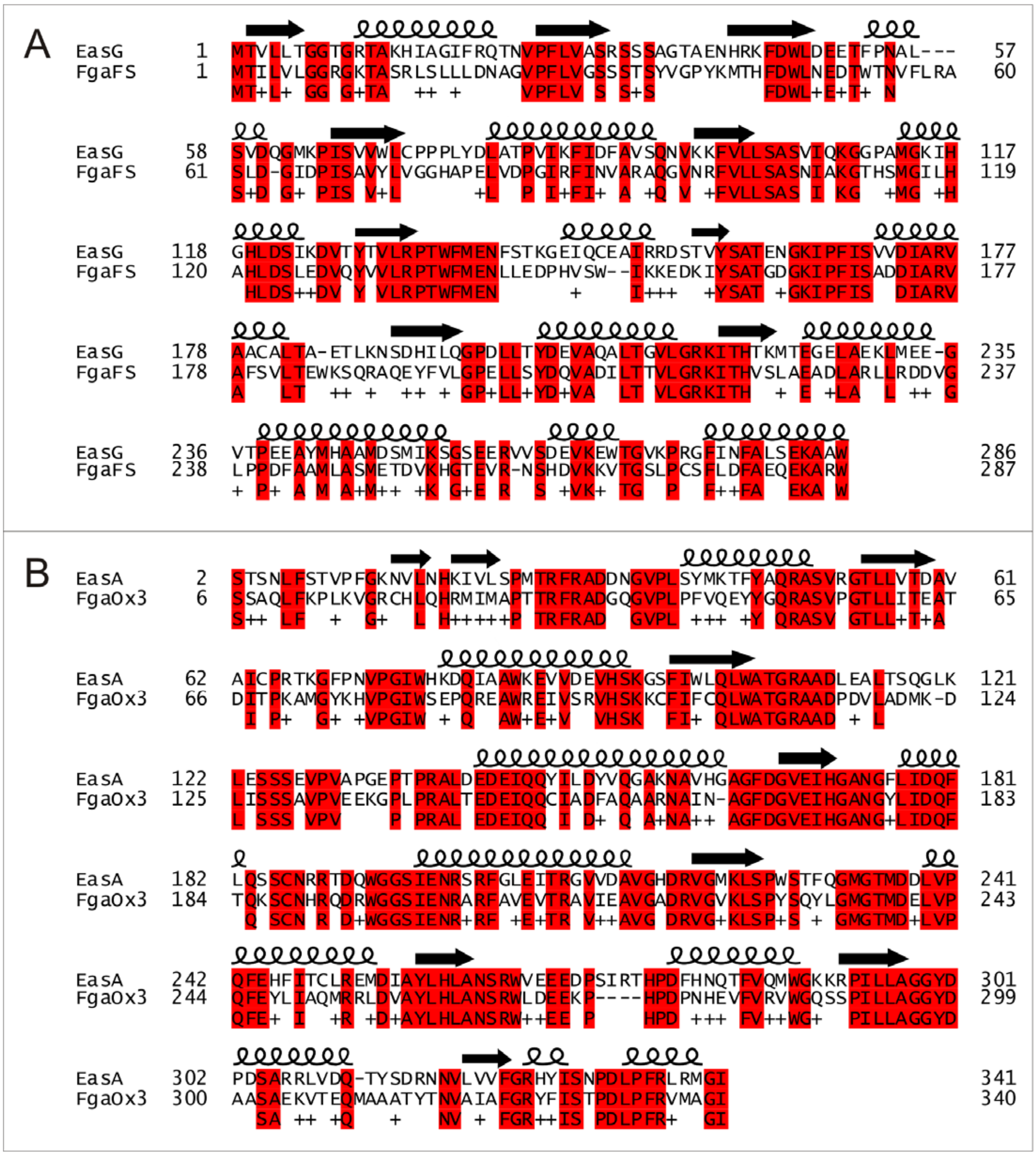

Figure S1: Alignments of the protein sequences of A) EasG and its homologue FgaFS and B) EasA and its homologue FgaOx3. Alignments were created by combination of Blast and SSEA.[1] The secondary structure was predicted by the program YASPIN.[2] Conserved amino acids are shown in red and conserved secondary structure elements are shown as helices and arrows. The score of the alignment of the secondary structures was in case of EasG and its homologue FgaFS 91 and in case of EasA and its homologue FgaOx3 94.

Reference List

1 P. Fontana et al., *Bioinformatics.*, 2005, **21**, 393-395.

2 K. Lin et al., *Bioinformatics.*, 2005, **21**, 152-159.

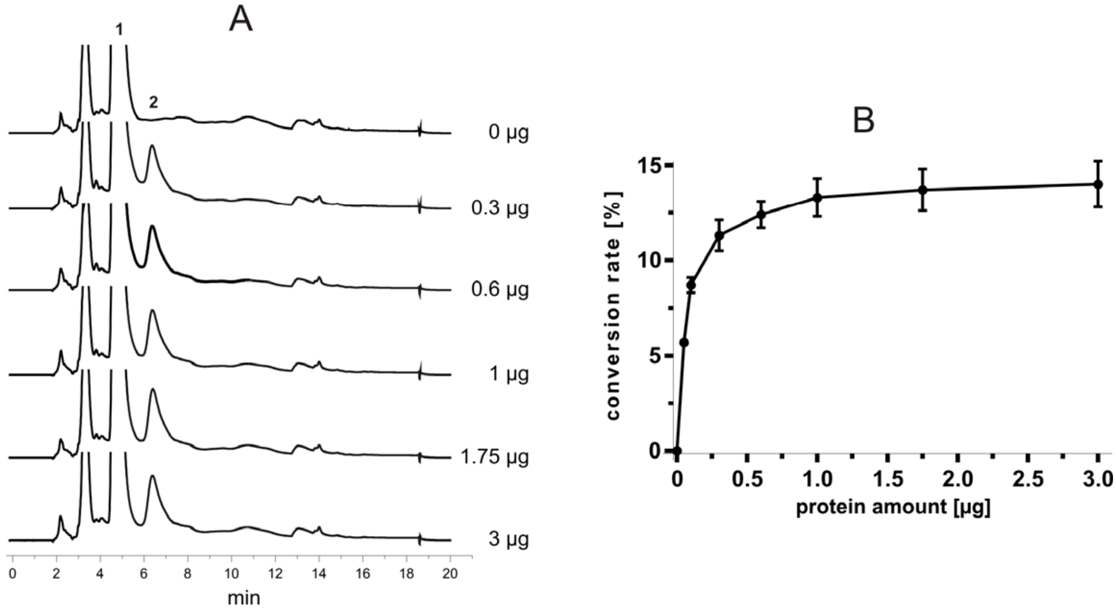

Figure S2: Dependence of the product formation of the EasG reaction on protein amounts. The assays (100 µl) contained 1 mM chanoclavine-I aldehyde, 1.5 mM NADPH, 1 mM GSH, and different amounts of EasG. The incubation time was 10 min.

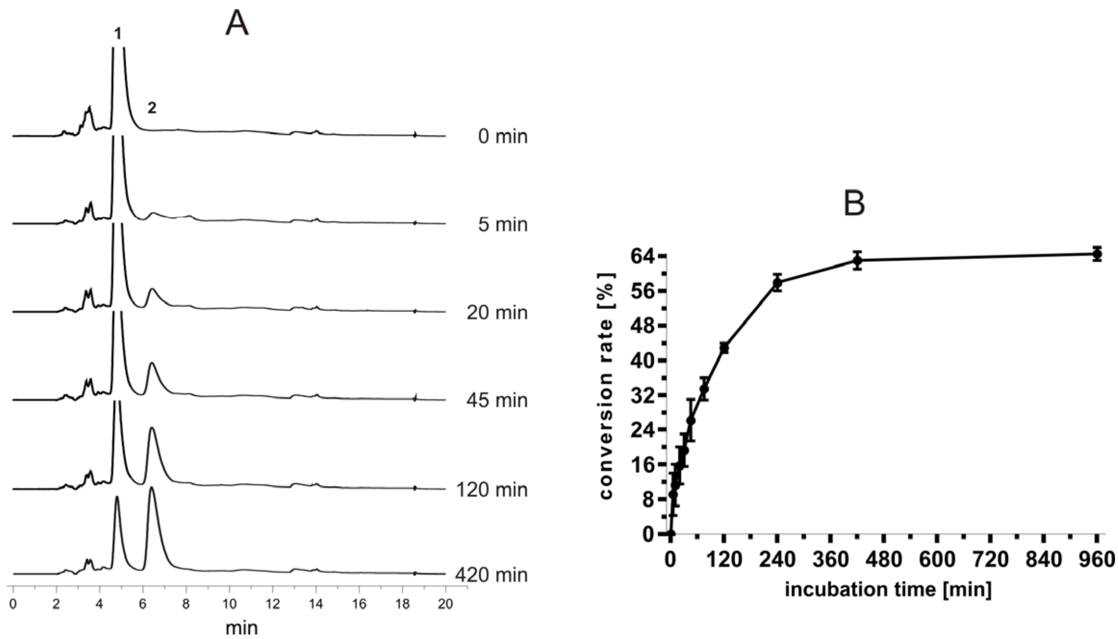

Figure S3: Dependence of the product formation of the EasG reaction on incubation time. The assays (100 µl) contained 1 mM chanoclavine-I aldehyde, 1.5 mM NADPH, 1 mM GSH and 0.1 µg (6.1 µM) of EasG.

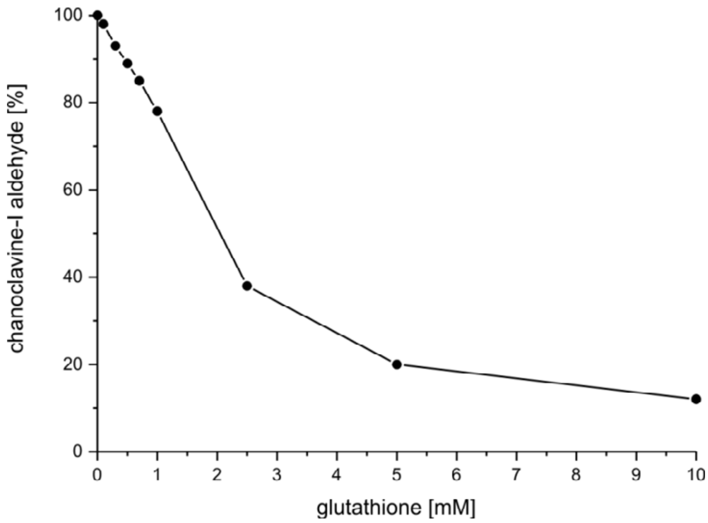

Figure S4: Dependence of the decreasing of chanoclavine-I aldehyde on the concentrations of GSH. The reaction mixtures (100 µl) contained 1 mM chanoclavine-I aldehyde and GSH at different concentrations. The incubation time was 80 min.

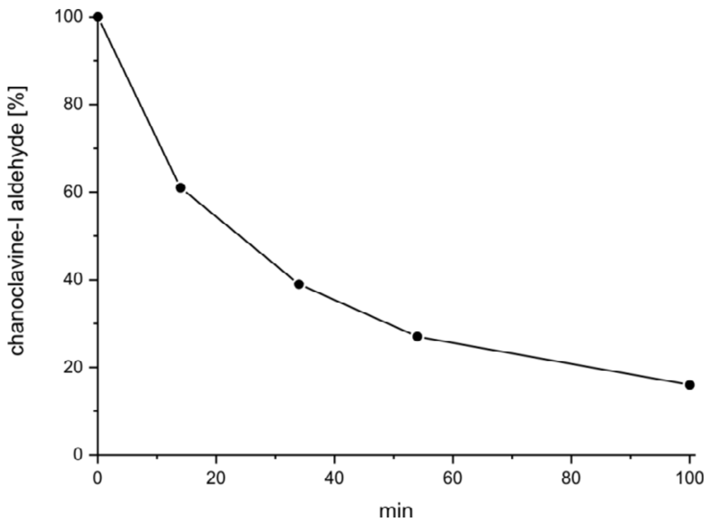

Figure S5: Dependence of the decreasing of chanoclavine-I aldehyde on reaction time with GSH. The assays (100 µl) contained 1 mM chanoclavine-I aldehyde and 5 mM GSH.

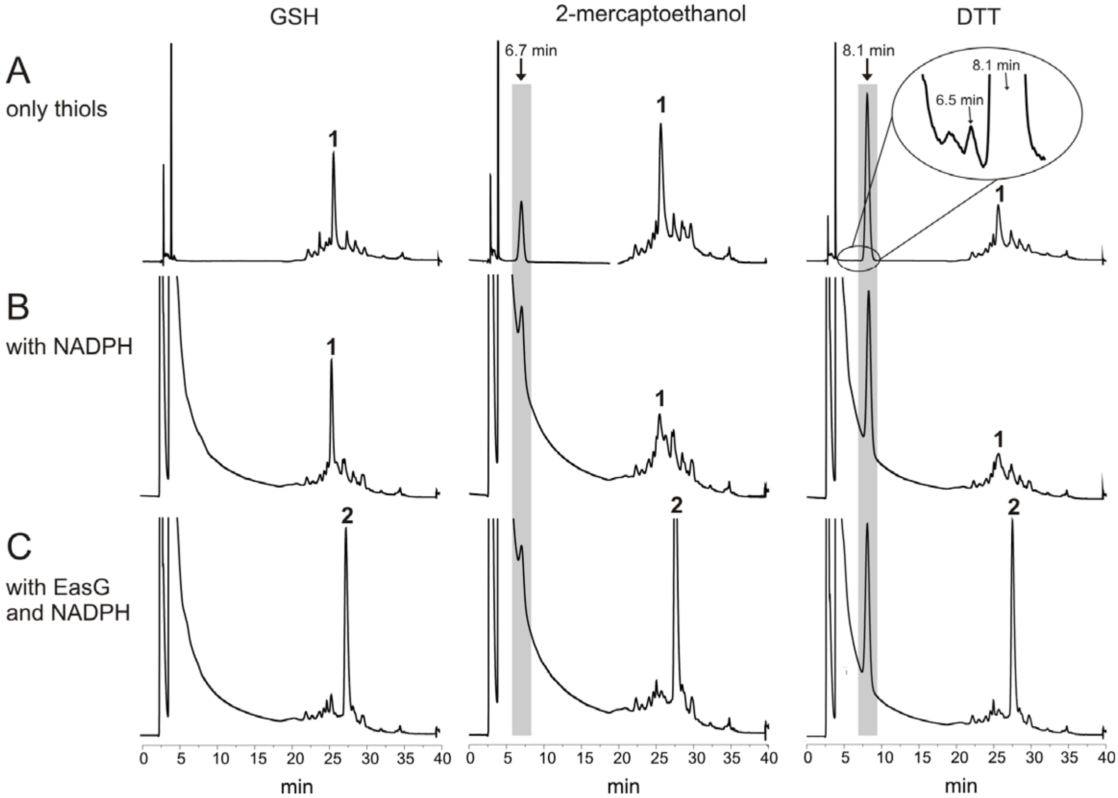

Figure S6: Formation of a non-enzymatic product with the different thiols. The assays (100 µl) contained 1 mM chanoclavine-I aldehyde and 1 mM of the corresponding thiol A) alone or with B) 1.5 mM NADPH or C) 1.5 mM NADPH and 2.5 µg of EasG . The incubation time was 16 h at 30 °C. **1**: chanoclavine-I aldehyde; **2**: agroclavine

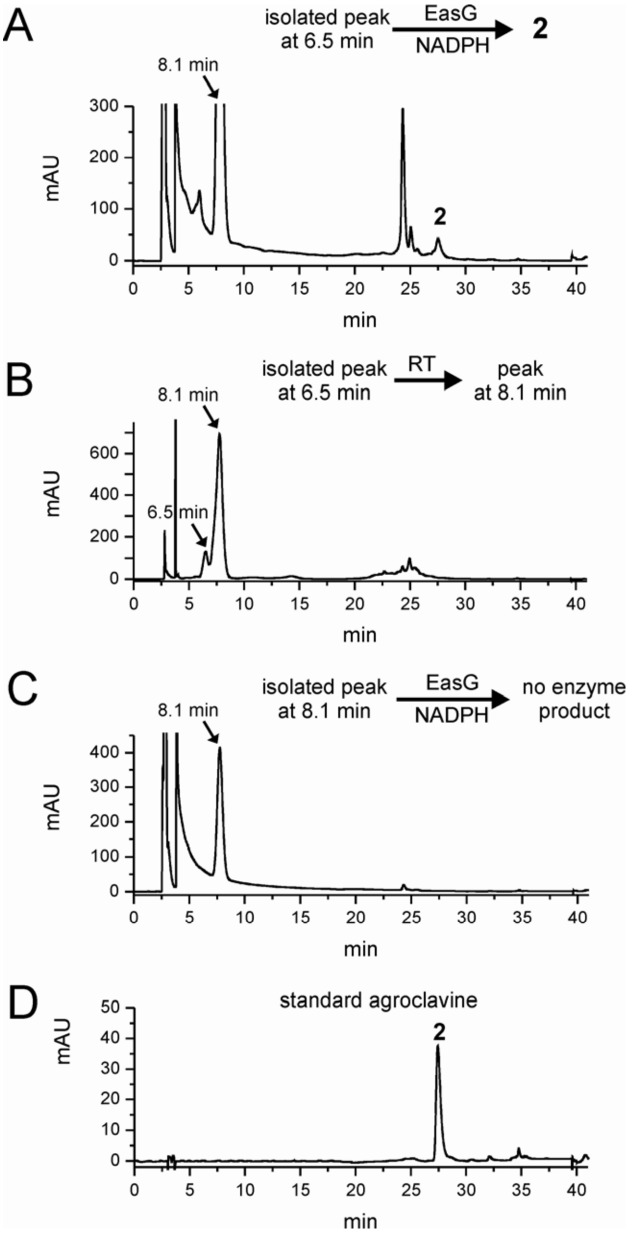

Figure S7: HPLC chromatograms of the isolated chanoclavine-I aldehyde-DTT intermediates after incubation with or without EasG. The enzyme assays (100 µl) contained the isolated chanoclavine-I aldehyde-DTT intermediate, 1.5 mM NADPH and 2.5 µg of EasG. The incubation time was 5 h at 30 °C.

Table S1 ^{1}H-NMR and ^{13}C-NMR data of agroclavine standard in deprotonated form ($CDCl_3$)

Position	δ_C	δ_H, multi., *J* in Hz	HMBC correlation
1	-	7.98, s	-
2	117.77	6.89, s	C-2 to H-4_α, H-4_β
3	112.47	-	C-3 to H-2, H-4_α, H-4_β
4_α	26.73	2.80, t, 13.0	C-4 to H-5
4_β		3.34, dd, 10.1, 4.6	-
5_β	63.92	2.55, m	C-5 to H-4_α, H-4_β, H-7_α, H-9_β, H-18
7_α	60.71	3.26, d, 16.1	C-7 to H-5, H-9, H-17, H-18
7_β		2.95, d, 16.0	-
8_α	132.32	-	C-8 to H-7_α, H-17
9	119.34	6.19, s	C-9 to H-7_α, H-17
10_α	41.01	3.76, d, 6.7	C-10 to H-4_α, H-4_β, H-9
11	132.58	-	C-11 to H-5, H-9, H-12, H-13, H-14
12	112.83	7.01, m	C-12 to H-13, H-14
13	122.97	7.17, m	C-13 to H-12
14	108.37	7.17, m	C-14 to H-12
15	133.58	-	C-15 to H-2, H-13, H-14
16	126.42	-	C-16 to H-2, H-4_β, H-12,H-14
17	20.78	1.79, s	C-17 to H-7_α, H-9
18	40.91	2.51, s	-

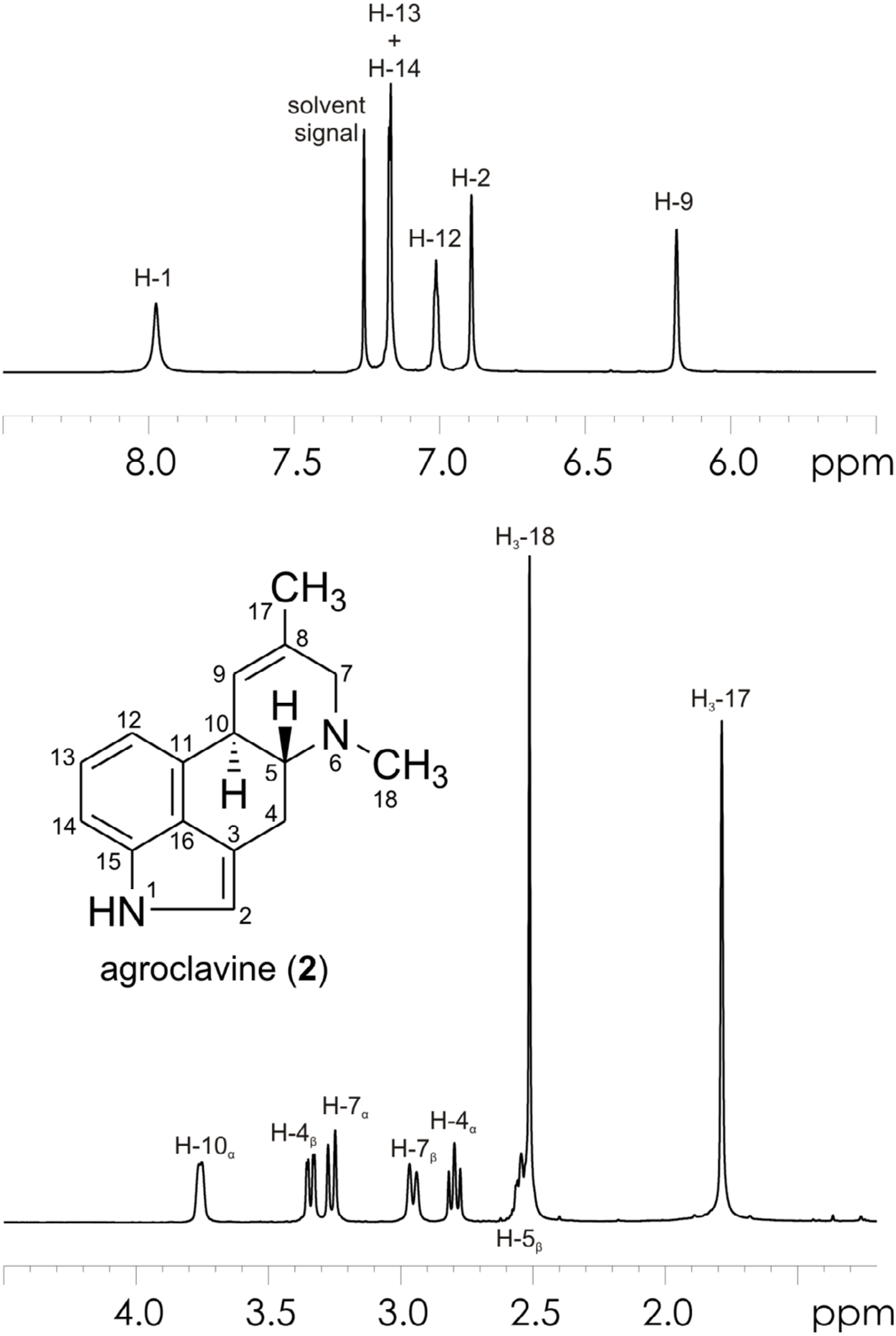

Figure S8 ^{1}H-NMR spectrum of authentic agroclavine as free base in $CDCl_3$

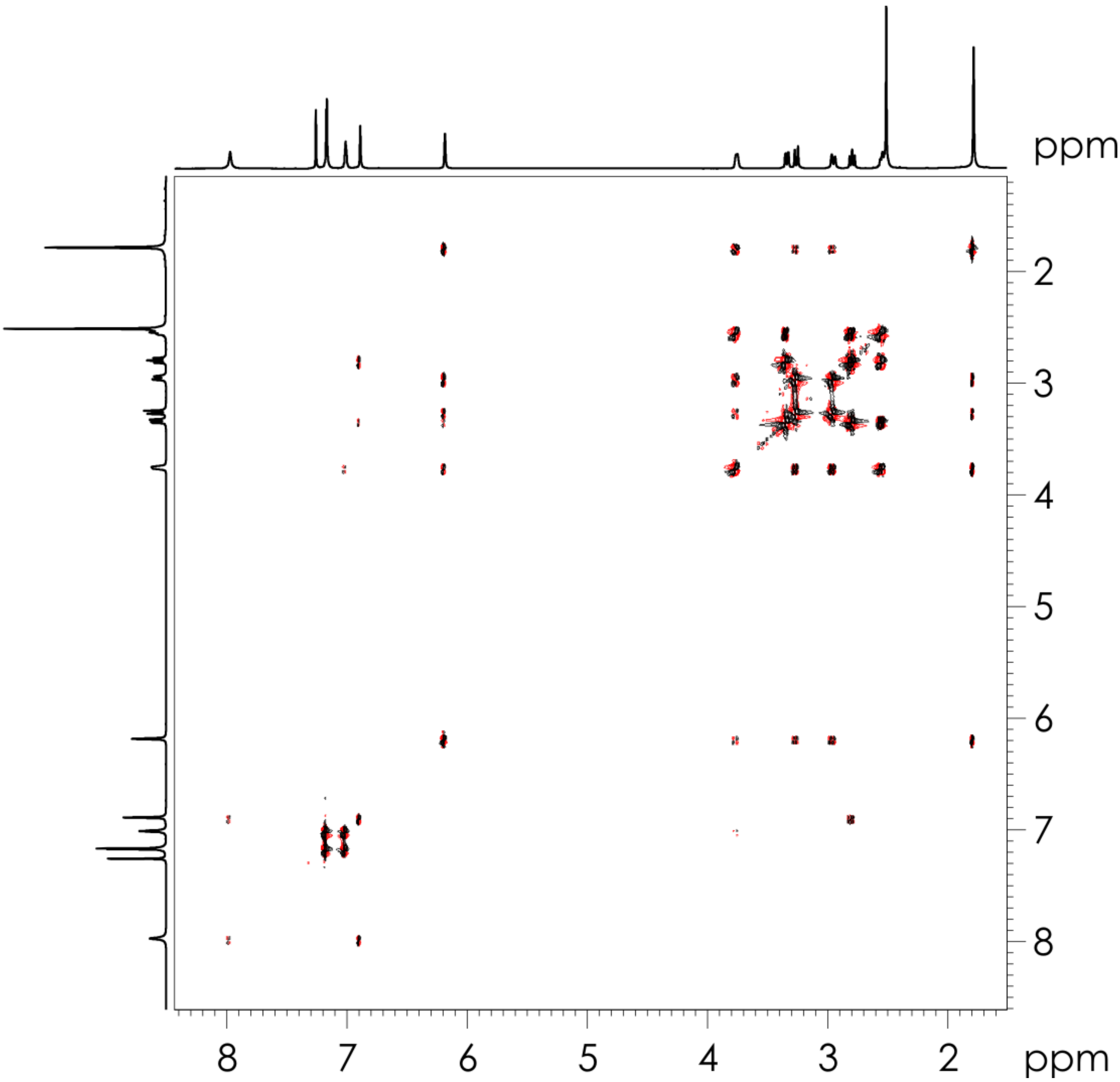

Figure S9 DQF-COSY spectrum of authentic agroclavine as free base in $CDCl_3$

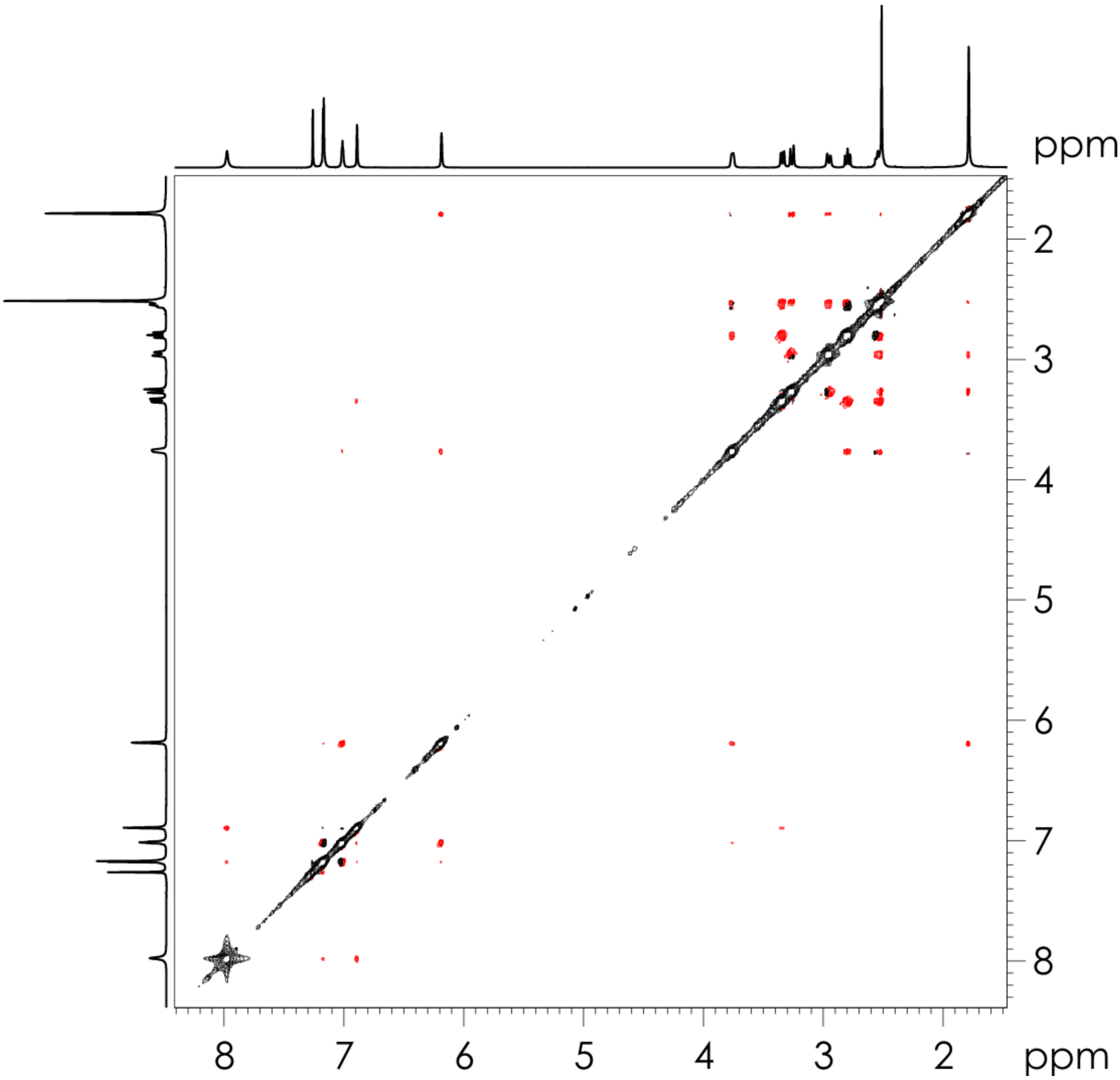

Figure S10 ROESY of authentic agroclavine as free base in $CDCl_3$

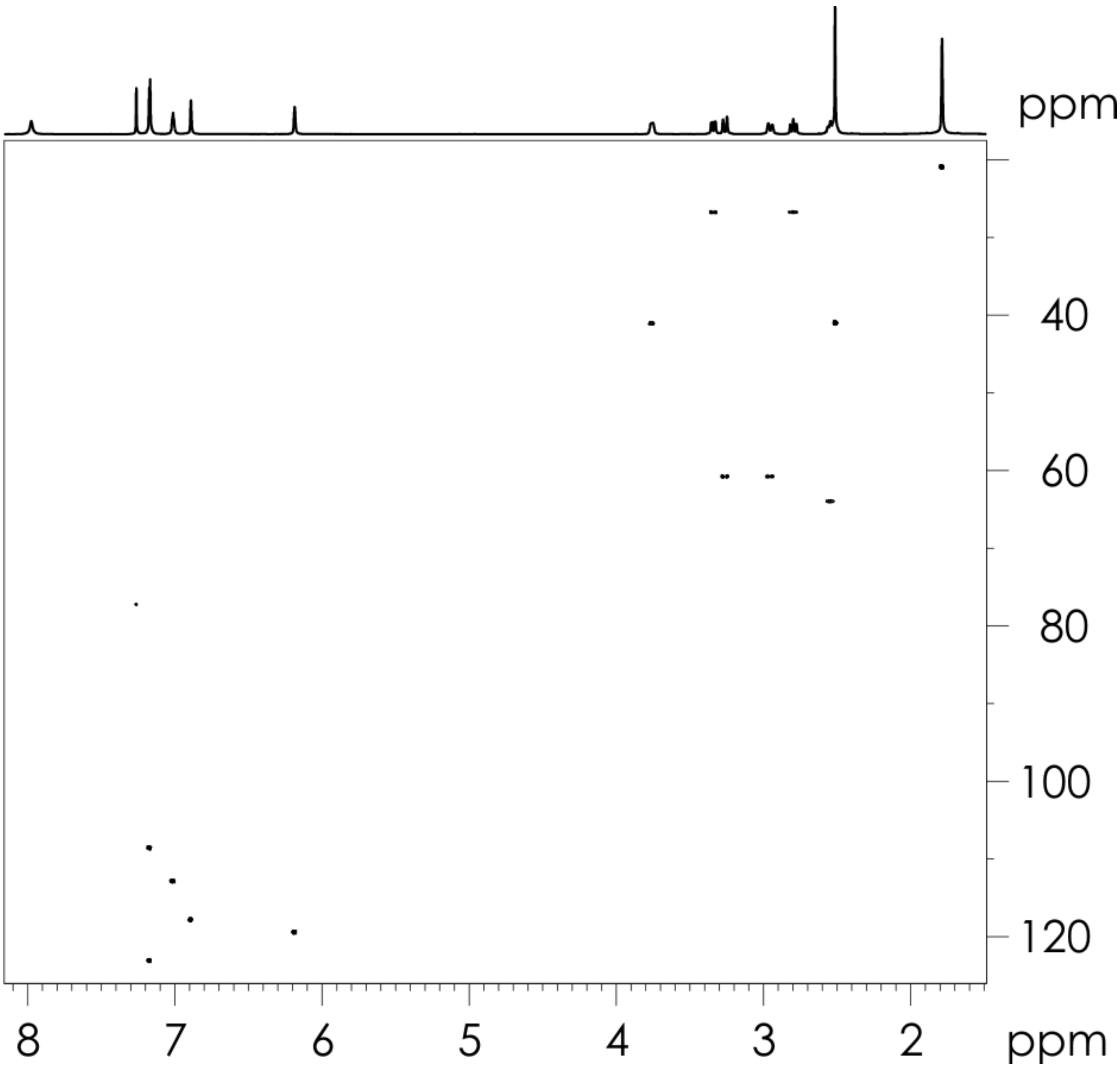

Figure S11 Enhanced parts of the HSQC spectrum of authentic agroclavine as free base in $CDCl_3$

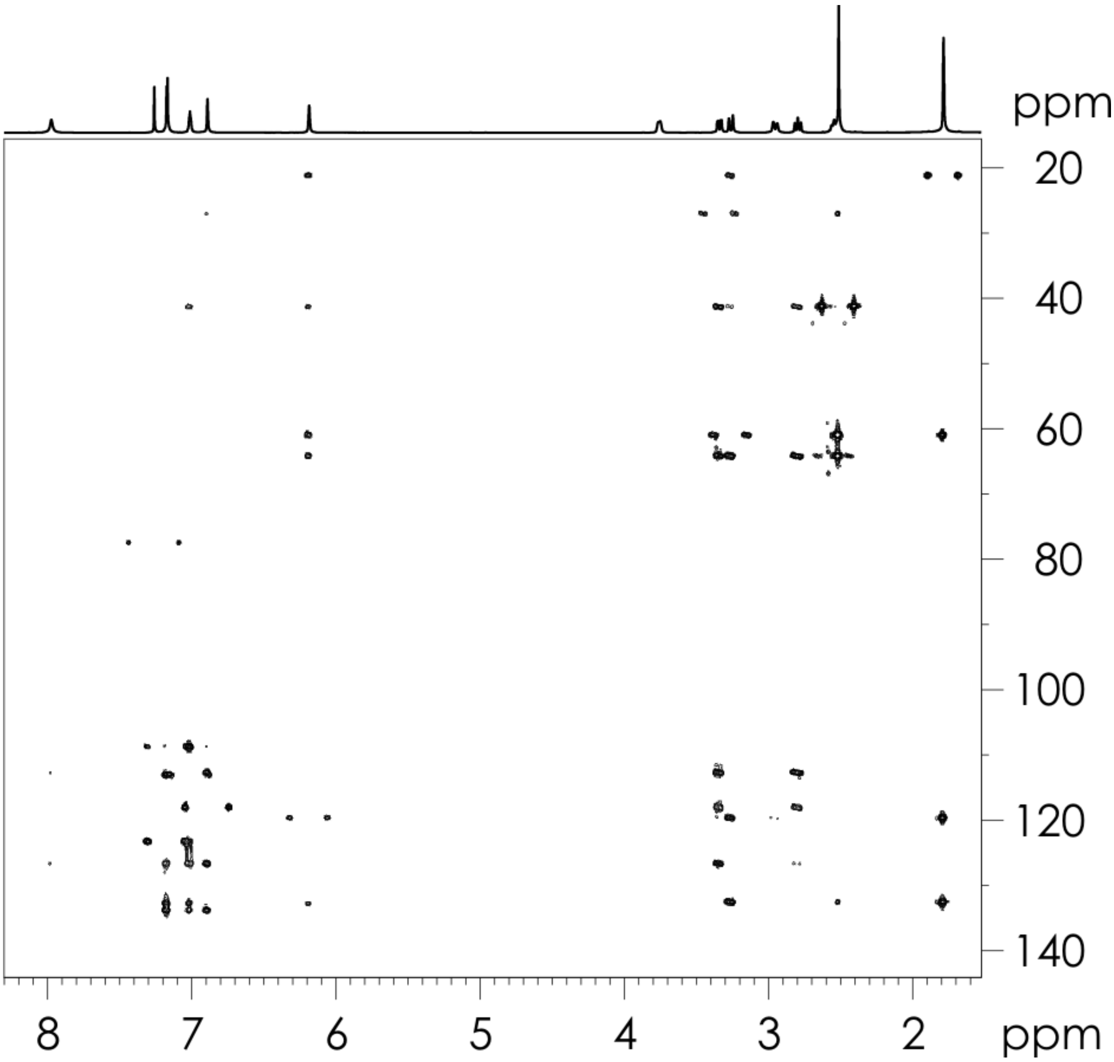

Figure S12 HMBC spectrum of authentic agroclavine as free base in $CDCl_3$

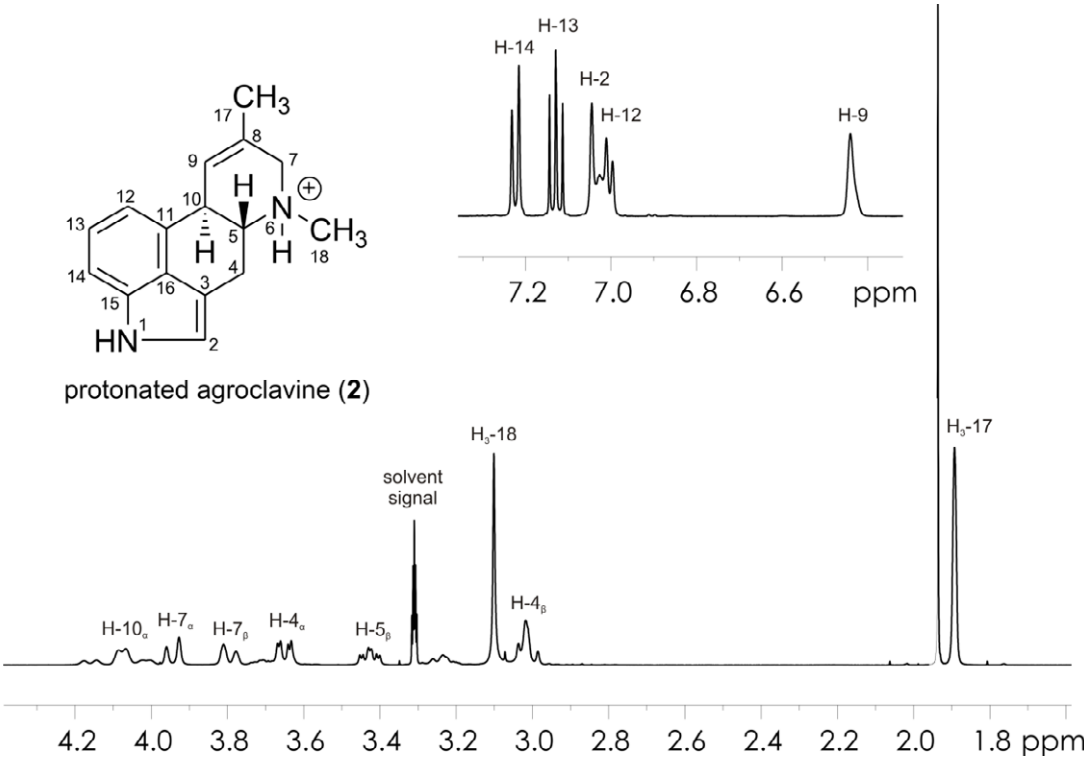

Figure S13 [1]H-NMR spectrum of authentic agroclavine in protonated form in CD_3OD
The sample of authentic agroclavine as free base was treated after NMR measurement in $CDCl_3$ with HPLC solvents containing CH_3CN and 0.5 % trifluoroacetic acid and evaporated to dryness.

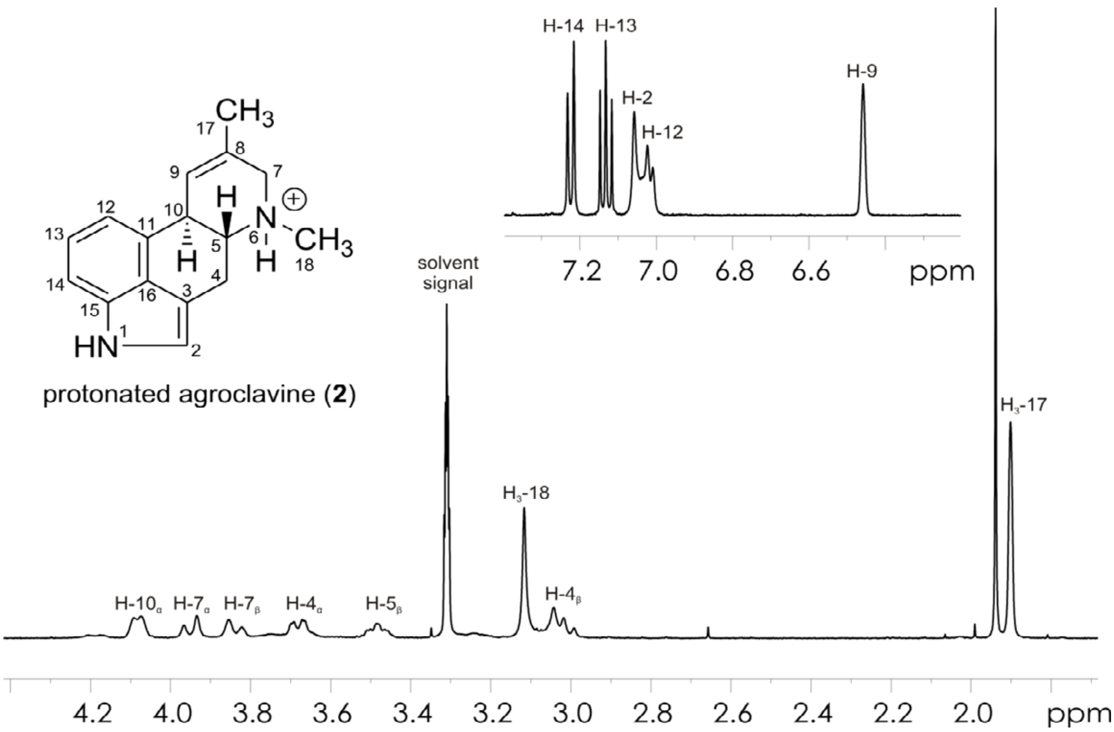

Figure S14 [1]H-NMR spectrum of isolated enzyme product of EasG reaction in protonated form in CD_3OD