The Enantioselective Transport of Z-Amino Acid (Z = Benzyloxycarbonyl) and Dipeptide K^+ Carboxylates by Dipeptide Derived Lariat Ethers

Mladen Žinić,*[a] Leo Frkanec,[a] Vinko Škarić,[a] Jack Trafton[b] and George W. Gokel[b]

[a] *Laboratory of Stereochemistry and Natural Compounds, Rudjer Bošković Institute, 41000 Zagreb, P.O.B. 1016, Croatia, Yugoslavia*

[b] *Department of Chemistry, University of Miami, Coral Gables, Florida 33124, USA*

The first enantioselective transport of Z-amino acid and dipeptide K^+ carboxylates has been realized using lariat ethers with dipeptide pendant arms as new 'symporter' models.

In nature, transport of amino acids through cell membranes is a highly specific process owing to the chiral recognition properties of natural carriers. For certain types of mammalian cells, transport of amino acids often depends upon the extracellular Na^+ concentration. This leads to the hypothesis that an amino acid and Na^+ ion use a common carrier, the 'symporter'.[1] Recently, Tsukube[2] reported on the artificial analogue of symporter mediated transport of potassium Z-amino acid carboxylates through a chloroform membrane using *N*,*N*′-didecyl-1,7,10,16-tetraoxa-4,13-diazacycloocta-

Table 1 Transport rates of Z-amino acid and dipeptide K^+ carboxylates through chloroform membrane[a]

		$10^6 \times$ Transport rates/mol h^{-1}			
Entry	Substrate	**2**	**3**	**5**	**6**
1[b]	Z-GlyOK	1.4	2.1	1.7	4.1
2[b]	Z-L-PheOK	1.4	40.3	0.4	20.5
3	Z-L-PheOK	1.59	27.2	0.2	14.9
4	Z-D-PheOK	1.42	38.1	0.3	9.4
5	Z-L-PhgOK	0.88	24.2	0.19	19.9
6	Z-D-PhgOK	0.81	25.1	0.25	11.7
7	Z-L-PhgGlyOK	0.07	15.5	0.02	4.2
8	Z-D-PhgGlyOK	0.08	16.1	0.03	4.8
9	Z-Gly-L-PhgOK	0.05	10.1	0.03	3.65
10	Z-Gly-D-PhgOK	0.05	14.6	0.03	2.48

[a] Transport conditions. Aqueous source phase (Aq I): substrate (0.15 mmol), KCl (1.0 mmol), 0.05 mol l^{-1} NaOH (3 ml) for entries 1 and 2 or 0.05 mol l^{-1} LiOH (3 ml) for entries 3–10. Membrane: $CHCl_3$ (8 ml), carrier (0.0372 mmol). Aqueous receiving phase (Aq II): distilled water (3 ml). [b] Transport rates for Z-GlyOK and Z-L-PheOK and *N*,*N*′-didecyl-1,7,10,16-tetraoxa-4,13-diazacyclooctadecane reported in ref. 2 were 0.5 and 8.1, respectively.

R

1 $PhCH_2OCONHCH_2CO$ (Z-Gly) **4**
2 $PhCH_2OCONHCHPhCONHCH_2CO$ (Z-PhgGly) **5**
3 $MeOCOCHPhNHCOCH_2$ (MeOPhgGly) **6**
7 H **8**

Z = $PhCH_2OCO$–; Phg = D-α-phenylglycine

decane, dibenzo-18-crown-6 and dibenzo[2.2.2]cryptand as the artificial potassium selective symporters. In this model system, however, the carriers were able to recognize the metal cation only, and Z-amino acid carboxylate anions were cotransported owing to ion-pairing with cationic carrier–K^+ complex. Therefore, different rates of transport found within the series of Z-amino acid and dipeptide carboxylates were more likely to be the consequence of different substrate lipophilicities than the recognition ability of such carriers.[3]

In this communication, we report on the synthesis and function of new symporter models **3** and **6** capable of transporting Z-amino acid and dipeptide K^+ carboxylates more efficiently than any known symporter model.[2] In addition, novel carriers **2**, **3**, **5** and **6** possess the ability of structural and chiral recognition of such substrates which allowed the first enantioselective transport of Z-amino acid and dipeptide carboxylate anions to be realized. New symporter models have been designed based on the lariat type ligands[4] containing the K^+ selective 18-membered azacrown ring bearing chiral dipeptide side arms. Such lariats were expected to bind K^+ ion by using the 18-membered azacrown ring and fix the amino acid or dipeptide carboxylate anion on one side of the macroring by ion-pairing interactions. Hydrogen bonding interactions between the lariat pendant dipeptide arms and fixed substrate anion may then be expected owing to the well known tendency of peptides to form hydrogen-bonded aggregates in lipophilic media.[5] Furthermore, it was recently shown that a tetraamidic 'selector' containing two (*S*)-phenylalanine units spaced by a 3,6,9-oxaumdecanoyl bridge was able to complex and recognize amino acid ester derivatives by formation of intermolecular hydrogen bonds.[6]

Onto the nitrogen atoms of the 18-membered monoaza-**7** and diaza-**8** macrocycles, dipeptide arms can be attached in two different ways giving lariats with amide (**2**, **5**) or amine (**3**, **6**) macroring junctions. The dipeptide-derived lariats of the latter type have been recently synthesized by the reaction of *N*-(chloroacetyl)amino acid esters with azacrowns in the presence of Na_2CO_3 and NaI.[7] Using the same method we have prepared the hitherto unknown lariats **3** and **6** having D-α-phenylglycine (Phg) as the chiral unit {**3**, oil, yield 55%, $[\alpha]_D$ −24.8° (*c* = 0.58, $CHCl_3$); **6**, m.p. 109–110 °C, yield 61%, $[\alpha]_D$ −81.5° (*c* = 0.75, $CHCl_3$)}. The lariats with amide type junctions **2** and **5** have been prepared in two steps. First Z-Gly was attached to nitrogen atoms of the parent aza-**7** or diaza-**8** macrocycle either by dicyclohexylcarbodiimide (DCC) condensation[8] or by use of Ph_3P–CCl_4–Et_3N[9] giving **1** and **4** in 60–80% yields. Hydrogenolytic removal of Z-groups (10% Pd/C, MeOH) gave *N*-glycyl-derived macrocycles in quantitative yields which were immediately subjected to reaction with Z-D-PhgOH and DCC or Ph_3P–CCl_4–Et_3N giving **2** {m.p. 96–98 °C, yield 50–60%, $[\alpha]_D$ −56.5° (*c* = 0.69, $CHCl_3$)} and **5** {m.p. 148–149 °C, yield 50–60%, $[\alpha]_D$ −73.2° (*c* = 0.64, $CHCl_3$)}.

The transport experiments with symporter models **2**, **3**, **5** and **6** were conducted under experimental conditions identical with those used by Tsukube[2] for the *N*,*N*′-didecyl analogue so the results obtained could be directly compared. The initial transport rates measured for different Z-amino acid and dipeptide carboxylates are shown in Table 1. On the basis of the results from Table 1, the following conclusions may be formulated: (*i*) lariats **3** and **6** are considerably more efficient symporters than the *N*,*N*-didecyl analogue, indicating the participation of dipeptide pendant arms in binding of substrates (Table 1, entries 1, 2 and footnote *b*).

(*ii*) The fact that **6** and **5** exhibited chiral recognition of enantiomeric substrates provides additional proof for interactions between the carrier's pendant arms and the bound substrate. The existence of hydrogen bonding interactions between the lariat pendant arms and bound Z-PheOK was proved by the specific NH proton shifts in the ^1H NMR spectra of 1 : 1 mixtures of **3** or **6** and Z-PheOK in $CDCl_3$. The amide NH protons of **3** and **6** underwent strong downfield shifts in the presence of Z-PheOK (from δ 8.44 to 9.61 for **3** and from δ 8.47, one NH doublet to 9.06 and 9.27, two NH doublets, for **6**).† On the other hand, the portionwise addition of **6** to a

† The same trend of ligand NH shifts was reported by Marchelli *et al.*[6] for their selector–amino acid ester complex, although the shifts were of much lower magnitude.

solution of Z-PheOK in $(CD_3)_2SO$–$CDCl_3$ (1:1.5) caused gradual upfield shifts of the substrate NH proton (from δ 6.47 to 5.99 for 1:0 and 1:1 molar ratios of **6** and Z-PheOK, respectively). The observed upfield shift suggests a position for the substrate between the lariat pendant arms where it is shielded from the exterior.

(*iii*) The two constitutionally isomeric dipeptide K^+ carboxylates, Z-D-PhgGlyOK and Z-Gly-D-PhgOK, were transported by **6** with rates different by a factor of 2. The enantioselection of enantiomeric dipeptide carboxylates was slightly better for enantiomeric Z-GlyPhgOK than for Z-PhgGlyOK having a chiral centre at greater distance from the carboxylate end.

(*iv*) The lariats **3** and **6** are more efficient carriers than **2** and **5**. This could be explained by stronger binding of K^+ by the former lariats. The stability constants (K_s) determined recently for a series of lariats of the same type as **3** and **6** and K^+ and Na^+ in MeOH were in the range of 10^4–10^5.[7] However, K values for **2** and **5** are only about 10^2 for both cations.‡ Apparently, the amide type junction of the dipeptide arms in **2** and **5** considerably diminishes their ability to complex K^+ and consequently, their ability to function as symporters.

Received, 30th August 1990; Com. 0/03955A

‡ The results of K_s measurements for a series of dipeptide-derived lariats having the amide-type arm junction will be published in a forthcoming full paper.

References

1 K. Ring, *Angew. Chem.*, 1970, **82**, 343.
2 H. Tsukube, *J. Chem. Soc., Perkin Trans. 1*, 1982, 2359.
3 J. Rebek Jr., B. Askew, D. Nemeth and K. Parris, *J. Am. Chem. Soc.*, 1987, **108**, 2432.
4 R. A. Schultz, B. D. White, D. H. Dishong, K. A. Arnold and G. W. Gokel, *J. Am. Chem. Soc.*, 1985, **107**, 6659.
5 N. Sugawara, E. S. Stevens, G. M. Bonora and C. Toniolo, *J. Am. Chem. Soc.*, 1980, **102**, 7044.
6 A. Spisni, R. Corradini, R. Marchelli and A. Dossena, *J. Org. Chem.*, 1989, **54**, 684; T. Kaneda, K. Hirose and S. Hisumi, *J. Am. Chem. Soc.*, 1989, **111**, 742.
7 B. D. White, J. Mallen, K. A. Arnold, F. R. Fronczek, R. D. Gandour, L. M. B. Gehrig and G. W. Gokel, *J. Org. Chem.*, 1989, **54**, 937.
8 N. G. Lukyanenko, A. V. Bogatsky, S. S. Basok, L. K. Ostrowskaya, N. J. Nazarova and L. P. Karpienko, *Zh. Org. Khim.*, 1984, **20**, 1580.
9 R. Appel, G. Bäumer and W. Strürer, *Chem. Ber.*, 1975, **108**, 2680.