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Development of a Supply Route for the Synthesis of an iNOS Inhibitor: Complications of the Key S_N2 Reaction

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Abstract:

The original medicinal chemistry synthesis of an iNOS inhibitor presented several challenges that had to be overcome in order to constitute a supply route suitable for operation on a multikilo scale. The key step in the synthesis is an S_N2 reaction that assembles the chiral carbon framework, but this reaction proved far more complex than we anticipated. Significant improvements were made to all stages. The modified route performed well over two pilot-plant campaigns and delivered over 250 kg of the active pharmaceutical ingredient (API).

1. Introduction

Nitric oxide (NO) is an important physiological mediator that plays a role in the regulation of blood pressure and blood flow, as a neurotransmitter in the central and peripheral systems and in the immune system.[1] However, the overproduction of NO by the inducible isoform of the NO synthetases (iNOS) is implicated in the pathophysiology of several disease states such as septic and endotoxic shock, neurodegenerative disorders, and inflammatory diseases such as asthma, rheumatoid arthritis, and multiple sclerosis. It is desirable, therefore, to develop selective inhibitors of iNOS for therapeutic use with a high degree of selectivity over other isoforms.[1] This paper describes our efforts to synthesise kilogram quantities of **1** (Figure 1), an iNOS inhibitor candidate, to support toxicology and clinical studies.

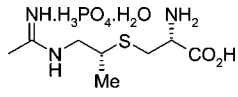

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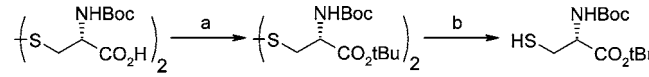
Figure 1

2. Results and Discussion

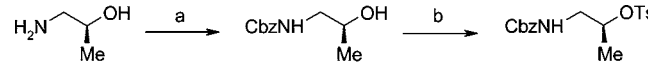
Synthesis of the S_N2 Reaction Partners. In our first campaign the synthesis began with the preparation of bis-*N*-Boc cystine bis-*tert*-butyl ester **3** from bis-*N*-Boc-cystine **2** and *tert*-butyl trichloroacetimidate (Scheme 1). For the campaigns that followed we purchased this material from a commercial source, and our synthesis started with the reduction of the bis-*N*-Boc cystine bis-*tert*-butyl ester **3** into the monomer **4** employing standard literature conditions.[2−4]

Scheme 1. **Synthesis of protected cysteine component[a]**



[a] Reagents and conditions: (a) *tert*-butyl trichloroacetimidate, DCM/heptane; (b) DCM, NEt_3, dithiothreitol, 80% from **2**.

Scheme 2. **Protection and activation of (*S*)-(+)-1-amino-2-propanol[a]**



[a] Reagents and conditions: (a) Aq K_2CO_3, CbzCl, DCM; (b)TosCl, NEt_3, NMe_3.HCl, 82% from **5**.

In a two-step process (Scheme 2), (*S*)-(+)-1-amino-2-propanol (**5**) was converted to tosylate **7** by protection with CbzCl followed by tosylation using a combination of *p*-toluenesulfonyl chloride, triethylamine, and trimethylamine hydrochloride according to a known method.[5−7] The trimethylamine generated in this process reacts with tosyl chloride to form the trimethylammonium adduct. This reactive intermediate acts as the tosylating agent and not only accelerates the reaction but also aids the hydrolytic decomposition of the excess tosyl chloride during the workup. A side reaction is the chlorodemethylation of the activated species, giving rise to *N*,*N*-dimethyltolylsulfonamide, but this is removed in the crystallisation of the product.

In total, more than 500 kg of each of **7** and **4** were prepared using the chemistry described above.

Development of the S_N2 Process. The reaction of **4** and **7** in an apparently simple S_N2 reaction formed the basis of the quality critical step in the preparation of **1**.

The original medicinal chemistry procedure for the preparation of **8** involved combination of equimolar amounts of **4** and **7** and anhydrous cesium carbonate, in 43 volumes of degassed

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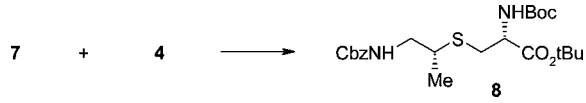
Scheme 3. **Reaction of 7 and 4 to afford 8 is the quality critical step**



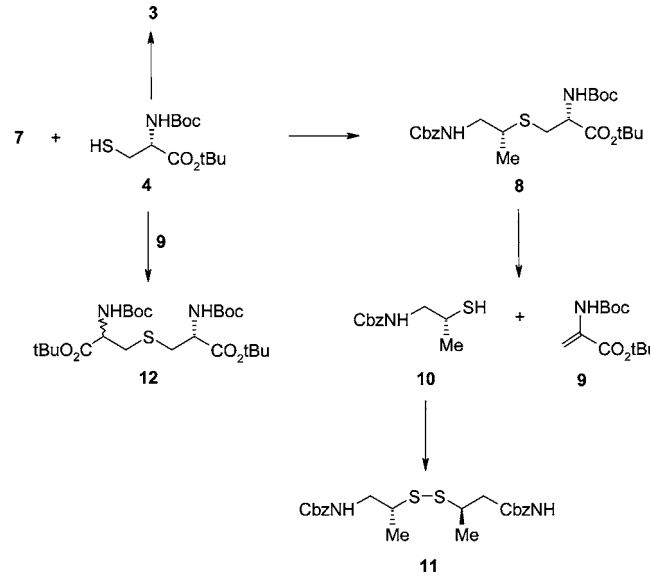
acetonitrile at 10 °C for 13 h (Scheme 3). Thioether **8** was obtained in 82% yield after column chromatography. Problems associated with this method included the large volumes of acetonitrile needed to be degassed in order to prevent dimerization of thiol **4** to disulfide **3**, and chromatography to remove unreacted **7** and other side products.

During the course of our investigation a crystallisation procedure was developed that allowed, for the first time, isolation of **8** without the need for chromatography. This process tolerated significant amounts of **4** (more than 1 equivalent in excess) but not of tosylate **7**; hence, our focus was turned to the stoichiometry of the reaction. In our hands there was complete consumption of the tosylate **7** when the reaction was carried out at ambient temperature with a 20% molar excess of both thiol **4** and cesium carbonate, to give **8** in good quality after crystallisation. Laboratory experiments on 5−10 g scale also showed that the volume of acetonitrile could be reduced from 43 volumes to 15 volumes. The cesium tosylate that precipitates out of the reaction mixture, however, causes thickening of the medium and poor mixing of the heterogeneous components at higher concentrations; thus, a 22-volume process was deemed optimum. The improved process was successfully demonstrated on 300 g scale in the kilo-lab and product **8** was obtained in 78% yield. Stretching experiments had shown that prolonged reaction times caused the product to degrade under the reaction conditions, and therefore, an assay method was also developed to establish the point at which the product concentration had reached a maximum. The revised conditions coupled with the assay method formed the protocol we adopted to prepare more than 6.0 kg of **8** in 79% yield using 50 L reactors in the plant. The apparent lack of robustness of this process however prompted us to look deeper into the details of this transformation.

Interestingly, during this work, some reactions unexpectedly failed to go to completion, with up to 60% **7** remaining unreacted. Addition of excess of each of the reagents and/or base failed to restart these reactions. From these runs **8** could only be isolated in low yield after chromatography because the presence of **7** compromises the crystallisation process. Since this is a heterogeneous reaction we initially looked into the particle size and water content of the hygroscopic Cs_2CO_3, speed of agitation and related mass transfer/mixing effects but despite our systematic approach the stalling effect not only persisted but also eluded our understanding.

In an attempt to understand the intrinsic aspects of this unusually sensitive S_N2 process, a stalled reaction was allowed to run for longer than usual, and its mass balance was subsequently examined. The first impurity isolated was acrylate **9** (Scheme 4), arising from base-induced elimination of thiol **10** from **8**.[8] The high HPLC response factor of **9** was

Scheme 4. **Degradation of 4 and 8 and mechanism of impurity formation**



misleading, as this was actually isolated in unexpectedly low amounts despite the severe decomposition of **8**. Not surprisingly, the Cbz-protected amino thiol component **10** was found in the reaction mixture together with its oxidised form namely disulfide **11**.

Since the elimination of the acrylate is a reversible process we expected that the corresponding conjugate addition of **10** to **9** could act as a mechanism of racemisation of the stereogenic centre α to the ester group. The optical purity of the surviving **8** however had not deteriorated appreciably (96% ee), implying that under these conditions when deprotonation α to the ester occurs, elimination is much faster than reprotonation (epimerization). The isolation of thiol **10** also indicates that its conjugate addition to acrylate **9** is not a facile process and in fact **10,** being an oil, remains in the liquors at the end of the process unless exposed to oxygen at elevated pH in which case it oxidatively dimerises to the corresponding disulfide **11** (Scheme 4). The major fate of **9** became evident upon characterisation of compound(s) **12** (Scheme 4), which accounted for 20% of the mass of the crude reaction mixture. The diastereomeric mixture of sulfides **12** arises from the conjugate addition of the excess primary thiol **4** to the eliminated acrylate **9** and had escaped our attention because of its poor UV absorbance. Apparently, the anion of **10**, being secondary and less reactive, cannot compete for **9** against the thiolate derived from the primary and more abundant **4**.[9,10] The latter pathway essentially renders **10** the sink for the overall process. Other impurities include disulfide **3**, arising from oxidative dimerization of the starting thiol **4** in the same way as **10** converts to **11**. Although **3** and **11** are formed in small amounts, they are not tolerated well in the crystallisation and therefore their formation had to be suppressed. Since the auto-oxidation of thiols takes place in the presence of oxygen and at high pH, the solvent had to be

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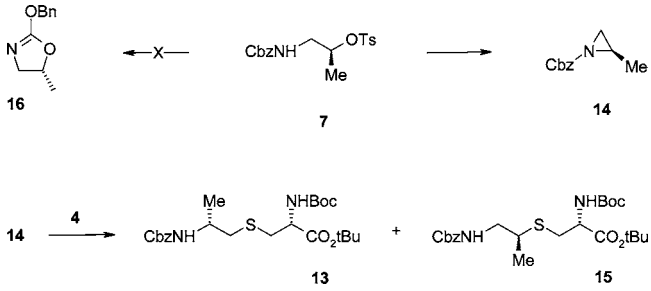
Scheme 5. **Degradation of 7 and mechanism for formation of impurities**



degassed and we found that refluxing the solvent under nitrogen or distilling 1−2 volumes prior to dissolving the thiol. Both of these methods are superior to degassing by sparging with nitrogen gas. Quenching the reaction with 15% aqueous NH_4Cl also helped to reduce disulfide formation.

The major problem however was the base sensitivity of the product and the side reactions of the degradants. At this point it became clear that the continuous exposure of the product to Cs_2CO_3 was not offering a sufficiently robust process for use in our next campaign. Thus a wide range of both inorganic and organic bases were screened in pursuit of a better coupling process but only K_3PO_4, Cs_2CO_3 and DBU appeared successful. The latter, although very effective in bringing about the desired transformation, caused extensive epimerization α to the ester group in product **8**. K_3PO_4 is comparable to Cs_2CO_3 in rate and profile but less consistent due to the irregular particle size and water content. Phase transfer catalysis offered no advantage with these heterogeneous systems; hence, we committed our efforts to kinetic organic bases. Thus, all Li, Na, and K salts of TMSOH, HMDS, and *t*-BuOH as well as the corresponding metal hydrides were tested. The reaction performance parallels the solubility of the metal thiolate, with the potassium species being completely soluble in a range of solvents unlike its Li and Na counterparts. The reaction is fastest in MeCN but not as clean as in acetone, THF, TBME, or mixtures of the latter solvents. We opted for stoichiometric quantities of *t*-BuOK (20% w/w in THF) with TBME as the bulk reaction solvent, and rewardingly, in contrast to the Cs_2CO_3 method, the reaction is homogeneous, never stalls, completes within 12 h, and can be left for several days without deterioration of either the ee or the chemical yield.

Some samples of **8** prepared by this method however contained a product-like impurity as high as 20% by NMR although no molecular ions were observed by LC/MS other than that corresponding to the product. This new impurity did not match any of the components corresponding to the four possible diastereoisomers of **8** previously prepared via the racemic synthesis. Fractional crystallisation of the enriched samples afforded good quantities of this new impurity, and ^1H NMR studies confirmed that this is the topological isomer **13** (Scheme 5), arising from the transposition of the methyl group to the adjacent carbon atom.

A rationale for the production of isomer **13** involved the ring-opening of aziridine **14**, formed by deprotonation of the CbzNH moiety and intramolecular displacement of the tosylate group within **7** (Scheme 5).[11,12] Consequently, **14** may be ring-opened by the (*R*)-thiolate at either the primary carbon atom, resulting in the unwanted isomer **13**, or at the secondary carbon atom with inversion, resulting in the also unwanted (*R*,*S*)-diastereoisomer **15** through an overall double inversion [note that **8** has the (*R*,*R*) stereochemistry]. We also looked for oxazoline **16** which could arise from the intramolecular displacement of the tosylate by the oxygen atom of the carbamate carbonyl group (Scheme 5), but none was detected.[13] Oxazoline **16** may be attacked at the benzyl group by nucleophiles (**4** being the best nucleophile in the reaction mixture) to generate the parent Evans-type oxazolidinone, but neither this nor the *S*-benzyl derivative of **4** was found. The absence of these impurities suggested that the pathway leading to **16** was not occurring.

The aziridine hypothesis was put to the test, and we were able to synthesise (*R*)-*N*-Cbz-2-methyl aziridine (**14**) by treating tosylate **7** with either Cs_2CO_3 or *t*-BuOK. Subsequent treatment of the aziridine with either K or Cs thiolate of **4** revealed an interesting trend. The kinetic base, which ensures product stability, was completely consumed in the reaction and gave an approximately 1:1 mixture of **15** and **13**, whereas by using the usual excess of Cs_2CO_3 reasonably pure **15** was isolated, albeit at half the overall yield. This suggests that the latter reaction probably exhibits the same regioselectivity for the ring-opening of the aziridine **14**, but isomer **13** is degraded faster than **15** by Cs_2CO_3 due to the elimination of a primary thiolate instead of the less likely leaving group secondary thiolate. This may explain the negligible amounts of **13** found in the re-examined crude samples of **8** prepared in our first campaign using the Cs_2CO_3 method.

The countermeasure we included in our new process was to preform the thiolate species and then add the tosylate **7**, thus avoiding exposure of **7** to excess of base. This was successful, provided that a short time was allowed for the deprotonation because the thiolate species decomposes with time into the Boc protected α-amino acrylate **9** and potassium hydrogen sulfide. In fact all of **4**, **7**, and **8** are sensitive to base, and their decomposition is concentration-, temperature-, and time-dependent. Thiolate formation is essentially complete at 10 °C by the time the base is added (over 10 min) as indicated by monitoring the exotherm which is actually allowed to raise the batch temperature to 22 °C. Rapid addition of the tosylate to the potassium thiolate at this point allows the reaction to proceed at a useful rate. At less than 15 °C the S_N2 reaction is slow, and proton transfer from the NHCbz group to the thiolate and subsequent aziridine formation becomes the dominant pathway. A tight control of *t*-BuOK between 1.05−1.10 equiv in the presence of 1.2 equiv of thiol offers a robust process with high conversion, minimum product degradation, and reaction completion within 10−12 h. Chiral analysis on the crude reaction mixtures indicates that the regioisomer **15** forms at about 3−5%, suggesting deprotonation of the NHCbz moiety by the thiolate species, and therefore aziridine formation occurs unavoidably

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(12) Hodgson, D. M.; Humphreys, P. G.; Miles, S. M.; Brierley, C. A. J.; Ward, J. G. *J. Org. Chem.* **2007**, *72* (26), 10009–10021.
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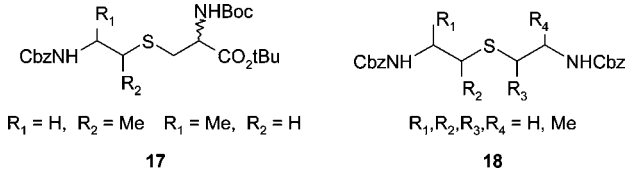


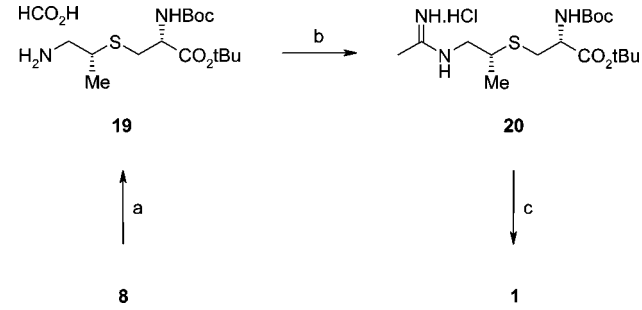
Figure 2

in the background. Subsequent crystallisation, however, furnishes the desired thioether **8** typically in 80% isolated yield with regioisomer levels consistently in the range of 1−2%. Moreover, the unwanted isomer **13** is not only tolerated but is completely removed in the next stages.

Finally, traces of impurities **17** (diastereoisomers of **8** and **13**) and **18** (Figure 2) were also isolated from the crystallisation liquors. This further supported our understanding which can be summarised in the following way: A family of product-like compounds **17** (all isomers, Figure 2) may result from **4** engaging both **7** and **14**. Elimination in isomers **17** can in turn provide **9** and several thiols that may also attack **7** and **14** to furnish the group of thioethers **18**. In addition, the reversibility of the elimination of isomers **17** via conjugate additions to **9** offers an epimerization pathway that enhances the population of group **17**. According to the argument above, the stalling effect in the Cs_2CO_3 method may be due to base starvation since part of the base is consumed in deprotonating the CbzNH group. Base added during the process induces elimination in the already formed base-sensitive product **8** (and/or **13**), while the resulting electrophile **9** may offer a more reactive partner than tosylate **7** to any thiolate anion present. This mechanistic proposal is in accord with the impurity profile and explains adequately both the stalling effect and why the reaction cannot be revived once derailed. To transform such a sensitive reaction into a robust process we believe is a remarkable achievement. More than 500 kg of **8** was produced in seven batches using the *t*-BuOK process in the plant.

Cbz Deprotection. In the next reaction we pursued the removal of the Cbz group in **8** via hydrogenolysis. Despite screening a variety of conditions and a number of catalysts, this transformation proved unusually problematic as it frequently required up to 1.2 wt equiv of Perlman's catalyst [20% $Pd(OH)_2$ on wet charcoal]. Below 1 wt equiv of the catalyst the reaction did not proceed to completion. In the past we had successfully carried out Pd-catalysed hydrogenation/lysis of sulfurous substrates; however, in this case we believe the nature of the reaction product **19** (1,2-amino sulfide with pendant amino acid moiety) may be responsible for strong ligation to palladium species, inhibiting thus the metal to re-enter the catalytic cycle. Even with catalysts known for their resistance to sulfur poisoning, we were unsuccessful in achieving a catalytic reaction superior to that obtained with palladium hydroxide. We examined alternative protocols for removal of the Cbz group, but these met either with failure or epimerization or required significant development work. In order to meet agressive project timelines we decided to develop the existing transfer hydrogenolysis process in ethanol using aqueous ammonium formate as the hydrogen source (Scheme 6). Next, we investigated a number of methods to remove the palladium residue from the reaction mixture, and the best way for doing this was to add Harbolite clay (filtering aid) in the rector to

***Scheme 6.* Final steps in the synthesis of 1**[a]



[a] Reagents and conditions: (a) $Pd(OH)_2$ EtOH, aq NH_4HCO_2, EtOAc, HCO_2H, 86%; (b) EtOAc, *S*-naphthyl methyl thioacetamidine hydrochloride, 85%; (c) toluene, K_2CO_3, then solid H_3PO_4, water, acetone, 82%.

absorb the catalyst and filter the reation mixture prior to aqueous workup. The solution of the crude amine obtained after extraction of the free base into ethyl acetate was found to be contaminated by approximately 2% of the *N*-ethyl analogue of the expected amine (possibly due to acetaldehyde generated by hydrogen transfer involving the solvent and the Pd species present). Because the desired free amine is not crystalline, after a screen of appropriate acids we decided to isolate the product as the formate salt **19** (Scheme 6). Rewardingly, the crystallisation of this salt from ethyl acetate removed the *N*-ethyl impurity completely, but the corresponding amine arising from Cbz removal from impurity **13** could not be eliminated (1−2%). The crystallisation of the formate salt proved sensitive to the water content carried over from the aqueous workup. In plant, we employed multiple distillations of ethyl acetate to azeotropically dry the solution of the amine prior to adding formic acid. In this way, more than 500 kg of **19** was made in five pilot-plant batches. The palladium levels in these batches were consistently below 20 ppm and were reduced to zero after the final stage.

Preparation of the Amidine Intermediate. In the subsequent stage the amidine hydrochloride **20** (Scheme 6) is prepared by the action of a slight excess of *S*-(1-naphthyl methyl) thioimidate on the amine formate **19**.[14] The reaction, as inherited from medicinal chemistry, required 18 h in 17 volumes of THF and excess of the amidinating agent followed by aqueous workup and chromatographic purification. Apart from the offensive odor of the thiol liberated during the destruction of *S*-(1-naphthylmethyl)thioacetimidate, the latter was also flagged as too toxic an impurity to carry forward in the final step. The same applied for acetamide which is also generated from the hydrolysis of the amidinating agent. For these reasons, significant efforts were devoted into limiting these undesirable impurities in our penultimate intermediate. We achieved this by using stoichiometric amounts of **19** and *S*-(1-naphthylmethyl)thioacetimidate suspended in ethyl acetate which allows direct precipitation of the amidine hydrochloride product **20**.[15] Addition of methanol at this stage decomposes the small amount of residual amidinating agent and helps to solubilise the impurities. The product is isolated via a simple

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(15) Box, D.; Colclough, D.; Gillies, I.; Loft, M. S.; Moore, R., PCT Int. Appl. 2002, WO 2002050021 A1 20020627, Appl. WO 2001-GB5596 20011217 CAN 137:47442, AN 2002:487518, 2002.

filtration in 85% yield and excellent purity, and more importantly, the amine and amidine derived from **13** are not detected.

Final Deprotection and Drug Substance Isolation. The final stage constitutes a combined global deprotection and salt-forming process where drug substance **1** is produced. The original version inherited from medicinal chemistry targeted the monohydrochloride salt via deprotection of the Boc group and hydrolysis of the *tert*-butyl ester with the use of anhydrous HCl in 1,4-dioxane. This process was undesirable due to the toxicity of 1,4-dioxane and the generation of the flammable isobutylene gas from the *tert*-butyl residues in the Boc and ester groups. Finally, the hygroscopic nature of both the mono- and dihydrochloride salts rendered these versions unacceptable for active pharmaceutical ingredient (API) development. From the version screen initiated, the monophosphate monohydrate salt emerged as the most suitable version for further development. Initially, 80% aqueous H_3PO_4 solution at 65 °C was used for the combined removal of the Boc and *tert*-butyl ester groups. During this process the desired phosphate salt was also generated. The use of solid phosphoric acid in the aqueous deprotection/salt-forming step allowed for greater control of the amount of reagent charged and consequently on the salt stoichiometry (API). Generation of the free base of **20** into toluene and treatment with a freshly prepared H_3PO_4 solution facilitated the desired reactions, and rewardingly no isobutylene gas evolution was detected in this process with online mass spectrometry. The fate of the *tert*-butyl group associated with the Boc and the ester functionalities is its conversion into *tert*-butanol.[15−17] Addition of the aqueous phase into acetone with seeding facilitates robust crystallisation of **1**. Upon drying, however, **1** dehydrates, and significant efforts were made to meet the challenge of consistent preparation of the monophosphate monohydrate salt. In collaboration with our Particle Science and Physical Properties departments no other hydrated forms were identified by standard solid form screening methods. Subsequently, the dehydration of the monohydrate was shown to be fully reversible by DSC, GVS, XRPD, and Raman spectroscopy. At scale, a reconditioning process was implemented using a Boltz dryer to achieve dehydration followed by the application of a moist nitrogen stream through the dryer to rehydrate to the monohydrate. This method proved robust and consistent for the manufacture of iNOS inhibitor **1** which was also analysed by a variety orthogonal techniques including chiral HPLC (99.9% PAR), metal analysis (Pd not detected), phosphate and water analysis (consistent with the monophoshate mononohydrate stoichiometry), and XRPD (consistent with desired polymorph and crystallinity).

3. Conclusions

In conclusion, we have described our efforts to deliver an efficient supply route for the iNOS inhibitor **1**. Although this route was capable of delivering multikilogram quantities of material for ongoing studies, we have further investigated alternative routes which address the current issues of reagent toxicity and the overall cost of goods. These are described in a separate communication.[18]

4. Experimental Section

^{1}H NMR and ^{13}C NMR data were acquired on a 400 MHz instrument.

Tosylate (7). *Phenylmethyl[(2S)-2-hydroxypropyl]carbamate-1-methyl-methylsulfonylbenzene.* Benzylchloroformate (72.64 kg, 2.27 wt, 1 equiv) was added to an aqueous solution (320 L, 10.0 vol) of (*S*)-1-amino-2-propanol **5** (32 kg, 1.0 wt) and potassium carbonate (64.96 kg, 2.03 wt) at <20 °C. The mixture was stirred at ambient temperature for 18 h and then extracted twice with dichloromethane (320 L, 10.0 vol and 128 L, 4.0 vol). The dichloromethane solution was washed with 5 M hydrochloric acid (128 L, 4.0 vol) and then diluted with additional dichloromethane (192 L, 6.0 vol). The organic phase was then concetrated by vacuum distillation, during which appoximately 200 L of dichloromethane were removed. The concentrated solution of the Cbz-protected amino alcohol **6** was then added in one charge to a suspension of tosyl chloride (137.92 kg, 4.31 wt) in dichloromethane (160 L, 5 vol), and the transfer line was washed with 32 L of the same solvent. Trimethylamine hydrochloride (48.96 kg, 1.53 wt) was added, follwed by the addition of triethylamine (160 L, 5.0 vol) at such a rate wherein the temperature would be maintained below 20 °C. The resultant slurry was stirred at ambient temperature for ∼0.5 h and then quenched by the addition of water (320 L, 10.0 vol). The phases were separated, and the organic phase was washed successively with 5 M hydrochloric acid (320 L, 10.0 vol), 10% w/w aqueous sodium carbonate (320 L, 10.0 vol), and water (320 L, 10.0 vol). The volume was reduced by vacuum distillation to 160 L, and the product was crystallised by the addition of heptane (480 L, 15.0 vol). The product was isolated by filtration and dried at 40 °C under vacuum; 130.2 kg, 84% th. yield, 99.2% pure by ^{1}H NMR.

^{1}H NMR in $CDCl_3$ ($CHCl_3$ as reference at 7.26 ppm) δ 1.24(3H) d J = 6.4 Hz, 2.40 (3H) s, 3.17−3.26 (1H) m, 3.39−3.47 (1H) m, 4.65−4.74 (1H) m, 4.99−5.11 (3H) m, 7.30 (2H) d J = 8.2 Hz, 7.31−7.39 (5H) m, 7.78 (2H) d J = 8.2 Hz. ^{13}C NMR ($CDCl_3$ as reference at 77.00 ppm) δ 18.14, 21.58, 45.72, 66.82, 78.72, 127.71, 127.92, 128.12, 128.50, 129.87, 133.75, 136.28, 144.82, 156.33. HRMS (Electrospray +) calculated for the protonated molecular ion (MH^+) $C_{18}H_{22}N_1O_5S_1$ 364.1219, found 364.1210.

Thioether (8). *1,1-Dimethylethyl-N-{[(1,1-dimethylethyl)oxy]carbonyl}-S-[(1S)-1-methyl-2-({[(phenylmethyl)oxy]carbonyl}amino)ethyl]-L-cysteinate.* TBME (1500 L, 20 vol) was degassed by distillation, and the total volume was adjusted to 1350 L, 18 vol. Thiol **4** (75 kg, 1 wt) was added, and the mixture was stirred under nitrogen to form a solution. The solution was cooled to 10 ± 3 °C, and potassium *tert*-butoxide in THF (20.5% w/w, 120 kg, 1.6 wt, 1.06 equiv) was added in less than 10 min (exothermic, ∼12 °C), and the reaction mixture was stirred for a maximum of 5 min to allow the exotherm to subside. Immediately after, a solution of tosylate **(7)** (75 kg, 1 wt) in THF (187.5 L, 2.5 vol), was then added as fast as possible, followed by a THF (37.5 L, 0.5 vol) line-wash. After

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the addition was complete, the reaction mixture was warmed to 22 ± 3 °C and stirred for 12 h before it was quenched with 600 L, (8 vol) of 15% w/v aqueous ammonium chloride solution. The mixture is stirred for ~10 min until a clear biphasic solution is formed and then allowed to settle. The aqueous layer was removed, and the organic phase was washed successively with 450 L (6 vol) of 8% w/v aqueous sodium bicarbonate solution and 450 L (6 vol) of 30% w/v aqueous sodium chloride solution. The aqueous layer was removed, and the organic phase was circulated for at least 30 min through a bed of sodium sulfate (~75 kg) before transfer to a clean vessel. TBME [225 L (3 vol)] was used as vessel rinse and transferred into the vessel containing the bulk of the filtrate. The filtrate was concentrated by vacuum distillation to approximately 187.5 L (2.5 vol) while the temperature was maintained below 50 °C. TBME (450 L, 6 vol) was added, and the solution was reconcentrated to approximately 187.5 L (2.5 vol) by vacuum distillation within the same temperature range. This dilution−distillation cycle was repeated once more, and the solution was finally diluted with TBME to a total of 300 L (4 vol). Isooctane (450 L, 6 vol) was then added at 20 ± 3 °C over 1 h, and the resulting suspension was aged at 20 ± 3 °C for 1.5 h. The product was filtered, washed with a 1:5 mixture of TBME/isooctane (225 L, 3 vol), and dried under vacuum at 30 ± 3 °C; 82.2 kg, 85% th. yield, 98.2% pure by ^{1}H NMR (1.4% of **13** present, solvents <0.2%).

^{1}H NMR in $CDCl_3$ ($CHCl_3$ as reference at 7.26 ppm) δ 1.26 (3H) d J = 6.9 Hz, 1.43 (9H) s, 1.47 (9H) s, 2.85−3.02 (3H) m, 3.18−3.27 (1H) m, 3.32−3.41 (1H) m, 4.35−4.42 (1H) m, 5.11 (2H) s, 5.27−5.34 (1H) m, 5.39 (1H) broad, 7.28−7.38 (5H) m. ^{13}C NMR ($CDCl_3$ as reference at 77.00 ppm) δ 19.10, 27.96, 28.27, 33.28, 41.40, 45.84, 53.89, 66.72, 77.21, 80.04, 82.72, 128.07*, 128.47, 136.53, 155.30, 156.48, 169.83 (* = two overlapping signals). HRMS (electrospray +) calculated for the sodiated molecular ion (MNa^+): $C_{23}H_{36}N_2O_6SNa$ 491.2192, found 491.2186.

Amine Formate Salt (19). *(2S)-2-{[(2R)-3-[(1,1-Dimethylethyl)oxy]-2-({[(1,1-dimethylethyl)oxy]carbonyl}amino)-3-oxopropyl]thio}-1-propanaminium Formate.* A mixture of thioether **8** (68 kg, 1 wt), 20% palladium hydroxide on carbon paste (68 kg, 1 wt) and IMS (680 L, 10 vol) was stirred and heated to 40−45 °C. A solution of ammonium formate (27.4 kg, 0.403 wt, 3 equiv) in 1:1 water/IMS (136 L, 2 vol) was added over ~30 min, and the reaction mixture was kept at 40−45 °C for a further 30 min before it was cooled to 20 °C. Harbolite 800 filter aid (40.8 kg, 0.6 wt) was added, and the mixture was stirred for 15−30 min before the solids (catalyst and filter aid) were removed by filtration. The filter cake was washed with IMS (340 L, 5 vol), and the combined filtrates were concentrated under vacuum to approximately (204 L, 3 vol). Ethyl acetate (578 L, 8.5 vol) was added, and the mixture was concentrated under reduced pressure vacuum to approximately (204 L, 3 vol). This dilution−distillation cycle was repeated once more before ^{1}H NMR was run to ensure that ethanol levels were acceptable (<5% w/w). Ethyl acetate (340 L, 5 vol) and 10% sodium carbonate solution (748 L, 11 vol) were added, and after a 10 min stirring period, the phases were separated. The aqueous phase was extracted with ethyl acetate (204 L, 3 vol), and then the combined organic solution was washed with water (408 L, 6 vol). The ethyl acetate solution was concentrated under reduced pressure to approximately 204 L (3 vol). Two cycles of diluting with ethyl acetate (578 L, 8.5 vol) and concentrating to approximately to 204 L and 408 L, respectively, were performed. KF analysis of the final solution confirmed acceptable water content (<2% w/w), the solution was then cooled to 5 °C, and formic acid (6.8 kg, 0.1 wt, 1 equiv) was added. The resulting slurry was aged for 1 h at 5 °C. The product was filtered, washed with ethyl acetate (2 × 102 L), and dried at 40 °C under vacuum to constant probe temperature; 45.48 kg, 86.0% th. yield, 97.6% pure by ^{1}H NMR (0.9% *N*-ethyl analogue and 1.1% of deprotected **13** present, solvents <0.2%).

^{1}H NMR in DMSO-d_6 (TMS as reference at 0.0 ppm) δ 1.22(3H) d J = 6.9 Hz, 1.39 (9H) s, 1.41 (9H) s, 2.68−2.85 (4H) m, 2.85−2.95 (1H) m, 3.92−4.00 (1H) m, 7.24 (1H) d J = 7.7 Hz, 8.34 (1H) s. ^{13}C NMR (DMSO-d_6 as reference at 39.50 ppm) δ 18.80, 27.60, 28.13, 30.85, 40.37, 45.09, 54.85, 78.29, 80.87, 155.31, 165.06, 170.09. HRMS (Electrospray +) calculated for the protonated molecular ion (MH^+) $C_{15}H_{31}N_2O_4S$ 335.2005, found 335.1994.

Amidine Hydrochloride (20). *(2S)-N-[(1Z)-1-Aminoethylidene]-2-{[(2R)-3-[(1,1-dimethylethyl)oxy]-2-({[(1,1-dimethylethyl)oxy]carbonyl}amino)-3-oxopropyl]thio}-1-propanaminium Chloride.* To a suspension of amine formate salt **19** (80.0 kg, 1 wt) in ethyl acetate (800 L, 10 vol) was added *S*-(1-naphthylmethyl)thioacetimidate hydrochloride (54.4 kg, 0.68 wt, 1.03 equiv) followed by an ethyl acetate (8 L, 0.1 vol) line wash. The mixture was stirred 2 h at 20 °C before it was quenched by the addition of methanol (0.4 L, 0.005 vol). Subsequently, the reaction mixture was heated to 55 °C for 2.5 h, cooled to 20 °C, and stirred for 1 h. The resulted slurry was filtered, and the cake was washed first with ethyl acetate (2 × 120 L) and then with MIBK (2 × 120 L). The product was dried at 45 °C under reduced pressure to constant probe temperature; 83.85%, 97% th. yield, 99.5% pure by ^{1}H NMR (solvents <0.2%). Chloride by IC 8.5% w/w, th. 8.6% w/w.

^{1}H NMR in DMSO-d_6. (TMS as reference at 0.0 ppm) δ 1.25(3H) d J = 6.8 Hz, 1.39 (9H) s, 1.41 (9H) s, 2.19 (3H) s*, 2.78−2.91 (2H) m, 3.00−3.09 (1H) m, 3.35 (2H) d J = 6.9 Hz, 3.92−3.99 (1H) m, 7.21 (1H) d J = 8.1 Hz, 8.85 (1H) broad, 9.37 (1H) broad, 9.57 (1H) broad (* = This signal has an associated minor rotameric peak at 2.24 ppm). ^{13}C NMR in DMSO-d_6 (DMSO-d_6 as reference at 39.5 ppm) δ 18.56, 18.91, 27.60, 28.14, 31.27, 38.53, 47.18, 54.85, 78.33, 80.92, 155.30, 164.23, 170.03. HRMS (Electrospray +) calculated for the protonated molecular ion (MH^+) $C_{17}H_{34}N_3O_4S$ 376.2270, found 376.2262.

Drug Substance (1). *(2S)-2-{[(2R)-2-Amino-2-carboxyethyl]-thio}-N-[(1Z)-1-aminoethylidene]-1-propanaminium Phosphate Monohydrate.* To a solution of 20 kg of amidine salt **20** in water (70 L, 3.5 vol) was added toluene (70 L, 3.5 vol) and the mixture was stirred vigorously and cooled to 5 ± 2 °C. A solution of potassium carbonate (24 kg, 1.2 wt) in water (24 L, 1.2 vol) was added over 15 min at 5 ± 2 °C, and the mixture was stirred at the same temperature for 30 min. The phases were allowed to settle, and the aqueous phase was removed.

To the organic phase was added a solution of phosphoric acid (5 kg, 0.25 wt) in water (48 L, 2.4 vol) over 10 min with the temperature being kept below 5 ± 3 °C. The biphase was then stirred at 72 ± 2 °C for 18 h and cooled to 20 °C, and then the layers were separated. The aqueous phase was added over 2 h to a preheated mixure of acetone (80 L, 4 vol) and water (16 L, 0.8 vol) at 40 ± 2 °C containing 60 g (0.003 wt) of seed of **1**. The temperature was maintained at 40 ± 2 °C throughout the addition, and the resulting suspension was stirred at 40 ± 2 °C for 15 min. Further acetone (48 L, 2.4 vol) was added over 60 min to the suspension at 40 ± 2 °C, and the mixture was stirred at 40 ± 2 °C for 15 min before it was cooled to 5 ± 2 °C and held at this temperature for 1 h. The slurry was filtered, and the cake was washed with acetone/water 3:1 (2 × 50 L) and then with acetone (3 × 40 L). The cake was deliquored for 60 min, and the product was dried in a Boltz dryer at 60 °C and at 10 mbar to constant probe temperature. Anhydrous **1** was then rehumidified over 2 h by "tumbling" in the presence of wet nitrogen (wet nitrogen stream was produced by passing nitrogen gas through 20% w/v aqueous sodium chloride solution). Isolated was 12.2 kg of **1**; 74.1% th. yield, 99.7% pure by ^{1}H NMR, >99.95% a/a by chiral HPLC, water by KF 5.35% w/w, th. 5.36 w/w, phoshate by IC 29.85% w/w, th. 29.22% w/w.

^{1}H NMR in D_2O (HDO as reference at 4.80 ppm) δ 1.35(3H) d J = 6.9 Hz, 2.31 (3H) s*, 3.14−3.29 (3H) m, 3.42−3.56 (2H) m, 4.00−4.04 (1H) m (* = This signal has an associated minor rotameric peak at 2.34 ppm). ^{13}C NMR in D_2O (TSP as reference at 0.00 ppm) δ 20.92, 21.35, 33.10, 40.73, 49.95, 56.60, 168.17, 175.62. HRMS (Electrospray +) calculated for the protonated molecular ion (MH^+) $C_8H_{18}N_3O_2S$ 220.1120, found 220.1111.

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