Lipase-Catalyzed Hydrolysis of Crowned Ester Substrates: Metal Cation-Enhanced Reactivity and Enantioselectivity of 12-Crown-4 Ester

Hiroshi Tsukube,*,† Akio Betchaku,‡ Yuji Hiyama,‡ and Toshiyuki Itoh*,‡

Department of Chemistry, College of Liberal Arts & Science, Okayama University, Okayama 700, Japan, and Department of Chemistry, Faculty of Education, Okayama University, Okayama 700, Japan

Received June 27, 1994®

Lipase from *Pseudomonas cepacia* was first applied in hydrolysis of ester substrates which had crown ether, azacrown, and acyclic polyether moieties as cation binding sites. The reaction behaviors of these crowned ester substrates were changed by addition of alkali metal salts. In particular, 12-crown-4 ester offered enhanced reactivity and enantioselectivity in the presence of sodium salt. FAB MS, ^{13}C NMR, and computational studies revealed that the ester formed a diastereomeric complex in which Na^+ cation was sandwiched between two chiral crowned substrates. The lipase reaction was effectively controlled by "host–guest chemistry" of the crowned ester substrate.

Introduction

Lipases have recently been recognized as effective catalysts in synthetic organic reactions as well as in biological reactions[1] and provide an interesting alternative method for resolution of enantiomers. They catalyze hydrolysis of various ester substrates having fluorinated, organometallic, and other exotic functional groups. Since their reaction rates and stereoselectivities are often modest, several approaches have been used to improve their reaction performances.[2] Optimization of reaction conditions, modification of substrates, use of nonaqueous media, and enantioselective inhibition have been investigated extensively.

We now report that lipase from *Pseudomonas cepacia* is an effective catalyst for a new series of ester substrates having cation binding sites such as crown ether and related polyether moieties.[3] We examined the effect of complexation between these crowned substrates and alkali metal cation on the lipase-catalyzed hydrolysis. Since reactivity and enantioselectivity of some 12-crown-4 derivative were specifically enhanced via crown–metal complexation, lipase reaction was controlled by "host–guest chemistry" of crowned ester substrate. Crown ethers have been reported to be complexing and solubilizing agents for several proteins[4] and to accelerate rates of some enzymatic reactions.[5] However, this is the first example of crowned ester derivative exhibiting enhanced reactivity and enantioselectivity in lipase-catalyzed hydrolysis. The present study, therefore, indicates that a combination of biological enzyme and synthetic crown ether offers new and promising possibilities in the optical resolution of racemic crown ethers and in the improvement of lipase catalysts.

Results and Discussion

Crowned Ester Substrates. We prepared three kinds of crowned ester substrates: racemic 12-crown-4 **1**, azacrown ethers **2**–**4**, and acyclic polyether **5** (Scheme 1). 12-Crown-4 derivative **1** and acyclic polyether **5** were obtained by acetylation of corresponding alcohols, while azacrown ethers **2**–**4** were prepared by reaction of unsubstituted azacrown ethers and 2-bromoethyl acetate.

Cation-binding properties of these crowned esters **1**–**5** were characterized using FAB MS competitive technique.[6] Table 1 summarizes relative peak intensities of [ester + metal]$^+$ ions which reflect the relative metal cation-binding abilities. Their complexation selectivities were clearly dependent on their polyether structures. For example, azacrown ethers **2**–**4** exhibited cation selectivities which were apparently controlled by size of the crown ring. 12-Membered crown ring is formally size-fitted to the Li^+ cation, and the aza-12-crown-4 **2** bound it more strongly than Na^+ and K^+ cations. On the other hand, larger 15- and 18-membered crowns **3** and **4** favored larger Na^+ and K^+ cations, respectively. Such a "cavity size-selectivity" has been frequently reported in the literature.[7] Crowned ester **1** showed somewhat different cation selectivity from the corresponding azacrown **2**. This has a 12-crown-4 ring but bound Na^+ cation more strongly than Li^+ cation. Since the signal due to the **1**–Na^+–**1** complex was weakly but definitely observed as was the signal of the **1**–Na^+ complex in the FAB MS spectrum, the crowned ester **1** was suggested to form a sandwich type 2:1 complex with Na^+ cation as did some 12-crown-4 derivatives.[8] Acyclic polyether **5** was employed for comparison. This has six ether-oxygen atoms

† College of Liberal Arts & Science.
‡ Faculty of Education.
® Abstract published in *Advance ACS Abstracts,* October 15, 1994.

(1) (a) Whiteside, G. M.; Wong, C.-H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 617. (b) Jones, J. B. *Tetrahedron* **1986**, *42*, 3351. (c) Chen, C.-S.; Sih, C. J. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 695. (d) Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114. (e) Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A. *Chem. Rev.* **1992**, *92*, 1071.

(2) Guo, Z.-W.; Sih, C. J. *J. Am. Chem. Soc.* **1989**, *111*, 6836. (b) Itoh, T.; Takagi, Y.; Nishiyama, S. *J. Org. Chem.* **1991**, *56*, 1521. (c) Kamat, S. V.; Beckman, E. J.; Russell, A. J. *J. Am. Chem. Soc.* **1993**, *115*, 8845.

(3) Preliminary communication: Tsukube, H.; Betchaku, A.; Hiyama, Y.; Itoh, T. *J. Chem. Soc., Chem. Commun.* **1992**, 1751.

(4) (a) Odell, B.; Earlam, G. *J. Chem. Soc., Chem. Commun.* **1985**, 359. (b) Reinhoudt, D. N.; Eendebak, A. M.; Nijenhuis, W. F.; Verboom, W.; Kloosterman, M.; Schoemaker, H. E. *J. Chem. Soc., Chem. Commun.* **1989**, 399. (c) Nagasaki, T.; Kimura, O.; Ukon, M.; Arimori, S.; Hamachi, I.; Shinkai, S. *J. Chem. Soc., Perkin Trans.* **1 1994**, 75.

(5) Itoh, T.; Hiyama, Y.; Betchaku, A.; Tsukube, H. *Tetrahedron Lett.* **1993**, *34*, 2617.

(6) (a) Bonas, B.; Bosso, C.; Vignon, M. R. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 88. (b) Sawada, M.; Okumura, Y.; Shizuoka, M.; Takai, Y.; Hidaka, Y.; Yamada, H.; Tanaka, T.; Kaneda, T.; Hirose, K.; Misumi, S.; Takahashi, S. *J. Am. Chem. Soc.* **1993**, *115*, 7381. (c) Tsukube, H. *Comprehensive Supramolecular Chemistry*; Pergamon Press: New York, Vol. 8, in press.

(7) Tsukube, H. *Crown Ethers and Analogous Compounds*; Elsevier: Amsterdam, 1992; p 100.

0022-3263/94/1959-7014$04.50/0

Scheme 1. Lipase-Catalyzed Hydrolysis of Crowned Esters 1–5

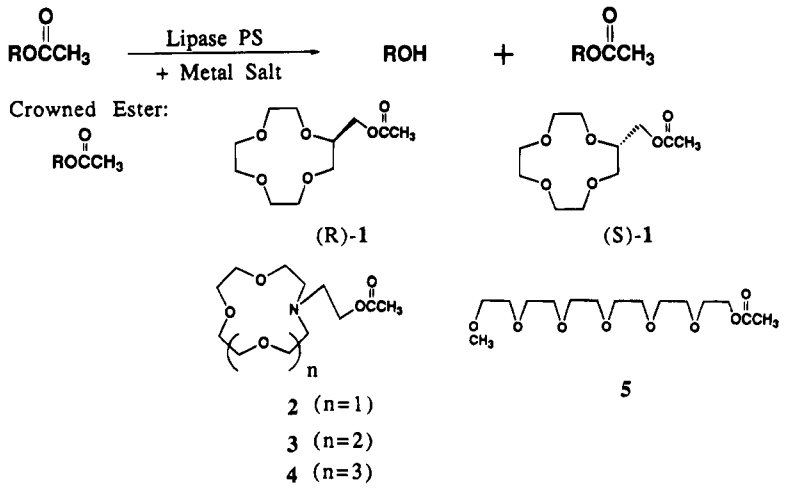


Table 1. Cation Binding Selectivities of Crowned Esters Assessed by FAB MS[a]

	relative peak intensity		
ester	ester + Li^+	ester + Na^+	ester + K^+
1	39	100	9
2	100	38	10
3	61	100	5
4	1	70	100
5	8	100	41

[a] Conditions: LiCl, 0.0083 mol/L; NaI, 0.0083 mol/L; KI, 0.0083 mol/L; crown, 0.0033 mol/L; in *m*-nitrobenzyl alcohol.

Table 2. Effect of Alkali Metal Cation on Hydrolysis Rates of Several Crowned Esters[a]

	relative rate[b]			
ester	none	+LiCl	+NaCl	+KCl
1[c]	2.9	1.7	5.8	4.3
2	5.3	5.5	5.9	5.1
3	3.2	2.1	3.1	2.5
4	3.6	3.9	2.8	1.6
5	2.3	2.1	1.8	1.4

[a] Hydrolysis was carried out in 0.1 mol/L of metal chloride. Details of reaction conditions are indicated in the Experimental Section. [b] Conversion (%)/reaction time (h). [c] Racemic substrate was examined.

and an ester terminal group and showed a Na^+ ion selectivity. We carried out lipase-catalyzed hydrolysis of these crowned ester substrates exhibiting characteristic cation binding properties and observed interesting effects of metal complexations on their reaction profiles.

Lipase-Catalyzed Hydrolysis of Crowned Ester Substrates. Lipase PS (Amano Pharmaceutical Co.) from *Pseudomonas cepacia* was chosen for this study, in which the enzyme showed a single band in an SDS disk gel electrophoresis experiment and had a molecular weight of 32 000. The enzyme content was estimated as less than 3.1×10^{-3} mmol/g, while the remainder was mostly amorphous Celite.[9] Esterase from porcine liver and lipase from *Aspergillus* were also examined. They slowly hydrolyzed crowned ester **1** and were not effective for chiral discrimination.

Table 2 summarizes hydrolysis profiles of crowned ester substrates **1**–**5** in the absence or presence of alkali metal salts. The conversion (%) of the crowned substrate/reaction time (h) was calculated as relative hydrolysis rate and used as a measure of reactivity. Lipase PS itself catalyzed hydrolysis of these nonbiological substrates, and their relative reaction rates depended on both crown structure of the substrate and nature of the metal salt additive. Among azacrown ethers, 12-membered ester **2** exhibited highest reaction rates under the various conditions. This may suggest that the 12-membered crown substrate easily draws toward the reaction center of the employed lipase. 15- and 18-membered esters **3** and **4** were slowly hydrolyzed, but their reactivities were interestingly changed by addition of alkali metal cations. LiCl delayed hydrolysis of aza-15-crown-5 derivative **3**, while KCl decreased reaction rate of aza-18-crown-6 **4**. Since the relative reaction rates of the aza-12-crown-4 **2** were rarely changed by addition of alkali metal salts, these salts were thought to have little influence on the activity of the lipase itself and to modify reactivity of the crowned substrate. 12-Crown-4 ester **1** exhibited characteristic cation dependent reaction behaviors. The relative reaction rate of this substrate was particularly increased by addition of NaCl and reached a comparable level to that of aza-12-crown-4 **2**. Acyclic polyether **5** was also examined. Its reaction was slow, and addition of metal salts lowered the rate further. Recently, KCl was reported to enhance catalytic activities of subtilisin and α-chymotrypsin in transesterification of Ac-Phe-OEt. In these cases, the salt was proposed to protect the enzyme by an organic solvent or to help in maintaining the enzyme's native structure.[10] In contrast, our observations suggest that NaCl enhanced reactivity of the crowned substrate **1** via crown–metal complexation.

Enantioselectivity of lipase-catalyzed hydrolysis of racemic ester **1** was, interestingly, influenced by metal cation complexation (Table 3). The *E* value indicated is called the "enantiomeric ratio" and calculated as shown in eq 1[11]

$$E = \ln[1 - c[1 + \mathrm{ee(P)}]]/\ln[1 - c[1 - \mathrm{ee(P)}]] \quad (1)$$

where *c* indicates conversion of the ester **1** and ee(P) means enantiomeric excess (%) of the product alcohol. The crowned ester **1** has a reactive ester separated by

(8) Izatt, R. M.; Pawlak, K.; Brashaw, J. S. *Chem. Rev.* **1991**, *91*, 1721.
(9) We thank Dr. Y. Hirose of Amano Pharmaceutical Co. for giving us this information.
(10) Khmelnitsky, Y. L.; Wilch, S. H.; Clark, D. S.; Dordick, J. S. *J. Am. Chem. Soc.* **1994**, *116*, 2647.
(11) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.

Table 3. Lipase-Catalyzed Hydrolysis of Racemic Crowned Ester 1

ester 1	additive (mol/L)	time (h)	conversion (%)	ee (%) of product[a]	E
racemic	none	7	20	44	3
	LiCl (0.1)	28	47	53	3
	LiCl (0.2)	10	44	50	4
	LiCl (0.3)	10	44	55	5
	NaCl (0.05)	7	54	41	4
	NaCl (0.1)	6	35	73	10
	NaCl (0.2)	9	32	54	4
	NaCl (0.3)	9	35	46	3
	KCl (0.1)	15	65	32	3
	Tris buffer (0.1, pH 7.2)	10	48	36	3
	Na^+ buffer (0.1, pH 7.2)[b]	8	46	42	4

[a] (S)-Alcohol was obtained as a main product. [b] 0.1 M NaH_2PO_4–0.1 M NaOH.

Table 4. Relative Reaction Rate of Crowned Ester 1

ester 1	additive (mol/L)	relative rate[a] (R)-1	relative rate[a] (S)-1
racemic	none	2.1	0.8
	LiCl (0.1)	1.3	0.4
	NaCl (0.05)	5.4	2.8
	NaCl (0.1)	5.0	0.8
	NaCl (0.2)	2.7	0.8
	NaCl (0.3)	2.8	1.1
	KCl (0.1)	2.9	1.5
(R)	none	2.2	
	NaCl (0.1)	8.7	
(S)	none		0.5
	NaCl (0.1)		3.1

[a] Conversion (%)/reaction time (h).

two atoms from asymmetric carbon, and high enantioselectivity was not expected in the hydrolysis of such a substrate. Indeed, the lipase catalyzed hydrolysis of the ester **1** to give (S)-alcohol with low enantioselectivity ($E = 3$). Addition of NaCl salt greatly increased the E value as well as reactivity. The highest E value observed was 10 when the hydrolysis was carried out in 0.1 mol/L NaCl solution. The lipase well recognized the chirality of the crowned ester **1** in the presence of NaCl and effectively promoted enantioselective hydrolysis. An enhanced E value was not observed in a Na^+-phosphate buffer solution, indicating that the counteranion is another important factor in determining enantioselectivity. As described above, KCl and LiCl influenced relative reaction rates but had no potential to enhance enantioselectivity. Therefore, crowned ester **1** was thought to form a ternary complex with Na^+ cation and Cl^- anion suitable for highly enantioselective recognition by the lipase.

Relative reaction rates of (R)-**1** and (S)-**1** were calculated from the data of Table 3 in which racemic substrate **1** was employed (see Table 4). Addition of NaCl salt significantly increased the reaction rate of (R)-**1**, while reactivity of (S)-**1** was slightly influenced in most cases. The greatest rate difference between (R)-**1** and (S)-**1** was observed in 0.1 M NaCl solution, which included 0.38 equiv of Na^+ cation to the ester **1**. Further addition of NaCl salt offered only slight enhancement for (R)-**1**, though adding a smaller amount of NaCl increased rates of both (R)-**1** and (S)-**1**. LiCl and KCl influenced reactivities of both (R)- and (S)-substrates to similar degrees, and rarely enhanced E value. Thus, Na^+ cation had a unique effect on lipase-catalyzed hydrolysis of the crowned ester **1**.

Chiral crowned esters (R)-**1** and (S)-**1** were separately subjected to lipase-catalyzed hydrolysis (see Table 4). The

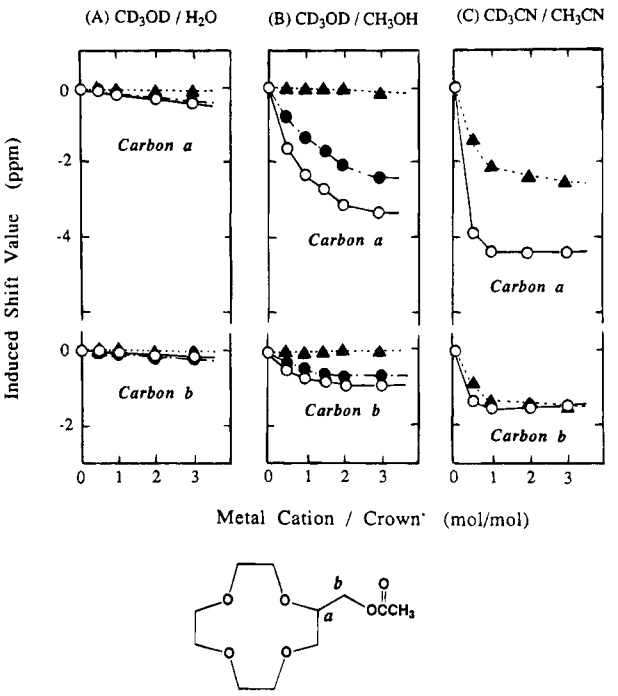
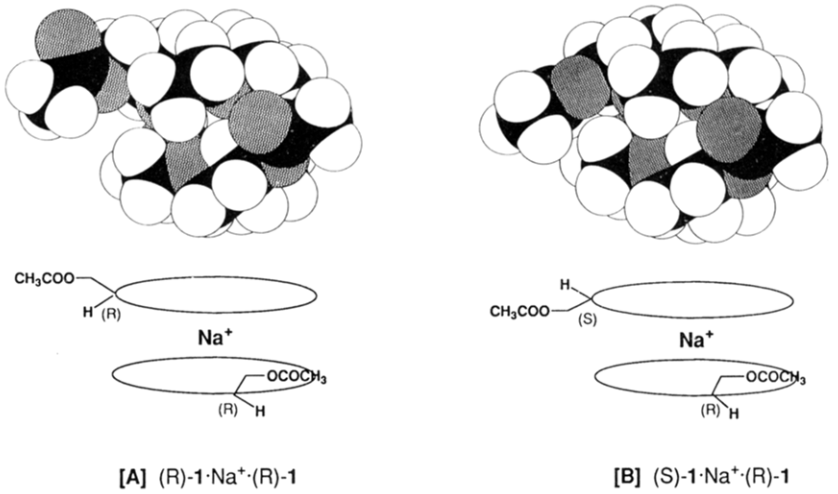


Figure 1. Metal cation-induced changes of ^{13}C NMR chemical shifts of crowned ester **1**. Reagents: ester **1**, 0.05 mmol; $LiClO_4$ (▲), $NaClO_4$ (○) or $KClO_4$ (●), 0–0.15 mmol.

lipase favored the (R)-**1** and gave the (S)-alcohol more efficiently than the (R)-alcohol. The hydrolysis of both (R)-**1** and (S)-**1** was notably promoted by NaCl, though greater rate enhancement (6.6 times) was obtained with (S)-**1** than that (3.9 times) with (R)-**1**. Such rate enhancements for chiral substrates were larger than expected from the results of racemic substrate. Although several possibilities should be considered, multicomponent complexation between ester **1** and Na^+ cation is probably involved in the enantioselective recognition of racemic substrate.

Cation-Binding Property of Crowned Ester 1. Cation binding of the crowned ester **1** was characterized in CD_3OD/H_2O, CD_3OD/CH_3OH, and CD_3CN/CH_3CN (2/3, v/v, each) by ^{13}C NMR spectroscopy. Figure 1 illustrates Li^+-, Na^+-, and K^+-induced changes in selected carbon signals, indicating that cation-binding characteristics of crowned ester **1** were largely dependent on the nature of the solvent. Indeed, addition of alkali metal salts offered significant shifts in CD_3OD/CH_3OH and CD_3CN/CH_3CN, while only slight spectral changes were recorded in CD_3OD/H_2O. The observed titration curves confirmed that the ester **1** preferred Na^+ cation to Li^+ and K^+ cations. Furthermore, this formed a 2:1 sandwich complex with Na^+ ion in less polar media, while 1:1 complexation occurred with Li^+ and K^+ ions. ^{23}Na-NMR spectra gave structural information on the sandwich complex. The chemical shifts of Na^+ cation complexed with ester **1** were almost the same as those complexed with unsubstituted 12-crown-4. The FT IR spectrum of CD_3CN/CH_3CN solution containing ester **1** and Na^+ salt was recorded.[12] Since the cation-induced shift in $\nu(C{=}O)$ was negligible (<3 cm^{-1}), the ester group of the substrate **1** did not offer effective coordination with the Na^+ cation. Cation-binding properties of the aza-

(12) We thank Professor T. Higashiyama of Okayama University for measurement of FT IR spectra.



[A] (R)-**1**·Na^+·(R)-**1** **[B]** (S)-**1**·Na^+·(R)-**1**

Figure 2. Possible structures of sandwich complexes with crowned ester **1** and Na^+ cation [A] (*R*)-**1**·Na^+·(*R*)-**1** and [B] (*S*)-**1**·Na^+·(*R*)-**1**. Sodium cation was omitted for simplified illustration, though the calculations were done for sodium complexes.

crown ethers **2**−**4** were similarly investigated by ^{13}C NMR titration experiments. They exhibited cation-induced spectral changes which were markedly different from those with the ester **1** and indicated 1:1 complexation even with Na^+ cation. Several lipases have recently been characterized by X-ray crystallographic analysis.[13] They have hydrophobic domains around the reaction sites and accommodate hydrophobic substrates. Although the detailed structure of the lipase PS employed here is not clear, this catalyzed enantioselective hydrolysis of hydrophobic substrates.[14] Thus, the crown ester **1** was suggested to specifically form a 2:1 sandwich complex with Na^+ cation and be hydrolyzed stereoselectively in the hydrophobic domain of the lipase.

When racemic ester **1** forms 2:1 complexes with Na^+ ion, there are three potential diastereomeric complexations which may occur: (*R*)-**1**·Na^+·(*R*)-**1**, (*S*)-**1**·Na^+·(*S*)-**1**, or (*R*)-**1**·Na^+·(*S*)-**1**. Further, each diastereomer has many isomers based on gearing of two crown rings. We carried out molecular mechanics calculation[15] for these 2:1 complexes, assuming that the two ester groups were not involved in the metal coordination. Figure 2 illustrates the optimized structures of two diastereomers, which are almost the same except for the geometry of the ester-functionalized side arm. This indicates that chirality of the crown ring carbon had a great influence on the spatial relationship between the two ester groups. The lipase was thought to primarily recognize two ester groups of the complex and to fix it in a restricted cavity. The 2:1 complexation may amplify the differences between enantiomers of the substrate **1** and facilitate enantioselective hydrolysis. Since the (*R*)-**1**·Na^+·(*S*)-**1** complex is not formed from chiral substrate, this may also explain the difference in rate enhancements between racemic and chiral substrates **1** (see Table 4).

The present study first revealed that the crown ether derivative has the potential to regulate enzymatic reaction via metal complexation. The crowned ester substrate was dynamically modified via noncovalent crown−metal interaction so that reactivity and/or enantioselectivity could be enhanced. Chiral crown ethers have been broadly utilized in asymmetric sensing, separation, and reaction processes[16] and have been prepared by elaborate stereoselective synthesis. Our approach can be considered a new and facile method for optical resolution of racemic crown ethers as well as for the improvement of enzyme-catalyzed organic syntheses.

Experimental Section

General. Racemic crowned ester **1** was prepared from 1,4,7,10-tetraoxacyclododecane-2-methanol (Janssen Chimica). (*R*)- and (*S*)-1,4,7,10-tetraoxacyclododecane-2-methanol were synthesized from (*R*)- and (*S*)-isopropylideneglycol (Merck) by the procedures reported[17] and then acetylated to yield chiral esters: $[\alpha]^{21}_D$ −6.7° (*c* 1.28, $CHCl_3$) for (*R*)-**1**; $[\alpha]^{21}_D$ +6.7° (*c* 1.11, $CHCl_3$) for (*S*)-**1**. Their spectroscopic data were exactly the same as racemic **1**. Azacrown ethers **2**−**4** were prepared by reaction of corresponding unsubstituted azacrown ethers (Aldrich) and 2-bromoethyl acetate.[18] Acyclic polyether analog **5** was derived from hexaethyleneglycol monomethyl ether (Parish Chem.). All of them were chromatographed (alumina; CH_2Cl_2/hexane) and had the correct elemental compositions determined by high-resolution mass spectroscopy (EI mode). The purity of all new compounds was established by ^1H and ^{13}C NMR spectroscopies (see supplementary material). Selected spectroscopic data of the newly obtained materials are summarized below.

1,4,7,10-Tetraoxacyclododecane-2-methyl acetate (1): oil, 90%; ^1H NMR ($CDCl_3$) δ 2.07 (s, 3H), 3.43−3.97 (m, 15H), 4.03−4.14 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 20.81, 64.13, 70.34, 70.61, 70.90, 71.10, 71.27, 71.46, 170.71; IR (neat) ν 1740 cm^{-1}; HRMS *m/e* calcd for $C_{11}H_{20}O_6$ 248.1259, found 248.1211.

1,4,7-Trioxa-10-azacyclododecane-10-ethyl acetate (2): oil, 70%; ^1H NMR ($CDCl_3$) δ 2.05 (s, 3H), 2.71−2.81 (t + t, 6H), 3.54−3.69 (m, 12H), 4.16 (t, 2H); ^{13}C NMR ($CDCl_3$) δ

(13) (a) Brady, L.; Brzozowski, A. M.; Derewenda, Z. S.; Dodson, E.; Dodson, G.; Tolley, S.; Turkenburg, J. P.; Christiansen, L.; Huge-Jensen, B.; Norskov, L.; Thim, L.; Menge, U. *Nature* **1990**, *343*, 767. (b) Cygler, M.; Grochulski, P.; Kazlauskas, R. J.; Schrag, J. D.; Bouthillier, F.; Robin, B.; Serreqi, A. N.; Gupta, A. K. *J. Am. Chem. Soc.* **1994**, *116*, 3180.

(14) Kim, M. J.; Choi, Y. K. *J. Org. Chem.* **1992**, *57*, 1605.

(15) "Extended MM2 program" (CAChe Scientific, version 3.0) was employed to calculate 32 kinds of isomers. Among them, complex B proved the most stable.

(16) (a) Cram, D. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1009. (b) Lehn, J. M. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 90. (c) Gokel, G. W. *Crown Ethers & Cryptands*; Royal Society of Chemistry; Cambridge, 1991; p 164. (d) Still, W. C.; Erickson, S.; Wang, X.; Li, G.; Armstrong, A.; Hong, J.-I.; Namgoong, S. K.; Liu, R. *Molecular Recognition: Chemical and Biochemical Problem II*; Royal Society of Chemistry: Cambridge, 1992; p 171.

(17) Miyazaki, T.; Yanagita, S.; Itoh, A.; Okahara, M. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2005.

(18) (a) Tsukube, H.; Uenishi, J.; Higaki, H.; Kikkawa, K.; Tanaka, T.; Wakabayashi, S.; Oae, S. *J. Org. Chem.* **1993**, *58*, 4389. (b) Tsukube, H.; Hori, K.; Inoue, T. *Tetrahedron Lett.* **1993**, *34*, 6749.

20.98, 55.06, 55.74, 62.69, 70.59, 71.49, 170.90; IR (neat) ν 1735 cm^{-1}; HRMS *m/e* calcd for $C_{12}H_{23}O_5N$ 261.1575, found 261.1568.

1,4,7,10-Tetraoxa-13-azacyclopentadecane-13-ethyl acetate (3): oil, 70%; ^1H NMR ($CDCl_3$) δ 2.04 (s, 3H), 2.82 (t, 6H), 3.50–3.75 (m, 16H), 4.14 (t, 2H); ^{13}C NMR ($CDCl_3$) δ 21.03, 54.96, 55.33, 62.88, 70.44, 70.73, 71.27, 170.95; IR (neat) ν 1735 cm^{-1}; HRMS *m/e* calcd for $C_{14}H_{27}O_6N$ 305.1837, found 305.1810.

1,4,7,10,13-Pentaoxa-16-azacyclooctadecane-16-ethyl acetate (4): oil, 60%; ^1H NMR ($CDCl_3$) δ 2.04 (s, 3H), 2.83 (t, 6H), 3.61–3.70 (m, 20H), 4.14 (t, 2H); ^{13}C NMR ($CDCl_3$) δ 21.00, 54.23, 54.89, 62.93, 70.34, 70.66, 70.98, 71.10, 170.93; IR (neat) ν 1730 cm^{-1}; HRMS *m/e* calcd for $C_{16}H_{31}O_7N$ 349.2099, found 349.2094.

2,5,8,11,14,17-Hexaoxanonadecanyl acetate (5): oil, 80%; ^1H NMR ($CDCl_3$) δ 2.07 (s, 3H), 3.37 (s, 3H), 3.56–3.68 (m, 22H), 4.16 (bt, 2H); ^{13}C NMR ($CDCl_3$) δ 20.93, 59.06, 63.69, 65.22, 69.29, 70.68, 70.78, 72.15, 166.32; IR (neat) ν 1735 cm^{-1}; HRMS *m/e* calcd for $C_{15}H_{30}O_8$ + H, 339.2017, found 339.1965.

Lipase-Catalyzed Hydrolysis. Determination of Hydrolysis Ratio (Table 2). A suspension of lipase PS (86 mg) is distilled water (1.0 mL) was centrifuged at 3000 rpm for 5 min at room temperature, and then the supernatant was immediately used as the enzyme solution. Ester **1** (0.045 mmol) in 0.1 M MCl aqueous solution (0.15 mL, M = Li, Na and K) and the enzyme solution (0.07 mL) were mixed and incubated at 35 °C for 6 h. The reaction was stopped by addition of small pieces of ice and then extracted with $CHCl_3$, dried over $MgSO_4$, and evaporated to dryness. The hydrolysis ratio was determined by ^1H NMR analysis. Other esters were similarly examined.

Determination of Enantiomeric Excess (Table 3). Ester **1** (50 mg, 0.20 mmol), lipase PS (25 mg), and additive in aqueous solution (0.75 mL) were incubated at 35 °C for 11 h. The mixture was extracted with $CHCl_3$, dried over $MgSO_4$, and evaporated to dryness. After determination of the hydrolysis ratio by ^1H NMR analysis, the crude product was separated by silica gel flash column chromatography (hexane: AcOEt = 1:6 to methanol) to give the alcohol and the recovered acetate **1**. Optical rotation measurement indicated that (*S*)-alcohol was a major product in every case. The optical purity of the alcohol was determined by ^{19}F NMR analysis of the corresponding (+)-MTPA ester.[19] The signal due to the trifluoromethyl group of the MTPA ester was split into two peaks (90.10 ppm and 90.14 ppm from C_6F_6 internal reference), and the enantiomeric excess of the product was calculated by comparison of the peak intensities.

FAB MS Experiments. Complexation of crowned ester (0.0033 mol/L) with LiCl, NaI, and KI (0.0083 mol/L, each) in *m*-nitrobenzyl alcohol was studied by measuring the relative peak heights of [ester + M^+] ions. FAB MS spectra were recorded with a JEOL DX 300 instrument, and the peak heights were averaged over at least 12 scans.

NMR Binding Experiments. ^{13}C NMR studies were carried out with a JEOL 90 A spectrometer, while ^{19}F and ^{23}Na NMR spectra were recorded with a Varian VXR-200 (SC-NMR Laboratory of Okayama University) and a Varian XL-300 (Kaneca Techno Research Co., Ltd). Crowned esters were dissolved in CD_3OD/H_2O, CD_3OD/CH_3OH, or CD_3CN/CH_3CN at a concentration of 0.05 mol/L.

Acknowledgment. The authors are grateful to Professors Koichiro Naemura of Osaka University and Kaoru Nakamura of Kyoto University for valuable discussions. They are also grateful to the SC-NMR Laboratory of Okayama University for ^{19}F NMR measurements. This research was supported in part by the Nishida Research Fund for Fundamental Organic Chemistry. The provision of lipases from Amano Pharmaceutical Co. is also appreciated.

Supplementary Material Available: ^1H and ^{13}C NMR spectra for compounds **1–5** (10 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfiche version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(19) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512.