Membrane extraction of organic compounds

2.* Transport of glycolic acid induced by α-aminophosphonates: kinetic study

I. S. Antipin, I. I. Stoikov, S. A. Repeikov, and A. I. Konovalov*

Kazan State University,
18 ul. Kremlevskaya, 420008 Kazan, Russian Federation.
Fax: +7 (843 2) 75 2253. E-mail: iantipin@ksu.ru

New lipophilic α-aminophosphonates containing cyclic or acyclic alkyl substituents at the carbon atom in the α position were synthesized by the Kabachnik—Fields reaction. Studies of the obtained compounds as carriers for transport of glycolic acid through polymer-supported liquid membranes demonstrated that the flux of glycolic acid through the membrane depends on the lipophilicity and the presence of substituents at the α-C atom of aminophosphonate. The structures of a number of α-aminophosphonate—glycolic acid complexes were calculated by the semiempirical PM3 method.

Key words: α-aminophosphonates, carriers; glycolic acid, transport, liquid membranes.

The transport of biologically active compounds, such as α-amino and α-hydroxy acids, through lipophilic organic membranes is of considerable interest from the viewpoint of its application in analysis and separation of complex multicomponent systems. In addition, this process can simulate some properties of biological systems, in particular, the selective permeability of phospholipid membranes. The permeability and selectivity of artificial membranes can be achieved by introducing lipophilic carrier molecules (or "receptors"), which can form complexes with the compound transported, into the membranes. In this connection, studies on the effect of carrier structure on the efficiency of transport of substrates is of fundamental importance for modeling and designing new selective "receptors."

Compounds that are "receptors" of the carboxyl group (tri-*n*-octylphosphine oxide,[2] tri-*n*-octylamine,[3] and substituted benzylamine[4]) are commonly used as carriers of α-hydroxy acids through liquid membranes. A characteristic feature of these compounds is the presence of only one potential center of binding of the substrate, which cannot provide high selectivity of interphase transfer of hydroxy acids because the special structural features of these acids are not taken into account by the carrier.

Recently, we have demonstrated that α-aminophosphonates can act as efficient carriers of α-hydroxy acids.[1,5] These compounds contain two proton-acceptor centers, namely, the P=O phosphoryl group and the lone electron pair of the N atom, which can be involved in specific interactions (hydrogen bonds) with hydroxyl and carboxyl groups of hydroxy acids. In addition, in the α-aminophosphonates—α-hydroxy acid system under consideration, the condition for complementarity (spatial correspondence) of the binding centers of interacting compounds is adequately fulfilled.

In this work, we report the results a kinetic study of the effect of the structure of α-aminophosphonate, which acts as a membrane carrier, on the rate of membrane transport of glycolic acid.

Experimental

The IR spectra of liquid films of the synthesized compounds formed between KBr plates were recorded on a Specord M-80 spectrometer in the region of 700—3600 cm^{-1}. The ^{31}P NMR spectra of α-aminophosphonates (in $CDCl_3$) were measured on a Varian XL-300 spectrometer relative to a 85% H_3PO_4 solution (external standard). The concentrations of the solutions under study were 3—5%. The conductivities of the solutions were measured on a Hydromat-LM 301 conductivity meter. Glycolic acid of analytical grade was used.

***O,O*-Dipentyl 1-(benzylamino)cyclopentylphosphonate (1)** was synthesized according to a known procedure,[1] d_4^{20} 1.1102, n_D^{20} 1.6847.

Synthesis of α-aminophosphonates 2—8 (general procedures). ***A.*** Freshly distilled amine (0.024 mol), dialkyl phosphite (0.022 mol), and a carbonyl compound (0.024 mol) 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 for at 60 °C for 4 h. The excess of the reagents was removed *in vacuo*. The mixture was twice chromatographed on a column with silica gel L 100/160 μm (a 10 : 1 $CHCl_3$—Pr^iOH mixture as the eluent).

B. Freshly distilled amine (0.022 mol), dialkyl phosphite (0.022 mol), and a carbonyl compound (0.022 mol) were placed in a 50-mL flask equipped with a magnetic stirrer and a reflux condenser and dissolved in a 0.1% TsOH solution in toluene (10 mL). The reaction solution was heated with stirring on a water bath at 80 °C for 12 h. The excess of the reagents and the solvent were distilled off *in vacuo*, and the residue was twice

---

* For Part 1, see Ref. 1.

Translated from *Izvestiya Akademii Nauk. Seriya Khimicheskaya*, No. 9, pp. 1746—1750, September, 1998.

1066-5285/98/4709-1697 $20.00

chromatographed on a column with silica gel L 100/160 μm (a 10 : 1 $CHCl_3$—Pr^iOH mixture as the eluent).

C. A suspension of paraformaldehyde (0.022 mol) in benzene (10 mL) was placed in a 50-mL two-neck 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 (0.022 mol) was added dropwise. After the precipitate disappeared, dialkyl phosphite (0.022 mol) was added to the reaction mixture. The mixture was refluxed for 12 h, and the solvent was removed *in vacuo*.

***O,O*-Dipentyl 1-(benzylamino)cyclohexylphosphonate (2)** was prepared according to procedure *A*. The yield was 6.76 g (75%), colorless viscous liquid, d_4^{20} 1.0304, n_D^{20} 1.6832. Found (%): C, 67 24; H, 9.42; P, 7.33. $C_{23}H_{40}NO_3P$. Calculated (%): C, 67.45; H, 9.84; P, 7.56. IR (KBr), ν/cm^{-1}: 990 (P—O—C); 1040, 1060 (P—O—C); 1190 (P—O—C_5H_{11}); 1240 (P=O); 1645, 3325 (N—H). ^{31}P NMR, δ: 29.

***O,O*-Di(2-ethylhexyl) 1-(benzylamino)cyclopentylphosphonate (3)** was prepared according to procedure *B*. The yield was 7.39 g (70%), colorless viscous liquid, d_4^{20} 0.9924, n_D^{20} 1.4884. Found (%): C, 69.92; H, 10.51; P, 6.33. $C_{28}H_{50}NO_3P$. Calculated (%): C, 70.11; H, 10.51; P, 6.46. IR (KBr), ν/cm^{-1}: 975 (P—O—C); 1025, 1050 (P—O—C); 1170 (P—O—C_8H_{18}); 1235 (P=O); 1645, 3350 (N—H). ^{31}P NMR, δ: 33.

***O,O*-Di(2-ethylhexyl) 1-(benzylamino)cyclohexylphosphonate (4)** was prepared according to procedure *B*. The yield was 7.82 g (72%), colorless viscous liquid, d_4^{20} 0.9932, n_D^{20} 1.4939. Found (%): C, 70.52; H, 10.73; P, 6.13. $C_{29}H_{52}NO_3P$. Calculated (%): C, 70.55; H, 10.62; P, 6.27. IR (KBr), ν/cm^{-1}: 975 (P—O—C); 1025, 1050 (P—O—C); 1170 (P—O—C_8H_{18}); 1235 (P=O); 1645, 3350 (N—H). ^{31}P NMR, δ: 32.

***O,O*-Di(2-ethylhexyl) 1-(benzylamino)-1-methylethylphosphonate (5)** was prepared according to procedure *B*. The yield was 8.68 g (87%), colorless viscous liquid, d_4^{20} 0.8497, n_D^{20} 1.6823. Found (%): C, 69.62; H, 10.64; P, 6.93. $C_{26}H_{48}NO_3P$. Calculated (%): C, 69.84; H, 10.66; P, 6.83. IR (KBr), ν/cm^{-1}: 990 (P—O—C); 1025, 1060 (P—O—C); 1170 (P—O—C_8H_{18}); 1235 (P=O); 1615, 3250 (N—H). ^{31}P NMR, δ: 34.

***O,O*-Didecyl 1-(benzylamino)-1-methylethylphosphonate (6)** was prepared according to procedure *B*. The yield was 10.09 g (90%), colorless viscous liquid, d_4^{20} 0.9734, n_D^{20} 1.4794. Found (%): C, 70.54; H, 10.93; P, 6.03. $C_{30}H_{56}NO_3P$. Calculated (%): C, 70.69; H, 11.07; P, 6.08. IR (KBr), ν/cm^{-1}: 975 (P—O—C); 1025, 1050 (P—O—C); 1170 (P—O—$C_{10}H_{21}$); 1250 (P=O); 1645, 3350 (N—H). ^{31}P NMR, δ: 30.

***O,O*-Di(2-ethylhexyl) 1-(benzylamino)pentylphosphonate (7)** was prepared according to procedure *B*. The yield was 6.57 g (62%), colorless viscous liquid, d_4^{20} 0.9646, n_D^{20} 1.4815. Found (%): C, 69.82; H, 10.99; P, 6.26. $C_{28}H_{52}NO_3P$. Calculated (%): C, 69.82; H, 10.88; P, 6.43. IR (KBr), ν/cm^{-1}: 980 (P—O—C); 1025, 1050 (P—O—C); 1115 (P—O—C_8H_{18}); 1240 (P=O); 1625, 3320 (N—H). ^{31}P NMR, δ: 28.

***O,O*-Di(2-ethylhexyl) (benzylamino)methylphosphonate (8)** was prepared according to procedure *C*. The yield was 8.90 g (95%), colorless viscous liquid, d_4^{20} 1.0173, n_D^{20} 1.4844. Found (%): C, 67.80; H, 10.80; P, 7.14. $C_{24}H_{44}NO_3P$. Calculated (%): C, 67.73; H, 10.42; P, 7.28. IR (KBr), ν/cm^{-1}: 975 (P—O—C); 1015, 1050 (P—O—C); 1115 (P—O—C_8H_{18}); 1250 (P=O); 1625, 3300 (N—H). ^{31}P NMR, δ: 28.

Experiments on membrane transport. The rates of transport of substrates through liquid impregnated membranes were measured in a glass temperature-controlled vertical diffusion cell with the use of a mobile drum.[6] Porous Teflon filters Millipore Type FA (thickness 1 μm, pore size 100 nm, porosity 85%; filters were reinforced with a capron net), which were impregnated with a liquid membrane, served as a hydrophobic matrix. The ratio of the volumes of the source and receiving phases was 5 : 1, which provided equal levels of the solutions to eliminate electroosmotic transport of the acid. The experiments on mass transport were carried out under normal conditions (25 °C).

The initial solutions of the substrates were prepared from precisely weighed samples with the use of water, which was twice distilled immediately before use. A pure solvent (*o*-nitrophenyl octyl ether) or a 1 *M* solution of α-aminophosphonate in the organic solvent was used as the liquid membrane.

The initial 0.1 *M* solution of the substrate was placed in an outer temperature-controlled vessel, and twice distilled water was placed in an inner vessel. The source and receiving solutions were mixed with magnetic stirrers. The concentrations of the compounds were determined from the conductivities of the solutions. The calibration dependence was reproduced three times. The experiments on membrane transport were carried out under identical conditions three times.

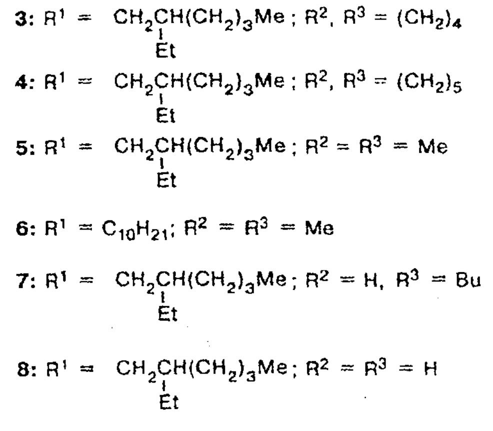
The membrane phases obtained after transport runs were analyzed by ^{31}P NMR spectroscopy. It was demonstrated that all carriers under study are stable in the systems used.

Statistical treatment of the results was carried out using the MICROCAL ORIGIN-4.10 application program package. The error of the flux determination was ≤10%.

Semiempirical quantum-chemical calculations of the complexes of α-aminophosphonates with glycolic acid were carried out by the PM3 method using the MOPAC 7.00 program package.

Results and Discussion

A series of lipophilic α-aminophosphonates **1—8** containing various substituents, R^1, R^2, and R^3, were synthesized by the Kabachnik—Fields reaction[7] in 62—95% yields.



1: $R^1 = C_5H_{11}$; $R^2, R^3 = (CH_2)_4$

2: $R^1 = C_5H_{11}$; $R^2, R^3 = (CH_2)_5$

3: $R^1 = CH_2CH(Et)(CH_2)_3Me$; $R^2, R^3 = (CH_2)_4$

4: $R^1 = CH_2CH(Et)(CH_2)_3Me$; $R^2, R^3 = (CH_2)_5$

5: $R^1 = CH_2CH(Et)(CH_2)_3Me$; $R^2 = R^3 = Me$

6: $R^1 = C_{10}H_{21}$; $R^2 = R^3 = Me$

7: $R^1 = CH_2CH(Et)(CH_2)_3Me$; $R^2 = H$, $R^3 = Bu$

8: $R^1 = CH_2CH(Et)(CH_2)_3Me$; $R^2 = R^3 = H$

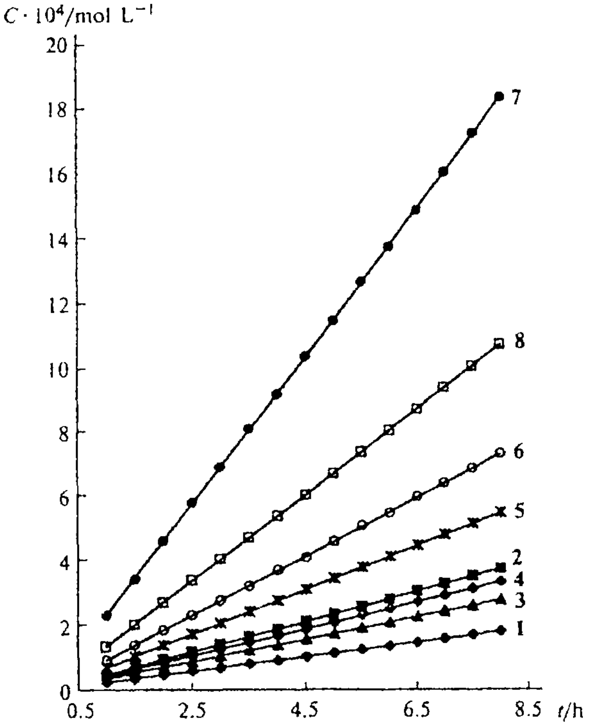
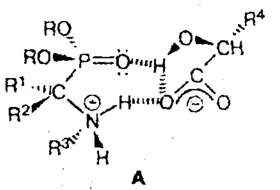


Fig. 1. Dependences of the concentration (C) of glycolic acid in the receiving phase on the time (t) for carriers **1—8**. The initial linear parts of the curves are given.

Solutions of α-aminophosphonates (1 mol L^{-1}) in *o*-nitrophenyl octyl ether, which were impregnated into pores of Millipore Type FA Teflon filters, were used as liquid membranes. A 0.1 *M* aqueous solution of glycolic acid was used as the source phase. The induced transport of the substrate through the membrane in the systems under study proceeded according to the scheme of dialysis. The kinetic curves for carriers **1—8** are shown in Fig. 1. Selected values of the fluxes (F) are shown below.

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
$F \cdot 10^6$/mol (h cm^2)$^{-1}$	2.4	4.0	3.7	4.5	7.2	9.6	24	14

A comparison of the values of mass transport determined by us with the data of the preliminary blank run, in which the value of the flux of glycolic acid through the membrane that contained only the pure solvent ($F = 6 \cdot 10^{-7}$ mol (h cm^2)$^{-1}$) was determined, demonstrated that introduction of α-aminophosphonate into the membrane leads to an increase in the rate of transport by factors of 4—40. This effect is associated with the formation of the complex of glycolic acid with α-aminophosphonate in the membrane phase (structure **A**), which was established by ^{1}H and ^{31}P NMR and IR spectroscopy and also by X-ray diffraction analysis using the complex of *O,O*-diethyl 1-methyl-1-(isopropylamino)ethylphosphonate with mandelic acid as an example.[8]



A

The obtained kinetic results indicate that there is no general dependence of the rate of transport of glycolic acid on the lipophilicity of the carrier. However, in the case of a series of structurally similar compounds (see pairs **1/3**, **2/4**, and **5/6**) in which only the lengths of alkoxyl radicals that are remote from centers of complexation are varied, a pronounced tendency is observed for an increase in the rate of transport as the lipophilicity of the carrier increases. Analogous dependences have been observed previously in Refs. 2, 9, and 10, where they have been attributed to an increase in the distribution coefficient of 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 affect substantially the ability of α-aminophosphonates to be involved in complexation. Based on the structure of the coordination centers of the complex (see structure **A**), we varied the number and the nature of alkyl groups at the α-C atom of aminophosphonate and established that *n*-alkyl substituents have the most substantial effect on the flux of glycolic acid through the membrane.

The smallest rate of transport is observed in the case of α-aminophosphonates containing cyclic substituents at the α-C atom, and the flux through the membrane decreases as the size of the ring decreases. Apparently, the five-membered ring prevents the carrier from adopting the conformation, which is optimum for interactions with α-hydroxy acid. The rate of transport increases as the number of alkyl substituents at the α-C atom in the molecule of the carrier decreases. Thus, in the case of monoalkyl-substituted aminophosphonate **7**, the flux of glycolic acid through the membrane is substantially larger than that in the case of dialkyl-substituted derivatives. The slight decrease in the rate of transport of glycolic acid in the case of α-unsubstituted aminophosphonate **8** compared to the rate observed for monosubstituted derivative **7** can be explained by the lower lipophilicity of the former carrier.

To elucidate the nature of the effect of alkyl substituents at the α position, we calculated the structures of the complexes of α-aminophosphonates with glycolic acid by quantum-chemical methods. Geometry optimization was carried out by the semiempirical PM3 method. The arrangement of the binding centers in the starting model of the complex corresponds to structure **A**.

Typical of the complex of α-unsubstituted aminophosphonate **8** with glycolic acid is the formation of the O(5)—H...N(1) and O(4)—H...O(1) hydrogen bonds (Fig. 2). However, unlike the experimental data obtained in solutions and in the solid phase, which indicate that the proton of the carboxyl group is almost completely transferred to the N atom of α-aminophosphonate (ion pair), the results of calcula-

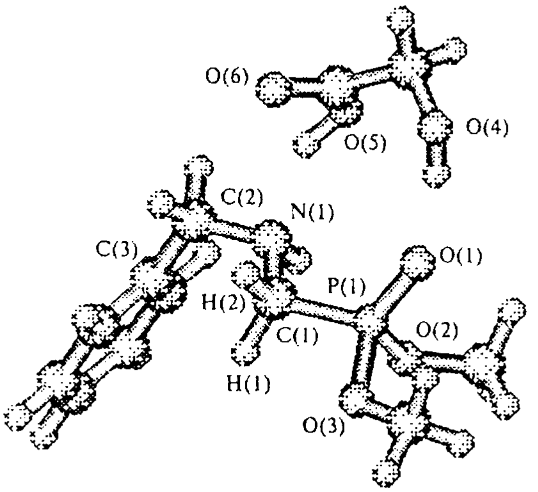


Fig. 2. Geometry of the complex of α-unsubstituted aminophosphonate (analog of compound **8**) with glycolic acid optimized by the PM3 method.

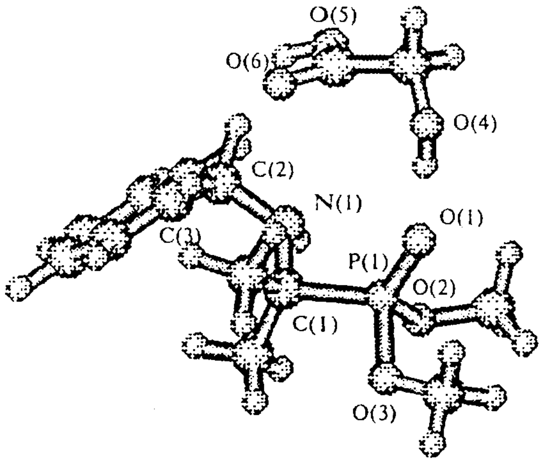


Fig. 3. Geometry of the complex of α-dimethyl-substituted aminophosphonate (analog of compound **5**) with glycolic acid optimized by the PM3 method.

tions indicate that only the molecular complex is formed. This difference is reasonable because the equilibrium between the molecular and ionic complexes upon hydrogen bonding AH...B $\rightleftharpoons$ A^-...HB^+ substantially depends on the solvating ability of the medium.[11] Our calculations correspond to the gaseous phase and ignore the possibility of stabilization of charges due to solvation.

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

In the case of the conformation that is most favorable for interaction with the hydroxy acid molecule, one hydrogen atom (H(1)) at the α-carbon atom (C(1)) of aminophosphonate is located in proximity to the aromatic ring. The distance between the C(3) and H(1) atoms is only 2.82 Å (C(3)—H(2) 3.51 Å). The half-thickness of the phenyl substituent is 1.85 Å, and the covalent radius of the hydrogen atom is 1.20 Å.[12] Consequently, the distance between the above-mentioned atoms is smaller that the sum of their van der Waals radii. Because of this, the attachment of a bulkier alkyl substituent at this position will cause strong repulsions between the aromatic ring and the substituent. Calculations demonstrated that in the last-mentioned case, the C(2)—N(1)—C(1) bond angle is distorted and the aromatic ring is rotated about the C(2)—N(1) bond (Table 1). In a series of aminophosphonates, namely, compounds **8** (α-unsubstituted), **5** (α-dialkyl-substituted), and **3** (α-disubstituted cyclic), the C(2)—N(1)—C(1) bond angle and the C(3)—C(2)—N(1)—C(1) torsion angle change monotonically. From the analysis of the C(3)—C(2)—N(1)—H and H—C(2)—N(1)—H torsion angles it follows that as steric hindrances at the α-C atom increase, the aminophosphonate molecule in the complex is forced to change the conformation from staggered (compound **8**) along the C(2)—N(1) bond to an energetically unfavorable eclipsed one (compound **3**). This leads to the cleavage of the hydrogen bond between the carboxyl group and the N atom (Fig. 3). As mentioned above, our calculations correspond to the gaseous phase and ignore the solvation component of the charged complex formed in the solution (see structure **A**). Therefore, the attachment of bulky substituents at the α position of aminophosphonate leads only to weakening of the above-mentioned hydrogen bond rather than to its complete cleavage.

Table 1. Selected bond angles (ω) and torsion angles (φ) in the complexes of glycolic acid with α-aminophosphonates **3**, **5**, and **8** calculated by the PM3 method

Angle	ω, φ/deg		
	3	5	8
C(2)—N(1)—C(1)	119.5	118.2	113.6
C(3)—C(2)—N(1)—C(1)	107.0	94.6	71.1
C(3)—C(2)—N(1)—H	18.9	30.4	50.3
H_A—C(2)—N(1)—H	101.8	90.7	64.6
H_B—C(2)—N(1)—H	144.4	155.2	179.8

The rate of membrane transport of glycolic acid by α-aminophosphonates changes in the same order: **8** > **5** > **3**. It is known that in this case, the limiting stage of transport is the transfer from the source phase to the membrane phase,[1] and hence, the activities of the carriers change in the observed order due to a decrease in the stability of the complexes formed in the membrane phase. Consequently, when carriers of hydroxy acids are constructed based on α-aminophosphonates, at least two factors, namely, their lipophilicity and steric hindrances at the α-C atom, should be taken into account.

This work was financially supported by the Russian Foundation for Basic Research (Project No. 98-03-33051).

References

1. I. S. Antipin, I. I. Stoikov, A. R. Garifzyanov, and A. I. Konovalov, *Zh. Obshch. Khim.*, 1996, **66**, 402 [*Russ. J. Gen. Chem.*, 1996, **66** (Engl. Transl.)].
2. Y. Syen, L. Gronberg, and J. A. Jonsson, *Anal. Chim. Acta*, 1994, **293**, 31.
3. R. S. Juang and L. J. Chen, *J. Membr. Sci.*, 1997, **123**, 81.
4. J.-M. Lehn, A. Moradpour, and J.-P. Behr, *J. Am. Chem. Soc.*, 1975, **97**, 2532.
5. I. S. Antipin, I. I. Stoikov, A. R. Garifzyanov, and A. I. Konovalov, *Dokl. Akad. Nauk*, 1996, **347**, 626 [*Dokl. Chem.*, 1996 (Engl. Transl.)].
6. S. Yu. Ivakhno, A. V. Afanas'ev, and G. A. Yagodin, *Itogi nauki i tekhniki. Neorganicheskaya khimiya*, [*Advances in Science and Technology: Inorganic Chemistry*], VINITY, Moscow, 1984, **13**, 3 (in Russian).
7. M. I. Kabachnik, T. Ya. Medved', N. M. Dyatlova, O. G. Arkhipova, and M. V. Rudomino, *Usp. Khim.*, 1968, 37, 1161 [*Russ. Chem. Rev.*, 1968, **37**, 503 (Engl. Transl.)].
8. I. I. Stoikov, Ph.D. (Chem.) Thesis, Kazan State University, Kazan, 1997 (in Russian).
9. M. P. Thien, T. A. Hatton, and D. J. C. Wang, *Biotechn. Bioeng.*, 1988, **32**, 604.
10. *Liquid Membranes: Applications*, Eds. T. Araki and H. Tsukube, CRC Press Inc., Boca Raton (Fla), 1990, 231 pp.
11. N. S. Golubev, G. S. Denisov, and V. M. Shraiber, in *Vodorodnaya svyaz'* [*Hydrogen Bonds*], Ed. N. D. Sokolov, Nauka, Moscow, 1981, 212 (in Russian).
12. J. F. Malone, C. M. Murray, M. H. Charlton, R. Docherty, and A. J. Lavery, *J. Chem. Soc., Faraday Trans.*, 1997, **93**, 3429.

Received February 27, 1998;
in revised form May 20, 1998