Molecular Recognition and Phase Transfer of Underivatized Amino Acids by a Foldable Artificial Host

Axel Metzger,[†] Karsten Gloe,[‡] Holger Stephan,[‡] and Franz P. Schmidtchen*[,†]

Institut für Organische Chemie und Biochemie, Technische Universität München, D-85747 Garching, Germany, and Institut für Anorganische Chemie, Technische Universität Dresden, Mommsenstrasse 13, D-01062 Dresden, Germany

Received August 3, 1995[⊗]

A novel molecular host **5** for amino acid zwitterions is described. The covalent connection of a chiral bicyclic guanidinium salt as an anchor function for the carboxylate and a triaza-crown ether as an ammonium binding moiety in combination with a strongly hydrophobic silyl ether serves to complex and transfer amino acids from water into dichloromethane with unprecedented efficiency. Quantitation of the extraction process by radiometry revealed a clean 1:1 stoichiometry and gives evidence for the zwitterion as the species undergoing phase transfer. Even small hydrophilic (Ser, Gly) but no charged amino acids were extracted better by factors up to 3000-fold than by a previously reported host of similar design (Galán, A.; Andreu, D.; Echavarren, A. M.; Prados, P.; de Mendoza, J. *J. Am. Chem. Soc.* **1992**, *114*, 1511−1512). Some enantioselection in the transfer of phenylalanine was observed (40% ee).

Amino acids are among the most preferred targets for molecular recognition by artificial host compounds. This is due to their relevance in the biological world and their rich chemistry which invites molecular architects to try host−guest binding of amino acids as an instrument for manipulation of their reactivity. Some applications (e.g., substrate selective sensors,[1] membrane transport and extraction[2,17] for enrichment, and optical resolution[3]) call for their selective transfer from the ordinary aqueous environment to some less polar organic phase. For this to be achieved the solvation shells around the highly hydrophilic and heavily hydrated carboxylate and ammonium moieties must be replaced by dedicated ligands (molecular solvents, hosts[4]) which specifically interact with these epitopes and compensate for the energetic cost of desolvation. By the same token, the host−guest complex formed should present a molecular surface that is readily solvated by the organic solvent resulting in good solubility. Of course, the overall lipophilicity is enhanced if the amino acid guest possesses a large hydrophobic side chain. But based on the extreme distribution in aqueous two-phase systems even of the most hydrophobic amino acids in favor of the water phase,[5] the essential task in amino acid phase transfer is the hydrophobic disguise of the α-amino carboxylate substructure. Any host capable of recognizing and binding to this combination of structural elements in water without requiring the additional interactions with the side chain would qualify as a potential candidate for extraction of amino acids as a class.[6] However, most hosts reported to complex underivatized amino acids in water rely on the binding of only these side chains[2a,7] with some notable exceptions: [2a,f,8,11] Reetz et al. recently realized an efficient carrier system for phenylalanine, taking advantage of termolecular complex formation with a boronic acid and a crown ether component.[2f] Aoyama et al.[8] used rhodium porphyrins to bind amino acids by two-point fixation in acidic solution and demonstrated carrier-mediated transport across a bulk liquid membrane. Following the concept of foldable receptors,[9,10] de Mendoza et al.[11] prepared an artificial chiral host composed of a linear arrangement of binding modules and reported the amazing nearly complete enantiodifferentiation of amino acid zwitterions in a one-step extraction experiment using this compound.

Drawing on a similar ensemble of building blocks, we embarked on the preparation of the polytopic host **5**

[†] Technische Universität München.

[‡] Technische Universität Dresden.

[⊗] Abstract published in *Advance ACS Abstracts,* February 15, 1996.

(1) (a) Kawabata, H.; Shinkai, S. *Chem. Lett.* **1994**, 375−378. (b) Morozumi, T.; Shinkai, S. *Chem. Lett.* **1994**, 1515−1518.

(2) (a) Rebek, J., Jr.; Askew, B.; Nemeth, D.; Parris, K. *J. Am. Chem. Soc.* **1987**, *109*, 2432−2434. (b) Mutihac, L.; Luca, C. *Rev. Roumaine Chim.* **1991**, *36*, 85−90. (c) Sunamoto, J.; Iwamoto, K.; Mokri, Y.; Kominato, T. *J. Am. Chem. Soc.* **1982**, *104*, 5502−5504. (d) Willner, I.; Marx-Tibbon, S. *J. Chem. Soc., Chem. Commun.* **1994**, 1261−1262. (e) Maruyama, K.; Tsukube, H.; Araki, T. *J. Am. Chem. Soc.* **1982**, *104*, 5197−5203. (f) Reetz, M. T.; Huff, J. Rudolph, J.; Töllner, K.; Deege, A.; Goddard, R. *J. Am. Chem. Soc.* **1994**, *116*, 11588−11589. (g) Behr, J.-P.; Lehn, J.-M. *J. Am. Chem. Soc.* **1973**, *95*, 6108−6110. (h) Mohler, L. K.; Czarnik, A. W. *J. Am. Chem. Soc.* **1993**, *115*, 7037−7038.

(3) (a) Peacock, S. S.; Walba, D. M.; Gaeta, F. C. A.; Helgeson, R. C.; Cram, D. J. *J. Am. Chem. Soc.* **1980**, *102*, 2043−2052. (b) Newcomb, M.; Toner, J. L.; Helgeson, R. C.; Cram, D. J. *J. Am. Chem. Soc.* **1979**, *101*, 4941−4947. (c) Konishi, K.; Yahara, K.; Toshishige, H.; Aida, T.; Inoue, S. *J. Am. Chem. Soc.* **1994**, *116*, 1337−1344. For the calculation of chiral discrimination of amino acid derivatives see: Burger, M. T.; Armstrong, A.; Guarnieri, F.; McDonald, D. Q.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 3593−3594.

(4) Cram, D. J. *Angew. Chem.* **1988**, *100*, 1041−1052.

(5) (a) Hecht, D.; Tadesse, L.; Walters, L. *J. Am. Chem. Soc.* **1994**, *115*, 3336−3337, and references cited therein. (b) Nozaki, Y.; Tanford, C. *J. Biol. Chem.* **1971**, *246*, 2211−2217.

(6) In terms of scope of applicability it seems more desirable to design molecular hosts that recognize a defined set of functional groups in a guest molecule than to strive for utmost selectivity in binding one particular guest species.

(7) (a) Lipkowitz, K. B.; Raghothama, S.; Yang, J. *J. Am. Chem. Soc.* **1992**, *114*, 1554−1562. (b) Tabushi, I.; Kuroda, Y.; Mizutani, T. *J. Am. Chem. Soc.* **1986**, *108*, 4514−4518. (c) Breslow, R.; Canary, J. W.; Varney, M.; Waddell, S. T.; Yang, D. *J. Am. Chem. Soc.* **1990**, *112*, 5212−5219 and references cited therein. (d) Rebek, J., Jr.; Nemeth, D. *J. Am. Chem. Soc.* **1985**, *107*, 6738−6739.

(8) (a) Aoyama, Y.; Asakawa, M.; Yamagishi, A.; Toi, H.; Ogoshi, H. *J. Am. Chem. Soc.* **1990**, *112*, 3145−3151. (b) **1988**, *110*, 4076−4077. (c) Aoyama, Y.; Nonaka, S.; Motomura, T.; Ogoshi, H. *Chem. Lett.* **1989**, 1877−1880.

(9) (a) Schmidtchen, F. P. *J. Am. Chem. Soc.* **1986**, *108*, 8249−8255. (b) Schmidtchen, F. P.; Gleich, A.; Schummer, A. *Pure Appl. Chem.* **1989**, *61*, 1535−1546.

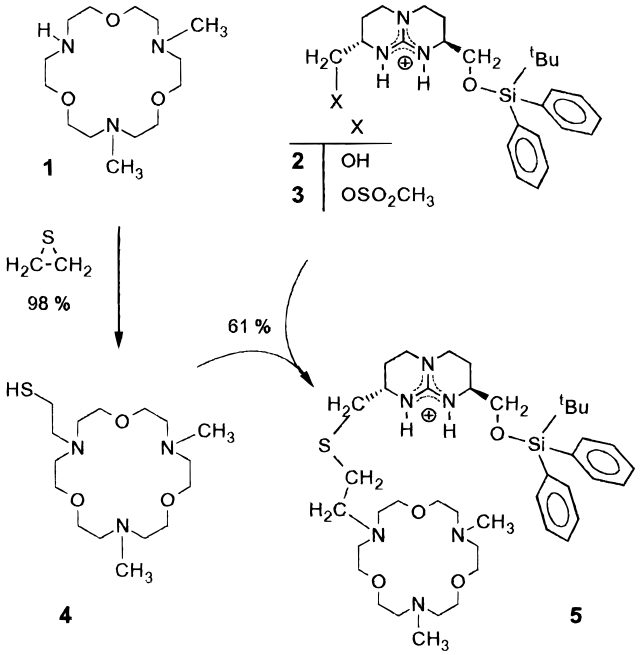
(10) (a) Schmidtchen, F. P. *J. Org.Chem.* **1986**, *51*, 5161−5168. (b) *J. Inclusion Phenom.* **1987**, *5*, 161−164. (c) *Z. Naturforsch.* **1987**, *42C*, 476−485.

(11) Galán, A.; Andreu, D.; Echavarren, A. M.; Prados, P.; de Mendoza, J. *J. Am. Chem. Soc.* **1992**, *114*, 1511−1512.

0022-3263/96/1961-2051$12.00/0

comprising two high specificity binding modules in addition to a bulky silyl ether conveying the necessary lipophilicity (Figure 1). A chiral bicyclic guanidinium unit[12] which is of proven utility in binding a carboxylate function of a guest[13] is attached to a triaza-crown ether[10a,14] for primary ammonium complexation. Both anchor groups are connected by a flexible but chemically stable and hydrophobic thioether bridge. Molecular modeling (see the caption of Figure 1, cf. ref 15) indicated that host **5** could easily fold to satisfy the ligation requirements of an α-amino carboxylate moiety.

The most successful out of several attempts to synthesize **5** started with the azacrown ether **1**[10a] which was treated with thiirane (10 h, 100 °C, toluene) to give the critically air-sensitive **4** in an almost quantitative yield. On the other hand, the chiral (*S*,*S*)-(hydroxymethyl)-guanidine **2**[12a,15] was converted to the mesylate **3**, which reacted smoothly in MeOH at room temperature with the thiolate generated by adding triazabicyclodecene (TBD, Fluka) to **4**. Purification of the mixture by gel filtration (fractogel HWS 40, 50 mM NH_4OAc/MeOH) gave the target host **5** in 61% yield.[16]



Initial studies in solid−liquid phase transfer of amino acids in methanol using **5** as the monochloride revealed that most of the hydrophobic guests tested (Phe, Trp, *p*-NO_2Phe) were solubilized to a greater extend than could be accounted for by a 1:1 host−guest complex formation. Moreover, differences in the extraction of

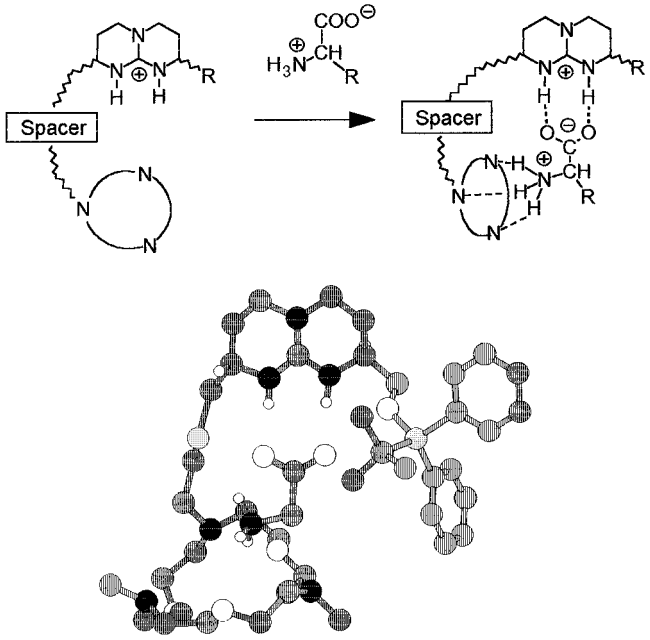


Figure 1. Energy-minimized model of the ensemble of amino acid zwitter ions with host **5** as obtained by Hyperchem 4.0, PM3 calculations.[20]

guest enantiomers were hardly visible. Apparently, the formation of more soluble salts of the basic host **5** and the solid zwitterionic amino acids governed this outcome.

Liquid−liquid partition of ^{14}C-labeled amino acids in a CH_2Cl_2/aqueous buffer system ought to give precise quantitative data on the extraction properties of host **5**. This method is far superior with respect to reliability, reproducibility, and sensitivity to the HPLC or NMR methods used in previous extraction protocols.[2,8,11] If extraction equilibrium[17] includes only one host−guest species of stoichiometry *n* (i.e., {aa∈$\mathbf{5}_n$}) a linear dependence according to eq 1 should be observed revealing the complex stochiometry *n* and the equilibrium constant for the phase transfer K_{ex} from the slope and intercept of a corresponding plot.

$$n\{\text{host}\}_{\text{org.phase}} + \{\text{aa}\}_{H_2O} \overset{K_{ex}}{\rightleftharpoons} \{\text{aa}\in\text{host}_n\}_{\text{org.phase}}$$

$$\log D_{aa} = n \log[\text{host}] + \log K_{ex}; \quad D_{aa} = \frac{[\text{aa}]_{\text{org.phase}}}{[\text{aa}]_{H_2O}} \tag{1}$$

As can be seen from Figure 2, the extraction behavior of host **5** and the monotopic guanidine **2** lacking the anchor group for the ammonium function of the guest with a set of representative amino acids very closely adheres to the suggested model. Clean 1:1 host−guest complex formation in the organic phase is observed for either host under conditions of fixed pH-value and ionic strength. In accord with expectations, the most hydrophobic amino acids are extracted best. But even quite hydrophilic amino acids (Gly, Ser) that were hardly extractable by any of the known artificial hosts[2,8,11] show respectable extraction efficiencies with **5**. Actually, only neutral amino acids were extracted under the experi-

(12) For the synthesis of chiral guanidinium anchor groups see: (a) Kurzmeier, H.; Schmidtchen, F. P. *J. Org. Chem.* **1990**, *55*, 3749−3755. (b) Gleich, A.; Schmidtchen, F. P. *Chem. Ber.* **1990**, *123*, 907−915. (c) Schmidtchen, F. P.; Oswald, H.; Schummer, A. *Liebigs Ann. Chem.* **1991**, 539−546. (d) Echavarren, A.; Galán, A.; de Mendoza, J.; Salmerón, A.; Lehn, J.-M. *Helv. Chim. Acta* **1988**, *71*, 685.

(13) (a) Müller, G.; Riede, J.; Schmidtchen, F. P. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1516−1518. (b) Gleich, A.; Schmidtchen, F. P.; Mikulcik, P.; Müller, G. *J. Chem. Soc., Chem. Commun.* **1990**, 55−57.

(14) Vierling, P.; Lehn, J.-M. *Tetrahedron Lett.* **1980**, *21*, 1323−1326. Experimentally, this aza-crown ether ring shows a marked preference of primary ammonium versus alkali metal cation binding. This is why the selection of this anchor group was done though the origin of this effect is debatable (see, e.g.: Kuleshova, L. N.; Zorkii, P. M. *Acta Crystallogr.* **1981**, *B37*, 1363).

(15) Kato, Y.; Conn, M. M.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1994**, *116*, 3279−3284.

(16) Schmidtchen, F. P. *J. Org. Chem.* **1986**, *51*, 5161−5168.

(17) (a) Hano, T.; Ohtake, T.; Matsumoto, M.; Kitayama, D.; Hori, F.; Nakashio, F. *Proc. Int. Solvent Extr. Conf.* 1990, Kyoto, Japan; Elsevier: Amsterdam, 1992; pp 1881−1886. (b) Teramoto, M.; Miyake, Y.; Matsunama, H.; Nara, H. *Ibid.* 1803−1808.

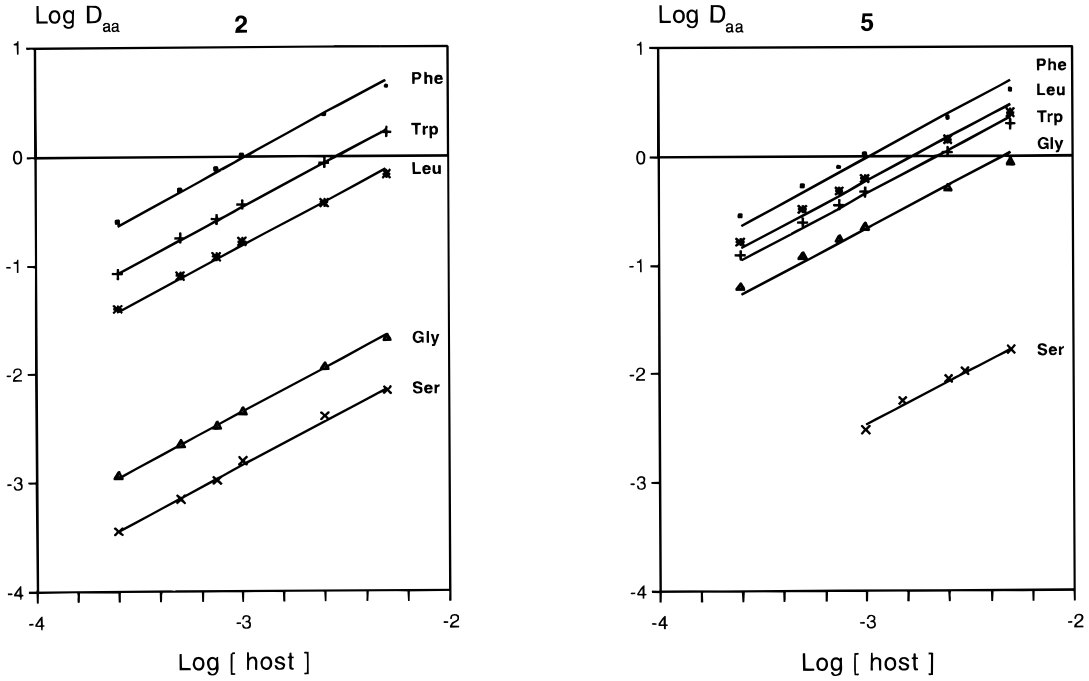


Figure 2. Distribution of underivatized ^{14}C-amino acids (1.0×10^{-4} M) between aqueous 0.05 M 2-amino-2-methylpropanol/HCl buffer pH 9.8 and CH_2Cl_2 in dependence on host concentration at ambient temperature; analysis by szintillation counting. The lines correspond to eq 1.

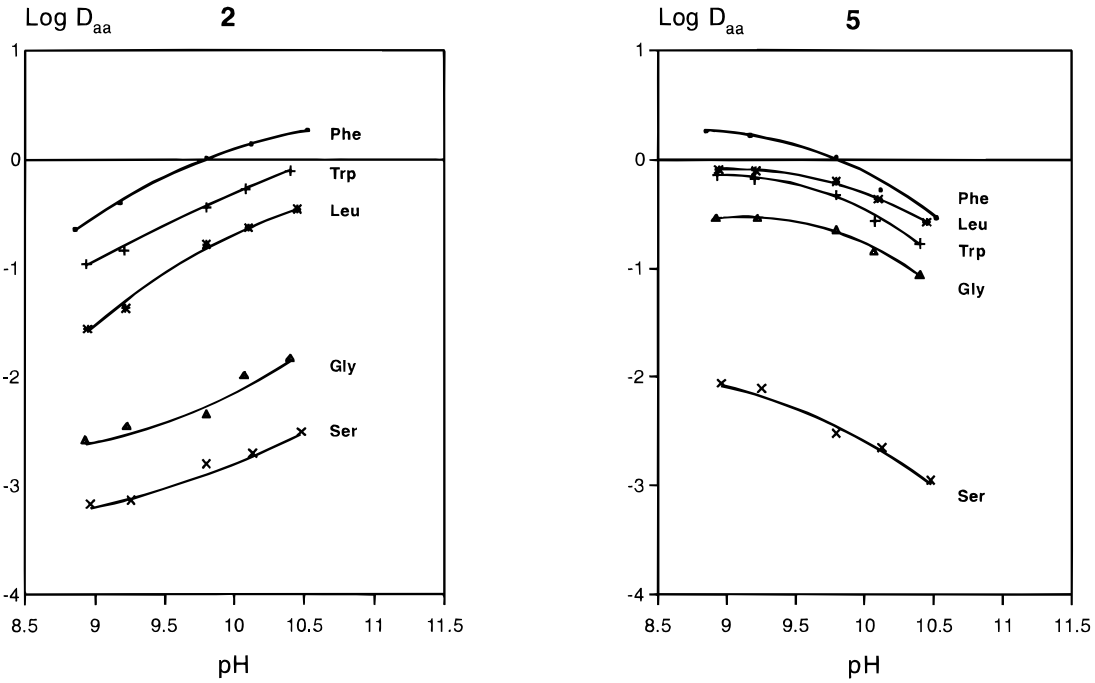


Figure 3. pH dependence of the amino acid distribution ratio; the conditions are the same as given in Figure 2; [host **5**] = 1×10^{-3} M.

mental conditions chosen. Studies with lysine and glutamic acid as basic and acidic species, respectively, revealed transfer to the organic phase of less than 0.2% ([**5**] = 0.001 M; pH = 8.9).

Figure 2 shows a clear distinction in the extraction behavior of **2** and **5**. The order of decreasing extractability is changed from Phe > Trp > Leu ≫ Gly > Ser for **2** to Phe > Leu > Trp > Gly ≫ Ser in the case of **5**. Remarkably, the ditopic host **5** extracts the highly hydrophilic glycine almost to the same extent as the more hydrophobic amino acids Phe, Leu, and Trp, indicating a common mode of interaction in which both anchor moieties of the host cooperate in substrate binding. Obviously, host **5** favors the extraction of less bulky amino acids. In consequence, leucine is extracted even better than tryptophan although the basic hydrophobicity scale of Tanford[5b] predicts the opposite order. The inspection of the dependence of extraction efficiency on pH value (Figure 3) suggests a clue for the binding pattern in the complexes extracted. With the strictly monotopic host **2** extractability is improved the more alkaline the aqueous phase becomes. Thus, amino acid anion is the most likely species to be extracted. Host **5**, on the contrary, displays higher extraction power with

Table 1. Extraction Constants K_{ex} (cf. Eq 1) of Representative Amino Acids with Monotopic (2) and Ditopic (5) Guanidinium Hosts. For Experimental Conditions See the Caption of Figure 2

	K_{ex} [M^{-1}]			
	2		**5**	
	pH 8.9[a]	pH 9.8[b]	pH 8.9[a]	pH 9.8[b]
Ser	0.7	1.55	8.6	3.32
Gly	2.6	4.45	290	222
Leu	27	155	810	613
Trp	110	354	710	466
Phe	228	1018	1810	1018

[a] Calculated from the data in Figure 3 [b] Calculated from Figure 2.

increasing acidity approaching an optimum around pH 9 of the aqueous phase. In view of the observation that complexes between aza-crown ethers and amino groups require one additional proton for stability[14] this trend meets our expectations and clearly points toward the participation of both anchor functions of **5** in binding the zwitterionic guest. However, this attractive interaction must be superimposed on some hidden repulsion, probably caused by unfavorable steric interactions in the complex, since there is considerable diminution, if the binding of amino acid anions is compared with **2** lacking the aza-crown ether substituent.

The extraction equilibrium constants K_{ex} are listed in Table 1.

At pH 8.9 near the maximum of amino acid, zwitterion complexation by **5** this host compares very favorably to earlier predecessors.[8,11] With phenylalanine and glycine spanning the entire hydrophobic range of nonfunctionalized amino acids, our host **5** is estimated to be a more efficient extracting agent than another guanidinium host[11,18] by factors of 1175 and 3000, respectively. Compared to the K_{ex} values reported for a rhodium−porphyrin host in the extraction of phenylalanine and leucine,[8] host **5** leads by 820- and 450-fold. These extractions with **5** are completely reversible since the amino acid guest can be recovered by a single back extraction of the organic phase using dilute acid. The phase transfer equilibrium is rapidly established in either direction, though host **5** cannot enter the aqueous layer as was confirmed by HPLC analysis.

Originally, host **5** was designed to function by two-point guest fixation which should not suffice for enantiodifferentiation of guests. The inherent chirality, however, might cause some adventitious chiral recognition. Using D,L-phenylalanine as a probe, we found **5** to favor L-phenylalanine in the extraction into CH_2Cl_2. The ee of 40% was almost independent of pH in the range of 9.1 to 10.5. Under identical conditions compound **2** having the same configurational layout as **5** showed no enantioselectivity at all.

In conclusion, the quick and easy assembly of specific guanidinium and aza-crown ether binding modules produces a flexible open-chain host **5** capable of transferring highly hydrophilic amino acid zwitterions to the organic phase. Improvement of this extraction process may be accomplished by some structural optimization of the host as well as from the addition of less coordinating anions.[19]

(18) The estimates given are based on recalculations of the original data. In ref 8 $CHCl_3$ is taken as the organic solvent for distribution instead of CH_2Cl_2. This difference should be of minor influence. The relation of extraction efficiencies to another amino acid host (cf. ref 2a) has not been possible.

Experimental Section

All chemicals were reagent grade and used as obtained. Solvents were dried by standard laboratory procedures but freshly distilled before use. The reactions in general were monitored by gradient HPLC using a commercial reversed-phase column: 250 × 4 mm Nucleosil RP-18, 5 mm (Macherey-Nagel). In addition to the organic modifier given with the individual preparations, all eluents contained 30 mM phosphoric acid and 30 mM sodium perchlorate. Multilayer-coil-countercurrent distribution (MLCC) used an Ito machine (Zinsser Analytic) and a preparative coil (370 mL). MS spectra were obtained using EI or CI (isobutane) ionization or preferably the FAB technique. Elemental microanalysis were obtained from the Microanalytical Laboratory, Institut für Org. Chemie und Biochemie, TU München.

1-(2-Mercaptoethyl)-7,13-dimethyl-1,7,13-triaza-4,10,16-trioxacyclooctadecane (4). A solution of 109 mg (377 μmol) of the secondary amine **1**[16] in 600 μL of toluene was treated with 22.7 mg (377 μmol, 23 μL) of thiirane in a 1 mL pressure vial at 100 °C overnight. The solvent was evaporated by a stream of N_2 in the hood, leaving a residue that was >98% pure by NMR analysis, and was immediately used as obtained in subsequent reactions. For storage the free amine was converted into the tris-hydrochloride salt in THF by addition of 0.1 M aqueous HCl. Evaporation of the solvent gave a white hygroscopic residue that was, however, reasonably stable to air oxidation: IR (×3 HCl, KBr) ν = 3600−3200 (NH), 3000 (CH), 2780 (SH); MS (FAB, glycerol/thioglycerol) m/z = 350 (30, M + H), 316 (18, M + H − H_2S); ^{1}H-NMR ($CDCl_3$ 200 MHz) δ = 3.61−3.51 (m, 12H, CH_2O), 2.80−2.53 (m, 16H, CH_2N, CH_2S), 2.30 (S, 6H, CH_3N); ^{13}C-NMR ($CDCl_3$) δ = 69.5, 69.0, 68.9 (*C*O); 58.2, 56.6, 53.8 (*C*H_2N); 43.5 (*C*H_3N); 22.8 (*C*H_2S).

(2*S*,8*S*)-8-[[(*tert*-Butyldiphenylsilyl)oxy]methyl]-2-[[[2-(7,13-dimethyl-1,7,13-triaza-4,10,16-trioxacyclooctadecyl)-ethyl]thio]methyl]-3,4,6,7,8,9-hexahydro-2*H*-pyrimido-[1,2-*a*]pyrimidine Hydrochloride 5. To a solution of 70 mg (500 μmol) of triazabicyclodecene **3**[21] in 500 μL of methanol was added a solution of 105 mg (300 μmol) of thiol **4** (free base) in 280 μL of methanol in portions under N_2 at 25 °C, and the reaction was monitored by HPLC. When all of **3** was consumed the mixture was quenched with 5% aqueous acetic acid and the solvent was evaporated. The residual oil was subjected to SEC chromatography (Fractogel HWS 40, column 2.5 × 70 cm, flow 1.3 mL/min) using 50 mM ammonium acetate in methanol for elution. All fractions containing the product were pooled, the solvent was stripped off, and the residue was taken up in water. Repeated lyophilization yielded 124 mg (123 μmol) of **5** (61%) as the tetraacetate salt: HPLC gradient 10% to 65% CH_3CN (8 min) then isocratic at 65% CH_3CN (8 min); flow 1.0 mL/min, R_v 15.6 mL; MS (FAB in glycerol/thioglycerol, monoperchlorate salt) m/z = 770 (6, M + H); IR (KBr, mono chloride salt) ν = 3418 (br, NH), 2932, 2877 (m, CH), 1621 (s, guanidinium); ^{1}H-NMR (360 MHz CD_3OD, tetraacetate salt) δ = 7.69−7.66 (m, 4H), 7.48−7.39 (m, 6H), 3.75−3.66 (m, 2H), 3.60−3.52 (m, 14H), 3.40−3.27 (m, 4H), 2.85−2.65 (m, 14H), 2.30 (s, 6H), 2.19−1.85 (m, 4H), 1.06 (s, 9H); ^{13}C-NMR (90.5 MHz, CD_3OD, teraacetate salt) δ = 152.7 (guanidinium-C); 137.0, 134.4, 131.4, 129.2 (arom C); 68.3, 67.7, 67.3 (*C*H_2O); 58.57, 58.51, 55.3, 51.8 (CH_2N, aza-crown moiety); 51.1, 50.0 (CHN); 46.4, 46.2 (CH_2N, guanidine); 38.8 (CH_3N); 37.9, 28.6 (CH_2S); 27.6 (CH_3C); 26.5, 24.3 (CH_2C, guanidine); 20.3 (SiC). The assignments are backed by standard COSY and DEPT experiments.

Liquid−Liquid Extraction. The extraction studies were performed at 25 ± 1 °C in 2 mL microcentrifuge tubes by means of mechanical shaking. The phase ratio $V_{(org)}$:$V_{(w)}$ was 1:1 (0.5 mL each); the shaking time was 30 min. The extraction equilibrium was achieved during this period. All samples were centrifuged after extraction. The determination

(19) Doxsee, K. M. *J. Org. Chem.* **1989**, *54*, 4712−4715.
(20) Stewart, J. J. P. *J. Comput. Chem.* **1991**, *12*, 320.
(21) Peschke,W.; Schiessl, P.; Schmidtchen, F. P.; Bissinger, P. Schier, A. *J. Org. Chem.* **1995**, *60*, 1039−1043.

of amino acid concentration in both phases was carried out radiometrically using the β-radiation measurement of ^{14}C-labeled amino acids (Sigma−Aldrich Chemie) in a liquid scintillation counter (Tricarb 2500, Canberra-Packard). The pH of aqueous solutions were adjusted using 0.05 M 2-amino-2-methylpropanol/HCl buffer in order to guarantee a constant species distribution of the amino acids.

Acknowledgment. This work was supported by Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie. We particularly thank Mrs. U. Stöckgen for competent technical assistance and T. Krüger for molecular modeling.

JO951436D