Experimental Section

X-ray structure determination of **1** ($C_{84}H_{114}N_6O_6$) at 220 K: The crystal contains chloroform and ethyl acetate and is stable at room temperature. Crystal size $0.24 \times 0.18 \times 0.17$ mm, Siemens SMART CCD area detector on a free axis platform, $Mo_{K\alpha}$ radiation, graphite monochromator. Cell dimensions from the positions of 5466 reflections: $a = 14.1464(1)$, $c = 42.8884(2)$ Å, $V = 7432.97(21)$ Å^3; trigonal, space group $P3_221$ (no. 154), $Z = 3$, $\rho_{calcd} = 1.062$ Mg m^{-3}, $\mu = 0.22$ mm^{-1}, empirical absorption correction based on redundant data (SADABS program, Siemens), max./min. transmission 1.000/0.816, R(merg) before/after correction 0.0633/0.0519, data collection $\Theta_{max} = 22.46^\circ$, detector distance 4.457 cm, half-sphere scan in ω with 0.3° spacing and four data sets of 636, 465, 636, and 465 pictures with $\phi = 0$, 88, and 180°, where more than 97% of the data are nominally recorded (data reduction with SAINT Program, V4.028, Siemens), decay correction (< 2%) by repetition of the measurements of the first 50 pictures at the end of the data sampling; of 49471 refections measured, 6471 were independent and 4174 observed ($I > 2\sigma(I)$); $R_{merg} = 0.0874$ (F^2). Structure solution with PATSEE[5] (no solution with direct methods) and refinement to F^2 (SHELXTL-Plus Ver. 97-1/Iris), 591 parameters, hydrogen atoms as riding groups with 1.2 or (for methyl groups) 1.5 times the isotropic displacement parameter of the corresponding carbon atom. $R = 0.0872$ ($I > 2\sigma(I)$), $wR2 = 0.2385$, GOF(F^2) = 1.068, $w^{-1} = \sigma^2(F_o^2) + (0.1074\,P)^2 + 4.41\,P$ with $P = [(\text{max. } F_o^2, 0) + 2F_c^2)]/3$, largest residual electron density 0.32 e Å^{-1}. Further details on the crystal structure investigation may be obtained from the Fachinformationszentrum Karlsruhe, D-76344 Eggenstein-Leopoldshafen, Germany (fax: (+49) 7247-808-666; e-mail: crysdata@fiz-karlsruhe.de), on quoting the depository number CSD-408718.

Received: May 18, 1998 [Z11868IE]
German version: *Angew. Chem.* **1998**, *110*, 2855 – 2857

Keywords: β turns · hydrogen bonds · peptides · steroids · structure elucidation

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The Conformation of a Peptidyl Methyl Ketone Inhibitor Bound to the Human Cytomegalovirus Protease**

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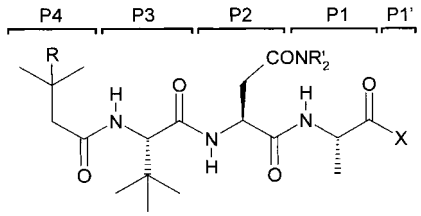
The transferred nuclear Overhauser effect (TRNOE) experiment represents a powerful NMR tool for the study of protein – ligand interactions.[1] Aided by computational chemistry, the method can provide valuable structural information concerning the conformation of a ligand when bound to a macromolecule. A key condition of the TRNOE method, however, is that the exchange between the free and complexed forms of the molecule to be investigated is rapid (faster than its spin-lattice relaxation rate) and reversible. In the present work, we provide the first structure of a peptidyl inhibitor bound to the human cytomegalovirus (HCMV) protease.[2] The use of the peptidyl methyl ketones MK1 and MK2 represents a novel approach to probing the structure of classical activated carbonyl inhibitors of serine proteases, which are themselves incompatible with the TRNOE method (Scheme 1). To date, such investigations of inhibitors of these enzymes have utilized, almost exclusively, peptide cleavage products or slowly cleaved substrates.[3]

There presently exists considerable interest in HCMV protease as a target for therapeutic agents against HCMV infection, which remains a serious clinical problem.[4] More-



MK1:	R = CH_3	R' = CH_3	X=CH_3
MK2:	R = H	R' = CH_3	X=CH_3
TFMK3:	R = CH_3	R' = CH_3	X=CF_3
PFEK4:	R = CH_3	R' = CH_3	X=CF_2F_3
AKA5:	R = CH_3	R'$_2$ = -$(CH_2)_4$-	X=C(O)N(H)CH_2(*p*-IC_6H_4)

Scheme 1. Various peptidyl inhibitors of HCMV protease. The numbers over the individual amino acids correspond to the notation of Schechter and Berger[10] for which a natural peptide substrate is cleaved between P1 and P1′.

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[**] Dr. L. Lagacé and M.-J. Massariol are gratefully acknowledged for providing the enzyme used in the present studies, as are G. Fazal, J. A. O'Meara, and C. Chabot for the synthesis of activated carbonyl inhibitors. We are grateful to Prof. L. Tong for providing the X-ray structure. We also thank Dr. F. Ni, Dr. Z. Chen, and Dr. P. Xu for valuable discussions and technical support.

over, the highly novel nature of this serine protease, as evidenced in its recently elucidated X-ray crystallographic structure,[5] has also fueled its investigation. Our efforts with respect to medicinal chemistry[6] have led to an array of activated peptidyl–carbonyl compounds such as trifluoromethyl ketone TFMK3 and pentafluoroethyl ketone PFEK4. Such molecules are classical serine protease inhibitors which act by reversibly forming stable covalent adducts with the serine residue at the active site.[7] However, the possibility of clarifying the protease-bound conformation of these types of inhibitors by TRNOE techniques is precluded by their very slow rates of exchange. Methyl ketones MK1 and MK2 were designed to circumvent this problem since, although they very closely mimic their potent activated carbonyl analogues, the exchange between the free and bound states is very rapid due to their inability to form covalent hemiketal adducts with the protease.[8] Another key feature of MK1 and MK2 is the presence of the ketonic CH_3 group, which is positionally equivalent to the P1′ amido NH group[10] and whose NMR resonance may provide information on the conformation in the P1–P1′ region of bound peptides.

Initial 1D NMR experiments[11] led to the observation of HCMV protease induced line broadening of the proton signals of MK1. Consistent with specific complexation by the enzyme, the ^{1}H NMR spectrum of the inhibitor upon titration with enzyme presented a nearly uniform broadening as compared to the resonances of the ligand in the uncomplexed state. The enzyme-dependent changes in the methyl signal of the alanine moiety at P1 shown in Figure 1 A are representative of the observed spectral changes as a whole. Upon subsequent addition of PFEK4 to the sample, the peak heights for the proton signals of unbound MK1 were restored as a consequence of the displacement of MK1 from the active site by its much more potent activated carbonyl analogue (Figure 1 B).

Transferred NOE studies were undertaken to obtain detailed structural information concerning the binding of the present inhibitors. Owing to the unfortuitous superposition of the resonances for *tert*-butyl groups at P3 and P4 of MK1 in 1D studies, TRNOE experiments were carried out with the isopropyl analogue MK2, whose side-chain signals were conveniently separated. The ^{1}H NOESY spectrum of MK2 in the presence of HCMV protease (selected regions of which are shown in Figure 2) presented negative NOE crosspeaks resulting from interproton cross-relaxation of the enzyme-bound inhibitor. Figure 2 A, which shows the NH-aliphatic

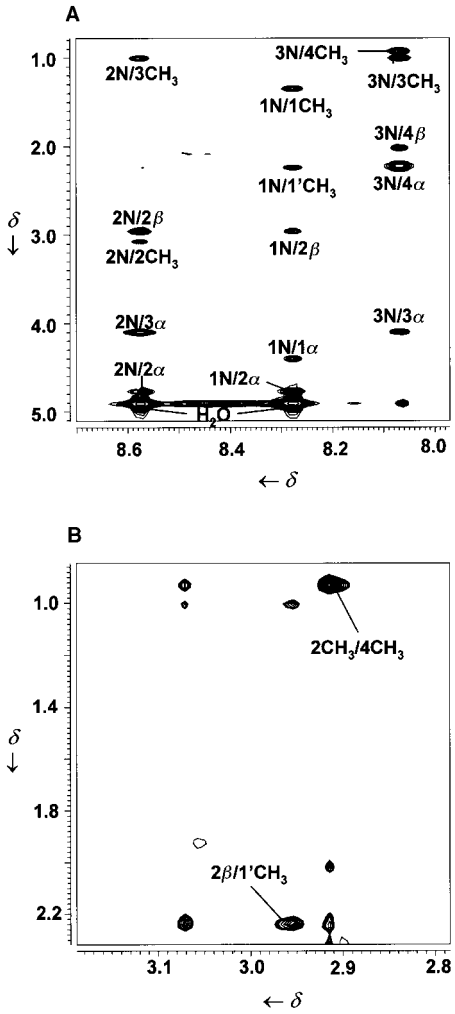

Figure 2. Selected regions of the 2D NOESY spectrum of 3.1 mM MK2 in the presence of 0.26 mM HCMV protease, recorded using a mixing time of 150 ms. A) The NH-aliphatic region. B) The aliphatic region with key interresidue side-chain correlations.

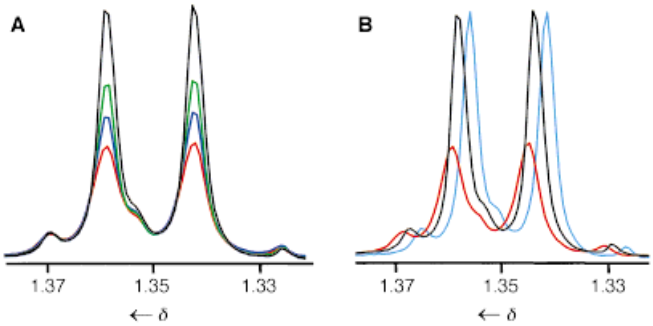

Figure 1. A) HCMV protease induced changes in the ^{1}H NMR signal of the P1-α-methyl group of MK1: MK1 alone (black) and in the presence of enzyme with molar excesses of inhibitor of 30-fold (green), 15-fold (blue), and 7-fold (red). B) The effect of PFEK4 on the protease-induced changes in the P1-α-methyl signal of MK1: inhibitor alone (black), a 7-fold excess in the presence of HCMV protease (red), and following subsequent addition of PFEK4 (cyan). (Signals have been skewed for the sake of clarity.)

region of the spectrum, presents a pattern of interresidue amide–C$^\alpha$H correlations between C$^\alpha$H(i) and NH($i+1$) of strong intensity alternating with weak intraresidue crosspeaks between C$^\alpha$H(i) and NH(i), which is characteristic of an extended peptide conformation.[12] This is corroborated by other key NOEs, indicated in Figure 2 B, including that between the methyl groups at P2 and P4. Also of importance is a crosspeak between one of the side-chain methyl groups at P2 and the ketonic methyl group, which indicates the presence of a "kink" at the C-terminal end of the chain.

The volumes of the NOE crosspeaks were subsequently scaled and converted[13] into interproton distance restraints for molecular modeling of enzyme-complexed MK2 using the method of restrained simulated annealing. These calculations[14] afforded a single family of structures, for which a final representative example consistent with the NMR data is

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presented in Figure 3. Both the extended backbone conformation and the kink between positions P1 and P1′ are clearly evident.

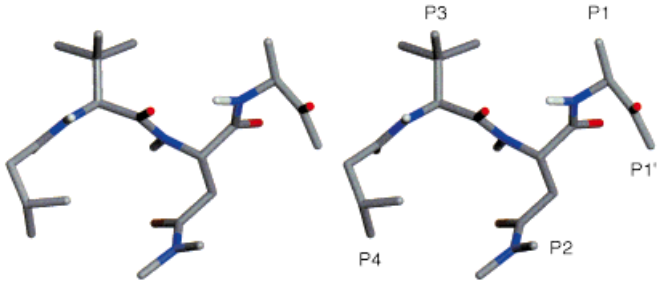

Figure 3. Stereoview of a representative structure derived from NMR data of MK2 when bound to HCMV protease, generated by restrained simulated annealing.

Finally, the subsequent elucidation of the crystal structure of the covalent complex of HCMV protease and the peptidyl α-ketoamide inhibitor AKA5[15] allows us to compare the conformations of bound inhibitors derived separately by NMR and crystallographic methods. As is evident in Figure 4,

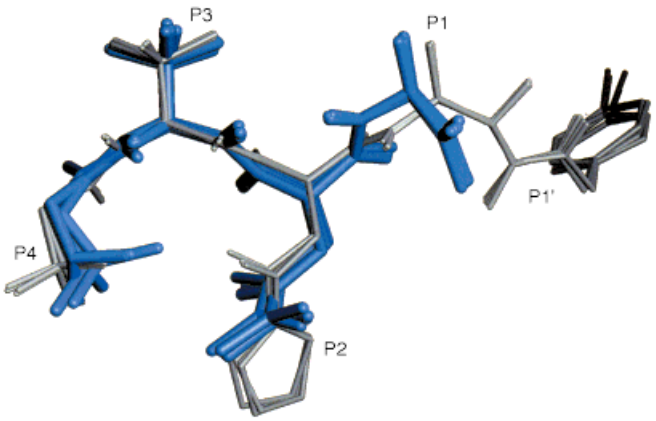

Figure 4. Comparison of the structures of HCMV protease bound inhibitors MK2 (blue, 29 low-energy conformers) obtained through transferred NOE methods and AKA5 (gray, four crystallographically unique conformers) derived from the X-ray crystallographic structure of the enzyme–inhibitor complex[15].

the four structures derived crystallographically and the superimposition of 29 structures determined from NMR experiments are in very good agreement.

The results described herein serve to underline the utility of peptidyl methyl ketones to probe the structure of activated carbonyl inhibitors of a serine protease using TRNOE methods. The bound conformation of MK2 will invariably aid in the rational design of inhibitors of HCMV protease and, potentially, the development of agents against HCMV disease.

Received: May 8, 1998 [Z11833IE]
German version: *Angew. Chem.* **1998**, *110*, 2858–2860

Keywords: conformation analysis · molecular modeling · NMR spectroscopy · peptidomimetics

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acquisition and relaxation delay (G. Lippens, C. Dhalluin, C. Wieruszeski, *J. Biomol. NMR* **1995**, *5*, 327–331).
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[14] The modeling of MK2 was carried out with the program Discover 95.0 and the CFF95 force field (Molecular Simulations Inc. San Diego, CA, USA). All calculations were performed without nonbonded or coulombic cutoffs and a dielectric constant of 1.0. Twenty-five NMR-derived distance restraints were generated from the NMR data using a method similar to that of Sykes et al.[13] The restraints were applied as strong (1.8–2.7 Å), medium (1.8–3.5 Å), or weak (1.8–5.0 Å) flat-bottomed potentials. A single, high-temperature unrestrained dynamics run was performed at 1000 K using a timestep of 1 fs, with 40 structures collected at intervals of 10 ps to generate a starting set of conformations. Each structure was cooled and minimized using the following simulated annealing protocol: The temperature was lowered to 500 K at a rate of 50 K ps^{-1}, where strong restraints were applied, and then to 250 K (5 K ps^{-1}). The remaining restraints were added, and cooling to 50 K (0.5 K ps^{-1}) was performed followed by restrained minimization to a final gradient of 0.01 kcal mol^{-1} Å^{-1}. Twenty-nine low-energy structures were isolated (average total restraint violation: 0.11 kcal mol^{-1}; root mean square deviation for all heavy atoms: 0.241), and a representative consistent with the NMR data was selected.
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Calixarene-Based Copper(I) Complexes as Models for Monocopper Sites in Enzymes**

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Metalloenzymes are fascinating natural "factories" adept at transforming organic molecules with high efficiency and selectivity.[1] A good chemical model for metalloenzymes is a key to the understanding of the fundamental mechanisms of the chemistry involved in the catalytic cycles and to the design of efficient and selective new tools for the synthetic chemist. Although mononuclear copper enzymes constitute an important class of proteins,[2] few, if any, chemical systems allow the specific chemistry at a monocopper site to be modeled. The first step for the elaboration of a biomimetic system is the conception of a synthetic model for the active copper(I) center (since the copper center in this oxidation state is the active form for the activation of small molecules).[2] For this purpose, three important elements are required:

- A polydentate ligand to coordinate to the cuprous ion and reproduce the geometry and the chemical properties of the amino acid residues involved in the coordination sphere of the metal.
- An appropriate environment to prevent the formation of binuclear species.
- A vacant site to allow the coordination and exchange of an external ligand.

The classic strategy to protect a redox metal center, is based on tuning the sterical environment provided by a tripodal ligand. This approach presents a dilemma: high steric hindrance will prevent the cuprous ion from reacting with an external substrate molecule; but on the other hand, a decrease in the steric bulk will drive the reaction with O_2 (or any other oxidant) to produce binuclear copper(II) complexes.[3]

Here we describe a new supramolecular model of the mononuclear cuprous sites, which takes advantage of the conic shape of a calix[6]arene that is appropriately functionalized with three pyridine (Py) moieties. The role of the pyridine groups is to provide an N_3 donor set for the copper ion, thereby mimicking the imidazole residues which coordinate the type II cuprous ion in enzymes such as dopamine β-hydroxylase, peptidylglycine α-amidating monooxygenase, copper amine oxidases, as well as nitrate reductases.[2] The geometry of the system is designed to orient the fourth coordination site toward the inside of the conic cavity provided by the calixarene skeleton. The role of the calixarene is thus to protect the metal center from any undesired interaction with another metal center, to facilitate the approach of an organic molecule, and finally to operate a means of selection based on its size and nature.

The new tridentate N-ligand $X_6Me_3Pic_3$ was synthesized in two steps from *p-tert*-butylcalix[6]arene (X_6H_6). The first step involved the alternate protection of three out of the six phenolic OH functions of the calixarene by a known selective methylation procedure.[4] Treatment of this key intermediate $X_6Me_3H_3$ with picolyl chloride in the presence of K_2CO_3 in DMF provided the desired tripod $X_6Me_3Pic_3$ in 87% yield (Scheme 1). The ^{1}H NMR spectrum of this ligand recorded at 297 K is characteristic of a major flattened *cone* conformation with C_3 symmetry. The methoxy groups are projected toward the inside of the cavity as indicated by the high field shift of the methyl protons in the spectrum. However, a low-temperature study revealed the presence of a minor conformer of lower symmetry, which is in equilibrium with the conic conformation.[5]

The addition of 1.1 equivalents of $[Cu(MeCN)_4]PF_6$ to a solution of $X_6Me_3Pic_3$ in $CDCl_3$ resulted in the formation of a

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[**] We thank Dr. A. van Dorsselaer and H. Nierengarten (LSMBO, Strasbourg, France) for the mass spectra of our complex.

  1433-7851/98/3719-2732 $ 17.50+.50/0