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The Efficient Synthesis and Simple Resolution of a Prolineboronate Ester Suitable for Enzyme-Inhibition Studies

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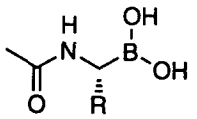
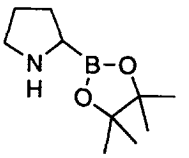
Abstract: A method for the preparation and resolution of the pinanediol ester of prolineboronic acid is described. The method takes advantage of the ease of both the lithiation and the reduction of boc-pyrrole to generate the desired compound rapidly and in high yield.

Interest in the boronic acid analogues of amino acids (**1**,**2**) has grown since the discovery that these compounds are effective inhibitors of many serine proteases.[1] It is believed that the empty p-orbital centered at boron interacts with an active-site hydroxyl group to form a tetrahedral adduct that mimics the transition state of enzymatic hydrolysis.[2] This hypothesis is supported by a wealth of physical data obtained through kinetic,[1a,1b,3] X-ray crystallographic,[4] ^{15}N-NMR[5] and ^{11}B-NMR[6] studies.



1

a R = Me
b R = iPr
c R = iBut
d R = Bn

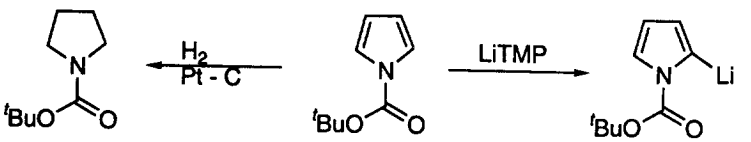


2

Previous routes to protected forms of aminomethylboronic acids rely on the procedure published in 1981 by Matteson *et al.*[1a] which follows the sequence of hydroboration, (asymmetric) homologation with chloromethyllithium, and aminolysis. In this way the boronic acid analogues of *N*-acetylalanine (**1a**), *N*-acetylvaline (**1b**), *N*-acetylleucine (**1c**), and *N*-acetylphenylalanine (**1d**) have been synthesized.[7a,b] The literature on these compounds reports enantiomeric ratios of only 9:1[7b] but recent improvements in the stereoselectivity of the asymmetric homologation step could boost this ratio to 200:1.[7c]

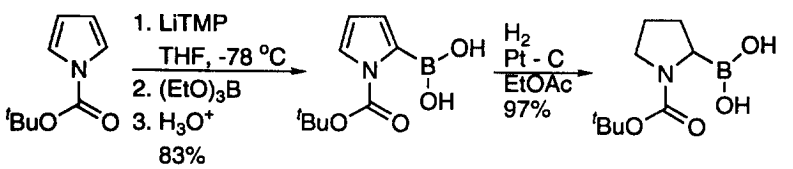
Prolineboronic acid is of special interest because peptides which incorporate this material have been shown to be potent inhibitors of certain post-proline cleaving enzymes implicated in both bacterial infection[8] and regulation of the immune response.[9] Efforts to explore further the biochemistry of these systems have been hampered by the lack of an efficient route to prolineboronic acid. The application of the Matteson procedure to the synthesis of **2** has been demonstrated[8,10] but the modifications required for the construction of the pyrrolidine ring render it unappealing. Furthermore, conditions for the preparation of a single enantiomer of **2**, although plausible by the above route, have not been reported.

A need for the optically active form of prolineboronic acid led us to explore alternate routes to this compound. Of prime interest were accounts in the literature that demonstrated the high efficiency of both the lithiation[11] and catalytic hydrogenation[12] of *N*-acylpyrroles. The combination of these two methods, coupled with reports on the behavior of boron electrophiles,[13] was thought to auger well for a short synthesis of the desired material.



(1)

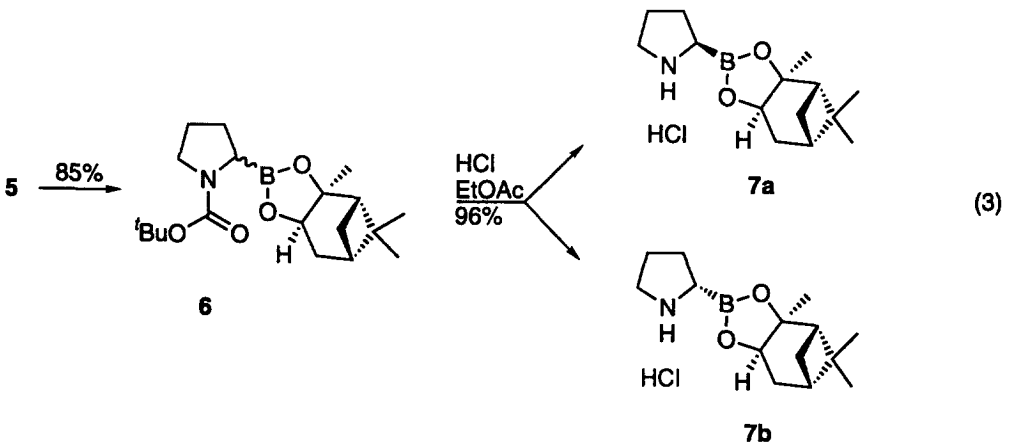
The synthesis was accomplished as shown below. Boc-pyrrole (**3**)[14] was treated at -78 °C in THF with lithium tetramethylpiperidide.[11] Subsequent addition of triethylborate followed by acid-catalyzed hydrolysis produced boc-pyrrole-2-boronic acid (**4**) in 83% yield.[15] Catalytic hydrogenation of this material in ethyl acetate over 5% Pt-on-carbon[12] efficiently reduced the ring to generate boc-prolineboronic acid (**5**, 97%).



3 **4** **5**

(2)

Boc-prolineboronic acid (**5**) was easily esterified with diols such as pinacol or optically active pinanediol by stirring the two components together in ether. When (1*S*,2*S*,3*R*,5*S*)-(+)-pinanediol was used, not only were the derivatives more stable to chromatography, but the two diastereomers were separable by HPLC thus providing a means to resolve the chiral center α to boron.[16]



(3)

Compound **6** can be deprotected using HCl in EtOAc (96%) to give a mixture of the amine hydrochlorides **7a** and **7b**. A slight modification of this technique (HCl in ether) produces a precipitate which is enriched in **7a** (62% yield; 6:4 **7a**:**7b**). Two recrystallizations produce **7a** in 80% recovery and greater than 99% diastereomeric purity.[17] Diastereomerically pure **7b** has been obtained by the deprotection of **6b** (91% yield).

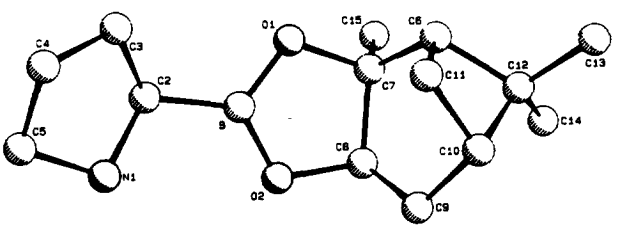


Figure 1. X-ray structure of **7a**.

An X-ray crystal structure[18] obtained on compound **7a** (Figure 1) provided the opportunity to assign the absolute stereochemistry of the pyrrolidine ring as *R*[19] by referencing it to the known stereochemistry of the (+)-pinanediol ring system. From this information it was then possible to make the stereochemical assignments of compounds **6a**, **6b**, and **7b**.

The amine hydrochlorides **7a** and **7b** are easily coupled to activated carboxylic acids such as those typically used in peptide synthesis.[8] Peptides containing a boronic acid are normally stored in the ester form and submitted for biological testing as such.

Analogues of amino acids that act as transition-state mimics are powerful tools for inhibiting enzymatic hydrolysis. The synthesis that has been described provides a method for the generation and resolution of multigram-quantities of an important analogue of proline. Biological data on this material and its derivatives will be reported in due course.

ACKNOWLEDGEMENT.

X-ray crystallography was performed by Gayle K. Schulte at Yale University. We thank Randall Barton and Charles Kennedy for helpful discussions. Additionally, we are grateful for the excellent work performed by members of the Analytical Sciences Department, in particular, Hal Butler and Arvind Shah for their work in developing the HPLC methods.

EXPERIMENTAL

General Methods: Melting points were obtained on a Buchi 510 melting point apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-NMR[20] spectra were recorded on a Bruker WM 250 MHz and a Bruker AF 270 spectrometer respectively. Chemical shifts are reported in ppm from tetramethylsilane. CIMS were measured with a Finnegan 4000 instrument using methane gas . HRMS were collected using a Kratos MS 25 operating at 70 eV. Microanalysis were performed by Midwest Microlab, Indianapolis, IN.

1-(1,1-Dimethylethoxycarbonyl)-pyrrole-2-boronic acid (4)[15]

To a solution of tetramethylpiperidine (8.8 mL, 52 mmol) in THF (275 mL) at -78 °C under an argon atmosphere was added a 2*M* solution of butyllithium in hexanes (26 mL, 52 mmol). After 15 min, 1-(1,1-dimethylethoxycarbonyl)-pyrrole (**3**)[14] in THF (10 mL) was added and the solution was stirred for 4 h at -78 °C. Triethylborate (30 mL, 176 mmol) was then added and the mixture was allowed to warm to room temperature over 3 h. After an additional 12 h the reaction mixture was diluted with ether (500 mL) and washed with 1*M* aqueous $KHSO_4$ (3 X 100-mL) followed by 1*M* aqueous $NaHCO_3$ (1 X 100-mL). Drying over $MgSO_4$ and rotary evaporation produced a brown solid that was purified by flash chromatography over silica gel (1:9 EtOAc:Hexane) to yield 8.7 g (83%) of a white crystalline solid (mp 101.0 - 101.5 °C).

^{1}H NMR ($CDCl_3$) δ 1.65 (s, 9 H), 6.26 (t, J = 3.3 Hz, 1 H), 7.10 (dd, J

= 1.6, 3.2 Hz, 1H), 7.15 (s, 2 H), 7.44 (dd, J = 1.6, 3.2 Hz, 1H); ^{13}C NMR[20] ($CDCl_3$) δ 27.9, 85.5, 112.0, 127.0, 128.7, 152.0; CIMS m/z (% rel int) 212 (MH+, 11), 156 (100), 138 (68); Anal. Calcd for $C_9H_{14}BNO_4$: C, 51.23, H, 6.69, N, 6.64. Found: C, 51.22, H, 6.51, N, 6.67.

1-(1,1-Dimethylethoxycarbonyl)-pyrrolidine-2-boronic acid (5)

A solution of 6.15 g (24 mmol) of compound **4** in EtOAc (100 mL) was hydrogenated over 5% Pt / C (ca. 500 mg) at 50 psi for 4 h. The resulting suspension was filtered through a pad of Celite and concentrated. This material was chromatographed on silica gel using sequential elutions of 9:1 hexanes:EtOAc then acetone. The acetone fractions were concentrated to produce 6.05 g (97%) of the desired compound as a clear glass that crystallized upon removal of trace solvents (mp 100-101°C).

^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9 H), 1.6 - 2.15 (m, 5 H), 3.1 - 3.6 (m, 2 H); ^{13}C NMR[20] ($CDCl_3$) δ 25.1, 25.7, 28.4, 45.6, 46.2, 78.6, 154.5; CIMS m/z (% rel int) 116 (100), 70 (46); Anal. Calcd for $C_9H_{18}BNO_4$: C, 50.27, H, 8.44, N, 6.51. Found: C, 50.52, H, 8.22, N, 6.58.

(1*S*,2*S*,3*R*,5*S*)-Pinanediol 1-(1,1-dimethylethoxycarbonyl)-pyrrolidine-2*R-boronate (6a) and (1*S*,2*S*,3*R*,5*S*)-Pinanediol 1-(1,1-dimethylethoxycarbonyl)-pyrrolidine-2*S**-boronate (6b).**

A solution of **5** (1.52 g, 7.1 mmol) and (1*S*,2*S*,3*R*,5*S*)-(+)-pinanediol (1.36 g, 8.0 mmol) was stirred at room temperature in ether(25 mL) for 2 h. Concentration and flash chromatography over silica gel (85:15 hexanes:EtOAc) produced 2.1 g (85%) of a 1:1 mixture of the two diastereomers of compound **6**. Normally the mixture was used as such, however it is possible to separate the two compounds by HPLC over a 300 X 3.9 mm column of microporasil A eluting with methyl *tert*-butyl ether:hexanes (1:9) using u.v. detection at 220 nm. Compound **6b** eluted first under these conditions.

6a: ^{1}H NMR (C_6D_6) δ 0.52 (s, 3 H), 1.08 (s, 3 H), 1.52 (s, 9 H), 1.61 (s, 3 H), 1.2 - 2.2 (m, 8 H), 3.1 - 3.6 (m, 3 H), 4.01 (m, 0.3 H), 4.25 (m, 0.7 H); ^{13}C NMR[20] (C_6D_6) δ 23.9, 26.6, 27.1, 27.3, 28.4, 28.7, 28.9, 35.8, 38.2, 39.6, 46.2, 51.8, 78.1, 78.5, 85.7, 154.5.

6b: ^{1}H NMR (C_6D_6) δ 0.55 (s, 3 H), 1.09 (s, 3 H), 1.52 (s, 9 H), 1.60 (s, 3 H), 1.2 - 2.2 (m, 8 H), 3.1 - 3.5 (m, 3 H), 4.11 (m, 0.3 H), 4.33 (m, 0.7 H); ^{13}C NMR[20] (C_6D_6) δ 23.9, 26.6, 27.1, 27.3, 28.4, 28.6, 28.8, 36.0, 38.2, 39.9, 46.1, 51.9, 78.3, 78.5, 85.7, 154.9.

(1*S*,2*S*,3*R*,5*S*)-Pinanediol pyrrolidine-2*R-boronate hydrochloride (7a).**

A solution of compound **6** (224g, 0.64 mol) in 900 mL of ether was treated with dry HCl at 0 °C for 35 min. The mixture was then stirred at 10 - 18 °C overnight. After cooling, the precipitate was collected and washed sequentially with 400 mL of ether and 200 mL of a 9:1 mixture of petroleum ether: ether. This produced 113 g of a white powder that analysis showed to be a 60:40 mixture of **7a:7b** (62% yield overall; 74% yield of **7a**).

A small amount of this material (1.18 g) was dissolved in 65 mL of dichloromethane with slight warming and the solution was filtered to remove a small amount of insoluble brown material. The filtrate was then diluted with 65 mL of ethyl acetate and crystallization began within a minute. The suspension was stirred for 1 to 2 h at room temperature and the first crop was collected (540 mg, 46%, 97:3 ratio of **7a:7b**). This material was then recrystallized from 10 mL of *iso*-propyl alcohol to afford 430 mg (80% recovery) of a material that was >99% **7a**.

The diastereomeric purity of **7a** and **7b** was assessed by treating an accurately weighed sample with a 2-fold molar excess of a 0.2 *M* solution of phenyl isothiocyanate in 9:1 dichloromethane-triethylamine. After 15 m at room temperature a 1 uL sample is diluted in 1.00 mL of HPLC-grade acetonitrile and 10 uL of this solution is analyzed by HPLC (column: YMC AQ-303 S-5 120A, 4.6 x 250 mm; mobile phase: 65% MeCN - 35% 25 m*M* ammonium phosphate,pH = 7.5; flow rate: 1 mL/min; detection: 254 nM). The phenyl thiourea derivatives of **7a** and **7b** elute at 6.4 m and 7.8 m, respectively. Unchanged phenyl isothiocyanate elutes at 12.2 m and acts as an internal standard.

7a: (mp 248 °C (dec)). ^{1}H NMR ($CDCl_3$) δ 0.82 (s, 3 H), 1.14 (d, 1 H), 1.30 (s, 3 H), 1.48 (s, 3 H), 1.85 - 2.15 (m, 6 H), 2.15 - 2.50 (m, 3 H), 3.20 (bs, 1 H), 3.45 (bs, 2 H), 4.40 (d, 1 H), 8.80 (bs, 1 H), 10.56 (bs, 1 H); ^{13}C NMR[20] ($CDCl_3$) δ 23.9, 24.5, 26.5, 27.0, 27.2, 28.5, 34.9, 38.1, 39.4, 45.8, 51.2, 79.0, 87.8; CIMS *m/z* (% rel int) 250 (MH+, 100); HRMS (EI) for $C_{14}H_{24}BNO_2$ calcd 249.1900, found 249.1903; Anal. Calcd for $C_{14}H_{25}ClBNO_2$: C, 58.87, H, 8.82, N, 4.90, Cl, 12.41. Found: C, 58.64, H, 8.79, N, 4.88, Cl, 12.66.

(1*S*,2*S*,3*R*,5*S*)-Pinanediol pyrrolidine-2*S-boronate hydrochloride (7b).**

A solution of compound **6b** (28.5 mg, 0.08 mmol) was stirred in a solution of dry HCL in EtOAc (app. 3*M*). After 2 h the solution was concentrated twice from EtOAc to produce 21.2 mg (91%) of the desired hydrochloride as a white powder. Attempts to obtain pure **7b** from the mother liquors obtained from the crystallization of **7a** have, as of yet, only been able to produce material that is 90% **7b**.

7b: (mp 204 °C (dec)). ^{1}H-NMR ($CDCl_3$): δ 0.81 (s, 3 H),1.14 (d, 1 H), 1.29 (s, 3 H), 1.45 (s, 3 H), 1.85 - 2.15 (m, 6 H), 2.15 - 2.50 (m, 3 H), 3.20 (bs, 1 H), 3.45 (bs, 2 H), 4.40 (d, 1 H), 8.80 (bs, 1 H), 10.56 (bs, 1 H); ^{13}C NMR[20] ($CDCl_3$) δ 23.9, 24.5, 26.5, 27.0, 27.2, 28.4, 34.9, 38.2, 39.4, 45.8, 51.2, 79.0, 87.6; CIMS *m/z* (% rel int) 250 (MH+, 100); HRMS (EI) for $C_{14}H_{24}BNO_2$ calcd 249.1900, found 249.1899.

REFERENCES.

1. (a) Matteson, D. S.; Sadhu, H. M.; Lienhard, G. E. *J. Am. Chem. Soc.* **1981**, *103*, 5241. (b) Kettner, C. A.; Shenvi, A. B.; *J. Biol. Chem.* **1984**, *259*, 15106. (c) Kinder, K. H.; Katzenellenbogen, J. A. *J. Med. Chem.* **1985**, *28*, 1917.

2. (a) Koehler, K. A.; Lienhard, G. E. *Biochemistry* **1971**, *10*, 2477. (b) Rawn, J. D.; Lienhard, G. E.; *Ibid.* **1974**, *13*, 3124.

3. Kettner, C. A.; Bone, R.; Agard, D. A.; Bachovchin, W. W. *Biochemistry* **1988**, *27*, 7682.

4. (a) Matthews, D. A.; Alden, R. A.; Birktoft, J. J.; Freer, S. T.; Kraut, J. *J. Biol. Chem.* **1975**, *250*, 7120. (b) Bone, R.; Shenvi, A. B.; Kettner, C. A.; Agard, D. A. *Biochemistry* **1987**, *26*, 7609. (c) Bone, R.; Kettner, C. A.; Agard, D. A. *Ibid.* **1989**, *28*, 7600. (d) Takahashi, L. H.; Radhakrishnan, R.; Rosenfield, R. E.; Meyer, E. F. *Ibid.* **1989**, *28*, 7610.

5. (a) Bachovchin, W. W.; Wong, W. Y. L.; Farr-Jones, S.; Shenvi, A. B.; Kettner, C. A. *Ibid.* **1988**, *27*, 7689. (b) Farr-Jones, S.; Smith, S. O.; Kettner, C. A.; Griffin, R. G.; Bachovchin, W. W. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 6922.

6. (a) Adebodun, F.; Jordan, F. *J. Am. Chem. Soc.* **1988**, *110*, 309. (b) Baldwin, J. E.; Claridge, T. D. W.; Derome, A. E.; Schofield, C. J.; Smith, B. D. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 9.

7. (a) Matteson, D. S. *Chem. Rev.* **1989**, *89*, 1535. (b) Matteson, D. S.; Jesthi, P. K.; Sadhu, K. M. *Organometallics* **1984**, *3*, 1284. (c) Matteson, D. S.; Sadhu, K. M.; Peterson, M. L. *J. Am. Chem. Soc.* **1986**, *108*, 810.

8. Bachovchin, W. W.; Plaut, A. G.; Flentke, G. R.; Lynch, M.; Kettner, C. A. *J. Biol. Chem.* **1990**, *265*, 3738.

9. Flentke, G. R.; Munoz, E.; Huber, B. T.; Plaut, A. G.; Kettner, C. A.; Bachovchin, W. W. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 1556.

10. Bachovchin, W. W.; Plaut, A. G.; Kettner, C. A. **World Patent: WO 89/03223**, April 1989.

11. Hasan, I.; Marinelli, E. R.; Lin, L.-C. C.; Fowler, F. W.; Levy, A. B. *J. Org. Chem.* **1981**, *46*, 157.

12. Kaiser, H.-P.; Muchowski, J. M. *Ibid.* **1984**, 4203.

13. (a) Brown, H. C.; Somayaji, V.; *Organometallics* **1983**, *2*, 1311. (b) Brown, H. C.; Cole, T. E. *Ibid.* **1983**, *2*, 1316.

14. Grehn, L.; Ragnarsson, U. *Angew. Chem., Int. Ed. Eng.* **1984**, *23*, 296.

15. After this work was completed a similar approach to compound **4** was reported. See: Martina, S.; Enkelman, V.; Wegner, G.; Schlüter, A.-D. *Synthesis,* **1991**, 613.

16. The diastereomeric purity of **6a** and **6b** could be assayed by integrating the ^{1}H-NMR spectra of these compounds obtained in C_6D_6. In this solvent the resonances of the methine protons α to the oxygen in the pinane system are clearly discernible AB-quartets appearing at δ 4.33 ppm (**6b**) and δ 4.25 ppm (**6a**). Due to conformational restriction in these molecules, each stereoisomer contains 30% of a minor rotamer. The resonances for the minor rotamers are observed at δ 4.01 ppm and δ 4.11 ppm for **6b** and **6a** respectively. This explanation is supported by a variable-temperature NMR experiment on **6b**.

17. Diastereomeric purity was assessed by means of an HPLC method on the phenylthiourea derivatives of the two compounds. See experimental.

18. Data have been submitted to the Cambridge Crystallographic Centre.

19. Due to the relative priorities of carbon and boron in the Cahn-Ingold-Prelog system of nomenclature, the stereochemistry of **7a** is *R* at the carbon α to boron whereas the analogous position in (L)-proline is *S*.

20. It is usually difficult to observe the carbon atom α to boron in a ^{13}C-NMR spectrum due to rapid quadrapole-induced relaxation.