Synthesis of 2-Amino-3-boronopropionic Acid: A Boron-Containing Analogue of Aspartic Acid†

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Received October 20, 1986

The boron-containing analogue of aspartic acid, 2-amino-3-boronopropionic acid (1), in which the side chain carboxyl group has been replaced by a boronic acid group, has been prepared. Two principal reactions were used for preparation of 1: a malonic ester alkylation with (chloromethyl)boronic esters, and, after saponification of one ethyl ester of the adduct, a modified Curtius rearrangement to introduce the amino group. Unlike α-amino boronic esters, the primary β-amino boronic acids and esters reported here are stable and do not undergo elimination reactions.

Introduction

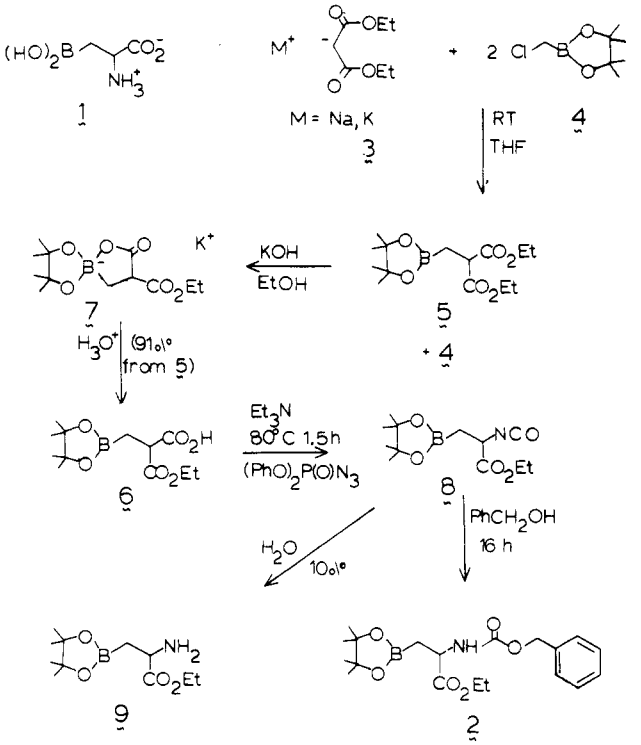
Boronic acid analogs of α-amino acids are effective inhibitors of serine proteases.[1-3] The analogues function by forming stable tetrahedral complexes with serine in the active site of the enzymes.[4] We have synthesized aspartic acid analogue 1 with boron substituted for the (γ)-carboxyl carbon as a precursor analogue inhibitor of dihydroorotase.[5,6] Biochemical and biological studies of 1 and related compounds will be reported elsewhere.[6]

Results and Discussion

The synthesis of 1 (Scheme I) centered on introduction of an amino group β to boron. We were initially concerned that the primary β-amino boronic acids and esters would not be stable, since it is known that nucleofugal groups β to boron will undergo facile elimination reactions.[7] Further, primary α-amino boronic acids and esters will proteodeboronate to form the primary amines and boric acid.[1,3] In a publication that appeared during the preparation of this manuscript, Matteson et al.[8] expressed a similar concern about the stability of the azido group β to boron (β-azido boronic esters appear to be stable molecules). The primary β-amino boronic esters and acids in this report were stable and did not require special handling precautions. Although a few examples of β-acylamino boronic acids have appeared in the literature,[9,10] this literature does not provide adequate methods for construction of this system. Thus, we devised a synthesis that utilized a Curtius arrangement as the key step for introduction of the amino function.[11] The sequence to the protected aspartate analogue **2** involved a malonic ester anion alkylation of chloroboronic ester followed by the Curtius rearrangement of the half ester.[12]

It has been shown that iodide of di-*n*-butyl (iodomethyl)boronate can be displaced by nucleophiles to produce alkylated product.[9] In this manner, we were able to cleanly alkylate either sodium or potassium diethylmalonate (**3**) in the presence of 2 equiv of pinacol (chloromethyl)boronate (**4**) to produce **5** in high yield (>90%). When 1 equiv of **4** was used, about 50% **5** was obtained with unreacted boronic ester **4** and diethylmalonate. We were not able to resolve the question of the requirement for the second equivalent of **4** to totally consume the malonic ester anion. Changes in solvent polarity, counterion on the malonate (Na, K, Li, Mg), stearic factors, or Lewis acid catalysts[13] had no effect on the reaction's

Scheme I



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(10) Butler, D. N.; Soloway, A. H. *J. Am. Chem. Soc.* **1966**, *88*, 484.
(11) Initial attempts to alkylate protected glycine esters (Djuric, S.; Venit, J.; Magnus, P. *Tetrahedron Lett.* **1988**, *22*, 1787) with (chloromethyl)boronic esters resulted in the isolation of glycine/boronic ester complexes. This lack of reactivity is consistent with the formation of an ate complex with the glycine enol oxygen (see ref 13).
(12) Sofia, M. J.; Katzenellenbogen, J. A. *J. Org. Chem.* **1985**, *50*, 2331.

† Work supported in part by Fraternal Order of Eagles National Cancer Fund, Eagles Grant 59.
‡ Supported in part by National Cancer Institute Training Grant CA09441, DHHS.
§ Recipient of NCI Research Career Development Award CA00755, DHHS.

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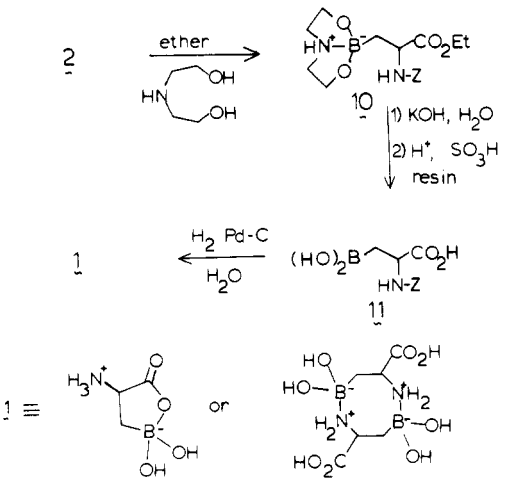
Scheme II



outcome. The extra equivalent of **4** was recovered in about 70% yield and recycled.

The amino group was introduced via a modified Curtius rearrangement on the half ester **6**. Saponification of the diester to the half ester salt **7** was accomplished by stirring overnight with 1 equiv KOH in ethanol at room temperature. The potassium salt **7** was more stable than the acid **6** and could be purified by recrystallization from ethanol–ethyl acetate mixtures. The salt **7** appeared to be cyclized as indicated by the upfield shift in the NMR spectrum (shielding by negative charge on boron) of the methylene protons α to boron to 0.5 ppm (from ~1.1) where they appear as two doublets. The cyclization (coordination to boron) probably accounts for the stability toward oxidation of the boronic ester group of **7** mentioned above. An aqueous solution of **7** was carefully acidified with 3 M HCl to produce **6** in 91% yield (from **5**, without purification of **7**) as an oil. The acid was prepared and used the same day to minimize decomposition and was sufficiently pure to use in the next reaction.

The Curtius reaction was carried out according to the literature procedure[12] in toluene, trapping the intermediate isocyanate with benzyl alcohol. The benzyloxycarbonyl (Z) amine **2** was isolated in 46–56% yield by flash chromatography.

Attempts were made to hydrolyze the isocyanate **8** directly to the free amine **9**. However, purification of the amine from the hydrolysis mixture was difficult and at best 10% yields were obtained. The sequence of reaction to produce **2** was thus preferred.

Scheme II shows the sequence for protective group removal to produce **1**. Removal of the pinacol ester involved a transesterification with diethanolamine.[14] The diethanolamine adduct **10** was isolated in an initial yield of 83%. Unreacted **2** was recovered from the mother liquor and recycled. Thus, yields >90% of **10** were realized.

Hydrolysis of the ethyl ester of **10** with 3 equiv of KOH in H_2O was complete in 10–15 min at 0 °C. The ethyl ester was particularly labile, possibly because of Lewis acid participation of the boron. After acidification of the reaction mixture with anion exchange resin, the Z-protected amino acid **11** was obtained as a solid after evaporation of the water. Attempts to recrystallize the acid **11** were not successful. Hydrogenolysis of **11** to produce **1** occurred readily in water (trace of HCl) with 5% Pd/C and was complete within 2 h at room temperature in 79% yield. Final product **1** could be purified only by repeated triturations with acetone. The NMR spectrum of **1** (D_2O) indicated that it exists either as a dimer or zwitterion in aqueous solution with the methine proton appearing as doublet of doublets at 3.8 ppm and the BCH_2 appearing separately as doublets of doublets centered at 0.9 ppm, indicating lack of free rotation.

Summary

We have produced a new class of unnatural amino acids by the successful introduction of an amino group β to boron via a Curtius rearrangement. Primary β-amino boronic esters/acids are stable molecules. The reactions presented here are not limited to the construction of β-amino boronic acids, and we are currently examining various intermediates for their potential as precursors for more complex system.

Experimental Section

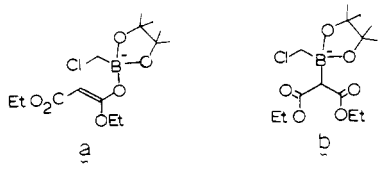
Reagents and solvents were purchased from Aldrich Chemical Co., Sigma, and Curtin Matheson Scientific Corp. and were of reagent grade or better. Necessary further purification is indicated with the specific experiment. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl just prior to use. Ether was distilled from CaH_2.

^1H and ^{13}C NMR spectra were recorded on an IBM NR80 spectrometer at 80.13 and 20.15 MHz, respectively, and are reported in ppm downfield from Me_4Si as internal standard (δ scale) unless otherwise indicated. IR spectra were recorded on a Beckman Acculab 8 and are reported as cm^{-1}. Mass spectra were obtained on a Kratos MS50 in electron impact mode, and data are reported as m/z (percent base). Elemental analysis was performed by Galbraith Labs, Knoxville, TN. Melting points were taken by using a Mel-Temp apparatus and are uncorrected.

Pinacol (chloromethyl)boronate (**4**)[15] was synthesized according to published procedures. Flash chromatography was performed by using N_2 as the carrier gas with Merck 230–400 mesh silica and UV detection at 254 nm.

Diethyl [(3,3,4,4-Tetramethyl-2,5,1-dioxaborolyl)-methyl]malonate (5). The reaction was usually carried out on the 0.05-mol scale. Diethyl malonate (14.4 g, 0.05 mol) was added carefully to 1.25 g NaH (0.055 mol as 50% oil suspension washed 3× with dry hexane) in 100 mL of THF cooled to 0 °C. After 15 min, pinacol (chloromethyl)boronate (**4**) (17.6 g, 0.1 mol) was added in 50 mL of THF. A white solid formed rapidly. After the mixture was stirred overnight, 10 mL of H_2O was added and the organic layer diluted with 100 mL of ether. The organic layer was separated and washed 2 × 15 mL with water and then 5 mL of brine. After drying ($MgSO_4$) and evaporation of the solvent, the residue was vacuum distilled. The first fraction gave pinacol (chloromethyl)boronate (50–70% recovery of the excess), bp 38 °C (0.05 torr), followed by **5**, bp 92–95 °C (0.05 torr), 85–90% yield based on malonic ester: NMR ($CDCl_3$) δ 4.2 (q, 4 H, CH_2CH_3, J = 8 Hz), 3.6 (t, 1 H, BCH_2CH, J = 8.5 Hz), ~1.2 (m, 20 H, CH_3, pinacol, CH_2B): mass spectrum, m/z 255 (7), 242 (74), 156 (100);

(13) Matteson et al. have reported that $ZnCl_2$ will promote migration of refractory alkyl groups in the ate complexes formed between chloroboronic esters and alkyl anions. In a personal communication, Prof. Matteson suggested that of the two possible ate complexes a and b, a rearranges to product, and b does not. In their own work, they have not



observed $ZnCl_2$ promoting the displacement of halide with oxyanions. The second equivalent of **6** would allow equilibration between a and b, driving the reaction to completion. Matteson, D. S.; Sadhu, K, M. *J. Am. Chem. Soc.* **1983**, *105*, 2078.

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exact mass calcd for $C_{14}H_{25}BO_6$ 300.2133, found 300.2097. Anal. Calcd: C, 56.02; H, 8.40; B, 3.60. Found: C, 56.15; H, 8.55; B, 3.67.

Potassium Ethyl [(3,3,4,4-Tetramethyl-2,5,1-dioxaborolyl)methyl]malonate (7). Ester **5** (10 g, 0.033 mol) was dissolved in 25 mL of absolute ethanol, and 1.8 g (0.033 mol) of KOH in 3 mL of H_2O was carefully added. The solution was stirred at room temperature for 16 h. The solvents were evaporated, and the potassium salt could be recrystallized from EtOH/EtOAc mixtures with 85–90% recoveries: mp >250 °C; NMR [$(CD_3)_2SO$] δ 4.0 (q, 2 H, CH_2CH_3, J = 8 Hz), 2.95 (t, 1 H, $CHCH_2B$, J = 9.3 Hz), 1.1 (t, 3 H, CH_3CH_2, J = 8 Hz), 0.95 (S, 12 H, pinacol), 0.6 (dd, 2 H, BCH_2, J = 9.5, 2.5 Hz). Characterization was completed by conversion to the acid **6**.

Ethyl [(3,3,4,4-Tetramethyl-2,5,1-dioxaborolyl)methyl]malonate (6). Potassium salt **7** was dissolved in H_2O (0.5 g/mL) and acidified with 3 M HCl, extracted into ether, and dried over $MgSO_4$. The solvent was evaporated to give the acid as an oil in 91% yield (from diester **5**), which was used within a few hours of synthesis in the next reaction: NMR ($CDCl_3$) δ 9.5 (brs, 1 H, OH), 4.25 (q, 2 H, CH_2CH_3, J = 8 Hz), 3.65 (t, 1 H, $CHCH_2B$, J = 9 Hz), ~1.2 (m, 17 H, CH_3, pinacol, CH_2B); mass spectrum, m/z 257 (22), 214 (92), 167 (57), 128 (100); exact mass calcd for $C_{12}H_{21}BO_6$ 272.1433, found 272.1432.

Ethyl 2-[*N*-(Benzyloxycarbonyl)amino]-3-(3,3,4,4-tetramethyl-2,5,1-dioxaborolyl)propionate (2). Acid **6** (8.2 g, 0.028 mol) was dissolved in 50 mL of toluene, and the solution was cooled to 0 °C. Triethylamine (2.9 g, 0.03 mol) was added followed by diphenyl phosphorazidate (8.2 g, 0.028 mol) in 10 mL of toluene. The solution was stirred at room temperature for 30 min and then heated to 80 °C. Bubbling occurred (N_2) when the temperature reached 75 °C; the reaction was timed from the appearance of the bubbles and was heated for 1.5 h. Benzyl alcohol (3.2 g, 0.028 mol, freshly distilled) was added and heating continued for about 16 h. Alternatively, the NCO stretch could be observed in the IR at 2240 cm^{-1}, and benzyl alcohol was added when no further increase in absorbance occurred. After the solution was cooled to room temperature, H_2O was added followed by 20 mL of ether. The layers were separated, and the organic layer was washed once with water. The product was purified by flash column chromatography with UV detection at 254 nm. The product was usually rechromatographed to removed residual benzyl alcohol. Alternatively (with up to 10% loss to ester hydrolysis, ~15 min exposure to base) the product was extracted from the organic layer with 4 × 15 mL of 1 m NaOH, and the combined basic extracts were back-washed with ether. After acidification of the aqueous phase with 3 M HCl, the product was extracted into ether (3 × 25 mL) and dried over $MgSO_4$. After evaporation of the ether, the product was purified by flash chromatography on silica, 25% EtOAc/hexane, to give 5–6 g (46–56% yield) of **4** as a colorless oil: NMR ($CDCl_3$) δ 7.3 (s, 5 H, Ar), 6.5 (br d, 1 H, NH, J = 10 Hz), 5.1 (s, 2 H, $ArCH_2$), 4.5 (m, 1 H, NCHCO), 4.2 (q, 2 H, CH_2CH_3, J = 8 Hz), 1.2 (m, 17 H, CH_3, pinacol, CH_2B); ^{13}C NMR ($CDCl_3$) δ 172.7, 155.6, 136.5, 128.3, 127.9, 83.6, 66.7, 61.2, 50.8, 24.8, 24.6, 15 (b); mass spectrum; m/z 304 (22), 260 (25), 83 (100); exact mass calcd for $C_{19}H_{28}BNO_6$ 377.2013, found 377.2069. Anal. Calcd C, 60.47; H, 7.48; N, 3.71; B, 2.87. Found: C, 60.07; H, 7.67; N, 3.33; B, 2.49.

Ethyl 2-amino-3-(3,3,4,4-tetramethyl-2,5,1-dioxaborolyl)-propionate (9): from hydrolysis of isocyanate with H_2O; mp 68–69 °C; NMR ($CDCl_3$) δ 3.7 (br t, 1 H, CHN, J = 9 Hz), 2.8 (br s, 2 H, NH_2), + ethyl, CH_2B, and pinacol resonances; mass spectrum; m/z 242 (3), 170 (100), 128 (30); exact mass calcd for $C_{11}H_{22}NBO_4$ 242.1334, found 242.1306.

Ethyl 2-[*N*-(Benzyloxycarbonyl)amino]-3-(5-aza-2,8-dioxa-1-boracyclooctanyl)propionate (10). The Z-amino ester **2** (1.0 g, 2.65 mmol) was dissolved in a minimal amount of EtOAc and treated with 0.30 g of diethanolamine. After 4 h, the solvent was removed on the rotary evaporator, and ether was added. A solid formed rapidly. The flask and contents were placed in the freezer for 24–72 h. The white solid was collected by filtration to give an 83% yield of **10**. The product could not be made to recrystallize but was sufficiently pure to use in the next experiment: mp 102–105 °C; NMR [$(CD_3)_2SO$] δ 0.8 (br d, 2 H, CH_2B, J = ~9 Hz) + ethanolamine multiplets at δ 3.9 and 3 and benzyl resonances.

2-[*N*-(Benzyloxycarbonyl)amino]-3-boronopropionic Acid (11). The diethanolamine ester **10** (0.36 g, 1 mmol) was treated with KOH (170 mg, 3 mmol) in 2 mL of H_2O for 1 h. The solution was acidified with Bio-Rad ion-exchange resin (sulfonic acid form). Evaporation of the water gave the product in 67% yield as hygroscopic oil, which crystallized after several hours under vacuum (0.1 torr). Acid **11** was converted to the amino acid **1** for purification: NMR [$(CD_3)_2CO$)] δ 7.3 (s, 5 H, Ar), 6.4 (brd, 1 H, NH, J = 9 Hz), 5.0 (s, 2 H, $ArCH_2$), 4.3 (m, 1 H, NCH), 1.2 (d, 2 H, CH_2B, J = 8 Hz), ~9 (br s, 1 -, CO_2H), ~2.5 (br s, ~2 H, $B(OH)_2$).

2-Amino-3-boronopropionic Acid (1). Z-amino acid **11** (250 mg, 0.94 mmol) was dissolved in 5 mL of H_2O with a trace of HCl. The reaction vessel was flushed with nitrogen. Pd–C (5%, 30 mg) was then added and H_2 bubbled through the reaction mixture for 2 h. The catalyst was removed by filtration through Celite, and 98 mg of the product was isolated (79% yield) as a white solid after evaporation of the solvent and tritration with acetone: mp 103–105 °C; NMR (D_2O, $(CH_3)_2CO$ internal reference) δ 3.8 (dd, 1 H, NCH, J = 9, 12 Hz), 0.9 (m, 2 H, CH_2B), 4.6 (s, ~5 H, OH); ^{13}C NMR (D_2O) 178.4, 54.3, 20 (br); IR (KBr) 1700 (C=O), 1325 (B–C) cm^{-1}; mass spectrum, m/z 133 (2), 134 (3). Anal. Calcd for $C_3H_8BNO_4$: C, 27.11; H, 6.07; N, 10.54; B, 8.13. Found: C, 27.17; H, 6.16; N, 10.41; B, 8.01.

Acknowledgment. We thank Professor John A. Katzenellenbogen for helpful suggestions during the course of this work, Professor D. S. Matteson for helpful comments during the preparation of this manuscript, and Wanda Rhodes for the expert typing of this manuscript.