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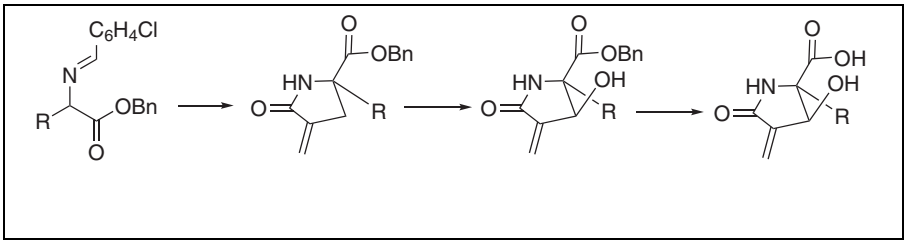

A concise protocol for the synthesis of α-methylene-β-hydroxy-γ-carboxy-γ-lactams has been described via alkylation of amino acid derived iminoesters with α-bromomethylmethacrylate, followed by allylic hydroxylation. All the synthesized compounds have been evaluated for their cytotoxicity on multiple myeloma cancer cell lines.

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INTRODUCTION

γ-Carboxy-γ-lactams (pyroglutamates) are important structural components found in many biologically important heterocyclic natural products [1]. The ester group as well as the lactam ring in pyroglutamates could be further manipulated to synthesize a variety of structurally complex molecules, and hence they serve as important synthetic intermediates in heterocyclic chemistry [1].

The clinical success of the proteasome inhibitor bortezomib (Velcade, **1**) led to the fast track approval of this drug in 2003 for treatment of multiple myeloma [2]. This molecule is also undergoing clinical trials for several other cancers. However, clinical use of this compound has revealed limitations such as intrinsic and acquired resistance [2]. In this regard, some of the pyroglutamate containing natural products such as omuralide **2**, lactacystin **3**, and cinnabaramides **4** were found to exhibit excellent anticancer activity by inhibiting the enzyme proteasome (Fig. 1) [3]. Whereas bortezomib is a reversible proteasome inhibitor, the corresponding natural products mentioned above are irreversible proteasome inhibitors. The mechanism of action for these molecules involves the hydrolytic attack of the proteasome onto the β-lactone unit resulting in the formation of a strong covalent bond and the irreversible inhibition of the proteasome. One potential problem with the use of these natural products as therapeutic agents is their short half-life in solution or in serum due to the highly unstable β-lactone unit and consequent loss of activity.

We envisaged that removing the labile β-lactone unit would afford aqueous stability to the pyroglutamate ring and establishing an α,β-unsaturated system in the pyroglutamate could provide a reactive site for potential nucleophilic interaction with the proteasome. In fact, similar phenomenon was observed for proteasome inhibition by macrocyclic lactam natural product syringolin A (**5**), wherein Thr1 of proteasome has been shown to undergo 1,4-addition with additional stabilization of the intermediate oxyanion by the G47 nitrogen leading to irreversible inhibition (Fig. 2) [4].

We have recently reported concise and stereoselective methodologies for the synthesis of functionalized pyroglutamates [5]. In continuation of this work, we have developed a novel protocol for the concise synthesis of α-methylene-β-hydroxy-γ-carboxy-γ-lactams.

RESULTS AND DISCUSSION

We initiated the synthesis of the target compounds with the esterification of representative amino acids valine **6a**, leucine **6b**, and phenylalanine **6c** as their corresponding benzyl esters **7a–c**. Imination of the α-amino esters **7a–c** was accomplished by reacting with *p*-chlorobenzaldehyde in the presence of $MgSO_4$ providing the imines **8a–c** in quantitative yield. These imines were utilized in the next step without further purification. Lithium enolates of imines **8a–c** were generated using LiHMDS, and they were reacted with α-bromomethylacrylate **9** [6].The acidic work up resulted in the hydrolysis of imines, and subsequent lactamization was realized upon refluxing the crude reaction mixture in HCl and toluene to provide pure α-methylenepyroglutamates **10a–c** in good yield (Scheme 1) [5].

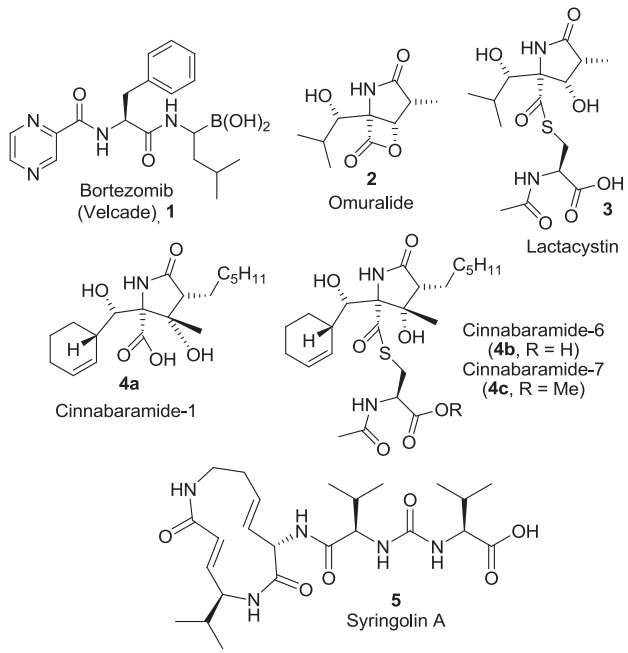


Figure 1. Reversible and irreversible proteasome inhibitors.

After obtaining the α-methylene pyroglutamates **10a–c**, we envisioned the introduction of β-hydroxyl group via allylic oxidation with SeO_2 [7]. Lactams **10a–c** were treated with catalytic selenium dioxide and *tert*-butyl hydroperoxide in ^{t}butanol. However, the reaction was very sluggish, and negligible (<5%) amount of the product was observed. Under these conditions, the benzyl ester underwent *trans*-esterification to afford the *t*-butyl ester containing pyroglutamates. Selenium dioxide and hydrogen peroxide in various aprotic solvents such as toluene, dichloromethane, and THF also proved futile. However, employing selenium dioxide in refluxing acetic acid proved optimal for the reaction as monitored by TLC. The reaction mixture was worked up with water and then $NaHCO_3$ and extracted with ethyl acetate. Concentration of the organic layer provided a dark colored product with many organoselenium impurities. The crude product was further dissolved in THF and treated with stoichiometric amount of hydrogen peroxide to reduce the amount of selenium impurities. The crude reaction product was purified by silica gel column chromatography using hexane and ethyl acetate. The purified product was further recrystallized in ethyl acetate to obtain pure

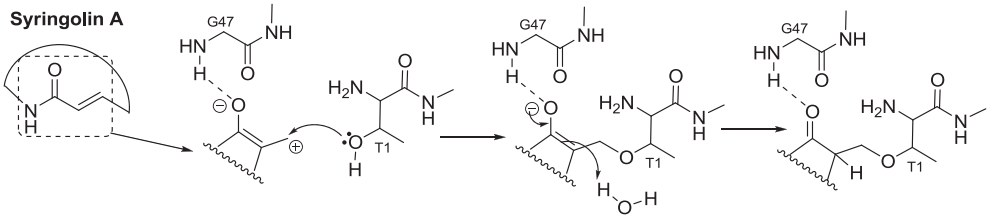
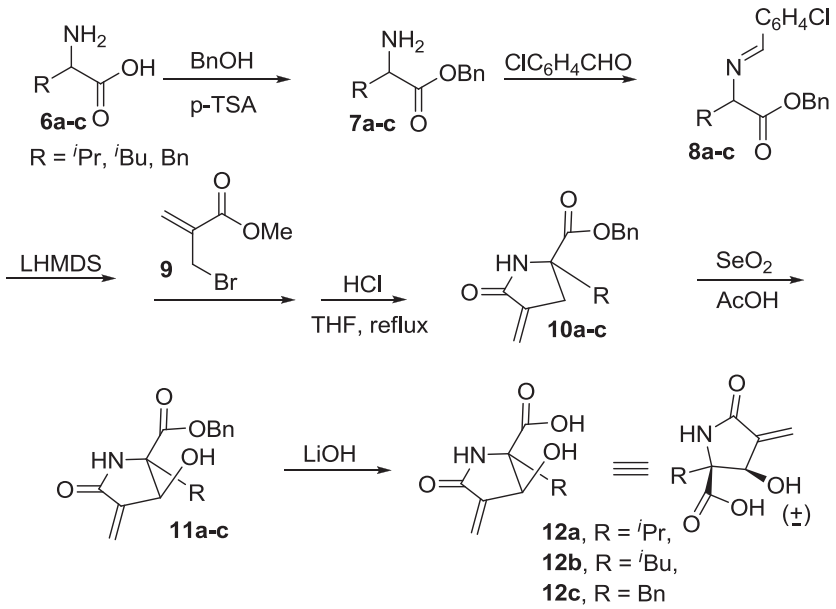
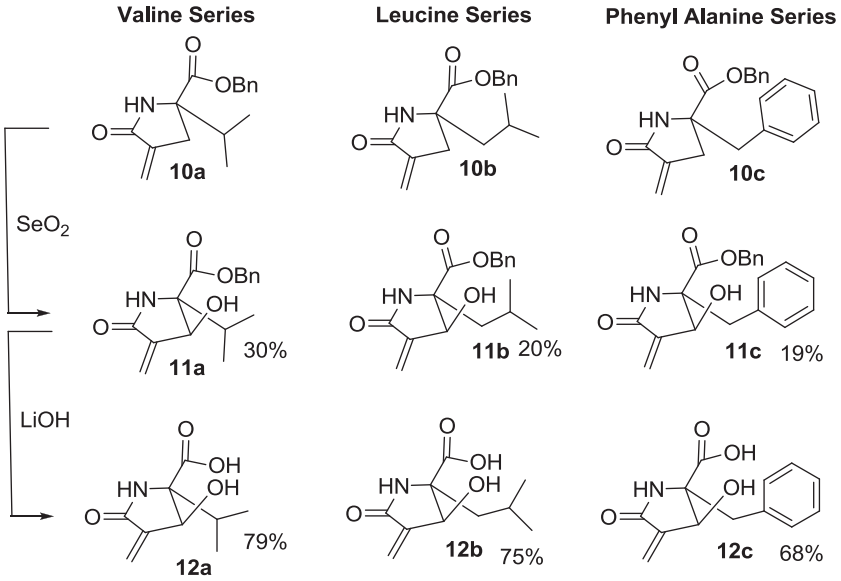


Figure 2. Mechanism of action for proteasome inhibition by syringolin A.

Scheme 1. Synthesis of α-methylene-β-hydroxypyroglutamates.



Scheme 2. Synthesis of α-methylene-β-hydroxypyroglutamates.

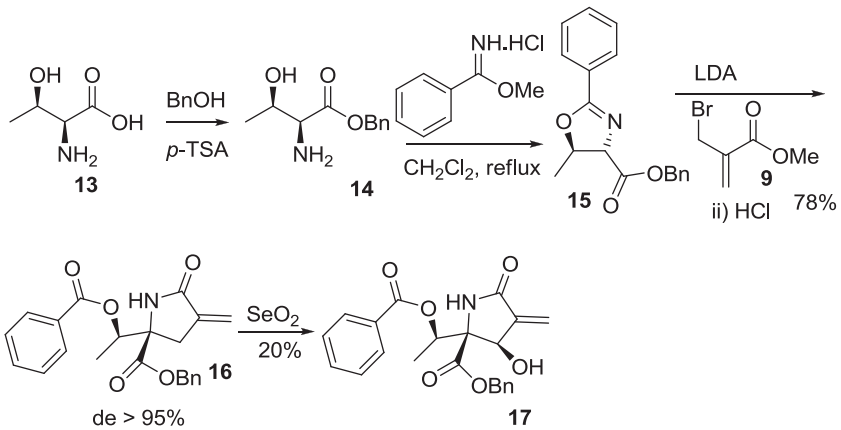


α-methylene-β-hydroxy-γ-carboxy-γ-lactams **11a–c** in modest yields (Scheme 2). High levels of diastereoselectivity was observed in all the examples, and as expected, the 1,2-*trans* isomer was obtained as the major product upon rigorous silica gel column chromatography. Hydroxylation of valine derived pyroglutamate **10a** essentially provided one diastereomer **11a** and the corresponding phenylalanine, and leucine based pyroglutamates afforded 20:1 dr (for **11b**) and 18:1 dr (for **11c**), respectively. The hydrolysis of esters **11a–c** was carried out using 0.1 *N* LiOH, and the hydroxy acids **12a**−**c** were obtained in good yields (68–79%) in all the cases (Scheme 2).

This methodology was further extended toward the enantioselective synthesis of α-methylene-β-hydroxy-γ-carboxy-γ-lactams. For this purpose, we chose to utilize a substrate controlled diastereoselective alkylation of a chiral oxazole followed by allylic hydroxylation. As a representative example, the requisite chiral oxazole **15** was obtained from L-threonine **13** in two steps involving esterification and subsequent treatment with benzimidate [8]. The alkylation of oxazole **15** was realized via lithiation with LDA followed by the addition of α-bromomethylmethacrylate **9** to furnish the α-methylenepyroglutamate **16** upon acidic hydrolysis [9]. Allylic hydroxylation of **16** with SeO_2 in acetic acid furnished the requisite β-hydroxypyroglutamate **17** in excellent diastereoselectivity (dr 15:1) upon silica gel column chromatography (Scheme 3).

Owing to the biological relevance of pyroglutamates, all the compounds synthesized were evaluated for their efficacy as potential anticancer agents utilizing multiple myeloma

Scheme 3. Enantioselective synthesis of α-methylene-β-hydroxypyroglutamates.



cancer cell lines (RPMI-8226). Briefly, the cancer cells were plated in 96 well plates and allowed to adhere for 3d. Cells were then treated with each compound (50 μ*M*) or with DMSO alone for 24 h. MTS assay was used for determining the number of remaining viable cells after exposure to compounds. MTS (20 μL) was added to 100 μL culture medium in each well. After incubation at 37°C for 3 h, absorbance was measured using an ELISA plate reader. Unfortunately, none of the compounds showed any appreciable cytotoxicity at this concentration. We believe that further studies are required to understand the structure activity relationship to identify a lead molecule.

CONCLUSIONS

In conclusion, we have developed a concise protocol for the synthesis of α-methylene-β-hydroxy-γ-carboxy-γ-lactams via iminoester alkylation followed by allylic hydroxylation with selenium dioxide. All the compounds synthesized were evaluated for their cytotoxity against multiple myeloma cancer cell lines. Owing to the importance of pyroglutamates in organic and medicinal chemistry, we anticipate this methodology to be useful for the synthetic community.

EXPERIMENTAL

General methods. All operations were carried out under an inert nitrogen atmosphere. THF, CH_2Cl_2, toluene, and ethyl acetate were purchased as anhydrous solvents and used as such. The ^{1}H- and ^{13}C-NMR spectra were plotted on a Varian Gemini-500 spectrometer (Agilent Varian, Santa Clara, CA). High resolution mass spectra were recorded using a Bruker BioTOF II ESI mass spectrometer (Bruker, Billerica, MA).

Representative procedure for allylic hydroxylation: α-methylene-β-hydroxy-γ-carboxy-γ-lactam 11a. SeO_2 (18.53 mmol) was added to the valine lactam **10a** (26.46 mmol) dissolved in glacial acetic acid (80 mL) and heated to reflux for 1 h. The reaction mixture was filtered, added with water (150 mL), and neutralized with solid $NaHCO_3$. The neutralized solution was extracted with ethyl acetate (3 × 50 mL) and evaporated *in vacuo* to obtain a dark yellow color residue. This residue was dissolved in THF (50 mL), added with H_2O_2 (30 mL), and stirred for 2 h at RT. The reaction mass was again extracted with ethyl acetate (3 × 50 mL), and combined organic extracts were washed with water (3 × 100 mL), dried over $MgSO_4$, and evaporated *in vacuo* to obtain a yellow color residue. Ethyl acetate (20 mL) was added and allowed to stand at room temperature for 2 days. The solid that crystallized out was washed with ice cold ethyl acetate to obtain pure product **11a** (30% yield); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.85 (br s, 1H), 7.33–7.25 (m, 5H), 6.04 (s, 1H), 5.65 (s, 1H), 5.21 (d, J = 12 Hz, 1H), 5.16 (d, J = 12 Hz, 1H), 4.69 (d, J = 9.5 Hz, 1H), 4.49 (d, J = 9 Hz, 1H), 2.40 (m, 1H), 0.78 (d, J = 7 Hz, 3H), 0.66 (d, J = 7 Hz, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 172.4, 169.4, 142.6, 135.4, 128.9, 128.8, 128.6, 121.6, 74.7, 74.0, 67.9, 34.5, 18.1, 16.0; ESI–MS: m/z 312 ($M + Na^+$) for $C_{16}H_{19}NO_4Na$.

Threonine α-methylene-β-hydroxy lactam 17. Procedure was as described above for **11a**. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.05 (br s, 1H), 7.78–7.76 (m, 2H), 7.46–7.27 (m, 8H), 5.93 (s, 1H), 5.73 (q, J = 6.5 Hz, 1H), 5.46 (s, 1H), 5.41 (d, J = 12.0 Hz, 1H), 5.33 (d, J = 12.0 Hz, 1H), 4.75 (d, J = 10.0 Hz, 1H), 4.64 (d, J = 9.5 Hz, 1H), 1.22 (d, J = 6.5 Hz, 3H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 170.0, 165.8, 165.6, 141.9, 134.9, 133.6, 130.1, 130.0, 129.4, 129.0, 128.8, 128.6, 121.5, 74.7, 73.7, 71.8, 68.6, 15.8.

Representative procedure for the preparation of hydroxy acid: valine α-methylene-β-hydroxy lactam acid 12a. To a solution of the hydroxy lactam **11a** (0.66 mmol) dissolved in 5 mL CH_3OH was added the aqueous LiOH (0.1 *N*, 5 mL) and stirred for 48 h at RT. It was then acidified with 2 *M* HCl and concentrated *in vacuo*. The crude product obtained was purified by column chromatography (MeOH/$CHCl_3$, 1:9) to obtain acid **12a** (79% yield). ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.44 (s, 1H), 5.81 (s, 1H), 5.47 (s, 1H), 4.49 (s, 1H), 2.29 (m, 1H), 0.87 (d, J = 6.5 Hz, 3H), 0.70 (d, J = 6.5 Hz, 3H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 172.9, 168.8, 145.5, 117.7, 72.9, 72.5, 33.5, 18.9, 16.7; ESI–MS: m/z 222 ($M + Na^+$) for $C_9H_{13}NO_4Na$.

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