# ChemInform Abstract: Jaspamides M—P: New Tryptophan Modified Jaspamide Derivatives from the Sponge Jaspis splendans

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# Jaspamides M–P: new tryptophan modified jaspamide derivatives from the sponge *Jaspis splendans*

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ABSTRACT

Four new jaspamide derivatives with antimicrofilament activity were isolated from the marine sponge *Jaspis splendans*. Their structures were elucidated by NMR and MS analysis. A structure–activity relationship analysis on all natural jaspamides is also reported here.



## 1. Introduction

In the frame of our long-term research program directed at discovery of anticancer compounds from marine organisms, we recently focused our attention towards jaspamide,[1] a cyclodepsipeptide with remarkable biological proprieties such as antifungal,[2] anthelminthic, insecticidal and ichthyotoxic activities.[1] It has been shown to be active against 36 human solid tumour cell cultures.[3] The potent antiproliferative activity is ascribable to the inhibition of the actin dynamics through the binding to F-actin with consequent extensive changes in microfilament morphology and eventual cell death. Considering that jaspamide is too toxic, as evidenced by preclinical trials,[4] it represents an interesting lead, as demonstrated by intense and still active experimentation devoted to the isolation of natural analogues of the parent compound,[5] total synthesis[6] and structural modification.[7] We have found that a *Jaspis* sponge, collected at the Vanuatu Islands, was a good source of jaspamide and congeners.

Previous chemical investigation on this sponge has led to the isolation of a small library of jaspamide-related derivatives,[8–10] which are useful for the investigation of the structure–activity relationships (Fig. 1).

In this paper, we describe the isolation from the same sponge, structure determination and biological activity of new jaspamide derivatives (jaspamides M–P), all characterised by a modification of the *N*-methylabrine (*N*-methyl-2-bromotrypthophan) residue (Fig. 2). The structures were elucidated by spectroscopic methods and the stereochemistry of the amino acids was determined by Marfey's method.[11]

## 2. Results and discussion

The lyophilised sponge was extracted with methanol and the crude methanolic extract was subjected to a modified Kupchan's[12,13] partitioning procedure. Chromatography of the $CHCl_3$ extract (ca. 3.2 g) by silica gel MPLC followed by repeated reversed phase HPLC yielded jaspamides M–P (**12**–**15**).

As suggested by the molecular formula, $C_{35}H_{43}BrN_4O_6$, jaspamide M (**12**) is a demethyl analogue of jaspamide. The analysis of the $^1H$ NMR spectrum indicated the absence of a methyl proton signal at $\delta_H$ 3.00, assigned to *N*-methyl group of the *N*-methylabrine residue. The $^1H$ NMR spectrum also evidenced the presence of an exchangeable proton signal at $\delta_H$ 6.11 (d, $J$=7.0 Hz) that, on the basis of the 2D NMR data was assigned to the NH proton of a 2-bromotryptophan unit. Therefore jaspamide M is congener of jaspamide having an abrine unit in the place of *N*-methylabrine. The similarity in $^1H$ and $^{13}C$ NMR shifts observed for jaspamide M and jaspamide implied that the stereogenic centres in polypropionate fragment had the same relative configurations.

The absolute configurations of the amino acid residues were determined by LC/MS analysis of the acid hydrolysate derivatised with Marfey's reagent ((1-fluoro-2,4-dinitrophenyl)-5-L-alaninamide; L-FDAA)[11] and comparison with appropriate amino acid standards, that secured the D-configuration for the alanine residue. Both aromatic residues, β-Tyr and abrine, were not recovered in the acid hydrolysate of jaspamide M; therefore they were both transformed into aspartate by ozonolysis followed by oxidative workup.[14] The presence of a single peak corresponding to D-Asp was observed by ion-selective monitoring for FDAA-Asp ($m/z$ 386) indicating that both β-Tyr and abrine residues had a D-configuration.

The HRESIMS analysis of jaspamide N indicated the presence of an additional oxygen atom in **13** in comparison with jaspamide (**1**). Indeed the $^1H$ NMR spectrum showed the presence of

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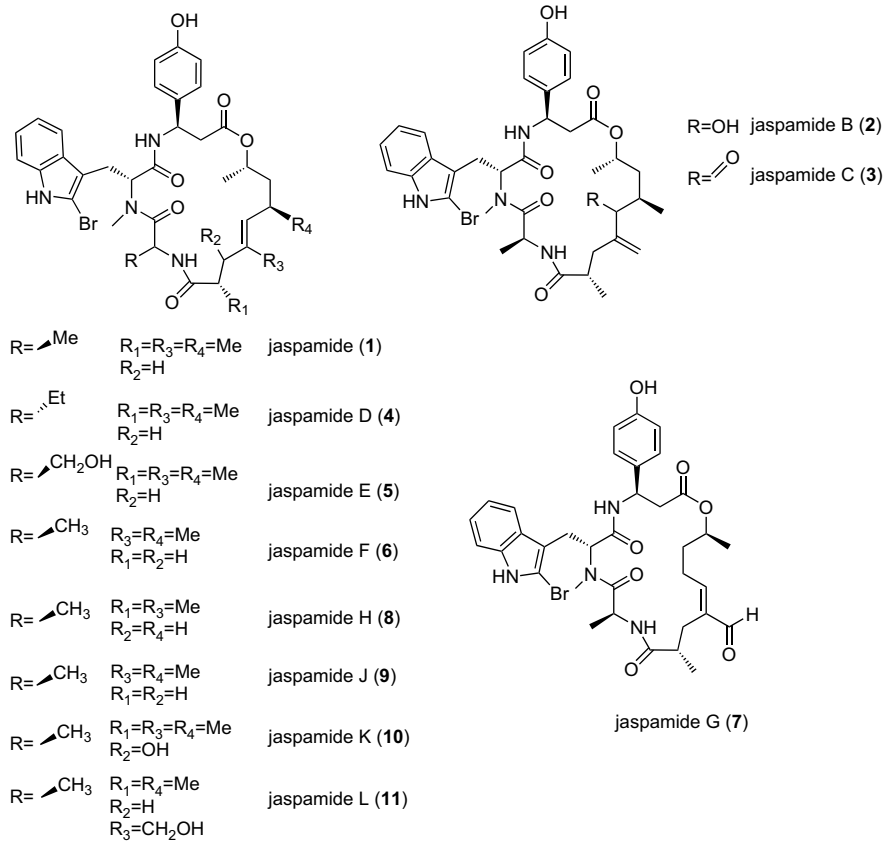


**Figure 1.** Jaspamide derivatives with antimicrofilament activity from *Jaspis splendans*.

a hydroxymethine doublet signal at $\delta_H$ 5.57 ($J$=9.6 Hz). The extra hydroxyl group was positioned at the β-carbon of the methylabrine residue on the basis of the HMBC correlations exhibited by the proton at $\delta_H$ 5.57 with C23, C24 and C30 carbon atoms of the indole nucleus and of the COSY correlation between the carbinol proton and the α-hydrogen of the *N*-methylabrine residue. β-Hydroxy tryptophan derivatives are well known as products of the biotransformation of the Trp residue.[15] A *N*-alkylated β-hydroxy tryptophan unit was found as a constituent of the cyclopeptide of marine origin cyclomarin,[16] and very recently, the cyclomarin biosynthetic gene cluster was identified including an oxygenase likely involved in the β-oxidation of the Trp unit.[17] The stereochemistry of D-alanine and D-β-Tyr residues was established as previously described for jaspamide M.

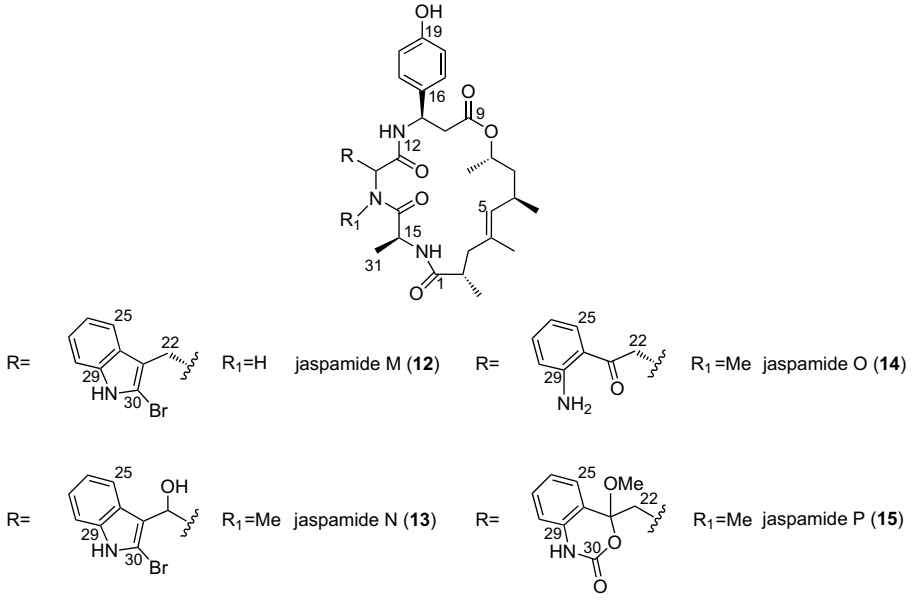


**Figure 2.** Jaspamides M–P (**12**–**15**) from *Jaspis splendans*.

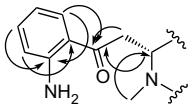


**Figure 3.** Key HMBC correlation for *N*-methylkynurenine residue in jaspamide O (**14**).

Jaspamide O (**14**) was obtained as colourless amorphous solid and showed the pseudomolecular ion $[M+H]^+$ peak at 635.3515 in the HRESIMS spectrum, corresponding to the molecular formula $C_{35}H_{46}N_4O_7$. The analysis of the NMR data revealed that compound differed from parent jaspamide with respect to the tryptophan moiety. COSY analysis revealed a spin system of four contiguous aromatic protons H-25 through H-28 that, from HMBC data (Fig. 3), were assigned to an *ortho*-disubstituted phenyl ring. The linkage of a carbonyl group ($\delta_C$ 198.3) to the aromatic moiety was inferred by the HMBC correlation from H-25 to C-23 (Fig. 3). The carbonyl carbon signal also showed HMBC correlation with two diastereotopic methylene protons at $\delta_H$ 3.28 (dd, $J$=7.6 and 17.2 Hz) and 3.74 (dd, $J$=7.6 and 17.2 Hz) and with an α-proton ($\delta_H$ 5.82, t, $J$=7.6 Hz; $\delta_C$ 53.0). The second substituent on the phenyl ring was assigned as an amino group on the basis of $^{13}C$ NMR chemical shift considerations and of the spectrometric data. The HMBC cross peak between the α-carbon and *N*-methyl protons at $\delta_H$ 2.98, and the analysis of the chemical shifts of the phenyl ring gave evidence for the presence of a *N*-methylkynurenine residue. The stereochemistry of the amino acid residues was determined as described for jaspamide M. The *N*-methylkynurenine residue afforded D-*N*-methylaspartic acid and therefore was assigned as D-configurated. The *N*-methylkynurenine residue, likely derived from tryptophan through oxidation is quite rare in cyclic peptides. To the best of our knowledge, it was only found in brunsvicamide C, a cyclic hexapeptide from the cyanobacterium *Tychonema* sp.[18]

Jaspamide P (**15**) was isolated as very minor component. The molecular formula $C_{37}H_{48}N_4O_9$ was deduced from the HRESIMS analysis of the pseudomolecular $[M+Na]^+$ ion. The analysis of the NMR data indicated, as for the previously described jaspamides M–O, a perturbation of the tryptophan nucleus. In the $^1H$ NMR spectrum an additional methoxy group at $\delta_H$ 3.19 was observed. COSY analysis indicated the presence of four contiguous phenyl protons, and therefore an *ortho*-disubstituted benzene ring. The linkage of a ketal carbon to the phenyl ring was deduced from the HMBC cross peak between one aromatic proton at $\delta_H$ 7.26 and a ketal carbon at $\delta_C$ 108.0. The same carbon showed HMBC correlations with the methoxy protons at $\delta$ 3.19 and with α and β protons of a *N*-methyl amino acyl subunit (Fig. 4). The second substituent on the phenyl ring was assigned as a nitrogen on the basis of the diagnostic $^{13}C$ NMR resonances of C24, C28 and C29 nuclei (see Table 3). Even if, due to the scarcity of the compound, the NMR resonance of the acyl group could not be revealed, the molecular formula clearly indicated the presence of a 4-methoxy-1,3-benzoxazine-2-one system. The proposed substructure was indirectly supported by biogenetic considerations. In fact it could arise from jaspamide O through the addition of a carboxylic-acid equivalent and subsequently ring closure to a carbamate. Therefore the structure of jaspamide P is that depicted in Figure 2. The stereochemistry of the Ala and β-Tyr residue was determined as previously described whereas the stereochemistry of 4-methoxy-1,3-benzoxazine-2-one system remained undetermined.

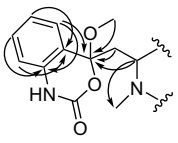


**Figure 4.** Key HMBC correlation for 4-methoxy-1,3-benzoxazine–2-one system in jaspamide P (**15**).

The $IC_{50}$ values of jaspamides M–P against HT-29 and MCF-7 tumour cell lines were reported in Table 1. In order to draw same conclusive considerations on the structure–activity relationship, the Table 1 reports the $IC_{50}$ values for all of the jaspamide compounds so far isolated from the marine sponge *Jaspis splendans*.

All tested jaspamide derivatives exhibited a consistent antiproliferative activity with a $IC_{50}$ value ranging from 0.01 to 10 μM. The antimicrofilament activity was also measured and parallels the observed in vitro cytotoxicity. An analysis of the structures depicted in Figures 1 and 2 indicated that the sole invariable residue is β-tyrosine whereas a wide variability of the alkylation and oxidation pattern of the polypropionate subunit as well as of the identity of the first two amino acid residues of the tripeptide portion was observed. Therefore the above residue appears the only strict feature essential for biological activity in these tumour cell lines, mainly assuring a β-turn motif for the folding of the entire molecule.

Also the modifications of the methylabrine residue, claimed as essential for the observed biological activity,[19] appears to have little influence (jaspamide M, O, P) on the observed antiproliferative effect with the exception of jaspamide N, where the β-hydroxylation of tryptophan unit causes a diminution of the biological activity.

As suggested by Maier,[7c,d] also the 1,3 methyl groups of the polypropionate subunit, imposing to the macrocycle conformational constraints through *syn* pentane interactions, play a significant role in assuming a correct folding of the entire molecule as suggested by the drop of activity for jaspamides F and H.

## 3. Experimental

### 3.1. General experimental procedures

For general experimental procedures see Gala et al.[10]

### 3.2. Sponge material and separation of individual peptides

For sponge material see Gala et al.[10]

The lyophilised material (360 g) was extracted with methanol (4×2.5 L) at room temperature and the crude methanolic extract (90 g) was subjected to a modified Kupchan's partitioning

**Table 1**
Cytotoxic activity of jaspamide B–P (**2**–**15**)

| Compound | Cell Line $IC_{50}$ (μM)[a] | |
|---|---|---|
| | MCF-7 | HT-29 |
| Jaspamide (**1**) | 0.019±0 | 0.035±0 |
| Jaspamide B (**2**) | 3.4±1.9 | 3.3±0.9 |
| Jaspamide C (**3**) | 2.0±0 | 2.6±0.3 |
| Jaspamide D (**4**) | 0.05±0 | 0.08±0 |
| Jaspamide E (**5**) | 0.02±0 | 0.02±0 |
| Jaspamide F (**6**) | 30.0±0 | Not tested |
| Jaspamide G (**7**) | 0.60±0.07 | 1.66±0.07 |
| Jaspamide H (**8**) | 30±0 | Not tested |
| Jaspamide J (**9**) | 5.0±0 | Not tested |
| Jaspamide K (**10**) | 0.48±0.09 | 0.90±0.07 |
| Jaspamide L (**11**) | 0.61±0 | Not tested |
| Jaspamide M (**12**) | 0.10±0 | 0.18±0 |
| Jaspamide N (**13**) | 33±0 | Not tested |
| Jaspamide O (**14**) | 0.38±0.09 | 0.30±0 |
| Jaspamide P (**15**) | 12.0±2.1 | 6.5±1.1 |

[a] MCF-7: human breast adenocarcinoma; HT-29: colon carcinoma.

procedure as follows. The methanol extract was dissolved in a mixture of MeOH/$H_2O$ containing 10% $H_2O$ and partitioned against *n*-hexane. The water content of the MeOH extract was adjusted to 30% (v/v) and partitioned against $CHCl_3$. The aqueous phase was concentrated to remove MeOH and then extracted with *n*-BuOH. The chloroform-soluble material (ca. 3.2 g) was chromatographed over silica gel MPLC (Macherey-Nagel 200–400 mesh, eluting with $CH_2Cl_2$/MeOH 0–10%), the fractions were collected on the basis of their TLC retention factors.

Fraction 4 was purified by HPLC on a μ-Bondapack C18 column (10 μ, 300×7.8 mm, 4.0 mL/min) with 65% MeOH/$H_2O$ as eluent to give 96.4 mg of jaspamide ($t_R$=20 min). The baseline was collected and further purified on a Phenomenex Luna C18 column (3 μ, 150×4.6 mm, 1.0 mL/min) with 65% MeOH/$H_2O$ as eluent to give 0.6 mg of jaspamide P ($t_R$=22.2 min) and 0.9 mg of jaspamide O ($t_R$=26.5 min).

Fraction 6 was purified by HPLC on Phenomenex Luna C18 column with 65% MeOH/$H_2O$ as eluent to give 23.2 mg of jaspamide ($t_R$=40.5 min) together with two peaks at 24 min and 26.2 min. The compound at $t_R$=26.2 min was purified on Phenomenex Luna C18 column with 63% MeOH/$H_2O$ as eluent to give 0.5 mg of jaspamide M ($t_R$=37.1 min) whereas compound at $t_R$=24 min was purified on Phenomenex Luna C18 column with 65% MeOH/$H_2O$ as eluent to give 0.7 mg of jaspamide N ($t_R$=19.5 min).

### 3.3. Characteristic data for each compound

#### 3.3.1. Jaspamide M

White amorphous solid; $[\alpha]_D^{25}$ +20.6 (*c* 0.03, chloroform); $^1H$ and $^{13}C$ NMR data in $CDCl_3$ given in Table 2; ESIMS: *m*/*z* 695.6–697.6 $[M+H]^+$, 717.4–719.4 $[M+Na]^+$. HRMS (ESI): calcd for $C_{35}H_{44}BrN_4O_6$ 695.2444; found 695.2494 $[M+H]^+$.

#### 3.3.2. Jaspamide N

White amorphous solid; $[\alpha]_D^{25}$ −41.4 (*c* 0.07, chloroform); $^1H$ and $^{13}C$ NMR data in $CDCl_3$ given in Table 2; ESIMS: *m*/*z* 725.5–727.5 $[M+H]^+$, 757.5–759.5 $[M+Na]^+$. HRMS (ESI): calcd for $C_{36}H_{46}BrN_4O_7$ 725.2550; found 725.2465 $[M+H]^+$.

#### 3.3.3. Jaspamide O

White amorphous solid; $[\alpha]_D^{25}$ −70.0 (*c* 0.02, chloroform); $^1H$ and $^{13}C$ NMR data in $CDCl_3$ given in Table 3; ESIMS: *m*/*z* 635.5 $[M+H]^+$, 657.3 $[M+Na]^+$. HRMS (ESI): calcd for $C_{35}H_{47}N_4O_7$ 635.3445; found 635.3515 $[M+H]^+$.

#### 3.3.4. Jaspamide P

White amorphous solid; $[\alpha]_D^{25}$ −85.0 (*c* 0.02, chloroform); $^1H$ and $^{13}C$ NMR data in $CDCl_3$ given in Table 3; ESIMS: *m*/*z* 737.5 $[M+Na]^+$. HRMS (ESI): calcd for $C_{37}H_{48}N_4NaO_9$ 715.3319; found 715.3257.

### 3.4. Determination of absolute stereochemistry

#### 3.4.1. Peptide hydrolysis

Peptide samples (200 μg) were dissolved in degassed 6 M HCl (0.5 mL) in an evacuated glass tube and heated at 160 °C for 16 h. The solvent was removed in vacuo and the resulting material was subjected to further derivatisation.

#### 3.4.2. LC/MS analysis of Marfey's (FDAA) derivatives[11]

A portion of the hydrolysate mixture (800 μg) or the amino acid standard (500 μg) was dissolved in 80 μL of a 2:3 solution of TEA/MeCN and treated with 75 μL of 1% 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (FDAA) in MeCN/acetone 1:2. The vials were heated at 70 °C for 1 h, and the contents were neutralised with 0.2 M HCl (50 μL) after cooling to room temperature. An aliquot of the L-FDAA (or D-FDAA) derivative was dried under vacuum, diluted with MeCN/5% HCOOH in $H_2O$ (1:1), and separated on a Vydac C18 (25×1.8 mm i.d.) column by means a linear gradient from 10% to 50% aqueous acetonitrile containing 5% formic acid and 0.05% trifluoroacetic acid, over 45 min at 1 mL/min. The RP-HPLC system was connected to the electrospray ion source by inserting a splitter valve and the flow going into the mass spectrometer source was set at a value of 100 μL/min. Mass spectra were acquired in positive ion detection mode (*m*/*z* interval of 320–900) and the data were analysed using the suite of programs Xcalibur (ThermoQuest, San José, California); all masses were reported as average values. Capillary temperature was set at 280 °C, capillary voltage at 37 V, tube lens offset at 50 V and ion spray voltage at 5 V.

Retention times of authentic FDAA-amino acids (min): L-Ala (16.6 min), D-Ala (20.5 min).

The hydrolysate of jaspamides M–P contained L-Ala (16.6 min).

#### 3.4.3. Determination of the absolute stereochemistry of aromatic amino acids

To determine the absolute configuration of abrine, *N*-methylkynurenine and β-Tyr residues, an authentic sample of jaspamide was used as standard.

A stream of ozone in $O_2$ was bubbled through cooled solutions of jaspamides M–P (0.1 mg) or of jaspamide (0.5 mg) in MeOH (0.5 mL) at −78 °C for 1 h. Hydrogen peroxide (35%, 10 drops) was

**Table 2**
$^1H$ and $^{13}C$ NMR data ($CDCl_3$, 700 MHz) for compounds **12** and **13**

| Position | Jaspamide M (**12**) | | Jaspamide N (**13**) | |
|---|---|---|---|---|
| | $\delta_H$[a] | $\delta_C$ | $\delta_H$[a] | $\delta_C$ |
| 1 | — | 175.5 | — | 174.8 |
| 2 | 2.52 m | 40.0 | 2.44 m | 39.2 |
| 3 | 1.90 d (16.2), 2.35 dd (11.2, 16.2) | 41.2 | 1.83 d (15.7), 2.38 m | 40.7 |
| 4 | — | 133.7 | — | 133.9 |
| 5 | 4.73 d (7.5) | 128.7 | 4.68 d (9.2) | 127.1 |
| 6 | 2.25 m | 29.7 | 2.26 m | 29.3 |
| 7 | 1.13 m, 1.13 m | 43.7 | 1.26 m, 1.26 m | 43.5 |
| 8 | 4.62 m | 71.4 | 4.64 m | 71.2 |
| 9 | — | 171.5 | — | 171.3 |
| 10 | 2.44 dd (4.4, 14.6), 2.56 dd (6.4, 14.6) | 40.7 | 2.67 ovl, 2.66 ovl | 40.2 |
| 11 | 5.13 m | 49.8 | 5.34 m | 48.9 |
| 12 | — | 170.1 | — | 170.4 |
| 13 | 4.74 q (7.0) | 53.6 | 6.01 d (9.6) | 59.3 |
| 14 | — | 173.0 | — | 174.4 |
| 15 | 4.47 m | 49.0 | 4.65 m | 45.6 |
| 16 | — | 132.6 | — | 132.2 |
| 17 | 6.95 d (8.3) | 127.6 | 7.07 d (8.5) | 127.4 |
| 18 | 6.70 d (8.3) | 115.7 | 6.84 d (8.5) | 115.7 |
| 19 | — | 154.9 | — | 155.3 |
| 20 | 6.70 d (8.3) | 115.7 | 6.84 d (8.5) | 115.7 |
| 21 | 6.95 d (8.3) | 127.6 | 7.07 d (8.5) | 127.4 |
| 22 | 3.30 d (7.0), 3.30 d (7.0) | 26.4 | 5.57 d (9.6) | 66.3 |
| 23 | — | 110.6 | — | 113.2 |
| 24 | — | 127.5 | — | 126.3 |
| 25 | 7.28 d (8.1) | 110.9 | 7.24 ovl | 110.5 |
| 26 | 7.12 dd (7.3, 8.1) | 121.3 | 7.15 dd (7.3, 7.8) | 123.2 |
| 27 | 7.19 dd (7.3, 7.8) | 123.2 | 7.12 dd (7.3, 7.8) | 120.9 |
| 28 | 7.59 d (7.8) | 118.8 | 7.82 d (7.8) | 119.6 |
| 29 | — | 136.8 | — | 136.7 |
| 30 | — | 108.0 | — | 109.5 |
| 31 | 1.17 d (6.8) | 19.1 | 0.44 d (7.0) | 17.4 |
| Me-2 | 1.13 d (6.8) | 20.3 | 1.10 d (6.6) | 20.6 |
| Me-4 | 1.60 s | 18.8 | 1.56 s | 18.7 |
| Me-6 | 0.81 d (6.6) | 22.3 | 0.83 d (6.6) | 22.0 |
| Me-8 | 1.01 d (6.3) | 19.3 | 1.08 d (6.4) | 19.5 |
| Me-Trp | | | 2.88 s | 32.2 |
| NH-Trp | 6.11 d (7.0) | — | | |
| NH-Ala | 6.41 d (6.6) | — | 6.50 d (6.8) | — |
| NH-Tyr | 7.60 d (7.8) | — | 8.01 d (8.3) | — |
| NH-29 | 8.24 s | — | 8.16 s | — |

Ovl: overlapped.
[a] Coupling constants are in parentheses and given in hertz. $^1H$ and $^{13}C$ assignments aided by COSY, TOCSY, HSQC and HMBC experiments.

**Table 3**
$^{1}H$ and $^{13}C$ NMR data ($CDCl_3$, 700 MHz) for compounds **14** and **15**

| Position | Jaspamide O (**14**) | | Jaspamide P (**15**) | |
|---|---|---|---|---|
| | $\delta_H$[a] | $\delta_C$ | $\delta_H$[a] | $\delta_C$ |
| 1 | — | 175.5 | — | 174.4 |
| 2 | 2.58 m | 40.6 | 2.57 m | 39.6 |
| 3 | 1.95 d (16.0), 2.40 dd (11.4, 16.0) | 40.5 | 1.90 d (16.0), 2.44 dd (11.3, 16.0) | 40.7 |
| 4 | — | 133.8 | — | 133.6 |
| 5 | 4.83 d (9.3) | 127.9 | 4.75 d (9.3) | 127.8 |
| 6 | 2.27 m | 29.0 | 2.23 m | 29.5 |
| 7 | 1.19 ovl, 1.34 ovl | 43.5 | 1.08 ovl, 1.21 ovl | 43.6 |
| 8 | 4.65 m | 70.4 | 4.60 m | 71.0 |
| 9 | — | 170.5 | — | 171.1 |
| 10 | 2.74 dd (4.6, 14.8), 2.62 dd (6.2, 14.8) | 40.1 | 2.59 dd (5.5, 14.8), 2.65 dd (4.9, 14.8) | 40.3 |
| 11 | 5.29 m | 48.9 | 5.14 m | 49.3 |
| 12 | — | 169.1 | — | 168.4 |
| 13 | 5.82 t (7.6) | 53.0 | 5.49 dd (4.0, 9.0) | 53.1 |
| 14 | — | 174.5 | — | 173.8 |
| 15 | 4.89 m | 46.2 | 4.90 m | 46.8 |
| 16 | — | 131.7 | — | 131.9 |
| 17 | 7.15 d (8.4) | 127.6 | 7.00 d (8.4) | 127.5 |
| 18 | 6.77 d (8.4) | 115.6 | 6.69 d (8.4) | 115.7 |
| 19 | — | 155.3 | — | 155.8 |
| 20 | 6.77 d (8.4) | 115.6 | 6.69 d (8.4) | 115.7 |
| 21 | 7.15 d (8.4) | 127.6 | 7.00 d (8.4) | 127.5 |
| 22 | 3.74 dd (7.6, 17.2), 3.28 dd (7.6, 17.2) | 36.6 | 2.47 dd (9.0, 15.5), 2.97 dd (4.0, 15.5) | 37.4 |
| 23 | — | 198.3 | — | 108.0 |
| 24 | — | 117.8 | — | 117.8 |
| 25 | 7.78 br dd (8.0, 1.2) | 131.1 | 7.26 ovl | 126.1 |
| 26 | 6.66 dd (8.0, 8.6) | 116.1 | 7.10 dd (7.8, 8.3) | 124.1 |
| 27 | 7.27 ovl | 134.9 | 7.29 dd (7.8, 8.5) | 131.1 |
| 28 | 6.64 d (7.6) | 117.6 | 6.77 d (8.5) | 114.6 |
| 29 | — | 150.7 | — | 135.2 |
| 30 | | | — | n.o. |
| Me-15 | 1.33 d (6.6) | 18.0 | 1.39 d (6.7) | 18.1 |
| Me-2 | 1.19 d (6.9) | 20.3 | 1.19 d (6.9) | 21.0 |
| Me-4 | 1.58 s | 18.5 | 1.60 s | 18.7 |
| Me-6 | 0.87 d (6.6) | 21.8 | 0.80 d (6.6) | 22.3 |
| Me-8 | 1.09 d (6.4) | 19.0 | 1.06 d (6.3) | 19.2 |
| Me-Trp | 2.98 s | 31.2 | 3.0 s | 31.2 |
| NH-Ala | 6.80 d (6.6) | — | 6.84 d (6.7) | — |
| NH-Tyr | 7.56 d (8.7) | — | 7.72 d (8.4) | — |
| NH-29 | | | 8.20 br s | — |
| $-OCH_3$ | | | 3.19 s | 51.2 |

Ovl: overlapped; n.o.: not observed.
[a] Coupling constants are in parentheses and given in hertz. $^{1}H$ and $^{13}C$ assignments aided by COSY, TOCSY, HSQC and HMBC experiments.

added to the reaction mixture, which was then allowed to stand at room temperature overnight. The solvent was removed under a stream of $N_2$. The ozonolysis products of jaspamides M–P and jaspamide were then dissolved in degassed 6 M HCl (0.5 mL) in an evacuated glass tube and heated at 160 °C for 16 h. The solvent was removed in vacuo. The resulting material from jaspamide was subjected to Marfey's derivatisation with L- and D-FDAA and LC/MS analysis.

Retention times of authentic FDAA-amino acids from jaspamide: L-MeAsp (14.2 min), D-MeAsp (16.3 min), L-Asp (15.6 min), D-Asp (16.8 min).

The hydrolysate of all jaspamides contained D-Asp (16.8 min).

The hydrolysate of jaspamide O also contained D-MeAsp (16.3 min).

### 3.5. Cytotoxicity assays

For cytotoxicity assays and immunofluorescence assays see Gala et al.[10]

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