Evidence for spontaneous, reversible paracyclophane formation. Aprotic solution structure of the boron neutron capture therapy drug, L-*p*-boronophenylalanine

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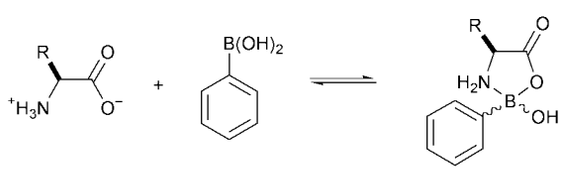
The simple, straightforward ^{1}H NMR spectrum of the neutron capture therapy drug, L-*p*-boronophenylalanine (L-*p*-BPA), in D_2O changes to a more complex one in DMSO-d_6 in which the ratio of new species observed is highly concentration dependent. The new species detected can only be explained by an additional stereocenter being formed at the boron center by intermolecular chelation of the amino acid of another molecule of L-*p*-BPA. This gives rise to the presence of an oligomeric species as well as another whose aromatic protons appear as a pair of sharp AB quartets centered further upfield at 6.66 and 6.84. Due to these shifts and couplings observed between the benzylic protons and the proton at the stereocenter of the amino acid, it is proposed that this species is a paracyclophane dimer of L-*p*-BPA in which one molecule of L-*p*-BPA chelates head-to-tail with another. This cyclophane dimer predominates in low concentrations (<50 mM) while the oligomer predominates at higher (>90 mM) concentrations. The formation of these two species is completely reversible, the addition of D_2O completely regenerating L-*p*-BPA. Variable temperature ^{1}H NMR found that the two pairs of aromatic protons of the cyclophane dimer coalesce at T_c = 141 °C, corresponding to a $\Delta G^{\ddagger}$ = 20.6 kcal mol^{-1}.

Introduction

Organoboronic acids have a wide diversity of uses in both synthesis as reagents,[1] key intermediates,[2] and molecular recognition (including saccharide transport)[3] as well as in biochemistry as antibiotics,[4] inhibitors,[5] and chemotherapeutic agents. The latter essentially consists of boron neutron capture therapy (BNCT),[6] and was first postulated by Lochner[7] and again by Sweet.[8] BNCT is a binary methodology for treating tumors in which a chemical agent containing at least one ^{10}B atom and thermal or isothermal neutrons are directed to a tumor where they combine to release a lethal dose of radiation to the cell. Early clinical attempts at BNCT in the 1960's[9] failed to show any therapeutic efficacy due to shortcomings in both the neutron beam sources as well as the boron compounds then available.[10] However, in the late 1980's and early 1990's encouraging clinical results were reported by Hatanaka[11] and Mishima[12] for the treatment of malignant gliomas and melanoma, respectively, using sodium mercaptoundecahydrododecaborate ($Na_2B_{12}H_{11}SH$) and L-*p*-boronophenylalanine (L-*p*-BPA). Due to its low water solubility (1.6 g L^{-1}), L-*p*-BPA was administered intravenously as its HCl salt[13] or as a complex with fructose,[14] now the standard method for its clinical use.



1a, R = H
1b, R = CH_3
1c, R = CH_2Ph

2

3a, R = H
3b, R = CH_3
3c, R = CH_2Ph

Scheme 1

Results and discussion

While analyzing samples of >99% ^{10}B enriched L-*p*-BPA by ^{1}H NMR, it was serendipitously found that changing solvents from D_2O to DMSO-d_6 resulted in a change from the expected, simple spectrum to a much more complex one. In a related study, Mohler and Czarnik[15] reported that glycine, alanine and phenylalanine (**1a**–**c**, respectively) formed an unprecedented 1:1 chelate with saturated solutions of phenylboronic acid (**2**) in DMSO-d_6. ^{11}B NMR indicated that the boron atom was tetracoordinate and ^{13}C NMR confirmed this by an approximately equimolar pair of complexes, consistent only with diastereomerism at a second stereocenter, which is possible only at the boron atom. It was expected that a similar phenomenon would be observed for the BNCT drug L-*p*-BPA (**4**), but with an oligomeric structure (**5**) or a 1:1 bimolecular complex (**6**) as the possible complexes. The ^{1}H NMR spectra of 18 mM L-*p*-BPA in DMSO-d_6 with varying amounts of D_2O indicate the presence of both species. At a 50% or less DMSO-d_6 solvent system, the only species observed is free L-*p*-BPA. However, when the portion of DMSO-d_6 is increased to 95% or greater, other species are observed (Fig. 1). As expected, one set of peaks appear at 7.29–7.36 and 7.08–7.17, similar in shift to that of L-*p*-BPA in pD 10 D_2O (doublets at 7.49 and 7.14 ppm) or the complex of L-*p*-BPA with fructose[16] at pD 7.4 (7.49 and 7.18 ppm), both of which indicate the presence of tetrahedral boronate formation.[17,18] The chemical shifts and complexity of these peaks are consistent with what would be expected for oligomer **5**. However, in 99:1 DMSO-d_6–D_2O, a pair of AB quartets centered at 6.66 and 6.84 was also observed and become the dominant species in the absence of added D_2O.[19] As in the case of Mohler and Czarnik, this pattern is consistent with the formation of a second stereocenter at the boron atom. The upfield shifts of these quartets are reminiscent of those observed for paracyclophanes[20] with two phenyl rings, whose aromatic protons are typically observed between 6.3 and 7.2 ppm,[21] due to the

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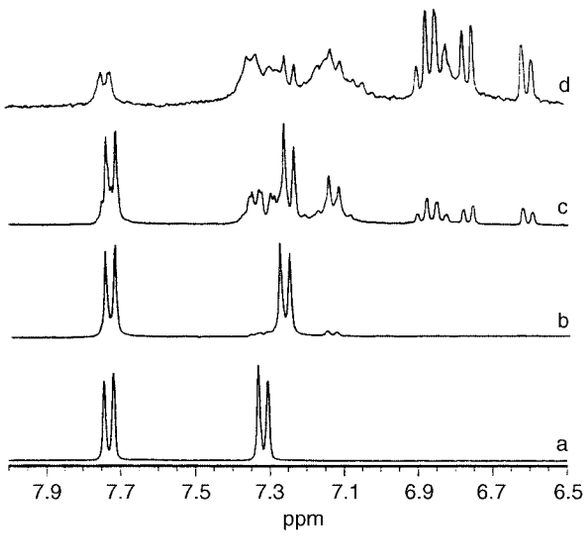
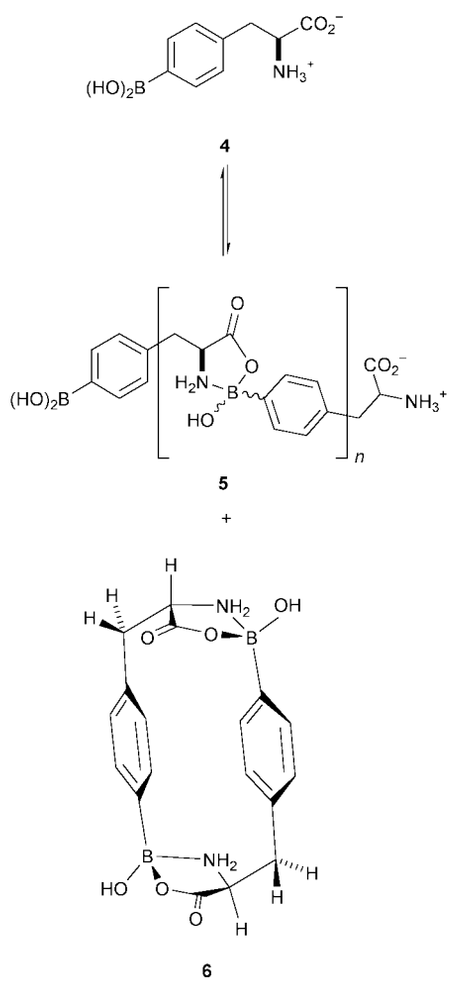


Fig. 1 Aromatic region of ^{1}H NMR spectrum of L-*p*-boronophenylalanine in DMSO-d_6 with (a) 10% D_2O, (b) 5% D_2O, (c) 1% D_2O and (d) DMSO-d_6 only.



Scheme 2

shielding effect that each phenyl ring has on the other's protons. Semiempirical (AM1) energy minimization calculations[22] of paracyclophane dimer **6** provided 41.1° and −75.7° for the dihedral angles between the benzylic protons and the proton at the stereocenter of the amino acid, corresponding to couplings of 5.8 (chemical shift of 2.64 ppm in DMSO-d_6) and 0.6 Hz (3.20 ppm), respectively. This is in good agreement with those found in the observed ^{1}H NMR spectrum (5.9 and 1.4 Hz). The

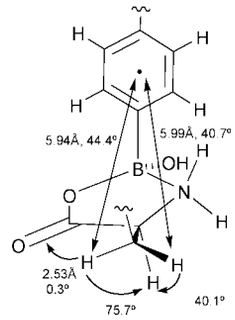


Fig. 2 Partial structure of dimer **6** with selected distances and angles shown.

change in the chemical shifts for these peaks from 2.85 ppm (in DMSO-d_6) for monomeric L-*p*-BPA cannot be explained solely by the effect exerted by the presence of the additional aromatic ring. The distance and angle to perpendicular of each of these protons to the centroid of the aromatic ring (5.94 Å and 44.4° for the 3.20 ppm peaks, 5.99 Å and 40.7° for the 2.64 ppm peaks, Fig. 2) correspond to −0.07 ppm and −0.09 ppm upfield shift, respectively[23] whereas the actual shifts for these peaks are +0.40 and −0.18, respectively. The 0.40 ppm downfield shift of the 3.20 ppm peaks is most likely due to a strong anisotropic effect imparted by the carbonyl of the carboxylate, in which this proton is only 2.53 Å from the centroid of the carbonyl and a dihedral angle of 0.3° (Fig. 2). These calculations also showed that the two phenyl rings of **6** were only 1.5° out-of-parallel and only 3.94 Å apart.

Other solvents were also investigated. Not surprisingly, L-*p*-BPA was not soluble in detectable amounts in $CDCl_3$, acetone-d_6, acetonitrile-d_3, dioxane-d_8, THF-d_8, benzene-d_6, bromobenzene-d_5, or pyridine-d_5. However, when DMF-d_7 was used as solvent, the spectra obtained were quite similar to that when DMSO-d_6 was used as solvent, except that very little oligomer and no monomeric L-*p*-BPA were present with the paracyclophane dimer. Although both DMSO and DMF are both good H-bond acceptors and solvate cations well, their very limited ability to solubilize ion pairs (including amino acids) is more dependent upon and is proportional to the size of the anion and charge delocalization.[24] Consequently, the formation of both the oligomer **5** and cyclophane dimer **6** in aprotic solvents DMSO and DMF is due to the lack of these solvents' ability to solvate the carboxylate anion, hence the association with the boronic acid functionality.

Variable-temperature ^{1}H NMR of an 18 mM DMSO-d_6 solution of L-*p*-BPA indicates that the temperature of coalescence (T_c)[25] for the aromatic protons of paracyclophane dimer **6** was 141 °C (Fig. 3). The free energy of activation for the exchange process at the coalescence temperature can be calculated by the Eyring equation[26] (1), where k_c is the rate constant of the

$$k_c = (kT_c/h)\exp(-\Delta G^{\ddagger}/RT_c) \quad (1)$$

chemical exchange. This is derived by the Gutowsky–Holm[27] relationship (2), in which $\Delta\nu_c$ is the difference, in hertz, of the

$$k_c = \pi\Delta\nu_c\sqrt{2} \quad (2)$$

two coalescing signals at T_c. By definition, this value cannot be measured directly and is usually determined at the slow-exchange limit. However, this treatment completely neglects the temperature dependency that the chemical shifts can have. Katritzky *et al.*[28] have recently stressed this point, and an excellent, detailed study by Fischer and Fettig[29] convincingly demonstrated the importance of accurately determining $\Delta\nu_c$ by plotting $\Delta\nu$ *versus* T (Fig. 4) and extrapolating from the linear region (temperatures somewhat lower than T_c) $\Delta\nu_c$ at T_c. Although two distinctly separate sets of peaks coalesce, both of

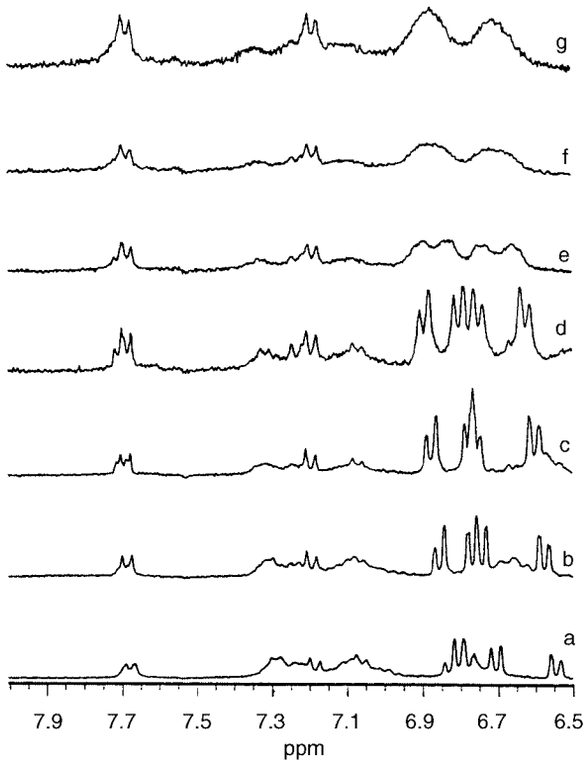


Fig. 3 Aromatic region of VT ^{1}H NMR spectrum of L-*p*-BPA in DMSO-d_6 (18 mM) at (a) 22 °C, (b) 50 °C, (c) 75 °C, (d) 100 °C, (e) 125 °C, (f) 135 °C and (g) 140 °C.

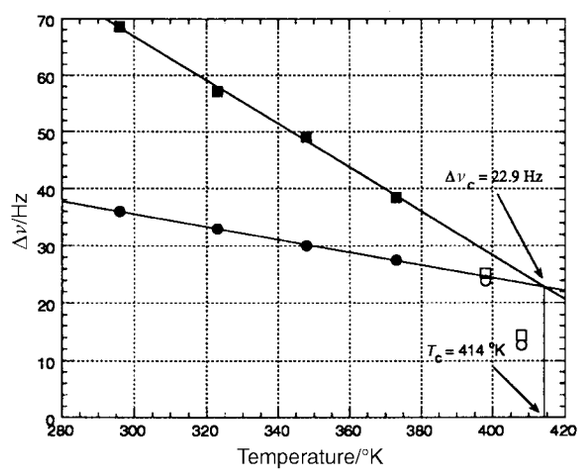


Fig. 4 Temperature dependence for the two pairs of protons that coalesce at 6.91 (●, correlation coefficient, $R = 0.999$) and 6.74 (■, $R = 0.998$) ppm. Only those data have been included in the regression analysis where the individual peaks do not significantly overlap.

which reside on the same phenyl ring, and two separate T_c's would be expected, both sets of peaks coalesced at the same temperature, 141 °C (Fig. 3). The plots of $\Delta\nu$ *versus* T (Fig. 4) for both sets of coalescing peaks also intersect at $T_c = 141$ °C, thus providing $\Delta\nu_c = 22.9$ Hz for each. These values thus give $\Delta G^{\ddagger}_c = 20.6$ kcal mol^{-1}. It should be noted here that this likely does not correspond to the energy of activation for phenyl ring rotation in **6** but rather a more complex process.

The amount of paracyclophane dimer *versus* oligomer was found to be highly concentration dependent. At concentrations less than 50 mM the paracyclophane dimer predominates, while the oligomer is the dominant species in concentrations greater than 90 mM. A small amount of free L-p-BPA (*ca.* <10%) is present in all solutions except when very dilute (0.4 mM or less), when it accounts for 25% of the equilibrium mixture. When a similar ^{1}H NMR study was performed on D,L-*p*-BPA, a new

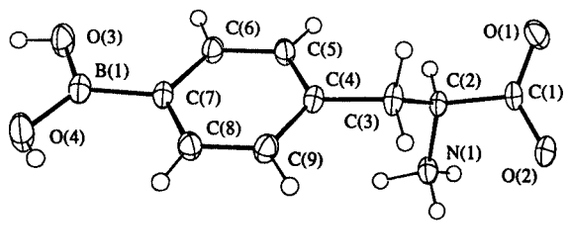


Fig. 5 ORTEP diagram of L-*p*-boronophenylalanine (L-*p*-BPA) **4**.

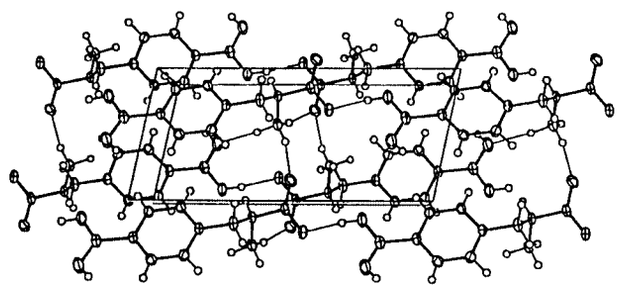


Fig. 6 Layered packing of L-*p*-BPA with intermolecular hydrogen bonding.

species was observed corresponding to the "mismatched" pairing of D-*p*-BPA with L-*p*-BPA, but with the "matched" pairs of D-BPA with D-BPA and L-*p*-BPA with L-*p*-BPA favored by 62 : 38.

Although the X-ray crystal structure of L-*p*-BPA† (Fig. 5, crystallized from 1 : 1 ethanol–water, pH 2) corresponds to what is observed in the D_2O NMR, its crystal packing (Fig. 6) shows interesting hydrogen bonding involving the boronic acid.[30] The boronic acid was found to be planar where one of the hydroxy groups [O(3)H(3)] of the boronic acid acts as a proton donor to a carboxylate anion [O(2), hydrogen bond length of 1.77 Å] and the other hydroxy [O(4)H(4)] of the boronic acid acts as a Lewis base with an ammonium proton (hydrogen bond length of 1.98 Å). Hence, the boronic acid acts simultaneously as both a proton donor as well as a proton acceptor. It is interesting to note that while the proton at O(4), designated H(4), does not participate in hydrogen bonding, it does participate in an O–H $\cdots$ π interaction with the phenyl ring of a neighboring molecule. The distance for this interaction H(4) to the centroid of the phenyl ring is 2.789 Å. Calculating the angle between the interaction H(4) $\cdots$ centroid vector and the phenyl plane perpendicular to H(4) vector yields an angle (γ) of only 13.9°, indicating that H(4) lies almost directly above the center of the phenyl ring. In addition, the hydrogen at C(6), H(6) also participates in a C–H(6) $\cdots$ π interaction with another neighboring phenyl ring. The H(6) $\cdots$ centroid distance is 2.795 Å and there is an angle γ of only 11.5°. This again indicates that this hydrogen sits almost directly below the phenyl ring on the opposite side of the O(4)–H(4) $\cdots$ π interaction.

Experimental

General

L-*p*-Boronophenylalanine was prepared by the method of Snyder[31] using either >99% ^{10}B enriched boric acid from Eagle Pitcher, Boulder, CO or natural abundance boric acid from Eastman Kodak Inc. All ^{1}H NMR spectra were recorded on a GE 300 MHz FT-NMR spectrometer in either DMSO-d_6, DMSO-d_6 + D_2O, DMF-d_7, D_2O buffered at a pD of 7.4 as the solvent. Chemical shifts are given in ppm downfield from an internal standard, either DSS when D_2O is used as the solvent, or TMS when DMSO-d_6, DMSO-d_6 + D_2O, or DMF-d_7 is used

† CCDC reference number 188/215. See http://www.rsc.org/suppdata/p2/a9/a906038c for crystallographic files in .cif format.

Table 1 Crystal data and structural refinement details for L-*p*-BPA

Empirical formula	$C_9H_{12}{}^{10}BNO_4$
Formula wt	208.20
Temp./K	298
Wavelength/Å	0.71073
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	
a/Å	5.3472(3)
b/Å	7.3229(6)
c/Å	12.1705(7)
β/deg	102.375(5)
V/Å^3	465.48(5)
Z	2
μ(Mo-Kα)/mm^{-1}	0.11
ρ_{calcd}/g cm^{-3}	1.491
Crystal size/mm	0.40 × 0.32 × 0.19
Color	Colorless
$2\theta_{max}$/deg	60
Index ranges:	$-7 \le h \le 7$, $-10 \le k \le 10$, $16 \le l \le 17$
R^a	0.031
wR^b	0.043
No. of reflections	2874
No. of unique reflections	2677 [2602 obs, $I > 1.0\sigma(I)$]
Refinement method	Full-matrix least squares on *F*
No. data/restraints/parameters	2602/1/184
Final shift/error	0.003
Largest diff; peak and hole, e Å^3	0.280; −0.170
Goodness-of-Fit	1.60

^{a} $R = \Sigma(F_o - F_c)/\Sigma F_o$. ^{b} $wR = [\Sigma w(F_o - F_c)^2/\Sigma wF_o^2]^{\frac{1}{2}}$.

as the solvent system. The ^{11}B NMR were recorded on a Bruker 300 MHz FT-NMR and the chemical shift is given in ppm down field from $BF_3{\cdot}Et_2O$ as an external standard.

L-*p*-Boronophenylalanine

^{1}H NMR (D_2O, pD = 7.4) δ_H 7.73 (d, *J* = 7.3 Hz, 2H), 7.33 (d, *J* = 7.3 Hz, 2H), 3.98 (dd, *J* = 8.1, 5.1 Hz, 1H), 3.26 (dd, *J* = 13.9, 5.1 Hz, 1H), 3.13 (dd, *J* = 13.9, 8.1 Hz, 1H); ^{1}H NMR (D_2O, pD = 10.0) δ_H 7.49 (d, *J* = 7.3 Hz, 2H), 7.14 (d, *J* = 7.3 Hz, 2H), 3.51 (dd, *J* = 7.3, 5.1 Hz, 1H), 2.96 (dd, *J* = 13.2, 5.1 Hz, 1H), 2.79 (dd, *J* = 13.2, 7.3 Hz, 1H); ^{13}C NMR (D_2O, pD = 10.0) δ_C 184.7 (s), 152.7 (br s), 137.7 (s), 134.0 (d, 2C), 130.9 (d, 2C), 59.9 (d), 42.9 (t); ^{11}B NMR (D_2O, pD = 7.4) δ_B 28.3.

Di-L-*p*-boronophenylalanine 6

^{1}H NMR (DMSO-d_6) δ_H 6.85 (2H) and 6.82 (2H) (ABq, J_{AB} = 8.0 Hz), 6.74 (2H) and 6.58 (2H) (ABq, J_{AB} = 7.3 Hz), 4.41 (N*H*, br d, *J* = 10.2 Hz, 2H), 4.049 (m, 2H), 3.91 (N*H*, br s, 2H), 3.20 (dd, *J* = 13.9, 1.4 Hz, 2H), 2.64 (dd, *J* = 13.9, 5.9 Hz, 2H); ^{1}H NMR (DMF) δ_H 7.03 (2H) and 6.91 (2H) (ABq, J_{AB} = 7.3 Hz), 6.94 (2H) and 6.75 (2H) (ABq, J_{AB} = 6.6 Hz), 4.42 (N*H*, m, 2H), 4.24 (m, 2H), 3.95 (N*H*, br s, 2H), 3.38 (d, *J* = 13.9 Hz, 2H), 2.64 (dd, *J* = 13.9, 6.3 Hz, 2H); ^{11}B NMR (DMSO) δ_B 7.2.

Acknowledgements

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17 Replacing benzeneboronic acid with *p*-tolueneboronic acid (*p*-TBA) in Mohler and Czarnik's study, the complexation of alanine with *p*-TBA in DMSO provided up-field shifts of the aromatic protons from 7.67 (d, *J* = 7.3 Hz, 2H) and 7.13 (d, *J* = 7.3 Hz, 2H) to 7.32 (d, *J* = 8.0, 1H), 7.29 (d, *J* = 7.3 Hz, 1H) and 7.04 (v br d, 2H).
18 This was also confirmed by ^{11}B NMR, in which the 28.3 ppm signal of L-*p*-BPA in D_2O shifts to 7.2 ppm in DMSO.
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