Paclitaxel Derivatives for Targeted Therapy of Cancer: Toward the Development of Smart Taxanes†

Ahmad Safavy,*,‡ Kevin P. Raisch, M. B. Khazaeli,§,‡ Donald J. Buchsbaum,‡ and James A. Bonner‡

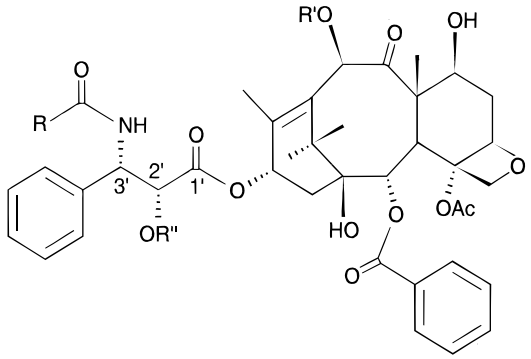
Departments of Radiation Oncology and Medicine, and The Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, Alabama 35294

Received July 12, 1999

The pharmacologic efficacy of the promising antitumor agent paclitaxel (Taxol) may be potentially enhanced through derivatization of the drug to a water-soluble tumor-recognizing conjugate. This work reports the design and synthesis of the first tumor-directed derivative of paclitaxel. A 7-amino acid synthetic peptide, BBN[7-13], which binds to the cell surface bombesin/gastrin-releasing peptide (BBN/GRP) receptor, was conjugated to the paclitaxel-2′-hydroxy function by a heterobifunctional poly(ethylene glycol) linker. The resulting conjugate, designated PTXPEGBBN[7-13], was soluble to the upper limit of tested concentrations (250 mg/mL). The conjugate completely retained the receptor binding properties of the attached peptide as compared with those of the unconjugated BBN[7-13]. In experiments with NCI-H1299 human nonsmall cell lung cancer cells, the cytotoxicity of the PTXPEGBBN[7-13] conjugate at a 15 nM dose was enhanced by a factor of 17.3 for 24 h and 10 for 96 h exposure times, relative to paclitaxel. The IC_{50} of the conjugate, tested against the same cell line, was lower than the free drug by a factor of 2.5 for both 24 h and 96 h exposures. These results describe, for the first time, the design and synthesis of a soluble tumor-directed paclitaxel prodrug which may establish a new mode for the utilization of this drug in cancer therapy.

Introduction

The diterpenoid molecule paclitaxel[1] (Taxol) (**1**, Figure 1), extracted in 1971 from the Western yew, *Taxus brevifolia*, and docetaxel[2] (**2**, Taxotere, Figure 1) are considered to be two of the most important and promising anticancer drugs currently in clinical use for breast and ovarian cancers. They have also shown promising activity for the treatment of skin, lung, and head and neck carcinomas.[3,4] Paclitaxel was approved by the FDA earlier this decade for the treatment of advanced ovarian cancer and breast cancer after showing substantial efficacy against these malignancies. Docetaxel, a semisynthetic derivative of paclitaxel, has also shown exceptional clinical results and was approved by the FDA for the treatment of breast cancer in 1996 and is now in phase II and III clinical trials for lung cancer and ovarian cancer.[3,4] Perhaps one of the most distinguishing properties of paclitaxel is its high activity against solid tumors compared to leukemias, when tested in animal models.[5,6] At the molecular level, paclitaxel exerts its antitumor activity through the stabilization of microtubule assemblies, thus interrupting mitosis and cellular division. The paclitaxel-induced stabilized microtubules have been shown to be resistant to depolymerization. On the basis of this mechanism and the fact that paclitaxel increases the fraction of cells in G_2 or M phase, it was hypothesized that the drug may enhance the DNA-damaging effects of radiation. To this end, Tishler et al.[7] and, more recently, O'Donnell et al.[8] reported the radiosensitizing effect of Taxol in G18 human astrocytoma cells and in nude mice bearing Burkitt's lymphoma xenografts, respectively. Based on these observations, many investigations have been initiated to explore combinations of paclitaxel and radiotherapy which may further expand the role of this agent in the armamentarium of the oncologist. Furthermore, Distefano et al. have shown apoptosis-inducing properties for Taxol and some of its derivatives.[9]



1, Paclitaxel: R = Ph, R' =Ac
2, Docetaxel: R = *tert*-BuO, R' = H
3, Paclitaxel -2'-succinate: R = Ph, R' = Ac, R'' = -$CO(CH_2)_2$ CO_2H

Figure 1. Structures of paclitaxel (Taxol), docetaxel (Taxotere), and paclitaxel-2′-succinate.

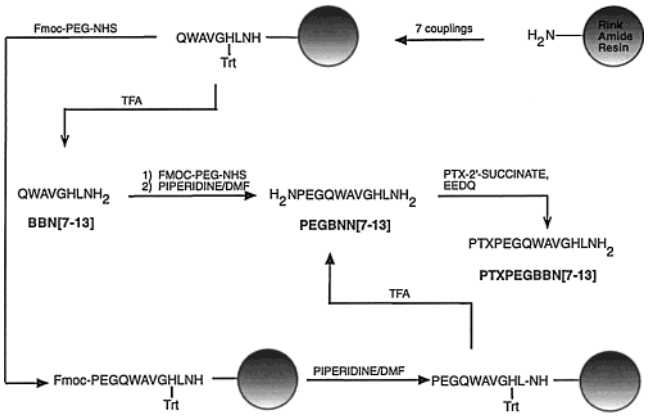
Despite these positive therapeutic features, paclitaxel suffers from significant drawbacks such as aqueous insolubility and dose-limiting toxicity at the clinically administered doses. The present formulation of this drug contains 30 mg of paclitaxel in 5 mL of a 50/50 mixture of Cremophore EL (polyethoxylated castor oil,

† Dedicated to the memory of Dr. Davood Safavy, M.D.

* Address for correspondence: University of Alabama at Birmingham, Room 674 WT, 1824 Sixth Avenue South, Birmingham, AL 35294-6832. E-mail: safavy@uab.edu.
‡ The Comprehensive Cancer Center.
§ Department of Medicine.

10.1021/jm990355x CCC: $18.00
Published on Web 11/18/1999

Scheme 1



a solubilizing surfactant) and ethanol. Cremophore EL has been reported to cause histamine release and to be associated with adverse effects such as severe allergic reactions.[10,11] Reported side effects of the drug include neutropenia, mucositis, and cardiac and neurological toxicities in addition to hypersensitivity.[12−16]

It would be highly desirable, therefore, to develop a mechanism through which a soluble paclitaxel derivative could be delivered specifically to the site of the disease in a targeted fashion. Possible advantages of such an approach may include (i) elimination of allergenic surfactants from the formulation due to increased solubility, (ii) efficient delivery of the drug to micrometastatic tumors, and (iii) reduction of the administered dose as a result of site-specific delivery of the drug. The latter advantage should, in turn, lead to a reduction of toxicity, enhancement in efficacy, lower drug doses, and thereby, more economic formulations.

Targeted therapy of malignancies has gained momentum during the past several years based on the simple rationale of efficient delivery of the oncolytic agent to the neoplastic tissue. Receptor-based targeted treatment of cancer through the application of tumor-recognizing molecules (TRMs) has advanced considerably since the development of monoclonal antibodies[17,18] and has been expanded by the introduction of small molecule peptide TRMs capable of binding to tumor cell surface receptors.[19] A number of drug, toxin, and radioisotope conjugates of TRMs have been developed, with some currently in clinical use. None of the designs of the large number of synthetic taxane derivatives, reported to this date, contain an element of specific tumor recognition, and despite the large body of work in this field, there are no reports of TRM conjugates of paclitaxel or of any derivative of this drug.

Accordingly, we synthesized a ternary conjugate consisting of paclitaxel (PTX), poly(ethylene glycol) (PEG), and a bombesin (BBN)/gastrin-releasing peptide (GRP) receptor-recognizing peptide (BBN[7-13])[20] as the first member of a series of soluble tumor-targeting paclitaxel derivatives (Scheme 1). These novel molecules were designed based on the premise that the soluble conjugate will be guided by the receptor-specific peptide to bind to tumor cell surface receptors, and that after internalization, the paclitaxel-PEG bond will be hydrolytically and/or enzymatically cleaved to release the cytotoxic agent.

Chemistry

The construction of the molecule began with the synthesis of the BBN[7-13] segment which, upon completion, was ligated to the PEG linker through both solid- and solution-phase techniques (Scheme 1). The BBN/GRP receptor ligand peptide was made by solid-phase peptide synthesis (SPPS) through the Fmoc protocol[21] and on a Rink amide resin. 1,3-Dicyclohexyl carbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) were used as coupling agents in DMF. Completion of each coupling was confirmed by the Kaiser ninhydrin test.[22] Histidine was used as the *im*-trityl (Trt)-protected amino acid. The Fmoc deprotection was carried out in a piperidine:DMF reagent, and the peptide−resin cleavage was performed using trifluoroacetic acid (TFA). The PEG linker was connected either through SPPS (route i, Scheme 1) or solution-phase synthesis (SPS, route v). In either procedure, commercially supplied *N*-hydroxysuccinimidyl Fmoc-amino-PEG-carboxylate (Fmoc-PEG-NHS) was used. In the solid phase, the standard Fmoc protocol of Fmoc peptide coupling was used. In solution, the cleaved peptide was attached to Fmoc-PEG-NHS in dry DMF and in the presence of *N*,*N*-diisopropyl ethylamine (DIEA). The homogeneity of the PEG-BBN[7-13] conjugate was determined unambiguously by matrix-assisted laser desorption−ionization time-of-flight mass spectrometry (MALDI MS) when a single group of peaks in the expected MW range (mean 4023 Da) was observed. Ligation of this molecule to paclitaxel pharmacophore (step vi) was carried out by activation of paclitaxel-2′-succinate (**3**) with 2′-ethoxy-1-ethoxycarbonyl-1, 2-dihydroquinoline (EEDQ)[23] and subsequent reaction with PEG-peptide in DMF. The reaction progress was monitored by MALDI MS which showed the formation of the target product. The peptide-free conjugate **7** was synthesized by the same procedure as for **6**, using paclitaxel-2′-succinate and amino-PEG-OH. Intermediate and final products were purified by reversed-phase (RP)-HPLC.

Results and Discussion

A high priority in the design of our paclitaxel conjugates was the induction of water solubility into the molecule. Water-soluble paclitaxl-PEG conjugates have been recently reported by Li et al., Greenwald et al., and Pendri et al.[23−26] During the course of the present study, we independently identified PEG as a potential "aqueous anchor" and considered the 2′-paclitaxel-PEG system as the soluble segment of the designed conjugate. This selection was made after screening a number of candidate linkers including several multimers of tetraethylene glycol with and without amino acid pendants.[27] Also, suitability of the paclitaxel-2′-succinate as a paclitaxel prodrug system has been documented[23,25,28] and was utilized for the construction of conjugates reported here. We prepared the paclitaxel-2′-succinate by the method of Deutsch et al.[29] Furthermore, interesting chemical and biological properties of PEGs have been extensively studied, and some useful characteristics of this polymer have been noted (see for example, the review article by Zalipsky[30]). These include aqueous as well as organic solubilities, lack of immunogenicity, favorable blood clearance patterns, and a favorable in vivo toxicity profile. Furthermore, in contrast to the

Table 1. [^{125}I]-Tyr^3-BBN Binding Data for BNR-11 and NCI-H1299 Cell Lines with BBN[7-13] Peptide **4** and PTXPEGBBN[7-13] Conjugate **6** as Inhibitiors

	% binding of [^{125}I]-Tyr^3-BBN		
cell line	no inhibitor	BBN[7-13]	PTXPEGBBN[7-13]
BNR-11	69 ± 1.6[a]	14 ± 1.2	5.4 ± 0.3
NCI-H1299	19 ± 0.5	3.6 ± 0.07	4.5 ± 0.6

[a] Standard deviation (n = 3).

reported paclitaxel-PEG derivatives,[23−25] we used in this synthesis a *heterobifunctional* derivative of the polymer which was placed as a linker between the peptide and the paclitaxel moieties.

Prior to the biological evaluation of this compound, three important issues needed to be addressed: (1) the overall water solubility, (2) the cell surface receptor binding ability of the conjugate, and (3) the drug release kinetics, i.e., the time during which the paclitaxel moiety is cleaved from the conjugate. The conjugate was readily soluble at a concentration of 250 mg/mL in such aqueous solvents as water, 0.9% saline, 50 mM PBS (pH 7.4), and 50 mM acetate buffer (pH 4.0), resulting in free-flowing, homogeneous solutions. Interestingly, the conjugate was also readily soluble in such organic solvents as methylene chloride, chloroform, methanol, ethanol, ethyl acetate, and dimethyl formamide (DMF). The unconjugated BBN[7-13] peptide was soluble only in DMF and methanol, and as such, the PEG linker seems to play the role of a *common universal solubilizer* for both paclitaxel and peptide moieties.

To evaluate the overall cell-binding efficiency of the PTXPEGBBN[7-13] conjugate, we compared the ability of the conjugate to inhibit the binding of [^{125}I]-Tyr^3-BBN to the BNR-11 cell line with that of the unconjugated BBN[7-13] peptide. This cell line is derived from murine 3T3 fibroblasts, stably transfected with *GRPr*,[31] and has been used for receptor affinity evaluations of this peptide.[20,32] We also used a human nonsmall cell lung carcinoma cell line (NCI-H1299) which has a significant but smaller number of BBN receptors.[33] The tracer peptide '[^{125}I]-Tyr^3-BBN' bound to both BNR-11 and NCI-H1299 cells in proportion to the number of available receptors (Table 1). Binding inhibition studies showed that the PTXPEGBBN[7-13] inhibition of [^{125}I]-Tyr^3-BBN binding to both cell lines was approximately 80−90%, which was the same as that of the unconjugated BBN[7-13] peptide (Table 1). Therefore, attachment of the 7-amino acid sequence to a relatively large molecule preserved the receptor binding ability of the peptide, and the presence of neither the PEG spacer nor the paclitaxel nucleus had any effect on its receptor affinity.

The release of the drug was studied by combination analysis using RP-HPLC and MALDI MS. The experiments were carried out at 37 °C and under two different conditions: in PBS at physiological pH (Figure 2A) and in freshly prepared human plasma (Figure 2B). The cleavage of paclitaxel from the conjugate showed a characteristic pattern[23−25] for the pseudo first-order decomposition of PTXPEGBBN[7-13], simultaneous with the formation of paclitaxel. These analyses indicated release half-lives of 154 and 113 min in PBS and human plasma, respectively, which may provide a rough measure for the in vivo behavior of these conjugates. Both

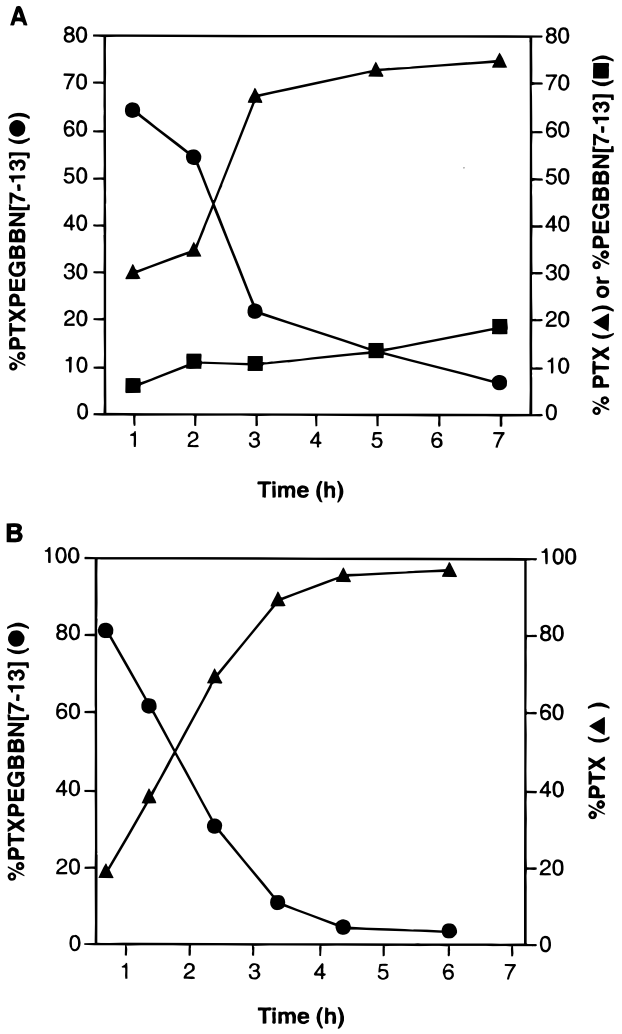


Figure 2. Drug release profile for the PTXPEGBBN[7-13] conjugate in PBS (A) and human plasma (B), showing the decomposition of the PTXPEGBBN[7-13] (●) and formation of paclitaxel (▲) and PEGBBN[7-13] (■). Formation of PEGBBN-[7-13] is shown only in PBS due to the obstruction of the corresponding HPLC signal by those of the plasma mixture. The lower absorbance intensities of the PEGBBN[7-13] compound may be due to the cleavage of the paclitaxel nucleus and, therefore, a lower molar absorptivity (ϵ) value.

drug release and tumor localization are important and determining factors in achieving high pharmacological efficacies for this conjugate. A drug release, which is considerably shorter or longer than the tumor localization time, will result in premature catabolism and excretion of either the cleaved drug or the intact conjugate.

The low receptor density NCI-H1299 cells were used to demonstrate the extent of the sensitivity of a native, untransfected cell line to this form of treatment. To evaluate the cytotoxic activity of the drug-peptide conjugate, 15 nM and 30 nM concentrations of paclitaxel and PTXPEGBBN[7-13] were incubated with the cells and the survival was assessed on day 4 (Figure 3). This assay showed a significant (p = 0.001) increase in cytotoxicity produced by the conjugate at both concentrations as compared to the unconjugated paclitaxel. Following exposure to 15 nM of the free drug, the cytotoxicity was 3% and 6% at 24 and 96 h, respectively, and 25% (24 h) and 32% (96 h) at 30 nM. In contrast, the conjugate resulted in 52% (24 h) and 60% (96 h)

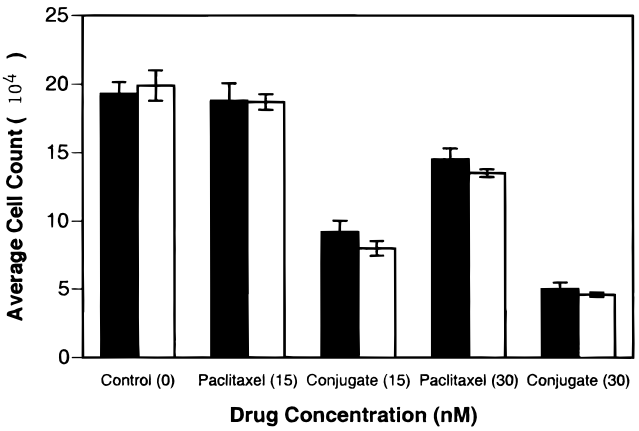


Figure 3. Cytotoxicities of paclitaxel and PTXPEGBBN[7-13] conjugate in NCI-H1299 cells at 24 h (■) and 96 h (□) posttreatment. Cells (10 000/well) were seeded in quadruplicate in 24-well plates and were allowed to grow for 24 h (day 0). All p values equal 0.001.

Table 2. IC_{50} Values for Unconjugated Paclitaxel (PTX), for PTXPEG-OH, and for PTXPEGBBN[7-13] Conjugates in NCI-H1299 Human Nonsmall Cell Lung Cancer Cells after 24 or 96 h Exposure to the Drug and Counted 96 h Posttreatment

	IC_{50} (nM)	
compound	24 h	96 h
PTX	35 ± 1.8[a]	15 ± 1.3
PTXPEG-OH	72 ± 5.9	44 ± 1.8
PTXPEGBBN[7-13]	14 ± 1.1	6 ± 0.9

[a] Standard deviation (n = 4).

cytotoxicity at 15 nM and 74% (24 h) and 77% (96 h) cytotoxicity at 30 nM.

The IC_{50} (concentration required for 50% growth inhibition) values were determined for the conjugate, at 24 and 96 h time points, through experiments designed to compare these values with those of underivatized paclitaxel and a paclitaxel-PEG conjugate which lacked the peptide moiety (PTXPEG-OH, **7**). The latter compound was synthesized to demonstrate that the improvement in cytotoxicity of PTXPEGBBN[7-13], as compared to unconjugated paclitaxel, is the result of peptide-guided receptor binding of the conjugate and not merely a consequence of the drug solubilization. For an isodynamic drug release, the PTX-PEG connection (an amide bond) and the length of the PEG linker (MW 3400) in this molecule were kept the same as in the PTXPEGBBN[7-13] conjugate. Cells were exposed to the PTXPEGBBN[7-13] at different concentrations (20−100 nM), and growth was monitored daily for up to 4 days and compared to untreated control cells. The cells were also exposed to the same range of concentrations of the underivatized drug and to the peptide-free PTXPEG-OH compound. The results (Table 2) indicated a decrease in the IC_{50} for the paclitaxel-peptide conjugate compared to paclitaxel alone or the PTXPEG-OH conjugate. Whereas the free drug showed an IC_{50} of 35 nM and 15 nM for 24 h and 96 h exposures, respectively, the values for the PTXPEGBBN[7-13] were 14 nM and 6 nM for the same exposure times. The IC_{50} values for PTXPEG-OH, against the NCI-H1299 cell line and under the same conditions, were higher than those of both the parent paclitaxel and the PTXPEGBBN[7-13] conjugate. This increase in the IC_{50} has been also reported by Greenwald et al. for paclitaxel-PEG derivatives in P388 and L1210 cells.[25] The fact that the peptide-conjugated paclitaxel showed improved cytotoxicity in a controlled, concentration-dependent manner may be indicative of the specific, receptor-mediated delivery of the drug to the tumor cells.

Paclitaxel and docetaxel are diterpenoid molecules with extremely high therapeutic potentials against breast, ovarian, and possibly other types of cancers with activity against refractory tumors. Although these compounds are currently in clinical use, a great deal of research is underway to improve their chemical, pharmacological, and pharmaceutical properties. Among these efforts are studies to develop compounds with optimal aqueous solubility and lowered toxicities. Water-soluble and tumor-directed paclitaxel derivatives which show enhanced cytotoxic properties may greatly improve the therapeutic outcome for patients treated with these agents. Additionally, these derivatives may be used to limit the systemic toxicity of the drug.

We have demonstrated the applicability of the prodrug approach to the design and synthesis of the first soluble tumor-recognizing paclitaxel derivative. The synthesis covalently linked a BBN/GRP receptor ligand peptide to a heterobifunctional poly(ethylene glycol) chain followed by the ligation of the PEG at the N-terminus to the 2′-hydroxy group of paclitaxel, resulting in a molecule containing the three segments at an equimolar ratio. The conjugate, designated PTXPEG-BBN[7-13], was readily water soluble as well as being soluble in a number of organic solvents. The molecule showed retention of the BBN/GRP receptor-binding property of its peptide moiety as well as the growth inhibition effects and cytotoxicity of the paclitaxel nucleus. The conjugate demonstrated improved cytotoxicity and IC_{50} in NCI-H1299 cells compared to the underivatized paclitaxel at equimolar concentrations. A paclitaxel-PEG compound, containing no peptide, showed an increased IC_{50} value with respect to paclitaxel.

The encouraging observations described in this report warrant further investigations of other congeners of the lead PTXPEGBBN[7-13] conjugate. Analogues with the paclitaxel moiety substituted at different sites and using different peptides, with affinities for different tumor cell surface receptors, as well as conjugates with other types of targeting moieties have been designed and are currently under investigation in this laboratory. The synthesis of these novel molecules may lead to new approaches to more effectively target tumors with the taxane class of antitumor agents. Additionally, since these agents have demonstrated radiosensitizing properties, it is possible that better tumor localization may enhance the use of these agents in combined therapy with radiation.

Experimental Section

Paclitaxel-2′-succinate. This compound was prepared from paclitaxel (Hande, Houston, TX) according to the procedure of Deutsch et al.,[29] except for an additional purification step with silica gel chromatography prior to crystallization. mp = 170.5−172.4 °C (lit.[29] 178−180 °C).

Solid-Phase Peptide Synthesis. The standard Fmoc procedure[34] was employed using Rink amide resin as the solid support. Coupling of amino acids was carried out manually in a standard Merrifield vessel, using HOBT and DCC as coupling reagents. Each amino acid and reagent was used at 2.5 molar excess with respect to the resin mole equivalents.

DMF was used as the reaction solvent. Completion of each coupling was ensured by the Kaiser ninhydrin test.[22] Deprotection reactions were carried out in piperidine:DMF (50:50, v/v) solutions.

HPLC Analyses. The HPLC experiments were performed on a Bio-Rad model 5000 Titanium system (Bio-Rad, Richmond, CA), equipped with model 1806 UV−vis detector and either a Vydac 4.6 × 250 mm C18 (analytical runs) or a 2.2 × 25 cm (preparative runs) reversed-phase column (Vydac, Hesperia, CA). For analytical runs, a 10−90% linear gradient of 40:60 (v/v) CH_3CN:H_2O over 40 min was used. Preparative runs used the same gradient system over a 70 min period. All runs used a UV detector wavelength of 280 nm. The retention times (t_Rs) for compounds reported below are from the analytical runs.

Electrospray MS and MS/MS. Analyses were performed on an API III triple quadrupole mass spectrometer (PE-Sciex, Concorde, Canada) by the flow injection method using electrospray ionization in the positive mode. MS/MS fragment ion spectra were obtained by passing the $(M + H)^+$ ion, selected with the first quadrupole, into the second quadrupole containing argon gas and analyzing the resulting fragment ions with the third quadrupole.

MALDI-TOF Mass Spectrometry. Samples were analyzed in the positive mode on a Voyager Elite mass spectrometer with delayed extraction technology (PerSeptive Biosystems, Framingham, MA). The acceleration voltage was set at 20 kV, and 50−100 laser shots were summed. α-Cyano-4-hydroxy cinnamic acid in a 1:1 (v/v) mixture of acetonitrile and 0.1% TFA was used as the matrix. Bovine serum albumin (1 pmol/μL) was used as an internal standard. Prior to each run, samples were diluted with the matrix and spotted on a smooth plate. The average of three measurements was used.

QWAVGHL-NH_2, 4. This peptide was prepared as reported previously[32] and through the Fmoc protocol as described above. After cleavage, the peptide was purified by preparative RP-HPLC. $t_R = 16.9$ min. ESMS: 809 $(M + 1)^+$. MS/MS: 809, 681 (-Q), 495 (-W), 424 (-A), 325 (-V), 268 (-G), 131 (-H) = L-NH_2 $(M + H)^+$.

PEGQWAVGHL-NH_2, 5. A. SPPS Method. *N*-Hydroxysuccinimidyl Fmoc-PEG-carboxylate (Fmoc-PEG-NHS, average MW 3400 Da, Shearwater Polymers, Huntsville, AL) was coupled to the resin-bound peptide by the Fmoc protocol described above.

B. SPS Method. To the solution of the peptide (3 mg, 0.004 mmol) in DMF (200 μL) was added DIEA (0.7 0 μL, 0.004 mmol) followed by the addition of Fmoc-PEG-NHS (34 mg, 0.01 mmol), and the reaction mixture was stirred at 25 °C for 1 h. The product was purified by RP-HPLC. $t_R = 24.7$ min. MALDI MS: 4023 $(M + H)^+$.

Paclitaxel-2′-PEGQWAVGHL-NH_2(PTXPEGBBN[7-13]), 6. To a solution of paclitaxel-2′-succinate (2 mg, 0.0021 mmol) in DMF (200 μL) was added EEDQ (1.04 mg, 0.0042 mmol). The solution was stirred at room temperature for 30 min and then added to the solution of the PEG-peptide (4.2 mg, 0.001 mmol) in 100 μL of DMF. The latter solution was stirred at ambient temperature for 2 h while the reaction progress was monitored by analytical HPLC. On completion, the final product was purified by RP-HPLC. $t_R = 29.6$ min. MALDI MS: 5300 $(M + H)^+$.

Paclitaxel-2′-PEG-OH, 7. A procedure identical to that for the preparation of **6** was used to conjugate PTX-2′-succinate to amino-PEG-OH (average MW of 3400 Da, Shearwater Polymers). HPLC: $t_R = 30.2$ min. ^1H NMR ($CDCl_3$, 400 MHz): δ 1.14 (s, 3H), 1.21 (s, 3H), 1.65 (s, 3H), 1.91 (s, 3H), 2.22 (s, 3H), 2.45 (s, 3H), 2.62 (t, 2H), 2.75 (m, 2H), 3.65 (s, PEG), 3.81 (d, 1H), 4.20 (d, 1H), 4.33 (d, 1H), 4.44 (m, 1H), 4.95 (d, 1H), 5.53 (d, 1H), 5.69 (d, 1H), 5.95 (m, 1H), 6.23 (t, 1H), 6.29 (s, 1H), 7.05 (d, 1H), 7.40−8.15 (set of m,s, 15H). MALDI: 4237 $(M + H)^+$.

Drug Release Kinetics. The conjugate in purified 18.2 MΩ water (Millipore, Bedford, MA) containing 0.05% TFA was added to three parts of 25 mM PBS (pH 7.4) or freshly prepared human plasma at t_0, and the solutions were incubated in argon-sealed vials at 37 °C in a dual chamber CO_2 incubator (Forma Scientific, Marietta, OH). Equally sized aliquouts were withdrawn at t_n and analyzed by reversed-phase analytical HPLC under the same conditions as described above in HPLC Analyses.

Cell-Binding Assay. Cells were harvested using EDTA/PBS and suspended in PBS with 1% bovine serum albumin (BSA) at 10^7 cells/mL. Cells were then aliquoted in triplicate for each peptide being tested at 1×10^6 cells/tube. The radiolabeled tracer peptide, [^{125}I]-Tyr^4-BBN, was added at 1×10^5 counts/min/tube. The unconjugated peptide BBN[7-13] (QWAVGHL-NH_2) was used as the inhibition control in the reference tube. Decreasing concentrations of the PTXPEG-BBN[7-13] were added to the sample tubes. All tubes were incubated at room temperature for 1 h and were then washed with 4 mL of PBS-1% BSA and 0.001 M EDTA. The cell pellets were counted in a well-type gamma counter for percent binding calculations. Nonspecific binding was assessed in the presence of ≥200-fold excess of the BBN[7-13] inhibitor.

Cytotoxicity Studies. NCI-H1299 cells (10 000/well) were seeded in quadruplicate in 24-well plates and were allowed to grow for 24 h (day 0). From stock solutions of paclitaxel (in DMSO) or PTXPEGBBN[7-13] (in water) were added volumes to each well to overall concentrations of either 15 or 30 nM in each reagent. The plates were incubated at 37 °C for either 24 or 96 h, and the drug was removed. All cells were counted (Coulter particle counter, Coulter, Hialeah, FL) on day 4. Underivatized paclitaxel was solubilized by DMSO with a DMSO concentration of <0.05% in the culture medium. At this concentration, DMSO was not cytotoxic as proven by a separate assay (not shown).

Evaluation of IC_{50} Values. NCI-H1299 cells (10 000/well) were exposed in quadruplicate to different concentrations of paclitaxel, the PTXPEG-OH, or the PTXPEGBBN[7-13] conjugate for 24 and 96 h, and counted on day 4 posttreatment. The IC_{50} values were determined using a linear regression model.

Supporting Information Available: MALDI mass spectra for **5**, **6**, and the corresponding starting material heterobifunctional PEG derivative as well as the synthetic scheme and MALDI spectrum for compound **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

Acknowledgment. We thank the UAB Department of Radiation Oncology for the partial support of this work in the form of a seed grant. Assistance of Dr. Ramin Arani (Biostatistics Unit, The UAB Comprehensive Cancer Center) with the calculation of *p* values is greatly acknowledged. We also acknowledge the assistance of the UAB Comprehensive Cancer Center Mass Spectroscopy and NMR Shared Facilities. Soneshia McMillan and Sally Lagan are thanked for manuscript preparation.

References

(1) Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. *J. Am. Chem. Soc.* **1971**, *93*, 2325−2327.
(2) Cortes, J. E.; Pazdur, R. Docetaxel. *J. Clin. Oncol.* **1995**, *13*, 2643−2655.
(3) Miller, K. D.; Sledge, G. W., Jr. Taxanes in the treatment of breast cancer: A prodigy comes of age. *Cancer Invest.* **1999**, *17*, 121−136.
(4) Arbuck, A.; Blaylock, B. Taxol: Clinical results and current issues in development. In *Taxol Science and Applications*; Suffness, M., Ed.; CRC: Boca Raton, FL, 1995; pp 379−415.
(5) Suffness, M. Taxol: from discovery to therapeutic use. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic Press: New York, 1993; pp 305−314.
(6) Rose, W. C. Taxol: A review of its preclinical in vivo antitumor activity. *Anti-Cancer Drugs* **1992**, *3*, 311−321.
(7) Tishler, R. B.; Geard, C. R.; Hall, E. J.; Schiff, P. B. Taxol sensitizes human astrocytoma cells to radiation. *Cancer Res.* **1992**, *52*, 3495−3497.

(8) O'Donnell, R. T.; DeNardo, S. J.; Miers, L. A.; Kukis, D. L.; Mirick, G. R.; Kroger, L. A.; DeNardo, G. L. Combined modality radioimmunotherapy with Taxol and ^{90}Y-LYM-1 for Raji lymphoma xenografts. *Cancer Biother. Radiopharm.* **1998**, *13*, 351−361.
(9) Distefano, M.; Scambia, G.; Ferlini, C.; Gaggini, C.; De Vincenzo, R.; Riva, A.; Bombardelli, E.; Ojima, I.; Fattorossi, A.; Panici, P. B.; Mancuso, S. Anti-proliferative activity of a new class of taxanes (14-beta-hydroxy-10-deacetylbaccatin III derivatives) on multidrug-resistance-positive human cancer cells. *Int. J. Cancer* **1997**, *72*, 844−850.
(10) Dorr, R. T. Pharmacology and toxicology of Cremophor EL diluent. *Ann. Pharmacother.* **1994**, *28*, S11−S14.
(11) Sharma, A.; Mayhew, E.; Bolcsak, L.; Cavanaugh, C.; Harmon, P.; Janoff, A.; Bernacki, R. J. Activity of paclitaxel liposome formulations against human ovarian tumor xenografts. *Int. J. Cancer* **1997**, *71*, 103−107.
(12) Roland, P. Y.; Barnes, M. N.; Niwas, S.; Robertson, M. W.; Alvarez, R.; Austin, J. M.; Kilgore, L. C.; Partridge, E. E. Response to salvage treatment in recurrent ovarian cancer treated initially with paclitaxel and platinum-based combination regimens. *Gynecol. Oncol.* **1998**, *68*, 178−182.
(13) Preston, N. J. Paclitaxel (Taxol): A guide to administration. *Eur. J. Cancer Care* **1996**, *5*, 147−152.
(14) Hajek, R.; Vorlicek, J.; Slavik, M. Paclitaxel (Taxol): A review of its antitumor activity in clinical studies. Minireview. *Neoplasma* **1996**, *43*, 141−154.
(15) Postma, T. J.; Vermorken, J. B.; Liefting, A. J.; Pinedo, H. M.; Heimans, J. J. Paclitaxel-induced neuropathy. *Ann. Oncol.* **1995**, *6*, 489−494.
(16) Maier-Lenz, H.; Hauns, B.; Haering, B.; Koetting, J.; Mross, K.; Unger, C.; Bauknecht, T.; du Bois, A.; Meerpohl, H. G.; Hollaender, N.; Diergarten, K. Phase I study of paclitaxel administered as a 1-hour infusion: Toxicity and pharmacokinetics. *Semin. Oncol.* **1997**, *24*, S19−16-S19−19.
(17) Goodwin, D. A. Stategies for antibody targeting. *Antib. Immunoconj. Radiopharm.* **1991**, *4*, 427−434.
(18) Kohler, G. M. C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **1975**, *256*, 495−497.
(19) Arap, W.; Pasqualini, R.; Ruoslahti, E. Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. *Science* **1998**, *279*, 377−380.
(20) Hoffman, T. J.; Sieckman, G. L.; Volkert, W. A. Targeting small cell lung cancer using iodinated peptide analogues. *J. Labeled Comp. Radiopharm.* **1995**, *37*, 321−323.
(21) Chang, A. Y.; Kim, K.; Glick, J.; Anderson, T.; Karp, D.; Johnson, D. Phase II study of taxol, merbarone, and piroxantrone in stage IV nonsmall-cell lung cancer: The Eastern Cooperative Oncology Group Results. *J. Natl. Cancer Inst.* **1993**, *85*, 388−394.
(22) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. Color test for detection of free terminal amino groups in the solid-phase synthesis of peptides. *Anal. Biochem.* **1970**, *34*, 595−598.
(23) Li, C.; Yu, D.; Inoue, T.; Yang, D. J.; Milas, L.; Hunter, N. R.; Kim, E. E.; Wallace, S. Synthesis and evaluation of water-soluble poly(ethylene glycol)-paclitaxel conjugate as a paclitaxel prodrug. *Anticancer Drugs* **1996**, *7*, 642−648.
(24) Greenwald, R.; Pendri, A.; Bolikal, D. Highly water soluble-taxol derivatives: 7-poly(ethylene glycol) carbamates and carbonates. *J. Org. Chem.* **1995**, *60*, 331−336.
(25) Greenwald, R. B.; Gilbert, C. W.; Pendri, A.; Conover, C. D.; Xia, J.; Martinez, A. Drug delivery systems: Water soluble taxol 2′-poly(ethylene glycol) ester prodrugs-design and in vivo effectiveness. *J. Med. Chem.* **1996**, *39*, 424−431.
(26) Pendri, A.; Conover, C. D.; Greenwald, R. B. Antitumor activity of paclitaxel-2′-glycinate conjugated to poly(ethylene glycol): a water-soluble prodrug. *Anticancer Drug Des.* **1998**, *13*, 387−395.
(27) Safavy, A. Unpublished results.
(28) Nicolaou, K. C.; Riemer, C.; Kerr, M. A.; Rideout, D.; Wrasidlo, W. Design, synthesis and biological activity of protaxols. *Nature* **1993**, *364*, 464−466.
(29) Deutsch, H.; Glinski, J.; Hernandez, M.; Haugwitz, R.; Narayanan, V.; Suffness, M.; Zalkow, L. Synthesis of congeners and prodrugs: Water soluable prodrugs of taxol with potent antitumor activity. *J. Med. Chem.* **1989**, *32*, 788−792.
(30) Zalipsky, S. Functionalized poly(ethylene glycol) for preparation of biologically relevant conjugates. *Bioconjugate Chem.* **1995**, *6*, 150−165.
(31) Battey, J. F.; Way, J. M.; Corjay, M. H.; Shapira, H.; Kusano, K.; Harkins, R.; Wu, J. M.; Slattery, T.; Mann, E.; Feldman, R. I. Molecular cloning of the bombesin/gastrin-releasing peptide receptor from Swiss 3T3 cells. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 395−399.
(32) Safavy, A.; Khazaeli, M. B.; Qin, H.; Buchsbaum, D. J. Synthesis of bombesin analogues for radiolabeling with Rhenium-188. *Cancer (Suppl).* **1997**, *80*, 2354−2359.
(33) Moody, T. W.; Zia, F.; Venugopal, R.; Fagarasan, M.; Oie, H.; Hu, V. GRP receptors are present in non small cell lung cancer cells. *J. Cell. Biochem. Suppl.* **1996**, *24*, 247−256.
(34) Chang, C.-D.; Meieinhofer, J. Solid-phase peptide synthesis using mild base cleavage of N^{α}-fluorenylmethyloxycarboxylamino acids emplified by synthesis of dihydrosomatostatin. *Int. J. Pept. Protein Res.* **1978**, *11*, 246−249.

JM990355X