α-Amino Acid Chelative Complexation by an Arylboronic Acid

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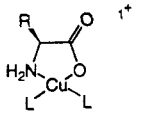
α-Amino acids are hydrophilic species, and as such their transfer from bulk water to binding proteins requires intricate polar stabilization by binding-site residues; the structure of the vancomycin–*N*-Ac-D-Ala-D-Ala complex offers an illuminating example.[1] While such receptors are accessible to synthesis,[2] the efficient design of wholly abiotic variants remains a continuing challenge.[3] Nevertheless, the transport of lipophobic amino acids through lipophilic phases (e.g., membranes, the blood–brain barrier) remains a topic of both theoretical and practical significance.[4] Lehn has reported that ammonium-phase transfer agents facilitate amino acid transport,[5a] but systems utilizing hydrophobic metal ion complexes have been notably unsuccessful in this regard.[5b,c] Our prediction of structural similarity between α-amino acid–Cu(II) complexes[6] (**1**) and their potential cyclic



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chelates with arylboronic acid (**4**) led us to evaluate the heretofore unreported, reversible complexation of amino acids with arylboronic acids. We now report α-amino acid chelation of phenylboronic acid and enhanced liquid membrane transport utilizing this mechanism for recognition of the α-amino acid group.

The passive diffusion of phenylalanine (**2a**) through dichloroethane is very slow, as shown in Figure 1. Both phenethylamine and phenylpropionic acid diffuse more rapidly, confirming the exceptional lipophobicity of the zwitterionic α-amino acid group. Upon introduction of 2.0 mM phenylboronic acid/trioctylmethylammonium bromide (PBA–TOMA),[7] the rate of phenylalanine transport is increased by ca. 100-fold in a typical "U-tube" experiment. Significantly, the rates of phenylethylamine and phenylpropionic acid transport are not increased under identical conditions. Furthermore, phenylalanine transport by PBA–TOMA displays saturation behavior, implicating a specific solubilizing interaction rather than a bulk one. While TOMA

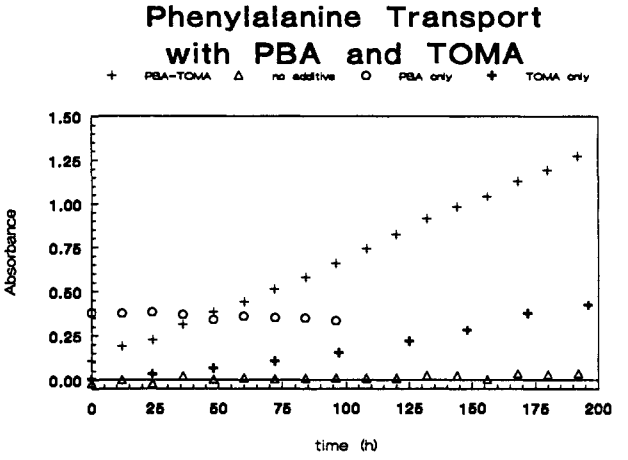
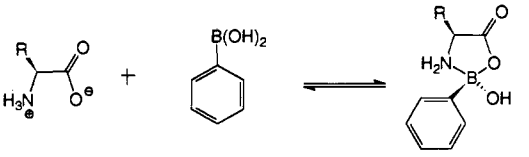


Figure 1. Effect of equilibrated PBA, TOMA, and PBA–TOMA (each 2 mM) on the transport of phenylalanine (**2a**, 20 mM), from pH 7.5 HEPES buffer (3.5 mL, 0.1 M) through $ClCH_2CH_2Cl$ (7.0 mL). The $ClCH_2CH_2Cl$ phase was stirred at a constant rate of 350 rpm, and phenylalanine transport was monitored in the receiving arm (3.5 mL of identical HEPES buffer) at 262 nm.

alone exhibits substantial transport ability (as anticipated),[5a] it is clearly and reproducibly less than that seen using PBA in conjunction.



2a, R=CH_2Ph
2b, R=CH_3
2c, R=H

3

4a, R=CH_2Ph
4b, R=CH_3
4c, R=H

We predict chelate **4**, which is charge-neutral, to be the lipophilic 1:1 complex and propose that it is the transported species.[8] While boronic acid–amine complexes (both 1:1 and 1:2) have substantial precedent,[9] chelate **4** does not. However, the idea is predicated on the work of Miller, who in 1970 proposed a cyclic structure for the *N*,*N*-dimethylglycinate–borane complex;[10] chelates of iminodiacetic acids with boronic acids[11] and of amino acids with diphenylborinic acid[12] have been described. We observe parent ions for solid 1:1 complexes between **2a–c** and **3** using EI mass spectrometry, but of course this cannot confirm chelate structure **4** as compared to a simple amine anate. ^{11}B NMR of **3** saturated with **2a**, **2b**, or **2c** in DMSO-d_6 solution reveals in each case a sharp new peak at 7 ppm, which is about 20 ppm upfield of **3** itself and most consistent with the formation of a tetracoordinate boronate species.[13]

(1) Williams, D. H. *Acc. Chem. Res.* **1984**, *17*, 364.
(2) For an approach to the synthesis of vancomycin, see: Evans, D. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1989**, *111*, 8912.
(3) For a beautiful, successful example of selective α-amino acid extraction under neutral aqueous conditions, see: Galán, A.; Andreu, D.; Echavarren, A. M.; Prados, P.; de Mendoza, J. *J. Am. Chem. Soc.* **1992**, *114*, 1511.
(4) For a review of biochemical amino acid transport systems, see: Ring, K. *Angew. Chem., Int. Ed. Engl.* **1970**, *9*, 345.
(5) (a) Behr, J.-P.; Lehn, J.-M. *J. Am. Chem. Soc.* **1973**, *95*, 6108. (b) Maruyama, K.; Tsukube, H.; Arake, T. *J. Am. Chem. Soc.* **1982**, *104*, 5197. (c) Belokon', Y. N.; Saporovokaya, M. B.; Belikov, V. M. *J. Am. Chem. Soc., Dalton Trans.* **1990**, 1873.
(6) Demaret, A.; Abello, L.; Fourati, M.; Lapluye, G. *J. Chem. Res.* **1978**, *9*, 354.
(7) The use of PBA–TOMA for ribonucleoside transport has been reported previously: Grotjohn, B. F.; Czarnik, A. W. *Tetrahedron Lett.* **1989**, *30*, 2325.
(8) As the predominant α-amino acid–phenylboronic acid complex in DMSO is found to be charge-neutral, it is somewhat counterintuitive that TOMA is still required for enhanced transport. By way of rationalization, there is no measurable formation of complex **4** in aqueous solution. Perhaps the amino acid must first be transported across the aqueous–organic interface by TOMA. Reaction with PBA to yield an internally charge-neutral chelate then creates a species more rapidly transported through the bulk organic phase.
(9) Steinberg, H. *Organoboron Chemistry*; Wiley: New York, 1964; Vol. 3, pp 345–351.
(10) Miller, N. E. *J. Am. Chem. Soc.* **1970**, *92*, 4564.
(11) Mancilla, T.; Contreras, R.; Wrackmeyer, B. *J. Organomet. Chem.* **1986**, *307*, 1.
(12) Strang, C. J.; Henson, M. E.; Okamoto, Y.; Paz, M. A.; Gallop, P. M. *Anal. Biochem.* **1989**, *178*, 276.
(13) Wrackmeyer, B. In *Annual Reports on NMR Spectroscopy*; Webb, G. A., Ed.; Academic Press: 1988; Vol. 20, pp 61–160. An external reference of BF_3-OEt_2 in C_6D_6 is used for ^{11}B NMR spectra.

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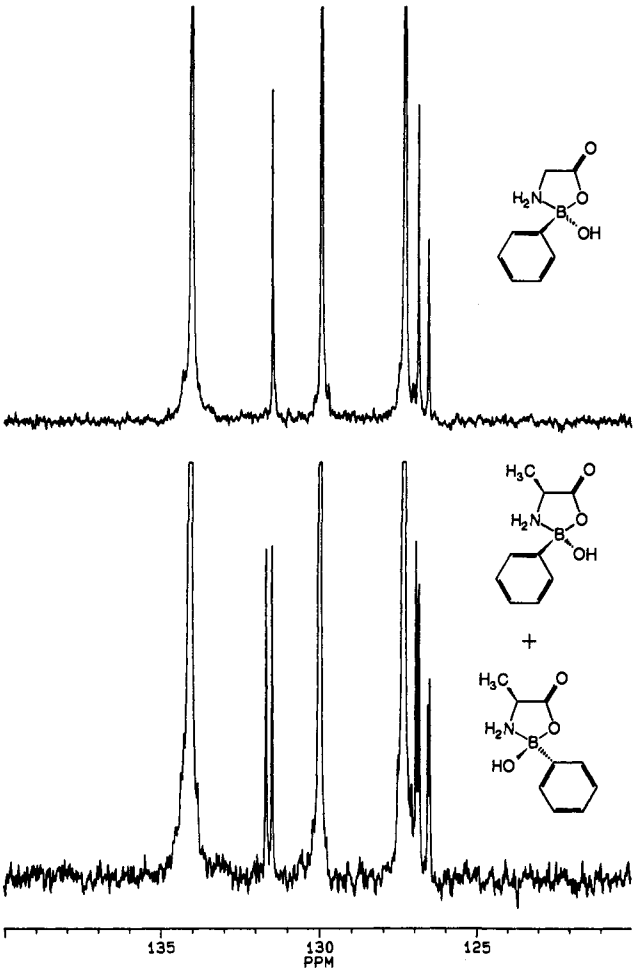

Figure 2. Aromatic regions of 500-MHz ^{13}C NMR spectra of PBA–amino acid complexes **4b** (bottom) and **4c** (top). Samples were prepared from solutions of PBA (40 mM) in DMSO-d_6 saturated with alanine (**2b**) and glycine (**2c**). Shifts are relative to TMS internal standard.

The ^{13}C NMR data are more persuasive. The spectrum of **2b** plus excess **3** in DMSO-d_6 solution (bottom, Figure 2; aromatic region shown) depicts an approximately equimolar pair of closely related amino acid complexes, as does the spectrum of **4a** (not shown). *The patterns are consistent with diastereomerism that can exist only if boron provides a second stereocenter.* In support of this interpretation, glycine (**2c**), which is virtually DMSO-insoluble, forms a DMSO-soluble complex with excess **3** whose ^{13}C NMR spectrum (top, Figure 2) gives similar chemical shifts but no diastereomers.[14] Because only glycine does not supply a carbon stereocenter, the observed behavior provides strong support for chelate structure **4**.

In summary, we report (1) that α-amino acids form chelated complexes with an organic boronic acid and (2) that such complexation is likely responsible for enhanced liquid membrane transport by a PBA–TOMA admixture. Arylboronic acid complexation, while not biomimetic, offers a nonetheless useful tool for the creation of α-amino acid receptors not derived from nature. Because arylboronic acids can be functionallized covalently, strategies may be envisioned for the design of related compounds with improved binding and transport properties.[15]

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(14) ^{13}C NMR chemical shifts are as follows: **4b**, δ 175.4 and 175.0 (C=O), 131.6 and 131.2 (*o*- or *m*-Ar C), 126.9 and 126.8 (*m*- or *o*-Ar C), 126.5 and 126.4 (*p*-Ar C), 50.5 and 49.8 (α-C), 16.2 and 15.4 (CH_3); **4c**, δ 172.7 (C=O), 131.4 (*o*- or *m*-Ar C), 126.8 (*m*- or *o*-Ar C), 126.5 (*p*-Ar C), 42.6 (α-C). Both solutions were 60 mM PBA in DMSO-d_6 (TMS reference); both spectra showed signals from the uncomplexed PBA, present in excess, at δ 134.0 (*o*- or *m*-Ar C), 129.9 (*p*-Ar C), 127.3 (*m*- or *o*-Ar C).

(15) The solubilization and enhanced cell membrane transport of hydrophobic boronic acids by amino alcohol complexation, termed "type I boradeption", has been described by Gallop: Gallop, P. M.; Paz, M. A.; Henson, E. *Science* **1982**, *217*, 166. Using this terminology, our work might be termed "type II boradeption", which was defined in the same article without example.