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Synthesis, Characterization, Mechanism of Decomposition, and Antiproliferative Activity of a Class of PEGylated Benzopolysulfanes Structurally Similar to the Natural Product Varacin

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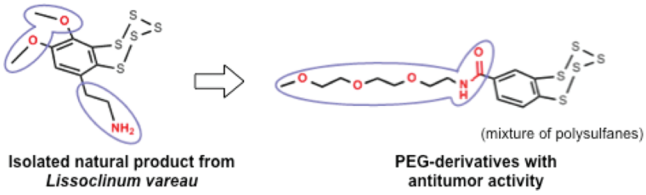

Benzopolysulfanes, 4-$CH_3(OCH_2CH_2)_3NHC(O)$-C_6H_4-1,2-S_x (x = 3−7 and 9) were synthesized with a PEG group attached through an amide bond and examined for water solubility, antitumor activity, and propensity to equilibrate and desulfurate. LCMS and HPLC data show the PEG pentasulfane ring structure predominates, and the tri-, tetra-, hexa-, hepta-, and nonasulfanes were present at very low concentrations. The presence of the PEG group improved water solubility by 50-fold compared to the unsubstituted benzopolysulfanes, $C_6H_4S_x$ (x = 3, 5, and 7), based on intrinsic solubility measurements. Polysulfur linkages in the PEG compounds decomposed in the presence of ethanethiol and hydroxide ion. The PEG pentathiepin desulfurated rapidly, and an S_3 transfer reaction was observed in the presence of norbornene; no S_2 transfer reaction was observed with 2,3-dimethylbutadiene. The antitumor activities of the PEG-substituted benzopolysulfane mixtures were analyzed against four human tumor cell lines PC3 (prostate), DU145 (prostate), MDA-MB-231 (breast), and Jurkat (T-cell leukemia). The PEG-conjugated polysulfanes had IC_{50} values 1.2−5.8 times lower than the parent "unsubstituted" benzopolysulfanes. Complete cell killing was observed for the PEG polysulfanes at 4 μM for PC3 and DU145 cells and at 12 μM for MDA-MB-231 cells. The results suggest that solubilization of the polysulfur linkage is a key parameter to the success of these compounds as drug leads.

Introduction

Tunicates or their associated microorganisms produce benzopolysulfanes, such as varacin (**1**), lissoclinotoxin A (**2**), and *N*,*N*-dimethyl-5-(methylthio)varacin (**3**) (Scheme 1).[1−6] Unnatural benzopolysulfanes have also been synthesized, e.g., 6-(2-aminoethyl)benzopentathiepin (**4**)[7] and the parent benzopentathiepin (**5B**)[8,9] (alphabetical labels will be given to some polysulfanes, as will be elaborated on below). Benzopolysulfanes represent an attractive target but are an understudied class of

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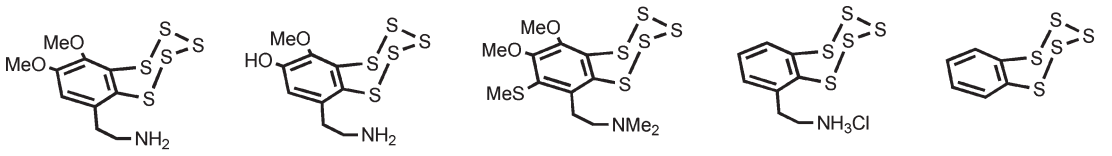
SCHEME 1. Natural and Unnatural Benzopolysulfanes



SCHEME 2. Equilibration Between Tri-, Penta-, and Heptasulfanes

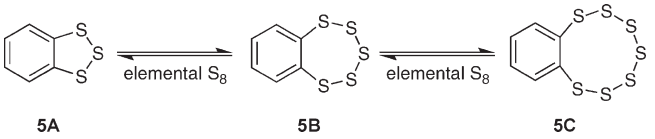


TABLE 1. GC−MS Detection of Parent Benzopolysulfanes 5A−C[a]

compound	no. of S atoms	MS t_R (min)[b]	molecular weights	
			calcd	found[c]
o-$C_6H_4S_3$ **5A**	3	14.2	172	172
o-$C_6H_4S_5$ **5B**	5	26.5	236	236
o-$C_6H_4S_7$ **5C**	7	39.1	300	300

[a]Reference 12. See also ref 13. [b]GC−MS retention time. [c]Low-resolution GC−MS. Support for the GC−MS peak assignments came from spectroscopic comparisons of **5A−C**, which were independently synthesized and examined shortly after their purification, i.e., before equilibration was pronounced, which took 1−3 days.

compounds for antitumor drug discovery. High nanomolar and low micromolar antiproliferative IC_{50} values have been reported for benzopolysulfanes.[1−7,10] It was suggested that the bioactivity of varacin derives from DNA damage because of an observed difference in toxicity toward the CHO cell line EM9 (chlorodeoxyuridine sensitive) compared to BR1.[1]

Benzopolysulfanes have not been studied widely in the context of drug discovery because of the instability of the polysulfur ring. Even determining the number of sulfur atoms in *o*-benzopolysulfanes has not been a trivial task.[11−13] MS fragmentation patterns can be difficult to interpret; for example, the structure of the natural product lissoclinotoxin A was assigned first as a trisulfane[3] and later revised as a pentasulfane.[11] In 2007, the synthesis and purification of parent *o*-$C_6H_4S_5$ (**5B**) revealed an equilibration involving elemental sulfur, S_8 (Scheme 2).[12] A facile equilibration took place between the pentasulfane and the tri- and heptasulfanes (*o*-$C_6H_4S_3$ and *o*-$C_6H_4S_7$): the ratio **5A**:**B**:**C** was 49:45:6 in CH_2Cl_2 over 1−3 days. In **5**, the labels A, B, and C correspond to compounds with 3, 5, and 7 sulfur atoms, respectively. Analysis of GC−MS retention times revealed that **5A** and **5B** differed by 12.3 min, and the retention times of **5B** and **5C** differed by 12.6 min (Table 1).

Because natural benzopolysulfanes are in short supply and their stability is difficult to assess, we synthesized benzopolysulfanes with a short-chain PEG, in which the benzene ring and PEG group replaced the naturally occurring dopamine core **1**−**3**. The aims of the present work were to determine (1) whether benzopolysulfanes could be synthesized with a PEG side group, (2) whether the pentasulfur species predominates, (3) whether the polysulfur linkage(s) are unstable to medium effects, (4) whether the PEGylated benzopolysulfanes decompose at different rates and transfer sulfur to norbornene and butadiene traps, (5) the extent the PEG group enhances water solubility, (6) whether the polysulfur ring is essential for bioactivity, and (7) whether enhanced benzopolysulfane water solubility is correlated with an enhanced pharmacological activity against human tumor cells. We synthesized a mixture of PEG-benzopolysulfane conjugates 4-$CH_3(OCH_2CH_2)_3NHC(O)$-C_6H_4-1,2-S_x ($x = 3-7$ and 9) **6A**−**F** and explored their stability and activity in a variety of tumor cell lines [PC3 (prostate), DU145 (prostate), MDA-MB-231 (breast), and Jurkat (T-cell leukemia)]. In **6**, the labels A−E and F correspond to compounds with 3−7 and 9 sulfur atoms, respectively. The octasulfane species was not detected.

Results and Discussion

Synthesis and Characterization. Pentasulfane **6C** was synthesized as the major constituent of a mixture of polysulfanes 4-$CH_3(OCH_2CH_2)_3NHC(O)$-C_6H_4-1,2-S_x (x = 3−7 and 9) in 8 steps and 1.5% overall yield. A procedure developed by Liénard et al.[14] was used for the conversion of 3,4-dihydroxybenzoic acid (**7**) to 3,4-disulfuranylbenzoic acid (**11**) (steps i−iv, Scheme 3). Dithiastannole-5-carboxylate anion **12** was generated under basic conditions by the reaction of dimethyltin chloride with **11** using a modified procedure by Sato et al.[15] Stannole **12** reacted with *p*-nitrophenol, DCC, and DMAP to yield 4-nitrophenyl ester stannole **13** in 72% yield. Stannole **13** reacted with disulfur dichloride giving 4-nitrophenyl ester benzopentasulfane (**14**) in 27% yield. Amino-terminated poly(ethylene glycol) (**15**) was prepared in 2 steps by the method of Dombi et al.[16] and PEGylated to benzopentasulfane **14** at the 7-position of the benzene ring. It is possible that other cyclic polysulfanes related to benzopentasulfane **14** were formed in ~1−10% yields, but this was not determined. The resulting mixture was purified by column chromatography to afford **6C** in 93% purity. In the ^{1}H NMR, ^{13}C NMR, COSY, HMBC, and HSQC spectra, the benzene and PEG portions of the structure were confirmed (Supporting Information). For example, in COSY NMR, a strong ^3J correlation was found between C9−H and C8−H, and between N−H and C13−H, and a weak ^4J-W correlation found between C8−H and C6−H (Scheme 4). The HMBC and HSQC NMR data further bolstered the structural assignment of the

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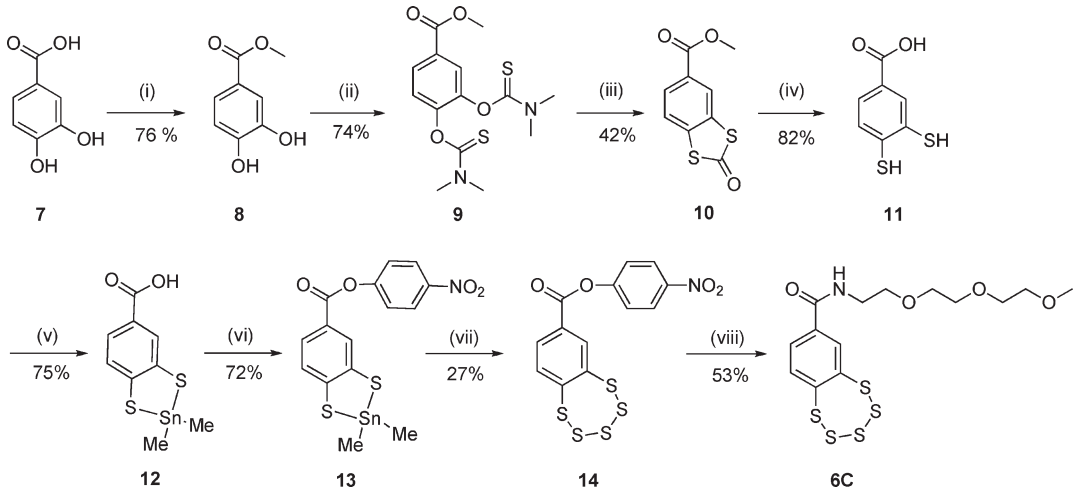
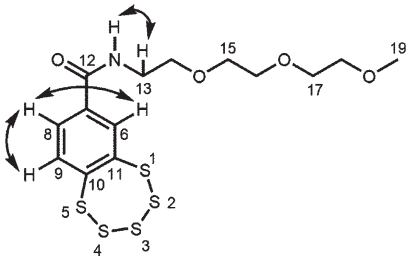
SCHEME 3. Synthesis of PEG Conjugated Benzopentasulfane 6C[a]

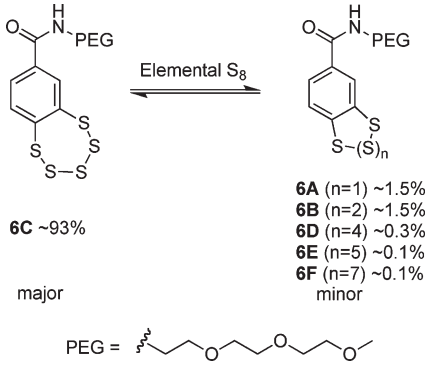


[a]Reagents and conditions: (i) HCl (cat.), MeOH, reflux 80 °C, 7 h; (ii) $Me_2NC(=S)Cl$, DABCO, DMF, rt, 30 min; (iii) Ph_2O, 230 °C, 40 min; (iv) (a) aqueous NaOH, under N_2, 70 °C, 4 h, (b) 1 N HCl; (v) (a) Me_2SnCl_2, KOH, EtOH, water, (b) 1 N HCl; (vi) *p*-nitrophenol, DCC, DMAP, CH_2Cl_2, 1 d; (vii) S_2Cl_2, CH_2Cl_2, 0 °C, 30 min, then warmed to rt, 24 h; (viii) H_2N-$(CH_2CH_2O)_3$-CH_3 **15** THF, rt, 12 h.

SCHEME 4. COSY NMR Couplings of Benzopentasulfane 6C



SCHEME 5. Equilibration of Pentasulfane 6C with Minor Amounts of Tri-, Tetra-, Hexa-, Hepta-, and Nonasulfanes in Methanol/Water (1:1 v/v)



nonsulfur portion of **6C**. LC/MS data indicated that **6C** contained five sulfur atoms (HRMS calcd for $C_{14}H_{19}NO_4S_5$ = 424.9918, found 424.9926).

Lability of the Pentasulfur Ring. Polysulfanes are challenging structures to study because of their instability. Few reports on benzopolysulfanes describe the distribution of the polysulfanes or their equilibration and often incorrectly assume the pentathiepin to be the sole compound present.[12] We found that over a 12−24 h period **6C** equilibrates S_8 and forms low concentrations of structurally related polysulfanes in aqueous methanol at room temperature (Scheme 5). Table 2 lists the

TABLE 2. Mass Spectrometry Data for PEGylated Benzopolysulfanes 6A−F

compound	formula	t_R[a] (min)	calculated mass	experimental mass [$(M + H^+) - H^+$][b]	error (ppm)
6A	$C_{14}H_{19}NO_4S_3$	4.6	361.0476	361.0478	0.62
6B	$C_{14}H_{19}NO_4S_4$	5.6	393.0197	393.0193	−1.00
6C	$C_{14}H_{19}NO_4S_5$	6.5	424.9918	424.9926	1.99
6D	$C_{14}H_{19}NO_4S_6$	7.1	456.9638	456.9636	−0.60
6E	$C_{14}H_{19}NO_4S_7$	7.8	488.9359	488.9354	−1.08
6F	$C_{14}H_{19}NO_4S_9$	9.2	552.8800	552.8787	−2.34

[a]LCMS retention time. Chromatography was performed on a SB-C18 3.5 μm column using water containing 0.1% formic acid and 5 mM ammonium formate (solvent A) and methanol containing 0.1% formic acid and 5 mM ammonium formate (solvent B) at a flow rate 0.5 mL/min. The gradient program was as follows: 15−85% B (0−13 min), 85% B (13−15 min), 85−15% B (1 min). [b]The experimental exact mass is calculated by the subtraction of a proton (H^+; 1.00728 Da) from the measured *m*/*z* value of the $[M + H^+]$ ion for the molecular formula of interest.

polysulfane masses and retention times to compare the number of sulfur atoms in the compounds. The LCMS spectra of polysulfanes **6A**−**F** contained peaks spaced by ~0.7 min per additional sulfur atom (Figure 1). For example, a minor amount of a sulfur-rich compound was assigned as nonasulfane **6F** (HRMS calcd for $C_{14}H_{19}NO_4S_9$ = 552.8800, found 552.8788). The ratio **6A**:**B**:**C**:**D**:**E**:**F** was 1.5:1.5:93:0.3:0.1:0.1 by HPLC (methanol/water), and there was ~2% uncharacterized material and ~1.5% of elemental sulfur. The moderate solubility of the elemental sulfur in methanol/water suggested it to be a residue or colloid particles of the orthorhombic cyclo-S_8 form, but neither the monoclinic S_8 ring form (usually found at ~95 °C) nor the polymeric, oligomeric, or amorphous forms (insoluble materials).[17−19] The overlaid-ion extracted LCMS of **6** in Figure 1 displayed a series of polysulfanes, analogous to that seen for parent **5** by SIM-GC−MS.[12] Preference for odd-membered ring

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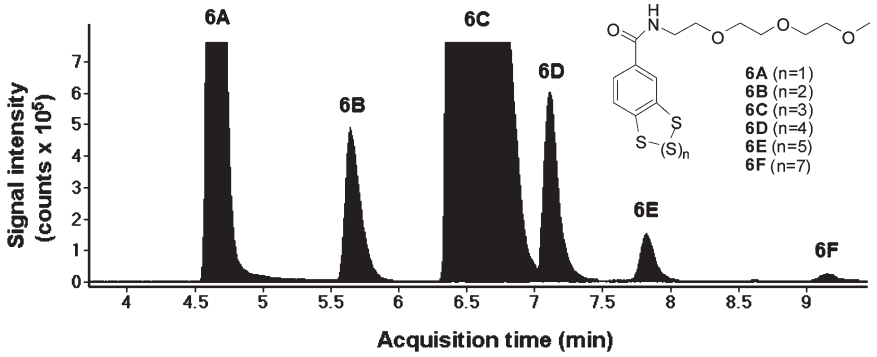

FIGURE 1. Ion extracted [M + H^+] LCMS spectra of compounds **6A**−**F**. Chromatograms were normalized to the largest peak **6C**. The "counts" on the *y*-axis are equal to the number of ions detected.

TABLE 3. Polysulfane Distribution as a Function of HPLC Solvent Elution Condition[a]

method[b]	$MeOH/H_2O$ ratio (v/v)	polysulfanes and elemental sulfur						
		6A	**6B**	**6C**	**6D**	**6E**	**6F**	S_8
1	50/50	2.2	1.8	93.5	0.4	0.3	0.2	1.6
2	60/40	2.5	2.1	94.7		0.6		
3	75/25	4.3	3.2	84.6	1.5	0.9	0.5	3.4
4	80/20	4.7	4.8	83.6	1.0	0.3	0.4	5.1
5	85/15	5.3	5.7	79.0	3.4	1.2	0.1	5.3

[a]HPLC analysis at 254 nm at room temperature with a flow rate of 1 mL/min in water/methanol mixtures. [b]HPLC methods: Method 1 consisted of a gradient of methanol from 10% to 90% over 53 min and maintained for 2 min before reverting to 10% methanol over 1 min. Method 2 consisted of 60% methanol run isocratically over 91 min. Method 3 consisted of a gradient of methanol from 60% to 95% over 30 min, which was maintained for 10 min before reverting to 15% methanol over 5 min. Method 4 consisted of a gradient of methanol from 15% to 95% over 30 min, which was maintained for 15 min before reverting to 15% methanol over 3 min. Method 5 consisted of a gradient of methanol from 15% to 95% over 15 min, which was maintained for 15 min before reverting to 15% methanol over 2 min. Method 6 consisted of water (0.1% formic acid and 5 mM ammonium formate in water) and methanol (0.1% formic acid and 5 mM ammonium formate in MeOH) with a gradient of methanol from 15% to 85% over 13 min, which was maintained for 2 min before reverting to 15% methanol over 1 min.

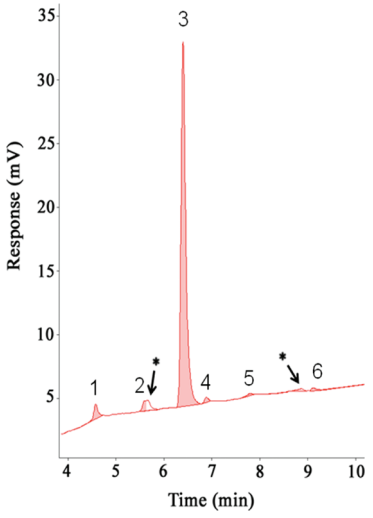

FIGURE 2. HPLC chromatogram of polysulfanes; peaks include 1, **6A**; 2, **6B**; 3, **6C**; 4, **6D**; 5, **6E**; 6, **6F**. Elemental sulfur S_8 elutes in the 11−13 min range. Peaks marked with an asterisk belong to unidentified substances.

compounds was observed experimentally for the parent system *o*-$C_6H_4S_x$ ($x = 3, 5, 7$) in CH_2Cl_2.[12] Gas-phase DFT calculations showed the odd-membered *o*-$C_6H_4S_x$ rings to be conformationally stable with gauche adjacent lone-pair electron interactions, whereas eclipsing lone-pair electron interactions occurred in the even-numbered cases.[13] It may be noted that Nakayama et al. also observed odd-membered ring products (namely, the trithiolane and penathiepan) in the reaction of elemental sulfur with a benzobarrelene compound and an acenaphthylene compound.[20,21] However, the preference for odd-membered polysulfur rings does not apply to the PEG benzopolysulfanes, which is likely because the equilibrium is at an early stage and has not been reached in aqueous methanol, even after 24 h. The effect of solvent or HPLC conditions on the equilibrium of **6A**−**F** and S_8 was investigated next.

Influence of Solvent on Equilibration of Polysulfanes 6A−**F.** To better understand the equilibration between **6A**−**F** and S_8, the HPLC elution methods were modified. When eluting at 1:1 methanol/water, the ratio **6A**:**B**:**C**:**D**:**E**:**F**:S_8 was 2.2:1.8:93.5:0.4:0.3:0.2:1.6 (Method 1, Table 3). Increasing the methanol concentration in water produces more pronounced polysulfane equilibration. When eluting at 85:15 methanol/water, the ratio **6A**:**B**:**C**:**D**:**E**:**F**:S_8 was 5.3:5.7:79.0:3.4:1.2:0.1:5.3 (Method 5, Table 3). The HPLC measurements do not ensure that thermodynamic conditions have been reached. Equilibrium at a new condition is established in 1−3 days in CH_2Cl_2[12] and probably much longer in methanol/water. Our results are similar to observations of 7-methylbenzopolysulfane equilibrations enhanced in polar solvents with higher solvation compared to nonpolar solvents.[22] Elemental S_8 also involves equilibration with S_6 and S_7, which was more pronounced in polar than nonpolar organic solvents,[23] and sulfur-reactive solvents such as pyridine or other amines that dissolve elemental sulfur readily.[18] The enhanced equilibration in methanol compared to that in methanol/water is likely a result of the increase in the solubility of S_8, which is pivotal to the reversible addition reactions leading to the ring compounds **6A**−**F** (Figure 2).

Desulfuration of 6A−**F in the Presence of Nucleophiles and Trapping Agents.** The PEGylated benzopolysulfanes **6A**−**F**

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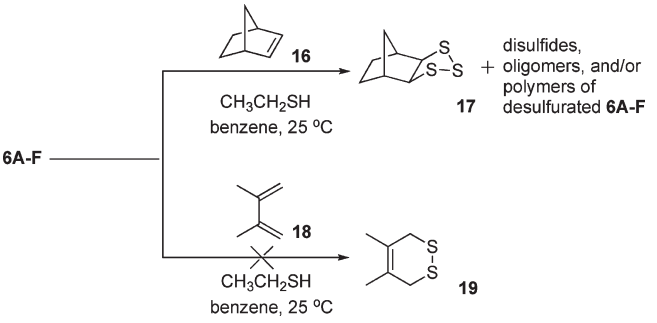
TABLE 4. Decomposition of PEGylated Benzopolysulfanes 6A−F as a Function of Hydroxide Ion Equivalents Added[a]

hydroxide ion equiv	internal standard	polysulfanes and elemental sulfur distribution[b]						
		6A	**6B** + X[c]	**6C**	**6D**	**6E**	**6F**	S_8
0.25	100	10.9	11.2	362.4	2.3	3.0	2.1	12.3
0.5	100	13.2	19.6	289.2	2.1	2.8	3.7	23.1
0.75	100	14.2	38.1	222.9	1.8	4.7	3.8	26.7
1.0	100	14.2	64.9	163.9	1.5	1.6	3.1	32.4
1.0[d]	100	14.9	85.5	113.6	0.7	2.2		3.1

[a]Reaction of benzopolysulfanes **6A−F** (2.5 mM) and hydroxide ion in the presence of internal standard acetanilide (0.25 mM) in methanol/water (99.9:0.1 v/v). [b]Polysulfane ratios were determined by HPLC monitoring at 254 nm at room temperature with the flow rate 1 mL/min using method 4 (Table 3). HPLC analysis was carried out after 1 h of equilibration between benzopolysulfanes **6A−F** and hydroxide ion. [c]X is an unidentified compound that was eluted with **6B**. [d]Analysis was done after 24 h.

SCHEME 6. PEGylated Benzopolysulfanes 6A−F as Sulfur-Transfer Reagents to Norbornene but Not to 2,3-Dimethylbutadiene



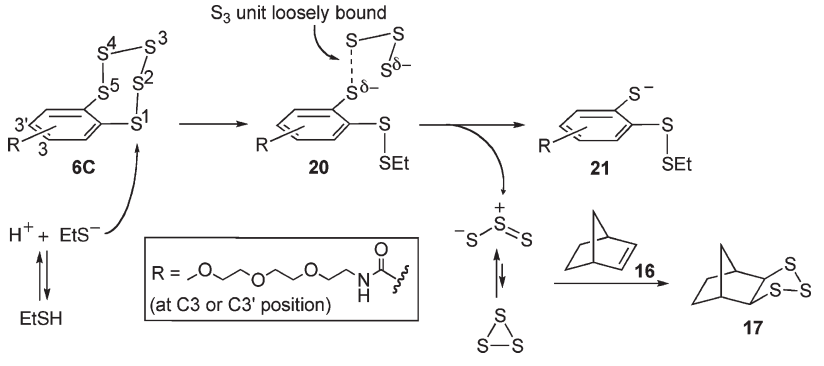
decomposed in the presence of NaOH or ethanethiol (Table 4), which led to an increase in elemental S_8 and uncharacterized products, such as oligomers and/or polymers of desulfurated PEGylated benzopolysulfanes. The reaction of hydroxide ion with benzopolysulfanes **6A−F** was comparatively slower than that with ethanethiol and could be monitored by HPLC. As can be seen in Table 4, the decomposition rate of pentasulfane **6C** was rapid, whereas the concentrations of the other benzopolysulfanes were barely reduced. Determining whether the concentration of tetrasulfane **6B** increased or decreased was not possible because it eluted along with an unidentified compound (X in Table 4), and the two peaks could not be deconvoluted. The data suggested that pentasulfane **6C** desulfurated more quickly compared to other polysulfanes, which led us to trapping studies to analyze the sulfur-transfer reaction with butadiene and norbornene traps.

The reaction of benzopolysulfanes **6A−F** with ethanethiol and norbornene (**16**) led to the formation of norbornenetrithiolane (**17**) and desulfurated or polymerized PEGylated benzopolysulfanes (Scheme 6). Unlike **6C**, the concentrations of **6A**, **6B**, and **6D−F** did not change significantly over the course of the trapping experiment. Thus, we propose that **6C** is the most reactive polysulfane and responsible for the S_3-transfer to norbornene. Interestingly, the decomposition of benzopolysulfanes **6A−F** did not show an S_2 transfer reaction. The sulfuration of 2,3-dimethylbutadiene (**18**) by **6A−F** with ethanethiol did not yield the disulfide product **19**. A possible mechanism for the desulfuration of pentathiepin **6C** is shown in Scheme 7, which begins with an apical attack of ethanethiolate ion to the sulfur atom (S1) adjoining the aryl ring. A previous DFT study showed that attack of the HS^- nucleophile at the S1 position of a pentathiepin was preferred to S2.[24] The resulting open-chain polysulfide anion (**20**) has the potential for thiozone (S_3) elimination driven by the delocalization of the negative charge in the remaining carbon−sulfur fragment (**21**)[24,25] followed by thiozonation of norbornene. Ab initio calculations predict the open C_{2v} zwitterionic form[26] of thiozone S_3 to be energetically preferred to the cyclic D_{3h} form.[27,28]

Intrinsic Solubilities. Elemental S_8 suffers from poor solubility.[19,29,30] We investigated the intrinsic solubilities of elemental S_8, **5A−C**, and **6A−F** (Table 5). Aliquots of methanol or water were added to 1−3 mg sample quantities, and the solutions were vortexed (2 min) and stirred (10−15 min) at room temperature. As expected, the presence of the PEG group at the 7-position of **6A−F** significantly increased their solubilities. By comparison to **6A−F**, elemental sulfur S_8 and **5A−C** had ~50-fold lower solubility. Our aqueous solubility measurements are consistent with previous values for elemental sulfur S_8 reported in the literature, namely, the solubility of S_8 in water was reported to be 0.4 μg/mL.[29] The solubility of S_8 in ethanol was reported to be 0.51 mg/mL.[30]

Antiproliferative Activities of Polysulfanes. We examined the effects of 1,2-benzenedithiol, *o*-benzopolysulfanes **5A−C**, and PEGylated benzopolysulfanes **6A−F** on the proliferation of several cancer cell lines. Their bioactivities are shown in Table 6 and Figure 3. 1,2-Benzenedithiol was nontoxic; it stimulated the growth of PC3 cells and had a minimal effect on the proliferation of MDA-MB-231 cells. However, at concentrations of 10 μM and above, it inhibited the proliferation of DU145 cells by ~40%. In a MTS assay (3-(4,5-dimethylthiazol-2-yl)-5-(3-car-

SCHEME 7. Potential Mechanism for the Triatomic Sulfur Transfer of Benzopentasulfane 6C



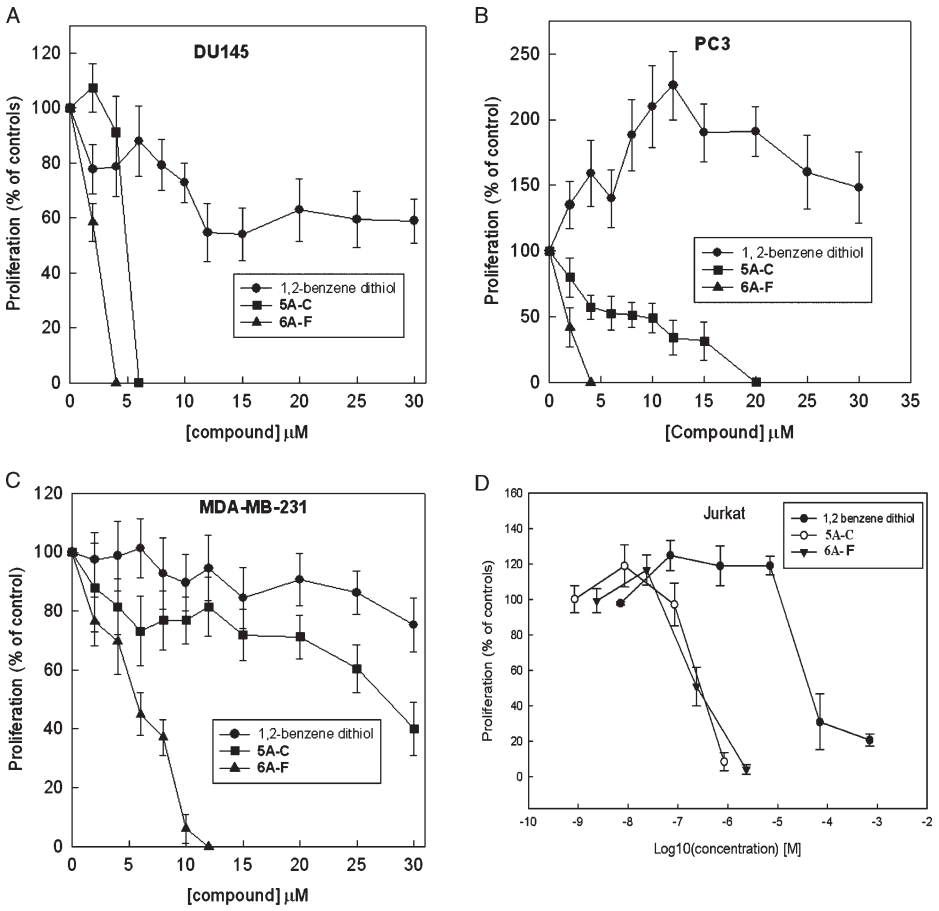


FIGURE 3. Effect of 1,2-benzenedithiol, *o*-benzopolysulfanes **5A**−**C**, and PEGylated benzopolysulfanes **6A**−**F** on the growth of human cancer cell lines. Prostate cancer cell lines DU145 and PC3 cells, breast cancer cell line MDA-MB-231, and Jurkat cells, a leukemia cell line, were grown in 96-well plates. When the cells were in exponential growth, they were incubated with different concentrations of 1,2-benzenedithiol, **5A**−**C**, or **6A**−**F** for 48 h (DU145, PC3, MDA-MB-231) or 72 h (Jurkat). The increase in cell numbers relative to controls was determined by the CyQuant assay (A−C) and by the MTS assay (D) as described in the Experimental Section. The values were expressed as the increase in cell numbers relative to controls without any compound. Data are the mean ± SE (n = 6−8).

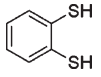
TABLE 5. Experimental Solubility Values[a]

	solvent	
reagent	methanol (mg/mL)	water (μg/mL)
elemental sulfur, S_8	0.33 ± 0.03	0.14 ± 0.02
o-benzopolysulfanes **5A**−**C** (97%)	0.37 ± 0.02	0.18 ± 0.02
PEGylated benzopolysulfanes **6A**−**F** (93%)	19.00 ± 0.05	8.70 ± 0.25

[a]Equilibrium time of 24 h was used in the solubility study. Measurements were conducted three times, and the solubility value was averaged.

boxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium in the presence of phenazine methosulfate),[31] 1,2-benzenedithiol was also not effective in inhibiting Jurkat cell growth (IC_{50} of 60 μM). The requirement of the polysulfur ring was also observed by Molinski et al.[5] in the natural lissoclinotoxin A system when judged against the corresponding benzenedithiol compound.

TABLE 6. Inhibition of Cancer Cell Gowth by 1,2-Benzenedithiol, *o*-Benzopolysulfanes 5A-C, and PEGylated Benzopolysulfanes 6A−F

	IC_{50} (μM)[a]			
compound	PC3	DU145	MDA-MB-231	Jurkat
	no effect	>30	>30	60
 1,2-benzenedithiol				
o-benzopolysulfanes **5A**−**C** (97% purity)	10.5 (20)[b]	4.9 (6)[b]	27	0.5
PEGylated benzopolysulfanes **6A**−**F** (~98% total purity)	1.8 (4)[b]	3 (4)[b]	5.5 (12)[b]	0.4

[a]IC_{50} values were obtained for 72 h incubations with Jurkat cells and 48 h for PC3, DU145 and MDA-MB-231 cells. [b]Concentrations that resulted in 100% cell kill are shown in parentheses. Experiments were conducted in 96-well plates with 8 replicates for each concentration (0−30 μM) of the indicated compounds.

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Parent polysulfanes **5A**−**C** had a moderate antiproliferative effect on MDA-MB-231 cells, inhibiting the proliferation with an IC_{50} value of 28 μM. PC3 cell proliferation was inhibited with an IC_{50} of 11 μM with complete cell killing at 20 μM. DU145 and Jurkat cells were sensitive to parent polysulfanes **5A**−**C**; 6 μM killed 100% of the DU145 cells, and the IC_{50} was 0.5 μM for Jurkat cells. PEGylated benzopolysulfanes **6A**−**F** were the most potent of the three compound classes for the four cell lines examined and were toxic for all four cell lines. Complete cell killing was observed for **6A**−**F** at 4 μM for PC3 and DU145 cells and at 12 μM for MDA-MB-231 cells. The PEG compounds **6A**−**F** were cytotoxic in Jurkat cells with an IC_{50} of 0.4 μM.

The data in Table 6 indicate that the polysulfur ring is significant in the enhancement of antiproliferative activity. The data also demonstrate that the PEG group leads to an increase in the antiproliferative effect. The PEGylated benzopolysulfanes **6A**−**F** have higher water solubility compared to that of the parent polysulfanes **5A**−**C** and produced the highest growth inhibition. It was previously reported that lissoclinotoxin A **2** was cytotoxic against L1210 leukemia cells (IC_{50} of 3.1 μM) and *N*,*N*-dimethylvaracin **3** was cytotoxic against MDA-MB-231 cells (IC_{50} of 3.6 μM).[10] Interestingly, varacin **1** showed greater cytotoxicity in HCT-116 colon cancer cells (IC_{90} of 0.15 μM).[1]

Potential of Benzopolysulfanes as Drug Candidates. Even though high nanomolar and low micromolar antiproliferative IC_{50} values for benzopolysulfanes were mentioned in the Introduction[1−7,10] and the above results for **6A**−**F** appear promising, in vivo stability and deliverability studies are needed to evaluate benzopolysulfane lability to glutathione, protein thiols, etc. Like some other drugs, benzopolysulfanes are reductively activated. Our in vitro studies showed that ethanethiol-induced desulfuration of **6C** in aqueous methanol took ~2 min but in benzene took 4 h, suggesting that greater thiol ionization/nucleophilicity increases the polysulfane lability. Thus, hydrophobic or mild acidic conditions that maintain the less reactive thiol form will preserve **6C**, but in the presence of thiolate ion **6C** decomposes rapidly. In the absence of thiol and thiolate ion, the solvent effect is in the opposite direction; benzopolysulfane equilibria in organic solvents such as CH_2Cl_2 were more facile than in methanol or methanol/water, driven by solvation of elemental sulfur and the benzopolysulfanes (vide supra). Lastly, although a DNA cleaving study of 7-methylbenzopentathiepin suggested a metal- and oxygen-dependent Fenton pathway,[32,33] more work is needed to evaluate a potential anaerobic S_3-transfer pathway that may underlie the antitumor activity.

Conclusion

The following conclusions can be made: (1) The synthesis of benzopolysulfanes **6A**−**F** was accomplished with attached PEG groups of 160 Da molecular weight via an amide linkage in 1.5% overall yield. (2) Pentathiepin **6C** was the main product, but even after its purification, benzopolysulfanes **6A**, **6B**, **6D**−**F** formed in very low concentrations. (3) The pentasulfur linkage of **6C** was sensitive to the solvent composition in the HPLC experiments. Methanol-rich elution conditions reduced the ratio of **6C** relative to the other polysulfanes. (4) Thiol-initiated reactions of **6A**−**F** led to an S_3-transfer to norbornene, but no S_2-transfer was observed to 2,3-dimethylbutadiene. (5) While the cause of the poor solubility of benzopolysulfanes is the polysulfur linkage itself, a key issue in the present study was the enhanced water solubility the PEG group provided, which did not reduce the prevalence of the pentasulfur ring species. (6) The results confirm the requirement of the polysulfur ring for low micromolar antiproliferative IC_{50} values; 1,2-benzenedithiol showed little or no antiproliferative activity. (7) The PEG polysulfanes **6A**−**F** were more water soluble and more active against four cancer cell lines than the parent polysulfanes **5A**−**C**, suggesting that enhanced solubilization of benzopolysulfanes holds promise for advancing these compounds as drug candidates.

Experimental Section

3,4-Dihydroxybenzoic acid, *N*,*N*-dimethylthiocarbamoyl chloride, DABCO, sodium hydroxide, potassium hydroxide, ethanethiol, acetanilide, formic acid, ammonium formate, dimethyltin chloride, *p*-nitrophenol, DCC, S_2Cl_2, triethylene glycol monomethyl ether, phthalimide, triphenylphosphine, DIAD, hydrazine monohydrate, elemental sulfur (S_8), norbornene, 2,3-dimethylbutadiene, DMAP, sodium sulfate (anhydrous), magnesium sulfate (anhydrous), NaCl, DMF, Na_2CO_3, $NaHCO_3$, THF, $CHCl_3$, CH_2Cl_2, methanol, ethanol, ethyl acetate, hydrochloric acid (12 M), diphenyl ether, benzene, acetone-d_6, $CDCl_3$, CD_3CN, CD_3OD, and hexanes were used as received without further purification. Purification of product mixtures was carried out by column chromatography using silica gel with 40−60 Å particle size. TLC was carried out using silica gel 60F 254 TLC plates. Proton NMR data were acquired at 400 MHz, and ^{13}C NMR data were acquired at 100.6 MHz. HRMS, GC−MS, HPLC, and melting point data were collected.

HPLC Instrumentation and Analysis. The HPLC instrument was equipped with an autosampler and diode array detector. The C18 column was 150 mm × 3.9 mm in size. The flow rate was 1 mL/min, and the injection volume was 50 μL. The mobile phase consisted of methanol and water. Compounds were detected by UV at 254 nm at rt.

LCMS Instrumentation and Analysis. The LCMS system consisted of a high-resolution TOF mass spectrometer attached to an HPLC equipped with an autosampler, diode array detector, and binary pump. The chromatography was conducted with a 2.1 mm × 30 mm SB-C18 3.5 μm column using water containing 0.1% formic acid and 5 mM ammonium formate (solvent A) and methanol containing 0.1% formic acid and 5 mM ammonium formate (solvent B) at a flow rate 0.5 mL/min. The gradient program was as follows: 15−85% B (0−13 min), 85% B (2 min), 85−15% B (1 min). The mass spectra were collected over a range of 100−1600 m/z. The reference masses used were purine with ($M + H^+$) ion at 121.05087 m/z and HP-922 with ion at 922.00980 m/z. They were infused into the spray chamber using a calibrant delivery system.

GC−MS Instrumentation and Analysis. GC−MS samples were ionized using the EI auto mode. The capillary column was a VF-5 ms 30 m × 0.25 mm × 0.25 μm (where ID = 0.25 mm, DF = 0.25 μm). The solvent delay was set to 3 min. Temperature program was as follows: 80 °C (0−5 min), 80−250 °C (5−22 min, at a rate 10 °C/min), 250 °C (22−28 min). Total run was 28 min. The instrument parameters were set as follows: injector temperature 200 °C, column flow rate 1 mL/min. Data were collected with the instrument set to mass range 40−650 m/z.

Equilibration and Solubility Determinations. To determine the extent of polysulfane equilibration, a 6.4 mg sample of dry **6A**−**F** was dissolved in 3 mL of methanol or methanol/water mixtures by stirring for 2 min at rt. Then 0.2 mL was placed in a vial and diluted to 1 mL with methanol. The solution was stirred

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for 1 min, in which the concentration of each sample was 0.42 mg/mL. Finally, 50 μL of the solution was injected into the HPLC autosampler and quantitated by the absorption signal at 254 nm. The solubilities of elemental S_8, **5A**−**C**, or **6A**−**F** were determined by adding 10−50 μL aliquots of methanol or water to 1−3 mg of the compounds. The solutions were vortexed for 2 min and then stirred 10−15 min at rt. Polysulfane decompositions were also conducted in the presence of sodium hydroxide. To a solution of polysulfanes **6A**−**F** (2.5 mM) and internal standard, acetanilide (0.25 mM), was added NaOH (0.625, 1.25, 1.875, or 2.5 mM) in 0.1 mL of methanol. After certain periods of time, the reaction was analyzed by HPLC by injecting 15 μL of the sample.

Sulfur Transfer and Trapping Studies. Trapping studies were carried out in 0.2 mL of benzene solution. To a solution of PEGylated benzopenthasulfanes **6A**−**F** (2.3 mM) and norbornene or 2,3-dimethylbutadiene (2.3 mM) was added ethanethiol (2.3 mM). The reaction mixture was vortexed for 2 min and analyzed by GC−MS by injecting 1 μL of the sample. Both scan and single ion mode (SIM) analyses were conducted on the sample.

Cell Proliferation Assays. The cell lines were grown from frozen stocks originally obtained from the American Type Culture Collection. The prostate cancer cell lines PC3 and DU145 cells were grown in F12K and DMEM, respectively. MDA-MB-231 breast cancer cells were grown in DMEM. All the media were supplemented with 10% FBS and penicillin/streptomycin. Cell proliferation was assessed by a cell proliferation assay as previously described.[34] Briefly, equal numbers of cells were distributed in 96-well plates and the cells were incubated at 37 °C in a 5% CO_2 incubator until they were in log phase growth. The medium was removed and replaced with a growth medium containing the sulfur-containing compounds (0−30 μM). After 48 h incubation, the media was removed, and the plates were frozen at −80 °C. The assay was performed by warming the plates to rt and followed by the addition of the reagent to the wells. The fluorescence was measured with a plate reader (excitation/emission, 485/530 nm).

The cytotoxicity assay was conducted as follows: Cells were cultured in RPMI-1640 medium with 10% fetal bovine serum and penicillin-streptomycin (100 U/mL and 100 μg/mL, respectively) and maintained in a 37 °C humidified 5% CO_2 incubator. On the day before the drug treatment, cells were plated onto each well of the 96-well plate at 2,000 cells/well (200 μL of the medium per well). After 24 h, cells were treated with different concentrations of the sulfur-containing compounds and incubated for 72 h. After the incubation, cell growth was evaluated using a 96-titer solution cell proliferation assay. UV absorption (490 nm) of each well was quantified with a microplate reader.

***N*-(2-(2-(2-Methoxyethoxy)ethoxy)ethyl)benzo[*d*][1,2,3]trithiole-5-carboxamide (6A).** HPLC (150 mm × 3.9 mm C18 column, 90% acetonitrile in water, flow rate 1 mL/min): t_R = 20.3 min. LCMS: t_R = 4.6 min; HRMS (+ESI) calcd for $C_{14}H_{19}NO_4S_3$ = 361.0476, found 361.0478.

***N*-(2-(2-(2-Methoxyethoxy)ethoxy)ethyl)benzo[*e*][1,2,3,4]tetrathiine-6-carboxamide (6B).** HPLC (150 mm × 3.9 mm C18 column, 90% acetonitrile in water, flow rate 1 mL/min): t_R = 22.6 min. LCMS: t_R = 5.6 min; HRMS (+ESI) calcd for $C_{14}H_{19}NO_4S_4$ = 393.0197, found 393.0193.

***N*-(2-(2-(2-Methoxyethoxy)ethoxy)ethyl)benzo[*f*][1,2,3,4,5]pentathiepine-7-carboxamide (6C).** Yield 9.6 mg (53%). A solution of 3,6,9-trioxadecylamine **15** (13.8 mg, 0.0847 mmol) in 1.5 mL of THF was added to a solution of 4-nitrophenyl benzo[*f*]-[1,2,3,4,5]pentathiepine-7-carboxylate **14** (17 mg, 0.0423 mmol) in 2.5 mL of THF. The reaction mixture was stirred under argon atmosphere overnight. The solvent was evaporated, and the residue was dissolved in 30 mL of CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ solution (3 × 30 mL), 1 M HCl (3 × 30 mL), and water (3 × 30 mL). The organic solvent was evaporated and the crude product was chromatographed ($CHCl_3$/MeOH, 10:1) to yield 9.6 mg of **6C** (93% purity): R_f = 0.57, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.30 (d, *J* = 1.9 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.78 (dd, *J* = 7.90, 1.9 Hz, 1H), 7.18 (br s, 1H), 3.66 (m, 10H), 3.54 (m, 2H), 3.32 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.4, 155.2, 150.3, 145.8, 1145.0, 137.3, 136.3, 131.5, 130.2, 125.4, 122.5. HPLC (150 mm × 3.9 mm C18 column, 90% acetonitrile in water, flow rate 1 mL/min): t_R = 28.7 min. LCMS: t_R = 6.5 min; HRMS (+ESI) calcd for $C_{14}H_{19}NO_4S_5$ = 424.9918, found 424.9926.

***N*-(2-(2-(2-Methoxyethoxy)ethoxy)ethyl)benzo[*g*][1,2,3,4,5,6]-hexathiocine-8-carboxamide (6D).** HPLC (150 mm × 3.9 mm C18 column, acetonitrile/water 1:9 to 9:1 over 53 min, flow rate at 1 mL/min): t_R = 30.0 min. LCMS: t_R = 7.1 min; HRMS (+ESI) calcd for $C_{14}H_{19}NO_4S_6$ = 456.9638, found 456.9636.

***N*-(2-(2-(2-Methoxyethoxy)ethoxy)ethyl)benzo[*h*][1,2,3,4,5,6,7]-heptathionine-9-carboxamide (6E).** HPLC (150 mm × 3.9 mm C18 column, acetonitrile/water 1:9 to 9:1 over 53 min, flow rate at 1 mL/min): t_R = 36.0 min. LCMS: t_R = 7.8 min; HRMS (+ESI) calcd for $C_{14}H_{19}NO_4S_7$ = 488.9359, found 488.9354.

***N*-(2-(2-(2-Methoxyethoxy)ethoxy)ethyl)benzo[*j*][1,2,3,4,5,6,7,8,9]nonathiacyclo-undecine-11-carboxamide (6F).** HPLC (150 mm × 3.9 mm C18 column, acetonitrile/water 1:9 to 9:1 over 53 min, flow rate 1 mL/min): t_R = 40.8 min. LCMS: t_R = 9.2 min; HRMS (+ESI) calcd for $C_{14}H_{19}NO_4S_9$ = 552.8800, found 552.8788. The identification of peak **6F** at 9.2 min in the spectrum is complicated because the former is a shoulder in the chromatogram of **3A**. Peak **6F** has been assigned on the basis of the extracted ion chromatogram feature of the software.

Methyl 3,4-Dihydroxybenzoate (8). Yield 1.018 g (76%). 3,4-Dihydroxybenzoic acid **7** (1.0 g, 6.49 mmol) was dissolved in 40 mL of MeOH. A catalytic amount of concentrated HCl (5 drops) was added to the methanol solution, which was then refluxed overnight at 80 °C. Water (300 mL) was added, and the mixture extracted with EtOAc/hexane (60:40) and dried over anhydrous $MgSO_4$. The solvent was removed, affording a light brown solid (mp 137−139 °C). ^{1}H NMR (acetone-d_6) δ 3.81 (s, 3H), 6.90 (d, *J* = 8.3 Hz, 1H), 7.45 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.50 (d, *J* = 2.0 Hz, 1H), 8.47 (br s, 2H); ^{13}C NMR (acetone-d_6) δ 51.9, 115.8, 117.2, 123.0, 123.4, 145.6, 150.8, 167.1; HRMS (+ESI) calcd for $C_8H_8O_4$ = 168.0423, found 168.0423.

Methyl 3,4-Bis[(dimethylamino carbothioyl)oxy]benzoate (9). Yield 0.546 g (74%). Methyl-3,4-dihydroxybenzoate **8** (738 mg, 4.39 mmol), DABCO (17.6 mmol), and *N*,*N*-dimethylthiocarbamoyl chloride (2.17 g, 17.6 mmol) were stirred in DMF (10 mL) for 30 min. The white solid that formed was dissolved in 50 mL of water. The product was extracted with EtOAc (3 × 30 mL) and dried over anhydrous $MgSO_4$. The solvent was removed, affording a yellow-green oil. Benzoate **9** was obtained by flash chromatography (EtOAc/hexanes, 2:3); R_f = 0.4 or via recrystallization in 95% EtOH as white crystals (mp 110−112 °C). ^{1}H NMR ($CDCl_3$) δ 8.01 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.85 (d, *J* = 2.0, 1H), 7.25 (d, *J* = 8.4, 1H), 3.90 (s, 3H), 3.43 (s, 3H), 3.30 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 186.6, 186.1, 165.6, 149.4, 145.6, 128.7, 128.1, 125.9, 124.4, 52.3, 43.4, 43.3, 38.9, 38.8; HRMS (+ESI) calcd for $C_{14}H_{18}O_4S_2N_2$ calcd 342.0708, found 342.0708. (lit. data for **9**, ref 14); ^{1}H NMR ($CDCl_3$) δ 8.00 (dd, *J* = 8.5, *J* = 2.0 Hz, 1H), 7.86 (d, *J* = 2.0 Hz, 1H), 7.25 (d, *J* = 8.5 Hz, 1H), 3.90 (s, 3H), 3.43, 3.42, 3.30, 3.29, 3.30 (4 × br s, 4 × 3H); ^{13}C NMR ($CDCl_3$) δ 186.4, 186.0, 165.6, 149.3, 145.5, 128.7, 128.2, 125.9, 124.4, 52.3, 43.4, 43.3, 38.9.

Methyl 2-Oxo-1,3-benzodithiole-5-carboxylate (10). Yield 0.079 g (40%). Methyl 3,4-bis([dimethylamino-carbothioyl]-oxy)benzoate **9** (300 mg, 0.876 mmol) was heated at 240 °C in 10 mL of diphenyl ether for 30 min. The reaction mixture was purified by flash chromatography using a gradient of EtOAc/

(34) Bittman, R.; Li, Z.; Samadder, P.; Arthur, G. *Cancer Lett.* **2007**, *251*, 53–58.

hexane (2:98 to 40:60) to afford 79 mg (40%) of **10** as a light brown solid. R_f = 0.75 (EtOAc/hexane 1:2), mp 140−142 °C. ^{1}H NMR ($CDCl_3$) δ 3.95 (s, 3H), 7.57 (d, J = 8.5 Hz, 1H), 7.98 (dd, J = 8.5, J = 2.0 Hz, 1H), 8.18 (d, J = 2.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 52.5, 122.9, 124.1, 128.2, 129.2, 133.0, 138.0, 165.7, 188.8; MS (EI): m/z = 226 (M), 198 (M − CO), 167 (M − CO_2Me) and 139 (M − CO_2Me − CO); molecular peak ($C_9H_6O_3S_2$, M = 226) isotopic ratio (calcd %, found %): [M + 1] 227 (11.5, 11.4), [M + 2] 228 (9.0, 9.8), and [M + 3] 229 (1.2, 1.5); HRMS (+ESI) calcd for $C_9H_6O_3S_2$ = 225.9758, found 225.9757 (lit. ref 14); ^{1}H NMR ($CDCl_3$) δ 3.95 (s, 3H), 7.57 (d, J = 8.5 Hz, 1H), 7.98 (dd, J = 8.5, J = 2.0 Hz, 1H), 8.18 (d, J = 2.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 52.6, 122.9, 124.1, 127.8, 129.1, 133.0, 137.9, 165.7, 189.0.

3,4-Disulfanylbenzoic Acid (11). Yield 0.52 mg (82%). An aqueous solution of NaOH (1 N, 3 mL) was added to **10** (79 mg, 0.35 mmol). The resulting mixture was heated at 70 °C under nitrogen atmosphere for 4 h. The reaction mixture was cooled to rt and acidified with HCl (1 N). The white precipitate was collected by filtration, washed several times with H_2O, and dried overnight to afford **11**, mp 218−220 °C. ^{1}H NMR (CD_3CN) δ 9.50 (br s, 1H), 7.98 (d, J = 1.8 Hz, 1H), 7.66 (dd, J = 8.1, 1.8 Hz, 1H), 7.45 (d, J = 8.1 Hz, 1H), 4.34 (br s, 2H). ^{1}H NMR (CD_3OD) δ 8.07 (d, J = 1.8 Hz, 1H), 7.73 (dd, J = 8.1, 1.8 Hz, 1H), 7.52 (d, J = 8.1 Hz, 1H), ^{13}C NMR (CD_3OD) δ 168.9, 140.4, 132.9, 131.5, 130.5, 129.5, 128.4. HRMS (−ESI): calcd for $C_7H_6O_2S_2$ = 185.9809, found 185.9811. (lit. data for **11**, ref 14); ^{1}H NMR (CD_3OD) δ 8.03 (d, J = 2 Hz, 1H), 7.70 (dd, J = 8, 2 Hz, 1H), 7.47 (d, J = 8 Hz, 1H), 4.05 (s, 1H), 3.67 (s, 1H); ^{13}C NMR (CD_3OD) δ 167.9, 139.4, 131.9, 130.4, 129.5, 128.5, 127.4.

2,2-Dimethylbenzo[*d*][1,3,2]dithiastannole-5-carboxylic Acid (12). Compound **12** was synthesized using a modified literature procedure by Lee et al.[35] To an ethanolic solution (10 mL) of **11** (116 mg, 0.623 mmol) was added an ethanolic solution of NaOH (5 mL, 0.2 M). After the mixture was stirred at rt for 15 min, an aqueous solution (5 mL) of dimethyltin chloride (273 mg, 1.24 mmol) was added. The solution was stirred for 1.5 h, acidified with 1 N HCl, and extracted with CH_2Cl_2 (30 mL × 3). The resultant organic layers were combined and washed with water and brine solution. The organic layer was dried over anhydrous $MgSO_4$, and the solvent was removed, affording 155 mg (75%) of **12**. ^{1}H NMR (CD_3CN) 7.95 (d, J = 1.6 Hz, 1H), 7.45 (d, J = 8.1 Hz, 1H), 7.40 (dd, J = 8.1, 1.6 Hz, 1H), 0.96 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 166.5, 146.5, 139.9, 129.7, 129.0, 125.1, 124.1 4.76; HRMS (+ESI) calcd for $C_9H_{10}O_2S_2Sn$ = 325.9170, found 325.9171.

4-Nitrophenyl 2,2-Dimethylbenzo[*d*][1,3,2]dithiastannole-5-carboxylate (13). Yield 149 mg (72%). Compound **12** (155 mg, 0.465 mmol) was dissolved in dry CH_2Cl_2 (5 mL). To this solution was added a catalytic amount of DMAP, with stirring for 5 min. DCC (113 mg, 0.51 mmol) was added. After the solution was stirred for 10 min, *p*-nitrophenol (138 mg, 0.93 mmol) was added, the solution was stirred overnight, and 30 mL of CH_2Cl_2 was added. The white precipitate of urea was removed by filtration. The organic layer was washed with concentrated citric acid solution, saturated aqueous $NaHCO_3$ solution, and water, and the organic layer was dried over anhydrous $MgSO_4$. The solvent was removed, affording crude compound **13**. The crude mixture was purified by column chromatography using EtOAc/hexanes (2:3) to afford **10**. R_f = 0.54, mp 198−199 °C; ^{1}H NMR ($CDCl_3$) δ 8.33 (d, J = 8.3 Hz, 2H), 8.30 (d, J = 1.6 Hz, 1H), 7.67 (dd, J = 8.1, 1.6 Hz, 1H), 7.59 (d, J = 8.1 Hz, 1H), 7.39 (d, J = 8.3 Hz, 2H), 1.09 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 163.6, 155.9, 147.4, 145.4, 139.7, 131.2, 130.0, 125.6, 125.2, 124.4, 122.6, 3.2; HRMS (−ESI) calcd for $C_{15}H_{13}NO_4S_2Sn$ = 446.9334, found 446.9324.

4-Nitrophenyl Benzo[*f*][1,2,3,4,5]pentathiepine-7-carboxylate (14). Yield 9.5 mg (27%). Compound **14** was synthesized using a modification of the procedure of Sato et al.[15] To a solution of **10** (40 mg, 0.088 mmol), in 7 mL of dry CH_2Cl_2 at 0 °C, was added dropwise a solution of S_2Cl_2 (23.8 mg, 0.176 mmol) in 3 mL of dry CH_2Cl_2 at 0 °C. The reaction mixture was allowed to reach rt and stirred for 24 h. After 15 mL of CH_2Cl_2 was added, the reaction mixture was washed with water (20 mL × 3). The organic layer was dried with anhydrous $MgSO_4$ and evaporated. The product was purified by column chromatography using EtOAc/hexanes (1:4) to afford **14**. R_f = 0.44 ($CHCl_3$/hexanes, 1:2); ^{1}H NMR ($CDCl_3$) δ 8.66 (d, J = 1.5 Hz, 1H), 8.36 (d, J = 8.1 Hz, 2H), 8.13 (dd, J = 8.1, 1.5 Hz, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.42 (d, J = 8.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 162.4, 155.2, 150.3, 145.8, 1145.0, 137.3, 136.3, 131.5, 130.2, 125.4, 122.5.

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Supporting Information Available: Spectroscopic data for compounds **6A**−**F** and **8**−**17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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