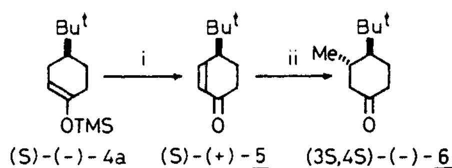
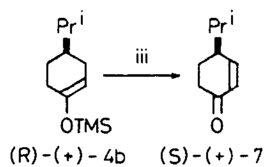
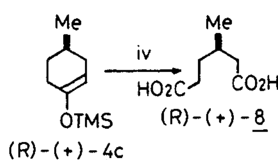
Scheme II[a]







[a] (i) $Pd(OAc)_2$, CH_3CN, 87%. (ii) Me_2CuLi, Et_2O, 84%. (iii) Pd-$(OAc)_2$, CH_3CN, 79%. (iv) $MoO_2(acac)_2$, t-BuOOH, C_6H_6, 60%.

1a was deprotonated with **2h** in the presence of HMPA in THF at −105 °C, **4a** was obtained in 97% ee.

Five-membered chelated structures are expected to be formed for the lithium amides **2a–j** as in **9**, where the isopropoyl group on nitrogen should be exclusively trans to the bulky substituent on the chiral carbon for steric reasons. This infers that the direction of the lone pair on chiral nitrogen to be used for deprotonation is fixed. It is known that axial α-hydrogens are lost in preference to equatorial α-hydrogens in enolization of cyclohexanones due to stereoelectronic effect.[7] For deprotonation to occur by synchronous proton and lithium ion transfer,[8] the carbonyl group in **1** will coordinate to the lithium from the same side as the lone pair. Correlation of configuration between the chiral center of the chiral base and that of the product may become possible as work advances. It should be noted that higher asymmetric induction was observed in the presence of HMPA. The effect of HMPA is likely to destroy aggregation of lithium amide bases and to generate more effective species[9] for selective deprotonation.

Interestingly, simple lithium amide bases **2k,l** having no additional ligation sites also caused fairly high asymmetric induction (entries 15–17, 19, 21). In these cases, however, higher asymmetric induction was observed in the absence of HMPA.[10]

The method outlined above represents a new approach to enantioselective asymmetric synthesis of chiral enol ethers **4**, which should be useful as synthons for the synthesis of optically active compounds.

Acknowledgment. Partial financial support from the Ministry of Education, Science and Culture, Japan (Grant-in-aid for Scientific Research, No. 60870075), is acknowledged.

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Structure of the Complex between an Unexpectedly Hydrolyzed Phosphonamidate Inhibitor and Carboxypeptidase A

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Recent high-resolution crystal structures of enzyme–inhibitor complexes[1] involving the metalloenzyme carboxypeptidase A (CPA)[2] have provided new three-dimensional insight as to potential catalytic conformations[3] of this zinc exopeptidase. Such X-ray crystallographic methods serve as powerful complements to studies of the enzyme in solution. We now report the structure of the complex between CPA and the unexpectedly hydrolyzed phosphonamidate inhibitor *N*-[[[(benzyloxycarbonyl)amino]-methyl]hydroxyphosphinyl]-L-phenylalanine (ZGP′; a possible transition-state analogue of the dipeptide substrate Cbz-Gly-L-Phe),[4] where the corresponding phosphonic acid occupies the S_1 subsite and phenylalanine occupies the S_1' subsite. The observed structure resembles a product/product analogue complex of the cleaved moieites bound to the enzyme, yet still resembles a possible transition-state analogue complex by virtue of the tetrahedral phosphorous moiety bound to the zinc ion.

Crystals of CPA were prepared and cross-linked as described,[1a] and then soaked in a buffer solution [0.2 M LiCl, 0.02 M Veronal-LiOH (pH 7.5)] containing a 7 mM concentration of ZGP′ for 5 days at 4° C. Data collection, reduction, and refinement procedures have been described.[1a] Model building was facilitated by the use of the Evans and Sutherland PS300 interfaced with a VAX 11/780, with graphics software developed by Jones[5] (FRODO). The final crystallographic R factor[6] was calculated to be 0.176 at 1.82-Å resolution.[7]

The carbobenzoxy carbonyl of the cleaved phosphonate does not make a hydrogen bond with Arg-71 in the S_2 subsite, as might be predicted from the interaction of CPA with the 39-amino acid inhibitor from the potato.[8] Instead, this portion of the inhibitor lies in the "aromatic" region of S_2 and S_3 (the area around Tyr-198 and Phe-279). An oxygen of the tetrahedral phosphonate moiety hydrogen bonds with Glu-270 (3.4 Å, yet still within experimental error of a hydrogen bond), and it is the only oxygen of the phosphonate to coordinate to zinc (the Zn–O distance is 2.2 Å). The long hydrogen bond to Glu-270 may reflect a particular state of ionization of the polyprotic phosphonic acid. Another phosphonate oxygen accepts a hydrogen bond from one of the guanidinium nitrogens of Arg-127 (2.7 Å; see Figure 1). This is the first observed interaction of Arg-127 with a zinc-bound inhibitor. Although this interaction does not involve the phosphonate oxygen which is also coordinated to zinc, it does support a role for Arg-127 proposed[1a] as a hydrogen bond donor to a transient intermediate in proteolytic reactions. Arg-127 might serve as an electrophilic catalyst through hydrogen bond donation, with or without the

[†] AT&T Bell Laboratories Scholar.

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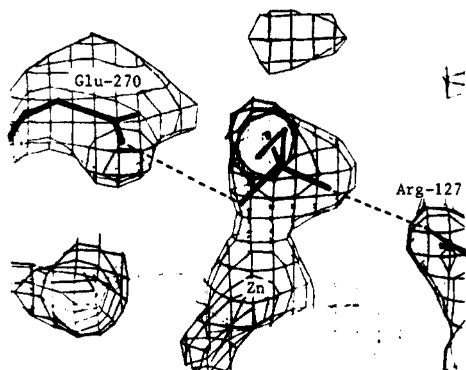

Figure 1. Portion of a $(5|F_o| - 4|F_c|)$ difference electron density map looking into the hydrophobic pocket of CPA. The bound phosphonate and phenylalanine moieties (ca. 60% occupancy), as well as the side chains of Glu-270 and Arg-127, were omitted from the structure factor calculation in order to obtain an unbiased map. Glu-270, the tetrahedral phosphonate above zinc, and the guanidinium moiety of Arg-127 are visible. The carboxylate group of the bound phenylalanine in the S_1' subsite is just visible in the upper background. Possible hydrogen bonds are denoted as dashed lines.

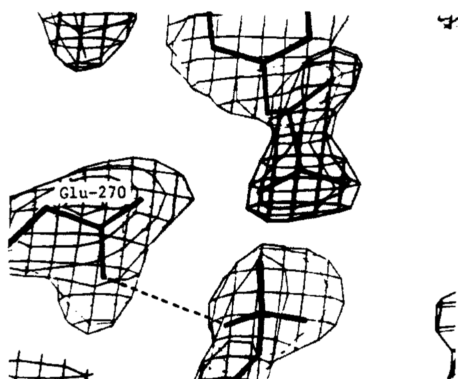

Figure 2. Same map as Figure 1, but viewed from a different angle. Note the relative positions of phosphonate and phenylalanine. Major reorientation occurred around the phenylalanine C_α–C_β and C_β–C_γ bonds in this observed conformation.

participation of zinc, at certain steps in substrate hydrolysis regardless of mechanism (promoted water/hydroxide or anhydride).

The phenylalanine bound in the S_1' subsite makes no hydrogen bond contacts with the enzyme. One oxygen of its carboxylate moiety, however, is within hydrogen bonding distance (2.7 Å) to a third phosphonate oxygen (i.e., the one not interacting with Glu-270 or Arg-127), yet it is in poor geometric orientation. Additionally, the molecule has endured significant rotations about C_α–C_β and C_β–C_γ (Figure 2) so that the amino nitrogen is directed out toward solution and the carboxylate has severed its salt link with Arg-145. The molecule may be tending toward a carboxyl-carboxylate interaction with Glu-270, similar to that observed in the product complex of CPA with the cleaved potato inhibitor (with C-terminal Gly),[8] yet is restrained by both the bulky phenyl group in the hydrophobic pocket of the enzyme and the three oxygens of the tetrahedral phosphonate. The observed orientation of phenylalanine may represent some step of product release,[9] which could occur through a carboxyl–carboxylate interaction with Glu-270. If, however, the observed orientation is merely the result of additional interactions provided by the tetrahedral phosphonate, the orientation of phenylalanine may be a nonproductive consequence.

It is uncertain whether CPA has actually participated in the cleavage of the phosphonamidate linkage in the crystal, but kinetic studies[4] in solution revealed no such effect. The reported[4] purity of synthetic inhibitor is about 86%; presumable contaminants are the hydrolyzed phosphonamidate moieties, neither of which bind to CPA with an affinity anywhere near that of the phosphonamidate itself ($K_i = 9.0 \times 10^{-8}$ M at pH 7.5). Except for travel time via the US mail, the inhibitor was stored at 4 °C, and all crystal chemistry, except data collection, was performed at 4 °C at pH 7.5. These are conditions that do not favor the immediate hydrolysis of the phosphonamidate, and at this pH at room temperature it has a reported[4] half-life of more than 8 days; certainly the lower temperature would only serve to increase its lifetime (at pH 8.5 at 4 °C it is reported to be indefinitely stable in stock solutions). It could well be that during the span of crystallographic data collection at room temperature (about 8 days), the bound phosphonamidate hydrolyzed enough, with or without the participation of the enzyme, to make the crystallographically observed time-averaged structure a cleaved inhibitor. Further study of this system at lower temperature and slightly higher pH may yield the structure of an intact complex.[10]

(9) The conformation of Tyr-248, a residue implicated in substrate binding, is disordered in the electron density maps, although the more continuous density puts it in the so-called "up" position. Such a conformation favors product release, since in this position Tyr-248 does not cover the active site.

(10) We thank Prof. Paul A. Bartlett for generously supplying us with ZGP'. Additionally, we acknowledge the National Institutes of Health for Grant GM 06920 in support of this research and the National Science Foundation for Grant PCM-77-11398 in support of the computational facility. D.W.C. thanks AT&T Bell Laboratories for a doctoral fellowship.

Photochemistry of *p*-Nitrophenyl Azide: Single-Electron-Transfer Reaction of the Triplet Nitrene

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Aromatic azides, nitro-substituted phenyl azides in particular, find widespread use as photolabeling agents of biological macromolecules.[1] In this report we describe the photochemistry of *p*-nitrophenyl azide (**1**).[2,3] Irradiation of **1** leads to loss of nitrogen and formation of (*p*-nitrophenyl)nitrene (**3**). Our findings reveal that the reaction of this triplet nitrene with tertiary amines proceeds by a single-electron-transfer (SET) route not by the hydrogen abstraction pathway generally associated with nitrenes.[3]

Photolysis of azide **1** at 77 K generates a transient species that exhibits an absorption maximum at ca. 375 nm. This absorption has previously been assigned[4] to triplet nitrene **3** on the basis of an EPR spectrum.[5] Irradiation of a 4.8×10^{-4} M solution of azide **1** in benzene (or chlorobenzene or benzonitrile) at room temperature with the output of a nitrogen laser[6] (15 ns, 337 nm, 7.0 mJ) creates a similarly adsorbing transient product. This species is generated within the rise time of the laser pulse and leads to formation of 4,4'-dinitroazaobenzene (**6**) by a second-order process with a rate constant of 1×10^9 M^{-1} s^{-1}. The yield of azobenzene **6** at low conversion is nearly quantitative. These observations assist in the assignment of the observed transient species to triplet nitrene **3**.

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