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Functional modification of naturally occurring ionophores as a new route to chiral receptors

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New chiral receptors characterized by acyclic polyether skeletons and neutral terminal substituents were derived from naturally occurring monensin, lasalocid, salinomycin and nigericin ionophores. Their chiral recognition ability was investigated by ion-selective electrode and ^{1}H-NMR spectroscopic methods. Monensin ester and amide derivatives specifically formed 1:1 complexes with chiral amine salts and exhibited excellent enantiomer selectivity, while parent monensin rarely discriminated between the enantiomers of examined amine salts. This review describes how chemical modification of naturally occurring ionophores provides unique molecular recognition functions and a new synthetic strategy for chiral receptor molecules.

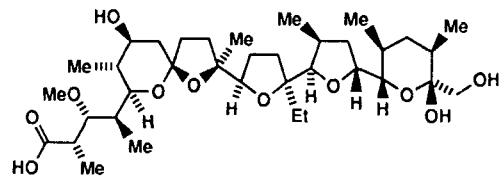
INTRODUCTION

Polyether antibiotics such as monensin and lasalocid form a well defined class of naturally occurring ionophores that transport alkali and alkaline earth metal cations across biomembranes.[1,2] They are composed of acyclic chiral polyether chains and carboxylate terminal groups, and have pseudo-cyclic conformations of their polyether chains via intramolecular head to tail hydrogen bonds. Crystallographic studies of their metal complexes show that guest metal cations are located at the centre of the pseudo-cavities and are co-ordinated by several oxygen atoms in a similar fashion to those in crown ether complexes. The stability constants of their metal complexes have been measured in organic media.[3] Interestingly, they form stable complexes with various cationic species other than biological guest cations. For example, monensin, a biological Na^+ ion carrier, shows high binding abilities for K^+, Rb^+, and Ag^+ ions; valinomycin prefers Rb^+ cation to biological guest K^+ cation. These unexpected features of polyether antibiotics suggest that they may have ligand arrangements flexible enough to adjust to various natural and unnatural guest cations.[4,5]

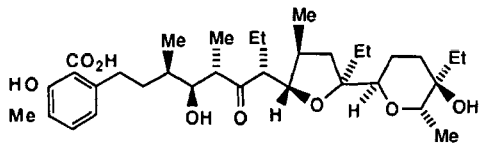
Naturally occurring ionophores also have potential abilities to form enantiomer-selective complexes. Westley *et al.*[6] first applied biological ionophores to the preferential crystallization of salts with racemic amines. In the crystal lattice, several organic ammonium cations specifically formed three N–H...O hydrogen bonds with pseudo-cyclic lasalocid. Recently, Lindoy and co-workers[7] reported this mediated enantiomer-selective transport of metal complexes. Enantiomers of some metal ammine and amine complexes were partially distinguished via liquid membrane transport. Although a limited number of successful examples has been reported to date, an excellent new chiral receptor may be derivable from naturally occurring ionophores.

We succeeded in deriving a new series of chiral receptors from naturally occurring ionophores that offered enantiomer-selective complexations and specific sensing functions towards several asymmetric amine salts. Various polyether antibiotics such as monensin, lasalocid, salinomycin, and nigericin are available for structural modification. The molecular structures are shown in Figure 1. Their ester and amide derivatives exhibited interesting chiral recognition ability, though they were themselves incapable of discriminating between optical isomers of amine salts. Enhanced chiral recognition abilities were observed especially with bis-monensin ester and monensin amide derivatives. Chemical functionalization of these polyether antibiotics significantly offered new and unique receptor functions. This review describes the synthetic strategy used in functional modification of naturally occurring ionophores and presents their chiral recognition behaviours as well as promising applications in sensory science. The basic chemistry and biochemical functions of each biological ionophore have been detailed in the literature.[1,2]

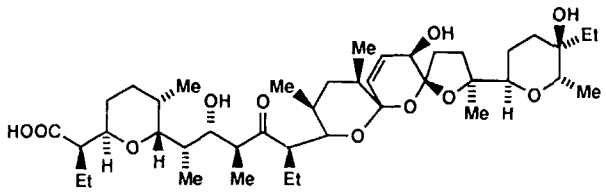
* To whom correspondence should be addressed.



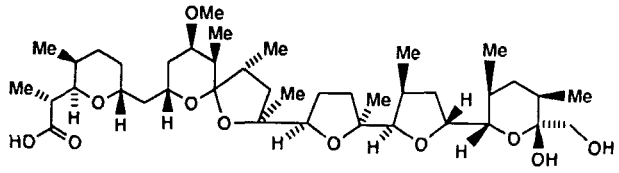
monensin



lasalocid



salinomycin



nigericin

Figure 1 Naturally occurring polyether ionophores available for structural modification.

RESULTS AND DISCUSSION

Structural modification of naturally occurring ionophore

Monensin, a typical polyether antibiotic, has a complicated molecular structure but one which can be chemically modified. It has a pseudo-cavity for accommodation of guest cations encircled by reactive OH and COOH groups as well as 17 asymmetric carbons. Lasalocid and other polyether antibiotics examined have similar structural features. Since their chain-lengths and pseudo-cavity sizes are varied, we modified them so that they have chiral, ordered pseudo-cavities specific for enantiomer-selective binding of ammonium cations.

Structural modifications of naturally occurring ionophores require mild reaction conditions and short reaction steps because they are unstable molecules. There are several studies on the structural modification of polyether antibiotics to determine the effect on their biological action.[8] We have shown that ester and amide derivatives were readily prepared from the parent ionophores via a one-step reaction. As shown in Figure 2, we confirmed that reaction with the sodium salt of a polyether antibiotic and bromide was effectively promoted by addition of cryptand [2.2.2], and we obtained new ester derivatives of polyether antibiotics in 80–98% yields.[9] Since the cryptand strongly bound the Na^+ cation, naked carboxylate anions of polyether antibiotics were generated and acted as potential nucleophiles in non-polar media. This esterification reaction was successfully applied to

Ester Formation

$$R\text{-}CO_2^-Na^+ + R'\text{-}Br \longrightarrow R\text{-}CO_2\text{-}R'$$

Amide Formation

$$R\text{-}CO_2H + R'\text{-}NH_2 \longrightarrow R\text{-}CONH\text{-}R'$$

Figure 2 Ester and amide formation.

the one-step synthesis of bis-monensin esters which had two monensin ester units.[10]

Monensin amides were prepared by a modification of Corey's lactonization reaction in 60–80% yields.[11] Corey *et al.*[12] prepared a macrocyclic monensin lactone in which terminal OH and CO_2H groups were linked intramolecularly. Suzuki *et al.*[5] employed this lactone as a sensing element of ion-selective electrodes and found a good Li^+ ion selectivity. Since this may have a reduced cavity for guest binding, we inserted glycine and β-alanine moieties into the macrocyclic skeleton to obtain large membered macrocycles suitable for binding organic ammonium cations. They were synthesized by debenzylation of acyclic monensin benzyl esters and successive cyclization.[11] The chiral recognition properties of these modified ionophores were characterized by ion-selective electrode and ^{1}H-NMR titration experiments.

Chiral recognition by ester derivatives of polyether antibiotics

The ion-selective electrode technique is recognized as a conventional and facile method for evaluating the metal binding ability of receptors.[13] We prepared sensor electrodes which incorporated the modified ionophores in a similar fashion to common metal-selective electrodes, and used them to estimate the enantiomer recognition ability. Near Nernstian responses for chiral ammonium salts were observed for the examined, chemically modified, ionophores in the range of 1×10^{-1} to 1×10^{-4} mol/l. Enantiomer selectivity coefficient $K_{S,R}$ is electrochemically defined as $10^{(E_S - E_R)/0.059}$, where E_S and E_R represent the potentials measured for the *S*- and *R*-guest containing solutions. As described below, this well correlates with the stability constants of the complexes between the employed ionophore and chiral guest ammonium cations. Typical results are summarized in Tables 1 and 2.

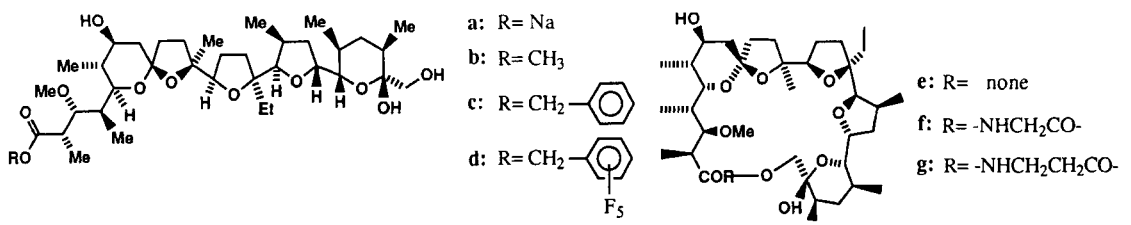
Ester derivatives of polyether antibiotics showed characteristic chiral recognition behaviour toward certain chiral ammonium cations.[9] Since the parent antibiotics examined exhibited little chiral discrimination, unnatural but interesting chiral recognition functions were attained by esterification. The enantiomer selectivity coefficients $K_{S,R}$ were largely dependent on the structures of the parent polyether antibiotics. Table 1 indicates that monensin ester showed higher chiral recognition ability than other polyether esters; enantiomer selectivity coefficient $K_{S,R}$ for phenylglycine methyl ester (Ph-GlyOMe) hydrochloride reached a high value of 4.0. The nature of the ester substituents influenced $K_{S,R}$ values; the $K_{S,R}$ values of various

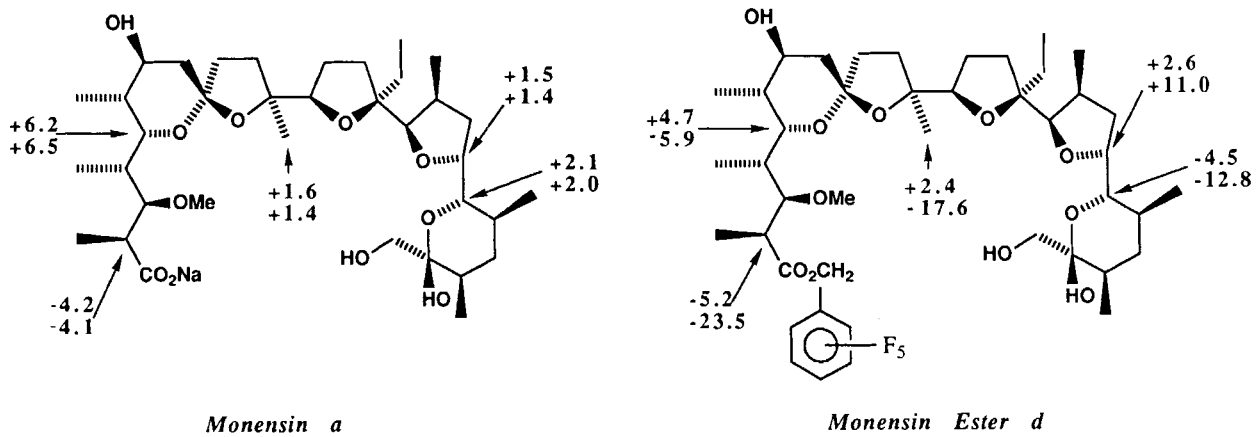
Table 1 Enantiomer selectivity coefficients ($K_{S,R}$) of naturally occurring ionophores and their benzyl esters

	$K_{S,R}$				
Ionophore	Ph-GlyOMe·HCl	PheOMe·HCl	LeuOMe·HCl	ProOMe·HCl	Naphthylethylamine·HCl
Monensin	1.8	1.6	1.6	1.00	0.76
Benzyl ester	4.0	2.2	2.5	0.77	0.48
Lasalocid	1.2	1.1	1.1	1.00	1.00
Benzyl ester	1.8	1.3	1.5	0.82	0.73
Salinomycin	1.4	1.1	1.2	1.20	0.91
Benzyl ester	1.7	1.2	1.3	1.00	0.83
Nigericin	1.0	1.0	1.0	1.10	1.00
Benzyl ester	1.1	1.8	1.6	1.00	1.00

Table 2 Enantiomer selectivity coefficients ($K_{S,R}$) of monensin ester derivatives

		$K_{S,R}$				
Ionophore		Ph-GlyOMe·HCl	PheOMe·HCl	LeuOMe·HCl	ProOMe·HCl	Naphthylethylamine·HCl
Monensin	**a**	1.8	1.6	1.6	1.00	0.76
Ester	**b**	3.7	2.1	2.4	0.90	0.47
	c	4.0	2.2	2.5	0.77	0.48
	d	4.4	2.5	3.4	1.00	0.37
Lactone	**e**	1.0	1.0	1.0	1.00	1.10
	f	1.0	1.0	1.0	1.00	1.00
	g	1.0	1.0	1.0	1.00	1.00





Monensin a *Monensin Ester d*

Figure 3 Guest-induced changes in ^{1}H-NMR chemical shift values (Hz) of monensin **a** and its pentafluorobenzyl ester **d** in $CDCl_3$. Shifted values in the prescence of 1 equiv. of *S*-1-(1-naphthyl)ethylamine acetate were shown upper; those in the presence of 1 equiv. of *R*-1-(1-naphthyl)ethylamine acetate were indicated lower.

monensin esters are summarized in Table 2. Monensin benzyl esters **c** and **d** seemed more effective than monensin methyl ester **b**, while monensin lactones **e**–**g** having different ring sizes offered no enantiomer-selective response. Since they had similar chiral polyether linkages and ester groups, it shows that the macrocyclic skeleton is not effective for chiral recognition. Monensin ester was demonstrated to have a neutral, acyclic (but pseudo-cyclic) skeleton suitable for chiral recognition of asymmetric ammonium cations.

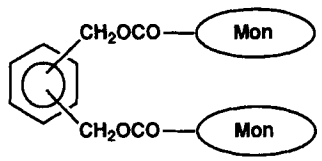
The enantiomer-selective complexation behaviour of monensin derivatives was investigated by ^{1}H-NMR spectroscopy. Figure 3 indicates guest-induced ^{1}H-NMR spectral changes of biological monensin **a** and ester **d**. Addition of *R*- or *S*-1-(1-naphthyl)ethylamine acetate to $CDCl_3$ solution of biological monensin caused definite changes in chemical shifts of the signals of several protons surrounding the pseudo-cavity. Although the shifted values were modest, the guest ammonium cation appeared to be in loose contact with the pseudo-cyclic polyether linkage in the complex. Since both enantiomers of the guest salt induced almost the same spectral changes, monensin **a** could not discriminate between these enantiomers. Electrostatic interaction between terminal carboxylate anion of monensin and guest ammonium cation is probably effective for binding the ammonium cation but not for enantiomer recognition. In contrast, addition of *S*- and *R*-1-(1-naphthyl)ethylamine salt to a $CDCl_3$ solution of monensin ester **d** exhibited remarkable spectral changes. When the *S*-guest cation was added, the shifted values of proton signals positioned at 5- and 31-carbons exhibited the opposite sign to the *R*-guest induced signals. This means that monensin ester **d** evidently forms complexes with enantiomers and the resulting complexes have different structures. The plots of the guest-induced changes in chemical shifts of the proton signals with changes in the concentration ratio of *S*- or *R*-1-(1-naphthyl)ethylamine acetate to monensin ester **d** gave good fits for 1:1 complexes. Thermodynamic stability constants were estimated as 36 l/mol for *R*-guest and 22 l/mol for *S*-guest. Since these spectroscopic observations are parallel to those obtained in the ion-selective electrode experiments, enantiomer-selective complexation on a molecular basis offered an electrochemical chiral recognition phenomenon.

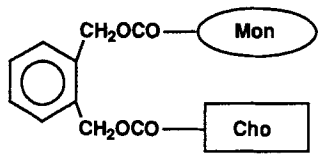
Chiral recognition by bis-monensin esters

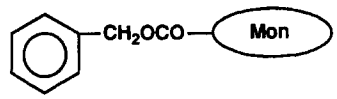
Several bis-crown ethers are known to show unique guest binding and recognition functions, in which two crown ether rings co-operatively bind one guest cation.[14] Lehn, Cram, and others[15] have reported interesting examples of chiral bis-crown ethers forming 1:1 complexes with monoammonium cations.

We characterized three kinds of bis-monensin derivatives which comprised two chiral monensin skeletons (Table 3).[10] One of them, *ortho*-bis-monensin, exhibited enhanced enantiomer selectivity relative to the mono-monensin benzyl ester. This favoured the *S*-isomers of several amino acid ester salts, and enantiomer selectivity coefficients $K_{S,R}$ were calculated to be 4.8–8.6. Other bis-monensin derivatives, *meta*- and *para*-isomers, displayed similar $K_{S,R}$ values to those with corresponding mono-monensin esters. Since binding to the external faces of the monensin moieties would not offer enhanced chiral recognition ability, *ortho*-bis-monensin may have a unique molecular cavity to accommodate chiral ammonium cations. A schematic illustration of their binding modes is shown in Figure 4. The enantiomer-selective complexation of bis-monensin ester was also supported by ^{1}H-NMR experiments. Titration curve analysis indicated that *ortho*-bis-monensin formed both 1:1 sandwich-like

Table 3 Enantiomer selectivity coefficients ($K_{S,R}$) of bis-monensins and their analogues

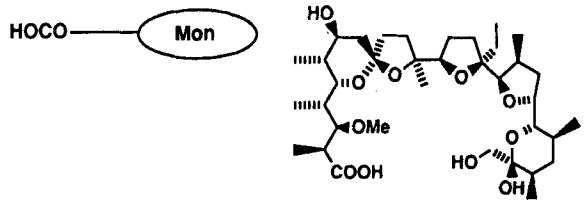




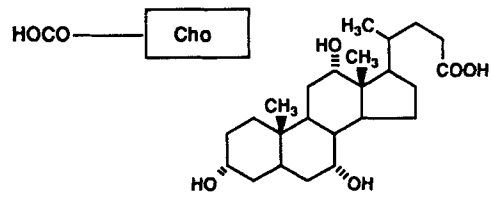


	Ortho	*Meta*	*Para*		
Ph-GlyOMe·HCl	5.9	3.5	2.8	2.9	4.0
PheOMe·HCl	8.6	2.4	1.9	1.8	2.2
LeuOMe·HCl	7.0	2.7	2.1	2.1	2.5
LysOMe·2HCl	4.8	2.2	2.8	2.1	2.3

Monensin:



Cholic acid:



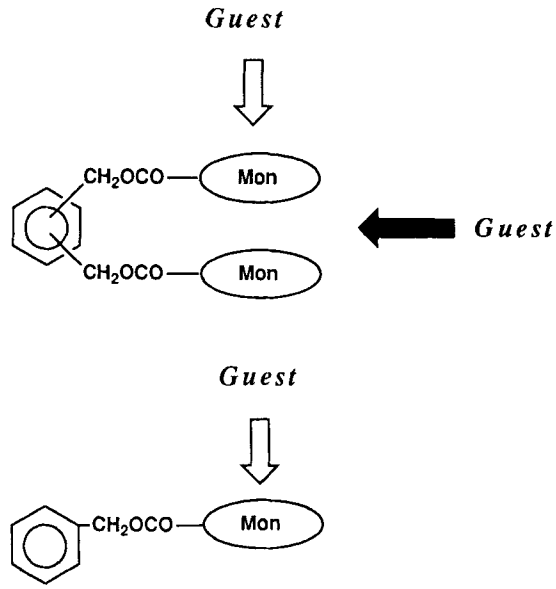


Figure 4 Binding modes of bis-monensin and monensin esters.

and 1:2 complexes (bis-monensin:guest mono-ammonium cation).

Chiral recognition by monensin amides

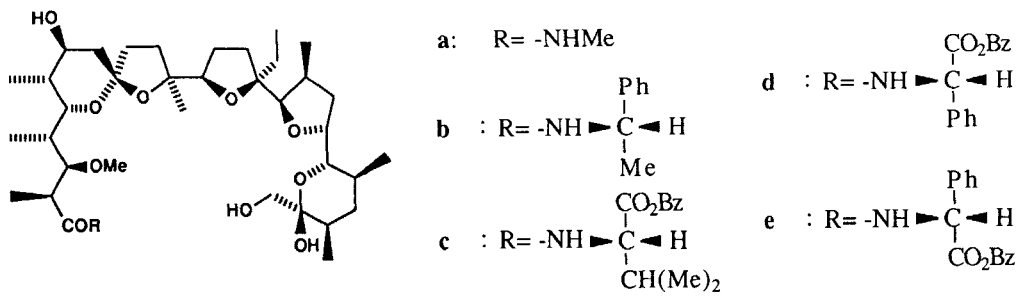
Acyclic monensin amides exhibited enantiomer-selective complexations with certain chiral ammonium cations.[11] Their chiral recognition abilities were similar to or higher than those of monensin ester and significantly depended on the nature of the amide substituents.

Table 4 summarizes the chiral recognition ability of monensin amides determined electrochemically. Monensin amides **b**–**e** having bulky groups showed larger $K_{S,R}$ values than amide **a** having a small group. The $K_{S,R}$ values for monensin amides **b**–**e** were calculated to be 3.3–7.6. A combination of pseudo-cyclic monensin skeleton, amide junction, and bulky residue provided excellent chiral recognition function. The stereochemistry of the introduced amide substituent influenced chiral recognition selectivity. Monensin amide **d** having an *S*-phenylglycine moiety gave larger $K_{S,R}$ values for all ammonium cations examined than monensin amide **e** with an *R*-phenylglycine moiety. ^{1}H-NMR binding experiments revealed that the amide junction acted as a potential binding site for ammonium guest cations and its participation enhanced enantiomer selectivity. A chiral crown ether was also examined under the same conditions (see Fig 5). This was originally developed by Cram *et al.* and recognized as one of the best chiral ionophores for asymmetric primary ammonium cations. As reported by Shinbo *et al.*,[16] it offered generally satisfactory enantiomer selectivity, but our monensin amides often exhibited higher enantiomer selectivities. Since these monensin amides have a great synthetic advantage over chiral crown ethers, chemical modification of biological ionophores can be considered to be a facile and effective methodology with which to develop a specific chiral receptor.

Table 5 compares thermodynamic stability constants K_S and K_R of monensin ester **b** and amide **c** determined

Table 4 Enantiomer selectivity coefficients ($K_{S,R}$) of monensin amides

Monensin amide	$K_{S,R}$				
	Ph-GlyOMe·HCl	PheOMe·HCl	LeuOMe·HCl	ProOMe·HCl	Phenethylamine·HCl
a	2.1	1.8	2.3	1.0	1.00
b	5.1	6.2	6.0	1.2	0.43
c	5.1	6.2	7.6	1.0	0.40
d	4.9	5.7	5.3	1.1	0.42
e	3.3	4.3	3.4	1.0	0.53



by ^{1}H-NMR titration with their enantiomer selectivity coefficients $K_{S,R}$. Monensin amide generally gave larger stability constants and higher enantiomer selectivity than monensin ester. As described above, further co-ordination with an amide junction enhanced both complex stability and chiral recognition ability. A similar relation between stability constant and electrochemical enantiomer selectivity has been reported by Bussman and Simon.[17]

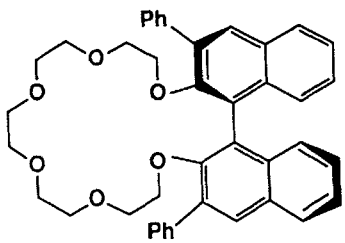


Figure 5 Chiral crown ether.

CONCLUSION

We successfully obtained a new series of chiral receptors from naturally occurring ionophores. In particular, several monensin esters and amides formed enantiomer-selective complexes with certain chiral ammonium cations. Guest cations were comfortably accommodated in the chiral and pseudo-cavity. A variety of beautiful host–guest complexes are known in nature other than ionophore–metal systems; typical examples are enzyme–substrate, co-enzyme–apo-enzyme, and antigen–antibody systems. These have complicated structures but the heart of their host–guest chemistry is similar to that of synthetic host–guest chemistry. We have already established several concepts and strategies in synthetic host–guest chemistry, and these make it possible to combine it with natural product chemistry. This may open up a route to a new and specific receptor molecule for a given guest species.

Table 5 Stability constants and selectivity coefficients of monensin derivatives

Ionophore	*Guest·AcOH*	*^{1}H-NMR method*[a]		*Electrode method*
		K_R or K_S	K_R/K_S	$K_{S,R}^{-1}$
Ester **b**	*R*-1-(1-Naphthyl)ethylamine·AcOH	13	2.3	2.1
	S-1-(1-Naphthyl)ethylamine·AcOH	6		
	R-Phenethylamine·AcOH	9	–	1.9
	S-Phenethylamine·AcOH	b		
Amide **c**	*R*-1-(1-Naphthyl)ethylamine·AcOH	89	3.0	2.6
	S-1-(1-Naphthyl)ethylamine·AcOH	30		
	R-Phenethylamine·AcOH	73	2.5	2.5
	S-Phenethylamine·AcOH	30		

[a] K_R and K_S indicate the stability constants with *R*- and *S*-enantiomers calculated by means of ^{1}H-NMR titration method.
[b] Could not be calculated because the changes in chemical shifts were too small.

EXPERIMENTAL SECTION

Materials

Monensin sodium salt and its methyl ester are commercially available (Wako and Calbiochem) and were used without further purification. Solvents and other reagents were used after usual purification. Amino acid esters are also commercially available (Sigma and Wako Pure Chemical Industries). Chiral crown ether was kindly provided by Dr. T. Yamaguchi of National Chemical Laboratory for Industry, Tsukuba, Japan. Other esters and amides of polyether antibiotics were prepared by methods described earlier.[9-11] These are 'hazardous compounds' and require careful handling and disposal. Typical preparation methods of *ortho*-bis-monensin and monensin amide **c** are described below.

Bis-monensin was synthesized by treatment of monensin sodium salt (58 mg) with α,α'-dibromo-*o*-xylene (10 mg) in the presence of cryptand [2.2.2] (47 mg) in CH_3CN. After stirring at room temperature for 1 day, chromatography on silica gel (CH_2Cl_2/$CH_3CO_2C_2H_5$) gave pure *ortho*-bis-monensin (38 mg).

Monensin amide **c** was obtained by the reaction of monensin free acid (30 mg) with L-ValOBz (100 mg, Bz = $-CH_2C_6H_5$) in the presence of 2,2'-dipyridyldisulphide (30 mg) and triphenylphosphine (45 mg) in CH_2Cl_2 at room temperature for 1 day. After refluxing for 1 day, the reaction mixture was washed with dilute HCl and then with saturated $NaHCO_3$ solution. Chromatography on silica gel (CH_2Cl_2/$CH_3CO_2C_2H_5$) gave pure amide **c** (24 mg).

Ion-selective electrode experiments

Ion selective electrode experiments were carried out as follows. Cell composition was Ag/AgCl, 0.01 M NaCl, membrane, sample solution, reference electrode (Orion 0.3 M NH_4NO_3, AgCl/Ag). Membrane potentials were measured with an EA-920 ion analyser (Orion) and recorded within ± 0.3 mV stability with a microcomputer system. We used a membrane electrode kit purchased from Denki Kagaku Kogyo (Tokyo, Japan). Potassium tetrakis(*p*-chlorophenyl)-borate (1 mg), ionophore (3 mg), poly(vinylchloride) (30 mg), and *o*-nitrophenyl octyl ether (66 mg) were dissolved in tetrahydrofuran (1 ml). After the solution had unified, 20 drops were spread on a Teflon sheet and put on the electrode tip. After being left for 12 h, the electrode tip was immersed in 0.01 M NaCl solution for 6 h and then mounted on an electrode body for measurement. The emf values measured at room temperature were used in the Nicolsky–Eisenman equation. Selectivity coefficients $K_{S,R}$ were calculated by the separate solution method. We confirmed near Nernstian response for each experiment.

^{1}H-NMR titration experiments

^{1}H-NMR titration experiments were carried out with a Varian VXR-500 spectrometer (SC-NMR Laboratory, Okayama University). We prepared 10 samples with different guest/ionophore ratios. The stability constant K_S or K_R for 1:1 complex formation is given by equation (1):

$$K = [C]/\{[I]_0 - [C]\}\{[G]_0 - [C]\} \quad (1)$$

where $[C]$, $[I]_0$, and $[G]_0$ represent the concentration of the complex in the equilibrated state, and initial concentrations of ionophore and guest, respectively. Since guest-induced change in the chemical shift of proton signal ($\Delta\delta$) can be related to $\Delta\delta_\infty$ (saturated $\Delta\delta$) as equation (2):

$$\Delta\delta = [C]\Delta\delta_\infty/[I]_0 \quad (2)$$

equation (3) can be obtained from equations (1) and (2):

$$\Delta\delta = [[I]_0 + [G]_0 + 1/K - \{([I]_0 + [G]_0 + 1/K)^2 - 4[I]_0[G]_0\}^{1/2}]/(2[I]_0/\Delta\delta_\infty) \quad (3)$$

Substituting measured $\Delta\delta$, $[I]_0$ and $[G]_0$ in equation (3), the stability constant was calculated by non-linear least squares treatment (Gauss–Newton method).

ACKNOWLEDGEMENTS

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