Organic Process Research & Development **2010**, *14*, 905–907

Shortcut to Selectivity: Make Them All and Let Preparative Chromatography Sort it Out

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Abstract:

Preparative chromatography was used to overcome the difficult selectivity challenge of accessing a single isomer of a mono *tert*-butyldimethylsilyl (TBDMS) derivative of an unsymmetrical diol. Chromatographic purification allowed rapid purification of more than 10 kg of the desired intermediate from the statistical mixture of undesired mono-TBDMS, bis-TBDMS, and very strongly retained, unreacted starting material. A noteworthy injection cycle strategy of performing three injections, then desorbing the accumulated strongly bound diol starting material with a strong solvent wash was employed in the separation.

Introduction

The control of chemical selectivity lies at the heart of modern synthetic chemistry. Recent years have seen the introduction of a vast arsenal of platform reaction technologies for chemoselective, regioselective, diastereoselective, or enantioselective formation of desired products.[1,2] Many of these platforms are so powerful and so predictable as to make possible the *de novo* design of selective syntheses that, following only limited optimization, can often be implemented at scale.[3] Nevertheless, numerous synthetic challenges facing today's pharmaceutical process chemist lie outside the realm of what can be addressed using these existing reaction platforms. In such cases, considerable investigation may be required to develop reaction conditions that afford the needed selectivity. Alternatively, platform purification tools such as crystallization or chromatography can provide the required selectivity, albeit often at the expense of product yield. The interplay between the use of selective reaction technologies and selective purification technologies in the preparation of challenging pharmaceutical targets is an evolving frontier where new strategies are constantly being created and put to the test.

Preparative chromatography is a useful tool for isomeric purification and is often used in early-stage process research in these laboratories, especially for the chromatographic separation of enantiomers.[4−7] A key advantage of the chromatographic approach arises from the ability to rapidly develop and execute a purification with minimal labor.[8,9] The wide variety of available chromatographic stationary phases means that selectivity challenges that are beyond the capabilities of any platform reaction technology can often be easily addressed using chromatography. Consequently, preparative chromatography provides a valuable complement to modern synthetic chemistry, augmenting existing platform reaction technologies to allow comprehensive access to almost any desired product. In addition, chromatography provides a valuable 'safety net', allowing recently developed chemistry to proceed at risk, with the knowledge that off-target selectivity or formation of unanticipated impurities can almost always be corrected, if needed. In this study we describe the integrated use of preparative chromatography and organic synthesis to provide rapid access to a demanding selectivity challenge, enabling the rapid production of more than 10 kg of an otherwise difficult-to-prepare regioisomer for use in the synthesis of a preclinical candidate.[10]

Results and Discussion

As part of the synthesis of a preclinical pharmaceutical candidate, we encountered a challenging reaction selectivity problem in which current platform reaction technologies were poorly suited to the transformation of diol, **1** (an intermediate available from a previous synthesis) to the desired mono-TBDMS derivative, **2** (Figure 1). Investigation of a number of reaction conditions confirmed that, at best, a statistical 1:1:1:1 mixture of desired and undesired TBDMS derivatives (**2** and **3**), bis-TBDMS derivative, **4**, and unreacted starting material, **1**, was obtained. The initial medicinal chemistry synthesis of the preclinical candidate used flash chromatography to access the desired regioisomer. While the expediency of flash chromatography is perfectly suitable for supporting an initial medicinal chemistry synthesis, we were initially reluctant to consider this possibility for a larger-scale synthesis, especially on the tens of kilograms scale.

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(10) Kromasil 5 μ particles 60 Å pore size silica stationary phase was obtained from Eka-Nobel, Bohus, Sweden. Amicon 20 μ irregular silica was obtained from Millipore, Inc., Billerica, MA. 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. The large-scale HPLC campaign was carried out using a Biotage preparative HPLC pumping skid with a 30 cm i.d. NovaSep ProChrom DAC column.

10.1021/op100095e
Published on Web 05/10/2010

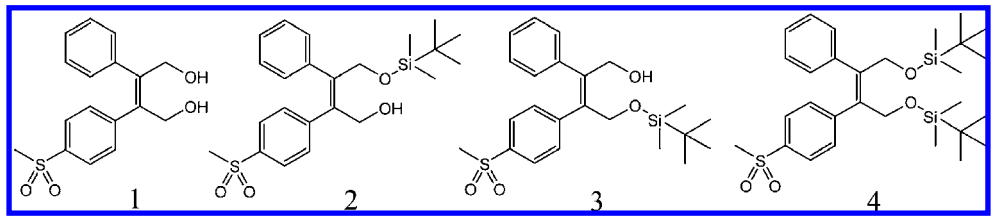

Figure 1. **Attempts to transform diol, 1 into selectively monoprotected TBDMS derivate 2 led to, at best, a 1:1:1:1 statistical mixture of compounds 1, 2, 3, and 4.**

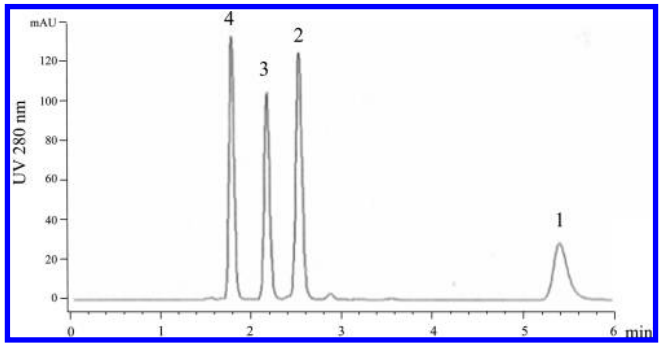

Figure 2. **Chromatographic separation of crude reaction mixture of products coming from the silation reaction. Conditions: Kromasil silica, 5 μ particles 60 Å pore size, 4.6 mm × 250 mm, 40% IPA/heptane, 2 mL/min, room temperature.**

Alternative synthetic routes to **2** that do not originate from **1** can be imagined; however, the ready availability of intermediate **1** and the lack of a truly compelling alternative made us concentrate our efforts on the selective formation of mono-TBDMS intermediate **2** from asymmetrical diol, **1**. Selective differentiation of subtly different diols such as **1** represents a significant synthetic challenge. Enzymes are often capable of such remarkable feats of stereoselectivity, and one could imagine that, in this case, selective acylation (or deacylation) using lipase-type enzymes could afford access to monoprotected **2**, or an equivalent. However, in this instance, initial screening of enzymatic approaches failed to identify viable conditions for accessing **2** in a reasonable time frame.

At this stage we turned our attention to a re-examination of the chromatographic purification of desired monoprotected intermediate, **2**. Figure 2 shows the separation of the four species of interest by HPLC on a silica column. The desired component, **2**, is eluted between two other peaks, which is typically an undesirable scenario. However, the diol starting material, **1**, is very strongly retained on the column in this separation. In fact, the eluent used in this example (40% IPA/heptane) was one of the *worst* conditions for the separation, with many eluents showing the elution of only peaks **2**, **3**, and **4** in a reasonable time frame and the diol peak being very strongly retained on the column. A variety of other columns and mobile phases were also investigated for this separation. While more exotic stationary phases such as Chiralpak AD, Chiralcel OF, Whelko, ES-NPI, ES-Nitro, and Chirobiotic T, showed promise, straightforward separation on inexpensive silica gel was among the best, with eluents such as EtOAc, MTBE, or EtOH in combination with heptane showing the most promising separations for **2**.

Chromatographic productivity, defined as the amount of purified component obtained from a given amount of chromatographic stationary phase in a given time, is a key metric in preparative chromatography, being closely associated with overall cost and complexity of a separation. Typically, the retention time of the most retained component dictates the overall injection cycle time in preparative chromatography, and longer injection cycle time typically leads to poorer chromatographic productivity. In the present case, dealing with the extreme retention of diol **1** required special consideration.

One possible approach to this problem would be to elute the first three components under isocratic conditions and then employ a gradient elution to desorb the fourth component. Employing gradients in preparative chromatography is to be avoided whenever possible, as significant time and solvent must be dedicated to reestablishing the original eluent prior to each new injection, which typically leads to an increase in cycle time and a decrease in productivity.

When one component of the mixture differs dramatically in retention from the others, the possibility of carrying out two different chromatographic separations is another possibility. In the present case, it would be relatively straightforward to chromatographically separate diol, **1**, from the remaining components, and then employ a subsequent chromatographic separation to resolve desired mono-TBDMS derivative **2** from components **4** and **3**. The principal disadvantage of such an approach stems from the simple fact that two different chromatographic steps must be carried out, rather than a single separation. While this represents a fairly small inconvenience on small scale, coordinating multiple pilot-plant campaigns for separations on the tens of kilogram scale is best avoided, if possible. In general, carrying out two chromatographic separations means that two waste streams are generated and two evaporations must be carried out, which again can be cumbersome and slow on pilot-plant scale.

In cases such as this where one of the components is very strongly retained, another option can sometimes be possible: Multiple injections under conditions required for resolution of the desired component from neighboring peaks, with desorption of the accumulated strongly retained component every few injections using a strong solvent system. This approach mitigates to some extent the problems of long cycle times and poor productivity that result if the strongly retained component must be desorbed for each injection. Our previous screening of chromatographic solvent combinations had made us aware of some eluents that afforded good resolution of desired component, **2**, without elution of diol, **1**, and other conditions that lead to fairly rapid elution of all components, including diol, **1**. One drawback to such a 'multiple-injections-then-desorb' approach is that the available binding sites of the column become saturated over several injections, ultimately leading to a decreased ability to perform the required separation, leading

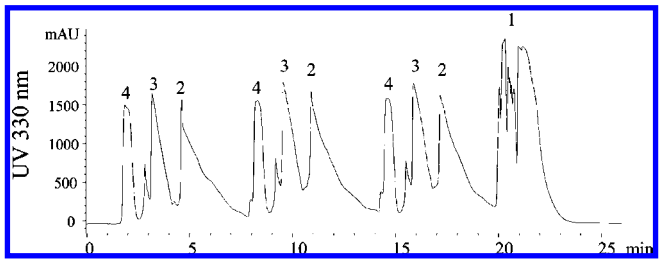

Figure 3. **Preparative chromatography modeled on an analytical column showing three injections of crude mixture under isocratic conditions, followed by desorption with a stronger solvent to remove adsorbed diol, 1. Conditions: Kromasil (4.6 mm × 250 mm), 5 μm/60 Å particles, 2 mL/min 40% EtOAc/heptane for 18 min, then 100% EtOAc for 4 min. Inject 500 μL @ 200 mg/mL (100 mg) in 1:1 EtOAc/heptane every 6 min, productivity ~2 kkd.**

to 'breakthrough' of the undesired component. We studied several possible solvent systems to develop conditions that would be robust enough to be used for the 40-kg campaign. Figure 3 shows one such method in which 100 mg of crude reaction mixture is injected onto a 4.6 mm i.d analytical column every 6 min for three injections using a mobile phase of 40% EtOAc in heptane, followed by a desorption of the strongly bound diol, **1**, at 18 min using 100% EtOAc. This method shows a chromatographic productivity of about 2 kkd[11] (kilograms of purified product per kilogram of stationary phase per 24 h day), and a specific solvent consumption of about 670 L of solvent per kilogram of desired product, which is quite good for a separation where the maximum possible recovery is only 25%. Furthermore, the strategy of employing a cycle of three injections between each desorption nearly doubles the productivity that would be obtained were the strongly retained diol desorbed on every injection. Interestingly, we found that with an 80% MTBE/heptane-based mobile phase, we could perform five injections before desorbing with EtOH or EtOAc, but the overall productivity of the EtOAc-based method was superior.

We next turned to a more detailed modeling in anticipation of the actual 40+ kg pilot plant campaign using a 30 cm. i.d. column. As this larger column requires more than 11 kg of stationary phase, and we did not at that time have a ready supply of the high-quality, small-particle, spherical silica used in our modeling study, we investigated the utility of inexpensive, larger-particle, irregular silica for the separation. Our studies showed that irregular Amicon 20 μm silica particles afforded much the same chromatography as we had already observed, although the decreased efficiency of this column forced us to inject only about half the sample per injection, while running at only half the flow rate. In addition, we utilized a feed solution of 200 mg/mL of the crude reaction product in a solvent mixture of 1:4:2 THF/IPAc/toluene, rather than the 1:1 EtOAc/heptane mixture that had been used in our initial studies. Despite these limitations, we were able to develop a very comparable method affording a productivity of about 0.57 kkd, which was implemented successfully in the pilot plant. Beginning with 31 kg of diol **1**, we were able to prepare the statistical mixture of TBDMS derivatives and employ the above-described preparative HPLC approach to obtain 10.8 kg of desired mono-TBDMS compound, **2**, in >98.5% LC area percent.

This chromatographic route was well suited for rapid preparation of the desired mono-TBDMS compound, **2**; for early clinical studies, however, a more efficient method would probably be required for larger-scale implementation. In addition to the exploration of completely new synthetic routes, further optimization of the chromatographic route may be possible. For example, the current route would most likely be improved by using the previously demonstrated more productive method employing smaller-particle, spherical silica. In addition, the maximum 25% yield of the current approach could potentially be improved by recycle and rederivatization of the undesired chromatographic fractions, potentially affording the ideal situation where all incoming starting material is eventually transformed into desired product (*cf.* the industrial chromatographic process for preparation of *p*-xylene[12]).

Conclusion

Preparative chromatography was used to overcome the difficult selectivity challenge of accessing a single isomer of a mono TBDMS derivative of an unsymmetrical diol. Chromatographic purification allowed rapid purification of more than 10 kg of the desired intermediate from the statistical mixture of undesired mono-TBDMS, bis-TBDMS, and strongly retained unreacted starting material. A noteworthy strategy of performing three injections, then desorbing the strongly bound diol starting material with a strong solvent wash was employed in the separation.

Received for review April 7, 2010.

OP100095E

(11) (3 injections per cycle × 100 mg per injection × 25% desired component per injection) /(2.10 g stationary phase in analytical column × 25 min per cycle) = 2.1 kkd.

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