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The Crocacins, Novel Antifungal and Cytotoxic Antibiotics from *Chondromyces crocatus* and *Chondromyces pediculatus* (Myxobacteria): Isolation and Structure Elucidation

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Four novel antifungal and highly cytotoxic metabolites, the crocacins A–D (**1**–**4**), were isolated in our screening of the myxobacterial genus Chondromyces from strains of *C. crocatus* and *C. pediculatus*. Crocacin A, B, and D (**1**, **2**, and **4**) are unusual dipeptides of glycine and a 6-aminohexenoic or -hexadienoic acid, which is *N*-protected by a complex polyketide-derived acyl residue. The latter is a multiply substituted phenylundecatrienoic acid, which is found as its primary amide crocacin C (**3**). Based on ^{1}H coupling constants, NOEs and MM$^+$ calculations the relative configuration of the asymmetric centers and their preferred conformation are proposed for the crocacins.

In continuation of our screening for biologically active metabolites from myxobacteria,[1] different strains of *Chondromyces crocatus*, i.e., strains Cm c2, Cm c3 and later Cm c5, were noticed for their high activity against fungi, yeast and animal cell cultures. Subsequently, these activities could be ascribed to several different metabolites that are simultaneously produced by these strains. The group of chondramides A−D[2] are depsipeptides structurally related to the sponge metabolites jaspamide (jasplakinolide).[3] They show only moderate antifungal activity but proved to be highly cytostatic in mammalian cell cultures,[4] where the occurrence of multinucleate cells and an interference with the actin polymerization was observed.[5] The biological activity of crocacin A (**1**), the main representative of the second group of metabolites, consists in an effective growth inhibition of fungi and yeasts, caused by inhibition of the electron flow within the cytochrome bc_1 segment (complex III) of the respiratory chain.[6] Here we report on the isolation and structure elucidation of the crocacins A−C (**1**−**3**) from *C. crocatus* and of crocacin D (**4**) from *C. pediculatus*, strain Cm p17.

The crocacins A−C (**1**−**3**) are a group of compounds which is regularly found in the extracts of *C. crocatus* strains. As a typical example the analytical diode-array-detected (DAD) RP HPLC of a cell extract from a large-scale fermentation of strain Cm c5 is shown in Figure 1. In shaken cultures the major component crocacin A (**1**) is produced in yields of about 20 mgL^{-1}.

The crocacins A−C (**1**−**3**) were isolated from the acetone extract of the wet cell mass of *C. crocatus* by an initial partition between methanol and heptane removing the lipophilic by-products, which is followed by consecutive chromatographic separations on Sephadex LH-20 and RP-18 silica gel. Because a serious destruction of further valuable co-metabolites occurs during silica-gel chromatography, which was previously applied for the isolation of the chondramides[2], this step was avoided in the new separation procedure.

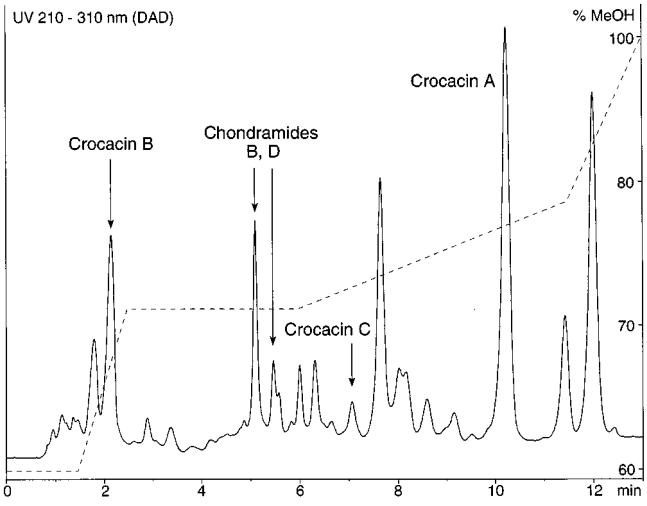
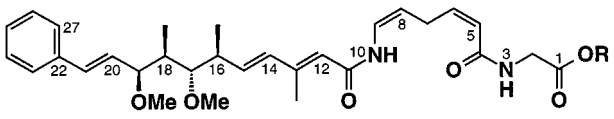


Figure 1. Typical RP-HPLC of an extract of *Chondromyces crocatus*; column 125 × 2 mm and pre-column 11 mm filled with Nucleosil 120-5 C_{18}, solvent gradient with solvent A = water and solvent B = MeOH as shown by dashed line, flow 0.3 mLmin^{-1}



Crocacin A (**1**) R = CH_3
Crocacin B (**2**) R = H
(relative configuration)

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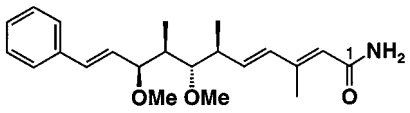
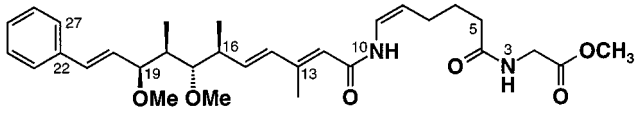


Crocacin C (**3**)
(relative configuration)



Crocacin D (**4**)
(relative configuration)

Crocacin D (**4**) was discovered by analytical RP-HPLC with DAD-UV and ESI-MS detection in extracts of *C. pediculatus*, strain Cm p17, as a by-product in addition to the jerangolides.[7] Compound **4** was isolated from shaken cultures by simultaneous extraction of cell mass and amberlite XAD 16 adsorbent resin, which had been present during fermentation. After lipophilic cell constituents had been eliminated by methanol/heptane partition, **4** was purified by RP-MPLC.

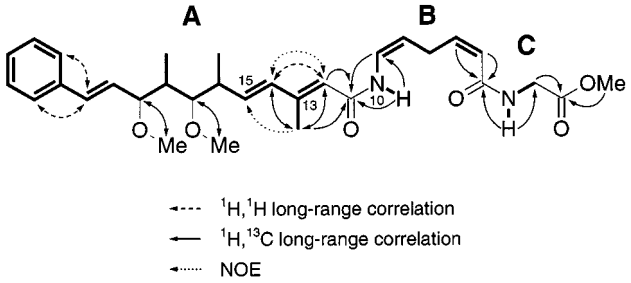


Figure 2. Structure parts and main correlations from NMR spectroscopy

The basic structure of the crocacins was elucidated with **1**, the main product from *C. crocatus*. HR-EI mass spectrometry of the molecular ion with *m/z* 538 furnished the elemental composition $C_{31}H_{42}N_2O_6$, which implies twelve double bond equivalents. Their assignment to different chromophores was suggested by the characteristic UV spectrum, which shows broad and structured absorption bands at 254 and 291 nm. Ester and amide groups were indicated in the IR spectrum of **1**[8] by intense carbonyl absorptions at 1747 as well as 1649 cm^{-1} and by broad NH bands around 3392 and 3252 cm^{-1}.

All signals in the ^{1}H- and ^{13}C-NMR spectra were assigned and correlated by ^{1}H,^{1}H-COSY, direct ^{1}H,^{13}C-correlation (HMQC) NMR spectroscopy to give the carbon backbone of three main structural elements (A−C), which are indicated by bold bonds in Figure 2. Their concatenation through secondary amide bonds and the attachment of the methyl ester and ether residues was provided by long-range correlation (HMBC) spectra (Figure 2). The (*E*) configuration of the Δ^{12} double bond was derived from nuclear Overhauser enhancements (NOEs) between the methyl group at C-13 and 15-H and between 12-H and 14-H. The configuration of the remaining double bonds in **1** was assigned from their vicinal coupling constants (Table 1).

Table 1. NMR data of crocacin A (**1**) in [D_6]acetone[a]

Position	δ_H	m	*J* [Hz]	δ_C	m
1	–	–	–	170.59	s
2	4.08	m[b]	–	41.64	t
3	7.97	t	5.4 br.	–	–
4	–	–	–	168.93	s
5	5.96	d	11.4	120.96	d
6	6.05	dt	11.4, 8.7	143.37	d
7	3.33	dd	8.7, 8.5 (br.)	26.27	t
8	4.78	dt	8.6, 8.5	104.47	d
9	6.83	ddt	8.6, 10.8, 1	125.82	d
10	10.05	d	10.8	–	–
11	–	–	–	164.46	s
12	5.83	d	1.0 (br.)	121.73	d
13	–	–	–	149.87	s
14	6.19	d	15.7	135.17	d
15	6.14	dd	8.1, 15.7	137.76	d
16	2.62	ddq	2.2, 8.1, 6.8,	40.84	d
17	3.19	dd	2.2, 9.5	87.14	d
18	1.58	ddq	2.5, 9.5, 7.0	43.39	d
19	4.08	m[b]	–	81.79	d
20	6.25	dd	7.1, 16.2	130.41	d
21	6.59	d	16.2	132.53	d
22	–	–	–	137.83	s
23	7.46	d	8.0 (br.)	127.21	d
24	7.31	dd	7.5, 8.0 (br.)	129.39	d
25	7.22	dd	7.5, 7.5 (br.)	128.23	d
26	7.31	dd	7.5, 8.0 (br.)	129.36	d
27	7.46	d	8.0 (br.)	127.21	d
1-OCH_3	3.69	s	–	52.28	q
13-CH_3	2.28	d	1.0	13.71	q
16-CH_3	1.20	d	6.8	19.21	q
17-OCH_3	3.52	s	–	61.44	q
18-CH_3	0.88	d	7.0	10.16	q
19-OCH_3	3.30	s	–	56.47	q

[a] ^{13}C NMR at 150 MHz; ^{1}H NMR at 600 MHz. – [b] Overlapping signals 2-H_2 and 19-H.

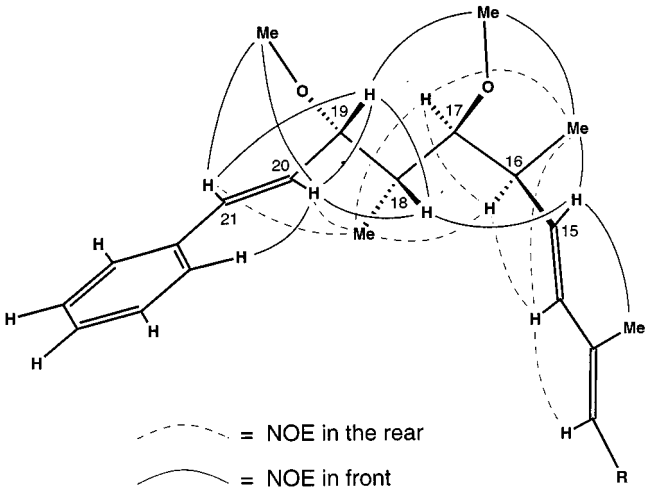


Figure 3. Partial view of the lowest energy conformer of crocacins and selected nuclear Overhauser enhancements; the NOEs between methyl and methoxy groups and their geminal chain protons are omitted for clarity

The vicinal coupling constants of **1**, combined with the information of NOESY and NOE difference spectra, further allow to propose a relative configuration as shown

Table 2. Calculated torsion angles[a] and observed vicinal ^{1}H coupling constants in the C-15 to C-20 segment of crocacin A (**1**)

Protons	15,16	16,17	17,18	18,19	19,20
φ [°]	−175	−55	−179	64	−37
$^3J_{H,H}$ [Hz]	8.1	2.2	9.5	2.5	7.1

[a] MM^+ calculation with HyperChem 5.1 and ChemPlus 2.0.

in Figure 3. It presents the four stereocenters of **1** and their neighborhood as a part of the lowest energy conformation found by MM^+ calculations with HyperChem. The observed coupling of 9.5 Hz between 17-H and 18-H is typical for an antiperiplanar (*anti*) relation of protons in a saturated carbon chain. In the model[9] a torsion angle of −179° is calculated for the lowest energy conformer (Table 2). Thus, both substituents at the central asymmetric carbon atoms of **1**, i.e. 17-OMe and 18-Me, also occupy the favored *anti* conformation. They control the spatial arrangement of the substituents at the adjacent asymmetric centers by avoidance of high-energy *syn*-pentane interactions. In the proposed configuration of **1** the pair of vicinal substituents on C-16, i.e. C-14 and 16-Me, are both directed by 17-OMe into a synclinal position. On the other side the pair of C-20 and 19-OMe similarly take a synclinal position to the 18-Me group. Consequently, the central *anti* protons 17-H and 18-H further have synclinal (gauche) relationships with their vicinal neighbor protons 16-H and 19-H. These *syn* positions were suggested by small couplings of 2.2 and 2.5 Hz and they are verified by corresponding torsion angles of −55° and 64° in the model. This arrangement of the asymmetric centers brings about a nearly straight carbon chain between 16-Me and C-20 of the crocacin skeleton. Because any *syn*-pentane interaction is only avoided by this low-energy conformer, as shown in Figure 3, it should be highly preferred and the segment may be treated as monoconformational.[10] An identically substituted C_4-chain section was previously reported only for one other natural product also isolated from myxobacteria, the stigmatellins.[11] The latter were shown to have the same configuration as shown here for **1** by comparison of a degradation product with the synthetic stereoisomeres.[12]

The crocacin chain is continued on both sides of the C-16 to C-19 section by sp^2-carbon atoms of (*E*)-double bonds, which adopt their lowest energy rotameres at calculated torsion angles of about 175° between 15-H and 16-H and −37° between 19-H and 20-H. Again, the observed vicinal couplings of $J_{15,16}$ = 8.1 and $J_{19,20}$ = 7.1 Hz are in good agreement with the model.

A series of NOE difference NMR spectra and the data of an ROESY-NMR spectrum of **1** were used to check the spatial arrangement of chain protons and methyl groups (Figure 3). Of course, NOEs between the chain protons and their geminal methyl or methoxy groups were observed, but they are omitted in Figure 3. As expected from the model, NOEs were found between 16-H and 17-H and between 18-H and 19-H, the *syn* pairs of protons, while no NOE was observed between the *anti* protons 17-H and 19-H. All further NOEs are in good agreement with the lowest energy conformation of crocacin A (**1**), which was calculated from the (16*S*,17*S*,18*R*,19*S*) configuration. The molecular skeleton with two conjugated, flat segments flanking a monoconformational C_4-chain segment results in a "U" shape of crocacin A (**1**) between phenyl ring and enamide residue.

The highly polar variant, crocacin B (**2**), was recognized as the free carboxylic acid from lack of its 1-methoxy group in the NMR spectra, which is corroborated by HR-EI mass spectrometry of the molecular ion *m*/*z* 524 with the elemental composition $C_{30}H_{40}N_2O_6$. As expected, the carboxylic acid **2** shows a pH-dependant retention in TLC and HPLC analysis.

Similarly, the structure of crocacin C (**3**) was derived from its elemental composition $C_{22}H_{31}NO_3$ corresponding to the molecular ion *m*/*z* 357 and from the absence of all NMR signals belonging to the unsaturated amino acid and the glycyl ester residues in **1**. The primary amide group in **3** gives rise to characteristic absorption bands in the IR spectrum at 1655 and 1600 cm^{-1}.

According to HR-EI mass spectrometry, crocacin D (**4**), the variant isolated from *C. pediculatus*, has an elemental composition $C_{31}H_{44}N_2O_6$ containing two hydrogen atoms more than crocacin A (**1**). The lack of the Δ^5-double bond is obvious from a comparison of the NMR spectra, where the C-5 and C-6 olefin signals are replaced in **4** by two new signals of methylene groups at δ_H = 2.26 and 1.67 corresponding to ^{13}C-NMR signals at δ_C = 34.54 and 26.12.

The crocacins represent a second novel group of modified peptides from *C. crocatus*. However, unlike the chondramides they are linear dipeptides. Crocacin C (**3**) is a structure fragment of **1**, **2**, and **4**. Although small amounts of **3** are regularly observed in extracts of *C. crocatus*, it may additionally be formed during the isolation process by cleavage of the acid-sensitive enamine bond.

With an MIC of 1.4 $ngmL^{-1}$ crocacin D (**4**) from *C. pediculatus* shows a distinctly higher biological activity against *Saccharomyces cerevisiae* compared to MICs of 10 mgL^{-1} for **1**, 100 mgL^{-1} for **3**, and 12.5 μgmL^{-1} for **2**. Another order is observed for the toxicity in L929 mouse fibroblast cell culture with an IC_{50} of 0.06 mgL^{-1} for **4** compared to 0.2 mgL^{-1} for **1**, 40 mgL^{-1} for **2** and 140 mgL^{-1} for **3**.

Experimental Section

UV: Shimadzu UV/VIS scanning spectrometer UV-2102, solvent methanol [Uvasol, (Merck)]. − IR: Nicolet FT-IR spectrometer 20 DXB. − NMR: Bruker spectrometer DMX 600 (^{1}H: 600.1 MHz; ^{13}C: 150.9 MHz), Bruker spectrometer ARX 400 (^{1}H: 400.1 MHz; ^{13}C: 100.6 MHz) or Bruker spectrometer AM 300 (^{1}H: 300.1 MHz; ^{13}C: 75.5 MHz); internal standard was the solvent signal. − Mass spectrometry: EI or DCI: Finnigan spectrometer MAT 95 (EI with 70 eV) (DCI with isobutane), resolution $M/\Delta M$ = 1000; high-resolution data from peak matching ($M/\Delta M$ = 10000).

Isolation of the Crocacins A−C (1−3): From 800 L of fermentation broth of *Chondromyces crocatus*, strain Cm c5, 6.3 kg of wet cell mass was harvested by centrifugation and extracted with 60 L of acetone in three portions of 30, 15 and 15 L. After the organic

solvent had been removed from the combined extracts by evaporation, the remaining water/oil mixture was extracted with dichloromethane at pH = 6.7. The extract was dried with sodium sulfate and concentrated to yield 92.4 g of a brown oil. This was dissolved in 1 L of methanol containing 3% of water and extracted with 2.4 L of heptane in three portions. Upon evaporation, the methanol layer yielded 49.9 g of a brown oily residue, which was separated by gel chromatography in two portions [column Superformance 1000−100 (Merck), bed height 850 mm of Sephadex LH-20 (Pharmacia), solvent methanol, flow 18 mLmin^{-1}; the fractions were taken according to TLC analysis]. A main fraction of 17.5 g, characterized by a number of TLC spots giving different color reactions with the vanillin/sulfuric acid spray reagent, contained the biologically active metabolites. This fraction was separated by RP-MPLC in two portions [column 300×60 mm (Kronlab); HD-Sil 18−30−60, RP-18, 20−45 μ, 60 Å; solvents, 70% aqueous methanol for 120 min, gradient to 80% methanol in 60 min, 80% methanol for 95 min, gradient to 100% methanol in 30 min, flow 24 mLmin^{-1}; detection UV absorption at 254 nm]. A crude mixture of chondramides (3.5 g) eluted from 52 to 85 min, an intermediate fraction (1.87 g) eluted from 85 to 190 min containing broad and overlapping peaks, and crude crocacin A (**1**) (0.62 g) eluted from 190 to 218 min. The intermediate fraction was further separated by a second RP-HPLC, however, with a buffered solvent gradient [column 320×30 mm, Eurosil Bioselect 100-20-C18, 15−25 μ (Knauer); solvent A: 50% aqueous methanol with 0.04 M ammonium acetate, solvent B: 100% methanol, 80% solvent A for 60 min, gradient of 80 to 70% A in 60 min, 70% A for 60 min; flow 20 mLmin^{-1}; detection UV absorption at 275 nm]. The organic solvent was removed by evaporation before the fractions were extracted with ethyl acetate and concentrated to give crocacin C (**3**) (120 mg, peak t_R = 150 min) and crude **2** (130 mg, main peak t_R = 65 min). Upon standing in methanol, 20 mg of crocacin B (**2**) was collected as white, amorphous solid from the crude fraction. − For analytical purposes crocacin C (**3**) was purified by preparative RP-HPLC [column 250×20 mm, Nucleosil 100-7 C_{18}, (100 Å, 7 μ) (Macherey-Nagel); solvent 67% aqueous methanol, flow 23 mLmin^{-1}, detection UV absorption at 226 nm]. − The crocacin A (**1**) containing fraction was separated by RP-MPLC [column: 480×30 mm (Kronlab); ODS AQ 120, 15 μ, (YMC); solvents: 70% aqueous methanol for 55 min, gradient to 80% methanol in 120 min, flow ca. 28 mLmin^{-1}; detection by UV absorption at 256 nm] to give a fraction at $t_R \approx$ 160 min furnishing 555 mg of pure, amorphous **1**.

Isolation of Crocacin D (4): 440 mL of cell mass and amberlite XAD 16 adsorbent resin from 22 L of shaken cultures of *Chondromyces pediculatus*, strain Cm p17, were extracted with methanol (three portions of 450 mL each) followed by acetone (450 mL). The first methanol extract was concentrated to give a water/oil mixture, which was extracted with dichloromethane. This extract and the remaining methanol and acetone extracts were combined, concentrated and partitioned between methanol and heptane. The crude product (2.8 g) from the methanol layer was separated by MPLC in three portions [column HPP-FPGC (Kronwald) 460 mm×40 mm, YMC-RP$_{18}$-120, 15−25 μ, solvent 80% aqueous methanol, flow ca. 14 mLmin^{-1}, detection by UV absorption at 254 nm] and gave 124 mg of crocacin D (**4**) at $t_R \approx$ 109 min.

Properties of the Crocacins: The crocacins were obtained as colorless amorphous solids and found to be pure according to TLC and HPLC analysis. Crocacin A (**1**) is soluble in methanol, acetone, chloroform and ethyl acetate, sparingly soluble in ether and unsoluble in hexane.

Analytical RP-HPLC: Column 125 × 2 mm and pre-column of 11 mm with Nucleosil 120-5 C_{18}, 5 μm (Macherey-Nagel); solvent gradient with aqueous methanol, 60% methanol for 4 min, gradient to 70% in 6 min, to 90% in 5 min, and to 100% methanol in 2 min, flow = 0.3 mLmin^{-1}; **1** t_R = 15.4 min; **2** t_R = 2.4 min; **3** t_R = 12.6 min; **4** t_R = 14.6 min.

Analytical TLC: Aluminum sheets with a layer of 0.2 mm silica gel 60 F_{254}, (Merck); solvent dichloromethane/methanol (9:1); detection by UV quenching at 254 nm and color reaction: sprayed with vanillin/sulfuric acid reagent and heated to 120°C the crocacins give blue violet spots, later changing to brown; **1**: R_f = 0.67; **2**: R_f = 0.12; **3**: R_f = 0.53.

Crocacin A (1): $[\alpha]_D^{22}$ = +109.6 (c = 1, in methanol). − UV (methanol): λ_{max} (lg ε) = 213 nm, 219 (sh), 254 (4.54), 261, 275 (sh), 282 (4.36), 291 (4.33), 298 (sh). − IR (KBr): $\tilde{\nu}$ = 3252 cm^{-1}, 3024, 2973, 2932, 2828 (m), 1750, 1653, 1611, 1522 (s), 1449, 1363 (m), 1265, 1202, 1182 (s), 1123 (m), 1089 (s), 974 (m), 750 (m), 694 (w). − NMR: Table 1. − MS (EI, 200°C): *m/z* (%) = 538 (100) $[M]^+$, 506 (15) $[M - CH_3OH]^+$, 449 (11) $[M - C_3H_7NO_2]^+$, 340 (16) $[M - C_9H_{14}N_2O_3]^+$, 308 (16), 277 (12), 276 (11), 259 (16), 249 (40), 248 (15), 233 (11), 209 (26), 169 (21), 165 (16), 147 (74), 115 (21), 109 (23), 91 (29). − $C_{31}H_{42}N_2O_6$: calcd. 538.3041 found 538.3042; $C_{28}H_{35}NO_4$ calcd. 449.2566, found 449.2579; $C_{22}H_{28}O_3$: calcd. 340.2038, found 340.2026; $C_{19}H_{21}$: calcd. 249.1643, found 249.1641 (EI-MS).

Crocacin B (2): $[\alpha]_D^{22}$ = + 99.0 (c = 0.5 in methanol). − UV (methanol): λ_{max} (lg ε) = 254 nm (4.48), 266, 276 (sh), 283 (4.31), 293, 300 (sh). − IR (KBr): $\tilde{\nu}$ = 1745 cm^{-1} (s), 1651 (s), 1610 (s), 1496 (s). − ^{1}H NMR (600.1 MHz, [D$_6$]acetone): similar to **1**, but δ = 4.07 (dd, J = 5.4/17.9 Hz, 1 H, 2a-H), 4.10 (dd, J = 5.9/17.9 Hz, 1 H, 2b-H), 7.87 (t, J = 5.4 Hz, br., 1 H, 3-H). − ^{13}C NMR (150.9 MHz, [D$_6$]acetone): similar to **1**, but δ = 170.91 (s, C-1), 41.46 (t, C-2). − MS [EI, 200°C]: *m/z* (%) = 524 (0.5) $[M]^+$, 506 (0.3), 492 (0.3), 357 (4), 325 (3), 293 (5), 249 (12), 187 (63), 147 (100); [(−)-DCI]: *m/z* (%) = 524 (100) $[M]^-$, 492 (79) $[M - CH_3OH]^-$, 460 (44) $[M - 2\ CH_3OH]^-$, 348 (9), 334 (27), 293 (52). − $C_{41}H_{57}NO_8$: calcd. 524.2886, found 524.2849 (EI-MS).

Crocacin C (3): $[\alpha]_D^{22}$ = + 52.2 (c = 0.3 in methanol). − UV (methanol): λ_{max} (lg ε) = 254 nm (4.64), 266, 277, 284, 293 (sh). − IR (KBr): $\tilde{\nu}$ = 3343 cm^{-1}, 3200 (m), 3081, 3057, 3024 (w), 2968, 2927 (m), 2824 (w), 1655, 1600 (s), 1494 (w), 1448, 1369, 1329 (m), 1205, 1191, 1160 (w), 1098 1084 (s), 1034 (w), 971 (s), 749, 694 (m). − ^{1}H NMR (400.1 MHz, [D$_6$]acetone): similar to corresponding signals in **1**, but δ = 6.65 (s, br., 2 H, NH_2), 6.09 (m, 1 H, 4-H), 6.07 (m, 1 H, 5-H), 5.80 (d, J = 1.1 Hz, 1 H, 2-H), 2.21 (d, J = 1.1 Hz, 3 H, 3-CH_3). − ^{13}C NMR (100.6 MHz, [D$_6$]acetone): similar to corresponding signals in **1**, but δ = 169.04 (C-1), 148.09 (C-3), 137.05 (C-5), 135.02 (C-4), 122.01 (C-2), 13.5 (3-CH_3). − MS [EI, 200°C]: *m/z* (%) = 357 (7) $[M]^+$, 325 $[M - CH_3OH]^+$ (3), 293 $[M - 2\ CH_3OH]^+$ (8), 249 $[M - 2\ CH_3OH - CH_2NO]^+$ (20), 217 (9), 187 (25), 148 (32), 147 (100), 115 (45), 91 (15); [(+)-DCI]: *m/z* (%) = 358 (82) $[M + H]^+$, 326 $[M + H\ CH_3OH]^+$. − $C_{22}H_{31}NO_3$: calcd. 357.2304, found 357.2309; $C_{20}H_{23}NO$: calcd. 293.1780, found 293.1781; $C_{19}H_{21}$: calcd. 249.1643, found 249.1633 (EI MS).

Crocacin D (4): $[\alpha]_D^{22}$ = +109.6 (c = 0.56 in methanol). − UV (methanol): λ_{max} (lg ε) = 255 nm (4.55), 262, 276, 280, 290 (sh). − IR (KBr): $\tilde{\nu}$ = 3302 cm^{-1} (m), 3167 (w), 3082 (w), 3057 (w), 3025 (w), 2970 (w), 2932 (s), 2828 (w), 1745 (s), 1651 (s), 1610 (s), 1496 (s), 1448 (m), 1438 (m), 1367 (m), 1263 (s), 1203 (s), 1183 (s), 1123 (m), 1091 (s), 973 (m), 750 (m), 694 (m). − ^{1}H NMR (300 MHz, [D$_6$]acetone): similar to **1**, but δ = 9.14 (d, J = 11 Hz, 1 H, 10-H), 7.59 (m, br., 1 H, 3-H), 6.78 (ddt, J = 9/11/1.5 Hz, 1 H, 9-

H), 4.67 (dt, J = 9/7.4 Hz, 1 H, 8-H), 3.96 (d, J = 5.8 Hz, 2 H, 2-H_2), 2.26 (t, J = 7.1 Hz, 2 H, 5-H_2), 2.12 (ddt, J = 1.5/7.4/6.1 Hz, 2 H, 7-H_2), 1.67 (tt, J = 6.1/7.1 Hz, 2 H, 6-H_2). − ^{13}C NMR (75 MHz, [D_6]acetone): similar to **1**, but δ = 174.53 (C-4), 171.21 (C-1), 123.82 (C-9), 109.64 (C-8), 41.57 (C-2), 34.54 (C-5), 26.12 (C-6), 25.36 (C-7). − MS [EI, 200°C]: *m/z* (%) = 540 (52) $[M]^+$, 525 (3), 508 (5), 493 (2), 431 (1), 378 (5), 249 (7), 248 (6), 201 (9), 187 (10), 165 (11), 156 (10), 147 (100); [(+)-DCI]: *m/z* (%) = 541 (100) $[M + H]^+$, 509 (16) $[M - CH_3OH + H]^+$; [(−)-DCI]: *m/z* (%) = 540 (100) $[M]^-$, 508 (10) $[M - CH_3OH]^-$, 441 (7). − $C_{31}H_{44}N_2O_6$: calcd. 540.3199, found 540.3179 (EI MS).

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