DOI: 10.1002/chem.200501098

Cyclodextrin-Derived Mimic of Glutathione Peroxidase Exhibiting Enzymatic Specificity and High Catalytic Efficiency

Ze-Yuan Dong, Xin Huang, Shi-Zhong Mao, Kai Liang, Jun-Qiu Liu,* Gui-Min Luo, and Jia-Cong Shen[a]

Abstract: To elucidate the relationships between molecular recognition and catalytic ability, we chose three assay systems using three different thiol substrates, glutathione (GSH), 3-carboxyl-4-nitrobenzenethiol (CNBSH), and 4-nitrobenzenethiol (NBSH), to investigate the glutathione peroxidase (GPx) activities of 2,2′-ditellurobis(2-deoxy-β-cyclodextrin) (2-TeCD) in the presence of a variety of structurally distinct hydroperoxides (ROOH), H_2O_2, *tert*-butyl peroxide (*t*BuOOH), and cumene peroxide (CuOOH), as the oxidative reagent. A comparative study of the three assay systems revealed that the cyclodextrin moiety of the GPx mimic 2-TeCD endows the molecule with selectivity for ROOH and thiol substrates, and hydrophobic interactions are the most important driving forces in 2-TeCD complexation. Furthermore, in the novel NBSH assay system, 2-TeCD can catalyze the reduction of ROOH about 3.4×10^5 times more efficiently than diphenyl diselenide (PhSeSePh), and its second-order rate constants for thiol are similar to some of those of native GPx. This comparative study confirms that efficient binding of the substrate is essential for the catalytic ability of the GPx mimic, and that NBSH is the preferred thiol substrate of 2-TeCD among the chosen thiol substrates. Importantly, the proposed mode of action of 2-TeCD imitates the role played by several possible noncovalent interactions between enzymes and substrates in influencing catalysis and binding.

Keywords: cyclodextrins · glutathione peroxidase · hydrogen bonds · hydrophobic interactions · specificity

Introduction

Enzymes exhibit high substrate-specificity and significantly accelerate reaction rates. The nature of substrate binding and the intracomplex interactions of enzymes plays an important role in strong catalytic ability. As one of a series of antioxidative selenoenzymes in living organisms, glutathione peroxidase (GPx) catalyzes the reduction of harmful hydroperoxide (ROOH) by glutathione (GSH) (Figure 1) to protect biological molecules from oxidative stress both inside and outside the cells.[1] The active site of GPx includes a selenocysteine residue which forms a catalytic triad with glutamine and tryptophan residues in a depression at the protein's surface, and some charged and hydrophobic amino acid residues (Phe, Trp, Asp) form a hydrophobic cavity.[2] In contrast to cytosolic GPx (cGPx), which uses GSH exclusively as cosubstrate, other enzymes, such as phospholipid hydroperoxide GPx (PHGPx), extracellular GPx (eGPx), and gastrointestinal GPx (GIGPx), readily accept many thiols as substrates. Indeed, the reactivity of these native enzymes differs considerably depending upon the nature of the hydroperoxides and thiols.[3] Several recent attempts have been made to produce synthetic selenium/tellurium compounds that mimic the properties of native GPx. These

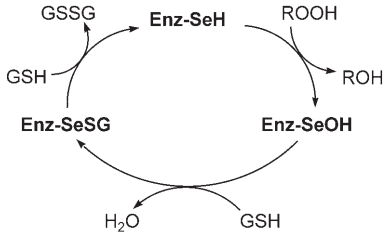

Figure 1. Catalytic cycle for GPx.

[a] Z.-Y. Dong, X. Huang, S.-Z. Mao, K. Liang, Prof. J.-Q. Liu, Prof. G.-M. Luo, Prof. J.-C. Shen
Key Laboratory for Supramolecular Structure and Materials of Ministry of Education
Jilin University, Changchun 130012 (China)
Fax: (+86) 431-519-3421
E-mail: junqiuliu@jlu.edu.cn

Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author.

compounds were designed by abstracting the structural and functional features of the native enzymes, however, they do not fulfil all of the expected prerequisites, such as containing Se···N or Se···O interactions and specific binding sites, and, thus, they show poor GPx activities.[4] An excellent GPx mimic, selenosubtilisin, reported by Hilvert et al., shows strong substrate specificity for 3-carboxyl-4-nitrobenzenethiol (CNBSH) by its evolved binding site.[5] We recently fabricated GPx mimics that use antibodies and proteins as receptors in which a binding site for the recognition of thiol substrate is generated.[6] However, the studies of structure–function relationships are blocked by the complicated nature of macromolecular proteins. Fabrication of small molecular GPx models offers an ideal alternative for elucidating the origin of substrate binding in enzyme catalysis.

Cyclodextrins, which bind hydrophobic substrates in cavities that have two rims of hydroxyl groups acting as anchors, have been exploited extensively as enzyme models and molecular receptors.[7] The interactions of cyclodextrins and guest molecules are directional, specific, and reversible, and a wealth of information is available concerning their binding strength and kinetics.[8] Consequently, these structurally delicate cyclodextrin molecules are useful implements for studying the catalytic nature of enzymes.

We recently reported that 2,2′-ditellurobis(2-deoxy-β-cyclodextrin) (2-TeCD, see Figure 2) acts as an efficient GPx

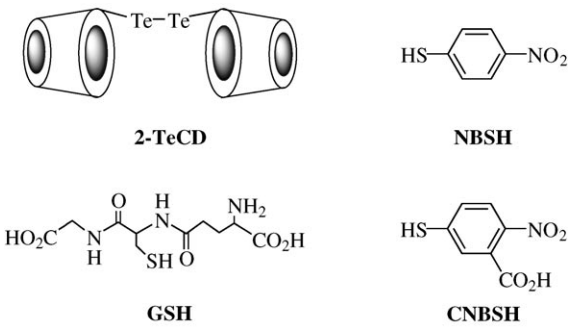

Figure 2. The structures of 2-TeCD and three thiol substrates (GSH, CNBSH, and NBSH).

mimic and employs the same catalytic mechanism as native GPx in the CNBSH assay system.[9] To elucidate further the relationships between molecular recognition and catalytic ability, we chose three assay systems using three different thiol substrates (Figure 2), GSH, CNBSH, and NBSH (4-nitrobenzenethiol), to investigate the GPx activities of 2-TeCD in the presence of a variety of structurally distinct hydroperoxides (ROOH), H_2O_2, *tert*-butyl peroxide (*t*BuOOH), and cumene peroxide (CuOOH), as the oxidative reagent. A comparative study of the three assay systems revealed that the cyclodextrin moiety of the GPx mimic 2-TeCD endows the molecule with selectivity for ROOH and thiol substrates, just as native GPx exhibits different ROOH and thiol specificity. Hydrophobic interaction is the most important driving force in 2-TeCD complexation.[9] Furthermore, in the novel NBSH assay system, 2-TeCD can catalyze the reduction of ROOH about 3.4×10^5 times more efficiently than diphenyl diselenide (PhSeSePh), and its second-order rate constants for thiol are similar to those of native GPx. The detailed kinetic analyses reported herein for the highly efficient GPx mimic demonstrate that efficient binding of substrate is essential for catalytic activity.

Results and Discussion

The synthesis of compound 2-TeCD is depicted in our previous literature.[9a] By using GSH as a thiol substrate in the coupled reductase assay system reported by Wilson et al.,[10] the GPx activity of 2-TeCD is only 24-fold and 27-fold higher than that of PhSeSePh and diphenyl ditelluride (PhTeTePh), respectively.[9c] However, we find that 2-TeCD can reduce H_2O_2, *t*BuOOH, and CuOOH effectively in the CNBSH assay system using CNBSH as a thiol substrate, and the peroxidase activity is almost 10^5 times greater than that of PhSeSePh.[9c] The rate enhancement is remarkable and reflects the recognition action for thiol substrate in 2-TeCD catalysis. The 2-TeCD scaffold seems to be preferred by the aromatic compounds rather than by the hydrophilic compound GSH. In our recent work, we reported the inclusion complexation of CNBSH and β-cyclodextrin with a binding constant of more than $10^3\,\text{M}^{-1}$.[9c] Although the thiol substrate CNBSH takes some advantage of the binding site of 2-TeCD and largely improves the catalytic efficiency of 2-TeCD, it seems unlikely that CNBSH is the optimal thiol substrate of 2-TeCD. Therefore, we used NBSH as another aromatic thiol substrate to investigate the relationships between specificity and activity of 2-TeCD.

By using NBSH as the substrate, a thorough analysis of the NBSH assay system was carried out (see Supporting Information). Compound NBSH exhibits particular UV spectroscopic properties ($\lambda_{max}=410$ nm, pH 7.0), similar to those of CNBSH. Furthermore, the corresponding disulfide does not interfere with the 410 nm absorbance of the thiol NBSH. The initial concentration of NBSH was measured from the 410 nm absorbance spectrum ($\varepsilon=14\,500\,\text{M}^{-1}\,\text{cm}^{-1}$, pH 7.0). From the disappearance of NBSH that can be easily followed spectrophotometrically at 410 nm, the initial rates of reduction of ROOH by NBSH in the presence of catalyst were determined and are listed in Table 1. The relative GPx activity was corrected for the respective control rate in the absence of catalyst and was calculated based on a catalytic activity of PhSeSePh equal to 1. Under the experimental conditions, the turnover numbers of the catalysts were calculated (Table 1) and the high value of 3018 for 2-TeCD was observed. In the NBSH assay system, the GPx activity of 2-TeCD was about 3.4×10^5 times more efficient than that of PhSeSePh. To study the origin of this impressive rate acceleration, we focussed on the binding of thiol substrate in the catalytic process of 2-TeCD. The investigation of the inclusion complexation of NBSH and β-cyclodextrin was performed by means of UV and ^{1}H NMR spectro-

Table 1. The initial rates (v_0)[a] and relative GPx activities of various catalysts (100 μM) for the reduction of ROOH (250 μM) by NBSH (100 μM) at pH 7.0 (50 mM PBS, 1 mM EDTA) and 25 °C in three assay systems.

Catalyst	Hydroperoxide	v_0 [M min^{-1}]	Relative activity[b]	Turnover number[c]
PhSeSePh	H_2O_2	$(0.11\pm0.01)\times10^{-7}$	≡1	<1
	*t*BuOOH	$(0.10\pm0.01)\times10^{-7}$	1	<1
	CuOOH	$(0.13\pm0.01)\times10^{-7}$	1	<1
PhTeTePh	H_2O_2	$(5.38\pm0.12)\times10^{-7}$	49	3.2
	*t*BuOOH	$(7.84\pm0.38)\times10^{-7}$	78	4.7
	CuOOH	$(8.51\pm0.42)\times10^{-7}$	65	5.1
β-cyclodextrin[d]	H_2O_2	ND		
	*t*BuOOH	ND		
	CuOOH	ND		
2-TeCD	H_2O_2	$(2.65\pm0.27)\times10^{-4}$	24091	180.0
	*t*BuOOH	$(1.26\pm0.14)\times10^{-3}$	126000	816.1
	CuOOH	$(4.48\pm0.18)\times10^{-3}$	344615	3018.0

[a] Mean of at least five values ± standard deviation. [b] Calculated based on a GPx activity of PhSeSePh equal to 1. [c] Under the experimental conditions, the turnover numbers of catalysts for one hour were calculated, as the controls were used in stoichiometric amounts. [d] In this assay system β-cyclodextrin has no detectable catalytic activity.

scopy. In the ^{1}H NMR spectra (Figure 3), the H^a and H^b protons of the aromatic region show significant downfield and upfield shifts, respectively, in the presence of β-cyclodextrin

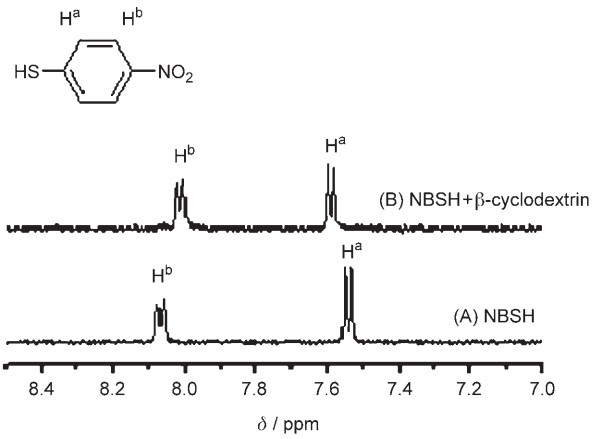

Figure 3. ^{1}H NMR spectra of NBSH in the absence (A) and presence (B) of β-cyclodextrin in D_2O.

compared to the spectrum of NBSH alone. This indicates that the H^a and H^b protons of the aromatic region were located in a different microenvironment in the presence of β-cyclodextrin in water. These experimental results (see Supporting Information) indicate that NBSH is easily bound to the hydrophobic cavity of β-cyclodextrin with a binding constant of 1860 M^{-1}. To further confirm the inclusion complexation in 2-TeCD catalysis, the GPx activity of 2-TeCD was assessed in the NBSH system in the presence of an inhibitor, 1-adamantaneethanol, that can compete with substrates for the hydrophobic pocket of β-cyclodextrin. As expected, addition of 1-adamantaneethanol strongly decreased the GPx activity of 2-TeCD (see Supporting Information). This observation clearly shows that hydrophobic interactions play a major role during the molecular recognition of 2-TeCD. In Table 1, the notable result is the specificity for reduction of aromatic peroxide CuOOH: CuOOH is reduced by thiol NBSH approximately 16 times faster than H_2O_2 in the presence of 2-TeCD. A similar observation was found recently.[9c,11] Furthermore, hydrophobic *t*BuOOH is also a better substrate than H_2O_2 for 2-TeCD, as evidenced by turnover numbers. It is apparent that the hydrophobic cavity provided by the cyclodextrin moiety of 2-TeCD acts as a binding site for the ROOH substrate.

Hydrogen bonding, van der Waals forces, and hydrophobic interactions depend on how the guest molecule fits into the host cavity, which is determined by the size and/or shape of the guest.[8] Therefore, the structurally diverse guest molecules drastically affect the molecular recognition ability of cyclodextrins. As expected, 2-TeCD, carrying the recognition properties of a cyclodextrin moiety, exhibits remarkably different GPx activities in the above three assay systems. Apparently, as cyclodextrins are neutral molecules, the interactions of 2-TeCD and thiol substrates are attributed mainly to a combination of hydrogen bonding and hydrophobic effects in catalysis. For the linear hydrophilic molecule GSH, weak complexation with β-cyclodextrin (association constant, 101 M^{-1})[12] may be responsible for the limited enhancement in catalytic efficiency of 2-TeCD. Although we know that aromatic thiol CNBSH has a relatively strong hydrophobic interaction with β-cyclodextrin,[9c] it is important to study the hydrogen-bonding interactions between them because hydrogen-bonding interactions are ubiquitous in natural enzyme systems. The ^{1}H and two-dimensional NMR spectra (Figure 4, and see Supporting Information) reveal that the two rims of hydroxyl groups of β-cyclodextrin can interact with the thiol and carboxyl groups of CNBSH by hydrogen bonds. This indicates that hydrogen-bonding interactions may function during the process of 2-TeCD catalysis. Moreover, we find that with CNBSH as thiol substrate, 2-TeCD shows approximately 2-fold lower catalytic activity than that with thiol NBSH. Considering the similar binding ability of β-cyclodextrin for CNBSH and NBSH (association constants 2010 M^{-1} and 1860 M^{-1}, respectively), the phenomenon suggests that hydrogen-bonding interactions between 2-TeCD and thiols may play an unexpected role in catalysis.

The second-order rate constants for the NBSH assay system are shown in Table 2. Saturation kinetics were observed for each of the peroxidase reactions at all the individual concentrations of NBSH and ROOH investigated. The rate constants of the control reaction between thiol and ROOH in the absence of catalyst vary in the order k (H_2O_2) > k (CuOOH) > k (*t*BuOOH). In contrast, the analo-

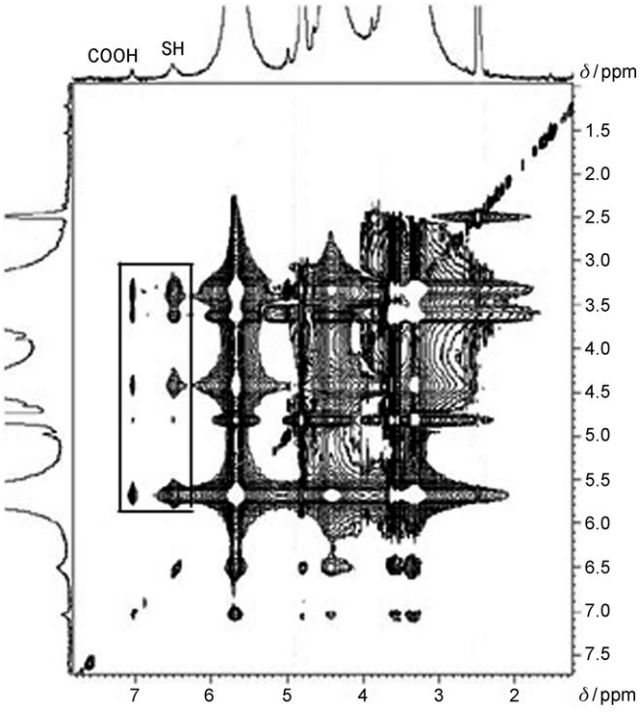

Figure 4. 2D NMR spectrum of CNBSH with β-cyclodextrin in [D_6]DMSO at ambient temperature for the observation of hydrogen-bonding interactions.

Table 3. Comparison of second-order rate constants for the GPx-like activity of 2-TeCD in the three assay systems, and binding constants (K_a) of thiol substrates and β-cyclodextrin.[a]

Thiol	Hydroperoxide	k_{max}/K_{ROOH} [$M^{-1}min^{-1}$]	k_{max}/K_{NBSH} [$M^{-1}min^{-1}$]
GSH[b]	H_2O_2	5.24×10^4	6.26×10^4
(K_a=101 M^{-1})	*t*BuOOH	7.99×10^4	6.28×10^4
	CuOOH	2.71×10^5	6.86×10^4
CNBSH[c]	H_2O_2	6.00×10^3	1.05×10^7
(K_a=2010 M^{-1})	*t*BuOOH	1.64×10^4	7.50×10^6
	CuOOH	1.61×10^5	5.92×10^6
NBSH	H_2O_2	1.01×10^4	4.56×10^6
(K_a=1860 M^{-1})	*t*BuOOH	5.86×10^4	3.59×10^6
	CuOOH	3.44×10^5	3.48×10^6

[a] Values are the reported means. [b] Data from reference [9a]. [c] Data from reference [9c].

Table 2. Kinetic parameters for the peroxidase activity of 2-TeCD in the NBSH assay system.[a]

Hydroperoxide	k_{max} [min^{-1}]	K_{ROOH} [mM]	K_{NBSH} [μM]	k_{max}/K_{ROOH} [$M^{-1}min^{-1}$]	k_{max}/K_{NBSH} [$M^{-1}min^{-1}$]
H_2O_2	210±16	20.78±1.03	46±4	$(1.01\pm0.07)\times10^4$	$(4.56\pm0.29)\times10^6$
*t*BuOOH	280±21	4.78±0.35	78±6	$(5.86\pm0.28)\times10^4$	$(3.59\pm0.32)\times10^6$
CuOOH	320±19	0.93±0.03	108±16	$(3.44\pm0.17)\times10^5$	$(3.48\pm0.53)\times10^6$

[a] Each value is the mean ± S.D.

gous second-order rate constants ($k=k_{max}/K_{ROOH}$) of 2-TeCD and ROOH vary as k (CuOOH) > k (*t*BuOOH) > k (H_2O_2). It is possible that the first series reflects the intrinsic rate of reaction between hydroperoxides and a thiolate in the absence of any significant binding effects, whereas the latter series indicates that CuOOH and *t*BuOOH are able to take advantage of the cyclodextrin scaffold, thereby raising their second-order rate constants above that of H_2O_2. Furthermore, the Michaelis–Menten constant (K_{ROOH}) values (Table 2) for 2-TeCD vary as $K_{CuOOH} < K_{tBuOOH} < K_{H_2O_2}$ in the aromatic thiol assay system. These observations reveal that the GPx mimic 2-TeCD exhibits substrate specificity for ROOH, similar to that reported previously.[9c] Table 2 illustrates that the second-order rate constants of 2-TeCD and NBSH are as high as $10^6\,M^{-1}min^{-1}$, similar to those of native eGPx and PHGPx ($10^6\,M^{-1}min^{-1}$), as determined previously.[3]

For comparison, the second-order rate constants of 2-TeCD in the three assay systems are illustrated in Table 3. For GSH, 2-TeCD shows low second-order rate constants ($10^4\,M^{-1}min^{-1}$), at least two orders of magnitude less efficient than for the aromatic thiols NBSH/CNBSH. This clearly indicates that the GPx mimic 2-TeCD exhibits substrate specificity for thiols. In particular, we observe that the specificity of 2-TeCD for the two aromatic thiols differs somewhat, which may arise from the unexpected hydrogen-bonding effect between CNBSH and β-cyclodextrin. In addition to ^1H and two-dimensional NMR spectra, we used molecular simulation to observe further the hydrogen-bonding effect. We found that a number of CNBSH molecules can easily sit around the cyclodextrin cavity, anchored by hydrogen bonds, with one CNBSH molecule encapsulated in the cavity (Figure 5). Due to steric hindrance arising from the adsorption of a number of CNBSH molecules, which blocks catalyst recognition for another substrate ROOH, 2-TeCD expectedly shows relatively low GPx activity and low second-order rate constants for ROOH in the CNBSH assay system. This is not, however, the case with NBSH, and, thus, the complexation of another substrate ROOH is facilitated. Indeed, in the

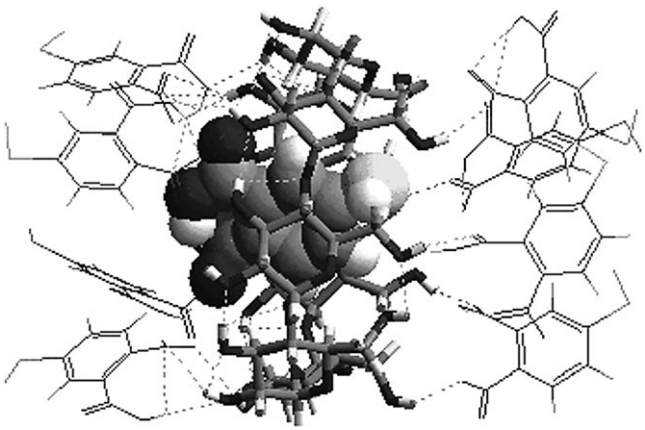

Figure 5. A model structure of complexation of many CNBSH molecules and one β-cyclodextrin.

NBSH assay system, 2-TeCD shows higher second-order rate constants for ROOH and higher catalytic activity.

Based on the structural understanding of GPx, as well as the nature of enzymes for molecular recognition and catalysis, we propose that the generation of specific and efficient binding of GPx models to thiol substrates should be important for efficient turnover. As observed from the catalytic activity, NBSH is a preferential thiol substrate of 2-TeCD among the chosen thiol substrates. This study confirms that efficient binding of substrate is essential for the catalytic activity of the GPx mimic. It is well known that steric and hydrogen-bonding interactions, as well as hydrophobic forces, are important in the operation of a vast range of different enzymes. The proposed mode of action of 2-TeCD represents the role played by several possible noncovalent interactions between enzymes and substrates in influencing catalysis and binding.

Conclusion

We show that the GPx mimic 2-TeCD is highly efficient in the reduction of ROOH by thiol NBSH in the NBSH assay system, and that its second-order rate constant for NBSH is similar to that of native GPx. This system corroborates that the effective binding of substrates is essential for the catalytic efficiency of enzymes. Furthermore, through a comparative study of three different assay systems, the GPx mimic 2-TeCD was shown to have similar properties to the native GPx, which exhibits different ROOH and thiol specificities. This study presents the substrate specificity of a small molecular GPx model and provides insight into a biocatalytic system. Furthermore, we show that during the catalytic process of enzymes, the hydrogen-bonding interactions between enzyme and substrates can sometimes have steric effects, and an ensemble of various noncovalent interactions determines the exact enzymatic nature of substrate specificity and rate acceleration.

Acknowledgements

We are grateful to the Natural Science Foundation of China (20534030, 20471023), the Program for Changjiang Scholars and Innovative Research Team in University (IRT0422), and the Ministry of Education of China for financial support.

[1] a) J. T. Roctruck, A. L. Pope, H. E. Ganther, A. B. Swanson, D. G. Hafeman, *Science* **1973**, *179*, 588; b) L. Flohé, W. A. Günzler, H. H. Schock, *FEBS Lett.* **1973**, *32*, 132.

[2] a) O. Epp, R. Ladenstein, A. Wendel, *Eur. J. Biochem.* **1983**, *133*, 51; b) B. Ren, W. Huang, B. Åkesson, R. Ladenstein, *J. Mol. Biol.* **1997**, *268*, 869.

[3] G. Takebe, J. Yarimizu, Y. Saito, T. Hayashi, H. Nakamura, J. Yodoi, S. Nagasawa, K. Takahashi, *J. Biol. Chem.* **2002**, *277*, 41254.

[4] a) G. Mugesh, H. B. Singh, *Chem. Soc. Rev.* **2000**, *29*, 347; b) G. Mugesh, W.-W. du Mont, *Chem. Eur. J.* **2001**, *7*, 1365; c) G. Mugesh, W.-W. du Mont, H. Sies, *Chem. Rev.* **2001**, *101*, 2125; d) L. Engman, D. Stern, I. A. Cotgreave, C. M. Andersson, *J. Am. Chem. Soc.* **1992**, *114*, 9737; e) M. Iwaoka, S. Tomoda, *J. Am. Chem. Soc.* **1994**, *116*, 2557; f) G. Mugesh, A. Panda, H. B. Singh, N. S. Punekar, R. J. Butcher, *J. Am. Chem. Soc.* **2001**, *123*, 839; g) G. Mugesh, A. Panda, S. Kumar, S. D. Apte, H. B. Singh, R. J. Butcher, *Organometallics* **2002**, *21*, 884; h) S. S. Zade, H. B. Singh, R. J. Butcher, *Angew. Chem.* **2004**, *116*, 4613; *Angew. Chem. Int. Ed.* **2004**, *43*, 4513; i) T. G. Back, Z. Moussa, M. Parvez, *Angew. Chem.* **2004**, *116*, 1288; *Angew. Chem. Int. Ed.* **2004**, *43*, 1268; j) T. G. Back, Z. Moussa, *J. Am. Chem. Soc.* **2003**, *125*, 13455; k) Y. You, A. Ahsan, M. R. Detty, *J. Am. Chem. Soc.* **2003**, *125*, 4918.

[5] a) Z.-P. Wu, D. Hilvert, *J. Am. Chem. Soc.* **1990**, *112*, 5647; b) I. M. Bell, M. L. Fisher, Z. P. Wu, D. Hilvert, *Biochemistry* **1993**, *32*, 3754; c) I. M. Bell, D. Hilvert, *Biochemistry* **1993**, *32*, 13969.

[6] a) G. Luo, X. Ren, J. Liu, Y. Mu, J. Shen, *Curr. Med. Chem.* **2003**, *10*, 1151; b) X. Ren, P. Jemth, P. G. Board, G. Luo, B. Mannervik, J. Liu, K. Zhang, J. Shen, *Chem. Biol.* **2002**, *9*, 789; c) X. Ren, S. Gao, D. You, H. Huang, Z. Liu, Y. Mu, J. Liu, Y. Zhang, G. Yan, G. Luo, T. Yang, J. Shen, *Biochem. J.* **2001**, *359*, 369.

[7] a) R. Breslow, S. D. Dong, *Chem. Rev.* **1998**, *98*, 1997; b) Y. Murakami, J. I. Kikuchi, Y. Hisaeda, O. Hayashida, *Chem. Rev.* **1996**, *96*, 721.

[8] a) J. Szejtli, *Chem. Rev.* **1998**, *98*, 1743; b) M. V. Rekharsky, Y. Inoue, *Chem. Rev.* **1998**, *98*, 1875.

[9] a) X. Ren, Y. Xue, J. Liu, K. Zhang, J. Zheng, G. Luo, C. Guo, Y. Mu, J. Shen, *ChemBioChem* **2002**, *3*, 356; b) X. Ren, Y. Xue, K. Zhang, J. Liu, G. Luo, J. Zheng, Y. Mu, J. Shen, *FEBS Lett.* **2001**, *507*, 377; c) Z. Dong, J. Liu, S. Mao, X. Huang, B. Yang, X. Ren, G. Luo, J. Shen, *J. Am. Chem. Soc.* **2004**, *126*, 16395.

[10] S. R. Wilson, P. A. Zucker, R.-R. C. Huang, A. Spector, *J. Am. Chem. Soc.* **1989**, *111*, 5936.

[11] M. McNaughton, L. Engman, A. Birmingham, G. Powis, I. A. Cotgreave, *J. Med. Chem.* **2004**, *47*, 233.

[12] X. Ren, J. Liu, G. Luo, Y. Zhang, Y. Luo, G. Yan, J. Shen, *Bioconjugate Chem.* **2000**, *11*, 682.

Received: September 6, 2005
Published online: February 21, 2006

