Molecular Recognition and Catalysis: Acceleration of Acyl Transfer Reactions by a Hydrogen-Bonding Receptor

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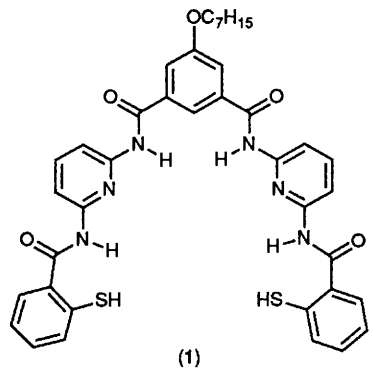
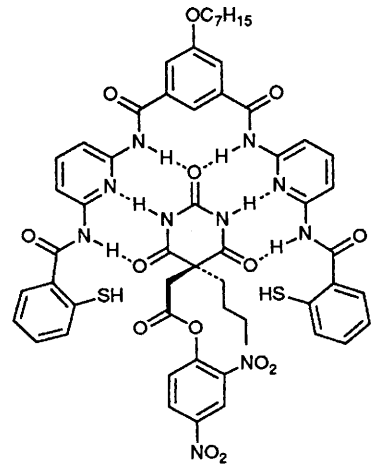
A hydrogen-bonding receptor containing an appended thiol has been synthesized and shown to cause large rate accelerations ($k_{obs}/k_{uncat} > 10^4$) in the thiolysis reaction of complementary barbiturate acetate derivatives.

The design of synthetic molecules that mimic elements of enzyme catalysis is of great interest.[1] In modelling the reactivity of the protease enzymes, a critical feature involves positioning a reactive nucleophile on a binding site in a suitable orientation for the facile transacylation reaction to take place with a non-covalently bound ester or amide substrate. A number of receptors containing such an arrangement has been reported, based primarily on the solvophobic binding of aromatic esters into cyclodextrins[2] and cyclophanes[3] or the strong association of alkylammonium ions with crown ethers[4] and spherands.[5] The binding interactions involved are either non- or weakly-directional resulting in considerable uncertainty in the position of the substrate in the receptor. Highly specialized substrate molecules[2b] or large and complex receptors[5] are required to achieve significant rate accelerations in these transacylation reactions. In the protease enzymes, however, it is hydrogen bonding that forms a critical binding force between catalyst and substrate.[6] These more directional interactions act both to bind the amide substrate and orientate it towards the catalytic groups in the active site. As part of a program aimed at the synthesis of new catalytic systems we sought to construct models for proteases in which a peptide-like substrate is held, solely by hydrogen bonds, in proximity to an appended thiol nucleophile.

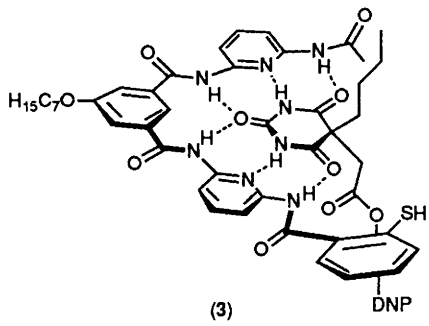
Our design for the new hydrogen-bonding catalyst is shown as (**1**). The binding environment is based on the strong hexa-hydrogen-bonding complementarity that exists between barbiturates and two 2,6-diamidopyridine units linked through an isophthalate spacer.[7] A nucleophile can be readily appended by acylation of the 6-amino groups by a mercaptobenzoic acid derivative. A barbiturate derivative with an acetate ester substituent in the 5-position will bind to (**1**) *via* six hydrogen bonds, as in (**2**). Molecular modelling studies[8] on (**2**) show a minimum energy conformation in which the thiol is directed into the cavity and positioned *ca.* 3.5 Å from the ester carbonyl on the substrate, as in (**3**).



(1)



(2)



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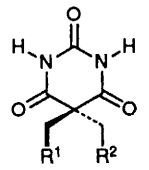
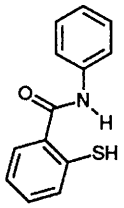
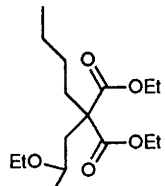


(4)

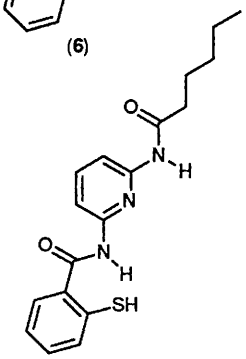
a: $R^1 = R^2 = CH_3$
b: R^1 = Pr, $R^2 = CO_2C_6H_4(NO_2)_2$-2,4
c: R^1 = Pr, $R^2 = CH(OEt)_2$
d: R^1 = Pr, $R^2 = CO_2H$



(6)



(5)



(7)

Receptor (**1**)† can be prepared in three steps by reacting 5-heptanoxyisophthaloyl dichloride with an excess of 2,6-diaminopyridine, followed by acylation of the free amino groups with *S*-benzoyl-2-mercaptobenzoyl chloride. Deprotection of the benzoyl groups (0.2 M NaOH, MeOH) gives (**1**) in 65% yield from the isophthaloyl dichloride. Addition of barbital (**4a**) (1 equiv.) to a $CDCl_3$ solution of (**1**) leads to large downfield shifts of the host amide and guest imide resonances consistent with a hexa-hydrogen-bonded complex of type (**2**).[7] Non-linear regression analysis[9] of the corresponding binding curve gave an association constant (K_a) of 1.9×10^4 dm^3 mol^{-1}. The required barbiturate ester (**4b**) was prepared by a modification of the method of Dickey.[10] Alkylation of diethyl malonate, firstly with 1-bromobutane (NaOEt, EtOH) and then with bromoacetaldehyde diethyl acetal (KOBu^t, toluene) gave diester (**5**) in 42% yield. Reaction of (**5**) with urea (NaOEt, EtOH, reflux) afforded barbiturate (**4c**) which was deprotected (HCl, acetone), oxidized ($KMnO_4$, pyridine) to acid (**4d**) and esterified with 2,4-dinitrophenol (DCC, dimethylaminopyridine, tetrahydrofuran) to form (**4b**) in 23% yield from (**4c**).

The rates of thiolysis of (**4b**) by different thiols in CH_2Cl_2 were followed through 5—6 half lives by observing spectrophotometrically at 350 nm the release of 2,4-dinitrophenol. The initial concentration of (**4b**) was 2.2×10^{-5} dm^3 mol^{-1} and the thiol (see Table 1) was present in excess. In all kinetic runs an excess of 2,6-lutidine (4.3×10^{-3} mol dm^{-3}) was added to assist in thiol deprotonation. The transacylations followed pseudo-first-order kinetics and their rate constants[11] and conditions are collected in Table 1.

The rate of release of 2,4-dinitrophenol is much faster in the presence of (**1**) reflecting the advantage of substrate association in the transacylation reaction (Figure 1). A comparison of

† All new compounds gave satisfactory spectroscopic and microanalytical or high resolution mass spectrometric data.

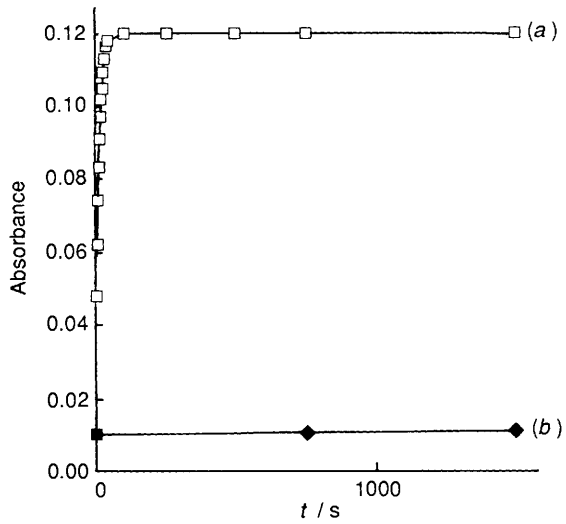


Figure 1. Release of 2,4-dinitrophenol in the reactions of (*a*): Receptor (**1**); (*b*): 2-mercaptobenzanilide, (**6**); with barbiturate ester (**4b**).

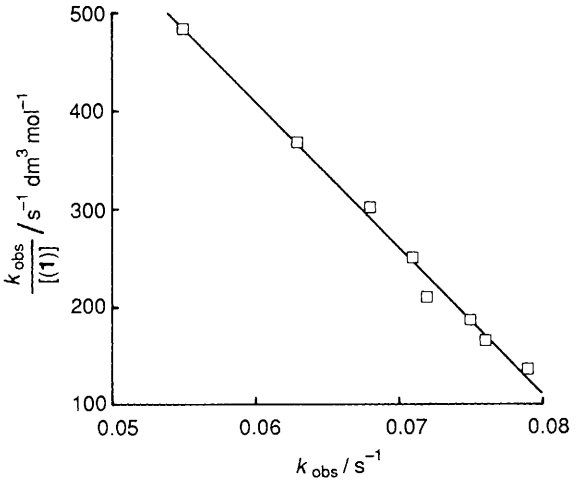


Figure 2. Eadie–Hofstee plot for the reaction between (**1**) and (**4b**).

Table 1.[a]

Thiol	10^4[Thiol] /mol dm^{-3}	k_{obs}/s^{-1}	k_{obs}/k_{uncat}
(**6**)	8.88	6.50×10^{-6}	1
(**7**)	4.40	5.78×10^{-5}	8
(**1**)	4.50	7.58×10^{-2}	11 700
(**1**)	0.57	3.95×10^{-2}	6 100
(**1**)	1.70	6.26×10^{-2}	9 600
(**1**)	2.84	7.10×10^{-2}	10 900
(**1**)	5.68	7.88×10^{-2}	12 100

[a] In CH_2Cl_2 (purged with argon) at 298 K [(**4b**)] = 2.2×10^{-5} mol dm^{-3}, [2,6-lutidine] = 4.3×10^{-3} mol dm^{-3}. The errors in rate-constant measurements were < 2%.

pseudo-first-order rate constants (k_{obs}/k_{uncat}) to the standard (uncatalysed) reaction between (**4b**) and 2-mercaptobenzanilide, (**6**), gives a rate acceleration of more than 10^4 for the reaction between (**1**) and (**4b**).[12] That this effect is due to the precise, hexa-hydrogen-bonding complementarity between (**1**) and (**4b**) is seen in the eightfold rate acceleration observed for compound (**7**) containing only three hydrogen-bonding sites. In addition, the reaction between (**1**) and (**4b**) follows Michaelis–Menten kinetics, and shows saturation behaviour as the host concentration is increased. An Eadie–Hofstee plot (Figure 2) gives $k_{cat} = 8.78 \pm 0.06 \times 10^{-2}$ s^{-1} and $K_M = 6.89 \pm 0.20 \times 10^{-5}$ mol dm^{-3}. The latter value corresponds to $K_a = 1.4 \times 10^4$ mol dm^{-3} and is consistent with the measured K_a values for related substrates. The product of the thiolysis reaction is the stable acylated receptor. We are currently modifying the receptor to promote the second, thioester cleavage, step in the process and to demonstrate turnover of the catalyst.

In summary, we have shown that an appropriately functionalised receptor can bind and orientate neutral substrates using only hydrogen-bonding interactions. This binding-enforced proximity between substrate and nucleophile leads to large accelerations in the rate of transacylation reactions.

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References

1 In 'Chemical Approaches to Understanding Enzyme Catalysis,' eds. B. S. Green, Y. Ashari, and D. Chipman, Elsevier, Amsterdam, 1982; H. Dugas, 'Bioorganic Chemistry,' Springer Verlag, New York, 1989, p. 172.
2 (a) M. L. Bender and M. Komiyama in 'Cyclodextrin Chemistry,' Springer, Berlin, 1978; (b) R. Breslow, G. Trainor, and A. Ueno, *J. Am. Chem. Soc.*, 1983, **105**, 2739; R. Breslow and S. Chung, *Tetrahedron Lett.*, 1990, 631.
3 Y. Murakami, *Top. Curr. Chem.*, 1983, **115**, 107; G. Schürmann and F. Diederich, *Tetrahedron Lett.*, 1986, 4249.
4 J. M. Lehn and C. Sirlin, *Nouv. J. Chem.*, 1987, **11**, 693; Y. Chao, G. R. Weisman, G. D. Y. Sogah, and D. J. Cram, *J. Am. Chem. Soc.*, 1979, **101**, 4948; K. Koga and S. Sasaki, *Pure Appl. Chem.*, 1988, **60**, 539.
5 D. J. Cram, H. E. Katz, and I. B. Dicker, *J. Am. Chem. Soc.*, 1984, **106**, 4987.
6 D. M. Blow, *Acc. Chem. Res.*, 1976, **9**, 145.
7 S. K. Chang and A. D. Hamilton, *J. Am. Chem. Soc.*, 1988, **110**, 1318.
8 Using the MACROMODEL program, W. C. Still, Columbia University.
9 Using the HOSTEST program, C. S. Wilcox, University of Pittsburgh.
10 J. B. Dickey and A. R. Gray, *Org. Synth.*, 1938, **18**, 8. For an alternative oxidative route see; O. Lafont, A. Talab, S. Menager, C. Cave, and M. Miocque, *Eur. J. Med. Chem.*, 1988, **23**, 427.
11 Determined by non-linear fit of the equation $(A_\infty\text{-}A_o)/(A_\infty\text{-}A_c) = e^{kt}$ using the ENZFITTER program, R. J. Leatherbarrow, Elsevier, Amsterdam, 1987.
12 For a discussion of rate comparisons see R. L. Schowen, *Chem. Eng. News*, 1983, 46; R. L. Schowen in 'Transition States of Biochemical Processes,' eds. R. D. Gandour and R. L. Schowen, Plenum Press, New York, 1978, pp. 77–114.