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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Through a careful de novo rational drug design process, we are developing a new class of high-fidelity estrogen mimics with intrinsic chemical properties conducive to potent binding to the estrogen receptor as well as to antiproliferation of human breast cancer cells. These mimics are constructed upon a stable boron heterocycle platform and can be made to bear a remarkable resemblance to the natural estrogens. Importantly, they can accommodate authentic A- and D-ring hydroxyl and ketone functionalities known to be essential to the intracellular recognition of estrogens by enzymes and receptor sites. The synthetic chemistry accomplishments made possible by this Award successfully removed all remaining obstacles to the facile generation of the boron heterocycle estrogen mimics. Accordingly, we are now in a position to easily and systematically produce analogs and examine them for bioactivities predictive of good breast cancer chemotherapeutic properties. The biological assay results obtained during this Award clearly reveal the covalently fused CD ring compounds as pure antagonists of the estrogen receptor, and we can now develop this subclass of compounds to produce potent lead candidates as novel boron heterocycle-based breast cancer chemotherapeutic agents.

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INTRODUCTION

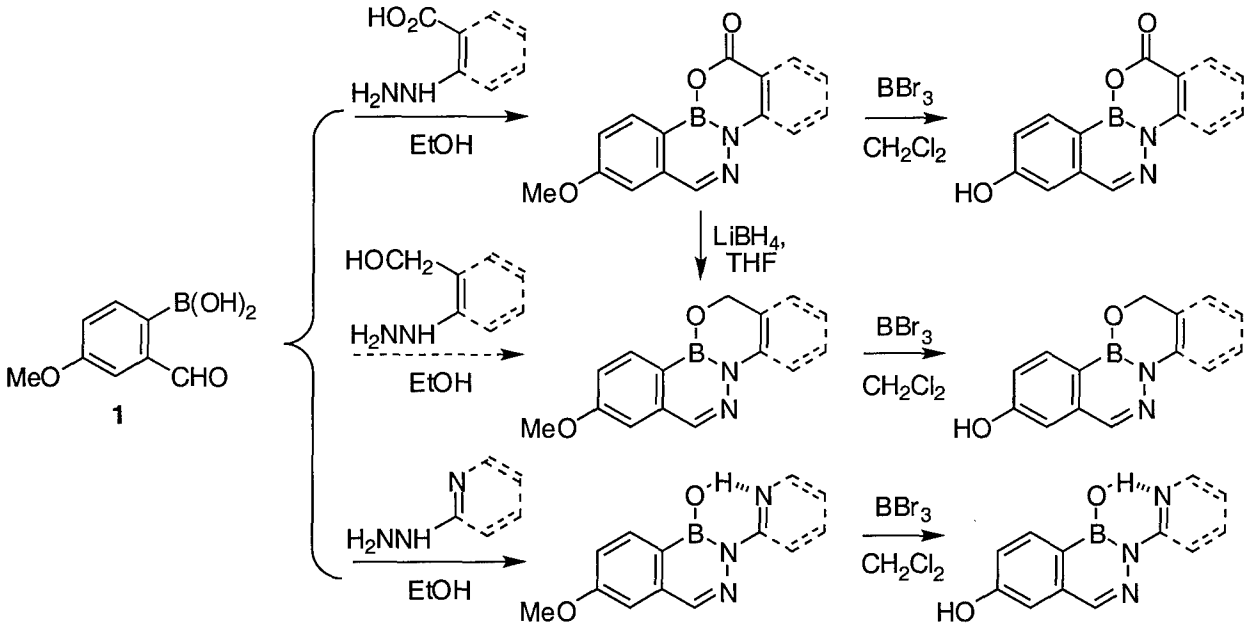
The goal of this Concept Award was to develop novel boron heterocycle estrogen mimics as antiestrogenic breast cancer therapeutics. These mimics, built upon a stable 2,3,1-benzodiazaborine heterocycle platform, can be made to bear a remarkable resemblance to natural estrogens. Their synthesis allows for a wide variation in structure, their good water solubility should produce good bioavailability, they can be equipped with A- and D-ring oxygen functionalities for proper intracellular recognition by enzymes and receptor sites, and the enhanced acidity of their A-ring hydroxyl group should lead to an enhanced association with estrogen-binding macromolecular targets. This work is based on our hypothesis that the unique intrinsic chemical properties and near estrogen-like shape of the boron heterocycle estrogen mimics would combine to produce potent ligands for key breast cancer protein targets such as the estrogen receptor (ER).

BODY

Chemistry

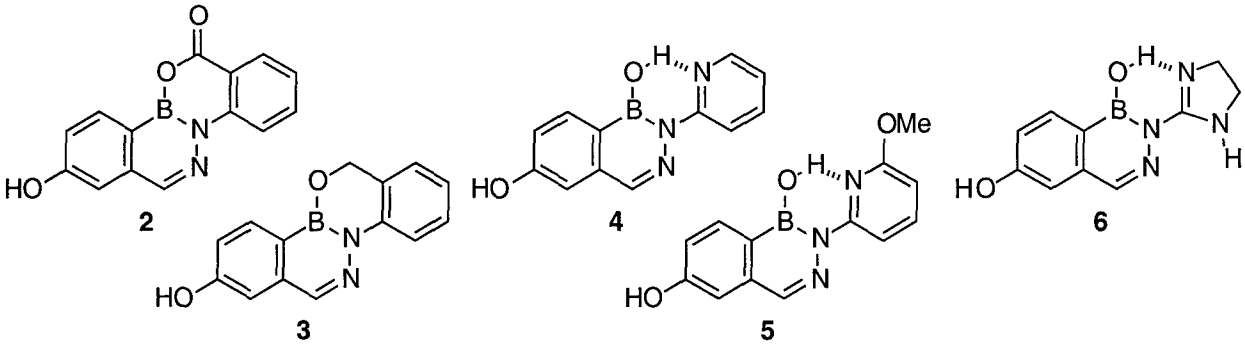
Initial Synthetic Route

Our first route to the three variants of the boron heterocycle estrogen mimics utilized commercial (Frontier Scientific, Inc.) 2-formyl-4-methoxybenzeneboronic acid—the most readily available A-ring precursor. As shown below, this boronic acid (**1**) was easily condensed with 2-hydrazinobenzoic acid and 2-hydrazinopyridine to give 2,3,1-benzodiazaborines directly. In theory, it is possible to condense it with 2-hydrazino benzyl alcohols, but this approach could not be verified because a suitable hydrazine reagent was not readily available.



The first set of boron heterocycle estrogen mimics, **2** through **6**, had been prepared prior to the Concept Award from **1** by using the above approach. In this project, they were resynthesized

and in many cases characterized more fully. The details of these syntheses and characterizations follow.



1,2-Dihydro-1,6-dihydroxy-2-(2-carboxyphenyl)-2,3,1-benzodiazaborine Lactone (2). A literature procedure(1) for a similar condensation was used. A solution of 2-formyl-4-methoxybenzeneboronic acid (Frontier Scientific) (900 mg, 5 mmol) in 10 mL of EtOH was added to a hot solution of 2-hydrazinobenzoic acid hydrochloride (1.04 g, 5.5 mmol) in 10 mL of water. A copious precipitate formed, and after it had cooled to 23 °C the mixture was suction filtered with the aid of 50 mL of 95% aqueous EtOH. The precipitate was washed with a small amount of 95% aqueous EtOH and dried under vacuum to give 1.215 g (87%) of 6-O-methylated **2**: mp 240-242 °C (EtOH). When 220 mg (5.5 mmol) of NaOH was used to neutralize the HCl in the hydrazine reagent, the yield was 1.165 g (84%). Both unrecrystallized products had mp 234-236 °C. ^{1}H NMR ($CDCl_3$) δ 8.39 (s, 1, H4), 8.38 (bs, 1, ArH), 8.34 (d, J = 5.4 Hz, 1, ArH), 8.31 (pseudo-t, 2, ArH), 7.80 (pseudo-t, 1, ArH), 7.37 (d of d, 1, ArH), 7.33 (d of d, 1, ArH), 7.21 (d, J = 2.3 Hz, 1, ArH), 3.97 (s, 3, OCH_3). Low-resolution DCI mass spectrum, *m/e* 279 (100%, MH^+). Anal. Calcd for $C_{15}H_{11}BN_2O_3$: C, 64.79; H, 3.99; N, 10.07. Found: C, 64.83; H, 4.05; N, 9.97.

A rapidly stirred suspension of 6-O-methylated **2** (300 mg, 1.08 mmol) in 15 mL of dry CH_2Cl_2 under argon was treated dropwise with neat BBr_3 (0.2 mL, excess). Chromatography on silica gel (5% MeOH/CH_2Cl_2 as eluent) gave **2** in a low yield: ^{1}H NMR [$(CD_3)_2SO$] δ 10.52 (s, 1), 8.53 (s, 1, H4), 8.26 (d, J = 8.4 Hz, 1, ArH), 8.13 (d, J = 7.8 Hz, 1, ArH), 8.08 (d, J = 8.1 Hz, 1, ArH), 8.86 (pseudo-t, 1, ArH), 7.38 (pseudo-t, 1, ArH), 7.30 (s, 1, ArH), 7.27 (d, J = 8.4 Hz, 1, ArH). Low-resolution DCI mass spectrum, *m/e* 265 (100%, MH^+). Anal. Calcd for $C_{14}H_9BN_2O_3$: C, 63.68; H, 3.44; N, 10.61. Found: analysis is in progress.

1,2-Dihydro-1,6-dihydroxy-2-[2-(hydroxymethyl)phenyl]-2,3,1-benzodiazaborine Anhydride (3). A suspension of 6-O-methylated **2** (300 mg, 1.08 mmol) in 20 mL of dry THF under argon was treated with $LiBH_4$ (100 mg, excess). The yellow mixture was stirred at 23 °C for 0.5 h, and then 3 mL of dry DMF was introduced and stirring was continued for 24 h. EtOAc (1 mL) was added, and the mixture was partitioned between water and EtOAc. The organic layer was separated, washed several times with water, and then dried (Na_2SO_4). The residue obtained upon evaporation was purified by passing a CH_2Cl_2 solution of it through SiO_2, and this gave analytically pure 6-O-methylated **3**: mp 122-124 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 8.10 (s, 1, H4), 8.01 (d, J = 8.5 Hz, 1, ArH), 7.93 (d, J = 8.3 Hz, 1, ArH), 7.35 (d of d, 1, ArH), 7.32 (pseudo-t, 1, ArH), 7.19 (d of d, J = 2.6, 8.4 Hz, 1, ArH), 7.09 (d, J = 2.4 Hz, 1, ArH), 7.05 (bs, 1, ArH), 5.32 (s, 2, CH_2), 3.93 (s, 3, OCH_3). Low-resolution DCI mass spectrum, *m/e* 265 (100%, MH^+). Anal. Calcd for $C_{15}H_{13}BN_2O_2$: C, 68.22; H, 4.96; N, 10.61. Found: C, 67.32; H, 5.00; N, 10.40.

A suspension of 6-O-methylated **3** (1 g, 3.6 mmol) in 40 mL of dry THF under argon was treated dropwise with 7.5 mL of 2 M $LiBH_4$ in THF (15 mmol, excess), and the yellow mixture was stirred at 23 °C for 5 h. EtOAc (50 mL) was introduced, and then water (5 mL) was added dropwise, cautiously. After the gas evolution had subsided, an additional 25 mL of EtOAc and 25

mL of water were added and the mixture transferred to a separatory funnel. The aqueous layer was separated, and the organic phase was washed twice more with 50 mL of water and then once with 10 mL of saturated aqueous NaCl before it was dried (Na_2SO_4). The solution was filtered and evaporated, and the residue was dissolved in a small amount of CH_2Cl_2 and filtered through a small pad of SiO_2, using CH_2Cl_2 as eluent. Rotary evaporation gave 100 mg of pure **3**. ^{1}H NMR ($CDCl_3$) δ 8.04 (s, 1, H4), 8.00 (d, J = 8.5 Hz, 1, ArH), 7.92 (d, J = 8.3 Hz, 1, ArH), 7.32 (pseudo-t, 1, ArH), 7.10 (d of d, 1, ArH), 7.06 (m, 3, ArH), 5.60 (bs, 1, OH), 5.31 (s, 2, CH_2). Anal. Calcd for $C_{14}H_{11}BN_2O_2$: C, 67.24; H, 4.43; N, 11.20. Found: C, 66.24; H, 4.39; N, 10.76.

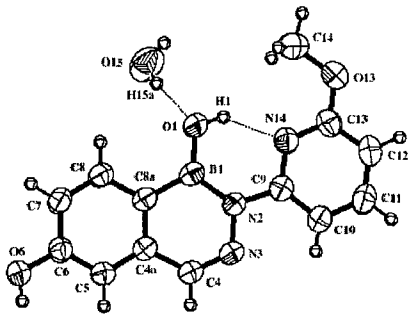
1,2-Dihydro-1-hydroxy-6-methoxy-2-(6-pyridinyl)-2,3,1-benzodiazaborine (4). A solution of **1** (450 mg, 2.5 mmol) in 2.5 mL of absolute EtOH was treated with a solution of 2-hydrazinopyridine (300 mg, 2.75 mmol) in 2.5 mL of EtOH, and the reaction mixture was allowed to stand at 23 °C overnight. The crystals that had deposited were collected by suction filtration and washed with a small amount of EtOH. Drying under vacuum gave 583 mg (92%) of 6-O-methylated **4** as yellow crystals: mp 256-258.5 °C (EtOH). ^{1}H NMR [$(CD_3)_2SO$] δ 7.92 (s, 1, H4), 7.86 (d, 1, ArH), 7.71 (pseudo-t, 1, ArH), 7.43 (d, J = 8.2 Hz, 1, ArH), 7.20 (s, 1, ArH), 7.02 (d of d, 1, ArH), 6.87 (d, 1, ArH), 6.64 (pseudo-t, 1, ArH), 3.85 (s, 3, OCH_3). Low-resolution DCI mass spectrum, *m/e* 489 (61%, anhydro dimer MH^+), 254 (100%, MH^+). Anal. Calcd for $C_{13}H_{12}BN_3O_2$: C, 61.70; H, 4.78; N, 16.60. Found: C, 61.76; H, 4.88; N, 16.57.

Exposure of 6-O-methylated **4** to BBr_3 removed of the phenolic protecting group, giving **4**: mp 223-227 °C (dec.) (EtOH). ^{1}H NMR [$(CD_3)_2SO$] δ 11.0 (s, 1, OH), 9.55 (s, 1, OH), 7.83 (m, 2, ArH), 7.70 (dt, J = 7.2, 1.8 Hz, 1, ArH), 7.32 (d, J = 8.0 Hz, 1, ArH), 6.96 (d, J = 2.0 Hz, 1, ArH), 6.88 (d, J = 1.4 Hz, 1, ArH), 6.86 (s, 1, ArH), 6.64 (dt, J = 6.0, 0.9 Hz, 1, ArH). Low-resolution DCI mass spectrum, *m/e* 240 (100%, MH^+). Anal. Calcd for $C_{12}H_{10}BN_3O_2$: C, 60.30; H, 4.22; N, 17.58. Found: C, 60.94; H, 4.94; N, 16.24.

1,2-Dihydro-1,6-dihydroxy-2-(2-methoxy-6-pyridinyl)-2,3,1-benzodiazaborine (5). A mixture of 2-chloro-6-methoxypyridine (2.87 g, 20 mmol) and anhydrous hydrazine (11 mL, excess) was heated under argon on a steam bath overnight. The mixture was allowed to cool to 23 °C, and then it was extracted with Et_2O (10 × 10 mL). The combined ether extracts were rotary evaporated to afford 1.58 g (57%) of 2-hydrazino-6-methoxypyridine as a yellow liquid that was ~95% pure by NMR: ^{1}H NMR ($CDCl_3$) δ 7.38 (pseudo-t, 1), 6.20 (d, J = 8.0 Hz, 1), 6.09 (d, J = 8.0 Hz, 1), 5.71 (bs, 1, exchanges upon addition of D_2O, NH), 3.84 (s, 3, OCH_3), 3.1 (bs, 2, exchanges upon addition of D_2O, NH_2). A solution of this hydrazine (1.58 g, 11 mmol) in 10 mL of absolute EtOH was combined with a solution of **1** (1.8 g, 10 mmol) in 20 mL of absolute EtOH, and the reaction mixture produced a precipitate within several minutes. The mixture was allowed to stand at 23 °C overnight. The solid was collected by suction filtration, washed with a small amount of EtOH, and dried under vacuum, giving 1.65 g of 6-O-methylated **5**. An additional 510 mg of product was obtained from the filtrate upon concentrating it to a small volume. Combined yield: 2.15 g (76%): mp 161-162 °C (EtOH). ^{1}H NMR [$(CD_3)_2SO$] δ 10.45 (s, 1, exchanges upon addition of D_2O, OH), 8.25 (s, 1, H4), 8.04 (d, J = 8.2 Hz, 1), 7.88 (pseudo-t, 1), 7.54 (d, J = 8.2 Hz, 1), 7.36 (s, 1), 7.26 (d, J = 8.3 Hz, 1), 6.68 (d, J = 7.9 Hz, 1), 6.64 (pseudo-t, 1), 3.92 (s, 3, OCH_3), 3.90 (s, 3, OCH_3). Low-resolution DCI mass spectrum, *m/e* 284 (100%, MH^+). Anal. Calcd for $C_{14}H_{14}BN_3O_3$: C, 59.40; H, 4.98; N, 14.84. Found: C, 58.95; H, 5.03; N, 14.89.

Exposure of 6-O-methylated **5** to BBr_3 in CH_2Cl_2 solution at 23 °C overnight effected a regioselective demethylation at the benzene ring, giving **5**: mp 198-199 °C (EtOH). ^{1}H NMR [$(CD_3)_2SO$] δ 10.41 (s, exchanges with D_2O, 1, OH), 10.26 (s, exchanges with D_2O, 1, OH), 8.17 (s, 1, H4), 7.98 (d, J = 8.9 Hz, 1, ArH), 7.88 (pseudo-t, J_{app} = 8.0 Hz, 1, ArH), 7.53 (d, J = 7.7 Hz, 1, ArH), 7.12 (m, 3, ArH), 6.67 (d, 1, ArH), 3.92 (s, 3, OCH_3). Low-resolution DCI mass spectrum, *m/e* 270 (100%, MH^+). Anal. Calcd for $C_{13}H_{12}BN_3O_3 \cdot H_2O$: C, 54.39; H, 4.92; N, 14.64. Found: C, 54.99; H, 4.90; N, 14.89.

An X-ray crystal structure determination of **5** was made (see ORTEP diagram below). This was published prior to the receipt of the Concept Award.(2) The remarkable estrogen-like shape makes **5** a close mimic of β-estradiol 17-*O*-methyl ether.

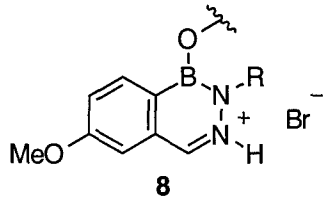


Exposure of 6-O-methylated **5** to molten C_5H_5N•HCl for 10 min demethylated it only at the pyridine ring, as verified by ^{1}H NMR: [$(CD_3)_2SO$] δ 8.24 (s, 1, H4), 8.08 (d, 1, ArH), 7.78 (t, 1, ArH), 7.37 (m, 2, ArH), 7.26 (dd, 1, ArH), 7.10 (s, 1, ArH), 6.47 (d, 1, ArH), 3.90 (s, 3, OCH_3).

1,2-Dihydro-1-hydroxy-2-(2-imidazolidinyl)-6-methoxy-2,3,1-benzodiazaborine (6). A solution of **1** (450 mg, 2.5 mmol) and 2-hydrazinoimidazoline hydrobromide (500 mg, 2.75 mmol) in 7.5 mL of absolute EtOH was treated with a solution of 100 mg of NaOH in 1.5 mL of water. The suspension was stirred at 23 °C for 24 h, and then the mixture was suction filtered and the solid washed with a small amount of EtOH. Drying under vacuum gave 470 mg (77%) of 6-O-methylated **6** as a white microcrystalline anhydro dimer: mp 281-282 °C (EtOH). ^{1}H NMR [$(CD_3)_2SO$] δ 7.57 (s, 1, exchanges upon addition of D_2O, NH), 7.49 (s, 1, H4), 7.38 (d, 1, ArH), 6.96 (s, 1, ArH), 6.94 (d, 1, ArH), 3.78 (s, 3, OCH_3), 3.29 (m, 2, CH_2), 2.97 and 2.73 (each pseudo-q, each 1, CH_2). Low-resolution DCI mass spectrum, *m/e* 471 (100%, anhydro dimer MH^+). Anal. Calcd for $C_{11}H_{13}BN_4O_2$: C, 54.13; H, 5.37; N, 22.96. Found: C, 54.10; H, 5.37; N, 22.68. Exposure of 6-O-methylated **6** to BBr_3 in CH_2Cl_2 solution at 23 °C overnight effected O-demethylation in low yield, giving **6**: ^{1}H NMR [$(CD_3)_2SO$] δ . Anal. Calcd for $C_{13}H_{12}BN_3O_3$•H_2O•HBr: C, 36.51; H, 4.29; N, 17.03. Found: C, 36.70; H, 4.37; N, 16.84.

An Improved A-Ring Precursor

While the commercial 2-formyl-4-methoxybenzeneboronic acid (**1**) was a convenient starting material in the first synthetic approach described above, the removal of its methyl protecting group from the A-ring hydroxyl proved problematic, likely because of the formation of the HBr salt **8** or the boron tribromide complex **9** during the BBr_3-mediated O-demethylation step. Precipitation of **8** or **9** from the CH_2Cl_2 solution was observed to occur during the reactions, effectively removing a large amount of the boron heterocycle from the demethylating media. It was obvious that a new route to our compounds was needed if we were to access them in an efficient manner.



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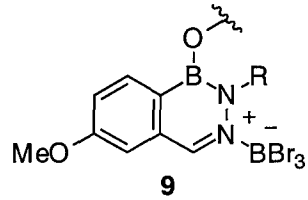

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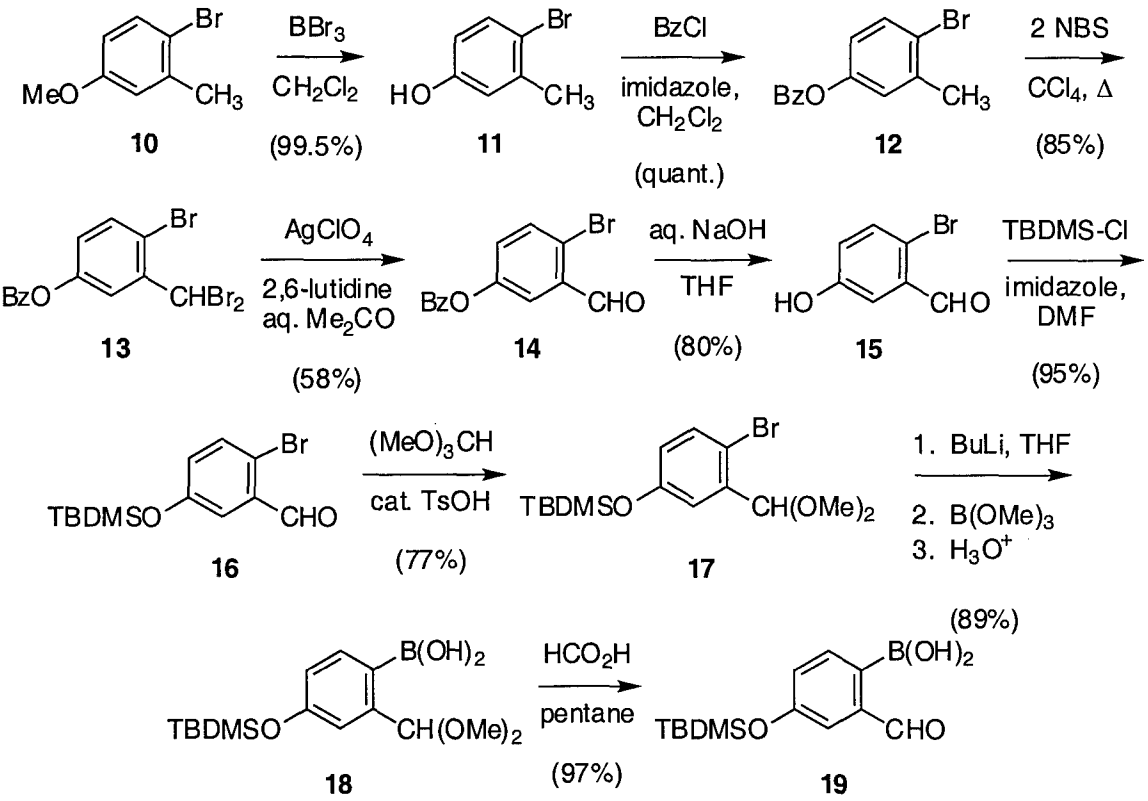
We devised and synthesized an improved A-ring precursor that could be used to prepare our targets along a route that had a mild and efficient ultimate O-deprotection step. The TBDMS group has been used to protect thiophenol-boronic acids,(3) but to our knowledge it has not been applied to the phenol-boronic acids until now. The synthesis of our desired A-ring precursor, 2-formyl-4-

(*tert*-butyldimethylsilyloxy)benzeneboronic acid (**19**), in 9 steps from commercial 2-bromo-5-methoxytoluene (**10**) is shown below, followed by the details of the synthesis.



4-Bromo-3-methylphenol (11). A 25.13 g (125 mmol) sample of 4-bromo-3-methyl-anisole (**10**, Aldrich Chemical Co.) in 250 mL of dry CH_2Cl_2 at -78 °C under argon was treated dropwise slowly with neat BBr_3 (11.8 mL, 31.3 g, 125 mmol), and the reaction mixture was kept at –78 °C for several hours before it was allowed to warm slowly to 23 °C overnight. It was kept at 23 °C for 2 days and then added slowly to a rapidly stirring mixture of 60 mL of concd. NH_4OH, 500 mL water, and 100 mL CH_2Cl_2. The biphasic mixture was stirred at 23 °C overnight, and then 12 M HCl was added until the aqueous phase reached neutral pH. The layers were separated and the aqueous phase extracted twice with 100 mL of CH_2Cl_2. All organic layers were combined and dried (Na_2SO_4). Evaporation gave, after pumping dry under vacuum, 23.27 g (99.5%) of **11** as a white powder. ^{1}H NMR ($CDCl_3$) δ 7.35 (d, J = 8.6 Hz, 1, ArH), 6.72 (d, J = 2.9 Hz, 1, ArH), 6.54 (d of d, J = 8.6, 2.9 Hz, 1, ArH), 4.78 (s, exchanges with D_2O, 1, OH), 2.33 (s, 3, $ArCH_3$). Anal. Calcd for C_7H_7BrO: C, 44.95; H, 3.77. Found: C, 44.99; H, 3.68.

4-Bromo-3-methylphenyl Benzoate (12). A solution of 23 g (0.123 mol) of **11** and 8.85 g (0.13 mol) of imidazole in 300 mL of CH_2Cl_2 was treated dropwise with BzCl (17.6 g, 14.5 mL, 125 mmol), and the mixture was stirred at 23 °C overnight. Extraction with water (2 × 100 mL, followed by drying (Na_2SO_4) and rotary evaporation, gave 36.6 g (100%) of **12** as a white powder: ^{1}H NMR ($CDCl_3$) δ 8.17 (d, J = 7.1 Hz, 2, ArH), 7.65-7.45 (m, 4, ArH), 7.10 (s, 1, ArH), 6.92 (d of d, 1, ArH), 2.40 (s, 3, $ArCH_3$). Anal. Calcd for $C_{14}H_{11}BrO_2$: C, 57.76; H, 3.81. Found: C, 57.69; H, 3.93.

4-Bromo-3-(dibromomethyl)phenyl Benzoate (13). A solution of 36 g (0.123 mol) of **12** in 550 mL of CCl_4 was heated at reflux under argon while 60 mL of CCl_4 was removed in three 20 mL portions via a Dean-Stark trap. Then, the heating was stopped, the Dean-Stark trap and condenser were removed, and 44.5 g (0.25 mol) of *N*-bromosuccinimide was added as a slurry in

60 mL of dry CCl_4 through a powder funnel to the hot stirring solution. The reflux condenser was reattached, and the mixture was heated at reflux while illuminated with a 300-W tungsten lamp. After 5 h, a small aliquot was removed for ^1H NMR ($CDCl_3$). Examination of the benzylic hydrogen resonances revealed a small amount of **12** (δ 2.4), a large amount of mono-bromo product (δ 4.6), and a small amount of **13** (δ 7.05). Therefore, heating with illumination was continued overnight. After 20 h at reflux, another aliquot was removed for ^1H NMR. This time, the mixture was mostly **13** with no **12** and only a small amount of mono-bromo product. The mixture was extracted with water (3 × 200 mL, emulsion) and then brine, then dried overnight (Na_2SO_4). Filtration through Celite gave a pale orange solution that was concentrated on the rotary evaporator. It was passed through a short pad of SiO_2 and eluted with CH_2Cl_2 until no more product emerged. Rotary evaporation gave 52.2 g of product as a yellow liquid. ^1H NMR showed 85:15 di-/mono-bromo products, for an 85% yield of **13**.

4-Bromo-3-formylphenyl Benzoate (14). The α-dibromomethyl compound **13** from the synthesis described above was hydrolyzed according to a modification of a literature procedure.(4) A solution of this crude dibromide (assumed to be 0.1 mol) in 1.6 L of acetone was treated with 80 mL of water, then 42 mL (0.363 mol) of 2,6-lutidine, and then 150 g (0.725 mol) of $AgClO_4$. The yellow suspension was stirred at 23 °C for 0.5 h, and then 92 g (1.06 mol) of LiBr was added and stirring was continued for another 0.5 h. The mixture was filtered through Celite, which was washed with more 20:1 acetone/water, and the filtrate and washing were combined and reduced in volume but not to dryness on the rotary evaporator. The mixture was then treated with EtOAc (800 mL), extracted with water (3 × 100 mL) and dried overnight (Na_2SO_4). Rotary evaporation gave a residue that still contained 2,6-lutidine, so the residue was redissolved in CH_2Cl_2 (400 mL) and was extracted with 5% aqueous HCl (3 × 30 mL), water (2 × 50 mL), and brine (1 × 50 mL), and then dried (Na_2SO_4). Rotary evaporation gave 40 g of a pale yellow solid. TLC (CH_2Cl_2) showed a solvent-front spot followed closely by a 2,4-DNP positive product spot. ^1H NMR shows mostly desired aldehyde. A CH_2Cl_2 solution of this was passed through SiO_2 to remove origin material. Rotary evaporation and vacuum drying overnight gave 34.44 g of a solid that was recrystallized from Et_2O/hexanes on a steam bath (starting with 300 mL Et_2O, gradually adding 200 mL of hexanes, and boiling until the volume was 200 mL). The first crop was 15.07 g (49%) of **14** as pale yellow needles. Anal. Calcd for $C_{14}H_9BrO_3$: C, 55.11; H, 2.97. Found: C, 55.07; H, 3.02. The second crop was 2.7 g of **14** (8.8%, for a total of 58%), as white needles.

2-Bromo-5-hydroxybenzaldehyde (15). A solution of 5.5 g (18 mmol) of **14** in 50 mL of THF was treated with 560 mg of 50% aqueous NaOH (280 mg NaOH, or 7 mmol) and the mixture stirred vigorously at 23 °C overnight. A yellow color soon developed. After 24 h, the saponification had proceeded only partially to completion, by TLC analysis. The mixture was warmed for several hours and then neutralized with HOAc, diluted with CH_2Cl_2, and washed several times with water. Drying (Na_2SO_4) gave, after evaporation, an oil that by TLC (CH_2Cl_2) was predominantly the phenol (R_f 0.20): 2.9 g (14.4 mmol, 80%) of **15**. ^1H NMR ($CDCl_3$) δ 10.30 (s, 1), 7.53 (d, J = 8.7 Hz, 1), 7.41 (d, J = 2.9 Hz, 1), 7.01 (d of d, J = 8.7, 2.9 Hz, 1), 5.43 (s, 1). Anal. Calcd for $C_7H_5BrO_2$: C, 41.82; H, 2.51. Found: C, 42.03; H, 2.57.

2-Bromo-5-(*tert*-butyldimethylsilyloxy)benzaldehyde (16). A solution of 2.9 g (14.4 mmol) of **15** and imidazole (1.02g, 15 mmol) in 15 mL of dry DMF was treated with 2.26 g (15 mmol) of TBDMS-Cl, and the solution was kept at 45 °C overnight. The solution was worked up by extraction (EtOAc, 8 × H_2O), dried (Na_2SO_4), and a trace of the starting phenol removed by filtering a 50% EtOAc/hexanes solution of the compound through a small pad of SiO_2. Yield: 4.3 g (95%) of **16** as a yellow oil. ^1H NMR ($CDCl_3$) δ 10.29 (s, 1), 7.50 (d, J = 8.4 Hz, 1), 7.36 (d, J = 2.7 Hz, 1), 6.96 (d of d, J = 8.4, 2.7 Hz, 1), 0.98 (s, 9), 0.21 (s, 6). Anal. Calcd for $C_{13}H_{19}BrO_2Si$: C, 49.52; H, 6.07. Found: C, 49.77; H, 6.09. A repeat of this procedure using 1.89 g (9.4 mmol) of **15**, 1.42 g (9.4 mmol) of TBDMS-Cl, and 640 mg (9.4 mmol) of imidazole in 10 mL of DMF gave 2.44 g (82%) of **16**. Both samples were pumped dry at 23 °C overnight.

2-Bromo-5-(*tert*-butyldimethylsilyloxy)-α-dimethoxytoluene (17). According to a literature report,(5) the dimethyl acetal group is effective at *ortho*-directing a BuLi halogen metal exchange. A solution of 1.34 g of **16** in 20 mL of $(MeO)_3CH$ was treated with 50 mg of pTsOH and kept at 23 °C overnight. The solution was diluted with 150 mL of CH_2Cl_2 and then stirred over 3 g of K_2CO_3 for 30 min. The mixture was filtered through Celite and evaporated to a yellow oil. ^1H NMR showed the oil to be the desired dimethyl acetal, contaminated with a trace of $(MeO)_3CH$. Since this procedure worked, another 3.45 g (10.94 mmol) sample of bromo-aldehyde was treated similarly, using 25 mL of $(MeO)_3CH$ and 100 mg of pTsOH. The crude products were worked up as described above, combined, and together purified by chromatography on SiO_2 using 33% CH_2Cl_2/hexanes. Rotary evaporation followed by pumping dry overnight gave 4.23 g (77%) of **17** as a pale yellow oil. Anal. Calcd for $C_{15}H_{25}BrO_3Si$: C, 49.86; H, 6.97. Found: C, 51.13; H, 7.13.

2-(Dimethoxymethyl)-4-(*tert*-butyldimethylsilyloxy)benzeneboronic Acid (18). The sample of **17** from the synthesis described above was dried under vacuum at 23 °C for 2 days. Trimethylborate was stirred under argon and over $Na_{(s)}$ at 23 °C overnight. (Evolution of hydrogen and the slow production of a small amount of a white solid indicated that the sample did indeed contain MeOH.) After 24 h, no more gas evolution was noted, and so the reagent was distilled from the sodium under argon immediately prior to use. A solution of 4.06 g (11.2 mmol) of the dimethyl acetal in 20 mL of dry THF under argon was cooled to –78 °C and treated dropwise with 7.0 mL of 1.6 M butyllithium (11.2 mmol). The orange solution was stirred at –78 °C for 20 min, and then 5.0 mL (45 mmol, 4 equiv.) of the freshly distilled trimethylborate was introduced dropwise, and the solution was stirred at –78 °C for at least 5 h, at which point no more dry ice was added to the cooling bath and the mixture was allowed to stir overnight. The translucent pale yellow suspension was then treated with 10 mL of water, stirred at 23 °C for 30 min, and then rotary evaporated to about one-third of the starting volume. The residue was stirred with 125 mL of diethyl ether, and the layers were separated and the organic phase washed once with 15 mL of water and then dried (Na_2SO_4). Rotary evaporation gave 4.8 g (> 100%) of **18** as a pale yellow semisolid that appeared to be a single, more polar spot on TLC (R_f 0.19, 5% MeOH/CH_2Cl_2), but when a CH_2Cl_2 solution was dried again, giving 4.2 g of a yellow oil, the product then showed more nonpolar character. ^1H NMR looked promising for the boroxine form of **18** but was complex due to restricted rotations: ^1H NMR ($CDCl_3$) δ 7.81 (d, J = 8.4 Hz, 1), 7.75 (d, J = 8.1 Hz, 1), 7.01 (d, J = 2.7 Hz, 1), 6.98 (d, J = 2.4 Hz, 1), 6.83 (d of d, J = 8.1, 2.4 Hz, 1), 6.80 (d of d, J = 8.4, 2.7 Hz, 1), 5.38 (s, 1), 5.37 (s, 1), 3.35 (s, 6), 3.34 (s, 6), 0.99 (s, 9), 0.98 (s, 9), 0.20 (s, 6), 0.19 (s, 6).

2-Formyl-4-(*tert*-butyldimethylsilyloxy)benzeneboronic Acid (19)—Small Scale Synthesis. Following a literature procedure,(6) a solution of 150 mg (0.46 mmol) of **18** in 12 mL of petroleum ether was stirred over 2 mL of 96% HCO_2H for 45 min, and then the layers were separated and the formic acid phase was washed with petroleum ether (5 × 15 mL). The combined organic phases were washed once with water, dried (Na_2SO_4), and rotary evaporated to give 125 mg (97%) of **19** as a pale yellow solid, which was kept under pentane under argon in the freezer: ^1H NMR ($CDCl_3$) δ 9.83 (s, 1), 8.20 (d, J = 8.4 Hz, 1), 7.93 (s, exchanges with D_2O, 2), 7.35 (d, J = 2.7 Hz, 1), 7.14 (d of d, J = 8.4, 2.7 Hz, 1), 1.01 (s, 9), 0.26 (s, 6). ^{13}C NMR ($CDCl_3$) δ 198.3, 158.4, 141.6, 140.6, 130.1, 125.3, 25.5, -4.4. As expected, the boron-bearing carbon was not observed. Anal. Calcd for $C_{13}H_{21}BO_4Si$: C, 55.72; H, 7.55. Found: C, 55.74; H, 7.49. Elemental microanalysis indicated that **19** was definitely the free boronic acid, and not the dimeric boronic anhydride or the trimeric boroxine. A large scale deacetalation was performed next.

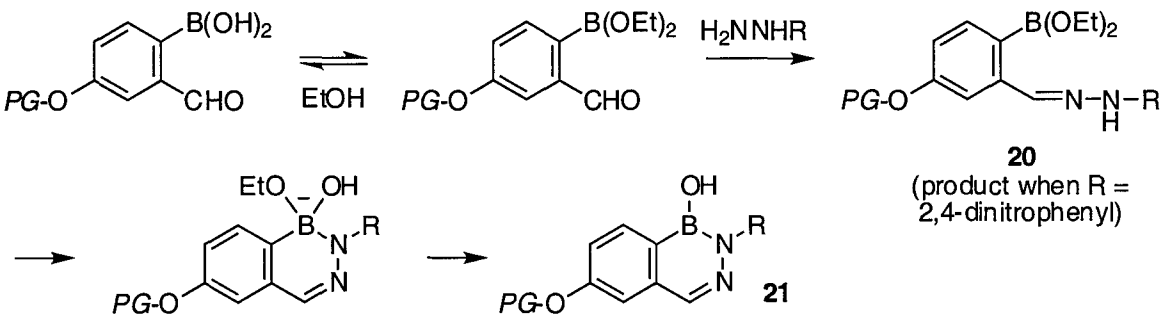
Large-Scale Synthesis of 19. A solution of 3.25 g (10.54 mmol monomer) of **18** in 250 mL of petroleum ether was stirred over 40 mL of 96% HCO_2H for 45 min, and then the layers were separated and the formic acid phase was washed with petroleum ether (6 × 200 mL). The combined organic phases were washed with water (3 × 150 mL), dried (Na_2SO_4), and rotary evaporated to afford 3.03 g (> 100%) of product as a pale yellow solid. Attempted recrystallization from hexanes (25 mL) failed. Rotary evaporation gave a thick yellow oil. Mini-scale recrystallization attempts

using Me_2CO, EtOAc, cyclohexane, and 95% EtOH failed. When a solution of **19** in 95% EtOH plus 1 drop of Et_3N was heated on a steam bath, TLC showed no reaction. Elution of the sample through SiO_2 using CH_2Cl_2 as eluent gave 0.5 g of the impurity 3-(TBDMSO)benzaldehyde. Elution with 5% MeOH/CH_2Cl_2 then gave 2.0 g of TLC-pure **19** as a dark red, thick oil/gum. The ^1H NMR spectrum in $CDCl_3$ solution showed **19** to exist as a mixture of monomer and dimer (or trimer), but the spectrum in CD_3OD solution clearly showed it to be the methyl hemiacetal. The sample of **19** was placed under vacuum at 23 °C for several hours and then dissolved in 125 mL of hexanes and placed in the freezer over 3 days to crystallize, but this failed. The sample was then evaporated to a dark gum, dissolved in 125 mL of pentane, washed once with water in a separatory funnel, and dried (Na_2SO_4). Shown to be pure by TLC (5% MeOH/CH_2Cl_2), this sample was rotary evaporated and immediately placed on pump at 23 °C, producing a solid along with a small amount of dark oil/gum. This material was covered with argon and treated with a small amount of pentane (10 mL), swirled, and placed in the freezer. After decanting, the solid was washed with a small amount of cold pentane and then air-dried on a large filter paper circle to give pure **19** as an off-white powder. The yield was slightly lower than that of the small scale deacetalation.

As described later below, we found the boronic acid **19** to be an excellent A-ring precursor to our boron heterocycle estrogen mimics. This boronic acid has recently attracted the interest of Frontier Scientific, Inc. (Logan, UT), one of the premier suppliers of boronic acid fine chemicals. Dr. Gary Allred, the CEO of Frontier Scientific, now intends to make **19** commercially available by setting it up as a new catalog item (personal communication). Once he does, a steady supply of **19** for the synthesis of boron heterocycle estrogen mimics will be assured.

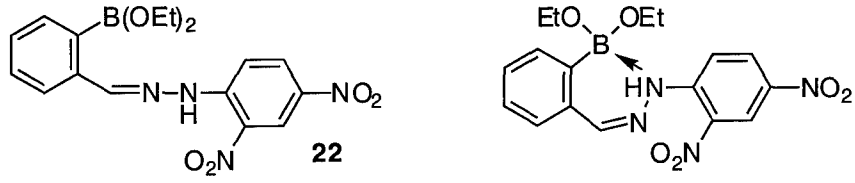
Scope of the Hydrazone Formation

The condensation of aromatic and heterocyclic hydrazines with 2-formylbenzeneboronic acids to form 2,3,1-benzodiazaborines, shown schematically below, is typically conducted in ethanol solution. During the course of this investigation, we encountered a phenomenon that appeared to prevent the reaction course from proceeding to completion when electron deficient hydrazine D-ring precursors were employed. Since this phenomenon could severely limit the variety of 2,3,1-benzodiazaborine estrogen mimics accessible, we decided to investigate its nature. With 2,4-dinitrophenylhydrazines in particular, the reaction stopped at the diethyl borate ester **20** rather than the boron heterocycle **21**. Surprisingly, we found that the 2,4-dinitrophenylhydrazone diethyl borate esters (**20**) were resistant to aqueous hydrolysis.



The arylboronic acids popularly used in Suzuki cross-coupling reactions are known to be stable to hydrolysis, but their borate esters are not. Considering the mechanisms that operate in phenylborate ester hydrolyses,(7) it is reasonable to anticipate that the interaction of an arylborate ester with a nucleophilic *ortho*-substituent might retard the rate of hydrolysis, but it is highly unusual to find that this type of interaction leads to stability in aqueous solution. This phenomenon was first observed by Snyder's group in 1964.(8) To explain the exceptional hydrolytic stability found for the diethyl borate ester of 2-formylbenzeneboronic acid 2,4-dintrophenylhydrazone (**22**), Tschampel and Snyder(8) proposed an intramolecular chelation of the NH group of a (Z)

hydrazone rotational isomer to the electron deficient boron to give the 6-6 heterobicyclic structure shown below at the right in which the boronate ester is structurally protected from facile hydrolysis.

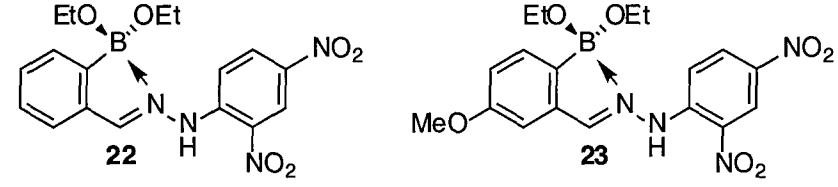


To investigate the structures of these stable borate esters and find a way to process them further to desired boron heterocycle estrogen mimics, we prepared both Snyder's compound **22** (the diethyl borate ester of the 2,4-dinitrophenylhydrazone of 2-formylbenzeneboronic acid) and its 4-methoxy derivative (**23**) under conditions of formation that typically gave rise to 2,3,1-benzodiazaborines in our hands.

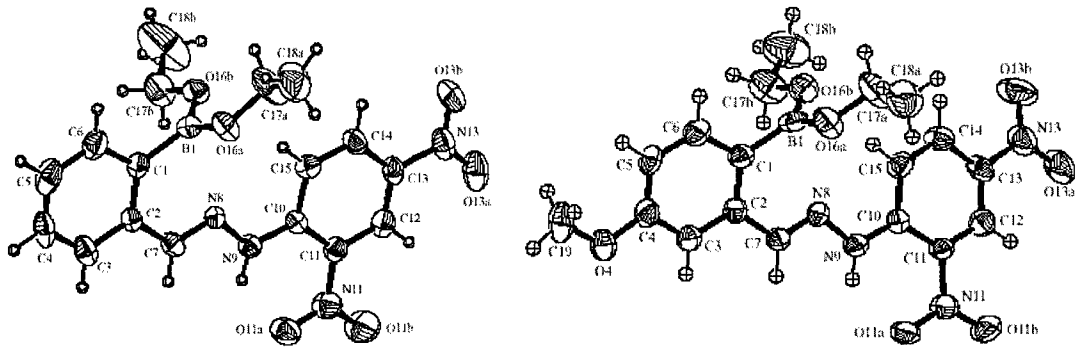
2-Formylbenzeneboronic Acid, 2,4-Dinitrophenylhydrazone Diethyl Ester (22). We prepared **22**, originally reported by Snyder's group, in 93% yield by heating 2,4-dinitrophenylhydrazine (191 mg, 0.97 mmol) in 20 mL of EtOH on a steam bath and then adding solid 2-formylbenzeneboronic acid (145 mg, 0.96 mmol, from Frontier Scientific, Inc.). Crystal formation began within minutes, and the mixture was allowed to cool to 23 °C and kept overnight. The orange crystals were collected by suction filtration, washed with a small amount of absolute ethanol, and air-dried to give 345 mg (93%) of **22** as orange needles: mp 223 °C (dec.) (EtOH); lit. 249-250 °C (Tschampel & Snyder, 1964). ^{1}H NMR ($CDCl_3$) δ 9.14 (bs, 1), 8.39 (d, 1), 8.17 (s, 1), 8.15 (d, 2), 7.46 (m, 4), 3.85 (q, J = 7.1 Hz, 4), 1.18 (t, J = 7.1 Hz, 6). Anal. Calcd for $C_{17}H_{19}BN_4O_6$: C, 52.87; H, 4.96; N, 14.51. Found: C, 52.03; H, 4.62; N, 14.93. X-ray quality crystals were grown via slow evaporation of an absolute ethanol solution under argon.

2-Formyl-4-methoxybenzeneboronic Acid, 2,4-Dinitrophenylhydrazone Diethyl Ester (23). This diethyl borate ester was obtained in 91% yield from a synthesis similar to that described above: mp 175-177 °C with re-solidification, and then mp 240 °C (dec.) (EtOH). The X-ray quality crystals were grown via slow evaporation of an absolute ethanol solution under argon. ^{1}H NMR ($CDCl_3$) δ 11.3 (bs, 1), 9.15 (bs, 1), 8.39 (d, J = 9.3 Hz, 1), 8.14 (d, J = 9.3 Hz, 1), 8.13 (s, 1), 7.37 (d, J = 8.0 Hz, 1), 7.06 (s, 1), 7.00 (d, J = 8.1 Hz, 1), 3.86 (s, 3), 3.85 (q, J = 7.1 Hz, 4), 1.18 (t, J = 7.1 Hz, 6). Anal. Calcd for $C_{18}H_{21}BN_4O_7$: C, 51.95; H, 4.94; N, 13.46. Found: C, 51.90; H, 4.94; N, 13.58.

The crystal structure determinations of **22** and **23** were conducted at no cost to this project in a collaborative effort with Paul D. Robinson, an X-ray crystallographer in the Department of Geology, Southern Illinois University. Although the diethyl borate group is orthogonal to the plane of the benzene ring, as Tschampel and Snyder had anticipated, we found **22** and **23** to be (*E*) rather than (*Z*) hydrazone rotational isomers, and the intramolecular chelation event affords 6-5 rather than 6-6 heterobicyclic structures:

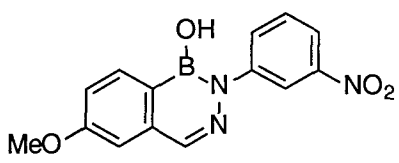


The ORTEP diagrams of the X-ray crystal structures of **22** and **23** are shown below. They both feature a diethyl borate group orthogonal to the benzene ring, and a hydrazone imine N with its lone electron pair oriented toward the B atom but too far away from it to permit more than weak interaction.

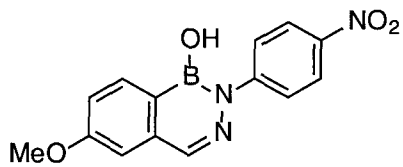


The hydrazone fragment in **23** is oriented such that its N8 imine lone electron pair is directed toward the boron atom, but the intramolecular B1-N8 distance [2.750 (7) Å] is indicative of only a weak N→B coordination. Nevertheless, upon careful inspection of its position with respect to the plane defined by C1, O16a, and O16b, the boron atom was found to be displaced toward N8 by 0.049 (9) Å. This provides additional evidence for the N→B interaction, and we conclude that this interaction must be responsible for the exceptional hydrolytic stability. The orthogonal relationship of the diethyl borate and benzene ring fragments is reminiscent of that found in 2-nitro-4-carboxyphenylboronic acid,(9) but it is surprising to find this orientation adopted by **23** because the boron atom's empty p-orbital is orthogonal to, and thus non-interacting with, the symmetry-adapted lone pair orbital on O4. A lack of conjugative interaction between O4 and the boron explains why the C1-B1 length in **23** (1.569 (7) Å) is essentially identical to that in **22** (1.580 (8) Å).

^{1}H NMR revealed that the solid state structures of **22** and **23** are preserved in solution as well. By studying other similar condensation products, it discovered that the electron deficient nature of the hydrazine component in the reaction plays a key role in determining the structural form of the hydrazone product generated. For example, both 4-nitro- and 3-nitrophenylhydrazone formation in 2-formyl-4-methoxybenzeneboronic acid (**1**) lead directly to benzodiazaborines (**24** and **25**, respectively), but unsubstituted phenylhydrazone formation produces an intramolecularly hydrogen bonded 'open' boronic acid (*E*) hydrazone product (**26**) instead. The condensations of 2-formyl-4-methoxybenzeneboronic acid (**1**) with 3-nitrophenyl-, 4-nitrophenyl-, and phenylhydrazine were conducted on a 1 mmol scale by our usual procedure. The 3- and 4-nitrophenylbenzodiazaborines **24** and **25** were obtained in 88% and 77% yield, respectively, and the 2-phenylbenzodiazaborine (**26**) was obtained in 72% yield.



24



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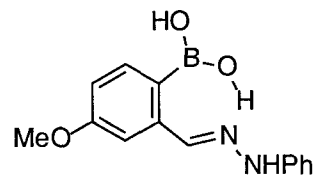


26

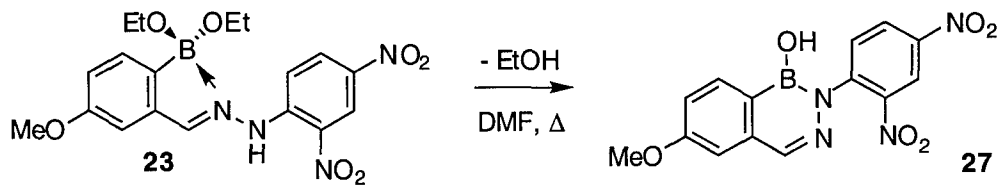
Although compounds **24** through **26** were not obtained as X-ray quality crystals, their structures were definitively established by their ^{1}H NMR spectra and elemental microanalysis. The mononitrated phenylhydrazones **24** and **25** were clearly 2,3,1-benzodiazaborines—i.e., they had undergone both diethyl borate hydrolysis and dehydrative ring closure. Curiously, phenylhydrazone **26** had undergone the borate hydrolysis but not the dehydrative ring closure. In the ^{1}H NMR spectrum of **26** in $(CD_3)_2SO$, one of the boronic acid OH resonances is located at δ 11.9 ppm, very far downfield from its normal position (δ 9.5 ppm), indicating that it participates in an intramolecular hydrogen bond with the hydrazone moiety. This type of 7-membered ring hydrogen

bond association has a precedent in the solid state structure of 2-formylbenzeneboronic acid O-methyl oxime.(10) Synthetic details for compounds **24** through **26** are provided next.

1,2-Dihydro-2-(3-nitro- and 4-nitrophenyl)-1-hydroxy-2,3,1-benzodiazaborines (24 and 25, respectively). The 3-nitro- and 4-nitrophenylhydrazones of 2-formylbenzeneboronic acid were prepared on a 1 mmol scale by condensing the aldehyde with 3-nitrophenylhydrazine hydrochloride or 4-nitrophenylhydrazine in 20 mL of EtOH, first on a steam bath, then at 23 °C, and then at 4 °C overnight. In this way, **24** was obtained in 88% yield as an off-white microcrystalline solid, and **25** was obtained in 77% yield as an orange microcrystalline powder. Immediate analysis by ^{1}H NMR using $(CD_3)_2SO$ as solvent revealed that diethyl borate ester hydrolysis was well under way for both compounds, so they were pumped dry at 23 °C overnight. ^{1}H NMR and elemental microanalysis showed both products to be ring-closed benzodiazaborine compounds. **24**: mp 162 °C (partial only); ^{1}H NMR [$(CD_3)_2SO$] δ 9.36 (bs, 1), 8.57 (t, J = 2.1 Hz, 1), 8.23 (s, 1), 8.17 (m, 1), 8.07 (m, 1), 7.70 (t, J = 8.2 Hz, 1), 7.37 (d, J = 2.4 Hz, 1), 7.29 (dd, J = 8.4, 2.4 Hz, 1), 3.91 (s, 3). Anal. Calcd for $C_{14}H_{12}BN_3O_4$: C, 56.60; H, 4.07; N, 14.14. Found: C, 56.06; H, 4.21; N, 13.92. **25**: mp > 275 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.46 (bs, 1), 8.35 (d, J = 8.4 Hz, 1), 8.29 (d, J = 9.3 Hz, 2), 8.25 (s, 1), 8.00 (d, J = 9.4 Hz, 2), 7.38 (d, J = 2.4 Hz, 1), 7.29 (dd, J = 8.4, 2.4 Hz, 1), 3.91 (s, 3). Anal. Calcd for $C_{14}H_{12}BN_3O_4$: C, 56.60; H, 4.07; N, 14.14. Found: C, 56.73; H, 4.15; N, 13.93.

2-Formylbenzeneboronic Acid, Phenylhydrazone (26). This unsubstituted phenylhydrazone was prepared on a 1 mmol scale by combining 2-formylbenzeneboronic acid and phenylhydrazine hydrochloride in 20 mL of warm EtOH , keeping the solution at 23 °C for several hours, and then at 4 °C overnight. When no crystallization occurred, the solution was reduced in volume to 10 mL on a rotary evaporator, at which point a small amount of solid appeared. The flask was then transferred to a steam bath and the volume reduced slightly before the solution was allowed to cool to 23 °C and then kept at 4 °C overnight: This produced small white crystals. The supernatant was decanted and the solid washed with a little Et_2O to give 85 mg of product. The supernatant and wash were combined and diluted with more Et_2O to 50 mL, whereupon more white solid precipitated. This was decanted, washed with Et_2O, and pumped dry to give 108 mg (for a total of 193 mg, 72% yield) of **26** as a white powder: mp 231-232 °C (dec.); ^{1}H NMR [$(CD_3)_2SO$] δ 11.9 (s, 1), 9.78 (s, 1), 9.36 (s, 1), 8.58 (d, J = 7.7 Hz, 1), 7.95 (m, 2), 7.83 (s, 1), 7.75 (m, 3), 7.67 (d, J = 7.7 Hz, 1), 3.94 (s, 3). The NMR data are consistent with an open hydrazone in which one of the $B(OH)_2$ hydroxyl groups is intermolecularly hydrogen bonded to the hydrazone imine N. Microanalytical data were consistent with a hydrate of this open benzeneboronic acid hydrazone: Anal. Calcd for $C_{14}H_{15}BN_2O_3 \cdot H_2O$ C, 58.36; H, 5.95; N, 9.72. Found: C, 58.28; H, 5.09; N, 9.55.

We were able to determine conditions that promoted the loss of ethanol from the stable borate esters. Heating **23** in DMF solution (90 °C, 15 min) liberated the ethanol and gave a precipitate that was characterized as the 2-(2,4-dinitrophenyl)-2,3,1-benzodiazaborine (**27**). Therefore, by adding this procedure as a workup in the protocol for condensing 2-formylbenzeneboronic acids with electron deficient hydrazines, we can bypass the stable diethyl borate esters and push the reaction through to the desired 2,3,1-benzodiazaborines, e.g., **27**.

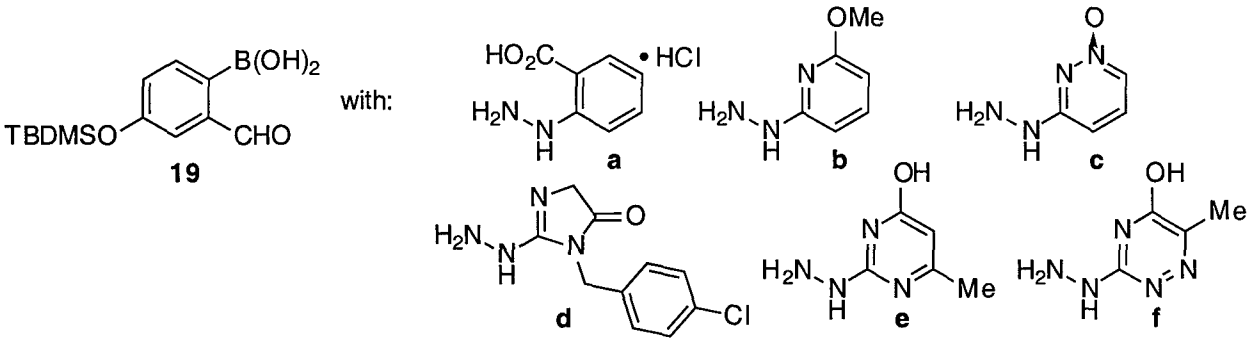


1,2-Dihydro-2-(2,4-dinitrophenyl)-1-hydroxy-4-methoxy-2,3,1-benzodiazaborine (27). Heating a DMF solution of **23** on a steam bath for 15 min liberated ethanol and promoted formation of the diazaborine. The solution was diluted with Et_2O and the precipitated product was

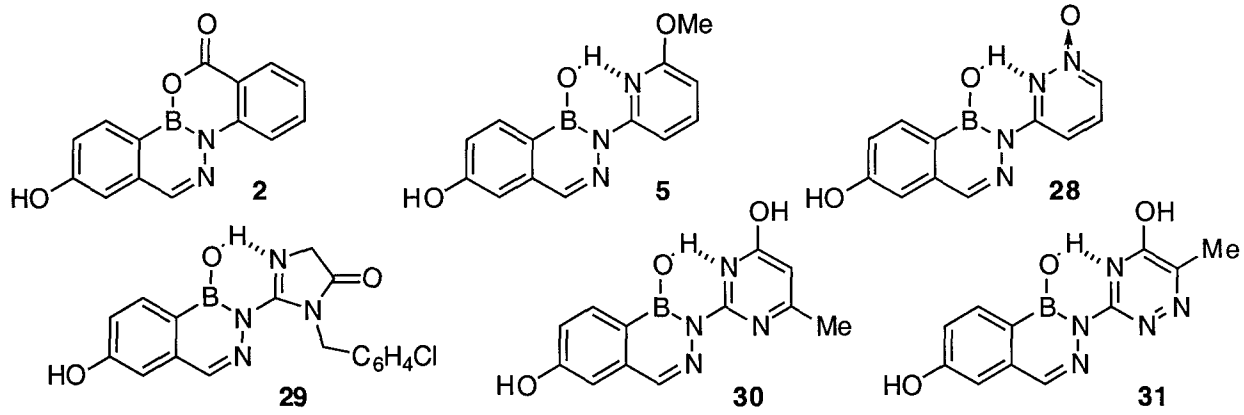
filtered to give **27** as a $CHCl_3$-insoluble orange solid, mp > 260 °C. ^1H NMR [$(CD_3)_2SO$] δ 11.67 (s, 1), 8.92 (s, 1), 8.84 (d, J = 3.0 Hz, 1), 8.34 (d of d, J = 9.9, 2.4 Hz, 1), 8.13 (d, J = 9.6 Hz, 1), 7.51 (d, J = 8.4 Hz, 1), 7.38 (d, J = 2.7 Hz, 1), 7.00 (d of d, J = 8.1, 2.7 Hz, 1), 3.83 (s, 3). Anal. Calcd for $C_{14}H_{11}BN_4O_7 \cdot H_2O$: C, 46.70; H, 3.64; N, 15.56. Found: C, 47.07; H, 3.77; N, 15.60.

Microwave Assisted Synthesis

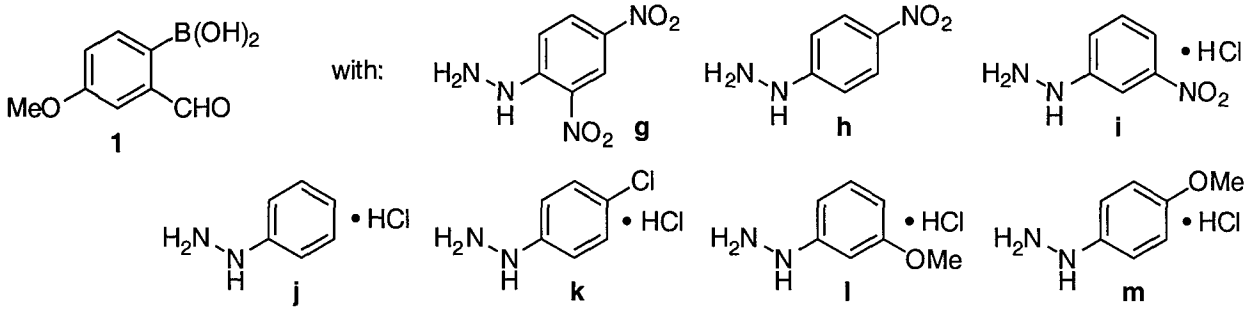
We examined the benefit of using microwaves to accelerate the hydrazone condensation step in the synthesis of our boron heterocycle estrogen mimics. Two sets of microwave-assisted reactions were conducted in 0.5-2.0 mL Smith Process Vials on a Smith Synthesizer from Personal Chemistry (Upsala, Sweden). A demonstration model of this instrument was installed for a short time in the labs here at SRI. The condensation of 2-formyl-4-(tert-butyldimethylsilyloxy)-benzeneboronic acid (**19**) with each compound (**a** through **f**) in the set of 6 hydrazines shown below was conducted on a small scale. The reaction mixtures were heated in 0.5 mL 95% EtOH at 150 °C for 3 min, and then 0.5 mL of MeOH and 4 equiv. of NH_4F were introduced and the mixtures were heated again at 150 °C for 3 min. Upon cooling, the last three reaction mixtures deposited crystals, but these solids were not collected. Instead, workup was performed by diluting the mixtures to 5 mL with CH_2Cl_2, passing the solutions through a short pad of SiO_2 and eluting with 5% MeOH/CH_2Cl_2 until the product had been completely removed (as verified by TLC). Rotary evaporation gave products as solids that were pumped dry overnight at 23 °C.



The A-ring deprotected product (**2**, below) of the condensation of **19** with 2-hydrazino-benzoic acid (**a**) was obtained in 77% yield, and its structure was verified by ^1H NMR. The similar condensation using 2-hydrazino-6-methoxypyridine (**b**) proceeded in 81% yield over two steps, and again the structure of the product (**5**) was verified by ^1H NMR. Isolation and characterization of the remaining condensation products (**28** through **31**) is pending. Importantly, this two-step, one-pot microwave assisted sequence is an improved synthetic approach for directly obtaining desired estrogen mimics in high yield.



In a similar fashion, the condensation of 2-formyl-4-methoxybenzeneboronic acid (**1**) with each compound (**g** through **m**) in the set of 7 phenylhydrazines shown below was conducted on a 0.5 mmol scale on the Smith Synthesizer. The reaction mixtures were heated in 0.5 mL 95% EtOH at 150 °C for 3 min. Products were isolated by diluting the solutions with Et_2O and decanting.



Biological Assays

Human Breast Cancer Cell Antiproliferation Assays

The boron heterocycle estrogen mimics **2** through **6** were re-evaluated for their ability to inhibit the proliferation of MCF-7 human breast cancer cells in vitro. These assays were conducted at no cost to the project, courtesy of Ms. Wan-Ru Chao, a biochemist colleague at SRI. The determinations were made in quadruplicate, and the results are shown in Table 1. The method of Liu et al.(11) was used with some modifications. MCF-7 cells were seeded in 24-well plates at a density of 5000 cells/well in 460 μL of phenol red-free DMEM-F12:Ham medium (1:1) containing 5% charcoal-treated (hormone-depleted) fetal calf serum, and the plates were incubated at 37 °C in humidified 5% CO_2/95% air for 24 h to allow the cells to attach. Each well was treated with test compound with or without 10^{-9} M of β-estradiol (E_2). The cells were grown for 7 days, with media and test solutions replaced every other day. Positive control wells contained 10^{-9} M of E_2 alone, and control wells only vehicle. Tamoxifen (TAM) was used as a reference compound. On Day 8, medium in each well was removed and replaced with fresh medium without test solutions, followed by 60 μL of tetrazolium dye solution (Promega) to determine cell viability. After 4 h of incubation, solubilization/stop reagent was added. During this incubation, viable cells convert the dye to blue formazan, which is detected at 575 nm on an ELISA plate reader. The absorbance reading for samples containing test compound plus E_2 relative to that for positive controls gives the percent inhibition.

Table 1. MCF-7 human breast cancer cell growth inhibition by compounds **2** through **6** and tamoxifen (TAM).*

	Concentration, M.				
Compound	**8×10^{-8}**	**4×10^{-7}**	**2×10^{-6}**	**1×10^{-5}**	**IC_{50}**
2	*na*	35	35	52	6.4 μM
3	*na*	*na*	36	100	1.7 μM
4	*na*	*na*	*na*	100	4.4 μM
5	*na*	22	34	100	1.8 μM
6	23	47	42	57	3.5 μM
TAM	2.0	29	54	95	1.1 μM

*Values are means of 4 determinations (*na* = not active).

Table 1 shows that our boron heterocycle estrogen mimics **2** through **6** are clearly almost as active as tamoxifen at inhibiting the E_2-stimulated growth of cultured MCF-7 human breast cancer cells.

Ishikawa Cell Assays

Ms. Chao also quite recently conducted Ishikawa cell assays on several of our compounds. Human (endometrial) Ishikawa cells are used in this assay of estrogenic and antiestrogenic activity.(12) These cells (a gift from Dr. Erlio Gurpide, Mount Sinai Medical Center) are very sensitive to estrogenic compounds. Estrogenics present in concentrations as low as 10^{-12} M induce alkaline phosphatase activity in them. The results for estrogen mimics **2** through **5** are shown in Table 2.

Table 2. Ishikawa cell assay of compounds **2**, **3**, **4**, and **5** at 10 μM.

Compound	% Estrogenicity	% Anti-estrogenicity
2	0	100
3	0	29
4	8	66
5	22	71

These results show that compounds **4** and **5** are mixed estrogen receptor (ER) agonists/antagonists but compounds **2** and **3** are "pure" ER antagonists. This distinction may be due to the structural characteristics of the D-ring and the nature of the C-D ring connection (i.e., whether it is a covalent attachment or simply a hydrogen bond connection). Both **4** and **5** are pyridine-based D-ring compounds with a hydrogen bonded CD-ring connection, but **2** and **3** are benzene-based D-ring compounds with a covalent CD ring connection. For "pure" ER antagonism, therefore, the boron heterocycle estrogen mimics might have to possess a covalent CD ring connection. Ishikawa cell assays of other mimics should reveal whether this pattern is a general one.

Review Article

Our Mimics in the Context of Other Therapeutics

In a review article published in the *American Journal of Therapeutics*, the PI placed the boron heterocycle estrogen mimics in proper context alongside other boron-containing therapeutics that are currently emerging.(13) A reprint of this review article is appended. Those other therapeutics include the boronic acid proteasome inhibitor known as PS-341, the β-lactamase inhibitors BZBTH2 and (1*R*)-1-benzamido-2-(3-carboxy-2-hydroxyphenyl)ethylboronic acid, the dipeptidyl peptidase IV inhibitor known as ProboroPro, the inositol 1,4,5-trisphosphate receptor modulator known as 2-APB, and the benzodiazaborine-based enoyl acyl carrier protein reductase inhibiting antibacterial agents.

KEY RESEARCH ACCOMPLISHMENTS

- We developed a new synthetic route to the boron heterocycle estrogen mimics that circumvents the previous difficulty of efficiently deprotecting the A-ring

methoxy functionality. The new A-ring precursor we synthesized, 2-formyl-4-(*tert*-butyldimethylsilyloxy)benzeneboronic acid (**19**), will soon be made available commercially by Frontier Scientific, Inc. Thus, the ready synthesis of a wide variety of boron heterocycle estrogen mimics is now assured.

- We examined the scope of the hydrazone condensation reaction that initially appeared problematic with electron-deficient D-ring hydrazine precursors. Examination of the structural features of the unusually stable borate ester products by X-ray crystallography led us to develop a post-condensation procedure for forcing diazaborine ring formation. Thus, even electron deficient D-ring hydrazine precursors can now be used to construct boron heterocycle estrogen mimics.

- We explored the use of microwaves to render the condensation reactions faster and more efficient. Using our improved A-ring precursor, we found that a two-step, one-pot microwave assisted sequence led directly to A-ring deprotected estrogen mimics in very high yield and a very short period of time. This now opens up the possibility of a high throughput generation of a wide variety of compounds for biological testing.

- We re-examined the antiproliferative activity of a set of boron heterocycle estrogen mimics against human MCF-7 (ER^+) breast cancer cells. Our compounds were almost as active as tamoxifen at inhibiting the β-estradiol stimulated growth of these cells, and they likely act as antiestrogens. The results of Ishikawa cell assays to establish their estrogenic/antiestrogenic activity indicate that the mimics with a hydrogen bond connection between the C and D rings are mixed ER agonists/antagonists while those with a covalently fused CD ring juncture are pure ER antagonists.

- We published a review article entitled "Boron Therapeutics on the Horizon" that places our boron heterocycle estrogen mimics in proper context with the handful of other boron-based agents that are being developed as therapeutic agents.

REPORTABLE OUTCOMES

Publications

Boron therapeutics on the horizon. Groziak, M. P. Am. J. Ther. **8**, 321-328 (2001). A reprint of this review article is appended.

Personnel

M. Groziak, Ph.D. (PI, Chemist): 721 hours (4.5 months).

R. Van Lengren (Technician): 9 hours.

R. Stuefloten (Technician): 6 hours.

D. Kreiss (Administrative Assistant): 1.4 hours.

M. Saunders (Technical Editor): 1 hour.

CONCLUSIONS AND FUTURE DIRECTIONS

The synthetic chemistry accomplishments made possible by the Concept Award successfully removed all obstacles to the facile generation of the boron heterocycle estrogen mimics. Accordingly, we are now in a position to test our hypothesis by reliably producing analogs in a systematic manner and examining them for bioactivities predictive of good breast cancer chemotherapeutic properties. The biological assay results obtained during the Concept Award clearly point to covalently fused CD ring compounds as pure antagonists of the estrogen receptor, and we can now pursue potent lead candidates for development as novel boron heterocycle-based breast cancer chemotherapeutic agents. Preliminary bioassays of some of our compounds for antiproliferative activity against cancer cells and for estrogenic activity have yielded promising results, and we will pursue these in future work.

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American Journal of Therapeutics 8, 321–328 (2001)

Boron Therapeutics on the Horizon

Michael P. Groziak

No pharmaceutical based on boron has yet made it to market, but this may soon change. The new millennium has brought with it some unique classes of bioactive boron compounds that are sufficiently mature in development to be considered significant and timely advances in their respective chemotherapeutic areas. Because boron is seldom seen as a constituent of a bioactive agent, this review relates some of the pertinent biologic and physiologic properties of boron and then describes in detail those boron-based agents clearly visible on the therapeutic horizon. Highlighted agents include boronic acids and boron heterocycles as potent proteasome inhibitors, β-lactamase inhibitors, dipeptidyl peptidase inhibitors, inositol trisphosphate receptor modulators, antibacterials, and antiestrogens. As these new agents are welcomed into the therapeutic armamentarium, others will surely follow and the prescribing clinician will already have an awareness and appreciation of the unique benefits that these compounds have to offer.

Keywords: boron, drug development, proteasome, β-lactamase, anticoagulant, antithrombotic, dipeptidyl peptidase IV, inositol trisphosphate receptor, antibacterial, antiestrogen.

INTRODUCTION

Boron, the element immediately to the left of carbon in the periodic table, has some unique and potentially valuable properties to offer to medicine, but unfortunately it has been greatly underutilized in therapeutic agent development to date. There are two principal reasons for this. The first is that very few boron-containing natural products are available to serve as an intellectual spark for medicinal chemists in their drug-design efforts, and to make matters worse, these turn out to be rather poor models. The ionophoric macrodiolide antibiotics boromycin,[1,2] aplasmomycin,[3–5] and tartrolon B[6–8] are all carbon/oxygen-based macrocycles that tightly yet reversibly complex boric acid in its borate conjugate base form through a network of four dissociable B-O bonds. These tetrahedral borate anion complexes are such potent potassium ion carriers that they are highly toxic to both bacteria and mammalian cells. They are the only boron-containing natural products known. No boronic acid natural product has ever been found, and it may be that nature simply lacks the biosynthetic enzyme machinery needed to form a carbon-boron bond. This, in turn, suggests that nature might also lack the metabolic enzymes needed to break them. If this turns out to be true, it would be highly significant to the development of boronic acid-based therapeutic agents.

The second reason for the underutilization of boron in drug development is that very few organic chemists have explored the construction of stable boron-containing molecular platforms on which new bioactive agents could be built.[9] The deliberate engineering of boron in a hydrolysis-resistant and charge-useful manner into such a platform requires considerable thought and planning in molecular design. For example, whereas anionic tetrahedral borates like those in the macrodiolide natural products are of very limited utility as molecular design fragments because of their charge, the carbon-substituted forms of boric acid $B(OH)_3$ are considerably more useful—but primarily just in monosubstituted form. This form is typified by the very weakly acidic boronic acids $RB(OH)_2$, which are water stable and neutral compounds at physiologic pH and thus are quite well suited for pharmaceutical agent design. Disubstitution, conversely, gives the borinic acids R_2BOH, which are

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acidic compounds existing largely as tetrahedral anionic borinates at physiologic pH. Trisubstitution gives the triorganoboranes R_3B, which are extremely acidic and for all practical purposes useless in drug design. Thus, it is not at all surprising to find that most of the boron-based therapeutics currently on the horizon are either boronic acids themselves or boron heterocycles that are simply internally complexed versions of boronic acids.

BIOLOGICAL ASPECTS OF BORON

Boronic acids are fairly common and easily prepared synthetic organic compounds. Many are commercially available, and none to date has been found to be unusually toxic. They are stable under physiologic conditions but can be induced under laboratory conditions to undergo chemical deboronation via either hydrolysis or oxidation. The former generates a hydrocarbon and the latter an alcohol from the organic fragment, but both produce boric acid. It follows, then, that no matter whether a boronic acid–based therapeutic agent is actively metabolized or simply undergoes chemical degradation in vivo, the production of boric acid is not to be unanticipated. It is therefore important to be aware of some of its biologic and physiologic properties.

Boron, present solely as boric acid or its borate salts in nature, is a micronutrient soil component essential for growth in vascular plants,[10,11] which take it up by the roots.[12] It is required by diatoms, but not fungi or bacteria, and it has been shown to stimulate growth in yeast.[13] A specific biochemical function for boron in mammals has yet to be determined, but dietary supplementation with it is known to increase significantly plasma steroid hormones,[14] alter plasma lipid metabolites, and improve bone strength,[15] thus having implications in clinical conditions like arthritis.[16] Boron nutriture may be important for brain and psychological function,[17] and indeed it may be important throughout the life cycle.[18] A recent study indicates that boron metabolism in humans may be subject to genetic regulation.[19] Evidence for its role as an essential nutrient for humans steadily accumulated during the past decade,[20–22] and it now seems likely that dietary boron merits some form of guidance under a recommended dietary allowance.[23–25] Earlier this year, the US Food and Nutrition Board set a Tolerable Upper Intake Level (UL) for boron at 20 mg per day.[25A] In the United States, coffee and milk account for the largest total boron intake because of their high consumption rate, but wine, raisins, and peanuts and other nuts actually have a higher boron content.[26] Interestingly, boron in drinking water was once thought to play a beneficial role in the pathology of dental caries,[27] it has attracted some attention in alternative medicine for treating osteoarthritis,[28] and it has been used by athletes as a nutritional supplement for building muscle mass.[29]

Boric acid (LD_{50} of 3450 mg/kg orally in the mouse and 2660 mg/kg orally in the rat) and its simple borate salts like borax have been studied in great detail and pose no significant toxicity threat,[30] even though there is considerable exposure from consumer products.[31] Serious poisoning of humans with boric acid is unlikely to result from a single acute ingestion,[32] and aggressive treatment is not necessary[33] unless there has been inadequate urine flow and dehydration for several days.[34] Boric acid does not associate strongly with serum proteins but instead rapidly diffuses to the extravascular space without accumulating in tissues and is excreted efficiently via glomerular filtration.[35] Pregnancy has been shown to have little or no effect on the renal clearance of boric acid in both rats[36] and humans.[37] Additional health-related information on boric acid and simple organoboron compounds can be found in the contributions to the International Symposia on the Health Effects of Boron and Its Compounds[38,39] and in specialized reviews of the chemistry and biology of simple inorganic and organic boron compounds.[40,41]

PROTEASOME INHIBITORS

Boronic acids interconvert with ease between a neutral trigonal planar form and an anionic tetrahedral borate one, and this property enables them to potently inhibit biochemical acyl group transfer reactions, especially hydrolyses like those catalyzed by the enzymes chymotrypsin, trypsin, thrombin, and other proteases. Boronic acid–based protease inhibition first emerged three decades ago with the discovery of good inhibitors of chymotrypsin.[42–45] A few of the boronic acids effect simple competitive inhibition from their anionic tetrahedral borate form, but most undergo a reversible yet strong covalent attachment from their neutral trigonal planar form to a protease active site nucleophile—usually a serine residue.

The proteasome is the major nonlysosomal endoprotease in cells, where it generates antigenic peptide ligands for the major histocompatibility complex (MHC) class I proteins. Suppressing the production of antigens for cytotoxic T cells by inhibiting the proteasome, therefore, is an important approach to modifying the cytotoxic immune response. This has obvious applications in the areas of transplant rejection and autoimmune disease.[46] Inhibiting the proteasome has applications in cancer chemotherapy as well. For ex-

ample, the dipeptide boronic acids like PS-341 (Fig. 1A) represent a new class of proteasome inhibitor[47] that are entering early-phase clinical trials as anticancer drugs.[48–50] PS-341, an *N*-pyrazinylcarbonylated derivative of the dipeptide boronic acid Phe-Leu-$B(OH)_2$, is potently cytotoxic against cultured MCF-7 human breast cancer cells with an IC_{90} of 50 nmol/L on 24 hours of exposure to the drug. The combination of proteasome inhibition with conventional chemotherapy may have significant potential in overcoming the high incidence of chemotherapy resistance.[51] In addition, the ability to suppresses β-amyloid peptide (A beta) secretion from cultured cells has been found to correlate extremely well with a peptide boronic acid's potency at inhibiting the proteasome. New boron-based chemotherapeutics for Alzheimer's disease may therefore be forthcoming.[52]

ANTICOAGULANTS

In the anticoagulant field, the clinically used heparins and the vitamin K antagonist warfarin may soon be joined by new boronic acid inhibitors of the trypsin-like protease thrombin and the coagulation factor Xa. Several of these types of compounds are now moving into clinical trials.[53] The inhibition of thrombin by boronic acids has been under development for nearly a decade,[54–59] with the aim to produce a drug to complement hirudin. The development of orally bioavailable boronic acid inhibitors of coagulation factor Xa is more recent,[60] and interest is high because cell signaling by Xa contributes to pro-inflammatory responses in vivo.

β-LACTAMASE INHIBITORS

Bacterial resistance to the β-lactam antibiotics has created a pressing need for new therapeutic agents for the treatment of β-lactam–resistant infections. Many β-lactamases depend on an essential active-site serine residue to effect catalysis, making them ideal targets for potent inhibition by boronic acids. Early on, simple boronic acids and even boric acid itself were found to competitively inhibit a β-lactamase from *Bacillus cereus*.[61] Soon afterward, boronic acids active against β-lactamases from *Pseudomonas aeruginosa* and *Escherichia coli*[62] and from *Citrobacter diversus* and *P. aeruginosa*[63] were uncovered. Using a rational design approach, the simple boronic acids were structurally elaborated to resemble the drugs penicillin G and methicillin, and these new compounds were found to potently inhibit β-lactamases from *B. cereus* and *P. aeruginosa*.[64]

An x-ray crystal structure of 3-aminophenylboronic acid bound by *E. coli* AmpC β-lactamase was obtained, and this was used as a guide to screen a large number of other boronic acids for activity.[65] One of the most potent uncovered in this manner was the benzothiophene boronic acid known as BZBTH2 (Fig. 1B), inhibitory with a K_i of 27 nmol/L against this intrinsically resistant β-lactamase.[66] In a follow-up study, the x-ray crystal structure of BZBTH2, itself bound by AmpC β-lactamase, was solved to assist in the structure-based development of third-generation boronic acid inhibitors. At the same time, BZBTH2 properties that are rather un-β-lactam in nature were discovered, namely, that this agent is unaffected by two common

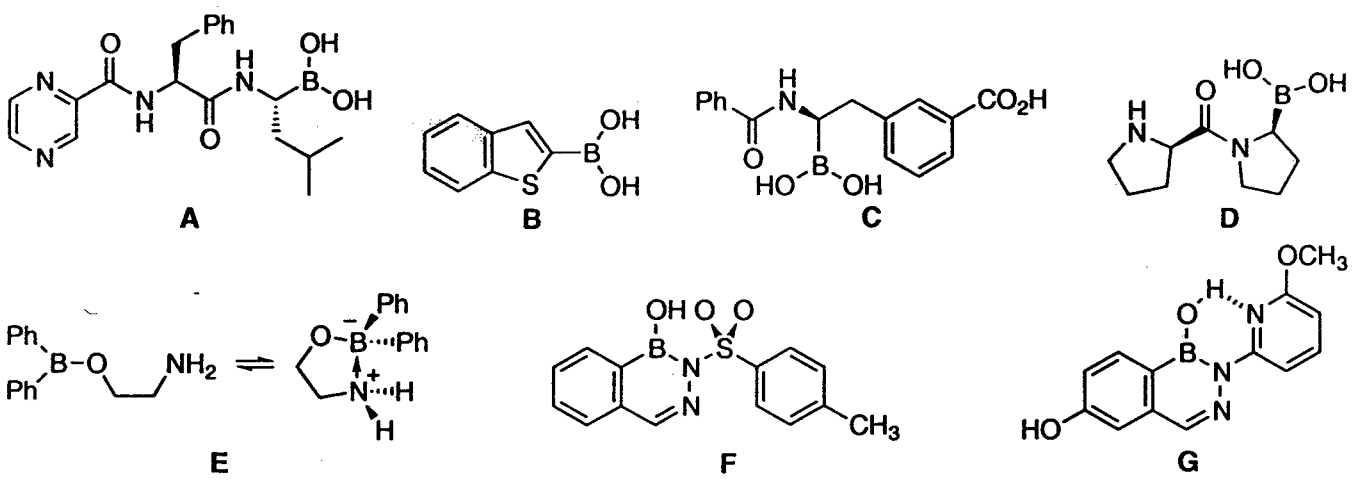

Fig. 1. Chemical structures of the proteasome inhibitor PS-341 **(A)**, the β-lactamase inhibitor BZBTH2 **(B)**, the β-lactamase inhibitor (1*R*)-1-benzamido-2-(3-carboxy-2-hydroxyphenyl)ethylboronic acid **(C)**, the dipeptidyl peptidase IV inhibitor ProboroPro **(D)**, the inositol 1,4,5-trisphosphate receptor modulator 2-APB **(E)**, the enoyl acyl carrier protein reductase inhibitor benzodiazaborine **(F)**, and a boron estrogen mimic **(G)**.

resistance mechanisms, and, furthermore, it does not induce the expression of AmpC.[67]

One of the first rationally designed boronic acid inhibitors of the TEM-1 β-lactamase from *E. coli* was a very potent compound, with a K_i of 110 nmol/L.[68] An x-ray crystal structure of the bound inhibitor was obtained, and even more potent compounds were designed based on that structure.[69] This effort produced (1*R*)-1-phenylacetamido-2-(3-carboxyphenyl)ethyl boronic acid (Fig. 1C), a compound closely resembling the best known TEM-1 β-lactamase substrate, benzylpenicillin (penicillin G).[70] This boronic acid is inhibitory with a K_i of 5.9 nmol/L, an amazing potency supporting the widespread contention that boronic acids are superior to all other kinds of inhibitor species, as was demonstrated for the class C β-lactamase of *Enterobacter cloacae* P99.[71]

DIPEPTIDYL PEPTIDASE IV INHIBITORS

The serine protease dipeptidyl peptidase IV (DP-IV, also known as CD26 and Tp103) is a 103-kd activating molecule expressed on the surface of human T lymphocytes. It is inhibited quite effectively by a boron compound known as ProboroPro, the dipeptide boronic acid (D)Pro-(D)Pro-$B(OH)_2$ (Fig. 1D).[72–76] In contrast to other serine proteases, DP-IV hydrolyzes the normally inhibitory *N*-peptidyl-O-acyl hydroxylamines, thus hinting at a catalytic mechanism for this proline-specific enzyme that is somehow different from the others. Proline-containing peptide boronic acids are usually somewhat unstable, but the acyl pyrrolidides like ProboroPro have a good stability and typically inhibit DP-IV in the micromolar and sometimes even nanomolar range. DP-IV is virtually absent on resting T cells, but after activation, it is strongly expressed and becomes involved in signal transduction during the immune response. Some of the newly developed boronic acid DP-IV inhibitors potently suppress T-cell proliferation.

INS(1,4,5)P3 RECEPTOR MODULATORS

The simple borinic acid 2-aminoethyl diphenyl borinate (2-APB, Fig. 1E) was discovered to be a novel membrane-permeable modulator of the inositol 1,4,5-trisphosphate receptor.[77] 2-APB inhibits the $Ins^{(1,4,5)}P3$-induced release of calcium[78–80] and appears to inhibit the store operated calcium channels (SOCs) in hepatocytes, but curiously by a mechanism that does not involve the $Ins^{(1,4,5)}P3$ receptor.[81] 2-APB is an unusually stable borinic acid compound because of the ethanolamine side chain. This side chain intramolecularly complexes to the acidic boron center and forms a five-membered ring zwitterion (see also Fig. 1E), which is quite stable. It is also nonpolar because, with no net charge, it is electrically neutral. Together with the presence of the two lipophilic phenyl groups, this explains why this molecule so readily penetrates membranes. At present, 2-APB is a valuable pharmacologic tool in sleep research since the $Ins^{(1,4,5)}P3$-induced release of calcium appears to play a role in the resetting of the mammalian circadian clock in the suprachiasmatic nucleus.[82] Clearly, the class of 2-APB-like compounds has the potential of producing valuable therapeutics for the treatment of sleep disorders.

ENOYL ACYL CARRIER PROTEIN REDUCTASE INHIBITORS

For more than two decades, it has been known that benzodiazaborine (Fig. 1F) and other ring-fused sulfonylated diazaborine heterocycles possess antibacterial properties, particularly against gram-negative organisms.[83] These endocyclic boron-containing compounds are simply aromatic boronic acids in which the boron is additionally held clamped by a covalent bond (the B-N) to a side chain. Early indications were that these compounds affected lipopolysaccharide biosynthesis,[84–92] and recent structural studies have established that the biomacromolecular target is in fact enoyl acyl carrier protein reductase (ENR), the NAD(P)H-dependent enzyme responsible for catalyzing a late step on the fatty acid biosynthetic pathway.[93–97] Diazaborine inhibitors of ENR form a covalent B-O bond with the 2'-hydroxyl group of the cofactor NAD's ribose unit, thus assembling a tightly yet noncovalently bound borate-based bisubstrate analog species at the active site. Interestingly, ENR is the very same biomacromolecular target of the commonly used broad-spectrum (bacteria, fungi, viruses) bacteriostatic germicide triclosan[98–101] and even, oddly enough, the well-known antituberculosis drug isoniazid.

ESTROGEN MIMICS

Our own laboratory has investigated the chemical and structural properties of a variety of benzodiazaborines formed via intramolecular dehydration in 2-acylamino- and amidino-substituted benzeneboronic acids[102] and in oximes and hydrazones of 2-formylbenzeneboronic acid.[103,104] By examining the structural

features of the latter compounds, we recognized the opportunity to design new variants of these boron heterocycles that would be ultra-high fidelity estrogen structural mimics. In particular, we saw that careful molecular engineering could produce very close structural mimics of A-ring aromatic estrogens like estradiol and estrone and AB-ring aromatic equilenins like some of the components of Premarin, the conjugated equine estrogens used extensively for estrogen replacement therapy and prevention of osteoporosis and cardiovascular disease in postmenopausal women. Our first prototype (Fig. 1G) was a boron heterocycle mimic of estradiol or dihydroequilenin O17-methyl ether featuring a strong intramolecular hydrogen bond that assembles a "virtual," flexible steroid-like C-ring. An x-ray crystal structure confirmed the remarkable estrogen-like shape adopted by this compound.[105] This and some of the other early prototypes that had a biorecognition-critical estrogen A-ring phenolic hydroxyl group were examined for their antiproliferative activity against cultured MCF-7 human breast cancer cells. The IC_{50} values near 5 μmol/L found for all these compounds provides a strong impetus for our current ongoing efforts to develop these unique boron-based compounds into therapeutics for treating breast cancer, likely as antiestrogens.

OTHERS

Other boron-based compounds exhibit intriguing in vitro activity and thus show some promise of evolving into useful therapeutics in the future. Examples include variants of benzodiazaborines found active against *Mycobacterium tuberculosis* $H_{37}R_v$,[106] acyclic nucleoside boronic acid derivatives targeted at HIV,[107] tetrapeptide boronic acids found to inhibit HIV-1 protease,[108] benzothiazoline boron complexes with antimicrobial properties,[109] and 3-aminophenylboronic acid (APBA), found to inhibit both the *Streptomyces griseus* NAD^+-glycohydrolase and ADP-ribosyltransferase enzymes.[110] The ongoing development of these bioactive agents will be well worth monitoring along side those that are further along in their development as useful therapeutics.

CONCLUSION

The novel classes of boron-based compounds described above are rapidly maturing into potent therapeutics in their own right, and prescribing clinicians need to be aware that they are coming on line. The fact that they are based on the unusual element boron is no cause for concern because it is only toward the end of the past millennium that organic chemists have learned to construct useful platforms with it and medicinal chemists have learned to appreciate its value.

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DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
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REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 28 Aug 02

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

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Encl

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