# Synthesis of (1*R*)-(1-Acetamido-2-phenylethyl)boronic Acid-1-$^{13}$C.

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## Summary

(1*R*)-(1-Acetamido-2-phenylethyl)boronic acid-1-$^{13}$C, which is the boron analogue of *N*-acetylphenylalanine, has been synthesized via a modification of the route previously established for the unlabelled compound. The $^{13}$C label is derived from dichloromethane-$^{13}$C. The previous conditions utilized excess dichloromethane, but it has now been found that a stoichiometric amount of dichloromethane suffices for the preparation of (dichloromethyl)lithium and its reaction with a boronic ester. Thus, the method has broad potential for the synthesis of compounds bearing $^{13}$C labels at chiral sites. The $^{1}$H and $^{13}$C NMR spectra of the target compound and the intermediates leading to it are discussed.

**Key words:** $^{13}$C label, boronic acid, (dichloromethyl)lithium

## Introduction

The efficient reaction of (dichloromethyl)lithium with pinanediol boronic esters to produce chiral α-chloro boronic esters [1,2] has obvious potential for the introduction of $^{13}$C labels at chiral sites. This process has been used to introduce stereospecific $^{2}$H labels into phenylalanine [3] and glycerol [4], but has not been tested previously with $^{13}$C. The cost of $^{13}$C provides strong incentive for maximizing the yield with respect to dichloromethane.

Amido boronic acids are of interest as inhibitors of chymotrypsin [5] and other serine proteases [6]. The present paper describes the efficient synthesis of (1*R*)-(1-acetamido-2-phenylethyl)boronic acid-1-$^{13}$C (**6**) from dichloromethane-$^{13}$C.

## Results

The synthesis of unlabeled (1*R*)-(1-acetamido-2-phenylethyl)boronic acid has been described previously [5,7]. Because dichloromethane is inexpensive, past practice was to use this reagent in large excess, often five-fold or more, and the effect of using a smaller amount was never tested.

A critical question was whether a chance excess of *n*-butyllithium over dichloromethane in the preparation of (dichloromethyl)lithium-$^{13}$C (**1**) would destroy this exceedingly unstable intermediate. The reason for concern was the pattern of instability of (dichloromethyl)lithium, which has been reported to be stable for hours at temperatures as high as –60 °C [8], and which can be generated and

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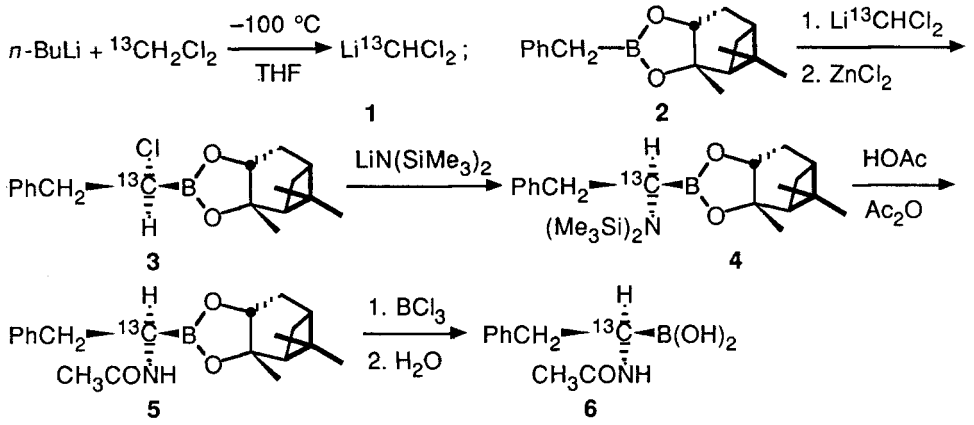
captured by addition of lithium diisopropylamide to a mixture of dichloromethane and substrate [9] such as a boronic ester [10], but which decomposes when preparation is attempted from *n*-butyllithium and dichloromethane in THF at –78 °C [10].

Fortunately, (dichloromethyl)lithium was found to be stable in the presence of butyllithium, and the unlabelled model system performed well with a stoichiometric amount of dichloromethane. Accordingly, we undertook the synthesis of (1*R*)-(1-acetamido-2-phenylethyl)boronic acid-1-$^{13}$C (**6**) from (*S*)-pinanediol [(phenyl)methyl]boronate (**2**) according to the previously established route for the unlabelled boronic acid [7]. Several of the experimental details were revised and improved.




The $^{13}$C bonded to boron and nitrogen in **6** appears as a broad peak at δ 49.5 in the $^{13}$C NMR spectrum in $D_2O$, illustrated in Figure 1. Impurities appear at δ 74.1, 75.8, 91.5, and 165.3. These were absent, though there was a different impurity at δ 83, in the $^{13}$C spectrum of the same sample in $CD_3OD$, not chosen for illustration because $^{13}CD_3OD$ obscures the major $^{13}$C peak of **6**. Freshly prepared **6** showed no impurity in $^1$H spectra, and no impurity was visible in $^{13}$C spectra of unlabelled material. Thus, the impurity peaks originate from the $^{13}$C label. Perhaps the gross impurity peak at δ 165.3 results from oxidation of ~1% of the boronic acid by a peroxide impurity in the dioxane standard, or by $O_2$ dissolved in $D_2O$, to form a carbonyl $^{13}$C labelled amide or related material.

The 500-MHz $^1$H NMR spectrum of the $CH_2{}^{13}CH$ group of **6** is shown in Figure 2. The $^{13}CH$ is seen as a pair of double doublets, $J_{CH}$ =132 Hz, $J_{HH}$ = 4.4 and 10.3 Hz, centered at δ 2.84.

## Conclusion

The efficient synthesis of (1*R*)-(1-acetamido-2-phenylethyl)boronic acid-1-$^{13}$C (**6**) illustrates a new and potentially general method of introducing a labelled carbon atom at a chiral center via the reaction of labelled (dichloromethyl)lithium with a chiral boronic ester.

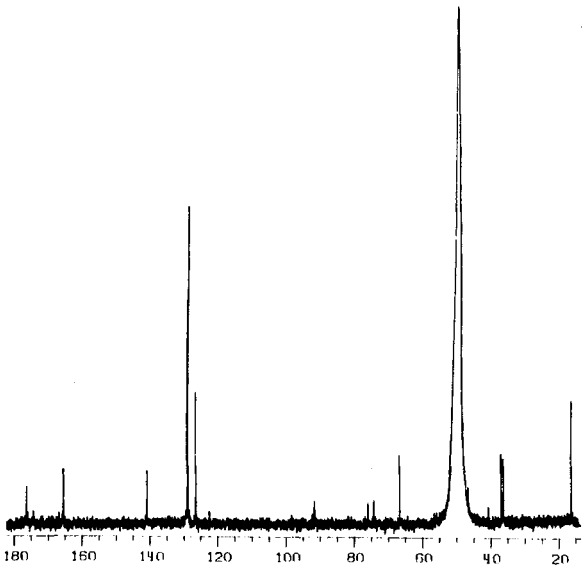



Figure 1. The 50-MHz $^{13}C$ NMR spectrum of (1*R*)-(1-acetamido-2-phenylethyl)boronic acid-1-$^{13}C$ (**6**) in $D_2O$ with 1,4-dioxane (δ 66.50) as internal standard.

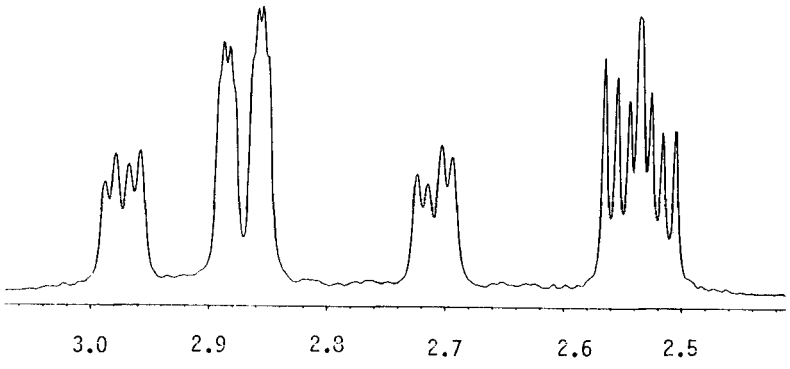



Figure 2. The 500-MHz $^1H$ NMR spectrum of the $CH_2{}^{13}CH$ protons of (1*R*)-(1-acetamido-2-phenylethyl)boronic acid-1-$^{13}C$ (**6**) in $CD_3OD$.

## Experimental

**General Data.** All reactions involving air sensitive reagents were carried out in oven-dried glassware under an argon atmosphere. Tetrahydrofuran (THF) was freshly distilled from benzophenone ketyl. Butyllithium (1.6 M in hexanes) and lithium diisopropylamide mono(tetrahydrofuran)

(1.5 M in cyclohexane) were titrated against 2-propanol to the 1,10-phenanthroline end point [11]. 1,1,1,3,3,3-Hexamethyldisilazane was freshly distilled before use. Dichloromethane-$^{13}C$ was purchased from Merck, Sharpe, and Dohme Isotopes of Canada with 99.7 atom % $^{13}C$. Powdered zinc chloride was prepared by heating reagent grade material between 70-80 °C under vacuum (0.1 torr) with vigorous stirring for 24-48 h.[1]. (1*S*,2*S*,3*R*,5*S*)-(+)-Pinanediol [(*S*)-pinanediol], 99%, was purchased from Aldrich Chemical Co. Flash chromatography refers to the technique of Still and coworkers [12] and was performed on Merck Silica Gel 60, 230-400 mesh. Melting points were determined in open-ended capillaries using a Thomas-Hoover melting point apparatus and are uncorrected. Optical rotations were measured in a one decimeter cell using a Jasco DIP-181 digital polarimeter. 200-MHz $^1H$ and 50.31 MHz $^{13}C$ NMR spectra were recorded on a Nicolet NT-200WB, 500-MHz $^1H$ spectra on a Varian VXR-500 spectrometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN.

**(*S*)-Pinanediol (Phenylmethyl)boronate (2).** This compound was prepared from (*S*)-pinanediol and dimethyl (phenylmethyl)boronate by the reported method [7,13], bp and 200 MHz NMR data in accord with those reported [7].

**(*S*)-Pinanediol (1*S*)-(1-Chloro-2-phenylethyl)boronate-1-$^{13}C$ (3).** The literature procedures [1,10,13] were followed except for the ratio of (dichloromethyl)lithium to starting boronic ester. A solution of 0.50 g (5.8 mmol) dichloromethane-$^{13}C$ and 20 mL of THF was stirred and cooled to –100 °C (EtOH/liq. $N_2$ bath). To the solution, 5.76 mmol of butyllithium (4.43 mL of 1.3 M butyllithium in hexanes) was added dropwise with care that the butyllithium was precooled by contact with the inside wall of the flask. The (dichloromethyl)lithium solution was stirred for 30 min at –100 °C, after which a solution of 1.57 g (5.81 mmol) of (*S*)-pinanediol (phenylmethyl)boronate (2) in 10 mL of THF at –78 °C was added dropwise. The solution was stirred for 10 min after which 0.55 g (4.1 mmol) of anhydrous zinc chloride was added. The solution was warmed slowly to room temperature. After 30 min the borate complex appeared as a white precipitate. After 6 h, the solvents were removed under reduced pressure and the residue was treated with 25 mL of petroleum ether (30-60 °C) and 25 mL of aqueous saturated ammonium chloride. The aqueous phase was removed and the organic phase was washed with 2 x 15 mL of aqueous saturated ammonium chloride. The organic phase was dried over sodium sulfate and filtered. The solvent was removed under reduced pressure to yield 1.95 g of crude **3** with 8% unchanged boronic ester **2**; 200 MHz $^1H$ NMR ($CDCl_3$) δ 0.81 (s, 3, $CH_3$), 0.85-2.33 (m, 12, with $CH_3$ singlets at 1.27 and 1.33, pinanyl), 3.08-4.04 (m, 3, $CH_2Ph$ and BC*H*Cl), 4.33 (dd, 1, C*H*OB), 7.19-7.28 (m, 5, $C_6H_5$).

**(*S*)-Pinanediol (1*R*)-(1-Acetamido-2-phenylethyl)boronate-1-$^{13}$C (5).** A solution of 0.992 g (6.15 mmol) of 1,1,1,3,3,3-hexamethyldisilazane in 20 mL of THF was stirred at –78 °C during dropwise addition of 6.09 mmol of butyllithium (4.70 mL of 1.3 M butyllithium in hexanes) followed by stirring for an additional 10 min. A solution of 1.81 g of (*S*)-pinanediol (1*S*)-(1-chloro-2-phenylethyl)boronate-1-$^{13}$C (**3**) in 10 mL of THF at -78 °C was added to the disilazane mixture and stirred at room temperature overnight (10-12 h) to form **4**. The solution of **4** was cooled to –78 °C, treated with 2.7 mL (24 mmol) of acetic anhydride followed by 0.42 mL (7.3 mmol) of glacial acetic acid, and stirred overnight (10-12 h) at room temperature. The solution was concentrated under reduced pressure and the residue of **5** was subjected to flash chromatography through a 12 cm by 5 cm (diameter) plug of silica gel with 4.5 L of diethyl ether (or 1.5 L of 5% ethyl acetate/hexanes) to yield 1.61 g (81%) of (5), mp 185-188 °C. A sample was recrystallized from dichloromethane, $[\alpha]^{22}_D$ –82.38° (*c* 5, $CHCl_3$), lit. [7] (unlabelled) $[\alpha]^{18}_D$ –82.43° (*c* 5, $CHCl_3$); 200 MHz $^1$H NMR ($CDCl_3$) δ 0.86 (s, 3, C*H*$_3$), 0.82-2.37 (m, 15, with C*H*$_3$ singlets at 1.27 and 1.37 and a COC*H*$_3$ singlet at 2.01, pinanyl), 2.62-3.34 (m, 3, C*H*$_2$Ph and BC*H*N), 4.18 (dd, 1, C*H*OB), 7.01 (br s, 1, N*H*, concentration dependent), 7.15- 7.34 (m, 5, $C_6H_5$). 50.3 MHz $^{13}$C NMR ($CDCl_3$) δ 19.03, 24.22, 26.53, 27.35, 29.11, 36.74 (d, *C*H$_2$Ph, $^1J_{CC}$=21.79 Hz), 37.60, 38.15, 40.01, 44.12 (B*$^{13}$C*HN, broad, width of peak at half-height 66 Hz), 52.24, 76.55, 77.00 (t, *C*$DCl_3$), 83.58, 83.60, 126.26, 128.65, 128.74, 140.52 (d, $C_1$ of $C_6H_5$,$^2J_{CC}$=1.46 Hz) 174.25. Anal. Calcd for $C_{19}{}^{13}CH_{28}BNO_3$: C, 70.23; H, 8.25; B, 3.16; N, 4.09 [14]. Found: C, 70.76; H, 8.26; B, 3.01; N, 4.09.

**(1*R*)-(1-Acetamido-2-phenylethyl)boronic Acid-1-$^{13}$C (6).** A solution of 1.51 g (4.41 mmol) of (*S*)-pinanediol (1*R*)-(1-acetamido-2-phenylethyl)boronate-1-$^{13}$C (**5**) in 15 mL of dichloromethane was added to excess liquid boron trichloride (~10 mL) at –78 °C and stirred at room temperature overnight (10-12 h). The black solution was concentrated under a stream of argon [13] and the residue was dissolved into 20 mL of water and 20 mL of diethyl ether. The organic phase was removed and the aqueous phase was washed with 3 x 10 mL of diethyl ether. The aqueous solution of boronic acid (**6**) was lyophilized. Any residual boric acid was removed by addition of 5-10 mL of methanol and distillation of methyl borate/methanol azeotrope. The boronic acid (**6**) methanol solution was lyophilized to give 0.89 g (97%) of (**6**); mp 206-209 °C; 500 MHz $^1$H NMR ($CD_3OD$) [14] δ 2.10 (s, 3, COC*H*$_3$), 2.535 (d of d of d's, $^2J_{CH}$ = 5.1, $^3J_{HH}$ = 10.1, $^2J_{HH}$ = 14.5 Hz, 1, C*H*HPh), 2.840 (d of d of d's, $^3J_{HH}$ = 4.4, $^3J_{HH}$ = 10.3, $^1J_{CH}$ = 132 Hz, 1, $^{13}$C*H*B), 2.871 (d of d of d's, $^2J_{CH} \cong 2$, $^3J_{HH} \cong 5$, $^2J_{HH}$ = 14.5 Hz, 1, CH*H*Ph), 3.30 (internal ref., m, 3, $CD_2HOD$), 4.90 (s, $CD_3OH$), 7.23 (m, 5, $C_6H_5$); 200 MHz $^1$H NMR ($D_2O$) δ 1.95 (s, 3, COC*H*$_3$), 2.38-3.12 (m, 3,

$CH_2$Ph and BC*H*N), 4.70 (*H*OD, internal ref.), 7.13-7.27 (m, 5, $C_6H_5$); $^1$H decoupled 50.3 MHz $^{13}$C NMR ($CD_3OD$) δ 16.86 ($COCH_3$), 37.95 (d, $CH_2Ph$,$^1J_{CC}$ 33.28), 49.00 (m, $CD_3OD$), 50.77 (br, BCHN), 83 (weak, imp.), 127.09, 129.49, 129.81, 141.97, 176.18; 50.3 MHz $^{13}$C NMR ($D_2O$, with 1,4-dioxane = 66.50 as internal standard) δ 16.65 ($COCH_3$), 36.82 (d, $CH_2PH$, $^1J_{CC}$=33.04 Hz), 50.07 (br, BCHN, width of peak at half-height 87 Hz), 74.1 (imp.), 75.8 (imp.), 91.5 (imp.) 126.74, 129.11, 129.27, 141.23 (d, $C_1$ of $C_6H_5$, $^2J_{CC}$=2.23 Hz), 165.3 (imp.), 176.76 (sometimes appears as a doublet at δ 176.77, $COCH_3$, $^2J_{CC}$=1.86 Hz). To help interpretation of the $^1$H NMR spectrum (proton assignments, H-C, and H-H coupling constants) a homodecoupling $^1$H NMR experiment was performed. The multiplets between δ 2.49 and 3.12 were irradiated at different frequencies within that range. Irradiation at δ 3.06 caused the multiplet at δ 2.65-2.85 to collapse to a less complicated doublet centered at δ 2.76 and the multiplet at δ 2.30-2.55 to become less complicated. Irradiation at δ 2.78 caused the broad doublet of doublets at δ 3.04 to collapse to a broad doublet and the multiplet at δ 2.30-2.55 to become less complicated. Irradiation at δ 2.44 caused the broad doublet of doublets at δ 3.04 to collapse to a broad doublet at δ 3.05 and the complicated multiplet at δ 2.65-2.85 to collapse to a broad singlet at δ 2.77. Anal. Calcd for $C_9{}^{13}CH_{14}BNO_3$: C, 57.81; H, 6.78; B, 5.20; N, 6.73 [15]. Found: C, 58.34; H, 6.71, B, 5.27; N, 6.63.

**Reaction Conditions and Observations of (Dichloromethyl)lithium.** A solution of 0.85 g (10.0 mmol) of dichloromethane in 30 mL of dry THF was stirred at –100 °C, 10.1 mmol of butyllithium (7.2 mL of 1.4 N butyllithium in hexanes) was added dropwise down the side of the flask. The temperature of the cold bath was monitored during the course of the reaction. After 30 min of stirring at –100 °C, the solution had a white precipitate, and another 1.0 mmol of butyllithium (0.7 mL of 1.4 M butyllithium in hexane) was added. No decomposition of the (dichloromethyl)lithium was seen after the addition of excess butyllithium as evidenced by the lack of the characteristic dark color. After stirring another 30 min at –100 °C, no color change of the solution was noticeable, and the white precipitate was still present. The solution was allowed to warm slowly. After a total stirring time of 2.5 h the bath temperature was –70 °C and the solution was clear. At a temperature of –25 °C (3.5 h), the solution was slightly darkened by decomposition of (dichloromethyl)lithium. At –10 °C (5 h), the solution was black in color.

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## References

(1) (a) Matteson D. S., Sadhu K. M., and Peterson M. L. – J. Amer. Chem. Soc. 108: 810 (1986).

(2) Matteson D. S. – Acc. Chem. Res. 21: 294 (1988).

(3) Matteson D. S., Beedle E. C., Christenson E., Dewey M. A., and Peterson M. L. – J. Labelled Compd. Radiopharm. 25: 675 (1988).

(4) Matteson D. S., Kandil A. A., and Soundararajan R. – J. Amer. Chem. Soc. 112: 3964 (1990).

(5) Matteson D. S., Sadhu K. M., and Lienhard G. E. – J. Amer. Chem. Soc. 103: 5241 (1981).

(6) (a) Kettner C. A. and Shenvi A. B. – J. Biol. Chem. 259 15106 (1984). (b) Shenvi A. B. – Biochemistry (Washington, D. C.) 25: 1286 (1986). (c) Kinder D. H. and Katzenellenbogen J. A. – J. Med. Chem. 28: 1917 (1985). (d) Philipp M., Maripuri S., Matteson D. S., Jesthi P. K., and Sadhu K. M. – Biochemistry (Washington, D. C.) 22: A13 (1983).

(7) Matteson D. S. and Sadhu K. M. – Organometallics 3: 614 (1984).

(8) (a) Köbrich G., Flory K., and Drischel W. – Angew. Chem. 76: 536 (1964); Angew. Chem. Int. Ed. Engl. 3: 513 (1964). (b) Köbrich G., Merkle H. R., and Trapp H. – Tetrahedron Lett.: 969 (1965).

(9) (a) Corey E. J., Jautelat M., and Oppolzer W. – Tetrahedron Lett.: 2325 (1967). (b) Taguchi H., Yamamoto H, and Nozaki H. – J. Amer. Chem. Soc. 96: 3010 (1974).

(10) Matteson D. S. and Majumdar D. – Organometallics 2: 1529 (1983).

(11) Watson S. C. and Eastham J. F. –J. Organomet. Chem. 9: 165 (1967).

(12) Still W. C., Kahn M., and Mitra A. – J. Org. Chem. 43: 2923 (1978).

(13) Matteson D. S., Ray R., Rocks R. R., and Tsai D. J. S. – Organometallics 2: 1536 (1983).

(14) This spectrum was taken on a sample which had been stored for 1 year at 25 °C and showed small impurity peaks at δ 1.25 (t), 1.89 (s), and 3.75 (m) which were not present in the 200-MHz spectrum of the fresh material.

(15) Calcd on the basis that the carbon is measured by thermal conductivity of the $CO_2$ produced, that the thermal conductivity of $^{13}CO_2$ is 98.88% $[(44/45)^{1/2}]$ that of $^{12}CO_2$, and that the analyst reports the result with no allowance for the $^{13}C$ content. Calcd for natural abundance $C_{20}H_{28}BNO_3$: C, 70.39; H, 8.27; B, 3.17; N, 4.10; for $C_{10}H_{14}BNO_3$: C, 58.01; H, 6.82; B, 5.22; N, 6.77.