Sapphyrin–Lasalocid conjugate: a novel carrier for aromatic amino acid transport

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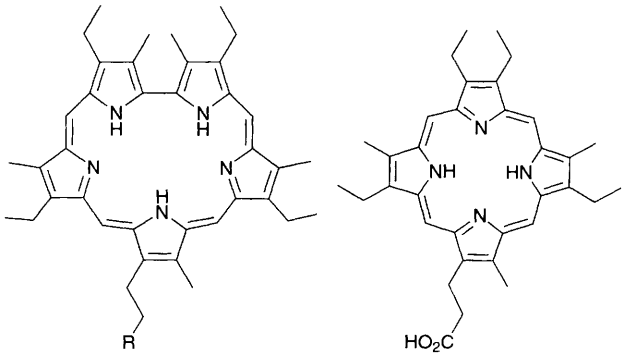
Sapphyrin, when connected covalently to lasalocid, acts as a selective carrier for the through-model-membrane transport of zwitterionic aromatic amino acids.

The binding and transport of amino acids constitutes an important problem in supramolecular chemistry from both the biomimetic and analytical points of view.[1] Presently, some information is available about the dynamics and regulation of amino acid transport in nature, but a detailed mechanistic or structural understanding remains lacking.[2] This paucity of information is providing an incentive to develop synthetic systems that are capable of carrying out amino acid transport.[3] In this paper we report the synthesis of novel sapphyrin[4]–lasalocid[5] conjugate, **6**. This species acts as an efficient receptor and carrier for aromatic amino acids.

The synthesis of conjugate **6** is shown in Scheme 1. Using similar chemistry, the structurally cognate porphyrin–lasalocid conjugate **7** was synthesized from **5** and the porphyrin mono acid **2**.[6] To the best of our knowledge, these compounds† represent the first examples of polypyrrolic macrocycles appended to lasalocid derivatives.

Aromatic amino acids, namely phenylalanine, tryptophan and tyrosine were chosen as the substrates for use in the requisite transport studies. This choice was made because it was considered likely that their side chains would provide for additional van der Waals interactions and thus favour transport. The transport experiments themselves were then carried out using a Pressman-type, H_2O–CH_2Cl_2–H_2O U-tube model membrane system. From these experiments (Table 1, Fig. 1), it was found that at neutral pH compound **6** acts as a very efficient carrier for phenylalanine and tryptophan. Further, in direct competition experiments, L-phenylalanine was found to be transported 4 times faster than L-tryptophan and 1000 times faster than L-tyrosine (Table 1, entry 1). On the other hand, none of the amino acids under investigation was transported effectively by the porphyrin–lasalocid conjugate **7** (entry 2) or parent lasalocid **3** (entry 4). Only modest transport (*ca.* 15–35% relative to **6**) was effected using single sapphyrin **1a**‡ either alone, (entry 3), or as a mixture with **3** (entry 5). Taken together, these results lead us to conclude that not only is system **6** an efficient carrier for aromatic α-amino acids, it is also an inherently selective one. Consistent with this hypothesis is the finding that some level of enantiomeric discrimination was attained when conjugate **6** was used as a carrier but not when any of the 'control' systems were employed (Table 1).

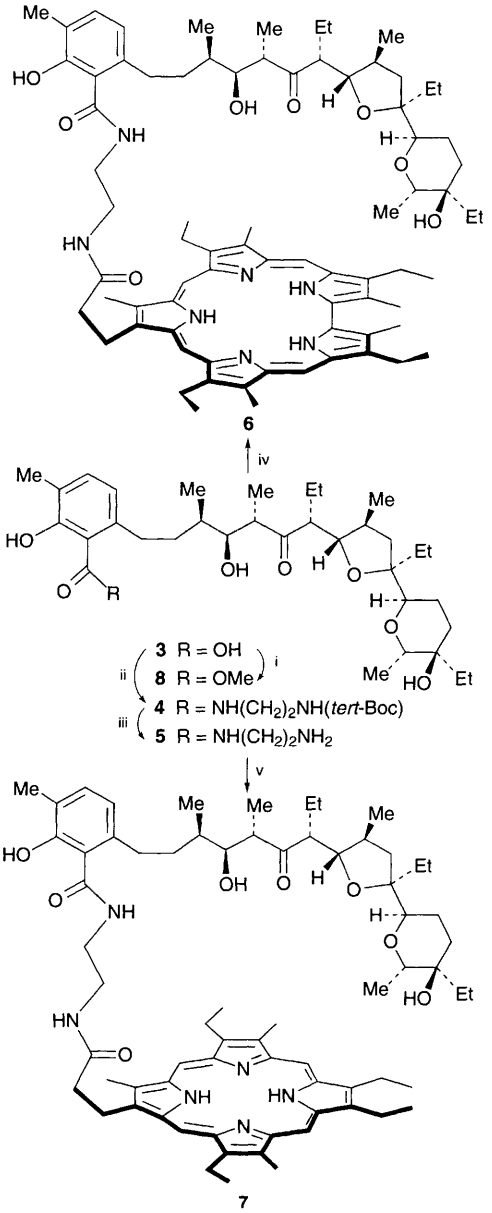
Further support for the contention that conjugate **6** can act as an amino acid binding agent came from quantitative visible



1 R = CO_2H

1a R = $CONH(CH_2)_2NH$(*tert*-Boc)

2



Scheme 1 *Reagents and conditions*: i, EDC, HOBt, MeOH, 48 h, 25 °C; ii, $H_2N(CH_2)_2NH$(*tert*-Boc), EDC, HOBt, Py, DMF, 24 h, 25 °C; iii, TFA, CH_2Cl_2; iv, **1**, EDC, HOBt, Py, DMF, 48 h, 25 °C; v, **2**, EDC, HOBt, Py, DMF, 48 h, 25 °C

Table 1 Initial amino acid transport rates (k_t)[a,b]

		k_t(10^{-5} mol cm^{-2} h^{-1})							
Entry	Carrier	L-Phe	D-Phe	L-Trp	D-Trp	L-Tyr	D-Tyr	$k_F:k_W:k_Y$(L)[c]	$k_F:k_W:k_Y$ (D)[c]
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	**1a**[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	**1a** + **3**[f]	3.2	3.1	0.9	0.9	0.2	0.2	16:4.5:1	15.5:4.5:1
6	**1a** + **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	none	0.05	na[e]	0.01	na[e]	<0.001	na[e]	5:1	na[e]

[a] Transport experiments were performed as described in ref. 4(*b*) under conditions of competitive transport. The initial rate values given for L-Phe, L-Trp and L-Tyr transport are thus derived from experiments involving mixtures of L-Phe (50 mmol dm^{-3}), L-Trp (50 mmol dm^{-3}) and L-Tyr (5 mmol dm^{-3}) in the initial aqueous phase, Aq. I. Likewise, those given for D-Phe, D-Trp and D-Tyr are from studies involving analogous mixtures of D-Phe (50 mmol dm^{-3}), D-Trp (50 mmol dm^{-3}) and D-Tyr (5 mmol dm^{-3}) in Aq. I. In all runs, 10 mmol dm^{-3} 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] F = Phe, *w* = Trp and Y = Tyr. In those instances where the initial Tyr transport rate was less than 10^{-8} mol cm^{-2} h^{-1}, the $k_F:k_W$ ratio is given rather than $k_F:k_W:k_Y$. [d] Concentration: 1 × 10^{-4} mol dm^{-3} in dichloromethane. [e] na = not measured; the carrier is achiral. [f] An organic (membrane) phase containing a mixture of **1a** (1 × 10^{-4} mol dm^{-3}) and **3** (1 × 10^{-4} mol dm^{-3}) in dichloromethane was used. [g] The organic phase consisted of a mixture of **1a** (1 × 10^{-4} mol dm^{-3}) and **8** (1 × 10^{-4} mol dm^{-3}) in dichloromethane.

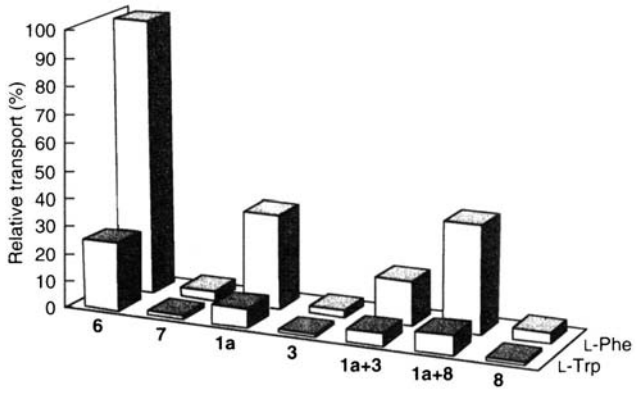


Fig. 1 Relative rates of L-Phe and L-Trp transport

spectroscopic titrations carried out in dichloromethane. These titrations were made by adding increasing quantities of the aromatic amino acids in question (as 10^{-5} mol dm^{-3} solutions in dichloromethane–methanol, 99 : 1) to dichloromethane solutions of **6** of initial 10^{-6} mol dm^{-3} concentration and recording the resulting changes in the Soret-like band of the sapphyrin portion of the conjugate. By following the increase in absorbance at 447 nm as a function of substrate-to-receptor ratio, both association constants (K_a) and stoichiometries of binding could be determined.§ In this way it was found that compound **6** forms 1 : 1 complexes with both phenylalanine and tryptophan. Also, it was found that the relevant association constants (K_a) are: 4.86 × 10^5 (L-Phe), 5.35 × 10^5 (D-Phe), 0.83 × 10^5 (L-Trp) and 0.94 × 10^5 dm^3 mol^{-1} (D-Trp) (estimated errors are ±15%).

While the higher affinity recorded for phenylalanine relative to tryptophan is consistent with the observation that this species is transported more efficiently, the findings that the L-enantiomers are bound with the same affinity (within error) as the D-congeners is somewhat puzzling at present. It could reflect the fact that binding affinities alone do not account for the observed transport rates, but rather, as has frequently been observed in other systems, off rates play a significant role in mediating the actual transport dynamics.[7] To the extent this is true, it leads us to suggest that slight modifications in the structure of **6** could lead to amino acid receptors displaying improved transport efficiency and/or enhanced enantioselectivity.

Footnotes

† Satisfactory spectroscopic and analytical data were obtained for all new compounds.

‡ Compound **1a** was prepared by coupling sapphyrin mono acid **1** with mono *tert*-butyloxycarbonylethylenediamine using EDC.†

§ Association constants and stoichiometries were determined by Benesi–Hildebrand plots and by standard non-linear curve-fitting protocols.[4b] In the case of L-Phe being complexed to receptor **6**, the 1 : 1 binding stoichiometry was confirmed by carrying out a Job analysis.

References

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