Efficient Transport of Aromatic Amino Acids by Sapphyrin – Lasalocid Conjugates

Jonathan L. Sessler* and Andrei Andrievsky

Abstract: The synthesis and characterization of several sapphyrin – lasalocid conjugates is reported. This family of receptors is capable of acting as efficient and selective carriers for aromatic α-amino acids, as judged from both U-tube and W-tube through-model-membrane transport experiments. The first member of this family, system **6**, was found to display an inherent preference for phenylalanine > tryptophan > tyrosine. Further, L-amino acids were shown to be transported with greater efficiency than the corresponding D-enantiomers by this particular carrier. The high level of amino acid carrier capability displayed by receptor **6** in dichloromethane solutions correlates well with the results of equilibrium binding studies carried out using visible-spectroscopic titrations. These latter studies revealed that system **6** does display significant affinity for zwitterionic amino acids in this organic solvent. These binding studies, as well as a number of control experiments involving, inter alia, porphyrin – lasalocid conjugate **7**, showed the importance of having both the sapphyrin and lasalocid subunits contained within the same overall receptor framework. The four other second-generation sapphyrin – lasalocid conjugates reported here (**11** – **14**) were also tested as carriers for the transport of Phe, Trp, and Tyr. It was found that the esterified systems **11** and **12** functioned well as amino acid carriers, while the free-acid compounds **13** and **14** did not. These latter conjugates, containing both carboxylic acid and sapphyrin subunits, presumably undergo self-assembly in organic solutions, a process that hampers their ability to act as effective carriers. In the case of the functioning systems **11** and **12**, the configuration of the stereogenic phenylalanine appendages could be varied such that either the L- or D-antipodes of the aromatic amino acid substrates being studied were transported at a greater rate.

Keywords: amino acids · ionophores · molecular recognition · sapphyrin · amino acid transport

Introduction

Nature uses amino acids as the building blocks for construction of proteins,[1] and as molecular messengers capable of transmitting information in living organisms.[2] Therefore, elaborate procedures for synthesizing, manipulating, and transporting these key biological molecules have developed. In all cases, the critical processes rely on molecular recognition of amino acids, which provides the basis for a precise, selective read out of the chemical information inherent in their structure and functionality.[3] Given the importance of the common amino acids, it is not surprising that supramolecular chemists have long been interested in designing abiotic receptors and carriers for this group of compounds.[4–6] Here, some of the motivating factors have come from a need to develop efficient methods of amino acid sensing and analysis,[7] and from a desire to extend what is learned with amino acids into the arena of peptide recognition.[8]

As a part of a program concerned with the generation of efficient methods for effecting the trans-membrane transport of important biological species,[9] we became interested in developing carriers capable of facilitating the transport of α-amino acids across lipophilic barriers. At present, only a minute amount of information is available concerning the dynamics and regulation of amino acid transport in nature.[10] Hence, our approach to the construction of abiotic amino acid carriers has been guided as much by intuition as by an analysis of natural amino acid binding systems. The very structure of amino acids, however, invites us to explore a variety of possible host – guest interactions. In particular, the zwitterionic form of this species contains a negatively charged carboxylate group, a positively charged ammonium moiety, and a side chain. Therefore, we came to appreciate that an ideal carrier for amino acids would a) have different, nonself-complementary binding sites capable of recognizing all three parts of a putative amino acid substrate, b) have enough extraction power to compensate for the energetic desolvation costs associated with bringing the heavily hydrated carbox-

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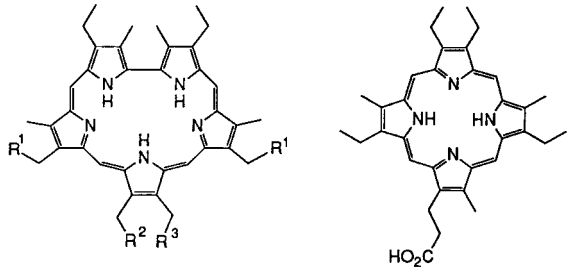
ylate and ammonium groups out of the aqueous environment, c) be able to release the substrates into an aqueous receiving phase rapidly, and, ideally, d) display stereochemically dependent interactions with the substrates, thereby allowing for their enantioselective transport.[11]

The above design requirements are, of course, very severe. Compounding the problem is the fact that the ammonium and carboxylate functionalities of α-amino acids are tied up in the form of a zwitterion. This makes each of these functional groups more difficult to chelate.[12] In view of this, much work in the area has been concerned with the problem of recognizing derivatized amino acids (i.e., those in which either the carboxylate or ammonium group is protected).[5,6] For the same reason, many of the amino acid transport studies have been carried out under conditions of either high or low pH.[13]

In this paper we report the synthesis of carriers designed to effect the selective transport of zwitterionic α-amino acids. We have shown previously that sapphyrins (cf. parent structure **1**), when protonated,[14] unlike their simple porphyrin congeners (e.g., **2**), act as excellent receptors for a variety of anions,[9b,d–h,15] including carboxylates.[16] Comprehensive solution and solid-state data[17] show that the bound carboxylate anion is located above the protonated sapphyrin plane, held in place by a combination of electrostatic interactions and NH-to-O hydrogen bonds. Given this, it was surmised that a sapphyrin subunit, once conjugated to an appropriate receptor for ammonium groups, would function as a receptor for zwitterionic amino acids.



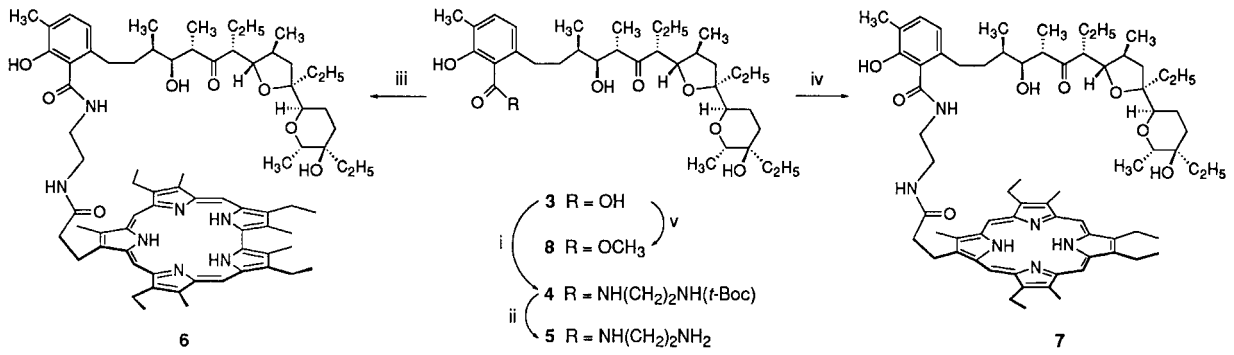
1a $R^1 = CH_3$; $R^2 = H$; $R^3 = CH_2CO_2H$
1b $R^1 = CH_3$; $R^2 = H$; $R^3 = CH_2CONH(CH_2)_2NH(t\text{-Boc})$
1c $R^1 = CO_2H$; $R^2 = R^3 = CH_3$

2

As our ammonium binding group, we have chosen the naturally occurring polyether ionophore lasalocid (cf. parent structure **3**, Scheme 1).[18–20] Lasalocid itself is composed of a conformationally constrained cyclic polyether chain terminated at one end by a carboxylic acid and at the other end by a hydroxyl group. These structural features allow lasalocid to form intramolecular head-to-tail hydrogen bonds, thereby generating a pseudocyclic cavity capable of binding cations. Indeed, lasalocid is known to bind and transport a range of cations across both biological and artificial membranes.[19,20] Importantly, lasalocid has been shown to effect the enantioselective recognition of chiral ammonium cations.[20a–c] On this basis it was thought that a covalently connected sapphyrin–lasalocid conjugate might indeed function as a bona fide amino acid receptor.[21] In particular, it was expected that the protonated sapphyrin portion of the conjugate would bind the carboxylate portion of an α-amino acid,[22] while the lasalocid subunit would simultaneously chelate the corresponding protonated amino group. We expected that such a receptor, featuring non-self-complementary binding sites, would be free of problems associated with internal collapse. Further, since it would contain an asymmetric cavity, this kind of system might function as an enantioselective amino acid binding agent. We have thus prepared and recently reported in communication form the synthesis of a prototypical sapphyrin–lasalocid conjugate **6**.[15e] In this paper full details as to the recognition and transport properties of this system are given. Also reported are several second-generation sapphyrin-lasalocid conjugates (**11**–**14**) which bear extra, chiral, phenylalanine-based covalent appendages. As expected, these systems do in fact bind and transport zwitterionic α-amino acids through model membrane barriers with good selectivity.

Results and Discussion

Synthesis of the sapphyrin–lasalocid conjugate 6 and porphyrin–lasalocid conjugate 7: The sapphyrin–lasalocid conjugate **6** was prepared from its constituents in a stepwise fashion as illustrated in Scheme 1. First, lasalocid (**3**) was coupled with mono-*tert*-butyloxycarbonylethylenediamine,[23] yielding the amide **4**. After deprotection with TFA, the resulting lasalocid amino derivative **5** was treated with the



Scheme 1. Synthesis of sapphyrin–lasalocid conjugate **6**, porphyrin–lasalocid conjugate **7**, and lasalocid methyl ester **8**. Reagents and conditions: i) $H_2N(CH_2)_2NH(t\text{-Boc})$, EDC, HOBt, Py, DMF, 72 h; ii) TFA, CH_2Cl_2, 2 h; iii) **1a**, EDC, HOBt, Py, DMF, 48 h; iv) **2**, EDC, HOBt, Py, DMF, 48 h; v) EDC, HOBt, MeOH, 48 h.

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sapphyrin monoacid **1a**[9d] in DMF, using EDC as a coupling agent. Compound **6** was isolated in approximately 73% yield after careful chromatographic purification. By means of the same approach, the structurally cognate porphyrin – lasalocid conjugate **7** was synthesized from the porphyrin monoacid **2**[24] as a control for compound **6**. To the best of our knowledge, these systems constitute the first examples of porphyrin and expanded-porphyrin appended lasalocids.[15e]

U-tube amino acid transport studies with conjugates 6 and 7: The aromatic α-amino acids phenylalanine, tryptophan, and tyrosine were selected for the transport studies, because a) the side chains of these amino acids could provide for additional, stereogenic van der Waals-type interactions involving both the aromatic surfaces of the sapphyrin macrocycle and the salicylate portion of the lasalocid subunit (thereby favoring selective transport), and b) the transport rates of these aromatic substrates can be monitored easily by HPLC analyses (by means of a UV detector). The transport experiments themselves were then run using a Pressman-type U-tube model membrane system.[25] Here, the initial phase, Aq. I, was charged with either a solution of the particular zwitterionic α-amino acid under study, or a mixture of all three amino acids being considered (Phe, Trp, Tyr), while the receiving phase, Aq. II, was made basic to increase the off-rates.[26]

It was found that at neutral pH compound **6** acts as a very efficient carrier for phenylalanine and tryptophan, but not tyrosine (Table 1, Figure 1). When this same system was tested under conditions of competitive transport, it was found that L-phenylalanine was transported four times faster than L-tryptophan and a thousand times faster than L-tyrosine (Table 1, entry 1). Interestingly, L-amino acids were transported with greater efficiency by this carrier than the corresponding D-antipodes. Taken together, these results are consistent with a high level of amino-acid-based selectivity, and provide clear evidence that the amino acid side chains actively participate in the binding and transport process.

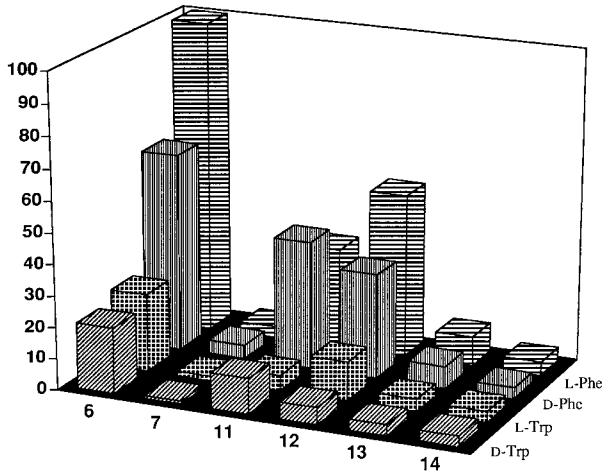

Figure 1. Relative rates of L-Phe and L-Trp transport as determined from U-tube model membrane experiments.

To help us understand more fully the above results, a number of control experiments were then carried out. These were designed to elucidate the roles the constituent parts of **6** could be playing in terms of mediating the overall transport phenomenon. For instance, in order to probe the role of carboxylate binding, the porphyrin – lasalocid conjugate **7** was tested. This system proved inefficacious for amino acid transport (entry 2). Likewise, lasalocid itself (**3**) proved incapable of effecting amino acid transport (entry 4). Taken together, these results support the contention that sapphyrin-based carboxylate binding plays a critical role in mediating the amino acid transport effected by conjugate **6**.

Interestingly, only modest transport was achieved by use of sapphyrin **1b**[27] either alone (entry 3), or as a mixture with **3** (entry 5).[28] In the latter case one could envision the formation of putative supramolecular complexes between protonated sapphyrin **1b** and lasalocid **3** as a result of lasalocid carboxylate chelation by sapphyrin. Formation of

Table 1. Amino acid transport rates measured in a U-tube (k_t) [a, b].

	Carrier	k_t (10^{-5} mol cm^{-2} h^{-1})						$k_F/k_W/k_Y$ (L) [c]	$k_F/k_W/k_Y$ (D) [c]
		L-Phe	D-Phe	L-Trp	D-Trp	L-Tyr	D-Tyr		
1	**6** [d]	20.0	12.7	5.0	4.2	0.02	0.02	1000/250/1	635/210/1
2	**7** [d]	0.7	0.9	0.3	0.2	< 0.001	< 0.001	2.3/1	4.5/1
3	**1b** [d]	6.9	NA [e]	1.4	NA [e]	< 0.001	NA [e]	4.9/1	NA [e]
4	**3** [d]	0.5	0.4	0.2	0.2	< 0.001	< 0.001	2.5/1	2/1
5	**1b** + **3** [f]	3.2	3.1	0.9	0.9	0.2	0.2	16/4.5/1	15.5/4.5/1
6	**1b** + **8** [g]	7.8	7.2	1.4	1.4	0.5	0.6	15.6/2.8/1	12/2.3/1
7	**8** [d]	0.8	0.8	0.2	0.2	< 0.001	< 0.001	4/1	4/1
8	**11** [d]	6.4	8.2	1.0	2.3	< 0.001	< 0.001	6.4/1	3.6/1
9	**12** [d]	10.5	6.7	2.5	1.1	< 0.001	< 0.001	4.2/1	6.1/1
10	**13** [d]	1.9	1.4	0.8	0.7	< 0.001	< 0.001	2.4/1	2/1
11	**14** [d]	0.9	0.7	0.7	0.7	< 0.001	< 0.001	1.3/1	1/1
12	none	0.05	0.05	0.01	0.01	< 0.001	< 0.001	NA [e]	NA [e]

[a] Transport experiments were performed as described in ref. [9d] under competitive conditions (with respect to amino acid substrates): the initial rate values given for L-Phe, L-Trp, and L-Tyr transport are thus obtained from experiments involving mixtures of L-Phe (50 mM), L-Trp (50 mM), and L-Tyr (5 mM) in the initial aqueous phase, Aq. I. Likewise, those given for D-Phe, D-Trp and D-Tyr are derived from studies involving analogous mixtures of D-Phe (50 mM), D-Trp (50 mM) and D-Tyr (5 mM) in Aq. I. In all runs 10 mM NaOH was used as the receiving phase, Aq. II. [b] Values are averages of two or three separate experimental runs; estimated errors are ±15%. [c] Single-letter symbols for amino acids are used: F = Phe, W = Trp, and Y = Tyr. When Tyr initial transport rate was less than 10^{-8} mol cm^{-2} h^{-1}, the k_F/k_W ratio was used instead of $k_F/k_W/k_Y$. [d] 1×10^{-4} M in dichloromethane. [e] NA = not measured due to achirality of the carrier. [f] Organic phase containing a mixture of **1b** (1×10^{-4} M) and **3** (1×10^{-4} M) in dichloromethane was used. [g] A mixture of **1b** (1×10^{-4} M) and **8** (1×10^{-4} M) in dichloromethane was used as the organic phase.

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such complexes would inevitably reduce the rate of amino acid transport by the mixed **1b** + **3** system. To exclude this possibility, a control experiment involving a mixture of sapphyrin **1b** and lasalocid methyl ester **8** (synthesized as shown in Scheme 1) was carried out (entry 6). Even in this case, however, no significant increase in transport rate was observed compared to the **1b** + **3** mixture.

Amino acid binding efficacy in dichloromethane was assessed quantitatively by visible-light spectroscopic titrations. Upon addition of aromatic amino acids, the Soret maximum of conjugate **6** underwent an approximate 5 nm blue shift from 452 nm to 447 nm that was accompanied by the growing-in of a new band also at 447 nm. By following the increase in absorbance at 447 nm as a function of substrate-to-receptor ratio, we determined both the relative association constants (K_a) and the stoichiometry of binding.[29] For instance, it was found that compound **6** forms 1:1 complexes with phenylalanine and tryptophan with association constants (K_a) as follows: K_a(L-Phe) = $4.86 \times 10^5\,M^{-1}$, K_a(D-Phe) = $5.35 \times 10^5\,M^{-1}$, K_a(L-Trp) = $0.83 \times 10^5\,M^{-1}$, K_a(D-Trp) = $0.94 \times 10^5\,M^{-1}$.[15e]

The high levels of absolute receptor-to-substrate binding affinity and amino acid binding selectivity are in accord with the transport results. However, the finding that the L-enantiomers are bound with the same affinity (within error) as the D-congeners may reflect the fact that not only binding affinities, but also the substrate release rates, are important in terms of mediating transport efficiency.[3b] Consistent with this contention is the fact that when water was used as a receiving phase (as opposed to a sodium hydroxide solution), the L/D ratio of the transport rates was found to be still further amplified (Table 2, entries 1, 2).

Table 2. Amino acid transport rates (k_t) determined from U-tube experiments using carrier **6** [a].

	Carrier	Aq. II	k_t (10^{-5} mol cm^{-2} h^{-1}) L-Phe	D-Phe
1	**6** [b]	10 mM NaOH	23.9	15.4
2	**6** [b]	H_2O	21.7	10.2
3	**6** + Cl^- [c]	10 mM NaOH	19.4	14.3
4	**6** + F^- [d]	10 mM NaOH	19.3	13.8

[a] In these experiments, the initial phase, Aq. I, contained a single amino acid: either L-Phe (50 mM) or D-Phe (50 mM). [b] 1×10^{-4} M in dichloromethane. [c] A mixture of **6** (1×10^{-4} M) and Bu_4NCl (1×10^{-4} M) in dichloromethane was used as the organic phase. [d] A mixture of **6** (1×10^{-4} M) and Bu_4NF (1×10^{-4} M) in dichloromethane was used as the organic phase.

Taken together, the above results lead us to propose the transport mechanism outlined in Figure 2. In particular, it is suggested that carrier **6**, featuring a monoprotonated sapphyrin macrocycle[22] and a deprotonated lasalocid,[30] binds zwitterionic amino acid at the Aq. I/CH_2Cl_2 interface, forming a neutral complex.[31] This complex then diffuses through the membrane and is released in the more basic receiving phase, Aq. II. The amino acid thus transported then reacts with NaOH to form an amino acid sodium salt. This transformation and sapphyrin deprotonation at the CH_2Cl_2/Aq. II interface facilitates product release. Once at this interface, the lasalocid

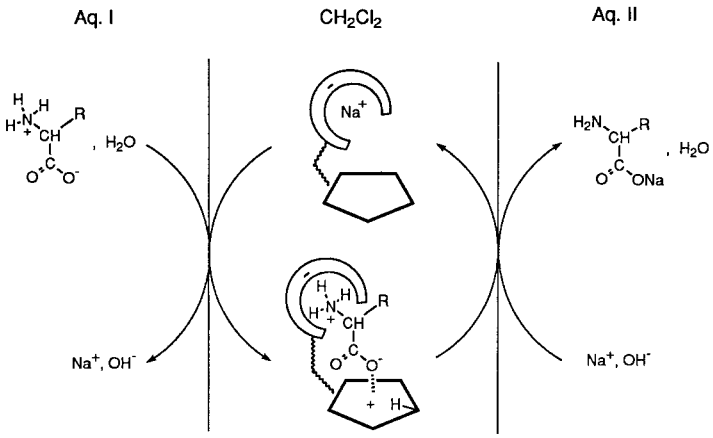

Figure 2. Proposed mechanism for amino acid transport catalyzed by the sapphyrin–lasalocid conjugate **6**. Pentagon, sapphyrin; horseshoe, lasalocid.

part of carrier **6** chelates a sodium cation and carries it back to the Aq. I/CH_2Cl_2 interface. Release of Na^+ and protonation of the sapphyrin macrocycle then occurs at this locus. This, in turn, regenerates carrier **6** in a form ready to effect amino acid transport again. Consistent with this mechanism is the fact that addition of anions (e.g., Cl^-, F^-)[32] to the organic phase did not result in a significant change in the transport rates (Table 2, entries 3, 4). This is most likely because the putative complex of the conjugate **6** with amino acids is a neutral, tightly bound entity, and does not require any extra anionic species for its efficient through-membrane transport. Also in accord with the proposed mechanism was the detection of countertransported Na^+ cations in Aq. I (see Experimental Section), and the observation of an increase in the pH of Aq. I ($\approx$0.3 pH units over 24 h) over the course of the transport experiments.[33,34]

Taken together, these results lead us to conclude that system **6** is an efficient carrier for aromatic α-amino acids and one that shows inherent selectivity within this limited substrate subset. This receptor and its amino acid substrates react to form lipophilic, well-defined supramolecular complexes that are stabilized by stereogenic multiple point interactions. The stable, neutral character of these complexes provides the basis for the fast, enantiomerically dependent amino acid transport observed in the model U-tube membranes.

Synthesis and U-tube amino acid transport of the second-generation sapphyrin–lasalocid conjugates 11–14: Encouraged by the successful transport results obtained using **6** as a carrier, we decided to take this methodology a step further and to prepare two new, second-generation sapphyrin–lasalocid conjugates **13** and **14**, featuring phenylalanine-derived carboxylate appendages. The reasons for this were threefold: first, we were interested to see whether the use of a zwitterionic carrier would be advantageous for the transport of zwitterionic amino acids. Secondly, we wanted to examine how the chirality of phenylalanine adjuvants affects the amino acid transport selectivity. Finally, on a more practical level, we considered that the carboxylate tail of **13** and **14** could provide

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a handle allowing these receptors to be attached to a solid support. These latter materials could act as chromatographic media for the separation and analysis of amino acid mixtures.[35] Here we realized, of course, that the protonated sapphyrin macrocycle and the anionic carboxylate of **13** are mutually complementary. This feature could result in the formation of the higher-order self-assembled aggregates of **13** in solution (e.g., non-covalent dimers), as has been shown previously with the zwitterionic sapphyrin monoacid **1a**[16b, 36] and with the other systems.[37] Therefore, we were interested to see which process (self-assembly versus amino acid binding) is dominant under conditions of our transport studies.

The synthesis of compound **13** is depicted in Scheme 2. The sapphyrin bis-acid **1c** was coupled with one equivalent of L-phenylalanine *tert*-butyl ester to yield monoacid monoamide **9** in 67% yield after careful chromatographic purification. Compound **9** was further conjugated with the lasalocid amino derivative **5** to yield the protected product **11**. It was then deprotected with TFA to produce the desired compound **13**. Transport studies using **13** showed, however, that it is a very poor carrier for aromatic zwitterionic α-amino acids (Table 1, entry 10, and Figure 1). This is because it self-assembles in organic media and this prevents it from binding an amino acid and effecting the through-membrane transport of these substrates.[38] Not so with its precursor **11**! This compound, when used in our standard U-tube model membrane transport studies, proved to be a much better carrier than **13** for both phenylalanine and tryptophan (Table 1, entry 8).

Interestingly, the enantiomeric selectivity of transport with carrier **11** was reversed compared to **6**. In particular, D-enantiomers of amino acid substrates were transported somewhat faster by **11** than the L-ones. This effect is clearly attributable to the presence of the L-phenylalanine appendage in **11**. Specifically, it was considered likely that the chirality of the lasalocid component and the L-phenylalanine *tert*-butyl ester moiety act to exert a contradictory effect on amino acid transport selectivity. It was thus thought that replacing the L-phenylalanine *tert*-butyl ester with its D-analogue would lead to a more synergistic effect.

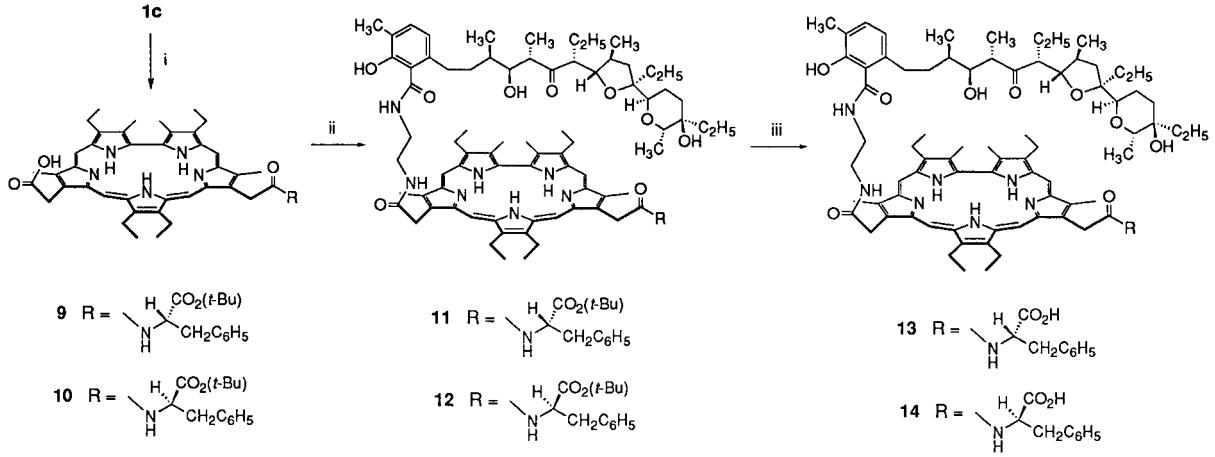
To test this idea, compounds **12** and **14** were prepared; these incorporate a D-phenylalanine residue instead of the L-phenylalanine subunit found in **11** and **13** (Scheme 2). This inversion of chirality in the phenylalanine appendage led, as expected, to a reversal (compared to compound **11**) of the observed amino acid transport selectivity. In particular, when the U-tube transport experiments were carried out with conjugate **12** (conditions as described in Table 1), L-Phe was found to be transported 1.6 times faster than D-Phe. Likewise, L-Trp was found to be transported 2.3 times faster than D-Trp (Table 1, entry 9).[39,40]

W-Tube transport experiments: To compare aromatic α-amino acid transport rates under conditions where two carriers were competing for two different amino acids (e.g. phenylalanine and tryptophan), transport experiments were carried out using a W-tube model membrane.[41] These W-tube experiments involve Aq. II/organic/Aq. I/organic/Aq. III arrangements and provide a better way of comparing efficiencies and selectivities under competitive conditions. They could also provide a better model for the chemical conditions of nature wherein a variety of ion-transporting entities are found to compete simultaneously for the same pool of ions. In the present instance, we chose to compare **6**, the most efficient carrier, with each of the second-best systems, **11** and **12**. The results obtained (Table 3) are consistent with what one would

Table 3. Amino acid transport rates (k_t) determined from W-tube experiments carried out under competitive conditions.

	Carrier		Amino acids	Left arm [a]		Right arm [a]	
	left arm	right arm		Phe	Trp	Phe	Trp
1	**6**	**11**	L-Phe, L-Trp [b]	10.3	2.1	3.0	1.1
2	**6**	**11**	D-Phe, D-Trp [c]	7.6	1.8	4.9	2.3
3	**6**	**12**	L-Phe, L-Trp [b]	11.5	2.3	8.7	1.9
4	**6**	**12**	D-Phe, D-Trp [c]	7.7	1.8	4.2	1.0

[a] Initial amino acid transport rates (k_t, 10^{-5} mol cm^{-2} h^{-1}) were measured by HPLC (UV detection). Two separated organic phases containing dichloromethane solutions of competing carrier compounds (1×10^{-4} M in each) were put in contact with the same initial phase, Aq. I. In all runs 10 mM NaOH was used as the two receiving phases, Aq. II and Aq. III. [b] The initial phase, Aq. I, contained mixtures of L-Phe (50 mM) and L-Trp (50 mM). [c] The initial phase, Aq. I, contained mixtures of D-Phe (50 mM) and D-Trp (50 mM).



Scheme 2. Synthesis of sapphyrin – lasalocid conjugates **11** – **14.** Reagents and conditions: i) L- or D-phenylalanine *tert*-butyl ester hydrochloride, EDC, HOBt, Py, DMF, 72 h; ii) **5**, EDC, HOBt, Py, DMF, 48 h; iii) TFA, CH_2Cl_2, 4 h.

expect based on the predicative U-tube studies (Table 1). In particular, phenylalanine was found to be transported faster than tryptophan by all carriers under investigation. Likewise, the enantiomeric selectivity of all carriers was found to correlate well with what was concluded from the U-tube experiments. In certain cases, however, the actual $k(L)/k(D)$ transport rate ratio was found to be enhanced in the W-tube experiments as compared to the U-tube ones.[42]

Conclusions

The present results have served to confirm that sapphyrin–lasalocid conjugates which feature binding sites for both carboxylate anion complexation and ammonium group recognition, can act as effective carriers for zwitterionic aromatic α-amino acids as judged from simple Pressman-type U-tube model membrane experiments. In the case of carrier **6**, selectivity for phenylalanine over tryptophan was observed. It was also found that tyrosine is not transported to any significant extent. Also, L-amino acids were found to be transported faster than their D-congeners using this carrier. In analogy to what is often observed in nature,[10c] the mechanism is thought to involve sodium-cation antiport. Specifically, the amino acid substrates from the initial aqueous phase are transported through the organic phase and into the receiving aqueous phase at the expense of sodium being carried back from Aq. II to Aq. I. The transport process itself is thought to be predicated upon formation of a tight supramolecular complex between **6** and its amino acid substrates as illustrated in Figure 2.

In the case of the second-generation conjugates **11**–**14** with chiral phenylalanine adjuvants (in addition to the sapphyrin and lasalocid moieties present in **6**), a clear difference was observed between the free acid (**13** and **14**) and esterified (**11** and **12**) forms. The former did not effect amino acid transport, presumably as the result of the carrier undergoing self-assembly in the organic phase. By contrast, the esterified systems **11** and **12** showed utility as amino acid transport enhancing agents. In fact, depending on the chirality of the phenylalanine appendage used, either L- or D-enantiomers of amino acid substrates were transported faster. This leads us to suggest that the approach illustrated here could provide a generalized basis for the design of other, module-based carriers capable of binding and transporting selectively a full range of chiral substrates.

Experimental Section

General materials and methods: ^{1}H and ^{13}C NMR spectra were recorded on General Electric QE-300 (300 MHz), GE GN-500 (500 MHz), and Bruker AM-500 (500 MHz) instruments. Visible spectra were recorded on a Beckman DU640 instrument with cuvettes of 1 cm path length. Sapphyrin mono- and bisacids **1a** and **1c** were prepared according to procedures described previously.[9d] Porphyrin monoacid **2** was synthesized as previously described.[24] Mono-*tert*-butyloxycarbonylethylenediamine was prepared according to a literature procedure.[23] The sodium salt of lasalocid, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 1,1′-carbonyldiimidazole, 1-hydroxybenzotriazole hydrate (HOBt), pyridine, trifluoroacetic acid, and dimethylformamide were purchased from Aldrich. Phenylalanine, tryptophan, tyrosine, and L-phenylalanine *tert*-butyl ester hydrochloride were purchased from Sigma. D-Phenylalanine *tert*-butyl ester hydrochloride was purchased from Advanced Chemtech. Transport experiments were performed using a standard glass U-tube, or modified W-tube at 293 K.[5a,25] The release of the amino acid substrates into the receiving phase was monitored as a function of time by HPLC product analysis (Varian, equipped with a UV detector; $\lambda =$ 209 nm). In all cases, control experiments were performed in the absence of the carrier. Errors are less than 15%. The presence of countertransported Na^+ ions in the Aq. I phase was confirmed by observation of the intense peaks corresponding to the [2.2.1] Na^+ cryptate in the FAB mass spectra of samples prepared from 4,7,13,16,21-pentaoxa-1,10-diazabicyclo[8.8.5]tricosane ([2.2.1] cryptand) mixed with solutions taken from Aq. I; these measurements were made 24 h after the transport experiments were commenced. Binding studies were effected by means of visible spectroscopic titrations (Beckman DU640) and were carried out at 293 K in dichloromethane. All receptors were prepared by washing organic solutions (dichloromethane containing 10% methanol) twice with 10% $NaOH_{aq}$ and three times with water. They were then taken to dryness on the rotorary evaporator and dried in vacuo.

Sapphyrin 1b: Sapphyrin monoacid **1a** (189 mg, 0.3 mmol) was dissolved under argon in anhydrous DMF (10 mL) containing dry pyridine (Py) (0.1 mL). A solution of 1,1′-carbonyldiimidazole (97 mg, 0.6 mmol) in anhydrous DMF (2 mL) was then added, followed by HOBt (13.5 mg, 0.1 mmol). The resulting mixture was stirred at room temperature under argon for 2 h. A solution of mono-*tert*-butyloxycarbonylethylenediamine (96 mg, 0.6 mmol) in anhydrous DMF (3 mL) was then added, and the resulting reaction mixture stirred at room temperature under argon for 24 h. The protected amine **1b** that resulted was then concentrated by means of a rotorary evaporator and purified by column chromatography on silica gel as the solid support and with a gradient of 2–7% methanol in dichloromethane as the eluent. Solvents were then evaporated off, and the residue redissolved in dichloromethane containing 20% methanol (100 mL). This solution was washed consecutively twice with 1M NaOH (30 mL), and three times with water. The organic phase was evaporated and the product dried in vacuo. The yield of compound **1b** was 185 mg (free base, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = -3.39$ (br s, 3H, pyrrole NH), 0.88 (s, 9H, CH_3), 1.81–1.97 (m, 12H, CH_3), 2.63 (br s, 2H, CH_2), 2.88 (br s, 2H, CH_2), 3.03 (br s, 2H, CH_2), 3.73 (s, 3H, CH_3), 3.74 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 3.86 (s, 6H, CH_3), 4.18 (m, 4H, CH_2), 4.35 (m, 4H, CH_2), 4.59 (t, ^3J (H,H) = 8 Hz, 2H, CH_2), 5.80 (br s, 1H, NH), 10.49 (s, 1H, CH), 10.56 (s, 1H, CH), 10.61 (s, 1H, CH), 10.63 (s, 1H, CH); ^{13}C NMR (75 MHz, $CDCl_3$, with 5% CD_3OD): $\delta = 12.12$, 12.50, 15.91, 17.59, 20.34, 20.45, 20.56, 23.24, 27.74, 39.14, 39.42, 39.51, 77.21, 78.87, 90.07, 90.34, 96.16, 126.92, 132.81, 132.97, 133.61, 133.94, 134.04, 134.17, 135.80, 135.92, 137.14, 138.38, 139.28, 139.44, 140.02, 140.35, 142.32, 156.23, 173.16; HRMS (FAB) calcd for $C_{47}H_{62}N_7O_3$ $[M+H]^+$ 772.4914, observed 772.4922.

***t*-Boc-protected aminolasalocid 4**: The sodium salt of lasalocid **3** (123 mg, 0.2 mmol), mono *tert*-butyloxycarbonylethylenediamine (64 mg, 0.4 mmol), and dry Py (0.1 mL) were dissolved in anhydrous DMF (10 mL) under argon. A solution of EDC (76 mg, 0.4 mmol) in anhydrous DMF (2 mL) was then added, followed by a catalytic amount of HOBt. The resulting reaction mixture was stirred at room temperature under argon for 72 h. The solvents were then evaporated, the solids dried in vacuo, and the product was purified by column chromatography on silica gel as the solid support and with a gradient of 2–5% methanol in dichloromethane as the eluent. The yield of compound **4** was 107 mg (Na^+ salt, 71%). ^{1}H NMR (300 MHz, CD_2Cl_2): $\delta = 0.74$–0.86 (m, 12H, CH_3), 0.93 (d, ^3J (H,H) = 7 Hz, 3H, CH_3), 1.02 (d, ^3J (H,H) = 6.7 Hz, 3H, CH_3), 1.12 (d, ^3J (H,H) = 7.7 Hz, 3H, CH_3), 1.20 (m, 3H), 1.39 (s, 9H, CH_3), 1.30–1.90 (m, 12H, CH and CH_2), 2.16 (s, 3H, CH_3), 2.20 (m, 1H, CH), 2.58–2.74 (m, 2H, CH_2), 2.89–2.95 (m, 2H, CH_2), 3.38 (m, 4H, CH and CH_2), 3.66 (m, 2H, CH), 3.91 (m, 1H, CH), 4.12 (m, 1H, CH), 5.60 (m, 1H, NH), 6.65 (d, ^3J (H,H) = 8.3 Hz, 1H, CH), 6.86 (s, 1H, NH), 7.07 (d, ^3J (H,H) = 8.6 Hz, 1H, CH), 10.34 (s, 1H, OH); ^{13}C NMR (75 MHz, CD_2Cl_2): $\delta = 6.47$, 8.98, 12.85, 13.72, 14.39, 15.56, 15.91, 17.07, 21.06, 28.53, 30.14, 30.81, 31.02, 31.60, 34.07, 35.04, 36.82, 38.78, 40.50, 40.83, 48.43, 54.67, 70.59, 71.26, 72.88, 77.74, 79.25, 85.15, 87.15, 118.19, 121.33, 123.77, 133.00, 138.84, 156.88, 157.08, 171.25, 213.62; HRMS (FAB) calcd for $C_{41}H_{69}N_2O_9$ $[M+H]^+$ 733.5003, observed 733.4987.

Aminolasalocid 5: *t*-Boc-protected **4** (90 mg, 0.12 mmol) was deprotected by treatment with 3:1 dichloromethane/TFA mixture (5 mL) for ca. 2 h

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(TLC control). Solvents were then evaporated off, and the residue purified chromatographically on silica gel as the solid support and 10% methanol (saturated with NH_3 gas) in dichloromethane as the eluent. Fractions with $R_f = 0.2 - 0.5$ were collected. The yield of compound **5** was 63 mg (84%). ^{1}H NMR (300 MHz, CD_2Cl_2): $\delta = 0.74 - 0.89$ (m, 15H, CH_3), 1.01 (d, ^3J (H,H) = 7 Hz, 3H, CH_3), 1.13 (d, ^3J (H,H) = 7.7 Hz, 3H, CH_3), 1.20 – 1.90 (m, 15H, CH and CH_2), 2.14 (s, 3H, CH_3), 2.20 (m, 1H, CH), 2.45 – 2.60 (m, 2H, CH_2), 2.75 – 2.94 (m, 4H, CH and CH_2), 3.33 – 3.42 (m, 2H, CH), 3.65 – 3.73 (m, 2H, CH), 3.87 (m, 1H, CH), 3.98 (m, 1H, CH), 6.59 (d, ^3J (H,H) = 8.7 Hz, 1H, CH), 7.01 (d, ^3J (H,H) = 8.3 Hz, 1H, CH); ^{13}C NMR (75 MHz, CD_2Cl_2): $\delta = 6.54$, 9.00, 12.84, 13.59, 14.40, 15.73, 16.15, 16.85, 20.61, 30.09, 30.50, 31.26, 31.31, 34.12, 35.36, 36.65, 39.05, 41.28, 48.52, 55.10, 70.82, 71.76, 72.15, 77.65, 84.80, 86.51, 120.41, 121.09, 123.73, 132.25, 138.72, 155.89, 171.26, 214.80; HRMS (FAB) calcd for $C_{36}H_{61}N_2O_7$ $[M+H]^+$ 633.4479, observed 633.4466.

Sapphyrin – lasalocid conjugate 6: The sapphyrin mono acid **1a** (189 mg, 0.3 mmol) and aminolasalocid **5** (379 mg, 0.6 mmol) were dissolved under argon in anhydrous DMF (10 mL) containing dry Py (0.1 mL). A solution of EDC (115 mg, 0.6 mmol) in anhydrous DMF (4 mL) was then added, followed by HOBt (13.5 mg, 0.1 mmol). The resulting reaction mixture was stirred at room temperature under argon for 48 h. The solvents were evaporated off, and the resulting solids were then dried in vacuo. The product was then purified chromatographically on silica gel as the solid support and with a gradient of 2 – 15% methanol in dichloromethane as the eluent. The yield of compound **6** was 272 mg (73%). ^{1}H NMR (300 MHz, CD_2Cl_2): $\delta = 0.49 - 1.70$ (m, 33H), 1.82 (br s, 2H), 1.91 – 2.15 (m, 13H), 2.13 (s, 3H, CH_3), 2.14 (m, 1H, CH), 2.47 (m, 2H), 2.69 (m, 2H), 3.16 (m, 4H), 3.13 (m, 2H), 3.45 (m, 2H), 3.64 (m, 4H), 3.84 (s, 3H, CH_3), 3.87 (s, 3H, CH_3), 4.01 (m, 3H, CH_3), 4.06 (m, 3H, CH_3), 4.08 (m, 3H, CH_3), 4.35 (m, 4H, CH_2), 4.57 (m, 4H, CH_2), 4.87 (m, 2H, CH_2), 6.37 (d, ^3J (H,H) = 8.7 Hz, 1H, CH), 6.72 (d, ^3J (H,H) = 8.3 Hz, 1H, CH), 10.51 (s, 1H, CH), 10.60 (s, 1H, CH), 10.64 (s, 1H, CH), 10.72 (s, 1H, CH); ^{13}C NMR (126 MHz, CD_2Cl_2): $\delta = 6.33$, 6.44, 8.44, 8.51, 11.96, 12.43, 12.60, 13.05, 13.14, 13.59, 13.83, 13.99, 14.10, 15.59, 15.67, 15.81, 16.42, 16.45, 17.45, 17.78, 18.08, 18.15, 20.61, 20.86, 20.97, 21.21, 23.57, 29.37, 29.51, 29.74, 29.96, 30.13, 30.71, 30.95, 31.88, 33.76, 34.29, 34.39, 35.29, 35.53, 35.86, 36.75, 38.82, 39.29, 39.98, 40.44, 48.42, 48.68, 55.06, 70.27, 70.43, 71.15, 71.71, 73.76, 74.23, 77.13, 77.35, 84.49, 84.66, 85.99, 86.07, 90.23, 90.34, 96.64, 122.10, 124.45, 127.51, 128.71, 130.84, 134.04, 135.49, 135.66, 136.27, 137.06, 140.00, 140.21, 141.09, 141.89, 144.19, 160.95, 169.66, 172.81, 214.28, 215.05; HRMS (FAB) calcd for $C_{76}H_{106}N_7O_8$ $[M+H]^+$ 1244.8103, observed 1244.8122.

Porphyrin – lasalocid conjugate 7: This material was made by the procedure used for the synthesis of conjugate **6,** with the exception that the starting material was the porphyrin monoacid **2**. The yield of compound **7** obtained this way was 86%. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = -3.91$ (br s, 2H, pyrrole NH), 0.20 – 2.24 (m, 49H), 2.13 (s, 3H, CH_3), 2.40 – 4.10 (m, 31H), 4.37 (br s, 2H, CH_2), 5.30 (s, 1H, NH), 6.43 (d, ^3J (H,H) = 7.3 Hz, 1H, CH), 6.53 (s, 1H, NH), 6.95 (d, ^3J (H,H) = 7.3 Hz, 1H, CH), 10.05 (m, 4H, CH); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 6.91$, 6.14, 6.31, 8.16, 8.18, 8.19, 9.01, 11.39, 11.43, 11.56, 11.99, 12.29, 12.41, 12.53, 12.96, 12.99, 13.14, 13.26, 13.35, 13.81, 15.08, 15.33, 15.53, 15.95, 16.07, 17.57, 17.59, 17.64, 18.51, 18.53, 18.66, 28.37, 28.99, 29.04, 29.33, 30.13, 30.43, 32.75, 33.47, 33.75, 35.39, 37.04, 37.40, 39.50, 48.30, 54.31, 55.21, 67.65, 69.99, 70.20, 70.58, 76.31, 82.36, 86.01, 86.76, 96.08, 96.22, 96.37, 119.32, 121.96, 122.39, 122.83, 124.27, 131.22, 131.57, 136.99, 143.02, 154.92, 160.69, 170.48, 173.45, 175.92, 218.39; HRMS (FAB) calcd for $C_{70}H_{99}N_6O_8$ $[M+H]^+$ 1151.7524, observed 1151.7518.

Lasalocid methyl ester 8: The sodium salt of lasalocid **3** (95 mg, 0.16 mmol) and dry Py (0.1 mL) were dissolved in anhydrous methanol (10 mL). EDC (59 mg, 0.31 mmol) and a catalytic amount of HOBt were then added. The resulting reaction mixture was stirred at room temperature under argon for 48 h. The solvents were then evaporated off, and the resulting solids dried in vacuo. The resulting crude product was then purified by column chromatography using silica gel as the solid support and 3% methanol in dichloromethane as the eluent. Compound **8** eluted off the column first, and was followed by trace quantities of various unidentified by-products. The yield of compound **8** was 72 mg (Na^+ salt, 74%). ^{1}H NMR (300 MHz, CD_2Cl_2): $\delta = 0.78 - 0.86$ (m, 9H, CH_3), 0.92 – 0.94 (m, 6H, CH_3), 1.01 (d, ^3J (H,H) = 7 Hz, 3H, CH_3), 1.15 (d, ^3J (H,H) = 7.6 Hz, 3H, CH_3), 1.20 – 1.90 (m, 15H, CH and CH_2), 2.19 (s, 3H, CH_3), 2.20 (m, 1H, CH), 2.72 (m, 1H, CH), 2.88 – 3.00 (m, 3H, CH), 3.25 (br s, 1H, CH), 3.42 (m, 1H, CH), 3.71 (m, 1H, CH), 3.87 (m, 1H, CH), 3.96 (s, 3H, OCH_3), 6.68 (d, ^3J (H,H) = 8.3 Hz, 1H, CH), 7.17 (d, ^3J (H,H) = 8.3 Hz, 1H, CH), 11.45 (s, 1H, OH); ^{13}C NMR (75 MHz, CD_2Cl_2): $\delta = 6.60$, 8.78, 12.36, 12.86, 13.85, 14.37, 15.79, 15.95, 17.53, 21.20, 29.93, 30.74, 31.16, 34.71, 35.04, 35.30, 37.14, 39.19, 48.95, 52.51, 54.96, 70.46, 71.69, 74.41, 77.78, 85.08, 86.51, 111.57, 122.11, 124.35, 135.37, 144.06, 161.13, 172.85, 214.35; HRMS (FAB) calcd for $C_{35}H_{57}O_8$ $[M+H]^+$ 605.4053, observed 605.4063.

Sapphyrin mono acids 9 and 10: The sapphyrin bis acid **1c** (205 mg, 0.31 mmol) and phenylalanine *tert*-butyl ester hydrochloride (80 mg, 0.31 mmol) (L- or D- for the synthesis of **9** and **10**, respectively) were dissolved under argon in anhydrous DMF (10 mL) containing dry Py (0.1 mL). A solution of EDC (89 mg, 0.47 mmol) in anhydrous DMF (2 mL) was then added, followed by HOBt (13.5 mg, 0.1 mmol). The resulting reaction mixture was stirred at room temperature under argon for 72 h. The progress of the reaction was followed by TLC. Three major sapphyrin-containing spots were observed: that of a bisamide (highest R_f), followed by the monoamide monoacid (**9** or **10**, depending on antipode used), and the starting material, **1c** (lowest R_f). When the reaction was deemed complete, solvents were evaporated off, the solids dried in vacuo, and the product (middle fraction) was carefully isolated by column chromatography on silica gel as the solid support and with a gradient of 2 – 15% methanol in dichloromethane as the eluent. The yield of compound **9** or **10** was found to be of the order of 180 mg (67%). ^{1}H NMR (300 MHz, $CDCl_3$ with 5% CD_3OD): $\delta = 1.29$ (br s, 9H, CH_3), 2.24 (m, 12H, CH_3), 2.4 – 3.9 (m, 4H), 4.13 (s, 3H, CH_3), 4.25 (s, 3H, CH_3), 4.38 (s, 3H, CH_3), 3.95 – 5.12 (m, 14H), 5.63 (m, 1H, NH), 6.60 – 7.10 (m, 5H, phenyl CH), 10.88 (s, 1H, CH), 11.16 (s, 1H, CH), 11.75 (s, 2H, CH); ^{13}C NMR (75 MHz, $CDCl_3$, with 5% CD_3OD): $\delta = 11.92$, 14.76, 14.97, 16.02, 16.59, 17.66, 18.20, 19.85, 20.16, 20.26, 26.79, 33.39, 36.87, 54.27, 81.42, 86.83, 93.24, 97.14, 99.21, 126.04, 127.20, 127.59, 128.46, 128.79, 129.78, 132.18, 133.25, 134.79, 136.13, 139.64, 141.95, 142.70, 142.80, 143.34, 170.28; HRMS (FAB) calcd for $C_{53}H_{63}N_6O_5$ $[M+H]^+$ 863.4860, observed 863.4849.

Sapphyrin – lasalocid conjugates 11 and 12: The sapphyrin monoamide monoacid **9** or **10**, for the synthesis of compounds **11** and **12** respectively, (60 mg, 0.07 mmol) and aminolasalocid **5** (88 mg, 0.14 mmol) were dissolved under argon in anhydrous DMF (10 mL) containing dry Py (0.1 mL). A solution of EDC (27 mg, 0.14 mmol) in anhydrous DMF (2 mL) was then added, followed by HOBt (6.8 mg, 0.05 mmol). The resulting reaction mixture was stirred at room temperature under argon for 48 h. The solvents were then evaporated off. After the resulting solids were dried in vacuo, the products **11** and **12**, as appropriate, were purified chromatographically on silica gel as the solid support and a solution of methanol (12% v/v) in dichloromethane as the eluent. The yield of compound **11** was 94 mg (92%), and the yield of compound **12** was 88 mg (86%). ^{1}H NMR (**11**, 300 MHz, $CDCl_3$): $\delta = 0.35$ (t, ^3J (H,H) = 7.3 Hz, 1H), 0.55 – 0.90 (m, 15H), 1.05 (s, 9H, CH_3), 1.1 – 2.2 (m, 37H), 2.3 – 3.2 (m, 10H), 3.84 (s, 3H, CH_3), 3.86 (s, 3H, CH_3), 3.91 (s, 3H, CH_3), 4.16 (s, 3H, CH_3), 3.3 – 4.7 (m, 19H), 5.37 (q, ^3J (H,H) = 18.7 Hz, 2H), 5.62 (s, 1H), 6.02 (t, ^3J (H,H) = 8 Hz, 2H, CH), 6.17 (d, ^3J (H,H) = 8.3 Hz, 1H, CH), 6.25 (m, 3H, CH), 6.78 (d, ^3J (H,H) = 8.3 Hz, 1H, CH), 10.43 (s, 1H, CH), 10.46 (s, 1H, CH), 10.84 (s, 1H, CH), 10.89 (s, 1H, CH); ^{13}C NMR (75 MHz, $CDCl_3$ with 5% CD_3OD): $\delta = 5.83$, 8.39, 12.01, 12.25, 12.43, 12.70, 12.75, 13.21, 15.43, 16.15, 16.43, 16.89, 17.67, 17.74, 18.31, 18.41, 19.65, 20.13, 20.48, 27.36, 28.91, 28.96, 30.46, 32.19, 33.65, 34.78, 34.89, 36.27, 37.27, 38.08, 38.79, 40.10, 49.29, 53.58, 54.91, 69.63, 70.37, 72.83, 76.19, 77.22, 81.98, 83.66, 86.03, 90.00, 90.68, 96.35, 96.47, 117.28, 119.03, 125.30, 125.91, 127.37, 128.40, 130.70, 130.96, 131.20, 132.48, 132.85, 133.09, 135.16, 135.30, 135.67, 135.85, 137.81, 138.75, 140.44, 142.41, 142.64, 143.06, 143.15, 163.34, 170.00, 170.55, 171.88, 172.20, 216.56; HRMS (FAB) calcd for $C_{89}H_{121}N_8O_{11}$ $[M+H]^+$ 1477.9155, observed 1477.9118. Compound **12** showed similar NMR characteristics.

Sapphyrin – lasalocid conjugates 13 and 14: *tert*-Butyl-protected **11** or **12** for the synthesis of compound **14** (59 mg, 0.04 mmol) was deprotected by treatment with 3:1 dichloromethane/TFA mixture (2 mL) for ca. 4 h at room temperature (TLC control). Solvents were then evaporated off, and the resulting solids were purified on a short chromatographic column, on silica gel as the solid support and dichloromethane – methanol (6:1 v/v) as the eluent. Small amounts of macrocyclic by-products (presumably, a mixture of esters obtained from the reaction of terminal carboxylic acid portion of the phenylalanine appendage with one or more of the free hydroxy groups present in the lasalocid appendage) were separated out during column chromatography. The yield of compound **13** obtained in this way was 40 mg (71%), while that of compound **14** was 43 mg (75%). ^{1}H

NMR (**13**, 500 MHz, $CDCl_3$): $\delta = 0.60-2.10$ (m, 52H), 2.3–3.7 (m, 10H), 3.99 (s, 3H, CH_3), 4.03 (s, 3H, CH_3), 4.05 (s, 3H, CH_3), 4.11 (s, 3H, CH_3), 4.3–4.7 (m, 16H), 4.8 (s, 1H), 5.46 (s, 2H), 5.56 (s, 1H), 6.40–7.00 (m, 7H), 11.35–11.65 (m, 4H, CH); ^{13}C NMR (126 MHz, $CDCl_3$ with 5% CD_3OD): $\delta = 5.91$, 6.03, 7.95, 8.43, 12.26, 12.29, 12.82, 13.12, 13.69, 13.93, 15.68, 16.13, 16.60, 17.31, 17.37, 17.89, 17.99, 18.24, 18.32, 19.92, 20.05, 20.19, 20.36, 20.55, 20.73, 20.77, 23.04, 23.18, 26.30, 28.46, 29.24, 29.46, 30.31, 30.71, 33.40, 35.04, 35.67, 36.26, 37.10, 38.68, 38.92, 45.17, 50.53, 54.25, 55.00, 70.46, 70.78, 72.38, 73.02, 83.59, 84.34, 85.70, 86.13, 91.11, 91.78, 92.13, 95.86, 120.26, 123.36, 125.58, 127.44, 127.79, 128.35, 128.68, 129.09, 129.28, 129.71, 131.61, 132.39, 132.53, 133.84, 134.37, 134.79, 137.73, 138.77, 140.74, 143.67, 150.88, 152.60, 169.33, 170.48, 170.60, 170.98, 215.00, 215.13; HRMS (FAB) calcd for $C_{85}H_{113}N_8O_{11}$ $[M+H]^+$ 1421.8529, observed 1421.8500. Compound **14** showed similar NMR characteristics.

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[21] Importantly, it was shown by Tsukube that chemically modified natural polyether ionophores (i.e., as their ester or amide derivatives) retain the ability to chelate ammonium cations. In most cases improved enantioselectivity is actually observed (see ref. [20b]).

[22] The central core of sapphyrin macrocycle is monoprotonated at neutral pH (ref. [14]).

[23] A. P. Krapcho, C. S. Kuell, *Synth. Commun.* **1990**, *20*, 2559 – 2564.

[24] J. L. Sessler, G. W. Genge, A. Urbach, P. I. Sansom, *Synlett* **1996**, 187 – 188.

[25] H. Tsukube, *Liquid Membranes: Chemical Applications* (Eds.: T. Araki, H. Tsukube), CRC, Boca Raton, FL, **1990**.

[26] In particular, increasing the pH of the receiving phase leads to deprotonation of the sapphyrin macrocycle. This, in turn should lead to weakening of the sapphyrin – amino acid carboxylate non-covalent interactions and thus result in a release of the amino acid substrate into Aq. II.

[27] Compound **1b** was chosen as a control because it has a large hydrophobic tail attached to the sapphyrin macrocycle. It was prepared by coupling the sapphyrin mono acid **1a** with mono-*tert*-butyloxycarbonylethylenediamine, using 1,1′-carbonyldiimidazole as the coupling agent (see Experimental Section).

[28] It appears, however, that the sapphyrin part of the conjugate plays greater role in enhancing amino acid transport than the lasalocid part.

[29] These spectroscopic titrations were carried out in dichloromethane with aliquots of the substrates in question (10^{-5} M in dichloromethane: methanol = 99:1) being added to a solution of receptor **6** at fixed (10^{-6} M) concentration. Association constants and the stoichiometric compositions of the complexes were determined by Benesi – Hildebrand plots and by standard nonlinear curve-fitting protocols: K. A. Connors, *Binding Constants*, Wiley, New York, **1987**. For a complete discussion, see supplementary material to ref. [9h]. Also, in the case of L-Phe, 1:1 stoichiometry was confirmed by a Job plot.

[30] The salicylic hydroxy group of lasalocid is acidic enough to be deprotonated by a strong base, which, in this instance, could be either sapphyrin or NaOH.

[31] Charge neutralization is required for efficient carrier-mediated transport (a corollary of Fick's first law, see ref. [25]). While we favor a model wherein the net neutralization process takes place as shown in Figure 2, a referee has suggested that it could occur in part as the result of proton transfer between oppositely charged species taking place within the lipophilic medium of the model membrane (e.g., from the primary ammonium group of an amino acid zwitterion to the salicylamide moiety of the lasalocid subunit). While at present it is difficult to distinguish between these interpretive extremes of what may well be a chemical continuum, it is important to point out that functionally (i.e., in terms of experimental prediction) they are the same.

[32] Fluoride anions have a much higher affinity for protonated sapphyrin than chloride (ref. [15d]).

[33] Importantly, the pH increase in question is not sufficient to change the predominant form of amino acid species in Aq. I from zwitterionic to anionic.

[34] Finally, in support of proposed mechanism are the results of two negative control experiments: When either a) the pH of the initial phase, Aq. I, was made basic by means of NaOH addition (pH = 13 and the other conditions as in entry 1 of Table 1) or b) the pH was lowered by adding HCl (pH = 1, with other conditions as in entry 1 of Table 1), no amino acid transport by carrier **6** was observed.

[35] a) B. L. Iverson, R. E. Thomas, V. Král, J. L. Sessler, *J. Am. Chem. Soc.* **1994**, *116*, 2663 – 2664; b) J. L. Sessler, J. W. Genge, V. Král, B. L. Iverson, *Supramol. Chem.* **1996**, *8*, 45 – 52.

[36] Analysis of CPK space-filling models indicated that the linker between the sapphyrin core and the carboxylate group in **13** is not long enough to allow for efficient, within-monomer carboxylate-to-sapphyrin binding. This precludes internal collapse.

[37] M. Berger, F. P. Schmidtchen, *J. Am. Chem. Soc.* **1996**, *118*, 8947 – 8948.

[38] The evidence for compound **13** self-dimerization was obtained from FAB mass-spectrometric analysis. An intense dimer peak at twice the molecular weight was found when carboxylate **13** was analyzed in this way. Such a peak, however, was not seen when the control *tert*-butyl ester **11** was subject to similar mass spectrometric analyses. Although these analyses were carried out in the gas phase, they can be safely extrapolated to solution conditions, as shown previously (see ref. [16b]). Further, in the case of **13**, the proposed aggregation effect was confirmed by ^{1}H NMR dilution experiments carried out in [D_2]dichloromethane.

[39] The deprotected, presumably zwitterionic substance **14** displayed poor transport capabilities, just as did its congener compound **13**.

[40] These results, not fully understood from a mechanistic point of view, somewhat resemble those of chiral amplification, a nonlinear phenomenon observed in asymmetric catalysis: R. Noyori, *Asymmetric Catalysis in Organic Synthesis*, Wiley, New York, **1994**.

[41] The W-tube was constructed in a fashion similar to Cram's Catalytic Resolving Machine (ref. [5a]). It has two organic phases (each containing competing carrier compounds) which share the same initial phase, Aq. I, (containing a mixture of the amino acids in question). Each organic phase was put in contact with its own receiving phase (Aq. II and Aq. III). The initial Aq. I phase and both of the organic phases were continuously stirred at equal rates. The increase in concentration of amino acids in phases Aq. II and Aq. III was monitored by HPLC analysis.

[42] For instance, the k_t(L-Phe)/k_t(D-Phe) ratio for compound **12** went up from 1.6 when measured in the U-tube to 2.1 when studied by means of the W-tube. The more complex equilibria relevant to the W-tube conditions are probably responsible for this effect.