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The Development of a Manufacturing Route for the GPIIb/IIIa Receptor Antagonist SB-214857-A. Part 2: Conversion of the Key Intermediate SB-235349 to SB-214857-A

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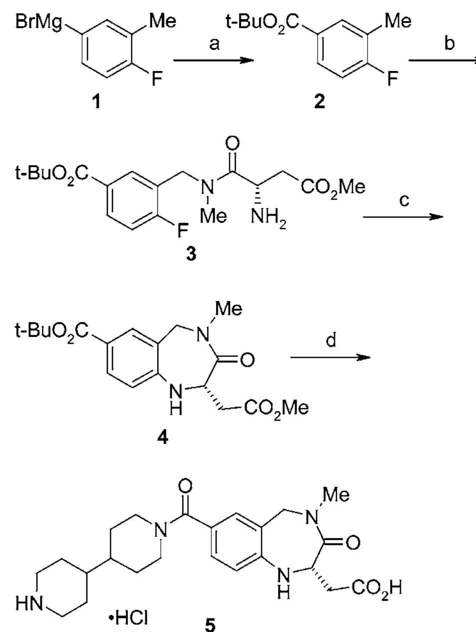
Abstract:

The process development to the manufacturing route to (2*S*)-7-([4,4′-bipiperidin]-1-ylcarbonyl)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1*H*-1,4-benzodiazepine-2-acetic acid hydrochloride (SB-214857-A, lotrafiban) is described. The starting point is the previously reported intermediate (2*RS*)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1*H*-1,4-benzodiazepine-2-acetic acid methyl ester. The first stage is a lipase-catalysed resolution of the racemic ester to (2*S*)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1*H*-1,4-benzodiazepine-2-acetic acid and subsequent iodination using a pyridine iodine monochloride complex to give (2*S*)-2,3,4,5-tetrahydro-7-iodo-4-methyl-3-oxo-1*H*-1,4-benzodiazepine-2-acetic acid. The unreacted (*R*)-enantiomer of the starting ester is recovered and recycled to the racemate by treatment with sodium methoxide. The next stage describes the palladium-catalysed aminocarbonylation of the aryl iodide with 4,4′-pyridylpiperidine to give (2*S*)-2,3,4,5-tetrahydro-4-methyl-3-oxo-7-[[4-(4-pyridinyl)-1-piperidinyl]carbonyl]-1*H*-1,4-benzodiazepine-2-acetic acid dihydrate. The third stage is the hydrogenation of the pyridine subunit over palladium on charcoal to obtain the zwitterionic (2*S*)-7-([4,4′-bipiperidin]-1-ylcarbonyl)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1*H*-1,4-benzodiazepine-2-acetic acid hexahydrate. The final stage is the formation of the hydrochloride salt to afford the drug substance.

SB-214857-A (lotrafiban) **5** is a potent nonpeptidic glycoprotein IIb/IIIa antagonist and consequently inhibits platelet aggregation.[1] The molecule has the (*S*)-stereochemistry and only this enantiomer is active. Clinical trials of SB-214857-A have been undertaken with a view to determine whether the repeat of secondary thrombolytic events such as heart attack or stroke could be prevented with such an agent.[2] Unfortunately the clinical programme was halted during phase III trials, by which stage the route for the manufacture of the drug substance had been established within Research and Development and was being transferred to the Manufacturing environment.

Scheme 1 [a]



[a] Reagents and conditions: a) i) CO_2, THF, 77%; ii) *i*-butylene, TfOH (5 mol %), Et_2O, 94%; b) i) NBS, $(PhCO)_2O_2$, CCl_4; ii) $MeNH_2$, THF, H_2O, 70% (over two steps); iii) Cbz-L-Asp-*β*-methyl ester, DCC, HOBt, DMF, 86%; iv) H_2, 10% Pd/C, MeOH, 98%; c) 3Å molecular sieves, DMSO, toluene, 47%; d) i) TFA, anisole, CH_2Cl_2, 95%; ii) *N*-BOC 4,4′-bipiperidine, EDC, $(i\text{-}Pr)_2NEt$, DMF, 94%; iii) NaOH, H_2O, MeOH, THF, 81%; iv) HCl, H_2O, dioxane, 62%.

Within R&D Chemical Development three routes were used to supply drug substance to fund preclinical and clinical development. The first route, Route A, Scheme 1, was an adaptation of the Medicinal Chemistry route, where the stereochemistry was derived from L-aspartic acid.[3] Although used to prepare kilogram quantities, the route was not suitable for further scale-up because of problems encountered on a 50-kg scale during the intramolecular fluoride displacement reaction to convert **3** to **4**. The reaction required 5 kg of 3Å molecular sieves per kg of **3** and produced only a 47% yield of **4**. In addition the product obtained had undergone racemisation such that the chiral purity was only 98%. Since upgrading the chiral purity of **4** or any of the later

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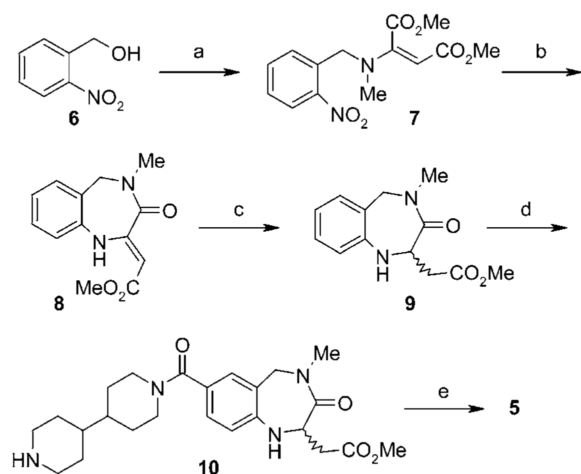
Scheme 2 [a]



[a] Reagents and conditions: a) i) MsCl, NEt_3, THF, rt; ii) $MeNH_2$, H_2O, rt; iii) dimethyl acetylenedicarboxylate, PhMe, rt; b) i) H_2, Raney Ni, MeOH, 50 psi, 50 °C; ii) AcOH, MeOH, 65 °C; iii) NaOMe, MeOH, 65 °C; c) H_2, Pd/C, MeOH, 60 psi, 60 °C, 70% (over seven steps); d) i) pyridine iodine monochloride complex, CH_2Cl_2, H_2O, 87%; ii) $PdCl_2(PPh_3)_2$, CO, (i-Pr)$_2$NEt, **22**, NMP, 10 psi, 95 °C, 85%; iii) HCO_2NH_4, 10% Pd/C, MeOH, 65 °C, 85%; e) i) Boehringer L-2 enzyme resin, H_2O, NH_3, pH 6.2, rt, 42%; ii) $C_5H_5N{\cdot}HCl$, EtOH, CH_2Cl_2, H_2O, 25 °C, 90%.

intermediates was not possible, Route A was discontinued. Subsequently, a group of independent workers have reported an alternative copper-catalysed procedure that is claimed to circumvent the racemisation problems.[4]

A second route, Route B, Scheme 2, was devised and again used to prepare kilogram quantities of drug substance.[5] The enzymatic resolution of **10** was carried out using *Candida antarctica* lipase B supplied in an immobilised form as Boehringer L-2.[6] Isolation of the zwitterionic form of SB-214857 required *N*-Cbz reprotection of the unreacted (*R*)-ester to facilitate a large-scale liquid−liquid separation of product acid from the unreacted ester. The drug substance produced from Route B had a chiral purity >99.5%. The major limitations with Route B were the low yield, 42%, for the preparation of mono-*N*-Cbz 4,4′-bipiperidine **22** from 4,4′-bipiperidine **21** (see Scheme 6) and the late-stage enzymatic resolution which led to low overall efficiency and a high cost of goods.

Route B evolved into Route C, Scheme 3, in which the enzyme resolution is carried out at an earlier stage, the unwanted (*R*)-enantiomer **11** is readily recycled, and 4,4′-pyridylpiperidine **20** is used as an efficient and cost-effective masked form of the bipiperidine subunit located in the target molecule.[7]

From the three routes used to make supplies and other routes that had been evaluated[8] Route C was chosen as the commercial route for the primary manufacture of the drug substance. From a logistical standpoint Route C was subdivided into five stages; stage 1 is the conversion of 2-nitrobenzyl alcohol **6** through to racemic ester **9,** and this is described in Part 1 of our work.[9] This is the same chemistry as used for Route B. Stage 2 is the enzymatic resolution of **9** and subsequent iodination of acid **12** to form **13**, as well as the recycling of the unwanted enantiomer **11**. Stages 3, 4, and 5 are the aminocarbonylation of **13** with pyridylpiperidine **20**, the hydrogenation of **14** to give the zwitterionic form of the drug substance **15** and conversion to the HCl salt **5**, respectively. In this contribution we describe the work carried out in stages 2−5 to enable successful chemistry qualification and validation campaigns to be carried out at the site of primary manufacture.

Stage 2: Enzymatic Resolution. Stage 2 had been successfully carried out in the pilot plant to prepare 3200 kg of **13**.[10] A degree of refinement, streamlining, and a fuller understanding of the process was required to enable the process to run efficiently within the confines of the production environment. In addition to this several other goals were set: (i) direct isolation of **13** within the purity specification required for stage 3: HPLC assay for purity >93%, total impurities by HPLC <3.0% peak area ratio (PAR), acid **12** <2.0% PAR, impurity **16** <0.5% PAR, impurity **17** <0.5% PAR and chiral purity >99.0%; (ii) maximise the use of Novozym 435, which is a significant cost centre at £500/kg; and (iii) recycle the unreacted ester **11** back to racemic ester **9**, again within the required purity specification.

To enable the resolution of racemic ester **9** to be undertaken, a screen of commercially available esterases, lipases, and proteases was carried out. Esterases and proteases which showed activity hydrolyzed the unwanted (*R*)-enantiomer with moderate selectivity (chiral purity 60−80%). The only lipase to show activity was *C. antarctica* lipase B, and it was highly selective for the hydrolysis of the (*S*)-enantiomer of **9** to give acid **12** with greater than 99.5% chiral purity. Unlike Route B where Boehringer L-2 resin was used as the enzyme source, this time Novozym 435 was preferred.[11]

A major focus of the work is to find the optimum conditions for this enzymatic resolution with respect to the following: (i) the chiral purity of **12**, (ii) reaction rate (a reaction time of longer than 24 h would affect plant throughput), and (iii) reuse of the resin.

Novozym 435 is a catalyst that has been designed for use in anhydrous systems the enzyme is absorbed onto a polyacryamide resin.[12] Factors which were thought to be important to extend its life span were the following: pH, water content of the solvent, and temperature.

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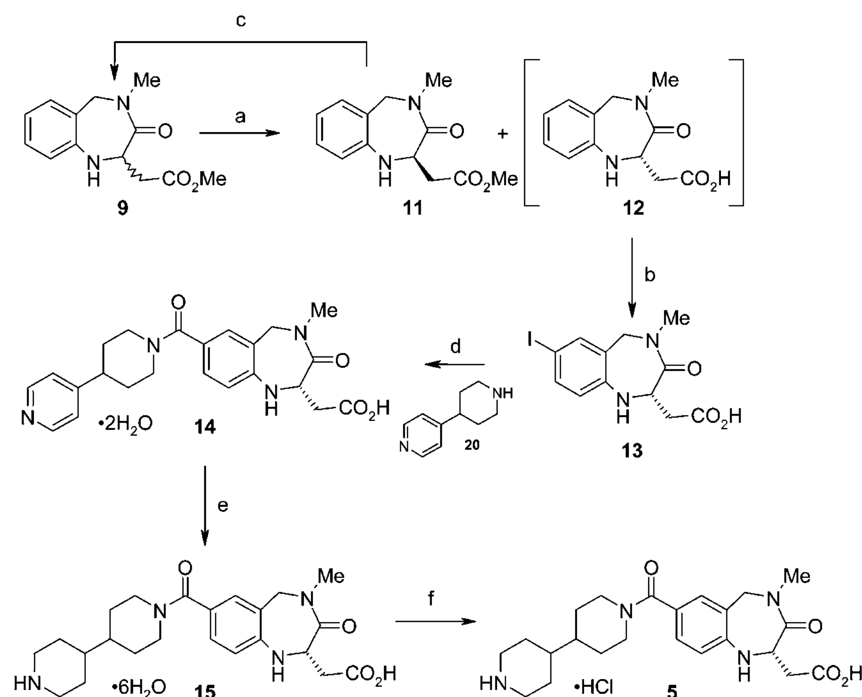
Scheme 3 [a]



[a] Reagents and conditions: a) Novozym 435, *t*-BuOH, H_2O, NH_3, pH 7.0, 50 °C; b) pyridine iodine monochloride complex, H_2O, NaOH, pH 7.0, 10 °C, 37.5% (over two steps); c) NaOMe, MeOH, $(MeO)_2CO$, 50 °C, 38.0% (over two steps); d) $PdCl_2(PPh_3)_2$, CO, **20**, anisole, dicyclohexylamine, 15 psi, 100 °C, 86%; e) H_2, Pd/C, *i*-PrOH, H_2O, 60 psi, 75 °C, 87%; f) $C_5H_5N.HCl$, EtOH, CH_2Cl_2, H_2O, 25 °C, 90%.

The operating premise for the resolution was to slurry the racemic ester **9** in a *t*-BuOH/H_2O mixture (concentration, 1 g in 10 mL), add the Novozym resin (30% dry weight with respect to **9**), apply heat, and maintain pH at 7.0 by addition of an ammonia titrant solution. Once the reaction was deemed to be complete (conversion to **12** >47% HPLC PAR relative to **9**), the Novozym 435 resin was removed by filtration, and the *t*-BuOH/H_2O solution of **11** and **12**, as ammonium salt, was processed further. Although the solubility of racemic ester **9** in *t*-BuOH/H_2O was low, 2.4% w/w at 50 °C, the solubility of the mixture of **11** and **12** was much greater, and no problems were encountered as long as the solution was filtered while still warm. For laboratory-scale experiments the reactions were carried out with an overhead stirrer to prevent attrition of the resin, and a Metrohm 718 Stat Titrino was used to maintain a constant pH by automated addition of the ammonia titrant.

The effect of running the enzyme resolution at different pH was measured, Table 1. Very satisfyingly, the results show that the chiral purity of acid **12** produced is unaffected by the reaction pH. What is apparent is that at low pH the rate of reaction drops off, and in further experiments it is shown that correcting the pH back to 7.0 causes the reaction to proceed to completion. This simply reflects a degree of reversible enzyme inhibition at lower pH values and follows the generally expected behavior of enzymes at different pH. At pH >7.5, there was a considerable increase in the amount of ammonia solution required. From a practical manufacturing perspective this meant that control of pH at this point was not a quality critical parameter. The recommended operating pH was 7.0, but the range pH 6.8−7.5 would be acceptable. Additionally, if there was an overcharge or undercharge of ammonia titrant due to mechanical problems then quality would not be affected.

Table 1. **Summary of effect of changing pH on enzyme resolution of 9 to 12**[a]

pH	chiral purity of **12** (%)	conversion after 20 h (%)	volume of titrant used (mL)
5.1	100	44	0
6.0	ND	45	5
6.5	99.7	47	21
6.8	99.8	48	38
7.0	99.8	48	37
7.5	99.8	49	50
8.0	99.6	49	65

[a] Reactions carried out on a 100 mmol scale at 50 °C, using 1.5 M NH_3 in *t*-BuOH as titrant and 88% *t*-BuOH as solvent. The % conversion to **12** was determined by HPLC. ND implies not determined.

The water content in the *t*-BuOH was expected to have a considerable effect on the life span of the Novozym 435. Since the enzyme is only absorbed onto the polyacryamide resin, it was anticipated that the enzyme could leech into the solvent if the water content was high. Any leeching would not be apparent on the first use of resin since the free enzyme would be expected to be as active as the resin-bound enzyme; thus, the resin would have to be reused to detect any long-term rate reduction, Table 2. The results show that the reusability of the Novozym 435 does decrease as the water level increases. For the manufacturing environment it was decided to use commercially available 88% *t*-BuOH (the other 12% being H_2O) as the solvent for the reaction and to make up the ammonia solution required as titrant. Not only is this composition of *t*-BuOH/H_2O acceptable from a reaction point of view, but it is also acceptable as a bulk

Table 2. Effect of water level in solvent upon catalyst reuse for the conversion of 9 to 12[a]

water content (%)	use	conversion after 20 h (%)
8	first	48
8	second	48
12	first	48
16	first	48
16	second	49
19	first	49
19	second	47
23	first	49
23	second	40

[a] Reactions carried out on a 100 mmol scale, pH 7.0, 50 °C, using 1.5 M NH_3 in 88% *t*-BuOH as titrant. Water content determined by Karl-Fisher titrimetry. The % conversion to **12** was determined by HPLC.

Table 3. Effect of reaction temperature upon catalyst reuse for the conversion of 9 to 12[a]

temperature (°C)	use	conversion after 20 h (%)
45	first	47
50	first	48
50	10th	45
55	first	48
55	fourth	45
60	first	48
60	second	12

[a] Reactions carried out on a 100 mmol scale, pH 7.0, 1.5 M NH_3 in 88% *t*-BuOH as titrant and 88% *t*-BuOH as solvent. The % conversion to **12** was determined by HPLC.

solvent since it remains liquid at temperatures down to −10 °C, unlike anhydrous *t*-BuOH which has a melting point of 25 °C and would have been very inconvenient to use as a bulk solvent.

The optimum operating temperature with respect to maximizing the life span of Novozym 435 was again determined by a series of resin reuse experiments, Table 3. The results show that reusability of the Novozym 435 did decrease quite markedly as the reaction temperature increased. The recommended temperature was 50 °C, but the range 48−52 °C would be acceptable.

Application of these parameters to the production environment proved very successful: control of pH was satisfactory. Upon the first use of the catalyst the reaction had passed the target end-point of >47% HPLC PAR of **12** after 9 h 50 min, Table 4. The one batch of catalyst was successfully reused 12 times, by which point the reaction end-point was achieved after 20 h 20 min, an outcome that was superior to the lab experiments, cf. Table 3. The only reason the catalyst was not used further was that the campaign had been completed.

At the end of the reaction the solution was filtered through an 80 μm screen in the base of the vessel, and the filtrate was transferred to another vessel for further processing. The resin was washed with a further portion of 88% *t*-BuOH and therefore remained in the vessel ready for reuse. No attrition of the resin was observed.

Table 4. Production plant results for the conversion of 9 to 12[a]

batch	reaction time	conversion (% PAR)
1	9 h 50 min	47.5
2	14 h 30 min	48.3
3	14 h	47.4
4	12 h 40 min	47.2
5	14 h 10 min	48.2
6	15 h 30 min	49.1
7	13 h 20 min	48.2
8	13 h 30 min	47.2
9	14 h 20 min	47.5
10	11 h 20 min	47.5
11	13 h 20 min	47.3
12	15 h 50 min	47.6
13	20 h 20 min	47.2

[a] Reactions carried out on 246 kg scale at 50 °C, pH 7.0, 1.5 M NH_3 in 88% *t*-BuOH as titrant and 88% *t*-BuOH as solvent.

To effect the physical separation of ester **11** from acid **12** the concept was to dissolve the acid in mild aqueous base and allow the insoluble ester to crystallise and be removed by filtration; the aqueous solution of **12** could then be used directly in the next reaction, the electrophilic aromatic iodination at C-7. The simple procedure of adding water to the *t*-BuOH solution of ester **11** and acid **12** (presumably as the ammonium salt) and removing the organic solvent by distillation under reduced pressure was unsuccessful. In these circumstances the acid cocrystallised with the ester, presumably due to dissociation of the ammonium salt and removal of ammonia under vacuum, leaving the insoluble parent acid. To alleviate this problem the acid **12** was converted to the sodium salt by adding 1.0 mol equiv (wrt **12**) of aqueous $NaHCO_3$. Concentration under reduced pressure and then addition of a further amount of water resulted in selective crystallisation of ester **11**, typically in 40% yield. Although this procedure worked well in the laboratory, it was not satisfactory when scaled up to 120-kg scale in the pilot plant. In the laboratory the solution yield of acid **12** was 45%, and the chiral purity was 99.6%, whereas in the pilot plant the yield of **12** was 49%, and the chiral purity reduced to 96.4%. The knock-on effect was that iodo-acid **13** was isolated with 97.8% chiral purity and required reworking before being progressed to drug substance. The problem with the procedure on scale-up was that the extended time (7 h) required to remove the *t*-BuOH (1500 L) at pH 8.3 was sufficient to cause some background hydrolysis of ester **11**. To circumvent this problem it was necessary to moderate the pH of the solution before removal of the solvent. The pH of the solution after the $NaHCO_3$ had been added was typically pH 8.3; this was reduced to pH 7.5 (range pH 7.3−7.8 was acceptable) by the addition of 1 M aqueous HCl. An overcharge of HCl to give a pH <6.8 would result in the cocrystallisation of **12** with **11**, whereas an undercharge of HCl (pH >7.8) could result in background hydrolysis of **11**. As an additional safeguard against background hydrolysis of **11** the internal vessel temperature was kept <50 °C during the vacuum distillation. On a 246-kg scale in the production plant this procedure worked well; the chiral purity of the intermediate acid **12** was >99.8%, even after the removal of 3000 L of

***Table 5.* Yield and analysis of ester 11 produced in production plant[a]**

batch	weight (kg)	yield (%)	HPLC assay of purity (%)	total impurities by HPLC (% PAR)	acid **12** % PAR
1	100.0	40.6	98.2	2.0	2.0
2	101.7	41.3	98.4	1.2	1.2
3	95.0	38.6	98.5	0.3	0.3
4	101.8	41.4	99.5	0.8	0.3
5	101.0	41.1	99.2	0.2	0.2
6	112.6	45.8	100.2	0.2	0.2
7	111.0	45.1	97.1	1.6	1.1
8	111.6	45.4	96.8	1.3	1.1
9	111.2	45.2	96.0	2.2	2.2
10	114.0	46.3	96.3	2.0	2.0
11	118.0	48.0	95.0	2.9	2.9
12	121.0	49.2	99.0	1.0	1.0
13	110.5	44.9	98.9	0.6	0.6

[a] Reactions carried out on 246 kg of ester **9**.

***Table 6.* Production plant batch data for the solution yields of acid 12 and the reaction with PIM[a]**

batch	acid **12** concn (% w/w)	mass flow for solution of acid **12** (kg)	solution yield for acid **12** (%)	charge of PIM (kg)	residual acid **12** after reaction with PIM (% PAR)
1	4.87	2124	44.6	128.3	0.4
2	5.45	1978	46.4	133.7	0.2
3	5.19	2146	48.0	138.1	0.8
4	5.27	1943	44.1	127.0	0.7
5	5.72	1866	46.0	132.4	0.2
6	5.65	1874	45.6	131.3	0.0
7	5.37	1849	42.8	123.1	0.8
8	5.57	1871	44.9	129.2	0.1
9	5.60	1794	43.3	124.6	0.5
10	5.66	1829	44.6	128.4	0.0
11	5.39	1910	44.4	127.7	0.0
12	5.13	2006	44.3	127.6	0.0
13	5.47	1925	45.4	130.6	0.4

[a] Reactions carried out on 246 kg of ester **9**.

solvent over a 12-h period. At the endpoint of the distillation (4 volumes remaining) additional warm water (3.2 volumes) was added to complete the crystallisation of **11**. Ester **11** was isolated in a centrifuge and the cake washed with further water (1.6 volumes). The wet cake was then dried in an agitated vacuum pan drier at 50 °C until the water level was <0.2% w/w. In the production environment ester **11** was isolated on average in 43.2% yield (range 38.6−49.2%, depending upon whether the heel was left in the drier), average HPLC assay of purity 98.1% (range 95.0−100.2%) and the only impurity in the HPLC profile was acid **12**, typically at 1.0% PAR, Table 5. The specification for ester **11** was total impurities by HPLC <5.0% PAR, acid **12** <4.0% PAR.

The aqueous filtrate from the centrifuge, containing the sodium salt of acid **12** was washed with CH_2Cl_2 to remove the residual ester **11** to levels <2% PAR. A solution yield of acid **12** was obtained by HPLC assay against a standard, average concentration = 5.4% w/w, mass flow = 1920 kg, and yield 44.7%, Table 6. This accurate information was required because the purity of the product isolated from the subsequent chemistry was sensitive to the charge of reagents.

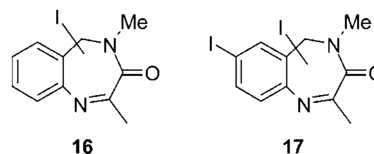


Figure 1.

It was felt that the effort required to do the solution assay for each batch would be considerably less than having to rework a batch due to an incorrect charge.

The aqueous solution of the sodium salt of acid **12** was used directly in the next stage of the procedure, the electrophilic iodination at C-7 using pyridine iodine monochloride complex (PIM).[13] Previous work on Route B[5] had shown that from a range of iodination reagents[14] the PIM complex could be very clean and selective for the iodination at C-7 of acid **12**. The PIM complex is a corrosive stable yellow solid which has only a slight odour of pyridine and was charged to the reaction mixture as a solid.[15] The initial reaction between acid **12** and PIM, although not exothermic, was very rapid. However, as the reagent was added, the pH of the medium fell to ca. pH 5, and both the substrate **12** and product **13** precipitated from solution. Since the PIM reagent was not stable in the reaction environment, complete conversion to product could not be achieved. To achieve complete conversion it was necessary to control the pH of the reaction medium by addition of aqueous 47% w/v NaOH whilst adding the PIM reagent. Running at the optimum pH of 7.0 kept both **12** and **13** in solution. In the lab a Metrohm 718 Stat Titrino was again used to maintain a constant pH, but in the pilot and production plant the pH could be controlled within the range pH 6.5−7.5. Control of pH at this point in the procedure was quality critical. At pH >8.0 very rapid decomposition of PIM occurred, leading to polymeric impurities and incomplete reaction, and at low pH there was the precipitation difficulty. In the event of an incomplete reaction it was never possible to improve the conversion of **12** to **13** by the addition of a further charge of PIM. The optimum charge of the PIM reagent was found to be 1.2 mol equiv with respect to the solution yield of acid **12**. Charges of PIM <0.7 mol equiv led to a low yield of **13** being obtained; this material contained high levels of **12**. The specification for iodo-acid **13** was for levels <2% PAR of acid **12**. With PIM charges within the range 0.7−1.1 mol equiv, the iodo-acid **13** was obtained with satisfactory quality but a diminished yield. At higher charges, >1.3 mol equiv of PIM, levels of uncharacterised oxidised and over-iodinated byproducts **16** and **17** (Figure 1) were obtained at levels >0.2% PAR in the HPLC profile of product **13**. The optimum reaction temperature was found to be 10 °C. At temperatures <5 °C the sodium salt of **12** precipitated from solution. At 20−25 °C incomplete reaction due to the rapid decomposition of PIM was observed. As alluded to, the rate of PIM addition needed to be rapid yet controlled, ideally less than 1 h. In the laboratory it was possible to add the

(13) Muathen, H. A. *J. Chem. Res. (S)* **1994**, 405.
(14) Merkushev, E. B. *Synthesis* **1988**, 923−937.
(15) The pyridine iodine monochloride complex was not stable as an aqueous solution.

Table 7. **Production plant batch data for acid 13**[a]

batch	weight (kg)	yield (% w/w)	HPLC assay for purity (%)	HPLC profile for **13** (% PAR)	level of impurity **16** (% PAR)	level of impurity **17** (% PAR)	chiral purity (%)
1	118.4	33.2%	99.2	99.5	0.06	0.01	99.94
2	138.2	38.8	99.1	99.3	0.01	0.01	99.93
3	136.9	38.4	99.1	99.5	0.09	0.01	99.92
4	128.3	36.0	98.9	99.6			99.95
5	131.4	36.8	98.3	99.4	0.10		99.95
6	135.0	37.8	99.2	99.5			99.94
7	140.9	39.5	98.4	99.4		0.01	99.95
8	147.7	41.4	99.0	99.4		0.03	ND
9	130.6	36.6	98.6	99.6	0.06		ND
10	141.9	39.8	98.0	99.5	0.06		ND
11	140.2	39.3	98.1	99.6	0.07	0.01	ND
12	126.9	35.6	98.6	99.5	0.08	0.01	ND
13	129.1	36.2	99.1	99.7	0.04		ND

[a] Reactions carried out on 246 kg of ester **9**. ND means not determined.

required few grams portionwise in a minute or two. In the production plant the time required to add a typical charge of 128 kg was 30 min. With the controls on pH and temperature in place, this rate of addition was more than satisfactory, and typically the residual levels of **12** seen in the HPLC in-process check prior to workup were <1% PAR, Table 6.

Once the PIM addition was complete, a reaction period of 30 min at 10 °C was allowed, still maintaining the pH within the range pH 6.5−7.5. After this time the reaction was worked up by the addition of CH_2Cl_2 and the mixture allowed to warm to ambient temperature so that the aqueous and organic layers would separate. The aqueous layer was given a second CH_2Cl_2 wash and then was acidified by using concentrated hydrochloric acid until the pH was within the range 3.5−4.0. The acidification caused acid **13** to crystallise from solution, and it could be isolated in a filter drier. The filtercake was washed with water and then *i*-PrOH and was finally dried under vacuum at 40 °C until the volatiles content was <0.5% w/w.

In the production environment 13 batches of Stage 2 were successfully completed on a 246-kg input of racemic ester **9**. Chiral iodo-acid **13** was isolated on average in 37.5% yield (range 33.2−41.4%, depending upon whether the heel was left in the filter drier); average HPLC assay for purity was 98.7% (range 98.0−99.2%), chiral purity >99.9%, and no single impurities were observed in the HPLC profile at levels >0.1% PAR, Table 7. The specification required for acid **13** was the following: HPLC assay for purity >93%, total impurities by HPLC <3.0% PAR, acid **12** <2.0% PAR, impurity **16** <0.5% PAR, impurity **17** <0.5% PAR, and chiral purity >99.0%.

Stage 2: Racemisation. To ensure that Route C would be a commercially viable route to SB-214857-A it was necessary to devise an efficient one-pot procedure for the recycling of the (*S*)-ester **11** back to the racemic ester **9**. Of course the quality of ester **9** prepared by this method would need to meet the high quality standards of de novo ester prepared by the stage 1 chemistry.[9]

Simple recycling of ester **11** by epimerisation of the potentially labile methine proton at C-2 proved to be very difficult to achieve. Extensive screening of either acidic or basic conditions for the racemisation of ester **11** or *N*-acetyl or *N*-trifluoroacetyl derivatives was undertaken.

Treatment of ester **11** with a range of protic acids (e.g., AcOH, TFA, $MeSO_3H$, HCl, H_2SO_4) or Lewis acids (e.g., $ZnCl_2$, $CuCl_2$, $AlCl_3$, $TiCl_4$, $BF_3{\cdot}OEt_2$, Ti(*i*-PrO)$_4$) in solvents such as CH_2Cl_2 or MeOH resulted in no racemisation.

Likewise a range of alkoxide bases (e.g., NaOMe, LiOMe, *t*-BuOLi, *t*-BuOK) were tried in solvents such as THF, toluene, *t*-BME, DMF, or diphenyl ether. Either slow racemisation or partial hydrolysis to the corresponding (*S*)-acid was observed, typically in the order of 10−30% even under strictly anhydrous conditions. The use of stronger bases such as NaH or LDA tended to lead to decomposition, whereas no reaction was observed with hindered amine bases such as (*i*-Pr)$_2$NEt or DBU.

The breakthrough came with the use of dimethyl carbonate as cosolvent with MeOH and NaOMe as base. The hope was that dimethyl carbonate would act as an in situ means of alkylating the acid back to the methyl ester. Although this type of mechanism was shown not to be operating, racemisation did occur, and the presence of dimethyl carbonate greatly reduced the levels of acid byproduct formed; typically levels <2% were seen. This procedure was then refined, scaled-up, and incorporated into the manufacturing route.

The preferred method used dimethyl carbonate as solvent and sodium methoxide as a solution in methanol as base. Given the high cost of dimethyl carbonate the process was optimised to minimise the quantity of solvent required. The ester **11** was slurried in dimethyl carbonate (5 volumes) at 40 °C, the sodium methoxide solution (1.25 mol equiv) was added, and complete racemisation as determined by chiral HPLC was achieved within 5 h. The level of acid byproduct was <2% if the total water content of the reaction solution prior to the sodium methoxide addition was <0.3% w/w. Any increase in water level gave an increase in hydrolysis. Whilst an increase in temperature led to the formation of polymeric impurities, any decrease in the charge of sodium methoxide resulted in a longer reaction time. Once the racemisation was complete, the reaction was quenched by the addition of glacial acetic acid. The use of a mineral acid such as HCl again led to partial hydrolysis. Maintaining the

Table 8. **Batch data for the racemisation of ester 11 to ester 9 in the production plant**[a]

batch	weight (kg)	yield (% w/w)	HPLC assay for purity (%)	level of acid **12** (% PAR)
1	59.0	65.6	98.5	0.10
2	80.0	88.9	97.9	
3	82.9	92.1	101.4	
4	77.9	86.6	98.0	0.11
5	79.0	87.8	96.6	0.08
6	80.0	88.9	97.5	0.05
7	78.0	86.7	98.3	0.08
8	79.0	87.8	97.3	0.04
9	82.0	91.1	97.8	0.09
10	75.2	83.6	98.8	0.04
11	86.5	96.1	97.8	0.11
12	74.0	82.2	99.8	0.12
13	79.8	88.7	99.8	0.11
14	75.0	83.3	99.9	0.07
15	86.0	95.9	100.3	0.11
16	88.0	97.8	99.1	0.07

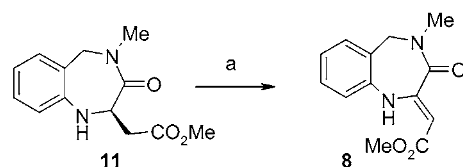
[a] Reactions carried out on 90 kg of ester **11**.

pH slightly basic at the end of the quench was essential to prevent any acid which had formed from coprecipitating with the ester **9** during the isolation. To isolate ester **9**, the dimethyl carbonate was removed by vacuum distillation with water added (5 volumes followed by 2 volumes), and then *i*-PrOH (2 volumes) was added as a cosolvent to solubilise a series of unknown minor byproducts (all <0.1%). The product **9** was isolated in a centrifuge, and the cake was washed with water (2 × 2 volumes) and then dried under vacuum in an agitated pan drier at 50 °C. The procedure was robust with respect to the levels of acid **12** found in the feedstock **11**. As noted above the typical level was 1%; at levels up to 8% the racemic ester could still be isolated in acceptable yield and quality. Only if the level of acid **12** were to exceed 15% would the procedure fail to deliver acceptable material. In the production environment 16 batches of stage 2 racemisation were successfully completed, using a 90-kg input of ester **11**. Racemic ester **9** was isolated on average in 88.0% yield (range 65.6−97.8%, depending upon whether the heel was left in the drier), average HPLC assay for purity was 98.7% (range 96.6−101.4%), and the only impurity in the HPLC profile was acid **12** typically at 0.1% PAR, Table 8. Material of this quality was more than acceptable as a feedstock for the resolution procedure; the specification was the following: HPLC assay for purity >95%, total impurities by HPLC <5.0% PAR, acid **12** <1.0% PAR.

In view of the initial difficulties experienced trying to recycle ester **11** by racemisation, alternative means of recycling back into the production route were investigated. Ester **11** could be oxidised to unsaturated ester **8** by treatment with aqueous *tert*-butyl hydrogen peroxide in the presence of a phase-transfer reagent and copper(II) chloride as catalyst in 70% yield (Scheme 4).[16] However, the quality of material produced did not meet specification, and the use of a peroxide

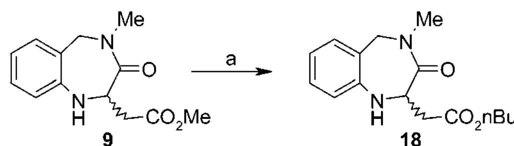
(16) Feldberg, L.; Sasson, Y. *Tetrahedron Lett.* **1996**, *37*, 2063−2066.

Scheme 4 [a]



[a] Reagents and conditions a) *t*-BuOOH, $CuCl_2$, tetra *n*-butylammonium hydrogen sulphate, CH_2Cl_2, H_2O, 20 °C, 70%.

Scheme 5 [a]



[a] Reagents and conditions a) Ti(*i*-PrO)$_4$, *n*-BuOH, 100 °C, 91%.

precluded this method from consideration as a candidate for scale-up.

Stage 2: Alternative Separation. In Route C the racemic ester **9** is resolved using Novozym 435, a reagent that was only available from a single source.[11] To avoid the commercial supply becoming compromised a second means of resolving the enantiomers of ester **9** was required. Chromatography on a chiral stationary phase was investigated. Although analytical methods could be found for the separation of the enantiomers of ester **9**, these methods were not applicable to a preparative scale due to the low solubility of ester **9** in the mobile phase. To improve the solubility, the methyl ester was transesterified using *n*-butanol and Ti(*i*-PrO)$_4$ [17] to the *n*-butyl ester **18** in 91% yield (Scheme 5). The solubility of **18** in the mobile phase was greatly improved, and details of a continuous process for the separation of enantiomers of **18** are disclosed elsewhere.[18] On a small scale the butyl ester **18** could be hydrolyzed to the acid **12** without any racemisation by using aqueous sodium hydroxide. This procedure was never scaled up.

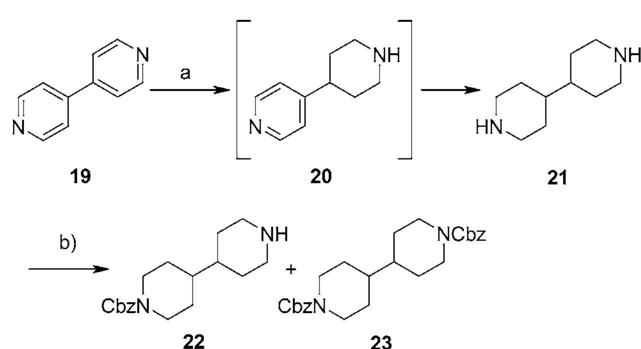
Stage 3: Palladium-Catalysed Aminocarbonylation. Stage 3 of the manufacturing route for SB-214857-A was the palladium-catalysed aminocarbonylation of aryl iodide **13** with a suitable 4,4′-bipiperidine equivalent.

The direct use of 4,4′-bipiperidine **21** in a carbonylation[5] with aryl iodide **13** led to the formation of product **15** in ca. 50% solution yield along with multiple unidentified byproducts. A satisfactory isolation procedure for **15** could not be developed; thus, this direct approach was discontinued.

In Route B (Scheme 2) *N*-Cbz 4,4′-bipiperidine **22** was used as the 4,4′-bipiperidine equivalent. The weakness in this approach was that the preparation of **22** from commercially available 4,4′-bipiperidine **21** was achieved in only 42% yield. A statistical mix of starting material, product, and bis-protected material **23** was always obtained (Scheme 6). 4,4′-Bipiperidine **21** can be prepared by the reduction of 4,4′-bipyridine **19** over platinum oxide.[19] The process is effectively stepwise via the intermediate 4,4′-pyridylpiperi-

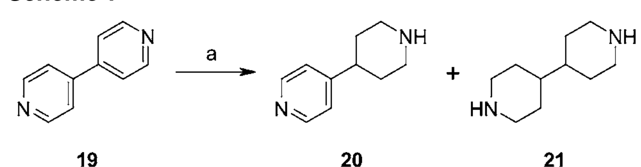
(17) Seebach, D.; Hungerbühler, E.; Naef, R.; Schnurrenberger, P.; Weidmann, B.; Züger, M. *Synthesis* **1982**, 138−141.
(18) Ludemann-Hombouger, O.; Pigorini, G.; Nicoud, R. M.; Ross, D. S.; Terfloth, G. *J. Chromatogr., A* **2002**, *947*, 59−68.
(19) Smith, C. R. *J. Am. Chem. Soc.* **1928**, *50*, 1936−1938.

Scheme 6 [a]



[a] Reagents and conditions a) for example see ref 19 b) Cbz-Cl, NaOH, EtOH, H_2O, **22**, 42%.

Scheme 7 [a]



[a] Reagents and conditions a) 10% Pd/C, H_2, 0.09 equiv citric acid, H_2O, 100 °C, 60 psi, 62%.

Table 9. **Reduction of 19 to 20 in the presence of different acid promotors**[a]

acid	equiv	% **21** formed
methanesulfonic	0.6	2.4
p-toluenesulfonic	0.6	2.6
phosphoric	0.2	3.9
trifluoroacetic	0.3	2.4
oxalic	0.25	2.5
tartaric	0.25	2.9
citric	0.17	2.7
sulfuric	0.25	2.9
hydrochloric	0.6	4.6
acetic	0.6	3.3

[a] Reactions carried out using a 5% Pd/5% Pt on carbon catalyst, 60 psi H_2, 75 °C until the level of **20** by HPLC < 5% PAR.

dine **20**, and this sparked the realisation that if 4,4′-pyridylpiperidine **20** could be isolated then perhaps it could act as an atom-efficient surrogate for the protected 4,4′-bipiperidine subunit.

The partial hydrogenation of 4,4′-bipyridine **19** to give pyridylpiperidine **20** has been reported (Scheme 7).[20] Two sets of conditions were disclosed, either Raney nickel in acetic acid at high pressure or platinum in hydrochloric acid at more moderate pressures. In our hands when we repeated the platinum procedure, we suffered from considerable over-reduction to bipiperidine **21**; levels upward of 20% were obtained. This prompted a screen of catalysts and acid promoters to try to optimise the process. Use of a mixed platinum/palladium on charcoal catalyst (5% Pt/5% Pd) decreased the level of over-reduction to **21** to 8% PAR by HPLC analysis. A range of different acids at substoichiometric levels (0.1−0.6 mol equiv) were tried, Table 9. The best results in terms of reaction rate (data not shown), cost,

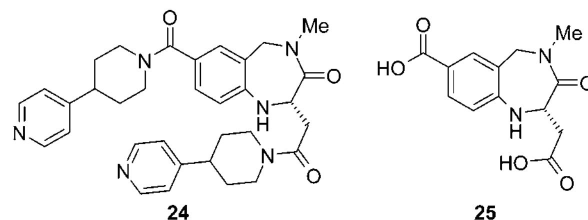


Figure 2.

ease of handling, and low level of **21** were achieved using citric acid (0.17 equiv). By using 0.09 equiv of citric acid as promoter it was possible to use either 5 or 10% palladium on charcoal as catalyst. Under hydrogenation conditions of 100 °C and 60 psi hydrogen pressure, a solution yield of 95% for **20** could be achieved, <2% PAR over reduction to **21**. Once the reduction was complete, the catalyst was removed by filtration and the product extracted into a mixture of *i*-PrOAc and *s*-BuOH. After a solvent swap to cyclohexane, the product **20** could be isolated by crystallisation in 62% yield; the remainder of the product remained in the aqueous layer. By using this procedure the specified quality of **20** could be readily obtained: <0.5% PAR **19**, <0.5% PAR **21** by HPLC analysis. The large-scale preparation of 4,4′-pyridylpiperidine **20** was not carried out in-house.

When the palladium-catalysed carbonylation conditions of $PdCl_2(PPh_3)_2$, 10 psi CO, 3 equiv of $(i\text{-Pr})_2NEt$, and *N*-methyl-2-pyrrolidinone as solvent, used in Route B[5] were applied to the reaction of aryl iodide **13** and pyridyl piperidine **20**, the desired product **14** was formed with an HPLC solution yield of 65%. From this promising starting position the process was refined and optimised with respect to solution yield, efficiency of workup, and ease of isolation of the product **14**.

The same catalyst system $PdCl_2(PPh_3)_2$ was retained at a loading of 0.02 mol equiv, 1.2 mol equiv of pyridylpiperidine **20** was employed, the pressure of carbon monoxide was increased slightly to 15 psi, and the solvent was switched to anisole (8 volumes). An improvement in reaction profile could be achieved if the amine base was changed from the tertiary amine $(i\text{-Pr})_2NEt$ to a secondary amine. Either diisopropylamine or dicyclohexylamine (2.6 mol equiv) could be employed, with similar results obtained for each. It is interesting and unexpected that no side products resulting from amino carbonylation of either of these secondary amines were detected. From a workup perspective it was decided to use dicyclohexylamine since its hydrogen iodide salt is insoluble and mostly removed by filtration. Under the optimised reaction conditions amide **14** was formed in 90% PAR by HPLC; the only significant impurity formed during the process was amide **24**, 3.9% PAR. Impurity **24**, Figure 2, is probably formed by the reaction of an intermediate anhydride with pyridylpiperidine **20**.[21]

Having established suitable reaction conditions it remained for us to either isolate amide **14** or to hydrogenate directly to amino acid **15**. The major obstacle to direct hydrogenation was the requirement for complete removal of iodide, since this acted as an extremely potent hydrogenation catalyst

(20) (a) Allport, D. C. Eur. Pat. Appl. 1130551, 1968. (b) Allport, D. C. Eur. Pat. Appl. 1129511, 1968.

(21) Grushin, V. V.; Alper, H. *J. Am. Chem. Soc.* **1995**, *117*, 4305−4315.

Table 10. **Pilot-plant batch data for stage 3, the preparation of amide 14**[a]

	batch 1	batch 2	batch 3
input of weight **13**	77.3 kg	69.8 kg	71.8 kg
wet weight of **14**	114.5 kg	90.0 kg	117.3 kg
assay	59.8% w/w	71.85%	70.3% w/w
water content	35.6% w/w	25.9% w/w	25.5%
weight of **14** at 100% purity	68.5 kg	64.7 kg	82.5 kg
yield	78%	79%	100%
HPLC profile for **14**	97.30% PAR	96.91% PAR	97.54% PAR
level of **24**	2.1%	1.4%	1.6%
level of **25**	0.4%	0.5%	0.4%

poison. It was found that excess iodide could be oxidised to iodine by using hydrogen peroxide at pH 5.0. The iodine could then be removed by extraction with an organic solvent. The excess hydrogen peroxide then needed to be reduced with the use of either palladium on carbon or the enzyme catalase on a polymer support.[22] Although workable procedures were developed, the overall process suffered from poor volume efficiency and inconsistent rates of hydrogenation of **14**.

The preferred option was to isolate amide **14** and wash out the iodide using water. To this end, once the carbonylation reaction was complete, water was added and the solution filtered. The product was extracted into aqueous sodium hydroxide, pH 10, and the aqueous solution was concentrated in volume by distillation. The pH of the solution was adjusted to pH 5.0 by the addition of aqueous citric acid. The temperature at which this pH adjustment takes place is crucial to the quality of the product obtained. If the temperature >60 °C then amide **14** crystallises as a monohydrate. The monohydrate proved slow to filter; 100 kg of product in 300 L of water took 18 h, and washing the filter cake with water to remove iodide was inefficient. However, if the pH adjustment took place at temperature <40 °C then amide **14** crystallised as a dihydrate. By contrast the dihydrate filtered well, 100 kg of product in 300 L of water took 1 h, and the water washes were rapid and readily removed the iodide to acceptable levels. The mono- and dihydrate forms of **14** were characterised by Karl Fischer titrimetry and X-ray powder diffraction, the crystal habits and particle sizes were similar, and the differences in filtration rates are empirical observations. The isolated amide **14** was not rigorously dried but used as a wet cake in the subsequent hydrogenation.

Three batches of these optimised reaction and workup conditions were run in the pilot-plant environment, Table 10. The yield obtained varied (77−100%), depending upon whether a heel was left in the filter drier. The average yield over the three batches was 86%. The main impurities present in amide **14** were bis-amide **24** and acid **25**. For all batches the chiral purity for **14** was >99.9%, and the levels of residual iodide were 0.1% w/w.

Stage 4: Hydrogenation. Early development of the hydrogenation of the pyridine ring of **14** to the fully saturated piperidine unit **15** was hindered by the presence of residual iodide. Initial experiments required high loadings of expensive platinum catalysts and suffered from variably long reaction times. Once the level of residual iodide in **14** could be reliably controlled to approximately 0.1% w/w, the hydrogenation became more robust. A full screen of different types of palladium and platinum catalysts was undertaken. From this screen it was found that Precious Metals Corporation Type 3310 catalyst gave the highest activity. The catalyst was 10% palladium on charcoal (shell, unreduced) and required a 30% w/w charge of wet catalyst with respect to dry substrate; the hydrogenation could be performed at a pressure of 60 psi of hydrogen at 75 °C. At the end of the reaction, typical reaction time 4 h, the reaction mixture was filtered while still hot, and the solvent (1:1 *i*-PrOH: H_2O) was partially removed by vacuum distillation. Upon cooling the zwitterionic amino acid **15** was isolated as the hexahydrate.

Table 11. **Pilot-plant batch data for stage 4, the preparation of amino acid 15**

	batch 1	batch 2	batch 3
input of weight **14**	68.5 kg	64.7 kg	82.5 kg
wet weight of **15**	78.1 kg	64.5 kg	96.8 kg
assay	81.2% w/w	79.5% w/w	77.8% w/w
water content	18.5% w/w	21.0% w/w	21.4% w/w
weight of **15** at 100% purity	63.4 kg	51.3 kg	75.3 kg
yield	92%	78%	90%
HPLC profile for **15**	99.73% PAR	99.81% PAR	99.74% PAR
level of **26**	0.09%	0.08%	0.11%

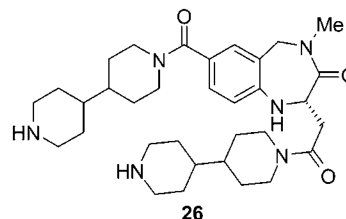


Figure 3.

Due to the lack of capacity for pressure hydrogenation reaction within our pilot plant each batch of amide **14** was divided into three portions for the hydrogenation and then recombined for crystallisation and isolation, Table 11. The yield obtained varied (78−92%), depending upon whether a heel was left in the filter, the average yield of **15** over three batches of **14** was 87%. Typical HPLC profile for amino acid **15** was 99.7% PAR, the only significant impurity present was bis-amide **26** (Figure 3) at 0.1% PAR, the chiral purity for **15** was >99.9%, and the level of residual palladium was 1 ppm.

Stage 5: Salt Formation and Crystallisation. The final stage in the preparation of the drug substance was conversion of the zwitterionic amino acid **15** into the hydrochloride salt **5** and effecting a crystallisation. The first procedure developed gave a 75% yield, the zwitterionic amino acid 15 was dissolved in a H_2O/absolute ethanol solvent mixture, and concentrated hydrochloric acid was added as the acid source. This procedure required large volumes (30 volumes), and the occasionally the product would be very slow to crystallise. In the next procedure developed, CH_2Cl_2 was added to help solubilise the salt and give better control over the crystallisation. This modification improved the yield to 85%, but a problem remained in that the drug substance degraded

(22) Alston, M.; Willetts, A.; Wells, A. *Org. Process Res. Dev.* **2002**, *6*, 505−508.

Table 12. **Pilot plant data for stage 5, preparation of the SB-214857-A 5**

	batch 1	batch 2	batch 3
input weight of **15**	63.4 kg	51.3 kg	75.3 kg
weight of **5**	64.3 kg	48.5 kg	72.7 kg
yield	93%	87%	89%
assay	100.06% w/w	100.19% w/w	100.46% w/w
chiral purity	100%	100%	100%
HPLC profile for **5**	99.86% PAR	99.88% PAR	99.86% PAR
level of **26**	0.06%	0.07%	0.08%

at low pH, something that was difficult to control with the use of concentrated hydrochloric acid. Pyridine hydrochloride was introduced in place of concentrated hydrochloric acid, and this gave much better control of the pH and minimised degradation. The final modification before the procedure was finalised prior to transfer to the manufacturing environment was to replace absolute ethanol with industrial methylated spirit (IMS). In the final procedure the zwitterionic hexahydrate amino acid **15** was dissolved in IMS and dichloromethane. The pyridine hydrochloride was dissolved in aqueous IMS and added to the solution of **15** to form the salt. To crystallise the product, CH_2Cl_2 and ethanol were removed by distillation at atmospheric pressure. Key to getting a good recovery was to control the level of water before distillation; the optimal range was 6.3−6.8% w/w. If the water content of the solution of **15** in IMS/CH_2Cl_2 was >12%, w/w then a new form of **15** is precipitated, and this would have consequences for the clarification filtration. This new form was identified as the tetrahydrate by Karl Fischer titrimetry and is significantly less soluble than the hexahydrated form; the tetrahydrate form of **15** also exhibited different characteristics by X-ray powder diffraction. However, a suspension of tetrahydrate in IMS/CH_2Cl_2 could still be taken through to hydrochloride salt **5** by the usual procedure.

When scaled-up to approximately 60 kg in the pilot plant, this procedure gave an average yield of 90%, Table 12. The only significant impurity was bis-amide **26**. The specification required for the drug substance included the following: HPLC assay for purity as the HCl salt was 98.0−102.0% w/w, chiral purity was >99.5%, total impurities by HPLC were <1.0% PAR, and the specific level for amide **26** was <0.2% PAR. As can been seen all three batches exceeded the specification.

Hydrochloride salt **5** was obtained as a nonhydrated, nonsolvated crystalline solid with primary particles about 40 μm in size which was acceptable for formulation purposes.

Considerable experimentation was performed by searching for different solid-state forms of the hydrochloride salt **5**, such as the following: amorphous material was prepared by lyophilisation and then slurried in a range of solvents at ambient or higher temperatures, or crystalline **5** was slurried in a range of solvents at ambient or higher temperatures, or **5** was recrystallised from various pure solvents or mixed-solvent systems, or finally **5** was dissolved in water and then precipitated by the addition of an anti solvent. In all cases only one solid-state form of hydrochloride salt **5** was ever produced.

Summary

In summary a reliable and efficient synthesis of lotrafiban **5** has been developed and demonstrated on scale. For stage 2 a successful validation campaign was run on full scale at the site of primary manufacture. For stages 3−5 successful pilot campaigns were carried out in the R&D pilot plant and at the primary manufacturing site. The chemistry highlights were the enzyme-catalysed enantioselective hydrolysis of racemic ester **9** to chiral acid **12** and the subsequent regioselective iodination to give iodo-acid **13** in 37.5% yield. The ester byproduct **11** from the enzyme hydrolysis reaction could be isolated in 43.2% yield and readily recycled back to the racemate **9** in 88.0% yield. The yield of iodo-acid **13** was 60.5%, based on recovered ester **9**. A palladium-catalysed amino carbonylation of iodo-acid **13** with 4,4′-pyridylpiperidine **20** gave late stage intermediate **14** in 86% yield. In this reaction 4,4′-pyridylpiperidine **20** was used as an atom efficient surrogate for the 4,4′-bipiperidine subunit found in the drug substance. Finally palladium-catalysed hydrogenation of the pyridine ring of **14** occurred in 87% yield and subsequent hydrochloride salt formation using pyridine hydrochloride gave the target drug substance **5** in 90% yield. The overall yield for the final four stages of the process was 41%.

Experimental Section

Stage 2: (2*R*)-2,3,4,5-Tetrahydro-4-methyl-3-oxo-1*H*-1,4-benzodiazepine-2-acetic Acid Methyl Ester (11), (2*S*)-2,3,4,5-Tetrahydro-4-methyl-3-oxo-1*H*-1,4-benzodiazepine-2-acetic Acid (12), (2*S*)-2,3,4,5-Tetrahydro-7-iodo-4-methyl-3-oxo-1*H*-1,4-benzodiazepine-2-acetic Acid (13). To a vessel were charged fresh Novozym 435 (74 kg), ester **9** (246 kg, 992 mol), and 88% aqueous *t*-BuOH (2460 L). The agitator was started, and the reactor contents were heated to 50 °C. The pH was maintained at pH 7.0 by the automated addition of 1.5 M ammonia in 88% *t*-BuOH (typically 300 L). Once the reaction was complete (as measured by HPLC: acid **12** >47% PAR, time 10 h), the reaction mixture was transferred to another vessel by filtration through the 80 μm mesh in the base of the vessel. The Novozym 435 was washed with 88% *t*-BuOH (345 L) and remained in the vessel ready for a subsequent batch. To the *t*-BuOH solution was added aqueous 8% w/v $NaHCO_3$ solution (535 L, 509 mol), and the pH was adjusted to pH 7.5 by the careful addition of 1 M aqueous hydrochloric acid (typically 250 L, 250 mol). The volume of the solution was concentrated to 980 L by vacuum distillation while maintaining the internal temperature at <50 °C. Water (790 L) was added while maintaining the internal temperature at >40 °C; the solution was then cooled to 20 °C and stirred for 1 h. The (*S*)-ester **11** was isolated in a centrifuge and the cake washed with water (395 L). The wet cake was transferred to an agitated pan drier and dried under vacuum at 50 °C until the water content by Karl Fischer titrimetry <0.2% w/w. The product **11** was isolated as a white solid (106 kg, 427 mol, 43% average yield): mp 132−134 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (td, J = 7.7, 1.4 Hz, 1 H), 6.93 (dd, J = 7.5, 1.1 Hz, 1 H), 6.67 (td, J = 7.4, 1.0 Hz, 1 H), 6.57 (dd, J = 8.1, 0.9 Hz, 1 H), 5.42 (d, J = 16.3 Hz, 1 H), 5.00 (q, J =

6.1 Hz, 1 H), 4.09 (d, J = 5.4 Hz, 1 H), 3.73 (s, 3 H), 3.72 (d, J = 16.4 Hz, 1 H), 3.07 (s, 3 H), 2.98 (dd, J = 16.0, 6.8 Hz, 1 H), 2.65 (dd, J = 16.0, 6.5 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 169.2, 145.1, 129.5, 129.0, 119.8, 118.2, 117.5, 53.3, 51.9, 51.8, 35.9, 34.6; IR (neat, cm^{-1}) 1733, 1639; LRMS (CI +ve) m/z 249 (M^+ + H).

The filtrate was transferred to another vessel and washed with CH_2Cl_2 (394 L). The CH_2Cl_2 was discarded, and the aqueous solution containing acid **12** was transferred to another vessel and the mass flow measured (1920 kg). An HPLC solution yield for acid **12** was determined (45%). This solution was used in the subsequent chemistry.

A sample of the intermediate acid **12** could be isolated for characterisation purposes by acidification of the aqueous solution with hydrochloric acid, filtration of the resulting precipitate, and then washing the filter cake with water and *i*-PrOH. Acid **12** was obtained as a white solid: mp 148−150 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 6.97 (t, J = 7.5 Hz, 1 H), 6.91 (d, J = 6.9 Hz, 1 H), 6.56−6.52 (m, 2 H), 5.51 (d, J = 16.4 Hz, 1 H), 5.06 (dd, J = 8.9, 5.1 Hz, 1 H), 3.80 (d, J = 16.5 Hz, 1 H), 3.01 (s, 3 H), 2.89 (dd, J = 16.7, 9.0 Hz, 1 H), 2.60 (dd, J = 16.7, 5.1 Hz, 1 H); ^{13}C NMR (100 MHz, CD_3OD) δ 175.2, 173.0, 147.6, 131.1, 130.3, 120.7, 118.8, 118.3, 54.7, 52.6, 36.9, 35.0; IR (neat, cm^{-1}) 1713, 1636; LRMS (CI +ve) m/z 235 (M^+ + H).

For the bulk solution the pH was adjusted to pH 7.0 by addition of 47% w/v aqueous NaOH (1 kg), and the internal temperature was cooled to 10 °C. Pyridine iodine monochloride (128 kg, 532 mol, 1.2 equiv) was added over 30 min while the pH was maintained within the range pH 6.5−7.5 by the addition of 47% w/v aqueous NaOH (typically 50 kg) and the temperature maintained at 10 °C. Once the addition was complete, the reaction mixture was stirred at 10 °C for 30 min and the pH maintained at pH 7.0 by the addition of 47% w/v aqueous NaOH (5 kg). CH_2Cl_2 (984 L) was added, the solution was warmed to 20 °C and the layers were allowed to settle. The organic layer was discarded and the aqueous layer washed with further CH_2Cl_2 (984 L). The organic layer was discarded, and the aqueous layer was acidified to the range pH 3.5−4.0 by the addition of concentrated hydrochloric acid (40 kg). The resulting slurry was stirred at 20 °C before being transferred to a Hastelloy filter drier and filtered. The filter cake was washed by displacement with water (670 L) then *i*-PrOH (670 L) and dried under vacuum at 50 °C until the level of volatile materials was <0.5% w/w. Iodo-acid **13** was isolated as a light tan solid (134 kg, 372 mol, 38% average yield): mp 205 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.18−7.14 (m, 2 H), 6.29 (d, J = 8.5 Hz, 1 H), 5.40 (d, J = 16.6 Hz, 1 H), 5.00 (dd, J = 8.9, 5.1 Hz, 1 H), 3.70 (d, J = 16.7 Hz, 1 H), 2.93 (s, 3 H), 2.81 (dd, J = 16.7, 9.0 Hz, 1 H), 2.51 (dd, J = 16.7, 5.1 Hz, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3, 169.7, 146.4, 137.7, 136.9, 122.2, 118.7, 76.7, 51.4, 50.3, 35.7, 34.0; IR (neat, cm^{-1}) 1709, 1630; LRMS (CI +ve) m/z 361 (M^+ + H).

HPLC in process-control method: Waters Symmetry C18 column 150 mm × 4.6 mm, 5 μm. Eluent A: 0.1% TFA in water. Eluent B: 0.1% TFA in acetonitrile. Gradient 25%-75% B over 10 min, run time 10 min. Flow rate 1 mL/min. Detector at 254 nm. Injection volume 20 μL. Sample preparation 0.1 mg/mL. Typical retention times: acid **12**, 4.4 min; esters **9**/**12**, 6.1 min; iodo-acid **13**, 7.3 min.

Chiral HPLC for acid **12**: Chiralcel OD-R column 250 mm × 4.6 mm. Eluent A 0.1 M $NaClO_4$ adjusted to pH 2 by $HClO_4$. Eluent B acetonitrile. Isocratic 20% B. Run time 20 min. Flow rate 0.6 mL/min. Detector at 250 nm. Column temperature 40 °C. Injection volume 20 μL. Sample preparation 0.5 mg/mL. Typical retention times: (*R*)-enantiomer, 11.1 min; (*S*)-enantiomer, 12.3 min.

Stage 2 Racemisation: (2*RS*)-2,3,4,5-Tetrahydro-4-methyl-3-oxo-1*H*-1,4-benzodiazepine-2-acetic Acid Methyl Ester (9). To a vessel were charged ester **11** (90 kg, 363 mol) and dimethyl carbonate (450 L), and the contents were heated to 40 °C; a sample, taken to ensure water content by Karl Fischer titrimetry, was <0.3% w/w; 30% w/v sodium methoxide in methanol (77 L) was added, and the contents were stirred at 40 °C for 5 h. The reaction was sampled for chiral HPLC analysis: reaction endpoint was (*S*)-enantiomer >48%. Once the reaction was deemed complete, the reaction mixture was transferred to another vessel and the previous vessel rinsed with dimethyl carbonate (30 L). Glacial acetic acid (14.5 L) was added and the pH measured to ensure it was within the range 8.5−9.5. Water (450 L) was added and the mixture concentrated by vacuum distillation while maintaining the internal temperature <45 °C until 310 L of distillate had been collected. Water (180 L) was added and the mixture concentrated by vacuum distillation while maintaining the internal temperature <45 °C until a further 310 L of distillate had been collected. While maintaining the internal temperature within the range 38−42 °C *i*-PrOH (180 L) was added, and the contents were transferred to another vessel; the previous vessel was rinsed with *i*-PrOH (74 L). The combined solution was stirred at 40 °C for 1 h and then at 20 °C for 4 h. The ester **9** was isolated in a centrifuge and the cake washed with water (2 × 180 L). The wet cake was transferred to an agitated pan drier and dried under vacuum at 50 °C until the water content by Karl Fischer titrimetry was <0.5% w/w. The product **9** was obtained as a white solid (79.2 kg, 319 mol, 88% average yield). Data were the same as those for ester **11** except mp 174−176 °C and IR (neat, cm^{-1}) 1728, 1650.

HPLC in process-control method: Kromasil KR-100-5-CHI (dimethyl DADT) column (25 cm × 0.46 cm). Isocratic eluent 98% *n*-hexanes, 2% *i*-PrOH. Run time 30 min. Flow rate 0.7 mL/min. Detection 215 nm. Typical retention times: (*S*)-enantiomer, 24.8 min; (*R*)-enantiomer **11**, 27.6 min.

(2*RS*)-2,3,4,5-Tetrahydro-4-methyl-3-oxo-1*H*-1,4-benzodiazepine-2-acetic Acid *n*-Butyl Ester (18). To a vessel were charged ester **9** (10 kg, 40.32 mol), *n*-butanol (76 kg), and titanium (IV) isopropoxide (0.50 kg, 1.76 mol) in *n*-butanol (5 kg). The reaction mixture was heated to 100 °C and the reaction monitored by HPLC. After 36 h the reaction was deemed complete when the level of ester **9** was <3.0% PAR by HPLC, and the reaction mixture was cooled to 5 °C and then stirred at 5 °C for 6 h. The ester **18** that

had crystallised was isolated in a centrifuge and the cake washed with cold *n*-BuOH (16.2 kg) and then cold *n*-hexanes (13.2 kg). The filtrate and wash were combined and returned to the vessel along with water (2.5 kg) and Celite (2.5 kg), and the solution was stirred at 25 °C for 2 h. The solution was filtered into another vessel and then concentrated by vacuum distillation until the residual volume was 3 L. The solution was cooled to 25 °C, and then petroleum ether (bp 80−100 °C, 17.25 kg) was added. The solution was cooled to 5 °C and then stirred at 5 °C for 2 h. The second crop of ester **18** was isolated in the same centrifuge, and the cake was washed with cold *n*-hexanes (3.3 kg). The combined wet ester **18** was dried in a tray drier under vacuum at 50 °C until the level of volatile materials was <0.5% w/w. The product **18** was isolated as a white solid (10.3 kg, 36.69 mol, 91%): mp 107−108 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (t, J = 7.6 Hz, 1 H), 6.93 (d, J = 7.4 Hz, 1 H), 6.67 (t, J = 7.4 Hz, 1 H), 6.56 (d, J = 8.0 Hz, 1 H), 5.42 (d, J = 16.3 Hz, 1 H), 5.00 (q, J = 6.3 Hz, 1 H), 4.15−4.11 (m, 2 H), 4.07 (d, J = 5.5 Hz, 1 H), 3.73 (d, J = 16.4 Hz, 1 H), 3.08 (s, 3 H), 2.97 (dd, J = 16.0, 6.6 Hz, 1 H), 2.64 (dd, J = 16.0, 6.7 Hz, 1 H), 1.67−1.60 (m, 2 H), 1.39 (sext, J = 7.5 Hz, 2 H), 0.94 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 169.7, 145.2, 129.6, 129.1, 119.8, 118.3, 117.5, 64.8, 53.4, 51.8, 36.1, 34.7, 30.5, 19.1, 13.7; IR (neat, cm^{-1}) 1724, 1644; LRMS (CI +ve) m/z 291 (M^+ + H).

HPLC in process-control method: Kromasil KR-100-5-CHI (Dimethyl DADT) column (25 cm × 0.46 cm). Isocratic eluent 98% *n*-hexanes, 2% *i*-PrOH. Run time 30 min. Flow rate 1.0 mL/min. Detection 215 nm. Typical retention times; **18**, 10.9 and 11.5 min; **9**, 15.2 and 16.7 min.

4,4′-Pyridylpiperidine (20). To a suitable pressure vessel were charged 4,4′-bipyridine (50.0 g, 320 mmol), citric acid monohydrate (6.15 g, 29 mmol), 10% palladium on charcoal (6.60 g, Precious Metals Corporation type 1910 lot 12194), and water (200 mL). The vessel was purged with nitrogen then purged with hydrogen and pressurised to 60 psi with hydrogen. The stirrer was started and the heating jacket set to 100 °C. After 6.5 h the reaction was determined to be complete: HPLC analysis showed the ratio of **20**:**19** = 87.5: 12.5 (which is equivalent to 96:4 after adjusting for relative absorbance). The reaction mixture was allowed to cool and then depressurised and purged with nitrogen. The palladium on charcoal was removed by filtration through Celite, and the filtercake was washed with water (50 mL). The aqueous phase was extracted twice with a mixture of *i*-PrOAc/*s*-BuOH (9:1 v/v, 2 × 250 mL). The combined organic extracts were concentrated by distillation at atmospheric pressure until 400 mL of distillate had been collected. Cyclohexane (400 mL) was added, and concentration by distillation at atmospheric pressure was continued until a further 300 mL of distillate had been collected. Upon cooling to ambient temperature the product **20** crystallised and was isolated by filtration as a white solid (31.5 g, 198 mmol, 62% yield): mp 77−78 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.45 (d, J = 6 Hz, 2 H), 7.36 (d, J = 6 Hz, 2 H), 3.17 (d, J = 12.6 Hz, 2 H), 2.81−2.72 (m, 3 H), 1.87 (d, J = 13.1 Hz, 2 H), 1.68 (qd, J = 12.5, 3.8 Hz, 2 H); ^{13}C NMR (100 MHz, CD_3OD) δ 156.6, 148.6, 122.6, 45.6, 41.6, 32.3; IR (neat, cm^{-1}) 3285, 2935, 1597, 1408; LRMS (CI +ve) m/z 163 (M^+ + H). HPLC in process-control method; Supelcosil ABZ column 75 mm × 4.6 mm. Eluent A 50 mM heptanesulfonic acid + 100 mM sodium perchlorate + 0.1% TFA in water. Eluent B acetonitrile. Isocratic 15% B. Run time 2 min. Flow rate 2.0 mL/min. Detector at 256 nm. Injection volume 10 μL. Sample preparation 0.5 mg/mL. Typical retention times: **19** 1.3 min; **20** 1.6 min.

Stage 3 (2*S*)-2,3,4,5-Tetrahydro-4-methyl-3-oxo-7-[[4-(4-pyridinyl)-1-piperidinyl]carbonyl]-1*H*-1,4-benzodiazepine-2-acetic Acid Dihydrate (14). To a glass-lined reactor were charged anisole (601.2 kg), 4,4′-pyridylpiperidine **20** (40.1 kg, at 98% purity, 242 mol), dicyclohexylamine (195.2 kg at 99.7% purity, 537 mol), palladium (II) chloride bis-triphenylphosphine (3.0 kg, 4.27 mol), and aryl iodide **13** (77.3 kg, at 98.9% purity, 209 mol). The reactor was purged three times with nitrogen then three times with carbon monoxide. On the final purge the reactor was pressurised to 15 psi, and the reaction mixture was heated to 97 °C over 57 min. The reaction mixture was stirred at 97−109 °C, maintaining the pressure at 15 psi. A sample was taken after 1 h 35 min and the reaction deemed complete (aryl iodide **13** <1% PAR). The excess carbon monoxide was vented and the reaction mixture cooled to 64 °C. Demineralised water (376 L) was charged and the mixture cooled to 35 °C. The mixture was then filtered through a bed of Celite (5.0 kg). The Celite bed was washed with demineralised water (226 L), and the liquor and water wash were combined. The mixture was stirred, and the layers were allowed to separate. The organic phase was discarded. Ethyl acetate (338 kg) was added, and the pH of the aqueous phase was adjusted to pH 11.0 with 5 M sodium hydroxide solution (49.6 kg). The mixture was stirred, and then the layers were allowed to separate. The organic phase was discarded. Further ethyl acetate (338 kg) was added to the aqueous phase, and the mixture was stirred and then allowed to separate. The organic phase was discarded. The aqueous phase was concentrated by vacuum distillation. Water (350 L) was removed to leave about 376 L of solution, and this mixture was cooled to 23−28 °C. The pH of the aqueous phase was adjusted from pH 8.14 to 5.13 with 33% w/w aqueous citric acid (65 L), and stirred at 23−28 °C for 12 h 30 min. The mixture was then cooled to 5 °C and stirred for 9 h at 0−5 °C. The product was filtered off and washed with demineralised water (2 × 376 L) and partially dried to give amide **14**, as a pale brown solid (114.5 kg at 59.8% assay, water content 35.6% w/w by Karl Fischer titrimetry, 68.5 kg at 100%, 162.1 mol, 78% from **13**). A residual amount of product remained in the filter drier and was incorporated into the subsequent batch. A small sample of the wet cake was dried for characterisation purposes: mp 163−165 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.34 (d, J = 3.0 Hz, 2 H), 7.27 (d, J = 4.2 Hz, 2 H), 7.06 (m, 2 H), 6.52 (d, J = 8.7 Hz, 1 H), 5.48 (d, J = 16.5 Hz, 1 H), 5.09 (m, 1 H), 4.40 (br s, 2 H), 3.81 (d, J = 16.6 Hz, 1 H), 3.00 (m, 2 H), 2.94 (s, 3 H), 2.84 (m, 2 H), 2.54 (dd, J = 16.7, 4.8 Hz, 1 H), 1.83 (m, 2 H), 1.62 (m, 2 H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.6, 171.9, 171.2, 156.3,

149.1, 148.6, 129.8, 128.7, 123.3, 123.2, 118.6, 115.9, 52.9, 51.0, 42.1, 35.4, 33.5, 32.6; IR (neat, cm^{-1}) 1643, 1610, 1449; LRMS (CI +ve) *m/z* 423 ($M^+ + H$); Karl Fischer titrimetry = 8.1% w/w H_2O.

HPLC in process-control method; Waters C18 column 150 mm × 4.6 mm. Eluent A 0.1% TFA in H_2O. Eluent B 0.1% TFA in acetonitrile. Gradient 5% B to 50% B over 20 min. Run time 20 min. Detector at 280 nm. Flow rate 1 mL/min. Sample preparation 0.1 mg/mL. Typical retention times: **14**, 7.7 min; **24**, 8.1 min; **13**, 17.9 min.

Chiral purity of amide **14** was determined by capillary electrophoresis. Capillary 50/60 cm, 75 μm. Detector 200 nm. Applied voltage 30 kV over 0.2 min. Current approximately 140 μA. Temperature 20 °C. Injection 5 s, 35 mBar pressure. Running buffer 100 nM phosphoric acid adjusted to pH 3.0 (4 M LiOH), 15 nm methyl-β-cyclodextrin (MW = 1350), 0.05% hydroxypropylmethylcellulose. Sample preparation 0.5 mg/mL in H_2O. Typical retention times: (*R*)-enantiomer, 26.1 min; (*S*)-enantiomer, 26.6 min.

Stage 4: (2*S*)-7-([4,4′-Bipiperidin]-1-ylcarbonyl)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1*H*-1,4-benzodiazepine-2-acetic Acid Hexahydrate (15). To a suitable pressure hydrogenation vessel were charged wet amide **14** (39.1 kg at 70.3% purity, 27.5 kg at 100%, 65.1 mol), 10% palladium on charcoal (Precious Metals Corporation Type 3310, 50% water wet, 8.3 kg), *i*-PrOH (66 kg), and demineralised water (83 L), and the reactor was purged with nitrogen and then hydrogen. The reactor was then pressurised to 60 psi with hydrogen and the reaction mixture heated under hydrogen to 75 °C and stirred for 7 h 30 min. A sample was taken for HPLC analysis, and the reaction was shown to be complete (pyridine **14** <1% PAR). The excess hydrogen was vented and the reactor purged with nitrogen. The mixture was filtered hot, and the filter and transfer lines were washed through with demineralised water (2 × 83 L) at 66 °C. The filtered solution (360 kg) was combined with two other reactions from the same batch of amide **14**. H_2O/*i*-PrOH was removed by vacuum distillation to leave about 740 L. The product crystallised from solution during the concentration. The slurry was cooled to 24 °C, stirred at 20−25 °C for 2 h, and then cooled and stirred for 4 h at 0−5 °C. The product was isolated by filtration and washed with demineralised water at 0−5 °C (248 L). The product was dried at 45−50 °C under vacuum for 64 h to a loss on drying of 25.1% w/w, to give amino acid hexahydrate **15** as a white solid (96.8 kg at 77.8% assay, water content 21.4% w/w by Karl Fischer titrimetry, 75.3 kg at 100%, 175.7 mol, 90.% yield from amide **14**).

HPLC in process-control method; Waters C18 column 150 mm × 4.6 mm. Eluent A 0.1% TFA in H_2O. Eluent B 0.1% TFA in acetonitrile. Gradient 5% B to 50% B over 20 min. Run time 20 min. Detector at 280 nm. Flow rate 1 mL/min. Sample preparation 0.1 mg/mL. Typical retention times: **14**, 7.7 min; **15**, 8.4 min.

Stage 5: (2*S*)-7-([4,4′-Bipiperidin]-1-ylcarbonyl)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1*H*-1,4-benzodiazepine-2-acetic Acid Hydrochloride (5). Industrial methylated spirit IMS (ethanol containing 1% MeOH) (300 kg), amino acid **15** (78.1 kg at 81.2% purity, 18.5% water content, 63.4 kg at 100%, 148.0 mol), and CH_2Cl_2 (505 kg) were charged to a reactor. The mixture was warmed to 23 °C and stirred at 23−28 °C for 30 min. The solution was transferred via a 0.9 μm in-line filter to a second clean reactor, washing the filter and transfer lines through with IMS (32 kg) and CH_2Cl_2 (54 kg). A filtered solution of pyridine hydrochloride (21.6 kg, 185.6 mol) in IMS (260 kg) and demineralised water (65 L) was added over 33 min. CH_2Cl_2/EtOH (510 L) was distilled off to a base temperature of 70 °C to leave a volume of about 850 L. The product crystallised out during the concentration, and the slurry was cooled to 4 °C and stirred at 0−5 °C for 4 h. The product was isolated by filtration and washed with filtered IMS (336 kg), at 3 °C and dried at 45−50 °C under vacuum for 13 h 25 min. The dry product was sieved through a 1400 μm screen to give hydrochloride salt **5** as a white solid (64.3 kg, 138.3 mol, 94% yield). The heel from this batch was left in the drier for inclusion with the next batch: mp 299 °C (DSC); $[\alpha]^{25}_D$ −182.6° (*c* 0.5, MeOH); ^1H NMR (400 MHz, DMSO-d_6) δ 11.81 (br s, 1 H), 8.96 (m, 1 H), 8.72 (m, 1 H), 7.06 (d, *J* = 1.8 Hz, 1 H), 7.03, (dd, *J* = 8.2, 1.9 Hz, 1 H), 6.56 (d, *J* = 8.3 Hz, 1 H), 6.18 (br s, 1 H), 5.44 (d, *J* = 16.4 Hz, 1 H), 5.05 (dd, *J* = 14.0, 5.1 Hz, 1 H), 4.13 (br s, 2 H), 3.86 (d, *J* = 16.7 Hz, 1 H), 3.24 (d, *J* = 11.9 Hz, 2 H), 2.92 (s, 3 H), 2.86−2.71 (m, 5 H), 2.57 (dd, *J* = 22.0, 5.0 Hz, 2 H), 1.79 (d, *J* = 9.7, 2 H), 1.67 (d, *J* = 11.9, 2 H), 1.42−1.27 (m, 4 H), 1.18−1.02 (m, 2 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.8, 169.3, 169.1, 147.5, 129.4, 127.9, 122.8, 118.0, 114.9, 51.6, 50.1, 43.2, 40.0, 37.8, 35.3, 33.5, 28.8, 25.5; IR (neat, cm^{-1}) 3308, 1737, 1663, 1650; LRMS (CI +ve) *m/z* 429.4 (M^+-Cl); Anal. Calcd for $C_{23}H_{33}ClN_4O_4$: C, 59.41; H, 7.15; N, 12.05. Found C, 59.37 H, 6.96; N, 11.94. HPLC method; Waters Symmetry C8 column, 5 μm, 250 mm × 4.6 mm, Eluent A 0.05 M aqueous formic acid, pH 4.2 with NH_3, eluent D 80:20 methanol/acetonitrile. Isocratic 15% B for 20 min. Run time 20 min. Flow rate 1 mL/min, Detector at 280 nm. Injection volume 10 μL. Typical retention time for **5** was 17.5 min.

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