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Production of (*R*)-Aminoglutethimide: A New Route from 1-Chloro-4-nitrobenzene

Michael J. Bunegar,[‡] Ulrich C. Dyer,[†] Graham R. Evans,[†] Richard P. Hewitt,[‡] Stephen W. Jones,*[,†] Neil Henderson,[†] Christopher J. Richards, Sivadasan Sivaprasad,[†] Benjamin M. Skead,[†] Mark A. Stark,[§] and Eric Teale[‡]

Celltech-Chiroscience p.l.c., Cambridge Science Park, Milton Road, Cambridge, CB4 0WE, UK, Hickson and Welch Ltd., Wheldon Road, Castleford, West Yorkshire, WF10 2JT, UK, and Department of Chemistry, University of Wales, Cardiff, P.O. Box 912, Cardiff, CF1 3TB, Wales

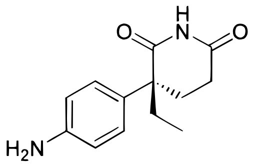
Abstract:

The development of a short, safe and enantioselective route for the preparation of (*R*)-aminoglutethimide is described. The process was designed for economic large-scale manufacture of the bulk drug substance to acceptable quality standards, to allow clinical evaluation of the single enantiomer over the existing racemate. (*R*)-Aminoglutethimide was prepared from 1-chloro-4-nitrobenzene using a six-stage synthetic sequence, via chemoresolution of key intermediate racemic 4-cyano-4-(4-nitrophenyl)hexanoic acid using (−)-cinchonidine. The process allowed for preparation of several kilograms of the precursor (*R*)-nitroglutethimide, to cGMP at pilot-plant scale, along with demonstration of the final hydrogenation step to (*R*)-aminoglutethimide in the laboratory. This route avoids the problems of hazardous nitration technology, and therefore regio-isomer contamination of the product, associated with other procedures. The resolution chemistry described represents an improvement on literature procedures. Optimisation of the asymmetric Michael addition offers an attractive alternative approach.

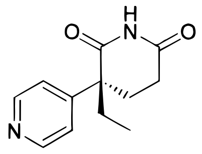
Introduction

Racemates of 3,3-di-substituted glutarimides such as aminoglutethimide (3-ethyl-3-(4-aminophenyl)-piperidine-2,6-dione) **(1)** and rogletimide (3-ethyl-3-(4-pyridyl)-piperidine-2,6-dione) **(2)** have been shown to be effective for the treatment of hormone-dependent breast cancer.[1,2] The mode of action of these compounds is considered to be by inhibition of the aromatase enzyme that catalyses the formation of oestrogens from androgens. Therefore, the compounds inhibit tumours whose growth is promoted by oestrogens.[3] Further studies have shown that the (*R*)-enantiomers of these compounds are much more potent aromatase inhibitors than their corresponding (*S*)-enantiomers.[4] In addition, medical evidence has also shown that some patients suffer acute CNS side effects during treatment, including lethargy, dizziness, and an all-over-body rash. However, it was unknown if these debilitating side effects could be reduced or even eliminated by treatment with the (*R*)-enantiomer as opposed to the racemate. If this were the case then the (*R*)-enantiomer would offer both increased efficacy and reduced side effects over the existing racemate.

Therefore, at Celltech-Chiroscience we established a drug development programme to establish proof of principle for the use of (*R*)-aminoglutethimide, with an improved therapeutic index over the racemate. Within the Process R & D department we were responsible for material supply for the programme. Initial supplies of the bulk drug were supplied by classical resolution of racemic aminoglutethimide with L-tartaric acid. This approach provided rapid access to material for clinical trials. However, for long-term supply this resolution was inefficient, requiring multiple crystallisations to ensure adequate optical purity. Furthermore, recycle of the unwanted enantiomer would be difficult. Therefore, we required a short, safe enantioselective route to (*R*)-aminoglutethimide, which could be developed for the economic large scale manufacture of the bulk single enantiomer drug substance.



(R)-Aminoglutethimide **(1)**



(R)-Rogletimide **(2)**

Synthetic Strategy

Previous preparations of (*R*)-aminoglutethimide have been reported utilising both biochemical[5] and chemical[6,7] resolution technology as well as synthetic approaches to heteroaryl[8] and fluoroaryl analogues.[9] The key issues to be addressed in the prior art were:

1. Introduction of the amino group via hydrogenation of a nitro function, incorporated using nitration methodology

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[†] Celltech-Chiroscience p.l.c.

[‡] Hickson and Welch Ltd.

[§] University of Wales.

(1) Smith, I. E.; Fitzharris, B. M.; McKinna, J. A.; Fahmy, D. R.; Nash, A. G.; Neville, A. M.; Gazet, J.-C.; Ford, H. T.; Powles, T. J. *Lancet* **1978**, *2*, 646.

(2) Foster, A. B.; Jarman, M.; Leung, C.; Rowlands, M. G.; Taylor, G. N.; Plevey, G. R.; Sampson, P. *J. Med. Chem.* **1985**, *28*, 200.

(3) Ogbunude, P. O. J.; Aboul-Enein, H. Y. *Chirality* **1994**, *6*, 623.

(4) Graves, P. E.; Salhanick, H. A. *Endocrinology* **1979**, *105*, 52.

(5) Fogliato, G.; Fronza, G.; Fuganti, C.; Grasselli, P.; Servi, S. *J. Org. Chem.* **1995**, *60*, 5693.

(6) Finch, N.; Dziemian, R.; Cohen, J.; Steinetz, B. G. *Experientia* **1975**, *31*, 1002; U.S. Patent No. 3,944,671.

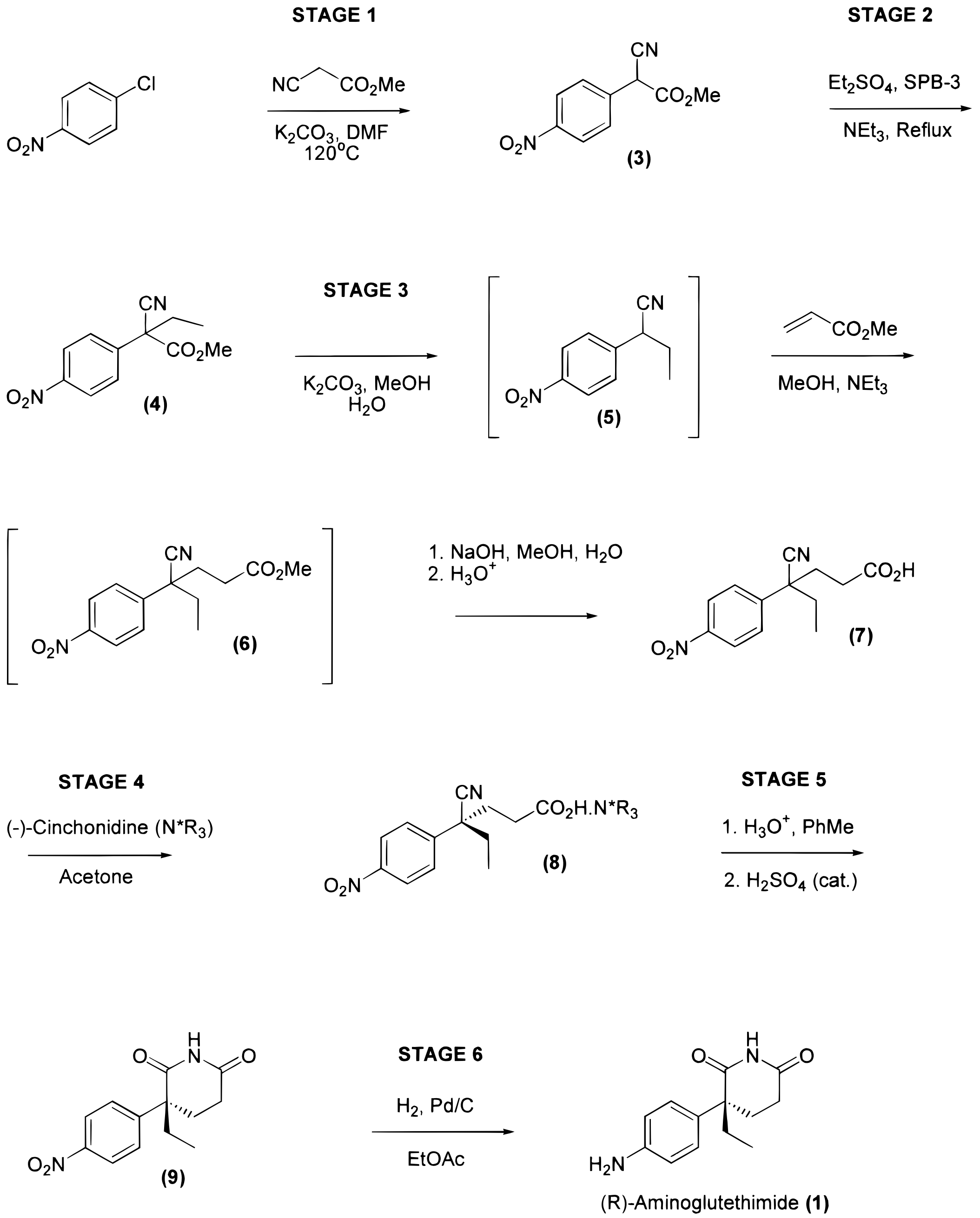
(7) Achmatowicz, O.; Malinowska, I.; Szechner, B.; Maurin, J. K. *Tetrahedron* **1997**, *53*, 7917.

(8) McCague, R.; Jarman, M.; Rowlands, M. G.; Mann, J.; Thickett, C. P.; Clissold, D. W.; Neidle, S.; Webster, G. *J. Chem. Soc., Perkin Trans. 1* **1989**, 196.

(9) Hammond, G. B.; Plevey, R. G.; Sampson, P.; Tatlow, J. C. *J. Fluorine Chem.* **1988**, *40*, 81.

10.1021/op9900075 CCC: $18.00

Scheme 1



which would be both nonregioselective and hazardous to operate at scale.[5]

2. Enantioselection via low-yielding resolution technology.

3. Lengthy synthetic sequences not suitable for operation at scale.

Therefore, we required a synthetic strategy which incorporated para selectivity along with an efficient asymmetric step, in a short sequence suitable for scale-up, from low-cost starting materials. This would be required as a credible material supply route upon completion of the pivotal clinical trials, which imposed challenging time constraints on the development programme.

After a retrosynthetic analysis we adopted a strategy aimed at developing a cost-effective racemic synthesis, incorporating a convenient resolution step. Our approach was developed using 1-chloro-4-nitrobenzene as a convenient starting material. This was available at low cost with less than 2% regioisomer contamination. Therefore the correct regiochemistry was established at the outset, and potentially hazardous nitration chemistry was avoided. Thus, (*R*)-aminoglutethimide **(1)** was prepared from 1-chloro-4-nitrobenzene using the six-stage synthetic sequence shown in Scheme 1. Aromatic nucleophilic substitution with methyl cyanoacetate, followed by ethylation, Michael addition and hydrolysis gave the key intermediate racemic 4-cyano-4-(4-nitrophenyl)hexanoic acid **(7)**. A screen of suitable resolving agents identified an efficient classical resolution and subsequent cyclisation and reduction gave (*R*)-aminoglutethimide **(1)**. The route utilised low-cost readily available materials and allowed for safe, manageable processing. The final product and all isolated intermediates are crystalline solids, and stages 1−5 of the process were successfully scaled up in the pilot plant.

Results and Discussion

Stage 1. Nucleophilic Substitution of 1-Chloro-4-nitrobenzene. Nucleophilic substitutions of substituted aryl nitro compounds with carbanions have been reported and product yields maximized when alkyl cyanoacetates were used in polar solvents in the presence of strong base.[9−11] Initial investigations into the nucleophilic substitution of 1-chloro-4-nitrobenzene revealed optimum conditions involved the use of 2 equivalents (equiv) of methyl cyanoacetate/2 equiv potassium carbonate/10 volumes (vol) DMF at elevated temperatures (>100 °C). However, product isolation was one of the major challenges. Recovered yields were good (80−90%) when the extractive work-up was replaced by an aqueous precipitation of the crystalline product, methyl 2-(4-nitrophenyl)cyanoacetate (**3**). This circumvented isolation problems caused by the use of ethyl cyanoacetate in early investigations, which gave the ethyl analogue as a difficult to handle semisolid product.

Further improvements to the work-up were required as neutralisation at ambient temperature with concentrated HCl caused extensive foaming due to carbon dioxide liberation, together with the formation of the byproduct, 4-nitrobenzylcyanide (15%), produced by decarboxylation. Both of these problems were overcome by slow neutralisation of the reaction mixture with dilute HCl at 10 °C. These combined process improvements allowed successful scale-up into 20 L laboratory glassware, based on an 800 g input of 1-chloro-4-nitrobenzene, to give methyl 2-(4-nitrophenyl)cyanoacetate **(3)** in 83−92% yield, 97−99% HPLC purity by peak area ratio (PAR).

Upon transfer to the pilot plant both the methyl cyanoacetate and potassium carbonate charge were reduced to 1.75 equiv, and the DMF charge was reduced to 4 vol. This improved the volume efficiency, and therefore batch size, aided the aqueous precipitation of product from DMF, and led to a further reduction in foaming of the reaction mixture on neutralisation. Dust explosion tests carried out on the product revealed it to be a class 2 dust hazard (medium risk) which required nitrogen purging, whilst discharging from the drier, and charging the product into anti-static bags.[12] Three consecutive 48 kg batches of 1-chloro-4-nitrobenzene were converted in 63, 73, and 94% yields, and 89−93% HPLC purity (PAR). The presence of unreacted 1-chloro-4-nitrobenzene (up to 3.4% by HPLC) caused excessive drying times, as it sublimed into the line connecting the filter/dryer to the vacuum system partially blocking it.

Stage 2. Alkylation of Methyl 2-(4-nitrophenyl)cyanoacetate (3). It had been reported that alkylation of analogues of methyl 2-(4-nitrophenyl)cyanoacetate **(3)** could be achieved with diethyl sulphate and potassium carbonate in refluxing acetone.[10] Early laboratory investigations using diethyl sulphate and potassium carbonate in 10 vol 2-butanone (MEK) at reflux (80 °C) allowed for isolation of methyl 2-cyano-2-(4-nitrophenyl)butyrate **(4)** as a crystalline solid. The product was isolated by an extractive work-up followed by evaporation to dryness under vacuum. However, the work-up was made difficult due to intensely dark-coloured organic/aqueous interfaces. Despite this the process was rapidly scaled to a 20 L working volume and gave yields of 41−55% and crude purities of 88−90% by HPLC (PAR), which increased to 97% after recrystallisation from 2-propanol.

Before the process could be operated in the pilot plant, product isolation issues had to be addressed. Therefore, a modified process was developed. Potassium carbonate was replaced with triethylamine which was added to a preheated two-phase mixture of the precursor methyl 2-(4-nitrophenyl)-cyanoacetate **(3)**, diethyl sulphate and 100−120 °C petroleum ether (SPB-3), at 90−95 °C. The reverse addition of triethylamine was beneficial, as it did not impact on reaction processing or product quality, and handling of the toxic alkylating agent was reduced to a minimum. The petroleum ether did not solubilise the reagents and was effectively used as an inert diluent to increase the mobility of the reaction mixture. This ensured efficient heat transfer and therefore safe operation of the process, as a hazard assessment had revealed thermal instability of both starting material and product.[13] Importantly, the lengthy aqueous work-up was also avoided by isolation of the product directly from the reaction mixture. Initially, excess diethyl sulphate was destroyed by the addition of water, followed by addition of 2-propanol and crystallisation of the product on cooling. Two 82.6 kg batches of methyl 2-(4-nitrophenyl)cyanoacetate **(3)** were processed. The optimisations resulted in improved yields of 59 and 73% of methyl 2-cyano-2-(4-nitrophenyl)butyrate **(4)**, purity 94% by HPLC (PAR), with no detrimental effect on impurity profile.

Stage 3. Conversion of Methyl 2-Cyano-2-(4-nitrophenyl)butyrate (4) to 4-Cyano-4-(4-nitrophenyl)hexanoic Acid (7). The hydrolysis and decarboxylation of methyl 2-cyano-2-(4-nitrophenyl)butyrate **(4)** to give 2-(4-nitrophenyl)butyronitrile **(5)** was extremely facile. This gave a product superior to that prepared by an alternative approach involving ethylation of 4-nitrobenzylcyanide. The alternative approach was attractive as it obviated stages 1 and 2. However 4-nitrobenzylcyanide was not commercially available and gave **5** contaminated with di-alkylated material. Michael addition of ethyl acrylate to 2-(4-nitrophenyl)-butyronitrile **(5)** gave ethyl 4-cyano-4-(4-nitrophenyl)hexanoate **(10)**. However, both products **5** and **10** were isolated

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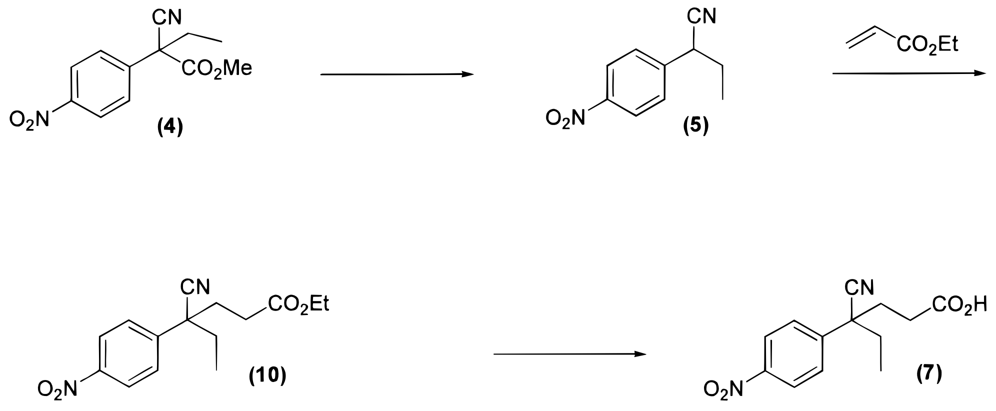
(10) Maeda, R.; Ohsugi, E.; Fujioka, T.; Hirose, K. *Chem. Pharm. Bull*. **1983**, *31*, 3424.

(11) Makosza, M.; Podraza, R.; Kwast, A. *J. Org. Chem*. **1994**, *59*, 6796.

(12) Dust explosion tests were carried out on a sieved sample of **3**, particle size <150 μm, in a Hartmann tube. Worst case results were maximum overpressure = 8.56 bar, maximum rate of pressure rise = 7128 bar sec^{-1}, lower explosive limit = 413 gm^{-3}, kst (dust explosion class standard) = 234 barmsec^{-1}, kst values of between 200 and 300 barmsec^{-1} are categorised as class 2, strongly explosible, by Lunn, G. *Guide to Dust Explosion Prevention and Protection Part 1 − Venting*, 2nd ed.; Gulf Publishing Company, 1992; Vol. 1. We thank Miss Sarah Birkby of Hickson and Welch for these results.

(13) DSC analysis (Mettler Toledo DSC 12E) of **3** revealed exothermic decomposition, onset 210 °C, −46 J/g, and also above 282 °C. DSC analysis of **4** revealed exothermic decomposition, onset 207 °C, −12 J/g, and also above 283 °C. We thank Dr. John O'Rourke of Chiroscience for these results.

Scheme 2

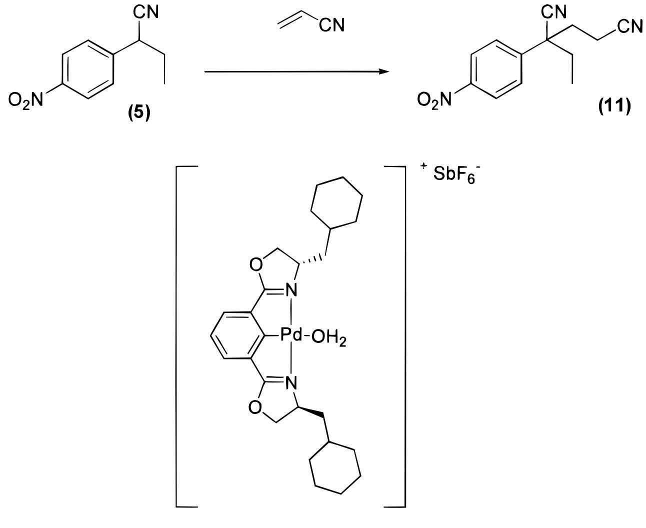


as oils which would not crystallise, and differential scanning calorimitry (DSC) had revealed decomposition on heating.[14] Therefore the materials were unsuitable for isolation or purification by large scale batch distillation. Hence a strategy of hydrolysis/decarboxylation of methyl 2-cyano-2-(4-nitrophenyl)butyrate **(4)**, followed by in situ Michael reaction and hydrolysis, was investigated. This gave 4-cyano-4-(4-nitrophenyl)hexanoic acid **(7)** as a granular crystalline solid in a single stage, avoiding isolation of 2-(4-nitrophenyl)butyronitrile **(5)** and ethyl 4-cyano-4-(4-nitrophenyl)hexanoate **(10)** (Scheme 2).

After considerable development the hydrolysis/decarboxylation reaction was performed in aqueous methanolic potassium carbonate solution over 10 min at 45 °C. The preferred Michael reagent methyl acrylate was then added directly to the butyronitrile intermediate, and the resultant reaction was complete after 5 h at 45 °C. A solution of sodium hydroxide was then added to promote hydrolysis of the intermediate methyl ester **(6)** to the sodium salt of the racemic acid **(7)**. A dichloromethane wash of the basic solution removed any non-acidic organic impurities. After acidification, followed by extraction into hot toluene, the racemic acid **(7)** was crystallised directly. The three-step process was scaled into 20 L glassware, and crystallised product yields were good, at 59−63%, with HPLC purities of 96−99% (PAR).

As a further improvement to the procedure the quantity of potassium carbonate was reduced, which significantly improved the impurity profile; however, this was at the expense of reaction time, which now took in excess of 15 h to reach completion. Therefore, a mixed base system involving potassium carbonate and triethylamine was developed which not only reduced reaction times to more workable levels (∼10 h) but also resulted in increased yields and purity. This combined three-step process was transferred to the pilot plant with typical yields in the region of 65−70% and product purity >98% by HPLC (PAR). A total of 71.5 kg of racemic acid **(7)** was manufactured from two 50.8 kg batches of methyl 2-cyano-2-(4-nitrophenyl)butyrate **(4)** using this procedure.

Scheme 3



(12)

Asymmetric Michael Addition. In an alternative approach an asymmetric Michael addition was proposed which would avoid the need for resolution-based technology. A series of cationic 2,6-bis(2-oxazolinyl)phenylpalladium(II) complexes had been developed by a group at Cardiff University that effectively catalysed the Michael addition of α-cyano carboxylates to methyl vinyl ketone.[15] Preliminary studies encouragingly revealed an ee of 30% was achieved in the addition of 2-(4-nitrophenyl)butyronitrile **(5)** to acrylonitrile, using the cationic (S,S)-2,6-bis(4′-methylenecyclohexyl-2′-oxazolinyl)phenyl-aquopalladium(II) hexafluoroantimonate complex **(12)**, to give 2-ethyl-2-(4-nitrophenyl)pentanedinitrile **(11)** (Scheme 3).[16] However, time constraints prevented further development of this approach.

*4-Cyano-4-(4-nitrophenyl)pentanoic Acid (**13**) Contamination.* It was observed that the racemic acid **(7)** prepared in the pilot plant contained a new impurity at a level of ∼2% by HPLC (PAR). This was of particular concern as it carried through subsequent processing to appear at ∼0.3% in the

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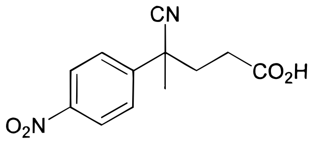
(14) DSC analysis of **5** (Mettler Toledo DSC 12E) revealed violent exothermic decomposition, onset 258 °C. DSC analysis of **10** revealed slow exothermic decomposition above 50 °C. We thank Dr. John O'Rourke of Chiroscience for these results.

(15) Stark, M. A., Richards, C. J. *Tetrahedron Lett.* **1997**, *38*, 5881. Stark, M. A., Jones, G., Richards, C. J. *Cationic 2,6-Bis(2-oxazolinyl)phenylpalladium-(II) Complexes: Catalysts for the Asymmetric Michael Reaction*, in press.

(16) Chiroscience funded LINK award to M. A. Stark, supervised by Dr. C. J. Richards, Department of Chemistry, University of Wales, Cardiff, P.O. Box 912, Cardiff, CF1 3TB.

final product, (*R*)-aminoglutethimide **(1)**, which was unacceptable from a quality standpoint. Exhaustive preparative HPLC effort, and high field NMR studies revealed the unknown impurity as the methyl analogue of racemic acid **(7)**, 4-cyano-4-(4-nitrophenyl)pentanoic acid **(13)**.[17]

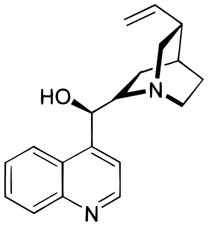


4-Cyano-4-(4-nitrophenyl)pentanoic acid **(13)**

It was initially thought that this impurity may have been derived from the stage 2 alkylation, by contamination of diethyl sulphate with dimethyl sulphate, or during the stage 1 alkylation by contamination of methyl cyanoacetate with methyl 2-cyanopropionate. However, NMR and HPLC analysis of these materials showed no contamination. We believe that the methyl impurity originated during the stage 1 nucleophilic substitution of 1-chloro-4-nitrobenzene. However, isotopic labeling studies will be required to confirm this. Removal of this impurity was achieved by crystallisation of the final product from aqueous ethanol, as described in the Stage 6 discussion.

Stage 4. Resolution of 4-Cyano-4-(4-nitrophenyl)hexanoic Acid (7). The resolution of 4-cyano-4-(4-nitrophenyl)hexanoic acid **(7)** was investigated with the use of a variety of chiral amines. (+)-α-Methylbenzylamine was found to give a crystalline product, but with a large variation in ee of regenerated acid. This was characteristic of a resolution which could only be controlled using kinetic conditions (i.e., accurate temperature and concentration control, controlled seeding with single diastereomer salt crystals, mechanical as opposed to magnetic stirring, and controlled crystallisation times). Therefore, we did not anticipate that an α-methylbenzylamine-based approach would provide a reproducible and scaleable resolution. This was in direct contrast with results reported by the Polish group.[7] They had prepared both (*R*)- and (*S*)-aminoglutethimide, via α-methylbenzylamine resolution of 4-cyano-4-(4-nitrophenyl)hexanoic acid **(7)** in acetonitrile, on a laboratory scale.

The resolution was also investigated using the alkaloid base (−)-quinine. This gave a crystalline salt with a high diastereomeric excess but unfortunately with the unwanted (*S*)-configuration. Therefore, the cinchona alkaloids quinidine, cinchonine and cinchonidine were also screened. It was found that (−)-cinchonidine **(14)** gave a crystalline diastereomeric salt, with the correct (*R*)-configuration. Therefore it was anticipated that, since it is commercially available in bulk quantities, a (−)-cinchonidine resolution would provide a practical approach to the synthesis of (*R*)-aminoglutethimide **(1)**.



(-)-Cinchonidine **(14)**

The (−)-cinchonidine resolution of racemic acid **(7)** was investigated on a small scale, using jacketed vessels for reliable temperature control, and external seeding. Chemical yield was determined by isolation of the crystalline salt, and optical purity by chiral HPLC analysis of a sample of acid regenerated from the diastereomeric salt. Fortunately, a large solubility differential was observed between the two diastereomeric salts. The (*R*)-acid cinchonidine salt had a solubility of 2.8 mg/mL in acetone at 15 °C, compared with 20.3 mg/mL for the diastereomer. Therefore, after investigating a variety of solvents the best results were achieved with 1 equiv of (−)-cinchonidine, and 30 vol of acetone with 1% added water to achieve complete dissolution, which gave a 36% yield of diastereomeric salt (72% of available), and 90% ee of regenerated acid. These results in acetone were improved by reducing the (−)-cinchonidine charge to 0.6 equiv, which gave comparative yields (∼35%) and ee's (∼90%) along with improved volume efficiency at 15 vol.

This resolution procedure was rapidly scaled up to 500 g racemic acid input, in 10 L laboratory glassware. However, the resolution did not perform satisfactorily on scale-up. The crystallised mixtures were very thick and difficult to stir and could only be removed from the vessel under pressure. The ee of the regenerated samples of (*R*)-acid at this stage was lower than expected at 70−80%. Fortunately this material was optically enriched, by slurrying in acetone at ambient temperature, to raise the ee to the required level of >94%. The resolution was further modified, using 0.5 equiv of (−)-cinchonidine in 20 vol of acetone. This typically gave material with an ee > 90% (*R*) in >30% yield. The required ee of >94% (*R*) was achieved with a further optical enrichment in acetone at reflux followed by an overnight stir, at 20 °C.

This procedure was used to resolve 52 kg of racemic acid **(7)** provided by the pilot plant campaign. Unfortunately a use-test gave poor optical quality material (40% ee regenerated (*R*)-acid), when compared with laboratory synthesised racemic acid, and required two further optical enrichments by slurry to produce acceptable quality material (97% ee regenerated (*R*)-acid, 21% yield). However, the time available necessitated that the racemic acid was resolved, using this procedure, in three 17 kg batches in the pilot plant. This gave a total of 40.6 kg of (*R*)-acid cinchonidine salt **(8)** in three batches (48−68% ee regenerated (*R*)-acid, 35−42% yield by loss on drying) which were then enriched in a single slurry, with batch integrity, to give 27.9 kg (84−91% ee regenerated (*R*)-acid, 24−27% yield). These three batches were combined and given a second optical enrichment in two batches, to give 23.2 kg of (*R*)-acid cinchonidine salt **(8)** (97 and 98% ee regenerated (*R*)-acid, 83 and 86% recovery).

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(17) We thank Mr. Iain Reid of Chiroscience for the isolation of this impurity by preparative HPLC.

Stage 5. Preparation of (*R*)-Nitroglutethimide (9) from *(R)*-Acid Cinchonidine Salt (8). Treatment of (*R*)-4-cyano-4-(4-nitrophenyl)hexanoic acid cinchonidine salt **(8)** with 1 M Hydrochloric acid and toluene at ambient temperature gave a toluene solution of (*R*)-4-cyano-4-(4-nitrophenyl)-hexanoic acid. This was azeotropically dried and cyclised in situ at reflux with catalytic sulphuric acid. The (*R*)-acid regeneration was facile, with (−)-cinchonidine hydrochloride removed in the aqueous acidic layer and aqueous washes, which were retained for regeneration of the resolving agent. The toluene solution was then azeotropically dried and cyclisation to (*R*)-nitroglutethimide **(9)** effected in 1−2 h using 0.2 equiv concentrated sulphuric acid. The azeotropic drying step was necessary, as in its absence the reaction took 5−6 h to complete, and resulted in partial decomposition of both substrate and product. The product was crystallised directly from the toluene solution as a pale yellow granular crystalline solid in 80−90% yield, with an ee value typically >98% compared with 95−98% ee of regenerated (*R*)-acid from input cinchonidine salt **(8)**. The achiral purity was >99.5%.

This process was operated successfully in the laboratory up to 250 g (*R*)-acid cinchonidine salt and taken into the pilot plant. Two batches of 11.4 and 11.7 Kg were processed giving 3.5 and 3.3 kg (65% and 60% yields) of (*R*)-nitroglutethimide, both 99.9% ee, and >99.7% achiral purity. The reaction had scaled up satisfactorily both in terms of optical and chemical purity. However, the yields of product isolated from both batches were below that obtained from laboratory batches, due to losses in the toluene mother liquors of recrystallisation.

Stage 6. Preparation of *(R)*-Aminoglutethimide (1) by Hydrogenation of *(R)*-Nitroglutethimide (9). Initial small-scale catalytic hydrogenation studies using dry palladium on carbon proved problematic due to the limited solubility of (*R*)-nitroglutethimide **(9)** in several common organic solvents. Methanol was investigated as hydrogenation solvent; however, the substrate was only sparingly soluble even in 15 vol. Dissolution was achieved by addition of a cosolvent such as tetrahydrofuran, or by performing the hydrogenation at 35−45 °C. However, reaction rates were modest at ambient temperature, and impurity profiles were unacceptable at elevated temperatures. Typically, product isolated by crystallisation from methanol/water contained 3−4% unidentified impurities, with the major one at a level of 0.7%.

Greater success was achieved when methanol was replaced by ethyl acetate as reaction solvent, and this process was rapidly scaled up to provide material which was urgently required for formulation studies. A total of 150 g of product was isolated in four separate batches in the laboratory. The reaction was complete within 2 h, and the product was readily precipitated from heptane. Yields were ∼93%, and the impurity profile was considerably improved compared to material prepared using methanol, with no individual impurity at a level >0.1%. However, to allow safe scale-up to the pilot plant, the use of wet palladium on carbon was investigated (5% w/w Pd/C containing 50% water). This gave ∼85% yield, comparable reaction times, and acceptable purities, but required a higher catalyst loading, equivalent to 10% w/w catalyst/substrate.

The previously described methyl analogue impurity was present at a level of 0.3% by HPLC (PAR) in (*R*)-nitroglutethimide produced in the pilot-plant batches, and remained at this level after hydrogenation, in laboratory use-tests. Attempts to remove the impurity by crystallisation/trituration indicated that alcoholic solvents were superior, however, only a modest purification was achieved. This led to the reinvestigation of crystallisation from alcohol/water mixtures. Problems were encountered with both methanol/water and propan-2-ol/water systems at elevated temperatures, as the product oiled out. Superior results were obtained with methanol/water, at ambient temperature, which gave optimum impurity removal and crystalline product. The current process would therefore require modification, involving a solvent exchange from ethyl acetate to methanol, or a suitable alcohol. The azeotrope of ethyl acetate/methanol is rather poor at 51:49; however, for ethyl acetate/ethanol it is considerably better at 74:26. Hence, ethanol was the solvent of choice and this process improvement was introduced.

Laboratory use-tests of (*R*)-nitroglutethimide **(9)** produced in the pilot-plant, using the optimum process, gave crystalline (*R*)-aminoglutethimide **(1)** in 77% yield, overall purity 99.8% by HPLC (PAR), methyl analogue impurity level 0.11% and ee > 99.9%. A second methanol/water crystallisation reduced the impurity level to <0.1%, with 82% recovery, thereby giving an overall yield of 63% from (*R*)-nitroglutethimide. In summary, this hydrogenation procedure was shown to be versatile, effective, and scaleable in the laboratory, with the product isolated as a crystalline solid, in moderate yield, with individual impurity levels within specification.

Conclusions

(*R*)-Aminoglutethimide **(1)** was prepared from 1-chloro-4-nitrobenzene using a six-stage synthetic sequence, which avoids the problems of hazardous nitration technology, and therefore regio-isomer contamination of final product. The process utilised low-cost, commercially available reagents and exploited the crystallinity of intermediates to overcome isolation difficulties. This, along with a number of key process improvements developed within the constraints imposed by time schedules, allowed safe and rapid scale-up from laboratory to pilot plant over stages 1−5. A total of 52 kg of the key intermediate racemic 4-cyano-4-(4-nitrophenyl)hexanoic acid **(7)** was produced from 144 Kg of 1-chloro-4-nitrobenzene. This material was prepared on a 1000 L scale over three stages in several batches and represents a 22% overall yield. A total of 6.8 kg of the stage five product (*R*)-nitroglutethimide **(9)** was prepared to cGMP on a 450 L scale, in a yield of 14%, from 50.3 kg of racemic acid **(7)**. A scaleable procedure for the Stage 6 hydrogenation to produce (*R*)-aminoglutethimide **(1)** was demonstrated in the laboratory. Acceptable yields were achieved on all stages except the resolution of racemic acid **(7)**. Therefore, future development work should be focused on optimising this key stage. Efforts to evaluate alternative resolving agents and solvent systems should be most productive. In addition the proposed synthetic approach via an asymmetric Michael addition was attractive. This would avoid resolution based technology and preliminary results were encouraging. Early clinical trial results in an oestrogen clearance study in postmenopausal breast cancer patients revealed both (*R*)-aminoglutethimide and the racemate suppressed all oestrogens measured to a similar extent and did not suggest any major clinical advantage from eliminating the (S)-enantiomer.[18]

Experimental Section

General. ^{1}H NMR spectra were recorded at 400 MHz on a Bruker AM 400 instrument, in $CDCl_3$ solution, using $SiMe_4$ as internal standard, unless otherwise stated. Melting points were recorded on a Mettler Toledo DSC 12E and are uncorrected. The IR spectrum was determined on a Perkin-Elmer 1600 FTIR spectrometer. The mass spectrum was determined on a VG Platform II quadrupole spectrometer. The accurate mass was determined on a VG ZAB magnetic sector spectrometer.

Methyl 2-(4-Nitrophenyl)cyanoacetate (3). 1-Chloro-4-nitrobenzene (48 kg, 305 mol, 1 equiv), K_2CO_3 (73.7 kg, 533 mol, 1.75 equiv) and DMF (206 L, 4.3 vol) were charged to the 1000 L glass-lined steel reaction vessel. The mixture was stirred and heated to 120 °C, at which temperature methyl cyanoacetate (52.8 kg, 533 mol, 1.75 equiv) was added over 3 h. The temperature was controlled at 118−125 °C during the methyl cyanoacetate feed, and during the subsequent 8 h stir. After the mixture was monitored for complete reaction by HPLC (<2% residual 1-chloro-4-nitrobenzene), it was cooled to 10−20 °C, and water (~144 L, 3 vol) was added. The pH was adjusted to 2 with 25% H_2SO_4. After acidification, further water (~398 L, 8.3 vol) was added to the mixture, and the slurry was stirred at 10 °C for 1 h to ensure complete precipitation of product. The mixture was filtered and the filter cake washed with water (2 × 180 L, 3.75 vol) and dried under vacuum at 55 °C. Yield of **3** = 45.5−70.3 kg, 63−93%, mp 95 °C, (89−93% assay by HPLC), 3 batches. ^{1}H NMR δ 3.85 (s, 3H), 4.90 (s, 1H), 7.70 (m, 2H), and 8.30 (m, 2H).

Methyl 2-Cyano-2-(4-nitrophenyl)butyrate (4). The 1000 L glass-lined steel reaction vessel was charged with **3** (82.85 kg, 340 mol, 1 equiv), SPB-3 (equivalent to 100−120 °C boiling petroleum ether) (99.4 L, 1.2 vol), and diethyl sulphate (57.7 kg, 374 mol, 1.1 equiv). The mixture was stirred and heated to 90−95 °C. NEt_3 (35.8 kg, 354 mol, 1.04 equiv) was added over 2 h, maintaining the reaction temperature at 90−95 °C. After the NEt_3 addition the reaction was stirred for 1 h at 90−95 °C and then cooled to 50−55 °C. Water (14.1 L, 0.17 vol) was added and the mixture heated to 70−75 °C for 1 h to destroy any residual diethyl sulphate. The reaction was then cooled to 50−55 °C, and *i*-PrOH (198.8 L, 2.4 vol) was added. The reactor contents were heated to 70−75 °C to ensure all the product was dissolved and cooled to 0−5 °C. To ensure complete crystallisation of the product the mixture was held at 0−5 °C for 2 h. The slurry was filtered and washed with cold *i*-PrOH (2 × 49.7 L, 2 × 0.6 vol), before the product was dried under vacuum at 45−55 °C. Yield of **4** = 65.5 kg, 73%, mp 92 °C, (94% assay by HPLC). ^{1}H NMR δ 1.10 (t, 3H), 2.22 (m, 1H), 2.50 (m, 1H), 3.85 (s,3H), 7.78 (m, 2H), and 8.30 (m, 2H).

4-Cyano-4-(4-nitrophenyl)hexanoic Acid (7). The 1000 L glass-lined steel reaction vessel was charged with **4** (50.9 kg, 193 mol, 1 equiv) and MeOH (407.2 L, 8 vol), and stirring commenced. To this suspension was added a solution of K_2CO_3 (28.0 kg, 203 mol, 1.05 equiv) in water (61.1 L, 1.2 vol). The contents were heated to 30−35 °C, and held for 30 min, until HPLC analysis indicated complete formation of **5**. NEt_3 (29.3 kg, 290 mol, 1.5 equiv) was added to the solution, which was heated to 50−55 °C. Methyl acrylate (18.3 kg, 212 mol, 1.1 equiv) was added over 1 h, maintaining the temperature at 50−55 °C. After stirring for 3 h, further methyl acrylate (5.0 kg, 58 mol, 0.3 equiv) was added, followed by a further 10 h stir to complete the reaction. The reactor contents were cooled to 10 °C, a solution of NaOH (12.4 kg, 309 mol, 1.6 equiv) in water (127.3 L, 2.5 vol) added, and the mixture heated to 40−45 °C and maintained at this temperature for 3 h to hydrolyze the intermediate **6**. MeOH was removed under vacuum and replaced with water to maintain the volume. The aqueous suspension was filtered to remove insoluble impurities and washed with CH_2Cl_2 (3 × 102 L, 2 vol) to remove any non-acidic organic impurities. The aqueous basic layer was acidified to pH 2.5 with 25% w/w H_2SO_4 to precipitate the product. Toluene (142.5 L, 2.8 vol) was added and the two-phase mixture heated to 70 °C to dissolve the product. The lower, aqueous layer was separated and the hot toluene layer washed with water (142.5 L, 2.8 vol) and separated. The toluene solution was then cooled to 0−5 °C to crystallise the product, before filtration, washied with cold toluene, and dried under vacuum at 40 °C. Yield of **7** = 43.7 kg, 84%, mp 125 °C, (>99.9% assay by HPLC). ^{1}H NMR δ 0.96 (t, 3H), 2.05 (m, 1H), 2.18 (m, 2H), 2.30 (m, 1H), 2.42 (m, 1H), 2.56 (m, 1H), 7.64 (m, 2H), 8.28 (m, 2H), and 10.32 (br s, 1H).

HPLC conditions for above procedures: column, Zorbax Silica, 250 mm × 4.6 mm; mobile phase, heptane 95, *i*-PrOH 5, TFA 0.1; wavelength, 254 nm; injection volume, 20 μL; flow rate, 1 mL/min; run time, 20 min. Retention times: **3**, 15.9 min; **4**, 4.8 min; **5**, 7.6 min; **6**, 8.7 min; **7**, 11.5 min.

2-Ethyl-2-(4-nitrophenyl)pentanedinitrile (11). To a suspension of (*S*,*S*)-2,6-bis(4′-methylenecyclohexyl-2′-oxazolinyl)phenyl-aquopalladium(II) hexafluoroantimonate **(12)** (0.0074 g, 9.6 μmol) in toluene (3 mL) under a nitrogen atmosphere was added **5** (0.14 mL, 0.96 mmol) and acrylonitrile (0.09 mL, 1.37 mmol). The resulting solution was cooled to −10 °C. *N*-Ethyldiisopropylamine (0.017 mL, 98 μmol) was added and the resulting mixture maintained at −10 °C for 24 h. The reaction mixture was warmed to room temperature and filtered through a short plug of neutral alumina (Grade IV) and washed through with CH_2Cl_2. The solvent was removed in vacuo and the residue purified by bulb-to-bulb distillation to give **11** as a brown oil. The instability of the product precluded further distillation. Yield

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[18] Geisler, J.; Lundgren, S.; Berntsen, H.; Greaves, J. L.; Lonning, P. E. *J. Clin. Endocrinol. Metab.* **1998**, *83*, 2687.

of **11** = 0.173 g, 74%, 30.6% ee by HPLC, 93.5% achiral purity by HPLC. HPLC conditions: achiral chromatography: column, Phenomenex Luna 2 C18, 150 mm × 4.6 mm; mobile phase, solvent A, 95:5, 20 mM KH_2PO_4 pH = 7.0: MeCN; solvent B, 25:75, 20 mM KH_2PO_4 pH = 7.0:MeCN; 0 min 100% A, 8.00 min 0% A, 11.50 min 0% A, 11.55 min 100% A, 14 min 100% A; wavelength, 210 nm; injection volume, 20 μL; flow rate, 2 mL/min; run time, 14 min. Retention time: **11**, 7.5 min: chiral chromatography: column, Chiralcel OD-H, 250 mm × 4.6 mm; mobile phase, heptane 70, *i*-PrOH 30; wavelength, 210 nm; injection volume, 20 μL; flow rate, 1 mL/min; run time, 20 min. Retention times: 14.5 min, 16.6 min. ^{1}H NMR (Bruker RPX 400, 400 MHz, $CDCl_3$) δ 0.90 (t, 3H), 2.00 (m, 1H), 2.10 (m, 2H), 2.25 (m,1H), 2.45 (m, 2H), 7.55 (m, 2H), and 8.25 (m, 2H). ^{13}C NMR (Bruker AMX 360) δ 10.0, 14.2, 34.5, 36.4, 49.1, 117.9, 120.1, 125.1, 127.6, 143.4, 148.4. IR (liquid film) ν_{max} 3055, 2985, 2306, 2253, 1608, 1527, 1422, 1348, 1265, 738 cm.$^{-1}$ *m*/*z* (EI) 243 (M^+, 11%), 215 (22), 203 (19), 189 (44), 175 (100), 69 (41). Found: M^+, *m*/*z* (EI) 243.1009, $C_{13}H_{13}N_3O_2$ requires 243.1008.

***(R)*-4-Cyano-4-(4-nitrophenyl)hexanoic Acid Cinchonidine Salt (8).** Acetone (340 L, 20 vol) and **(7)** (17.0 kg, 64.8 mol, 1 equiv) were charged to the 450 L glass-lined steel reaction vessel with stirring, and the cloudy solution was filtered through a 5 μm filter. (−)-Cinchonidine (9.54 kg, 32.4 mol, 0.5 equiv) was added, the mixture heated to reflux (57 °C), and reflux maintained for 30−35 min, ensuring complete dissolution. The solution was cooled to 40 °C, seeded with **(8)** (17 g, 0.1% w/w), and allowed to crystallise at 40 °C for 4−5 h. The mixture was cooled to 30 °C over 1−2 h and held at this temperature for 15 h. The mixture was then cooled to 20 °C over 1−2 h, held at this temperature for 5 h, and isolated by centrifugation to give the product as an acetone damp cake (15.25 kg, 42% yield by loss on drying, 48% ee of regenerated acid). Acetone (340 L, 20 vol) and the acetone damp product from above were charged to the vessel, and the mixture was taken to reflux (57 °C). The reflux was maintained for 30−35 min. The mixture was cooled to 20 °C over 2−3 h and stirred out for 15−18 h. The product was isolated by centrifugation, washed with acetone (170 L, 10 vol), and dried under vacuum at 40 °C, to give **8** (9.8 kg, 27% yield, 84% ee of regenerated acid). Acetone (235 L, 17 vol) and dry enriched **8** (13.8 kg) from above were charged to the vessel, the mixture was taken to reflux (57 °C), and reflux was maintained for 30−35 min. The mixture was cooled to 20 °C over 2−3 h and then stirred for 15−18 h. The product was isolated by centrifugation, washed with acetone (117 L, 8.5 vol), and then dried under vacuum at 40 °C, to give **8** (11.48 kg, 83% recovery, 97% ee of regenerated acid). HPLC conditions: achiral chromatography: as for preparation of **7**. Chiral chromatography: column, Chiralpak AD, 250 mm × 4.6 mm; mobile phase, heptane 90, *i*-PrOH 10, TFA 0.1; wavelength, 254 nm; injection volume, 20 μL; flow rate, 1 mL/min; run time, 40 min. Retention times: (*R*)-**(7)**, 18.0 min; (S)-**(7)**, 26.5 min.

***(R)*-Nitroglutethimide (9).** Toluene (69.2 kg, 7 vol) was charged to the 450 L glass-lined steel reaction vessel, followed by **8** (11.43 kg, 20.5 mol). A solution of 36% HCl (5.2 kg) in water (60 L, 5.2 vol) was added, and the contents were adjusted to 20 °C and stirred until the solids dissolved. The lower, aqueous acidic layer was separated and extracted with toluene (30 L, 2.6 vol). The combined toluene extracts were washed with water (57.2 L, 5 vol) and separated. The toluene layer was cooled to 0−5 °C, activated charcoal (286 g, 2.5% w/w) added, and the mixture stirred at this temperature for 1 h. The suspension was filtered, returned to the vessel, and dried by azeotropic distillation. The solution was heated to reflux (111−112 °C), and 96% H_2SO_4 (419 g, 0.2 equiv) was added. Reflux was maintained until all of the (*R*)-**(7)** was consumed by HPLC. The contents were cooled to 50−60 °C and, whilst this temperature was maintained, were washed with water (35.2 L, 3 vol), a solution of $NaHCO_3$ (1825 g, 1.06 equiv) in water (21.9 L, 1.9 vol), and further water (35.2 L, 3 vol). The toluene solution was hot-filtered through a 1 μm filter to remove particulates and concentrated to 9−10 vol, with respect to expected product, by distillation at reflux. The solution was cooled to 40 °C and seeded with **(9)** (5.7 g). The crystallising mixture was then cooled to 0−5 °C and stirred at this temperature for 1 h. The product was isolated by centrifugation, washed with cold toluene (9.3 L), and dried under vacuum at 55 °C. Yield of **9** = 3.5 kg, 65% yield, mp 136 °C, 99.9% ee by HPLC, 99.7% achiral purity by HPLC. HPLC conditions: achiral chromatography: column, Zorbax Silica, 250 mm × 4.6 mm; mobile phase, heptane 85, *i*-PrOH 15, TFA 0.1; wavelength, 254 nm; injection volume, 20 μL; flow rate, 1 mL/min; run time, 20 min. Retention times: **7**, 5.0 min; **9**, 10.0 min; chiral chromatography: column, Chiralpak AD, 250 mm × 4.6 mm; mobile phase, heptane 80, *i*-PrOH 20, TFA 0.1; wavelength, 254 nm; injection volume, 20 μL; flow rate, 1 mL/min; run time, 40 min. Retention times: (*S*)-Nitroglutethimide, 29.0 min; **9**, 31.6 min. ^{1}H NMR δ 0.92 (t, 3H), 1.98 (m, 1H), 2.10 (m, 1H), 2.30−2.48 (m, 3H), 2.70 (m, 1H), 7.52 (m, 2H), 8.24 (m, 2H), and 8.80 (br s, 1H).

***(R)*-Aminoglutethimide (1).** To a solution of **9** (20.0 g, 76 mmol) in EtOAc (300 mL, 15 vol) in a 2-L Parr pressure vessel was added 5% Pd/C containing approximately 50% water (4.7 g). The system was stirred under an atmosphere of hydrogen until complete removal of the substrate was observed by HPLC. The catalyst was filtered off and washed with EtOAc (40 mL, 2 vol). The EtOAc solution was concentrated to 5 vol by distillation at atmospheric pressure and the EtOAc replaced by successive additions of EtOH (124 mL, 6.2 vol), followed by concentration to 5 vol each time, until EtOAc was removed. The solution was cooled to 25−30 °C, water (140 mL, 7 vol) added until the cloud point was reached, and the mixture seeded with **1** (0.2 g, 0.1% w/w). After crystallisation, water (60.0 mL, 3 vol) was added and the mixture cooled to 0−5 °C for 1 h. The product was filtered, washed with cold water (100 mL, 5 vol), and pulled dry on the filter. The product was then dissolved in MeOH (80 mL, 5 vol). Water (65 mL, 4 vol) was added to this solution, maintaining the temperature between 20 and 25 °C, and the solution was seeded with **1** (0.2 g, 0.1% w/w). The crystallising mixture was stirred for 30 min, water (160 mL, 10 vol) added, and the mixture cooled to 0−5 °C. The mixture was stirred at 0−5 °C for 1 h, and the product was filtered, washed with cold water (80.0 mL, 5 vol), and dried under vacuum. Yield of **1** = 33.6 g, 82% yield, mp 111 °C, >99.9% ee by HPLC, 99.9% achiral purity by HPLC. HPLC conditions: achiral chromatography: column, Nova-Pak C18, 150 mm × 3.9 mm; mobile phase, MeOH 25%, pH 5 Phosphate Buffer 75%; wavelength, 254 nm; injection volume, 20 μL; flow rate, 1 mL/min; temperature, 40 °C; run time, 45 min. Retention times: **9**, 29.0 min; **1**, 7.5 min; chiral chromatography: column, Chiralcel OJ, 50 mm × 4.6 mm; mobile phase, EtOH 25%, hexane 75%; wavelength, 242 nm; injection volume, 20 μL; flow rate, 1 mL/min; temperature, 40 °C; run time, 12 min. Retention times: **1**, 3.0 min; (*S*)-aminoglutethimide, 6.0 min. ^{1}H NMR δ 0.86 (t, 3H), 1.88 (m, 1H), 1.98 (m, 1H), 2.16 (m, 1H), 2.30 (m, 1H), 2.44 (m, 1H), 2.58 (m, 1H),3.72 (br s, 2H), 6.66 (m, 2H), 7.04 (m, 2H), and 8.18 (br s, 1H).

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