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SUPPORTING INFORMATION

Title: **Isolation and Structure Elucidation of Cruentarens A and B — Novel Members of the Benzolactone Class of ATPase Inhibitors from the Myxobacterium *Byssovorax cruenta***

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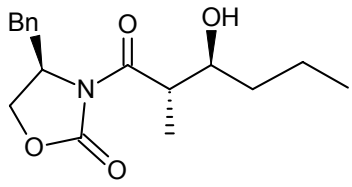
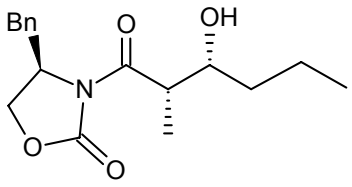
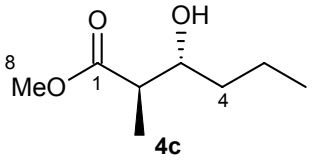
Ref. No.: O200600421

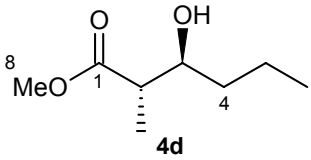




(4'*R*)-benzyl-3-(3-hydroxy)-2-methylhexanoyl)-1,3-oxazolidin-2-one: (4'*R*)-4-benzyl-3-(2-bromopropanoyl)-1,3-oxazolidin-2-one (100 mg, 0.32 mmol) was condensed with *n*-butyraldehye as described above to give after chromatography 95 mg (97%) of a slightly yellow oil. R_f : 0.62 (PE / Et_2O = 6 : 4). UV (MeOH): λ_{max} (lg ε) = 207 (4.3), 252 nm (2.7).
IR (KBr): 2980 (m), 2940 (m), 1781 cm^{-1}(s). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 1.22 (m, 6H, H6, H7), 1.37-1.63 (m, 4H, H4, H5), 2.73 (m, 1H, H4'b), 2.95 (m, 1H, H2), 3.30 (m, 1H, H4'a), 3.64-3.91 (m, 1H, H3), 4.02-4.21 (m, 2H, H2'), 7.25 (m, 5H, H6'-H10'). MS (EI): m/z (%):305 (15, [M^+]), 244 (76) 178 (100). HRMS (EI) for $C_{17}H_{23}O_4NNa$: calcd. 328.153; found 328.153.



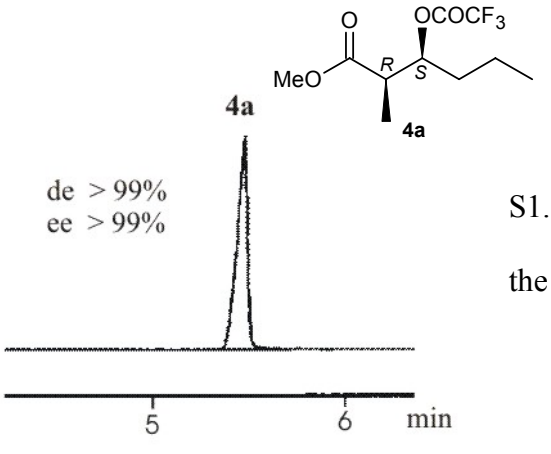
4c



4d

(2*S*,3*R*)/(2*R*,3*S*)-3-Hydroxy-2-methylhexanoic acid methylesters (4c/4d): The diastereomeric mixtures of oxazolidinones (30 and 20 mg) obtained above were hydrolyzed by treatment with hydrogenperoxide/lithium iodide. The resulting hexanoic acids were esterified with methanol/hydrochloric acid to give mixtures of **4a/4c** and **4b/4d** (6 and 8 mg).

General procedure for trifluoroacetylation: Ca. 1 mg samples of the 3-hydroxy-2-methylhexanoic acid methylesters **4a-d** were dissolved in trifloroacetic acid anhydride (0.1ml) and heated to 100 °C for 30 seconds. The solution was evaporated in a stream of nitrogen and the residue dissolved in dichloromethane (1 mL) for GC analysis.



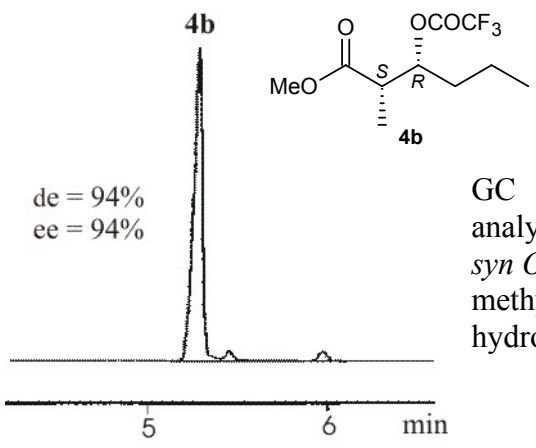


Figure S1. GC analysis of *syn O*-TFA methyl 3-hydroxy-2-methylhexanoates from the *Evans* aldol synthesis.

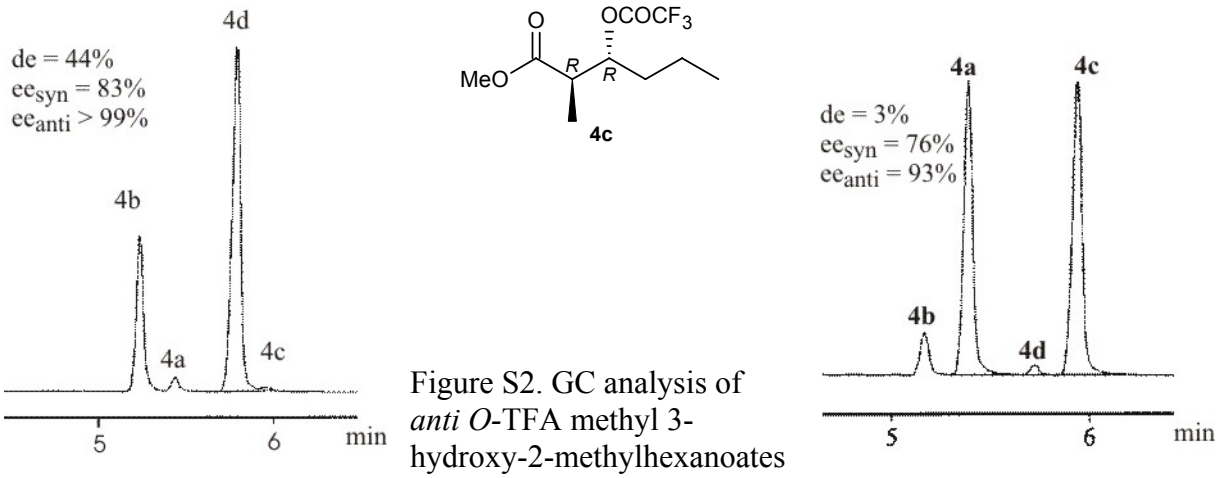


Figure S2. GC analysis of *anti O*-TFA methyl 3-hydroxy-2-methylhexanoates from the *Wessjohann* chromium *Reformatsky* reaction.

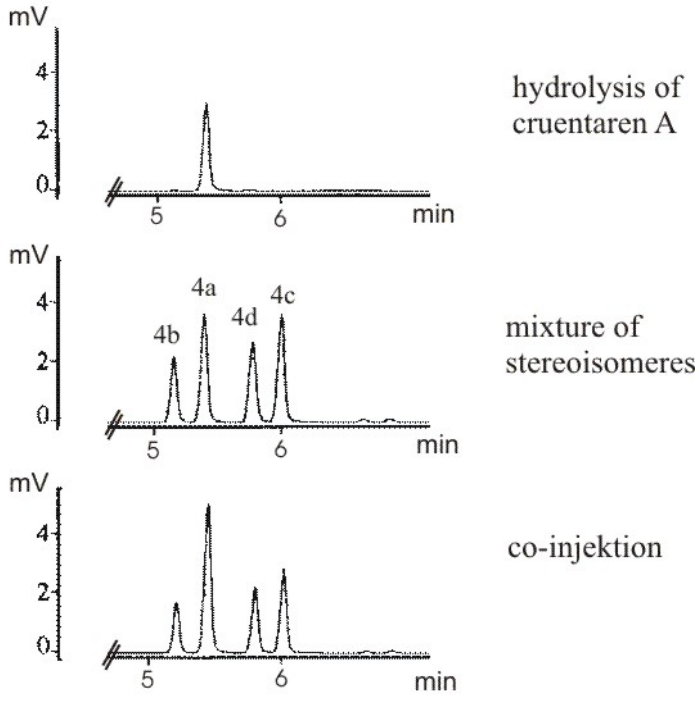
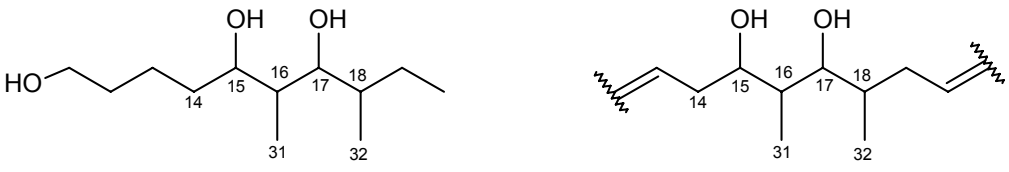


Figure S3. GC analysis of *O*-TFA methyl 3-hydroxy-2-methylhexanoates from the degradation of cruentaren A (**1**) and co-chromatography with the four synthetic stereoisomers.



model compound **15** cruentaren B (**2**)

Table S1. ^{13}C NMR chemical shifts predicted for model compound **15** [1] and predicted, observed and adjusted for cruentaren B (**2**).[2]

	C-14	C-15	C-16	C-17	C-18	C-19	C-31	C-32
δ (**15**) (predicted)	35.7	70.6	40.1	74.6	37.3	24.1	8.0	13.9
δ (**2**) (predicted)	38.7	70.6	40.3	75.1	35.1	34.0	8.1	14.3
Δδ	3.0	0.0	0.2	0.5	-2.2	9.9	0.1	0.4
δ (**2**) (observed)	33.4	77.0	37.2	80.2	36.5	30.7	4.4	15.8
δ (**2**) (adjusted)	30.4	77.0	37.0	79.7	38.7	20.8	4.3	15.4

Table S2. Orientation of C-15 to C-18 substituents in model compounds **15a – 15h**. [1]

15a : αααβ	**15b** : αααα
15c : ααβα	**15d** : αααβ
15e : βααβ	**15f** : βααα
15g : βαβα	**15h** : βααβ

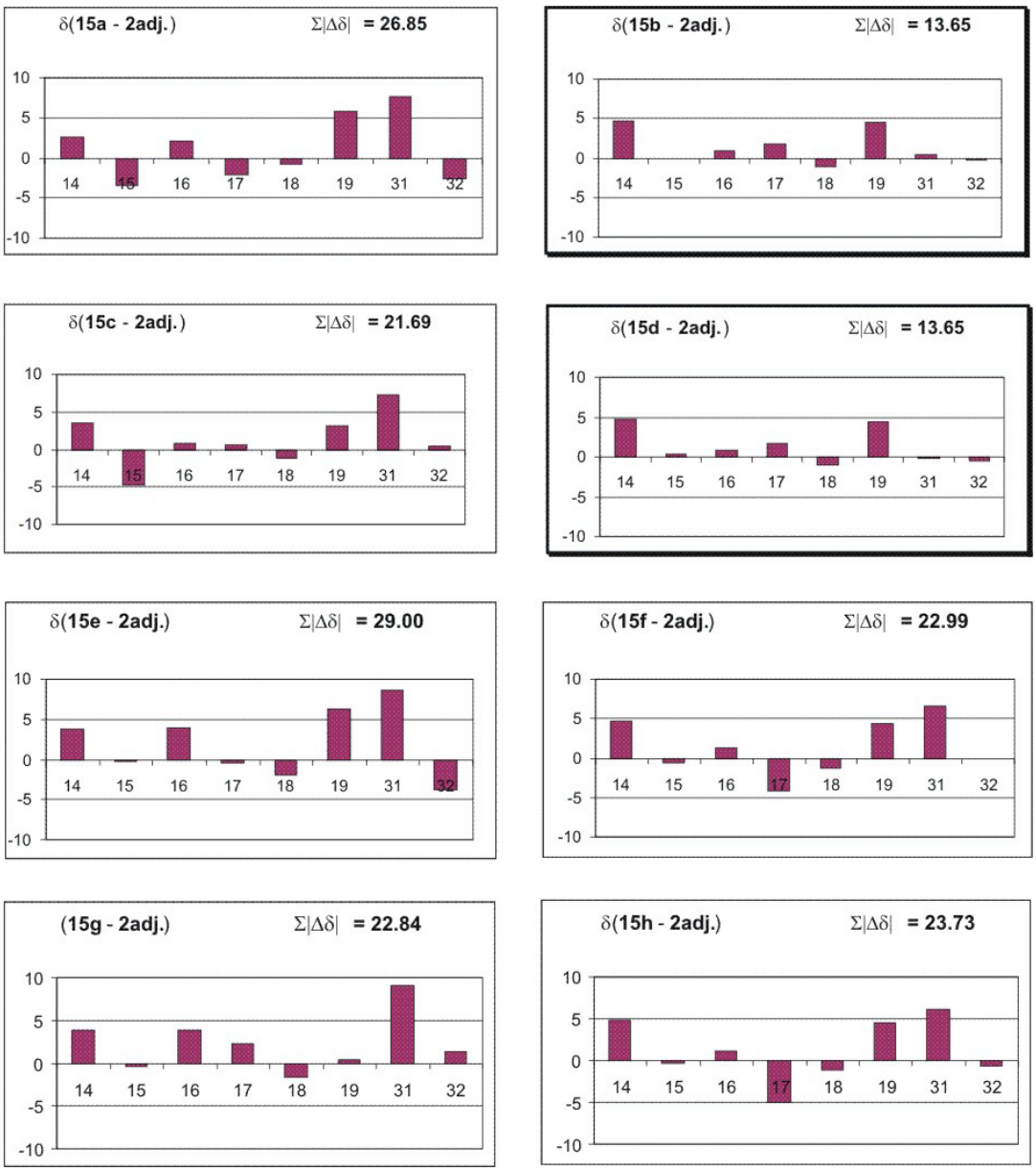


Figure S4. Differences in carbon chemical shifts of model compounds **15a – 15h** and adjusted values of cruentaren B (**2**). The x and y axes represent carbon numbers and chemical shift differences in ppm.

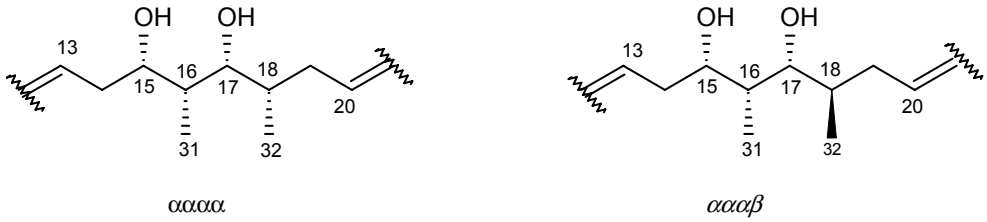
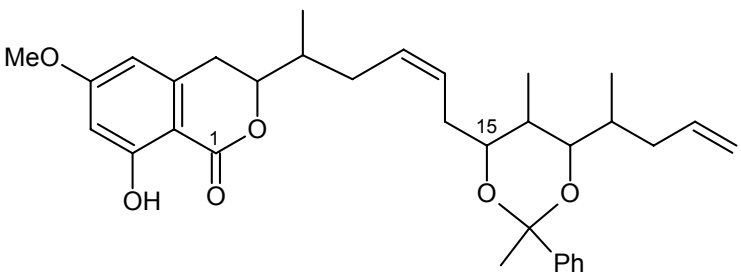
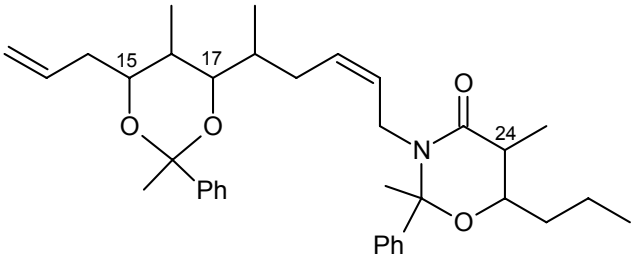


Figure S5. Relative configurations of C-15 – C-18 of cruentaren B (**2**) predicted from Figure S4.



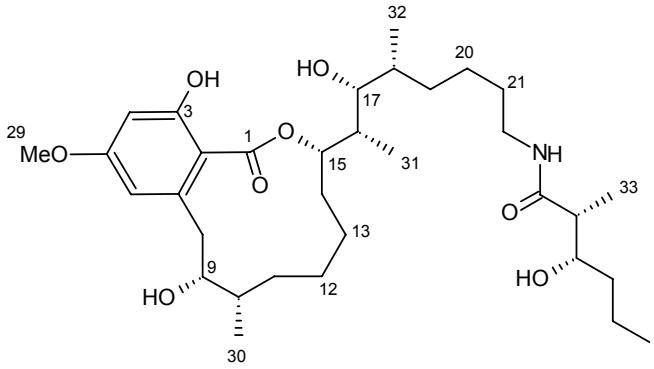
21,22-*seco*-Cruentaren B-monoacetal 10:

MS (DCI): m/z (%): 577 (73), [M+NH_4^+], 560 (100), (M^+). HRMS (EI) for $C_{36}H_{49}N_1O_4$: calcd. 559.3661; found 559.3676.



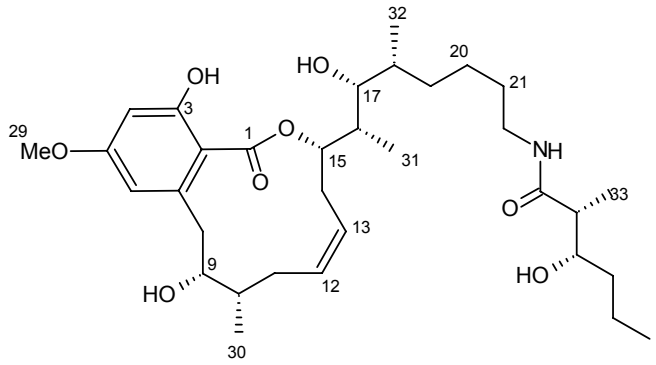
11,12-*seco*-Cruentaren B-bisacetal 11:

MS (DCI): m/z (%): 553.0 (73), (M+NH_4^+), 560 (100), (M^+). HRMS (EI) for $C_{33}H_{42}O_6$:calcd. 534.2981; found 534.2976.



12,13,20,21-Tetrahydrocruentaren A: Cruentaren A (**1**) (10 mg) was dissolved in methanol (1 mL), 10 % Palladium/C (10 mg) was added and stirred in an atmosphere of hydrogen for 3 h. The product was purified by prep. TLC on silica gel plates (petroleum ether/ diethylether 6:4) to give 4 mg (40%) of a α_D^{20} colorless oil. R_f: 0.37 (EE). UV (MeOH): λ_{max} (lg ε) = 217 (4.2), 266 (4.0), 304 nm (3.6). = + 46 (c = 0.5 , MeOH). IR (KBr): 3413 (m), 2933 (m), 2871 (w), 1645 (s), 1615 cm^{-1}(m). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 0.75 (d, *J*=6.78 Hz, 3 H, H32), 0.92 (d, *J*=7.16 Hz, 3 H, H31), 0.92 (t, *J*=6.88 Hz, 3 H, H28), 0.95 (d, *J*=6.78 Hz, 3 H, H30), 1.06 (m, 2 H, 19b,H20b), 1.14 (d, *J*=7.16 Hz, 3 H, H33), 1.22 - 1.38 (m, 8 H, H12, H19a, H20a, H21, H26b, H27b), 1.39 - 1.49 (m, 4 H, H11, H26a, H27a), 1.50 - 1.65 (m, 3 H, H13, H18), 1.70 – 1.85(m, 2H, H10,H14b), 1.99 (dd, *J*=16.20, 7.16 Hz, 1 H, H14a), 2.12 (ddd, *J*=7.21, 4.29, 3.01 Hz, 1 H, H16), 2.18 - 2.29 (m, 2 H, H8b, H24), 3.14 (d, *J*=3.96 Hz, 1 H, H22), 3.42 (dd, *J*=7.16, 3.01 Hz, 1 H, H17), 3.71 - 3.79 (m, 1 H, H9), 3.80 (s, 3 H, H29), 3.84 (m, 1 H, H25), 3.89 (d, 1 H, H8a), 5.12 (dd, *J*=9.42, 4.71 Hz, 1 H, H15), 5.75 (t, *J*=5.65 Hz, 1 H, 22-N<u>H</u>), 6.34 (d, 1 H, H6), 6.37 (d, 1 H, H4), 11.45 (s, 1 H, 3-O<u>H</u>). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ

= 9.9 (C31), 11.2 (C33), 14.1 (C28), 15.1 (C30), 16.0 (C32), 19.3 (C27), 23.5 (C11), 23.9 (C20), 25.7 (C14), 26.5 (C13), 29.8 (C21), 30.1 (C12), 31.6 (C19), 35.9 (C26), 36.8 (C8), 37.3 (2C, C10, C18), 38.6 (C16), 39.0 (C22), 44.9 (C24), 55.4 (C29), 71.8 (C25), 75.1 (C17), 75.2 (C9), 82.2 (C15), 99.8 (C4), 105.5 (C2), 111.9 (C6), 144.3 (C7), 163.6 (C5), 165.7 (C3), 171.8 (C1), 176.6 (C23). MS (EI): m/z (%): 593.3 (27, M^+). MS (DCI): m/z (%): 611 (4), (M+ NH_4^+), 594.3 (34), (M+H^+), 219.1 (100). HRMS (EI) for $C_{33}H_{55}N_1O_8$: calcd. 593.3928; found 593.3915.



20,21-Dihydrocruentaren A: To cruentaren A (**1**) (5 mg) dissolved in methanol (0.2 mL) 10 % Palladium/C (5 mg) was added and stirred in an atmosphere of hydrogen for 3 h. The product was purified by prep. TLC on silica gel plates (petroleum ether/ diethylether 6:4) to give 2.5 mg (50%) of a light yellow oil. UV (MeOH): λ_{max} (lg ε) = 216 (4.2), 264 (3.9), 305 nm (3.6). ^{1}H-NMR ($CDCl_3$, 600 MHz): δ = 0.77 (d, *J*=6.70 Hz, 3 H, H32), 0.92 (t, *J*=7.06 Hz, 3 H, H28), 0.92 (d, *J*=7.08 Hz, 3 H, H31), 1.02 (d, *J*=6.85 Hz, 3 H, H30), 1.14 (d, *J*=7.13 Hz, 3 H, H33), 1.17 (m, 2H, H19b, H20b), 1.29 - 1.35 (m, 3 H, H27b, H26b, H21b), 1.39 (m, 2 H, H20a, H21a), 1.45 - 1.55 (m, 4 H, H18, H19a, H26a, H27a), 1.96 (d, *J*=14.49 Hz, 1 H, H11b), 1.99 - 2.07 (m, 2 H, H10, H16), 2.19-2.22 (m, 2 H, H8b, H14b), 2.23 - 2.27 (dq, 1 H, H24), 2.32 (dd, *J*=14.21, 11.94 Hz, 1 H, H11a), 2.84 (dt, *J*=14.35, 11.50 Hz, 1 H, H14a), 3.16 - 3.22 (m, *J*=6.85 Hz, 2 H, H22), 3.40 - 3.45 (m, 1 H, H17), 3.64 (d, *J*=11.76 Hz, 1 H, H9), 3.74 (d, 1H, H8a), 3.80 (s, 3 H, H29), 3.85 (m, 1 H, H25), 5.23 (ddd, *J*=11.66, 4.82, 1.79 Hz, 1 H, H15), 5.40 - 5.46 (m, *J*=11.11, 4.69, 2.12 Hz, 1 H, H13), 5.50 (td, *J*=11.31, 2.27 Hz, 1 H, H12), 5.78 (t, *J*=5.57 Hz, 1 H, 22.N<u>H</u>), 6.30 (d, *J*=2.69 Hz, 1 H, H6), 6.36 (d, *J*=2.64 Hz, 1 H, H4), 11.55 (s, 1 H, 3-O<u>H</u>). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 9.1 (C31), 11.2 (C33), 14.1 (C28), 14.2 (C30), 15.9 (C32), 19.3 (C27), 23.8 (C20), 29.5 (C14), 29.8 (C21), 31.6 (C11), 31.8 (C19), 35.8 (C26), 36.8 (C8), 37.0 (C18), 38.3 (C10), 38.8 (C16), 38.9 (C22), 44.9 (C24), 55.4 (C29), 71.8 (C25), 73.0 (C9), 75.5 (C17), 78.2 (C15), 99.8 (C4), 104.8 (C2), 112.4 (C6), 125.7 (C13), 132.3 (C12), 143.7 (C7), 163.6 (C5), 165.9 (C3), 171.5 (C1), 176.5 (C23). MS (EI): m/z (%): 591.2 (17), (M^+), 258.1 (100). HRMS (EI) for $C_{33}H_{53}N_1O_8$: calcd. 591.3771, found 591.3746.

Table S3. ^{1}H- und ^{13}C-NMR spectral data of cruentaren B (**2**) in $CDCl_3$ (400/100 MHz)

H-atom	δ (ppm) multi.	J [Hz]	C-atom	δ (ppm)
3 - OH	11.35 s br		C - 1	170.0
4 - H	6.35 d	2.3	C - 2	101.8
6 - H	6.26 d	2.3	C - 3	164.6
8 - H_a	2.81 dd	16.0, 3.3	C - 4	99.5
8 - H_b	2.91 dd	16.0, 11.7	C - 5	165.9
9 - H	4.35 ddd	11.6, 6.4, 3.2	C - 6	106.4
10 - H	1.95-2.02 m		C - 7	141.1

11 – H_2	2.15-2.40 m		C - 8	30.1
12 - H	5.45-5.67 m		C - 9	82.3
13 - H	5.45-5.67 m		C - 10	37.3
14 – H_b	2.30-2.45 m		C - 11	30.1
14 – H_a	2.10-2.25 m		C - 12	129.7
15 - H	3.80-3.90 m		C - 13	127.7
16 - H	1.65-1.75 m		C - 14	33.4
17 - H	3.47 dd	9.8, 1.9	C - 15	77.3
18 - H	1.65-1.75 m		C - 16	37.2
19 – H_2	2.20 - 2.35 m		C - 17	80.2
20 - H	5.62 - 5.68 m		C - 18	36.5
21 - H	5.45 - 5.67 m		C - 19	30.7
22 – H_2	3.80 - 3.90 m		C - 20	131.5
22 - NH	6.35 br		C - 21	126.4
24 - H	2.25 - 2.35 m		C - 22	36.2
25 - H	3.80 - 3.90 m		C - 23	176.3
26 – H_a	1.30 - 1.35 m		C - 24	44.9
26 - H_b	1.40 - 1.50 m		C - 25	71.9
27 - H_a	1.30 - 1.35 m		C - 26	35.8
27 - H_b	1.45 - 1.50 m		C - 27	19.2
28 - CH_3	0.91 t	7.1	C - 28	14.1
29 - OCH_3	3.80 s		C - 29	55.6
30 - CH_3	1.02 d	6.9	C - 30	15.1
31 - CH_3	0.92 d	7.1	C - 31	4.4
32 - CH_3	0.84 d	6.9	C - 32	15.8
33 - CH_3	1.13 d	6.9	C - 33	11.2

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[1] J. Lee, Y. Kobayashi, K. Tezuka, Y. Kishi, *Org. Lett.*, **1999**, *13*, 2181-2184.

[2] L. Vollbrecht, *Doctoral Thesis*, Technical University of Braunschweig, **2005**.