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Improvement of chemical instability of prostacyclin in aqueous solution by complexation with methylated cyclodextrins

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Summary

Effects of various methylated cyclodextrins on the hydrolysis rate of prostacyclin (PGI_2) were investigated and compared with natural cyclodextrins (CyDs). All CyDs decelerated the hydrolysis of PGI_2. Among them, heptakis (2,3-di-O-methyl)-β-cyclodextrin (DM-β-CyD) had a marked stabilization effect, by factors of about 30 and 10 at pHs 4.0 and 7.0, respectively. Analysis of the rate–pH profiles indicated that the ionization of the terminal carboxylic acid moiety of PGI_2 is suppressed upon binding to CyDs. Thermodynamic activation parameters suggested that the deceleration effect of CyDs generally resulted from the increase in the activation enthalpy term rather than the decrease in entropy term.

Introduction

Prostacyclin (PGI_2) is of great interest as a therapeutic agent in the treatment of cardiovascular and thrombotic disorders because of its extremely potent vasodilatory and platelet anti-aggregatory activities (Moncada and Vane, 1980). However, one of the difficulties associated with development of pharmaceutical dosage forms comes from the hydrolytic lability (see Scheme 1); for example, the half-life of PGI_2 in aqueous solution at pH 7.0 and 25°C is about 2 min. Kurono et al. (1982) reported that the hydrolysis of PGI_2 was about 10 times slower in the presence of human serum albumin. Wynalda and Fitzpatrick (1980) found that various vertebrate albumins retard the hydrolysis by factors of 3–9. We have previously reported that the stability of PGI_2 and its methyl ester can be improved about 2–3 and 6–8 times, respectively, by complexation with natural cyclodextrins (CyDs) (Uekama et al., 1981). In this study, effects of methylated CyDs, particularly heptakis (2,6-di-O-methyl)-β-CyD (DM-β-CyD) and heptakis(2, 3, 6-tri-O-methyl)-β-CyD (TM-β-CyD), on the hydrolysis of PGI_2 were kinetically investigated in the hope of achieving greater hydrolytic stability than for those observed when complexed with natural CyDs and albumins.

Materials and Methods

Materials

PGI_2 sodium salt was a gift of Ono Pharma-

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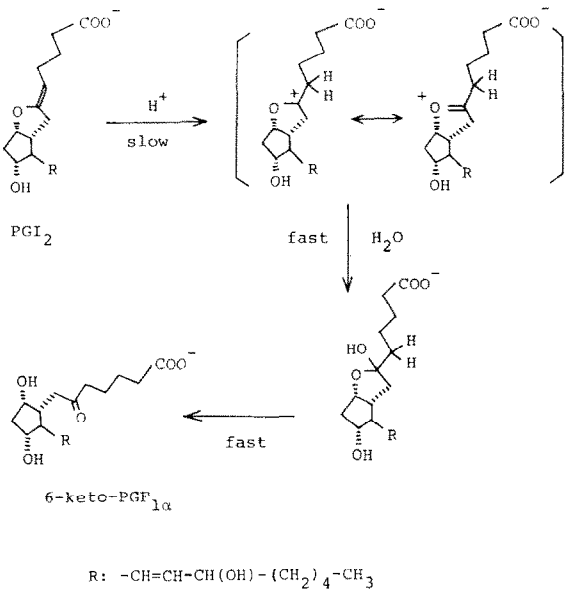


Scheme 1

ceutical Co., Japan. α-, β- and γ-CyDs were donated from Nihon Shokuhin Kako Co., Japan. DM-α- and DM-β-CyDs were supplied by Toshin Chemical Co., Japan and used after recrystallization from water. TM-α-, TM-β-, and TM-γ-CyDs were prepared according to the method reported (Uekama et al., 1985). DM-γ-CyD was prepared according to the method of Szejtli et al. (1980). All other materials and solvents were of analytical reagent grade and deionized double-distilled water was used throughout the study.

Kinetics

The hydrolysis rate of PGI_2 in the absence and presence of CyDs was spectrophotometrically monitored by measuring the decrease in absorbance at 230 nm (Uekama et al., 1981). The reaction was initiated by addition of stock solution of PGI_2 sodium salt in water into phosphate buffer at constant temperature. The final concentration of PGI_2 was adjusted to 3.0×10^{-4} M. pH of the sample solution was ascertained to be identical before and after the reaction. First-order plots for the hydrolysis of PGI_2 were linear for 3 or more half-lives, from which the hydrolysis rate constants were calculated.

Results and Discussion

Effects of various methylated CyDs on the hydrolysis rate of PGI_2

Table 1 summarizes the effects of various methylated CyDs on the hydrolysis rate of PGI_2. All CyDs studied retarded the hydrolysis rate. Among these CyDs, DM-β-CyD exhibited the largest stabilization effect. In a subsequent study, effects of methylated β-CyDs were investigated in detail in comparison with natural β-CyD, because the deceleration was generally greater in the β-CyD system than in the α- and γ-CyD systems.

Effects of CyDs and ethanol concentrations

pK_a of the terminal carboxylic acid of PGI_2 is known to be about 5.0 (Chiang et al., 1979), as is also described later. Fig. 1 shows the effects of concentration of three β-CyDs on the hydrolysis rate of PGI_2 at pHs 4.0 and 7.0, where the substrate exists predominantly, although not completely, in unionized and ionized forms, respectively. These pH conditions were chosen also for convenience of kinetic measurement due to the moderate reaction rate. In both pH regions, the hydrolysis rate was hyperbolically decreased with increasing CyD concentration. These concentration dependencies of the apparent rate constants (k_{obs}) were quantitatively treated by Eqn. 1 (Bender and Komiyama, 1978) to obtain the stability constants (K_{eq}) and rate constants (k_c) of

TABLE 1

Effects of various CyDs (5.0×10^{-3} M) on hydrolysis rate (k_{obs})[a] of PGI_2 in phosphate buffer (pH 7.0, $\mu = 0.2$) at 15°C

System	k_{obs} ($\times 10^3$ s^{-1})
Without CyDs	2.58
With α-CyD	1.54
With β-CyD	1.23
With γ-CyD	1.76
With DM-α-CyD	1.06
With DM-β-CyD	0.488
With DM-γ-CyD	2.04
With TM-α-CyD	1.16
With TM-β-CyD	2.08
With TM-γ-CyD	2.49

[a] Average of the values for duplicate measurements, which coincide with each other within ±1%.

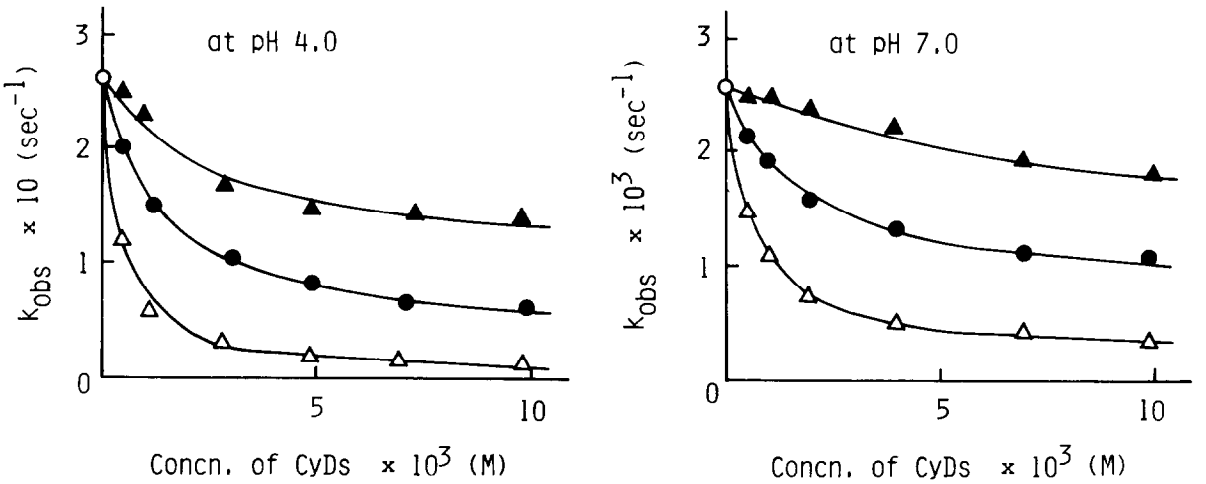


Fig. 1. Observed rate constants for hydrolysis of PGI_2 as a function of CyD concentration in phosphate buffer (pHs 4.0 and 7.0, $\mu = 0.2$) at 15°C. o, PGI_2 alone; ●, with β-CyD; Δ, with DM-β-CyD; ▲, with TM-β-CyD.

the complexes, assuming the 1 : 1 complexation scheme as reported previously (Uekama et al., 1981; Hirayama et al., 1984):

$$\frac{[CyD]_t}{k_0 - k_{obs}} = \frac{1}{k_0 - k_c}[CyD]_t + \frac{1}{K_{eq}(k_0 - k_c)} \quad (1)$$

where k_0 and $[CyD]_t$ are the rate constant in the absence of CyDs and the total concentration of CyDs, respectively. The plots of Eqn. 1 resulted in the straight line with a correlation coefficient (r) greater than 0.999. The results on k_0, k_0/k_c, and K_{eq} are summarized in Table 2. The K_{eq} values at pH 4.0 were larger than those at pH 7.0, supporting that unionized PGI_2 interacts more strongly with the CyD cavity than its ionized form, and in both pH regions PGI_2 molecule had a higher affinity to DM-β-CyD than to the other CyDs. Fig. 2 shows the relationship between the deceleration ratio (k_0/k_c) and K_{eq} value. The $\log(k_0/k_c)$ value increased linearly with increasing the $\log(K_{eq})$ value, although the points for the TM-β-CyD complexes were somewhat deviated from the straight line. The linear regression equation for the β- and DM-β-CyD systems was given by Eqn. 2 or by the linear free energy relationship of Eqn. 3;

$$\log(k_0/k_c) = 1.16 \log(K_{eq}) - 2.76 \quad (r = 0.991) \quad (2)$$

$$\Delta G_0^{\ddagger} - \Delta G_c^{\ddagger} = 1.17 \Delta G + 3.67 \quad (r = 0.992) \quad (3)$$

where ΔG is change in free energy of the complexation and $\Delta G_0^{\ddagger}$ and $\Delta G_c^{\ddagger}$ are changes in free energy of activation for the hydrolysis of free and complexed PGI_2 molecules, respectively. This result indicates that a tighter binding leads to a

TABLE 2

Rate constants (k_c) and stability constants (K_{eq})[a] of PGI_2–CyD complexes

System	k_0 ($\times 10^3$ s^{-1})	k_c ($\times 10^3$ s^{-1})	k_0/k_c	K_{eq} (M^{-1})
Hydrolysis at pH 4.0				
PGI_2	258.3	–	–	–
PGI_2-β-CyD	–	45.1	5.72	940
PGI_2-DM-β-CyD	–	8.91	29.0	4500
PGI_2-TM-β-CyD	–	131.0	1.97	980
Hydrolysis at pH 7.0				
PGI_2	2.58	–	–	–
PGI_2-β-CyD	–	0.868	2.97	690
PGI_2-DM-β-CyD	–	0.227	11.4	1900
PGI_2-TM-β-CyD	–	1.09	2.37	100

[a] Accuracy of ±3%. Kinetic conditions were the same as in Fig. 1.

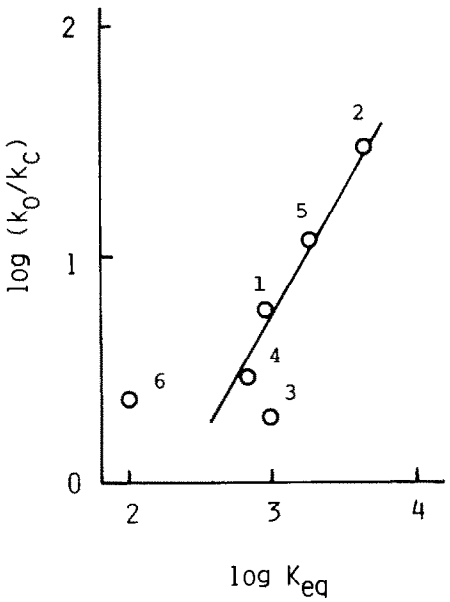


Fig. 2. Relationship between $\log(k_0/k_c)$ and $\log K_{eq}$. Numbers 1, 2 and 3 refer to the β-CyD, DM-β-CyD and TM-β-CyD complexes at pH 4.0, and numbers 4, 5 and 6 to those at pH 7.0, respectively.

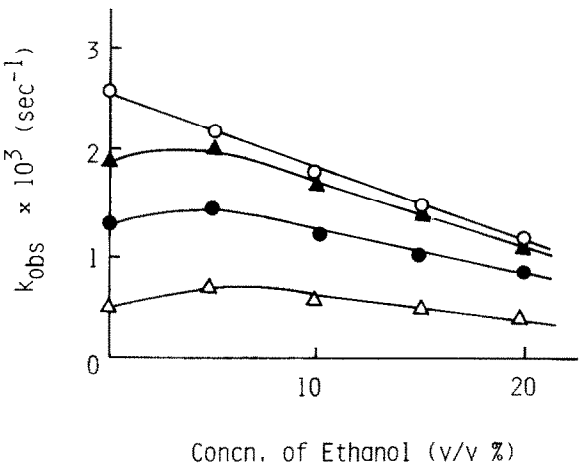


Fig. 3. Effects of ethanol concentration on hydrolysis rate of PGI_2 in the absence and presence of CyDs (5.0×10^{-3} M) in phosphate buffer (pH 7.0, $\mu = 0.2$) at 15°C. ○, PGI_2 alone; ●, with β-CyD; △, with DM-β-CyD; ▲, with TM-β-CyD.

larger stabilization. Indeed, the marked stabilization by a factor of about 30 was observed in the DM-β-CyD complex having the large K_{eq} value. The deviation of the TM-β-CyD system from the straight line might be due to little penetration of the bulky guest molecule and/or different inclusion mode, because the macrocyclic conformation of TM-β-CyD is significantly distorted from the regular heptagonal symmetry of β-CyD and DM-β-CyD (Harata et al., 1984).

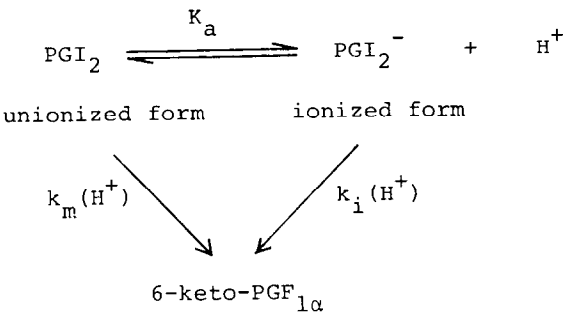
Fig. 3 shows the effects of ethanol concentration on the hydrolysis rates of PGI_2 in the absence and presence of CyDs. In the absence of CyDs, the hydrolysis rate decreased with increasing ethanol concentration, i.e., with decreasing the polarity of the solution. In the presence of CyDs, however, the hydrolysis rate increased initially, and then approached to the rate of the free guest molecule on further addition of ethanol. These kinetic behaviors, particularly in the low ethanol concentration range, may be ascribed to competitive inclusion of the guest and solvent molecules toward the host cavity, since ethanol molecule is known to form inclusion complexes with CyDs (Matsui and Mochida, 1979; Tokuoka et al., 1980; Buvári et al., 1983). Of course, other factors such as the medium effect should be considered to interpret these kinetic features, since the presence of as much as 20% ethanol can significantly change the inclusion equilibrium.

Effects of pH

Fig. 4 shows the log k_{obs}–pH profiles for the hydrolysis of PGI_2 in the absence and presence of CyDs. These profiles were biphasic with a slope of −1 below and above pHs about 2.0 and 5.5, respectively. The hydrolysis rates at low acidities (pH 4–7) were significantly faster than those (the dashed line in Fig. 4, as an example) extrapolated from the rates at high acidities. This extra lability is known to come from the ionization of the terminal carboxylic acid moiety of PGI_2 (Chiang et al., 1979). Therefore, these pH profiles were analyzed in terms of Eqn. 4 on the basis of Scheme 2, in order to obtain the apparent dissociation constants (K_a) of the terminal carboxylic acid in the presence of CyDs.

$$k_{obs} = \frac{k_m \cdot [H^+]^2 + k_i \cdot K_a \cdot [H^+]}{[H^+] + K_a} \qquad (4)$$

In Eqn. 4, k_m and k_i are the hydrolytic rate



Scheme 2

constants for unionized and ionized PGI_2, respectively, and $[H^+]$ is hydrogen ion concentration. The apparent pK_a values refined by a non-linear least squares method are listed in Table 3. Values in parentheses in Table 3 are the intrinsic pK_as bound to CyDs, which were calculated by the equation of $K_a^c = K_a^f \cdot K_{eq}^i / K_{eq}^m$ (Uekama and Hirayama, 1978). In this equation, K_a^c is the intrinsic acid dissociation constant of PGI_2 in the complexed form, K_a^f is that in the absence of CyDs, and K_{eq}^m and K_{eq}^i are the stability constants of the complex with unionized and ionized PGI_2, respectively. The K_{eq}^m and K_{eq}^i values were approximated by those obtained at pHs 4.0 and 7.0, respectively. The apparent and intrinsic pK_as in the β-CyD and DM-β-CyD systems coincided each other adequately within experimental error, since the guest molecule is predominantly in the complexed form due to the large K_{eq} values. As is apparent from Table 3, there is a trend of the protolytic dissociation of PGI_2 being suppressed upon binding to CyDs. This suppressing effect was particularly large in the TM-β-CyD complex in spite of its small deceleration effect, suggesting that the interaction mode is somewhat different between the TM-β-CyD complex and β- or DM-β-CyD complexes, as was described before.

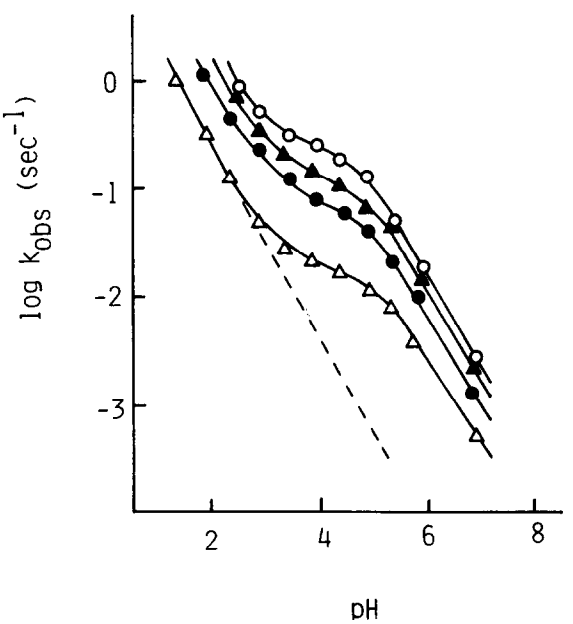


Fig. 4. pH profiles for hydrolysis rate of PGI_2 in the absence and presence of CyDs (5.0×10^{-3} M) at 15°C. ○, PGI_2 alone; ●, with β-CyD; △, with DM-β-CyD; ▲, with TM-β-CyD.

TABLE 3

Apparent catalytic rate constants (k_m and k_i) and protolytic dissociation constants (pK_a)[a] of PGI_2 in the absence and presence of CyDs (5.0×10^{-3} M) at 15°C

System	k_m ($\times 10^{-2}$ $s^{-1}M^{-1}$)	k_i ($\times 10^{-4}$ $s^{-1}M^{-1}$)	pK_a
PGI_2	1.45	2.51	4.99
PGI_2-β-CyD	1.38	1.19	5.21 (5.12) [b]
PGI_2-DM-β-CyD	0.356	0.521	5.50 (5.36) [b]
PGI_2-TM-β-CyD	1.40	2.05	5.24 (5.98) [b]

[a] Accuracy: $pK_a \pm 0.08$.

[b] The values in parenthesis represent the intrinsic pK_as bound to CyDs (see text).

Effects of temperature

Fig. 5 shows Arrhenius plots for the hydrolysis of PGI_2 in the absence and presence of CyDs at pHs 4.0 and 7.0. The thermodynamic activation parameters calculated from these linear plots are listed in Table 4. It is apparent that the CyD-induced deceleration generally resulted from the increase in activation enthalpy term ($\Delta H^{\ddagger}$). This appears to be particularly true for the case of unionized PGI_2 molecule. At pH 4.0, for example, the favorable change in activation entrophy term ($\Delta S^{\ddagger}$) was cancelled out by the large unfavorable change in $\Delta H^{\ddagger}$. The rate-determining step of the hydrolysis of PGI_2 is known to be the proton-catalyzed formation of the charged alkoxycar-

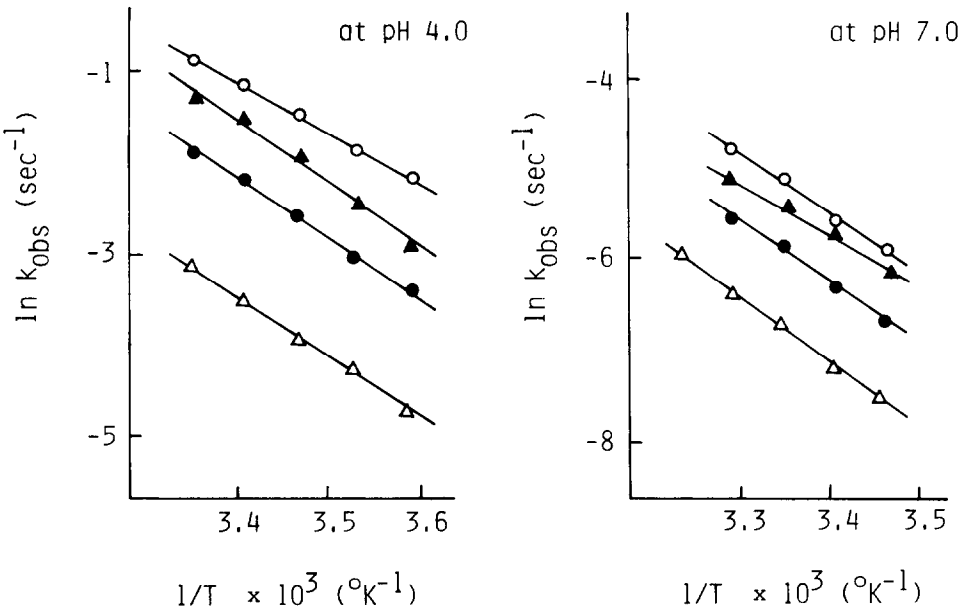


Fig. 5. Arrhenius plots for hydrolysis of PGI_2 in the absence and presence of CyDs (5.0×10^{-3} M) in phosphate buffer (pHs 4.0 and 7.0, $\mu = 0.2$). ○, PGI_2 alone; ●, with β-CyD; △, with DM-β-CyD; ▲, with TM-β-CyD.

bonium ion intermediate, as shown in Scheme 1 (Cho and Allen, 1978; Chiang et al., 1979). Therefore, these results suggest that more energy is required to develop the charged intermediate in the hydrophobic CyD cavity, leading to the increase in $\Delta H^{\ddagger}$, although further study should be done to interpret thoroughly the activation parameters.

In conclusion, the extremely high hydrolytic lability of PGI_2 was significantly improved by β-CyD complexation, where the deceleration ratio was well correlated with the magnitude of the stability constant of the complexes. Among the three β-CyDs, DM-β-CyD provided the largest stabilization (about 30 times), which is greater than those observed when complexed with albumins. This kind of knowledge will provide both a rational basis for formulation design and a means for stabilizing PGI_2 in pharmaceutical dosage forms.

TABLE 4

Thermodynamic activation parameters [a] *for hydrolysis of* PGI_2 *in the absence and presence of CyDs*

System	$\Delta G^{\ddagger}_{288}$ (kcal/mol)	$\Delta H^{\ddagger}_{288}$ (kcal/mol)	$\Delta S^{\ddagger}_{288}$ (e.u.)
Hydrolysis at pH 4.0			
PGI_2	17.6	11.2	−22.2
PGI_2-β-CyD	18.3	12.9	−18.5
PGI_2-DM-β-CyD	19.1	13.2	−20.6
PGI_2-TM-β-CyD	17.9	12.7	−18.1
Hydrolysis at pH 7.0			
PGI_2	20.2	13.3	−24.1
PGI_2-β-CyD	20.6	13.5	−24.8
PGI_2-DM-β-CyD	21.1	14.0	−24.8
PGI_2-TM-β-CyD	20.4	12.2	−28.5

[a] Accuracy: $\Delta H^{\ddagger}$, ±0.2 kcal/mol; $\Delta S^{\ddagger}$, ±0.7 e.u. Kinetic conditions were the same as in Fig. 5.

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