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A facile route to paclitaxel C-10 carbamates

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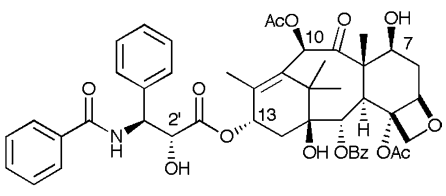
Abstract—A general protocol for the synthesis of paclitaxel C-10 carbamates is described. The method entails MeI-mediated activation of 2′-*O*-TBS-7-*O*-TES-10-*O*-deacetyl-paclitaxel-10-*O*-carbonylimidazole prior to reaction with amines. This method is effective for the synthesis of paclitaxel C-10 derivatives, including bifunctional molecules.

Paclitaxel (Fig. 1), a natural product isolated from *Taxus brevifolia*,[1] has been used widely to treat solid tumours. The mechanism of action involves mitotic arrest in cancer cells through stabilization of microtubules via an inhibition of depolymerization.[2] Recent pioneering studies by Trojanowski and co-workers at the University of Pennsylvania have also shown that paclitaxel may hold considerable promise for the treatment of neuro- degenerative diseases, such as Alzheimer's disease, by replacing the protein tau, which binds to and stabilizes tubulin polymerization, and which is subject to misfolding.[3] Other microtubule binding drugs such as the epothilones, eleutherobine and discodermolide may hold similar clinical potential.[4]

Although an effective clinical agent in cancer chemotherapy, disadvantages associated with the use of paclitaxel include significant toxicity due to the high doses required, the lack of selectivity towards normal and tumour cells, and the poor water solubility, combined with the rapid onset of drug resistance and the inability to cross the blood–brain barrier. In an attempt to address these issues, numerous bifunctional conjugates have been designed and synthesized over the past few years to improve the overall biological profile of paclitaxel by linking the taxane skeleton to an auxiliary molecule. These molecules include, for example, targeting moieties such as monoclonal antibodies for selective delivery of the drug to tumours.[5] Other tactics have been used to increase solubility, and thereby the bioavailability of the drug, by attaching PEGs, carbohydrates and/or phosphates.[6–8] In addition, fluorescent probe molecules have been linked to paclitaxel to generate bifunctional conjugates to be employed as imaging agents able to visualize microtubules in living cells.[9]



Paclitaxel

Figure 1.

The vast majority of bifunctional taxane-conjugates reported to date have been constructed exploiting the C-2′ or C-7 positions. Since the hydroxyl moiety at C-2′ is known to be essential for biological activity, C-2′ conjugates are typically designed to release the parent drug. The C-7 position, on the other hand, is known to be relatively tolerant to structural modifications, thus conjugates at C-7 can be designed to be hydrolytically stable. However, the size of the substituents attached at C-7 may play a significant role in determining the biological activity of the drug. For example, hydrolytically stable conjugates containing large substituents such as folic acid moieties were found to be inactive.[10]

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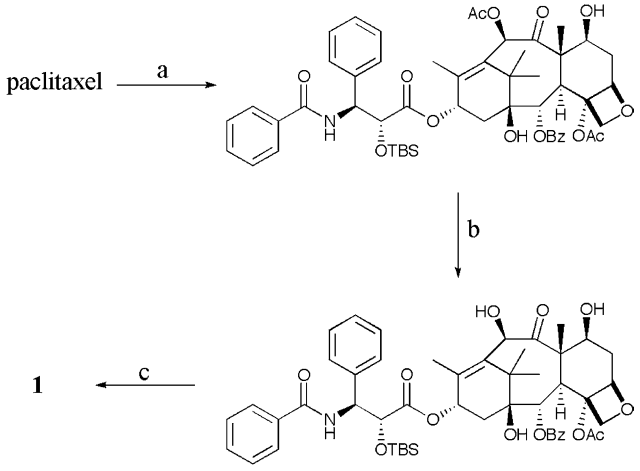


Figure 2. Reagents and conditions: (a) TBS–Cl, dimethylformamide, imidazole, 60 °C, 1.5 h; (b) hydrazine, ethanol, rt, 1.5 h; (c) TES–Cl, DMAP, dichloromethane, rt, 1 h.

Recently, our group and others have examined the C-10 hydroxyl as an alternative point of attachment that might be more tolerant of conjugation with large molecules. We were particularly interested in developing a general protocol that would allow easy access to C-10 bifunctional constructs with hydrolytically stable carbamate linkages. In 2004, Miller et al.[11] reported an example of direct carbamoylation of a paclitaxel analogue via a C-10 carbonylimidazole intermediate.[12] We have taken a similar tack beginning with 2′-*O*-TBS-7-*O*-TES-10-*O*-deacetyl-paclitaxel (**1**),[13] available in three steps and in high overall yield from paclitaxel (Fig. 2).

Conversion of **1** into the C-10 carbonylimidazole derivative (**2**) was next achieved quantitatively upon reaction with carbonyl-diimidazole (CDI) (Fig. 3). Compound **2** was obtained in high purity as determined by NMR and LC–MS, and could be employed directly without purification, or stored at −20 °C for several weeks without noticeable decomposition.[14] For derivatization of **2**, we first explored a protocol, which called for addition of 5–7 equiv of various amines (Table 1) in *tert*-butanol (0.04 M) at 82 °C (Fig. 3, Method A). Greenwald et al.[6] had successfully employed nearly identical reaction conditions for the synthesis of several paclitaxel C-7 carbamates from the corresponding C-7 carbonylimidazole. The data presented in Table 1 indicate this protocol is, however, not generally applicable. For example, carbamate formation was found to be dependent upon concentration, temperature and amount of amine employed. In addition, the nucleophilicity and

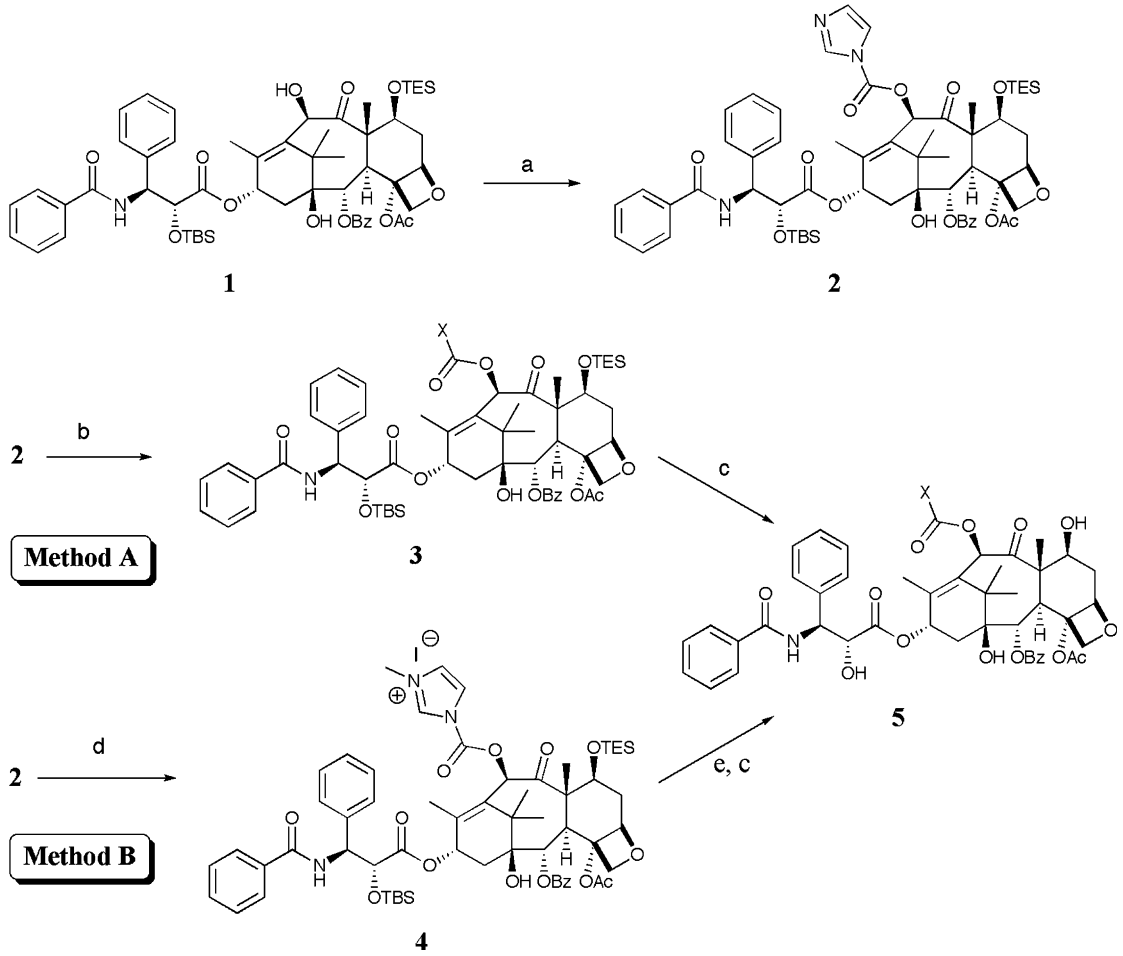
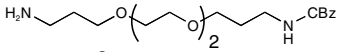
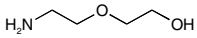
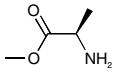
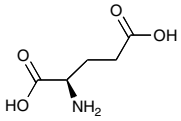
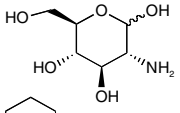
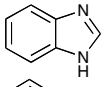
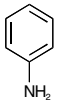
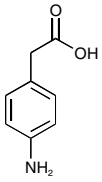
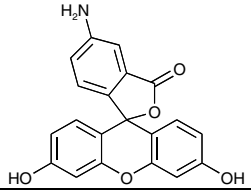


Figure 3. Reagents and conditions: (a) dichloromethane/carbonyl-diimidazole, rt, 16 h; (b) 5–7 equiv of amine in *tert*-butanol, 82 °C, 16 h; (c) acetonitrile/pyridine (1:1), HF/Py, 0 °C to rt, 4 h; (d) methyl iodide/acetonitrile, 55 °C, 3 h, (e) 2–3 equiv amine, rt.

Table 1. Examples of paclitaxel C-10 carbamates

Entry	Compd	Amine	% Conversion Method A[a]	% Conversion Method B[b]	Yield[c] (%)	MPA[d] (%)
1	**5a**		>95		59	81
2	**5b**		>95		57	75
3	**5c**		>95		57	67
4	**5d**		<5[e]	>95[e]	45	117
5	**5e**		Undetectable	>95	42	92
6	**5f**		>95		80	59
7	**5g**		Undetectable	>95	69	94
8	**5h**		Undetectable[e]	>95[e]	59	60
9	**5i**		Undetectable	>95	45	65
10	**5j**		Undetectable[e]	>95[e]	71	148
11	**5k**		Undetectable[e]	>95[e]	45	17

[a] Conversion of **2** to compounds of general structure **3** (Fig. 3), as determined by LC–MS.
[b] Conversion of **4** to compounds of general structure **3** (Fig. 3), as determined by LC–MS.
[c] Overall yield over two steps, after HPLC purification.
[d] Ability of the compounds of general structure **5** to promote polymerization of tubulin, expressed as percentage of the activity of the parent drug. The microtubule polymerization assay was done using the fluorescence based kit (cat. # BK011) produced by Cytoskeleton (Denver, CO).
[e] Reaction carried out in slightly basic condition by addition of DIEA.

steric encumbrance of the amine was observed to play a significant role in the success or failure of the derivatization.

Although primary aliphatic amines (entries 1–3) and reactive secondary amines such as piperidine (entry 6) provide the corresponding C-10 carbamates in high yield, less reactive amines such as morpholine (entry 7) fail to react. Likewise, aniline derivatives (entries 9–11) and benzimidazole (entry 8) fail to produce the desired carbamate analogues under the reaction conditions. Moreover, if the amines are not completely soluble at the relatively high concentration (0.2–0.3 M) required for coupling (e.g., glutamic acid and glucosamine HCl, entries 4 and 5, respectively), the reaction is either unsuccessful or proceeds too slowly to be synthetically useful.

To improve the reaction protocol leading to these and other 'difficult' to form carbamates, we treated carbonylimidazole derivative **2** with methyl iodide (Fig. 3, Method B). The activated C-10 carbonyl methylimidazolinium salt (**4**) was obtained in near quantitative yield.[15] Reaction of **4** with 2–3 equiv of amine at room temperature then furnished the desired adduct in good to excellent yield. Moreover, all reactions were complete within 20–30 min. Removal of the protecting groups in situ led to the desired final adduct (e.g., **5h**)[16] after HPLC purification[17] (Table 1); overall yields were in general very good.

The derived paclitaxel C-10 carbamates were subsequently evaluated for their ability to promote polymerization and stabilize microtubules in vitro, in direct comparison with paclitaxel (Table 1). With the exception of compound **5k** (entry 11), the carbamates retain good to excellent activity in the biochemical assay, with two paclitaxel C-10 carbamates showing enhanced activity (entries 4 and 10). Taken together, these results support the thesis that the C-10 position is quite tolerant to structural modifications.

In summary, we demonstrate in this letter that activation of carbonylimidazole derivatives such as **2** with methyl iodide (Method B) permits construction of sterically encumbered C-10 paclitaxel carbamate analogues under quite mild conditions, and as such can be employed for the construction of hydrolytically stable bifunctional molecules possessing intricate architectures. We further believe this activation protocol holds considerable promise for the construction of sterically highly encumbered carbamates in general.

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14. Synthetic and selected spectroscopic data for compound **2**: To 2′-*O*-TBS, 7-*O*-TES, 10-*O*-deacetyl-paclitaxel (**1**, 845 mg, 0.81 mmol), in anhydrous DCM (6 mL) was added carbonyl-diimidazole (530 mg, 400 mol %). The reaction mixture was allowed to stir for 16 h at room temperature under nitrogen atmosphere, and then extracted with water (5 mL). The organic layer was dried over sodium sulfate, filtered and concentrated to give 890 mg (96% yield) of **2**, which was subsequently used without purification; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.26 (s, 1H), 8.18 (d, J = 9 Hz, 2H), 7.77 (d, J = 8 Hz, 2H), 7.53 (m, 11H), 7.14 (s, 1H), 7.09 (d, J = 9 Hz, 1H), 6.59 (s, 1H), 6.32 (app t, J = 9 Hz, 1H), 5.78 (m, 2H), 5.02 (d, J = 8 Hz, 1H), 4.72 (d, J = 2 Hz, 1H), 4.56 (m, 1H), 4.38 (d, J = 8 Hz, 1H), 4.25 (d, J = 8 Hz, 1H), 3.88 (d, J = 7 Hz, 1H), 2.62 (s, 3H), 2.45 (m, 1H), 2.2 (m, 1H), 2.08 (s, 3H), 1.98 (m, 1H), 1.78 (s, 3H), 1.62 (s, 3H), 1.32 (m, 3H), 1.22 (s, 3H), 0.95 (m, 9H), 0.83 (s, 9H), 0.62 (m, 6H), 0.1 (s, 3H), −0.2 (s, 3H); Electrospray (LC–MS) *m/z* 1134 ($M+H^+$, $C_{61}H_{80}N_3O_{14}Si_2$ requires 1134). Retention time 9.92 min (1–99% B, Method 2).[17]
15. Synthetic procedure for compound **4**: to a solution of 2′-*O*-TBS, 7-*O*-TES, 10-*O*-deacetyl-paclitaxel-10-*O*-carbonyl-imidazole (**2**, 200 mg, 0.176 mmol) in acetonitrile (2 mL) in a sealable tube was added methyl iodide (1 mL) and the resulting mixture was stirred for 3 h at 55 °C. A stream of nitrogen was then used to remove the volatiles and the residue was held under high vacuum for 4 h. The residue was then redissolved in anhydrous DMSO or another anhydrous aprotic solvent and used directly for carbamate formation.
16. Synthetic procedure for **5 h**: To a solution of 2′-*O*-TBS, 7-*O*-TES, 10-*O*-deacetyl-paclitaxel-10-*O*-carbonyl-methyl-imidazolinium iodide (**4**, 45 mg, 0.035 mmol), in anhydrous DMSO (0.4 mL) was added benzimidazole (13 mg, 300 mol %) followed by DIEA (6.1 μL, 300 mol %). The reaction mixture was stirred at room temperature for 30 min until complete decolouration. The mixture was then diluted with pyridine (0.4 mL), cooled to 0 °C and treated with HF/pyridine (0.35 mL). The resulting solution was stirred for 3 h allowing the temperature to rise to room temperature. The mixture was then diluted with EtOAc (10 mL) and extracted with a saturated solution of $CuSO_4$ (3 × 2 mL) and water (3 × 2 mL). The organic solution was then dried over sodium sulfate, filtered and concentrated. The residue so obtained was purified by preparative RP-HPLC (Method 1).[17] Fractions containing the appropriate mass, as determined by analytical HPLC–MS (Method 2) were pooled and CH_3CN removed under reduced pressure. The remaining aqueous mixture was then lyophilized obtaining 20 mg of **5h** (59% overall yield). ^{1}HNMR ($CDCl_3$, 300 MHz): δ 8.62 (s, 1H), 8.18 (d, J = 7 Hz, 2H), 8.06 (d, J = 7 Hz, 1H), 7.82 (d, J = 7 Hz, 1H), 7.77 (d, J = 7 Hz, 2H), 7.62 (m, 1H), 7.53 (m, 11H), 7.09 (d, J = 9 Hz, 1H), 6.59 (s, 1H), 6.32 (app t, J = 9 Hz, 1H), 5.78 (m, 2H), 5.02 (d, J = 8 Hz, 1H), 4.82 (d, J = 3 Hz, 1H), 4.56 (m, 1H), 4.38 (d, J = 8 Hz, 1H), 4.25 (d, J = 8 Hz, 1H), 3.88 (d, J = 7 Hz, 1H), 2.62 (m, 1H), 2.38 (m, 5H), 1.95 (m, 4H), 1.78 (s, 3H), 1.38 (s, 3H), 1.22 (s, 3H); Electrospray (LC–MS) *m/z* 956 ($M+H^+$, $C_{53}H_{54}N_3O_{14}$ requires 956). Retention time 6.55 min (1–99% B, Method 2).
17. Preparative RP-HPLC purification was conducted on YMC-Pack ODS-A columns (S-5 μM, 300 × 20 mm ID) with gradient elution between 0% B and 50% B or 0% B and 100% B (A = 0.05% TFA in H_2O; B = 0.05% TFA in CH_3CN) with gradient times of 10 min and a flow rate of 25 mL/min with UV 220 nm detection (Method 1). Analytical HPLC–MS was conducted on a YMC Combi-Screen ODS-A column (S-5 μM, 50 × 4.6 mm ID) with gradient elution of 0% B to 100% B (A = 0.05% TFA in H_2O; B = 0.05% TFA in CH_3CN) with gradient times of 10 min and a flow rate of 3.5 mL/min with UV 220 nm and electrospray MS detection (Method 2).