ence of asymmetry and asymmetric preferences in the quinoline anti-infectives. Studies to more clearly define the significant spatial relationships on the quinolone nucleus are underway.

**Acknowledgment.** We are grateful for the expert work of our analytical groups under Dr. F. Mackellar, especially M. Stickney. We also thank Dr. C. Heifetz and J. Sesnie of our Microbiology staff and M. Solomon for providing the gyrase data.

**Townley P. Culbertson, John M. Domagala***
**Jeffery B. Nichols, Stephen Priebe, Richard W. Skeean**
*Warner-Lambert/Parke-Davis Research*
*Ann Arbor, Michigan 48105*
*Received April 6, 1987*

# *Articles*

## Synthesis and Biological Properties of $N^3$-(4-Methoxyfumaroyl)-L-2,3-diaminopropanoic Acid Dipeptides, a Novel Group of Antimicrobial Agents

Ryszard Andruszkiewicz,* Henryk Chmara, Sławomir Milewski, and Edward Borowski

*Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, 80-952 Gdańsk, Poland.*
*Received June 20, 1986*

A series of dipeptides with $N^3$-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP), the irreversible inhibitor of glucosamine-6-phosphate synthetase from bacteria and fungi, have been synthesized and their antibacterial and antifungal properties in vitro evaluated. The results demonstrate that these peptides inhibit the growth of a number of the tested microorganisms, especially pathogenic fungus *Candida albicans*. The results of competitive antagonism studies indicate specific peptide transport of the peptides via peptide permeases as drug delivery system and gives evidence for the high selectivity of the action upon the cells, as a result of the inhibition of generation of glucosamine.

In our previous studies we have demonstrated that $N^3$-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP),[1] a novel glutamine analogue, is a strong irreversible inhibitor and inactivator of glucosamine-6-phosphate irreversible inhibitor and inactivator of glucosamine-6-phosphate synthetase (EC 2.6.1.16) of bacterial[2] ($K_i = 2.7 \times 10^{-7}$ M, $K_{inact} = 1.8 \times 10^{-6}$ M) and fungal[3] ($K_i = 1.0 \times 10^{-7}$ M, $K_{inact} = 5.13 \times 10^{-6}$ M) origin,[4] a key enzyme in the biosynthesis of amino sugars. Inhibition of this enzyme by a probable covalent modification of its sulfhydryl group in the active center with FMDP prevents the formation of amino-sugar-containing macromolecules of the microbial cell wall. Therefore this enzyme can be considered as a valuable target for antimicrobial agents. Since FMDP itself is poorly active against whole microbial cells, we have presumed that this new inhibitor could be transported into the cells when incorporated in a peptide chain and hydrolyzed by intracellular peptidase releasing free FMDP as the "warhead" component of the peptide, which can react with the target enzyme, i.e., glucosamine-6-phosphate synthetase.

In recent years the idea of antimicrobial agent transport, called also "portage transport",[5] "smugglin transport",[6] or "warhead" delivery transport system,[7] for delivery of toxic, impermeant amino acids into targeted cells, using peptide carrier systems, has been the subject of numerous investigations.[8-10] This approach to selective drug delivery into microbial cells led to the discovery of a number of natural and synthetic peptides that contain growth inhibitory amino acids.[7] Antimicrobial peptides of this type are transported by the specific peptide permeases into the cells and hydrolyzed rapidly by intracellular peptidases, resulting in the release of the "warhead" component of peptides, so that it can react on a target enzyme. The most extensive study for exploitation of this concept for design of new chemotherapeutical agents has been carried out by the Roche group.[11] The investigators at Roche have

(1) Andruszkiewicz, R.; Chmara, H.; Milewski, S.; Borowski, E. *Book of Abstracts*, Postsymposium of IUPAC 14th International Symposium on the Chemistry of Natural Products, Gdańsk, 16–18 July 1984; p 43; Andruszkiewicz, R.; Chmara, H.; Milewski, S.; Borowski, E. *Int. J. Pept. Protein Res.* **1986**, *27*, 449.
(2) Chmara, H.; Andruszkiewicz, R.; Borowski, E. *Biochim. Biophys. Acta* **1986**, *870*, 357.
(3) Milewski, S.; Chmara, H.; Andruszkiewicz, R.; Borowski, E. *Biochim. Biophys. Acta* **1985**, *828*, 247.
(4) Abbreviations used in the text are as follows: $K_i$, inhibition constant for steady-state experiment; $K_{inact}$, inactivation constant, according to Meloche, P. (*Biochemistry* **1967**, *6*, 2237); MIC, minimum inhibitory concentration; cfu, colony-forming units; Nva, norvaline; Nle, norleucine; Abu, $\alpha$-aminobutyric acid; DPPA, diphenylphosphoryl azide; DMF, *N,N*-dimethylformamide; TFA, trifluoroacetic acid; TLC, thin-layer chromatography.
(5) Gilvarg, C. In *The Future of Antibiotherapy and Antibiotic Research*; Ninet, L., Bost, P. E., Bouanchaud, D. H., Florent, J., Eds.; Academic: New York, 1981.
(6) Payne, J. W. *Adv. Microb. Physiol.* **1976**, *13*, 55.
(7) Ringrose, P. S. In *Microorganisms and Nitrogen Sources*; Payne, J. W., Ed.; Wiley: New York, 1980; pp 641–692 and references therein.
(8) Cheung, K. S.; Wasserman, S. A.; Dudek, E.; Lerner, S. A.; Johnson, M. *J. Med. Chem.* **1983**, *26*, 1733.
(9) Kingsbury, W. D.; Boehm, J. C.; Metha, R. J.; Grappel, S. F. *J. Med. Chem.* **1983**, *26*, 1725.
(10) Kingsbury, W. D.; Boehm, J. C.; Metha, R. J.; Grappel, S. F.; Gilvarg, C. *J. Med. Chem.* **1984**, *27*, 1447.

0022-2623/87/1830-1715$01.50/0 © 1987 American Chemical Society

**Table I.** Analytical and Physical Data of Protected Peptides

| no. | compound | prep meth | yield, % | mp, °C | $[\alpha]^{25}_D$ (MeOH) | anal. C, H, N |
|---|---|---|---|---|---|---|
| 1 | Boc-Ala-FMDP | A | 81 | 92–93 | +31.2 (*c* 0.5) | $C_{16}H_{25}N_3O_8$ |
| 2 | Boc-Met-FMDP | A | 79 | 98–100 | +36.4 (*c* 0.5) | $C_{18}H_{29}N_3O_8S$ |
| 3 | Boc-Gly-FMDP | A | 70 | 120–121 | +15.5 (*c* 0.6) | $C_{15}H_{23}N_3O_8$ |
| 4 | Boc-Val-FMDP | A | 84 | 74–76 | 315.1 (*c* 0.3) | $C_{18}H_{29}N_3O_8$ |
| 5 | Boc-Leu-FMDP | A | 84 | 92–94 | +23.4 (*c* 0.4) | $C_{19}H_{31}N_3O_8$ |
| 6 | Boc-Phe-FMDP | A | 87 | 144–146 | –57.2 (*c* 0.7) | $C_{22}H_{29}N_3O_8$ |
| 7 | Boc-Tyr-FMDP | A | 80 | 140–142 | –34.2 (*c* 0.6) | $C_{22}H_{29}N_3O_9$ |
| 8 | Boc-Nva-FMDP | A | 83 | 68–70 | +22.6 (*c* 0.5) | $C_{18}H_{29}N_3O_8$ |
| 9 | Boc-Nle-FMDP | A | 79 | 78–80 | +20.9 (*c* 0.5) | $C_{19}H_{31}N_3O_8$ |
| 10 | Boc-Abu-FMDP | A | 76 | 50–60 | +27.3 (*c* 0.5) | $C_{17}H_{27}N_3O_8$ |
| 11 | Boc-Lys-(Boc)-FMDP | A | 68 | oil | not deter. | $C_{24}H_{40}N_4O_{10}$ |
| 12 | Boc-FMDP-Gly | B | 61 | 75–76 | –38.1 (*c* 0.5) | $C_{15}H_{23}N_3O_8$ |
| 13 | Boc-FMDP-Val | B | 70 | 77–80 | –41.2 (*c* 0.5) | $C_{18}H_{29}N_3O_8$ |
| 14 | Boc-FMDP-Leu | B | 72 | 85–87 | –37.6 (*c* 0.4) | $C_{19}H_{31}N_3O_8$ |
| 15 | Boc-FMDP-Phe | B | 74 | 91–93 | –62.3 (*c* 0.4) | $C_{22}H_{29}N_3O_8$ |
| 16 | Boc-FMDP-Tyr | B | 73 | 96–98 | –26.2 (*c* 0.2) | $C_{22}H_{29}N_3O_9$ |
| 17 | Boc-FMDP-Nva | B | 74 | 73–75 | –18.4 (*c* 0.5) | $C_{18}H_{29}N_3O_8$ |
| 18 | Boc-FMDP-FMDP | B | 70 | 108–110 | +11.2 (*c* 0.5) | $C_{21}H_{30}N_4O_{11}$ |
| 19 | Boc-FMDP-Ala-O-*t*-Bu | C | 84 | 105–106 | –21.3 (*c* 0.5) | $C_{20}H_{33}N_3O_8$ |
| 20 | Boc-FMDP-Met-O-*t*-Bu | C | 88 | 72–73 | –38.2 (*c* 0.5) | $C_{22}H_{37}N_3O_8S$ |

**Table II.** Analytical and Physical Data of Deprotected Peptides

| no. | compound | depr meth | yield, % | mp, °C | $[\alpha]^{25}_D$ (c 1, MeOH) | anal. C, H, N |
|---|---|---|---|---|---|---|
| 21 | Ala-FMDP·HCl | D | 90 | 183–185 | –42.8 | $C_{11}H_{18}N_3O_6Cl$ |
| 22 | Met-FMDP·HCl | D | 89 | 172–174 | –66.1 | $C_{13}H_{22}N_3O_6ClS$ |
| 23 | Gly-FMDP·HCl | D | 84 | 185–188 | –37.2 | $C_{10}H_{16}N_3O_6Cl$ |
| 24 | Val-FMDP·HCl | D | 88 | 186–188 | –26.8 | $C_{13}H_{22}N_3O_6Cl$ |
| 25 | Leu-FMDP·HCl | D | 86 | 189–190 | –30.2 | $C_{14}H_{24}N_3O_6Cl$ |
| 26 | Phe-FMDP·HCl | D | 88 | 200–202 | –36.8 | $C_{17}H_{22}N_3O_6Cl$ |
| 27 | Tyr-FMDP·HCl | D | 90 | 199–200 | –28.8 | $C_{17}H_{22}N_3O_7Cl$ |
| 28 | Nva-FMDP·HCl | D | 94 | 192–194 | –39.2 | $C_{13}H_{22}N_3O_6Cl$ |
| 29 | Nle-FMDP·HCl | D | 92 | 189–191 | –41.5 | $C_{14}H_{24}N_3O_6Cl$ |
| 30 | Abu-FMDP·HCl | D | 90 | 180–182 | –36.4 | $C_{12}H_{20}N_3O_6Cl$ |
| 31 | Lys-FMDP·2HCl | D | 82 | 205–207 | –54.1 | $C_{14}H_{26}N_4O_6Cl_2$ |
| 32 | FMDP-Gly·HCl | D | 87 | 188–190 | –23.2 | $C_{10}H_{16}N_3O_6Cl$ |
| 33 | FMDP-Val·HCl | D | 82 | 172–174 | –29.6 | $C_{13}H_{22}N_3O_6Cl$ |
| 34 | FMDP-Leu·HCl | D | 87 | 175–178 | –36.8 | $C_{14}H_{24}N_3O_6Cl$ |
| 35 | FMDP-Phe·HCl | D | 89 | 183–185 | –28.4 | $C_{17}H_{22}N_3O_6Cl$ |
| 36 | FMDP-Tyr·HCl | D | 92 | 174–177 | –38.2 | $C_{17}H_{22}N_3O_7Cl$ |
| 37 | FMDP-Nva·HCl | D | 88 | 189–190 | –45.2 | $C_{13}H_{22}N_3O_6Cl$ |
| 38 | FMDP-FMDP·HCl | D | 90 | 153–157 | –78.4 | $C_{16}H_{23}N_4O_9Cl$ |
| 39 | FMDP-Ala | E | 84 | 248–250 | –38.8[a] | $C_{11}H_{17}N_3O_6$ |
| 40 | FMDP-Met | E | 83 | 242–244 | –66.2[a] | $C_{13}H_{21}N_3O_6S$ |

[a] In 1 N HCl.

synthesized a large number of alaphosphono di- and oligopeptides containing a carboxy terminal L-(1-aminoethyl)phosphonic acid residue,[11] which is a powerful inhibitor of alanine racemase.[12] It has been shown that from the synthesized peptides alafosfalin was selected as a wide spectrum antibacterial agent, and more recently, L-norvalyl-L-(1-aminoethyl)phosphonic acid exhibited significantly higher activity against a variety of organisms when compared with alafosfalin.

According to this concept we have prepared a number of $N^3$-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid dipeptides and report here on their antimicrobial properties against some selected microorganisms.

## Chemistry

Synthesis of the FMDP peptides was accomplished by using standard peptide synthetic procedures as described in detail in the Experimental Section. The other peptides were prepared according to the same procedures, unless otherwise stated. The protected peptides prepared in this work are summarized in Table I. Dipeptides that contain FMDP as the C-terminus were prepared in the following manner. *N*-(*tert*-Butoxycarbonyl)-L-amino acids in the form of their *N*-hydroxysuccinimide esters[13] were combined with $N^3$-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP)[1] in a water–methanol solution to give the protected dipeptides **1–11** in good yields. Removal of the Boc groups from the N-terminal amino functions by acidolysis with 2 N hydrogen chloride in dioxane furnished the desired dipeptides in the form of their hydrochlorides (Table II).

Peptides with FMDP as the N-terminal amino acid were prepared either by coupling $N^2$-(*tert*-butoxycarbonyl)-$N^3$-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid

(11) Atherton, R. F.; Hall, M. J.; Hassall, C. H.; Lambert, R. W.; Ringrose, P. S. *Antimicrob. Agents Chemother.* **1979**, *15*, 677. Allen, J. G.; Atherton, R. F.; Hall, M. J.; Hassall, C. H.; Holmes, S. W.; Lambert, R. W.; Nisbet, L. J.; Ringrose, P. S. *Antimicrob. Agents Chemother.* **1979**, *15*, 684. Atherton, R. F.; Hall, M. J.; Hassall, C. H.; Lambert, R. W.; Lloyd, W. J.; Ringrose, P. S. *Antimicrob. Agents Chemother.* **1979**, *15*, 696. Atherton, R. F.; Hall, M. J.; Hassall, C. H.; Holmes, S. W.; Lambert, R. W.; Lloyd, W. J.; Ringrose, P. S. *Antimicrob. Agents Chemother.* **1980**, *18*, 897. Atherton, R. F.; Hall, M. J.; Hassall, C. H.; Lambert, R. W.; Lloyd, W. J.; Ringrose, P. S.; Westmacott, D. *Antimicrob. Agents Chemother.* **1982**, *22*, 571.

(12) Lambert, M. P.; Neuhaus, F. C. *J. Bacteriol.* **1972**, *110*, 978.

(13) Anderson, G. W.; Zimmerman, I. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1963**, *86*, 1839.

**Table III.** Activity of FMDP Dipeptides against Selected Microorganisms

| compound | MIC, μg/mL | | | | |
|---|---|---|---|---|---|
| | *Candida albicans* AMB 25 | *Staphylococcus aureus* 209P[a] | *Bacillus pumilus*[a] 1697 | *Shigella sonnei*[a] 433 | *Escherichia coli*[b] ATCC 9637 |
| Ala-FMDP | 0.8 | 50 | 1.56 | 3.12 | 100 |
| Met-FMDP | 0.8 | 50 | 3.12 | 3.12 | 100 |
| Gly-FMDP | 12.5 | >200 | 25 | 50 | >100 |
| Val-FMDP | 1.56 | 12.5 | 0.8 | 1.56 | 100 |
| Leu-FMDP | 0.8 | 12.5 | 1.56 | 1.56 | 100 |
| Phe-FMDP | 12.5 | 100 | 6.25 | 12.5 | >200 |
| Tyr-FMDP | 50 | 100 | 12.5 | 25 | >200 |
| Nva-FMDP | 0.4 | 12.5 | 0.4 | 3.12 | 100 |
| Nle-FMDP | 0.8 | 25 | 0.4 | 3.12 | 100 |
| Abu-FMDP | 1.56 | 25 | 0.8 | 6.25 | 100 |
| Lys-FMDP | >200 | >200 | 100 | >200 | >200 |
| FMDP-Gly | >200 | >200 | >200 | >200 | >200 |
| FMDP-Val | 6.25 | 100 | >200 | >200 | >200 |
| FMDP-Leu | 3.12 | 100 | >200 | >200 | >200 |
| FMDP-Phe | 1.56 | >200 | >200 | >200 | >200 |
| FMDP-Tyr | 25 | >200 | >200 | >200 | >200 |
| FMDP-Nva | 6.25 | 100 | >200 | >200 | >200 |
| FMDP-FMDP | >200 | >200 | >200 | >200 | >200 |
| FMDP-Ala | 3.12 | >200 | >200 | >200 | >200 |
| FMDP-Met | 1.56 | >200 | >200 | >200 | >200 |
| FMDP | >200 | >200 | >200 | >200 | >200 |

[a] Inhibition of growth was observed for FMDP dipeptides (with N-terminal FMDP) at high concentrations (~1 mg/mL). [b] Growth of this microorganism was generally inhibited by FMDP dipeptides (with C-terminal FMDP) at high concentrations (~1 mg/mL), but not by their structural isomers.

succinimido ester (Boc-FMDP-OSu) with corresponding amino acids, yielding peptides **12–17**, or by condensation of $N^2$-(*tert*-butoxycarbonyl)-$N^3$-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid with the *tert*-butyl esters of alanine[14] or methionine[14] respectively by the use of the DPPA[15] method, giving the fully protected dipeptides **19–20**. Treatment of the Boc protected peptides **12–17** with 2 N hydrogen chloride in dioxane afforded the deprotected peptides **32–37**. Peptides **19** and **20**, which contain both Boc and *tert*-butyl esters, were deprotected with trifluoroacetic acid to obtain deprotected dipeptides in the form of their hygroscopic trifluoroacetate salts, which were converted into corresponding acetate salts by ion exchange chromatography on a Dowex 1×2 (acetate form). After the removal of acetic acid by evaporation to dryness, peptides **39** and **40** were obtained as the compounds with zwitterionic structures. Additionally, we have obtained, for comparative purposes, dipeptides that consist of two FMDP residues, peptides **18** and **38**, respectively. This synthesis was performed as described for the synthesis of compound **12** (method B in Experimental Section).

All peptides were identified by $^1$H NMR spectra and elemental analysis. All compounds showed also a single spot on TLC utilizing various solvent systems.

## Results and Discussion

All synthesized compounds containing FMDP as the "warhead" component of the dipeptides in either the amino or carboxy terminal position show effective activity againt several tested microorganisms, including the pathogenic yeast *Candida albicans*. The MIC values estimated for a selected strain of *Candida albicans*, AMB 25, range from 0.4 to 200 μg/mL (Table III). These values are substantially lower for the peptides containing the "warhead" in the C-terminal position and for amino acids with an aliphatic side chain (Met, Ala, Leu, Nva, Nle). Peptides composed of these amino acid residues are known to be very effectively transported by bacteria and fungi.[16] As can be seen from the table, the most active dipeptide was Nva-FMDP·HCl (**28**) with a MIC of 0.4 μg/mL. Similar results were earlier observed for alaphosphono dipeptides containing a norvalyl residue. On the other hand, the peptide containing a lysine residue (Lys-FMDP) shows no anticandidal activity up to 200 μg/mL. This result is consistent with a general observation that peptides with a basic amino acid residue, i.e., Lys, Orn, are transported into microbial cells at low rates.[16] Dipeptides having FMDP in the N-terminal position are, to some extent, less active against *Candida albicans* than are their structural isomers. Thus, the most active peptide within this group (FMDP-Met) is four-fold less active than its isomer Met-FMDP (MIC 1.6 μg/mL vs. 0.4 μg/mL, respectively). These differences probably reflect, to some degree, differing rates of peptide transport and characteristic specifities of fungal peptide permeases. It is of some interest to note that the dipeptide consisting of two FMDP residues (FMDP-FMDP·HCl, **38**) exhibits no detectable biological activity (MIC > 200 μg/mL). This lack of activity may be account for by either ineffective transport of the dipeptide or resistance of the peptide bond to the action of intracellular peptidases.

These similarities and differences in the biological activities were also observed for the activity of the synthesized peptides against the selected strains of bacteria (Table III). We have also found that peptides with the C-terminal FMDP as a "warhead" were more potent against all bacteria tested than their structural isomers. Interestingly, *Escherichia coli* and *Shigella sonnei* are completely resistant to peptides containing FMDP residue in the N-terminal position in contrast to *Staphylococcus aureus* and *Bacillus pumilus*, which are also sensitive to a few FMDP peptides within this group.

Several selected dipeptides with FMDP in either the C-terminal or N-terminal positions were also examined against five various strains of *Candida albicans*, including ATCC 26278, by using the disk diffusion method (data not

(14) Roeske, R. W. *J. Org. Chem.* **1963**, *28*, 1251.
(15) Shioiri, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203.
(16) Becker, J. M.; Naider, F. In *Microorganisms and Nitrogen Sources*; Payne, J. W., Ed.; Wiley: New York, 1980; pp 257–279 and references therein.

**Table IV.** Activity of Selected Antifungal Agents and FMDP Dipeptides against *C. albicans* ATCC 26278

| compound | zone size, mm: 20 μg/disk | zone size, mm: 10 μg/disk |
|---|---|---|
| Leu-FMDP | 41 | 39 |
| Nva-FMDP | 42 | 40 |
| FMDP-Met | 35 | 31 |
| nikkomycin X/Z | 28 | 25 |
| 5-fluorocytosine | 39 | 35 |
| clotrimazole | 21 | 17 |
| miconazole | 32 | 27 |

**Table V.** Antagonism of FMDP Dipeptides by Dipeptides and GlcNAc

| compound[a] | control | Met-Val,[b] 10 nmol/disk | Leu-Met,[b] 10 nmol/disk | GlcNAc,[b] 10 nmol/disk |
|---|---|---|---|---|
| | Against *C. albicans* ATCC 26278 | | | |
| FMDP-Met | 17 | 0 | 0 | 0 |
| Leu-FMDP | 18 | 0 | 0 | 0 |
| | Against *B. pumilus* CCM 1697 | | | |
| Nle-FMDP | 22 | 0 | 0 | 0 |
| | Against *Shigella sonnei* 433 | | | |
| Nle-FMDP | 20 | 10 (hazy) | 11 (hazy) | 0 |

[a] 1.5 pmol/disk of FMDP dipeptide. [b] Peptides and GlcNAc were codisked on FMDP dipeptide disks.

shown). No remarkable differences in susceptibility to the action of FMDP peptides were noted. Results indicate that peptides with FMDP as the C-terminal amino acid are generally more active than are compounds with N-terminal FMDP. A comparison of the biological effects exhibited by the FMDP peptides with other commonly used antifungal agents indicates (Table IV) that some of the peptides are as active as or even more active than the selected antifungal agents. However, FMDP-Met displayed a weaker effect against *C. albicans* than did 5-fluorocytosine.

Table V shows the results of a series of disk diffusion assays in which *Candida albicans*, *Bacillus pumilus*, and *Shigella sonnei* as test organisms were treated with disks containing the inhibitory peptide and noninhibitory peptide or *N*-acetylglucosamine. The activity of the selected FMDP peptides against *C. albicans* and bacteria is antagonized by the addition of *N*-acetylglucosamine. Antagonism of inhibitory FMD peptides by dipeptides Met-Val and Leu-Met was more potent for *C. albicans* and *B. pumilus* than for *S. sonnei* where inhibition zones were markedly reduced.

Antagonism studies point to the peptide transport mediated by peptide permeases as drug delivery mechanism. They also evidence the high selectivity of action of peptides on microbial cells, confined only to the inhibition of the process of metabolic generation of glucosamine.

We postulate that the mechanism of action of all the active dipeptides described here involves peptide transport into cells mediated by permeases, intracellular hydrolysis of the peptide by peptidases with the liberation of FMDP as the "warhead", followed by the inactivation of glucosamine-6-phosphate synthetase.

Results of detailed studies on the mechanism of action of FMDP dipeptides will be the subject of a forthcoming paper.

## Experimental Section

Melting points were determined in open glass capillaries and are uncorrected. $^1$H NMR spectra were recorded on a Tesla BS-487 60-MHz spectrometer with hexamethyldisiloxane as the internal standard. TLC was carried out on Kieselgel 60F254 plates (Merck). The location of spots was detected by spraying with ninhydrin reagent or by illumination with a UV lamp. The elemental analyses (C, H, N) for the new substances were within =0.4% of the theoretical values. Optical rotations were determined on a Hilger-Watts polarimeter. The *N*-hydroxysuccinimide esters of *N*-(*tert*-butoxycarbonyl)amino acids[13] were prepared by the previously described methods with the exception of a new compound. Typical examples of the various methods employed for the synthesis of peptides are given below.

**Method A. $N^2$-(*N*-(*tert*-Butoxycarbonyl)-L-alanyl)-$N^3$-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic Acid (1).** To a cooled solution of $N^3$-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid hydrochloride[1] (1.26 g, 5 mmol) and triethylamine (1.01 g, 10 mmol) in methanol (20 mL) was added *N*-(*tert*-butoxycarbonyl)-L-alanine succinimido ester (1.43 g, 5 mmol). The mixture was stirred at 5 °C for 30 min and then overnight at room temperature. The solvent was evaporated, and the foamy residue that remained was triturated with petroleum ether or crystallized from an ethyl ether–petroleum ether mixture to give the peptide 1: $^1$H NMR ($CDCl_3$) δ 1.28 (d, 3 H, $CH_3$, $J$ = 7.0 Hz), 1.39 (s, 9 H, *tert*-butyl), 3.68 (s, 3 H, $OCH_3$), 4.10–4.20 (m, 3 H, CH and $CH_2$), 4.52 (q, 1 H, CH, $J$ = 7.0 Hz), 5.42 (br s, 1 H, NH), 6.65–6.85 (m, 1 H, CH=CH), 7.25–7.65 (m, 2 H, 2 × NH).

Yield and analytical data are summarized in Table I. According to this procedure peptides 2–11 were also obtained.

**Method B. a. $N^2$-(*tert*-Butoxycarbonyl)-$N^3$-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic Acid Succinimido Ester (Boc-FMDP-OSu).** $N^2$-(*tert*-Butoxycarbonyl)-$N^3$-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (1.83 g, 5.8 mmol) and *N*-hydroxysuccinimide (0.67 g, 5.8 mmol) were dissolved in ethyl acetate (25 mL) cooled to 5 °C, and dicyclohexylcarbodiimide (1.32 g, 6.4 mmol) dissolved in ethyl acetate (10 mL) was added. The mixture was stirred overnight, the urea was filtered off, and the filtrate evaporated to dryness, leaving a crystalline residue, which was crystallized from an ethyl acetate–ethyl ether mixture to yield 1.7 g (70%) of the ester: mp 118–120 °C; $^1$H NMR ($CDCl_3$) δ 1.3 (s, 9 H, *tert*-butyl), 2.7 (s, 4 H, $(CH_2)_2$), 3.7–3.9 (d, 2 H, $CH_2$ and s, 3 H, $OCH_3$), 4.7 (t, 1 H, CH), 5.9 (m, 1 H, NH), 6.8 (s, 2 H, CH=CH), 7.15 (br s, 1 H, NH). Anal. ($C_{17}H_{23}N_3O_9$) C, H, N.

**b. *N*-($N^2$-(*tert*-Butoxycarbonyl)-$N^3$-(4-methoxyfumaroyl)-L-2,3-diaminopropanoyl)glycine (12).** To a chilled solution of glycine (0.165 g, 2.2 mmol) and $NaHCO_3$ (0.187 g, 2.2 mmol) in 10 mL of water was added $N^2$-(*tert*-butoxycarbonyl)-$N^3$-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid succinimido ester (0.93 g, 2 mmol) dissolved in methanol (20 mL). After the mixture was stirred overnight, the solvent was removed in vacuo and the residue was dissolved in 20 mL of water and then extracted with ethyl acetate (2 × 50 mL). The water phase was acidified with 10% citric acid and extracted with ethyl acetate again (2 × 50 mL). The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the solvent afforded the crystalline residue, which was crystallized from an ethyl ether–petroleum ether mixture or triturated with petroleum ether to yield the title compound: $^1$H NMR ($CDCl_3$) δ 1.33 (s, 9 H, *tert*-butyl), 3.40–3.60 (m, 2 H, $CH_2$), 3.65 (s, 3 H, $OCH_3$), 3.75 (s, 2 H, $CH_2$), 4.33 (m, 1 H, CH), 5.95 (br s, 1 H, NH), 6.55–6.85 (m, 1 H, CH=CH), 7.25–7.45 (m, 2 H, 2 × NH).

Yield and analytical data are collected in Table I. Compounds 13–18 were prepared analogously to this procedure.

**Method C. *N*-($N^2$-(*tert*-Butoxycarbonyl)-$N^3$-(4-methoxyfumaroyl)-L-2,3-diaminopropanoyl)-L-alanine *tert*-Butyl Ester (19).** $N^2$-(*tert*-Butoxycarbonyl)-$N^3$-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (0.79 g, 2.5 mmol) was dissolved in DMF (10 mL) and cooled to −5 °C. The L-alanine *tert*-butyl ester[14] (0.43 g, 3 mmol), DPPA[15] (0.65 mL, 3 mmol), and triethylamine (0.83 mL, 6 mmol) were added. The reaction mixture was left at room temperature for 24 h, diluted with water (20 mL), and extracted with ethyl acetate (2 × 50 mL). The organic layer was washed with water, 10% citric acid, 5% $NaHCO_3$ solution, and water again and dried over $MgSO_4$. After evaporation of the solvent, the residue was crystallized from an ethyl ether–petroleum ether mixture to give compound 19 (Table I): $^1$H NMR ($CDCl_3$) δ 1.29 (d, 3 H, $CH_3$, $J$ = 7.0 Hz), 1.42 (s, 9 H,

*tert*-butyl), 1.47 (s, 9 H, *tert*-butyl), 3.72 (s, 3 H, $OCH_3$), 4.10–4.30 (m, 3 H, CH and $CH_2$), 4.46 (q, 1 H, CH, $J = 7.0$ Hz), 5.55 (br s, 1 H, NH), 6.60–6.80 (m, 1 H, CH=CH), 7.0–7.25 (m, 2 H, 2 × NH).

Compound **20** was prepared in the same manner.

**Method D. $N^2$-L-Alanyl-$N^3$-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic Acid Hydrochloride (21).** $N^2$-(*N*-(*tert*-Butoxycarbonyl)-L-alanyl)-$N^3$-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (0.77 g, 2 mmol) was dissolved in cold 2 N HCl in dioxane (19 mL) and left for 4 h. The solvent was removed in vacuo, and the residue crystallized from methanol–ethyl ether, affording peptide **21** (Table II). In this manner, peptides **22**–**38** were prepared, and their analytical data are collected in Table II.

**Method E. $N^3$-(4-Methoxyfumaroyl)-L-2,3-diaminopropanoyl-L-alanine (39).** The protected peptide **19** (0.886 g, 2 mmol) was dissolved in cold TFA (20 mL) and stirred for 2 h. The trifluoroacetic acid was removed in vacuo and the residue dissolved in water (20 mL), and passed through a column of Dowex 1 × 2 (acetate form) (5 mL), and eluted with water. The fractions containing peptide **39** were collected, evaporated to dryness, and crystallized from a water–methanol mixture. Similarly peptide **40** was also prepared. For analytical data of these peptides, see Table II.

**Biological Assays. A.** The minimum inhibitory concentration (MIC) of each peptide was determind by the serial twofold dilution method.

Determination for *C. albicans* was performed on ligand YNB medium (Difco) containing 1% glucose. The compounds were diluted from 200 to 0.2 μg/mL. The medium was inoculated with *C. albicans* AMB 25 cells from overnight culture on the same medium to a concentration of $10^4$ cfu/mL and incubated at 30 °C for 48 h. Results were determined by using a turbidimetric method.

Determination for bacteria was performed on solid minimal agar Davis medium (Difco) containing 0.1% yeast extract (Difco) and 0.1% casamino amino (Difco). Twenty-milliliter portions of prewarmed medium containing tested compounds (from 200 to 0.2 μg/mL) were poured into petri dishes. Inocula of bacterial cells diluted to $10^5$ cfu/mL were applied on the surface of agar plates by using a platinum loop. Plates containing inoculated agar were inoculated at 37 °C for 24 h. Results were read visually. The MIC was defined as the lowest concentration of peptide that resulted in complete inhibition of growth of yeasts or bacteria.

**B.** Disk diffusion assay for *C. albicans* strains was performed as follows: YNB medium containing 1% glucose and 1% agar was inoculated with $10^4$ cfu/mL *C. albicans* cells from overnight culture on Sabouraud medium. Inoculated media were poured into petri dishes. The peptides were dissolved in water, and 6.35-mm Schleicher and Schuell disks were saturated with solutions containing 20 μg of the tested compound and placed on the agar surface. After 48-h incubation at 30 °C, zones of inhibition were measured.

**C.** Disk diffusion assay for bacteria was carried out as follows: Medium containing 20 mL of minimal agar Davis (Difco) and yeast extract (Difco) supplemented with 0.1% of casamino acids (Difco) was inoculated with $10^4$ cfu/mL of bacteria cells from 18-h culture and incubated at 37 °C for 30 min. Twenty microliters of solution containing tested compound was poured onto 6.35-mm Schleicher and Schuell disks and placed on the prepared plates. After incubation at 37 °C for 24 h, the diameters of inhibition zones were determined.

**D.** For antagonism studies, dipeptides and GlcNAc solutions were applied on the same disks as FMDP dipeptides. Conditions for the disk diffusion assay were the same as described above.

**Acknowledgment.** We gratefully acknowledge the financial support of these studies by the Technical University of Wrocław (Project No. CPBR 3.13.6).

**Registry No.** 1, 108232-97-9; **2**, 108232-98-0; **3**, 108232-99-1; 4, 108233-00-7; **5**, 108233-01-8; **6**, 108233-02-9; **7**, 108233-03-0; **8**, 108233-04-1; **9**, 108233-05-2; **10**, 108233-06-3; **11**, 108233-07-4; **12**, 108233-08-5; **13**, 108233-09-6; **14**, 108233-10-9; **15**, 108233-11-0; **16**, 108233-12-1; **17**, 108233-13-2; **18**, 108233-14-3; **19**, 108233-15-4; **20**, 108233-16-5; 21, 108233-17-6; **21** (free base), 108340-63-2; **22**, 108233-18-7; **22** (free base), 108340-64-3; **23**, 108233-19-8; **23** (free base), 108233-38-1; **24**, 108233-20-1; **24** (free base), 108340-65-4; **25**, 108233-21-2; **25** (free base), 108340-66-5; **26**, 108233-22-3; **26** (free base), 108340-67-6; **27**, 108233-23-4; **27** (free base), 108340-68-7; **28**, 108233-24-5; **28** (free base), 108340-69-8; **29**, 108233-25-6; **29** (free base), 108340-70-1; **30**, 108233-26-7; **30** (free base), 108340-71-2; **31**, 108233-27-8; **31** (free base), 108340-72-3; **32**, 108233-28-9; **32** (free base), 108233-39-2; **33**, 108233-29-0; **33** (free base), 108340-73-4; **34**, 108233-30-3; **34** (free base), 108340-74-5; **35**, 108233-31-4; **35** (free base), 108340-75-6; **36**, 108233-32-5; **36** (free base), 108340-76-7; **37**, 108233-33-6; **37** (free base), 108340-77-8; **38**, 108233-34-7; **38** (free base), 108340-78-9; **39**, 108233-35-8; **40**, 108233-36-9; FMDP, 96920-07-9; FMDP·HCl, 104371-44-0; BOC-Ala-ONSu, 3392-05-0; BOC-Met-ONSu, 3845-64-5; BOC-Gly-ONSu, 3392-07-2; BOC-Val-ONSu, 3392-12-9; BOC-Leu-ONSu, 3392-09-4; BOC-Phe-ONSu, 3674-06-4; BOC-Tyr-ONSu, 20866-56-2; BOC-Nva-ONSu, 108233-37-0; BOC-Nle-ONSu, 36360-61-9; BOC-Abu-ONSu, 103290-07-9; BOC-Lys(BOC)-ONSu, 30189-36-7; H-Gly-OH, 56-40-6; H-Val-OH, 72-18-4; H-Leu-OH, 61-90-5; H-Phe-OH, 63-91-2; H-Tyr-OH, 60-18-4; H-Nva-OH, 6600-40-4; H-Ala-OBu-*t*, 21691-50-9; Hh-Met-OBu-*t*, 4976-72-1; BOC-FMDP-ONSu, 108233-40-5; BOC-FMDP, 104302-84-3; *N*-hydroxysuccinimide, 6066-82-6; glucosamine-6-phosphate synthetase, 9030-45-9.

# Synthesis of Angiotensin II Antagonists with Variations in Position 5[†]

Olga Nyéki,* Katalin Sz. Szalay,[‡] Lajos Kisfaludy, Egon Kárpáti, László Szporny, Gábor B. Makara,[‡] and Bertalan Varga[‡]

*Chemical Works of Gedeon Richter, Ltd., H-1475 Budapest, Hungary, and Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1450 Budapest, Hungary.* *Received September 29, 1986*

Six angiotensin II antagonists containing cyclohexylglycine (Chg) or cyclopentylglycine (Cpg) in position 5 were synthesized by stepwise elongation in solution, using the pentafluorophenyl ester method. The influence of substitution on the inhibitory properties of the analogues was studied in four different bioassays. [Sar$^1$,Chg$^5$,Lac$^8$]AII proved to be the most potent antagonist with low intrinsic activity in both the in vitro and in vivo tests.

Investigation of a large number of angiotensin II (AII) analogues affords the possibility of defining the role played by each amino acid residue in the biological activity of the hormone.[1–4] Analogues substituted in positions 1 and 8 are antagonists with high affinity to hormone receptors,

[†] Presented in part at the Bayliss and Starling Society National Scientific Meeting, "Regulatory Peptides", London, U.K., 1985. Published in part as an abstract: *Regul. Pept.* **1985**, *13*, 71.

[‡] Hungarian Academy of Sciences.

(1) Regoli, D.; Park, W. K.; Rioux, F. *Pharmacol. Rev.* **1974**, *26*, 69.
(2) Khosla, M. C.; Smeby, R. B.; Bumpus, F. M. In *Handbook of Experimental Pharmacology*; Page, I. H., Bumpus, F. M., Eds.; Springer-Verlag: Berlin, 1974; pp 126–161.

0022-2623/87/1830-1719$01.50/0 © 1987 American Chemical Society