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Preparative Chromatography with Extreme Productivity: HPLC Preparation of an Isomerically Pure Drug Intermediate on Multikilogram Scale

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Abstract:

A highly productive method for HPLC preparation of 7.5 kg of an isomerically pure drug intermediate is described. The method employs an IPA/heptane eluent with a Chromegabond Nitro stationary phase, and affords an unusually large productivity of 5.5 kkd (kilograms of desired product per kilogram of stationary phase per day). Details relating to the development and execution of the separation are provided, and possibilities for further improvements are discussed.

Introduction

Preparative chromatography is a valuable tool for isomeric purification and is routinely used in early-stage process research in these laboratories, especially for the chromatographic resolution of enantiomers.[1,2] The principal advantage of the chromatographic approach stems from the ability to rapidly develop and execute a purification with minimal labor.[3,4] At times, preparative chromatography can form the basis for manufacturing processes, the defining example perhaps being the separation of *p*-xylene from related C-8 isomers, *o*-xylene and ethylbenzene. In this process, a single simulated moving bed (SMB) chromatographic installation with an 8 m i.d. column containing 1800 t of adsorbent is used to produce more than 700,000 t of highly purified *p*-xylene per year[5,6] which translates to a productivity of about 1 kg of final product per kilogram of stationary phase per day (1 kkd). In these laboratories, pulse injection chromatographic separations of isomer mixtures with productivity as low as 0.05 kkd are occasionally used to support early development, with more productive methods in the 0.3−1.0 kkd range being generally preferred, and rare examples in the 2−3 kkd range being occasionally encountered. In this study, we report the development of a pulse injection HPLC method with productivity in excess of 5.5 kkd, and use of this method in the preparation of 7.5 kg of a stereoisomerically pure intermediate for a pharmaceutical development compound.

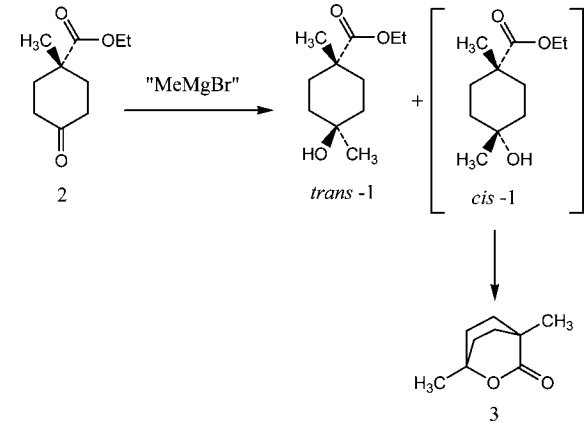
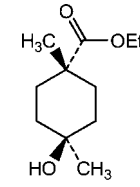


Figure 1. **Methyl Grignard addition to cyclohexanone, 2, affords 1 as a 2.2:1 mixture of *trans/cis* isomers, with cis-1 spontaneously lactonizing upon workup. Optimal conditions: conventional addition of MeMgCl to a THF/toluene solution of the cyclohexanone at −30 °C.**

Results and Discussion

As part of a recent development project, we were faced with the challenge of preparing multikilogram quantities of isomerically pure *trans*-**1**, an early intermediate in the preparation of a pharmaceutical development candidate. Rapid access to this relatively simple molecule in isomerically pure form proved a somewhat formidable stereochemical challenge.



trans -1

Retrosynthetic analysis of *trans*-**1** suggests a simple preparation via addition of a methyl Grignard equivalent to readily available ketone, **2** (Figure 1). Investigation of a variety of reagents and conditions for this transformation afforded at best a 2.2:1 ratio of the *trans*/*cis* isomers, the relatively modest stereoselectivity likely being attributed to the lack of a defined conformational preference in cyclohexanone, **2**. Upon workup, undesired *cis*-**1** spontaneously cyclizes to afford bicyclic lactone **3**, offering the interesting possibility of accessing isomerically pure *trans*-**1** by removal of the lactone, **3**, a compound that could be expected to have significantly different physical properties.

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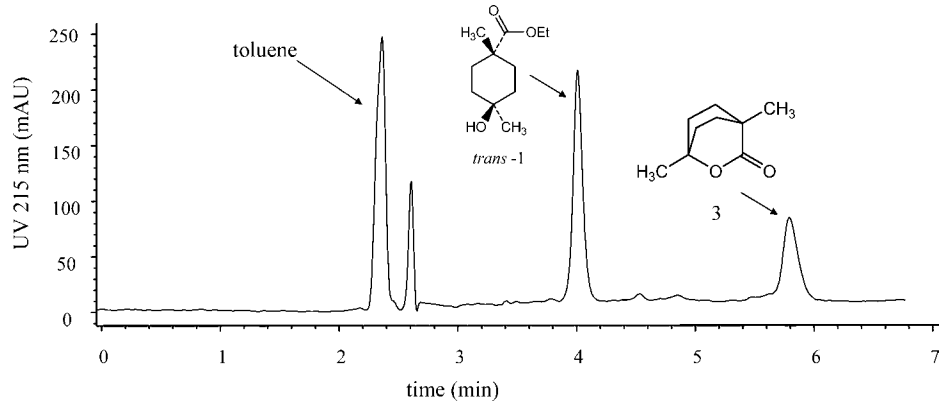

Figure 2. **Gradient analytical SFC screening affords excellent separation of crude mixture of *trans*-1 and bicyclic lactone, 3, on Chromegabond Nitro stationary phase. Conditions: outlet pressure 200 bar, column temperature 35 °C; 1.5 mL/min methanol in carbon dioxide, gradient of 4% methanol for 4 min, then ramp to 40% methanol @ 2%/min with 3 min hold.**

Conventional purification approaches such as silica or reversed phase chromatography proved fairly ineffective for the separation of *trans*-**1** and bicyclic lactone, **3**; however, screening of the mixture by supercritical fluid chromatography (SFC) showed an unusually large separation with the Chomegabond Nitro stationary phase (Figure 2). A small amount of residual toluene in the crude reaction mixture appears as a large, early-eluting peak owing to the comparatively poor UV chromophores of *trans*-**1** and lactone **3** at the observe wavelength of 215 nm. Interestingly, lactone **3** is more strongly retained on the stationary phase than *trans*-**1**, opposite what might be expected based on a simple accounting of polar groups of the two molecules. This result is somewhat surprising, and may stem from the fact that the polar ester and alcohol groups in *trans*-**1** are relatively inaccessible, owing to steric encumbrance, with little possibility for simultaneous interaction of both groups with the stationary phase. In contrast, the oxygen lone pair electrons of bicyclic lactone, **3**, are considerably more exposed and available for interaction with the stationary phase, perhaps with the electron-deficient dinitrobenzene ring of the stationary phase. Whatever the reason underlying the greater retention of bicyclic lactone, **3**, on this stationary phase, the results clearly indicate the possibility of facile chromatographic purification of the desired *trans*-**1**.

Small-scale semipreparative SFC purification of 50 g of the crude mixture of *trans*-**1** and bicyclic lactone, **3**, was carried out by isocratic elution with 10% EtOH/CO_2 on a 2.1 cm i.d. column, using automated injection and fraction collection, and conveniently afforded *trans*-**1**, with high purity (Figure 3). An extremely high productivity of 3.5 kkd (kilograms of purified product per kilogram of stationary phase per day) was obtained in this SFC purification, with every indication that loading could be further increased. A chromatographic productivity of 3.5 piqued our interest in the possibility of larger-scale implementation of this separation, given the difficulty in otherwise accessing pure *trans*-**1**.

Our preferred approach for carrying out separations of developmental compounds at kilogram scale is to use SFC when possible, owing to the advantages in speed and solvent savings often observed relative to the corresponding HPLC approaches. However, at the time that this study was conducted, we had not yet built our capabilities for carrying out SFC separations on larger scale, and such purifications were carried out by HPLC. Suitable conditions were identified using an eluent of 5% IPA/heptane, with a step gradient to 60% IPA/heptane, which improved peak shape and helped to desorb the strongly retained bicyclic lactone, **3**. This HPLC method was employed in a cGMP preparation of 7.5 kg of *trans*-**1** (Figure 4). In this campaign, an 11 cm diameter HPLC column filled with 1.5 kg of the Chromegabond Nitro stationary phase was used to purify 12 kg of crude mixture, affording 7.5 kg of pure *trans*-**1** in 98% purity with 97% recovery. The separation took place over a period of three work days (~22 h instrument time) using a total of 1200 L of solvent, 170 L of which were evaporated to recover the desired product.

A chromatographic productivity of 5.5 kkd was obtained during the campaign, with clear indications that further improvements could be possible. For example, nearly 20% of each cycle is spent simply applying sample to the column using an injector pump with 100 mL/min maximum flow rate − well suited for general separations, but clearly undersized for this separation. Simply applying sample at the higher flow rate of 750 mL/min would decrease cycle time by nearly 4 min, resulting in a productivity of 6.6 kkd. In addition, the step gradient could almost certainly begin at least 2 min earlier in the chromatogram, resulting in a further cycle-time reduction and a corresponding increase in productivity.

Despite the extraordinary productivity of this method, the inability to recycle the undesired isomer makes this process somewhat less than ideal for implementation at the manufacturing scale. Nevertheless, the method did allow rapid and convenient access to multikilogram quantities of the desired *trans*-**1**, effectively eliminating a significant development challenge for this project.

Experimental Section

Chemicals. Methanol, 2-propanol, and *n*-heptane were obtained from EMD Chemicals (Gibbstown, NJ, U.S.A.). Compounds **1**−**3** were obtained from Merck Process Research (Merck Research Laboratories, Rahway, NJ, U.S.A.).

Chromatographic Stationary Phases. The chiral stationary phases used for the column screening study were Chiralpak AD, Chiralpak AS, Chiralcel OD, Chiralcel OJ, and Chiralcel OF (Chiral Technologies, Inc., West Chester, PA, U.S.A.). The CSP for the method optimization, loading studies, and final preparative separation was Chiralpak AD (particle size 20 μm).

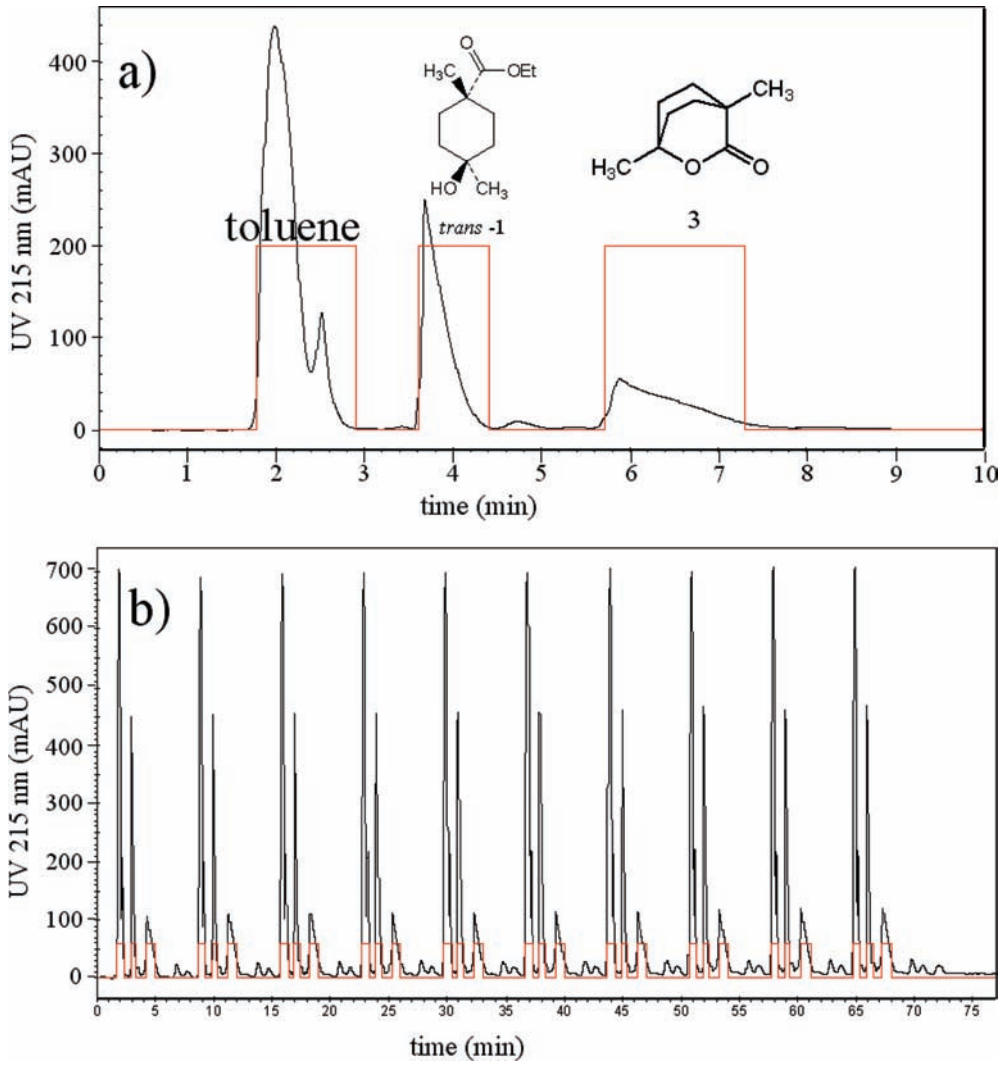

Figure 3. **(a) Semipreparative SFC separation of crude ~1:1 mixture of *trans*-1 and bicyclic lactone, 3. Conditions: Chromegabond Nitro column (2 cm × 25 cm); 50 mL/min; 10% EtOH/CO_2; 100 bar outlet pressure; 35 °C; UV 215 nm; inject 2 mL 2:1 mixture of *trans*-1 and bicyclic lactone, 3, @ 650 mg/mL in ethanol. (b) Automated injections and fraction collection allow separation of 50 g of crude mixture in a single afternoon to afford 20 g of product with >99% purity and a demonstrated productivity of 3.5 kkd.**

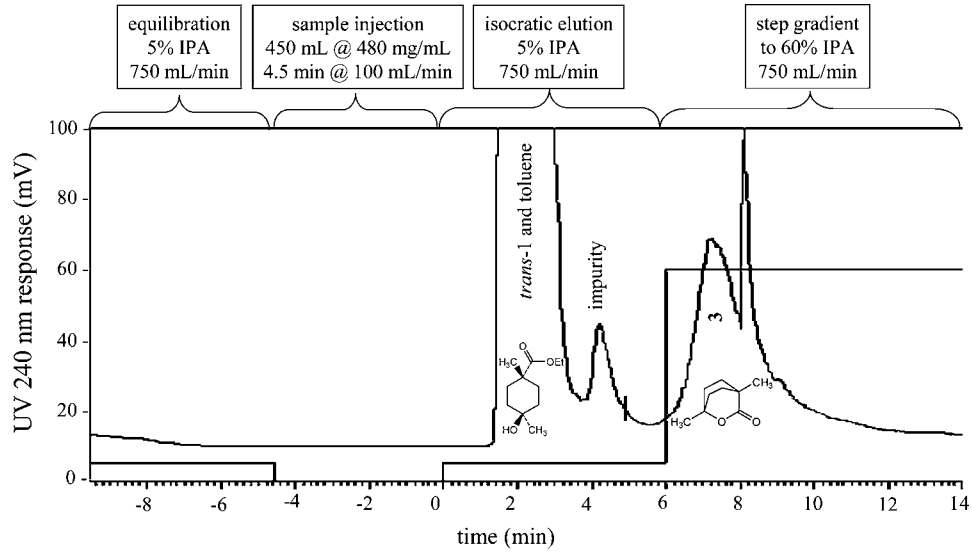

Figure 4. **Preparative HPLC separation of ~2:1 mixture of *trans*-1 and bicyclic lactone, 3, Conditions: Chromegabond Nitro column (11 cm × 25 cm); 5% IPA/heptane equilibration for 5 min, inject 450 mL crude feed product mixture @ 480 mg/mL in heptane (4.5 min @ 100 mL/min), elute with 5% IPA/heptane for 6 min, then step to 60% IPA/heptane for 8 min, flow 750 mL/min except during injection segment, UV 240 nm.**

Chromegabond Nitro stationary phase was obtained from ES Industries, Inc., West Berlin, NJ, U.S.A.

Equipment. *SFC Method Development Screening.* SFC method development screening was carried out using a Berger-Thar analytical supercritical fluid chromatograph (Thar Instruments, Inc., Pittsburgh, PA, U.S.A.) fitted with six-position column-selection valve and Agilent model 1100 diode array UV−visible detector (Agilent Technologies, Palo Alto, CA, U.S.A.). Column screening was carried out using a standard gradient approach described previously.[7]

Preparative HPLC Method Development and Loading Studies. Preparative method development and loading studies were conducted on an Agilent 1100 system with a G1311A quaternary pump, G2260 Prep ALS autosampler, and a G1315B diode array UV−vis detector.

Preparative HPLC. Preparative HPLC separations were carried out using a Varian preparative HPLC system (Varian, Palo Alto, CA, U.S.A.) with PrepStar SD-1 binary pumps, 210 injection pump, 320 UV detector, and a ReSzonator Fluid Module. A Prochrom dynamic axial compression column with dimensions of 11 cm i.d. × 25 cm (Novasep, Boothwyn, PA, U.S.A.) was used for carrying out the kilogram-scale preparative separation.

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Conclusion

A highly productive HPLC method was developed and used in the cGMP production of 7.5 kg of an isomerically pure drug intermediate. The exceptionally high productivity (5.5. kkd) obtained in this separation stems from the spontaneous conversion of the undesired isomer to a bicyclic lactone byproduct, which is strongly retained on the Chromegabond Nitro stationary phase. Several additional opportunities for further improvement in productivity are presented.

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