for applications in organic synthesis, solutions are being sought to the unfavorable deinsertions of these complexes relative to their annulations with acetylenes, and also based on the successful stereoselective akylation of the benzyl complex **8**, the possibility of asymmetric induction in reactions at the carbon–nitrogen bond of the imino ligand are being examined.

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Supplementary Material Available: Spectral and physical data for all new compounds and X-ray crystallographic data for compounds **7**, **9**, and **15a** (25 pages). Ordering information is given on any current masthead page.

^{11}B Nuclear Magnetic Resonance Studies of the Structure of the Transition-State Analogue Phenylboronic Acid Bound to Chymotrypsin[1]

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Several solution kinetic studies have suggested that the serine protease class of enzymes is subject to reversible inhibition by boric acid and boronic acid derivatives.[2] X-ray crystallographic studies of boronic acids attached to α-chymotrypsin (α-CHT)[3] and subtilisin[4] demonstrated that the boron atom is in a tetrahedral environment covalently bonded to the active center serine. Raman spectroscopic studies further supported this finding in the solid state.[5]

We report ^{11}B NMR studies on phenylboronic acid (PBA) in the absence and presence of α-CHT at pH 7.2, 22 °C, and our ability to deduce the solution structure around the boron atom when enzyme-bound. Under conditions of fast exchange of the boron nucleus between the bound and free states, both the chemical shift and the relaxation rates of the nucleus can be deduced from a "titration" of the boron resonance with limiting amounts of α-chymotrypsin. For ^{11}B (I, the nuclear spin is 3/2) in the limit of extreme narrowing ($\omega_I^2\tau_c^2 \ll 1$),[6] the quadrupole relaxation rate ($1/T_q$) is given by

$$R_q = 1/T_q = (2\pi^2/5)(1 + \eta^2/3)(e^2qQ/h)^2\tau_c \qquad (1)$$

where (e^2qQ/h) is the quadrupole coupling constant in Hz, τ_c is the correlation time, and η is the asymmetry parameter—a measure of the deviation of the electric field gradient from axial symmetry.

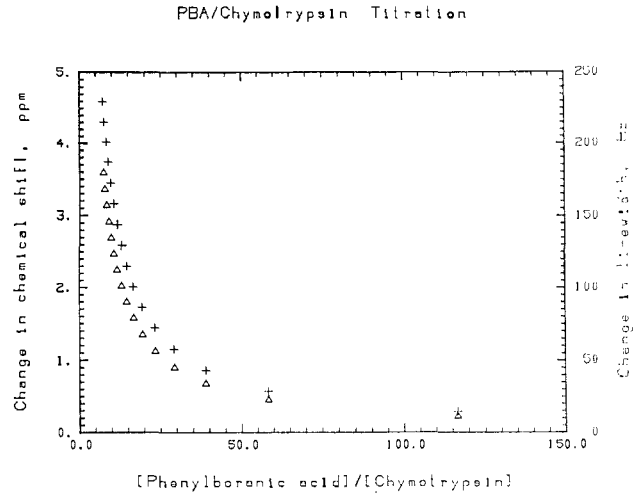

Figure 1. Dependence of the ^{11}B chemical shift (Δ) and line width (+) on the molar ratio of phenylboronic acid (fixed at 2.9 mM) to α-chymotrypsin (0.025–0.4 mM, 3× recrystallized from Worthington) at pH 7.2, 22 °C, in 0.05 M total phosphate buffer. Measurements were performed on an IBM WP-200 SY instrument operating at 64.2 MHz for ^{11}B. Chemical shifts are reported relative to external trimethylborate; there is an upfield chemical shift of the ^{11}B resonance on addition of α-chymotrypsin. The line width was corrected for viscosity induced effects by subtraction of the line width of ^{11}B in 2.9 mM phenylboronic acid but in the presence of the same concentration of diisopropylphosphoryl-α-chymotrypsin as used for native enzyme.

A comparison of the spin-lattice relaxation time (T_1) of 2.9 mM PBA (1.28 ms, measured by the inversion-recovery method) to the spin–spin relaxation time (T_2, 1.30 ms from the line width) demonstrates that the condition of extreme narrowing applies,[7] the principal relaxation mechanism is quadrupolar, and the contribution of field inhomogeneity to the line width is negligible. Addition of small, limiting amounts of α-CHT[8] broadened the boron resonance and shifted it upfield relative to free PBA. The chemical shift of the free PBA is 13.42 ppm relative to external $B(OMe)_3$. The "pK_a" of PBA is 8.85 for the midpoint of the trigonal-to-tetrahedral transition, hence at pH 7.2 there is a large preponderance of trigonal species. As a control, diisopropylphosphoryl-α-CHT[9] was added to PBA producing smaller broadening than did active enzyme and no change in chemical shift. This control served to subtract out the effect of viscosity induced broadening and the effects, if any, of nonspecific binding. The fast exchange condition was confirmed by a study indicating that the excess line width, after correction for the line width observed in the control, decreases with increasing temperature. From the dependence of the chemical shift and line width on α-CHT concentration, the chemical shift of the enzyme bound ^{11}B (−12.9 ± 0.2 ppm) and the $K_{dissociation}$ [(2.6 ± 0.3) × 10^{-5} M] could be calculated,[10] as well as the line width (1932 ± 14 Hz) of the resonance of the bound ^{11}B atom (Figure 1).

In the presence of 2.91 mM PBA and 0.2 mM α-CHT, a $1/T_1$ of 2160 s^{-1} and $1/T_2$ of 6068 s^{-1} were determined for the enzyme-bound ^{11}B atom, indicating nonextreme narrowing conditions.[11] The ratio R_2/R_1 is 2.81 and implies that $\omega_I\tau_c < 1.5$, thus

(1) Supported by the Rutgers University Busch Fund and the Rutgers Research Council. Presented in part at the 1987 meeting of the American Society of Biological Chemists, Philadelpha. Abbreviations: α-CHT, α-chymotrypsin; PBA, phenylboronic acid; T_1, spin–lattice relaxation time; T_2, spin–spin relaxation time; R_1, spin–lattice relaxation rate; R_2, spin–spin relaxation rate.

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(9) Preparation described by Jordan et al. (Jordan, F.; Polgar, L.; Tous, G. *Biochemistry* **1985**, *24*, 7711–7717).

(10) Under fast exchange conditions and assuming a 1:1 complex between the PBA and α-CHT, $x_{obsd} = (1 - n)x_{free} + nx_{bound}$ where x is the observed line width or chemical shift or relaxation rate, n is the mole fraction of bound species, and x_{free} and x_{bound} are the quantities for uncomplexed and complexed material. A standard solution is $PBA_0 = (\Delta X_m/\Delta X)[\alpha\text{-CHT}]_0 - K_d$ (Dwek, R. A. *NMR in Biochemistry*; Clarendon Press: NY, 1983; pp 136–138), where PBA_0 and $[\alpha\text{-CHT}]_0$ are the initial total concentrations of PBA and enzyme, ΔX_m is the difference between fully bound and free inhibitor property, and ΔX is the difference between observed and totally free property.

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enabling us to calculate τ_c and (e^2qQ/h) from a linear approximation[12] as 3.14×10^{-9} s/rad and 0.87 MHz, respectively. A Stokes-Einstein-Debye calculation gave a $\tau_{rotational}$ of 1.2×10^{-8} s/rad for α-CHT.[13] Therefore, the boron environment at the active center is much more mobile than the gross tumbling rate of the enzyme.

The quadrupolar coupling constant estimated for the active center-bound ^{11}B is 0.87 MHz characteristic of a tetrahedral boronate,[15] as is the bound chemical shift of -12.9 ppm (compared to 13.4 for the unbound PBA at this pH).[16] Both quantities confirm a transition statelike structure in solution.

This study demonstrates the potential of ^{11}B NMR to study the active center of enzymes in solution.

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$$R_1 = \frac{1}{T_1} = \frac{2\pi^2}{5}(e^2qQ/h)^2\left[\frac{0.2\tau_c}{1+\omega_1^2\tau_c^2} + \frac{0.8\tau_c}{1+4\omega_1^2\tau_c^2}\right]$$

$$R_2 = \frac{1}{T_2} = \frac{2\pi^2}{5}(e^2qQ/h)^2\left[0.3\tau_c + \frac{0.5\tau_c}{1+\omega_1^2\tau_c^2} + \frac{0.2\tau_c}{1+4\omega_1^2\tau_c^2}\right]$$

(13) Reference 6, p 39, an effective radius of 22.5 Å was used in calculation. Under our experimental conditions of large excess of PBA over α-CHT (<0.4 mM), ionic strength (0.15), and temperature, the fraction of dimerized enzyme is estimated to be small,[14a,b] and the effect on the calculated result negligible.

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Asymmetric Hetero-Diels-Alder Reaction Catalyzed by Chiral Organoaluminum Reagent

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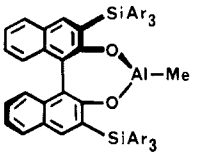
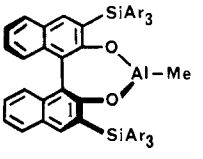
The importance of chiral Lewis acid catalysts in organic synthesis has been tremendously demonstrated in recent years.[1] However, the asymmetric hetero-Diels-Alder reaction (Danishefsky reaction), which is quite useful in natural product syntheses,[2] has never been developed to a useful level due to the lack of the well-designed asymmetric catalysts.[3] Here we wish to report a first solution to this problem by using the newly devised chiral organoaluminum catalyst of type (*R*)-**1** and (*S*)-**1**.[4]



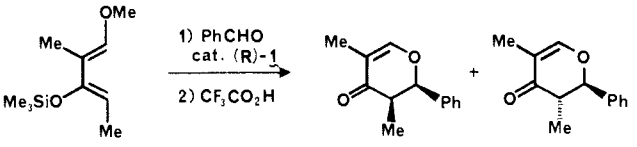
(R)-1



(S)-1

The optically pure (*R*)-(+)-3,3′-bis(triarylsilyl)binaphthol[5] ((*R*)-**2**) requisite for preparation of (*R*)-**1** can be synthesized in two steps from (*R*)-(+)-3,3′-dibromobinaphthol.[6] Reaction of (*R*)-**2** in toluene with Me_3Al produced the chiral organoaluminum reagent (*R*)-**1** as a pink to wine-red solution. Its molecular weight, found cryoscopically in benzene, corresponds closely with the value calculated for monomeric species of **1** (Ar = Ph).

Treatment of a mixture of benzaldehyde and siloxydiene **3** in toluene under the influence of catalytic (*R*)-**1** (Ar = Ph: 10 mol%) at -20 °C for 2 h furnished, after exposure of the resulting hetero-Diels-Alder adducts to trifluoroacetic acid in CH_2Cl_2, *cis*-dihydropyrone **4** (77%) and its trans isomer **5** (7%).[7] The



3 4 5

major cis adduct **4** was shown to be 95% ee.[8] Further, use of sterically more hindered aluminum reagent (*R*)-**1** (Ar = 3,5-xylyl) has proved to exhibit the excellent cis and enantioselectivity (93% yield; cis/trans = 30:1; 97% ee in **4**).

Some examples are listed in Table I. The present catalytic method is applicable to various siloxydienes[9] and aldehydes with high enantioselectivity. The new chiral organoaluminum reagent **1** disclosed herein exhibited the following characteristic features. (1) The optical yield appeared to be independent of the amount (5-100 mol%) of **1** but increased gradually by lowering the reaction temperature (entries 1-3, 7, and 8). (2) Choice of the bulky triarylsilyl moiety in **1** is crucial for obtaining the high enantioface differentiation of prochiral aldehydes, and switching the triarylsilyl

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(5) (*R*)-**2** (Ar = Ph): $[\alpha]_D$ +125° (*c* 1.10, THF); (*S*)-**2** (Ar = Ph): $[\alpha]_D$ -125° (*c* 1.04, THF); (*R*)-**2** (Ar = 3,5-xylyl): $[\alpha]_D$ +135° (*c* 1.02, THF).

(6) (*R*)-(+)-3,3′-Dibromobinaphthol was converted with Ar_3SiCl/imidazole in DMF to bis-silyl ether (>95% yield), which on treatment with *t*-BuLi underwent a remarkably smooth 1,3-rearrangement to furnish optically pure (*R*)-**2** in 80-95% yield. The details of this process and its application to other phenol derivatives will be reported in due course. For preparation of the starting dibromobinaphthol, see: Lingenfelter, D. S.; Helgeson, R. C.; Cram, D. J. *J. Org. Chem.* **1981**, *46*, 393.

(7) A typical experimental procedure is exemplified by the reaction of benzaldehyde with the diene **3** (entry 2). To a degassed solution of (*R*)-(+)-3,3′-bis(triphenylsilyl)binaphthol (*R*)-**2** (Ar = Ph) (88 mg, 0.11 mmol) in dry toluene (5 mL) was added a 0.5 M hexane solution of Me_3Al (0.2 mL, 0.1 mmol), and the resulting wine-red solution was stirred at room temperature for 1 h. After having been cooled to -20 °C, benzaldehyde (0.102 mL, 1 mmol) and the diene **3** (220 mg, 1.1 mmol) were added. The mixture was stirred at -20 °C for 2 h, poured into 10% HCl, and extracted with ether. The combined extracts were concentrated in vacuo to give the crude adducts which were redissolved in CH_2Cl_2 (30 mL) and treated with trifluoroacetic acid (0.092 mL, 1.2 mmol) at 0 °C for 1 h. The reaction mixture was then poured into saturated $NaHCO_3$, extracted with CH_2Cl_2, and dried over Na_2SO_4. Evaporation of solvent and column chromatography of the residue on silica gel, eluting with 1:3 ether/hexane, gave a mixture of *cis*-dihydropyrone **4** (156 mg, 77%; $[\alpha]_D$ +7.1° (*c* 1.0, $CHCl_3$)) and the trans isomer **5** (14 mg, 7%; $[\alpha]_D$ -27.3° (*c* 0.75, $CHCl_3$)).

(8) The optical purity of the trans adduct **5** was 52% ee.

(9) The isomeric ratios of the dienes in Table I are as follows: **6** (*E*/*Z* = 84:16); **7** (*E*/*Z* = 1:1).

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