The complete stereochemistry of the antibiotic candicidin A3 (syn. ascosin A3, levorin A3)

Julia Borzyszkowska-Bukowska[a†] & Paweł Szczeblewski,[a†] Agnieszka Konkol,[a] Jakub Grynda,[a] Katarzyna Szwarc-Karabyka[b] and Tomasz Laskowski[a]*

SUPPLEMENTARY INFORMATION

[†]*These authors contributed equally.*

[a]*Department of Pharmaceutical Technology and Biochemistry, Faculty of Chemistry, Gdańsk University of Technology, G. Narutowicza Street 11/12, 80-233 Gdańsk, Poland.* [b]*Nuclear Magnetic Resonance Laboratory, Faculty of Chemistry, Gdańsk University of Technology, G. Narutowicza Street 11/12, 80-233 Gdańsk, Poland.*

*Corresponding author: Tomasz Laskowski, Department of Pharmaceutical Technology and Biochemistry, Faculty of Chemistry, Gdańsk University of Technology, G. Narutowicza Street 11/12, 80-233 Gdańsk, Poland. Phone: +48 58 347 20 79, fax: +48 58 347 11 44, e-mail: tomasz.laskowski@pg.edu.pl

ABSTRACT: Herein, the stereostructure of the aromatic heptaene macrolide (AHM) antifungal antibiotic candicidin A3 (syn. ascosin A3, levorin A3) has been established upon the 2D NMR studies, consisting of DQF-COSY, TOCSY, ROESY, HSQC & HMBC experiments, as well as upon extensive molecular dynamics simulations. The geometry of the heptaenic chromophore was defined as: (22*E*, 24*E*, 26*Z*, 28*Z*, 30*E*, 32*E*, 34*E*). The previously unreported absolute configuration of the chiral centres of candicidin A3 was established as: (3*R*, 9*R*, 11*S*, 13*S*, 15*R*, 17*S*, 18*R*, 19*S*, 21*R*, 36*S*, 37*R*, 38*S*, 40*S*, 41*S*).

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^{1}H AND ^{13}C NMR DATA FOR METHYL ESTER OF 3'-N-ACETYLCANDICIDIN A3 (A3*)

Table S1. ^{1}H and ^{13}C NMR data for **A3***. The coupling constants labelled by an asterisk (*) could not be measured due to severe signal overlap and higher order effects.

No.	***δ_C (p.p.m.), type***	***δ_H (p.p.m.)***	***$J_{H,H}$ (Hz)***	***ROE contacts***
Aglycone				
1	171.42, C	-	-	-
2a	43.58, CH_2	2.50	11.3 (2b), 2.5 (3)	4b, 5b, 5a
2b		2.69	11.3 (2a), 10.3 (3)	3, 4b, 4a, 5b, 5a
3	67.84, CH	4.36	2.5 (2a), 10.3 (2b), 3.0 (4a), 9.5 (4b)	2b, 4b, 4a, 5b, 5a
4a	37.63, CH_2	1.53	3.0 (3), 14.2 (4b), * (5a), 10.0 (5b)	2b, 3, 4b, 5b, 5a, 6a
4b		1.69	9.5 (3), 14.2 (4a), * (5a), 2.0 (5b)	2b, 2a, 3, 4a, 5b, 6a, 6b
5a	19.88, CH_2	1.68	* (4a), * (4b), 15.3 (5b), 3.5 (6a), 8.0 (6b)	2b, 2a, 3, 4a, 5b, 6a, 6b
5b		1.89	10.0 (4a), 2.0 (4b), 15.3 (5a), 8.0 (6a),3.5 (6b)	2b, 2a, 3, 4b, 4a, 5a, 6a
6a	43.97, CH_2	2.41	3.5 (5a), 8.0 (5b), 15.0 (6b)	4b, 4a, 5b, 5a, 8b
6b		2.48	8.0 (5a), 3.5 (5b), 15.0 (6a)	4b, 5a, 8b
7	209.04, C	-	-	-
8a	50.57, CH_2	2.49	13.0 (8b), 0.0 (9)	8b, 10a
8b		2.77	13.0 (8a), 10.0 (9)	6a, 6b, 8a, 10b
9	67.59, CH	4.60	0.0 (8a), 10.0 (8b), 0.0 (10a), 10.0 (10b)	11
10a	43.82, CH_2	1.50	0.0 (9), 13.0 (10b), 0.0 (11)	8a, 10b, 11, 12b
10b		1.80	10.0 (9), 13.0 (10a), 11.0 (11)	8b, 10a, 12a
11	73.07, CH	4.30	0.0 (10a), 11.0 (10b), 0.0 (12a), 11.0 (12b)	9, 10a, 12a, 13, 25
12a	44.13, CH_2	1.49	0.0 (11), 14.0 (12b), 4.0 (13)	10b, 11, 12b, 13, 14a
12b		1.72	11.0 (11), 14.0 (12a), 11.4 (13)	10a, 12a, 14b
13	69.12, CH	4.76	4.0 (12a), 11.4 (12b), 3.5 (14a), 11.0 (14b)	11, 12a, 14a, 22
14a	46.84, CH_2	1.78	3.5 (13), 15.0 (14b)	12a, 13, 14b, 16b
14b		1.98	11.0 (13), 15.0 (14a)	12b, 14a, 16b, 16a
15	98.02, C	-	-	-
16a	45.11, CH_2	1.76	10.2 (16b), 11.1 (17)	14b, 16b, 18
16b		2.56	10.2 (16a), 4.0 (17)	14b, 14a, 16a, 17
17	66.38, CH	5.03	11.1 (16a), 4.0 (16b), 11.0 (18)	16b, 18
18	58.60, CH	2.85	11.0 (17), 10.5 (19)	16a, 17, 20b, 20a
19	66.12, CH	5.08	10.5 (18), 11.0 (20a), 0.0 (20b)	20b, 20a, 22
20a	37.81, CH_2	2.05	11.0 (19), 11.7 (20b), 0.0 (21)	18, 19, 20b, 21
20b		2.44	0.0 (19), 11.7 (20a), 7.5 (21)	1', 18, 19, 20a, 21
21	76.18, CH	4.92	0.0 (20a), 7.5 (20b), 6.4 (22)	1', 20b, 20a, 22, 23
22	137.19, CH	6.48	6.4 (21), 15.3 (23)	13, 19, 21, 24
23	130.10, CH	6.45	15.3 (22), 10.8 (24)	21
24	130.61, CH	6.34	10.8 (23), 15.0 (25)	22, 26
25	124.88, CH	6.59	15.0 (24), 11.3 (26)	11, 28

26	130.41, CH	6.77	11.3 (25), 11.0 (27)	24, 27
27	127.88, CH	6.95	11.0 (26), 11.6 (28)	26, 30
28	125.11, CH	7.08	11.6 (27), 12.0 (29)	25, 29
29	133.14, CH	7.35	12.0 (28), 11.5 (30)	28, 31
30	129.10, CH	6.47	11.5 (29), 16.0 (31)	27, 32
31	132.83, CH	6.64	16.0 (30), 10.5 (32)	29, 33
32	135.02, CH	6.73	10.5 (31), 15.5 (33)	30, 34
33	133.64, CH	6.37	15.5 (32), 11.4 (34)	31, 35
34	132.56, CH	6.31	11.4 (33), 15.6 (35)	32, 36
35	137.57, CH	5.60	15.6 (34), 7.9 (36)	33, 37, Me36
36	40.30, CH	2.60	7.9 (35), 9.3 (37), 6.7 (Me36)	34, Me36, Me38
37	79.50, CH	5.10	9.3 (36), 2.4 (38)	35, 38, 39a, Me36
38	31.23, CH	2.15	2.4 (37), 0.0 (39a), 9.5 (39b), 6.8 (Me38)	37, 39b, 39a, Me36, Me38, Me40
39a	37.42, CH_2	1.54	0.0 (38), 11.1 (39b), 7.1 (40)	37, 38, 39b
39b		1.87	9.5 (38), 11.1 (39a), 0.0 (40)	38, 39a, Me38
40	36.79, CH	2.03	7.1 (39a), 0.0 (39b), 4.2 (41), 7.9 (Me40)	41, 42a, Me38, Me40
41	71.89, CH	4.52	4.2 (40), 3.8 (42a), 8.0 (42b)	40, 42a, Me40
42a	42.05, CH_2	3.16	3.8 (41), 13.0 (42b)	40, 41, 42b, Me40, B
42b		3.40	8.0 (41), 13.0 (42a)	42a, Me40, B
43	198.03, C	-	-	-
Me36	16.51, CH_3	0.99	6.7 (36)	35, 36, 37, 38
Me38	12.53, CH_3	1.07	6.8 (38)	36, 38, 39b, 40
Me40	15.77, CH_3	1.11	7.9 (40)	38, 40, 41, 42a, 42b
COOMe	173.87, C	-	-	-
COO**Me**	51.59, CH_3	3.81	-	2', 3'
Aromatic carbons				
A	113.21, CH	6.98	8.7 (B)	B
B	131.28, CH	8.15	8.7 (A)	42a, 42b, A
C*CO	154.21, C	-	-	-
C*NH2	126.71, C	-	-	-
3'-N-acetylmycosamine moiety				
1'	98.47, CH	4.97	1.4 (2')	2’, 3’, 5’, 20b, 21
2'	70.83, CH	4.47	1.4 (1'), 3.1 (3')	1’, 3', COOMe
3'	56.02, CH	4.70	3.1 (2'), 10.3 (4'), 7.5 (NH)	1’, 2', 4', 5’, COOMe, NH
4'	71.79, CH	4.05	10.3 (3'), 10.2 (5')	3', 5', 6', NH
5'	74.81, CH	3.78	10.2 (4'), 6.4 (6')	1’, 3’, 4', 6’
6'	18.43, CH_3	1.58	6.4 (5')	4', 5’
NH	-	8.93	7.5 (3')	3', 4’, COMe
COMe	170.81, C	-	-	-
CO**Me**	22.89, CH_3	2.11	-	NH

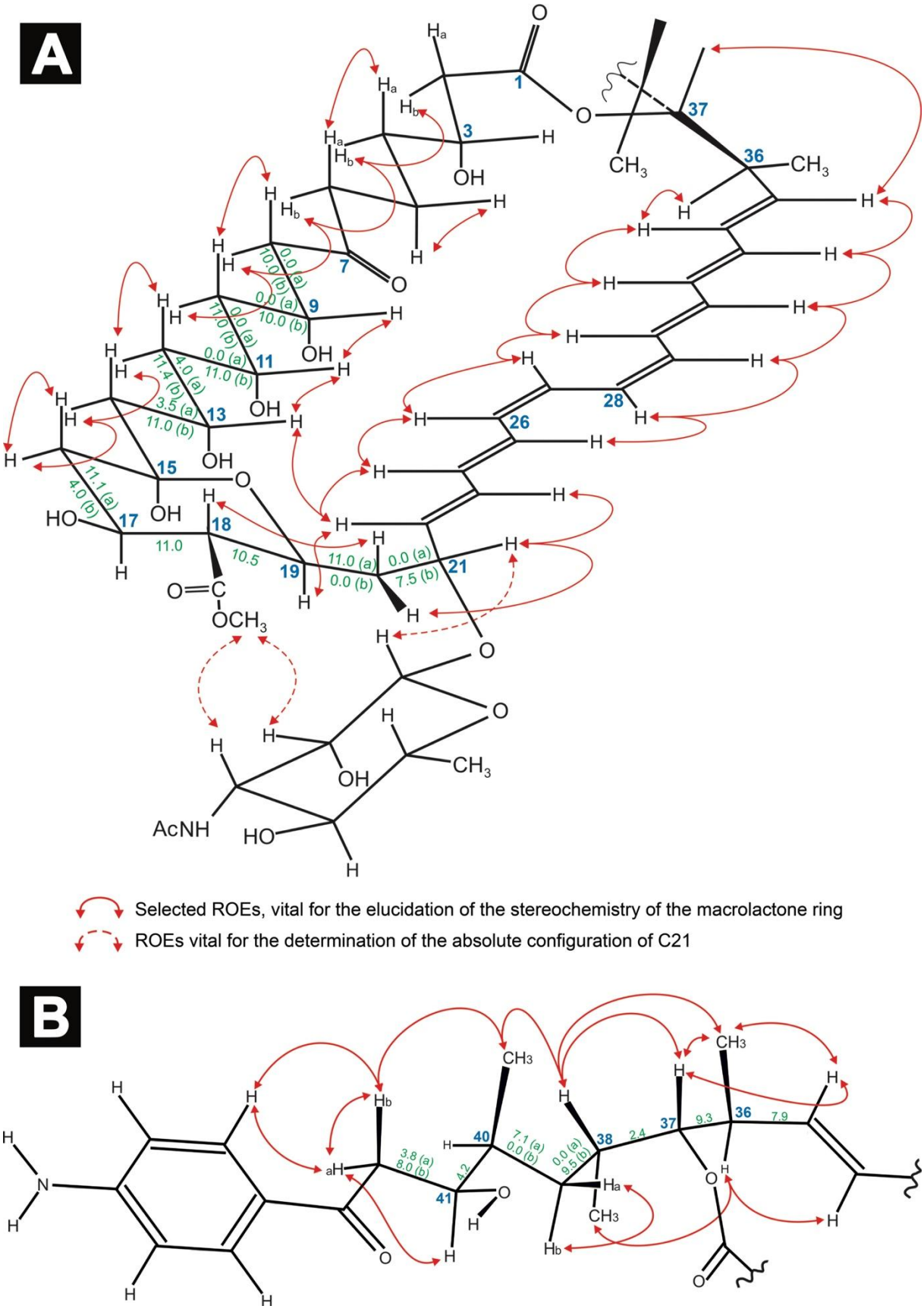

Figure S1. The stereochemistry of the methyl ester of 3'-N-acetylcandicidin A3, **A3***: the macrolactone ring (A) and the aromatic sidechain (B). Selected vicinal coupling constants are given along the C-C bonds. Selected ROEs are depicted as red, bidirectional arrows.

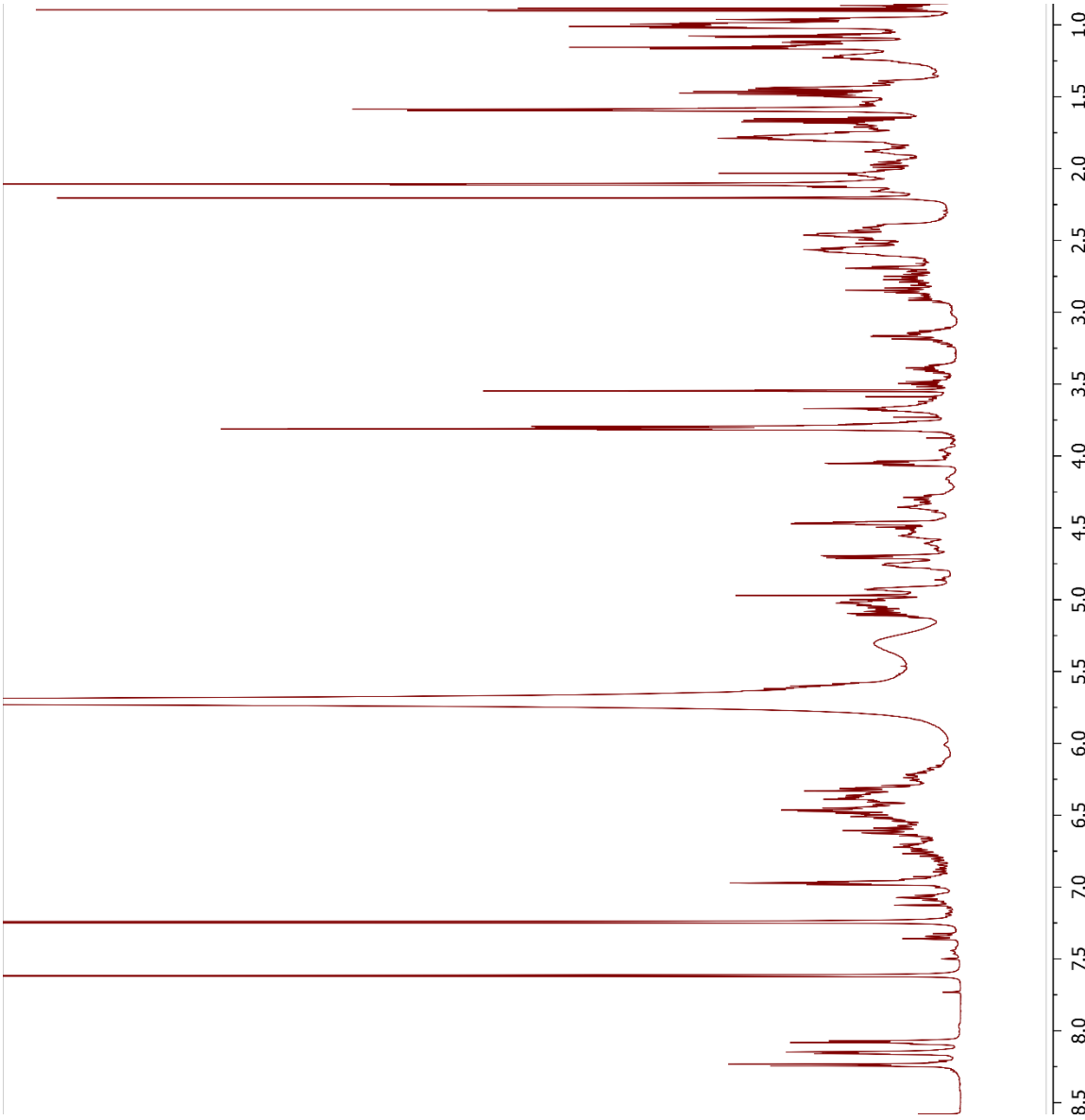

Figure S2. ^{1}H NMR spectrum of **A3***.

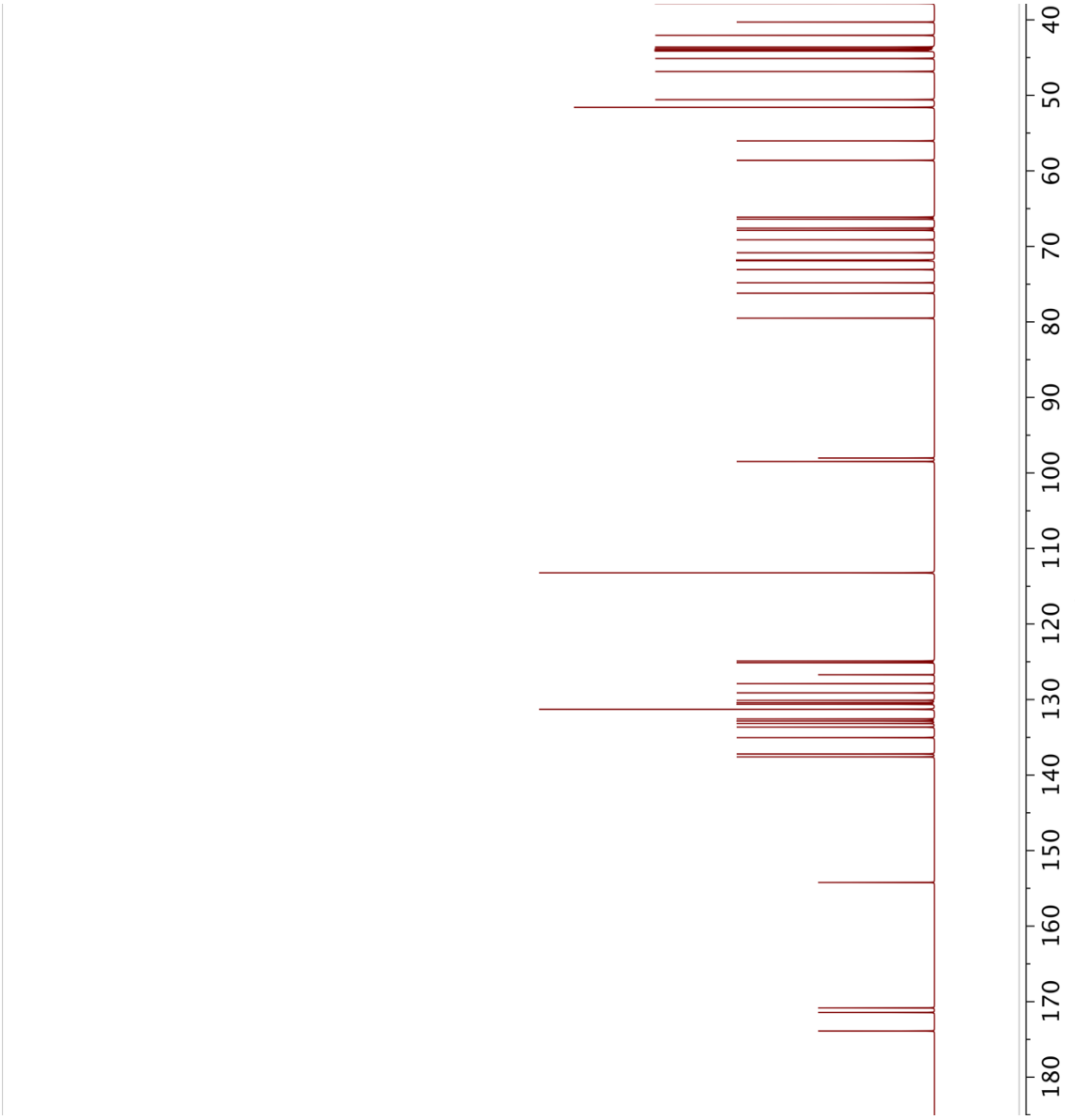

Figure S3. Simulated ^{13}C NMR spectrum of **A3***, prepared on the basis of the ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC data.

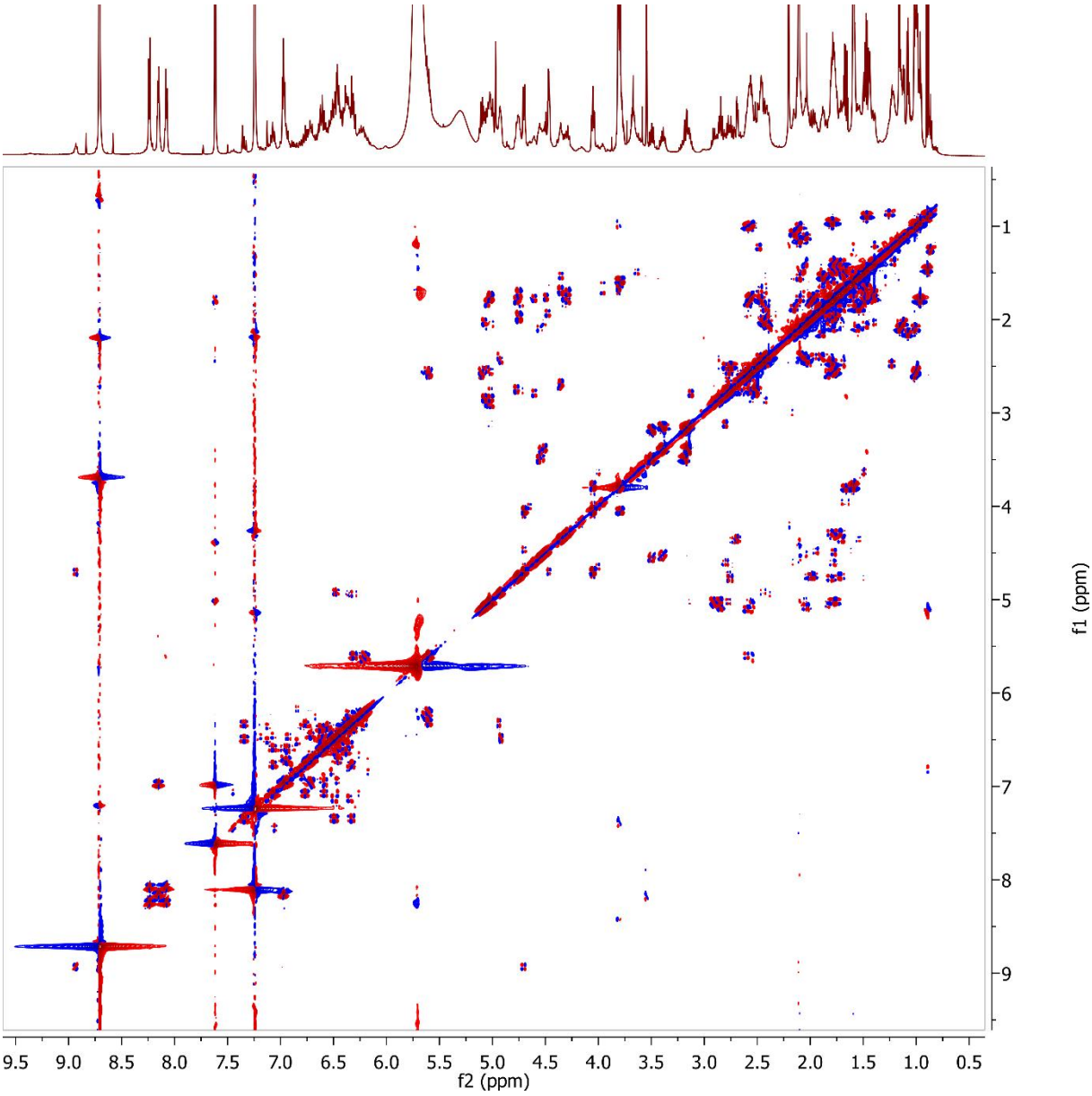

Figure S4. DQF-COSY spectrum of **A3***.

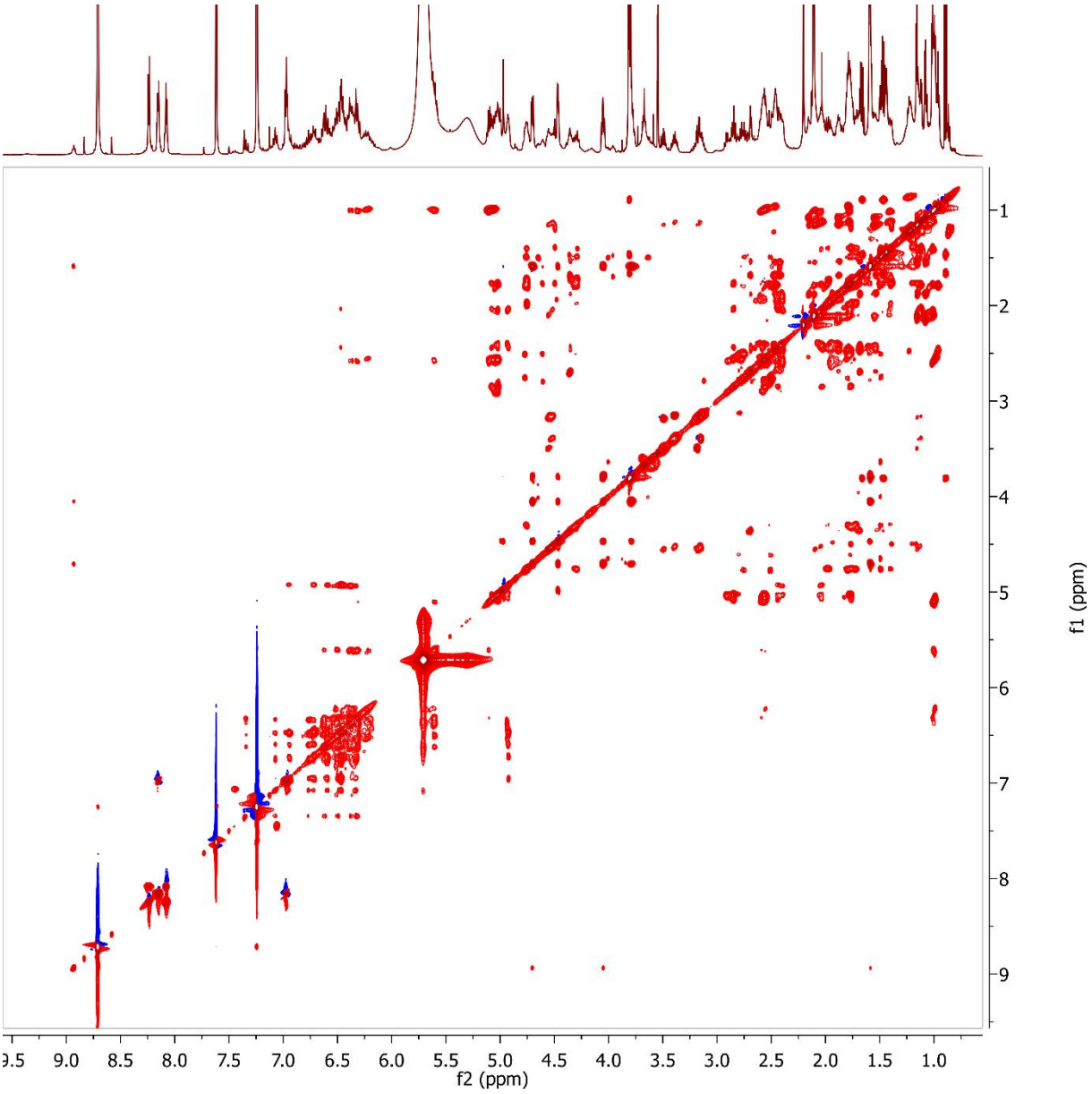

Figure S5. TOCSY spectrum of **A3***. Mix time = 110 ms.

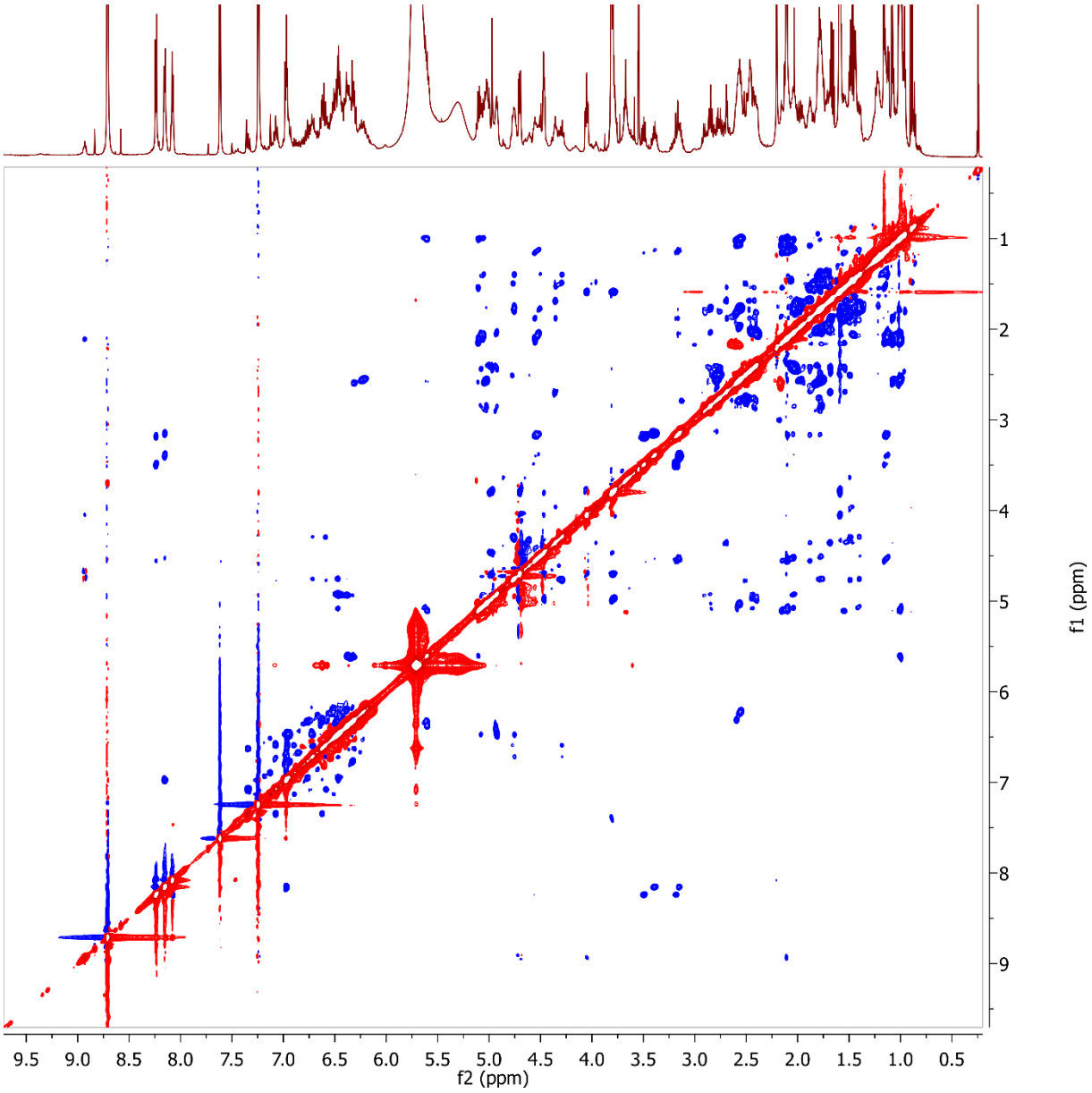

Figure S6. ROESY spectrum of **A3***. Mix time = 300 ms.

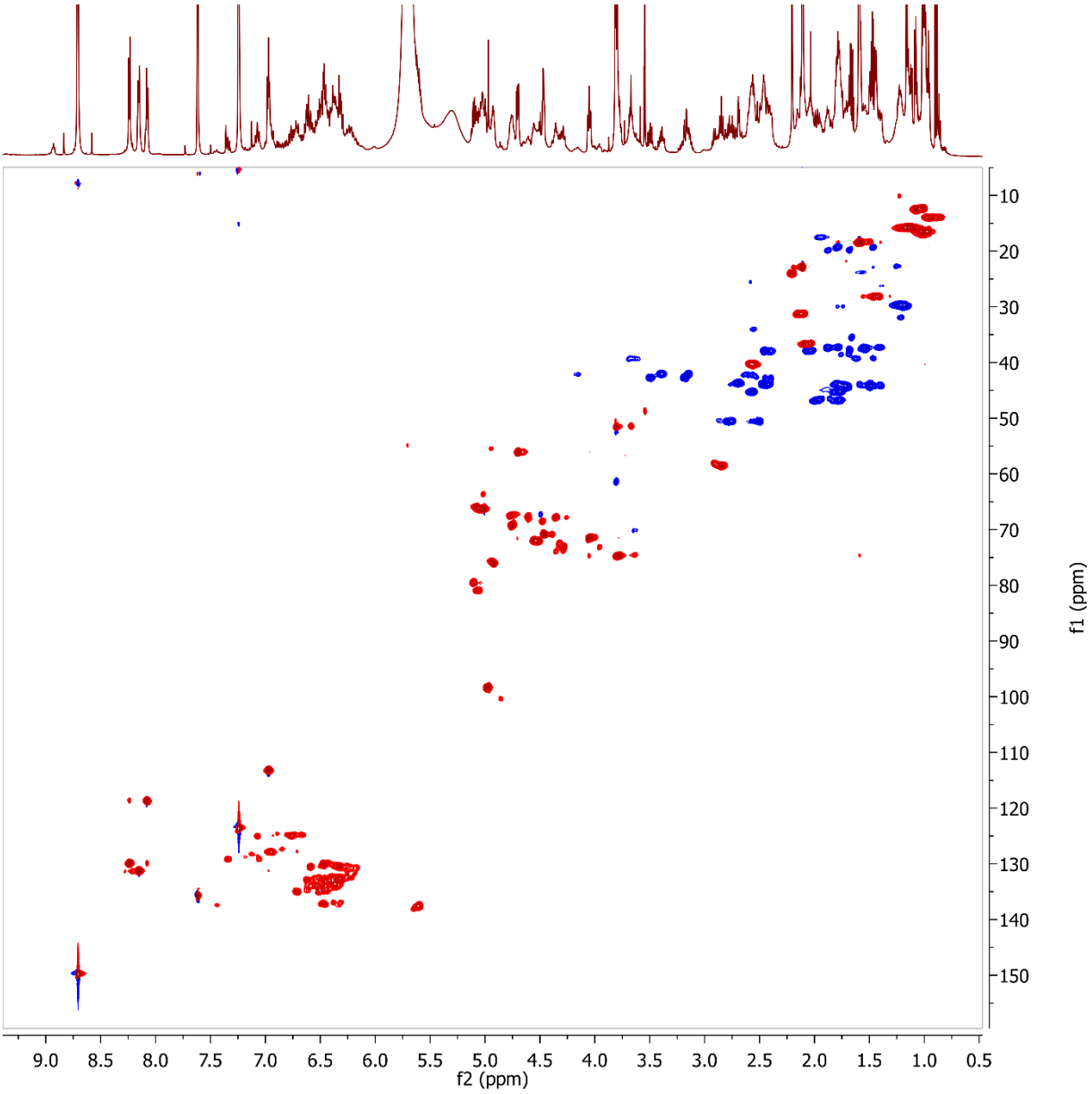

Figure S7. Edited-HSQC spectrum of **A3***.

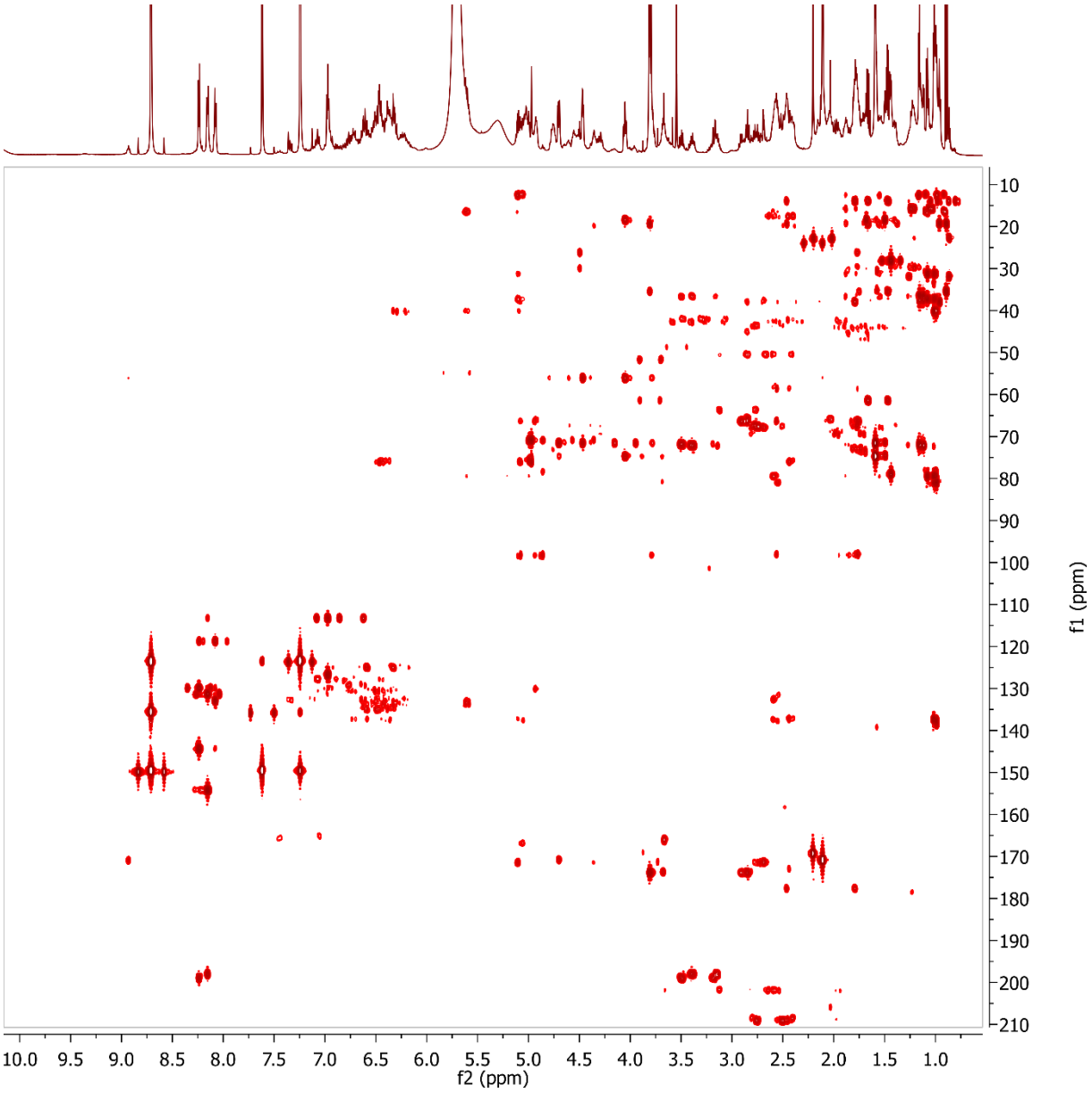

Figure S8. HMBC spectrum of **A3***.

SUPPLEMENTARY INFORMATION FOR THE HLA EQUATION

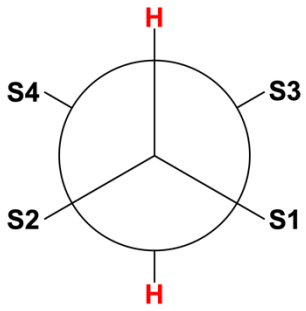

Figure S9. The assignment of the values of the ξ_i parameters of the HLA equation. For the calculation of the $^3J_{H/H}$ coupling constant in the alignment shown above, the ξ_i of the S1 and S3 substituents are equal to 1, whereas for the S2 and S4 substituents they are equal to -1.

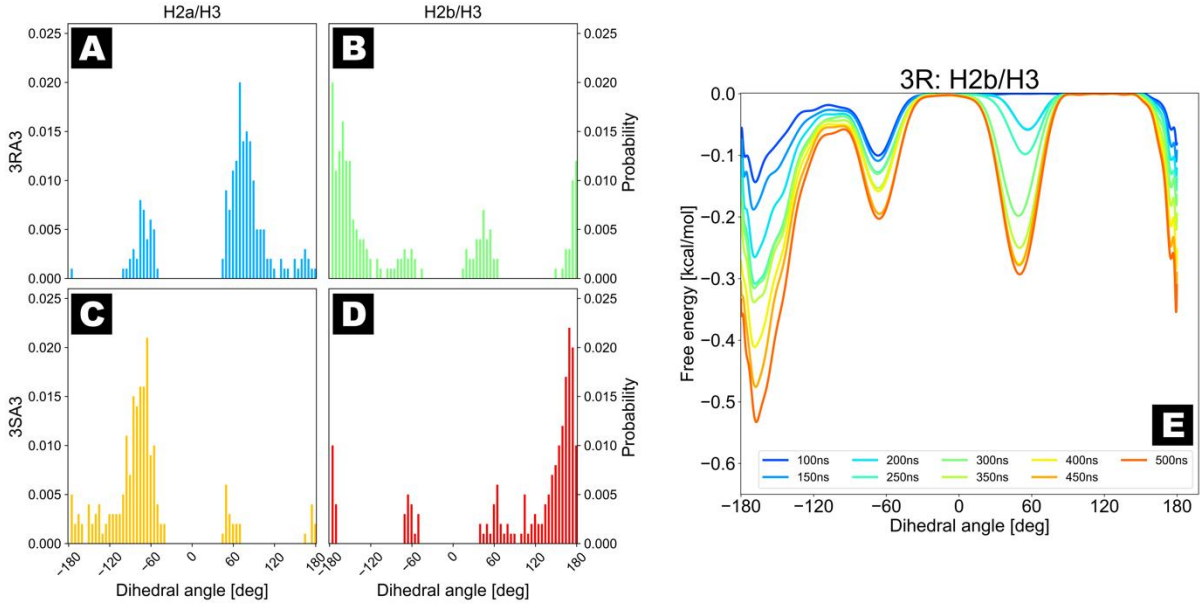

Figure S10. Distribution of the dihedral angles during the 500 ns equilibrium MD simulations of the both possible candicidin A3 diastereoisomers, presented as histograms: H2a/H3 and H2b/H3 of the **3RA3** (**A** and **B**, respectively), H2a/H3 and H2b/H3 of the **3SA3** (**C** and **D**, respectively). On the right side: free energy profile of the H2b/H3 dihedral of **3RA3** (**E**).

Table S2. Measured and calculated vicinal coupling constants for H2a/H3, H2b/H3, H3/H4a and H3/H4b dihedral angles.

Dihedral	Measured $^{3}J_{H/H}$ [Hz]	Calculated averaged $^{3}\hat{J}_{H/H} \pm$ standard deviation [Hz]	
		3RA3	3SA3
H2a/H3	2.5	2.73 ± 1.46	4.63 ± 1.58
H2b/H3	10.3	9.75 ± 1.79	7.72 ± 1.84
H3/H4a	3.0	3.27 ± 1.31	3.43 ± 1.14
H3/H4b	9.5	9.65 ± 1.80	8.46 ± 1.69