# Isolation and Structure of the Cancer Cell Growth Inhibitor Dictyostatin 1

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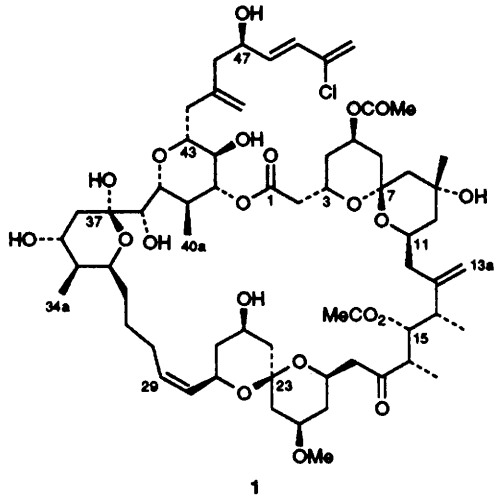
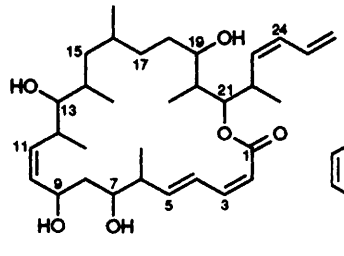
Dictyostatin 1 **2**, a new type of macrocyclic lactone bearing a 22-membered ring system, has been isolated ($3.4 \times 10^{-7}$% yield) from a Republic of Maldives marine sponge in the genus *Spongia* and found to strongly inhibit growth of the murine P388 lymphocytic leukaemia.

Marine invertebrates are proving, as originally predicted,[1,2] to be exceptionally important sources of structurally unique antineoplastic substances. Among such biosynthetic products already under preclinical or clinical development, macrocyclic lactones of the bryostatin,[3] spongistatin 1[4] and halichondrin/halistatin[5] types provide useful illustrations. Other interesting cell-growth inhibitory substances include the marine sponge jaspisamides A–C,[6] and miyakolide,[7] the sea pen lituarines A–C,[8] the dinoflagellate amphidinolides B,[9] G and H,[10] all incorporating 16- to 25-membered lactone rings, and the related open-chain marine sponge constituent discodermolide.[11,12] We herein report the discovery and structural elucidation of a unique macrocyclic lactone designated dictyostatin 1 **2** with strong cancer-cell growth inhibitory properties.

Specimens of the dark marine Spongia sp. (order Dictyoceratida, class Demospongiae, family Spongiidae) collected in the Republic of Maldives in 1986 and recollected (400 kg, wet mass) in 1988 were originally employed to isolate spongistatins 1–3[4] in $10^{-6}$ to $10^{-8}$% yields. Further vigorous investigation of a murine P388 lymphocytic leukaemia cell line (PS system) active fraction (dichloromethane derived)[4] afforded a mixture of trace constituents. The most promising (PS active) of these were separated by a series of column chromatographic steps utilizing Sephadex LH-20, silica gel with pressure (to 3.5 atm with a hexane–dichloromethane–methanol → methanol gradient) and reversed phase HPLC with Prepex C8 followed by LiChrospher 100 RP-18 using methanol–acetonitrile–water or acetonitrile–water as mobile phase to yield ($3.4 \times 10^{-7}$%) 1.35 mg of dictyostatin 1 **2**: colourless and amorphous; PS $ED_{50}$ $3.8 \times 10^{-4}$ μg/ml); mp 87–88 °C; $[\alpha]^{22}_D$ −20 (*c* 0.12, MeOH); UV (MeOH) λ/nm (log ε) 225, (4.3) 263 (4.2); IR (film) ν/cm$^{-1}$ 3412, 2926, 1693, 1638, 1597, 1379, 1277, 1180, 964; high resolution FAB MS, *m/z* 555.36621 [M + Na]$^+$ corresponding to $C_{32}H_{52}O_6Na$ (calc. 555.36621).



**1**



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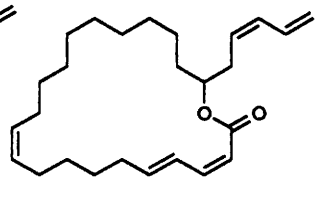

**3**

The structure of dictyostatin 1 was primarily deduced on the basis of high field (400 and 500 MHz) 2D NMR data that included $^1$H, $^{13}$C, APT, $^1$H–$^1$H COSY, heteronuclear multiple quantum coherence (HMQC),[13] $^1$H-detected heteronuclear multiple-bond correlation (HMBC)[14] and NOE experiments. Presence of an ABX spin system in the $^1$H NMR spectrum of dictyostatin 1 at δ 5.21 (br d, *J* 17 Hz), 5.11 (br d, *J* 11 Hz), and 6.67 (dt, 17, *J* 11 Hz) indicated a terminal unit. A broad singlet at δ 5.10 correlating with a $^{13}$C signal at δ 78.63 and a carbonyl signal at δ 168.10 in the HMBC spectrum suggested a macrolide. The coupling relationships of signals corresponding to H-2, H-3, H-4 and H-5 were established and extended to H-13. Although some of the signals were obscured, coupling of H-13 to H-19 was also established and extended to H-23 and H-26. Analysis of the HMQC and HMBC spectra supported structure **2**, the signal assignments (Table 1) and especially those attributed to the six methyl proton signals, which showed strong HMBC correlations. A six-membered ring, formed by bonding C-9 to C-13, was eliminated by the NOE observed between H-9 and H-12. Dreiding models illustrated that an NOE between H-9 and H-12 would not be possible if a C-9 to C-13 dihydropyran ring was present. Also, the mass spectra supported absence of a dihydropyran ring.

The geometry of the $\Delta^2$, $\Delta^{10}$, and $\Delta^{23}$ double bonds was assigned the *cis* (*Z*) configuration based on the 11 Hz coupling constants found for each of the respective sets of olefin hydrogen atoms. The $\Delta^4$ double-bond hydrogens each exhibited a 16 Hz coupling constant. Thus, the $\Delta^4$-olefin was assigned the *trans* (*E*) geometry. The NOE difference spectroscopy experiments were recorded in both $CD_3OD$ and $CD_3CN$ and the results (Table 1) favoured the solution configuration depicted. Many of the single bonds in **2** are flexible. Therefore, this assignment of relative stereochemistry needs to be considered only preliminary. A definitive assignment of the relative and absolute configurations will require a future X-ray crystal structure determination.

Presumably dictyostatin 1 **2** represents the first member of a new series of cancer-cell growth inhibitors and dictyoene **3** is proposed for the parent ring system. Presently, experiments are in progress to markedly increase the availability of dictyostatin 1 through biosynthetic and/or total synthetic approaches. Increased availability of this promising substance will allow the absolute configuration to be ascertained and extended biological evaluations to proceed. The possible biosynthetic relationship of dictyostatin 1 to the spongistatins[4] presents another interesting challenge.

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**Table 1** NMR assignments for dictyostatin 1 **2** recorded in $CD_3OD$, and coupling constants in Hz (in parentheses): the n and p are APT results. Mixing time for the HMBC was set at 60 μs

| Postn | $^{13}C$(100 MHz) | $^{1}H$(400 MHz) | HMBC(500 MHz) | NOE(400 MHz) |
|---|---|---|---|---|
| 1 | 168.10p | | H-21, H-2, H-3 | |
| 2 | 118.03n | 5.52 d(11) | | |
| 3 | 144.89n | 6.62 t(11) | H-5 | H-2, H-5 |
| 4 | 128.58n | 7.17 dd(11, 16) | H-2 | H-27 |
| 5 | 146.42n | 6.14 dd(6.7, 16) | H-3, H-27 | H-3, H-7 |
| 6 | 44.05n | 2.57 brm | H-27 | H-7, H-5 |
| 7 | 70.37n | 4.02 dt(3.1, 10.7) | H-27 | H-6 |
| 8 | 40.59p | 1.47[a]; 1.38[a] | | |
| 9 | 65.50n | 4.62 brdd(4.8, 8.7) | H-11 | H-12 |
| 10 | 134.89n | 5.37 brdd(8.7, 11) | | |
| 11 | 131.32n | 5.52 brt(11) | H-28 | |
| 12 | 35.74n | 2.72 brm | H-28, H-10 | H-9, H-13 |
| 13 | 80.37n | 3.06 dd(2.9, 8.2) | H-28, H-29 | |
| 14 | 35.32n | 1.58[a] | H-29 | |
| 15 | 42.26p | 1.22 m; 0.88 m | H-29, H-30 | |
| 16 | 31.22n | 1.50 m; | H-30 | |
| 17 | 32.74p | 1.56 m; 0.68 m | H-30 | |
| 18 | 32.50p | 1.82 m; 1.08 m | | |
| 19 | 73.72n | 3.33 m | H-31 | |
| 20 | 40.82n | 1.86 m | H-31 | H-19, H-21 |
| 21 | 78.63n | 5.10 dd(5,7)[b] | H-31, H-32 | H-22 |
| 22 | 35.82n | 3.13 m | H-32, H-24 | H-25 |
| 23 | 134.53n | 5.30 t(11) | H-32 | H-24, H-32 |
| 24 | 131.22n | 6.02 t(11) | | H-23, H-26 |
| 25 | 133.43n | 6.67 dt(17, 11) | H-23 | H-22 |
| 26 | 118.58p | 5.21 brd(17); 5.11 brd(11) | H-24 | |
| 27 | 13.75n | 1.11 d(7.0) | H-5 | H-4 |
| 28 | 19.35n | 1.09 d(7.1) | | |
| 29 | 15.97n | 0.92 d(6.4) | | H-13 |
| 30 | 21.81n | 0.89 d(6.5) | | H-15 |
| 31 | 10.39n | 1.03 d(6.8) | | H-21 |
| 32 | 18.06n | 0.98 d(6.7) | | |

[a] These are overlapping signals. [b] The coupling pattern and coupling constants were measured in $CD_3CN$.

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