Supporting Information

for

Structure and conformational analysis of spiroketals from 6-*O*-methyl-9(*E*)-hydroxyiminoerythronolide A

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Observed nOe contacts (Table SI1–4), proton vicinal coupling constants used for molecular modelling calculations (Table SI5) and accurate mass measurements (Table SI7) for compounds 2–4, as well as HRMS fragmentation for compound 2 (Figures SI1 and SI2, Table SI6). Details of the reaction kinetics calculation.

Table SI1. nOe interactions of compound **2** in DMSO-d_6 at 25 °C.

	2	2Me	3	3OH	4	4Me	5	6Me	6OMe	7a	7b	8	8Me	10	10Me	11	11OH	12Me	13	14a	14b	15
2	●	ss		s			ss							ss								
2Me	ss	●	ss	s																		m
3		ss	●		ss	ss	s															
3OH	m	s	ss	●	ss			ww														
4			ss	ss	●		ss	ss														
4Me			ss		ss	●	w															
5	ss			s	m	w	●	ss	s		s				s							
6Me				w	ss		ss	●	ss	m	m	ww										
6OMe						m	ss	ss	●	s	ww	m	ww									
7a								ss	ss	●			m									
7b							ss	ss	ww		●		s		ss							
8									m			●										
8Me									w	m	w	ss	●		ss	s						
10	ss													●	ss		s					
10Me							s				s		ss	ss	●	s	s					
11													s		ss	●		ss				
11OH														s	s	ss	●	m		s		
12Me																ss	m	●	s	m	ss	
13																		ss	●	s	w	ss
14a																	s	m		●	ss	ss
14b																ss			w	ss	●	
15		m															s	s				●

ss - very strong, s - strong, m - medium, w - weak, ww - very weak, blue shading – most probably nOe signals of 6OMe, overlap with 10, green shading – key nOe's for conformational analysis

Table SI2. nOe interactions of compound **2** in $CDCl_3$ at 25 °C.

	2	2Me	3	3OH	4	4Me	5	6Me	6OMe	7a	7b	8	8Me	10	10Me	11	11OH	12Me	13	14a	14b	15
2	●						ss							ss								
2Me		●	ss		w																	w
3		ss	●		ss	ss																
3OH				●																		
4			ss		●		ss	ss														
4Me			ss			●			s	w												
5	ss				ss		●	ss			s			ss								
6Me					ss		ss	●	ss	s	s											
6OMe								ss	●	ss		m										
7a								ss	ss	●			s									
7b					m		s	s			●		m		ss							
8												●	ss									
8Me										m	m	ss	●		ss	m		m				
10	ss						ss			ww				●		s						
10Me										m	ss	ww	ss		●	ss						
11													s		ss	●		ss				
11OH																	●					
12Me																ss		●	s	m	ss	
13																		ss	●			m
14a																		s		●		
14b																		ss			●	
15		w																	ss		s	●

ss - very strong, s - strong, m - medium, w - weak, ww - very weak

Table SI3. nOe interactions of compound **3** in $CDCl_3$ at 25 °C.

	2	2M	3	3OH	4	4M	5	6Me	6OMe	7a	7b	8	8M	10	10Me	11	11OH	12Me	13	14a	14b	15
2	●		m				ss															
2M		●																				
3	m	s	●		s	m																
3OH				●																		
4			ss		●			s	w													
4M			m			●	ww		w													
5	m				s		●	ss			w				ss							
6Me					s		ss	●	ss	m		s										
6OMe					w	ww		ss	●	m		ww										
7a								s	s	●			m									
7b					m		s	s			●		m		s							
8								s	w			●	m									
8Me										m	w	s	●		ss							
10														●								
10Me	ww						ss				m		s		●	s						
11															m	●	s				ww	
11OH																	●					
12Me															s			●	m			
13																		m	●	ww	w	w
14a																			m	●		
14b																m					●	
15																w			m	s	s	●

ss - very strong, s - strong, m - medium, w - weak, ww - very weak, blue shading – overlap 4 and 8

Table SI4. nOe interactions of compound **4** in DMSO-d_6 at 25 °C.

	2	2Me	3	3OH	4	4Me	5	6Me	6OMe	7a	7b	8	8Me	10	10Me	11	12Me	13	14a	14b	15
2	●	s	w				s														
2Me	s	●	m																		w
3	w	s	●		m	s	m														
3OH				●																	
4			m		●	s	s		m												
4Me			m		s	●	w		w		s							m			
5	m				s		●	s	w												
6Me							s	●	s	m		s									
6OMe					w	w	w	s	●	m	w										
7a								m	m	●	s	m	s								
7b						s			w	s	●		m								
8								s		m		●	s		s						
8Me										m	s	m	●		m	w	s				
10														●							
10Me												m			●	s	s				
11															s	●	s			s	
12Me													s			s	●	s	m		
13						m											s	●			m
14a																			●	s	s
14b																s			s	●	
15		w															w	m	s		●

s - strong, m - medium, w - weak, blue shading – signals used in molecular modelling calculations

Table SI5. Proton vicinal coupling constants (^3J) for compound **4** in DMSO-d_6 at 25 °C with corresponding angle constraints.

Protons	^{3}J/Hz	Angle constrains	
		0° ≤ α ≤ 90°	**90° ≤ α ≤ 180°**
2, 3	10.3		180
3, 4	3.0	51	125
4, 5	2.0	60	120
7a, 8	3.7	46	130
7b, 8	12.7		180
13, 14a	2.4	56	122
13, 14b	11.3		180

LC–HRMS ANALYSIS

LC–HRMS analysis of compound **2** revealed a chromatographic peak at t_R = 12.6 min to be the spiroketal. HRMS results confirm the proposed structure based on three signals: protonated molecule $(M + H)^+$, ammonium adduct $(M + NH_4)^+$ and ammonium adduct of dimmer molecule $(2M + NH_4)^+$ (Figure SI1 and Table SI6). According to these results, fragmentation scheme was proposed (Figure SI2). Furthermore, H/D exchange experiment confirmed two exchangeable protons.

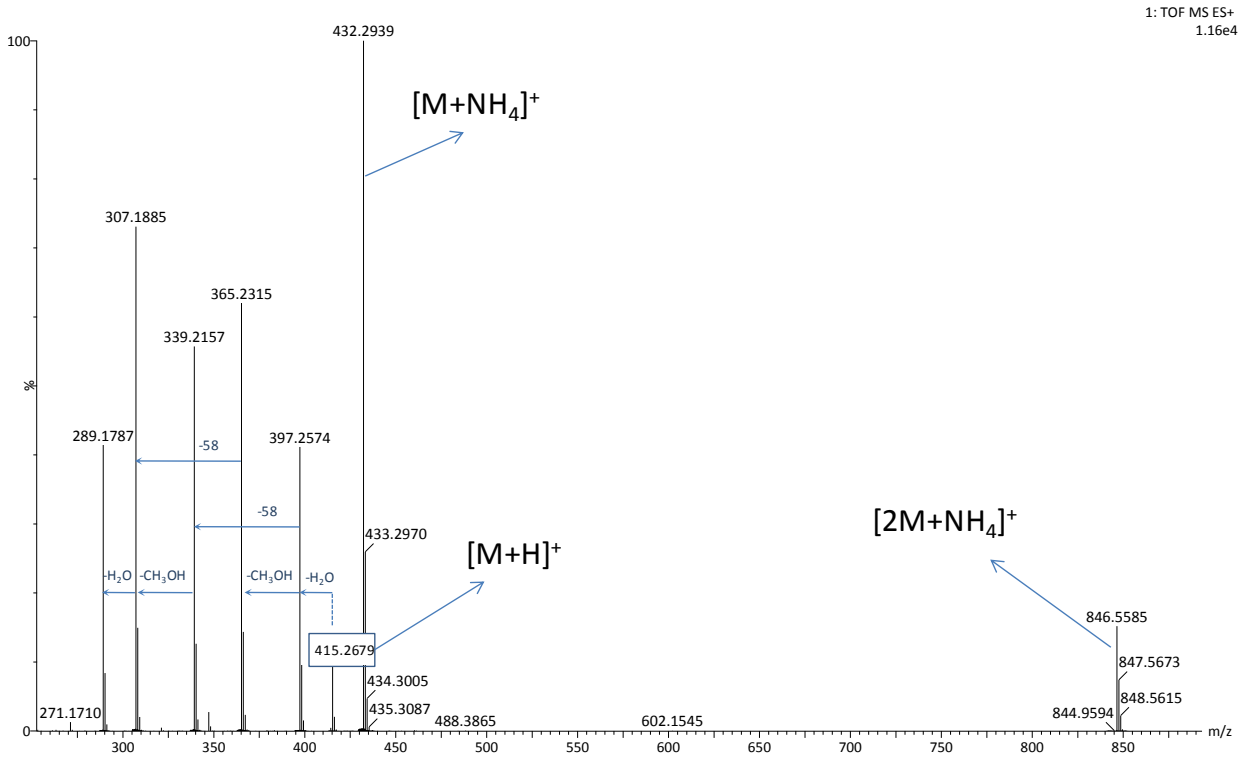

Figure SI1. Full scan HRMS spectrum and signal assignment of compound **2**.

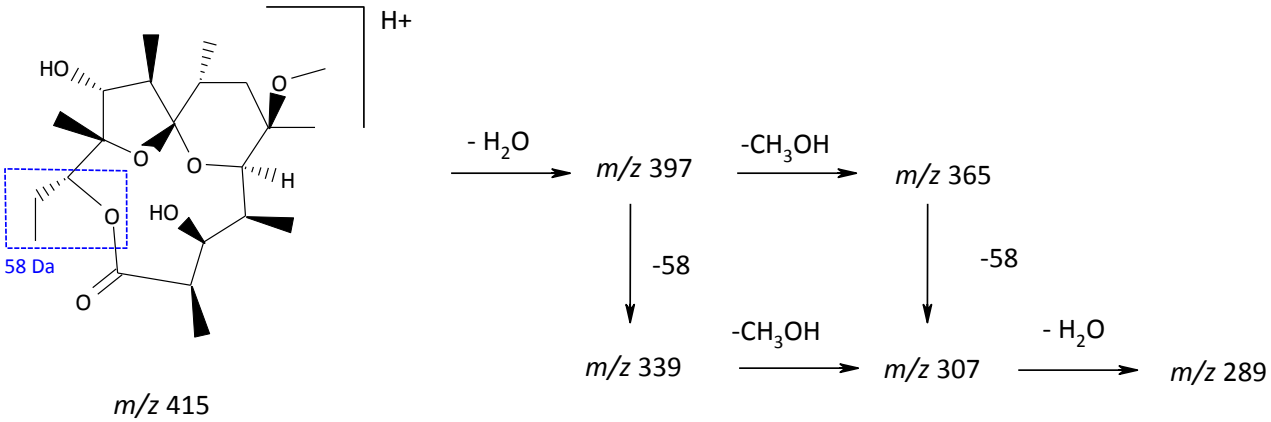

Figure SI2. Proposed fragmentation scheme for a compound **2**; elimination of neutral fragment 58 Da corresponds to part of molecule marked with box on scheme.

Table SI6. Elemental composition results for the most significant signals in MS spectra of compound **2**.

Ion	Molecular formula	Calculated mass	Measured mass	Error (ppm)
$[M+H]^+$	C22H39O7	415.2696	415.2679	-4.1
$[M+NH_4]^+$	C22H42NO7	432.2961	432.2939	-5.1
$[2M+NH_4]^+$	C44H80NO14	846.5579	846.5585	0.7

Table SI7. Accurate mass measurements for determination of molecular formula of **2**–**4.**

Compound	Molecular formula	Calculated mass	Measured mass	Error (ppm)
2	C22 H38 O7 Na	437.2515	437.2504	-2.5
3	C22 H37 O6	397.2590	397.2586	-1.0
4	C22 H37 O6	397.2590	397.2588	-0.5

REACTION KINETICS

The rate constants were calculated using the Microsoft Excel 2013 (32 bit) for Microsoft Windows 8.1 (64 bit) with Solver add-in. The time dependence of [A], [B] and [C] concentrations for reaction scheme presented in Eq. 1 is given by the following equations (see the reference 32):

$$[\mathrm{A}] = [\mathrm{A}]_0 \exp(-k_1 t)$$

$$[\mathrm{B}] = [\mathrm{B}]_0 + \alpha_2 + \frac{1}{k_2 + k_3 - k_1}\left\{[\mathrm{A}]_0 (k_1 - k_3)\exp(-k_1 t) - [\alpha_2 k_2 - \alpha_3 (k_3 - k_1)]\exp(-(k_2 + k_3)t)\right\}$$

$$[\mathrm{C}] = [\mathrm{C}]_0 + \alpha_3 + \frac{1}{k_2 + k_3 - k_1}\left\{[\mathrm{A}]_0 k_2 \exp(-k_1 t) - [\alpha_2 k_2 - \alpha_3 (k_3 - k_1)]\exp(-(k_2 + k_3)t)\right\}$$

where $\alpha_1 = [\mathrm{A}]_\infty - [\mathrm{A}]_0$, $\alpha_2 = [\mathrm{B}]_\infty - [\mathrm{B}]_0$ and $\alpha_3 = [\mathrm{C}]_\infty - [\mathrm{C}]_0$, while subscripts 0 and ∞ present the values of corresponding concentrations at times t = 0 and $t = \infty$. The experimental data was fitted by non-linear least squares fit method i.e. simulated data vs. experimental data, by varying the constants k_1, k_2 and k_3, with R^2 as the criterion for the goodness of fit. The algorithm used by Solver to find the optimal solutions was the GRG Nonlinear Solving Method for nonlinear optimization (uses the Generalized Reduced Gradient (GRG2) code), followed by the Evolutionary Solving Method for non-smooth optimization which uses a variety of genetic algorithm and local search methods. The lines on Figure 3 present the best fit obtained for the concentration values calculated as stated above and the rate constants are determined from these functions.