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Purification of Difluoromethylornithine by Global Process Optimization: Coupling of Chemistry and Chromatography with Enantioselective Crystallization

Scott R. Perrin,*,† Wilhelm Hauck,† Elias Ndzie,‡ Jean Blehaut,§ Olivier Ludemann-Hombouger,§ Roger-Marc Nicoud,§ and William H. Pirkle||

NOVASEP, Inc., 23 Creek Circle, Boothwyn, Pennsylvania 19061, Novartis Pharma AG, Litchstrasse 35, CH-4056, Basel, Switzerland, NOVASEP SAS, Site Eiffel, Boulevard de la Moselle, F54340, Pompey, France, and School of Chemical Sciences, University of Illinois, Urbana, Illinois 61801, U.S.A.

Abstract:

An industrial process for the purification of metric tons of enantiomerically pure difluoromethylornithine (DFMO HCl) is described. The amino acid DFMO HCl is cyclized to form the lactam, which is acylated with pivaloyl chloride to form *rac*-*N*-pivaloyl-DFMO lactam (4). The lactam 4 provides enhanced separation compared to a direct resolution of racemic DFMO HCl (1). A hybrid chiral resolution process is proposed to separate the enantiomers of 4. This process involves a multicolumn continuous enantioselective chromatographic process (VARI*COL*) coupled with enantioselective crystallization of (D)-*N*-pivaloyl-DFMO lactam 5. The interest of this hybrid process is based on a favorable eutectic point providing a higher productivity of the VARI*COL* process and lower purification costs than the chromatographic process alone. A final chemical modification (hydrolysis) is used to form the single enantiomers of both (D)-DFMO (6) and (L)-DFMO in high chemical purity and enantiomeric excess. A global optimization approach is applied to design an economical industrial process, which is based on a parametric study of the VARI*COL* process and enantioselective crystallization to obtain maximum recovery and purity while significantly lowering the cost of manufacturing the single enantiomers. A detailed description of the global process optimization is presented.

Introduction

Difluoromethylornithine (DFMO) (**1**) also known as Eflornithine, Ornidyl, or RMI-71782 is an irreversible inhibitor of the enzyme ornithine decarboxylase (ODC), which decarboxylates ornithine into putrescine and is one of the key enzymes for the biosynthesis of polyamines. Enzyme inhibitors have been developed for several enzymes involved in polyamine metabolism. Both enantiomers form enzyme-inhibitor complexes with ODC. The rate of the irreversible reaction in ODC inactivation is similar for both the (L)- and (D)-enantiomers. The (D)-enantiomer **5** may have advantages, such as decreased normal tissue toxicity.[1] DFMO was one of a series of analogues that were prepared by Merrell Dow.[2–4]

At the clinical level, interest in the exploration of DFMO as either a chemopreventive agent or chemotherapeutic drug [5,6] has recently increased markedly. Several clinical trials using DFMO are under investigation for breast, esophageal, cervix, prostate, skin, and colon cancer. Currently, clinical trials using DFMO are under investigation: breast (C. Fabian, University of Kansas), Barrett's esophagus (D. Brenner, University of Michigan), cervix (M. Follen Mitchell, M. D. Anderson Cancer Center, Houston, TX), and prostate (A. Simoneau, University of California—Irvine). Additionally, DFMO is being studied in combination with piroxican in a phase II nonmelanoma skin cancer trial (P. Carbone, University of Wisconsin) and with sulindac in a phase II colon cancer prevention study (F. Meyskens, University of California—Irvine, and E. Gerner, University of Arizona).

DFMO is used for the treatment of hirsutism (excessive facial hair) in women. Ornithine decarboxylase is important in the human hair growth cycle by altering ODC activity, and the rate and character of hair growth may be influenced. This application is used to decrease the growth of unwanted hair in men or may even eventually be used as a substitute for the razor to control facial hair growth. Bristol-Myers Squibb Company and the Gillette Company announced the FDA approval of Vaniqa (Eflornithine) as a topical cream for the treatment of hirsutism in women.[7–9]

DFMO was also investigated for the treatment of *Trypanosoma brucei*, the parasite that causes African sleeping sickness.[10]

The therapeutic effect has been demonstrated to be associated with both the (D)- and (L)-DFMO enantiomers. Therefore, a need to produce enantiomerically pure enantiomers is required on an industrial scale to supply both of the enantiomers for clinical trials and future commercial manufacturing.

* To whom correspondence should be addressed: E-mail: scott.perrin @novasep.com.
† NOVASEP, Inc.
‡ Novartis Pharma AG.
§ NOVASEP SAS.
|| University of Illinois.

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The preparation of the individual enantiomers of DFMO is well documented.[11–15] These preparations were comprised of forming a 2-substituted piperidinone and forming either a diastereomeric salt or by an enzymatic process. The main issue with these modes of preparation is the cost at industrial scale. This paper describes a global process optimization for the preparation of the individual enantiomers of DFMO HCl.

Chiral chromatography has obtained substantial interest during the last decade with the application of efficient multicolumn processes at industrial scale.[16] After only a few years, the industrial cumulated capacity of multicolumn chromatographic equipment dedicated to chiral or enantioselective separations exceeded 1500 t in 2004. Coupling direct crystallization of the enriched enantiomer with the chromatographic process has been described as an efficient way to improve the process performances when a favorable eutectic point was observed from a phase diagram for the enantiomers.[17] This concept has been applied for the separation of *rac*-DFMO HCl.

To couple chemistry and chromatography with enantioselective crystallization, a new analogue *rac*-*N*-pivaloyl-DFMO lactam (**4**) is prepared by chemical modification of *rac*-DFMO HCl (**1**). A multicolumn continuous chromatographic process (VARI*COL*)[18] coupled with enantioselective crystallization yields very high enantiomeric purity of (D)-*N*-pivaloyl-DFMO lactam (**5**). Hydrolysis of **5** produces enantiopure (D)-DFMO (**6**) as the hydrochloride salt. Furthermore, using the same global process (L)-DFMO HCl is obtained with the same enantiomeric purity after subsequent hydrolysis.

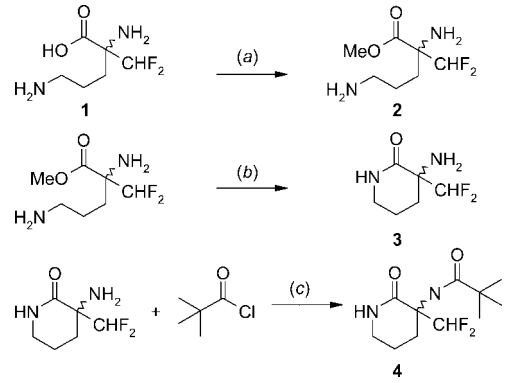
The process was optimized for a 50 t a year production of each purified (D)- and (L)-DFMO HCl enantiomers. To produce 50 t a year of each enantiomer of DFMO requires the production of 100 t of **4**.

Results and Discussion

Chemistry Step I. As shown in (Scheme 1), **4** is prepared in three steps in a single reactor without isolation of intermediates. *rac*-Difluoromethylornithine monohydrochloride monohydrate (**1**) reacts with thionyl chloride in the presence of methanol to form the methyl ester of DFMO (**2**). Subsequent treatment with pyridine and catalytic amounts of DMAP is used for cyclization to *rac*-3-amino-difluoromethyl-2-piperidinone (DFMO lactam) (**3**). The resulting lactam is readily acylated with pivaloyl chloride to furnish **4**.

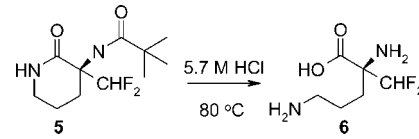
Chemistry Step II. After purification by the VARI *COL* process and subsequent enantioselective crystallization, the resulting enantiomers of **4** are hydrolyzed by heating in an aqueous solution of hydrochloric acid to provide the individual

Scheme 1. **Preparation of *rac*-*N*-pivaloyl-DFMO lactam 4 starting with *rac*-DFMO (1)[a]**



[a] Reagents and conditions: (*a*) MeOH, $SOCl_2$, 5 °C, toluene; (*b*) ACN, pyridine, reflux, DMAP; (*c*) pivaloyl chloride, reflux.

Scheme 2. **Preparation of (D)-DFMO HCl (5) by hydrolysis of (D)-DFMO lactam (6)**



enantiomers of (D)- and (L)-DFMO HCl in high enantiomeric excess and chemical purity.

Scheme 2 shows the hydrolysis of (D)-*N*-pivaloyl DFMO lactam (**5**) to form the pure enantiomer of (D)-DFMO HCl (**6**) by heating an aqueous solution of 5.7 M hydrochloric acid at 80 °C. This process can also be used for (L)-*N*-pivaloyl DFMO lactam.

Continuous Chromatography (VARI*COL* Process). Continuous chromatographic processes such as simulated moving bed (SMB) has proven to be an efficient chromatographic separation technique for the industrial scale production of enantiomers. Recently, a new continuous chromatographic process (VARI*COL*) was introduced and has demonstrated, in general, better performance than SMB.[18,19]

A more efficient use of a chiral stationary phase (CSP) for the separation can be achieved compared to the SMB process with the VARI*COL* process with an asynchronous switching of the inlet and outlet lines. With this new process, higher flexibility is achieved compared to the SMB process and enhanced productivity and performances can be expected.[20]

By use of the HELP*CHROM* software, optimum operating conditions for the VARI*COL* process could be obtained.

Physicochemical Data. A complete screening of various CSPs with different solvents has been completed. The best chiral separation was obtained on the CSP (Chiralpak 61161, 20 μm) eluted with an isocratic mixture of acetonitrile/methanol 90/10 (v/v) at a temperature of 25 °C.

Figure 1 shows the chromatogram corresponding to a dilute or analytical injection of **4** on an analytical column (250 × 4.6 mm i.d.) at a flow rate of 1 mL/min.

The retention times corresponding to an analytical injection of **4** (**5** is the second eluting enantiomer) and several overloaded

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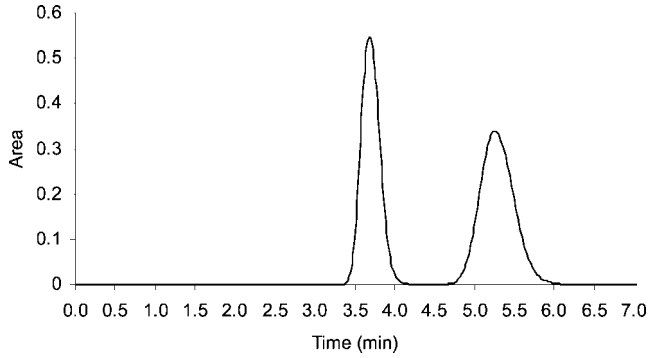

Figure 1. **Analytical injection of 4 on a Chiralpak 61161 column: FR, 1.0 mL/min; V_{inj}, 20 μL; C_{inj}, 1.0 g/L; eluent, isocratic mixture of acetonitrile/methanol 90/10 (v/v) at a temperature of 25 °C; UV detection, 200 nm.**

injections provided the adsorption isotherm parameters.[21] Good agreement was obtained between the experimental results and computer simulations using the HELPCHROM software by choosing a modified competitive Langmuir adsorption isotherm to describe the equilibrium between the liquid and solid phase. The simulation models are verified by comparing experimental elution profiles obtained during mass overloaded experiments with simulated elution profiles generated by the software. The results from our simulation models were in agreement with experimental results. Thus, the curve fitting resulted in the following parameters for the adsorption isotherms (eqs 1 and 2):

$$\overline{C}_1 = 0.7C_1 + \frac{0.1095C_1}{1 + 0.0243C_1 + 0.1643C_2} \qquad (1)$$

$$\overline{C}_2 = 0.7C_2 + \frac{0.7393C_2}{1 + 0.0243C_1 + 0.1643C_2} \qquad (2)$$

In these equations C is the concentration of the species in the liquid phase and $\overline{C}$ is in the solid phase (concentration in g/L).

The pressure drop was measured to determined the Darcy's law coefficient (eq 3):

$$\frac{\Delta P}{L}\ (\text{bar/cm}) = 0.65u\ (\text{cm/s}) \qquad (3)$$

The column efficiency was determined by measuring the influence of the linear fluid velocity on the height equivalent to theoretical plate (HETP) (eq 4). The following simplified Van Deemter equation was obtained:

$$\text{HETP (cm)} = 4.46dp\ \text{(cm)} + 0.27u\ \text{(cm/s)} \qquad (4)$$

Enantioselective Crystallization. The solid/liquid equilibrium (SLE) through phase diagrams forms the basis for the initial design of a global process optimization for enantioselective crystallization. The phase behavior of a system (two enantiomers or two enantiomers and a solvent) is well understood using phase diagrams which are powerful tools for the description of binary and ternary systems. For ternary systems, it should be noted that interactions between the enantiomers remain quasi-ideal and therefore the symmetrical monovariant valleys (starting from the symmetrical binary eutectics down to the two symmetrical ternary eutectic points) are straight.

From the binary phase diagrams of racemic mixtures three main associations are observed.[22–24] Conglomerates of a racemic mixture (racemate) consist of an equal amount of crystals composed of only pure enantiomers, in this case the melting point of the racemic mixture will always be lower than that of the pure enantiomers. This category represents approximately 5–10% of the total solid racemic mixtures.

Racemic compounds consist of crystals composed of both enantiomers within the same unit cell. The melting point of the racemic mixture can be greater or lower than that of the pure enantiomers depending on the position of the eutectic point. This category represents approximately 90% of the total solid racemic mixtures encountered. Solid solutions represent less than 1% of the total solid racemic mixtures.

The characterization of the phase diagram is a critical aspect for the selection of the optimized enantioselective chromatographic separation process. A fast and efficient method can be used, based on the measurement of thermodynamical characteristics of both the pure enantiomer and the racemic mixture. The application of thermodynamic equations can be used to predict the position or nature of the association type of the two enantiomers.[22]

The Schroeder–van Laar equation (eq 5), in its simplified form, relates the mole fraction (x) of a conglomerate to its melting point T^f at a given composition, where $T^f_{enantiomer}$ and $\Delta H^f_{enantiomer}$ are the melting point and the heat of fusion of the pure enantiomer, respectively, and R is the gas constant.

$$\ln\ x = \frac{\Delta H^f_{enantiomer}}{R}\left(\frac{1}{T^f_{enantiomer}} - \frac{1}{T^f}\right) \qquad (5)$$

The Prigogine–Defay–Mauser equation (eq 6) is used for chiral compounds crystallizing as racemic compounds where ΔH^f_{rac} relates to the heat of fusion and the term T^f_{rac} relates to the melting point of the racemic compound.

$$\ln\ 4x(1-x) = \frac{2\Delta H^f_{rac}}{R}\left(\frac{1}{T^f_{rac}} - \frac{1}{T^f}\right) \qquad (6)$$

Considering a racemate, these two equations can be used to calculate the position of the eutectic point. Starting from an enriched mixture of enantiomers (obtained for example with a chromatographic purification process), an enantiopure compound can be crystallized when the starting enantiomeric purity of the solution is exceeding the eutectic composition. The enantiomeric purity and composition of the eutectic may be used to calculate the

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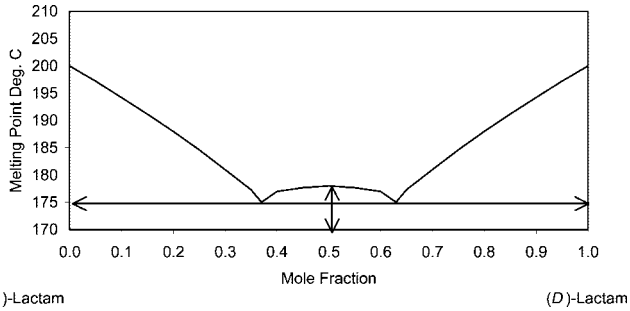

Figure 2. **Binary phase diagram (melting point and mole fraction) derived from the Schroeder–van Laar curves from the pure enantiomer of 5 and the Prigogine–Defay–Mauser equation for 4.**

Table 1. **Enthalpies of fusion, and onset of point and peak of melting points for (D)-*N*-pivaloyl DFMO (6) and *rac*-*N*-pivaloyl DFMO lactam (5)**

$\Delta H^{f}_{(R)}$ (J/g)	$\Delta H^{f}_{(Rac)}$ (J/g)	$T^{f}_{(R)}O$	$T^{f}_{(Rac)}O$	$T^{f}_{(R)}$ (°C)	$T^{f}_{(Rac)}$ (°C)
135.277	144.199	198.12	176.42	200.03	178.02

maximum recovery (Max_{rec}) of an enantiopure compound from an enriched mixture of enantiomers assuming equilibrium crystallization and ideal behavior (eq 7).

$$Max_{rec} = \frac{(X_{pur} - X_{eut})}{(1 - X_{eut})} \qquad (7)$$

The maximum recovery (Max_{rec}) is calculated by a known initial enantiomeric purity of the enriched mixture (X_{pur}) and the determination of the composition of the eutectic point (X_{eut}) from the phase diagram.

The graphic determination of the phase diagram is shown in Figure 2 (Table 1). The eutectic composition or mole fraction as determined from the phase diagram for **5** was 63%.

Enantioselective crystallization was possible by recrystallization in acetonitrile, which will enantioselectively control nucleation and crystal growth of **5** from supersaturated solutions.

Global Process Optimization. The design of an economically successful process requires a global optimization approach. The goal of any process optimization for an industrial process should be to reduce costs of manufacturing and to reach an economic optimum. This still requires that specific conditions be met. Conditions that should be addressed before process optimization include possible chemical modification of the molecule to obtain enantioselectivity on a given CSP and optimization of the chromatographic process, which includes the optimization of the eluent composition. Ideally, one should determine if the chiral molecule can be racemized to improve yields. All of these conditions govern the overall productivity of the global system and cost of manufacturing. After these requirements are investigated, then a parametric study is performed to reach the global optimization requirements. In order to do so, one cannot optimize each single unit operation, but a global optimization has to be achieved.

The first requirement, in the presented case, was the chemical modification of **1**. Initial screening of **1** did not provide a promising direct resolution by a chiral chromatographic process. Direct separation by a reversed-phase high-performance liquid chromatographic (HPLC) method using a C_{18} for the separation of the enantiomers of α-substituted ornithine and other amino acids is possible based on mobile phase additives, such as either (L)-proline and copper or (*R,R*)-tartaric acid mono-*n*-octylamide.[25,26] However, this process requires removal of such additives, which is not easily applicable at industrial scale.

Investigation into the feasibility of using a CSP with copper sulfate as a chromatographic process was investigated, and the preparation of Mono-Boc *rac*-DFMO using a Chiralpak WH was successful. We chose not to use this route based on unfavorable thermodynamic and hydrodynamic data. Numerical optimization using the HELP*CHROM* software to develop a VARI*COL* chromatographic process gave low productivity. This process was not the most efficient process on an industrial scale, and the economics or cost of manufacturing was much higher than the current process being presented.

We found that this method was acceptable for the determination of enantiomeric excess of the single enantiomers of DFMO as an analytical assay.[1]

Several analogues of **1** (not reported in this paper) were investigated, and an extensive chromatographic screening study of a wide variety of CSPs finally resulted in optimal chromatographic conditions for the VARI*COL* process with **4**. **5** is collected in the extract outlet of the VARI*COL* process. A simple and economical approach was developed to prepare **4**. The results of this study confirmed that chemical modification was the most favorable way to perform the enantioselective separation of the two enantiomers. This coupled the chemical modification of DFMO with an enantioselective chromatographic separation process.

The next study was to investigate if enantioselective crystallization could be applied to improve the enantiomeric purity of **5** from the extract outlet of the VARI*COL* process, which would allow one to reduce the targeted enantiomeric purity of the chromatographic process and thus increase productivity. Differential scanning calorimetry (DSC) measurements were made, and a favorable eutectic point was obtained for **5**.

To derive the highest productivity at the lowest cost from the purification process, extrapolation of data by a parametric study was conducted. This study takes into account the different parameters of both the VARI*COL* and crystallization process.

Chromatography and crystallization are coupled such that the extract stream of the VARI*COL* process, containing **5** at a given enantiomeric purity, enters directly into a crystallizer. Enantioselective crystallization is achieved and leads to enantiopure crystals of **5** and mother liquors at the composition of the eutectic point, which can be recycled back into the VARI*COL* process.

In a parametric study, investigation on the influence of the extract enantiomeric purity on the VARI*COL* productivity was conducted. Determination of the mass of noncrystallized **5** to be recycled back into the VARI*COL* process at the composition of the eutectic point was established using the phase diagram

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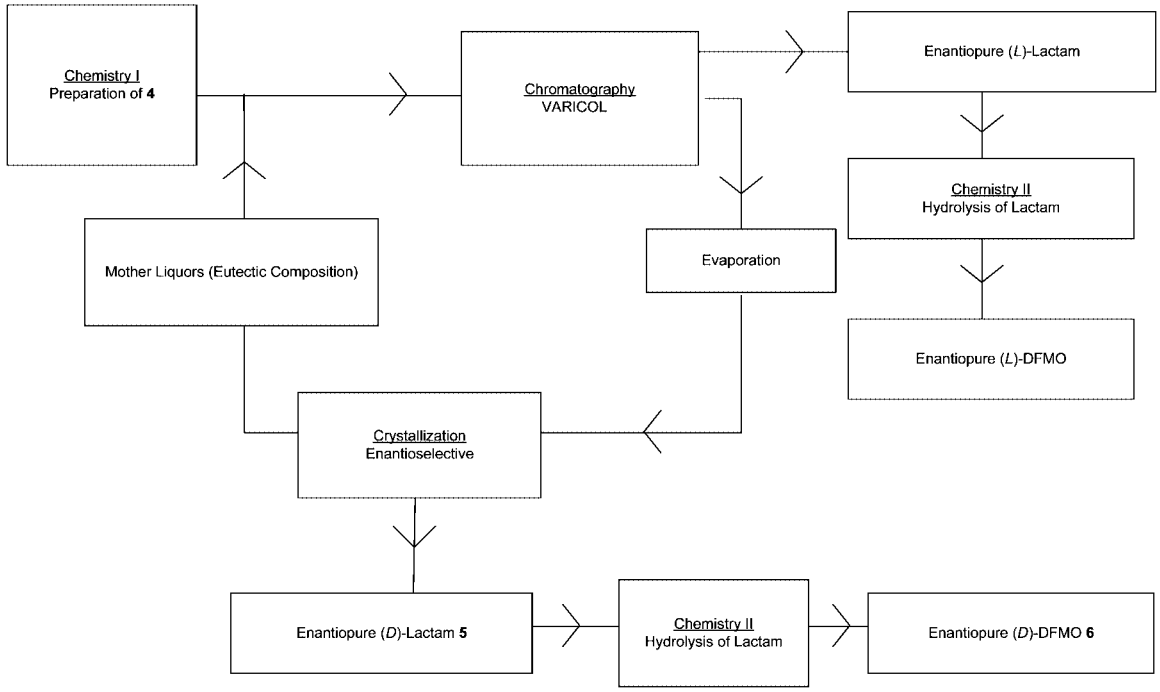
Scheme 3. **Global optimization for the production of metric tons of the single enantiomers of DFMO**



by DSC experiments. The lower the enantiomeric purity out of the VARI*COL* process result in a greater mass of enantioenriched **5** to be recycled. Thus, increasing the size of the equipment needed in order to produce the desired output of enantiopure **5**. At the same time, the number of columns required and operating conditions will change depending on the targeted outlet enantiomeric purity from the VARI *COL* process as a reduction of the targeted purity improves the overall productivity of the chromatographic process.

The target enantiomeric purity in the extract stream of the VARI*COL* process was varied, and the process was numerically optimized for target enantiomeric purity. For each of these enantiomeric purity points, a cost analysis was performed and the global manufacturing cost was plotted against the corresponding enantiomeric purity of **5** at the outlet of the chromatographic unit. Scheme 3 presents the global process optimization for the production of metric ton quantities of both **5** and (L) DFMO HCl.

The global optimization process minimizes the manufacturing costs, which includes the chromatographic and enantioselective crystallization steps. In this process, enantiopure (L)-DFMO lactam is obtained directly from the VARI*COL* process. After evaporation, **5** was subjected to enantioselective crystallization to provide the required enantiomeric purity and the enantioenriched material at the composition of the eutectic point is fed back into the continuous chromatography process. Measurable factors such as maximum recovery and eutectic composition obtained from enantioselective crystallization studies provide data for the global optimization of the process.

Influence of VARI*COL* Extract Enantiomeric Purity during Enantioselective Crystallization. From the phase diagram (Figure 2), maximum recovery was evaluated based on a given enantiomeric purity of the enantioenriched mixture obtained from the VARI*COL* system. From eq 7, it was determined that an enantiomeric purity of 90% at the extract outlet of the chromatographic process, which contains the enantiomer **5**, would lead to a maximum recovery of 73% from enantioselective crystallization.

Maximum recovery from the crystallization step increases as enantiomeric purity at the extract outlet of the VARI*COL* increases. This shows the importance of a favorable eutectic point and coupling chromatography with enantioselective crystallization. Having a higher eutectic composition is unfavorable as it reduces maximum recovery for a given enantiomeric enrichment and thus reduces productivity of the global purification process.

Influence of VARI*COL* Extract Purity. Directly linked to the maximum crystallization recovery is the mass to be recycled into the VARI*COL* system to get a high yield for the global purification process. The lower the enantiomeric purity, the higher the mass of enantioenriched material which is returned to the VARI*COL* process, thus increasing the size of the equipment and the cost of manufacturing.

Figure 3 shows the evolution of the mass of injected **5** from the VARI*COL* process with the variation of the enantiomeric purity at the extract outlet. The injected DFMO lactam stream

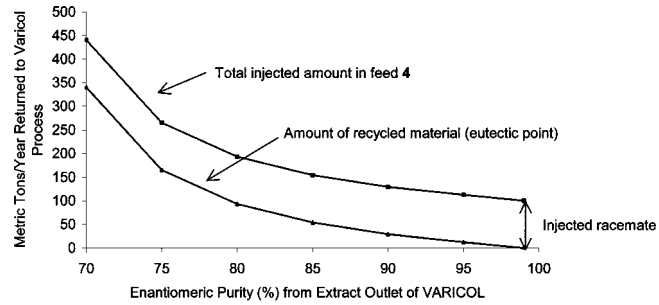


Figure 3. **Total injected amount and amount of recycled material into the VARI*COL* process as a function of VARI*COL* extract purity.**

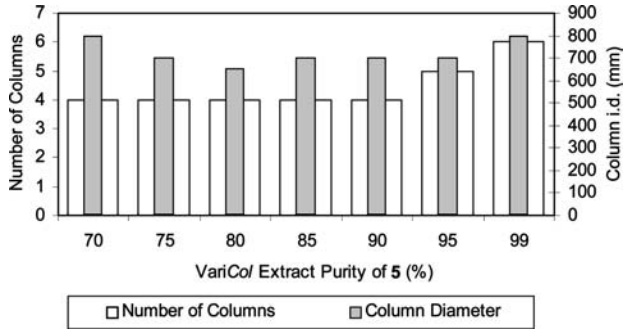


Figure 4. **Variation of column numbers and diameters as a function of VARI*COL* outlet enantiomeric purity.**

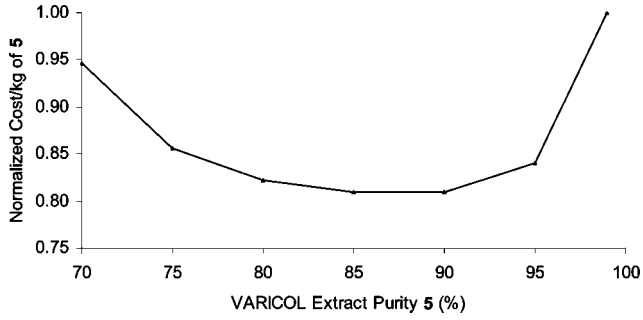


Figure 5. **Cost of manufacturing as a function of the enantiomeric purity from the VARI *COL* process.**

included the newly injected racemate and the recycled material from the mother liquor of the coupled crystallization step.

A decrease in the enantiomeric purity from 99% to 70% from the VARI*COL* process increases the mass of enantioenriched **5** returned from 0 to 350 t a year. Even if the VARI*COL* productivity can be significantly increased when reducing the enantiomeric purity of the extract from 99% to 70%, the large amount of recycled material increases the size of the chromatographic system.

Influence of Extract Purity on Column Numbers and Diameter. The lower the enantiomeric purity requirements for the VARI*COL* process are, the lower the number of columns required. However, at the same time the recycled amount of enantioenriched **5** into the VARI*COL* system increases, which increases the total amount of material to be processed (Figure 4).

The diameter of the column is large at 70% due to the mass of material to be recycled and decrease until approximately 80%. The number of columns increases as the enantiomeric purity increases due to the number of plates required to achieve high enantiomeric purity above 90%. As the enantiomeric purity requirements for the VARI*COL* process increase, the need for column efficiency increases, which leads to longer columns. In order to not exceed the maximum pressure drop in the system, the column diameters have to be increased in order to lower the linear velocity.

Normalized Global Cost of Manufacturing Based on Enantiomeric Purity. Having established the influence of the VARI*COL* extract purity and equipment configuration, size, and operating conditions, a cost analysis can be performed for each case. Figure 5 shows a graph of the normalized cost of manufacturing and the enantiomeric purity of **5** from the outlet of the VARI*COL* process.

The normalized cost decreases as the enantiomeric purity from the VARI*COL* process increases and reaches a minimum at around 85–90%. This point reflects a global purification optimum between throughput of the VARI*COL* process and recovery out of the enantiomeric crystallizer. Higher purities from the VARI*COL* process will lead to a higher recovery out of the crystallization process and less **5** to be recycled. However, this gain is consumed by the much higher need for chromatographic efficiency for the VARI*COL* process and increased investment cost due to a need for a higher number of columns of larger diameter.

A decrease of the enantiomeric purity out of the VARI*COL* process would lead to a reduction in the number of columns and diameters. This effect is counterbalanced by the decrease of recovery during the crystallization step, which leads to an increase of recycled **5** in the feed stream to be purified. In order to be able to process the total amount of enantioenriched **4** in the VARI*COL* system, the column diameter has to be increased, which leads again to higher investment costs.

Global Optimization Scheme. The global optimized process (Scheme 4) shows the manufacture of 100 t per year of **4**, which contains 50% of **5** by definition.

Chromatographic separation occurs and produces 50 t per year with 100% yield and high enantiomeric purity of (L)-DFMO lactam. From the extract outlet of the VARI*COL* system with an enantiomeric purity of 90%, (D)-DFMO lactam is placed into the crystallizer to produce an enantiomeric purity of greater than 99%. The remaining (D)-DFMO lactam is returned to the chromatography unit with a molar composition of 63%.

Solvent recycling streams from the VARI*COL* system extract and raffinate outlets and to the crystallizer back to the chromatographic system is greater than 99%.[27,28]

CONCLUSION

This study points out that a global process optimization strategy, which couples chemistry with chromatography and enantioselective crystallization can produce 50 t a year of both enantiomers of DFMO HCl based on 100 t of *rac-N*-pivaloyl-DFMO lactam.

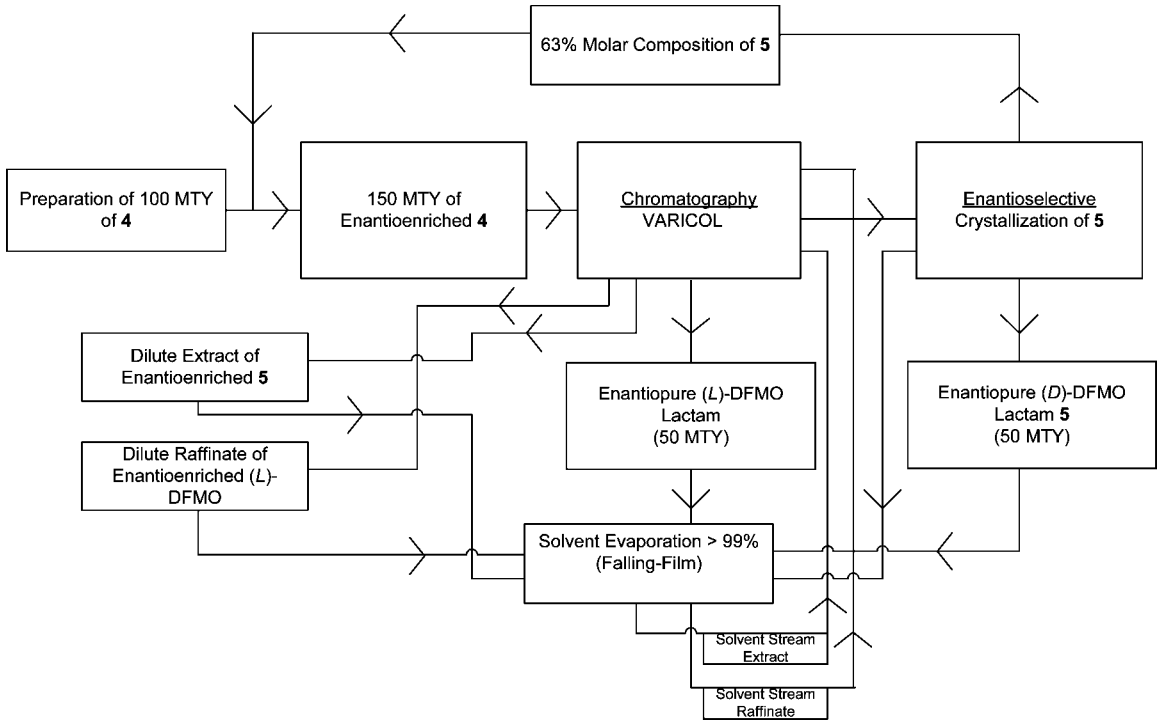
When developing an industrial process, it is important to consider a global process optimization and develop unit studies in each of the respective areas to obtain an economical process. Every aspect from the most efficient process for resolving chiral molecules by chromatography, even if it requires chemical modification or evaluating different synthetic pathways during the preparation of an active pharmaceutical ingredient (API) is essential to developing a cost-effective process. It is vital to the success of producing industrial quantities (metric tons) of an enantiopure chiral molecule to investigate the potential of coupling chemistry with chromatography and enantioselective crystallization.

Experimental Section

rac-DFMO and the single enantiomers as the monohydrochloride monohydrate were obtained from Ilex Oncology of

(27) Hamende, M.; UCB Pharma, plenary lecture at SPICA, (2004), Aachen.
(28) McCormick, D. In the loop: continuous chromatography for chial (and other) separations. *Pharm. Technol.* **2006**, *30* (5), 54–68.

Scheme 4. **Shows the global process starting with 100 metric tons of *rac*-DFMO lactam 4**



San Antonio, TX. Pivaloyl chloride (trimethylacetyl chloride), pyridine, 4-(dimethylamino)pyridine (DMAP), di-*tert*-butyl dicarbonate (DTBC), copper(II) sulfate ($CuSO_4$), acetonitrile (ACN), methanol (MeOH), sodium phosphate monobasic, sodium dodecyl sulfate, and hydrochloric acid (HCl) were products of Sigma of St. Louis, MO. Galbraith Laboratories, Inc., of Knoxville, TN, performed melting point and elemental analysis. Differential scanning calorimetry (DSC) was performed on a Setaram 131.

Chromatographic Analysis of DFMO HCl Enantiomers. The single enantiomers of DFMO HCl were analyzed using reversed-phase high-performance liquid chromatography (HPLC). The column was Spherisorb ODS 2 (250 mm × 4.6 mm i.d. with a 5.0 μm particle size) from Phenomenex of Torrance, CA, thermostated at 30 °C. The flow rate was 1 mL/min. UV detection was monitored at a wavelength of 200 nm. The isocratic mobile phase consisted of 78% phosphate buffer with 22% acetonitrile. The phosphate buffer was prepared by adding 38.7 mM of monobasic phosphate buffer adjusted to pH 2.8 with phosphoric acid (85%). To this buffer, 1.9 mM of sodium dodecyl sulfate was added and the solution was mixed well.

Chromatographic Analysis of *N*-Pivaloyl DFMO Lactam Enantiomers. Analysis of the enantiomers of *rac*-*N*-pivaloyl-DFMO lactam was performed on a Chiralpak 61161 (250 mm × 4.6 mm i.d. with 20 μm particle size) from Chiral Technologies, Inc., of West Chester, PA. The column was thermostated at a temperature of 25 °C. UV was monitored at a wavelength of 200 nm. The isocratic mobile phase was 90% acetonitrile and 10% methanol.

Chromatographic Analysis of Mono-BOC (*tert*-butyloxycarbonyl)-DFMO Enantiomers. Analysis of the enantiomers of Mono-BOC-DFMO was performed on a Chiralpak WH (250 × 4.6 mm i.d. with 10 μm particle size) of Chiral Technologies, West Chester, PA. The column was thermostated to 50 °C. The isocratic mobile phase was a 10 mM $CuSO_4$. The flow rate was 1.0 mL/min. The wavelength is 254 nm.

Preparation of the Mono-BOC-DFMO Enantiomers. To a solution of di-*tert*-butyl dicarbonate (DTBC) (55 mg, 0.25 mM) in methanol (500 μL) was added either the racemate or single enantiomers of DFMO HCl (50 mg, 0.25 mM) and triethylamine (60 μL). The suspension was shaken for 20 min. The resulting suspension was filtered through a 0.2 μm syringe filter into a fresh test tube. A 10 mM solution of $CuSO_4$ (1 mL) was added, and the resulting solution was filtered and directly injected for chromatographic analysis.

The results of the chromatographic analysis of (D)-DFMO HCl and (L)-DFMO HCl were addressed in another publication.[1]

Preparation of *rac*-*N*-Pivaloyl-DFMO Lactam. In the same flask, 80 mL of methanol and 12.2 g (102.5 mmol) of thionyl chloride are added while maintaining the temperature at 5 °C. To the solution, 20 g (84.51 mmol) of *rac*-difluoromethylornithine monohydrochloride monohydrate (**1**) is added. The solution is heated to reflux for 4 h and concentrated in vacuo. Toluene is added to remove the water as an azeotrope to form the methyl ester of DFMO (**2**). To the residue is added 100 mL of ACN, 21 mL of pyridine, and 2.2 g of DMAP. The suspension is refluxed overnight to form *rac*-3-amino-difluoromethyl-2-piperidinone (DFMO lactam) (**3**). Pivaloyl chloride (18.5 mL, 150.6 mmol) was slowly added to the suspension with stirring. The suspension is refluxed overnight or until the solution is clear. The solution is concentrated in vacuo, and the residue is extracted into ethyl acetate (100 mL) and was washed several times with water (20 mL).

The crude product was crystallized from ACN and allowed to cool to room temperature. After being dried in vacuo at 50 °C, 13.0 g of **4** was obtained as a clear crystalline solid: mp 179.5–179.9 °C. Anal. Calcd for $C_{11}H_{18}F_2N_2O_2$: C, 53.22; H,

7.31; N, 11.28. Found: C, 53.26; H, 7.33; N, 11.34. By HPLC analysis the chemical purity was 99.1% AUC.

Preparation of either the (D) or (L) Enantiomers of DFMO HCl. To 4 g (16.1 mmol) of either (D) or (L)-*N*-pivaloyl-DFMO lactam, 37% HCl (7.5 mL, 91.2 mmol) was added. The suspension was heated to 80 °C and stirred overnight. The resulting clear solution was cooled to 60 °C, and 70 mL of propionic acid was added. The pH of the solution was adjusted to 6.0 and cooled to 20 °C. Triethylamine (3.4 mL) was added, and the product precipitated, was filtered off, and washed with ethanol (20 mL). After drying in vacuo the white to tan solid afforded 2.75 g. The chemical purity by HPLC was 98.1% AUC.

Experimental results of thermal analysis by differential scanning calorimetry (DSC) are given in Table 1.

The DSC heating rate was 2 K/min with airflow of 50 mL/min. Approximately 6–10 mg of each solid component was place in to a 30 μL aluminum crucible.

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Supporting Information Available

DSC data for **4** and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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