CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200700269

Thuggacins, Macrolide Antibiotics Active against *Mycobacterium tuberculosis*: Isolation from Myxobacteria, Structure Elucidation, Conformation Analysis and Biosynthesis

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Abstract: Two novel antibiotics, thuggacin A (**1**) and B (**2**), were isolated from the myxobacterium *Sorangium cellulosum*. **1** and **2** are unique thiazole-containing macrolides with side chains on both sides of the lactone group. Upon standing in solution, thuggacin A (**1**) rearranges by acyl migration of the lactone group to give a mixture with thuggacins B (**2**) and C (**3**). NOEs and vicinal coupling constants within the lactone ring provided distinct data for the generation of a structure model by PM3 calculations, which allowed an analysis of the conformation in solution and the relative configuration of six asymmetric centres. A minor sorangium metabolite was identified as 13-methyl-thuggacin A (**4**). Furthermore, two natural thuggacin variants, **5** and **6**, were found in another myxobacterium, *Chondromyces crocatus*. In these variants, one side chain is replaced by a methyl group and a hydroxy group is repositioned to give a primary alcohol at the former methyl site, in an α position with respect to the thiazole ring. **1** proved to be active against clinical isolates and reference strains of *Mycobacterium tuberculosis*. Preliminary studies on the mechanism of action indicate inhibition of the cellular electron-transport chain.

Keywords: antibiotics · biosynthesis · conformation analysis · myxobacteria · structure elucidation

Introduction

Although tuberculosis (TB) can be effectively treated by antibiotic therapy, it remains a life-threatening infection not only in third world countries. The increasing antibiotic resistance of *Mycobacterium tuberculosis*, the causative pathogen of TB, requires long and extensive chemotherapy by second-line antibiotics. The ability to persist as a latent infection, which can be reactivated in immune-impaired people, and the enhanced susceptibility of the latter to novel infections make TB a major cause of death among HIV patients. The development of novel TB drugs should be focussed particularly on antibiotics with alternative or synergistic mechanisms of action.[1] Recently phenothiazine drugs, that are bactericidal for replicating and non-replicating mycobacteria, identified mitochondrial respiration as a possible target for new antibiotics that might shorten TB therapy.[2]

In the course of our ongoing screening for biologically active compounds from myxobacteria, the extract of *Sorangium cellulosum*, strain So ce895, attracted particular attention because it showed antibacterial activity against *Mycobacterium phlei* and *M. chitae*, as well as against Corynebacteria, *Nocardia corallina* and *Micrococcus luteus*.[3] Bioactivity-guided isolation led to the unique novel macrolides thuggacins A (**1**) and B (**2**), which are simultaneously produced by this strain along with the antifungal ambruticins.[4,5] When tested against the standard test strain *Mycobacterium tuberculosis* H37RV, thuggacin A (**1**) inhibited the growth at a concentration of 8 $\mu g\,mL^{-1}$.[6] Initial studies towards the

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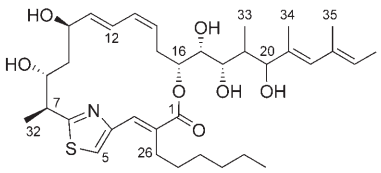

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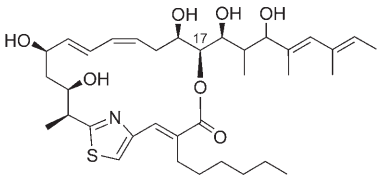

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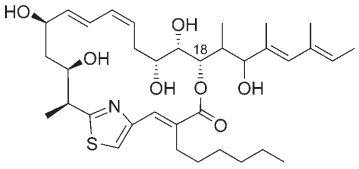

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mechanism of action showed complete inhibition of the oxygen consumption at a concentration of 2.5 ng mL^{-1} and inhibition of reduced nicotinamide adenine dinucleotide (NADH) oxidation at the cytoplasmic membranes with *Micrococcus luteus*. Herein, we report the isolation, structure elucidation, rearrangement and conformation analysis of the thuggacins, as well as the results of feeding experiments with ^{13}C-labelled biosynthetic precursors.

Results and Discussion

Isolation: The thuggacins were isolated from a mixture of XAD 1180 adsorber resin and wet cell mass, which was obtained by sieving the fermentation broth of *Sorangium cellulosum*, strain So ce895. From 300 L of fermentation broth, about 4.5 L of resin and cell mass were harvested and extracted with acetone. After evaporation of the acetone, the remaining water was extracted with dichloromethane to give, after drying and evaporation, 26.6 g of crude material. Partition between methanol and heptane reduced the weight of the more polar fraction to 15.6 g. This was dissolved in ice-cold ethyl acetate and extracted three times with cold aqueous sodium carbonate (1 %) and then with cold saturated sodium chloride solution to yield 5.3 g of crude thuggacin. A similarly crude mixture containing, among others, the carboxylic acids ambruticin F and S (5.8 g) was re-extracted from the water layer after acidification to pH 4.[5]

Silica gel flash chromatography of the thuggacins with a gradient of increasing polarity provided an enriched mixture of thuggacins (1.5 g), which was separated by reversed-phase (RP) chromatography to give thuggacin A (**1**; 244 mg) and thuggacin B (**2**; 61 mg). Thuggacin A (**1**) gave a microcrystalline powder from diethyl ether/petroleum ether.

Structure elucidation: The basic structure of the thuggacins was elucidated with the main component A (**1**). The UV spectrum showed a maximum at $\lambda = 224$ nm and a less abundant band at $\lambda = 289$ nm. The IR spectrum indicated an ester or lactone group by a prominent absorption at 1707 cm^{-1} and hydroxy groups by a broad band at 3418 cm^{-1}. The presence of sulfur in the elemental composition of **1** was recognised from the isotope pattern of the molecular ion at m/z 631 in the EI and (−)DCI mass spectra and was confirmed by EI-HRMS, to provide an empiric formula of $C_{35}H_{53}NSO_7$.

Well-separated signals in the ^{13}C NMR spectrum of **1** in [D$_6$]dimethylsulfoxide ([D$_6$]DMSO) confirmed the number of carbon atoms (35) and enabled the unambiguous correlation of 48 directly carbon-bound protons from the ^{1}H,^{13}C HMQC spectrum (Table 1). Thus, five H/D-exchangeable protons must be present. By taking the direct correlations in the ^{1}H,^{1}H-COSY NMR spectrum into account, two main structural parts were initially recognised (Figure 1). The larger one—ranging from the C32 methyl group to the C20 oxymethine unit—was derived from vicinal correlations, including all four clearly visible correlations with OH protons. According to the integration result of the ^{1}H NMR spectrum, the fifth hydroxy proton signal, belonging to the OH group on C17, is located in the signal group at $\delta = 4.88$ ppm and overlaps with the signals for H16 and H10. Thus, in the ^{1}H,^{1}H-COSY NMR spectrum, a correlation signal of H17 with the OH group overlaps with the correlation signal of H17 with H16. The latter vicinal correlation is still present after H/D exchange. The structure of the chain end from C21 to C25 was deduced from long-range correlations in the ^{1}H,^{1}H-COSY and ^{1}H,^{13}C-HMBC NMR spectra of **1** (Figure 1).

The second structure part, a C$_6$ aliphatic chain from the C26 methylene group to the C31 methyl moiety, contains all of the remaining methylene groups. Its position as a side chain at the olefinic quaternary C2 atom ($\delta = 131.90$ ppm[7]) was apparent from examination of the long-range correlations (Figure 1) in the HMBC spectrum, especially from the mutual correlations between the C26 methylene and C3 olefin methine groups. Further correlations with the carbonyl signal at $\delta = 166.48$ ppm indicated the position of the C1 carboxyl group, which was additionally correlated to the C16 oxymethine moiety. Since H16 showed a typical downfield shift ($\delta = 4.86$ ppm), an ester or lactone group must be present.

While the ^{1}H singlet of H3 at $\delta = 7.77$ ppm showed a typical olefin-type ^{1}H,^{13}C coupling constant of $^1J_{HC} = 160$ Hz in the HMBC spectrum, the second ^{1}H singlet signal at $\delta = 7.72$ ppm had a one-bond coupling constant of $^1J_{HC} = 190$ Hz. Such large values characterise methine groups in heteroaromatic rings. With this methine group (C5), with the nitrogen and sulfur atoms remaining from the molecular formula, and with two quaternary carbon atoms with appro-

Table 1. NMR spectroscopic data for thuggacin A (**1**) and thuggacin C (**3**) in [D_6]DMSO.[a]

	Thuggacin A (**1**)[b]						Thuggacin C (**3**)[b]					
atom no.	δ_H [ppm]	mult.	J [Hz]	ROESY[c]	δ_C [ppm]	mult.	δ_H [ppm]	mult.	J [Hz]	ROESY[c,d]	δ_C [ppm]	mult.
1	–	–	–	–	166.48	s	–	–	–	–	166.84	s
2	–	–	–	–	131.90	s	–	–	–	–	133.65	s
3	7.77	s	–	H12	132.94	d	7.69	s	–	$H15_a$, H12 ≫ H20, OH17, H18, $H15_b$	130.64	d
4	–	–	–	–	149.04	s	–	–	–	–	149.89	s
5	7.72	s	–	$H26_b > H26_a > H27$	118.94	d	7.84	s	–	$H26_b ≫ H27 > H26_a$	121.64	d
6	–	–	–	–	171.24	s	–	–	–	–	172.30	s
7	3.38	dq	9.2, 6.8	H10	43.30	d	3.38	dq	8.0, 6.8	H10	43.77	d
8	3.52	dt	9.4, 3.6	H32 ≫ H11	75.70	d	3.58	ddd	3.7, 4.8, 8.0	H32	74.08	d
OH8	(5.00	d	6.6)	–			(5.12	d	6.4)	H32, H10		
9_a	1.69	ddd	14.9, 9.1, 3.8	H11	39.87	t	1.63	m	10.2, 5.3[e]	H11 > H7	39.62	d
9_b	0.61	dd	13.6, 3.0, br	–			1.05	dt	14.1, 3.3	H12, H11, OH8		
10	4.85	dd	9.2, 9.1	H7, H12 ≫ 8	69.69	d	4.18	ddd	3.4, 7.7, 10.1	H7, H12 > OH8	69.07	d
OH10	(5.01	d	3.1)	–			(5.06	d	3.8)	H11, $H9_a$		
11	5.39	dd	9.2, 14.9	$H13 ≫ H9_a > H9_b$	137.33	d	5.46	dd	7.9, 15.1	H13, $OH10 > H9_a > H9_b$	136.76	d
12	6.41	dd	10.9, 15.1	H10, $H15_a$, H3	124.76	d	6.09	dd	11.3, 15.1	H10, $H15_a$ > H3 > $H9_b$	124.92	d
13	5.84	t	10.9	H11	130.89	d	5.83	tt	11.1, 1.6	H11	127.65	d
14	5.24	dt	5.3, 11.3	H16, ($H15_b$, no $H15_a$) > H17	127.85	d	5.48	dt	3.9, 10.0	H16, OH16 > $H15_b$ (no $H15_a$)	130.98	d
15_a	2.79	ddd	11.5, 11.3, 14.1	H12 > H18	29.89	t	2.79	dd	9.1, 16.4	H12, H3, OH17	29.53	t
15_b	2.24	dd	5.3, 14.3, br	H17, H18			1.53	ddt	16.4, 10.8, 2.9	H18 > OH16 ≫ H3		
16	4.86	ddd	11.0, 3.5, 1	H14, H19 > H18, ($H15_b$) > H33	74.24	d	3.52	dd	5.3, 10.9	H14 > OH17 > H18	72.14	d
OH16	–	–	–	–			(4.93	d	4.5)	H14, H17 > H18		
17	3.58	dd	3.8, 6.4	H20, H19, H33, $H15_b$	72.05	d	3.76	d	5.5 (br)	H20, OH16 > H19, H33	70.89	d
OH17	(4.87	m	–)	–			(5.29	d	5.3)	H20, H16, OH20 > $H15_a$, H19		
18	3.46	dd	3.0, 6.4	H20 > H19 > ($H15_b$, H34, $H15_a$)	71.44	d	5.18	d	7.2	H33, H20, OH16, OH20 > 15b, H22	73.47	d
OH18	(4.30	d	5.5)	–			–	–	–	–		
19	1.85	ddq	3.0, 6.4, 6.8	H16, H17, H34	37.17	d	2.20	ddq	7.0, 3.4, 7.2	H34, OH17 > H17, H22	36.88	d
20	3.87	d	6.0	H22 > H18 > H34, H17	77.43	d	4.05	d	2.6 (br)	H17, H22, H34 > H18, OH17, H3	73.74	d
OH20	(4.62	d	3.0)	–			(5.00	d	4.1)	H22 > OH17, H18, H33	–	–
21	–	–	–	–	135.82	s	–	–	–	–	135.65	s
22	5.78	s	br	H20 ≫ H24, H35 > H18	128.35	d	5.89	s	br	OH20, H20, H35 > H24 > 33, 34	127.80	d
23	–	–	–	–	133.07	s	–	–	–	–	133.03	s
24	5.24	dq	1.0, 7.2	H22, H34	123.01	d	5.32	q	6.7 (br)	H22	123.07	d
25	1.55	d	6.8	H35	13.42	q	1.63	d	6.7	–	13.50	q
26_a	2.48	m	–[f]	H5	26.66	t	3.02	ddd	6.6, 8.7, 12.6	H5, H28	27.19	t
26_b	2.30	ddd	7.1,7.1,13.3	H5			2.59	ddd	6.4, 8.5, 12.6	H5, H28		
27	1.31	m	–	–	27.60	t	1.43	m	–	H29, H30	28.14	t
28_a	1.31	m	–	–	28.37	t	1.31	m	–	–	28.61	t
28_b	1.21	m	–	–								
29	1.17	m	–	–	31.10	t	1.23	m	–	–	31.08	t
30	1.17	m	–	–	21.93	t	1.23	m	–	–	21.93	t
31	0.78	m	–	–	13.85	q	0.80	m	–	–	13.88	q
32	1.44	d	6.8	H8, H7	17.39	q	1.42	d	6.8	H8 > OH8	17.44	q
33	0.77	m	–	H17, H20, H18, H16, H22	8.21	q	0.74	d	7.2	H18, H22, H17, OH20 > H34	9.40	q
34	1.60	s	–	H20, H24, H19 > H18	14.18	q	1.62	s	–	H20, H19	15.34	q
35	1.64	s	–	H22, H25	16.68	q	1.69	s	br	H22	16.75	q

[a] ^{1}H NMR: 600 MHz, ^{13}C NMR: 150 MHz. [b] Data after H/D exchange; selected data of the OH-form are given in parentheses. [c] Without vicinal proton signals. [d] Data from the OH form. [e] Partially under the C34 methyl signal. [f] With the solvent signal.

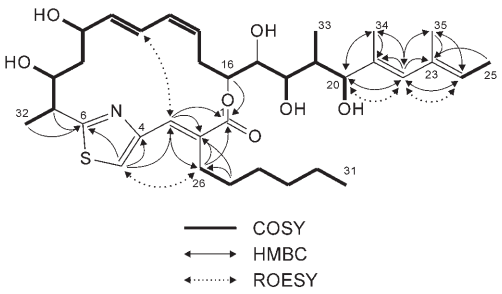


Figure 1. Structure elements from NMR spectroscopy of thuggacin A (**1**).

priate ^{13}C NMR shifts,[8] the thiazole ring was assembled. Its orientation in the structure of **1** followed from the $^{1}H,^{13}C$ and $^{1}H,^{1}H$ long-range correlations presented in Figure 1.[9]

The configuration of the double bonds and their s-*trans* spatial orientation in the 11*E*,13*Z* diene of **1** was derived from the vicinal coupling constants of about 15 and 11 Hz, respectively. In the case of the olefin singlet signals of H3 and H22, correlations in the $^{1}H,^{1}H$-ROESY (Table 1) and NOE difference NMR spectra allowed the determination of the configuration of the double bonds. These spectra were taken in [D_6]acetone after H/D exchange as the H3 and H5 signals were well separated, by 0.26 ppm, in this solvent. The spectra indicated NOEs between H5 and the methylene protons on C26 and between H3 and H12, a long-range interaction across the lactone ring. Since no effect was seen between H3 and the C26 methylene protons, the *E* configuration of the $\Delta^{2,3}$ double bond was assigned unambiguously. Although the olefin signals of H13 and H22 overlap, their NOEs could be reliably used, as the protons are spatially well separated in the structure of **1**. Thereby, the *E*,*E* configuration of the second diene system in thuggacin A (**1**) was apparent from the observed NOEs (Figure 1).

The second antibiotic metabolite **2** was easily recognised in the extracts as a thuggacin variant from the identical UV and IR spectra and elemental composition. Analysis of the NMR data of thuggacin B (**2**) proved that both compounds share an identical carbon skeleton. However, in the NMR spectra of **2**, the H17 signal shows an acylation shift of 1.3 ppm downfield to $\delta = 4.92$ ppm compared to the same signal for **1**, whereas H16 ($\delta = 4.86$ ppm) had approximately the same shift. Together with the $^{1}H,^{13}C$-HMBC correlation between H17 and C1, the acylation shift indicates closure of the lactone ring between C17 and C1 in **2**. The C16 position of **2** is now characterised as bearing a secondary alcohol group from the upfield shift of H16 ($\delta = 3.71$ ppm) and its coupling to an H/D-exchangeable OH proton doublet at $\delta =$ 5.03 ppm. The ring expansion of just one carbon accounts for the high similarity of the spectroscopic data of **1** and **2**. While both compounds behave very similarly during TLC on silica gel, a shorter retention time was observed for **2** in RP-HPLC on a Nucleosil C18 column.

Rearrangement of thuggacins: Thuggacin B (**2**) was detected by analytical HPLC in fresh extracts of fermentation broths of *S. cellulosum* or of XAD 1180, which was harvested from fermentations. However, in repeated analyses of samples stored in methanol, the proportion of **2** gradually grew while that of **1** decreased. The conversion was studied in more detail by an NMR experiment with an initial concentration of about 8 mg mL^{-1} of thuggacin A (**1**) in [D_4]methanol. Integration of the low-field singlet signals of H3 (**1**: $\delta =$ 8.01 ppm; **2**: $\delta = 8.27$ ppm) provided the data presented in Figure 2.[10] With a half life of 16.4 h at room temperature,

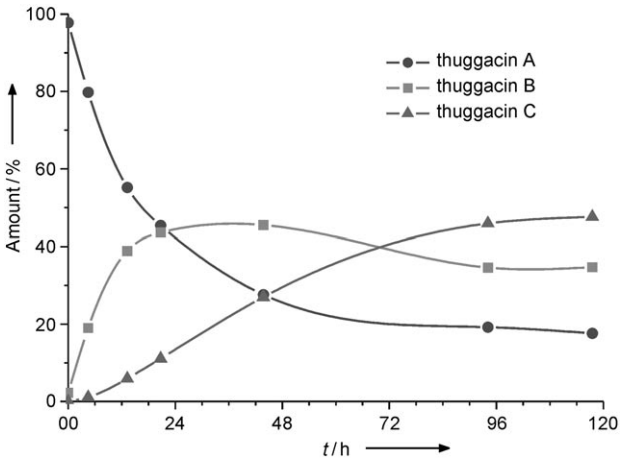


Figure 2. Rearrangement of thuggacins in methanol (8 mg mL^{-1}).

thuggacin A (**1**) underwent a rearrangement to other thuggacin-type compounds. Thuggacin B (**2**) appeared as the first product. Unexpectedly, a third H3 singlet of a further product, thuggacin C (**3**), slowly emerged at $\delta = 7.90$ ppm and became the major product of about 48% (**1**: 17%, **2**: 35%). After 117 h this experiment was very close to a steady state, in which reverse transesterification reactions establish the equilibrium. Repeated experiments revealed that the reaction rate varied with the quality of the starting material. Recrystallised material had an extremely low reaction rate. Traces of acid, such as, acetic acid, completely inhibited the rearrangement of **1**. Such acyl-group migrations are well known, for example, with acetate blocking groups in synthetic chemistry. They occur under basic and acidic conditions via orthoester intermediates with retention of chirality.[11] An enforced transesterification of another macrolide was observed with chondropsin A, which could be rearranged to the chondropsin D isomer by prolonged standing in pyridine.[12]

Accordingly, the second rearrangement product **3** was confirmed as a thuggacin variant from the nearly identical UV, IR, and MS data. In the NMR spectra of thuggacin C (**3**), the H18 signal ($\delta = 5.18$ ppm) now had an acylation shift of 1.7 ppm downfield compared to the euqivalent signal for **1** and it had lost the coupling to the OH proton, while the $^{1}H,^{13}C$-HMBC correlation between H18 and C1 additionally indicated the closure of the enlarged lactone ring between C18 and C1 in **3** (Table 1).

Conformation analysis: The thuggacins contain eight stereocentres clustered in two structural regions. As they are partially incorporated within the lactone ring, their relative configuration should be accessible from NOEs and vicinal ^{1}H coupling constants.

In view of the complex structure, including two semi-fixed structure elements, that is, the thiazolyl acrylic acid lactone and the diene, a predominant conformation for the thuggacin lactone ring can be expected. Conspicuous NOEs between protons that are separated at least by four bonds and distinct vicinal coupling constants supported the predominant existence of a well-defined conformation of the lactone ring in thuggacin A (**1**). The NOEs were studied by ^{1}H,^{1}H-ROESY NMR spectroscopic analysis of **1** in the OH and OD forms in [D$_6$]DMSO (Table 1).

A stereo-model of the relative configuration of thuggacin A (**1**) was calculated by using the HyperChem program, initially with mm+ but finally with the semi-empirical PM3 method. As the *E*,*Z* diene fixes five bonds and the thiazolyl acrylic acid part fixes a further seven bonds of the 17-bond lactone ring, only five possibly flexible bonds remain in the ring. Thus, a bare lactone ring with these structural elements was created initially, which had to meet the constraints set by the ring-bridging NOE between H3 and H12, as well as the NOEs between H12 and both H$_a$15 and H10 and, especially, the NOE between H7 and H10, which is the strongest of all the NOEs in the ROESY spectrum of **1**. The structure was then completed by addition of the *n*-hexyl, methyl and hydroxy substituents, with consideration of further NOEs and the vicinal coupling constants from the H/D-exchanged ^{1}H NMR spectrum of **1**. In the calculated model (Figure 3), the *E*,*Z* diene adopts the expected planar s-*trans* arrangement while the conjugated thiazolyl acrylic lactone is distorted with an angle of +32° for C2–C3–C4–C5, which twists C2 below the plane of C3–C4–C5. However, this twist is not enforced by the lactone ring strain. Without the repulsion between thiazole H5 and the alkyl side chain, the angle is recalculated to −28° and C2 is located slightly above the plane.[13]

The final PM3 model of **1** takes all of the NOE constraints into account. The conspicuously strong NOE between H7 and H10, which is only possible if the bonds of the ring segment between C7 and C10 form a loop, thereby bringing their protons into close vicinity, is explained by the very short distance of 1.84 Å in the model. In this loop, a series of $^3J_{H,H}$ coupling constants of about 9 Hz indicated s-*trans* arrangements of the vicinal proton pairs H7/H8, H$_a$9/H10 and H10/H11, which are characterised in the model by torsion angles of −177, −160 and −179°. On the right-hand side of the lactone ring, *trans* orientations were similarly required to account for couplings of about 11 Hz between H14 and H$_a$15 and between H$_a$15 and the lactone proton H16 (179° and 147°). Together, these orientations provide a conformation in which H14 and H16 are in the "*cisoidal*" position necessary for their mutual NOEs (2.39 Å). The relative configuration given in Figure 3 shows the thuggacin A model with 7*S**, 8*R**, 10*R**, 16*R** stereochemistry. The configuration of C17 and C18 in the side-chain of this model was derived from the analysis of compound **3**.

By using analogous arguments, the relative configuration of thuggacin A (**1**) can be extended to thuggacin C (**3**), which incorporates two further asymmetric centres (C17 and C18) in the lactone ring. However, due to the higher flexibility of the ring, the observed vicinal coupling constants are less accentuated in **3**. On the left-hand side, the PM3 model of **3** is close to the model of **1** (−173, −165, 159° for H7/H8, H$_a$9/H10, H10/H11; Figure 4). On the other side, the extension of the lactone ring led to distinct differences: Although NOEs crossing the ring, that is, between H3/H12 and H3/H$_a$15, are present (2.95 and 2.24 Å, respectively), H$_b$15 is now directed to the front and H16 to the rear. These positions allow the observed NOEs for H14/H16 (2.90 Å) and, as expected, H14/H$_b$15, while the observed vicinal coupling constants fit the calculated dihedral angles in the model:

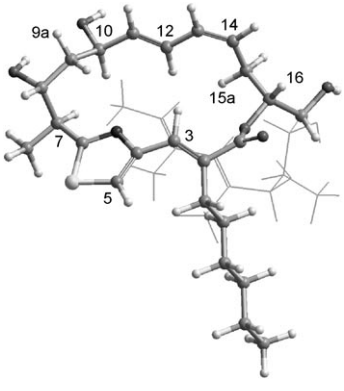


Figure 3. PM3-calculated relative configuration of thuggacin A (**1**). Selected proton distances [Å]: H3–H12: 2.62, H3–H15$_a$: 3.10, H7–H10: 1.84, H10–H12: 2.48, H12–H15$_a$: 1.87, H14–H16: 2.39; torsion angles (H–C–C–H) [°]: H7–H8: −177, H9$_a$–H10: −160, H10–H11: −179, H11–H12: 180, H12–H13: −174, H13–H14: 1, H14–H15$_a$: 179, H15$_a$–H16: −147. The side chain at the back is rendered as a stick representation.

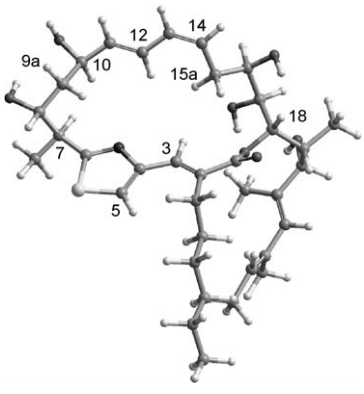


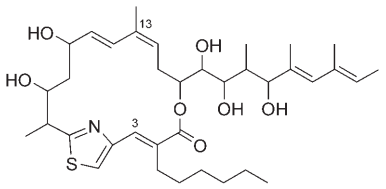
Figure 4. PM3-calculated relative configuration of thuggacin C (**3**). Selected proton distances [Å]: H3–H12: 2.95, H3–H15$_a$: 2.24, H3–H15$_b$: 2.84, H7–H10: 1.79, H10–H12: 2.49, H12–H15$_a$: 1.81, H14–H16: 2.99; H15$_b$–H18: 2.63; torsion angles (H–C–C–H) [°]: H7–H8: −173, H9$_a$–H10: −165, H10–H11: 160, H11–H12: 180, H12–H13: 175, H13–H14: −1, H14–H15$_a$: −170, H15$_b$–H16: −171, H16–H17: 55, H17–H18: 92.

$J_{15b,16} \approx 11$ and $J_{14,15a} \approx 9$–10 Hz agree with the angles of −171 and 170° as does $J_{14,15b} \approx 3$–4 Hz with 74°. The most intense NOE of H_b15 is with H18, which not only substantiates the calculated frontal orientation of H_b15, but also that of the lactone proton H18. Their distance in the model is 2.6 Å. The vicinal coupling constants $J_{16,17} \approx 5.4$ and $J_{17,18} \approx 0$ Hz are also in good agreement with modelling results which give dihedral angles of 55 and 92°, respectively. With these findings, the relative configuration of six asymmetric centres was established as shown in Figure 4 for thuggacin C (**3**) with 7*S**, 8*R**, 10*R**, 16*R**, 17*S**, 18*S** stereochemistry. This relative configuration was used to present the structures of **1**–**3**.

Calculation of all possible configurations of the remaining two stereocentres of the diene side chain of **3** was expected to afford their relative configuration too. However, even after extensive use of the conformation search in HyperChem, none of the energy-minimised models were in good agreement with the NMR spectroscopic data. Several of the observed NOEs could not be explained by the existence of only one major conformation. Thus, the NMR spectroscopic data apparently represent the mean values of a population of various rotamers. This conclusion was substantiated when NMR spectra of thuggacin C (**3**) were recorded at lower temperatures in [D_4]methanol. Most of the initially sharp ^{1}H NMR signals lost their fine resolution. At −24 and −47 °C, only a few signals of the methyl groups were still present as sharp signals. The doublet signal of the C33 methyl group—right at the beginning of the side chain—appeared as a triplet with $J = 7.3$ Hz at $\delta = 0.99$ ppm, corresponding to two partially overlapping doublets of equally abundant rotamers. By contrast, the doublet signal of the C32 methyl group on the opposite side of the lactone ring was not affected.

Thuggacin variants: During the separation of a 60-L fermentation broth, a compound represented by a small peak following that of thuggacin A (**1**) in the RP-MPLC was collected and refined by preparative RP-HPLC. The main component, **4**, had a thuggacin-type UV chromophore while the MS data indicated a molecular mass of 645, which was 14 mass units higher than that of **1**. As expected, the corresponding elemental composition of $C_{36}H_{55}NO_7S$ obtained by EI-HRMS additionally contained one carbon atom and two protons more. The extra structural element appeared as an allylic methyl group at $\delta = 1.68$ ppm in the ^{1}H NMR spectrum. Analysis of COSY, HMQC and HMBC spectra indicated that this group replaced the H13 proton of the diene system in the lactone ring. In the ^{1}H,^{1}H-ROESY NMR spectrum, the C13 methyl group showed correlations with H11 and H14, while H12 correlated with H3 and the C15 methylene protons. Thus, the s-*trans*-*E*,*Z* double-bond geometry of thuggacins **1**–**3** was retained in 13-methyl-thuggacin A (**4**).

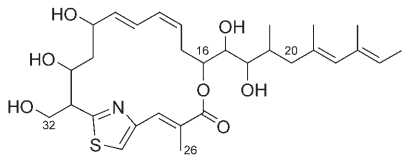
Previous screenings of the myxobacteria species *Chondromyces crocatus*, strain Cm c5, afforded the novel chondramides,[14] crocacins,[15] crocapeptins, ajudazoles[16] and chondrochlorens.[17] Besides these main antifungal and cytotoxic



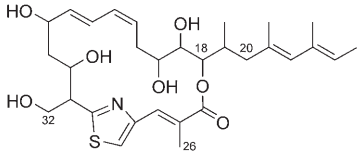
4

compounds, this strain produces further metabolites that were discovered by HPLC analyses of culture extracts with UV-diode-array detection. Surprisingly, the UV data of some extracts contained peaks with the typical UV spectrum of the thuggacins, which had previously only been seen in extracts of *Sorangium cellulosum*. Subsequently, two new variants were isolated by RP-HPLC from pooled side fractions of separations of *Chondromyces* extracts.

EI-HRMS analyses of the molecular ions and the prominent dehydrated $[M-18]^+$ ions suggested the elemental composition $C_{30}H_{43}NSO_7$ for variants **5** and **6**, which indicat-



5



6

ed a decrease of C_5H_{10} compared to the composition of thuggacins A–C (**1**–**3**). As expected, this difference resulted in the loss of all signals of the *n*-hexyl chain in the NMR spectra (Table 2). Instead, a new singlet assignable to a methacryl methyl group ($\delta = 1.95$ ppm, C26) appeared in the NMR spectra of **5**. Additionally, two further differences were observed: the signal for the methyl group at C32[18] in thuggacin A (**1**), a prominent ^{1}H doublet signal at $\delta =$ 1.44 ppm, was replaced by a broad multiplet of a new hydroxymethylene group at $\delta_H = 3.94$ ($\delta_C = 59.80$ (t), $\delta_{OH} =$ 5.17 ppm) in thuggacin cmc-A (**5**). As a consequence of the unchanged number of oxygen atoms, one of the secondary hydroxy groups must be replaced by a proton. The signals of the new allylic methylene protons at C20 appeared as two double doublet ^{1}H signals at $\delta = 2.12$ and 1.77 ppm and the corresponding ^{13}C signal at $\delta = 45.21$ ppm in the NMR spectra of **5**.

While the lactone ring size in **5** is similar to **1**, as indicated by the ^{1}H NMR shift of H16 at $\delta = 4.90$ ppm, the second

Table 2. NMR spectroscopic data for thuggacins cmc-A (**5**) and cmc-C (**6**) in [D_6]DMSO.[a]

atom no.	Thuggacin cmc-A (**5**)					Thuggacin cmc-C (**6**)				
	δ_H [ppm]	mult.	J [Hz][b]	δ_C [ppm]	mult.	δ_H [ppm]	mult.	J [Hz][b]	δ_C [ppm]	mult.
1	–	–	–	166.86	s	–	–	–	167.33	s
2	–	–	–	125.99	s	–	–	–	127.90	s
3	7.87	s	–	133.46	d	7.80	s	(br)	131.33	d
4	–	–	–	148.98	s	–	–	–	149.63	s
5	7.82	s	–	121.01	d	7.93	s	–	123.14	d
6	–	–	–	167.97	s	–	–	–	169.31	s
7	3.44	m	(3.6, 5.5, 9.5)	51.37	d	3.48	m	–	51.54	d
8	3.88	m	(9.7, 3.5, 3.5)	70.09	d	3.86	m	–	69.86	d
OH8	5.00	d	6.5, br			5.18	d	5.0		
9_a	1.64	m	–	39.26	t	1.60	ddd	3.7, 10.5, 14.5	39.17	t
9_b	0.43	dd	14.3, 2, br			0.78	m	–		
10	4.94	dd	9.2, 9.5	69.55	d	4.73	dd	8.0, 10.0 (br)	69.35	d
OH10	5.05	d	2.0, br			5.25	s	(br)		
11	5.39	dd	15.1, 9.2	137.16	d	5.48	dd	8.0, 15.0	136.67	d
12	6.46	dd	15.1, 11.1	125.03	d	6.19	dd	11.3, 15	124.66	d
13	5.87	dd	10.0, 11.0	130.95	d	5.83	dd	10.9, 11.3	127.52	d
14	5.30	ddd	11.4, 11.4, 5.4	128.02	d	5.50	ddd	3.5, 9.7, 10.9	131.08	d
15_a	2.86	ddd	13.7, 11.6, 11.5	29.97	t	2.87	dd	9.7, 16.0	29.24	t
15_b	2.24	dd	13.7, 5.1			1.44	ddt	11.5, 16, 3		
16	4.90	dd	10.8, 3.3, br	74.25	d	3.46	m	–	72.45	d
OH16	–	–	–			4.96	m	–		
17	3.57	dd	6.8, 3.5 (m, br)	72.10	d	3.71	t	5.4	71.43	d
OH17	4.82	d	6.0, br			5.04	d	6.0		
18	3.33	dd	6.8, 3 (m, br)	71.84	d	4.96	m	–	74.81	d
OH18	4.31	d	6.0, br			–	–	–		
19	1.94	m	–	32.33	d	2.20	dddq	4.0, 6.8, 10.3, 6.8	32.51	d
20_a	2.12	dd	12.9, 6.5	45.21	t	2.32	dd	4.0, 13.2	44.40	t
20_b	1.77	dd	12.9, 7.8			1.80	dd	10.3, 13.2		
21	–	–	–	133.27	s	–	–	–	132.96	s
22	5.59	s	–	130.19	d	5.63	s	(br)	130.37	d
23	–	–	–	133.27	s	–	–	–	133.15	s
24	5.22	q	7.0, br	122.64	d	5.30	q	6.8 (br)	122.87	d
25	1.59	d	7.0	13.46	q	1.63	d	6.8	13.44	q
26	1.95	s	br	13.35	q	2.26	s	–	14.54	q
32	3.94	m	–	59.80	t	3.98	ddd	4.0, 5.0, 10.4 (br)	60.55	t
						3.89	m	–		
OH32	5.17	t	4.3, br			5.12	t	4.0		
33	0.76	d	6.5	13.64	q	0.79	d	6.8	15.28	q
34	1.70	s	br	17.67	q	1.69	s	–	17.41	q
35	1.63	s	–	16.68	q	1.68	s	–	16.68	q

[a] ^{1}H NMR: 600 MHz, ^{13}C NMR: 150 MHz. [b] Values after H/D exchange are given in parentheses.

isomer **6** has an enlarged ring similar to that of thuggacin C (**3**). In thuggacin cmc-C (**6**), the signal for H18 appears in the ^{1}H NMR spectrum at $\delta = 4.96$ ppm as a multiplet overlapping with the signal for the hydroxy group on C16 (Table 2).

According to these alterations, the thuggacins from *Chondromyces crocatus* showed more structural differences than initially expected: besides the replacement of the *n*-hexyl side chain by a methyl group, a primary alcohol function was introduced at C32 while a secondary alcohol group was removed from the side chain.

Biosynthesis: From their structures, the thuggacins are polyketide-derived secondary metabolites. As the insertion of amino acids during polyketide biosynthesis is often observed with myxobacteria and as the biosynthetic origin of thiazole rings from cysteine[19] is well known, we only checked the incorporation of [1-^{13}C]acetate, [1,2-$^{13}C_2$]acetate, [1-^{13}C]propionate and [$^{13}CH_3$]methionine in feeding experiments with *Sorangium cellulosum*. The incorporation of the precursors was analysed by ^{13}C NMR spectroscopy of the labelled thuggacin A (**1**) in [D_6]DMSO (Table 3) and was found to be consistent with previous signal assignments.

[1-^{13}C]acetate feeding gave a ^{13}C enrichment of 3.64%, calculated from the signal intensities of the aliphatic side chain C26 to C31.[20] This allowed unambiguous differentiation between the ten labelled carbon signals from [1-^{13}C]acetate and the remaining carbon atoms (Figure 5, Table 3). [1,2-^{13}C]acetate feeding gave an enrichment of 1.6% ^{13}C in labelled positions compared to the signal of natural ^{13}C abundance (1.1%). The spectra showed intense singlet signals (s in Table 3) for unlabelled positions.[21] The magnitude of the coupling constants $J_{C,C}$ from the doublets of labelled carbon atoms were all in the expected ranges

Table 3. ^{13}C NMR data[a] for thuggacin A (**1**) from feeding experiments with ^{13}C-labelled acetates and propionate.

C	δ_C [ppm]	mult.	[1-^{13}C]acetate (intensity[b])	[1,2-^{13}C]acetate (J_{CC} [Hz])	[1-^{13}C]propionate (intensity[b])
1	166.48	s	→2.00	71.2	0.06
2	131.90	s	0.71	71.2	0.06
3	132.94	d	0.52	s	0.05
4	149.04	s	n.o.[c]	s	0.05
5	118.94	d	0.71	s	n.o.[c]
6	171.24	s	1.11	s	→3.55
7	43.30	d	0.76	s	n.o.
8	75.70	d	→2.11	36.3	n.o.
9	39.87	t	solvent[d]	solvent[d]	solvent[d]
10	69.69	d	→1.80	48.7	0.12
11	137.33	d	0.71	48.7	0.03
12	124.76	d	→2.78	54.8	0.05
13	130.89	d	0.62	54.8	0.05
14	127.85	d	→3.02	42.5	0.07
15	29.89	t	0.73	42.5	0.09
16	74.24	d	→2.52	41.4	0.12
17	72.05	d	0.58	m[e]	n.o.
18	71.44	d	0.78	s	→3.43
19	37.17	d	0.81	s	n.o.[c]
20	77.43	d	0.60	s	→2.69
21	135.82	s	0.52	s	0.04
22	128.35	d	1.72	s	→3.28
23	133.07	s	0.52	s	n.o.[c]
24	123.01	d	→5.01	43.2	0.07
25	13.42	q	0.78	43.2	0.07
26	26.66	t	→3.00	m[e]	n.o.[c]
27	27.60	t	0.91	m[e]	n.o.[c]
28	28.37	t	→6.51	34.7	0.09
29	31.10	t	1.33	34.7	0.09
30	21.93	t	→5.40	43.9	0.12
31	13.85	q	1.22	43.9	0.13
32	17.39	q	1.44	s	0.11
33	8.21	q	1.15	s	0.08
34	14.18	q	0.85	s	0.07
35	16.68	q	0.85	s	0.09

[a] ^{13}C NMR: 75 MHz in [D_6]DMSO. [b] Intensity of solvent signal at 39.51 ppm = 100; labelled carbon atoms are marked by →. [c] n.o. = not observed. [d] Obscured by solvent signals. [e] Overlapping signals.

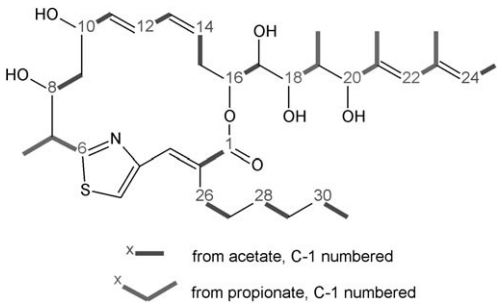


Figure 5. Incorporation of acetate and propionate in the biosynthesis of thuggacin A (**1**).

and unambiguously indicated the intact incorporation of all ten acetate precursors. The experiment with [1-^{13}C]propionate gave a high ^{13}C enrichment of 69.6%, calculated for C22 compared to the mean intensity of all other double-bond methine carbon signals. The spectrum unmistakably showed four nearly equally abundant carbon signals for C6, C18, C20 and C22.[22] Thus, the propionate units provide the four methyl groups of **1**. Consequently, no incorporation of label from [$^{13}CH_3$]methionine was observed. The data showed that the starter for the biosynthesis of **1** (Figure 5) is acetate, which is elongated by three propionate units. Five acetate units are then added before the last propionate is introduced. Its carboxyl group is condensed with cysteine to provide the thiazole ring. As expected the former cysteine carbon atoms were not labelled in our feeding experiments. The remaining part of thuggacin with the hexyl side chain is derived by a rarely occurring condensation with a second oligoketide chain, in this case containing four acetate units. The closure of the lactone ring occurs in the final stage of the polyketide synthesis. Presumably the only direct product of the biosynthesis is thuggacin A (**1**). The small amount of thuggacin B (**2**) in fresh extracts can readily be explained by the observed rearrangement of the thuggacins, which is not completely inhibited even though the fermentation products are stabilised by adsorption to the XAD resin present in the broth.

Owing to their selective activity against different mycobacteria species and related bacteria, the novel macrolactone antibiotics **1**–**6** are valuable additions to the series of highly active natural products from myxobacteria, including amongst others the antifungal soraphenes,[23] antimicrobial sorangicins[24] and antineoplastic epothilones.[25] Their mode of action, the inhibition of cell respiration, still has to be explored more thoroughly. The inhibition of this essential cell function may offer the chance to strengthen the power of the drug combinations used against tuberculosis.[26] Further chemical studies are necessary to complete the structure elucidation for all stereocentres, that is, their absolute configuration, to synthesise structural variants, and to evaluate the structure–activity relationship for this novel structural type.

The metamorphosis-like rearrangement of thuggacins A (**1**), B (**2**) and C (**3**) may be seen as a handicap for antibiotics, as structural rigidity is usually preferred in drug design. However, as **1** and **2** were similarly active and only **3** lost some activity, the structural flexibility may be an advantage with regard to the drug resistance of bacteria, which is often caused by target-site modification. As was shown with the RNA polymerase inhibitor sorangicin A, some slight flexibility allows the antibiotic to adapt to small changes at the target site and thus retain the activity.[27] When the structural space of active variants is considered, the thuggacin skeleton seems to offer a good chance of increasing the antibiotic properties by chemical derivatisation of the natural product or by total synthesis of more complex modified thuggacins. A good example of a modified but active variant is the *Chondromyces* product thuggacin cmc-A (**5**), which is as active as thuggacin A (**1**). Thuggacin A (**1**) not only showed activity against different mycobacteria used in our screening but also proved to be active against clinical isolates and the reference strain of *Mycobacterium tuberculosis*.

Experimental Section

General: UV: Shimadzu UV-2102 UV/Vis scanning spectrometer, solvent methanol (Uvasol from Merck). IR: Nicolet 20 DXB FTIR spectrometer. NMR: Bruker DMX 600 (^{1}H: 600.1 MHz, ^{13}C: 150.9 MHz), ARX 400 (^{1}H: 400.1 MHz; ^{13}C: 100.6 MHz) or AM 300 (^{1}H: 300.1 MHz; ^{13}C: 75.5 MHz) spectrometers, the internal standard was the solvent signal. Mass spectrometry: EI or DCI: Finnigan MAT 95 spectrometer (EI with 70 eV; DCI with isobutane), resolution $M/\Delta M = 1000$; high-resolution data from peak matching ($M/\Delta M = 10000$). Molecular modelling: HyperChem Professional Version 7.51, mm+ and PM3 (Polak-Robiere), gradient 0.005. ^{13}C content of precursors in feeding experiments: [1-^{13}C]acetate 99%; [1,2-^{13}C]acetate 99%; [1-^{13}C]propionate 99%; [$^{13}CH_3$]methionine 98%.

Isolation of thuggacin A (1) and B (2): Adsorber resin XAD 1180 (3.0 L) and cell mass (1.5 L) were separated from a fermentation broth (300 L) of *Sorangium cellulosum*, strain So ce895 by sieving and were extracted with acetone (18 L) in three portions. After evaporation of the acetone, the remaining water (3 L) was extracted with CH_2Cl_2. Drying and evaporation of the solvent yielded crude material (26.6 g). Partition between methanol (500 mL) and two portions of heptane (500 mL) reduced the weight of the polar material to 15.6 g after evaporation of the methanol. The residue was dissolved in ice-cold ethyl acetate (500 mL) and extracted with 2 portions of a cold aqueous solution of sodium carbonate (1%) and with cold saturated sodium chloride solution. For faster separation of the liquid layers, the mixtures were centrifuged under cooling. The organic layer was dried (sodium sulfate) and evaporated to yield enriched thuggacins (5.3 g). Acidification of the water layer to pH 4.8 and extraction with ethyl acetate recovered a mixture enriched with ambruticins (5.8 g). The thuggacin-containing residue was dissolved in CH_2Cl_2 and transferred to a column of silica gel (200 mL, 0.063–0.200 mm), which was eluted with CH_2Cl_2 (500 mL), CH_2Cl_2/acetone (9:1; 400 mL), CH_2Cl_2/acetone/methanol (90:9:1; 400 mL), CH_2Cl_2/acetone/methanol (90:5:5; 400 mL) and CH_2Cl_2/methanol (9:1; 300 mL). The last fraction provided raw thuggacins (1.5 g), which were separated by RP chromatography (column: 40×530 mm, Eurosil Bioselect C18 100–20, 15–25 Å; solvents: A = water, B = methanol; gradient: 65% B for 76 min, from 65→80% B in 120 min, 80% B for 100 min, from 80→100% B in 60 min; flow: 17 mL min^{-1}; detection: UV absorption at 226 nm) to give thuggacin A (**1**; 244 mg; $t_R = 242$ min) and thuggacin B (**2**; 61 mg; $t_R = 203$ min). Compound **1** was crystallised from diethyl ether/petroleum ether.

Thuggacin A (**1**): M.p. 92–94 °C; $[\alpha]_D^{22} = -148.4$ ($c = 0.4$ in methanol); ^{1}H and ^{13}C NMR: see Table 1; IR (KBr): $\tilde{\nu} = 3417$ (s), 2926 (s) 1707 (s), 1223 (s), 1043 cm^{-1}; UV (methanol): λ_{max} (lg ε) = 224 (4.74), 231, 239 (sh), 289 nm (4.11); EI MS (200 °C): *m*/*z* (%): 631 (6), 614 (14), 613 (29), 595 (21), 577 (17), 560 (6), 546 (6), 542 (7), 528 (6), 506 (8), 460 (13), 418 (15), 388 (11), 355 (11), 348 (15), 320 (16), 304 (30), 296 (21), 278 (19), 268 (38), 267 (55), 251 (21), 250 (100), 249 (65), 248 (14), 224 (15), 223 (10), 222 (20), 221 (17), 206 (12); (−)DCI-MS (isobutane): *m*/*z* (%): 631 (100); (+)DCI-MS (isobutane): *m*/*z* (%): 632 (19), 614 (100), 596 (30); EI-HRMS: calcd for $C_{35}H_{53}NSO_7$: 631.3543; found: 631.3553.

Thuggacin B (2): $[\alpha]_D^{22} = +88.3$ ($c = 1.1$ in methanol); ^{1}H NMR (^{1}H 600 MHz, [D$_6$]DMSO, after H/D exchange, selected data of OH form given in square brackets): $\delta = 7.98$ (s; H3), 7.73 (s; H5), 3.30 (dq, $J = 6.2$, 6.8 Hz; H7), 3.76 (brdt, $J = 6.2$, 4.9 Hz; H8), [4.87 (d, $J = 5.5$ Hz; OH8)], 1.68 (m; H$_a$9), 1.62 (m; H$_b$9), 3.69 (m (with H$_2$O signal); H10), [4.61 (d, $J = 5.6$ Hz; OH10)], 5.63 (dd, $J = 5.5$, 15.3 Hz; H11), 6.10 (dd, $J = 11.1$, 15.3 Hz; H12), 5.96 (t, $J = 11.1$, 11.1 Hz; H13), 5.43 (dt, $J = 5.5$, 10.3 Hz; H14), 2.47 (dt, $J = 14.3$, 10.4 Hz; H$_a$15), 1.93 (ddt, $J = 14.3$, 5.6, 1.9 Hz; H$_b$15), 3.71 (dt, $J = 11.0$, 2.2 Hz; H16), [5.03 (d, $J = 6.1$ Hz; OH16)], 4.92 (dd, $J = 6.6$, 2.5 Hz; H17), 3.85 (dd, $J = 3.4$, 6.8 Hz; H18), [4.47 (d, $J = 5.5$ Hz; OH18)], 1.82 (ddq, $J = 3.4$, 7.2, 6.8 Hz; H19), 3.92 (d, $J = 7.2$ Hz; H20), [4.62 (d, $J = 5$ Hz; OH20)], 5.82 (brs; H22), 5.35 (tq, $J = 1.1$, 6.7 Hz; H24), 1.62 (d, $J = 6.8$ Hz; CH$_3$25), 2.66 (ddd, $J = 6.6$, 8.8, 12.9 Hz; H$_a$26), 2.52 (m (with solvent signal); H$_b$26), 1.34 (m; CH$_2$27), 1.46 (m; CH$_2$28), 1.24 (m; CH$_2$29), 1.24 (m; CH$_2$30), 0.82 (brt, $J = 7.2$ Hz; CH$_3$31), 1.31 (d, $J = 6.8$ Hz; CH$_3$32), 0.87 (d, $J = 6.8$ Hz; CH$_3$33), 1.64 (s; CH$_3$34), 1.69 ppm (s; CH$_3$35); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 167.72$ (s; C1), 133.12 (s; C2), 132.24 (d; C3), 149.69 (s; C4), 119.31 (d; C5), 170.19 (s; C6), 44.33 (d; C7), 70.76 (d; C8), 39.20 (t; C9), 66.27 (d; C10), 138.57 (d; C11), 123.51 (d; C12), 129.55 (d; C13), 128.90 (d; C14), 31.84 (t; C15), 69.96 (d; C16), 77.90 (d; C17), 70.07 (d; C18), 37.07 (d; C19), 77.90 (d; C20), 136.18 (s; C21), 128.90 (d; C22), 133.12 (s; C23), 123.34 (d; C24), 13.48 (q; C25), 27.63 (t; C26), 28.74 (t; C27), 27.63 (t; C28), 31.10 (t; C29), 21.98 (t; C30), 13.91 (q; C31), 17.85 (q; C32), 8.57 (q; C33), 13.62 (q; C34), 16.73 ppm (q; C35); IR (KBr): $\tilde{\nu} = 3422$ (s), 2923 (s), 1706 (s), 1455 (s) 1224, 1126 (s), 1060 cm^{-1} (s); UV (methanol): λ_{max} (lg ε) = 226 (4.70), 231, 240 (sh), 287 nm (4.13); EI-MS (200 °C): *m*/*z* (%): 631 (12), 614 (17), 613 (40), 595 (17), 577 (13), 560 (8), 546 (5), 542 (8), 528 (5), 506 (8), 489 (14), 460 (13), 436 (11), 418 (16), 388 (14), 348 (12), 344 (12), 320 (15), 304 (20), 296 (22), 278 (19), 268 (38), 267 (80), 251 (18), 250 (100), 249 (60), 248 (13), 224 (12), 222 (18), 206 (11); EI-HRMS: calcd for $C_{35}H_{53}NSO_7$: 631.3543; found: 631.3535; calcd for $C_{35}H_{51}NSO_6$: 613.3437; found: 613.3434.

Thuggacin C (3): Compound **3** was isolated from a thuggacin (50 mg) equilibrium mixture after rearrangement at room temperature in methanol by preparative RP-HPLC (column: 22×250 mm, Nucleodur 100–10 C18 EC (Macherey-Nagel), 10 Å; solvent A: 50% methanol, solvent B: methanol; gradient: 40% B (70% MeOH) for 15 min, from 40→60% B (80% MeOH) in 45 min, 60% B for 20 min; flow: 18 mL min^{-1}; detection: UV absorption at 225 nm). The thuggacin fractions were acidified with 1–2 drops of acetic acid, evaporated and the remaining water was extracted twice with dichloromethane. After evaporation of the solvent, the samples were dried under high vacuum to give thuggacin B (**2**; 11 mg; $t_R = 42$ min), thuggacin A (**1**; 7.4 mg; $t_R = 60$ min) and thuggacin C (**3**; 8.6 mg; $t_R = 65$ min). $[\alpha]_D^{22} = -35.7$ ($c = 0.7$ in methanol); ^{1}H and ^{13}C NMR: see Table 1; UV (methanol): λ_{max} (lg ε) = 228 (4.555), 286 nm (4.054); EI-HRMS: calcd for $[C_{35}H_{53}NSO_7+H]^+$: 632.3615; found: 632.3623.

13-Methyl-thuggacin A (4): Compound **4** was isolated from the fraction (38 mg) eluting directly after **1** during RP-MPLC of the thuggacin-containing crude extract from a fermentation (60 L) of strain So ce895. The fraction was purified further by RP-HPLC with a gradient of 80→90% methanol in 15 min on a column (250×21 mm) of Nucleodur 100–10 C18 (Macherey-Nagel). The main peak (UV detection at 232 nm) of two injections was collected and evaporated to give **4** (16 mg). $[\alpha]_D^{22} = -127.7$ ($c = 0.94$ in methanol); ^{1}H NMR (^{1}H 600 MHz, [D$_6$]acetone): $\delta = 7.98$ (s; H3), 7.69 (s; H5), 3.53 (m; H7), 3.64 (brm; H8), 1.91 (ddd, $J = 14.6$, 9.4, 3.6 Hz; H$_a$9), 0.80 (m; H$_b$9), 5.21 (m; H10), 5.54 (dd, $J = 15.5$, 9.1 Hz; H11), 6.71 (d, $J = 15.5$ Hz; H12), 1.68 (t, $J = 0.9$ Hz; CH$_3$13), 5.34 (dd, $J = 11.3$, 5.3 Hz; H14), 3.05 (ddd, $J = 14.5$, 11.3, 11.1 Hz; H$_a$15), 2.29 (m, $J = 5.2$, 1.4, 1.4 Hz; H$_b$15), 5.06 (brd, $J = 10.6$ Hz; H16), 3.81 (m; H17 and H18), 2.15 (ddd, $J = 6.9$, 4.6, 2.1 Hz; H19), 4.18 (brs; H20), 5.95 (brs; H22), 5.32 (m; H24), 1.64 (d, $J = 6.8$ Hz; H25), 2.61 (ddd, $J = 13.1$, 8.4, 7.2 Hz; H$_a$26), 2.43 (ddd, $J = 13.1$, 8.0, 6.8 Hz; H$_b$26), 1.33 (m, CH$_2$27), 1.27 (m, CH$_2$28, CH$_2$29 and CH$_2$30), 0.87 (t, $J = 6.9$ Hz; CH$_3$31), 1.56 (d, $J = 6.8$ Hz; CH$_3$32), 0.89 (d, $J = 6.8$ Hz; CH$_3$33), 1.75 (d, $J = 1.1$ Hz; CH$_3$34), 1.71 ppm (t, $J = 1.0$ Hz; CH$_3$35); ^{13}C NMR (150 MHz, [D$_6$]acetone): $\delta = 167.14$ (s; C1), 132.79 (s; C2), 133.84 (d; C3), 150.40 (s; C4), 118.32 (d; C5), 171.95 (s; C6), 44.51 (d; C7), 77.00 (d; C8), 40.57 (t; C9), 71.52 (d; C10), 133.93 (d; C11), 127.13 (d; C12), 134.51 (s; C13), 21.07 (q; C13-CH$_3$), 127.40 (d; C14), 30.53 (t; C15), 74.72 (d; C16), 73.37 (d; C17), 74.41 (d; C18), 37.48 (d; C19), 79.25 (d; C20), 135.79 (s; C21), 128.80 (d; C22), 133.77 (s; C23), 123.34 (d; C24), 13.21 (q; C25), 27.43 (t; C26), 28.47 (t; C27), 29.92 (t; C28), 31.96 (t; C29), 22.70 (t; C30), 13.79 (q; C31), 17.32 (q; C32), 6.65 (q; C33), 14.96 (q; C34), 16.47 ppm (q; C35); UV (methanol): λ_{max} (lg ε) = 227 (4.622), 286 nm (4.038); EI-MS: (200 °C): *m*/*z* (%): 645 (9), 627 (34), 609 (18), 591 (19), 384 (9), 320 (16), 304 (26), 267 (60), 250 (100); (−)DCI-MS (NH$_3$): *m*/*z* (%): 645 (100); (+)DCI-MS (NH$_3$): *m*/*z* (%): 646 (100); EI-HRMS: calcd for $C_{36}H_{55}NSO_7$: 645.3699; found: 645.3649; calcd for $C_{36}H_{53}NSO_6$: 627.3593; found: 627.3573.

Isolation of thuggacins from extracts of *Chondromyces crocatus*: The crude acetone extract of cells, which were harvested from the fermentation broth (690 L) of strain Cm c5 by centrifugation, was evaporated to give an oily residue (about 180 g), which was subjected to a methanol/

heptane partition (methanol (3% water; 800 mL) and heptane (600 mL) in three portions) in order to remove lipophilic byproducts. Oily material (about 65 g) was recovered after evaporation of the methanol layer. Large-scale RP chromatography with a stepwise gradient of 75, 80, 85 and 100% MeOH provided fractions that were combined according to HPLC analyses. The first fraction, eluted with 75% MeOH, contained the polar compounds (for example, the chondramides) and thuggacins and was followed by a fraction containing chondrochlorens. The first fraction (about 8.6 g) was again separated by RP chromatography with a solvent gradient of between 50 and 70% aqueous methanol containing 0.2% acetic acid. After HPLC analyses, similar fractions were combined and provided three consecutive fractions containing different thuggacin variants (I ($\approx$70 mg), II (280 mg) and III (220 mg)), which eluted between the more polar chondramides and more lipophilic chondrochlorens. These fractions were separated further by LH-20 chromatography (solvent: methanol) to give thuggacin cmc-B ($\approx$30 mg) from I.[28] From the LH-20 fractions of II and III, a further separation by silica gel PSC provided thuggacin cmc-C (**6**; 38 mg) and cmc-A (**5**; 70 mg), respectively.

Analytical RP-HPLC: Column: 125×2 mm, Nucleosil C18, precolumn 10×2 mm; solvent A: water, solvent B: methanol; gradient: 60% B for 3 min, 60→70% B in 6 min, 70→90% B in 3 min, 90→100% B in 3 min, 100→60% B in 0.5 min, 60% B for 3 min; flow: 0.3 mL min^{-1}; detection: UV absorption at 220–224 nm; $t_R \approx 8.3$–9.5 min for thuggacin cmc-B; $t_R \approx 9.9$–10.9 min for thuggacin cmc-C; $t_R \approx 10.5$–11.6 min for thuggacin cmc-A.

Thuggacin cmc-A (5): $[\alpha]_D^{22} = -160.6$ ($c = 1.24$ in methanol); ^{1}H and ^{13}C NMR: see Table 2; UV (methanol): λ_{max} (lg ε) = 224 (4.672), 285 nm (4.085); EI-MS (200 °C): *m*/*z* (%): 561 (37), 543 (66), 525 (22), 452 (24), 382 (23), 294 (27), 196 (62), 178 (100); (−)DCI-MS (NH_3): *m*/*z* (%): 561 (100); EI-HRMS: calcd for $C_{30}H_{43}NSO_7$: 561.2760; found: 561.2894; calcd for $C_{30}H_{41}NSO_6$: 543.2654; found: 543.2649.

Thuggacin cmc-C (6): $[\alpha]_D^{22} = -47.4$ ($c = 0.5$ in methanol; ^{1}H and ^{13}C NMR: see Table 2; UV (methanol): λ_{max} (lg ε) = 227 (4.640), 285 nm (4.135); EI-MS (200 °C): *m*/*z* (%): 561 (28), 543 (63), 525 (14), 452 (24), 382 (26), 294 (27) 178 (100); (−)DCI-MS (isobutane): *m*/*z* (%): 561 (100); (+)DCI-MS (isobutane): *m*/*z* (%): 562 (100), 544 (74); EI-HRMS: calcd for $C_{30}H_{43}NSO_7$: 561.2760; found: 561.2802; calcd for $C_{30}H_{41}NSO_6$: 543.2654; found: 543.2646.

Acknowledgements

We thank S. Knöfel, N. Dankers and K. Schober for their skilful assistance in the isolation work, A. Roß, H. Schüler, R. Krützfeld and coworkers for their contribution in the large-scale fermentation and downstream processing, B. Jaschok-Kentner and C. Kakoschke for recording the NMR spectra, R. Christ for measuring the mass spectra and S. Rüsch-Gerdes, Research Centre Borstel (National Reference Centre for Mycobacteria), for testing thuggacin A against *Mycobacterium tuberculosis*.

[1] J. D. McKinney, *Nat. Med.* **2000**, *6*, 1330–1333.

[2] a) Z. Xie, N. Siddiqi, E. J. Rubin, *Antimicrob. Agents Chemother.* **2005**, *49*, 4778–4780; b) E. Weinstein, T. Yano, L.-S. Li, D. Avarbock, D. Helm, A. A. McColm, K. Duncan, J. T. Lonsdale, H. Rubin, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 4548–4553.

[3] H. Irschik, R. Jansen, G. Höfle, H. Reichenbach, unpublished results.

[4] a) D. T. Connor, R. C. Greenough, M. von Strandtmann, *J. Org. Chem.* **1977**, *42*, 3664–3669; b) D. T. Connor, M. von Strandtmann, *J. Org. Chem.* **1978**, *43*, 4606–4607.

[5] G. Höfle, H. Steinmetz, R. Jansen, K. Gerth, H. Reichenbach, *Liebigs Ann. Chem.* **1991**, 941–945.

[6] Test method: BACTEC 460TB; S. Rüsch-Gerdes, Research Centre Borstel, National Reference Centre for Mycobacteria, personal communication.

[7] Carbon shifts are given with two decimal places in order to enable easier discrimination between close signals, although the last value may vary between repeated NMR spectroscopic measurements.

[8] Compared to the equivalent signal in unsubstituted thiazole, the signal of C6 in **1** is shifted from $\delta = 153$ to 171.24 ppm due to the substitution effects by the larger structural element. Carbon C4 on the other side is only moderately shifted from $\delta = 143$ to 149.04 ppm due to substitution by the double bond.

[9] Due to a very similar ^{1}H chemical shift for the singlets of the methine protons on C5 and C3, a mutual long-range correlation cannot be detected in the ^{1}H,^{1}H-COSY spectrum. The allylic coupling constant $J_{3,5}$ is expected be very small and not visible in the 1D NMR spectrum because the protons are in one plane.

[10] From repeated experiments, it was recognised that the reaction time depends on the quality of the starting material and solvent. Recrystallised material needed longer reaction times.

[11] M. A. Rangelov, G. N. Vayssilov, D. D. Petkov, *Int. J. Quantum Chem.* **2006**, *106*, 1346–1356, and references cited therein.

[12] M. A. Rashid, C. L. Cantrell, K. R. Gustafson, M. R. Boyd, *J. Nat. Prod.* **2001**, *64*, 1341–1344.

[13] Therein, the hexyl chain was replaced by a hydrogen atom.

[14] a) R. Jansen, B. Kunze, H. Reichenbach, G. Höfle, *Liebigs Ann.* **1996**, 285–290; b) B. Kunze, R. Jansen, F. Sasse, G. Höfle, H. Reichenbach, *J. Antibiot.* **1995**, *48*, 1262–1266; c) F. Sasse, B. Kunze, T. M. A. Gronewold, H. Reichenbach, *J. Natl. Cancer Inst.* **1998**, *90*, 1559–1563.

[15] a) R. Jansen, P. Washausen, B. Kunze, H. Reichenbach, G. Höfle, *Eur. J. Org. Chem.* **1999**, 1085–1089; b) B. Kunze, R. Jansen, G. Höfle, H. Reichenbach, *J. Antibiot.* **1994**, *47*, 881–886.

[16] a) R. Jansen, B. Kunze, H. Reichenbach, G. Höfle, *Eur. J. Org. Chem.* **2002**, 917–921; b) B. Kunze, R. Jansen, G. Höfle, H. Reichenbach, *J. Antibiot.* **2004**, *57*, 151–155.

[17] R. Jansen, B. Kunze, H. Reichenbach, G. Höfle, *Eur. J. Org. Chem.* **2003**, 2684–2689.

[18] For comparison with the respective carbon atoms in thuggacins A–C, the numbers 27–31 are skipped in thuggacins cmc-A and cmc-C.

[19] W. Trowitzsch-Kienast, V. Wray, K. Gerth, H. Reichenbach, G. Höfle, *Liebigs Ann. Chem.* **1986**, 93–98.

[20] % Enrichment = 1.1% × (I_l/I_n) − 1.1% (I_l: signal intensity of labelled compound, I_n: natural signal intensity); due to the 98–99% ^{13}C content of the precursors, the enrichment nearly equals the specific incorporation rate [(% enrichment) × 100/(% enrichment of the precursor)].

[21] Carbon atoms in labelled positions had a smaller singlet in the centre of a doublet from one-bond coupling within the former labelled acetate units. The doublets showed a small isotope shift.

[22] The signals of natural abundance ^{13}C carbon atoms could not be detected completely.

[23] a) N. Bedorf, D. Schomburg, K. Gerth, H. Reichenbach, G. Höfle, *Liebigs Ann. Chem.* **1993**, 1017–1021; b) K. Gerth, N. Bedorf, H. Irschik, G. Höfle, H. Reichenbach, *J. Antibiot.* **1994**, *47*, 23–31.

[24] a) H. Irschik, R. Jansen, G. Höfle, H. Reichenbach, *J. Antibiot.* **1987**, *40*, 7–13; b) R. Jansen, H. Irschik, H. Reichenbach, D. Schomburg, V. Wray, G. Höfle, *Liebigs Ann. Chem.* **1989**, 111–119.

[25] a) G. Höfle, N. Bedorf, H. Steinmetz, D. Schomburg, K. Gerth, H. Reichenbach, *Angew. Chem.* **1996**, *108*, 1671–1673; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1567–1569; b) K. Gerth, N. Bedorf, G. Höfle, H. Irschik, H. Reichenbach, *J. Antibiot.* **1996**, *49*, 560–563; c) G. Höfle, H. Reichenbach in *Anticancer Agents from Natural Products* (Eds.: G. M. Cragg, D. G. I. Kingston, D. J. Newman), CRC Press, Taylor & Francis, Boca Raton, **2005**, 413–450.

[26] Y. Zhang, K. Post-Martens, S. Denkin, *Drug Discovery Today* **2006**, *11*, 21–25.

[27] E. A. Campbell, O. Pavlova, N. Zenkin, F. Leon, H. Irschik, R. Jansen, K. Severinov, S. A. Darst, *EMBO J.* **2005**, *24*, 674–682.

[28] The sample of thuggacin cmc-B underwent rearrangement during spectroscopy. Thus, only a ^{1}H NMR spectrum and a nominal molecular mass (*m*/*z*: 561 [*M*−H]$^-$) were obtained from a pure sample. The shifts of the ^{1}H NMR spectroscopic signals for H3 and H5 are

characteristic of thuggacin variants with different lactone ring sizes. Comparable to the signals for thuggacin B ($\delta = 8.23$ and 7.65 ppm), the corresponding singlet signals of thuggacin cmc-B are at $\delta = 8.29$ and 7.78 ppm in [D_4]methanol. Furthermore, the lactone H17 signal of thuggacin cmc-B at $\delta = 5.05$ ppm (dd, $J = 7.5$, 1.6 Hz, 1H) can be compared with the corresponding signal at $\delta = 5.07$ (dd, $J = 7.7$, 1.7 Hz, 1H) for thuggacin B (**2**).

Received: February 15, 2007
Published online: May 15, 2007

