Organic Process Research & Development **2001**, *5*, 226−233

Development of a Scalable Process for CI-1020, A Novel Endothelin Antagonist[1]

James E. Ellis,* Edward M. Davis, Gary J. Dozeman, Edward A. Lenoir, Daniel T. Belmont, and Phillip L. Brower

Pfizer Global Research and Development, Holland Laboratories, Pfizer Inc., 188 Howard Avenue, Holland, Michigan 49424, U.S.A.

Abstract:

The process development of a route for preparing CI-1020 on pilot-plant scale is described in 55% overall yield. Hydrocyanation conditions are described which use acetone cyanohydrin catalyzed by tetramethylammonium hydroxide and which provide the desired ketonitrile intermediate (4) in 85% yield with excellent quality. The penultimate intermediate (7), a hydroxybutenolide, is prepared in a two-step process using an aldol condensation followed by acid-catalyzed ring closure to give product in 86.8% yield. The active pharmaceutical ingredient (API) (8) is prepared by ring-opening of the hydroxybutenolide with sodium carbonate to provide the sodium salt. The use of ReactIR to monitor the API reaction is described. ReactIR was required to determine an endpoint for the reaction. The use of chromatographic analysis to determine the endpoint was not possible. The API and the penultimate hydroxybutenolide are not separable by chromatographic methods.

Introduction

In 1985 initial reports were published concerning isolation of peptidic compounds with very potent vasoconstrictor activity from endothelial tissues. These compounds were designated endothelins and were found to be peptides of 21 amino acids. Since then this class of compounds has been identified in tissues throughout the body. Elevated levels of endothelins have been found to be associated with several disease states. They have possible effects on ischemic stroke, congestive heart failure, pulmonary hypertension, and a number of other cardiovascular conditions.[2]

A series of novel hydroxybutenolide compounds with activity as endothelin antagonists was discovered recently in our Discovery endothelin research program.[3,4] These compounds are non-peptides and have selective activity at nanomolar concentrations with endothelin A receptors. Kilogram-scale quantities of CI-1020 (**8**) were needed for the testing program. This article describes the development of a scalable synthesis of CI-1020.

Original Synthesis. Our Discovery group using the synthetic route shown in Scheme 1 developed a very useful synthesis of CI-1020.

This synthetic route was based on earlier work for preparation of hydroxybutenolides.[5] The first step of the synthesis was the preparation of the chalcone. A standard Claisen−Schmidt reaction was run in which *p*-methoxyacetophenone (**1**) was reacted with piperonal (**2**) in ethanol with a catalytic amount of base to give the desired chalcone (**3**) in 95% yield.[6] The chalcone was hydrocyanated using the Lapworth procedure of sodium cyanide with acetic acid to give the desired ketonitrile (**4**) in 86% yield.[7] The ketoester (**5**) was prepared in 95% yield by alcoholysis of **4** using *p*-toluenesulfonic acid monohydrate in refluxing dioxane/methanol.[8] The penultimate hydroxybutenolide intermediate (**7**) was prepared in a two-step sequence. The first step was an aldol reaction between **5** and 3,4,5-trimethoxybenzaldehyde (**6**) catalyzed with sodium methoxide to give a mixture of double bond isomers of CI-1020 (**8**). The reaction mixture was then treated with excess acetic acid at reflux providing the ring-closed hydroxybutenolide structure (**7**) in 59% yield. The last step of the synthesis was reaction of **7** with aqueous sodium hydroxide solution followed by lyophilization to provide the desired sodium salt CI-1020 (**8**) in 88% yield.

Development of a Revised Synthesis. A very efficient synthesis for preparing CI-1020 on small scale had been developed by our Discovery group. However, several potential problems in the synthesis were identified as the process was reviewed from a scale-up perspective. In solving these problems a revised synthesis was developed, which is shown in Scheme 2.

Synthesis of the Ketonitrile (4). As noted above, the synthesis of the chalcone (**3**) was quite straightforward giving the desired product in nearly quantitative yield. However, the original Claisen−Schmidt reaction conditions gave a product slurry that was quite thick and difficult to transfer from the reaction flask. Because the solubility of **3** in ethanol was very low, the reaction could be diluted 3- to 5-fold and still obtain the chalcone in 98% yield on pilot scale.

* Author for correspondence. Telephone: 616 392-2375, ext. 2387. Fax: 616 392-8916. E-mail: James.Ellis3@Pfizer.com.

(1) Based in part on presentation given at 8th Midwest Pharmaceutical Process Chemistry Consortium Symposium, West Lafayette, Indiana, October 1999.

(2) For a review: Ellis, R. L. *N. Engl. J. Med.* **1995**, *333*, 356.

(3) Doherty, A. M.; Patt, W. C.; Edmunds, J. J.; Berryman, K. A.; Reisdorph, B. R.; Plummer, M. S.; Shahripour, A.; Lee, C.; Cheng, X.; Walker, D. M.; Haleen, S. J.; Keiser, J. A.; Flynn, M. A.; Welch, K. M.; Hallak, H.; Taylor, D. G.; Reynolds, E. E. *J. Med. Chem.* **1995**, *38*, 1259.

(4) Reynolds, E. E.; Kaiser, J. A.; Haleen, S. J.; Walker, D. M.; Olszewski, B.; Shroeder, R. L.; Taylor, D. G.; Hwang, O.; Welch, K. M.; Flynn, M. A.; Thompson, D. M.; Edmunds, J. J.; Berryman, K. A.; Lee, C.; Reisdorph, B. R.; Cheng, X. M.; Patt, W. C.; Doherty, A. M. *J. Pharmacol. Exp. Ther.* **1995**, *273*, 1410.

(5) Allen, C. F. H.; Frame, G. F.*Can. J. Res.* **1932**, *6*, 605.

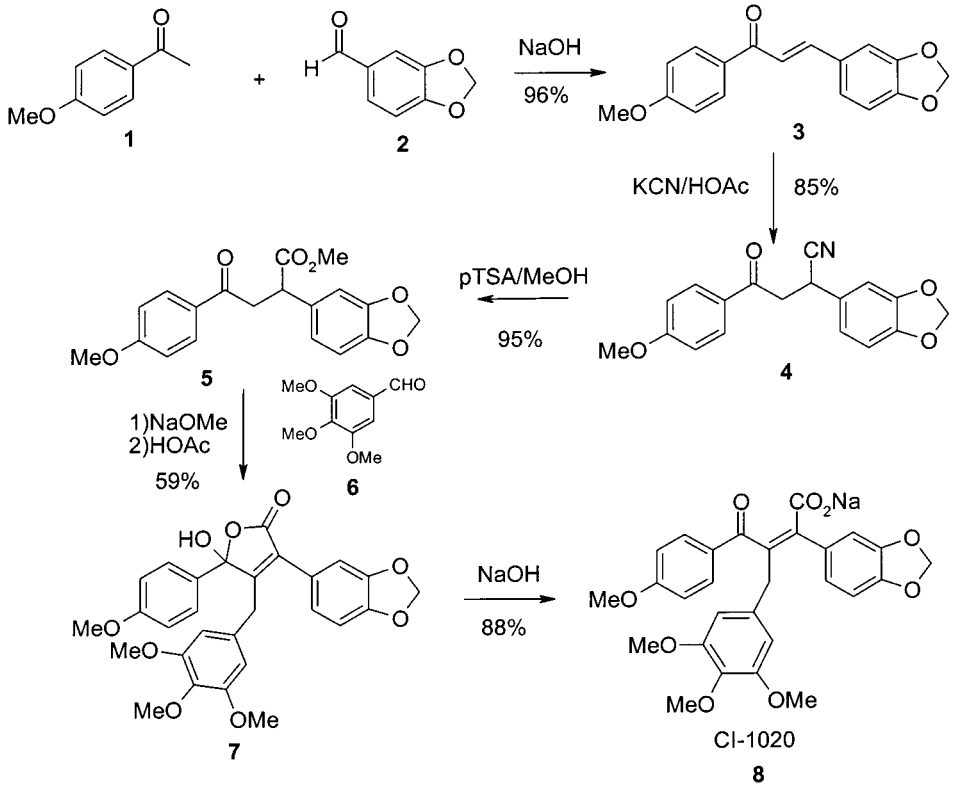
(6) Kohler, E. P.; Chadwell, H. M. *Organic Syntheses*, 2nd ed. ; Wiley: New York, 1941; Collect. Vol. I, p 78.

(7) Ishii, H.; Chen, I.; Ishikawa, T. *J. Chem. Soc., Perkin Trans. 1* **1987**, 671; Davey, W.; Tivey, D. J. *J. Chem. Soc.* **1958**, 1230; Lapworth, A.; Wechsler, E. *J. Chem. Soc.* **1910**, *97*, 38.

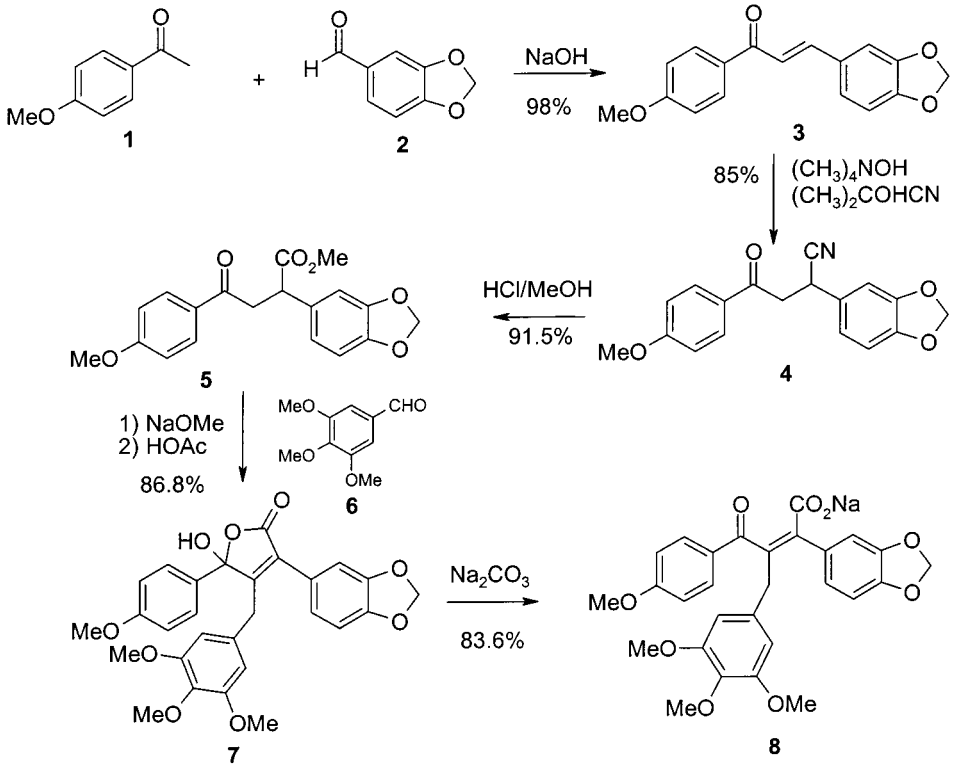
(8) For a review: Sandler, S. R.; Karo, W. *Organic Functional Group Preparations*, 2nd ed.; Academic Press: New York, 1986; Vol. 3, pp 314−350.

 10.1021/op010200a CCC: $20.00
Published on Web 04/19/2001

Scheme 1. **Original synthesis of CI-1020**



Scheme 2. **Revised process of CI-1020**



The initial preparation of the ketonitrile (**4**) used the Lapworth procedure of potassium cyanide and acetic acid to hydrocyanate the chalcone. The hydrocyanation reaction was run in ethoxyethanol because it was an excellent solvent for chalcones. The reaction proceeded to completion in approximately 1 h at reflux using 2 mol of potassium cyanide and 1.5 mol of acetic acid per mole of substrate.

Two major problems hindered the scale-up of this process. The first significant problem with the Lapworth method from a scale-up perspective was the generation of large amounts

of hydrogen cyanide from partial neutralization of the excess potassium cyanide. This process would have required special equipment to prevent release of hydrogen cyanide.

The second problem was that the reaction mixture became highly colored as the reaction proceeded. Since the color carried through the remainder of the synthesis to the bulk pharmaceutical compound, it was necessary to purify the ketonitrile by recrystallization and carbon treatment to remove the colored impurities. Attempts to directly isolate **4** that was not colored were unsuccessful. Consequently, we began to look for a suitable alternative method to overcome these problems.

There are a number of potentially useful alternative methods available in the literature for hydrocyanation of α,β-enones. Some of the methods, such as using hydrogen cyanide or trialkylaluminum cyanides, raise significant safety issues on scale.[9,10] The method which appeared to be the most practical for scale-up was the base-catalyzed Nazarov hydrocyanation using acetone cyanohydrin as the cyanide source.[11]

The process evaluation was begun using a sodium carbonate-catalyzed hydrocyanation with acetone cyanohydrin. In the literature, this reaction is typically run in alcoholic solvents. Therefore, several different solvents were evaluated: methanol, ethanol, ethoxyethanol, and also acetone. In all of the solvents, except acetone, the reaction mixture darkened considerably at reflux. Since minimization of color in the isolated product was desired, acetone was chosen for further studies.

Initially, a reaction stoichiometry of 2 mol of acetone cyanohydrin and 0.25 mol of a 10% aqueous solution of base per mole of **3** was used, which Betts and Davey had described as optimal.[11b] Under these conditions, the sodium carbonate-catalyzed hydrocyanation with acetone as solvent proceeded smoothly to the desired product with good purity and excellent color. However, the reaction rate was slow with the sodium carbonate-catalyzed method, requiring more than 40 h to complete the reaction on scale. This problem was partly due to the reaction in acetone starting out as a slurry, since the chalcone and sodium carbonate have low solubility in acetone. On pilot scale, the solid sodium carbonate presented additional problems. It settled into the vortex under the reactor agitator, making it less available for reaction and acted to block the bottom valve of the reactor.

The slow reaction rate was also a function of the electron-donating characteristics of the substituents on the chalcone, which are known to slow the reaction.[12] The electron-donating substituents decrease the electrophilicity of the chalcone double bond, resulting in a decreased rate of Michael addition by the cyanide anion.

Since the slow reaction rate using sodium carbonate catalyst was also related in part to the low solubility of the base in the reaction solvent, several other bases were evaluated, which might have better solubility in the reaction system. Catalytic triethylamine did not give complete reaction even after 40 h of reaction time. Aqueous potassium hydroxide gave no improvement in rate and also gave an oily product. Catalytic aqueous potassium cyanide did not significantly improve the reaction rate. Aqueous potassium carbonate did improve the reaction rate but still required 20−24 h for complete reaction.

At this point, 25% tetrabutylammonium hydroxide in water was tried as a soluble form of hydroxide. This base could also function as a phase transfer catalyst to assist in the delivery of the cyanide in the Michael reaction. The reaction proceeded very well, reaching completion after about 4 h. However, the presence of water in the reaction system appeared to adversely affect the reaction by reducing the solubility of the chalcone in acetone. The next base evaluated was 25% tetramethylammonium hydroxide in water, which also gave complete reaction in about 4 h. Further studies used this reagent, since it has nearly twice the molar concentration compared to the tetrabutylammonium salt. Thus, the reaction could be run with the same stoichiometry and only half the amount of added water.

As the reaction conditions were optimized, it was found that both the amount of cyanohydrin and base could be reduced substantially. However, as the amounts of cyanohydrin and base were reduced, the reaction times increased. A maximum desirable reaction time of 10 h was chosen, which was obtained using 1.25 mol of cyanohydrin per mole of substrate with 5 mol % of tetramethylammonium hydroxide. The reduction in the amount of cyanohydrin in the reaction was a very significant improvement from a development perspective since it facilitated the waste treatment by reducing the amount of excess cyanide to be destroyed. In addition, the increase in concentration gave an increase in throughput.

An additional benefit in using acetone as the reaction solvent is that it simplified the waste treatment. The use of acetone avoided mixed organic solvents in the waste stream since acetone is released in the reaction of acetone cyanohydrin. More importantly, the acetone could be separated from the water phase easily by atmospheric distillation, since it does not azeotrope with water. This allowed the treatment of the water phase with sodium hypochlorite to destroy the residual cyanide.[13] This distillation separation was used successfully on pilot scale using a reactor connected to an atmospheric scrubber containing sodium hypochlorite to trap any HCN vapors. The acetone distillate contained less than 10 ppm of hydrogen cyanide. The residual cyanide in the water was treated with sodium hypochlorite without any problems. By avoiding alcoholic solvents, the possibility of the aqueous layer containing residual alcohol was eliminated, which could have been dangerous since alcohols can form explosive alkyl hypochlorites.[14]

(9) For a review: Nagata, W.; Yoshioka, M. In *Organic Reactions*; Dauben, W. O., Ed.; John Wiley and Sons Inc.: New York, 1977; Vol. 25, p 255.
(10) Liotta, C. L.; Dabdoub, A. M.; Zalkow, L. H. *Tetrahedron Lett.* **1977**, 1117.
(11) (a) Nazarov, I. N.; Zav'yalov, *J. Gen. Chem. USSR (Engl. Transl.)* **1954**, *24*, 475; *Chem. Abstr*. **1955**, *49*, 6139f. (b) Betts, B. E.; Davey, W. *J. Chem. Soc.* **1958**, 4193. (c) Acetone cyanohydrin is a commercially available raw material. An estimated 500,000 metric tons were used in the U.S. in 1989: *Encyclopedia of Chemical Technology*; Howe-Grant, M., Ed., Wiley: New York, 1993; Vol. 7, p 829.
(12) Ellis, J. E.; Davis, E. M.; Brower, P. L. *Org. Process Res. Dev.* **1997**, *1*, 250.
(13) Jenks, W. R. In *Encyclopedia of Chemical Technology*; Grayson, M., Ed.; Wiley: New York, 1979; Vol. 7, p 320.

The optimized hydrocyanation process resulted in a process that ran quite consistently on scale-up. The process was combined with the chalcone process. The hydrocyanation conditions on pilot scale were 1.25 equiv of acetone cyanohydrin with 5 mol % of tetramethylammonium hydroxide. The reaction was complete in 9 h at reflux and provided excellent quality product in 85−95% yield for the two steps.

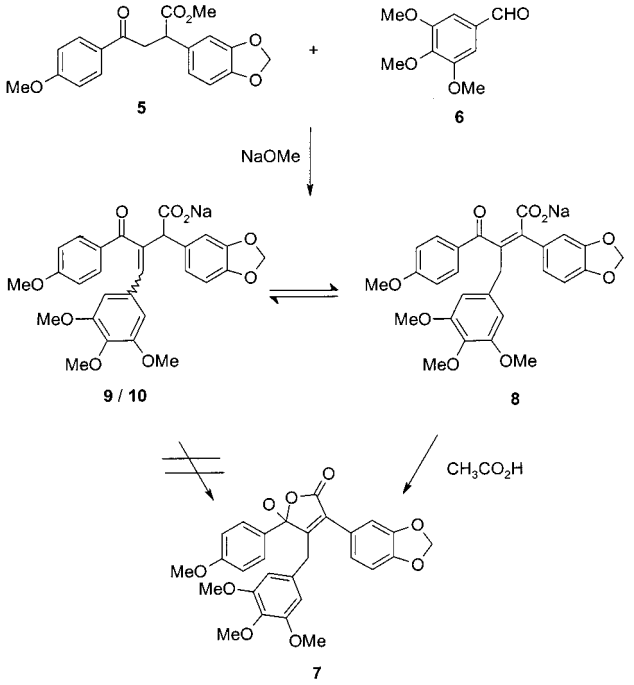
Synthesis of the Ketoester (5). The alcoholysis of the ketonitrile (**4**) to the ketoester (**5**) using the Pinner reaction/hydrolysis process ran quite well on small scale but had some problems on scale-up. Under the initial reaction conditions, the ketonitrile was refluxed with *p*-toluenesulfonic acid monohydrate in a 1:1 mixture of dioxane and methanol for 16 h. The isolated crude product was reslurried with water to remove the by-product ammonium *p*-toluenesulfonate that had cocrystallized with the product, giving a 59% yield of a blue/green solid. The color of this product carried through to the active pharmaceutical ingredient (API).

Several changes were made to improve the reaction. Since methanol was necessary for the Pinner reaction, the first process change was to eliminate dioxane as one of the reaction solvents, leaving only methanol. The use of dioxane was undesirable on-scale since it is a suspected carcinogenic agent. Since Pinner reactions are most commonly run using hydrogen chloride gas, the use of hydrogen chloride was evaluated for the reaction.[8] Hydrogen chloride was easier to use than *p*-toluenesulfonic acid, and the by-product ammonium chloride was more soluble in methanol and less likely to coprecipitate with the product than ammonium *p*-toluenesulfonate. The reaction was run at reflux for 16 h, and then water was added to hydrolyze the acetimidate intermediate. The changes worked quite well, giving better isolated yields and purity. However, the isolated product still was quite colored, varying from blue to green. We believe this color may result from trace amounts of highly colored quinone methide impurities arising from partial cleavage of the ether functionalities in the substrate or product under the Pinner reaction conditions.[15]

At this point, the process was changed to use aqueous hydrochloric acid in methanol to further simplify the process. The ketonitrile (**4**) was refluxed in aqueous methanolic HCl for 8−18 h and then cooled to crystallize a very lightly colored product. The small amount of color now present did not carry through to the API. The optimized process ran very well on pilot scale, providing ketoester (**5**) in 91−95% yield with 98% purity by HPLC.

Synthesis of the Hydroxybutenolide (7). The next step in the synthetic route was preparation of the penultimate hydroxybutenolide (**7**). This process has two distinct reaction phases as shown in Scheme 3 below. The first reaction was an aldol coupling of 3,4,5-trimethoxy benzaldehyde to **5** in the presence of strong base.[5] The second reaction was neutralization of the mixture of isomeric aldol adducts with acetic acid and isomerization of the isomeric double bonds

Scheme 3. **Hydroxybutenolide formation**



of **9** and **10** into conjugation between the two carbonyl groups, with rapid concomitant cyclization to the stable hydroxybutenolide.

The original process used freshly prepared sodium methoxide in methanol for the aldol reaction. The reaction mixture was heated at reflux for 16 h and then acidified with acetic acid. The acidified reaction mixture was heated at reflux for an additional 10 h. The mixture was cooled to crystallize the hydroxybutenolide in 70−75% yield.

In the initial optimization of this process, sodium hydroxide, potassium hydroxide, sodium ethoxide, and sodium methoxide were evaluated as basic catalysts for the aldol reaction. When the reaction was run in water with sodium hydroxide, the main product observed was the carboxylic acid resulting from hydrolysis of the ketoester. The use of potassium hydroxide in ethanol gave a low yield of the desired product in low purity. The best base/solvent combination for the aldol reaction in terms of both yield and purity was sodium ethoxide, freshly prepared with sodium metal, in ethanol. Commercial sodium ethoxide gave a highly colored product in poor yield. Since the use of sodium metal was undesirable on scale-up, the use of commercial sodium methoxide in alcohols was evaluated and found to be superior to commercial sodium ethoxide. Either dry sodium methylate or sodium methoxide in methanol were found to work equally well in this reaction. Dry sodium methylate was chosen for use on scale-up to minimize the amount of methanol in the reaction.

The solvents for the aldol reaction that were studied included water, methanol, ethanol, and 2-propanol (IPA). As noted above, water with sodium hydroxide caused hydrolysis of the ketoester. The hydroxybutenolide (**7**) was more soluble in methanol compared to higher alcohols, thus potentially producing lower yields. The range of solubility of **7** in the alcohols was methanol > ethanol > IPA with roughly 4 g

(14) Brethereck, L. *Handbook of Reactive Chemical Hazards*, 2nd ed.; Butterworths: London, 1979; p 805.
(15) Filar, L. J.; Winstein, S. *Tetrahedron Lett.* **1960**, *25*, 9.

of **7** per liter in methanol to roughly 2.4 g of **7** per liter in IPA. On the basis of the solubility data, initially IPA was thought to be the ideal solvent for this reaction. However, this was not the case because the sodium salts of the isomers **9** and **10** formed during the aldol reaction were so insoluble that they formed an unstirrable reaction mixture. Addition of acid did not make the mixture stirrable. Ethanol was chosen as the reaction solvent because **7** had moderate solubility in that solvent. The minimum amount of ethanol for the reaction was that which would completely dissolve the sodium methylate. The practical reaction concentration was found to be about 1.25 M ketoester (**5**) in ethanol.

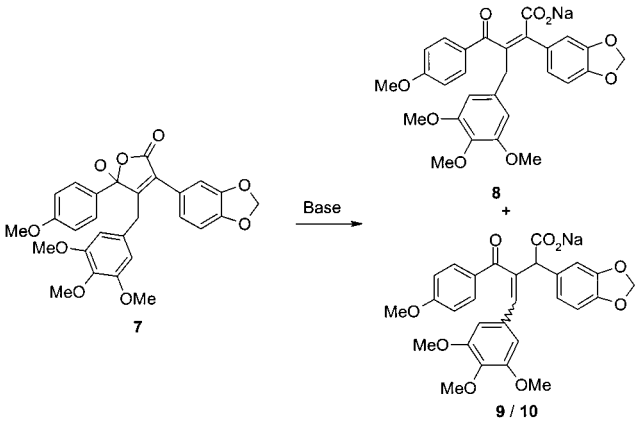
Initially, it was presumed the aldol reaction required reflux temperatures of about 70 °C. However, during the optimization study it was found that when the reaction was run at lower temperatures it produced far fewer by-products, thus providing higher yield and purity. At 30 °C the reaction was complete after about 3 h, the same time it takes at reflux. The in-process HPLC purity improved from about 80% to almost 90% by area normalization.

The order of addition was also critical to minimizing formation of by-products. The sodium alkoxide must be preformed or predissolved in the solvent. Reactions in which we tried to form the alkoxide in the presence of the other reagents gave little or no yield. The best reaction conditions were to add the alkoxide solution to a vessel containing both **5** and 3,4,5-trimethoxybenzaldehyde (**6**). When base was added to a solution of only ketoester without 3,4,5-trimethoxybenzaldehyde, a large impurity peak appeared in the in-process assay, particularly at temperatures >40 °C.

The neutralization was accomplished with acetic acid. No other acids were studied for the neutralization. However, from experiments directed at converting CI-1020 (**8**) back to hydroxybutenolide, strong acids such as hydrochloric acid did not allow isomerization of **9** and **10** to hydroxybutenolide to occur. The components of the reaction mixture precipitated immediately upon adding the strong acids. In the case of acetic acid, the acidified reaction mixture remained in solution, allowing the isomerization to **7**. No advantage was found from cooling the reaction during addition of the acetic acid. Temperatures below 65 °C did not produce isomerization to any great extent, and thus the scaled-up runs were isomerized at reflux. Studies on the amount of acetic acid required indicated a practical minimum of about 50% excess to do the isomerization and to neutralize the excess methoxide. When less than 50% excess of acetic acid was used, the isomerization either was very slow or did not go to completion. On-scale, a 60% excess of acetic acid was used, which gave an isomerization completion time of 10 to 11 h based on the in-process assay.

Upon completion of the isomerization, the reaction was cooled to 0 °C over several hours, aged at that temperature, and then filtered. The crude cake was washed with cold ethanol followed by water. In the initial work on this step large amounts of sodium acetate were found to coprecipitate with the product. The sodium acetate was easily removed using a water reslurry since **7** is very insoluble in water.

Scheme 4. **API formation**



The optimized process was successfully piloted, providing an improved yield of hydroxybutenolide to 80−85% compared to the 59% obtained with the original process. The improved process also provided HPLC purities of 90−95% by area normalization.

Synthesis of the API (8). The final step of the synthesis of CI-1020 was preparation of the sodium salt, which was water-soluble and could be used in IV formulation of the API. The hydroxy lactone ring of **7** was opened by reaction with a base to give the API (**8**) as shown in Scheme 4. The main impurities of concern in this reaction were the geometric isomers **9** and **10** shown in Scheme 4, which resulted from movement of the double bond out of conjugation with the ester carbonyl.

The choice of base was the first parameter in the development of this step that was studied. A base was needed which was strong enough to form the sodium salt but weak enough to minimize olefin isomerization. The bases tried included sodium hydroxide, sodium methoxide, sodium carbonate, and sodium bicarbonate. Sodium hydroxide had been used in the Discovery preparation of **8**. However, when sodium hydroxide was used for larger-scale runs, significant amounts of the olefin isomers were obtained in the API prepared. The first larger-scale experiments of the API process used sodium methoxide. Careful analysis determined that this material was contaminated with as much as 40% of the isomers. The major breakthrough on this reaction was the use of sodium carbonate, as the base that had the balance of properties needed. The sodium carbonate was used in equimolar quantities because it was converted in the reaction to sodium bicarbonate, which precipitated since it was nearly insoluble in methanol. The sodium bicarbonate precipitate was filtered off to give a clear solution of the API sodium salt. In subsequent reactions, 1.2 equiv of sodium carbonate was used to achieve a reasonable reaction rate with complete conversion to product.

The choice of reaction solvent was determined primarily by two major factors. First, a solvent was needed in which the product was reasonably soluble because a pre-crystallization filtration was necessary to remove the insoluble carbonates and other particulates at the end of the reaction to ensure the product was suitable for use in IV formulations. Second, the product needed to be isolated by crystallization. In the original work, lyophilization had been used to isolate

the product and was undesirable for large-scale work. The crystallization from a range of solvents including water, methanol, ethanol, isopropyl alcohol (IPA), methyl *tert*-butyl ether (MTBE), ethyl acetate, and toluene was evaluated. Of these solvents, only ethanol showed any promise for use in a single-solvent system. Either the API was so soluble in a solvent that it would not crystallize, as with water and methanol, or it was very insoluble, making a pre-crystallization filtration impossible. Several mixed-solvent systems were evaluated in which the primary solvent, that the product was very soluble in, could be removed using an azeotropic distillation, leaving the product as a slurry in a solvent in which it is essentially insoluble, for example, water/MTBE, water/ethyl acetate, methanol/ toluene, and methanol/ethyl acetate. In all of these systems, the product failed to crystallize. Instead it usually formed a gum during the distillation. Solvent-exchange of IPA for methanol was also studied. This showed the most promise of any system. Finally it was found that if the reaction was run in a minimum amount of methanol, the product could be precipitated simply by adding IPA, eliminating the need for a distillation.

The next critical part was determining the minimum volume of methanol the reaction could be run in. Early in our scale-up work it was discovered that using larger volumes of methanol and then distilling off the excess solvent prior to crystallization with IPA could be problematic. In several runs the product crystallized during the methanol distillation. The resulting product appeared to be a methanol solvate that was very difficult to dry. The solvated material required several days of drying using vacuum oven temperatures greater than 110 °C to obtain product with less than 0.5% methanol. Although a slight color is introduced with these temperatures, no degradation was detected.

The premature crystallization could be avoided by dissolving the product formed in a minimum volume of methanol. A solution of **8** in methanol of about 0.55 molarity was used for the pilot-scale lots. The product could then be crystallized by the addition of 2.5 to 3 vol of IPA to the methanol reaction solution.

Initially, the API reaction was run at reflux but gave quite inconsistent results at that temperature. As the reaction was studied more thoroughly, higher reaction temperatures were found to favor formation of the impurities **9** and **10**. The API reaction must be run at temperatures less than 30 °C to minimize these impurities.

The most significant problem in the development of the API process was the determination of reaction completion. This was very difficult to this point since no chromatographic method capable of separating hydroxybutenolide from the sodium salt was found. Hydroxybutenolide and the sodium salt readily interconverted in solution in a pH-dependent equilibrium. The two compounds gave a single peak in the chromatograms for all HPLC systems studied to date. Without a good method for completion of reaction, alternative methods for determining the reaction rate had to be evaluated. However, HPLC was useful for monitoring the levels of **9** and **10**, which form during the sodium salt formation reaction. The levels of the two impurities grew steadily during the reaction with the rate of formation of **10** approximately twice that of **9**. In the case of **10**, concentrations up to 0.5% (area normalization) were acceptable in-process since the concentration was reduced 5- to 10-fold when the API is isolated. However, the concentration of **9** was of concern since it was usually present in the isolated product in the same percentage as the crude reaction. This problem was easily monitored by HPLC. If the reaction was stopped prior to completion because of high levels of **9**, the main effect was decreased yield with the unreacted **7** filtered off with the sodium bicarbonate by-product.

Since an HPLC method was not found that was able to resolve penultimate intermediate from the final product, other methods for determining the hydroxybutenolide content of the API were evaluated. Methods using IR were evaluated because of significant differences in the IRs of **7** and **8**. A quantitative IR method was developed. In this method an analyte peak characteristic for hydroxybutenolide was identified which initially appeared to have few interferences from other compounds in the reaction mixture. This method was useful for isolated API but was not useful as an in-process assay because isolation of the API from each sample was required before running the assay. In addition, once pure samples of the double-bond isomers, **9** and **10** (the other major impurities in the API reaction), were isolated they were found to have an IR band in the same region as the hydroxybutenolide analyte peak. Thus, the utility of the quantitative IR for assay of hydroxybutenolide content was diminished.

However, the ReactIR was found to be an effective method for monitoring reaction progress. In this method, sampling at intervals during the reaction allowed monitoring of the formation of **8** in the reaction mixture. The other main components of the reaction mixture, **7**, Na_2CO_3, and $NaHCO_3$, have low solubility in methanol and consequently do not contribute much to the absorbance spectra obtained. The end point of the reaction was determined by the plateauing of the absorbance of several peaks in the IR spectrograms. The IR band at 1598 cm^{-1} was the primary analytical band monitored since it is associated with the carbonyl stretch of the forming carboxylate. At least one other peak was monitored to avoid being fooled by early plateauing in a single peak. This method was initially studied on lab-scale experiments. The reactions were run directly in the ReactIR. Several parameters were studied in these experiments: powdered versus granular sodium carbonate, presence of water, reaction temperature, and reaction time. The type of sodium carbonate had a slight impact on the reaction. The powdered carbonate, which was used in the laboratory, ran slightly faster than the granular carbonate that was typically used in the pilot plant. The powdered form was used in the subsequent pilot-plant work. The presence of a small amount of water was found to have no effect on the reaction. Initially, it had been thought that water might help to solubilize the sodium carbonate and thus facilitate the reaction, but this was not the case.

As expected, the reaction proceeded faster at temperatures up to 50 °C. However, the rate of formation of the isomeric

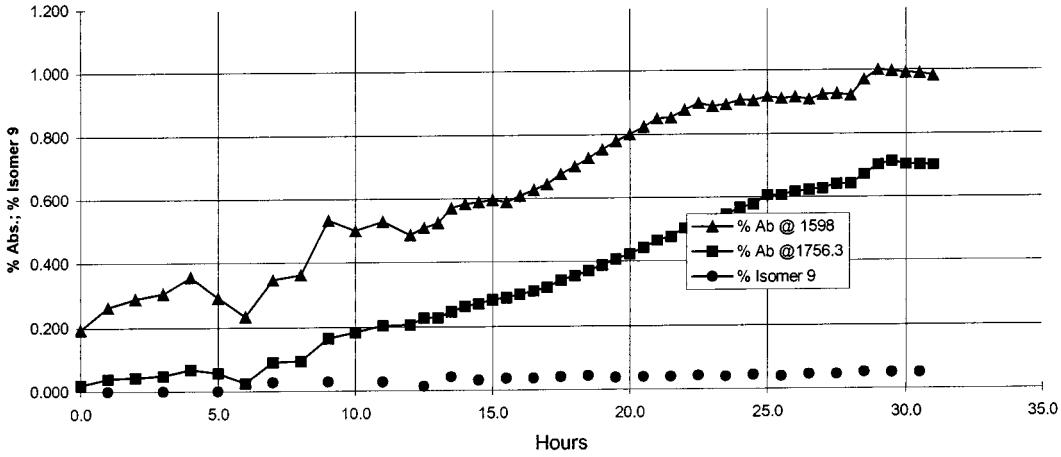


Figure 1. **ReactIR study.**

impurities was also found to be much higher. Consequently, the optimization experiments were run at 20−25 °C. The laboratory experiments run at 20−25 °C were generally done in 42−44 h. Using these data, a lot in the pilot plant was run using a 44 h reaction time for the batch. Analysis of the isolated product determined that it contained higher amount of the isomers than had been seen previously. This suggested that the reaction was complete well before the 44 h stop time since the rate of isomerization increases as the amount of hydroxybutenolide decreases. In the next pilot-plant run, in-process samples of the reaction were taken at regular intervals, and the samples were run on the ReactIR to gather data to determine completion of reaction. In addition, the samples were assayed by HPLC to determine the isomer content. The collected data is shown in Figure 1. As the data were being collected, a plateau developed for both analyte peaks at about 28 h, suggesting that the reaction might be done. However, after about 0.5 h the peaks started to increase in size again briefly and then plateaued again for several hours. It is not clear what caused the increase in peak size at 28.5 h, but since the reaction is heterogeneous, the remaining hydroxybutenolide may have gone into solution and reacted at that point. The reaction was finally considered to be complete at about 30 h, a significantly shorter reaction time than was used in the previous run. The material obtained was the best quality product produced. The shorter reaction times on scale-up were attributed to much better mixing of the heterogeneous reaction mixture in pilot-plant reactors compared to the lab-scale reactors.

The process optimization done to date provided a process for CI-1020, which ran successfully on pilot scale. However, more work using ReactIR® needs to be done to have a fully optimized manufacturing process for CI-1020.

Summary

Overall, the original route was significantly improved into a scalable process that was run successfully on pilot-plant scale. The synthesis used only common solvents for all processing steps—ethanol, methanol, acetone, and IPA. The cyanide waste generated in hydrocyanation step was minimized, contained, and easily destroyed in the workup for that step. The yields for all of the steps of the synthetic route were improved to greater than 85% on-scale.

Several issues remain to be resolved on this synthesis of CI-1020. The most important of these involve the API. Further work would need to be done to develop an analysis that would determine the hydroxybutenolide content in API. This work is necessary only if an IV formulation remains the formulation of choice. If an oral formulation were to be developed, that formulation would probably use the hydroxybutenolide. This would obviate the need for an assay to separate the hydroxybutenolide from the current API. More work also needs to be done on control of the crystallization of the API to avoid formation of the methanol solvate that has been obtained in some small-scale lots.

Unfortunately, work on this project has been terminated. Toxicology studies on CI-1020 demonstrated that it caused arteriopathy in dogs and monkeys. No safe dose of CI-1020 was found which would have allowed clinical studies to proceed.

Experimental Section

All of the reagents used in this study were purchased from the Aldrich Chemical Co. Solvents were purchased from commercial bulk sources. All of the reactions were run under nitrogen atmosphere. The ^{1}H spectra were taken on a Varian 200 MHz spectrometer; chemical shifts are expressed in ppm from tetramethylsilane as an internal standard. The melting points were determined on a Buchi melting point apparatus and are uncorrected. HPLC for the in-process assay and final product assay were performed on a Perkex 5 μ C-18 125 × 4.6 mm column with a mobile phase of 45% acetonitrile and 55% 0.1 M sodium acetate buffer adjusted to pH 6 with the detector at 254 nm. The analyses by HPLC are expressed by % area normalization for individual components.

2-(Benzo[1,3]dioxo-5-yl)-4-(4-methoxyphenyl)-4-oxobutyronitrile (4). 3-(Benzo[1,3]dioxo-5-yl)-1-(4-methoxyphenyl)-prop-2-en-1-one (9.3 kg, 32.9 mol) was slurried into 32 L of acetone at room temperature. Acetone cyanohydrin (3.5 kg, 41.1 mol) and a 25% aqueous solution of tetramethylammonium hydroxide (0.75 L, 2.08 mol) were added to the slurry. The reaction was heated at reflux for 10 h. The mixture was cooled, and 18 L of water was added to

crystallize the product. The resulting slurry was then cooled to 0−10 °C for at least 3 h. The product was collected by centrifugation and dried in vacuo at 40−50 °C. The desired product (8.6 kg, 85% theory) was obtained with a purity of 99% (area normalization) by HPLC. mp 93.9−94.9 °C; ^{1}H NMR (200 MHz, d_6-DMSO) 3.4(1H, dd, CHCH_aHCO), 3.6-(1H, dd, CHCH_bHCO), 3.9(3H, s, OMe), 4.5(1H, t, ArCHCH$_2$), 5.95(2H, s, OCH$_2$O), 7.3(1H, d, ArH), 7.35−7.5(4H, m, ArH), 7.9(2H, d, ArH).

2-(Benzo[1,3]dioxol-5-yl)-4-(4-methoxyphenyl)-4-oxobutyric Acid Methyl Ester (5). 2-(Benzo[1,3]dioxo-5-yl)-4-(4-methoxyphenyl)-4-oxobutyronitrile (8.5 kg, 27.5 mol) and 37% hydrochloric acid (7.2 kg, 72 mol) were slurried into methanol (43 L) at room temperature. The reaction mixture was heated to reflux for 18 h and then cooled to 0 ± 5 °C and held at that temperature range for 12 h. The product was collected by centrifugation and washed with methanol. The wet product was dried in vacuo at 50 °C to give 2-(benzo[1,3]dioxol-5-yl)-4-(4-methoxyphenyl)-4-oxobutyric acid methyl ester (8.6 kg, 91.5%) as an off-white solid with a purity of 97.9% (area normalization) by HPLC. ^{1}H NMR (200 MHz, CDCl$_3$) 3.2(1H, dd, CHCH_aHCO), 3.7(3H, s, CO$_2$CH$_3$), 3.9(3H, s, OCH$_3$), 3.9(1H, dd, CHCH_b-HCO), 4.1(1H, m, ArCHCH$_2$), 5.95(2H, s, OCH$_2$O), 6.7−6.9(5H, d, ArH), 7.9(2H, d, ArH).

Preparation of 3-(Benzo[1,3]dioxol-5-yl)-5-hydroxy-5-(4-methoxyphenyl)-4-[(3,4,5-trimethoxyphenyl)methyl]-2(5h)-furanone (7). Sodium methylate (2.3 kg, 42.6 mol) and ethanol SD3A (16 kg) were charged to a reactor, allowed to warm to near reflux until the methylate had dissolved, and then cooled to 35 °C. To a separate reactor was added 2-(benzo[1,3]dioxol-5-yl)-4-(4-methoxyphenyl)-4-oxobutyric acid methyl ester (8.6 kg, 25.1 mol) and 3,4,5-trimethoxy benzaldehyde (4.9 kg, 25 mol). The sodium methylate/ethanol solution was then transferred to a second reactor. The reaction was held at 27−32 °C for 3 h, first by cooling, later by heating. After 3 h, glacial acetic acid (4.2 kg, 69.9 mol) was added and the reaction heated to 68−72 °C. After 10 h at 70 °C, the reaction was allowed to cool to 0−10 °C over 3 h and then held at that temperature range for 8 h. The resulting slurry was collected by centrifugation and washed with cold ethanol and then with water. The filter cake was immediately reslurried at 20 °C in water (30 L) for 0.5 h, filtered, and washed with water and then with cold ethanol. The white solid cake was dried in vacuo at 50−55 °C for 18−20 h to give 3-(benzo[1,3]dioxol-5-yl)- 5-hydroxy-5-(4-methoxyphenyl)-4-[(3,4,5-trimethoxyphenyl)-methyl]-2(5h)-furanone (11.2 kg, 86.8%) with a purity of 97.6% (area normalization) by HPLC. mp 182.4−183.5 °C; ^{1}H NMR (200 MHz, d_5-pyridine) 3.6(6H, s, OCH$_3$), 3.7(3H, s, OCH$_3$), 3.8(3H, s, OCH$_3$), 4.1(2H, s, ArCH$_2$), 5.95(2H, s, OCH$_2$O), 6.3(2H, s, ArH), 6.95−7.25(5H, m, ArH) 7.9(2H, d, ArH), 10.6(1H, broad, OH).

(*Z*)-Sodium 2-(benzo[1,3]dioxol-5-yl)-4-(4-methoxyphenyl)-4-oxo-3-(3,4,5-trimethoxyphenyl)-but-2-enoate (8). 7 (7.0 kg, 13.8 mol) and sodium carbonate powder (1.7 kg, 16.0 mol) were slurried into methanol (25 L). This slurry was stirred for 31 h at 18−22 °C. The reaction mixture was filtered to remove the sodium bicarbonate by-product and the filter rinsed with methanol (10 L). IPA (120 L) was added to crystallize the product at 18−22 °C. The slurry was cooled at 0 ± 5 °C for 12 h. The slurry was collected by centrifugation and washed with IPA. The wet product cake was dried in vacuo at 40−50 °C for 26 h to give 5.9 kg (83.6%) of CI-1020 as a white solid with a purity of 99.92% (area normalization) by HPLC. mp 258.3−259.3 °C; ^{1}H NMR (200 MHz, d_5-pyridine) 3.38(2H, s, ArCH$_2$), 3.52(3H, s, OCH$_3$), 3.56(6H, s, OCH$_3$), 3.76(3H, s, OCH$_3$), 6.0(2H, s, OCH$_2$O), 6.2(2H, s, ArH), 6.9−7.0(5H, m, ArH) 7.85(2H, d, ArH). Anal. Calcd for $C_{28}H_{25}O_9Na$: C, 63.64; H, 4.77. Found: C, 63.65; H, 4.60. Na assay Calcd: 4.35 Found: 4.15.

Received for review January 3, 2001.

OP010200A