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The Chemical Development and Scale-Up of Sampatrilat[1]

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Abstract:

The discovery and scale-up of two routes to sampatrilat are described. The first Chemical R and D route used a side product from another development project to accelerate drug supply and expedite the early development programme. The second, more efficient Chemical R and D route had the potential for commercialisation and used an environmentally friendly variant of the Baylis−Hillman reaction, and an asymmetric Michael addition as key steps. Full preparative details for the aminomethacrylate 4, a potentially useful chiral synthon, are given for the first time, along with full experimental details of the asymmetric Michael addition to make the chiral glutarate 5. Finally, a striking polymorph case history is described.

Introduction

During the 1990s Pfizer conducted clinical trials on two geminal-cycloalkylglutaramide derivatives candoxatrilat **(1)**, or its indanyl ester prodrug candoxatril, and sampatrilat **(2)**. These compounds are inhibitors of the zinc metalloprotease, neutral endopeptidase 24.11, and potentiate the natriuretic factor actions of the peptide hormone, atrial natriuretic factor (ANF) in animals and man.[2] In addition, sampatrilat also inhibits the angiotensin converting enzyme (ACE) and was thus a dual inhibitor (Figure 1).

Medicinal Chemistry Route. The medicinal chemistry route, outlined in Scheme 1, provided access to a good range of derivatives during the medicinal chemistry programme by functionalisation of intermediates such as **5**, **6**, or **7**. Although this route was used to prepare the first 500 g in Chemical R and D, it suffered from a number of problems:

(1) Several chromatographic purifications were required because there were only two crystalline intermediates.

(2) The synthesis was 15 steps from commercially available materials and proceeded in 2% overall yield from diethyl malonate using either chiral or achiral variants.

(3) The formation of the bromomethacrylate **3** had extremely poor atom utilisation.

(4) The use of large volumes of constant boiling HBr in a slow reaction (step 2) was a scale-up and corrosion problem.

(5) The bromomethacrylate **3** was a severe lachrymator.

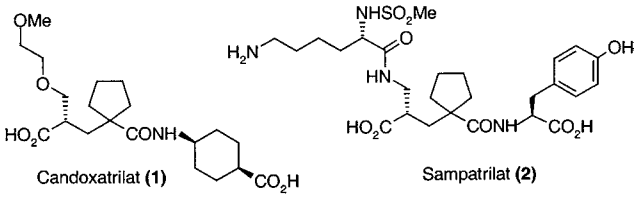
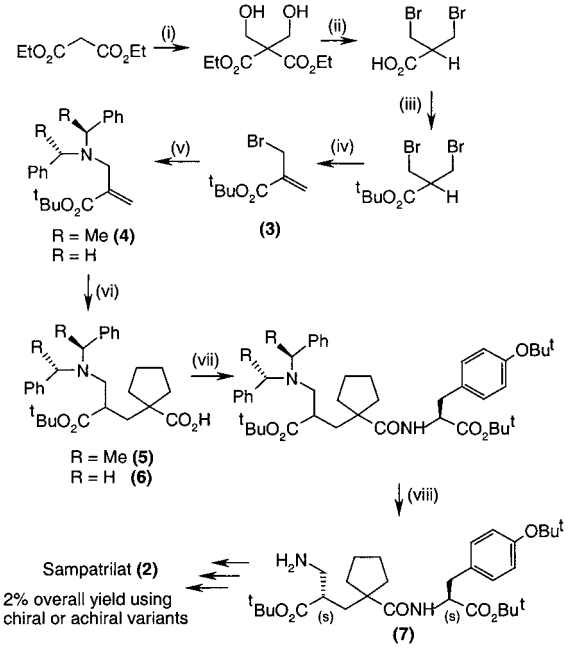


Figure 1.

Scheme 1. **Medicinal Chemistry route to sampatrilat**[a]



[a] Conditions: (i) $KHCO_3$, CH_2CO. (ii) HBr (aq), heat. (iii) 2-Methyl-1-propene, *p*-TSA (0.1 equiv), CH_2Cl_2, rt. (iv) *N*-Ethyldiisopropylamine (1.2 equiv), toluene 40 °C, 5 h. (v) K_2CO_3 (1.2 equiv), CH_3CN 60−70 °C, 18 h. (vi) Cyclopentanecarboxylic acid, LDA (2.2 equiv), 0 °C, 1 h, then cool to −50 °C. Add acrylate (1 equiv) −50 to −20 °C, 6 h. Quench into ice cold ether/1 M HCl. (vii) **5** or **6** (1 equiv) *S*-*tert*-butyl-*O*[4]-butyltyrosinate (1.1 equiv), CH_2Cl_2 0 to 25 °C. (viii) H_2, $Pd(OH)_2$, ethanol, 20 °C, 60 psi.

Although the Michael addition reaction to give **5** proceeded with a high level of diastereoselectivity, it was unreliable. The achiral variant proceeding through compound **6** was preferred by medicinal chemistry for compound supply.

At an early stage it was found that the intermediate **7** was extremely crystalline and high melting (130 °C compared with the diastereoisomeric aminomethyl compound which melted at 5 °C). This intermediate provided significant purification, and this was a strong driver for syntheses to proceed though this intermediate. Indeed all three syntheses that were scaled up used **7** as an intermediate.

(1) This chemistry was presented at the First International Conference on Organic Process Research and Development, San Francisco, CA, U.S.A., November 5, 1997.

(2) James, K.; Alabaster, C. T.; Barclay, P. L.; Barnish, I. T.; Blackburn, K. J.; Brown, D.; Cambell, S. F.; Cussans, N. J.; Danilewicz, J. C.; Palmer, M. J.; Terrett, N. K.; Samuels, G. M. R.; Wythes, M. J. *Perspect. Med. Chem.* **1993**, 45−60.

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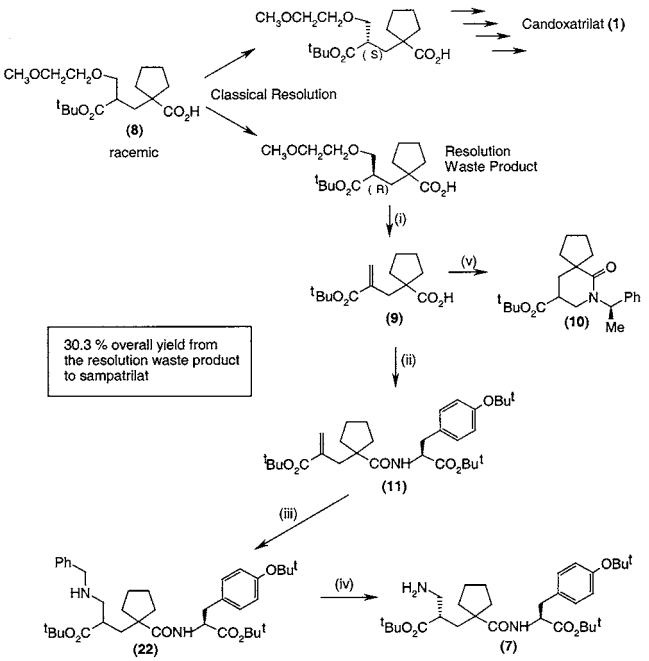
Scheme 2. **Chemical R & D route 1**[a]



[a] Conditions: (i) KOBu^t (2 equiv), Bu^{t}OH, 25 °C. (ii) (*S*)-*tert*-Butyltyrosinate (PPACA) (2.5 equiv), two-phase, 25 °C. (iii) $BnNH_2$ (4 equiv), BF_3·OEt_2 (1.1 equiv), ΔPrOH. (iv) H_2 (55−60 psi), Pd/C, EtOH, 30−40 °C. (v) (*S*)-α-Methylbenzylamine (4 equiv), ΔEtOH.

Chemical R and D Route 1. The preparation of a 500 g batch via the Medicinal Chemistry route was extremely labour intensive, and in parallel with this activity an alternative route for early drug supply was sought. At the time, candoxatril was being prepared by a classical resolution from the racemic glutarate **8** (Scheme 2). The desired (*S*)-isomer was converted into candoxatril, but a stock of 200 kg of the unwanted *(R)*-isomer was available for potential conversion to sampatrilat (Scheme 2). Base-induced elimination of methoxyethanol gave the acrylate **9**. An attempted asymmetric Michael addition of *(S)*-α-methylbenzylamine to the acrylate **9** gave some diasteroselectivity (2.7:1) but resulted in formation of the lactam **10**. To avoid this lactamisation the tyrosine moiety was introduced early to give the acrylate **11**. Michael additions to **11** were examined using both chiral α-methylbenzylamine and also achiral benzylamine. The chiral reaction gave encouraging selectivity in the initial stages of the reaction (3:1), but this dropped to 1.4:1 as the reaction proceeded. It was harder to push the chiral reaction to completion and more difficult to hydrogenolyse the α-methylbenzyl protecting group. As the overall yield from **11** to **7** was the same using either reagent, the cheaper, benzylamine variant was selected for scale-up. However, unacceptable levels of epimerisation of the tyrosine chiral centre were encountered with either amine. A large number of Lewis acids were screened for their ability to accelerate the desired Michael addition reaction without increasing the rate of tyrosine epimerisation, and boron trifluoride was found to be the best catalyst. The addition of boron trifluoride diethyl etherate complex eliminated the epimerisation problem and gave a 4-fold increase in reaction rate. Following hydrogenation, the low melting (*R*,*S*)-diastereomer was easily removed from the desired (*S*,*S*)-product **7** by crystallisation.

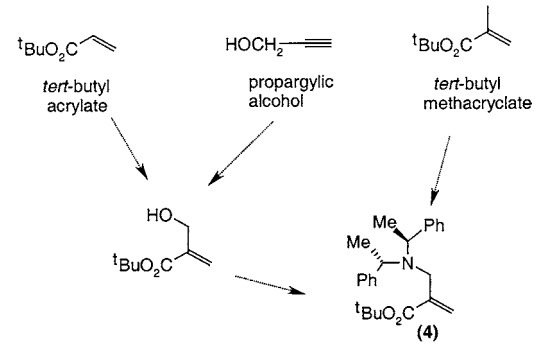


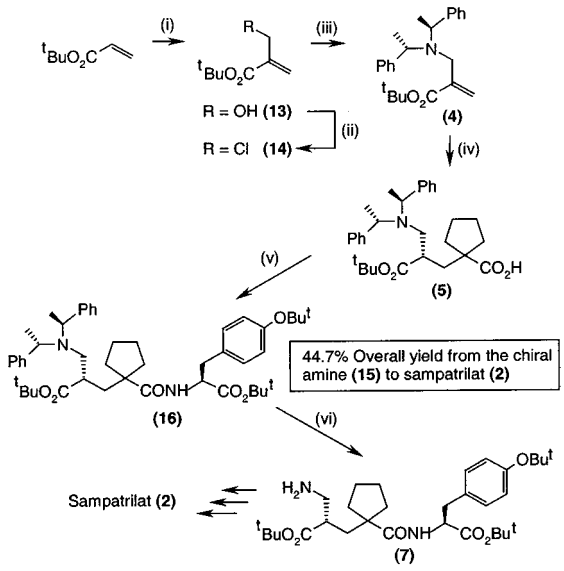
Figure 2. **Potential cheap starting materials for the synthesis of 4.**

This chemistry was scaled up in the pilot plant to provide multikilogram batches of sampatrilat; however, colleagues in Chemical R and D working on the candoxatril project developed an enantiospecific synthesis of the glutarate intermediate **8** using an asymmetric hydrogenation reaction.[3,4] Hence, the ready supply of the unwanted enantiomer dried up. In addition the low bioavailability of sampatrilat and the relatively low selling price in the hypertension market meant that a more cost-effective, enantiospecific synthesis was required.

Chemical R and D Route 2. Most of the problems with the Medicinal Chemistry route are associated with the inefficient synthesis and use of the bromomethacrylate **3**. An efficient synthesis of the chiral aminomethacrylate **4** in combination with some salt formations to reduce the chromatographic purification would alleviate most of the problems. In addition to the conventional disconnection approach, we also tried to identify cheap raw materials that had some structural similarity with the target. Three such materials (see Figure 2) were *tert*-butyl methacrylate (which although not a standard item in many western catalogues is available at low cost on a large scale[5]), propargylic alcohol, and *tert*-butyl acrylate. Our attempts to activate the methyl group of the methacrylate by radical bromination led to radical polymerisation. At Pfizer it has been shown that the methyl group could be activated by an iodosulphonation−dehydroiodination−isomerisation sequence,[3] but this chemistry was too expensive to be used for sampatrilat. Carbonylation of inexpensive propargylic alcohol is not always regioselective.[6] Hence, the best approach seemed to be to start from cheap *tert*-butyl acrylate and use the Baylis−Hillman reaction[7] to provide the additional carbon atom. The successful realisation of this route is shown in Scheme 3.

(3) Challenger, S.; Derrick, A.; Mason, C. P.; Silk, T. V. *Tetrahedron Lett.* **1999**, *40*, 2187−2190.
(4) Burk, M. J.; Bienewald, F.; Challenger, S.; Derrick, A.; Ramsden, J. A. *J. Org. Chem.* **1999**, *64*, 3290−3298.
(5) Available from Tokyo Kasei Organic Chemicals and Mitsubishi Chemicals.
(6) Rosenthal, R. W.; Schartzman, L. H.; Greco, N. P.; Proper, R. *J. Org. Chem.* **1963**, *28*, 2835−2838.
(7) Baylis, A. D.; Hillman, M. A. D. German Patent 2 155 133 (1972); Drewes, S. E.; Roos G. H. P. *Tetrahedron* **1988**, *44*, 4653; Ciganek, E. *Org React.* **1997**, *51*, 201; Morita, K.; Suzuki, Y.; Hirose, H.; *Bull. Chem. Soc. Jpn.* **1968**, *41*, 2815; Mathias, L. J.; Kosefoglu, S. H.; Kress, A. O. *Macromolecules* **1987**, *20*, 2326.

Scheme 3. **Chemical R & D route 2 to sampatrilat**[a]



[a] Conditions: (i) CH_2O (1.6. equiv), 3-quinuclidinol (0.25 equiv), ΔH_2O/CH_3CN. (ii) $SOCl_2$ (0.88 equiv), Et_3N (1.02 equiv), Py (0.1 equiv). (iii) (*S*,*S*)-Bis(α-methylbenzyl)amine (**15**) (0.66 equiv). (iv) Cyclopentanecarboxylic acid (1.1 equiv), LDA (2.2 equiv), THF −30 to 20°C, add **4**/hexane, quench citric acid. (v) CDI (1.05 equiv), HOBT (0.1 equiv), *tert*-butyl-*O*[4]-*tert*-butyltyrosine (1.05 equiv), toluene 90−95 °C. (vi) H_2 50−60 psi, Pd/C, IMS, 25−35 °C.

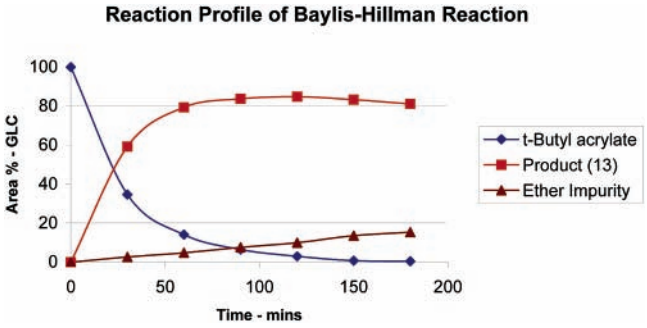


Figure 3. **Reaction profile of Baylis−Hillman reaction.**

The Baylis−Hillman reaction in nonaqueous solvents was complex, leading to a range of ethers and acetals. However, it was found that by using aqueous acetonitrile (or other aqueous solvents) and 1 equiv of 3-quinuclidinol the reaction was clean, and 85−90% yields were obtained. 3-Quinuclidinol was by far the most expensive component of this reaction; hence, we looked at making the reaction catalytic. This reduced the chemical yield to 80−85%, but overall it was still preferred on an economic basis. A typical profile is shown in Figure 3. As can be seen from the graph the level of the ether by-product increases with time, and the maximum yield of product is obtained after 2 h. On the plant, the preferred reaction period was 90 min due to the longer heat-up and sampling times. There was one last frustrating problem to overcome. It was discovered that the Baylis−Hillman product mixture did not have a sufficient margin of safety for scale-up into the pilot plant. Its DSC showed an exotherm starting at 110 °C, and we wanted to run the reaction at reflux (77 °C) as we had found that lower-temperature reactions were less clean. Exothermic polymerisations are a well-known safety hazard, but strangely the starting *tert*-butyl acrylate did not show evidence of exothermic decomposition (by DSC) until 200 °C. Finally the "penny dropped" when it was noted that the chromatographed Baylis−Hillman products polymerised and solidified faster than the crude materials. This led to the realisation that the acidic phenolic stabilisers (such as dihydroquinone and monomethylhydroquinone) that are present in *tert*-butyl acrylate were being removed on aqueous work up by the basic 3-quinuclidinol. The addition of 50−100 ppm of fresh stabiliser before the toluene solution was stripped restored sufficient margin of safety (DSC in the presence of stabiliser showed an exotherm at 177 °C which in combination with other standard tests gave us confidence to transfer to the pilot plant with no safety concerns). The Baylis−Hillman reaction is an inherently environmentally friendly, atom-efficient reaction in which all the atoms of the two starting materials get incorporated into the product. The process developed by Pfizer has added environmental benefit since it allows simple reuse of the 3-quinuclidinol as shown in Figure 4 and generates no waste stream. A small amount of acetonitrile is added back into the aqueous layer before it is reused in the next reaction. This is to replace the acetonitrile which is partitioned into the toluene layer. It is critical to have enough acetonitrile to maintain a homogeneous reaction, otherwise side reactions are much greater.

The hydroxymethacrylate **13** was chlorinated with thionyl chloride in acetonitrile and reacted with the chiral amine **15** to give the desired aminomethacrylate **4** in 90% yield. However, on the plant scale it was decided to use a slightly lower-yielding but more practical option. The process selected for scale-up used the second-best reaction solvent, toluene, which was used to extract the Baylis−Hillman product **13**. The dried toluene extract was then used directly in a highly streamlined process to the desired product **4**, in good yield and avoiding isolation of the lachrymatory chloromethacrylate **14**. Compound **4** was conveniently purified by 5-sulphosalicylic acid salt formation, providing an expedient large-scale synthesis of a potentially useful chiral synthon **4**.

Once the chiral synthon **4** was obtained in a pure crystalline form, the problems which had been associated with the unpredictability of the asymmetric Michael addition went away. The original medicinal chemistry procedure used cryogenic conditions at −78 °C, but as can be seen in Table 1, very good selectivities can be obtained using plant-friendly temperatures. The conditions highlighted in bold in Table 1 were selected for scale-up as these conditions gave the maximum yield in standard chemical plant. It has been proposed[8] that the excellent stereoselectivity is due to a six-membered lithium chelate in which the lithium counterion is complexed between the enolate oxygen and the β-nitrogen atom. This chelate facilitates transmission of stereochemical information present in the two (*S*)-α-methylbenzyl protecting groups to the newly developing carbon−hydrogen bond.

The coupling reaction to give the amide **16** was originally scaled up in the pilot plant using 2−3 equiv of propanephosphonic acid cyclic anhydride (PPACA), but a cheaper

(8) Barnish, I. T.; Corless, M.; Dunn, P. J.; Ellis, D.; Finn, P. W.; Hardstone, D. J.; James, K. *Tetrahedron Lett.* **1993**, *34*, 1323−1326.

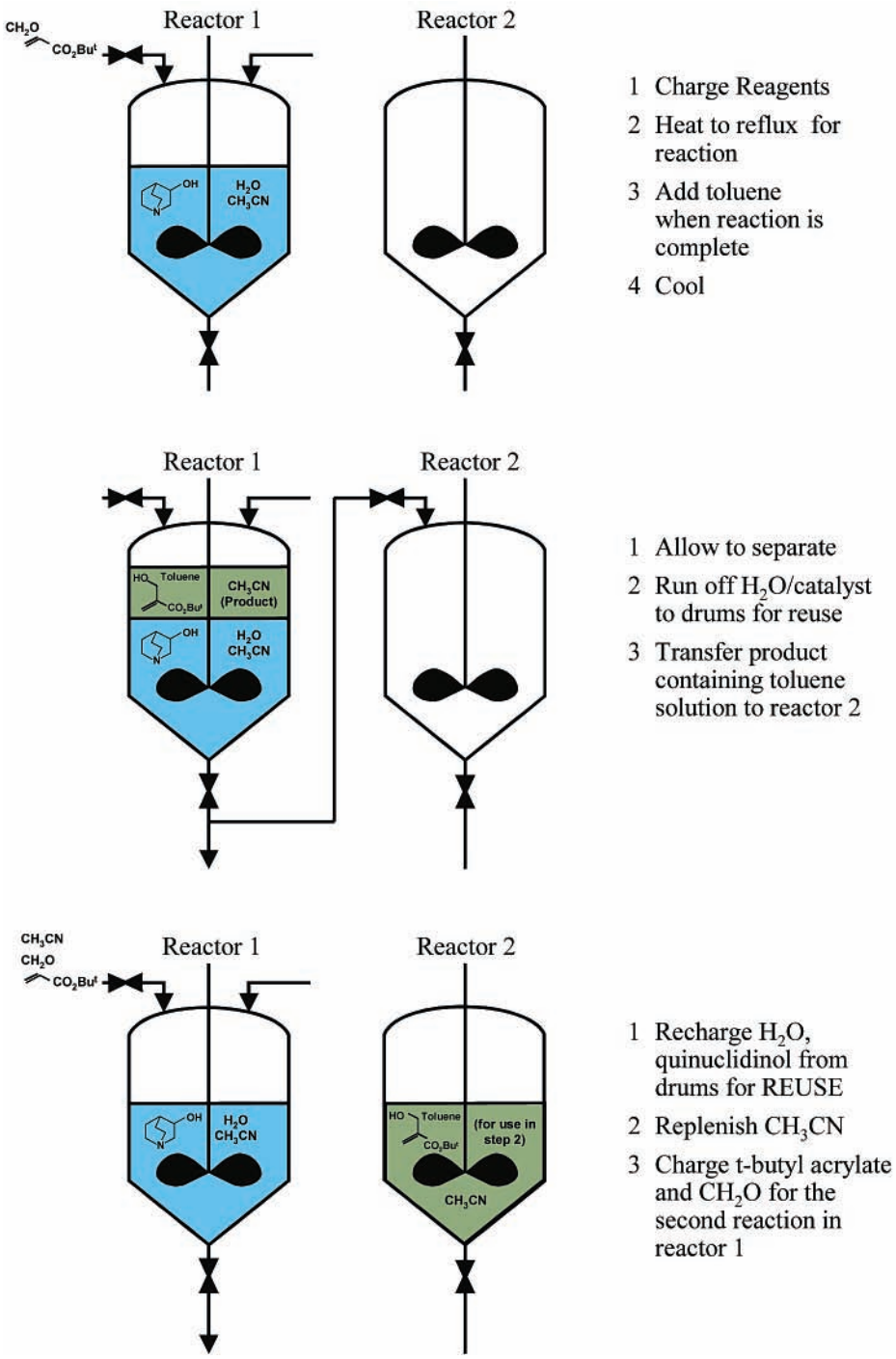


Figure 4.

alternative was sought. It was shown that the reaction of the glutarate **5** with *N*,*N*′-carbonyldiimidazole (CDI) cleanly gave the imidazolide **17** but that this imidazolide would not react with di-*tert*-butyl tyrosine even at elevated temperatures. Presumably the imidazolide is too hindered due to the geminal cyclopentyl group. The addition of a catalytic quantity of 1-hydroxybenzotriazole solved this problem, and the imidazolide reacted cleanly with the tyrosine derivative to give the desired amide **16**. CDI is an extremely water-sensitive, reagent and the solution of the glutarate **5** needed to be dry following the salt-breaking operation. It was found that on scale-up the glutarate **5** was exquisitely sensitive to pH, heat, and especially both (see Figure 5). Heating a solution of the glutarate **5** in toluene, washed with water at pH 7, at reflux for 17 h gave a 70% conversion to the diacid **18**. If the pH of the wash was pH 4, there was a 95% conversion to the diacid **18** under the same conditions. One solution to the problem, which worked on scale-up, was to wash the toluene solution with brine which gave a low water content and then charge an extra 2 or 3% of CDI to complete the drying process.

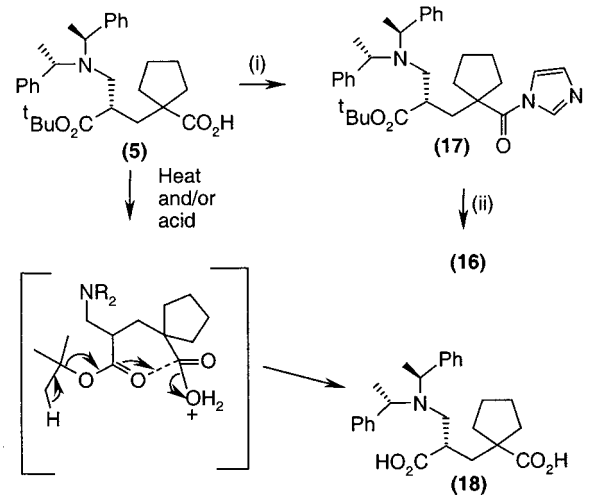
It is proposed that the sensitivity of the glutarate **5** to pH and heat is due to an intramolecular process such as that shown in Figure 5.

Subsequent hydrogenation of **16** gave the common intermediate **7**. It was found that Johnson−Matthey catalyst

Table 1. **Effect of Temperature on the Michael Addition Reaction to give 5**

reaction temperature	yield[a] (%)	diasteroeselectivity of reaction mixture
−78 °C	75	99:1
−30 °C	82	98:2
−5 °C (−10 to 0 °C)	**88**	**97:3**[b]
+20 °C	88	95:5

[a] The yield after isolation as salt. [b] Diasteroselectivity during the reaction. The selectivity was improved to 99.2:0.8 after salt formation.



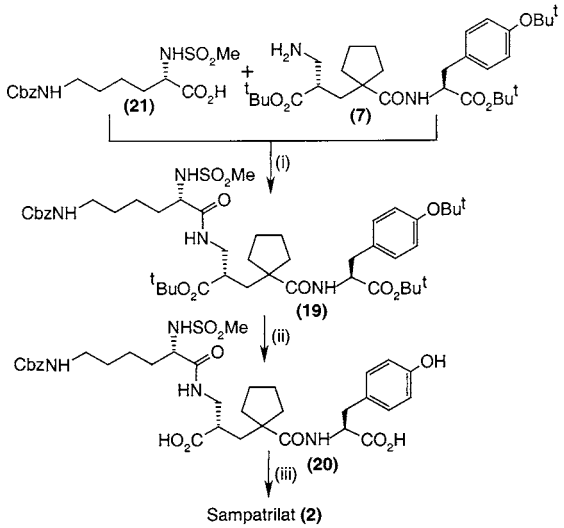
(i) *N,N'*-carbonyldiimidazole (1.05eq), HOBT (0.1 eq) toluene, 40°C
(ii) *tert*-butyl *O*⁴-*tert*-butyltyrosine (1.05eq), toluene, 95°C.

Figure 5.

type 5R39 (5%Pd/C) was more effective that several other Pd catalysts including Pearlman's catalyst [20% $Pd(OH)_2$/C]. Pearlman's catalyst is commonly used for difficult debenzylation reactions but was clearly inferior for this substrate. In our view the 4-fold lower palladium content of the type 39 catalyst compared with that of Pearlman's catalyst also gave a greater margin of safety (with respect to flammability) when the catalyst was filtered off.

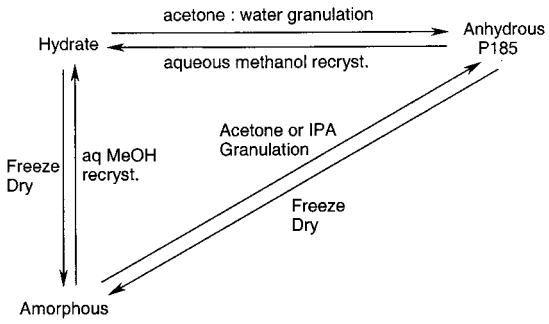
End-Game Chemistry and a Striking New Polymorph. All three routes conclude with the same final three steps (Scheme 4). The first coupling step to give **19** was left unchanged from the Medicinal Chemistry method. However, on scale-up of the second step, the trifluoroacetic acid induced deprotection of **19**, severe problems were found during the increased distillation time cycles used to remove the trifluoroacetic acid. The increased exposure time led to a variety of degradation reactions, and in some reactions less than 50% of the desired product was obtained. The problem was solved by the addition of water after the reaction was complete to remove the TFA by washing rather than distillation. This led to a three-layer system and a complex extraction process, which, while unconventional, worked well over many batches. Hydrogenolysis of the Cbz group was originally achieved in an ethanol or ethyl acetate solution, and the Medicinal Chemistry preparations involved isolating sampatrilat as an amorphous freeze-dried solid. This was replaced by the discovery of a crystalline hydrate which was good for purification by recrystallisation from aqueous

Scheme 4. **Endgame chemistry**[a]

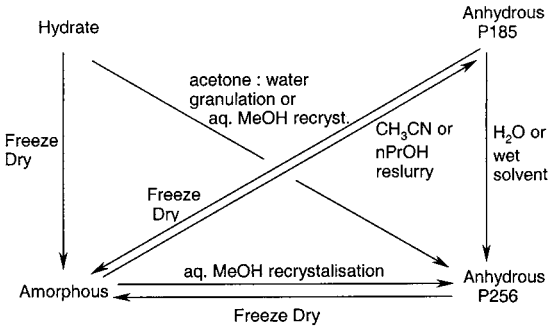


[a] Conditions: (i) WSCDI (1.4 equiv), HOBT (1.05 equiv), CH_2Cl_2, 10−20 °C. (ii) TFA (22 equiv), anisole (16 equiv), CH_2Cl_2, 35 °C. (iii) NaOH (2 equiv), Pd/C, H_2 (60 psi), 21−29 °C, then HCl (aq) to pH = 4.

Scheme 5. **Polymorph interconversion before the discovery of the new polymorph**



Scheme 6. **Polymorph interconversion after the discovery of the new polymorph**



methanol. The purified hydrate was dehydrated in acetone, containing less than 3% water, to give an anhydrous polymorph, which melted at 185 °C. This material was used for early toxicology and clinical work, but after making several batches of sampatrilat, a routine purification rework using the aqueous methanol recrystallisation on a 25 L scale set solid. It took 45 min to carefully chip out the thermometer from the solid mass so that the material could be recovered—a new polymorph had been formed!!

The new form melted at 256 °C and was designated P256. This melting point was over 70 °C higher than that of the previous anhydrous form that was designated P185. From

Table 2. **Comparison of Polymorphs P185 and P256**

polymorph	mp (DSC)	heat of fusion	solubility in water
P185 (original polymorph used in clinical trials)	185 °C	50−70 J/g	600 mg/mL
P256 (new polymorph)	256 °C	240 J/g	1 mg/mL

the melting point, heats of fusion and solubility data given in Table 2 it was obvious that the new form was significantly more stable. The interconversion diagram before the discovery of the new form is shown in Scheme 5. On appearance of the new form the interconversion diagram changed dramatically and is shown in Scheme 6. Several of the transformations in Scheme 5 are now historical even though they were previously run on a multikilogram scale before the discovery of the new form. It has been asserted that a polymorph once formed can always be made again,[9] and indeed a new way of preparing P185 was found by freeze-drying the more stable P256 to give amorphous material and then reslurrying the amorphous material in 1-propanol or acetonitrile. However, thus far we have been unable to find a new way of making the hydrate. In the current interconversion diagram (Scheme 6) all arrows lead away from the hydrate. Even though a 100 g sample of this material still exists we have not yet identified a way of making further material.

It was now found that the old process could no longer be used to make sampatrilat, as the new form was so insoluble that it crystallised out onto the catalyst, stopping the reaction. The process was therefore redesigned by dissolving the starting material **20** in aqueous sodium hydroxide for hydrogenation to give the disodium salt of sampatrilat. After the catalyst was removed by filtration, the resulting solution was acidified to its isoeletric point (pH 4) to precipitate sampatrilat (polymorph P256) which was collected by filtration. The discovery of the new form had three advantages.

(i) The new form was very insoluble, and this resulted in a 15% yield improvement.

(ii) Biological performance over the old form was unchanged, and we were able to file for a new polymorph patent which has been granted.[10]

(iii) The new form (P256) was significantly less hygroscopic than P185.[10]

Conclusions

The hypertension market is cost competitive; as a result efficient, convergent, asymmetric chemistry was required for sampatrilat. An atom-efficient Baylis−Hillman reaction was devised and converted into an environmentally friendly process. A scalable, efficient synthesis of the chiral aminomethacryalte **4** was developed, and this material was used in an asymmetric Michael addition reaction to prepare the glutarate **5**. The overall yield for preparing sampatrilat was improved from 2 to 44.7%.

This case history re-emphasises the importance of polymorph control. Following this project and others like it, Pfizer undertook screening for polymorphs at an even earlier stage in the development process and to use automated, high-throughput screening to maximize the chances of finding all polymorphs.

(9) Dunitz, J. D.; Bernstein, J. *Acc. Chem. Res*. **1995**, *28*, 193−200.
(10) Dunn, P. J.; Hughes, M. L. World Patent WO 95/15308, 1994.

Experimental Section

Proton and carbon-13 NMR data were recorded on a Varian Unity 300 spectrometer operating at 300 and 62.9 MHz, respectively, unless otherwise stated. Microanalytical data were performed by Reginald English at Pfizer. Melting points were determined on a Buchi melting point apparatus. Ethanol refers to industrial methylated spirits (95% ethanol, 5% methanol).

1-[2-(*tert*-Butoxycarbonyl)-2-propenyl]-1-cyclopentanecarboxylic acid (9). The glutarate **8**[3] (as its α-methylbenzylamine salt) (42 kg, 107.2 mol) was subjected to a salt-break operation between hexane (100 L) and 2 M hydrochloric acid solution (100 L). The hexane phase was washed with 2 M hydrochloric acid (2 × 50 L) followed by water (50 L). The resulting hexane layer was concentrated to a yellow oil, dissolved in *tert*-butyl alcohol (210 kg), and treated with potassium *tert*-butoxide (24.18 kg, 215.5 mol). The reaction was stirred for 20 h at 25 °C to give a very thick slurry. Dichloromethane (437.5 L) and 1 M hydrochloric acid (267 L) were added, keeping the temperature below 30 °C. After stirring for 30 min the phases were separated, and the organic phase was washed with water (267 L). The dichloromethane layer was stripped to a low volume and then stripped and replaced with ethyl acetate so that the final volume was around 200 L. The ethyl acetate solution was washed with a solution of sodium bicarbonate (made by dissolving 200 g of $NaHCO_3$ in 32 L of water) to remove the diacid biproduct. Finally, the organic layer was washed with water (32 L) and concentrated to an oil which solidified on standing. Yield of the title compound (27.35 kg, 99.8%). Purity by main band HPLC = 93.5%.

The crude product was recrytallised from isooctane (crystallisation at −10 °C in a freezer) to give a white solid mp 50−51 °C. Found C, 66.16; H, 8.89. $C_{14}H_{22}O_4$ requires C, 66.11; H, 8.72%. ^{1}H NMR ($CDCl_3$) 1.48 (9H, s), 1.58−1.80 (6H, m), 2.07−2.20 (2H, m), 2.70 (2H, s), 5.52 (1H, s), 6.15 (1H, s).

Attempted Asymmetric Michael Addition of (*S*)-α-Methylbenzylamine to the Acrylate (9). The acrylate **9** (508 mg, 2 mmol), *(S)*-α-methylbenzylamine (1.04 mL, 8 mmol), and ethanol (2 mL) were heated at reflux for 14 h. The reaction was stripped and the residue purifed by column chromatography on silica; 10% ethyl acetate in hexane eluted the lactam **10** (240 mg) as a 6:1 mixture of diastereoisomers. This material was recrystallised from a small volume of ethyl acetate hexane to give **10** as a 7.5:1 mixture of diastereoisomers as a white solid. Found C, 73.67; H, 8.55; N, 3.53.

$C_{22}H_{31}NO_3$ requires C, 73.92; H, 8.74; N, 3.92%. ^{1}H NMR ($CDCl_3$, 250 MHz) 1.43 (9H, s), 1.56 (3H, d), 1.6−2.1 (2H, m), 2.40−2.68 (2H, m), 3.05 (1H, d, d, d), 3.33 (1H, t), 6.17 (1H, q), 7.27−7.45 (5H, m). Further elution gave a (200 mg) of oil shown by NMR to be a 10:12 ratio of diasteroisomers favouring the minor diastereoisomer. Overall the combined yield of the two isomers was 61.5% and an overall ratio of 2:1.

***tert*-Butyl *(S)*-*N*-{1-[2-(*tert*-Butyloxycarbonyl)-2-propenyl]-1-cyclopentylcarbonyl}-*O*^{4}-*tert*-butyltyrosinate (11).** Dichloromethane (131.5 kg), water (66.8 L), acrylate **9** (17.65 kg, 93.5% purity, 54.5 mol), and *tert*-butyl *O*^{4}-*tert*-butyltyrosinate hydrochloride[11] (21.4 kg, 64.9 mol) were charged to a reactor equipped for pH monitoring and simultaneous addition of two reagents. The pH was adjusted to pH 8.5 to 9.0 with 20% sodium hydroxide solution. A 50% solution of propanephosphonic acid cyclic anhydride in ethyl acetate (124 kg) was added over about 1 h with simultaneous addition of 20% sodium hydroxide solution (approximately 132 kg) maintaining the pH in the range 8.5 to 9.0. During these additions the temperature was maintained at 25 °C. The reaction was stirred at 25 °C for 3 h. The phases were allowed to separate (product containing phase on the top). The aqueous layer was extracted twice with dichloromethane (2 × 52 kg) and the combined organic phase washed with water (40 L). The organic phase was concentrated to low volume and ethyl acetate (55 kg) added along with hexane (90.4 kg). The organic phase was washed with 1 M hydrochloric acid (2 × 34.3 L) (to remove any unreacted di-*tert*-butyl tyrosine), then with 10% potassium bicarbonate solution (2 × 34.3 L), and finally with water (35 L). The organic phase was concentrated to an oil (35.8 kg, 104.1% weight yield) which was dissolved in 1-propanol (34.3 L) ready for the next step.

A small portion was purified for analysis by medium-pressure column chromatography over silica using hexane as eluant. This gave a colourless oil which solidified on standing, mp 66−68 °C. Found C, 70.24; H, 8.94; N, 2.87. $C_{31}H_{47}NO_6$ requires C, 70.29; H, 8.94; N, 2.64%. ^{1}H NMR ($CDCl_3$, 250 MHz) 1.32 (s, 9H), 1.39 (s, 9H), 1.50 (s, 9H), 1.52−1.74 (6H, m), 1.79−1.98 (2H, m), 2.60 (2H, s), 3.02 (2H, s), 4.70 (1H, q, J = 7 Hz), 5.41 [1H, s(br)], 6.06 (1H, d, J = 7 Hz), 6.08 [1H, s (br)], 6.90 (2H, d, J = 8 Hz), 7.08 (2H, d, J = 8 Hz).

***tert*-Butyl *(S)*-*N*-{*(R,S)*-2-*tert*-Butoxycarbonyl)-3-benzylaminopropyl}-1-cyclopentylcarbonyl)-*O*^{4}-*tert*-butyltyrosinate (22).** The acrylate **11** (48.2 kg, 91 mol) was dissolved in 1-propanol (70.5 L in total). Benzylamine (42.2 kg, 393.8 mol) was added, followed cautiously by boron trifluoride diethyl etherate (15.44kg, 108.8 mol). The mixture was heated at reflux for 6.75 h then allowed to cool naturally overnight. The reaction was concentrated under vacuum to remove 85 L of distillate, cooled to ambient, and diluted with ethyl acetate (171.6 kg) and hexane (125.9 kg). The resulting solution was washed with 1 M hydrochloric acid (2 × 286 L) [to remove excess benzylamine] followed by 5% aqueous sodium bicarbonate solution (114 L) then water (286 L). The organic layer was concentrated to an oil (55.9 kg, 96.4%) then dissolved in ethanol (45.8 kg) ready for the next step.

A small portion was purified for analysis by medium-pressure chromatography over silica, eluting with ethyl acetate/hexane mixtures. Ethyl acetate (20%) in hexane eluted the title compound as a 1:1 mixture of diastereoisomers as a colourless oil. Found C, 71.59; H, 8.77; N, 4.20. $C_{38}H_{56}N_2O_5$ requires C, 71.67; H, 8.86; N, 4.40%. ^{1}H NMR ($CDCl_3$) 1.35 (9H, s), 1.40 [9H, 2 × s, (two diasteroisomers)], 1.45 (9H, s), 1.5−2.02 (10H, m), 2.32−3.0 (4H, m), 3.04 (2H, d, J = 8 Hz), 3.75 (2H, m), 4.68 (1H, m), 6.80 and 6.42 [1H, 2 × d, J = 8 Hz, (two diastereoisomers)], 6.92 (2H, d, J = 8 Hz), 7.06 (2H, d, J = 8 Hz), 7.32 (5H, m). ^{13}C NMR ($CDCl_3$) [Where splitting is observed, it is reported as 2 × CH (etc.), for peaks in the region 33−55 ppm, diasteromeric pairs are less obvious and all resonances are reported.] 23.6 and 23.9 (2 × CH_2), 24.3 and 24.5 (2 × CH_2), 27.6 (CH_3), 28.6 (CH_3), 33.3 (CH_2), 33.9 (CH_2), 36.8 (CH_2), 37.1 (CH_2), 37.8 (CH_2), 38.0 (CH_2), 39.1 (CH_2), 44.1 (CH), 44.4 (CH), 52.1 (CH), 52.3 (CH_2), 53.4 (CH_2), 53.6 (CH), 53.9 (CH_2), 77.9 (C), 79.9 and 80.0 (2 × C), 81.4 and 81.5 (2 × C), 123.9 (CH), 126.6 and 126.8 (2 × CH), 127.9−128.1 (multiple CH resonances), 129.5 and 129.6 (2 × CH), 131.3 and 131.5 (2 × C), 140.0 and 140.1 (2 × C), 153.9 and 154.0 (2 × C), 170.6 and 171.2 (2 × C), 174.6 and 174.7 (2 × C), 176.1 and 176.3 (2 × C).

***tert*-Butyl *(S,S)*-*N*-{1-[3-amino-2-(*tert*-butoxycarbonyl)-propyl]-1-cyclopentylcarbonyl}-*O*^{4}-*tert*-butyltyrosinate (7)** The amine **(22)** (55.9 kg, 87.8 mol dissolved in ethanol (45.8 kg) was diluted with further ethanol (176.5 kg) and hydrogenated over 5% palladium/carbon (60% water wet paste) (11.2 kg) at 30−40 °C and 55−60 psi pressure for about 8 h. When the reaction was complete, the catalyst was removed by filtration through filteraid and washed with ethanol, and the resulting ethanol solution was concentrated to low volume (approximately 70 L). Hexane (120 L) was added, the mixture was cooled to 5−10 °C, and the product was collected by filtration and washed with hexane (40 L). The wet cake was reslurried with hexane (70 L), refiltered, and washed with hexane (23 L). The resulting damp cake (17.8 kg as two crops) was recrystallised from cyclohexane (89 L), cooled to 5−10 °C, and granulated for 3 h. The title product was collected by filtration, washed with cyclohexane (18 L), and dried at 50 °C in vacuo. Yield 15.53 kg, 32.4% (theoretical yield 50%) mp 129−130 °C (lit.[12] 122−127 °C). ^{1}H NMR ($CDCl_3$) 1.32 (9H, s), 1.40 (9H, s), 1.45 (9H, s), 1.55−1.75 (6H, m), 1.78−2.06 (4H, m), 2.23 (1H, m), 2.62 (1H, dd, J = 12.8, 6.6 Hz), 2.64 (1H, dd, J = 12.8, 6.8 Hz), 3.02 (2H, d, J = 7 Hz), 4.73 (1H, q, J = 7 Hz), 6.60 (1H, d, J = 8 Hz), 6.91 (2H, d, J = 8.4 Hz), 7.08 (2H, d, J = 8 Hz). The relative stereochemistry of **(7)** has previously been established.[8]

***tert*-Butyl 2-(hydroxymethyl)propenoate (13).** (**SAFETY NOTE:** Severe contact dermatitis has been reported after exposure to reaction products from formaldehyde and methyl

(11) Beyerman, H. C.; Bontekoe, J. S. *Receuil* **1962**, *81*, 691−698.

(12) Danilewicz, J. C.; James, K.; Kobylecki, R. J. European Patent 0358398, 1990.

acrylate.[7] No issues were noted at Pfizer using the *tert*-butyl ester, but given the structural similarity care should be taken.) Water (459 kg) and 3-quinuclidinol (12.65 kg, 99.4 mol) were charged to a reactor and stirred to give a clear solution. Paraformaldehyde (19.1 kg, 636.7 mol) was added and the solution heated to 40 °C before adding acetonitrile (201.5 kg). The resulting mixture was heated to reflux (77 °C) and *tert*-butyl acrylate (51kg, 397.9 mol) added cautiously. (**SAFETY NOTE:** The boiling point will drop during the addition and vigorous boiling may occur if the addition rate is too high.) The *tert*-butyl acrylate was washed in with acetonitrile (50 L). The reaction was heated at reflux for 90 min, and then toluene (255 L) was cautiously added. On completion of the toluene addition the mixture was cooled to 30 °C, and the layers were separated. The bottom aqueous layer was re-extracted with toluene (76.5 L) and then retained for reuse of the 3-quinuclidinol. The combined toluene layers were washed with saturated sodium chloride solution (2 × 150 L). Water (100 L) was added and the pH of the aqueous phase adjusted to pH = 4 with 2 M citric acid solution. After separating the phases, the toluene solution was washed with saturated sodium chloride solution (2 × 100 L), and monomethylhydroquinone (150 g) was added. (**SAFETY NOTE:** It is important this stabiliser addition is made, see text.) The toluene solution was stripped in vacuo at a temperature of up to 50 °C to give a final volume of 120 L (108.2 kg), which, by GC assay, was shown to contain 39.4 kg of the title compound (64.1% yield). A small portion of the solution was purified by column chromatography on silica, eluting with hexane and then 10% ethyl acetate in hexane, to give the title compound **13** which was further purifed by Kugelrohr distillation. Bp 80−100 °C/0.5 mmHg. Found C, 60.74; H, 9.14. $C_8H_{14}O_3$ requires C, 60.74; H, 8.92%. ^{1}H NMR ($CDCl_3$) 1.45 (9H, s), 2.9 [1H, m (br)], 4.21 (2H, s), 5.70 (1H, s), 6.10 (1H, s). ^{13}C NMR 27.9 (CH_3), 62.1 (CH_2), 81.1 (C), 124.3 (CH), 140.8 (CH), 165,5 (C). *m*/*z* 159, MH^+, 103 (M − isobutylene).

***tert*-Butyl *(S,S)*-1-[2-Bis(1-phenylethyl)amino]propanoate 5-sulphosalicylate (4).** *Part A: Prepartion of Bis(*α*-methylbenzyl)amine (**15**) Free Base Solution.* Water (82.8 L) was charged to the reactor followed by toluene (82.8 L), *(S,S)*-bis(α-methylbenzyl)amine hydrochloride (27.6 kg, 168.6 mol), and then 11.1 kg of 40% w/w sodium hydroxide solution. When all the material had dissolved, the pH of the mixture was approximately pH = 10. The phases were separated, and the aqueous phase was re-extracted with toluene (27.6 L). The combined toluene phases were washed with water (2 × 20 L) and then concentrated to approximately 60 L by atmospheric distillation to remove any water present. The resulting amine solution in toluene was used directly in the next procedure.

Part B: Chlorination and Alkylation. The toluene concentrate of the hydroxymethacrylate **(13)** (108.2 kg, containing 254.7 mol) prepared above was charged to the reactor along with further toluene (354 L), pyridine (1.96 kg, 24.8 mol), and triethylamine (26.4 kg, 260.9 mol) washed in with toluene (10 L). The resulting solution was cooled to 0 to −5 °C, and a solution of thionyl chloride (26.7 kg, 224.4 mol) in toluene (83 L) was added over 1 h, keeping the temperature below 15 °C. The resulting suspension was then stirred for 1 h without cooling and then was degassed by heating the mixture to 40−50 °C under vacuum to remove any SO_2. The mixture was cooled to 25 °C, and the toluene solution of *(S,S)*-bis(α-methylbenzyl)amine **(15)** prepared above was added over 30 min, keeping the temperature below 40 °C, followed by a toluene line wash (20 L). The reaction was stirred overnight at 35−45 °C at which time an HPLC sample showed 6% of the starting amine remained. Water (520 kg) was added, and the phases were separated. The aqueous layer was re-extracted with toluene (104 L), and the combined toluene extracts were washed with water (125 L), adjusting to pH = 4 with 2 M citric acid. Finally, the toluene solution was washed with water (2 × 125 L). Monomethylhydroquinone (5 g) was added (**SAFETY NOTE:** It is important that this is done; see text.) and the toluene solution concentrated to 360 L in vacuo. The toluene solution was diluted with 2-butanone (317 L), and a solution of 5-sulphosalicylic acid dihydrate (38.6 kg, 151.8 mol) in 2-butanone (106 L) was added followed by a seed of the desired product. The resulting slurry was cooled to 0−5 °C and granulated overnight. The title compound **4** was collected by filtration, washed with 1:1 2-butanone:ethyl acetate (2 × 30 L) and then ethyl acetate alone (2 × 50 L), and dried in vacuo at 40 °C. Yield 79.83 kg, 81.1% of a white crystalline solid, 97% pure by HPLC. A small sample was recrystallised from ethyl acetate: 2-propanol (4:1) for microanalysis. Mp 139−141 °C. Found C, 63.83; H, 6.55; N, 2.48; S, 5.54. $C_{31}H_{37}NO_8S$ requires C, 63.79; H, 6.39; N, 2.40; S, 5.49.

***(S,S,S)*-1-{3-[Bis(1-phenylethyl)aminomethyl]-2-(*tert*-butoxycarbonyl)propyl}-1-cyclopentylcarboxylic Acid 5-Sulphosalicylic Acid Salt (5).** Sodium hydroxide solution (1 M, 7.784 L), the amine salt (2.273 kg, 3.894 mol) **4**, and hexane (5.15 L) were mixed and stirred until two clear layers were obtained. The phases were separated, and the aqueous phase was re-extracted with hexane (1.03 L) with the two hexane extracts kept separate. The first hexane solution was washed with water (2 × 1.5 L) and stirred with magnesium sulphate (140 g) to remove entrained water. The magnesium sulphate was filtered off and the second hexane extract passed slowly through the magnesium sulphate so that it also was dry.

THF (2.59 L) was charged to a 25-L vessel followed by LDA solution (2895 g, 28.8%w/w in heptane ex FMC lithium, 7.784 mol), and the resulting solution was cooled to −30 °C. Cyclopentanecarboxylic acid (**STENCH**) (444.4 g, 3.895 mol) was added at −10 °C over 15 min and washed in with THF (400 mL). The resulting slurry was stirred overnight, allowing the temperature to rise to 20 °C to form the dianion. The resulting red-ochre-coloured slurry was cooled to −12 °C, and the hexane solution of the free base **(4)** was added over 50 min, keeping the temperature between −10 and 0 °C. The second hexane extract (see above) was used to wash in the amine **4**. The reaction was stirred at −5 °C for 30 min. The reaction was cooled to −15 °C and then quenched with citric acid solution (2 M, 9.07 L) with continuous cooling over 16 min (the resulting pH = 4). The

layers were separated, and the aqueous phase was re-extracted with hexane (1.5 L). The combined organics were washed with water (3 × 2 L), and the organic phase was concentrated to half volume in vacuo. 2-Butanone (6 L) was added and the evaporation continued again to half volume. The concentrate was diluted with 2-butanone (10 L) and cooled to −10 °C. A solution of 5-sulphosalicylic acid dihydrate (854.5 g, 3.362 mol) in 2-butanone (5 L) was added over 30 min, keeping the temperature below 0 °C. The resulting slurry was granulated at 0−5 °C for 2.5 h. The title product **5** was collected by filtration, washed with 2-butanone, and dried in vacuo to give an off-white granular solid (2.38 kg, 87.6%). Purity by HPLC was 98.4% with 0.8% of the diastereoisomer and 0.2% of the starting material **4** present. A small sample was recrystallised from ethyl acetate/2-propanol to give material for microanalysis. Mp 172−174 °C. Found C, 63.45; H, 6.90; N, 2.07. $C_{37}H_{47}NO_{10}S$ requires C, 63.68%; H, 6.79; N, 2.01%.

***tert*-Butyl *(S,S,S,S)*-*N*-(1-{3-{Bis[1-phenylethyl)aminomethyl]-2-(*tert*-butoxycarbonyl)propyl}-1-cyclopentylcarbonyl)-*O*^{4}-*tert*-butyltyrosinate (16).** *Method 1 with 1-Propanephosphonic Acid Cyclic Anhydride (PPACA).* Water (177 L), sodium hydroxide solution (40% w/w, 19 kg) the glutarate **(5)** (64.4 kg, 92.1 mol) and dichloromethane (275.6 kg) were stirred to give two clear layers. The aqueous layer was run off, and the dichloromethane layer was washed with brine (2 × 65 L). The resulting glutarate solution was transferred to a reactor equipped for simultaneous and synchronous addition of two reagents with on-line pH monitoring, and water was added (168 L). *tert*-Butyl *O*^{4}-*tert*-butyltyrosine hydrochloride (30.4 kg, 92.1 mol) was added and the pH adjusted to 8.5−9.0 with 20% sodium hydroxide solution. PPACA (50% in ethyl acetate, 83.4 kg) was added, whilst maintaining the pH in the range 8.5 to 9.0 by the simultaneous addition of 20% sodium hydroxide solution (approximately 75 kg added). During the additions of PPACA and sodium hydroxide the temperature was maintained below 25 °C. The reaction was stirred for 30 min before adding the second portion of PPACA (50% in ethyl acetate, 83.4 kg), again keeping the pH in the region 8.5−9.0. The reaction was stirred for 5.5 h and then a further 66.5 kg of PPACA solution added, again keeping the pH 8.5−9.0.[13] The reaction was left to separate overnight and the bottom aqueous layer run off. Hexane (300 L) was added and the organic solution washed with 1 M hydrochloric acid (2 × 84 L) (to wash out any unreacted tyrosine derivative) followed by water (2 × 84L with adjustment to pH 7 with sodium bicarbonate). The organic layer was concentrated to low volume by atmospheric distillation. Ethanol (55.2 kg) was added to give 127.4 kg of concentrated solution containing 72.2 kg of crude title product. Crude yield 104.3%.

A sample was stripped for NMR spectroscopy. ^{1}H NMR ($CDCl_3$) 1.31 (9H, s), 1.33 (6H, d), 1.36 (9H, s), 1.37 (9H, s), 1.4−1.7 (6H, m), 1.8−2.0 (4H, m), 2.55−2.80 (3H, m), 2.98 (2H, dd, J = 13.5, 6.7 Hz), 3.11 (2H, dd, J = 13.5, 4.8 Hz), 3.93 (2H, q, J = 6.7 Hz), 4.70 (1H, q, 6 Hz), 6.10 (1H, d, J = 7.8 Hz), 6.89 (2H, d, J = 6.9 Hz), 7.08 (2H, d, J = 6.9 Hz), 7.14−7.25 (10H, m).

***tert*-Butyl *(S,S,S,S)*-*N*-(1-{3-{Bis[1-phenylethyl)aminomethyl]-2-(*tert*-butoxycarbonyl)propyl}-1-cyclopentylcarbonyl)-*O*^{4}-*tert*-butyltyrosinate (16).** *Method 2 with N,N′-Carbonyldiimidazole.* The glutarate **(5)** (174.25 g, 0.25 mol), sodium hydroxide (20.0 g, 0.5 mol) dissolved in water (700 mL) and toluene (870 mL) were mixed and stirred for 15 min. The aqueous layer was separated and the organic layer washed with water (250 mL) then brine (520 mL). An alternative method to drying by brine washing is stripping out 1 mL/g of toluene, but this only worked well on a laboratory scale (see text). 1-Hydroxybenzotriazole (low water grade, 3.37 g, 0.025 mol) was added followed by *N,N′*-carbonyldiimidazole (42.5 g, 0.2625 mol). The reaction was stirred at 40 °C for 2 h to give a clear, pale-yellow solution. *tert*-Butyl-*O*^{4}-*tert*-butyltyrosine hydrochloride (86.6 g, 0.2625 mol) was added in one portion, and the reaction was heated to 90−95 °C for 3 h to give a cloudy yellow solution. The reaction was cooled to room temperature and washed with hydrochloric acid (2 M, 175 mL). The two clear phases separated, and the organic phase was washed with water (200 mL). The organic phase was evaporated to give the title compound **16** as a pale yellow gum (191.6 g, 101.5%). ^{1}H NMR ($CDCl_3$) in agreement with that prepared by method 1.

***tert*-Butyl *(S,S)*-*N*-{1-[3-Amino-2-*tert*-butoxycarbonyl)-propyl]-1-cyclopentylcarbonyl}-*O*^{4}-*tert*-butyltyrosinate (7)**. A portion of the above ethanol solution of the amine **16**, prepared by method 1, 107 kg containing 60.5 kg of crude product was diluted with further ethanol (400 L) and hydrogenated over 5% Pd/C type 5R39 at 50−60 psi and 25−35 °C until hydrogenation was complete. The catalyst was filtered off and washed with ethanol. The filtrate was concentrated to low volume (approximately 70 L) and hexane (106.5 L) added. The resulting slurry was cooled to 5−10 °C, granulated overnight then collected by filtration, and washed with hexane (43 L). The resulting damp cake (34.5 kg) was recrystallised from cyclohexane (174 L), granulated for 3 h at 5−10 °C, collected by filtration, and dried in vacuo to give the title compound **7** 27.2 kg [70.2% yield from the glutarate **5**] mp 129−130 °C (lit.[12] 122−127 °C). ^{1}H NMR ($CDCl_3$) identical to that prepared by CRD route 1 (see above).

***tert*-Butyl *(S,S,S)*-*N*-{1-[3-(*N*^{6}-Benzyloxycarbonyl-*N*^{2}-mesyllysylamino)-2-(*tert*-butoxycarbonyl)propyl]-1-cyclopentylcarbonyl}-*O*^{4}-*tert*-butyltyrosine (19).** The amine **7** (20.81 kg, 38.06 mol) and the acid **21**[10] (13.64 kg, 38.06 mol) were dissolved in dichloromethane (166.2 kg). 1-Hydroxybenzotriazole hydrate (6.19 kg, 40.46 mol) was added and the reaction cooled to 10 °C. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (10.25 kg, 53.47 mol) was added over about 15 min allowing the temperature to rise to 20 °C and the reaction stirred overnight. The reaction solution was washed with saturated sodium bicarbonate

(13) The number of equivalents of PPACA required for complete reaction in these heterogeneous amide coupling reactions depends heavily on the efficiency of mixing in the reactor. For reactors with efficient stirring 2 equiv of PPACA can be used, and the third charge used in this preparation would be unnecessary.

solution (2 × 29 L), followed by 1 M hydrochloric acid (2 × 29 L), and finally with water (29 L, adjusting the pH to 7.0). Dichloromethane (100 L) was stripped out, and further dichloromethane (50 L) was added and then stripped out again to give the title product **19** in dry dichloromethane (107.2 kg of solution that was used directly in the next step). A stripped aliquot indicated the yield was quantitative.

(*S*,*S*,*S*) -*N*-{1-[3-(*N*^{6}-Benzyloxycarbonyl-*N*^{2}-mesyllysyl-amino)-2-carboxypropyl]-1-cyclopentylcarbonyl}tyrosine (20). The product from the last reaction (107.2 kg of dichlormethane solution containing **19** (38.06 mol) was charged to a reactor and washed in with dichloromethane (16.8 kg) followed by anisole (64.5 kg). Trifluoroacetic acid (97 kg, 850.7mol) was added to the stirred solution over a period of 1 h allowing, the temperature to rise to (but not exceed) 35 °C. When the addition was complete, the reaction was stirred for 6 h at 30−35 °C and then allowed to cool naturally overnight. Water (84.4 L) was added to the reaction to give **three phases.** The phases were separated, and the bottom oil phase was combined with the upper organic phase and diluted with ethyl acetate (169 L). Saturated brine solution (67.5 L) was added, and the bottom aqueous phase was run off. Saturated brine solution (47 L) was added followed by sodium bicarbonate (47 L, 10% w/w). The pH of the aqueous phase was equal to 3. The mixture was stirred for 15 min, and the **three phases** were allowed to separate. The bottom oil phase was run off and dissolved in ethyl acetate (47 L). The middle aqueous phase was run off and the ethyl acetate solution of the oil added back to the reactor. The product was extracted out of the aqueous layer as the disodium salt by adding brine (47 L) and sodium bicarbonate solution (210 L, 10%w/w). The layers were separated, and the product containing aqueous phase was washed with ethyl acetate (2 × 33 L). Finally, the product containing aqueous phase was adjusted to pH 3 by the addition of 28 L of concentrated hydrochloric acid and the product extracted back into ethyl acetate (2 × 27.5 L). The ethyl acetate layer was washed once with brine (23.5 L) to give 120.8 kg of product **20** containing solution. An aliquot was stripped to show the crude yield was 100%.

Sampatrilat (2). The product containing solution from the last reaction (120.8 kg) was extracted with a sodium hydroxide solution (3.01 kg of sodium hydroxide in 56 L of water) followed by a water wash (2 L). The combined organic layers were hydrogenated over 5% Pd/C (1.5 kg, 60% wet) at 60 psi and 21−29 °C for 7 h. The catalyst was removed by filtration and washed with water (40 L). The solution of the disodium salt was then neutralised with 5 M hydrochloric acid to the isoelectric point, pH = 4, and granulated for 24 h at room temperature. The product was collected by filtration, washed with water (4 × 15 L) and then acetone (15 L), and dried in vacuo to give the title compound **2** as a white crystalline solid as polymorph (P256) (19.12 kg, 85.9% from compound **7**. Mp 250 °C (lit.[10] 248−250 °C).

An earlier preparation performed on a 0.3 mol scale[10] gave microanalysis as follows C, 53.47; H. 7.25, N, 9.50. $C_{26}H_{40}N_4O_6S$ requires C, 53.41; H, 6.90; N, 9.58%.

Polymorph Preparations and Interconversions. *Historical Preparation of the Hydrate.* A solution of Cbz−Sampatrilat **(20)** (351 g, 0.49 mol) in ethyl acetate (1300 mL) was added to water (385 mL) and the two-phase mixture hydrogenated at 60 psi and room temperature over a 5% palladium-on-carbon catalyst (35 g) for 20 h. The catalyst was filtered off, and the aqueous phase was separated and concentrated to low volume under reduced pressure. The viscous solution was poured into methanol (2.85 L) and stirred at room temperature for 18 h during which time there was a slow precipitation of a solid. The solid was granulated at 5−10 °C for 2 h, filtered, washed with methanol, and dried to give the title compound as a white solid (178.1 g, 60.5%) mp 168−171 °C. Found C, 51.37; H, 7.47; N, 9.06. $C_{26}H_{40}N_4O_9S\cdot xH_2O$ (where $x = 1$) requires C, 51.81; H, 7.02; N, 9.30%. Water content 3.6 wt % as determined by Karl Fischer analysis ($x = 1$ requires 3.0 wt %).

Historical Coversion of the Hydrate to Polymorph (P185). Sampatrilat hydrate (847.0 g, 1.41 mol) was dissolved in water (762 mL) and diluted with acetone (1.0 L). This solution was added to vigorously stirred acetone (18.05 L) at room temperature, and a white solid precipitated. The mixture was stirred at room temperature for 18 h, and the solid was collected by filtration, washed with acetone, and dried to give the title compound as a white solid (775 g, 94%), mp 179−181 °C (DSC 185 °C). Found C, 53.42; H, 6.88; N, 9.37; S, 5.49. $C_{26}H_{40}N_4O_9S$ requires C, 53.41; H, 6.90; N, 9.58; S, 5.48%. This preparation had been scaled up to 5.4 kg before the discovery of the new form made it invalid.

Preparation of the Amorphous Form. Sampatrilat (P256) (4 g, 6.84 mmol) was added to water (200 mL) and the mixture stirred at 90−95 °C for 30 min. Insoluble material was filtered off and the filtrate diluted further with water (50 mL) and cooled to room temperature, After filtration to remove a slight haze, the clear filtrate was frozen by using a solid carbon dioxide/acetone bath. The solid mass obtained was freeze-dried to yield the title compound as a white solid (3.0 g, 75%). PXRD showed the material was amorphous.

Conversion of the Amorphous Form to Polymorph (P185). The amorphous form prepared above (0.3 g) was stirred in 1-propanol (10 mL) for 5 days. The resulting white solid was collected by filtration and dried under reduced pressure to give the title compound (0.26 g) mp 175−180 °C.

Further data (PXRD, IR) for all four physical forms can be found in ref 10.

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