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Exploring sources of catalysis in the basic elimination of 5-nitrobenzisoxazole

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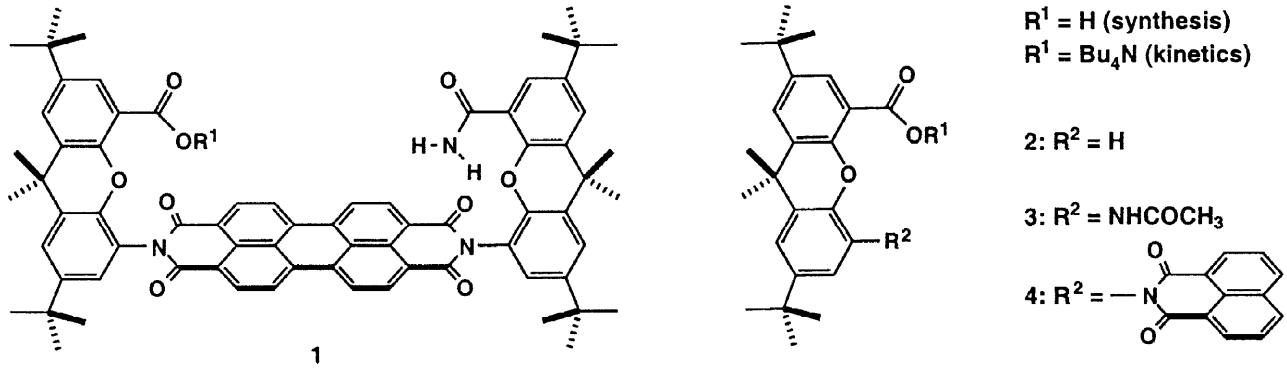
Abstract

A C-shaped, bifunctional host with a highly preorganized cleft was used as a host system to investigate the sources of catalysis in the base-promoted conversion of 5-nitrobenzisoxazole to 2-cyano-5-nitrophenolate. Kinetic studies with compounds that partly conserve structural elements of the host suggest that π-stacking interactions and solvation effects mainly contribute to the observed rate acceleration of the host compared to the acetate promoted reaction.

Keywords: benzimidazoles, elimination reaction, host compounds, catalysis

Compound **1** (Figure 1) belongs to a family of C-shaped molecules with highly preorganized clefts that have been designed to provide an asymmetric environment with convergent active groups [1, 2]. The ability of such molecular clefts to bind smaller molecules [1] with complementary functional groups allows for the study of the mechanisms of molecular recognition and their application in the design of synthetic catalysts.

Figure 1



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We used the base-promoted decomposition of 5-nitrobenzisoxazole to 2-cyano-5-nitrophenolate (Scheme 1) to study the catalytic behavior of receptor **1** and related simpler

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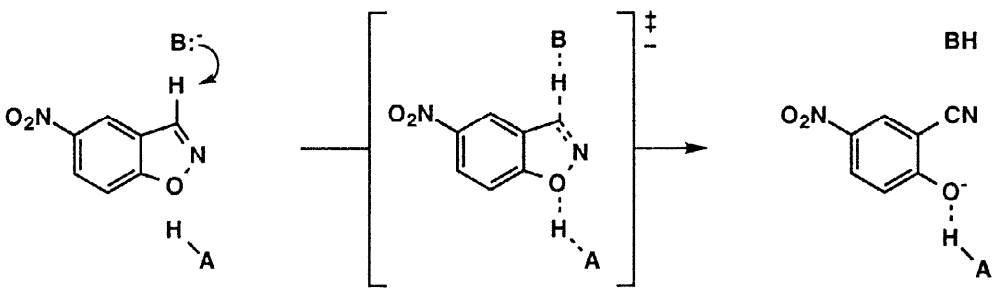
systems (**2** - **4**). This elimination reaction has become a popular target in the design of artificial enzymes [3 - 7]. Monoclonal antibodies [3], BSA [4] and a xylene-bridged host system [5] have been shown to catalyze this reaction with relatively high efficiency, but the sources of the observed rate enhancements are still the subject of discussion.

Scheme 1



Kinetic studies with compounds **1** - **4** (Figure 1) allowed to evaluate the factors that contribute to the catalysis of this solvent dependent reaction. The nitrobenzisoxazole elimination reaction was followed by UV/VIS spectroscopy at 380 nm ($\Delta\varepsilon$ = 16000 $M^{-1}s^{-1}$)[2]. The kinetic data (Table 1) were calculated as apparent second order rates ($k_{app.}$) from the initial rates in different solvents with varying polarity, a factor known to influence the elimination reaction [8 - 10]. Attempts to saturate the receptor were unsuccessful due to the low solubility of the substrate.

The carboxyxanthene **2** was synthesized from the corresponding 4,5-dibromoxanthene [11]. Selective halogen-metal exchange reactions (nBuLi, THF, - 78 °C; NCS, -78 → 23 °C, NH_4Cl quench, 95%; nBuLi, THF, -78 °C; $CH_3OC(O)Cl$, 78%) led to the chloroxanthene carboxylic acid methyl ester, which was readily hydrolyzed ($LiOH{\cdot}H_2O$, H_2O/THF, Δ, 98%) and dechlorinated (H_2, 10 % Pd-C, MeOH/EtOAc, 38%) providing the desired material **2**[3]. Acetamidocarboxyxanthene **3** was prepared by acetylation of the aminocarboxyxanthene [1] with acetic anhydride (CH_2Cl_2, pyridine, 23 °C, 73%)[4].

The elimination in the presence of compounds **2** and **3** was significantly accelerated relative to the acetate-promoted reaction, which has been used as reference in the kinetic studies of catalytic antibodies [3]. Better solvation of the smaller acetate compared to the aromatic carboxylates of **2** and **3**, in which the carboxylates' negative charge is dispersed over the

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[2] Methanol was distilled over $CuSO_4$ before use. Assays were performed at 25±0.5 °C. Stock solutions of **1** to **4** were prepared in CH_2Cl_2 by addition of 1 equ. of tetrabutylammonium hydroxide. The concentration of 5-nitrobenzisoxazole varied between 160 and 192 µM. The reactions were initiated by the addition of the xanthene compounds **1** - **4** and tetrabutylammonium acetate respectively (final concentrations: 8.5 to 9.2 µM for **1** - **4**, 3 mM for acetate). The $k_{app.}$ for compound **2** in CH_3OH/H_2O was 0.17 $M^{-1}s^{-1}$.

[3] Chloroxanthene carboxylic acid methyl ester: R_f= 0.4 (10% EtOAc/hexanes); mp 126 - 128 °C; IR (thin film) 1715, 1575, 1450, 1365, 1265 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, *J* = 2.4 Hz, 1H), 7.65 (d, *J* = 2.4 Hz, 1H), 7.29 (AB, J_{AB} = 2.2 Hz, $\Delta\nu_{AB}$ = 4.3 Hz, 2H), 4.00 (s, 3H), 1.63 (s, 6H), 1.34 (s, 9H), 1.31 (ds, 9H); HRMS (FAB) *m/e* calcd for $C_{25}H_{31}O_3ClNa$ $(M+Na)^+$ 437.1859, found 437.1873. Chloroxanthene carboxylic acid: R_f= 0.3 (30% EtOAc/hexanes); mp 232-233 °C; IR (thin film) 3300-2400 (br), 1695, 1605, 1570, 1455, 1265, 1245 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 10.95 (br, 1H), 8.18 (d, *J* = 2.4 Hz, 1H), 7.67 (d, *J* = 2.4 Hz, 1H), 7.36 (s, 2H), 1.67 (s, 6H), 1.36 (s, 9H), 1.34 (s, 9H); HRMS (FAB) *m/e* calcd for $C_{24}H_{29}O_3ClNa$ $(M+Na)^+$ 423.1703, found 423.1714. Carboxyxanthene **2**: R_f= 0.2 (30% EtOAc/hexanes); IR (thin film) 3500-2500 (br), 1695, 1610, 1500, 1460, 1405, 1365 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 10.62 (br, 1H), 8.10 (d, *J* = 2.2 Hz, 1H), 7.68 (d, *J* = 2.2 Hz, 1H), 7.46 (d, *J* = 2.1 Hz, 1H), 7.29 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H), 1.68 (s, 6H), 1.36 (s, 9H), 1.35 (s, 9H); HRMS (FAB) *m/e* calcd for $C_{24}H_{31}O_3$ $(M+H)^+$ 367.2272, found 367.2264.

[4] R_f = 0.6 (10% $MeOH/CH_2Cl_2$); IR (thin film) 3500–2500 (br), 3360, 1690, 1625, 1590, 1550, 1440 cm^{-1}; ^1H NMR (600 MHz, DMSO–d_6) δ 13.40 (br, 1H), 9.20 (br, 1H), 8.20 (d, *J* = 2.0 Hz, 1H), 7.80 (d, *J* = 2.2 Hz, 1H), 7.72 (d, *J* = 1.7 Hz, 1H), 7.19 (d, *J* = 1.9 Hz, 1H), 2.17 (s, 3H), 1.60 (s, 6H), 1.31 (s, 9H), 1.28 (s, 9H); ^1H NMR (600 MHz, $CDCl_3$) d 9.07 (br, 1H, NH); HRMS (FAB) *m/e* calcd for $C_{26}H_{33}NO_4Na$ $(M+Na)^+$ 446.2307, found 446.2291.

aromatic ring, may contribute to the decreased reactivity of acetate [12]. This explanation is consistent with the fact that larger rate enhancements (Table 1) were observed in aqueous methanol solution in the reactions between **2** and acetate than in pure methanol and ethanol, in accord with their expected solvating ability [10]. Furthermore, a decreased rate was observed in the presence of the acetamide group (**3**). The ^{1}H NMR spectrum of compound **3**[4] revealed a downfield shift of the amide proton at δ 9.07 [13], indicating that the hydrogen forms a bridge to the carboxy group in its neighborhood decreasing its availability for the base-promoted reaction.

Table 1. Rate constants for the conversion of 5-nitrobenzisoxazole to 2-cyano-5-nitrophenolate with different catalysts at 25 °C

	rel. $k_{app.}$ ($M^{-1}s^{-1}$)		
catalyst	CH_3OH/H_2O 1/1	CH_3OH	CH_3CH_2OH
AcO^-	0.017	0.46	0.77
2	1	9.9	14
3	-	-	9.2
4	-	-	24
1	2.6	17	38

The carboxynaphthylimidoxanthene receptor **4**, which was prepared from the xanthene aminoacid with naphthalic anhydride and zinc acetate (220 °C, 5h) in 76% yield[5] juxtaposes the catalytic base with a hydrophobic floor. The introduction of the naphthalene ring caused a nearly 2-fold rate acceleration relative to the carboxyxanthenes **2** and **3** (Table 1). It is clear that π-stacking interactions of the guest (benzisoxazole) with the naphthalene ring, which is nearly perpendicular to the xanthene plane because of restricted rotation about the C_{aryl}-N_{imide} bonds [1], influences the catalytic behavior of the receptor significantly even in competititve H-bonding solvents.

The final receptor **1**[6] contains a second xanthene ring providing an amide as an additional functional group. This system had a modest additional effect on the rate of the elimination reaction compared to compound **4** (Table 1). The amide group has been introduced as hydrogen-bond donor to stabilize the negative charge of the oxygen in position 1 of the transition state (Scheme 1) that has been predicted as additional source of catalysis by quantum mechanical calculations [6]. Such acid-base catalysis is known to contribute to the efficiency of many enzymes [14]. The observed rate difference of **4** to **1** suggests that the bifunctional (base-acid) catalysis is either not as important as predicted or its effect is counterbalanced by rate decreasing factors. For instance, the two functional groups in the cleft of the host **1** may be not optimally positioned towards the substrate. Alternatively,

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[5] R_f = 0.3 (2% MeOH/CH_2Cl_2); IR (thin film) 3500–2500 (br), 1715, 1675, 1585, 1455, 1355, 1275, 1240 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61 (dd, *J* = 7.0, 1.3 Hz, 2H), 7.75–7.64 (m, 5H), 7.60 (d, *J* = 2.2 Hz, 1H), 7.38 (d, *J* = 2.4 Hz, 1H), 7.27 (d, *J* = 2.3 Hz, 1H), 1.82 (s, 6H), 1.41 (s, 9H), 1.40 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.09, 164.17, 147.88, 146.41, 144.97, 143.61, 133.90, 131.68, 131.39, 131.07, 129.97, 128.36, 127.72, 126.94, 126.74, 125.01, 122.98, 122.86, 122.46, 117.18, 34.98, 34.69, 34.50, 32.08, 31.48, 31.42; HRMS (FAB) *m/e* calcd for $C_{36}H_{35}NO_5Cs$ $(M+Cs)^+$ 694.1570, found 694.1590.

[6] The receptor **1** was prepared by condensation of ammonia with the corresponding previously reported mono-mixed anhydride (NH_3, CH_2Cl_2, 30%). R_f= 0.5 (3% MeOH/CH_2Cl_2); IR (thin film) 3600-2900 (br), 1705, 1670, 1595, 1450, 1360, 1260 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.70 (apparent AB, J_{AB} = 7.8 Hz, $\Delta\nu_{AB}$ = 41.2 Hz, 8H), 7.84 (d, *J* = 2.3 Hz, 1H), 7.83 (d, *J* = 2.4 Hz, 1H), 7.65 (d, *J* = 2.4 Hz, 1H), 7.58 (m, 3H), 7.33 (d, *J* = 2.0 Hz, 1H), 7.30 (d, *J* = 2.1 Hz, 1H), 6.77 (br s, 1H), 5.09 (br s, 1H), 1.75 (s, 12H), 1.37 (s, 18H), 1.33 (s, 9H), 1.32 (s, 9H); HRMS (FAB) *m/e* calcd for $C_{72}H_{67}N_3O_9Cs$ $(M+Cs)^+$ 1250.3932, found 1250.3987; UV/VIS (1.28 mM in 20% CH_2Cl_2/CH_3OH; nm [log ε]) 526 (2.6), 491 (2.5), 461 (2.1), 258 (sh, 2.5).

binding of the benzisoxazole in the cleft of **1** may be energetically unfavorable, resulting in the low affinity for the substrate.

Further modifications of the receptor will be necessary to optimize the host system. Comparison with more efficient catalysis by another synthetic host molecule [5] and by proteins such as BSA [4] and catalytic antibodies [3] indicates that a better defined binding site presenting the required functional groups will be needed for continued systematic evaluation of bifunctional catalysis in the benzisoxazole elimination reaction.

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