# Design and Synthesis of Brefeldin A Sulfide Derivatives as Prodrug Candidates with Enhanced Aqueous Solubilities

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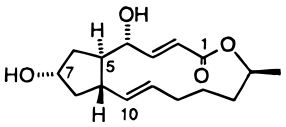
*Received November 4, 1997*

The addition of a variety of thiols to the α,β-unsaturated lactone functionality present in brefeldin A has been carried out, and the resulting sulfides have been oxidized to the corresponding sulfoxides. These sulfoxides have the potential to undergo syn elimination to regenerate brefeldin A. The sulfoxides were more active than the sulfides as cytotoxic agents in a variety of human cancer cell cultures with the activities of the sulfoxides approaching that of brefeldin A itself. The cytotoxicities of the sulfoxides may be due to their conversion back to brefeldin A. The kinetics of sulfoxide elimination to form brefeldin A were studied in four cases, and the results indicate that substantial amounts of brefeldin A are likely to be generated during the cytotoxicity assays of the sulfoxide derivatives. Since the oxidation of sulfides to sulfoxides is a common metabolic reaction, the sulfides derived from brefeldin A can be considered as potential brefeldin A prodrugs. Several of the sulfide derivatives were determined to have enhanced aqueous solubilities relative to brefeldin A itself. A number of brefeldin A succinates, glutarates, oxidation products, and sulfone derivatives were also prepared and evaluated for cytotoxicity in cancer cell cultures. Some of the more active brefeldin A derivatives were tested in an in vivo animal model in which hollow fibers containing cancer cell cultures were implanted subcutaneously (SC) and intraperitoneally (IP), and the compounds were administered IP. Greater cytotoxic activity was observed at the SC site than at the IP site for the majority of these compounds, an observation which is consistent with the hypothesis that they are acting as brefeldin A prodrugs in vivo.

Brefeldin A is a macrolide antibiotic first isolated from the fungus *Penicillium decumbens*.[1] The structure **1**




**1**

was subsequently established by X-ray crystallography.[2] It has been known for a long time that brefeldin A possesses a number of interesting biological properties of potential therapeutic interest, including antitumor,[3,4] antiviral,[5−6] antifungal,[7−10] nematocidal,[11] and antimitotic[12] effects. Studies of the mode of action of brefeldin A have revealed that it inhibits protein transport from the endoplasmic reticulum to the Golgi apparatus,[13−15] causes reversible disassembly of the Golgi complex,[16,17] and blocks protein transport beyond the Golgi complex.[18,19] In addition, the ability of brefeldin A to induce DNA fragmentation associated with apoptosis in cancer cells has stimulated a great deal of recent interest in its preclinical development as an anticancer agent.[20,21] However, the potential clinical use of brefeldin A is severely limited by its undesirable pharmacokinetic properties including negligible bioavailability after oral administration and rapid clearance from the blood plasma after intravenous administration.[22] Formulation of brefeldin A is also complicated by its low aqueous solubility.[22] In view of these problems, additional work in the area of brefeldin A congener and prodrug synthesis is indicated.

Brefeldin A prodrugs would ideally be water soluble compounds which would be readily absorbed after oral administration and would be metabolized back to brefeldin A after systemic distribution in the blood plasma. As a possible strategy for accomplishing these goals, the Michael addition of thiols to the α,β-unsaturated lactone system present in brefeldin A has been contemplated. The resulting sulfides might then be metabolized to sulfoxides after absorption,[23] and the sulfoxides could then conceivably undergo syn elimination back to the α,β-unsaturated lactone system present in brefeldin A.[24,25] The metabolic oxidation of sulfides to sulfoxides is a facile and well-documented reaction in mammalian systems. Recent examples include oxidations of aryl alkyl sulfides,[26−30] dialkyl sulfides,[31−33] substituted alk-

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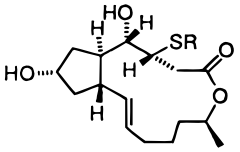
S0022-2623(97)00746-2 CCC: $15.00 
Published on Web 08/11/1998

enyl alkyl sulfides,[26] as well as cyclic sulfides.[27−34] In vivo studies of certain drugs have demonstrated the metabolic interconversion of sulfides and sulfoxides. For example, the sulfides metiamide and cimetidine undergo reversible metabolism with their sulfoxide metabolites,[35−37] while the sulfoxides sulindac and sulfinpyrazone interconvert metabolically with their sulfides.[37−40] In general, the sulfone metabolites of sulfoxides are not metabolized back to the sulfoxides, even in cases in which the sulfide−sulfoxide interconversion has been demonstrated to occur.[26,30−32]

To investigate the potential utility of sulfide derivatives of brefeldin A as prodrugs, it was planned to carry out the addition of a variety of thiols to brefeldin A, oxidize the resulting sulfides to sulfoxides, and investigate the stabilities of several of the sulfoxides. The cytotoxicities of the resulting sulfides and sulfoxides could also be investigated in human cancer cell cultures in order to gain information about the possible validity of the proposed brefeldin A prodrug design strategy.

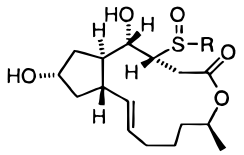
## Results and Discussion

Thiol addition products **2**−**12** were prepared by react-



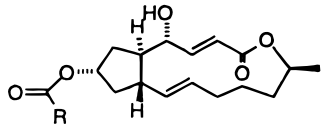
**2** R = $CH_2CH_2COOCH_3$
**3** R = *p*-$C_6H_4OH$
**4** R = $CH_2CH_2N(CH_3)_2$
**5** R = $CH_2CH_2OH$
**6** R = $CH_2COOH$
**7** R = $CH_2COOCH_3$
**8** R = $CH_2CH(OH)CH_2OH$
**9** R = $CH_2CH_2NH_2$
**10** R = *p*-$C_6H_4OCH_3$
**11** R = $CH_2CH(NH_2)COOH$
**12** R = $CH_2CHNHCOCH_2CH_2CH(NH_2)COOH$
$CONHCH_2COOH$

ing brefeldin A with the corresponding thiols in aqueous methanol in the presence of Proton Sponge [1,8-bis-(dimethylamino)naphthalene]. The reactions occurred readily and were found to be highly diastereoselective. The *R* configuration at C-3 in these products was assigned on the basis of the X-ray structure of a crystalline bis(3,5-dinitrobenzoate) derivative of the adduct formed from brefeldin A and 2-mercaptoethanol.[41] The X-ray and NMR data indicate that there is a conformational change in the macrocycle in going from brefeldin A to the adducts. In brefeldin A, C-2 and C-5 are anti, whereas in the products, they are gauche.[41] The specific thiol addition products prepared were chosen to incorporate a variety of polar, acidic, and basic functional groups that would impart additional aqueous solubility. Compounds **11** and **12** were synthesized because studies in Chinese hamster ovary cells have indicated that brefeldin A is secreted as these glutathione and cysteine conjugates.[42] The biological activities of these two compounds is of interest because of the possibility that the glutathione-*S*-transferase system is responsible for the inactivation of the antibiotic in mammalian cells.[42] Nine of the sulfides were oxidized to the corresponding sulfoxides **13**−**21**.



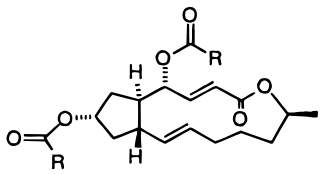
**13** R = $CH_2CH_2COOCH_3$
**14** R = *p*-$C_6H_4OH$
**15** R = $CH_2CH_2OH$
**16** R = $CH_2COOCH_3$
**17** R = $CH_2CH(OH)CH_2OH$
**18** R = $CH_2CH_2NH_2$
**19** R = *p*-$C_6H_4OCH_3$
**20** R = $CH_2CH_2N(CH_3)_2$
**21** R = $CH_2CH(NH_2)COOH$

Certain mono- and diesters of brefeldin A, with polar groups in the side chain, were synthesized by the reaction of brefeldin A with succinic anhydride and glutaric anhydride. These succinate and glutarate derivatives might also act as brefeldin A prodrugs and be hydrolyzed back to brefeldin A by esterases present in the blood plasma.[23] The monosuccinate **22** of brefel-



**22** R = $CH_2CH_2COOH$
**23** R = $CH_2CH_2CH_2COOH$

din A was obtained by the reaction of **1** with 1.5 equiv of succinic anhydride in pyridine at 60 °C. However, when glutaric anhydride was employed using this method, there was no product formation. To force the reaction, 4-dimethylaminopyridine was added to the reaction mixture. This led to the formation of both the monoester **23** and the diester **25** along with unreacted



**24** R = $CH_2CH_2COOH$
**25** R = $CH_2CH_2CH_2COOH$

starting material. The diester **25** could be separated from the mixture by column chromatography on silica gel, but the monoester **23** co-eluted with some impurities and brefeldin A. The reaction was repeated again with 3 equiv of glutaric anhydride and 2 equiv of DMAP to afford the desired diester **25** as the major product. A single recrystallization from hexanes and ethyl acetate afforded a pure sample of the diglutarate **25**. This method was then applied to the synthesis of the disuccinate **24** of brefeldin A. Reaction of **1** with 3 equiv of succinic anhydride in the presence of 2 equiv of 4-dimethylaminopyridine in pyridine at 60 °C afforded the desired compound **24** in moderate yield after column chromatographic purification on silica gel and recrystallization.

To synthesize and isolate the monoglutarate **23** in pure form, we attempted the reaction with pyridine as the solvent. Since addition of 4-dimethylaminopyridine

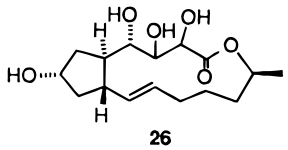
**Table 1.** Cytotoxicities of Brefeldin A Analogues[a]

| 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[b] |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 0.070 | 0.029 | 0.040 | 0.022 | 0.032 | 0.090 | 0.13 | 0.041 | 0.040 ± 0.019 |
| **2** | 5.8 | 2.0 | 3.5 | 4.7 | 2.7 | 6.6 | 24 | 3.6 | 4.1 ± 0.76 |
| **3** | 3.2 | 2.5 | 5.1 | 4.8 | 2.4 | 4.4 | 6.3 | 3.3 | 3.2 ± 0 |
| **4** | 0.54 | 0.75 | 0.39 | 0.43 | 0.54 | 1.40 | 2.4 | 0.50 | 0.68 ± 0.062 |
| **5** | 34 | 19 | 23 | 40 | 27 | 33 | 30 | 34 | 20 ± 1.8 |
| **6** | 30 | 23 | 34 | 33 | 30 | 39 | 38 | 42 | 33 |
| **7** | 28 | 19 | 24 | 6.8 | 27 | 32 | 30 | 30 | 17 ± 0 |
| **8** | 33 | 36 | 32 | 16 | 22 | 34 | 18 | 36 | 23 |
| **9** | 3.9 | 3.5 | 4.9 | 2.7 | 2.2 | | | 3.3 | 2.5 |
| **10** | 24 | 33 | 26 | 29 | 22 | 30 | 36 | 19 | 31 ± 5.3 |
| **11** | 3.8 | 2.6 | 2.4 | 2.4 | 1.5 | 3.5 | 3.5 | 1.0 | 1.8 ± 0.18 |
| **12** | 0.87 | 0.24 | 0.42 | 0.28 | 0.29 | 0.55 | 2.48 | 0.52 | 0.46 ± 0.12 |
| **13** | 0.40 | 0.32 | 0.41 | 0.083 | 0.20 | 0.61 | 0.45 | 0.29 | 0.35 |
| **14** | 0.088 | 0.026 | 0.017 | 0.030 | 0.025 | 0.050 | 0.118 | 0.042 | 0.037 ± 0.007 |
| **15** | 0.29 | 0.21 | 0.058 | 0.064 | 0.19 | 0.65 | 0.26 | 0.090 | 0.11 ± 0.07 |
| **16** | 0.44 | 0.48 | 0.38 | 0.24 | 0.27 | 1.46 | 1.25 | 0.39 | 0.68 |
| **17** | 0.37 | 0.26 | 0.19 | 0.24 | 0.22 | 0.36 | 0.88 | 0.38 | 0.28 ± 0.12 |
| **18** | 0.15 | 0.086 | 0.020 | 0.021 | 0.082 | 0.12 | 0.17 | 0.055 | 0.055 ± 0.028 |
| **19** | 0.49 | 0.65 | 0.41 | 0.32 | 0.25 | | | 0.42 | 0.41 |
| **20** | 0.35 | 0.28 | 0.30 | 0.071 | 0.14 | 0.15 | 0.48 | 0.41 | 0.20 ± 0.01 |
| **21** | 0.70 | 0.089 | 0.039 | 0.038 | 0.050 | 0.16 | 0.43 | 0.050 | 0.096 ± 0.077 |
| **22** | 3.4 | 17 | 0.54 | 1.9 | 3.3 | 4.3 | 6.8 | 1.1 | 2.5 ± 0.62 |
| **23** | 33 | 21 | 22 | | 20 | 32 | 30 | 11 | 13 |
| **24** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | 89 |
| **25** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | 98 |
| **26** | 5.0 | 3.4 | 0.68 | 1.6 | 2.4 | 2.7 | 6.7 | 3.8 | 2.2 ± 0.35 |
| **27** | 5.1 | 3.7 | | 5.0 | 3.2 | 3.3 | 6.0 | 5.5 | 3.5 |
| **28** | 54 | 37 | 38 | 53 | 25 | 28 | 70 | 38 | 31 |

[a] The cytotoxicity $GI_{50}$ values expressed in $\mu$M are the concentrations corresponding to 50% growth inhibition. [b] Mean graph midpoint for growth inhibition of all human cancer cell lines successfully tested. The entries with population standard deviations are the results of two determinations, and the ones without population standard deviations are the result of single determinations.

causes the formation of the diester **25** and the reaction does not occur at 60 °C, the reaction temperature was raised to 110 °C for 36 h, and 4-dimethylaminopyridine was omitted. The TLC of the reaction mixture indicated the formation of the monoglutarate **23** as the major product along with some unreacted starting material. A very minor amount of the undesired diester **25** was also present. Column chromatography on silica gel followed by recrystallization from diethyl ether and hexanes afforded the desired compound **23**.

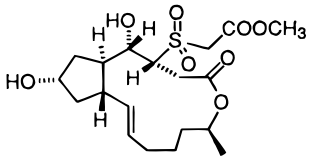
An attempt was also made to introduce additional hydroxyl groups into the brefeldin A system to increase aqueous solubility and to provide additional structure−activity information. To this end, the reaction of brefeldin A with osmium tetroxide was investigated. Thus, the reaction of (+)-brefeldin A with two equiv of *N*-methylmorpholine oxide (NMO) and a catalytic amount of osmium tetraoxide ($OsO_4$) in a mixture of *t*-BuOH/$H_2O$ was attempted at room temperature. After the mixture was stirred for 4 h at room temperature, the formation of a product was observed on TLC. After the usual work up and purification, the dihydroxylation product **26** was isolated in 89% yield. $^1$H NMR analysis




**26**

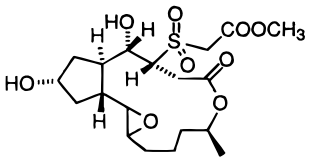
of the reaction product **26** showed disappearance of C-2 and C-3 olefinic protons from **1** and presence of C-10 and C-11 olefinic protons, indicating a regioselective dihydroxylation. At this stage, the stereochemistry of the dihydroxylation product **26** at C-2 and C-3 has not been determined.

Some attention was also directed toward the determination of the structures of the reaction products obtained from the oxidation of the methyl thioglycolate addition product **7** under forcing conditions. The oxidation of **7** with 1.1 equiv of *m*-chloroperbenzoic acid in methylene chloride at 0 °C for 2 min afforded the corresponding sulfoxide **16** in good yield. However, the treatment of sulfide **7** with 2.2 equiv of *m*-chloroperbenzoic acid in methylene chloride at room temperature for 4 h gave the desired sulfone **27** in 46% isolated yield.




**27**

When the sulfide **7** was treated with 4.4 equiv of *m*-chloroperbenzoic acid in methylene chloride for 7 h, the sulfur was oxidized to the sulfone and the C-10, C-11 double bond was also oxidized, resulting in compound **28**. The stereochemistry of the epoxide has not yet been




**28**

determined.

The 28 newly synthesized brefeldin A prodrug candidates and analogues were examined for antiproliferative activity against human cancer cell lines in the National Cancer Institute screen, in which the activity of each compound was evaluated with 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 summarized in Table 1. The MGM is based on a calculation of the average $GI_{50}$ for all of the cell lines tested (approximately 55) in which $GI_{50}$ values below and above 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.[43]

It is apparent from the data in Table 1 as well as from the more extensive data in approximately 55 cell lines (data not shown) that neither brefeldin A nor any of the new derivatives prepared in the present study have significant selectivity for any particular subpanel of cancer cell lines. It is also clear that the sulfide derivatives **2**−**12** (MGM 0.46−33 $\mu$M) are, in general, much less active than brefeldin A (MGM 0.040 $\mu$M). The $\alpha,\beta$-unsaturated lactone moiety therefore appears to be an important structural determinant for cytotoxicity. This point is also borne out by the cytotoxic activity of **26** (MGM 2.2 $\mu$M) relative to **1** (MGM 0.040 $\mu$M), and it is also consistent with a recent report documenting the importance of the $\alpha,\beta$-unsaturated lactone moiety for induction of apoptotic DNA fragmentation.[21]

The more active of the sulfide derivatives were **12** (MGM 0.46 $\mu$M), **4** (MGM 0.68 $\mu$M), **11** (MGM 1.8 $\mu$M), and **9** (MGM 2.5 $\mu$M). It is worth noting that all of these more active sulfides have side chains containing basic amino groups that could possibly catalyze the elimination of the sulfide to regenerate brefeldin A. Included in this list are the brefeldin A secondary metabolites **11** and **12**, assuming the metabolites, in fact, have the same configuration as the major thiol addition products.[42]

Turning to the sulfoxides **13**−**21**, it is evident that these compounds are the most active set investigated, with MGM values ranging from 0.037 $\mu$M for compound **14** to 0.68 $\mu$M for compound **16**. The cytotoxicity of **14** is essentially equipotent with that of brefeldin A (MGM 0.040 $\mu$M). The average MGM value for the set of sulfoxides **13**−**21** is 0.24 $\mu$M, which is much lower than the value of 12.43 $\mu$M found for the sulfides **2**−**12**. It seems likely that the sulfoxides undergo elimination during cytotoxicity testing and that the observed biological results are at least in part due to the presence of brefeldin A. This would account for the difference in activity seen between the sulfides and sulfoxides.

In order to investigate the proposed sulfoxide elimination reaction to regenerate brefeldin A, compounds **13**, **14**, **15**, and **20** were placed in a $D_2O/CD_3OD$ buffer, pH 7.4, containing sodium bicarbonate and sodium acetate at 37 °C, and the $^1H$ NMR spectra were recorded at various time intervals. The conversion of each of these sulfoxides to brefeldin A was monitored by observing the disappearance of the C-2 protons in the starting material and the appearance of the C-2 and C-3 alkene protons in brefeldin A. During this process, no other compounds beside these sulfoxides and brefeldin A were detected. Under these conditions, the half-life for the conversion of the sulfoxide **13** to brefeldin A was 4.4 h, while those of **14**, **15**, and **20** were 22.8 h, 72 min, and 87 min, respectively. It is therefore clear that there is likely to be substantial conversion of the sulfoxides to regenerate brefeldin A during the in vitro cell culture cytotoxicity experiments, which are conducted using an incubation period of 72 h.

There is considerable variability in elimination rates among the four sulfoxides **13**, **14**, **15**, and **20**. If our hypothesis is correct that the cytotoxicities of the sulfoxides result appreciably from their conversion back to brefeldin A, then one would expect the cytotoxicites of these four sulfoxides to reflect their conversion rates. This is decidedly not the case since all four sulfoxides have similar $GI_{50}$ values. The situation is complicated by the following factors: (1) the cytotoxicities were determined after a 3-day incubation period, so that even with a 22.8 h half-life, appreciable amounts brefeldin A would still form during the incubation period; (2) the rate of elimination in the buffer used to study the elimination rate by NMR may not reflect the elimination rate in the cell-culture medium; (3) the elimination might occur intracellularly as well as extracellularly, and if intracellular rates are appreciable, the overall rate of brefeldin A production would be influenced by potentially dissimilar rates of cellular penetration; and (4) the sulfoxides themselves are likely to have low-level cytotoxicities as indicated by those of the corresponding sulfides. However, the fact that the sulfoxides are significantly more cytotoxic than the corresponding sulfides and are converted to brefeldin A in buffer still argues that the cytotoxicities of the sulfoxides are at least partially the result of their conversion back to brefeldin A in the culture medium. It seems unlikely that the difference in activity between the sulfides and sulfoxides would be due to a greater rate of cellular uptake of the sulfoxides which then exert a biological effect as the sulfoxides themselves, since an intact $\alpha,\beta$-unsaturated carbonyl system is probably required for activity. This would be in agreement with the prior observation that tetrahydrobrefeldin A is inactive in inducing differentiation and apoptotic DNA fragmentation in HCT116 human colon cancer cells.[21] There is also no reason to expect that the sulfoxides would penetrate cell membranes faster than the sulfides. In fact, in the case of the sulfoxide ML-1035, it has been demonstrated that the sulfide metabolite is better absorbed orally than the more polar parent compound.[31]

To determine whether or not the conversion of brefeldin A to various sulfide derivatives in fact has an appreciable effect on their solubilities, saturated solutions of brefeldin A and several of the derivatives were prepared in distilled water at room temperature, and the concentrations were determined gravimetrically after evaporation of all of the water by azeotropic distillation with ethanol. The solubility of brefeldin A determined in this way was 2.8 mg/mL, while those of the sulfides were as follows: **3**, 12 mg/mL; **4**, 10 mg/mL; **5**, 12 mg/mL; **6**, 40 mg/mL; and **11**, 35 mg/mL. The increased solubilities of these derivatives will facilitate their formulations for biological evaluation.

From the data reported in Table 1, compound **21**, the sulfoxide of one of the more soluble sulfides **11**, is nearly as active as brefeldin A in some of the cell lines but not in others. In this regard, it should be pointed out that the 0.70 $\mu$M $GI_{50}$ observed for **21** in the HOP-62 cell line is the average of two widely disparate values, 0.042 and 1.36 $\mu$M. If one were to ignore the 1.36 $\mu$M value as a spurious outlier, the remaining 0.042 $\mu$M value would be more in line with the numbers observed in the remaining cell lines. The other two "high" $GI_{50}$ values

**Table 2.** Anticancer Activities of Various Brefeldin A Derivatives in the Hollow Fiber Assay[a]

| compd | IP score[b] | SC score[b] | cell kill[c] |
|---|---|---|---|
| **2** | 4 | 6 | Y |
| **3** | 4 | 10 | N |
| **4** | 6 | 2 | N |
| **10** | 6 | 6 | N |
| **12** | 0 | 4 | N |
| **14** | 4 | 8 | Y |
| **15** | 2 | 12 | Y |
| **17** | 2 | 6 | N |
| **26** | 2 | 2 | N |

[a] Polyvinylidene fluoride hollow fibers containing various cancer cell lines were implanted intraperitoneally (IP) or subcutaneously (SC) in mice, and compounds were injected IP using a qd × 4 treatment schedule. A total of 12 IP and 12 SC cell lines were tested in triplicate at two dosage levels, and each cell line with a 50% or greater reduction in viable cell mass was given a score of 2. [b] The IP and SC scores listed are the sums of all of the IP and SC scores for each compound. [c] A net cell kill at one or more of the implant sites in indicated with a Y.

of 0.16 and 0.43 $\mu$M were the results of single determinations, in contrast to the remaining values, which were all the results of duplicate determinations.

The succinates and glutarates **22**−**25** ranged from being moderately cytotoxic to essentially inactive, and there was a difference in activity between the monoacylated and diacylated products. Of these four compounds, the monosuccinate **22** was the most active, displaying an MGM value of 2.5 $\mu$M. This was followed by the monoglutarate **23** (MGM 13 $\mu$M). The disuccinate **24** and diglutarate **25** were much less active, displaying MGMs of 89 and 98 $\mu$M, respectively.

The effect of sulfone vs sulfoxide substitution can be seen by comparing the activities of **27** (MGM 3.5 $\mu$M) and **16** (MGM 0.68 $\mu$M). In this particular case, the sulfoxide is more cytotoxic. Oxidation of the C-10 to the C-11 double bond in **27** to form **28** (MGM 31 $\mu$M) resulted in a significant loss of activity. This is consistent with the observation that epoxidation of the C-10 to the C-11 double bond of brefeldin A results in a substantial loss of potency for induction of apoptotic DNA fragmentation.[21]

Overall, the increase in biological activity seen with the conversion of the sulfides to the sulfoxides, along with the documented elimination of several of the sulfoxides to regenerate brefeldin A, provides strong support for the general strategy of employing the sulfide products formed from the addition of thiols to brefeldin A as prodrug candidates. Sulfides attached to acidic or basic functional groups appear to be particularly attractive, since these compounds can be converted to salts having increased solubility in an aqueous environment.

Several of the more active brefeldin A derivatives were evaluated as anticancer agents in an in vivo animal model in which polyvinylidene fluoride hollow fibers containing various cancer cell cultures were implanted intraperitoneally (IP) and subcutaneously (SC) into mice and compounds were administered by the IP route. The effects of the compounds on reduction in viable cancer cell mass compared to those of controls were determined. The results, listed in Table 2, show that six of nine compounds showed greater anticancer activity at the SC implant site than at the IP implant site after the compounds were injected IP. Of the remaining three compounds, derivative **26** was not designed as a prodrug, sulfide **10** showed equal activity at both sites, and **4** was more active at the IP site. Greater activity at the more remote implant site (SC) relative to that at the injection site (IP) is characteristic of an inactive compound which is converted to an active one after administration (i.e., the classical definition of a prodrug). While this provides only preliminary evidence of in vivo activity, follow-up studies in additional animal models are planned.

## Experimental Section

$^1$H and $^{13}$C NMR spectra were recorded on a 300 MHz instrument. Merck silica gel 60-$F_{254}$ thin-layer chromatography plates of 0.25-mm thickness were used and visualized with *p*-anisaldehyde stain. Flash chromatography was conducted using 60−200 mesh silica gel. (+)-Brefeldin A was supplied by the National Cancer Institute. Unless otherwise indicated, all reagents were commercially available and used without further purification.

**2,3-Dihydro-(3*R*)-(2′-methoxycarbonylethylthio)brefeldin A (2).** Methyl mercaptopropionate (0.036 g, 0.3 mmol) was added to a solution of (+)-brefeldin A (0.056 g, 0.2 mmol) and Proton Sponge [1,8-bis(dimethylamino)naphthalene, 0.085 g, 0.4 mmol] in a mixture of MeOH (3 mL) and water (1 mL) at room temperature. The reaction mixture was stirred at ambient temperature for 2 h, and then diluted with distilled water (10 mL). The aqueous solution was extracted with *n*-hexanes (3 × 15 mL) to remove Proton Sponge and excess thiol. The resulting aqueous solution was then extracted with $CHCl_3$ (4 × 30 mL). The organic extract was dried over anhydrous $MgSO_4$, and the solvent was removed under a reduced pressure. The residue obtained was purified by means of flash column chromatography (silica gel, 1−3% EtOH/$CHCl_3$) to obtain the desired product **2** (0.075 g, 94%) as an oil: TLC $R_f$ 0.42 (10% EtOH/$CHCl_3$); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 5.52 (m, 1 H), 5.39 (m, 1 H), 4.87 (m, 1 H), 4.30 (m, 1 H), 3.70 (s, 3 H), 3.55 (dd, 1 H, $J$ = 1.8 and 7.9 Hz), 3.42 (dt, 1 H, $J$ = 2.2, 2.8 and 7.6 Hz), 2.90−2.72 (m, 2 H), 2.68 (dd, 1 H, $J$ = 3.3 and 16.6 Hz), 2.60 (m, 3 H), 2.25 (dd, 1 H, $J$ = 10.7 and 16.6 Hz), 2.15 (m, 3 H), 1.90 (m, 3 H), 1.65 (m, 3 H), 1.60−1.30 (m, 3 H), 1.15 (d, 3 H, $J$ = 6.1 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 172.40, 170.29, 135.54, 129.81, 76.57, 72.99, 71.65, 51.90, 45.76, 45.42, 44.39, 43.24, 40.72, 34.94, 34.85, 33.42, 31.27, 25.98, 25.10, and 20.32; IR ($CHCl_3$) 3439, 1725, 1629, and 1262 cm$^{-1}$; CIMS *m*/*z* 401 ($MH^+$); HRMS calcd for $C_{20}H_{32}O_6S$ 401.1998, found 401.1986.

**2,3-Dihydro-(3*R*)-(4′-hydroxyphenylthio)brefeldin A (3).** 4-Hydroxythiophenol (0.038 g, 0.3 mmol) was added to a solution of (+)-brefeldin A (0.056 g, 0.2 mmol) and Proton Sponge [1,8-bis(dimethylamino)naphthalene] (0.085 g, 0.4 mmol) in a mixture of MeOH (3 mL) and water (1 mL) at room temperature. The reaction mixture was stirred at ambient temperature for 12 h and then diluted with distilled water (10 mL). The aqueous solution was extracted with *n*-hexanes (3 × 15 mL) to remove Proton Sponge and excess thiol. The resulting aqueous solution was then extracted with $CHCl_3$ (4 × 30 mL). The organic extract was dried over anhydrous $MgSO_4$, and the solvent was removed under a reduced pressure. The residue obtained was purified by means of flash column chromatography (silica gel, 1% EtOH/$CHCl_3$) to obtain the desired product **3** (0.056 g, 70%) as an oil: TLC $R_f$ 0.28 (10% EtOH/$CHCl_3$); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.38 (dd, 2 H, $J$ = 2.1 and 8.7 Hz), 6.82 (dd, 2 H, $J$ = 2.1 and 8.7 Hz), 5.47 (m, 1 H), 5.34 (m, 1 H), 4.94 (m, 1 H), 4.23 (m, 1 H), 3.70 (dd, 1 H, $J$ = 3.2 and 10.9 Hz), 3.49 (dt, 1 H, $J$ = 1.38 and 10.8 Hz), 2.72 (dd, 1 H, $J$ = 3.3 and 15 Hz), 2.37 (dd, 1 H, $J$ = 10.8 and 15 Hz), 2.70−1.90 (m, 6 H), 1.78−1.56 (m, 6 H), 1.44 (br m, 1 H) 1.30 (d, 3 H, $J$ = 6.13 Hz); $^{13}$C NMR ($CD_3OD$) $\delta$ 174.48, 160.67, 138.69, 138.34, 132.06, 126.55, 118.61, 74.93, 74.75, 53.29, 49.68, 48.79, 47.08, 45.56, 42.35, 37.12, 35.93, 34.19, 27.94, 22.16; IR ($CHCl_3$) 3398, 1768, 1700, 1282, and 1042 cm$^{-1}$; HRMS calcd for $C_{22}H_{30}O_5S$ 406.1813, found 406.1821. Anal. ($C_{22}H_{30}O_5S\cdot0.9H_2O$) C, H, S.

**2,3-Dihydro-(3*R*)-(2′-*N*,*N*-dimethylaminoethylthio]-brefeldin A (4).** *N*,*N*-Dimethylaminoethanethiol hydrochlo-

ride (0.105 g, 0.75 mmol) was added to a solution of (+)-brefeldin A (0.140 g, 0.5 mmol) and Proton Sponge [1,8-bis(dimethylamino)naphthalene] (0.214 g, 1.0 mmol) in a mixture of MeOH (9 mL) and water (3 mL) at room temperature. The reaction mixture was stirred at ambient temperature for 2 h and then diluted with distilled water (30 mL). The aqueous solution was extracted with *n*-hexanes (3 × 30 mL) to remove Proton Sponge and excess thiol. The resulting aqueous solution was then extracted with EtOAc (4 × 60 mL). The organic extract was dried over anhydrous $MgSO_4$, and the solvent was removed under a reduced pressure. The residue obtained was purified by means of flash column chromatography (silica gel, 1−5% EtOH/$CHCl_3$) to obtain the desired product **4** (0.180 g, 94%) as an oil: TLC $R_f$ 0.2 (20% MeOH/$CHCl_3$); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 5.57 (m, 1 H), 5.37 (dd, 1 H, $J$ = 9, 15.3 Hz), 4.87 (m, 1 H), 4.31 (m, 1 H), 3.67 (dd, 1 H, $J$ = 1.8, 8.7 Hz), 3.49 (m, 1 H), 2.76−2.60 (m, 5 H), 2.41 (dd, 1 H, $J$ = 10.5, 16.2 Hz), 2.32 (s, 6 H), 2.26−2.08 (m, 3 H), 2.06−1.97 (m, 3 H), 1.68−1.82 (m, 3 H), 1.62−1.45 (m, 2 H), 1.26 (d, 3 H, $J$ = 6 Hz), 1.18 (m, 1 H); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.54, 135.55, 129.63, 72.86, 71.53, 60.29, 45.50, 45.37, 44.40, 43.13, 40.51, 34.94, 33.36, 31.31, 28.31, 25.06, 20.33; IR ($CHCl_3$) 3395, 1727, 1454, 1262, 1062 cm$^{-1}$; MS *m*/*z* 386 ($MH^+$); HRMS calcd for $C_{20}H_{35}O_4NS$ 386.2365, found 386.2376.

**2,3-Dihydro-(3*R*)-(2′-hydroxyethylthio)brefeldin A (5).** 2-Mercaptoethanol (0.021 mL, 0.3 mmol) was added to a solution of (+)-brefeldin A (0.056 g, 0.2 mmol) and Proton Sponge [1,8-bis(dimethylamino)naphthalene] (0.085 g, 0.4 mmol) in a mixture of MeOH (3 mL) and water (1 mL) at room temperature. The reaction mixture was stirred at ambient temperature for 2 h and then diluted with distilled water (10 mL). The aqueous solution was extracted with *n*-hexanes (3 × 15 mL) to remove Proton Sponge and excess thiol. The resulting aqueous solution was saturated with NaCl and then extracted with EtOAc (4 × 30 mL). The organic extract was dried over anhydrous $MgSO_4$, and the solvent was removed under a reduced pressure. The residue obtained was purified by means of flash column chromatography (silica gel, 1−2% EtOH/$CHCl_3$) to obtain the desired sulfide **5** (0.066 g, 93%) as an oil: TLC $R_f$ 0.29 (10% EtOH/$CHCl_3$); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 5.55 (m, 1 H), 5.39 (m, 1 H), 4.90 (m, 1 H), 4.32 (m, 1 H), 3.78 (t, 2 H, $J$ = 6.1 and 5.5 Hz and dd, 1 H, for the C-4 proton are merged), 3.58 (t and dt, 3 H), 2.90−2.65 (dd and m, 4 H), 2.35 (dd, 1 H, $J$ = 10.8 and 10.8 Hz), 2.22−1.90 (m, 6 H), 1.85−1.40 (m, 3 H), 1.22 (d, 3 H, $J$ = 6.4 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.70, 135.58, 129.72, 72.71, 72.15, 45.61, 44.42, 43.19, 40.58, 33.32, 31.25, 25.24, 20.32; IR ($CHCl_3$) 3398, 1768, 1700, 1282, and 1043 cm$^{-1}$; HRMS calcd for $C_{18}H_{30}O_5S$ 359.1892, found 359.1999.

**2,3-Dihydro-(3*R*)-(carboxymethylthio)brefeldin A (6).** Mercaptoacetic acid (0.042 mL, 0.6 mmol) was added to a solution of (+)-brefeldin A (0.140 g, 0.5 mmol) and Proton Sponge [1,8-bis(dimethylamino)naphthalene] (0.214 g, 1.0 mmol) in a mixture of MeOH (9 mL) and water (3 mL) at room temperature. The reaction mixture was stirred at ambient temperature for 48 h and then diluted with distilled water (10 mL). The aqueous solution was extracted with *n*-hexanes (3 × 30 mL) to remove Proton Sponge and excess thiol. The resulting aqueous solution was diluted with MeOH (50 mL), and the methanolic solution was azeotroped. The residue obtained was purified by means of flash column chromatography (silica gel, 5−20% MeOH/$CHCl_3$) to obtain the desired sulfide **6** (0.115 g, 62%) as an oil: TLC $R_f$ 0.32 (35% MeOH/$CHCl_3$); $^1$H NMR ($D_2O$, 300 MHz) $\delta$ 5.65 (m, 1 H), 5.33 (m, 1 H), 4.69 (m, 1 H), 4.23 (m, 1 H), 3.68 (bd, 1 H, $J$ = 8.9 Hz), 3.42 (bd, 1 H, $J$ = 9.8 Hz), 3.24 (bs, 1 H), 2.85−2.65 (m, 3 H), 2.46 (m, 1 H), 2.40−1.50 (m, 10 H), 1.39 (m, 1 H), 1.20 (d, 3H, $J$ = 6.1 Hz); $^{13}$C NMR ($D_2O$) $\delta$ 180.48, 173.95, 135.01, 130.63, 73.97, 71.96, 45.45, 44.02, 43.71, 42.26, 37.84, 34.89, 32.50, 30.77, 27.72, 24.66, 23.17, 19.38; IR ($CHCl_3$) 3469, 1701, 1282, 1128 cm$^{-1}$; MS *m*/*z* 373 ($MH^+$); HRMS calcd for $C_{18}H_{28}O_6S$ 373.1685, found 373.1673.

**2,3-Dihydro-(3*R*)-(methoxycarbonylmethylthio)brefeldin A (7).** Methyl mercaptoacetate (0.025 g, 0.24 mmol) was added to a solution of (+)-brefeldin A (0.056 g, 0.2 mmol) and Proton Sponge [1,8-bis(dimethylamino)naphthalene, 0.085 g, 0.4 mmol] in a mixture of MeOH (3 mL) and water (1 mL) at room temperature. The reaction mixture was stirred at ambient temperature for 2 h and then diluted with distilled water (10 mL). The aqueous solution was extracted with *n*-hexanes (3 × 15 mL) to remove Proton Sponge and excess thiol. The resulting aqueous solution was then extracted with EtOAc or $CHCl_3$ (4 × 30 mL). The organic extract was dried over anhydrous $MgSO_4$, and the solvent was removed under a reduced pressure. The residue obtained was purified by means of flash column chromatography (silica gel, 1−3% EtOH/$CHCl_3$) to obtain the desired product **7** (0.070 g, 91%) as an oil: TLC $R_f$ 0.57 (10% EtOH/$CHCl_3$); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 5.54 (m, 1 H), 5.34 (m, 1 H), 4.85 (m, 1 H), 4.30 (m, 1 H), 3.75 (s, 3 H), 3.70 (dt, 1 H, $J$ = 2.4 and 9.6 Hz), 3.67 (dd, 1 H, $J$ = 1.6 and 8.3 Hz), 3.37 (d, 1 H, $J$ = 14.7 Hz), 3.26 (d, 1 H, $J$ = 14.8 Hz), 2.75 (dd, 1 H, $J$ = 3.2 and 16.7 Hz), 2.39 (dd, 1 H, $J$ = 10.7 and 16.7 Hz), 2.28 (m, 1 H), 2.15 (m, 2 H), 2.05−1.45 (m, 9 H), 1.20 (d, 3 H, $J$ = 6.3 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 171.25, 170.38, 135.45, 129.64, 72.58, 71.71, 52.51, 45.71, 45.60, 43.87, 43.06, 40.51, 34.57, 33.17, 32.33, 31.24, 24.99, 20.16; IR ($CHCl_3$) 3443, 1729, and 1280 cm$^{-1}$; CIMS *m*/*z* 387 ($MH^+$); HRMS calcd for $C_{18}H_{30}O_6S$ 387.1841, found 387.1832.

**2,3-Dihydro-(3*R*)-(2′,3′-dihydroxypropylthio)brefeldin A (8).** 2,3-Dihydroxypropanethiol (0.063 mL, 0.75 mmol) was added to a solution of (+)-brefeldin A (0.140 g, 0.5 mmol) and Proton Sponge [1,8-bis(dimethylamino)naphthalene, 0.214 g, 1.0 mmol] in a mixture of MeOH (9 mL) and $H_2O$ (3 mL) at room temperature. The reaction mixture was stirred at an ambient temperature for 2 h and then diluted with distilled water (10 mL). The aqueous solution was extracted with *n*-hexanes (3 × 30 mL) to remove Proton Sponge and excess thiol. The resulting aqueous solution was saturated with NaCl and then extracted with EtOAc (4 × 60 mL). The organic solution was dried over anhydrous $MgSO_4$, and solvent was removed under a reduced pressure. The residue obtained was purified by means of flash column chromatography (silica gel, 1−10% EtOH/$CHCl_3$) to obtain the desired sulfide **8** (0.150 g, 79%) as an oil: TLC $R_f$ 0.5 (20% EtOH/$CHCl_3$); $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 5.54 (m, 1 H), 5.29 (m, 1 H), 4.71 (m, 1 H), 4.11 (m, 1 H), 3.58−3.75 (m, 1 H), 3.48−3.55 (m, 2 H), 3.52 (m, 2 H), 3.42 (b d, 1 H, $J$ = 10.4 Hz), 2.50−2.80 (m, 3 H), 2.10−2.30 (m, 2 H), 1.45−2.10 (m, 10 H), 1.35 (d, 3 H, $J$ = 5.8 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 171.08, 135.31, 129.88, 72.44, 72.22, 71.56, 70.96, 65.12, 58.35, 45.70, 44.25, 43.06, 35.05, 34.27, 34.08, 33.23, 31.36, 25.16, 20.25, 18.35; IR ($CHCl_3$) 3401, 1765, 1705, 1280, 1041 cm$^{-1}$; MS *m*/*z* 389 ($MH^+$); HRMS calcd for $C_{19}H_{32}O_6S$ 389.1998, found 389.2006.

**2,3-Dihydro-(3*R*)-(2′-aminoethylthio)brefeldin A (9).** 2-Aminoethanethiol hydrochloride (0.085 mL, 0.75 mmol) was added to a solution of (+)-brefeldin A (0.140 g, 0.5 mmol) and Proton Sponge [1,8-bis(dimethylamino)naphthalene (0.214 g, 1.0 mmol) in a mixture of MeOH (9 mL) and $H_2O$ (3 mL) at room temperature. The reaction mixture was stirred at an ambient temperature for 2 h and then diluted with distilled water (10 mL). The aqueous solution was extracted with *n*-hexanes (3 × 30 mL) to remove Proton Sponge and excess thiol. The resulting aqueous solution was diluted with MeOH (50 mL), and the methanolic solution was evaporated. The residue obtained was purified by means of flash column chromatography (silica gel, 5−20% MeOH/$CHCl_3$) to obtain 0.170 g (95%) of the desired sulfide **9**. TLC $R_f$ 0.13 (50% EtOH/$CHCl_3$); $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 5.56 (m, 1 H), 5.29 (m, 1 H), 4.76 (m, 1 H), 4.17 (m, 1 H), 3.70 (bd, 1 H), 3.30 (bd, 1 H), 3.22 (m, 2 H), 3.10−3.00 (m, 2 H), 2.90−2.75 (m, 2 H), 2.64 (dd, 1 H, $J$ = 3.8 and 16.2 Hz), 2.40 (m, 1 H), 2.20−1.84 (m, 5 H), 1.80−1.45 (m, 6 H), 1.36 (m, 1 H), 1.21 (m and d, 4 H, $J$ = 6.3 Hz); $^{13}$C NMR ($CD_3OD$) $\delta$ 172.39, 136.90, 130.88, 73.47, 73.13, 47.45, 46.69, 46.46, 45.52, 43.99, 41.01, 40.11, 36.20, 34.13, 32.46, 29.65, 26.45, 20.54; IR ($CHCl_3$) 3377, 1704, 1629, 1508, 1458, 1267 cm$^{-1}$; MS *m*/*z* 358 ($MH^+$); HRMS calcd for $C_{18}H_{31}NO_4S$ 358.2052, found 358.2059.

**2,3-Dihydro-(3*R*)-(4′-methoxyphenylthio)brefeldin A (10).** Mercaptoanisole (0.092 mL, 0.75 mmol) was added to a solution of (+)-brefeldin A (0.140 g, 0.5 mmol) and Proton Sponge [1,8-bis(dimethylamino)naphthalene] (0.214 g, 1.0 mmol) in a mixture of MeOH (9 mL) and $H_2O$ (3 mL) at room temperature. The reaction mixture was stirred at an ambient temperature for 12 h and then diluted with distilled water (20 mL). The aqueous solution was extracted with *n*-hexanes (3 × 30 μL) to remove Proton Sponge and excess thiol. The resulting aqueous solution was saturated with NaCl and then extracted with $CHCl_3$ (4 × 60 mL). The organic solution was dried over anhydrous $MgSO_4$, and solvent was removed under a reduced pressure. The residue obtained was purified by means of flash column chromatography (silica gel, 1−3% EtOH/$CHCl_3$) to the desired sulfide **10** (0.056 g, 67%) as an oil: TLC $R_f$ 0.62 (10% EtOH/$CHCl_3$); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.43 (dd, 2 H, $J$ = 2.8 and 8.7 Hz), 6.87 (dd, 2 H, $J$ = 2.7 and 8.7 Hz), 5.45 (m, 1 H), 5.35 (m, 1 H), 4.92 (m, 1 H), 4.29 (m, 1 H), 3.82 (s, 3 H), 3.76 (dt, 1 H, $J$ = 1.7 and 10.2 Hz), 3.45 (dd, 1 H, $J$ = 1.6 and 8.5 Hz), 2.75 (dd, 1 H, $J$ = 3.1 and 16.5 Hz), 2.35 (dd, 1 H, $J$ = 10.8 and 16.5 Hz), 2.20−1.85 (m, 5 H), 1.80−1.35 (m, 7 H), 1.25 (d, 3 H, $J$ = 6.28 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.50, 159.77, 137.06, 135.60, 135.35, 129.52, 124.16, 114.92, 114.76, 76.59, 72.80, 71.56, 55.32, 50.96, 45.22, 44.11, 43.03, 40.77, 34.13, 33.42, 31.26, 24.96, and 20.42; IR ($CHCl_3$) 3433, 1716, 1593, and 1488 cm$^{-1}$; MS *m*/*z* 420 ($M^+$); HRMS calcd for $C_{23}H_{32}O_5S$ 420.1970, found 420.1965.

**2,3-Dihydro-(3*R*)-(*S*-L-cysteinyl)brefeldin A (11).** (*R*)-(*L*)-Cysteine (0.816 g, 6.75 mmol) was added to a solution of (+)-brefeldin A (2.1 g, 7.5 mmol) and Proton Sponge [1,8-bis-(dimethylamino)naphthalene] (3.21 g, 15 mmol) in a mixture of MeOH (80 mL) and $H_2O$ (40 mL) at room temperature. The reaction mixture was stirred at an ambient temperature for 5 h and diluted with MeOH (200 mL). The methanolic solution was concentrated. To the resulting residue was added $CHCl_3$ (200 mL), and the mixture was stirred for 5 min, filtered, and washed with $CHCl_3$ (2 × 50 mL) to obtain the requisite sulfide **11** (1.8 g, 65%): mp 178 °C; TLC $R_f$ 0.25 (30% MeOH/$CHCl_3$); $^1$H NMR ($D_2O$, 300 MHz) $\delta$ 7.6 (bm, 2 H), 5.60 (m, 1 H), 5.22 (m, 1 H), 4.70 (m, 1 H),4.14 (m, 1 H), 3.76 (m, 1 H), 3.66 (bd, 1 H, $J$ = 7.3 Hz), 3.44 (bd, 1 H, $J$ = 8.6 Hz), 3.09 (m, 2 H), 2.70 (bd, 1 H, $J$ = 16.4 Hz), 2.40−2.10 (m, 2 H), 2.10−1.80 (m, 4 H), 1.75−1.50 (m, 5 H), 1.29 (m, 1 H), 1.13 (d, 3 H, $J$ = 6.3 Hz); $^{13}$C NMR ($D_2O$) $\delta$ 174.46, 172.94, 135.70, 131.95, 74.98, 72.83, 55.05, 46.61, 46.34, 44.49, 42.94, 39.18, 36.28, 33.36, 33.30, 31.49, 25.66, 20.27; IR (neat) 3341, 1703, 1631, 1391, 1343, and 1270 cm$^{-1}$; MS *m*/*z* 402 ($MH^+$); HRMS calcd for $C_{19}H_{31}O_6NS$ 402.1950, found 402.1951.

**2,3-Dihydro-(3*R*)-(*S*-glutathionyl)brefeldin A (12).** Glutathione (0.138 g, 0.4 mmol) was added to a solution of (+)-brefeldin A (0.140 g, 0.5 mmol) and Proton Sponge (0.214 g, 1.0 mmol) in a mixture of MeOH (2 mL) and $H_2O$ (2 mL) at room temperature. The reaction mixture was stirred at an ambient temperature for 5 h and diluted with MeOH (40 mL). The methanolic solution was concentrated. To the resulting residue was added $CHCl_3$ (75 mL), and the mixture was stirred for 5 min, filtered, and washed with $CHCl_3$ (2 × 25 mL) to obtain the requisite sulfide **12** (0.130 g, 44%): mp 195 °C; TLC $R_f$ 0.13 (40% MeOH/$CHCl_3$); $^1$H NMR ($CD_3OD$ + $D_2O$, 300 MHz) $\delta$ 5.56 (m, 1 H), 5.19 (m, 1 H), 4.69 (m, 1 H, partially merged with solvent peak), 4.45 (m, 4 H), 4.06 (m, 1 H), 3.70−3.45 (m, 3 H), 3.40−3.10 (m, 3 H), 2.80−2.50 (m, 2 H), 2.40 (m, 1 H), 2.40−2.00 (m, 2 H), 1.80 (m, 2 H), 1.80−1.50 (m, 4 H), 1.20 (m, 1 H), 1.10 (d, 3 H, $J$ = 6.4 Hz), 1.05 (m, 1 H); $^{13}$C NMR ($D_2O$) $\delta$ 174.66, 173.73, 173.51, 172.24, 172.05, 134.88, 130.71, 74.04, 71.83, 53.76, 53.29, 45.68, 45.58, 44.92, 43.81, 42.22, 41.70, 35.39, 33.57, 32.45, 31.34, 31.17, 30.60, 26.20, 24.70, 19.48; IR (neat) 3367, 1705, 1700, 1680, 1638, 1355, 1259 cm$^{-1}$; HRMS calcd for $C_{26}H_{41}O_{10}N_3S$ 610.2410, found 610.2435.

**2,3-Dihydro-(3*R*)-(2′-methoxycarbonylethylsulfinyl)-brefeldin A (13).** To a solution of sulfide **2** (0.180 g, 0.45 mmol) in $CH_2Cl_2$ (45 mL) was added *m*-CPBA (85% Aldrich, 0.100 g, 0.5 mmol) in one portion at 0 °C under $N_2$ atmosphere. TLC analysis of the reaction mixture showed that the reaction was completed within 3 min. The reaction mixture was then neutralized with a saturated aqueous solution of $NaHCO_3$ (5 mL) and extracted with EtOAc (3 × 75 mL). The organic extract was dried over anhydrous $MgSO_4$, solvent was removed under a reduced pressure, and the resulting residue was purified by flash column chromatography (silica gel, 2% EtOH/$CHCl_3$) to afford the desired sulfoxide **13** (0.150 g 80%) as an oil: TLC $R_f$ 0.35 (10% EtOH/$CHCl_3$); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 5.40 (m, 2 H), 4.94 (m, 1 H), 4.32 (m, 1 H), 4.18 (br d, 1 H, $J$ = 10.16 Hz), 3.74 (s, 3 H), 3.44 (dd, 1 H, $J$ = 3.1 and 8.5 Hz), 3.09 (br t, 3 H), 2.95 (dd, 1 H, $J$ = 3.4 and 17.8 Hz), 2.88 (t, 1 H, $J$ = 7.1 Hz), 2.80 (br d, 1 H, $J$ = 8.8 Hz), 2.66 (dd, 1 H, $J$ = 8.3 and 8.3 Hz), 2.30−1.80 (m, 6 H), 1.80−1.40 (m, 5 H), 1.25 (m, 1 H), 1.17 (d, 3 H, $J$ = 6.3 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 171.41, 169.42, 136.25, 129.71, 75.88, 72.97, 72.52, 71.87, 52.27, 45.49, 44.52, 43.21, 40.70, 33.45, 30.33, 29.87, 27.42, 24.54, 20.29; IR ($CHCl_3$) 3387, 1725, 1649, 1438, 1358, 1263 cm$^{-1}$; MS *m*/*z* 417 ($MH^+$); HRMS calcd for $C_{20}H_{32}O_7S$ 417.1947, found 417.1955.

**2,3-Dihydro-(3*R*)-(4′-hydroxyphenylsulfinyl)brefeldin A (14).** To a solution of sulfide **3** (0.050 g, 0.123 mmol) in $CH_2Cl_2$ was added *m*-CPBA (85% Aldrich, 0.028 g, 0.135 mmol) in one portion at 0 °C under $N_2$ atmosphere. TLC analysis of the reaction mixture showed that the reaction was completed within 3 min. The reaction mixture was then neutralized with a saturated aqueous solution of $NaHCO_3$ (5 mL) and extracted with EtOAc (3 × 50 mL). The organic extract was dried over anhydrous $MgSO_4$, solvent was removed under a reduced pressure, and the resulting residue was purified by flash column chromatography (silica gel, 2% EtOH/$CHCl_3$) to afford the desired sulfoxide **14** (0.035 g, 68%) as an oil: TLC $R_f$ 0.18 (10% EtOH/$CHCl_3$); $^1$H NMR ($CDCl_3$ + $CD_3OD$, 300 MHz) $\delta$ 7.43 (d, 2 H, $J$ = 8.8 Hz), 6.95 (d, 2 H, $J$ = 8.6 Hz), 5.23 (m, 2 H), 4.78 (m, 1 H), 4.04 (m, 1 H), 3.78 (br d, 1 H, $J$ = 10.2 Hz); 3.32 (dd, 1 H, $J$ = 3.3 and 8.3 Hz), 2.82 (dd, 1 H, $J$ = 3.5 and 17.6), 2.63 (dd, 1 H, $J$ = 17.6 and 17.6 Hz), 2.10−1.70 (m, 5 H), 1.70−1.30 (m, 6 H), 1.25 (m, 1 H), 1.03 (d, 3 H, $J$ = 6.2 Hz). Anal. ($C_{22}H_{30}O_6S$) C, H, S.

**2,3-Dihydro-(3*R*)-(2′-hydroxyethylsulfinyl)brefeldin A (15).** To a solution of dialkyl sulfide **5** (0.072 g, 0.2 mmol) in a mixture of THF (2.5 mL) and $CH_2Cl_2$ (2.5 mL) was added *m*-CPBA (85% from Aldrich, 0.049 g, 0.24 mmol) in one portion at 0 °C under $N_2$ atmosphere. TLC analysis showed that the reaction was completed within 3 min. The reaction mixture was neutralized with a saturated aqueous $NaHCO_3$ solution (5 mL), the aqueous layer was separated and diluted with EtOH (20 mL), and the precipitated salt was filtered. The filtrate was concentrated, and the resulting residue was flash chromatographed (silica gel, $CHCl_3$ to 5% EtOH/$CHCl_3$) to afford the desired product **15** (0.046 g, 62%) as an oil: TLC $R_f$ 0.44 (15% EtOH/$CHCl_3$); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 5.40 (m, 2 H), 4.96 (m, 1 H), 4.30 (m, 1 H), 4.14 (m, 3 H), 3.54 (dd, 1 H, $J$ = 3.6 and 7.9 Hz), 3.02 (m, 2 H), 2.93 (dd, 1 H, $J$ = 3.7 and 17.3 Hz), 2.66 (dd, 1 H, $J$ = 16.4 and 17.4 Hz), 2.15−1.88 (m, 6 H), 1.80−1.45 (m, 5 H), 1.25 (m, 1 H), 1.19 (d, 3 H, $J$ = 6.3 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 169.94, 135.66, 129.95, 77.19, 71.87, 71.70, 58.28, 55.85, 51.81, 45.23, 44.15, 43.21, 40.17, 33.46, 33.46, 30.13, 29.00, 23.86, 20.19; IR ($CHCl_3$) 3377, 1720, 1443, 1263, 1058, 987 cm$^{-1}$; MS *m*/*z* 375 ($MH^+$); HRMS calcd for $C_{18}H_{30}O_6S$ 375.1841, found 375.1856.

**2,3-Dihydro-(3*R*)-(methoxycarbonylmethylsulfinyl)-brefeldin A (16).** To a solution of sulfide **7** (0.145 g, 0.38 mmol) in $CH_2Cl_2$ (8 mL) was added *m*-CPBA (85% Aldrich, 0.084 g, 0.41 mmol) in one portion at 0 °C under $N_2$ atmosphere. TLC analysis of the reaction mixture showed that the reaction was completed within 3 min. The reaction mixture was then neutralized with a saturated aqueous solution of $NaHCO_3$ (5 mL) and extracted with EtOAc (3 × 75 mL). The organic extract was dried over anhydrous $MgSO_4$, solvent was removed under a reduced pressure, and the resulting residue was purified by flash column chromatography (silica gel, 2% EtOH/$CHCl_3$) to afford the desired sulfoxide **16** (0.125 g, 83%) as an oil: TLC $R_f$ 0.34 (10% EtOH/$CHCl_3$); $^1$H NMR ($CDCl_3$,

300 MHz) $\delta$ 5.56−5.30 (m, 2 H), 4.94 (m, 1 H), 4.31 (m, 1 H), 4.12 (dt, 1 H, $J$ = 3.7 and 9.8 Hz), 3.95 (d, 1 H, $J$ = 13.9 Hz), 3.82 (s, 3 H), 3.77 (d, 1 H, $J$ = 13.8 Hz), 3.69 (dd, 1 H, $J$ = 3.6 and 7.9 Hz), 2.95 (dd, 1 H, $J$ = 3.9 and 17.4 Hz), 2.70 (dd, 1 H, $J$ = 8 and 17.5 Hz), 2.35−1.82 (m, 7 H), 1.80−1.40 (m, 4 H), 1.25 (m, 1 H), 1.19 (d, 3 H, $J$ = 6.3 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 169.48, 165.40, 135.98, 129.98, 72.65, 72.31, 71.87, 56.78, 53.36, 53.05, 45.69, 44.03, 43.19, 40.61, 33.33, 30.25, 29.22, 24.27, 20.20; IR ($CHCl_3$) 3377, 1740, 1725, 1704, 1639, 1433, 1358, 1263 cm$^{-1}$; MS $m/z$ 403 ($MH^+$); HRMS calcd for $C_{19}H_{30}O_7S$ 403.1791, found 403.1787.

**2,3-Dihydro-(3*R*)-(2′,3′-dihydroxypropylsulfinyl)brefeldin A (17).** To a solution of dialkyl sulfide **8** (0.057 g, 0.15 mmol) in THF/$CH_2Cl_2$ (1:1 v/v, 8 mL) was added *m*-CPBA (85% from Aldrich, 0.032 g, 0.16 mmol) in one portion at 0 °C under $N_2$ atmosphere. TLC analysis showed that the reaction was completed within 3 min. The reaction mixture was neutralized with aqueous saturated $NaHCO_3$ solution (5 mL), the aqueous layer was separated and diluted with EtOH (20 mL), and the precipitated salt was filtered. The filtrate was concentrated, and the resulting residue was flash chromatographed (silica gel, 5% EtOH/$CHCl_3$) to afford 0.030 g (50%) of the desired product **17** as a colorless oil: TLC $R_f$ 0.27 (20% EtOH/$CHCl_3$); $^1$H NMR ($D_2O$) $\delta$ 5.58 (m, 1 H), 5.37 (m, 1 H), 4.95 (m, 1 H), 4.28 (m, 2 H), 4.05 (m, 1 H), 3.50−3.75 (m, 3 H), 3.10−2.70 (m, 3 H), 2.50 (m, 1 H), 2.30−1.60 (m, 9 H), 1.60−1.30 (m, 3 H), 1.24 (d, 3 H, $J$ = 6.2 Hz); $^{13}$C NMR ($D_2O$) $\delta$ 172.12, 134.70, 131.55, 73.39, 71.52, 71.27, 67.66, 64.12, 63.96, 58.64, 58.64, 52.48, 44.89, 43.89, 42.38, 38.99, 32.79, 29.22, 28.50, 23.09, 19.20; MS $m/z$ 405 ($MH^+$); HRMS calcd for $C_{19}H_{32}O_7S$ 405.1947, found 405.1926.

**2,3-Dihydro-(3*R*)-(2′-aminoethylsulfinyl)brefeldin A (18).** HCl (4 M, dioxane, 1.4 mL, 5.6 mmol) was added over a period of 5 min at 0 °C to a solution of sulfide **9** (1.0 g, 2.8 mmol) in anhydrous THF (50 mL). The reaction mixture was kept at 0 °C for 48 h, treated with *m*-CPBA (85%, Aldrich, 0.69 g, 3.36 mmol), and stirred for 10 min at 0 °C. The precipitated sulfoxide was filtered when it was cold and washed with cold diethyl ether (2 × 50 mL) to give 1.07 g (93%) of the desired sulfoxide **18**: $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.24 (m, 1 H), 5.54−5.10 (m, 2 H), 4.85 (m, 1 H, overlapping with solvent peak), 4.09 (m, 1 H), 3.84 (br d, 1 H, $J$ = 10.3 Hz), 3.50 (dd, 1 H, overlapping with the ethanol peak), 3.37 (m, 2 H), 3.19 (m, 2 H), 2.85 (dd, 1 H, $J$ = 3.5 and 15.6 Hz), 2.58 (dd, 1 H, $J$ = 15.0 and 17.0 Hz), 2.35−1.25 (m, 12 H), 1.13 (d, 3 H, $J$ = 6.3 Hz); MS $m/z$ 374 ($M^+$ − Cl); HRMS calcd for $C_{18}H_{32}NO_5SCl$ 375.2079, found 375.2078.

**2,3-Dihydro-(3*R*)-(4′-methoxyphenylsulfinyl)brefeldin A (19).** To a solution of sulfide **10** (0.100 g, 0.24 mmol) in $CH_2Cl_2$ (10 mL) was added *m*-CPBA (85% Aldrich, 0.053 g, 0.26 mmol) in one portion at 0 °C under $N_2$ atmosphere. TLC analysis of the reaction mixture showed that the reaction was completed within 3 min. The reaction mixture was then neutralized with saturated aqueous solution of $NaHCO_3$ (5 mL) and extracted with EtOAc (3 × 75 mL). The organic extract was dried over anhydrous $MgSO_4$, solvent was removed under a reduced pressure, and the resulting residue was purified by flash column chromatography (silica gel, 5% EtOH/$CHCl_3$) to afford the desired sulfoxide **19** (0.090 g, 87%) as an oil: TLC $R_f$ 0.45 (10% EtOH/$CHCl_3$); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.55 (d, 2H, $J$ = 8.4 Hz), 7.13 (d, 2 H, 7.9 Hz), 5.40−5.20 (m, 2 H), 5.02 (m, 1 H), 4.15 (m, 1 H), 3.90 (s, 3 H), 3.84 (br d, 1 H, $J$ = 10.9 Hz), 3.50−2.95 (m, 2 H), 2.20−1.35 (m, 12 H), 1.28 (d, 3 H, $J$ = 6.2 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 169.41, 161.95, 136.58, 131.88, 129.16, 125.74, 114.97, 73.11, 72.45, 71.86, 60.23, 55.54, 45.36, 44.10, 42.87, 40.23, 33.69, 30.53, 30.02, 25.08, 20.69; IR ($CHCl_3$) 3418, 1722, 1593, 1488, 1248, 1213, 1085 cm$^{-1}$; MS $m/z$ 437 ($MH^+$); HRMS calcd for $C_{23}H_{32}O_6S$ 437.1998, found 437.2007.

**2,3-Dihydro-(3*R*)-[2′-*N*,*N*-dimethylaminoethylsulfinyl]-brefeldin A (20).** *m*-CPBA (85% from Aldrich, 0.22 g, 1.2 mmol) was added in one portion at 0 °C under $N_2$ atmosphere to a solution of sulfide **4** (0.35 g, 0.92 mmol) in THF/$CH_2Cl_2$ (1:1 v/v; 40 mL). TLC analysis showed that the reaction was completed within 3 min. The reaction mixture was neutralized with aqueous saturated $NaHCO_3$ solution (10 mL), the aqueous layer was separated and diluted with EtOH (80 mL), and the precipitated salt was filtered. The filtrate was concentrated, and the resulting residue was flash chromatographed (silica gel, $CHCl_3$ to 5% MeOH/$CHCl_3$) to afford 0.27 g (75%) of the desired product **20**: TLC $R_f$ 0.42 (30% MeOH/$CHCl_3$); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 5.45 (m, 2 H), 5.01 (m, 1 H), 4.33 (m, 1 H), 4.16 (d, 1 H, $J$ = 10.6 Hz), 3.51 (dd, 1 H, $J$ = 3.5 and 8.5 Hz), 3.10−2.90 (m, 4 H), 2.85−2.75 (m, 2 H), 2.42 (s, 6 H), 2.30 (m, 1 H), 2.20−1.90 (m, 5 H), 1.85−1.45 (m, 6 H), 1.30 (m, 1 H), 1.22 (d, 3 H, $J$ = 6.3 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 169.66, 136.13, 129.54, 72.77, 72.25, 71.65, 56.53, 52.29, 47.51, 45.43, 44.96, 44.26, 43.10, 40.51, 33.40, 30.30, 29.66, 24.34, 20.29; IR ($CHCl_3$) 3380, 1724, 1559, 1451, 1376, 1264, 1040, 987 cm$^{-1}$; MS $m/z$ 402 ($MH^+$); HRMS calcd for $C_{20}H_{35}O_5NS$ 402.2314, found 402.2289.

**2,3-Dihydro-(3*R*)-(*S*-L-cysteinyl)brefeldin A sulfoxide (21).** HCl (4 M, dioxane, 2.24 mL, 8.96 mmol) was added over a period of 5 min at 0 °C to a solution of sulfide **11** (1.8 g, 4.48 mmol) in anhydrous THF (50 mL). The reaction mixture was kept at 0 °C for 24 h, treated with *m*-CPBA (85%, Aldrich, 1.06 g, 5.38 mmol), and stirred for 10 min at 0 °C. The precipitated sulfoxide was filtered when it was cold and washed with cold THF (2 × 50 mL) to give 1.6 g (86%) of the desired sulfoxide **21**: mp 120 °C; TLC (Whatman 250-$\mu$m silica gel glass plates) $R_f$ 0.22 (30% MeOH/$CHCl_3$); $^1$H NMR ($CD_3$-OD, 300 MHz) $\delta$ 7.69 (partially exchanged s, 2 H), 5.63 (m, 1 H), 5.37 (m, 1 H), 4.99 (m, 1 H), 4.55 (m, 1 H), 4.31 (m, 1 H), 4.10−3.75 (m, 2 H), 3.70−3.35 (m, 2 H), 2.85 (m, 1 H), 2.50 (m, 1 H), 2.40−1.40 (m, 12 H), 1.23 (d, 3 H, $J$ = 6.2 Hz); MS $m/z$ 418 ($MH^+$); HRMS calcd for $C_{19}H_{31}O_7NS$ 418.1900, found 418.1904.

**Brefeldin A 8*O*-Succinate (22).** Brefeldin A (0.056 g, 0.2 mmol) was dissolved in pyridine (4 mL), and succinic anhydride (0.025 g, 0.25 mmol) was added. The reaction mixture was stirred at 60 °C for 48 h. The reaction mixture was then diluted with water (40 mL), and the mixture was extracted with chloroform (3 × 25 mL). The combined organic layers were washed with 1 N HCl (2 × 25 mL) and brine, dried ($MgSO_4$), and filtered. The filtrate was evaporated to afford an oil which was flash chromatographed on silica gel, eluting with $CHCl_3$ to 5% MeOH in $CHCl_3$. Fractions showing a single spot corresponding to that of the product were pooled and evaporated to dryness under reduced pressure to afford an oil which was crystallized from a mixture of ethyl ether and hexanes to afford the ester **22** (0.035 g, 46%) as a white solid: mp 107−9 °C; TLC $R_f$ 0.66 ($CHCl_3$/MeOH, 4:1, silica gel); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.35 (dd, 1 H, $J$ = 3, 15.6 Hz), 5.92 (dd, 1 H, $J$ = 1.8, 15.6 Hz), 5.72 (m, 1 H), 5.17−5.33 (overlapping m and dd, 2 H, $J$ = 9, 15.3 Hz), 4.87 (m, 1 H), 4.12 (ddd, 1 H, $J$ = 2.1, 2.4, 9 Hz), 2.63 (m, 5 H), 2.20−2.42 (m, 2 H), 2.02 (br m, 1 H), 1.68−1.91 (m, 5 H), 1.48−1.63 (m, 2 H), 1.26 (d, 3 H, $J$ = 6.3 Hz), 0.89−1.0 (br m, 1 H); FABMS $m/z$ 403 ($M + Na^+$, 5), 381 ($MH^+$, 20), 363 ($MH^+ - 18$, 15); HRMS calcd $MH^+$ 381.1913, found 381.1900.

**Brefeldin A 8*O*-Glutarate (23).** Brefeldin A (0.056 g, 0.2 mmol) was dissolved in pyridine (4 mL), and glutaric anhydride (0.04 g, 0.35 mmol) was added. The reaction mixture was heated at 110 °C under argon for 36 h. The TLC ($CHCl_3$/MeOH, 4:1, silica gel) indicated the presence of a major spot at $R_f$ 0.58 and a very minor spot at $R_f$ 0.19 along with some unreacted starting material. The reaction mixture was cooled to room temperature and diluted with water (40 mL). Workup and isolation as reported above for the synthesis of **22** afforded the desired monoglutarate derivative **23** (0.033 g, 42%) as an oil; TLC $R_f$ 0.58 ($CHCl_3$/MeOH, 4:1, silica gel); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.27 (dd, $J$ = 3 and 15 Hz, 1 H), 5.84 (dd, $J$ = 3 and 15 Hz, 1 H), 5.64 (m, 1 H), 5.22 (m, 1 H), 5.10 (m, 1 H), 4.78 (m, 1 H), 4.04 (m, 1 H), 2.40−2.00 (m, 6 H), 1.90−1.30 (m, 12 H), 1.17 (d, $J$ = 6 Hz, 3 H); MS $m/z$ 417 ($M + Na^+$), 395 ($MH^+$).

**Brefeldin A Disuccinate (24).** In a manner similar to that reported below for the synthesis of **25**, the reaction of

brefeldin A (0.056 g, 0.2 mmol) and succinic anhydride afforded the desired diester **24** (0.051 g, 53%) as an off-white solid: mp 85−87 °C; TLC $R_f$ 0.24 ($CHCl_3$/MeOH, 4:1, silica gel); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.19 (dd, 1 H, $J$ = 3.6, 15.6 Hz), 5.70 (m, 2 H), 5.33 (br m, 2 H), 5.19 (dd, 1 H, $J$ = 9, 15 Hz), 4.90 (m, 1 H), 2.85−2.93 (m, 1 H), 2.54−2.74 (m, 8 H), 2.32−2.47 (m, 2 H), 1.94−2.05 (m, 3 H), 1.69−1.89 (m, 3 H), 1.47−1.64 (m, 2 H), 1.25 (d, 3 H, $J$ = 6 Hz), 0.96−1.04 (m, 1 H); FABMS $m/z$ 503 ($M + Na^+$), 481 ($MH^+$); HRMS calcd $MH^+$ 481.2074, found 481.2084.

**Brefeldin A Diglutarate (25).** Brefeldin A (0.056 g, 0.2 mmol) was dissolved in pyridine (4 mL), and glutaric anhydride (0.07 g, 0.6 mmol) was added to this solution, followed by the addition of DMAP (0.05 g, 0.4 mmol). The reaction mixture was heated at 60 °C for a period of 48 h under argon when the TLC ($CHCl_3$/MeOH, 4:1, silica gel) indicated the formation of a major product spot at $R_f$ 0.19. The reaction mixture was cooled, diluted with water, and the pH of the solution was adjusted to 4. This solution was extracted with $CHCl_3$ (3 × 25 mL). The $CHCl_3$ extracts were washed with 1 N HCl (2 × 30 mL) and brine (50 mL), dried ($MgSO_4$) and filtered. The filtrate was evaporated to dryness under reduced pressure. The residue was flash chromatographed on a silica gel column eluting with 1% to 6% MeOH in $CHCl_3$. The fractions showing a single spot corresponding to that of the product were pooled and evaporated to afford a gum. This material was recrystallized from a mixture of ethyl acetate and hexanes to afford the diester **25** (0.041 g, 40%) as a white solid: mp 100−101 °C; TLC $R_f$ 0.19 ($CHCl_3$/MeOH, 4:1, silica gel); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.23 (dd, 1 H, $J$ = 3.6, 15.9 Hz), 5.68−5.77 (overlapping m and d, 2 H, $J$ = 1.8, 15.9 Hz), 5.28 (m, 1 H), 5.21 (dd, 1 H, $J$ = 9.3, 15.3 Hz), 5.15 (m, 1 H), 2.25−2.55 (overlapping m and t, 11 H, $J$ = 7.2 Hz), 1.93−2.10 (overlapping m and t, 8 H, $J$ = 7.2 Hz), 1.79−1.91 (m, 1 H), 1.65−1.74 (m, 1 H), 1.50−1.63 (m, 2 H), 1.25 (d, 3 H, $J$ = 6.3 Hz), 0.86−0.99 (br m, 1 H); FABMS $m/z$ 531 ($MNa^+$), 509 ($MH^+$); HRMS calcd $MH^+$ 509.2387, found 509.2397.

**2,3-Dihydro-2,3-dihydroxybrefeldin A (26).** To a solution of (+)-brefeldin A (0.056 g, 0.2 mmol) in *t*-BuOH/$H_2O$ (2:1 v/v, 6 mL) at room temperature was added NMO (0.047 g, 0.4 mmol) followed by $OsO_4$ (1 wt% solution in $H_2O$, 4 drops). The reaction progress was monitored by TLC. The reaction mixture was stirred at room temperature for 4 h, quenched with brine (5 mL), extracted with ethyl acetate (3 × 40 mL), and the ethyl acetate extract was dried over anhydrous $MgSO_4$ and filtered, and the solvent was evaporated. The resulting residue was crystallized from MeOH/EtOAc/*n*-hexanes (1:1:3, v/v) solvent system to afford the product **26** (0.056 g, 89%); mp 183 °C; TLC $R_f$ 0.45 (20% EtOH/$CHCl_3$); $^1$H NMR ($CDCl_3$ + $CD_3OD$, 300 MHz) $\delta$ 5.40−5.22 (m, 2 H), 4.80 (m, 1 H), 4.08 (m, 1 H), 3.58 (m, 1 H), 3.28 (m, 1 H), 2.82 (m, 1 H), 2.60 (m, 2 H), 2.45−2.20 (m, 3 H), 2.10−1.80 (m, 6 H), 1.60−1.20 (m, 4 H), 1.20 (d, 3 H, $J$ = 6.2 Hz); $^{13}$C NMR ($CD_3OD$) $\delta$ 174.14, 136.78, 131.34, 74.29, 73.32, 72.67, 71.40, 47.66, 46.39, 45.30, 44.27, 34.47, 33.35, 26.60, 25.97, 20.35; IR (MeOH) 3317, 1730, 1438, 1237, 1132, 1102 cm$^{-1}$; $m/z$ 315 ($MH^+$); HRMS calcd for $C_{16}H_{20}O_6$ 315.1808, found 315.1814.

**2,3-Dihydro(3*R*)-(methoxycarbonylmethylsulfonyl)-brefeldin A (27).** To a dry reaction flask equipped with a rubber septum and a magnetic stirring bar was placed sulfide **7** (0.087 g, 0.2 mmol) and $CH_2Cl_2$ (10 mL) at room temperature under $N_2$. The reaction solution was cooled to 0 °C and to it was added *m*-CPBA (85% from Aldrich, 0.091 g, 0.44 mmol) in one portion and further stirred for 4 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution (10 mL), stirred for an additional 20 min, and extracted with $CHCl_3$ (3 × 40 mL). The organic extract was dried over anhydrous $MgSO_4$ and filtered, and the filtrate was evaporated. The resulting residue was purified by silica gel flash column chromatography using $CHCl_3$ to 2% EtOH/$CHCl_3$ solvent system. The fractions containing product at TLC $R_f$ 0.64 (10% EtOH/$CHCl_3$) were combined, and the solvent was removed to give sulfone **27** (0.032 g, 40%) as an oil: $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 5.66 (m, 1 H), 5.44 (m, 1 H), 4.90 (m, 1 H), 4.46 (dd, 1 H, $J$ = 2.9 and 9.5 Hz), 4.36 (m, 1 H), 4.22 (d, 1 H, $J$ = 14.4 Hz), 4.08 (d, 1 H, $J$ = 14.4 Hz), 4.08 (m, 1 H), 3.88 (s, 3 H), 3.13 (bs, 1 H), 2.96 (dd, 1 H, $J$ = 17.6 and 17.7 Hz), 2.84 (dd, 1 H, $J$ = 3.0 and 17.7 Hz), 2.36 (m, 1 H), 2.30−1.95 (m, 5 H), 1.85−1.45 (m, 7 H), 1.22 (d, 3 H, $J$ = 6.3 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 168.741, 163.382, 135.774, 129.604, 77.217, 73.759, 72.774, 72.428, 62.128, 56.501, 53.421, 46.115, 43.213, 40.948, 33.048, 31.118, 28.261, 24.804, 19.984; IR ($CHCl_3$) 3518, 3387, 1735, 1438, 1318, 1278, 1147, 1112 cm$^{-1}$; MS $m/z$ 419 ($MH^+$); HRMS calcd for $C_{19}H_{30}O_8S$ 419.1740, found 419.1757.

**2,3-Dihydro-l0,11-epoxy-(3*R*)-(methoxycarbonylmethylsulfonyl)brefeldin A (28).** To a dry reaction flask equipped with a rubber septum and a magnetic stirring bar was placed sulfide **7** (0.064 g, 0.165 mmol) and $CH_2Cl_2$ (10 mL) at room temperature under $N_2$. The reaction solution was cooled to 0 °C, and to it was added *m*-CPBA (85% from Aldrich, 0.150 g, 0.72 mmol) in one portion. The reaction mixture was stirred for 7 h, quenched with a saturated aqueous $NaHCO_3$ solution (10 mL), stirred for an additional 20 min, and extracted with $CHCl_3$ (3 × 30 mL). The organic extract was dried over anhydrous $MgSO_4$ and filtered, and the filtrate was evaporated. The resulting residue was purified by silica gel flash column chromatography using $CHCl_3$ to 2% EtOH/$CHCl_3$ solvent system. The fractions containing product at TLC $R_f$ 0.53 (10% EtOH/$CHCl_3$) were combined, and the solvent was removed to give epoxysulfone **28** (0.035 g, 50%) mp 180 °C TLC $R_f$ 0.53 (10% EtOH/$CHCl_3$); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 5.25 (m, 1 H), 4.35−4.28 (m, 2 H), 4.10 (d, 1 H, $J$ = 14.6 Hz), 4.03 (d and m, 3 H, $J$ = 14.7 Hz), 3.85 (s, 3 H), 3.24 (m, 1 H), 3.10−2.85 (m, 3 H), 2.30 (m, 1 H), 2.20 (m, 1 H), 2.00−1.30 (m, 12 H), 1.26 (d and m, 4 H, $J$ = 6.0 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 168.17, 163.44, 73.71, 73.33, 72.68, 62.19, 61.60, 58.68, 55.72, 53.58, 43.65, 42.76, 40.67, 39.07, 32.61, 30.09, 28.35, 21.80, 20.42; IR ($CHCl_3$) 3367, 1730, 1438, 1257, 1147, 1122 cm$^{-1}$; MS $m/z$ 435 ($MH^+$); HRMS calcd for $C_{19}H_{30}O_9S$ 435.1689, found 435.1711.

**Preparation of pH 7.4 Buffer for Study of Sulfoxide Elimination Reactions.** Sodium bicarbonate (21 mg) was dissolved in a mixture of $D_2O$ (2 mL) and $CD_3OD$ (1 mL). Sodium acetate (3 mg) was added, and the pH was measured at 7.4 using pH paper.

**Kinetics of the Conversion of Sulfoxides 13, 14, 15, and 20 to Brefeldin A.** The sulfoxides (0.04 mmol) were dissolved in buffer solution (0.63 mL) at 37 °C under $N_2$ atmosphere. The $^1$H NMR spectra were recorded at fixed time intervals, and the reaction progress was monitored, observing the disappearance of the C-2 protons of the starting materials and the appearance of the C-3 and C-4 alkene protons of brefeldin A. The log % sulfoxide remaining values were plotted vs time to obtain lines that were used to estimate the half-life values. No compounds other than the starting materials and brefeldin A were detected during the course of the kinetics experiments. The time (% sulfoxide remaining) values were as follows: **13**, 0 h (100), 0.5 h (88.5), 1.0 h (80.0), 1.5 h (71.8), 2.0 h (67.0), 2.5 h (57.6), 3.0 h (56.8), 3.5 h (53.0), 4.0 h (52.0), 4.5 h (50.0), 5.0 h (47.3), 5.5 h (45.0), 6.0 h (43.2), 6.5 h (41.5), 7.0 h (37.9), 7.5 h (26.2); **14**, 0 h (100), 0.5 h (87.2), 1.0 h (85.8), 1.5 h (85.7), 2.0 h (85.3), 2.5 h (84.1), 3.0 h (82.6), 3.5 h (81.6), 4.0 h (80.1), 4.5 h (79.2), 5.5 h (77.8), 6.0 h (76.4), 7.0 h (74.2), 7.5 h (73.4); **15**, 0 min (100), 11 min (84.8), 26 min (72.5), 41 min (63.7), 56 min (59.5), 71 min (5.20), 86 min (48.0), 101 min(35.5); **20**, 0 min (100), 5 min (95.0), 15 min (91.8), 25 min (86.6), 35 min (85.0), 45 min (80.6), 55 min (71.0), 65 min (62.4), 75 min (60.4), 85 min (55.6), 95 min (51.0), 105 min (49.2), 115 min (36.0), 125 min (25.5).

**Determination of Solubilities.** An excess of brefeldin A or sulfide derivative was added to distilled water at room temperature, and the suspension was stirred for 1 h. The insoluble material was filtered off, and ethanol (1 mL) was added to the filtrate. The solvent was removed by evaporation, and the residue was dried under vacuum until the weight under vacuum was constant. The residue was then weighed. The solubility of brefeldin A determined in this way was 2.8

mg/mL, while those of the sulfides were as follows: **3**, 12 mg/mL; **4**, 10 mg/mL; **5**, 12 mg/mL; **6**, 40 mg/mL, and **11**, 35 mg/mL.

**Acknowledgment.** We are grateful to the National Cancer Institute for providing a generous supply of (+)-brefeldin A. We also gratefully acknowledge the National Institutes of Health (NIH) for support of this work with Contract NO1-CM-67260 and Training Grant 5T32-09634.

**Supporting Information Available:** $^{1}H$ and $^{13}C$ NMR spectra (47 pages). Ordering information is given on any current masthead page.

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JM970746G