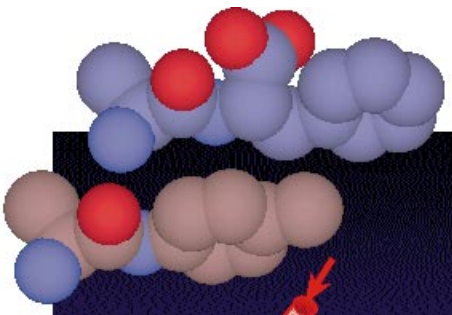
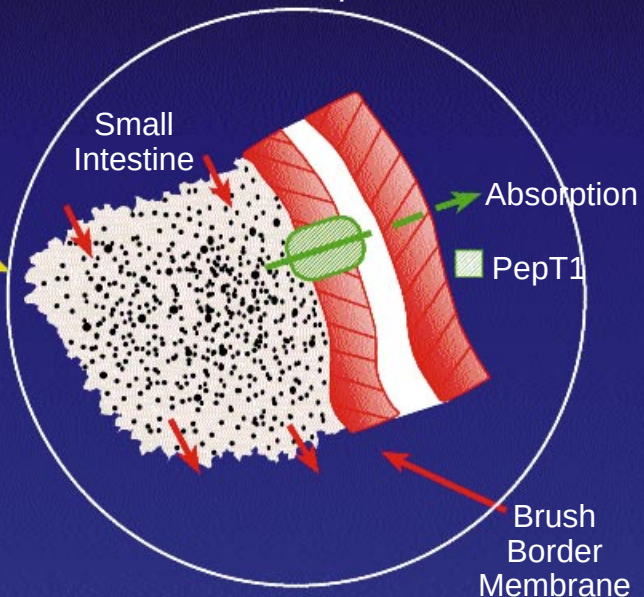


PepT1 Transporter

Di/Tri-peptides

Small Intestine

Digested protein and some drugs (penicillins) use PepT1 mechanism.

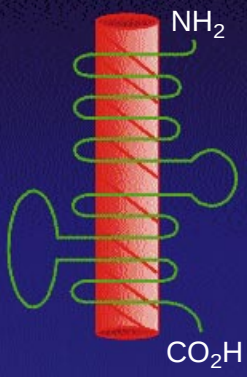


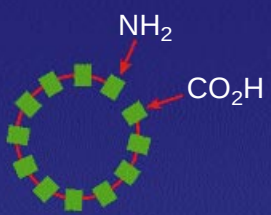
PepT1 is a protein in the wall of the small Intestine; billions of them allow certain molecules to enter the circulation.

How Does PepT1 Work?

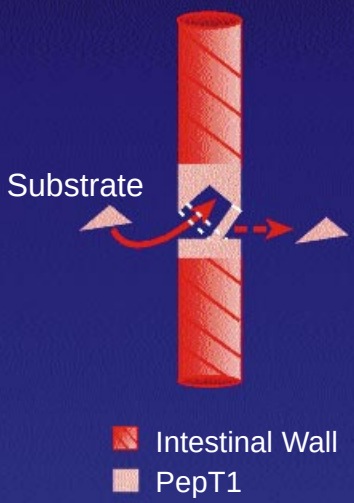
PepT1

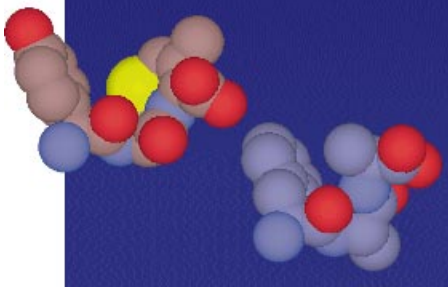
- *Long protein (707AAS)*
- *Crosses membrane 12 times*
- *12 trans-membrane domain channel*
- *Di-/tri-peptides transported*
- *Analogues.....?*





Cattle-gate mechanism





Find out more on the following pages.

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How to Make Drugs Orally Active: A Substrate Template for Peptide Transporter PepT1**

Patrick D. Bailey,* C. A. Richard Boyd, J. Ramsey Bronk, Ian D. Collier, David Meredith, Keith M. Morgan, and Catherine S. Temple

PepT1 is an essential mammalian protein that actively transports small peptides.[1] It is found in the wall of the small intestine of mammals, and provides the main pathway for the absorption of dietary nitrogen.[2] Of particular importance medicinally is the ability of PepT1 to transport certain drugs into the circulatory system,[3–5] which allows them to be taken orally instead of by injection.

Although PepT1 is generally accepted to be a transmembrane (TM) protein possessing 12 TM domains,[6] its 3-D structure is still not known. Nevertheless, data on several hundred PepT1 substrates have now been published, and some features for efficient transport have been put forward within certain sub-classes of substrates.[7] However, no general model for the affinity/transport of substrates by PepT1 has been proposed.

In order to try to generate a template for substrate specificity we have considered the binding and transport properties of virtually all of the published PepT1 substrates. We have also carried out in vitro binding and transport studies on around 100 substrates ourselves, initially using brush border membrane vesicles (BBMV),[8] and more recently using *Xenopus* oocytes in which PepT1 expression has been induced by injection with cRNA;[9] our work has also included in vivo experiments with intact rat jejenum (part of the small intestine), which allowed us to confirm that an excellent correlation exists between the in vitro and real-life situations.[10]

By considering different combinations of potential binding features in 3-D space we have been able to identify a template for PepT1 substrates (Figure 1). This simple model would appear to account for all of the substrate specificities relating to PepT1, and provides an indication of whether substrates will be transported with high, medium, or low efficiency.

The template has the following features, illustrated in Figure 1, and is described below from the N- to the C-terminus: 1) a strong binding site for an N-terminal NH_3^+ group; 2) preference for the stereochemistry shown (usually L) to accommodate R^1; 3) an extended planar backbone from the N-terminal C_α atom to R^2; 4) a hydrogen bond to the carbonyl group of the first peptide bond; 5) alkylation of N^2 is

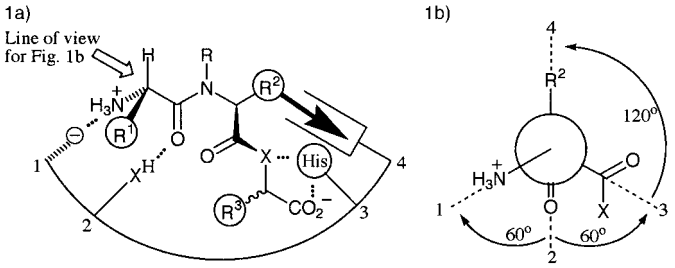

Figure 1. a) Substrate template for binding to PepT1. Free dipeptides terminate with O^- at X, whilst tripeptides are extended by X = NH; possible complementarity to PepT1 is indicated for the 4 main binding features; plain bonds (——) lie close to the plane of the paper. b) The orientation of the four main binding features, viewed as indicated in Figure 1a.

permitted; 6) specific orientation of three groups at the second residue (usually L); 7) a hydrophobic pocket, which possesses a strong directional vector as indicated; 8) a carboxylate binding site; 9) available space for the side-chain R^3; 10) a second carboxylate binding site, with stereochemical consequences on the adjacent chiral center (usually L).

This model effectively identifies four key binding sites (1, 4, 7, and 8/10), whilst the 3-D layout of the template is defined by the other stereochemical and conformational features. Thus, high-affinity substrates can generally adopt the correct 3-D conformation without paying a high energetic price. It is the aggregate effect of all of these factors that governs whether substrates have high, medium, or low affinity for PepT1.

The strong structure – activity correlation is emphasized by looking at eight diverse classes of compound, for which selected data are provided in Table 1: a) amino acid derivatives (entries 1 – 2); b) dipeptides (entries 3 – 16); c) β-amino acid dipeptides (entries 17 – 18); d) peptide space mimics (entries 19 – 25); e) alanyl anilides (entries 26 – 29); f) tripeptides (entries 30 – 33); g) β-lactams (entries 34 – 36); h) other drugs (entries 37 – 42).

Particularly notable is the affinity of PepT1 for molecules possessing amino and carboxylic acid groups separated by about 6 Å (see **1**, **2**, and **3** in Figure 2), even in non-peptidic substrates, whereas poor binding is observed for β-Ala-Gly and D-Phe-L-Pro (**4** and **5**), which cannot readily adopt appropriate conformations. The template successfully predicts the stereochemical and side-chain selectivity of PepT1 for dipeptides (**6**); the previously unexplained high affinity of 4-substituted alanyl anilides (**7** and Figure 3) is also consistent with the template, as is the observed low affinity of alanyl benzylamide (**8**). The template explains the selectivity in the binding of tripeptides (**9**) and, remarkably, how β-lactam antibiotics (**10**) can also bind with high affinity; for the latter, the amide group of the β-lactam does not follow the template backbone, but the C–N bond must instead be rotated by 180° (**11** and Figure 3), which leads to the D-stereochemistry of the penicillins/cephalosporins matching the L-stereochemistry of tripeptides.

In conclusion, the template described here provides a model for the orientation of the key binding features of substrates for PepT1. We can therefore, for the first time, predict whether substrates will have high, medium, or low affinity for PepT1, and the template can be used to place a set

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Table 1. Predicted and observed affinity of selected substrates for PepT1.

Entry	Substrate	Key site[a]	Prediction[b]	K_i [mM][c]	Method[c]
1	Phe	N^1	L	> 50	B, [12]
2	Ac-Phe	C^2	M +	2	B, [12]
3	Phe-Tyr	N^1	H +	0.1	B, [12]
4	Ac-Phe-Tyr	C^3	M	8.5	B, [12]
5	L-Ala-Gly	N^1	H	0.027	[11]
6	D-Ala-Gly	N^1	H –	1.9	[11]
7	Gly-L-Ala	N^1	H	0.032	[11]
8	Gly-D-Ala	N^1	M	12	[11]
9	Gly-Asp	N^1	H –	0.15	[11]
10	Gly-Lys	N^1	H –	0.15	[11]
11	Gly-Phe	N^1	H +	0.019	[11]
12	Gly-Sar	N^1	H	0.37	[11]
13	Pro-Gly	N^1	M	6.0	[11]
14	Gly-Pro	N^1	H	0.38	[11]
15	Ala(*trans*-ψS)-Pro	N^1	H	0.3	[13]
16	D-Phe-Pro	N^1	L	21	B
17	Gly-β-Ala	N^1	H –	1.3	[11]
18	β-Ala-Gly	N^1	M	5.2	[11]
19	H_2N-$(CH_2)_3$-CO_2H	N^1	L	> 50	[15]
20	H_2N-$(CH_2)_4$-CO_2H	N^1	M	1.14	[15]
21	$H_2NCH_2C(=O)(CH_2)_2CO_2H$	N^1	M +	0.4	[16]
22	4-H_2N-C_6H_4-CO_2H	N^1	M –	11	A
23	3-H_2N-C_6H_4-CH_2CO_2H	N^1	M	6	A
24	4-H_2N-C_6H_4-CH_2CO_2H	N^1	M	6.5	A, [14]
25	4-H_2NCH_2-C_6H_4-CO_2H	N^1	H –	1.4 (±0.4)	A, [9]
26	Ala-NH-Ph	N^1	M +	2.9	[17], B
27	Ala-NH-C_6H_4(4-Me)	N^1	H –	0.34	[17], B
28	Ala-NH-C_6H_4(4-Ph)	N^1	H +	0.03	[17]
29	Ala-NH-CH_2-Ph	N^1	M –	14	[17]
30	Ala-Ala-Ala	N^1	H	0.54	B
31	D-Ala-Ala-Ala	N^1	M –	15	B
32	Ala-D-Ala-Ala	N^1	L	16	B
33	Ala-Ala-D-Ala	N^1	M	4.1	B
34	amoxycillin (a penicillin)	N^1	H –	2	[18]*
35	cefadroxil (a cephalosporin)	N^1	H –	0.6	A
36	lorabarbef (a carba-ceph.)	N^1	H –	1.8	[18]
37	captopril	C^2	M	5	A
38	enalapril	C^2	H	1	A
39	acyclovir	?	L	> 50	[4]*
40	Val-acyclovir	N^1	M	4.1	[5]
41	L-dopa	N^1	L	> 50	[5]*
42	L-dopa-Phe	N^1	H	0.03	[5]*

[a] The key site identifies the key locating feature for the substrate on the template; $N^1 = NH_3^+$ site, C^2 = dipeptide carboxylate site, C^3 = tripeptide carboxylate site, ? = no identifiable match. [b] Predicted binding, see text for further information. [c] K_i values were obtained by us (see Experimental Section for A/B) or by others (ref. given in parenthesis; * indicates K_i was estimated from the data).

of related substrates in order of increasing affinity. The template should be of immediate value in designing and improving medicinally important compounds by increasing their oral bioavailablity through the PepT1 transport mechanism.

Experimental Section

For all data used in this analysis, representative examples of substrate K_is determined by other groups were also analyzed under our experimental conditions (see A and B below) at an external pH value of 5.5 to ensure comparability of data. New data reported herein use either A) inhibition of ^{3}H-labeled D-Phe-L-Gln (10 mM) uptake in rat BBMV at pH 5.5[8] or B) Michaelis – Menten analysis of the inhibition of ^{3}H-labeled D-Phe-L-Gln uptake at pH 5.5 into *Xenopus laevis* oocytes expressing PepT1 from microinjected cRNA.[9]

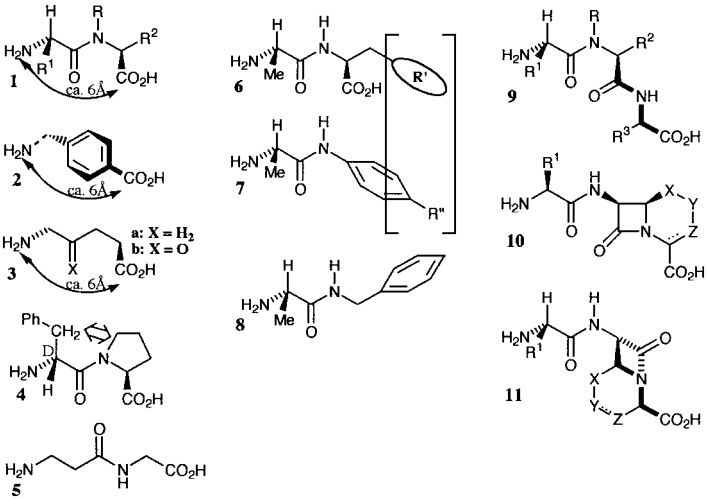

Figure 2. Structures **1** – **5**: Dipeptide analogues showing: the standard L-L dipeptide (**1**) in the template conformation; space mimics 4-(aminomethyl)benzoic acid (**2**), 5-aminopentanoic acid (**3a**), and laevulinic acid (**3b**), all of which show expected medium/high affinity for PepT1; D-Phe-L-Pro (**4**) with the steric clash identified; β-Ala-Gly (**5**) with the template mismatch apparent. Structures **6** – **8**: Dipeptide analogues showing: standard L-L dipeptide (**6**), emphasising the location of the hydrophobic part (R′) of side-chain R^2; the high-affinity alanyl anilides (**7**), showing R″ in the hydrophobic pocket; low-affinity alanyl benzylamide (**8**). Structures **9** – **11**: Tripeptide analogues showing: standard L-L-L tripeptide (**9**) in the template conformation; the general structure of L-α-amino penicillin/cephalosporin β-lactam antibiotics (**10**); **10** presented in the template conformation **11** to explain the medium/high affinity for PepT1 of the orally active β-lactam substrates. (X = S or CH_2 (carba-analogues); Y = nothing and Z = CMe_2 (penicillins); Y = CH and Z = CMe (cephalosporins)).

The predicted affinity was determined by assuming an overlay onto the template (examples shown in Figure 3). Structures were produced in XED98 using extended electron distribution molecular mechanics,[19] and the default dielectric constant of 4. For template structures, rotations of less than 10° were permitted about the angles identified in Figure 1, and acceptable conformations were required to be within 10 kcal mol^{-1} of the global minima, which were determined by the rigorous fast dynamics/Monte Carlo conformational search routine within XED98.[20] All of the ten factors identified in the template were given a weighting, and hydrophobicity and entropy factors were also included. This process allowed semi-quantitative assignment of high (H, < 1 mM), medium (M, 1 – 20 mM), or low (L, > 20 mM) affinity, although this is usually clear by simple inspection; the relative affinity could also be predicted within a group of related substrates (designated by + for higher affinity, and – for lower affinty, in Table 1).

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[1] D. Meredith, C. A. R. Boyd, *J. Membrane Biol.* **1995**, *145*, 1.
[2] a) D. M. Matthews, *Protein Absorption: Development and Present State of the Subject*, Wiley-Liss, New York, **1991**; b) V. Ganapathy, M. Brandsch, F. H. Leibach in *Physiology of the Gastro-intestinal Tract*, 3rd ed. (Ed.: L. R. Johnson), Raven, New York, **1994**, pp. 1773 – 1794.
[3] G. L. Amidon, H. J. Lee, *Ann. Rev. Pharmacol. Toxicol.* **1994**, *34*, 321.
[4] P. V. Balimane, I. Tamai, A. Guo, T. Nakanishi, H. Kitada, F. H. Leibach, A. Tsuji, P. J. Sinko, *Biochem. Biophys. Res. Commun.* **1998**, *250*, 246.
[5] I. Tamai, T. Nakanishi, H. Nakahara, Y. Sai, V. Ganapathy, F. H. Leibach, A. Tsuji, *J. Pharm. Sci.* **1998**, *87*, 1542.
[6] Y.-J. Fei, Y. Kanai, S. Nussberger, V. Ganapathy, F. H. Leibach, M. F. Romero, S. K. Singh, W. F. Boron, M. A. Hediger, *Nature* **1994**, *368*, 563.

  0570-0833/00/3903-0507 $ 17.50+.50/0

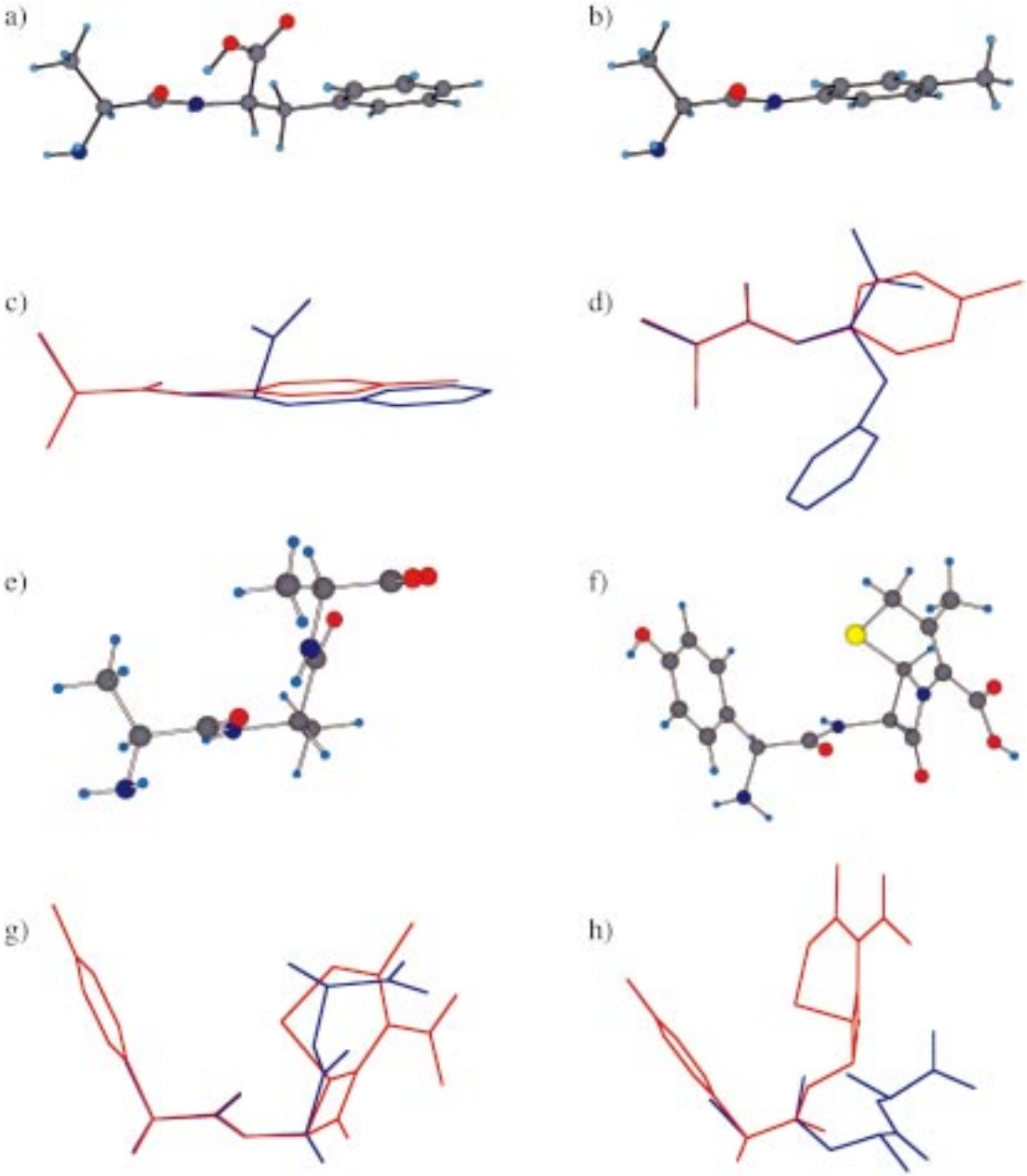

Figure 3. a) L-Ala-L-Phe and b) alanyl (4-methyl)anilide (see structures **6** and **7**), in their template conformations; c) overlay of the backbones from (a) and (b), showing good overlap; d) overlay of global minima conformations for (a) and (b), demonstrating poor overlap; e) L-Ala-L-Ala-L-Ala and f) L-cefadroxil (see structures **9** and **11**), both in their template conformations; g) overlay of the backbones from (e) and (f), showing good overlap; h) overlay of global minima conformations for (e) and (f), demonstrating poor overlap.

[7] a) J. B. Li, I. J. Hidalgo, *J. Drug Targeting* **1996**, *4*, 9; b) P. W. Swaan, J. J. Tukker, *J. Pharm. Sci.* **1997**, *86*, 596.

[8] C. S. Temple, P. D. Bailey, J. R. Bronk, C. A. R. Boyd, *J. Physiol.* (*London*) **1996**, *494*, 795.

[9] D. Meredith, C. A. R. Boyd, J. R. Bronk, P. D. Bailey, K. M. Morgan, I. D. Collier, C. S. Temple, *J. Physiol.* (*London*) **1998**, *512*, 629.

[10] N. Lister, A. P. Sykes, P. D. Bailey, C. A. R. Boyd, J. R. Bronk, *J. Physiol.* (*London*) **1995**, *484*, 173.

[11] H. Daniel, E. L. Morse, S. A. Adibi, *J. Biol. Chem.* **1992**, *267*, 9565.

[12] N. Guha, C. A. R. Boyd, D. Meredith, *J. Physiol.* (*London*) **1999**, *517P*, 57.

[13] M. Brandsch, F. Thunecke, G. Kullertz, M. Schutkowski, G. Fischer, K. Neubert, *J. Biol. Chem.* **1998**, *273*, 3861.

[14] C. S. Temple, A. K. Stewart, D. Meredith, N. A. Lister, K. M. Morgan, I. D. Collier, R. D. Vaughan-Jones, C. A. R. Boyd, P. D. Bailey, J. R. Bronk, *J. Biol. Chem.* **1998**, *273*, 20.

[15] F. Döring, J. Walter, J. Will, M. Föcking, M. Boll, S. Amasheh, W. Clauss, H. Daniel, *J. Clin. Invest.* **1998**, *101*, 2761.

[16] F. Döring, J. Will, S. Amasheh, W. Clauss, H. Ahlbrecht, H. Daniel, *J. Biol. Chem.* **1998**, *273*, 23211.

[17] V. Börner, Y.-J. Fei, B. Hartrodt, V. Ganapathy, F. H. Leibach, K. Neubert, M. Brandsch, *Eur. J. Biochem.* **1998**, *255*, 698.

[18] U. Wenzel, D. T. Thwaites, H. Daniel, *Br. J. Pharmacol.* **1995**, *116*, 3021.

[19] J. G. Vinter, *J. Comput. Aided Mol. Des.* **1996**, *10*, 417.

[20] S. D. Morley, D. E. Jackson, M. R. Sanders, J. G. Vinter, *J. Comput. Chem.* **1992**, *13*, 693.

  0570-0833/00/3903-0508 $ 17.50+.50/0