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O–N Intramolecular Acyl Migration Strategy in Water-Soluble Prodrugs of Taxoids

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Abstract—We synthesized a highly water-soluble canadensol prodrug **6** that formed canadensol **3** by a simple pH-dependent chemical mechanism via the O–N intramolecular acyl migration of the isobutyryl group. This prodrug, a 2′-*O*-isobutyryl isoform of **3**, has no additional functional auxiliaries released during the conversion to **3**. This is a significant advantage in toxicology and medical economics, since the potential side effects of reported water-soluble auxiliaries and the use of detergent for solubilization can be avoided. The solubility of **6** was 2.26 mg mL^{-1} and only the parent drug **3** was released under physiological conditions (pH = 7.4) while, in acidic medium, the release of **3** slowed until migration was completely obstructed at pH = 2. In further consideration of this strategy, we elucidated the use of an 'O–N acyl-like' migration reaction of the Boc group in the design of a docetaxel prodrug. Both O–N migration and undesired hydrolysis of the Boc group occurred under physiological conditions, although no oxazolidinone formation was observed, suggesting the limitation of our water-soluble prodrug strategy to docetaxel.

The introduction of anti-cancer agents paclitaxel (Taxol®, **1**)[1] and docetaxel (Taxotere®, **2**)[2] has revolutionized the treatment of cancer and markedly improved the survival time of patients. More recently, other taxoids, such as canadensol (**3**),[3] have been developed, with improved potency against cancer showing multi-drug resistance (MDR).[4] However, despite the hope and promise that these agents have engendered, their poor water-solubility, based on their common taxane ring structure, is a serious problem in intravenous administration. Although this has prompted many researchers to develop water-soluble paclitaxel prodrugs through the introduction of hydrophilic moieties to C2′ or/and C7 positions,[5] the released auxiliary moieties may have negative effects in vivo, suggesting a need for novel approaches to water-soluble prodrugs.

Along the same lines, we previously designed and synthesized a novel water-soluble paclitaxel prodrug, isotaxel **4** that realized a higher water-solubility (0.45 mg mL^{-1}), due to the ionized 3′-amino group, than the parent drug, paclitaxel (0.00025 mg mL^{-1}), and the formation of paclitaxel through a simple pH-dependent chemical mechanism via the O–N intramolecular acyl migration of the benzoyl group.[6] This prodrug, a 2′-*O*-benzoyl isoform of paclitaxel, releases no additional functional auxiliaries during its conversion to paclitaxel. Isotaxel had a practical conversion time under physiological conditions ($t_{1/2}$ = 15 min) and sufficient stability in 0.035% citric acid saline (pH 4.0), one of the possible injection conditions for practical clinical use. These characteristics therefore promise a significant advantage in toxicology and medical economics and the diversified application of the O–N acyl migration prodrug to other important water-insoluble taxoids such as **2** and **3** is highly valuable.

In this paper, we studied the viability of the water-soluble prodrugs of docetaxel (**2**) and canadensol (**3**) based on O–N acyl migration. Both taxoids include an α-hydroxy-β-amino acid structure in the phenylisoserine part, as does paclitaxel (Fig. 1), but the functional groups at the 3′-position differed, with *t*-butyloxycarbonyl (Boc) and isobutyryl groups in **2** and **3**, respectively, instead of the benzoyl group in **1**. Hence, the water-soluble prodrugs **5** and **6**, which are 2′-*O*-Boc

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and 2′-*O*-isobutyryl isoforms of **2** and **3**, respectively, were designed to increase water solubility and allow conversion to their corresponding parent drugs via O–N acyl migration under physiological conditions.

Firstly, to examine the capability of O–N migration of the Boc group, we synthesized a model compound **10**, which includes the Boc group at the 2-position in the phenylisoserine part and a bulky cyclohexyl structure instead of the taxane ring in **5** (Scheme 1). An amino group of commercially available (2*R*,3*S*)-phenylisoserine·HCl **7** was protected by the benzyloxycarbonyl (*Z*) group in a conventional manner, then the *O*-Boc group was introduced by coupling with Boc_2O. The resulting compound **8** was coupled with cyclohexanol using a DCC/DMAP method to afford **9**. Finally, the *Z* group of **9** was deprotected by hydrogenation, followed by ion-exchange HPLC to give compound **10** as a hydrochloride salt.[7] This compound was dissolved in phosphate buffered saline (PBS, pH 7.4), incubated at 37 °C and the kinetic profile was examined. As Figure 2 shows, **10** was consumed completely and the time-dependent formation of parent compound **11** with *N*-Boc was observed, indicating that 'O–N acyl-like' migration of Boc occurred through the formation of a five-membered ring transition state. To the best of our knowledge, the formation of *t*-butyloxycarbamate from corresponding carbonate through intramolecular migration is unprecedented. Moreover, we did not observed any oxazolidinone formation, which was reported in other carbonates under basic conditions.[8] However, compound **12** was also produced by simple hydrolysis of the carbonate bond in **10**. Since the anti-tumor activity of **2** without the Boc group was drastically lower than that of **2**,[2] further application of our prodrug strategy to docetaxel **5** was abandoned.

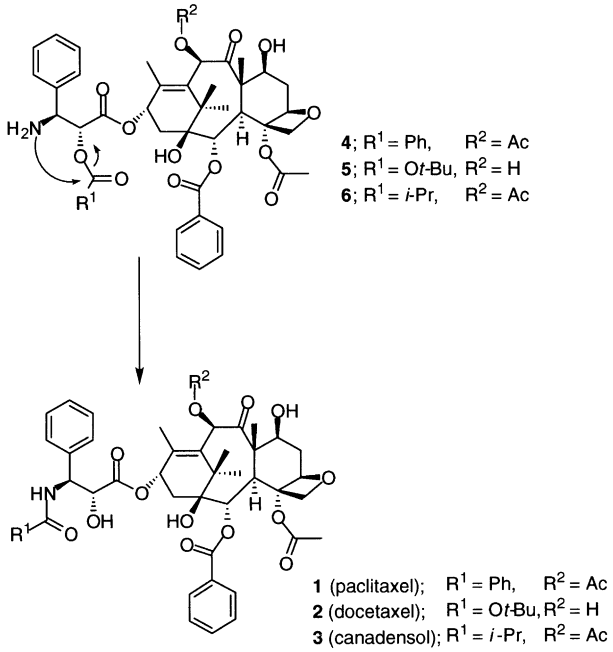
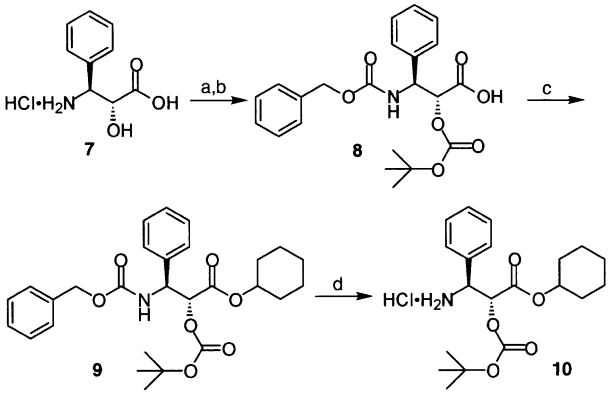


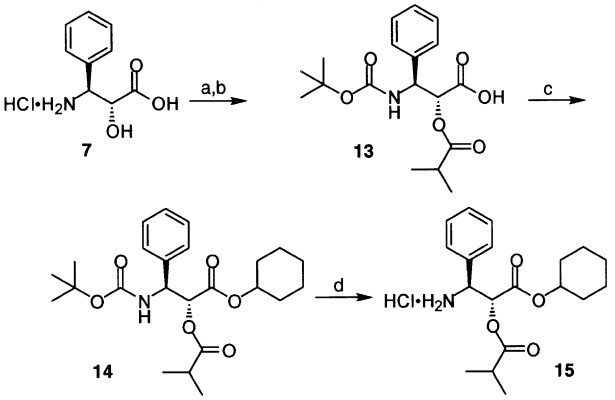
Figure 1. The O–N acyl migration reaction of taxoid prodrugs to their corresponding parent drugs under physiological conditions (pH 7.4).

Next, the effect of the isobutyryl group on the kinetics of the O–N acyl migration was examined using a model compound **15** which has a cyclohexyl structure instead of the taxane ring in **6**. Compound **15** was synthesized through the *N*-Boc-protection of (2*R*,3*S*)-phenylisoserine, followed by acylation with isobutyryl chloride, coupling with cyclohexanol by the DCC/DMAP method and final deprotection of the Boc group with 4 M HCl in dioxane (Scheme 2). The conversion time of **15** to the corresponding *N*-acyl compound was analyzed in a similar manner as for **10**. Compound **15** was completely and promptly converted to its *N*-acyl isomer with a $t_{1/2}$ value of 3.7 min with no disruptions, such as hydrolysis. This faster migration in **15** could be explained by the relatively higher electrophilic nature of the carbonyl carbon of the isobutyryl group compared to the benzoyl group in isotaxel.[6] However, this is compatible with our previous study on prodrugs of HIV-1 protease inhibitors.[9,10]

This promising result prompted us to apply the strategy to the water-soluble prodrug of canadensol **3**. The prodrug **6** was synthesized according to Scheme 3. Compound **16**, prepared according to the procedures described previously,[6] was coupled with isobutyryl



Scheme 1. Synthesis of model compound **10**: (a) benzyloxycarbonyl chloride, $NaHCO_3$, H_2O/Et_2O (1:1), 0 °C to rt; (b) Boc_2O, DMAP, THF, rt; (c) cyclohexanol, DCC, DMAP, CH_2Cl_2, rt; (d) Pd/C, H_2, EtOAc, then ion exchange HPLC with 12 mM HCl.



Scheme 2. Synthesis of model compound **15**: (a) Boc_2O, Et_3N, THF/H_2O, 0 °C to rt; (b) isobutyryl chloride, pyridine, CH_2Cl_2, 0 °C to rt; (c) cyclohexanol, DCC, DMAP, CH_2Cl_2, rt; (d) 4 M HCl in dioxane, anisole, 0 °C to rt.

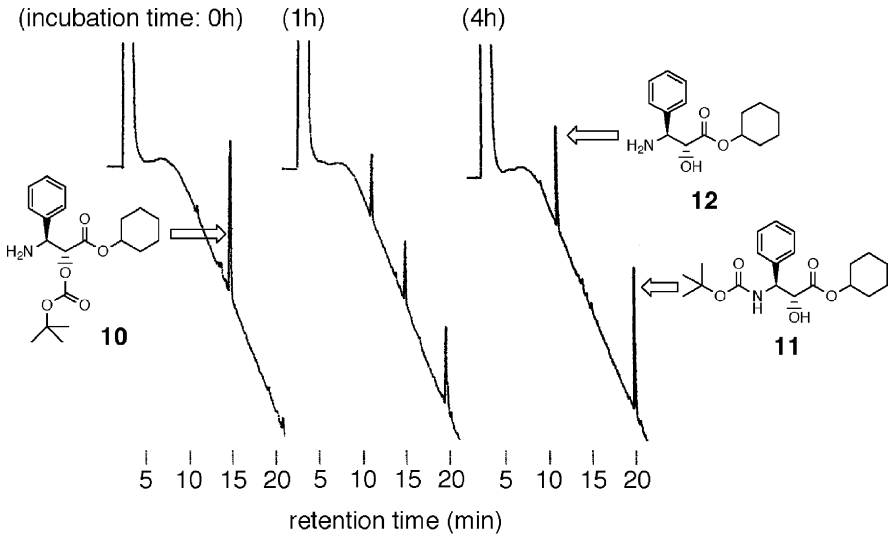
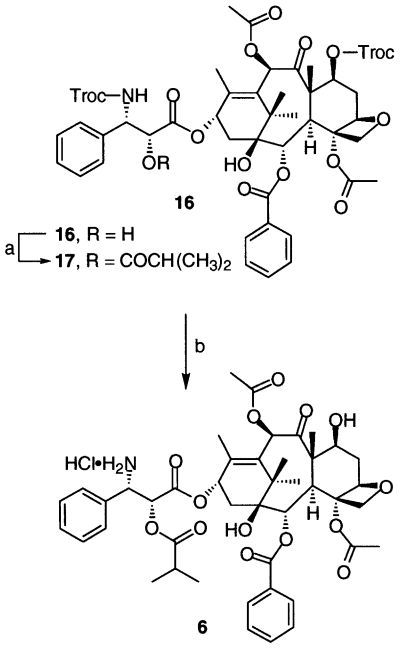


Figure 2. The HPLC profiles of model compound **10** with the Boc group in PBS (pH 7.4, 37 °C).



Scheme 3. Syntheses of canadensol prodrug **6**: (a) isobutyryl chloride, pyridine, CH_2Cl_2, 0 °C; (b) Zn, AcOH, AcOEt, rt, then ion exchange HPLC with 12 mM HCl.

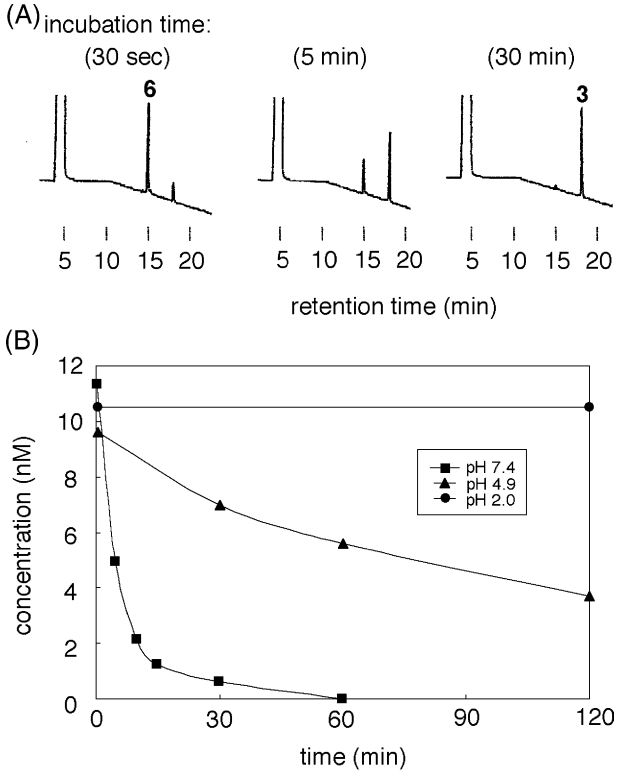


Figure 3. (A) HPLC profiles of prodrug **6** in PBS (pH 7.4, 37 °C); (B) migration of prodrug **6** in different pH at 37 °C.

chloride to give ester **17**. The deprotection of both the 2,2,2-trichloroethyloxycarbonyl (Troc) groups of this ester and the following purification with ion exchange by HPLC gave prodrug **6** as an HCl salt.[11] The water-solubility and migration rate of **6** at pH 7.4 (37 °C) were examined (Fig. 3). The water-solubility was determined as 2.26 mg mL^{-1}, 10-fold higher than that of canadensol (0.22 mg mL^{-1}).[12] Complete migration was observed at pH 7.4 with a $t_{1/2}$ value of 4.3 min similar to that in model compound **15**, indicating that the highly bulky taxane ring did not influence the migration rate. In addition, slower migration was observed at pH 4.9 with a value of 82.0 min and no migration at pH 2.0 after 6 h incubation. These findings suggested that the kinetics of migration from **6** to parent drug **3** was clearly pH-dependent. The prodrug **6** was stable in acidic aqueous media (pH = 2.0) for at least 6 h. In addition, a solid of **6**·HCl was also stable for at least 1 month at 4 °C. Only slight migration of **6** (1% of canadensol was released after 1 h) was observed during incubation in 0.035% citric acid saline (pH 4.0) at room temperature, suggesting a possible injection condition for practical clinical use.

In conclusion, the *O*-acyl isoform of canadensol **6**, having no auxiliary and no by-product during conversion to parent drug **3**, was synthesized and the water-solubility and conversion time at various pH conditions were

examined. Prodrug **6** had a 10-fold higher water solubility than **3** and was converted to parent **3** through O–N acyl migration with clear pH-dependency. This finding supported the premise that this prodrug strategy based on the O–N intramolecular acyl migration reaction has significant advantages in toxicity and medical economics. Summarized, our studies of water-soluble prodrugs in both paclitaxel and canadensol suggest that this strategy can be applied to other 3′-*N*-acyl taxoids with similar promising results. However, it is important to note that 'O–N acyl-like' migration for the docetaxel-type prodrug is difficult to apply using our present prodrug strategy.

Acknowledgements

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7. Selected spectroscopic data for **10**, mp 156–159 °C, ^{1}H NMR (CD_3OD, 400 MHz): δ 7.50–7.43 (m, 5H), 5.16 (d, J = 8.3 Hz, 1H), 4.68 (d, J = 8.4 Hz, 1H), 4.69–4.62 (m, 1H), 1.76–1.58 (m, 2H), 1.54–1.18 (m, 7H), 1.48 (s, 9H), 1.06–0.96 (m, 1H). HRMS (FAB+): calcd For $C_{20}H_{30}NO_5$ [M^+ + H]: 364.2124, found: 364.2128.
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11. Selected spectroscopic data for **6**, mp 156–160 °C, ^{1}H NMR (CD_3OD, 400 MHz): δ 8.08–8.06 (m, 2H), 7.76–7.71 (m, 1H), 7.66–7.50 (m, 6H), 7.38–7.34 (m, 1H), 6.41 (s, 1H), 5.93 (t, J = 8.0 Hz, 1H), 5.59 (d, J = 7.3 Hz, 1H), 5.33 (d, J = 9.0 Hz, 1H), 4.97 (dd, J = 9.6, 1.9 Hz, 1H), 4.82 (d, J = 9.0 Hz, 1H), 4.31 (dd, J = 6.5, 11.0 Hz, 1H), 4.17, 4.14 (2d, J = 8.2 Hz, 2H), 3.72 (d, J = 7.1 Hz, 1H), 2.84 (septet, J = 7.0 Hz, 1H), 2.48–2.41 (m, 1H), 2.27 (s, 3H), 2.16 (s, 3H), 1.92–1.85 (m, 1H), 1.85 (d, J = 1.3 Hz, 3H), 1.81–1.74 (m, 1H), 1.62 (s, 3H), 1.46–1.40 (m, 1H), 1.28 (d, J = 7.0 Hz, 3H), 1.25 (d, J = 6.8 Hz, 3H), 1.12 (s, 3H), 1.10 (s, 3H). HRMS (FAB+): calcd For $C_{44}H_{54}NO_{14}$ [M^+ + H]: 820.3544, found: 820.3542. Purity was higher than 98% (HPLC analysis at 230 nm).
12. Canadensol **3** was synthesized via simple acylation (*i*-PrCOOH, HOBt, EDC·HCl) of 3′-amine group of Troc-deprotected (Zn, AcOH, AcOEt) compound **16**. Spectroscopic data are identical with data from ref 3.