Drug Design

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Total Synthesis of Chondramide C and Its Binding Mode to F-Actin**

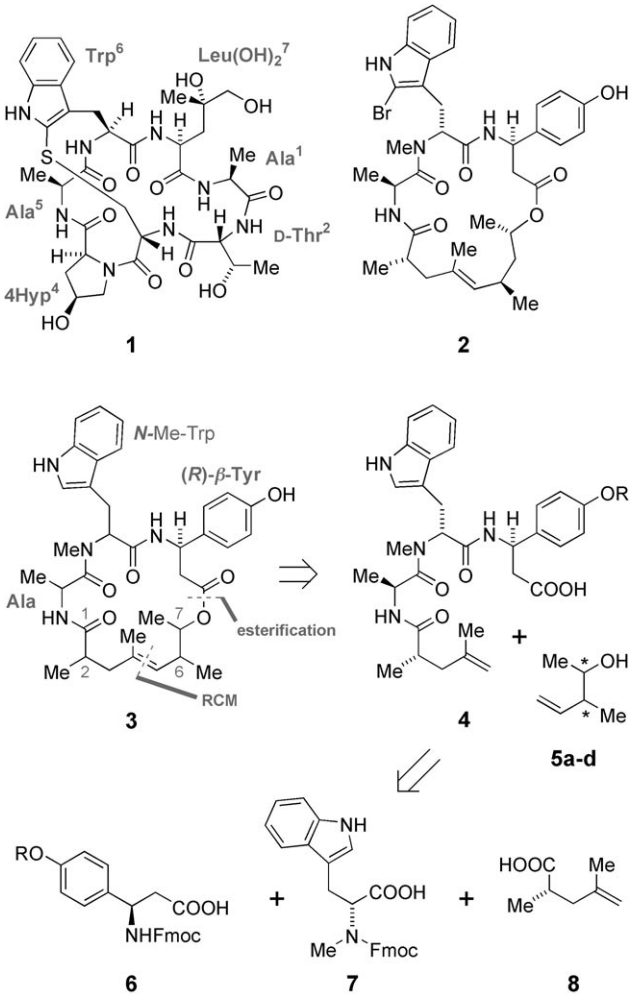
Herbert Waldmann, Tai-Shan Hu, Steffen Renner, Sascha Menninger, René Tannert, Toshiro Oda, and Hans-Dieter Arndt**

Dedicated to Professor Reinhard W. Hoffmann on the ooccasion of his 75th birthday

The actin cytoskeleton maintains the cell's shape and is essential for cell movement, phagocytosis, and cytokinesis.[1] Small molecules that interfere with the dynamic assembly and disassembly of actin have hence proven to be invaluable tools for chemical biology and medicinal chemistry research.[1a,2] In particular, natural products have been uncovered that either inhibit or induce F-actin polymer formation from the monomeric G-actin and thereby modulate the maintenance of the cytoskeleton.[1a,2]

Phalloidin (**1**),[3] jasplakinolide (**2**),[4] and chondramide C (**3**)[5] (Scheme 1) stabilize F-actin by a similar mode of action.[6] In contrast to **1**,[7] jasplakinolide (**2**) and chondramide C (**3**) are cell-permeable and display potency against tumor cell lines which renders them interesting target structures for drug discovery. While several total syntheses of **2** have been described,[8] the commercially not available 18-membered ring cyclodepsipeptide **3** has not been prepared so far, and its stereochemistry had remained unresolved. Here we unveil a successful total synthesis of chondramide C (**3**) that has allowed us to easily access diastereoisomers and firmly assign the configuration of all its stereogenic centers.[9] Furthermore, initial biological investigations and results of computationally docking phalloidin (**1**) and chondramide C (**3**) to its molecular target site on F-actin are reported.[6]

In a retrosynthetic sense it was planned to synthesize **3** via the peptide acid **4** from acids **6**–**8**, Fmoc-Ala-OH (**9**), and homoallylic alcohols **5a**–**d** (Scheme 1). For ease of operation and synthetic flexibility we envisioned assembling the *N*-acylated tripeptide **4** on solid support. Ring-closing metathesis (RCM) as the key step could provide the 18-membered cyclodepsipeptide ring after esterification of the released peptide acid **4** with alcohols **5a**–**d**. Such a strategy would allow all building blocks to be varied and pave the way for a synthesis of a diverse collection of chondramide C analogues.[10] The structural similarity of **3** and **2** as well as



Scheme 1. Structures of phalloidin (**1**), jasplakinolide (**2**), and chondramide C (**3**), and retrosynthetic disconnection of chondramide C. Important amino acids are annotated. R = TIPS (triisopropylsilyl); Fmoc = 9-fluorenylmethoxycarbonyl.

[*] Prof. Dr. H. Waldmann, Dr. T.-S. Hu, Dr. S. Renner, S. Menninger, R. Tannert, Dr. H.-D. Arndt
Max-Planck-Institut für Molekulare Physiologie
Abteilung Chemische Biologie
Otto-Hahn-Strasse 11, 44227 Dortmund (Germany)
Fax: (+49) 231-133-2499
E-mail: herbert.waldmann@mpi-dortmund.mpg.de
hans-dieter.arndt@mpi-dortmund.mpg.de
and
Technische Universität Dortmund, Fakultät Chemie
Otto-Hahn-Strasse 6, 44221 Dortmund (Germany)
Dr. T. Oda
RIKEN, SPring-8 Center, Structural Physiology Research Group
Kouto 1-1-1, Sayo (Japan)

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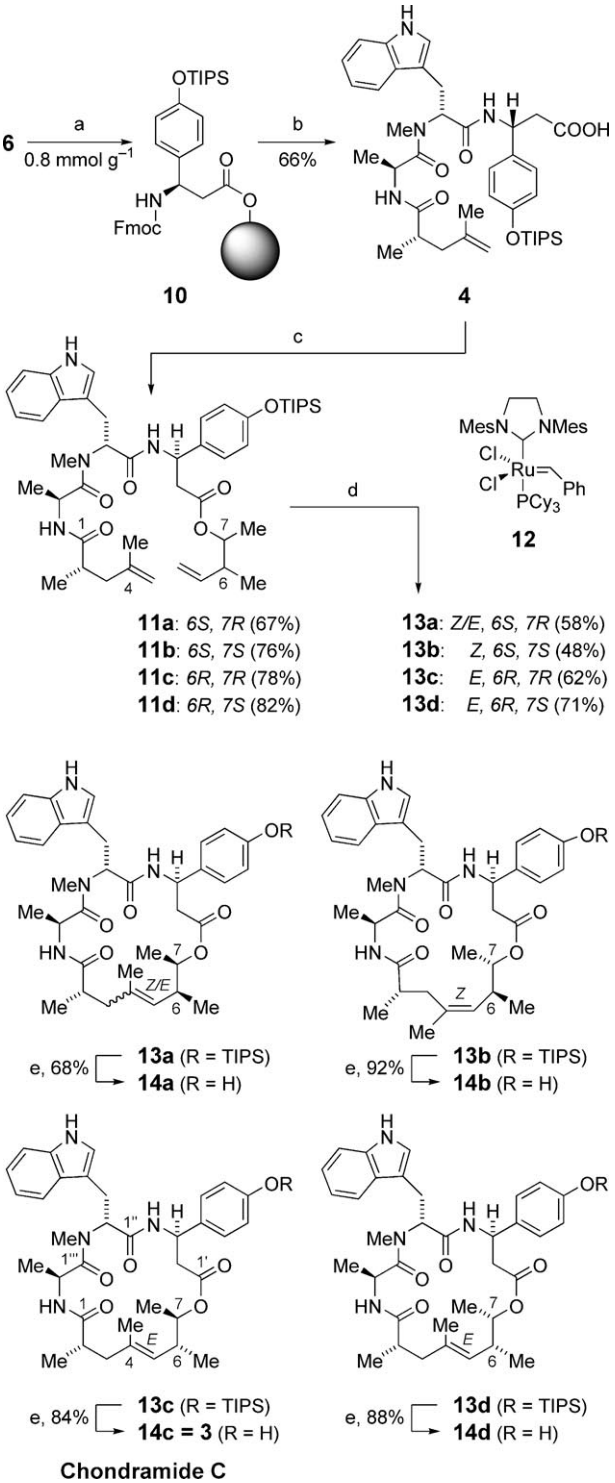
biosynthetic considerations[11] suggested that the stereogenic centers in the peptide fragment and C2 might feature the same absolute configurations. Consequently (*R*)-β-tyrosine,[11] D-*N*-methyl tryptophan (D-abrine), and L-alanine as well as *S*-configured acid **8** were chosen, whereas the configuration of the two stereocenters of the secondary alcohols **5** were unclear. Therefore our synthetic work began with the preparation of all four stereoisomers **5a–d** using Brown's asymmetric crotylboration methodology.[12] *O*-TIPS-protected (*R*)-Fmoc-β-tyrosine **6** was synthesized by a diastereoselective addition of (*S*)-*N*-benzyl-1-phenylethylamine[13] to the corresponding cinnamic acid benzyl ester followed by hydrogenolysis and Fmoc-introduction (Supporting Information).[8e] Fmoc-D-abrine **7** was obtained from D-tryptophan by two reductive aminations (PhCHO, HCHO)[14] and protecting group exchange (Supporting Information). Acid **8** was prepared by stereoselective alkylation using Seebach's oxazolidinone.[15] The *S*-configuration of the newly formed stereocenter of **8** was unambiguously deduced from an X-ray crystal structure analysis.[16] With all required building blocks in hand, acid **6** was attached to 2-Cl-trityl resin to give solid-supported ester **10**, which was elongated by alternating Fmoc deprotection and peptide coupling steps (Scheme 2). Release of **4** under mildly acidic conditions and Steglich esterification with alcohols **5a–d** in solution afforded the pure diastereomers **11a–d** in excellent overall yields (44–54% from **10**).

The formation of α-branched trisubstituted olefins embedded in macrocycles by ring-closing metathesis has previously been found to be challenging and strongly dependent on structural features of the substrates.[17] After careful experimentation it was found that treatment of **11** with 25–30 mol% of catalyst **12** in refluxing toluene gave consistent results and delivered the macrocycles **13** in 48–71% yield, if a constant purging flow of argon was applied throughout.[18] The cyclodepsipeptides **13** were then easily deprotected to give the desired final products **14a–d** (Scheme 2). The stereoselectivity of the metathesis reactions depended significantly on the configuration of the starting materials. For example, **11a** cyclized to give an inseparable 1.4:1 mixture of (*Z*)- and (*E*)-**13a**, but macrocycle **13b** was formed as the *Z* isomer exclusively. The macrocyles **13c** and **13d** were formed as pure *E* isomers (NOE data). Although the reasons for the observed stereoselectivity currently remain unclear, we note that the isomer ratios did apparently not change during the reaction course. We hence assume that the double bond is formed under kinetic control and that individual geometries result from distinct ruthenacyclobutane intermediate conformations.[19]

Careful analysis of the NMR spectra and HPLC traces of the cyclization products **14a–d** and comparison with data obtained from an authentic sample[20] clearly revealed (*E*)-**14c** as the naturally occurring chondramide C (Table 1 and Supporting Information).[9] Furthermore, the identity was evident from similar biological activity profiles of authen-



Scheme 2. Preparation of ring-closing metathesis precursors **11a–d** and synthesis of chondramide C and its stereoisomers. a) 2-chlorotrityl chloride resin (1.4 mmol g^{-1}), EtN(*i*Pr)$_2$ (4 equiv), CH_2Cl_2, RT, 2 h; b) 1. 20% piperidine, DMF (2×20 min); 2. **7** (2.6 equiv), DIC (2.6 equiv), HOBt (2.6 equiv), DMF, 2.5 h; 3. 20% piperidine, DMF (2×20 min), 4. **9** (2.3 equiv), HATU (2.3 equiv), HOAt (2.3 equiv), EtN(*i*Pr)$_2$ (4.6 equiv), DMF (2×2.5 h); 5. 20% piperidine, DMF (2×20 min); 6. **8** (2.3 equiv), HATU (2.3 equiv), HOAt (2.3 equiv), EtN(*i*Pr)$_2$ (4.6 equiv), DMF, 2.5 h; 7. HOAc/trifluoroethanol/CH_2Cl_2 (1:1:8), 2×1.5 h; c) **5** (3 equiv), EDC (2 equiv), DMAP (2 equiv), EtN(*i*Pr)$_2$ (2 equiv), CH_2Cl_2/DMF (20:1), 14 h; d) catalyst **12** (25–30 mol%), Ar purging, toluene, 110°C, 2 h; e) TBAF (2 equiv), THF, 0°C, 1 h. DIC = Diisopropylcarbodiimide; DMF = dimethylformamide; HATU = *O*-(7-azabenzotriazol-1-yl)tetramethyluronium hexafluorophosphate; EDC = *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide; DMAP = 4-dimethylaminopyridine; TBAF = tetrabutylammonium fluoride.

Table 1: Characteristic ^{1}H NMR chemical shifts (at 600 MHz, in ppm) of compounds **3** and **14a–d**, and their effective concentrations against actin.

Compd	5-H	7-H	3′-H	2″-H	2‴-H	c_{eff} [μM][a]
3[b]	4.71	4.34	5.23	5.50	4.61	0.2
(*Z*/*E*)-**14a**	5.00[c]	4.56[c]	5.17[c]	5.46[c]	4.69[c]	10[e]
	4.56[d]	4.89[d]	5.11[d]	5.56[d]	4.56[d]	
(*Z*)-**14b**	4.96	4.56	5.12	5.32	4.69	5
(*E*)-**14c**	4.72	4.35	5.23	5.51	4.61	0.2
(*E*)-**14d**	4.67	4.19	5.20	5.48	4.59	10

[a] Concentration at which the actin-stabilizing phenotype was fully developed. [b] Data taken from ref. [5], see Scheme 2 for numbering. [c] For *Z* olefin. [d] For *E* olefin. [e] Apparent c_{eff} of the *Z*/*E* mixture.

tic **3** and **14c**, whereas the other polyketide stereoisomers were much less active (vide infra). Therefore the natural product **3** embodies (*R*)-β-tyrosine, D-*N*-methyltryptophan, L-alanine, and its polyketide configurations are 2*S*, 6*R*, and 7*R*.[21]

Whole-cell microscopy in BSC-1 cells was employed to evaluate F-actin stabilization properties, using jasplakinolide (**2**) as control (Figure 1 and Supporting Information). Cell shrinkage, reduction or disappearance of F-actin fibers, formation of large F-actin clumps mainly in the perinuclear region, and binucleation of cells was prominent for both synthetic chondramide C (**14c**) and an authentic sample (**3**, data not shown)[20] already at $c = 200$ nM. This phenotype is in full accord with earlier studies on actin-stabilizing compounds[4,5] and was indistinguishable from **2** at $c = 100$ nM (Figure 1 B and E). In contrast, the other isomers studied (**14a**, **14b**, **14d**) induced a comparable phenotype only at $c =$ 5–10 μM (Figure 1 C, D, and F), which demonstrates that the configuration at C7 is a major determinant of the F-actin stabilizing activity of **3** and of similar importance to the double bond geometry.

These results confirm that chondramide C (**3** = **14c**) is a competitive ligand for phalloidin (**1**) on F-actin.[4,5] Compound **1** binds a cavity formed at the point of contact of three actin protein monomers in the polymeric filament.[6,22] Molecular docking simulations on the best currently available

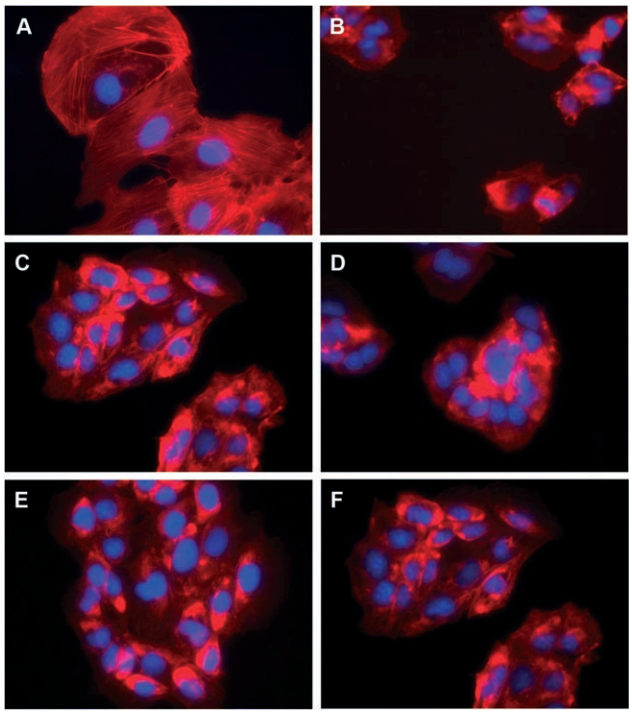


Figure 1. Actin stabilization phenotypes in BSC-1 cells monitored by whole-cell fluorescence microscopy (magnification 40×) after staining for actin (red, TRITC-phalloidin, Sigma) and chromatin (blue, DAPI, Sigma). A) DMSO only (negative control); B) 100 nM jasplakinolide (**2**, positive control); C) 10 μM (*Z*/*E*)-**14a**; D) 5 μM (*Z*)-**14b**; E) 200 nM (*E*)-**14c** (=**3**); F) 10 μM (*E*)-**14d**. TRITC = tetramethylrhodamin isothiocyanate. DAPI = 4′,6-diamidino-2-phenylindole.

data[6c] were therefore initiated to gain deeper insight into potential binding modes. The binding of phalloidin (**1**) to its target was re-evaluated first.[6a,c,23] The [Ala^7]-phalloidin crystal structure[24] was used as input for a conformational search in MOE,[25] and the conformational ensemble obtained in this way was then subjected to unbiased computational docking onto the actin polymer structural data[6c] using GOLD.[26]

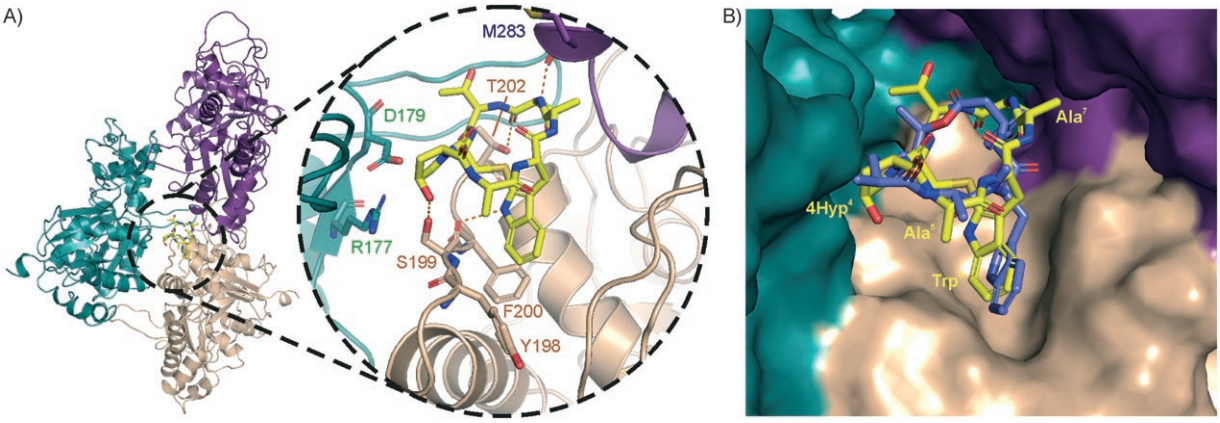


Figure 2. Proposed binding modes of [Ala^7]-phalloidin and chondramide C on F-actin. A) 3D-orientation of three actin monomers in the F-actin filament with a binding mode proposition for [Ala^7]-phalloidin (yellow) derived from unbiased computational docking experiments. Proposed hydrogen bonds are indicated by dashed lines. B) Surface representation of F-actin with the binding mode prediction of chondramide C (blue) overlaid with [Ala^7]-phalloidin (yellow).

The top scoring docking results clearly suggested a binding mode for [Ala^7]-**1** (Figure 2A), which is characterized by the indole moiety being in close contact with aromatic residues (Y198, F200) and *cis*-Pro(OH)^4 pointing towards the actin R177/D179 salt bridge. 4Hyp^4 interacts with S199 by hydrogen-bonding, and T202 forms a hydrogen bond to the backbone carbonyl group of Ala^1. This model refines earlier proposals[6a,c,23] and orients phalloidin roughly similar to the binding mode anticipated by Lorenz et al.[6a] More specifically, all SAR data available on **1** are in agreement with the binding geometry found here by unbiased docking.[6c,23,24,27,28] Interestingly, dye attachment to Leu(OH)$_2^7$ of **1** does not affect affinity.[7] This is in excellent agreement with our binding model, where the Ala^7-side chain extends into an accessible cavity (Figure 1B) in the same region in which a phalloidin-attached dye was located experimentally.[6c]

The binding of chondramide C (**3** = **14c**) was investigated in a similar fashion. Owing to the larger conformational freedom of **14c**, the size of the cavity, and limitations of the available data (8 Å resolution),[6c] binding modes were preferred that showed key interactions similar to the phalloidin pharmacophore.[29] In our best solution (Figure 1B) the Trp side chain of **14c** similarly interacts with aromatic amino acid residues, and the Ala in **14c** overlays with the respective Ala^5 of **1**. The polyketide segment aligns well with the 4Hyp^4 and Cys^3 of **1**, and the Tyr-OH group interacts with T202, which has been predicted to contribute to binding of **1**. In this binding model of **14c** the order of the residues is identical to **1**, despite the opposite Trp stereochemistry in phalloidin (L) and chondramide C (D). Importantly, the model explains well the influence of the stereogenic centers in the polyketide backbone of **3**. Inversion of configuration at C7 attenuated activity by 100-fold, presumably by populating unfavorable conformations[30] in the peptide segment of chondramide C.

In summary, a total synthesis of chondramide C (**3**) was accomplished featuring a rewarding *E*-selective ring-closing metathesis as the key step. The excellent overall yield (34% from **10**) highlights the benefit of our swift solid-phase based synthesis strategy, and chondramide C analogues were rapidly assembled (19–38% from **10**). Phenotypic actin assays revealed C7 and the double bond in **3** as crucial stereogenic elements for determining F-actin-stabilizing activity. Computational docking studies substantiated a pharmacophore model for phalloidin (**1**) and provided a binding mode for chondramide C (**3** = **14c**). These results are expected to guide further developments of actin-stabilizing agents in the future.

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