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The large scale synthesis of (*S*)-*N*-Boc *bis*(4-fluorophenyl)alanine

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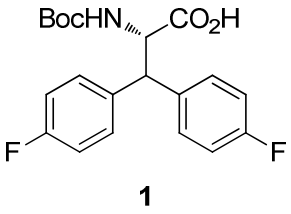
Abstract

The synthesis of (*S*)-*N*-Boc *bis*(4-fluorophenyl)alanine, an intermediate in the synthesis of Denagliptin, is described from the synthesis of a 12g proof of principle sample to a >900 Kg cGMP manufacturing campaign. The chiral centre was established by the asymmetric hydrogenation of the sterically crowded precursor ethyl 2-acetamido-3,3-*bis*(4-fluorophenyl)acrylate. The ability to isolate the various intermediates in a physical form that would readily allow filtration, washing and ultimately purification

underpinned the successful manufacturing campaign.

INTRODUCTION



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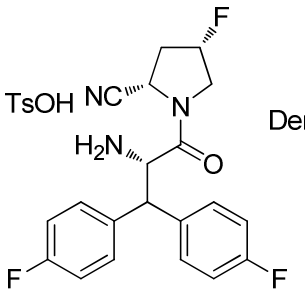

Denagliptin tosylate **2**

(*S*)-*N*-Boc-3,3-*bis*(4-fluorophenyl)alanine, (*S*)-**1** is a key raw material in the synthesis of Denagliptin tosylate **2**, a dipeptidyl peptidase IV (DPPIV) inhibitor developed for the treatment of type II diabetes. The final supply route selected by the innovator was based on the coupling of (4*S*)-fluoro-L-proline and *N*-Boc-(*S*)-difluorophenyl amino acid **1**.[1]

The synthesis of small, highly substituted chiral molecules such as **1** is an enduring challenge. The various substituents need to be brought together in an efficient manner while achieving the desired high stereoselectivity. Within a commercial environment, this must be accomplished with due regard for the overall process efficiency and cost. Furthermore, all this must be completed within a market-driven time frame with the resources available. In developing an asymmetric synthesis of Boc-3,3-*bis*(4-fluorophenyl)alanine, we considered the use of resolution of diastereomeric salts, biocatalysis and asymmetric hydrogenation. Initial evaluation of these routes quickly led us to focus on asymmetric hydrogenation as the means of introducing the key chiral centre.

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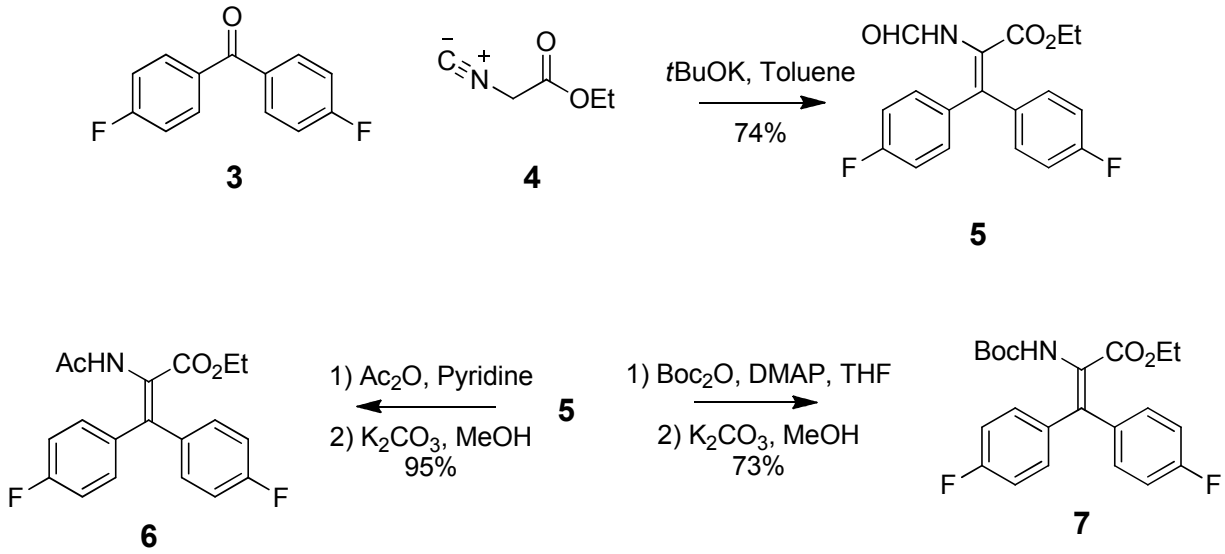
[1] Patterson, D. E.; Powers, J. D.; LeBlanc, M.; Sharkey, T.; Boehler, E.; Irdam, E.; Osterhout, M. H. *Org. Process Res. Dev.* **2009**, *13*, 900.

During the initial route selection process it became clear that our chosen route still posed some significant challenges. We had a great deal of experience in the synthesis of unnatural amino acids using asymmetric hydrogenation; typically, this involved an Erlenmeyer condensation and the use of catalysts such as [(EtDuPhos)Rh(COD)]BF_4.[2] However, this synthetic approach was not be suitable in this case.

RESULTS AND DISCUSSION

A number of hydrogenation substrates were prepared using chemistry outlined in Scheme 1. Reaction of ethyl isocyanoacetate **4** with 4,4'-difluorobenzophenone **3** in the presence of base afforded the required *N*-formyl dehydroamino ester **5**.[3]

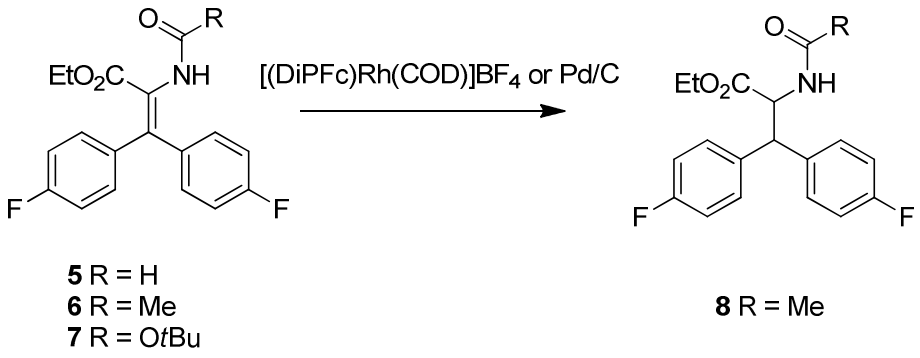


Scheme 1. The synthesis of the *N*-formyl **5**, *N*-acetyl **6** and *N*-Boc **7** hydrogenation substrates.

[2] Cobley, C.J.; Johnson, N.B.; Lennon, I.C.; McCague, R.; Ramsden, J.A.; Zanotti-Gerosa, A. *The application of DuPHOS Rhodium(I) Catalysts for Commercial Scale Asymmetric Hydrogenation*, In *Asymmetric Catalysis on Industrial Scale* Eds Blaser and Schmidt. Wiley-VCH: Weinheim, Germany, **2004**. Page 269.

[3] Antihypercholesterolemic Tetrazol-1-yl Compounds, Sit, S.-Y.; Wright J.J. US patent 4870187, **1988**.

The coupling reaction was conducted using potassium *tert*-butoxide to give ethyl 3,3-*bis*(4-fluorophenyl)-2-formamidoacrylate **5** in 74% yield. Ethyl 2-acetamido-3,3-*bis*(4-fluorophenyl)acrylate **6** and ethyl 2-((*tert*-butoxycarbonyl)amino)-3,3-*bis*(4-fluorophenyl)acrylate **7** were prepared by forming the diamide with the appropriate anhydride followed by treatment with methanolic potassium carbonate. Preliminary attempts at achiral hydrogenation of **5** and **7** using [DiPFc Rh COD]BF_4 gave no conversion.[4] However, hydrogenation of **6** with the same catalyst gave racemic ethyl *N*-Ac-*bis*(4-fluorophenyl)alanine **8**. This became the focus of subsequent asymmetric hydrogenation studies *vide infra*. Further studies on **5**, **6** and later **7** with both [DiPFc Rh COD]BF_4 and Pd/C showed that fairly forcing conditions were required to hydrogenate the double bond. For example, at a molar S/C = 1,000 with [DiPFc Rh COD]BF_4 the reaction took several hours at 50 °C. Similarly high temperatures were required for reductions with 10% Pd/C at a 4% w/w loading.



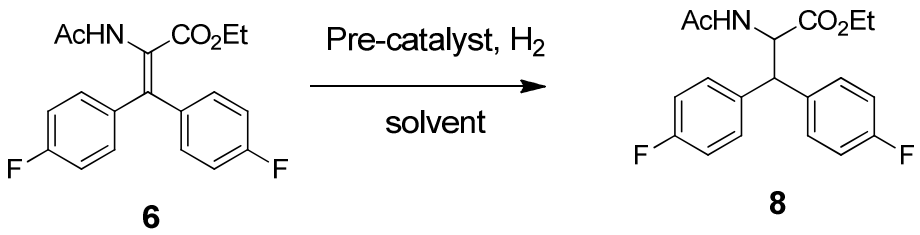
Scheme 2. Synthesis of racemic *N*-Boc and *N*-acetyl *bis*(4-fluorophenyl)alanine.

Having demonstrated the non-stereoselective hydrogenation of the *N*-acetyl derivative with [DiPFc Rh COD]BF_4, we embarked on a screen of asymmetric hydrogenation catalysts. We chose to use similar

[4] Smith, M.E.B.; Derrien, N.; Substituted cyclopentenes, their preparation and their use for chiral scaffolds WO2001017952. Smith, M.E.B.; Derrien, N.; Lloyd, M.C.; Taylor, S.J.C.; Chaplin, D.A.; McCague, R.; *Tetrahedron Letters*, **2001**, *42*, 1347. Sperry, J.; Moody, C.J.; *Tetrahedron* **2010**, *66*, 6483.

reaction conditions (10 Bar H_2, 50 °C) as we expected the reaction to be very slow at lower pressures and temperatures. Selected results are shown in Table 1.

Table 1. Results of screen of hydrogenation precatalysts against *N*-acetyl substrate **6**.



6 **8**

	Pre-catalyst	Solv	S/C	Press (bar)	Temp (ºC)	Conv (%)[#]	e.e. (%)
1	[(*R,R*)-Et-DuPhos Rh COD]BF_4[5]	MeOH	250	10	50	24	nd
2	[(*S,S*)-Ph-BPE Rh COD]BF_4[6]	MeOH	250	10	50	>95	91.8
3	[(*R,R*)-Ph-BPM Rh COD]BF_4[7]	MeOH	250	10	50	16	nd
4	[(*R,R*)-Me-BPE Rh COD]BF_4	MeOH	250	10	50	94	52.8
5	[(*R*)-PhanePhos Rh COD]BF_4[8]	MeOH	250	10	50	>95	92.7
6	[(*R,R*)-Me-5-Fc Rh COD]BF_4[9]	MeOH	250	10	50	34	nd
7	[(*R,R*)-^{i}Pr-5-Fc Rh COD]BF_4	EtOH	250	10	50	>95	50.3
8	[(*R,R*)-Me-5-Fc Rh COD]BF_4	EtOH	250	10	50	>95	74.2
9	[(*R,R*)-Et-5-Fc Rh COD]BF_4	EtOH	250	10	50	>95	72.4
10	[(*S,S,R,R*)-TangPhos Rh COD]BF_4[10]	EtOH	250	10	50	57	55.5

[5] Burk, M.J.; Feaster, J.E.; Nugent, W.A.; Harlow, R.L. *J. Am. Chem. Soc.* **1993**, *115*, 10125

[6] Pilkington, C.J.; Zanotti-Gerosa, A.; *Org. Lett.* **2003**, *5*, 1273.

[7] Jackson, M.; Lennon, I.C. *Tetrahedron Letters*, **2007**, *48*, 1831.

[8] Pye, P.J.; Rossen, K.; Reamer, R.A.; Tsou, N.N.; Volante, R.P.; Reider P.J. *J. Am. Chem. Soc.* **1997**, *119*, 6207.

[9] Burk, M.J.; Gross, M.F. *Tetrahedron Letters*, **1994**, *35*, 9363.

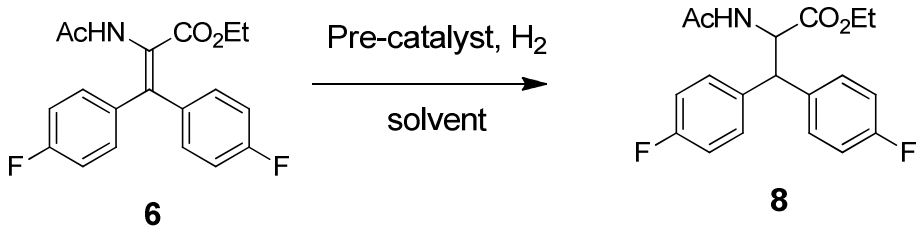
[10] Tang, W.; Zhang, X. *Angew. Chem., Int. Ed. Eng. I**2002**,41*, 1612 ref

11	[(*R*)-^{i}Pr-PhanePhos Rh COD]BF_4	EtOH	250	10	50	96	24.2
12	[(*R,R*)-Et-BPE Rh COD]BF_4	EtOH	250	10	50	91	10.3
13	[(*R,R*)-Me-DuPhos Rh COD]BF_4	EtOH	250	10	50	14	nd
14	[(*S,S*)-2,5-diMe-1-Ph-Phospholane]$_2$ [Rh COD]BF_4	EtOH	250	10	50	94	52.3

[#]Conversion assessed by analysis of ^{1}H NMR spectrum of the crude material. nd: Not determined.

Quite clearly [Ph-BPE Rh COD]BF_4 and [PhanePhos Rh COD]BF_4 (entries 2 and 5) provide both high conversion and selectivity. Further evaluation of these two catalyst systems was conducted with the intention of increasing the substrate to catalyst (S/C) ratio and enantiomeric excess. The results are shown in Table 2. Initial results at S/C = 1,000 showed [PhanePhos Rh COD]BF_4 gave slightly higher activity, particularly in ethanol where 41% conversion was achieved (entry 6). We continued to modify the process in order to target full conversion at a reasonably low catalyst loading. Unfortunately at this stage we had to concede that high conversion could only be achieved by increasing the amount of catalyst used (Entry 10).

Table 2: Preliminary optimization of the hydrogenation of **6**.



6 **8**

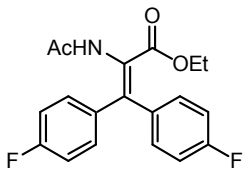
	Pre-catalyst	Solv	S/C	Pressure (bar)	Temp. (ºC)	Conv. (%)[a]	e.e. (%)
1	[(*R,R*)-Ph-BPE Rh COD]BF_4	MeOH	1000	10	50	13	96.8
2	[(*R,R*)-Ph-BPE Rh COD]BF_4	EtOH	1000	10	50	15	95.3
3	[(*R*)-PhanePhos Rh COD]BF_4	MeOH	1000	10	50	20	95.4

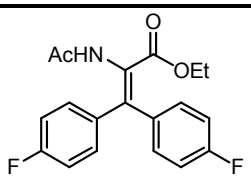
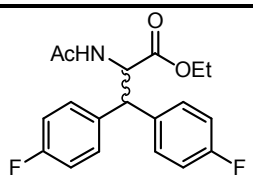
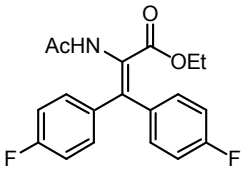
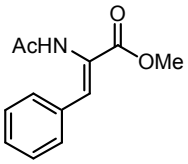
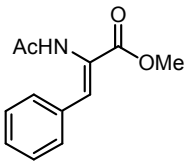
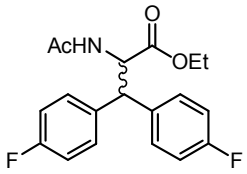
4	[(*R*)-PhanePhos Rh COD]BF_4	EtOH	1000	10	50	54	94.3
5	[(*R*)-PhanePhos Rh COD]BF_4	MeOH	1000	10	70	10	94.5
6	[(*R*)-PhanePhos Rh COD]BF_4	EtOH	1000	10	70	41	94.1
7	[(*R*)-PhanePhos Rh COD]BF_4	MeOH	1000	10	30	15	96.2
8	[(*R*)-PhanePhos Rh COD]BF_4	EtOH	1000	10	30	43	95.6
9	[(*R*)-PhanePhos Rh COD]BF_4	EtOH	1000	20	50	69	94.5
10	[(*R*)-PhanePhos Rh COD]BF_4	EtOH	350	14	50	96	94.9
11	[(*S*)-PhanePhos Rh COD]BF_4	PhMe	1000	10	50	86	83.9
12	[(*S*)-PhanePhos Rh COD]BF_4	PhMe/EtOH	1000	10	50	18	42.4
13	[(*S*)-PhanePhos Rh COD]BF_4	PhMe/MeOH	1000	10	50	35	65.7

[a]Conversion assessed by analysis of ^{1}H NMR spectrum of the crude material.

Some competition reactions were conducted in order to try to determine why the asymmetric hydrogenation reaction required a relatively high catalyst loading even though a significant amount of conversion was observed after a few minutes. Firstly, the reaction was conducted in which equimolar amounts of reduced material was added to the reaction to check for product inhibition (Table 3, entry 2),

Table 3: Results of competition studies in the hydrogenation of **6**.

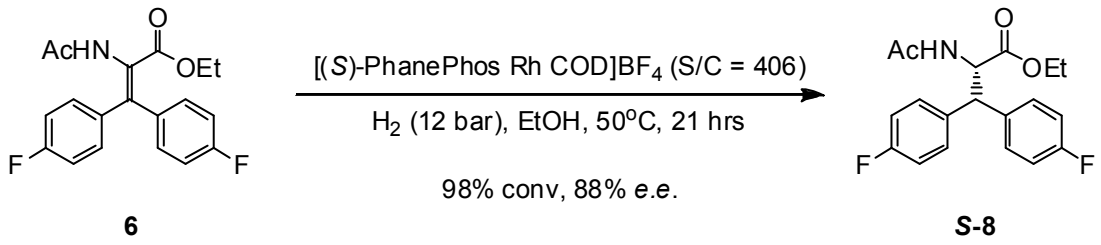
	Substance A	Substance B	Conv Sub A (%)[#]	Conv Sub B (%)[#]
1			79	

2			70	N/A
3			85	>95
4			>95	N/A

[#]Conversion assessed by analysis of ^{1}H NMR spectrum of the crude material.

The reactions were performed at S/C = 500. At this loading, Entry 2 would be expected to proceed to between 80 and 90% conversion. Entry 2 suggests that the addition of product to the reaction causes some inhibitory effect albeit rather small. In Entry 3, the reaction was run in the presence of another hydrogenation substrate, methyl acetamidocinnamate (MAC). This was completely reduced in the presence of **6** and **8**. While these results are not exhaustive, they do indicate that product inhibition of the catalyst had a fairly minor effect.

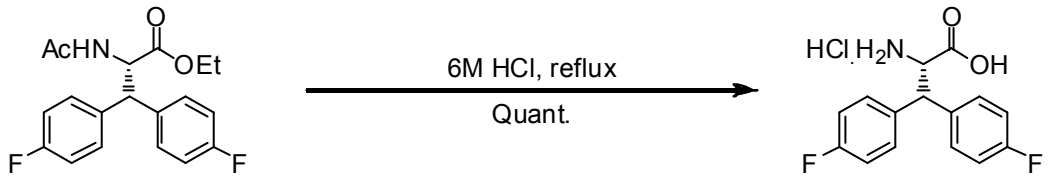
Taking the results we had in hand, we scaled up the hydrogenation reaction to 15 g (Scheme 3). The hydrogenation of the substrate **6** was initiated at a molar substrate to catalyst ratio (S/C) of 500, but additional catalyst was added after 18 hours in order to achieve complete conversion, giving a final substrate to catalyst ratio of 406. Interestingly, on this scale the reaction was observed to proceed to 88% conversion after a relatively short time (45 minutes), and the remaining material took a considerable time to convert. The reason for the apparent drop in enantiomeric excess compared to the results obtained during the screen was unclear.



6 **S-8**

Scheme 3. The asymmetric hydrogenation of **6** with [(*S*)-Phanephos Rh(COD)]BF_4.

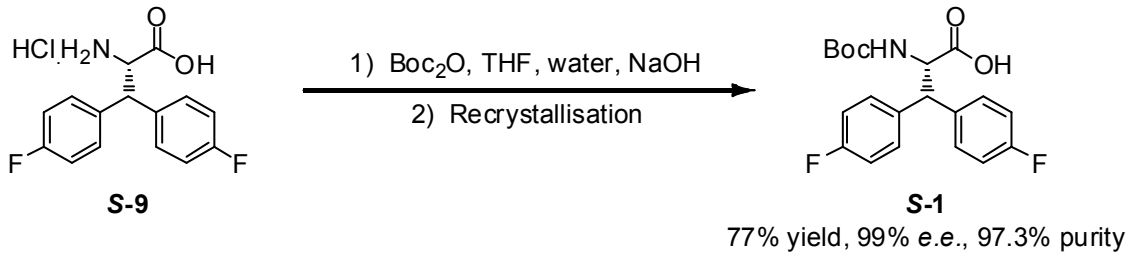
Complete deprotection and formation of the hydrochloride salt was accomplished by refluxing in 6M hydrochloric acid (Scheme 4).



***S*-8** ***S*-9**

Scheme 4. Hydrolytic deprotection of **(*S*)-8**.

The crude hydrochloride salt (*S*)-**9** was then dissolved in water, the pH was adjusted and the nitrogen was Boc protected to provide the desired material (Scheme 5). Although the enantiomeric excess achieved in the hydrogenation step was lower than expected, the crystallization of (*S*)-**1** gave material of high e.e.



***S*-9** ***S*-1**

77% yield, 99% *e.e.*, 97.3% purity

Scheme 5. *N*-Boc formation and final crystallization of (*S*)-**1**.

The chemistry discussed above gave rise to a putative process for the synthesis of **(S)-1**. The next step was to establish a synthesis which could be operated on a multi Kg scale. With a route established and economically evaluated, it became apparent that the key step was the hydrogenation (Stage 4, Scheme 3). Not only was the [(S)-PhanePhos Rh COD]BF_4 catalyst the main raw material cost, at the 15-g scale an extra aliquot of catalyst was required to drive this reaction to completion (final S/C 406:1). For the development of an economical and robust commercial process, optimisation of this particular step was a necessity.

A design of experiment plan was set up to determine the important factors governing the hydrogenation. Design parameters at a substrate to catalyst ratio (S/C) of 500:1.were:

Substrate Concentration: Low= 20 g/L; High= 100 g/L

H_2 Pressure: Low= 4 Bar; High = 12 Bar

Reaction Temperature: Low= 20 °C; High = 50 °C

Umetrics software was used to process the data.[11] As the product e.e. did not vary significantly during these experiments (from 94 to 96%), the substrate conversion was used as the significant response. The generated model showed good reproducibility, the model validity was correct (above 0.25) and the ANOVA (analysis of variance) plot also showed a good model. The main factor was determined to be the substrate concentration, followed by H_2 pressure. The model predicted that better substrate conversion would be obtained under conditions of high substrate concentration, high H_2 pressure and high reaction temperature

[11] Version MODDE 6.0. www.umetrics.com

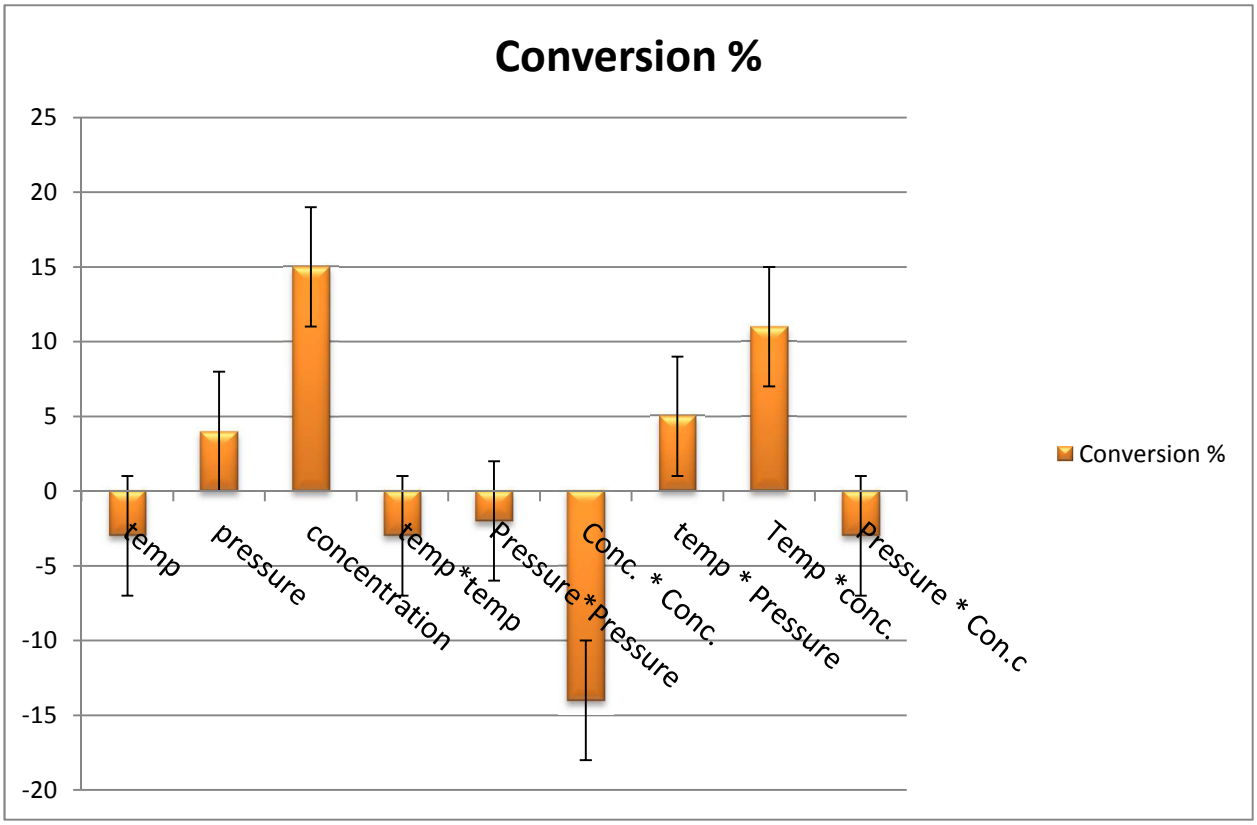


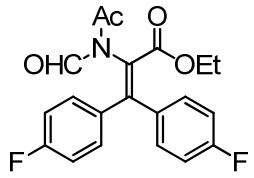
Figure 1. Coefficient plots to determine the effects of three factors (Temperature, Pressure of H_2 and Substrate Concentration) on the hydrogenation of **6**.

As large scale hydrogenation would be carried out at a maximum pressure of 8 Bar due to equipment limitations, it was decided to fix the pressure at this value for further experiments. The next set of experiments was used to determine the effect of different catalyst loadings. S/C ratios of 500, 600, 750 and 1000:1 were used at 120 g/L substrate concentration at 50 °C and 8 Bar H_2. At both S/C 500 and 600:1, >98% conversion was obtained. A slight drop to 95% conversion was observed at 750:1 and about 87% at 1000:1. This suggests that the inhibition of the catalyst would prevent use at higher S/C ratios.

Further studies at S/C of 1000:1 were carried out to investigate the effects of the use of a co-solvent (toluene, ethyl acetate, MTBE, THF), higher concentration, or temperature on conversion. Unfortunately, it soon became apparent that the reproducibility of the hydrogenation at this catalyst

loading was very poor; for example at 120 g/L, 50 °C, 8 Bar, the conversion varied from 70 to 92%.

The effect of the presence of residues from previous steps in the route was investigated by the addition of small amounts of these compounds to hydrogenations which were run using the standard conditions used previously (120 g/L substrate concentration, 50 °C, 8 Bar H_2). It was found that when 4,4'-difluorobenzophenone **3** or the *N*-Formyl product **5** from stage 1 were added, > 98% conversion was obtained; however, only 4% conversion was obtained when the *N*-Formyl-*N*-Acetyl compound **10** (the product of Stage 2) was added. This last experiment was repeated and confirmed that the presence of this compound inhibited the hydrogenation.



10

Figure 2. The *N*-Formyl-*N*-Acetyl intermediate **10**.

One batch of substrate could not be hydrogenated at all. As the initial analysis (NMR and SFC) did not identify an impurity (it was free of **10** contamination), it was thought that an inorganic contaminant might be responsible for the poor quality hydrogenation. Consequently, a reaction with good substrate containing 5% of potassium carbonate was run, and no reaction at all was observed. It is thus thought that the presence of small amounts of potassium carbonate, which is used in the previous stage, also prevented the hydrogenation of **6**.

The troublesome batch was hydrogenated in presence of acetic acid (3% v/v in ethanol) at S/C 477 but this did not give any conversion. However, after purification by recrystallization from ethanol/acetic acid (8/1) this batch could be hydrogenated.

As we were concerned that the catalyst could be deactivated by impurities in the substrate, we examined the process conditions that were likely to arise at commercial scale, and particularly how they differed from small scale lab conditions. Of particular concern was the longer time required at larger scale for the reactor to reach operating pressure. In order to replicate this, a series of laboratory experiments were carried out in which the reactor was brought to operating pressure over the course of 1h. This was repeated at various catalyst loadings and reaction temperatures (Table 4).

Table 4. Results of hydrogenations run under pilot plant conditions.

Entry	temp	S/C	Time	Conv	ee
1	50 °C	600	+1Hr	93%	92%
2	35 °C	600	+1Hr	99%	94%
3	35 °C	900	+1Hr30	82% *	
			+4Hr41	88%	93%
4	35 °C	750	+1Hr23	97%	97%

* The in-process sample was not homogeneous as the substrate is not completely soluble in ethanol.

The results, shown in Table 4, suggest that at 50 °C the catalyst was not sufficiently stable, since under laboratory conditions at a S/C 750:1, complete conversion was obtained within 20 minutes. Higher conversions were obtained at 35 °C. At S/C = 900, 750 and 600, conversions were 88%, 97%, and 99%, respectively.

Hydrogenation of **6** containing 0.02% of **10** at 35 °C and a S/C = 595 gave only 65% conversion after 2 hours at 8 Bar. Additional catalyst (S/C of 433) with slow pressurization gave 76% conversion within

1 hr at 8 Bar and no further uptake of hydrogen after 30 minutes. Pressurization to 9 Bar drove the reaction to 91% conversion (95% ee) overnight at 35 °C.

The experiments carried out demonstrate that variations in substrate concentration and temperature both effect the hydrogenation; high concentration (>100 g/L) coupled with high temperature (>35 °C) gave better conversion of substrate to product. However, at 50 °C the catalyst became unstable leading to poor overall conversion despite an initial higher reaction rate.

Proposed pilot plant conditions were 35 °C, 8 Bar hydrogen pressure, at a concentration of 120 g substrate/L.

PILOT PLANT CAMPAIGN

Although we had only made slight additional improvements to the overall process and to the hydrogenation step in particular, it was felt that we had enough confidence in the process overall to scale up the process to a multi Kg pilot plant campaign.

Problems were experienced with stage 1 of the reaction. The longer contact times between 4,4'-difluorobenzophenone and potassium *tert*-butoxide lead to the formation of significant amounts of 4,4'-*bis*-(*tert*-butoxy)benzophenone. Re-examination of samples previously made in the lab also showed small amounts of 4,4'-*bis*(*tert*-butoxy)benzophenone, but not in the amounts observed in the pilot plant. Modification of the order of addition (i.e. gradual addition of potassium *tert*-butoxide in THF solution to a mixture of 4,4'-difluorobenzophenone and ethyl isocyanoacetate in THF) gave a much improved product purity and yield.

It also became very clear that the physical form of some of the intermediates were going to create

problems. Typically the materials formed thick slurries which were difficult to stir and to isolate by filtration. The slurries with *N*-Formyl **5** and *N*-Acetyl **6** acrylates were both thick pastes. A consequence of the poor physical form was the extended drying time and high residual solvent content of the products. The loss on drying for **6** was approximately 20%; in order to save time, the product was discharged from the dryer and analyzed to determine ethanol and water content. This was then used to adjust the solvent charges in the product recrystallization. Fortunately, after recrystallization the material was more tractable. The recrystallization step proceeded smoothly to generate a product which filtered and dried very rapidly.

The full scale hydrogenation batch ran very smoothly, with hydrogen uptake being complete within 1 hour. Reaction occurred almost immediately after hydrogen addition, and it was estimated that when the desired 8 bar pressure was achieved, the reaction was at approximately 80% complete based on gas absorption. In process analysis showed 98.7% conversion and 94.1% ee, as expected.

The hydrolysis of the ester and amide groups was somewhat slow, requiring additional charges of 28% hydrochloric acid and extended reflux to obtain complete conversion. No new impurity peaks were detected as a result of this.

The Boc protection step worked very well, being complete within 2 hours. Further problems, however, occurred when acidifying the mixture as the product separated out as an oil. After a short laboratory investigation, the batch was treated with 32% sodium hydroxide to give a two phase mixture, and the lower aqueous layer discarded. The organic layer was diluted with water and partially re-acidified, giving a thick, unstirrable slurry. Due to the difficulty in obtaining a consistent pH throughout the slurry, the mixture was filtered and washed with water and then the filtrates acidified in three aliquots which were filtered and washed separately. In all, the crude material was obtained in four parts.

The problems with product physical form were repeated on the purification step, during which the cooled slurry became very thick and then immobile. The mixture was transferred to the filter with

difficulty, and although the filtration rate was reasonable, when the flow of filtrates stopped, LOD analysis was approximately 70%.

Despite the physical form difficulties, the recrystallization gave significant enhancement of enantiomeric excess; analysis of the filtrates showed that the product waste stream had a composition of 50% e.e. (*S*).

MANUFACTURING CAMPAIGNS

Progressing to a 300 Kg campaign, more care was given to controlling cooling profiles for the recrystallization of the hydrogenation substrate **6**. While hydrogen pressure was expected to be an important parameter, laboratory batches showed that the processing could be completed at 60 psig rather than the 90 psig that had been used in the previous campaign. Laboratory studies of the hydrogenation reaction using representative batches of **6** indicated that a catalyst loading of S/C = 1,200 could be achieved. The first plant batch was carried out at S/C = 800. After implementation of handling improvements, subsequent batches were run with S/C = 1,000 – 1,200. During process development studies for this campaign, it was discovered that purification of the final product was best accomplished by carrying out a preliminary partial purification of **1** as a mixed salt; adjusting the pH in the final crystallization then gave the product in excellent purity. The *N*-Boc crude product could be difficult to handle during filtration and washing steps, but this could be circumvented by the concurrent addition of 32% HCl and the basic Boc protected intermediate solution to a pH 3 water solution. The concurrent addition precipitated the product to provide an easily isolated wet cake.

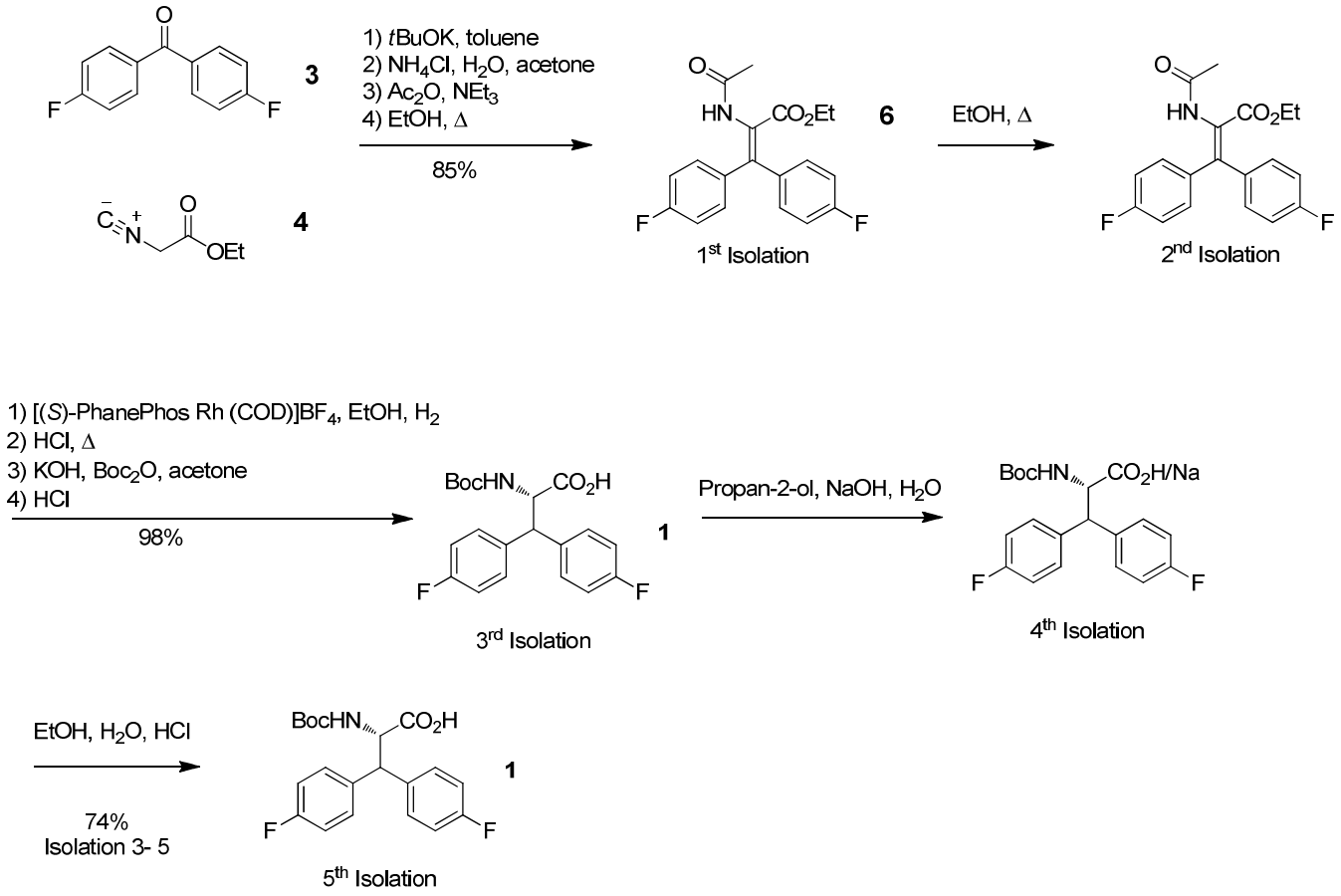
In the subsequent 900 Kg campaign, significant improvements were made to the process (Scheme 6). By switching to a mixture of toluene and THF for the first step, it was possible to telescope the first two steps. In previous runs after the acetylation step, the reaction mixture was quenched with water and the intermediate *N*-formyl-*N*-acetyl **10** was isolated. A rapid water addition with insufficient cooling led to

a temperature rise to 86 °C. HPLC analysis showed partial formation of the *N*-acetyl **6,** indicating an acidic hydrolysis of the *N*-formyl group. By adding water and heating to 70 °C, 60% conversion to **6** could be achieved. Using ethanol for the acetic anhydride quench, the hydrolysis product **6** may be isolated directly by crystallization, affording a one pot procedure for the conversion of the *N*-formyl **5** to the *N*-acetyl **6** (isolation # 1). This was followed by the recrystallization of the hydrogenation substrate **6** (isolation # 2). The overall yield for these first stages including the crystallization was slightly higher than achieved in the previous campaigns. Studies of the downstream chemistry and purification found that conversion in the hydrogenation reaction as low as 90% could be tolerated. In order to minimize the amount of catalyst used, the first batch was run at S/C = 1,800 compared to the S/C = 1,200 used in the previous 300 Kg campaign. The reaction proceeded to 93 % conversion and the downstream processed as expected. Ten subsequent hydrogenation batches were run with catalyst loadings of between 1,600 and 2,700; all proceeded to 97 – 100 % conversion. The hydrogenation, hydrolysis and Boc protection steps (**6** → **1**) were telescoped (isolation # 3). It had been previously noted that when sodium hydroxide was used as base in the Boc addition step, the resulting solid was difficult to process, it was troublesome to isolate on the centrifuge, and it had a very high loss on drying (35-40%). Furthermore, it gave an inconsistent mixture of carboxylic acid and sodium salt. This complicated unit ratio calculation for the next step and gave an inconsistent recovery in the crystallization. The process was improved by switching to aqueous potassium hydroxide. The resulting solid, which was predominantly the carboxylic acid form, was more easily handled. This eliminated the concurrent addition of acid and isolation of the basic **1** intermediate used in the 300 Kg campaign.

It was further discovered that if the subsequent crystallization was carried out as a mixture of the sodium salt and carboxylic acid, a polar mixture of water and 2-propanol could be used as solvent. This solvent mixture greatly facilitated the removal of organic impurities, and the residual rhodium level from the hydrogenation catalyst was typically reduced from 160 to 3 ppm. It was found that the optimal recovery was achieved when 0.5 equivalents of sodium hydroxide was used (isolation # 4). In the final

crystallization, the pH was again adjusted to below 2.0 to give exclusively the carboxylic acid form (ROI <0.1%) (isolation # 5). This material was essentially free of any rhodium residue (<1 ppm).

Unfortunately, after successfully completing this project, Phase III clinical trials of Denagliptin were suspended.



Scheme 6. Overall scheme for the manufacture of 900 Kg of (*S*)-*N*-Boc *bis*(4-fluorophenyl)alanine **(*S*)-1**.

CONCLUSIONS

The synthesis of (*S*)-*N*-Boc *bis*(4-fluorophenyl)alanine, (*S*)-**1** has been developed from a lab sample to a >900 Kg cGMP manufacturing campaign. The overall synthetic route, including the choice of asymmetric hydrogenation catalyst, remained essentially unchanged throughout the process. With

hindsight it is clear that we had a sound chemical synthesis in place. The success of the project depended on minimising the formation of process related impurities such as 4,4'-*bis* (*tert*-butoxy)benzophenone and ensuring complete conversion of intermediates particularly the *N*-Formyl-*N*-Acetyl compound **10**. While selection of reaction conditions helped minimise the amount of these impurities and intermediates, their presence could not be completely avoided. It was therefore necessary to further lower the amount of these materials to a level that the rhodium catalyst would tolerate and the final product would meet the customer's specification. Critical to this was the identification of crystalline intermediates with physical properties that readily allowed filtration and washing to give material of high quality without sacrificing yield. These purifications were carried out at key stages in the sequence. A purification immediately prior to the asymmetric hydrogenation step allowed the amount of catalyst required to be lowered to an economically viable level. Careful selection of bases during the isolation of the final product gave material of sufficient purity to satisfy the customer specifications. With these successful isolations, it was possible to telescope several steps, reducing the number of synthetic steps and additional purification steps from twelve in the preliminary sample synthesis to five in the final manufacturing campaign.

EXPERIMENTAL SECTION

2-Acetylamino-3,3-*bis*(4-fluorophenyl)acrylic acid ethyl ester 6.

A 400 L reactor was loaded with 4,4'-difluorobenzophenone (159.7 Kg, 731.8 mol) followed by toluene (756 Kg) and ethyl isocyanoacetate (95.9 Kg, 848 mol), and the load lines were rinsed with toluene (47 Kg). The reactor contents were cooled to < 5 °C, and a solution of potassium *tert*-butoxide (20 % in THF, 431 Kg, 768 mol) was added at a rate to maintain the internal temperature at less than 10 °C. The reactor contents were warmed to 15 °C and held for 2 hours before sampling and analyzing for conversion (0.15% unreacted 4,4'-difluorobenzophenone). A solution of ammonium chloride (10 %,

887 Kg) was added to the reaction mixture while maintaining the temperature at < 30 °C. The organic phase was diluted with acetone (638 Kg), and the mixture was heated to 40 °C to complete the dissolution of the intermediate. The aqueous phase was removed and discarded. The organic phase was washed with 10 % sodium chloride solution (887 Kg). The organic phase was concentrated, ca 1450 Kg of distillates were removed leaving the reactor contents as a thick slurry. The reactor was charged with acetic anhydride (302 Kg, 2959 mol), and the reactor was cooled with a jacket temperature of 0 °C. The load line was rinsed with toluene (9 Kg). Triethylamine (146 Kg, 1443 mol) was loaded over 30 – 60 min maintaining the reaction temp at < 10 °C, and the load line was rinsed with toluene (10 Kg). The reactor contents were warmed to 20 °C and held for 5-6 hours. The reactor was sampled and analyzed for conversion (0.7% stage 1 material). With the jacket temperature of 20 °C, the initial portion of ethanol was added slowly. There was an initial exotherm to 60 °C and once this subsided the remaining ethanol was added (823 Kg total). The reaction mixture was heated to reflux for 28 hours. The mixture was sampled and analyzed for conversion (0.14% stage 2 intermediate). The mixture was cooled to 2 °C over 7 h and then held at <5 °C for 4 h. The slurry was transferred to a centrifuge in two lots, washing each load with chilled ethanol (118 Kg). About 220 Kg of wet cake was isolated (LOD 2.5%) and used in the next step (dry weight 214 Kg, 620 mol, 85% yield).

Crystallization

A 2000 L reactor was charged with the wet cake (219 Kg, 210 Kg dry weight, 608 mol) and ethanol (1158 Kg) and heated to reflux. The contents were cooled to 80 °C at 5 °C/min. After verifying that no solids were present the mixture was cooled to 5 °C over 5 hr. The slurry was held at < 5 °C for at least 1 hr. The slurry was transferred to a centrifuge in three loads, and each load was washed with chilled ethanol (56 Kg). A total of 209 Kg of wet cake was isolated (3% LOD, 587 mol, 96% yield) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24-7.12 (m, 2H), 7.12-7.04 (m, 4H), 7.02-6.96 (m, 2H), 6.78 (s, 1H), 4.03 (q, J = 7Hz, 2H), 2.02 (s, 3H), 0.97 (t, J = 7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.5, 165.6, 162.7 (J = 249Hz), 162.6 (J = 250Hz), 135.4 (J = 4Hz), 134.3, 134.2 (J = 4Hz), 131.7 (J = 8Hz), 130.0 (J = 8Hz),

126.0, 116.0 (J = 22Hz), 115.3 (J = 22Hz), 61.4, 22.8, 13.5. ^{19}F NMR (376 MHz, $CDCl_3$) δ -113.1, -111.6. HPLC Phenomenex LUNA 5µ, Phenyl-hexyl 4.6 x 150 mm, temp 24 °C, Eluent A 10/90 MeCN/water 0.1 % TFA, Eluent B 90/10 MeCN/water 0.1% TFA, gradient: 70% A to 30% A over 30 minutes. Flow rate 2 mL/min, injection vol 5µL. Wavelength 254 nm. Retention times 2-Acetylamino-3,3-*bis*(4-fluorophenyl)acrylic acid ethyl ester **6**, 9.9 min; ethyl 3,3-*bis*(4-fluorophenyl)-2-formamidoacrylate **5**, 10.4 min; 4,4'-difluorobenzophenone **3** 12.8 min; ethyl 3,3-*bis*(4-fluorophenyl)-2-(*N*-formylacetamido)acrylate **10**, 16.0 min. The purity for the three loads from the centrifuge was 100 area %, 100 area% and 99.7 area %. The liquors contained 83 area % of **6** and 16 area % **5**. The material was use tested in a hydrogenation reaction before progressing to the next stage.

(*S*)-2-*tert*-Butoxycarbonylamino-3,3-*bis*(4-fluorophenyl)propionic acid, *S*-1.

A 2000 L pressure reactor was charged with 2-acetylamino-3,3-*bis*(4-fluorophenyl)acrylic acid ethyl ester **6** wet cake (120.3 Kg, 2.5% LOD, 348 mol) and ethanol 3C (530 Kg). The reactor was sealed and leak tested.[12] [(*S*)-PhanePhos Rh COD]BF_4 (115g, 0.131 mol, molar S/C = 2,650) was charged to a shot tube in a glove box and then attached to the reactor. The catalyst charge was dropped into the reactor and the charging apparatus was flushed with degassed ethanol (59 Kg). Addition of hydrogen to the reactor was initiated, and the uptake of hydrogen was monitored as an indication of reaction progress. The mixture was maintained at 40 °C, 60 psig hydrogen; conversion was 98.9% complete by HPLC after 12 h. The reactor was vented and purged with nitrogen. The reaction mixture was transferred to a 1200 L vessel, rinsing with additional ethanol (118 Kg). The majority of the ethanol (614 Kg) was removed by distillation. Water (307 Kg) and hydrochloric acid (32%, 186 Kg, 1,632 mol) were added and rinsed in with additional water (12 Kg), and the mixture was heated to above 80 °C to allow the removal of the ethanol by-product. Two additional water charges (113 Kg) were added. Distillation

[12] The reactor was inerted by two cycles of evacuating to 200 mm/Hg then backfilling with nitrogen. This was followed by three cycles of pressurising the vessel to 60 psig then venting.

continued until the reaction was complete (>95% by HPLC, 32 hr). The pH was adjusted to between 9 and 10 using aqueous potassium hydroxide (45%, 217 Kg, 1,740 mol), keeping the temperature below 25 °C. A solution of Boc anhydride (82 Kg, 377 mol) in acetone (93 Kg) was added. Additional aqueous potassium hydroxide was added (82 Kg, 658 mol) to maintain the solution at pH 9 – 10. The solution was checked for reaction completion (>99 % by HPLC) after 1 hr. The pH was lowered to 2.6 using 32% HCl (96 Kg, 843 mol) and rinsed in with water (23 Kg). The product began to precipitate at a pH below 4.5 with significant carbon dioxide evolution. The slurry was filtered by centrifugation in three loads and washed with water (380 Kg). The product was collected as a wet cake (216 Kg, LOD 40%, 129 Kg dry weight, 342 mol, 98 % yield).

Crystallizations of (*S*)-2-*tert*-Butoxycarbonylamino-3,3-*bis*(4-fluorophenyl)propionic acid, *S*-1:

A 4500 L nitrogen-inerted reactor was charged with 2-propanol (816 Kg) and 392 Kg of crude **S-1** wet cake (equivalent to 235 Kg, 623 mol dry weight) was added. After sealing and inerting the reactor, a additional 627 Kg of 2-propanol was added. The mixture was heated to above 65 °C. Sodium hydroxide (50%, 29 Kg, 363 mol) was added over 1 hour followed by water (29 Kg). The mixture was heated to 80 °C for 1 h, then cooled to -5 °C over 16 h. The resulting slurry was held at -5 °C for about 1 hr. The mixed salt wet cake was collected and washed with chilled 2-propanol (236 Kg) to give 348 Kg of wet cake.

A 1200 L reactor was loaded with ethanol (526 Kg) and cooled to less than 4 °C. Water (72 Kg) and the mixed salt wet cake (339 Kg) were added. The solution was heated to about 75 °C and aqueous hydrochloric acid (32%, 30.9 Kg, 271 mol) was added in portions until the pH dropped below pH 2. The solution was passed through a preheated polish filter into a 2000 L reactor at 65 °C and rinsed through with ethanol (73 Kg). Water (658 Kg) was added and cooled to -5 °C over 14 h and the slurry was held 2 hr. The wet cake was collected and washed with ethanol/water (50/50 w/w, 228 Kg). Analysis found a chemical purity 99.7%, and an optical purity 99.6% e.e.,for the 175 Kg (dry basis) of final isolated

product (464 mol, 74% yield). The wet cakes from 2 to 3 centrifuge loads were dried under vacuum (50 °C 70 – 180 mmHg) to generate (S)-2-*tert*-Butoxycarbonylamino-3,3-*bis*(4-fluorophenyl)propionic acid, S-**1**, average yield 77%. ^{1}H NMR (400 MHz, DMSO) δ 12.4 (1H), 7.40-7.31 (m, 4H), 7.29 (d, *J* = 9Hz, 1H) 7.15-7.04 (m, 4H), 4.78 (dd, *J* = 9 & 11 Hz, 1H), 4.31 (d, *J* = 12 Hz, 1H), 1.28 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 160.9 (*J* = 243Hz), 160.8 (*J* = 243Hz), 155.1, 137.41, 137.38, 130.1 (*J* = 8Hz), 130.0 (*J* = 8Hz), 115.1 (*J* = 21Hz), 114.8 (*J* = 21Hz), 78.2, 56.9, 51.0, 28.0. ^{19}F NMR (376 MHz, $CDCl_3$) δ -116.7, -116.2. Distinct NMR signals associated with the minor rotamer (ca 15%) were observed at: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.90-6.84 (m), 4.70-4.61 (m), 4.25 (d, *J* = 11Hz), 1.31 (s). ^{13}C NMR (101 MHz, $CDCl_3$) δ 78.6, 27.8. ^{19}F NMR (376 MHz, $CDCl_3$) δ -116.5, -116.1. HPLC Column Zorbax Eclipse XDB-C18, 3.5 µ, 4.6 x 150 mm, eluent A water 0.05% TFA, eluent B MeCN 0.05% TFA. Gradient 100% A to 0% A over 25 min, flow rate 1 mL/min, detection 254 nm, column temp 40 °C injection vol 1µL. The sample was prepared at 1 mg/mL in 9:1 MeCN:water. Retention time (S)-**1** 11.2 min, Purity 99.7 area %, impurities at RRT 0.98 0.1 area % and RRT 1.16, 0.2 area %. Loss on drying <0.1 % residue on ignition <0.1%, water by Karl Fisher 0.1% Chiral HPLC method Column OJ-RH, 5µ, 4.6 x 150 mm, eluent A water 0.05% TFA, eluent B MeCN 0.05% TFA; isocratic 35 % B over 20 min, flow rate 1 ml/min, detection 254 nm, column temp 40 °C injection vol 1µL. The sample was prepared at 1 mg/ml in 9:1 MeCN:water. Retention time (S)-**1** 11.2 min, (R)-**1** 10.1 min, 99.5% e.e.

Acknowledgements

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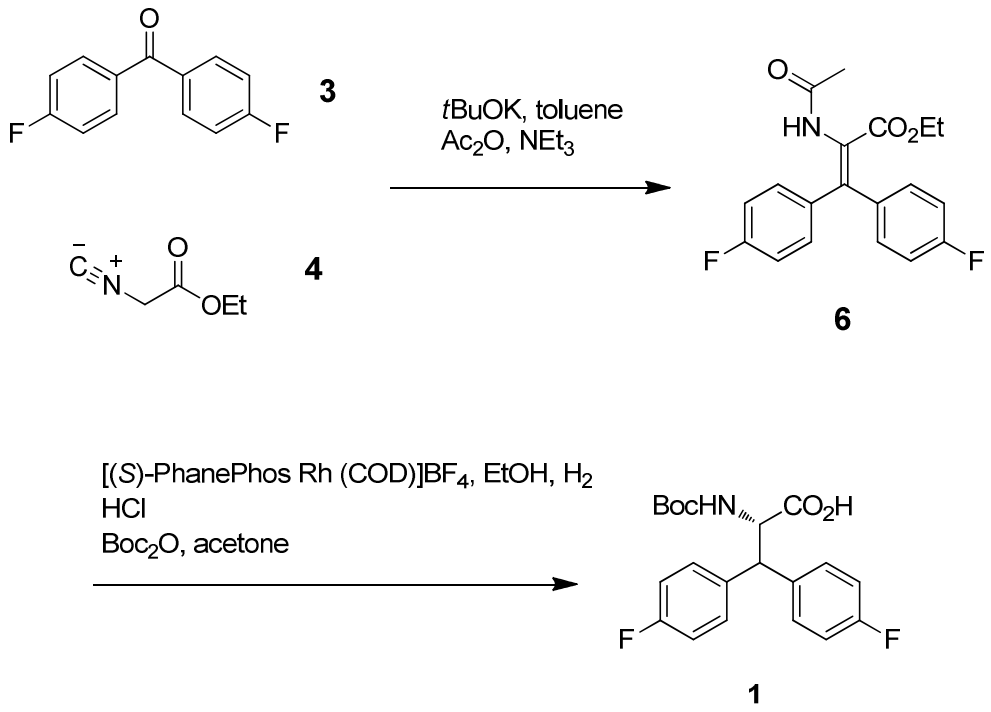
TOC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4
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1