A Novel Approach of Water-Soluble Paclitaxel Prodrug with No Auxiliary and No Byproduct: Design and Synthesis of Isotaxel

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Abstract: A novel water-soluble paclitaxel prodrug, isotaxel **2**, that realizes a higher water-solubility and the formation of paclitaxel through a simple pH-dependent chemical mechanism via the O−N acyl migration was synthesized and showed promising results in water-solubility and kinetics. This prodrug, a 2′-*O*-benzoyl isoform of paclitaxel, has no additional functional auxiliaries released during conversion to paclitaxel, which would be a great advantage in toxicology and medical economics.

Paclitaxel (Taxol, **1**) is one of the most important chemotherapeutic agents with promising antitumor activity, especially against ovarian, breast, and lung cancers.[1] However, its sparing water-solubility (0.00025 mg mL^{-1})[2] requires coinjection of a detergent, Cremophor EL, which was suggested to cause hypersensitivity reactions, and patients receiving this drug require premedication.[3] To resolve these problems, many attempts such as water-soluble prodrugs,[2,4] nonprodrug water-soluble analogues,[5] or orally active analogues[6] were reported. In water-soluble prodrugs, the hydroxyl groups at the C-2′ and/or C-7 were extensively modified with hydrophilic and/or charged solubilizing moieties. The targeting of paclitaxel using hydrophilic enzymatically cleavable groups[4e] and tumor targeting moieties[4f−i] has also been reported. None of these applications are presently in clinical use, although some of them are currently undergoing clinical evaluation.[4j−m] In addition, the released auxiliary moieties may have some unfavorable effects in vivo. These factors suggest that novel approaches for water-soluble prodrugs are needed.

We report herein a new prodrug isotaxel **2** (Figure 1) with improved water-solubility. This prodrug, having no additional water-solubilizing auxiliaries and forming no byproduct during conversion to the parent drug, is a 2′-*O*-benzoyl isoform of **1**, was designed to increase water-solubility with an ionized 3′-amino group and allows for conversion to **1** via O−N acyl migration of the benzoyl group under physiological conditions.[7]

N−O intramolecular acyl migration is known as a side reaction of Ser- or Thr-containing peptides.[8] The β-hydroxyl groups are acylated by the N−O shift under acidic conditions and the resulting *O*-acyl products can be readily converted to *N*-acyl compounds under neutral or slightly basic conditions in aqueous buffer. The liberated ammonium ion enhances the water-solubility of *O*-acyl products. Considering these features, we designed a novel class of "O−N intramolecular acyl migration"-type water-soluble prodrugs of HIV-1 protease (PR) inhibitors having allophenylnorstatine (Apns, (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoic acid).[9] Hurley et al. also reported a study on the O−N acyl migration of renin inhibitors.[10] The *O*-acyl prodrug of HIV-1 PR inhibitors at the 2-hydroxyl group of the Apns moiety completely released their parent drug in a few minutes under physiological conditions.

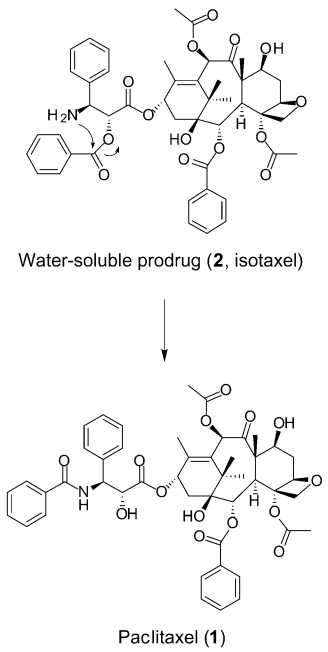

Figure 1. The O−N acyl migration of isotaxel **2** to paclitaxel.

First, we examined the effect of the benzoyl group and stereochemistry of α-hydroxy-β-amino acids on the kinetics of the O−N acyl migration using a series of model compounds (see Supporting Information). It was revealed that the migration of *O*-benzoylphenylisoserine derivative corresponding to the amino acid part of paclitaxel showed relatively slower migration with a $t_{1/2}$ value of 12 min under physiological conditions. This value appeared to be suitable for systemic distribution after injection and not long enough for metabolism and elimination. Moreover, under the acidic aqueous conditions, these compounds were maintained stably without any migration. These promising results prompted us to apply this strategy to paclitaxel. Hence, we next investigated the synthesis of the *O*-acyl form of paclitaxel, namely isotaxel **2**.

As depicted in Scheme 1, N^{β}-Troc-phenylisoserine methyl ester **4**,[11] was prepared from commercially available (2*R*,3*S*)-phenylisoserine·HCl **3**. 1,3-Oxazolidine derivative **5** was obtained from ester **4** in the reaction with 4-methoxybenzaldehyde dimethyl acetal in the presence of a catalytic amount of PPTS. Hydrolysis of **5** gave carboxylic acid **6** which was used in the next step without further purification. The coupling of **6** with 7-Troc-baccatin III[12] in the presence of DCC afforded the corresponding ester **7** in a nearly quantitative yield without any detectable epimerization at the

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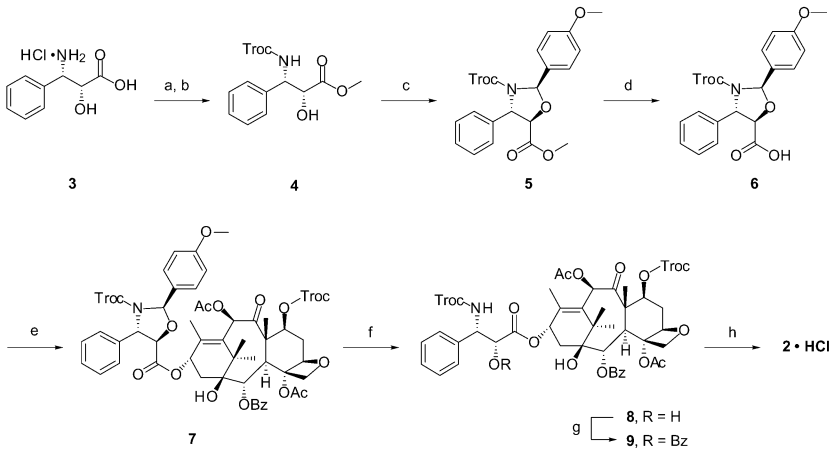
Scheme 1[a]



[a] Reagents and conditions: (a) succinimidyl-2,2,2-trichloroethyl carbonate, NaOH, $NaHCO_3$, dioxane, rt, 1 h; (b) $SOCl_2$, MeOH, 0 °C to room temperature, 14 h, 97% over two steps; (c) 4-methoxybenzaldehyde dimethyl acetal, PPTS, toluene, distillation 30 min, 92%; (d) KOH, MeOH, rt, 30 min. 99%; (e) 7-Troc-baccatin III, DCC, DMAP, toluene:CH_2Cl_2 2:1, rt, 3 h, 98%; (f) PTS, MeOH, rt, 24 h, 94%; (g) benzoic acid, EDC·HCl, DMAP, CH_2Cl_2, rt, 2 h, 92%; (h) Zn (dust), MeOH:AcOH 1:1, rt, 4 h, then HCl, 77%.

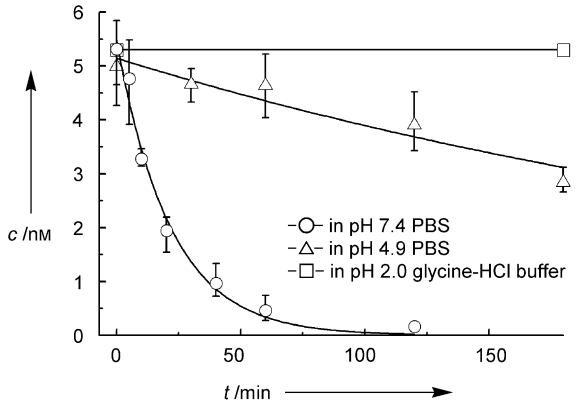


Figure 2. Migration of prodrug **2** in different pH conditions at 37 °C; c = concentration, t = incubation time.

C-2′ position.[13] The oxazolidine ring in **7** was cleaved with PTS. Finally, after benzoylation of the 2′-hydroxyl group with benzoic acid by the EDC−DMAP method, deprotection of both Troc groups using Zn−AcOH, and following purification and ion-exchange by HPLC eluted with 12 mM aq HCl gave isotaxel **2** as a HCl salt with a good total yield (58%).

The water-solubility of **2**·HCl was determined as a value of 0.45 ± 0.04 mg mL^{-1}, which was 1800-fold higher than that of paclitaxel (0.00025 ± 0.00004 mg mL^{-1}). To study the kinetics of O−N benzoyl migration, **2**·HCl was dissolved in PBS at different pH and incubated at 37 °C. The migration was monitored by HPLC (see Supporting Information). A complete migration was observed at pH 7.4 with a $t_{1/2}$ value of 15.1 ± 1.3 min (Figure 2), and this value is suggested to be appropriate for the systemic distribution. On the other hand, a slower migration was observed at pH 4.9 with a $t_{1/2}$ value of 252.2 ± 37.7 min and no migration at pH 2.0 after 6 h of incubation.

These results indicated that the kinetics of migration from **2**·HCl to parent drug **1** were clearly pH-dependent, a faster migration could be obtained under physiological conditions (pH 7.4) than under acidic conditions, and the prodrug **2** was stable in pH 2.0. In addition, a solid of **2**·HCl, which is the expected storage form, was stably maintained for one month at 4 °C with no migration or decomposition. Moreover, incubation in 0.035% citric acid saline (pH 4.0) at room temperature showed very slow migration of **2**·HCl (<3% of paclitaxel was released after incubation for 3 h), suggesting a possible condition of the injectable solution for practical clinical use.[4c,14] In addition, the 2′-*O*-benzoyl ester of **2** was biologically stable since this bond did not cleave at all in the experiment using porcine liver esterase (see Supporting Information).

In conclusion, we synthesized a water-soluble paclitaxel prodrug, isotaxel **2**, that realized a higher water-solubility and the formation of paclitaxel through a simple pH-dependent chemical mechanism via the O−N acyl migration. This prodrug, a 2′-*O*-benzoyl isoform of paclitaxel, has no additional functional auxiliaries released during conversion to paclitaxel. This would be a great advantage in toxicology and medical economics, since the potential side effects caused by reported auxiliaries and the use of detergent for solubilization can be omitted.

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Supporting Information Available: The synthetic and spectroscopic data of **2**, **4**−**9** and *O*-benzoyl-α-hydroxy-*β*-amino acid derivatives, O−N benzoyl migration study of *O*-benzoyl-α-hydroxy-*β*-amino acid derivatives, the modeling of migration intermediates of model compounds, and water-solubility and stability studies of isotaxel. This material is available free of charge via the Internet at http://pubs.acs.org.

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