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# An allosteric crown ether-induced activity control for the cleavage of a phosphodiester bond

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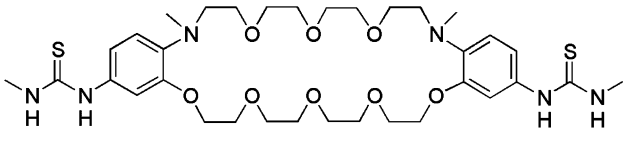
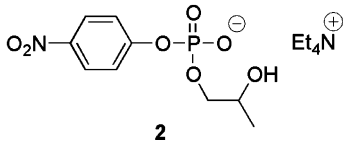
**Abstract**—Bis (thiourea) receptor **1** based on a highly flexible dibenzo-diaza-30-crown-10 scaffold represented a $K^+$-organized microenvironment to markedly accelerate the cleavage of the phoshodiester linkage of the RNA model substrate (2-hydroxypropyl-*p*-nitrophenyl phosphate). 

In current supramolecular chemistry, much attention has focused on the development of molecular systems possessing 'dynamic' function of molecular recognition. Indeed switched functionalized systems[1] and artificial allosteric molecules[2] would be applicable for the basic design of molecular machines.[3] Such regulatory systems are also essential in biological process,[4] where an intriguing fact is that the group I monovalent cations (e.g. $Na^+$, $K^+$) and $NH_4^+$ are important effectors of catalytic activity for a variety of enzymes.[5] The phenomena have prompted us to adroitly combine metal-induced allostery and enzyme functions, the approach of which would open the way to develop a new type of 'supramolecular catalysts.'[6] In this context, a rational design of catalysis-tunable model that can cleavage phosphodiester bond is one of fundamental challenges.[7] Surprisingly, the development of the relevant systems, however, is quite limited, where a trinuclear metal complex skeleton has been employed.[8] Our motivation regarding this subject is to explore an alternative approach based on crown ether, the numerous insights of which[9] would be most welcome to the design of a variety of activity-controllable supramolecular catalysts.

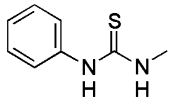
As a part of our ongoing program to function-tunable molecular systems,[10] a dynamic topological change of large-size crown ether has allowed us to design a new type of allosteric systems. Indeed, a highly flexible regioselectively bis(thioura) substituted dibenzo-diaza-30-crown-10 **1** displayed $K^+$-assisted diphenylphosphate binding coupled with a dynamically conformational change,[10b] where a cleft-type geometry is organized to create a bis(thiourea)-based microenvironment. The intrigued phenomena might be applicable for an activity control for phosphodiester bond cleavage because $K^+$ could tune the active site. As detailed below, system **1**, to the best of our knowledge, is first rationalized crown ether-derived system for such a desired property.



**1**



**2**



**3**

We employed 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNP) as a tetraethylammonium salt, **2**, toward this end. The phosphodiester cleavage of **2** (0.85 mM) was carried out in MeCN under a basic condition by adding excess amount of $NEt_3$ (0.1 M)[11] at 25°C, being monitored by increasing the absorption intensity at 400 nm caused by release of the ionized *p*-nitrophenol via a transesterification mechanism, and then followed pseudo-first-order kinetics. In this way, the kinetic data[12] were collected under several conditions.[13] In contrast with a much lower cleavage reactivity of **2** under an uncatalytic condition,[14] adding **1** (4.5 mM) and 1 equiv. of potassium tetrakis (*p*-chlorophenyl)

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borate (K-TCPB)[15] to the solution allowed the cleavage reaction to proceed significantly,[16] the pseudo-first order reaction rate constant ($k_{obs}$) being 8.0 (±0.7)×$10^{-5}$ $s^{-1}$. Interestingly, under identical condition using $K^+$ free-**1**, the cleavage rate resulted in quite decrease [$k_{obs}$ = 2.0 (±0.1)×$10^{-7}$ $s^{-1}$], the value being 400-fold lower than that of the case of the 1:1 mixture of $K^+$ and **1**. On the other hand, use of other monovalent metal ions [$Na^+$ and $Cs^+$ as tetraphenylborate salts] in a similar condition resulted in slower reaction rates [2.8 (±0.2)×$10^{-5}$ $s^{-1}$ for $Na^+$ and 1.9 (±0.1)×$10^{-5}$ $s^{-1}$ for $Cs^+$, respectively] in comparison with the case in the presence of $K^+$. These insights are indicative of the role of $K^+$ as an efficient allosteric effector toward the catalytic reaction. However, the acceleration was not obtained in the cases of *N*-phenyl-*N*′-methyl thiourea **3** (9.0 mM) in the presence of $K^+$ (4.5 mM) [$k_{obs}$ = 1.3 (±0.1)×$10^{-6}$ $s^{-1}$] as well as dibenzo-30-crown-10 (4.5 mM) as a macrocyclic control compound in the presence of $K^+$ (4.5 mM) [$k_{obs}$ = 2.9 (±0.7)×$10^{-7}$ $s^{-1}$].[17] It means that the cooperative association of $K^+$ with **1** could lead a suitable microenvironment for the cleavage of phosphodiester bond. Fig. 1 shows the results of rate acceleration compared to **1** which possess with a highly flexible structure as shown Fig. 2a.[18] From these results, the quite enhanced acceleration effect in the case of (c) in Fig. 1 indicates that $K^+$-assisted organization of **1** could be well-suited to position the two thiourea unit, in the manner depicted in Fig. 2b,[18] to activate the substrate, where the phosphorus atom is allowed to be more electrophilic.[19] Subsequently, the intramolecular nucleophilic attack based on deprotonated OH group of HPNP would be promoted.

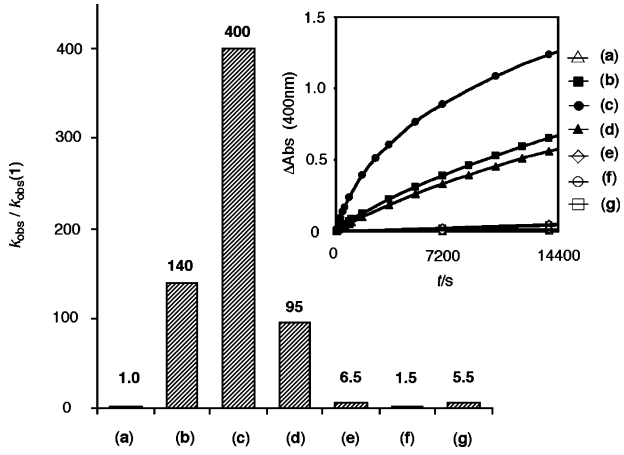


**Figure 1.** Acceleration effects $k_{obs}/k_{obs}$ (**1**) on the cleavage of **2** (0.85 mM) in a MeCN solution involving 0.1 M $NEt_3$ at 25°C. (a) **1** (4.5 mM); (b) **1** (4.5 mM)+$Na^+$ (4.5 mM); (c) **1** (4.5 mM)+$K^+$ (4.5 mM); (d) **1** (4.5 mM)+$Cs^+$ (4.5 mM); (e) **3** (9.0 mM)+$K^+$ (4.5 mM); (f) dibenzo-30-crown-10 (4.5 mM)+$K^+$ (4.5 mM); (g) $K^+$ (4.5 mM). Insertion data: time course of the reaction in above conditions, (a) (△); (b) (■); (c) (●); (d) (▲); (e) (◇); (f) (○); (g) (□). The kinetic data have been averaged over at least individual three runs.

As the next stage, remarkable rate enhancement using the $K^+$·**1** complex-contained system for the HPNP-cleavage led us to determine Michaels–Menten kinetics parameters. Upon increasing both concentrations of **1** and $K^+$ ([**1**]=[$K^+$]), the reaction rate showed a saturation behavior (Fig. 3). We thus decided to employ an Eadie–Hofstee plot to calculate Michaels–Menten constant ($K_m$) and the catalytic constant ($k_{cat}$) as a catalytic system in the presence of 1 equiv. of $K^+$, the values being 7.1×$10^{-3}$ M and 2.1×$10^{-4}$ $s^{-1}$, respectively.[20] These

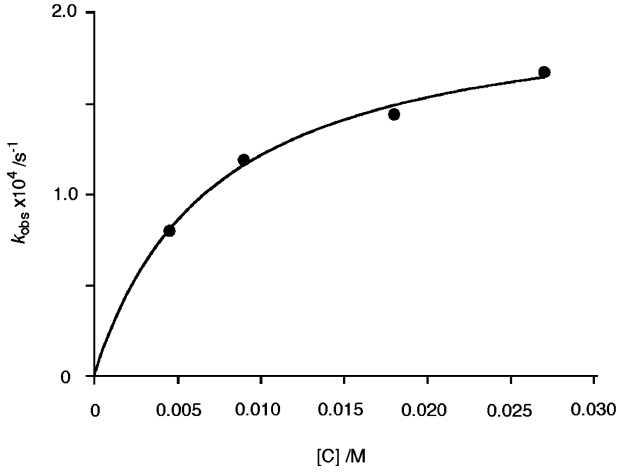


**Figure 3.** Saturation kinetics curve for the cleavage reaction of **2** (0.85 mM) with several concentrations **1** and $K^+$ in the presence of $NEt_3$ (0.1 M) in MeCN at 25°C. [C] means the concentration of a 1:1 mixture of **1** and $K^+$. The kinetics data are averaged over at least three runs.

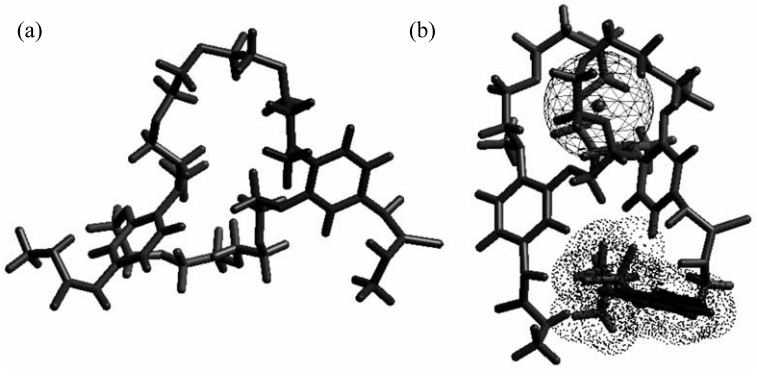


**Figure 2.** An energy-minimized structure of **1** (a) and complex structure [**1**-$K^+$-HPNP] (b).

correspond to the $k_{cat}/K_m$ value of $3.0\times10^{-2}$ $M^{-1}$ $s^{-1}$. It is noteworthy that the catalytic activity might be almost similar to that of a cleft-type bis (acylguanidinium) receptor which have been reported by Hamilton et al.[21] Taken together, an efficient off–on switching for the activity control for the cleavage of **2** could be achieved using allosteric receptor **1** coupled with $K^+$ as a metal cofactor.

We conclude that use of a highly flexible crown ether scaffold allowed us to represent a simplified activity-controllable catalytic system for phosphodiester bond cleavage. The approach would be feasible for strategy to develop tunable microenvironment at the nano-scale level. We feel that such possibilities warrant future exploration.

## Acknowledgements

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11. No deprotonation of the thiourea units was occurred even in an excess amounts of $NEt_3$ (>100 equiv.), being checked by $^1H$ NMR experiment.
12. The $k_{obs}$ ($s^{-1}$) values were estimated from initial rates (<ca. 15% conversion).
13. In order to assess the amount of *p*-nitrophenol conveniently, we conducted a calibration between [absorbance] at 400 nm versus [*p*-nitrophenol] in the presence of 0.1 M of $NEt_3$ so that the solution system was provided by the observation that ca. 54% of *p*-nitrophenol was ionized to be *p*-nitrophenolate in this medium. Alternatively, under the conditions where $K^+$-**1** complex was present in excess relative to **2** we should consider an interaction the ionized *p*-nitrophenol and $K^+$-**1**; the process may be expressed by *p*-nitrophenol+$K^+$-**1**+$NEt_3$⇄*p*-nitrophenolate+$K^+$-**1**+$HN^+Et_3$. The distribution of [*p*-nitrophenolate-$K^+$-**1**] in mainly due to the concentration of $K^+$-**1** complex. Subsequently, aforementioned calibrations were carried out for each condition: [**1**]=[$K^+$]=4.5, 9.0, 18, 27 mM, respectively. Thus, the [slope] values were obtained from the correlation between absorbance at 400 nm and [*p*-nitrophenol]. In this way, [*p*-nitrophenol] could be estimated based on these calibrations. The calibrations in both cases of [**1**]=[$Na^+$]=4.5 mM and [**1**]=[$Cs^+$]=4.5 mM were also conducted, respectively.
14. The cleavage reaction was too slow to estimate the pseudo-first order rate constants accurately.
15. A stoichiometric 1:1 complex formation of **1** with $K^+$ has been determined in this medium.[10b]
16. The cleavage reaction could be also monitored in terms of $^1H$ NMR measurements. The hydroxyl group of HPNP played a significantly role for the cleavage reaction: indeed, bis(*p*-nitrophenyl) phosphate tetraethylammonium salt with no hydroxyl group was inactive in the presence of 1:1 mixture of $K^+$ (4.5 mM) and **1** (4.5 mM) in MeCN at 25°C.
17. The $k_{obs}$ value in the only presence of $K^+$ (4.5 mM) is 1.1 $(\pm0.1)\times10^{-6}$ $s^{-1}$.
18. The energy minimization (ESFF force field) with the Quasi Newton–Rapton algorithm was performed using InsightII version 2000/Discover Release 3.0.0 version 98.0 (Accelrys) on a Silcon Graphics COMTEC 4D O2 workstation.
19. This inspection has been supported by the downfield shift of $^{31}P$ NMR signal of diphenylphosphate as a HPNP analogue upon addition of 1:1 mixture of $K^+$ and **1** in $CD_3CN$.
20. Obtained $K_m$ and $k_{cat}$ values are apparent; to address the catalytic activity of $K^+$-**1** complex the complexation ratio of each condition ([**1**]=[$K^+$]=4.5, 9.0, 18, 27 mM) should be calculated. Based on $^1H$ NMR titrations, the value in the case of [**1**]=[$K^+$]=4.5 mM was 78%. However, at higher concentrations ($\geq$9.0 mM), the estimations were failed because of the solubility of **1**.
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