$NH_4CH_3CO_2$) at 2 mL/min and peak areas measured. Yields (Figure 1) are given as area (product)/area (all peaks).

Determination of Specific Activities and Radiochemical Purities. An aliquot of the solution was transferred to a thin glass vial and its radioactivity measured with a dose calibrator. Another aliquot (of the same volume) was removed by the same syringe and injected onto the analytical HPLC column and the UV peak area of the product measured. The mass of the product was calculated by comparison to a standard curve. The sensitivity of the analyses was maximized by setting the wavelength of the UV detector to 239 nm—the λ_{max} of **21**. The specific activity in mCi/μmol was then calculated by dividing the number of millicuries in the aliquot by the number of micromoles in the same aliquot. The radiochemical purity of the final product was always >99%; some hydrophilic radioactive impurities (13%) were detected in ^{125}I-labeled **21** following storage for 3 months at −10 °C.

Binding Assays. Male CD-1 mice were killed, and the whole brain minus cerebellum was rapidly homogenized in 50 mM phosphate-buffered saline (pH = 7.4, 37 °C). The tissue homogenate (8 mg/mL buffer) was then incubated with 1 nM [^{3}H]-*N*-methylscopolamine (New England Nuclear, 70 Ci/mmol) alone or in the presence of 1.0 μM atropine and in the presence of increasing concentrations of the compounds listed in Table I. Samples were incubated for 30 min at 37 °C in a shaking water bath to achieve equilibrium. All samples were incubated in triplicate. Samples containing atropine defined nonspecific binding.

Following the incubation period samples were rapidly filtered over Whatman glass fiber filters under vacuum and rapidly washed with 15 mL of ice-cold phosphate-buffered saline. Filters were then counted in the presence of 10 mL of Formula 963 scintillation fluid (New England Nuclear) by using standard techniques at efficiencies of ca. 40%. Radioactivity in the presence of atropine (nonspecific binding) was subtracted from that in other samples to compute net specific binding. Net specific binding at each drug concentration was then expressed as percent of control specific binding. Log-logit analysis was then used to compute the 50% inhibitory concentrations (IC_{50}) for each test compound. Dexetimide was always studied in parallel with other compounds to assure experimental reproducibility. The values shown in Table I represent average results from two to three experiments; values varied less than 20%. Saturation experiments using [^{125}I]- and [^{123}I]iododexetimide were carried out in a similar fashion using 10^{-6} M atropine to define nonspecific binding.

Biodistribution studies were carried out on male CD-1 mice according to literature procedures.[11]

Acknowledgment. We thank Dr. Pierre Laduron for samples of authentic dexetimide and levetimide and Annslie Smith for carrying out the binding and biodistribution studies. This work was supported by USPHS Grant No. CA32845 and NS-15080.

Registry No. (*S*)-**1**, 21888-98-2; (*R*)-**2**, 21888-99-3; (*R,S*)-**3**, 119391-55-8; (*R,S*)-**3**·HCl, 6767-69-7; (*S*)-**4**, 115216-87-0; (*R*)-**5**, 115216-88-1; (*R,S*)-**6**, 119477-43-9; (*R,S*)-**7**, 119391-56-9; (*R,S*)-**7**·HCl, 119391-73-0; (*R,S*)-**8**, 119391-57-0; (*R,S*)-**8**·HCl, 119391-74-1; (*R,S*)-**9**, 119391-58-1; (*R,S*)-**9**·HCl, 119391-75-2; (*R,S*)-**10**, 119391-59-2; (*R,S*)-**10**·HCl, 119391-76-3; (*R,S*)-**11**, 119391-60-5; (*R,S*)-**11**·HCl, 119391-77-4; (*R,S*)-**12**, 119391-61-6; (*R,S*)-**12**·HCl, 119391-78-5; (*R,S*)-**13**, 119391-62-7; (*R,S*)-**13**·HCl, 119391-79-6; (*R,S*)-**14**, 119391-63-8; (*R,S*)-**14**·HCl, 119391-80-9; (*R,S*)-**15**, 119391-64-9; (*R,S*)-**15**·HCl, 119391-81-0; (*R,S*)-**16**, 119391-65-0; (*R,S*)-**16**·HCl, 119391-82-1; (*R,S*)-**17**, 119391-66-1; (*R,S*)-**17**·HCl, 119391-83-2; (*R,S*)-**18**, 119413-94-4; (*R,S*)-**18**·HCl, 119391-84-3; **19**, 17903-42-3; **20**, 16004-15-2; **20** (^{125}I), 105644-30-2; **20** (^{123}I), 105644-31-3; **21**, 119478-57-8; ^{125}I-**21**, 119391-69-4; ^{123}I-**21**, 119391-70-7; **22**, 119477-44-0; ^{125}I-**22**, 119391-71-8; ^{123}I-**22**, 119391-72-9; (*S*)-**23**, 119391-67-2; (*R*)-**24**, 119391-68-3; 4-iodotoluene, 624-31-7; 3-fluorobenzyl bromide, 456-41-7; 3-iodobenzyl bromide, 49617-83-6; 3-bromobenzyl bromide, 823-78-9; 3-chlorobenzyl bromide, 766-80-3; 4-chlorobenzyl bromide, 622-95-7; 2-fluorobenzyl bromide, 446-48-0; 4-bromobenzyl bromide, 589-15-1; 4-fluorobenzyl bromide, 459-46-1; 2-chlorobenzyl bromide, 611-17-6; 2-bromobenzyl bromide, 3433-80-5; 2-iodobenzyl bromide, 40400-13-3.

Aromatic Dienoyl Tetramic Acids. Novel Antibacterial Agents with Activity against Anaerobes and Staphylococci[1]

Terry Rosen,*[,†] Prabhavathi B. Fernandes, Mary A. Marovich, Linus Shen, James Mao, and André G. Pernet

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Streptolydigin (**1**) and tirandamycin A (**2**) are typical members of the naturally occurring class of 3-dienoyl tetramic acids. These compounds, which possess potent antibacterial activity particularly against anaerobes, have been shown to inhibit bacterial RNA polymerase. In contrast, tenuazonic acid (**5**), which lacks a complex dioxabicyclononane moiety and diene chromophore present in **1** and **2**, exhibits essentially no antimicrobial activity and has no effect on bacterial RNA polymerase, suggesting that one or both of these structural features may be critical for antibacterial activity. In this paper, we report on a novel series of synthetic dienoyl tetramic acids that lack a complex dioxabicyclononane unit. Several of these compounds, particularly **8T–W**, exhibit potent antimicrobial activity against Gram-positive and Gram-negative anaerobes as well as staphylococci. We will discuss the structure–activity relationship for this series of compounds which, in contrast to their natural counterparts, do not inhibit significantly RNA polymerase. We will also discuss preliminary results on the biochemical and microbiological properties of this series of compounds, several of which moderately inhibit supercoiling by DNA gyrase isolated from *E. coli* H560, although this enzyme has not been established as their target in whole cells. Compound **8W**, which is not cross-resistant with DNA gyrase subunit A or B inhibitors or tirandamycin, has also been demonstrated to be rapidly bactericidal.

Streptolydigin (**1**),[2] tirandamycin A (**2**),[3] BU2313A (**3**), and BU2313B (**4**)[4] are members of the naturally occurring class of 3-dienoyl tetramic acids. These compounds possess potent antibacterial activity, particularly against anaerobes and some Gram-positive aerobes. Streptolydigin and tirandamycin A have been shown to be inhibitors of bacterial RNA polymerase.[3,5] In contrast, tenuazonic acid

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(1) This work was presented, in part, at the 1987 ICAAC. Rosen, T.; Fernandes, P. B.; Shen, L.; Marovich, M. A.; Mao, J.; Pernet, A. *Abstracts of the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy* **1987**, Abstract No. 1003.

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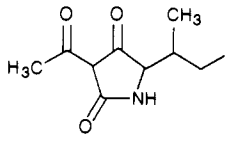
(5) exhibits essentially no antimicrobial activity and has



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no effect on bacterial RNA polymerase.[3,6] It has been postulated that the dioxabicyclononane skeleton or the diene unit present in 1–4 may be critical to the antibiotic properties of these compounds.[7] Although tirandamycin was reported initially to be ineffective for the treatment of bacterial infections in mice, more recently tirandamycin and the BU2313 compounds have been shown to be effective in mouse protection tests.[8] We have also demonstrated that tirandamycin is effective in a mouse abscess model for *Bacteroides fragilis*.[9]

There has been a structure–activity study on a series of tetramic acids lacking the diene chromophore, and these compounds are generally devoid of substantial antibacterial activity.[10] In this paper, we report our preliminary results on the synthesis and biological activity of a series of dienoyl tetramic acids. Several of these compounds possess potent antibacterial activity. However, unlike their natural counterparts, these compounds do not inhibit appreciably RNA polymerase. These compounds show moderate inhibition of supercoiling by DNA gyrase, although this enzyme has not been established as the target of these agents in whole cells.

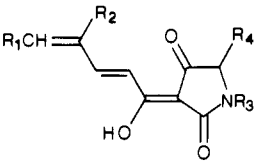
Results and Discussion

Chemistry. The tetramic acids in this study were synthesized by employing general methodology analogous to that reported by Schlessinger et al. in their synthesis of tirandamycin A.[11] The key step in this convergent methodology involves Wadsworth–Emmons condensation of a tetramic acid keto phosphonate **7** and an aldehyde **6** (Scheme I, eq 1). In Schlessinger's synthesis of **2**, it was necessary to employ a protecting group on the nitrogen of the tetramic acid **7** (R_3 = 2,4-dimethoxybenzyl, R_4 = H) in order to better facilitate the coupling reaction. Deblocking is accomplished with neat trifluoroacetic acid to afford **8** ($R_3 = R_4$ = H). We used this sequence to prepare compounds in which R_3 in **8** is hydrogen. However, we also utilized the keto phosphonate **7** (R_3 = CH_3, R_4 = H), which affords compounds with the *N*-CH_3 moiety present in BU2313A (**3**), obviating the need for a subsequent deprotection step.

The general route to the keto phosphonate reagents (**7**) is shown in Scheme I (eq 2). Treatment of the bromo acid bromide **9** with an amino ester **10** gives the adduct **11**. Exposure of **11** to the potassium salt of diethyl phosphite results in displacement of bromide and intramolecular cyclization to furnish **7**. The reagent **7** (R_3 = CH_3, R_4 = H) is obtained from *N*-methylglycine ethyl ester (**10**, R_3 = CH_3, R_4 = H, R_5 = C_2H_5) (see the Experimental Section).

The aldehydes employed in the condensation reaction were obtained either from commercial sources or by a homologation sequence employing an aldehyde and an appropriate stabilized Wittig reagent ((carbethoxyethylidene)triphenylphosphorane or (carbethoxymethylene)triphenylphosphorane, Aldrich) followed by manipulation to the desired oxidation state (Scheme I, eq 3).[15,16] The structures of the 3-acyltetramic acids (**8**) thus

Table I. Structures of Compounds



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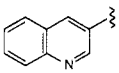
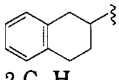
8[a]	R_1	R_2	R_3	R_4	formula
A	*i*-C_3H_7	CH_3	CH_3	H	$C_{14}H_{19}NO_3$
B[b]	*n*-$C_{11}H_{23}$	CH_3	CH_3	H	$C_{22}H_{35}NO_3$
C	C_6H_{11}	CH_3	CH_3	H	$C_{17}H_{23}NO_3 \cdot {}^1/_2H_2O$
D	$C_6H_{11}CH_2$	CH_3	CH_3	H	$C_{18}H_{25}NO_3$
E	$C_6H_{11}CH(CH_3)$	CH_3	CH_3	H	$C_{19}H_{27}NO_3$
F		CH_3	CH_3	H	$C_{16}H_{21}NO_4$
G	*p*-$(CH_3)_2NC_6H_4$	H	CH_3	H	$C_{18}H_{20}N_2O_3$
H	*o*-$NO_2C_6H_4$	H	CH_3	H	$C_{16}H_{14}N_2O_5$
I[11a]	C_6H_5	H	H	H	
J	C_6H_5	H	CH_3	H	$C_{16}H_{15}NO_3$
K	C_6H_5	Br	H	H	$C_{15}H_{12}BrNO_3$
L[b]	C_6H_5	Br	CH_3	H	$C_{16}H_{14}BrNO_3$
M[b]	C_6H_5	Cl	H	H	$C_{15}H_{12}ClNO_3$
N	C_6H_5	Cl	CH_3	H	$C_{16}H_{14}ClNO_3$
O	C_6H_5	CH_3	H	H	$C_{16}H_{15}NO_3$
P[b]	C_6H_5	CH_3	CH_3	H	$C_{17}H_{17}NO_3$
Q	C_6H_5	*n*-C_5H_{11}	CH_3	H	$C_{21}H_{25}NO_3$
R	$C_6H_5CH(CH_3)$	CH_3	CH_3	H	$C_{19}H_{21}NO_3$
S	2-C_5H_4N	CH_3	CH_3	H	$C_{16}H_{16}N_2O_3$
T	1-$C_{10}H_7$	CH_3	H	H	$C_{20}H_{17}NO_3$
U	1-$C_{10}H_7$	CH_3	CH_3	H	$C_{21}H_{19}NO_3 \cdot {}^1/_3H_2O$
V	2-$C_{10}H_7$	CH_3	H	H	$C_{20}H_{17}NO_3$
W	2-$C_{10}H_7$	CH_3	CH_3	H	$C_{21}H_{19}NO_3 \cdot {}^1/_2H_2O$
X[b]	*p*-MeO-1-$C_{10}H_6$	CH_3	CH_3	H	$C_{22}H_{21}NO_4$
Y	*p*-F-1-$C_{10}H_6$	CH_3	CH_3	H	$C_{21}H_{18}NO_3F$
Z	*p*-$C_6H_5C_6H_4$	CH_3	CH_3	H	$C_{23}H_{21}NO_3$
AA		CH_3	CH_3	H	$C_{20}H_{18}N_2O_3$
BB[b]		CH_3	CH_3	H	$C_{20}H_{18}N_2O_3$
CC		CH_3	CH_3	H	$C_{21}H_{23}NO_3 \cdot {}^1/_6H_2O$
DD	2-$C_{10}H_7$	CH_3	$(CH_2)_3$		$C_{23}H_{21}NO_3$
EE	2-$C_{10}H_7$	CH_3	CH_3	CH_3	$C_{22}H_{21}NO_3$
FF	*o*-HO-1-$C_{10}H_6$	CH_3	CH_3	H	$C_{21}H_{19}NO_4$
GG	*o*-MEMO-1-$C_{10}H_6$[c]	CH_3	CH_3	H	$C_{25}H_{27}NO_5 \cdot {}^1/_4H_2O$

[a] Unless noted otherwise, all new compounds had high field ^1H NMR spectra, mass spectra, and combustion analysis (C, H, N ± 0.4% of theoretical value) and/or high-resolution mass spectra that were consistent with the indicated structures. [b] Compound characterized by high field ^1H NMR spectrum and low-resolution mass spectrum. [c] MEM = methoxyethoxymethyl.

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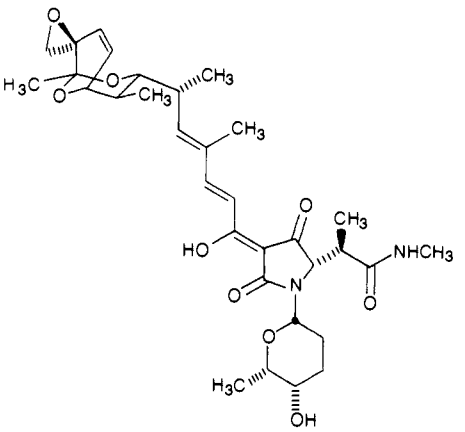

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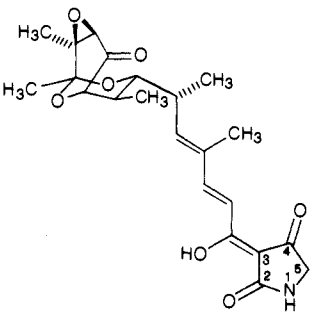

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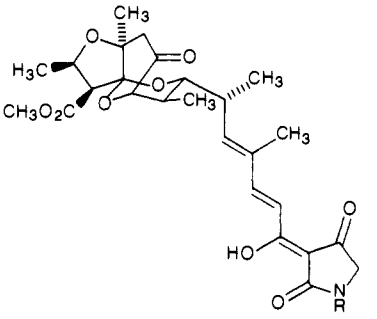


3: R = CH_3
4: R = H

Table II. Minimum Inhibitory Concentrations of Tetramic Acids 8

organism	MIC, μg/mL A	B	C	D	E	F	G	H	I	J	K	L	M
B. fragilis ATCC 25285	31	125	8	8	8	125	125	62	50	62	8	16	8
B. thetaomicron ATCC 29741	62	125	8	31	62	125	62	62	50	62	8	16	8
C. perfringens ATCC 13124	31	31	4	2	2	125	31	62	25	125	31	8	4
C. difficile ATCC 9689	31	4	4	2	2	125	31	62	25	16	8	16	4

organism	MIC, μg/mL N	O	P	Q	R	S	T	U	V	W	X	Y	Z
B. fragilis ATCC 25285	16	4	4	62	8	62	1.0	≤0.5	≤0.5	≤0.5	62	2	8
B. thetaomicron ATCC 29741	16	4	8	125	16	125	1.0	2.0	≤0.5	31	125	2	125
C. perfringens ATCC 13124	8	8	8	16	125	31	1.0	≤0.5	≤0.5	2	2	62	31
C. difficile ATCC 9689	8	4	4	31	125	8	1.0	≤0.5	≤0.5	≤0.5	4	31	≤0.5

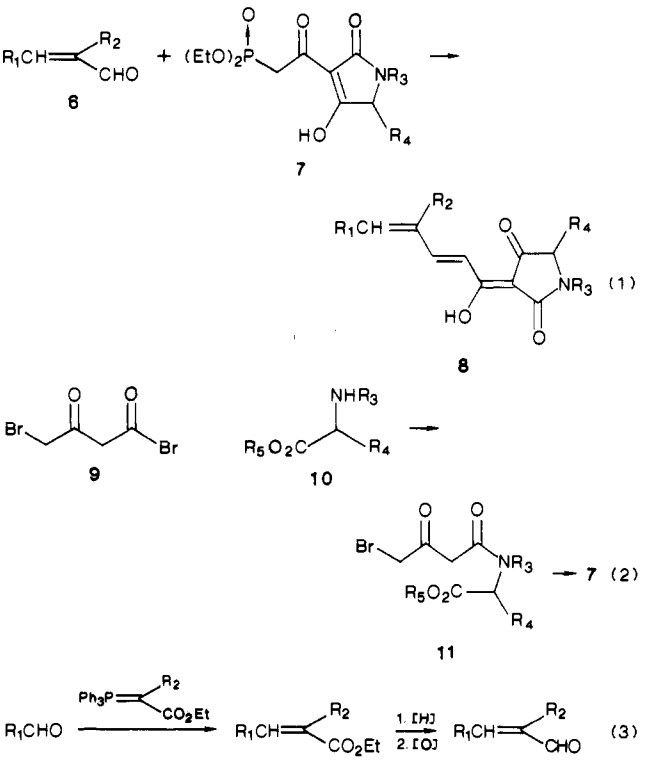
organism	MIC, μg/mL AA	BB	CC	DD	EE	FF	GG	TIR	CLIN
B. fragilis ATCC 25285	16	2	125	31	62	31	125	0.1–0.8	2
B. thetaomicron ATCC 29741	31	4	62	62	62	31	250	0.2–0.8	8
C. perfringens AtCC 13124	8	2	125	31	31	16	125	≤0.05–0.1	≤0.5
C. difficile ATCC 9689	8	2	125	16	62	16	8	1.6–3.1	1–2

obtained are shown in Table I, and further physical and spectral properties are provided in the Experimental Section.

Biological Evaluation of Compounds

In Vitro Antibacterial Activity. The compounds of

Scheme I. Synthesis of 3-Dienoyl Tetramic Acids[a]



[a] For R_3 = 2,4-dimethoxybenzyl, R_4 = H, see Schlessinger, R. L.; et al. *J. Org. Chem.* **1985**, *50*, 1344.

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(16) In the condensation of aldehydes with the keto phosphonate reagents 8, we observed NMR data indicative of the formation of trans double bonds (coupling constants of ~16 Hz); these results are consistent with those reported for the synthesis of **2**.[11] The bromo and chloro derivatives **8K–N** were derived from condensations with *cis*-α-bromocinnamaldehyde and *cis*-α-chlorocinnamaldehyde (Aldrich), respectively; **8K–N** are the only compounds in this study derived from aldehydes possessing cis geometry. Furthermore, exposure of the *N*-CH_3 tetramic acid **8L** to TFA (conditions required to cleave the dimethoxybenzyl protecting group to give *N*-H tetramic acids)[11] afforded material that had ^{1}H NMR spectral data (in both C_6D_6 and $CDCl_3$) identical with untreated material, indicating a lack of lability of the diene system to these acidic conditions.

Table III. Activity of Dienoyl Tetramic Acids against Aerobes

organism	MIC, μg/mL 8T	8U	8V	8W
S. aureus ATCC 6538P	3.1	3.1	0.78	1.56
S. aureus CMX 686B	3.1	3.1	0.78	1.56
S. aureus A5177	3.1	3.1	1.56	1.56
S. aureus 45	3.1	3.1	0.78	1.56
S. epidermis 3519	6.2	3.1	3.1	1.56
M. luteus ATCC 4698	12.5	6.2	6.2	1.56
S. bovis A5169	6.2	50	12.5	200
S. pyogenes EES61	12.5	12.5	12.5	50
E. coli Juhl	100	>100	>100	200
E. coli H560	>100	>100	>100	200
P. aeruginosa A5007	100	>100	100	100

this study were initially evaluated against the organisms shown in Table II. The minimum inhibitory concentrations (MICs) are shown relative to clindamycin (CLIN). The corresponding values for tirandamycin (TIR) are given for comparison. Several trends emerge with respect to structure–activity. A methyl group on the carbon adjacent to the terminus of the diene appears to enhance activity relative to the corresponding unsubstituted analogue (**8O** and **8P** versus **8I** and **8J**). The activities of the corresponding chloro and bromo derivatives (**8K–N**) as well as the deleterious effect of the *n*-pentyl group in **8Q** suggest that the effect of this substituent is spatial (versus electronic) in nature, on the basis of the similar van der Waals radii of the chloro, bromo, and methyl substituents. The

substit	van der Waals radius, Å
CH_3	2.0
Br	1.9
Cl	1.8
H	1.2

first analogue that we found to have definite, although marginal, activity was the phenyl derivative **8I**.[11a] As mentioned above, addition of a methyl group to the diene (**8O**), in the same position relative to the tetramic acid as it occurs in the natural antibiotics **1–4**, resulted in significantly improved antibacterial activity. Addition of a second fused aromatic ring affords the most potent agents in this study, the 1- and 2-naphthyl derivatives **8T–W**, which show excellent in vitro potency against anaerobes similar to that of tirandamycin and clindamycin. These agents also show good activity versus staphylococci (Table III), although they are less active against streptococci (in contrast to their natural counterparts) and show no activity against Gram-negative aerobes. Several modifications on the naphthalene moiety (**8X**, **8Y**, **8FF**, **8GG**) result in a significant decrease in antibacterial activity. The deleterious effects of these modifications may be electronic in nature. The quinoline analogue **8BB** also shows good in vitro activity although somewhat less than the more active naphthalene derivatives. *p*-Biphenyl (**8Z**) is not an effective replacement for the naphthyl moiety. Although the cyclohexyl and phenyl analogues **8C** and **8P** have quite similar activity, fusing an aromatic ring on **8C** to give the tetralin **8CC** results in greatly diminished activity in contrast to the improved activity derived from the same modification on **8P** (to give **8W**).

Compound **8E** (racemic) is identical with BU2313A (**3**) in the tetramic acid and dienoyl side chain portions of the molecule, however possessing a cyclohexyl group in place of the complex dioxabicyclononane moiety. Although it possesses antibacterial activity, it is appreciably less active than BU2313A; the related aromatic derivative **8R** is even less active. Several acyclic dienoyl derivatives were prepared (**8A** and **8B** are representative of this class), all of which are only weakly antibacterial; branching at the position α to the diene (**8A**) is not a suitable replacement for a full ring (**8C** and **8P**).

Table IV. Evaluation of Tetramic Acids as Inhibitors of Bacterial RNA Polymerase

compound 8	concn, μG/mL	% inhibn of RNA polymerase activity
K	100	7.3
M	100	3.2
O	100	4.5
Q	100	5.6
V	100	13.1
W	100	12.3
tirandamycin	80	50
rifampicin	0.05	57

Table V. Cross-Resistance Studies

organism	MIC, μg/mL 8W	diflox-acin	coumer-mycin
S. aureus 730A	0.25	0.25	0.008
S. aureus 337-113 (norfloxacin-resistant)	0.25	4.0	0.008
S. aureus 45	0.25	0.25	0.008
S. aureus 45CR-1 (coumermycin-resistant)	0.25	0.25	4.0
B. fragilis ATCC 25285	0.5		
B. fragilis TirR (tirandamycin-resistant)	0.5		

It should be noted that a methyl group on the nitrogen (1-position) of the tetramic acid has little effect on antibacterial activity, perhaps reducing MICs by one $\log_2$ dilution in general. This trend is the same seen for the BU2313 compounds. The *N*-methyl compounds are generally easier to work with from a practical standpoint, having better solubility properties. Introduction of a methyl group at the 5-position of the tetramic acid (**8EE**), however, dramatically reduces activity as does bridging the nitrogen to the 2-position with a propylene unit (**8DD**); thus, the activity of this series of compounds is quite sensitive to structural modifications.

Evaluation of Compounds as Inhibitors of RNA Polymerase and Cross-Resistance Studies. Several of the agents in this study were evaluated as inhibitors of RNA polymerase isolated from *Escherichia coli* K12 (Sigma); tirandamycin and rifampicin were used as standards. As can be seen in Table IV, these compounds show no appreciable inhibition of this enzyme. We did, however, observe that several of these synthetic compounds inhibit supercoiling by DNA gyrase isolated from *E. coli* H560, using norfloxacin as a standard (I_{50} = 1 μg/mL).[12] The I_{50} values (μg/mL) obtained for **8V**, **8W**, **8T**, **8K**, **8C**, **8U**, **8FF** and BU2313B are 3.5, 10.3, 17, 30, 31, 49, 64, and ~100, respectively. Compound **8W** was studied further to determine whether it shows cross-resistance with the quinolones (inhibitor of DNA gyrase subunit A), coumermycin (inhibitor of DNA gyrase subunit B), or tirandamycin. The quinolones and coumermycin are the only reported inhibitors of DNA gyrase.[13] As can be seen from Table V, **8W** is not cross-resistant with any of these agents.

Quinolone antibacterials are well known to trap the gyrase–DNA intermediate during the enzyme-inhibition step, resulting in cleaved DNA products upon addition of a protein denaturant to the reaction medium.[14] Compound **8W** was evaluated for such cleavage activity, and the result was negative, indicating that the DNA gyrase inhibitory properties of this agent do not resemble the mode of inhibition by the quinolones.

Bactericidal Activity. The killing kinetics of **8W** were determined versus *Staphylococcus aureus* and *Bacteroides*

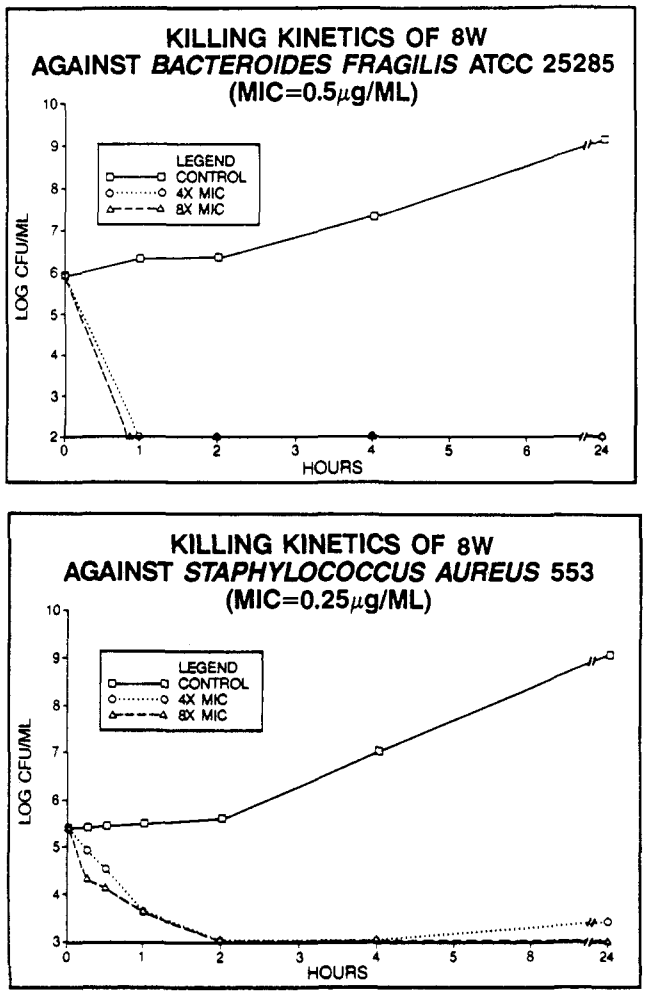


Figure 1. Killing kinetics of compound **8W**.

fragilis at 4 and 8 times the MIC values. The killing curves are shown in Figure 1. It can be seen that **8W** is rapidly bactericidal versus both organisms.

Summary of Results. A series of dienoyl tetramic acids has been prepared, and several structure–activity parameters have emerged from their biological evaluation. Several of these agents show good in vitro activity against Gram-positive and Gram-negative anaerobes as well as staphylococci and as such are the first tetramic acids lacking a complex dioxabicyclononane moiety reported to have these properties. The most potent agents in this series (**8T–W**) have an unsubstituted naphthalene group attached to the terminus of the diene. A methyl group on the carbon adjacent to the terminus of the diene enhances antibacterial activity, and this effect appears to be spatial in nature. The activities of these agents are quite sensitive to structural modification.

These compounds do not show appreciable activity against bacterial RNA polymerase, in contrast to streptolydigin. Although several of these compounds show moderate inhibition of DNA gyrase, this enzyme has not been established as the target of these agents in whole cells; compound **8W** does not show cross-resistance with known DNA gyrase inhibitors (coumermycin or the quinolones) or tirandamycin. This compound has also been demonstrated to be rapidly bactericidal. Further biochemical studies concerning the mode of action and properties of these novel compounds are in progress.

Experimental Section

General Methods. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Aldehydes were either commercially available or obtained from the corresponding carboxylic acid by sequential reduction (diborane) and oxidation ($(ClCO)_2$, dimethyl sulfoxide (DMSO); triethylamine). Melting points are uncorrected. ^1H NMR spectra were determined on a General Electric GN-300 spectrometer operating at 300.1 MHz. Chemical shifts are expressed in ppm downfield form internal tetramethylsilane. Significant ^1H NMR data are tabulated in the order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons, coupling constant(s) in hertz. Mass spectra were obtained with a Hewlett-Packard 5985A mass spectrometer or a Kratos MS-50 instrument with EI source (70 eV). Column chromatography was done with Merck silica gel 60 (70–230 mesh ASTM). Elemental analyses were performed by the Microanalytical Laboratory, operated by the Analytical Department, Abbott Laboratories, North Chicago, IL.

The keto phosphonate reagent **7** ($R_3 = CH_3$, $R_4 = H$) was prepared by using protocol similar to that described previously by Schlessinger for the synthesis of **7** (R_3 = 2,4-dimethoxybenzyl, $R_4 = H$);[11] full experimental details are provided below. Full experimental procedures for the synthesis of **8W** and **8AA** are given and are representative of the general methodology employed for the preparation of compounds for this study; spectral and physical properties for other tetramic acids **8** are also given below. Assignments for the signals in the ^1H NMR spectra of compounds **8A** and **8V** are also given in this tabulation of data.

Diethyl 2-(2,4-Dioxo-1-methylpyrrolidin-3-yl)-2-oxoethylphosphonate (7; $R_3 = CH_3$, $R_4 = H$). Under a nitrogen atmosphere, in a three-neck 2-L round-bottom flask equipped with a mechanical stirrer, a low-temperature thermometer, and a pressure-equalizing addition funnel were placed 24.8 mL (316 mmol) of diketene and 200 mL of dichloromethane. To the system (cooled in a dry ice bath, −65 °C internal temperature) was added 15.1 mL (295 mmol) of bromine in 45 mL of dichloromethane over a period of 20 min; the internal temperature was maintained at ca. −60 °C. The reaction mixture was stirred at −60 °C until it was colorless. To this resulting solution of the bromo acid bromide **9** was added 61.7 mL (443 mmol) of triethylamine dropwise over a period of 15 min; the internal temperature was not allowed to exceed −55 °C. To the system was added dropwise 24.6 g (211 mmol) of sarcosine ethyl ester in 80 mL of CH_2Cl_2, while the internal temperature was maintained at −55 °C. After this addition was complete, the reaction mixture was stirred at −55 to −60 °C for 1 min. The solids were removed by suction filtration and rinsed well with ethyl acetate. The filtrate was concentrated with a rotary evaporator, ~500 mL of ethyl acetate was added to the system, and solids were again removed by suction filtration. The filtrate was washed with 250 mL of 1 M aqueous H_3PO_4 and three 250-mL portions of brine, dried (Na_2SO_4), and concentrated to give a viscous red oil. The crude material was subjected to column chromatography (350 g of silica gel) using 2:1 hexanes/ethyl acetate as the eluant (collecting 20-mL fractions) to obtain 40.0 g (68% yield, fractions 49–129) of pure **11** ($R_3 = CH_3$, $R_4 = H$, $R_5 = CH_2CH_3$) as an oil, which was used immediately for the next transformation.

Into a dry 1-L round-bottom flask, flushed with N_2, were placed 36.0 g (315 mmol) of KH/mineral oil dispersion and a magnetic stirbar. The KH was rinsed with two 10-mL portions of hexanes. The system was charged with 165 mL of tetrahydrofuran (THF) and cooled to 0 °C. To the system was added slowly (via addition funnel, over a period of 45 min) 42.4 mL (329 mmol) of diethyl phosphite. The resulting suspension was stirred at 0 °C for 30 min. To the system was added 40 g (143 mmol) of bromide **11** ($R_3 = CH_3$, $R_4 = H$, $R_5 = CH_2CH_3$) prepared above in 90 mL of THF over a period of 10–15 min. The mixture was stirred at 0 °C for 1 h and at room temperature overnight and partitioned between 350 mL of ether and 100 mL of saturated aqueous sodium bicarbonate. The layers were separated, the ether phase was extracted with three 50-mL portions of saturated aqueous sodium bicarbonate, and the combined aqueous fractions were washed with 100 mL of ether. The aqueous layer was sequentially extracted with 50 mL of CH_2Cl_2, acidified to pH 7.5, extracted with three 50-mL portions of CH_2Cl_2, acidified to pH 7, extracted with two 50-mL portions of CH_2Cl_2, acidified to pH 6, extracted with two 50-mL portions of CH_2Cl_2, acidified to pH 2.5, and extracted with two 100-mL portions of CH_2Cl_2. The CH_2Cl_2 extracts were

analyzed by TLC, and the pure fractions were concentrated to afford 22.2 g (53% yield) of **7** (R_3 = CH_3, R_4 = H) as an orange viscous oil: ^{1}H NMR ($CDCl_3$) δ 1.33 (t, 6 H, J = 7), 3.04 (s, 3 H), 3.56 (d, 2 H, J = 24), 3.77 (s, 2 H), 4.18 (m, 4 H); mass spectrum, m/z 291 (parent). Anal. ($C_{11}H_{18}NO_6P$) C, H, N.

The keto phosphonate reagents **7** (R_3 = CH_3, R_4 = CH_3) and **7** (R_3, R_4 = $(CH_2)_3$) (precursors to **8EE** and **8DD**, respectively) were prepared in an analogous manner, replacing sarcosine ethyl ester with *N*-methyl-D-alanine ethyl ester and D,L-proline methyl ester, respectively, and these reagents had the following properties.

7 (R_3 = CH_3, R_4 = CH_3): ^{1}H NMR ($CDCl_3$) δ 1.34 (overlapping d and t, 9 H), 3.00 (s, 3 H), 3.53 (d, 1 H, J = 24), 3.56 (d, 1 H, J = 24), 3.74 (q, 1 H, J = 7.5), 4.18 (m, 4 H).

7 (R_3, R_4 = $(CH_2)_3$): ^{1}H NMR ($CDCl_3$) δ 1.33 (t, 6 H, J = 7.5), 1.54 (m, 1 H), 2.17 (m, 4 H), 3.29 (m, 1 H), 3.52 (d, 1 H, J = 24), 3.57 (d, 1 H, J = 24), 3.72 (m, 1 H), 4.00 (dd, 1 H, J = 6, 10), 4.18 (m, 4 H); mass spectrum, m/z 317 (parent); exact mass calcd for $C_{13}H_{20}NO_6P$ 317.1028, found 317.1030.

Ethyl 3-Naphth-2-ylmethacrylate. Under a nitrogen atmosphere, in a round-bottom flask were placed 3.65 g (23.4 mmol) of 2-naphthaldehyde and 125 mL of benzene. To the system was added 10.2 g (28.1 mmol) of (carbethoxyethylidene)triphenylphosphorane, and the reaction mixture was stirred at room temperature for 4.5 h and concentrated with a rotary evaporator. The crude product was purified by column chromatography (300 g of silica gel) using 1:5 ether/hexanes as the eluant to obtain 5.65 g (100% yield) of ethyl 3-naphth-2-ylmethacrylate as a solid: mp 42–44 °C; ^{1}H NMR ($CDCl_3$) δ 1.38 (t, 3 H, J = 7.5), 2.21 (d, 3 H, J = 1), 4.31 (q, 2 H, J = 7.5), 7.51 (m, 3 H), 7.85 (m, 5 H); mass spectrum, m/z 240 (parent). Anal. ($C_{16}H_{16}O_2$) C, H.

2-Methyl-3-naphth-2-ylprop-2-en-1-ol. Under a nitrogen atmosphere, in a round-bottom flask were placed 4.93 g (20.5 mmol) of the α,β-unsaturated ester prepared above and 10 mL of CH_2Cl_2. To the system, at 0 °C, was added 44.2 mL (44.2 mmol) of 1 M diisobutylaluminum hydride (Dibal) in CH_2Cl_2, and the reaction mixture was stirred at 0 °C for 30 min. To the system was added a mixture of 6.9 mL of methanol and 108 mL of ether, and the mixture was stirred at 0 °C for 5 min and at room temperature for 1 h. The solids were removed by suction filtration through a Celite pad and rinsed well with ether. The filtrate was concentrated with a rotary evaporator, and the crude product was purified by column chromatography to obtain 1.8 g of pure 2-methyl-3-naphth-2-ylprop-2-en-1-ol as a crystalline solid: mp 85–86 °C; ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 3 H), 4.24 (d, 2 H, J = 2), 6.68 (s, 1 H), 7.45 (m, 3 H), 7.78 (s, 1 H), 7.81 (m, 3 H); mass spectrum m/z 198 (parent).

2-Methyl-3-naphth-2-ylprop-2-enal (6; R_1 = naphth-2-yl, R_2 = CH_3). Under a nitrogen atmosphere, in a round-bottom flask were placed 1.20 mL (13.8 mmol) of oxalyl chloride and 30 mL of CH_2Cl_2. To the system, at −70 °C, was added a solution of 1.67 mL (23.6 mmol) of DMSO in 6 mL of CH_2Cl_2, and the mixture was stirred at −70 °C for 2 min. To the system was added 1.95 g (9.84 mmol) of the allylic alcohol prepared above, and the reaction mixture was stirred at −70 °C for 30 min. To the system was added 4.94 (49 mmol) of triethylamine, and the mixture was stirred at −70 °C for 10 min and at room temperature for 30 min. The reaction mixture was diluted with 250 mL of CH_2Cl_2 and washed with 500 mL of 1 M aqueous $NaHCO_3$ and 250 mL of brine, dried, and concentrated to afford 1.90 g of crude product. The crude material was purified by column chromatography (100 g of silica gel) using 1:2 ether/hexanes as the eluant to obtain 1.77 g (91% yield) of pure aldehyde **6** (R_1 = 2-naphthyl, R_2 = CH_3) as a solid: mp 91–93 °C; ^{1}H NMR ($CDCl_3$) δ 1.98 (d, 3 H, J = 1), 7.42 (s, 1 H), 7.54 (m, 2 H), 7.64 (dd, 1 H, J = 2,9), 7.88 (m, 3 H), 8.02 (s, 1 H), 9.65 (s, 1 H).

2,4-Dioxo-1-methyl-3-(4-methyl-5-naphth-2-ylpenta-2,4-dienoyl)pyrrolidine (8W). Under a nitrogen atmosphere, in a round-bottom flask were placed 500 mg (1.72 mmol) of the keto phosphonate **7** (R_3 = CH_3, R_4 = H) and 1.75 mL of THF. To the system, at 0 °C, was added 405 mg (3.61 mmol) of potassium *tert*-butoxide, the mixture was stirred at 0 °C for 30 min, and 350 mg (1.8 mmol) of **6** (R_1 = 2-naphthyl, R_2 = CH_3) was added to the system. The reaction mixture was stirred at 0 °C for 1 min and partitioned between 25 mL of 1 M aqueous phosphoric acid and ca. 50 mL of CH_2Cl_2. The layers were separated, and the organic phase was washed with brine, dried (Na_2SO_4), and concentrated to afford 650 mg of crude material. The crude product was purified by column chromatography (125 g of silica gel) using 3:97 methanol/chloroform as the eluant to obtain 225 mg of pure **8W** as a yellow solid: mp 182 °C; ^{1}H NMR[17] ($CDCl_3$) δ 2.28 (d, 3 H, J = 1), 3.06 (s, 3 H), 3.77 (s, 2 H), 7.12 (s, 1 H), 7.33 (d, 1 H, J = 15), 7.51 (m, 3 H), 7.74 (d, 1 H, J = 15), 7.84 (m, 4 H); mass spectrum, m/z 333 (parent); exact mass calcd for $C_{21}H_{19}NO_3$ 333.1365, found 333.1365. Anal. ($C_{21}H_{19}NO_3 \cdot {}^1/_2H_2O$) C, H, N.

Ethyl 3-Quinolin-4-ylmethacrylate. Under a nitrogen atmosphere, in a round-bottom flask were placed 1.00 g (6.37 mmol) of 4-quinolinecarboxaldehyde and 2 mL of benzene. To the system was added 2.77 g (7.64 mmol) of (carbethoxyethylidene)triphenylphosphorane, and the reaction mixture was stirred at room temperature for 0.5 h and concentrated with a rotary evaporator. The crude product was purified by column chromatography (50 g of silica gel) using 1:1 ether/hexanes as the eluant to obtain 1.43 g (93% yield) of pure ethyl 3-quinolin-4-ylmethacrylate as an oil: ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3 H, J = 7.5), 1.97 (d, 3 H, J = 1), 4.35 (q, 2 H, J = 7.5), 7.27 (m, 1 H), 7.5 (dt, 1 H, J = 1, 8), 7.76 (dt, 1 H, J = 1, 8), 7.92 (d, 1 H, J = 9), 8.07 (s, 1 H), 8.15 (d, 1 H, J = 9), 8.94 (d, 1 H, J = 5); mass spectrum, m/z 241. Anal. ($C_{15}H_{15}NO_2$) C, H, N.

2-Methyl-3-quinolin-4-ylprop-2-en-1-ol. Under a nitrogen atmosphere, in a round-bottom flask were placed 1.42 g (5.89 mmol) of the quinoline α,β-unsaturated ester prepared above and 3.0 mL of CH_2Cl_2. To this stirring solution, at −50 °C, was added 12.7 mL (12.7 mmol) of 1 M Dibal/hexanes. After 0.5 h, additional 1 M Dibal/hexanes (3 mL, 3 mmol) was added to the system, and the reaction mixture was stirred at −50 °C for 15 min. To the system were added cautiously 1.15 mL of methanol and 1.9 mL of water. The mixture was stirred at −50 °C for 2 min, diluted with 100 mL of ether, stirred at −50 °C for 5 min, and stirred at room temperature for 1 h. Sodium sulfate was added to the mixture, the mixture was stirred at room temperature for 1 h, and the solids were removed by suction filtration. The filtrate was concentrated with a rotary evaporator to obtain 1.33 g of a yellow oil. The crude product was subjected to column chromatography (25 g of silica gel) using 2.5:1 ether/hexanes as the eluant to obtain 0.95 g (81% yield) of pure 2-methyl-3-quinolin-4-ylprop-2-en-1-ol as a crystalline solid: mp 104–106 °C; ^{1}H NMR ($CDCl_3$) δ 1.76 (d, 3 H, J = 1), 4.36 (s, 2 H), 6.98 (br s, 1 H), 7.22 (d, 1 H, J = 5), 7.52 (m, 1 H), 7.71 (m, 1 H), 7.96 (dd, 1 H, J = 1, 9), 8.13 (d, 1 H, J = 9), 8.86 (d, 1 H, J = 4); mass spectrum, m/z 199 (parent). Anal. ($C_{13}H_{13}NO$) C, H, N.

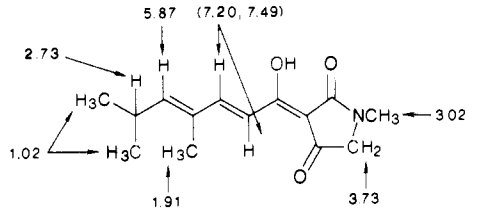
2-Methyl-3-quinolin-4-ylprop-2-enal (6; R_1 = quinolin-4-yl, R_2 = CH_3). Under a nitrogen atmosphere, in a round-bottom flask were placed 0.57 mL (6.54 mmol) of oxalyl chloride and 8.0 mL of CH_2Cl_2. To this stirring solution, at −60 °C, was added 0.8 mL (11.2 mmol) of DMSO in 0.2 mL of CH_2Cl_2, and the mixture was stirred at −60 °C for 2 min. To the system was added 0.93 g (4.67 mmol) of the quinoline allylic alcohol prepared above (as a solid, 3 mL of CH_2Cl_2 rinse), and the reaction mixture was stirred at −60 °C for 45 min. To the system was added 3.25 mL (23.4 mmol) of triethylamine, and the mixture was stirred at −60 °C for 2 min and at room temperature for 45 min. The mixture was diluted with 50 mL of ether, washed with two 20-mL portions of 1 M aqueous phosphoric acid and 20 mL of brine, dried (Na_2SO_4), and concentrated. The acidic washes were made basic with solid $NaHCO_3$ and extracted with organic solvent. The extract was washed with brine, dried, and concentrated. The crude concentrates were subjected to column chromatography (20 g of silica gel) using 2:1 ether/hexanes as the eluant to obtain pure **6** (R_1 = quinolin-4-yl, R_2 = CH_3) as a crystalline solid: mp 91–94 °C; ^{1}H NMR ($CDCl_3$) δ 1.93 (d, 3 H, J = 1), 7.33 (dd, 1 H, J = 1, 4), 7.62 (m, 1 H), 7.79 (m, 1 H), 7.82 (m, 1 H), 7.92 (dd, 1 H, J = 1,

(17) As has been discussed in detail previously,[18-21] several tautomers of tetramic acids may exist in solution, and these populations may be observed in the NMR spectra of these compounds. In our series of compounds, we generally observed one major tautomer accompanied by a minor tautomeric component (approximately 10%), which was most apparent by observation of the signals in the ^{1}H NMR spectrum for the *N*-methyl and/or methylene substituents on the tetramic acid nucleus. For example, these minor peaks were present in the spectrum of **8Y** at δ 3.04 (N-CH_3) and δ 3.87 (CH_2).

9), 8.18 (d, 1 H, J = 9), 8.98 (d, 1 H, J = 5), 9.82 (s, 1 H); mass spectrum, m/z 197 (parent). Anal. ($C_{13}H_{11}NO$) C, H, N.

2,4-Dioxo-methyl-3-(4-methyl-5-quinolin-4-ylpenta-2,4-dienoyl)pyrrolidine (8AA). Under a nitrogen atmosphere, in a round-bottom flask were placed 137 mg (0.47 mmol) of the keto phosphonate **7** (R_3 = CH_3, R_4 = H) and 0.47 mL of THF. To this stirring solution, at 0 °C, was added 111 mg (0.99 mmol) of potassium *tert*-butoxide, and the mixture was stirred at 0 °C for 40 min. To the system was added 91 mg (0.46 mmol) of **6** (R_1 = quinolin-4-yl, R_2 = CH_3), and the reaction mixture was stirred at 0 °C for 15 min. The reaction mixture was partitioned between CH_2Cl_2 and 1 M aqueous phosphoric acid. The layers were separated, and the aqueous phase was washed with CH_2Cl_2. The aqueous phase was made neutral with solid $NaHCO_3$, extracted with dichloromethane, made basic with solid $NaHCO_3$, and extracted with dichloromethane. The combined dichloromethane fractions were dried (Na_2SO_4) and concentrated (rotary evaporator) to afford ca. 120 mg of a yellow solid which was rinsed with several portions of ether (ca. 35 mL total) to give pure **8AA**:[22] ^{1}H NMR ($CDCl_3$)[17] δ 2.08 (d, 3 H, J = 1), 3.08 (s, 3 H), 3.78 (s, 2 H), 7.28 (d, 1 H, J = 5), 7.38 (m, 2 H), 7.58 (m, 1 H), 7.78 (m, 2 H), 7.92 (dd, 1 H, J = 1, 9), 8.16 (d, 1 H, J = 9), 8.94 (d, 1 H, J = 5); mass spectrum, m/z 334 (parent); exact mass calcd for $C_{20}H_{18}N_2O_3$ 334.1317, found 334.1318. Anal. ($C_{20}H_{18}N_2O_3$) C, H, N.

^{1}H NMR Spectral Data Tetramic Acids 8.[17] **A**: mp 116–119 °C; ^{1}H NMR ($CDCl_3$) δ 1.02 (d, 6 H, J = 7), 1.91 (d, 3 H, J = 1), 2.73 (m, 1 H), 3.02 (s, 3 H), 3.73 (s, 2 H), 5.87 (d, 1 H, J = 10), 7.20 (d, 1 H, J = 16), 7.49 (d, 1 H, J = 16).



B: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3 H, J = 7), 1.2–2.25 (complex), 1.87 (s, 3 H), 3.03 (s, 3 H), 3.73 (s, 2 H), 6.12 (t, 1 H, J = 8), 7.09 (d, 1 H, J = 16), 7.52 (d, 1 H, J = 16).

C: mp 153–155 °C; ^{1}H NMR ($CDCl_3$) δ 1.22 (m, 5 H), 1.68 (m, 5 H), 1.89 (d, 3 H, J = 1), 2.41 (m, 1 H), 3.02 (s, 3 H), 3.72 (s, 2 H), 5.78 (d, 1 H, J = 9), 7.08 (d, 1 H, J = 16), 7.48 (d, 1 H, J = 16).

D: mp 122–124 °C; ^{1}H NMR ($CDCl_3$) δ 0.9–1.73 (complex), 1.88 (s, 3 H), 2.14 (t, 2 H, J = 7.5), 3.03 (s, 3 H), 3.72 (s, 2 H), 6.09 (t, 1 H, J = 7.5), 7.08 (d, 1 H, J = 16), 7.52 (d, 1 H, J = 16).

E: ^{1}H NMR ($CDCl_3$) δ 0.84–1.83 (complex), 0.97 (d, 3 H, J = 7), 2.38 (m, 1 H), 3.03 (s, 3 H), 3.77 (s, 2 H), 5.91 (d, 1 H, J = 10), 7.08 (d, 1 H, J = 16), 7.53 (d, 1 H, J = 16).

F: mp 146–148 °C; ^{1}H NMR ($CDCl_3$) δ 1.53 (m, 5 H), 1.89 (m, 1 H), 1.92 (d, 3 H, J = 1), 3.03 (s, 3 H), 3.51 (m, 1 H), 3.72 (s, 2 H), 4.01 (m, 1 H), 4.21 (m, 1 H), 5.95 (d, 1 H, J = 8), 7.18 (d, 1 H, J = 16), 7.48 (d, 1 H, J = 16).

G: mp 215–220 °C; ^{1}H NMR ($CDCl_3$) δ 3.03 (s, 9 H), 3.74 (s, 2 H), 6.67 (d, 2 H, J = 9), 6.90 (m, 2 H), 7.18 (d, 1 H, J = 16), 7.41 (d, 2 H, J = 9), 7.67 (dd, 1 H, J = 11, 16).

H: ^{1}H NMR ($CDCl_3$) δ 3.07 (s, 3 H), 3.78 (s, 2 H), 7.03 (dd, 1 H, J = 11, 16), 7.36 (d, 1 H, J = 16), 7.45–7.75 (complex, 5 H), 8.00 (d, 1 H, J = 10).

J: ^{1}H NMR ($CDCl_3$) δ 3.06 (s, 3 H), 3.76 (s, 2 H), 7.02 (m, 2 H), 7.35 (m, 4 H), 7.49 (m, 2 H), 7.64 (dd, 1 H, J = 10, 16).

L: mp 192–195 °C; ^{1}H NMR ($CDCl_3$) δ 3.06 (s, 3 H), 3.78 (s, 2 H), 7.39 (m, 5 H), 7.65 (s, 1 H), 7.84 (m, 2 H).

M: ^{1}H NMR (DMSO-d_6) δ 3.81 (s, 2 H), 7.47 (m, 5 H), 7.66 (s, 1 H), 7.77 (d, 1 H, J = 15), 7.86 (d, 1 H, J = 8).

O: mp 117–120 °C; ^{1}H NMR (DMSO-d_6) δ 2.09 (s, 3 H), 3.76 (s, 2 H), 7.19 (s, 1 H), 7.4 (m, 6 H), 7.66 (d, 1 H, J = 16).

P: mp 82–85 °C; ^{1}H NMR ($CDCl_3$) δ 2.19 (d, 3 H, J = 1), 3.06 (s, 3 H), 3.77 (s, 2 H), 6.97 (s, 1 H), 7.35 (m, 6 H), 7.64 (d, 1 H, J = 16).

Q: ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3 H, J = 7.5), 1.42 (m, 4 H), 1.63 (m, 2 H), 2.58 (m, 2 H), 3.06 (s, 3 H), 3.76 (s, 2 H), 6.92 (s, 1 H), 7.34 (m, 6 H), 7.60 (d, 1 H, J = 16).

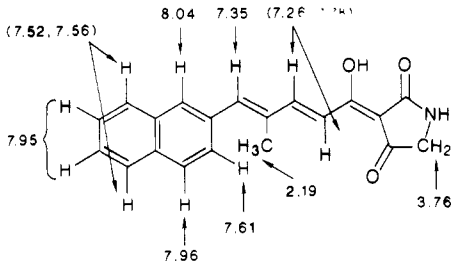
R: mp 171–173 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (d, 3 H, J = 7), 1.98 (s, 3 H), 3.02 (s, 3 H), 3.72 (s, 2 H), 3.88 (m, 1 H), 6.14 (d, 1 H, J = 9), 7.12 (d, 1 H, J = 16), 7.25 (m, 5 H), 7.50 (d, 1 H, J = 16).

S: mp 206–208 °C; ^{1}H NMR ($CDCl_3$) δ 2.45 (d, 3 H, J = 1), 3.07 (s, 3 H), 3.70 (s, 2 H), 6.90 (s, 1 H), 7.20 (m, 1 H), 7.35 (m, 2 H), 7.70 (m, 3 H).

T: mp 226 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.99 (s, 3 H), 3.79 (s, 2 H), 7.2–8.2 (complex).

U: mp 161–163 °C; ^{1}H NMR ($CDCl_3$) δ 2.07 (d, 3 H, J = 1), 3.07 (s, 3 H), 3.77 (s, 2 H), 7.38 (d, 1 H, J = 16), 7.5 (m, 5 H), 7.85 (d, 1 H, J = 16), 7.9 (m, 3 H).

V: mp 226 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.19 (s, 3 H), 3.76 (s, 2 H), 7.26 (d, 1 H, J = 16), 7.35 (s, 1 H), 7.52 (d, 1 H, J = 4), 7.56 (d, 1 H, J = 4), 7.61 (dd, 1 H, J = 1, 9), 7.78 (d, 1 H, J = 16), 7.95 (m, 2 H), 7.96 (d, 1 H, J = 9), 8.04 (s, 1 H).



W: see experimental procedures.

X: mp 191–193 °C; ^{1}H NMR ($CDCl_3$) δ 2.11 (d, 3 H, J = 1), 3.06 (s, 3 H), 3.76 (s, 2 H), 4.04 (s, 3 H), 6.86 (d, 1 H, J = 9), 7.30 (d, 1 H, J = 16), 7.38 (d, 1 H, J = 8), 7.46 (s, 1 H), 7.53 (m, 2 H), 7.86 (d, 1 H, J = 16), 7.92 (m, 1 H), 8.32 (m, 1 H).

Y: mp 195–197 °C; ^{1}H NMR ($CDCl_3$) δ 2.07 (d, 3 H, J = 1), 3.07 (s, 3 H), 3.77 (s, 2 H), 7.17 (dd, 1 H, J = 8, 11), 7.33 (d, 1 H, J = 16), 7.35 (m, 1 H), 7.41 (s, 1 H), 7.58 (m, 2 H), 7.82 (d, 1 H, J = 16), 7.93 (m, 1 H), 8.15 (m, 1 H).

Z: mp 210–212 °C; ^{1}H NMR ($CDCl_3$) δ 2.22 (s, 3 H), 3.04 (s, 3 H), 3.76 (s, 2 H), 6.99 (s, 1 H), 7.11 (d, 1 H, J = 16), 7.35–7.67 (m, 4 H), 7.62 (m, 5 H), 7.72 (d, 1 H, J = 16).

AA: see experimental procedures.

BB: mp 207–209 °C; ^{1}H NMR ($CDCl_3$) δ 2.28 (d, 3 H, J = 1), 3.06 (s, 3 H), 3.78 (s, 2 H), 7.08 (s, 1 H), 7.38 (d, 1 H, J = 16), 7.59 (m, 1 H), 7.73 (d, 1 H, J = 16), 7.75 (m, 1 H), 7.87 (d, 1 H, J = 9), 8.13 (d, 1 H, J = 9), 8.19 (m, 1 H), 8.92 (d, 1 H, J = 2).

CC: mp 130–132 °C; ^{1}H NMR ($CDCl_3$) δ 1.64 (m, 2 H), 1.97 (d, 3 H, J = 1), 2.62 (dd, 1 H, J = 12, 18), 2.88 (m, 4 H), 3.02 (s, 3 H), 3.73 (s, 2 H), 6.01 (d, 1 H, J = 9), 7.10 (m, 5 H), 7.53 (d, 1 H, J = 16).

DD: mp 172–174 °C; ^{1}H NMR ($CDCl_3$) δ 1.59 (m, 1 H), 2.3 (m, 3 H), 2.28 (s, 3 H), 3.30 (m, 1 H), 3.78 (m, 1 H), 4.01 (dd, 1 H, J = 10), 7.12 (s, 1 H), 7.34 (d, 1 H, J = 16), 7.52 (m, 3 H), 7.76 (dd, 1 H, J = 1, 16), 7.85 (m, 4 H).

EE: mp 162–164 °C; ^{1}H NMR ($CDCl_3$) δ 1.38 (d, 3 H, J = 7), 2.26 (d, 3 H, J = 1), 3.02 (s, 3 H), 3.74 (q, 1 H, J = 7), 7.11 (s, 1 H), 7.34 (d, 1 H, J = 16), 7.50 (m, 3 H), 7.73 (d, 1 H, J = 16), 7.85 (m, 4 H).

FF: mp 178–180 °C; ^{1}H NMR ($CDCl_3$) δ 1.92 (d, 3 H, J = 1), 3.08 (s, 3 H), 3.78 (s, 2 H), 7.14 (s, 1 H), 7.21 (d, 1 H, J = 10), 7.35 (d, 1 H, J = 16), 7.41 (m, 1 H), 7.46 (m, 1 H), 7.61 (d, 1 H, J = 9), 7.79 (t, 2 H, J = 8), 7.86 (d, 1 H, J = 16).

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(22) We have found that trituration of the crude products with ether is generally superior to chromatographic purification. In our early studies, compounds that were homogeneous by TLC analysis (after silica gel column chromatography) occasionally gave broadened signals in their NMR spectra; dissolving the compound in solvent and washing with dilute aqueous phosphoric acid subsequently afforded material that gave sharp signals in its NMR spectrum. We speculate that this line broadening may be related to inadvertent exposure of the tetramic acid to trace quantities of chelatable species during the chromatography.

GG: mp 116–118 °C; ^{1}H NMR ($CDCl_3$) δ 1.82 (s, 3 H), 3.07 (s, 3 H), 3.37 (s, 3 H), 3.54 (m, 2 H), 3.77 (s, 2 H), 3.84 (m, 2 H), 5.35 (s, 2 H), 7.21 (s, 1 H), 7.32 (d, 1 H, J = 16), 7.43 (m, 2 H), 7.51 (d, 1 H, J = 9), 7.69 (d, 1 H, J = 9), 7.87 (m, 3 H).

Microbiological Evaluation of Compounds. The minimum inhibitory concentrations (MICs) were determined by using the 2-fold dilution method. The aerobic MIC values were determined on brain–heart infusion agar, and the anaerobic MIC values were determined with Wilkens-Chalgren broth. All values are expressed in micrograms/milliliter.

DNA Gyrase Supercoiling Inhibition Assay. The DNA gyrase supercoiling inhibition assay was performed by an agarose gel electrophoresis technique in a manner similar to that described previously.[12] Test compounds were dissolved in methanol (100%). The final concentration of methanol in each reaction mixture, including the control tube without the drug, was 12.5%. Methanol, at this concentration, does not affect the enzyme activity nor change the inhibitory potency of the norfloxacin control.

Registry No. **6** (R_1 = i-C_3H_7; R_2 = CH_3), 57691-99-3; **6** (R_1 = n-$C_{11}H_{23}$; R_2 = CH_3), 118895-40-2; **6** (R_1 = C_6H_{11}; R_2 = CH_3), 100764-16-7; **6** (R_1 = $C_6H_{11}CH_2$; R_2 = CH_3), 96227-85-9; **6** (R_1 = $C_6H_{11}CH(CH_3)$; R_2 = CH_3), 118895-41-3; **6** (R_1 = tetrahydropyran-2-yl; R_2 = CH_3), 118895-42-4; **6** (R_1 = p-$(CH_3)_2NC_6H_4$; R_2 = H), 20432-35-3; **6** (R_1 = o-$NO_2C_6H_4$; R_2 = H), 66894-06-2; **6** (R_1 = C_6H_5; R_2 = H), 14371-10-9; **6** (R_1 = C_6H_5; R_2 = Br), 33603-90-6; **6** (R_1 = C_6H_5; R_2 = Cl), 33603-89-3; **6** (R_1 = C_6H_5; R_2 = CH_3), 15174-47-7; **6** (R_1 = C_6H_5; R_2 = n-C_5H_{11}), 78605-96-6; **6** (R_1 = $C_6H_5CH(CH_3)$; R_2 = CH_3), 118895-41-3; **6** (R_1 = 2-C_5H_4N; R_2 = CH_3), 75102-17-9; **6** (R_1 = 1-$C_{10}H_7$; R_2 = CH_3), 75102-13-5; **6** (R_1 = 2-$C_{10}H_7$; R_2 = CH_3), 118895-36-6; **6** (R_1 = p-MeO-1-$C_{10}H_6$; R_2 = CH_3), 118895-43-5; **6** (R_1 = p-F-1-$C_{10}H_6$; R_2 = CH_3), 118895-44-6; **6** (R_1 = p-$C_6H_5C_6H_4$; R_2 = CH_3), 75102-04-4; **6** (R_1 = quinolin-4-yl; R_2 = CH_3), 118895-39-9; **6** (R_1 = quinolin-3-yl; R_2 = CH_3), 118895-45-7; **6** (R_1 = 1,2,3,4-tetrahydronaphthalen-2-yl; R_2 = CH_3), 118895-46-8; **6** (R_1 = o-HO-1-$C_{10}H_6$; R_2 = CH_3), 118895-47-9; **6** (R_1 = o-MEMO-1-$C_{10}H_6$; R_2 = CH_3), 118895-48-0; **7** (R_3 = CH_3; R_4 = H), 118895-32-2; **7** (R_3 = R_4 = CH_3), 118920-49-3; **7** (R_3, R_4 = $(CH_2)_3$), 118895-33-3; **7** (R_3 = 2,4-dimethoxybenzyl; R_4 = H), 95218-33-0; **8A**, 118894-99-8; **8AA**, 118895-24-2; **8B**, 118895-00-4; **8BB**, 118895-25-3; **8C**, 118895-01-5; **8CC**, 118895-26-4; **8D**, 118895-02-6; **8DD**, 118895-27-5; **8E**, 118895-03-7; **8EE**, 118895-28-6; **8F**, 118895-04-8; **8FF**, 118895-29-7; **8G**, 118895-05-9; **8GG**, 118895-30-0; **8H**, 118895-06-0; **8I**, 118895-07-1; **8J**, 118895-08-2; **8K**, 118895-09-3; **8L**, 118895-10-6; **8M**, 118895-11-7; **8N**, 118895-12-8; **8O**, 118895-13-9; **8P**, 118895-14-0; **8Q**, 118895-15-1; **8R**, 118920-48-2; **8S**, 118895-16-2; **8T**, 118895-17-3; **8U**, 118895-18-4; **8V**, 118895-19-5; **8W**, 118895-20-8; **8X**, 118895-21-9; **8Y**, 118895-22-0; **8Z**, 118895-23-1; **9**, 52148-44-4; **11** (R_3 = CH_3; R_4 = H; R_5 = CH_2CH_3), 118895-31-1; diketene, 674-82-8; sarcosine ethyl ester, 13200-60-7; N-methyl-D-alanine ethyl ester, 118895-34-4; D,L-proline methyl ester, 52183-82-1; 2-naphthaldehyde, 66-99-9; (carbethoxyethylidene)triphenylphosphorane, 54356-04-6; ethyl 3-naphth-2-ylmethacrylate, 100510-32-5; 2-methyl-3-naphth-2-ylprop-2-en-1-ol, 118895-35-5; 4-quinolinecarboxaldehyde, 4363-93-3; ethyl 3-quinolin-4-ylmethacrylate, 118895-37-7; 2-methyl-3-quinolin-4-ylprop-2-en-1-ol, 118895-38-8; RNA polymerase, 9014-24-8.

Synthesis of 8-Substituted Derivatives of the 2-Deoxy Analogue of 3-Deoxy-β-D-*manno*-2-octulopyranosonic Acid (2-Deoxy-β-KDO) as Inhibitors of 3-Deoxy-D-*manno*-octulosonate Cytidylyltransferase[1]

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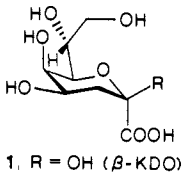
Received March 4, 1988

The 2-deoxy analogue of 3-deoxy-β-D-*manno*-2-octulopyranosonic acid (2-deoxy-β-KDO, **2**) is a potent inhibitor of the enzyme 3-deoxy-D-*manno*-octulosonate cytidylyltransferase, which is involved in the biosynthesis of lipopolysaccharide, an essential component of the outer membrane of Gram-negative bacteria. Since compound **2** lacks antibacterial activity, a series of 8-substituted derivatives of **2** has been synthesized in an attempt to find enzyme inhibitors suitable for modification as antibacterials. Compounds **9**, 11, and **13**, in which the 8-hydroxy group of **2** is replaced by F, H, and NH_2, respectively, were as potent inhibitors of the enzyme as **2**, but were devoid of antibacterial activity, with the exception of the amino acid **13**, which showed weak activity against some strains of *Salmonella typhimurium*.

Recent work in these laboratories aimed at finding novel antibacterial agents has been focused on the design and synthesis of potential inhibitors of the biosynthesis of the lipopolysaccharide (LPS) of Gram-negative bacteria.[2-4] Enzymes involved in the incorporation of the sugar 3-deoxy-D-*manno*-2-octulosonic acid[5] (KDO, **1**) into LPS have been considered as attractive targets since bacterial mutants defective in KDO biosynthesis are not viable.[6]



1, R = OH (β-KDO)
2, R = H

KDO provides the link between the hydrophilic polysaccharide part of LPS and the hydrophobic lipid A moiety, an acylated and phosphorylated β-(1→6)-linked glucosamine disaccharide which is embedded in the outer bacterial membrane.[7] The enzyme 3-deoxy-D-*manno*-octulosonate cytidylyltransferase (CMP-KDO synthetase; EC 2.7.7.38) converts KDO (**1**) to the nucleotide sugar cytidine 5′-monophosphate KDO (CMP-KDO), which in turn serves as substrate for a series of KDO-lipid A

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