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Selection and Development of a Route for Cholesterol Absorption Inhibitor AZD4121

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Supporting Information

ABSTRACT: The development of a synthetic route to the cholesterol absorption inhibitor AZD4121 is presented. Key steps are a highly enantioselective CBS reduction, a stereospecific Staudinger reaction, an amine/lithium chloride mediated ester hydrolysis, and a resolution of a 50:50 diastereomeric mixture by recrystallization. The synthesis was accomplished in 10 linear steps, and the overall yield, when compared with the lead optimization (LO) route, was improved from 1% to 20%. All purifications of intermediates through preparative HPLC or silica gel chromatography were avoided. This was possible since many of the intermediates along the route could be used as such in the next step until an intermediate with suitable crystalline properties could be identified and purified through crystallization.

INTRODUCTION

Hypercholesterolemia is a metabolic disorder that is associated with several diseases, most notably atherosclerosis, which is often followed by early death. Cholesterol absorption inhibitors (CAI) is a class of compounds that have attracted attention for the treatment of hypercholesterolemia. The mechanism of CAI is based on blocking the uptake of cholesterol. The azetidinone (β-lactam) structure is common to the class of CAIs to which also ezetimibe[1] (Figure 1) belongs. A feature of AZD4121 dissimilar to ezetimibe is that it has a low bioavailability, which was thought to give a low systemic exposure and low risk of side effects. Much synthetic experience has been gained from the synthesis of ezetimibe and its analogues, and a number of syntheses have been devised.[2] Well known routes include catalytic asymmetric synthesis,[2a] asymmetric induction by chiral auxiliary,[2b−d] and chiral pool based chemistry.[2e] In the present work, we have optimized a synthesis of a CAI, *viz*. AZD4121 **1** (Figure 1).[3]

Unlike ezetimibe, AZD4121 incorporates a sulfur atom which potentially can stabilize a vicinal carbocationic center, i.e. the benzylic one bearing the hydroxyl group. Thus, we were not surprised to hear from the lead optimization (LO) group that epimerization of this center occurred when treated with strong acids such as formic acid and HCl. Moreover, the sulphur atom probably has a pK_a lowering effect of the adjacent methine proton. This increases the risk of epimerization of this position when treated with strong bases. It was actually reported from the LO group that bases such as LiOH and NaOH efficiently effected this transformation, and consequently, mild conditions had to be employed in every single operation when handling this sensitive scaffold.

RESULTS AND DISCUSSION

First-Generation Synthesis. The LO route utilized a strategy similar to one of the published procedures for ezetimibe.[1b,c] The route, shown in Scheme 1, was used for producing the first milligrams of material for in vivo studies. Reaction between 2-bromo-4′-fluoroacetophenone and ethyl thioglycolate furnished sulfide **4**, which sequentially was protected as its ketal and then hydrolyzed to compound **5** (Scheme 1B). For the ring closure to give the azetidinone, the LO route was based on a condensation of chiral acyloxazolidinone **6** with a Schiff's base **3** (prepared according to Scheme 1A) in the presence of $TiCl_4$ and $Ti(OiPr)_4$ followed by cyclization to give the azetidinone scaffold **8** via intermediate **7**. The azetidinone **8** could be obtained diastereomerically pure after silica gel chromatography. After deprotection of the ketal to ketone **9**, the ester was hydrolyzed to the corresponding acid **10** followed by coupling with *tert*-butyl 2-aminoacetate to give *tert*-butyl ester **11**. Cleavage of the *tert*-butyl ester followed by $NaBH_4$ reduction gave acid **12** in a low diastereoselectivity, which then was coupled with (*R*)-2-amino-3-cyclohexylpropanoic acid to give the acid **13** in low yield. This was initially purified by preparative HPLC followed by freeze-drying to obtain a solid with suitable properties. Later, it was found that the acid **13** nicely crystallized as its *tert*-butylammonium salt, which also turned out to be the preferred solid state form for the API **1** (AZD4121). The synthesis was completed in 10 steps and substeps, of which the cyclization step of **6** to **8** did not give reproducible results. The sequence also included several low-yielding steps. This, in combination with several challenging separations by flash chromatography or preparative HPLC, made it clear that the sequence was not attractive for scale up.[4,5] Thus, in order to supply the project with the first hundred grams of API material for toxicity studies, further efforts to improve the overall yield and robustness of the route were necessary.

Second-Generation Synthesis. Retrosynthesis. At the outset of our scaling up, we identified **14** as a strategic target. The retrosynthesis of **14** is outlined in Figure 2. Our synthetic strategy was based on the formation of the azetidinone scaffold from two appropriately protected building blocks, **A** and **B**. We hoped the Staudinger reaction between chiral building block **A**

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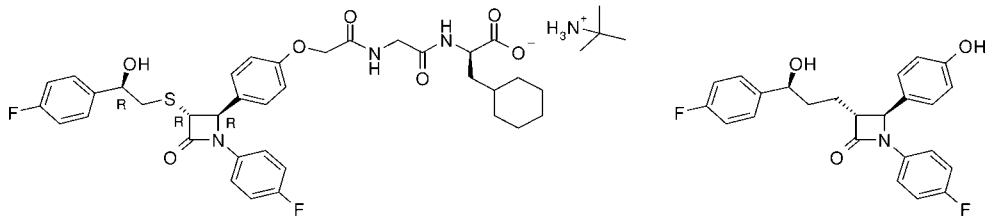

Figure 1. Structures of AZD4121 and ezetimibe.

and imine **B** would furnish a diastereomeric mixture of azetidinone **14**. We believed that this mixture at some stage of the synthesis could be resolved into one pure diastereomer.

Building block **A** should be easily accessible via coupling of **C** with methyl thioglycolate. Chiral building blocks such as **C** are easily obtained via asymmetric CBS-reduction of the corresponding halo-ketone.[6] Imine **B** can be obtained from condensation of 4-fluoroaniline with aldehyde **D**.

Much experience had been gained during the LO-phase in the synthesis of **14**, and several issues had to be addressed. It had been found that, upon formic acid catalyzed removal of a *tert*-butyl ester of **14**, 5−10% epimerization of the benzylic OH position took place. Moreover, the sulfur substituted position of the azetidinone was found to epimerize in the presence of various bases, and furthermore, ring-opening of the azetidinone could take place with nucleophilic bases. Another concern was that the thioether function would probably interfere in catalytic hydrogenations, e.g. debenzylation. Thus, our avenue of protective groups was narrow in terms of conditions for deprotection, and we concluded that protective groups were to be avoided if possible.

Since it had been reported from the LO group that most of their intermediates in the first generation synthesis were noncrystalline and, thus, had to be purified through chromatography, we were aware of the risk of ending up in the same situation with our second generation route. In that case and in order to avoid chromatography, our strategy was to identify a route that included steps that were both high yielding and which could give intermediates that were pure enough to be processed in the next step without further purification. This would enable us to telescope the intermediates until material with suitable crystalline properties could be identified and purified through crystallization.

The synthesis of the acid **14** proceeded through eight intermediates, of which seven turned out to be oils or amorphous solids, and thus, to avoid chromatography, we had to use all these intermediates as such with no purifications other than extractions.

Building Block A and B Synthesis. Scheme 2 outlines our synthesis for securing the first 0.8 mol of building block **14**. The synthesis commenced on a 3 mol scale by the introduction of the chiral benzylic alcohol in **15** via an asymmetric (*R*)-(+)-2-methyloxazaborolidine catalyzed borane-dimethyl sulfide reduction[6] of 2-bromo-4′-fluoroacetophenone. After successfully having isolated **15** as an oil in >99% ee, the material was next reacted with methyl thioglycolate in the presence of triethylamine and cesium carbonate to give **16** as an oil. The protection of the resulting benzylic alcohol had to be carefully balanced, since mild deprotection conditions later on were essential based on the previously mentioned known lability of the molecule, yet withstand the ester hydrolysis which was necessary for the completion of building block **18**. Among many silyl protective groups available, both TES and TMS were tried but rejected because of their lability in the later hydrolysis step. The TBDMS was found to fulfill our requirements, and standard protection conditions using TBDMS-chloride in DMF in the presence of imidazole gave building block **17** as an oil which was pure enough to be used as such in the next step. Simple alkaline hydrolysis of the methyl ester **17** gave us the chiral building block **18** in good yield over 4 steps. This viscous acid was used as such in the next step without further purification.

The Schiff base **3** was prepared from 4-hydroxybenzaldehyde by alkylation of this with methyl bromoacetate to give aldehyde **2** followed by the condensation of this with 4-fluoroaniline using azeotropic removal of water, according to Scheme 1A. The pure Schiff base **3** was obtained in 62% overall yield over the 2 steps after a crystallization from acetonitrile and methanol.

Staudinger Reaction. With the two building blocks **18** and **3** (corresponding to building blocks A and B, Figure 2) in hand, the ring closure was effected using 1-methyl-2-chloropyridinium iodide to give azetidinone *trans*-**19** (Scheme 2B).[7] During the optimization of the reaction, it was found that *slow* addition of the acid **18** as a solution in DCM to a mixture of imine **3**, triethylamine, and 1-methyl-2-chloropyridinium iodide in DCM gave superior results. The resulting product **19** was obtained as an oil, a 50:50 diasteromeric mixture of only trans isomers.[8] The crude product was of sufficient purity to be used as such in the next step.

Hydrolysis and Deprotection. Hydrolysis of the methyl ester in **19** was a major challenge. Standard conditions using alkali hydroxide all resulted in ring-opening and/or epimerization of the sulfur substituted position in the azetidinone. Other conditions tried were reflux in toluene with magnesium iodide, which, apart from a messy mixture, led to 35% formation of cis isomers.[9] Lewis acid catalysis, such as ferric(III) chloride in wet solvents, did give hydrolysis to various extents but was not possible to balance against side reactions on the benzylic OH position. Mild hydrolysis using Me_3SnOH[10] was considered but abandoned due to toxicity and waste issues. After much experimentation, a novel hydrolysis method was developed using a mixture of triethylamine and lithium chloride in wet acetonitrile at 20 °C.[11] After having completed the ester hydrolysis, as little as 1−2% cis isomers was detected as a result of epimerization at the sulfur substituted position of the azetidinone. Removal of the TBDMS group was also a slightly delicate problem. The benzylic position bearing the hydroxyl group was found to epimerize quite readily under acidic conditions. After some optimization work using various additives, we finally found that mild, albeit slow, deprotection

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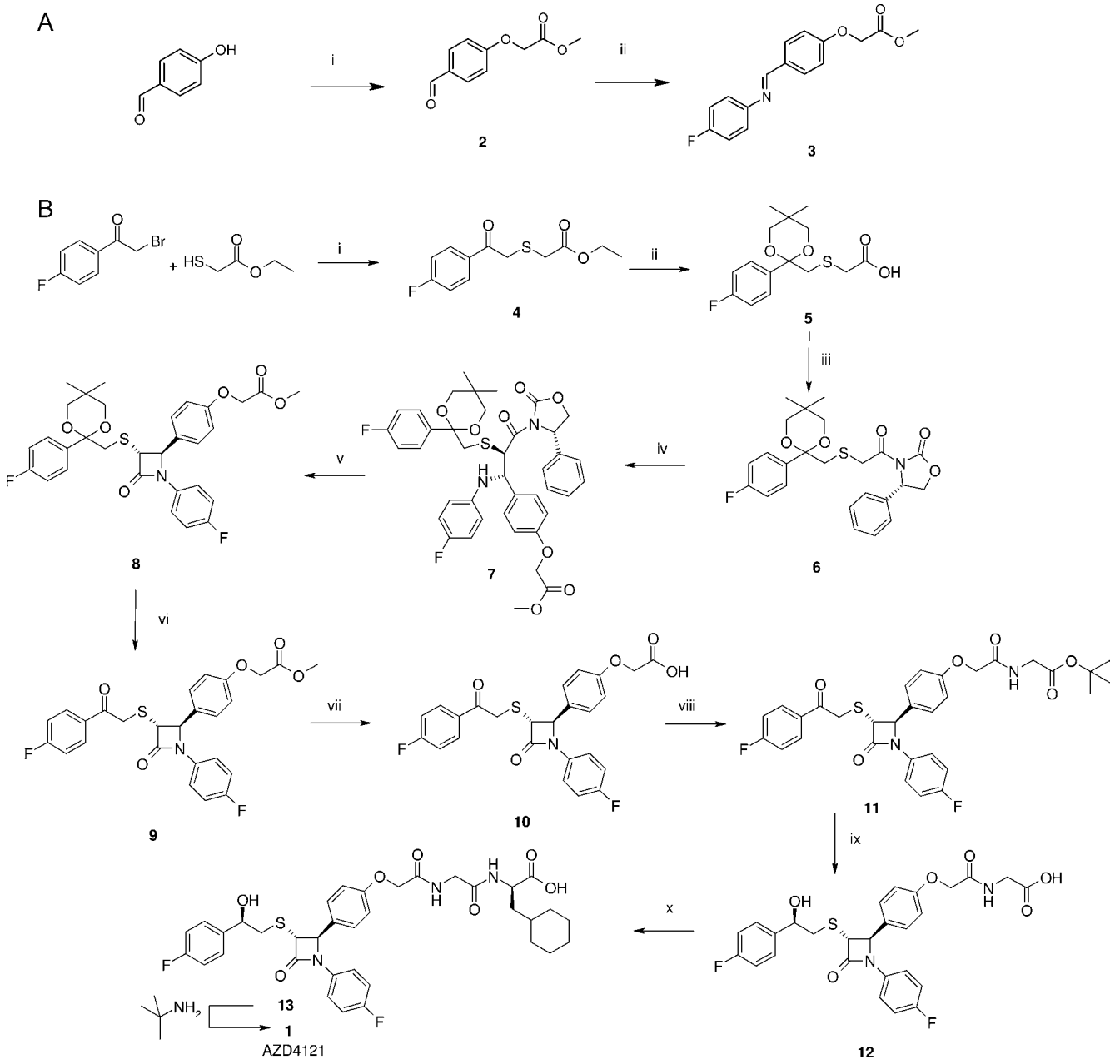
Scheme 1. (A) Preparation of Imine 3[a] and (B) First Generation Synthesis of AZD4121[b]



[a]Reaction conditions: (i) K_2CO_3, methyl bromoacetate, acetone, reflux; (ii) 4-fluoroaniline, toluene, reflux, 62% overall yield. [b]Reaction conditions: (i) K_2CO_3 (1 equiv), acetone, reflux (2 h), 94%; (ii) (a) 2,2-dimethyl-1,3-propanediol (7.5 equiv), p-TSA (cat.), benzene; (b) LiOH, H_2O, THF, 20 °C, 19 h, 87%; (iii) DCC (1.1 equiv), DCM, 0 °C, (*S*)-4-phenyloxazolidin-2-one (1 equiv), 72 h, flash chrom. 74%; (iv) (a) $Ti(OiPr)_4$ (0.25 equiv), $TiCl_4$ (1 equiv) DCM, 10 min; (b) **3** (2 equiv), −30 °C, 20 min; (c) DIPEA (2.5 equiv), −30 °C, 90 min; (d) iPrOH, −78 to 0 °C; (e) cryst. (MeOH), 67%; (v) (a) *N*,*O*-bis(trimethylsilyl)acetamide (3 equiv), toluene, 90 °C, 1 h; (b) TBAF, 45 °C, 21 h, flash chrom. 52%; (vi) HCOOH/DCM 25:75, 20 °C, 16 h, flash chrom. 43%; (vii) Et_3N, LiCl, MeCN, H_2O, 20 °C, 18 h, 56%; (viii) *tert*-butyl 2-aminoacetate·HCl (1.2 equiv), TBTU (1.3 equiv), NMM (3.6 equiv), DCM 20 °C, 1 h, flash chrom. 87%; (ix) (a) HCOOH, 35 °C, 6 h; (b) $NaBH_4$, MeOH, prep HPLC, 36%; (x) (a) NMM, TBTU (1.2 equiv), DMF, 20 °C, 60 min; (b) H-(*R*)-cyclohexylalanine-OH, 20 °C, 60 min, prep. HPLC, 43%.

could be accomplished by using a mixture of acetic acid, water, and lithium chloride at 25 °C to give compound **20**.

Resolution. The resolution of the resulting diastereomeric mixture of the foamlike carboxylic acid, **20**, was tried as the corresponding ammonium salt with various amines. With initial slightly positive results using *tert*-butylamine, we then found that the salt with adamantylamine had favorable properties. After crystallization, a 40% yield of **21** over four steps was obtained with a diastereomeric ratio of 92:8 that in our hands was not possible to improve further.[12] After isolation of free acid **14**, only attachment of the dipeptide **23** remained.

Peptide Coupling. With the inherent sensitivity of our scaffold in mind, we sought a coupling with the dipeptide without having to resort to further deprotection procedures. The dipeptide was synthesized by uneventful peptide synthesis as depicted in Scheme 3A.

The final coupling of dipeptide **23** with carboxylic acid **14** to give the API AZD4121 is outlined in Scheme 3B:

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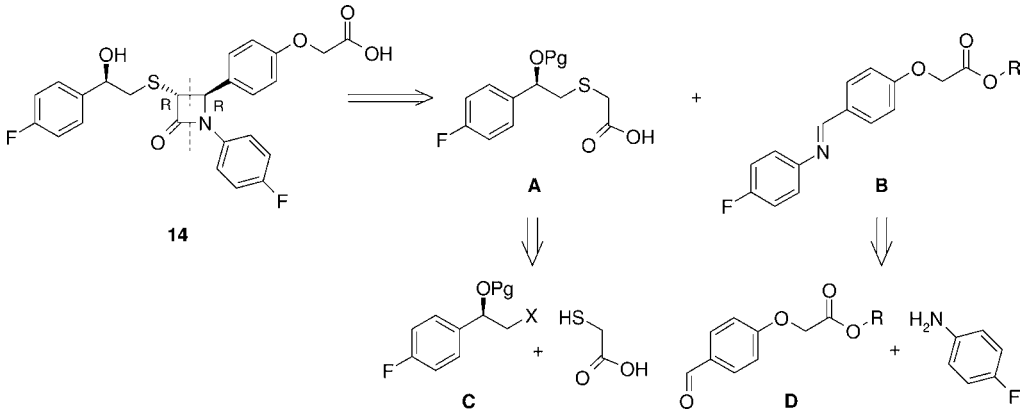
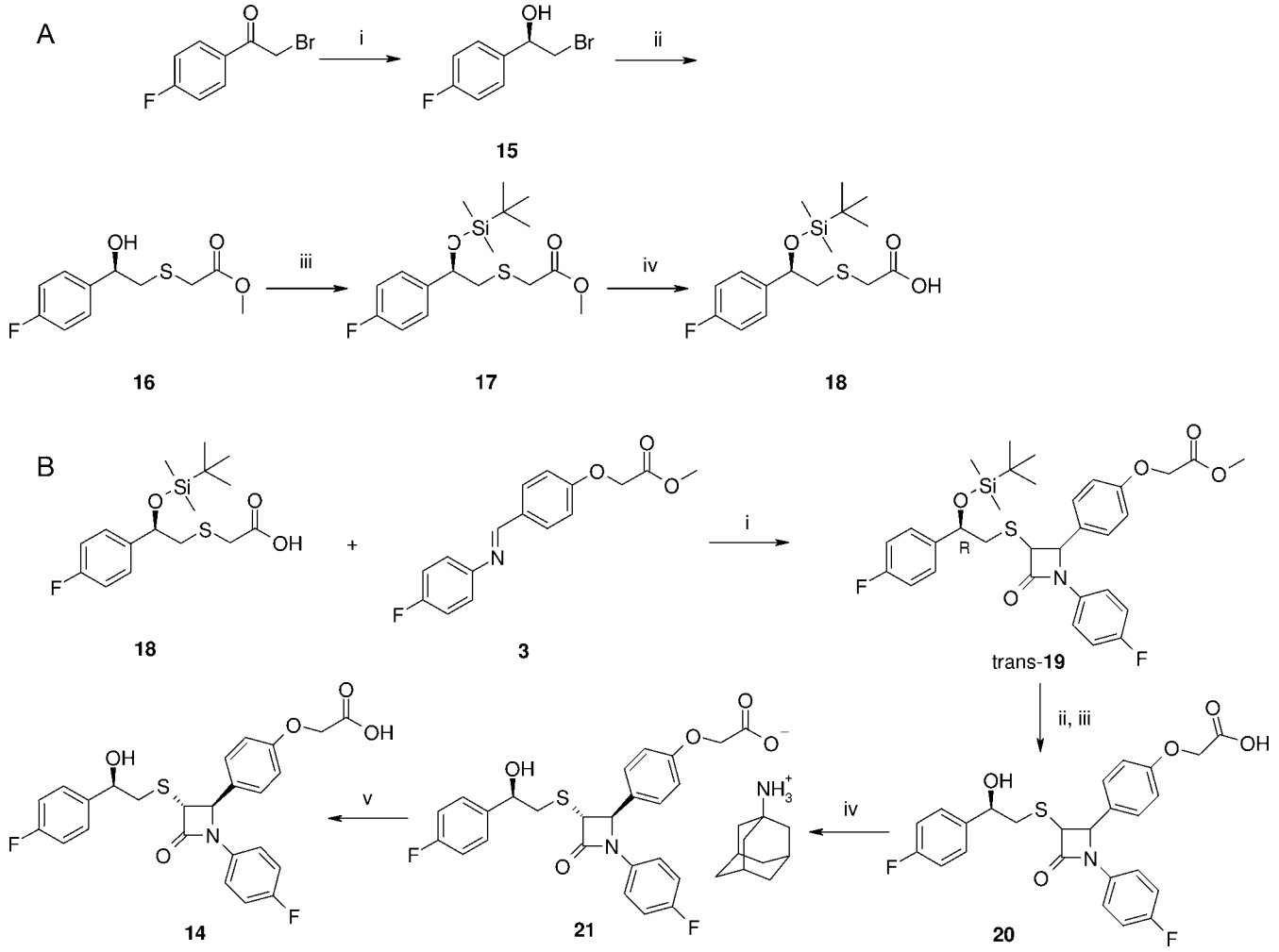


Figure 2. Retrosynthesis of **14**.

Scheme 2. (A) Preparation of Building Block 18[a] and (B) Second Generation Route to Compound 14[b]



[a]Reaction conditions: (i) (*R*)-(+)-2-Me-CBS-oxazaborolidine (0.05 equiv), BH_3-DMS (0.6 equiv), THF, 0 °C, 99% ee; (ii) $HSCH_2COOMe$, Et_3N, Cs_2CO_3, 0 °C, DMF; (iii) TBDMSCl, imidazole, DMF, 25 °C; (iv) NaOH, MeOH, 92% effective yield over 4 steps. [b]Reaction conditions: (i) 2-chloro-1-methylpyridinium iodide, Et_3N, CH_2Cl_2, 23 °C; (ii) Et_3N, LiCl, MeCN/water 95:5, 10−25 °C; (iii) LiCl, HOAc/water 10:1, 23 °C; (iv) adamantylamine (0.7 equiv), acetone/MeOH 3:1, 40% over four steps; (v) 0.2 M HCl, EtOAc, 100%.

We wanted an active ester of **14** that was resistant enough toward intermolecular attack of the free benzylic alcohol, resulting in dimers but at the same time reactive toward attack of the dipeptide. After having tried different[13] active esters on similar structures, our experience was that the 4-chlorophenyl ester of **14** would be of suitable reactivity to fulfill the requirements. TBTU mediated esterification between 4-chlorophenol and **14** in the presence of triethylamine at −15 to 0 °C gave us the desired intermediate active ester **24** while the competing intermolecular esterification could be minimized

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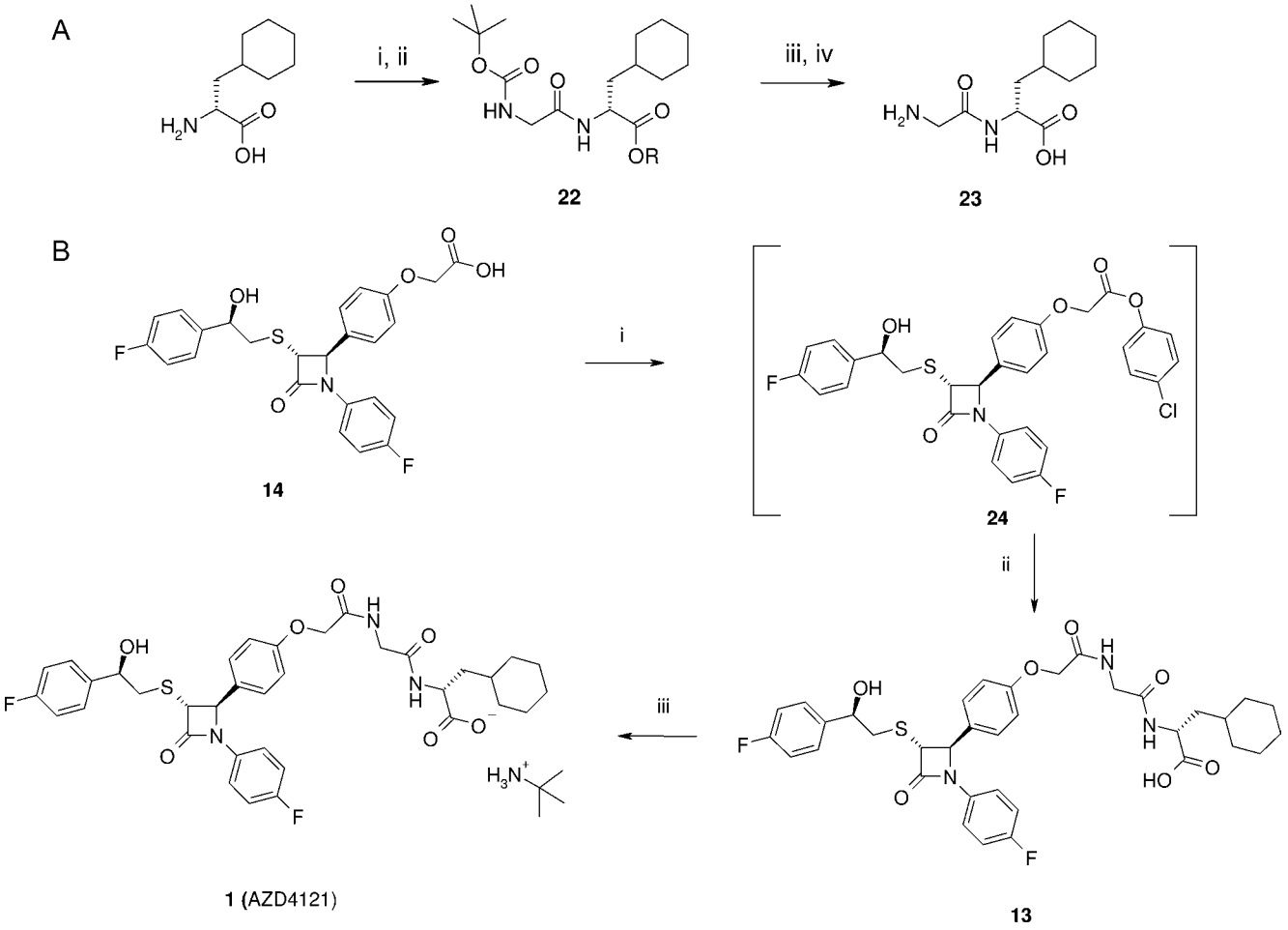
Scheme 3. (A) Synthesis of Peptide Fragment 23[a] and (B) Activation and Coupling of 14 with Peptide 23[b]



[a]Reaction conditions: (i) thionyl chloride, methanol, 83%; (ii) N-Boc-Gly-OH, TBTU, NMM, DCM, 23 °C, 85%; (iii) NaOH, MeOH, 20 °C; (iv) HCOOH, 40 °C, 71% (two steps). [b]Reaction conditions: (i) 4-chlorophenol (2 equiv), Et_3N (2.1 equiv), TBTU (1.3 equiv), DMF, −15 to 0 °C, 17 h; (ii) **23** (1.1 equiv), LiCl (15 equiv), 25−30 °C, 5 days; (iii) *tert*-butylamine (1.1 equiv), acetone, 35 °C, 2 days, 44% + 10% (three steps).

as a result of less reactivity of the benzylic OH as compared with 4-chlorophenol.[14] Even though isolation of the 4-chlorophenyl ester was possible, the simplest procedure was found to be direct addition of peptide **23** to the reaction mixture. The dipeptide had a very low solubility in the reaction mixture, leading to a slow reaction. With the aim of increasing the rate of reaction, we investigated if additives such as lithium salt could have a beneficial effect on the rate. Fortunately, we found that concomitant addition of lithium chloride facilitated dissolution of the dipeptide and also increased the speed of the reaction significantly. After workup, *tert*-butylamine was added to the crude **13** in order to convert the final API to a crystalline isolable salt. Residual 4-chlorophenol, which was found to interfere with the crystallization, was removed by washing of the salt with MTBE. The *tert*-butylammonium salt of AZD4121 was finally crystallized from acetone in 44% yield[15] over three steps to give a material with a diastereomeric ratio of 95:5.[16] This material was acceptable for our initial toxicological studies. Apart from the diastereomeric impurity, the main impurity is the dehydrated product depicted in Figure 3 (1.3%, the structure is suggested on the basis of the HRMS).[17]

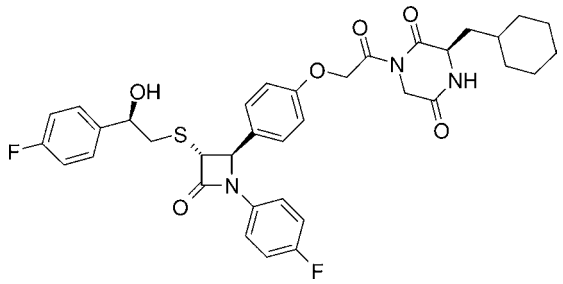


Figure 3. Main contaminant in the API.

CONCLUSION

We have shown a route to prepare the cholesterol absorption inhibitor AZD4121 without the use of chromatographic purifications. This was possible since the route included steps that were both high yielding and furnished intermediates that were pure enough to be telescoped into the next step. Only two purification steps through crystallization were used in the 10 linear step sequence. The accomplishments also include the following: (1) the use of very mild methods for ester hydrolysis and TBDMS deprotection; (2) partial resolution of a diastereomeric mixture of acids by use of adamantylamine; and (3) coupling of the resulting acid with a dipeptide without

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the use of protective groups. For final isolation, the API was isolated as its *tert*-butylammonium salt.

EXPERIMENTAL SECTION

General. All materials were purchased from commercial suppliers and used as such without further purification. All reactions were performed under an atmosphere of nitrogen. Reactions were performed in glass reactors equipped with an overhead stirrer and connected to an external Huber aggregate. IPCs were recorded either by HPLC or ^{1}H NMR analysis of the crude reaction mixture. Determination of the enantiomeric purity of **15** was made by HPLC on a (R,R) Whelk-01 column and using heptane/2-propanol (99/1) as eluent. The diastereomeric purities of the intermediates were recorded using ^{1}H NMR integration. High resolution mass spectrometry was performed on a TOF-MS instrument LCT (Waters), mass precision ±5 ppm. NMR measurement was performed using a Varian Unity spectrometer. HPLC analyses were recorded using a Waters LC 2000 instrument, column Kromasil C_8, 7 μm, eluent MeCN/ammonium acetate buffer. LC/MS analyses were recorded on a Waters ZMD, LC column x Terra MS C_8 (Waters); detection with a HP 1100 MS-detector diode array.

*Methyl 2-(4-Formylphenoxy)acetate (**2**).*[18] With the mantle temperature set at 20 °C, 4-hydroxy benzaldehyde (200 g, 1.64 mol) was dissolved in acetone (0.8 L), and potassium carbonate (278 g, 2.01 mol) was added followed by portionwise addition of methyl bromoacetate (308 g, 2.01 mol). The mixture was heated to reflux for 15 h and cooled to 20 °C, followed by addition of water (1 L) and diethyl ether (1 L). The aqueous phase (also containing a heavy precipitate, presumably potassium hydrogen carbonate) was removed, and the organic phase was washed with more water (2 × 150 mL). By coevaporation with toluene, the water, acetone, excess methyl bromoacetate, and ether were removed under reduced pressure, yielding a white solid (318 g, 1.64 mol). The crude product is used as such in the next step. The yield is assumed to be quantitative. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.77 (s, 3H), 4.69, (s, 2H), 6.97 (d, 2H), 7.80 (d, 2H), 9.85 (s, 1H). ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 52.5, 65.2, 115.0, 130.9, 132.1, 162.6, 168.6, 190.8. HRMS (ESI) m/e 193.0503 [$(M - H)^-$, calcd for $C_{10}H_9O_4$ 193.0501].

*Methyl 2-[4-[(E)-(4-Fluorophenyl)iminomethyl]phenoxy]-acetate (**3**).* To a solution of the crude methyl 2-(4-formylphenoxy)acetate **2** (318 g, 1.64 mol) from the previous step in toluene (1.4 L) was added 4-fluoroaniline (182 g, 1.64 mol), and the mixture was heated to reflux. Water was azeotropically removed using a Dean−Stark apparatus. When the aldehyde had been consumed (about 1 h, monitored by HPLC analysis), the toluene was distilled off under reduced pressure. To the remaining solid was added acetonitrile (400 mL). Addition of methanol (1.2 L) resulted in precipitation of the product, which was isolated by filtration. Yield 292 g (62% over 2 steps). ^{1}H NMR (400 MHz, d_6-DMSO) δ 3.72 (s, 3H), 4.90 (s, 2H), 7.06, (d, 2H), 7.19−7.32 (m, 4H), 7.87 (d, 2H), 8.53 (s, 1H). ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 52.5 (d), 65.3, 114.9, 115.9 (d, J = 23 Hz), 122.3 (d, J = 8 Hz), 130.2, 130.6, 148.3 (d, J = 3 Hz), 159.3 (d, J = 1 Hz), 161.0 (d, J = 244 Hz), 160.4, 169.0. MS: m/e 288 $[M + H]^+$. HRMS (ESI) m/e 288.1046 $[(M + H)^+$, calcd for $C_{16}H_{15}FNO_3$ 288.1036].

*(1R)-2-Bromo-1-(4-fluorophenyl)ethanol (**15**).* Borane dimethylsulfide complex (135 g, 1.8 mol) was added over a 5 min period to a solution of the (*R*)-(+)-2-methyl-CBS-oxazaborolidine (157 g of a 1 M solution in toluene, 0.165 mol) in THF (1 L) at a temperature of 0 °C. After 30 min a solution of the 2-bromo-4′-fluoroacetophenone (662 g, 3.05 mol) in THF (2.5 L) was added over a 3 h period at a reaction temperature of +4 to +7 °C. Fifteen minutes after the addition was complete, HPLC analysis of the mixture indicated full conversion. Methanol (400 mL) was added over 10 min with a jacket temperature set at 0 °C. Due care should be taken during the quench, as the reaction is effervescent and exothermic! The temperature was set to +10 °C, and after 45 min the solvent was removed in vacuo at an inner temperature of 25 °C (the jacket temperature was 35 °C, and the pressure was 120 mmHg) over a 3 h period to give a total volume of 1.7 L. Diethyl ether (3 L) was added followed by the addition of hydrochloric acid (1 M, 1 L) at 20 °C. A precipitate started to form almost immidiately. The mixture was cooled to +5 °C and then stirred for another 45 min. The precipitate was removed by filtration, and the organic phase was separated and washed once with brine (1.2 L). DMF (2 L) was added, and the ether was removed in vacuo to give the desired compound as a solution in DMF which was used as such in the next step. The yield was assumed to be quantitative. The enantiomeric excess (ee) was measured to be 99% by chiral HPLC analysis. A small sample was concentrated to give an analytical sample. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.73 (s, br, 1H), 3.50 (dd, 1H), 3.60 (dd, 1H), 4.90 (dd, 1H), 7.03−7.09 (m, 2H), 7.33−7.39 (m, 2H).

*Methyl 2-[(2R)-2-(4-Fluorophenyl)-2-hydroxyethyl]-thioacetate (**16**).* To the crude DMF solution of (1*R*)-2-bromo-1-(4-fluorophenyl)ethanol (**15**) from the previous step + 85 g (0.39 mol) of a smaller batch was added methyl thioglycolate (370 g, 3.48 mol). The mixture was chilled to 0 °C and then the triethylamine (383 g, 3.78 mol) was added over a 10 min period, while keeping the inner temperature at approximately 0 °C. Very soon after the addition of triethylamine was complete, a precipitate started to form. Stirring was continued at 0 °C for 15 h. ^{1}H NMR analysis of the mixture showed 13% unreacted starting bromide. Cesium carbonate (61 g, 0.19 mol) was added, and the mixture was then stirred at 20 °C for an additional 18 h. At this point, the reaction was complete and ethyl acetate (2.5 L) and water (2 L) were added. After separation of the phases, the organic phase (~4 L) was washed with water (2 × 1 L). The pooled aqueous phases were extracted with extracted with ethyl acetate (2 × 1 L), and the combined organic extracts were washed with water (2 × 1 L). All organic layers were combined, dried ($MgSO_4$), and concentrated to give an oil (930 g). The crude product containing 11% DMF by weight was used as such in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.81 (dd, 1H), 2.97 (dd, 1H), 3.25 (d, 1H), 3.29 (d, 1H), 3.73 (s, 3H), 4.78 (dd, 1H), 6.99−7.03 (m, 2H), 7.31−7.35 (m, 2H).

*Methyl 2-[(2R)-2-[tert-Butyl(dimethyl)silyl]oxy-2-(4-fluorophenyl)ethyl]thioacetate (**17**).* With a mantle temperature set at 20 °C, to the crude alcohol **16** (840 g, 3.44 mol, assuming quantitative yield in the previous step) from the previous step was added DMF (1.25 L) followed by the addition of imidazole (585 g, 8.6 mol) and TBDMS-Cl (673 g, 4.5 mol). The reaction temperature rose from 18 to 27 °C. Full conversion (as judged by HPLC analysis) had been obtained after stirring over the weekend. The mixture was diluted with MTBE (2.5 L) and then washed with water (2 L). The organic phase was washed with water (1 L) and concentrated in vacuo at 40 °C to give the crude product as a pale yellow oil (1250 g). According to ^{1}H NMR analysis, the crude product was contaminated with TBDMS-OH as one major impurity (~60

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g calculated from ^{1}H NMR), giving a corrected yield of 97%. The mixture was used as such in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.11 (s, 3H), 0.06 (s, 3H), 0.87 (s, 9H), 2.82 (dd, 1H), 2.92 (dd, 1H), 3.12 (d, 1H), 3.18 (d, 1H), 3.71 (s, 3H), 4.80 (dd, 1H), 6.98−7.04 (m, 2H), 7.28−7.33 (m, 2H).

*2-[(2R)-2-[tert-Butyl(dimethyl)silyl]oxy-2-(4-fluorophenyl)-ethyl]thioacetic Acid (**18**).* The crude ester **17** (1250 g) from the previous step was dissolved in MeOH (1000 mL). The mixture was cooled to 17 °C (the mantle temperature was set at 0 °C). NaOH (180 g, 4.5 mol) in MeOH (1300 mL) was added during 10 min. A 90% conversion to the desired product had been obtained after 10 min of stirring. The mixture was allowed to stir at 10 °C for 16 h, after which full conversion had been obtained. The temperature of the reaction mixture was increased to 30 °C, and most of the MeOH was distilled off under reduced pressure until a total volume of ∼2 L was achieved. Diethyl ether (7 L) and water (5 L) were added. After separation of the phases, the aqueous phase was acidified to pH 1 using 2.5 M HCl (aqueous, 2 L). Diethyl ether (2 L) was added. The organic phase was dried ($MgSO_4$) and concentrated to give the desired acid as a pale yellow oil (1144 g). The crude product was contaminated with TBDMS-OH as the main impurity (∼10 mol %, 4% w/w), giving a corrected yield of 92%. The acid was pure enough to be used as such in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.11 (s, 3H), 0.06 (s, 3H), 0.88 (s, 9H), 2.85 (dd, 1H), 2.94 (dd, 1H), 3.17 (d, 1H), 3.23 (d, 1H), 4.83 (dd, 1H), 6.98−7.04 (m, 2H), 7.28−7.33 (m, 2H). MS: *m/e* 343 $[M - H]^-$. HRMS (ESI) *m/e* 343.1189 $[(M - H)^-$, calcd for $C_{16}H_{24}FNO_3SSi$ 343.1199].

*Methyl 2-[4-[(2RS, 3RS)-3-[(2R)-2-[tert-Butyl(dimethyl)-silyl]oxy-2-(4-fluorophenyl)ethyl]thio-1-(4-fluorophenyl)-4-oxo-azetidin-2-yl]phenoxy]acetate (trans-**19**).* Under an atmosphere of nitrogen, a 10 L reactor was charged with the imine **3** (963 g, 3.35 mol), CH_2Cl_2 (3 L), and triethylamine (969 g, 9.58 mol) followed by the addition of 2-chloro-1-methylpyridinium iodide (856 g, 3.35 mol) in one portion. The mantle temperature was set at +23 °C, and the crude acid **18** (1100 g from above) dissolved in CH_2Cl_2 (3 L) was slowly added via syringe pump at a rate of approximately 5 mL/min. The mixture turned red-brown immediately after the addition of acid **18** had started. The temperature of the reaction mixture was maintained at 23 °C during the whole reaction. After approximately 16 h, all the acid **18** had been added. HPLC analysis of the mixture indicated full conversion of the starting acid, **18**. 2 M HCl (aqueous 3.5 L) was added. After separation, the organic phase was extracted with $NaHCO_3$ (aqueous saturated, 3 L), brine (2.5 L), and water (2 L). The organic phase was then concentrated to give the title compound (2018 g, brown viscous oil) as a 50:50 mixture of two diastereomeric trans-isomers. The compound was pure enough to be used as such in the next step. The main impurities were the aldehyde derived from the imine and 1-methylpyridone. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.13 and −0.10 (s, 3H), 0.01 and 0.08 (s, 3H), 0.84 and 0.88 (s, 9H), 2.85−3.13 (m, 2H), 3.83 (s, 3H), 3.84−3.86 (m, 1H), 4.58−4.63 (m, 1H), 4.66 (s, 2H), 4.83−4.93 (m, 1H), 6.90−7.02 (m, 6H), 7.20−7.35 (m, 6H).

*2-[4-[(2RS,3RS)-1-(4-Fluorophenyl)-3-[(2R)-2-(4-fluoro-phenyl)-2-hydroxyethyl]thio-4-oxoazetidin-2-yl]phenoxy]-acetic Acid (**20**).* The crude ester **19** (1400 g) was dissolved in acetonitrile (4.5 L) and triethylamine (1.5 L, 10.8 mol) followed by the addition of water (260 mL). The mixture was chilled to 13 °C, and then lithium chloride (500 g, 11.8 mol) was added in one portion. The mixture was then stirred vigorously at 20 °C for 24 h. At this point the ester hydrolysis was complete according to HPLC. EtOAc (3.5 L) and water (3.5 L) were added. The mixture was stirred for 10 min, and the phases were then separated. The organic phase was concentrated in vacuo at 30 °C, toluene (2 L) was added, and the solvent was removed in vacuo at 25 °C. ^{1}H NMR analysis of the mixture revealed that 1−2% cis-isomer was present in the crude product. The 50:50 diastereomeric mixture was used as such in the next step. An analytical sample of the acid could be obtained through extraction with 1 M HCl/EtOAc followed by concentration in vacuo. ^{1}H NMR (500 MHz, DMSO-d_6) δ −0.18 and −0.15 (s, 3H), −0.04 and 0.03 (s, 3H), 0.77 and 0.81 (s, 9H), 2.83−3.01 (m, 2H), 4.27 and 4.32 (d, 1H), 4.66 (s, 2H), 4.89−4.99 (m, 1H), 5.00 and 5.09 (d, 1H), 6.91−7.40 (m, 12 H). HRMS (ESI) *m/e* 600.2062 $[(M + H)^+$, calcd for $C_{31}H_{36}F_2NO_5SSi$ 600.2052]. To remove the TBDMS group from the intermediate acid, AcOH (4.5 L) and water (450 mL) were added. At a temperature of 10 °C, LiCl (1500 g, 35 mol) was added portionwise over a period of 10 min. The mixture was allowed to stir vigorously at 25 °C. After several days of stirring, the conversion had reached 97.5% by HPLC.[19]

The temperature of the reaction mixture was lowered to 13 °C, and water (2 L) and MTBE (4 L) were added. The temperature rose from 13 to 25 °C during this addition. The organic phase was washed with water (3 × 2 L). The organic layer was concentrated under reduced pressure at 26 °C and then coevaporated with isopropanol (2 × 2.5 L), heptane (2.5 L), and finally 2-propanol/toluene (50%, 3 L). This was done in order to remove most of the AcOH. The crude product **20** was obtained as a brown semicrystalline solid (a 50:50 mixture of two diastereomeric trans-isomers). The crude product was used as such in the next step. The material contained approximately 30 mol % of AcOH.

*1-Adamantylammonium; 2-[4-[(2R,3R)-1-(4-Fluorophen-yl)-3-[(2R)-2-(4-fluorophenyl)-2-hydroxyethyl]thio-4-oxoaze-tidin-2-yl]phenoxy]acetate (**21**).* To the crude acid **20** above was added acetone (3.9 L) and methanol (2.2 L). The solution was heated to 27 °C, and adamantan-1-amine (200 g, 1.3 mol) dissolved in methanol (400 mL) was added over 1 min to give a solution with a temperature of 30 °C. Stirring was halted, seeding crystals were added, and a temperature gradient was set from 35 to 23 °C over 3 h with no stirring. The mixture was allowed to stand for 18 h at 23 °C.[12] A sample of the crystals was taken which showed a diastereomeric ratio of 80:20. The supernatant was allowed to drain off, and the crystals were subsequently suspended in 40% methanol in acetone (4 L). Stirring at 30 °C for 45 min was followed by filtration (without previous cooling). The diastereomeric ratio was now 90:10. The crystals were again suspended in 40% methanol in acetone (2 L), and the mixture was stirred at 50 °C for 1.5 h. The warm mixture was filtered, and the solid collected was dried overnight under reduced pressure at 55 °C to give 377 g (0.78 mol, 40% effective yield over three steps) of the chemically pure salt, diastereomeric ratio 92:8. ^{1}H NMR (500 MHz, d_6-DMSO) δ 1.50−1.64 (m, 6H), 1.72 (d, 6H), 2.00−2.04 (m, 3H), 2.88−2.95 (m, 2H), 4.15 (s, 2H), 4.24 (d, 1H), 4.26 (d, 1H, cis-isomer), 4.71 (t, 1H), 5.01 (d, 1H, cis-isomer), 5.03 (d, 1H), 6.79−6.82 (m, 2H), 7.09−7.17 (m, 4H), 7.22−7.36 (m, 6H). ^{13}C NMR (150.9 MHz, DMSO-d_6) δ 28.4, 35.2, 38.8, 40.2, 50.2, 58.4, 62.0, 67.4, 71.3, 114.7 (d, J = 14 Hz), 114.9, 116.0 (d, J = 22 Hz), 118.80 (d, J = 8 Hz), 127.2, 127.6, 128.0 (d, J = 8 Hz), 133.4 (d, J = 2 Hz), 140.4 (d, J = 3 Hz), 158.4 (d, J = 241 Hz), 159.3, 161.3 (d, J = 242 Hz), 163.8, 170.7.

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*2-[4-[(2R,3R)-1-(4-Fluorophenyl)-3-[(2R)-2-(4-fluorophenyl)-2-hydroxyethyl]thio-4-oxo-azetidin-2-yl]phenoxy]acetic Acid (**14**).* The "free acid" **14** was obtained through extraction of the salt **21** with EtOAc and 0.2 M HCl and washing of the organic layer with water followed by drying ($MgSO_4$) and concentration of the organic layer to a white solid (diastereomeric ratio 92/8 of the two trans-isomers). ^{1}H NMR (600 MHz, d_6-DMSO) δ 2.88−2.95 (m, 2H), 4.27 (d, 1H), 4.67 (s, 2H), 4.70−4.74 (m, 1H), 5.08 (d, 1H), 5.64−5.68 (m, 1H), 6.92−6.94 (m, 2H), 7.09−7.39 (m, 10H). HRMS (ESI) *m/e* 484.1035 [(M − H)$^-$, calcd for $C_{25}H_{20}F_2NO_5S$ 484.1030].

*tert-Butylammonium; (2R)-3-Cyclohexyl-2-[[2-[[2-[4-[(2R,3R)-1-(4-fluorophenyl)-3-[(2R)-2-(4-fluorophenyl)-2-hydroxyethyl]thio-4-oxoazetidin-2-yl]phenoxy]acetyl]-amino]acetyl]amino]propanoate (**1**) (AZD4121).* The acid **14** (122 g, 0.25 mol), 4-chlorophenol (65 g, 0.35 mol), and triethylamine (53 g, 0.53 mol) were dissolved in DMF (1 L). The solution was cooled to −15 °C. TBTU (107 g, 0.33 mol) was added in three portions over a 10 min period. After stirring for 16 h at −15 °C, an HPLC analysis of the mixture revealed that there was still 15% starting material left in the mixture. Another portion of TBTU (10 g) was added. The temperature was set to 0 °C, and after a further 1.25 h, more TBTU (9 g) was added. After 1.5 h, full conversion to the intermediate *p*-chlorophenyl ester **24** had been obtained. The dipeptide **23** (63 g, 0.28 mol) was added in one portion followed by the addition of lithium chloride (160 g, 3.77 mol). The mantle temperature was then set to 30 °C. After 4 days of stirring, full conversion to the acid **13** had been obtained. The temperature was lowered to 16 °C, and ethyl acetate (550 mL) and water (550 mL) were added. The aqueous phase was extracted with EtOAc (3 × 250 mL). The pooled organic phases were washed with 0.5 M hydrochloric acid (0.5 L), brine (250 mL), and, last, water (250 mL). Drying ($MgSO_4$), filtration through Celite, and removal of the solvent in vacuo at 25 °C gave 280 g of crude **13** as a pale brown oil (contaminated with 4-chlorophenol, tetramethylurea, and DMF). This was dissolved in acetone (1.5 L). To this solution was added *tert*-butylamine (18 g, 0.25 mol). The solution was left for 24 h at 35 °C without any proper precipitation observed. Therefore, most of the solvent was removed in vacuo to leave a syrup which was washed with MTBE (2 × 300 mL) to remove most of the DMF and 4-chlorophenol. The washed syrup was partitioned between ethyl acetate (500 mL) and 0.5 M hydrochloric acid (500 mL). The aqueous phase had a pH of 2. The phases were separated, and the ethyl acetate phase was washed with water (2 × 250 mL) and brine (2 × 100 mL), followed by drying ($MgSO_4$) and concentration to give a viscous oil. This was dissolved in acetone (1.2 L) and heated to 35 °C, and then *tert*-butylamine (16.7 g, 0.22 mol) was added. The mixture was left without stirring with a mantle temperature of 35 °C for 46 h. The suspension formed was filtered, and the solid collected was washed with acetone (500 mL), followed by drying of the salt to give **1** (88 g, 0.11 mol, 44% yield from **14**). The mother liquor was purified through preparative HPLC followed by crystallization as described above to give an additional 20 g (26 mmol, an additional 10%) of **1**. Purity by HPLC (210 nm) = 96%, >95:5 diastereomeric purity. ^{1}H NMR (500 MHz, d_6-DMSO) δ 0.70−0.87 (m, 2H), 1.03−1.15 (m, 3H), 1.20 (s, 9H), 1.25−1.38 (m, 2H), 1.47−1.77 (m, 6H), 2.92 (dd, 1H), 2.95 (dd, 1H), 3.70 (dd, 1H), 3.76 (dd, 1H), 3.92−3.99 (m, 1H), 4.29 (d, 1H), 4.53 (s, 2H), 4.72 (t, 1H), 5.07 (d, 1H), 6.98−7.54 (m, 13H), 8.34 (t, 1H). ^{13}C NMR (150.9 MHz, d_6-DMSO) δ 25.8, 25.9, 26.2, 27.6, 32.5, 33.3, 33.7, 38.9, 40.0, 40.7, 41.9, 50.1, 52.0, 58.4, 61.9, 66.8, 71.3, 114.7 (d, J = 21.1 Hz), 115.2, 116.0 (d, J = 22.7 Hz), 118.8 (d, *J* = 8.1 Hz), 128.0 (d, *J* = 7.9 Hz), 128.9, 133.3 (d, *J* = 2.3 Hz), 140.4 (d, *J* = 2.8 Hz), 158.0, 159.2 (d, *J* = 379.8 Hz), 160.5 (d, *J* = 443.5 Hz), 163.7, 167.1, 167.7, 174.7. HRMS (ESI) *m/e* 696.2568 [(M + H)$^+$, calcd for $C_{36}H_{40}F_2N_3O_7S$ 696.2555].

*Methyl (2R)-2-[[2-(tert-butoxycarbonylamino)acetyl]-amino]-3-cyclohexylpropanoate (**22**).* *N*-BOC-Glycine (84.5 g, 0.48 mol) and *N*-methylmorpholine (146 g, 1.44 mol) were dissolved in CH_2Cl_2 (500 mL). TBTU (168 g, 0.52 mol) was added, and the mixture was allowed to stir at 23 °C for 1 h. (*R*)-Cyclohexylalanine methylester hydrochloride (107 g, 0.48 mol)[20] was added, and the mixture was stirred for 1 h at 23 °C. After a wash with water (300 mL), the organic layer was filtered and then concentrated under reduced pressure. The residue was triturated with heptane (500 mL), and the resulting suspension was then stirred for 1 h with ice-cooling. Filtration of the mixture and washing of the solid with heptane gave after drying 140 g (85%) of the desired product. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82−1.85 (m, 13H), 1.45 (s, 9H), 3.72 (s, 3H), 3.77 (dd, 1H), 3.85 (dd, 1H), 4.59−4.69 (m, 1H), 5.12−5.26 (m, 1H), 6.52 (d, 1H).

*(2R)-2-[[2-Aminoacetyl]amino]-3-cyclohexylpropanoate (**23**).* The ester **22** (140 g, 0.41 mol) was dissolved in MeOH (600 mL). NaOH (20 g, 0.5 mol) dissolved in water (50 mL) was added, and the mixture was allowed to stir overnight, after which full hydrolysis of the ester had been obtained. The solution was neutralized with CO_2(s) followed by concentration of the mixture. Trace of water was azeotropically removed with formic acid (50 mL). To the residue was added formic acid (500 mL), and the mixture was allowed to stir at 40 °C for 17 h. The formic acid was removed under reduced pressure followed by addition of water (500 mL). The pH of the solution was adjusted to ∼5 using 25% NH_3 solution. The suspension formed was stirred at 23 °C for 6 h followed by filtration of the mixture and washing of the solid with water (100 mL). The wet solid (110 g) was suspended in hot (50 °C) acetone (400 mL), and the mixture was then allowed to attain 23 °C followed by filtration of the mixture and washing of the solid with acetone (200 mL). After drying under reduced pressure, the title compound was obtained as a white solid (67.3 g, 72%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.30−2.35 (m, 13H), 4.44 (d, 1H), 4.51 (d, 1H), 5.03−5.10 (m, 1H), 12.02 (br s, 4H). ^{13}C NMR (126 MHz, CD_3COOD) δ 27.5, 27.7, 27.9, 33.7, 35.1, 35.6, 40.7, 42.7, 52.5, 168.8, 178.4. MS: *m/e* 229 [M + H]$^+$. HRMS (ESI) *m/e* 229.1557 [(M + H)$^+$, calcd for $C_{11}H_{21}N_2O_3$ 229.1552].

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR spectra (compounds **1**, **2**, **3**, **14**, **15**, **16**, **17**, **18**, **19**, **21**, **22**, and **23**), ^{13}C NMR spectra (compounds **1**, **21**, and **23**), HPLC purity analysis (compound **1**), LC-MS analysis (during syntheses of **15**, **19**, and the ester hydrolysis product of **19**), chiral HPLC analysis (**15**), and HRMS (**1**, **2**, **3**, **14**, **18**, the ester hydrolysis product of **19**, **23**, and the major contaminant in **1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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(12) It was found that crystallization under nonstirring conditions was paramount for a successful resolution of the diastereomers.

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(14) About 4% intermolecular dimerization through ester formation was observed. Reaction between 4-chlorophenol and TBTU gave rise to small amounts of 2-(4-chlorophenyl)-1,1,3,3-tetramethylisouronium byproduct also.

(15) Purification of the mother liquor by preparative HPLC and conversion to the *tert*-butylammonium salt followed by crystallization raised the yield of the last three steps to 54%.

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