Electronic Supporting Information to:

In vitro conversion of chanoclavine-I aldehyde to the stereoisomers festuclavine and pyroclavine was controlled by the second reduction step

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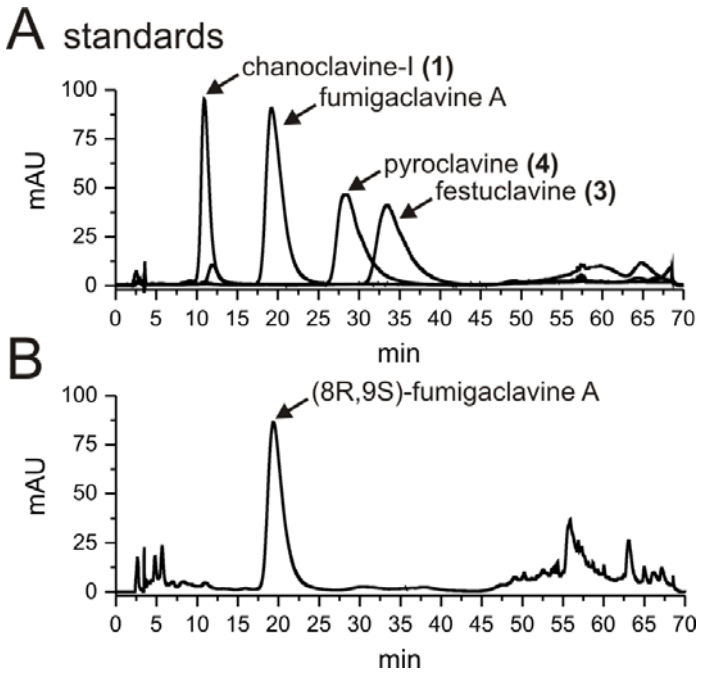

Figure S1 HPLC analysis of the cultural extract of *P. commune* NRRL 2033 (B) by using ergot alkaloids or precursors as standards (A). The substances were detected with a Photodiode Array detector and illustrated for absorption at 282 nm.

Table S1 ^{1}H-NMR and ^{13}C-NMR data of (8R,9S)-fumigaclavine A in protonated form (CD_3OD)

Position	δ_C	δ_H, multi., *J* in Hz	HMBC correlation
2	120.69	7.07, d, 1.3	C-2 to H-14
3	107.80	-	C-3 to H-2
4_α	25.15	3.03, ddd, 1.2, 11.7, 12.9	-
4_β		3.74, m	
5_β	64.16	3.80, m	C-5 to H-18
7_α	58.25	3.49, d, 13.0	C-7 to H-17, H-18
7_β		3.66, dd, 12.9, 4.0	
8_β	33.26	2.47, m	C-8 to H-17
9_α	69.89	5.78, br s	C-9 to H-17, H-7_α
10_α	39.39	3.73, m	-
11	126.91	-	C-11 to H-13
12	113.69	6.76, d, 7.2	C-12 to H-14
13	123.89	7.10, t, 7.7	C-13 to H-14
14	110.84	7.22, d, 8.1	C-14 to H-12
15	135.72	-	C-15 to H-2
16	127.67	-	C-16 to H-2, H-12, H-14
17	15.40	1.41, d, 7.6	C-17 to H-7_β
18	42.42	3.15, s	-
19	172.08	-	C-19 to H-20
20	20.66	1.88, s	-

Table S2 NOE contacts for proving the stereochemistry of (8R,9S)-fumigaclavine A

Protons	Strength
H-5_β to H-7_β	Medium
H-10_α to H-4_α	Medium
H-9_α to H-17	Medium
H-9_α to H-10_α	Medium
H-17 to H-10_α	Medium
H-17 to H-7_α	Medium

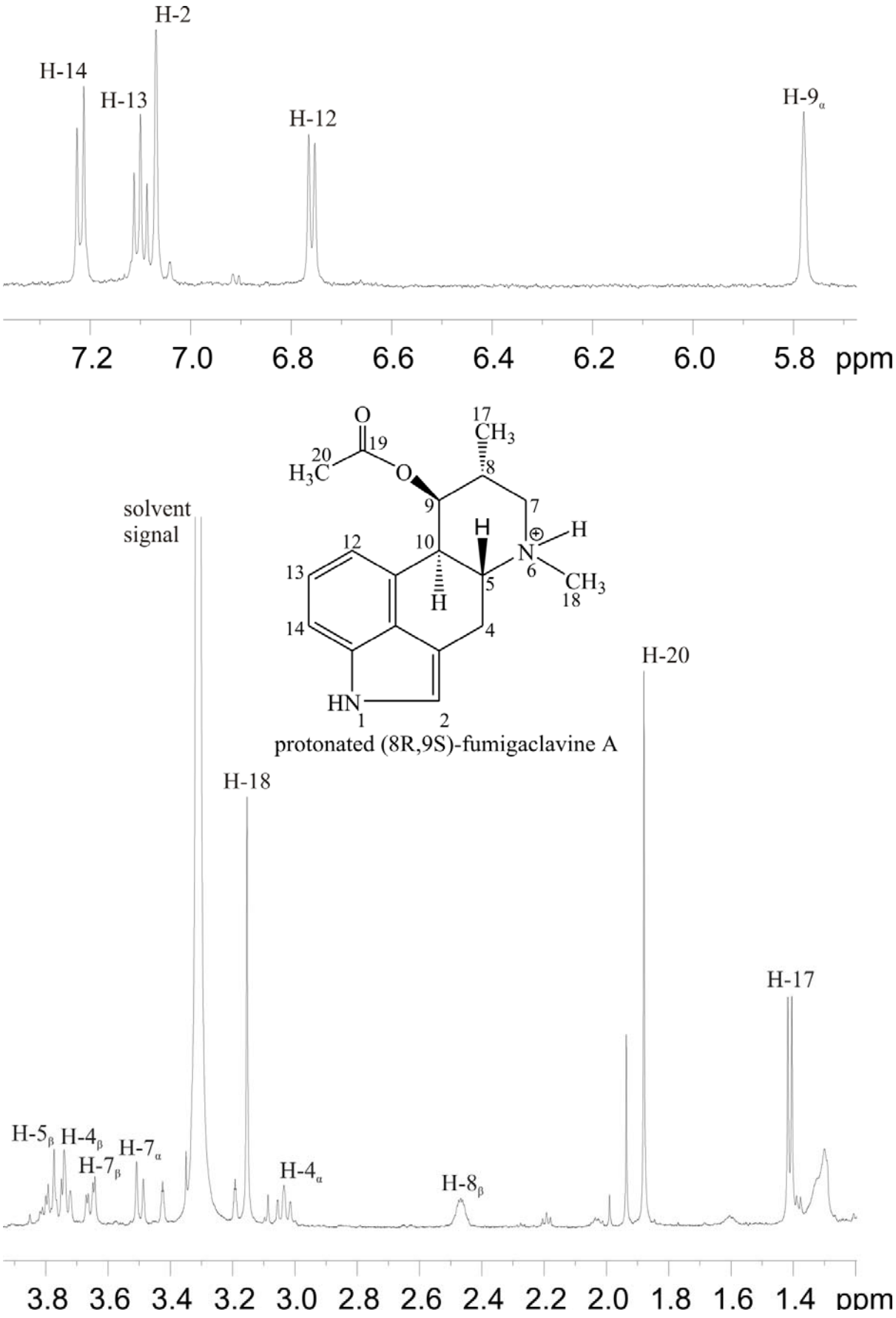

Figure S2.1 ^{1}H-NMR spectrum of (8R,9S)-fumigaclavine A in protonated form in CD_3OD

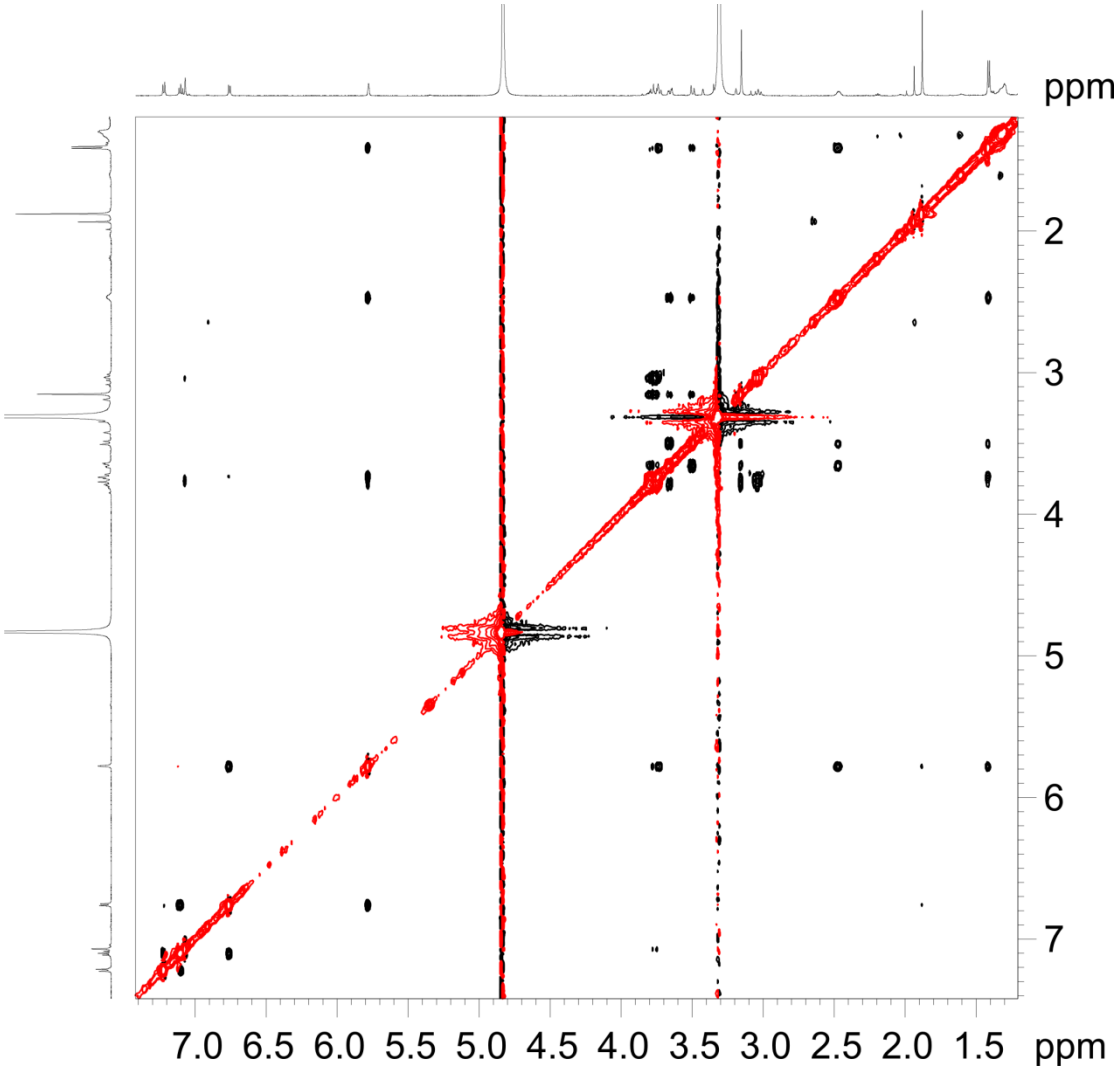

Figure S2.2 NOESY spectrum of (8R,9S)-fumigaclavine A in protonated form in CD_3OD

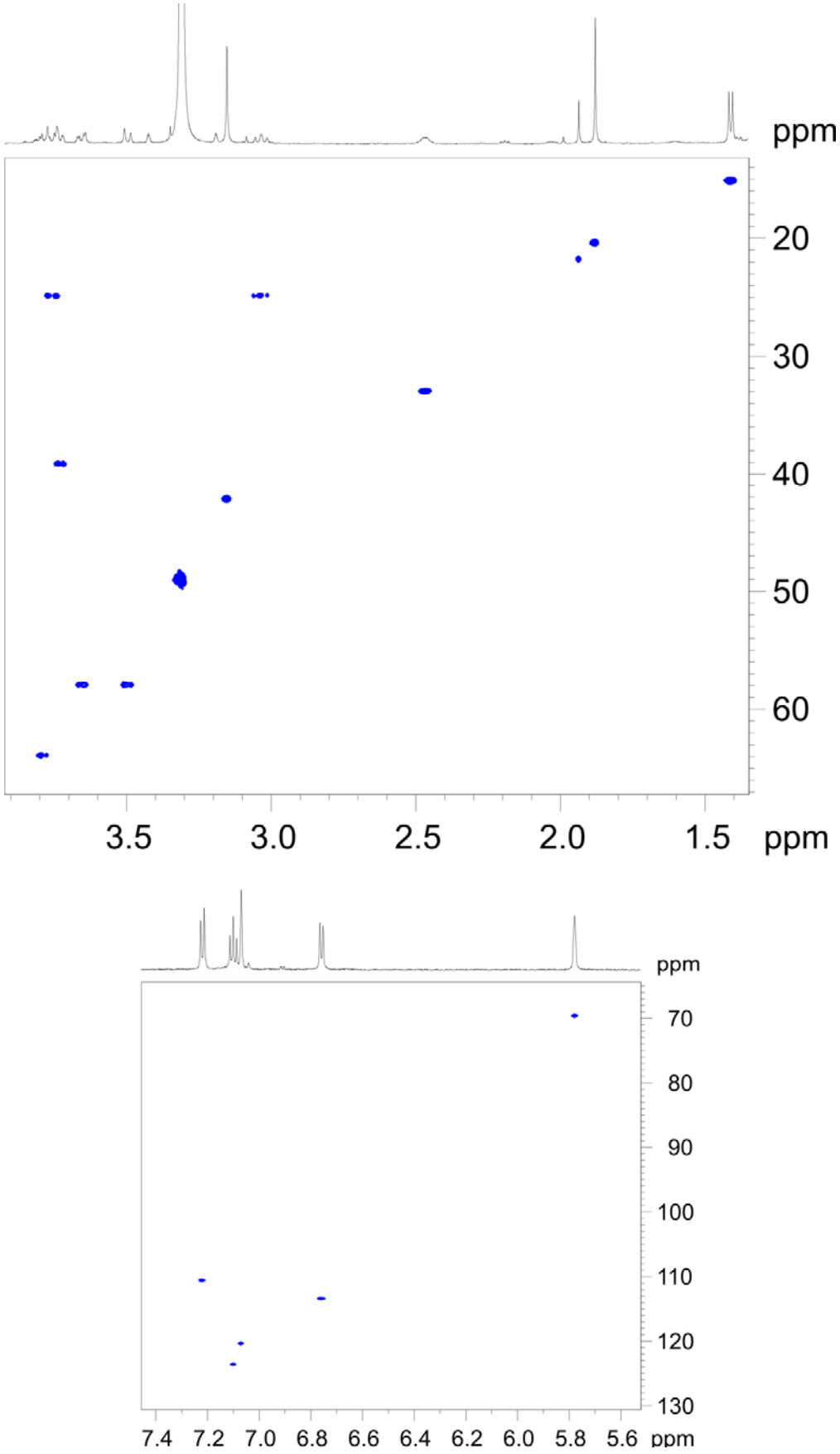

Figure S2.3 Enhanced parts of the HSQC spectrum of (8R,9S)-fumigaclavine A in protonated form in CD_3OD

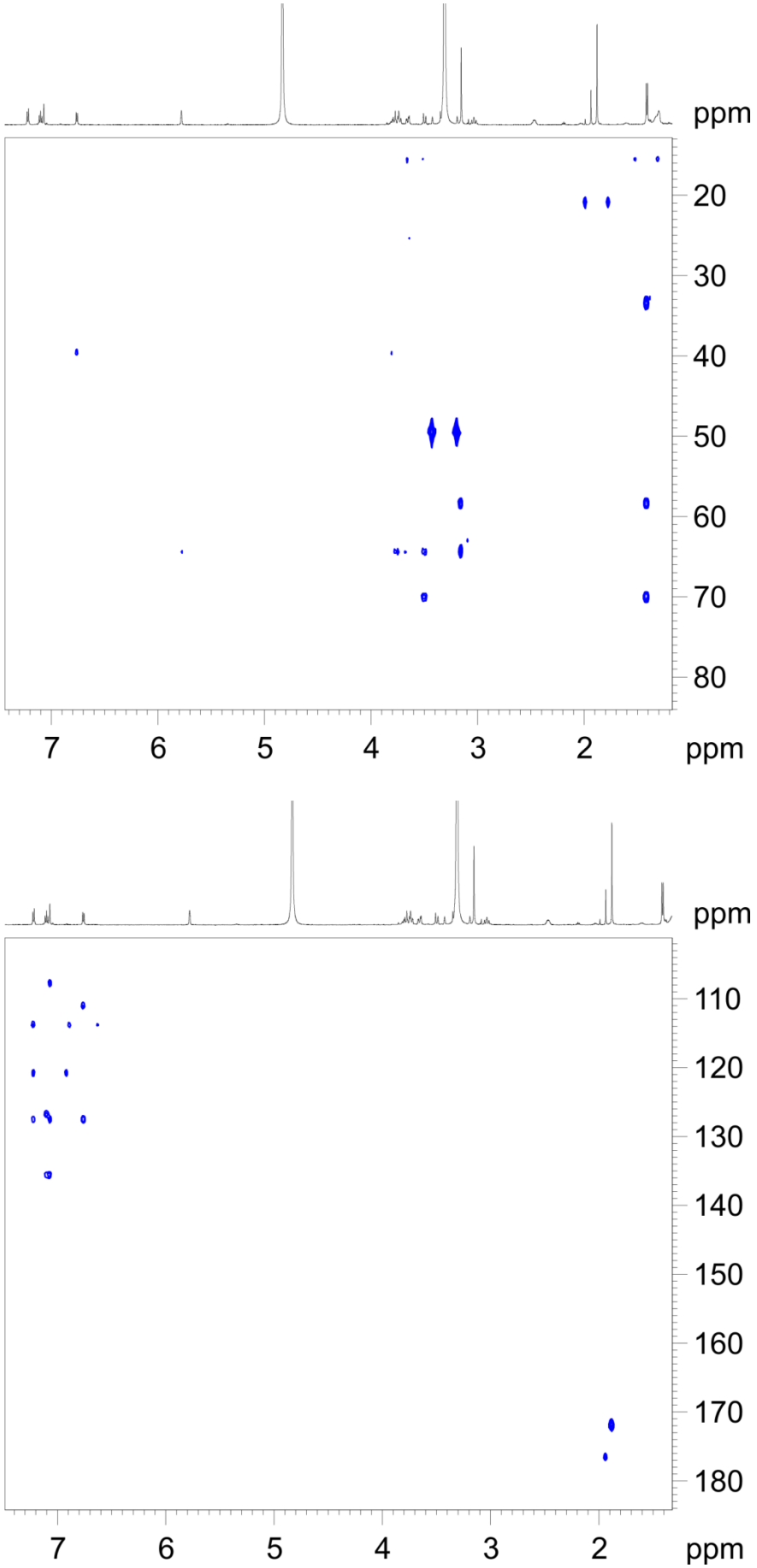

Figure S2.4 HMBC spectrum of (8R,9S)-fumigaclavine A in protonated form in CD_3OD

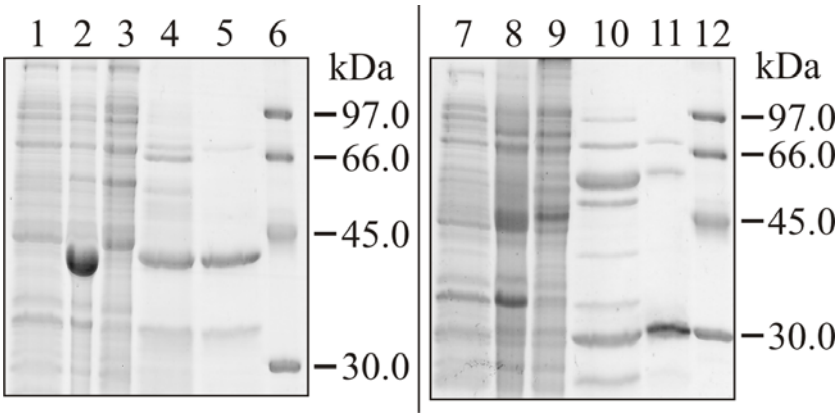

Figure S3 Analysis of the overproduction and purification of His_6-$FgaOx3_{pc}$ (lanes 1-5) and $FgaFS_{pc}$ (lanes 7-11). The proteins were separated on a 15% SDS-polyacrylamide gel and stained with Coomassie Brilliant Blue R-250. Lane 1: Total proteins before induction; 2: Total proteins after induction; 3: Soluble proteins after induction; 4: Ni-NTA elution of His_6-$FgaOx3_{pc}$; 5: Co-resin elution of His_6-$FgaOx3_{pc}$; 6: Molecular weight standard; Lane 7: Total proteins before induction; 8: Total proteins after induction; 9: Soluble proteins after induction; 10: Co-resin elution of His_6-$FgaFS_{pc}$; 11: Ni-NTA elution of His_6-$FgaFS_{pc}$; 12: Molecular weight standard

Table S3 ^{1}H-NMR and ^{13}C-NMR data of pyroclavine in protonated form (CD_3OD)

Position	δ_C	δ_H, multi., *J* in Hz	HMBC correlation
2	120.29	7.03, d, 1.5	C-2 to H-4$_\alpha$, H-4$_\beta$, H-12
3	108.10	-	C-3 to H-2, H-4$_\alpha$, H-4$_\beta$
4$_\alpha$	25.55	2.99, ddd, 14.0, 11.6, 1.7	C-4 to H-5, H-18
4$_\beta$		3.69, dd, 14.2, 4.5	-
5$_\beta$	69.84	3.30**, td, 11.0, 4.5	C-5 to H-4$_\alpha$, H-4$_\beta$, H-7$_\alpha$, H-9$_\beta$, H-12, H-18
7$_\alpha$	62.86	3.51, dt, 12.8, 1.5	C-7 to H-17, H-18
7$_\beta$		3.41*, dd, 12.8, 3.9	-
8$_\beta$	28.12	2.49, m	C-8 to H-7$_\alpha$, H-7$_\beta$, H-9$_\beta$, H-17, H-18
9$_\alpha$	32.72	2.63, ddt, 13.7, 3.6, 2.1	C-9 to H-7$_\alpha$, H-17
9$_\beta$		1.91, td, 13.1, 4.9	-
10$_\alpha$	35.56	3.41*, dt, 4.2, 11.6	C-10 to H-4$_\beta$, H-9$_\beta$,
11	130.42	-	C-11 to H-9$_\beta$, H-10$_\alpha$, H-13
12	113.95	6.90, dd, 7.2, 0.8	C-12 to H-2, H-13, H-14
13	123.89	7.11, dd, 8.2, 7.2	C-13 to H-12
14	110.65	7.20, d, 8.2	C-14 to H-12
15	135.07	-	C-15 to H-2, H-13
16	126.81	-	C-16 to H-2, H-4$_\beta$, H-12, H-14
17	17.29	1.37, d, 7.5	C-17 to H-7$_\beta$, H-9$_\beta$
18	42.46	3.07, s	-

* = overlapped signals, *J* measured from DQF-COSY cross peaks, ** = under the solvent signal

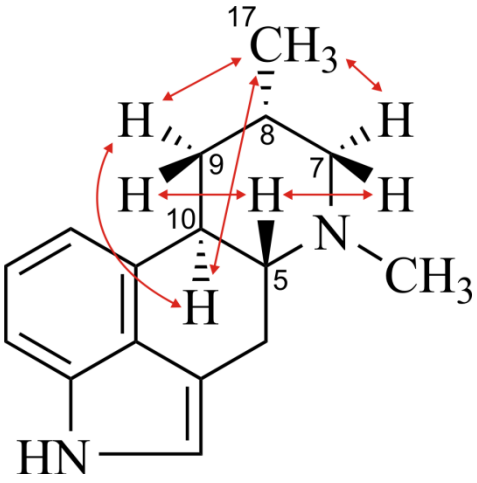

Figure S4 NOE contacts (red arrows) for proving the stereochemistry of pyroclavine

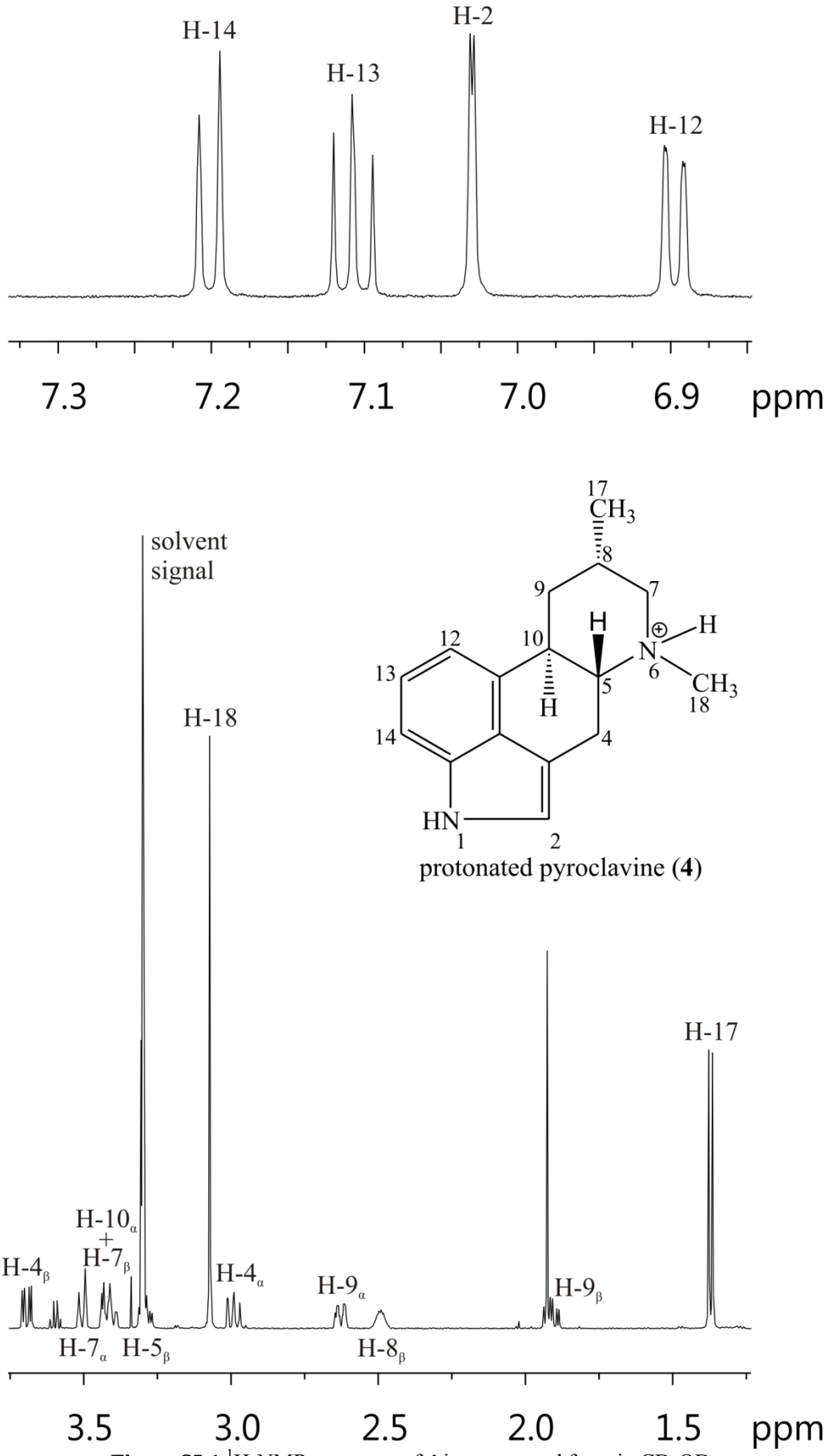

Figure S5.1 ^{1}H-NMR spectrum of **4** in protonated form in CD_3OD

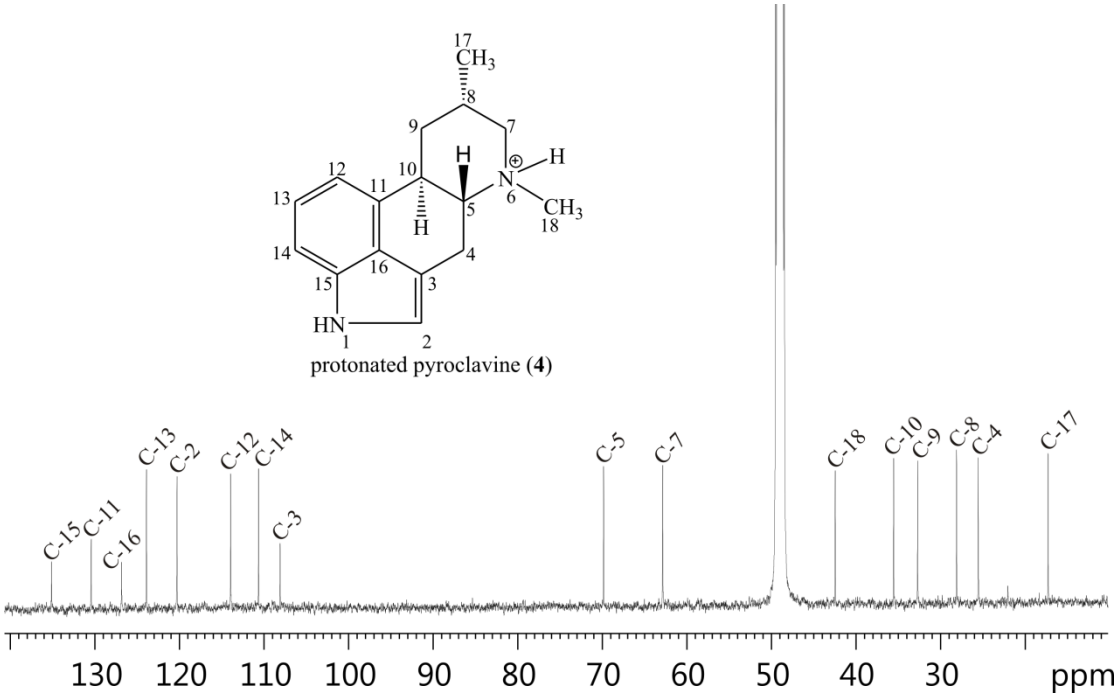

Figure S5.2 ^{13}C-NMR spectrum of **4** in protonated form in CD_3OD

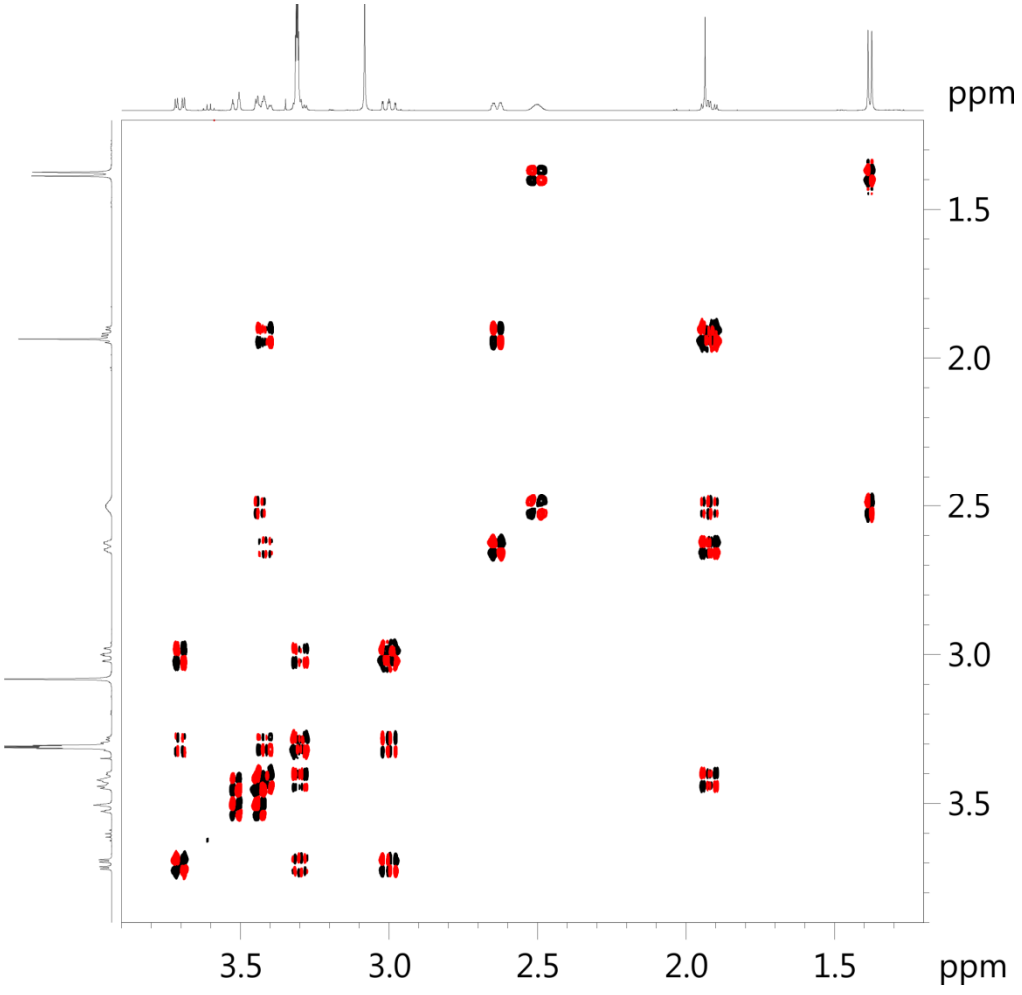

Figure S5.3 DQF-COSY spectrum of **4** in protonated form in CD_3OD

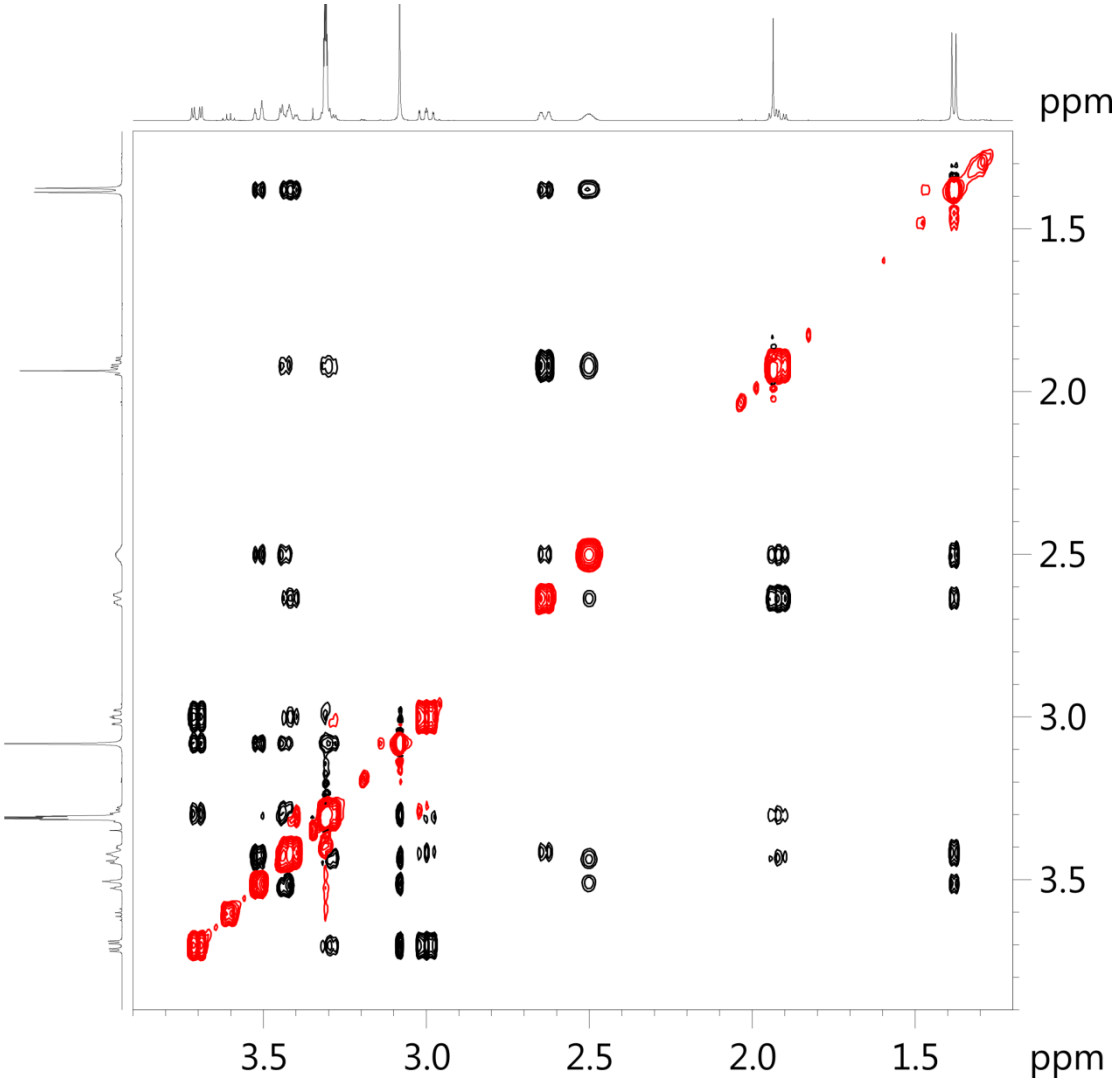

Figure S5.4 NOESY spectrum of **4** in protonated form in CD_3OD

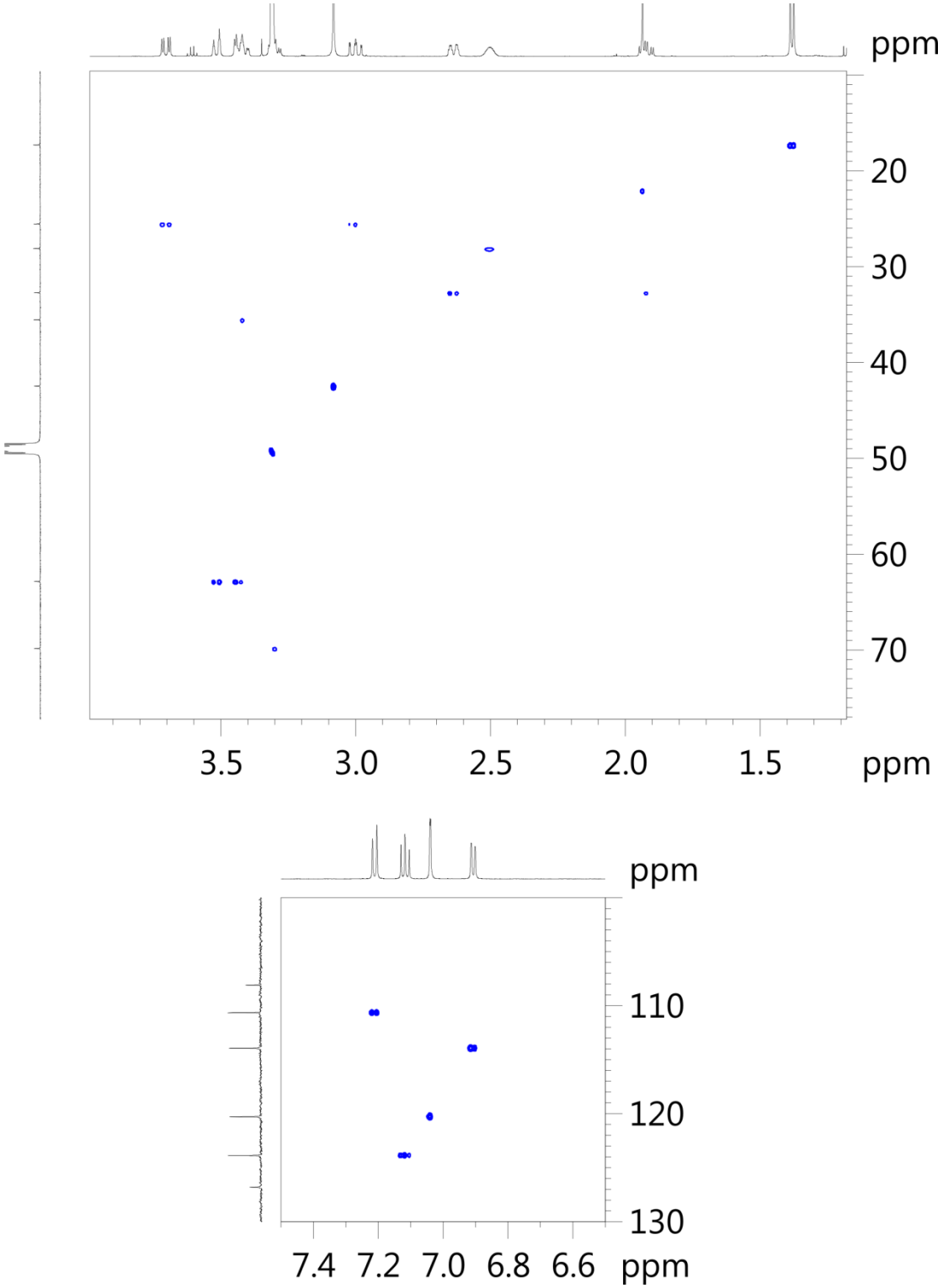

Figure S5.5 Enhanced parts of the HSQC spectrum of **4** in protonated form in CD_3OD

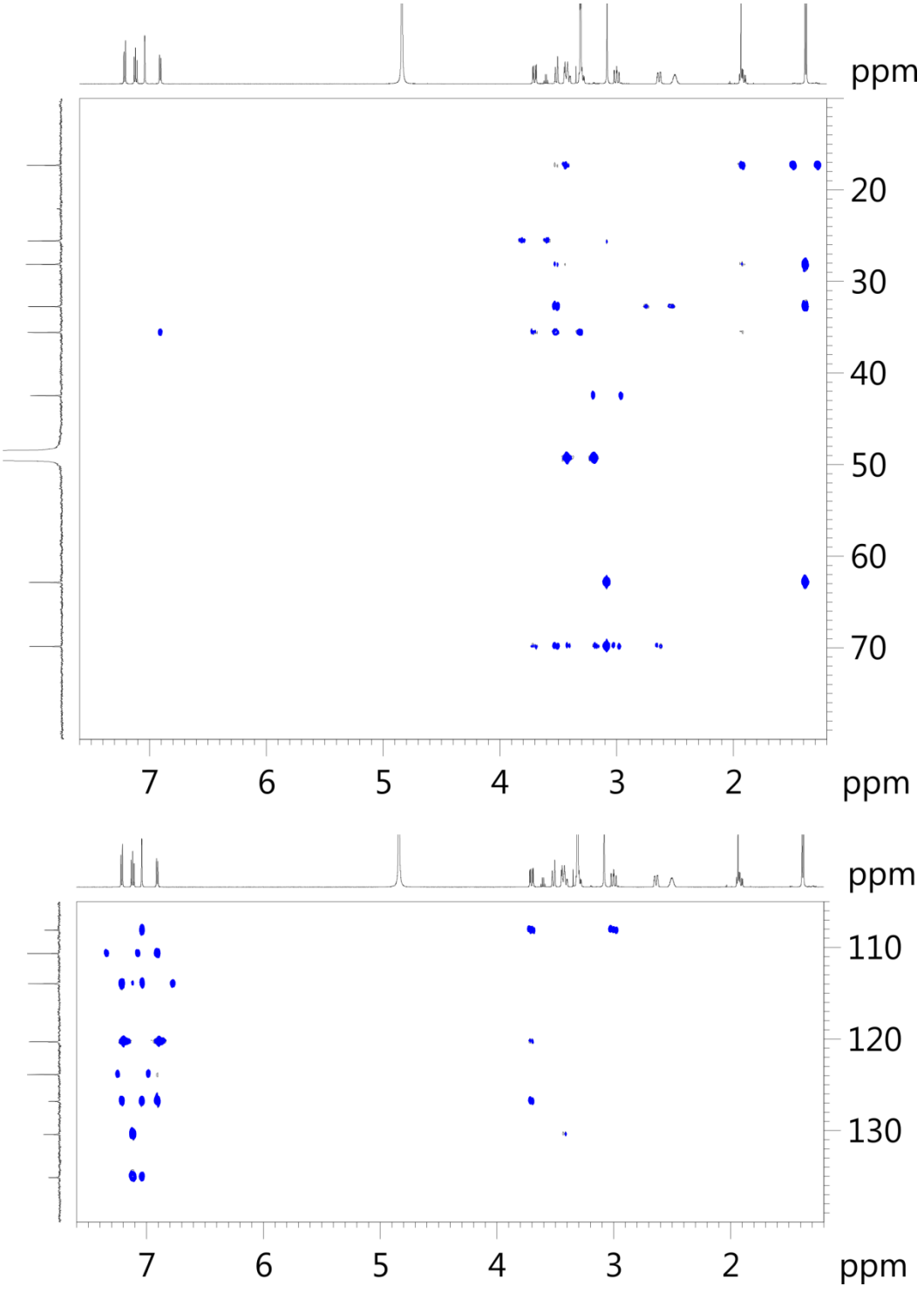

Figure S5.6 HMBC spectrum of **4** in protonated form in CD_3OD

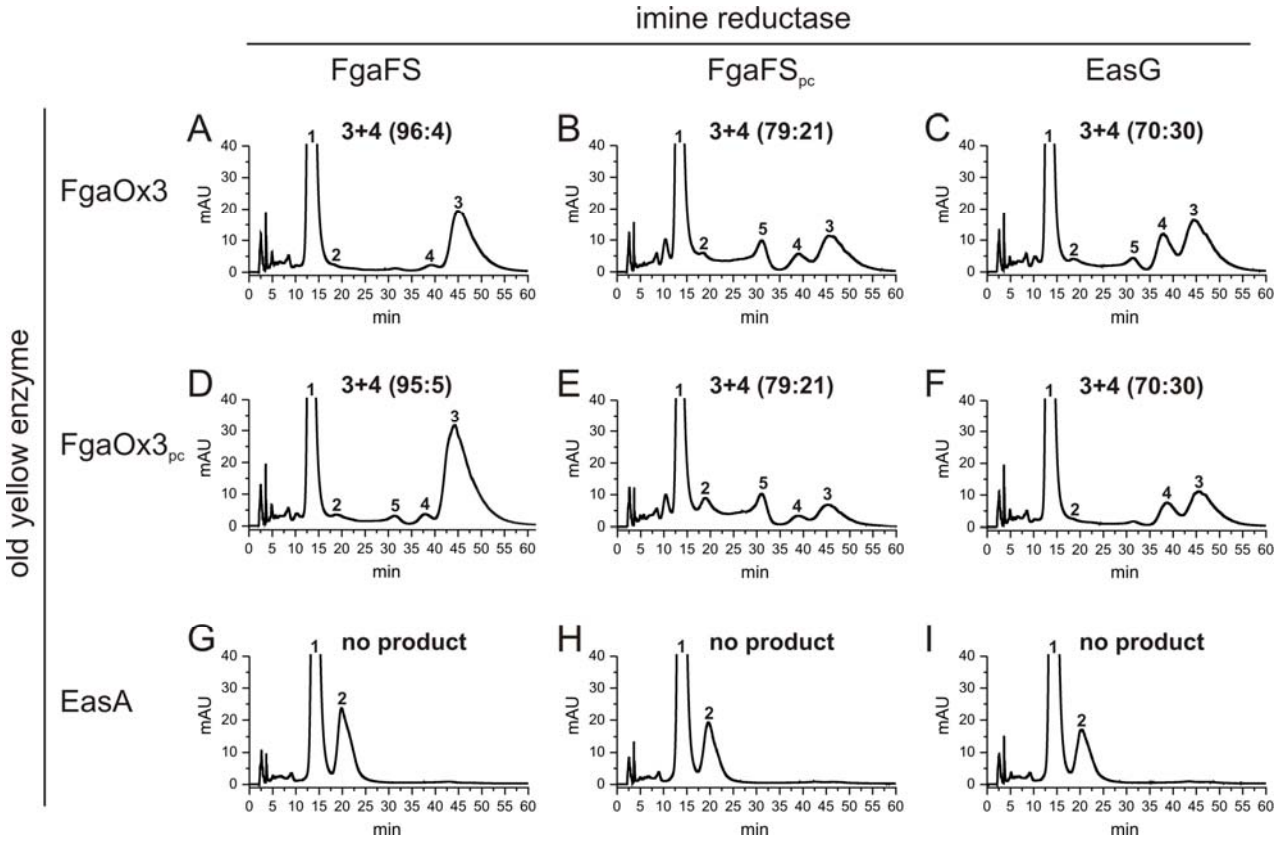

Figure S6 HPLC chromatograms of dichloromethane extracts of the incubation mixtures with different enzyme combinations. The reaction mixtures contained 5 mM of the cofactors NAD^+, FMN, NADPH, 1 mM chanoclavine-I and 5 µg of FgaDH, one old yellow enzyme and one imine reductase and were incubated at 30°C for 15 h. The substances were detected with a Photodiode Array detector and illustrated for absorption at 282 nm. **1**: chanoclavine-I; **2**: chanoclavine-I aldehyde; **3**: festuclavine; **4**: pyroclavine; **5**: shunt product

Table S4 Conversion of chanoclavine-I aldehyde to festuclavine and pyroclavine in the presence of NADPH or NADH. The assays contained 10 µg of the recombinant enzymes each and were incubated at 30°C for 15 h.

enzyme combination	incubation with NADPH			incubation with NADH		
		ratio of products			ratio of products	
	chanoclavine-I consumption (%)	festuclavine **(3)**	pyroclavine **(4)**	chanoclavine-I consumption (%)	festuclavine **(3)**	pyroclavine **(4)**
FgaOx3$_{pc}$ + FgaFS	78	95	5	28	88	12
FgaOx3$_{pc}$ + FgaFS$_{pc}$	19	80	20	5	73	27
FgaOx3$_{pc}$ + EasG	70	72	28	12	63	37
FgaOx3 + FgaFS	79	96	4	96	92	8
FgaOx3 + FgaFS$_{pc}$	29	81	19	28	78	22
FgaOx3 + EasG	75	71	29	63	66	34