Platelet Aggregation Inhibiting and Anticoagulant Effects of Oligoamines, Part 32

Antimicrobial Effects of Oligoamines

Klaus Rehse[a]*, Christian Woyke[a], Arne Rodloff[b], and Helmut Hahn[c]

[a] Institut für Pharmazie der Freien Universität Berlin, Königin-Luise-Str. 2+4, 14195 Berlin, Germany

[b] Institut für Medizinische Mikrobiologie und Infektionsimmunologie der Freien Universität Berlin

[c] Present address: Institut für Medizinische Mikrobiologie und Infektionsepidemiologie der Universität Leipzig

Key Words: *oligoamines; antimicrobial effects; structure activity relationships; S. aureus; E. coli; platelet aggregation*

Summary

Twenty-four oligoamines belonging to six (**1–6**) structurally different types were tested *in vitro* for their antibacterial activity against 14 different bacterial species comprising a total of 187 strains. Ten compounds were able to inhibit growth of at least one strain at concentrations ≤10 µmol/L. For three compounds, minimum inhibitory concentrations for some strains were even below 1 µmol/L. Clear structure-activity relationships showed that the inhibitory effect depended on the bridge connecting the nitrogen atoms, the substitution of the nitrogens, and the number of nitrogen atoms present in one molecule. Substitutions like *N*-4-phenylbutyl, *N*-octyl, and *N*-nonyl were most active, while short (butyl) and long (dodecyl) substituents diminished or abolished the activity. The antimicrobial spectrum of the oligoamines tested here covered gram-positive (e.g. *Staphylococcus aureus, Listeria monocytogenes, Bacillus subtilis*) and gram-negative (e.g. *Escherichia coli, Citrobacter spp., Acinetobacter spp.*) microorganisms. The type of action was classified as bactericidal. As the inhibition of growth is complete immediately after the addition of the oligoamines, an interaction with the bacterial cell-membrane is probable.

Introduction

In recent years we have recognized that certain oligoamines (e.g those in Table 1) are able to inhibit the aggregation of platelets and the formation of fibrin *in vitro*. Suitable prodrugs of these compounds have shown to exhibit antithrombotic properties *in vivo* even after oral administration. This class of compounds therefore might have considerable benefit in the prophylaxis of thrombo-embolic events[7]. When elucidating the mechanism of action, we found that these drugs were able to bind strongly to negatively charged phospholipids like phosphatidylserine[2]. As this phospholipid plays a crucial role in the platelet membrane during the activation process, it seemed probable that the membranes of other cells could also be effected. In rat thymocytes, we consequently observed an inhibition of transport processes[3]. Using fluorescent probes, we were able to show that the oligoamines increased lipid motility and membrane permeability[3]. The elastomechanical properties of red blood cell membranes were influenced as well[4,5]. The growth of L1210 leukemic cells was inhibited in concentrations between 4–10 µmol/L. Viability measurements showed that the cells were killed in a time dependent manner[6]. These results prompted us to look for antimicrobial effects in this newly developed class of compounds.

For this purpose six different classes of oligoamines (Table 1) including 24 individual compounds were selected according to the following criteria: (i) strong inhibition of platelet aggregation, (ii) clear cut structure activity relationships concerning this parameter, (iii) good inhibition of growth of L1210 cells, (iv) significant structural variability concerning the core of the compound. Compound **1b** was was included because of its especially good solubility in aqueous media. The syntheses of the compounds in part already have been reported[7–12]. The preparation of **1e–1j, 2d** and **2f–2h** is described in the experimental part.

Results and Discussion

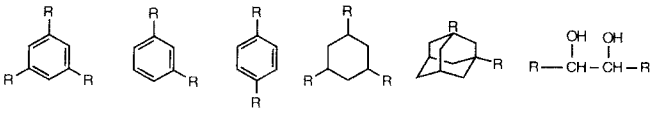
The compounds selected here were assayed for their antimicrobial activity employing eight different gram-positive (g^+) species (1 to 44 strains each) and ten gram-negative (g^-) species (2 to 17 strains each) and using conventional microtitration procedures to determine minimum inhibitory concentrations (MIC). The minimum concentrations inhibiting growth of 50% of the strains (MIC_{50}) are compiled in Table 1 for *S. aureus* as an example of g^+ bacteria and for *E. coli* as a representative of g^- microorganisms. The IC_{50} for platelet aggregation is also given for comparison. Moreover inhibitory concentrations for L1210 cells are included where data were available[6]. In general, the MIC_{50} values cover a range of more than three orders of magnitude suggesting pronounced structure-activity relationships. The most active compounds (e.g. **1a, 1g, 1h, 4a**) are effective in concentrations below 1 µmol/L while others are ineffective even in concentrations above 100 µmol/L (e.g. **1c, 1d, 2a, 2c, 6a**). With regard to the microorganisms, we found among the more susceptible ones most of the g^+ bacteria (*S. aureus, L. monocytogenes, B. subtilis, M. luteus*) but few of the g^- organisms (*Citrobacter spp., Acinetobacter* spp.).

In four types of compounds investigated here (**1,2,4,5**) at least one species showed considerable antibacterial effects. It appeared that type **6** compounds (**6a–c**) are too hydrophilic and thus unable to anchor in the bacterial membrane. Type **3** compounds exhibited only small or none antibacterial effects. The most "active" compound was **3a**. The lack of activity shows that substitution in the para positions of the aromatic core is unfavourable.

Compound **1b** is the methanosulfonate of **1a**. This class of compounds is known to decompose in a retro Mannich like reaction to the parent amine, hydrogen sulfite, and formalde-

  0365-6233/96/0303-0155 $5.00 + .25/0

Table 1: Antimicrobial activities in oligoamines compared to antiplatelet effects. For the legend of (a)–(o) see Fig. 1. n.t. = not tested. The references for synthesis and antiplatelet effects are given in brackets[ref].



1 2 3 4 5 6

Compound	R	MIC$_{50}$ [µmol/L] *S.aureus*	*E.coli*	most sensitive	IC$_{50}$ [µmol/L] Platelet	L1210
1 a	CH_2-NH-$(CH_2)_4$-Ph	0.7	2.8	0.2 (g)	3[11]	4
1 b	CH_2-N-$(CH_2)_4$-Ph CH_2SO_3Na	1.9	30.0	1.9 (a)	7[12]	n.t.
1 c	CH_2-NH-$(CH_2)_3$-CH_3	>200	>200	>200 (a)	35	n.t.
1 d	CH_2-NH-$(CH_2)_4$-CH_3	>200	>200	100 (h)	11	n.t.
1 e	CH_2-NH-$(CH_2)_5$-CH_3	16.0	25.0	12.5 (g,h)	5	n.t.
1 f	CH_2-NH-$(CH_2)_6$-CH_3	2.5	3.1	1.6 (g)	5	n.t.
1 g	CH_2-NH-$(CH_2)_7$-CH_3	2.5	1.6	0.4 (g)	5	n.t.
1 h	CH_2-NH-$(CH_2)_8$-CH_3	2.5	6.3	0.4 (g)	88	n.t.
1 i	CH_2-NH-$(CH_2)_9$-CH_3	12.5	12.5	3.1 (g)	200	n.t.
1 j	CH_2-NH-$(CH_2)_{11}$-CH_3	200	>200	200 (a)	125	n.t.
2 a	CH_2-NH-$(CH_2)_2$-Ph	>100	>100	>100 (a)	25[8]	n.t.
2 b	CH_2-NH-$(CH_2)_3$-Ph	100	>100	100 (e)	20[8]	n.t.
2 c	CH_2-NH-$(CH_2)_3$-CH_3	>200	>200	>200 (a)	30[8]	n.t.
2 d	CH_2-NH-$(CH_2)_5$-CH_3	200	200	100 (o)	5	n.t.
2 e	CH_2-NH-$(CH_2)_6$-CH_3	50.0	50.0	25.0 (o)	19[8]	n.t.
2 f	CH_2-NH-$(CH_2)_7$-CH_3	12.5	12.5	6.3 (o)	>500	n.t.
2 g	CH_2-NH-$(CH_2)_8$-CH_3	5.0	6.3	3.1 (o)	45	n.t.
2 h	CH_2-NH-$(CH_2)_9$-CH_3	3.1	6.3	3.1 (a,g,o)	90	n.t.
3 a	$(CH_2)_2$-NH-$(CH_2)_4$-Ph	25.0	50.0	25.0 (a)	10[7]	n.t.
4 a	CH_2-NH-$(CH_2)_4$-Ph	0.4	25.0	0.1 (b)	2[9]	4
5 a	(CH_2)-NH-$(CH_2)_4$-Ph	3.1	50.0	3.1 (a,c)	10[9]	5
6 a	CH_2-NH-$(CH_2)_4$-Ph	100	100	100 (b)	23[10]	n.t.
6 b	CH_2-NH-$(CH_2)_5$-CH_3	100	100	100 (a)	9[10]	n.t.
6 c	CH_2-NH-$(CH_2)_7$-CH_3	25.0	25.0	25.0(a,b,d)	8[10]	8

hyde[14,15]. The effects stated in Table 1 for **1b** therefore might be due to the formation of **1a** from **1b**. In compounds **1c–1j**, the chain length of the alkyl group at the nitrogen atoms is variable. Here, a very clear dependence of the antibacterial effect on the number of carbon atoms was observed. While **1c** (*N*-butyl) was inactive, a rising number of methylene groups decreased the MIC$_{50}$ rapidly. The optimum effects were achieved with the *N*-octyl derivative **1g** and the nonyl homologue **1h**. A further increase in chain length stepwise decreased the antimicrobial activity: The dodecyl compound **1j** displayed no detectable activity. These differences in effectiveness fairly corresponded to the observations made for the antiplatelet effect. In this case, short (**1c**) and long (**1h,1j**) carbon chains also resulted in inactive compounds. In contrast to the antibacterial effect the optimum antiplatelet activity was found with slightly shorter carbon chains, i.e. the heptyl (**1f**) and octyl derivatives (**1g**).

Similar observations were made with the different alkyl substituents in type **2** compounds. While the optimum antiplatelet effect was measured for the hexyl derivative **2c**, the best antibacterial effects were found with longer carbon chains (see **2f–2h**). The high activity of **4a** clearly demonstrates that an aromatic core is not a prerequisite for a strong antibacterial or antiplatelet effect (compare with **1a**). The adamantyl derivative **5a** shows that even space consuming cores are accepted and retain considerable activity.

In general the described observations held true irrespective of bacterial species tested and their particular susceptibility (Table 1). However, there is one striking difference: **1a**, **1g**, **4a**, and **5a** were nearly equipotent in their activity against *S. aureus*. In contrast, **1a** and **1g** (which both have an aromatic

core) exhibited low MIC_{50} values for *E.coli*, while **4a** and **5a** (which possess an alicyclic core) showed only poor activity. The same held true for other g^- bacteria like *Klebsiella pneumonia, Proteus mirabilis, Enterobacter spp., and Pseudomonas aeroginosa* (results not shown). The aromatic connection of the nitrogen functions seemed to be essential for high activity in g^- bacteria.

Because of its broad antibacterial spectrum, the *in vitro* activities of **1a** against various microorganisms are depicted in Fig. 1. The concentrations which inhibited the growth of 50% of the strains tested are given in the boxes. The bars represent the range of the MIC-values for different strains of individual species. The results underline the broad spectrum of activity of **1a**. Most MICs were found well below 10 µmol/L.

In order to characterize the type of activity, not only were MIC determined but, in addition, minimum bactericidal concentrations (MBC) were also established. The results showed little or no difference for MIC and MBC values (data not shown).

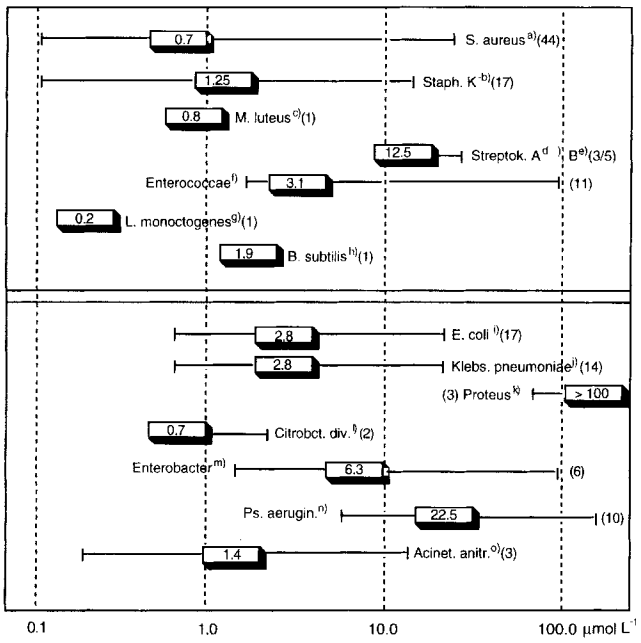


Fig. 1: Antimicrobial spectrum of **1a**, gram-positive germs (upper panel) and gram-negative germs (lower panel). The number of strains tested is given in parentheses. In the boxes are the concentrations which inhibit 50% of the strains (MIC_{50}) tested. The small letters with one parenthesis are used instead of names in Table 1.

Finally, growth kinetics of an *E. coli* strain were investigated in presence of **1a** using nephelometric (Fig. 2, left panel) or protein determination methods (right panel). It is obvious that growth is inhibited immediately after the addition of **1a** (Fig. **2a** and **2b**). When the bactericidal activity of **1a** was evaluated by plating aliquots on inhibitor free media and colony counting after appropriate incubation, it became apparent that the bactericidal activity could be detected after as little as 5 minutes of incubation. This suggests that an interaction with the bacterial membrane occurs. Otherwise a lag phase should be observed.

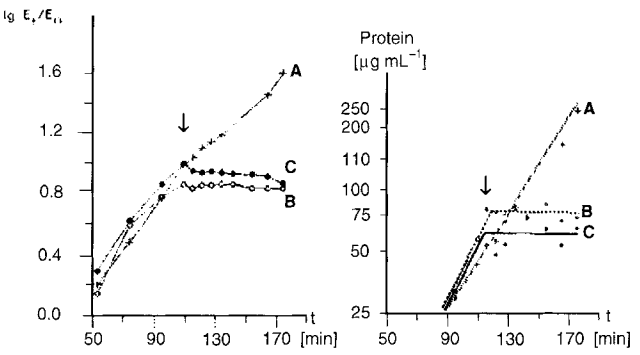


Fig. 2: Time dependence of growth inhibition in *E. coli* by **1a**. Photometric (left) and protein determination (right). A: control; B: 20 µmol/L; C: 4 µmol/L (MIC = 3.1 µmol/L). The arrow indicates the time when **1a** is added

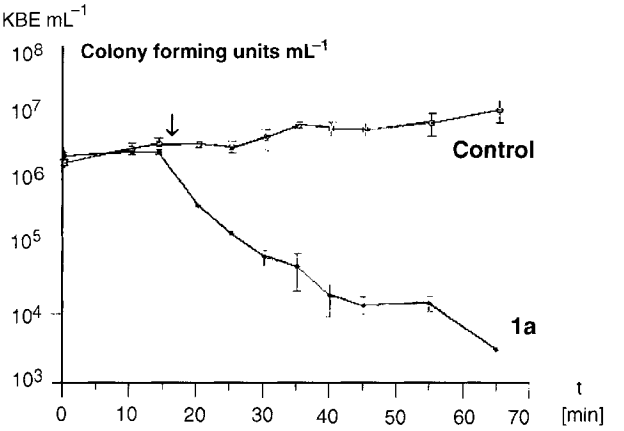


Fig. 3: Determination of survival of *E. coli* after addition of a bactericidal concentration (21 µmol/L) of **1a**.

Because of certain structural similarities to disinfectants like benzalkonium chlorides, hexetidine, chlorohexidine or Tego 103 S, one might guess that the antibacterial effects stated above are due to a tenside effect, i.e. to a decrease in surface tension. This, however, is not the case. Compound **1a** in a concentration of 1 µmol/L did not alter the surface tension of water (72.5 mN m^{-1}). When the concentration was raised to 10 µmol/L a slight decrease to 67.4 mN m^{-1} was measured. At a concentration of 100 µmol/L a tension of 64.0 mN m^{-1} was determined. True tensides, however, at this concentration decrease the surface tension to about 30 mN m^{-1}.

Furthermore we found that the *N,N,N',N',N'',N''*-hexamethyl-derivative of **1a**, i.e. the quaternary ammonium salt, showed MICs between 500 µmol/L (*B. subtilis, S. areus*) and > 2000 µmol/L (*E. coli*). This also contrasts with tensides such as the benzalkonium chlorides. In addition, we found that *N*-phenylbutyl-benzylamine – the monoamino partial structure of **1a** – was devoid of any antibacterial activity. Therefore it is probable that at least two amino groups in one molecule are a prerequisite also for the antibacterial effects. In conclusion, the antibacterial effects are not evoked by tenside properties of the test compounds.

Acknowledgement

We thank Dr. W. Mehnert, Institut für Pharmazie I der FU Berlin for the surface tension measurements.

Experimental Part

Syntheses

General Procedure for **1c–1j**

To a solution of 30 mmol of amine in 100 ml dioxane is added 10.0 g dried Na_2CO_3. A solution of 10 mmol 1,3,5-benzenetricarboxylic acid chloride in 50 ml dioxane is then added dropwise. The mixture is refluxed for 1 h, cooled to room temp., and poured into 300 ml ice water. The suspension is adjusted with dild. HCl to pH 5. The precipitate is filtered under suction and recrystallized from the solvent stated.

N,N′,N″-Tributyl-1,3,5-benzene-tricarboxylic Acid Amide

Crystals (ethanol), mp. 220 °C, yield 2.63 g (70%).– Anal. $C_{21}H_{33}N_3O_3$.– IR (KBr): ν = 3250 cm^{-1} (NH), 1640 (CO).– ^{1}H-NMR ($CDCl_3$): δ = 0.93 (t, *J* = 7 Hz, 9H, CH_3), 1.34 (tq, *J* = 7/7 Hz, 6H, CH_2-CH_3), 1.54 (tt, *J* = 7/7 Hz, 6H, NH-CH_2-CH_2), 3.33 (dt, *J* = 7/6 Hz, 6H, NCH_2), 7.41 (s, 3H, D_2O exchange, NH), 7.57 (s, 3H, aromatic H).– MS (70 eV): *m/z* (%) = 375 (57) [M^+], 303 (100) [$M^+ - NHC_4H_9$].

N,N′,N″-Tripentyl-1,3,5-benzene-tricarboxylic Acid Amide

Crystals (ethanol), mp. 198 °C, yield 2.93 g (70%).– Anal. $C_{24}H_{39}N_3O_3$.– IR (KBr): ν = 3300 cm^{-1} (NH), 1640 (CO).– ^{1}H-NMR ($CDCl_3$): δ = 0.99 (t, *J* = 7 Hz, 9H, CH_3), 1.36–1.87 (m, 18H, $(CH_2)_3$-CH_3), 3.01–3.59 (m, 6H, NH-CH_2), 7.44 (br. s, 3H, D_2O exchange, NH), 7.66 (s, 3H, aromatic H).– MS (70 eV): *m/z* (%) = 417 (82) [M^+], 360 (98) [M – C_4H_9], 331 (100) [M^+ – NHC_5H_{11}].

N,N′,N″-Trihexyl-1,3,5-benzene-tricarboxylic Acid Amide

Crystals (acetone), mp. 191 °C, yield 6.13 g (75%).– Anal. $C_{27}H_{45}N_3O_3$.– IR (KBr): ν = 3252 cm^{-1} (NH), 1642 (CO).– ^{1}H-NMR ($CDCl_3$): δ = 0.94 (t, *J* = 7 Hz, 9H, CH_3), 1.33–1.49 (m, 18H, $(CH_2)_3CH_3$), 1.53–1.58 (m, 6H, $NHCH_2CH_2$), 3.37 (dt, *J* = 7/6 Hz, 6H, $NHCH_2$), 7.44 (br. s, 3H, D_2O exchange, NH), 7.76 (s, 3H, aromatic H).– MS (70 eV): *m/z* (%) = 459 (26) [M^+], 359 (100) [$M^+ - NHC_6H_{13}$].

N,N′,N″-Triheptyl-1,3,5-benzene-tricarboxylic Acid Amide

Crystals (acetone), mp. 196 °C, yield 3.51 g (70%).– Anal. $C_{30}H_{51}N_3O_3$.– IR (KBr): ν = 3300 cm^{-1} (NH), 1644 (CO).– ^{1}H-NMR ($CDCl_3$): δ = 0.89 (t, *J* = 7 Hz, 9H, CH_3), 1.29–1.32 (m, 24H, $(CH_2)_4CH_3$), 1.57–1.62 (m, 6H, $NHCH_2CH_2$), 3.42 (dt, *J* = 7/6 Hz, 6H, $NHCH_2$), 6.86 (br. s, D_2O exchange, NH), 8.16 (s, 3H, aromatic H).– MS (70 eV): *m/z* (%) = 501 (52) [M^+], 387 (100) [$M^+ - NHC_7H_{15}$].

N,N′,N″-Trioctyl-benzene-1,3,5-tricarboxylic Acid Amide

Crystals (ethanol), mp. 196 °C, yield 3.54 g (65%).– Anal. $C_{33}H_{57}N_3O_3$.– IR (KBr): ν = 3308 cm^{-1} (NH), 1645 (CO).– ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, *J* = 7 Hz, 9H, CH_3), 1.28–1.33 (m, 30H, $(CH_2)_5CH_3$), 1.57–1.62 (m, 6H, $NHCH_2CH_2$), 3.42 (dt, *J* = 7/6 Hz, 6H, $NHCH_2$), 6.54 (br. s, 3H, D_2O exchange, NH), 8.34 (s, 3H, aromatic H).– MS (70 eV): *m/z* (%) = 543 (52) [M^+], 415 (100) [$M^+ - NHC_8H_{17}$].

N,N′,N″-Trinonyl-benzene-1,3,5-tricarboxylic Acid Amide

Crystals (ethanol), mp. 197 °C, yield 4.10 g (70%).– Anal. $C_{36}H_{63}N_3O_3$.– IR (KBr): ν = 3303 cm^{-1} (NH), 1644 (CO).– ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, *J* = 7Hz, 9 H, CH_3), 1.13–1.88 (m, 42H, $(CH_2)_7CH_3$), 3.27–3.59 (m, 6H, $NHCH_2$), 7.06 (br. s, 3H, D_2O exchange, NH), 8.08 (s, 3H, aromatic H).– MS (70 eV): *m/z* (%) = 585 (51) [M^+], 443 (100) [$M^+ - NH(CH_2)_8CH_3$].

N,N′,N″-Tridecyl-benzene-1,3,5-tricarboxylic Acid Amide

Crystals (ethanol), mp. 196 °C, yield 4.71 g (75%).– Anal. $C_{39}H_{61}N_3O_3$.– IR (KBr): ν = 3242 cm^{-1} (NH), 1641 (CO).– ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, *J* = 7 Hz, 9H, CH_3), 1.27–1.32 (m, 42H, $(CH_2)_7CH_3$), 1.58 (m, 6H, $NHCH_2CH_2$), 3.39 (dt, *J* = 7/6 Hz, 6H, $NHCH_2$), 7.11 (br. s, 3H, D_2O exchange, NH), 8.78 (s, 3H, aromatic H).– MS (70 eV): *m/z* (%) = 627 (70) [M^+], 471 (100) [$M^+ - NHC_{10}H_{21}$].

N,N′,N″-Tridodecyl-benzene-1,3,5-tricarboxylic Acid Amide

Crystals (ethanol), mp. 189 °C, yield 5.0 g (70%).– Anal. $C_{45}H_{81}N_3O_3$.– IR (KBr): ν = 3247 cm^{-1} (NH), 1641 (CO).– ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, *J* = 7 Hz, 9H, CH_3), 1.26–1.32 (m, 54H, $(CH_2)_9CH_3$), 1.57–1.62 (m, 6 H,$NHCH_2CH_2$), 3.40 (dt, *J* = 7/6 Hz, 6H, $NHCH_2$), 6.98 (br. s, 3H, D_2O exchange, NH), 8.09 (s, 3H, aromatic H).– MS (70 eV): *m/z* (%) = 711 (86) [M^+], 527 (74) [$M^+ - NHC_{12}H_{25}$], 43 (100) [$C_3H_7^+$].

General Procedure for the Preparation of the Triamines **1c–1j** *from the Above Amides*

5 mmol triamide is dissolved in 15 ml $POCl_3$, kept 2 h at 60°C and 14 h at room temp. The excess of $POCl_3$ is removed at 40 °C *in vacuo*. The residue is taken up in 120 ml diglyme. Then 5.67 g (150 mmol) $NaBH_4$ is added with stirring and kept at 70 °C overnight. While cooling with ice 80 ml HCl (10%) is added carefully. The mixture is concentrated *in vacuo*, made alkaline with conc. NaOH, and extracted several times with ether. The ethereal layers are dried with Na_2SO_4, filtered, and the hydrochloride precipitated with an excess of etheral HCl. The crystals are filtered off under suction and recrystallized from the solvent stated.

*N,N′,N″-Tributyl-benzene-1,3,5-trimethanamine Trihydrochloride (***1c***)*

Crystals (ethanol), mp. 300 °C (dec.), yield 1.0 g (45%).– Anal. $C_{21}H_{39}N_3$• 3HCl • 0.25 H_2O.– IR (KBr): ν = 2871 cm^{-1}, 2780, 2573, 2422 (all NH).– ^{1}H-NMR ([D_6]DMSO): δ = 0.89 (t, *J* = 7 Hz, 9H, CH_3), 1.31 (tq, *J* = 8/7 HZ, 6H, CH_2CH_3), 1.67 (tt, *J* = 8/7 Hz, 6H, $NH_2^+CH_2CH_2$), 2.87 (m, 6H, $NH_2^+CH_2$), 4.12 (m, 6H, arCH_2), 7.81 (s, 3H, aromatic H), 9.51 (m, 6H, D_2O exchange, NH_2^+).– MS (70 eV): *m/z* (%) = 333 (2) [M^+], 290 (100) [$M^+ - C_3H_7$].

*N,N′,N″-Tripentyl-benzene-1,3,5-trimethanamine Trihydrochloride (***1d***)*

Crystals (ethanol), mp. 298 °C (dec.), yield 0.6 g (25%).– Anal. $C_{24}H_{45}N_3$• 3HCl.– IR (KBr): ν = 3433 cm^{-1}, 2869, 2783, 2585, 2395 (all NH).– ^{1}H-NMR ([D_6]DMSO): δ = 0.86 (t, *J* = 7 Hz, 9H, CH_3), 1.26 (m, 18H, $(CH_2)_3CH_3$), 1.67 (m, 6H, $NH_2^+CH_2CH_2$), 2.85 (m, 6H, $NH_2^+CH_2CH_2$), 4.11 (m, 6H, arCH_2), 7.81 (s, 3H, aromatic H), 9.52 (m, 6H, D_2O exchange, NH_2^+).– MS (70 eV): *m/z* (%) = 375 (2) [M^+], 318 (100) [$M^+ - C_4H_9$].

*N,N′,N″-Trihexyl-benzene-1,3,5-trimethanamine Trihydrochloride (***1e***)*

Crystals (ethanol), mp. 298 °C (dec.), yield 1.3 g (50%).– Anal. $C_{27}H_{51}N_3$• 3HCl.– IR (KBr): ν = 3186 cm^{-1}, 2857 (both NH).– ^{1}H-NMR ([D_6]DMSO): δ = 0.86 (t, *J* = 7 Hz, 9H, CH_3), 1.26 (m, 18H, $(CH_2)_3CH_3$), 1.67 (m, 6H, $NH_2^+CH_2CH_2$), 2.85 (m, 6H, $NH_2^+CH_2CH_2$), 4.11 (m, 6H, arCH_2), 7.81 (s, 3H, aromatic H), 9.52 (m, 6H, D_2O exchange, NH_2^+).– MS (70 eV): *m/z* (%) = 417 (2) [M^+], 346 (100) [$M^+ - C_5H_{11}$].

*N,N′,N″-Triheptyl-benzene-1,3,5-trimethanamine Trihydrochloride (***1f***)*

Crystals (DMSO), mp. 292 °C (dec.), yield 0.9 g (30%).– Anal. $C_{30}H_{57}N_3$• 3HCl • 0.5 H_2O.– IR (KBr): *n* = 2855 cm^{-1}, 2784, 2416 (all N^+H).– ^{1}H-NMR ([D_6]DMSO): δ = 0.86 (t, *J* = 7 Hz, 9H, CH_3), 1.25 (m, 24H, $(CH_2)_4CH_3$), 1.67 (m, 6H, $NH_2^+CH_2CH_2$), 2.85 (m, 6H, $NH_2^+CH_2CH_2$), 4.11 (m, 6H, arCH_2), 7.82 (s, 3H, aromatic H), 9.56 (m, 6H, D_2O exchange, NH_2^+).– MS (70 eV): *m/z* (%) = 459 (2) [M^+], 374 (100) [$M^+ - C_6H_{13}$].

*N,N′,N″-Trioctyl-benzene-1,3,5-trimethanamine trihydrochloride (***1g***)*

Crystals (ethanol), mp. 286 °C (dec.), yield 1.5 g (50%).– Anal. $C_{33}H_{63}N_3$• 3HCl.– IR (KBr): ν = 2852 cm^{-1}, 2782 (both NH).– ^{1}H-NMR ([D_6]DMSO):

δ = 0.86 (t, *J* = 7 Hz, 9H, CH_3), 1.25 (m, 30H, $(CH_2)_5CH_3$), 1.65 (m, 6H, $NH_2^+CH_2CH_2$), 2.85 (m, 6H, $NH_2^+CH_2CH_2$), 4.12 (m, 6H, arCH_2), 7.78 (s, 3H, aromatic H), 9.42 (m, 6H, D_2O exchange, NH_2^+).– MS (70 eV): *m/z* (%) = 501 (4) [M^+], 402 (100) [$M^+ - C_7H_{15}$].

N,N′,N″-Trinonyl-benzene-1,3,5-trimethanamine Trihydrochloride (**1h**)

Crystals, mp. 286 °C (dec.), yield 2.0 g (60%).– Anal. $C_{36}H_{69}N_3$ • 3HCl • 0.5 H_2O.– IR (KBr): ν = 2852 cm^{-1}, 2780 (both NH).– ^{1}H-NMR ([D_6]DMSO): δ = 0.86 (t, *J* = 7 Hz, 9H, CH_3), 1.25 (m, 36H, $(CH_2)_6CH_3$), 1.65 (m, 6H, $NH_2^+CH_2CH_2$), 2.85 (m, 6H, $NH_2^+CH_2CH_2$), 4.13 (m, 6H, arCH_2), 7.78 (s, 3H, aromatic H), 9.40 m, 6H, D_2O exchange, NH_2^+).– MS (70 eV): *m/z* (%) = 543 (3) [M^+], 430 (100) [$M^+ - C_8H_{17}$].

N,N′,N″-Tridecyl-benzene-1,3,5-trimethanamine Trihydrochloride (**1i**)

Crystals (ethanol), mp. 280 °C (dec.), yield 1.5 g (45%).– Anal. $C_{39}H_{75}N_3$• 3HCl.– IR (KBr): ν = 2854 cm^{-1} (NH).– ^{1}H-NMR (CF_3COOD): δ = 0.90 (m, 9H, CH_3), 1.34 (m, 42H, $(CH_2)_7CH_3$), 1.89 (m, 6H, $NH_2^+CH_2CH_2$), 3.44 (m, 6H, $NH_2^+CH_2CH_2$), 4.49 (m, 6H, arCH_2), 8.01 (s, 3H, aromatic H).– MS (70 eV): *m/z* (%) = 585 (5) [M^+], 458 (100) [$M^+ - C_9H_{19}$].

N,N′,N″-Tridodecyl-benzene-1,3,5-trimethanamine Trihydrochloride (**1j**)

Crystals (ethanol), mp. 276 °C (dec.), yield 1.35 g (35%).– Anal. $C_{45}H_{87}N_3$• 3HCl.– IR (KBr): ν = 2850 cm^{-1} (NH).– ^{1}H-NMR (CF_3COOD): δ = 0.91 (m, 9H, CH_3), 1.33–1.43 (m, 54H, $(CH_2)_9CH_3$), 1.89 (m, 6H, $NH_2^+CH_2CH_2$), 3.34 (m, 6H, $NH_2^+CH_2CH_2$), 4.48 (m, 6H, arCH_2), 8.01 (s, 3H, aromatic H).– MS (70 eV): *m/z* (%) = 669 (7) [M^+], 514 (100) [$M^+ - C_{11}H_{23}$].

General Procedure for the Synthesis of **2d,f,g,h**

4.02 g (30 mmol) isophthalaldehyde, 60 mmol of the appropriate amine, and a catalytic amount of *p*-toluenesulfonic acid are dissolved in 100 ml toluene and refluxed with a water separator for 6 h. The toluene is removed, the residue dissolved in 100 ml ethanol, and 4.6 g $NaBH_4$ (120 mmol) added. The mixture is refluxed for 5 h.

After cooling to room temp. 160 ml H_2O are poured in. The mixture is extracted with $CHCl_3$, washed with water, dried over Na_2SO_4, and the solvent removed. The residue is dissolved in ether and ethereal HCl is added dropwise. The precipitate is filtered off under suction and dried *in vacuo* or recrystallized from the solvent stated.

N,N′-Dihexyl-benzene-1,3-dimethanamine Dihydrochloride (**2d**)

Crystals, mp. 184 °C (dec.), yield 4.23 g (37%).– Anal. $C_{20}H_{36}N_2$ • 2 HCl• 0.25 H_2O.– IR (KBr): ν = 2857 cm^{-1}, 2795, 2416 (all NH).– ^{1}H-NMR ([D_6]DMSO): δ = 0.86 (t, *J* = 7 Hz, 6H, CH_3), 1.26 (m, 12H, $(CH_2)_3CH_3$), 1.67 (m, 4H, $NH_2^+CH_2CH_2$), 2.83 (t, *J* = 7 Hz, 4H, $NH_2^+CH_2CH_2$), 4.11 (s, 4H, arCH_2), 7.49 (t, *J* = 8 Hz, 1H, 5-H), 7.65 (d, *J* = 8 Hz, 2H, 4, 6-H), 7.74 (s, 1H, 2-H), 9.5 (br. s, 4H, NH_2^+).– MS (70 eV): *m/z* (%) = 304 (5) [M^+], 233 (100) [M^+-C_5H_{11}].

N,N′-Dioctyl-benzene-1,3-dimethanamine Dihydrochloride (**2f**)

Light yellow crystals (ethanol), mp. 170 °C, yield 7.3 g (55%).– Anal. $C_{24}H_{44}N_2$ • 2 HCl • 0.5 H_2O.– IR (KBr): ν = 2853 cm^{-1}, 2789, 2419 (all NH).– ^{1}H-NMR ([D_6]DMSO): δ = 0.86 (t, *J* = 6.5 Hz, 6H, CH_3), 1.25 (m, 20H, $(CH_2)_5CH_3$), 1.67 (m, 4H, $NH_2^+CH_2CH_2$), 2.84 (m, 4H, $NH_2^+CH_2CH_2$), 4.10 (m, 4H, arCH_2), 7.55 (t, *J* = 7.5 Hz, 1H, 5-H), 7.70 ('d', *J* = 7.5 Hz, 2H, 4, 6-H), 7.80 (s, 1H, 2-H), 9.8 (br. s, 4H, NH_2^+).– MS (70 eV): *m/z* (%) = 360 (4) [M^+], 261 (100) [$M^+ - C_7H_{15}$].

N,N′-Dinonyl-benzene-1,3-dimethanamine Dihydrochloride (**2g**)

Crystals (ethanol), mp. 190 °C, yield 10.2 g (72%).– Anal. $C_{26}H_{48}N_2$ • 2 HCl • 0.5 H_2O.– IR (KBr): ν = 2852 cm^{-1}, 2788, 2423 (all NH).– ^{1}H-NMR ([D_6]DMSO): δ = 0.86 (t, *J* = 7 Hz, 6H, CH_3), 1.25 (m, 24H, $(CH_2)_6CH_3$), 1.66 (m, 4H, $NH_2^+CH_2CH_2$), 2.85 (m, 4H, $NH_2^+CH_2CH_2$), 4.14 (br. s, 4H, arCH_2), 7.52 (t, *J* = 7.5 Hz, 1H, 5-H), 7.69 ('d', *J* = 7.5 Hz, 2H, 4, 6-H), 7.80 (s, 1H, 2-H), 9.51 (br. s, 4H, NH_2^+).– MS (70 eV): *m/z* (%) = 388 (3) [M^+], 275 (95) [$M^+ - C_8H_{15}$].

N,N′-Didecyl-benzene-1,3-dimethanamine Dihydrochloride (**2h**)

Crystals (ethanol), mp. 194 °C, yield 11.2 g (75%).– Anal. $C_{28}H_{52}N_2$ • 2 HCl • 0.5 H_2O.– IR (KBr): ν = 2852 cm^{-1}, 2794, 2409 (all NH).– ^{1}H-NMR ([D_6]DMSO): δ = 0.86 (t, *J* = 7 Hz, 6H, CH_3), 1.25 (m, 28H, $(CH_2)_7CH_3$), 1.66 (m, 4H, $NH_2^+CH_2CH_2$), 2.85 (m, 4H, $NH_2^+CH_2CH_2$), 4.14 (br. s, 4H, arCH_2), 7.54 (t, *J* = 7.5 Hz, 1H, 5-H), 7.69 ('d', *J* = 7.5 Hz, 2H, 4, 6-H), 7.89 (s, 1H, 2-H), 9.54 (br. s, 4 H, NH_2^+).– MS (70 eV): *m/z* (%) = 416 (6) [M^+], 289 (95) [$M^+ - C_8H_{19}$].

Biological Experiments

Materials

1. Culture Media

Blood agar: 1.5% No.1 (Oxid); 1% Lab-Lemco (Oxid); 1% neutral peptone (Oxid); 0.5% NaCl; 5% defibrinated sheep blood; pH 7.2.

Dextrose broth: 1% dextrose; 1% neutral peptone (Oxid); 1% Lab-Lemco (Oxid); 0.25% NaCl; 0.2% Na_2HPO_4; pH 7.2.

Iso-Sensitest-agar: pH 7,4 ± 0,1, CM 471 (Oxid)

Iso-Sensitest-broth: pH 7,4 ± 0,1 CM 473 (Oxid)

Hepes buffer 0.01 mol/L

238.3 mg Hepes 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethane-sulfonic acid (Sigma) disolved in 100 ml H_2O.

PPTW buffer: 2.27 g KH_2PO_4, 8.9 g Na_2HPO_4 and 2.5 ml Tween 80 dissolved in 1L H_2O.

2. Bacteria

Staphylococus aureus ATCC 25923, *Micrococcus luteus* ATCC 9341, *E. coli* ATCC 25927 and 184 wild strains isolated from infected patients.

3. Apparatus

Autodiluter II (Dynatech); automatic pipettes (Eppendorf); incubator B5060 E (Heraeus); digital multichannel pipette 50-200 µl (Titertek®), inoculator MIC-2000 (Cooke laboratories), laminar flow bench NP 481 (Celaire), Micur-viewer®, microtitration plates 96 wells (Greiner no. 655101), Millipore filters 0.2 µm; Petri plates, plastic, 8 or 13 cm diameter; pipette tips, sterile; div. vials (Eppendorf); whirl mix VF-1 (IKA).

4. Determination of MIC and MBC (Modified According to DIN 58940[13]*)*

The compound to be tested was disolved in 0.01 M Hepes buffer and a stock solution was prepared to contain twice the initial concentration to be tested. The stock solution was sterile filtered and added in aliquots of 100 µl each to the first row of microtitration plates previously filled with 100 µl Iso-Sensitest per well. By means of an autodiluter two-fold dilututions were obtained using the next 10 rows of each plate. The 12th row served as growth control. The plates were inoculated by transfering 10 µl of a bacterial suspension. The inoculum was prepared by suspending 200 µl of an 18 h dextrose broth culture in 20 ml of PPTW buffer (whirlmix). This procedure resulted in an initial bacterial concentration of 5×10^5 colony forming units (CFU) mL^{-1} in each well of the microtitration plates. Purity and concentration of the bacterial inoculum was controled by plating 20 µl on blood agar plates. After incubation for 18 ± 1 h at 37 °C the plates were read employing a mirror (Micur) and MIC were recorded.

MBC were established by subculturing each well of the microtitration plates (used for MIC determination) onto inhibitor free Iso-sensitest agar plates and additional incubation.

5. Materials for Kinetic Studies

Semimicro vials, 1 cm, Sarstedt no. 67.742; lyophilizer Leybold-Heraeus GT2; photometer 6118, Eppendorf, with filter Hg 578 nm; mixing bench Certomat® HK, B. Braun; varifuge RF, Heraeus; bovine serum albumin, fr V, Cabiochem; Comassie blue protein assay reagent, Pierce no. 23200.

6. Growth Kinetics

80 ml of an Iso-sensitest broth was inoculated with 1 ml of an 18 h dextrose broth culture of *E. coli* ATCC 25927 and incubated (mixing bench 80 rpm, 37 °C). After defined time intervals (see Fig. 2) 1 mL aliquots were removed and the absorption was measured at 578 nm. In addition, 600 µl were transferred into an ice-cold reaction vial (for protein determination). When the bacterial growth reached the exponential phase **1a** was added at a final concentration of 4 or 20 µmol/L. After defined time intervals samples were taken for protein determination.

6a. Protein Determination

The samples are washed twice with water by centrifugation (3000 rpm, 5 min, 4 °C), frozen at –20 °C, lyophilized and taken up in 1 mL H_2O. 900 mL of this solution are mixed with 900 µL Comassie reagent. The absorption is measured at 578 nm. For standardization a curve from bovine serum albumine is used (run in duplicate, $r = 0.99$).

6b. Determination of the Kill Kinetics

The bacteria were grown as described above (see 6.) until an absorption of 0.5 was reached. Then, 0.5 ml of the culture were transferred to 15 ml prewarmed broth. After 15 min blank (NaCl solution 0.9%) or test solution (final concentration 21.5 µmol/L) were added. After defined time intervals 100 µL were removed, mixed with 900 µL ice-cold NaCl solution (0.9%), and serial dilutions with a factor of 1:10 were prepared. From each dilution aliquots were plated on blood agar plates and incubated. CFU were counted after 24 h of incubation and concentrations were calculated.

References

[1] K. Rehse, *Drugs Fut.* **1988**, *13*, 941–950.

[2] K. Rehse, D. Piechocki, *Arch. Pharm. (Weinheim)* **1994**, *327*, 687–690.

[3] A. Kesselhut, H. Ebel, F. v. Bruchhausen, K. Rehse, *Arch. Pharm. (Weinheim)* **1995**, *328*, 21–27.

[4] M. Neumann, S.Müller, J. Grosse, D. Lerche, K. Rehse, *Arch. Pharm. (Weinheim)* **1994**, *327*, 497–501.

[5] K. Rehse, S. Müller, *Arch. Pharm. (Weinheim)* **1995**, *328*, 339–342.

[6] K. Rehse, B. Noack, R. Maurer, P. Hilgard, *Arch. Pharm. (Weinheim)* **1991**, *324*, 797–801.

[7] K. Rehse, U. Lükens, *Arch. Pharm. (Weinheim)* **1986**, *319*, 603–607.

[8] K. Rehse, U. Lükens, S. Leißring, G. Claus, *Arch. Pharm. (Weinheim)* **1987**, *320*, 228–233.

[9] K. Rehse, A. Carstensen, H.-J. Ernst, *Arch. Pharm. (Weinheim)* **1987**, *320*, 829–836.

[10] K. Rehse, G. Claus, S. Leißring, *Arch. Pharm. (Weinheim)* **1987**, *320*, 1155–1161.

[11] K. Rehse, U. Lükens, G. Claus, *Arch. Pharm. (Weinheim)* **1987**, *320*, 1283–1286.

[12] K. Rehse, M. Kämpfe, *Arch. Pharm. (Weinheim)* **1989**, *322*, 811–815.

[13] Deutsches Institut für Normung DIN 58940-Md.

[14] E. Knoevenagel, E. Mercklin, Ber. Dtsch. Chem. Ges. **1904**, *37*, 4087–4094.

[15] J. Halberkann, F. Tretwurst, Hoppe-Seyler's Z. Physiol. Chem. **1950**, *185*, 97–127.

Received: November 9, 1995 [FP072]