Total Synthesis

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Chondramide C: Synthesis, Configurational Assignment, and Structure–Activity Relationship Studies**

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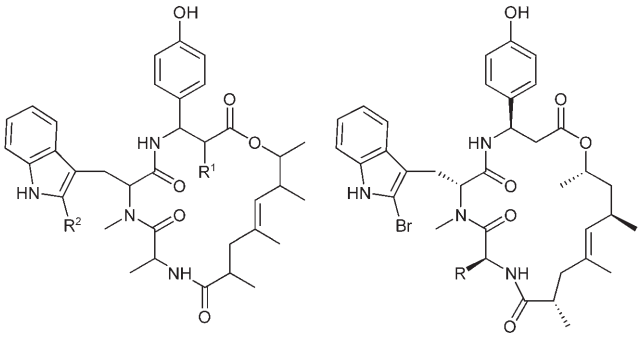
In 1995 the groups of Reichenbach and Höfle isolated the family of chondramides.[1] Three years later it was reported that chondramides induce the polymerization of G-actin.[2] In contrast, other natural products isolated from myxobacteria such as rhizopodin[3] or chivosazol[4] lead to destabilization and reorganization of actin filaments. In both cases a collapse of actin-dependent cellular processes is the result. Additionally, cytokinesis at the end of cell division cannot be completed and enlarged polynuclear cells develop.

The structure elucidation unraveled an 18-membered macrocycle without providing the configuration of the six stereocenters included. The family of chondramides is characterized by different substituents at R^1 and R^2 (Scheme 1). Compared to the structurally related jaspamide (jasplakinolide) an obvious similarity is the amino acid sequence of the tripeptide. The apparent concurrence was reinforced by the fact that the β-tyrosine subunit has the same configuration as in jaspamide.[5] One significant difference compared to jaspamide is the ring size (18- versus 19-membered) and a different secondary hydroxy group in the polyketide segment of chondramide. Parallel to our contribution the group of Waldmann and Arndt completed the total synthesis of chondramide C.[6]

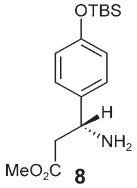
First members of the jaspamide family were isolated by Zabriskie et al.,[7] Crews et al.,[8] and Braekman et al.[9] and attracted significant attention due to their ability to induce the polymerization of the actin skeleton.[10] A thorough investigation of their anti-proliferative activity revealed activity against 36 solid tumors,[11] and consequently initiated a variety of synthetic contributions.[12] Besides total synthesis, research groups targeted simplified jaspamide analogues. Riccio et al.[13] substituted the polyketide segment by an amino acid linker. In a different approach, Maier et al.[14] investigated the effect of allylic strain by impeding the preferred conformation with the aid of arenes. Nevertheless, all synthetic derivatives showed reduced biological activities. Recently, extended structure–activity relationship (SAR) studies became available when Zampella et al.[15] isolated additional natural jaspamide derivatives from *Jaspis splendans*. Nevertheless, a detailed investigation of the structural significance of the polyketide segment of the jaspamides–chondramides family still has not been reported.



Scheme 1. Members of the chondramide–jaspamide family.

Based on the structural similarities of the two classes we started a synthesis program aimed at providing derivatives of chondramide C that would help to determine the absolute configuration of the chondramides and to provide first SAR data. Furthermore, we proposed that the polyketide segment determines the conformation of the tripeptide and is thus the pivotal structural element for the biological activity.

The synthesis of the tripeptide segment is based on procedures published in the context with the jaspamide synthesis.[7,16,17] and commenced with compound **8** (Scheme 2).[16] The synthesis of tripeptide **14** has been described for jaspamide by Kocienski et al. and provides with exception of the bromination step the basis for the tripeptide synthesis of chondramide C (Scheme 3). The adjustments necessary for our synthesis include the synthesis of **10** according to Hirai et al.[18] Here, in particular the use of DMF as the solvent in the methyl-



Scheme 2. The β-tyrosine unit **8**. TBS = *tert*-butyldimethylsilyl.

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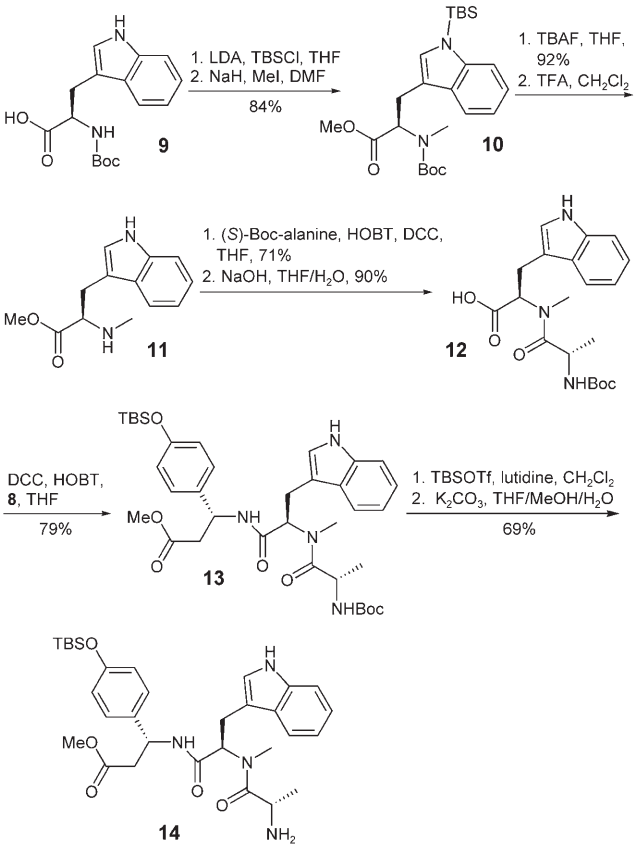


Scheme 3. Synthesis of the tripeptide of chondramide C based on the route devised by Kocienski et al.[16] Boc = *tert*-butoxycarbonyl, DCC = *N*,*N*′-dicyclohexylcarbodiimide, HOBT = 1-hydroxy-1*H*-benzotriazole, LDA = lithium diisopropylamide, OTf = trifluoromethanesulfonate, TBAF = tetrabutylammonium fluoride, TFA = trifluoroacetic acid.

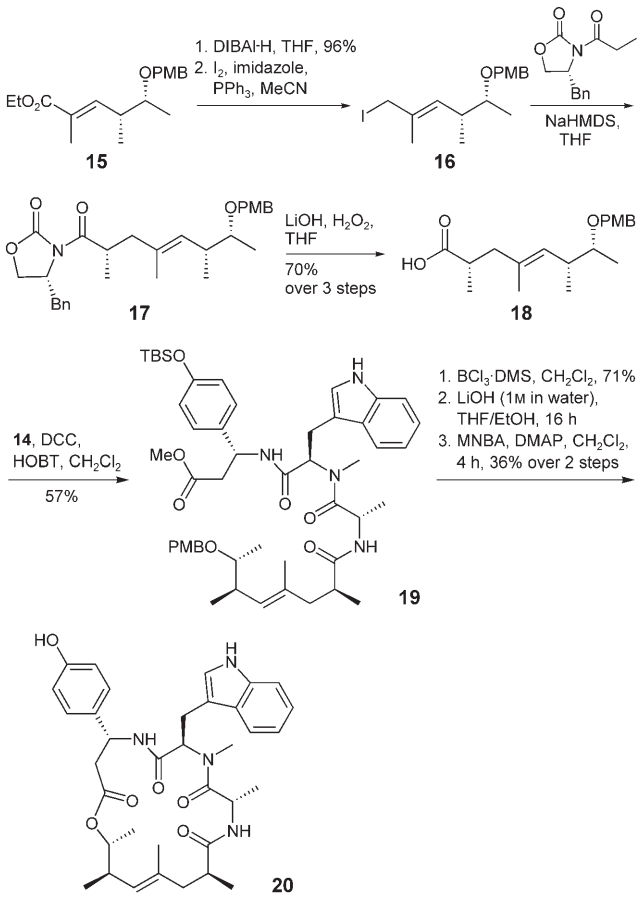
ation step increases the yields to 84% over two steps. To improve the non-reproducible yield in the deprotection step (**13**→**14**) the sequence reported by Grieco et al.[12e] was employed. In this two-step sequence, intermediate **13** was first protected with TBS and then treated with two equivalents of K_2CO_3 in THF/MeOH/H_2O (2:1:1) at room temperature to provide the liberated amine **14** in good yield.

The synthesis of the polyketide segment commences with a *syn*-selective crotyl boration based on the protocol devised by Brown et al.,[19,20] followed by protection by PMB and ozonolysis. The resulting aldehyde was subjected to a Wittig olefination and provided unsaturated ester **15**. Reduction with DIBAl-H and treatment with iodine, imidazole, and PPh_3 provided the iodide **16**, which was employed in an Evans alkylation to furnish **17**. Liberation of the acid can be achieved under standard conditions by using LiOH and H_2O_2 (Scheme 4).

Tripeptide **14** was then coupled with acid **18** using DCC and HOBT and the PMB group was removed by using BCl_3. Remarkably, attempts to perform this transformation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) failed and provided oxidized tryptophan as the sole product. Liberation of the corresponding seco acid was achieved by using LiOH in EtOH/THF/H_2O. Initial experiments to achieve the macrocyclization failed and to exclude the possibility that the observed problems were due to unfavorable isomers we optimized the cyclization protocol on the seco acid derived from authentic chondramide C.

The macrolactonization provided unexpected challenges because established protocols such as the Keck–Boden method,[21] the Yamaguchi lactonization,[22] as well as the Mukaiyama or Mitsunobu reaction[23] proved unsuccessful. Other strategies such as transesterification using [(nBu$_2$ClSn)O] according to Giannis et al. and Otera et al.[24] were similarly unsuccessful. Fortunately, the Shiina[25] method using 2-methyl-6-nitrobenzoic anhydride (MNBA) to activate the carboxylic acid provided acceptable yields of all four stereoisomers (Scheme 4). Following the route described above, the diastereomers differing in the configurations of the polyketide segment were obtained and comparison of their NMR spectra clearly identified isomer **20** to be identical with natural chrondramide C.

With these four isomers in hand we began to investigate their biological activity. By comparing their anti-proliferative effects with the ability to induce the polymerization of actin, valuable information can be obtained not only in terms of SAR studies, but also as to whether an additional cellular



Scheme 4. Synthesis of the polyketide segment **18** and coupling with the tripeptide. Bn = benzyl, DIBAl-H = diisobutylaluminum hydride, DMAP = 4-dimethylaminopyridine, DMS = dimethyl sulfide, MNBA = 2-methyl-6-nitrobenzoic anhydride, NaHMDS = sodium hexamethyldisilazanide, PMB = *para*-methoxybenzyl.

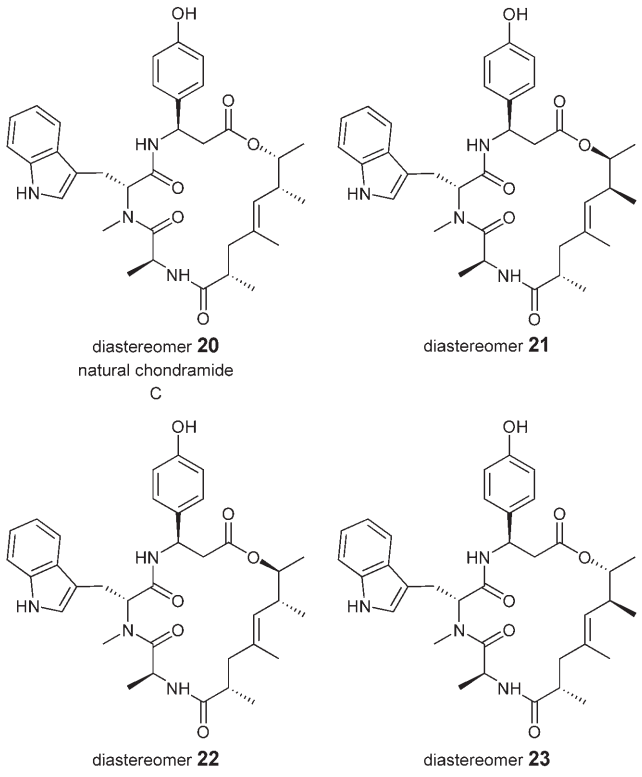
Table 1: Antiproliferative activity of **20**, **21**, **22**, and **23** on different mammalian cell lines, recorded as IC_{50} values in nM.[a]

Cell line	Origin	**20**	**21**	**22**	**23**
L-929	murine connective tissue fibroblasts	55 ± 24	81 ± 6	1670 ± 390	2400 ± 115
A-431	human epidermoid carcinoma	55 ± 5	49 ± 3	620 ± 280	1200 ± 45
A-498	human kidney carcinoma	24 ± 3	32 ± 2	500 ± 130	920 ± 270
A-549	human lung carcinoma	26 ± 5	23 ± 2	510 ± 195	930 ± 60
SK-OV-3	human ovary adenocarcinoma	16 ± 5	19 ± 2	320 ± 60	1040 ± 160

[a] Data are means ± standard deviations of two independent IC_{50} value determinations.

target might be addressed that at least in part would account for the observed biological activity.

To assess the anti-proliferative effects of chondramide C, five different cell lines were investigated. As shown in Table 1 diastereomer **21** had a strong growth inhibitory activity on cultured mammalian cells. The data obtained were nearly the same as those observed for the natural chondramide C (**20;** Scheme 5) isolated from *Chondromyces crocatus*. The diastereomers **22** and **23** were clearly less efficacious in inhibiting cell propagation; on the average their IC_{50} values were 20 and 36 times higher, respectively.



Scheme 5. Chondramide C diastereomers.

In a subsequent set of experiments the ability of chondramide C to induce actin polymerization was determined. Figure 1 shows that the natural compound **20** is more efficacious than the other isomers, but also in these cell-free experiments, diastereomer **21** was more active than **22** and **23**. Polymerization curves with different concentrations of the diastereomers (see Supporting Information) gave the following approximate EC_{50} values: 2 (**20**), 3 (**21**), 5 (**22**), and 6 μM (**23**). The EC_{50} data are related to the maximum value obtained by a sigmoidal fit of the polymerization curve with **20** (40 μM). As shown in Figure 1 with different concentrations of **20,** the relation between the concentration

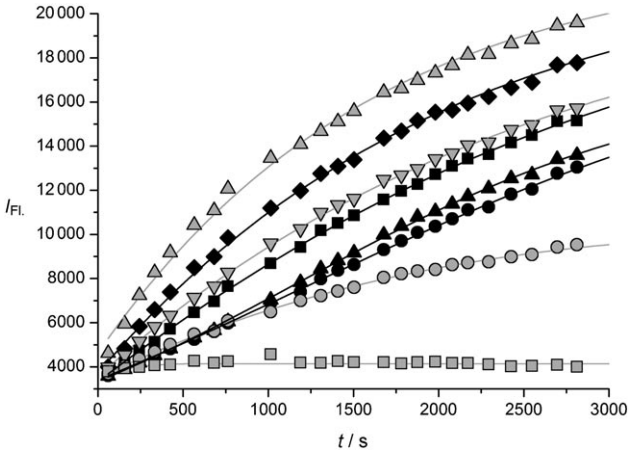


Figure 1. Induction of G-actin polymerization by chondramide. Pyrene-actin in a nonpolymerizing buffer was incubated with chondramide C diastereomers, and actin polymerization was monitored by measuring increasing fluorescence. Each diastereomer was applied at a concentration of 10 μM (depicted by black symbols: ◆ **20**, ■ **21**, ▲ **22**, and ● **23**). For chondramide C the induction efficacy at additional concentrations is shown (gray symbols: ▲ 40 μM, ▼ 5 μM, and ● 1 μM; ■ methanol).

and the percentage of polymerization induction is strongly exponential. This has to be taken into account when comparing the EC_{50} data.

With isomer **21** at a concentration of 160 nM, we clearly observed effects on the actin cytoskeleton of kidney cancer cells (Figure 2). Reorganization of the actin structures of the cells during mitosis seemed to be impaired, and in the interphase cells stronger stress fibers, spots, and flakes of F-actin were formed. After 18 h the first cells with two nuclei were observed. We could also demonstrate these phenotypes with diastereomer **22** but only at much higher concentration (8 μM).

The superimposition of the four diastereomers shows that **20** and **21** exhibit similar conformations in the peptidic part even though two of the three configuration centers in the polyketide segment display opposite configurations. On the other hand the conformations of **22** and **23** deviate significantly from those of **20** and **21** (Figure 3).

The unexpected similar biological profile of compounds **20** and **21** can be rationalized by a similar geometric constrain imposed by the polyketide segment of both isomers. Scheme 6 depicts the conformation of the segments incorporated, and it can be seen that the torsion angles and distances in **20** and **21**

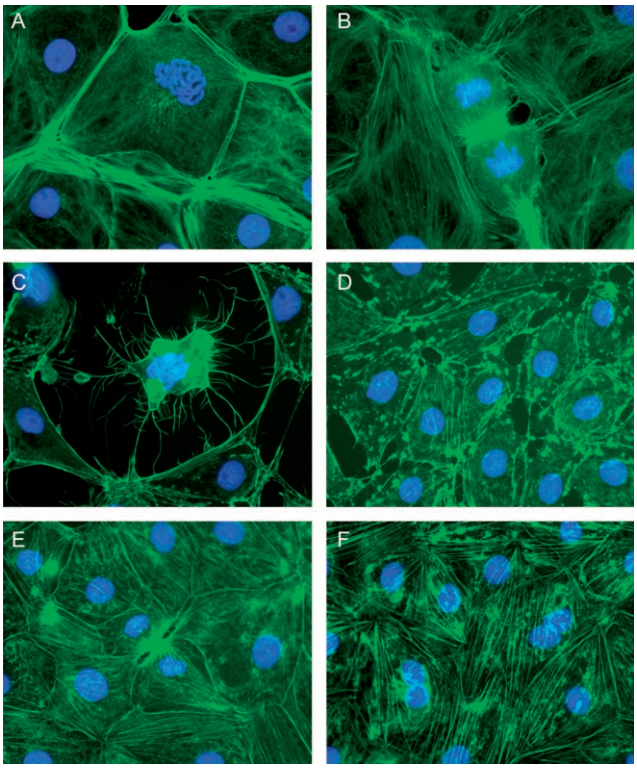


Figure 2. Influence of **21** on the actin cytoskeleton of A-498 kidney cancer cells after different incubation times. F-actin was labeled green, nuclei and chromosomes blue. A, B: control cells with a mitotic cell in the center; metaphase (A), and telophase (B). Cells that were incubated with **21** (160 nM) showed abnormal metaphase cells (C, after 2 h), and a strengthened contractile ring in the late telophase (E, after 18 h). Spots of F-actin became visible especially at focal adhesion points (D, after 4 h), stress fibers became stronger and flakes of actin appeared (E and F, after 18 h).

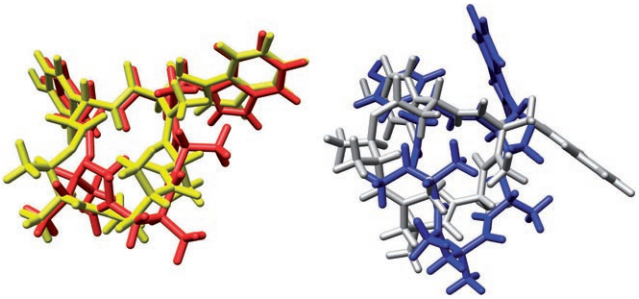
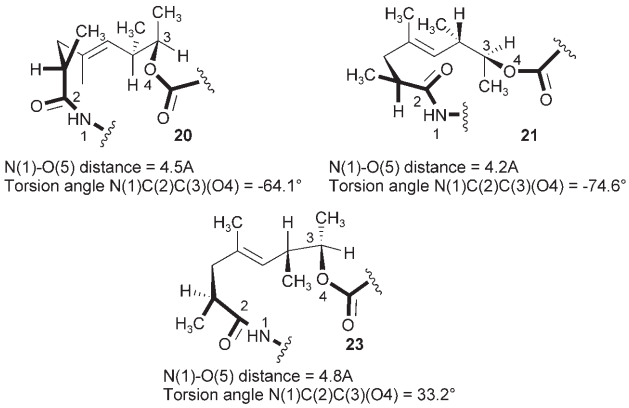


Figure 3. Superimposition of the chondramide C isomers. **20** red, **21** yellow, **22** gray, **23** blue. For compounds **20** and **21** the tripeptide has the same conformation.

are similar to each other but significantly different from the values for **23**.

In summary, we have reported on the total synthesis of chondramide C and its absolute configuration. The synthesis of three additional diastereomers enabled first SAR studies, in which the ability to induce actin polymerization was compared with the anti-proliferative effects. The results show that the potential antitumor activity is derived through interaction with the actin skeleton. Surprisingly, two compounds, the natural product **20** and isomer **21** exhibit nearly the same biological activity. This can be explained through a conformational analysis of the isomers, which clearly shows that **20** and **21** exhibit nearly the same conformation of the tripeptide. This analysis clearly supports the hypothesis that the polyketide segment acts as a structural element that performs a fine-tuning on the conformation of the amino acids in the molecule.



Scheme 6. Polyketide segments of compounds **20**, **21**, and **23**.

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