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Stereochemical determination of dictyostatin, a novel microtubule-stabilising macrolide from the marine sponge *Corallistidae sp.*†

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The relative stereochemistry of the 22-membered marine macrolide dictyostatin, a Taxol-like antimitotic agent, was determined based on a combination of extensive high field NMR studies, including *J*-based configuration analysis, and molecular modelling.

In recent years, marine macrolides have gained prominence as potent disrupters of cell cycle events. Dictyostatin is an antimitotic macrolide, originally isolated in 1994 by Pettit and co-workers from an Indian Ocean sponge.[1] More recently, it was isolated from a Caribbean sponge (*Corallistidae* sp.) and demonstrated to inhibit human cancer cell proliferation at nanomolar concentrations, retaining activity against multidrug-resistant cell lines and displaying a Taxol-like mechanism of action, by binding to tubulin and promoting microtubule assembly.[2] Dictyostatin now joins an elite group of microtubule-stabilising polyketides of marine origin that includes laulimalide,[3] peloruside A[4] and discodermolide,[5] as natural product leads for the development of improved anti-cancer drugs.[6] As a prelude to initiating a synthetic campaign to enhance the supply of dictyostatin for biological evaluation, we now report the determination of its full stereostructure through the application of Murata's method of *J*-based configuration analysis[7] combined with extensive NOESY experiments.

The planar structure **1** (Fig. 1) of dictyostatin, featuring a 22-membered macrolactone ring with five alkenes (2*Z*,4*E*,10*Z*,23*Z*) and a characteristic sequence of methyl and hydroxyl-bearing stereocentres, was confirmed by comparison of spectroscopic data (^{1}H and ^{13}C NMR, COSY and HMQC) obtained for our *Corallistidae* derived sample (1 mg) to that reported previously. Optimum ^{1}H signal dispersion was realised in CD_3OD at the highest available field strength (700 and 800 MHz). The $^3J_{H,H}$ coupling constants were extracted from a combination of 2D *J*-resolved spectra and homonuclear decoupling experiments, while measurement of heteronuclear coupling constants ($^{2,3}J_{C,H}$) relied on analysis of HSQC-HECADE[8] spectra.† A series of 1D and 2D NOESY experiments proved invaluable in defining relationships between the three isolated stereoclusters indicated in **1**. Notably, the coupling constants and NOESY correlations suggested the C-2 to C-16 region is relatively rigid, while at least two rapidly interconverting conformations must be considered for the C-17 to C-21 region (*vide infra*).

As depicted in Fig. 2, *J*-based configuration analysis indicated a 6,7-*anti*-7,9-*anti* relationship within the C-5 to C-10 segment **2**.† The small coupling between H-6 and H-7 suggested a *gauche* relationship, while a large heteronuclear coupling from H-6 to C-7 and a relatively large coupling from H-7 to Me-6 supported an *anti* relationship between the adjacent methyl and hydroxyl substituents, confirmed by a number of strong NOESY correlations. The diastereotopic methylene protons at C-8 could be related to H-7 and H-9 through large dipolar couplings, H-8a to H-7 and H-8b to H-9, and small couplings, H-8a to H-9 and H-8b to H-7. A large coupling, H-8a to C-7, and a small coupling, H-8a to C-9, established the relationship between H-8a and the two carbinol stereocentres. Couplings of similar magnitude were observed from H-8b to C-9 and H-8b to C-7, securing the 7,9-*anti* diol relationship.

The homo- and heteronuclear coupling constants observed for the C-11 to C-16 subunit of dictyostatin **3** are listed in Fig. 3.† Assignment of an *anti* relationship between the adjacent methyl and hydroxyl substituents at C-12 and C-13 was determined by the small couplings observed from H-12 to C-13, H-12 to H-13 and H-13 to Me-12. A large coupling between H-13 and H-14 suggested an antiperiplanar relationship between these protons, supported by

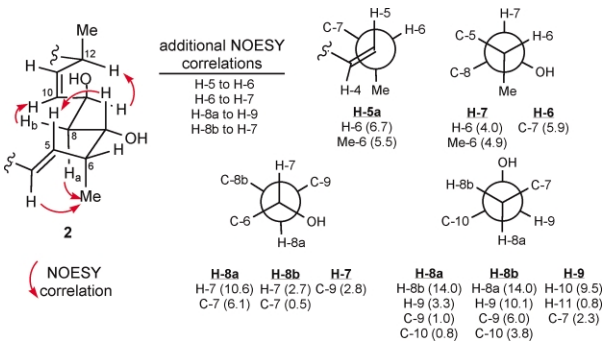

Fig. 2 Rotamers determined for C-5 to C-10 subunit **2**; coupling constants, $^{2,3}J_{H,H}$ and $^{2,3}J_{H,C}$ (Hz), in parentheses.

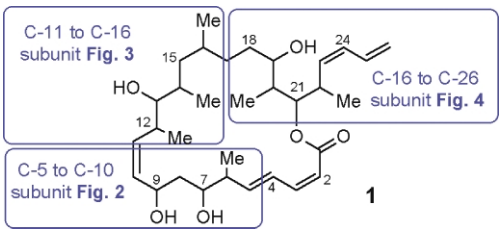

Fig. 1 Planar structure of dictyostatin.

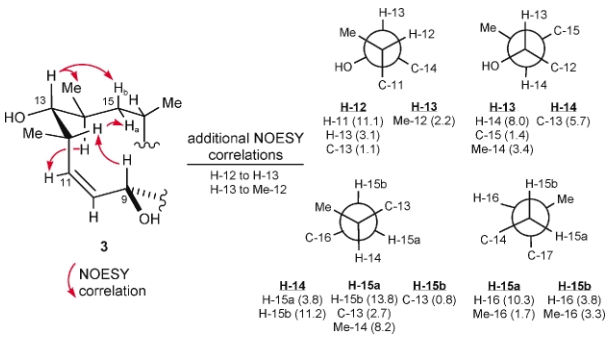

Fig. 3 Rotamers determined for C-11 to C-16 subunit **3**.

† Electronic supplementary information (ESI) available: copies of 1D and 2D NMR spectra, tables of spectral data and calculated torsion angles. See http://www.rsc.org/suppdata/cc/b3/b316390c/

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small couplings from H-13 to C-15 and H-13 to Me-14. A series of NOESY correlations (H-12 and H-15a, H-13 and H-15b, and H-13 and Me-14) confirmed the 13,14-*syn* relationship. Connectivity between the 1,3-related methyl-bearing stereocentres at C-14 and C-16 relied on a confident assignment of the diastereotopic methylene protons at C-15. Large dipolar couplings, H-15a to H-16 and H-15b to H-14, and small couplings, H-15a to H-14 and H-15b to H-16, together with small heteronuclear couplings between both methylene protons and C-13 and Me-16 supported the relative orientation depicted (14,16-*syn*). Analysis of the NOESY spectra revealed a series of correlations, H-14 to H-11, H-11 to H-10 and H-10 to H-8b, indicating these protons are oriented on the same face of the macrolide ring. Additional correlations from H-15a to H-12, H-15b to H-13 and H-12 to H-9, in combination with the relative stereochemistry determined by *J*-based configuration analysis established the connectivity between the isolated C-6 to C-9 and C-12 to C-16 stereoclusters.

As depicted in Fig. 4, both the homo- and heteronuclear couplings observed for the C-17 to C-21 segment of dictyostatin suggested the contribution of two or more rapidly interconverting conformations.† In particular, medium couplings from H-19 to H-18a, H-19 to H-20, H-19 to Me-20 and H-20 to H-21 supported a degree of conformational flexibility. Establishing a relationship between the substituents at C-21 and C-22 relied on both *J*-based configuration analysis and relevant NOESY correlations. A relatively large homonuclear coupling between H-21 and H-22 and small couplings, H-21 to C-23 and H-21 to Me-22, supported an antiperiplanar relationship. Two key NOESY correlations, between H-20 and Me-22 and between H-23 and H-4, supported the relative assignment as depicted. Analysis of models representing potential C-19 and C-20 stereoisomers suggested this was most consistent with an all-*syn* relationship for the substituents at C-19, C-20 and C-21. The strong NOESY correlations from H-17b to H-20 and H-18a to H-21, along with the observed couplings in this region, were rationalised by the conformational reorganisation required to accommodate both the C-1/C-2 *s-trans* and *s-cis* rotamers **4A** and **4B** indicated in Fig. 4. While the determination of the relationship between this isolated stereocluster and the C-1 to C-16 segment was not possible through *J*-based configuration analysis, molecular modelling of the two possible stereochemical permutations favoured the assignment of structure **5** (Fig. 5).

Using Macromodel (Version 7.2),[9] a 10,000 step Monte Carlo search was performed with the MM2* force field and the generalised Born/surface area (CB/SA) water solvent model.[10] For structure **5**, a series of discrete families of low energy conformations were found, with only two conformers within 2.00 kcal mol^{-1} of the global minimum. The lowest energy conformation **4A**, in which the lactone adopts a C-1/C-2 *s-trans* arrangement, accounted

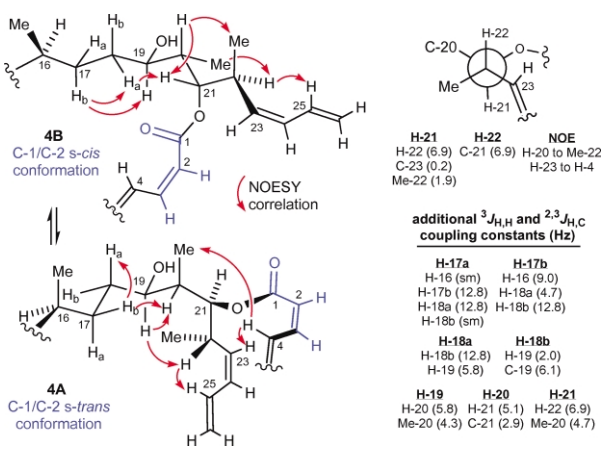

Fig. 4 Conformations **4A/4B** determined for the C-16 to C-26 subunit.

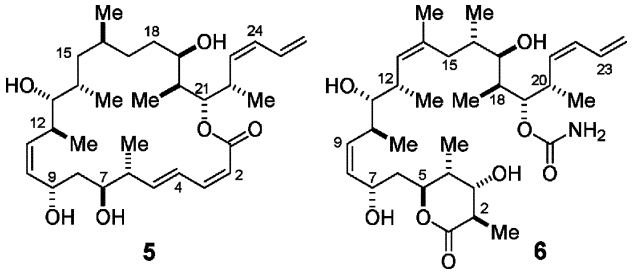

Fig. 5 Stereostructures for dictyostatin (**5**) and discodermolide (**6**).

for the observed NOESY correlations from H-17b to H-20, H-19 to H-22, and H-22 to H-25.[11] Furthermore, examination of the calculated dihedral angles† and correlation to a corresponding series of $^{3}J_{H,H}$ coupling constants, resulted in an acceptable match with the experimental NMR data. Finally, the remarkable homology between the relative stereochemistry, as assigned by these studies, and that of the structurally related polyketide discodermolide (**6**)[5] suggests the assignment of the full absolute configuration for dictyostatin, as shown in **5**, based on a common biogenesis.

In conclusion, a full stereochemical assignment for the antimitotic macrolide dictyostatin is proposed as **5** (2*Z*,4*E*,6*R*,7*S*,9*S*,12*S*,13*R*,14*S*,16*S*,19*R*,20*S*,21*S*,22*S*,23*Z*), based on the results of extensive high field NMR studies, including *J*-based configuration analysis, and molecular modelling. This assignment is consistent with a common biogenesis for discodermolide and suggests that both these sponge-derived polyketides interact in a similar fashion[12] with the Taxol binding site on β-tubulin. Confirmation of this proposal will rely on the stereocontrolled total synthesis of dictyostatin.

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