

Tumor-Specific Novel Taxoid–Monoclonal Antibody Conjugates

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Abstract: Taxoids bearing methyldisulfanyl(alkanoyl) groups for taxoid–antibody immunoconjugates were designed, synthesized and their activities evaluated. A highly cytotoxic C-10 methyldisulfanylpropanoyl taxoid was conjugated to monoclonal antibodies recognizing the epidermal growth factor receptor (EGFR) expressed in human squamous cancers. These conjugates were shown to possess remarkable target-specific antitumor activity in vivo against EGFR-expressing A431 tumor xenografts in severe combined immune deficiency mice, resulting in complete inhibition of tumor growth in all the treated mice.

Current cancer chemotherapy is based on the premise that rapidly proliferating tumor cells are more likely to be killed by cytotoxic drugs. Unfortunately, the difference in activity of current drugs against tumor tissues in comparison to healthy tissues is relatively small. The amount of a drug required to achieve a clinically effective level of cell kill often causes severe damage to actively propagating nonmalignant cells such as cells of the gastrointestinal tract and bone marrow, resulting in a variety of undesirable side effects.¹ Therefore, it is very important to develop new chemotherapeutic agents with improved tumor specificity.

The discovery of antigens that are particularly overexpressed on the surface of cancer cells suggests that by using certain antibodies to selectively “mark” tumor cells, malignant tissues could be distinguished from normal tissues.¹ Monoclonal antibodies (mAb's), which have shown high binding specificity for tumor-specific antigens, could fulfill this task. In fact, these mAbs could be used as vehicles to deliver cytotoxic drugs selectively to tumor cells.^{1–3} A drug–mAb conjugate would target the tumor cells by binding to the antigens on their surfaces. The conjugate is then internalized and releases the original cytotoxic agent in its active form.^{4–6} This type of immunoconjugates can be categorized as a “tumor-activated prodrug” (TAP).¹ Ideally, a TAP should be stable during circulation (no premature release of the drug) and should not bind to normal tissue cells.

The practical efficacy of such immunoconjugates heavily depends on the nature of the cytotoxic agents as well as the tumor specificity of mAb's. An mAb–calicheamicin conjugate “Mylotarg” has been approved for clinical use.⁷ Maytansinoids and CC-1065 analogues have been conjugated to mAb's for treatment of tu-

mors^{2,3} and showed encouraging potency and selectivity in preclinical models.

Paclitaxel and docetaxel have brought about significant impact on the current cancer chemotherapy mainly because of their unique mechanism of action⁸ but seriously suffer from the lack of tumor specificity and multidrug resistance (MDR). Thus, it is beneficial to develop immunoconjugates of these drugs. Indeed, two research groups have recently reported paclitaxel–mAb conjugates^{9,10} as potential tumor-specific anticancer agents. However, one of the conjugates was only tested in vitro¹⁰ and the other showed only limited efficacy in vivo.⁹ It is clear from the current understanding of the requirements for effective immunoconjugates that the cytotoxicity level of paclitaxel or docetaxel is not sufficient as the cytotoxic component of the conjugate for human clinical use.¹ In addition, those conjugates are anticipated to be inactive against tumors expressing MDR phenotype.

On the basis of our structure–activity relationship study of taxoids,^{11,12} we have developed a series of highly potent second-generation taxoids.^{13–18} Most of these taxoids exhibited 2–3 orders of magnitude higher potency than that of paclitaxel and docetaxel against drug-resistant cell lines expressing MDR phenotypes. One of these second-generation taxoids, SB-T-110131 (IDN5109; BAY59-8862), exhibited excellent pharmacological profile in preclinical studies and is currently undergoing phase II human clinical trials sponsored by Bayer Corporation. Accordingly, in principle we should be able to develop novel chemotherapeutic agents with high potency and exceptional tumor specificity by linking these second-generation taxoids with mAb's. We report here our promising preliminary results on the design, synthesis, and evaluation of mAb–taxoid immunoconjugates.

Design of mAb–Taxoid Conjugates. Use of an appropriate linker between a taxoid and an mAb is crucial for the efficacy of the resulting immunoconjugate. It is required that the linker be stable for an extended period of time upon storage and also in circulation in vivo, while it is readily cleavable inside cancer cells. Among possible linker units reported, we chose to employ a disulfide linker unit because of its favorable characteristics.^{1–3} It is expected that the mAb component of the conjugate binds to the specific antigens on tumor surfaces and the whole conjugate is internalized via endocytosis. The disulfide bond is then cleaved by an intracellular thiol such as glutathione¹ to release taxoid in its active form.

To synthesize a mAb–taxoid conjugate, both a taxoid and a mAb need to be modified to form a disulfide linkage by disulfide–thiol exchange reaction. Since the necessary modification of mAb had been worked out in one of these laboratories, the critical issue was to find highly potent second-generation taxoids modified with a sulfhydrylalkanoyl group, which would be the actual cytotoxic agent in the target cancer cells. Because the logical precursor (or synthon) for the sulfhydrylalkanoyl group is the methyldisulfanyl (MDS) alkanoyl group, we decided to synthesize MDS-alkanoyltaxoids. It has been

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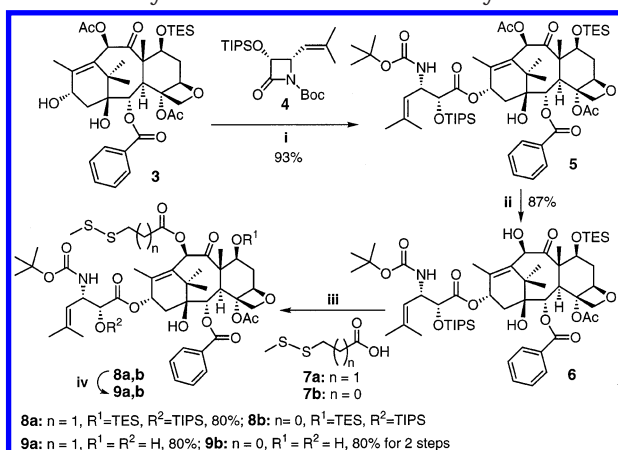
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shown that the number of tumor-associated antigens on the cancer cell surface is limited (estimated to be typically 10^5 molecules/cell). Thus, the cytotoxic agents that can be effectively used in these conjugates must have an IC_{50} value of 10^{-10} – 10^{-11} M against target cancer cells.¹ Two of the second-generation taxoids possess cytotoxicity in the required range.¹³ Thus, these two taxoids were chosen for modification with an MDS-alkanoyl group.

Since incorporation of an MDS-alkanoyl group into these taxoids may well affect the cytotoxicity of the resulting taxoids, an SAR study was necessary to determine the optimal position for the introduction of an MDS-alkanoyl group. Thus, we have synthesized new taxoids bearing an MDS-alkanoyl group at the C-10, C-7, C-2' and C-2 positions, and their cytotoxicity was assayed.

Syntheses of MDS-alkanoyltaxoids. The syntheses of 10-MDS-alkanoyltaxoids are shown in Scheme 1. We planned the syntheses of taxoids using the β -lactam ring-opening coupling protocol^{11,12,19,20} with appropriately modified baccatins. However, we found that the introduction of 3-MDS-propanoyl group at the C-10 position of taxoids is not a trivial matter. After the attempted acylation of 7-TES-10-deacetylbaccatin with 3-MDS-propanoyl chloride/LiHMDS failed, we chose the acetyl group as the protecting group for 10-OH, which could be selectively removed after the attachment of the C-13 isoserine moiety. As Scheme 1 shows, 7-TES-baccatin (**3**)^{17,18,20,21} was coupled with enantiopure β -lactam **4**^{13–15,17,18} to give taxoid **5**²² in excellent yield. After several attempts, we found that the 10-Ac group of taxoid **5** could be cleanly cleaved using a procedure reported by Georg et al.²³ with modifications. Hydrazinolysis of **5** using hydrazine-hydrate in EtOH afforded 10-deacetyltaxoid **6** in high yield. Subsequently, **6** was coupled with acids **7a** and **7b**,²⁴ followed by removal of the silyl groups by HF-pyridine to give 10-MDS-taxoids, **9a** and **9b**, respectively, in high overall yields.

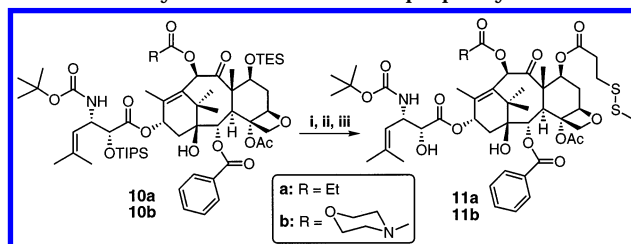
Scheme 1. Syntheses of C-10-MDS-alkanoyltaxoids^a



^a (i) LiHMDS (1.5 equiv), **4** (1.5 equiv), THF, -40°C , 40 min; (ii) $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$, EtOH, 3 h; (iii) **7a** or **7b** (10 equiv), DIC (11 equiv), DMAP, CH_2Cl_2 , overnight; (iv) HF-pyridine, pyridine, CH_3CN , overnight.

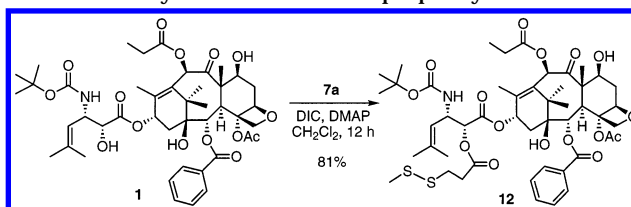
Synthesis of 7- and 2'-MDS-alkanoyltaxoids **11** and **12** are shown in Schemes 2 and 3. In a similar manner,²² we have synthesized two more analogues **13** and **14** shown in Scheme 4 (see Supporting Information for detailed synthetic procedure).

Scheme 2. Syntheses of C-7-MDS-propanoyltaxoids **11a**^a

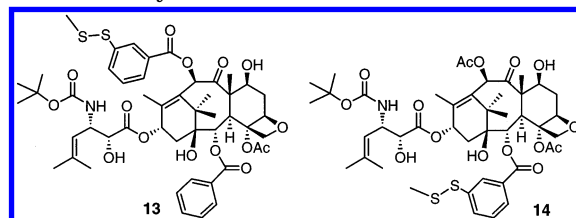


^a (i) 0.1 N HCl in EtOH, overnight (**a** 88%, **b** 85%); (ii) **7a** (5 equiv), DIC (10 equiv), DMAP, CH_2Cl_2 , 2 h (**a** 85%, **b** 88%); (iii) HF-pyridine, CH_3CN , pyridine, overnight (**a** 86%, **b** 90%).

Scheme 3. Synthesis of 2'-MDS-propanoyltaxoid **12**



Scheme 4. 10-(3-MDS-benzoyl)taxoid **13** and 2-(3-MDS-benzoyl)taxoid **14**



Evaluation of Biological Activities. (a) In Vitro Cytotoxicity Assay. Cytotoxicity of new taxoids thus synthesized was assayed against A431 (epidermoid), A549 (non-small-cell lung), MCF7 (breast), and MCF7-MDR (breast) cancer cell lines. Their parent taxoids were also assayed for comparison. The IC_{50} values of these taxoids are summarized in Table 1. As Table 1

Table 1. In Vitro Cytotoxicity of Taxoids

taxoid	IC_{50} , ^a nM			
	A431 ^b	A549 ^c	MCF7	MCF7-MDR
paclitaxel		3.0 (3.6 ^d)	1.7 ^d	299 ^d
docetaxel		(1.0 ^d)	1.0 ^d	235 ^d
1	0.09	0.1	0.18 ^d	2.2 ^d
2	0.2	0.6	0.09 ^d	12.5 ^d
9a	0.5	0.8		
9b	0.7	0.8		
11a	2.0			
11b	> 3.0			
12	> 3.0	0.9		
13	> 3.0	> 3.0		
14	> 3.0	> 3.0		

^a The concentration of compound that inhibits 50% of the growth of cancer cell line after 72 h of drug exposure. ^b Human epidermoid carcinoma. ^c Non-small-cell lung carcinoma. ^d See ref 13.

shows, the 10-MDS-alkanoyltaxoids **9a** and **9b** retain subnanomolar IC_{50} values, making each of them very promising as a cytotoxic component for taxoid-mAb conjugates.

Previous SAR studies of taxoids have shown that the C-7 position is well tolerated for modifications.²⁶ To our surprise, taxoids **11a** and **11b**, bearing an MDS-propanoyl group at the C-7 position, exhibit compromised cytotoxicity. Interestingly, when the MDS-propanoyl

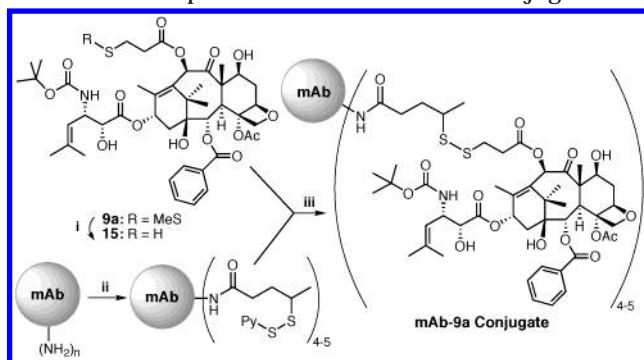
group is attached to 2'-OH, the resulting taxoid **12** is much less active against the A431 cell line while maintaining its potency against the A549 cell line. Taxoids **13** and **14**, bearing a 3-MDS-benzoyl group at C-10 and C-2, respectively, show substantial loss of activity.

Thus, the SAR study has clearly demonstrated that cytotoxicity can be retained when an MDS-propanoyl group is attached to the C-10 position of a taxoid. This is an important finding, which is totally unexpected.

(b) Conjugation of Taxoid with mAb. On the basis of the in vitro study described above, **9a** was selected for linking to mAb's to form immunoconjugates.

The epidermal growth factor receptor (EGFR) is known to be overexpressed in several human squamous cancers such as head, neck, lung, and breast cancers. Murine monoclonal antibodies directed against the human EGFR were used as the tumor-targeting moieties in immunoconjugates. Three such immunoglobulin G class monoclonal antibodies, KS61 (IgG2a), KS77 (IgG1), and KS78 (IgG2a), were linked to **9a** via disulfide bonds. The preparation of mAb-**9a** conjugates is illustrated in Scheme 5. Taxoid **9a** was treated with

Scheme 5. Preparations of mAb-Taxoid Conjugates^a



^a (i) dithiothreitol (DTT); (ii) *N*-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP, 10 equiv in ethanol), 50 mM potassium phosphate buffer, pH 6.5, NaCl (50 mM), EDTA (2 mM), 90 min; (iii) 50 mM potassium phosphate buffer, pH 6.5, NaCl (50 mM), EDTA (2 mM), **15** (1.7 equiv per dithiopyridyl group in EtOH), 24 h.

dithiothreitol to generate HS-taxoid **15** bearing a free thiol functionality. The anti-EGFR mAb was modified with *N*-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP) to attach 4-pyridyldithio (PDT) pentanoyl groups. Recovery of the antibody was about 90%, with four to five PDT-propanoyl groups linked per antibody molecule on average. Then, the modified mAb was conjugated with HS-taxoid **15** (2 equiv in aqueous EtOH) at pH 6.5 in a buffer solution. The mAb-taxoid conjugates were purified by gel filtration over Sephacryl S300, which separated aggregates from monomeric species, and only the fractions corresponding to the monomeric conjugates were collected. Recovery of the conjugate was 65–70% (see Supporting Information for detailed procedures). Preliminary MALDI-TOF analyses of the KS77-**9a** conjugate (peaked at *m/z* 151 784 with the half peak width of 3,650) in comparison with KS77 (peaked at *m/z* 148 500) strongly support that four to five taxoids, on average, are attached to the mAb. The final formulation of the conjugate in phosphate-buffer saline (PBS) contained 20% propylene glycol and 0.1% Tween 80 (v/v). Three immunoconjugates, KS61-**9a**, KS77-**9a**, and

KS78-**9a**, were thus prepared. A conjugate of **9a** with monoclonal antibody mN901 that does not bind to EGFR was also prepared in a similar manner for comparison.

(c) In Vitro Cytotoxicity Assays. In vitro cytotoxicity was determined in a clonogenic assay²⁷ after continuous exposure of the cells to the conjugates. It is expected that antigen-expressing cancer cells could only be "targeted" by an immunoconjugate bearing an mAb specific to the antigen. In fact, mN901-**9a** exhibits no cytotoxicity against the A431 cell line, expressing EGFR. In sharp contrast, KS78-**9a** shows high potency ($IC_{50} = 1.5$ nM) against the same A431 cell line (see Supporting Information for details). It should be noted that the addition of an excess of unconjugated anti-EGFR antibody, e.g., KS-61 at 3×10^{-8} M to the KS-61-**9a** conjugate, abolished its cytotoxicity against A-431 cells, indicating that cytotoxicity depended on the specific binding of the conjugate to the antigen on cells and that the preparation of conjugate had little unconjugated taxoid. If there were free taxoid present, addition of unconjugated antibody should not have blocked the cytotoxic effect.

These results demonstrate that the binding of anti-EGFR mAb-taxoid conjugate to EGFR is highly specific. Moreover, it is strongly indicated that the immunoconjugate KS78-**9a** generates highly cytotoxic agent **15** upon binding to EGFR, followed by internalization and the subsequent cleavage of the disulfide linkage.

(d) In Vivo Tumor Growth Inhibition Assays. The antitumor activities of two anti-EGFR mAb-taxoid conjugates, KS61-**9a** and KS77-**9a**, were evaluated against human tumor xenografts in severe combined immune deficiency (SCID) mice (Figure 1). Each mouse

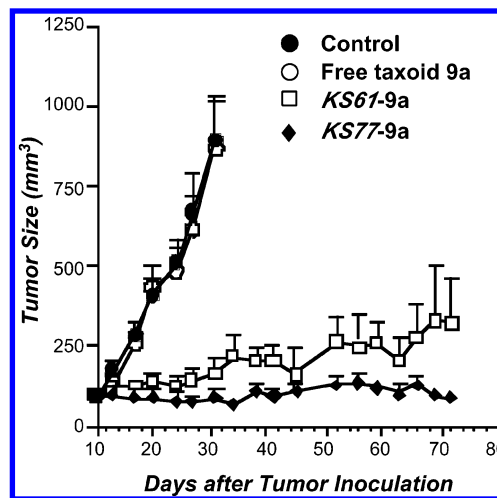


Figure 1. Antitumor activity of anti-EGFR mAb-taxoid conjugates against A-431 xenografts in SCID mice.

was inoculated with 1.5×10^6 A431 human squamous cancer cells, and the tumors were allowed to grow for 11 days to an average size of 100 mm³ (range of 54–145 mm³). The mice were then randomly divided into four groups. The first group received KS61-**9a** conjugate (10 mg/kg, qd \times 5, administered iv). The second group received KS77-**9a** conjugate in the same manner. The third group received free taxoid **9a** (0.24 mg/kg, qd \times 5, iv) at the same dose as that present in the conjugate. A control group of mice received phosphate-buffered saline using the same treatment schedule as in groups 1–3.

The weights of the mice and tumors sizes were measured twice weekly, and the tumor volumes were calculated with the formula $\frac{1}{2}(\text{length} \times \text{width} \times \text{height})$. The results are shown in Figure 1. The tumors in the control group of mice grew to a size of nearly 1000 mm³ in 31 days. Treatment with free taxoid **9a** showed no therapeutic effect, and the tumors in this group grew at essentially the same rate as in the untreated control group of mice. In contrast, both anti-EGFR mAb–taxoid conjugates showed remarkable antitumor activity, resulting in complete inhibition of tumor growth in all the treated animals for the duration of the experiment. Necropsy on day 75, followed by histopathological examination, showed residual calcified material at the tumor site but no evidence of tumor cells. These data also indicate that mAb-mediated delivery of the taxoid using tumor-selective mAbs leads to more pronounced antitumor activities than systemic drug treatment. However, further experiments that include nonbinding control mAb conjugates are required to determine if the effects are due to immunologically specific drug delivery. Notably, the doses of antibody–taxoid conjugates used are nontoxic to the mice, as demonstrated by the absence of any weight loss (see Supporting Information).

Conclusion. The results described above clearly indicate that the TAP approach incorporating second generation taxoids to mAbs highly specific to the antigen on tumor cell surfaces is very promising for producing potential chemotherapeutic agents with few side effects.

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Supporting Information Available: Synthesis procedures, characterization data, and biological assay methods. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Chari, R. V. J. Targeted delivery of chemotherapeutics: tumor-activated prodrug therapy. *Adv. Drug Delivery Rev.* **1998**, *31*, 89–104.
- (2) Liu, C. N.; Tadayoni, B. M.; Bourret, L. A.; Mattocks, K. M.; Derr, S. M.; Widdison, W. C.; Kedersha, N. L.; Ariniello, P. D.; Goldmacher, V. S.; Lambert, J. M.; Blattler, W. A.; Chari, R. V. J. Eradication of large colon tumor xenografts by targeted delivery of maytansinoids. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8618–8623.
- (3) Chari, R. V. J.; Jackel, K. A.; Bourret, L. A.; Derr, S. M.; Tadayoni, B. M.; Mattocks, K. M.; Shah, S. A.; Liu, C. N.; Blattler, W. A.; Goldmacher, V. S. Enhancement of the Selectivity and Antitumor Efficacy of a CC-1065 Analog through Immunoconjugate Formation. *Cancer Res.* **1995**, *55*, 4079–4084.
- (4) Sunada, H.; Magun, B. E.; Mendelsohn, J.; MacLeod, C. L. Monoclonal antibody against epidermal growth factor receptor is internalized without stimulating receptor phosphorylation. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 3825–3829.
- (5) Vollmar, A. M.; Bunker, D. E.; Mendelsohn, J.; Herschman, H. R. Toxicity of ligand and antibody-directed ricin A-chain conjugates recognizing the epidermal growth factor receptor. *J. Cell Physiol.* **1987**, *131*, 418–425.
- (6) Bender, H.; Takahashi, H.; Adachi, K.; Belser, P.; Liang, S.; Prewett, M.; Schrappe, M.; Sutter, A.; Rodeck, U.; Herlyn, D. Immunotherapy of human glioma xenografts with unlabeled ¹³¹I, or ¹²⁵I-labeled monoclonal antibody 425 to epidermal growth factor receptor. *Cancer Res.* **1992**, *52*, 121–126.
- (7) Hamaan, P. R.; Hinman, L. M.; Hollander, I.; Beyer, C. F.; Lindh, D.; Holcomb, R.; Hallett, W.; Tsou, H.-H.; Upeslakis, J.; Shochat, D.; Mountain, A.; Flowers, D. A.; Bernstein, I. Gemtuzumab Ozogamicin, a potent and selective anti-CD33 antibody–calicheamicin conjugate for treatment of acute myeloid leukemia. *Bioconjugate Chem.* **2002**, *13*, 47–58.
- (8) Schiff, P. B.; Fant, J.; Horwitz, S. B. Promotion of Microtubule Assembly in Vitro by Taxol. *Nature* **1979**, *277*, 665–667.
- (9) Guillemand, V.; Saragovi, H. U. Taxane–antibody conjugates afford potent cytotoxicity, enhanced solubility, and tumor target selectivity. *Cancer Res.* **2001**, *61*, 694–699.
- (10) Jaime, J.; Page, M. Paclitaxel immunoconjugate for the specific treatment of ovarian cancer in vitro. *Anticancer Res.* **2001**, *21*, 1119–1128.
- (11) Ojima, I.; Kuduk, S. D.; Chakravarty, S. In *Advances in Medicinal Chemistry*; Maryanoff, B. E., Reitz, A. B., Eds.; JAI Press: Greenwich, CT, 1998; Vol. 4, pp 69–124.
- (12) Ojima, I.; Lin, S.; Wang, T. The Recent Advances in the Medicinal Chemistry of Taxoids with Novel β -Amino Acid Side Chains. *Curr. Med. Chem.* **1999**, *6*, 927–954.
- (13) Ojima, I.; Slater, J. C.; Michaud, E.; Kuduk, S. D.; Bounaud, P.-Y.; Vrignaud, P.; Bissery, M.-C.; Veith, J.; Pera, P.; Bernacki, R. J. Syntheses and Structure–Activity Relationships of the Second Generation Antitumor Taxoids. Exceptional Activity against Drug-Resistant Cancer Cells. *J. Med. Chem.* **1996**, *39*, 3889–3896.
- (14) Ojima, I.; Slater, J. S.; Kuduk, S. D.; Takeuchi, C. S.; Gimi, R. H.; Sun, C.-M.; Park, Y. H.; Pera, P.; Veith, J. M.; Bernacki, R. J. Syntheses and Structure–Activity Relationships of Taxoids Derived from 14 β -Hydroxy-10-deacetylbaccatin III. *J. Med. Chem.* **1997**, *40*, 267–278.
- (15) Ojima, I.; Kuduk, S. D.; Pera, P.; Veith, J. M.; Bernacki, R. J. Synthesis of and Structure–Activity Relationships of Non-Aromatic Taxoids. Effects of Alkyl and Alkenyl Ester Groups on Cytotoxicity. *J. Med. Chem.* **1997**, *40*, 279–285.
- (16) Ojima, I.; Lin, S. Efficient Asymmetric Syntheses of β -Lactams Bearing a Cyclopropane or an Epoxide Moiety and Their Application to the Syntheses of Novel Isoleucines and Taxoids. *J. Org. Chem.* **1998**, *63*, 224–225.
- (17) Ojima, I.; Wang, T.; Miller, M. L.; Lin, S.; Borella, C. P.; Geng, X.; Pera, P.; Bernacki, R. J. Syntheses and Structure–Activity Relationships of New Second-Generation Taxoids. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3423–3428.
- (18) Lin, S.; Geng, X.; Qu, C.; Tynebor, R.; Gallagher, D. J.; Polina, E.; Rutter, J.; Ojima, I. Synthesis of Highly Potent Second-Generation Taxoids through Effective Kinetic Resolution Coupling of Racemic β -Lactams with Baccatins. *Chirality* **2000**, *12*, 431–441.
- (19) Holton, R. A.; Biediger, R. J.; Boatman, P. D. In *Taxol: Science and Applications*; Suffness, M., Ed.; CRC Press: New York, 1995; pp 97–121.
- (20) Ojima, I.; Habus, I.; Zhao, M.; Zucco, M.; Park, Y. H.; Sun, C. M.; Brigaud, T. New and Efficient Approaches to the Semisynthesis of Taxol and Its C-13 Side-Chain Analogs by Means of Beta-Lactam Synthon Method. *Tetrahedron* **1992**, *48*, 6985–7012.
- (21) Ojima, I.; Duclos, O.; Zucco, M.; Bissery, M.-C.; Combeau, C.; Vrignaud, P.; Riou, J. F.; Lavelle, F. Synthesis and Structure–Activity Relationships of New Antitumor Taxoids. Effects of Cyclohexyl Substitution at the C-3' and/or C-2 of Taxotère (Docetaxel). *J. Med. Chem.* **1994**, *37*, 2602–2608.
- (22) Ojima, I.; Duclos, O.; Kuduk, S. D.; Sun, C.-M.; Slater, J. C.; Lavelle, F.; Veith, J. M.; Bernacki, R. J. Synthesis and Biological Activity of 3'-Alkyl- and 3'-Alkenyl-3'-dephenyldocetaxels. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2631–2634.
- (23) Datta, A.; Hepperle, M.; Georg, G. I. Selective Deesterification Studies on Taxanes. Simple and Efficient Hydrazinolysis of C-10 and C-13 Ester Functionalities. *J. Org. Chem.* **1995**, *60*, 761–763.
- (24) Chari, R. V. J.; Goldmacher, V. S.; Lambert, J. M.; Blattler, W. A. Cytotoxic agents comprising maytansinoids and their therapeutic use. U.S. Patent 5,208,020, 1993.
- (25) Kingston, D. G. I.; Chaudhary, A. G.; Chordia, M. D.; Gharpure, M.; Gunatilaka, A. A. L.; Higgs, P. I.; Rimoldi, J. M.; Samala, L.; Jagtap, P. G.; Giannakakou, P.; Jiang, Y. Q.; Lin, C. M.; Hamel, E.; Long, B. H.; Fairchild, C. R.; Johnston, K. A. Synthesis and biological evaluation of 2-acetyl analogues of paclitaxel (Taxol). *J. Med. Chem.* **1998**, *41*, 3715–3726.
- (26) Chen, S. H.; Kant, J.; Mamber, S. W.; Roth, G. P.; Wei, J. M.; Marshall, D.; Vyas, D. M.; Farina, V.; Casazza, A.; Long, B. H.; Rose, W. C.; Johnston, K.; Fairchild, C. Taxol Structure–Activity Relationships–Synthesis and Biological Evaluation of Taxol Analogs Modified at C-7. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2223–2228.
- (27) Han, J.-w.; Dionne, C. A.; Kedersha, N. L.; Goldmacher, V. S. Status Affects the Rate of the Onset but not the Overall Extent of Doxorubicin-Induced Cell Death in Rat-1 Fibroblasts Constitutively Expressing c-Myc. *Cancer Res.* **1997**, *57*, 176–182.