Preparation and Evaluation of Sulfide Derivatives of the Antibiotic Brefeldin A as Potential Prodrug Candidates with Enhanced Aqueous Solubilities

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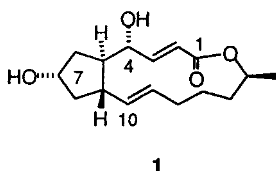
Several sulfide (+)-brefeldin A (BFA) analogues were prepared through the Michael addition of various thiols. Many of the sulfides were also oxidized to the corresponding sulfoxide with *m*-CPBA. The sulfides were designed to act as BFA prodrugs via the metabolic oxidation to the sulfoxide and subsequent syn elimination. Kinetic experiments were used to prove that the syn elimination of the sulfoxides prepared did in fact take place. Five selenide BFA prodrugs were also prepared that are envisioned to act in the same manner as the sulfides. As expected, when oxidation of the selenide to selenoxide was attempted, in situ syn elimination was observed. All of the compounds prepared were evaluated for antiproliferative activity against human cancer cell lines in the National Cancer Institute screen. The sulfoxides were much more potent than either the sulfides or selenides. Especially notable were sulfoxide **21**, which possessed a cytotoxicity mean graph midpoint value (MGM) value lower than BFA itself, and sulfoxide **22**, which possessed an MGM value slightly less potent than that of BFA. The sulfide analogues were shown to possess increased aqueous solubilty with respect to BFA.

Introduction

(+)-Brefeldin A (BFA) is a macrocyclic lactone antibiotic that was first isolated in 1958 from *Penicillium decumbens*.[1] Structure **1** was determined by X-ray



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crystallography over 10 years later.[2] Brefeldin A possesses a diverse array of biological activities including antiviral,[3,4] antifungal,[5−8] antitumor,[9,10] nematocidal,[11] and antimitotic[12] effects. It has been demonstrated that BFA rapidly and reversibly blocks traffic from the endoplasmic reticulum (ER) to the Golgi apparatus but not from the Golgi apparatus to the ER, resulting in the redistribution of Golgi proteins into the ER.[13] BFA causes the reversible disassembly of the Golgi apparatus[13,14] and was found to inhibit protein traffic from the distal Golgi apparatus to the cell surface.[15,16] Furthermore, BFA has been shown to affect the cell cycle, causing the accumulation of PC-3 and DU-145 cells in G_1.[17] The ability of BFA to induce apoptosis in cancer cells has stimulated interest in its development as an anticancer agent.[18−23] Unfortunately, BFA possesses a number of undesirable pharmacokinetic properties, including poor bioavailability after oral administration and rapid clearance from blood plasma after intravenous administration, that would limit its use clinically.[24] Additionally, the low aqueous solubility of BFA will impede formulation.[24] To address these issues, we set out to design and synthesize a number of potential BFA prodrugs.

Several sulfide prodrugs were prepared via a Michael addition of respective thiols to the α,β-unsaturated lactone of BFA. The set of sulfide prodrugs was chosen to have a wide range of lipophilicity so that structural effects on bioavailability, clearance, and solubility could be clearly discerned. It is envisioned that after administration, the sulfides would be oxidized to the corresponding sulfoxides,[25] which could then undergo syn elimination to regenerate BFA.[26,27] The oxidation of sulfides to sulfoxides by cytochrome P450 and FMO is well documented[28−32] and has been shown to occur with arylalkyl sulfides,[29−33] dialkyl sulfides,[34−36] and cyclic sulfides.[29] Furthermore, in vivo disposition studies have shown that the sulfides metiamide and cimetidine, as well as the sulfoxides sulindac and sulfinpyrazone, undergo metabolic sulfide−sulfoxide interconversion.[37−42]

Our prior studies with BFA sulfide derivatives revealed that the most cytotoxic analogues contained side chains with amino and hydroxyl groups.[43] Therefore, the BFA derivatives having these functionalities were examined in more detail in the present investigation.

Results and Discussion

Sulfide analogues **11**−**19** were prepared by conjugate addition of the corresponding thiols with **1** in the presence of 1,8-bis(dimethylamino)naphthalene (proton sponge) (Scheme 1). Reactions of the alkanethiols proceeded readily at ambient temperature, whereas the aromatic thiols required extended reaction times and occasionally increased temperatures. The reactions proceeded with good stereoselectivity to provide the *R* isomer in approximately 30-fold excess. The stereochemistry of the major addition product was determined by obtaining the single-crystal X-ray structure of **18**

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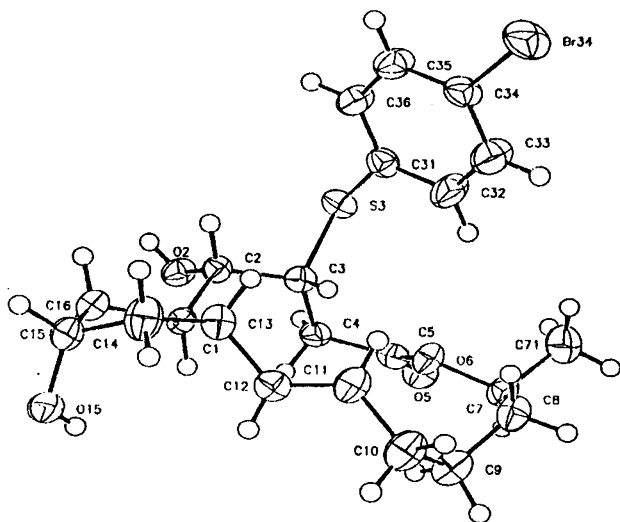
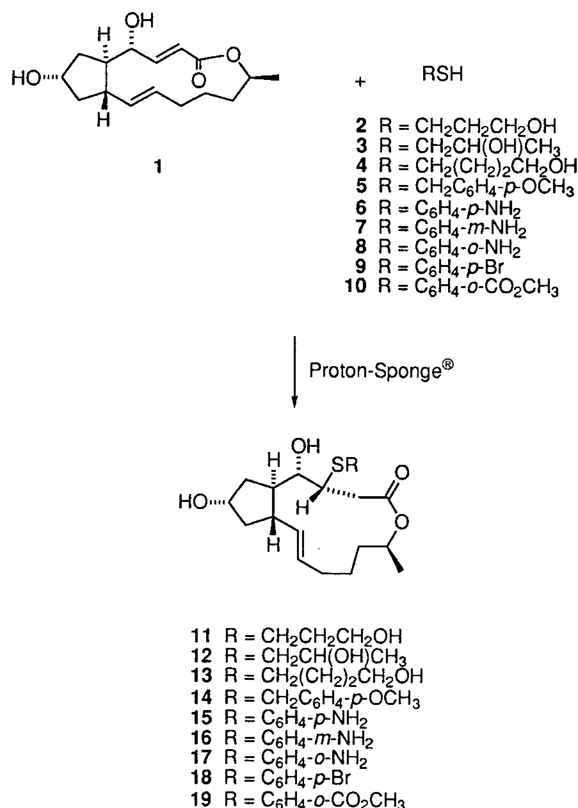


Figure 1. Single-crystal X-ray structure of **18** demonstrating *R* configuration of carbon−sulfur bond.

Scheme 1



(Figure 1). Oxidation of the corresponding sulfides with *m*-CPBA provided sulfoxide analogues **20**−**27** (Scheme 2). Oxidation occurred in a diastereoselective manner, providing a single diastereomer. This results in *S* stereochemistry of the S−O bond for compound **24**, and the other oxidations are assumed to occur with the same stereochemical outcome. The stereochemistry of the sulfoxides was assigned by X-ray crystallography of **24**. The resulting ORTEP diagram of the crystal structure is displayed in Figure 2. Sulfides **17** and **19** were also oxidized, but the resulting sulfoxides were unstable and eliminated to provide BFA on standing.

Sulfide analogue **12** was prepared from the corresponding racemic thiol and was tested as a mixture of

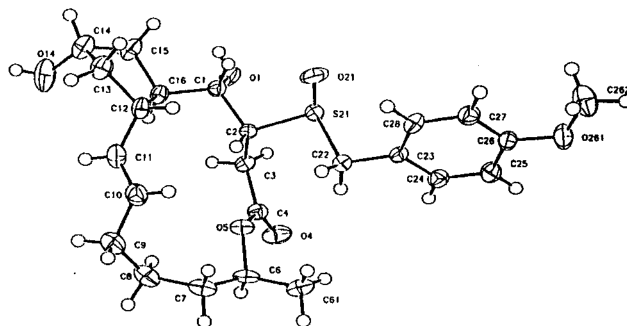
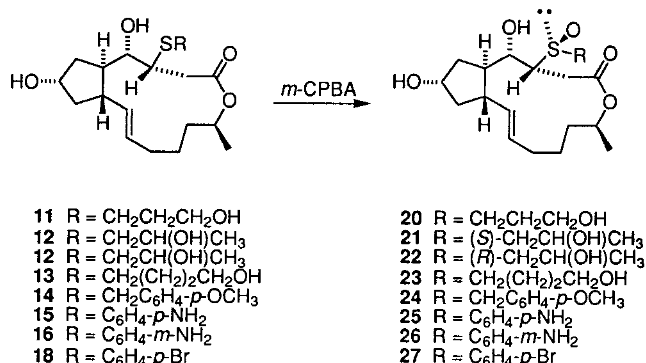


Figure 2. Single-crystal X-ray structure of **24** demonstrating the *S* configuration of the sulfur−oxygen bond.

Scheme 2



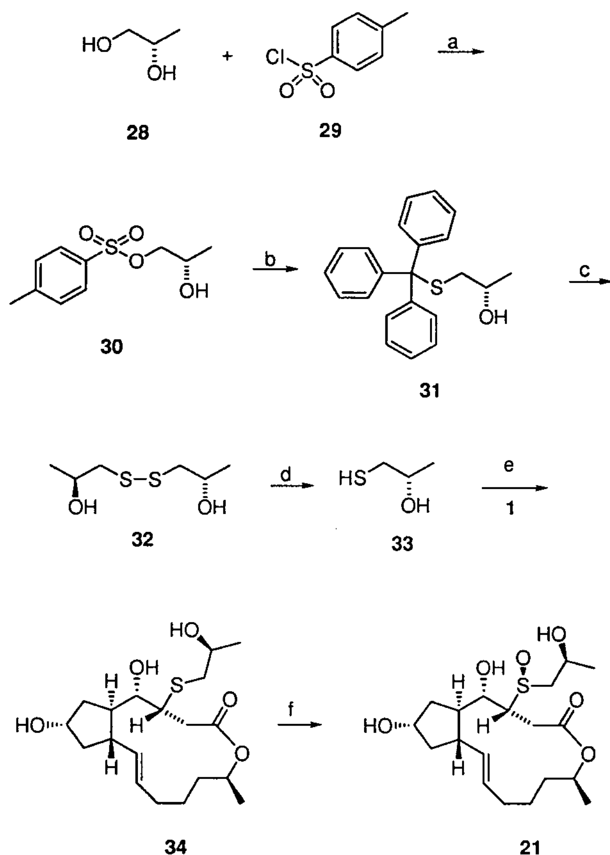
epimers at C2′. After oxidation to the sulfoxides, the diastereomers **21** and **22** were separated by flash chromatography. When tested in the in vitro assay, sulfoxide analogues **21** and **22** were found to be our most potent brefeldin analogues to date (Table 1). To determine the stereochemistry at C2′ of these compounds, the *S* isomer was independently synthesized from (*S*)-(+)-propanediol (Scheme 3). (*S*)-(+)-2-hydroxypropyl *p*-toluenesulfonate (**30**) was prepared from (*S*)-(+)-propanediol (**28**) as described.[44] Conversion of sulfonate **30** to (*S*)-(+)-2-hydroxypropyl triphenylmethylsulfide (**31**) was achieved by treatment of **30** with triphenylmethanethiol.[45] The trityl group was removed with iodine in methanol, resulting in disulfide **32**.[46] Disulfide **32** was reduced to the corresponding thiol **33** with $NaBH_4$. Thiol **33** was used immediately in the Michael addition with BFA. The resulting sulfide **34** was oxidized with *m*-CPBA to produce the corresponding sulfoxide **21**. The TLC R_f and the ^{1}H NMR spectrum of the 2′-*S* isomer synthesized as shown in Scheme 3 are identical to those of sulfoxide **21**. Consequently, the stereochemistry at C2′ of **21** and **22** could be assigned.

In addition to the sulfide analogues synthesized, five selenide analogues were prepared. Selenides are commonly used to generate α,β-unsaturated carbonyl compounds by oxidation and subsequent in situ syn elimination. The preparation of selenides might therefore represent a viable strategy for the design of BFA prodrugs. Starting with commercially available diselenides in an ethanol solution, sodium borohydride was added in portions, resulting in a clear solution of sodium selenide. Treatment with acetic acid led to the corresponding selenol. Addition of **1** and heating the reaction mixture at reflux provided the desired selenides **35**−**38** in good yields (Scheme 4).[47−49] Selenide **41** was prepared from diethyl (2-bromoethyl)phosphonate **39** by

Table 1. Cytotoxicities of (+)-Brefeldin A Prodrugs in μM

compd	lung HOP-62	colon HCT-116	CNS SF-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12C	prostate DU-145	breast MDA-MB-435	MGM
1	0.070	0.029	0.040	0.022	0.032	0.090	0.13	0.041	0.040 ± 0.019
11	85.4	>100	NT	35.5	>100	>100	>100	43.7	72.9 ± 20.4
12	75.9	22.4	35.5	31.6	37.2	41.7	49.0	34.7	38.0
13	77.6	>100	53.7	30.9	46.8	72.4	>100	58.9	50.1
14	79.4	63.1	25.1	33.9	63.1	37.2	>100	40.7	36.3
15	6.61	2.75	5.37	4.42	3.13	4.17	9.32	3.97	3.76 ± 0.04
16	6.49	2.30	3.30	2.66	3.52	2.65	6.34	4.91	2.72 ± 0.03
17	13.0	9.62	6.46	3.17	3.68	11.9	42.2	4.86	6.07 ± 1.17
18	3.21	2.19	2.54	2.36	2.75	5.89	9.16	3.24	3.00 ±0.31
19	35.5	35.5	20.4	20.0	30.9	45.7	53.7	32.4	28.8
20	0.62	0.40	0.59	0.24	0.26	0.83	3.30	0.44	0.42 ± 0.26
21	0.041	0.024	0.045	0.026	0.025	0.04	0.24	0.032	0.028 ± 0.017
22	0.11	0.49	0.71	0.028	0.03	0.093	0.48	0.03	0.048 ± 0.023
23	0.90	0.37	0.60	0.27	0.28	1.02	1.91	0.29	0.35 ± 0.16
24	2.60	1.83	5.37	1.48	1.49	2.51	6.78	0.96	1.47 ± 0.88
25	2.18	1.08	2.82	0.30	0.40	1.39	5.13	0.52	0.74 ± 0.33
26	1.04	1.13	0.93	0.32	0.30	1.06	4.23	0.43	0.59 ± 0.19
27	1.84	1.21	2.88	0.097	0.49	1.71	6.49	0.37	0.84 ± 0.61
35	11.5	12.9	>100	2.51	6.51	26.7	31.9	11.6	10.3 ± 3.2
36	3.82	2.34	4.47	2.4	0.99	5.83	9.97	3.76	3.39 ± 0.00
37	6.21	3.35	4.07	3.63	3.49	9.69	14.9	4.18	5.43 ± 0.06
38	2.23	1.5	2.43	5.25	1.75	2.69	2.72	2.91	1.84 ± 0.06
41	1.8	0.51	0.54	1.13	0.30	2.19	1.97	0.6	0.695 ± 0.06

Scheme 3[a]

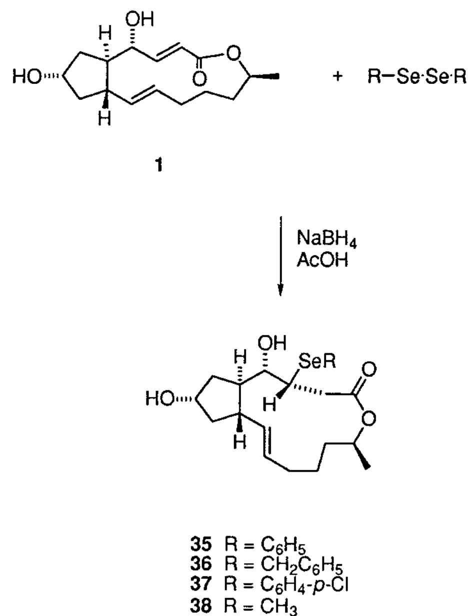


[a] (a) NEt_3, CH_2Cl_2; (b) TritSH, *n*-BuLi; (c) I_2, MeOH; (d) $NaBH_4$, *i*-PrOH; (e) 3:1 MeOH/H_2O, 1,8-bis(dimethylamino)naphthalene; (f) *m*-CPBA, CH_2Cl_2, THF.

preparation of diselenide **40** followed by conjugate addition using the previously described conditions (Scheme 5). The stereochemistry of the conjugate additions was based on precedence of the sulfur derivatives and comparison of the ^{1}H NMR spectra of the selenide and sulfur analogues.

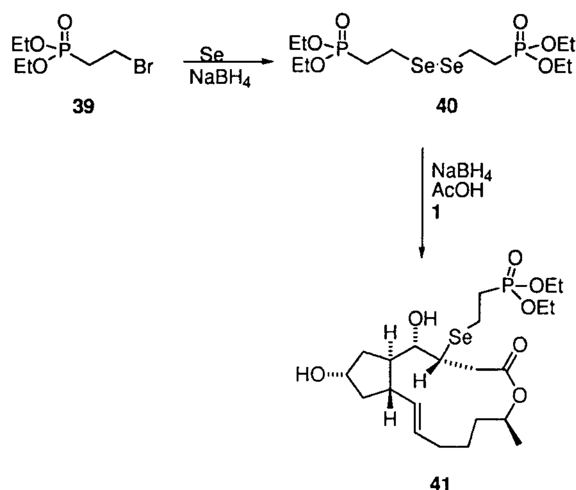
Oxidation of the selenide **35** to the corresponding selenoxide using *m*-CPBA resulted in a 61% yield of BFA and 38% recovered starting material as the only compounds isolated from the reaction mixture. The in situ syn elimination to regenerate **1** demonstrates the potential of the selenides to function as efficient prodrugs of BFA.

Scheme 4



The 23 BFA prodrug analogues were tested for antiproliferative activity against human cancer cells in the National Cancer Institute screen, in which the activity of each compound was evaluated in approximately 55 different cancer cell lines of diverse tumor origins. The GI_{50} values obtained with selected cell lines, along with the mean graph midpoint (MGM) values, are shown in Table 1. The MGM is determined from a calculation of the average GI_{50} for all of the cell lines tested in which GI_{50} values above and below the test range (10^{-4}–10^{-8} M) are taken as the minimum (10^{-8} M) and maximum (10^{-4} M) drug concentrations used in the screening test.

Scheme 5



It is evident from the data in Table 1 that the sulfide derivatives (MGM 2.72−72.9 μM) and the selenide derivatives (MGM 0.70−10.3 μM) are much less active than BFA (MGM 0.040 μM). This is in agreement with our previous findings[43] and supplies further evidence that the α,β-unsaturated double bond is required for growth inhibitory activity.[19,20] In contrast, the sulfoxide analogues (MGM 0.028−1.47 μM) have activity comparable to that of BFA. In fact, **21** (MGM 0.028 μM) was shown to be more active than BFA. It is possible that the sulfoxide analogues are eliminated during the in vitro assay to regenerate BFA. This would explain the increased activity compared to the sulfides, which are unable to regenerate BFA during the in vitro assay.

To demonstrate the ability of the sulfoxides to undergo syn elimination, the kinetics of elimination of eight sulfoxide prodrugs were investigated by ^{1}H NMR. The procedure is fully explained in the Experimental Section. Briefly, the sulfoxides were dissolved in a pH 7.4 buffer solution and the elimination of sulfoxide to BFA was monitored by following the disappearance of the C-3 proton of the sulfoxide and the appearance of the C-2 and C-3 protons of BFA. The half-lives of the sulfoxides determined in this manner are as follows: **20**, 285 min; **21**, 23 min; **22**, 26 min; **23**, 148 min; **24**, 305 min; **25**, 686 min; **26**, 440 min; and **27**, 440 min. The half-lives of the sulfoxides clearly show that substantial amounts of BFA should be regenerated during the National Cancer Institute (NCI) in vitro assay, which takes place over a 48 h incubation time. The regeneration of BFA would explain the increased activity of the sulfoxides relative to that of the sulfide prodrugs, which are unable to regenerate BFA in this assay. The data presented in Table 2 are organized in an attempt to correlate the rate of sulfoxide elimination with cytotoxicity. The rank order of elimination rate for the first three compounds (**22**, **23**, and **20)** is the same as the corresponding rank order of cytotoxicity, suggesting that a correlation does exist. However, the correlation does not appear to hold at elimination half-lives of greater than 285 min, since compounds **24** and **25** are clearly "out of order".

One of the goals of this work was to prepare prodrugs that possessed increased aqueous solubility compared to BFA. The aqueous solubilities of nine sulfide analogues were obtained as detailed in the Experimental Section. The solubility of **1** determined in this manner was 0.079 mg/mL, whereas the solubilities of the sulfide analogues are as follows: **11**, 12.1 mg/mL; **13**, 11.3 mg/mL; **14**, 0.8 mg/mL; **15**, 0.6 mg/mL; **16**, 0.9 mg/mL; **17**, 1.0 mg/mL; **18**, 3.3 mg/mL; **19**, 0.5 mg/mL; and **34**, 15.5 mg/mL. All of the sulfide analogues had increased aqueous solubility with respect to **1**. Especially soluble were analogues **11**, **13**, and **34** that contain a hydroxyl group in the side chain. The use of BFA sulfide derivatives with alcohols in the side chain therefore does constitute a practical strategy for obtaining BFA prodrugs with enhanced aqueous solubilities. It is noteworthy that sulfoxide **21** is the most active compound and it has the shortest elimination half-life, and its sulfide precursor **34** is the most soluble analogue.

Table 2. Correlation of Sulfoxide Elimination Rates with Cytotoxicity

compd	elimination $t_{1/2}$ (min)	cytotoxicity (MGM, μM)
21	23	0.028 ± 0.017
22	26	0.048 ± 0.023
23	148	0.35 ± 0.16
20	285	0.42 ± 0.16
24	305	1.47 ± 0.88
26	440	0.59 ± 0.19
27	440	0.84 ± 0.61
25	686	0.74 ± 0.33

Experimental Section

^{1}H NMR spectra were recorded on an ARX300 300 MHz Bruker NMR spectrometer. Kinetic experiments were conducted using a DMX500 500 MHz Varian NMR spectrometer. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. IR spectra were obtained using a Perkin-Elmer 1600 series FTIR spectrometer. Flash chromatography was performed with 230−400 mesh silica gel. Thin-layer chromatography was performed using Merck silica gel 60-F_{254} plates of 0.25 mm thickness and was visualized with *p*-anisaldehyde stain. Preparative thin-layer chromatography was performed using Analtech silica gel GF plates of 1000 μm thickness. Melting points were taken in capillary tubes and are uncorrected. Microanalyses were performed at the Purdue University Microanalysis Laboratory. (+)-Brefeldin A was supplied by the National Cancer Institute.

2,3-Dihydro-(3*R*)-(3′-hydroxypropylthio)brefeldin A (11). To a solution **1** (155 mg, 0.554 mmol) in methanol (9 mL) was added 3-mercapto-1-propanol (**2**) (72.0 μL, 0.830 mmol), 1,8-bis(dimethylamino)naphthalene (238.0 mg, 1.11 mmol), and water (3 mL). The mixture was stirred at room temperature for 7 h, at which point it was diluted with water (20 mL) and washed with hexanes (4 × 50 mL). The aqueous layer was extracted with ethyl acetate (4 × 50 mL), and the organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting colorless oil was purified by flash chromatography (silica gel, 10% EtOH/$CHCl_3$) to provide **11** (202.1 mg, 98%) as a white foam: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.50 (m, 1 H), 5.37 (dd, J = 8.47 and 15.27 Hz, 1 H), 4.85 (m, 1 H), 4.30 (m, 1 H), 3.75 (t, J = 5.95 Hz, 2 H), 3.65 (d, J = 7.48 Hz, 1 H), 3.45 (dt, J = 2.61 and 9.94 Hz, 1 H), 2.73 (t, J = 3.41 Hz, 2 H), 2.69 (m, 1 H), 2.35 (dd, J = 10.39 and 16.33 Hz, 1 H), 2.25−2.05 (m, 3 H), 2.05−1.90 (m, 3 H), 1.90−1.65 (m, 6 H), 1.65−1.40 (m, 2 H), 1.24 (d, J = 6.18 Hz, 3 H); IR (film) 3386, 1709, 1273, and 1068 cm^{-1}; LRMS (PDMS) *m/z* (rel intensity) 373.6 (38, MH^+), 355.5 (100, $MH^+ - H_2O$). Anal. ($C_{19}H_{32}O_5S$) C, H, S.

2,3-Dihydro-(3*R*)-(2′-hydroxypropylthio)brefeldin A (12). To a solution of **1** (158 mg, 0.564 mmol) in methanol (30 mL) was added 1-mercapto-2-propanol (**3**) (74.4 μL, 0.85 mmol), 1,8-bis(dimethylamino)naphthalene (241.0 mg, 1.13 mmol), and water (10 mL). The mixture was stirred at room

temperature for 4 h, after which it was diluted with water (40 mL) and washed with hexanes (4 × 50 mL). The aqueous layer was extracted with ethyl acetate (3 × 100 mL), and the organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting colorless oil was purified by flash chromatography (silica gel, 5% EtOH/$CHCl_3$) to afford **12** (200.1 mg, 95%) as a diastereomeric mixture of alcohols at the 2′ carbon: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.50 (m, 2 H), 5.40 (dd, J = 8.78 and 15.28 Hz, 2 H), 4.90 (m, 2 H), 4.32 (m, 2 H), 3.91 (m, 2 H), 3.59 (m, 4 H), 2.90−2.60 (m, 4 H), 2.60−2.30 (m, 4 H), 2.20−2.10 (m, 4 H), 2.05−1.90 (m, 6 H), 1.85−1.65 (m, 10 H), 1.60−1.40 (m, 4 H), 1.27 (d, J = 6.10 Hz, 6 H, and d, J = 6.18 Hz, 6 H are merged); IR (film) 3384.1, 2929.5, 1708.7, 1438.2, 1355.6, 1271.4, and 1061.2 cm^{-1}; LRMS (PDMS) m/z (rel intensity) 373.0 (33, MH$^+$), 355.5 (100, MH$^+$ − H_2O). Anal. ($C_{19}H_{32}O_5S$) C, H, S.

2,3-Dihydro-(3*R*)-(4′-hydroxybutylthio)brefeldin A (13). To a solution of **1** (300 mg, 1.07 mmol) in methanol (24 mL) was added 4-mercapto-1-butanol (**4**) (221 μL, 2.14 mmol), 1,8-bis(dimethylamino)naphthalene (686.9 mg, 3.21 mmol), and water (8 mL). The mixture was stirred at room temperature for 4 h, at which point it was diluted with water (20 mL) and washed with hexanes (3 × 80 mL). The aqueous layer was extracted with ethyl acetate (4 × 90 mL), and the organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting colorless oil was purified by flash chromatography (silica gel, 5% EtOH/$CHCl_3$) to provide **13** (369.6 mg, 89%) as a white foam: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.51 (m, 1 H), 5.37 (dd, J = 8.35 and 15.23 Hz, 1 H), 4.85 (m, 1 H), 4.29 (m, 1 H), 3.66 (t, J = 5.12 Hz, 2 H), 3.61 (d, J = 8.19 Hz, 1 H), 3.45 (dt, J = 2.37 and 9.80 Hz, 1 H), 2.77 (bs, 1 H), 2.71 (dd, J = 3.42 and 16.41 Hz, 1 H), 2.59 (m, 2 H), 2.34 (dd, J = 10.34 and 16.41 Hz, 1 H), 2.20−1.85 (m, 7 H), 1.80−1.40 (m, 8 H), 1.23 (d, J = 6.23 Hz, 3 H), 1.19 (m, 1 H); IR (film) 3383.9, 2930.0, 1708.3, 1449.4, 1356.0, 1270.6, and 1062.2 cm^{-1}; LRMS (PDMS) m/z (rel intensity) 387.0 (27, MH$^+$), 369.5 (100, MH$^+$ − H_2O). Anal. ($C_{20}H_{34}O_5S$) C, H, S.

2,3-Dihydro-(3*R*)-(*p*-methoxybenzylthio)brefeldin A (14). To a solution of **1** (300 mg, 1.07 mmol) in methanol (36 mL) was added 4-methoxy-α-toluenethiol (**5**) (224 μL, 1.61 mmol), 1,8-bis(dimethylamino)naphthalene (687 mg, 3.21 mmol), and water (12 mL). The reaction mixture was stirred at room temperature for 4 h, at which point it was quenched with water (20 mL) and washed with hexanes (4 × 80 mL). The aqueous layer was extracted with ethyl acetate (4 × 125 mL), and the organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting colorless oil was purified by flash chromatography (silica gel, 5% EtOH/$CHCl_3$), resulting in **14** as a colorless glass (551.3 mg, 98%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.25 (d, J = 8.53 Hz, 2 H), 6.90 (d, J = 8.61 Hz, 2 H), 5.25 (m, 2 H), 4.80 (m, 1 H), 4.27 (quin, J = 4.53 Hz, 1 H), 3.81 (s, 3 H), 3.74 (d, J = 13.81 Hz, 1 H), 3.71 (d, J = 13.81 Hz, 1 H), 3.46 (bd, J = 8.48 Hz, 1 H), 3.34 (dt, J = 2.72 and 10.39 Hz, 1 H), 2.64 (dd, J = 3.09 and 16.53 Hz, 1 H), 2.27 (dd, J = 10.80 and 16.54 Hz, 1 H), 2.10−1.85 (m, 7 H), 1.80−1.55 (m, 3 H), 1.50−1.25 (m, 4 H), 1.19 (d, J = 6.25 Hz, 3 H); IR (film) 3421.9, 2931.1, 1722.8, 1609.7, 1511.5, 1451.5, 1248.5, and 1035.6 cm^{-1}; LRMS (PDMS) m/z (rel intensity) 434.7 (100, MH$^+$). Anal. ($C_{24}H_{34}O_5S$) C, H, S.

2,3-Dihydro-(3*R*)-(*p*-aminophenylthio)brefeldin A (15). A solution of **1** (445 mg, 1.59 mmol) and 1,8-bis(dimethylamino)naphthalene (681 mg, 3.18 mmol) in methanol (100 mL) and water (25 mL) was degassed by bubbling argon through the solution. A solution of 4-aminothiophenol (**6**) (299 mg, 2.39 mmol) in methanol (25 mL) was degassed in a similar manner. After 30 min, the thiol solution was introduced via cannula. The reaction mixture was heated at reflux under an argon atmosphere for 48 h, at which point it was quenched with water (125 mL) and washed with hexanes (4 × 150 mL). The aqueous layer was extracted with ethyl acetate (4 × 200 mL), and the organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting green oil was purified by flash chromatography (silica gel, 5% EtOH/$CHCl_3$) to provide **15** as a white foam (523.7 mg, 81%): ^{1}H NMR (CD_3OD, 300 MHz) δ 7.30 (d, J = 8.54 Hz, 2 H), 6.70 (d, J = 8.54 Hz, 2 H), 5.45 (m, 1 H), 5.30 (dd, J = 9.05 and 15.28 Hz, 1 H), 4.80 (m, 1 H), 4.05 (m, 1 H), 3.60 (d, J = 10.24 Hz, 1 H), 3.50 (d, J = 9.23 Hz, 1 H), 2.65 (dd, J = 2.80 and 16.50 Hz, 1 H), 2.25 (dd, J = 10.76 and 16.41 Hz, 1 H), 2.05−1.50 (m, 9 H), 1.40−1.10 (m, 1 H), 1.25 (d, J = 6.19 Hz, 3 H); IR (film) 3364.6, 2929.9, 1713.9, 1622.3, 1598.6, 1495.7, 1450.5, 1273.6, and 1063.7 cm^{-1}; LRMS (PDMS) m/z (rel intensity) 405.4 (100, MH$^+$). Anal. ($C_{22}H_{31}NO_4S$) C, H, N.

2,3-Dihydro-(3*R*)-(*m*-aminophenylthio)brefeldin A (16). A solution of **1** (500 mg, 1.79 mmol) and 1,8-bis(dimethylamino)naphthalene (764 mg, 3.57 mmol) in methanol (100 mL) and water (25 mL) was degassed by bubbling argon through the solution. After 30 min, 3-aminothiophenol (**7**) (380 μL, 3.57 mmol) was introduced by syringe. The reaction mixture was heated at reflux for 48 h, at which point it was quenched with water (100 mL) and washed with hexanes (3 × 150 mL). The aqueous layer was then extracted with ethyl acetate (4 × 200 mL), and the organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting yellow oil was purified by flash chromatography (silica gel, 5% EtOH/$CHCl_3$) to provide **16** as a white foam: ^{1}H NMR (CD_3OD, 300 MHz) δ 7.00 (t, J = 7.76 Hz, 1 H), 6.80 (m, 2 H), 6.60 (ddd, J = 0.64, 2.03, and 7.92 Hz, 1 H), 5.60 (m, 1 H), 5.30 (dd, J = 7.97 and 15.39 Hz, 1 H), 4.75 (m, 1 H), 4.05 (m, 1 H), 3.90 (d, J = 10.66 Hz, 1 H), 3.55 (d, J = 8.63 Hz, 1 H), 2.70 (dd, J = 2.82 and 16.48 Hz, 1 H), 2.30 (dd, J = 10.89 and 16.41 Hz, 1 H), 2.10−1.50 (m, 10 H), 1.40 (m, 1 H), 1.25 (m, 1 H), 1.15 (d, J = 6.19 Hz, 3 H); IR (film) 3365.3, 2928.5, 1712.9, 1591.9, 1480.2, 1441.3, 1269.0, and 1060.6 cm^{-1}; LRMS (PDMS) m/z (rel intensity) 405.2 (100, MH$^+$). Anal. ($C_{22}H_{31}NO_4S{\cdot}0.25H_2O$) C, H, N.

2,3-Dihydro-(3*R*)-(*o*-aminophenylthio)brefeldin A (17). A solution of **1** (500 mg, 1.79 mmol) and 1,8-bis(dimethylamino)naphthalene (764 mg, 3.52 mmol) in methanol (75 mL) and water (25 mL) was degassed by bubbling argon through the solution. A solution of 2-aminothiophenol (**8**) (447 mg, 3.57 mmol) in methanol (10 mL) was degassed in a similar manner. After 30 min, the thiol solution was introduced via cannula. The reaction mixture was heated at reflux under an argon atmosphere for 48 h, at which point it was quenched with water (150 mL) and washed with hexanes (3 × 250 mL). The aqueous layer was extracted with ethyl acetate (3 × 300 mL), and the organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting green oil was purified by flash chromatography (silica gel, 5% EtOH/$CHCl_3$), resulting in **17** as a pale-yellow oil (101.1 mg, 40%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.35 (dd, J = 1.44 and 7.64 Hz, 1 H), 7.15 (dt, J = 1.55 and 7.93 Hz, 1 H), 6.75 (dd, J = 1.1 and 8.00 Hz, 1 H), 6.70 (dt, J = 1.32 and 8.02 Hz, 1 H), 5.50 (m, 1 H), 5.30 (dd, J = 8.20 and 15.06 Hz, 1 H), 4.90 (m, 1 H), 4.15 (m, 1 H), 3.85 (dt, J = 1.75 and 10.15 Hz, 1 H), 3.33 (d, J = 9.74 Hz, 1 H), 2.84 (d, J = 2.82 Hz, 1 H), 2.75 (dd, J = 2.77 and 17.1 Hz, 1 H), 2.35 (dd, J = 10.90 and 17.04 Hz, 1 H), 2.10−1.85 (m, 6 H), 1.80−1.35 (m, 6 H) 1.30 (d, J = 6.18 Hz, 3 H); IR (film) 3358.3, 2928.4, 1712.8, 1610.8, 1479.3, 1447.2, 1267.4, and 1061.0 cm^{-1}; LRMS (ESMS) m/z (rel intensity) 406.3 (100, MH$^+$). Anal. ($C_{22}H_{31}NO_4S{\cdot}0.5H_2O$) C, H, N.

2,3-Dihydro-(3*R*)-(*p*-bromophenylthio)brefeldin A (18). A solution of **1** (500 mg, 1.79 mmol) and 1,8-bis(dimethylamino)naphthalene (764 mg, 3.57 mmol) in methanol (100 mL) and water (25 mL) was degassed by bubbling argon through the solution. A solution of 4-bromothiophenol (**9**) (675 mg, 3.57 mmol) in methanol (10 mL) was degassed in a similar fashion. After 30 min, the thiol solution was introduced into the reaction flask via cannula. The reaction mixture was heated at reflux for 48 h under an argon atmosphere, at which point it was quenched with water (100 mL) and washed with hexanes (3 × 200 mL). The aqueous layer was extracted with ethyl acetate (4 × 200 mL). The organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting yellow oil was purified by flash chromatography

(silica gel, 10% EtOH/$CHCl_3$), yielding a colorless oil. After the oil stood at ambient temperature for 24 h, colorless cubic crystals formed (589.4 mg, 77%): mp 111−112 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.47 (d, J = 8.49 Hz, 2 H), 7.35 (d, J = 8.52 Hz, 2 H), 5.50 (m, 1 H), 5.39 (dd, J = 8.84 and 15.34 Hz, 1 H), 4.93 (m, 1 H), 4.25 (m, 1 H), 3.92 (dt, J = 2.67 and 9.93, 1 H) 3.47 (dd, J = 1.57 and 7.94 Hz, 1 H), 2.77 (dd, J = 3.19 and 16.71 Hz, 1 H), 2.35 (m, 1 H), 2.20−1.95 (m, 7 H), 1.80−1.35 (m, 6 H), 1.24 (d, J = 6.28 Hz, 3H); IR (film) 3399.8, 2929.8, 1725.4, 1473.6, 1385.1, 1357.8, 1268.2, and 1068.4 cm^{-1}; LRMS (FABMS) m/z (rel intensity) 470.8 (25, MH$^+$), 472.8 (25, MH$^+$), 454.8 (100, MH$^+$ − H_2O). Anal. ($C_{22}H_{29}BrO_4S$·0.5H_2O) C, H.

2,3-Dihydro-(3*R*)-(*o*-methylbenzoatethio)brefeldin A (19). A solution of **1** (208 mg, 0.741 mmol) and 1,8-bis-(dimethylamino)naphthalene (316 mg, 1.48 mmol) in methanol (30 mL) and water (10 mL) was degassed by bubbling argon through it. A solution of methylthiosalicylate (**10**) (186.5 mg, 1.11 mmol) in methanol (6 mL) was degassed in a similar manner and after 30 min was introduced into the reaction flask via cannula. The reaction mixture was stirred at room temperature for 48 h, at which point it was quenched with water (50 mL) and washed with hexanes (4 × 30 mL). The aqueous layer was extracted with ethyl acetate (4 × 50 mL), and the organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting oil was purified by flash chromatography (silica gel, 10% EtOH/$CHCl_3$), yielding **19** as a white foam (282.2 mg, 63%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.80 (dd, J = 1.46 and 7.80 Hz, 1 H), 7.63 (d, J = 7.96 Hz, 1 H), 7.50 (dt, J = 1.47 and 7.61 Hz, 1 H), 7.32 (dt, J = 0.92 and 7.58 Hz, 1 H), 5.55 (m, 1 H), 5.40 (dd, J = 8.25 and 15.21 Hz, 1 H), 4.92 (m, 1 H), 4.25 (m, 1 H), 4.10 (m, 1 H), 3.93 (s, 3 H), 3.45 (d, J = 9.58 Hz, 1 H), 2.83 (dd, J = 3.43 and 16.78 Hz, 1 H), 2.45 (dd, J = 10.20 and 16.86 Hz, 1 H), 2.20−1.95 (m, 6 H), 1.80−1.40 (m, 7 H), 1.20 (d, J = 6.17 Hz, 3 H); IR (film) 3409.5, 2930.3, 1720.8, 1435.2, 1252.8, and 1058.6 cm^{-1}; LRMS (FABMS) m/z (rel intensity) 449.5 (100, MH$^+$). Anal. ($C_{24}H_{32}O_6S$·H_2O) C, H, S.

2,3-Dihydro-(3*R*)-[3′-hydroxypropyl-(*S*)-sulfinyl]brefeldin A (20). *m*-CPBA (115.3 mg, 0.668 mmol) was added to a solution of **11** (226.2 mg, 0.607 mmol) in CH_2Cl_2 (10 mL) and THF (10 mL) at 0 °C under an argon atmosphere. After 10 min, the reaction was quenched with saturated $NaHCO_3$ (30 mL) and the layers were separated. The aqueous layer was extracted with ethyl acetate (4 × 40 mL), and the organic extracts were combined. The organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting oil was purified by flash chromatography (silica gel, 10% EtOH/$CHCl_3$), providing **20** as a white foam (128.6 mg, 55%): ^{1}H NMR (CD_3OD, 300 MHz) δ 5.50 (m, 1 H), 5.35 (dd, J = 8.37 and 15.69 Hz, 1 H), 4.95 (m, 1 H), 4.20 (quin, J = 4.62 Hz, 1 H), 3.97 (d, J = 10.22 Hz, 1 H), 3.70 (m, 2 H), 3.52 (m, 1 H), 3.08 (m, 1 H), 2.85 (m, 2 H), 2.40 (dd, J = 6.24 and 16.93 Hz, 1 H), 2.30−1.30 (m, 15 H), 1.22 (d, J = 6.27 Hz, 3 H); IR (film) 3416.3, 2932.0, 1708.6, 1646.8, 1438.9, 1263.1, and 1056.2 cm^{-1}; LRMS (ESMS) m/z (rel intensity) 389.2 (100, MH$^+$). Anal. ($C_{19}H_{32}O_6S$·1.1H_2O) C, H, S.

2,3-Dihydro-(3*R*)-[(2′*S*)-hydroxypropyl-(*S*)-sulfinyl]-brefeldin A (21). *m*-CPBA (102.0 mg, 0.591 mmol) was added to a solution of **12** (200.0 mg, 0.537 mmol), a mixture of diastereomeric alcohols at the 2′ carbon, in CH_2Cl_2 (10 mL) and THF (10 mL) at 0 °C under an argon atmosphere. After 10 min, the reaction was quenched with saturated $NaHCO_3$ (30 mL) and the layers were separated. The aqueous layer was extracted with ethyl acetate (4 × 40 mL), and the organic extracts were combined and washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting colorless oil was purified by flash chromatography (silica gel, 10% EtOH/$CHCl_3$), providing two diastereomers in a combined yield of 44%. Sulfoxide **21** eluted first and was isolated as a white foam (21.0 mg): TLC R_f 0.13 (silica gel, 10% EtOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.43 (m, 2 H), 4.97 (m, 1 H), 4.52 (m, 1 H), 4.35 (m, 1 H), 4.24 (d, J = 10.37 Hz, 1 H), 3.49 (dd, J = 3.17 and 8.87 Hz, 1 H), 3.08 (dd, J = 8.95 and 12.90 Hz, 1 H), 2.92 (dd, J = 3.66 and 17.76 Hz, 1 H), 2.84 (dd, J = 9.03 and 17.85 Hz, 1 H), 2.64 (dd, J = 2.47 and 12.85 Hz, 1 H), 2.40−1.90 (m, 12 H), 1.38 (d, J = 6.30 Hz, 3 H), 1.19 (d, J = 6.31 Hz, 3 H); IR (film) 3402.5, 2931.2, 1716.8, 1265.3, and 1048.3 cm^{-1}; LRMS (ESMS) m/z (rel intensity) 389.2 (100, MH$^+$). Anal. ($C_{19}H_{32}O_6S$·0.5H_2O) C, H, S.

2,3-Dihydro-(3*R*)-[(2′*R*)-hydroxypropyl-(*S*)-sulfinyl]-brefeldin A (22). *m*-CPBA (102.0 mg, 0.591 mmol) was added to a solution of **12** (200.0 mg, 0.537 mmol), a mixture of diastereomeric alcohols at the 2′ carbon, in CH_2Cl_2 (10 mL) and THF (10 mL) at 0 °C under an argon atmosphere. After 10 min, the reaction was quenched with saturated $NaHCO_3$ (30 mL) and the layers were separated. The aqueous layer was extracted with ethyl acetate (4 × 40 mL), and the organic extracts were combined, washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting colorless oil was purified by flash chromatography (silica gel, 10% EtOH/$CHCl_3$), providing two diastereomers in a combined yield of 44%. Sulfoxide **22** eluted second and was isolated as a white foam (67.5 mg): TLC R_f 0.10 (silica gel, 10% EtOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.40 (m, 2 H), 4.97 (m, 1 H), 4.45 (m, 1 H), 4.30 (m, 1 H), 4.15 (d, J = 10.39 Hz, 1 H), 4.00 (m, 1 H), 3.50 (m, 2 H), 2.90 (m, 3 H), 2.65 (dd, J = 8.07 and 17.51 Hz, 1 H), 2.30−1.40 (m, 10 H), 1.39 (d, J = 6.24 Hz, 3 H), 1.20 (d, J = 6.27 Hz, 3 H); IR (film) 3377.8, 2929.8, 1716.9, 1266.7, and 1044.9 cm^{-1}; LRMS (ESMS) m/z (rel intensity) 411.1 (100, MNa$^+$). Anal. ($C_{19}H_{32}O_6S$·1.7H_2O) C, H, S.

2,3-Dihydro-(3*R*)-[4′-hydroxybutyl-(*S*)-sulfinyl]brefeldin A (23). *m*-CPBA (118.6 mg, 0.687 mmol) was added to a solution of **13** (205.3 mg, 0.531 mmol) in CH_2Cl_2 (10 mL) and THF (10 mL) at 0 °C under an argon atmosphere. After 10 min, the reaction was quenched with saturated $NaHCO_3$ (30 mL) and the layers were separated. The aqueous layer was extracted with ethyl acetate (4 × 40 mL), and the organic extracts were combined and washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting yellow oil was purified by flash chromatography (silica gel, 10% EtOH/$CHCl_3$), yielding **23** as a white foam (153.7 mg, 72%): TLC R_f 0.08 (silica gel, 10% EtOH/$CHCl_3$); ^{1}H NMR (CD_3OD, 300 MHz) δ 5.50 (m, 1 H), 5.35 (dd, J = 7.77 and 15.25 Hz, 1 H), 4.97 (m, 1 H), 4.20 (m, 1 H), 3.98 (d, J = 10.00 Hz, 1 H), 3.61 (t, J = 6.20 Hz, 2 H), 3.51 (m, 1 H), 2.85 (m, 3 H), 2.37 (dd, J = 6.13 and 16.83 Hz, 1 H), 2.30−1.30 (m, 16 H), 1.22 (d, J = 6.27 Hz, 3 H); IR (film) 3407.2, 2931.5, 1718.1, 1646.2, 1449.5, 1265.4, and 1055.2 cm^{-1}; LRMS (ESMS) m/z (rel intensity) 403.3 (27, MH$^+$), 425.2 (100, MNa$^+$). Anal. ($C_{20}H_{34}O_6S$·1.1H_2O) C, H.

2,3-Dihydro-(3*R*)-[*p*-methoxybenzyl-(*S*)-sulfinyl]brefeldin A (24). *m*-CPBA (127 mg, 0.736 mmol) was added to a solution of **14** (167 mg, 0.385 mmol) in CH_2Cl_2 (5 mL) and THF (5 mL) at 0 °C under an argon atmosphere. After 10 min, the reaction was quenched with saturated $NaHCO_3$ (20 mL) and the layers were separated. The aqueous layer was extracted with ethyl acetate (4 × 50 mL), and the organic extracts were combined and washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting yellow oil was purified by flash chromatography (silica gel, 5% EtOH/$CHCl_3$) followed by recrystallization from ethyl acetate and hexanes by the solvent diffusion method to provide **24** as white needles (91.0 mg, 53%): mp 150 °C (dec); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.27 (d, J = 8.58 Hz, 2 H), 6.96 (d, J = 8.63 Hz, 2 H), 5.33 (dd, J = 9.01 and 15.39 Hz, 1 H), 5.18 (m, 1 H), 4.83 (m, 1 H), 4.31 (m, 2 H), 4.21 (d, J = 13.28 Hz, 1 H), 4.19 (d, J = 13.26 Hz, 1 H), 3.82 (s, 3 H), 3.29 (dd, J = 2.89 and 10.02 Hz, 1 H), 3.02 (dd, J = 10.08 and 18.21 Hz, 1 H), 2.94 (dd, J = 3.01 and 18.16 Hz, 1 H), 2.30−2.00 (m, 4 H), 1.95−1.70 (m, 3 H), 1.67−1.40 (m, 3 H), 1.20−1.05 (m, 2 H), 1.02 (d, J = 6.29 Hz, 3 H); IR (film) 3396.9, 2931.3, 1723.6, 1512.9, 1441.1, 1252.0, and 1032.4 cm^{-1}; LRMS (PDMS) m/z (rel intensity) 451.3 (100, MH$^+$). Anal. ($C_{24}H_{34}O_6S$) C, H, S.

2,3-Dihydro-(3*R*)-[*p*-aminophenyl-(*R*)-sulfinyl]brefeldin A (25). *m*-CPBA (56.2 mg, 0.325 mmol) was added to a solution of **15** (45.5 mg, 0.123 mmol) in CH_2Cl_2 (5 mL) and

THF (5 mL) at 0 °C under an argon atmosphere. After 4 h the reaction mixture was heated to room temperature. After 20 h, the reaction was quenched with saturated aqueous $NaHCO_3$ (20 mL) and the mixture was extracted with ethyl acetate (4 × 30 mL). The organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting clear oil was purified by flash chromatography (silica gel, 10% EtOH/$CHCl_3$), providing **25** (47.1 mg, 91%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.50 (d, J = 8.69 Hz, 2 H), 6.70 (d, J = 8.69 Hz, 2 H), 5.25 (m, 2 H), 4.75 (m, 1 H), 4.10 (m, 1 H), 3.95 (m, 1 H), 3.85 (d, J = 10.56 Hz, 1 H), 2.85 (m, 2 H), 2.15−1.80 (m, 5 H), 1.80−1.30 (m, 6 H), 1.15 (d, J = 6.22 Hz, 3 H); IR (film) 3361.7, 2928.5, 1715.4, 1636.2, 1435.3, 1266.6, and 1085.8 cm^{-1}; LRMS (ESMS) m/z (rel intensity) 422.2 (92, MH^+), 444.2 (100, MNa^+). HRMS calcd for $C_{22}H_{31}NO_5S$: 422.2001. Found: 422.2000.

2,3-Dihydro-(3*R*)-[*m*-aminophenyl-(*R*)-sulfinyl]brefeldin A (26). *m*-CPBA (56.2 mg, 0.325 mmol) was added to a solution of **16** (46.2 mg, 0.114 mmol) in CH_2Cl_2 (5 mL) and THF (5 mL) at 0 °C under an argon atmosphere. After 4 h, the reaction mixture was heated to room temperature. After 20 h, the reaction was quenched with saturated aqueous $NaHCO_3$ (20 mL) and the mixture was extracted with ethyl acetate (4 × 30 mL). The organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting clear oil was purified by flash chromatography (silica gel, 10% EtOH/$CHCl_3$), providing **26** (39.4 mg, 82%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.37 (t, J = 7.79 Hz, 1 H), 7.20 (m, 2 H), 6.95 (dd, J = 1.58 and 7.4 Hz, 1 H), 5.30 (m, 2 H), 5.03 (m, 1 H), 4.13 (m, 1 H), 3.83 (d, J = 10.21 Hz, 2 H), 3.36 (dd, J = 1.97 and 10.56 Hz, 1 H), 3.16 (dd, J = 10.63 and 18.33 Hz, 1 H), 2.75 (m, 1 H), 2.30−1.45 (m, 11 H), 1.30 (m, 1 H), 1.26 (d, J = 6.23 Hz, 3 H); IR (film) 3427.5, 2929.2, 1709.8, 1638.0, 1438.6, 1449.3, 1265.9, and 1017.0 cm^{-1}; LRMS (ESMS) m/z (rel intensity) 422.1 (100, MH^+). Anal. ($C_{22}H_{31}NO_5S\cdot H_2O$) C, H, N.

2,3-Dihydro-(3*R*)-[*p*-bromophenyl-(*R*)-sulfinyl]brefeldin A (27). *m*-CPBA (97.6 mg, 0.566 mmol) was added to a solution of **18** (205.1 mg, 0.437 mmol) in CH_2Cl_2 (10 mL) and THF (10 mL) at 0 °C under an argon atmosphere. After 10 min, the reaction was quenched with saturated $NaHCO_3$ (30 mL) and the layers were separated. The aqueous layer was extracted with ethyl acetate (4 × 40 mL), and the organic extracts were combined. The organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting yellow oil was purified by flash chromatography (silica gel, 10% EtOH/$CHCl_3$), yielding **27** as a white solid (161.3 mg, 76%): mp 118−120 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 7.80 (d, J = 8.57 Hz, 2 H), 7.70 (d, J = 8.65 Hz, 2 H), 5.37 (m, 1 H), 5.25 (dd, J = 8.21 and 15.46 Hz, 1 H), 4.70 (m, 1 H), 4.16 (t, J = 4.60 Hz, 1 H), 4.00 (d, J = 10.64 Hz, 1 H), 3.45 (t, J = 5.18 Hz, 1 H), 2.85 (dd, J = 4.67 and 16.80 Hz, 1 H), 2.40 (dd, J = 5.78 and 16.76 Hz, 1 H), 2.20−1.20 (m, 12 H), 0.91 (d, J = 6.25 Hz, 3 H); IR (film) 3384.5, 2928.9, 1724.2, 1437.7, 1261.8, and 1028.3 cm^{-1}; LRMS (ESMS) m/z (rel intensity) 485.0 (97, MH^+), 487 (100, MH^+). HRMS calcd for $C_{22}H_{29}BrO_5S$: 485.0997. Found: 485.0975.

(*S*)-(−)-2-Hydroxypropyl Triphenylmethylsulfide (31). A 50 mL round-bottomed flask equipped with a magnetic stirring bar, reflux condenser, and septa was flame-dried. Triphenylmethanethiol (1.30 g, 4.69 mmol) and THF (15 mL) were added. The solution was cooled to 0 °C, and *n*-BuLi (1.91 mL, 4.78 mmol) was added dropwise with stirring. A deep-red color persisted. A solution of tosylate **30**[44] (1.0 g, 4.34 mmol) in THF (15 mL) was added dropwise. The red color disappeared and was replaced with a yellow color. The reaction mixture was allowed to warm to room temperature and then heated at 40 °C for 12 h. The reaction was quenched with 20% AcOH in methanol (20 mL), and the mixture was concentrated in vacuo. The resulting solid was purified by flash chromatography (silica gel, 8:1 hexane/EtOAc) to provide **31** as a white solid (1.00 g, 69%): mp 92.5−94 °C; $[\alpha]_D^{20}$ −27.5° (c 3.35, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (m, 6 H), 7.27 (m, 6 H), 7.20 (m, 3 H), 3.39 (m, 1 H), 2.34 (m, 2 H), 1.03 (d, J = 6.19 Hz, 3 H); IR (film) 3385.6, 3055.5, 2968.2, 2921.3, 1594.0, 1488.4, 1443.9, 1182.9, 1121.4, 1079.0, and 1033.5 cm^{-1}; LRMS (ESMS) m/z (rel intensity) 357.0 (100, MNa^+). Anal. ($C_{22}H_{22}OS$) C, H, S.

(*S*)-(+)-2-Hydroxypropyl Disulfide (32). I_2 (8.26 g, 0.033 mol) was added to a solution of **31** (10.88 g, 0.033 mol) in methanol (800 mL) in portions over 30 min. The reaction mixture was stirred at room temperature for 10 min and then quenched with 10% aqueous sodium thiosulfate (50 mL). The mixture was concentrated in vacuo to 300 mL and then diluted with water (200 mL). The solution was extracted with EtOAc (3 × 300 mL). The organic layers were pooled, washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to provide a dark-brown solid. Purification by flash chromatography (silica gel, 5% EtOH/$CHCl_3$) provided **32** as a colorless oil (1.51 g, 51%): $[\alpha]_D^{20}$ +199.3° (c 1.96, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.06 (m, 2 H), 2.85 (dd, J = 3.44 and 13.78 Hz, 2 H), 2.66 (dd, J = 8.37 and 13.65 Hz, 2 H), 2.29 (bs, 2 H), 1.27 (d, J = 6.26 Hz, 6 H); LRMS (GC−MS) m/z (rel intensity) 182 (34, M^+), 45 (100, CH_3CHOH^+). Anal. ($C_6H_{14}O_2S_2$) C, H, S.

2,3-Dihydro-(3*R*)-[(2′*S*)-hydroxypropylthio]brefeldin A (34). A solution of **32** (1.51 g, 8.29 mmol) in 2-propanol (30 mL) was cooled to 0 °C and degassed by bubbling argon through it for 30 min. $NaBH_4$ (313 mg, 8.29 mmol) was added, and the reaction mixture was stirred at 0 °C for 30 min. The reaction mixture was warmed to room temperature and stirred for 1.5 h and then quenched with AcOH (1 mL) and poured into water (50 mL). The solution was extracted with ether (3 × 50 mL), and the organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to provide **33** as a pale-yellow liquid. This liquid was added to a solution of **1** (2.0 g, 7.14 mmol) and 1,8-bis(dimethylamino)naphthalene (3.06 g, 14.3 mmol) in methanol (200 mL) and water (50 mL). The reaction mixture was stirred at room temperature for 4 h. TLC (silica gel, 5% $CHCl_3$/EtOH) indicated the complete disappearance of thiol **32**. It was found previously that BFA **1** and sulfide **34** coelute during flash chromatography. Therefore, 4-methoxy-α-toluenethiol (**5**) (702 μL, 5.05 mmol) was added to consume any remaining BFA. Thiol **5** was chosen because the resulting sulfide **14** has an R_f value much larger than that of **34**, allowing for easy purification. The reaction mixture was stirred at room temperature for an additional 4 h and then diluted with water (100 mL) and washed with hexanes (3 × 100 mL). The aqueous layer was extracted with EtOAc (3 × 200 mL). The EtOAc layers were pooled, washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to provide a white solid. Purification via flash chromatography (silica gel, 5%, 6%, 7%, and 8% EtOH/$CHCl_3$) provided **34** as a clear colorless oil (471 mg, 18%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.53 (m, 1 H), 5.38 (m, 1 H), 4.91 (m, 1 H), 4.31 (m, 1 H), 3.94 (m, 1 H), 3.56 (d, J = 8.83 Hz, 1 H), 2.80 (dd, J = 3.19 and 13.99 Hz, 1 H), 2.72 (d, J = 16.94 Hz, 1 H), 2.43 (dd, J = 7.96 and 13.99 Hz, 1 H), 2.32 (dd, J = 11.06 and 16.98 Hz, 1 H), 2.20−2.10 (m, 3 H), 2.05−1.95 (m, 3 H), 1.85−1.45 (m, 7 H), 1.28 (d, J = 6.07 Hz, 6 H).

2,3-Dihydro-(3*R*)-[(2′*S*)-hydroxypropyl-(*S*)-sulfinyl]-brefeldin A (21). *m*-CPBA (240 mg, 1.39 mmol) was added to a solution of **34** (471 mg, 1.27 mmol) in CH_2Cl_2 (15 mL) and THF (15 mL) at 0 °C under argon. The reaction mixture was stirred at 0 °C for 15 min. TLC (silica gel, 10% EtOH/$CHCl_3$) indicated that some **34** remained. Additional *m*-CPBA (44 mg, 0.253 mmol) was added, and the reaction mixture was stirred at 0 °C for 15 min and then quenched with saturated aqueous $NaHCO_3$ (30 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 25 mL). The organic layers were pooled, washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to provide a colorless oil. Purification by flash chromatography (silica gel, 5%, 6%, 7%, 8%, 9%, and 10% EtOH/$CHCl_3$) provided **21** as a clear, colorless oil (455 mg, 93%). The R_f value and ^{1}H NMR spectra are identical with those for compound **21** made previously from racemic thiol.

General Procedure for the Conjugate Addition of Selenides. Commercially available diselenides (1.43 mmol, 4.0

equiv) were placed in a 50 mL round-bottomed flask equipped with a magnetic stirring bar, reflux condenser, and argon line. They were dissolved in EtOH (10 mL), and then sodium borohydride (111 mg or 2.86 mmol) was added in portions. After 10 min, glacial acetic acid (330 μL, 343 mg, 5.71 mmol) was added. Brefeldin A (**1**) (100 mg, 0.36 mmol) was added, and the reaction mixture was heated to 80 °C. After 24 h, the reaction was quenched with water (40 mL), extracted with EtOAc (5 × 50 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude material was purified via flash chromatography (40:1 silica gel/product), eluting with 5% EtOH/$CHCl_3$, to yield the desired selenide analogues **35**−**38**.[48−50]

2,3-Dihydro-(3*R*)-(phenylseleno)brefeldin A (35). The general procedure afforded **35** in 88% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.61 (m, 2 H), 7.33 (m, 3 H), 5.49 (m, 1 H), 5.38 (d, J = 8.2 Hz, 1 H), 4.92 (m, 1 H), 4.23 (dt, J = 3.7 and 7.4 Hz, 1 H), 3.97, (d, J = 11.0 Hz, 1 H), 3.72 (ddd, J = 4.5, 7.0, and 11.5 Hz, 2 H), 3.59 (d, J = 9.3 Hz, 1 H), 2.87 (ddd, J = 3.3, 3.5, and 16.8 Hz, 1 H), 2.50 (m, 2 H), 2.05 (m, 5 H), 1.70 (m, 2 H), 1.45 (m, 1 H), 1.24 (d, J = 6.3 Hz, 3 H); IR ($CHCl_3$) 3406, 2927, 1721, 1440, 1263, and 1130 cm^{-1}; LRMS (FAB) m/z (rel intensity) 437.8 (55, M$^+$), 245.0 (100, M$^+$ − $2H_2O$ and C_6H_5Se). Anal. ($C_{22}H_{30}O_4Se\cdot0.25H_2O$) C, H.

2,3-Dihydro-(3*R*)-(benzylseleno)brefeldin A (36). The general procedure afforded **36** in 77% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.36 (s, 2 H), 7.34 (s, 3 H), 5.27 (d, J = 5.3 Hz, 1 H), 4.80 (m, 1 H), 4.24 (dt, J = 3.8 and 7.5 Hz, 1 H), 3.85 (s, 2 H), 3.43 (m, 1 H), 2.75 (dd, J = 2.8 and 13.8 Hz, 1 H), 2.35 (dd, J = 11.5 and 16.0 Hz, 1 H), 2.07 (m, 2 H), 1.93 (s, 2 H), 1.68 (m, 1 H), 1.41 (m, 1 H), 1.25 (dd, J = 6.7 and 13.9 Hz, 1 H), 1.18 (d, J = 6.1 Hz, 3 H); IR ($CHCl_3$) 3290, 2928, 1719, 1443, 1337, 1265, and 1129 cm^{-1}; LRMS (FAB) m/z (rel intensity) 434.8 (50, M$^+$ − H_2O), 244.8 (100, M$^+$ − $2H_2O$ and C_7H_7Se). Anal. ($C_{23}H_{32}O_4Se\cdot0.5H_2O$) C, H.

2,3-Dihydro-(3*R*)-(*p*-chlorophenylseleno)brefeldin A (37). The general procedure afforded **37** in 87% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.54 (d, J = 8.0 Hz, 2 H), 7.30 (d, J = 8.2 Hz, 2 H), 5.44 (ddd, J = 7.3, 12.2, and 17.0 Hz, 1 H), 4.90 (m, 1 H), 4.26 (dd, J = 3.6 and 4.9 Hz, 1 H), 3.94 (m, 1 H), 3.72 (d, J = 8.2 Hz, 1 H), 3.58 (d, J = 8.8 Hz, 1 H), 2.88 (dd, J = 3.3 and 17.1 Hz, 1 H), 2.50 (dd, J = 11.0 and 16.7 Hz, 1 H), 2.07 (m, 2 H), 1.77−1.47 (m, 2 H), 1.24 (d, J = 6.3 Hz, 3 H); IR ($CHCl_3$) 3381, 3184, 2928, 1720, 1385, 1337, 1264, 1130, and 1089 cm^{-1}; LRMS (FAB) m/z (rel intensity) 471.5 (25, M$^+$), 244.8 (100, M$^+$ − $2H_2O$ and C_6H_4ClSe). Anal. ($C_{22}H_{29}O_4SeCl$) C, H.

2,3-Dihydro-(3*R*)-(methylseleno)brefeldin A (38). The general procedure afforded **38** in 79% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.55 (dd, J = 5.7 and 14.3 Hz, 1 H), 5.39 (dd, J = 5.7 and 11.4 Hz, 1 H), 4.88 (m, 1 H), 4.32 (m, 1 H), 3.74 (d, J = 7.1 Hz, 1 H), 3.55 (m, 1 H), 2.84 (dd, J = 3.7 and 16.4 Hz, 1 H), 2.22 (m, 1 H), 2.06 (s, 3 H), 2.03 (m, 2 H), 1.73 (m, 5 H), 1.55 (m, 1 H), 1.25 (d, J = 6.3 Hz, 3 H), 0.93 (d, J = 6.8 Hz, 1 H); IR ($CHCl_3$) 3213, 2925, 1721, 1420, 1336, 1264, and 1135 cm^{-1}; LRMS (FAB) m/z (rel intensity) 358.8 (60, M$^+$ − H_2O), 244.8 (100, M$^+$ − $2H_2O$ and CH_3Se). Anal. ($C_{17}H_{28}O_4Se$) C, H.

Oxidation of 2,3-Dihydro-(3*R*)-(phenylseleno)brefeldin A (35). Selenide **35** (10.5 mg, 0.024 mmol) was dissolved in CH_2Cl_2/THF (1:1; 2 mL) in a 10 mL round-bottomed flask equipped with a magnetic stirring bar and argon line. The solution was cooled to 0 °C, and *m*-CPBA (5.0 mg, 0.027 mmol) was added. After 3 min, TLC showed that **1** had begun to form. After 7 min, only trace amounts of selenide **35** could be seen by TLC. After 10 min and no further change in the reaction mixture, the mixture was concentrated in vacuo. The residue was purified via preparative TLC, eluting with 10% EtOH/$CHCl_3$. Two cuts were obtained. The first corresponded to unreacted starting material **35** (4.0 mg, 38% recovery). The second was **1** (4.1 mg, 61%).

Bis[2-(diethylphosphono)ethyl]diselenide (40). Sodium borohydride (437 mg, 11.5 mmol) and selenium powder (1.36 g, 17.25 mmol) were placed in a 100 mL round-bottomed flask equipped with a magnetic stirring bar, condenser, and Ar line. The mixture was cooled to 0 °C, and ethanol (30 mL) was added. After 15 min, the reaction mixture was heated to reflux with Ar bubbling through the solution. After 2 h, diethyl (2-bromoethyl)phosphonate (**39**) (910 μL, 1225 mg, 5.0 mmol) was added. The reaction was heated at 40 °C for 16 h. After the mixture cooled to room temperature, the reaction was quenched with saturated $NaHCO_3$ (100 mL). The resulting mixture was extracted with EtOAc (4 × 150 mL). The combined organic layers were washed with water (1 × 100 mL) and with brine (1 × 100 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo to provide **40** (889 mg, 73%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.12 (m, 2 H), 3.03 (dd, J = 8.7 and 8.9 Hz, 2 H), 2.24 (m, 2 H), 1.35 (t, J = 9.2 Hz, 3 H); LRMS (FAB) m/z (rel intensity) 491.2 (100, MH$^+$). HRMS (FAB) calcd for $C_{12}H_{28}O_6P_2Se_2$ (M$^+$): 489.0639. Found: 489.0637.

2,3-Dihydro-(3*R*)-[2-(diethylphosphono)ethylseleno]-brefeldin A (41). Diselenide **40** (750 mg, 1.54 mmol) was dissolved in EtOH (10 mL) in a 100 mL round-bottomed flask equipped with a magnetic stirring bar, condenser, and argon line. Sodium borohydride (235 mg, 6.2 mmol) was added over 1 h, followed by acetic acid (700 μL, 740 mg, 12.3 mmol). After 30 min, **1** (105 mg, 0.36 mmol) was added. The reaction mixture was heated at reflux for 18 h. After the mixture was cooled to room temperature, the reaction was quenched with water (50 mL). The resulting mixture was extracted with EtOAc (3 × 125 mL). The combined organic layer was washed with brine (1 × 100 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude material was purified via flash chromatography (25:1 silica gel/product), eluting with 10% EtOH/$CHCl_3$, to afford the desired product **41** (143 mg, 73%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.55 (dd, J = 5.7 and 14.3 Hz, 1 H), 5.39 (dd, J = 5.7 and 11.4 Hz, 1 H), 4.88 (m, 1 H), 4.32 (m, 1 H), 4.11 (m, 2 H), 3.74 (t, J = 7.1 Hz, 1 H), 3.60 (d, J = 9.9 Hz, 1 H), 2.81 (m, 4 H), 2.45 (dd, J = 10.7 and 16.6 Hz, 1 H), 2.12 (m, 10 H), 2.00 (m, 2 H), 1.73 (m, 4 H), 1.35 (t, J = 7.0 Hz, 1 H), 1.25 (d, J = 6.3 Hz, 3 H), 0.93 (d, J = 6.8 Hz, 1 H); LRMS (FAB) m/z (rel intensity) 523.5 (100, M$^+$). Anal. ($C_{22}H_{37}O_7PSe$) C, H.

Solubility Determination. An excess of sulfide analogues (**11**, **13**−**19**, and **34**) were mechanically stirred in 2 mL of milli-Q water for 24 h. The mixture was then filtered through a 0.45 μm syringe filter, and the filtrate was concentrated in vacuo. The resulting residue was dissolved in CD_3OD, and a known quantity of piperonal was added to serve as an internal standard. ^{1}H NMR spectra were then recorded, and integrations for the sulfide analogue were compared to those of piperonal in order to determine the quantity of sulfide analogue. The solubility of BFA was also determined in this manner. All values obtained from ^{1}H NMR compared favorably with those obtained gravimetrically.

Determination of the Kinetics of Elimination of Sulfoxides To Afford (+)-Brefeldin A. The buffer solution was prepared by combining D_2O (8 mL), CD_3OD (4 mL), NaOAc (12 mg), and $NaHCO_3$ (84 mg). Testing by pH paper confirmed a pH of 7.4. Sulfoxides (**20**−**27**) (10 mg) were dissolved in 0.8 mL of buffer, the solutions were heated at 37 °C, and ^{1}H NMR spectra were recorded at fixed time intervals. The elimination reaction was monitored by observing the disappearance of the C-3 proton of the sulfoxide and the formation of the C-2 and C-3 protons of BFA. The natural log of the sulfoxide mole fraction was plotted vs time to obtain a straight line. The equation of the line was then used to determine the half-lives of the sulfoxides.

In Vitro Cytotoxicity Values. The cell line panel consists of 60 lines from nine disease-related subpanels including leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer. The cell lines are inoculated onto 96-well microtiter plates typically at 20 000 cells/well and preincubated at 37 °C for 24 h in the absence of drug. The drug is then administered in five 10-fold dilutions and incubated for 48 h at 37 °C. After incubation the cells are fixed to the wells by addition of trichloroacetic acid followed by incubation for 60 min at 4 °C. The supernatants are discarded, and the plates are washed with deionized water and dried. A sulforhodamine B (SRB) solution is added to each well and

incubated for 10 min at room temperature. Unbound SRB is removed by washing with 1% acetic acid. The plates are then dried, and the SRB is solubilized with Tris buffer and measured spectophotometrically.[50,51]

X-ray Crystallographic Analysis of 18. A colorless chunk of **18**, $C_{22}H_{29}BrO_4S$ [0.40 mm × 0.35 mm × 0.30 mm], was mounted on a glass fiber in a random orientation. Preliminary examination and data collection were performed with Mo Kα radiation ($\lambda = 0.710\,73$ Å) on a Nonius KappaCCD diffractometer. The cell constants and an orientation matrix for data collection were obtained from least-squares refinement, using the setting angles of 16 103 reflections in the range $4° < \theta < 30°$. The data were collected at a temperature of 193 ± 1 K. A total of 16 103 reflections were collected, of which 6292 were unique. The structure was solved by direct methods using SIR97. The remaining atoms were located in succeeding difference Fourier syntheses. Hydrogen atoms were included in the refinement but restrained to ride on the atom to which they are bonded. The structure was refined in full-matrix least squares where the function minimized was $\sum w(|F_o|^2 - |F_c|^2)^2$. Refinement was performed on a AlphaServer 2100 using SHELX-97. Crystallographic drawings were done using programs ORTEP.

X-ray Crystallographic Analysis of 24. A colorless chunk of **24**, $C_{24}H_{34}O_6S$ [0.30 mm × 0.28 mm × 0.22 mm], was mounted on a glass fiber in a random orientation. Preliminary examination and data collection were performed with Mo Kα radiation ($\lambda = 0.710\,73$ Å) on a Nonius KappaCCD diffractometer. The cell constants and an orientation matrix for data collection were obtained from least-squares refinement, using the setting angles of 8504 reflections in the range $4° < \theta < 26°$. The data were collected at a temperature of 203 ± 1 K. A total of 8504 reflections were collected, of which 4653 were unique. The structure was solved by direct methods using SIR97. The remaining atoms were located in succeeding difference Fourier syntheses. Hydrogen atoms were included in the refinement but restrained to ride on the atom to which they are bonded. The structure was refined in full-matrix least squares where the function minimized was $\sum w(|F_o|^2 - |F_c|^2)^2$. Refinement was performed on a AlphaServer 2100 using SHELX-97. Crystallographic drawings were done using programs ORTEP.

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