supernatant with distilled water so that 0.2 mL of supernatant gave an average reaction rate for the control reaction of 0.0100 + 0.0010 absorbance units per min. The effects of the inhibitors on enzyme activity were determined by including 0.2 mL of an aqueous solution of the inhibitor at the desired concentration in the reaction mixture. Each compound was tested at least three different concentrations with a minimum of two determinations per concentration. The percent inhibition for each compound at all concentrations was then calculated by comparing the reaction rate of the solutions containing inhibitor to that of control reactions with no inhibitor and log dose–response curves constructed. Inhibitor IC_{50} values were then obtained by least-squares analyses of the linear portions of the log dose–response curves with use of the LINEFIT linear regression program of Barlow.[19]

Kinetic studies were performed with four concentrations (10, 5.0, 2.5, and 1.0 μM) of inhibitor **5m**. For these studies, the concentrations of the substrate DL-glyceraldehyde were varied (1.25, 0.625, 0.313, 0.156, 0.078 mM) while inhibitor and cofactor concentrations (0.104 mM) were held constant. The nature of inhibition produced by each concentration of **5m** was then determined by analyzing double-reciprocal plots of enzyme velocity versus glyceraldehyde concentration. The double reciprocal plots were generated by least-squares fit of the data using the LINEFIT program of Barlow.[19]

Acknowledgment. This research was supported in part by a Faculty Research Grant from the University of Mississippi, by the Research Institute of Pharmaceutical Sciences, and by a grant from the Vicksburg Hospital Medical Foundation. The technical assistance provided by R. L. Peden, G. M. Worsham, B. E. Swearingen, Kevin Kerr, Jackie Johnson, and Tina Artale is gratefully acknowledged.

Registry No. **1a**, 5398-96-9; **1b**, 1080-44-0; **1c**, 13029-74-8; **1d**, 13514-59-5; **1e**, 109065-68-1; **1f**, 5616-30-8; **1g**, 13029-71-5; **1h**, 13029-72-6; **1i**, 15054-42-9; **1j**, 15054-44-1; **1k**, 92740-48-2; **1l**, 109065-69-2; **5a**, 59724-82-2; **5b**, 80271-12-1; **5c**, 117309-27-0; **5d**, 117309-28-1; **5e**, 117309-29-2; **5f**, 92192-48-8; **5g**, 117309-30-5; **5h**, 92290-91-0; **5i**, 117309-31-6; **5j**, 117309-32-7; **5k**, 117309-33-8; **5l**, 117309-34-9; **5m**, 117309-35-0; **5n**, 117309-36-1; **5o**, 117309-37-2; **5p**, 117309-38-3; **5q**, 117309-39-4; **5r**, 117309-40-7; **5s**, 117309-41-8; **5t**, 117309-42-9; **5u**, 117309-43-0; **5v**, 117309-44-1; **5w**, 117309-45-2; **6a**, 96686-11-2; **6b**, 105441-57-4; **6c**, 60712-47-2; **6d**, 111524-98-2; **6e**, 96686-12-3; **6f**, 117309-46-3; **6g**, 96686-13-4; **6h**, 117309-47-4; **6i**, 117309-48-5; **6j**, 117309-49-6; **6k**, 117309-50-9; **6l**, 117309-51-0; **6m**, 96686-17-8; **6n**, 117406-01-6; **7a**, 34837-67-7; **7b**, 6311-23-5; **7c**, 38957-44-7; **7d**, 530-73-4; **7e**, 51012-31-8; **7f**, 51012-29-4; **7g**, 38957-45-8; **7h**, 117309-52-1; **7i**, 117309-53-2; **7j**, 117309-54-3; **7k**, 117309-55-4; **7l**, 117309-56-5; **14a**, 117309-57-6; **14b**, 117309-58-7; **14i**, 117309-59-8; **14j**, 117309-60-1; $PhSO_2Cl$, 98-09-9; 4-$MeC_6H_4SO_2Cl$, 98-59-9; 4-$MeOC_6H_4SO_2Cl$, 98-68-0; 4-$ClC_6H_4SO_2Cl$, 98-60-2; 4-$FC_6H_4SO_2Cl$, 349-88-2; 4-$O_2NC_6H_4SO_2Cl$, 98-74-8; 2-naphthalenesulfonyl chloride, 93-11-8; (*R*)-2-phenylglycine, 875-74-1; (*S*)-2-phenylglycine, 2935-35-5; (*R*)-α-methylbenzylamine, 3886-69-9; aldose reductase, 9028-31-3.

Synthesis and Activity of Nonhydrolyzable Pseudomonic Acid Analogues

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Several series of pseudomonic acid analogues have been prepared that incorporate modified functionalities in place of the C1–C3 α,β-unsaturated ester group. The inhibition of isoleucyl-*t*RNA synthetase and the in vitro activity of these compounds against various Gram-positive and Gram-negative strains are described. Several derivatives showed enzyme inhibition equivalent to or better than that of methyl pseudomonate (**3**), while lacking the hydrolyzable ester group at C1. These analogues include the corresponding phenyl ketone and the ether **12**. The long-chain ketone **24** exhibited similar in vitro activity as the parent ester.

Pseudomonic acid A, **1**, is a novel Gram-positive antibiotic that was isolated in 1971 from *Pseudomona fluorescens*.[1] Its structure was determined[2] in 1977, and a year later the absolute stereochemistry was defined by Alexander.[3] In 1982 Beecham Co. marketed the sodium salt **2** as a topical agent under the trade name Bactroban.[4] Studies of the in vivo efficacy of this antibiotic showed its short half-life was due to metabolic inactivation. The major metabolite was discovered to be monic acid A, **4** (Scheme I), which itself shows little antibacterial activity and is rapidly cleared in the urine.[5] Since that time several attempts to slow or halt the enzymatic hydrolysis by varying the structure and function of the C1–C3 fragment have been carried out, including preparation of the 2-halo and 2-alkyl derivatives and the formation of amides at C1.[6] Synthesis of the corresponding alkyl ketones by Beecham led to compounds retaining good in vitro activity.[7] We report in this paper our attempts to study the effect of several functional and structural modifications of the C1–C3 moiety on the enzyme inhibition and in vitro activity in hopes of deriving an in vivo active analogue of this antibiotic.

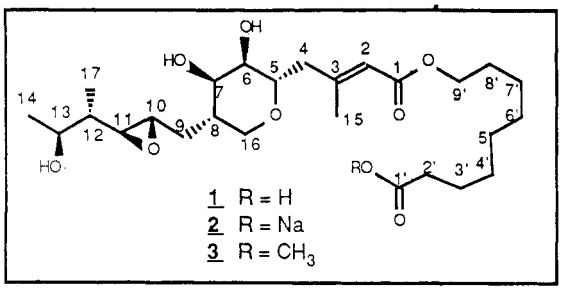
Chemistry

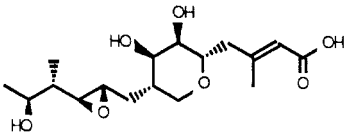
Initially our approach involved ascertaining the general structure–activity relationships of the various segments of pseudomonic acid. A description of standard substitution of the hydroxyl groups with hydrogen, fluoro, and amino groups will be published in due course. However, since the functionality paramount to activity is thought to be unsaturated grouping at C1–C3, we focused our efforts there, and this paper will disclose results from this study. Previous reports indicated a marked sensitivity to change of this molecular unit. For example, 1,2- or 1,4-reduction of this group produced the C2–C3 dihydro ester

(1) Fuller, A. T.; Mellows, G.; Woolford, M.; Banks, G. T.; Barrow, K. D.; Chain, E. B. *Nature (London)* **1971**, *234*, 416.
(2) Chain, E. B.; Mellows, G. *J. Chem. Soc., Perkin Trans. 1* **1977**, 294.
(3) Alexander, R. G.; Clayton, J. P.; Luk, K.; Rogers, N. H.; King, T. J. *J. Chem. Soc., Perkin Trans. 1* **1978**, 561.
(4) *Bactroban; Proceedings of an International Symposium*; Dobson, R. L., Leyden, J. J., Noble, W. C., Price, J. D., Eds.; Excerpta Medica: Amsterdam, The Netherlands, 1985.
(5) Reference 4, pp 7–8 (G. Mellows).
(6) (a) Crimmin, M. J.; O'Hanlon, P. J.; Rogers, N. H. *J. Chem. Soc., Perkin Trans. 1* **1985**, 549. (b) Amides: Rogers, N. H. U.S. Patent 4 200 635, Apr 1980 (Beecham Group Ltd.).
(7) Rogers, N. H.; Coulton, S. U.S. Patent 4 312 874, Jan 1982 (Beecham Group Ltd.).

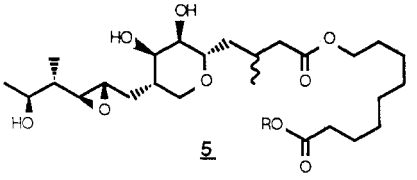
0022-2623/89/1832-0151$01.50/0

Scheme I





4



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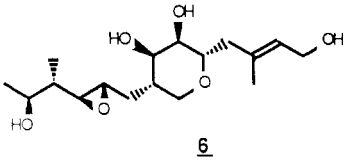

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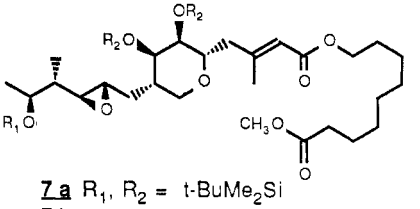
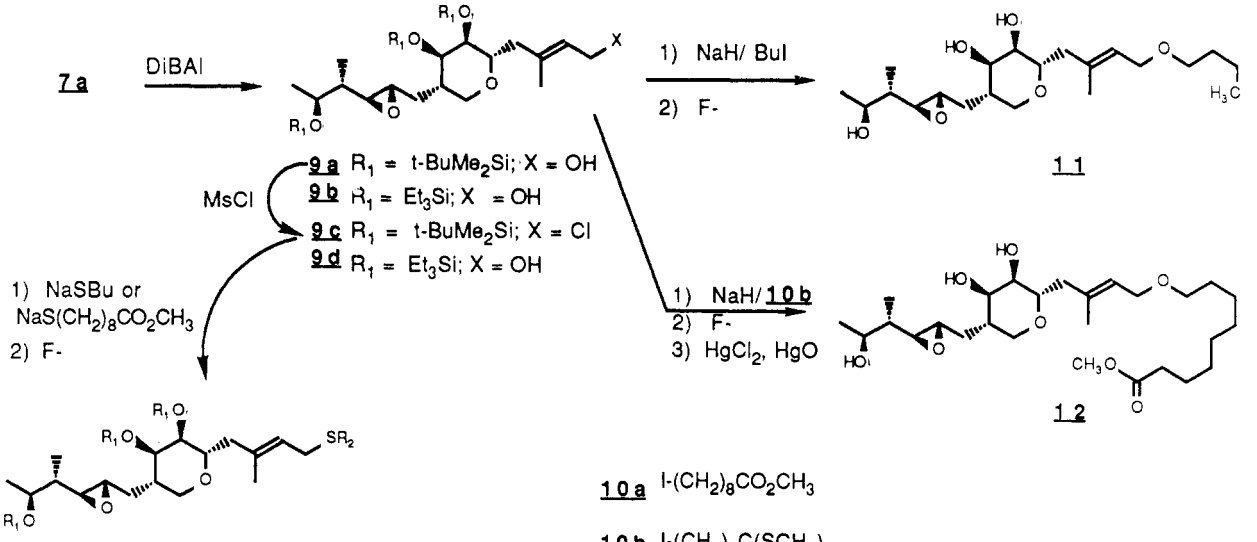


7a R_1, R_2 = t-$BuMe_2Si$
7b R_1, R_2 = Et_3Si
7c R_1, R_2 = Me_3Si
8 R_1 = H; R_2 = -C(O)-

Scheme II

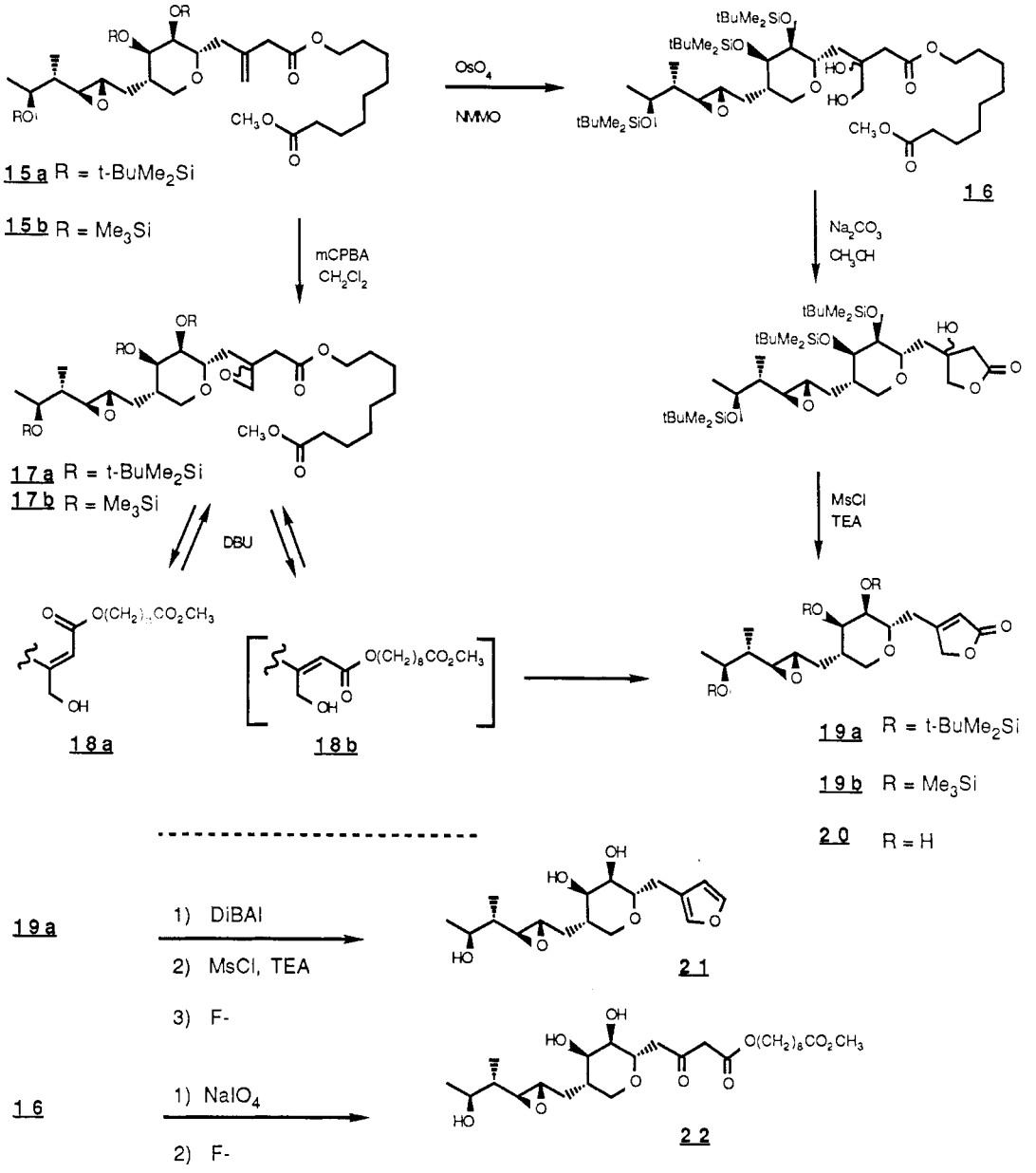


5[2] and the allylic alcohol 6,[8] respectively, and both were found to be inactive.

Pseudomonic acid A was isolated from fermentation broths of *Pseudomonas fluorescens*[9] and was methylated prior to purification. Since the ester **3** displayed enzyme inhibition and in vitro values similar to those of the parent acid, all the ensuing carboxyl analogues were retained as the corresponding methyl esters. The parent acid **1** and ester **3** had been shown to undergo an intramolecular epoxide opening process at certain pH levels ($4 > pH > 9$),[10] and so protection of the hydroxyl groups was in order. To allow for maximal choice of reaction conditions we required other protecting groups in addition to the trimethylsilyl group that had been employed in earlier chemistry. The tris(*tert*-butyldimethylsilyl), tris(triethylsilyl), and the tris(trimethylsilyl) methyl pseudomonate intermediates **7a**, **7b**, and **7c** were prepared in a straightforward manner. Through the use of tetrabutylammonium fluoride solution, **7b**, was completely desilylated in 15 min while **7a** required 18 h. The sluggishness of this latter reaction allowed for selective deprotection of the C6 and C7 silyl residues from **7a**, and after 1 h a good yield of methyl 13-(*tert*-butyldimethylsilyl)pseudomonate was obtained. In contrast, formation of the C6–7 carbonate as described previously[11] gave access to the complementary protected system, **8**.

(8) Alcohol: Rogers, N. H.; Coulton, S.; O'Hanlon, P. J. *J. Chem. Soc., Perkin Trans. 1* **1982**, 729.
(9) We are grateful to the CAPD division at Abbott for their efficient production of this antibiotic.
(10) Clayton, J. P.; Oliver, R. S.; Rogers, N. H.; King, T. J. *J. Chem. Soc., Perkin Trans. 1* **1979**, 838.
(11) Coulton, S.; O'Hanlon, P. J.; Rogers, N. H. *Tetrahedron* **1987**, *43*, 2165.

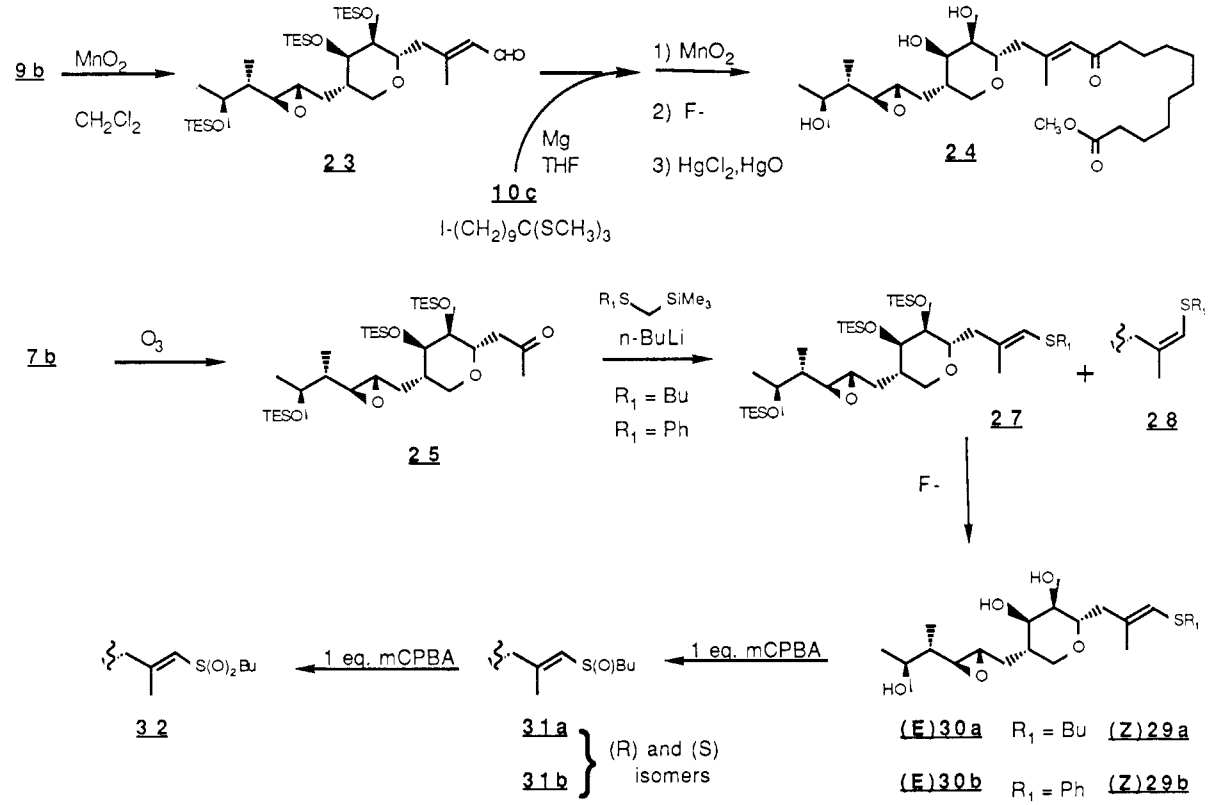
Scheme III



(a) Allyl Ethers and Thioethers. In order to study the structural aspects of the C1–C3 grouping more closely, we sought to prepare ether **12** (Scheme II). This target resembles the structure of the parent ester **3** while lacking its conjugated electronic system. Reduction of the ester **7a** with Dibal led to the allylic alcohol **9a**, which when combined with NaH and butyl iodide yielded model ether **11**. Under similar conditions, alkylation of **9a** with the iodo ester **10a** failed, leading instead to acylation of **9a** by the carboxyl group (**9a**; X = $OCO(CH_2)_8I$). Therefore, we prepared the tris(methylthio) iodide **10b** as a protected iodo ester chain from 1,8-diiodooctane. Again, with use of the allylic alkoxide from **9a** as the nucleophile, alkylation with **10b** went smoothly. Deprotection of the silyl groups followed by mercuric ion hydrolysis to the methyl ester afforded the desired ether, **12**. Alcohol **9a** was also treated with mesyl chloride to give the corresponding allylic chloride, **9c**. When this chloride was treated with sodium butyl mercaptide or the sodium salt of methyl 9-mecaptononanoate, the greater nucleophilicity of these anions allowed for straightforward alkylation to the silylated thioethers **13a**, **14a**. Subsequent deprotecton afforded the thioethers **13b**, **14b**.

(b) Cyclic Analogues. Since the electronics of the C1–C3 system was apparently the key to activity, we thought to retain the ester, albeit in a more hydrolytically stable butenolide form, **20** (Scheme III). Although these systems are known to exist primarily as the nonaromatic lactone tautomer, we expected the less electrophilic character of the carbonyl to disfavor the enzymatic hydrolysis. Workers at Beecham had previously deconjugated monate esters and trapped the intermediate anions with electrophiles with some success.[6a] We found that by using the appropriate amount of base, one could deconjugate the parent methyl (trisilyl)pseudomonate **7a** directly to obtain a 1:2 ratio of **7a**:C3–C15 olefin **15a**. Following chromatographic separation, the starting material was recycled and the olefin **15** was oxidized with *m*-chloroperbenzoic acid to give **17** or treated with osmium tetraoxide. The cis-hydroxylation of **15a** led to the C3–C15 diol **16**, presumably as a mixture of diastereomers. Without separation, this mixture was lactonized, and **19a** was obtained directly by mesylation and in situ elimination. Alternatively, it was discovered that direct treatment of epoxide **17a**, again as a presumed mixture of diastereomers, with diazabicycloundecene (DBU) produced a 71% yield

Scheme IV



of butenolide **19a**. We assume that eliminative epoxide opening occurs to give α,β-unsaturated esters **18a** and **18b** with the latter undergoing concomitant lactonizaton. When *E* isomer **18a** was isolated and resubmitted to these reaction conditions, it was also found to lead to desired butenolide **19a** via reversible closure to **17a**. Unfortunately, attempted removal of the *tert*-butyldimethylsilyl protecting groups of **20a** led only to decomposition products. Treatment of **19a** with Dowex in methanol removed ony the C13 silyl group, which when subsequently treated with fluoride also led to intractable materials.

To circumvent this problem, the trimethylsilyl-protected deconjugated ester **15b** was utilized as starting material, and the same described chemistry was carried out to give butenolide **19b**. Removal of this less stable protecting group with DMAP–HCl led to a 81% yield of the desired trihydroxy butenolide **20**. The tris(*t*-BDMS) butenolide is useful though, as a precursor to other systems such as furan **21**, which was prepared through standard treatment of **19a** with Dibal. Dehydration of the crude reducton product with mesyl chloride followed by subsequent desilylation smoothly afforded furan **21**. The intermediate diol **16** could also be oxidized with sodium periodate and deprotected to the keto ester **22**. This intermediate is useful in preparing various heterocyclic systems which will be reported at a later date.

(c) Unsaturated Sulfur Analogues. Previous SAR studies[2,8] of the C1–C3 unit promoted the need for an electron-withdrawing function at C1. Preparation of a variety of compounds having an electron-withdrawing substituent at C2 was therefore carried out so as to retain the electronic character of the parent system. We used ketone **24** (Scheme IV) as a standard for this class of compounds in that it is structurally similar to methyl pseudomonate A, yet incapable of hydrolysis at C1. The synthesis of ketone **24** and its biological data was not described in previous reports;[7] therefore it was prepared as shown. We found the alkyl bromide **10c** useful as a Grignard reagent precursor. Aldehyde **23**, obtained by manganese oxide oxidation of allylic alcohol **9b**, reacted smoothly at −78 °C with the reagent formed from **10c** and magnesium. Following oxidation of the incipient alcohols and deprotection, ketone **24** was obtained. The phenyl ketone analogue[12] prepared in similar fashion with phenylmagnesium bromide is listed in Tables I and II for the purpose of comparison. This ketone exhibited in vitro activity similar to that of methyl pseudomonate.

The synthesis of the vinyl sulfide **30** commenced from tris(triethylsilyl) ketone **25**, which in turn was obtained from ozonolysis of ester **7b**. Treatment of ketone **25** with anions formed from silyl sulfide reagents **26** led to 1:1 mixtures of isomeric vinyl sulfides **27** and **28**. The latter *Z* isomer was separated through chromatographic means and was carried on to the (*Z*)-butyl and the (*Z*)-phenyl sulfides **29**. Deprotecton of the *E* isomer **27** in a similar manner led to sulfide **30**. Oxidaton with 1 equiv or 2 equiv of mCPBA afforded sulfoxide **31** and sulfone **32**, respectively. The sulfoxide **31** was shown by TLC and spectral data to be approximately a 1:1 mixture of components, assumedly diastereomers at sulfur.

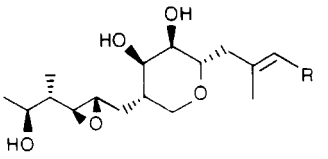
Biological Results and Discussion

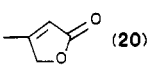
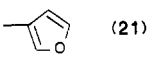
Isoleucyl-tRNA synthetase was isolated from *Escherichia coli* and purified to homogeneity by the method described by Kawakami et al.[13a] The ATP–pyrophosphate exchange assay[13b] was employed to determine inhibitory concentrations (IC_{50}) of the synthesized analogues. The bacterial strains used for our in vitro assay either were clinical isolates maintained frozen in our culture collection

(12) Experimental details of the synthesis of this and 10 other ketones along with bioactivity data will be reported at a later date. Klein, L. L. Unpublished results.

(13) (a) Kawakami, M.; Miyazaki, M.; Yamada, H.; Mizushima, S. *FEBS* **1985**, *185*, 162. (b) Berg, P.; Bergmann, F. H.; Ofengard, E. J.; Dieckmann, M. *J. Biol. Chem.* **1961**, *236*, 1726.

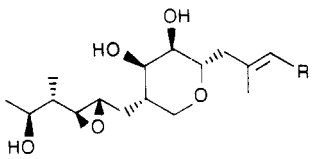
Table I. Inhibition of Isoleucyl-tRNA Synthetase by Pseudomonic Acid Derivatives

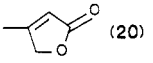


entry	structure	IC_{50}, μg/mL	entry	structure	IC_{50}, μg/mL
1	$CO(CH_2)_8CO_2Na$ (**2**)	0.1	12	$S(CH_2)_3CH_3(E)$ (**30a**)	2.1
2	$CO(CH_2)_8CO_2CH_3$ (**3**)	0.6	13	$S(CH_2)_3CH_3(Z)$ (**29a**)	289
3	$CH_2O(CH_2)_3CH_3$ (**11**)	20	14	$S(O)(CH_2)_3CH_3(E)$[a] (**31a**)	69
4	$CH_2O(CH_2)_8CO_2CH_3$ (**12**)	0.85	15	$S(O)(CH_2)_3CH_3(E)$[a] (**31b**)	>400
5	$CH_2S(CH_2)_3CH_3$ (**13b**)	157	16	$S(O)_2(CH_2)_3CH_3(E)$ (**32**)	76
6	$CH_2S(CH_2)_8CO_2CH_3$ (**14b**)	9.5	17	$COCH_2CO_2(CH_2)_8CO_2CH_3$ (**22**)	>400
7	$CO(CH_2)_9C(SCH_3)_3$	9			
8	$CO(CH_2)_9CO_2CH_3$ (**24**)	1.7	18	 (**20**)	272
9	COC_6H_5	0.2			
10	$SC_6H_5(E)$ (**30b**)	4.5	19	 (**21**)	>400
11	$SC_6H_5(Z)$ (**29b**)	19			

[a] The absolute stereochemistry of these isomers has not been proven.

Table II. Antibacterial Activities of Pseudomonic Acid Derivatives



		minimum inhibitory concentration (μg/mL)					
entry no.	R	*Staph. aureus* ATCC 6535P	*Staph. epidermidis* 3519	*Strep. agalactiae* CMX508	*Strep. pyogenes* EES61	*E. coli* Juhl	*E. coli* SS
1	$CO(CH_2)_8CO_2Na$ (**2**)	0.1	0.1	0.78	0.1	>100	0.2
2	$CO(CH_2)_8CO_2CH_3$ (**3**)	0.2	0.39	0.78	0.1	50	0.05
3	$(CH_2O(CH_2)_3CH_3$ (**11**)	100	100	>100	50	>100	12.5
4	$CH_2O(CH_2)_8CO_2CH_3$ (**12**)	>100	>100	>100	100	>100	12.5
5	$CH_2S(CH_2)_3CH_3$ (**13b**)	>100	>100	>100	100	>100	50
6	$CH_2S(CH_2)_8CO_2CH_3$ (**14b**)	100	>100	>100	50	>100	25
7	$CO(CH_2)_9C(SCH_3)_3$	1.56	1.56	>100	1.56	>100	6.2
8	$CO(CH_2)_9CO_2CH_3$ (**24**)	0.39	0.39	3.1	0.1	50	0.05
9	COC_6H_5	0.2	0.39	3.1	0.39	100	0.2
10	$SC_6H_5(E)$ (**30b**)	3.1	6.2	6.2	3.1	>100	1.56
11	$SC_6H_5(Z)$ (**29b**)	25	50	50	12.5	>100	12.5
12	$S(CH_2)_3CH_3(E)$ (**30a**)	1.56	0.78	3.1	0.78	>100	0.78
13	$S(CH_2)_3CH_3(Z)$ (**29a**)	>100	>100	>100	>100	>100	100
14	$S(O)(CH_2)_3CH_3(E)$[a] (**31a**)	>100	>100	>100	>100	>100	100
15	$S(O)(CH_2)_3CH_3(E)$[a] (**31b**)	>100	>100	>100	100	>100	100
16	$S(O)_2(CH_2)_3CH_3(E)$ (**32**)	>100	>100	>100	25	>100	>100
17	$COCH_2CO_2(CH_2)_8CO_2CH_3$ (**22**)	>100	>100	>100	>100	>100	>100
18	 (**20**)	100	100	100	6.2	>100	25
19	 (**21**)	>100	>100	>100	>100	>100	100

[a] The absolute stereochemistry of these isomers has not been proven.

or were obtained from the American Type Culture Collection. Minimum inhibitory concentrations (MICs) were determined by the agar dilution method on brain heart infusion agar with 10^4 cfu/spot as the inoculum.

Pseudomonic acid and its active analogues are active against Gram-positive bacteria though inactive against Gram-negative bacteria. The reason for this lack of activity is probably related to permeability since these compounds are active against strains that have a defective lipopolysaccharide layer such as *Escherichia coli* SS and *Pseudomonas aeruginosa* K799/61. It is also interesting to note that, among Gram-positive bacteria, *Micrococcus luteus* is resistant to these compounds.

The isoleucyl-tRNA synthetase inhibition of the ether and thioether derivatives (entries 3–6, Table I) is interesting in that the two examples containing the nonanoate chain were 20-fold better inhibitors than their butyl analogues. Furthermore, ether **12** exhibited IC_{50} values comparable to those of the corresponding parent ester **3**. This is the first evidence that inhibitory effects are possible without a carbonyl group at the C1 position. None of these examples, however, exhibited measurable in vitro activity against the strains listed in Table II. The long-chain ketone **24** also showed good to excellent IC_{50} as expected, but

in this case the corresponding phenyl ketone along with other ketones[12] were equally as active. The antibacterial activities for this series were similarly as good as those described for other ketone derivaties in the patent literature.[7] The tris(methylthio) ketone (entry 7) showed weak enzyme inhibitory and antibacterial activity, perhaps denoting the ester function as unnecessary.

Keto ester derivative **22**, butenolide **20**, and furan **21** (entries 17, 18, 19) showed little or no enzyme inhibitory activity or antibacterial activity. The fact that the ^{1}H NMR resonances of the C2 and C4 protons of butenolide **20** were quite similar to that of the parent ester **3** reflects the functional similarity in these two systems. Therefore, we suspect that other characteristics other than the electronics of the conjugated system are necessary for activity, for example the conformation of the lactone carbonyl. A published X-ray crystal structure of the simple derivative ethyl monate C[14] shows the carbonyl oxygen in close proximity to the ring oxygen of **20**. Furthermore, the higher oxidation state of the C15-methyl may modify the mode of action of this compound. These and other differences between the structures of **20** and **3** will be investigated through further modification.

Finally, the vinylthio compounds and their oxygenated analogues **29–32** were studied so as to vary the electron-withdrawing effects of the "C1" function, and in fact, this series produced a wide range of IC_{50} values. The vinyl sulfides were relatively active against isoleucyl-tRNA synthetase with the *E* isomers again showing 5–100-fold improvement over the *Z* isomers. The vinyl sulfides also exhibited good in vitro data although they were 10-fold less potent than the ketone derivatives. No direct evidence was obtained to determine the absolute configuration of sulfoxides **31a** and **31b** (entries 14 and 15) although the former showed better inhibition than did the latter. The reason for this difference is unclear since the corresponding sulfone **32** showed similar inhibition to **31a**. These oxidized derivatives were considered to be important in the series since in vivo administration of the vinyl sulfides could be expected to lead to such materials via metabolic oxidation. Unfortunately as shown in Table I the inhibition was weak and none of these oxygenated derivatives showed reasonably good in vitro data.

The in vivo efficacies of a number of derivatives in these classes were measured through standard mouse protection protocol. Although several analogues mentioned above exhibited good in vitro activities, the in vivo activities, though measurable, were not of sufficient magnitude to encourage further study.

Experimental Section

^{1}H NMR spectra were recorded on a General Electric QE300 spectrometer using Me_4Si as an internal standard. Elemental analyses were performed by Abbott Laboratories, North Chicago, IL. The high-resolution MS were obtained on a Kratos MS50 instrument at Abbott Laboratories. E. Merck silica gel (230–400 mesh) obtained from VWR Scientific was used for column chromatography. CH_2Cl_2 was distilled from P_2O_5 and THF was distilled from sodium and benzophenone. All other solvents were HPLC grade and were not purified prior to use.

Methyl Pseudomonate Tris(triethylsilyl ether) 7b. To a solution of 20 g (33.8 mmol) of methyl pseudomonate (**3**) in 50 mL (379 mmol) of collidine at −15 °C in 10 min was added 36 mL (160 mmol) of triethylsilyl triflate. After 15 min, 230 mL of saturated NH_4Cl solution and 150 mL of Et_2O was added to the reaction mixture and stirring was continued for 16 h at room temperature. The Et_2O layer was separated and evaporated under high vacuum. The oily residue (38 g) was chromatographed on silica gel with a gradient elution of 2–8% EtOAc/hexane. **7b**: yield, 24 g (72%) as a clear colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.74 (br s, 1, H2), 4.06 (t, 2, J = 6.62 Hz, H9′), 3.82–3.92 (m, 4, H13,7,5,6), 3.67 (s, 3, OCH_3), 3.54 (br d, 1, H16), 3.38 (br d, 1, H16), 2.66 (m, 2 H10,11), 2.55 (br d, 1, H4), 2.30 (t, 2, J = 7.53 Hz, H2′), 2.18 (s, 3, H15), 2.05 (dd, 1, H4), 1.8 (m, 3, H8,9), 1.59–1.66 (m, 4, H3′,8′), 1.31–1.39 (m, 9, H12,4′,5′,6′,7′), 1.19 (d, 3, J = 6.25 Hz, H14), 0.87–1.04 (m, 30, H17, $SiCH_3$), 0.55–0.69 (m, 18, $SiCH_2$). Anal. ($C_{45}H_{88}O_9Si_3$) C, H.

Tri-TES Allylic Alcohol 9b. To a solution of methyl pseudomonate tris(triethylsilyl ether) (**7b**) (16.45 g, 19.2 mmol) in 70 mL of CH_2Cl_2 at −78 °C was added in 10 min 70 mL of 1 M Dibal/CH_2Cl_2. After 15 min, 5.25 mL of MeOH was added followed by 8.76 mL of H_2O. The mixture was stirred for 16 h at room temperature and the resulting slurry was filtered. The oily residue obtained after solvent evaporation was chromatographed on silica gel with 20% EtOAc/hexane. **9b**: yield, 10.9 g (84.4%); ^{1}H NMR ($CDCl_3$) δ 5.5 (br t, 1, H2), 4.16 (d, 2, J = 7.0 Hz, H1), 3.78–3.92 (m, 4, H13,7,5,6), 3.53 (d, 1, J = 14 Hz, H16), 3.37 (dd, 1, H16), 2.65–2.67 (m, 2, H10,11), 2.45 (br d, 1, H4), 1.95 (m, 1, H4), 1.80 (m, 1, H8), 1.71 (s, 3, H15), 1.45–1.60 (m, 2, H9), 1.34–1.42 (m, 1, H12), 1.19 (d, 3, J = 6.62 Hz, H14), 0.93 (m, 30, H17, $SiCH_3$), 0.55–0.66 (m, 18, $SiCH_2$). Anal. ($C_{35}H_{72}O_6Si_3$) C, H.

Tri-tBDMS Methyl Pseudomonate Tris(*tert*-butyldimethylsilyl ether) (7a). **7a** was prepared according to the procedure outlined in the synthesis of **7b**: yield, 81% starting with 7 g of **3**; ^{1}H NMR ($CDCl_3$) δ 5.73 (br s, 1, H2), 3.67 (s, 3, OCH_3), 2.18 (d, 3, J = 0.64 Hz, H15), 1.19 (d, 3, J = 6.25 Hz, H14), 0.89 (m, 30, H17, Si-*t*-Bu), 0.07 (m, 18, $SiCH_3$). Anal. ($C_{45}H_{88}O_9Si_3$) C, H.

Tri-tBDMS Allylic Alcohol 9a. **9a** was prepared according to the procedure outlined in the synthesis of **9b**: yield, 78% starting with 6 g of **7b**: ^{1}H NMR ($CDCl_3$) δ 5.49 (br t, 1, H2), 4.18 (d, 2, J = 6.99 Hz, H1), 1.70 (s, 3, H15), 1.19 (d, 3, J = 6.25 Hz, H14), 0.89 (m, 30, H17, Si-*t*-Bu), 0.07 (m, 18, $SiCH_3$). Anal. ($C_{35}H_{72}O_6Si_3$) C, H.

Tri-tBDMS Chloride 9c. To a solution of alcohol **9a** (3.5 g, 5.2 mmol) in 15 mL of DMF at room temperature were added LiCl (0.66 g, 3 equiv), collidine (2.27 mL, 3.3 equiv), and MsCl (1.33 mL, 3.3 equiv). After $2^1/_2$ h, the mixture was quenched with saturated NH_4Cl solution and extracted with hexane. The oily residue obtained after solvent removal was chromatographed on silica gel (150 g) with 20% Et_2O/hexane: yield, 2.95 g (82%); ^{1}H NMR ($CDCl_3$) δ 5.52 (t, 1, H2), 4.12 (m, 2, H1), 3.78–3.90 (m, 4, H13,7,5,6), 3.55 (d, 1, H16), 3.36 (dd, 1, H16), 2.68 (m, 2, H10,11), 2.50 (d, 1, H4), 1.92 (dd, 1, H4), 1.7 (m, 2, H9), 1.75 (s, 3, H15), 1.52 (m, 1, H8), 1.35 (m, 1, H12), 1.19 (d, 3, H14), 0.9 (m, 30, H17, Si-*t*-Bu), 0.07 (m, 18, $SiCH_3$); exact mass (FAB) calcd for $C_{35}H_{72}Si_3O_5Cl$ 691.4376, found 691.4361.

Tri-tBDMS Butyl Thioether 13a. A solution of allylic chloride **9c** (0.6 g, 0.87 mmol) and sodium butanethiol (105 mg, 1 equiv) in 10 mL of MeOH was heated to reflux for 1 min and cooled to room temperature. The mixture was partitioned between Et_2O and saturated NH_4Cl solution. The oily residue obtained after solvent evaporation was chromatographed on silica gel (75 g) with 5% acetone/hexane: yield, 0.59 g (91.2%); ^{1}H NMR ($CDCl_3$) δ 5.30 (br t, 1, H2), 3.75–3.9 (m, 4, H7, 13, 5, 6), 3.53 (d, 1, J = 11.03 Hz, H16), 3.55 (dd, 1, J = 11.03 and 0.73 Hz, H16), 3.18 (m, 2, H1), 2.68 (m, 2, H10,11), 2.44–2.49 (br t, 3, H4′,4), 1.89 (m, 1, H4), 1.78 (m, 1, H8), 1.69 (s, 3, H15), 1.45–1.60 (m, 4, H3′, 9), 1.48 (m, 3, H2′,12), 1.19 (d, 3, J = 6.25 Hz, H14), 0.91 (m, 33, H17, 1′, Si-*t*-Bu), 0.06 (m, 18, $SiCH_3$); exact mass (FAB) calcd for $C_{39}H_{80}Si_3O_5SNa$ 767.4932, found 767.4939.

Butyl Thioether 13b. A solution of tri-tBDMS thioether **13a** (0.49 g, 0.66 mmol) and 2.3 mL of 1 M Bu_4NF/THF (3.4 equiv) was stirred at room temperature for 18 h. The solvent was evaporated and the resulting oil was chromatographed on silica gel (100 g) with 5% MeOH/$CHCl_3$. **13b**: yield, 0.21 g (79%); ^{1}H NMR ($CDCl_3$) δ 5.40 (br t, 1, H2), 3.72–3.88 (m, 4, H16,7,13,5), 3.51–3.55 (m, 2, H16,6), 3.17 (d, 2, J = 7.73 Hz, H1), 2.82 (m, 1, H10), 2.71 (dd, 1, J = 1.21 and 10.3 Hz, H11), 2.48 (t, 2, J = 7.35 Hz, H4′), 2.43 (dd, 1, H4), 2.28 (dd, 1, H4), 2.02 (m, 1, H8), 1.73 (s, 3, H15), 1.52–1.75 (m, 4, H9,3′), 1.3–1.5 (m, 3, H2′,12), 1.22 (d, 3, J = 6.25 Hz, H14), 0.89–0.96 (m, 6, H17, 1′); exact mass

(14) Rogers, N. H.; O'Hanlon, P. J.; Clayton, J. P.; King, T. J. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2827.

(FAB) calcd for $C_{21}H_{39}O_5S$ 403.2518, found 403.2534.

Methyl 9-Mercaptononanoate. A solution of methyl oleate (22 g, 74.2 mmol) in 100 mL of CH_2Cl_2 and 25 mL of MeOH at room temperature was ozonized at a rate of 0.5 for 2 h. $NaBH_4$ (2 g, 53 mmol) was added portionwise at 15 °C. After the mixture was stirred for 1 h, the solvent was evaporated and the resulting oil was chromatographed on silica gel (400 g) with 20% EtOAc/hexane to give 8.6 g (61.6%) of oil. To a solution of 8.6 g of hydroxyl ester (45.7 mmol) in 100 mL of CH_2Cl_2 and Et_3N (10.8 mL, 1.7 equiv) was added MsCl(4.96 mL, 1.4 equiv) dropwise at 0 °C. After $^1/_2$ h, the reaction mixture was quenched with saturated NH_4Cl solution and the organic layer was evaporated and eluted through silica gel (20 g) with Et_2O to give 12.13 g of oil. The oil was dissolved in 150 mL of acetone and NaI (20.51 g, 3 equiv) was added. After the mixture was stirred for 1 h, the solvent was evaporated and the resulting oil was partitioned between Et_2O and saturated NH_4Cl solution. The Et_2O layer was separated, evaporated, and eluted through silica gel (25 g) with 50% hexane/Et_2O to give 13.23 g of oil. To a solution of iodononanoate (13.23 g, 44.4 mmol) in 50 mL of DMF at room temperature was added potassium thioacetate (6.59 g, 1.3 equiv). After $^1/_2$ h, the solvent was evaporated and the resulting oil was partitioned between Et_2O and saturated NH_4Cl solution. The Et_2O layer was evaporated and the oily residue was chromatographed on silica gel (25 g) with 30% Et_2O/hexane to give 10.69 g of oil. The 10.69 g of thioacetate was hydrolyzed in 50 mL of MeOH with NaH (60 wt % in oil) (1.54 g, 1.5 equiv) which had been washed with hexane at 0 °C for 10 min. The solvent was evaporated and the resulting oil was chromatographed on silica gel (250 g) with 30% Et_2O/hexane: yield, 5.18 g (34.2% overall) as an oil; ^1H NMR (CDC_3) δ 3.67 (s, 3, OCH_3), 2.52 (q, 2, J = 6.99 Hz, H9), 2.31 (t, 2, J = 7.72 Hz, H2), 1.55–1.65 (m, 4, H8, 3), 1.25–1.42 (m, 8, H4,5,6,7). Anal. ($C_{10}H_{20}O_2S$) C, H. S.

Tri-tBDMS Thioether 14a. A solution of allylic chloride **9c** (1 g, 1.45 mmol), mercaptononanoate (0.58 g, 2 equiv), and NaH (60 wt % in oil) (0.74 g, 2.5 equiv) in 2 mL of DMF and 2 mL of THF was stirred for 1 h at −78 °C. The reaction mixture was quenched with saturated NH_4Cl solution and extracted with Et_2O. The oily residue after solvent evaporation was chromatographed on silica gel (75 g) with 5% EtOAc/hexane. **14a**: yield, 0.92 g (74%); ^1H NMR ($CDCl_3$) δ 5.3 (br t, 1, H2), 3.74–3.89 (m, 4, H7,13,5,6), 3.67 (s, 3, OCH_3), 3.53 (d, 1, H16), 3.36 (dd, 1, H16), 3.18 (m, 2, H1), 2.68 (m, 2, H10,11), 2.46 (br t, 3, H9′,4), 2.30 (t, 2, J = 7.35 Hz, H2′), 1.89 (m, 1, H4), 1.78 (m, 1, H8), 1.67 (s, 3, H15), 1.45–1.65 (m, 6, H8′,3′,9), 1.30–1.42 (m, 9, H12,4′,5′,6′,7′), 1.19 (d, 3, J = 6.61 Hz, H14), 0.91 (m, 30, H17, Si-t-Bu), 0.06 (m, 18, $SiCH_3$); exact mass (FAB) calcd for $C_{45}H_{91}O_7SSi_3$ 859.5793, found 859.5802.

Thioether 14b. A solution of tri-tBDMS thioether **14a** (0.64 g, 0.74 mmol) and 2.5 mL of 1 M Bu_4NF/THF (3.4 equiv) was stirred at room temperature for 18 h. The solvent was evaporated and the oily residue was chromatographed on silica gel (100 g) with 5% MeOH/$CHCl_3$. **14b**: yield, 0.28 g (74%); ^1H NMR ($CDCl_3$) δ 5.39 (br t, 1, H2), 3.7–3.88 (m, 4, H16,7,13,5), 3.67 (s, 3, OCH_3), 3.53 (m, 2, H16,6), 3.16 (d, 2, J = 7.73 Hz, H1), 2.82 (m, 1, H10), 2.71 (dd, 1, H11), 2.35–2.59 (m, 3, H9′,4), 2.24–2.33 (m, 3, H2′,4), 2.02 (m, 1, H8), 1.73 (s, 3, H15), 1.5–1.7 (m, 6, H8′,3′,9), 1.30 (m, 9, H12,4′,5′,6′,7′), 1.22 (d, 3, J = 6.25 Hz, H14), 0.95 (d, 3, J = 6.98 Hz, H17); exact mass (FAB) calcd for $C_{27}H_{49}O_7S$ 517.3199, found 517.3199.

Butyl Ether 11. A suspension of the allylic alcohol **9c** (1.5 g, 2.23 mmol) and NaH (60 wt % in oil) (0.18 g, 2 equiv) which had been washed with hexane in 8 mL of DMF was stirred for 10 min at room temperature. Butyl iodide (0.5 mL, 2 equiv) was then added and the mixture was stirred for 18 h at room temperature. The solvent was evaporated and the oily residue was partitioned between saturated NH_4Cl solution and Et_2O. The Et_2O layer was evaporated and the oily residue was chromatographed on silica gel (50 g) with 7% EtOAc/hexane: yield, 0.92 g (57%); ^1H NMR ($CDCl_3$) δ 5.43 (t, 1, H2), 3.98 (m, 2, H1), 3.78–3.94 (m, 4, H13,7,5,6), 3.54 (d, 1, H16), 3.42 (t, 2, J = 6.62 Hz, H4′), 3.35 (dd, 1, H16), 2.68 (m, 2, H10,11), 2.48 (d, 1, H4), 1.9 (m, 1, H4), 1.77 (m, 1, H8), 1.69 (s, 3, H15), 1.45–1.62 (m, 4, H3′,9), 1.37 (m, 3, H2′,12), 1.19 (d, 3, J = 6.25 Hz, H14), 0.9 (m, 33, H17, 1′, Si-t-Bu), 0.06 (m, 18, $SiCH_3$). Anal. ($C_{39}H_{80}Si_3O_6$) C, H.

A solution of above tri-tBDMS ether (0.47 g, 0.64 mmol) and 2.25 mL of 1 M Bu_4NF/THF (3.5 equiv) was stirred at room temperature for 18 h. The solvent was evaporated and the oily residue was chromatographed on silica gel (100 g) with 10% MeOH/$CHCl_3$: yield, 0.2 g (80%); ^1H NMR ($CDCl_3$) δ 5.48 (br t, 1, H2), 3.93 (br d, 2, H1), 3.73–3.89 (m, 4, H16,7,13,5), 3.50–3.58 (m, 2, H16,6), 3.42 (t, 2, J = 6.62 Hz, H4′), 2.67–2.83 (m, 2, H10,11), 2.42 (dd, 1, H4), 2.28 (dd, 1, H4), 2.02 (m, 1, H8), 1.74 (s, 3, H15), 1.72 (m, 2, H9), 1.56 (m, 2, H3′), 1.25–1.42 (m, 3, H2′,12), 1.22 (d, 3, J = 6.25 Hz, H14), 0.95 (m, 6, H17, 1′); exact mass (FAB) calcd for $C_{21}H_{39}O_6$ 387.2747, found 287.2743.

1-Iodo-9-tris(methylthio)nonane (10b). To a solution of tris(methylthio)methane (4 mL, 30 mmol) in 350 mL of THF was added 2.6 M n-BuLi/hexane (17 mL, 1.2 equiv) at −78 °C. The mixure was stirred for 1 h and 1,8-diiodooctane (6 mL, 30 mmol) was added all at once. After 1 h, the mixture was quenched with 100 mL of saturated NH_4Cl solution and extracted with EtOAc. The oily residue obtained after solvent evaporation was chromatographed on silica gel (200 g) with 13% $CHCl_3$/hexane. **10b**: yield, 2.3 g (19.5%); ^1H NMR ($CDCl_3$) δ 3.19 (t, 2, J = 6.94 Hz, H1), 2.11 (s, 9, SCH_3), 1.76–1.90 (m, 2, H7,8), 1.64 (m, 2, H2), 1.29–1.44 (m, 8, H3,4,5,6). Anal. ($C_{12}H_{25}IS_3$) C, H.

Ether 12. A suspension of allylic alcohol **9a** (1.2 g, 1.78 mmol) and NaH (60 wt % in oil) (0.14 g, 2 equiv) which had been washed with hexane in 10 mL of DMF was stirred for 10 min at room temperature. The iodononane **10b** (1.44 g, 2 equiv) was then added and the mixture was stirred at room temperature for 18 h. The solvent was evaporated and the oily residue was partitioned between saturated NH_4Cl solution and Et_2O. The Et_2O layer was evaporated and the oily residue was chromatographed on silica gel (50 g) with 7% EtOAc/hexane: yield, 0.74 g (64%); ^1H NMR ($CDCl_3$) δ 5.44 (br t, 1, H2), 3.97 (m, 2, H1), 3.78–3.93 (m, 4, H13,7,5,6), 3.54 (d, 1, H16), 3.41 (t, 2, H9′), 3.35 (dd, 1, H16), 2.65–2.72 (m, 2, H10,11), 2.48 (d, 1, H4), 2.10 (s, 9, SCH_3), 1.83–1.95 (m, 3, H4,2′), 1.75 (m, 1, H8), 1.69 (s, 3, H15), 1.45–1.65 (m, 6, H8′,3′,9), 1.25–1.40 (m, 8, H7′,4′,5′,6′), 1.19 (d, 3, H14), 0.89 (m, 30, H17, Si-t-Bu), 0.06 (m, 18, $SiCH_3$). Anal. ($C_{47}H_{96}O_6S_3Si_3$) C, H.

Tris(methylthio) ether v: a solution of tri-tBDMS ether iv (0.47 g, 0.5 mmol) and 1.7 mL of 1 M Bu_4NF/THF (3.4 equiv) was stirred at room temperature for 20 h. The solvent was evaporated and the oily residue was chromatographed on silica gel (100 g) with 10% MeOH/$CHCl_3$: yield, 0.22 g (74%); ^1H NMR ($CDCl_3$) δ 5.48 (br t, 1, H2), 3.95 (d, 2, H1), 3.72–3.89 (m, 4, H13,7,6,5), 3.55 (m, 2, H16), 3.41 (t, 2, H9′), 2.81 (m, 1, H10), 2.63–2.73 (m, 2, H11,4), 2.30 (m, 1, H4), 2.10 (s, 9, SCH_3), 2.02 (m, 1, H8), 1.87 (m, 2, H2′), 1.74 (s, 3, H15), 1.52–1.70 (m, 6, H3′,8′,9), 1.32 (m, 8, H7′,4′,5′,6′), 1.22 (d, 3, H14), 0.94 (d, 3, H17); exact mass (FAB) calcd for $C_{29}H_{54}O_6S_3Na$ 617.2980, found 617.2980.

To a solution of tris(methylthio) ether v (0.12 g, 0.2 mmol) in 15 mL of MeOH at −20 °C were added $HgCl_2$ (0.2 g, 0.74 mmol) and HgO (0.07 g, 0.32 mmol). The suspension was stirred for 50 min and filtered through Celite with $CHCl_3$ into a cold saturated NH_4Cl solution. The organic layer was evaporated and the resulting oil was chromatographed on silica gel (25 g) with 7% MeOH/$CHCl_3$: yield, 90 mg (63%); ^1H NMR ($CDCl_3$) δ 5.48 (br t, 1, H2), 3.97 (br t, 2, H1), 3.72–3.89 (m, 4, H13,7,6,5), 3.67 (s, 3, OCH_3), 3.5–3.6 (m, 2, H16), 3.40 (t, 2, J = 6.62 Hz, H9′), 2.81 (dt, 1, H10), 2.70 (dd, 1, J = 2.21 and 1.00 Hz, H11), 2.40 (dd, 1, H4), 2.30 (t, 2, J = 7.77 Hz, H2′), 2.25–2.35 (m, 1, H4), 2.03 (m, 1, H8), 1.74 (s, 3, H15), 1.52–1.77 (m, 6, H9,3′,8′), 1.30 (m, 9, H12,4′,5′,6′,7′), 1.22 (d, 3, J = 6.62 Hz, H14), 0.94 (d, 3, J = 6.98 Hz, H17); exact mass (FAB) calcd for $C_{27}H_{49}O_8$ 501.3427, found 501.3422.

Methyl Pseudomonate Tris(trimethylsilyl ether) (7c). To a solution of methyl pseudomonate (3) (8 g, 15.5 mmol) in 50 mL of THF at 0 °C were added Et_3N (9.7 mL, 4.5 equiv), TMSCl (8.9 mL, 4.5 equiv), and DMAP (0.1 g, 0.6 mmol). After 1 h, the mixture was filtered and the solvent was evaporated. The resulting oil was chromatographed on silica gel (250 g) with 10% EtOAc/hexane: yield, 10.7 g (94%); ^1H NMR ($CDCl_3$) δ 5.74 (s, 1, H2), 3.67 (s, 3, OCH_3), 2.19 (d, 3, J = 1.10 Hz, H15), 1.20 (d, 3, J = 6.32 Hz, H14), 0.87 (d, 3, J = 6.25 Hz, H17), 0.14 (m, 27, $SiCH_3$). Anal. ($C_{36}H_{70}O_9Si_3$) C, H.

Deconjugated Methyl Pseudomonate Tris(trimethylsilyl ether) (15b). LDA solution was generated in 1 h with use of 2.6

M *n*-BuLi/hexane (10 mL, 2.1 equiv) and diisopropylamine (3 mL, 2.5 equiv) in 40 mL of THF at −78 °C. A solution of TMS pseudomonate **7c** (10 g, 13.7 mmol) in 10 mL of THF was added to the above solution, and the mixture was stirred for $^1/_2$ h at −78 °C. The mixture was then quenched with saturated NH_4Cl solution and extracted with Et_2O. The oily residue obtained after solvent removal was chromatographed on silica gel (300 g) with 9% EtOAc/hexane: yield, 4 g (40%) of **15b** with starting material recovery (**7c**; 1.3 g, 13%); ^1H NMR ($CDCl_3$) δ 5.00 (d, 2, *J* = 15.07 Hz, H15), 4.06 (t, 2, *J* = 6.68 Hz, H9′), 3.74–3.91 (m, 4, H7,13,5,6), 3.67 (s, 3, OCH_3), 3.52 (br d, 1, H16), 3.36 (dd, 1, *J* = 2.57 and 8.82 Hz, H16, 3.10 (s, 2, H2), 2.66–2.70 (m, 2, H10,11), 2.55 (br d, 1, H4), 2.30 (t, 2, *J* = 7.36 Hz, H2′), 2.04 (m, 1, H4), 1.80 (m, 3, H9,8), 1.6 (m, 4, H3′,8′), 1.3 (m, 9, H12,4′,5′,6′,7′), 1.20 (d, 3, *J* = 6.25 Hz, H14), 0.90 (d, 3, *J* = 6.98 Hz, H17), 0.11 (m, 27, $SiCH_3$); exact mass (FAB) calcd for $C_{36}H_{71}O_9Si_3$ 731.4405, found 731.4401.

Methyl 3,15-Epoxypseudomonate Tris(trimethylsilyl ether) (17b). To a solution of deconjugated pseudomonate **15b** (4 g, 5.5 mmol) in 60 mL of $CHCl_3$ at room temperature was added 2 g of 80% MCPBA (1.7 equiv). After the mixture was stirred for $1^1/_2$ h, the solvent was evaporated and the resulting oil was chromatographed on silica gel (250 g) with 15% EtOAc/hexane: yield, 2.4 g (59%); ^1H NMR ($CDCl_3$) δ 4.08 (t, 2, *J* = 6.62 Hz, H9′), 3.70–3.93 (m, 4, H7,13,5,6), 3.66 (s, 3, OCH_3), 3.58 (m, 1, H16), 3.36 (m, 1, H16), 2.50–2.90 (m, 7, H15,2,10,11,4), 2.30 (t, 2, *J* = 7.72 Hz, H2′), 2.08 (m, 1, H4), 1.7–1.95 (m, 3, H9,8), 1.5–1.68 (m, 4, H3′,8′), 1.3 (m, 9, H12,4′,5′,6′,7′), 1.20 (d, 3, *J* = 6.62 Hz, H14), 0.89 (d, 3, *J* = 6.89 Hz, H17), 0.11 (m, 27, $SiCH_3$). Anal. ($C_{36}H_{70}O_{10}Si_3$) C, H.

Tri-TMS Butenolide 19b. A solution of epoxy pseudomonate **17b** (2 g, 2.6 mmol) and DBU (0.37 mL, 1 equiv) in 5 mL of toluene was heated at 90 °C for 3 h. The oily residue obtained after solvent evaporation was chromatographed on silica gel (125 g) with 15% EtOAc/hexane: yield, 0.99 g (66%) of **19b** with 0.11 g of the intermediate, 15-OH enol ester **18b** (7.4%); ^1H NMR ($CDCl_3$) 5.92 (br s, 1, H2), 4.80 (2 dd, 2, H15), 3.73–3.93 (m, 4, H16,13,7,5), 3.58 (d, 1, H16), 2.88 (dd, 1, H6), 2.86 (br d, 1, H4), 2.7 (m, 2, H10,11), 2.32 (dd, 1, H4), 1.7 (m, 2, H9), 1.6 (m, 1, H8), 1.4 (m, 1, H12), 1.20 (d, 3, *J* = 6.62 Hz, H14), 0.91 (d, 3, *J* = 7.35 Hz, H17), 0.11 (m, 27, $SiCH_3$); exact mass (FAB) calcd for $C_{26}H_{51}O_7Si_3$ 559.2942, found 559.2953. **18b**: ^1H NMR ($CDCl_3$) δ 5.93 (dd, 1, H2), 4.16–4.21 (m, 2, H15), 4.08 (dt, 2, H9′), 3.75–3.94 (m, 4, H5,13,6,7), 3.67 (s, 2, OCH_3), 3.40–3.57 (m, 2, H16), 3.16 (br t, 1, 15-OH), 2.63–2.72 (m, 3, H10, 11, 4), 2.50 (dd, 1, H4), 2.30 (t, 2, *J* = 7.36 Hz, H2′), 1.83 (m, 1, H8), 1.52–1.62 (m, 6, H9′,3′,8′), 1.3–1.45 (m, 9, H12,4′,5′,6′,7′), 1.20 (d, 3, *J* = 6.25 Hz, H14), 0.90 (d, 3, *J* = 7.36 Hz, H17), 0.11 (m, 27, $SiCH_3$); exact mass (FAB) calcd for $C_{36}H_{71}O_{10}Si_3$ 747.4355, found 747.4360.

Butenolide 20. A solution of DMAP·HCl (85 mg, 3 equiv) and tri-TMS butenolide **19b** (0.1 g, 0.18 mmol) in 4 mL of MeOH was stirred at room temperature for 4 h. The oily residue obtained after solvent evaporation was chromatographed on silica gel (25 g) with 10% MeOH/$CHCl_3$: yield, 50 mg (81%); ^1H NMR ($CDCl_3$) δ 5.96 (br s, 1, H2), 4.82 (d, 2, *J* = 1.84 Hz, H15), 3.99 (br d, 1, H7), 3.91 (dd, 1, *J* = 2.58 and 11.76 Hz, H16), 2.8 (m, 1, H13), 3.60–3.72 (m, 2, H5,16), 3.45 (m, 1, H6), 2.94 (dd, 1, H4), 2.70–2.85 (m, 2, H10,11), 2.6 (dd, 1, H4), 2.05 (m, 1, H8), 1.65–1.70 (m, 2, H9), 1.45 (m, 1, H12), 1.23 (d, 3, *J* = 6.25 Hz, H14), 0.94 (d, 3, *J* = 6.98 Hz, H17); exact mass (FAB) calcd for $C_{17}H_{27}O_7$ 343.1756, found 343.1751.

Deconjugated Methyl Pseudomonate Tris(*tert*-butyldimethylsilyl ether) (15a). **15a** was prepared according to the procedure outlined for the synthesis of **15b**. Starting with 5 g of **7a**, the yield is 54% (**15a**) with 26% recovery of **7a**: ^1H NMR ($CDCl_3$) δ 5.01 (d, 2, H15), 3.67 (s, 3, OCH_3), 3.09 (s, 2, H2), 1.19 (d, 3, *J* = 6.62 Hz, H14); exact mass (FAB) calcd for $C_{45}H_{89}O_9Si_3$ 857.5814, found 857.5803.

Methyl 3,15-Dihydroxypseudomonate Tris(*tert*-butyldimethylsilyl ether) (16). To a solution of deconjugated pseudomonate **15a** (1.6 g, 1.86 mmol) in 10 mL of 20% H_2O/THF and *N*-methylmorpholine *N*-oxide (0.44 g, 2 equiv) was added 2.5 wt % OsO_4/*t*-BuOH (0.4 mL, 0.03 equiv). After stirring for 5 h at room temperature, the mixture was quenched with Celite/$NaHSO_3$ (12/1 by wt) and filtered. The oily residue obtained after solvent removal was chromatographed on silica gel (90 g) with 30% EtOAc/hexane: yield, 1.47 g, (91.6%); ^1H NMR ($CDCl_3$) δ 3.97–4.18 (m, 4, H15,9′), 3.80–3.93 (m, 4, H7,13,5,6), 3.66 (s, 3, OCH_3), 3.57 (m, 1, H16), 3.36 (m, 1, H16), 2.57–2.75 (m, 5, H2,10,11,4), 2.30 (t, 2, *J* = 7.70 Hz, H2′), 2.10 (m, 1, H4), 1.70–1.84 (m, 3, H9,8), 1.50–1.68 (m, 4, H3′,8′), 1.3 (m, 9, H12,4′,5′,6′,7′), 1.20 (d, 3, *J* = 6.62 Hz, H14), 0.89 (m, 30, H17, Si-*t*-Bu), 0.08 (m, 18, $SiCH_3$); exact mass (FAB) calcd for $C_{45}H_{91}O_{11}Si_3$ 891.5869, found 891.5862.

Furan 21. To a solution of trisilyl butenolide **19a** (0.1 g, 0.15 mmol) in 10 mL of CH_2Cl_2 at 78 °C was added 1.44 mL of 1 M Dibal/hexanes. After 30 min, 0.072 mL of CH_3OH was added, followed by 0.12 mL of H_2O. The mixture was stirred vigorously for 1 h at room temperature, and the resulting slurry was filtered. The oily residue obtained after solvent evaporation was dissolved in 10 mL of CH_2Cl_2 and cooled to 0 °C. To this solution was added 0.5 mL of triethylamine followed by 0.2 mL of methanesulfonyl chloride. After 30 min the reaction was quenched with saturated NH_4Cl solution. The organic layer was separated, washed with saturated NaCl solution, dried over anhydrous Na_2SO_4, and evaporated. The crude furan was dissolved in 5 mL of THF, and to this solution was added 0.5 mL of 1 M Bu_4NF/THF and stirring was continued for 8 h. The solvent was evaporated and the resulting oil was chromatographed on silica gel with 5–10% MeOH/CH_2Cl_2: yield, 25.5 mg (51%); ^1H NMR δ 7.38 (t, 1 H2), 7.33 (br s, 1, H15), 6.37 (br s, 1, H1), 3.93 (br d, 1, H7), 3.91 (dd, 1, H16a), 3.82 (dq, 1, H13), 3.72 (dt, 1, H5), 3.6 (dd, 1, H16b), 3.51 (dd, 1, H6), 2.85 (dd, 1, H4a), 2.7 (dt, 1, H10), 2.68 (dd, 1, H11), 2.65 (dd, 1, H4b), 2.05 (m, 1, H8), 1.7–1.8 (m, 2, H9), 1.39 (m, 1, H12), 1.21 (d, 3, H17), 0.93 (d, 3, H14).

Keto Ester 22. To a solution of diol **16** (1.47 g, 1.71 mmol) in 25 mL of MeOH at 0 °C was added portionwise a solution of $NaIO_4$ (1.1 g, 3 equiv) in 5 mL of H_2O. After stirring for 4 h at 0 °C, the mixture was evaporated to dryness and the resulting oil was chromatographed on silica gel (110 g) with 10% EtOAc/hexane: yield, 1.24 g (86.5%); ^1H NMR (CCl_3) δ 4.01 (t, 3, H9′), 3.8–3.95 (m, 4, H5,6,13,7), 3.67 (s, 3, OCH_3), 3.55 (d, 1, H16), 3.50 (s, 2, H2), 3.42 (dd, 1, H16), 2.82 (dd, 1, H4), 2.63–2.72 (m, 2 H10,11), 2.5 (dd, 1, H4), 2.30 (t, 2, H2′), 1.8 (m, 3, H9,8), 1.58–1.66 (m, 4, H3′,8′) 1.70–1.80 (m, 9, H12,4′,5′,6′,7′), 1.19 (d, 3, *J* = 6.25 Hz, H14), 0.89 (m, 30, H17, Si-*t*-Bu), 0.08 (m, 18, $SiCH_3$). Anal. ($C_{44}H_{86}O_{10}Si_3$) C, H.

A solution of tri-tBDMS ester (0.97 g, 1.13 mmol) and 1 M Bu_4NF/THF (4 mL, 3.5 equiv) was stirred at room temperature for 18 h. The oily residue obtained after solvent removal was chromatographed on silica gel (125 g) with 7% MeOH/$CHCl_3$: yield, 0.46 g (79%) of **22** with recovery of the C-13 tBDMS keto ester, 0.1 g (13%); ^1H NMR ($CDCl_3$) δ 4.13 (t, 2, *J* = 6.62 Hz, H9′), 3.78–4.00 (m, 4, H13,7,5,6), 3.67 (s, 3, OCH_3), 3.59 (br d, 1, H16), 3.53 (s, 2, H2), 3.50 (m, 1, H16), 2.94 (dd, 1, *J* = 4.78 and 16.17 Hz, H4), 2.48–2.82 (m, 3, H4,10,11), 2.31 (t, 2, *J* = 7.35 Hz, H2′), 2.03 (m, 1, H8), 1.56–1.76 (m, 6, H9,3′,8′), 1.35 (m, 9, H12,4′,5′,6′,7′), 1.22 (d, 3, *J* = 6.25 Hz, H14), 0.94 (d, 3, *J* = 6.99 Hz, H17). Anal. ($C_{26}H_{44}O_{10}$) C, H.

Tri-TES Allylic Aldehyde 23. To a solution of allylic alcohol **9b** (2.7 g, 4 mmol) in 70 mL of CH_2Cl_2 was added 6 wt equiv of MnO_2 (12 g). After the mixture was stirred for 16 h at room temperature, the suspension was filtered through Celite. The oily residue (2.4 g, 88% yield) obtained after solvent evaporation was used immediately for the next step.

1-Bromo-10-tris(methylthio)decane (10c). To a solution of tris(methylthio)methane (10 mL, 75 mmol) in 400 mL of THF was added 2.6 M *n*-BuLi/hexane (34.6 mL, 1.2 equiv) at −78 °C. The mixture was stirred for 1.5 h and 1,9-dibromononane (15.3 mL, 75 mmol) was added all at once. After 45 min, the reaction mixture was quenched with 100 mL of saturated NH_4Cl solution and extracted with Et_2O. The oily residue obtained after solvent removal was chromatographed on silica gel (450 g) with 13% $CHCl_3$/hexane: yield, 13.7 g (50.7%); ^1H NMR ($CDCl_3$) δ 3.41 (t, 2, *J* = 6.8 Hz, H1), 2.11 (s, 9, SCH_3), 1.8–1.9 (m, 4, H8,9), 1.58–1.68 (m, 2, H2), 1.4–1.5 (m, 2, H3), 1.31 (m, 8, H4,5,6,7). Anal. ($C_{13}H_{27}BrS_3$) C, H.

Methyl Pseudomonate Ketone (24). Tri-TES tris(methylthio) allylic alcohol i: To a suspension of 300 mg of Mg turnings (12.3 mmol) in 20 mL of THF at room temperature were added portionwise the bromodecane **10c** (3.8 mL, 12.3 mmol) and a few drops of 1,2-dibromomethane. After stirring for 5 h, 16 mL of

this Grignard reagent was added to a solution of allylic aldehyde **23** (1.2 g, 1.8 mmol) in 10 mL of THF at −78 °C. After 1/2 h, the mixture was quenched with 200 mL of saturated NH_4Cl solution and extracted with 200 mL of Et_2O. The oily residue after solvent removal was chromatographed on silica gel (75 g) with 20% EtOAc/hexane: yield, 1.37 g (80%); ^1H NMR ($CDCl_3$) δ 5.23 (d, 1, H2), 4.48 (m, 1, H1), 3.73–3.95 (m, 4, H13,7,5,6), 3.52 (m, 1, H16), 3.47 (m, 1, H16), 2.67 (m, 2, H10,11), 2.52 (m, 1, H4), 2.10 (s, 9, SCH_3), 1.75–1.95 (m, 7, H8,2′,3′,9), 1.71 (d, 3, J = 0.7 Hz, H15), 1.62 (m, 6, H8′,9′,10′), 1.25–1.42 (m, 9, H12,4′,5′,6′,7′), 1.19 (d, 3, J = 6.25 Hz, H14), 0.92–1.00 (m, 30, H17, $SiCH_3$), 0.55–0.66 (m, 18, $SiCH_2$). Anal. ($C_{48}H_{98}O_6SSi_3$) C, H.

Tri-TES tris(methylthio) enone ii: To a solution of allylic alcohol i (1.1 g, 1.15 mmol) in 50 mL of CH_2Cl_2 was added 6 wt equiv of MnO_2 (6.6 g). After the mixture was stirred for 16 h at room temperature, the suspension was filtered through Celite. The oily residue obtained after solvent removal was chromatographed on silica gel (75 g) with 10% EtOAc/hexane: yield, 1.01 g (92%); ^1H NMR ($CDCl_3$) δ 6.11 (s, 1, H2), 3.82–3.92 (m, 4, H13,7,5,6), 3.54 (d, 1, J = 11.4 Hz, H16), 3.38 (dd, 1, H16), 2.67 (m, 2, H10,11), 2.52 (m, 1, H4), 2.42 (t, 2, J = 7.35 Hz, H10′), 2.15 (s, 3, H15), 2.10 (s, 9, SCH_3), 1.80–2.02 (m, 7, H8,2′,3′,9), 1.62 (m, 4, H8′,9′), 1.25–1.42 (m, 9, H12,4′,5′,6′,7′), 1.19 (d, 3, J = 6.25 Hz, H14), 0.92–1.00 (m, 30, H17, $SiCH_3$), 0.55–0.69 (m, 18, $SiCH_2$). Anal. ($C_{48}H_{96}O_6Si_3S_3$) C, H.

Tris(methylthio) enone iii: A solution of tri-TES enone ii (1 g, 1.05 mmol) and 1 M Bu_4NF/THF (3.4 mL, 3.2 equiv) was stirred at room temperature for 1/2 h. The oily residue obtained after solvent removal was chromatographed on silica gel (120 g) with 5% MeOH/$CHCl_3$; yield, 0.62 g (97%); ^1H NMR ($CDCl_3$) δ 6.14 (s, 1, H2), 3.93 (br t, 1, H7), 3.88 (dd, 1, H16), 3.82 (m, 1, H13), 3.74 (dt, 1, H5), 3.57 (dd, 1, H16), 3.45 (m, 1, H6), 2.81 (dt, 1, H10), 2.72 (dd, 1, H11), 2.58 (dd, 1, H4), 2.42 (t, 2, H10′), 2.2–2.3 (m, 1, H4), 2.18 (d, 3, J = 0.73 Hz, H15), 2.10 (s, 9, SCH_3), 2.02 (m, 1, H8), 1.83–1.9 (m, 2, H2′), 1.73 (m, 2, H9), 1.55–1.68 (m, 6, H3′,8′,9′), 1.29 (m, 9, H4′,5′,6′,7′,12), 1.22 (d, 3, J = 6.25 Hz, H14), 0.95 (d, 3, J = 6.91 Hz, H17); exact mass (FAB) calcd for $C_{30}H_{54}O_6S_3Na$ 629.2980, found 629.2980.

To a solution of tris(methylthio) enone iii (0.45 g, 0.74 mmol) in 12 mL of MeOH at −40 °C were added $HgCl_2$ (0.6 g, 2.2 mmol) and HgO (0.21 g, 0.7 mmol). After stirring for 40 min, the mixture was filtered through Celite with $CHCl_3$ into a cold saturated NH_4Cl solution. The organic layer was separated and evaporated, and the resulting oil was chromatographed on silica gel (75 g) with 5% MeOH/$CHCl_3$: yield, 0.15 g (41%); ^1H NMR ($CDCl_3$) δ 6.14 (s, 1, H2), 3.93 (m, 1, H7), 3.87 (dd, 1, J = 3.12 and 11.77 Hz, H16), 3.82 (m, 1, H13), 3.74 (dt, 1, J = 2.94 and 8.83 Hz, H5), 3.67 (s, 3, OCH_3), 3.55 (dd, 1, J = 2.21 and 11.77 Hz, H16), 3.45 (m, 1, H6), 2.81 (dt, 1, J = 2.21 and 6.26 Hz, H10), 2.71 (dd, 1, J = 2.21 and 8.09 Hz, H11), 2.6 (m, 1, H4), 2.42 (t, 2, J = 7.35 Hz, H10′), 2.30 (t, 2, J = 7.36 Hz, H2′), 2.25 (m, 1, H4), 2.18 (s, 3, H15), 2.02 (7, 1, H8), 1.75 (m, 2, H9), 1.55–1.75 (m, 6, H3′,8′,9′), 1.28–1.38 (m, 9, H12,4′,5′,6′,7′), 1.22 (d, 3, J = 6.25 Hz, H14), 0.95 (d, 3, J = 6.98 Hz, H17); exact mass (FAB) calcd for $C_{28}H_{48}O_8$ 513.3427, found 513.3427.

Tri-TES Methyl Ketone 25. A solution of pseudomonate **7a** (11.2 g, 13.08 mmol) in 125 mL of CH_2Cl_2 and 31 mL of MeOH at −78 °C was ozonized for 40 min. An excess of ozone was blown off by N_2, $P(OEt)_3$ (2.85 mL, 16.62 mmol) was added, and the mixture was stirred for 2 h at room temperature. The oily residue (24 g) obtained after solvent removal was chromatographed on silica gel (400 g) with 10% EtOAc/hexane: yield, 7.3 g (86%); ^1H NMR ($CDCl_3$) δ 4.12 (dt, 1, J = 2.7 and 9.7 Hz, H5), 3.82–3.96 (m, 3, H13,7,6), 3.55 (d, 1, J = 11.03, H8,16), 3.41 (dd, 1, J = 1.84 and 9.20 Hz, H16), 2.65–2.71 (m, 3, H10,11,4), 2.41 (dd, 1, H4), 2.19 (s, 1, H15), 1.80 (br s, 2, H9), 1.5 (m, 1, H8), 1.48 (m, 1, H12), 1.19 (d, 3, H14), 0.94 (m, 30, H17, $SiCH_3$), 0.52–0.66 (m, 18, $SiCH_2$); exact mass (FAB) calcd for $C_{33}H_{68}O_6Si_3Na$ 667.4221, found 667.4215.

Tri-TES Butyl Vinyl Sulfide 27. A solution of [(butylthio)methyl]trimethylsilane (0.74 g, 4.22 mmol) in 5 mL of THF and 2.6 M n-BuLi/hexane (1.62 mL, 4.22 mmol) was stirred at 0 °C for 1 h. The tri-TES ketone **25** (2 g, 3.1 mmol) in 3 mL of THF was then added and the mixture was stirred for 40 min. The mixture was quenched with saturated NH_4Cl solution and extracted with 50 mL of Et_2O. The oily residue obtained after solvent removal was chromatographed on silica gel (200 g) with 5% EtOAc/hexane: yield, Z isomer (less polar fraction) 0.68 g (30%), E isomer 0.7 g (31%); E isomer, ^1H NMR ($CDCl_3$) δ 5.70 (s, 1, H2), 3.72–3.95 (m, 4, H13,7,5,6), 3.52 (d, 1, H16), 3.47 (m, 1, H16, 2.6–2.7 (m, 4, H10,11,4′), 2.45 (d, 1, H4), 1.95 (m, 1, H4), 1.76–1.90 (m, 3, H9,8), 1.75 (s, 1, H15), 1.6 (m, 2, H3′), 1.34–1.5 (m, 3, H2′,12), 1.19 (d, 3, J = 6.25 Hz, H14), 0.89–1.02 (m, 33, H17,1′, $SiCH_3$), 0.52–0.67 (m, 18, $SiCH_2$); exact mass (FAB) calcd for $C_{38}H_{78}O_5Si_3S$ 730.4878, found 730.4882. Z-isomer: ^1H NMR δ 5.73 (s, 1, H2), 1.82 (d, 3, J = 1.11 Hz, H15); exact mass (FAB) found 730.4882.

Butyl Vinyl Sulfide 30a. A solution of tri-TES (E)-sulfide **27** (0.5 g, 0.68 mmol) and 2.26 mL of 1 M Bu_4NF/THF (3.3 equiv) was stirred at room temperature for 1/2 h. The solvent was evaporated and the resulting oil was chromatographed on silica gel (80 g) with 8% i-PrOH/$CHCl_3$: yield, 242 mg (91%); E isomer (**30a**), ^1H NMR ($CDCl_3$) δ 5.77 (d, 1, J = 0.73 Hz, H2), 3.77–3.90 (m, 3, H7,16,13), 3.67–3.75 (m, 1, H5), 3.48–3.58 (m, 2, H16,6), 2.82 (m, 1, H10), 2.68–2.72 (dd, 1, H11), 2.63–2.67 (t, 2, J = 7.36 Hz, H4′), 2.26–2.48 (m, 2, H4), 1.96–2.05 (m, 1, H8), 1.78 (d, 3, J = 0.73 Hz, H15), 1.65–1.77 (m, 2, H9), 1.6 (m, 2, H3′), 1.3–1.48 (m, 3, H2′,12), 1.22 (d, 3, J = 6.25 Hz, H14), 0.89–0.96 (m, 6, H17,1′); exact mass (FAB) calcd for $C_{20}H_{36}O_5S$ 388.2283, found 388.2279. Z isomer **29a**: yield is 88% with use of the above procedure; ^1H NMR ($CDCl_3$) δ 5.75 (d, 1, J = 0.74 Hz, H2), 1.87 (d, 3, J = 0.74 Hz, H15); exact mass (FAB) found 388.2289.

Butyl Vinyl Sulfoxide 31. A solution of sulfide **30a** (0.2 g, 0.5 mmol) in 10 mL of $CHCl_3$ at room temperature was expoxidized with MCPBA (80%) (0.1 g, 1.1 equiv) in 1/2 h. The solvent was evaporated and the resulting oil was chromatographed on silica gel (25 g) with 9% i-PrOH/$CHCl_3$: yield of the less polar isomer (**31a**), 49 mg (23.5%), and of the more polar isomer (**31b**), 51 mg (24.5%); ^1H NMR ($CDCl_3$) (**31a**) δ 6.15 (s, 1, H2), 3.88–3.93 (m, 2, H7,16), 3.69–3.79 (m, 2, H5,13), 3.51–3.56 (m, 2, H16,6), 2.79–2.91 (m, 2, H10,11), 2.62–2.72 (m, 2, H4′), 2.53–2.59 (dd, 1, J = 15.21 and 2.21 Hz, H4), 2.40–2.48 (dd, 1, J = 15.23 and 6.62 Hz, H4), 2.07 (d, 3, J = 0.74 Hz, H15), 2.03 (m, 1, H8), 1.83 (m, 2, H9), 1.65 (m, 2, H3′), 1.4–1.55 (m, 3, H2′,12), 1.23 (d, 3, J = 6.25 Hz, H14), 0.96 (t, 3, J = 7.17 Hz, H1′), 0.87 (d, 3, J = 7 Hz, H17); exact mass (FAB) calcd for $C_{20}H_{37}O_6S$ 405.2311, found 405.2303; ^1H NMr (**31b**) δ 6.09 (s, 1, H2), 2.04 (d, 3, J = 0.74 Hz, H15); exact mass found 405.2303.

Butyl Vinyl Sulfone 32. A solution of sulfide **30a** (0.22 g, 0.56 mmol) in 10 mL of $CHCl_3$ at room temperature was epoxidized with MCPBA (80%) (0.21 g, 2.2 equiv) in 1/2 h. The solvent was removed and the resulting oil was chromatographed on silica gel (50 g) with 9% i-PrOH/$CHCl_3$: yield, 0.1 g (42%); ^1H NMR ($CDCl_3$) δ 6.12 (d, 1, H2), 3.96 (m, 1, H7), 3.88 (dd, 1, H16), 3.82 (m, 1, H13), 3.68 (m, 1, H5), 3.58 (br d, 1, H16), 3.45 (m, 1, H6), 2.95–3.02 (m, 2, H4′), 2.80 (dt, 1, H10), 2.72 (dd, 1, H11), 2.64 (br d, 1, H4), 2.29 (dd, 1, H4), 2.20 (d, 3, H15), 2.02 (m, 2, H8), 1.70–1.82 (m, 4, H9,3′), 1.42–1.53 (m, 3, H2′,12), 1.21 (dd, 3, H14), 0.93 (m, 6, H1′,17); exact mass (FAB) calcd for $C_{20}H_{37}O_7S$ 421.2260, found 421.2269.

Phenyl Vinyl Sulfide 30b. A solution of [(phenylthio)methyl]trimethylsilane (0.45 mL, 1.05 equiv), 2.6 m n-BuLi/hexane (0.87 mL, 0.98 equiv), and 2 mL of THF was stirred at 0 °C for 1/2 h. The tri-TES ketone **25** (1.37 g, 2.12 mmol) in 1 mL of THF was then added and the mixture was stirred for 20 min. The mixture was quenched with saturated NH_4Cl solution and extracted with Et_2O. The oily residue obtained after solvent evaporation was chromatographed on silica gel (100 g) with 3% EtOAc/hexane: yield of both isomers, 1.24 g (78%); ^1H NMR ($CDCl_3$) Z isomer δ 1.93 (dd, 3, H15), E isomer δ 1.88 (dd, 3, H15). To the above tri-TES sulfide (1.24 g) was added 5.1 mL of 1 M Bu_4NF/THF (3.1 equiv) and the mixture was stirred for 11/2 h. The oily residue obtained after solvent removal was chromatographed on silica gel (150 g) with 8% i-PrOH/$CHCl_3$: yield of Z isomer 0.24 g (35%), E isomer 0.27 g (40%); ^1H NMR ($CDCl_3$) E isomer δ 7.17–7.33 (m, 5, ArH), 6.05 (d, 1, J = 0.74 Hz, H2), 3.74–3.90 (m, 4, H7,16,13,5), 3.48–3.60 (m, 2, H16,6), 2.81 (dt, 1, H10), 2.70 (dd, 1, J = 2.3 and 7.89 Hz, H11), 2.56 (dd, 1, H4), 2.48 (dd, 1, H4), 2.03 (m, 1, H8), 1.91 (d, 3, J = 0.74 Hz, H15), 1.73 (m, 2, H9), 1.43 (m, 1, H12), 1.22 (d, 3, J = 6.25 Hz, H14), 0.94 (d, 3, J = 6.99 Hz, H17); exact mass (FAB) calcd for $C_{22}H_{33}O_5S$ 409.2049, found 409.2036. ^1H NMR ($CDCl_3$) Z isomer δ 6.03 (d,

1, J = 1.11 Hz, H2), 1.97 (d, 3, J = 1.11 Hz, H15); exact mass (FAB) found 409.2036.

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Registry No. **3**, 40980-52-7; **7a**, 117407-59-7; **7b**, 117407-79-1; **7c**, 117407-80-4; **8**, 117407-60-0; **9a**, 117407-61-1; **9b**, 117407-81-5; **9c**, 117407-82-6; **9** (R_1 = Et_3Si, X = OBu), 117407-90-6; **10a**, 75452-47-0; **10b**, 117407-85-9; **10c**, 117407-86-0; **11**, 117407-62-2; **12**, 117438-17-2; **13a**, 117407-63-3; **13b**, 117407-83-7; **14a**, 117407-64-4; **14b**, 117407-84-8; **15a**, 117407-65-5; **15b**, 117407-93-9; **16** (isomer 1), 117407-66-6; **16** (isomer 2), 117467-95-5; **17a** (isomer 1), 117407-67-7; **17a** (isomer 2), 117556-67-9; **17b** (isomer 1), 117407-94-0; **17b** (isomer 2), 117556-63-5; **18a**, 117407-68-8; **18b**, 117467-91-1; **19a**, 117407-69-9; **19a** (lactol), 117407-96-2; **19b**, 117407-95-1; **20**, 117407-70-2; **21**, 117407-71-3; **21** (tris t-$BuMe_2Si$ ether), 117407-97-3; **22**, 117407-72-4; **22** (tris t-$BuMe_2Si$ ether), 117407-98-4; **23**, 117407-73-5; **24**, 117407-74-6; **25**, 117407-75-7; **27**, 117438-18-3; **27** (R_1 = Ph), 117467-94-4; **28**, 117556-66-8; **28** (R_1 = Ph), 117408-03-4; **29a**, 117407-76-8; **29b**, 117408-02-3; **30a**, 117467-90-0; **30b**, 117467-92-2; (*R*)-**31**, 117407-77-9; (*S*)-**31**, 117467-93-3; **32**, 117407-99-5; i (isomer 1), 117407-99-5; i (isomer 2), 117556-68-0; ii, 117408-00-1; iii, 117408-01-2; iv, 117407-91-7; v, 117407-92-8; $HO(CH_2)_8CO_2CH_3$, 34957-73-8; $MeSO_2O(CH_2)_8CO_2CH_3$, 117407-87-1; $AcS(CH_2)_8CO_2CH_3$, 117407-88-2; $HS(CH_2)_8CO_2CH_3$, 117407-89-3; $HC(SCH_3)_3$, 5418-86-0; $BuSCH_2SiMe_3$, 18236-28-7; $PhSCH_2SiMe_3$, 17873-08-4; methyl oleate, 112-62-9; isoleucyl-tRNA synthetase, 9030-96-0.

Quinazoline Antifolates Inhibiting Thymidylate Synthase: Synthesis of Four Oligo(L-γ-glutamyl) Conjugates of N^{10}-Propargyl-5,8-dideazafolic Acid and Their Enzyme Inhibition

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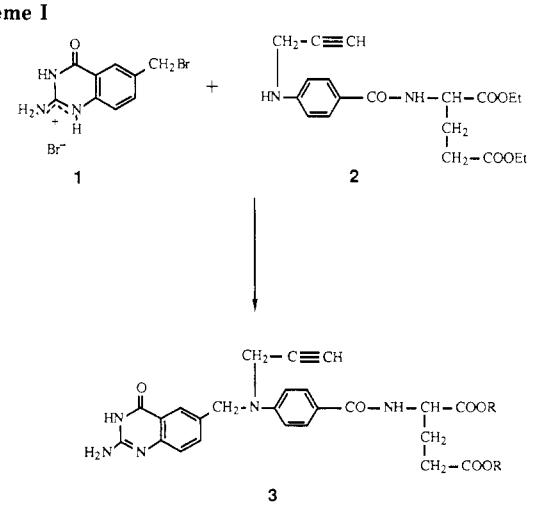
The synthesis is described of four oligo(γ-glutamyl) conjugates of N^{10}-propargyl-5,8-dideazafolic acid containing a total of two, three, four, and five L-glutamic acid residues. The *tert*-butyl group was chosen as the carboxyl protecting group in order to obviate the use of alkali and thus the possibility of γ → α transpeptidation. The starting material, di-*tert*-butyl glutamate, was coupled to *N*-(benzyloxycarbonyl)-L-glutamic acid α-*tert*-butyl ester via a mixed anhydride with isobutyl chloroformate. Hydrogenolysis of the benzyloxycarbonyl group in the product gave a carboxyl-protected diglutamate, which either was acylated with 4-[(benzyloxycarbonyl)amino]benzoyl chloride to give a protected aminobenzamide or was cycled further by using the above mixed anhydride/hydrogenolysis sequence into tri-, tetra-, and pentaglutamates. Each of the last named was also acylated, as above, to give a benzamide. The benzyloxycarbonyl group in the benzamides was removed by hydrogenolysis and the amino groups thus exposed were N-alkylated with propargyl bromide. The resulting proparglyamines were further alkylated with 2-amino-6-(bromomethyl)-4-hydroxyquinazoline hydrobromide to give the antifolate poly(t-Bu) esters. Deprotection with trifluoroacetic acid in the final step delivered the desired antifolates as their trifluoroacetate salts. The di- to pentaglutamates were, respectively, 31-, 97-, 171-, and 167-fold more inhibitory to WI-L2 human thymidylate synthase than the parent compound.

N^{10}-Propargyl-5,8-dideazafolic acid, CB3717,[1] is a novel tight-binding antifolate inhibitor[2] of the enzyme thymidylate synthase (EC 2.1.1.45) that has recently undergone clinical evaluation.[3] Polyglutamation is a known metabolic pathway for both natural folates[4] and classical antifolates,[5] resulting in their increased intracellular retention and enhanced binding to certain folate-metabolizing enzymes. The latter phenomenon has been amply demonstrated for thymidylate synthase.[6] Biochemical and pharmacological studies[7-9] of the polyglutamate derivatives of CB3717—extent of formation, transport characteristics, and role in the antitumor activity of the drug—led to a need for pure reference samples. The synthesis of four conjugates (**30–33**) of CB3717 and their inhibition of human TS is described herein. While our work was in progress, the preparation of these conjugates, by a different synthetic route, and a description of some of their biochemical properties was published by others.[10,11]

Scheme I



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