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Lipophilic Aminophosphonates and Their Calix[4]arene Derivatives: Synthesis and Membrane Transport of Biorelevant Species

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ABSTRACT: *Many new lipophilic α-aminophosphonates including macrocyclic (on calix[4]arene platform) and chiral ones were synthesized by the Kabachnik-Fields reaction. Some of them were examined as carriers for membrane transport of biorelevant species such as hydroxy, amino acids, and amino alcohols. Transport measurements showed that these compounds exhibited remarkable efficiency and selectivity of the transport of very hydrophilic substances across lipophilic membranes. The structures of α-aminophosphonate-substrate complexes were investigated by spectral and theoretical methods.* © 2000 John Wiley & Sons, Inc. Heteroatom Chem 11:518–527, 2000

INTRODUCTION

The main aspect investigated in supramolecular chemistry is the construction of highly selective receptors for molecular recognition and membrane transport of ionic, neutral, and zwitterionic species [1]. Recently, much attention has been drawn to the complexation of carboxylates, carboxylic acids, and their derivatives, such as hydroxy, amino acids, and small peptides [2,3]. Liquid membranes are frequently used to evaluate complexation and transport properties of novel receptors and to mimic important cell processes. In this case, the transport is facilitated by carrier interaction with transported substrate and formation of a lipophilic complex in the membrane phase. But a strong solvation of very hydrophilic species in water makes the membrane transport through a lipophilic membrane difficult because an enormous energy cost has to be paid to desolvate such substrates prior to complexation and extraction into the membrane phase. Although many receptors for binding ammonium, carboxyl, and carboxylate moieties have been reported [2,3], only a few successful examples of membrane transport of hydrophilic hydroxy and zwitterionic amino acids by artificial carriers are known [4,5].

The carboxyl and hydroxyl groups of hydroxy acids as well as carboxylate and ammonium moieties of amino acids are expected to act as binding sites for hydrogen bonding and electrostatic interactions. So, the receptors for such compounds have to contain either two hydrogen bond acceptor groups in the first case or hydrogen bond donor and acceptor groups in the second case. For these reasons, we have chosen α-aminophosphonates as carriers for the membrane transport of such compounds. Actu-

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ally, α-aminophosphonates can bind the ammonium, hydroxy, carboxyl moieties due to H-bonding with the phosphoryl group or/and nitrogen lone pair and bind the carboxylate group due to interaction with the N–H bond. Moreover, these interaction sites are complimentary to each other.

RESULTS AND DISCUSSION

A series of the new lipophilic α-aminophosphonates containing various substituents, R^1, R^2, R^3 and R^4, were synthesized by the Kabachnik-Fields reaction [6] in 62–95% yields.

The structures of the obtained compounds were confirmed by NMR and IR spectroscopy. The nature of substituents, phosphorus chemical shifts, and yields of α-aminophosphonates are summarized in Table 1.

To estimate quantitatively the ability of aminophosphonates to act as carriers for hydroxy and amino acids, their enthalpies of specific interaction with protonodonor and protonoacceptor solvents were determined. For this purpose the solution enthalpies of three aminophosphonates **1, 2, 3** in chloroform, $\Delta H_s^{A_i/CHCl_3}$ (pronodonor), pyridine $\Delta H_s^{A_i/C_5H_5N}$ (protonoacceptor), and tetrachloromethane $\Delta H_s^{A_i/CCl_4}$ (inert solvent) were measured by the calorimetric method and are presented in Table 2. From these data, the enthalpies of specific interaction $\Delta H_{sp}^{A_i}$ were calculated using Equations 1 (for chloroform) and 2 (for pyridine) [7,8].

$$\Delta H_{sp.}^{A_i} = \Delta H_s^{A_i/CHCl_3} - \Delta H_s^{A_i/CCl_4} \quad (1)$$

$$\Delta H_{sp.}^{A_i} = (\Delta H_{solv}^{A_i/C_5H_5N})_{exp} - (\Delta H_{solv}^{A_i/C_5H_5N})_{nonsp} \quad (2)$$

where $(\Delta H_{solv}^{A_i/C_5H_5N})_{exp}$ is the experimental solvation enthalpy and $(\Delta H_{solv}^{A_i/C_5H_5N})$ is the enthalpy of nonspecific solvation of aminophosphonates with pyridine calculated according to Equation 3 [9,10].

$$(\Delta H_{solv}^{A_i/C_5H_5N})_{nonsp.} = 17.0 + 1.01\ MR_D \quad (3)$$

One can see that all values are very negative. So, α-aminophosphonates act as rather strong protonodonors and protonoacceptors in H-bonding interactions. It is necessary to emphasize that these values are essentially more negative than the H-bonding energy of chloroform with the protonoacceptor molecules such as dipropyl methylphosphonate (−14.3 kJ/mol) [10], cyclohexylamine (−15.0 kJ/mol), and pyridine with protonodonor molecules such as ethanol (−15.9 kJ/mol) and pyrrole (−20.9 kJ/mol) [11]. The obtained results allow us to conclude that α-aminophosphonates are very promising host molecules for binding of hydroxy and amino acids.

The less negative value of the specific interaction enthalpy of compound **3** with pyridine indicates that N-alkyl aminophosphonates are less effective for binding of electron-rich groups (for example, carboxylate moieties) than N-benzyl derivatives. This can be explained by the electron acceptor effect of the benzyl group on NH-bond acidity. N-phenyl substituted α-aminophosphonates are significantly stronger prononodonor molecules than N-alkyl or N-benzyl derivatives. However, they are not hydrolytically stable and easily undergo hydrolysis at water contact [12]. For these reasons, N-benzyl derivatives of aminophosphonates only were used as carriers in our investigation of the membrane transport.

In this work, polymer-supported liquid membranes (SLM) impregnated with the carrier's solution in *o*-nitrophenyl *n*-octyl ether ($\varepsilon_r = 24$) were used. It is more convenient to use SLM in which the organic phase containing the carrier is immobilized in a microporous polymer film that separates two aqueous phases. The geometry of the cell is described in detail in the Experimental section.

The influence of the α-aminophosphonate structure on the α-hydroxy acid membrane transport has been investigated for glycolic acid [13,14]. Measured fluxes of glycolic acid across a membrane and carriers used are presented in Table 3 where X is the substituent at the α-carbon atom and RO is the alkoxyl radical at the phosphorus atom. The carrier concentration was 1 mol L^{-1}. The comparison of fluxes with and without carrier indicates that introduction of α-aminophosphonates in the membrane phase leads to an increase of glycolic acid flux. This effect may be associated with the complex formation of glycolic acid with the α-aminophosphonate in the

TABLE 1 Phosphorus Chemical Shifts and Yields of α-Aminophosphonates.

N	*R*[1]	*R*[2]	*R*[3]	*R*[4]	δ^a (*ppm*)	*Yields* (%)
1	CH_2Ph	$-(CH_2)_5-$		C_5H_{11}	29	75
2	CH_2Ph	$-CH_3$	$-CH_3$	C_2H_5	32	83
3	$CH(CH_3)_2$	$-CH_3$	$-CH_3$	C_2H_5	31	79
4	CH_2Ph	$-(CH_2)_4-$		C_5H_{11}	31	88
5	CH_2Ph	$-CH_3$	$-CH_3$	C_5H_{11}	34	87
6	CH_2Ph	$-(CH_2)_4-$		$CH_2CH(C_2H_5)C_4Hg$	33	70
7	CH_2Ph	$-(CH_2)_5-$		$CH_2CH(C_2H_5)C_4Hg$	32	72
8	CH_2Ph	$-CH_3$	$-CH_3$	$CH_2CH(C_2H_5)C_4Hg$	34	87
9	CH_2Ph	$-CH_3$	-H	$CH_2CH(C_2H_5)C_4Hg$	28	67
10	CH_22Ph	-H	-H	$CH_2CH(C_2H_5)C_4Hg$	28	73
11	CH_2Ph	C_4H_9	-H	$CH_2CH(C_2H_5)C_4Hg$	28	62
12	CH_2Ph	CH_3	CH_3	$C_{10}H_{21}$	30	90
13	CH_2Ph	-H	-H	$C_{10}H_{21}$	30	57
14	CH_2Ph	$-(CH_2)_5-$		$c-C_6H_{11}$	28	76
15	CH_2Ph	H	H	$c-C_6H_{11}$	28	78

[a]Chemical shifts ^{31}P NMR ($CDCl_3$).

TABLE 2 Enthalpies of Solution and Specific Interactions of α-Aminophosphonates with Some Solvents (298K, kJ mol^{-1}).

		Compounds		
	Solvent	**1**	**2**	**3**
$\Delta H^{A/S}_{S.}$	CCl_4	16.0	15.4	9.5
	$CHCl_3$	−18.8	−18.4	−20.1
	C_5H_5N	−9.5	−8.4	−2.6
$\Delta^{A}_{SP.}$	$CHCl_3$	−34.8	−34.2	−29.6
	C_5H_5N	−22.6	−21.0	−8.7

membrane phase. In order to detect α-aminophosphonate-hydroxy acid complexes, NMR and IR investigations were performed [15].

NMR chemical shifts are very indicative and characteristically depend on the complex structures. The proton signals of aminophosphonates in the complexes are shifted to lower field. The low-field shifts of the different protons are in the range of 0.1–0.2 ppm (Figure 1). On the other hand, an upfield shift was observed for CH protons of a hydroxy acid. The formation of a positive charge on the aminophosphonate and a negative charge on the hydroxy acid may explain these changes. IR spectra confirm the presence of ammonium NH_2^+ (νNH_2^+ 2250–2700 cm^{-1}, δNH_2^+ 1575 cm^{-1}) and carboxylate ($\nu_{as}COO^-$ 1600–1625 cm^{-1}, $\nu_s COO^-$ 1395 cm^{-1}) groups in the complex. The shifted band of the phosphoryl group (60 cm^{-1}) as well as the broad band νOH at 3300–3430 cm^{-1} indicates the formation of H-bonds between the aforementioned groups.

Moreover, the structure of one crystal complex of *O,O*-diethyl-1-methyl-1-(*i*-propylamino)ethylphosphonate with mandelic acid was investigated by the X-ray method [15]. A solid complex between α-aminophosphonates and α-hydroxy acids forms due to a proton transfer from the carboxyl group to the nitrogen atom of the aminophosphonate and the resultant electrostatic interactions of the forming counterions (structure **A**). Besides, the formation of the hydrogen bonds array with the participation of phosphoryl, hydroxyl, ammonium, and carboxylate groups is observed.

A

The obtained kinetic results indicate that two factors have an influence on the flux. These are the carrier lipophilicity and steric repulsion of α-alkyl and N-benzyl substituents in the complexes. Actually, there is no general dependence of the transport rate of glycolic acid on the aminophosphonate lipophilicity. But the comparison of the structurally similar compounds (see pairs **4/6, 1/7, 8/12, 9/11,** and **10/13**) allows us to establish that the carrier possessing more of the larger alkyl substituents, either in the alkoxy groups or at the α-carbon, performs a more intensive transport of glycolic acid across the membrane. Analogous dependencies have been observed previously in Refs. [16–18], where they have

been attributed to an increase in the distribution coefficient of the more lipophilic complexes in the water–organic phase system.

The steric effects of the substituents located in the immediate vicinity of the binding centers also substantially affect the ability of α-aminophosphonates to be involved in complexation. Based on the structure of the coordination centers of the complex (see structure A), the number and the nature of alkyl groups at the α-C atom of the aminophosphonates were varied. It was found that the order of a carrier's efficiency is as follows: α-unsubstituted > α-monosubstituted > α-disubstituted. The smallest rate of transport is observed in the case of aminophosphonates containing cyclic substituents at the α-C atom, and the flux through the membrane decreases as the size of the ring decreases. Introduction of α-unsubstituted aminophosphonates leads to the increase of flux by a factor of 20–50.

In order to understand the structural details of the α-substitution influence on flux, the PM3 simulation of complex structures between aminophosphonates and glycolic acid was performed [14]. The arrangement of the binding centers in the starting model of the complex corresponds to structure A. The result of this optimization for α-unsubstituted aminophosphonates is presented in Figure 2.

Three principal observations should be pointed

TABLE 3 Initial Glycolic Acid Fluxes through a Supported Liquid Membrane Impregnated by Carrier

Carrier	X	R	$J \cdot 10^6$, mol h^{-1} cm^{-2}
No carrier	-	-	0.6
4	C (five-membered ring)	C_5H_{11}	2.4
6	C (five-membered ring)	$CH_2CH(C_2H_5)C_4H_9$	3.7
1	C (six-membered ring)	C_5H_{11}	4.0
7	C (six-membered ring)	$CH_2CH(C_2H_5)C_4H_9$	4.5
14	C (six-membered ring)	c-C_6H_{11}	3.6
8	H_3C, CH_3	$CH_2CH(C_2H_5)C_4H_9$	7.2
12	H_3C, CH_3	$C_{10}H_{21}$	9.6
9	H, CH_3	$CH_2CH(C_2H_5)C_4H_9$	11.0
10	H, H	$CH_2CH(C_2H_5)C_4H_9$	13.5
11	H, C_4H_9	$CH_2CH(C_2H_5)C_4H_9$	24
15	H, H	c-C_6H_{11}	1.4
13	H, H	$C_{10}H_{21}$	30

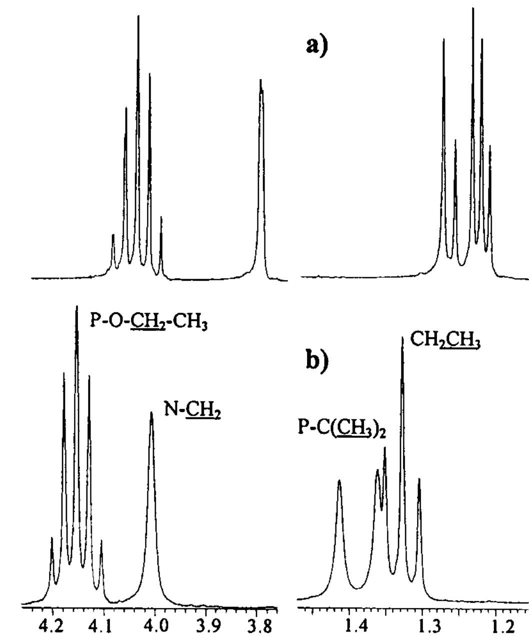

FIGURE 1 Portions of the ^{1}H NMR spectrum (CD_2Cl_2) of (a) α-aminophosphonate **2,** (b) complex of **2** with mandelic acid.

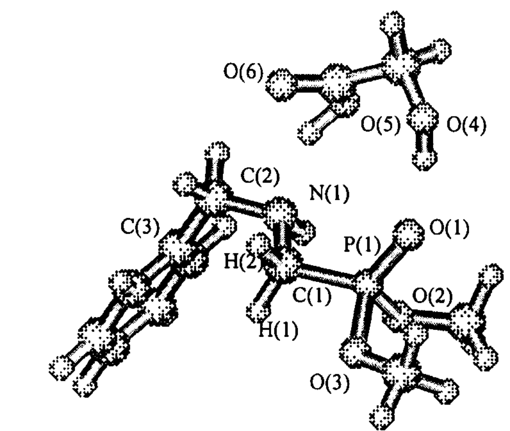

FIGURE 2 Geometry of the complex of α-unsubstituted aminophosphonate (analog of compounds **10** and **13**) with glycolic acid optimized by the PM3 method.

out. First, the PM3 simulations are in reasonable agreement with X-ray, IR, and NMR data on the investigated complex structures. There are two attractive intermolecular interactions leading to complex formation: H-bonding of the carboxylate moiety with nitrogen and of the hydroxyl group with the phosphoryl group.

Second, in the conformation that is most favorable for hydrogen bonding, the Ph group (the C(2)–C(3) bond) of the N-benzyl fragment of the aminophosphonate is in the *anti* position with respect to the lone electron pair of the N(1) atom, and correspondingly, with respect to the glycolic acid. The rotation of the Ph group about the C(2)–N(1) bond, that is, the transition to the *gauche* conformation relative to the lone electron pair of the N atom, leads to the cleavage of the 0(5)–H · · · N(1) hydrogen bond.

Third, in this conformation one hydrogen atom at the α-carbon is close to the phenyl ring. The distance between the C(3) and H(1) atoms is only 2.82 A (C(3)–H(2) 3.51 A). The half-thickness of the phenyl substituent is 1.85 A, and the covalent radius of the hydrogen atom is 1.20 A [12]. Consequently, the distance between the C(3) and H(1) atoms is smaller than the sum of their van der Waals radii. So, attachment of a bulky alkyl substituent at this position will cause a very strong repulsion between the aforementioned groups and thus destabilization of the optimal complex conformation. As a result, the complexation and extraction constants, as well as the fluxes, of glycolic acid across SLM have to be decreased [19], and these computational data result.

The significance of steric hindrance minimization of complex formation is reflected in the rates of glycolic acid transport mediated by carrier **14** and **15** containing cyclohexoxy substituents at the phosphorus atom. The attachment of bulky groups to α-unsubstituted aminophosphonate **15** leads to a considerable decreasing of the flux, but had no effect in the case of the α-disubstituted derivative **14.** According to the semiempirical calculations, an α-unsubstituted aminophosphonate adopts an optimal conformation for a two-point interaction with a hydroxy acid. So, any steric obstacles (at the α-carbon or the phosphorus atoms) destabilizes the complex. In carrier **14,** the cyclic substituent at the α-carbon atom doesn't permit, in principle, an effective interaction with both binding centers of the substrate, and, for this reason, the appearance of additional steric hindrance can not have an influence on the complex stability and flux.

The carrier-mediated transport of other biorelevant species (amino acids and amino alcohols) was studied for D,L-valine and ethanolamine hydrochloride [13,20,21]. The carrier was the aminophosphonate **4** (see the structure at the top of Table 3), and the obtained data are summarized in Table 4. The linear dependence of substrate fluxes on the substrate concentration in the source phase indicates that the limiting stage of membrane transfer is substrate extraction into the membrane. So, the dependence of the flux across the membrane on the stability of the substrate-carrier complexes can be expected.

Examination of Table 4 shows that α-aminophosphonate addition into the membrane increases the flux of the amino acids but has no effects on the flux of the amino alcohol hydrochloride. The data of blank experiments (without a carrier in the membrane) are presented in the brackets. Such behaviour may be rationalized in terms of the different stability of the complexes forming into the membrane. The possible arrangement of binding centers leading to the two-points interaction may be presented by structures **B** (for amino acids) and **C, D** (for amino alcohol hydrochlorides).

B **C** **D**

Both substrates contain the ammonium NH_3^+ cation which will preferably form a hydrogen bond with the electron-rich P=O group of α-aminophosphonates. The comparison of the specific interaction energies of typical protonodonors (phenol, methanol, and chloroform) with phosphonate and amine shows that the former are stronger hydrogen bond acceptors. For example, the complexation constants

TABLE 4 The Initial Fluxes of Organic Substrates through a SLM (298K)

	J^a (J^b) mol h^{-1} cm^{-2}	
C, mol L^{-1}	D,L-Valine	Ethanolamide hydrochloride
1.00	—	$3.8 \cdot 10^{-5}$ ($2.0 \cdot 10^{-5}$)
0.50	$2.4 \cdot 10^{-4}$ ($<10^{-7}$)	$1.0 \cdot 10^{-5}$ ($9.9 \cdot 10^{-6}$)
0.10	$7.3 \cdot 10^{-5}$ ($<10^{-7}$)	$2.2 \cdot 10^{-6}$ ($1.8 \cdot 10^{-6}$)
0.05	$2.5 \cdot 10^{-5}$ ($<10^{-7}$)	$1.1 \cdot 10^{-6}$ ($8 \cdot 10^{-7}$)

[a]SLM contains 1 M solution of α-aminophosphonate **4**.
[b] Blank experiment.

of phenol are equal to 310 L mole^{-1} with *O,O*-diethylmethylphosphonate $CH_3P(O)(OEt)_2$ and 96 L mole^{-1} with diethylamine [11]. Moreover, the basicity of the nitrogen atom in α-aminophosphonates is decreased compared with that of the aliphatic secondary amines by a factor of 10^4–10^5 [22]. So, the ability of the nitrogen atom to act as a hydrogen bond acceptor is sharply reduced as well.

However, in any case, this type of interaction cannot cause a discrimination of complexation constants. So, the complex stability differences come from another moiety of the molecules, namely, the carboxyl and hydroxyl groups. Obviously, the hydrogen bonding energy of the NH bond with a charged carboxylate COO^- is significantly larger than with an uncharged hydroxyl (OH) group. For this reason, complex **B** can be more stable than **C** or **D.** Moreover, the transport experiments (Table 4) give evidence that interactions of amino alcohol hydrochlorides, either of the OH···N or NH···O types, are not sufficiently strong to form a stable lipophilic complex which can be extracted into the membrane phase. Only two charged centres, COO^- and $NH^{+}{}_3$, in the zwitterionic form of amino acids can effectively interact with α-aminophosphonates.

Biological receptors consist of large linear molecules that form three-dimensional structures and can selectively recognize guests due to multipoint specific interactions with them. A possible strategy for the synthesis of artificial three-dimensional receptors can consist of the functionalization of available natural or synthetic preorganized macrocycles by suitable functional groups, which are properly arranged in the receptor so as to achieve effective interaction with the substrate in the complex.

Linear aminophosphonates can provide two-point interactions with the substrate. Obviously, the higher substrate selectivity of transport can be achieved by the formation of an additional (third) binding site which is able to recognize the substituents at the α-carbon atom of the amino and hydroxy acids due to electrostatic, van der Waals, hydrophobic attractive interactions or even to steric repulsion.

It is well known that organic molecules (alkanes, aromatics, etc.) can be incorporated into the cavity of a calix[4]arene with the formation of stable, host-guest complexes [23,24]. So, a calixarene cavity may act as a third recognition center of a side-chain substituent. To increase the strength of the host-guest interaction, and to achieve more selectivity, the new macrocyclic receptors **17** and **19** containing α-aminophosphonate fragments at the lower and upper rim of the calix[4]arene were synthesized [25,26].

The synthesis of **17** was performed by the Kabachnik-Fields reaction of 1,3-bis-(2-aminoethoxy)-calix[4]arene **16** with diethylphosphite in refluxing acetone (the carbonyl compound acting also as the solvent) (Scheme 1).

Compounds **19** were synthesized analogously from **18** (Scheme 2). All macrocyclic receptors were isolated by column chromatography (silica gel). According to ^{1}H NMR spectroscopy, both macrocycles adopt a cone conformation.

Obtained receptors were examined as carriers for the transport of aromatic amino acids. For comparison, the same experiments were performed with the α-aminophosphonate **8,** which is not anchored on a calixarene platform. The results are summarized in Table 5.

Unlike the membrane transport of glycolic acid, the hydrophilicity of the amino acids doesn't have an effect on the flux. There is no dependence between the flux J and log$P$ values that is a quantitative measure of hydrophilic/lipophilic properties of organic compounds [27]. For example, the transport of tryptophane (the most lipophilic of these amino acids) exhibits lower flux values through the organic membrane, whereas higher fluxes of the hydrophilic histidine were observed.

The linear aminophosphonate **8** does not demonstrate an essential transport selectivity. The higher selectivity was established for phenylalanine relative to tryptophane (flux ratio is 7.3). Compound **8,** with practically the same rates, transports other investigated amino acids. The attachment of aminophosphonate moieties to the lower and upper rim of the calix[4]arene lead to the different changes in the rate and selectivity of amino acid transport. Calixarene **17** (substituted in the lower rim) and **8** are similar in efficiency as carriers, with the exception of histidine which shows a surprisingly high transport rate through the hydrophobic membrane. It seems likely that the imidazolyl side chain of histidine can also present the additional sites for interaction with aminophosphonate units of **17.**

Unlike carrier **17,** the molecular cavity of calixarene **19** (substituted in the upper rim) can participate in complexation and recognize the aromatic side chains of the amino acids. As a result, the selectivity of membrane transport for some amino acids is enhanced. For example, **19** transports phenylalanine 40 times faster than tryptophane (flux ratio for **8** = 7.3, for **17** = 4.9). This proves that (1) the molecular cavity of calixarene **19** is involved in the complexation and (2) the three-point interaction of amino acid (due to carboxylate, ammonium groups, and side chain) with the carrier leads to enhancement of transport efficiency and selectivity.

SCHEME 1

SCHEME 2

The approach of multipoint interaction of aminophosphonates was further applied to enantioselective transport. Using a chiral *O,O*-diamyl-1-methyl-1-[*N*-(1-bornyl)amino]ethylphosphonate as the carrier, notable enantioselectivity of hydroxy and amino acid transport was achieved [28,29]. The best result was found for membrane transport of mandelic acid. Coefficient of chiral discrimination was 4.5. The reason for chiral discrimination may be steric repulsion between the bulky chiral substituents in the α-aminophosphonate and the transported compound.

There are three sites where chirality can be introduced. These are alkoxy groups at the phosphorus atom, the α-carbon atom, and the substituent at the nitrogen atom. α-Aminophosphonates with one, two, or three chiral fragments can be synthesized using commercial chiral reagents such as (*R*) and (*S*) α-methylbenzylamine, (1*S*)-*endo*-(−)-borneol, (*R*)-(+)-bornylamine, (+)- and (−)-menthol, and some others [30,31]. So, these compounds are good models for evaluation of transport enantioselectivity–structure relationships.

EXPERIMENTAL

Synthesis of α-Aminophosphonates (General Procedures)

A. Freshly distilled amine (24 mmol), dialkyl phosphite (22 mmol), and a carbonyl compound (24 mmol) were stirred in a 25 mL flask equipped with a magnetic stirrer and a reflux condenser. The reaction mixture was heated with stirring on a water bath at 60°C for 4 hours. The excess of the reagents was removed in vacuo. The mixture was chromatographed twice on a column with silica gel L100/160 (10:1 $CHCl_3$ − PriOH mixture as the eluent).

B. Freshly distilled amine (22 mmol), dialkyl phosphite (22 mmol), and a carbonyl compound (22 mmol) were placed in a 50 mL flask equipped with

TABLE 5 Initial Amino Acid Fluxes across a SLM (J 10^6 $mol \cdot cm^{-2} \cdot h^{-1}$)[a],b

Amino Acid	logP[c]	Carrier 8	17	19
D,L-Phenylalanine	−1.45	4.8	3.1	9.7
D,L-Dihydroxyphenylalanine	—	2.9	3.0	5.7
D,L-Tyrosine	−2.11	1.7	2.8	4.5
D,L-Histidine	−2.85	2.4	6.6	5.1
D,L-Tryptophane	−1.16	0.66	0.63	0.24

[a]Amino acid concentration in source phase, 10^{-3} M.
[b]Blank (without carrier) fluxes of the all amino acids were less than 10^{-7} $mol \cdot cm^{-2} \cdot h^{-1}$.
[c]Octanol/water partition coefficient [27]; more negative value of log$P$ corresponds to less lipophilic compound.

a magnetic stirrer and a reflux condenser and dissolved in 0.1% TsOH solution in toluene (10 mL). The reaction solution was heated with stirring for 12 hours in a water bath at 80°C. The excess of the reagents and the solvent were distilled in vacuo, and the residue was chromatographed twice on a column with silica gel L100/160 (10:1 $CHCl_3$ − PriOH mixture as the eluent).

C. A suspension of paraformaldehyde (22 mmol) in benzene (10 mL) was placed in a 50 mL two-necked flask equipped with a magnetic stirrer, a Dean–Stark trap, a reflux condenser, and a dropping funnel, and brought to reflux. Then, freshly distilled amine (22 mmol) was added dropwise. After the precipitate disappeared, the dialkyl phosphite (22 mmol) was added to the reaction mixture. The mixture was refluxed for 12 hours, and the solvent was removed in vacuo.

D. A mixture of macrocyclic amine **16** [32] or **18** [33] (1.4 mmol), dialkyl phosphite (5.6 mmol), and a carbonyl compound (42 mmol) in toluene (10 mL) was stirred at 80°C for 24 hours. The excess of the reagents was removed in vacuo. The mixture was chromatographed twice on a column with silica gel L100/160 (10:1 $CHCl_3$ − PriOH mixture as the eluent).

O,O-Dipentyl-1-(benzylamino)cyclohexylphosphonate **(1).** Procedure **A,** yield 75%. Colorless viscous liquid, d_4^{20} 1.0304, n_D^{20} 1.6832. IR (KBr), ν/cm^{-1}: 990 (P–O–C); 1040, 1060 (P–O–C): 1190 (P–O–R); 1240 (P=O); 1645, 3325 (N–H).

O,O-Diethyl-1-(benzylamino)-1-methylethylphosphonate **(2).** Procedure **B,** yield 83%. Colorless viscous liquid, n_D^{20} 1.4890. IR (KBr), ν/cm^{-1}: 960 (P–O–C); 1020, 1055 (P–O–C): 1160 (P–O–R); 1240 (P=O); 1640, 3325 (N-H). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19–7.32 (m, 5H, Ar-H), 4.18 (dq, $^3J_{PH}$ 7.1 Hz, $^3J_{HH}$ 7.1 Hz, 4H, P-O-C$\underline{H}_2$-CH$_3$), 3.85 (s, 2H, Ph-C$\underline{H}_2$-N), 1.51 (s, 1H, N-$\underline{H}$), 1.35 (d, $^3J_{PH}$ 15.4 Hz, 6H, P-C(C$\underline{H}_3$)$_2$), 1.31 (t, $^3J_{HH}$ 7.7 Hz, 6H, P–O–CH$_2$-C$\underline{H}_3$).

O,O-Diethyl-1-(isopropylamino)-1-methylethylphosphonate **(3).** Procedure **B,** yield 79%. Colorless viscous liquid, n_D^{20} 1.4324. IR (KBr), ν/cm^{-1}: 960 (P–O–C); 1030, 1055 (P–O–C): 1160 (P–O–R); 1240 (P=O); 1640, 3330 (N–H).

O,O-Dipentyl-1-(benzylamino)cyclopentylphosphonate **(4).** Procedure **A,** yield 88%. Colorless viscous liquid, d_4^{20} 1.1102, n_D^{20} 1.6847 IR (KBr), ν/cm^{-1}: 985 (P-O–C); 1040, 1060 (P–O–C); 1170 (P–O–R), 1240 (P=O); 1645, 3310 (N–H).

O,O-Dipentyl-1-(benzylamino)-1-methylethylphosphonate **(5).** Procedure **B,** yield 87%. Colorless viscous liquid, d_4^{20} 1.4887, n_D^{20} 1.6847. IR (KBr), νcm^{-1}: 985 (P–O–C); 1040, 1070 (P–O–C); 1170 (P–O–R), 1240 (P=O); 1645, 3310 (N–H).

O,O-Di-(2-ethylhexyl)-1-(benzylamino)cyclopentylphosphonate **(6).** Procedure **B,** yield 70%. Colorless viscous liquid, d_4^{20} 0.9924, n_D^{20} 1.4884. IR (KBr) ν/cm^{-1}: 975 (P–O–C); 1025, 1050 (P–O–C): 1170 (P–O–R); 1235 (P=O); 1645, 3350 (N–H).

O,O-Di-(2-ethylhexyl)-1-(benzylamino)cyclobexylphosphonate **(7).** Procedure **B,** yield 72%. Colorless viscous liquid, d_4^{20} 0.9932, n_D^{20} 1.4939, IR (KBr) ν/cm^{-1}: 975 (P–O–C); 1025, 1050 (P–O–C); 1170 (P-O-R); 1235 (P=O); 1645, 3350 (N–H).

O,O-Di-(2-ethylhexyl)-1-(benzylamino)-1-methylethylphosphonate **(8)**). Procedure **B,** yield 87%. Colorless viscous liquid, d_4^{20} 0.8497, n_D^{20} 1.6823. IR (KBr) ν/cm^{-1}: 990 (P–O–C); 1025, 1060 (P–O–C); 1170 (P–O–R); 1235 (P=O); 1615, 3250 (N–H).

O,O-Di-(2-ethylhexyl)-1-(benzylamino)ethylphosphonate **(9).** Procedure **A,** yield 67%. Colorless viscous liquid, d_4^{20} 1.0183, n_D^{20} 1.48443. IR (KBr) ν/cm^{-1}: 990 (P–O–C); 1025, 1075 (P–O–C); 1170 (P–O–R); 1230 (P=O); 1610, 3250 (N–H).

O,O-Di-(2-ethylhexyl)-1-(benzylamino)methylphosphonate **(10).** Procedure **C,** yield 95%. Colorless viscous liquid, d_4^{20} 1.0173, n_D^{20} 1.4844. IR (KBr), ν/cm^{-1}: 975 (P–O–C); 1015, 1050 (P–O–C); 1115 (P–O–R); 1250 (P=O); 1625, 3300 (N–H).

O,O-Di-(2-ethylhexyl)-1-(benzylamino)pentylphosphonate **(11).** Procedure **B,** yield 62%. Color-

less viscous liquid, d_4^{20} 0.9646, n_D^{20} 1.4815. IR (KBr) ν/cm^{-1}: 980 (P–O–C); 1025, 1050 (P–O–C); 1115 (P–O–R); 1240 (P=O); 1625, 3320 (N–H).

O,O-Didecyl-1-(benzylamino)-1-methylethylphosphonate **(12).** Procedure **B,** yield 90%. Colorless viscous liquid, d_4^{20} 0.9734, n_D^{20} 1.4794. IR (KBr), ν/cm^{-1}: 975 (P–O–C); 1025, 1050 (P–O–C); 1170 (P–O–R); 1250 (P=O); 1645, 3350 (N–H).

O,O-Didecyl-1-(benzylamino)methylphosphonate **(13).** Procedure **C,** yield 57%. Colorless viscous liquid, d_4^{20} 0.9734, n_D^{20} 1.4794. IR (KBr), ν/cm^{-1}: 975 (P–O–C); 1025, 1050 (P–O–C); 1170 (P–O–R); 1250 (P=O); 1645, 3350 (N–H).

O,O-Di-(cyclohexyl)-1-(benzylamino)cyclohexylphosphonate **(14).** Procedure **B,** yield 76%. Colorless crystals, m.p. 78°C. IR (KBr) ν/cm^{-1}: 980 (P–O–C); 1020, 1060 (P–O–C); 1160 (P–O–R); 1235 (P=O); 1650, 3300 (N–H).

O,O-Di-(cyclohexyl)-1-(benzylamino)-1-methylethylphosphonate **(15).** Procedure **C,** yield 78%. Colorless viscous liquid, n_D^{20} 1.5240. IR (KBr), ν/cm^{-1}: 990 (P–O–C); 1015, 1050 (P–O–C); 1160 (P–O–R); 1235 (P=O); 1615, 3300 (N–H).

5,11,17,23-Tetra-tert-butyl-25,27-dihydroxy-26,28-bis-{N-[1-(O,O-diethyl-phosphoryl)-1-methylethyl]-2-aminoethoxy}calix[4]arene **(17).** Procedure **D,** yield 52%. Amorphous solid. IR (KBr) ν/cm^{-1}: 3100–3400 (OH, NH), 1220 (P=O), 1020, 1060 (P–O–C), 960 (P–O–Et); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (s, 4H, ArH), 6.75 (s, 4H, ArH), 4.34 (d, $^2J_{HH}$ 13.2 Hz, 4H, ArC$\underline{H}_2$Ar), 4.20–4.11 (m, 8H, P-O-C$\underline{H}_2$-CH$_3$), 4.06 (t, $^3J_{HH}$ 5.0 Hz, 4H, O-C$\underline{H}_2$-CH$_2$N), 3.29 (d, $^2J_{HH}$ 13.2 Hz, 4H, ArC$\underline{H}_2$Ar), 3.27 (t, $^3J_{HH}$ 5.0 Hz, 4H, O–CH$_2$–C$\underline{H}_2$N), 1.51 (d, $^3J_{PH}$ 15.4 Hz, 12H, P-C(C$\underline{H}_3$)$_2$), 1.31 (t, $^3J_{HH}$ 7.7 Hz, 12H, P–O–CH$_2$–C$\underline{H}_3$), 1.28 (s, 18H, *t*-Bu), 0.89 (s, 18H, *t*-Bu).

5,11-Bis-{N-[1-(O,O-diethylphosphoryl)-1-methylethyl]-aminomethyl}-25,26,27,28-tetrakispropoxycalix[4]arene **(19).** Procedure **D,** yield 64%. Amorphous solid. IR (KBr) ν/cm^{-1}: 3400–3200 (NH), 1240 (P=O), 1010, 1040 (P–O–C), 960 (P–O–Et); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.94 (s, 4H, ArH), 6.29–6.20 (m, 6H, ArH), 4.41 (d, $^2J_{HH}$ 13.2 Hz, 4H, ArC$\underline{H}_2$Ar), 4.23–4.15 (m, 8H, P–O–C$\underline{H}_2$-CH$_3$), 3.93 (t, $^3J_{HH}$ 8.2 Hz, 4H, O–C$\underline{H}_2$–CH$_2$–CH$_3$), 3.82 (s, 4H, ArC$\underline{H}_2$N), 3.70 (t, $^3J_{HH}$ 8.2 Hz, 4H, O-C$\underline{H}_2$–CH$_2$–CH$_3$), 3.10 (d, $^2J_{HH}$ 13.2 Hz, 4H, ArC$\underline{H}_2$Ar), 1.98–1.80 (m, 8H, O-CH$_2$-C$\underline{H}_2$-CH$_3$), 1.37 (t, $^3J_{HH}$ 6.9 Hz, 12H, P–O–CH$_2$–C$\underline{H}_3$), 1.26 (d, $^3J_{PH}$ 12.6 Hz, 12H, P-C(C$\underline{H}_3$)$_2$), 1.06 (t, $^3J_{HH}$ 7.4 Hz, 6H, O–CH$_2$–CH$_2$–C$\underline{H}_3$), 0.89 (t, $^3J_{HH}$ 7.4 Hz, 6H, O–CH$_2$–CH$_2$–C$\underline{H}_3$).

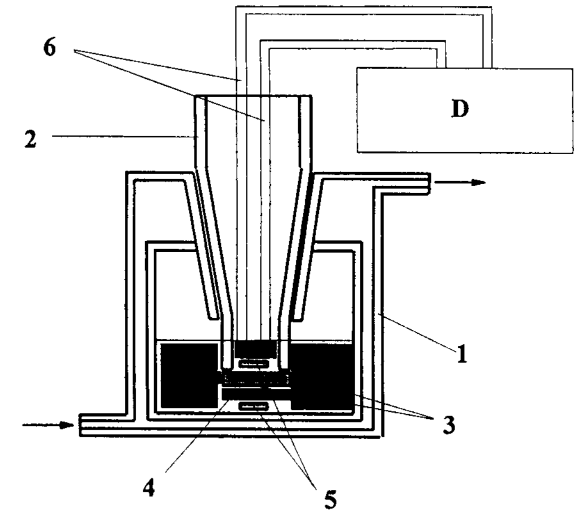

FIGURE 3 Glass thermostated cell for membrane extraction: 1, external thermostated vessel with source phase; 2, internal vessel with receiving phase; 3, ring; 4, supported liquid membrane (SLM); 5, magnetic stir bars; 6, flow-through cell; D, spectrophotometer.

Experiments on Membrane Transport

The transport experiments were performed in a glass temperature-controlled vertical diffusion cell that consists of two concentric glass tubes (Figure 3). The membrane was a microporous Teflon film (Millipore Type FA; thickness 100 μm, pore size 1 μm, porosity 85%) impregnated with a solution of carrier in *o*-nitrophenyl octyl ether. It is positioned on the bottom of the inner tube and separates two aqueous phases. Stirring of both phases was accomplished by a magnetic stirrer (rate of 900 rpm). The outer tube was double-walled for thermostating. The measurements were performed at 298 K. All obtained receptors are hydrolytically stable. ^{31}P NMR analysis of the membrane phase after the transport experiments showed 100% recovery of the carrier.

In the case of UV-vis control of the substrate concentration in the receiving phase a sipper system composed of a peristaltic pump and a quartz flow cell was used. Conductometric control was directly carried out in the cell by the placing of electrodes into the receiving solution.

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