Articles

Synthesis and Anti-Tubulin Activity of a 3′-(4-Azidophenyl)-3′-dephenylpaclitaxel Photoaffinity Probe

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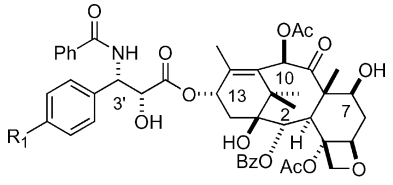
The synthesis and biological evaluation of a novel paclitaxel photoaffinity probe is described. The synthesis involved the preparation of an azide-containing C13 side chain through a Staudinger cycloaddition followed by a lipase-mediated kinetic resolution to obtain the azetidinone in 99% ee. Coupling of the enantiopure side chain precursor to 7-TES-baccatin III and subsequent silyl ether deprotection afforded 3′-(4-azidophenyl)-3′-dephenylpaclitaxel, which was shown to be as active as paclitaxel in tubulin assembly and cytotoxicity assays.

Introduction

Paclitaxel (**1**, Taxol; Figure 1) is regarded as one of the clinically most effective chemotherapeutic agents. It is currently employed in the treatment of a variety of cancers including drug-refractory ovarian cancer, Kaposi's sarcoma, small-cell lung cancer, and metastatic breast cancer.[1] Paclitaxel is believed to block mitosis by suppressing microtubule dynamics that leads to apoptosis.[2] Even though paclitaxel is known to bind to the β-subunit of tubulin, little is known of its interactions at the molecular level. Insight into specific residue interaction has been gained through the use of photoaffinity probes,[3−8] fluorescent probes,[9] electron crystallography,[10] tubulin mutations,[11] and computational studies.[12−14]

Recently, there has been much discussion on the biologically active orientation of paclitaxel bound to tubulin. Three hypotheses have emerged with respect to the bioactive conformation of the highly flexible C2 and C3′ side chains. The first involves an aggregation where the groups of the C2 benzoyl and C3′ benzamide side chains are in proximity to one another as is seen in the solid state.[15] The second conformation aligns the C2 benzoyl and C3′ phenyl side chains as is observed by NMR in polar solvents and is termed "hydrophobic collapse".[12,16] A third hypothesis orients the side chains of paclitaxel in a T-shape with the C2 benzoyl group equidistant to both the phenyl rings at C3′ and the *N*-benzoyl group.[13]

Since its first use by Knowles et al. in 1969,[17] the arylazide moiety has become one of the most commonly used photoactivatable functionalities. Azide-based paclitaxel photoaffinity analogues with the labels attached to paclitaxel via an ester or amide linkage through the N3′,[3,4,6] O2,[4,7] and O7[5,6] positions have provided insight



Paclitaxel **1**: R_1 = H
2: $R_1 = N_3$,

Figure 1. Structure of paclitaxel and 3′-azidophenylpaclitaxel.

(along with the electron crystallograph)[10] to the paclitaxel binding site. We hypothesized that photolabeling experiments with a photoaffinity probe at the 3′-phenyl group should provide additional detailed information on the paclitaxel binding site and the bound conformation of the phenylisoserine side chain.

On the basis of known paclitaxel SAR,[18] we expected that placing an azide group at the para position of the C3′ phenyl ring should retain activity of the analogue while allowing for satisfactory labeling of the tubulin protein. Herein, we report the synthesis and biological evaluation of the first paclitaxel photoaffinity label carrying an azide moiety at the C3′ phenyl group.

Chemistry and Synthesis

The semisynthesis of paclitaxel and analogues requires the asymmetric synthesis of the phenylisoserine side chain and its coupling to naturally occurring baccatin III.[19,20] Synthetic routes to this side chain include chiral ester−imine cyclocondensation reactions[21−24] and the asymmetric aminohydroxylation of *trans*-cinnamic esters.[25] The stability of the azide functionality to these reactions was of major concern, and attempts to synthesize an azide-containing phenylisoserine using these methodologies were unsuccessful.

Requiring a milder route to the side chain, we turned to the Staudinger reaction, a [2 + 2] ketene−imine cycloaddition.[26,27] At the time of this project's undertaking, there were few reports of catalytic, asymmetric

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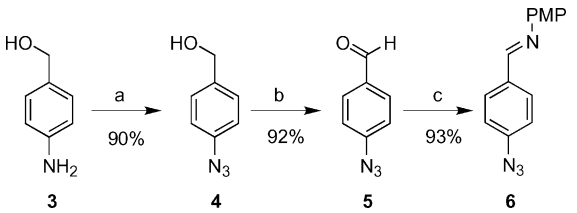
Scheme 1[a]



[a] (a) (i) H_2SO_4, $NaNO_2$, H_2O; (ii) NaN_3, H_2O; (b) PCC, CH_2Cl_2; (c) *p*-anisidine, EtOH.

Staudinger (or Staudinger-like) reactions;[28] however, ample research on chiral auxiliary mediated versions had been published.[27,29,30] Chiral auxiliaries connected to the nitrogen of the imine generally proceed with lower levels of diastereoselectivity compared to those found on the carbon portion of the imine or on the ketene itself.[30] However, specific substitutions required at C2′ and C3′ of paclitaxel (corresponding to C3 and C4 of the azetidinone precursor, respectively) allowed only the use of *N*-imine auxiliaries. One example of a chiral benzaldimine from enantiopure (*S*)-(−)-1-(*p*-methoxyphenyl)propyl-1-amine was reported in good chemical yield and, after recrystallization, excellent enantiopurity (>99%).[31] Notably this *N*-benzyl derivative could be cleaved under oxidative conditions.[32] This system was examined using the acid chloride derived from bis(trimethylsilyl)glycolate **8** (vide infra), and analysis of the products indicated a 1:1 mixture of inseparable diastereomers.

We therefore turned to resolution techniques reported previously by Brieva et al.[32] They reported the use of lipases to resolve racemic 3-hydroxy-4-aryl β-lactams as a route to the enantiopure paclitaxel side chain.[32] The high stereochemical and substrate specificity reported for this class of enzymes was an attractive solution to our problem.

The Staudinger cyclization precursor **6** was made via known synthetic transformations as outlined in Scheme 1. 4-Aminobenzyl alcohol was converted to the azide by diazotization of amine **3**, followed by displacement with sodium azide. The resultant azido alcohol **4** was oxidized to the aldehyde with PCC followed by addition of freshly sublimed *p*-anisidine, producing imine **6** in excellent yield.

To obtain the other Staudinger reactant, an amount of 2 equiv of TMSCl was added to glycolic acid (**7**) to give bis(trimethylsilyl)-glycolate (**8**) (Scheme 2). This ester was not isolated but was used immediately in the next step.

The Staudinger cyclization was envisioned to occur by generating the ketene in situ from **8** (Scheme 2). This was accomplished with the addition of a 2 M solution of oxalyl chloride in the presence of a catalytic amount of DMF to form the acid chloride.[33] Ketene formation and spontaneous cyclization ensued, following cannulation of the acid chloride solution to a mixture of triethylamine and imine **6**. The crude solid was taken up into methanol and aqueous citric acid, deprotecting the TMS ether, affording β-lactam **10** in excellent yield. The predicted cis relationship at C3 and C4 was material in setting the required configuration of the phenylisoserine side chain. This stereochemical outcome was anticipated on the basis of the conrotatory [2 + 2] pathway predicted for the Staudinger cyclization.[34] None of the trans isomer was detected.[35] Acetylation of **10** with acetyl chloride provided **11**, setting the stage for the lipase-mediated kinetic resolution. A more direct route to **11** involved ketene generation from commercially available acetoxyacetyl chloride and subsequent Staudinger cyclization with imine **6** in 83% yield.[36] Both methods proved to be valid entries to the (±)-lactam **11**.

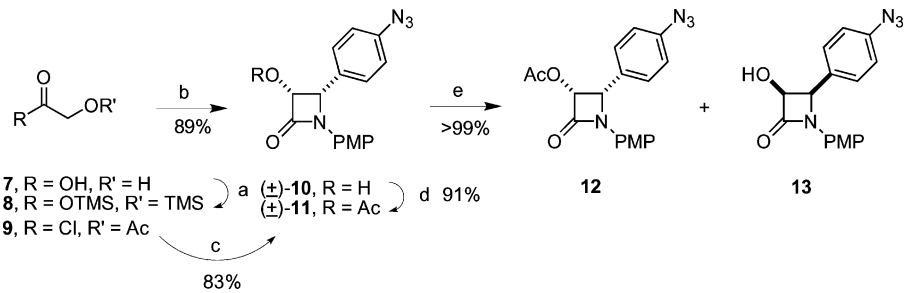
The resolution proceeded smoothly using *Pseudomonas cepacia* lipase in a 0.2 M phosphate buffer with 10% acetonitrile as a cosolvent,[32] affording **12** in >99% yield, based on the desired enantiomer, and 99% enantiomeric excess as determined by HPLC. Saponification of the enantiopure ester **12** with lithium hydroxide and subsequent protection of the resultant alcohol with TBSCl gave (+)-**15** in 84% over two steps (Scheme 3).

Oxidative deprotection of the *p*-methoxyphenyl group with ammonium cerium(IV) nitrate (CAN)[37] provided compound **16** in 78% yield.[38] Imidation of the lactam nitrogen of **16** with benzoyl chloride[22] served to activate the lactam toward nucleophilic attack from baccatin III. Azetidinone **17** was obtained in 73% yield after chromatography and used immediately in the coupling with 7-TES-baccatin III (**19**) (Scheme 4).

The absolute stereochemistry of the azetidinone was determined by X-ray crystallography of the chloroacetyl derivative **18**. An ORTEP drawing (Figure 2) shows the *R*,*S* configuration of C3 and C4, respectively.

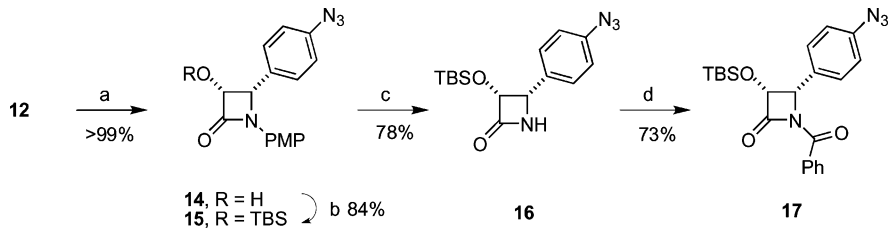
Coupling procedures for N-acylated β-lactams typically involve strong lithiated amine salts serving as the base for baccatin III deprotonation.[39] The stability of the azide to these nucleophilic amines was of concern. We therefore turned to coupling procedures that do not involve the use of nucleophilic bases.[23,24] An amount of 50 equiv of sodium hydride[23] was added to a solution of

Scheme 2[a]



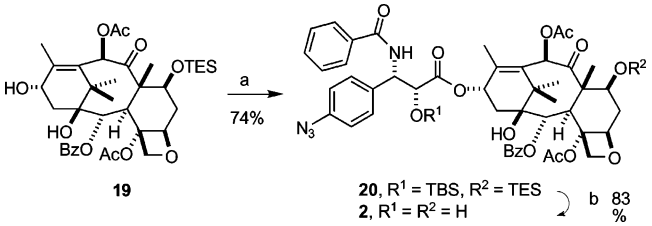
[a] (a) TMSCl, DMAP, pyridine, CH_2Cl_2; (b) (i) $(ClCO)_2$, DMF, CH_2Cl_2, 0 °C; (ii) 0.2 equiv of **6**, TEA, CH_2Cl_2, 0 °C to room temp; (iii) MeOH, aqueous citric acid; (c) **6**, TEA, CH_2Cl_2, 0 °C to room temp; (d) AcCl, DMAP, TEA, THF; (e) 0.2 M NaH_2PO_4 buffer solution with pH at 7.00/CH_3CN, *Pseudomonas* cepacia lipase.

Scheme 3[a]



[a] (a) LiOH, H_2O/acetone 0 °C; (b) TBDMSCl, DMAP, imidazole, CH_2Cl_2; (c) CAN, CH_3CN/H_2O; (d) benzoyl chloride, TEA, CH_2Cl_2, 0 °C to room temp.

Scheme 4[a]



[a] (a) NaH, **17**, THF, 0 °C, room temp; (b) HF/pyridine, pyridine, 0 °C to room temp.

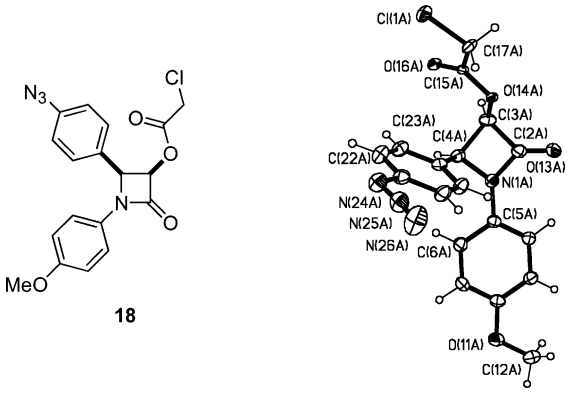


Figure 2. Structure and ORTEP drawing of derivative **18**.

7-TES-baccatin III[19] and **17**, affording the protected paclitaxel derivative **20** (Scheme 4) in 74% yield. Deprotection of the silyl protecting groups in **20** under standard conditions gave **2** as a white powder in 83% yield.

Anti-Tubulin Activity

Compound **2** was evaluated for its ability to interact with tubulin in a microtubule assembly assay and was found to be as active as the parent compound **1** with an effective dose ratio ($ED_{50}/ED_{50(paclitaxel)}$) of 1.1. An in vitro study against MCF-7 and MCF7-ADR (multidrug-resistant phenotype) breast cancer cell lines showed activity similar to that of paclitaxel with an $ED_{50}/ED_{50(paclitaxel)}$ of 2.7 and 1.5, respectively.[40] These results showed that compound **2** had activity comparable to that of the parent paclitaxel. These findings suggest that **2** holds promise for future photolabeling studies.

Conclusions

An efficient synthesis of the 3′-(4-azidophenyl)-3′-dephenylpaclitaxel photoaffinity probe was achieved. The azide moiety survived all synthetic transformations. A lipase kinetic resolution was used to resolve the enantiomers of the phenylisoserine precursor. This resolution is scalable to gram quantities. The cytotoxicity and microtubule assembly studies indicate that a photoaffinity analogue has been obtained that is of similar potency compared to paclitaxel. The photolabeling studies will be performed and presented in due course. Information obtained from these studies is expected to provide details on the paclitaxel binding site and to give insight into the tubulin-bound conformation of the phenylisoserine side chain of paclitaxel.

Experimental Section

(4-Azidophenyl)methan-1-ol (4). Alcohol **3** (1.55 g, 12.6 mmol) was taken up in 4 N H_2SO_4 (15 mL) at 0 °C, affording a reddish suspension. $NaNO_2$ (1.30 g, 18.9 mmol) was added as a solution in water (10 mL) open to the air. The reaction mixture was maintained at 0 °C for 15 min with the solution clearing. NaN_3 (1.23 g, 18.9 mmol) in water (10 mL) was added slowly with gas evolution. The reaction mixture was stirred at room temperature for 1 h, resulting in a brownish-white precipitation. The reaction mixture was extracted with Et_2O, and the organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. Flash chromatography with EtOAc/hexanes (30% → 40%) afforded 1.69 g (90%) of a yellowish solid: mp 27−28 °C; IR (neat) 3333, 2107 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.69 (d, J = 5.68 Hz, 2H), 7.04 (d, J = 8.38 Hz, 2H), 7.38 (d, J = 8.27 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 64.6, 119.0, 128.5, 137.5, 139.3; HRMS (FAB+) m/z calcd for $C_7H_7N_3O$ $[M^+]$ 149.0589, found 149.0567.

4-Azidobenzaldehyde (5). To a solution alcohol **4** (1.69 g, 11.3 mmol) in CH_2Cl_2 (50 mL) was added PCC (4.15 g, 19.3 mmol) in one portion. The resulting mixture was stirred at room temperature for 1 h. The reaction mixture was then filtered over a pad of silica gel on a fritted funnel. The solvent was removed, affording 1.53 g (92%) of a yellow liquid: IR (neat) 2119, 1696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.17 (d, J = 8.54 Hz, 2H), 7.89 (d, J = 10.62 Hz, 2H), 9.95 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 119.4, 131.5, 133.2, 146.2, 190.5; HRMS (FAB+) m/z calcd for $C_7H_5N_3O$ $[M^+]$ 147.0433, found 147.0439.

(4-Azidobenzylidene)-(4-methoxyphenyl)amine (6). To a solution of freshly sublimed *p*-anisidine (4.01 g, 31.3 mmol) in EtOH (7 mL) at room temperature open to the air, was added a solution of aldehyde **5** (3.41 g, 23.1 mmol) in EtOH (2 mL) in one portion. A dense white precipitate formed immediately. The reaction mixture was stirred for an additional hour. The solid was collected by vacuum filtration, affording 5.34 g (93%) of bright-yellow crystals: mp 121−123 °C; IR (neat) 3418, 2120, 1620 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.91 (d, J = 8.50 Hz, 2H), 7.26 (d, J = 11.90 Hz, 2H), 7.13 (d, J = 8.46 Hz, 2H), 6.96 (d, J = 8.71 Hz, 2H), 3.86 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.4, 114.3, 119.2, 122.1, 130.0, 133.4, 142.5, 144.6, 156.8, 158.3; HRMS (FAB+) m/z calcd for $C_{14}H_{13}N_4O$ $[MH^+]$ 253.1089, found 253.1093.

(+)-4-(4-Azidophenyl)-3-hydroxy-1-(4-methoxyphenyl)-azetidin-2-one (10). A 0.27 M solution of bis-silylester **8** (15.1 mmol) in CH_2Cl_2 was cooled to 0 °C, and a catalytic amount of DMF (0.328 g, 4.50 mmol) was added followed by dropwise addition of oxalyl chloride as a 2 M solution in CH_2Cl_2 (16.0 mmol). The reaction mixture was stirred 1 h at 0 °C and 1 h at room temperature. Meanwhile, a solution of triethylamine

(3.60 g, 35.6 mmol) and imine **6** (0.892 g, 3.35 mmol) in CH_2Cl_2 (13 mL) was cooled to 0 °C. The imine solution was then cannulated to the acid chloride solution (recooled to 0 °C). The reaction mixture was allowed to warm to room temperature over 4 h. The reaction mixture was then poured into 100 mL of water and extracted with CH_2Cl_2. The combined organic layers were washed with $NaHCO_3$ and brine. The organic fraction was then concentrated under reduced pressure. The resulting residue was taken up in methanol (50 mL), and to it was added citric acid (614 mg, 6.67 mmol) as a solution in water. The resulting mixture was allowed to react 30 min at room temperature. The methanol was removed under reduced pressure, and the residue was taken up in CH_2Cl_2 (50 mL) and washed with $NaHCO_3$ and brine. The organic fraction was concentrated and the crude residue was subjected to column chromatography in EtOAc/hexanes (3:7), affording 925 mg (89%) of an off-white solid: mp 135−137 °C; IR (neat) 3432, 2124, 2097, 1723 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.05 (d, $J = 6.93$ Hz, 1H) 3.78 (s, 3H), 5.22 (br s, 1H), 5.26 (d, $J = 5.06$ Hz, 1H), 6.83 (d, $J = 8.92$ Hz, 2H), 7.09 (d, $J = 8.36$ Hz, 2H), 7.29 (d, $J = 8.87$ Hz, 2H), 7.36 (d, $J = 8.33$ Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.4, 61.7, 77.6, 114.4, 118.7, 119.6, 129.0, 129.8, 130.2, 140.7, 156.5, 165.3; HRMS (FAB+) *m/z* calcd for $C_{16}H_{15}N_4O_3$ [MH^+] 311.1139, found 311.1139.

(+)-4-(4-Azidophenyl)-1-(4-methoxyphenyl)-2-oxoazetidin-3-yl Acetate (11). Procedure A from 10. To a solution of DMAP (0.910 g, 7.45 mmol), **10** (2.11 g, 6.81 mmol), and triethylamine (3.71 g, 36.7 mmol) in THF (60 mL) was added dropwise neat acetyl chloride (2.21 g, 28.1 mmol), immediately forming a slurry. The ice bath was removed, and the reaction mixture was stirred at room temperature for 1 h, then poured into $NaHCO_3$ (50 mL) and extracted with Et_2O. The combined organic extracts were washed with brine and dried over $MgSO_4$, and the solvent was removed under reduced pressure. Column chromatography with EtOAc/hexanes (3:7) gave 2.174 g of a white solid (91%).

Procedure B from Commercially Available Acetoxyacetyl Chloride (9). To a solution of **6** (0.042 g, 0.166 mmol) in CH_2Cl_2 (1 mL) was added a solution of **9** (0.033 g, 0.240 mmol) and TEA (0.028 g, 0.280 mmol) in CH_2Cl_2 (1 mL) via cannula at 0 °C. The yellow solution was allowed to warm to room temperature over 12 h. The solution was quenched with water and poured into $NaHCO_3$ and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 three times. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Flash chromatography with EtOAc/hexanes (3:7) afforded 49 mg (83%) of a white solid: mp 159−160 °C; IR (neat) 2130, 2100, 1757, 1603 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.74 (s, 3H), 3.75 (s, 3H), 5.32 (d, $J = 4.79$ Hz, 1H), 5.90 (d, $J = 4.80$ Hz, 1H), 6.80 (d, $J = 8.96$ Hz, 2H), 7.01 (d, $J = 8.45$ Hz, 2H), 7.26 (d, $J = 8.95$ Hz, 2H), 7.30 (d, $J = 8.45$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.3, 55.8, 61.3, 76.9, 114.9, 119.2, 119.5, 129.4, 129.9, 130.5, 141.0, 157.0, 161.5, 169.6; HRMS (FAB+) *m/z* calcd for $C_{18}H_{17}N_4O_4$ [MH^+] 353.1250, found 353.1247.

(4*S*,3*R*)-4-(4-Azidophenyl)-1-(4-methoxyphenyl)-2-oxoazetidin-3-yl Acetate (12). A 0.2 M NaH_2PO_4 buffer solution was prepared (Millipore water), and the pH was adjusted to 7.00 using 0.2 M Na_2HPO_4. Racemic lactam **11** (1.60 g, 4.54 mmol) was dissolved in a buffer/acetonitrile mix (150 mL/25 mL) open to the air. *Pseudomonas cepacia* lipase was added in one portion (1.78 g), and the mixture was stirred vigorously for 24 h. The aqueous mixture was extracted with EtOAc, and the combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Flash chromatography with EtOAc/hexanes (3:7) afforded 799 mg (>99% yield of enantiomer **12**) of a white solid: mp 120−121 °C; $[\alpha]^{22}_D$ +22 (*c* 0.55, $CHCl_3$); HPLC, Chiralpak AD-RH, 20% *i*-PrOH/hexane, 0.5 mL/min, 254 nm, retention time for the major isomer = 16.7 min, 99% ee, retention time for the minor isomer = 15.6 min. ^1H and ^{13}C NMR, IR, and HRMS data are identical to those of **11** and are found in the Supporting Information. **13** was isolated as 790 mg (99%) of a white powder, and spectral data are found in the Supporting Information.

(4*S*,3*R*)-4-(4-Azidophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one (14). To a stirred solution of **12** (0.025 g, 0.07 mmol) in ACS grade acetone (2 mL) at 0 °C open to the air was added a 2 N solution of LiOH (0.14 mmol) in water. The reaction mixture was allowed to warm to room temperature over 1 h. The reaction mixture was then diluted with EtOAc (12 mL) and washed with water. The organic layer was washed with brine, dried with $MgSO_4$, and concentrated under reduced pressure. Flash chromatography with EtOAc/hexanes (1.5:5) afforded 21.7 mg (>99%) of an off-white solid: mp 132−134 °C; $[\alpha]^{22}_D$ +220 (*c* 1.0, $CHCl_3$). ^1H and ^{13}C NMR, IR, and HRMS data are identical to **10** and are found in the Supporting Information.

(4*S*,3*R*)-4-[4-(Azidophenyl)]-3-*tert*-butyldimethylsiloxy-1-(4-methoxyphenyl)azetidin-2-one (15). A solution of **14** (0.113 g, 0.365 mmol), TBSCl (0.149 g, 0.986 mmol), imidazole (0.067 g, 0.986 mmol), and DMAP (0.121 g, 0.986 mmol) in CH_2Cl_2 (3 mL) was stirred under argon at room temperature for 1 h. The reaction was quenched with water (15 mL) after the mixture was diluted with CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2, and the combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Flash chromatography with EtOAc/hexanes (1:5) afforded 130 mg (84%) of an off-white solid: mp 117−118 °C; IR (neat) 2121, 2098, 1732 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ −0.09 (s, 3H), 0.11 (s, 3H), 0.71 (s, 9H), 3.75 (s, 3H), 5.12−5.13 (overlapped, 2H), 6.80 (d, $J = 9.02$ Hz, 2H), 7.03 (d, $J = 8.49$, 2H), 7.27 (d, $J = 9.01$, 2H), 7.33 (d, $J = 8.51$, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.4, −4.9, 17.8, 25.2, 55.3, 62.2, 77.6, 114.3, 118.6, 118.8, 129.7, 130.7, 130.9, 140.0, 156.2, 165.2; HRMS (FAB+) *m/z* calcd for $C_{22}H_{29}N_4O_3Si$ [MH^+] 425.2009, found 425.2010; $[\alpha]^{22}_D$ +115 (*c* 1.05, $CHCl_3$).

(4*S*,3*R*)-4-[4-(Azidophenyl)]-3-*tert*-butyldimethylsiloxyazetidin-2-one (16). A solution of **15** (0.104 g, 0.245 mmol) in HPLC-grade acetonitrile (6 mL) was cooled to −20 °C (external temperature) for 30 min. An ice-cold solution of CAN (0.390 g, 0.711 mmol) in water (2.5 mL) was added with fast dropwise addition, carefully ensuring that the internal temperature did not rise above −10 °C. The reaction mixture was maintained at −15 to −10 °C for 2 h. The aqueous layer was extracted three times with Et_2O. The combined organic extracts were washed with water, $NaHCO_3$, and brine and dried over $MgSO_4$, and the solvent was removed under reduced pressure. Flash chromatography with EtOAc/hexanes (1:5) afforded 61 mg (78%) as an off-white solid: mp 80−81 °C; IR (neat) 2127, 2094, 1750, 1712 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ −0.12 (s, 3H), 0.07 (s, 3H), 0.69 (s, 9H), 4.80 (d, $J = 4.71$ Hz, 1H), 5.07 (dd, $J = 4.68$ and 2.86 Hz, 1H), 6.39 (s, 1H), 7.04 (d, $J = 8.50$ Hz, 2H), 7.32 (d, $J = 8.45$ Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.4, −5.0, 17.8, 25.2, 58.6, 79.5, 118.5, 129.4, 133.1, 140.0, 169.8; HRMS (FAB+) *m/z* calcd for $C_{15}H_{23}N_4O_2Si$ [MH^+] 319.1590, found 319.1574; $[\alpha]^{22}_D$ +45.1 (*c* 1.10, CH_2Cl_2).

(4*S*,3*R*)-4-[4-(Azidophenyl)]-3-*tert*-butyldimethylsiloxy-1-(phenylcarbonyl)azetidin-2-one (17). A solution of **16** (0.025 g, 0.079 mmol), DMAP (0.003 g, 0.024 mmol), and TEA (0.016 g, 0.158 mmol) in CH_2Cl_2 (1 mL) was cooled to 0 °C under argon for 5 min. Neat benzoyl chloride (0.017 g, 0.120 mmol) was added in one portion, and the ice bath was removed. After 90 min, another 1.5 equiv of benzoyl chloride was added and the reaction mixture was allowed to stir at room temperature. The reaction was quenched with ice-cold brine after another 90 min. The reaction mixture was diluted with CH_2Cl_2, washed three times with brine, and dried over $MgSO_4$, and the solvent was removed under reduced pressure. Flash chromatography with EtOAc/hexanes (1:10) afforded 24 mg (73%) as an off-white solid: mp 80−83 °C; IR (neat) 2125, 1800, 1677 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ −0.06 (s, 3H), 0.12 (s, 3H), 0.75 (s, 9H), 5.15 (d, $J = 6.13$ Hz, 1H), 5.41 (d, $J = 6.11$ Hz, 1H), 7.06 (d, $J = 8.5$ Hz, 2H), 7.39 (d, $J = 8.48$ Hz, 2H), 7.51 (t, $J = 7.93$ Hz, 2H), 7.62 (t, $J = 7.47$ Hz, 1H), 8.05 (d, $J = 7.2$ Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.4, −5.0,

17.9, 25.2, 60.1, 76.3, 118.7, 128.1, 129.5, 129.8, 130.6, 131.8, 133.4, 140.0, 164.9, 166.2; HRMS (FAB+) *m/z* calcd for $C_{22}H_{27}N_4O_3Si$ [MH^+] 423.1852, found 423.1847; $[\alpha]^{22}_D$ +135 (*c* 1.40, $CHCl_3$).

(4*S*,3*R*)-Chloroacetic Acid 2-(4-Azidophenyl)-1-(4-methoxyphenyl)-4-oxoazetidin-3-yl Ester (18). A solution of chloroacetyl chloride (0.0096 g, 0.090 mmol) in THF (1 mL) at room temperature was added to a solution of DMAP (0.0010 g, 0.0040 mmol), TEA (0.020 g, 0.20 mmol), and alcohol **18** (0.012 g, 0.040 mmol) via syringe. The reaction mixture was stirred for 1 h, and the reaction was monitored by TLC. The solution was quenched with NH_4Cl, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with $NaHCO_3$ and brine. The organic layer was dried with brine and concentrated under reduced pressure. Flash chromatography with EtOAc/hexanes (1:5) gave 5 mg (33%, unoptimized) as a white solid: mp 140−143 °C; IR (neat) 2125, 2095, 1757, 1634 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.60 (1/2 ABq, J = 15.21 Hz, 1H), 3.78 (s, 3H), 3.87 (1/2 ABq, J = 15.21 Hz, 1H), 5.39 (d, J = 4.82 Hz, 1H), 6.00 (d, J = 4.84 Hz, 1H), 6.83 (d, J = 9.04 Hz, 2H), 7.04 (d, J = 8.54 Hz, 2H), 7.27 (d, J = 7.27 Hz, 2H), 7.32 (d, J = 8.51 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.2, 55.9, 61.2, 77.5, 114.9, 119.2, 119.7, 128.7, 129.8, 130.3, 141.4, 157.3, 160.4, 166.2; HRMS (FAB+) *m/z* calcd for $C_{18}H_{15}N_4O_4Cl$ [MH^+] 387.0860, found 387.0854; $[\alpha]^{22}_D$ +4.0 (*c* 0.25, $CHCl_3$). An X-ray crystal structure has been obtained confirming the absolute configuration. See Supporting Information.

3′-(4-Azidophenyl)-2′-*tert*-butyldimethylsiloxy-3′-dephenyl-7-triethylsiloxypaclitaxel (20). To a cold (0 °C) solution of 7-TES-baccatin III (**19**, 0.027 g, 0.038 mmol) and **17** (0.024 g, 0.057 mmol) in THF (1 mL) was added NaH (0.046 g, 1.90 mmol) in one portion. The reaction mixture was stirred at 0 °C for 1 h and then warmed to room temperature and stirred for an additional 3 h. The mixture was then cooled to 0 °C and quenched with slow, dropwise addition of acetic acid. The solution was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Flash chromatography with EtOAc/hexanes (1:5) gave 32 mg (74%) as a white solid: mp 186−189 °C; IR (neat) 3426, 2122, 1725, 1665 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ −0.23 (s, 3H), 0.03 (s, 3H), 0.58−0.63 (m, 6H), 0.85 (s, 9H), 0.95 (t, J = 7.90 Hz, 9H), 1.20 (s, 3H), 1.28 (s, 3H), 1.72 (s, 3H), 1.90−1.97 (m, 1H) 2.04 (s, 3H), 2.07−2.13 (m, 2H), 2.19 (s, 3H), 2.37−2.43 (m, 1H), 2.58 (s, 3H), 3.85 (d, J = 6.96 Hz, 1H), 4.22 (1/2 ABq, J = 8.42 Hz, 1H), 4.34 (1/2 ABq, J = 8.37 Hz, 1H), 4.47−4.52 (m, 1H), 4.66 (br s, 1H), 4.97 (d, J = 8.6 Hz, 1H), 5.69−5.73 (overlapped, 2H), 6.28 (t, J = 8.85 Hz, 1H), 6.47 (s, 1H), 7.06−7.1 (m, 3H), 7.35 (d, J = 8.39 Hz, 2H), 7.41−7.45 (m, 2H), 7.50−7.56 (m, 3H), 7.61−7.65 (m, 1H), 7.75 (1/2 ABq, J = 7.46 Hz, 2H), 8.15 (1/2 ABq, J = 7.48 Hz, 2H); ^{13}C NMR (100 MHz) δ −5.2, −4.7, 5.6, 7.7, 10.5, 14.7, 18.6, 21.3, 21.9, 23.5, 25.6, 26.9, 30.1, 35.9, 38.2, 44.6, 47.1, 55.8, 58.8, 71.9, 72.6, 75.3, 79.2, 81.7, 84.6, 119.7, 127.5, 128.5, 129.2, 130.6, 132.3, 134.1, 134.2, 135.7, 140.2, 140.5, 167.3, 167.6, 169.7, 170.5, 171.7, 202.1; HRMS (FAB+) *m/z* calcd for $C_{59}H_{78}N_4O_{14}Si_2$ [MH^+] 1123.5232, found 1123.5132; $[\alpha]^{22}_D$ −55 (*c* 0.75, CH_2Cl_2).

3′-(4-Azidophenyl)-3′-dephenylpaclitaxel (2). To a solution of **20** (0.007 g, 0.006 mmol) in pyridine (0.5 mL) at 0 °C was added four drops of HF/pyridine solution dropwise. The ice bath was removed after 30 min, and the reaction was monitored by TLC (approximately 4 h). The reaction mixture was then diluted with EtOAc, washed with saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The organic fraction was concentrated under reduced pressure. Flash chromatography with EtOAc/hexanes (1:1) afforded 5 mg (83%) of a white solid: mp 150−151 °C; IR (neat) 3419, 2112, 1724, 1651 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.17 (s, 3H), 1.27 (s, 3H), 1.72 (s, 3H), 1.84 (s, 3H), 1.89−1.94 (m, 1H), 2.27−2.42 (overlapped, 8H), 2.48 (d, J = 3.98 Hz, 1H), 2.55−2.61 (m, 1H), 3.62 (d, J = 4.36 Hz, 1H), 3.83 (d, J = 6.80 Hz, 1H), 4.22 (1/2 ABq, J = 8.42 Hz, 1H), 4.34 (1/2 ABq, J = 8.60 Hz, 1H), 4.42−4.45 (m, 1H), 4.79 (br d, 1H), 4.97 (d, J = 9.13 Hz, 1H), 5.70 (d, J = 6.93 Hz, 1H), 5.81 (d, J = 8.85 Hz, 1H), 6.26−6.30 (overlapped, 2H), 6.98 (d, J = 8.91 Hz, 1H), 7.10 (d, J = 8.27 Hz, 2H), 7.41−7.44 (m, 2H), 7.51−7.56 (m, 5H), 7.63−7.66 (m, 1H), 7.75 (1/2 ABq, J = 7.89 Hz, 2H), 8.16 (1/2 ABq, J = 7.94, 2H); ^{13}C (125 MHz) δ 9.5, 14.9, 20.8, 21.6, 22.6, 26.8, 35.6, 43.1, 45.6, 54.2, 58.6, 72.2, 72.4, 72.9, 74.8, 75.5, 79.0, 81.2, 84.3, 119.5, 127.0, 128.7, 129.0, 130.1, 132.0, 133.3, 133.7, 134.7, 140.1, 141.7, 166.8, 167.0, 170.3, 171.2, 172.4, 203.5; HRMS (FAB+) *m/z* calcd for $C_{47}H_{51}N_4O_{14}$ [MH^+] 895.3402, found 895.3418; $[\alpha]^{22}_D$ −21 (*c* 1.0, CH_2Cl_2); HPLC, IProSIL C18 AQ, 1:1 CH_3CN/H_2O, 1.0 mL/min, 254 nm, retention time = 5.5 min, 97.5% purity.

Tubulin Assembly Assay. The ED_{50} value is the concentration of the compound that causes 50% of the maximum reduction in supernatant protein concentration. Maximum reduction was obtained using 25 μM paclitaxel. A mixture of 10 μM tubulin bovine brain (1 mg/mL) with varying concentrations of **2** in 100 μL of buffer (with 4% DMSO from the analogue solution) containing 0.1 M Pipes, pH 6.9, 1 mM $MgSO_4$, 1 mM EGTA, and 0.5 mM GTP at 37 °C was incubated for 15 min, and the samples were centrifuged in a Beckman TL-100 ultracentrifuge for 4 min at 35 000*g*. Protein concentrations of the supernates were determined, and under these conditions, tubulin did not assemble in the absence of paclitaxel or **2**.

Cell Cytotoxicity Assay. MCF-7 and MCF7-ADR human breast cancer cells were plated in 96-well culture plates at a density near 2000 cells per well in Dulbecco's MEM/F12 containing 10% fetal calf serum. The cells were incubated for 24 h, after which the medium was replaced with fresh medium with **2** at varying concentrations. The cultures were grown an additional 72 h, and the degree of proliferation was measured using sulforhodamine B.

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Supporting Information Available: General methods, experimental procedures, and spectral data for those compounds not provided in the Experimental Section and X-ray structural information for compound **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

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