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Dipeptide-derived lariat ethers as enantioselective carriers of Z-amino acid and dipeptide carboxylates

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Lariat ethers bearing *N*-pivot dipeptide arms have been designed and synthesized as models of natural amino acid and Na^+ cation carriers, the "symporters". Two types of dipeptide derived lariats have been prepared: those having amino terminal dipeptide arms (ATD-lariats, 6, 7, 10, 11, 16–18) and those having carboxylic group terminal dipeptide arms (CTD-lariats, 12, 19). ATD-lariats were synthesized by *N*-acylation of aza-15-crown-5, aza-18-crown-6, and 4,13-diaza-18-crown-6 with Z-GlyOH or Z-PhgOH (Phg = D-α-phenylglycine) in the presence of DCC or $Ph_3P/CCl_4/Et_3N$ condensation agents, subsequent hydrogenolytic removal of Z-protecting groups, and the introduction of a second Z-amino acid unit using the same condensation reagents. CTD-lariats 12 and 19 were obtained by *N*-alkylations of aza-18-crown-6 and 4,13-diaza-18-crown-6 with methyl *N*-(chloro-acetyl)-D-α-phenylglycinate (acetonitrile, Na_2CO_3, NaI). The binding affinities of ATD-lariats toward Na^+ and K^+ assessed in anh. MeOH and compared with those previously determined for CTD-lariats were found to be approximately 100-fold lower than those of CTD-lariats. Transport studies with 10, 16, 12, and 19 and a series of Z-amino acid and dipeptide K^+-carboxylate guests showed that CTD-lariats 12 and 19 are efficient carriers with chiral and constitutional recognition properties. ^{1}H-NMR studies of 19-Z-PheO$^-K^+$ complex revealed formation of intra-complex hydrogen bonds between lariat pendant arms and bound substrate.

INTRODUCTION

In the not too distant past it was thought that only biological macromolecules could exhibit the property of molecular recognition. However, contemporary studies in supramolecular[2a] or host-guest[2b] chemistry have shown that ion or molecule recognition is possible for carefully designed structures. Synthetic molecular receptors[2ab] may exist in a variety of shapes and sizes depending on the substrate to be recognized.[3] The design and syntheses of receptors able to recognize and bind such biologically relevant molecules as amino acids and peptides[4] or nucleic acid components[5] have proved to be of special interest. These synthetic receptors may serve to mimic important biological processes.[6] In addition, they may substitute for the original structures that inspired their synthesis *in vivo* and lead to important biological effects.

The related problems of binding and transporting amino acids and small peptides by synthetic molecular receptors and carriers has recently attracted considerable attention.[4a–n] Model systems have been based upon crown ethers,[4d,e,k] crown ethers and aza-crown-ethers bearing amino acid side arms.[4c,f,g] Aliquat 336,[4b] a merocyanine dye,[4h] Rebek's molecular clefts,[4a] the rigid receptor containing guanidinium subunit,[4l] a bifunctional metalloporphyrin receptor[4m] and calix[6]arene[4n] have all been studied. Based upon the molecular receptors and carriers reported so far, three types of binding of amino acid or peptide substrates may be recognized: (i) binding the substrate's primary ammonium group by crown receptors or carriers, (ii) ion-pairing of substrate carboxylates with the cationic binding site of a carrier such as Aliquat 336[4b] and guanidinium receptor[4l] or with the crown metal cation complex[4f,i,j] and (iii) binding of substrate in the zwitterionic form (Rebek's molecular cleft).[4a]

Proteins primarily utilize hydrogen bonding between amide groups to bind substrates.[7] Model studies have also been reported that use to advantage the strength and directionality of hydrogen bonds to engage substrates.[8] Novel molecular receptors and carriers for amino acids and peptides that use supramolecular (intra-complex) amidic hydrogen bonds are of obvious interest. One approach to such receptors or carriers may be the construction of lariat ether derivatives bearing peptidic pendant arms. Lariats of this type, possessing a 15- or 18-membered crown ether ring, would be able to anchor the amino acid or small peptide guest in the form of ammonium or carboxylate salts (binding types i and ii in the preceding paragraph). Then, the hydrogen bonding interactions

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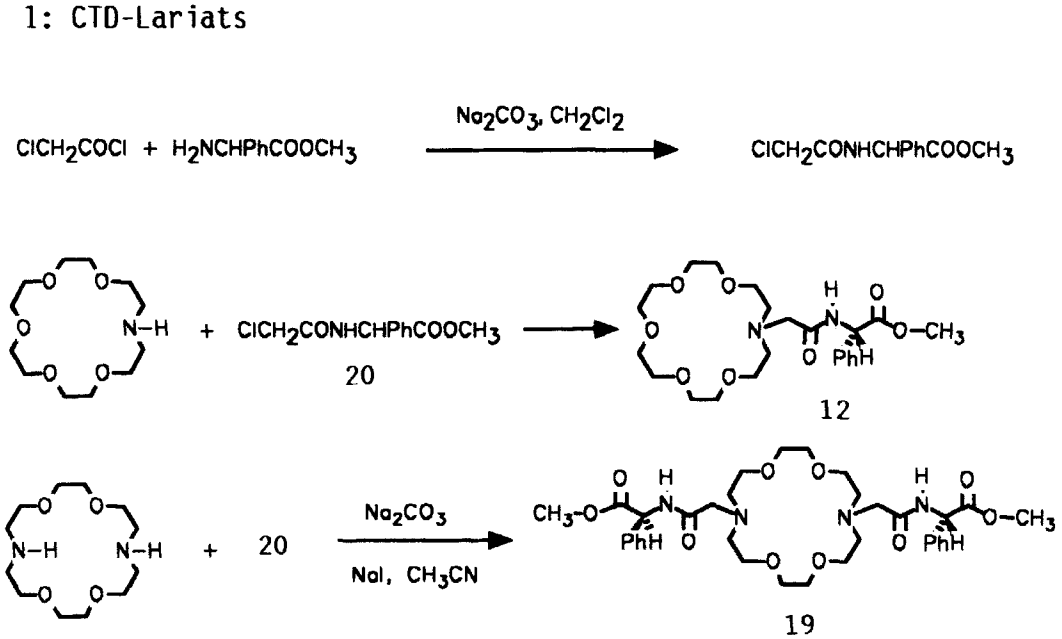
between the pendant peptidic sidearms and the anchored substrate may be anticipated. The expectation is based on the well known ability of small, open-chained peptides to form hydrogen bonded aggregates in lipophilic media.[9a,b,10] Such lariat type receptors may possess recognition properties originating from the structural and stereochemical characteristics of their pendant, peptidic arms. In this paper we report the syntheses and binding and transport properties of amino acid, dipeptide, and some metal ion guests.

RESULTS AND DISCUSSION

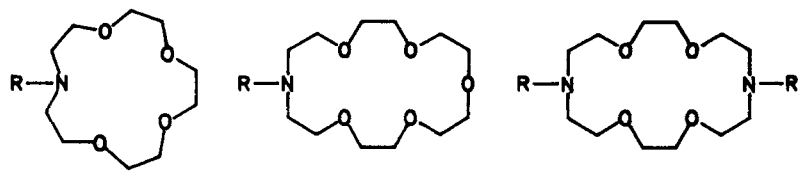
Syntheses

Dipeptide sidearms could be attached to the nitrogen atoms of 15-membered (⟨15N⟩, **1**), 18-membered aza-(⟨18N⟩, **2**) and diaza-(⟨N18N⟩, **3**) crown ethers in two different ways giving amino terminal (**ATD**-lariats) or carboxylic group terminal dipeptide lariat ethers (**CTD**-lariats) (Chart 1). Recently, the series of **CTD**-lariats have been synthesized by reaction of *N*-(chloroacetyl)amino acid esters with azacrowns **2** (⟨18N⟩) and **3** (⟨N18N⟩) in the presence of Na_2CO_3 and NaI.[11a-c] Using the same method we have prepared the hitherto unknown **CTD**-lariats **12** and **19** having D-α-phenylglycine (Phg) as the second (Gly is the first) amino acid (Scheme 1). **ATD**-lariats **10**, **11**, **16**, **17**, and **18** were prepared in two steps. The first approach was to attach one *N*-benzyloxycarbonyl(Z)-amino acid unit to the macroring (**1**–**3**) nitrogen atoms using the usual peptide bond forming reactions (DCC condensation, mixed anhydride, activated ester).[12a] The mixed anhydride method (isobutyl chloroformate/*N*-methylmorpholine) with Z-D-PhgOH and **1** gave **5** in 78% yield and a byproduct identified as *N*-(isobutyloxycarbonyl)-aza-15-crown-5. The same reaction with diaza-18-crown-6 (**3**) gave a mixture of **15** and *N*-(isobutyloxycarbonyl)-*N'*-(Z-D-Phg)-4,13-diaza-18-crown-6 which was very difficult to separate. The same product mixture was formed even when the reaction time for formation of the mixed anhydride was prolonged. Thus, formation of the byproducts is likely to be the consequence of the concurrent macroring nitrogen attack on the carbonic carbonyl of the mixed amino acid-carbonic anhydride.[12b] The reaction of **2** or **3** with activated ester Z-PhgOpNP required 2 days at 55° to give 55% and 62% yields of **9** and **15**, respectively.

More satisfactory results were obtained by using DCC and $Ph_3P/CCl_4/Et_3N$ condensations[13] giving **4**, **5**, **8**, **13**, **14**, and **15** in yields ranging from 55–80%. Hydrogenolytic removal of Z-blocking groups using 10% Pd/C catalyst gave the corresponding *N*-aminoacyl derived azamacromolecules in quantative yields. The second Z-amino acid unit was introduced *via* either the DCC or Ph_3P method giving the dipeptide-derived lariats **10**, **16**, and **18** in 50–60% yields. The more liphophilic *N*-benzoyl(Bz)-**ATD**-lariats **7**, **11**, and **17** have been prepared from **6**, **10**, and **16** by hydrogenolytic removal of Z groups and subsequent reaction with benzoyl chloride. Lariats **10** and **16** were also prepared in one step by $Ph_3P/CCl_4/Et_3N$ condensation of Z-D-PhgGlyOH



Scheme 1 Formation of **CTD**-lariats.



R			
H	1	2	3
$PhCH_2OCONHCH_2CO$ (Z-Gly)	4	8	13
$PhCH_2OCONHCHPhCO$ (Z-Phg)	5	9	14
$PhCH_2OCONHCHCH_2PhCO$ (Z-Phe)	-	-	15
$PhCH_2OCONHCHPhCONHCH_2CO$ (Z-PhgGly)	6	10	16
$PhCONHCHPhCONHCH_2CO$ (Bz-PhgGly)	7	11	17
$PhCH_2OCONHCHPhCONHCHPhCO$ (Z-PhgPhg)	-	-	18
$MeOCOCHPhNHCOCH_2$ (MeOPhgGly)	-	12	19

Z=$PhCH_2OCO$- ; Bz=PhCO- ; Phg=D-α-phenylglycine ; Phe=D-phenylalanine

Chart 1

and **2** or **3**. The yields were 57% and 13% respectively for **10** and **16**. The one step synthesis using the same reactants and DCC was unsuccessful, affording only small amounts of **10** and **16**.

Dipeptides Z-Gly-D-PhgOH, Z-Gly-L-PhgOH, Z-D-PhgGlyOH and Z-L-PhgGlyOH needed for transport studies were prepared in two steps. First the dipeptide methyl esters were prepared by DCC condensations of the respective Z- and methyl ester blocked amino acid derivatives in yields exceeding 90%. The dipeptide methyl esters were then hydrolysed using a small excess of NaOH in a dioxane/water mixture[14] giving dipeptides in 90–95% yields. The sequence is illustrated below.

$$PhCH_2\text{-}O\text{-}CO\text{-}CHR\text{-}COOH +$$
$$H_2N\text{-}CHR'\text{-}COOCH_3 \rightarrow PhCH_2\text{-}O\text{-}CO\text{-}NH\text{-}$$
$$CHR\text{-}CO\text{-}NH\text{-}CHR'\text{-}COOCH_3 \rightarrow PhCH_2\text{-}O\text{-}$$
$$CO\text{-}NH\text{-}CHR\text{-}CO\text{-}NH\text{-}CHR'\text{-}COOH$$

Cation binding studies

Cation binding constants were determined (see Table 1) as previously described.[11] Three groups of structures were studied. These are *N*-substituted derivatives of aza-15-crown-5, aza-18-crown-6, and 4,13-diaza-18-crown-6. All of the derivatives except **12** and **19** have an amide sidearm macroring linkage. The macroring nitrogen electrons are involved in resonance with the carbonyl group. This places a formal positive charge on nitrogen, diminishing the Lewis basicity of the macroring hole. This difference was apparent in the observed binding constants. The Na^+ and K^+ cation binding constants ($\log_{10} K_S$) for $\langle$18N$\rangle CH_2COOEt$ ($\langle$18N$\rangle$Gly-OEt) are, respectively, 4.10 and 4.03.[11] The corresponding values for $\langle$18N$\rangle COCH_2$-$NHCOOCH_2Ph$ ($\langle$18N$\rangle$Gly-Z) are 2.63 and 2.14. These reductions in binding strength (by nearly 100-fold for K^+) reflect the formal positive charge of the >N–CO– group and the increased rigidity of the macroring. When two amino acids are present in the sidearm, cation binding strength is also enhanced relative to amides. Thus, $\langle$18N$\rangle$-GlyGly-OMe has the following stability constants: Na^+, 3.50; K^+, 4.53.[11]

Transport studies

Transport of amino acids through cell membranes *in vivo* is a very discriminating process due to the highly selective recognition properties of natural carriers. For certain types of mammalian cells, this process depends upon the extracellular Na^+ concentration which implies that the amino acid and sodium cation use a common carrier, "the symporter".[15] Recently, Tsukube[4j] reported that an artificial symporter analog mediated transport of potassium Z-amino acid carboxylates through a $CHCl_3$ membrane. The *N*,*N*-didecyl derivative of **3**, dibenzo-18-crown-6 and dibenzo-[2.2.2]-cryptand were the potassium selective symporter models. In this model system, however, the symporters recognized the metal cation rather than the amino acid selectively. Differences in transport rates observed for a series of Z-amino and dipeptide carboxylates were probably the consequence of different substrate lipophilicities.[4a]

Because both peptide side arms and crown macrorings

Scheme 2 Formation of **ATD**-lariats

Cpd. No.	Reaction	Conditions
4	⟨15N⟩-H + Z-Gly-OH → ⟨15N⟩-Gly-Z	$Ph_3P/CCl_4/Et_3N$
5	⟨15N⟩-H + Z-Phg-OH → ⟨15N⟩-Phg-Z	$Ph_3P/CCl_4/Et_3N$
6	⟨15N⟩-Gly-OH → → ⟨15N⟩-Gly-Phg-Z	(1) H_2, Pd/C, CH_3OH (2) Z-PhgOH, $Ph_3P/CCl_4/Et_3N$
7	⟨15N⟩-Gly-Phg-Z → ⟨15N⟩-GlyPhg-Bz	(1) H_2, Pd/C, CH_3OH (2) PhCOCl, C_5H_5N
8	⟨18N⟩-H + Z-Gly-OH → ⟨18N⟩-Gly-Z	DCC, CH_2Cl_2
9	⟨18N⟩-H + Z-Phg-O-pNP → ⟨18N⟩-Phg-Z	See experimental
10	⟨18N⟩-Gly-Z(**8**) → ⟨18N⟩-Gly-Phg-Z	(1) H_2, Pd/C, CH_3OH (2) Z-PhgOH, $Ph_3P/CCl_4/Et_3N$
11	⟨18N⟩-Gly-Phg-Z → ⟨18N⟩-Gly-Phg-Bz	(1) H_2, Pd/C, CH_3OH (2) PhCOCl, C_5H_5N
13	H-⟨N18N⟩-H + 2Z-Gly-OH → Z-Gly-⟨N18N⟩-Gly-Z	$Ph_3P/CCl_4/Et_3N$ or DCC, CH_2Cl_2
15	H-⟨N18N⟩-H + 2Z-Phg-OH → Z-Phg-⟨N18N⟩-Phg-Z	$ClCOCH_2CHMe_2$/*N*-methylmorpholine
16	**13** → Z-Phg-Gly-⟨N18N⟩-Gly-Phg-Z	(1) H_2, Pd/C, CH_3OH (2) Z-PhgOH, $Ph_3P/CCl_4/Et_3N$
17	**16** → Bz-Phg-Gly-⟨N18N⟩-Gly-Phg-Bz	(1) H_2, Pd/C, CH_3OH (2) PhCOCl, C_5H_5N
18	**15** → Z-Phg-Phg-⟨N18N⟩-Phg-Phg-Z	(1) H_2, Pd/C, CH_3OH (2) Z-PhgOH, $Ph_3P/CCl_4/Et_3N$

Table 1 Cation binding by one and two-sidearm lariat ethers

		Log K_S in MeOH[a]	
Cpd. No.	*Structure*	Na^+	K^+
1	aza-15-crown-5[b]	1.70	1.60
2	aza-18-crown-6[b]	2.69	3.98
3	diaza-18-crown-6[c]	<1.5	<1.5
4	$PhCH_2OCONHCH_2CO⟨N15⟩$[d](Z-Gly[e])	1.7	1.9
5	$PhCH_2OCONHCHPhCO⟨N15⟩$(Z-Phg)	1.8	2.1
6	$PhCH_2OCONHCHPhCONHCH_2CO⟨N15⟩$(Z-PhgGly)	ND	ND
7	$PhCONHCHPhCONHCH_2CO⟨N15⟩$(Bz-PhgGly)	2.37	<1.5
8	$PhCH_2OCONHCH_2CO⟨N18⟩$(Z-Gly)	2.63	2.14
9	$PhCH_2OCONHCHPhCO⟨N18⟩$(Z-Phg)	2.50	2.53
10	$PhCH_2OCONHCHPhCONHCH_2CO⟨N18⟩$(Z-PhgGly)	2.4	2.48
11	$PhCONHCHPhCONCH_2CO⟨N18⟩$(Bz-PhgGly)	2.70	2.19
12	$MeOCOCHPhNHCOCH_2⟨N18⟩$(MeOPhgGly)	ND	ND
13	$PhCH_2OCONHCH_2CO⟨N18N⟩R$(Z-Gly)	INS	INS
14	$PhCH_2OCONHCHPhCO⟨N18N⟩R$(Z-Phg)	<1.5	<1.5
15	$PhCH_2OCONHCHCH_2PhCO⟨N18⟩R$(Z-Phe)	<1.5	<1.5
16	$PhCH_2OCONHCHPhCONHCH_2CO⟨N18N⟩$(Z-PhgGly)	INS	INS
17	$PhCONHCHPhCONHCH_2CO⟨N18N⟩R$(Bz-PhgGly)	INS	INS
18	$PhCH_2OCONHCHPhCONHCHPhCO⟨N18N⟩R$(Z-PhgGly)	1.8	1.8
19	$MeOCOCHPhNHCOCO_2⟨N18N⟩R$(MeOPhgGly)	ND	ND

[a] 25.0 ± 0.1 °C. [b] Izatt, R.M., Bradshaw, J.S., Nielsen, S.A., Lamb, J.D. and Christensen, J.J. (1985). *Chem. Reviews* **85**, 271. [c] Gatto, V.J. and Gokel, G.W. (1984). *J. Am. Chem. Soc.* **106**, 8340. [d] The bracketed symbols refer to crowns. Thus, ⟨N15⟩ and ⟨N18⟩ refer to aza-15-crown-6 and aza-18-crown-6, respectively, with sidearms attached to macroring nitrogen. ⟨N18N⟩R indicates 4,13-diaza-18-crown-6 in which the R group on the right is identical to that illustrated at the left. [e] Z = benzyloxycarbonyl, i.e., $PhCH_2OCO$-. Abbreviations: ND, not determined; INS, insoluble.

are present, the dipeptide derived lariats **10**, **12**, **16**, and **19** might be effective symporter models. They should be able to recognize both the metal cation (macroring interaction) and the amino acid or peptide carboxylate anion by hydrogen bonding and other interactions using peptide recognition sites from lariat side arms.

The results of symport studies are collected in Table 2. Entries 1 and 2 show the initial symport rates of Z-GlyO$^-$K$^+$ and Z-L-PheO$^-$K$^+$ by **10**, **12**, **16**, and **19** determined under experimental conditions identical to those used by Tsukube[4j] for *N*,*N*-bis(decyl)-**3**. The reported symport rates for Z-GlyO$^-$K$^+$ and Z-L-PheO$^-$K$^+$ by *N*,*N*-bis(decyl)-**3** are 0.5 and 8.1 × 10^{-6}

(mol/hour), respectively.[4j] Compounds **12** and **19** transported Z-GlyO$^-$K$^+$ faster by factors of 4 and 8 than did *N,N*-bis(decyl)-3. For more lipophilic Z-L-PheO$^-$K$^+$ the symport rates for **12** and **19** were by factors 5 and 2.5-fold greater than those observed with *N,N*-bis(decyl)-**3**. The **ATD**-lariats **10** and **16** were much less efficient symporters than **CTD**-lariats **12** and **19** in these experiments. Still, **10** was a somewhat better symporter than *N,N*-bis(decyl)-**3** for Z-GlyO$^-$K$^+$. The considerably higher symport rates obtained with **12** and **19** [compared to *N,N*-bis(decyl)-**3**] indicate formation of complexes having additional binding interactions between lariat pendant arms and the substrates.

Further proof of lariat ether sidearm participation in interactions with the symported substrates is provided by experiments with enantiometric Z-amino acid and dipeptide carboxylates (entries 3–10, Table 2). **CTD**-Lariats **12** and **19** exhibited different symport rates for enantiometric substrates. The rate differential was presumably the consequence of interactions between their chiral pendant arms and the bound substrate. The highest enantioselection was measured for **19** and D,L-Z-PhgO$^-$K$^+$ (entries 5 and 6, Table 2) giving the ratio of symport rates sym_L/sym_D of 1.6. It is interesting to note that one-armed lariat **12** showed higher symport rates than two-armed **19** for all substrates examined except Z-GlyO$^-$K$^+$. Nevertheless, **19** exhibited better chiral recognition ability than one-armed **12**. The difference in symport rates between **12** and **19** may be understood in terms of the difference in number of oxygen donors present in **12** compared to **10**. It is well known that crown ethers bind alkali and alkaline earth metal cations more strongly than their equally large aza- or diaza-analogs.[16] The same relation between symport rates and chiral recognition abilities holds for **ATD**-lariats **10** and **16** but both **ATD**-lariats possess much lower symport abilities than **CTD**-lariats **12** and **19**.

Table 2 Transport rates for Z-amino acid and dipeptide K$^+$—Carboxylates through a chloroform membrane

Entry No.	Substrate	*Transport rates* ($\times 10^6 \cdot mol \cdot h^{-1}$) *with carrier* 10	12	16	19
1[a]	Z-GlyOK	1.4	2.1	1.7	4.1
2[a]	Z-L-PheOK	1.4	40.3	0.4	20.5
3	Z-L-PheOK	1.6	27.2	0.2	14.9
4	Z-D-PheOK	1.4	38.1	0.3	9.4
5	Z-L-PheOK	0.9	24.2	0.2	19.9
6	Z-D-PheOK	0.8	25.1	0.2	11.7
7	Z-L-PhgGlyOK	0.1	15.5	0	4.2
8	Z-D-PhgGlyOK	0.1	16.1	0	4.8
9	Z-Gly-L-PhgOK	0	10.1	0	3.6
10	Z-Gly-D-PhgOK	0	14.6	0	2.4

Transport conditions were as follows. Aqueous source phase (Aq I): substrate 0.15 mmol, KCl, 1.0 mmol, 0.05 N NaOH 3 mL for entries 1, 2 or 0.05 N LiOH for entries 3–10. Membrane: $CHCl_3$, 8 mL, carrier, 0.0372 mmol. Aqueous receiving phase (Aq II): distilled water, 9 mL. a. Transport rates for Z-GlyOK and Z-L-PheOK and *N,N*-didecyl-4,13-diaza-18-crown-6 reported in reference 4j were 0.5 and 8.1, respectively.

Table 3 Chemical shifts and chemical shift differences for Z-PheOK, 12 and 19 NH protons

Host	*Guest*	*Chemical shifts (ppm, δ) and chemical shift differences (Δδ)* δ_{Host}	$\Delta\delta_{Host}$	δ_{Guest}	$\Delta\delta_{Guest}$
none	Z-PheOH	—	—	5.13	0
12	none	8.44	0	—	—
12	Z-PheOK	9.61	1.17	6.09	0.96
19	none	8.47	0	—	—
19	Z-PheOK	9.10	0.63	5.79	0.66
		9.27	0.80		

Z-Dipeptide carboxylates (entries 7–10) were transported less readily than Z-amino acid carboxylates by both **12** and **19** and especially by **10** and **16**. Isomeric Z-PhgGlyO$^-$K$^+$ and Z-GlyPhgO$^-$K$^+$ were clearly discriminated by **12** and **19**; the ratio of symport rates for Z-D-PhgGlyO$^-$K$^+$, Z-Gly-D-PhgO$^-$K$^+$ and **19** was approximately 2 (entries 8 and 10, Table 2). The stereoselection of enantiomeric dipeptide carboxylates by **19** was slightly better for Z-GlyPhgO$^-$K$^+$ than for Z-PhgGlyO$^-$K$^+$ the chiral center of which is more remote from the carboxylate end.

Generally, **19** symports L-substrates more readily than D-substrates. The same preference also holds for Z-amino acid carboxylates without the phenyl group. The measured symport rates ($\times 10^{-6}$ mol/hour) for enantiomeric Z-AlaO$^-$K$^+$ and Z-ValO$^-$K$^+$ were: Z-L-AlaO$^-$K$^+$ 0.82; Z-D-AlaO$^-$K$^+$ 0.58; Z-L-ValO$^-$K$^+$ 3.26; Z-D-ValO$^-$K$^+$ 2.65. However, the opposite holds for one-armed **12** and two-armed **16** where a slight preference for D-substrates was noted.

^{1}H-NMR studies of symporter-substrate complexes

The results of symport studies suggested participation of lariat dipeptide side arms in binding of Z-amino acid carboxylates by **12** and **19**. To learn more about the nature of binding interactions within such complexes we examined the ^{1}H-NMR spectra of lariat-substrate equimolar mixtures in relation to spectra of pure components. Generally, the spectra of these mixtures showed broadened resonances for both components differently shifted with respect to their positions in spectra of pure components. The broadening of the macrocycle methylene resonances is especially pronounced upon complexation with K$^+$ ion. The largest shifts were observed for the amide NH protons of **12**, **19** and the substrate NH proton (Table 3).

For **12**, NH was shifted downfield by 1.17 ppm ($\Delta\delta$),

while the NH protons of **19** showed two doublets shifted downfield by 0.80 and 0.63 ppm in the presence of equimolar Z-D-PheO$^-$K$^+$. These large downfield shifts suggest formation of hydrogen bonds between lariat pendant arms and the guest. In addition the substrate NH was also shifted downfield compared with its chemical shift in Z-D-PheOH in $CDCl_3$. The temperature dependence of the NH shifts ($\Delta\delta/\Delta T$; 0.05 M for **19** and Z-D-PheO$^-$K$^+$ in $CDCl_3$; temp. range 299–323 K) gave values of 7.1 and 4.4 $\times$ 10^{-3} (ppm/K) for two lariat NH protons while the substrate NH chemical shift remained essentially constant during the temperature variation. The relatively large NH temperature coefficients for **19** are in accord with their involvement in intra-complex hydrogen bonding which underwent changes during the temperature variation.[9b]

On the contrary, the constancy of the substrate NH chemical shift during the temperature variation suggested a buried hydrogen bond.[9b] The additional proof for such a buried position of the substrate NH in **19**-Z-D-PheO$^-$K$^+$ complex was provided by the following experiment. The addition of increasing amounts of **19** to 0.5 M solution of Z-D-PheO$^-$K$^+$ in DMSO-d_6/$CDCl_3$ 1:1.5 (v/v) mixture, caused gradual upfield shifts of the substrate NH proton from 6.47 ppm to 5.99 ppm for **19** to Z-D-PheO$^-$K$^+$ molar ratio variation from 1:0 to 1:1, respectively. This experiment clearly shows that upon complexation the substrate NH changes from a solvent-exposed to a buried position (probably between the lariat's pendant arms) where it is efficiently shielded from solvent molecules.

Titration experiments were performed by adding increasing amounts of each enantiomer of Z-PheO$^-$K$^+$ to 0.1 M solution of **19** in $CDCl_3$ at room temperature. Dependence of lariat **19** and the substrate NH chemical shifts upon the variation of **19** to Z-PheO$^-$K$^+$ molar ratio is shown in Fig. 1. Plotting of **19**-NH chemical shifts *vs.* substrate to lariat molar ratios gave two slightly different curves for D- and L-Z-PheO$^-$K$^+$ (curves a and b, Fig. 1).

For Z-D-PheO$^-$K$^+$ (curve a) the **19**-NH protons were shifted downfield slightly more than for the L-enantiomer (curve b). Both curves reach a plateau after an equivalent of substrate is added to **19**; this suggests a 1:1 complex stoichiometry. The D- and L-substrate NH chemical shifts relationship to the variation of substrate to **19** molar ratio gave two clearly distinct curves c and d (Fig. 1). Here too, the greater downfield shifts for D-substrate NH protons (curve c) were obtained. The more significant difference between curves c and d is likely to be the consequence of greater differences in the local magnetic environments experienced by buried substrate NH's of two diastereomeric

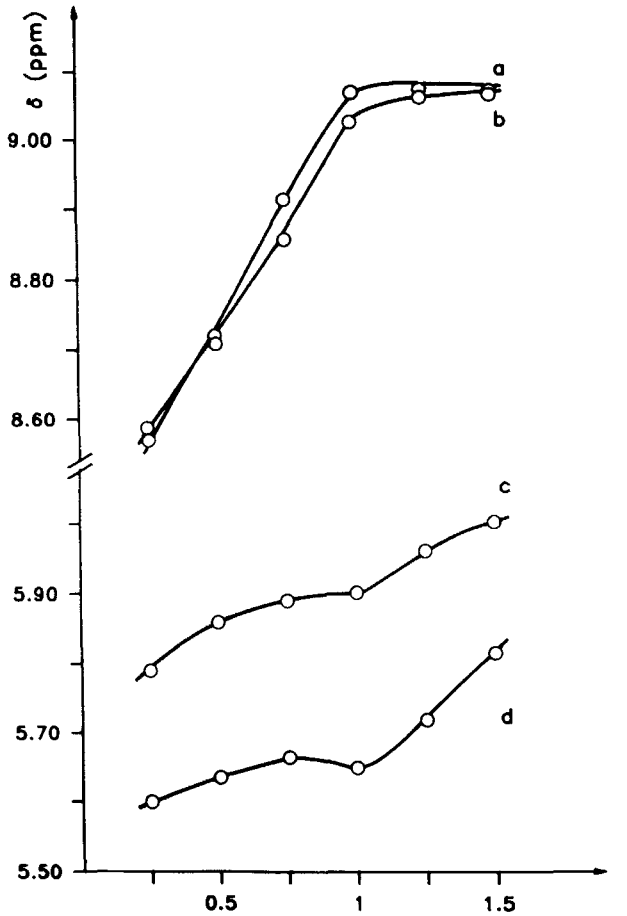


Figure 1 Dependences of lariat **19** (curves **a** and **b**), Z-D-PheO$^-$K (curve **c**) and Z-L-PheO$^-$K$^+$ (curve **d**) NH chemical shifts on the variation of the guest šsć to lariat šlć molar ratio.

complexes **19**-Z-D-PheO$^-$K$^+$ and **19**-Z-L-PheO$^-$K$^+$. In contrast to curves a and b for **19**-NH's showing plateau after 1:1 substrate to **19** molar ratio, the curves c and d of substrate NH's after reaching plateau at 1:1 molar ratio showed an increase of the slope. Apparently, some further binding between the complex and the substrate is possible.

The ^{1}H-NMR spectrum of the equimolar mixture of **19** and racemic Z-PheO$^-$K$^+$ shows the simultaneous existence of two diastereoisomeric complexes (Fig. 2b). In the spectrum, two well separated NH doublets at 5.79 and 5.65 ppm correspond to D- and L-complexed substrate, respectively, as can be concluded from their chemical shifts in complexes **19**-Z-D-PheO$^-$K$^+$ (Fig. 2a) and **19**-Z-L-PheO$^-$K$^+$ (Fig. 2c). In addition the two less well resolved signals of AB spin system corresponding to Z-methylene protons can be observed near 5 ppm. Apparently, lariat **19** functions as a chiral shift reagent able to differentiate the enantiomers of Z-PheO$^-$K$^+$. By lowering the temperature the differences between the diastereoisomeric complexes become more pronounced (Fig. 3). At 25 °C, the two

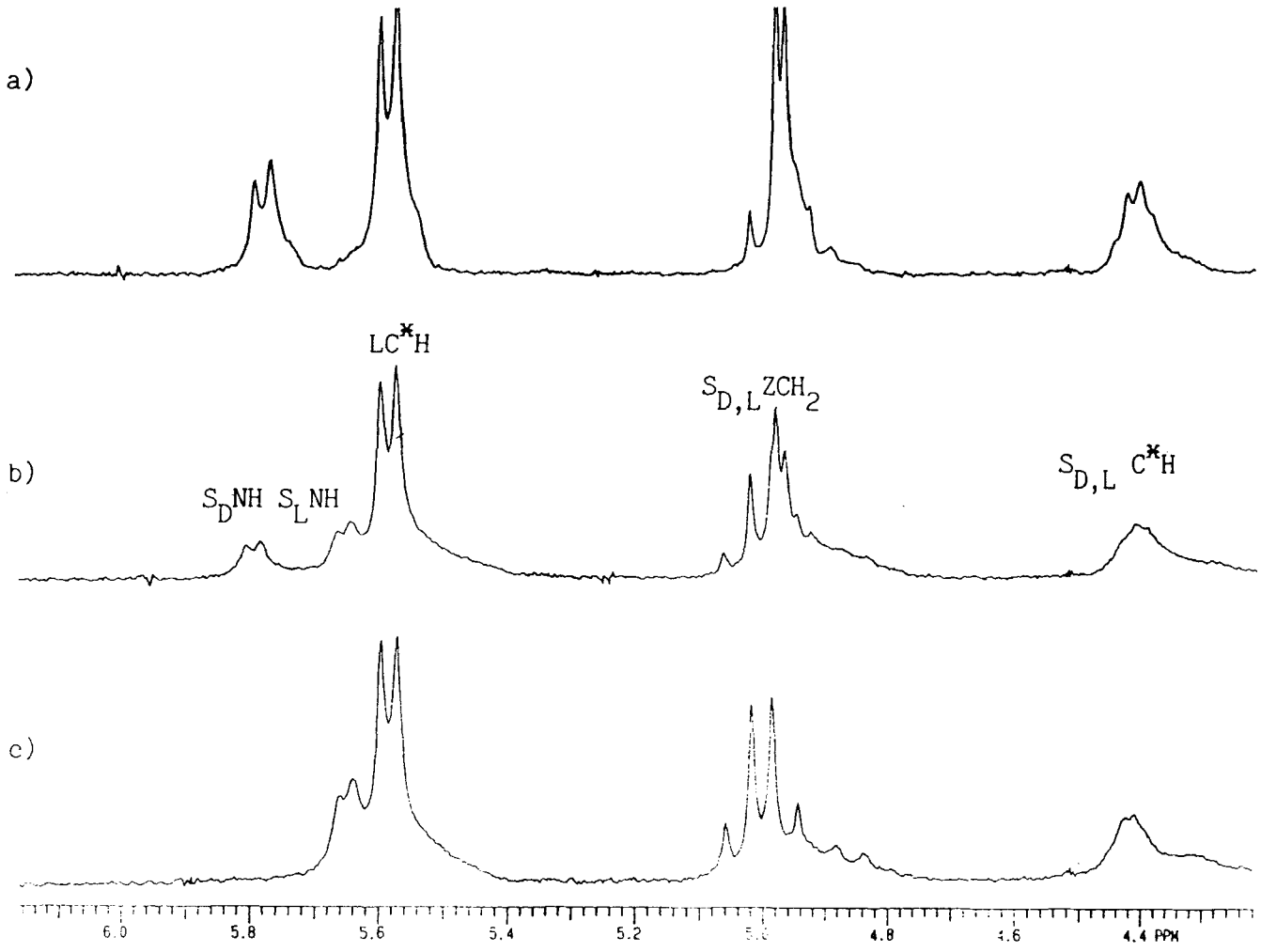


Figure 2 The ^{1}H-NMR spectra ($CDCl_3$; 25 °C; δ4.2–6.0 ppm region shown) of **19** to Z-D-PheO$^-$K$^+$ (a), Z-D,L-PheO$^-$K$^+$ (b) and Z-L-PheO$^-$K$^+$ (c) equimolar mixtures. L and S denote lariat and substrate resonances, respectively.

methine protons of **19** Phg fragments appear as doublet at δ5.57 ppm (Fig. 3a) which at −35 °C splits to two not well resolved doublets centered at δ5.57 and 5.52 ppm (Fig. 3b). Also the singlet of **19** methyl ester protons at δ3.68 gives at −35 °C two singlets at 3.73 and 3.66 ppm.

The structural details of the diastereomeric complexes may only be clarified by additional studies, perhaps involving intramolecular NOE experiments. Nevertheless, the present results with **19** and D- and L-Z-PheO$^-$K$^+$ in combination with symport experiments provide clear evidence that the lariat pendant arms participate in binding of the substrates by forming intra-complex hydrogen bonds.

EXPERIMENTAL SECTION

Melting points (Kofler hot stage) are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 297 spectrophotometer. IR spectral bands are reported in cm^{-1}. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded in $CDCl_3$ (unless otherwise noted) at 100 MHz on a JEOL FX100Q or at 300 MHz on Varian Gemini-300 instrument. Chemical shifts [part per million (δ) downfield from internal Me_4Si] are reported in the following order: chemical shift, spin multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet) and integration. Proton-decoupled ^{13}C-NMR spectra were recorded at 25.2 MHz on a JEOL FX100Q; chemical shifts (δ, ppm downfield from Me_4Si) are reported. The mass spectrum (MS) was recorded on a Shimadzu GCMS-QP 1000 spectrometer. Masses (m/z) of the molecular ion (M^+), the base peak and some prominent peaks are reported. Optical rotations were determined using an AA-10 automatic polarimeter (Optical Activity Ltd., England).

All reagents were the best grade commercially available. Solvents were purified and dried in the usual

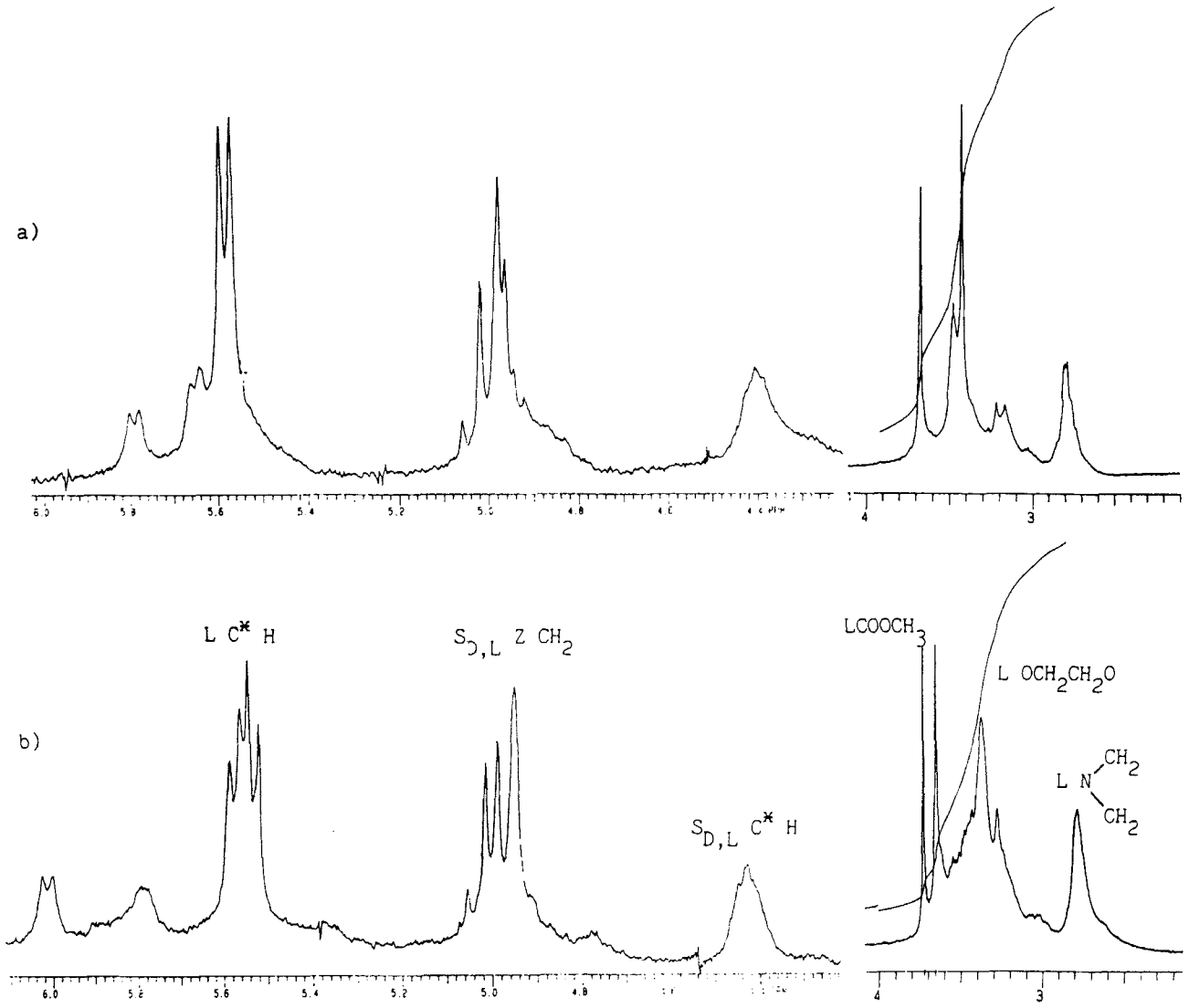


Figure 3 The ^{1}H-NMR spectra ($CDCl_3$; δ2.5–4.0 and 4.2–6.0 ppm regions shown) of the equimolar mixture of **19** and Z-D,L-PheO$^-$K$^+$ at 25 °C (a) and −35 °C (b). L and S the same as on Fig. 2.

manner before use. Chromatographic columns were filled with Merck silica gel 60(70–230 mesh). Merck Fertigplatten F-254 were used for TLC.

N-[(N'-benzyloxycarbonyl)-glycyl]aza-15-crown-5(4)

To a solution of dry MeCN (5.8 mL) and Z-GlyOH (610 mg; 2.89 mmol) was added triphenylphosphine (910 mg; 3.47 mmol) CCl_4 (0.28 mL; 2.89 mmol), aza-15-crown-5 (1,633 mg; 2.89 mmol) and triethylamine (0.4 mL, 2.89 mmol) and the reaction mixture stirred at room temperature for 18 hours. The precipitated salts were removed by filtration and acetonitrile by rotary evaporation. The residue was purified by column chromatography (EtOAc) giving 942 mg (80%) of 4, heavy oil, R_f = 0.26 (EtOAc). IR (film): 3410, 3320, 2840, 1720, 1650, 1470, 1120. ^{1}H-NMR: 3.61, m, 20H; 4.09, s, 2H; 5.11, s, 2H; 5.78, br s, 1H; 7.34, s, 5H. ^{13}C-NMR: 42.72, 49.49, 66.65, 69.02, 70.09, 70.32, 70.54, 71.45, 127.94, 128.39, 136.57, 156.21, 168.63. *Anal.* Calcd. for $C_{20}H_{30}N_2O_7$: C, 58.52; H, 7.37; N, 6.83%. Found: C, 58.59; H, 7.45; N, 6.66.

Racemic-N-[(N'-benzyloxycarbonyl)-α-phenyl-glycyl)]aza-15-crown-5(5)

To a stirred and cooled (−15 °C) solution of (rac)-Z-α-phenylglycine (PhgOH) (1.16 g, 4.06 mmol) in dry THF (20 mL) was added *N*-methylmorpholine (0.45 mL, 4.06 mmol) and isobutyl chloroformate (0.53 mL, 4.06 mmol). The reaction mixture was stirred for 4 min at −15 °C. Then, a solution of aza-15-crown-

5 (1.0 g, 4.56 mmol) in THF (2.0 mL) was added. After 1 min the cooling bath was removed and the reaction mixture then stirred for 1 hour. The solvent was removed by rotary evaporation and the residue dissolved in a mixture of $CHCl_3$ (66 mL), EtOAc (34 mL) and H_2O (4 mL). The aqueous layer was removed and the organic layer was washed with 1 M HCl (4 mL) and twice with water (8 mL). After drying and removal of solvents, the residue was Flash-chromatographed ($CHCl_3$/EtOAc, 4:1) giving the mixture of **5** and *N*-(isobutyloxycarbonyl)aza-15-crown-5 in ratio of 2:1, respectively as calculated from the ^{1}H-NMR spectrum. Pure **5**, 1.12 g (57%) was obtained by repeated column chromatography as a heavy oil, R_f = 0.45 (EtOAc). IR (film): 3310, 3050, 1712, 1645, 1512, 1110, 1118. ^{1}H-NMR: 3.57, m, 20H; 5.05, d-AB(J_{AB} = 12.3 Hz), 2H; 5.68, d(J = 7.9 Hz), 1H; 6.32, d(J = 7.9 Hz), 1H; 7.31, m, 10H. ^{13}C-NMR: 49.66, 55.81, 66.70, 68.96, 69.47, 70.20, 70.31, 70.60, 71.50, 127.93, 128.39, 128.95, 136.46, 137.93, 155.30, 170.09. *Anal.* Calcd. for $C_{26}H_{34}N_2O_7$: C, 64.18; H. 7.04; N, 5.76%. Found: C, 64.41; H, 7.26; N, 5.64.

N-[(N'-benzyloxycarbonyl)-D-α-phenylglycyl]aza-15-crown-5(6)

Compound **4** (1.71 g, 4.17 mmol) dissolved in abs. EtOH (50 mL) was hydrogenated in a Parr apparatus at 60 psi in the presence of 10% Pd/C catalyst (117 mg) for 3 hours. After removal of the catalyst and the solvent, oily *N*-(glycyl)aza-15-crown-5 was obtained and used immediately in condensation with D-Z-PhgOH (1.07 g, 3.76 mmol) in the presence of triphenylphosphine (1.18 g, 4.5 mmol), CCl_4 (0.36 mL, 3.76 mmol) and Et_3N (0.52 mL, 3.76 mmol) following the procedure described for the preparation of **4**. Purification by column chromatography (EtOAc) and recrystallization from EtOAc/petroleum ether gave 1.26 g (62%) of **6**, m.p. 108–110 °C; R_f = 0.22 (EtOAc). $[\alpha]_D = -71.06$ (c = 0.39, $CHCl_3$). IR (KBr): 3310, 2870, 1690, 1640, 1540, 1250, 1130. ^{1}N-NMR: 3.56, m, 20H; 3.76, m, 2H; 5.07, s, 2H; 5.25, d(J = 6.5 Hz), 1H; 6.19, d(J = 7.0 Hz, 1H; 7.16, br-d 1H; 7.31, s, 5H; 7.35, s, 5H. ^{13}C-NMR: 41.70, 49.60, 49.72, 59.03, 66.99, 68.96, 69.36, 70.15, 70.43, 70.66, 71.62, 127.20, 128.05, 128.50, 129.12, 136.29, 138.15, 155.53, 168.06, 169.36. *Anal.* Calcd. for: $C_{28}H_{37}N_3O_8$: C, 61.86; H, 6.86; N, 7.73%. Found: C, 61.99; H, 6.72; N, 7.65%. Racemic **6** was also prepared by reaction of succinimidic ester of (rac)-α-phenylglycine (662 mg, 1.7 mmol) and *N*-(glycyl)aza-15-crown-5 (469 mg, 1.7 mmol) in dry dimethoxyethane (5 mL). After stirring for 1 hour at room temperature and addition of water (15 mL) the precipitate was collected and recrystallized from MeOH-Et_2O giving 344 mg (37%) of (rac)-**6**.

N-[(N'-benzoyl)-D-α-phenylglycylglycyl]aza-15-crown-5(7)

To an ice-cooled solution of *N*-(D-α-phenylglycylglycyl)-aza-15-crown-5 (224 mg, 0.55 mmol, obtained from **6** by hydrogenolytic removal of the Z-group) in dry pyridine (8 mL) was added benzoyl chloride (0.07 mL, 0.06 mmol). After 2 hours stirring in an ice-bath and 10 hours at room temperature, the pyridine was evaporated, the residue dissolved in $CHCl_3$ (10 mL) and extracted with 0.1 M HCl and water. After removal of solvent, the residue was purified by preparative TLC (silica gel, EtOAc). Recrystallization from an EtOAc/petroleum ether mixture gave 132 mg (47%) of **7**, m.p. 86–87 °C; R_f = 0.65 (acetone). $[\alpha]_D = -25.73$ (c = 0.272, $CDCl_3$). IR (KBr): 3320, 2900, 2860, 1690, 1530, 1120. ^{1}H-NMR: 3.61, m, 20H; 4.14, m, 2H; 5.68, d(J = 6.4 Hz), 1H; 6.92, br-d, 1H; 7.38, m, 8H; 7.59, br-d, 1H; 7.83, m, 2H. ^{13}C-NMR: 41.70, 49.60, 57.45, 68.91, 69.36, 70.03, 70.37, 70.54, 71.50, 127.20, 128.27, 128.44, 128.95, 131.60, 133.92, 138.09, 166.48, 168.06, 169.75. *Anal.* Calcd. for $C_{27}H_{35}H_3O_7$: C, 63.14; H, 6.87; N, 8.18. Found: C, 62.94; H. 7.17; N, 8.38. Compound **7** was also prepared from D-Bz-PhgOH (585 mg, 2.29 mmol) and *N*-(glycyl)aza-15-crown-5 (632 mg, 2.29 mmol, prepared from **4** by hydrogenolytic removal of Z-group) in the presence of triphenylphosphine (7.19 mg, 2.75 mmol), CCl_4 (0.22 mL, 2.29 mmol) and Et_3N (0.32 mL, 2.29 mmol) by the procedure described for **4**. Purification by Flash-chromatography gave 370 mg (32%) of **7**.

N-[(N'-benzyloxycarbonyl)-glycyl]aza-18-crown-6(8)

To an ice cooled solution of Z-GlyOH (188 mg, 0.9 mmol) and aza-18-crown-6 (**2**, 217 mg, 0.82 mmol) in dry CH_2Cl_2 (3.5 mL) was added DCC (186 mg, 0.9 mmol). After stirring for 1 hour in ice and 16 hours at room temperature the precipitated dicyclohexylurea was removed by filtration and the filtrate extracted with 1 M HCl, 1 M NH_4OH, and then water. Evaporation of the solvent and purification by column chromatography (EtOAc) gave 255 mg (68%) of oily **8**. IR (film): 3470, 3330, 2880, 1730, 1650, 1250, 1100. ^{1}H-NMR: 3.64, m, 24H; 4.12, d(J = 4.4 Hz), 2H; 5.12, s, 2H; 5.12, s, 2H; 5.87, br, 1H; 7.34, s, 5H. ^{13}C-NMR: 42.66; 46.73; 48.25, 66.59, 69.36, 69.59, 70.43, 70.60, 70.99, 127.88, 128.39, 136.57, 156.15, 168.51. *Anal.* Calcd. for $C_{22}H_{34}N_2O_8$: C, 58.13; H, 7.54; N, 6.16%. Found: C, 58.37; H, 7.78; N, 6.11%. Compound **8** was also prepared using the triphenylphosphine method as described for **4** in 58% yield.

N-[(N'-Benzyloxycarbonyl)-D-α-phenylglycyl]aza-18-crown-6(9)

To dry pyridine (7.0 mL) at 55 °C was added dropwise

a solution of the *p*-nitrophenyl ester of D-α-phenylglycine (1.59 g, 3.92 mmol) and aza-18-crown-6 (**2**) (859 mg, 3.26 mmol) in the same solvent (7.0 mL), under N_2. The reaction mixture was stirred at 55 °C for 48 hours. Pyridine was evaporated, the residue dissolved in $CHCl_3$ and extracted with 1 M aqueous NaOH, 1 M HCl, and H_2O. Evaporation of $CHCl_3$, purification of the residue by column chromatography (EtOAc) and recrystallization from CH_2Cl_2-Et_2O-petroleum-ether gave 1.17 g (67%) of **9**, m.p. 60–62 °C, $R_f = 0.28$ (EtOAc), $[\alpha]_D = -13.59$ (c = 0.48, $CHCl_3$). IR (KBr): 3290, 2850, 1710, 1640, 1530, 1230, 1120. ^{1}H-NMR: 3.59, m, 24H; 5.06, s, 2H; 5.69, d(J = 7.9 Hz), 1H; 6.31, d(J = 7.9 Hz), 1H; 7.36, m, 10H. ^{13}C-NMR: 47.33, 48.47, 55.64, 66.59, 69.24, 70.37, 70.54, 70.65, 70.77, 127.82, 127.93, 128.22, 128.33, 128.89, 136.46, 137.98, 155.31, 169.92. *Anal.* Calcd. for $C_{28}H_{38}N_2O_8$: C, 63.38; H, 7.22; N, 5.28%. Found: C, 63.28; H, 7.01; N, 5.35%.

N-([*N'*-Benzoyloxycarbonyl)-D-α-phenylglycylglycyl]-aza-18-crown-6(10)

Using the procedure described for the preparation of **6**, **4** (455 mg, 1.42 mmol) and Ph_3P (448 mg, 1.71 mmol), CCl_4 (0.14 mL, 1.42 mmol) and Et_3N (0.19 mL, 1.42 mmol) gave, after column chromatography (EtOAc) and recrystallization from MeOH/Et_2O, 502 mg (60%) of 10, m.p. 96–98 °C, $R_f = 0.11$ (EtOAc), $[\alpha]_D = -56.5$ °(c = 0.69, $CHCl_3$). IR (KBr): 3420, 3340, 2880, 1740, 1680, 1630, 1490, 1120, 1100. ^{1}H-NMR: 3.62, m, 24H; 4.13, m, 2H; 5.06, s, 2H; 5.24, d(J = 5.9 Hz), 1H; 6.15, d(J = 6.4 Hz), 1H; 6.83, br, 1H; 7.35, s, 5H; 7.31, s, 5H. ^{13}C-NMR: 41.70, 46.89, 48.42, 59.03, 66.98, 69.52, 70.60, 70.61, 71.22, 127.20, 128.05, 128.44, 129.06, 136.34, 138.26, 155.47, 168.00, 169.30. *Anal.* Calcd. for $C_{30}H_{41}N_3O_9$: C, 61.31; H, 7.03; N, 7.15%. Found: C, 61.03; H, 7.24; N, 7.04%. Compound **10** has also been prepared by condensation of **4** (483 mg, 1.51 mmol) and Z-D-PhgOH (431 mg, 1.51 mmol) in the presence of DCC (344 mg, 1.66 mmol) following the procedure described for **8**. Purification by column chromatography (EtOAc) and recrystallization gave 389 mg (44%) of **10**. The triphenylphosphine method using dipeptide Z-D-PhgOH (343 mg, 1.30 mmol), Ph_3P (411 mg, 1.56 mmol), CCl_4 (0.13 mL, 1.30 mmol) and Et_3N (0.18 mL, 1.30 mmol) gave 438 mg (56%) of **10**.

N-([*N'*-Benzoyl)-D-α-phenylglycylglycyl]aza-18-crown-6(11)

Using the procedure described for the preparation for **10**, compound **11** was prepared from **4** (766 mg, 2.39 mmol), Bz-D-PhgOH (611 mg, 3.39 mmol), triphenylphosphine (752 mg, 2.87 mmol), CCl_4 (0.23 mL, 2.39 mmol) and triethylamine (0.33 mL, 2.39 mmol). Purification by column chromatography (EtOAc) and recrystallization from EtOAc/petroleum ether mixture gave 509 mg (38%) of **11**, m.p. 95–97 °C, $R_f = 0.47$ (acetone), $[\alpha]_D = -17.5$ (c = 0.40, $CHCl_3$). IR (KBr): 3440, 2870, 1630, 1520, 1110. ^{1}H-NMR: 3.62, m, 24H; 4.26, m, 2H; 6.58, d(J = 6.4 Hz), 1H; 6.93, br, 1H; 7.38, m, 8H; 7.58, d(J = 6.4 Hz), 1H; 7.88, m, 2H. *Anal.* Calcd. for $C_{29}H_{39}N_3O_8$: C, 62.49; H, 7.05; N, 7.54%. Found: C, 62.61; H, 7.30; N, 7.46%.

N-(O-Methyl-D-α-phenylglycylglycyl)aza-18-crown-6(12)

The previously reported[10c] procedure for the preparation of similar **CTD**-lariats was followed. From D-PhgOMe·HCl (7.44 g, 36.93 mmol) and chloroacetyl chloride (2.9 mL, 36.93 mmol) in the presence of Na_2CO_3 was obtained, after purification by column chromatography (CH_2Cl_2) and recrystallization from EtOAc/hexane, 7.62 g (86%) of *N*-(chloroacetyl-D-α-phenylglycine methyl ester (**20**), m.p. 55–56 °C, $R_f = 0.46$ (CH_2Cl_2), $[\alpha]_D = -153.7°$ (c = 0.40, $CHCl_3$). IR (KBr): 3310, 2960, 1740, 1660, 1540, 1220, 1180. ^{1}H-NMR: 3.75, s, 3H; 4.06, s, 2H; 5.56, d(J = 7.0 Hz), 1H; 7.37, s, 5H; 7.52, br, 1H. ^{13}C-NMR: 42.32, 52.93, 56.60, 127.20, 128.78, 129.06, 135.78, 165.40, 170.66. *Anal.* Calcd. for $C_{11}H_{12}ClNO_3$: C, 54.89, H, 5.03; N, 5.82%. Found: C, 55.11; H, 5.24; N, 5.63%. Reaction of aza-18-crown-6 (**2**) (1.2 g, 4.56 mmol) and **20** (1.16 g, 4.79 mmol) in the presence of Na_2CO_3 (0.53 g, 5.02 mmol) gave after purification by column chromatography (5% MeOH in CH_2Cl_2) 1.19 g (56%) of oily **12**, $R_f = 0.32$ (5% MeOH in CH_2Cl_2) $[\alpha]_D = -24.8°$, (c = 0.58, $CHCl_3$). IR (film): 3320, 2880, 1750, 1680, 1510, 1455, 1350, 1250, 1110, 950, 840. ^{1}H-NMR: 2.84, t, 4H; 3.29, s, 2H; 3.63, m, 20H; 3.73, s, 3H; 5.59, d(J = 7.9 Hz), 1H; 7.27, s, 5H; 8.47, br, 1H. ^{13}C-NMR: 52.48, 55.08, 56.26, 59.25, 69.47, 70.26, 70.82, 127.54, 128.33, 128.83, 136.51, 171.21, 171.72. MS: $M^+ + 1$, 469 (calcd. 468); 277, 276 base peak; 120, 100, 70, 56.

The syntheses of compounds **13**, **14**, and **15** have been reported by Bogatskii[4g] using DCC condensation of Z-GlyOH, Z-PhgOH and Z-PheOH and diaza-18-crown-6 (**3**). In order to compare the DCC and triphenylphosphine methods with respect to possible racemization, we prepared compound **14** by the latter method (yield 73%) and found $[\alpha]_D = -103.8°$ (c = 0.13, EtOH). Compound **14** prepared by DCC condensation[4g] had $[\alpha]_D = -73.7°$ (same solvent and concentration) indicating greater racemisation by the latter method.

N,N'-[(*N''*-Benzyloxycarbonyl)-D-α-phenylglycylglycyl)-4,13-diaza-18-crown-6(16)

Compound **16** has been prepared by triphenylphosphine

procedure from *N,N'*-bis(glycyl)-4,13-diaza-18-crown-6 (obtained from **13** by hydrogenolytic removal of the Z groups) (172 mg, 0.46 mmol), Z-D-PhgOH (261 mg, 0.91 mmol), Ph_3P (287 mg, 1.09 mmol), CCl_4 (0.1 mL, 0.91 mmol) and Et_3N (0.13 mL, 0.91 mmol), yield 238 mg (57%), m.p. 148–149 °C, $R_f = 0.13$ (EtOAc), $[\alpha]_D = -73.2°$ (c = 0.64, $CHCl_3$). IR (KBr): 3310, 2930, 2870, 1730, 1700, 1640, 1540, 1140, 1110, 1100. ^{1}H- and ^{13}C-NMR spectra of **16** are solvent dependent, in $CDCl_3$, some resonances and especially the macroring *N*-methylene resonances in ^{13}C-NMR spectra are doubled due presumably to hindered rotation about arm to macroring amide bond juncture. ^{1}H-NMR: 3.56, m, 24H; 4.26, m, 4.33, d, 4.48, d, 2H; 5.02, 5.05, s, 4H; 5.35, m, 2H; 6.20, d, 6.43, d, 2H; 7.34, m, 20H; 7.66, br, 2H. (DMSO-d_6): 3.50, m, 24H; 3.96, m, 4H; 5.03, s, 4H; 5.35, d(J = 8.5 Hz), 2H; 7.30, m, 20H; 7.69, br, 2H: 8.13, br, 2H. ^{13}C-NMR: 41.47, 41.70, 47.85, 48.42, 49.21, 49.54, 58.91, 66.81, 69.18, 69.30, 69.64, 70.54, 70.82, 72.23, 127.26, 127.99, 128.39, 128.72, 128.95, 136.40, 138.09, 138.43, 155.47, 167.83, 168.60, 169.90. ^{13}C-NMR(DSMO-d_6): 46.84, 47.57, 58.12, 65.63, 68.68, 69.41, 70.03, 127.43, 127.65, 127.82, 128.22, 128.38, 137.02, 138.71, 155.58, 168.17, 169.86. *Anal.* Calcd. for $C_{48}H_{58}N_6O_{12}$: C, 63.28; H, 6.41; N, 9.22%. Found: C, 63.11; H, 6.64; N, 9.42%.

N,N'-[(*N''*-Benzoyl)-D-α-phenylglycylglycyl]-4,13-diaza-18-crown-6(17)

Compound **17** has been prepared as **16** from *N,N'*-bis(glycyl)-4,13-diaza-18-crown-6 (361 mg: 0.97 mmol), Bz-D-PhgOH (498 mg, 1.95 mmol), triphenylphosphine (614 mg, 2.34 mmol), CCl_4 (0.19 mL, 1.95 mmol) and triethylamine (0.27 mL, 1.95 mmol). Obtained after recrystallization from chloroform/ether 258 mg (31%) of **17**, m.p. 186–189 °C, $R_f = 0.68$ (acetone): $[\alpha]_D = -3.1°$ (c = 0.82, $CHCl_3$). IR (KBr): 3320, 2900, 1640, 1520, 1480, 1110. ^{1}H- and ^{13}C-NMR in $CDCl_3$ showed the presence of two isomers due to the hindered rotation about the amide bond (see **16**). ^{1}H-NMR: 3.53, m, 24H; 4.09, m, 4H; 5.82, m, 2H; 7.07, br, 2H; 7.38, m, 16H; 7.73, m, 4H; 8.05, br, 2H. ^{13}C-NMR: 41.53, 47.96, 48.53, 49.32, 49.72, 57.45, 57.84, 69.24, 69.69, 70.60, 70.93, 72.34, 127.37, 127.71, 128.10, 128.27, 128.44, 128.71, 129.01, 131.43, 133.91, 138.15, 138.54, 166.30, 166.59, 168.00, 168.56, 168.96, 170.09, 170.37. *Anal.* Calcd. for $C_{46}H_{54}N_6O_{10}$: C, 64.92; H, 6.40; N, 9.88%. Found: C, 65.12; H, 6.50; N, 10.16%.

N,N'-[(*N''*-Benzyloxycarbonyl)-D-α-phenylglycyl-D-α-phenylglycyl]-4,13-diaza-18-crown-6(18)

Compound **18** was prepared from **14** (1.50 g; 1.88 mmol), Z-D-PhgOH (1.08 g, 3.77 mmol) and DCC (788 mg, 3.77 mmol) using the procedure described for the preparation of **8**. Purification by column chromatography (EtOAc/petroleum ether 3:1) and recrystallization from a CH_2Cl_2/Et_2O/petroleum ether mixture gave 710 mg (36%) of **18**; m.p. 90–92 °C: $R_f = 0.41$ (EtOAc): $[\alpha]_D = -74.4°$ (c = 0.82, $CHCl_3$). IR (KBr): 3290, 2940, 2860, 1710, 1640, 1540, 1120. ^{1}H-NMR: 3.36, m, 24H, 5.00, s, 4H; 5.24, br, 2H; 5.76, m, 2H; 7.31, m, 30H. ^{13}C-NMR: 47.40, 48.30, 54.12, 58.46, 66.70, 69.29, 70.43, 126.92, 127.09, 127.54, 127.82, 128.27, 128.72, 128.87, 136.28, 136.79, 137.30, 137.98, 138.26, 155.36, 168.79, 169.30, 169.46. *Anal.* Calcd. for $C_{60}H_{66}N_6O_{12}$: C, 67.78; H, 6.26; N, 7.90%. Found: C, 67.78; H, 6.48; N, 7.85%.

N,N'-bis(O-Methyl-D-α-phenylglycylglycyl)-4,13-diaza-18-crown-6(19)

Compound **19** was prepared from 4,13-diaza-18-crown-6 (**3**) (1.12 g, 4.25 mmol), and **20** (991 mg, 9.35 mmol) following a published procedure.[11c] Yield: 1.72 g (60%), m.p. 109–119 °C after recrystallization from MeOH/Et_2O solvent mixture, $R_f = 0.29$ (5% MeOH/CH_2Cl_2), $[\alpha]_D = -81.5°$ (c = 0.75, $CHCl_3$). IR (KBr): 3460, 3320, 2960, 2880, 1750, 1690, 1510, 1130. ^{1}H-NMR: 2.81, t, 8H; 3.26, s, 4H; 3.46, m, 16H; 3.70, s, 6H; 5.57, d(J = 7.6 Hz), 2H; 7.35, s, 10H; 8.45, br, 2H. ^{13}C-NMR: 52.37, 54.85, 56.03, 59.20, 69.24, 70.48, 127.31, 128.22, 128.67, 136.34, 171.04, 171.39. MS: M^+ 672 (calcd. 672): 480, 289 base peak, 275, 162, 144, 91.

Symport experiments

Symport experiments have been performed using the cell dimensions and experimental conditions reported by Tsukube[4j] so the results could be directly compared. The aqueous source phase was prepared by dissolving 0.15 mmol of substrate and 223.8 mg (3.00 mmol) of KCl in 3 mL of 0.05 M NaOH or LiOH (Table 2). The $CHCl_3$ membrane phase (8 mL) contained 3.72×10^{-2} mmol of a lariat ether carrier. The aqueous receiving phase was distilled water (9 mL). The symport rates have been calculated from the initial rates of appearance of substrate anion in the aqueous receiving phase which were determined spectrophotometrically. The molar extinction coefficients (ε) determined at $\lambda = 257$ nm for substrates studied are: Z-PhgO$^-$K$^+$ (400.0); Z-GlyO$^-$K$^+$ (166.1); Z-PheO$^-$K$^+$ (353.5); Z-AlaO$^-$K$^+$ (204.0); Z-ValO$^-$K$^+$ (192.0); Z-GlyGlyO$^-$K$^+$ (209.0); Z-GlyPheO$^-$K$^+$ (442.9); Z-PhgGlyO$^-$K$^+$ (483.0); Z-GlyPhgO$^-$K$^+$ (419.5).

^{1}H-NMR titration experiments

Z-D or L-PheOH (29.9 mg; 0.1 mmol) was dissolved

in aqueous 0.1 M KOH (1.0 mL) and evaporated to dryness. The residue was dissolved in dry MeOH and the solvent evaporated to dryness again. Then, the salt was dissolved in exactly 2 mL of dry MeOH. A portion of this solution (0.25 mL) was pipetted into a $CHCl_3$ solution (2 mL) of **19** (33.6 mg; 0.05 mmol), evaporated to dryness, and additionally dried using a vacuum pump. The residue was then dissolved in $DCCl_3$ (0.5 mL) and the ^{1}H-NMR spectrum determined. This procedure was repeated after each addition of the substrate until a substrate to **19** molar ratio of 1.5 was reached.

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NOTES AND REFERENCES

1. *Preliminary communication:* Žinić, M., Frkanec, L., Škarić, V., Trafton, J. and Gokel, G.W. (1990). *J. Chem. Soc., Chem. Commun.* 1726.
2. (a) Lehn, J.M. (1988). *Angew. Chem. Int. Ed. (Engl)* 1726. (b) Cram, D.J. (1988). *Ibid.* **27,** 1009.
3. (a) Gutsche, C.D., Iqbal, M., Nam, K.S., See, K. and Alam, I. (1988). *Pure Appl. Chem.* **60,** 483. (b) Diederich, F. (1988). *Angew. Chem. Int. Ed. (Engl)* **27,** 362. (c) Rebek, Jr. J. (1987). *Science* **235,** 1478. (d) Gokel, G.W., Arnold, K.A., Delgado, M., Echeverria, L., Gatto, H.J., Gustowski, D.A., Hernandez, J., Kaifer, A.E., Miller, S.R. and Echegoyen, L. (1988). *Pure Appl. Chem.* **60,** 476. (f) Odell, B., Reddington, M.V., Slawin, A.M.Z., Spencer, N., Stoddart, J.F. and Williams, D.J. (1988). *Angew. Chem. Int. Ed. (Engl)* **28,** 753. (h) Sheridan, R.E. and Whitlock, Jr. H.W. (1988). *J. Am. Chem. Soc.* **110,** 4071.
4. (a) Rebek. Jr. J., Askew, B., Nemeth, D. and Parris, K. (1987). *J. Am. Chem. Soc.* **109,** 2432. (b) Behr, J.-P. and Lehn, J.-M. (1973). *Ibid.* **95,** 6108. (c) Behr, J.-P., Lehn, J.-M. and Vierling, P. (1982). *Helv. Chim. Acta* **65,** 1835. (d)Newcomb, M., Helgeson, R.C. and Cram, D.J. (1974). *J. Am. Chem. Soc.* **96,** 7367. (e) Knobler, C.B., Gaeta, F.C.A. and Cram, D.J. (1988). *J. Chem. Soc., Chem. Commun.* 330. (f) Bogatskii, A.V., Lukyanenko, N.G., Nazarova, N.Yu., Basok, S.S., Lobach, A.V. and Kuzimina, T.V. (1984). *Dokl. Akad. Nauk. SSSR.* 633. (g) Lukyanenko, N.G., Bogatskii, A.V., Basok, S.S., Ostrovskaya, L.K., Nazarova, N.Yu. and Karpienko, L.P. (1984). *Zh. Org. Khim.* **20,** 1580. (h) Sunamoto, J., Iwamoto, K., Mohri, Y. and Kominato, T. (1982). *J. Am. Chem. Soc.* **104,** 5502. (i) Tsukube, H. (1981). *Tet. Lett.* **22,** 3981. (j) Tsukube, H. (1982). *J. Chem. Soc. Perkin Trans. I,* 2359. (k) Prelog, V. (1987). *Pure Appl. Chem.* **50,** 893. (l) Echavarren, A., Galan, A., Lehn, J.-M., deMendoza, J. (1989). *J. Am. Chem. Soc.* **111,** 4994. (m) Aoyama, Y., Asakawa, M., Yamagishi, H.T. and Ogoshi, H. (1990). *J. Am. Chem. Soc.* **112,** 3145. (n) Chang, S.-K., Hwang, H.-S., Son, H., Youk, J. and Kang, Y.S. (1991). *J. Chem. Soc., Chem. Commun.* 217.
5. (a) Hamilton, A.D., Pant, N. and Muehldorf, A. (1988). *Pure Appl. Chem.* **60,** 533. (b) Askew, B., Ballester, P., Buhr, C., Jeong, K.S., Jones, S., Parris, K., Williams, K. and Rebek, Jr. J. (1989). **111,** 1082. (c) Hosseini, M.W., Blacker, A.J. and Lehn, J.-M. (1988). *J. Chem. Soc., Chem. Commun.* 596.
6. (a) Bergman, N. and Schmidtchen, F.P. (1988). *Tet. Lett.* **29,** 6235. (b) Blackburn, G.M., Tatcher, G.R.J., Hosseini, M.W. and Lehn, J.-M. (1987). *Tet. Lett.* **28,** 2779. (c) Hosseini, M.W. and Lehn, J.-M. (1988). *J. Chem. Soc., Chem. Commun.* 397. (d) Huft, B.J., Askew, B., Duff, J. and Rebek, Jr. J. (1988). *J. Am. Chem. Soc.* **110,** 5908. (e) Whitlock, B.J., Whitlock, Jr. H.W. (1988). *Tet. Lett.* **29,** 6047. (f) Diederich, F., Schuermann, G. and Chao, I. (1988). *J. Org. Chem.* **53,** 2744. (g) Koga, K. and Sasaki, S. (1988). *Pure Appl. Chem.* **60,** 539. (h) Cram, D.J., Yuk-Sun Lam, P. and Ho, S.P. (1986). *J. Am. Chem. Soc.* **108,** 839. (i) Lehn, J.-M. and Sirlin, C. (1987). *J. Chem. Soc., Chem. Commun.* 949.
7. Schultz, G.E. and Schirmer, R.H. (1979). "*Principles of Protein Structure*", Springer-Verlag: New York.
8. (a) Shinkai, S., He, G., Matsuda, T., Hamilton, A.D. and Rosenzwig, H.S. (1989). *Tet. Lett.* **30,** 5895. (b) Chang, S.-K. and Hamilton, A.D. (1988). *J. Am. Chem. Soc.* **110,** 1318. (c) Bell, T.W. and Lin, J. (1988). *J. Am. Chem. Soc.* **110,** 3673. (d) Kelly, T.R. and Maguire, M.P. (1987). *J. Am. Chem. Soc.* **109,** 6549. (e) Kim, M.S. and Gokel, G.W. (1987). *J. Chem. Soc., Chem. Commun.* 1686. (f) Medina, J.C., Li, C., Bott, S.G., Atwood, J.L. and Gokel, G.W. (1991). *J. Am. Chem. Soc.* **113,** 366. (g) Gokel, G.W., Medina, J.C. and Li, C. (1991). *SYNLETT* 677.
9. (a) Sugawara, N., Stevens, E.S., Bonora, G.M. and Toniolo, C. (1980). *J. Am. Chem. Soc.* **102,** 7044. (b) Stevens, E.S., Sugawara, N., Bonora, G.M. and Toniolo, C. (1980). *Ibid.* **102,** 7050.
10. It was recently shown that a tetraamidic selector containing two L-phenylalanine units spaced by a 3,6,9-oxadecanoyl bridge was able to complex and recognize amino acid ester derivatives by formation of intramolecular hydrogen bonds; Spisni, A., Corradini, R., Marchelli, R. and Dossena, A. (1989). *J. Org. Chem.* **54,** 684.
11. White, B.D., Arnold, K.A. and Gokel, G.W. (1987). *Tet. Lett.* 1749. (b) White, B.D., Fronzek, F.R., Gandour, R.D. and Gokel, G.W. (1987). *Ibid.* 1753. (c) White, B.D., Mallen, J., Arnold, K.A., Fronczek, F.R., Gandour, R.D., Gehrig, L.M.B. and Gokel, G.W. (1989). *J. Org. Chem.* **54,** 937.
12. (a) "Synthese von Peptiden II", Houben-Weyl, Methoden der Organischen Chemie, G. Thieme Verlag, 4th Ed., 1974. Stuttgart. (b Leister, N.A. and Tarbell, D.S. (1958). *J. Org. Chem.* **23,** 1152.
13. Appel, R., Baumer, G. and Sturer, W. (1975). *Chem. Ber.* **108,** 2680.
14. Goldschmidt, S. and Gupta, K.K. (1965). *Chem. Ber.* **98,** 2831.
15. Ring, K. (1970). *Angew. Chem.* **82,** 343.
16. Weber, E. and Vogtle, F. (1981). *Topics in Current Chem.* **21,** Springer-Verlag: Berlin.