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# Conformational studies of free and $Li^+$ complexed jasplakinolide, a cyclic depsipeptide from the Fijian marine sponge *Jaspis splendens*†

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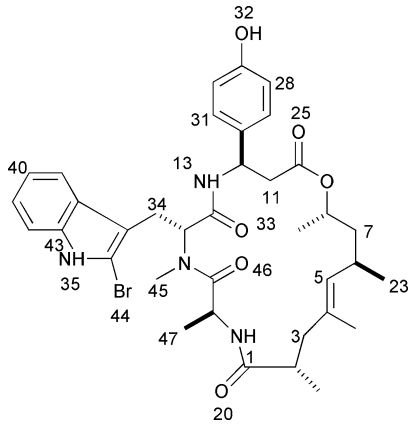
The complexation of $Li^+$ to jasplakinolide, a marine sponge derived cyclic depsipeptide showed preferential binding to two out of four carbonyl oxygens (C-10, C-14) and the electrons of the aromatic system of the β-tyrosine amino acid residue. This is in contrast to previous results obtained by others who proposed complexation to three out of four available carbonyl oxygens (C-1, C-10, C-14). The structure of the complex in $CD_3CN$ was determined by NOE restrained molecular dynamic calculations. Structures of the uncomplexed jasplakinolide were calculated in $CDCl_3$ and $CD_3CN$ for comparison.

## Introduction

Biological activities of macrocycles such as cyclic peptides are usually related to their conformation in biological media.[1] However, poor solubility in aqueous media is often a problem. LiCl complexed cyclic peptides have been suggested to better reflect conformational properties in aqueous solution compared to those carried out in conventional solvents alone.[2] The availability of these conformations will be invaluable in future investigations of the various biological activities displayed by jasplakinolide, such as its anticancer activity and its stabilisation of actin polymerisation. In addition to our interest in the conformations of jasplakinolide in aqueous media we were interested if there were any interactions between the aromatic rings of the amino acid residues of jasplakinolide and the $Li^+$ ion. Cation–π interactions are now recognised to play important roles in stabilizing protein–DNA complexes[3] as well as in molecular recognition processes.[4]

Jasplakinolide (jaspamide) is a member of a small family of four residue cyclic depsipeptides that share a common twelve carbon hydroxy acid fragment linked to three variable amino acids. It was originally isolated from a sponge of the genus *Jaspis* sp. from Fiji.[5,6] It was later isolated from a sample of *Jaspis johnstoni* collected from Papua New Guinea.[7] It has been the subject of several total syntheses.[8–11] Jasplakinolide has many interesting biological properties including anthelminthic,[6] catatonic,[5] insecticidal[5] and ichtytotoxic[7] activities. Jasplakinolide has been shown to be active against 36 human solid tumour cell cultures.[12] The mechanism of action is known to be through the stabilisation of cell actin filaments.[13–18] Pre-clinical trials performed by the NCI as an anti-actin agent showed it to be too toxic. It has, however, been investigated as a potential warhead for antibody delivery and as a molecular probe for the study of actin polymerisation.[19]




Previous studies performed by Inman and Crews on the conformation of jasplakinolide[12] and its lithium complexation[20] suggested the preferential binding of the $Li^+$ to three of the four carbonyl oxygens which are oriented on the same side of the macrocycle (C-1, C-10, C-14). The $Li^+$ binding site in their studies[20] was ascertained using molecular mechanics calculations where $Li^+$ was docked close to the three carbonyl oxygens and then minimized. The minimized structure was then compared to experimental NMR titration data. In this study we use a complementary approach. The solution structure of jasplakinolide in the absence and presence of $Li^+$ was calculated using NOE-restrained molecular dynamics calculations without the use of an explicit $Li^+$ model.[21–23] A molecular mechanics conformational search of the jasplakinolide structure using a continuum solvent model and an explicit $Li^+$ gave several conformational families, one of which mirrored that found by Inman and Crews.[20] One of our calculated conformational

† Electronic supplementary information (ESI) available: Table S1: $^1H$ and $^{13}C$ NMR data of jasplakinolide in $CDCl_3$ and $CD_3CN$ at 400/100 MHz. Table S2: $^1H$, $^{13}C$ NMR, $^1H$–$^1H$ COSY and HMBC data at 400/100 MHz and literature $^{13}C$ NMR data[20] of jasplakinolide–$Li^+$ complex in $CD_3CN$. Table S3: $^{13}C$ Chemical shift differences between jasplinolide and jasplakinolide–$Li^+$ complex in $CD_3CN$. Table S4: Restraints used for NOE restrained structure calculation of jasplakinolide in $CDCl_3$. Table S5: Torsion angle restraints for jasplakinolide in $CDCl_3$. Table S6: Energies (kcal $mol^{-1}$) for jasplakinolide minimum energy structure in $CDCl_3$. Table S7: Statistics for 22 lowest energy structures of jasplakinolide in $CDCl_3$. Table S8: Restraints used for NOE restrained structure calculation of jasplakinolide in $CD_3CN$. Table S9: Torsion angle restraints for jasplakinolide in $CD_3CN$. Table S10: Energies (kcal $mol^{-1}$) for jasplakinolide minimum energy structure in $CD_3CN$. Table S11: Statistics for the 19 lowest energy structures of jasplakinolide in $CD_3CN$. Table S12: Restraints used for NOE restrained structure calculation of the jasplakinolide–$Li^+$ complex in $CD_3CN$. Table S13: Torsion angle restraints for jasplakinolide–$Li^+$ complex in $CD_3CN$. Table S14: Energies (kcal $mol^{-1}$) for jasplakinolide–$Li^+$ complex minimum energy structure in $CD_3CN$. Table S15: Statistics for the 35 lowest energy structures of jasplakinolide–$Li^+$ complex in $CD_3CN$. See http://www.rsc.org/suppdata/ob/b4/b416839a/

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families was consistent with our NOE-derived structure for the jasplakinolide–$Li^+$ complex indicating that this might be the situation in solution. Differences between our NMR data and that of Inman and Crews indicate that the two complexes observed are different, possibly as a result of concentration or other more subtle differences in sample preparation.

## Results and discussion

Any solution conformational studies rely heavily on accurate assignments of the $^1$H NMR resonances, otherwise structures with high energy and a large number of NOE violations are obtained.[22] Jasplakinolide data was collected in $CDCl_3$ and $CD_3CN$ at 26 °C. Assignments were based on 1D and 2D spectroscopic data and by comparison of experimental with published data (Fig. 1(a), Table S1, ESI†). For the complexation experiment, 16 mM jasplakinolide in $CD_3CN$ was complexed with an equivalent amount of LiBr and protons and carbons were reassigned based on both 1D and 2D NMR data as before (Fig. 1(b), Table S2, ESI†). The presence of the $Li^+$ complex was confirmed by LRESIMS with $m/z$ of 709.28, 711.28 at 94 and 100% intensity, respectively, indicating the presence of one bromine atom in the molecule.

### Solution conformation

Solution state conformations for jasplakinolide were determined in both $CDCl_3$ and $CD_3CN$ while the jasplakinolide–$Li^+$ complex was determined only in $CD_3CN$. NOE restraints were obtained from T-ROESY spectra ($t_{mix}$ = 150 and 300 ms) and classified as weak (<5 Å), medium (<3.5 Å) or strong (<2.5 Å). Restrained molecular dynamics calculations were carried out using X-PLOR,[24] using previously reported conditions.[25] Statistics for the three structures are given in Table 1 and in the supporting information. All structures had low target energy functions and gave few restraint violations (Fig. 2) and good backbone RMSD values (Table 1).

The NOE based structure of jasplakinolide in solution is open with a lot of flexibility in the backbone particularly with respect to the 12 carbon hydroxy fragment (C-1–C-9) as shown in the structures in $CDCl_3$ and $CD_3CN$ (Fig. 1(a) and (b)). The solution structure predicts the absence of hydrogen bonding which is in agreement with the temperature gradient data of the NH groups obtained by Inman and Crews.[20] There are similarities between the solution conformation and the crystal structure[5] in terms of the overall openness of the backbone despite the presence of a hydrogen bond in the crystal structure (Fig. 3(a)). The change in the dielectric constant of the solvents from $CDCl_3$ to $CD_3CN$ (nonpolar to polar) affects the conformation of the twelve carbon hydroxy acid fragment, and also the $\phi$ and $\psi$ of the L-Ala-D-BrTrp fragment (Fig. 1(a) and (b) and Fig. 2(a)). Peptide backbone dihedral angles $\phi$ and $\psi$ values of the D-BrTrp-L-βTyr segment in these two solvents are very similar (Table 2). This is consistent with results obtained by other workers which indicate that the jasplakinolide Ala-D-BrTrp region is responsible for the cytotoxic activity of jasplakinolide while the D-BrTrp-L-βTyr region gives it the conformation that is responsible for its insecticidal and anti actin microfilament activities.[26] The backbone dihedral angles for the XRD structure,[5] and the conformations found in this study are given in Table 2.

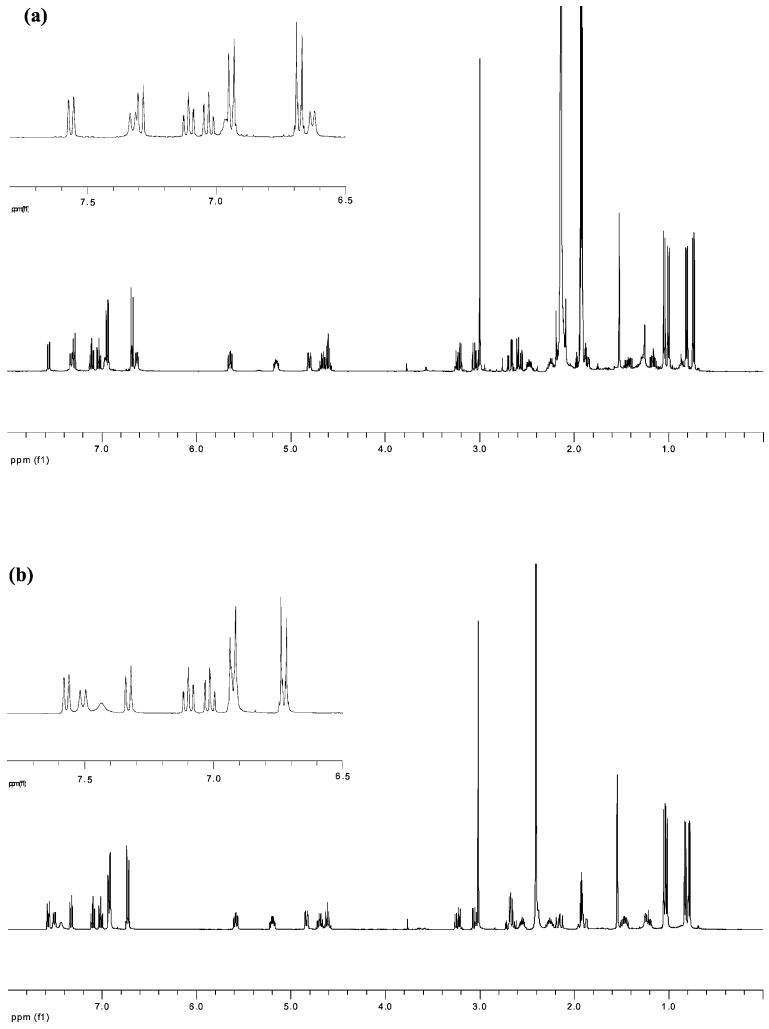


**Fig. 1** $^1$H NMR spectra at 400 MHz of (a) jasplakinolide in $CD_3CN$ and (b) jasplakinolide–LiBr (1 : 1) in $CD_3CN$.

**Table 1** Statistics for the solution structures determined in this study

| | Jasplakinolide | Jasplakinolide | Jasplakinolide–$Li^+$ complex |
|---|---|---|---|
| Solvent | $CDCl_3$ | $CD_3CN$ | $CD_3CN$ |
| Structures with same backbone (out of 100) | 22 | 19 | 35 |
| $E_{total}$/kcal $mol^{-1}$ | 1.46 | 1.25 | 10.0 |
| Backbone RMSD/Å | 0.18 ± 0.10 | 0.15 ± 0.09 | 0.10 ± 0.05 |
| Heavy atom RMSD/Å | 0.70 ± 0.21 | 1.43 ± 0.40 | 0.53 ± 0.27 |

**Table 2** Peptide backbone dihedral angles $\phi$ and $\psi$ in degrees for the L-Ala-D-BrTrp-L-βTyr segment of jasplakinolide for this study (NMR) and the published crystal structure (XRD)[5]

| Method/structure | $\phi_i$ | $\psi_i$ | $\phi_{i+1}$ | $\psi_{i+1}$ |
|---|---|---|---|---|
| XRD | −156.5 | +160.0 | +109.4 | −26.7 |
| NMR ($CDCl_3$) | −121.6 | +42.9 | +131.5 | −31.8 |
| NMR ($CD_3CN$) | +76.2 | +59.3 | +132.3 | −21.8 |
| NMR ($CD_3CN$ + Li) | −159.1 | −45.7 | +80.8 | 28.4 |

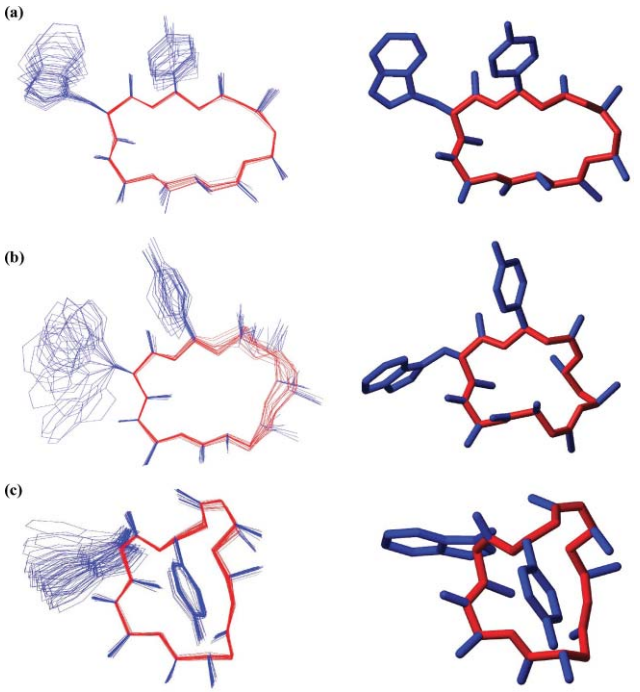


**Fig. 2** Figure showing ensemble of minimum energy structures (left column) and representative minimum energy structures (right column) for (a) jasplakinolide in $CDCl_3$, (b) jasplakinolide in $CD_3CN$ and (c) jasplakinolide–LiBr (1 : 1) in $CD_3CN$. For numbers of structure in each ensemble refer to Table 1. The backbone is coloured in red and the sidechains are shown in blue. Heavy atoms only shown.

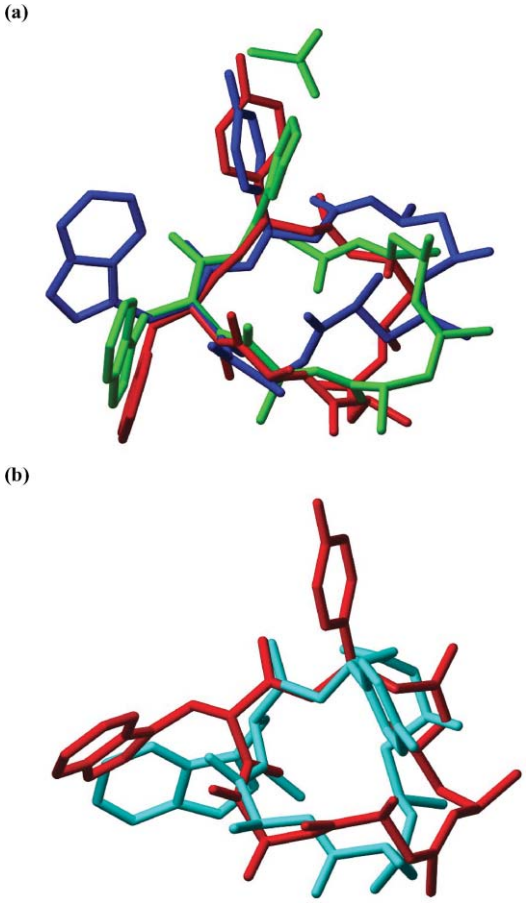


**Fig. 3** (a) Overlay of the jasplakinolide acetate crystal structure in green and representative minimum energy structures of jasplakinolide in $CD_3CN$ (red) and $CDCl_3$ (blue). The C, N, $C_\alpha$ of the Ala-BrTrp-βTyr have been overlaid with an RMSD of 0.412 Å. (b) Overlay of the representative minimum energy structures of jasplakinolide in $CD_3CN$ (red) and $Li^+$ complexed jasplakinolide in $CD_3CN$ (cyan). Heavy atoms only shown.

The addition of LiBr to jasplakinolide yielded some changes to the $^1H$ NMR spectrum (Fig. 1) notably the de-shielding ($\Delta\delta$ = 0.07 ppm) of one of the diastereotopic protons of H-11, the shielding of H-6 ($\Delta\delta$ = −0.41 ppm) and significant changes in the two amide and indole NH (Fig. 1, Tables S1 and S2, ESI†). The deshielding of one of the diastereotopic protons at H-11 can be explained by its increased proximity to the amide carbonyl at C-14 due to the twist of the skeletal backbone from C-8–C-9–C-10–C-11 causing the two carbonyl groups to be oriented close to each other (Fig. 2(b) and (c)). The observed shielding of H-6 can be explained similarly by its being brought into a shielding zone due to a change in conformation upon complexation of the macrocycle.

It was observed that the addition of $Li^+$ to jasplakinolide affects the orientation of the bromo-tryptophan group outside the ring system of the macrocycle, mirrored by the change in shift for C-34 (Table S3, ESI,† Fig. 3(b)). The β-tyrosine group underwent a major change in conformation in the presence of $Li^+$. It shifted from being oriented outside the macrocycle in $CD_3CN$ to adopt the conformation shown in Fig. 2(c) in the presence of $Li^+$. The model presented here suggests that the β-tyrosine group together with the amide carbonyl group at C-14 and the ester carbonyl group in close proximity at C-10 form the binding sites of the $Li^+$ ion. Although the C-10 carbonyl is deshielded by 3.3 ppm corroborating this as a binding site, conclusive evidence from $^{13}C$ chemical shift change for C-14 was less significant, compared to the results obtained by Inman and Crews[20] (Table S3, ESI†). Rigorous comparison of the differences in the $^{13}C$ NMR data for the carbonyl groups between this study and the published data is made difficult as the $^{13}C$ NMR data for jasplakinolide in $CD_3CN$ are not reported.[20]

Docking calculations of $Li^+$ to jasplakinolide were performed using the MMFF force field with the low-mode conformational searching algorithm as implemented in Macromodel v8.1.[27] The $Li^+$ ion was introduced near the carbonyl oxygens at C-14 and C-17 as they were on the same side of the ring.[20] The cation

was also introduced near the oxygen of the ester carbonyl (C-10) and the benzene ring of the β-tyrosine group. After minimization, the lowest energy structures were compared to the NOE-restrained structure. The most favourable structure is shown in Fig. 4. The consistency between the results obtained from the NOE restrained molecular dynamics calculations and the conformational search suggests that $Li^+$ binds to the outside of the macrocycle utilising electrons of the oxygen atoms of the carbonyl groups of C-10 and C-14 and the π-electrons of the β-tyrosine group. π-Systems have been known to bind alkali metals in biological systems[28,29] and even compete with known metal coordinating groups like amines and alcohols.[30] Tyrosine has been known to contribute to the allosteric binding of $Na^+$ ions in a class of serine protease enzymes.[31] Other examples in the literature include the positioning of the $Na^+$ ion over the aromatic face of the tryptophan side chain of the egg white lysozyme crystal structure.[32] The distance from $Li^+$ to the centre of the π-system of the tyrosine group is about 2.5 Å which reasonably agrees with that predicted by Nicholas *et al.* using *ab initio* techniques.[33]

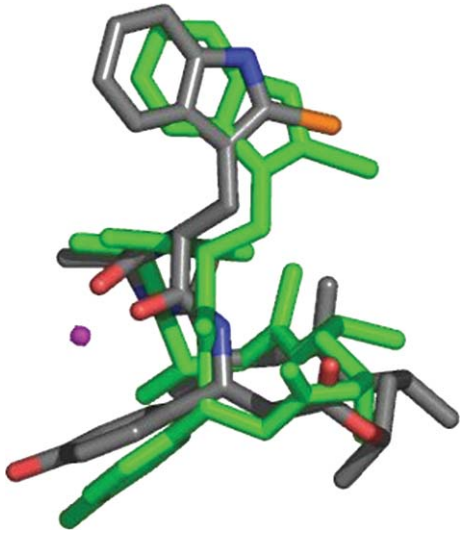

**Fig. 4** Overlay of the NOE-derived representative minimum energy structure of $Li^+$ complexed jasplakinolide (green) with the $Li^+$-docked minimized structure obtained by conformational searching (CPK colours). Lithium is depicted in magenta (heavy atoms only shown).

## Conclusion

Jasplakinolide has a flexible backbone structure due to the flexibility and the various possible conformations[12] adopted by the 12-carbon fragment hydroxy fragment of the peptide. The binding of $Li^+$ is observed to bind outside the macrocycle utilising the π-electron cloud of the β-tyrosine residue and the lone pairs electrons of the oxygens at C-14 and C-10. This is in contrast to the findings of Inman *et al.*[20] who suggested that the $Li^+$ anion bound to the inside of the macrocycle utilising the oxygen atoms of C-1, C-10 and C-14. This study indicates the importance of the D-BrTrp-L-βTyr segment of the backbone and the aromatic electrons of the β-tyrosine residue in the binding of jasplakinolide to other positively charged species like metal ions and positively charged amino acid residues of proteins. Such binding could cause conformational changes in both jasplakinolide and proteins and would hence affect their properties.

## Experimental

### General

$^1H$ and all 2D NMR experiments were recorded using a Varian Unity INOVA 400 MHz spectrometer in chloroform-*d* ($CDCl_3$) and acetonitrile-$d_3$ ($CD_3CN$) and were referenced to tetramethylthysilane (TMS) at 0.00 ppm. $^{13}C$ NMR were recorded at 100 MHz and referenced to 77.0 ppm in $CDCl_3$ and 118.3 in $CD_3CN$. HPLC purifications were carried out using a Spectra series P100 isocratic pump a Phenomenex Luna C18 (10 × 250, 10 μ) column and monitored using an Agilent 1100 series diode array detector (DAD).

### Extraction

The sample of *Jaspis splendens* was collected from Suva Harbour, Fiji Islands and extracted with MeOH and dichloromethane, dried and transported to Aberdeen. The sample was fractionated on Sephadex LH-20 using $CH_2Cl_2$–MeOH (1 : 1). The fraction containing jasplakinolide was purified by C18 HPLC using acetonitrile–water (80 : 20) as solvent.

### Structure calculation

Restraints were derived from the T-ROESY spectrum ($t_{mix}$ = 150 and 300 ms) and classified as weak, medium or strong by contour counting. The T-ROESY sequence minimises any unwanted TOCSY correlations in the ROESY sequence. Restrained molecular dynamics calculations were carried out with XPLOR 3.851[24] using a G-force field with repulsive non-bonded terms. *Ab initio* simulated annealing calculations (YASAP 3.0: 120 ps total time simulated annealing from 2000 to 100 K, 200 steps of minimization) were used to calculated structures from 100 starting conformations with randomised $\phi$ and $\psi$ angles. From this ensemble structures were refined using a simulated annealing with slow cooling protocol (600 ps, cooling 1500 to 100 K, 4000 steps of minimization). The lowest energy structures from the ensemble were selected to represent the final structure. The overlay and display of structure was achieved with MolMol.[34]

Molecular mechanics calculations were performed using Macromodel v8.1 from Schrödinger Inc.[35] The Low-Mode Conformational Searching (LMCS) method was employed for explicit investigation of the $Li^+$ binding. The LMCS method was selected as it is designed for efficient searching of 'soft' degrees of freedom, such as those expected to exist between jasplakinolide and $Li^+$. The parameter set used for these calculations was obtained from the MMFF force field. The cation was introduced into several positions near the peptide and 1000 LMCS steps were made from these initial geometries. The resulting sampled structures were energy-minimised with the Polak–Ribier conjugate gradient method using default settings for the termination of this step.

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## References

1 P. Wipf, *Chem. Rev.*, 1995, **95**, 2115–34.
2 D. L. Boger, M. A. Patane and J. Zhou, *J. Am. Chem. Soc.*, 1995, **117**, 7357–7363.
3 R. Wintjens, J. Lievin, M. Rooman and E. Buisine, *J. Mol. Biol.*, 2000, **302**, 395–410.
4 J. L. Sussman, M. Harel, F. Frolow, C. Oefner, A. T. Goldman and L. I. Silman, *Science*, 1991, **253**, 872–879.
5 T. M. Zabriskie, J. E. Klocke, C. M. Ireland, A. H. Marcus, T. F. Molinski, D. J. Faulkner, C. Xu and J. C. Clardy, *J. Am. Chem. Soc.*, 1986, **108**, 3123–3124.
6 P. Crews, L. V. Manes and M. Boehler, *Tetrahedron Lett.*, 1986, **27**, 2797–2800.
7 J. C. Braekman, D. Daloze and B. Moussiaux, *J. Nat. Prod.*, 1987, **50**, 994–995.

8 I. Takayuki, Y. Hamada and T. Shioiri, *Tetrahedron Lett.*, 1994, **35**, 591–594.
9 K. S. Chu, G. R. Negrete and J. P. Konopelski, *J. Org. Chem.*, 1991, **56**, 5196–5202.
10 P. A. Grieco, Y. S. Hon and A. Perez-Medrano, *J. Am. Chem. Soc.*, 1988, **110**, 1630–1631.
11 U. Schmidt, W. Siegel and M. K., *Tetrahedron Lett.*, 1988, **28**, 1269.
12 W. Inman and P. Crews, *J. Am. Chem. Soc.*, 1989, **111**, 2822–2829.
13 A. M. S. Mayer, *Pharmacology*, 1999, **41**, 159–164.
14 M. R. Bubb, I. Spector, B. B. Beyer and M. K. Fosen, *J. Biol. Chem.*, 2000, **275**, 5163–5170.
15 I. Fabian, D. Halperin, S. Lefter, L. Mittelman, R. T. Altstock, O. Seaon and I. Tsarfaty, *Blood*, 1999, **93**, 3994–4005.
16 B. Tian and P. L. Kaufman, *Invest. Ophth. Vis. Sci.*, 2000, **41**, S510–S510.
17 L. P. Cramer, *Curr. Biol.*, 1999, **9**, 1095–1105.
18 M. R. Bubb, A. M. J. Senderowicz, E. A. Sausville, K. L. K. Duncan and E. D. Korn, *J. Biol. Chem.*, 1994, **269**, 14869–14871.
19 R. P. Haugland, *Handbook of Fluorescent Probes and Research Chemicals*, Molecular Probes, Inc, Eugene, OR, USA, 6th edn., 1996, p. 252.
20 W. D. Inman, P. Crews and R. Macdowell, *J. Org. Chem.*, 1989, **54**, 2523.
21 J. N. Tabudravu, M. Jaspars, L. A. Morris, J. J. Kettenes-van den Bosch and N. Smith, *J. Org. Chem.*, 2002, **67**, 8593–8601.
22 J. N. Tabudravu, L. A. Morris, J. J. Kettenes-van den Bosch and M. Jaspars, *Tetrahedron*, 2002, **58**, 7863–7868.
23 L. A. Morris, J. J. K. van den Bosch, K. Versluis, G. S. Thompson and M. Jaspars, *Tetrahedron*, 2000, **56**, 8345–8353.
24 A. T. Brunger *X-PLOR a system for X-ray crystallography and NMR 3.851*, Yale University, New Haven, CT, 1993.
25 J. Tabudravu, M. Jaspars, L. Morris, J. Kettenes-van den Bosch and N. Smith, *J. Org. Chem.*, 2002, **67**, 8593–8601.
26 S. Terraciano, I. Bruno, G. Bifulco, J. E. Copper, C. D. Smith, L. Gomez-Paloma and R. Riccio, *J. Nat. Prod.*, 2004, **67**, 1325–1331.
27 F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caulfield, G. Chang, T. Hendrickson and W. C. Still, *J. Comput. Chem.*, 1990, **11**, 440.
28 L. S. De Wall, E. S. Meadows, L. J. Barbour and G. W. Gokel, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 6271–6276.
29 J. C. Ma and D. A. Dougherty, *Chem. Rev.*, 1997, **97**, 1303–1324.
30 J. B. Nicholas, B. P. Hay and D. A. Dixon, *J. Phys. Chem. A.*, 1999, **103**, 1394–1400.
31 Q. D. Dang, E. R. Guinto and E. Dicera, *Nat. Biotechnol.*, 1997, **15**, 146–149.
32 J. Wouters, *Protein Sci.*, 1998, **7**, 2472–2475.
33 D. Feller, D. A. Dixon and J. B. Nicholas, *J. Phys. Chem. A.*, 2000, **104**, 11414–11419.
34 R. M. B. Koradi and K. Wuthrich, *J. Mol. Graph.*, 1994, **14**, 51–59.
35 F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson and W. C. Still, *J. Comput. Chem.*, 1990, **11**, 440.