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Short Research Article

Synthesis of four isotopically labeled forms of a proteasome inhibitor, bortezomib†

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Abstract: [D_2](1*R*)-3-methyl-1-[[(2S)-1-oxo-3-phenyl-2-[(pyrazinylcarbonyl)amino]propyl]-amino]butyl] boronic acid ([D_2]bortezomib), a proteasome inhibitor, was synthesized in 11 steps from *iso*butyryl chloride. Key steps in the synthesis included formation of the *iso*butyryl boronic acid via Grignard reaction and preparation of the chiral chloride using Matteson reaction. [$^{13}C_9$]bortezomib, [D_5]bortezomib, and [D_1]bortezomib were similarly synthesized from appropriate labeled precursors. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: [D_2]*iso*butyl alcohol; [D_2]*iso*butyl bromide; [D_2]*iso*butyl boronic acid; [D_1](α*S*,3a*S*,4*S*,6*S*,7a*R*)-hexahydro-3a,5,5-trimethyl-α-(2-methylpropyl)-4,6-methano-1,3,2-benzodioxaborole-2-methanamine; [D_2](α*S*,3a*S*,4*S*,6*S*,7a*R*)-hexahydro-3a,5,5-trimethyl-α-(2-methylpropyl)-4,6-methano-1,3,2-benzodioxaborole-2-methanamine; [$^{13}C_9$]bortezomib; [D_1]bortezomib; [D_2]bortezomib; [D_5]bortezomib

Introduction

The 26S proteasome is a multicatalytic proteolytic complex located in both the nuclei and cytoplasm of eukaryotic cells.[1] The proteasome hydrolyzes proteins (e.g. misfolded proteins) marked for degradation through the ubiquitin pathway and plays an essential role in cellular homeostasis and cell cycle regulation.[2] Importantly, various hematological cancers are dependent on the amplified proteasome activity for cell survival. While the reasons are not completely understood, healthy cells do not exhibit this same dependency, and therefore, inhibition of the proteasome may represent a therapeutic advantage in targeted cancer therapy.[3] Bortezomib **5** (VELCADE®, formerly known as PS-341, Scheme 1) is a first-in-class proteasome inhibitor that has been approved in Europe and the United States for the treatment of patients with relapsed multiple myeloma.[4] Four isotopically labeled forms of bortezomib were prepared to support multiple biotransformation and pharmacokinetic studies. [D_1]bortezomib **5C** was synthesized to aid in the elucidation of the mechanisms mediating the P450-catalyzed deboronation of bortezomib. [D_2]Bortezomib **5D** was required to assist in determination of the structures of two metabolites. [$^{13}C_9$]Bortezomib **5A** and [D_5]bortezomib **5B** were prepared as internal standards for the liquid chromatographic/tandem mass spectrometric (LC/MS/MS) bioanalysis of nonclinical and clinical studies.

Results and discussion

Synthesis of ($^{13}C_9$)bortezomib 5A and (D_5)bortezomib 5B

Preparation of [$^{13}C_9$]bortezomib 5A: Commercially available BOC-[$^{13}C_9$]*L*-phenylalanine **2A** was chosen as the labeled starting material (Scheme 1). The boronate ester **1** was prepared by the procedure of Kettner *et al.*[5] Coupling of **1** with **2A** followed by deprotection of the resulting **3A** led to the amine which was further coupled with 2-pyrazine carboxylic acid to afford **4A**. Compared to HATU and EDCI, TBTU was found to be the best coupling agent for these two coupling steps.[6,7] [$^{13}C_9$]bortezomib **5A** was prepared by the acidic catalytic transesterification of **4A**. The overall yield of

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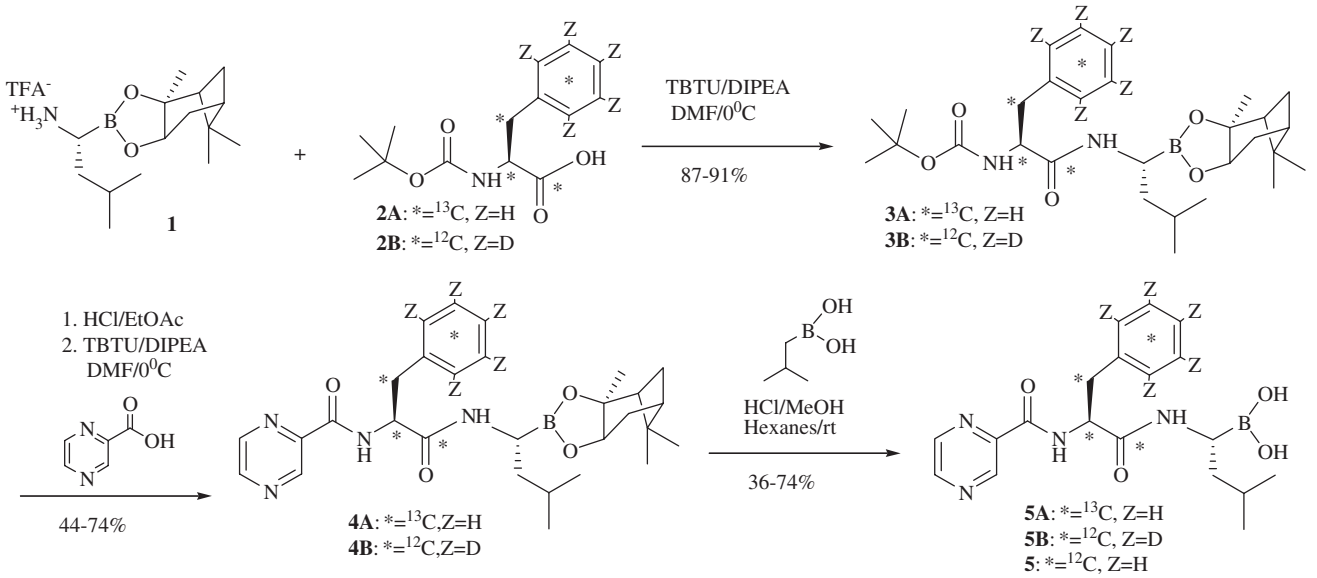
5A was 49%. [D_5]bortezomib **5B** was prepared in a similar manner using commercially available BOC-[D_5]*L*-phenylalanine **2B**. The overall yield of **5B** was 14%. The lower yield of **5B** compared to **5A** was due to the inefficient crystallization of the final compound. **5B** was found to be equally effective compared to **5A** as an internal standard for bioanalytical studies.

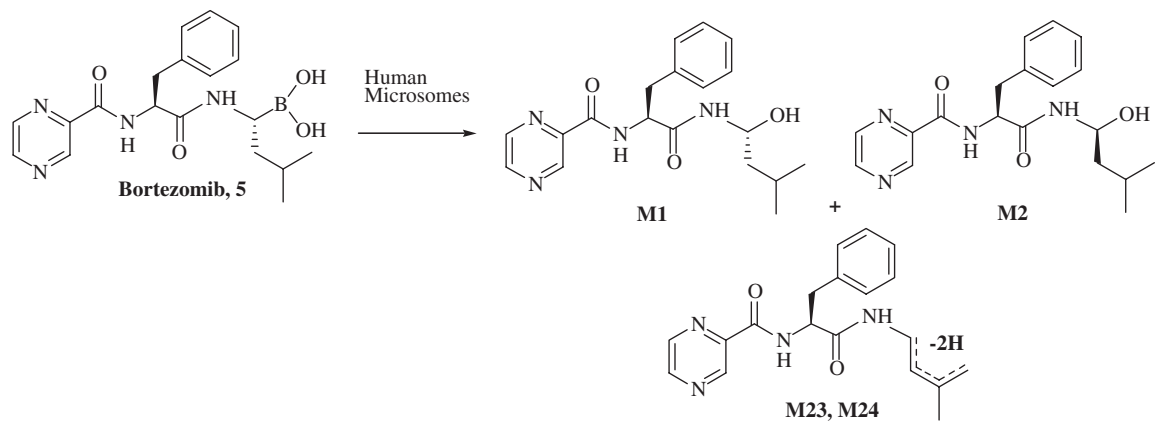
Synthesis of (D_2)bortezomib 5D and (D_1)bortezomib 5C

Inhibition of the 26S proteasome involves a dative bond between the *N*-terminal threonine residue of the proteasome and the boron atom of bortezomib.[3,8] Hence, deboronation represents a deactivation pathway and is the principal route of metabolism of this chemotherapeutic agent.[9] A near equal mixture of diastereomeric carbinolamide metabolites (**M1** and **M2**) was observed following metabolism in human hepatic microsomes (Scheme 2). Metabolites **M23** and **M24** were observed at minor levels and were tentatively assigned as bearing an unsaturation of the *iso*butyl side chain.[9]

In order to assist in the structural elucidation of **M23** and **M24**, [D_2]bortezomib **5D** was prepared. *Iso*butyryl chloride **6** was reduced by lithium aluminum deuteride to provide [D_2]*iso*butyl alcohol (Scheme 3).[10] Bromination of this alcohol with PBr_3 yielded [D_2]isobutyl bromide **7**.[11] The low yield of this bromination step was due to the decomposition of **7** during its distillation. The reaction of Grignard **7** with trimethylborate,



Scheme 1



Scheme 2

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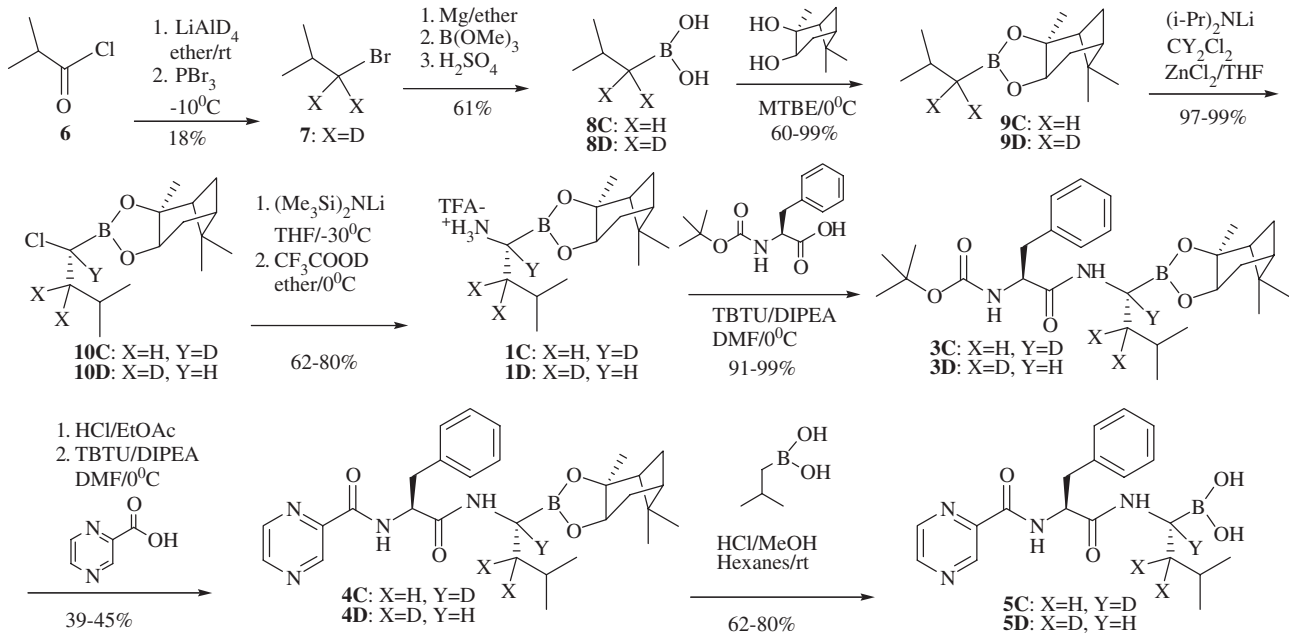
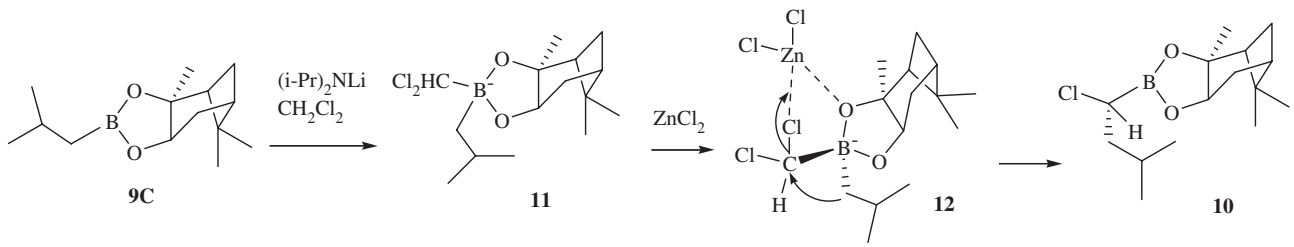


Scheme 3



Scheme 4

followed by subsequent hydrolysis, resulted in the formation of the desired [D_2]*iso*butyl boronic acid, **8**.[12] Coupling of **8** with (1*S*,2*S*,3*R*,5*S*)-(+)-pinanediol afforded the boronate ester **9**. Capitalizing on the exceptional enantioselectivity of the Matteson rearrangement, the reaction of the boronate ester (**9**) with dichloromethane and $ZnCl_2$ provided the key chiral chloride **10D** in an excellent yield (99%).[13,14] Nucleophilic substitution of **10D** with lithium hexamethyldisilazide followed by the deprotection (TFA) of two trimethylsilyl groups afforded the chiral amine **1D**. The subsequent four steps followed the same methods described in Scheme 1 to get the final product **5D**, [D_2]bortezomib. The overall yield of the 11-step synthesis of **5D** was 1%.

[D_1]bortezomib **5C** was instrumental in the mechanistic investigation into the unusual P450-catalyzed deboronation of bortezomib. Compound **5C** was synthesized in a manner analogous to the later stage preparation of [D_2]bortezomib **5D**. The overall yield of the eight-step synthesis of **5C** was 9%.

The enantioselectivity of the Matteson reaction may be explained by the formation of two intermediates (Scheme 4).[15] The first intermediate **11** is formed by coordinating the Lewis acid (the boronate ester **9C**) with the carbanion produced from CH_2Cl_2. The second intermediate **12** is formed by subsequent coordination of a Lewis acid catalyst ($ZnCl_2$) with an oxygen and a chlorine atom of **11**. The enantioselectivity one observes from the Matteson rearrangement may be best explained via the proposed transition state chemistry depicted in Scheme 4. The spacial arrangement of coordinate complex **12** is such that the critical carbon-bond migration is favored, thus resulting in the desired precursor, **10**.

Mechanism of P450-catalyzed deboronation of bortezomib

There are two possible mechanisms to explain the P450-catalyzed deboronation of bortezomib.[9] The first mechanism is the peroxidation by iron-peroxo species

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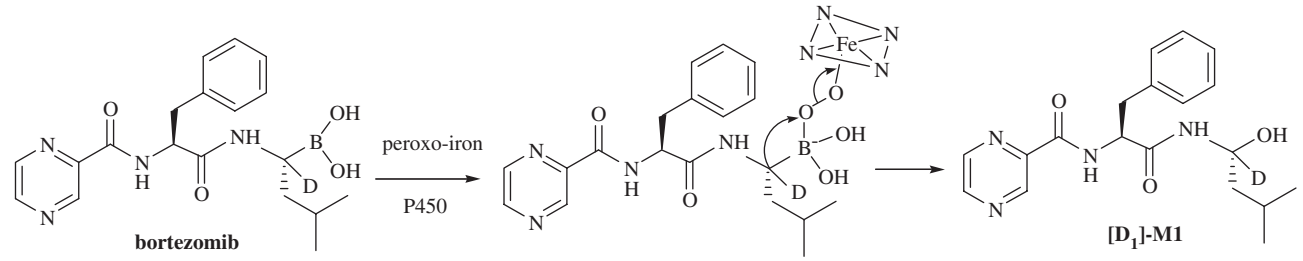
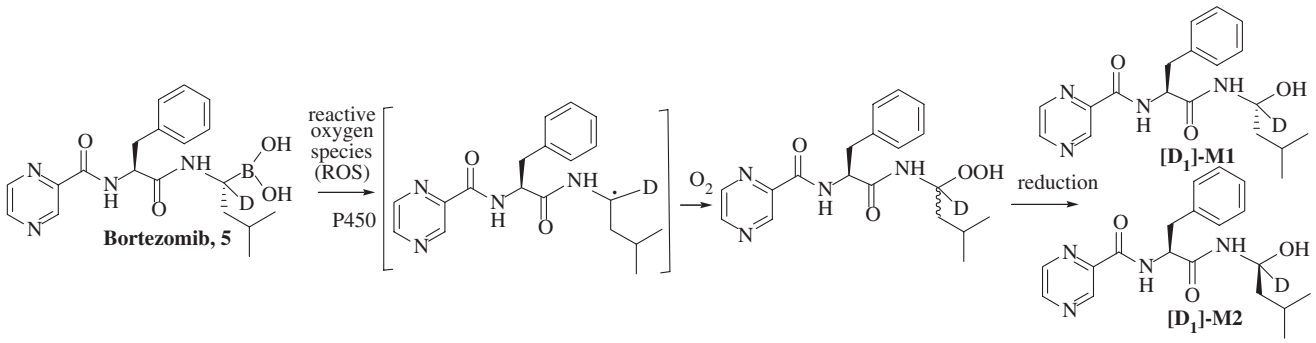


Scheme 5



Scheme 6

produced in the P450 catalytic cycle (Scheme 5), where the iron-peroxo mediated reaction would result in the stereocontrolled oxidation of **5** (i.e. retention of stereochemistry at the migrating carbon center producing a single diastereomer). Consequently, two metabolites (**M1** and **M2**) were produced as a result of human metabolism of **5**. These data would indicate an alternative mechanism contributing to the deboronation of this chemotherapeutic agent. Importantly, incubation of isolated carbinolamide **M1** with human liver microsomes *did not* afford a mixture of **M1** and **M2**;[9] this finding eliminated the possible role of reversible dehydration as a means of producing the metabolite mixture.

Given the numerous historical references of boronate autoxidation, another probable mechanism considered was deboronation via the varied reactive oxygen species (ROS) produced as byproducts of P450 catalysis (Scheme 6).[9] Formation of an epimerizing carbon-centered radical is implicated in the lack of stereochemical control historically observed in the autoxidation of boronates, i.e. the stereochemical approach of molecular oxygen with the said radical is indiscriminate. Importantly, the autoxidation mechanism is proposed to be initiated via the homolytic fragmentation of the carbon-boron bond. Therefore, substitution of deuterium at the *alpha* boronate carbon should have no effect on the overall deboronation reaction. Indeed, human liver metabolism of **5C** resulted in the same metabolite profile, with no apparent alteration of the ratios of **M1** or **M2**. Furthermore, no loss of deuterium label was observed during the metabolism of **5C**, thus providing additional evidence for a homolytic-autoxidation mechanism mediating the deboronation of **5** (Daniels JS *et al.* unpublished results).[16,17]

Conclusion

In summary, a practical method for the preparation of the D_1 chiral amine **1C** and D_2 chiral amine **1D** was developed. Grignard reaction of D_2 *iso*butyl bromide **7** with trimethyl borate followed by hydrolysis provided a convenient method of preparing D_2 isobutyl boronic acid **8D** in a satisfactory yield. The Matteson reaction of **9D** (or **9C**) with CH_2Cl_2 (or CD_2Cl_2) and $ZnCl_2$ provided a practical method of preparing D_2 chiral chloride **10D** (or D_1 chiral chloride **10C**) in an excellent yield. Chemical purities of labeled compounds were assessed by HPLC, LC/MS/MS, and NMR.

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REFERENCES

1. Adams J. *Proteasome Inhibitors in Cancer Therapy*. Humana: Totowa, 2004; 17–38.
2. Boccadoro M, Morgan G, Cavenagh J. *Cancer Cell Int* 2005; **5**: 1–9.
3. Pekol T, Daniels JS, Labutti J, Parsons I, Nix D, Baronas E, Hsieh F, Gan LS, Miwa G. *Drug Metab Dispos* 2005; **33**: 771–777.
4. Hideshima T, Richardson P, Chauhan D, Palombella VJ, Elliott PJ, Adams J, Anderson KC. *Cancer Res* 2001; **61**: 3071–3076.
5. Kettner CA, Shenvi AB. *J Biol Chem* 1984; **259**: 15106–15114.
6. Li Y, Prakash SR. *J Label Compd Radiopharm* 2005; **48**: 323–330.
7. Li Y, Plesescu M, Prakash SR. *J Label Compd Radiopharm* 2006; **49**: 789–799.
8. McCormack T, Baumeister W, Grenier L, Moomaw C, Plamondon L, Pramanik B, Slaughter C, Soucy F, Stein R, Zuhl F, Dick L. *J Biol Chem* 1997; **272**: 26103–26109.
9. Labutti J, Parsons I, Huang R, Miwa G, Gan LS, Daniels JS. *Chem Res Toxicol* 2006; **19**: 539–546.
10. Furniss BS, Hannaford AJ, Smith PWG, Tatchell AR. *Vogel's Textbook Practical Organic Chemistry* (5th edn). Pearson: Harlow, 1989; 530–531.
11. Blatt AH. *Org Synth Coll* 1943; **II**: 358–359.
12. Caldwell CG, Rupprecht KM, Bondy SS, Davis AA. *J Org Chem* 1990; **55**: 2355–2361.
13. Matteson DS. *Acc Chem Res* 1988; **21**: 294–300.
14. Matteson DS, Hurst GD. *Heteroatom Chem* 1990; **1**: 65–74.
15. Corey EJ, Barnes-Seeman D, Lee TW. *Tetrahedron: Asymm* 1997; **8**: 3711–3713.
16. Cadot C, Dalko PI, Cossy J, Ollivier C, Chuard R, Renaud P. *J Org Chem* 2002; **67**: 7193–7202.
17. Mirviss SB. *J Org Chem* 1967; **32**: 1713–1717.

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