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An Efficient, Economical Synthesis of the Novel Anti-tumor Agent CPI-613

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ABSTRACT: An efficient and practical synthesis of the novel anti-tumor compound 6,8-dithiobenzyl octanoic acid, CPI-613 (**2**), was developed and executed on a practical scale. CPI-613 can be made in a single vessel from (±)-lipoic acid (**1**) via reductive opening of the disulfide ring followed by benzylation of the sulfhydryls with benzyl bromide. CPI-613 was isolated by simple crystallization in high yield and purity. The process is scaleable and has been demonstrated at up to 100 kg.

INTRODUCTION

One of Cornerstone Pharmaceutical's current research programs is focused on developing new anti-tumor agents that exploit the differences between normal cell and cancer cell energy metabolism. This program seeks to discover unique small-molecule analogues of lipoic acid that exploit its known role in cellular energy metabolism[2] and might be effective in selectively disrupting the energy metabolism of cancer cells, while leaving normal healthy cells largely unaffected (see Figure 1). This could be an effective means to treat a variety of cancers.

An early drug candidate, (±)CPI-613 (**2**) was shown to be effective in killing cancer cells both in vitro and in vivo while being essentially nontoxic to healthy cells.[3] Production of **2** on a kilogram scale became necessary to support accelerated timelines for preclinical toxicity evaluations and eventual phase I trials.

DISCUSSION

Compound **2** was initially made on gram scale by dissolving inexpensive and commercially available racemic **1** into aqueous $NaHCO_3$, and then reductively opening the disulfide ring with an excess of $NaBH_4$, followed by acidification with conc. HCl. Extraction of the reaction mixture with CH_2Cl_2 and evaporation of the solvent delivered intermediate dithiol **3** as a thick oil. Subsequent reaction of **3** with an excess of freshly made sodium ethoxide, followed by alkylation of the two resulting sulfur anions with benzyl bromide (BnBr), produced crude **2**,[4] which was then isolated as an oil after acidification and extraction with ethyl acetate. Clean **2** was finally isolated and purified by column chromatography (see Scheme 1).

This early synthetic route suffered from a number of problems that would impede the efficient and cost-effective scale up of the chemistry, and these issues needed to be addressed before pilot-plant production of **2** on a kilogram scale could commence. Below is described our development of a scalable process to prepare **2** and validation of the work on multikilogram scale.

For the dissolution of **1**, the use of aqueous NaOH was superior to that of $NaHCO_3$ since it was easier to control the stoichiometry of addition and did not release CO_2 during acidification, minimizing the risk of excess foaming during the quench. Only a single equivalent of $NaBH_4$ was needed to quickly and completely reduce the disulfide linkage in 1 h at 50 °C. $NaBH_4$ was added in portions to control the temperature and minimize foaming. Solutions of **3** at this stage were stable at ambient temperature under nitrogen for at least three days. The isolation of **3** was troublesome, as it was fairly sensitive to air oxidation and prone to reverting back to **1** upon concentration. This suggested that we examine telescoping the solution into the next step. Unfortunately, treatment of the crude reduction reaction mixture with 2 equiv of BnBr led to the production of monobenzylated intermediate **4** as the major product. Further addition of BnBr did not improve the conversion to **2**, nor did extended reaction times or heating.

Instead we achieved rapid and complete bisthiobenzylation at ambient temperature by the addition of 2 equiv of NaOH in place of NaOEt, followed by 2 equiv of BnBr. Use of NaOH in place of NaOEt delivered a cleaner product, while being less costly, and eliminated the use of flammable ethanol. Careful control of the BnBr addition was necessary to minimize the formation of the tribenzyl impurity **5**, produced rapidly upon the addition of >2 equiv of BnBr. Less than 1 area % of either **1** or **4** was typically seen after the benzylation was complete in gram-scale reaction runs, but 4 area % of tribenzylated impurity **5** was typically seen (Figure 2).

Attempts to minimize **5** by reducing the amount of BnBr charged or shortening the reaction time invariably led to increases in **4**. This was disadvantageous given the fact that **5** was easily purged during crystallization, while **4** was more difficult to remove. The sodium salt of **2** precipitated from the reaction mixture upon addition of BnBr to deliver a fine, mobile slurry that could be held at ambient temperature for at least three days (Scheme 2).

However, attempts to filter the crude **2** as its sodium salt directly from the benzylation reaction proved unmanageable, as the collected solids became a sticky, gummy semisolid of low purity. The reaction mixture containing the slurry of crude **2** sodium salt was instead diluted with 2 vol of ethyl acetate, and acidified with conc. HCl to destroy any lingering $NaBH_4$ and to fully protonate the product, carboxylic acid **2**. A slow addition of

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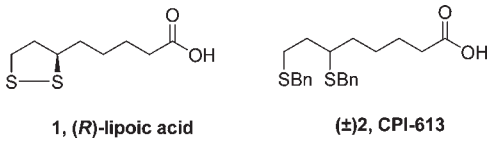


Figure 1. Lipoic acid and CPI-613.

Scheme 1. Early synthetic route

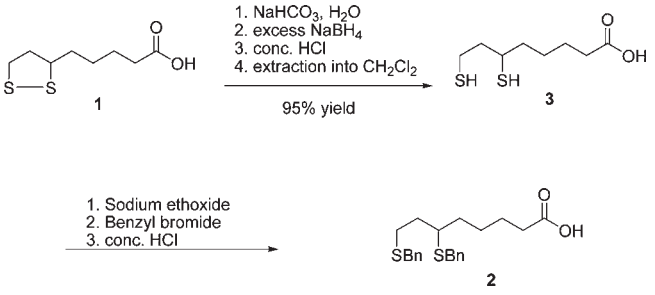
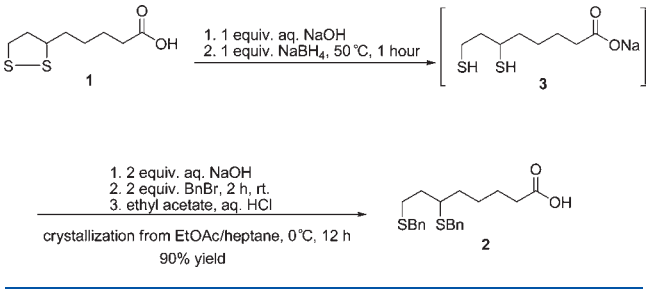


Figure 2. Key in-process impurities.

Scheme 2. Scaled process



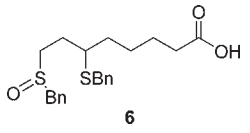


Figure 3. Sulfoxide impurity.

acid controlled the temperature and foaming. At pH <2, extraction of the product into the ethyl acetate layer was essentially quantitative. The product-rich ethyl acetate layer was then washed with dilute HCl and water.

After drying and concentration by distillation, heptane was added, and the solution was seeded to initiate crystallization while cooling to 0−5 °C. White, crystalline **2** was isolated after 12 h by filtration. The product was unhydrated, unsolvated, and a single-crystal polymorph.

The improved process was scaled to 100-g scale and produced CPI-613 of >99.5 HPLC area %, and 88% yield. The solid crystalline **2** was a free-flowing solid that was stable and easily handled. No single impurity was detected with an area % >0.2.

A 4-kg batch was produced in 90% yield using the streamlined process with no significant issues arising. HPLC quality was improved to 99.8 area %, with <0.16% of any single impurity. The only impurity detected having >0.1 area % by HPLC was the sulfoxide **6** (Figure 3). None of the bis-sulfoxide nor any other monosulfoxide was detected.

Of note is that GC/MS analysis indicated no residual BnBr, a potential genotoxic impurity, was detected in the crystalline product. This material was of sufficient quality to be formulated into final drug product.

CONCLUSION

A safe, reliable, one-pot process for the large-scale synthesis of the novel anti-cancer agent CPI-613 was developed and demonstrated on a pilot scale. All scale problems were solved, and the process may now be conducted in a single vessel. The material produced was of exceptionally high quality with only trace impurities. The cost of goods was reduced over 80% on the basis of the cost of the initial process. The process described has been scaled successfully to 100 kg.

EXPERIMENTAL SECTION

General. The reactions were monitored using a Waters Corporation Alliance HPLC system with a photoarray detector, and run with Empower 2 software. The column used was a Waters Symmetry C18, 4.6 mm × 75 mm, 3.5 μm. The mobile phases were acetonitrile/water with 0.1% H_3PO_4. Detection was at 205 nm.

(±)6,8-Bis-thiobenzyl Octanoic Acid, CPI-613, (2). A mixture of racemic lipoic acid **1** (2.77 kg, 13.5 mol) and 27 L of 0.5 M aqueous NaOH was stirred at 20−25 °C until all of **1** had dissolved. The reaction mixture was heated to 40 °C, and 513 g of solid $NaBH_4$ (13.5 mol) was added in portions over 1 h, at <60 °C. HPLC analysis indicated that, after an additional 1 h of reaction, less than 1 area % of starting **1** remained. A solution of 1 M NaOH (27 L) was charged, followed by BnBr (4.62 kg, 27 mol) over 1 h, at <50 °C. HPLC analysis indicated that <1 area % of **3** remained after an additional hour of reaction. Ethyl acetate (54 L) was charged, followed by conc. HCl (2 L), until the pH of the aqueous layer was <2. The aqueous layer was discarded, and the organic layer was washed with 0.1 M HCl (20 L), followed by water (20 L).

The rich ethyl acetate layer was azeotropically dried and reduced in volume by vacuum distillation to approximately 25 L. Heptane (50 L) was added, seeds of pure **2** were added (5 g), and the reaction mixture was cooled to 0−5 °C and stirred for 12 h. The resulting crystalline slurry was vacuum filtered and the wet cake dried at 20−25 °C for 12 h under vacuum. CPI-613 (**2**) was isolated [4.7 kg (90%)] with an HPLC purity of 99.8 area %. Mp 66−67 °C. IR: 3050, 1710, 1400, 668 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.40−7.20 (m, 10 H), 3.80−3.60 (m, 4 H), 2.60−2.50 (m, 2 H), 2.44 (t, J = 8.7, 2 H), 2.23 (t, J = 8.1, 2 H) 2.03−1.30 (m, 8 H). Anal. Calc for $C_{22}H_{28}O_2S_2$: C, 68.00; H, 7.26; S, 16.50. Found: C, 67.99; H, 7.31; S, 16.37.

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