Synthesis and Structural Properties of the Benzopentathiepins Varacin and Isolissoclinotoxin A

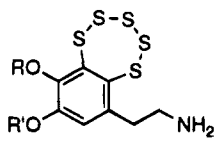
Paul W. Ford, Mathew R. Narbut, Jack Belli, and Bradley S. Davidson*

Department of Chemistry, University of Hawaii at Manoa, Honolulu, Hawaii 96822

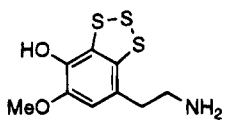
Received June 7, 1994®

The unique pentathiepin-containing compounds varacin (**1**) and isolissoclinotoxin A (**3**) have each been synthesized in eight steps from vanillin. Formation of the pentathiepin ring in varacin was accomplished by treatment of the dithiolate anion, generated from the Na/NH_3 reduction of bis-butyl sulfide intermediate **10**, with 2 equiv of S_2Cl_2. Placement of the sulfur atoms on the aromatic ring was accomplished by treatment of 5,6-dibromovanillin (**6**) with cuprous *n*-butylmercaptide in pyridine/quinoline at 160 °C. The structure of the penultimate intermediate, TEOC-protected varacin (**11**), was confirmed by X-ray diffraction analysis. Isolissoclinotoxin A was prepared in an analogous manner, using a MOM group to protect the phenol. The structure of varacin has been confirmed; however, reductive acetylation of **3** yielded tetraacetate derivative **20**, which was different than that reported as a product of lissoclinotoxin A acetylation. Because the data recorded for **20** did not match that previously reported, it is likely that the structure of lissoclinotoxin A should be reassigned to regioisomer **4**, which has the phenol and methoxy group interchanged.

Organic polysulfides have generated significant interest among organic chemists, initially for their interesting physical properties and potential for synthetic utility[1] and more recently because of the discovery of several unprecedented classes of biologically active, polysulfide-containing natural products. Within the past few years, several unusual dopamine-derived cyclic polysulfides, including varacin (**1**)[2] and lissoclinotoxin A (**2**),[3] have been isolated from *Lissoclinum* tunicates, a genus better known as a source of cyclic peptides.[4] While the structure of varacin (**1**), which bears the first reported naturally occurring pentathiepin ring, was confirmed based on the results of two recently communicated syntheses,[5] the structure proposed for lissoclinotoxin A (**2**) has been questioned. In fact, there have been suggestions that lissoclinotoxin A also bears a pentathiepin ring, as in structure **3**.[5a,6,7]



1 R = R' = Me
3 R = H, R' = Me
4 R = Me, R' = H



2

In addition to having unique structures, varacin and lissoclinotoxin A exhibit significant biological activities. Varacin (**1**) was originally reported to have antifungal and cytotoxic activities, while lissoclinotoxin A was reported to have antimicrobial and antifungal activity.[3] Furthermore, a differential cytotoxicity toward the CHO cell lines EM9 (chlorodeoxyuridine sensitive) versus BR1 (BCNU resistant) indicated that varacin's mechanism of action may involve the formation of single stranded DNA breaks.[2,8]

The challenge in securely assigning the structures of varacin and lissoclinotoxin A is 2-fold. First, it is difficult to determine the number of sulfur atoms in the polysulfide ring, both because sulfur is not easily detected by methods such as NMR and because pentathiepin rings readily lose S_2 in the mass spectrum. This problem is especially significant considering that pentathiepin and trithiole ring systems have been shown to equilibrate under polar protic or basic conditions.[9] Secondly, the ^1H and ^{13}C NMR data provide limited information about the substitution pattern around the aromatic ring, forcing the reliance on either long-range $^1H-^{13}C$ coupling or NOE data for the few protons in the molecule.

In an effort to corroborate the chemical structures proposed for these interesting dopamine-derived polysulfides, as well as to allow further investigation of their chemical and biological properties, we undertook the syntheses of varacin and lissoclinotoxin A. This paper provides complete details of our synthesis of varacin (**1**) along with the synthesis of compound **3**. The results provide unambiguous confirmation of the structures proposed for varacin; however, the spectral data for compound **3** do not match that reported for lissoclinotoxin A, indicating that its structure needs to be reassigned, perhaps as regioisomer **4**.[7] Synthetic compound **3** will be referred to as isolissoclinotoxin A.

Results and Discussion

Pentathiepins have been synthesized using several strategies.[10] Our general approach (see Scheme 1), which easily allows the preparation of both **1** and **3** or a variety of structural analogs, involves the formation of the

® Abstract published in *Advance ACS Abstracts,* September 1, 1994.

(1) (a) Sato, R.; Akutsu, Y.; Goto, T.; Saito, M. *Chem. Lett.* **1987**, 2161. (b) Sato, R.; Onodera, A.; Goto, T.; Saito, M. *Chem. Lett.* **1989**, 2111. (c) Sato, R.; Satoh, S.; Saito, M. *Chem. Lett.* **1990**, 139.

(2) Davidson, B. S.; Molinski, T. F.; Barrows, L. R.; Ireland, C. M. *J. Am. Chem. Soc.* **1991**, *113*, 4709.

(3) Litaudon, M.; Guyot, M. *Tetrahedron Lett.* **1991**, *32*, 911.

(4) Davidson, B. S. *Chem. Rev.* **1993**, *93*, 1771.

(5) (a) Ford, P. W.; Davidson, B. S. *J. Org. Chem.* **1993**, *58*, 4522. (b) Behar, V.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1993**, *115*, 7017.

(6) Faulkner, D. J. *Nat. Prod. Rep.* **1993**, 497.

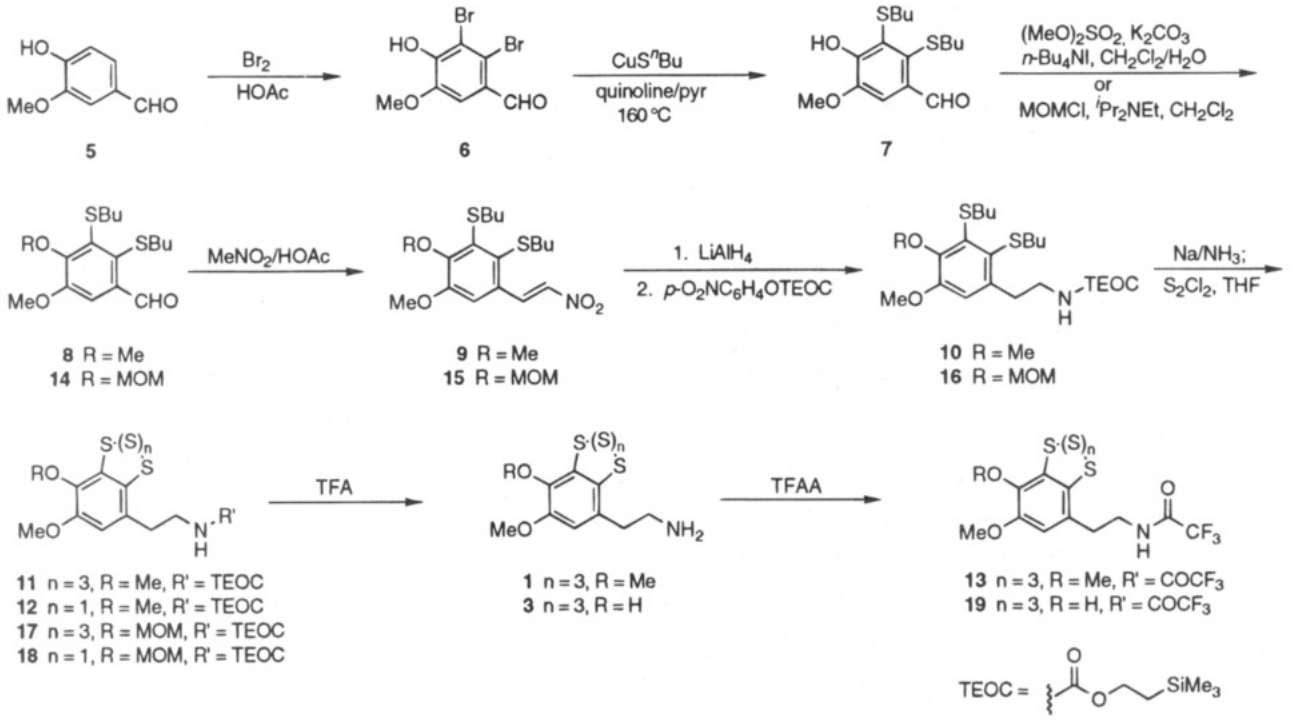
(7) While this manuscript was in review Guyot and co-workers reassigned the structure of lissoclinotoxin A to compound **4**, which not only bears the pentathiepin ring, but also has the positions of the phenol and methoxy groups interchanged with respect to the originally proposed structure (**2**) (Litaudon, M.; Trigalo, F.; Martin, M.-T.; Frappier, F.; Guyot, M. *Tetrahedron* **1994**, *50*, 5323).

(8) Barrows, L. R.; Paxton, M. B.; Kennedy, K. A.; Thompson, L. H. *Carcinogenesis* **1991**, *12*, 805.

(9) Chenard, B. L.; Harlow, R. L.; Johnson, A. L.; Vladuchick, S. A. *J. Am. Chem. Soc.* **1985**, *107*, 3871.

0022-3263/94/1959-5955$04.50/0

Scheme 1



pentathiepin ring by addition of sulfur monochloride (S_2Cl_2) to an appropriate dithiol or dithiolate precursor.[9,10a] Although our initial attempts to place both sulfur atoms on the ring involved electrophilic chemistry of the electron-rich aromatic ring, our most successful methodology involved introduction of the sulfur atoms onto the aromatic ring as sulfides by the nucleophilic substitution of an aromatic dibromoarene with a cuprous *n*-alkylmercaptide.[11]

Synthesis of Varacin. 5,6-Dibromovanillin (**6**) was prepared from vanillin (**5**) in a single step (52%).[12] A NOE experiment showing dipolar coupling between the aromatic proton and the —OMe signal confirmed that the product (**6**) was the desired regioisomer. Treatment of **6** with cuprous *n*-butylmercaptide in quinoline/pyridine at 160 °C[11] then gave compound **7** in 63% yield. Aldehyde **7** served as the branch point for the syntheses of varacin and isolissoclinotoxin A. Methylation of the phenol of **7** followed by treatment of the resulting dimethoxybenzaldehyde (**8**) with nitromethane/NH_4OAc gave nitrostyrene **9** in 79% yield from **7**. Reduction of **9** with $LiAlH_4$ completed installation of the phenethylamine side chain, providing the primary amine, which was protected with a [β-(trimethylsilyl)ethoxy]carbonyl (TEOC) group,[13] giving **10** in 57% from **9**. Treatment of **10** with Na/NH_3 cleanly removed the *n*-butyl groups to give a dithiolate anion,[14] which, upon addition of 2 equiv of S_2Cl_2, provided the major product (**11**) in 47% yield.

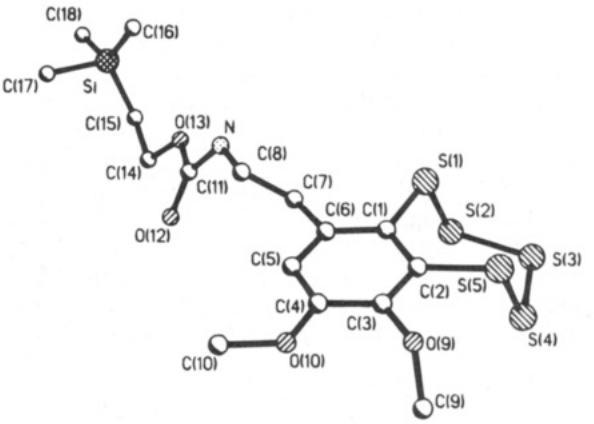


Figure 1. Crystal structure of compound **11**. Hydrogens are omitted for clarity.

Although the structure of compound **11** was assigned on the basis of HRMS data, which indicated a molecular formula of $C_{16}H_{25}NO_4S_5Si$, ultimate confirmation was provided by X-ray diffraction analysis.[15] The computer representation (Figure 1) clearly shows the presence of the pentathiepin ring which exists in the expected chair conformation.[16] The ^1H NMR spectrum of **11** showed the expected signals, but also provided some particularly interesting information. The signals assigned to the side chain methylene protons were observed as complex multiplets, rather than simple multiplets as would be expected. Our initial explanation for this unforeseen signal complexity was that the large pentathiepin ring

(10) (a) Feher, F.; Langer, M. *Tetrahedron Lett.* **1971**, 2125. (b) Feher, F.; Langer, M.; Volkert, R. *Z. Naturforsch. B* **1972**, *27*, 1006. (c) Chenard, B. L.; Miller, T. J. *J. Org. Chem.* **1984**, *49*, 1221. (d) Sato, R.; Saito, S.; Chiba, H.; Goto, T.; Saito, M. *Chem. Lett.* **1986**, 349. (e) Gronowitz, S.; Moses, P.; Hornfeldt, A. *Ark. Kemi* **1960**, *17*, 237.

(11) Adams, R.; Reifschneider, W.; Ferretti, A. *Organic Syntheses;* Wiley: New York, 1973; Collect. Vol. 5, p 107.

(12) Kubo, I.; Ochi, M.; Shibata, K.; Hanke, F. J.; Nakatsu, T.; Tan, K.-S.; Taniguchi, M.; Kamikawa, T.; Yamagiwa, Y.; Arizuka, M.; Wood, W. F. *J. Nat. Prod.* **1990**, *53*, 50.

(13) (a) Carpino, L. A.; Tsao, J.-H.; Ringsdorf, H.; Fell, E.; Hettrich, G. *J. Chem. Soc., Chem. Commun.* **1978**, 358. (b) Wunsch, E.; Moroder, L.; Keller, O. *Hoppe-Seyler's Z. Physiol. Chem.* **1981**, *362*, 1289.

(14) Adams, R.; Rerretti, A. *J. Am. Chem. Soc.* **1959**, *81*, 4939.

(15) The author has deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB 1EZ, UK.

(16) Chenard, B. L.; Dixon, D. A.; Harlow, R. L.; Roe, D. C.; Fukunaga, T. *J. Org. Chem.* **1987**, *52*, 2411–2420.

hindered the rotations around the side-chain bonds;[5a] however, we have recently provided evidence that a high barrier to interconversion of the low-energy chair conformations of the pentathiepin ring induces asymmetry into the molecule, causing these protons to become diastereotopic.[17,18]

Typically, treatment of the dithiolate anion with S_2Cl_2 yielded a second compound (**12**), which was related to benzopentathiepin **11**. While the NMR spectra for **11** and **12** were similar, significant differences allowed them to be easily distinguished. For example, the chemical shift of the lone aromatic proton in the spectrum of **12** was δ 6.44, upfield of the analogous proton in the spectrum of **11** (6.77 ppm). Also, the phenethylamine side-chain methylene protons in the spectrum of **12** exhibited the expected coupling patterns, compared to the complex diastereotopic signals observed for **11**. In addition, the UV spectra were characteristic, with pentathiepin **11** showing an absorption maximum at 209 nm, with a strong shoulder at 245 nm, while the spectrum of **12** shows an absorption at 206 nm, but lacks any significant absorption at a wavelength greater than 235 nm. Finally, the HRMS data indicated a molecular formula of $C_{16}H_{25}NO_4S_3Si$, confirming that compound **12** was the corresponding trithiole.

Exposure of **11** to TFA in chloroform provided the trifluoroacetate salt of varacin (**1**) in 86% yield. The spectroscopic data obtained for the salt of synthetic **1** matched that reported for the natural product.[2,19] Significant differences were observed in the ^{1}H NMR spectra of the salt versus the free base, which could be prepared by treatment of the TFA salt with solid K_2CO_3 in a $CHCl_3$ solution, although extended treatment often resulted in the formation of small amounts of the trithiole analog. Further confirmation of the pentathiepin ring was provided by HRMS of *N*-trifluoroacetamide derivative **13**, formed by treatment of **1** with TFAA in CH_2Cl_2 at 160 °C, which gave a molecular ion consistent with a molecular formula of $C_{12}H_{12}NO_3S_5F_3$.

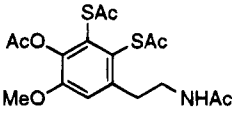
In contrast, deprotection of **12** yielded a product, which was unstable, rapidly degrading to several uncharacterized products. The observed lability of the trithiole ring system upon deprotection of the primary amine is consistent with reports that the equilibration of pentathiepin/trithiole ring systems can be base-catalyzed.[8] It is likely that, once deprotected, the free amine may autocatalyze such an equilibration; however, in the absence of external sulfur or a pentathiepin ring, it is not clear to what the trithiole is degrading.

Synthesis of Isolissoclinotoxin A. The synthetic methodology developed for varacin could be easily adapted for the synthesis of isolissoclinotoxin A (**3**). In fact, the only difference is the use of a MOM group for the protection of the phenol, which is required for compound **3**. Treatment of **7** with MOM chloride and diisopropylethylamine in CH_2Cl_2 provided protected phenol **14** in 97% yield. Extension of the side chain was accomplished in the same manner as before. Treatment of the aldehyde with CH_3NO_2 gave nitrostyrene **15** (81%), which was reduced with $LiAlH_4$ and protected to give **16** in 48% yield from **15**. Reduction of the SBu groups with Na/NH_3 followed directly by treatment of the resulting dithiolate anion with S_2Cl_2 provided a 3:2 mixture of **17** and **18** in 80% yield that could be separated using reversed-phase HPLC.

Compounds **17** and **18** exhibited many spectral characteristics which paralleled those observed for TEOC-protected varacin intermediates **11** and **12**, respectively. For example, the ^{1}H NMR spectrum of **18** showed simple multiplets for the phenethylamine side chain protons, while the analogous signals in the spectrum of **17** were more complex. Interestingly, the methylene protons of the MOM protecting group were observed as an AB spin system, supporting the hypothesis that the rigidity of the pentathiepin ring induces asymmetry into the molecule.[17]

Simultaneous deprotection of the phenol and the primary amine of **17** with TFA yielded the TFA salt of isolissoclinotoxin A (**3**) in 94% yield. Further confirmation of the pentathiepin ring was obtained by treatment of **3** with TFAA, under conditions identical to those used for varacin, providing the *N*-trifluoroacetamide derivative **19** with only a trace of the diacetylated product. HRMS of compound **19** indicated a molecular formula of $C_{11}H_{10}NO_3S_5F_3$, consistent with the presence of the pentathiepin ring.

Because the ^{1}H NMR data of synthetic **3** were also very similar to that reported for lissoclinotoxin A, compound **3** was treated with $NaBH_4$ followed by acetic anhydride to yield tetraacetate **20**, for which a more complete set



20

of spectral data had been reported.[3] While the ^{1}H NMR data were similar, the ^{13}C NMR data clearly distinguished compound **20** from the lissoclinotoxin A derivative formed through treatment with acetic anhydride and DMAP in DMF.[3] The most significant difference was the chemical shift of the protonated aromatic carbon which occurs at δ 115.4 in the spectrum of **20** and was reported at δ 127.4 for the tetraacetate of lissoclinotoxin A.[3]

Conclusions

We have developed a convenient route useful for the preparation of varacin (**1**), isolissoclinotoxin A (**3**), and a variety of related benzopentathiepin analogs. Confirmation of the proposed structure of varacin was accomplished by complete spectral characterization of two derivatives of **1** (**11** and **13**), including an X-ray diffraction analysis of TEOC-protected varacin **11** and a direct comparison of penultimate pentathiepin precursor **11** with the protected trithiole **12**. Removal of the amino protecting group with TFA provided the ammonium salts of two easily distinguishable compounds, of which one (**1**) matched the natural product, while the other proved to be unstable. The structure of isolissoclinotoxin A was also established by spectral analysis of derivatives **17** and

(17) Davidson, B. S.; Ford, P. W.; Wahlman, M. *Tetrahedron Lett.*, in press.

(18) The ^{1}H NMR spectra for acyclic trisulfides having the general structure $-CH_2SSS-$, including natural products such as the esperamicins (Golik, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B. Ohkuma, H.; Saitoh, K. Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3462) and calichemicins (Lee, M. L.; Dunne, T. S.; Chang, C. C.; Ellestad, G. A.; Siegel, M. M.; Morton, G. O.; McGahren, W. J.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3466), also contain diastereotopic methylene signals presumably resulting from conformational stablility within the polysulfide chain (Block, E.; Iyer, R.; Grisoni, S.; Saha, C.; Belman, S.; Lossing, F. P. *J. Am. Chem. Soc.* **1988**, *110*, 7813).

(19) It is likely that the data reported for varacin are actually for the TFA salt, because final HPLC purification was performed using a solvent system containing aqueous TFA.

19. Reductive acetylation of **3** yielded tetraacetate derivative **20**, which was previously reported as a product of lissoclinotoxin A acetylation. Because the data recorded for **20** did not match that previously reported, it is likely that the structure of lissoclinotoxin A should be reassigned to **4**.[7]

Experimental Section

General. Unless otherwise noted, materials and solvents were obtained from commercial suppliers and used without further purification. THF and ethyl ether were distilled from Na/benzophenone immediately prior to use. Anhydrous solvents and solutions were added using syringes. Chromatography was carried out using silica gel, Merck 60 (60 A), 230–400 mesh, according to the procedure described by Still.[20] Reactions and chromatography fractions were analyzed using TLC on 2 × 5 cm aluminum-backed plates covered with a 0.20 mm layer of silica gel 60 F_{254}, Art. 5554 (E. Merck, Darmstadt). UV light and ninhydrin–ethanol solution followed by heating were used for visualization. Melting points were determined on a Laboratory Devices Mel-Temp II apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR spectrophotometer. UV spectra were obtained on either a Shimadzu UV-101 PC or a Perkin-Elmer 280 spectrophotometer in the solvent indicated at 25 °C. NMR spectra were recorded on either a General Electric QE-300 or GE GN-Omega 500 spectrometer in $CDCl_3$ (7.26 and 77.0 ppm) or in other solvents as indicated. Mass spectra were recorded on a VG Analytical 70SE (EI) mass spectrometer. For new compounds purity was ascertained by both proton and ^{13}C NMR spectra on samples obtained by preparative chromatographic purification.

5,6-Dibromo-4-hydroxy-3-methoxybenzaldehyde (6). To a solution of vanillin (**5**) (100 g, 0.6 mol) in glacial acetic acid (200 mL) was added 1 equiv of Br_2 (33.8 mL). The reaction was stirred for 45 min, during which time a fine white precipitate formed. Following the addition of a catalytic amount of powdered iron (200 mg), an additional 2.1 equiv of bromine (56.1 mL) was slowly added, causing the precipitate to eventually dissolve. The mixture was heated at reflux overnight, cooled to room temperature, and filtered. The air-dried precipitate was dissolved in acetone and filtered to remove the catalyst. Evaporation of the solvent and recrystallizaton of the product in methanol yielded the desired product (**6**) as a white solid (74.5 g, 52%, mp 230 °C): IR ν_{max} (film) 3423, 2340, 1653, 1587, 1558 cm^{-1}; UV (MeOH) λ_{max} 292, 238, 216 nm; ^1H NMR δ (CD_3SOCD_3) 11.20 (s, 1H), 10.03 (s, 1H), 7.35 (s, 1H), 3.89 (s, 3H); ^{13}C NMR δ (CD_3SOCD_3) 190.71, 151.19, 147.36, 126.21, 122.17, 113.80, 110.27, 56.35; HREIMS calcd for $C_8H_6Br_2O_3$ 309.8664, found 309.8605.

Reaction of Cuprous *n*-Butylmercaptide with 5,6-Dibromovanillin (6). Dibromovanillin (**6**) (4.0 g, 12.9 mmol) and cuprous *n*-butylmercaptide (6.96 g, 45.2 mmol) were added to a well-stirred solution of quinoline/pyridine (4:1, 200 mL), and the resulting white slurry was heated to 160 °C. The reaction darkened and, after approximately 2 h of heating, became homogeneous. When the starting material (**6**) was consumed, as monitored by TLC, the reaction mixture was cooled and poured into ice cold 50% aqueous HCl (750 mL). The resulting mixture was extracted with EtOAc (4 × 250 mL), and the combined organic extract was washed successively with 25% HCl, H_2O, and brine. It was then dried (Na_2SO_4) and concentrated to give a dark brown oil. Flash chromatography over silica gel (3:1, hexane/EtOAc) provided **7** (2.65g, 63% yield) as a clear brown oil that solidified to a waxy solid but could not be recrystallized: IR ν_{max} (film) 3305, 2958, 1672, 1580, 1454, 1370 cm^{-1}; UV (MeOH) λ_{max} 278, 245, 228 nm; ^1H NMR δ ($CDCl_3$) 10.68 (s,1H), 7.58 (s, 1H), 7.47 (s, 1H), 3.98 (s, 3H), 2.84 (q, 4H, J = 6.8 Hz), 1.51 (sextet, 4H, J = 7.8 Hz), 1.39 (septet, 4H, J = 7.4 Hz), 0.86 (q, 6H, J = 6.8 Hz); ^{13}C NMR δ ($CDCl_3$) 192.23, 152.77, 147.82, 137.89, 132.43, 126.15, 110.67, 56.19, 38.74, 36.28, 31.64, 31.41, 21.92 (×2C), 13.58 (×2C); HREIMS found 328.1175, calculated for $C_{16}H_{24}O_3S_2$ 328.1167.

2,3-Bis(*n*-butylthio)-4,5-dimethoxybenzaldehyde (8). Compound **7** (100 mg, 0.03 mmol) was combined with dimethyl sulfate (115 mg, 0.91 mmol), K_2CO_3 (46.3 mg, 0.34 mmol), tetra-*n*-butylammonium iodide (29.5 mg, 0.08 mmol), dichloromethane (5 mL), and H_2O (5 mL). The resulting heterogeneous mixture was stirred vigorously at room temperature for 3 h, at which time the reaction appeared complete, as monitored by TLC. The organic layer was decanted, and the aqueous layer was extracted with dichloromethane (2 × 50 mL). The combined organic extract was concentrated, and the residue was resuspended in H_2O and extracted with diethyl ether (3 × 50 mL). The combined ether extract was washed with 2 N ammonia solution, H_2O, and brine. It was then dried (Na_2SO_4) and concentrated to give **8** (103 mg, 99% yield) as a brown oil: IR ν_{max} (film) 2957, 2931, 2871, 1685, 1570, 1458, 1364, 1076 cm^{-1}; ^1H NMR δ ($CDCl_3$) 10.67 (s, 1H), 7.37 (s, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 2.93 (t, 2H, J = 7.3 Hz), 2.77 (t, 2H, J = 7.3 Hz), 1.44 (m, 4H), 1.33 (m, 4H), 0.80 (dt, 6H, J = 7.2, 3.3 Hz); ^{13}C NMR δ ($CDCl_3$) 192.47, 155.03, 153.27, 137.02, 135.63, 135.29, 110.14, 60.14, 55.71, 38.03, 35.10, 31.64, 31.19, 21.74 (×2C), 13.41 (×2C); HREIMS calcd for $C_{17}H_{26}O_3S_2$ 342.1323, found 342.1301.

3,4-Bis(*n*-butylthio)-1,2-dimethoxy-5-(2-nitroethenyl)-benzene (9). A mixture of **8** (1.35 g, 3.95 mmol), CH_3NO_2 (20 mL), and NH_4OAc (0.30 g, 3.90 mmol) was stirred at 100 °C for 6 h, at which time the solution was poured into ice-water (75 mL). The aqueous mixture was extracted with diethyl ether (2 × 50 mL), and the combined organic extracts were washed with H_2O and brine and dried (Na_2SO_4). Purification with flash chromatography (3:1 Hex/EtOac) provided **9** as a brown/yellow oil (1.22 g, 80% yield): IR ν_{max} (film) 2958, 2872, 1519, 1339, 1080 cm^{-1}; ^1H NMR δ ($CDCl_3$) 8.83 (d, 1H, J = 13.7 Hz), 7.46 (d, 1H, J = 13.7 Hz), 7.00 (s, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 2.92 (t, 2H, J = 7.3 Hz), 2.71 (t, 2H, J = 7.1 Hz), 1.41 (m, 4H), 1.34 (m, 4H), 0.79 (sextet, 6H, J = 3.5 Hz); ^{13}C NMR δ ($CDCl_3$) 153.17, 138.75, 137.57, 137.40, 134.14, 133.84, 130.99, 109.63, 60.08, 55.77, 37.54, 35.10, 31.63, 31.12, 21.67 (×2C), 13.37, 13.34; HREIMS calcd for $C_{18}H_{27}NO_4S_2$ 385.1382, found 385.1417.

2,3-Bis(butylthio)-1,2-dimethoxy-5-[2-[[[2-(trimethylsilyl)ethoxy]carbonyl]amino]ethyl]benzene (10). To a stirred slurry of LAH (152 mg, 4.0 mmol) in anhydrous diethyl ether (40 mL) under an atmosphere of argon was added a solution of β-nitrostyrene **9** (440 mg, 1.14 mmol) in ether (2 mL). After heating for 1 h at reflux, the reaction was cooled in an ice bath. After the excess LAH was quenched with H_2O (carefully!), 3 N HCl was added until most of the salts dissolved. The solution was stirred for 30 min at which time it was extracted with ether (2 × 50 mL) and ethyl acetate (3 × 50 mL), providing the crude amine salt (300 mg) after concentration of the combined organic extracts. The crude product was dissolved in dry THF (20 mL), and to the resulting solution was added Et_3N (0.5 mL) and 2-(trimethylsilyl)ethyl *p*-nitrophenyl carbonate (216 mg, 0.76 mmol). The reaction mixture was stirred under argon at room temperature for 16 h. The THF was removed under vacuum and the resulting yellow oil chromatographed over silica gel (3:1 Hex/EtOac) to give **10** as a colorless oil (325 mg, 57% yield from **9**): IR ν_{max} (film) 3346, 2955, 1703, 1459, 1249, 837 cm^{-1}; ^1H NMR δ ($CDCl_3$) 6.71 (s, 1H), 4.80 (br t, 1H), 4.11 (t, 2H, J = 8.3 Hz), 3.81 (s, 3H), 3.79 (s, 3H), 3.34 (q, 2H, J = 6.6 Hz), 3.07 (t, 2H, J = 7.2 Hz), 2.97 (t, 2H, J = 7.2 Hz), 2.73 (t, 2H, J = 7.2 Hz), 1.49 (hex, 4H, J = 7.1 Hz), 1.36 (hex, 4H, J = 7.0 Hz), 0.93 (t, 3H, J = 8.3 Hz), 0.84 (dt, 6H, J = 7.3, 2.5 Hz), −0.01 (s, 9H); ^{13}C NMR δ ($CDCl_3$) 156.64, 152.93, 148.96, 139.95, 136.60, 129.10, 112.75, 62.75, 59.83, 55.64, 42.19, 37.12, 36.14, 35.24, 31.80, 31.37, 22.06, 21.91, 17.68, 13.59 (×2C), −1.60 ppm; HREIMS calcd for $C_{24}H_{43}NO_4S_2Si$ 501.2403, found 501.2384.

6,7-Dimethoxy-9-[2-[[[2-(trimethylsilyl)ethoxy]carbonyl]amino]ethyl]benzopentathiepin (11) and 4,5-Dimethoxy-7-[2-[[[2-(trimethylsilyl)ethoxy]carbonyl]amino]-ethyl]benzotrithiole (12). Ammonia (40 mL) was condensed into a 100 mL two-necked round bottom flask outfitted with a

(20) Still, W. C.; Kahn, M.; Mitra, J. *J. Org. Chem.* **1978**, 43, 2923.

dry-ice condenser, which contained a solution of TEOC-protected amine **10** (140 mg, 0.28 mmol) in anhydrous ethyl ether (3 mL). The reaction was cooled using a CH_3CN/dry ice bath (−42 °C), and good stirring was provided. Small pieces of sodium metal were added until the solution remained blue for 20 min (5−6 equiv of Na). The reaction was then quenched by the careful addition of solid NH_4Cl until the blue color dissipated. The ammonia was allowed to evaporate under a stream of argon, and the white, solid residue was dissolved in dry THF and cooled to 0 °C under argon with stirring. A solution of S_2Cl_2 (79 mg, 0.59 mmol) in methylene chloride was added dropwise over 10 min. The reaction was then allowed to warm to room temperature, and stirring was continued for an additional 45 min. The THF was removed under reduced pressure to give a yellow residue, which was dissolved in CH_2Cl_2 and filtered through Celite. The clear yellow solution was then washed with 5% $NaHCO_3$ (50 mL), H_2O (50 mL), and brine (50 mL), dried (Na_2SO_4), and concentrated to give the crude product as a viscous yellow oil. Purification of the products could be accomplished using either flash chromatography (hexane/EtOAc, 2:1) or reversed-phase HPLC (Rainin Microsorb C18, 5 μm, 10 × 250 mm, 100% CH_3CN) to provide pure **11** (64 mg, 47%) and **12** (23 mg, 20%) as a pale yellow oil and a dark yellow oil, respectively. **11**: UV (MeOH) λ_{max} 208, 249 (shoulder) nm; ^1H NMR δ ($CDCl_3$) 6.77 (s, 1H), 4.74 (br s, 1H), 4.13 (dd, 2H, J = 8.5, 8.5 Hz), 3.87 (s, 3H), 3.83 (s, 3H), 3.36 (m, 2H), 3.11 (m, 2H), 0.95 (t, 2H, J = 8.5 Hz), 0.02 (s, 9H); ^{13}C NMR δ ($CDCl_3$) 156.72, 154.77, 149.53, 151.07, 134.96, 115.52, 63.15, 61.89, 56.20, 42.19, 37.00, 17.79, −1.47; HREIMS found 483.0149, calcd for $C_{16}H_{25}O_4NS_5Si$ 483.0156. **12**: UV (MeOH) λ_{max} 205, 230 (shoulder) nm; ^1H NMR δ ($CDCl_3$) 6.44, (s, 1H), 4.69 (br s, 1H), 4.13 (dd, 2H, J = 8.5, 8.5 Hz), 3.85 (s, 3H), 3.81 (s, 3H), 3.36 (q, 2H, J = 6.5 Hz), 2.79 (t, 2H, J = 6.9 Hz), 0.95 (t, J = 8.4 Hz), 0.01 (s, 9H); ^{13}C NMR δ ($CDCl_3$) 156.68, 152.42, 132.56, 130.31, 112.24, 63.15, 60.64, 56.36, 40.97, 37.60, 17.79, −1.47; HREIMS calcd for $C_{16}H_{25}O_4NS_3Si$ 419.0715, found 419.0706.

X-ray Analysis of Compound 11. A crystal of **11** measuring approximately 0.5 × 0.4 × 0.1 mm^3 was selected and aligned on a Nicolet P3 diffractometer system. Preliminary X-ray photographs displayed monoclinic symmetry, and accurate lattice constants of a = 15.908(12) Å, b = 17.328(13) Å, and c = 8.784(3) Å were determined from a least-squares fit of 16 diffractometer-measured 2q values. The emperical formula was $C_{16}H_{25}NO_4S_5Si$. The crystal density, 1.335 g/cm^3, indicated that four molecules of **11** made up the unit cell. Systematic extinctions were consistent with the space group $P2_1/c$ (with four molecules per unit cell). All data were collected using variable speed (1.00−10.00°/min in ω) scans and a graphite-monochromated Mo Kα radiation (λ = 0.710 73 Å). Of the 2574 reflections collected, 1456 were judged observed ($F > 6.0\sigma(F_o)$) after corrections. Employing the Siemens Shelxtlplus (VMS) program all non-hydrogen atoms were located by direct methods. Full-matrix least-squares refinements (with anisotropic non-hydrogen atoms) converged to a crystallographic residual of 0.0606 (R_w = 0.0596) for the observed data.

Deprotection of 11 To Give the Trifluoroacetate Salt of 1. To a solution of compound **11** (52.4 mg, 0.11 mmol) in $CHCl_3$ (3 mL) was added TFA (200 μL). The reaction was stirred at room temperature for 1 h, at which time the solvent and excess TFA were removed under vacuum, yielding the TFA salt of **1** (42.4 mg, 86%) as a colorless glass. TFA salt of **1**: UV (MEOH) λ_{max} 212, 244 (sh) nm; ^1H NMR δ (CD_3OD) 7.06 (s, 1H), 3.94 (s, 3H), 3.81 (s, 3H), 3.32 (m, 1H), 3.22 (m, 1H), 3.14 (m, 2H); ^{13}C NMR δ (CD_3OD) 156.56, 151.38, 142.10, 140.26, 136.16, 117.17, 62.19, 56.91, 41.67, 35.14.

Formation of Free Base of 1. The free base of **1** was prepared by treating the TFA salt of **1** with solid K_2CO_3 in $CHCl_3$, followed by filtration and solvent removal. **1** (free base): ^1H NMR δ (CD_3OD) 7.05, (s, 1H), 3.93 (s, 3H), 3.80 (s, 3H), 3.19 (ddd, 1H, J = 12.9, 9.3, 6.2 Hz), 3.08 (ddd, 1H; J = 12.9, 8.8, 6.6 Hz), 2.93 (m, 2H); ^{13}C NMR δ (CD_3OD) 156.47, 150.84, 142.50, 141.66, 136.00, 117.26, 62.16, 56.82, 43.59, 38.97.

6,7-Dimethoxy-9-[2-[*N*-(trifluoroacetyl)amino]ethyl]-benzopentathiepin (13). The TFA amide (**13**) was obtained by heating a CH_2Cl_2 solution of **1** with excess TFAA for 5 min at 160 °C in a sealed pressure tube. Evaporation of the solvent and excess trifluoracetic anhydride provided pure **13**: ^1H NMR δ ($CDCl_3$) 6.74 (s, 1H), 6.42 (br s, 1H), 3.88 (s, 1H), 3.86 (s, 1H), 3.59, (m, 2H), 3.20 (m, 2H); ^{13}C NMR δ ($CDCl_3$); HREIMS calcd for $C_{12}H_{12}O_3NS_5F_3$ 434.9373, found 434.9370.

2,3-Bis(*n*-butylthio)-4-(methoxymethoxy)-5-methoxybenzaldehyde (14). Aldehyde **7** (270 mg, 0.82 mmol) was dissolved in CH_2Cl_2 (10 mL) along with diisopropylethylamine (425 mg, 3.3 mmol), and the resulting solution was cooled to 0 °C in a NaCl/ice bath. Chloromethyl methyl ether (263 mg, 3.3 mmol) was added dropwise over 5 min, and the solution was stirred for 20 min, at which time the reaction appeared complete by TLC. The reaction mixture was then concentrated and subjected to flash chromatography (silica gel, 3:1 Hex/EtOac), yielding desired product **14** (297 mg, 97%) as a tan oil: IR ν_{max} (film) 2959, 2872, 1685, 1570, 1364, 1160, 1076, 946, 756 cm^{-1}; ^1H NMR δ ($CDCl_3$) 10.74 (s, 1H), 7.44 (s, 1H), 5.27 (s, 2H), 3.91 (s, 3H), 3.66 (s, 3H), 2.99 (t, 2H J = 7.3 Hz), 2.83 (t, 2H J = 7.3 Hz), 1.50 (m, 4H), 1.40 (m, 4H), 0.87 (dt, 6H J = 7.2 and 2.2 Hz); ^{13}C NMR δ ($CDCl_3$) 192.59, 153.12, 152.18, 137.28, 136.25, 135.48, 110.26, 98.69, 57.81, 55.81, 38.27, 35.29, 31.62, 31.27, 21.83 (×2C), 13.50 (×2C); HREIMS calculated for $C_{18}H_{28}O_4S_2$ 372.1411, found 372.1409.

3,4-Bis(*n*-butylthio)-1-methoxy-2-(methoxymethoxy)-5-(2-nitroethenyl)benzene (15). The same procedure for the preparation of nitrostyrene **9** was employed for the synthesis of compound **15**. Thus, from 297 mg (0.80 mmol) of **8** was obtained 269 mg (81% yield) of compound **15** as a dark yellow oil after flash chromatography (3:1 hex/EtOac): IR ν_{max} (film) 2960, 2931, 2873, 1626, 1519, 1340, 1265, 1080, 949, 738 cm^{-1}; ^1H NMR δ ($CDCl_3$) 8.91 (d, 1H J = 13.7 Hz), 7.47 (d, 1H J = 13.7 Hz), 6.99 (s, 1H), 5.24 (s, 2H), 3.91 (s, 3H), 3.66 (s, 3H), 2.99 (t, 2H J = 7.1 Hz), 2.78 (t, 2H J = 7.1 Hz), 1.49 (m, 4H), 1.41 (m, 4H), 0.88 (dt, 6H J = 7.1, 1.3 Hz); ^{13}C NMR δ ($CDCl_3$) 153.11, 150.25, 138.95, 137.54, 134.63, 131.29, 109.68, 98.71, 57.87, 55.90, 37.82, 35.37, 31.66, 31.28, 21.87, 21.83, 13.54, 13.51; EIMS m/z (rel intensity) 415 (M^+, 1), 383 (38), 372 (55), 369 (100), 328 (25), 281 (80), 255 (55), 225 (82), 211 (90), 211 (90).

3,4-Bis(*n*-butylthio)-1-methoxy-2-(methoxymethoxy)-5-[2-[[[2-(trimethylsilyl)ethoxy]carbonyl]amino]ethyl]-benzene (16). To a slurry of LAH (41.7 mg, 1.1 mmol) in dry ethyl ether (20 mL) under an atmosphere of argon was added a solution of β-nitrostyrene **15** (265 mg, 0.64 mmol) in ether (3 mL). After being heated for 1.5 h at reflux, the reaction mixture was cooled in an ice bath. Sodium fluoride (1 g, 23.8 mmol, powdered) was added followed by dropwise addition of H_2O (0.3 g, 16.7 mmol). The reaction mixture was then stirred vigorously for 45 min. The crystalline precipitate was removed by filtration and washed with ether (4 × 25 mL), and the filtrate was concentrated to give a clear oil (150 mg). The crude product was dissolved in dry THF (10 mL), and to the resulting solution was added triethylamine (157 mg, 1.6 mmol) and 2-(trimethylsilyl)ethyl p-nitrophenyl carbonate (121 mg, 0.43 mmol). The mixture was stirred under argon at room temperature for 16 h. The THF was then removed under vacuum and the resulting yellow oil chromatographed on silica gel (Merck 60) with 3:1 hex/EtOac . The product **16** was obtained as a colorless oil (164 mg, 48% yield from **15**): IR ν_{max} (film) 3345, 2955, 1711, 1530, 1453, 1249, 964, 837 cm^{-1}; ^1H NMR δ ($CDCl_3$) 6.73 (s, 1H), 5.13 (s, 2H), 4.78 (br t, 1H), 4.13 (t, 2H J = 7.8 Hz), 3.82 (s, 3H), 3.64 (s, 3H), 3.36 (q, 2H J = 6.3 Hz), 3.09 (t, 2H J = 7.0 Hz), 2.97 (t, 2H J = 7.2 Hz), 2.74 (t, 2H J = 7.2 Hz), 1.49 (sextet, 4H J = 7.5 Hz), 1.38 (m, 4H), 0.95 (t, 2H J = 8.3 Hz), 0.86 (dt, 6H J = 7.3, 3.1 Hz), 0.01 (s, 9H); ^{13}C NMR δ ($CDCl_3$) 156.71, 152.92, 145.93, 140.24, 136.90, 129.93, 112.95, 98.51, 62.84, 57.72, 55.70, 42.15, 37.34, 36.26, 35.42, 31.73, 31.44, 22.11, 21.98, 17.74, 13.64 (×2C), −1.54 (×3C); HREIMS calcd for $C_{25}H_{45}O_5S_2NSi$ 531.2508, found 531.2493.

6-Methoxy-7-(methoxymethoxy)-9-[2-[[[2-(trimethylsilyl)ethoxy]carbonyl]amino)ethyl]benzopentathiepin (17) and 4-Methoxy-5-(methoxymethoxy)-7-[2-[[[2-(trimeth-

ylsilyl)ethoxy]carbonyl]amino]ethyl]benzotrithiole (18). The same procedure used for formation of compounds **11** and **12** was utilized in the synthesis of **17** and **18**. Thus, 164 mg (0.31 mmol) of **16** provided a mixture of **17** and **18** (127 mg, 80% yield after flash chromatography, 3:1 Hex/EtOac) in a 3:2 ratio. Final purification was accomplished using reversed-phase HPLC (Rainin Microsorb C18, 5 μm, 10 × 250 mm, 100% CH_3CN) yielding **17** (50.1 mg) as a pale yellow oil and **18** (34.2 mg) as a deep yellow film. **17**: ^1H NMR δ ($CDCl_3$) 6.78 (s, 1H), 5.12 (dd, 2H J = 12.1, 5.9 Hz), 4.79 (br t, 1H), 4.15 (dd, 2H J = 8.5, 8.5 Hz), 3.87 (s, 3H), 3.63 (s, 3H), 3.37 (m, 2H), 3.10 (m, 2H), 0.97 (t, 2H J = 8.5 Hz), 0.03 (s, 9H); ^{13}C NMR δ ($CDCl_3$) 156.72, 154.30, 146.42, 141.34, 135.09, 115.44, 99.49, 63.10, 58.06, 56.20, 42.13, 37.02, 17.77, −1.50 (×3C); HREIMS calcd for $C_{17}H_{27}O_5NS_5Si$ 513.0263, found 513.0278. **18**: ^1H NMR δ ($CDCl_3$) 6.47 (s, 1H), 5.14 (s, 2H), 4.72 (br t, 1H), 4.15 (dd, 2H J = 8.5, 8.5 Hz), 3.81 (s, 3H), 3.60 (s, 3H), 3.37 (q, 2H J = 6.5 Hz), 2.81 (t, 2H J = 6.9 Hz), 0.97 (t, 2H J = 8.6 Hz), 0.33 (s, 9H); ^{13}C NMR δ ($CDCl_3$) 156.71, 152.07, 140.87, 137.29, 132.89, 130.58, 112.19, 98.55, 63.12, 57.84, 56.35, 40.91, 37.62, 17.77, −1.49 (×3C); HREIMS calcd for $C_{17}H_{27}O_5NS_3Si$ 449.0821, found 449.0830.

Isolissoclinotoxin A Trifluoroacetate Salt (3). Compound **3** was prepared utilizing the same procedure as described for varacin trifluoroacetate salt (**1**). Thus, 26.8 mg (0.05 mmol) of compound **17** yielded 23.7 mg (94%) of pure **3** as a clear film: ^1H NMR δ (CD_3OD) 6.92 (s, 1H), 3.94 (s, 3H), 3.27 (m, 1H), 3.17 (m, 1H), 3.11 (m, 2H); ^{13}C NMR δ (CD_3OD) 151.53, 150.13, 136.56, 134.59, 131.67, 115.30, 56.96, 41.93, 34.88.

6-Hydroxy-7-methoxy-9-[2-[*N*-(trifluoroacetyl)amino]-ethyl]benzene (19). To a solution of **3** in CH_2Cl_2 was added an excess of trifluoroacetic anhydride. The mixture was heated in a pressure tube at 160 °C for 10 min providing the singly acetylated product **19** together with some unreacted **3**. Chromatography over silica gel with CH_2Cl_2 as eluent yielded pure **19**: ^1H NMR δ (300 MHz, CD_3OD) 6.67 (s, 1H), 6.38 (s, 1H), 3.91 (s 3H), 3.55 (m, 2H), 3.16 (m, 2H); HREIMS found 420.9199, calcd for $C_{11}H_{10}O_3S_5NF_3$ 420.9216.

2-Acetoxy-1-methoxy-3,4-bis(thioacetoxy)-5-[2-(*N*-acetylamino)ethyl]benzene (20). To a solution of isolissoclinotoxin A (**3**) (11 mg, 0.02 mmol) in methanol (3 mL) was added $NaBH_4$ (3 mg, 0.08 mmol) with stirring. After 20 min, acetic anhydride (500μL) was added, and the reaction mixture was stirred for an additional 30 min. The mixture was then diluted with H_2O and extracted with ethyl ether (3 × 15 mL). The combined ether extracts were dried (Na_2SO_4), filtered, and concentrated. Silica gel chromatography (4:1 Hex/EtOac) provided 3 mg (33% yield) of compound **20** as a clear oil: ^1H NMR δ ($CDCl_3$) 7.00 (s, 1H), 5.82 (br t, 1H), 3.86 (s, 3H), 3.47 (br m, 2H), 3.01 (br t, 2H), 2.41 (s, 3H), 2.39 (s, 3H), 2.30 (s, 3H), 1.92 (s, 3H); ^{13}C NMR δ ($CDCl_3$) 194.37, 191.83, 170.42, 167.93, 153.36, 143.44, 140.93, 129.45, 124.08, 115.38, 56.23, 39.70, 35.03, 30.03 (x2C), 23.20, 20.40; HREIMS calcd for $C_{17}H_{21}O_6S_2N$ 399.0810, found 399.0798.

Acknowledgement is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research. We would like to thank Professor Craig Jensen for his help in obtaining the X-ray analysis. Wesley Yoshida and Mike Berger are acknowledged for their help in recording NMR and mass spectral data, respectively.

Supplementary Material Available: ^1H NMR spectra of compounds **1** (TFA salt), **1** (free base), **3**, and **6-20** (18 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.