buffer (10 mM Tris, pH = 7.4 at room temperature, 10% glycerol). Solid ammonium sulfate was added slowly to a final concentration of 40% and the precipitate was pelleted by centrifugation at 40000*g* for 10 min. The pellet was gently resuspended in TG buffer to a final dilution of 1:10. Dry charcoal was added (2 mg/mL) and the mixture was incubated overnight at 4 °C. The charcoal was removed by centrifugation at 15000*g* for 20 min followed by another centriguation at 135000*g* for 1 h. Samples were frozen and stored at -70 °C. The hSHBG preparation was thawed at room temperature and diluted an additional 1:4 with TG buffer. Duplicate aliquots were incubated for 2 h at 4 °C with [1,2-^{3}H(N)]-5α-dihydrotestosterone (1 nM final concentration) in either the absence or the presence of increasing concentrations (10^{-9}–10^{-4} M) of DHT or test compounds. Following the incubation period, a suspension of dextran-coated charcoal (0.5% charcoal, 0.05% dextran T-70) was added to the ligand–cytosol mixture and incubated for 2 min. The charcoal was pelleted by centrifugation at 1500*g* for 10 min and the supernatant (protein-bound [^{3}H]DHT) was counted. Relative binding affinities were calculated as described for rABP. Compounds that did not inhibit [^{3}H]DHT binding by 50% at a competitor concentration of 10 μM were considered to be inactive (RBA $\leq$ 0.01).

Rat Prostate Androgen Receptor Competition Assay. This procedure was run as previously described.[14] Cystosol, prepared with the prostates from castrated adult male Sprague–Dawley rats, was incubated with 17α-methyl-[^{3}H]R1881 (New England Nuclear; methyltrienolone, 5 nM final concentration) in either the absence or the presence of increasing concentrations (10^{-9}–10^{-5} M) of R1881 (New England Nuclear) or test compounds for 18 h at 4 °C. After the incubation period, a suspension of dextran-coated charcoal (1% charcoal, 0.05% dextran T-70) was added to the ligand–cytosol mixture and incubated for 5 min. The charcoal-bound ^{3}H-R1881 was removed by centrifugation and the supernatant was counted. Relative binding affinities were calculated as the ratio of the concentration required to inhibit [^{3}H]R1881 specific binding by 50% (with R1881 arbitrarily set at 100). Compounds that did not inhibit [^{3}H]R1881 binding by 50% at a competitor concentration of 10 μM were considered to be inactive (RBA < 0.01). A Lineweaver–Burk analysis of the binding of **11** to rat ABP was run as previously described.[15]

Registry No. **1**, 446-52-6; **2**, 105-45-3; **3**, 86408-08-4; **4**, 104431-82-5; **5**, 104431-76-7; **6**, 104431-77-8; **6**·HCl, 104431-83-6; **7**, 104431-78-9; **8**, 104431-72-3; **9**, 104431-73-4; **10**, 87-13-8; **11**, 104431-74-5.

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Anticandidal Properties of N^3-(4-Methoxyfumaroyl)-L-2,3-diaminopropanoic Acid Oligopeptides

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Tri-, tetra-, and pentapeptides containing N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP), an inactivator of glucosamine 6-phosphate synthase of fungal origin (a key enzyme in the biosynthesis of macromolecular components of the fungal cell wall) have been synthesized and investigated as anticandidal agents. Structure–activity relationships of a series of peptides revealed that tripeptides were generally more active than the other peptides examined. In this study, the lysyl peptide, Lys-Nva-FMDP has been found to be the most active compound in the series.

Our previous report demonstrated that dipeptides composed of aliphatic amino acids and N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP), especially in the C-terminal position, exhibited remarkable antimicrobial activity against a range of fungal organisms, including the pathogenic fungus *Candida albicans*.[1] The anticandidal properties of this peptides are connected with the presence of the FMDP residue, which, after release from dipeptide by intracellular peptidases, irreversibly inactivates glucosamine 6-phosphate synthase from *C. albicans*.[2] This enzyme catalyzes the formation of D-glucosamine 6-phosphate from L-glutamine and D-fructose 6-phosphate. D-Glucosamine 6-phosphate is a key molecule in the biosynthetic formation of amino–sugar-containing macromolecules of the microbial cell wall.[3] Therefore, glucosamine 6-phosphate synthase inhibitors offer a potentially useful approach to the rational design of anticandidal agents. It has been shown that dipeptides with FMDP as the "warhead" utilize a peptide transport system for entry into the cell according to the "portage transport", a concept described by Gilvarg.[4] Moreover, we have reported that FMDP tripeptides, in contrast to dipeptides with FMDP residues, are carried into *C. albicans* cells via two permeases. The first is specific for di- and tripeptides and the second permease transports peptides containing three through six amino acid residues.[5] This finding prompted us to synthesize a set of FMDP-containing tripeptides in order to establish a structure–activity rela-

Table I. Analytical and Physical Data of Protected Peptides

no.	compd	% yield	mp, °C	$[\alpha]^{25}_{578}$ [a]	anal.
1	Boc-Ala-FMDP-Ala	73	142–145	-29.5	$C_{19}H_{30}N_4O_9$
2	Boc-Met-FMDP-Met	51	148–150	-26.2	$C_{23}H_{38}N_4O_9S_2$
3	Boc-Met-Ala-FMDP	82	74–76	-23.2	$C_{21}H_{34}N_4O_9S$
4	Boc-Met-Met-FMDP	88	162–163	-27.0	$C_{23}H_{38}N_4O_9S_2$
5	Boc-Met-Nva-FMDP	88	152–154	-24.2	$C_{23}H_{38}N_4O_9S$
6	Boc-FMDP-Met-Ala	75	102–104	-16.8	$C_{21}H_{34}N_4O_9S$
7	Boc-FMDP-Met-FMDP	72	105–107	-15.2	$C_{26}H_{39}N_5O_{12}S$
8	Boc-Sar-Nva-FMDP	82	145–150	-28.2	$C_{21}H_{34}N_4O_9$
9	Boc-Nva-Nva-FMDP	92	82–84	-34.4	$C_{23}H_{38}N_4O_9$
10	Boc-Nva-FMDP-Nva	62	172–174	-27.2	$C_{23}H_{38}N_4O_9$
11	Boc-Lys-Boc-Nva-FMDP	84	oil	*b*	$C_{29}H_{49}N_5O_{11}$
12	Boc-Met$_3$-FMDP	86	114–116	-28.5	$C_{28}H_{47}N_5O_{11}S_3$
13	Boc-Met-FMDP$_2$	58	182–184	-19.8	$C_{31}H_{48}N_6O_{13}S_2$
14	Boc-FMDP-Met$_2$-FMDP	61	144–146	-19.6	$C_{31}H_{48}N_6O_{13}S_2$
15	Boc-Met$_4$-FMDP	88	152–155	-29.8	$C_{33}H_{56}N_6O_{11}S_4$

[a] In degrees (c = 1, MeOH). [b] Not determined.

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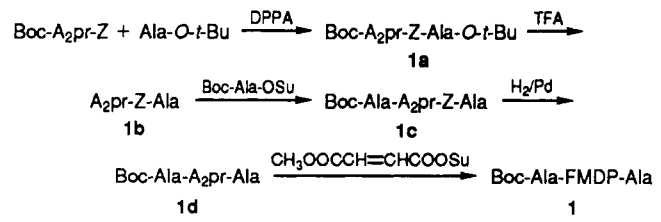
Scheme I



tionship within this group and to evaluate their anticandidal potency. We reasoned that tripeptides would display higher anticandidal effects than the dipeptides and that the formation of spontaneous mutants resistant to FMDP tripeptides would also be reduced. We present here the results of both synthetic and microbiological studies which support our hypothesis. We have also prepared tetra- and pentapeptides for comparison purposes.

Chemistry

Starting with the previously reported procedure employed for FMDP dipeptides, the conventional *N*-hydroxysuccinimide ester method has been used in the preparation of all obtained peptides. The protected peptides obtained in this work are summarized in Table I. Tripeptides with the FMDP residue in the C-terminal position, **3–5**, **8**, **9**, and **11**, were prepared by coupling the *N*-hydroxysuccinimide ester of *N*-(*tert*-butoxycarbonyl)-L-amino acids with FMDP dipeptides in a water–methanol solution. Yields varied from 92% for compound **9** to 82% for **3**. Tripeptide **1** was obtained in the following reaction sequences (Scheme I): N^2-(*tert*-butoxycarbonyl)-N^3-(benzyloxycarbonyl)-L-2,3-diaminopropanoic acid (Boc-A_2pr-Z)[6] was combined with *tert*-butyl alaninate (Ala-*O*-*t*-Bu)[7] with the aid of DPPA reagent[8] to give peptide **1a**. Deprotection of both groups, the *tert*-butyl ester and Boc group, was accomplished almost quantitatively with trifluoroacetic acid. Treatment of the resultant peptide **1b** with Boc-Ala-OSu[9] gave peptide **1c**. Conventional reductive removal of benzyloxycarbonyl group from tripeptide **1c** in the presence of Pd on charcoal afforded compound **1d**, which was acylated with the *N*-hydroxysuccinimide ester of monomethyl fumarate[10] to yield the final peptide **1**. Peptides containing nonterminal FMDP residue (**2** and **10**) were similarily prepared by using the same coupling procedures described earlier, although in moderate yields. Peptides **6** and **7** with FMDP in the N-terminal position were synthesized by coupling *N*-hydroxysuccinimide ester of N^2-(*tert*-butoxycarbonyl)-N^3-(4-methofumaroyl)-L-2,3-diaminopropanoic acid (Boc-FMDP-OSu)[1] with corresponding dipeptides, yielding peptides in good yields. Tetrapeptides containing two FMDP residues (**13** and **14**) were also prepared by activated ester coupling of corresponding fragments. Pentapeptide **15** was obtained from the appropriate tetrapeptide, Met_3-FMDP, and N-protected methionine by the active ester route with 88% yield. Final deprotection was achieved by acidolysis with 4 N HCl in dioxane to yield the desired peptides **16–30** (Table II) as their hydrochlorides.

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Table II. Analytical and Physical Data of Deprotected Peptides

no.	compd	% yield	mp, °C	$[\alpha]^{25}_{578}$[a]	anal.
16	Ala-FMDP-Ala·HCl	92	193–196	+8.2	$C_{14}H_{23}N_4O_7Cl$
17	Met-FMDP-Met·HCl	87	205–207	+15.2	$C_{18}H_{31}N_4O_7S_2Cl$
18	Met-Ala-FMDP·HCl	88	185–187	+12.2	$C_{16}H_{27}N_4O_7SCl$
19	Met-Met-FMDP·HCl	90	177–179	+4.8	$C_{18}H_{31}N_4O_7S_2Cl$
20	Met-Nva-FMDP·HCl	86	182–184	−13.5	$C_{18}H_{31}N_4O_7SCl$
21	FMDP-Met-Ala·HCl	86	166–168	−22.0	$C_{16}H_{27}N_4O_7SCl$
22	FMDP-Met-FMDP·HCl	92	173–175	−16.2	$C_{21}H_{32}N_5O_{10}SCl$
23	Sar-Nva-FMDP·HCl	94	212–214	−18.2	$C_{16}N_{27}N_4O_7Cl$
24	Nva-Nva-FMDP·HCl	88	175–177	+6.8	$C_{18}H_{32}N_4O_7Cl$
25	Nva-FMDP-Nva·HCl	90	228–230	+16.5	$C_{18}H_{22}N_4O_7Cl$
26	Lys-Nva-FMDP·2HCl	92	253–257	−7.4	$C_{19}H_{35}N_5O_7Cl_2$
27	Met_3-FMDP·HCl	84	185–187	−8.8	$C_{23}H_{40}N_6O_8S_3Cl$
28	Met-$FMDP_2$·HCl	90	189–192	+4.8	$C_{26}H_{41}N_6O_{11}S_2Cl$
29	FMDP-Met_2-FMDP·HCl	91	186–188	−29.6	$C_{26}H_{41}N_6O_{11}S_2Cl$
30	Met_4-FMDP·HCl	86	192–194	−18.6	$C_{28}H_{49}N_6O_9S_4Cl$

[a] In degrees (*c* = 1, MeOH).

Table III. Antifungal in Vitro Activity of FMDP Peptides

	MIC, μg/mL			
peptide[a]	*C. albicans* ATCC 262	*C. albicans*[b]	*C. epidermidis* OBS 6023	*S. cerevisiae* ATCC 9763
Leu-FMDP	0.8	0.25	100	62.5
Nva-FMDP	0.4	0.1	75	NT[c]
Ala-FMDP-Ala	0.75	0.25	80	37.5
Met-FMDP-Met	0.3	0.15	110	50
Met-Ala-FMDP	0.75	0.25	125	7.5
Met-Met-FMDP	0.25	0.1	125	37.5
Met-Nva-FMDP	0.125	0.05	130	7.5
FMDP-Met-Ala	0.25	0.1	50	15
FMDP-Met-FMDP	0.75	0.25	75	150
Sar-Nva-FMDP	1.0	0.5	130	150
Nva-Nva-FMDP	0.1	0.05	125	12.5
Nva-FMDP-Nva	0.05	0.01	125	50
Lys-Nva-FMDP	0.025	0.01	125	NT
Met_3-FMDP	0.75	0.25	75	100
Met-$FMDP_2$	0.75	0.25	125	150
FMDP-Met_2-FMDP	0.25	0.25	75	75
Met_4-FMDP	0.5	0.25	75	37.5

[a] Tested as hydrochlorides. [b] Sixteen clinical strains. [c] Not tested.

Table IV. Rate of Transport and Intracellular Cleavage of FMDP Peptides in *C. albicans* ATCC 26278

peptide	transport rate, nmol/min per mg	cleavage rate, nmol/min per mg
Leu-FMDP	1.8	15.4
Nva-FMDP	2.0	35.3
Ala-FMDP-Ala	0.9	5.4
Met-FMDP-Met	2.0	4.8
Met-Ala-FMDP	1.2	15.2
Met-Met-FMDP	1.4	4.9
Met-Nva-FMDP	1.4	17.3
FMDP-Met-Ala	1.0	4.9
FMDP-Met-FMDP	1.4	3.0
Nva-Nva-FMDP	1.7	20.9
Nva-FMDP-Nva	2.9	8.9
Lys-Nva-FMDP	3.6	51.0
Met_3-FMDP	0.9	23.7
Met-$FMDP_2$	1.9	10.0
FMDP-Met_2-FMDP	1.2	5.8
Met_4-FMDP	1.3	18.6

Results and Discussion

To compare structure–activity relationships, we classified the peptides into three groups according to their structures (tri-, tetra-, and pentapeptides). The results obtained in the serial dilution method for 16 fresh clinical isolates of *C. albicans* collected at the Medical Academy of Gdańsk and for the standard strain of *C. albicans* ATCC 26278 are presented in Table III. These findings demonstrate that the peptides possess excellent activity against all the test strains of *C.* albicans. As can be seen from the

Table V. Number of Resistant Colonies Obtained from 10^5 Inoculum Cells of *C. albicans* ATCC 26278

peptide tested (10 μg/mL)	no. of colonies
Nva-FMDP	40
Leu-FMDP	30
Met-Met-FMDP	3
Nva-Nva-FMDP	2
Lys-Nva-FMDP	2
Met_3-FMDP	12

table, the MIC's range from 0.025 to 1.0 μg/mL. Tripeptide Lys-Nva-FMDP has been shown to be the most active compound tested in this study with the MIC = 0.025 μg/mL for *C. albicans* ATCC 26278 and 0.01 μg/mL for clinical isolates of *C. albicans*. This high activity may be explained by exceptionally high rates of peptide uptake into fungal cells (Table IV, resulting in higher levels of FMDP inside the cell compared with other peptides (values of 3.6 and 51.0 nmol/min per mg, respectively). Although the lysyl dipeptides are transported into *C. albicans* cells at good rates,[11] their rates of intracellular hydrolysis are very low. Lys-Ala and Lys-FMDP were cleaved by peptidases from *C. albicans* ATCC 26278 cell free extract with rates of 1, 3, and 0.2 nmol/min per mg respectively.[12] Taking into account a very good transport rate of Lys-X dipeptides and a good cleavage rate of Nva-X di- and tripeptides, we rationally designed the structure Lys-Nva-FMDP. We expected this peptide to be transported and cleaved very fast; these assumptions were fortunately confirmed. The least active peptide was found to be the sarcosyl peptide Sar-Nva-FMDP (MIC ranged from 0.5 to 1.0 μg/mL). It should be noted, however, that the position of FMDP has little or no influence on the biological activity. Peptides with the N-terminal or C-terminal FMDP exhibited almost identical activity. Previously, we reported that dipeptides with the C-terminal FMDP displayed higher anticandidal activity than their structural isomers.[1] Moreover, the presence of two FMDP residues in the peptide molecule did not enhance their anticandidal potency. Rates of peptide transport into the cells and intracellular cleavage of these peptides were comparable with those for peptides with one FMDP residue.

All peptides were also tested against *Candida epidermidis* OBS 6023 and *Saccharomyces cervisiae* ATCC 9763 (see Table III). Results indicate that FMDP peptides, in general, displayed a weak effect in vitro.

Table V shows that for the selected tripeptides (Met-Met-FMDP, Nva-Nva-FMDP, and Lys-Nva-FMDP) a number of resistant colonies of *C. albicans* ATCC 26278 is remarkably reduced in comparison to those of di- and tetrapeptides. The phenomenon of the spontaneous *C. albicans* resistance to antifungal oligopeptides was described by many authors, including Payne and Shallow.[13] They found the average frequency of resistant-colony formation to about 1 per 10^4 inoculum cells for dipeptides. In our previous paper concerning the specifity of peptide permeases in *C. albicans*, we reported that only tripeptides were transported by two permeases. This is the obvious reason for a lower frequency of spontaneous resistance to tripeptides. It seems to be sufficiently confirmed that a tripeptide is the optimal size for an anticandidal drug candidate of oligopeptide structure.

The peptide Nva-FMDP was previously shown to exhibit promising anticandidal in vivo activity when tested on murine general candidiosis model infection, being practically nontoxic for animals ($LO_{50} \gg 300$ mg/kg).[14] Some preliminary data indicate that the most active tripeptides should show much better chemotherapeutic properties. The particular results will be published as soon as all the "in vivo" tests are completed.

Conclusions

The data indicate that a tripeptide is the optimal size of peptide carrier for FMDP delivery into *C. albicans* cells. It is probably connected with the lower frequency of spontaneous resistance observed for FMDP tripeptides. The lysyl tripeptide, i.e. Lys-Nva-FMDP, was found to be the most active compound synthesized and evaluated in this study. Both transport and intracellular cleavage rates influenced the anticandidal activity of FMDP peptides. Moreover, tripeptides containing norvaline residue appeared to be most efficient antifungal peptides.

Experimental Section

Melting points were determined in open capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded on a Tesla BS-487 60-MHz spectrometer and shifts are presented in ppm from internal hexamethyldisiloxane as a standard. Homogenity of the products was determined by TLC on Kieselgel 60F 254 plates (Merck). Products were visualized by successive exposure (where appropriate) to ultraviolet light (UV), ninhydrin, and cerium sulphate reagents. Optical rotations were measured in a Polmat (Carl Zeiss Jena) polarimeter. The *N*-hydroxysuccinimide esters of *N*-(*tert*-butoxycarbonyl)amino acids were prepared by the previously described methods.[9] Dipeptide Met-Ala was purchased from Sigma, Boc-FMDP-Osu and dipeptides containing FMDP were prepared according to the previously described procedures.[1]

N^2-(*tert*-Butoxycarbonyl)-N^3-(benzyloxycarbonyl)-L-(2,3-diaminopropanoyl)-L-alanine *tert*-Butyl Ester (1a). To a solution of Boc-L-A_2pr-Z (1.6 g, 5 mmol) and L-Ala-*o*-*t*-Bu (0.87 g, 6 mmol) in DMF (10 mL) at −5 °C were added DPPA (1.3 mL, 5.5 mmol) and Et_3N (0.83 mL, 6 mmol). The reaction mixture was stirred at room temperature for 12 h, diluted with water (20 mL), and extracted with EtOAc (3 × 50 mL). The organic layer was washed with H_2O, 10% citric acid, 5% $NaHCO_3$, and H_2O again and dried over $MgSO_4$. After evaporation of the solvent, an oily peptide (**1a**) was obtained (2.13 g, 92%): ^{1}H NMR δ = 1.28 (d, 3 H), 1.38 (s, 9 H) 1.42 (s, 9 H), 3.2–3.5 (m, 2 H), 3.8–4.3 (m, 2 H), 5.12 (s, 2 H), 5.4 (br s, 1 H), 7.25 (s, 5 H), 7.3–7.6 (m, 2 H). Anal. ($C_{23}H_{35}N_3O_7$) C, H, N.

N^3-(Benzyloxycarbonyl)-L-(2,3-diaminopropanoyl)-L-alanine Trifluoroacetate Salt (1b). Peptide **1a** (1.85 g, 4 mmol) was dissolved in ice-cold TFA and stored at room temperature for 2 h. The solvent was removed in vacuo at 35 °C. The residue was taken up in $CHCl_3$ and reevaporated. The procedure was repeated; the residue was triturated with Et_2O, filtered, and dried over KOH in a dessicator to yield title product **1b** (1.69 g, 82%): mp 86–88 °C; $[\alpha]^{25}_{578} = -6.8°$ (c = 0.5, MeOH–H_2O (1:1)). Anal. ($C_{16}H_{20}N_3O_7F_3$) C, H, N.

N^2-[(*N*-*tert*-Butoxycarbonyl)-L-alanyl]-N^3-(benzyloxycarbonyl)-L-(2,3-diaminopropanoyl)-L-alanine (1c). To a cooled solution of **1b** (1.26 g, 3 mmol) and $NaHCO_3$ (0.54 g, 6 mmol) in H_2O (20 mL) was added *N*-hydroxysuccinimide ester of *N*-(*tert*-butoxycarbonyl)-L-alanine (0.85 g, 3 mmol) dissolved in CH_3OH (20 mL). The mixture was stored overnight, the solvent was evaporated, and the residue was dissolved in H_2O (5 mL), acidified with 10% citric acid, and extracted with EtOAc (3 × 50 mL). The organic extract was washed with H_2O, dried over $MgSO_4$, and evaporated to give **1c** (1.21 g, 84%) as an amorphous powder: ^{1}H NMR δ = 1.23 (d, 3 H), 1.30 (d, 3 H), 1.43 (s, 9 H), 3.3–3.6 (m, 2 H), 4.21 (m, 1 H), 4.4 (m, 1 H), 4.7 (m, 1 H), 5.10

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(s, 2 H), 5.5 (br s, 1 H), 7.3 (s, 5 H), 7.3–7.8 (m, 2 H). Anal. ($C_{22}H_{32}N_4O_8$) C, H, N.

N^2-[(N-*tert*-Butoxycarbonyl)-L-alanyl]-L-(2,3-diaminopropanoyl)-L-alanine (1d). Protected peptide 1c (0.96 g, 2 mmol) was dissolved in CH_3OH (40 mL) and 10% Pd/C (10% by weight of peptide) was added. Then the mixture was hydrogenolyzed for 2 h until TLC indicated complete deprotection. The catalyst was filtered and the residue was crystallized from CH_3OH–Et_2O to give **1d** (0.64 g, 92%): mp 142–144 °C; $[\alpha]^{25}_{578}$ = −16.1° (c = 1, MeOH). Anal. ($C_{14}H_{26}N_4O_6$) C, H, N.

N^2-(N-*tert*-Butoxycarbonyl)-N^3-(4-methoxyfumaroyl)-L-(2,3-diaminopropanoyl)-L-alanine (1). To a cooled solution of **1d** (0.51 g, 1.5 mmol) and $NaHCO_3$ (0.126 g, 1.5 mmol) was added the N-hydroxysuccinimide ester of monomethyl fumarate (0.34 g, 1.5 mmol) in CH_3OH (5 mL) with stirring. After usual workup, the title compound was obtained (0.51 g, 73%). Analytical data are summarized in Table I.

General Procedure A. Coupling Reaction Using N-Hydroxysuccinimide Active Ester. The appropriate dipeptide, tripeptide, or tetrapeptide hydrochloride (1.5 mmol) was dissolved in a mixture of CH_3OH–H_2O (1:1, v/v, 10 mL) containing Et_3N (0.45 mL, 3 mmol) and the mixture was cooled to 0 °C. To this solution were added the N-hydroxysuccinimide esters of the following N-(*tert*-butoxycarbonyl)amino acids and N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (1.5 mmol) dissolved in THF (5 mL) with stirring: L-alanine, L-methionine, sarcosine, L-norvaline, L-lysine. The resultant residue was stored at room temperature overnight. The solvents were removed, and the residue was dissolved in H_2O (10 mL), acidified with 10% citric acid, and extracted with EtOAc (5 × 20 mL). The organic layer was washed with saturated NaCl solution, dried over $MgSO_4$, and evaporated. The crude peptides **1–15** were crystallized from EtOAc–petroleum ether. Yields and analytical data are summarized in Table I.

General Procedure B. Removal of the *tert*-Butoxycarbonyl Group. The protected peptide (**1–15**, 1 mmol) was dissolved in cold 4 N HCl in dioxane (10 mL). The mixture reacted for 2 h and the solvent was evaporated at room temperature, leaving the deprotected peptides (**16–30**), which were crystallized from CH_3OH–Et_2O. Yields and analytical data are collected in Table II.

Preparation of Cell-Free Extract. Cells of *C. albicans* ATCC 26278 were cultivated on Sabouraud medium overnight at 30 °C with vigorous shaking. Then cells were harvested by centrifugation at 4 °C and washed twice with 0.05 M phosphate buffer, pH = 6.5. Cells were disrupted and cell-free extract was prepared as previously;[2] however, extraction of the cell paste was performed with 0.05 M phosphate buffer, pH = 6.5. Protein concentration in cell-free extract was determined according to Layne's modification of the Lowry procedure.[15]

Determination of Cleavage Rate. 200 μM solutions of FMDP peptides in 0.005 M phosphate buffer were incubated with 0.1 mL of cell-free extract in a total volume of 5 mL. Incubation was carried out at 30 °C for 30 min. At 10-min intervals, 0.5-mL samples were collected. Amino acid concentration in collected samples was determined by Cd–ninhydrin method[16] using a positive blank (without peptide) and a negative blank (without cell-free extract). Concentrations were read from standard curves obtained for a mixture of equimolar amounts of amino acids constituting respective FMDP peptide.

Determination of Transport Rate. Transport studies were performed according to the method described previously[5] using *C. albicans* ATCC 26278.[17]

Sensitivity Tests. The minimum inhibitory concentration (MIC) of each peptide was determined on a liquid YNB medium containing sodium glutamate.[5] The medium was inoculated with 10^4 cells/mL of various *C. albicans* strains. Results were determined after 24 h at 30 °C by using a turidimetric method at 660 nm.

Isolation of Mutants Resistant to FMDP Peptides. Conditions used for the isolation of *C. albicans* ATCC 26278 colonies, resistant to FMDP peptides, were the same as described elsewhere.[5]

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Registry No. **1**, 122593-02-6; **1a**, 122593-28-6; **1b**·TFA, 122593-32-2; **1c**, 122593-35-5; **1d**, 122593-38-8; **2**, 122593-03-7; **3**, 122593-04-8; **4**, 122622-42-8; **5**, 122593-05-9; **6**, 122593-06-0; **7**, 122593-07-1; **8**, 122593-08-2; **9**, 122593-09-3; **10**, 122622-43-9; **11**, 122622-44-0; **12**, 122593-10-6; **13**, 122593-11-7; **14**, 122593-12-8; **15**, 122593-13-9; **16**·HCl, 122593-14-0; **16** (free base), 122672-74-6; **17**·HCl, 122593-15-1; **17** (free base), 122672-75-7; **18**·HCl, 122593-16-2; **18** (free base), 122672-76-8; **19**·HCl, 122672-73-5; **19** (free base), 115083-98-2; **20**·HCl, 122593-17-3; **20** (free base), 122672-77-9; **21**·HCl, 122593-18-4; **21** (free base), 122672-78-0; **22**·HCl, 122593-19-5; **22** (free base), 122672-79-1; **23**·HCl, 122674-59-3; **23** (free base), 122593-26-4; **24**·HCl, 122593-20-8; **24** (free base), 122672-80-4; **25**·HCl, 122593-21-9; **25** (free base), 122672-81-5; **26**·2HCl, 122593-22-0; **26** (free base), 122672-82-6; **27**·HCl, 122674-60-6; **27** (free base), 115083-99-3; **28**·HCl, 122593-23-1; **28** (free base), 122672-83-7; **29**·HCl, 122593-24-2; **29** (free base), 122672-84-8; **30**·HCl, 122593-25-3; **30** (free base), 122672-85-9; Leu-FMDP·HCl, 108233-21-2; Nva-FMDP·HCl, 108233-24-5; Leu-FMDP, 108340-66-5; Nva-FMDP, 108340-69-8; Ala-FMDP, 108340-63-2; Met-FMDP, 108340-64-3; Met-Ala, 3061-96-9; BOC-FMDP-OSu, 108233-40-5; BOC-Met-FMDP, 108232-98-0; BOC-FMDP-Met, 122593-27-5; BOC-Met-OSu, 3845-64-5; BOC-Sar-OSu, 80621-90-5; BOC-Nva-OSu, 108233-37-0; BOC-Lys(BOC)-OSu, 30189-36-7; BOC-A_2pr(Z), 65710-57-8; Ala-OBu-*t*, 21691-50-9; Met-OBu-*t*, 4976-72-1; Nva-OBu-*t*, 15911-75-8; A_2pr(Z)-Met-OBu-*t*, 122593-29-7; A_2pr(Z)-Nva-OBu-*t*, 122593-30-0; A_2pr(Z)-Me·TFA, 122593-34-4; A_2pr(Z)-Nva·TFA, 122622-46-2; BOC-Ala-OSu, 3392-05-0; BOC-Met-A_2pr(Z)-Met, 122593-36-6; BOC-Nva-A_2pr(Z)-Nva, 122593-37-7; BOC-Met-A_2pr-Met, 122593-39-9; BOC-Nva-A_2pr-Nva, 122593-40-2; (*E*)-MeOCOCH=CHCOOSu, 104302-78-5.

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(16) Doi, E.; Shibata, D.; Matoba, T. *Anal. Biochem.* **1981**, *118*, 173.
(17) The chromatographic analysis of the yeast suspension in phosphate buffer containing 1% glucose during peptide-transport determination proved that the tested peptide was the only amino-containing product present in the medium. Thus, there were no compounds interfering in the TNBS assay method.