mmol) using sodium methoxide was performed as described to obtain **4**. The crude product was purified by flash chromatography using dichloromethane–methanol (7:3) to yield 0.45 g (48%) of **19**: mp >300 °C; ^{1}H NMR δ 2.15 (s, 3 H, CH_3), 4.54 (d, J = 8.1 Hz, 1 H, C_1'H), 6.09 (s, 2 H, NH_2 exchangeable), 10.31 and 10.85 (2 br, 2 H, N_3H and N_7H both exchangeable). Anal. ($C_{12}H_{16}N_4O_5$) C, H, N.

Antiviral Studies. Semliki Forest Virus Model. Swiss Webster female mice (Charles River Labs, Wilmington, MA) weighing about 20 g each at the beginning of the experiment were inoculated intraperitoneally with test compounds (or placebo) in aqueous 2% sodium bicarbonate solution at −24 and −18 h relative to virus inoculation. The optimal dose of 7-thia-8-oxoguanosine (the positive control compound) was established in previous experiments.[28] The dosing schedule indicated here was also found to be optimum for all guanosines tested. A lethal dose (10 × LD_{50}) of the Semliki Forest virus (original strain) was administered by ip injection to groups of 12 mice. These procedures have been detailed elsewhere.[39]

Acknowledgment. We are indebted to Dr. Arthur Lewis and his staff for large-scale preparation of 9-deaza- and 7-deazaguanine.

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Antimicrobial Properties of N^3-(Iodoacetyl)-L-2,3-diaminopropanoic Acid–Peptide Conjugates

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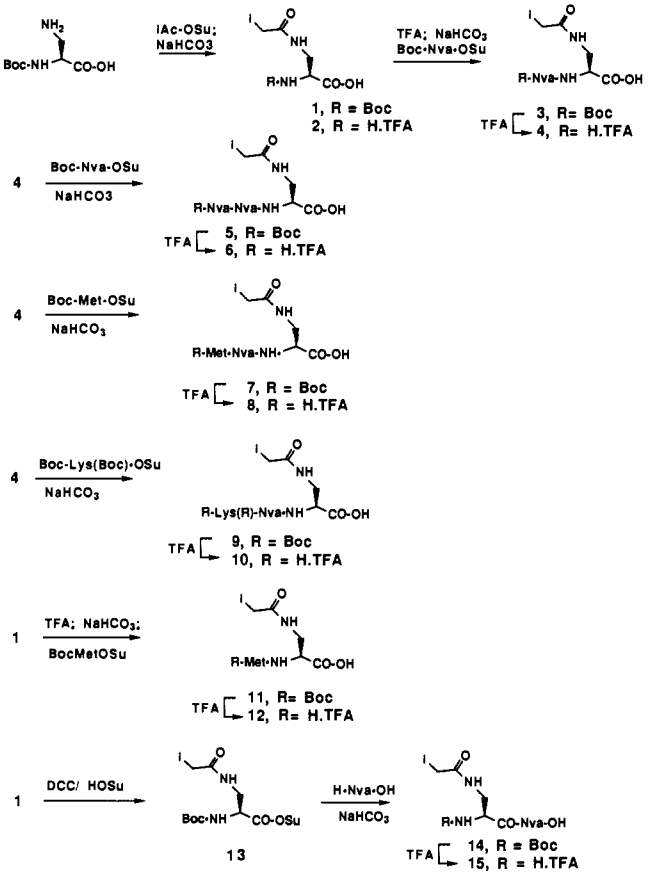
Six peptide conjugates consisting of either norvaline, methionine, or lysine and N^3-(iodoacetyl)-L-2,3-diaminopropanoic acid—a strong, irreversible inactivator of bacterial and fungal glucosamine-6-phosphate synthase—were synthesized and their antibacterial and antifungal activities were evaluated. Antimicrobial potencies of these peptides were correlated with their transport and cleavage rates inside the cells. Bacteriolysis of *Bacillus pumilus* cells and inhibition of [^{14}C]glucose incorporation into cell-wall polysaccharides of *Candida albicans* as a result of glucosamine 6-phosphate inactivation were also observed. Reversal of growth inhibitory effect of these peptides by *N*-acetylglucosamine in bacteria and fungi suggests the effective delivery of N^3-iodoacetyl-L-2,3-diaminopropanoic acid into the cell by a peptide-transport system.

Previous reports from our laboratory[1,2] have described the synthesis and biological properties of a series of peptides containing a new glucosamine-6-phosphate synthase inhibitor, i.e. N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid. Structure–activity relationship studies revealed that many of these peptides, beside antibacterial activity, also displayed remarkable anticandidal activity. It has been shown that inactivation and inhibition of glucosamine-6-phosphate synthase in fungi results in the fungistatic and fungicidal effects as a consequence of cell-wall mannoproteins and chitin biosynthesis inhibition.[3] Inactivation of glucosamine-6-phosphate synthase in bacteria leads to inhibition of peptidoglycan synthesis.[4] These peptides were found to be transported into microbial cells by peptide permeases and then cleaved by intracellular peptidases with the liberation of inhibitor inside the cells that may react with the target enzyme. Therefore glucosamine-6-phosphate synthase may be proposed as a promising target for the rational design of antimicrobial agents. As part of our continuing program aimed at the search for more active inhibitors of glucosamine-6-phosphate synthase, we have synthesized N^3-(haloacetyl) derivatives of L-2,3-diaminopropanoic acid[5,6] and found that

Scheme I



N^3-bromo- and especially N^3-(iodoacetyl)-L-2,3-diaminopropanoic acids were superior to N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid as inhibitors of bacterial and fungal glucosamine-6-phosphate synthase.

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Table I. Antimicrobial in Vitro Activity of Some Peptides Containing A_2pr(IAc) in Regard to Bacterial and Fungal Strains

(1) Nva-A_2pr(IAc) (2) A_2pr(IAc)-Nva

(3) Nva-Nva-A_2pr(IAc) (4) Lys-Nva-A_2pr(IAc)

	MIC,[a] μg/mL			
	1	2	3	4
Bacteria				
Staphylococcus aureus 163 J	1	4	4	2
Staphylococcus epidermidis 154 J	4	16	8	8
Streptococcus faecalis CCM 1875	8	32	2	2
Streptococcus β-haemolyticus A 800	4	16	2	2
Microsporum luteus NCTC 2665	16	32	4	32
Corynebacterium sp. 239	2	2	2	8
Bacillus pumilus CCM 1697	0.25	2	1	4
Fungi				
Geotrichum candidum	15	17.5	10	5
Candida albicans 4477	10	15	7.5	2.5
Candida tropicalis	12.5	15	8	3
Trychophyton nanum	7.5	10	5	2
Rhizopus arhizus	>50	>50	>50	>50
Aspergillus nidulans 590	>50	>50	>50	>50

Since these derivatives were not active when tested against whole microbial cells, they may be transported into the cells in the form of peptide conjugates which may then undergo intracellular hydrolysis with the formation of an active inhibitor. This concept, known as "illicit transport",[7] is utilized to enable a specific inhibitor to reach its intracellular site of action and has been applied for the design of antimicrobial agents.[8]

In this paper we wish to report on the synthesis and antimicrobial properties of a series of peptides with N^3-iodoacetyl-L-2,3-diaminopropanoic acid residue as well as the consequences of glucosamine-6-phosphate synthase inhibition on the living microbial cells.

Results and Discussion

Chemistry. The compounds prepared for this study were synthesized by application of previously published procedures[1,2] (Scheme I). The active esters method (*N*-hydroxysuccinimide esters) was employed for the preparation of all di- and tripeptides. Removal of the Boc protecting group was accomplished almost quantitatively with the use of trifluoroacetic acid in the presence of anisole, yielding compounds **2**, **4**, **8–10**, **12**, and **15**. Deprotection of the Boc group with the aid of hydrogen chloride in dioxane was not suitable because it gave a mixture of two peptides containing N^3-(iodoacetyl)- and N^3-(chloroacetyl)-L-2,3-diaminopropanoic acid residues in a ratio of 1:1.

Biological Activity. As can be seen from Table I, the obtained peptides exhibited antimicrobial activity against a variety of Gram-positive bacteria. The MIC values ranged from 0.25 to 32 μg/mL. The peptides with the C-terminal N^3-(iodoacetyl)-L-2,3-diaminopropanoic acid residue were more potent than peptides with the N-terminal iodoacetyl derivative. The antibacterial activity of the dipeptides was slightly higher than that of the tripeptides. Unfortunately, none of the peptides displayed any activity against Gram-negative bacteria (data not shown). The peptides were also tested against selected fungal strains. In general, tripeptides were found to be more potent than dipeptides. The peptide with an N-terminal lysine residue was determined to be the most efficient compound tested in this study with MIC = 2.5 μg/mL for *Candida albicans* 4477. Our earlier studies had suggested that norvaline and lysine residues were essential for peptide molecules to be efficiently transported into fungal cells.[2] These results are substantiated by the present study. Peptides containing a methionine residue were tested against various strains of Gram-positive bacteria using the disk diffusion method (Table II). The results indicate that the methionyl peptides were less active than compounds with norvalyl residue. Structure–activity relationship studies have shown that antifungal efficiency was dependent on rates of transport of peptides into the cells as well as intracellular cleavage (Table III). It is interesting to note that methionyl peptides are transported and cleaved at much lower rates than norvalyl ones. This observation confirmed our earlier results concerning the effective transport of the norvalyl peptides into fungi. Despite the very high enzyme inhibitory properties of N^3-(iodoacetyl)-L-2,3-diaminopropanoic acid, peptides containing this inhibitor show moderate antimicrobial activity. MIC's for these peptides, however, are higher than those of the corresponding N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid peptides.[2] The reason for the lower antifungal activity is not quite clear. N^3-(iodoacetyl)-L-2,3-diaminopropanoic acid itself contains a very reactive electrophilic group which may react with other targets inside the cell. Moreover, nonspecific interactions cannot be ruled out and they may decrease the effectiveness of the active component inside the cell.

Table II. Activity of Selected Peptides Using the Disk Diffusion Method[a]

(1) Nva-A_2pr(IAc) (2) Met-A_2pr(IAc)

(3) Met-Nva-A_2pr(IAc)

	zone, mm		
	1	2	3
S. epidermidis 154 J	39	26	23
S. faecalis CCM 1875	36	23	20
S. β-haemolyticus A 800	38	27	24
M. luteus NCTC 2665	40	28	28
B. pumilus CCM 1697	40	28	20

[a] Disk assays were performed as previously described.[1]

Table III. Rate of Transport and Intracellular Cleavage of Peptides in *C. albicans* ATCC 26278[a]

peptide	transport rate, μmol/min per mg	cleavage rate, μmol/min per mg
Nva-A_2pr(IAc)	1.3	32.4
Met-A_2pr(IAc)	1.9	3.1–6.5
A_2pr(IAc)-Nva	0.4	27.0
Nva-Nva-A_2pr(IAc)	1.0	18.3
Met-Nva-A_2pr(IAc)	0.5	3.8
Lys-Nva-A_2pr(IAc)	4.4	17.1

[a] Transport rates were carried out as described earlier[17] and cleavage rate determinations were performed according to the published procedure.[2]

Effects of *N*-Acetylglucosamine on the Growth of *Bacillus subtilis* and *C. albicans*. The addition of the dipeptide Nva-A_2pr(IAc) (2 μM) to a logarithmically growing culture of *Bacillus pumilus* CM1697 in MBD LYC medium (Difco) caused significant cell lysis (Figure 1).

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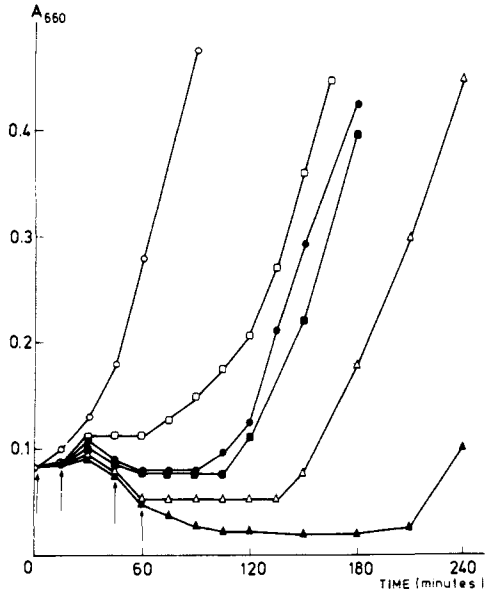

Figure 1. Reversal of bacteriolysis induced by Nva-A_2pr(IAc) in *B. pumilus* CCM 1697 by GlcNAc: ○, control; □, Nva-A_2pr(IAc) (2 μM) and GlcNAc (5 mM) added at the same time; ●, Nva-A_2pr(IAc) (2 μM) and GlcNAc (1 mM) added at the same time; Nva-A_2pr(IAc) (2 μM) added at zero time and GlcNAc (1 mM) added after 15 min (■), 45 min (△), and 60 min (▲); the arrows indicate the addition of GlcNAc. Experimental conditions were taken from the published procedure.[4]

This was confirmed by microscopic examination of a sample treated with dipeptide, which showed a high level of cell debris and lysed cells compared to a control sample. These observations are in accord with the expectation that inhibition of amino sugar biosynthesis[12-14] will lead to the lysis of growing cells. Simultaneous addition of the dipeptide Nva-A_2pr(IAc) (2 μM) and *N*-acetylglucosamine (5 mM) caused only the growth inhibition of bacteria (without lysis) and after 60 min the new growth of the cells was observed. *N*-acetylglucosamine (GlcNAc) added at lower concentration (1 mM) together with the dipeptide (2 μM) partially protected the cells against lysis, and rapid growth was observed after 90 min. When the growing culture of bacteria was treated with GlcNAc (1 mM), after 60 min a slowdown of lysis followed by the growth of the cells after a further 150 min was seen. It is interesting that addition of GlcNAc to the peptide-treated cells induced rapid growth, comparable to that of untreated sample with GlcNAc. Moreover, the fact that GlCNAc itself reversed the bacteriolytic effect of the dipeptide suggested that the enzyme glucosamine-6-phosphate synthase is the target for the inhibitor which is released upon the action of peptidases on the active peptide. The same effects were also observed when *C. albicans* ATCC 26278 cells from logarithmic phase of growth were treated with a tripeptide, Nva-Nva-A_2pr(IAc) (10 μM), and GlcNAc (10 mM) (Figure 2). Addition of the peptide together with GlcNAc at zero time had no effect on the growth. GlcNAc protects the cells completely against the action of inhibitory peptide. As shown in Figure 2, addition of GlcNAc at 90 and 180 min reversed the growth-inhibition effect exerted by the tripeptide.

Influence of Peptides on the Incorporation of Radioactive Precursors into Microbial Cell-Wall Macromolecules. As shown in Figure 3, the incorporation of radioactive [^{14}C]-DL-Ala into bacterial peptidoglycan was a linear process for 60 min. The peptide Nva-A_2pr(IAc) inhibits this incorporation by 58% and 90% at the concentration of 1 and 10 μM, respectively. Inhibition of petidoglycan synthesis by the peptide proceeded quickly and was observed after 15 min of incubation. This effect

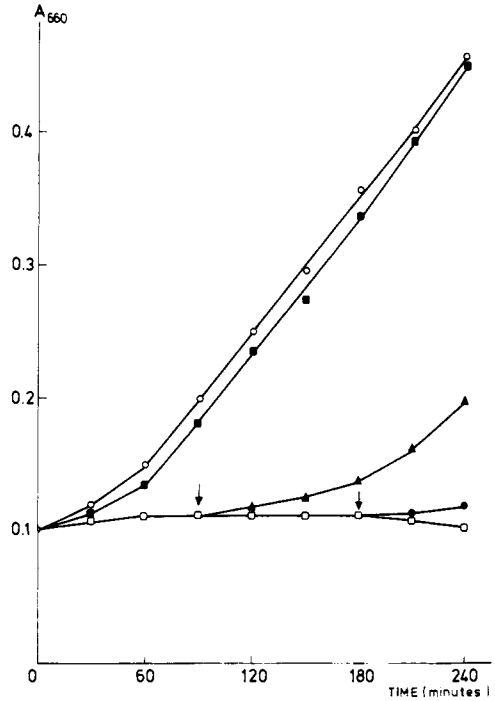

Figure 2. Reversal of growth inhibition effect of Nva-Nva-A_2pr(IAc) by GlcNAc: ○, control; ■, Nva-Nva-A_2pr(IAc) (10 μM) and GlcNAc (10 mM) added at the same time; □ Nva-Nva-A_2pr(IAc) (10 μM) added at zero time and GlcNAc added after 60 and 180 min (arrows indicate GlcNAc addition).

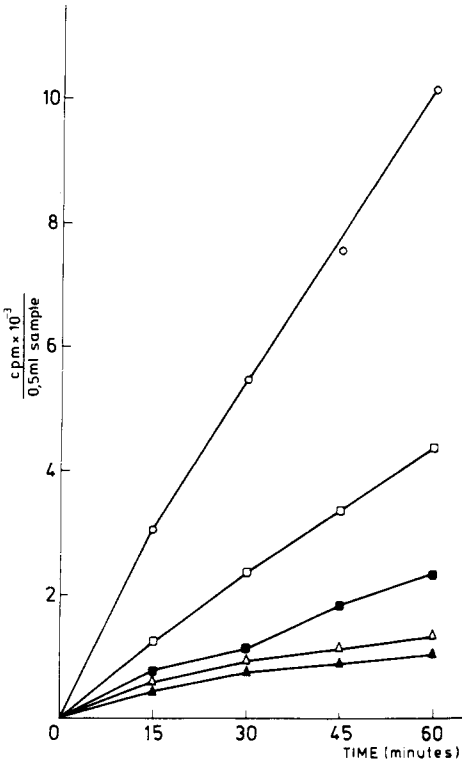

Figure 3. Influence of Nva-A_2pr(IAc) on incorporation of [^{14}C]-DL-Ala into peptidoglycan of *B. pumilus* CCM 1697: ○, control; Nva-A_2pr(IAc) at concentration of 1 μM (□), 2 μM (■), 5 μM (△), 10 μM (▲).

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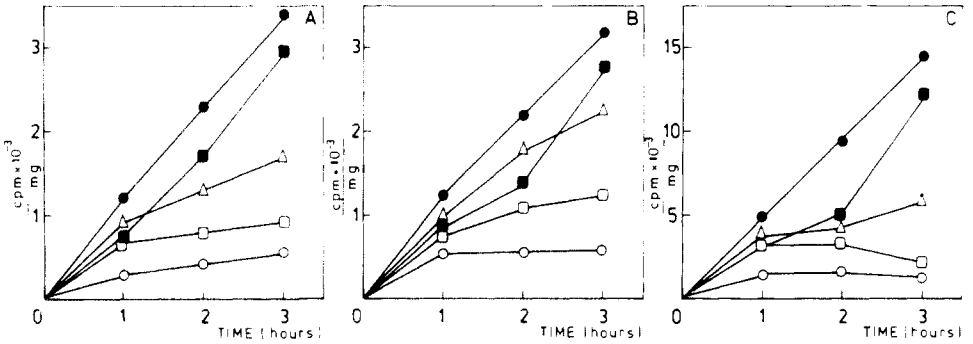

Figure 4. Incorporation of [^{14}C]glucose into cell-wall polysaccharides of *C. albicans* ATCC 26278: A, chitin; B, mannoproteins; C, alkali-insoluble glucan; ●, control; ○, 100 μM Nva-Nva-A_2pr(IAc); □, 10 μM Nva-Nva-A_2pr(IAc); △, 1 μM Nva-Nva-A_2pr(IAc); ■, 10 μM Nva-Nva-A_2pr(IAc) and 10 mM GlcNAc. Experiments were performed according to the published procedure.[18]

supports our hypothesis that the rate of formation of peptidoglycan is decreased due to the shortage of its amino sugar precursors, namely UDP-GlcNAc and UDP-MurNAc, which is a direct result of the inhibition of the enzyme glucosamine-6-phosphate synthase.

Experiments with the inhibition of the incorporation of [^{14}C]glucose into cell-wall polysaccharides of the fungal C. albicans ATCC 26278 were performed with the use of the tripeptide Nva-Nva-A_2pr(IAc) (Figure 4). Incorporation of glucose into all components of fungal cell wall, namely chitin, mannoproteins, and alkali-insoluble glucan, was strongly inhibited. Unexpectedly, glucan biosynthesis was also strongly reduced. A recently published article[15] suggested that covalent bonding exists between chitin and glucan during the biosynthesis of these macromolecules. It is therefore possible that the precursors of both may be inhibited to the same extent. However, such a strong effect was not observed with tetaine, a dipeptide antibiotic which is also a strong inhibitor of glucosamine-6-phosphate synthase.[16] GlcNAc was shown to be a strong antagonist of inhibition of [^{14}C]glucose incorporation into cell-wall macromolecules.

Conclusions

The results supported the intermediacy of the microbial peptide-transport system in the intracellular delivery of N^3-(iodoacetyl)-L-2,3-diaminopropanoic acid in the form of its peptide conjugates. All of the peptides tested in this study exhibited moderate antibacterial and relatively higher antifungal activities. *N*-acetylglucosamine, due to its protective effect on glucosamine-6-phosphate synthase, reversed the action of antimicrobial peptides. Thus, the inhibition of peptidoglycan synthesis in bacteria and chitin and mannoproteins in fungal cells can be directly linked to the inhibition of glucosamine-6-phosphate synthase.

Experimental Section

Melting points were measured in open capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded at 60 MHz on a Telsa BS-487 spectrometer and shifts are presented in ppm from internal hexamethyldisiloxane as a standard. Optical rotations were measured in a Polamat (Carl Zeiss Jena) polarimeter. N^2-(*tert*-Butoxycarbonyl)-L-2,3-diaminopropanoic acid,[9] succinimidoyl iodoacetate,[10] and the *N*-hydroxysuccinimide esters of N-(*tert*-butoxycarbonyl)amino acids (Nva, Met, and Lys) were synthesized by previously described methods.[11] Homogeneity of the final products was determined by TLC on Kieselgel 60 plates (Merck) using *n*-BuOH–AcOH–H_2O (4:1:1) as the solvent system. Deprotected peptides in the form of their trifluoroacetate salts were hygroscopic compounds without reliable melting points.

N^2-(*tert*-Butoxycarbonyl)-N^3-(iodoacetyl)-L-2,3-diaminopropanoic Acid (1). N^2-(*tert*-Butoxycarbonyl)-L-2,3-diaminopropanoic acid[9] (2.04 g, 10 mmol), $NaHCO_3$ (0.84 g, 10 mmol), and *N*-succinimidoyl iodoacetate[10] (2.84 g, 10 mmol) were dissolved in a mixture of water (25 mL) and methanol (25 mL) and kept at room temperature for 24 h. Then, the reaction mixture was concentrated under reduced pressure to 10 mL, acidified with 10% citric acid to pH = 2, and extracted with ethyl acetate (3 × 50 mL). The organic layer was washed with water and saturated NaCl solution, dried over $MgSO_4$, and evaporated to give 1. Crystallization from ethyl ether gave 3.15 g (81%) of 1. Mp: 140–143 °C. ^{1}H NMR ($CDCl_3$): δ 1.4 (s, 9 H), 2.8 (s, 2 H), 3.4–3.6 (m, 2 H), 4.1–4.2 (m, 1 H), 6.0 (m, 1 H), 7.1 (m, 1 H). Anal. ($C_{10}H_{17}N_2O_5$): C, H, N.

General Deblocking Procedure. A cold trifluoroacetic acid (10 mL) solution containing anisole (1 mL) was added to the neat Boc-protected compound and the solution was stirred for 2 h at room temperature. Excess TFA was removed under reduced pressure. The oily residue was triturated with dry ethyl ether to yield the trifluoroacetate salt which was filtered and dried over KOH in a dessicator.

N^3-(Iodoacetyl)-L-2,3-diaminopropanoic Acid Trifluoroacetate Salt (2). Compound 2 was prepared by using the general deblocking procedure to yield 2.6 g (92%) as an amorphous powder. ^{1}H NMR (D_2O): δ 2.8 (s, 2 H), 3.6–3.8 (m, 2 H), 4.1–4.3 (m, 1 H). Anal. ($C_7H_{10}N_3O_5F_3I$): C, H, N.

N^2-[*N*-(*tert*-Butoxycarbonyl)-L-norvalyl]-N^3-(iodoacetyl)-L-2,3-diaminopropanoic acid (3) was prepared from 2 in 92% yield by the procedure used to prepare 1. The product was crystallized from ethyl acetate–ethyl ether. Mp: 99–101 °C. ^{1}H NMR ($CDCl_3$): δ 0.9–1.1 (br m, 7 H), 1.4 (s, 9 H), 2.85 (s, 2 H), 3.6–3.8 (m, 2 H), 4.0–4.2 (m, 1 H), 4.5–4.7 (m, 1 H), 5.8 (br s, 1 H), 7.2 (br s, 1 H). Anal. ($C_{15}H_{26}N_3O_6I$): C, H, N.

N^2-L-Norvalyl-N^3-(iodoacetyl)-L-2,3-diaminopropanoic acid trifluoroacetate salt (4) was prepared in 95% yield by the procedure used for preparation of 2, as an amorphous powder. R_f: 0.32. $[\alpha]^{25}_{578}$ = −2.3° (*c* = 1, H_2O). Anal. ($C_{12}H_{19}N_3O_6F_3I$): C, H, N.

N^2-[[*N*-(*tert*-Butoxycarbonyl)-L-norvalyl]-L-norvalyl]-N^3-(iodoacetyl)-L-2,3-diaminopropanoic Acid (5). Peptide 5 (0.97 g, 88%) was obtained from 4 according to the procedure applied for the synthesis of compound 1. Mp: 156–158 °C. Anal. ($C_{20}H_{35}N_4O_7I$): C, H, N.

N^2-(L-Norvalyl-L-norvalyl)-N^3-(iodoacetyl)-L-2,3-diaminopropanoic acid trifluoroacetate salt (6) was obtained as an amorphous powder in 92% yield from 5 by the procedure used to prepare 2. R_f: 0.35. $[\alpha]^{25}_{578}$ = +4.2° (*c* = 1, H_2O). Anal. ($C_{17}H_{28}N_4O_7F_3I$): C, H, N.

N^2-[[*N*-(*tert*-Butoxycarbonyl)-L-methionyl]-L-norvalyl]-N^3-(iodoacetyl)-L-2,3-diaminopropanoic Acid (7). Compound 7 was obtained as described for 1 (0.96 g, 80%). Mp: 132–134 °C. Anal. ($C_{20}H_{35}N_4O_7IS$): C, H, N.

N^2-(L-Methionyl-L-norvalyl)-N^3-(iodoacetyl)-L-2,3-diaminopropanoic acid trifluoroacetate salt (8) was prepared

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from **7** as an amorphous powder in 90% yield by the procedure used to prepare **2**. R_f: 0.24. $[\alpha]^{25}_{578}$ = +13.2° (c = 1, H_2O). Anal. ($C_{17}H_{28}N_4O_7F_3IS$): C, H, N.

N^2-[[N^α,N^ϵ-Bis(*tert*-butoxycarbonyl)-L-lysyl]-L-norvalyl]-N^3-(iodoacetyl)-L-2,3-diaminopropanoic Acid (9). Compound **9** was obtained as described for **1**, as an oil (1.22 g, 90%). Anal. ($C_{26}H_{46}N_5O_9I$): C, H, N.

N^2-(L-Lysyl-L-norvalyl)-N^3-(iodoacetyl)-L-2,3-diaminopropanoic acid bis(trifluoroacetate salt) (10) was obtained from **9** as an amorphous powder in 94% yield by the procedure used to prepare **2**. R_f: 0.12. $[\alpha]^{25}_{578}$ = −6.2° (c = 1, H_2O). Anal. ($C_{20}H_{32}N_5O_9F_6I$): C, H, N.

N^2-[N-(*tert*-Butoxycarbonyl)-L-methionyl]-N^3-(iodoacetyl)-L-2,3-diaminopropanoic Acid (11). According to the methodology described for **1**, peptide **11** was obtained (3.13 g, 90% yield). Mp: 122–124 °C. ^{1}H NMR ($CDCl_3$): δ 1.1–1.3 (m, 2 H), 1.4 (s, 9 H), 2.1 (s, 3 H), 2.2–2.4 (m, 2 H), 2.8 (s, 2 H), 3.6–3.8 (m, 2 H), 4.0–4.3 (m, 1 H), 4.6–4.8 (m, 1 H), 5.6 (br s, 1 H), 7.1 (br s, 1 H). Anal. ($C_{15}H_{26}N_3O_6SI$): C, H, N.

N^2-(L-Methionyl)-N^3-(iodoacetyl)-L-2,3-diaminopropanoic acid trifluoroacetate salt (12) was obtained from **11** as an amorphous powder in 92% yield by the procedure used for preparation of **2**. R_f: 0.30. $[\alpha]^{25}_{578}$ = +15.1° (c = 1, H_2O). Anal. ($C_{12}H_{19}N_3O_6F_3IS$): C, H, N.

N-Succinimidoyl N^2-(*tert*-butoxycarbonyl)-N^3-(iodoacetyl)-L-2,3-diaminopropanoate (13) was synthesized according to the procedure described earlier.[1] Crystallization from ethyl acetate–petroleum ether gave 1.8 g (77% yield) of **13**. Mp: 123–125 °C. ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 9 H), 2.7 (s, 4 H), 2.8 (s, 2 H), 3.6–3.8 (m, 2 H), 4.1–4.3 (m, 1 H), 5.8 (br s, 1 H), 7.2 (br s, 1 H). Anal. ($C_{14}H_{20}N_3O_7I$): C, H, N.

[N^2-(*tert*-Butyloxycarbonyl)-N^3-(iodoacetyl)-L-2,3-diaminopropanoyl]-L-norvaline (14). Peptide **14** was obtained with use of the procedure described for **1** and crystallized from ethyl acetate–hexane to give 0.61 g (65%). Mp: 129–131 °C. ^{1}H NMR ($CDCl_3$): δ 0.8–1.2 (br m, 7 H), 1.45 (s, 9 H), 2.7 (s, 2 H), 3.6–3.8 (m, 2 H), 4.1–4.3 (m, 1 H), 4.5–4.7 (m, 1 H), 5.6 (br s, 1 H), 7.0 (br s, 1 H). Anal. ($C_{15}H_{26}N_3O_6I$): C, H, N.

[N^3-(Iodoacetyl)-L-2,3-diaminopropanoyl]-L-norvaline trifluoroacetate salt (15) was prepared from **14** in 90% yield as an amorphous powder by the procedure used for preparation of **2**. R_f: 0.39. $[\alpha]^{25}_{578}$ = 2.4° (c = 1, H_2O). Anal. ($C_{12}H_{19}N_3O_6F_3I$): C, H, N.

Estimation of Peptidoglycan Synthesis. Bacteria were grown overnight with aeration at 37 °C in MBD LYC medium (Difco). Then, the cultures were transferred into similar medium and grown with aeration until an absorbance of approximately 0.6 at 660 nm was reached. An absorbance of 1.0 was an equivalent of 340 μg of dry weight per mL. Cultures were harvested by centrifugation and resuspended in the same medium supplemented with chloramphenicol (100 μg/mL) and prewarmed to 37 °C. Samples (10 mL) were then incubated for 30 min, [^{14}C]-DL-Ala (0.5 μCi/mL) was added to each of the 10-mL cultures, the appropriate amount of the peptide was added, and 0.5-mL aliquots were removed at intervals and diluted into 5 mL of an ice-cold trichloroacetic acid (10%, v/v). After storage on ice for at least 15 min, the samples were heated at 80 °C for 15 min to remove teichoic acid and then filtered through a glass-fiber filter (Whatman GF/C). The filters were washed with cold 5% trichloroacetic acid, ethanol, and ethyl ether and counted for radioactivity.

Reversal of the Growth Inhibition Effect of Tripeptide on *C. albicans* ATCC 26278 by *N*-Acetylglucosamine. *C. albicans* ATCC 26278 from logarithmic phase of growth on Sabouraud medium at 30 °C were harvested, washed with 0.9% saline, and suspended in YNB medium (Difco) at about 10^5 cells per mL. After 10 min of preincubation, a solution of peptide was added to obtain a final concentration of 10 μM, then GlcNAc (10 mM) was added at 90 and 180 min, and the optical density of the culture was measured at 660 nm in a Zeiss spectrocolorimeter.

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4-Heterocyclyloxy-2*H*-1-benzopyran Potassium Channel Activators

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The reaction of 2,4-dihydroxypyridine (**2**) with 3,4-epoxy-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (**1**) yielded the 4-[(1,2-dihydro-2-oxo-4-pyridyl)oxy] compound **3a**, accompanied by small amounts of the isomeric 4-(1,2-dihydro-4-hydroxy-2-oxo-1-pyridyl) compound **4**. This could also be prepared by hydrogenation of the benzyloxy derivative **5**. Reaction of 3,6-pyridazinediol (**10**) with **1** (R = CN) gave the 4-[(1,6-dihydro-6-oxo-3-pyridazinyl)oxy] compound **11a**, which in turn rearranged on heating with NaH in DMSO into the 4-(1,6-dihydro-3-hydroxy-6-oxo-1-pyridazinyl) compound **12**. A series of 6-substituted analogues (R = CO_2Me, $CSNH_2$, NO_2, Br) of **3a** and **11a** were synthesized. N-Alkylation led to compounds **14a–c** (R = Me, Et, $CHMe_2$). The 4-heterocyclyloxychromenes **9** and **16a** were obtained by alkaline hydrolysis of their 3-camphorsulfonates. The racemic pyridazinyloxy compounds **11a** and **14a** could be resolved via their diastereomeric camphorsulfonates or camphanates. The differences between the 4-heterocyclyloxychromanols and the isomeric N-substituted compounds **4** and **12** were elucidated in the course of extensive NMR investigations. While in DMSO the former appeared to be conformationally flexible molecules the latter were rigid. All compounds were tested for oral antihypertensive activity in spontaneously hypertensive rats, using doses of 1 mg/kg. High and long lasting activities were found for the pyridyloxy compounds **3a** and **3d**, the pyridazinyloxy compound **11a**, and its N-alkylation products, as well as for the 3*S*,4*R*-enantiomers **20a** and **22a**. (−)-(3*S*,4*R*)-3,4-Dihydro-4-[(1,6-dihydro-1-methyl-6-oxo-3-pyridazinyl)oxy]-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (**22a**) was selected for further development.

Angina pectoris, bronchial asthma, and essential hypertension are disorders in which intermittent or permanent narrowing of the vascular or bronchial lumina as a result of vaso- or bronchoconstriction is encountered. A growing number of people suffer from these disorders attributed to modern civilization, which cannot be treated adequately by current therapeutics. It would be desirable to have available agents acting more selectively on the coronary or bronchial systems combined with a smooth muscle relaxing effect. The recently characterized potassium channel activators may be helpful here.[1] At

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