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Synthesis of Chondramide A Analogues with Modified β-Tyrosine and Their Biological Evaluation

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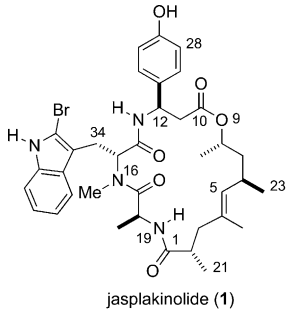
Abstract: Starting from cinnamates **9**, obtained by Wittig reaction or Heck coupling, the diols **17** were prepared by asymmetric dihydroxylation. This was followed by a regioselective substitution of the 3-OH group with hydrazoic acid under Mitsunobu conditions. Methylation of the 2-OH group and reduction of the azide group led to the β-tyrosine derivatives **8**. Condensation with the dipeptide acid **6** furnished the tripeptide part of the chondramides. The derived acids **21** were combined with the hydroxy ester **7** to the esters **22**. Cleavage of the *tert*-butyl groups and intramolecular lactam formation gave rise to the chondramide A analogues **2b–k**. Growth inhibition assays showed most of the analogues to be biologically active. Some of them even reach the activity of jasplakinolide. It can be concluded that the 4-position of the aryl ring in the β-tyrosine of chondramide A tolerates structural modifications quite well.

Keywords: chondramides · cyclopeptides · Mitsunobu reaction · total synthesis · tyrosine analogues

Introduction

Cyclodepsipeptides comprise a unique class of secondary metabolides. They frequently contain unusual amino acids, like D-amino acids or N-methylated amino acids and hydroxy acids that typically originate from the polyketide pathway. Incorporation of the hydroxy acid results in an ester bond, explaining the term "depsi".[1,2] Many cyclodepsipeptides have been isolated from marine sponges and found to display interesting biological activities.[3] Thus, cyclodepsipeptides with anti-HIV or anti-tumor activity are known. With the peptide subunit cyclodepsipeptides are clearly protein-like and therefore it is not surprising that they very often modulate protein–protein interactions.[4]

Very prominent examples of such cyclodepsipeptides, displaying anti-tumor activity are the jasplakinolides and the chondramides. Jasplakinolide (**1**) was isolated from the marine sponge *Jaspis splendans* many years ago (Figure 1).[5]



	R^1	R^2
chondramide A (**2**)	OMe	H
chondramide B (**3**)	OMe	Cl
chondramide C (**4**)	H	H
chondramide D (**5**)	H	Cl

Figure 1. Structures of jasplakinolide (**1**) and the chondramides A–D (**2**–**5**).

Independently, it was also found in a *Jaspis* sponge and named jaspamide.[6] In the meantime jasplakinolide was isolated from many other sponges. Furthermore, several additional jasplakinolides could be isolated by the groups of Zampella[7] and Crews.[8] In contrast, so far only four natural chondramides are known, namely chondramides A–D (**2**–**5**) (Figure 1). They were isolated by the Höfle/Reichenbach group from myxobacteria.[9] The chondramides are quite similar in structure to jasplakinolide, in that they contain a tripeptide subunit consisting of an L-alanine, an N-methyl-D-tryptophan, and an L-β-tyrosine. Jasplakinolide and the

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chondramides differ essentially in the hydroxy acid that bridges the tripeptide part to form the cyclodepsipeptide. Whereas jasplakinolide contains an 8-hydroxy acid, in the chondramides the corresponding sector is a 7-hydroxy acid. Accordingly, jasplakinolide features a 19-membered macrocycle, while the chondramides are 18-membered.

Over the years several total syntheses of jasplakinolide have been achieved.[10] Most of them close the macrocyclic ring by intramolecular amide or ester formation. The Waldmann/Arndt synthesis[10g] relies on a relay ring-closing metathesis reaction. Furthermore, syntheses of simplified jasplakinolide analogues have been reported.[11,12] Surprisingly, syntheses of chondramides were achieved only recently. The reason was that the configuration at the stereogenic centers was not given in the original article. Even though it could be assumed that the configurations in the peptide part and even the overlapping hydroxy acid might be similar to the ones in jasplakinolide. The Waldmann[13] and Kalesse[14] groups independently secured the configuration of the hydroxy acid in the chondramides by synthesizing various stereoisomers of chondramide C. Our group had developed a concise synthesis of the hydroxy acid through an asymmetric vinylogous aldol reaction.[15] Subsequently, we reported the total synthesis of chondramide A (chon A).[16] Instead of a β-tyrosine, this chondramide contains a 3-amino-2-methoxy-3-arylpropanoic acid, whose configuration was determined to be (2*S*,3*S*). The ester bond could be established either by Yamaguchi esterification or via a Mitsunobu reaction[17] that was then followed by macrolactam formation. While the β-tyrosine derivative in chondramide A seems more complicated on first sight, it is easily available by asymmetric dihydroxylation of a cinnamic ester precursor.

The potent antitumor activity of these cyclodepsipeptides is due to their stabilizing effect on F-actin filaments.[18] Because of this property jasplakinolide became an important tool in cell biology. Together with microtubules and the intermediate filaments, actin filaments (F-actin) make up the cytoskeleton which has a key role in cell shape and division. According to a binding model recently refined by the Waldmann group, chondramide C is located in a shallow binding pocket made up by three independent actin subunits in the filament.[19] The binding site is identical to the one of the bicyclic heptapeptide phalloidin.[20] There seems to be a hydrophobic interaction of the indole moiety with a loop region of one of the actin subunits (subunit X). The phenolic group of the β-tyrosine occupies a larger cavity with the hydroxyl group close to an Asp of G-actin monomer Y. It seems that the hydroxy acid not only serves to provide a scaffolding role, but rather parts (C1–C3) of it are close to the protein surface of subunit Z and contribute substantially to efficient binding. This model was supported by the activity of the available jasplakinolide and chondramide analogues (Figure 2).[12] For example, omitting the phenolic ring essentially results in an inactive jasplakinolide analogue. With regard to the hydroxy acid, it turned out that the configuration at C2 of the methyl-bearing carbon and the *E*-configuration of the C4–C5 double bond are crucial. Jasplakinolide analogues with this feature are still active even if they lack some of the other methyl groups of the hydroxy acid. For chondramide C analogues it could be shown that the configuration at C6 and C7 of the hydroxy acid is less critical.

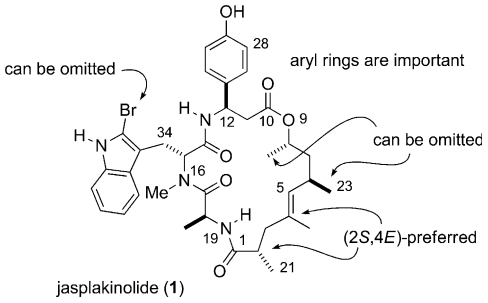


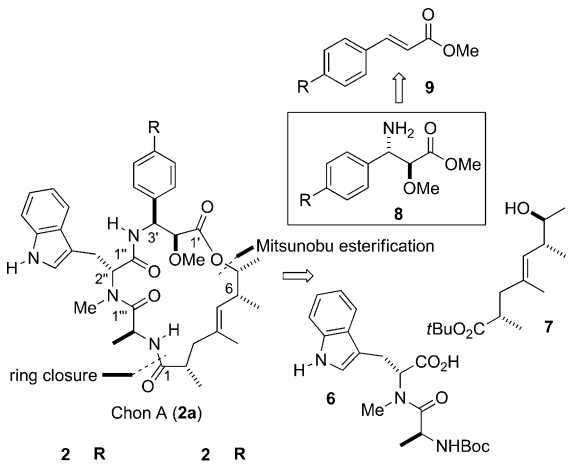
Figure 2. Key structure-activity relationships for good biological activity of jasplakinolides and chondramides illustrated with the jasplakinolide structure.

Due to their interesting biology and structural similarity analogues of the above-mentioned cyclodepsipeptides should help to understand protein–protein interactions and further advance the binding site model.[12] With an efficient route to chondramide A we embarked on the synthesis of chondramide A analogues with modified β-tyrosine derivatives. In this paper we report on the synthesis of ten analogues and their biological activity.

Results and Discussion

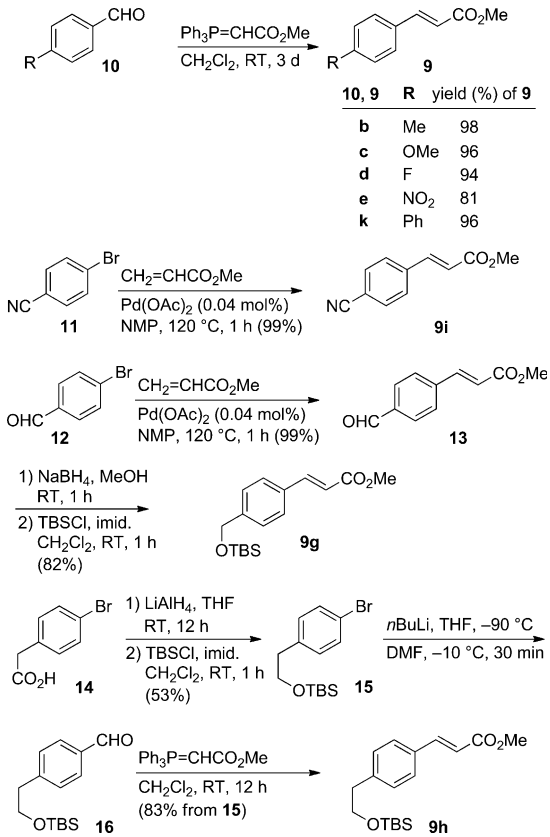
Synthesis of the analogues: The synthetic route towards natural chondramide A (**2a**), recently disclosed from our laboratory,[16] was put as a basis for synthesis of new chondramide analogues, containing variations in the β-tyrosine unit.[16] Accordingly, this implied the use of differently substituted β-amino esters **8** that had to be synthesized from substituted *trans*-cinnamic esters **9**. At this point, taking into account the compatibility of the substituents at the aromatic ring with all the synthetic steps, we have chosen eight cinnamic esters **9b–e**, **9g–i**, and **9k** as precursors for the desired chondramide analogues (Scheme 1).

All the *trans*-cinnamic esters **9** were synthesized from easily accessible commercial starting materials as outlined in Scheme 2. Enoates **9b–e**, **9k** were prepared by Wittig reaction of the corresponding aldehydes **10** with the stabilized ylide [(carbomethoxy)methylene]triphenylphosphorane[21] in dichloromethane. Enoate **9e** could also be obtained by Knoevenagel condensation with subsequent esterification in high 93% yield. In case of enoate **9k**, its low solubility in methanol, allowed for efficient isolation of the product by simple filtration. The 4-cyanocinnamate **9i** was synthesized by Heck reaction from 4-bromobenzonitrile under low catalyst loading and ligand-free conditions, using $Pd(OAc)_2$ as catalyst.[22] This method is especially efficient for electron deficient aromatic bromides and enoate **9i** was obtained in



2	R	2	R
a	OH	g	CH_2OH
b	Me	h	CH_2CH_2OH
c	OMe	i	CN
d	F	j	$CONH_2$
e	NO_2	k	Ph
f	NH_2		

Scheme 1. Synthetic strategy and key fragments for the synthesis of chondramide A analogues **2b**-**k**.

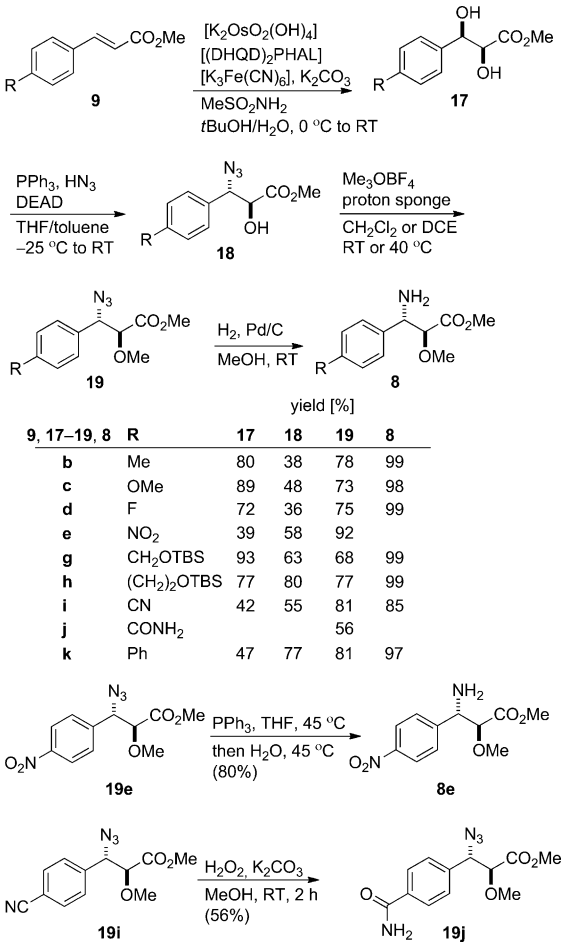


10, 9	R	yield (%) of 9
b	Me	98
c	OMe	96
d	F	94
e	NO_2	81
k	Ph	96

Scheme 2. Synthesis of cinnamic acid building blocks **9** by Wittig reaction or Heck coupling; NMP = *N*-methyl-2-pyrrolidone.

essentially quantitative yield. This result stipulated the choice of 4-bromobenzaldehyde as a precursor for preparation of the CH_2OTBS-substituted enoate **9g**, that was finally done in three steps in high overall yield. Thus, the Heck reaction to (*E*)-methyl 4-formylcinnamate (**13**) was followed by $NaBH_4$ reduction of the aldehyde group and silylation of the primary alcohol. Cinnamate **9h** with a CH_2CH_2OTBS substituent in 4-position was prepared from the arylacetic acid **14** through reduction with $LiAlH_4$ and silylation of the resulting primary alcohol. A subsequent halogen-metal exchange on aryl bromide **15** and quenching the anion with DMF furnished aldehyde **16** which was extended via Wittig reaction in good yield. The synthesis of the last derivative, enoate **9h** initially was also attempted using Heck reaction, however, the corresponding substrate **15** did not react neither under ligand free conditions nor in the presence of PPh_3. Here, formation of palladium black was observed and starting material was recovered unchanged. This is explained by the low reactivity of the aromatic bromide, which is not activated by an electron-withdrawing substituent. In all cases the cinnamates **9** were obtained configurationally pure and no *cis*-isomers could be detected by NMR in the products.

With several cinnamic esters in hands we next turned to the synthesis of amino esters **8** (Scheme 3). Sharpless asymmetric dihydroxylation (AD)[23] under standard conditions



9, 17–19, 8	R	17	18	19	8
		yield [%]			
b	Me	80	38	78	99
c	OMe	89	48	73	98
d	F	72	36	75	99
e	NO_2	39	58	92	
g	CH_2OTBS	93	63	68	99
h	$(CH_2)_2OTBS$	77	80	77	99
i	CN	42	55	81	85
j	$CONH_2$			56	
k	Ph	47	77	81	97

Scheme 3. Conversion of the cinnamates **9** to the β-tyrosine derivatives **19**, respectively; DCE = dichloroethane, DEAD = diethyl azodicarboxylate.

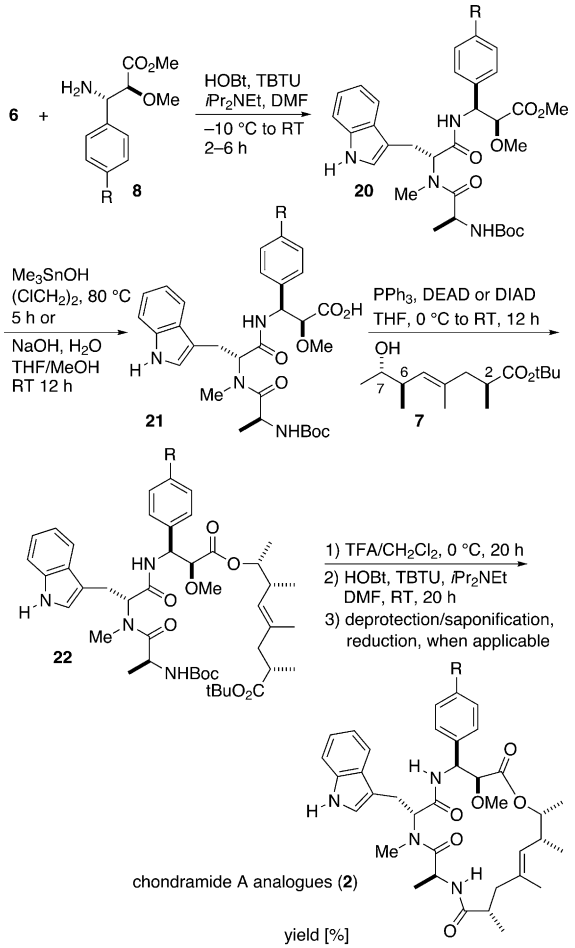
(0.4 mol% Os catalyst, 2 mol% ligand) was used initially, but this proved problematic providing the diols only in moderate yield (30–68%). After some experimentation, possible reasons for these problems became clear. Due to the electron-deficient nature of the alkenes, especially of the nitro and cyano derivatives, dihydroxylation occurred with reduced rate and saponification of the methyl ester became a significant side reaction. In the case of the nitro-substituted diol **17e** the corresponding acid was partially isolated and characterized (see the supporting information). The dihydroxylation reactions with the NO_2-, Ph-, and CN-substituted cinnamates were additionally hampered by very limited solubility of the alkenes in the reaction medium. As soon as the ester saponification seemed to be the only problem and otherwise the reaction was clean, attempts were made to improve the yield by addition of $KHCO_3$ to the reaction mixture, to make it less alkaline, but this was again not sufficiently successful. Finally, the situation was significantly improved when increased catalyst loading was used together with buffering the reaction mixture with $KHCO_3$. Indeed, in the presence of 1 mol% $K_2OsO_2(OH)_4$/1.5 mol% chiral ligand, the reaction required somewhat shorter time and the diol **17g** was obtained in 93% yield. Although the synthesis of other diols was not attempted under the optimized conditions, we believe that increasing the catalyst loading (e.g., up to 2 mol%) should be the most efficient way to increase the yield in the AD of these relatively unreactive and hardly soluble alkenes. This is in line with the observations given in the literature.[23] For three of the diols (**17e**, **17g**, **17i**) the optical purity was assessed by means of HPLC on a chiral column (see the Supporting Information), and in no case the opposite enantiomer could be detected, which implies at least 98% *ee* for these compounds.[16] For the other diols the enantiomeric purity was tentatively assessed to be "high" by the observation of no significant side peaks in the ^1H and ^{13}C NMR spectra on later steps when diastereomers would appear.

Diols **17** were next transformed to β-azides **18** by means of a Mitsunobu reaction with hydrazoic acid.[24] Initial experiments revealed only moderate conversion of the diols, accompanied by significant nitrogen evolution. This side reaction was caused by hydrazoic acid decomposition of the ternary mixture in the presence of triphenylphosphine and DEAD. Mixing all three components together caused rapid nitrogen evolution, while the binary mixture of HN_3 and PPh_3 was stable at room temperature. This undesired side reaction was diminished when the DEAD solution was introduced into the reaction as the last component at −25°C rather than at 0°C. Thereafter, the mixture was allowed to stand at room temperature until the reaction stopped. Under these conditions, the desired azides were obtained in better yields as pure stereoisomers in accordance with literature data.[24] The structure and purity were additionally confirmed by careful comparison of NMR spectra with previously described compounds (see supporting information). In these Mitsunobu reactions, diols **17e** (NO_2), **17i** (CN), and **17k** (Ph) were used as hardly separable mixtures, slightly contaminated with $MeSO_2NH_2$, resulting from the previous step. We found $MeSO_2NH_2$ to have no influence on the reaction.

Subsequently, azides **18** were smoothly methylated by trimethyloxonium tetrafluoroborate in the presence of proton sponge to give the α-methoxy propanoates **19**. The cyano derivative **19i** was hydrolyzed with H_2O_2/K_2CO_3 to provide amide **19j**, the precursor for chondramide analogue **2j**. The hydrolysis was performed in DMSO, MeOH and DMF media and was found to be the fastest in DMSO (complete conversion was observed already in 1–2 min), but in this case the product was isolated only as inseparable mixture with dimethyl sulfone as by-product. Pure amide **19j** was obtained in MeOH, but in this case the reaction took much longer time and gave the product in reduced yield most likely due to hydrolysis of the ester.

Azides **19** were conveniently hydrogenated in presence of Pd/C to afford the amino esters **8**. Nitro derivative **19i** was selectively reduced by treatment with PPh_3 followed by hydrolysis of the iminophosphorane intermediate to afford amine **8e**, that was used without further purification.

In several steps amino esters were transformed into chondramide analogs **2b–k** (Scheme 4). First, peptide coupling with the known dipeptide acid,[11c,25] **6** afforded tripeptides **20** in good yields. Subsequent saponification of the methyl ester, either with Me_3SnOH (**20b–d**) or simply with NaOH [**20e** (NO_2), **20i** (CN), **20j** ($CONH_2$), **20k** (Ph)] occurred in quantitative yields and without epimerization. But saponification of the CH_2OTBS- and $(CH_2)_2$OTBS-substituted tripeptide esters **20g** and **20h** was accompanied by partial deprotection of the alcohol functions. Still, pure acids **21g** and **21h** were successfully isolated by chromatography in reduced, but good yields (~80%). Thus, there was no need to call upon the milder Me_3SnOH procedure. On the next stage, acids **21b–d** were initially esterified with hydroxy ester 7-*epi*-**7** under Yamaguchi conditions to give the *seco*-compounds **22b–d** in reasonable yields. However, later we found it more convenient to perform the esterification reaction under Mitsunobu conditions since the synthesis of hydroxy ester **7** is shorter than the one for 7-*epi*-**7**.[15] Therefore, the acids **21** were esterified with alcohol[15] **7** under Mitsunobu conditions in quite good yields. Here, the correct use of either DEAD or DIAD reagents in each case is important for successful separation of the esterification product. Otherwise, the corresponding hydrazodicarboxylate by-product would be inseparable (or hardly separable) from the product. Unfortunately, the acid **21j** ($CONH_2$) could not be esterified under Mitsunobu conditions. Other methods (Yamaguchi esterification, DCC/DMAP conditions) were unsuccessful either. Eventually, the corresponding chon A derivative was prepared by nitrile hydrolysis on the macrocycle **2j** (see below). For macrolactam formation, the NBoc protected *tert*-butyl esters **22** were subjected to double deprotection under acidic conditions. Initially, the reaction was carried out in a mixture of TFA/CH_2Cl_2 (1:30) as previously described.[16] The progress of the deprotection was easily monitored by TLC and NMR and under these conditions



20–22, 2	R	yield [%] 20	21	22	2
b	Me	75	–	67	33
c	OMe	77	–	61	31
d	F	68	–	64	39
e	NO_2	76	–	86	48
f	NH_2				47
g[a]	CH_2OTBS	88	82	73	20
h[a]	$(CH_2)_2OTBS$	86	80	66	60
i	CN	86	–	78	45
j	$CONH_2$				67
k	Ph	83	–	56	54

Scheme 4. Synthesis of chondramide A analogues **2b–j**. Esters **22b–d** were also prepared by Yamaguchi esterification using hydroxy ester 7-*epi*-**7**; TBTU = 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate, HOBt = 1-hydroxy-benzotriazole, DIAD = diisopropyl azodicarboxylate. [a] For compound **2**, R = CH_2OH and $(CH_2)_2OH$, respectively.

the conversion was not complete even after 2 d. In particular, the carboxylic ester cleavage is quite slow. However with more concentrated acid (1:10) complete and clean deprotection was observed for each compound after about 20 h. Interestingly, under these reaction conditions the TBS groups of **22g** and **22h** were quantitatively transformed to the corresponding TFA esters, as suggested by NMR of the crude products. In each case the crude amino acids were subjected to macrolactamization in the presence of TBTU, which afforded chondramide A analogs **2e** (NO_2), **2i** (CN), and **2k** (Ph) directly. In order to obtain the analogues **2g** (CH_2OH) and **2h** [$(CH_2)_2OH$] the crude cyclization products were stirred in aqueous K_2CO_3/MeOH for 5–10 min before purification. This resulted in complete hydrolysis of the corresponding TFA esters. Unfortunately, the presence of the benzylic trifluoroacetate negatively affected cyclization of the amino acid generated from **22g** (CH_2OTBS), yielding after short hydrolysis with aqueous K_2CO_3/MeOH two products as judged by TLC. The more polar substance turned out to be the desired chondramide A analogue **2g**. The second substance was shown to be the corresponding benzotriazolyl ether that was characterized by ^1H NMR and HRMS analysis (see Supporting Information for details). Thus, for this analogue it can be concluded that the TBS group is not ideal and revision of the synthetic scheme would be required in this case for an optimized synthesis. But for the other cases the described synthetic Scheme is considered reliable. With the NO_2-substituted analogue **2e** we attempted reduction of the nitro group to obtain the NH_2 derivative **2f**. This was tried with catalytic hydrogenation and sodium dithionite. Both methods afforded the desired amino chondramide **2f** in moderate yield. Finally, the $CONH_2$-substituted analogue **2j** was prepared by hydrolysis of the cyano group of **2i** with H_2O_2/K_2CO_3/DMSO under mild conditions in good unoptimized yield.

Biology: The biological activity of the chondramide A (chon A) analogues **2** was evaluated in a cytotoxicity assay on human primary foreskin fibroblast (HFF) cells. These data are included in Table 1. We also included the natural products jasplakinolide and other chondramides in this assay. As can be seen, several of the chon A analogues are slightly more active than the natural product itself. Three of the analogues [**2b** (Me), **2d** (F), **2i** (CN)] are even comparable to jasplakinolide (**1**) itself (EC_{50} in the range of 30 nM). These are characterized by small substituents. Other analogues show intermediate activity, being about half as active as jasplakinolide (EC_{50} in the range of 60 nM). These analogues

Table 1. Cytotoxicity of jasplakinolide (**1**) and chondramide A derivatives **2**.[a]

Entry	Compound	EC_{50} (HFF) [μM]	IC_{50} (L-929) [μM]
1	jasplakinolide_np (**1**)	0.028 ± 0.004	
2	chon A_np (**2a**)	0.118 ± 0.023	0.056 ± 0.013
3	chon B_np (**3**)	0.044 ± 0.005	
4	chon C_np (**4**)	0.036 ± 0.007	
5	chon A_synth (**2a**)	0.109 ± 0.007	
6	chon A_Me (**2b**)	0.027 ± 0.002	0.0079 ± 0.0013
7	chon A_OMe (**2c**)	0.066 ± 0.008	0.0083 ± 0.0021
8	chon A_F (**2d**)	0.029 ± 0.002	0.0080 ± 0.0013
9	chon A_NO_2 (**2e**)	0.040 ± 0.006	0.017 ± 0.005
10	chon A_NH_2 (**2f**)	0.057 ± 0.001	0.031 ± 0.002
11	chon A_CH_2OH (**2g**)	0.052 ± 0.010	0.035 ± 0.002
12	chon A_ $(CH_2)_2OH$ (**2h**)	0.059 ± 0.010	0.033 ± 0.004
13	chon A_CN (**2i**)	0.027 ± 0.001	0.011 ± 0.001
14	chon A_$CONH_2$ (**2j**)	1.424 ± 0.017	0.223 ± 0.042
15	chon A_Ph (**2k**)	0.213 ± 0.016	0.025 ± 0.002

[a] np = natural product (isolated), synth = synthetic compound.

include **2c** (OMe), **2e** (NO_2), **2f** (NH_2), **2g** (CH_2OH), and **2h** ($(CH_2)_2OH$). Somewhat surprising is the activity of analogue **2k** (Ph) ($EC_{50}=213$ nM). With this large substituent one would not expect that the analogue would fit in the binding pocket.[12] Although the amide substituent in **2j** is not very large, this compound is much less active. A similar trend in activity can be seen on the mouse fibroblast cell line L-929, which is more sensitive to these cyclodepsipeptides.

In general, it can be concluded that the aryl ring on the tyrosine derivative accepts a range of substituents. One explanation could be that the 4-position of the aryl ring protrudes out of the binding pocket. Thus, some of the prepared derivatives might be used for the preparation of labeled derivatives. The recent finding that jasplakinolide V with a catechol in place of the phenol substituent is also quite active shows this region to be less sensitive to structural modifications than the other parts.[8b]

Conclusion

Based on our previous synthesis of chondramide A, a range of analogues was prepared that feature modifications in the 4-position of the β-tyrosine derivative. The required building blocks, 3-aryl-3-amino-2-methoxypropanoates **8** were prepared from cinnamates **9** by asymmetric dihydroxylation, regioselective Mitsunobu substitution with hydrazoic acid, O-methylation and reduction of the azide group. Subsequently, amino esters **8** were condensed with dipeptide acid **6**. After hydrolysis of the methyl ester function, esterification of the tripeptide acids **21** with the 7-hydroxy ester **7** or 7-*epi*-**7**, respectively furnished the *seco*-compounds **22**. Deprotection of both the amino and the ester group was followed by macrolactam formation to give analogues **2**.

In cell growth assays several of the derivatives surpassed natural chondramide A in its biological activity. Among the derivatives only the amide derivative **2j** was not very active. Otherwise, it appears that the aryl ring of the β-tyrosine tolerates a broad range of substituents in the 4-position. These derivatives should be useful for probing a number of cellular questions in different systems that rely on actin filaments for important aspects of biology.

Experimental Section

Only part of the experimental material is given here. For further details and for compound characterization see Supporting Information. Procedures are given for the sequence leading to chondramide A analogue **2e** (NO_2) and the reduction of **2e** to analogue **2f** (NH_2).

(*E*)-Methyl 4-nitrocinnamate (9e) (by Knoevenagel reaction): A solution of nitrobenzaldehyde (1.51 g, 0.01 mol), malonic acid (1.14 g, 1.1 equiv), and pyridine (0.25 mL, 0.31 equiv) in ethanol (2 mL) was heated at reflux. Already after 10–15 min a white solid began to precipitate. After 1.5 h, when TLC (EtOAc/petroleum ether, 1:2) indicated complete consumption of the aldehyde, the reaction mixture was cooled and acidified with diluted aqueous HCl. The precipitate was filtered, washed with water and dried in vacuo to afford crude but pure (2*E*)-3-(4-nitrophenyl)acrylic acid in quantitative yield (Note: the free acid is soluble in acetone, DMSO, but hardly soluble in CH_2Cl_2, EtOAc). It was taken up in methanol (20 mL), thionyl chloride (1.3 equiv) was carefully added and the resulting suspension was heated at reflux for 1 h. At this time TLC (EtOAc/petroleum ether, 1:2) indicated complete consumption of the acid and the volume of precipitates visually increased. The product was obtained by recrystallization directly from the reaction mixture as follows. The reaction mixture was concentrated in vacuo till a thick suspension resulted, that was filtered on a glass frit and the precipitate was washed with a small amount of cold methanol to afford 1.52 g (93%) of pure methyl (2*E*)-3-(4-nitrophenyl)acrylate (**9e**) as a slightly yellow solid. Analytical data were in agreement with literature data.[26]

(2*S*,3*R*)-Methyl 3-(4-cyanophenyl)-2,3-dihydroxypropanoate (17e): A mixture of $K_3Fe(CN)_6$ (7.22 g, 22.0 mmol, 3 equiv), K_2CO_3 (3.04 g, 22.0 mmol, 3 equiv), $MeSO_2NH_2$ (0.70 g, 7.37 mmol, 1 equiv), $K_2OsO_2(OH)_4$ (10 mg, 0.027 mmol, 0.0037 equiv), and the ligand $(DHQD)_2PHAL$ (57 mg, 0.073 mmol, 0.01 equiv) was stirred in a mixture of water (35 mL) and *t*BuOH (35 mL) until dissolved and then the solution was cooled to 0°C in an ice bath. At this point cinnamate **9e** (1.52 g, 7.34 mmol) was added and the reaction mixture was allowed to reach room temperature slowly while being stirred overnight, at which time a yellow suspension was formed and complete or almost complete conversion was observed according to TLC (petroleum ether/EtOAc, 1:1). Then solid Na_2SO_3 (9.2 g, 73.4 mmol, 10 equiv) was added and the mixture was stirred for several min. The suspension was filtered, and the filter cake was washed with EtOAc. The filtrate was transferred to a separatory funnel, the organic phase was separated, and the water phase was extracted twice with EtOAc. The combined organic extracts were washed with saturated NaCl solution, dried with Na_2SO_4, filtered, and evaporated. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5) to afford pure diol **17e** (0.698 g, 39%) as a colorless solid. Sometimes it was difficult to purify the diol from $CH_3SO_3NH_2$ (as a contaminant in different compounds comes at $\delta=3.08$–3.10 (s, 3H), 4.67–4.79 ppm (brs, 2H)), but the sulfone amide impurity did not influence the next Mitsunobu azidation as was established later. Also, a better procedure but for another substrate (**17g**) utilizing 1% of the catalyst is described in the Supporting information. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=$ 2.81 (brs, 1H, OH), 3.13 (brs, 1H, OH), 3.86 (s, 3H, OCH_3), 4.39 (brs, 1H, 2-H), 5.14 (brs, 1H, 3-H), 7.59 (d, $J=8.4$ Hz, 2H, Ar), 8.23 ppm (d, $J=8.4$ Hz, 2H, Ar).

Preparation of hydrazoic acid solution: **CAUTION**: Hydrazoic acid is a highly volatile, toxic and explosive liquid in individual state. However, in solution it is stable and safe. In this study solutions up to 5M were used. Sodium azide (3.0 g, 46 mmol) was mixed with water (1.5 mL) and toluene (10 mL). The suspension was cooled to near 0°C and concentrated H_2SO_4 (~2 g, ~1.09 mL, 20 mmol) was carefully added while cooling and shaking the round bottom flask (stirring with magnetic stirring bar is not sufficient). Crystals were kneaded with a spatula shortly after the addition of the acid. Then the mixture was filtered under positive pressure and dried with Na_2SO_4 (there was no water phase remained, and no need to separate it from toluene solution). To rapidly estimate the resulting concentration of hydrazoic acid, a known amount of NaOH was dissolved in a small amount of water, phenolphthalein was added and the pink solution was titrated with the hydrazoic acid solution from an analytical pipette (the concentration was found to be 3.3M against theoretical 4.0M).

(2*S*,3*S*)-Methyl 3-azido-2-hydroxy-3-(4-nitrophenyl)propanoate (18e): To a stirred solution of diol **17e** (0.228 g, 0.946 mmol), triphenylphosphine (0.297 g, 1.14 mmol, 1.2 equiv), hydrazoic acid (0.86 mL, 3.3M in toluene, 3 equiv) in THF (2.0 mL) at −25°C was added DEAD (0.56 mL, 0.535 g, 1.23 mmol, 40% wt. solution in toluene, 1.3 equiv), then the cooling bath was removed (slight evolution of N_2 was observed) and the resulting mixture was stirred overnight at ambient temperature (TLC control: petroleum ether/EtOAc, 1:1; NMR control: a sample portion was taken from the reaction mixture, evaporated and directly analyzed by NMR). Then the reaction mixture was concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/EtOAc, 4:1 to 2:1) to yield

18e (0.145 g, 58%) as a slightly orange oil which solidified into a waxy solid upon standing. R_f (petroleum ether/EtOAc, 1:1) = 0.55; $[\alpha]_D^{20} = +101.1$ (c = 1.00, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.11$ (br s, 1H, OH), 3.73 (s, 3H, OCH_3), 4.58 (d, 1H, 2-H), 5.08 (d, $J = 3.8$ Hz, 1H, 3-H), 7.53 (d, $J = 8.7$ Hz, 2H, Ar), 8.23 ppm (d, $J = 8.7$ Hz, 2H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 53.1$ (OCH_3), 66.4 (C-3), 73.7 (C-2), 123.7 (C_{ar}), 128.7 (C_{ar}), 141.8 (C_{ar}), 148.1 (C_{ar}), 171.3 ppm (CO_2CH_3); HMRS (ESI): m/z calcd for $C_{10}H_{10}N_4O_5$ $[M+Na]^+$: 289.05434; found: 289.05431. *Note.* The product **18e** was slightly contaminated with diethyl hydrazodicarboxylate (~3–5 mol%), having signals $\delta = 1.28 \pm 0.01$ (t, 6H), 4.22 ± 0.01 ppm (q, 4H). This impurity did not cause problems in the subsequent steps.

(2*S*,3*S*)-Methyl 3-azido-2-methoxy-3-(4-nitrophenyl)propanoate (19e): To a solution of α-hydroxy ester **18e** (0.495 g, 1.86 mmol) in dry 1,2-dichloroethane (1.9 mL) was added trimethyloxonium tetrafluoroborate (0.495 g, 3.35 mmol, 1.8 equiv) and proton sponge (0.876 g, 4.09 mmol, 2.2 equiv). The flask was covered with alumina foil. After stirring the suspension at 40°C overnight, a small probe was taken from the reaction mixture and quenched with EtOAc/HCl_{aq} for TLC (petroleum ether/EtOAc, 1:1), that indicated full conversion. The reaction mixture was cooled, treated with EtOAc/H_2O, and acidified with 1N HCl to pH 2–3. The precipitate was filtered off and the filtrate was separated. The aqueous phase was extracted once with EtOAc and the combined organic extracts were washed with water, and saturated NaCl solution, dried with Na_2SO_4, filtered, and concentrated in vacuo. The residue was chromatographed (petroleum ether/EtOAc, 3:1 to 2:1) to yield **19e** (0.48 g, 92%) as a slightly orange oil. R_f (petroleum ether/EtOAc, 2:1) = 0.54; $[\alpha]_D^{20} = +49.5$ (c = 1.00, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.38$ (s, 3H, OCH_3), 3.75 (s, 3H, CO_2CH_3), 3.99 (d, $J = 6.1$ Hz, 1H, 2-H), 4.89 (d, $J = 6.1$ Hz, 1H, 3-H), 7.55 (d, $J = 8.7$ Hz, 2H, Ar), 8.22 ppm (d, $J = 8.7$ Hz, 2H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 52.5$ (OCH_3), 59.3 (OCH_3), 65.0 (C-3), 83.0 (C-2), 123.8 (C_{ar}), 129.0 (C_{ar}), 142.4 (C_{ar}), 148.1 (C_{ar}), 169.6 ppm (CO_2CH_3); HMRS (ESI): m/z calcd for $C_{11}H_{12}N_4O_5$ $[M+Na]^+$: 303.06999; found: 303.07002.

Reduction of azide 19e and coupling of amine 8e with acid 6 to tripeptide 20e (NO_2): A solution of azide **19e** (89.6 mg, 0.32 mmol) and PPh_3 (92.2 mg, 0.352 mmol, 1.1 equiv) in of THF (1 mL) was stirred at 40–50°C for 1 h for clean and complete conversion to the corresponding iminophosphorane (TLC control: CH_2Cl_2/MeOH/NH_3, 10:1:0.1, R_f 0.5 for the iminophosphorane). Selected ^{1}H NMR (400 MHz, $CDCl_3$) data for the iminophosphorane: $\delta = 7.91$ (d, 2H), 3.67 (s, 3H), 3.13 ppm (s, 3H). Then water (0.1 mL) was added and the mixture was further stirred at 40–50°C for ~8 h. Because the R_f values of the iminophosphorane, Ph_3P=O and the resulting amine were all the same, the reaction progress was conveniently monitored by analyzing small evaporated probes taken from the reaction mixture by NMR. Selected ^{1}H NMR (400 MHz, $CDCl_3$) data for the amine: $\delta = 8.15$ (d, 2H), 3.65 (s, 3H), 3.39 ppm (s, 3H). When appropriately clean and high (~86%) conversion was achieved, the mixture was evaporated to yield 0.166 g of a sticky oil, containing ~36% w/w amine **8e** (assuming the conversion was 80% as the lowest). Then, to a solution of this crude mixture (108 mg), containing amine **8e** (approx. 38.9 mg, 0.153 mmol, 1.24 equiv of the amine) in DMF (2.3 mL) were added acid **6** (47.7 mg, 0.123 mmol), HOBt (24.9 mg, 0.184 mmol, 1.5 equiv), iPr$_2$NEt (0.064 mL, 0.369 mmol, 3 equiv). At −10°C TBTU (59 mg, 0.184 mmol, 1.5 equiv) was added and the reaction was stirred for 5–6 h at room temperature. The mixture was diluted with water (5 mL) and extracted with ethyl acetate (3×8 mL). The combined organic layers were washed with 1N $NaHSO_4$ solution (5 mL), saturated $NaHCO_3$ solution (5 mL), saturated NaCl solution (5 mL), dried with Na_2SO_4, filtered, and concentrated in vacuo. Purification of the residue by flash chromatography (petroleum ether/EtOAc, 2:1) gave tripeptide **20e** (58.7 mg, 76%) as a white foam. R_f (petroleum ether/EtOAc, 2:1) = 0.48; $[\alpha]_D^{20} = +4.0$ (c = 1.00, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.97$ (d, $J = 6.9$ Hz, 3H, Ala CH_3), 1.40 (s, 9H, tBu), 2.98 (s, 3H, NCH_3), 3.17 (dd, $J = 15.5$, 9.4 Hz, 1H, CH_2), 3.38 (s, 3H, OCH_3), 3.38 (m, 1H, CH_2), 3.61 (s, 3H, CO_2CH_3), 4.07 (d, $J = 5.1$ Hz, 1H, C*H*OCH_3), 4.48–4.54 (m, 1H, Ala CH), 5.33 (d, $J = 7.4$ Hz, 1H, Ala NH), 5.44 (dd, $J = 8.1$, 5.1 Hz, 1H, β-Tyr CH), 5.53 (dd, $J = 8.9$, 7.4 Hz, 1H, Trp CH), 6.89 (s, 1H, Trp H_{Ar}), 7.08 (ddd, $J = 7.9$, 7.1, 0.8 Hz, 1H, Trp H_{Ar}), 7.16 (ddd, $J = 7.9$, 7.1, 0.8 Hz, 1H, Trp H_{Ar}), 7.21 (d, $J = 8.1$ Hz, 1H, β-Tyr NH), 7.31 (d, $J = 7.9$ Hz, 1H, Trp H_{Ar}), 7.34 (d, $J = 8.3$ Hz, 2H, Ar), 7.57 (d, $J = 7.9$ Hz, 1H, Trp H_{Ar}), 8.06 (d, $J = 8.3$ Hz, 2H, Ar), 8.21 ppm (s, 1H, Trp NH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 17.8$ (Ala CH_3), 23.5 (CH_2), 28.3 (C-(CH_3)$_3$), 30.9 (NCH_3), 46.7 (Ala CH), 52.1 (OCH_3), 53.8 (β-Tyr CH), 56.8 (Trp CH), 59.3 (OCH_3), 79.7 (C(CH$_3$)$_3$), 81.8 (*C*HOCH_3), 110.5 (quat. Trp), 111.2, 118.5, 119.5, 122.2 (2C, Trp), 123.4 (2C, Ar), 127.1 (quat. Trp), 128.7 (2C, Ar), 136.1 (quat. Trp), 144.3 (quat. Ar), 147.5 (quat. Ar), 155.2 (Boc), 169.5, 169.6, 174.5 ppm; HMRS (ESI): m/z calcd for $C_{31}H_{39}N_5O_9$ $[M+Na]^+$: 648.26400; found: 648.26502.

Tripeptide acid 21e (NO_2): To a solution of methyl ester **20e** (55.3 mg, 0.0884 mmol) in THF (0.4 mL) were added water (0.6 mL), methanol (0.3 mL) and NaOH (7.5 mg, 0.188 mmol, 2.1 equiv). The initial biphasic mixture became homogeneous with progressing saponification. After being stirred for 1 h at room temperature until complete conversion (controlled by TLC), the mixture was diluted with water (5 mL) and ethyl acetate (8 mL). It was carefully acidified with 1M $NaHSO_4$ to pH ~2 before the layers were separated and the aqueous phase extracted once with ethyl acetate (8 mL). The combined organic layers were washed with water, saturated NaCl solution, dried with Na_2SO_4, filtered, and concentrated in vacuo to afford the crude acid **21e** as a colorless foam. R_f (EtOAc/AcOH 100:1) = 0.4; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.83$ (d, $J = 6.9$ Hz, 3H, Ala CH_3), 1.39 (s, 9H, tBu), 2.93 (s, 3H, NCH_3), 3.18 (dd, $J = 15.3$, 9.9 Hz, 1H, CH_2), 3.28–3.34 (m, 1H, CH_2), 3.34 (s, 3H, OCH_3), 3.98 (d, $J = 6.3$ Hz, 1H, C*H*OCH_3), 4.42–4.49 (m, 1H, Ala CH), 5.40 (dd, $J = 7.6$, 7.1 Hz, 1H, β-Tyr CH), 5.46 (d, $J = 7.4$ Hz, 1H, Ala NH), 5.59 (dd, $J = 9.9$, 7.4 Hz, 1H, Trp CH), 6.88 (s, 1H, Trp H_{Ar}), 7.05 (app t, $J = 7.0$ Hz, 1H, Trp H_{Ar}), 7.13 (app t, $J = 7.0$ Hz, 1H, Trp H_{Ar}), 7.21 (d, $J = 8.6$ Hz, 1H, β-Tyr NH), 7.28 (d, $J = 7.9$ Hz, 1H, Trp H_{Ar}), 7.41 (d, $J = 8.4$ Hz, 2H, Ar), 7.53 (d, $J = 7.9$ Hz, 1H, Trp H_{Ar}), 8.04 (d, $J = 8.4$ Hz, 2H, Ar), 8.36 ppm (s, 1H, Trp NH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 17.0$ (Ala CH_3), 23.2 (CH_2), 28.3 (C(*C*H$_3$)$_3$), 30.6 (NCH_3), 46.6 (Ala CH), 54.0 (β-Tyr CH), 56.7 (Trp CH), 58.8 (OCH_3), 80.8 (C(CH$_3$)$_3$), 82.5 (*C*HOCH_3), 110.2 (quat. Trp), 111.2, 118.4, 119.5, 122.1, 122.3, 123.5 (2C, Ar), 127.1 (quat. Trp), 128.4 (2C, Ar), 136.1 (quat. Trp), 145.2 (quat. Ar), 147.4 (quat. Ar), 156.3 (Boc), 169.5, 170.7, 174.8 ppm; HMRS (ESI): m/z calcd for $C_{30}H_{37}N_5O_9$ $[M+Na]^+$: 634.24835; found: 634.24819.

Depsipeptide 22e (NO_2): The crude acid **21e** (52 mg, 0.0851 mmol) and alcohol **7** (32.8 mg, 0.128 mmol, 1.5 equiv) were dissolved in THF (1 mL) and Ph_3P (40 mg, 0.152 mmol, 1.8 equiv) was added at 0°C. This was followed by the dropwise addition of DIAD (0.030 mL, 0.152 mmol, 1.8 equiv). The cooling bath was removed and the mixture stirred overnight at room temperature. The reaction mixture was concentrated in vacuo and the residue purified by flash chromatography (petroleum ether/EtOAc 2:1 to 1:1) to give ester **22e** (62 mg, 86%) as a colorless foam. R_f (petroleum ether/EtOAc 1:1) = 0.30; $[\alpha]_D^{20} = -4.0$ (c = 1.00, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.77$ (d, $J = 6.6$ Hz, 3H, CH_3), 0.91 (d, $J = 6.1$ Hz, 3H, CH_3), 0.96 (d, $J = 6.8$ Hz, 3H, Ala CH_3), 1.01 (d, $J = 6.8$ Hz, 3H, CH_3), 1.40 (s, 9H, tBu), 1.41 (s, 9H, tBu), 1.56 (s, 3H, CH_3), 1.92 (dd, $J = 13.9$, 7.6 Hz, 1H, CH_2), 2.33 (dd, $J = 13.9$, 6.8 Hz, 1H, CH_2), 2.40–2.51 (m, 2H, 2 CH), 2.97 (s, 3H, NCH_3), 3.17 (dd, $J = 15.4$, 9.6 Hz, 1H, CH_2), 3.37 (dd, $J = 15.4$, 7.1 Hz, 1H, CH_2), 3.40 (s, 3H, OCH_3), 4.06 (d, $J = 4.5$ Hz, 1H, C*H*OCH_3), 4.46–4.61 (m, 2H, Ala CH, CO_2CH), 4.84 (d, $J = 9.9$ Hz, 1H, =CH), 5.34 (d, $J = 7.3$ Hz, 1H, Ala NH), 5.43 (dd, $J = 8.3$, 4.5 Hz, 1H, β-Tyr CH), 5.52 (dd, $J = 9.6$, 7.1 Hz, 1H, Trp CH), 6.88 (s, 1H, Trp H_{Ar}), 7.07 (ddd, $J = 7.8$, 7.1, 0.8 Hz, 1H, Trp H_{Ar}), 7.15 (ddd, $J = 8.1$, 7.1, 0.8 Hz, 1H, Trp H_{Ar}), 7.21 (d, $J = 8.3$ Hz, 1H, β-Tyr NH), 7.30 (d, $J = 8.1$ Hz, 1H, Trp H_{Ar}), 7.39 (d, $J = 8.6$ Hz, 2H, Ar), 7.56 (d, $J = 7.8$ Hz, 1H, Trp H_{Ar}), 8.04 (d, $J = 8.6$ Hz, 2H, Ar), 8.25 ppm (s, 1H, Trp NH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 16.4$, 16.6, 17.1, 17.6, 17.9, 23.5 (CH_2), 28.0 (C(*C*H$_3$)$_3$), 28.3 (C(*C*H$_3$)$_3$), 30.9 (NMe), 37.5, 38.6, 43.4 (CH_2), 46.7 (Ala CH), 53.8 (β-Tyr CH), 56.8 (Trp CH), 59.3 (OCH_3), 76.4 (CO_2*C*H), 79.6 (C(CH$_3$)$_3$), 79.9 (C(CH$_3$)$_3$), 81.4 (*C*HOCH_3), 110.5 (quat. Trp), 111.1, 118.4, 119.5, 122.1, 122.2, 123.3 (2C, Ar), 127.1 (quat. Trp), 127.5 (=CH), 129.1 (2C, Ar), 134.0 (=C<), 136.1 (quat. Trp), 144.3, 147.4, 155.2 (Boc), 168.6, 169.5, 174.5, 175.7 ppm; HMRS (ESI): m/z calcd for $C_{45}H_{63}N_5O_{11}$ $[M+Na]^+$: 872.44163; found: 872.44212.

Chondramide 2e (NO_2): To a stirred solution of compound **22e** (62.7 mg, 0.0738 mmol) in CH_2Cl_2 (2.2 mL) was added TFA (0.22 mL, 0.34 g, 2.98 mmol) at 0°C. The reaction mixture was allowed to warm to room temperature and after stirring for 22 h, the solvent was removed in vacuo (TLC control: CH_2Cl_2/MeOH/NH_3, 10:1:0.1). For azeotropic removal of TFA the residue was taken up in toluene (3×0.5 mL) and concentrated in vacuo each time. The crude product was dissolved in DMF (30 mL) and *i*Pr_2NEt (64 μL, 47.7 mg, 0.369 mmol, 5 equiv), HOBt (19.9 mg, 0.147 mmol, 2 equiv) and TBTU (47.4 mg, 0.147 mmol, 2 equiv) were added. The solution was stirred at room temperature for 20 h and then diluted with water (20 mL) and EtOAc (20 mL). The aqueous layer was extracted with EtOAc (3×20 mL) and the combined organic layers were washed with 5% aqueous $KHSO_4$ solution (20 mL), water (20 mL), saturated $NaHCO_3$ solution (20 mL), water (2×20 mL) and saturated NaCl solution (20 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (petroleum ether/EtOAc, 1:3 to 0:1) to give depsipeptide **2e** (27.3 mg, 48%) as a colorless foam. R_f (EtOAc) = 0.41; $[\alpha]_D^{20} = +18.5$ (c = 1.00, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.84 (d, J = 6.6 Hz, 3H, CH_3), 0.85 (d, J = 6.1 Hz, 3H, CH_3), 1.07 (d, J = 6.8 Hz, 3H, CH_3), 1.17 (d, J = 6.8 Hz, 3H, CH_3), 1.64 (s, 3H, CH_3), 1.88 (d, J = 13.1 Hz, 1H, CH_2), 2.36–2.51 (m, 3H, 2 CH, CH_2), 2.95 (s, 3H, NCH_3), 3.17 (dd, J = 15.2, 8.6 Hz, 1H, CH_2), 3.22 (s, 3H, OCH_3), 3.31 (dd, J = 15.2, 7.8 Hz, 1H, CH_2), 3.75 (d, J = 8.1 Hz, 1H, C*H*OCH_3), 4.77–4.83 (m, 2H, Ala CH, CO_2CH), 4.90 (d, J = 9.1 Hz, 1H, =CH), 5.30 (t, J = 8.3 Hz, 1H, β-Tyr CH), 5.60 (t, J = 8.1 Hz, 1H, Trp CH), 6.50 (d, J = 7.3 Hz, 1H, Ala NH), 6.82 (s, 1H, Trp H_{Ar}), 7.06 (d, J = 8.8 Hz, 1H, β-Tyr NH), 7.10 (ddd, J = 8.1, 7.1, 0.8 Hz, 1H, Trp H_{Ar}), 7.17 (ddd, J = 8.1, 7.1, 0.8 Hz, 1H, Trp H_{Ar}), 7.24 (d, J = 8.6 Hz, 2H, Ar), 7.32 (d, J = 8.1 Hz, 1H, Trp H_{Ar}), 7.59 (d, J = 8.1 Hz, 1H, Trp H_{Ar}), 8.06 (d, J = 8.6 Hz, 2H, Ar), 8.11 ppm (s, 1H, Trp NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 15.8, 16.6, 17.3, 18.6, 20.0, 23.6 (CH_2), 30.2 (NCH_3), 37.0, 40.2, 44.1 (CH_2), 45.2 (Ala CH), 54.4 (β-Tyr CH), 56.0 (Trp CH), 58.2 (OCH_3), 76.8 (CO_2*C*H), 81.9 (*C*HOCH_3), 110.2 (quat. Trp), 111.2, 118.5, 119.6, 122.2, 122.3, 123.4, 127.1 (quat. Trp), 127.8 (=CH), 127.9, 134.3 (=C<), 136.1 (quat. Trp), 144.5, 147.3, 169.77, 169.82, 174.1, 174.5 ppm; HMRS (ESI): m/z calcd for $C_{36}H_{45}N_5O_8$ $[M+Na]^+$: 698.31603; found: 698.31672.

Chondramide 2f (NH_2): *By catalytic hydrogenation*: A solution of chondramide **2e** (6.1 mg, 9.03 μmol) in methanol (0.5 mL) was hydrogenated overnight in a round bottom flask connected to a hydrogen filled balloon, using 10% Pd on carbon (TLC control: CH_2Cl_2/MeOH, 10:1). Upon complete conversion, the solvent was evaporated and the residue purified by flash chromatography (CH_2Cl_2/MeOH, 30:1 to 20:1) to afford chondramide **2f** (2.7 mg, 47%) as white foam. A mixed fraction, containing **2f** and another unknown compound as major component was also isolated. Complete conversion was observed, but the reaction was not very clean, likely because of formation of RNO and/or RNHOH, as suggested by LC/MS examination of the mixed fraction. No evidence for competitive hydrogenation of the double bond was found (by ^{1}H NMR).

By reduction with sodium dithionite: Alternatively, reduction of chondramide **2e** (5.8 mg, 8.58 μmol) was carried out in THF (0.5 mL), H_2O (0.5 mL) in the presence of $Na_2S_2O_4$ (20 mg, 0.115 mmol) overnight at room temperature. Conversion was high but not complete. The reaction mixture was diluted with saturated NaCl solution and EtOAc with addition of some saturated $NaHCO_3$ solution. The water phase was extracted once more with EtOAc. The combined organic extracts were dried with Na_2SO_4, filtered, and evaporated. The desired NH_2-chondramide **2f** (2 mg, 36%) was isolated successfully by flash chromatography (CH_2Cl_2/MeOH 30:1 to 20:1) as a white foam. R_f (CH_2Cl_2/MeOH 20:1) = 0.26; $[\alpha]_D^{20} = +24.9$ (c = 0.35, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.80 (d, J = 6.3 Hz, 3H, CH_3), 0.85 (d, J = 6.8 Hz, 3H, CH_3), 1.03 (d, J = 6.8 Hz, 3H, Ala CH_3), 1.15 (d, J = 7.1 Hz, 3H, CH_3), 1.63 (s, 3H, CH_3), 1.80 (d, J = 13.9 Hz, 1H, CH_2), 2.33–2.40 (m, 2H, 2 CH), 2.53 (d, J = 10.4, 13.6 Hz, 1H, CH_2), 2.94 (s, 3H, NCH_3), 3.14 (dd, J = 15.5, 9.2 Hz, 1H, CH_2), 3.23 (s, 3H, OCH_3), 3.27 (dd, J = 15.5, 6.8 Hz, 1H, CH_2), 3.68 (br s, 2H, NH_2), 3.75 (d, J = 7.3 Hz, 1H, C*H*OCH_3), 4.77–4.84 (m, 2H, Ala CH, 7-H), 4.90 (d, J = 8.8 Hz, 1H, =CH), 5.25 (dd, J = 7.3, 9.1 Hz, 1H, β-Tyr CH), 5.60 (dd, J = 7.1, 9.1 Hz, 1H, Trp CH), 6.58 (d, J = 7.3 Hz, 1H, Ala NH), 6.58 (d, J = 8.3 Hz, 2H, Ar), 6.81 (d, J = 2.2 Hz, 1H, Trp H_{Ar}), 6.92–6.96 (m, 3H, β-Tyr NH, Ar), 7.11 (ddd, J = 8.1, 7.8, 1.0 Hz, 1H, Trp H_{Ar}), 7.17 (ddd, J = 8.1, 7.8, 1.0 Hz, 1H, Trp H_{Ar}), 7.30 (d, J = 7.8 Hz, 1H, Trp H_{Ar}), 7.90 (d, J = 7.8 Hz, 1H, Trp H_{Ar}), 7.87 ppm (s, 1H, Trp NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 15.3, 16.6, 17.6, 18.6, 20.5, 23.3 (CH_2), 30.1 (NCH_3), 37.1, 40.3, 43.8 (CH_2), 45.3 (Ala CH), 53.9 (β-Tyr CH), 55.7 (Trp CH), 58.0 (OCH_3), 77.2 (CO_2*C*H), 82.6 (*C*HOCH_3), 110.7 (quat. Trp), 111.0, 115.0 (2C, Ar), 118.6, 119.4, 122.05, 122.11, 127.2 (quat. Trp), 127.7 (quat. Ar), 127.9 (2C, Ar), 128.4 (=CH), 134.1 (=C<), 136.1 (quat. Trp), 145.9, 169.6, 170.6, 174.0, 174.5 ppm; HMRS (ESI): m/z calcd for $C_{36}H_{47}N_5O_6$ $[M+Na]^+$: 668.34186; found: 668.34224.

Cytotoxicity assay and EC_{50} determination: Human foreskin fibroblast (HFF) cells grown in Dulbecco's modified Eagle's medium with 10% fetal bovine serum and 10 μg mL^{-1} gentamicin were seeded at 2000 cells per well in 96-well microtiter plates. After 2 h of incubation at 37°C in a humidified atmosphere with 5% CO_2, jasplakinolide and chon A analogues were added to reach the final concentrations of 0.0049–5 μM (in two-fold increments). The number of viable cells was determined 72 h later using the CellTiter 96 AQ_{ueous} One Solution Cell Proliferation Assay (Promega). Absorbance at 570 λ (A_{570}) measured was normalized to percentage of control (untreated) and plotted against logarithmic conversions of compound concentrations using Prism 5.0 (GraphPad Software). The log (inhibitor) vs. response curve with variable slope was generated from triplicates of data to calculate EC_{50} values with Prism 5.0.

MTT test with the L-929 mouse fibroblasts: Growth inhibitory activity on L-929 mouse fibroblasts were determined after incubation with serial dilutions of the compounds for 5 d using an MTT assay.[27,28] The IC_{50} was estimated from the concentration dependent activity curves.

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