SUPPORTING INFORMATION RESULTS

Content.

Suppl. Table 1. Proton Chemical Shift Values for Salivaricin A2

Amino Acid	NH	Hα	Hβ	Side Chain
Lys1	8.09	3.80	1.72	Hγ 1.30, Hδ 1.52, Hε 2.75, Hζ 7.63
Arg2	8.59	4.39	1.72	Hγ2 1.58, Hγ3 1.66, Hδ, 3.13, Hε 7.49 HH 6.82
Gly3	8.31	3.84		
Thr4	7.85	4.24	3.99	Hγ1 4.94, Hγ2 1.02
Gly5	7.97	3.66, 3.74		
Trp6	8.01	4.47	2.82, 3.02	Hδ1 7.05, Hε1 10.75, Hε3 7.54, Hζ2 7.34 Hζ3 6.63
Phe7	8.09	3.97	3.05,2.77	Hδ1, Hδ2, Hε1, Hε2, Hζ1 7.28
Ala8	8.86	4.67	1.30	
Abu9	8.09	4.68	2.74	Hγ1 1.50
Ile10	n.d.	3.83	1.93	Hγ12 1.30, Hγ13 1.02, Hγ2 0.67, Hδ1 0.37
Abu11	9.06	4.13	2.79	Hγ1 1.08
Asp12	n.d.	4.67	3.20, 2.68	Hδ1 4.97
Asp13	n.d.	2.89	1.79, 1.50	Hδ1 5.18
AlaS14	8.10	4.46	3.02, 2.75	
Pro15	-	4.38	2.02, 1.90	Hγ2 1.96, Hγ3 1.83, Hδ 3.61
Asn16	7.83	4.43	2.88, 2.67	Hδ21 7.23, Hδ22 6.85
AlaS17	n.d.	4.30	3.56	
Val18	n.d.	4.18	1.90	Hγ 0.80
Phe19	8.08	4.45	3.02, 2.81	Hδ1, Hδ2, Hε1, Hε2, Hζ1 7.23
Val20	7.84	4.21	1.72	Hγ1 0.81
AlaS21	7.41	3.93	3.12	
AlaS22	n.d.	4.74	3.41	

n.d. Values were not detected or were not assignable due to peak overlap

Suppl. Table 2. Proton Chemical Shift Values for Salivaricin A2 (3-22).

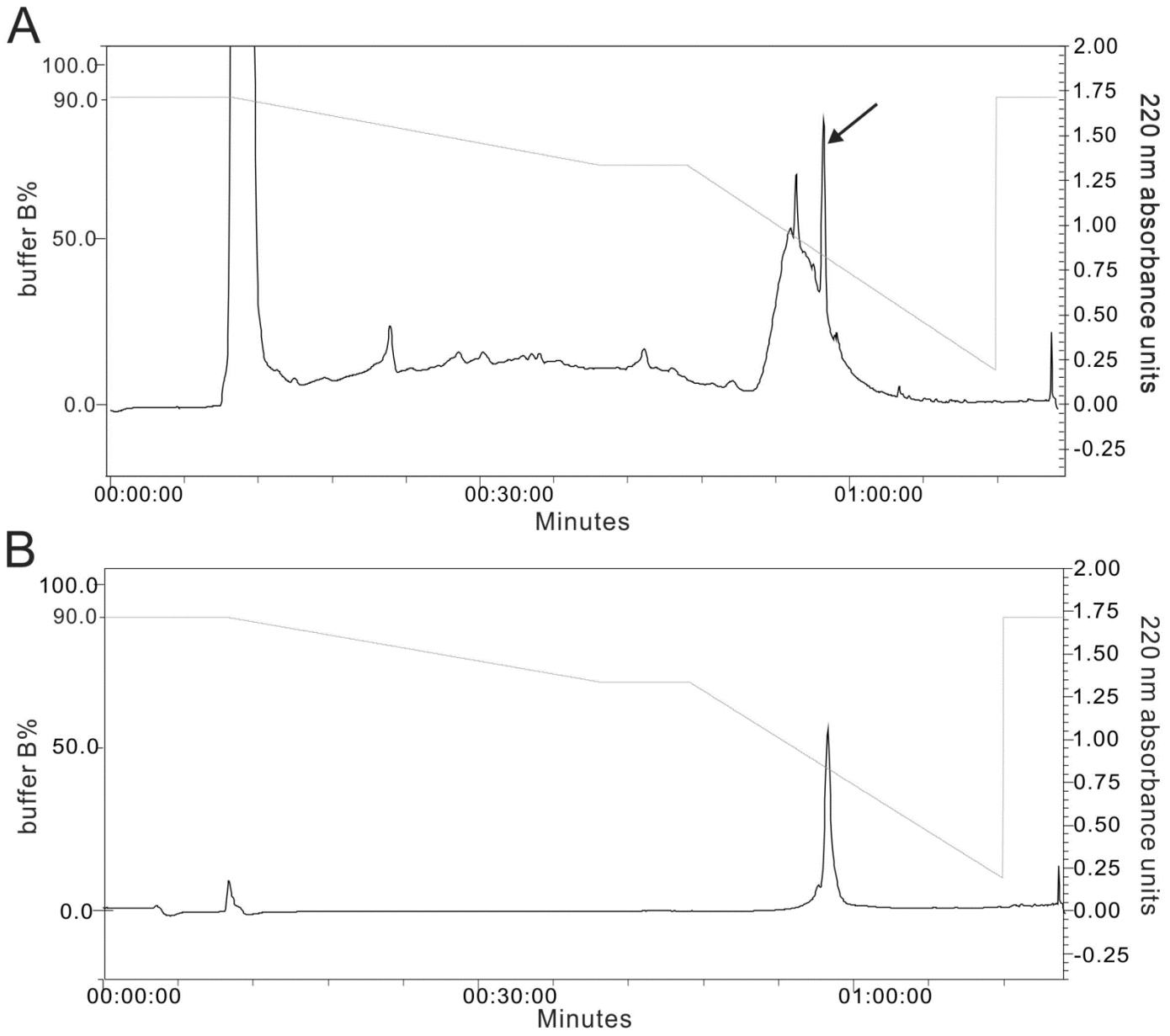
Amino Acid	NH	Hα	Hβ	Side Chain
Gly3	7.93	3.65		
Thr4	8.39	4.29	3.99	Hγ1 5.00, Hγ2 1.06
Gly5	8.05	3.79, 3.62		
Trp6	8.02	4.50	2.81, 3.02	Hδ1 7.07, Hε1 10.75, Hε3 7.56, Hζ2 7.32 Hζ3 6.96
Phe7	8.25	4.53	3.02,2.81	Hδ1, Hδ2, Hε1, Hε2, Hζ1 7.23
Ala8	8.06	4.43	1.24	
Abu9	7.85	4.39	3.64	Hγ1 1.20
Ile10	n.d.	3.81	1.93	Hγ12 1.30, Hγ13 1.02, Hγ2 0.80, Hδ1 0.39
Abu11	9.0	4.09	3.46	Hγ1 1.13
Asp12	7.28	4.59	3.23, 2.70	Hδ1 4.97
Asp13	7.62	2.77	1.79, 1.50	Hδ1 5.18
AlaS14	8.22	4.44	3.04, 2.88	
Pro15	-	4.38 (4.17)	2.01 (1.96)	Hγ 1.89 (Hγ 1.83), Hδ 3.59 (Hδ 3.26)
Asn16	7.98	4.44	2.88, 2.64	Hδ21 6.98, Hδ22 6.62
AlaS17	8.36	4.40	3.61, 3.78	
Val18	7.58	4.39	2.35	Hγ1 0.91, Hγ2 1.01
Phe19	8.08	4.45	3.02, 2.81	Hδ1, Hδ2, Hε1, Hε2, Hζ1 7.23
Val20	7.87	4.23	1.72	Hγ1 0.81
AlaS21	7.44	3.94	3.09	
AlaS22	n.d.	4.75	3.40	

n.d. Values were not detected or were not assignable due to peak overlap

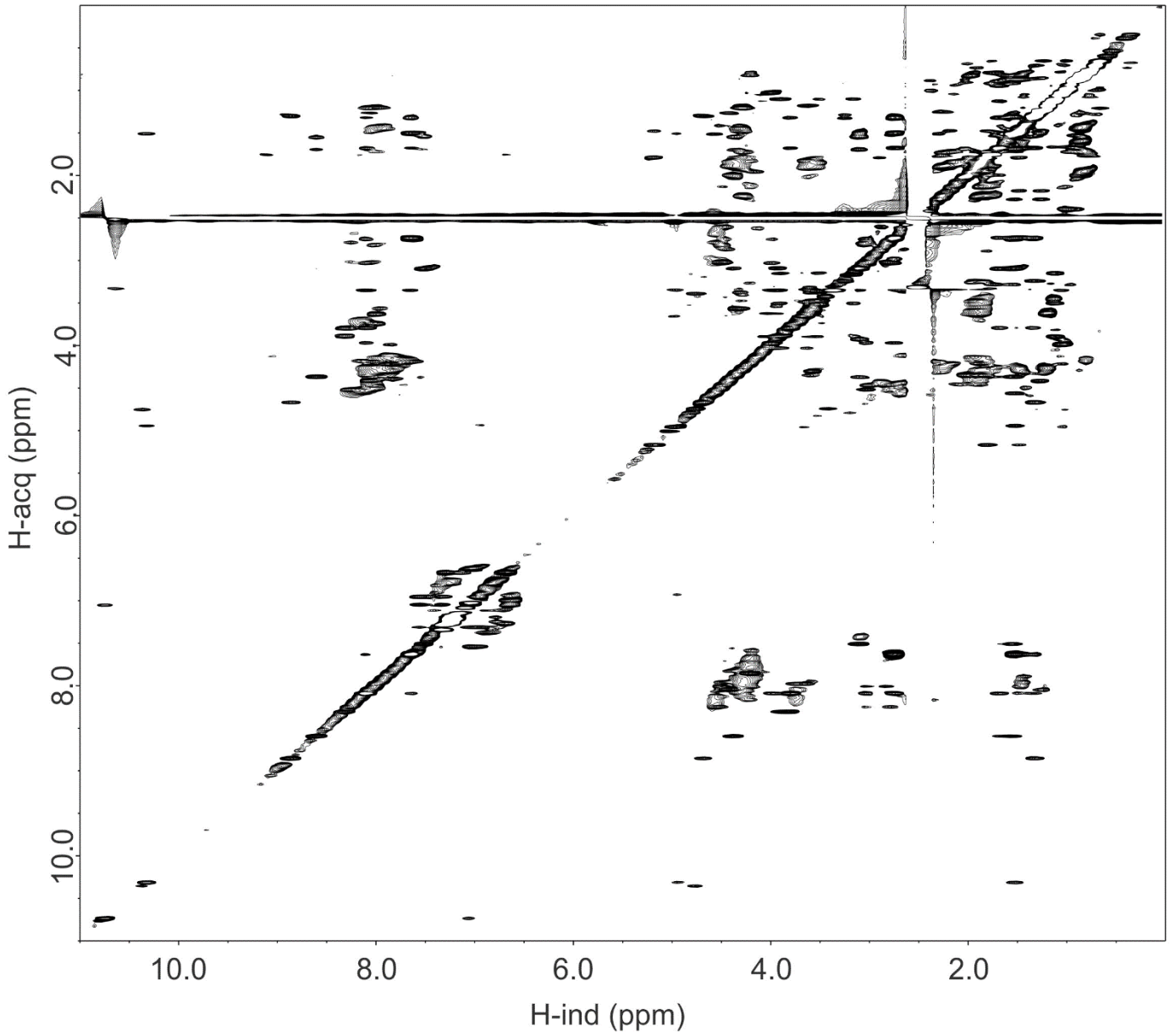
Suppl. Table 3. Protease stability of salivaricin A2.

	pepsin stability (10^3 CFU/mL)			trypsin stability (10^3 CFU/mL)		
	treatment 1	treatment 2	average	treatment 1	treatment 2	average
untreated A2	30	26	28	31	37	34
treated A2	28	33	30.5	3100	3600	3350
control 1	2300	2600	2450	2900	3100	3000
control 2	2100	2700	2400	2700	3300	3000
control 3	2900	3300	3100	2900	3400	3150
control 4	2600	2900	2750	3300	2800	3050

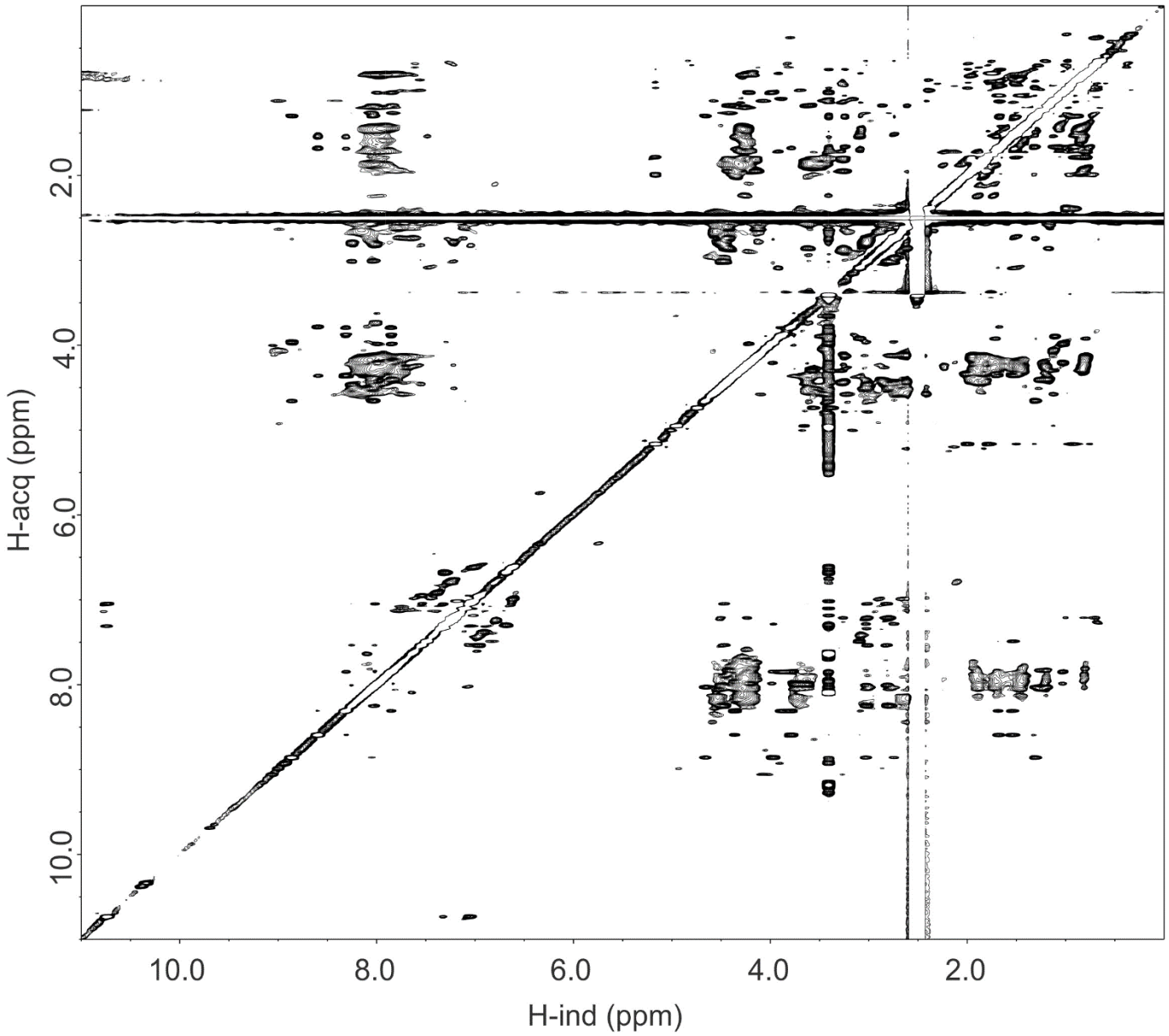
Activity of protease treated salivaricin A2 was determined by viable cell counts (CFU/ml). Salivaricin A2 was exposed to pepsin for 120 min and trypsin for 30 min. Control 1, control 2, control 3, and control 4 are the reaction buffer with protease and protease inhibitors, the reaction buffer with protease, the reaction buffer alone, and the THyex broth, respectively.



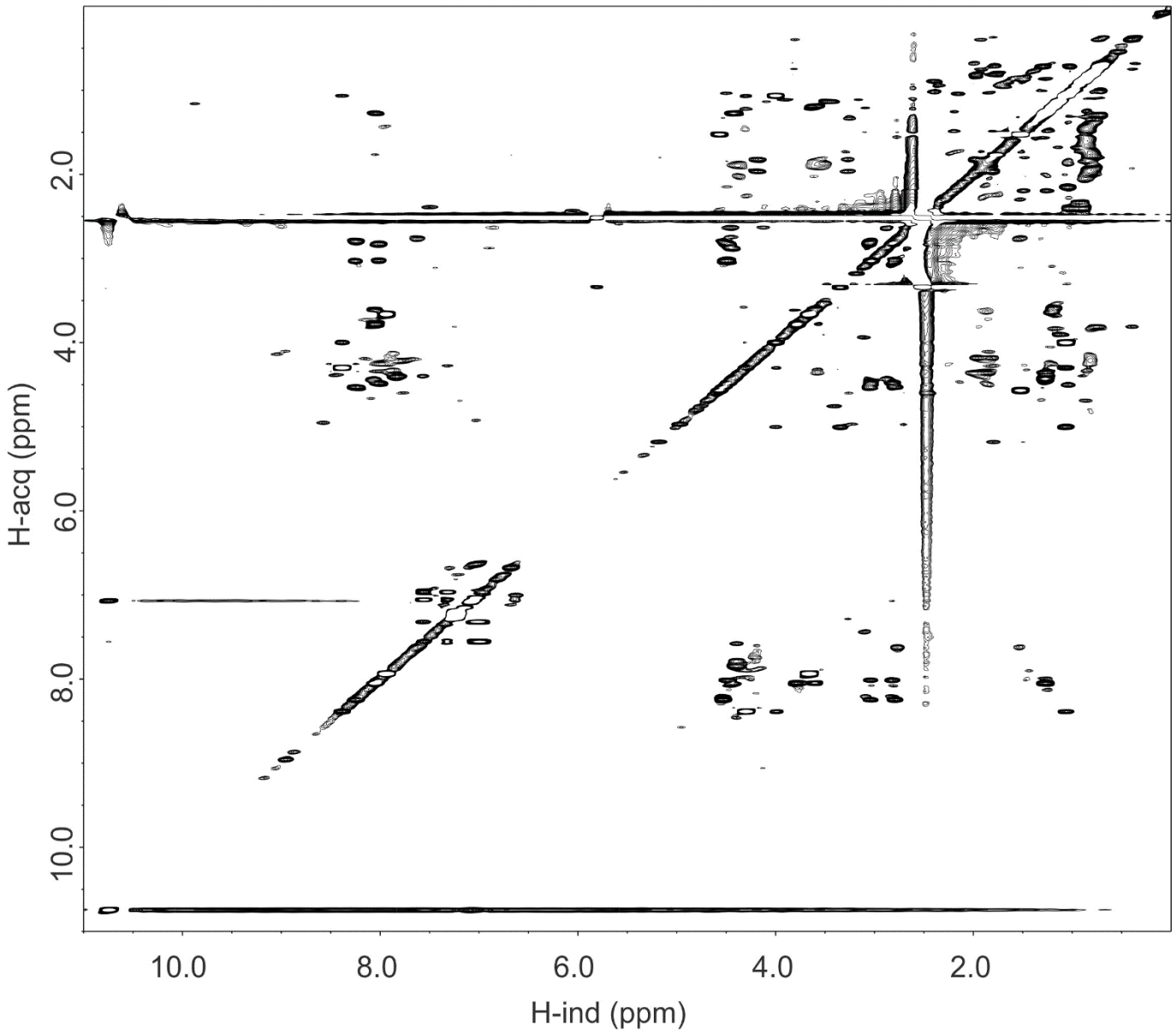
Suppl. Figure 1 HPLC purification of salivaricin A2. *S. salivarius* HS0302 was inoculated into modified THyex agar and incubated for 72 h at 37 ºC before chloroform extraction. Extraction was applied on a semi-prep column (A) and the fraction indicated by arrow was collected, applied on the same column for further purification (B). Grey lines represent the gradient of buffer B (water with 0.1%TFA), while black lines represent the absorbance unit at 220 nm.



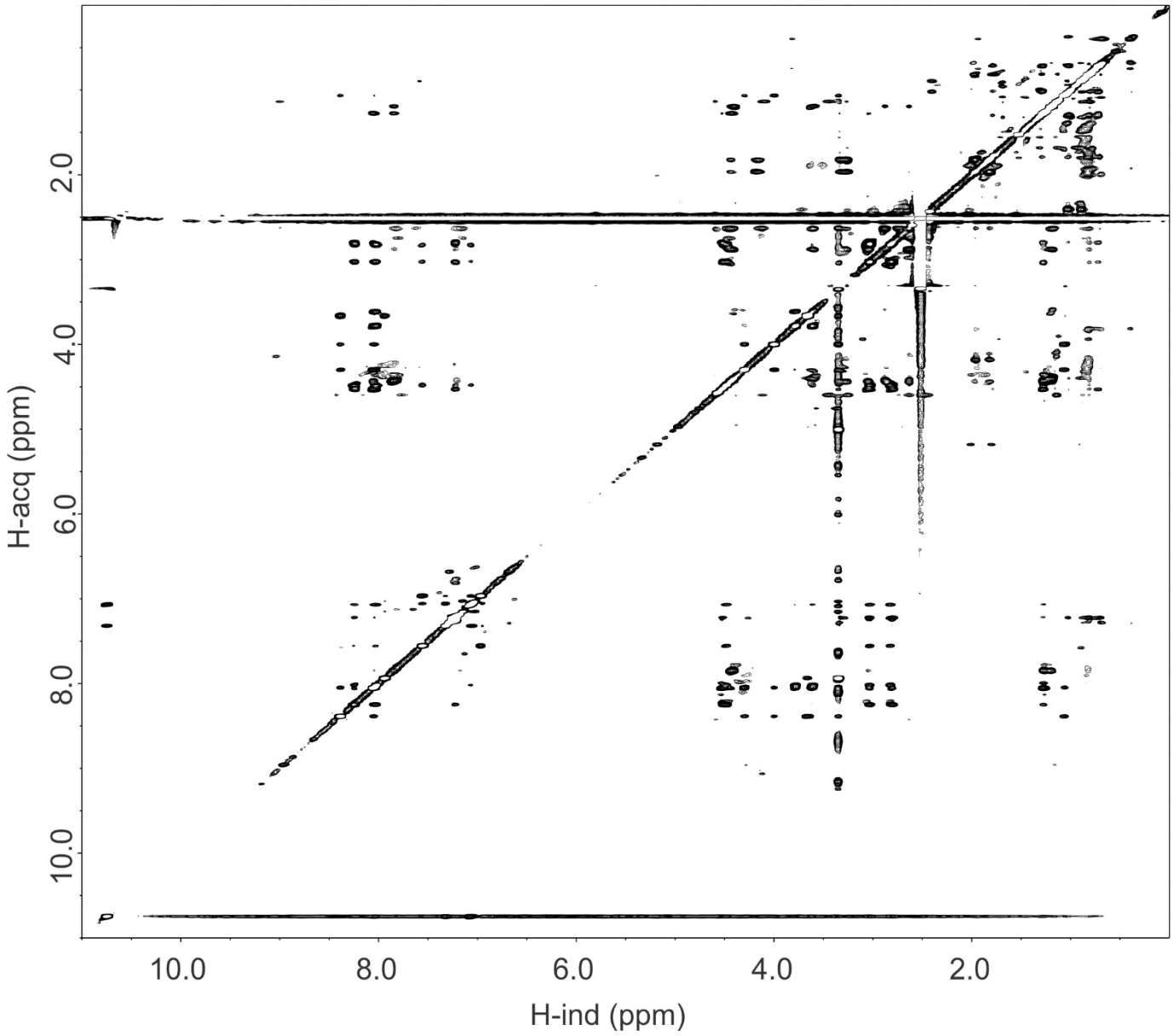
Suppl. Figure 2. TOCSY 60ms NMR Spectrum of salivaricin A2 in DMSO-d6 recorded at proton frequency of 850 MHz, using a TCI Cryoprobe.



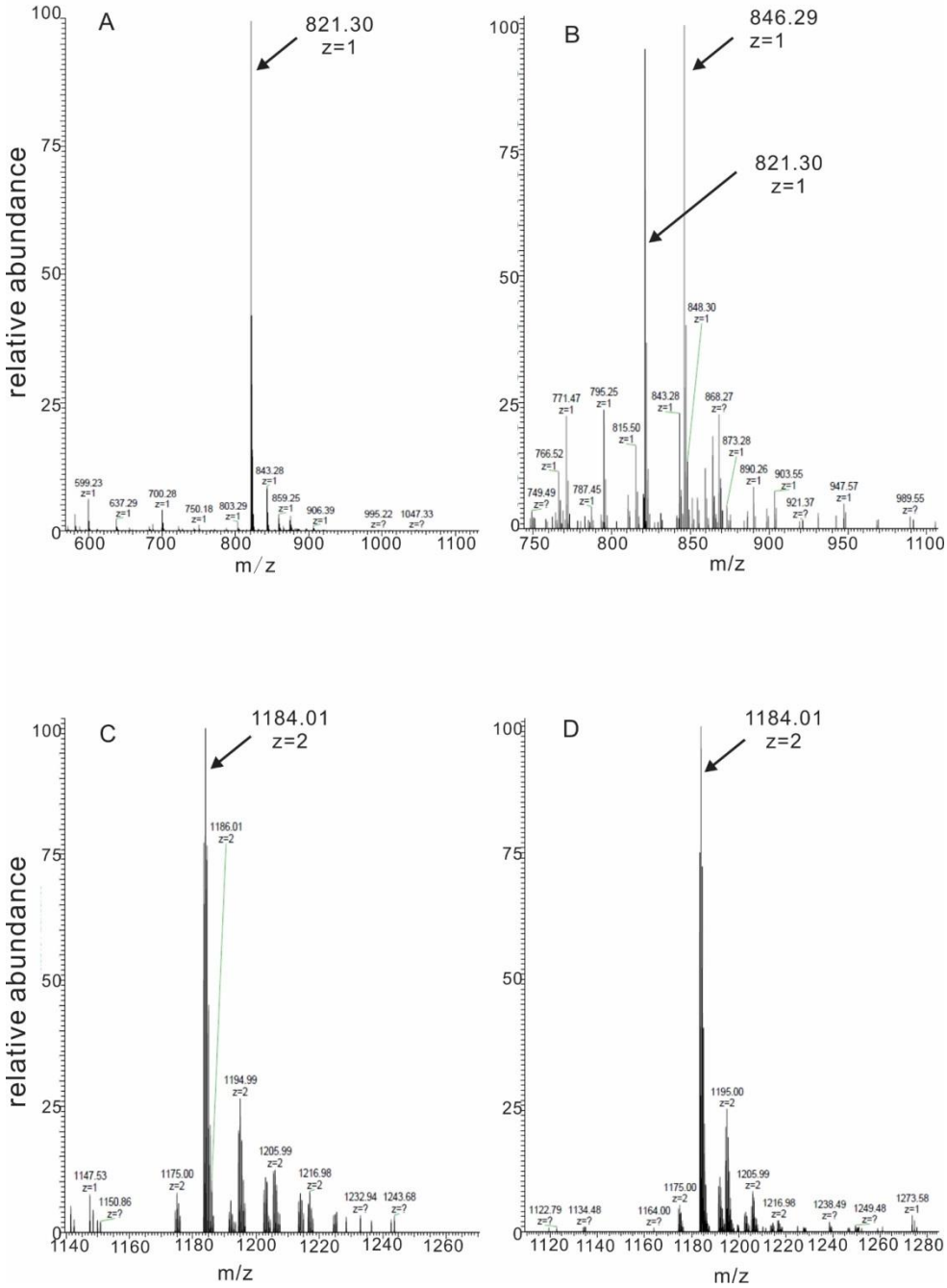
Suppl. Figure 3. NOESY 500ms NMR Spectrum of salivaricin A2 in DMSO-d6 recorded at proton frequency of 850 MHz, using a TCI Cryoprobe.



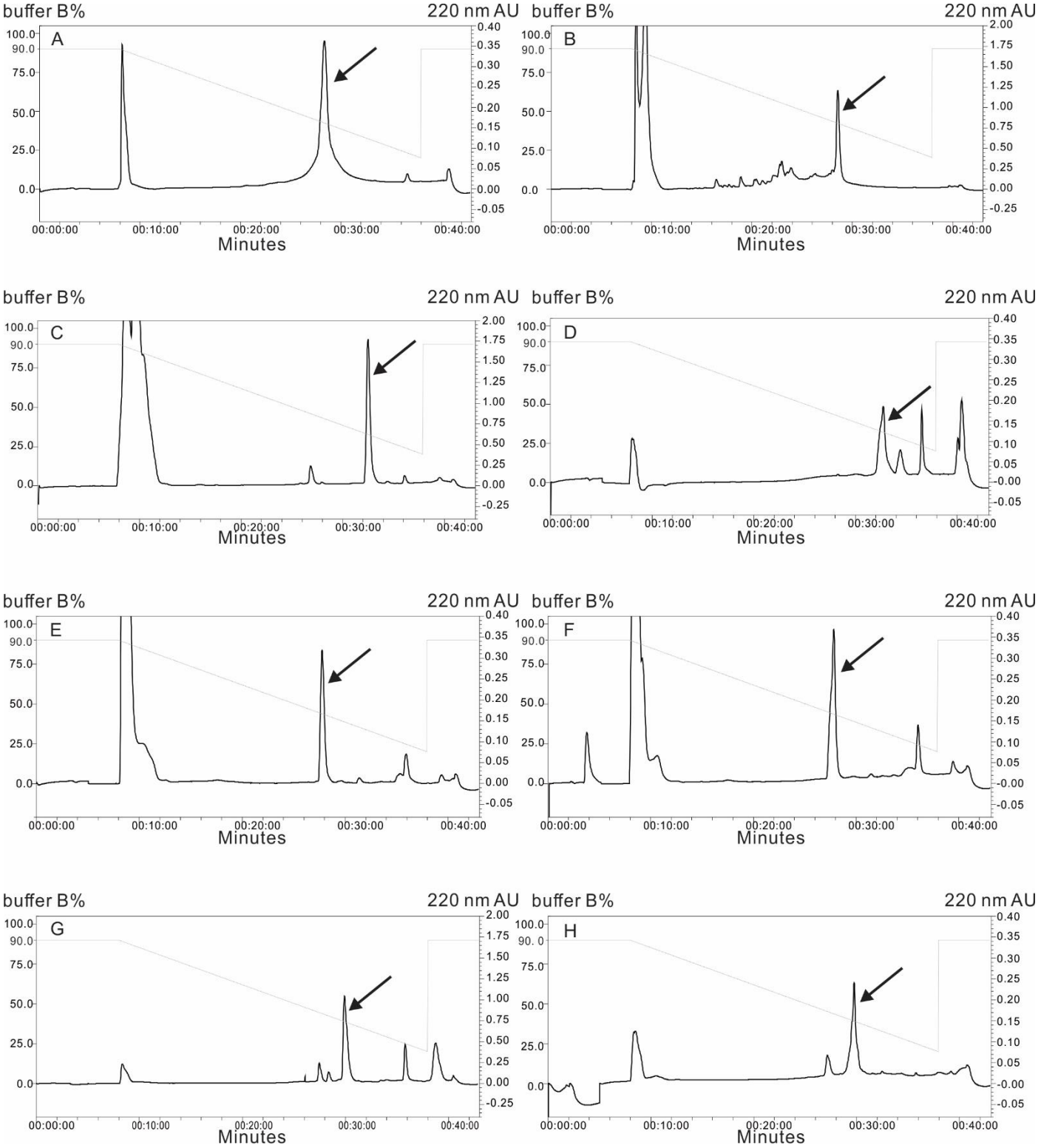
Suppl. Figure 4. TOCSY 60ms NMR Spectrum of salivaricin A2(3-22) in DMSO-d6 recorded at proton frequency of 850 MHz, using a TCI Cryoprobe.



Suppl. Figure 5. NOESY 500ms NMR Spectrum of salivaricin A2(3-22) in DMSO-d6 recorded at proton frequency of 850 MHz, using a TCI Cryoprobe.



Suppl. Figure 6. Masses of salivaricin A2 and control peptide SFNSYTC before and after cyanylation by CDAP. (A) control peptide with one free cysteine; (B) control peptide after cyanylation; (C) salivaricin A2 before cyanylation; (D) salivaricin A2 after cyanylation.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B
C
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F
G
H
Minutes
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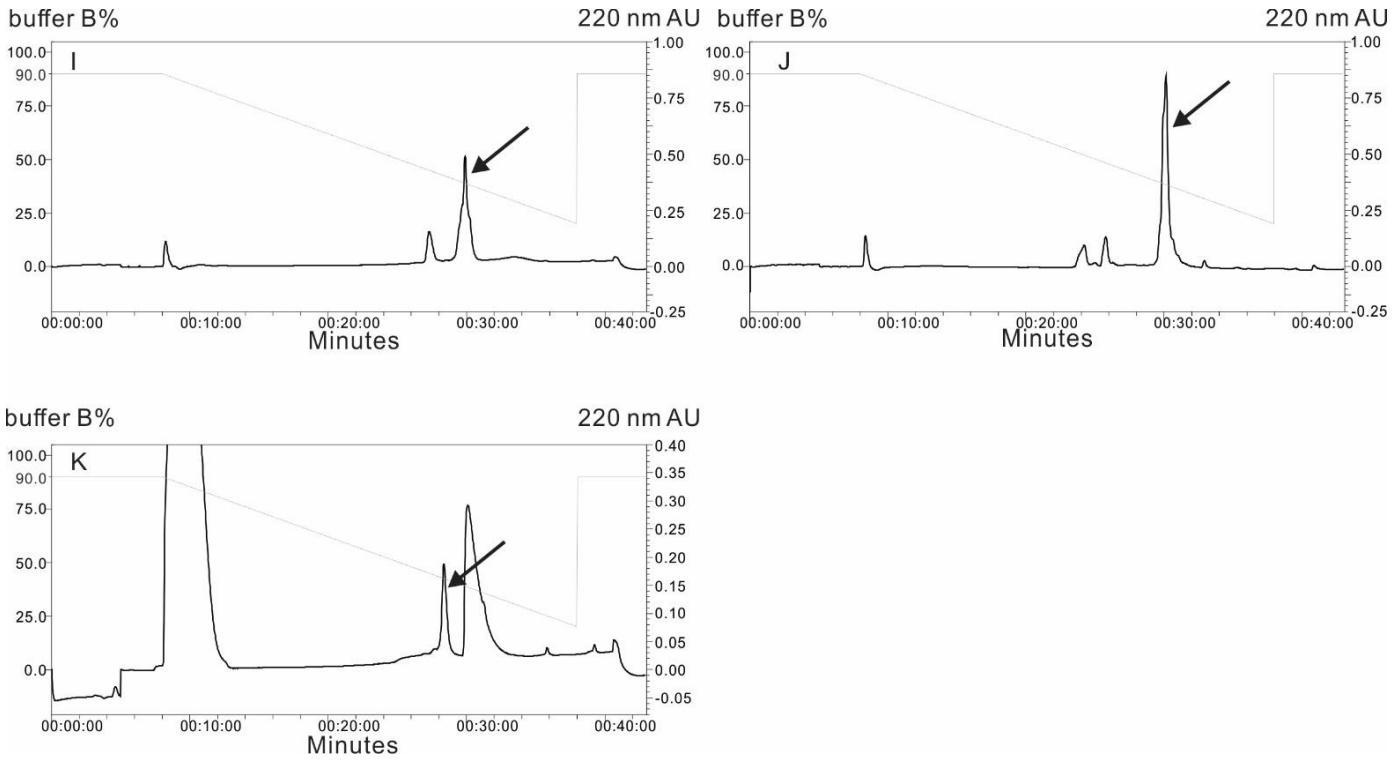
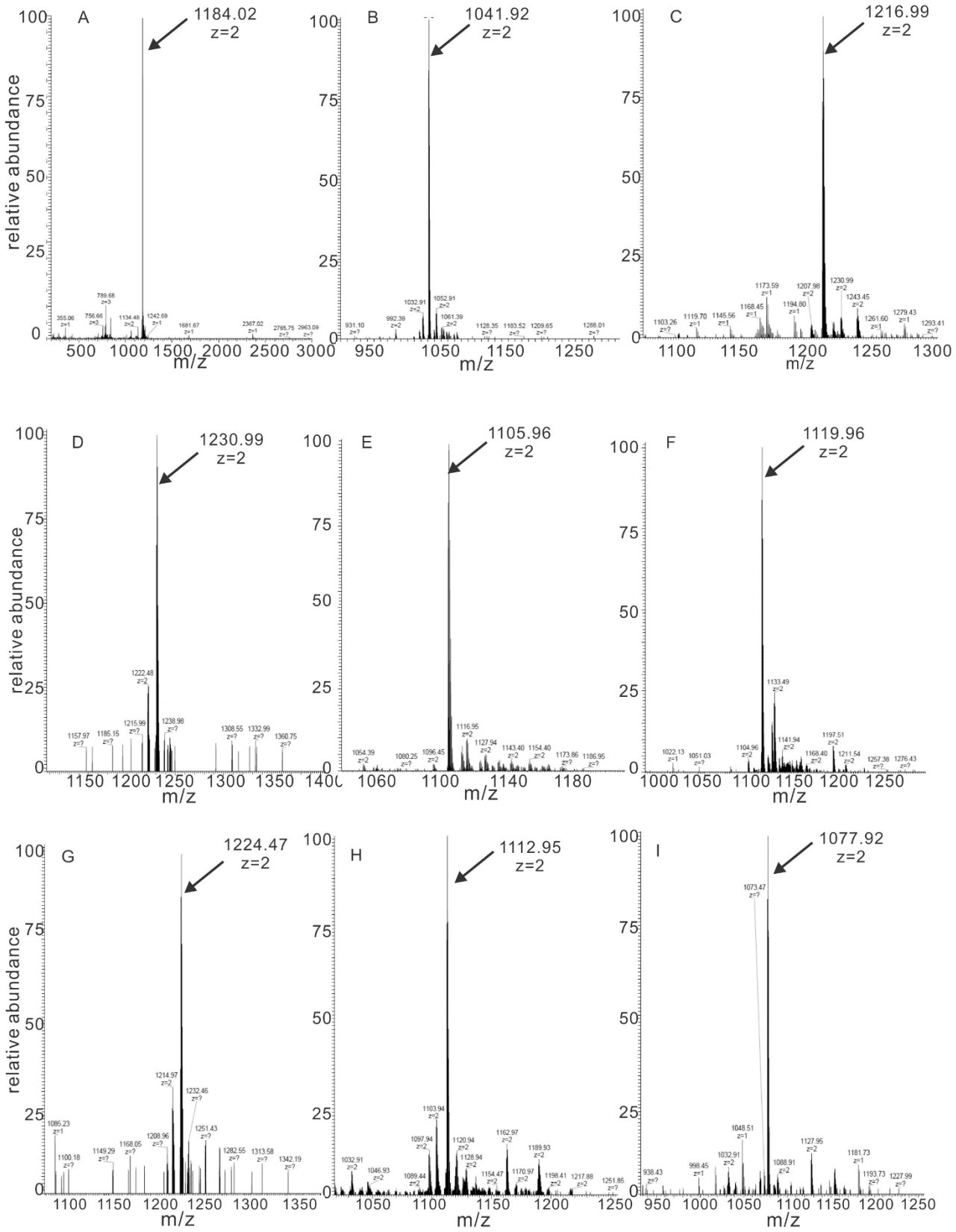


Suppl. Figure 7. HPLC chromatograms of reactions from HOAt/EDC couplings of salivaricin A2(3-22) and amines. Grey lines represent the gradient of buffer B (water with 0.1%TFA), while black lines represent the absorbance unit (AU) at 220 nm. (A) 100 µg salivaricin A2; (B) trypsin digestion of salivaricin A2; (C) salivaricin A2(3-22) coupled with Fmoc-Lys; (D) salivaricin A2(3-22) coupled with Fmoc-Arg; (E) salivaricin A2(3-22) coupled with Lys; (F) salivaricin A2(3-22) coupled with Arg; (G) salivaricin A2(3-22) coupled with Fmoc-Ala-Ala; (H) salivaricin A2(3-22) coupled with Ala-Ala; (I) salivaricin A2(3-22) coupled with Ala; (J) salivaricin A2(3-22) coupled with 5(6)-carboxyfluorescein; (K) salivaricin A2(3-22) coupled with Lys-Arg.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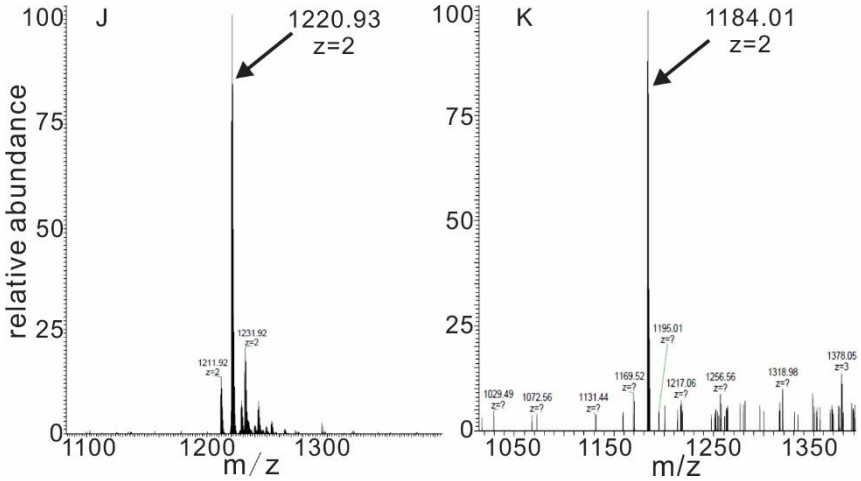
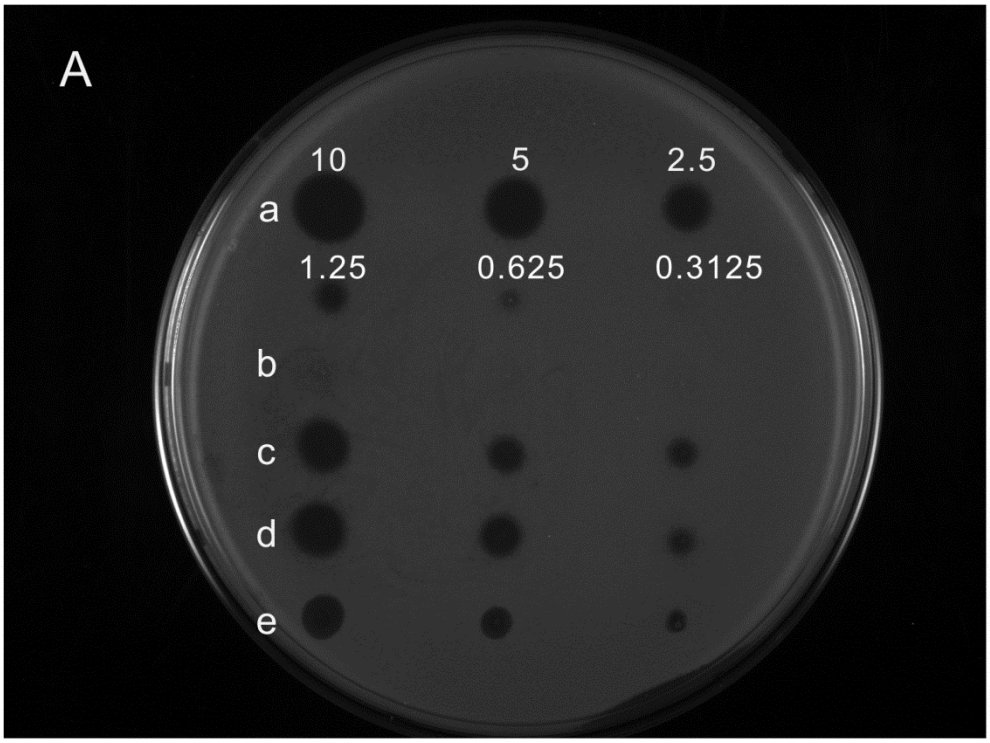
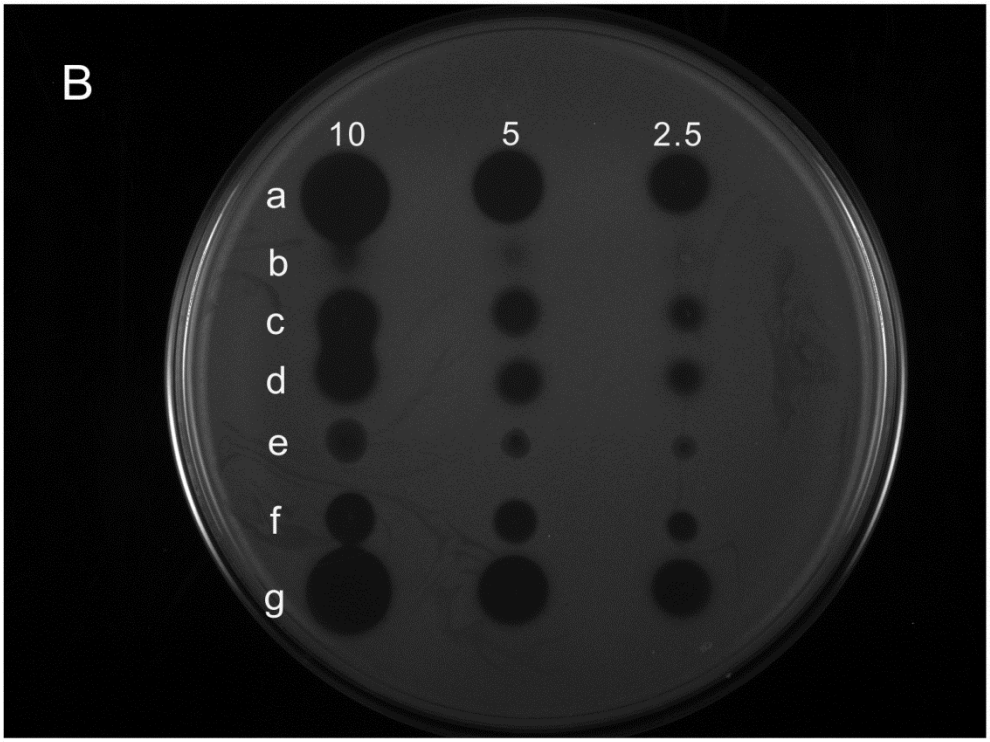
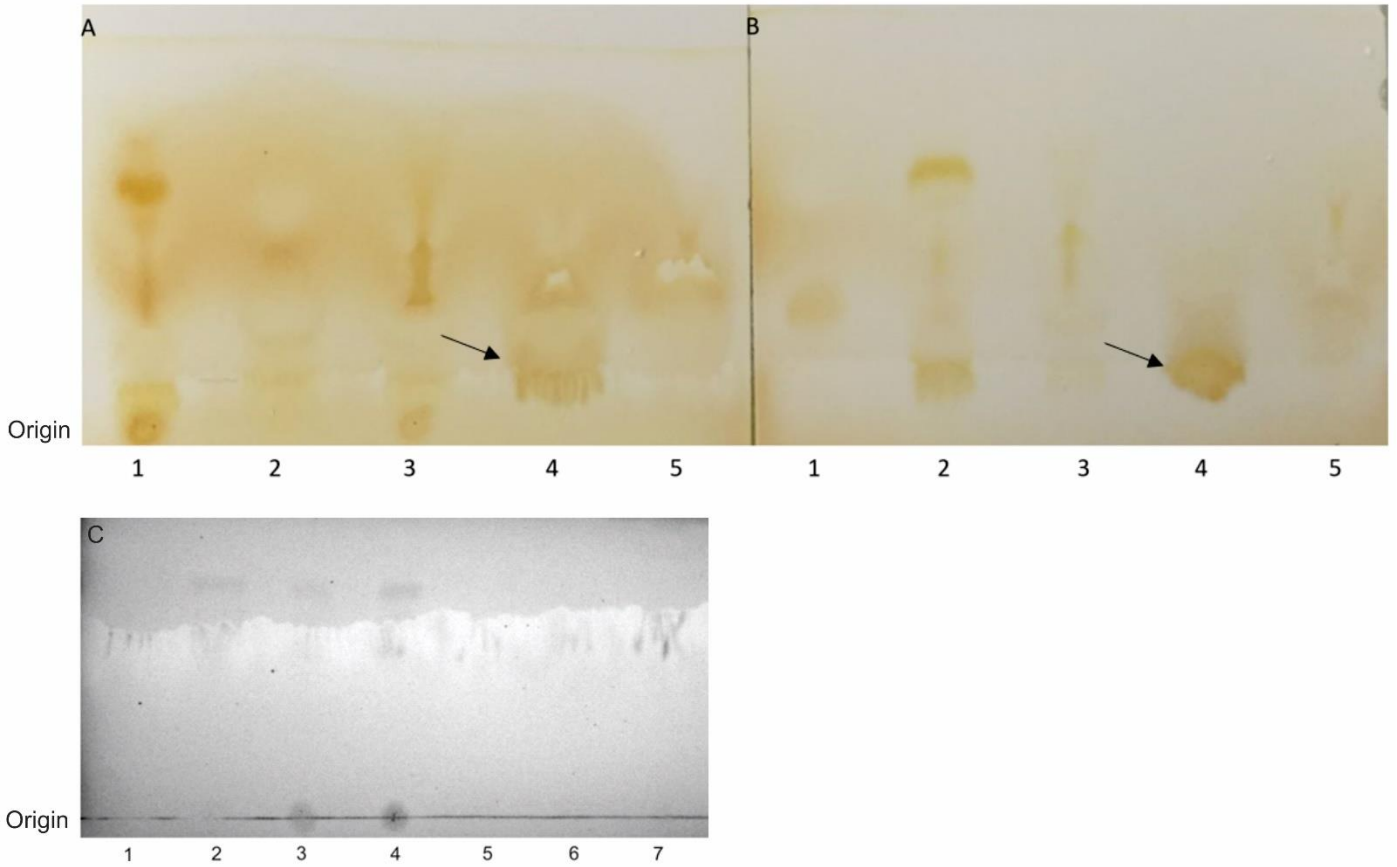


Suppl. Figure 8. ESI-MS data of novel analogues of salivaricin A2. (A) native salivaricin A2; (B) salivaricin A2(3-22); (C) salivaricin A2(3-22) coupled with Fmoc-Lys; (D) salivaricin A2(3-22) coupled with Fmoc-Arg; (E) salivaricin A2(3-22) coupled with Lys; (F) salivaricin A2(3-22) coupled with Arg; (G) salivaricin A2(3-22) coupled with Fmoc-Ala-Ala; (H) salivaricin A2(3-22) coupled with Ala-Ala; (I) salivaricin A2(3-22) coupled with Ala; (J) salivaricin A2(3-22) coupled with 5(6)-carboxyfluorescein; (K) salivaricin A2(3-22) coupled with Lys-Arg.





Suppl. Figure 9. Plates for overlays of novel analogues of salivaricin A2. The numbers on top of each spot specify the amount of material (in micrograms) spotted to give the zones of inhibitions. Plate A: A: native salivaricin A2, spotted at 10, 5, 2.5, 1.25, 0.625, and 0.3125 µg; B: salivaricin A2(3-22), spotted at 10, 5, and 2.5 µg. There is no sign of any inhibitory activity. The other analogues were spotted at 10, 5, and 2.5 µg of material. C: Fmoc-Lys+A2(3-22); D: Fmoc-Arg+A2(3-22); E: Fmoc-Ala-Ala+A2(3-22). 2: A: native salivaricin A2; B: salivaricin A2(3-22); C: Lys+A2(3-22); D: Arg+ A2(3-22); E: Ala+ A2(3-22); F: Ala-Ala+ A2(3-22); G: Lys-Arg+ A2(3-22).



Suppl. Figure 10. TLC plates. A: Lane 1: mutacin 1140 and lipid II, 12:1 ratio; Lane 2: salivaricin A2 and lipid II, 12:1 ratio; Lane 3: salivaricin A2 and lipid II, 24:1 ratio; Lane 4: vancomycin and lipid II, 12:1 ratio; lane 5: digested salivaricin A2 and lipid II, 24:1 ratio. B: Lane 1: lipid II; Lane 2: mutacin 1140; Lane 3: salivaricin A2; Lane 4: vancomycin; Lane 5: digested salivaricin A2. Mutacin 1140 is known for retaining lipid II at the origin and is served as a positive control. Vancomycin targets the terminal D-Ala-D-Ala moiety of lipid II. The antibiotic migrates on the TLC plate (as demarcated by the arrow), thus it is not able to retain lipid II at the origin. The antibiotic served as a negative control. C: Lane 1: lipid II; Lane 2: Mu1140; Lane 3 and 4: Mu1140 to lipid II 3:1 and 12:1 ratio, respectively; Lane 5: salivaricin A2; Lane 6 and 7: salivaricin A2 to lipid II, 3:1 and 12:1 ratio, respectively.

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cctcctttct aaggaa-

Suppl. Figure 11. Representative 16S rRNA sequence of *S. salivarius* HS0302. Based on the genome sequence of the bacterium, it has at least four copies of 16S rRNA, all of which possess higher than 99% identity with *S. salivarius* ATCC 27945, thus we name it as *S. salivarius* HS0302. The only different nucleotide (A-G) in the 16S rRNA sequence shown above is highlighted in red.