Synthesis of Varacin, a Cytotoxic Naturally Occurring Benzopentathiepin Isolated from a Marine Ascidian

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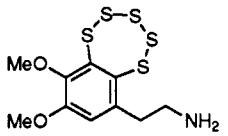
Summary: The total synthesis of varacin (1), a cytotoxic metabolite isolated from a marine ascidian, has been accomplished in 11 steps from vanillin, providing unambiguous confirmation of the unique dopamine-related benzopentathiepin structure.

Organic polysulfides have generated significant interest during the past few years, initially because of 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. Considerable effort has also been invested in the preparation of organic analogs of sulfur (S_8). In fact, syntheses of cyclic polysulfides containing three,[2] five,[3] seven,[4] and nine[5] contiguous sulfur atoms have been reported. Of these ring systems, the 1,2,3,4,5-pentathiepins have received the greatest attention. Recently, varacin (1),[6] the first naturally-occurring



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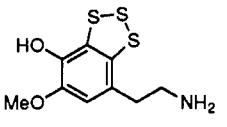

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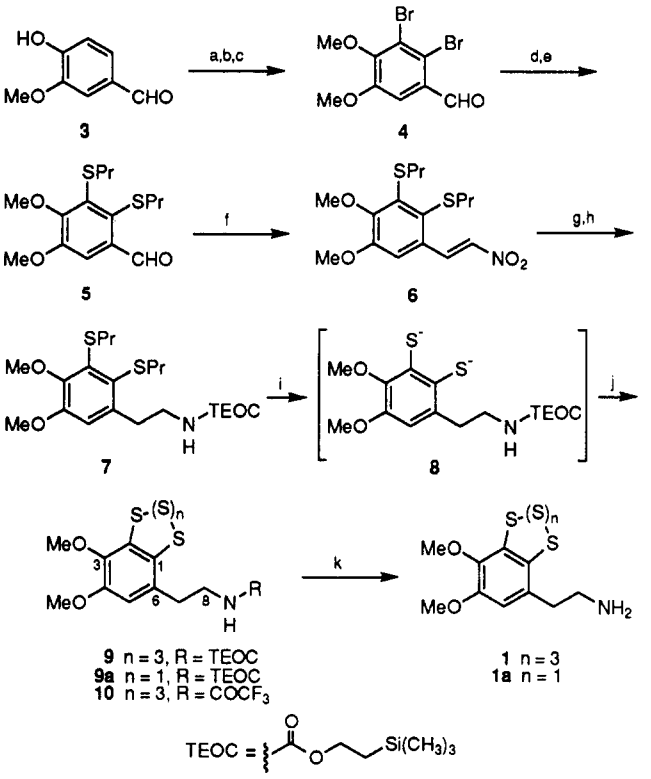
compound determined to contain this ring system, was isolated from a marine ascidian. Varacin exhibited significant antifungal and cytotoxic activities, and was as much as 100 times more potent than 5-fluorouracil toward the human colon cancer cell line HCT 116. Furthermore, preliminary assays suggested that varacin's cytotoxicity may be due to DNA damaging activity.

Because the structure of varacin was proposed solely on the basis of spectroscopic data, some question remained as to whether the compound was present only as a pentathiepin, or as a mixture of pentathiepin and trithiane ring systems. The equilibration of pentathiepin and trithiane ring systems has been reported[3] and is relevant when comparing varacin (1) with lissoclinotoxin A (2),[7] a closely related tunicate metabolite proposed to contain a trithiane ring. We would now like to report a synthesis of 1 which provides conclusive evidence that varacin exists as the proposed pentathiepin structure. These results pave the way for more detailed investigations into varacin's interesting physical and biological properties.

Scheme I[a]



[a]*Key*: (a) Br_2, HOAc; (b) MeI, aqueous K_2CO_3, *n*-Bu_4NI, THF; (c) Br_2, HOAc, 60 °C; (d) CuS^nPr, quinoline/pyr, 160 °C; (e) $(MeO)_2SO_2$, aqueous K_2CO_3, *n*-Bu_4NI, CH_2Cl_2/H_2O; (f) $MeNO_2$, NH_4OAc/HOAc; (g) $LiAlH_4$, THF, reflux; (h) *p*-$O_2NC_6H_4OCO_2CH_2CH_2Si(CH_3)_3$, Et_3N, CH_2Cl_2; (i) Na/NH_3; (j) S_2Cl_2, THF; (k) TFA, $CHCl_3$.

Pentathiepins have been synthesized using several strategies.[8] Our general approach (see Scheme I) involves the formation of the pentathiepin ring by addition of sulfur monochloride (S_2Cl_2) to an appropriate dithiol or dithiolate precursor.[3,8a] The sulfur atoms are introduced onto the aromatic ring as sulfides by the nucleophilic substitution of an aromatic dibromoarene with cuprous *n*-propylmercaptide.[9]

2,3-Dibromoveratraldehyde (4) was prepared from vanillin (3) in three steps. This procedure, which included an initial bromination of the 5-position (85%),[10] followed by methylation of the phenol (99%) and a second bromination (51%), was more efficient than incorporating both bromines onto the ring in a single step.[11] A NOE experiment

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showing dipolar coupling between the aromatic proton and one of the –OMe signals, which, together with literature precedence for the first bromination reaction,[10] confirmed that the product (**4**) was the desired regioisomer. Treatment of **4** with cuprous *n*-propylmercaptide in quinoline/pyridine at 160 °C[9] yielded a mixture of **5** and the two possible monodemethylated products, which could be methylated using phase-transfer conditions[12] to give **5** in 52% from **4**. Extension of the side chain was accomplished in two steps, involving treatment of aldehyde **5** with nitromethane in HOAc/NH_4OAc to give **6** (74%),[13] which was reduced with $LiAlH_4$ to give the phenethylamine side chain. The crude primary amine reduction product was protected with a β-(trimethylsilyl)ethoxycarbonyl (TEOC) group[14] giving **7** in 32% from **6**.

While treatment of **7** with Na/NH_3 cleanly removed the *n*-propyl groups to give dithiolate **8**,[15] the subsequent addition of S_2Cl_2 to intermediate **8** provided two main products (**9** and **9a**), which could be separated using flash chromatography[16] or reversed-phase HPLC, in 28% and 32% yields, respectively.[17] Compounds **9** and **9a** gave very similar NMR spectra;[18] however, there were some significant differences which allowed the compounds to be easily distinguished. The ^1H chemical shift of the lone aromatic proton in the spectrum of **9** was δ 6.77, while the analogous proton in the spectrum of **9a** was shifted upfield to δ 6.44. Furthermore, while the phenethylamine side chain methylene protons in the spectrum of **9a** exhibited the expected coupling patterns, the corresponding protons in the spectrum of **9** were complex multiplets, indicating that rotations around the side chain bonds are restricted. Both ring systems also give characteristic UV spectra.[3] Pentathiepin **9** gives an absorbance maximum at 209 nm, with a strong shoulder at 245 nm, while the trithiane spectrum shows an absorption at 206 nm but lacks any significant absorbance at a wavelength greater than 235 nm. Finally, HRMS analyses confirmed conclusively that **9** corresponded to the benzopentathiepin (483.0149, Δ 0.7 mmu, calculated for $C_{16}H_{25}NO_4S_5Si$), while **9a** was the benzotrithiane (419.0706, Δ 0.9 mmu, calculated for $C_{16}H_{25}NO_4S_3Si$).

Exposure of **9** to TFA[14a] in chloroform provided the trifluoroacetate salt of varacin (**1**) in 86% yield. The spectroscopic data obtained for the salt of synthetic **1**[19] matched that reported for the natural product.[6,20] Significant differences were observed for the ^1H NMR spectra of the salt versus the free base. Interestingly, in the spectrum for the free base,[21] the H7 protons are downfield of the H8 protons and they are diastereotopic, indicating hindered rotation of the side chain. Further confirmation of the pentathiepin ring was provided by HREIMS of *N*-trifluoroacetamide derivative **10**,[22] which gave a molecular ion at *m/z* 435 (HREIMS 434.9370, Δ 0.3 mmu, calculated for $C_{12}H_{12}NO_3S_5F_3$).

In contrast, deprotection of **9a** yielded a product (**1a**), clearly different than **1**, which was unstable, rapidly degrading to several uncharacterized products. The observed lability of the trithiane ring system in **1a** provides evidence supporting the pentathiepin structure proposed for varacin. The increased stability of **9a**, relative to **1a**, in view of reports that the equilibration of pentathiepin/trithiane ring systems can be base-catalyzed,[3] suggests the possibility that, once deprotected, the free amine in either **1** or **1a** may autocatalyze such an equilibration; however, in the absence of external sulfur or a pentathiepin ring, it is not clear to what the purified trithiane **1a** is degrading.

In summary, the unusual benzopentathiepin natural product varacin has been synthesized. Confirmation of the proposed structure was accomplished by complete spectral characterization, including MS, of two derivatives (**9** and **10**) of **1** and a direct comparison of penultimate precursor **9** with the protected trithiane **9a**. 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 (**1a**) proved to be unstable. In view of these results, it is likely that lissoclinotoxin A (**2**) also contains a pentathiepin ring and that mass spectral evidence for this would be observed only for a suitable derivative. A synthesis of **2**, along with a more detailed investigation into physical and biological properties of these interesting natural products, is underway and will be reported at a later date.

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Supplementary Material Available: Experimental procedures, ^1H and ^{13}C NMR spectra of compounds **1** (TFA salt), **1** (free base, only H^1), **4–7**, **9**, **9a**, and **10** (only ^1H), and mass spectral data for **9**, **9a**, and **10** (26 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.

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(17) Subsequent runs of this reaction have provided significantly different product ratios, ranging from the almost equal amounts of **9** and **9a**, as reported above, to yielding almost exclusively the pentathiepin **9**.
(18) **9**: ^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, –1.4; EIMS *m/z* (rel intensity) 483 (M^+, 2), 419 ($M^+ - S_2$, 35), 391 (7), 301 (39), 245 (21), 213 (18), 75, (68), 73 (100). **9a**: ^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, –1.79; EIMS *m/z* (rel intensity) 419 (M^+, 36), 391 (6), 301 (24), 213 (20), 75 (100), 73 (83).
(19) 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.
(20) It is likely that the data reported for varacin are actually for the TFA salt, because final HPLC purification was performed using a solvent containing aqueous TFA.
(21) 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.
(22) The TFA amide (**10**) was obtained by heating a CH_2Cl_2 solution of **1** with excess trifluoroacetic anhydride for 5 min at 160 °C in a sealed pressure tube. Evaporation of the solvent and excess trifluoracetic anhydride provided pure **10**: ^1H NMR δ ($CDCl_3$) 6.73 (s, 1H), 6.41 (br s, 1H), 3.87 (s, 1H), 3.85 (s, 1H), 3.58, (m, 2H), 3.19 (m, 2H); EIMS *m/z* (rel intensity) 435 (M^+, 4), 371 ($M^+ - S_2$, 100), 245 (62).