Briefly, rats were given intraperitoneal injections of 2 mL of a 1:5 dilution of the rat antiserum in isotonic saline. Two hours later 0.3 mL of a 5% solution of Pontamine Sky Blue (Raymond A. Lamb, London) in isotonic saline was injected intravenously, and followed 30 s later by an intraperitoneal injection of 5 mL of Tyrode solution containing 50 μg/mL of heparin and 0.4 mg/mL of ovalbumin. Exactly 5 min after challenge, the rats were stunned and bled and their peritoneal fluids were collected. Animals in control groups received a dilution of normal rat serum instead of antiserum at the time of sensitization (not sensitized) or were given Tyrode solution free of antigen at the time of challenge (not challenged). Compounds were given intraperitoneally in 1 mL of saline, 30 s before the antigen in 5 mL of Tyrode solution. Doses of the compounds are quoted as their concentrations in the 6 mL of fluid injected intraperitoneally.

Assay of Peritoneal Fluids. Collected peritoneal fluids were immediately cooled to 0 °C and centrifuged and the supernatant fluids assayed for dye within 2 h. The supernatant, 0.5 mL, was added to 1 mL of 12% trichloroacetic acid and stored at −20 °C and used to assay for histamine.

Dye Assay. The optical densities (OD) at 625 nm of the supernatants were determined.

Histamine Assay. Histamine was assayed by using an automated spectrofluorimetric system (Technicon Autoanalyser) as described.[23] At the concentrations used, the compounds tested did not interfere with the assay.

The concentrations of histamine and extravasated dye in the peritoneal fluids collected from nondrug-treated control rats were similar to those described;[23] i.e., the mean values obtained ± SEM (n = 19–36) were for passively sensitized and challenged rats 2.03 ± 0.08 μg/mL of histamine and 0.88 ± 0.06 OD (625 nm) for dye. For negative control rats, passively sensitized and no challenge or not sensitized and challenged, the mean values were up to 0.2 μg/mL for histamine and 0.12 OD for dye. For each drug studied, each dose was given to five to seven animals, and at least two doses were given that produced some but a less than maximum inhibition. The percentage inhibition in each animal was calculated from the concentration in that animal × 100 over the mean concentration in five to seven positive control animals treated at the same time from the same group. Negative controls were not taken into account. Regression lines were fitted to each data set plotted against the log of the dose. The median effective dose and associated confidence limits were then estimated as the doses corresponding to an inhibition of 50%, as calculated from the equations of the regression line and the 95% confidence limits of the mean response to any dose.

Registry No. 4, 120-43-4; 6, 55037-87-1; 7 (R_1 = Cl), 23145-88-2; 8 (R_1 = Cl), 39577-03-2; 8 (R_1 = H), 23253-99-8; 9 (R_1 = Cl, n = 2), 91860-37-6; 10, 63360-23-6; 11, 69076-27-3; 12, 75590-39-5; 13, 75590-43-1; 14, 75590-40-8; 15, 75590-44-2; 16, 75590-35-1; 17, 75590-48-6; 18, 91860-38-7; 18·2HCl, 75590-49-7; 19·2HCl, 75590-59-9; 20, 75590-53-3; 21, 75590-54-4; 22, 75590-64-6; 22·2HCl, 75590-55-5; 23·2HCl, 75590-60-2; 24, 75590-36-2; 25·2HCl, 75590-50-0; 26·2HCl, 75590-56-6; 27, 75590-41-9; 28·2HCl, 91860-39-8; 29, 75590-61-3; 30, 75590-37-3; 30·2HNO_3, 75590-38-4; 31, 75590-51-1; 32, 75590-57-7; 33, 75590-42-0; 34, 75590-46-4; 35, 70744-27-3; 4-chlorobenzyl chloride, 104-83-6; 1-bromo-3-chloropropane, 109-70-6; 4,7-dihydroxycoumarin, 1983-81-9; 4-hydroxy-7-methylcoumarin, 18692-77-8.

Bispyridinamines: A New Class of Topical Antimicrobial Agents as Inhibitors of Dental Plaque

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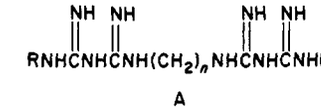
A series of *N,N'*-polyalkylenebis[4-(substituted-amino)pyridines] has been prepared, and members have been evaluated as potential anti-dental plaque agents. From among the most active members of the series, one compound, *N,N'*-[1,10-decanediyldi-1(4*H*)-pyridinyl-4-ylidene]bis(1-octanamine) dihydrochloride, octenidine, was selected as a candidate for clinical study.

Periodontal disease constitutes the leading cause of tooth loss in man and is present in almost all persons who retain natural teeth.[1] It is widely accepted that dental plaque, a dense bacterial matrix that adheres avidly to tooth surfaces, plays an important role in the initiation of caries and periodontal diseases.[2,3] The control of dental plaque by prudent application of chemotherapeutic agents therefore constitutes a potentially effective means of controlling these common disease states.[4-6]

An effective antiplaque chemotherapeutic agent should possess a number of important characteristics. It should (1) be active at low minimal inhibitory concentrations against pathogenic plaque-forming species, (2) be substantive to tooth surfaces, and (3) retain efficacy in the presence of saliva.

Much attention has been directed toward the antimicrobial bisbiguanides A, as potential agents for controlling and/or preventing the formation of dental plaque,[7-18] and a number of structural studies have been published.[19-24]



A

Chlorhexidine, (A, R = 4-ClC_6H_4, n = 6) and alexidine (A, R = 2-ethylhexyl, n = 6) salts are the most studied com-

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δ Deceased.

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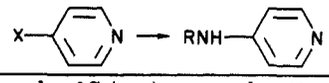
Table I. 4-Pyridinamines

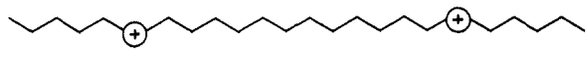


no.	R	mp, °C, or bp, °C (torr)	solvent[a]	X	formula[b]
1	n-C_6H_{13}	66–68	A	Br	$C_{11}H_{18}N_2$
2	n-C_7H_{14}[c]	49–51	B	Br	$C_{12}H_{20}N_2$
3	n-C_8H_{17}	70–73	B	C_5H_5N	$C_{13}H_{22}N_2$
4	n-C_9H_{19}	55–57	A	C_5H_5N	$C_{14}H_{14}N_2$
5	n-$C_{10}H_{21}$	71–73	C	C_5H_5N	$C_{15}H_{26}N_2$
6	n-$C_{12}H_{25}$	83–85	C	C_5H_5N	$C_{17}H_{30}N_2$
7	C_6H_{11}[d]	146–148	A, C, D	C_5H_5N	$C_{11}H_{16}N_2$
8	C_8H_{17}[e]	145–150 (0.9)		C_5H_5N	$C_{13}H_{22}N_2$
9	4-ClC_6H_4	250–252	D	C_5H_5N	$C_{11}H_9ClN_2$
10	4-FC_6H_4	200–201	A, C, D	Cl	$C_{11}H_9FN_2$

[a] A, Et_2O; B, hexane; C, MeCN; D, MeOH. [b] New compounds of Table I analyzed within ±0.4% of the calculated values for C, H, and N. [c] Yakhonotov, L. N.; Marshalkin, M. F. *Dokl. Akad. Nauk. SSSR* **1971**, *199*, 625. [d] Cyclohexyl, Jerchel, D.; Jakob, L. *Chem. Ber.* **1958**, *91*, 1266. [e] 2-Ethylhexyl.

pounds within this class, but neither has received full clinical acceptance.[25] We have prepared a series of N,N'-polyalkylenebis[4-(substitutedamino)pyridines] ("bispyridinamines") and examined them for in vitro antibacterial activities and for their ability to inhibit plaque formation on a synthetic tooth surface.

Chemistry. In their protonated forms, the bisbiguanides can be looked upon as alternating units of lipid and cationic fragments:



Because of the availability of extensive antimicrobial SAR data on compounds with this arrangement,[21,24,26] we elected to mimic this molecular form using 4-pyridinamine units as the cationic elements.

4-Pyridinamine and N-methyl-4-pyridinamine are strongly basic substances with pK_a values of 9.29 and 9.66, respectively.[27] The 4-pyridinamines have been shown to undergo alkylation on the ring nitrogen[28,29] and the products of alkylation with polymethylene dihalides have been described.[29,30] In a similar manner, the N-substituted-4-pyridinamines of Table I underwent alkylation on the ring nitrogen to give the compounds of Tables II and III.

The substances of this investigation were prepared by treating 2 equiv of the appropriate N-substituted-4-pyridinamine with 1 equiv of a 1,n-disubstituted normal alkane of n carbon atoms in an aprotic solvent at elevated

Scheme I

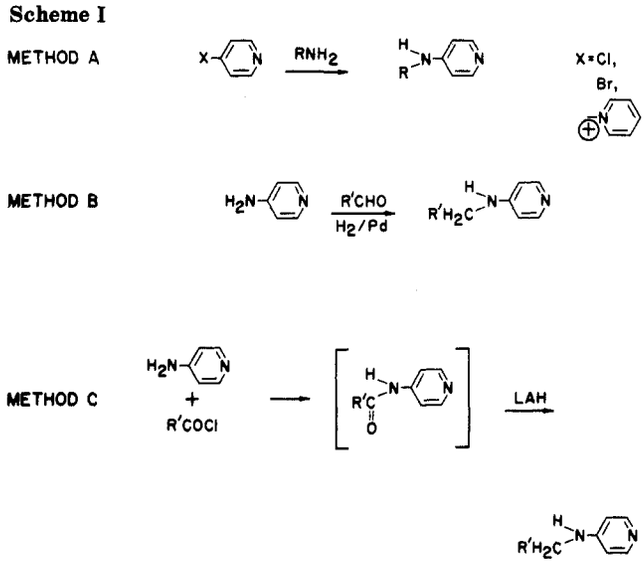
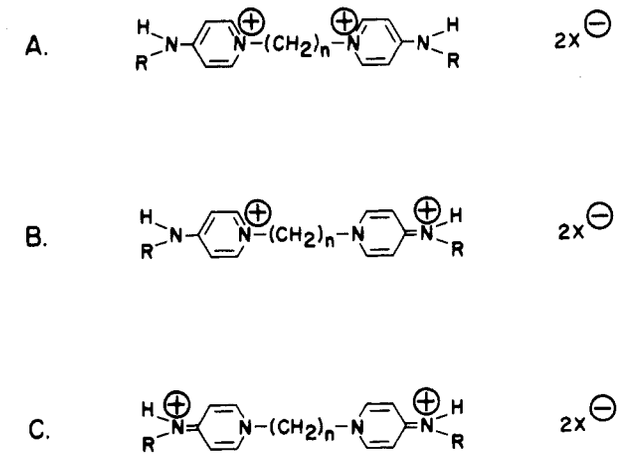


Chart I



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temperatures. The substrate pyridinamines could be prepared by the procedures shown in Scheme I.

The alkylation of the substituted pyridinamines could theoretically occur on either the ring or exocyclic nitrogen and the products of ring alkylation can exist in a number of resonance forms (Chart I).[31]

NMR evidence indicated that alkylation occurred on the ring nitrogen. Thus, in **47**, the two NH's appear as a triplet (coupled with the adjacent CH_2 of the octyl group) and exchange upon treatment with D_2O.[32] The CH_2's adjacent to the exocyclic nitrogens appear as a quartet at δ 3.28 (coupled to both the NH and the adjacent CH_2). Upon exchange of the NH, this signal collapses to a triplet. The CH_2's adjacent to the ring nitrogens occur as a triplet at δ 4.21.

Results and Discussions

As a first broad screen for antiplaque activity, all compounds were examined for their ability to inhibit plaque formation by a standard test organism, *Streptococcus mutans* OMZ-61. Substances were applied in solution to a standardized polycrystalline ceramic hydroxylapatite plate (durapatite),[33] and subsequent to rinsing with water, the plate was incubated with the test organism. Antiplaque activity was indicated by inhibition of plaque formation after visualization by staining with the dye FD and C Red No. 3. Plaque scores could thereby be assigned by visually ranking the degree of dye accumulation on the plate surface. Inhibition of growth of the *S. mutans* in the culture medium was an indication that excessive amounts of the antiplaque agent were leaching off the hydroxylapatite surface—an undesirable characteristic for an effective antiplaque agent. The results of this preliminary screen are recorded in Tables II and III as the minimum concentration of test substance that completely prevented plaque formation under the assay conditions.

The compounds were also profiled against chlorhexidine and alexidine for their minimum inhibitory concentrations (MICs) vs. a variety of Gram-negative and Gram-positive bacteria not indigenous to the oral cavity. Interestingly, the preliminary antiplaque activity appeared not as sensitive to structural changes as the MICs for the nonoral bacteria and were generally maximized when the side chains contained seven, eight, or nine carbon atoms. In the alkyl series (Table II), the sum of the side chain and polymethylene carbon atoms for all of the compounds with minimum plaque inhibitory concentrations (MPICs) of $\leq$0.01% fell in the range 22–30. More than half (16/28) of the test substances in this class contained 24, 26, or 28 carbon atoms in the chains with the largest number of active compounds (6) occurring in the 26 carbon atom group. By comparison, the compounds of the aryl series (Table III) were relatively inactive against the *S. mutans* test organism.

With use of the preliminary data as a guide, compounds were selected for concentration-range studies against two plaque-forming organisms, *S. mutans* NCTC 10449 and *A. viscosus* M-100. Bactericidal activity was examined in the concentration range of 0.3–20 mM by using preformed plaques and a 30-min immersion time at 37 °C. The results for compounds bactericidal at concentrations of $\leq$10 mM are shown in Table IV along with their respective stain indexes. The latter figure represents the ratio of the concentration of test substance that produced staining of the ceramic plate in the absence of plaque to the MPIC of the test substance when both assays were run under the same conditions. Increasing concentrations of antiplaque agents (bispyridinamines, chlorhexidine, alexidine) or an increase in exposure time deposit proportionally more drug on the ceramic surface and, even in the absence of plaque, provide a substrate for the absorption of the test dye. In actual human use, chlorhexidine and alexidine have been shown to produce tooth staining, possibly by interacting with chromophores from food or beverages.[25] This staining liability was the major impediment to the development of the latter materials, and staining potential, therefore, became a critical issue in the development of the bispyridinamine series.

Finally, to examine the effect of saliva on the new antiplaque agents, selected compounds were processed through the assay procedure in the presence of either 5% or 50% pooled saliva. Cetylpyridinium chloride (CPC), a substance reported to have antiplaque activity in vitro,[34] was run in this assay as a control. In the absence of saliva, CPC was effective as an antiplaque agent on test surfaces, but antiplaque activity was completely inhibited by 5% saliva even at 20 times the aqueous MPIC. Loss of efficacy by CPC against *S. mutans* in the presence of saliva has been noted previously.[35] By contrast, none of the bispyridinamine compounds screened lost antiplaque activity even in 50% saliva. Alexidine and chlorhexidine demonstrated a slight loss of activity under these conditions (Table V).

Among the several candidate compounds, **47**, octenidine, was selected for further study. This agent was studied for its effects on other plaque-forming microorganisms under conditions that are considered to approximate clinical usage and for its inhibition of extracellular polysaccharide producing enzymes of *S. mutans* OMZ-61. For these enzyme inhibition studies the procedure described by Robrish et al.[36] was used with alexidine and CPC being used as positive controls. The results shown in Table VI demonstrate that **47** is an effective inhibitor of the plaque-forming enzymes of *S. mutans* OMZ-61. The concentration of **47** inhibiting this enzymatic process by 50% (I_{50}) was approximately one-fifth and one-third that of alexidine and CPC, respectively. Also, the I_{50} for **47** was 31 times the MIC for *S. mutans*, whereas the I_{50}s for alexidine and CPC were approximately 312 and 95 times the corresponding MICs, respectively. Thus, **47** was not only a more potent inhibitor of the polysaccharide synthesizing enzymes than alexidine and CPC but also had a more favorable I_{50}:MIC ratio.

In experiments designed to estimate conditions of clinical use of an antiplaque agent, **47** was assessed for bactericidal activity against preformed in vitro 3-day-old pure culture plaques.[37] These plaques were treated for 2 min once daily on successive days with a standardized con-

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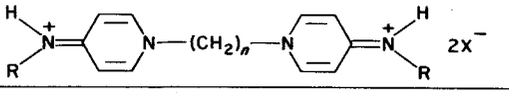
(37) We are grateful to Dr. Jason Tanzer of the University of Connecticut Health Center, Farmington, CT, for providing these cultures.

Table II. Alkyl-Substituted "Bispyridinamines"

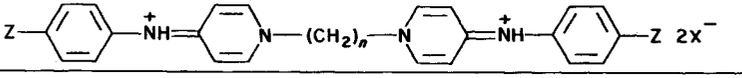


compd	R	n	X	mp, °C	solvent[a]	formula[b]	antimicrobial minimum inhibitory concn (MIC), μg/mL: *S. aureus* Smith	*E. coli* Vogel	*K. pneumo* 39645	*P. mirab* MGH-1	*Ps. aerug* MGH-2	*E. coli* AB1932-1	*E. coli* 100/B22	minimum plaque inhibitory concn, %
alexidine-2HCl							0.5	2.0	3.9	31.3	15.6	1.0	1.0	0.005
chlorhexidine digluconate							0.2	0.5	3.9	15.6	15.6	0.5	1.0	0.005
11[c]	H	4	Br	275–276	A, B, C	$C_{14}H_{20}Br_2N_4$	125	500	>500	>500	>500			>1.0
12[c]	H	6	Br	305–306	A, C	$C_{16}H_{24}Br_2N_4$	250	500	>500	>500	>500			>1.0
13[d]	H	8	Br	302–304	A, C	$C_{18}H_{28}Br_2N_4$	31.3	125	>500	>500	>500	125	250	>1.0
14[c]	H	9	Br	228–229	A, C	$C_{19}H_{30}Br_2N_4$	31.3	250	500	>500	>500			>1.0
15[d]	H	10	Br	249–250	A, C	$C_{20}H_{32}Br_2N_4$	7.8	62.5	250	>500	500	31.3	62.5	>1.0
16[c]	H	12	Br	216–217	A, C	$C_{22}H_{36}Br_2N_4$	2.0	>62.5	>62.5	>125	>62.5			>1.0
17	n-C_6H_{13}	4	Br	199–201	A, B	$C_{26}H_{44}Br_2N_4$	7.8	125	>125	>125	>250	62.5	125	
18	n-C_6H_{13}	5	Br	155–156	A, B	$C_{27}H_{46}Br_2N_4$	2.0	125	>125	>250	>125	31.3	62.5	
19	n-C_6H_{13}	6	Br	178–179	A, B, C	$C_{28}H_{48}Br_2N_4$	0.5	31.3	125	>250	>125	7.8	7.8	1.0
20	n-C_6H_{13}	7	Br	157–158	A, B	$C_{29}H_{50}Br_2N_4$	0.4	15.6	15.6	>125	>125	7.8	7.8	>1.0
21	n-C_6H_{13}	8	Br	180–181	A, B	$C_{30}H_{52}Br_2N_4$	0.2	3.9	7.8	>250	125	2.0	3.9	>1.0
22	n-C_6H_{13}	9	Br	114–115	A, B	$C_{31}H_{54}Br_2N_4$	0.4	2.0	3.9	125	125	2.0	2.0	>1.0
23	n-C_6H_{13}	10	Br	149–149	A, C	$C_{32}H_{56}Br_2N_4$	0.2	2.0	3.9	31.3	31.3	1.0	2.0	>1.0
24	n-C_6H_{13}	12	Cl	86–88	A, C	$C_{34}H_{60}Cl_2N_4$	0.2	1.0	1.0	7.8	15.6	1.0	0.5	0.01
25	n-C_6H_{13}	14	Cl	94–95	A, C	$C_{36}H_{64}Cl_2N_4$	0.5	1.0	1.0	3.9	3.9	0.5	0.5	0.004
26	n-C_7H_{15}	4	Br	229–230	A	$C_{29}H_{48}Br_2N_4$	1.0	31.3	>125	>250	>250	7.8	15.6	>1.0
27	n-C_7H_{15}	5	Br	153–154	A, C	$C_{29}H_{50}Br_2N_4$	0.5	31.3	31.3	>125	125	3.9	7.8	>1.0
28	n-C_7H_{15}	6	Cl	176–178	B, C	$C_{30}H_{52}Cl_2N_4$	0.2	3.9	3.9	>125	>62.5	2.0	2.0	>1.0
29	n-C_7H_{15}	7	Br	142–143	A	$C_{31}H_{54}Br_2N_4$	0.2	2.0	3.9	125	125	2.0	2.0	1.0
30	n-C_7H_{15}	8	Cl	204–206	A, C	$C_{32}H_{56}Cl_2N_4$	0.5	2.0	2.0	62.6	31.3	1.0	2.0	0.1
31	n-C_7H_{15}	9	Cl	154–155	A, C	$C_{33}H_{58}Cl_2N_4$	0.2	1.0	2.0	31.3	31.3	1.0	1.0	0.004
32	n-C_7H_{15}	10	Cl	209–210	A, C	$C_{34}H_{60}Cl_2N_4$	0.2	0.5	1.0	7.8	7.8	1.0	0.5	0.004
33	n-C_7H_{15}	12	Cl	109–112	A, C	$C_{36}H_{64}Cl_2N_4$	0.2	0.5	2.0	2.0	3.9	1.0	0.5	0.003
34	n-C_7H_{15}	14	Cl	113–116	A, C	$C_{38}H_{68}Cl_2N_4$	1.0	2.0	3.9	3.9	2.0	1.0	1.0	0.01
35	C_8H_{17}[e]	4	Br	245–246	A, C	$C_{30}H_{52}Br_2N_4$	1.0	62.5	>125	>125	125	7.8	31.3	>1.0
36	C_8H_{17}[e]	5	Br	150–151	A, C	$C_{31}H_{54}Br_2N_4$	0.5	15.6	>125	>125	125	7.8	15.6	>1.0
37	C_8H_{17}[e]	6	Br	208–209	A, C	$C_{32}H_{56}Br_2N_4$	0.5	7.8	125	>125	>250	2.0	3.9	>1.0
38	C_8H_{17}[e]	7	Br	219–220	A, C	$C_{33}H_{58}Br_2N_4$	0.5	3.9	7.8	>125	125	3.9	3.9	>1.0
36	C_8H_{17}[e]	8	Br	163–161	A, C	$C_{34}H_{60}Br_2N_4$	0.3	3.9	7.8	125	125	1.0	2.0	0.004
40	C_8H_{17}[e]	9	Br	158–159	A, C	$C_{35}H_{62}Br_2N_4$	0.3	1.0	7.8	125	125	1.0	1.0	0.004
41	C_8H_{17}[e]	10	Br	162–163	A, C	$C_{36}H_{64}Br_2N_4$	0.2	1.5	3.0	12.1	48.4	0.4	0.8	0.003
42	C_8H_{17}[e]	12	Br	146–147	A	$C_{38}H_{68}Br_2N_4$	0.5	1.0	2.0	3.9	3.9	1.0	1.0	0.004
43	n-C_8H_{17}	6	Cl	189–191	A, C	$C_{32}H_{56}Cl_2N_2$	0.2	1.0	2.0	15.6	31.3	0.5	0.5	0.004
44	n-C_8H_{17}	7	Br	129–131	A, C	$C_{33}H_{58}Br_2N_4$	0.5	2.0	1.0	7.8	31.3	1.0	1.0	0.005
45	n-C_8H_{17}	8	Cl	210–211	A, C	$C_{34}H_{60}Cl_2N_4$	0.2	1.0	1.0	3.9	7.8	1.0	0.5	0.005
46	n-C_8H_{17}	9	Cl	161–162	A, C	$C_{31}H_{62}Cl_2N_4$	1.0	1.0	2.0	15.6	3.9	1.0	1.0	0.003
47	n-C_8H_{17}	10	Cl	213–214	A, C	$C_{36}H_{64}Cl_2N_4$	1.0	1.0	2.0	2.0	3.9	1.0	1.0	0.005
48	n-C_8H_{17}	12	Br	119–120	A, C	$C_{38}H_{68}Br_2N_4$	0.5	2.0	1.0	2.0	2.0	2.0	2.0	0.005
49	n-C_8H_{17}	14	Br	113–115	A, C	$C_{40}H_{72}Br_2N_4$	1.0	7.8	7.8	3.9	3.9	3.9	3.9	0.005
50	n-C_9H_{19}	6	Cl	194–195	A, C	$C_{34}H_{60}Cl_2N_4$	1.0	2.0	3.9	7.8	15.6	2.0	2.0	0.01
51	n-C_9H_{19}	7	Br	132–134	A, C	$C_{35}H_{62}Br_2N_4$	0.5	1.0	2.0	3.9	15.6	2.0	2.0	0.004
52	n-C_9H_{19}	8	Br	178–179	A, C	$C_{36}H_{64}Br_2N_4$	1.0	3.9	2.0	3.9	7.8	2.0	2.0	0.004
53	n-C_9H_{19}	9	Br	121–122	A, C	$C_{37}H_{66}Br_2N_4$	2.0	2.0	3.9	3.9	3.9	3.9	2.0	0.004

54		n-C_9H_{19}	10	Br	172–173	A, C	$C_{38}H_{68}Br_2N_4$	2.0	2.0	3.9	3.9	3.9	3.9	3.9	0.004
55		n-C_9H_{19}	12	Br	105–106	A, C	$C_{40}H_{72}Br_2N_4$	2.0	3.9	3.9	3.9	3.9	7.8	3.9	0.01
56		n-$C_{10}H_{21}$	6	Br	138–140	C, D	$C_{36}H_{64}Br_2N_4$	1.0	2.0	3.9	2.0	7.8	2.0	7.8	0.01
57		n-$C_{10}H_{21}$	7	Br	145–147	C, D	$C_{37}H_{66}Br_2N_4$	1.0	3.9	7.8	3.9	7.8	3.9	3.9	0.01
58		n-$C_{10}H_{21}$	8	Br	180–182	C, D	$C_{38}H_{68}Br_2N_4$	1.0	2.0	3.9	7.8	7.8	7.8	3.9	0.01
59		n-$C_{10}H_{21}$	9	Br	124–126	C, D	$C_{39}H_{70}Br_2N_4$	1.0	2.0	15.6	7.8	7.8	7.8	3.9	0.01
60		n-$C_{10}H_{21}$	10	Br	173–174	C, D	$C_{40}H_{72}Br_2N_4$	2.0	7.8	31.3	7.8	15.6	15.6	15.6	0.01
61		n-$C_{10}H_{21}$	12	Br	102–106	C, D	$C_{42}H_{76}Br_2N_4$	2.0	7.8	31.3	15.6	15.6	31.3	15.6	0.1
62		n-$C_{12}H_{25}$	6	Br	162–165	C, E	$C_{40}H_{72}Br_2N_4$	7.8	31.3	>62.5	>62.5	>62.5	>62.5	62.5	0.1
63		n-$C_{12}H_{25}$	7	Br	148–150	C, E	$C_{41}H_{74}Br_2N_4$	3.9	62.5	>62.5	62.5	62.5	62.5	62.5	0.1
64		n-$C_{12}H_{25}$	8	Br	184–185	C, E	$C_{42}H_{76}Br_2N_4$	7.8	31.3	>62.5	62.5	>62.5	62.5	>62.5	0.1
65		n-$C_{12}H_{25}$	9	Br	134–135	C, E	$C_{43}H_{78}Br_2N_4$	7.8	62.5	>62.5	62.5	>62.5	>62.5	62.5	0.1
66		n-$C_{12}H_{25}$	10	Br	178–180	C, E	$C_{44}H_{80}Br_2N_4$	15.6	>62.5	>62.5	>62.5	>62.5	>62.5	>62.5	0.1
67		n-$C_{12}H_{25}$	12	Br	75–77	C, E	$C_{46}H_{84}Br_2N_4$	31.3	>62.5	>62.5	>62.5	>62.5	>62.5	>62.5	1.0
68		C_6H_{11}[f]	4	Br	288–290	A, C	$C_{26}H_{40}Br_2N_4$	125	125	>500	>500	>500	125	125	>1.0
69		C_6H_{11}[f]	5	Br	255–256	A, C	$C_{27}H_{42}Br_2N_4$	125	>500	>500	>500	>500	>500	>500	>1.0
70		C_6H_{11}[f]	6	Br	307–308	A, C	$C_{28}H_{44}Br_2N_4$	15.6	>125	>125	>125	>125	125	>125	>1.0
71		C_6H_{11}[f]	7	Br	311–313	A, C	$C_{29}H_{41}Br_2N_4$	7.8	>125	>125	>125	125	125	>125	>1.0
72		C_6H_{11}[f]	8	Br	270–271	A, C	$C_{30}H_{48}Br_2N_4$	3.9	62.5	>125	>125	250	31.3	62.5	>1.0
73		C_6H_{11}[f]	9	Br	149–151	A, C	$C_{31}H_{50}Br_2N_4$	1.0	31.3	125	>500	500	31.3	31.3	>1.0
74		C_6H_{11}[f]	10	Br	226–227	A, C	$C_{32}H_{52}Br_2N_4$	0.2	15.6	>125	>125	>125	7.8	15.6	>1.0

[a] A, MeCN; B, Me_2CO; C, Et_2O; D, MeOH; E, EtOH. [b] All compounds of Table II analyzed within ±0.4% of the calculated values for C, H, and N. [c] U.S. Patent 3055902. [d] Walker, G. N.; et al. *J. Org. Chem.* **1961**, *26*, 2740. [e] 2-Ethylhexyl. [f] Cyclohexyl.

Table III. Aryl-Substituted "Bispyridinamines"



							antimicrobial minimum inhibitory concn (MIC), μg/mL							minimum plaque inhibitory concn, %
no.	Z	N	X	mp, °C	solvent[a]	formula[b]	*S. aureus* Smith	*E. coli* Vogel	*K. pneumo* 139645	*P. mirab* NGH-1	*Ps. aerug* MGH-2	*E. coli* AB1932-1	*E. coli* 100/B22	
75	Cl	4	CH_3SO_3[c]	245–257	C, D	$C_{28}H_{32}Cl_2N_4O_6S_2$	7.8	15.6	>250	>250	>125	7.8	15.6	>1.0
76	Cl	5	Br	166–168	A, D	$C_{27}H_{28}Br_2Cl_2N_4$	7.8	125	>125	>125	>125	31.3	125	>1.0
77	Cl	6	CH_3SO_3[c]	108–110	B, D	$C_{30}H_{36}Cl_2N_4O_6S_2$	3.9	15.6	500	500	500	3.9	15.6	>1.0
78	Cl	7	Br	202–204	A, E	$C_{29}H_{32}Br_2Cl_2N_4$	1.0	3.9	>125	>125	>125	125	3.9	>1.0
79	Cl	8	CH_3SO_3[c]	164–165	A, B.	$C_{32}H_{40}Cl_2N_4O_6S_2$	0.4	3.1	31.3	125	125	1.6	3.1	>1.0
80	Cl	9	Br	178–179	A, E	$C_{31}H_{36}Br_2Cl_2N_4$	0.6	1.0	7.8	>125	125	1.0	2.0	>1.0
81	Cl	10	CH_3SO_3[c]	202–204	A, B	$C_{34}H_{44}Cl_2N_4O_6S_2$	0.4	1.6	3.1	>125	62.5	0.8	1.6	>1.0
82	F	6	Br	220–222	A, B, D	$C_{28}H_{30}Br_2F_2N_4$	15.6	31.3	>125	>125	>62.5	15.6	15.6	>1.0
83	F	8	Br	226–228	A, B, D	$C_{30}H_{34}Br_2F_2N_4$	2.0	15.6	>62.5	>125	>62.5	7.8	15.6	>1.0

[a] A, Et_2O; B, hexane; C, MeCN; D, MeOH. [b] All compounds of Table III analyzed within ±0.4% of the calculated values of C, H, and N. [c] Methanesulfonate.

Table IV. Bactericidal Activity and Stain Index of Selected Bispyridinamines[a]

compd	minimal bactericidal concn,[b] mM, for *S. mutans*	*A. viscosus*	stain index
25	1.40	2.80	1
32	0.73	1.46	10
33	2.81	2.81	10
45	8.80	0.88	10
47	3.20	1.60	10
48	2.70	1.35	10
49	1.30	2.60	2
50	7.30	7.30	1
51	2.86	7.16	1
52	2.50	2.50	1
53	1.37	1.37	1
54	1.35	2.70	1
55	1.30	2.60	1
chlorhexidine	3.20	1.60	1

[a] See Experimental Section for details. [b] Plaques were run in quadruplicate.

Table V. Effect of Saliva upon the Antiplaque[a] Activity and Staining Potential of 47, Alexidine, Chlorhexidine, or Cetylpyridinium Chloride in Aqueous Solutions

compd run for 48 h[b]	% saliva	% compd	aqueous solution: plaque accum[c]	aqueous solution: staining potential[d]
inoculated control	0		++	-
	5		++	-
	50		++	-
47	0	0.01	-	-
		0.025	-	-
		0.05	-	+
	50	0.01	-	-
		0.025	-	-
		0.05	-	+
alexidine	0	0.01	-	+
		0.025	-	+
		0.05	-	+
	50	0.01	+	-
		0.025	-	+
		0.05	-	+
chlorhexidine	0	0.01	-	+
		0.025	-	+
		0.05	-	+
	50	0.01	+	-
		0.025	-	+
		0.05	-	+
cetylpyridinium chloride	0	0.001	+	-
	0	0.005	-	-
	0	0.01	-	+
	0	0.1	-	+
	5	0.01	+	-
	5	0.005	+	-
	5	0.01	+	-
	5	0.1	+	-

[a] Plaque produced by *S. mutans*. [b] Two 1-min exposures for each 24 h of testing. [c] No plaque accumulation (-), slight plaque accumulation (+), moderate accumulation (++). [d] No staining (-), slight staining (+).

Table VI. Inhibition of the Plaque-Forming Enzyme of *S. mutans* OMZ-61 by 47, Alexidine, and Cetylpyridinium Chloride

compd	enzyme inhib, I_{50}, μg of base/mL	growth inhib, MIC, μg of base/mL
47	31	1.0
alexidine	156 ± 45.5[a]	0.5
cetylpyridinium chloride	95	1.0

[a] Mean + SD of 17 assays.

centration of test agent (3.2 mM) until the plaques were killed as judged by the cessation of acid production. This treatment regimen may be viewed as being analogous to single daily use of a mouthrinse. The data can be expressed in terms of a plaque bactericidal index (PBI)[21] that represents the integration of the effects of concentration (mM), duration (daily exposure time in minutes), and frequency of treatments (number of daily treatments required to kill the plaques) required to achieve a plaque bactericidal effect. The results of these tests are shown in Table VII.

Compound **47**, octenidine, is under study by the National Caries Program, National Institute of Dental Research, NIH.[38,39] The results of a clinical trial have been reported elsewhere.[40]

Experimental Section

Melting points were taken in capillary tubes and are uncorrected. NMR spectra were recorded with a Varian HA100 spectrometer. Elemental analyses were performed by Instranal Laboratories, Rensselaer, NY, and Galbraith Laboratories, Inc., Knoxville, TN.

***N*-Hexyl-4-pyridinamine (1). Method A.** A mixture containing 100.0 g (0.51 mol) of 4-bromopyridine hydrochloride and 100 g (0.8 mol) of *n*-hexylamine hydrochloride was heated in an oil bath. When the bath temperature reached 175–180 °C, the reaction mixture began to melt and stirring was begun. The temperature of the bath was then raised to 227 °C and the stirring continued 3.5 h. After cooling to room temperature, the reaction mixture was dissolved in hot H_2O, and the resulting solution was cooled with ice, made alkaline with dilute aqueous NaOH, and extracted with $CHCl_3$. The $CHCl_3$ extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was triturated with Et_2O and cooled. The resulting solid was collected by filtration and washed with cold Et_2O. Evaporation of the filtrate afforded a second crop of solid. The crops were combined, dissolved in $CHCl_3$, and treated with decolorizing carbon, and the suspensions were filtered. The filtrate was evaporated under reduced pressure, and the residue was triturated with cold Et_2O. The product was collected by filtration, washed with cold Et_2O, and dried to give 63.6 g, 75% yield, of product, mp 66–68 °C. Anal. ($C_{11}H_{18}N_2$) C, H, N.

***N*-Octyl-4-pyridinamine (3). Method B.** A mixture containing 34 g (1 mol) of 4-aminopyridine, 384 g (3 mol) of octaldehyde, 7 g of 10% palladium-on-carbon hydrogenation catalyst, and sufficient absolute EtOH to give a total volume of 1.2 L was hydrogenated 4.5 h at 70–90 °C under an initial hydrogen pressure of 45 psi. After cooling of the mixture, the hydrogenation catalyst was removed by filtration and the filtrate was evaporated to dryness under reduced pressure. The residual oil crystallized on standing, and the solid product was triturated with hexane, collected by filtration, washed with fresh hexane, and dried at 40 °C under vacuum to give 182 g, 88% yield, of product, mp 70–72 °C. Anal. ($C_{13}H_{22}N_2$) C, H, N.

***N*-(4-Fluorophenyl)-4-pyridinamine (10). Method A.** A mixture containing 64.0 g (0.43 mol) of *p*-fluoroaniline hydrochloride and 4-chloropyridine hydrochloride was heated gradually to an internal temperature of 170 °C, whereupon the solid mixture began to melt and stirring was begun. Upon continued heating, the melt began to resolidify. Stirring was stopped and heating was continued until no further change was evident. The mixture was cooled and dissolved in 1 L of H_2O and ice, and the solution was made alkaline with 35% NaOH. The resulting precipitate was collected, washed with H_2O, and redissolved in hot MeOH. The MeOH solution was treated with decolorizing carbon, filtered, and evaporated to dryness. The residue was recrystallized from MeOH–MeCN–Et_2O to give after drying 47.3 g, 62% yield, of material with mp 200–201 °C. Anal. ($C_{11}H_9FN_2$) C, H, N.

(38) Shern, R. J.; Monell-Torrens, E.; Bowen, W. H.; Kingman, A. *J. Dent. Res.* **1980**, *59*, 314.
(39) Emilson, C. G.; Bowen, W. H.; Robrish, S. A.; Kemp, C. W. *J. Dent. Res.* **1980**, *59*, 390.
(40) Patters, M. R.; Anerud, K.; Trummel, C. L.; Kornman, K. S.; Nalbandian, J.; Robertson, P. R. *J. Periodontal Res.* **1983**, *18*, 212.

Table VII. Plaque Bactericidal Index (PBI) of 47 and Chlorhexidine

	concn × dur × freq = PBI					
agent	*S. mutans* 10449	*S. sanguis* 10558	*S. mitis* 90557	*A. viscosus* M-100	*A. viscosus* T14V	*A. naeslundii* 631
47	3.2 × 2 × 4 = 25.6	3.2 × 2 × 5 = 32	3.2 × 2 × 5 = 32	3.2 × 2 × 3 = 19.2	3.2 × 2 × 3 = 19.2	3.2 × 2 × 3 = 19.2
chlorhexidine	3.2 × 2 × 4 = 25.6	3.2 × 2 × 5 = 32	nt[a]	32.2 × 2 × 3 = 19.2	nt	3.2 × 2 × 3 = 19.2

[a] Not tested.

***N*-(2-Ethylhexyl)-4-pyridinamine (8). Method C.** To a stirred solution containing 800 g (8.4 mol) of 4-aminopyridine and 1500 mL of Et_3N in 6.4 L of CH_2Cl_2 was added over 3 h a solution containing 1610 g (10.0 mol) of 2-ethylhexanoyl chloride in 1.6 L of CH_2Cl_2. Throughout the addition the temperature was maintained at 15 °C, after which the mixture was warmed on a steam bath 2 h. After cooling, the reaction mixture was washed thoroughly with H_2O, dried over anhydrous Na_2SO_4, treated with decolorizing carbon, and filtered. Evaporation of the filtrate afforded 1843 g, 100% crude yield, of *N*-(4-pyridyl)-2-ethylhexanamide.

To a mixture containing 100 g (2.63 mol) of LAH in 2 L of THF was added a solution containing 570 g (2.62 mol) of the above crude (*N*-(4-pyridyl)-2-ethylhexanamide in 4 L of THF at a sufficient rate to maintain gentle reflux. When the addition was complete (approximately 3 h), the reaction mixture was heated under reflux for 7 h. After cooling, the mixture was treated successively with 100 mL of H_2O, 100 mL of 15% aqueous NaOH, and 100 mL of H_2O. The solids were removed by filtration, and the solvent was evaporated from the filtrate under reduced pressure. Vacuum distillation of the residual oil gave 420 g, 78% yield from crude amide, of product, bp 145–150 °C (0.9 torr). Anal. ($C_{13}H_{22}N_2$) C, H, N.

***N*,*N*-[1,10-Decanediyldi-1(4*H*)-pyridinyl-4-ylidene]bis(1-octanamine) Dihydrochloride (47).** A mixture containing 61.8 g (0.3 mol) of 4-(octylamino)pyridine and 31.5 g (0.15 mol) of 1,10-dichlorodecane was stirred and heated slowly to 120 °C. The heat source was removed and the temperature of the now exothermic reaction continued to rise to 180 °C. As soon as the reaction mixture began to crystallize, 250 mL of DMF was rapidly added. The resulting mixture was heated to give a clear homogeneous solution and then cooled to 0 °C. The precipitated product was collected by filtration, washed with Et_2O, and dried 24 h under vacuum at 60 °C to give 73 g, 78% yield, of product, mp 215–217 °C. Anal. ($C_{36}H_{64}Cl_2N_4$) C, H, N.

***N*,*N*-(1,5-Pentanediyl)bis[4-[(4-chlorophenyl)amino]-pyridinium Dibromide (76).** To a stirred warm solution containing 10.0 g (0.049 mol) of 4-[(*p*-(chlorophenyl)amino]pyridine in a mixture of 275 mL of MeCN and 100 mL of DMF was added dropwise a solution containing 5.75 g (0.025 mol) of 1,5-dibromopentane in 25 mL of MeCN, and the resulting mixture was heated 24 h under reflux. The reaction mixture was then evaporated to dryness under reduced pressure and the residue triturated with a mixture of Et_2O and MeCN. The resulting pale yellow solid was redissolved in EtOH, treated with decolorizing carbon, and filtered. The filtrate was evaporated to dryness under reduced pressure and the residual oil was crystallized from Et_2O–MeCN. The colorless solid was crystallized from MeOH–MeCN and dried 48 h at 115 °C (0.1 torr) to give 8.1 g, 51% yield of product, mp 166–168 °C. Anal. ($C_{27}H_{28}Br_2Cl_2N_4$) C, H, N.

Antimicrobial Spectrum. MICs for the various test compounds were determined against a variety of Gram-positive and Gram-negative bacteria and compared to those for alexidine and chlorhexidine. MICs were determined by serial twofold tube dilutions with use of tryptose phosphate broth for all bacterial genera except *Streptococcus* for which brain–heart infusion broth containing 1% normal horse serum was used.

A stock solution of each compound containing 1000 μg/mL was prepared in distilled water and serially diluted in double-strength broth before 2×10^5 cells of an appropriate culture was added. Tubes containing bacterial species were incubated aerobically at 37 °C for 24 h. The lowest concentration of test substance that inhibited visible growth was considered to be the MIC.

Plaque Assays. Ceramic hydroxylapatite plates[33] ca. 9 × 12 × 1.5 mm fitted with a 0.75-mm drill hole to accommodate a nichrome wire hanger were used for all plaque assays. The initial antiplaque assays were accomplished by using a modification of a procedure previously detailed by Turesky et al.[41]

Stock cultures of *S. mutans* OMZ-61 were maintained in fluid thioglycollate medium (BBL) supplemented with 20% beef heart infusion (dehydrated Difco). The growth medium used in the antiplaque-activity determinations consisted of the following: beef extract paste (BBL), 1.5 g; sodium chloride, 5 g; dehydrated trypticase, 10 g; sucrose, 5 g; distilled water, to 1000 mL. the pH of the medium was adjusted to 7.0 and sterilized by membrane filtration using an HA (0.45 μm) Millipore filter. The medium was then dispensed aseptically in 10-mL amounts into sterile, plastic capped 16 × 150 mm tubes and stored at 4 °C until used.

One hundred milligrams of each compound was put into solution in 1 mL of water, 0.1 N NaOH, and Me_2SO or 10% DMF and then diluted to 10 mL with distilled water. This 1.0% stock solution was diluted in distilled water to concentrations of 0.1%, 0.01%, 0.005%, andd 0.001% for testing. All solutions were sterilized by membrane filtration prior to use.

A sterile plate of ceramic hydroxylapatite was suspended in each concentration of the appropriate test compound for two 1-min periods, each followed by a 1-min air-drying period. The treated plate was then suspended and agitated for 5 min in individual test tubes containing sterile distilled water. After the rinse, each piece was suspended in a test tube containing 10 mL of liquid beef extract medium to which had been added 0.3 mL of a late log culture of *S. mutans* OMZ-61. The tubes were incubated anaerobically at 37 °C for 24 h. At the end of that incubation period, the same process of two 1-min soaks in the proper concentration of the compound with air-drying and rinsing was repeated. Once again, the treated plate was suspended in fresh growth medium innoculated with the organism and incubated anaerobically for another 24 h at 37 °C. At the end of the second 24-h incubation period, each plate was rinsed for 1 min in each of three successive tubes of distilled water. It was then suspended for 1 min in a 1% solution of FD & C Red No. 3 dye, which stains plaque but does not stain natural teeth or ceramic hydroxylapatite. This staining procedure was used to identify and score the development of plaque after 48 h of exposure to *S. mutans*. The staining period was followed by another short rinse in distilled water to remove excessive dye. Plaque formation stains brilliant pink and test results can be read rapidly as plaque inhibition or absence of plaque inhibition (plaque formation). With some compounds, the inhibition of plaque production is caused by the inhibition of *S. mutans* growth in culture medium rather than on the surface of the plate. This result may occur because the compound is antibacterial but somewhat less substantive than necessary to bind to the ceramic hydroxylapatite surface. Concentrations were considered active when no plaque was formed on the plates *and* the density of the growth of *S. mutans* in the test medium was visibly equal to that of the control culture. By conducting the above procedure, omitting the addition of *S. mutans* culture, the ceramic plates could be scored to determine the minimum staining concentration of the test substance itself.

Plaque-inhibition studies using this procedure were modified by the addition of 50% pooled normal human saliva. Saliva was collected from normal human volunteers (paraffin stimulated) and centrifuged for 20 min at 2500 rpm. The supernatant fluid was filtered through a 5.0-μm filter paper (Gelman), sterilized by membrane filtration (Millipore HA, 0.45 μm), and added to the growth medium.

Preformed Plaque Model: In Vitro Plaque Formation. In vitro plaques were grown on ceramic hydroxylapatite slabs (1 cm × 1 cm) which were individually suspended on No. 26 gauge nichrome wire, using a modification of methods previously de-

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tailed.[21] Fluid thioglycollate cultures were used to inoculate a complex growth medium[41] which was supplemented with 5% (w/v) sucrose and 0.5% (w/v) Na_2CO_3. The wire-attached ceramic hydroxylapatite pieces were transferred daily to fresh medium three times. All cultures were incubated at 37 °C under an anaerobic atmosphere (GasPak, BBL). In vitro plaques formed by this method were graded, with those with similar girth and mass being selected for assessment of agent efficacy.

Assessment of Efficacy of Bispyridinamines. Efficacy of the bispyridinamines was assessed by using techniques previously detailed.[21] Briefly, in vitro plaques were immersed in 10 mL of a solution of the control or test agents for various durations and frequencies. Following treatment, the in vitro plaques were rinsed twice by immersion in 15 mL of sterile distilled H_2O for 10 min and transferred to fresh broth containing the pH indicator bromocresol purple. The in vitro plaques were judged to be killed by the cessation of culture acid production, lack of turbidity increase, and the failure of 48-h posttreatment plaque samples to grow when plated on appropriate agar media.

Inhibition of Extracellular Polysaccharide Formation. *S. mutans* OMZ-61 was grown in 0.15% brain–heart infusion medium, 2% glucose, 0.5% NaCl, and 1% trypticase and incubated at 37 °C for 24 h under anaerobic conditions. No plaque was produced when glucose was the carbon source, but the constitutive enzymes dextran sucrase and levan sucrase were synthesized and released into the medium. Cells were separated from the spent culture medium by filtration through a Millipore membrane filter (0.45-μm pore size). The filtrate was adjusted to pH 5.5, divided into convenient aliquots, and stored at −20 °C until use.

The assay procedure employed to determine enzymatic activity was that of Robrish et al.[36] The reaction mixture comprised 0.2 mL of the treated spent culture medium test compound, 3 μmol of sucrose containing 0.3 μCi of sucrose-4-^{14}C, and citrate phosphate buffer, pH 5.5, in a total volume of 0.5 mL. The reaction was initiated by the addition of sucrose. After 2 h of incubation at 37 °C, the reaction was terminated by the addition of absolute MeOH in which any labeled dextan and levan formed would be insoluble. The precipitated polysaccharides were collected on glass filter disks (0.1-μm pore size) and extensively washed with MeOH. Zero time values were obtained by adding the MeOH to the incubation tube prior to the addition of the substrate. The filter disks were placed in vials containing scintillation fluid and counted in a liquid scintillation counter. The compounds were sparingly soluble in H_2O but were soluble in EtOH. Incorporation of sucrose was lowered approximately 40% or 60% by the presence of 20% or 40% EtOH, respectively, while EtOH concentrations of 4% or less had no significant effect on activity of the various enzymes. Thus, 4% v/v EtOH in the incubation mix was routinely used. The compounds were solubilized in EtOH and diluted with H_2O at 37 °C.

The compounds were tested for inhibition at concentrations of 50, 160, and 500 μg of base/mL. The I_{50} was estimated graphically. If the I_{50} was less than 50 μg/mL, the compound was retested at lower concentrations. Alexidine, an active antiplaque agent and known inhibitor of this enzyme system, was used as reference.

Registry No. **1**, 64690-14-8; **2**, 35036-87-4; **3**, 64690-19-3; **4**, 64690-27-3; **5**, 64690-61-5; **6**, 64690-59-1; **7**, 34844-87-6; **8**, 64690-39-7; **9**, 35488-08-5; **10**, 72358-71-5; **11**, 91389-28-5; **11** (base), 91389-94-5; **12**, 91389-29-6; **12** (base), 85966-36-5; **13**, 91389-24-1; **13** (base), 91389-95-6; **14**, 91389-25-2; **14** (base), 85966-37-6; **15**, 91389-26-3; **15** (base), 91389-96-7; **16**, 91389-27-4; **16** (base), 85966-38-7; **17**, 91389-22-9; **17** (base), 85990-12-1; **18**, 91389-23-0; **18** (base), 85966-39-8; **19**, 91389-30-9; **19** (base), 85966-40-1; **20**, 91389-31-0; **20** (base), 85966-42-3; **21**, 91389-32-1; **21** (base), 85966-45-6; **22**, 91389-33-2; **22** (base), 85966-48-9; **23**, 91389-34-3; **23** (base), 91389-97-8; **24**, 91389-35-4; **24** (base), 91389-98-9; **25**, 91389-36-5; **25** (base), 85966-60-5; **26**, 91389-37-6; **26** (base), 91389-99-0; **27**, 91389-38-7; **27** (base), 91390-00-0; **28**, 91389-39-8; **28** (base), 91390-01-1; **29**, 91389-40-1; **29** (base), 91390-02-2; **30**, 91389-41-2; **30** (base), 91390-03-3; **31**, 91389-42-3; **31** (base), 85966-49-0; **32**, 91389-43-4; **32** (base), 85966-54-7; **33**, 91389-44-5; **33** (base), 85966-57-0; **34**, 91389-45-6; **34** (base), 91390-04-4; **35**, 91389-46-7; **35** (base), 91390-05-5; **36**, 91409-26-6; **36** (base), 91390-06-6; **37**, 91389-47-8; **37** (base), 91390-07-7; **38**, 91389-48-9; **38** (base), 91409-29-9; **39**, 91389-49-0; **39** (base), 91390-08-8; **40**, 91389-50-3; **40** (base), 91390-09-9; **41**, 91389-51-4; **41** (base), 91390-10-2; **42**, 91389-52-5; **42** (base), 91390-11-3; **43**, 91389-53-6; **43** (base), 91390-12-4; **44**, 91389-54-7; **44** (base), 91390-13-5; **45**, 91389-55-8; **45** (base), 91390-14-6; **46**, 91389-56-9; **46** (base), 85966-50-3; **47**, 70775-75-6; **47** (base), 71251-02-0; **48**, 91389-57-0; **48** (base), 91390-15-7; **49**, 91389-58-1; **49** (base), 85966-61-6; **50**, 91389-59-2; **50** (base), 85966-41-2; **51**, 91389-60-5; **51** (base), 85966-43-4; **52**, 91389-61-6; **52** (base), 85966-46-7; **53**, 91389-62-7; **53** (base), 85966-51-4; **54**, 91389-63-8; **54** (base), 85966-55-8; **55**, 91389-64-9; **55** (base), 85966-58-1; **56**, 91389-65-0; **56** (base), 91390-16-8; **57**, 91389-66-1; **57** (base), 91390-17-9; **58**, 91389-67-2; **58** (base), 91390-18-0; **59**, 91389-68-3; **59** (base), 85966-52-5; **60**, 91389-69-4; **60** (base), 91390-19-1; **61**, 91389-70-7; **61** (base), 91390-20-4; **62**, 91389-71-8; **62** (base), 85990-13-2; **63**, 91389-72-9; **63** (base), 85966-44-5; **64**, 91389-73-0; **64** (base), 85966-47-8; **65**, 91389-74-1; **65** (base), 85966-53-6; **66**, 91389-75-2; **66** (base), 85966-56-9; **67**, 91389-76-3; **67** (base), 85966-59-2; **68**, 91389-77-4; **68** (base), 91390-21-5; **69**, 91389-78-5; **69** (base), 91390-22-6; **70**, 91389-79-6; **70** (base), 91390-23-7; **71**, 91389-80-9; **71** (base), 91390-24-8; **72**, 91389-81-0; **72** (base), 91390-25-9; **73**, 91389-82-1; **73** (base), 91390-26-0; **74**, 91389-83-2; **74** (base), 91390-27-1; **75**, 91389-84-3; **75** (base), 64690-68-2; **76**, 91389-85-4; **76** (base), 91390-28-2; **77**, 91389-87-6; **77** (base), 91389-86-5; **78**, 91389-88-7; **78** (base), 91390-29-3; **79**, 91389-90-1; **79** (base), 91389-89-8; **80**, 91409-27-7; **80** (base), 91390-30-6; **81**, 91389-92-3; **81** (base), 91389-91-2; **82**, 91409-28-8; **82** (base), 91390-31-7; **83**, 91389-93-4; **83** (base), 91409-30-2; 4-bromopyridine hydrochloride, 19524-06-2; *n*-hexylamine hydrochloride, 142-81-4; 4-aminopyridine, 504-24-5; octaldehyde, 124-13-0; *p*-fluoroaniline hydrochloride, 2146-07-8; 4-chloropyridine hydrochloride, 7379-35-3; 2-ethylhexanoyl chloride, 760-67-8; *N*-(4-pyridyl)-2-ethylhexanamide, 64690-40-0; 1,10-dichlorodecane, 2162-98-3; 1,5-dibromopentane, 111-24-0.