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Natural Products Synthesis

Total Synthesis and Configurational Assignment of (–)-Dictyostatin, a Microtubule-Stabilizing Macrolide of Marine Sponge Origin**

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Dictyostatin (1, Scheme 1) is a potent cytotoxic macrolide first isolated by Pettit et al.^[1] from a marine sponge of the genus Spongia sp., collected in the Republic of Maldives, and, subsequently, isolated by Wright and co-workers^[2] from a lithistida sponge of the family Corallistidae, harvested at great depths off the Jamaican coast. The planar structure of this unsaturated 22-membered macrolactone, featuring 11 stereogenic centers, an endocyclic 2Z,4E dienoate and a pendant Zdiene moiety at C21, was deduced by the Pettit group, primarily on the basis of 2D NMR spectroscopic data.^[1] Recently, we proposed a full stereochemical assignment for dictyostatin, as indicated in 1, based on the use of extensive high-field NMR experiments, including the Murata J-based configuration analysis, in combination with molecular modeling.^[3] Although it was not possible to ascertain the absolute configuration, our assignment was based on dictvostatin being biogenetically related to discodermolide.^[4]

Notably, dictyostatin displays powerful growth-inhibitory activity against a number of murine and human cancer cells at low nanomolar concentrations and, importantly, retains

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Scheme 1. Retrosynthetic analysis for dictyostatin (1) leading to key building blocks **3**, **6**, and **7**.

activity against taxol-resistant cancer cells that express active P-glycoprotein. Preliminary studies into the mechanism of action demonstrated that dictyostatin, in a similar manner to taxol (paclitaxel), arrests cells in the G2/M phase by potently inducing tubulin polymerization and suppressing normal microtubule dynamics, leading to apoptosis.^[2] Hence, dictyo-statin currently represents a promising antimitotic natural product lead for development in cancer chemotherapy, joining an elite group of microtubule-stabilizing polyketides that include laulimalide,^[5] peloruside A,^[6] and discodermo-lide.^[7] Unfortunately, evaluation of its antitumor properties has been precluded so far by the very low natural abundance.

Herein, we disclose the first total synthesis of dictyostatin (1) and establish unequivocally the relative and absolute configuration of this rare polyketide metabolite. Furthermore, the modular synthetic approach employed is flexible, highly convergent, and stereocontrolled, and thus offers the potential to provide useful quantities of dictyostatin as well as a range of structural derivatives to initiate structure–activity relationship studies.

As outlined retrosynthetically in Scheme 1, our approach relies on a late-stage reduction of the enone 2, controlled by the macrocyclic conformation adopted by the 22-membered ring; molecular modeling studies^[8] indicated that hydride addition should occur selectively to install the requisite C9 hydroxyl-bearing stereocenter. The 10Z alkene moiety in 2 was planned to arise from a Still-Gennari-type olefination between the β -keto phosphonate 3 and aldehyde 4, in conjunction with a Stille cross-coupling with vinyl stannane 5 to deliver the 2Z, 4E dienoate. In principle, either of these two reactions could be employed to close the macrocycle as an alternative to a more conventional Yamaguchi macrolactonization, thus offering considerable flexibility in the synthesis. In turn, the C11-C26 subunit 4 should be accessible by a Horner-Wadsworth-Emmons (HWE) coupling between aldehyde 6 and phosphonate 7, which contains the terminal Z diene. As these latter segments share a common stereochemical triad, it was anticipated that both 6 and 7 could be prepared from common intermediate 8, which is available in multigram quantities through efficient aldol methodology reported previously by our group.^[9]

Synthesis of the C11-C26 subunit 4, as shown in Scheme 2, commenced with conversion of 1,3-diol 8 into bis-TBS ether 9 and liberation of the primary hydroxy group under mildly acidic conditions. The resulting alcohol 10 was readily converted into iodide 11 through a modified Mitsunobu protocol (PPh₃, I₂, imid).^[10] Subsequent alkylation of the lithium enolate of the Myers propionamide 12^[11] effected a three-carbon homologation, delivering 13 with excellent diastereoselectivity and yield (19:1 d.r., 88%).^[12] Reductive removal of the pseudoephedrine auxiliary (LiNH₂BH₂)^[11] afforded a primary alcohol, which was, in turn, oxidized with Dess-Martin periodinane to provide aldehyde 6. The HWE coupling partner 7 was synthesized by addition of (MeO)₂P(O)CH₂Li to aldehyde 14, which was prepared from 1,3-diol 8 as described previously.^[9,13] The resulting epimeric mixture of alcohols was converted into β -ketophosphonate 7 (Dess-Martin periodinane, 83% over two steps). Following a HWE coupling between aldehyde 6 and phosphonate 7, employing Ba(OH)₂ in wet THF, enone 15 was isolated in 92% yield.^[14] With the entire C11–C26 backbone assembled, we next focused on the stereocontrolled reduction of the C19 ketone directed by the proximal hydroxy-bearing stereocenter through metal chelation. To this end, conjugate reduction of enone 15 with the Stryker reagent {[Ph₃PCuH]₆}^[15] and oxidative removal of both PMB groups with DDQ provided the intermediate β -hydroxyketone, which underwent 1,3-syn-selective reduction when treated with a cold $(-30 \,^{\circ}\text{C})$ ethereal solution of $\text{Zn}(\text{BH}_4)_2$,^[16] generating triol 16 (>20:1 d.r.).^[17] Finally, the synthesis of subunit 4 was completed by a three-step sequence involving



Scheme 2. Synthesis of C11-C26 subunit 4. a) TBSOTf, 2,6-lutidine, CH₂Cl₂, 0°C→RT, 15 min; b) *p*-TSA (30 mol%), 20:1 THF/H₂O, 24 h; c) PPh₃, I₂, imid, PhMe, 0°C, 15 min; d) 1) 12, LiCl, LDA, THF, –78°C→RT; 2) **11**, THF, 0°C→RT, 18 h; e) 1) LDA, BH₃NH₃, THF, 0°C; 2) 13, THF, 0°C \rightarrow RT, 18 h; f) DMP, NaHCO₃, CH₂Cl₂, 0°C, 2 h; g) 1) *n*BuLi, (MeO)₂P(O)Me, THF, -78 °C, 30 min; 2) **14**, THF, -78 °C ; h) DMP, CH₂Cl₂, room temperature, 10 min; i) 1) 7, Ba(OH)₂·8H₂O, THF, 1 h; 2) 6, 40:1 THF/H₂O, 20 h; j) [Ph₃PCuH]₆, PhH, H₂O, room temperature, 2 h; k) DDQ, pH 7 buffer, CH₂Cl₂, 0°C, 6 h; l) Zn(BH₄)₂, Et₂O, -30°C, 2 h; m) TBSOTf, 2,6-lutidine, -78→0°C, 30 min; n) TBAF, AcOH, THF, room temperature, 24 h; o) TEMPO, PhI(OAc)₂, CH_2Cl_2 , room temperature, 18 h. TBS = tert-butyldimethylsilyl, Tf = trifluoromethanesulfonyl, p-TSA = p-toluenesulfonic acid, imid = imidazole, LDA = lithium diisopropylamide, DMP = Dess-Martin periodinane, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoguinone, TBAF = tetrabutylammonium fluoride, TEMPO = 2,2,6,6-tetramethyl-1-piperidinyloxy, free radical.

selective TBS protection of the C11 and C19 hydroxy groups over that at C21 (TBSOTf), cleavage (TBAF, AcOH) of the primary TBS ether in **17**, and oxidation of resulting alcohol **18** (TEMPO/PhI(OAc)₂).^[18]

As our primary objective was an efficient and convergent synthesis of dictyostatin, it was crucial to install the 10Z alkene function by employing advanced coupling partners. Although a number of protocols exist to effect such coupling, most require strongly basic conditions. Our concerns regarding the epimerizable center at C12 in aldehyde **4** led us to investigate the mild Still–Gennari modification of the HWE olefination.^[19] The requisite F_3CCH_2O -substituted phosphonate **3** was prepared from alcohol **19**, which was obtained with 95% *ee* and 20:1 d.r. through a Brown asymmetric crotylation of aldehyde **20** (Scheme 3).^[20] Silyl ether formation



Scheme 3. Synthesis of C4–C10 subunit 3. a) TBSOTf, 2,6-lutidine, CH_2Cl_2 , -78 °C, 30 min; b) 1) O₃, CH_2Cl_2 , -78 °C; 2) Ph_3P , -78 °C \rightarrow RT, 2 h; c) $CrCl_2$, CHI_3 , THF, dioxane, 0 °C, 18 h; d) TBAF, AcOH, THF, room temperature, 14 h; e) DMP, py, CH_2Cl_2 , room temperature, 3 h; f) NaClO₂, NaH₂PO₄, 10:1 tBuOH/H₂O, 0 °C, 2 h; g) 1) Me₂C = C(Cl)NMe₂, CH_2Cl_2 , room temperature, 15 min; 2) (F₃CCH₂O)₂P(O)CH₂Li, THF, -100 °C, 1 h.

(TBSOTf), ozonolysis, and Takai methylenation^[21] of the intermediate aldehyde provided *E* vinyl iodide **21** in 71% yield. After selective cleavage of the primary silyl ether, oxidation of the resulting alcohol afforded acid **22**, which was transformed into its acid chloride by using the Ghosez reagent $(Me_2C = C(Cl)NMe_2)$.^[22] Next, the acid chloride was added to a solution of $(F_3CCH_2O)_2P(O)CH_2Li$ in THF,^[23] generated at -100 °C, to afford phosphonate **3** containing the required functionality for the pivotal fragment assembly.

To our delight, when a mixture of aldehyde **4** and phosphonate **3** was subjected to an excess of K_2CO_3 in the presence of [18]crown-6 in toluene (Scheme 4),^[19] the HWE coupling proceeded smoothly to produce Z enone **23** efficiently, with good levels of selectivity (Z/E = 5:1, 77%). Notably, this constitutes one of the first examples of a Z-selective *intermolecular* Still–Gennari olefination employing such an elaborate β -ketophosphonate. Construction of the dictyostatin carbon backbone was completed through a copper-mediated, Liebeskind-type Stille coupling^[24] (CuTC in NMP) of vinyl iodide **23** and Z alkenyl stannane **5**, which is readily accessible from known acid **24**.^[25] Finally, TIPS ester **25** was converted into acid **26** (KF, THF/MeOH) in 83% overall yield.

With **26** in hand, attention was now directed towards the completion of the synthesis, as shown in Scheme 5. Seco acid **26** was subjected to a standard Yamaguchi macrolactonization protocol (2,4,6-trichlorobenzoyl chloride, NEt₃, DMAP, PhMe, 60 °C, 2 h) to furnish the 22-membered macrocycle **2** cleanly in 77 % yield.^[26] Next, we investigated the reduction of the C9 ketone, in a bid to exploit macrocyclic stereocontrol.^[8]

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Scheme 4. Synthesis of seco acid **26.** a) K_2CO_3 (10 equiv), [18]crown-6 (25 equiv), toluene, room temperature, 48 h; b) CuTC, NMP, room temperature, 1 h; c) KF, THF, MeOH, room temperature, 2 h; d) TIPSCI, Et₃N, CH₂Cl₂, room temperature, 45 min. CuTC = copper(1) thiophenecarboxylate, NMP = 1-methyl-2-pyrrolidinone, TIPS = triiso-propylsilyl.



Scheme 5. Completion of the synthesis of dictyostatin (1). a) 2,4,6-trichlorobenzoyl chloride, NEt₃, DMAP, PhMe, 60 °C, 2 h; b) NaBH₄, CeCl₃·7 H₂O, EtOH, -30 °C; c) HCl (3 N), MeOH, room temperature, 6 h. DMAP = 4-dimethylaminopyridine.

Gratifyingly, Luche reduction of enone **2** (NaBH₄, CeCl₃, EtOH)^[27] provided the expected alcohol **27** (70%). Finally, global deprotection with HCl (3N) in methanol gave dictyostatin (**1**) in 87% yield. The spectroscopic data of our synthetic material were in complete agreement with those of an authentic sample obtained from the *Corallistidae* sponge source (¹H (700 MHz), ¹³C NMR (125 MHz), MS), and the specific rotation of $[\alpha]_D = -32.7$ (c = 0.22, MeOH) agreed with that measured in our laboratory for the authentic sample,^[28] thus allowing confident assignment of the relative and absolute configuration of dictyostatin and validating our earlier proposal.^[3a]

Significantly, this configurational assignment for dictyostatin is fully consistent with a common biogenesis for the structurally related, but open-chain, polyketide discodermolide (**28**, Figure 1), presently in clinical development as a new-



Figure 1. Overlay of preferred solution conformation^[3a] of dictyostatin

(in yellow, C1/C2 s-*trans* rotamer) in water with conformation of discodermolide (**28**) obtained from the X-ray crystal structure (in blue).

generation microtubule-stabilizing anticancer agent.^[29] There is an exact configurational match of the C19–C26 and C6–C14 regions of dictyostatin with those at C17–C24 (shown in red) and C4–C12 (shown in blue) of discodermolide, respectively. As shown by the overlay in Figure 1, the preferred conformation for dictyostatin in solution^[3a] resembles closely that determined for discodermolide in the solid state, suggesting that they may interact in a similar fashion with the taxolbinding site on β -tubulin.^[30] As a conformationally constrained macrocyclic analogue of discodermolide, dictyostatin represents a highly attractive template for rational drug design.

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In conclusion, this expedient total synthesis of (-)dictyostatin proceeds in 27 steps and 3.8% overall yield (longest linear sequence from the Roche ester^[9]) and establishes unequivocally the relative and absolute configuration. Key transformations include an unprecedented Still– Gennari-type HWE coupling with a complex β -ketophosphonate, a Liebeskind-type Stille cross-coupling to install the 2*Z*,4*E* dienoate, a facile Yamaguchi macrolactonization to construct the macrocycle, and subsequent reduction of the C9 ketone under macrocyclic stereocontrol. Importantly, this modular, convergent synthesis should be amenable to producing useful quantities and designed analogues of this novel microtubule-stabilizing agent, enabling extensive exploration of its anticancer properties.

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