Synthesis of Thrombin Inhibitor DuP 714

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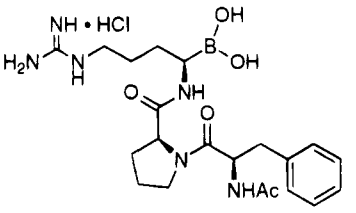
The asymmetric synthesis of thrombin inhibitor DuP 714 (**1**) is described. The route uses the Matteson boronic ester homologation to prepare the key intermediate, α-aminoboronic acid **4**. New methodology was developed for the formamidination of boroornithine peptides and for pinanediol boronate ester cleavage.

Introduction

Thrombin is a serine protease that plays a key role in the process of coagulation. As the last protease in the coagulation cascade, thrombin catalyzes the conversion of fibrinogen to fibrin. Additionally, it is a potent activator of platelets and other coagulation factors. Thrombin is an appealing target for therapeutic intervention in thrombotic disease states, with potential for the treatment of pulmonary embolism, deep vein thrombosis, unstable angina, thrombolysis, and the thromboembolic complications of coronary bypass surgery.

In 1990, a series of boropeptide thrombin inhibitors based upon the D-Phe-Pro-Arg sequence was described.[1] These modified peptides have a C-terminal boronic acid moiety in place of the carboxyl terminus and are among the most potent inhibitors of this enzyme known. Boronic acids interact with serine proteases through an attack of the active site serine on boron, resulting in the formation of a tetrahedral boron ate-complex.[2] This complex may be regarded as an analog of the tetrahedral intermediate formed during hydrolysis of a natural substrate. As part of an ongoing program for the development of thrombin inhibitors, DuP 714 (**1**) was identified as an agent with high potency and oral activity. While we had previously reported a synthesis of **1**, the sequence contained formamidination and boronic acid deprotection steps that were low-yielding and therefore unsuitable for scaleup. We now describe in full detail an improved synthesis of DuP 714 which has overcome these synthetic problems and allowed the preparation of hundreds of grams of this material.

Synthesis of α-Amino Boronic Ester 4. The synthesis of α-amino boronic ester **4** relies upon Matteson's elegant asymmetric homologation methodology.[3] Allyl bromide was hydroborated neat, using catecholborane at 100 °C. The resulting catechol ester **2** was then transesterified with (+)-pinanediol, giving the pinanediol ester **3** in 60% yield over the two steps (Scheme 1).



DuP 714, **1**

Pinanediol was introduced by Matteson for the asymmetric control of boronic ester homologations.[4] The advantages of pinanediol are the stability of its esters, the high level of stereoinduction resulting from homologation, and its availability in either enantiomeric form from commercial (+)- or (−)-α-pinene. Furthermore, we have shown that (+)-pinanediol may be prepared in >98% ee,[5] using literature methods[6] from low-cost, technical grade (+)-α-pinene.

The homologation of **3** was highly stereoselective (>98% de) when conducted at −100 °C and using *n*-BuLi as the base to generate lithium dichloromethide. However, due to the problems anticipated with conducting large-scale reactions at low temperatures, we hoped to run this reaction at a much higher temperature upon scaleup. Using LDA to generate the lithium dichloromethide, and working at −20 °C, the homologation resulted in a 93% combined yield of 1(*S*)-chloride **5** and a small amount of what was likely to be the 1(*R*)-chloride **6**. To confirm the presence of diastereomer **6** in the reaction mixture, a 1:1 mixture of **5** and **6** was prepared by conducting the homologation on the achiral pinacol ester **7** followed by transesterification with (+)-pinanediol (Scheme 2). Formation of the 1(*R*)-diastereomer **6** was unequivocally confirmed and the stereoselectivity assigned using ^{1}H NMR spectroscopy (see Experimental Section), indicating that **5** was formed with >80% de (10:1). While greater diastereoselectivity in the homologa-

[⊗] Abstract published in *Advance ACS Abstracts,* May 15, 1995.

(1) Kettner, C.; Mersinger, L.; Knabb, R. *J. Biol. Chem.* **1990**, *265*, 18289.

(2) (a) Baldwin, J. E.; Claridge, D. W.; Derome, A. E.; Schofield, C. J.; Smith, B. D. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 9. (b) Farr-Jones, S.; Smith, S. O.; Kettner, C. A.; Griffin, R. G.; Bachovchin, W. W. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 6922. (c) Bone, R.; Frank, D.; Kettner, C. A.; Agard, D. A. *Biochemistry* **1989**, *28*, 7600. (d) Takahashi, L. H. Radhakrishnan, R.; Rosenfield, R. E.; Meyer, E. F. *Biochemistry* **1989**, *28*, 7610. (e) Bachovchin, W. W.; Wong, W. Y. L.; Farr-Jones, S.; Shenvi, A. B.; Kettner, C. A. *Biochemistry* **1988**, *27*, 7689. (f) Bone, R.; Shenvi, A. B.; Kettner, C A.; Agard, D. A. *Biochemistry* **1987**, *26*, 7609.

(3) (a) Matteson, D. S.; Jesthi, P. K.; Sadhu, K. M. *Organometallics* **1984**, *3*, 1284. (b) Matteson, D. S. *Tetrahedron* **1989**, *45*, 1859. (c) Matteson, D. S. *Chem. Rev.* **1989**, *89*, 1535. The homologation methodology is protected under US 4,525,309.

(4) Matteson, D. S.; Ray, R. *J. Am. Chem. Soc.* **1980**, *102*, 7590.

(5) As determined by Mosher's ester analysis: Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 3543.

(6) (a) Jacobson, E. N.; Marko, I.; Mungall, W. S.; Schroder, G.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 1968. (b) Erdik, E.; Matteson, D. S. *J. Org. Chem.* **1989**, *54*, 2742.

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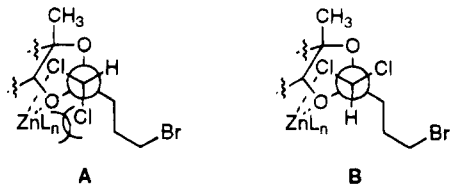
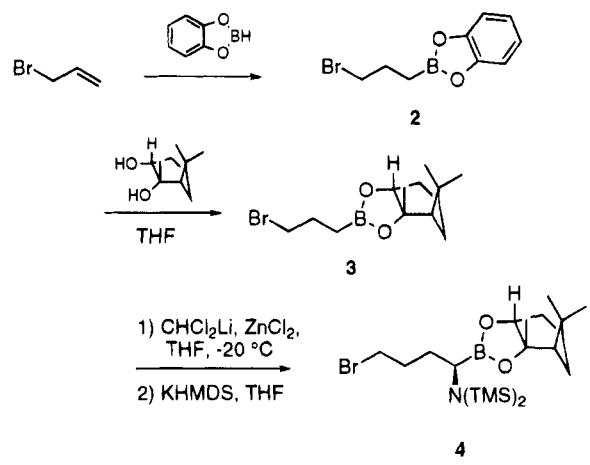
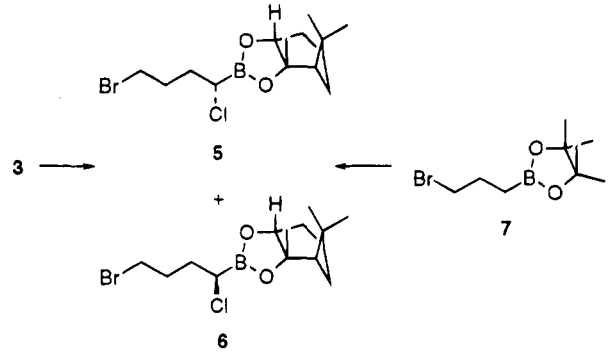


Figure 1. Homologation transition states.

Scheme 1



Scheme 2

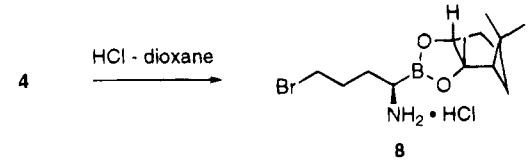


tion is desirable, purification of the epimeric mixture was conveniently accomplished two steps later, through precipitation of amine **8**. Although the new high temperature homologation conditions worked well on a laboratory scale, the low temperature conditions were used upon scaleup, to minimize epimerization of the newly formed stereocenter.[7]

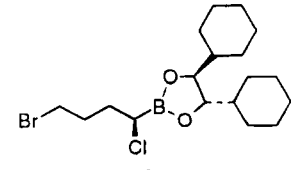
Based upon the probable mechanism, the stereochemical outcome of the homologation may be rationalized by attack of lithium dichloromethide from the less hindered convex face of the chiral auxiliary (i.e., the same side as the carbinol methyl group). This affords an ate-complex in which zinc complexation of the leaving chloride may result in two conformations from which an S_N2 displacement upon rearrangement is possible. These conformations are represented by Newman projections **A** and **B** (Figure 1). From a comparison of the two transition states, it is likely that the combination of nonbonded interactions present in conformation **A** (zinc coordination sphere–chlorine) raises its energy relative to **B** (zinc coordination sphere–hydrogen). Rearrangement from conformation **B** leads to the desired 1(*S*)-isomer **5**.

(7) Epimerization was observed from kilogram scale reactions run at −100 °C. It was reported that prolonged contact with chloride ion is the apparent cause of epimerization in boronic ester homologations, see: Matteson, D. S.; Majumdar, D. *J. Am. Chem. Soc.* **1980**, *102*, 7590.

The boron-assisted displacement of the secondary chloride using lithium bis(trimethylsilyl)amide was found to work smoothly at −20 °C, giving with inversion of configuration protected amine **4**. The chemoselective displacement of chloride is greatly facilitated by the α-boro substituent, which is known to cause a rate acceleration on the attack of nucleophiles of approximately 2 orders of magnitude relative to a primary halide.[8] Related amine nucleophiles gave yields inferior to LHMDS, although changing the counterion from lithium to potassium eased the separation of inorganic salts. Rather than isolating bis(trimethylsilyl)amine **4**, the silyl protecting groups were cleaved using anhydrous HCl in dioxane to afford the 1(*R*)-amine hydrochloride **8**. After precipitation from hexanes, diastereomerically pure **8** was obtained in 53% overall yield.



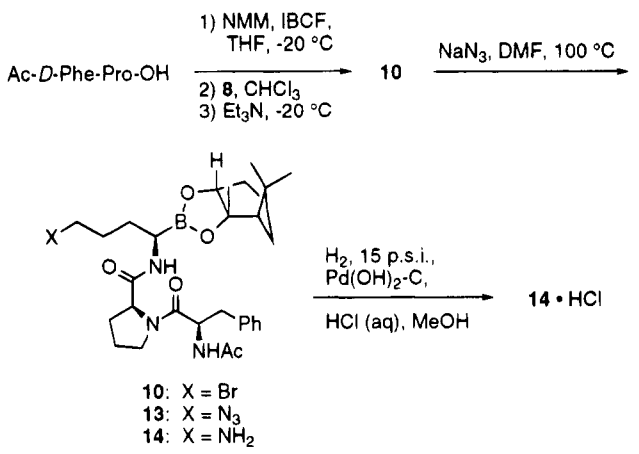
Homologation: Evaluation of Alternatives to Pinanediol. Boronic acid pinanediol esters are typically amorphous solids having broad melting ranges. Other auxiliaries were therefore investigated with the intention of arriving at a crystalline series. In this regard, 1,2-dicyclohexylethanediol was investigated as one possibility, since this auxiliary was known to afford excellent stereoselection in the addition of allylboronic esters to aldehydes.[9] Additionally, the corresponding 1,2-diisopropylethanediols[10] and 2,3-butanediols[11] had been used successfully as chiral directors in boronic ester homologations. We therefore prepared the (*S*,*S*)-1,2-dicyclohexylethanediol ester **9** using standard methods. Unfortunately, the ^1H NMR spectra in this series did not provide a diastereotopic marker suitable for the evaluation of isomers. Furthermore, the transesterification of the racemic pinacol ester with (*S*,*S*)-1,2-dicyclohexylethanediol failed to produce proton resonances representative of a 1:1 epimeric mixture, preventing an unequivocal assignment of stereoselection. In addition, the crystallinity of this series was no better than in the pinanediol series. The 2,3- and 3,4-caranediols and (+)-hydrobenzoin were also investigated with similarly disappointing results.



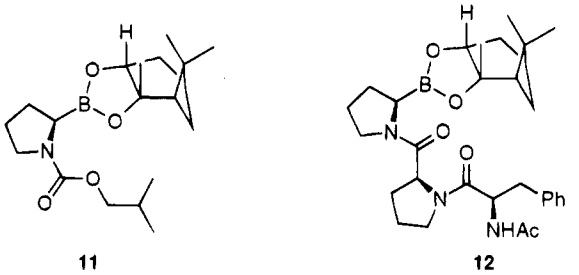
Synthesis of Amine 14. The coupling of **8** to *N*-acetyl-D-phenylalanylproline was accomplished *via* the isobutyl mixed anhydride[12] to give tripeptide **10** in 94% yield. A high-yield outcome of this reaction required the free-basing of amine **8** only when in the presence of a

(8) Matteson, D. S.; Schaumberg, G. D. *J. Org. Chem.* **1966**, *31*, 726.
(9) Stürmer, R.; Hoffmann, R. W. *Synlett* **1990**, 759.
(10) Matteson, D. S.; Kandil, A. A. *Tetrahedron Lett.* **1986**, *27*, 3831.
(11) Sadhu, K. M.; Matteson, D. S.; Hurst, G. D.; Kurosky, J. M. *Organometallics* **1984**, *3*, 804.
(12) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1967**, *89*, 5012.

Scheme 3



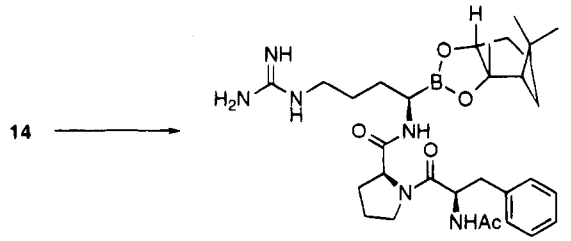
sufficiently reactive acylating agent. Free-basing prior to exposure to the mixed anhydride resulted in a complex mixture from which boroproline derived products **11** and **12** were isolated.[13]



11 **12**

Displacement of bromide from **10** using NaN_3 in DMF then gave azide **13** (92%). This displacement appeared to require a soft nucleophile,[14] as a variety of amine nucleophiles were unsuccessful. The use of phthalimide anion in this regard was successful, but the resulting imide could not be converted to the amine using standard conditions. The reduction of azide **13** using $NaBH_4$ or PPh_3 failed to give the desired amine **14**; however, catalytic hydrogenations over PtO_2 (22%) or Pd/C (42%) afforded **14**, albeit with the recovery of substantial quantities of unreacted azide. Eventually, it was found that catalytic hydrogenation over Pearlman's catalyst ($Pd(OH)_2/C$) gave the desired hydrochloride salt of **14** in 69% yield. The increase in yield of **14** upon going to Pearlman's catalyst likely resulted from reduced complexation of the product to the catalyst (Scheme 3).

Formamidination of Amine 14. Prior to this work, formamidination of **14** to afford guanidine **15** was accomplished by reacting the benzenesulfonic acid salt of **14** with an excess of cyanamide. Forcing conditions were necessary to drive the reaction to completion, resulting in epimerization and formation of other unidentified byproducts. Formamidination of **14** was therefore attempted using a variety of common formamidinating reagents, including *O*-methylisourea, *S*-methylisothiourea, and 3,5-dimethyl-1-formamidinopyrazole; however, when followed by HPLC, none of these reactions afforded guanidine **15**.



Since amine complexes of boranes and boronic esters are well-precedented, it was thought that the low reactivity of amine **14** was due to complexation of the boroornithine δ-amino group to boron. The ^{11}B NMR spectrum of the amine hydrochloride exhibited a rather broad signal centered at 6 ppm ($B(OMe)_3$ = 0 ppm) coincident with a sharp signal at 4.03 ppm (Figure 2). This spectral behavior was suggestive of a dynamic exchange process and a less-ordered structure in solution. The ^{11}B NMR spectrum of the free-base was uncomplicated by comparison, resulting in a single broad line at −9.30 ppm. This upfield shifted signal was consistent with a more-ordered tetrahedral boronate species.[15] In addition, this behavior was independent of concentration in the range of 1−100 mM. Upon formation of the free-base, spectral simplification to a single, broad, upfield shifted and concentration independent signal was compelling evidence for the formation of an intramolecular ate-complex.

The intramolecular complexation in **14** necessitated the use of a very reactive species for the formamidination. Recently, the use of formamidine sulfonic acid (FSA)[16] and *N*-substituted formamidine sulfonic acids[17] were described. The use of FSA under the conditions of Mosher[16a] afforded a 62% conversion (by HPLC) of free-base **14** to guanidinium **15** at room temperature. Additional equivalents of FSA and/or triethylamine failed to improve the outcome of the reaction.

As an alternative to isolating the free-base of **14**, formation of the free-base *in situ* from the hydrochloride salt of **14** was investigated. In the absence of an external base, **14**·HCl was completely unreactive with FSA at room temperature, however, a 40% conversion to **15** was obtained in the presence of 1 equiv of Et_3N. Additional investigation indicated that DMAP and DABCO were superior bases for this reaction, each providing 65% conversion to **15**. Further improvement was made by using 2 equiv of both FSA and DMAP, which provided an 84% conversion after 20 h. When the reaction was then run at a temperature of 78 °C (EtOH reflux), complete conversion was achieved in 1 h. Under the optimized conditions, **15** was obtained in 62% isolated yield following preparative HPLC purification.

Pinanediol Cleavage. In its dual role as protecting group and chiral auxiliary, pinanediol esters perform quite well, their only liability surfacing when one attempts deprotection of the boronic acid moiety.[18] Pinanediol esters may be cleaved using BCl_3 or through equilibration with aqueous boric acid;[19] however, the

(13) Structures assigned based upon ^1H NMR and mass spectral data.

(14) Among reagents tried without success were benzylamine, dibenzylamine, LHMDS, guanidine, and tosylguanidine. In related studies, thiols reacted particularly well in the displacement of bromide from **10**.

(15) Kidd, G. R. In *NMR of Newly Accessible Nuclei*; Lazlo, P., Ed.; Academic Press: New York, 1983; Vol. 2, pp 49−77.

(16) (a) Kim, K.; Lin, Y.-T.; Mosher, H. A. *Tetrahedron Lett.* **1988**, *29*, 3183. (b) Miller, A. F.; Bischoff, J. J. *Synthesis* **1986**, 777.

(17) Maryanoff, C. A.; Stanzione, R. C.; Plampin, J. N.; Mills, J. E. *J. Org. Chem.* **1986**, *51*, 1882.

(18) Matteson, D. S. *Tetrahedron* **1989**, *45*, 1859.

(19) Philipp, M.; Maripuri, S.; Matteson, D. S.; Jesti, P. K.; Sadhu, K. M. *Biochemistry* **1983**, *22*, A13.

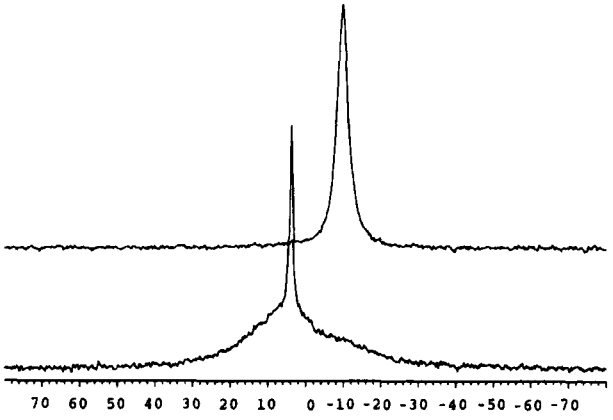


Figure 2. ^{11}B NMR spectra of **14** free-base (top) and **14**·HCl (bottom).

former conditions are strongly Lewis acidic and destructive to fragile substrates, while the latter conditions provide boronic acids "satisfactorily for purposes of testing for enzyme inhibition".[3] Due to these shortcomings, we have developed a synthetically useful two-phase ether/water system in which excess phenylboronic acid is used to affect transesterification of the boronic ester under mild conditions.[20] The ether solubility of the pinanediol ester of phenylboronic acid allows it to be easily separated from the desired water-soluble boronic acid. Using this procedure, pinanediol ester **15** afforded DuP 714 (**1**) in yields of up to 90%.

Conclusion

Starting from allyl bromide, our synthesis of DuP 714 proceeded in an overall yield of 7%. Noteworthy features were the development of mild conditions for the *N*-formamidination of boroornithine peptides using FSA and a new method for the deprotection of boronic acid pinanediol esters using phenylboronic acid. These improvements have allowed us to prepare large batches of this material, and to more efficiently carry out analog work in this promising series of novel boropeptide thrombin inhibitors.

Experimental Section

General Experimental. Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. Analytical HPLC were run on a Waters Delta Prep 3000 using a Waters 484 detector set to 210 nm, 10 μL loop, Novapak C18, 1.5 mL/min flow rate, 74:26 = MeOH:H_2O containing 0.005% sodium octanesulfonate. Proton, ^{13}C, and ^{11}B NMR data were obtained using Varian Unity 300, Unity 400, or VXR400 spectrometers and are referenced to TMS, residual HOD, or trimethyl borate. Mass spectra were obtained on either VG 70-VSE (FAB, high res FAB, high res DCI) or Finnigan MAT 8230 (DCI) mass spectrometers. Combustion analyses were performed by Quantitative Technologies, Inc., Bound Brook, NJ. Solvents and reagents were used as purchased from Aldrich Chemical Co. unless otherwise stated. Yields quoted in this paper are isolated yields.

Preparation of (+)-Pinanediol. In a 2 L, three-necked, round-bottom flask, fitted with a mechanical stirrer, reflux condenser, and heating mantle were combined (+)-α-pinene[21] (136.34 g, 1 mol), *N*-methylmorpholine *N*-oxide (207 mL of 60% in water, 1.2 equiv), water (90 mL), acetone (1 L), pyridine (1 mL), and OsO_4 (1.0 g, 3.9 mmol). The mixture was heated to reflux while stirring rapidly. After 2–3 days at reflux, the reaction mixture became homogeneous and all of the starting material was consumed (monitored by TLC on silica gel, 9:1 hexane–EtOAc, R_f = 0.95, stains with iodine). After cooling in an ice bath, sodium metabisulfite (40 g), Magnesol (20 g), and Na_2SO_4 (80 g) were added. The mixture was warmed to room temperature while stirring vigorously for 2 h and filtered through a pad of Celite. After concentration of the filtrate on a rotary evaporator to remove the acetone, the residue was partitioned between Et_2O (1.5–2 L) and water (500–1000 mL). The organic layer was washed with saturated $Na_2S_2O_3$, 2 N HCl, water, saturated $NaHCO_3$, and brine. The solution was dried ($MgSO_4$) and concentrated to a brown oil. The crude product was distilled bulb-to-bulb under vacuum (bp ~100 °C/1 Torr) to obtain a clear to pale yellow oily solid (107 g). It was crystallized from heptane (300 mL) to obtain 80 g (47% yield) of pure product: $[\alpha]_D$ = +7.91° (*c* = 0.354, toluene); mp 58–59 °C.

Determination of the Optical Purity of Pinanediol. To a solution of (+)-pinanediol (511 mg, 3 mmol), Et_3N (0.50 mL 3.6 mmol), and DMAP (5 mg, 0.04 mmol) in CH_2Cl_2 (20 mL) was added (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*S*)-(+)-MTPACl, 0.73 M in CCl_4, 4.93 mL, 3.6 mmol] with stirring. After stirring overnight, the reaction mixture was partitioned between water (50 mL) and CH_2Cl_2 (50 mL). The organic layer was washed with water, saturated $NaHCO_3$, and then dried ($MgSO_4$). After evaporation of the solvent, the crude material was purified *via* column chromatography on silica gel (4:1 hexane:EtOAc). The diastereomeric purity was determined through examination of the 400 MHz ^1H NMR spectrum to be >98% ee. The ester from (+)-pinanediol exhibits characteristic peaks at δ 3.55 (s, 3H, OCH_3), 1.65 (m, 1H), and 1.40 (d, *J* = 10 Hz, 1H), and was easily distinguishable from the ester from (−)-pinanediol: δ 3.58 (s, 3H, OCH_3), 1.78 (m, 1H), and 1.46 (d, *J* = 10 Hz, 1H).

Preparation of (+)-Pinanediol Ester 3. To neat catecholborane (24 g, 0.200 mmol) in a 100 mL, three-necked round-bottomed flask was added allyl bromide (17.3 mL, 0.200 mol). As the reaction mixture was heated, it began to reflux at about 75–85 °C and then was warmed to 100 °C. The reaction was exothermic on this scale and was cooled with a water bath when necessary. The mixture was held at 100 °C for 4 h and then cooled to room temperature. To a solution of (+)-pinanediol (34.9 g, 0.205 mol) in THF (100–200 mL) at 0 °C was added *via* cannula the brown solution of **2**. After transfer was complete, the reaction was warmed to room temperature and stirred overnight. The reaction mixture was concentrated *in vacuo* to remove most of the THF and poured into a 1 L separatory funnel containing hexane (400–500 mL) and Na_2CO_3 (10.8 g in 200 mL water). The organic layer was washed with saturated $NaHCO_3$, water, and brine and dried (Na_2SO_4). The solution was rapidly filtered through silica (150 g, wetted with hexane) in a 6.5 cm (diameter) × 15 cm (length) fritted funnel (medium frit), and rinsed with 9:1 hexane:EtOAc (200–400 mL). After concentration *in vacuo*, the resulting liquid was distilled *via* Kugelrohr (bp 80–90 °C/345 mTorr), affording **3** as a colorless liquid (37 g, 60% yield): $[\alpha]_D$ = +21.45° (*c* = 0.676, $CHCl_3$) [lit.[3] $[\alpha]_D$ = +21.9° (*c* = 3, $CHCl_3$)]; ^1H NMR (300 MHz, $CDCl_3$) δ 4.26 (dd, *J* = 1.8, 8.8 Hz, 1H), 3.44 (t, *J* = 7 Hz, 2H), 2.34 (m, 1H), 2.22 (m, 1H), 1.95–2.07 (m, 3H), 1.92 (m, 1H), 1.84 (m, 1H), 1.38 (s, 3H), 1.29 (s, 3H), 1.09 (d, *J* = 11 Hz, 1H), 0.94 (t, *J* = 8 Hz, 1H), 0.84 (s, 3H).

Preparation of Chloride 5. Pinanediol ester **3** (31.2 g, 103.7 mmol) was dissolved in a mixture of cyclohexane (240 mL) and THF (120 mL) containing CH_2Cl_2 (8.12 mL, 126.7 mmol, dried over K_2CO_3 before use). The solution was cooled in a dry ice/CCl_4 bath to maintain the temperature at −20 °C. LDA (Lithco 9505, 1.92 M in heptane–THF, 60 mL, 115.2 mmol) was added dropwise *via* an addition funnel, such that the internal temperature of the reaction stayed between −15

(20) Kettner, C. A. US 5,384,410, 1995. During the preparation of this manuscript a similar procedure for the cleavage of boroproline pinanediol esters was published, see: Coutts, S. J.; Adams, J.; Krolikowski, D.; Snow, R. J. *Tetrahedron Lett.* **1994**, *35*, 5109.

(21) Aldrich No. 20,545-1 (85%) (+)-α-pinene was treated with Darco overnight and then filtered down a short column of basic alumina prior to use.

to −20 °C. Immediately after the addition of LDA was complete, a solution of zinc chloride (1.0 M in THF, 173 mL, 173 mmol) was added and the internal temperature of the reaction was maintained between −15 to −20 °C. After the addition was complete, the reaction mixture was allowed to warm to room temperature and stirred overnight. The mixture was diluted with hexane (500 mL), and poured into a flask containing hexane (1.5 L) and cold 1 N H_2SO_4 (600 mL). The reaction flask was washed with an additional 500 mL of hexane to affect quantitative transfer. The mixture was stirred for 5 min, and the layers were separated. The aqueous layer was washed with an additional 150 mL of hexane. The combined hexane solution was washed with water, saturated $NaHCO_3$, and brine, and was dried ($MgSO_4$). Concentration *in vacuo* gave the crude product as a yellow oil (40.8 g). The product was purified by rapid filtration through silica (150 g, wetted with hexane) in a 6.5 cm (diameter) × 15 cm (length) fritted funnel (medium frit), rinsed with 9:1 hexane−EtOAc (200−400 mL). The solvent was pulled though the column quickly using a vacuum adapter. Removal of solvent provided 33.7 g (93%) of the products **5** and **6** (10:1 ratio) as a very pale yellow oil: $[\alpha]_D$ = +25.86° (*c* = 0.754, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 4.38 (dd, *J* = 1.8, 8.8 Hz, 1H), 3.49 (m, 1H), 3.45 (t, *J* = 7.0 Hz, 2H), 2.36 (m, 1H), 2.25 (m, 1H), 1.84−2.20 (m, 7H), 1.43 (s, 3H), 1.30 (s, 3H), 1.16 (d, *J* = 11 Hz, 1H), 0.85 (s, 3H). The isomer ratio was determined by analysis of the 400 MHz ^1H NMR spectrum. The doublet centered at 1.20 ppm provided a direct measurement of the diastereomeric ratio. The ^{13}C NMR spectrum is also diagnostic in the range 30−33 ppm, but it is less reliable.

Preparation of α-Amino Boronic Ester 8. To a 0.5 M solution of KHMDS in toluene (14.3 mL, 7.15 mmol) at −20 °C was added dropwise over 20 min a solution of chloride **5** (2.50 g, 7.15 mmol) in THF (20 mL). The mixture was then allowed to warm to room temperature overnight (18 h). The reaction mixture was concentrated to dryness, and hexanes (30 mL) was added. The resulting suspension was filtered through a bed of Celite followed by hexanes wash. The combined filtrate was cooled to −10 °C and 4 M HCl in dioxane was added (6 mL, 24 mmol) and the mixture allowed to warm to room temperature. After 36 h, concentration *in vacuo* gave a brown oil. The oily residue was dissolved in hexanes (30 mL), resulting in the immediate precipitation of the hydrochloride salt. Filtration, concentration of the filtrate *in vacuo*, and pumping under vacuum to constant weight afforded 1.39 g (53%) of **8**: $[\alpha]_D$ = +17.2° (*c* = 1.0, EtOH); mp 149−150 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 8.27 (bs, 3H), 4.42 (d, *J* = 8.0 Hz, 1H), 3.44 (m, 2H), 3.00 (m, 1H), 2.1 (m, 9H), 1.43 (s 3H), 1.29 (s, 3H), 1.15 (d, *J* = 11.0 Hz, 1H), 0.83 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 87.8, 78.8, 51.0, 39.4, 38.1, 36.8, 35.0, 33.0, 29.6, 28.4, 28.2, 26.9, 26.6, 23.9; IR (Nujol mull) 3370, 2900, 1600, 1530, 1460, 1410, 1380, 1290 cm^{-1}. Anal. Calcd for $C_{14}H_{25}ClBBrNO_2$: C, 45.88; H, 7.15; N, 3.82. Found: C, 46.05; H, 7.22; N, 3.77.

Preparation of Bromide 10. To a suspension of Ac-D-Phe-Pro-OH (166.0 g, 0.545 mol) in THF (700 mL) was added *N*-methylmorpholine (NMM, 55.09 g, 0.545 mol) and the mixture heated at reflux until dissolution (30 min). After cooling to −8 °C, isobutyl chloroformate (IBCF, 0.26 mL, 2.00 mmol) was added, resulting in immediate formation of a white precipitate. After 10 min, a cooled (−15 °C) solution of **8** (200 g, 0.546 mol) in $CHCl_3$ (585 mL) was added followed by the addition of Et_3N (55.08 g, 0.544 mol) at a rate such that the internal temperature of the reaction did not exceed −5 °C. After stirring at −10 °C for 1 h, the cooling bath was removed, and the mixture stirred for an additional 2 h. The mixture was filtered (THF wash) and the combined filtrate concentrated *in vacuo*. After dissolving the oily residue in ethyl acetate, it was washed with 0.1 M HCl, saturated $NaHCO_3$, and brine and was dried ($MgSO_4$). Concentration *in vacuo* and removal of residual solvent under high vacuum resulted in 322.1 g (94%) of the desired bromo tripeptide: $[\alpha]_D$ = −106.6° (*c* = 0.47, $CHCl_3$); mp 77−87 °C; ^1H NMR (400 MHz, $CDCl_3$, 55 °C, major conformer) δ 7.71 (bs, 1H), 7.24 (m, 5H), 6.41 (bs, 1H), 4.61 (m, 1H), 4.54 (dd, *J* = 8.6, 2.2 Hz, 1H), 4.26 (dd, *J* = 8.8, 2.2 Hz, 1H), 3.62 (dt, *J* = 9.0, 3.0 Hz, 1H), 3.39 (m, 2H), 2.88 (m, 2H), 2.31 (m, 1H), 2.16 (m, 1H), 2.03 (s, 3H), 1.8 (m, 12H), 1.38 (s, 3H), 1.36 (m, 1H), 1.26 (s, 3H), 0.94 (dd, *J* = 11.7, 6.6 Hz, 1H), 0.83 (s, 3H); IR ($CHCl_3$) 2990, 1655, 1450 cm^{-1}; HRMS (CI, NH_3) *m/z* 616.2543 [(M + H)$^+$ calcd for $C_{30}H_{44}BBrN_3O_5$: 616.2557].

Preparation of Azide 13. To a solution of **10** (143.4 g, 0.2326 mol) in DMF (110 mL) was added NaN_3 (19.4 g, 0.279 mol) and the mixture heated at 100 °C for 10 min. After cooling to room temperature and dilution with EtOAc, the mixture was washed with water (4 × 100 mL) and brine and was dried ($MgSO_4$) and concentrated *in vacuo*. The resulting viscous oil was placed under vacuum until constant weight was acheived, affording 131.4 g (97%) of the desired azide **13** as an amorphous solid: $[\alpha]_D$ = −120.0° (*c* = 0.47, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, 55 °C, major conformer) δ 7.36 (bs, 1H), 7.24 (m, 5H), 6.12 (bs, 1H), 4.63 (m, 1H), 4.49 (dd, *J* = 8.0, 1.5 Hz, 1H), 4.26 (dd, *J* = 8.8, 2.0 Hz, 1H), 3.59 (dt, *J* = 9.0, 2.9 Hz, 1H), 3.25 (m, 2H), 3.00 (m, 2H), 2.31 (m, 1H), 2.16 (m, 1H), 1.99 (s, 3H, coincident with m, 1H), 1.85 (m, 3H), 1.6 (m, 8H), 1.36 (s, 3H, coincident with m, 1H) 1.27 (s, 3H), 0.94 (dd, *J* = 11.7, 6.6 Hz, 1H), 0.84 (s, 3H); IR ($CHCl_3$) 2990, 2930, 2100, 1655, 1450 cm^{-1}; CIMS (NH_3) *m/z* 579 (M + H$^+$, 100). HRMS (CI, NH3) *m/z* 579.3454 [(M + H)$^+$ calcd for $C_{30}H_{44}BN_6O_5$: 579.3466].

Preparation of Boroornithine 14·HCl. To a suspension of Pearlman's catalyst (122 g) in 1 M HCl (2.1 L, 2.10 mol) was added MeOH (10 L) and **13** (1.220 kg, 2.11 mol) and the mixture hydrogenated at 15 psi and ambient temperature for 21 h. The mixture was filtered through Celite and washed with MeOH (4.6 L), and the filtrate was concentrated *in vacuo* at 50 °C to give an oily residue (1.631 kg). This material was dissolved in water (6.1 L) and washed with Et_2O (3 × 6.1 L), and the aqueous portion was concentrated *in vacuo*. The resulting oil (1.132 kg) was dissolved in $CHCl_3$ (2 L), dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was dissolved in $CHCl_3$ (100 mL) and precipitated by the addition of hexanes (7.5 L), resulting in an amorphous solid. After the solvent was decanted, the material was again precipitated, affording 822.5 g (69%) of amine hydrochloride **14** as a white powder: $[\alpha]_D$ = −118.8° (*c* = 0.49, $CHCl_3$); mp 189.6−193.1 °C; ^1H NMR (400 MHz, $CDCl_3$, 60 °C) δ 7.98 (bs, 1H), 7.22 (m, 5H), 7.00 (bs, 1H), 4.57 (m, 1H), 4.42 (dd, *J* = 8.0, 2.0 Hz, 1H), 4.11 (m, 1H), 3.58 (m, 1H), 3.05 (m, 5H), 2.56 (q, *J* = 7.8 Hz, 1H), 2.28 (m, 1H), 2.11 (m, 2H), 2.07 (s, 3H), 1.95 (t, *J* = 5.4 Hz, 1H), 1.67 (m, 9H), 1.38 (d, *J* = 10.3 Hz, 1H), 1.29 (s, 3H), 1.25 (s, 3H), 0.83 (s, 3H); IR ($CHCl_3$) 3235, 3030, 2960, 1640, 1450 cm^{-1}; HRMS (CI, NH3) *m/z* 553.3557 [(M + H)$^+$ calcd for $C_{30}H_{46}BN_4O_5$: 553.3561].

Preparation of DuP 714 Pinanediol Ester 15. To a solution of **14**·HCl (235.0 g, 0.399 mol) in EtOH (3 L) was added DMAP (97.7 g, 0.799 mol). After 15 min at room temperature, formamidinesulfonic acid (98.9 g, 0.797 mol) was added, and the resulting suspension stirred at reflux for 3 h. The reaction was cooled to room temperature and filtered, the precipitate washed with $CHCl_3$, and the combined filtrate concentrated *in vacuo*. The resulting foam (421.2 g) was dissolved in $CHCl_3$ (7.6 L) and washed with chilled 0.1 M HCl (2 × 275 mL), chilled water (275 mL), and brine (275 mL) and was dried ($MgSO_4$). Concentration *in vacuo* afforded 240.5 g of crude product. Precipitation of this material from a $CHCl_3$ solution by the addition of hexanes gave an amorphous, hygroscopic solid (207.4 g). A second batch of crude **15** (154.46 g) was obtained in a similar fashion from 198.8 g of **14**·HCl. The combined product (361.86 g) then underwent final purification using preparative HPLC (Waters Prep LC 4000, 3−25 × 50.8 mm columns, neutral alumina, activity 1 (ICN), gradient elution of 90:10 $CHCl_3$−MeOH through 50:50 $CHCl_3$−MeOH ramped over 45 min, 210 nm detection) in 1.6 g lots to afford 278.1 g (62%): $[\alpha]_D$ = −102.5° (*c* = 0.56, $CHCl_3$); mp 110 °C, gas evolution at 128.1 °C; ^1H NMR (400 MHz, $CDCl_3$, 60 °C, major conformer) δ 8.42 (bd, *J* = 3.9 Hz, 1H), 7.91 (bs, 1H), 7.48 (bm, 1H), 7.20 (m, 7H), 4.45 (m, 1H), 4.39 (dd, *J* = 7.8, 2.4 Hz, 1H), 4.23 (dd, *J* = 8.5, 1.4 Hz, 1H), 3.65 (m, 1H), 3.11 (m, 4H), 2.27 (m, 1H), 2.13 (m, 1H), 2.06 (s, 3H), 1.87 (m, 8H),

1.58 (m, 5H), 1.36 (d, J = 10.5 Hz, 1H), 1.32 (s, 3H), 1.26 (s, 3H), 0.90 (m, 1H), 0.82 (s, 3H); IR ($CHCl_3$) 3340, 2990, 1645, 1450, 1215 cm^{-1}; HRMS (CI, NH3) m/z 595.3765 [$(M + H)^+$ calcd for $C_{31}H_{48}BN_6O_5$: 595.3779].

Preparation of *N*-Acetyl-D-phenylalanyl-prolyl-boroarginine Hydrochloride (1). To a solution of **15** (75.0 g, 0.118 mol) in 1:1 Et_2O-H_2O (4.5 L) was added phenylboronic acid (60.0 g, 0.49 mol). The resulting mixture was rapidly stirred for 3.5 h, after which time the layers were allowed to separate. The aqueous phase was washed with Et_2O (3 × 2.2 L), concentrated *in vacuo* at 40 °C to one-tenth volume, and then passed through a 3–4 in. diameter column of AG1-X8 resin (chloride form, 200–400 mesh, 600 g) using water elution. Combination of the appropriate fractions and concentration *in vacuo* afforded a white powder, which was placed under vacuum over P_2O_5 until constant weight was achieved, affording 48.4 g (82%) of an amorphous solid; $[\alpha]_D = -135.0°$ (c = 0.588, H_2O); ^1H NMR (400 MHz, D_2O) δ 7.29 (m, 5H), 4.45 (m, 1H), 4.39 (dd, J = 7.8, 3.9 Hz, 1H), 3.64 (m, 1H), 3.14 (t, J = 6.8 Hz, 2H), 2.99 (m, 2H), 2.76 (dd, J = 17.6, 7.8 Hz, 1H), 2.58 (t, J = 6.8 Hz, 1H), 1.95 (s, 3H), 1.89 (m, 2H), 1.75 (m, 1H), 1.51 (m, 5H); ^{13}C NMR (100 MHz, D_2O) δ 175.37, 174.00, 172.74, 156.74, 135.57, 129.30, 128.91, 128.83, 128.71, 127.53, 58.16, 53.85, 47.62, 44.81, 41.10, 36.51, 28.80, 27.18, 26.01, 23.76, 21.41; IR (KBr) 3258, 3176, 2950, 1648, 1540, 1448 cm^{-1}.

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Supplementary Material Available: ^1H NMR for adducts **10**, **13**, **14**·HCl, and **15**, ^1H NMR expansions for the diastereomers **5** and **6** and for the Mosher esters of (+)- and (−)-pinanediol, an analytical HPLC chromatogram from adduct **15**, and ^1H, ^{13}C COSY, NOESY, DEPT and ^1H-^{13}C HETCOR spectral data from adduct **1** (19 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of this journal, and may be ordered from the ACS; see any current masthead page for ordering information.

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