No Auxiliary, No Byproduct Strategy for Water-Soluble Prodrugs of Taxoids: Scope and Limitation of O−N Intramolecular Acyl and Acyloxy Migration Reactions[†]

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Since numerous new taxoids active against multidrug resistant (MDR) tumors have been developed and their poor water-solubility is a very real problem in intravenous administration, we have designed and synthesized a series of novel water-soluble taxoid prodrugs (isotaxoids). These prodrugs, a 2′-*O*-isoform of taxoids, showed promising results with higher water solubility (0.8−1.1 mg/mL) and proper kinetics for parent drug release by a simple pH-dependent chemical mechanism via O−N intramolecular acyl migration. No additional functional auxiliaries are released during the conversion to parent drugs, which would be an advantage in toxicology and general pharmacology, and the cost for the evaluations of auxiliary units in these fields could be saved in prodrug development. In addition, we demonstrate for the first time the successful application of the O−N intramolecular acyloxy migration reaction in the prodrug design, with the exception of the *tert*-butyloxycarbonyl group, and that this reaction can be provided with no organic solvent and no side products.

Introduction

The introduction of the anticancer agents paclitaxel (Taxol, **1**)[1] and docetaxel (Taxotere, **2**)[2] has revolutionized the treatment of cancer and markedly improved the survival time of patients.[3−5] However, despite the hope and promise that taxoids such as paclitaxel and docetaxel have engendered, their lack of activity against multidrug resistant (MDR) tumors as well as dose-limiting toxicity corresponding to the side toxicity of taxoids are significant drawbacks. Additionally, their poor water solubility, based on their hydrophobic common taxane ring structure, is a very real problem in intravenous administration. Since paclitaxel can be dissolved with a detergent, Cremophor EL, at low concentrations, a prolonged intravenous administration time is required. Significant side effects associated with hypersensitivity to Cremophor EL have also been observed and a premedication schedule that includes a corticosteroid and antihistamine is required.[6] Moreover, it was recently reported that both Cremophor EL and Tween 80 used in the formulation of paclitaxel and docetaxel reduce the antitumor efficacy of these drugs by reducing their antiangiogenic activity.[7] To overcome these problems, two main strategies can be distinguished: the design of new paclitaxel derivatives and the temporary modification of paclitaxel for a prodrug form. Thus, numerous new taxoids have been designed and synthesized with improved properties,[8−15] and some of them are in clinical trials.[11−15] Also, water-soluble paclitaxel prodrugs and targeted prodrugs with reduced systemic toxicity through the introduction of hydrophilic

or targeting moieties to C-2′or/and C-7 positions have been developed,[16−31] some also currently undergoing clinical evaluation[27−31] (Chart 1). It would therefore be more attractive to connect these two strategies, namely, the utilization of a prodrug strategy to novel taxoids.

In all standard prodrug strategies, auxiliary moieties are employed and their release may have negative effects in vivo.[32] Hence, prodrug designs that avoid the use of additional moieties are very promising. Since the O−N intramolecular acyl migration reaction proceeded under mild basic or neutral condition with no byproduct formation,[33−40] we anticipate that this reaction would be ideal. Previously, we demonstrated the utilization of the O−N intramolecular acyl migration strategy in paclitaxel and canadensol prodrugs, isotaxoids **4** and **6**, respectively (Figure 1).[41−43] These prodrugs, 2′-*O*-acyl isoforms of **1** and **3**, showed (1) higher water solubility than that of parent drugs and (2) complete and prompt conversion to parent drugs under physiological conditions (pH = 7.4), while being stable in stock solution and in storage conditions as solid HCl salts as well as during synthesis. Therefore, this strategy was demonstrated to be effective.

In this present paper, based on these promising results, we successfully expanded the O−N intramolecular acyl migration strategy to a number of taxoids that are active against MDR tumors, since they all possess the α-hydroxy-β-amino acid moiety necessary for migration. Herein, we also demonstrate the first successful application of the O−N intramolecular acyloxy migration reaction in a prodrug design. We designed water-soluble prodrugs of 3′-*N*-carbamate taxoids and considered the possibility of utilizing this strategy in a docetaxel prodrug, **5**. Despite problems in the application of this strategy to **2**, the O−N intramolecular

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Chart 1

Protaxel

XYOTAX (CT-2103)

Taxoprexin (DHA-paclitaxel)

PNU166945
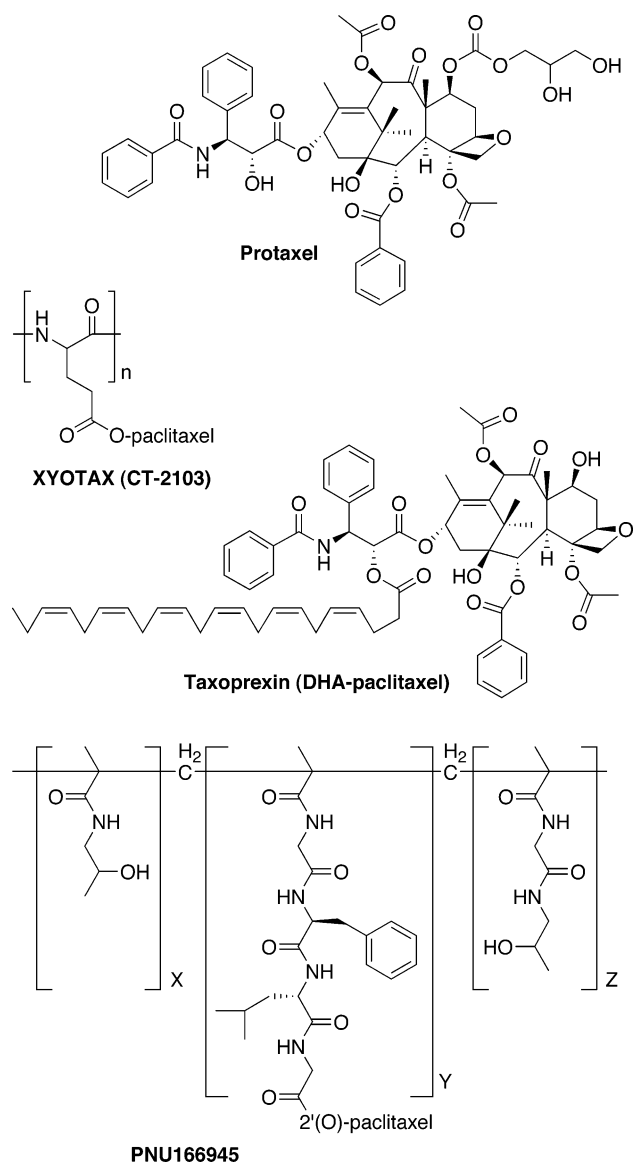
acyloxy migration reaction for all other prodrugs of 3′-
N-carbamate taxoids proceeded promptly and with no
side product formation under physiological conditions.
Thus, we demonstrated the successful application of the
O−N intramolecular acyloxy migration reaction in pro-
drug design for the first time. This is also the first report
that showed that this reaction can be provided with no
organic solvent and with no side products. In addition,
both O−N acyl and acyloxy migration reactions were
pH-dependent, and the migration rate was correlated
to the structure of acyl/acyloxy groups. In contrast, no
relationship was observed between the water solubility
of the prodrug and the structure of the acyl/acyloxy
moieties. However, all prodrugs showed significantly
increased water solubility compared to the parent drug.

Chemistry

The synthesis of the prodrugs **8**−**13** is shown in
Scheme 1. Compound **7**, which was synthesized by a
previously described method,[41] was coupled with a
corresponding carboxylic acid by the EDC−DMAP
method (EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbo-
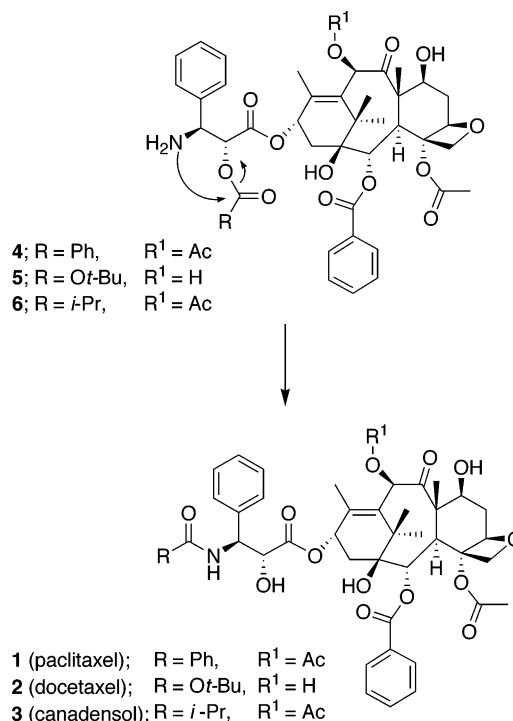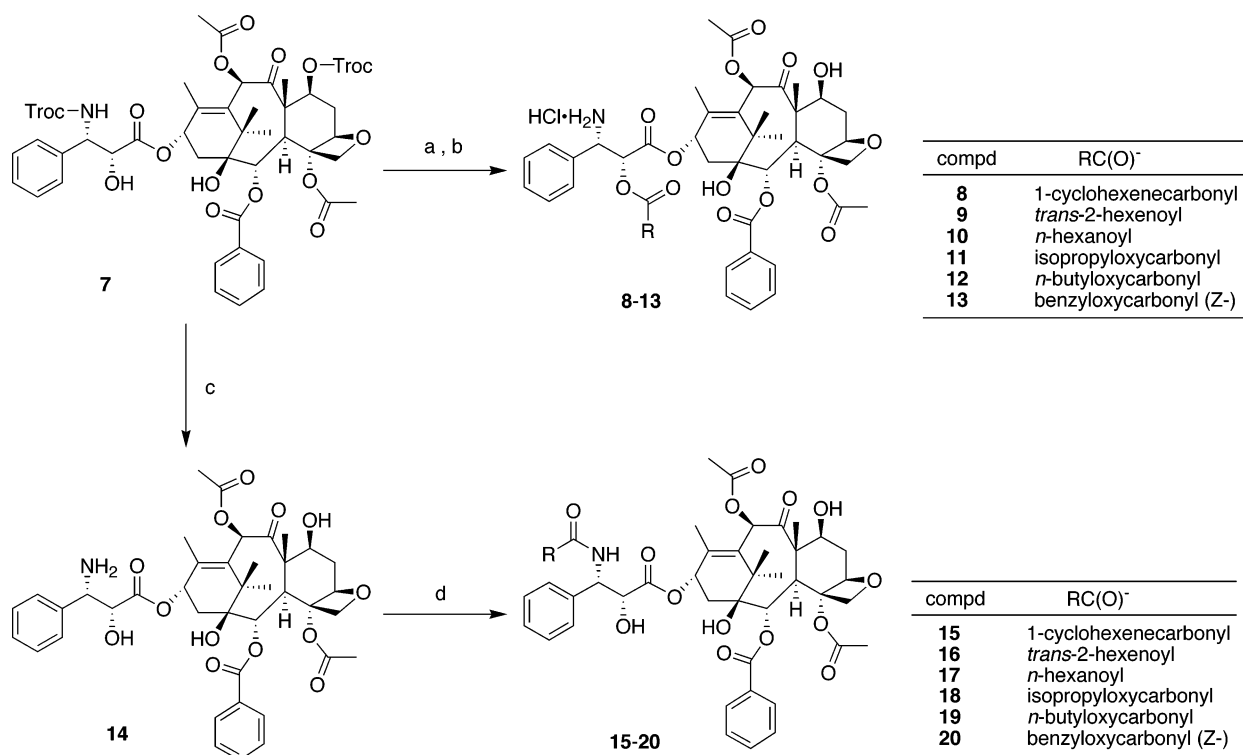diimide; DMAP, 4-(dimethylamino)pyridine) or chloro-

4; R = Ph, R^1 = Ac
5; R = Ot-Bu, R^1 = H
6; R = i-Pr, R^1 = Ac

1 (paclitaxel);   R = Ph, R^1 = Ac
2 (docetaxel);   R = Ot-Bu, R^1 = H
3 (canadensol); R = i-Pr, R^1 = Ac

Figure 1. The O−N acyl/acyloxy migration reaction of taxoid
prodrugs to their corresponding parent drugs under physi-
ological conditions.

formate in pyridine/CH_2Cl_2 solution, and subsequent
deprotection of both 2,2,2-trichloroethyloxycarbonyl (Troc)
groups using Zn−AcOH and the following purification
with ion exchange by HPLC gave prodrugs **8**−**13** as a
HCl salt. Additionally, parent drugs were synthesized
by the deprotection of both Troc groups of **7** to afford
compound **14** (Scheme 1), then the product of this
reaction was coupled with the desired acid by the EDC−
HOBt method (HOBt, 1-hydroxybenzotriazole) in DMF
or chloroformates in CH_2Cl_2/saturated NaHCO$_3$ solu-
tion, resulting in the formation of the parent drugs **15**−
20.

The synthesis of docetaxel prodrug **5** (Scheme 2) was
carried out using a similar strategy to that described
previously for isotaxel **4**.[41] Commercially available
(2R,3S)-phenylisoserine·HCl **21** after protection of an
amine moiety by the benzyloxycarbonyl group (Z) and
formation of the methyl ester was allowed to react with
4-methoxybenzaldehyde dimethyl acetal in the presence
of a catalytic amount of pyridinium p-toluenesulfonate
(PPTS) under kinetic control to afford 1,3-oxazolidine
derivative **22** as a diastereomeric mixture with 14% of
the minor and undesired diastereomer. The formation
of the minor, S-diastereomer of 1,3-oxazolidine, as
expected according to the literature,[44] was also observed
in the previously described synthesis of isotaxel **4** (data
not showed), although the desired R-diastereomer was
isolated after careful chromatographic purification.[41] As
the minor diastereomer of **22** was difficult to separate
by column chromatography, **22** was used for the next
step as a mixture. Compound **22**, after hydrolysis to the
coresponding carboxylic acid without further purifica-
tion (due to high lability in an acidic medium, even to
silica gel), was coupled with 10-deacetyl-7,10-dibenzyl-
oxycarbonylbaccatin III[45] in the presence of DCC to give
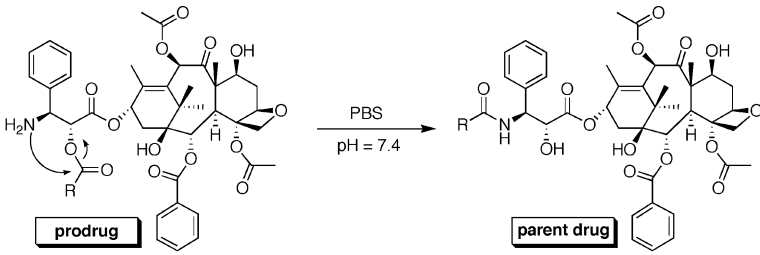the corresponding crude ester **23**, which was immedi-

Scheme 1[a]

compd	RC(O)⁻
8	1-cyclohexenecarbonyl
9	*trans*-2-hexenoyl
10	*n*-hexanoyl
11	isopropyloxycarbonyl
12	*n*-butyloxycarbonyl
13	benzyloxycarbonyl (Z-)

compd	RC(O)⁻
15	1-cyclohexenecarbonyl
16	*trans*-2-hexenoyl
17	*n*-hexanoyl
18	isopropyloxycarbonyl
19	*n*-butyloxycarbonyl
20	benzyloxycarbonyl (Z-)

[a] Reagents and conditions: (a) RCOOH, EDC·HCl, DMAP, CH₂Cl₂, rt (for **8**−**10**), or RCOCl, CH₂Cl₂, pyridine, rt (for **11**−**13**); (b) Zn, AcOH, MeOH, rt, then ion exchange HPLC with 12 mM HCl, (**8**, 76%; **9**, 47%; **10**, 62%; **11**, 65%; **12**, 71%; **13**, 53%) over two steps; (c) Zn, AcOH, AcOEt, rt, 60%; (d) RCOOH, EDC·HCl, HOBt, DMF, rt, for **15**−**17**, or RCOCl, CH₂Cl₂, sat. NaHCO₃, rt, for **18**−**20**, (**15**, 77%; **16**, 56%; **17**, 56%; **18**, 87%; **19**, 93%; **20**, 95%).
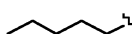
Scheme 2[a]

24, R = H
25, R = C(O)OC(CH₃)₃
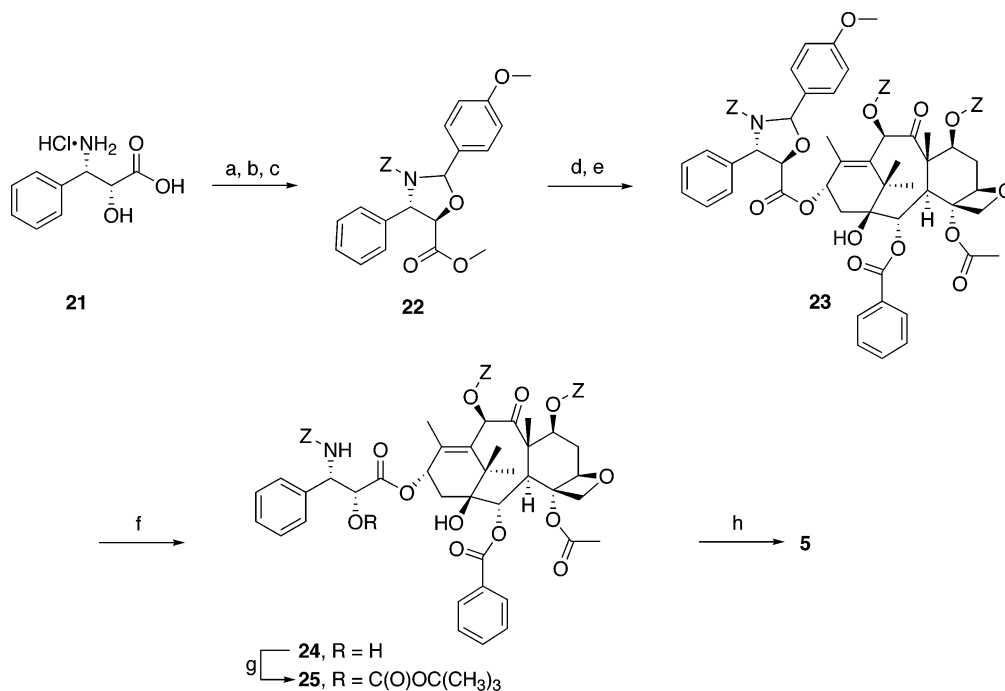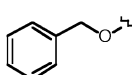
[a] Reagents and conditions: (a) SOCl₂, MeOH, 0 °C to rt, overnight; (b) benzyloxycarbonyl chloride, CH₂Cl₂, sat. NaHCO₃, 0 °C to rt, 3 h; (c) 4-methoxybenzaldehyde dimethyl acetal, PPTS, toluene, distillation, 40 min, 96% over three steps; (d) KOH, MeOH, rt, 2 h.; (e) 10-deacetyl-7,10-dibenzyloxycarbonylbaccatin III, DCC, DMAP, CH₂Cl₂, rt, 2 h; (f) PTS, MeOH, rt, 5 h, 80% over two steps; (g) Boc₂O, DMAP, CH₂Cl₂:pyridine 1:1, rt, overnight, 84%; (h) Pd/C, H₂, AcOEt, rt, 8 h, then 0.1 M HCl in methanol, 99%.

ately allowed to react with PTS to afford **24**. Although the oxazolidine ring of **23** was easily cleaved with the major diastereomer, the minor diastereomer was stable even with excess PTS and a long reaction time. Despite

this, the yield of **24** formation was good (80%). The Boc group was then introduced to **24** to afford compound **25**. Finally, three Z groups were deprotected under standard conditions, and HCl was added to afford the final

Table 1. Water Solubility and Conversion Time of Prodrugs

| prodrug | parent drug | R | water solubility | | | $t_{1/2}$ (min)[c] |
			prodrug (mg/mL)	parent drug (mg/mL)	ratio	
4[a]	**1**		0.45	0.00025	1800	15.1
6[b]	**3**		2.26	0.22	10	4.3
8	**15**		0.8	0.021	38	60.0
9	**16**		1.1	0.0013	850	29.3
10	**17**		0.56	0.008	70	6.0
11	**18**		1.7	0.018	90	19.2
12	**19**		0.8	0.004	200	3.8
13	**20**		1.0	<0.00025	>4000	< 1

[a] Data from ref 41. [b] Data from ref 42. [c] $t_{1/2}$ is the time required for 50% release of the parent drug from prodrug at 37 °C in phosphate-buffered saline (pH = 7.4).

prodrug **5** as a HCl salt quantitatively with a good total yield (67% calculated from baccatin III derivative coupling). It is noteworthy that prodrug **5** is unstable, even under aqueous mildly acidic conditions, due to the hydrolysis of the Boc group; even reverse-phase HPLC purification could not be employed. However, the deprotection of three Z groups and hydrochloride salt formation required no purification, as described in the Experimental Section.

Results

In our previous study, we synthesized water-soluble prodrug **4**[41] and **6**[42] on the basis of an O−N intramolecular acyl migration reaction, which is well-known in peptide chemistry.[46] To diversify this strategy and to understand the effect of acyl structures on the O−N acyl migration reaction, three reported taxoids were chosen. Taxoids **15** and **16**, which have 1-cyclohexenecarbonyl and *trans*-2-hexenoyl moieties, respectively, instead of

3′-*N*-benzoyl in **1**, show high activity against MDR tumors,[47,48] and **17**, which has a hexanoyl moiety at the same position, is also more potent than paclitaxel.[49] The prodrugs, isotaxoids **8**−**10**, *O*-acyl isoforms of parent taxoids **15**−**17**, were synthesized, and the water solubility and kinetic profile of the prodrugs were evaluated. As shown in Table 1, prodrugs **8**−**10** showed practical water solubility with values from 0.56 to 1.1 mg/mL, 38−850-fold higher than of parent drugs **15**−**17** (0.021−0.0013 mg/mL). To study the kinetics of O−N intramolecular acyl migration, prodrugs were dissolved in phosphate-buffered saline (PBS, pH = 7.4) and incubated at 37 °C, and the migration profile was monitored periodically by HPLC. The migration rate was significantly different among the three isotaxoids **8**−**10**. Sterically hindered prodrug **8** with the cyclohexene moiety showed the longest migration time ($t_{1/2}$ = 60.0 min), four times longer than that of isotaxel **4** ($t_{1/2}$ = 15.1 min), while prodrug **9** with the *trans*-2-*n*-hexenoyl
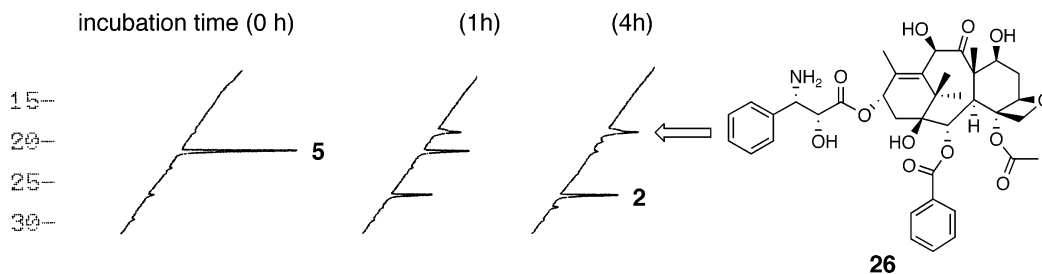
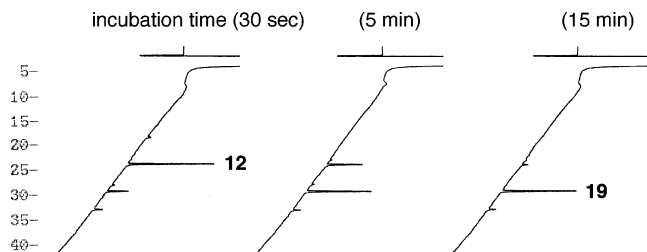**Figure 2.** HPLC profiles of O−N intramolecular acyloxy migration in prodrug **5** in PBS (pH 7.4, 37 °C).

Figure 3. HPLC profiles of O−N intramolecular acyloxy migration of prodrug **12** in PBS (pH 7.4, 37 °C).
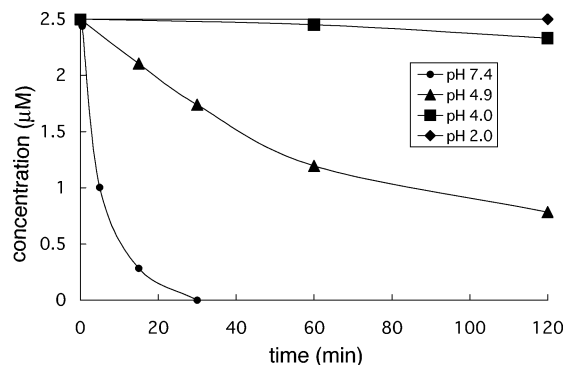

Figure 4. Migration of prodrug **12** at different pH values at 37 °C: pH 7.4 and 4.9 (PBS), pH 4.0 (0.035% citric acid saline), and pH 2.0 (glycine-HCl buffered saline).

group migrated for twice as long as **4**. Prodrugs **6** and **10** with simple aliphatic acyl groups, isobutanoyl and hexanoyl, respectively, released the parent drugs fastest ($t_{1/2}$ = 4.3 and 6.0 min, respectively). Importantly, in each case, the migration reaction proceeded smoothly with no side reactions, such as the simple hydrolysis of the newly introduced ester bond in the prodrugs.

In addition, on the basis of these promising results in prodrugs **8**−**10**, we considered that O−N intramolecular migration could be applied to important and clinically contributing docetaxel **2**, which possesses a *tert*-butyloxycarbonyl (Boc) group in place of the benzoyl group in paclitaxel. Namely, the O−N intramolecular acyloxy migration in docetaxel prodrug **5** was expected. Hence, prodrug **5**, which is the 2′-*O*-acyloxy isoform of docetaxel **2**, was designed and synthesized. This prodrug **5** showed a high water solubility of more than 6 mg/mL. However, in the conversion profile in PBS (pH 7.4, 37 °C), undesired hydrolysis of the Boc group, resulting in the production of compound **26**, was detected, while the desired O−N acyloxy migration from **5** to **2** was also observed (Figure 2).

On the basis of these observations, we assumed that this strategy should be considered for other carbonates more stable than Boc. Therefore, for further examination of the O−N acyloxy migration strategy, three reported taxoids, i.e., **18**,[50] **19**,[51] and **20**,[52] with structurally different acyloxy groups (isopropyloxycarbonyl, *n*-butyloxycarbonyl, and benzyloxycarbonyl, respectively) were chosen. Prodrugs **11**−**13**, including newly formed carbonate groups, were synthesized as HCl salts, and their water solubility was determined in the range from 0.8−1.7 mg/mL, 90−4000-fold higher than those of the parent drugs **18**−**20** (Table 1). Interestingly, complete migration was observed in all cases with no byproduct formation in PBS (pH 7.4, 37 °C) (Figure 3), and in contrast to compound **5**, no hydrolysis was detected. Prodrug **13** had the fastest migration in this group ($t_{1/2}$ < 1 min). Prodrug **11**, having the bulky isopropyloxy group, showed a slower migration ($t_{1/2}$ = 19.2 min) than isotaxoid **12** with the *n*-butyloxy group ($t_{1/2}$ = 3.8 min). On the other hand, the release of the

parent drug from prodrug **12** slowed at pH 4.9 to $t_{1/2}$ = 55.5 min, and no O−N acyloxy intramolecular migration was observed at pH 2 (Figure 4). Moreover, compound **12** was almost stable in 0.035% citric acid saline (pH 4.0) at room temperature (<2% of the parent drug was released after 1 h of incubation), suggesting a possible condition for injection.[20] These results indicate that the kinetics of O−N acyloxy migration are clearly pH-dependent, and a faster migration could be obtained under physiological conditions (pH 7.4) than under acidic conditions. This result is comparable to that observed previously in O−N intramolecular acyl migration.[38−40]

Discussion

Prodrugs are bioreversible derivatives of the drug molecule used to overcome some barriers in the parent drug utilities, such as solubility, permeability, oral absorption, stability, toxicity, and targeting.[32] In 2001 and 2002, 14% of all new approved chemical drugs were prodrugs.[53] This trend caused a dramatic increase in the number of submitted prodrug patents (>2000% increase in 2002 compared to 1993), with claims for cancer treatment comprising 37%,[53] which clearly shows the importance of this field in the development of anticancer agents. However, auxiliary units are used in all standard prodrug strategies, and the release of these moieties may cause some undesired side effects.[32] Therefore, the potential side effects of the auxiliary unit should be evaluated; otherwise, safe and nontoxic auxiliary units already accepted by the registration authorities should be used. However, even commonly used pivalic acid showed some toxicity connected with changes in carnitine homeostasis,[54] as well as the phosphate group (popularly used in prodrug design), which was reported to cause hypocalcemic effects, and special caution should be implemented, for example, for

patients with renal impairment.[55] Also, the formaldehyde released upon hydrolysis of some linker can be a toxicological concern.[32] In consideration, prodrug strategies that avoid any additional auxiliary moieties are very promising.

Along these lines, we previously developed isotaxel **4**, a 2′-*O*-acyl isoform of paclitaxel **1**. This prodrug **4** showed 1800-fold higher water solubility (0.45 mg/mL) than **1** and released parent drug **1** via an O−N intramolecular acyl migration reaction with no side reaction under physiological conditions (pH 7.4).[41] In a similar manner, we designed and synthesized the first water-soluble prodrug of canadensol **3**,[42] a promising candidate as a new taxoid drug. In both cases, the production of parent drugs was pH-dependent, while prodrugs were stable in the solid form and under acidic conditions, which suggested a possible injection condition for practical clinical use. Thus, the O−N acyl migration reaction could be one of the leading strategies in prodrug design, since isotaxoids **4** and **6** showed higher water solubility and there was no byproduct formation during parent drug formation.

On the other hand, it was demonstrated that paclitaxel prodrug 7-(2″,3″-dihydroxypropylcarbonoxy)-paclitaxel (protaxel),[20] which had improved water solubility (0.05 mg/mL) and released **1** in a similar pH-dependent manner (pH 7.4, $t_{1/2}$ = 10 min,) via intramolecular cyclization, showed lower systemic toxicity than paclitaxel. Therefore, it can be administered in higher doses. Protaxel is currently in clinical trials.[28] In relation to those results, we also expected the water solubility and kinetics of our prodrugs to be promising in practice.

In this paper, prodrugs **8**−**10** of important taxoids, which have different acyl groups and can be converted to parent drugs by the O−N intramolecular acyl migration reaction, were synthesized and evaluated. All showed significant increases in water solubility (38−800-fold) compared to the parent drugs. The water-soluble prodrug **8** with the cyclohexene carbonyl group showed the slowest migration rate ($t_{1/2}$ = 60 min), which is thought to result from its steric hindrance. Indeed, the less sterically hindered prodrug **4** with the flat benzoyl group showed faster migration ($t_{1/2}$ = 15.1 min), and prodrug **10** with linear hexanoyl moiety showed even faster migration ($t_{1/2}$ = 6.0 min). However, in contrast to these results, the significantly longer migration time of prodrug **9**, with the hexenoyl moiety ($t_{1/2}$ = 29.3 min), than **10** ($t_{1/2}$ = 6.0 min) was rather unexpected. The decelerating influence of the double bond on the migration rate seems difficult to explain by the steric or electrical effects of only this bond. Moreover, the faster migration rate of prodrug **6** with the isopropyl group ($t_{1/2}$ = 4.3 min) similar to **10** with the hindered hexanoyl group is surprising. These complicated results suggest the difficulty of precise theoretical prediction of O−N intramolecular acyl migration kinetics among various acyl moieties. However, the important conclusions are that (1) the obtained $t_{1/2}$ values of a series of synthetic taxoids prodrugs (4−60 min) are though to be in not too short time for systemic distribution and not too long for metabolism, although the optimal migration rate should be estimated from in vivo evaluation, and (2) no byproduct formation based on like acyl group

hydrolysis was observed, which is a very important factor for practical use of the prodrugs. Additionally, these prodrugs, *O*-acyl isoforms of taxoids, are not expected to be active, based on previous SAR studies on paclitaxel derivatives.[56,57] Namely, it is known that both *N*-acyl and free 2′-OH groups are required for taxoid activity.[57]

Taxoids can be divided into two groups, 3′-*N*-acyl derivatives such as paclitaxel (**1**) and 3′-*N*-acyloxy derivatives such as docetaxel (**2**). Therefore, our prodrug strategy was also applied to an O−N intramolecular "acyl-like" (acyloxy) migration to docetaxel (**2**). A model prodrug of **2** was designed in which the baccatin III moiety was replaced by a bulky cyclohexyl ester. However, a simple hydrolysis of the Boc group, besides the desired O−N acyloxy migration for the production of the *N*-acyl parent model compound, was observed under physiological conditions.[42] On the other hand, this strategy was recently used for the prodrug design of HIV-1 protease inhibitors and no hydrolysis of carbonate moieties (3-tetrahydrofuranyloxycarbonyl) was observed.[58] Considering this feature, we decided to reconsider this strategy for the synthesis of 3′-*N*-acyloxy taxoid prodrugs, including **2**, a docetaxel prodrug **5**. However, the undesired hydrolysis of the Boc group in PBS (pH 7.4, 37 °C) was replicated in this real taxoid prodrug (Figure 2). We suggested that replacement of the *tert*-butyl group in the primary or secondary aliphatic groups could avoid undesired hydrolysis. Hence, the application of new acyloxy groups in our prodrug strategy has been demonstrated. Three isotaxoids, **11**−**13**, prodrugs of the reported 3′-*N*-carbamate taxoids **18**−**20**,[50−52] based on the O−N acyloxy migration strategy, were synthesized and showed practical water solubility, ranging from 0.8 to 1.7 mg/mL, than isotaxel **4** (Table 1). The prodrugs **11** and **12** showed promising kinetic data. Prodrug **11**, having the bulky isopropyloxy group, due to steric hindrance, clearly showed a slower migration ($t_{1/2}$ = 19.2 min) than prodrug **12** ($t_{1/2}$ = 3.8 min). Prodrug **13** showed the fastest migration in this group ($t_{1/2}$ < 1 min), due to the electron-withdrawing effect of the phenyl group. This suggests that the steric effect of the phenyl ring on acyl migration appears only in cases when the phenyl ring is directly connected to the carbonyl carbon (**4**, $t_{1/2}$ = 15.1 min). As expected, no byproduct formation, such as hydrolysis, was observed. Interestingly, in the previously mentioned prodrugs of HIV-1 protease inhibitors using the hydroxyethylamine core structure,[58] a byproduct of oxazolidinone was produced during the parent drug formation (Figure 5A). The lack of oxazolidinone formation in our case was probably due to the electron-withdrawing influence of a neighboring ester group in the phenylisoserine moiety (Pis), and thus the hydroxyl group of Pis is a good leaving group, as shown in Figure 5B. Thus, herein, we reported the first successful atom economical O−N intramolecular acyloxy migration reaction under aqueous conditions without the formation of side products, as we reported previously (hydrolysis),[42] as did GlaxoSmithKline (oxazolidone formation).[58] In addition, it was shown that there is no major difference in kinetics between the previously reported model compounds with the cyclohexanol moiety instead of baccatin III and real prodrugs.[41−43] This suggests that the time of parent
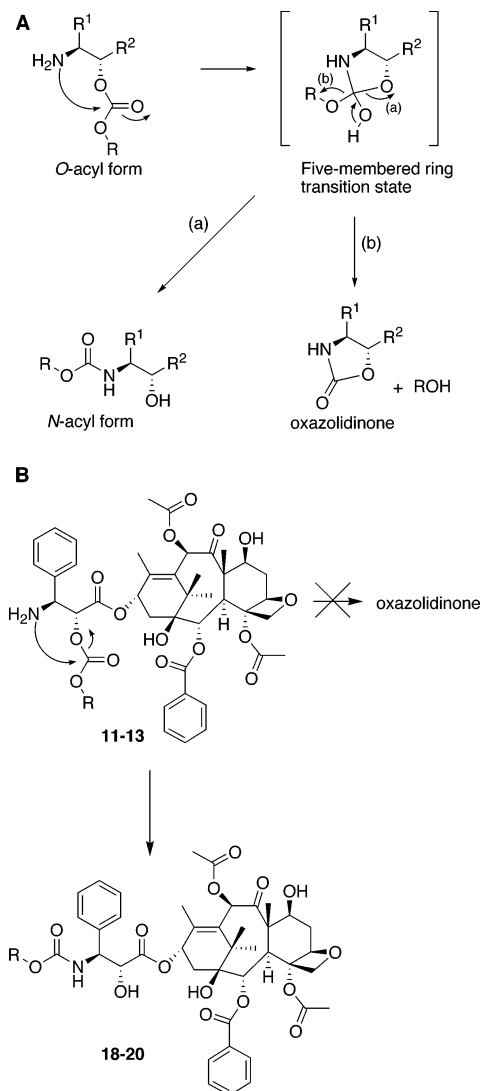
Figure 5. Possible products of amine group nucleophilic attack on carbonate moiety (A) and pure O−N intramolecular acyloxy migration reaction observed in prodrugs **11**−**13** (B).

drug formation does not necessarily depend on the modification of the baccatin III structure, namely the kinetics of migration observed for prodrugs **4**, **6**, **8**−**13** can be expected to replicate without significant differences among all prodrugs that have the same phenylisoserine derivative moiety. Thus, for example, similar kinetic behavior can be expected for other taxoids with isopropyloxycarbonyl and *n*-butyloxycarbonyl groups, which showed very promising anticancer activity,[51,59,60] different from drugs **18** and **19** only in baccatin III moiety. Additionally, differences in migration rates between prodrug **10** ($t_{1/2}$ = 6.0 min) and its isoster prodrug **12** ($t_{1/2}$ = 3.8 min) demonstrated that O−N acyloxy migration is kinetically similar to O−N acyl migration. There is no major difference in $t_{1/2}$ values between these two compounds (Table 1).

In addition, pH-dependent kinetics observed in both types of migration reactions suggest a design for the injectable solution. We demonstrated previously that isotaxel **4** was almost stable for several hours at pH 4.0,[41] which is an acceptable value for injection in practical clinical use.[20] The prodrug of canadensol **6** was also stable at pH 4.0 for at least 1 h.[42] In this study, prodrug **12**, which also had a very small $t_{1/2}$ value at

pH 7.4 (3.8 min), showed only slight migration (<2%) after 1 h of incubation in 0.035% citric acid saline (pH 4.0). Naturally, prodrugs with higher $t_{1/2}$ values under physiological conditions can remain stable for a longer time under these injectable pH 4.0 conditions. Therefore, it should be possible to prepare a stable prodrug solution just before injection. One exception is prodrug **13**, which has a very small $t_{1/2}$ value for parent drug release, and it would be difficult to adopt this pH 4.0 and even lower pH conditions because the precipitate would be formed just after injection in the body without complete distribution.

Interestingly, the water-solubility ratios of prodrugs compared to parent drugs exist over a wide range (10−4000-fold), but the resulting water solubility of the prodrugs is very similar over the range of 0.45−2.3 mg/mL. This suggests that the solubility of prodrugs is related much more to the whole structure of isotaxoids and the ionized amine group than to the R acyl/acyloxy groups, in contrast to the solubility of parent drugs, which is highly dependent on the nature of acyl/acyloxy groups. However, in all cases, the improved water solubility of prodrugs suggests that the utility of the O−N acyl/acyloxy intramolecular migration strategy for water-soluble taxoid produgs can be generalized.

Conclusion

We developed water-soluble prodrugs of taxoids based on the O−N acyl migration reaction. All prodrugs showed a significant increase in water solubility and promising kinetic data. These prodrugs, a 2′-*O*-isoform of taxoids, have no additional functional auxiliaries released during conversion to the parent drugs. This would be an advantage in toxicology and general pharmacology, since the detergent for solubilization, which has some side effects, can be omitted and the potential side effects caused by reported auxiliaries can be avoided. In addition, no costly toxicological and general pharmacological evaluation of released auxiliary units is required. Therefore, we can recommend this strategy as a first choice for water-soluble prodrug design for other drugs if O−N acyl intramolecular migration is possible. Additionally, this study demonstrates for the first time the pure O−N intramolecular acyloxy migration reaction, which proceeds under aqueous conditions with no side product formation. The first successful application of this reaction in a prodrug strategy was demonstrated with the same promising data as for acyl migration. Thus, our prodrug strategy can be extended to carbamate derivatives of taxoids, except for Boc. Moreover, our success with O−N intramolecular acyloxy migration will open the door for the application of this atom economical reaction in water-soluble prodrug design of other drugs which have a carbamate moiety and the neighboring hydroxy group.

Experimental Section

Chemistry. Reagents and solvents were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan), Nacalai Tesque (Kyoto, Japan), and Aldrich Chemical Co. Inc. (Milwaukee, WI) and used without further purification. Column chromatography was performed on Merck 107734 silica gel 60 (70−230 mesh). TLC was performed using Merck silica gel 60F$_{254}$ precoated plates. Analytical HPLC was performed using a C18 reverse phase column (4.6 × 150 mm; YMC Pack ODS

AM302) with a binary solvent system: a linear gradient of CH_3CN in 0.1% aqueous TFA at a flow rate of 0.9 mL min^{-1}, detected at 230 nm. Preparative HPLC was carried out on a C18 reverse phase column (20 × 250 mm; YMC Pack ODS SH343−5) with a binary solvent system: a linear gradient of CH_3CN in 12 mM aqueous HCl at a flow rate of 5.0 mL min^{-1}, detected at 230 nm. Solvents used for HPLC were of HPLC grade. All other chemicals were of analytical grade or better. ^{1}H NMR spectra were obtained using a 400 MHz Varian UNITY INOVA 400NB spectrometer with TMS as an internal standard at 25 °C. FAB-MS was performed on a JEOL JMS−SX102A spectrometer equipped with the JMA-DA7000 data system. ESI-MASS was performed on a Finnigan SSQ-7000 spectrometer.

2′-O-(1-Cyclohexenecarbonyl)-3′-N-debenzoylpaclitaxel Hydrochloride (8). A solution of **7** (21 mg, 0.019 mmol), 1-cyclohexenecarboxylic acid (2.9 mg, 0.023 mmol), DMAP (0.7 mg, 0.006 mmol), and EDC·HCl (4.4 mg, 0.023 mmol) in dry CH_2Cl_2 (1 mL) was stirred under an argon atmosphere at room temperature for 5 h. Then the reaction mixture was diluted with AcOEt and successively washed with 10% citric acid three times and water and saturated $NaHCO_3$ twice. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The resulting oil was dissolved in a mixture (2 mL) of MeOH:AcOH (1:1), zinc dust was added in three portions (a total of 38 mg, 0.57 mmol) within 30 min, and the reaction mixture was stirred for an additional 15 min at room temperature. The reaction mixture was directly applied to preparative HPLC, which was eluted with a linear gradient of 10−60% CH_3CN in 12 mM aqueous HCl over 100 min at a flow rate of 5 mL/min. The desired fraction was collected and lyophilized to give a white powder of **8** as an HCl salt (12.9 mg, 14.4 μmol, 76%). ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 8.08−8.06 (m, 2 H, CH), 7.75−7.71 (m, 1 H, CH), 7.65−7.49 (m, 6 H, CH), 7.39−7.35 (m, 2 H, CH), 6.41 (s, 1 H, CH), 5.93 (bt, ^3J(H,H) = 9.3 Hz, 1 H, CH), 5.60 (d, ^3J(H,H) = 7.1 Hz, 1 H, CH), 5.40 (d, ^3J(H,H) = 9.0 Hz, 1H, CH), 4.97 (bd, ^3J(H,H) = 7.7 Hz, 1H, CH), 4.85 (d, 1 H, CH, partially overlapping with signal from CD$_3$OD), 4.30 (dd, ^3J(H,H) = 6.6, 11.0 Hz, 1 H, CH), 4.17, 4.14 (2d, ^2J(H,H) = 8.2 Hz, 2 H, CH$_2$), 3.72 (d, ^3J(H,H) = 7.1 Hz, 1 H, CH), 2.48−2.41 (m, 1 H, CH$_2$), 2.34−2.31 (m, 4H, CH$_2$), 2.27 (s, 3 H, CH$_3$), 2.16 (s, 3 H, CH$_3$), 1.92 (dd, ^3J(H,H) = 9.3 Hz, ^2J(H,H) = 15.4 Hz, 1 H, CH$_2$), 1.85 (d, ^4J(H,H) = 1.1 Hz, 3 H, CH$_3$), 1.82−1.68 (m, 5 H, CH$_2$), 1.62 (s, 3 H, CH$_3$), 1.47 (dd, ^3J(H,H) = 8.8 Hz, ^2J(H,H) = 15.5 Hz, 1 H, CH$_2$), 1.12 (s, 3 H, CH$_3$), 1.10 (s, 3 H, CH$_3$). HRMS (FAB+): calcd for $C_{47}H_{56}NO_{14}$ [M$^+$ + H] 858.3701, found 858.3705. Anal. ($C_{47}H_{56}ClNO_{14}$·3.5H$_2$O) C, N, H: calcd, 6.63; found, 6.05.

2′-O-(*trans*-2-Hexenoyl)-3′-N-debenzoylpaclitaxel Hydrochloride (9). The above procedure with *trans*-2-hexenoic acid afforded **9** (47%): ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 8.08−8.05 (m, 2 H, CH), 7.75−7.71 (m, 1 H, CH), 7.65−7.48 (m, 6 H, CH), 7.37−7.30 (m, 1 H, CH), 7.26(dt, ^3J(H,H) = 7.0, 15.6 Hz, 1H, CH), 6.41 (s, 1 H, CH), 6.09 (dt, ^4J(H,H) = 1.5 Hz, ^3J(H,H) = 15.6 Hz, 1 H, CH), 5.93 (bt, ^3J(H,H) = 8.9 Hz, 1 H, CH), 5.59 (d, ^3J(H,H) = 7.3 Hz, 1 H, CH), 5.33 (d, ^3J(H,H) = 9.0 Hz, 1H, CH), 4.96 (bd, ^3J(H,H) = 9.5 Hz, 1H, CH), 4.80 (d, ^3J(H,H) = 8.6 Hz, 1H, CH), 4.30 (dd, ^3J(H,H) = 6.5, 10.9 Hz, 1 H, CH), 4.16, 4.13 (2d, ^2J(H,H) = 8.9 Hz, 2 H, CH$_2$), 3.72 (d, ^3J(H,H) = 7.1 Hz, 1 H, CH), 2.48−2.40 (m, 1 H, CH$_2$), 2.34−2.28 (m, 2H, CH$_2$), 2.26 (s, 3 H, CH$_3$), 2.15 (s, 3 H, CH$_3$), 1.90 (dd, ^3J(H,H) = 6.0 Hz, ^2J(H,H) = 15.6 Hz, 1 H, CH$_2$), 1.85 (d, ^4J(H,H) = 1.1 Hz, 3 H, CH$_3$), 1.82−1.74 (m, 1 H, CH$_2$), 1.62 (s, 3 H, CH$_3$), 1.62−1.53 (m, 2 H, CH$_2$), 1.44 (dd, ^3J(H,H) = 8.7 Hz, ^2J(H,H) = 15.5 Hz, 1 H, CH$_2$), 1.12 (s, 3 H, CH$_3$), 1.09 (s, 3 H, CH$_3$), 1.00 (t, ^3J(H,H) = 7.4 Hz, 3 H, CH$_3$). HRMS (FAB+): calcd for $C_{46}H_{56}NO_{14}$ [M$^+$ + H] 846.3701, found 846.3698. Anal. ($C_{46}H_{56}ClNO_{14}$·2.5H$_2$O) C, H, N.

2′-O-Hexanoyl-3′-N-debenzoylpaclitaxel Hydrochloride (10). The above procedure with hexanoic acid afforded **10** (62%): ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 8.08−8.06 (m, 2 H, CH), 7.76−7.71 (m, 1 H, CH), 7.66−7.48 (m, 6 H, CH), 7.37−7.33 (m, 1 H, CH), 6.41 (s, 1 H, CH), 5.93 (bt, ^3J(H,H) = 8.5

Hz, 1 H, CH), 5.59 (d, ^3J(H,H) = 7.3 Hz, 1 H, CH), 5.31 (d, ^3J(H,H) = 9.0 Hz, 1H, CH), 4.97 (dd, ^3J(H,H) = 2.0, 9.7 Hz, 1H, CH), 4.78 (bd, ^3J(H,H) = 8.4 Hz, 1H, CH), 4.30 (dd, ^3J(H,H) = 6.5, 11.1 Hz, 1 H, CH), 4.16, 4.14 (2d, ^2J(H,H) = 8.4 Hz, 2 H, CH$_2$), 3.72 (d, ^3J(H,H) = 7.2 Hz, 1 H, CH), 2.57 (t, ^3J(H,H) = 7.2 Hz, 1 H, CH), 2.48−2.40 (m, 1 H, CH$_2$), 2.26 (s, 3 H, CH$_3$), 2.16 (s, 3 H, CH$_3$), 1.93−1.88 (m, 1H, CH$_2$), 1.86 (d, ^4J(H,H) = 1.1 Hz, 3 H, CH$_3$), 1.81−1.74 (m, 1 H, CH$_2$), 1.73−1.66 (m, 2 H, CH$_2$), 1.62 (s, 3 H, CH$_3$), 1.44 (dd, ^3J(H,H) = 9.0 Hz, ^2J(H,H) = 15.4 Hz, 1 H, CH$_2$), 1.39−1.31 (m, 4 H, CH$_2$), 1.12 (s, 3 H, CH$_3$), 1.10 (s, 3 H, CH$_3$), 0.93 (t, ^3J(H,H) = 7.0 Hz, 3 H, CH). HRMS (FAB+): calcd for $C_{46}H_{58}NO_{14}$ [M$^+$ + H] 848.3857, found 848.3854. Anal. ($C_{46}H_{58}ClNO_{14}$·2.5H$_2$O) C, H, N.

2′-O-Isopropyloxycarbonyl-3′-N-debenzoylpaclitaxel Hydrochloride (11). Isopropyloxycarbonyl chloride (5 μL, 43.6 μmol) was added to the stirring solution of **7** (24.0 mg, 21.8 μmol) in dry CH_2Cl_2 (1 mL) and dry pyridine (1 mL), and the mixture was stirred under an argon atmosphere at room temperature for 30 min. The reaction mixture was diluted with AcOEt and then washed with water, 1 N hydrochloric acid twice, and then brine. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The resulting oil was dissolved in a mixture (2 mL) of MeOH:AcOH (1:1), zinc dust was added in three portions (a total of 42 mg, 0.65 mmol) within 30 min, and the reaction mixture was stirred for an additional 15 min at room temperature. The reaction mixture was directly applied to preparative HPLC, which was eluted with a linear gradient of 30−70% CH_3CN in 12 mM aqueous HCl over 40 min at a flow rate of 5 mL/min. The desired fraction was collected and lyophilized to give a white powder of **11** as an HCl salt (12.3 mg, 14.1 μmol, 65%). ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 8.08−8.06 (m, 2 H, CH), 7.74−7.70 (m, 1 H, CH), 7.64−7.50 (m, 6 H, CH), 7.40−7.36 (m, 1 H, CH), 6.41 (s, 1 H, CH), 5.99−5.95 (m, 1 H, CH), 5.60 (d, ^3J(H,H) = 7.3 Hz, 1 H, CH), 5.34 (d, ^3J(H,H) = 8.8 Hz, 1H, CH), 4.99−4.80 (m, 3 H, CH, partially overlapping with signal from CD$_3$OD), 4.30 (dd, ^3J(H,H) = 6.6, 11.0 Hz, 1 H, CH), 4.17, 4.16 (2d, ^2J(H,H) = 8.4 Hz, 2 H, CH$_2$), 3.72 (d, ^3J(H,H) = 7.1 Hz, 1 H, CH), 2.49−2.41 (m, 1 H, CH$_2$), 2.29 (s, 3 H, CH$_3$), 2.16 (s, 3 H, CH$_3$), 1.94 (dd, ^3J(H,H) = 9.6 Hz, ^2J(H,H) = 15.3 Hz, 1 H, CH$_2$), 1.86 (d, ^4J(H,H) = 1.3 Hz, 3 H, CH$_3$), 1.82−1.75 (m, 1 H, CH$_2$), 1.63 (s, 3 H, CH$_3$), 1.53 (dd, ^3J(H,H) = 8.9 Hz, ^2J(H,H) = 15.3 Hz, 1 H, CH$_2$), 1.36 (d, ^3J(H,H) = 6.2 Hz, 3 H, CH$_3$), 1.35 (d, ^3J(H,H) = 6.2 Hz, 3 H, CH$_3$), 1.13 (s, 3 H, CH$_3$), 1.11 (s, 3 H, CH$_3$). HRMS (FAB+): calcd for $C_{44}H_{54}NO_{15}$ [M$^+$ + H] 836.3493, found 836.3502. Anal. ($C_{44}H_{54}ClNO_{15}$·4.5H$_2$O) C, H, N.

2′-O-n-Butyloxycarbonyl-3′-N-debenzoylpaclitaxel Hydrochloride (12). The above procedure with *n*-butyloxycarbonyl chloride (6 equiv) afforded **12** (71%): ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 8.08−8.06 (m, 2 H, CH), 7.75−7.70 (m, 1 H, CH), 7.64−7.49 (m, 6 H, CH), 7.39−7.36 (m, 1 H, CH), 6.42 (s, 1 H, CH), 5.96 (bt, ^3J(H,H) = 9.5 Hz, 1 H, CH), 5.60 (d, ^3J(H,H) = 7.3 Hz, 1 H, CH), 5.31 (d, ^3J(H,H) = 8.8 Hz, 1H, CH), 4.96 (dd, ^3J(H,H) = 1.8, 9.7 Hz, 1H, CH), 4.82 (d, 1 H, CH, partially overlapping with signal from CD$_3$OD), 4.34−4.22 (m, 3 H, CH, CH$_2$), 4.17, 4.14 (2d, ^2J(H,H) = 8.4 Hz, 2 H, CH$_2$), 3.73 (d, ^3J(H,H) = 7.3 Hz, 1 H, CH), 2.48−2.41 (m, 1 H, CH$_2$), 2.27 (s, 3 H, CH$_3$), 2.16 (s, 3 H, CH$_3$), 1.92 (dd, ^3J(H,H) = 9.4 Hz, ^2J(H,H) = 15.5 Hz, 1 H, CH$_2$), 1.87 (d, ^4J(H,H) = 1.1 Hz, 3 H, CH$_3$), 1.82−1.75 (m, 1 H, CH$_2$), 1.75−1.67 (m, 2H, CH$_2$), 1.63 (s, 3 H, CH$_3$), 1.53−1.40 (m, 3 H, CH, CH$_2$), 1.13 (s, 3 H, CH$_3$), 1.11 (s, 3 H, CH$_3$), 0.97 (t, ^3J(H,H) = 7.3 Hz, 3 H, CH$_3$). HRMS (FAB+): calcd for $C_{45}H_{56}NO_{15}$ [M$^+$ + H] 850.3650, found 850.3655. Anal. ($C_{45}H_{56}ClNO_{15}$·3.5H$_2$O) C, H, N.

2′-O-Benzyloxycarbonyl-3′-N-debenzoylpaclitaxel Hydrochloride (13). The above procedure with benzyloxycarbonyl chloride (20 equiv) afforded **13** (53%): ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 8.09−8.05 (m, 2 H, CH), 7.74−7.70 (m, 1 H, CH), 7.63−7.59 (m, 2 H, CH), 7.57−7.34 (m, 10 H, CH), 6.41 (s, 1 H, CH), 5.94 (dt, ^4J(H,H) = 1.3 Hz, ^3J(H,H) = 9.2 Hz, 1 H, CH), 5.60 (d, ^3J(H,H) = 7.3 Hz, 1 H, CH), 5.35 (d, ^3J(H,H) = 8.6 Hz, 1H, CH), 5.31, 5.27 (2d, ^2J(H,H) = 12.0 Hz, 2 H,

CH$_2$), 4.97 (dd, ^3J(H,H) = 1.9, 9.6 Hz, 1H, CH), 4.81 (d, ^3J(H,H) = 8.8 Hz, 1 H, CH), 4.30 (dd, ^3J(H,H) = 6.6, 11.0 Hz, 1 H, CH), 4.17, 4.14 (2d, ^2J(H,H) = 9.0 Hz, 2 H, CH$_2$), 3.72 (d, ^3J(H,H) = 7.3 Hz, 1 H, CH), 2.50–2.42 (m, 1 H, CH$_2$), 2.27 (s, 3 H, CH$_3$), 2.17 (s, 3 H, CH$_3$), 1.91 (dd, ^3J(H,H) = 9.3 Hz, ^2J(H,H) = 15.4 Hz, 1 H, CH$_2$), 1.81 (d, ^4J(H,H) = 1.3 Hz, 3 H, CH$_3$), 1.82–1.76 (m, 1 H, CH$_2$), 1.63 (s, 3 H, CH$_3$), 1.49 (dd, ^3J(H,H) = 9.0 Hz, ^2J(H,H) = 15.4 Hz, 1 H, CH$_2$), 1.13 (s, 3 H, CH$_3$), 1.10 (s, 3 H, CH$_3$). HRMS (FAB+): calcd for C$_{48}$H$_{54}$NO$_{15}$ [M$^+$ + H] 884.3493, found 884.3486. Anal. (C$_{48}$H$_{55}$ClNO$_{15}$·1.5H$_2$O) C, H, N.

3′-*N*-Debenzoylpaclitaxel (14). Zinc dust was added in three portions (a total of 0.71 g, 10.9 mmol) to the stirring solution (9 mL) of **7** (400 mg, 0.363 mmol) in AcOEt:AcOH (8:1) within 5 h and the reaction mixture was stirred for an additional 3 h at room temperature. The zinc dust was filtered, and the resulting mixture was diluted with AcOEt and washed with saturated NaHCO$_3$ three times. The organic layer was dried over MgSO$_4$ and the solvent was removed under reduced pressure. The resulting oil was purified by silica gel column chromatography (MeOH:CHCl$_3$ 10:1) to yield a white powder **14** after lyophilization (164 mg, 0.219 mmol, 60%). ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 8.07–8.05 (m, 2 H, CH), 7.67–7.63 (m, 1 H, CH), 7.54–7.50 (m, 2H, CH), 7.38–7.36 (m, 4 H, CH), 7.28–7.21 (m, 1 H, CH), 6.27 (s, 1 H, CH), 6.14 (dt, ^4J(H,H) = 1.3 Hz, ^3J(H,H) = 8.9 Hz, 1 H, CH), 5.63 (d, ^3J(H,H) = 7.1 Hz, 1 H, CH), 4.93 (dd, ^3J(H,H) = 2.0, 9.5 Hz, 1H, CH), 4.40 (dd, ^3J(H,H) = 6.7, 10.9 Hz, 1 H, CH), 4.31–4.26 (m, 3H, CH$_2$, CH), 4.14 (d, ^3J(H,H) = 8.2 Hz, 1H, CH), 3.75 (d, ^3J(H,H) = 7.0 Hz, 1 H, CH), 2.57–2.49 (m, 1 H, CH$_2$), 2.24 (s, 3 H, CH$_3$), 2.13 (s, 3 H, CH$_3$), 2.19–1.82 (m, 3 H, CH$_2$), 1.87 (d, ^4J(H,H) = 1.3 Hz, 3 H, CH$_3$), 1.65 (s, 3 H, CH$_3$), 1.24 (s, 3 H, CH$_3$), 1.13 (s, 3 H, CH$_3$). HRMS (FAB+): calcd for C$_{40}$H$_{48}$NO$_{13}$ [M$^+$ + H] 750.3126, found 750.3120. Anal. (C$_{40}$H$_{47}$NO$_{13}$·H$_2$O) C, H, N.

3′-*N*-(1-Cyclohexenylcarbonyl)-3′-*N*-debenzoylpaclitaxel (15). A solution of **14** (10.4 mg, 0.014 mmol), 1-cyclohexenecarboxylic acid (2.1 mg, 0.017 mmol), HOBt (2.5 mg, 0.017 mmol), and EDC·HCl (3.2 mg, 0.017 mmol) in DMF (1 mL) was stirred at room temperature for 3 h. Then DMF was removed under reduced pressure and the reaction mixture was dissolved with AcOEt. After washing with 10% citric acid three times and water and saturated NaHCO$_3$ twice, the organic layer was dried over MgSO$_4$ and the solvent was removed under reduced pressure. The crude product was applied to preparative HPLC, which was eluted with a linear gradient of 40%–80% CH$_3$CN in 12 mM aqueous HCl over 80 min at a flow rate of 5 mL/min. The desired fraction was collected and lyophilized to give a white powder of **15** (9.2 mg, 10.7 μmol, 77%). ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 8.13–8.10 (m, 2 H, CH), 7.64–7.60 (m, 1 H, CH), 7.53–7.49 (m, 2H, CH), 7.41–7.38 (m, 4 H, CH), 7.37–7.31 (m, 1 H, CH), 6.65–6.63 (m, 1H, CH), 6.47 (d, ^3J(H,H) = 8.8 Hz, 1 H, NH) 6.27 (s, 1 H, CH), 6.20 (bt, ^3J(H,H) = 8.2 Hz, 1 H, CH), 5.67 (d, ^3J(H,H) = 7.0 Hz, 1 H, CH), 5.61 (dd, ^3J(H,H) = 2.8, 8.8 Hz, 1H, CH), 4.95–4.93 (m, 1H, CH), 4.71 (dd, ^3J(H,H) = 2.8, 5.4 Hz, 1 H, CH), 4.43–4.37 (m, 1 H, CH), 4.30, 4.19 (2d, ^2J(H,H) = 8.3 Hz, 2 H, CH$_2$), 3.78 (d, ^3J(H,H) = 7.0 Hz, 1 H, CH), 3.57 (d, ^3J(H,H) = 5.5 Hz, 1 H, OH) 2.59–2.51 (m, 1 H, CH$_2$), 2.47 (d, ^3J(H,H) = 4.0 Hz, 1 H, OH), 2.35 (s, 3 H, CH$_3$), 2.29 (dd, ^3J(H,H) = 5.2 Hz, ^2J(H,H) = 8.9 Hz, 1 H, CH$_2$), 2.25 (s, 3 H, CH$_3$), 2.22–2.11 (m, 4H, CH$_2$), 1.91–1.85 (m, 1 H, CH$_2$), 1.85 (s, 1H, OH), 1.79 (d, ^4J(H,H) = 1.1 Hz, 3 H, CH$_3$), 1.68 (s, 3 H, CH$_3$), 1.66–1.63 (m, 5 H, CH$_2$, partially overlapping with signal from H$_2$O), 1.26 (s, 3 H, CH$_3$), 1.15 (s, 3 H, CH$_3$). HRMS (FAB+): calcd for C$_{47}$H$_{56}$NO$_{14}$ [M$^+$ + H] 858.3701, found 858.3696.

3′-*N*-(*trans*-2-Hexenoyl)-3′-*N*-debenzoylpaclitaxel (16). The above procedure with *trans*-2-hexenoic acid afforded **16** (56%). ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 8.14–8.12 (m, 2 H, CH), 7.64–7.60 (m, 1 H, CH), 7.55–7.31 (m, 7 H, CH), 6.79 (dt, ^3J(H,H) = 7.0, 15.4 Hz, 1H, CH), 6.27 (s, 1H, CH), 6.25–6.17 (m, 2 H, CH, NH), 5.79 (bd, ^3J(H,H) = 15.6 Hz, 1 H, CH), 5.67 (d, ^3J(H,H) = 7.0 Hz, 1H, CH), 5.62 (dd, ^3J(H,H) = 2.6, 8.6 Hz, 1H, CH), 4.94 (dd, ^3J(H,H) = 2.0, 7.3 Hz, 1 H, CH), 4.71 (bs, 1 H, CH), 4.43–4.38 (m, 1H, CH), 4.30, 4.19 (2d,

^2J(H,H) = 8.8 Hz, 2 H, CH$_2$), 3.79 (d, ^3J(H,H) = 7.0 Hz, 1 H, CH), 3.58 (d, ^3J(H,H) = 5.3 Hz, 1 H, OH) 2.59–2.51 (m, 1 H, CH$_2$), 2.46 (bs, 1 H, OH), 2.37 (s, 3 H, CH$_3$), 2.33–2.27 (m, 1 H, CH$_2$), 2.25 (s, 3 H, CH$_3$), 2.13–2.08 (m, 2H, CH$_2$), 1.91–1.84 (m, 1 H, CH$_2$), 1.81 (d, ^4J(H,H) = 1.1 Hz, 3 H, CH$_3$), 1.68 (s, 3 H, CH$_3$), 1.64–1.59 (m, 1 H, CH$_2$, partially overlapping with signal from H$_2$O), 1.44–1.35 (m, 2H, CH$_2$), 1.26 (s, 3 H, CH$_3$), 1.15 (s, 3 H, CH$_3$), 0.87 (t, ^3J(H,H) = 7.3 Hz, 3 H, CH$_3$). HRMS (FAB+): calcd for C$_{46}$H$_{56}$NO$_{14}$ [M$^+$ + H] 846.3701, found 846.3707.

3′-*N*-(Hexanoyl)-3′-*N*-debenzoylpaclitaxel (17). The above procedure with hexanoic acid afforded **17** (56%). ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 8.12–8.10 (m, 2 H, CH), 7.64–7.60 (m, 1 H, CH), 7.53–7.32 (m, 7 H, CH), 6.28 (s, 1 H, CH), 6.23–6.20 (m, 2 H, CH, NH), 5.68 (d, ^3J(H,H) = 7.1 Hz, 1 H, CH), 5.58 (dd, ^3J(H,H) = 2.3, 8.9 Hz, 1H, CH), 4.94 (dd, ^3J(H,H) = 1.8, 9.5 Hz, 1 H, CH), 4.68 (bs, 1 H, CH), 4.40 (dd, ^3J(H,H) = 6.4, 11.0 Hz, 1 H, CH), 4.30, 4.19 (2d, ^2J(H,H) = 8.3 Hz, 2 H, CH$_2$), 3.79 (d, ^3J(H,H) = 7.0 Hz, 1 H, CH), 3.47 (bs, 1 H, OH) 2.58–2.50 (m, 1 H, CH$_2$), 2.47 (bs, 1 H, OH), 2.34 (s, 3 H, CH$_3$), 2.32–2.25 (m, 1 H, CH$_2$), 2.25 (s, 3 H, CH$_3$), 2.19 (t, ^3J(H,H) = 7.6 Hz, 2H, CH$_2$), 1.93–1.84 (m, 1 H, CH$_2$), 1.82 (d, ^4J(H,H) = 1.13 Hz, 3 H, CH$_3$), 1.68 (s, 3 H, CH$_3$), 1.65–1.53 (m, 3 H, CH, CH$_2$), 1.28–1.22 (m, 4H, CH$_2$), 1.27 (s, 3 H, CH$_3$), 1.15 (s, 3 H, CH$_3$), 0.83 (t, ^3J(H,H) = 7.0 Hz, 3 H, CH$_3$). HRMS (FAB+): calcd for C$_{46}$H$_{58}$NO$_{14}$ [M$^+$ + H] 848.3857, found 848.3864.

3′-*N*-Debenzoyl-3′-*N*-isopropyloxycarbonylpaclitaxel (18). 3′-*N*-Debenzoylpaclitaxel **14** (10.0 mg, 13.3 μmol) was dissolved in CH$_2$Cl$_2$ (0.5 mL) and a saturated solution of NaHCO$_3$ (0.5 mL) was added. Then isopropyloxycarbonyl chloride (1.7 μL, 17 μmol) was added with vigorous stirring. After 1 h, the next portion of isopropyloxycarbonyl chloride (1.7 μL, 17 μmol) was added, and after an hour, the reaction mixture was diluted with EtOAc and washed with water. The organic layer was dried over MgSO$_4$ and the solvent was removed under reduced pressure. The crude product was applied to preparative HPLC, which was eluted with a linear gradient of 30%–70% CH$_3$CN in 12 mM aqueous HCl over 80 min at a flow rate of 5 mL/min. The desired fraction was collected and lyophilized to give a white powder of **18** (9.7 mg, 11.6 μmol, 87%). ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 8.10 (d, ^3J(H,H) = 7.3 Hz, 2 H, CH), 7.68–7.64 (m, 1 H, CH), 7.58–7.54 (m, 2 H, CH), 7.39 (d, ^3J(H,H) = 4.6 Hz, 4 H, CH), 7.28–7.24 (m, 1 H), 6.46 (s, 1 H, CH), 6.15 (t, ^3J(H,H) = 10.0 Hz, 1 H, CH), 5.64 (d, ^3J(H,H) = 7.3 Hz, 1 H, CH), 5.15 (bs, 1H, CH), 5.00–4.97 (m, 1 H, CH), 4.81–4.75 (m, 1 H, CH), 4.52 (d, ^3J(H,H) = 4.4 Hz, 1 H, CH), 4.32 (dd, ^3J(H,H) = 6.6, 11.0 Hz, 1 H, CH), 4.18 (bs, 2 H, CH$_2$), 3.82 (d, ^3J(H,H) = 7.1 Hz, 1 H, CH), 2.50–2.42 (m, 1 H, CH$_2$), 2.33 (s, 3 H, CH$_3$), 2.28–2.19 (m, 1 H, CH$_2$), 2.17 (s, 3 H, CH$_3$), 2.00–1.95 (m, 1 H, CH$_2$), 1.92 (s, 3 H, CH$_3$), 1.84–1.77 (m, 1 H, CH$_2$), 1.66 (s, 3 H, CH$_3$), 1.19 (d, ^3J(H,H) = 6.4 Hz, 6 H, CH$_3$), 1.17 (s, 3 H, CH$_3$), 1.16 (s, 3 H, CH$_3$). HRMS (FAB+): calcd for C$_{44}$H$_{54}$NO$_{15}$ [M$^+$ + H] 836.3493, found 836.3502.

3′-*N*-*n*-Butyloxycarbonyl-3′-*N*-debenzoylpaclitaxel (19). The above procedure with *n*-butyloxycarbonyl chloride afforded **19** (93%). ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 8.10 (d, ^3J(H,H) = 7.5 Hz, 2 H, CH) 7.68–7.64 (m, 1 H, CH), 7.58–7.54 (m, 2 H, CH), 7.39 (d, ^3J(H,H) = 4.6 Hz, 4 H, CH), 7.28–7.24 (m, 1 H), 6.46 (s, 1 H, CH), 6.16 (bt, ^3J(H,H) = 8.5 Hz, 1 H, CH), 5.64 (d, ^3J(H,H) = 7.3 Hz, 1 H, CH), 5.17 (d, ^3J(H,H) = 4.6 Hz, 1 H, CH), 4.99 (dd, ^3J(H,H) = 1.8, 9.5 Hz, 1H, CH), 4.54 (d, ^3J(H,H) = 4.0 Hz, 1H, CH), 4.33 (dd, ^3J(H,H) = 6.6, 11.0 Hz, 1 H, CH), 4.19, 4.17 (2d, ^2J(H,H) = 8.8 Hz, 2 H, CH$_2$), 4.05–3.94 (m, 2 H, CH, CH$_2$), 3.82 (d, ^3J(H,H) = 7.3 Hz, 1 H, CH), 2.50–2.42 (m, 1 H, CH$_2$), 2.34 (s, 3 H, CH$_3$), 2.29–2.18 (m, 1 H, CH$_2$), 2.17 (s, 3 H, CH$_3$), 2.03–1.97 (m, 1H, CH$_2$), 1.92 (s, 3H, CH$_3$), 1.84–1.77 (m, 1 H, CH$_2$), 1.66 (s, 3 H, CH$_3$), 1.61–1.55 (m, 2 H, CH$_2$), 1.38–1.29 (m, 2H, CH$_2$), 1.18 (s, 3 H, CH$_3$), 1.16 (s, 3 H, CH$_3$), 0.90 (t, ^3J(H,H) = 7.2 Hz, 3 H, CH$_3$). HRMS (FAB+): calcd for C$_{45}$H$_{55}$NO$_{15}$ [M$^+$ + H] 872.3469, found 884.3477.

3′-*N*-Benzyloxycarbonyl-3′-*N*-debenzoylpaclitaxel (20). The above procedure with benzyloxycarbonyl chloride afforded

20 (95%). ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 8.10 (d, ^3J(H,H) = 9.5 Hz, 2 H, CH), 7.67–7.63 (m, 1 H, CH), 7.57–7.52 (m, 2 H, CH), 7.45–7.24 (m, 10 H), 6.44 (s, 1 H, CH), 6.15 (t, ^3J(H,H) = 8.9 Hz, 1 H, CH), 5.64 (d, ^3J(H,H) = 7.1 Hz, 1 H, CH), 5.20 (d, ^3J(H,H) = 4.6 Hz, 1H, CH), 5.08, 5.05 (2d, ^2J(H,H) = 12.6 Hz, 2 H, CH$_2$), 4.98 (dd, ^3J(H,H) = 1.9, 9.6 Hz, 1H, CH), 4.55 (d, ^3J(H,H) = 4.7 Hz, 1 H, CH), 4.30 (dd, ^3J(H,H) = 6.6, 11.0 Hz, 1 H, CH), 4.19, 4.17 (2d, ^2J(H,H) = 9.2 Hz, 2 H, CH$_2$), 3.81 (d, ^3J(H,H) = 7.1 Hz, 1 H, CH), 2.50–2.42 (m, 1 H, CH$_2$), 2.34 (s, 3 H, CH$_3$), 2.34–2.18 (m, 1 H, CH$_2$), 2.18 (s, 3 H, CH$_3$), 1.99–1.90 (m, 1 H, CH$_2$), 1.90 (s, 3 H, CH$_3$), 1.83–1.76 (m, 1 H, CH$_2$), 1.66 (s, 3 H, CH$_3$), 1.16 (s, 3 H, CH$_3$), 1.15 (s, 3 H, CH$_3$). HRMS (FAB+): calcd for C$_{48}$H$_{54}$NO$_{15}$ [M$^+$ + H] 884.3493, found 884.3498.

(4S,5R)-5-Methoxycarbonyl-2-(4-methoxyphenyl)-4-phenyl-3-benzoxycarbonyl-1,3-oxazolidine (22). (2R,3S)-3-Phenylisoserine hydrochloride **21** (1.00 g, 4.59 mmol) was dissolved in anhydrous MeOH (25 mL), and SOCl$_2$ (0.50 mL, 6.89 mmol) was added dropwise at 0 °C under an argon atmosphere. The reaction mixture was stirred overnight at room temperature and then quenched with NaHCO$_3$, evaporated under reduced pressure, and diluted with H$_2$O. The water phase was extracted with AcOEt three times, the combined organic solution was dried over MgSO$_4$, and the solvent was removed under reduced pressure. Benzyloxycarbonyl chloride (1.3 mL, 9.18 mmol) was added dropwise at 0 °C to the resulting oil dissolved in CH$_2$Cl$_2$ (30 mL) and saturated NaHCO$_3$ (30 mL). The whole mixture was stirred vigorously at room temperature for 3 h. The desired compound was extracted with AcOEt. The organic solution was dried over MgSO$_4$ and the solvent was evaporated under reduced pressure to yield 1.42 g of white solid. A portion of the resulting solid (360 mg) was dissolved in anhydrous toluene (30 mL) and pyridinium p-toluenesulfonate (PPTS) (2.7 mg, 0.011 mmol) was added. Toluene distillation was then started and 4-methoxybenzaldehyde dimethyl acetal (0.22 mL, 1.31 mmol) was added dropwise under an argon atmosphere. During 40 min of azeotropic distillation, approximately half of the solvent was removed and then the reaction mixture was allowed to cool to ambient temperature and diluted with Et$_2$O. The organic layer was washed with water, saturated NaHCO$_3$, water, and brine and dried over MgSO$_4$, and the solvent was removed under reduced pressure. The resulting oil was purified by silica gel column chromatography (AcOEt:hexane 1:4) to yield a product **22** (469 mg, 1.05 mmol, 96%) as a mixture of diastereomers in the ratio 17:3 (detected by ^{1}H NMR and HPLC analysis). ^{1}H NMR (CDCl$_3$, 400 MHz), major diastereomer: δ = 7.42–7.32 (m, 7 H, CH), 7.22–7.06 (m, 3 H, CH), 6.92–6.84 (m, 2 H, CH), 6.75 (d, ^3J(H,H) = 7.3 Hz, 2 H, CH), 6.42 (bs, 1 H, CH), 5.50 (bs, 1 H, CH), 4.92, 4.77 (2d, ^2J(H,H) = 12.3 Hz, 2 H, CH$_2$), 4.59 (d, ^3J(H,H) = 4.0 Hz, 1 H, CH), 3.82 (s, 3 H, CH$_3$), 3.59 (s, 3 H, CH$_3$); minor diastereomer: δ = 7.42–7.32 (m, 7 H, CH), 7.22–7.06 (m, 3 H, CH), 6.92–6.84 (m, 2 H, CH), 6.75 (d, ^3J(H,H) = 7.3 Hz, 2 H, CH), 6.50 (bs, 1 H, CH), 5.34 (bs, 1 H, CH), 5.09, 5.05 (2d, ^2J(H,H) = 12.4 Hz, 2 H, CH$_2$), 4.88 (d, ^3J(H,H) = 3.5 Hz, 1 H, CH), 3.83 (s, 3 H, CH$_3$), 3.59 (s, 3 H, CH$_3$). HRMS (FAB+): calcd for C$_{26}$H$_{26}$NO$_6$ [M$^+$ + H] 448.1760, found 448.1763.

13-(3′-N-Benzyloxycarbonylphenylisoserine)-10-deacetyl-7,10-dibenzyloxycarbonylbaccatin III (24). A solution of KOH (66 mg, 1.18 mmol) in water (10 mL) was added slowly at room temperature to a stirring solution of **22** (440 mg, 0.983 mmol) in MeOH (30 mL). The reaction mixture was stirred for 2 h and then MeOH was evaporated under reduced pressure. The residual mixture was diluted with water, washed with Et$_2$O, acidified with 1 N HCl, and extracted with AcOEt. This organic phase was dried over MgSO$_4$ and evaporated under reduced pressure. The resulting oil, 10-deacetyl-7,10-dibenzyloxycarbonylbaccatin III (399 mg, 0.491 mmol), and DMAP (12 mg, 0.098 mmol) were dissolved in anhydrous CH$_2$Cl$_2$ (20 mL), DCC was added, and the reaction mixture was stirred for 2 h at room temperature under an argon atmosphere. The reaction mixture was diluted with AcOEt and washed with saturated NH$_4$Cl, water, satu-

rated NaHCO$_3$, and brine. The organic layer was dried over MgSO$_4$ and the solvent was removed under reduced pressure. The resulting oil was dissolved in MeOH (25 mL) and a solution of p-toluenesulfonic acid (112 mg, 0.589 mmol) in MeOH (10 mL) was added. After stirring for 5 h at room temperature, the reaction mixture was diluted with AcOEt and washed three times with saturated NaHCO$_3$ and brine. The organic layer was dried over MgSO$_4$ and the solvent was removed under reduced pressure. The resulting oil was purified by silica gel column chromatography (AcOEt:hexane 2:3), and unreacted minor diastereomer was discarded. The desired product was dissolved in a few milliliters of CH$_3$CN, the remaining dicyclohexylurea was filtered off, and solution was lyophilized to give a white powder of **24** (438 mg, 0.395 mmol, 80%). ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 8.10 (d, ^3J(H,H) = 7.7 Hz, 2 H, CH), 7.67–62 (m, 1 H, CH), 7.57–7.53 (m, 2 H, CH), 7.42–7.22 (m, 20 H, CH), 6.24 (s, 1 H, CH), 6.17 (t, ^3J(H,H) = 8.7 Hz, 1H, CH), 5.65 (d, ^3J(H,H) = 7.1 Hz, 1 H, CH), 5.52 (dd, ^3J(H,H) = 7.2, 10.5 Hz, 1 H, CH), 5.22 (d, ^3J(H,H) = 4.6 Hz, 1 H, CH), 5.20–4.99 (m, 7 H, CH, CH$_2$), 4.57 (d, ^3J(H,H) = 4.8 Hz, 1 H, CH), 4.20, 4.18 (2d, ^2J(H,H) = 9.2 Hz, 2 H, CH$_2$), 3.90 (d, ^3J(H,H) = 7.0 Hz, 1 H, CH), 2.60–2.52 (m, 1 H, CH$_2$), 2.36 (s, 3 H, CH$_3$), 2.27–2.21 (m, 1 H, CH$_2$), 2.05–1.99 (m, 1 H, CH$_2$), 1.93 (s, 3 H, CH$_3$), 1.90–1.83 (m, 1 H, CH$_2$), 1.79 (s, 3 H, CH$_3$), 1.82 (s, 3 H, CH$_3$), 1.16 (s, 3 H, CH$_3$), 1.11 (s, 3 H, CH$_3$). HRMS (FAB+): calcd for C$_{62}$H$_{64}$NO$_{18}$ [M$^+$ + H] 1111.4123, found 1111.4127.

13-(3′-N-Benzyloxycarbonyl-2′-*tert*-butoxycarbonyl-phenylisoserine)-10-deacetyl-7,10-dibenzyloxycarbonyl-baccatin III (25). To a solution of **24** (200 mg, 0.180 mmol) were added dry CH$_2$Cl$_2$ (1 mL) and pyridine (3 mL), DMAP (4.4 mg, 0.036 mmol), and then Boc$_2$O (59 mg, 0.27 mmol) in dry CH$_2$Cl$_2$ (2 mL) at 0 °C under an argon atmosphere. The mixture was stirred at room temperature overnight. The reaction mixture was diluted with AcOEt and successively washed with 10% citric acid three times, water, saturated NaHCO$_3$, and brine. The organic layer was dried over MgSO$_4$ and the solvent was removed under reduced pressure. The resulting oil was purified by silica gel column chromatography (AcOEt:hexane 1:2) to yield a white solid **25** (182 mg, 0.150 mmol, 84%). ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 8.12–8.10 (m, 2 H, CH), 7.64–7.60 (m, 1 H, CH), 7.53–7.50 (m, 2 H, CH), 7.43–7.20 (m, 20 H, CH), 6.27 (s, 1 H, CH), 6.23 (bt, ^3J(H,H) = 8.9 Hz, 1H, CH), 5.71 (bd, 1 H, CH, partially overlapping with next signal), 5.68 (d, ^3J(H,H) = 7.1 Hz, 1 H, CH), 5.56 (dd, ^3J(H,H) = 7.0, 10.7 Hz, 1 H, CH), 5.47 (bd, ^3J(H,H) = 9.0 Hz, 1 H, CH), 5.28 (bs, 1 H, NH), 5.21, 5.16 (2d, ^2J(H,H) = 11.9 Hz, 2 H, CH$_2$), 5.14 (s, 2 H, CH$_2$), 5.07, 5.00 (2d, ^2J(H,H) = 12.5 Hz, 2 H, CH$_2$), 4.95 (d, ^3J(H,H) = 7.9 Hz, 1H, CH), 4.32, 4.18 (2d, ^2J(H,H) = 8.5 Hz, 2 H, CH$_2$), 3.93 (d, ^3J(H,H) = 7.0 Hz, 1 H, CH), 2.64–2.56 (m, 1 H, CH$_2$), 2.43 (s, 3 H, CH$_3$), 2.36–2.28 (m, 1 H, CH$_2$), 2.05 (s, 3 H, CH$_3$), 2.01–1.94 (m, 2 H, CH$_2$), 1.83 (s, 3 H, CH$_3$), 1.44 (s, 9 H, CH$_3$), 1.21 (s, 3 H, CH$_3$), 1.17 (s, 3 H, CH$_3$). HRMS (FAB+): calcd for C$_{67}$H$_{72}$NO$_{20}$ [M$^+$ + H] 1210.4648, found 1210.4642.

2′-O-*tert*-Butoxycarbonyl-3′-N-de(*tert*-butoxycarbonyl)docetaxel Hydrochloride (5). Pd/C was added (16 mg) to the stirring solution of **25** (31 mg, 0.0256 mmol) in EtOAc (1 mL), and the reaction mixture was vigorously stirred for 8 h at room temperature under a hydrogen atmosphere. The catalyst was filtered off and 1 equiv of 0.1 M HCl in methanol (260 μL, 0.026 mmol) was added. The solvent was removed under reduced pressure to give a white powder of **5** as a HCl salt (21.4 mg, 0.0253 mmol, 99%). The product was pure, required no purification, and remained stable under refrigeration in solid form for at least 2 weeks. ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 8.08–8.06 (m, 2 H, CH), 7.74–7.70 (m, 1 H, CH), 7.64–7.60 (m, 2 H, CH), 7.57–7.50 (m, 4 H, CH), 7.39–7.34 (m, 1 H, CH), 5.98 (dt, ^4J(H,H) = 1.1 Hz, ^3J(H,H) = 9.2 Hz, 1 H, CH), 5.59 (d, ^3J(H,H) = 7.1 Hz, 1 H, CH), 5.28 (d, ^3J(H,H) = 8.8 Hz, 1H, CH), 5.22 (s, 1 H, CH), 4.97 (dd, ^3J(H,H) = 2.0, 9.7 Hz, 1H, CH), 4.76 (d, ^3J(H,H) = 8.8 Hz, 1 H, CH), 4.22–4.14 (m, 3 H, CH, CH$_2$), 3.78 (d, ^3J(H,H) = 7.1 Hz, 1 H, CH), 2.47–2.39 (m, 1 H, CH$_2$), 2.29 (s, 3 H, CH$_3$), 1.93 (dd, ^3J(H,H)

= 9.5 Hz, ^2J(H,H) = 15.4 Hz, 1 H, CH$_2$), 1.88−1.77 (m, 1 H, CH$_2$), 1.84 (d, ^4J(H,H) = 1.3 Hz, 3 H, CH$_3$), 1.66 (s, 3 H, CH$_3$), 1.55−1.47 (m, 1 H, CH$_2$), 1.52 (s, 9 H, CH$_3$), 1.11 (s, 3 H, CH$_3$), 1.09 (s, 3 H, CH$_3$). HRMS (FAB+): calcd for C$_{43}$H$_{54}$NO$_{14}$ [M$^+$ + H] 808.3544, found 808.3550. Anal. (C$_{43}$H$_{54}$ClNO$_{14}$·3H$_2$O) C, H, N.

3′-*N*-De(*tert*-butoxycarbonyl)docetaxel Hydrochloride (26). Pd/C was added (16 mg) to the stirring solution of **24** (31 mg, 0.0279 mmol) in EtOAc (1 mL), and the reaction mixture was vigorously stirred for 8 h at room temperature under a hydrogen atmosphere. The catalyst was filtered off and 0.1 M HCl in methanol (280 μL, 0.028 mmol) was added. The solvent was removed under reduced pressure to give **26** as the HCl salt, a white powder (20.6 mg, 0.0277 mmol, 99%). The product was pure and required no purification. ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 8.05−8.02 (m, 2 H, CH), 7.73−7.68 (m, 1 H, CH), 7.62−7.50 (m, 6 H, CH), 7.44−7.39 (m, 1 H, CH), 6.07 (dt, ^4J(H,H) = 1.3 Hz, ^3J(H,H) = 9.0 Hz, 1 H, CH), 5.59 (d, ^3J(H,H) = 7.1 Hz, 1 H, CH), 5.23 (s, 1 H, CH), 4.97 (dd, ^3J(H,H) = 2.1, 9.6 Hz, 1H, CH), 4.58 (d, ^3J(H,H) = 8.6 Hz, 1H, CH), 4.55 (d, ^3J(H,H) = 8.4 Hz, 1 H, CH), 4.22−4.14 (m, 3 H, CH, CH$_2$), 3.80 (d, ^3J(H,H) = 7.1 Hz, 1 H, CH), 2.46−2.39 (m, 1 H, CH$_2$), 2.21 (s, 3 H, CH$_3$), 1.96 (dd, ^3J(H,H) = 9.2 Hz, ^2J(H,H) = 15.2 Hz, 1 H, CH$_2$), 1.87 (d, ^4J(H,H) = 1.5 Hz, 3 H, CH$_3$), 1.85−1.78 (m, 1 H, CH$_2$), 1.72 (dd, ^3J(H,H) = 9.0 Hz, ^2J(H,H) = 15.2 Hz, 1 H, CH$_2$), 1.67 (s, 3 H, CH$_3$), 1.12 (s, 3 H, CH$_3$), 1.09 (s, 3 H, CH$_3$). HRMS (FAB+): calcd for C$_{38}$H$_{46}$NO$_{12}$ [M$^+$ + H] 708.3020, found 708.3024 Anal. (C$_{38}$H$_{46}$ClNO$_{12}$·2H$_2$O) C, H, N.

Water Solubility. The parent drugs (**2**, **15−20**) and the prodrugs (**5**, **8−13**) were saturated in distilled water and shaken vigorously. The saturated solutions were sonicated for 15 min at 25 °C and passed through a centrifugal filter (0.45 μm filter unit, Ultrafree-MC, Millipore). The filtrate was analyzed using RP-HPLC.

Stability Studies of 8−13 in PBS Buffer. The conversion profiles of **5**, **8−13** were determined in phosphate-buffered saline (PBS, pH 7.4). To 990 μL of PBS (pH 7.4) was added 5 μL of DMSO and 5 μL of solution including **5**, **8−13** (1 mM in DMSO), and the mixture was incubated at 37 °C. At the desired time points, 1 mL of MeOH was added to the samples to dissolve parent drugs completely, and 1 mL of the mixture was directly analyzed by RP-HPLC. In the case of **12**, PBS (pH 4.9) and HCl/glycine-buffered saline (pH 2.0) were also employed to evaluate the conversion profiles. HPLC was performed using a C18 (4.6 × 150 mm; YMC Pack ODS AM302) reverse phase column with a binary solvent system: linear gradient of CH$_3$CN (0−100%, 40 min) in 0.1% aqueous TFA at a flow rate of 0.9 mL/min, UV detection at 230 nm.

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