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A convergent approach to synthesis of bortezomib: the use of TBTU suppresses racemization in the fragment condensation

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ABSTRACT

Bortezomib is a first-in-class therapeutic antineoplastic agent used for treating patients with multiple myeloma and mantle cell lymphoma. In this paper we report an improved method for synthesis of the title compound using a convergent approach. TBTU was found to efficiently suppress racemization in the fragment condensation. In comparison with the original synthesis, the presented one is shorter by two steps, higher in yield, and provides better atom economy.

1. Introduction

Bortezomib (originally PS-341) **1** (Fig. 1) is the first therapeutic proteasome inhibitor, marketed by Millennium Pharmaceuticals as Velcade. It is approved in the U.S. for treating relapsed multiple myeloma and mantle cell lymphoma.[1] Bortezomib acts as a reversible inhibitor of 26S proteasome, an ATP-dependent 2.4 MDa multicatalytic proteinaceous machine, located in both the nuclei and cytoplasm of eukaryotic cells. It is involved in cellular homeostasis and cell cycle regulation by degrading a large variety of proteins and polypeptides marked through the ubiquitin pathway. By blocking the proteolysis, normally performed by the proteasome, bortezomib disrupts various cell signaling cascades, leading to cell death.[2–4]

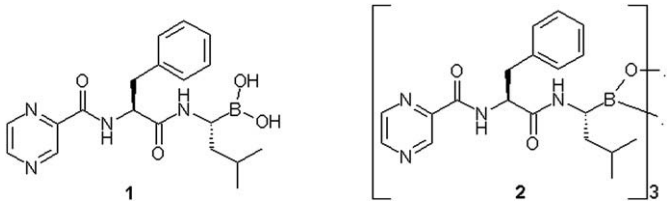

Figure 1. The forms of bortezomib.

The essence of bortezomib is produced in the form of the trimeric boronic anhydride **2**. During lyophilization performed for preparing the drug product, the boroxine **2** forms a diester with mannitol used as an excipient. From this ester, the active boronic acid **1** is obtained by reconstitution of the drug product in saline solution for injection.[5]

2. Results and discussion

Retrosynthetically, the molecule of bortezomib consists of the three structural units: pyrazinoyl, L-phenylalanyl, and L-boronoleucine, which are linked together by peptide bonds. Scheme 1 outlines the initial sequential approach to synthesis of bortezomib.[6–8] The enantiopure L-boronoleucine unit is synthesized by using (1*S*,2*S*,3*R*,5*S*)-pinanediol ester as a chiral director. Alkylation of boronic ester **3** with dichloromethyllithium, generated in situ from methylene dichloride (DCM) and lithium diisopropylamide (LDA), leads to 'onium' salt **4**, which undergoes a stereoselective rearrangement, promoted with $ZnCl_2$, to form (1*S*,2*S*,3*R*,5*S*)-pinanediol (*R*)-1-bis(trimethylsilyl)amino-3-methylbutane-1-boronate **5**. This transformation is a highly efficient method for synthesis of chiral α-substituted boronic esters often referred to as the Matteson rearrangement.[9–11] In the next step chlorine atom in the intermediate **5** is exchanged with a bis(trimethylsilyl)amino group by the action of lithium bis(trimethylsilyl)amide (LiHMDS). A nucleophilic substitution reaction proceeds with inversion of the stereogenic center. Since α-aminoboronic compounds are prone to

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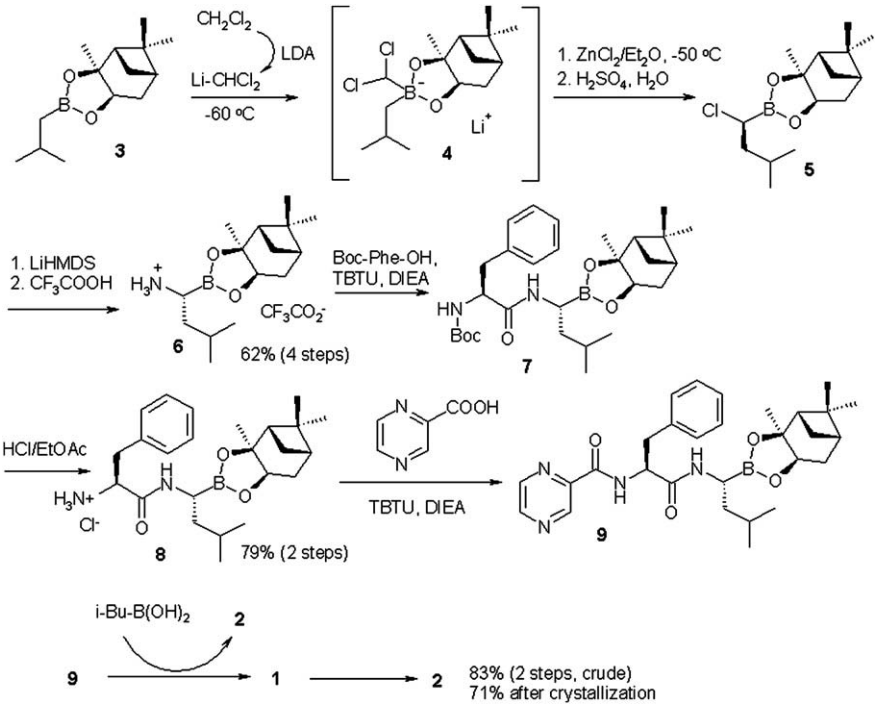
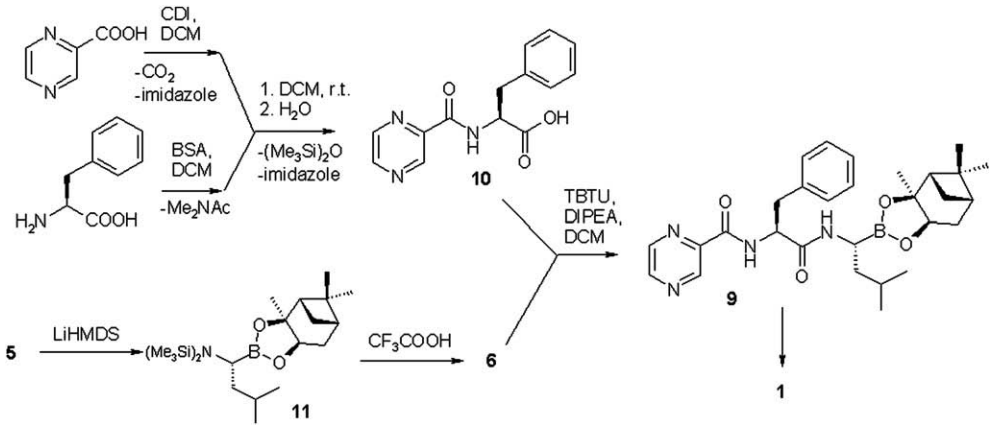


Scheme 1. Linear synthesis of bortezomib.

spontaneous rearrangement if the amino group is not protonated, acylated or otherwise derivatized,[9] the silylated amino group of the bis(trimethylsilyl)amino intermediate is transformed to the corresponding ammonium trifluoracetate **6** by treating with trifluoracetic acid (TFA). After recrystallization from TFA, chiral amine salt **6** is coupled with *N*-Boc-L-phenylalanine using TBTU as the condensing reagent to yield Boc-protected dipeptide **7**. Cleavage of the Boc group with hydrochloric acid gives salt **8**. The latter is coupled with pyrazinecarboxylic acid to form pinanediol ester of bortezomib **9**. Lastly, the pinanediol chiral auxiliary is removed and regenerated by a transesterefication reaction between **9** and isobutylboronic acid in a biphasic mixture of hexane and aqueous methanol under strongly acidic conditions. After concentrating the basified organic solution and recrystallization, bortezomib is isolated in its trimeric anhydride form **2**.

Despite the rather high overall yield (38% of pure substance), the described process suffers from few drawbacks connected with the sequential strategy of synthesis and the use of a protecting group, which adds to the step count, yield and the overall process complexity. In many cases rational design enables partial or complete omission of protecting groups. This principle is especially fruitful when applied to the synthesis of complex molecules and scale-up production.[12,13] With the aim of developing a straightforward and more efficient method for synthesis of bortezomib, we designed an alternative approach to the target compound. *N*-pyrazinoyl-L-phenylalanine **10** and the aminoboronic ester salt **4** had been identified as the pivotal intermediates for a convergent assembly of bortezomib molecule. A potential difficulty in performing a condensation between these two parts was connected with the known susceptibility of chiral N^{α}-acylamino acids to racemization after activation of the carboxylic group.[14] In order to probe the feasibility of the racemization free convergent synthesis we had to prepare both the intermediates first.

The synthesis of *N*-pyrazinoyl-L-phenylalanine methyl ester was achieved by a diphenylphosphoryl azide (DPPA) mediated coupling of pyrazinecarboxyic acid with L-phenylalanine methyl ester hydrochloride, followed by TLC-purification of the product.[15] The tedious isolation procedure and an extra step necessary for conversion of

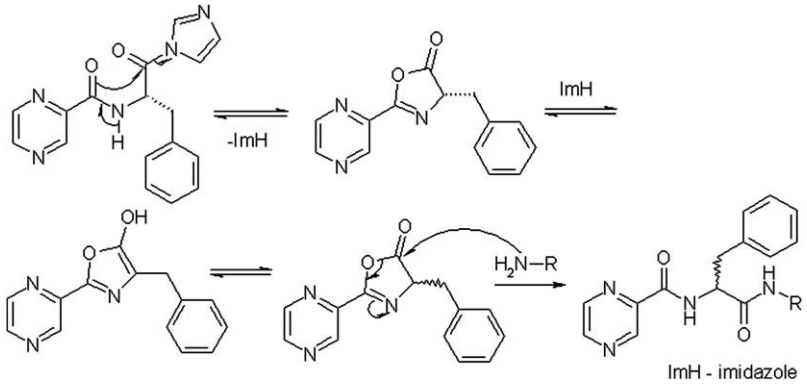


Scheme 2. Convergent synthesis of bortezomib.

methyl ester to carboxylic acid **10** prompted us to develop an alternative method. We synthesized intermediate **10** through a simple *N*,*O*-bis-silylation of L-phenylalanine with *N*,*O*-bis(trimethylsilyl)-acetamide (BSA) with subsequent acylation with pyrazinecarboxylic acid imidazolide (prepared from pyrazinecarboxylic acid and *N*,*N*′-carbonyldiimidazole (CDI)) (Scheme 2). In this process silylation protected the carboxylic group as trimethylsilyl ester, while the silylated amino group of phenylalanine retained its reactivity towards the acylating agent. Besides, converting the amino acid in zwitterionic form to a derivative soluble in methylene dichloride enabled further reactions to proceed under homogenous conditions. The product was isolated in pure form and high yield after the aqueous work-up.

While making the other key intermediate, chiral ammonium salt **6**, according to the known procedure,[8] we found it difficult to achieve appropriate purity even after crystallization. That prompted us to modify the original protocol. The bis(trimethylsilyl)amino derivative **11**, prepared by reaction of compound **5** with LiHMDS, was subjected to extractive workup and isolated in pure form. The ^{1}H and ^{11}B NMR spectra of compound **11** confirmed that the silyl groups remained unaffected despite the aqueous workup. Mild acidolysis with TFA under the inert atmosphere afforded pure salt **6**, which could be utilized without additional purification.

Attempted coupling of the ammonium salt **6** with carboxylic acid **10**, pre-activated with CDI, at 30 °C led to a 45:55 mixture of diastereomeric products distinguishable by non-chiral normal phase HPLC. Obviously, the stereogenic centre of *N*-acylamino acid **10** underwent almost complete racemization after activation of the carboxylic group. This process is believed to proceed via an oxazolone intermediate, and is favored by polar solvents and electron-withdrawing N^{α}-acyl groups, such as pyrazinoyl[14] (Scheme 3).



Scheme 3. Putative racemization mechanism via oxazolone.

In order to suppress racemization, we employed TBTU as the coupling agent in this reaction. TBTU is widely adopted in peptide synthesis and usually gives low percentage of racemization due to the 1-oxybenzotrialole unit incorporated into its molecule. Slow addition of $^{i}Pr_2NEt$ to a mixture of chiral amine salt **6**, *N*-pyrazinoyl-L-phenylalanine, and TBTU in DCM at −5 °C resulted in formation of the coupling product with retention of L-phenylalanine configuration. LC–MS analysis of the crude product showed only small peak of the diastereomeric compound (de 97%). The pinanediol boronic ester **9** was then transformed to bortezomib by the transesterefication reaction with isobutylboronic acid.[8] After crystallization from ethyl acetate, bortezomib was obtained in its trimeric form **2** with a purity of 99.83% by HPLC (UV). The structure of the product obtained was confirmed by spectral analyses including ESI-MS, ^{1}H and ^{13}C NMR, APT and COSY experiments. ESI-MS spectrum of compound **2** showed the same characteristic peaks in the positive ion mode with *m*/*z* 367 ($MH^{+}-H_2O$) and 423 (MK^{+}) as reported for bortezomib[16] (M—molecular weight of bortezomib in its boronic acid form **1**).

3. Experimental section

3.1. General

(*S*)-1-Chloro-3-methylbutane-1-boronate **7** was synthesized by the known method.[8] Isobutylboronic acid was prepared in-house from isobutyl bromide.[17] All reagents were obtained from commercial sources. Zinc chloride was dried by fusion at ca. 300 °C. Diisopropylamine and diisopropylethylamine were distilled from sodium hydroxide. Diethyl ether was distilled from sodium benzophenone ketyl. Argon was dried by passing through column containing sulfuric acid. Elemental analysis was performed on an automated Carlo Erba EA1108 microanalyzer. FTIR spectra were registered with Purge In. IR200 instrument. NMR spectra were recorded using Bruker AMX-360 and Bruker DPX-300 spectrometers. Chemical shifts are quoted in parts per million downfield from $Si(CH_3)_4$ (δ=0 ppm). The hydrogen, carbon and silicon spectra were referred to $Si(CH_3)_4$ or residual solvent signals, and boron spectrum to boron trifluoride etherate as standard. LC–MS analysis was performed on Agilent 1200 instrument with Agilent 6310 ion trap LC–MS detector. We were not able to obtain spectral data for salt **8**.

3.2. (1*S*,2*S*,3*R*,5*S*)-Pinanediol (*R*)-1-bis(trimethylsilyl)amino-3-methylbutane-1-boronate (11)

A solution of *n*-butyllithium in hexane (1.6 M, 132 mL) was added to a stirred solution of hexamethyldisilazane (41.1 g, 255 mmol) in diethyl ether (160 mL) at −40 °C. The mixture was stirred for 10 min, then a solution of (1*S*,2*S*,3*R*,5*S*)-pinanediol (*S*)-1-chloro-3-methylbutane-1-boronate **7** (53 g, 129 mmol) in diethyl ether (180 mL) was added during 45 min at −25 to −30 °C. The temperature was allowed to rise to 0 °C during 1 h. The reaction mixture was washed with ice-cold water, and dried over sodium sulfate. The organic solution was filtered and evaporated under vacuum. The residual oil was dried under vacuum ($\sim 1\times10^{-2}$ mmHg) at 35 °C to give compound **11** (62.98 g, 87%) as viscous oil. [Found: C, 63.21; H, 9.29. $C_{15}H_{26}BClO_2$ requires C, 63.30; H, 9.21.] δ_H (360 MHz, $CDCl_3$) 4.27 (dd, 1H, *J* 8.6, 1.8 Hz, CH–O), 2.65 (dd, 1H, *J* 6.8 Hz, CH–N), 2.31 (m, 1H), 2.20 (m, 1H), 2.03 (t, 1H), 1.74–1.95 (m, 3H), 1.65 (m, 1H), 1.37 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.25 (m, 1H), 1.14 (d, 1H, *J* 11.2 Hz), 0.89 (dd, 6H, *J* 5.8 Hz), 0.84 (s, 3H, CH_3), 0.11 (s, 18H, $2Me_3Si$); δ_C (90 MHz, $CDCl_3$) 3.1 (6C, $2Me_3Si$), 22.7, 23.5, 23.9, 25.3, 26.3, 27.1, 28.4, 35.4, 38.0, 39.5, 45.3, 51.4, 78.1, 85.1; δ_B (116 MHz, $CDCl_3$) 32.96 (br s, B); δ_{Si} (72 MHz, $CDCl_3$) 4.92 (s, $SiMe_3$).

3.3. (1*S*,2*S*,3*R*,5*S*)-Pinanediol 1-ammonium-3-methylbutane-1-boronate trifluoroacetate (8)

Bis(trimethylsilyl)amine **11** (10.00 g, 62 mmol) was dissolved in diethyl ether (90 mL), and the resulting solution was added during 1 h to a stirred solution of trifluoroacetic acid (7.5 mL, 98 mmol) in diethyl ether (100 mL) at −15 to −10 °C under argon. The mixture was stirred for another 1.5 h, and the precipitate formed was filtered, washed with diethyl ether (50 mL) and dried under vacuum at 35 °C to give 7.2 g (78%) of the white crystalline salt **8**, mp 197–198 °C. The product was stored in the fridge under argon until used on the next step.

3.4. *N*-Pyrazinoyl-L-phenylalanine (10)

N,*O*-Bis(trimethylsilyl)-L-phenylalanine solution was perepared by adding BSA (50.7 g, 250 mmol) to a suspension of L-phenylalanine (20.5 g, 125 mmol) in DCM (200 mL), and stirring the resulting solution at room temperature overnight.

A solution of pyrazinecarboxylic acid imidazolide was obtained by adding CDI (26.5 g, 250 mmol) to a stirred suspension of pyrazinecarboxylic acid (24.0 g, 194 mmol) in DCM (400 mL). The mixture was stirred at room temperature overnight, cooled to −30 to −40 °C, and then *N*,*O*-bis(trimethylsilyl)-(L)-phenylalanine solution was added dropwise during 30 min. The temperature was raised to 20 °C during 2 h and the mixture was stirred for another 17 h. The reaction solution was washed with aqueous solution of citric acid (60 g of citric acid monohydrate in 400 mL of water), the aqueous phase was separated, washed with methylene dichloride (100 mL), and the combined organic solutions were diluted with diethyl ether (200 mL), and dried over sodium sulfate. The solvent was removed under vacuum at 35 °C to give 3.1 g (97%) of yellow powder, mp 142–146 °C. [Found: C, 61.85; H, 4.90; N, 15.32. $C_{14}H_{13}N_3O_3$ requires C, 61.99; H, 4.83; N, 15.49.] $[\alpha]_D^{20}$ +13.5 (*c* 1.0, MeOH); ν_{max} (KBr): 1522, 1653, 1680, 2925, 2952, 3325; δ_H (360 MHz, acetone-d_6) 9.22 (d, 1H, *J* 1.4 Hz, CHpyrazine), 8.83 (d, 1H, *J* 2.5 Hz, CHpyrazine), 8.63 (dd, 1H, *J* 2.5, 1.44 Hz, CHpyrazine), 8.39 (br d, 1H, NH), 7.40–7.10 (m, 5H, CHPh), 5.00 (m, 1H, αCHPhe), 3.34 (m, 2H, CH_2Ph); δ_C (90 MHz, DMSO-d_6) 172.4, 162.6, 147.8, 144.1, 143.5, 143.4, 137.4, 129.1 (2C), 128.2 (2C), 126.5, 53.5, 36.3.

3.5. (1*S*,2*S*,3*R*,5*S*)-Pinanediol *N*-pyrazinoyl-L-phenylalanine-L-boronoleucine (9)

Ammonium salt **6**, (7.1 g, 18.7 mmol), *N*-pyrazinoyl-L-phenylalanine **10** (5.08 g, 18.7 mmol), and TBTU (6.62 g, 20.6 mmol) were suspended in DCM (75 mL), and the mixture was cooled to −5 °C while stirring. After that a solution of $^{i}Pr_2NEt$ (9.5 mL) in DCM (35 mL) was added dropwise during 2 h to a stirred reaction mixture maintaining the temperature between −10 and −5 °C. The mixture was stirred for another 1.5 h, and then slowly heated to room temperature. The solvent was evaporated under vacuum, and the residue was dissolved in ethyl acetate (110 mL), washed with water (75 mL), with 3% aqueous potassium carbonate (3×50 mL), with water (75 mL), with 3% aqueous citric acid (3×50 mL), and lastly, with water (75 mL). The organic phase was dried over sodium sulfate, the solvent was removed under vacuum, the residue dissolved in diethyl ether (50 mL) and filtered through a pad of silica gel (washed with 100 mL of ether). Ether was evaporated under vacuum to give 8.14 g (84%) of viscous oil, which slowly crystallized upon standing, mp 58–59.5 °C. [Found: C, 67.30; H, 7.62; N, 10.59. $C_{29}H_{39}BN_4O_4$ requires C, 67.18; H, 7.58; N, 10.81.] $[\alpha]_D^{20}$ −40.0 (*c* 1.0, MeOH); ν_{max} (KBr): 1518, 1685, 1714, 3265, 3386; *m*/*z* (ESI-MS) 519.8 (MH^+), 1038.1 ($2MH^+$); δ_H (360 MHz, acetone-d_6) 9.20 (d, 1H, *J* 1.44 Hz, CHpyrazine), 8.82 (d, 1H, *J* 2.2 Hz, CHpyrazine), 8.63 (m, 1H, CHpyrazine), 8.40 (br d, 1H, NH), 7.61 (br s, 1H, NH), 7.10–7.33 (m, 5H, CHPh), 4.93 (m, 1H, αCHPhe), 4.30 (dd, 1H, *J* 8.6, 1.8 Hz, CHpinane–O), 3.2 (t, 2H, CH_2Ph), 3.05–0.7 (m, 25H, αCH–B, $5CH_3$, $3CH_2$, 3CH); δ_C (75 MHz, $CDCl_3$) 170.5, 162.5, 147.1, 144.0, 142.5, 136.3, 129.2 (2C), 128.2 (2C), 126.6, 85.3, 77.5, 53.7, 51.3, 39.8 (CH_2), 39.4, 38.5 (CH_2), 38.3, 37.9, 35.7, 35.4 (CH_2), 28.4, 26.9, 26.1 (CH_2), 25.9, 23.8, 22.7, 21.8.

3.6. Bortezomib (2)

Boronic ester **9** (68 g, 13.12 mmol) was dissolved in methyl alcohol (0.5 L). To that solution hexane (0.5 L) and 1 N hydrochloric acid (290 mL) were added under stirring, and the biphasic mixture was cooled to 10 °C. Isobutylboronic acid (24 g, 235 mmol) was added, and the mixture was stirred for 17 h at room temperature. The layers were separated in the separating funnel. The methanolic layer was washed with hexane (3×0.1 L) and the combined hexane solutions were evaporated to dryness. From the residual oil boronic ester **3**, incorporating valuable (1*S*,2*S*,3*R*,5*S*)-pinanediol chiral auxhiliary, was regenerated by chromatography. The aqueous methanolic layer was neutralized with sodium hydrocarbonate (30 g) and extracted with ethyl acetate (0.85 L). The solution was dried over sodium sulfate, filtered through a plug of silica gel, and recrystallized twice from ethyl acetate to give 26.55 g (26.3 mmol, 60%) of the white amorphous solid 99.83% pure by HPLC (UV). ν_{max} (KBr): 701, 750, 1021, 1155, 1201, 1400, 1522, 1657, 2954, 3385; δ_H (360 MHz, $CDCl_3$) 9.08 (d, 1H, *J* 1.2 Hz), 8.82 (d, 2H, *J* 2.4 Hz), 8.80 (s, 1H), 8.70 (m, 1H), 7.10–7.30 (m, 5H, CHPh), 4.86 (m, 1H, αCHPhe), 3.15 (m, 2H, CH_2Ph), 2.64 (br m, 1H, αCH–B), 1.52 (m, 1H, $CH(CH_3)_2$), 1.30, 1.17 (2m, 2H, CH_2–^{i}Pr), 0.76 (d, 6H, *J* 6.60 Hz, $2CH_3$); δ_C (90 MHz, $CDCl_3$) 172.5, 162.3, 147.5, 143.9, 143.3, 143.2, 143.1, 136.6, 129.1, 127.9, 127.8, 126.2, 51.5, 42.7, 39.8 (CH_2), 37.2 (CH_2), 24.9, 22.8, 22.3.

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