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Selective Enzymatic Acylation of 10-Deacetylbaccatin III

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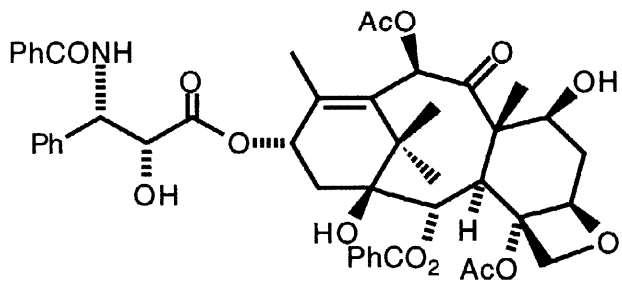
Abstract: 10-Deacetylbaccatin III (10-DAB) and 7-TES-10-DAB are selectively acylated using *Pseudomonas cepacia* lipase as the catalyst and dichloroacetic anhydride or tricholoracetic anhydride as the acylating agent.

Paclitaxel (**1**) isolated from the Pacific yew, *Taxus brevifolia*, has attracted considerable attention in many research areas due to its potent anticancer activities and complex structure.[1] Although the total synthesis of paclitaxel have been recently reported by several groups,[2] more practical synthesis is the semi-synthesis from 10-deacetylbaccatin III (10-DAB, **2**), available in larger quantities from the needles of the European yew *Taxus baccata L.*, and chemically synthesized *N*-benzoylphenylisoserine (BPI).[3] The semi-synthesis first requires the selective protection of 10-DAB which has three secondary alcohols at C-7, C-10, and C-13. It has been reported that the 7- and 10-hydroxyl groups are similarly more reactive than the 13-hydroxyl group and each of them can be selectively acylated or silylated.[4] Recently, an enzymatic method using the crude extracts of *Taxus baccata* has been described for the C-10 acetylation of 10-DAB.[5] To the best of our knowledge, no method have been so far described for the selective protection of the least reactive 13-hydroxyl group in the presence of the more reactive 7- and 10-hydroxyl groups. We herein wish to report for the first time a lipase-based approach for the selective acylation of the 13-hydroxyl group as well as for that of the 7- and 10-hydroxyl groups.



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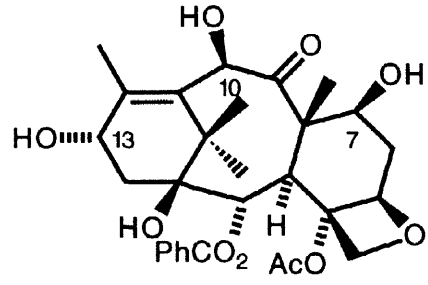

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To search for the selective acylation system, more than twenty hydrolases and several acylating reagents were screened with 10-DAB. Among the enzymes screened, the *Pseudomonas cepacia* lipase (PCL) showed more promising results in reactivity and selectivity. Among the acylating agents screened, moderately activated anhydrides, dichlroacetic anhydride

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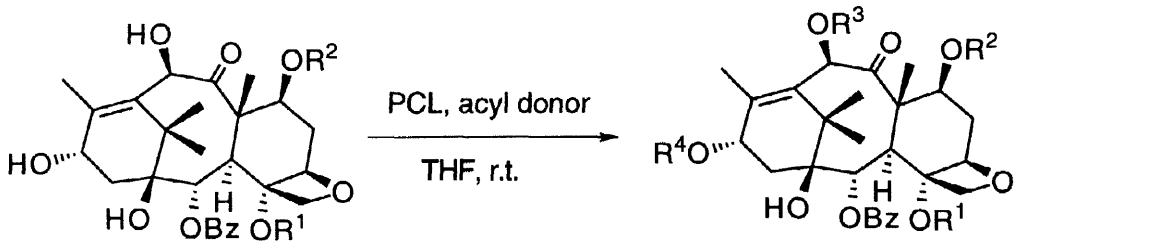
(DCAA) and trichloroacetic anhydride (TCAA), showed favorable reactivity and selectivity. Less activated vinyl acetate and acetic anhydride were unreactive and more activated trifluoroacetic anhydride was too reactive to be selective. Thus, we chose PCL as the catalyst and DCAA and TCAA as the acylating agents for further systematic studies.



2 R^1 = Ac; R^2 = H
3 R^1 = Ac; R^2 = TES
4 R^1 = H; R^2 = TES

5 R^1 = Ac; R^2 = R^3 = DCA; R^4 = H
6 R^1 = Ac; R^2 = TCA; R^3 = R^4 = H
7 R^1 = Ac; R^2 = TES; R^3 = H; R^4 = DCA
8 R^1 = Ac; R^2 = TES; R^3 = H; R^4 = TCA
9 R^1 = H; R^2 = TES; R^3 = H; R^4 = DCA

In a typical experiment,[6] the heterogeneous solution containing substrate (**2-4**), acylating agent (9 equiv.), triethylamine (0-12 equiv.), and enzymes (1 mg/mg substrate) in THF was stirred at r.t. and the reaction progress was followed by tlc analysis. When the reaction was complete, the products (**5-9**) were isolated by silica gel chromatography.[7] The acylated positions of the products were identified on the basis of the analysis by ^{1}H NMR spectroscopy including the *HH* and *CH* COSY experiments. The results are described in Table 1.

Table 1. PCL-catalyzed acylation of **2-4**.[a]

substrate	acyl donor	additive	rxn time (h)	product	selectivity	yield (%)
2	DCAA	-	12	**5**	7,10	88
	TCAA	-	8	**6**	7	73
3	DCAA	Et_3N	12	**7**	13	86
	TCAA	Et_3N	12	**8**	13	84
4	DCAA	-	12	**9**	13	98

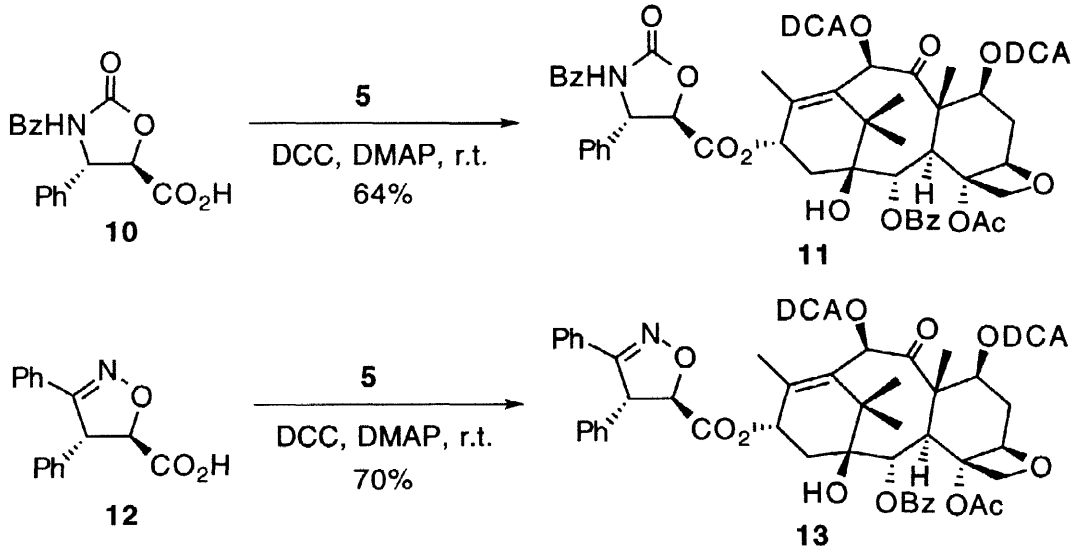
[a]TES, triethylsilyl; DCA, dichloroacetyl; TCA, trichloroacetyl; DCAA, dichloroacetic anhydride; TCAA, trichloroacetic anhydride.

In the enzymatic reaction of 10-DAB (**2**) using DCAA as the acylating agent, both 7- and 10-hydroxyl groups were acylated and the 13-hydroxyl group remained unreacted. It was observed that the diacylation reaction took place in a random sequence, indicating that the two hydroxyl groups at C-7 and C-10 are similarly reactive with DCAA. In the reaction using TCAA as the acylating agent, however, only the monoacylated products were obtained: the 7-acylate,

the major product (yield, 73 %), and the 10-acylate, the minor product (yield, less than 10 %). This result suggests that the 7-hydroxyl group is significantly more reactive than the 10-hydroxyl group with TCAA. At this time, the reason is unclear why the second acylation is depressed when more activated TCAA is used as the acylating agent.

In the enzymatic reactions of 7-silylated **3**, surprisingly, the chemically least reactive 13-hydroxyl group was acylated and the 10-hydroxyl group remained unreacted with both DCAA and TCAA. It is noted that these acylation reactions require triethylamine as an additive to promote the reaction rate.[8] The C-13 selective acylation was also observed in the enzymatic reaction of 7-silylated and 4-deacetylated **4**. These results indicate that when C-7 is protected with a sterically bulky group such as TES the 10-acylation is depressed and the 13-acylation activated. However, it is unclear why the 13-acylation is strongly favored. Overall, these studies have shown that the 13-hydroxyl group can be selectively protected in the presence of the unprotected 10-hydroxyl group.

Enzymatically acylated 10-DAB can be utilized in the synthesis of paclitaxel analogs. As illustrative examples, the diacylated product **5** was coupled with a C-13 side chain equivalent **10** and its isostere **12**, respectively, in the presence of DCC and DMAP to afford **11** and **13** in 64% and 70% yields.



In summary, the work described in this manuscript has demonstrated that 10-DAB and 7-TES-10-DAB can be selectively acylated using the lipase from *Pseudomonas cepacia* and DCAA or TCAA as the acylating agent. The 7-selective acylation of 10-DAB can be achieved using TCAA as the acylating agent. The 7,10-selective diacylation can be achieved using DCAA. The 13-selective acylation of 7-TES-10-DAB, which is difficult to achieve chemically, can be accomplished using either DCAA or TCAA. We have also shown that the 7,10-diacylated product can be coupled chemically with C-13 side chain equivalents for the synthesis of paclitaxel analogs. Finally, it is noted that it will be interesting if the coupling can be performed enzymatically. Our research efforts toward this end are under progress in our laboratory.

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6. **A representative experimental procedure**: to a solution of **3** (52.7mg, 0.08 mmol) in 1 mL of distilled THF was added PSL (50mg), Et_3N (0.134mL, 12 eq.), and DCAA (0.115mL, 6 eq.). The resulting heterogeneous mixture was allowed to stirr at room temperature until the starting material was disappeared on TLC. The solution was filterd on celite to remove the enzymes and then subjected to flash chromatography to yield **7** (53.1mg, 0.07mmol) in 86% yield.
7. All the ^1H and ^{13}C NMR data are satisfactory. HRMS: **5** Calcd for $C_{33}H_{36}O_{12}Cl_4Cs$ (M + Cs): 897.0015. Found: 897.0052; **6** Calcd for $C_{31}H_{36}Cl_3O_{11}Cs$ (M + Cs): 689.1323. Found: 689.1328; **7** Calcd for $C_{37}H_{51}O_{11}C_{12}Si$ (M + H): 769.2578 Found: 769.2570; **8** Calcd for $C_{37}H_{49}O_{11}Cl_3SiNa$ (M + Na): 805.2489 Found: 805.2522.
8. No detectable chemical acylations due to the presence of triethylamine were observed for 1 day in the control reactions without enzymes.