Antitumor Compounds Based on a Natural Product Consensus Pharmacophore

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We report the design and highly enantioselective synthesis of a potent analogue of the spliceosome inhibitor FR901464, based on a non-natural product scaffold. The design of this compound was facilitated by a pharmacophore hypothesis that assumed key interaction types that are common to FR901464 and an otherwise unrelated natural product (pladienolide). The synthesis allows for the preparation of numerous novel analogues. We present results on the in vitro activity for this compound against several tumor cell lines.

Introduction

A novel mechanism of antitumor activity was recently elucidated for two structurally distinct fermentation products (Figure 1)[1] pladienolide[2] (a set of closely related compounds that have been designated pladienolides A−G, isolated from *Streptomyces platensis*)[3] and FR901464 (isolated from *Pseudomonas* sp. no. 2663).[4−6] These natural products have previously been reported to have a similar novel effect on the cell cycle in mammalian cell lines, cell cycle arrest at the G1 and G2/M phases.[3,4] This effect on the cell cycle distinguished these compounds from other antitumor agents that also inhibit proliferation in tumors and act at previously identified molecular targets.[3−5] This interaction with the spliceosome leads to the inhibition of mRNA splicing and the translation of unspliced mRNA, which ultimately results in the observed effects on the cell cycle.[2,7] The first of these natural products to be discovered, FR901464, showed activity in in vivo cancer animal models but showed a relatively narrow therapeutic window.[4] On the other hand pladienolide-based compounds (first reported in 2004) have shown a very broad therapeutic window.[2] Of the seven pladienolides, pladienolide B was reported to have the most promising anticancer activity in vivo, and it has recently been reported that a derivative of pladienolide B has entered human clinical trials for cancer.[2]

Discussion

Motivated by the recognition that FR901464 and pladienolide have a very similar (or possibly identical) mechanism of action, we compared overlays of molecular models of the two natural products. Upon inspection of these overlays we recognized that despite the gross dissimilarities between FR901464 and pladienolide, both molecules share certain common key features (which are known to be critical to their activity) that can overlap in low energy conformations in three dimensions (Figure 2). It is known from previous studies that the orientation of the acetyl group and the presence of the epoxide are important in conferring activity to FR901464 analogues[8,9] but that the glycosidic hydroxyl group can be replaced by an alkoxy[10] or a methyl group[11] to give more stable and more active compounds.

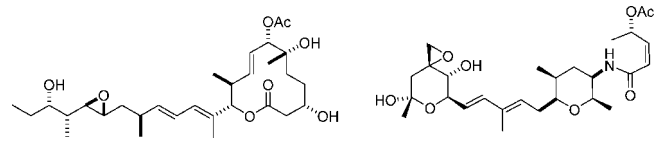

Figure 1. Structures of pladienolide B (left) and FR901464 (right).

Active analogues of pladienolide also contain similar functionality,[2,3] though less structure−activity information has been reported on these more recently discovered compounds. This led us to propose that the three critical requirements for activity of FR901464 and pladienolide are an appropriately positioned epoxy and carbonyloxy groups connected via an appropriately constrained "linker" group of the right shape and length. We also assumed that these groups would need to be conformationally constrained in a way similar to that seen in the natural products. Information regarding the requirement of the linker group is not available, but both natural products have significant similarities in the structure of their diene linker group. In FR901464, the linking group includes conformational constraints consisting of two pyran 6-membered rings, whereas in pladienolide the common diene constraint is an unsaturated 12-membered macrocyclic lactone. Using this information, we developed a hypothetical 3D pharmacophore model that allowed us to propose that compounds of the general structures **1**−**3** (Figure 3) could have similar biological activity and could therefore serve as starting points for the development of compounds with potential for human cancer therapy. The proposed **1** contains the unchanged methylpenta-2,4-diene group and 5-oxopent-3-en-2-yl acetate group along with the modified deoxy-spiro-epoxide group, with the pyran ring replaced by a cis-1,4-substituted cyclohexyl group, whereas **2** and **3** include additional polar modifications to the methylpenta-2,4-diene group that are very synthetically assessable. These changes (particularly for **1**) are expected to impart similar conformational presentation of the linker, the epoxy, and the carbonyloxy groups as that found in FR901464.

It is noted that these modifications (conversion of the pyran ring oxygen to carbon, removal of two methyl groups, and removal of a hydroxyl oxygen) eliminate six chiral centers when compared to FR901464, which made the synthesis of all of our target compounds readily achievable. At the outset of our project it was not clear that compounds containing all of these substantial modifications would be active, since there have been no reports providing detailed structural information regarding

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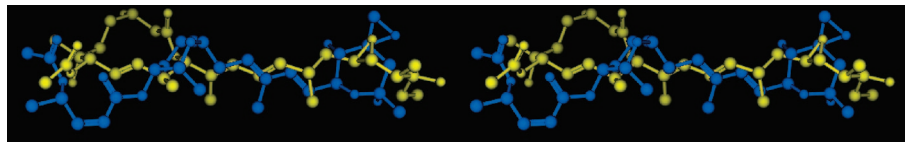


Figure 2. A 3D representation of an overlay of the presumed key interaction groups in a low energy conformation of pladienolide and FR901464, showing that the epoxy group and the carbonyloxy groups are the same distance in both molecules and that the methyl-2,4-pentadienyl groups can readily adopt a similar positioning. The overlays were prepared using the Molecular Operating System (MOE 2007.09, Chemical Computing Group, Inc.) using the Flexible Alignment function with both molecules following a conformational minimization using MOE default settings.

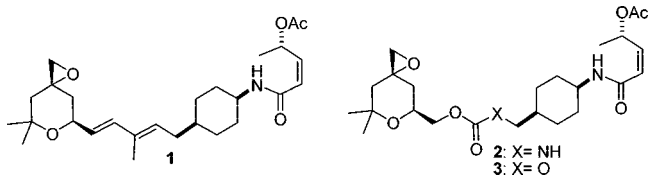
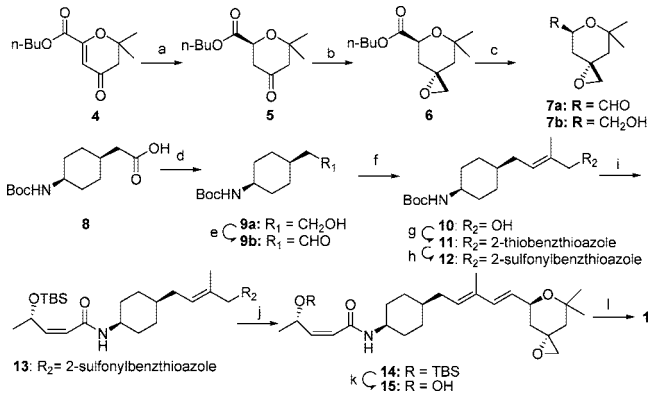


Figure 3. Initial synthetic targets in our study that matched our hypothetical pharmacophore.

Scheme 1. Synthesis of Analogue **1**[a]

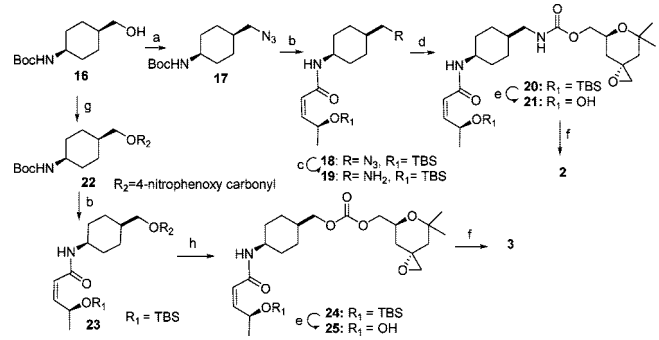


[a] Reagents and conditions: (a) (2*R*,5*R*)-5-benzyl-3-methyl-(5-methylfuran-2ylimidazolidin-4-one, *tert*-butyl Hantzsch ester, Cl_3CCO_2H, Et_2O, 4 °C; (b) NaH, $(CH_3)_3SOI$, DMSO; (c) DIBALH, THF, −78°C; (d) BH_3·THF, THF; (e) oxalyl chloride, DMSO, Hunig's base, −78 °C, CH_2Cl_2; (f) (i) $Ph_3PC(CH_3)CO_2Et$, benzene; (ii) DIBALH, −78°C, toluene; (g) 2-mecaptobenzothiazole, DIAD, THF; (h) ammonium molybdate, H_2O_2, EtOH; (i) (i) TFA, CH_2Cl_2; (ii) (*S*,*Z*)-4-(TBSO)pent-2-enoic acid, HBTU, Hunig's base, CH_3CN; (j) LHMDS, **7a**, THF, −78°C to room temp; (k) Bu_4NF, THF, 0°C; (l) Ac_2O, Et_3N, CH_2Cl_2.

the interaction of FR901464 and pladienolide with the spliceosome SF3b subunit or of the synthesis of compounds that are similar to **1**−**3**. We believed, however, that there was a reasonable likelihood that one or more of our compounds could demonstrate substantial activity, especially given that there is an example of a FR901464 analogue with a cell-based growth inhibition IC_{50} as low as 10 pM.[11]

There have been numerous synthetic approaches to FR901464; however, since these synthetic approaches target the much more complex natural product, these routes were not highly instructive for the construction of our synthetic targets **1**−**3**. Our approach to these analogues is shown in Schemes 1 and 2. We found the readily available inexpensive **4** (which has commercial application as an insect repellant) to be an attractive starting material for the left-hand portion of our synthetic targets **1**−**3**. To introduce the desired stereochemistry, we explored numerous transition metal catalysts for the enantioselective reduction of **4** to ketoester **5**; for example, we investigated the ruthenium(II) BINAP complex as a chiral hydrogenation catalyst, using the published conditions, but found no reduction or at higher temperatures the undesired reduction of the ketone carbonyl in preference to the carbon−carbon double bond.[12] However, we

Scheme 2. Synthesis of Analogues **2** and **3**[a]



[a] Reagents and conditions: (a) (i) MsCl, Et_3N, 0°C, CH_2Cl_2; (ii) NaN_3, DMF, 70°C; (b) (i) TFA, CH_2Cl_2; (ii) (*S*,*Z*)-4-(TBSO)pent-2-enoic acid, HBTU, Hunig's base, CH_3CN; (c) Ph_3P, H_2O, THF; (d) **7b**, 4-nitrophenyl chloroformate, Et_3N, THF; (e) Bu_4NF, THF, 0°C; (f) Ac_2O, Et_3N, CH_2Cl_2; (g) 4-nitrophenyl chloroformate, Et_3N, THF; (h) **7b**, DMAP, CH_3CN.

found that application of the appropriate MacMillan conditions[13] gave the desired product in good yield (though contaminated with the starting material **4**, which coeluted on silica chromatography) and in high enantiomeric excess (91% based on chiral SFC chromatography of a derivative; see Supporting Information). The absolute stereochemistry of our ketoester product **5** is based on numerous published examples of the stereochemistry obtained using this organocatalyst on similar substrates.[13,14] We then turned our attention to the diastereoselective introduction of the desired spiroepoxide; we expected that the addition of dimethylsulfoxonium methylide[15] would occur from an axial approach on the ketone carbonyl of **5** to give primarily the desired diastereomer (epoxyester **6**). We found that this was indeed the case; in fact, we did not detect any of the undesired epoxide in the crude reaction mixture, and the product was obtained in excellent yield (see Supporting Information for details on the NOE experiments used for assigning the relative stereochemistry). This approach allowed us to set two of the three chiral centers in our targets and provided intermediates that were appropriately functionalized for conversion to our ultimate targets **1**−**3**.

The synthesis of the **1** could then be accomplished starting with the aldehyde **9** derived from the commercially available cis-cyclohexane derivative **8**, followed by homologation to give trans alcohol **10** cleanly in high overall yield.[16] This compound was converted to the corresponding 2-thiobenzthioazole derivative **11** using Mitsunobu conditions[17] and subsequently oxidized to the corresponding 2-sulfonylbenzothioazole derivative **12**.[10] Removal of the BOC group in **12** and coupling of the optically pure (*S*,*Z*)-4-(dimethyl-*tert*-butylsilyloxy)pent-2-enoic acid (prepared according to the known procedure)[18] gave the desired functionalized amide **13** in excellent overall yield. Coupling of this reagent with epoxyaldehyde **7a**[10] to give diene **14** followed by deprotection and acylation of **15** gave final compound **1** cleanly in acceptable yield (the overall yield of **1** from **4** is 2.7%).

Table 1. In Vitro Cytotoxicity of Compound **1** after a 72 h Exposure

cell line	published IC_{50} (μM) pladienolide B[2,3]	published IC_{50} (μM) FR901464[5]	$IC_{50} \pm$ SEM (μM)[a] **1**
A549	0.0014	0.0013	4.65 ± 0.37
JeKo-1	NA[b]	NA[b]	0.10 ± 0.002
JVM-2	NA[b]	NA[b]	0.13 ± 0.03
MCF-7	0.0004	0.0018	2.29 ± 0.38
OVCAR-3	0.0004	NA[b]	1.98 ± 0.63
PC-3	0.0023	NA[b]	2.12 ± 0.57
WiDr	0.00086	NA[b]	2.03 ± 0.20

[a] Experimental IC_{50} values represent the mean $\pm$ SEM from three independent experiments. [b] NA = data not available.

In order to obtain the carbamate **2** and carbonate **3**, as shown in Scheme 2, we used the commercially available alcohol **16** to prepare the azide **17**, which could be deprotected and converted to the amide azide **18** in excellent yield. This derivative could be reduced with triphenylphosphine on solid support to cleanly give amine **19**,[19] which was then converted to the carbamate **2** in excellent yield[20] (the overall yield of **2** from **16** is 12.6%). In a similar way the alcohol **16** was converted to the *p*-nitrophenylcarbonate intermediate **22**, which was deprotected and underwent amidation with (*S*,*Z*)-4-(dimethyl-*tert*-butylsilyloxy)pent-2-enoic acid (prepared according to a published method)[10] to give amide **23** in good yield. This amide could then be coupled with alcohol **7b** (from Scheme 1) to give the carbonate **3** (the overall yield of **3** from **16** is 14.2%).

Seven human cancer cell lines were then used to evaluate the cytotoxic potential of **1**−**3**; however, only **1** showed potent activity. Compound **2** did not show any significant cytotoxic activity in our initial tumor line screen (in A549, COS-7, MCF-7, WiDr, and NIH3T3 cells) at concentrations up to 5 μM. In this screen, **3** inhibited cell growth by only 10−20% at the highest concentration tested of 5 μM, but only in the A549 and MCF-7 lines. Compound **1** was then screened in an expanded tumor panel consisting of the following cell lines: A549 lung cancer, WiDr colorectal cancer, MCF-7 breast cancer, PC-3 prostate cancer, OVCAR-3 ovarian cancer, and JeKo-1 and JVM-2 mantle cell lymphoma cell lines. An XTT colorimetric cytotoxicity assay was employed to estimate the IC_{50} values (Table 1). The cytotoxicity profile of **1** against the A549, MCF-7, OVCAR-3, PC-3, and WiDr cancer cell lines revealed IC_{50} values of 4.65, 2.29, 1.98, 2.12, and 2.03 μM, respectively (Table 1). Although **1** is substantially less potent than the natural products against several of the cell lines tested, it shows potent and selective killing of two tumor cell lines (JeKo-1 and JVM-2, IC_{50} values of 100 and 130 nM, respectively).

To further investigate the biological activity of **1**, the effect of the compound on the cell cycle was evaluated. Following an 18 h incubation of **1** with the A549 lung cancer, JeKo-1 mantle cell lymphoma, and WiDr colorectal cancer cell lines, all three lines accumulated in the G_2/M phases of the cell cycle (Figure 4). The degree of G_2/M cell cycle arrest correlated with the sensitivity of the cell lines to the cytotoxic activity of **1** (JeKo-1 > WiDr > A549). In addition to the increased fraction of cells in the G_2/M phase of the cell cycle, a concomitant decrease in the S-phase fraction was observed for all three cell lines following treatment with **1**. As reported in the literature,[3,4,7] the spliceosome inhibitor compounds pladienolide B, FR901464, and spliceostatin A all also induce a similar accumulation of cells in the G_2/M phases of the cell cycle, with a simultaneous decrease in the fraction of cells in S phase.

Conclusion

We report the enantioselective and diastereospecific synthesis of **1** that shows an activity profile similar to those of FR901464 and pladienolide but with reduced potency. The design of this compound is based on insights from the common positioning of similar interacting groups from these unrelated natural products. By judicious modification of atom types and the removal of groups that did not appear to impart interaction features or conformational constraints, we were able to develop compounds that showed significant antitumor activity but that have only three stereocenters (compared to nine in FR901464) and are therefore much more synthetically attractive. Since **1** displays a similar bioactivity profile, it is quite possible that it also acts in a manner analogous to FR901464 and pladienolide, by targeting the SF3b subunit of the spliceosome. The synthetic scheme that we have developed allows for the introduction of numerous modifications that will allow us to fully elucidate the structure−activity relationships in this series and will also allow us to prepare compounds that have an improved in vivo profile, in order to develop lead compounds as potential human therapeutics.

Experimental Section

(*S*)-Butyl 6,6-Dimethyl-4-oxotetrahydro-2*H*-pyran-2-carboxylate (5). A solution of commercially available butopyronoxyl **4** (230 mg, 1.01 mmol) and (2*R*,5*R*)-5-butylmethyl-2-(5-methylfuran-2-yl)imidazolidin-4-one (55 mg, 0.20 mmol) in anhydrous Et_2O (4.6 mL) was stirred and cooled at ice bath temperature (0−4 °C) as di-*tert*-butyl 2,6-dimethyldicarboxylate (346 mg, 1.11 mmol) and trichloroacetic acid (TCA) (32 mg, 0.20 mmol) were sequentially added. The resulting yellow solution was allowed to stir for 3 days at 4 °C. The reaction mixture was passed through a short pad of silica gel and eluted with Et_2O (60 mL). Concentration of the Et_2O and purification of the residue by flash chromatography (20% EtOAc in hexane) gave 204 mg (88%) of keto ester **5** as an oil. $[\alpha]_D^{25}$ +15.7° (*c* 2.1 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.46 (dd, J = 4.0, 11.0 Hz, 1H), 4.19 (td, J = 2.7, 6.7 Hz, 2H), 2.64−2.47 (m, 3H), 2.32 (dd, J = 1.6, 14.0 Hz, 1H), 1.68−1.59 (m, 2H), 1.46 (s, 3H), 1.43−1.33 (m, 2H), 1.23 (s, 3H), 0.94 (t, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 205.2, 170.1, 75.2, 70.0, 65.4, 52.9, 43.5, 30.6, 30.5, 23.7, 19.0, 13.6; IR (neat film) 2965, 2875, 1734, 1725, 1464, 1379, 1241, 1181 cm^{-1}; HRMS (FAB) *m*/*z* calcd for $C_{12}H_{21}O_4$ $(M + 1)^+$ 229.1440, found 229.1440.

(3*R*,5*S*)-Butyl 7,7-Dimethyl-1,6-dioxaspiro[2.5]octane-5-carboxylate (6). A suspension of sodium hydride (96 mg, 2.40 mmol, 60% in mineral oil) in anhydrous DMSO (4.2 mL) was stirred at room temperature as trimethylsulfoxonium iodide (491 mg, 2.40 mmol) was added. After 30 min, the keto ester **5** (500 mg, 2.19 mmol) in DMSO (1.0 mL) was added dropwise. After 1 h at room temperature, the reaction was quenched with ice cold H_2O (5 mL). The product was then extracted with EtOAc (3 × 30 mL), and the combined extracts were washed with H_2O (2 × 15 mL) and brine (20 mL) and dried over $MgSO_4$. Evaporation of the solvent and purification by flash chromatography (20% EtOAc in hexane) gave 430 mg (81%) of epoxide derivative **6** as a liquid. $[\alpha]_D^{25}$ +41.5° (*c* 2.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.53 (dd, J = 2.7, 12.1 Hz, 1H), 4.14 (t, J = 6.7 Hz, 2H), 2.61 (dd, J = 4.5, 13.9 Hz, 2H), 2.14−2.02 (m, 2H), 1.65−1.58 (m, 2H), 1.47 (ddd, J = 4.2, 6.7, 10.9 Hz, 1H), 1.39 (s, 3H), 1.38−1.33 (m, 2H), 1.34 (s, 3H), 1.16 (dd, J = 2.0, 14.0 Hz, 1H), 0.93 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.5, 74.0, 68.3, 64.9, 55.2, 51.1, 42.2, 35.2, 31.0, 30.5, 23.3, 19.0, 13.7; IR (neat film) 2966, 2874, 1756, 1465, 1378, 1218; 1180 cm^{-1}; HRMS (FAB) *m*/*z* calcd for $C_{13}H_{23}O_4$ $(M + 1)^+$ 243.1596, found 243.1595.

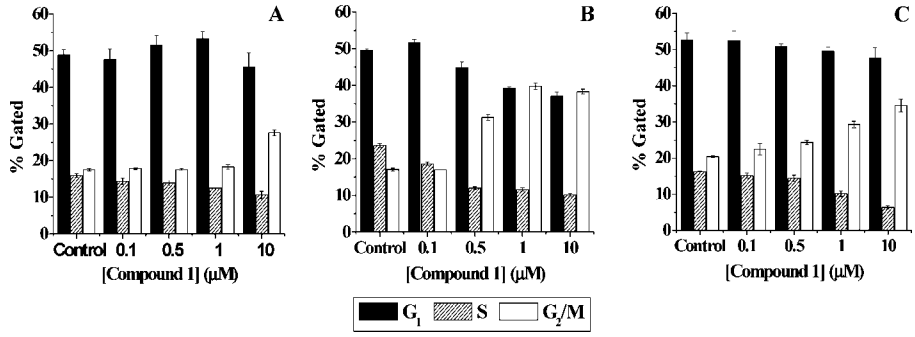


Figure 4. Cell cycle analysis following treatment with **1**. The (A) A549, (B) JeKo-1, and (C) WiDr cancer cell lines were treated with **1** for 18 h, then permeabilized and stained with propidium iodide for cell cycle analysis by flow cytometry.

(3*R*,5*S*)-7,7-Dimethyl-1,6-dioxaspiro[2.5]octane-5-carbaldehyde (7a). To a stirred solution of ester **6** (1 g, 4.1 mmol) in anhydrous hexane (15 mL) and THF (9 mL) was added DIBALH (4.7 mL, 4.75 mmol, 1.0 M in hexane) at −78 °C. The resulting solution was allowed to stir for 30 min at −78 °C, by which time TLC indicated that the alcohol has been consumed. MeOH (1.5 mL) was added, and the mixture was diluted with saturated aqueous sodium/potassium tartarate (30 mL) and EtOAc (80 mL). The solution was stirred for 15 min at room temperature to get a clear solution. The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with brine (45 mL) and dried over Na_2SO_4. Evaporation of the solvent and purification of the residue by flash chromatography (50% EtOAc in hexane) gave 390 mg (56%) of aldehyde as a liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (s, 1H), 4.35 (dd, J = 3.0, 12.0 Hz, 1H), 2.60 (q, J = 4.6 Hz, 2H), 2.04−1.86 (m, 2H), 1.46−1.40 (m, 1H), 1.39 (s, 3H), 1.33 (s, 3H), 1.20 (dd, J = 2.0, 14.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.7, 74.0, 73.6, 54.7, 51.1, 42.5, 32.6, 30.6, 30.9, 23.3; IR (neat film) 2970, 2913, 1732, 1184, 1084 cm^{-1}; HRMS (FAB) *m*/*z* calcd for $C_9H_{14}O_3$ (M^+) 170.0943, found 170.0943.

7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)methanol (7b). A solution of epoxy ester **6** (320 mg, 1.32 mmol) in anhydrous THF (5.0 mL) was stirred and cooled at −78 °C as DIBALH (4.6 mL, 1.0 M in hexane) was slowly added dropwise. The solution was stirred at the same temperature for 3 h. Saturated aqueous NH_4Cl (4 mL) was introduced to destroy excess reagent at −78 °C. This mixture was allowed to warm to room temperature and was stirred for 30 min to get a clear mixture. The organic layer was then separated, and the aqueous layer was extracted with EtOAc (3 × 30 mL). The combined layers were washed with saturated aqueous $NaHCO_3$ (15 mL) and brine (15 mL) and dried over Na_2SO_4. Concentration and purification by flash chromatography (50% EtOAc in hexane) gave the alcohol **7b** in quantitative yield as a liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.07−3.98 (m, 1H), 3.70−3.61 (m, 1H), 3.53−3.43 (m, 1H), 2.58 (q, J = 4.6 Hz, 2H), 2.15 (t, J = 5.8 Hz, 1H), 1.99−1.88 (m, 2H), 1.38 (s, 3H), 1.25 (s, 3H), 1.17 (dd, J = 2.0, 13.9 Hz, 1H), 1.08−1.01(m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 73.1, 68.7, 65.9, 55.5, 51.0, 42.7, 33.7, 31.3, 23.8; IR (neat film) 3435, 2973, 2914, 1376, 1250, 1221, 1186, 1093, 1052 cm^{-1}; HRMS (FAB) *m*/*z* calcd for $C_9H_{16}O_3$ $(M)^+$ 172.1100, found 172.1099.

(*S*,*Z*)-4-(*tert*-Butyldimethylsilyloxy)-*N*-((1*R*,4*R*)-4-((2*E*,4*E*)-5-((3*R*,5*S*)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dienyl)cyclohexyl)pent-2-enamide (14). A solution of sulfone derivative **13** (430 mg, 0.75 mmol) in anhydrous THF (3 mL) was stirred and cooled at −78 °C as LHMDS (0.92 mL, 0.92 mmol, 1.0 M in THF) was added dropwise. The solution turned orange during the addition. After the mixture was stirred for 10 min at −78 °C, aldehyde **7a** (75 mg, 0.75 mmol) in anhydrous THF (2 mL) was introduced dropwise. The resulting suspension was again stirred for 1 h at −78 °C. The mixture was then slowly allowed to warm to room temperature and stirred for 2 h. Saturated aqueous NH_4Cl solution (15 mL) was added, and the product was extracted with EtOAc (3 × 35 mL). The combined extracts were washed with brine (40 mL) and dried over $MgSO_4$. Evaporation of the solvent and purification of the residue by flash chromatography (25% EtOAc in hexane) gave 70 mg of diene **14** as a liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.27 (d, J = 15.7 Hz, 1H), 5.99 (dd, J = 7.8, 11.6 Hz, 1H), 5.61−5.45 (m, 5H), 4.48−4.44 (m, 1H), 4.03 (s, 1H), 2.57 (s, 2H), 2.08 (t, J = 7.2 Hz, 2H), 2.01−1.89 (m, 2H), 1.72 (s, 3H), 1.57−1.53 (m, 6H), 1.46−1.36 (m, 1H), 1.40 (s, 3H), 1.29−1.27 (m, 6H), 1.18−1.08 (m, 4H), 0.87 (s, 9H), −0.01 (d, J = 5.3 Hz, 6H); IR (neat film) 3394, 2926, 2856, 1735, 1666, 1629, 1532, 1448, 1320, 1244, 1049 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{31}H_{54}NO_4Si$ $(M + 1)^+$ 532.3822, found 532.3825.

(*S*,*Z*)-*N*-((1*R*,4*R*)-4-((2*E*,4*E*)-5-((3*R*,5*S*)-7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dienyl)cyclohexyl)-4-hydroxypent-2-enamide (15). A solution of TBS protected diene **14** (13 mg, 0.02 mmol) in THF (1.0 mL) was stirred and cooled at 0 °C as TBAF (49 μL, 0.04 mmol, 1.0 M in THF) was added. The resulting yellow solution was allowed to stir for 2 h at room temperature, by which time the TLC showed that all of starting material has been consumed. Evaporation of THF and purification of the residue by flash chromatography (60−80% EtOAc in hexane) gave 9.5 mg (95%) of **15** as a liquid. $[\alpha]_D^{25}$ +78° (*c* 0.86, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.27 (d, J = 15.7 Hz, 1H), 6.15 (dd, J = 5.4, 11.9 Hz, 1H), 5.87 (bd, 1H), 5.75 (dd, J = 1.6, 12.0 Hz, 1H), 5.56 (dd, J = 6.7, 15.6 Hz, 1H), 5.47 (t, J = 7.7 Hz, 1H), 4.80−4.73 (m, 1H), 4.50−4.43 (m, 1H), 4.09−4.02 (m, 1H), 2.57 (s, 2H), 2.09 (t, J = 7.3 Hz, 2H), 2.04−1.88 (m, 2H), 1.72 (s, 3H), 1.69−1.57 (m, 6H), 1.52−1.44 (m, 1H), 1.40 (s, 3H), 1.34 (d, J = 6.7 Hz, 3H), 1.28 (s, 3H), 1.26−1.23 (m, 2H), 1.18−1.11 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.9, 150.0, 136.1, 134.1, 131.6, 127.1, 123.1, 73.0, 69.6, 64.6, 55.7, 51.0, 45.8, 42.4, 38.6, 36.4, 34.1, 31.5, 29.2, 27.8, 27.7, 23.8, 22.8, 12.5; IR (neat film) 3307, 2926, 2857, 1656, 1622, 1536, 1447, 1372, 1258, 1183, 1108 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{25}H_{40}NO_4$ $(M + 1)^+$ 418.2957, found 418.2948.

(*S*,*Z*)-5-((1*R*,4*R*)-4-((2*E*,4*E*)-5-((3*R*,5*S*)-7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dienyl)cyclohexylamino)-5-oxopent-3-en-2-yl Acetate (1). A solution of alcohol **15** (7 mg, 0.01 mmol) in CH_2Cl_2 (0.9 mL) was allowed to stir at 0 °C as Et_3N (16 μL, 0.11 mmol) and DMAP (2.0 mg, 0.01 mmol) were sequentially added. After 5 min, acetic anhydride (5.4 μL, 0.05 mmol) was added using a micropipet. The solution was stirred for 1 h at 0 °C. Evaporation of the solvent and purification of the residue by flash chromatography (50% EtOAc in hexane) gave 6.1 mg (79%) of **1** as a viscous oil. $[\alpha]_D^{25}$ −1.0° (*c* 0.21, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 (br d, 1H), 6.27 (d, J = 15.7 Hz, 1H), 5.81 (m, 2H), 5.66 (dd, J = 9.2, 11.6 Hz, 1H), 5.55 (dd, J = 6.7, 15.7 Hz, 1H), 5.49 (t, J = 7.5 Hz, 1H), 4.50−4.43 (m, 1H), 4.12−4.06 (m, 1H), 2.57 (s, 2H), 2.12−2.06 (m, 2H), 2.07 (s, 3H), 2.01−1.88 (m, 2H), 1.72 (s, 3H), 1.72−1.66 (m, 1H), 1.64−1.57 (m, 6H), 1.40 (s, 3H), 1.36 (d, J = 6.4 Hz, 3H), 1.28 (s, 3H), 1.27−1.19 (m, 3H), 1.15 (dd, J = 2.0, 13.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 164.8, 137.9, 136.2, 133.8, 131.9, 126.9, 125.4, 73.0, 69.6, 69.2, 55.6, 51.0, 45.5, 42.4, 38.6, 36.4, 34.2, 31.5, 29.5, 29.4, 27.8, 27.6, 23.7, 21.2, 20.3, 12.5;[21] IR (neat film) 3310, 2928, 2856, 1660, 1626, 1534, 1445, 1365, 1252, 1219,

1185, 1116, 1072 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{27}H_{42}NO_5$ $(M + 1)^+$ 460.3063, found 460.3049.

(2*S*,*Z*)-5-((1*s*,4*R*)-4-((((7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)methoxy)carbonylamino)methyl)cyclohexylamino)-5-oxopent-3-en-2-yl Acetate (2). Acetylation of **21** (6 mg, 0.01 mmol) used the same procedure as for conversion of **15** to the corresponding acetate **1**, to give 5.9 mg (90%) of **2** as an oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.19 (br d, 1H), 5.82−5.74 (m, 2H), 5.65 (dd, $J = 9.1$, 11.8 Hz, 1H), 4.89 (br t, 1H), 4.17−4.04 (m, 4H), 3.16−3.08 (m, 2H), 2.57 (q, $J = 4.6$ Hz, 2H), 2.09 (s, 3H), 1.97 (d, $J = 13.9$ Hz 1H), 1.84 (dd, $J = 11.6$, 13.5 Hz, 1H), 1.77−1.69 (m, 2H), 1.68−1.59 (m, 4H), 1.37−1.34 (m, 6H), 1.28−1.24 (m, 6H), 1.18−1.11 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.9, 152.6, 137.3, 125.8, 73.1, 69.1, 67.1, 55.4, 50.9, 45.8, 45.2, 42.5, 36.2, 31.3, 29.7, 29.1, 25.4, 25.1, 23.6, 21.3;[21] IR (neat film) 3334, 2928, 2859, 1717, 1666, 1533, 1452, 1371, 1247, 1122, 1047 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{24}H_{39}N_2O_7$ $(M + 1)^+$ 467.2757, found 467.2759.

(2*S*,*Z*)-5-((1*S*,4*R*)-4-((((7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)methoxy)carbonyloxy)methyl)cyclohexylamino)-5-oxopent-3-en-2-yl Acetate (3). Acetylation of **25** (7 mg, 0.01 mmol) was achieved following the same procedure used for conversion of **15** to the corresponding acetate **1**, to give 6 mg (78%) of **3** as an oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.20 (br d, 1H), 5.84−5.74 (m, 2H), 5.65 (dd, $J = 9.1$, 11.8 Hz, 1H), 4.20−4.08 (m, 4H), 4.02 (d, $J = 6.9$ Hz, 2H), 2.57 (q, $J = 4.6$ Hz, 2H), 2.06 (s, 3H), 2.04−1.85 (m, 2H), 1.84−1.80 (m, 1H), 1.78−1.63 (m, 6H), 1.44−1.35 (m, 8H), 1.25 (s, 3H), 1.20−1.12 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 164.9, 155.3, 137.4, 125.6, 73.3, 71.7, 70.4, 69.1, 66.4, 55.4, 50.9, 45.3, 42.4, 35.1, 34.3, 31.2, 28.9, 28.8, 24.4, 24.2, 23.5, 21.2, 20.3;[21] IR (neat film) 3326, 2932, 1742, 1668, 1631, 1532, 1451, 1371, 1252, 1119, 1048 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{24}H_{38}NO_8$ $(M + 1)^+$ 468.2597, found 426.2584.

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Supporting Information Available: General experimental methods, procedures for the synthesis of **9**−**13** and **17**−**25**, and full experimental details for the cell cycle analysis and the in vitro cytotoxicity assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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