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Process Development and Scale-Up of a Novel Atypical DAT Inhibitor (*S*)-CE-123

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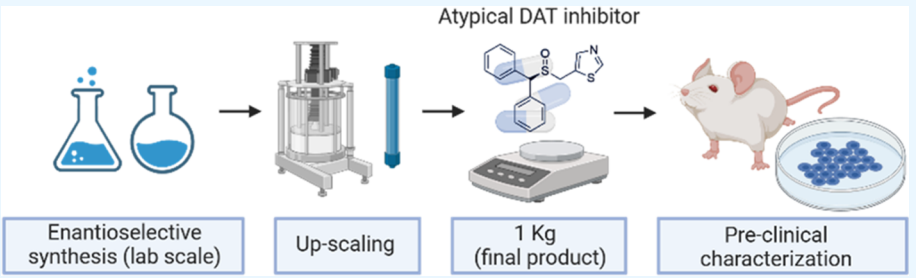

ABSTRACT: Large-scale syntheses of small molecules and kilo laboratories are crucial steps in drug development, especially in advanced stages. (*S*)-5-((Benzhydrylsulfinyl)methyl)thiazole, (*S*)-CE-123, a potent, selective, and novel atypical DAT inhibitor, has undergone iterative testing as part of the preclinical evaluation step. This required the process transfer, scale-up, and synthesis of a 1 kg preclinical batch. The Kagan protocol for asymmetric sulfide to sulfoxide oxidation was successfully applied within a four-step synthetic process for the successful upscaling of (*S*)-CE-123. During the scale-up of the last step, several changes were made to the original synthetic procedure, as with every increase in batch size, new problems had to be overcome. These include, among others, the workup optimization of the last step, the simplification of chromatographic purification, elution modification to improve the purity of the product and saving of workup time. Two washing steps were added to the original procedure to enhance both the yield and the enantiomeric excess value of the final product. The modifications introduced allowed access to a 1 kg (*S*)-CE-123 batch with a purity >99% and an enantiomeric excess value of 95%.

INTRODUCTION

Despite advances in the field of biologics, small molecules, conceptualized and synthesized by medicinal chemists, still remain the backbone for modern drug discovery with 18 of them (out of total thirty seven from all drug modalities) being approved by the US Food and Drug Administration (FDA) in 2022.[1,2] To enter this drug discovery space, a certain compound must undergo thorough investigation in the pharmaceutical industry, where the goals of synthetic chemistry significantly vary, depending on the stage of the development and a scale on which a compound needs to be prepared.[3] Upon identification of a lead candidate, a typical preclinical development program aims to fulfill the following six goals: manufacture of the drug substance/active pharmaceutical ingredient; drug formulation (dosage design); analytical and bioanalytical method development and validation; metabolism and pharmacokinetics; safety and genetic toxicology and possibly safety pharmacology; and good manufacturing practice (GMP) manufacture and documentation of the drug product for use in clinical trials.[4] During this process of drug transition, from the medicinal chemistry laboratory to kilo-scale facilities, large-scale synthesis plays a crucial role.[5] Hence, kilo laboratories are rather important tools that support process development in the pharmaceutical industry with the main objective to manage a dynamic portfolio of potential products while meeting all environmental, health, and safety aspects, ensuring product quality and enabling business requirements.[6]

(*S*)-5-((Benzhydrylsulfinyl)methyl)thiazole, (*S*)-CE-123, is a novel modafinil analogue (Figure 1) with improved specificity and efficacy for dopamine transporter inhibition that improves cognitive and motivational processes in experimental animals.[7−12] Substantial chemical modifications of modafinil over the years have led to the discovery of (*S*)-CE-123, but the structure−activity relationship (SAR) studies clearly indicated the necessity of preserving a sulfoxide moiety within the core structure for obtaining an active moiety.[13,14] (*S*)-CE-123 belongs to a class of atypical DAT inhibitors, with

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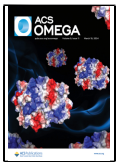


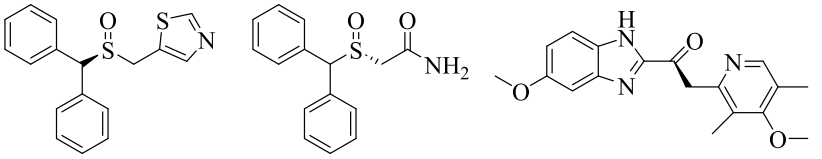
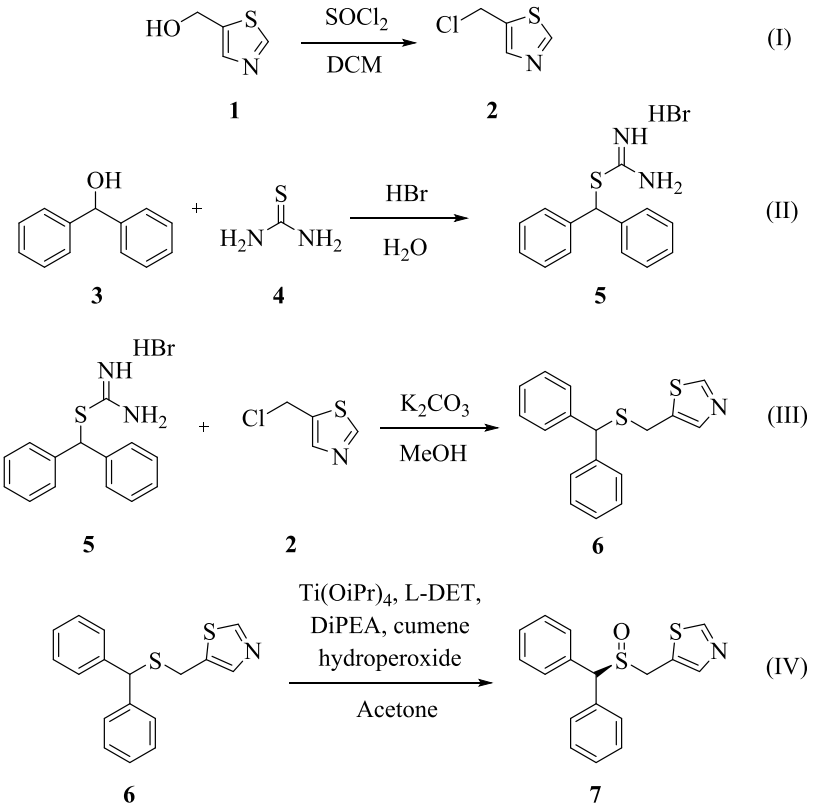


Figure 1. Structures of (*S*)-CE-123 (left), R-modafinil (middle), and esomeprazole (right).

Scheme 1. Steps of the Synthesis Pathway for Compound 7



a pharmacological profile that can be clearly distinguished from those of cocaine and amphetamine.[10]

Atypical DAT inhibitors are being extensively tested and strong efforts are being made to develop them as therapeutics for treatment of drug addiction.[15−19] The necessity of bringing in drugs against addiction is also reflected by the number of deaths of young and middle-aged adults in the US and Canada due to the current opioid crisis: 15.8 and 6.4 per 100,000 people in 2019, respectively.[20] In the US, programs have been conducted to develop potential pharmacotherapies, which have led to the identification of a promising lead molecule, JJC8-091, that exhibits a novel binding mode at DAT.[15] JJC8-091, (*S*)-CE-123, and *R*- and *S*-modafinil exert a unique binding profile to DAT that is clearly distinguished from the binding of cocaine and thus exert a pharmacological profile of atypical DAT inhibitors.[10,15,21]

For initial pharmacological profiling, (*S*)-CE-123 was produced in house via separations on a chiral phase, which was later on replaced by total synthesis on a small scale with the enantioselective oxidation of sulfide to a sulfoxide moiety in the last step.[12] For enantioselective catalysis of the oxidation of sulfides, Modena and co-workers investigated several complexes comprising a metal and a chiral tartrate, finding out that a species made of titanium(IV) tetraisopropoxide (TTIP) with the formula $Ti\{OCH(CH_3)_2\}_4$ and (*R*,*R*)-diethyl tartrate in a 1 to 4 molar ratio provided high enantioselectivity.[22] In parallel, Kagan and co-workers discovered that high enantioselectivity is achieved using a complex consisting of TTIP, (*R*,*R*)-diethyl tartrate, and water in a 1:2:1 molar ratio.[23]

The original Kagan protocol requires special conditions and has previously been associated with two fundamental limitations (employment of stoichiometric amounts or near-stoichiometric amounts of a chiral Ti complex and exerting very weak or no induction at all in the case of oxidation of sulfides substituted at sulfur with two large groups), which were later on overcome as showcased on the example of the highly enantioselective synthesis of esomeprazole (Figure 1).[24] This was achieved by the preparation of the titanium complex, including $Ti(OiPr)_4$, (*S*,*S*)-DET, and water, in the presence of sulfide, increasing the temperature and reaction time of the complex formation, and addition of the amine, such as DIPEA.[24] Based on their work, Cephalon (France) developed and patented a process for the asymmetric synthesis of single enantiomers of modafinil (EP1516869A1, WO2005028428A1).

In the present work, we describe the process development toward scaling up the complete synthesis of the 1 kg preclinical batch of (*S*)-CE-123. In the last of the four synthetic steps, we also successfully apply the modified Kagan protocol in obtaining the preclinical bsatch with high enantioselectivity

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(>95%). Lastly, the synthesized compound has been unambiguously characterized.

RESULTS AND DISCUSSION

Route Design. The planned synthetic procedure was scaled up from a previously reported 1−2 g scale to provide 1 kg of the final material. Scheme 1 depicts the planned synthetic route. In the first step, 5-thiazolylmethanol (**1**) is chlorinated by thionyl chloride in dichloromethane. The solvent is evaporated, and the crude product (**2**) is used without further purification. In the second step, hydrogen bromide is added to diphenylmethanol (**3**) and thiourea (**4**) in methanol. The reaction mixture is refluxed followed by evaporation of the solvent. The residue is washed with dichloromethane and water. After drying, **5** is obtained as a white solid. The building blocks (**2**) and (**5**) are dissolved in methanol, then potassium carbonate is added, and the mixture is heated. The mixture is filtered, and the solvent is evaporated. The residue is dissolved in water and extracted with ethyl acetate. The combined organic phases are dried and the solvent is removed under reduced pressure. Chromatographic purification and recrystallization yield (**6**). In the final step, (**6**) is oxidized under Sharpless−Kagan conditions ($Ti(OiPr)_4$), L-DET, DiPEA, and cumene hydroperoxide.[25] Removal of the solvent and chromatographic purification yielded the final product (**7**). This procedure was repeated in two batches that are blended at the end to obtain 1 kg of compound **7**.

Scale-Up of the First Step (I). The first reaction step is depicted in Scheme 2.

Scheme 2. First Reaction Step

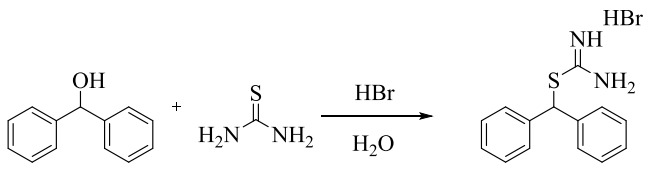
1 **2**

Only minor changes were made to the original procedure.[12] As the temperature increase was rather high, even when a very slow rate of addition was used, thionyl chloride was diluted with dichloromethane. This enabled a better control of the reaction temperature while simultaneously allowing a faster rate of addition. The only problem encountered during the scale-up was the formation of big lumps during the reaction. These impaired stirring, but variation of the stirring rate and regular mechanical breakup proved to be a simple solution to this problem. The yield of the reaction was between 95 and 100% in seven batches ranging in size from 0.5 to 500 g.

Scale-Up of the Second Step (II). The second reaction step is depicted in Scheme 3.

The first scale-up batches of the second step were performed using methanol as a solvent, which was evaporated after the reaction. This approach proved to be difficult when larger batch sizes were tested. Even a 5 g batch took 4 h to evaporate due to residual hydrogen bromide. Therefore, the solvent was changed to water. As the product is insoluble in cold water, it precipitated when the reaction mixture was cooled after completion. The cooling was done slowly, and the mixture was thoroughly stirred to prevent the formation of lumps. The workup was thereby reduced to a simple filtration step. After thoroughly drying, yields between 76 and 97% were obtained in eight batches ranging in size from 1.0 to 1000 g.

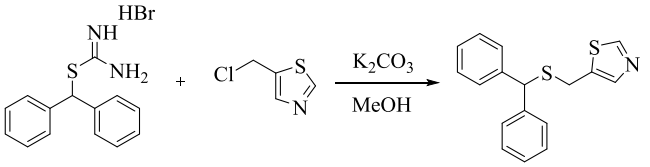
Scheme 3. Second Reaction Step



3 **4** **5**

Scale-Up of the Third Step (III). The third reaction step is depicted in Scheme 4.

Scheme 4. Third Reaction Step



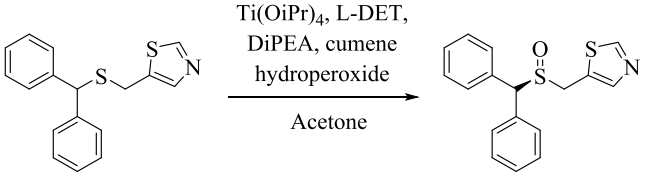
5 **2** **6**

The reaction was performed according to the literature, but larger batch sizes led to the formation of side products with an obnoxious smell that could not be separated satisfactorily by the workup procedure. The reason for these side reactions was investigated, and test reactions under TLC control indicated that the reaction time might be the problem. According to the tests, the reaction was finished after 2−2.5 h, and when this time was not exceeded, no smelly byproducts were formed. Thus, the reaction time was reduced in the following batches.

The workup was simplified as well. The reaction mixture was filtered hot and after cooling, filtered again to remove precipitated potassium carbonate. The filter cake was washed with methanol, and the solvent was evaporated. The residue was dissolved in dichloromethane and filtered through a silica column. In the rare cases when the purity of the product was not sufficient, the product could be recrystallized from cyclohexane/toluene 10:1. Yields from 76 (with recrystallization) to 95% were obtained in seven batches ranging in size from 1.5 to 1300 g.

Modifications of the Original Lab Method and Scale-Up of the Fourth Step (IV). The fourth reaction step is depicted in Scheme 5.

Scheme 5. Fourth Reaction Step



6 **7**

During the scale-up of the last step, several changes were made to the original synthetic procedure, as with every increase in batch size, new problems had to be overcome. The first of these problems occurred at small batch sizes (5−10 g) when peroxide tests (Merck MQuant) showed that traces of peroxides were left after the reaction. As the reaction mixture had to be evaporated before purification could take place, this posed an explosive hazard that could not be ignored.

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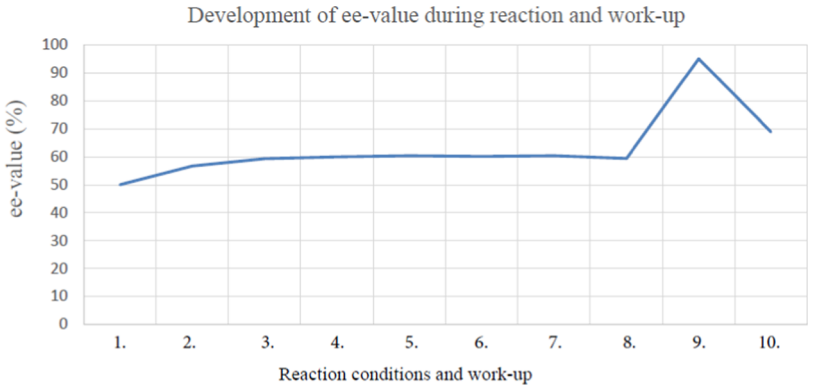

Figure 2. Graphical representation of the development of the ee-values. 1. 0.5 h after hydroperoxide addition, 2. 1 h after hydroperoxide addition, 3. 17 h after hydroperoxide addition, 4. 20 h after hydroperoxide addition, 5. after $FeSO_4$ quenching, 6. after evaporation of the solvent, 7. after washing with citric acid and NaOH, 8. after recrystallization from cyclohexane/toluene, 9. chromatography column first half of the batch, and 10. chromatography column second half of the batch.

Therefore, a way to destroy the remaining peroxides had to be found, especially as later batches would require several 100 g of peroxide. In the first experiment, the reaction mixture was filtered over basic aluminum oxide, but the peroxide content was only diminished marginally. In the next experiment, an aqueous sodium thiosulfate solution was added, and the mixture was stirred.

The peroxide test still showed a positive result after several hours of stirring. As a third possibility, an aqueous iron(II) sulfate solution was added. After 10 min of stirring, no peroxides could be detected, but after the addition of dichloromethane, phase separation was very slow and was hampered further by the formation of a fine white precipitate. This problem could be overcome by using only a few milliliters of the aqueous iron(II) sulfate solution, which was evaporated together with the rest of the solvent. The residue could then be purified by column chromatography, as described in the original literature. This column chromatography was later optimized by using a short silica column and changing the eluent from dichloromethane/methanol to dichloromethane/ethyl acetate (7:3). This became necessary as larger batches took days and up to 100 L of eluent for single chromatography. This could be reduced to 6−8 h and 20 L of eluent by applying the changes described above. At the same time, the change in the eluent gave a better separation of the product and impurities, thus reducing the number of mixed fractions significantly.

During the scale-up to 50−100 g, it was noticed that an additional purification step before column chromatography was necessary. Evaporating the solvent left a dark brown, oily residue that clogged the column and did not give pure fractions. Therefore, the residue was extracted with hot cyclohexane/toluene 10:1. After the solution was cooled, a yellow wax was obtained that was easier to purify by column chromatography and gave significantly fewer mixed fractions. For the 1 kg batches, the hot cyclohexane/toluene extract was poured onto cold cyclohexane to accelerate the precipitation.

Despite all of the improvements, the yield of the final scale-up to 1 kg was unsatisfactory. Chromatography produced many impure fractions (300 g of product fractions and 450 g of mixed fractions) containing DET, an unknown impurity, and traces of the starting material, which had to be chromatographed up to three times to yield another 160 g of pure product. Further investigations showed that parts of the product remained in the residues of evaporation and recrystallization. Thus, an improved workup had to be devised to improve the yield. The main goal of this workup had to be the removal of as much DET and unknown impurity as possible before chromatographic purification. Tests were conducted to wash the residues with an acid and a base to remove the impurities. Therefore, the residues were dissolved in dichloromethane, and the dichloromethane solutions were washed with diluted hydrochloric acid, citric acid, aqueous sodium hydroxide solution, and aqueous sodium hydrogen carbonate solution. NMR analysis showed that washing with an aqueous citric acid solution and 0.1 M sodium hydroxide solution gave the best results for the removal of DET. This was combined with a washing step, using cyclohexane/toluene, derived from the recrystallization used before, to remove the unknown impurity. This strategy was used for the workup of the residues/mixed fractions of both 1 kg batches, yielding an additional 160−200 g of product for both batches.

Development of the ee-Values. During the workup of the 1 kg batches, a drastic inhomogeneity of the ee-values was noticed. Samples from different chromatography columns (each batch was chromatographed up to four times) showed ee-values ranging from 68 to 97%. It turned out later on that even samples from the same fraction showed different ee-values, depending on the sampling location in the flask after evaporation, indicating different rates of crystallization for both enantiomers. At first, this was attributed to the basic washing procedure, but tests showed that exposure of the product to the base for up to 48 h did not decrease the ee-value significantly. To gain insight into the development of the ee-value during the reaction and the workup, a small batch was synthesized and the ee-value was measured after every step. The graph shown in Figure 2 shows the development of the ee-values. As may be observed on the graph, the ee-value rose steeply at the beginning of the reaction and evened out at 60% after about 17 h of reaction time. It also did not change during the workup, and prior to crystallization, it remained 60%. However, chromatographic purification influenced and improved the ee-value significantly. The first fractions had an ee-value over 90% that dropped constantly afterward. As the first fractions were the ones most likely to be contaminated by DET, the ee-value of the pure fractions was less than that of the mixed fractions, making a preliminary removal of DET even more important.

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In a parallel investigation, the stability of the product regarding the ee-value was tested under different conditions (Table 1).

Table 1. ee-Values under Different Conditions

condition	ee-value [%]
reference sample	89.2
stirred in 0.1 M HCl/DCM for 48 h	90.4
stirred in 0.1 M NaOH/DCM for 48 h	88.8
stirred in silica gel DCM/EtOAc 7/3 for 48 h	81.8 (some material crystallized and showed an ee-value of 97.3)
stirred in aq. $FeSO_4$ solution/DCM for 48 h	80.1
solid heated to 70 °C for 3 h	84.5
addition of extra cumene hydroperoxide to the reaction	56.7 starting from 60.1

All of the tests confirmed the observations made during the synthesis of the batch performed under ee-value control. Acid and base washing proved to have no significant influence on the ee-value, while drying and the addition of $FeSO_4$ solution had. However, as the quenching with $FeSO_4$ was done in the shortest time possible (around 10 min) and drying at an elevated temperature could be avoided altogether, both did not pose a problem. The same applied to the addition of extra amounts of cumene hydroperoxide, which is not normally part of the synthetic procedure. Silica gel, on the contrary, seemed to be the major factor. The tests seemed to indicate that the desired enantiomer slowly crystallized during chromatography, leading to an enrichment of the "wrong" enantiomer in later fractions and thus a decreased ee-value.

These results indicated that a recrystallization procedure to increase the ee-value should be possible. Therefore, different solvents and mixtures were tested. These experiments are summarized in the following table. On top of the ee-values for the recrystallized material and the mother liquor, an ee-value for the residue is given, as most of the samples did require huge amounts of solvent (300 mL of cyclohexane per g, for example). To some of the samples, additional DET was added, as it was the only chiral substance present during the chromatographic purification that influenced the ee-value. One sample was recrystallized three times to test if the ee-value can be further increased by multiple recrystallizations and the results are summarized in Table 2.

Analysis of the obtained data showed some interesting findings:

First, the ee-value could be increased by most solvents, but the yield is greatly diminished in most cases. The ee-value of the mother liquor indicated that a large amount of the desired enantiomer was dissolved. This problem was magnified by the low overall solubility in most solvents and the large amounts of solvent that were required to completely dissolve the material.

Second, if the product was not completely dissolved during recrystallization, the ee-value of the residue was increased, while the ee-value of the mother liquor and the recrystallized material was decreased. This seemed to indicate that the "wrong" enantiomer dissolved quicker in the solvent, thus enriching the mother liquid and the resulting crystals, while at the same time depleting the residual solid.

Third, additional DET in the sample seemed to have little or no influence on the ee-value.

Lastly, multiple recrystallizations increased the ee-value even further, but the increase became smaller each time.

As the "wrong" enantiomer seemed to dissolve faster, there should be a possibility to increase the ee-value by simply washing the product with an appropriate solvent mixture (Table 3). To test this, a product sample was suspended in the solvent and stirred under different conditions. The mixture was filtered, and the ee-value of the residual solid and the mother liquor was analyzed.

Table 3. ee-Values after Washing under Different Conditions

conditions	start ee (%)	solid ee (%)	mother liquor ee (%)
cyclohexane/toluene 12/1 1 h at 20 °C	89.2	93.6	5.3
cyclohexane/toluene 12/1 1 h at 40 °C	93.6	96.8	20.6
cyclohexane/toluene 12/1 17 h at 20 °C	96.8	97.2	84.6

The test showed that washing gave the same increase in the ee-value as recrystallization but required less than half the amount of solvent. Washing with hot solvent worked even better than the use of cold solvent. However, as the mother liquor contained more of the "wanted" enantiomer after each washing, the efficiency of the washing decreased with every step and a limit, after which no further increase in the ee-value will be observed, must be expected. This washing procedure

Table 2. ee-Values after Different Recrystallizations

conditions	start ee (%)	recrystallized ee (%)	mother liquor ee (%)	residue ee (%)	yield (%)
cyclohexane/toluene 8/1	89.2	85.5	24.8	96	~90
cyclohexane/toluene 8/1 additional DET in sample	89.2	81.1	9.9	95.3	~90
cyclohexane	89.2	69.5	34.7	95.9	~80
cyclohexane additional DET in sample	89.2	74.3	40.3	95.6	~80
cyclohexane/toluene 12/1	94.2	96.0	61.1		85
cyclohexane	94.2	96.2	64.9		87
EtOAc	89.2	96.3	74.1		58
heptane	89.2	79.3	29		~100
cyclohexane/EtOAc 10/1	89.2	95.1	12		76
DCM/EtOAC 7/3	89.2	95.6	23.7		61
cyclohexane/toluene 12/1 1st recrystallization	89.2	93.1	18.4 "wrong" enantiomer		
cyclohexane/toluene 12/1 2nd recrystallization	93.1	94.1	24.5		
cyclohexane/toluene 12/1 3rd recrystallization	94.1	96.6	47.1		57 in 3 steps

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was applied to both 1 kg batches and the ee-value of several fractions could be increased from <85 to 95% by multiple washing steps. The yield could be considerably improved as fractions could be purified that would have been discarded previously.

Finally, the synthesis was successfully scaled up by adding some improvements. First, a method to destroy residual peroxides was devised, thus preventing any explosion hazard during the workup. Second, the workup was redesigned by incorporating a washing procedure to diminish the number of impurities before the chromatographic purification. Due to this washing procedure, residues that previously could not be purified could be worked up, thus improving the overall yield.

Lastly, a washing procedure was established to significantly increase the ee-value of the product, thus enabling the use of the material that previously would have been discarded due to not meeting the specifications for the ee-value.

Overall, 19 batches ranging in size from 1.3 to 1150 g were synthesized, yielding yields from 50 (with recrystallization) to 65%.

The purification was improved as well by shortening the column and changing the eluent to improve the separation of the components while at the same time saving time and eluent.

CONCLUSIONS

All steps of the reaction could be successfully scaled up to batch sizes of between 500 and 1300 g. The first three steps gave good yields >90%, while the yield of the last step was diminished, as the focus was on obtaining a material of excellent purity, with a high ee-value. Therefore, the impure material and the material with low ee-values had to be discarded, thus diminishing the yield.

During the scale-up, several improvements to the original synthetic procedures were made to overcome the problems encountered during the synthesis:

(1) By changing the solvent for the second step, the workup could be simplified, thus increasing the yield and saving time and additional solvents.

(2) The third step was improved by reducing the reaction time to diminish the amount of side products, as well as reducing the workup to a short silica column.

(3) The workup of the last step was reworked, changing it into an elaborate purification procedure. First, the chromatographic purification was simplified by shortening the column and changing the eluent to improve the purity of the product while at the same time drastically saving time and the eluent. Second, two washing steps were added, which improved the yield and the ee-value of the product, respectively. Due to the new workup procedure, a product with a purity >99% and an ee-value of 95% could be obtained.

EXPERIMENTAL SECTION

5-(Chloromethyl)thiazole (2). 5-Thiazolylmethanol **1** (495 g; 4.3 mol) was dissolved in dichloromethane (2 L) under an argon atmosphere. The solution was cooled to 0−5 °C and thionyl chloride (312 mL, 4.3 mol) diluted with dichloromethane (500 mL) was added dropwise. The reaction was slowly warmed to ambient temperature and stirred for 15 h. The solvent was evaporated, and the crude product was dried. The product **2** (713 g, 4.2 mol, 98% yield) was obtained as a yellow solid.

[(Diphenylmethyl)sulfanyl]methanimidamide hydrobromide (5). Diphenylmethanol **3** (1000 g, 5.4 mol) and thiourea (471 g, 6.2 mol) were suspended in water (2.5 L) and the suspension was heated to 90 °C. At around 60 °C, the solid was completely dissolved and the suspension turned clear. Hydrobromic acid (2.8 L, 25 mol) was slowly added over 60 min at 90 °C and the mixture was stirred at this temperature for another 30 min. To ensure a clean crystallization and to avoid the formation of large lumps, the reaction mixture was slowly cooled over 5 h to 5 °C. The precipitate was filtered out and washed with water (2 × 1L). The resulting solid was dried under vacuum for 4 days. The product (**5**) (1690 g, 5.2 mol, 97% yield) was obtained as a white solid.

5-((Benzhydrylthio)methyl)thiazole (6). The building blocks (**5**) (1288g, 4.0 mol) and (**2**) (744 g, 4.4 mol) were dissolved in methanol (20 L). Potassium carbonate (2764 g, 20.0 mol) was added portionwise, and the reaction mixture was heated under reflux for 2.5 h. The suspension was filtered hot to remove excess potassium carbonate. The solvent was evaporated, and the residue was purified by filtration over a short silica column using dichloromethane as the eluent. The product (**6**) (1090 g, 3.7 mol, 92% yield) was obtained as an off-white solid.

(S)-5-((Benzhydrylsulfinyl)methyl)thiazole (7). (**6**) (1032 g, 3.5 mol) was dissolved in dry acetone (10 L). Titanium isopropoxide (309 mL, 1.0 mol) and L-(+)-diethyl tartrate (357 mL, 2.1 mol) were added, and the mixture was stirred for 10 min at ambient temperature. Water (9 mL, 0.5 mol) was added, and the reaction mixture was stirred for another 5 min. The reaction mixture was stirred at 65 °C for a further hour. After cooling back to ambient temperature, *N*,*N*-diisopropylethylamine (121 mL, 0,7 mol) was added and the mixture was stirred for 10 min. Cumene hydroperoxide (80% solution in cumene, 648 mL, 3.4 mol) was slowly added, and the reaction was stirred for 20 h at ambient temperature. The reaction was quenched by the addition of an aqueous iron(II) sulfate solution (10 g in 200 mL of water). The solvent was evaporated, and the residue was dissolved in a mixture of cyclohexane (16 L) and toluene (2 L) by heating. The hot solution was added onto cold cyclohexane (12 L) and allowed to cool to ambient temperature. The obtained precipitate was filtered out. The oily residue that did not dissolve in the cyclohexane/toluene mixture was dissolved in dichloromethane (2 L) and washed twice with an aqueous citric acid solution (200 g in 2 L of water) and twice with an aqueous sodium hydroxide solution (0.1 M, 2 L). The solvent was evaporated. Cyclohexane/toluene (10:1, 2 L) was added, and the mixture was stirred under reflux for 2 h. After the mixture cooled, the precipitate was filtered out. The solid fractions were purified by chromatography on a short silica gel column with 7:3 dichloromethane/ethyl acetate as the eluent. Mixed fractions were chromatographed up to four times to increase the yield. The purified fractions were stirred at 40 °C with cyclohexane/toluene 10:1 (10 mL/g of product) to increase the ee-value. The solid was filtered out and dried under vacuum for 2 h. This process was repeated up to 23 times until the ee-value was high enough. The product (**7**) (540 g, 1.7 mol, 50% yield) was obtained as a white solid.

Both ∼1 kg batches of (**7**) were blended by dry mixing them for 2 h using a Mixomat A by Fuchs AG. The blended batch was washed with cyclohexane/toluene 10/1 (10 mL per g of product) five additional times. 1040 g of the blended product (**7**) was obtained. The final product batch was additionally

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characterized upon batch delivery as reported earlier (please refer to the Supporting Information).

Measurements. ^{1}H NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer. The resonance frequency for ^{1}H NMR was 400.13 MHz. All measurements were performed for a solution in fully deuterated methanol at 300 K. Chemical shifts for proton and carbon measurements are referenced internally to residual, nondeuterated maleic acid's signal for ^{1}H (δ = 6.33 ppm). HPLC, ICP-MS, qNMR, and IR measurements during the development of synthetic steps and for batch characterization were performed on instruments available at the ChemCon manufacturing site, Freiburg, Germany.

HRESIMS spectra of the final batch were obtained on a maXis HD ESI-Qq-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). Samples were dissolved to 20 μg/mL in MeOH and directly infused into the ESI source at a flow rate of 3 μL/min with a syringe pump. The ESI ion source was operated as follows: capillary voltage: 0.9 to 4.0 kV (individually optimized), nebulizer: 0.4 bar (N_2), dry gas flow: 4 L/min (N_2), and dry temperature: 200 °C. Mass spectra were recorded in the range of m/z 50−1550 in the positive-ion mode. The sum formulas were determined using Bruker Compass DataAnalysis 4.2 based on mass accuracy ($\Delta m/z \leq 2$ ppm) and isotopic pattern matching (SmartFormula algorithm).

General compound purity of the final batch was determined by HPLC on an UltiMate 3000 series system equipped with a VWD detector (Dionex/Thermo Fisher Scientific, Germering, Germany). Separation was carried out on an Acclaim 120 C18, 2.1 mm × 150 mm, 3 μm HPLC column (Thermo Fisher Scientific) using LC-MS-grade water and acetonitrile as mobile phases A and B, respectively. The sample components were separated and eluted with a linear gradient from 10 to 90% B in 25 min, followed by an isocratic column cleaning and re-equilibration step. The flow rate was 0.2 mL/min, and the column oven temperature was set to 25 °C. The purity was determined from the UV chromatogram (254 nm) as the ratio of the peak area of the compound to the total peak area (i.e., the sum of the areas of all peaks that were not present in the solvent blank).

The enantioselectivity of asymmetric syntheses of the final compound (**7**) batch was assessed via an HPLC system (Diacel Inc., Tokyo, Japan) using a Chiralpack IA analytical column (4.6 × 250 mm^2, 5 μm) with the column oven temperature set to 25 °C. HPLC-grade ethyl acetate at 1 mL/min flow rate was used as the mobile phase, and the running time was set to 20 min.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c09348.

^{1}H NMR spectra, chiral- and reversed-phase HPLC spectra, and HRESIMS spectra. (PDF)

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Notes

The authors declare no competing financial interest.

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