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Efficient and selective transport of ω-amino acids across a bulk chloroform membrane by a macrocyclic dicopper(II) complex

Umberto Pichler,[a] Paolo Scrimin,[a,*] Paolo Tecilla,[b,*] Umberto Tonellato,[a,*] Andrea Veronese[a] and Massimo Verzini[a]

[a]*Dipartimento di Chimica Organica e ITM-CNR, Sezione di Padova, Università di Padova, Via Marzolo 1, I-35131 Padova, Italy*
[b]*Dipartimento di Scienze Chimiche, Università di Trieste, Via Licio Giorgieri 1, I-34127 Trieste, Italy*

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Abstract—The Cu(II) complex of a ditopic macrocyclic ligand efficiently transports ω-amino acids of different length across a bulk chloroform membrane with promising selectivity.

Molecular recognition by natural proteins involves the cooperative participation of several subsites and the interplay of different energetic factors.[1] It is not surprising that the design of molecular receptors with improved selectivity should consider multipoint recognition.[2] Amino acids, as bifunctional compounds, appear to be good candidates as potential substrates for ditopic receptors. Examples of molecular receptors featuring convergent recognition sites for α-amino acids have indeed been reported.[3] Among them, examples of lipophilic receptors able to transport amino acids across a bulk liquid membrane have also been described.[4]

Following our interest in the recognition and, eventually, modification of amino acid derivatives by metal complexes[5] we thought that a macrocyclic ligand able to bind two transition metal ions with a well-defined geometry could recognize ω-amino acids of different length with selectivity related to their size. This led us to the synthesis of the molecular receptor **1** and model compound **2** (Scheme 1).

The common starting material for the synthesis of compounds **1** and **2** is the lipophilic pyridine dialdehyde **3**, which was prepared as described in Scheme 2. Chelidamic acid diethyl ester **6** was alkylated with dodecyl bromide, the ester moieties were reduced to alcohols and, eventually, oxidized to aldehydes. Macrocyclic receptor **1**[6] was assembled by reductive amination of compound **3** with 4,4′-di-(aminomethyl)-diphenylmethane[5e] **4** under high dilution conditions (Scheme 1). A similar procedure using benzylamine **5** afforded reference compound **2**.[7]

In receptor **1** the two diaminomethylpyridine units, spaced by two diphenylmethane groups, provide the complexation sites for two transition metal ions which, due to the rigidity of the macrocycle, are placed at a defined distance from each other. The two hydrocarbon chains ensure a good lipophilicity to the system. Binding experiments, in water/ethanol 1:1 at pH 5.5, with Cu(II) clearly showed that **1** forms a stable complex involving two metal ions; in contrast the open ligand **2** (corresponding to only half the macrocycle **1**) binds only one Cu(II) ion.[8]

Exploiting the lipophilicity of the two ligands **1** and **2** and the known ability of lipophilic complexes of Cu(II) to act as carriers of amino acids across a bulk liquid membrane,[4d] we tested our systems as carriers of α-, β- and γ-amino acids across a chloroform membrane from a source to a receiving water solution. Our interest was on efficiency and also on the selectivity of transport on the assumption that the geometrical requirements for

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* Corresponding authors. Tel.: +39-049-8275276; fax: +39-049-8275239 (P.S.); tel.: +39-040-5583925; fax: +39-040-5583903 (P.T.); tel.: +39-049-8275269; fax: +39-049-8275239 (U.T.); e-mail addresses: paolo.scrimin@unipd.it; tecilla@dsch.univ.trieste.it; umberto.tonellato@unipd.it

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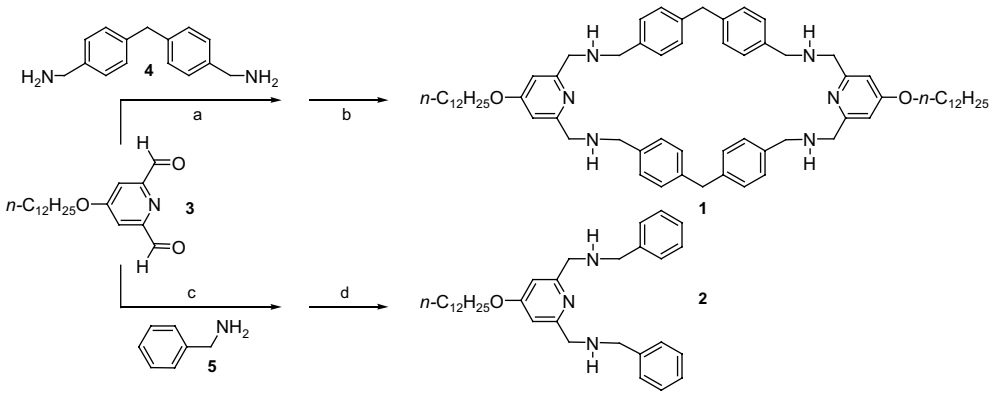
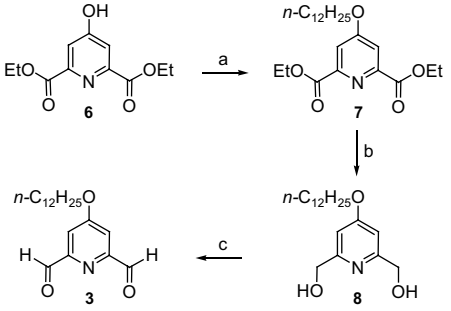


Scheme 1. Reagents and condition. (a) CH_2Cl_2, molecular sieves, 15 h. (b) $NaBH_4$, EtOH, 6 h, 24% from **3**. (c) Benzene, reflux, 3 h, 91%. (d) $NaBH_4$, EtOH, 6 h, 80%.



Scheme 2. Reagents and conditions. (a) Dodecyl bromide, *t*-BuOK, K_2CO_3, DMF, 90 °C, 1 h, 70%. (b) $NaBH_4$, $CaCl_2$, EtOH, 3 h, 92%. (c) SeO_2, dioxane, reflux, 6 h, 80%.

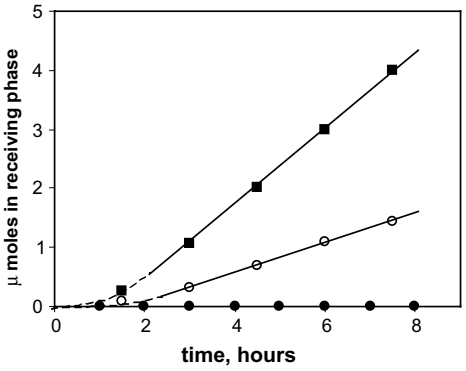


Figure 1. Micromoles of phenylalanine (○), 3-amino-3-phenylpropionic acid (■) and 4-aminobutanoic acid (●) transported in the receiving phase as a function of time by 0.5 mM **1** at 25 °C. The intercept of the lines is not zero because of the induction time (dotted lines) necessary to reach saturation of the chloroform phase. We take the slope of these lines as the initial rate (v_i) of transport.

binding of the ditopic ligand **1** would be able to select preferentially one of the three types of amino acids. A classical U-shaped tube apparatus was set up[9] and experiments were performed using chloroform solutions of ligands **1** and **2**. For the source phase a buffered (pH = 5.5, 2-morpholinoethanesulphonic acid) water solution was used containing the amino acid (2.5 mM) and $Cu(NO_3)_2$ (20 mM). The receiving phase was a 50 mM solution of ethylenediaminetetraacetic acid (EDTA) at pH = 6.5. The transport experiments were started by stirring the chloroform solution (magnetic stirring) and the two water phases (mechanical stirring). Visually the occurrence of transport is highlighted by the fading colour of the blue source phase accompanied by the colouring of the receiving phase. Analytical determination of the amino acid and the Cu(II) content of the receiving phase was performed by the ninhydrin method and by atomic absorption spectroscopy, respectively. The time course of the transport experiment for the three amino acids in the presence of the macrocyclic ligand **1** is illustrated in Figure 1.

In such an experiment, the ligands transport both Cu(II) and amino acid.[10] While the transport of Cu(II) also occurs in the absence of the amino acid, the transport of the latter needs the presence of the metal ion. The role of EDTA in the receiving phase is to strip off the copper ions from the complex. Consequently, this allows the release of the amino acid and its quantitative translocation in the receiving phase. Figure 1 reveals that **1** is quite efficient in the transport of the α- and β-amino acids while it performs very poorly in the case of the γ-amino acid. In Table 1 are collected the initial rates of transport of the three amino acids studied with macrocycle **1** and its open ligand model **2**. The concentration of the macrocycle used in the experiments was half that of **2** to allow for the different number of binding sites for Cu(II) ions.[11]

The absolute rates of transport of α-Phe by carriers **1** and **2** compare well with those found for lipophilic diamine ligands and exploiting the same transport mechanism.[4d] Thus the Cu(II) complexes of **1** and **2** are good carriers of this amino acid and β-Phe as well. In contrast, the less hydrophobic Gaba is transported more slowly and this is in accord with the previous observations that the rate of transport of substrates across a bulk membrane is related to their lipophilicity.[4d]

An important question here is whether the dinuclear complex of macrocycle **1** shows any selectivity in the recognition of the amino acids and in their transport rates. Transport rates depend on a number of factors,

Table 1. Initial rate of transport (v_i, μmol h^{-1}) of ω-amino acids across a bulk chloroform membrane mediated by ligands **1** and **2**[a]

Receptor[b]	v_iα-Phe[c]	v_iβ-Phe[d]	v_i Gaba[e]
1	0.28	0.57	0.001
2	0.12	0.29	0.036

[a] For conditions see text.
[b] [**1**] = 0.5 mM, [**2**] = 1.0 mM.
[c] Phenylalanine.
[d] 3-Amino-3-phenylpropionic acid.
[e] 4-Aminobutanoic acid.

including the lipophilicity of the amino acid. Accordingly, in order to have a fair evaluation of the selectivity only relative numbers have to be compared, taking as the reference the mononuclear ligand **2**. Of course, the strength of binding is also important. Apparent binding constants under the transport conditions were determined by measuring the initial rate of transport as a function of the concentration of the ligand in the chloroform phase. The profile obtained for β-Phe and **1** is shown in Figure 2.[12]

Figure 3 reports the values of the relative binding constants (back row) and initial transport rates for the three amino acids (front row). In terms of binding constant, there is a clear selectivity for the β-amino acid in accord with molecular models that indicate a good match between this amino acid and the dinuclear Cu(II)

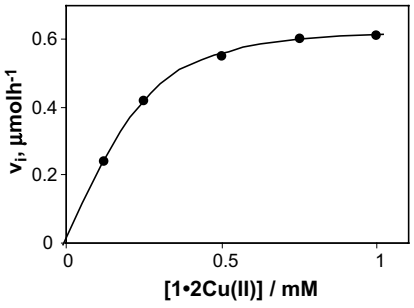


Figure 2. Initial rate of transport (v_i, μmol h^{-1}) of β-Phe as a function of the concentration of the Cu(II) complex of ligand **1**. The line has been generated by fitting the experimental points with a Michaelis–Menten-like equation.

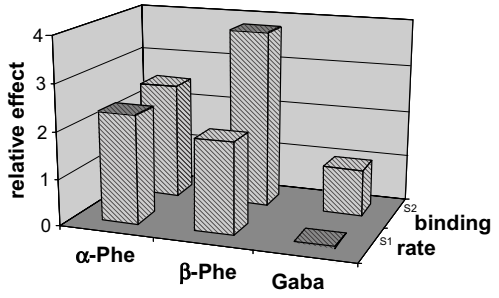


Figure 3. Ratio between the initial rate of transport of ω-amino acid measured in the presence of **1** (0.5 mM) and **2** (1.0 mM) (front row) and the ratio between the apparent formation constant of the ternary complex ligand–Cu(II)–amino acid measured for ligands **1** and **2** (back row).

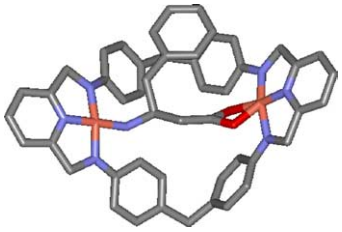


Figure 4. Proposed mode of binding of β-Phe to the Cu(II) complex of macrocycle **1**.

complex of **1**. This complex should be similar to that reported in Figure 4: the α- and γ-amino acids are too short or too long to insert properly between the two metal ions and take advantage of their contemporaneous complexation. However, the selectivity vanishes when the transport rates are considered. This might imply the formation, in the case of α-Phe, of a complex with two amino acids (one for each metal ion) that makes the transport process somewhat more efficient. The limited number of data used for the determination of the apparent binding constants does not allow differentiation between these two different stoichiometries of binding. Once again molecular models suggest that the formation of such a complex is indeed possible.

In conclusion, we have described a macrocyclic ditopic receptor able to bind and transport efficiently amino acids in their zwitterionic form across a bulk chloroform membrane. Due to its geometrical requirements the receptor preferentially binds β-amino acids over the shorter or longer α- and γ-analogue. However, this selectivity is not paralleled by the kinetic data, which show that the α-amino acid is transported slightly more favorably than the β-amino acid and that both are strongly preferred over the γ-analogue. This discrepancy highlights the complexity of the transport process, which depends not only on the strength of binding but on other factors such as the stoichiometry of binding, the lipophilicity of the resulting complex and its rate of formation and destruction. Thus, besides the promising selectivity of binding, the present results indicate that such a property will be paralleled by an analogous transport selectivity only if a more stringent control of the parameters that govern the transport process is present in the carrier. Work in this direction is currently being pursued in our laboratory.

Acknowledgements

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References and notes

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6. **1**: mp = 118–120 °C; ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 0.87$ (t, $J = 7.2$ Hz, 6H), 1.26 (m, 36H), 1.77 (m, 8H), 3.74 (s, 8H), 3.84 (s, 8H), 3.91 (s, 4H), 3.99 (t, J 6.9 Hz, 4H), 6.66 (s, 4H), 7.05 and 7.19 (AB quartet, $J_{AB} = 8.0$ Hz, 16H); ^{13}C NMR ($CDCl_3$) $\delta = 14.12$, 22.57, 25.92, 28.93, 29.32, 29.57, 29.62, 31.90, 41.24, 53.04, 54.42, 67.91, 107,15, 138.35, 138.90, 137.81, 139.71, 160.58, 166.07; FABMS (NBA) m/z 1027 [MH^+]; Calcd for $C_{68}H_{94}N_6O_2$: C, 79.49; H, 9.22; N, 8.18; Found: C, 79.02; H, 9.31, N, 8.09.
7. **2**: pale yellow oil; ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 0.88$ (t, $J = 7.2$ Hz, 3H), 1.26 (m, 18H), 1.78 (quint, $J = 7.0$, 2H), 2.12 (br s, 2H); 3.83 (s, 4H), 3.85 (s, 4H), 3.98 (t, $J = 6.4$ Hz, 2H), 6.72 (s, 2H), 7.21 (m, 10H); ^{13}C NMR ($CDCl_3$) $\delta = 14.11$, 22.67, 25.92, 28.93, 29.32, 29.57, 29.61, 31.90, 53.52, 54.60, 67.89, 106.88, 126.93, 128.24, 128.36, 140.20, 160.88, 166.22; Calcd for $C_{33}H_{47}N_3O$: C, 79.00; H, 9.44; N, 8.37; Found: C, 78.72; H, 9.48, N, 8.26.
8. Association constants for Cu(II) have not been determined. However, the association constants measured in water for a macrocycle consisting of two 2,6-diaminomethyl pyridine units are $10^{13.5}$ M^{-1} (for the LCu complex) and 10^{19} M^{-2} (for the LCu_2 complex). See: Arnaud-Neu, F.; Sanchez, M.; Schwing-Weill, M. J. *Helv. Chim. Acta* **1985**, *68*, 840–845.
9. The tube used had an internal diameter of 1.6 cm and the area of the surfaces between the different phases was 2.0 cm^2. The tube was immersed in a thermostatic bath kept at 25 °C. The stirring speed of the magnetic bar was >200 rpm. Above this value the rate of transport does not depend on the stirring speed.
10. In these experiments the metal ion is transported at a higher rate than the amino acids and this is probably related to the formation of a ternary complex comprising two ligands and two metal ions (or two ligand and one metal ion in the case of **2**). This ternary complex is productive as a Cu(II) carrier but not as an amino acid carrier and competes with the ternary complex comprising ligand, copper and amino acid. This behaviour has already been observed. It has been ascribed to a lower affinity for the metal ion of the amino acid with respect to the ligand (see Ref. 4d).
11. This is based on the rough assumption that the binding constant of the second Cu(II) ion by the macrocycle is the same as that of the first one. Quite likely it is lower (see Ref. 8) and, as a consequence, the transport efficiency of the macrocycle is underestimated.
12. The apparent binding constants of the carriers for the three amino acids were obtained by interpolation of the rate of transport as a function of carrier concentration (see Fig. 2 for **1** and β-Phe) plots by using a Michaelis–Menten-like equation. In the case of α- and γ-amino acids the curvature of the profiles was less defined than that of the β-analogue and, therefore, the error in the determination of the formation constant is probably higher. However, since we are considering ratios of constants the effect of the error is somehow minimized.