Published in final edited form as:

Angew Chem Int Ed Engl. 2009 ; 48(37): 6817–6819. doi:10.1002/anie.200903390.

Boron Mimetics: 1,2-Dihydro-1,2-azaborines Bind inside a Non-polar Cavity of T4 Lysozyme**

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The element boron has not received much attention in biomedical applications compared to its periodic table neighbors carbon, nitrogen and oxygen. Arguably, this might be due to the apparent “insignificance” of boron in Nature’s evolution of life.^[1,2] Boron has however useful elemental and chemical features that include nuclear spin, large cross section for neutron capture, and Lewis acidity. If boron could be incorporated into biologically relevant molecules^[3] it might benefit biomedical research by being used as a marker,^[4] as a new pharmacophore,^[5] or in cancer therapy.^[6] We are interested in synthetic approaches that will allow the incorporation of boron into such molecules^[7] with minimal perturbation of their structures. 1,2-Dihydro-1,2-azaborines (abbreviated as 1,2-azaborines) serve as a unique structural platform to accomplish this goal because of their isostructural relationship with arenes, a ubiquitous motif in living organisms and in pharmaceuticals. Furthermore, the bonding of arenes with cations and other arenes through cation- π ^[8] and π - π ^[9] interactions have been demonstrated to be vital in biological systems. Thus, the broad utility and fundamental importance of arenes in biomedical research combined with the unique elemental/chemical features of boron, and the potential of expanding the diversity of arene structures through CC/BN isosterism^[10] make 1,2-azaborines attractive targets for biomedical investigation (Scheme 1).

Dewar and White pioneered the chemistry of monocyclic and ring-fused polycyclic 1,2-azaborine derivatives in the 1960s.^[11] Since 2000, contributions by Ashe,^[12] Piers,^[13] and Paetzold,^[14] have further advanced the preparation of novel BN heterocycles and sparked a renewed interest in the chemistry and properties of these compounds.^[15] We have recently developed synthetic methods that expand the scope of accessible 1,2-azaborines^[16] and enable the preparation of the long sought-after parent compound of this family of heterocycles.^[17] We have also shown through X-ray crystallographic studies that 1,2-azaborines possess delocalized structures consistent with aromaticity.^[18] While the development of a versatile synthetic toolbox for 1,2-azaborines has been improved, their investigation in a biological context has remained elusive. In this communication we provide the first experimental evidence for the interaction of 1,2-azaborines with a biological system. Specifically, we describe the binding of *N*-Et-1,2-azaborine and the parent 1,2-azaborine (hereafter EtAzB and HAzB, respectively) inside an engineered non-polar cavity of T4 lysozyme *via* single-crystal X-ray crystallography.

The mutation Leu99 $\rightarrow$ Ala (L99A) in T4 lysozyme^[19] creates a disc-shaped apolar cavity of volume $\sim 160 \text{ \AA}^3$. Although this cavity is internal it readily binds various arenes and other

** [] The authors thank Dr. Walter A. Baase for his helpful comments and discussion. Support has been provided by the University of Oregon and NIH grant GM21967. The authors also thank Drs. Derek Yoder and Craig Ogata at Beamline IB-23-D, Advanced Photon Source, and Drs. Jeff Dickert and Peter Zwart at Beamline 5.0.2, Advanced Light Source, for their help in data collection.

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Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

ligands.^[20,21] We are interested in addressing the following questions: (1) Do EtAzB and HAzB bind to L99A lysozyme and (2) if so, do they bind in the same fashion as their all-carbon analogues.

EtAzB^[16] and HAzB^[17] were prepared according to our reported procedure. L99A lysozyme was purified and crystallized as described.^[19,22] The protein crystals and their soaking solution (2.2 M sodium/potassium phosphate, pH 6.9, 50 mM 2-mercaptoethanol, 50 mM hydroxyethylidisulfide) were then deoxygenated. The respective complexes of L99A with EtAzB, ethylbenzene, HAzB, and benzene were prepared by vapor-diffusion of the ligand into the protein crystals in a sealed oxygen-free glass tube at 277K for three days. Diffraction data (see Supporting Information) were collected from frozen crystals (100K) to 1.25 Å resolution. Crystallographic refinement was carried out using the CCP4^[23] package.

Figures 1(a) and 1(b) show, respectively, the $F_o - F_c$ electron density maps for EtAzB and ethylbenzene bound in the L99A cavity. As is immediately apparent, both ligands bind in a similar fashion. In both cases the aromatic ring occupies the classic benzene binding site.^[19,20] Also for both ligands the ethyl group occupies two alternative positions. In one of these positions the ethyl moiety protrudes into the “bulge” region of the cavity and makes a van der Waals contact (~ 3.8 Å) with the sulfur atom of Met102. In the alternative conformation the ethyl group still maintains a van der Waals contact (~ 3.8 Å) with Met102 but occupies space between Met102 and Val111. This causes the side-chain of Val111 to rotate $\sim 120^\circ$ into a different conformer. The crystallographic refinement suggests that EtAzB binds to L99A with essentially 100% occupancy, whereas the occupancy for ethylbenzene is about 60%. In a prior lower-resolution study of ethylbenzene binding to L99A at room temperature,^[20] the geometry of binding was similar to Figure 1(b) except that the “bulge” region binding mode was predominant.

The difference electron density (Figure 1(a)) and the subsequent crystallographic refinement indicate that the atoms in the azaborine ring as well as the benzylic carbon of the ethyl group are coplanar. This is in agreement with the prior observations using small-molecule crystallography.^[16] The present analysis suggests that EtAzB is stable in the cavity, at least during the three days at room temperature preparation time plus much shorter X-ray data collection time at 100K. There is no obvious electron density suggesting that the ligand might have chemically modified some site on the protein.

Although EtAB and ethylbenzene bind similarly, there are some differences. As can be seen in Figure 1(b), the phenyl rings of ethylbenzene in its two alternative modes of binding superimpose within about 0.2 Å. For EtAB, however (Fig. 1(a)), the azaborine rings are offset relative to each other by about 0.6 Å. The difference between EtAB and ethylbenzene may be due in part to the C-B bond (~ 1.5 Å) which is approximately 0.1 Å longer than the aromatic C-C bond.^[18] It should be noted that due to the alternative positions occupied by EtAzB it is not possible to reliably identify the specific site(s) occupied by the boron atom. No putative close contacts between the N or B atoms of EtAzB and the surrounding atoms of the protein are apparent. This suggests that polar interactions do not have a dominant effect in the binding of this ligand, which again is consistent with the previous observation that the 1,2-azaborine ring is aromatic and has considerable hydrophobicity.

In contrast to EtAzB, the parent 1,2-azaborine HAzB contains a polar and protic N–H bond that readily undergoes isotopic exchange in the presence of deuterated methanol CD_3OD .^[17] Consistent with this protic bond, HAzB has a significantly lower R_f value of 0.4 on silica gel (using pentane as eluent) compared to benzene's R_f value of close to 1.0 under the same conditions. It has been shown that a number of ligands of polar character including pyridine, phenol, and aniline do not bind significantly to the L99A cavity.^[20a] Thus, an efficient

binding of HAZB to the hydrophobic cavity of L99A was questionable due to the dichotomous characteristics exhibited by HAZB (Scheme 2).

Crystals of L99A lysozyme in the presence of HAZB were prepared and analyzed in the same manner as for EtAzB. Gratifyingly, we observed that the resultant difference electron density map (Figure 2(a)) has a strong disc-shaped feature clearly indicating the binding of HAZB in the cavity. As a control, the binding of benzene, previously shown at room temperature,^[19b] was redetermined (Figure 2(b)) under the low-temperature conditions used for HAZB and EtAzB. There is essentially no change in the cavity ($< 0.1 \text{ \AA}$) when either HAZB or benzene is bound.

The electron density for the benzene complex (Figure 2(b)) suggests that the six carbons occupy well-defined sites. For HAZB, however, the density in the vicinity of Met102 and Val111 suggests that HAZB may bind in two conformations, the first with the nitrogen $4.0 \text{ \AA}$ from sulfur atom of Met102 and the second “flipped over” with the nitrogen $3.7 \text{ \AA}$ from the carbonyl oxygen of Ala99. In neither case is there any indication of a strong hydrogen-bonding interaction. This is consistent with HAZB having apolar rather than polar characteristics inside this specific protein environment. Also consistent with this, HAZB appears to bind to L99A lysozyme with essentially 100% occupancy. This structure of HAZB bound to L99A represents the first crystallographic characterization of the parent 1,2-azaborine in its metal-free form.

In summary, we furnished the first experimental evidence for the binding of 1,2-azaborines, including that of the parent HAZB, to a biological system. We demonstrate that unnatural 1,2-azaborines bind inside the cavity of T4 lysozyme L99A, and that they bind in a very similar fashion compared to their “natural” all-carbon isosteres. The observed binding of the parent HAZB is consistent with its non-polar characteristics despite its protic N–H functionality. Our studies provide the proof of concept that 1,2-azaborines can serve as boron-containing, hydrophobic arene mimics in a biological context. In view of the abundance and importance of arene structures in biomedical applications and the unique elemental and chemical features of boron, we believe that our studies will encourage the development of 1,2-azaborines in biologically relevant applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Binding of EtAzB (a) and ethylbenzene (b) in the T4 lysozyme L99A cavity. The difference maps (blue) have coefficients $(F_o - F_c)\exp(i\alpha_c)$ where F_o is the amplitude observed for crystals of the complex, and F_c and α_c are the amplitudes and phases, respectively, calculated from the refined model prior to including the ligand. The maps are contoured at 3σ and 2σ for the EtAzB and ethylbenzene, respectively, at resolution of $1.25\text{ \AA}$. The nitrogen atom on the 1,2-azaborine ring of EtAzB is colored blue. The sites for the boron atoms could not be specified unambiguously (see text).

Figure 2.

Binding of HAZB (a) and benzene (b) in the T4 lysozyme L99A cavity. The difference electron density maps (blue), both contoured at 3 σ level, are calculated from coefficients $(F_o - F_c)\exp(i\alpha_c)$ where F_o is the amplitude observed for crystals of the complex, and F_c and α_c are the amplitudes and phases, respectively, calculated from refined model prior to the incorporation of ligands. The nitrogen atom on the 1,2-azaborine ring is colored blue. Boron atom sites are included (orange).

Scheme 1.
1,2-Azaborines as boron-containing arene mimetics.

Scheme 2.

The dichotomy of the parent HAzB.