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Trifluoroacetic Acid-Mediated Cleavage of a Triethylsilyl Protecting Group: Application in the Final Step of the Semisynthetic Route to Paclitaxel (Taxol)

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Abstract:

The final step of the semisynthetic route to paclitaxel involves cleavage of the triethylsilyl (TES) protecting group from the C-7 hydroxyl group. Paclitaxel is an extremely complex molecule, and standard deprotection conditions led to formation of several impurities. Trifluoroacetic acid in aqueous acetic acid was found to be very effective in the cleavage of the TES group without compromising the quality of the product.

Introduction

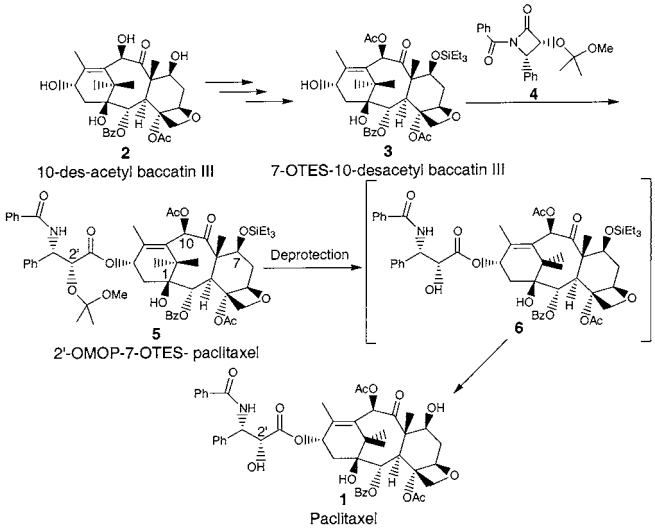
Paclitaxel **1** (Taxol) is a diterpene natural product extracted from the bark of the pacific yew tree, *Taxus brevifolia*.[1] Taxol is an established anticancer drug whose mode of action involves inhibition of cell replication in the mitotic phase of the cell cycle by promoting polymerization of microtubules, which are abnormally stable to depolymerization.[2]

The original manufacturing route was based on Holton's work on the semisynthesis of paclitaxel.[3] In this route, 10-desacetyl baccatin III (10-DAB **2**) is acetylated and silylated to give intermediate **3**. Coupling with the protected β-lactam **4** produces the penultimate **5**. The final step involves the removal of C-7 TES and C-2′ methoxypropyl (MOP) groups to afford paclitaxel **1** (Scheme 1). Herein, we describe the development of the deprotection reaction conditions leading to the manufacture of high-quality paclitaxel in good yield.

Results and Discussion

While the MOP protecting group is easily removed under very mild acidic conditions, yielding intermediate **6**, more stringent reaction conditions are required to remove the TES group from C-7. Although several methods for cleaving silyl ethers have been reported,[4] when applied to **5**, most of these procedures generated significant amount of impurities.

Scheme 1



Several of these (compounds **7**−**12** in Figure 1) have been isolated and characterized.

Development of Reliable Deprotection Conditions. Numerous conditions for the conversion of **5** to **1** were screened. From this screening, three suitable reaction conditions were identified and used to prepare small amounts of paclitaxel: (i) 1.5 M HCl/EtOH−THF at 0 to 5 °C, (ii) 2 M HCl/MeOH at 0−5 °C, and (iii) 48% HF−pyridine/acetonitrile at 0−5 °C.

These procedures were unsuitable for scale-up and in the manufacturing setting. The HCl/EtOH−THF and HCl/MeOH procedures afforded good-quality product after crystallization, but they suffered from the following disadvantages: (i) the levels of **6** (partially deprotected intermediate) and **7** (over-reacted product) in the reaction mixture ranged from 0.5 to 1%, and any attempt to further lower the level of **6** resulted in an increase in the level of **7**; (ii) the reaction was very sensitive to any changes in temperature—for example, a slight increase in the reaction temperature resulted in higher levels of **7**; and (iii) The reaction time (>22 h) was less than optimal for scale-up. The 48% HF−pyridine procedure provided very good quality product; however, engineering and safety issues related to the use of HF on a manufacturing scale precluded its consideration for scale-up.

Since deacetylation (impurity **7**) was the major degradation pathway, we hypothesized that by using acetic acid

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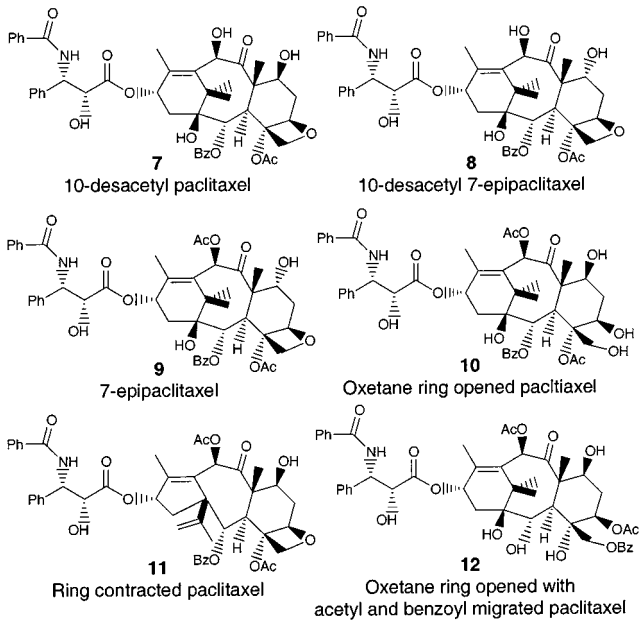

Figure 1. **Structures of impurities in the acid-catalyzed desilylation of 5.**

Table 1. **Effect of various acids and solvents on the HPLC profile of the reaction mixture**

entry	acid	equiv	solvent	time (h)	AP[a] of **1**	AP of **6**	AP of **7**
1	**TFA**	**5**	**HOAc**	**6**	**97.2**	**0.19**	**0.24**
2	TFA	10	EtOH	30	95.3	1.9	1.1
3	TFA	10	Acetone	24	97.2	0.1	0.95
4	**TCA**	**5**	**HOAc**	**4**	**95.4**	**1.7**	**0.3**
5	TCA	10	HOAc	4	94.7	0.46	0.47
6	TCA	10	Acetone	21	95.9	0.4	1
7	**HCl**	**0.5**	**HOAc**	**5**	**96.2**	**0.72**	**0.23**
8	HCl	1	HOAc	4	93.5	0.62	0.22
9	HCl	5	Acetone	18	92.6	0.34	1.2
10	H_2SO_4	0.7	HOAc	9	95.3	0.4	0.6

[a] AP = HPLC area %.

(HOAc) as the reaction solvent, this side reaction could be suppressed, as per le Chatalier's principle.[5] Aqueous acetic acid has been used for the deprotection of silyl ethers at higher temperatures.[4] However, in our hands, these reaction conditions did not afford acceptable-quality product. We screened a number of strong organic and mineral acids under a variety of reaction conditions, using HOAc, EtOH, and acetone as reaction solvents. All reactions were conducted at ambient temperature. Table 1 provides those reaction conditions where the pacltitaxel HPLC area % was at least 92.

The results show that the reaction time was shorter when the reaction was conducted in HOAc (entries 1, 4, 5, 7, and 8). In other solvents, the reaction time was much longer, even with additional acid (entries 2, 3, 6, and 9). While the formation of **7** was suppressed when trifluoroacetic acid (TFA), trichloroacetic acid (TCA), or 1 M HCl in acetic acid

(5) le Chatelier's principle states that, "if, to a system in equilibrium, a stress is applied, the system will react so as to relieve the stress". (a) Thomsen, V. E. *J. Chem. Educ.* **2000**, *77*, 173. (b) Raviolo, A. *J. Chem. Educ.* **2001**, *78*, 629.

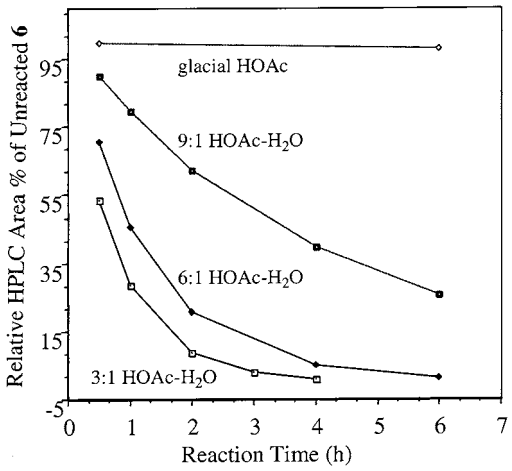

Figure 2. **Effect of water on the rate of deprotection.**

were used (entries 1, 4, and 7), a somewhat higher level of **7** was observed with 1 M H_2SO_4 (entry 10). The data confirmed the hypothesis that the deacetylation pathway would be suppressed in aqueous HOAc. To the best of our knowledge, this simple and novel use of TFA (or TCA) in aqueous HOAc for the deprotection of TES has not been reported.[4,6]

Since equipment trains in many manufacturing sites are made of stainless steel, TFA- and H_2SO_4-based processes were considered more suitable for manufacturing because both acids are noncorrosive. Since the TFA process afforded a cleaner reaction profile than the H_2SO_4 process, efforts were directed toward developing the former process.

Effect of Water on the Rate of Deprotection. The reaction was conducted in the presence of 5 equiv of TFA using various ratios of acetic acid to water. Figure 2 shows that the rate of reaction is directly proportional to the amount of water present in the reaction mixture. However, if the amount of water exceeded a 3:1 (v/v) ratio of HOAc−H_2O, the intermediate **6** precipitated out of the reaction mixture, and the reaction stopped. Thus, we selected 3:1 (v/v) HOAc−H_2O for further development work.

Quenching of the Reaction Mixture. The deprotection reaction mixture degrades with the formation of **7** as the major impurity and different amounts of impurities **8**−**12** (Figure 1) if it is not neutralized at the end of the reaction. For scale-up, it is important to have a stable reaction mixture to allow sufficient time for further processing. Therefore, experiments were conducted to find a suitable additive to quickly neutralize the reaction mixture at the end of the reaction. While the use of sodium carbonate or sodium bicarbonate led to formation of impurities **8** and **9**, sodium acetate (NaOAc), triethanolamine, and diisopropylamine were found to quench the reaction effectively. Addition of these reagents at the end of the reaction prevented further degradation of the product for at least 24 h at ambient temperature. NaOAc was preferred as the reaction quench due to its innocuous nature. The graph in Figure 3 shows the relationship between the amount of NaOAc used as the

(6) Singh, A. K.; Weaver, R. E.; Powers, G. L.; Rosso, V. R. U.S. Patent 6,-184,395, 2001.

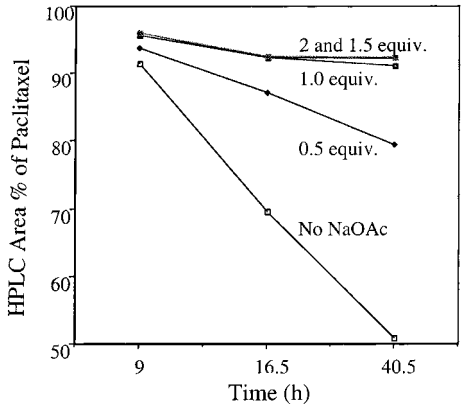

Figure 3. **Stability of paclitaxel in the reaction mixture in the presence of different amounts of NaOAc at 35 °C.**

quenching reagent and the HPLC area % of paclitaxel. The optimal amount of NaOAc required to quench the reaction mixture was 1 equiv with respect to the moles of acid used.

Conclusions

Although many reaction conditions are known for the cleavage of silyl ethers, when applied to paclitaxel penultimate, they led to formation of several impurities. A novel desilylation reaction condition using trifluoroacetic acid in aqueous acetic acid was identified. This reaction condition was optimized to afford paclitaxel in high yield and quality.

Experimental Section

General. Glacial acetic acid, TFA, and $NaOAc{\cdot}3H_2O$ are commercially available and were used as-is. Dichloromethane and IPA were used without any further purification. Moisture content was determined by coulometric titration on a Mitsubishi CA-06 moisture meter on a weight/volume basis. Proton and carbon NMR were run on a Bruker AC-300 spectrometer at 300 MHz for proton and 75 MHz for carbon in $CDCl_3$. HPLC was run under the following conditions: Apex octadecyl, 5 μ, 150 mm × 4.6 mm i.d.; column temperature 30 °C; flow rate 1.0 mL/min; λ 227 nm; injection volume 20 μL; mobile phase A, 50:50 CH_3CN−water; mobile phase B, CH_3CN; gradient program 0−10 min (100% A), 10−30 min (linear gradient to 100% B), 30−35 min (100% B), 35−45 min (linear gradient to 100% A); retention times in minutes 7 (**1**), 32 (**5**), 28 (**6**), 4 (**7**), 9 (**9**).

Safety Considerations. Paclitaxel is a cytotoxin, mutagen, and a potential carcinogen. All operations must be conducted in a containment room. A solution of KOH in methanol has been found to effectively degrade paclitaxel.

Preparation of 1. Penultimate **5** (40 g, 37.3 mmol, corr. for purity) was dissolved in glacial acetic acid (355 mL) at ambient temperature. Separately, a solution of TFA (23.4 g, 205.1 mmol, 5.5 equiv) was prepared in glacial acetic acid (89 mL) and water (120 mL). The TFA solution was added to the solution of **5** in acetic acid over a few minutes. The progress of the reaction was followed by an in-process reversed phase HPLC method. At the end of the reaction (ca. 7 h), the reaction mixture was quenched with a solution of $NaOAc{\cdot}3H_2O$ (30.5 g, 228.3 mmol, 6 equiv) in water (66 mL). Dichloromethane (400 mL) and water (266 mL) were added, and the biphasic solution was stirred for 15 min. After the phases were separated, dichloromethane (268 mL) was added to the upper spent aqueous phase and stirred for 15 min. After phase separation, the rich organic layers were combined and washed with water (3 × 475 mL). The rich dichloromethane solution was solvent-exchanged into IPA to a final volume of 625 mL. The solvent exchange was conducted as follows. The rich dichloromethane solution was concentrated to a minimum volume (about 100 mL) under atmospheric pressure. About 800 mL of IPA was added, and the distillation was continued at pot temperature no more than 40 °C until the final volume of about 625 mL was reached. The moisture content of the solution was checked and then adjusted to 2−4% (w/v) by adding water. The solution was heated to 50−55 °C to dissolve any product which may have come out during solvent exchange. The solution was cooled to 40−45 °C to initiate crystallization. The thin crystal slurry was further cooled to 30−35 °C and stirred for several hours. The crystal slurry was cooled to 0−5 °C and stirred for at least 2 h. The slurry was filtered, washed with cold IPA, and dried under vacuum at <50 °C to a constant weight (yield 26.3 g, 80 M%, corr. for purity, HPLC area % >99). The proton and carbon NMR spectra were identical to those of an authentic sample.

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