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Structure-Based Design of a Novel Synthetic Spiroketal Pyran as a Pharmacophore for the Marine Natural Product Spongistatin 1

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Abstract—SPIKET-P, a novel synthetic spiroketal pyran, was rationally designed as a pharmocophore for the tubulin depolymerizing marine natural product Spongistatin 1. SPIKET-P was prepared from the commercially available benzyl (*R*)-(−)-glycidyl ether using a versatile 11-step synthetic scheme in a stereocontrolled fashion. At nanomolar concentrations, SPIKET-P caused tubulin depolymerization in cell-free turbidity assays and exhibited potent cytotoxic activity against cancer cells as evidenced by destruction of microtubule organization, and prevention of mitotic spindle formation in human breast cancer cells.

Microtubules play a pivotal role in mitotic spindle assembly and cell division.[1–5] These cytoskeletal elements are formed by the self-association of the αβ tubulin heterodimers.[1–5] Recently, the structure of the αβ tubulin dimer was resolved by electron crystallography of zinc-induced tubulin sheets.[6] According to the reported atomic model, each 46×40×65 Å tubulin monomer is made up of a 205 amino acid N-terminal GTP/GDP binding domain with a Rossman fold topology typical for nucleotide-binding proteins, a 180 amino acid intermediate domain comprised of a mixed β sheet and five helices which contains the taxol binding site, and a predominantly helical C-terminal domain implicated in binding of microtubule-associated protein (MAP) and motor proteins.[2,5]

Spongistatins are macrocyclic lactone compounds containing six pyran-type rings and four of the six pyran rings are incorporated into two spiro[5.5]ketal moieties (Fig. 1A).[7] Spongistatin 1 (SP) (R = Cl; Fig. 1A) is a potent tubulin depolymerizing natural product which has been isolated from an Eastern Indian Ocean sponge in the genus *Hytrios*.[7] SP exhibited potent cytotoxicity with subnanomolar IC_{50} values against the members of the NCI panel of 60 human cancer cell lines.[7] In a rational drug design effort intended to determine the minimal molecular architecture of the SP structure necessary for biologic activity, we used the 3-D atomic model of the αβ tubulin dimer for the identification of the potential tubulin binding sites for SP. Our integrated effort to identify the SP binding site involved 'cavity searching', analysis of the binding environment, docking procedures based on the electron crystallographic coordinates of tubulin, and the calculation of the binding constant by a modified score function (LUDI score function).

The large molecular volume of SP, which has a >10 Å length and >10 Å width, prompted us to hypothesize that it likely binds to a deep pocket on the surface of tubulin, which provides a highly hydrophobic environment for molecular interactions. We examined the electron crystallographic structure of tubulin using graphics programs including GRASP and INSIGHT II to identify a possible binding site for SP that would have suitable dimensions and contain a cluster of hydrophobic residues near the protein surface. The systematic search resulted in the discovery of a candidate binding pocket on the tubulin surface that is large enough to accommodate SP and provides an environment for extensive hydrophobic interactions. The SP molecule was then docked into this candidate binding site using the Affinity module within the Insight II program. The initial coordinates of SP, representing several different conformations, were modeled and energy-minimized using the DISCOVER program. The docking results, which utilized different conformations of SP, were analyzed and ranked based on their interaction scores. The SP model that was assigned the highest interaction score and lowest potential energy was chosen for further

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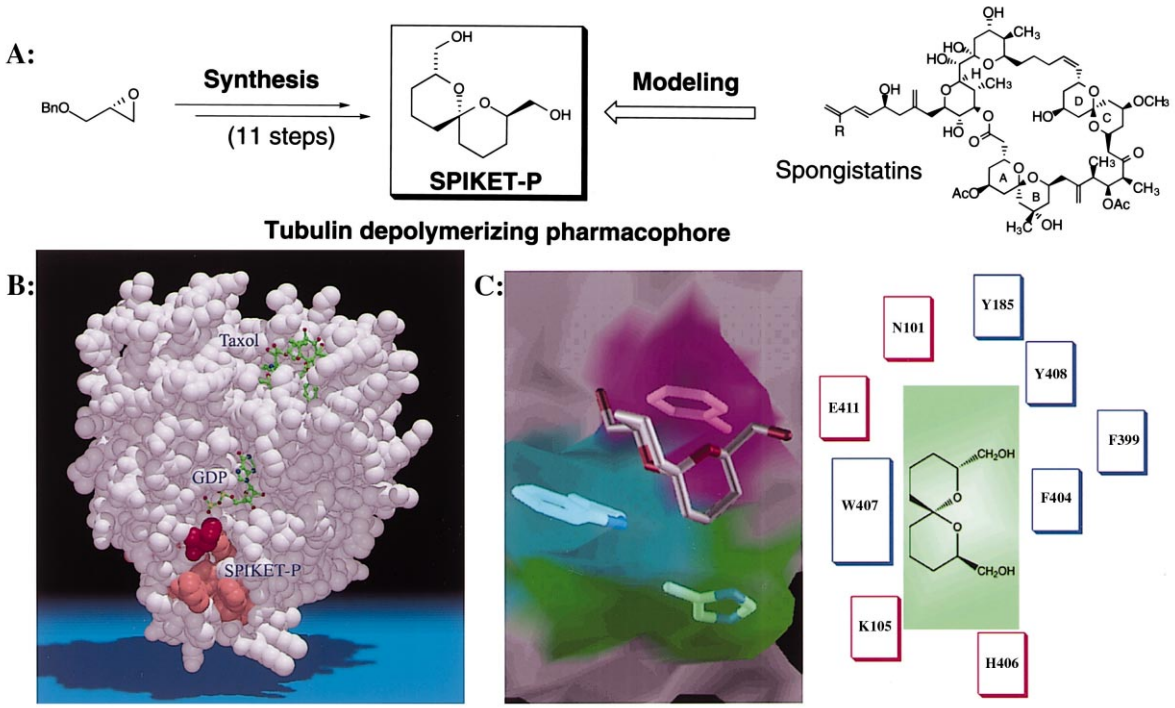
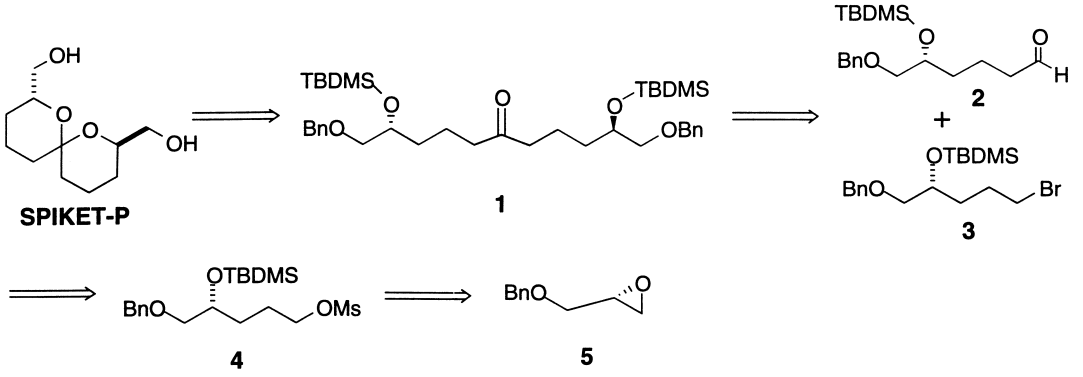


Figure 1. Modeling design of SPIKET-P as a new pharmocophore of tubulin depolymerizing agents: A, structures of Spongistatin and SPIKET-P; B: the space-filling model of β tubulin shown with ball-and-stick models of GDP and taxol molecules binding to the different sites as labeled. Spongistatin binding pocket (SBP) is marked in orange color for all aromatic residues in a close range. The residues N101 (colored in red) is located near the GDP exchange site (see text); C, SPIKET-P as a Spongistatin pharmacophore. SPIKET-P interaction with the putative SBP illustrated in a surface model of the SBP and stick-and-ball model for SPIKET-P.

calculations. The docking simulation results indicated that the putative SP binding pocket which is located on the surface of tubulin is approximately 8 Å wide×18 Å long×11 Å deep (Fig. 1B). This unique pocket consists of an unusual cluster of 10 aromatic residues situated in close proximity that includes Y108, W103, Y185, W407, Y408, F399, F404, F395, F418 and H406 (Fig. 1C).

The proposed tubulin binding pocket for SP is in close proximity to the GDP exchange site on the β subunit of the tubulin heterodimer (Fig. 1B). This location for the SP binding pocket would provide a cogent explanation for the ability of SP to inhibit the displacement of the bound GDP molecules from tubulin.[8,9] Furthermore, according to the recently published high-resolution model of the microtubule structure,[10] this binding pocket contacts the longitudinal interdimer interface of the microtubule. The binding of SP to this pocket may therefore hinder interdimer interactions of tubulin and contribute to the tubulin depolymerizing activity of SP. Advanced modeling studies of the interactions of SP with this putative SP binding pocket indicated that the two spiroketal groups of SP are in close contact with protein residues lining the binding pocket and may therefore serve as the critical binding components of SP. The identification of the spiroketal subunits as the likely tubulin binding elements of SP prompted the hypothesis that a synthetic spiroketal pyran (SPIKET-P) representing these subunits (Fig. 1A) could serve as a pharmacophore for SP. Docking studies indicated that, when bound to tubulin, the spiroketal ring of SPIKET-P would be sandwiched between aromatic residues F404



Scheme 1. Retro-synthetic analysis.

and W407 in the binding pocket and would provide favorable hydrophobic interactions and van der Waals contacts with these residues (Fig. 1C). The spiroketal group of SPIKET-P has a molecular surface of 218 Å,[2] 75% of which would be covered by the aforementioned two aromatic rings. Therefore, SPIKET-P was selected as a synthetic target.

Retro-synthetic analysis (Scheme 1) was started by converting the spiroketal group in SPIKET-P to the carbonyl and hydroxy groups in **1**, which was further converted to two segments **2** and **3**. Both **2** and **3** could be derived from the commercially available benzyl (*R*)-(−)-glycidyl ether **5**. The synthesis was initiated by opening the commercially available epoxide **5** using vinylmagnesium bromide to obtain the alcohol **6** which was protected as *tert*-butyldimethylsilyl ether to form **7** (Scheme 2). Hydroboration of the terminal olefin in **7** yielded the primary alcohol as in **8**, which was then converted to the mesylate **4**. The mesylate group in **4** was substituted by cyanide to form **9** which was further converted to aldehyde **2** by DIBAL reduction followed by acid catalyzed hydrolysis. Alternatively, the mesylate group in **4** was substituted by bromide to form **3**. Compound **3** was coupled with **2** by first converting **3** to a Grignard reagent and then reacting it with aldehyde **2**

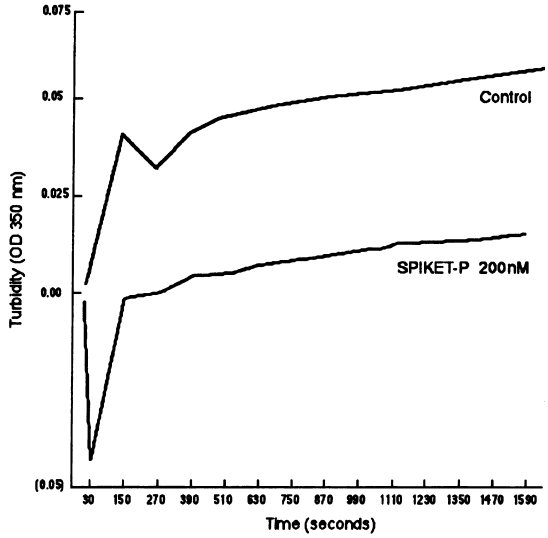
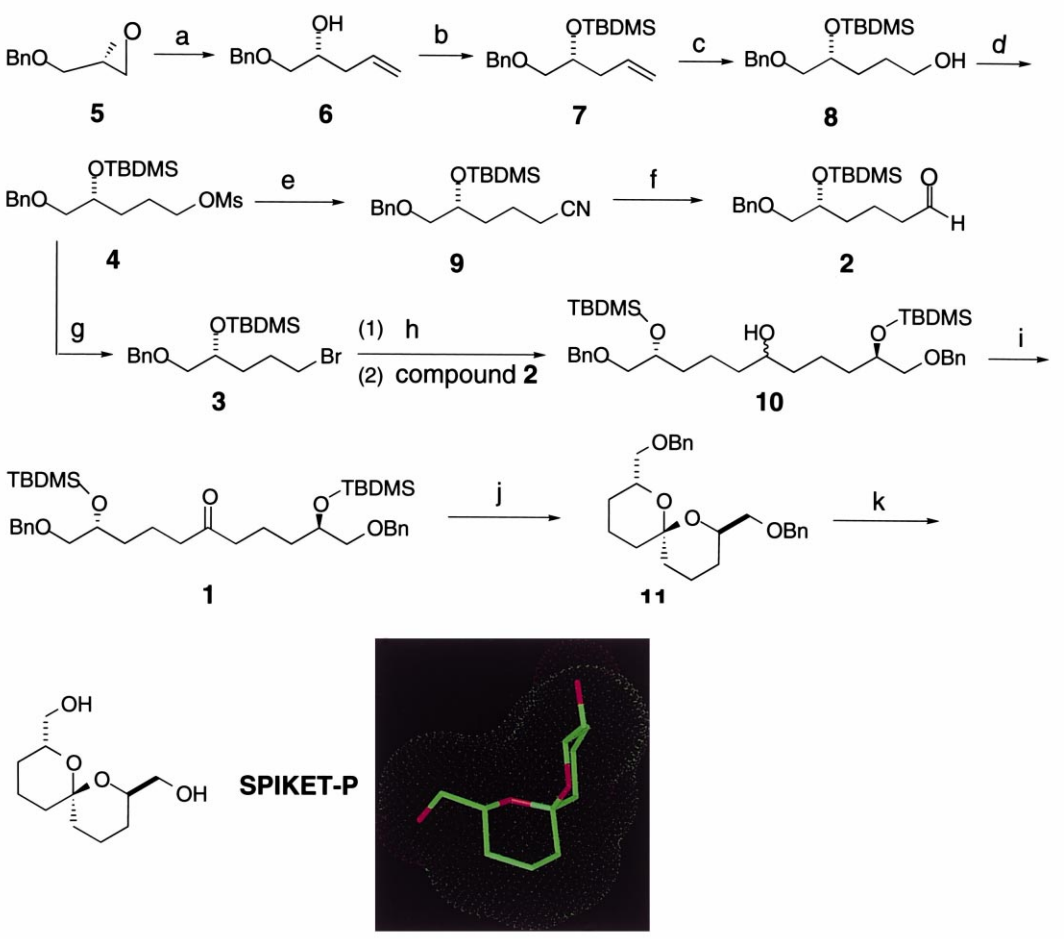


Figure 2. Turbidity measurements. Compounds (in 1% DMSO) were added to tubulin (1 mg/mL, 0.1 M MES, 1 mM EGTA, 0.5 mM $MgCl_2$, 0.1 mM EDTA, 2.5 M glycerol, 1 mg/mL leupeptin, 1 mg/mL aprotinin, pH 6.5) followed by stimulation of polymerization with 1 mM GTP at 2 min. Optical density was measured using a Becton Dickinson UV spectrophotometer (350 nm) using a thermostated cuvette holder to keep the reaction at 37 °C. Readings obtained from the spectrophotometer were standardized by subtracting the background absorbance of the compound in water from the sample reading following drug addition.



Scheme 2. Stereocontrolled synthesis of the SP pharmacophore SPIKET-P**:** (a) vinylMgBr, CuBr, 2 h, 0 °C; 81%; (b) TBDMSCl, imidazole, DMAP, 1 h at 0 °C, 3 h at rt; 98%; (c) (1) BH_3-THF, (2) H_2O_2, NaOH; 70%; (d) MsCl, Et_3N, 2 h, 0 °C; 94%; (e) NaCN, DMSO, 15-Crown-5, 40 °C, overnight; 95%; (f) DIBAL-H, −78 °C 3 h, 10% tartaric acid; 93%; (g) LiBr, acetone, reflux, 0.5 h; 80%; (h) Mg/Et_2O; 45%; (i) oxalyl chloride, DMSO, Et_3N, −78 °C; 83%; (j) 5% HF/CH_3CN, 0.5 h, rt; 100%; (k) LDBB, 0 °C; 50%.

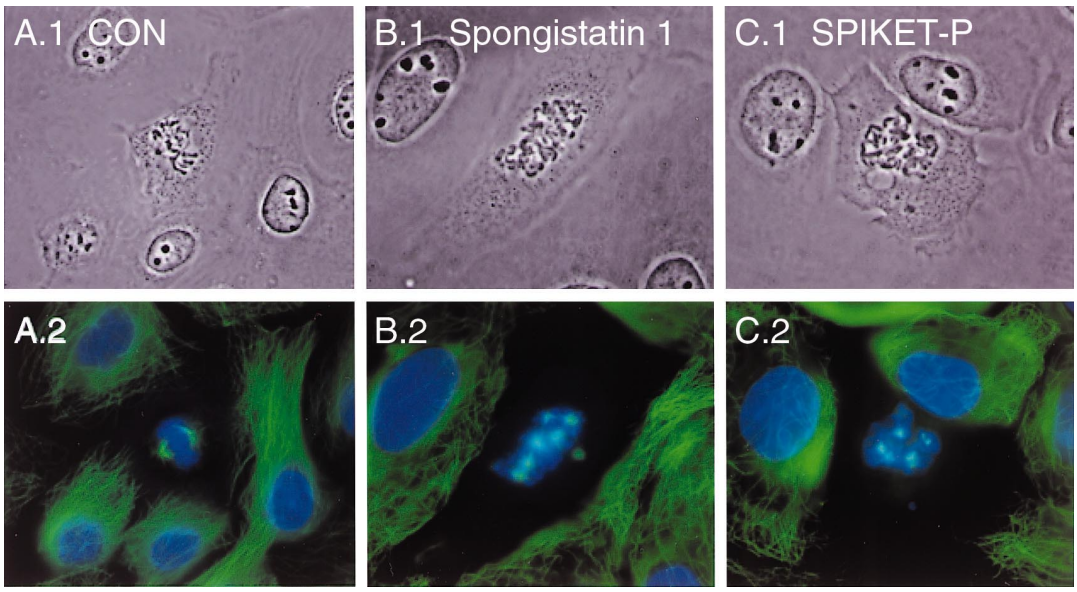


Figure 3. Effects of SPIKET-P1 on mitotic spindle assembly in human breast cancer cells. BT-20 cells in log phase were trypsinized and seeded onto sterile 22 mm^2 coverslips in six-well plates. Cells on coverslips were returned to the incubator for 24 h prior to treatment. The following day SPIKET-P (or Spongistatin 1) was added to yield a final concentration of 10 nM. Final DMSO concentration was 0.025%. Cells were returned to a 37 °C incubator for 24 h. Cells on coverslips were extracted prior to fixation by a 10 min treatment with 4% CHAPS detergent in 1.0× PHEM buffer (60 mM Pipes, 25 mM Hepes, 10 mM EGTA, 4 mM $MgCl_2$, pH 6.95). Cells were then fixed for 10 min in 1.0× PHEM buffer + 0.15% glutaraldehyde and 2% formaldehyde. This was followed by a 15 min incubation in phosphate buffered saline + 0.1% Triton X-100(PBS + Tx). Coverslips were next incubated for 40 min at 37 °C with a primary antibody recognizing α-tubulin (Sigma, St Louis, MO) in a humidified chamber. Coverslips were washed for 15 min in PBS-Tx followed by a 40 min incubation with a fluorescently labeled secondary antibody (Jackson Immunoresearch, West Grove, PA). The coverslips were again rinsed in PBS-Tx and incubated with 5 μM Dapi (Sigma, St Louis, MO) for 20 min to label the DNA. Coverslips were immediately inverted onto slides in Vectashield (Vector Labs, Burlingame, NH) to prevent photobleaching, sealed with nail polish and stored at 4 °C. Slides were examined using a Nikon TE200 inverted microscope equipped for epifluoresence and with high numerical aperture objectives. Digital images were collected with a Princeton Instruments Micromax digital camera using Metamorph software (Universal Imaging, West Chester, PA). Digital data was prepared for publication using Photoshop (Adobe Systems, Mountain View, CA) software and printed on a Pictrography printer (Fuji Photo, Elmsford, NY).

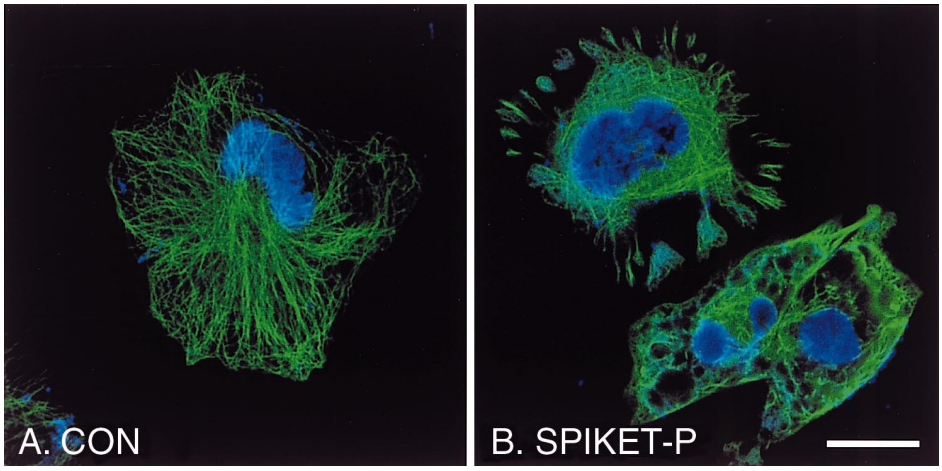


Figure 4. SPIKET-P effects on MDA-MB-231 breast tumor cells. Vehicle-treated control cells are adherent with a well organized microtubule cytoskeleton (A, green). In contrast, cells treated with 500 nM SPIKET-P (B) for 24 h have fewer, less organized microtubules. Additionally, membrane blebbing and nuclear fragmentation is observed. Large vacuoles are present within treated cells. Green = microtubules; blue = DNA; bar = 20 μm.

to form **10** (Scheme 2). Swern oxidation converted **10** to **1**. Deprotection of the two *tert*-butyldimethylsilyl protected hydroxyl groups in **1** followed by acid catalyzed acetal formation to give the immediate benzyl-protected precursor of SPIKET-P, **11**, was carried out by treating **1** with 5% HF in acetonitrile at room temperature for 30 min. After the unsuccessful attempt of removing the two benzylic protecting groups in **11** by platinum catalyzed hydrogenation, SPIKET-P was obtained by treating **11** with lithium 4,4′-di-*tert*-butylbiphenylide (LDBB).[11]

We first examined the ability of SPIKET-P to cause tubulin depolymerization or prevent tubulin polymerization. Bovine brain tubulin (Sigma, St. Louis, MO) was used in standard turbidity assays to test the effect of SPIKET-P on GTP-induced tubulin polymerization. As shown in Figure 2, SPIKET-P caused partial depolymerization of tubulin and inhibited GTP-induced tubulin polymerization. We next examined the effects of SPIKET-P on human breast cancer cells (Fig. 3) using confocal laser scanning microscopy.[12] At low nanomolar concentration, SPIKET-P abrogated cell division in human breast cancer cells by preventing mitotic spindle assembly. Whereas 2.3% of vehicle-treated control cells were in mitosis at 24 h, none of the SPIKET-P treated cells was. Confocal images of SPIKET-P treated breast cancer cells were virtually identical to those of Spongistatin 1 treated breast cancer cells. As shown in Figure 4, breast cancer cells treated with higher concentrations of SPIKET-P showed destruction of the microtubule organization, membrane blebbing, and nuclear fragmentation consistent with apoptosis.

In summary, SPIKET-P, a new pharmocophore of the tubulin depolymerizing marine natural product Spongistatin 1, has been rationally designed based upon the recently resolved electron crystallographic structure of tubulin. SPIKET-P, which was synthesized in a stereocontrolled fashion from the commercially available benzyl (*R*)-(−)-glycidyl ether in 11 steps,[13] caused tubulin depolymerization in turbidity assays, prevented mitotic spindle formation, destroyed the microtubule organization, and induced apoptosis in human breast cancer cells.

References and Notes

1. Avila, J. *Life Sci*. **1992**, *50*, 327.
2. Hyams, J. S.; Lloyd, C. W. *Microtubules*; New York, 1994.
3. Hyman, A.; Karsenti, E. *J. Cell Sci*. **1998**, *111*, 2077.
4. Downing, K. H.; Nogales, E. *Curr. Opin. Cell Biol*. **1998**, *10*, 16.
5. Kozielski, F.; Arnal, I.; Wade, R. H. *Curr. Biol*. **1998**, *8*, 191.
6. Nogales, E.; Wolf, S. G.; Downing, K. H. *Nature* **1998**, *391*, 199.
7. Pettit, G. R.; Cichacz, Z. A.; Gao, F.; Herald, C. L.; Boyd, M. R. *J. Org. Chem*. **1993**, *58*, 1302.
8. Bai, R.; Cichacz, Z. A.; Herald, C. L.; Pettit, G. R.; Hamel, E. *Molecular Pharmacology* **1993**, *44*, 757.
9. Bai, R.; Taylor, G. F.; Cichacz, Z. A.; Herald, C. L.; Kepler, J. A.; Pettit, G. R.; Hamel, E. *Biochemistry* **1995**, *34*, 9714.
10. Nogales, E.; Whittaker, M.; Milligan, R. A.; Downing, K. H. *Cell* **1999**, *96*, 79.
11. Cohen, T.; Jeong, I.-H.; Mudryk, B.; Bhupathy, M.; Awad, M. M. A. *J. Org. Chem*. **1990**, *55*, 1528.
12. Vassilev, A.; Ozer, Z.; Navara, C.; Mahajan, S.; Uckun, F. M. *J. Biol Chem*. **1999**, *274*, 164616.
13. Characterization data: [(2*R*,8*R*)-8-(hydroxymethyl)-1,7-dioxaspiro[5,5]undec-2-yl]methan-1-ol (SPIKET-P): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26–1.53 (m, 6H), 1.63 (m, 4H), 1.88 (m, 2H), 2.14 (bs, 2H), 3.52 (dd, J = 11.0, 7.0 Hz, 2H), 3.62 (dd, J = 11.0, 3.5 Hz, 2H), 3.75 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 96.0, 69.7, 66.2, 35.2, 26.3, 18.2; GC/MS m/z 216 (M^+); IR (neat): 3373, 2939, 2871 cm^{-1}; $[\alpha]_D^{22}$ −59.4 (c 0.7, $CHCl_3$); X-ray: Space group: $P2_1$, unit cell: a = 11.4510(15) Å, b = 8.9628(11) Å, c = 22.942(3) Å, α = 90°, β = 93.396(3)°, γ = 90°, volume = 2350.5(5) Å^3, Z = 8, θ range for data collection = 1.78 to 28.32° (λ = 0.71073 Å), total reflections collected = 12,806, independent reflections = 9044, data/restraints/parameters = 9044/1/542, R1 [$I > 2\sigma(I)$] = 0.139, wR2 = 0.187, goodness of fit on F^2 = 1.009.