11 may provide an improved profile for the treatment of schizophrenia.

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Enantiomers of 1-Ethyl-7-[3-[(ethylamino)methyl]-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid: Preparation and Biological Activity

Sir:

We have recently reported the synthesis of a new quinolone antibacterial agent, 1-ethyl-7-[3-[(ethylamino)-methyl]-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid, CI-934 (**1**), which is unusually active against a wide spectrum of aerobic and anaerobic bacteria, especially against streptococcus and staphylococcus species, and is an effective inhibitor of bacterial gyrase.[1,2] We have also demonstrated that this unique activity against the Gram-positive strains (streptococci and staphylococci) is directly related to the 3-(aminomethyl)pyrrolidinyl side chain **2**.[1]

Virtually all of the significant quinolones reported to date either are achiral or are being developed as racemic mixtures.[3] Recently, however, several cases have been reported in which the enantiomers of certain quinolones were separated, and a substantial difference in potency was observed between the chiral forms.[3c] In particular, flumequine (**3**),[4] ofloxacin (**4**),[3c,5] and S-25930 (**5**)[6] all have asymmetry at the methyl-substituted carbon in the benzoxazine or quinolizine rings. In all cases, most if not all of the activity (10–100-fold!) was present in just one enantiomer. Since fewer quinolones contain chiral side chains, much less has been reported on the influence of side-chain asymmetry on antibacterial activity. In just one case, the enantiomeric quinolones with 2-substituted pyrrolidinyl side chains were synthesized. Once again, one isomer (**6**) possessed a substantial share of the potency (10–60-fold).[7] Because of the excellent antibacterial properties of **1** and the asymmetric center present in its *N*-ethyl-3-pyrrolidinemethanamine side chain, we report the synthesis and biological activity (in vivo, in vitro, and at the enzyme level) of the pure enantiomeric forms of this agent.

Attempts at classical resolution of 5-oxo-1-(phenylmethyl)-3-pyrrolidinecarboxylic acid,[8] a key intermediate in the reported synthesis of **1**,[1] were tedious and only partially successful (80% enantiomeric excess achievable). Instead, we turned our attention to the use of (*R*)-(+)-α-methylbenzylamine as both a resolving agent and a protecting group for the chiral synthesis. The chiral benzylamine **8** (Scheme I) was added to either itaconic acid or its dimethyl ester **7** to produce a near 50:50 mixture of the acids **9ab** or the esters **10ab**, respectively. The acids and esters were readily interconvertible under standard conditions.[9] The diastereomeric esters **10ab** were separated cleanly by column chromatography on silica gel using ethyl acetate–pentane. The 3*S* isomer **10b** was isolated as a white solid, while the epimeric 3*R* isomer **10a** was a thick syrup. The properties and reaction conditions of these and the other products in Scheme I are given in Table I. Prior to chromatography the isomer **10b** could be seeded and crystallized in pure form from the 50:50 mixture in ~14% yield.

To establish the absolute configuration at the 3-position, **10a** and **10b** were each reduced to the alcohols **12a** and **12b**. Deprotection of the pyrrolidine nitrogen gave the (*R*)-(+)-**15a** and *S*)-(−)-**15b** pyrrolidinemethanols. The absolute configuration of the (*S*)-(−)-pyrrolidinemethanol

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Table I. Physical Properties of the Chiral Compounds Prepared in Scheme I

no.	prepd from	rctn condtns:[a] reagent/solvent/temp/time	% yield	config at C_3	mp[b]	rotation[c] (solvent)	ratio of **a**/**b**[d] (method)	elemental formula (anal. for)
9ab	7 (H)	xylene/reflux/24 h	63	*RS*	126–182	113.6 (EtOH)	48/52 (GLPC)	$C_{13}H_{15}NO_3$ (C, H, N)
9a	**10a**	6 N HCl/100 °C/3 h	70	*R*	202–204	102.1 (MeOH)	100/0 (GLPC)	(C, H, N)
9b	**10b**	same as above	66	*S*	127–133	119.0 (MeOH)	0/100 (GLPC)	(C, H, N)
10ab	**9ab**	*p*TSA/MeOH/reflux/24 h	98	*RS*	oil	–	48/52 (GLPC)	–
10a	**10ab**	Si gel chromatography	41	*R*	oil	84.1 (MeOH)	100/0 (GLPC)	$C_{14}H_{17}NO_3$ (C, H, N)
10a	7 (CH_3)	toluene, 4A sieves, Si gel chromatography	34	*R*	oil	79.4 (MeOH)	100/0 (HPLC)	(C, H, N)
10b	**10ab**	Si gel chromatography	16	*S*	69–71	116.2 (MeOH)	0/100 (GLPC)	(C, H, N)
10b	7 (CH_3)	toluene, 4A sieves Si gel chromatography	25	*S*	67–69	124.2 (MeOH)	0/100 (HPLC)	(C, H, N)
11a	**10a**	70% $EtNH_2$/−10 °C/1 h	98	*R*	oil	–	99/1 (HPLC)	$C_{15}H_{20}N_2O_2$ (C, H, N)
11b	**10b**	same as above	100	*S*	108–116	89.9 (MeOH)	0/100 (HPLC)	(C, H, N)
12a	**10a**	LAH/THF/reflux/24 h	87	*R*	86–88	51.3 (MeOH)	–	$C_{13}H_{19}NO$ (C, H, N)
12b	**10b**	same as above	51	*S*	oil	46.6 (MeOH)	–	(C, H, N)
13a·HCl	**12a**	$SOCl_2/C_2H_4Cl_2$/reflux/3 h	100	*R*	140–146	27.8 (MeOH)	–	$C_{13}H_{19}NCl_2$ (C, H, N, Cl)
13b·HCl	**12b**	same as above	94	*S*	184–186	20.0 (MeOH)	–	(C, H, N, Cl)
14a	**13a**	70% $EtNH_2$/100 °C/24 h	96	*S*	oil	45.6 (MeOH)	99/1 (GPLC)	$C_{15}H_{24}N_2$ (C, H, N)
14a·2HCl	**13a**	2-propanol, 6 N HCl	67	*S*	267–268	20.5 (MeOH)		$C_{15}H_{26}Cl_2N_2$ (C, H, N, Cl)
14b	**13b**	70% $EtNH_2$/100 °C/24 h	94	*R*	oil	45.0 (MeOH)	2/98 (GPLC)	$C_{15}H_{24}N_2$ (C, H, N)
14b·2HCl	**13b**	2-propanol, 6 N HCl	63	*R*	244–245	13.9 (MeOH)		$C_{15}H_{26}Cl_2N_2$ (C, H, N, Cl)
15a	**12a**	H_2/Pd, MeOH, 50 psi/17 h	74	*R*	92–95	26.6 (EtOH)	–	$C_7H_{13}NO_5$ (C, H, N)
15b	**12b**	same as above	69	*S*	oil	−26.4 (EtOH)	–	(C, H, N)
						see ref 16		
15b oxalate	**12b**	EtOH, oxalic acid	63	*S*	95–97	−9.5 (H_2O)	–	–
						see ref 16		
16a·2HCl	**14a**	1. H_2/Pd, MeOH, 50 psi 2. ether, HCl	66	*S*	184–185	5.4 (1 N NaOH)	–	$C_7H_{18}Cl_2N_2$ (C, H, N, Cl)
16a·2HCl	**11a**[e]	1. LAH/THF/reflux/24 h 2. H_2/Pd, MeOH, 50 psi 3. ether, HCl	68	*S*	184–185	5.9 (1 N NaOH)	98/2 (HPLC)	(C, H, N)
16b·2HCl	**14b**	same as **16a** from **14a**	80	*R*	181–183	−5.1 (1 N NaOH)	–	(C, H, N, Cl)
16b·2HCl	**11b**[e]	same as **16a** from **11a**	76	*R*	184–185	−5.5 (1 N NaOH)	1/99 (HPLC)	(C, H, N)
17a·HCl	**16a**	1. ref 1 2. HCl, H_2O/freeze-dried	66	*S*	280	−99.1 (1 N NaOH) −153.2 (H_2O)	–	$C_{19}H_{24}ClF_2N_3O_3$ (C, H, N, Cl)
17a·$MeSO_3H$	**16a**	1. ref 1 2. $MeSO_3H$, H_2O/freeze-dried	86	*S*	236–242	−84.0 (1 N NaOH) −138.7 (H_2O)	100/0 (HPLC)	$C_{20}H_{27}F_2N_3O_6S$ (C, H, N)
17b·HCl	**16b**	same as **17a**·HCl	71	*R*	288	92.8 (1 N NaOH) 159.6 (H_2O)	0/100 (HPLC)	$C_{19}H_{24}ClF_2N_3O_3$ (C, H, N, Cl)
17b·$MeSO_3H$	**16b**	same as **17a**·$MeSO_3H$	87	*R*	236–242	84.4 (1 N NaOH) 141.0 (H_2O)	0/100 (HPLC)	$C_{20}H_{27}F_2N_3O_6$·0.5H_2O (C, H, N, H_2O)

[a] Room temperature unless specified; silica gel was 70–230 mesh. [b] Uncorrected. [c] Degrees. Concentrations were all 1.0–1.2%. [d] GLPC was performed on a Varian 6000 instrument using capillary columns of 30M DB-1 plus 30M DB-5 or 50M silica gel with RSL-310 coating. [e] Conversion of **11** to **16** without purification of **14**.

Table II. Biological Testing Results from the Drug-Induced DNA Cleavage Assay, Antibacterial Screening, and Mouse Protection Assay

	minimum inhibitory concentrations (MIC),[a] μg/mL											protective dose (PD_{50})[b] in mouse, mg/kg								
	Gram-negative organisms					Gram-positive organisms						*E. coli* Vogel		*S. pyogenes* C203		*S. pneumoniae* SV-1		gyrase–drug-induced cleavage,[c] μg/mL: *E. coli*	GYR IC_{50}, μg/mL
compd	*E. cloacae* HA 2646	*E. coli* Vogel	*E. coli* H560	*K. pneumoniae* MGH-2	*P. retigeri* H1771	*P. aeruginosa* UI-18	*S. aureus* H228	*S. aureus* UC-76	*S. faecalis* MGH-2	*S. pneumoniae* SV-1	*S. pyogenes* C203	po	sc	po	sc	po	sc		
1 (17ab)	0.2	0.1	0.1	0.2	0.4	1.6	0.1	0.05	0.1	0.1	0.1	12	2	9	3	20	4	2.5	13.8
17a	0.2	0.1	0.05	0.2	0.8	3.1	0.1	0.025	0.1	0.1	0.05	10	3	10 (HCl)	4	11	3	2.5	13.8
17b	0.4	0.2	0.1	0.4	1.6	3.1	0.2	0.05	0.4	0.2	0.2	15	7	30	14	31	8	2.5	13.8
norfloxacin	0.1	0.025	0.1	0.05	0.025	0.2	0.8	0.05	1.6	1.6	0.8	1	0.7	25 (HCl) >100	45	150	>100	1.0	5.5

[a] Standard microdilution techniques; see ref 10. All in vitro experiments were performed in duplicate or triplicate with an error of one serial dilution. In vivo experiments were performed in duplicate with an error of ~15%. [b] Dose required to protect 50% of mice from lethal infection. [c] Minimum concentration of drug needed to produce linear DNA at an intensity relative to oxolinic acid at 10 μg/mL.

has been published.[8,16] The ethylamino group was introduced by two distinct methods with equal success. In the first method, the alcohols **12a** and **12b** were each chlorinated with thionyl chloride to give **13a** and **13b**. The chlorines were cleanly displaced with ethylamine in a pressure bottle to give **14a** and **14b**. Subsequent deprotection yielded the desired side chains (*S*)-**16a** and (*R*)-**16b**. The entire process was attended with no more than a 2% loss in enantiomeric purity (Table I, compounds **16a**, **16b** and **17a**, **17b**).

The second approach for preparing the side chains **16a** and **16b** was more applicable to scale up and involved treating the pure diastereoisomeric esters **10a** and **10b** with ethylamine in water. A very rapid amidation occurred producing **11a** and **11b** without epimerization at C_3. When alcoholic ethylamine was used, some epimerization was observed by thin-layer analysis. Reduction of the amides **11** produced **14a** and **14b**, which were deprotected without purification. By this route, the *S* side chain **16a** was 98% enantiomerically pure while **16b** displayed 99% enantiomeric purity.

The enantiomeric side chains were appended to the quinolone nucleus as previously described[1] to give (*S*)-(−)-**17a** and the enantiomer (*R*)-(+)-**17b**, which were characterized as the hydrochloride and methanesulfonate salts. When the HPLC derivatization procedure employed for the side chains was used (see analytical methods), both enantiomers were shown to be 100% optically pure. Apparently, the last traces of enantiomeric impurities were removed during the water workup or salt formation.

The chiral forms of **1** were tested side by side with racemic material against an assortment of 10 organisms by using standard microtitration techniques,[10a] and their minimum inhibitory concentrations (MICs) are reported in Table II.

The compounds were also tested for their inhibition of the target enzyme DNA gyrase,[11] which was obtained from *Escherichia coli* H560 cells.[11a,12] The assay employed for this study measured the concentration of drug (micrograms/milliliter) required to produce linear DNA from closed circular DNA by the denaturation of the drug–gyrase–DNA complex.[13] The linear DNA was resolved and visualized by gel electrophoresis and ethidium bromide stain. Aqueous stock solutions were prepared with use of 0.1 N sodium or potassium hydroxide. The in vivo potency, expressed as the median protective dose (PD_{50}, milligrams/kilogram), of these compounds was determined in acute, lethal systemic infections in 18-22-g female Charles River CD-1 mice. Challenges were accomplished by the intraperitoneal injection of an estimated 100 median lethal dose in 0.5-mL volumes of 5% hog gastric mucin (*E. coli*, *Staphylococcus aureus*) or tryptic soy broth (*Streptococcus pneumoniae*). Single doses of compound, in twofold rising incremental series, were administered concurrently with challenge in 0.5-mL volumes, subcutaneously as aqueous solutions and orally by gavage in 5% gum acacia. Survival percentages among groups of eight mice at each dose interval were used to estimate the median protective doses by the log probit method.[10b] The combined results from all assays are given in Table II.

The enantiomeric purities of the side chain **16ab** and the quinolones **17ab** were determined by HPLC[14] using a derivatization technique.[15] The derivatization of *N*-ethyl-3-pyrrolidinemethanamine (**16a,b**) was achieved with

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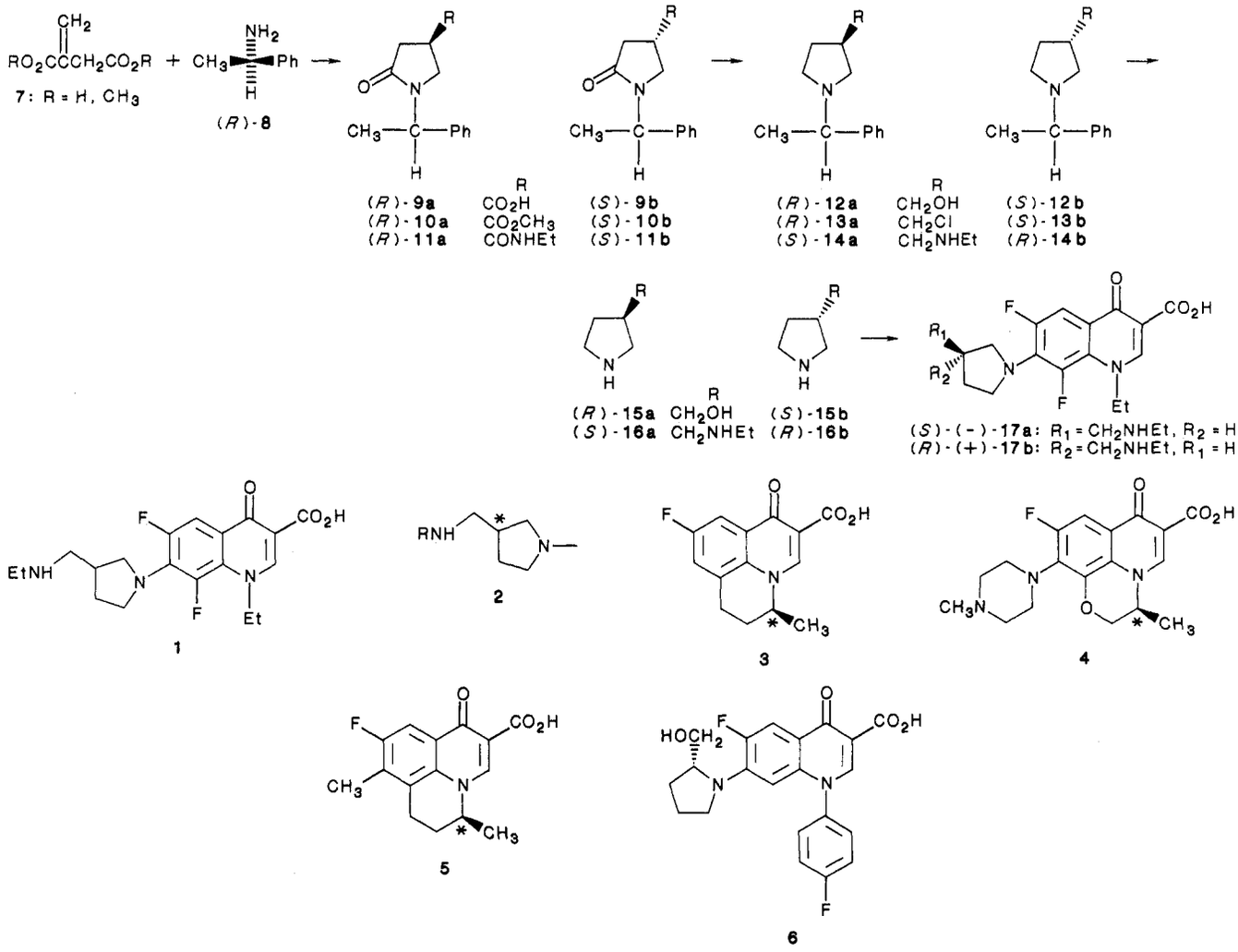
(16) The reported rotations (ref 8) for **15b** and its oxalate salt are −19.1° (*c* 4.5, EtOH) and −6.8° (*c* 3.1, H_2O).

Scheme I



2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) in acetonitrile. The derivative was diluted in the HPLC mobile phase [methanol–water (50:50), 0.05 M NaH_2PO_4]. With a flow rate of 1.5 mL/min and detection wavelength of 254 nm, the retention times of the *R* and *S* derivatives were 36 and 39 min, respectively.

The derivatization of racemic **1** and the enantiomers **17a** and **17b** was accomplished in DMF with GITC and triethylamine. The mobile phase was THF (25%), methanol (15%), and 60% 0.08 M diethylamine in water with the pH adjusted to 3.0 with formic acid. At 1.0 mL/min and detection at 290 nm, the *R* and *S* derivatives eluted at 24 and 27 min. Base-line separation was possible in both cases.

The chemical pathways chosen in Scheme I have permitted the synthesis of chiral **1** in an unambiguous manner. The results in Table II clearly indicate that there is no significant difference between the (*S*)-(−)-quinolone **17a** and its enantiomer (*R*)-(+)-**17b** at the enzyme or bacterial levels. Although (*S*)-(−)-**17a** does show a consistent trend toward increased potency against Gram-positive organisms, the magnitude of the difference is within the margin of experimental error. The gyrase inhibition values are identical. Thus, the target enzyme in *E. coli* cannot distinguish between the subtle structural differences in the side chain. The small improvements witnessed for the Gram-positive organisms may be due to differences in their gyrase or cell wall permeability. In vivo, in the mouse protection tests, **17a** is ~3 times more active than **17b** against *S. pneumoniae* and *Streptococcus pyogenes* induced infections. This three-fold superiority may not necessarily be solely a function of the slightly lower MIC values for **17a** but could also reflect better pharmacokinetics, metabolism, or permeability differences in the animal model. No distinctions were observed between the HCl or methanesulfonate salts in vitro (not shown) or in vivo. Smaller in vivo differences were observed vs. the Gram-negative organisms. It is also interesting to note that the racemic form is almost as active as the most active component **17a**, indicating that even in vivo the differences between the enantiomers is not sufficient to alter the efficacy of the racemic material.

In all of the previous cases reported above, chirality had a profound impact on activity, and the chiral group was in close proximity to the quinolone nucleus at N_1 or C_7. Our results suggest that chiral differences at the 3-position of the pyrrolidine, more remote to the quinolone nucleus, are not recognized at the enzyme level, are barely distinguished at the cellular level, and are most significant in vivo due in part to how the body handles the individual enantiomers. Our results are in direct contrast to those previously reported, especially when compared to the 2-substituted pyrrolidinyl **6**, where major in vitro differences were reported.[7] In the present case, movement of the substituent to the 3-position greatly reduces enantiomeric recognition despite the increased bulk of the (ethylamino)methyl group. Flexibility in the bulk of the side chain[13] and its influence on in vivo activity[1] have already been reported. The results in this work confirm such findings and indicate a possible spatial limit to the influ-

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.

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Articles

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

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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

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