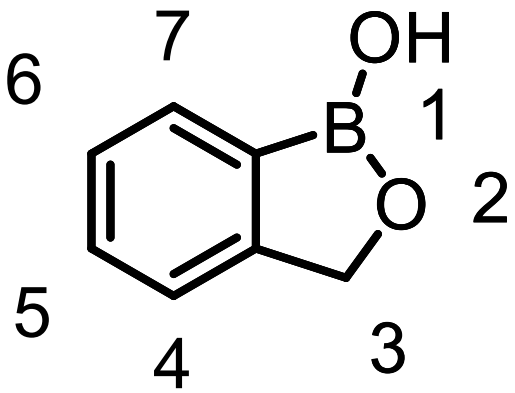
Benzoxaboroles

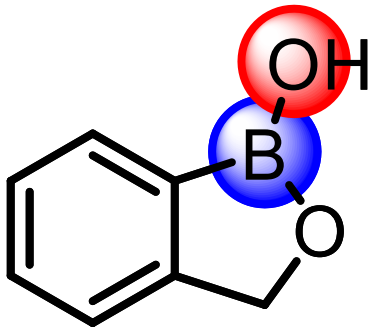
REBECCA M. LEISING

SED GROUP MEETING

JANUARY 19, 2016

What are benzoxaboroles?





1957 – First synthesized and characterized by Torssell

Other names used in the literature:

- Boronophthalides
- Arylboronolactones
- Benzoboroxoles

Hemi boronic ester derived from phenyl boronic acids

Contains a Lewis acidic center and free hydroxyl group

Adamczyk-Woźniak, A.; Borys, K. M.; Sprozyński, A. *Chem Rev* **2015**, *115*, 5224–5247.

Overview

Physicochemical Properties

Biological Properties

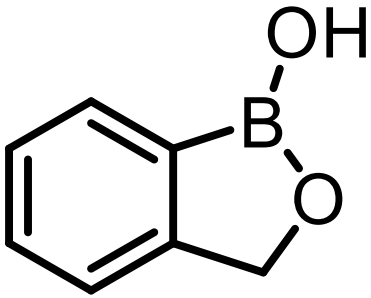
Synthetic Routes

- Formation of benzoxaborles
- Further complexation
- Uses for synthetic Chemists

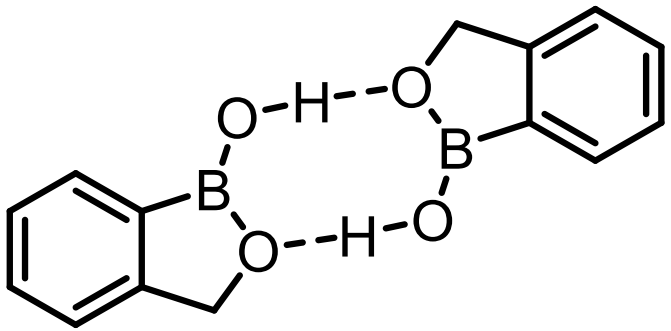
Medicinal Applications

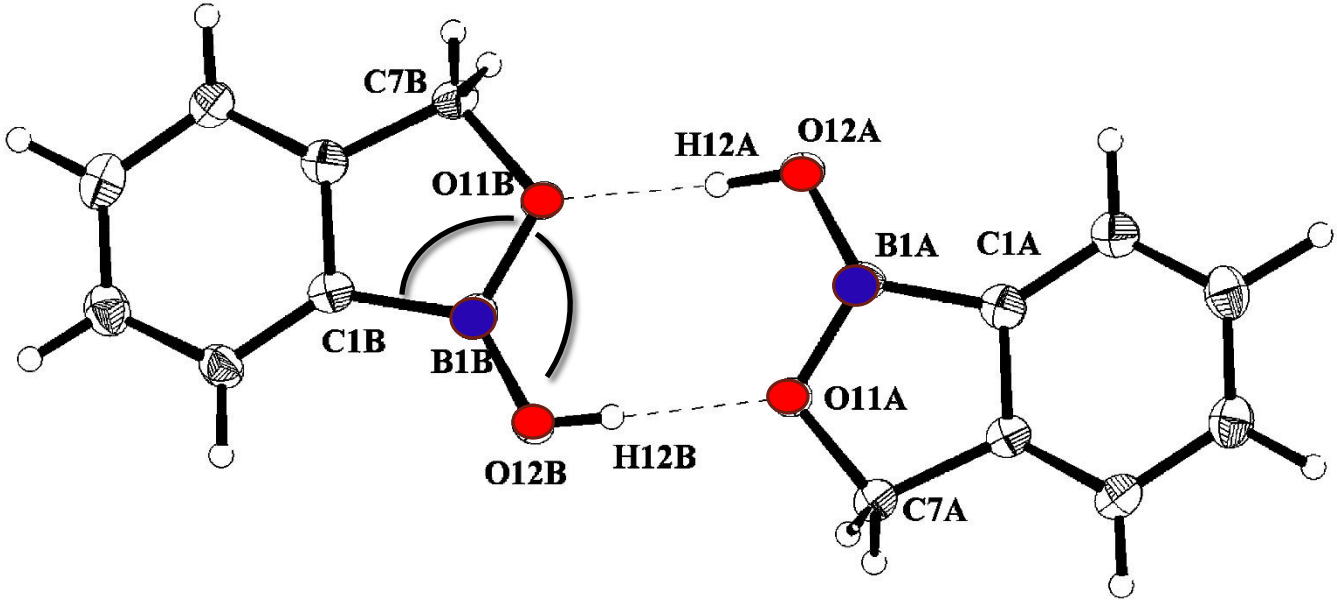
- Anti-fungal
- Anti-protoazoal
- Anti-bacterial
- Anti-inflammatory

Future Directions



X-ray Crystal Structure





Planar dimeric pairs that are centiosymmetric

Medium to strong intermolecular hydrogen bonds

Can form infinite 2D and 3D networks through lateral hydrogen bonding with additional substitution

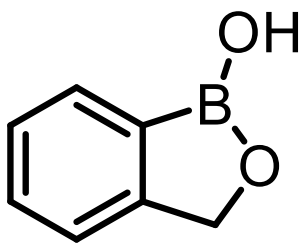
BOO fragment is coplanar with the phenyl fragment

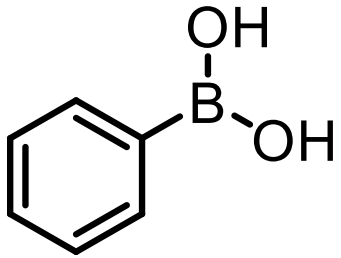
Slight distortion of bond angles and bond lengths around Boron – strained oxaborole

CBO Angle	Bond Angle (°)
Exocyclic	133.1
Endocyclic	108.6
BO Bond	**Bond Length (Å)**
Exocyclic	1.350
Endocyclic	1.394

Adamczyk-Woźniak, A. et al. *J. Organomet. Chem.* **2009**, *694*, 3533–3541.
Adamczyk-Woźniak, A. et al. *J. Phys. Chem.* **2010**, *114*, 2324–2330.

Chemical behavior – Stability of oxaborole





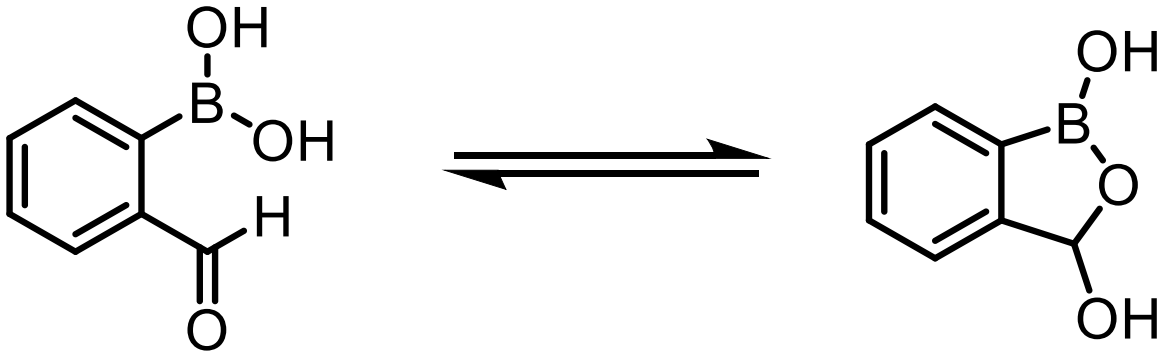
PBA

B-O bond is resistant to hydrolysis

- 3h refluxing with 10% HCl – quantitative recovery
- 1.5h – 90% of PBA decomposes

Quantitatively recovered benzoxaborole after:

- 3h refluxing with 15% NaOH
- Refluxing with thionyl chloride

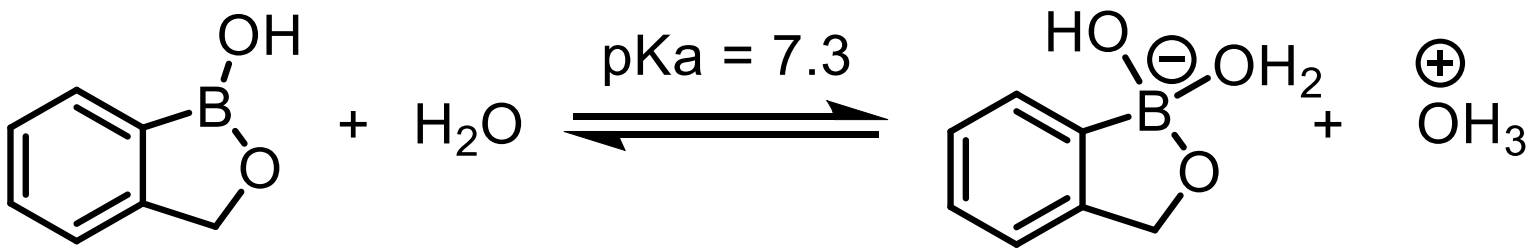


Tautomerization of o-formyl phenyl boronic acid to the more stable benzoxaborole has been observed

- First observation by Snyder in 1958
- Later confirmed by NMR

Zhang, J.; Zhu, M. Y.; Lin, Y.; Zhou, H. *Sci. China Chem.* **2013**, *56*, 1372–1381.
Adamczyk-Woźniak, A.; Borys, K. M.; Sprozyński, A. *Chem Rev* **2015**, *115*, 5224–5247.
Adamczyk-Woźniak, A. et al. *J. Organomet. Chem.* **2009**, *694*, 3533–3541.

Lewis Acidity



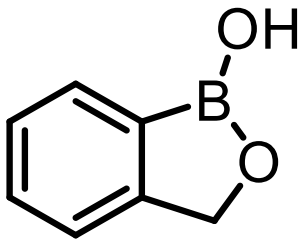
Formation of a tetrahedral anion alleviates strain of oxaborole ($sp^2 \rightarrow sp^3$)

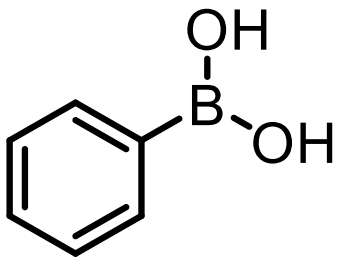
- Bond angle is reduced from 120° to 109°

pK_a is 1-2 lower than phenylboronic acids

Allows for increase solubility in aqueous media at physiological pH

- Benzoxaborole – 50% of anionic form present
- Phenylboronic acid – <1% of anionic form present

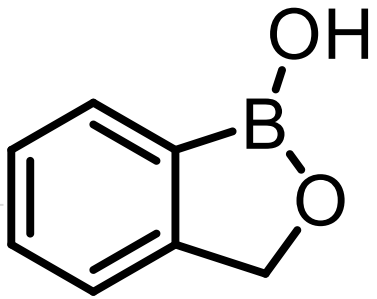


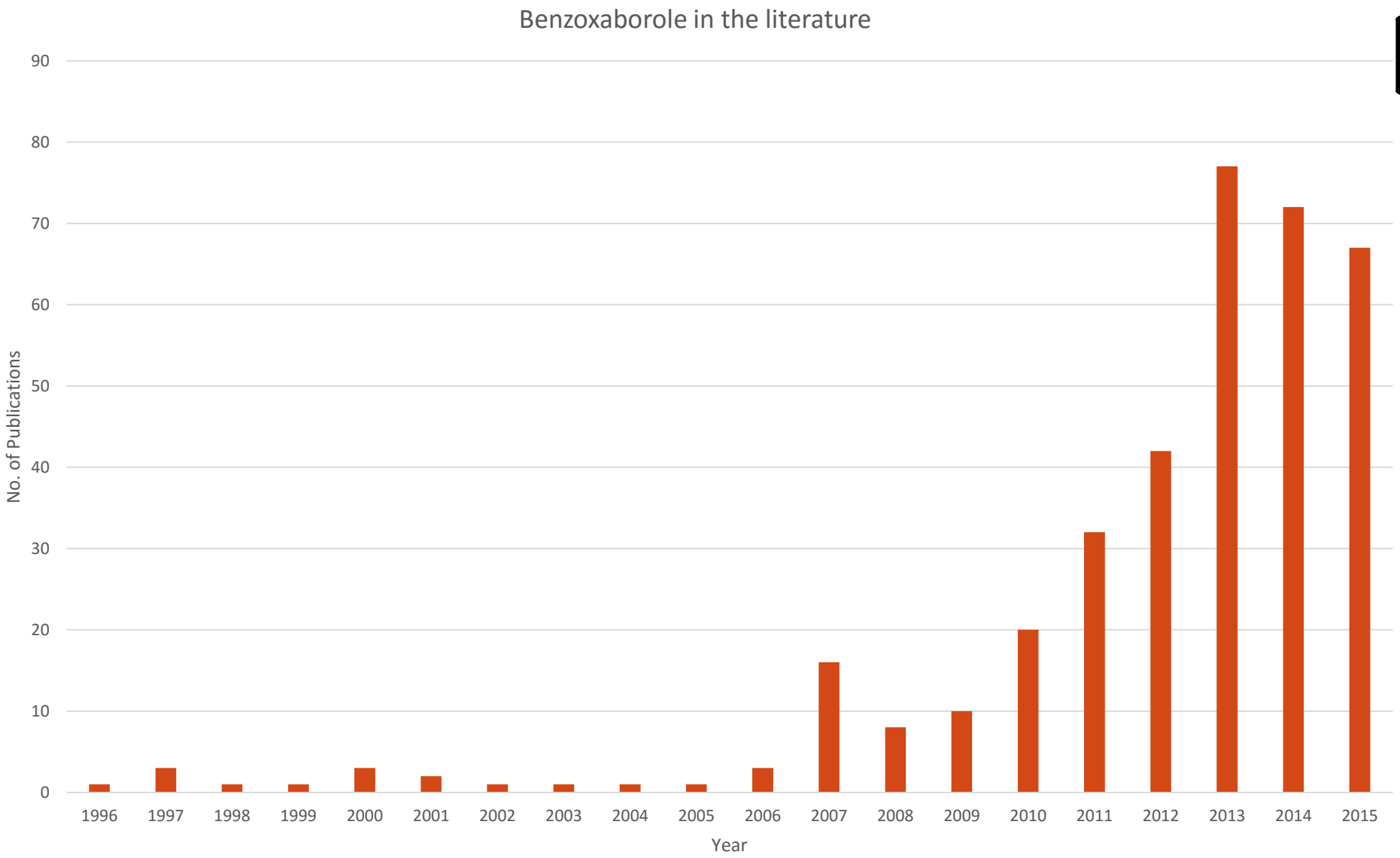


Structure	pKa
Benzoxaborole	7–8
Phenylboronic acid	8–9

Zhang, J.; Zhu, M. Y.; Lin, Y.; Zhou, H. *Sci. China Chem.* **2013**, *56*, 1372–1381.
Adamczyk-Woźniak, A.; Borys, K. M.; Sprozyński, A. *Chem Rev* **2015**, *115*, 5224–5247.

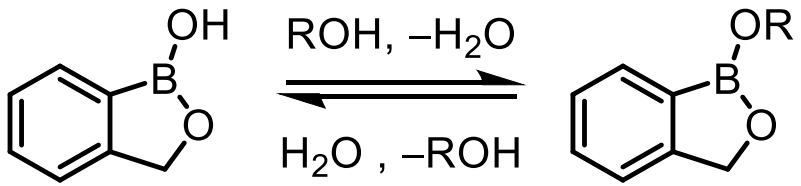
Revival of Benzoxaborole





Scopus. http://www.scopus.com/ (accessed 12 Jan 2016).

Formation of Cyclic Esters with Diols

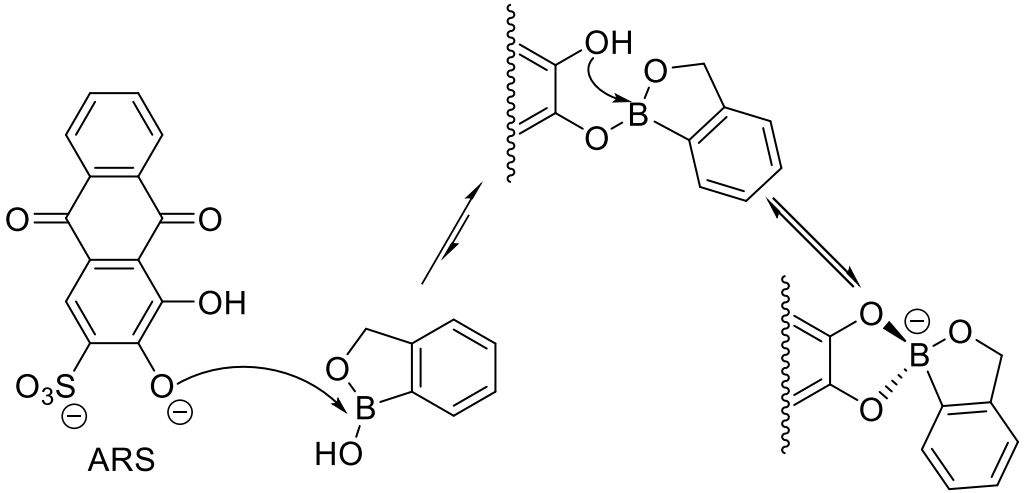


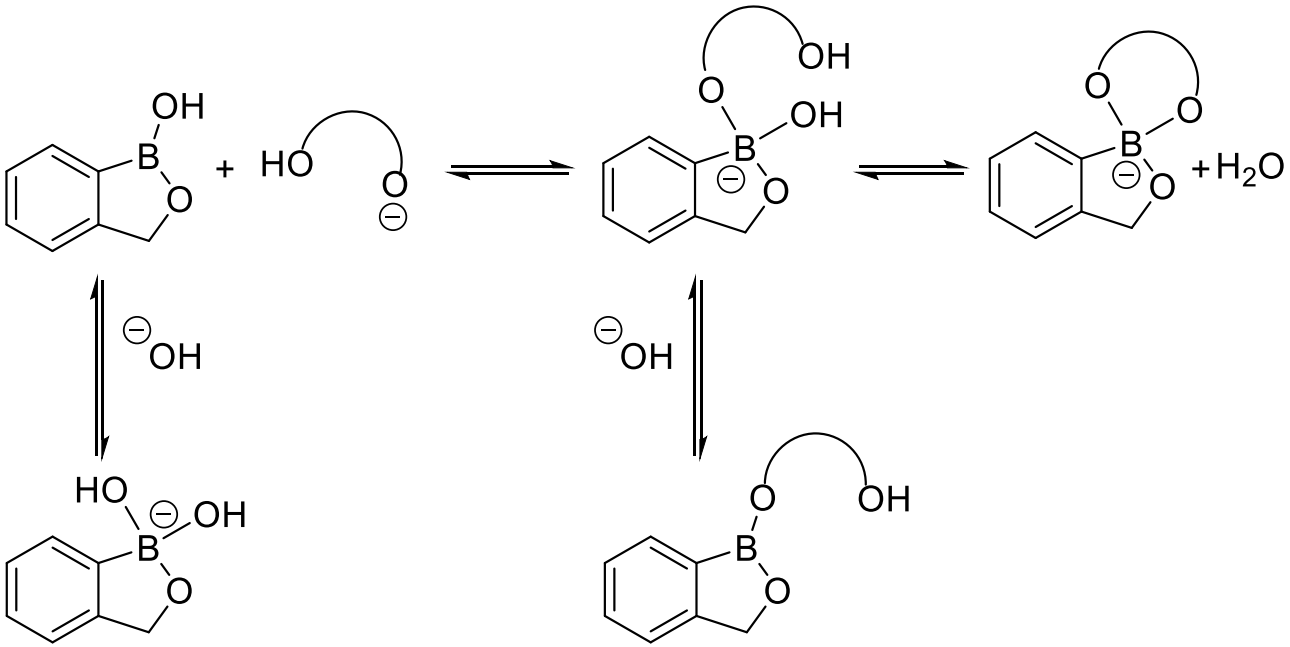
Benzoxaboroles can form monoester with alcohols

- Spontaneous hydrolysis in air

Due to improved solubility in aqueous solution, benzoxaboroles demonstrate enhanced binding of diols at neutral pH

Stepwise formation of spiro ester, starting from the neutral, trigonal form of benzoxaboroles





Mechanism studied by Benkovic and Tomsho with Alizarin Red S (ARS) by UV/Vis and Fluoresence

Adamczyk-Woźniak, A. et al. *J. Organomet. Chem.* **2009**, *694*, 3533–3541.
Tomsho, J. W.; Benkovic, S. J. *J. Org. Chem.* **2012**, *77*, 11200–11209.
Adamczyk-Woźniak, A.; Borys, K. M.; Sprozyński, A. *Chem Rev* **2015**, *115*, 5224–5247.

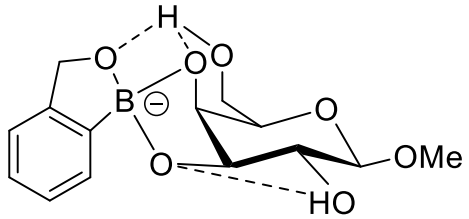
Binding of Sugars

Rediscovery of benzoxaborole in 2006 as sugar-binding agents

- Non-reducing hexopyranosides

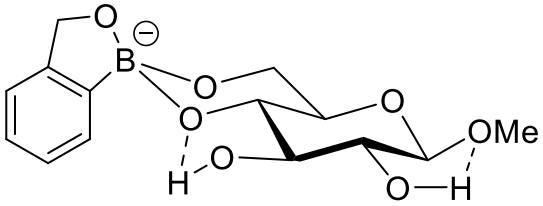
Used ^{1}H – NMR spectroscopy to observed strong sugar binding with galactopyranosides

- Preferred 3,4-diol binding

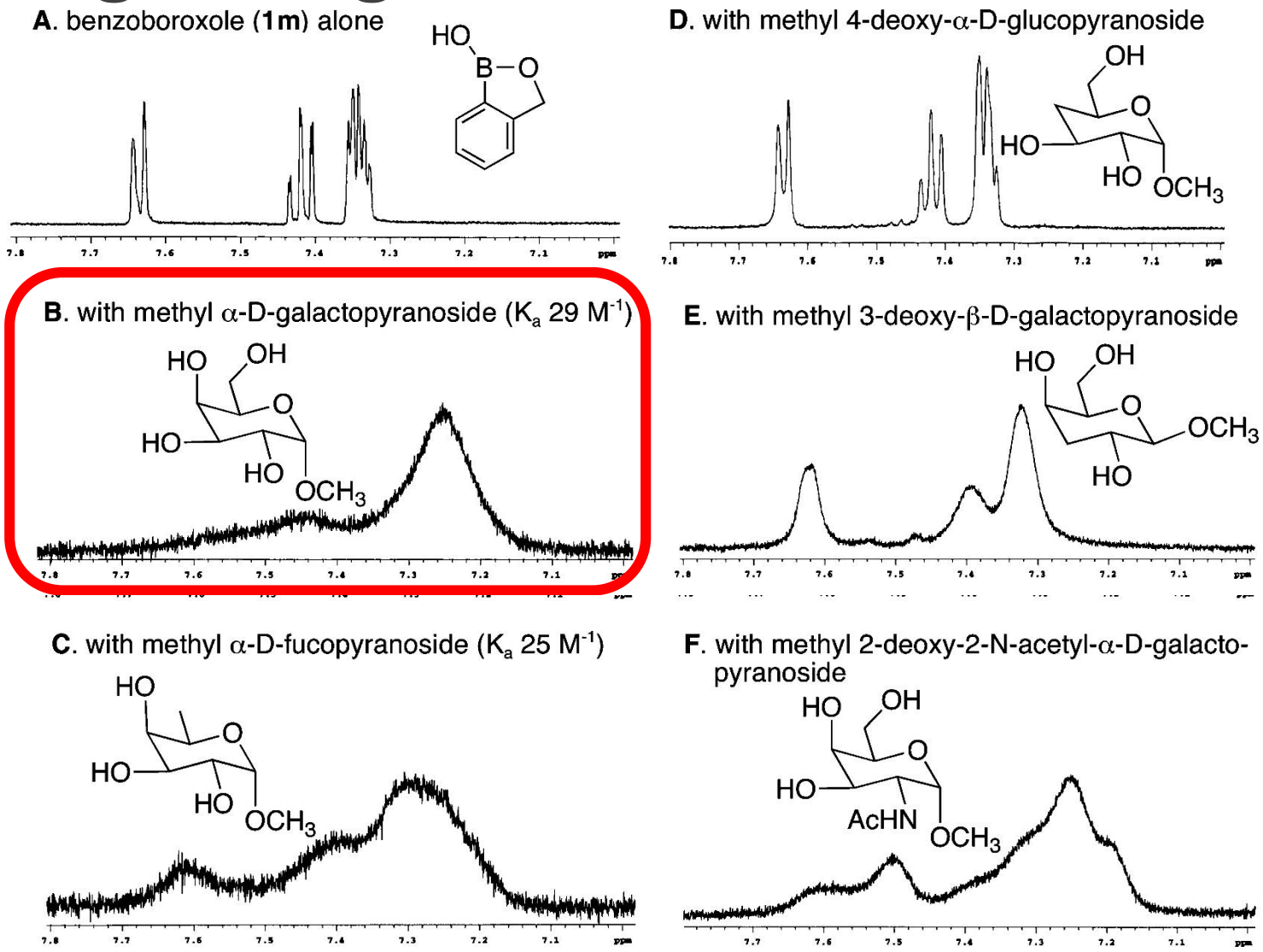


Weaker complexation with glucopyranosides resulted in the use of UV spectrophotometry with ARS assay

- Preferred 4,6-diol binding



Determined by MS a 1:1 binding – *M* = 309.1



Dowlut, M.; Hall, D. G. *J. Am. Chem. Soc.* **2006**, *128*, 4226–4227.
Bérubé, M.; Dowlut, M.; Hall, D. G. *J. Org. Chem.* **2008**, *73*, 6471–6479.
Adamczyk-Woźniak, A.; Borys, K. M.; Sprozyński, A. *Chem Rev* **2015**, *115*, 5224–5247.

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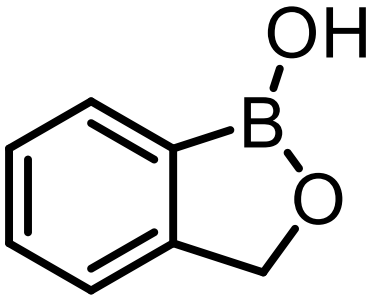
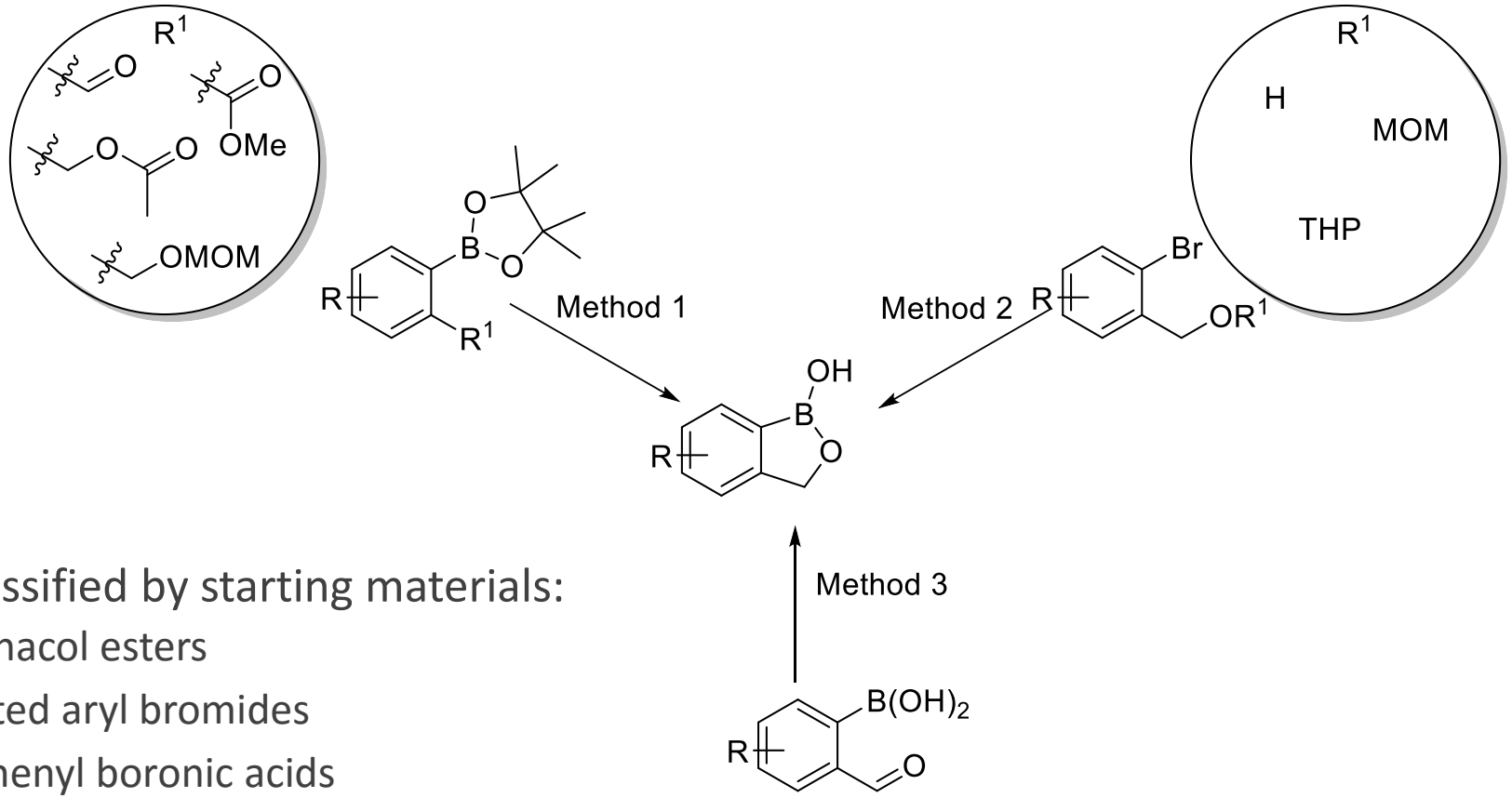


Overall Routes for Cyclization

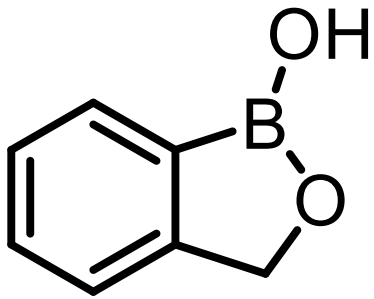
The last step in the formation of benzoxaboroles is a spontaneous cyclization with a boronic moiety and neighboring hydroxymethyl group



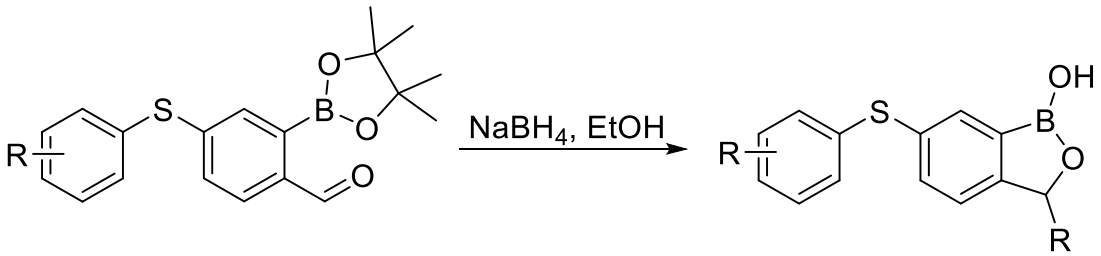
Methods classified by starting materials:

- Boronic pinacol esters
- *o*-substituted aryl bromides
- 2-formylphenyl boronic acids

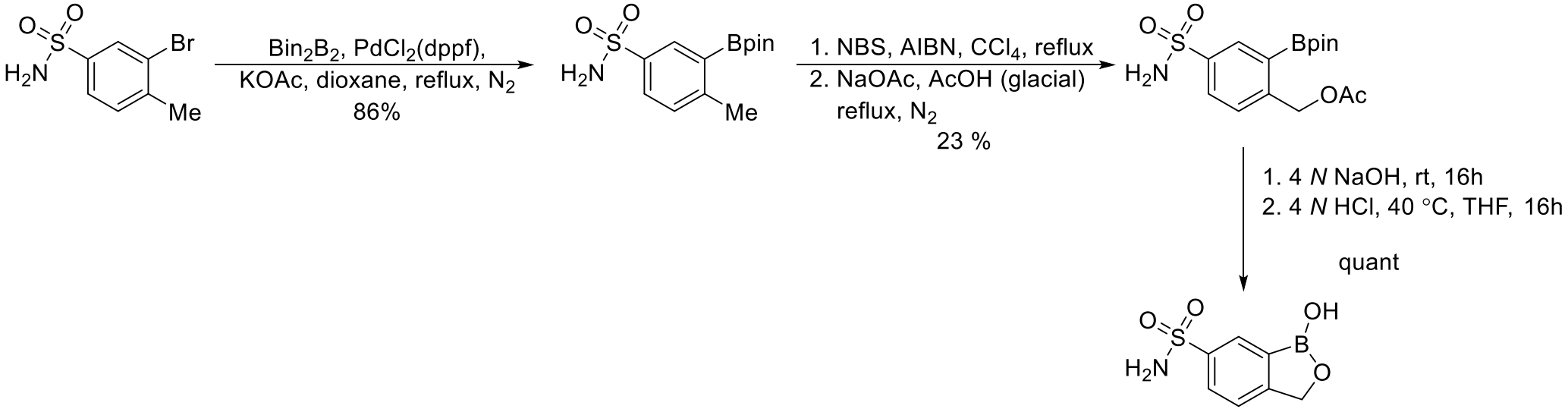
Method 1: Boronic Pinacol Esters



Hydrolysis of pinacol is necessary before cyclization



R = CH_2CO_2H, CH_2NO_2, CN or R^1R^2NH



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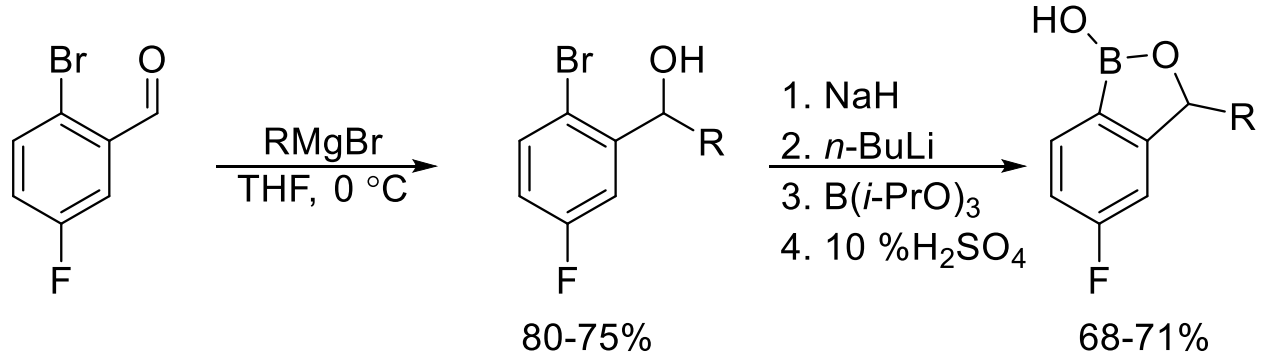
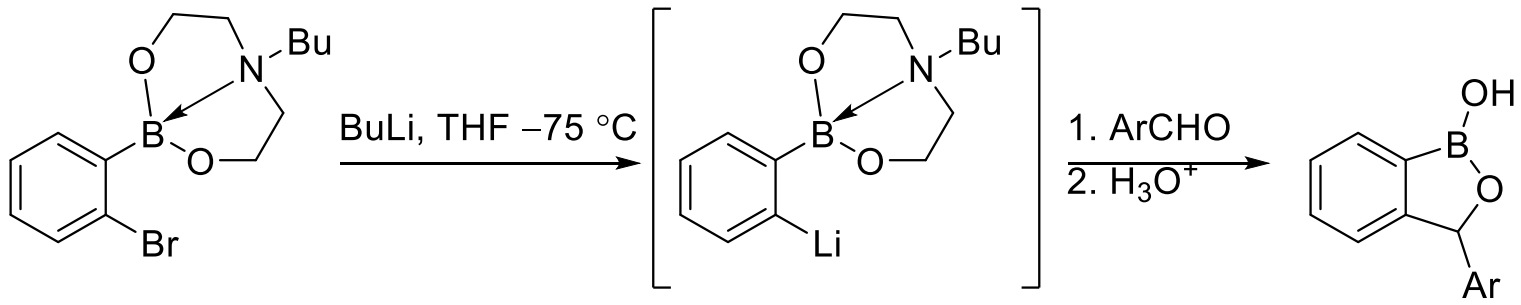
Zhou, H.-C. et. al. *Sci. China Chem.* **2013**, *56,* 1372–1381.
Kiprof, P.; Zhdankin, V. V.; Reddy, M. V. R. *Tetrahedron*, **2007**, *63*, 9401–9405.

Method 2: Aromatic Bromides



Can synthesize both 5- and 3-substituted benzoxaboroles

Boron introduction through Li-halogen exchange and transmetalation with boron



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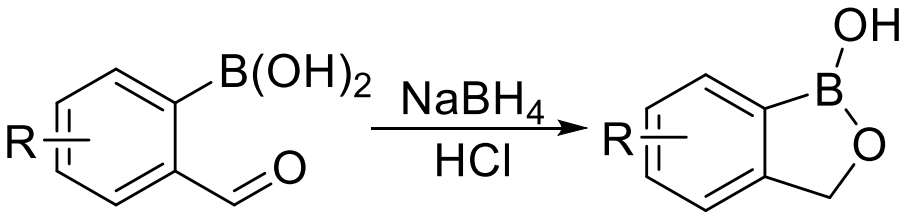
Kiprof, P.; Zhdankin, V. V.; Reddy, M. V. R. *Tetrahedron*, **2007**, *63*, 9401–9405.
Luliński, S. et al. *Appl. Organomet. Chem.* **2007**, *21*, 234–238.

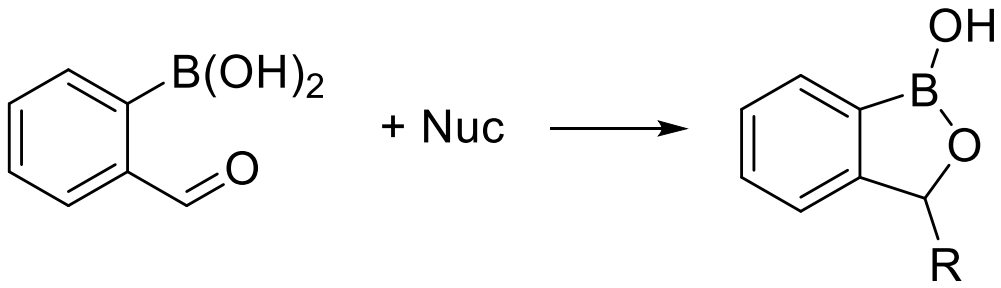
Method 3: Formylphenyl boronic acid

1. Reduction



R = H, 5-F, 6-OBz

2. Addition with nucleophiles for 3-substitution
   - Malonic acid
   - Nitromethane
   - Sodium cyanide



R = CH_2CO_2H, CH_2NO_2, CN or R^1R^2NH

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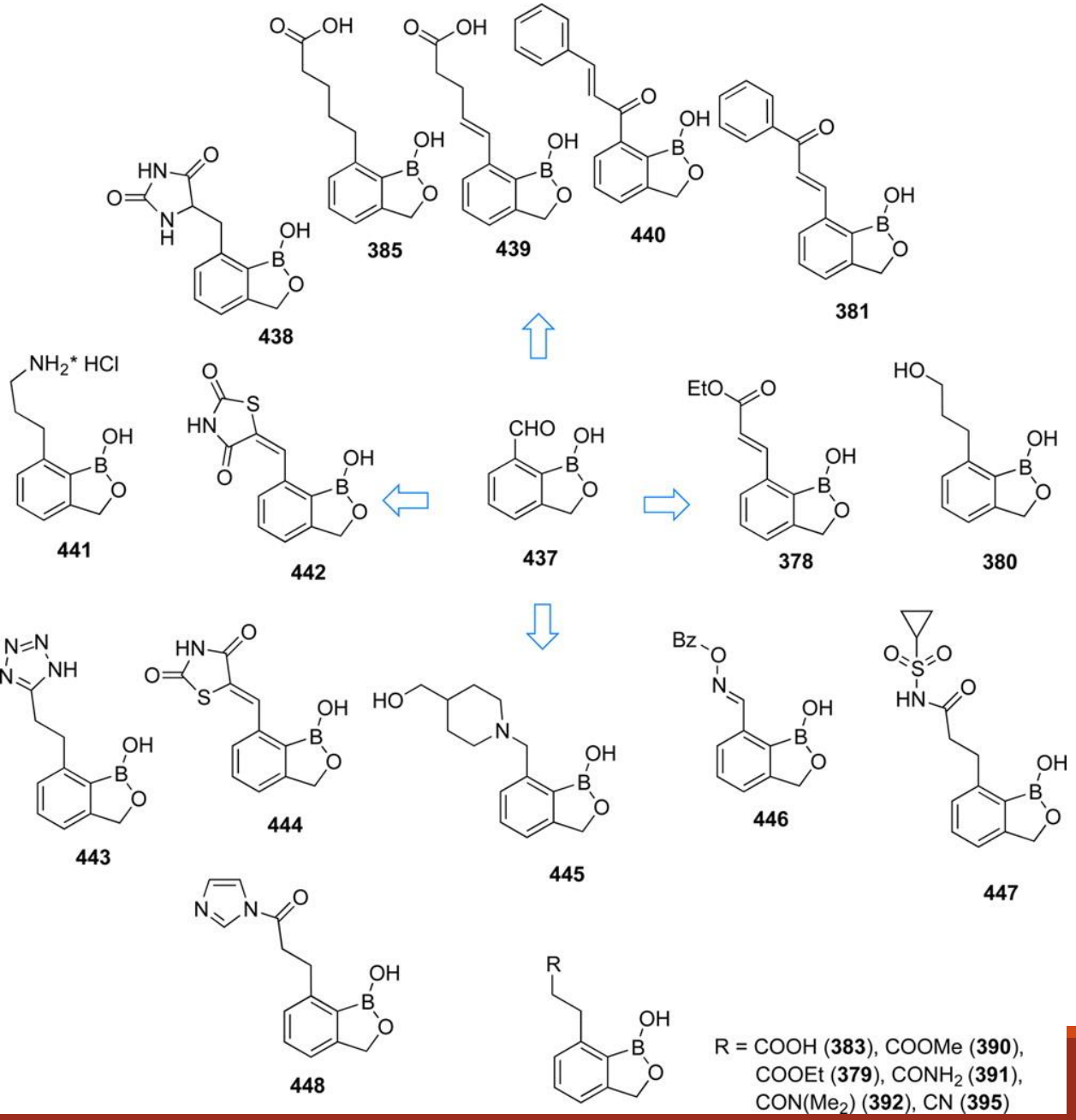
Further Functionalization



Benzoxaborole stable under nitration (fuming HNO_3), hydrogenation (Pd/C or Raney Ni), oxidation (CrO_3), and reduction ($LiAlH_4$)

7-formyl benzoxaborole shown immense functionalization

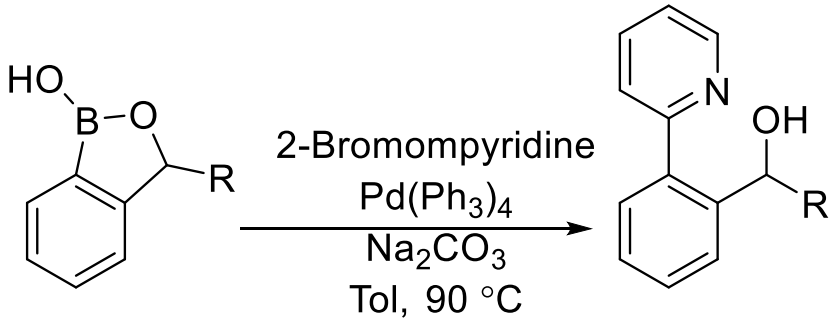
Formation of 7-formyl benzoxaborole by various methods

Adamczyk-Woźniak, A.; Borys, K. M.; Sprozyński, A. *Chem Rev* **2015**, *115*, 5224–5247. Reproduced (adapted) with permission from Adamczyk-Woźniak, A.; Borys, K. M.; Sprozyński, A. *Chem Rev* **2015**, *115*, 5224–5247. Copyright 2013 American Chemical Society.

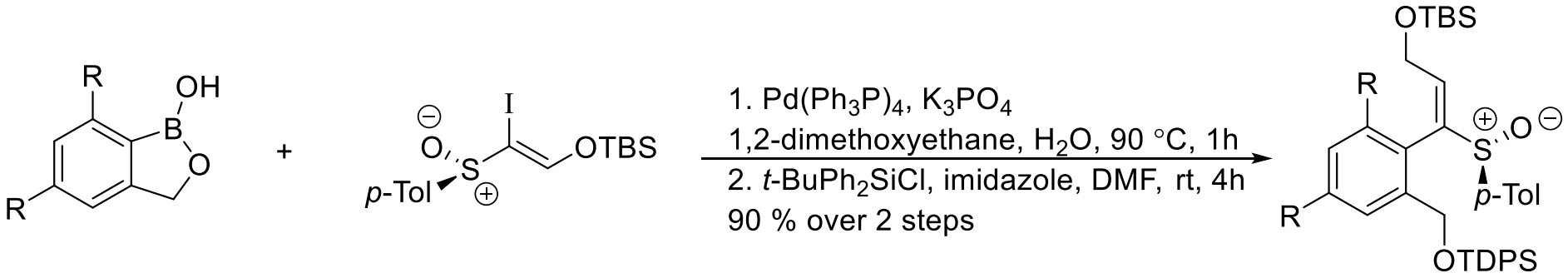
Removal of Benzoxaborole – Cross Coupling

Benzoxaborole can be used as a coupling partner in the Suzuki-Miyaura

Formation of primarily amino alcohols and biaryls



R = H, Vinyl, Allyl, Phenyl, *n*-Decyl 89-95%



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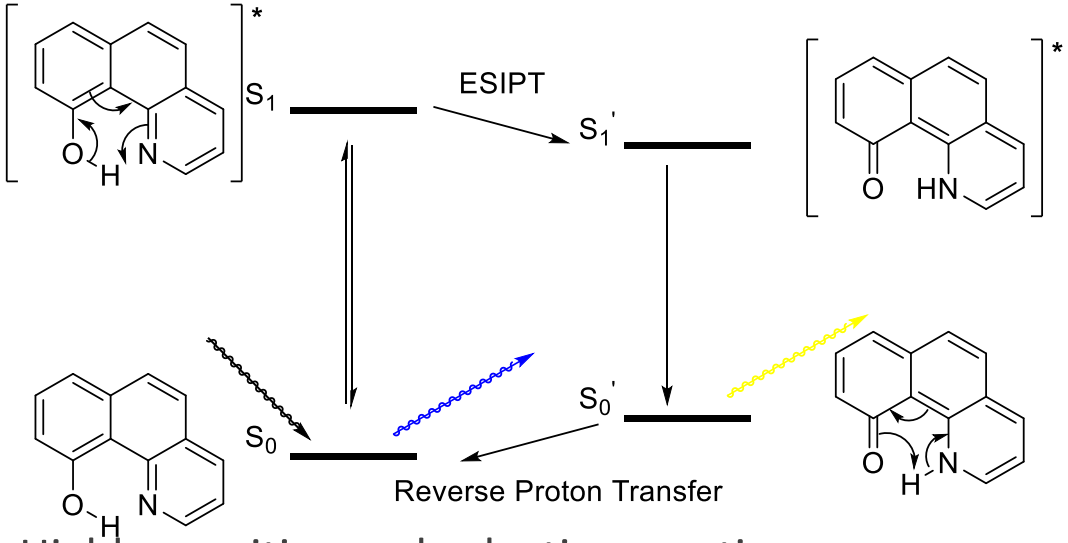
Visualization of Benzoxaborole

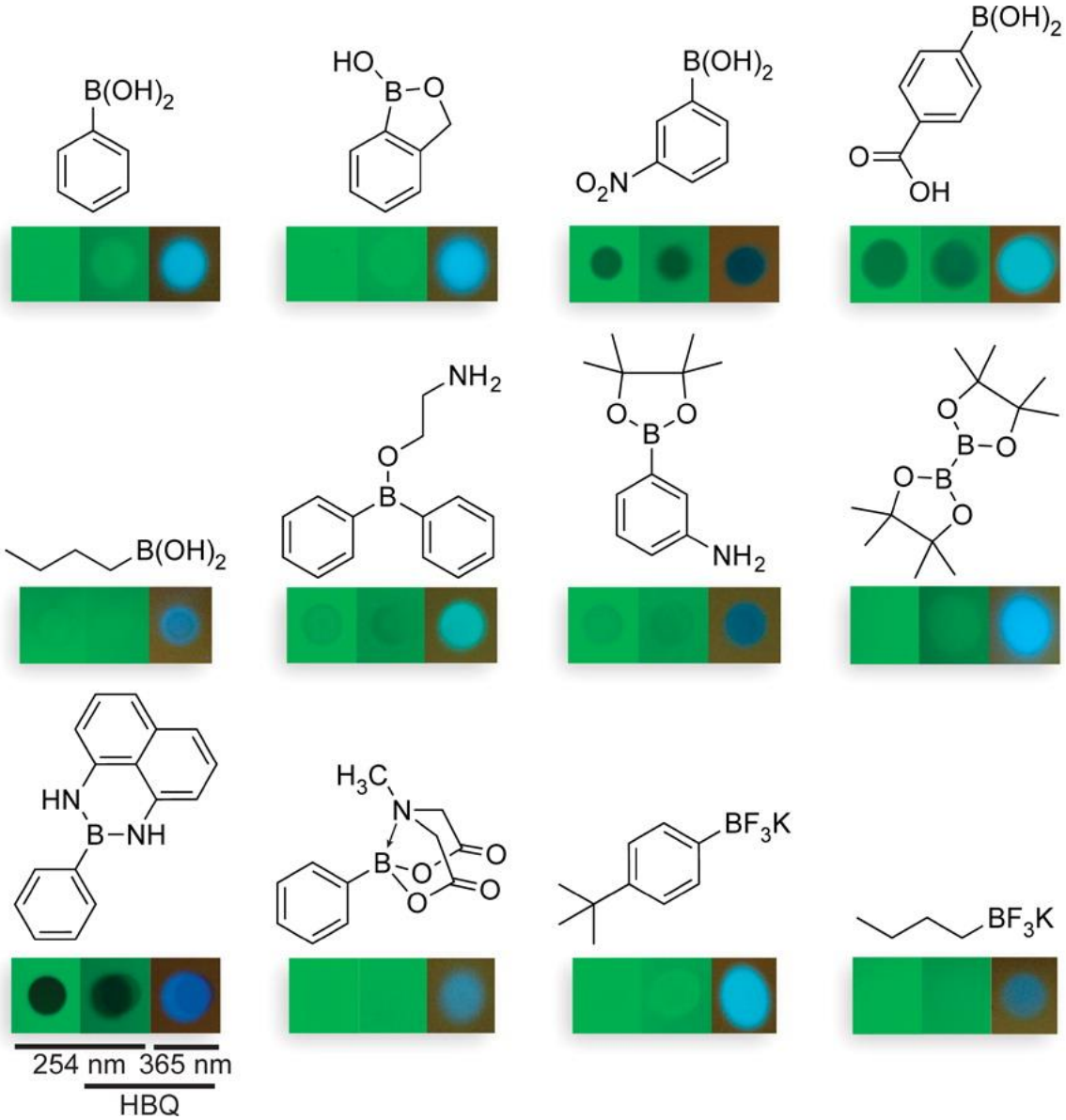
Benzoxaboroles can interrupt the excited-state intramolecular proton transfer (ESIPT) of 10-hydroxybenzo[*h*]quinolone



Highly sensitive and selective reaction

Only false positives were with highly electrophilic functional groups (acyl chlorides and sulfonyl chlorides)

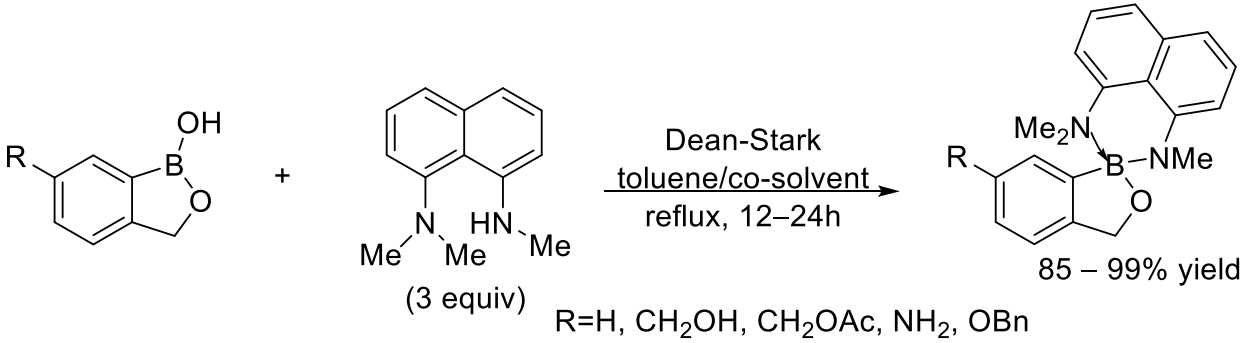
Can use 0.1 nM solution as a TLC stain with spots fluoresce bright blue-green on a yellow background with a UV light

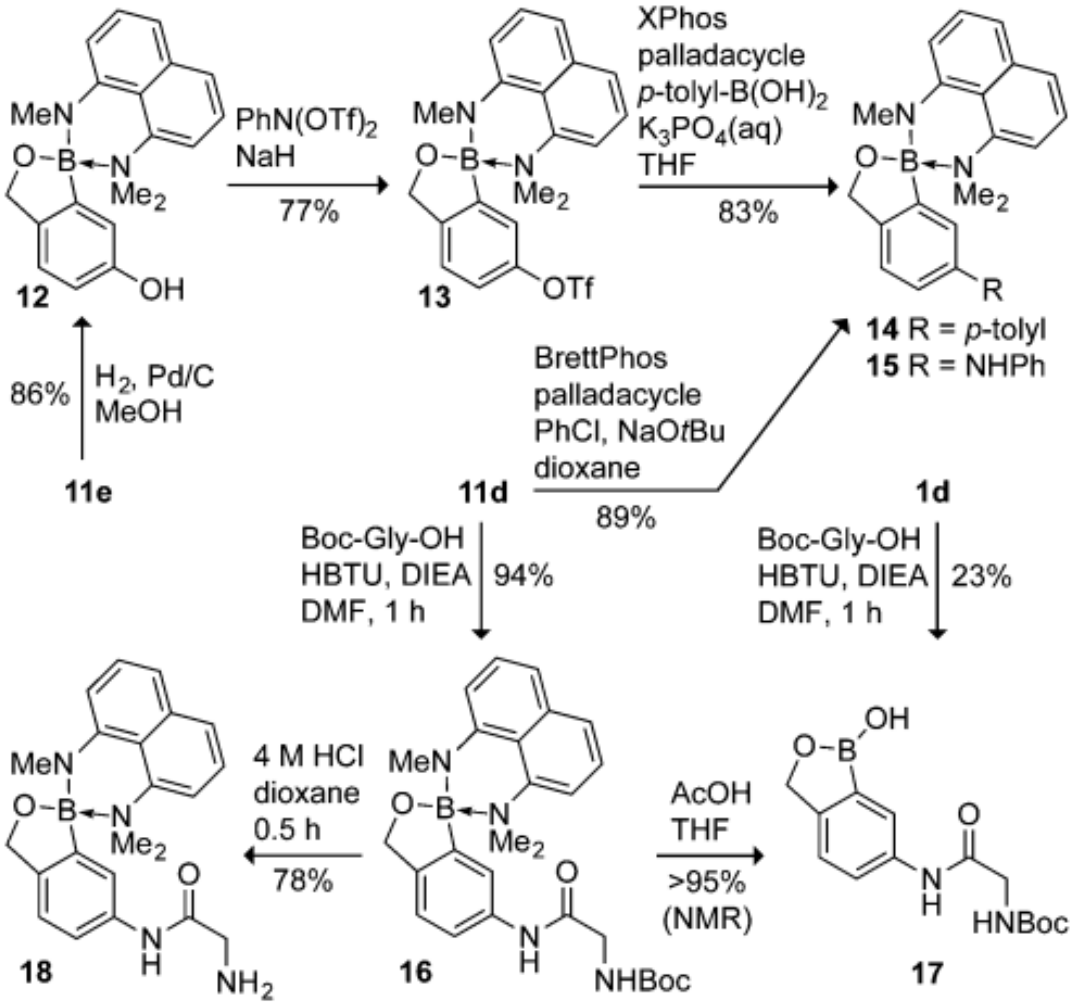


Aronoff, M. R.; VanVeller, B.; Raines, R. T. et al. *Org. Lett.* **2013**, *15*, 5382–5385.

Protection of Benzoxaborole

1-Dimethylamino-8-methylaminonaphthalene as a benzoxaborole protecting group





Stable under basic conditions, Suzuki-Miyaura and Buchwald-Hartwig Pd cat couplings, reducing agents, and peptide synthesis

Fluorescent and chromatographically purification

Demonstrated functionalization of 6-substituent

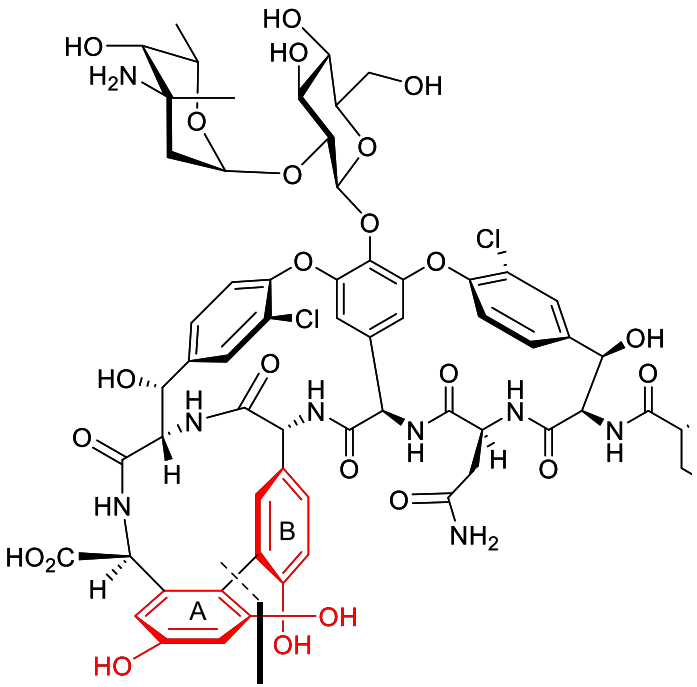
Moderately stable under anhydrous acid

Not stable to oxidations

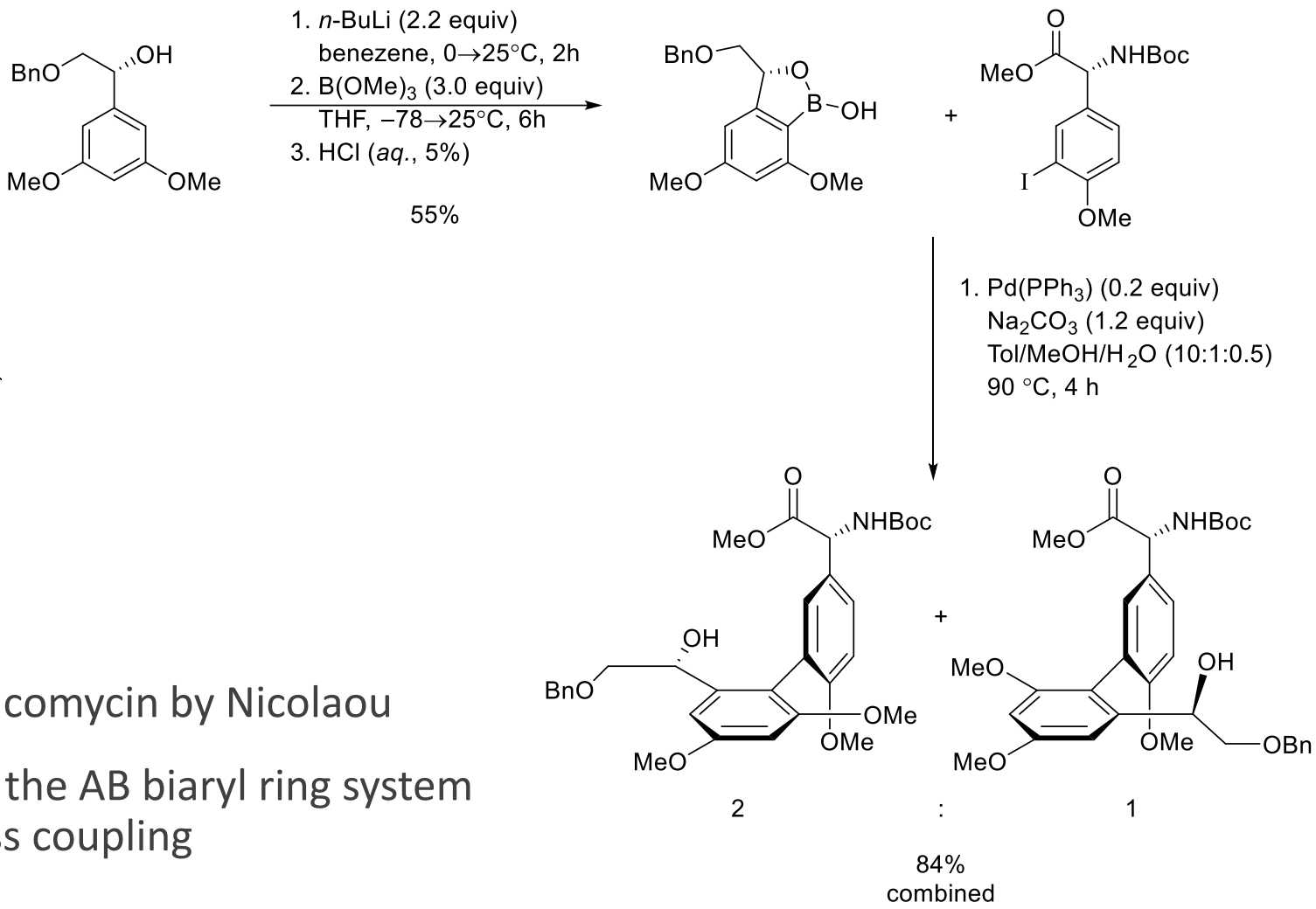
Removed by aqueous acidic conditions

Aronoff, M. R.; VanVeller, B.; Raines, R. T. *RCS Adv.* **2013**, *3*, 21331–21334.

Benzoxaborole in Total Synthesis



Suzuki-Myra Coupling



1999 – Total synthesis of vancomycin by Nicolaou

Benzoxaborole used to form the AB biaryl ring system through Suzuki-Miyaura cross coupling

Nicolaou, K. C. et al. *Chem.–Eur. J.* **1999**, *5*, 2584–2601.
Nicolaou, K. C. et al. *Chem.–Eur. J.* **1999**, *5*, 2602–2621.

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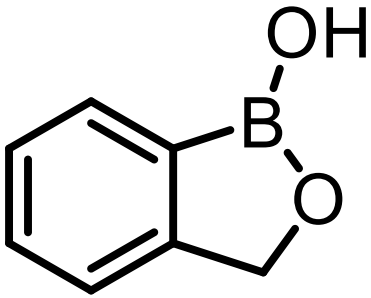


Anti-fungal activity – Inhibition of LeuRS

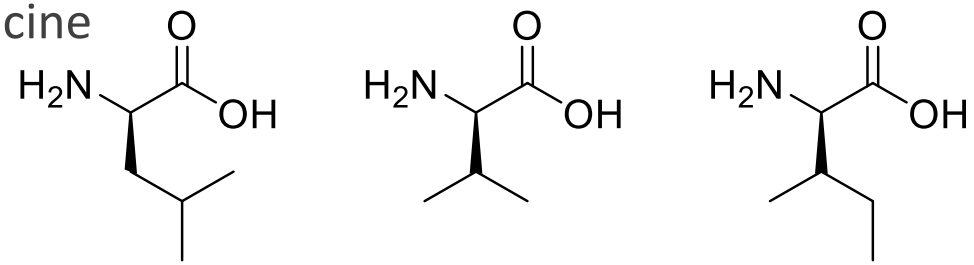
Inhibition of a fungal leucyl-tRNA synthetase (LeuRS)

Aminoacyl-*t*RNA synthetase is crucial for gene translation

7 out of the 20 aminoacyl tRNA synthetase have two domains for synthesis and editing

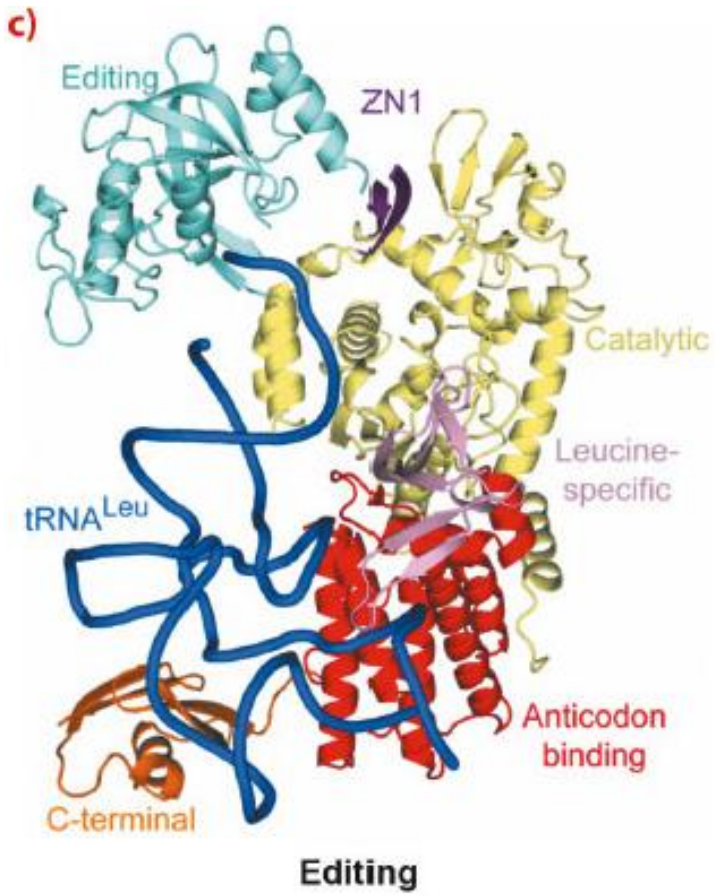
- Each contain a unique active site

LeuRS charges leucine, but is not able to selective for leucine over valine or isoleucine



Editing domain hydrolyzes mis-charged tRNA

- Binding pocket for Val and Ile, but not Leu



The European Synchrotron. http://www.esrt.eu/UsersAndScience/Publications/Highlights/2012/sb/sb13 (Accessed 16 Jan 2016).
Baker, S. J.; Tomsho, J. W.; Benkovic, S. J. *Chem. Soc. Rev.* **2011**, *40*, 4279–4285.

Inhibition of LeuRS Mechanism

Editing domain is targeted of LeuRS, with tRNA is necessary for inhibition

Benzoxaboroles are non-competitive inhibitors with respect to leucine and ATP

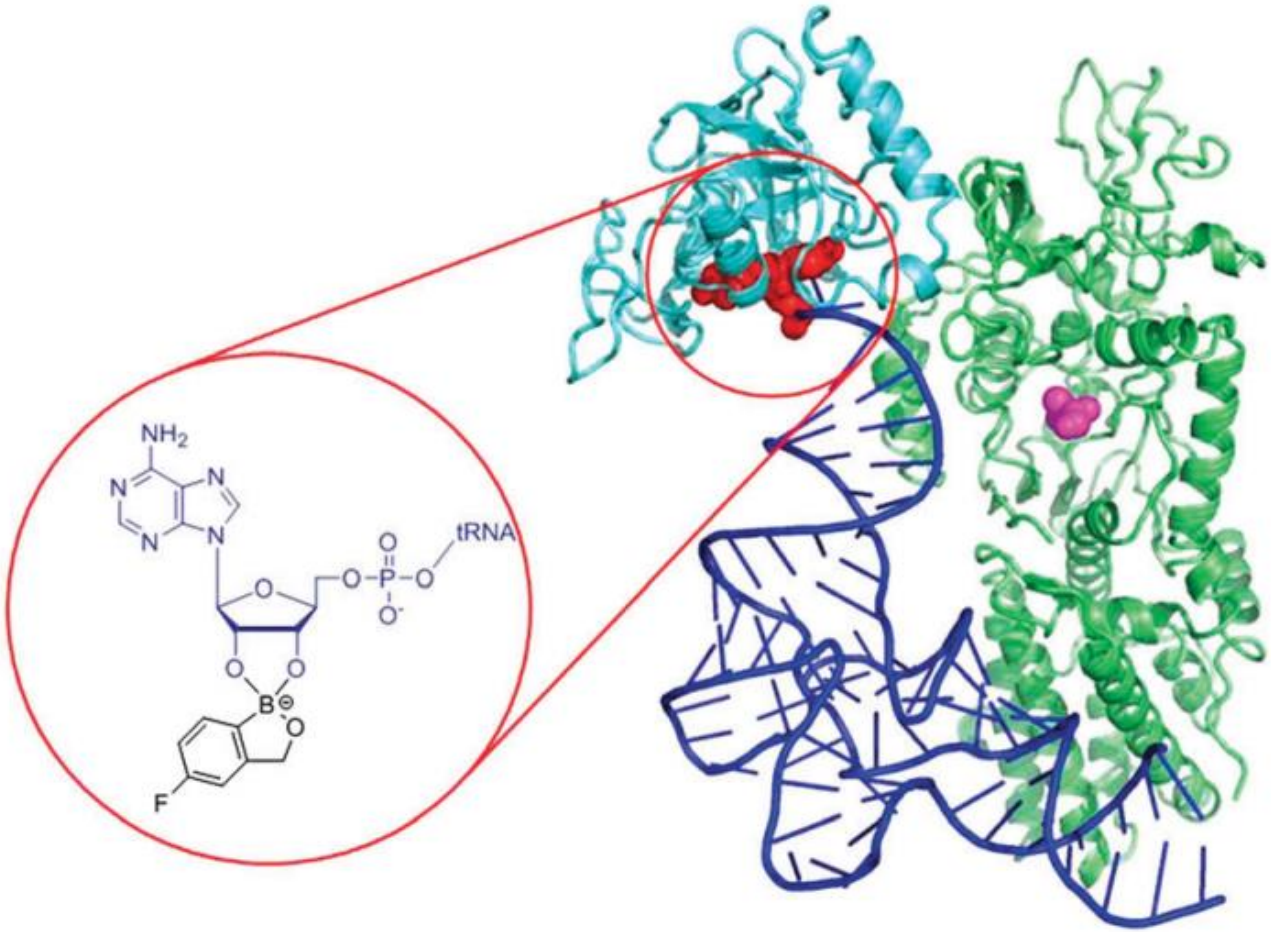
Slow-tight binding inhibitor, with a $t_{1/2}$ = 5h

Formation of spiro ester complex with 3'-terminal adenosine of $tRNA^{LEU}$

Locks tRNA in editing active site which prevents catalytic turn over

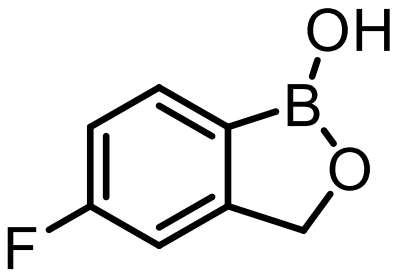
Other tRNA are also blocked from binding to the enzyme through this spiro complex

Oxaborole tRNA trapping (OBORT) mechanism



Baker, S. J.; Tomsho, J. W.; Benkovic, S. J. *Chem. Soc. Rev.* **2011**, *40*, 4279–4285.

First benzoxaborole pharmaceutical



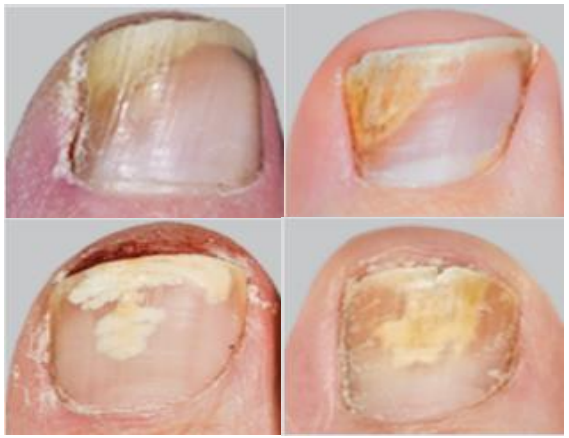
KERYDIN™ – topical solution approved by FDA (2014) for the treatment of onychomycosis

- Estimated 35 million people in US are afflicted

Penetrates the nail plate to treat both the nail and nail bed

After approx. 52 weeks:

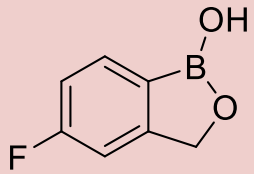
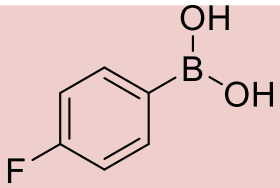
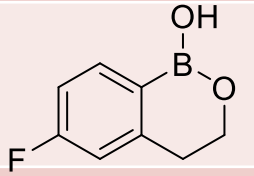
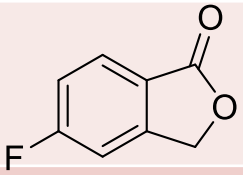
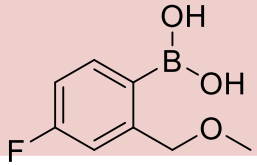
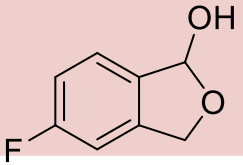
- 31.1% of patients were at "Complete or Almost Complete Cure"
- 35.9% of patient were at "Mycological Cure" (<10% affected area)



X-ray structure guided SAR studies

Boron as well as oxaborole were found to be necessary for anti-fungal activity

Due to a small binding pocket, only small substituents were successful

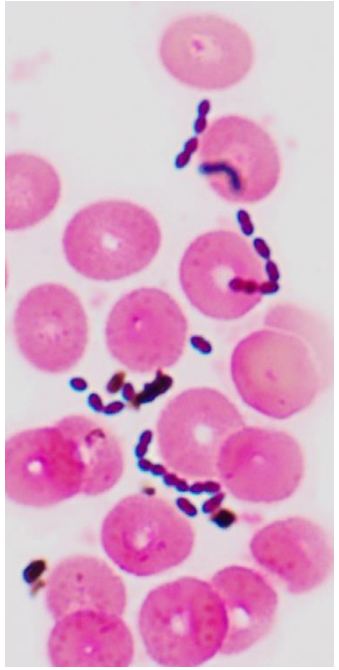
Compound	IC_{50} (µM)	Compound	IC_{50} (µM)
	2.1		>100
	96		>100
	>100		>100

Shapiro, L.; Benkovic, S. J. et al. *Science* **2007**, *316*, 1759–1761.

Kerydin™ (Tavaborole) Topical Solution, 5%. Toenail Fungus Can Make You A Toe Tucker; http://www.kerydin.com/about-toe-nail-fungus.html

Anacor Pharmaceuticals. FDA Approves Anacor Pharmaceuticals' KERYDIN™ (Tavaborole) Topical Solution, 5% for the Treatment of Onychromycosis of the Toenails. Press Release, July 8, 2014; http://investor.anacor.com/.

Anti-bacterial activity: Motif Combination



Glycopeptide antibiotics inhibit enzymatic reactions related to the synthesis of the cell wall

Antibiotics bind in a highly selective manner to the terminal D-Ala-D-Ala of a peptidoglycan precursor

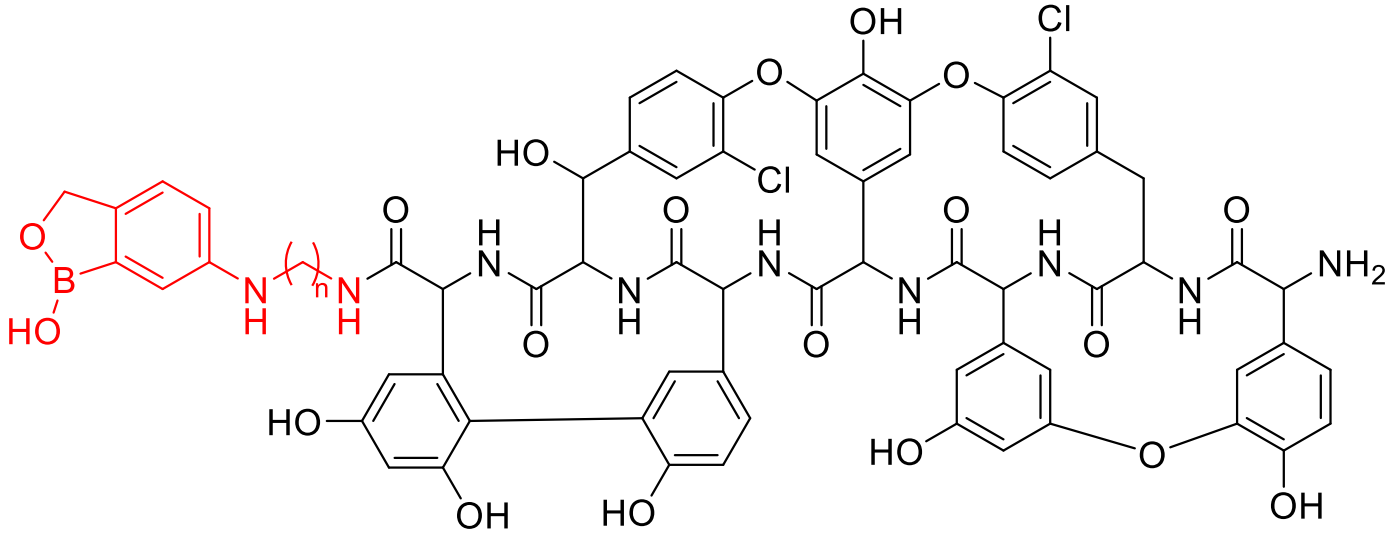
Resistance occurs when bacterium express resistance genes of a D-Ala-D-lactate instead

Enterococcus causes: UTIs, bloodstream infections as well as infection of hospital wounds

Incorporation of benzoxaborole into well-known antibiotics (vancomycin, eremomycin, and teicoplanin aglycone) could demonstrate activity against vancomycin-resistant enterococci

Teicoplanin aglycone benzoxaborole derviatives separated by an amide linker demonstrated 4-16 µg/mL activity against vancomycin-resistant enterococci

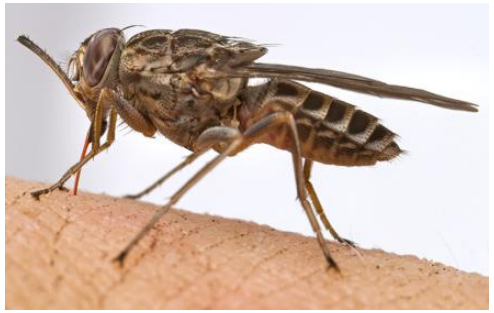
- Compared to >32 µg/mL for the antibiotics



n	*E. faecium* (µg/mL)
2	16
3	4
5	16

Adamczyk-Woźniak, A.; Borys, K. M.; Sprozyński, A. *Chem Rev* **2015**, *115*, 5224–5247.
Preobrazhenskaya, M. N. et al. *Future Med. Chem.* **2013**, *5*, 641–652.
Labmanual/clinlab/micro_images/bacteriology/climincal gram stains/blood cultures. http://labmedx.ucsfmedicalcenter.org (Accessed 17 Jan 2016).

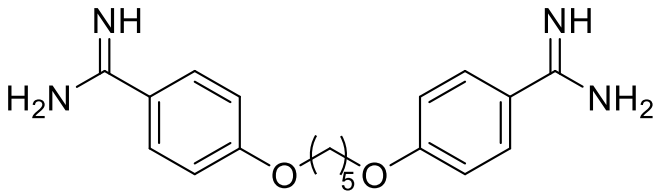
Anti-protoazoal activity – HAT



Human African trypanosomiasis (African sleeping sickness)

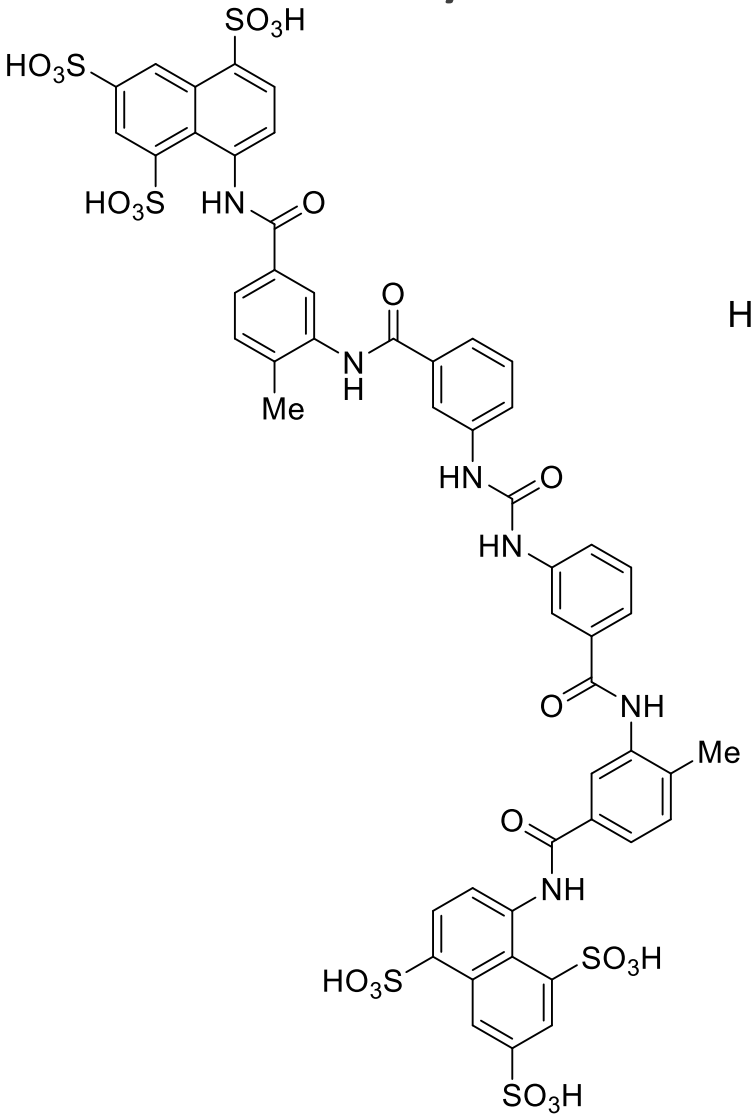
Fatal disease caused by *T. brucei* transmitted by a bite from the testse fly

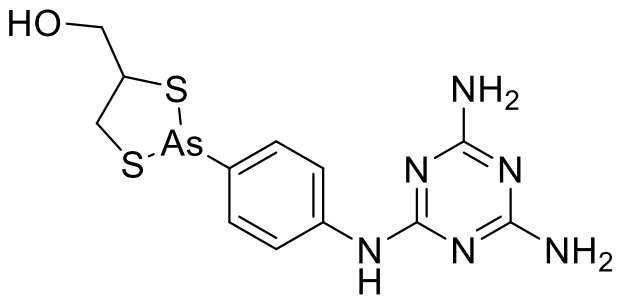
Stage 1 HAT – bloodstream; treated with pentamidine and suramin

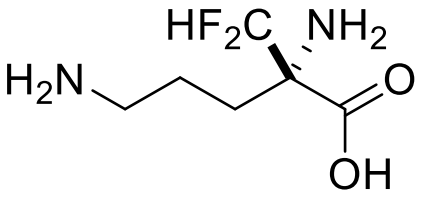


Stage 2 HAT – parasites migrate across blood-brain barrier (hallucinations, coma and death); treated with melarsoprol and eflornithine

Current medications are expensive, toxic and low efficacy







Jacobs, R T. et al. *Future Med. Chem.* **2011**, *3*, 1259–1278.
Zhou, H.: Jacobs, R. T. et al. *ACS Med. Chem. Lett.* **2010**, *1*, 165–169.
Jacobs, R. T.; Plattner, J. J.; Don, R. *Curr. Top. Med. Chem.* **2011**, *11*, 1301–1303.
The Smaller Majority. Mozambique Diary: Not all flies fly. http://thesmallermajority.com/2014/11/25/

Stage 2 HAT – *T. brucei* inhibition

Mechanism of action is unknown for benzoxaboroles

SAR studies highlighted 3,3-disubstituted benzoxaborole 6-carboxamides as targets for stage 2 CNS HAT

Aqueous solubility and lipophilicity (logD) were within the acceptable range for oral availability

Predicted good permeability across blood-brain barrier

Predicted little interaction with P-gylcoprotein (Pgp)-efflux transporter

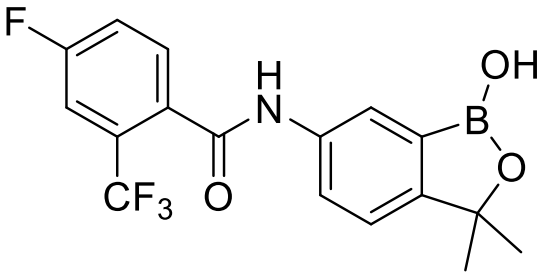
- Flushes foreign objects from cells

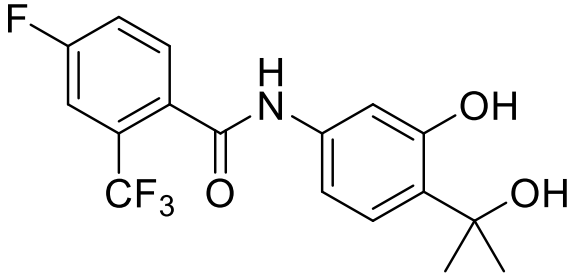
Good *in vitro* and *in vivo* (mice, monkeys and dogs) activity

For CNS HAT – dosing started 21 days after infection (25 mg/kg, qd, oral administration) and lasted a week

- 100% cured – lack of paraistemia in blood for 180 days after final dose

Slowly metaboilized – only 1-5% of original drug underwent oxidative deboronation





Assay (Units)	Key Properties
T. b. brucei 427 (µg/mL)	IC_{50} = 0.29
Solubility, pH 7.4 PBS (µM)	25
Lipophilicity (logD)	3.51
Permeability, MDCKII-hMDR1 monolayer (nm/sec)	Papp = 415
Pgp efflux liability, MDCKII-hMDR1 monolayer (none)	AQ = 0.03
Metabolic stability, mouse S9 fraction	$t_{1/2}$ > 350

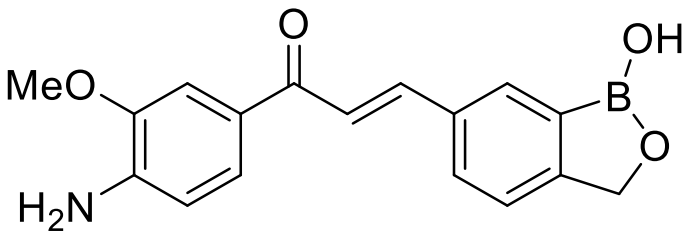
Jacobs, R. T.; Plattner, J. J.; Don, R. *Curr. Top. Med. Chem.* **2011**, *11*, 1301–1303.
Jacobs, R T. et al. *Future Med. Chem.* **2011**, *3*, 1259–1278.
Zhou, H.; Jacobs, R. T.; et al. *ACS Med. Chem. Lett.* **2010**, *1*, 165–169.

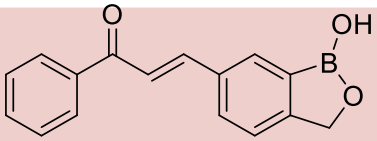
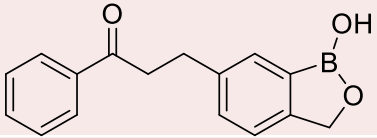
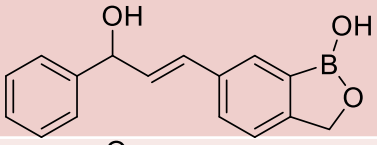
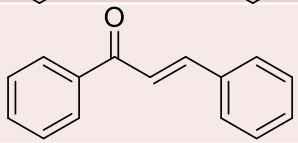
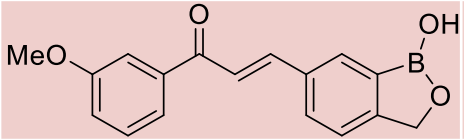
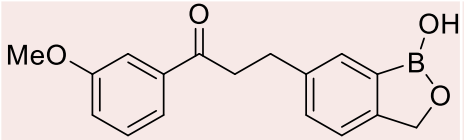
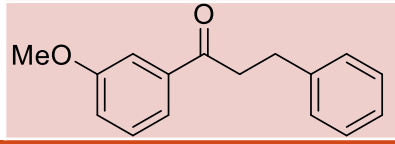
Synergistic Inhibition: Chalcone-Benzoxaborole

Both benzoxaboroles and chalcones have separately demonstrated antiprotozoal activity

Chalcone-benzoxaboroles hybrid molecules were an example of synergy inhibition

Forty compounds were tested with the highest activity of 10 ng/mL with 4-amino-3-methoxyphenyl chalcone



Compound	*T. brucei* IC_{50} (µg/mL)	L929 IC_{50} (µg/mL)	Decrease in activity
	**0.089**	**>10**	**Parent Compound**
	0.331	>10	4-fold
	0.239	2.88	3-fold
	0.473	6.92	5-fold
	**0.022**	**>10**	**Parent Compound**
	0.690	>10	31-fold
	0.395	5.70	18-fold

Adamczyk-Woźniak, A.; Borys, K. M.; Sprozyński, A. *Chem Rev* **2015**, *115*, 5224–5247.
Zhou, H.; Jacobs, R. T. et al. *J. Med. Chem.* **2012**, *55*, 3553–3557.

Synergistic Inhibition: Chalcone-Benzoxaborole

Demonstrated vitro studies using first stage HAT with bloodstream *T. brucei*

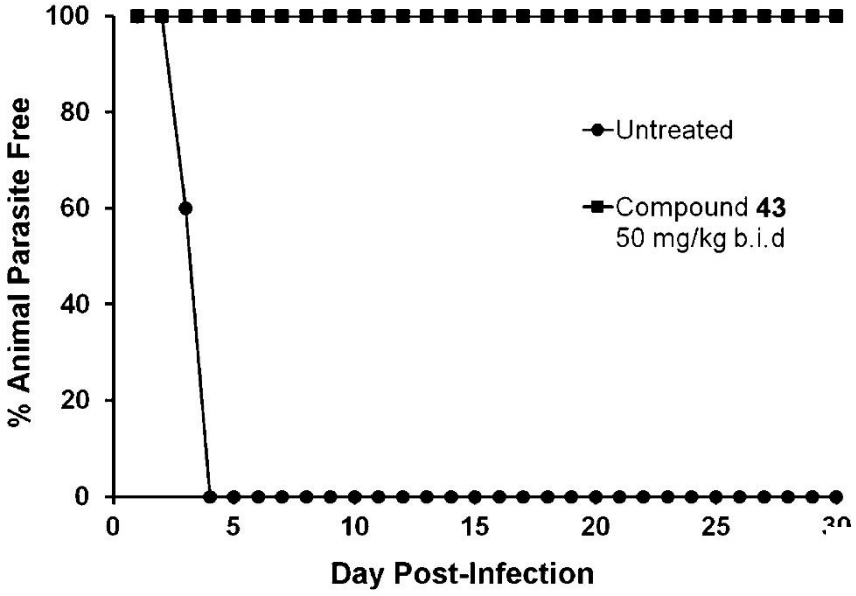
Showed in vivo studies in a murine model of first stage HAT

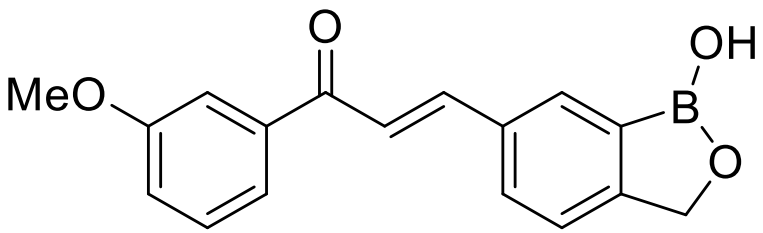
Mice were infected with 600 *T. b. brucei* 221 parasites

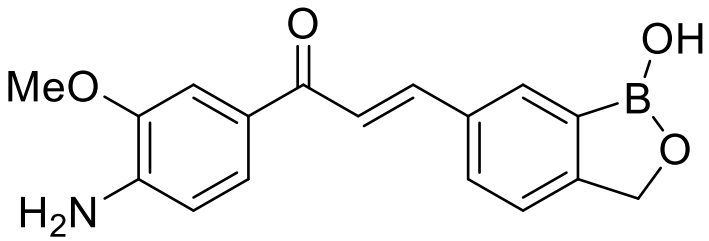
24 h postinfection – started 50 mg/kg bid, ip, dosage for five days

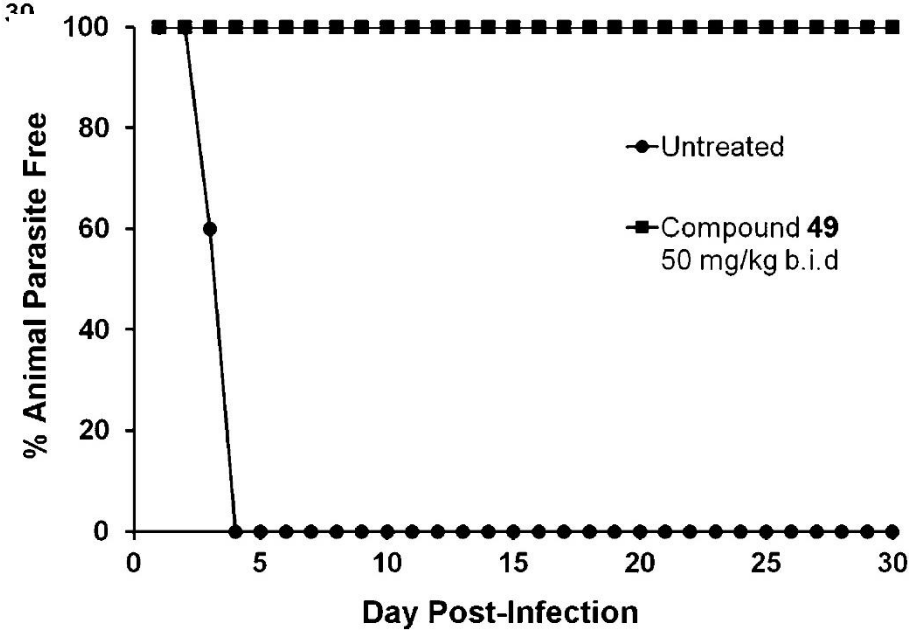
- Intraperitoneal injection

100% survival rate was observed 30 days after infection









Zhou, H.; Jacobs, R. T. et al. *J. Med. Chem.* **2012**, *55*, 3553–3557.

Anti-Inflammatory activity: PDE4 Inhibition

Inflammatory diseases: psoriasis, inflammatory bowel disease, asthma, and rheumatoid dermatitis

Inflammation and immune responses are promoted by excess cytokine production

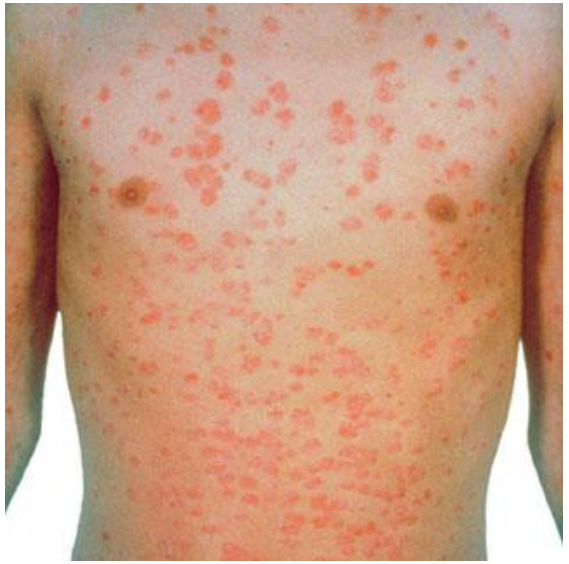
Mechanism of action – inhibition of cyclic nucleotide phosphodiesterase 4 (PDE4)

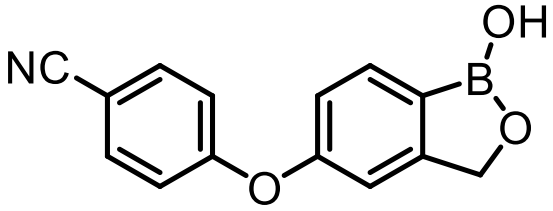
PDE4 catalyzes hydrolysis of cyclic adenosine monophosphate (cAMP)

- PDE4 inhibition → accumulation of cAMP → activation of protein kinase A→ inhibition of cytokines

Common clinical problem with therapeutics are side effects, such as emesis

SAR of phenoxybenzoxaboroles found AN2728 as the most promising lead





AN2728

Freund, Y. R. et al. *FEBS Letters* **2012**, *586*, 3410–3414.
Akama, T. et al. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2129–2132.
Adamczyk-Woźniak, A.; Borys, K. M.; Sprozyński, A. *Chem Rev* **2015**, *115*, 5224–5247.
Baker, S. J.; Ding, C. Z.; Akama, T.; Zhang, Y.-K.; Hernandez, V.; Xia, Y. *Future Med. Chem.* **2009**, *1*, 1275–1288.
National Psoriasis Foundation. Guttate Psoriasis. https://www.psoriasis.org/about-psoriasis/types/guttate (Accessed 12 Jan 16).

PDE4 inhibition

2014 – AN2728 (Crisaborole) entered into phase III of clinical trials for treatment of atopic dermatitis

Crisaborole inhibition of PDE4 was IC_{50} = 0.49 µM

SAR studies revealed 5-phenoxy groups with *para*-electron withdrawing groups were crucial

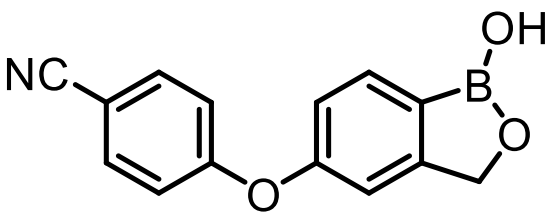
An addition of a cyano group to the *meta*-position increased inhibitory activity and is also in clinical trials

Co-crystallized PDE4 and AN2928 showed a tetrahedral boron bound to a water molecule held between a zinc and magnesium cation by x-ray crystallography

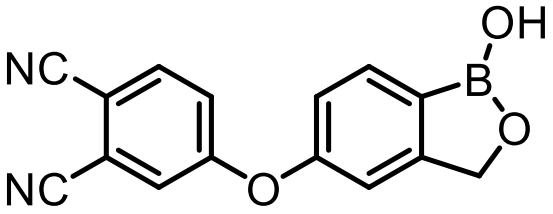
Fused benzo group interacts with Met and Leu

3,4-dicyanophenoxy π-stacks with Phe at the entrance of the hydrophobic pocket

5-phenoxy benzoxaboroles were found to be reversible, substrate-competitive binders



AN2728



AN2928

Freund, Y. R. et al. *FEBS Letters* **2012**, *586*, 3410–3414.
Akama, T. et al. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2129–2132.
Adamczyk-Woźniak, A.; Borys, K. M.; Sprozyński, A. *Chem Rev* **2015**, *115*, 5224–5247.
Baker, S. J.; Ding, C. Z.; Akama, T.; Zhang, Y.-K.; Hernandez, V.; Xia, Y. *Future Med. Chem.* **2009**, *1*, 1275–1288.

ROCK Inhibition: Novel Binding Mode

5- and 6-(aminomethylphenoxy)benzoxaboroles were subjected to a kinome-wide screen

- Resulted in discovered inhibition of Rho-activated kinases (ROCK)

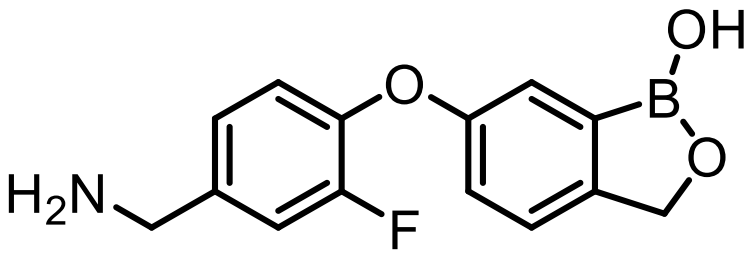
ROCK strongly affects inflammation and contraction of soft muscle tissue (i.e. asthma)

AN3484 – Strongly inhibited ROCK1 (IC_{50} = 0.81μM) and ROCK2 (IC_{50} = 0.54μM)

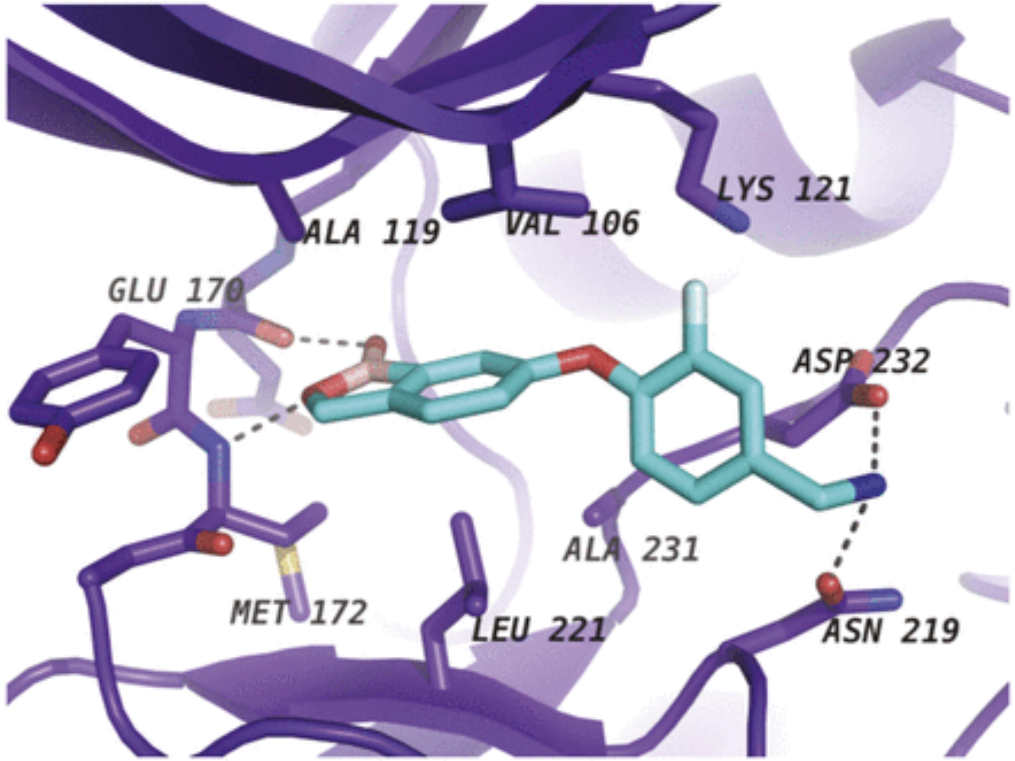
XRD of cocrystallized ROCK2 and AN3484 showed novel binding mode of oxaborole

Boron **stayed trigonal planar**: oxaborole formed two hydrogen bonds with methionine and glutamic acid in active site

Hydrogen bonds between asparagine and aspartic acid with the aminomethyl group



AN3484



Adamczyk-Woźniak, A.; Borys, K. M.; Sprozyński, A. *Chem Rev* **2015**, *115*, 5224–5247.
Akama, T. et al. *J. Pharmacol. Exp. Ther.* **2013**, *375*, 615–625.

Toxicity

O'Donovan tested 13 boronic acids with an Ames assay

- 12/13 were found to be weakly mutagenic

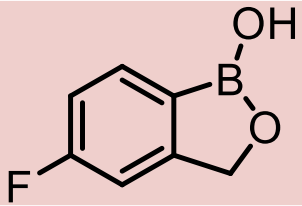
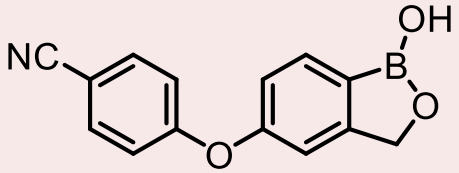
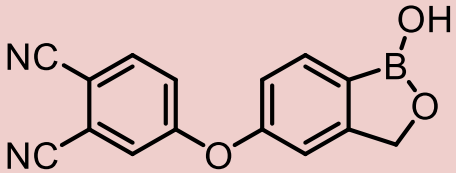
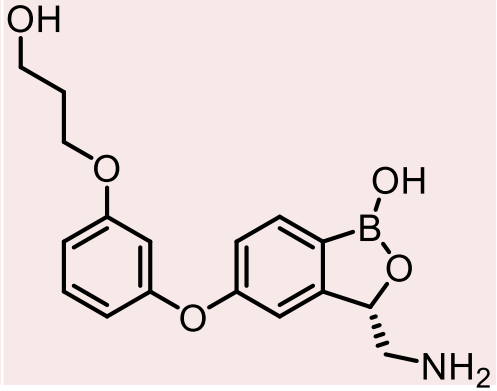
Safety of benzoxaborole therapeutic agents:

- Baterial reverse mutation (Ames) assay
- *In vitro* chromosome aberration assay with peripheral human lymphocytes
- *In vivo* rat micro nucleus study

All four compounds were found to negative in all three genotoxicity assays

No carcinogenic potential was also found for AN2690 after 2 year bioassays in mice and rats

Anacor Pharmaceuticals is releasing Long-Term Safety Study with Crisaborole (AN2728)

Compound	Structure	Therapeutic (administration)
AN2690		Onychomycosis (Topical)
AN2728		Atopic Dermatitis Psoriasis (Topical)
AN2898		Atopic Dermatitis Psoriasis (Topical)
AN3365		Gram-negative bacterial infection (oral and intravenous)

O'Donovan, M. R. et al. *Mutat. Res.* **2011**, *724*, 1–6.
Ciaravino, V.; Plattner, J.; Chanda, S. *Environ. Mol. Mutagen.* **2013**, *54*, 338–346.

Anacor Pharmaceuticals. Anacor Pharmaceuticals to Present Data From Long-Term Safety Study of Cisaborole Topical Ointment, 2% in Patients with Mild-to-Moderate Atopic Dermatitis at Upcoming Medical Conference. Press Release, January 8, 2016; http://investor.anacor.com/.

Future Directions

No report of anticancer activity

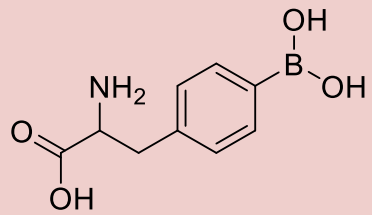
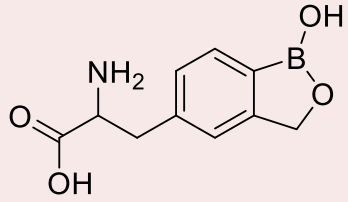
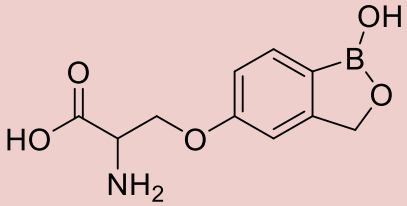
p-Boronophenylalanine (BPA):

- High affinity for tumor cells
- Low solubility at physiological pH

Benzoxaboroles analogs showed improved solubility

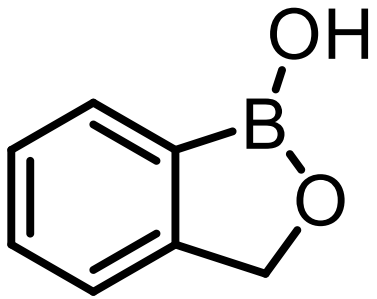
Possible boron neutron capture therapy (BNCT) application

- Irradiation with thermal neutrons →BPA absorbs neutrons → self destruction to ^{7}Li and α-particles → cell death

Structure	Solubility (mg/mL)
	1.6
	5.2
	3.6

Adamczyk-Woźniak, A.; Borys, K. M.; Sprozyński, A. *Chem Rev* **2015**, *115*, 5224–5247.
Li, X. et al. *Tet. Lett.* **2011**, *52*, 4924–4926.

SED Group Connection



Dr. Robert Jacobs

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Post Doc from Sept. 1985 to Aug. 1987