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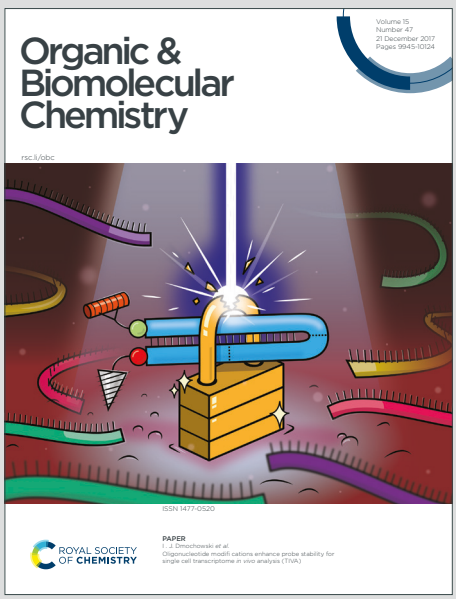

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Design, Synthesis and Structure of a Frustrated Benzoxaborole and its Applications in the Complexation of Amines, Amino Acids, and Protein Modification

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Jasmine Bhangu,[a] Randy M. Whittal,[b] Dennis G. Hall,*[a]

This study describes the design and synthesis of arylboronic acid **2**, the first example of a permanently open "frustrated" benzoxaborole, along with an exploration of its application in bioconjugation. An efficient and high yielding seven-step synthesis was optimized. NMR experiments confirmed that compound **2** exists in the open ortho-hydroxyalkyl arylboronic acid structure **2-I**, a form that is effectively prevented to undergo a dehydrative cyclization as a result of unfavorable geometry. Compound **2-I** conjugates effectively with amines to form stable hemiaminal structures, including a highly effective and irreversible reaction with lysozyme. Complexation with cysteine induces an open structure containing a free hydroxymethyl arm, with the amino and thiol groups reacting preferentially with the formyl group to form a N,S-acetal.

Introduction

Boronic acids and their cyclic hemiboronic acid derivatives have recently emerged as new and unconventional chemotypes in drug discovery efforts.[1] These compounds offer desirable properties as mild Lewis acids, with the unique ability to undergo reversible exchange of their boron-hydroxy bonds with alcohols and carboxylate groups on target biomolecules. In particular, benzoxaboroles are a class of stable cyclic 5-membered hemiboronic acids that has received significant attention owing to the recent approval of two new drugs, tavaborole and crisaborole (Figure 1a), which are marketed for the treatment of onychomycosis and psoriasis, respectively.[2,3] Other derivatives have shown potential towards a range of health issues,[4] along with significant success and further promise in other applications like carbohydrate recognition, catalysis, and as biomaterials.[4,5] To fully exploit the potential of benzoxaboroles, complementary studies must be conducted to understand their structural and physicochemical properties. In this regard, our group recently demonstrated, through NMR spectroscopic evidence, that benzoxaborole exists largely in its closed form in aqueous media, yet it can undergo rapid and reversible hydrolysis to the open form (Figure 1b).[6]

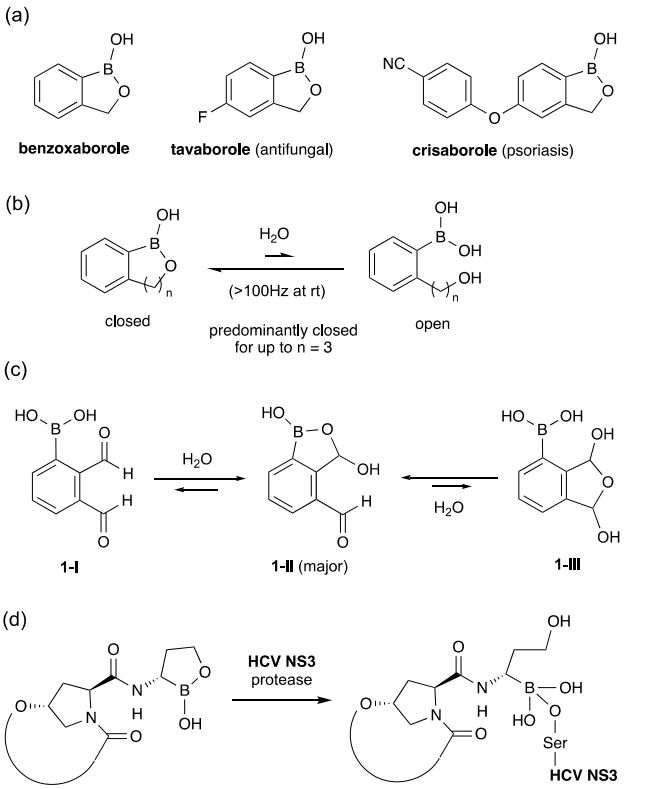

Fig. 1. Benzoxaborole derivatives (a); and various, closed and open hydrated forms (b)-(d).

This sort of new and important knowledge can help provide a better understanding of the pharmacokinetic and

[a] Department of Chemistry, University of Alberta, 4-010 Centennial Centre for Interdisciplinary Science, Edmonton, Alberta, Canada T6G 2G2.
[b] Mass Spectrometry Laboratory, Department of Chemistry, University of Alberta.
† Footnotes relating to the title and/or authors should appear here.
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pharmacodynamic behaviour of benzoxaborole drugs. Remarkably, there appears to be no example of a permanently open benzoxaborole derivative. Lulinski and Serwatowski studied the ortho-phtalaldehyde derivative **1**-**I**, which was found to exist predominantly in its closed form **1**-**II** in wet organic solvent, with **1**-**III** as a minor open form (Figure 1c).[7] The only reported instance of a temporary open member of this general class of boron heterocycles is an aliphatic α-amino oxaborole bound to the NS3 serine protease (Figure 1d).[8] Thus it remains unknown what unique properties a predominantly and permanently open benzoxaborole would possess, and how these properties can guide the development of new applications in chemical biology and catalysis. Herein, we present the design and synthesis of compound **2**, a geometrically restricted ortho-hydroxyalkyl arylboronic acid that is incapable of undergoing a dehydrative cyclization to afford its closed form (Figure 2). Being prevented from existing in the closed form of benzoxaboroles normally preferred, ortho-hydroxyalkyl boronic acid **2** thus can be viewed as a "frustrated benzoxaborole". Moreover, it was recognized that **2**-**I**, a hemiacetal, may interconvert with its hydroxy-aldehyde form **2-IV**, leading to an efficient complexation of biological amines and amino alcohols to give adducts **3** (Figure 2a). Herein, the structure of compound **2** is investigated along with its potential applications in the complexation of amines and amino acids, and protein modification.

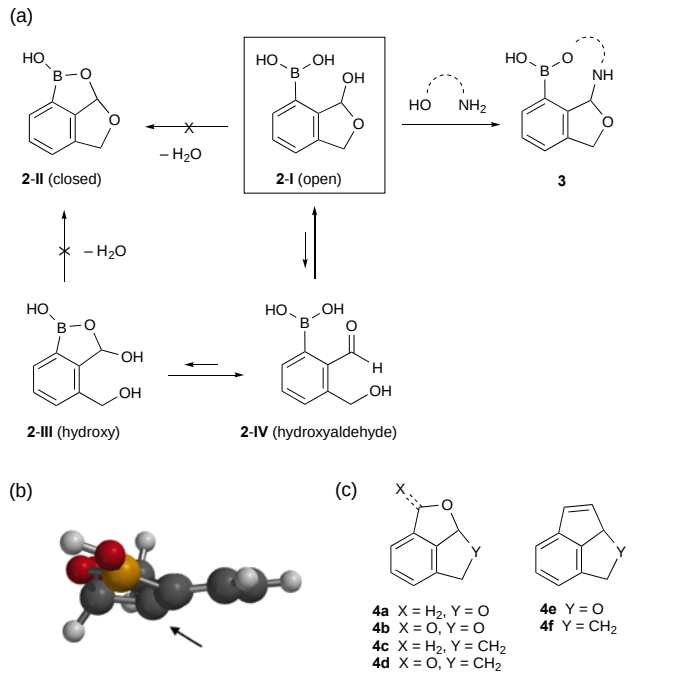

Fig. 2. (a) Design of a permanently open, "frustrated" benzoxaborole compound, **2**-**I**; (b) energy-minimized model of the putative closed form **2**-**II** showing severe distortion of the arene unit; (c) corresponding carbon isosteres of **2**-**II**.

Results and Discussion

Design and Synthesis

To favor the open form of an ortho-hydroxyalkyl arylboronic acid, the intrinsic energy of the closed form (i.e., a benzoxaborole ring) needs to be raised significantly. It was anticipated that such a frustrated benzoxaborole could be designed by imposing ring strain into the putative closed form. In line with this stratagem, the α-hydroxyalkyl unit of arylboronic acid **2**-**I** is expected to be geometrically restricted as a result of its incorporation into a strained 5-membered hemiacetal. Presumably, the secondary hydroxy group of **2**-**I** would be incapable of undergoing a dehydrative cyclization to the corresponding benzoxaborole, **2**-**II**, a tricyclic fused ring system rendered highly unfavorable due to the extreme angle strain imposed on the fused arene unit. Molecular modeling (AM1) of both forms **2**-**I** and **2**-**II** provides strong support for this proposal. Even when normalized for bond enthalpy due to the loss or addition of water, compared to benzoxaborole the closed form **2**-**II** was found to be significantly higher in energy.[§] Indeed, the energy-minimized bowl-shaped structure of **2**-**II** shows significant distortion of the arene ring with the central arene carbon laying out of plane with bond angles that deviate significantly (up to 13°) from the ideal planar configuration of such an sp^2 carbon (Figure 2b). Also presaging of the unlikelihood of form **2**-**II** is the lack of precedence for most of the analogous C–C isosteres, such as **4a**-**f**, which are presumably too unstable to prepare or even exist.[9]

The synthesis of target compound **2** from commercial material **5** involved the preparation of the known dibromide **7** with small modifications to the published procedures.[10] From **7**, formation of aldehyde **8** was achieved in high yield using a standard lithiation/formylation reaction. A classical Miyaura borylation of **8** installed the requisite boronyl group leading to intermediate **9** in a moderate yield. The formyl group was then reduced to the corresponding alcohol **10** without difficulties. Attempts to transform this intermediate directly into **2** by a concomitant acidic hydrolysis of both the acetal and the boronic ester led to issues of product isolation. Instead, the conversion of boronate **10** into the corresponding trifluoroborate salt is effectively achieved using aqueous KHF_2, releasing HF in the reaction medium, which in turn cleaves the acetal in situ to yield **11** as a readily isolable precipitate. The final hydrolytic step in this synthesis proved to be tricky as it was predicted that the target compound **2** would be highly water-soluble and therefore difficult to isolate and purify. This problem was easily overcome by using a strong, solid fluorophile, in this case silica gel, a procedure developed by Molander and co-workers to extract the fluorides from boron without any contamination of the aqueous phase with salts.[11] However, since silica gel is partially soluble in water, the clean product from the aqueous phase was contaminated with a small amount of silica gel. To fix this issue, the reaction is instead diluted with acetone, then silica gel is easily separated by filtration and the filtrate concentrated *in vacuo*. For purification, the white crude solid was dissolved in water, and any intractable organic impurities are separated by filtration. Product **2** is obtained in good yield with only minor impurities (<5% according to ^1H NMR) after a simple evaporation of the aqueous phase.

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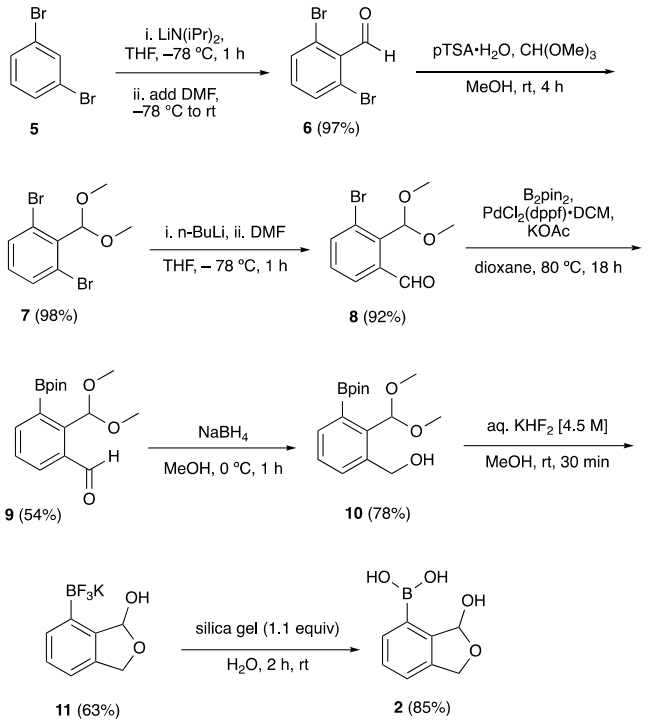
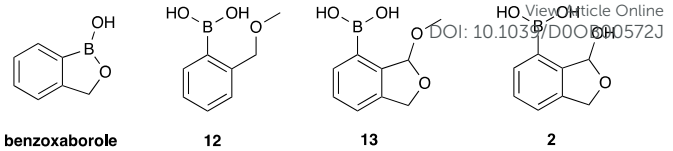


Scheme 1. Synthesis of target compound **2**.



benzoxaborole	12	13	2
^{11}B : 32.3 ppm	**^{11}B = 29.5 ppm**	**^{11}B = 28.7 ppm**	**^{11}B = 30.3 ppm**
OH : 9.15 ppm	OH = 8.03 ppm	OH = 7.86 ppm	OH = 8.09 ppm
CH_2O : 4.97 ppm	CH_2O : 4.50 ppm	CH_2O : 4.98, 4.90 ppm	CH_2O : 5.09, 4.90 ppm

Fig. 3. Diagnostic NMR signals (DMSO-d6, with one drop of H_2O) for benzoxaborole, derivatives **12**-**13**, and target compound **2**.

Structure Elucidation

With the synthesis of **2** complete, the next task was to conclusively address its equilibrium position in aqueous and non-aqueous media and whether or not it exists under form **2**-**I** (cf. Figure 2). To this end, product **2** was analyzed initially by ^{1}H NMR spectroscopy using several organic solvents. In very dry DMSO, the ^{1}H NMR spectrum revealed an extensive formation of boroxine and other oligomeric anhydrides.§ To hydrolyze these species, one drop of H_2O was added to the NMR tube, which considerably cleaned the spectrum. The absence of resonances associated with a diastereotopic methylene and aldehydic hydrogens ruled out the existence of forms **2-III** and **2-IV**. One way to assess if a benzoxaborole structure is closed or open is to compare NMR shifts of relevant derivatives. Chosen for this purpose were benzoxaborole, along with 2-methoxymethylphenyl boronic acid (**12**) and intermediate **13**, a methylated thus permanently open derivative of **2** (Figure 3). When ran in DMSO-d6 (with one drop of H_2O to suppress boronic anhydrides), benzoxaborole displays a ^{11}B chemical shift of 32.3 ppm compared to 29.5 ppm and 28.7 ppm, respectively, for **12** and **13**. The newly designed frustrated benzoxaborole **2** displays a ^{11}B chemical shift of 30.3 ppm, which suggests its structure is similar to the open control compounds **12**-**13**.

The ^{1}H chemical shift of the protons on the B(OH) moiety also act as a diagnostic signal. In wet DMSO, these B(OH) protons do not appear to exchange rapidly, thus providing sharp and distinct resonances. With benzoxaborole, the B(OH) resonance shows up at 9.15 ppm while all the other B(OH) ^{1}H shifts on open boronic acid compounds, **12**, **13** and **2**, appear at ~8.0 ppm. The AB resonances of the diastereotopic CH_2O unit within the benzofuran unit are also in line with those assumptions. Compound **2** displays chemical shifts of approximately 4.9–5.1 ppm that are very similar to that of **13**, and about 0.5 ppm higher than the corresponding resonances in **12** and the open derivative **10**.

The ^{1}H NMR spectrum of **2** in slightly wet acetone (again to prevent the dimerization and oligomerization of B(OH) groups) was also very informative (Figure 4). Under these conditions, all hydroxy protons were found to exchange with the solvent at a rate slower than the spectrometer frequency, and are thus observable as distinct, sharp peaks. The singlet at 7.6 ppm, with an integration of 2H, was attributed to the boronic acid resonances, whereas the doublet at 6.6 ppm was tentatively assigned to the hemiacetal OH group (coupled with the benzylic methine). These assignments were confirmed by a deuterium-exchange experiment; not only did these peaks disappear, the doublet at 6.6 ppm became a singlet, thus confirming this resonance belongs to the methine of the benzylic hemiacetal. Altogether, these NMR spectroscopic studies in wet polar solvent provide overwhelming evidence that compound **2** exists exclusively in its open form, **2-I**, not in the closed oxaborole form **2-II**.

A key feature of a free boronic acid moiety is the ability to dehydrate trimolecularly to form a 6-membered boroxine under dry solvent conditions or in the gas phase. With this knowledge in mind, the behavior of compound **2** was examined by high-resolution ESI-MS. A trimeric boroxine was not observed.§ Instead, compound **2** displays the loss of only two molecules of water to form the putative dimer **2**-**V**, which can only originate through the involvement of the alcohol unit in the open form **2**-**I** (Figure 5a). Such a doubly dehydrated dimer does not occur with either benzoxaborole or the blocked o-hydroxymethyl arylboronic acid **12** (Figure 5b). Therefore, mass spectrometric analysis corroborates the above NMR evidence in support of the open, "frustrated" boroxole form (**2**-**I**) of compound **2**.

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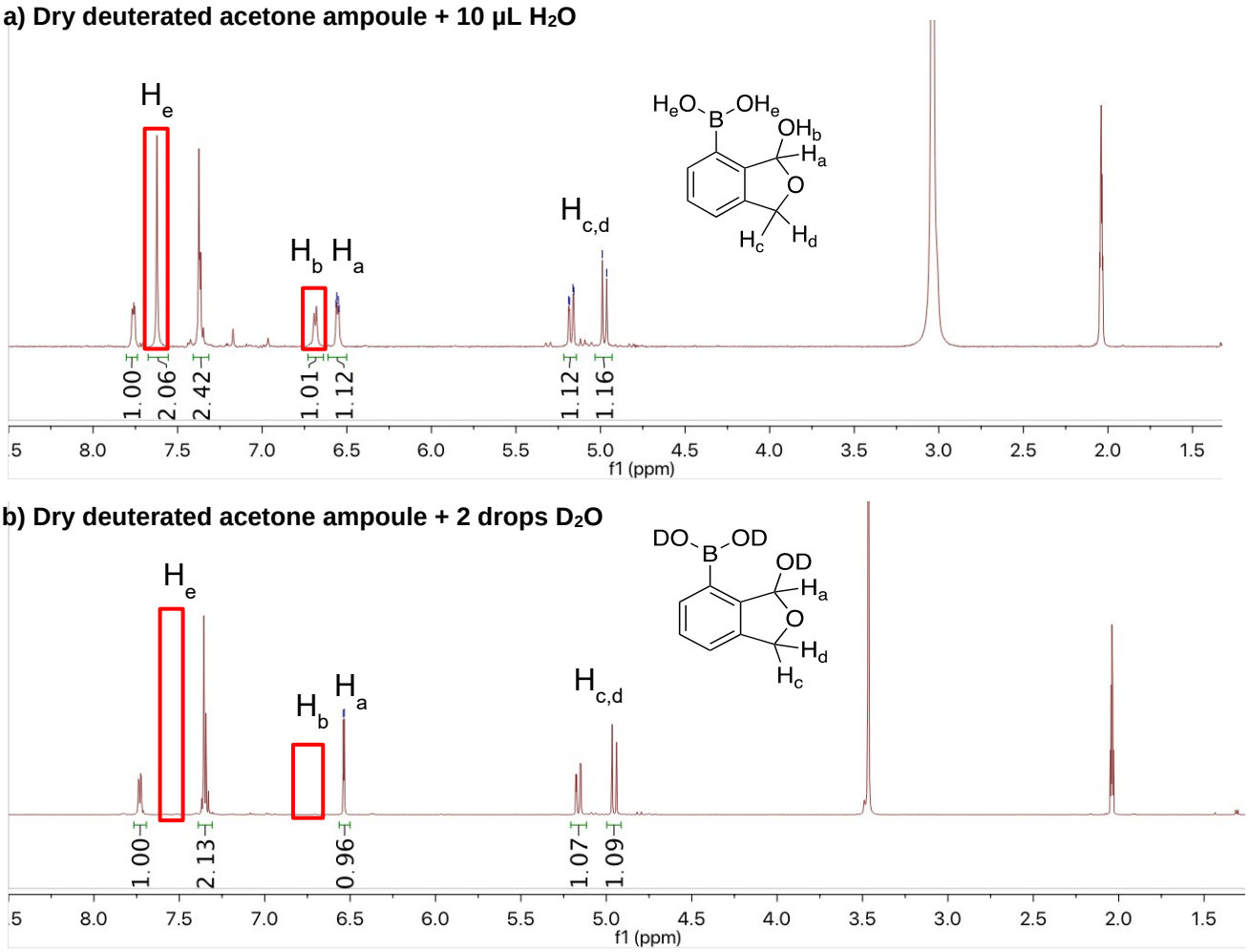

Fig. 4. ^{1}H NMR spectra of compound **2** in acetone-d6 with (a) 10 µL H_2O; (b) one drop of D_2O.

Equilibrium Dissociation in Water

Having convincingly demonstrated that compound **2** exists predominantly in its open "frustrated benzoxaborole" form **2-I**, we turned towards a preliminary examination of the reactivity of this unique molecule, starting with its equilibrium dissociation in water. Boronic acids behave as Brønsted acids through indirect ionization of water leading to the formation of a tetrahedral hydroxyborate anion as the conjugate base. It is well established that benzoxaboroles generally display a pKa approximately one to two units lower than the corresponding arylboronic acids. For example, the reported pKa of 7.2 for benzoxaborole is almost two units lower than that of phenylboronic acid (8.9).[12] Here, the pKa of compound **2** was measured using a ^{11}B NMR titration method (Figure 6). To our surprise, the resulting pKa of 7.5 is closer to that of benzoxaborole (reproducibly measured at 7.2 in our hands) compared to a normal boronic acid. Moreover, the titration curve for **2-I** is significantly steeper than the broader transition curve displayed by benzoxaborole,[§] with less than one pH unit difference from the first to the second plateau. This difference

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in the transition suggests that benzoxaborole and **2-I** may feature different a mechanism of capturing and stabilizing hydroxy ions. Whereas benzoxaborole's low pKa is likely due to favorable release of angle strain upon hydroxy coordination to afford a tetrahedral sp^3 boron, the low pKa of **2-I** may be explained by a stabilization of the conjugate trihydroxyborate conjugate base **2-VI** by the hydrogen-bonding effect of the ortho hydroxyalkyl unit (Fig. 6a).

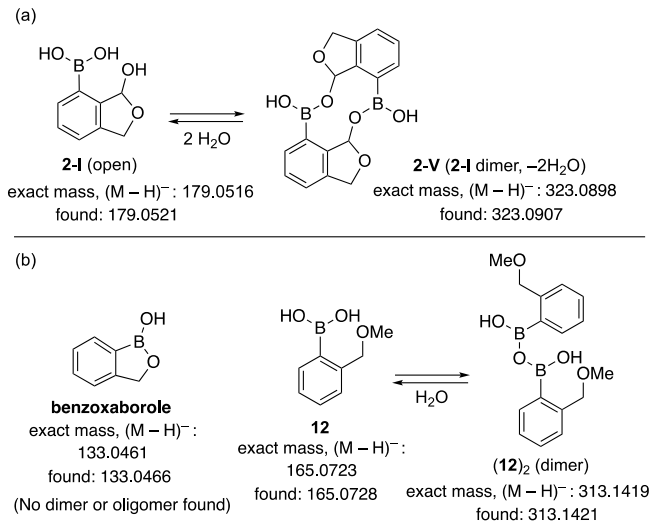

Fig. 5. Major species observed in the electrospray (neg. mode) mass spectral analysis of: (a) compound **2**; (b) control compounds: benzoxaborole, and **12**.

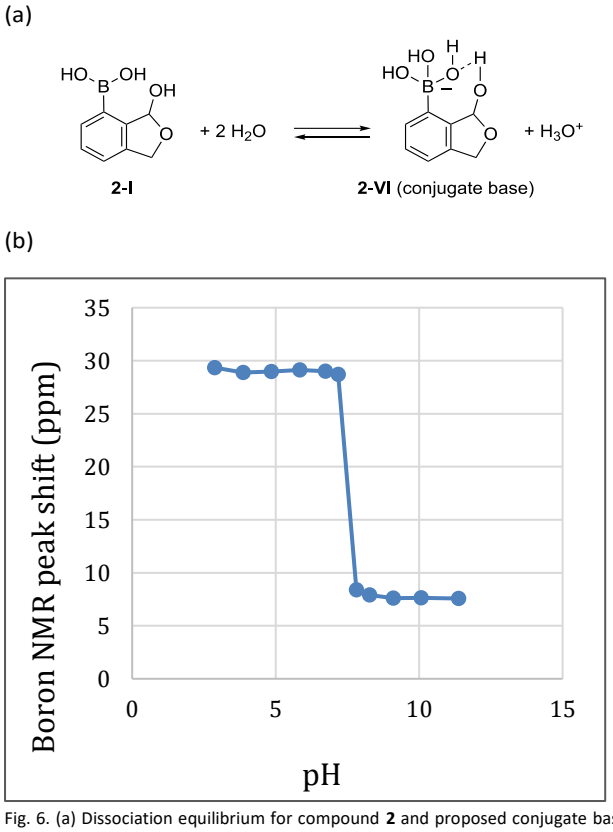

Fig. 6. (a) Dissociation equilibrium for compound **2** and proposed conjugate base **2-VI**; and (b) Acid-base titration by ^{11}B NMR in NH_4OAc buffer.

Reactivity with Monoamines

It is well established that the carbonyl groups of 2-formylphenylboronic acid (2-FPBA)[13] and 2-acetylphenylboronic acid (2-APBA)[14] can condense readily with amines in aqueous media to form the corresponding imines, which are stabilized by a dative B–N bond with the adjacent boronic acid (Figure 7a). These types of structures, termed “iminoboronates”, provide reversible bioconjugates.[15] The potential ability of **2-I** to undergo reversible exchange with the hydroxy-aldehyde form **2-IV** could provide a method for ligating amines in aqueous conditions through formation of aminal derivatives **3** (Figure 2). By analogy with the "water-inserted" ortho-dimethylaminoalkyl benzeneboronate structure described by Anslyn and co-workers,[16] these complexes may be particularly favorable in water. The dynamic nature of the hydroxymethyl arm on compound **2-I**, however, could potentially result in a more stable and possibly irreversible hemiaminal structure, which could be favored over the imine isomer (Figure 7b).

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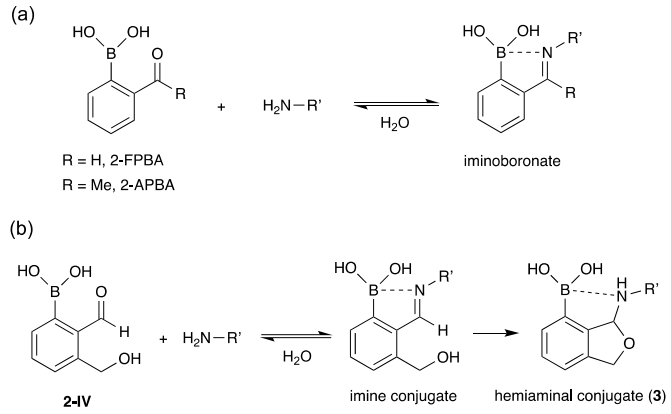

Fig. 7. Amine conjugation with 2-FPBA, 2-APBA (a); and with reagent **2** (b).

Initial studies conducted to study the binding of **2** with benzylamine were performed in acetone and monitored via NMR and LC-MS for any signs of conjugation. Shown in Figure 8 is the ^{1}H NMR spectrum of compound **2-I** in deuterated acetone, after the addition of one equivalent of benzylamine (Figure 8). The most important change in the spectrum is the upfield shift of the hemiacetal proton H_a, from 6.54 ppm in the unbound compound **2-I** (c.f., Fig. 4), to 6.05 ppm after addition of benzylamine. This observation confirms that the hemiacetal hydroxy group on compound **2-I** has been replaced by benzylamine to form the aminal **3a**. This result provides strong evidence that structure **2-I** is dynamic in water and affords enough of the ring-opened isomer **2-IV** to enable the formation of an imine with benzylamine. Subsequent ring closing of the hydroxymethyl group onto the C=N bond (c.f., Fig. 7b) provides the 5-membered hemiaminal structure **3a**. The ^{11}B NMR spectrum of isolated **3a** shows a major resonance with a chemical shift of 28.9 ppm consistent with a neutral trigonal boronic acid. A minor species (δ ~3 ppm) is likely associated with its partial ionization into the trihydroxyboronate conjugate base.

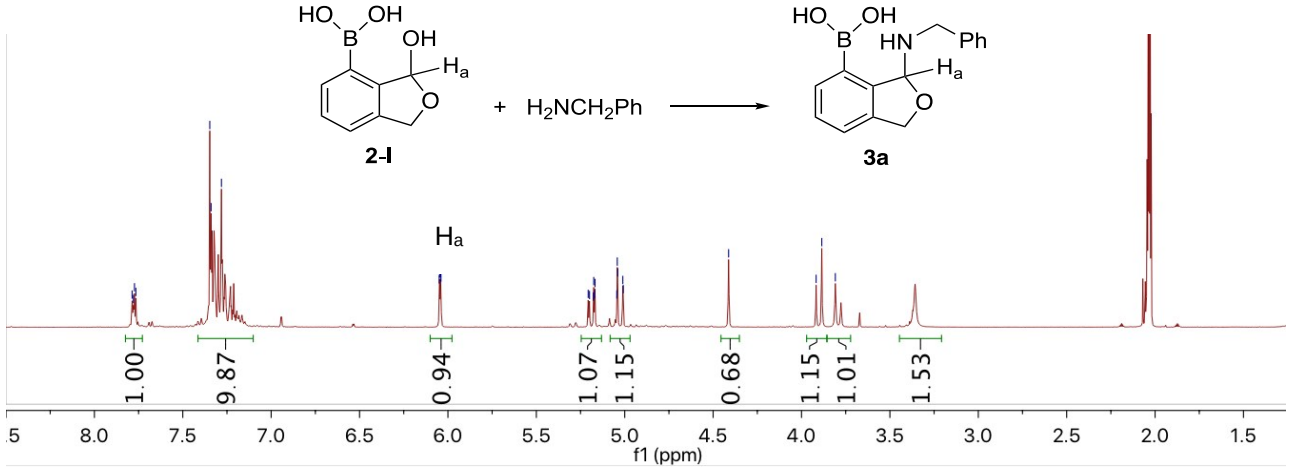

Fig. 8 ^{1}H NMR spectra of conjugate of compound **2-I** with benzylamine in acetone-d_6. (See Fig. 4 for the spectrum of free **2-I**)

The outcome of this NMR analysis was corroborated by the LC-MS chromatogram of the benzylamine addition product, which shows mostly the mass of the hemiaminal product **3a** after 0.5 h, with only a small amount of leftover starting material.§ As expected, 2-hydroxybenzylamine also forms an aminal with reagent **2**. According to mass spectrometric analysis, it undergoes further dehydration likely to form a hemiboronic ester with the phenolic hydroxy group. §

Complexation with Amino Acids and Esters

The complexation of bifunctional molecules such as amino acid derivatives was examined next. 'Click' chemistry has become a valuable tool in the area of bioconjugation as it can provide a quick, easy and stable method for labeling amino acids and peptides. Relatively few methods exist where a compound can undergo a chemoselective bioconjugation reaction with a naturally occurring functionality on a peptide. One of these methods utilizes 2-FPBA and 2-APBA, which can form relatively stable imines with N-terminal residues on proteins.[13-15] As described by Gao and Gois, 2-FPBA and 2-APBA are also known to bind to *N*-terminal cysteines and form a thiazolidino boronate (TzB) structure (Figure 9a).[17,18] TzB structures, unlike iminoboronates, are stable to competing biomolecules present in solution, although they are also known to be reversible in slightly acidic or basic solutions. It was proposed that compound **2** would form a similar type of structure, but instead have enhanced stability due to the additional adjacent hydroxymethyl arm that may form an additional ring on the conjugate structure. Therefore, binding with compound **2** could potentially form a fused 3-membered ring conjugate that would be more stable and irreversible within a wide range of pH (Figure 9b).

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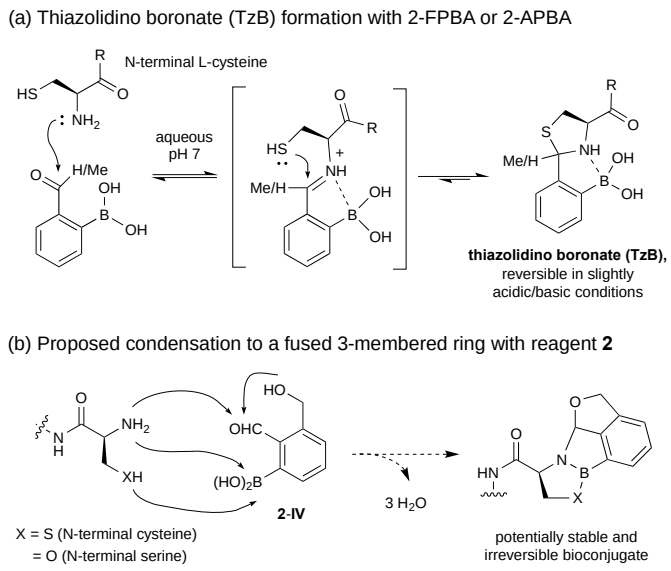

Fig. 9. *N*-terminal amino acid conjugation with 2-FPBA, 2-APBA and compound **2**.

As an initial test for cysteine and serine conjugation, compound **2** was dissolved in ammonium acetate buffered aqueous solution with one equivalent of each amino acid and left to stir at room temperature. In the case of *N*-terminal cysteine, a white solid material crashed out of solution within 5 minutes. This material was collected by filtration and analyzed by ^{1}H NMR spectroscopy. Some of the possible condensation adducts are shown in Figure 10a. In addition to the strained triple-dehydration tricycle **14**, a double-dehydration aminal, **15**, may also form, along with a ring-open thiazolidine **16** similar to that observed by Gao and Gois.[17,18]

There are a few notable details in the resulting ^{1}H NMR spectrum of Figure 10b. Firstly, similar to the benzylamine conjugation described above, in the presence of cysteine the hemiacetal proton H_a has once again shifted upfield, from 6.5 ppm in free **2-I** (c.f. Fig. 4b) to around 6.3 ppm. Secondly, the methylene protons, H_c and H_d, have shifted vastly from 5.0 ppm to 4.5 ppm. By analogy with the model compounds of Figure 3 (and also compound **10**), this chemical shift most likely indicates that the methylenoxy arm is open. Together this information strongly suggests that the conjugate between cysteine with compound **2** has a structure with an open –CH_2OH arm, which rules out structures such as **14** and **15**. Thus the proposed thiazolidine adduct **16** is consistent with the formation of TzB adducts observed with 2-FPBA and 2-APBA (c.f. Fig. 9a), not with a tricyclic adduct such as **14**, which is presumably too strained to form. Moreover, the ^{11}B NMR chemical shift of 11.3 ppm[§] for isolated **16** is in line with data obtained for the simpler TzB adducts (~10 ppm) and supports a neutral, tetrahedral species with N–B coordination promoted by the strained nature of this acyloxy adduct.[17] The HRMS data of **16** further supports this structure.[§] Attempts to observe a conjugate between serine and compound **2** met with minimal success, with only starting materials and a few minor peaks appearing in the ^{1}H NMR spectrum after 24 hours.[19]

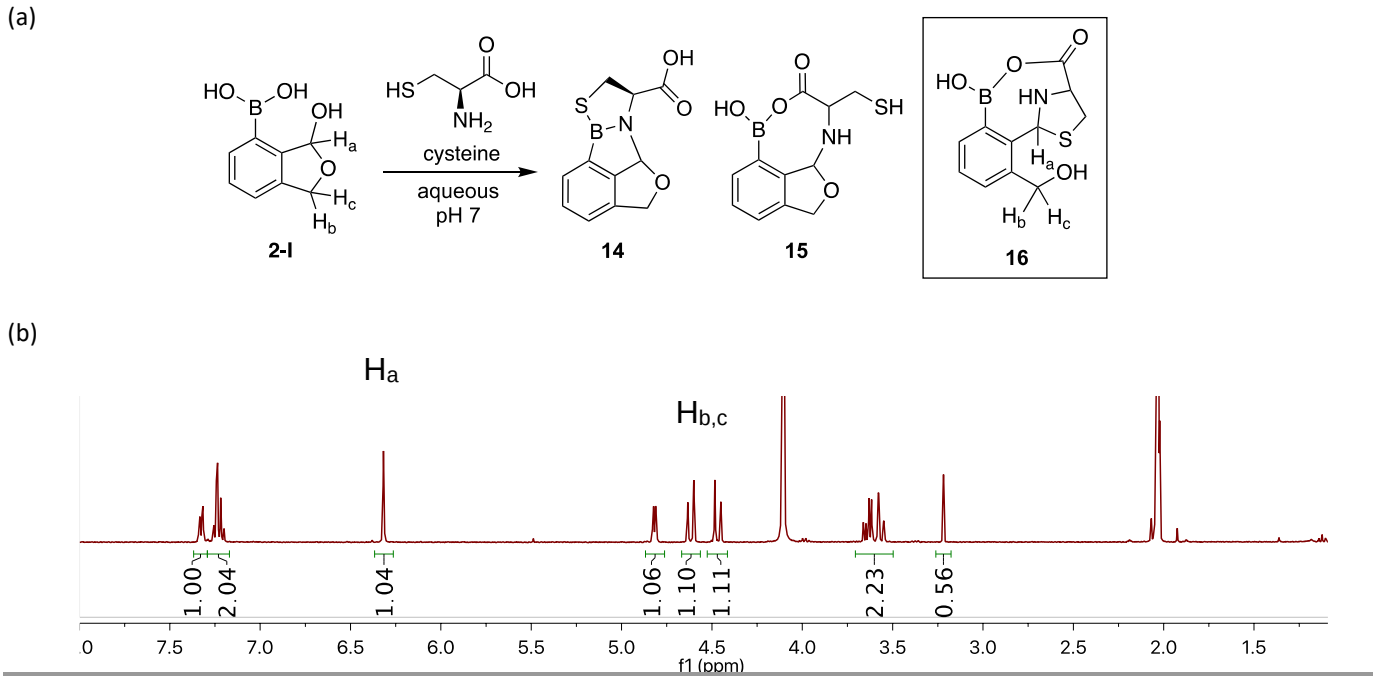

Fig. 10. (a) Possible structures of the equimolar adduct between compound **2** and free cysteine. (b) ^{1}H NMR spectrum of cysteine conjugate of compound **2** in in acetone-d_6 with one drop H_2O. (See Fig. 4 for the spectrum of free **2-I**)

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To emulate a Cys-terminal peptide, the *O*-protected L-cysteine ethyl ester was reacted with reagent **2** under the same conditions (Figure 11). Surprisingly, despite the lack of a free carboxylate, again a white solid material crashed out of solution within 5 minutes. Signals from the ethyl group of protected L-cysteine were not found in the ^{1}H NMR spectra of the resulting white solid. Spectra in both acetone-d6 and DMSO-d6 were identical to that of adduct **16** with free cysteine.§ The HRMS spectrum of the white solid material further confirmed the loss of ethoxide. Therefore, during the reaction, the ethyl ester group was readily hydrolyzed, possibly as a result of internal activation by the Lewis acidic boron unit.

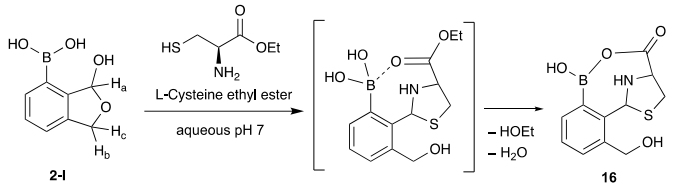

Fig. 11. Formation of adduct **16** by condensation and hydrolysis of cysteine ethyl ester with compound **2**.

Lysozyme Conjugation

To assess the potential of compound **2** as a bioconjugation reagent for biologically relevant targets, we utilized as a model protein, lysozyme (aka, muramidase), an antimicrobial enzyme previously utilized to evaluate the 2-FPBA reagent in protein bioconjugation.[13] Functionalization of amino groups of up to the six lysine residues on lysozyme was monitored using ESI-Fourier transform ion cyclotron resonance (FTICR)-MS. The conjugation of reagent **2** with lysozyme was compared to that of 2-FPBA and 2-APBA after stirring with an excess of the boron reagent for 30 min in 50 mM ammonium acetate buffer at room temperature. Under these conditions, reagent **2** was found to modify the model protein multiple times (Figure 12). Although all three compounds show up to six modifications of the lysozyme protein, 2-FPBA shows the least amount of free lysozyme, suggesting a superior binding efficacy.§ To test the reversibility of the boronic acid/lysozyme conjugates, fructose was added as a biomolecular competitor, and the qualitative change in the proportion of free lysozyme and modified peaks was monitored qualitatively.§ In the event, with the addition of excess fructose, the reversibility of 2-FPBA conjugation was evident. Not only did a significant increase of the free lysozyme peak was observed, but most of the other peaks in the mass spectrum changed from 2-FPBA-lysozyme adducts to fructose-bound lysozyme adducts.§ In contrast, both 2-APBA and reagent **2** (Figure 12) showed no appearance of reversibility, as indicated by the similar appearance of the mass spectra before and after the addition of fructose. Although 2-APBA also displays strong amine binding, it is poorly soluble in water in comparison with reagent **2**. Thus, with excellent reactivity and increased aqueous solubility, frustrated benzoxaborole **2** presents attractive attributes as a bioconjugation reagent.

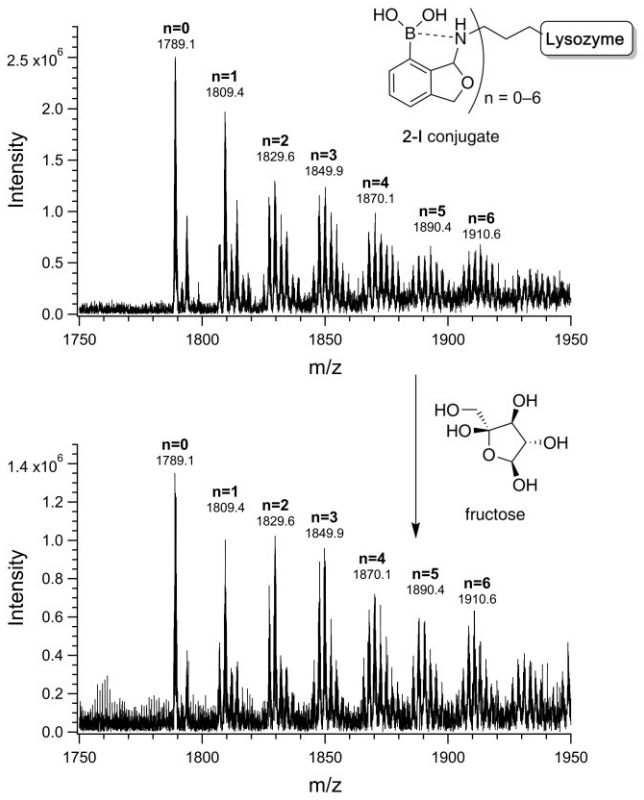

Fig. 12. ESI-FTICR-MS lysozyme binding assays showing the $(M+8H)^{8+}$ charge state of lysozyme with excess reagent 2 (top) and competition experiment with excess fructose (bottom).

Conclusion

This study details the design and synthesis of arylboronic acid **2**, the first example of a permanently open "frustrated" benzoxaborole, along with an exploration of its application in bioconjugation. An efficient and high yielding seven-step

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synthesis was optimized, which exploits the hydrophilicity of the final product with a simple purification that circumvents the need for chromatography. Through several NMR experiments, compound **2** was found to exist in the open ortho hydroxyalkyl arylboronic acid structure **2**-**I**, a form that is effectively prevented to undergo a dehydrative cyclization as a result of unfavorable geometry. Boronic acid **2**-**I** was found to have a pKa of 7.5 that is similar to that of benzoxaborole (7.2), and significantly lower than traditional boronic acids. The relatively low pKa of compound **2**-**I** is proposed to be the result of stabilization of the trihydroxyborate conjugate base by the hydrogen-bond donor ability of the ortho hydroxyalkyl unit. This low pKa favors solubility in water, which increases the potential to use compound **2**-**I** in aqueous biological systems. In this regard, compound **2**-**I** was found to conjugate effectively with amines to form stable hemiaminal structures, including highly effective and irreversible reaction with lysozyme. Complexation of reagent **2** with cysteine was also demonstrated. Surprisingly, cysteine induced an open structure containing a free hydroxymethyl arm, as the amino and thiol group bind preferentially onto the formyl group to form a N,S-acetal. As a first-of-its-kind permanently open "frustrated benzoxaborole", compound **2**-**I** demonstrates unique properties that can be further explored to meet the ever-growing needs and applications of novel boron containing drugs and bioconjugates.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

§ See Supporting Information for details.

1 For general reviews on boron therapeutics: (a) S. J. Baker, C. Z. Ding, T. Akama, Y.-K. Zhang, V. Hernandez, Y. Xia, *Future Med. Chem.* 2009, **1**, 1275-1288; (b) A. Nocentini, C. T. Supuran, J.-Y. Winum, *Expert Opinion on Therapeutic Patents* 2018, 28, 493; (c) G. F. S. Fernandes, W. A. Denny, J. L. Dos Santos, *Eur. J. Med. Chem.* 2019, **179**, 791-804.

2 S. J. Baker, Y.-K. Zhang, T. Akama, A. Lau, H. Zhou, V. Hernandez, W. Mao, M. R. K. Alley, V. Sanders, J. J. Plattner, *J. Med. Chem*. 2006, **49**, 4447–4450.

3 T. Akama, S. J. Baker, Y.-K. Zhang, V. Hernandez, H. Zhou, V. Sanders, Y. Freund, R. Kimura, K. R. Maples, J. J. Plattner, *Bioorg. Med. Chem. Lett.* 2009, **19**, 2129–2132.

4 For reviews focused on benzoxaboroxoles: (a) J. Zhang, M. Zhu, Y. Lin, H. Zhou, *Sci. China Chem*. 2013, **56**, 1372-1381; (b) C. T. Liu, J. W. Tomsho, S. J. Benkovic, *Bioorg. Med. Chem.* 2014**,** **22**, 4462-4473; (c) A. Adamczyk-Woźniak, K. M. Borys, A. Sporzyński, *Chem. Rev.* 2015**,** **115**, 5224-5247.

5 For recent examples of applications in: Catalysis: (a) S. Kusano, S. Miyamoto, A. Matsuoka, Y. Yamada, R. Ishikawa, O. Hayashida, *Eur. J. Org. Chem.* 2020, Early Access, DOI: 10.1002/ejoc.201901749. Materials science: (b) Y. Chen, W. D. Wang, D. Wu, H. B. Zeng, D. G. Hall, R. Narain, *ACS Appl. Mater. Interfaces* 2019, **11**, 44742-44750; (c) D. Wu, W. D. Wang, D. Diaz-Dussan, Y. Y. Peng, Y. J. Chen, R. Narain, D. G. Hall, *Chemistry of Materials* 2019, **31**, 4092-4102; (d) Y. J. Chen, D. Diaz-Dussan, D. Wu, W. D. Wang, Y. Y. Peng, A. B. Asha, D. G. Hall, K. Ishihara, R. Narain, *ACS Macro Letters* 2018, **7**, 904-908.

6 S. Vshyvenko, M. L. Clapson, I. Suzuki, D. G. Hall, *ACS Med. Chem. Lett.* 2016, **7**, 1097−1101.

7 S. Luliński, J. Serwatowski, *J. Organomet. Chem.* 2007, **692**, 2924.

8 X. Li, Y.-K. Zhang, Y. Liu, C. Z. Ding, Y. Zhou, Q. Li, J. J. Plattner, S. J. Baker, S. Zhang, W. M. Kazmierski, L. L. Wright, G. K. Smith, R. M. Grimes, R. M. Crosby, K. L. Creech, L. H. Carballo, M. J. Slater, R. L. Jarvest, P. Thommes, J. A. Hubbard, M. A. Convery, P. M. Nassau, W. McDowell, T. J. Skarzynski, X. Qian, D. Fan, L. Liao, Z.-J. Ni, L. E. Pennicott, W. Zou, J. Wright, *Bioorganic & Medicinal Chemistry Letters* 2010, **20**, 5695.

9 According to our survey of the literature, none of derivatives **4a**-**f** were isolated and characterized. Only fluoradene compounds similar to **4f** were reported: B. McDowell, H. Rapoport, *J. Org. Chem.* 1972, **37**, 3261.

10 S. Luliński, I. Madura, J. Serwatowski, H. Szatyłowicz, J. Zachara, *New J. Chem*. 2007, **31**, 144–154.

11 G. A. Molander, S. L. J. Trice, S. M. Kennedy, S. D. Dreher, M. T. Tudge, *J. Am. Chem. Soc.* 2012, **134**, 11667.

12 M. Dowlut, D. G. Hall, *J. Am. Chem. Soc.* 2006, **128**, 4226-4227.

13 P. M. S. D. Cal, J. B. Vicente, E. Pires, A. V. Coelho, L. F. Veiros, C. Cordeiro, P. M. P. Gois, *J. Am. Chem. Soc.* 2012, **134**, 10299–10305.

14 A. Bandyopadhyay, J. Gao, *Chem. Eur. J.* 2015, **21**, 14748–14752.

15 For Reviews: (a) B. Akgun, D. G. Hall, *Angew. Chem. Int. Ed.* 2018, **57**,13028–13044; (b) S. Cambray, J. Gao, *Acc. Chem. Res.* 2018, **51**, 2198-2206; (c) J. P. M. Antonio, R. Russo, C. P. Carvalho, P. M. S. D. Cal, P. M. P. Gois, *Chem. Soc. Rev.* 2019, **48**, 3513-3536.

16 L. Zhu, S. H. Shabbir, M. Gray, V. M. Lynch, S. Sorey, E. V. Anslyn, *J. Am. Chem. Soc.* 2006, **128**, 1222.

17 A. Bandyopadhyay, S. Cambray, J. Gao, *Chem. Sci.* 2016, **7** 4589.

18 H. Faustino, M. J. S. A. Silva, L. F. Veiros, G. J. L. Bernardes, P. M. P. Gois, *Chem. Sci.* 2016, **7**, 5052.

19 For a successful example of 1,2-aminoalcohol complexation using tris(hydroxymethyl)aminomethane, see: K. Li, M. A. Kelly, J. Gao, *Org. Biomol. Chem.* 2019, **17**, 5908-5912.

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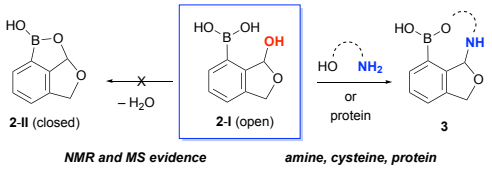
DOI: 10.1039/D0OB00572J

TOC Abstract



Design and synthesis of arylboronic acid **2**, the first example of a permanently open "frustrated" benzoxaborole, is described along with an exploration of its application in the complexation of amines and amino acids, and protein modification.