Organic Process Research & Development **2003**, *7*, 58–66

A New Approach to Rapid Parallel Development of Four Neurokinin Antagonists. Part 2. Synthesis of ZD6021 Cyano Acid

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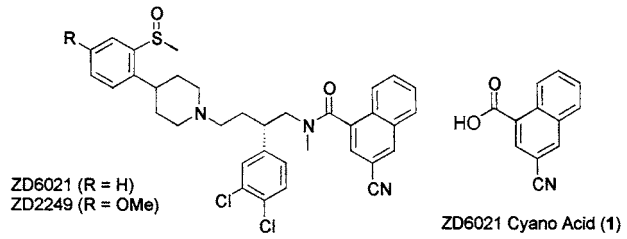
Abstract:

The manufacture of ZD6021 cyano acid (1) using a new project approach is described. Research Department processes were scaled up to 100 L if process safety and robustness were not compromised; other factors were treated according to the new approach. By using this strategy, we were able to manufacture a key intermediate on sufficient scale to support delivery of 1 kg quantities of bulk drug within 6 months of the start of lab work.

Introduction

The preceding contribution[1] presented a new approach by Zeneca Pharmaceuticals to the rapid parallel development of several candidate drugs. An overview of the strategy was given and illustrated by examples taken from across the neurokinin (NK) project.[2] This contribution and the next[3] discuss in more detail the experiences of manufacture of key fragments of molecules required for the NK programme and seek to assess the application and success of this new approach in more detail.



The first compounds in the NK programme, ZD6021 and ZD2249, both required the cyano acid (**1**) as a common structural unit. The Research Department route built on that of Dewar,[4] and since a key aspect of the new approach was to apply the Research-based route in all possible cases, provided that process safety considerations permitted, this was the route we sought to develop. The route to cyano acid used the same intermediates as the Research route[2] with some changes of reagents as shown in Scheme 1.

The simple structure of **1** disguises the fact that 1,3-disubstituted naphthalenes are difficult to make. This substitution pattern defies the standard directing rules for naphthalenes, namely that activating groups are ortho/para-directing in the same ring while deactivating groups are meta-directing in the other ring. Fortunately, 3-bromo-1-naphthoic acid is described in the literature,[5] and its synthesis relies on the introduction of a temporary group to effect the substitution. Naphthalic anhydride is brominated in the 3-position, and mercuric oxide-mediated decarboxylation gives predominantly the desired 1,3-isomer.[6] Conversion of this compound to the desired cyano acid relies on more straightforward chemistry.[2,4] Given the absence of other routes, successful preliminary scale-up of the chemistry in the Research Department facilities, and an acceptance that mercury waste could be disposed of reliably on a small scale, it was decided to use this route for the first delivery. However, given the undesirable nature of the process from an environmental viewpoint, a project was initiated at Zeneca Agrochemicals (Huddersfield) to investigate a longer-term manufacturing route which is reported in this issue.[7] It will be helpful to know in the discussion that follows that we remanufactured cyano acid for ZD2249 by the same route as for ZD6021, on which most of the discussion is focused.

Results and Discussion

Bromo Anhydride Stage (3). The bromo anhydride stage provided an early and multifaceted example of the application of the new project approach. The process used by Research started with 1,8-naphthoic anhydride (**2**) in 70% concentrated nitric acid to which 0.75 equiv of bromine was added at 50 °C.[5] A typical yield of 17% was achieved with acceptable quality, residual starting material at the 3−4% level being the main impurity, which was readily removed in the following stages. Attempts to push the reaction further invariably resulted in multiple bromination products and a poor impurity profile.[5] The process was assessed by our Hazards Group and deemed to be safe with appropriate precautions (mainly adequate gas disengagement capacity). We therefore decided this was a Research-based process

† Large Scale Laboratory, Macclesfield.

(1) Moseley, J. D.; Moss, W. O. *Org. Process Res. Dev.* **2003**, *7*, 53−57.
(2) Bernstein, P. R.; Aharony, D.; Albert, J. S.; Andisik, D.; Barthlow, H. G.; Bialecki, R.; Davenport, T.; Dedinas, R. F.; Dembofsky, B. T.; Koether, G.; Kosmider, B. J.; Kirkland, K.; Ohnmacht, C. J.; Potts, W.; Rumsey, W. L.; Shen, L.; Shenvi, A.; Sherwood, S.; Stollman, D.; Russell, K. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2769. Albert, J. S.; Aharony, D.; Andisik, D.; Barthlow, H.; Bernstein, P.; Bialecki, R. A.; Dedinas, R.; Dembofsky, B. T.; Hill, D.; Kirkland, K.; Koether, G. M.; Kosmider, B. J.; Ohnmacht, C.; Palmer, W.; Potts, W.; Rumsey, W.; Russell, K.; Shen, L.; Shenvi, A.; Sherwood, S.; Warwick, P. J. *J. Med. Chem.* **2002**, *45*, 3972.
(3) Parker, J. S.; Bowden, S. A.; Firkin, C. R.; Moseley, J. D.; Murray, P. M.; Welham, M. J.; Wisedale, R.; Young, M. J.; Moss, W. O. *Org. Process Res. Dev.* **2003**, *7*, 67−73.
(4) Dewar, M. J. S.; Grisdale, P. J. *J. Am. Chem. Soc.* **1962**, *84*, 3541.
(5) Rule, H. G.; Thompson, S. B. *J. Chem. Soc.* **1937**, 1764.
(6) Whitmore, F. C.; Fox, A. L. *J. Am. Chem. Soc.* **1929**, *51*, 3363.
(7) Ashworth, I.; Bowden, M. C.; Dembofsky, B.; Levin, D.; Moss, W. O.; Robinson, E.; Szczur, N.; Virica, J. *Org. Process Res. Dev.* **2003**, *7*, 74−81.

Published on Web 12/17/2002
10.1021/op020065h CCC: $25.00

Scheme 1

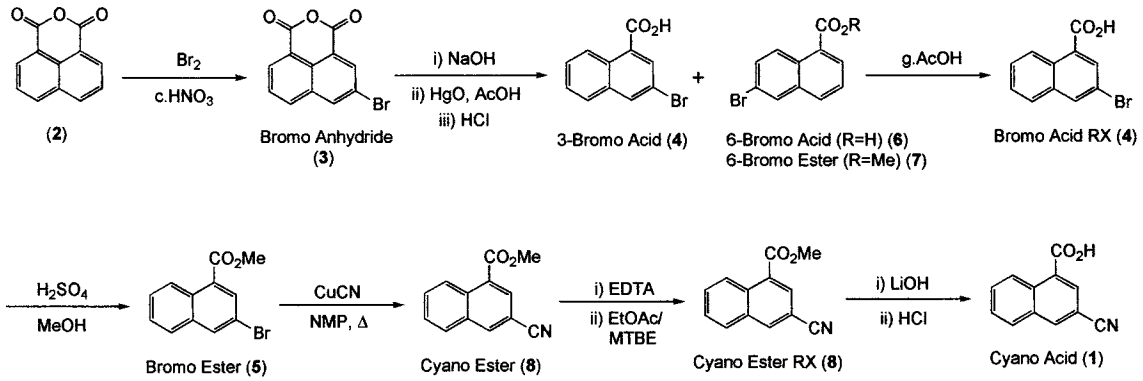


Table 1. **FED parameters for bromo anhydride stage**

parameter	low level	high level
temperature (°C)	50	65
bromine charge (equiv)	0.75	0.95
bromine addition time (min)	10	60
nitric acid charge (vols)	15	20
reaction time (min)	30	240

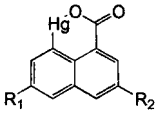
which could be operated in the Large Scale Laboratory (LSL)[8] for the following reasons: (a) process safety was acceptable, (b) cheap and available reagents meant that the low output could be tolerated, (c) the volume was not so prohibitively high that efficiency would be compromised, (d) the process was operationally simple (except for the handling of bromine, which could be accommodated due to the flexibility of the LSL), and (e) product quality was satisfactory.

We performed a small factorial experimental design (FED) to check for robustness and to determine if we could improve the modest yield. We investigated five parameters at two levels in eight experiments with three centre points, giving a quarter factorial overall. The parameters investigated with their high and low values are shown in Table 1.

A higher charge of bromine gave an apparently higher yield, but quality was much worse. A reduction to 15 vols was possible, but not to 10. However, we were advised by Hazards Group not to go below 20 vols for safety reasons. Overall, no improvements were made compared to the Research process, but we did establish that the process was robust within wide ranges for several key parameters. Having met the specified key criteria[1] of process safety and robustness with acceptable quality, seven batches of **3** were manufactured in the LSL for ZD6021 in an overall yield of 16.7%,[9] with quality in the range 92−96%.

This process also left us with large quantities of acidic waste containing a high level of bromine. We had taken the decision to accept the costs of disposal and also reasoned that the quantities would not be prohibitive on this scale. However, we soon discovered that the liquors were unstable and resulted in gassing. It was necessary to dilute the liquors 2-fold with water to suppress this gassing to an acceptable level as determined by our Hazards Group. We had also stored some of the waste in HDPE containers, but the residual bromine attacked the plasticisers, making the containers brittle; thus, the waste was transferred to glass bottles with venting caps. Given these additional considerations and the additional quantities of acidic waste we generated, it is doubtful that we would have repeated this process on the pilot-plant scale (i.e., >10 kg). However, we did repeat the synthesis of ZD6021 cyano acid (**1**) using this process to make material for ZD2249, and achieved an identical yield of 16.7% over five batches with no processing issues. We therefore felt we had been justified in our intention to take this process directly from Research and accommodate it in the LSL on this scale. (For a discussion of the alternative silver sulfate-mediated bromination, see below.)

Bromo Acid Stage (4/6). Again, the Research method was followed for this stage with only minor changes.[6] The process proceeded by digestion of the bromo anhydride (**3**) with NaOH followed by insertion of Hg into the anhydride with loss of one of the carboxylate groups as CO_2. The organo−Hg intermediates **9a** and **9b** were formed in a 3:1



9a R_1 = H, R_2 = Br
9b R_1 = Br, R_2 = H

ratio and could be isolated and oven dried. Acidic hydrolysis with concentrated HCl liberated 1 mol of CO_2 and yielded the desired 3-bromonaphthoic acid (**4**) (ZD6021 bromo acid) and its 6-bromo regioisomer (**6**), still in a 3:1 ratio.[10] The product was isolated by filtration from the cooled reaction mixture, washed, and dried to give a quantitative yield of the 2 isomers **4** and **6**.

(8) The Macclesfield LSL is a cGMP manufacturing facility for synthesis of bulk drug for clinical studies and uses all-glass vessels. It is typically where the first significant scale-up of a process occurs, and commonly delivers tens of kilograms of intermediates and kilograms of bulk drug. It consists of a range of glass reactors 10−100 L in scale, fully contained with other ancillary equipment in fume cupboards. Operating ranges vary from −78 to +130 °C. Atmospheric hydrogenations can be performed, and a 20-L rotary evaporator is available for distillations if required. Product is generally isolated as a solid on Nutsches. AstraZeneca has several other LSLs at different sites which operate in a similar fashion.

(9) The first batch gave poor-quality product which was not used and is not included in the yield figures. The reasons for this failure were never established, but poor-quality naphthoic anhydride (**2**) was suspected, and this batch of input was not used again.

(10) We had to develop a separate HPLC method to determine the regioisomer ratio at this stage. Details are given in the Experimental Section.

We made some initial improvements to the Research process by cutting down the reaction time for the Hg insertion reaction from 4 to 2 days. This was not easy to determine as the organo−Hg adducts (**9**) had very poor solubility as noted by Leuck,[11] and so each analytical sample taken had to be heated in concentrated HCl for several hours to liberate the bromo acids (**4/6**) before analysis. However, 2 days was found to be adequate for complete reaction in all cases. We were reluctant to isolate the organo−Hg intermediates (**9**) on the larger scale for handling and health hazard reasons, and indeed it was found unnecessary to do so; the process could be acidified directly after completion of the Hg insertion reaction. This also avoided an unnecessary filtration and drying procedure, as well as the handling issues. The acidic hydrolysis was complete in 4 h, with most of the CO_2 being driven off during heating.[12]

We also investigated the NaOH charge, which at 4.0 equiv was potentially all neutralised by the glacial acetic acid used to dissolve the HgO. An "all-in" reaction with no pretreatment of the anhydride by NaOH unsurprisingly failed to go to completion. However, overcharging the NaOH to 5 or 6 equiv quenched the reaction altogether, the vivid orange colour of the HgO persisting through-out. We speculated whether Hg might coordinate directly to the anhydride without the need for base, but this reaction too failed. With 2.5 equiv, the reaction was reliable with only a brief induction period to open up the anhydride of **2**, even though not all of it appeared to have reacted/dissolved during this period. With these modest improvements made, we then attempted to progress the regioisomeric mixture **4/6** through the subsequent stages in the hope of removing the 6-bromo acid (**6**) or its derivatives by selective crystallization. This, however, was unsuccessful, and we therefore decided to investigate the front half of the process more thoroughly with the aim of improving the isomer ratio in favour of the desired 3-bromo acid (**4**).

We decided to conduct an FED using our large Zymark robot which would allow us extensive coverage of the parameters investigated. We chose six parameters at two levels and conducted 32 test tube-scale reactions (with five centre points) which gave a half factorial overall. The parameters investigated with their high and low values are shown in Table 2.

All temperatures were at or just off reflux since given the long reaction time there was no point investigating lower values if we were to have a viable process. We took samples at 6-, 12-, 24-, and 48-h time points in each case, and assayed the reaction both for completion and regioisomer ratio. Despite this industry (greatly facilitated by the use of the robot), we could not find any factors that improved the ratio beyond 3:1. We did learn that the process was slightly more robust with the higher-charge of HgO and benefited from the 2-day reflux but was insensitive to the other factors. This gave us confidence for the manufacture, and only one batch was needed for ZD6021. This was repeated for ZD2249, and yields of 97% were achieved in both cases with no processing issues.

Table 2. **FED Parameters for bromo acid stage**

parameter	low level	high level
NaOH charge (equiv)	2.5	4.0
water charge 1 (vols)	6.75	13.25
HgO charge (equiv)	1.08	1.15
acetic acid charge (vols)	1.0	2.0
HCl charge (equiv)	10	20
water charge 2 (vols)	0	12.5

Bromo Acid RX Stage (4). After the failure of the FED investigation, we reluctantly turned our attention to the recrystallisation employed by Research, which we had been hoping to avoid. This proved to be reproducible on scale-up with no changes. A simple crystallization of the crude bromo acids (**4/6**) from 10 vols of hot glacial acetic acid reliably gave a 60−62% yield of the desired 3-bromo acid (**4**) with the regiosiomer ratio >100:1 (i.e., effectively >80% yield of available **4**).[10] Other impurities were also reduced to well below 1%, being mainly 1-naphthoic acid derived from Hg-mediated decarboxylation of unreacted **2** carried through from the first stage. Bromo anhydride (**3**) proved more difficult to remove if it carried through above 4%, but we were able to ensure that this did not occur.

We attempted to crystallise the water-wet paste of crude bromo acids directly (to avoid handling and drying this material potentially contaminated with Hg residues), but the paste retained about 2 vols of water which compromised the crystallization process. We did investigate azeotropic drying, but the process was not developed before it was needed, and we did not review the situation for the repeat manufacture for ZD2249. The first time through, two batches of bromo acid RX (**4**) were manufactured in 61% overall yield with 0.7% of the 6-bromo acid (ratio 143:1) and 0.2% of 1-naphthoic acid. For ZD2249, one double-size batch was made in 60% yield with the 6-bromo acid (**6**) undetected. Mercury analysis was performed as discussed below.

Bromo Ester Stage (5). The Research process reacted the bromo acid (**4**) with oxalyl chloride to form the acid chloride in dichloromethane, which was then concentrated to dryness and redissolved in methanol to give bromo ester (**5**). The reaction was catalysed by DMF so that generation of carcinogenic dimethylcarbamoyl chloride was a potential issue.[13] Clearly we could have accommodated this process, since this method was used for the later amide bond-forming reaction,[3] but we felt in this case that a simpler procedure could just as easily be developed within the time we had set ourselves, that is, 4 weeks per stage.

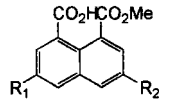
Initially, crude bromo acid (**4/6**) was slurried in methanol with concentrated H_2SO_4 and the mixture heated to reflux overnight. The methyl esters (**5/7**) were smoothly formed and, after an aqueous drown-out, extracted with a variety of

(11) Leuck, G. J.; Perkins, R. P.; Whitmore, F. C. *J. Am. Chem. Soc.* **1929**, *51*, 1831.

(12) The reaction mixture foamed alarmingly at reflux, and it was initially thought that the mixture was effervescing constantly. However, the hazard study showed that most of the CO_2 generated was driven off during the heating phase, and the foaming was attributed to the properties of the reaction. The process was run off reflux at 95 °C to avoid foaming and hence material being caked onto the vessel walls.

(13) Irving Sax, N. *Dangerous Properties of Industrial Materials*, 5th ed.; Van Nostrand Reinhold Company: New York, 1979.

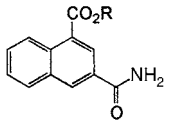
solvents to give 70−75% yields on concentration. The presence of the 6-bromo ester (**7**) in up to 25% of the crude product mixture thwarted the direct crystallization of the desired bromo ester (**5**). However, once bromo acid RX was available, a reverse drown-out of the screened hot methanolic solution into water gave product of good form and quality in >90% yields, with no extraction needed. We briefly investigated other acids (CF_3SO_3H and CF_3CO_2H) and the charge of H_2SO_4, but found no advantage. Minor impurities present included 1-methyl naphthoate and the half ester/acids (**10a/b**) formed by methanolysis of unreacted bromo anhydride. All of these were removed to insignificant levels by



10a (R_1 = H, R_2 = Br)
10b (R_1 = Br, R_2 = H)

slurry-washing the wet product cake with a dilute bicarbonate wash, which also removed any unreacted **4**. Only one batch of each was needed for ZD6021 and ZD2249, giving 97 and 95% yields, respectively, and with quality >98.5%. Overall, this process was sufficiently simple to be developed within our target 4-week time frame and was operationally easier than the Research method. However, it was a rare luxury to be able to develop a new process on this project, rather than simply modify the Research one.

Cyano Ester Stage (8). The Research process heated bromo ester (**5**) and copper (I) cyanide in NMP at 180 °C to achieve displacement of the bromide by cyanide.[4] Obvious concerns with this process were the ability to handle cyanide on the large scale, the high temperature required, and the need to remove copper from the product. The use of cyanide seemed unavoidable, and the copper could be dealt with, but the high temperature reaction could not be achieved in the LSL except in a heating mantle. This was deemed unacceptable for safety reasons in combination with cyanide, although we did later successfully make use of a heating mantle for the *S*-thiocarbamate stage of ZD2249.[14] We were therefore limited to 130 °C in this case, which consequently extended the reaction time from 4 to 5 h for the Research process to 48 h at 130 °C. Initial lab reactions also revealed that an impurity identified as the amide ester (**11a**) was being produced in up to 25%, presumably by adventitious moisture hydrolyzing the nitrile group under the forcing conditions. Rigorous exclusion of water from the apparatus and the use of anhydrous reagents reduced this to a tolerable level of 4%. However, unreacted bromo ester (**5**) also remained at 4% which was too high for subsequent processing. A slight under-charge (0.98 equiv) of CuCN had been used initially to ensure that no cyanide remained during the workup. With a slight over-charge (1.02 equiv), less than 2% **5** remained after 48 h which was acceptable. "Catalytic" pyridine had also been used previously,[4] presumably the idea being to accelerate the reaction by binding to the copper and thus solubilising the cyanide anion. We investigated the use of pyridine and 2,6-lutidine in the hope of improving the reaction rate but were surprised to find that the reaction was, if anything, faster with no additive.



11a R = Me
11b R = H

Finally, to remove the copper, we used experience gained on a similar process in another Zeneca project rather than the dilute drown-out using ammonium hydroxide reported by Dewar.[4] The reaction mixture was first diluted with an equal volume of NMP to prevent it from solidifying on cooling to room temperature. A brine solution was added which allowed removal of most of the copper as the soluble cuprate complex and simultaneously precipitated the crude product. This process typically gave a near quantitative yield with organic purity >90% as determined by HPLC.[15] Colour, however, varied from light to dark brown, and later results confirmed that, relative to the desired metal specification, there was still much copper to remove. We had already determined that a separate crystallization stage would be required, though.

Cyano Ester RX Stage (8). No process was available for this stage as generally intermediates were purified by chromatography in the Research Department. The main purpose was to allow further removal of copper residues down to a sufficiently low level for continued processing through to the bulk drug. The crude cyano ester was slurried in ethyl acetate at 20 °C and washed with a brine and 0.15 M solution of EDTA disodium salt. The crude material was not completely soluble at this temperature, and the separations were not ideal, but they could not be improved by heating either. Three washes were planned on the lab scale, but during manufacture up to six or seven washes were needed until the characteristic blue colour of the copper salts was no longer discernible. After a final brine wash, the ethyl acetate solution was concentrated to dryness to give a 90% yield of the cyano ester. This was redissolved in hot ethyl acetate in a clean vessel and MTBE added to aid crystallization. Cooling to 5−10 °C gave a white solid in typically 70% yield with the organic purity >98% and copper levels in the ppm range (see below).

The yield for the cyano ester and cyano ester RX stages combined was 69% overall for ZD6021 manufacture. A slight mischarge of the crystallization solvents during the second manufacture led to only a 59% yield for ZD2249, with a small second crop being isolated in addition. Attempts to avoid concentrating to dryness or to use a single solvent throughout (toluene and MTBE were investigated) could not be made to work in the short time available. Had time

(14) Bowden, S. A.; Burke, J. N.; Gray, F.; McKown, S.; Moseley, J. D.; Moss, W. O.; Murray, P. M.; Welham, M. J. *Org. Process Res. Dev.* Manuscript in preparation.

(15) In addition to unreacted **5** and hydrolysis product **11a**, there were other minor impurities (<1% each). None was identified, although the debrominated reduction product would be a possible candidate. The levels were not troublesome to further processing, and no further work was needed.

allowed, the ethyl acetate/MTBE ratio would have been further optimised to improve the recovery.

Cyano Acid Stage (1). Cyano ester was hydrolyzed with LiOH in water/THF at 20 °C overnight. The use of LiOH was made to minimise hydrolysis of the cyano group, but even with an under-charge (0.98 equiv), typically 2% of the amide acid (**11b**) was formed. Acidification with HCl precipitated generally good yields (90−95%) of product with 93−95% purity by LC. The solvent ratio of water:THF (5:1) was found to be important for good recovery; at a ratio of 1:1 the crystallization failed completely. A volume of methanol equal to that of THF was also found to be advantageous, in this case for suppressing additional hydrolysis of the nitrile group. More was not added in case of perturbing the crystallization. The reaction could also be run faster at a higher temperature, but predictably levels of the amide acid (**11b**) also increased. By this stage in the manufacturing sequence, development time was very short, and the process was judged fit-for-purpose without further work. This was a good example of an unoptimised stage which required minimal effort and was achieved within the 4-week time frame.

Overall, the process performed smoothly in the LSL for both campaigns, giving 88 and 89% yields with quality ∼94%. The amide acid was controlled at 2%, but on scale-up, 3% of another impurity was detected which could not be identified by LC−MS at the time. Fortunately it processed out in the later stages without complication, and we did not return to this issue.

Cyano Acid RX Stage (1). Due to the low final purity achieved for ZD6021 (94.8%), it was decided for the next compound (ZD2249) to maximise the input quality of the three key intermediates prior to their coupling.[1,3] Cyano acid quality was ∼94%, and it was felt that this could be improved. A series of 16 test tube-scale scale crystallizations was conducted, covering a range of solvents and solvent ratios. A blended batch of cyano acid (93.6% purity by LC) was used throughout, but quality was only improved by 2% in most cases (i.e., almost within experimental error), with recoveries in the range 50−90%. An IPA/isohexane combination (1:1) gave a marked improvement to 98.3% by LC, although recovery was disappointing at 55%. Further adjustments of the solvent ratio to 2:1 and cooling to 0 °C for several hours improved the recovery to 78% with quality reliably around 99%.

This process however was never used in the LSL. Use of cyano acid RX in the following amide alcohol coupling stage[3] gave only a marginal improvement in overall quality of 2%, and there was no improvement in yield, allowing for the different input strengths. By this stage of ZD2249 manufacture, the yield loss on crystallising cyano acid in 78% far out-weighed the marginal 2% improvement in quality obtained and could not be tolerated. Cyano acid was judged to be fit-for-purpose at 94% and was used accordingly, with no issues.

Metals Analysis. A consequence of the new project approach was the acceptance that more metals, and probably more toxic heavy metals such as Hg, would have to be tolerated in the manufacture. This in turn required, first, their removal from intermediates and bulk drug (as discussed above for Cu) and, second, analysis to prove that they had been removed to acceptable levels in the final bulk drug. For ZD6021, the manufacture out-ran our ability to perform the appropriate analysis in time, and thus most results were performed retrospectively. Final figures for Hg by XRF were 2 ppm at cyano ester RX and 1 ppm at cyano acid for ZD6021 manufacture, with Cu not determined. A fuller set of data for both Cu and Hg was available for the second manufacture supporting ZD2249, and this is collected in Table 3.

Table 3. **Hg and Cu analysis**[a]

stage	Hg by XRF	Hg by AFS	Cu by XRF
bromo acid RX	1100	1057	n.d.[c]
bromo ester	850	846	n.d.
cyano ester	31	31	n.d.
cyano ester RX	6	6	175
cyano acid[b]	9	9	55
ZD2249 pure	0	n.d.	1

[a] All figures in ppm; see ref 16 also. [b] Mean for two batches. [c] n.d. = not determined.

Mercury analysis was conducted by our analytical department at Macclesfield using XRF and locally by Avlon Works Environmental Group using AFS, and the results showed a remarkable level of agreement, given the experimental error expected on these techniques.[16] Crude bromo acid (**4**/**6**) itself was not assayed, but the level in bromo acid RX after the acetic acid crystallisation was far too high at 1100 ppm. There was effectively little reduction during the bromo ester stage, but the aqueous drown-out of the NMP solution at the crude cyano ester stage gave an impressive reduction (nearly 30-fold). The EDTA washes in the recrystallisation stage appeared to bring the level down a further 5-fold, but there was no improvement beyond this.

Copper analysis was determined using XRF alone, and was only relevant from the cyano ester stage, although again not determined for crude **8**. The EDTA washes had already reduced the level to 175 ppm in the crystallised cyano ester RX, and there was a further drop to 55 ppm in the cyano acid stage. The Hg specification was to be set at 5 or 10 ppm in the bulk drug, and for Cu at possibly up to 50 ppm for initial toxicity testing. Given the molecular weight increase from the cyano acid to the bulk drug, no further clean up was actually required. However, for both metals, further reductions were observed during downstream processing, leading to final levels in ZD2249 bulk drug of Hg as not detected and Cu at 1 ppm (similar results were achieved for ZD6021). Our decision to use Research-based chemistry more reliant on stoichiometric heavy metals had therefore paid off, although it did reveal that we needed more analytical resources to support these activities using this strategy.

(16) The error range for the XRF results (allowing for calibration of the apparatus, sample matrix, and sample collection parameters) was estimated to be ±2 ppm at the 10 ppm level for heavy metals such as Hg and Pd, and ±5 ppm in this range for first-row transition metals such as Cu, Fe, and Zn, in non-halogen-containing samples.

Alternative Bromination Reaction. Given the very low yield of bromination of naphthoic anhydride, we naturally considered other processes. Another process in the literature for this conversion was that of Mitchell,[17] which used bromine in concentrated H_2SO_4 with 0.5 equiv of silver sulfate (presumably to precipitate AgBr and hence drive the reaction to completion). Mitchell quoted an 81% yield and in our hands the yield was apparently quantitative. Unfortunately, the quality was obviously poor, with typically up to 15% unreacted **2** contaminating the product. The melting point was also significantly depressed against crystallised material by nearly 30 °C. A further determination of strength by NMR showed the level to be about 60%, giving an overall yield of typically 60%. This was much improved on 16%, but there were significant processing problems also.

First, the AgBr salt had to be screened off from concentrated H_2SO_4, which was judged to be inoperable in the LSL because the solvent would have destroyed the paper filters. The acid solution was then diluted with a vast quantity of water, which was highly exothermic. This also limited the output, even allowing for the higher yield per batch. The form of the product was very poor, leading to extended filtration and drying times. Finally, the product was only about 60% strength, which meant the impurities were carried into the next stage. A separate crystallization stage would almost certainly have been needed. Overall, this process was judged to have too many problems to be worth pursuing in the time available, and thus we focused our efforts on the existing process described above. The cost of the silver sulfate was not considered on this scale.

We did briefly review the decision before the start of ZD2249 manufacture, but decided that this process was still very problematic. The existing process, despite its 16% yield, was robust and operable on this scale with reasonable output. We felt the effluent could be managed on this scale and therefore ran the manufacture again with no problems for ZD2249.

Conclusions

ZD6021 cyano acid manufacture proved to be an excellent example of our intended new project strategy,[1] in particular our revised approach to traditional long-term manufacturing factors such as environmental, health, manufacturability, and output/yield criteria. Although we did manage to avoid the chromatography option for quality targets, we did need two separate crystallizations to deal with specific cases (the refractory regioisomer ratio of the bromo acid stage, and removal of copper at the cyano ester RX stage). As planned, safety was not compromised in any way, and robustness was also demonstrated in that only one batch had to be quarantined from both manufactures.[9]

We achieved our aim of demonstrating that a Research-type route could be successfully operated on up to 100-L scale with minimal development to deliver 1 kg of bulk drug. This included one process (bromo anhydride) that we would have judged inoperable for a larger-scale delivery (and later did),[7] and two others which would have been highly undesirable (bromo acid and cyano ester). Interestingly, the overall yield was only modestly improved over the Research route from 4.0% to around 5.4%, but this had not been our primary goal. We made use of the robot for a large screening study on the bromo acid stage and performed two FEDs for the first two stages. We did expend some effort later in trying to improve stages (bromo anhydride and cyano acid RX) since **1** was an intermediate common to two compounds. Most stages were developed sufficiently to pass our safety and robustness criteria within 4−6 weeks as intended; bromo ester and cyano acid required only 3.

We also gained much valuable learning from this project. In particular, we learnt that we needed more analytical support, especially LC−MS and metals analysis, to support product development at the rate of 4 weeks per stage, and although we managed to handle the SHE issues of some unpleasant reagents and demanding conditions, we realised that more effort was required to accommodate these less well developed stages. This is likely to be a continuing trend within the industry.

In summary, ZD6021 cyano acid manufacture provided challenging examples to our new project strategy in almost every aspect. Even so, we delivered the desired quality and quantity of material to manufacture bulk drug within the demanding 6-month deadline. That we were able to re-manufacture a second campaign for a 1-kg delivery of bulk drug without incident supports the conclusion that this approach was both safe and robust.

Experimental Section

General Procedures. Reaction mixtures and products were analysed by reverse phase HPLC on Hewlett-Packard 1050 or 1100 instruments according to the following conditions. **Method A (general):** column, HiChrom (or Waters Spherisorb) S5ODS-1, 250 mm × 4.6 mm i.d.; eluent, 550:450 acetonitrile:water with 0.1% v/v TFA; flow rate 1.0 mL/min; wavelength 230 nm; injection volume 10 μL. Typical retention times (t_r's) were: 1,8-naphthalic anhydride, 6.0; 1-naphthoic acid, 5.2; bromo anhydride, 10.1; 3- and 6-bromo acids, 8.6; 3-bromo ester, 28.4; 6-bromo ester, 27.3; cyano ester, 10.4; amide ester, 3.9; 1-methyl naphthoate, 12.9; half acid-esters, 5.5; cyano amide, 2.8; cyano acid, 4.6 min. **Method B (regioisomer ratio):** column, Zorbax SB-Phenyl, 250 mm × 4.6 mm i.d.; eluent, 700:200:100 water:acetonitrile:THF with 0.1% v/v TFA; flow rate 1.5 mL/min; wavelength 230 nm; injection volume 10 μL. Typical retention times (t_r's) were: bromo anhydride, 19.2; 6-bromo acid, 20.3; 3-bromo acid, 21.1 min. HPLC purities/strengths are area % normalised, except where noted otherwise. Melting points were determined using a Griffin melting point apparatus (aluminium heating block) and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova 400 spectrometer at 400 and 100.6 MHz, respectively, with chemical shifts given in ppm relative to TMS at $\delta = 0$. Electrospray (ES^+) mass spectra were determined on a Micromass LCT with time-of-flight and electron impact (EI) mass spectra were determined on a Micromass Autospec. Analytical TLC was carried out on commercially prepared plates coated with 0.25 mm of self-indicating Merck Kiesel-

(17) Mitchell, W. J.; Topsom, R. D.; Vaughan, J. *J. Chem. Soc.* **1962**, 2526.

gel 60 F_{254} and visualised by UV light at 254 nm. Preparative-scale silica gel flash chromatography (for lab work) was carried out by standard procedures using Merck Kieselgel 60 (230−400 mesh). Where not stated otherwise, assume standard practices have been applied. Note well: Yields and strengths given in the text above are those which can be expected from the processes operated under normal conditions; figures below are quoted for individual experiments and may vary slightly from those quoted in the text.

Preparation of ZD6021 BromoAnhydride (3). 1,8-Naphthalic anhydride (50.0 g, 252 mmol) was slurried in concentrated nitric acid (1000 mL of 70% solution, sp gr 1.42) and heated to 50 °C. Bromine (9.7 mL, 189 mmol, 0.75 equiv) was added evenly over 10 min, and the resulting brown solution was held at 50 °C for 4 h before cooling to 20 °C. A cream-coloured solid precipitated during this cooling period which was isolated by filtration at a water pump. (*Note well: Acidic gases present!*) The filter cake was slurry washed with water three times (130 mL each) or until the final water wash was neutral as determined by pH papers. (*Note well: The damp product is unstable if dried in the presence of residual acidic liquors*.) The isolated product was dried in vacuo at 60 °C to yield the title compound as a white-to-pale cream-coloured crystalline solid (14.2 g, 19.1% corrected for strength (based on naphthalic anhydride)). HPLC (Method A: t_R 6.0 min, strength 94%); mp 238−240 °C (lit.,[5] 242−243 °C); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.85 (1H, s), 8.53 (1H, d, J = 7.6 Hz), 8.48 (2H, d, J = 7.6 Hz), 7.95 (1H, t, J = 7.8 Hz); ^{13}C NMR (100.6 MHz, d_6-DMSO) δ 160.12, 159.57, 136.73, 134.31, 133.88, 132.65, 132.61, 128.68, 128.32, 121.33, 120.07, 119.38; MS (EI$^+$) 276/278 (M^+, 1:1, 94%), 232/234 ($(M - CO_2)^+$, 1:1, 100%). (*Caution: waste disposal*. The liquors from this reaction are acidic and are known to decompose with gassing on standing. It was found necessary to dilute the liquors at least 2-fold with water before further disposal. Neither diluted nor undiluted liquors should be stored in plastic containers even in the short term, as the residual bromine can attack the plasticisers making the containers brittle. Glass containers with a venting cap were used in this case.)

Preparation of ZD6021 Bromo Anhydride (3) by Silver Sulfate Method.[17] 1,8-Naphthalic anhydride (10.0 g, 50.5 mmol) and silver sulfate (7.9 g, 25.25 mmol, 0.50 equiv) were slurried in concentrated sulfuric acid (200 mL) at ambient temperature to give a dark yellow/green slurry. Bromine (3.2 mL, 63.0 mmol, 1.26 equiv) was added over 30 min, and then heated to 60 °C for 6−12 h, before cooling back to 20 °C. The solid silver bromide byproduct was filtered off at a water pump to give a clear orange solution (*Note well: acidic gases present!*). This was added dropwise over 45 min to a second vessel containing water (1600 mL) and standing in an ice bath. (*Caution: a substantial exotherm occurs on this scale!*) An off-white solid precipitates during the addition. This was isolated by filtration either immediately or after several hours, but in both cases, the filtration rate was slow. The filter cake was washed once by displacement with water (50 mL) and twice with cold ethanol (100 mL each) and dried in vacuo at 60 °C to constant weight to yield the title compound as an white solid (15.5 g, 111% uncorrected for strength). HPLC purity 72% by area, with 14−16% unreacted naphthoic anhydride remaining. ^{1}H NMR strength against maleic acid internal standard, 56%. Corrected yield using NMR strength, 8.7 g, 62%. Mp (crude) 215−217 °C (lit.,[15] 246−252 °C). Other data as recorded above.

Preparation of ZD6021 Bromo Acid (4/6). The preceding bromo anhydride (22.25 g, 80.3 mmol) was slurried in sodium hydroxide solution (201 mL of 1.0 M solution, 201 mmol, 2.5 equiv) and heated to 50 °C with agitation for 15 min. During this period most of the bromo anhydride dissolved, but complete solution is not required for successful reaction. In a second vessel, yellow mercury (II) oxide (18.91 g, 87.0 mmol, 1.09 equiv) was charged followed by water (57.3 mL) and then glacial acetic acid (19.1 mL). The contents are heated to 50 °C with gentle agitation during which time the mercury (II) oxide readily dissolved to give a colourless solution. (Note well: The order of addition of the solvents, water then acetic acid, affects the ease of solution.) This freshly prepared mercury (II) acetate solution was transferred to a heated addition funnel at 50 °C and added dropwise over 15 min to the bromo anhydride solution. (Note well: The mercury acetate will crystallise if not kept warm.) A milky white suspension forms immediately in a pale orange solution, the colour of which fades to white during heating. The reaction mixture was heated at reflux for 2 days, or just off reflux (95 °C) on the larger scale if preferred, and then cooled to 80 °C. Concentrated HCl (158 mL, 1.98 mol, 24.6 equiv) was added dropwise over 10 min and the reaction mixture reheated to reflux for a further 4 h. The reaction mixture was cooled to 20 °C and the resulting solid isolated by filtration. The wet cake is slurry-washed three times with water (225 mL each) and then dried in vacuo at 70 °C to yield a 3:1 mixture of the 3- and 6-bromo acids as a white solid (18.7 g, 93% corrected for strength for the combined bromo acids). HPLC (Method A: t_R = 8.6 min for both isomers, strength 89%); mp (crude) 195−198 °C. Other data reported in the bromo acid RX section below.

Preparation of ZD6021 Bromo Acid RX (4). The preceding crude ZD6021 bromo acid (4.98 g, 19.8 mmol) was slurried in glacial acetic acid (50 mL) and heated to reflux with stirring. The reaction mixture was held at reflux for 3 h to ensure that all the solid had dissolved; it was then cooled evenly at 10 °C/h to 20 °C and stirred overnight at this temperature. The resulting solid was isolated by filtration and dried in vacuo at 70 °C to yield the title compound as a white solid (3.22 g, 58% corrected for strength). HPLC (Method A: t_R 8.6 min, strength 90%; Method B: 6-bromo acid, t_R 20.3 min; 3-bromo acid, t_R 21.1 min; isomer ratio of 3:6-bromo acid = typically >100:1). **Data on pure 3-bromo acid (4):** mp 230−231 °C (lit.,[5] 231−232 °C); ^{1}H NMR (400 MHz, d_6-DMSO) δ 13.55 (1H, br s), 8.82 (1H, d, J = 8.4 Hz), 8.48 (1H, d, J = 1.2 Hz), 8.18 (1H, J = 2.0 Hz), 8.02 (1H, d J = 7.6 Hz), 7.63−7.72 (2H, m); ^{13}C NMR (100.6 MHz, d_6-DMSO) δ 167.25, 134.78, 134.37, 131.96, 130.05, 129.13, 128.08, 127.88, 127.36, 125.58, 117.57. **Data on pure 6-bromo acid (6)** (obtained by base hydrolysis of an

analytically pure sample of 6-bromo ester): mp 180−181 °C (lit.,[4] 185−186 °C); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.82 (1H, d, J = 9.2 Hz), 8.31 (1H, d, J = 2.0 Hz), 8.18 (1H,d, J = 7.2 Hz), 8.14 (1H, d, J = 8.0 Hz), 7.76 (1H, dd, J = 9.2, 2.0 Hz), 7.64 (1H, t, J = 7.6 Hz); ^{13}C NMR (100.6 MHz, d_6-DMSO) δ 168.47, 135.03, 132.36, 130.67, 130.63, 130.56, 129.45, 128.10, 128.06, 126.43, 119.75.

Preparation of ZD6021 Bromo Ester (5). The preceding bromo acid RX (100 g, 0.40 mol) was slurried in methanol (1200 mL) at 20 °C with mechanical agitation, and concentrated sulfuric acid (10.9 mL of 98% strength, 0.20 mol, 0.5 equiv) was added dropwise to the slurry over 4 min. The reaction mixture was heated to reflux (65 °C) for 18−24 h. The resulting solution was cooled to 60 °C and filtered through preheated apparatus to remove any fines. The filter apparatus was rinsed with methanol (50 mL) and warmed to 60 °C, and the wash was combined with the main reaction solution in a jacketed addition funnel at 55 °C. The warm solution of bromo ester was added dropwise over 1 h to a second vessel containing water (1500 mL) and stirred at 25 °C which precipitated the bromo ester product. (Note well: The drown-out vessel may need additional cooling on this scale.) The addition funnel was washed with warm methanol (50 mL) and the washings were added to the drown-out vessel. The resulting white precipitate was stirred at 25 °C for 1 h and then isolated by filtration. The damp cake was slurry-washed sequentially with a dilute solution of $NaHCO_3$ (300 mL of 0.05 M solution) and then twice with water (300 mL each), pulled as dry as possible, and dried in vacuo at 30 °C (Note well: low-melting solid) to yield the title compound as a white solid (103.7 g, 96.3% corrected for strength). HPLC (Method A: t_R 28.4 min, strength 98.5%; 6-bromo ester, t_R 27.3 min; the isomer ratio can also be determined at this stage using Method A at 295 nm if required). **Data on pure 3-Bromo Ester (5):** mp 58−59 °C (lit.,[5] 59 °C); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.69 (1H, d, J = 8.4 Hz), 8.51 (1H, s), 8.16 (1H, d, J = 2.0 Hz), 8.04 (1H, d, J = 8.0 Hz), 7.60−7.73 (2H, m), 3.96 (3H, s); ^{13}C NMR (100.6 MHz, d_6-DMSO) δ 165.94, 134.78, 134.69, 132.03, 128.90, 128.86, 128.36, 127.98, 127.55, 125.21, 117.49, 52.58; MS (ES$^+$) 306/308 (M + CH_3CNH^+, 100%), 265/267 (M + H^+, 61%), 233/235 (M − CH_3OH^+, 48%). **Data on pure 6-bromo ester (7)** (obtained by flash chromatography of a crude mixture of the 3- and 6-bromo esters and eluting with 5% MTBE in isohexane): mp 62−63 °C (lit.,[4] 63−64 °C); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.67 (1H, d, J = 9.2 Hz), 8.30 (1H, d, J = 2.0 Hz), 8.15 (2H, m), 7.75 (1H, dd, J = 9.4, 2.2 Hz), 7.63 (1H, t, J = 7.8 Hz), 3.92 (3H, s); ^{13}C NMR (100.6 MHz, d_6-DMSO) δ 166.86, 134.71, 132.52, 130.72, 130.43, 130.42, 128.91, 127.47, 126.73, 126.18, 119.71, 52.35.

Preparation of ZD6021 Cyano Ester (8). The preceding bromo ester (20.0 g, 75.4 mmol), copper (I) cyanide (6.89 g, 77.0 mmol, 1.02 equiv) and anhydrous NMP (40 mL) were charged to a nitrogen-inerted vessel with stirring at 20 °C. The resulting mixture was heated to 130 °C for 48 h under a gentle stream of nitrogen. A yellow solution was formed which became progressively darker during this period. On completion of the reaction as determined by HPLC, the reaction mixture was cooled to 30−40 °C and diluted further with NMP (40 mL) to prevent the product from crystallising prematurely. The solution was cooled to 25 °C and a saturated solution of brine (200 mL) added dropwise over 1 h. The resulting slurry was stirred for 3 h at 20 °C and the solid isolated by filtration. The filter cake was washed sequentially, once by displacement with saturated brine (40 mL) and twice with water (40 mL each), and dried in vacuo at 50 °C to yield the crude title compound as a light to mid-brown solid (15.24 g, 87% corrected for strength). HPLC (Method A: t_R 10.4 min, strength 91%); mp 101−103 °C. Other data reported in cyano ester RX section below.

Preparation of ZD6021 Cyano Ester RX (8). The preceding crude cyano ester (5.0 g, 23.7 mmol) was slurried in ethyl acetate (60 mL) at 20 °C. Saturated brine solution (20 mL) and EDTA solution (20 mL of 0.15 M EDTA-disodium salt) were added to the ethyl acetate slurry of cyano ester and stirred vigorously at 20 °C for 1 h. The layers were allowed to settle, and the blue-coloured lower aqueous layer was separated. This procedure was repeated once more, or on the larger scale, until the aqueous layer no longer retained the characteristic blue colour of solvated copper ions (up to six times). The ethyl acetate solution was washed with a saturated brine solution (20 mL), and the ethyl acetate layer concentrated under reduced pressure to give cyano ester as a brown solid. In a clean vessel, this solid was redissolved in ethyl acetate (4.3 mL) and MTBE (17.2 mL) with stirring by heating to 60−65 °C for 1 h to achieve a solution. The reaction mixture was then cooled at 14 °C/h over 4 h down to 5−10 °C, and stirred at this temperature for 1.5 h. The resulting solid was isolated by filtration, washed twice by displacement with cold MTBE (8.6 mL each), and dried in vacuo at 50 °C to yield the title compound as a white solid (2.77 g, 58% on this scale). HPLC (Method A: t_R 10.4 min, strength 100%); mp 109−110 °C (lit.,[4] 108−109 °C); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.84 (1H, s), 8.75 (1H, d, J = 8.8 Hz), 8.29 (1H, s), 8.17 (1H, d, J = 8.4 Hz), 7.87 (1H, t, J = 7.8 Hz), 7.77 (1H, t, J = 7.6 Hz), 3.98 (3H, s); ^{13}C NMR (100.6 MHz, d_6-DMSO) δ 165.88, 138.93, 132.53, 131.37, 131.04, 129.72, 129.48, 128.42, 128.04, 125. 27, 118.14, 107.67, 52.69; MS (ES$^+$) 253 (M + CH_3CNH^+, 100%) 211 (M^+, 20%).

Preparation of ZD6021 Cyano Acid (1). The preceding cyano ester RX (2.0 g, 9.47 mmol) was dissolved in THF (10 mL) to give a coloured solution. Lithium hydroxide monohydrate (389 mg, 9.28 mmol, 0.98 equiv) was dissolved in water (40 mL) to give a clear solution which was added to the cyano ester RX over several minutes. A pale precipitate formed which disappeared as the reaction proceeded. A line wash of water (10 mL) was added followed by methanol (10 mL) and the reaction mixture stirred at 20 °C overnight (16−18 h). The solution was screened through a filter to remove any residual solid before the addition of dilute aqueous HCl (2.0 M, 9.5 mL, 18.9 mmol, 2.0 equiv). A solid crystallised during this addition and was isolated by filtration, slurry-washed twice with water (20 mL each), and dried in vacuo at 60 °C to yield the title compound as a white solid

(1.44 g, 72% on this scale but typically 90−95%). HPLC (Method A: t_R 4.6 min, strength 94%); mp 209−210 °C (lit.,[4] 210−212 °C); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.88 (1H, d, J = 8.4 Hz), 8.81 (1H, s), 8.29 (1H, s), 8.16 (1H, d, J = 8.4 Hz), 7.85 (1H, t, J = 7.6 Hz), 7.76 (1H, t, J = 7.4 Hz); ^{13}C NMR (100.6 MHz, d_6-DMSO) δ 167.33, 138.37, 132.61, 131.71, 130.70, 130.11, 129.39, 129.36, 127.82, 125.72, 118.35, 107.67; MS (ES^+) 198 ($M + H^+$, 100%), 154 ($(M - CO_2)^+$, 45%).

Preparation of ZD6021 Cyano Acid RX (1). The preceding crude cyano acid (4.0 g crude, 19.0 mmol corrected for strength) was slurried in 2-propanol (28 mL) with stirring and heated to reflux (82 °C) to achieve a complete solution. The solution was cooled back to 60 °C and isohexane (14 mL) added dropwise from an addition funnel over 10 min. The solution was cooled to 20 °C evenly at 20 °C/h and stirred at 20 °C overnight (for convenience). The product crystallised in the range 40−50 °C. The slurry was further cooled to 0 °C and stirred for 2 h, and the solid was isolated by filtration. The filter cake was washed by displacement twice with cold isohexane (10 mL each) and dried in vacuo at 40 °C to yield the title compound as a white solid (2.92 g, 78% corrected for strength). HPLC (Method A: t_R 4.6 min, strength 99%); mp 215−216 °C (lit.,[4] 210−212 °C). Other data as noted above.

Acknowledgment

We thank Steve Hallam and Paul Gillespie (Process Safety Group, Blackley, now at Macclesfield) for sterling work on the hazard studies in support of this project; Bob Osborne (PR&D Avlon) for the use of the Zymark robot and both him and Rob Shaw (Macclesfield) for help with the FEDs and statistical analysis; Jo Brenton (Avlon Works Environmental Group) and Gordon Plummer (Analytical Department, Macclesfield) for Cu and Hg analyses; Steve Lake (PR&D Avlon) for mass spectra; and Caroline Wright and Roger Crosby for additional experimental work on the bromo anhydride stage. We also benefited from excellent cooperation with our Research colleagues at Wilmington, DE, in particular, Jim Hulsizer and his team.

Received for review July 22, 2002.

OP020065H