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From bead to flask: Synthesis of a complex β-amido-amide for probe-development studies

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Abstract

A concise synthesis of benzimidazole-substituted β-amido-amide LLW62 is presented. The original synthesis of compounds related to LLW62 was developed on Rink resin as part of a "one-bead, one-compound" combinatorial approach for on-bead screening purposes. The current synthesis is carried out in solution and is amenable to scale-up for follow-up studies on LLW62 and investigations of related structures. The key step involves the use of a β-amino acid-forming three-component reaction (3CR), the scope of which defines its role in the synthetic strategy.

Introduction

Library syntheses and high-throughput screening can often be combined to enable the discovery of new small-molecule probes that modulate biological phenomena [1]. Although the use of solid-phase, split-pool combinatorial synthesis for the preparation of solutions of small-molecule libraries has declined, the use of these compounds for on-bead screening has resulted in recent screening innovations [1,2]. The Lam and Kurth groups have published several "one-bead, one-compound" (OBOC) library syntheses of heterocyclic structures for a variety of screening endeavors [3-12]. Some of these compounds were identified as inhibitors of p21, which is a protein that modulates the activity of cyclin kinases [13-15]. One function of p21 is that it acts downstream of p53 to repair DNA-damaged cells and may function to convey anti-apoptotic activity to cancer cells (Figure 1) [13]. As such, an inhibitor of p21 could sensitize malignant cells to DNA-damaging chemical and radiation therapy by subverting this p21-mediated DNA repair process [14-17]. In this study, we developed a synthesis of LLW62 (**1**, Figure 2), which is a complex benzimidazole-substituted β-amido-amide similar in structure to inhibitors of p21 that were reported previously to support studies of this compound as a biological probe [14,15].

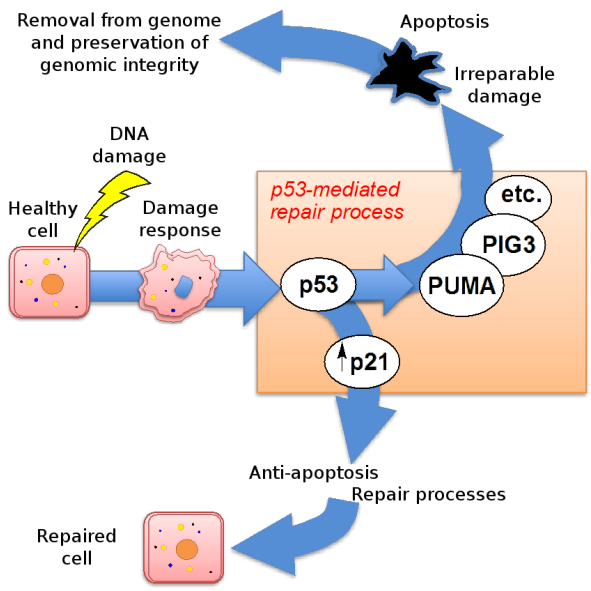


Figure 1: p21 determines the fate of DNA-damaged [13] cells.

The synthesis of **1** emanates from a one-pot, three-component reaction (3CR) of an arylaldehyde, malonic acid (**5**), and ammonium acetate, which assembles the β-amino acid core (Figure 2) [14,15,18]. In the reported synthesis of **1**, a protected β-amino acid core was attached to Rink-amide resin and carried through to **1** by a series of elaboration and tagging steps [14,15]. Synthetic intermediates in this route were not characterized, and **1** was ultimately purified by high-performance liquid chromatography and partially characterized by matrix-assisted laser desorption/ionization mass spectrometry [14]. In the current synthesis, we set out to develop a concise and scalable solution-phase route to **1** and provide characterization data for **1** and all intermediate compounds.

In our retrosynthetic analysis, we envisioned **1** coming from acylation of benzimidazole **3** with isocyanate **2** (Figure 2). We initially sought to avoid nitration, protection and deprotection steps and access this intermediate by performing a late-stage 3CR with benzimidazole **4**, which would be available from nitrile **6** or acid **7** (Figure 2, A). Although synthesis of **4** proceeded without difficulty from acid **7**, this route was unsuccessful at a late stage for a reason that we describe below. We next envisioned benzimidazole **3** emanating from β-amino ester **10,** which could be accessed in a few steps starting with an early stage 3CR of aldehyde **11**, malonic acid (**5**), and ammonium acetate (Figure 2, B). Gratifyingly, **10** was converted to the requisite benzimidizole **3** in three steps and carried through to **1**.

Results and Discussion

Our initial target was benzimidazole **4**, which we envisioned originating from nitrile **6** or acid **7**, each of which is commercially available (Figure 2). We first attempted to synthesize **4** from **6**, which would lead to the shortest possible synthesis of **1**. Nitrile **6** was treated with *N*-(3-aminopropyl)pyrrolidine (**8**) to produce aniline **12** in 81% yield (Scheme 1) [19]. This compound was reduced to aniline **13** in 79% yield and converted to the benzimidizole **14** in 63% yield with aldehyde **9** under oxidative conditions. The resultant nitrile proved to be extremely

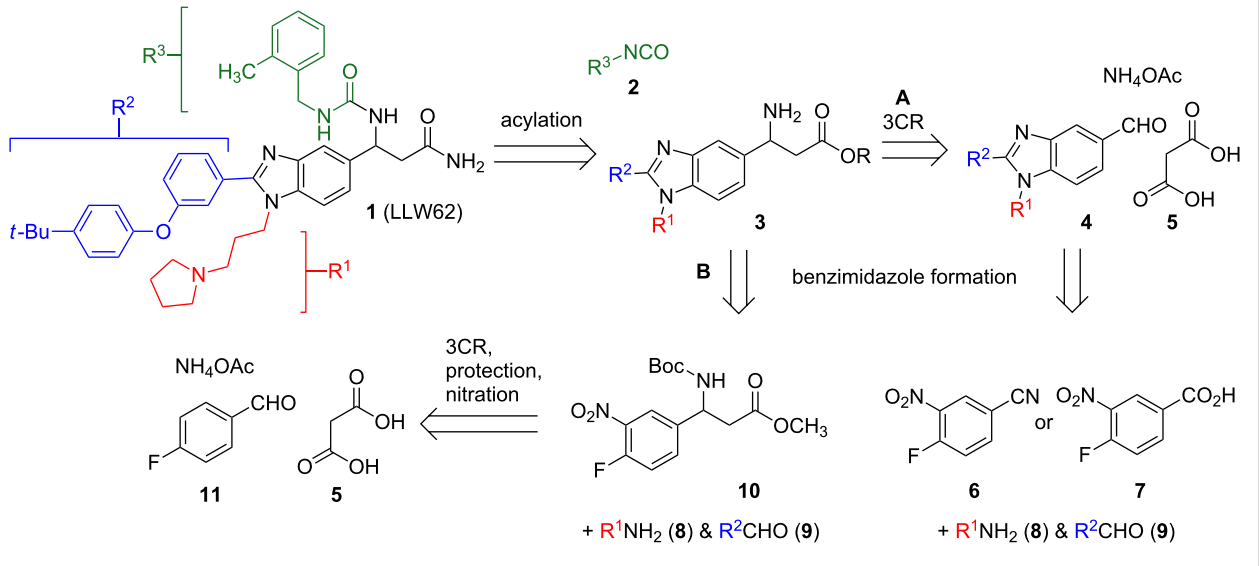
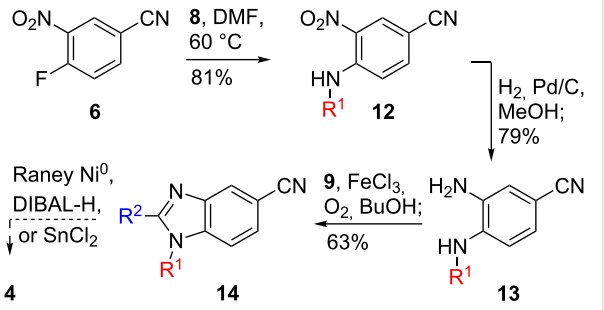


Figure 2: Retrosynthetic analysis of LLW62 (**1**) from acid **7** (A) or aldehyde **11** (B).

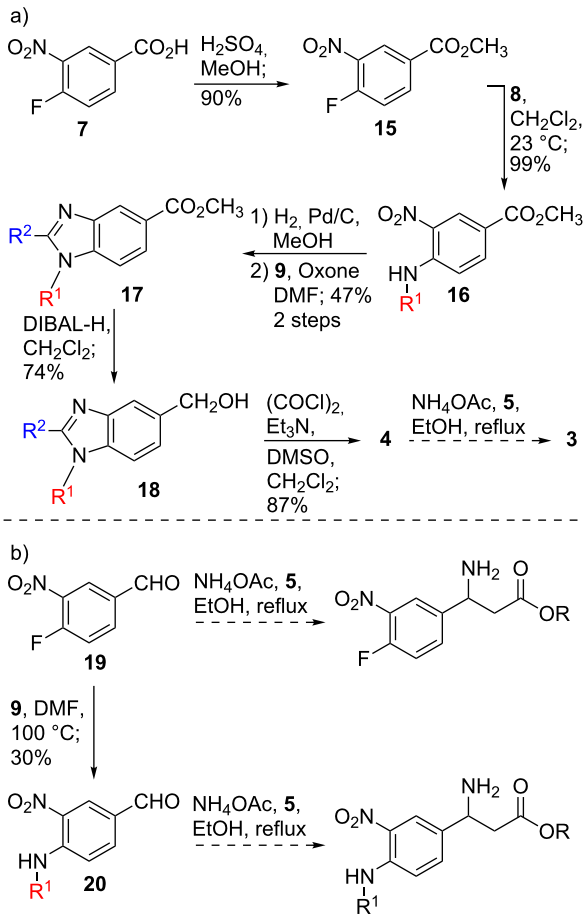
insoluble and difficult to handle. Several reduction conditions were attempted to produce benzimidazole **4** with no success. In addition, attempts to use the nitrile in a Blaise-type reaction or similar nucleophilic addition were also unsuccessful (not shown). Although nitrile **6** would have provided the shortest, most direct entry into the requisite β-amino core structure, we turned our attention to another route to **4**.



Scheme 1: Attempted synthesis of aldehyde **4** from nitrile **6**.

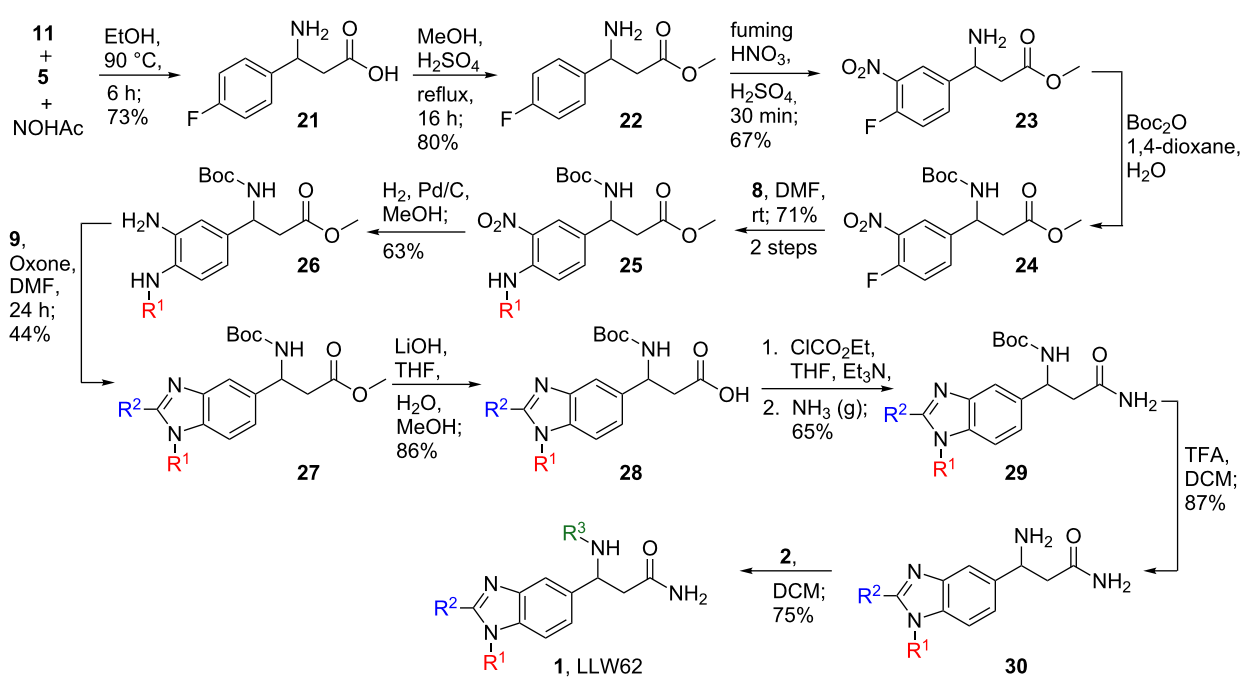
Acid **7** was immediately more promising as a starting material for benzimidazole **4**. Commercially available **7** was converted to methyl ester **15** in 90% yield, due to its ease of handling (Scheme 2) [20]. Next, S_NAr displacement of the fluoride of **15** by *N*-(3-aminopropyl)pyrrolidine (**8**) proceeded in high yield, 99%, to give aniline **16** [20]. Reduction of the nitro group was nearly quantitative and subsequent benzimidazole formation with Oxone furnished benzimidazole **17** in 47% yield over two steps [20,21]. The ester of **17** was smoothly reduced to the alcohol **18**, in 74% yield, and immediately oxidized to the aldehyde **4**, in 87% yield. Unfortunately, **4** produced none of the desired β-amino acid **3** under several different variants of the 3CR with malonic acid (**5**) and ammonium acetate. Tan and Weaver demonstrated previously that the β-amino acid forming 3CR works best for electron-rich aldehydes and poorly for electron-deficient aldehydes [18]. Thus, we suspected that aldehyde **4** may be too electron poor for the 3CR to work efficiently. Our suspicions were supported by attempting 3CRs on aldehydes **19** and **20**, each of which has a single nitro group, and neither was successful in this transformation.

An early-stage 3CR enabled the use of the least electron-poor aldehyde in this key step (Scheme 3). Heating of 4-fluorobenzaldehyde (**11**), malonic acid and ammonium acetate under reflux proceeded smoothly, as previously described, to furnish β-amino acid **21** in 73% yield [18]. Methylation of **21** (80%) followed by nitration of **22** (67%), boc protection of **23** and S_NAr displacement of the fluoride in **24** with amine **8** (71% over two steps), and finally reduction of the nitro group of **25**



Scheme 2: (A) Synthesis of **4** from acid **7** and (B) attempted β-amino acid-forming 3CRs.

(63%) provided aniline **26** as our key intermediate for forming the benzimidazole core of **1**. We next attempted to produce **27** under the higher yielding oxidative conditions described for the formation of nitrile containing benzimidazole **14**. The yield for this reaction was significantly lower, less than 50%, compared to the reaction to produce **14**, and we observed some transesterification of the methyl ester with butanol to produce a mixture of **27** and the butyl ester of **27** as the major products (not shown). We thus turned to using Oxone, and benzimidazole formation proceeded in acceptable yield (44%) from aniline **26** to furnish **27.** Benzimidazole **27** was then saponified under basic conditions to give acid **28** (86%) [21]. Installation of the primary amide of **1** was then achieved in a single pot by treatment of **28** with ethyl chloroformate to make the mixed anhydride followed by displacement with ammonia gas to produce **29** in 65% yield [22]. Final Boc deprotection of **29** with TFA (87%) and subsequent acylation of the free amine of **30** with isocyanate **2** (75%) provided the desired compound **1** in 11 total steps and 3% overall yield.



Scheme 3: Synthesis of LLW62 by using an early stage 3CR.

Conclusion

We have completed a solution-phase synthesis of **1** and thus provided a common route to related compounds that may emerge from future on-bead screening experiments. The key step was the 3CR to form the β-amino acid core structure. Although the electronic requirements of this reaction limit it to electron-rich, or at least not excessively electron poor, aromatic aldehydes, application of this transformation early in the synthesis ultimately proved successful. Although this route is not suitable for large-scale production of **1**, multigram quantities of this compound and benzimidazoles of comparable complexity are easily accessible for early stage studies of these compounds in vitro and in vivo using model organisms.

Supporting Information

Supporting Information File 1

Experimental procedures and compound characterization.
[http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-9-31-S1.pdf]

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