# Application of Immobilized Lipase in Production of Camptosar (CPT-11)

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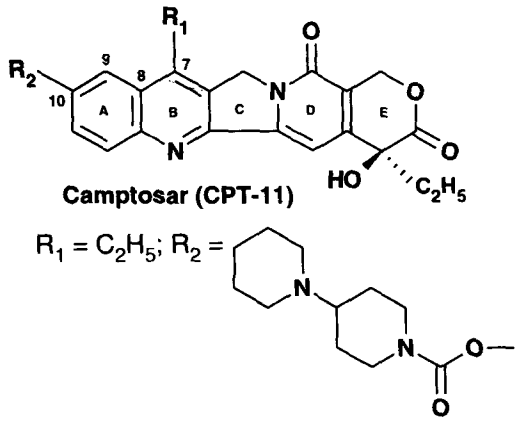
**ABSTRACT:** Lipase from *Pseudomonas cepaica* (Amano, PS-30) was immobilized on celite and used in organic solvent for the selective acylation of a key alcohol intermediate. The compound was transformed in the synthesis to the anticancer drug Camptosar (CPT-11). Catalyst activity was influenced by the water content and method used to dry the catalyst. This resolution has been conducted on production scale with equal weight of recyclable catalyst.
*JAOCS 73,* 1377–1378 (1996).

**KEY WORDS:** Anticancer drug, Camptosar, immobilized PS-30 lipase, selective acylation.

Camptosar (CPT-11, irinotecan hydrochloride) is currently being used in Japan by intravenous injection for the treatment of ovarian cancer. In 1994, Upjohn acquired a license from Yakult Pharmaceuticals to sell and manufacture this drug in the United States. At the present time, the bulk drug is obtained semisynthetically from the natural source, camptothecin ($R_1 = R_2 = H$) (see Scheme 1).

In the literature, several syntheses of camptothecin are described. We adapted Shen *et al.*'s approach (1) for the synthesis of CPT-11 (see Scheme 2). In this route, the chiral tricyclic lactone **1** became the target molecule. In our synthetic route (2), we realized that diol **2** could be readily transformed in three steps to **1**. The remaining steps leading from **1** to CPT-11 would then follow published procedures (1,3).




SCHEME 1

Therefore, we focused our attention on the lipase-mediated resolution of diol **2**. The reaction conditions are: (i) catalyst 100 g—PS-30 (Amano) + Celite 521, 1:1 by weight; (ii) 100 g (0.25 mol) substrate, 2.5 L MTBE, 75 mL (0.68 mol) IPA; (iii) reaction time 1 d (reaction time increased by 1 d with each recycle; the catalyst was recycled three times), room temperature; (iv) isolated yield of (S)-10 CPT alcohol 40 g, ee >99% (Scheme 3). The percentage of product and substrate was determined on a Chiralpak AD column, with 10% isopropanol in hexane at a flow rate of 1 mL/min and UV detection at 294 nm.

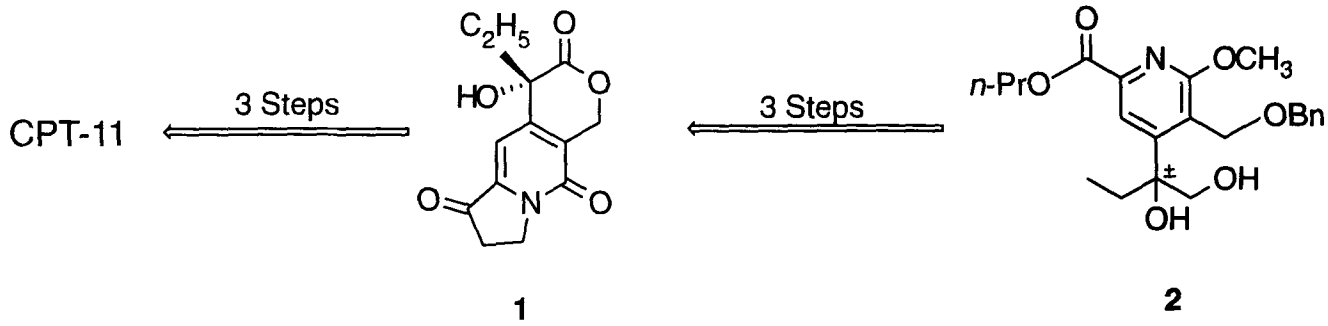
The celite-supported PS-30 catalyst was prepared in drying tray(s) by thoroughly mixing 2 kg of Celite 521 and 2 kg of PS-30 (Amano). The resulting paste was first placed in an oven at 40°C under nitrogen purging to evaporate some of the water and then dried under vacuum at 40°C for 2–3 d. We have discovered that the drying time is not critical for catalyst activity. PS-30 celite-supported catalyst can be stored under vacuum and 40–50°C for several weeks without loss in activity. The only times at which we have observed a decrease in catalyst activity (slower reaction rate) were in those reactions in which the catalyst contained >1% water by KF analysis. In our hands, our celite PS-30 catalyst cannot be deactivated by drying at 40–45°C with conventional laboratory techniques, i.e., nitrogen and house vacuum.

With regard to catalyst drying technique, we have found a notable difference. If our PS-30-celite water mixture was dried by lyophilization (freeze-drying) to the same water content as obtained by vacuum or nitrogen drying, the catalyst was nearly inactive, i.e., when using the conditions in Scheme 3, <5% acylation occurred after 1 d reaction time.

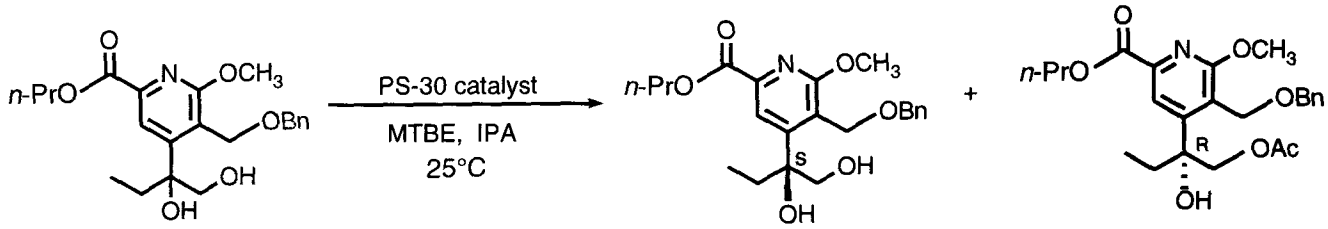
**TABLE 1**
**Increase in Selectivity as Measured by Enantiometric Ratio (E value)**

| Percentage water added | Reaction time | $ee_s$[a] | $ee_p$ | Percentage conversion | E value |
|---|---|---|---|---|---|
| 0.00 | <1 d | 0.72 | 0.87 | 45 | 32 |
| 0.32 | <2 d | 0.79 | 0.93 | 46 | 68 |
| 0.64 | <2 d | 0.65 | 0.94 | 41 | 66 |
| 0.96 | <4 d | 0.74 | 0.90 | 45 | 44 |

[a]ee = enantiomeric excess.




SCHEME 2




SCHEME 3

The addition of water in the lipase acylation reaction led to a modest increase in selectivity, as measured by the enantiomeric ratio [E value (4)]. The results are shown in Table 1. We reached maximum selectivity when 0.32–0.64 mol% of water (with respect to substrate) was added to the reaction. There are practical advantages in production for this increase in selectivity. With the addition of exogeneous water, reaction times can be controlled, and the acylation reaction can be run for several days without significant overreaction.

There are several advantages in using lipase technology for the resolution of chiral drugs in the pharmaceutical industry. Lipase-mediated acylation of alcohols is easy to carry out on kilogram quantities and does not require special equipment or handling. The biocatalyst is cost effective (can be recycled several times), and unlike chemical catalysts, which frequently utilize hazardous and difficult-to-dispose heavy metals, immobilized lipases present no environmental problems.

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[Received July 10, 1996; accepted August 7, 1996]