Highly Efficient Diastereoselective Michael Addition of Various Thiols to (+)-Brefeldin A

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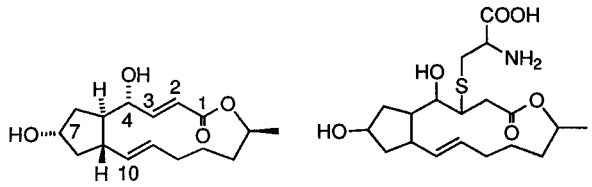
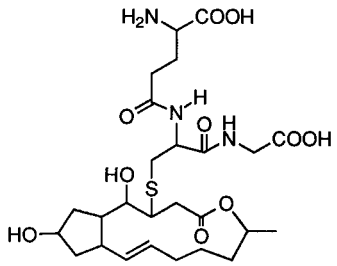
The Michael addition of thiols to brefeldin A occurs with high diastereoselectivity, affording ratios of major to minor diastereomers of at least 30:1. On the basis of the X-ray structure of a crystalline dibenzoyl derivative, the major diastereomers are assigned the 3*R* configuration, while the minor diastereomers have the 3*S* configuration.

Brefeldin A (**1**), a macrolide antibiotic whose structure has been established by X-ray crystallography, was first isolated from the fungus *Penicillium decumbens*.[1,2] Recent interest in brefeldin A has been stimulated by its ability to induce DNA fragmentation associated with apoptosis in cancer cells, and it is in preclinical development as an anticancer agent.[3] Studies in Chinese hamster ovary cells have indicated that brefeldin A is secreted as glutathione and cysteine conjugates, and the glutathione-*S*-transferase system may be responsible for the inactivation of the antibiotic in mammalian cells.[4] Based on FAB mass spectroscopy and UV evidence, structures **2** and **3** were assigned to the metabolites.[4] The present investigation was undertaken in order to study the stereochemistry of the reaction of thiols with brefeldin A. The addition products might be valuable as a starting point for the design of water-soluble prodrugs of brefeldin A, which could be important because the insolubility of the antibiotic itself is one of the factors limiting its potential clinical utility.



1 **2**



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Results and Discussion

Treatment of brefeldin A (**1**) with methyl mercaptoacetate in the presence of proton sponge [1,8-bis(dimethylamino)naphthalene] in aqueous methanol for 2 h at room-temperature resulted in a diastereomeric mixture of adducts **4** and **5**. These epimers could be separated by silica gel column chromatography, affording the major diastereomer in 91% yield and the minor diastereomer in 2.8% yield. The coupling constant J_{ab} between the C-3 and C-4 methine protons in the ^{1}H NMR spectrum of the major diastereomer was 1.6 Hz, while that of the minor diastereomer was 7.5 Hz. These coupling constants do not allow the assignment of the structures of the major and minor diastereomers, however, because of the conformational freedom of the 13-membered ring. It was therefore necessary to consider the preparation of conformationally restricted derivatives which might allow stereochemical assignment by NMR analysis.

To restrict rotation around the single bond between C-3 and C-4, the two diastereomers **4** and **5** were converted into bis-lactones **6** and **7**, respectively, by treatment with trimethylsilyl triflate. Two chair forms can be considered for the lactone derived from epimer **4**. These are conformer **6a**, in which the methine protons H_a and H_b are both equatorial, and **6b**, in which these protons are both axial. The corresponding bis-lactones derived from **5** are **7a**, in which H_a is equatorial and H_b is axial, and **7b**, in which H_a is axial and H_b is equatorial. The coupling constants J_{ab} between H_a and H_b observed in the ^{1}H NMR spectra of the lactones were 1.2 Hz (derived from the major Michael addition product) and 1.7 Hz (derived from the minor Michael addition product). This rules out conformer **6b**, in which H_a and H_b are both axial. Although the coupling constants of vicinal diequatorial protons in six-membered rings are generally slightly smaller than the corresponding axial−equatorial coupling

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(1) Singleton, V. L.; Bohonos, N.; Ullstrupp, A. J. *Nature* **1958**, *181*, 1072−1073.
(2) Weber, H. P.; Hauser, D.; Sigg, H. P. *Helv. Chim. Acta* **1971**, *54*, 2763−2766.
(3) Shao, R.-G.; Shimizu, T.; Pommier, Y. *Exptl. Cell Res.* **1996**, *227*, 190−196.
(4) Brüning, A.; Ishikawa, T.; Kneusel, E.; Matern, U.; Lottspeich, F.; Wieland, F. T. *J. Biol. Chem.* **1992**, *267*, 7726−7732.

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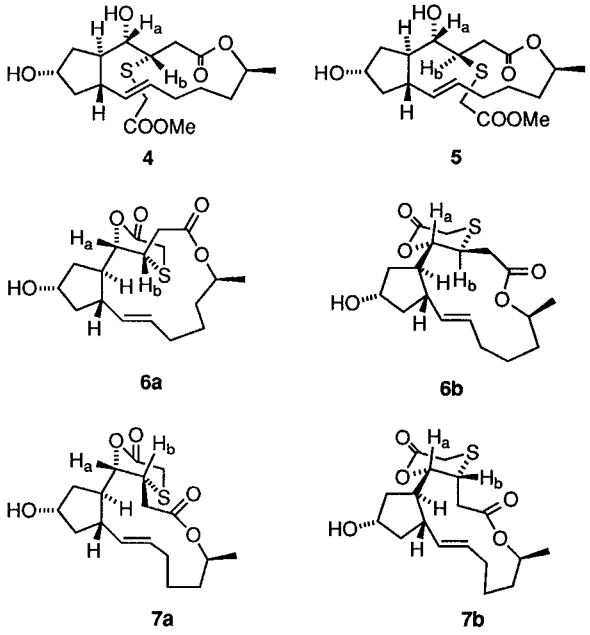
constants,[5−8] the very small difference in the J_{ab} coupling constants between the two diastereomeric lactones in the present case does not allow an unequivocal configurational assignment.

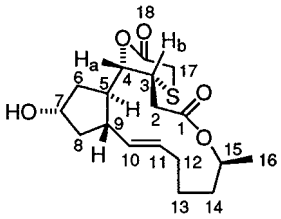


In lactone **6a**, carbons C-2 and C-5 are anti to one another, whereas in either lactone **7a** and **7b**, they are gauche. The C-2 and C-5 carbons would be expected to be shifted upfield in **7a** and **7b** relative to **6a** on the basis of the γ-gauche effect.[9,10] Complete carbon and proton assignments were made for both lactones on the basis of HMQC (heteronuclear multiple quantum coherence) and HMBC (heteronuclear multiple bond correlation) spectra (Tables 1 and 2).[11] The difference in the chemical shifts between the two bis-lactones at C-2 (δ 37.47 and δ 38.47 for the major and minor diastereomers) and at C-5 (δ 44.15 and δ 43.48 for the major and minor diastereomers) also did not allow firm stereochemical assignments to be made. The differences are certainly lower than the 5 ppm generally observed for a γ-gauche effect.[9,10]

The directing effect of adjacent hydroxyl groups on the stereochemistry of Michael additions to enones is well documented,[12] but cannot be relied on in the present case to predict the stereochemistry of the addition reaction because the hydroxyl group is near the plane of the double bond in question as indicated by the published X-ray structure of brefeldin A (Figure 1).[13] In this regard, it is interesting to note that the bis-TBDMS ether **8** was completely unreactive under the conditions used to prepare **4** and **5** from brefeldin A (**1**), which indicates that the hydroxyl group may be playing a role in the addition.

In view of the generally frustrating attempts to determine the structures of the addition products based on NMR analysis of conformationally restricted derivatives, considerable effort was directed toward obtaining a crystalline derivative that could be subjected to X-ray analysis. An acceptable quality crystalline derivative

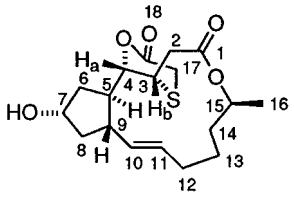
Table 1. Assignments of HMQC NMR Signals for the Bis-lactone Derived from the Major Diastereomer



position	^{13}C signal (δ ppm)	^{1}H signal (δ ppm)
1	168.97[a]	
2	37.47	2.93, 2.48
3	36.28	4.01
4	86.40	4.25
5	44.15	2.45
6	43.00	2.20, 1.51
7	72.15	4.30
8	40.79	2.15, 1.52
9	44.03	2.25
10	134.70	5.37
11	130.50	5.56
12	33.33	1.53, 1.35
13	30.93	1.59 (2 H)
14	24.93	1.54, 1.02
15	71.96	4.92
16	20.38	1.21 (3 H)
17	25.34	3.58, 3.29
18	167.52[a]	

[a] Assignment based on HMBC data.

Table 2. Assignments of HMQC NMR Signals for the Bis-lactone Derived from the Minor Diastereomer



position	^{13}C signal (δ ppm)	^{1}H signal (δ ppm)
1	168.97[a]	
2	38.25	2.87, 2.70
3	37.53	3.40
4	78.39	4.86
5	43.48	2.35
6	33.80	1.86, 1.59
7	72.25	4.36
8	32.49	1.67, 1.42
9	45.40	2.15
10	135.45	5.34
11	131.19	5.52
12	41.51	2.25, 1.40
13	29.47	2.00, 1.77
14	26.40	1.65, 1.15
15	73.05	4.82
16	19.62	1.23 (3 H)
17	26.38	3.51
18	168.37[a]	

[a] Assignment based on HMBC data.

(5) Jackman, L. M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*; 2nd ed.; Perhamon Press: Oxford, 1969; p 288.
(6) Pretsch, E.; Simon, W.; Seibl, J.; Clerc, T. *Tables of Spectral Data for Identification of Organic Compounds*; 2nd ed.; Springer-Verlag: Berlin, 1989; p H185.
(7) Harris, R. K. *Nuclear Magnetic Resonance Spectroscopy*; Longman Scientific and Technical: Essex, 1986; p 227.
(8) Dyke, S. F.; Floyd, A. J.; Sainsbury, M.; Theobald, R. S. *Organic Spectroscopy. An Introduction*; Penguin Books: Middlesex, 1971; p 136.
(9) Grant, D. M.; Cheney, B. V. *J. Am. Chem. Soc.* **1967**, *89*, 5315−5318.
(10) Dalling, D. K.; Grant, D. M. *J. Am. Chem. Soc.* **1967**, *89*, 6612−6622.
(11) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565−569.
(12) Hoveyda, A. H.; Evans, D. A.; Fu, G. C. *Chem. Rev. (Washington, D.C.)* **1993**, *93*, 1307−1370.
(13) Ryu, S.-E.; Truneh, A.; Sweet, R. W.; Hendrickson, W. A. *Structure* **1994**, *2*, 59−74.

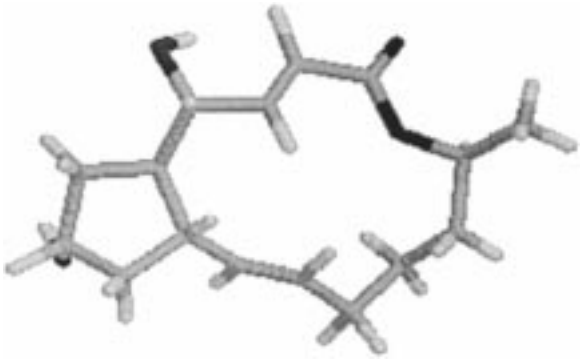
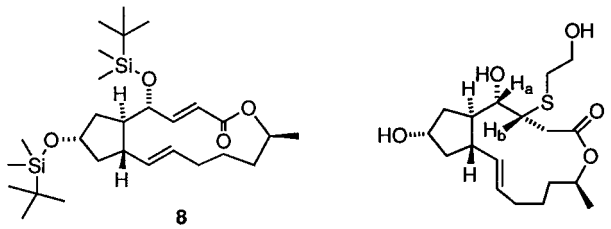


Figure 1. Structure of brefeldin A derived from the crystal coordinates.



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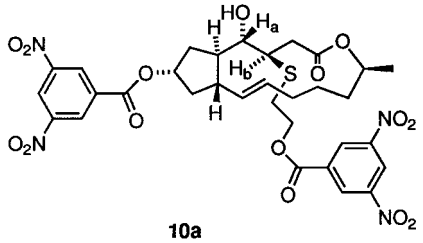
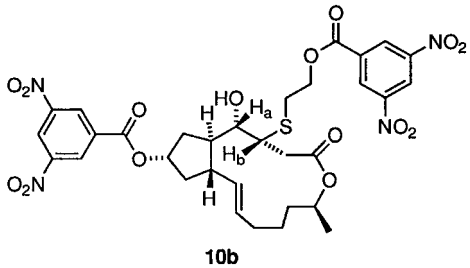
9

was eventually obtained by acylation of the major (β-hydroxyethyl)thiol adduct **9** with 3,5-dinitrobenzoyl chloride, which afforded a diacylation product. The product was crystallized in small plates using the solvent diffusion method, with the compound dissolved in ethyl acetate and with hexane diffusion into the solution. Crystallography of one of these small plates established the 3*R* configuration indicated in structure **10** for the product. Since the ^{1}H NMR spectrum of the major product **9** was similar to the major product derived from addition of methyl mercaptoacetate to brefeldin A, the major product in fact has structure **5**, and the minor product has structure **4**.

The ORTEP drawing (Figure 2) shows that drawing **10a** does not represent the solid state conformation of the product, since in **10a** C-2 and C-5 are anti, whereas in the ORTEP they are gauche. This can be seen more



10a



10b

readily in the stick stereodrawing of the crystal structure of the product (Figure 3). This places the C-3 substituent facing outward away from the macrocycle in an equatorial orientation, which is different from the solid state conformation of brefeldin A itself (Figure 1), in which C-2 and C-5 are anti. The conformation of the bis(dinitrobenzoate) product is more closely represented by structural drawing **10b**.

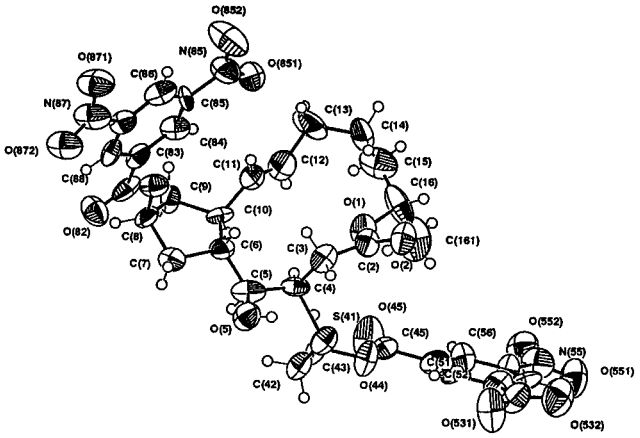


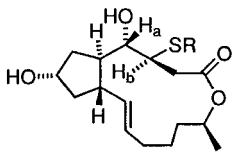
Figure 2. ORTEP plot of compound **10**.

Table 3. Michael Additions of Thiols to Brefeldin A[a]

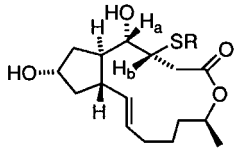
compds	yield (%)[b]	rxn time	ratio (*R*:*S*)[c]
4/**5**	94	2	33:1
9	93	2	
11/**12**	96	2	38:1
13/**14**	69	12	34:1
15/**16**	72	12	35:1

[a] Reactions were performed in 3:1 MeOH/H_2O at room temperature. [b] Isolated yield of the diastereomeric mixture. [c] Based on NMR integrations of the isolated mixture of diastereomers.

To investigate the scope of the reaction and determine the stereochemical outcome in a limited number of additional cases, three more thiols were reacted with brefeldin A, resulting in compounds **11**−**16** (Table 3). In all of these cases, the reaction was found to be highly diastereoselective, affording 3*R* to 3*S* ratios of at least 30:1. In these cases, the J_{ab} coupling constants of the minor diastereomers were approximately 8 Hz, whereas in the major diastereomers they were approximately 2 Hz. Based on these coupling constants and the X-ray data for **10**, the major and minor diastereomers can be assigned the macrocyclic conformations indicated for structures **11**−**16**. Structures **4** and **5**, which were originally drawn to emphasize correspondence with brefeldin A, do not represent the most stable conformations of the macrocycles accurately, and should be revised along the lines indicated for structures **11**−**16** in order to accomplish this. We are presently in the process of preparing a large number brefeldin thiol adducts as possible prodrug candidates and have consistently observed the same stereochemical preference in this larger array of compounds.



minor



major

11 R = $CH_3OOCCH_2CH_2$
13 R = *p*-$CH_3OC_6H_4$
15 R = *p*-HOC_6H_4

12 R = $CH_3OOCCH_2CH_2$
14 R = *p*-$CH_3OC_6H_4$
16 R = *p*-HOC_6H_4

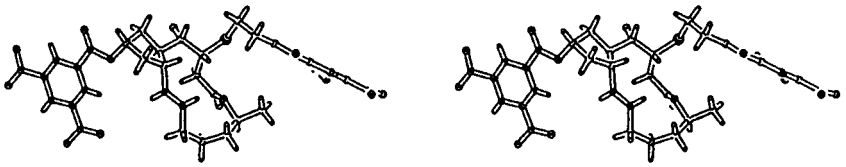


Figure 3. Stick stereodrawing (walleye) derived using the crystal coordinates for compound **10**.

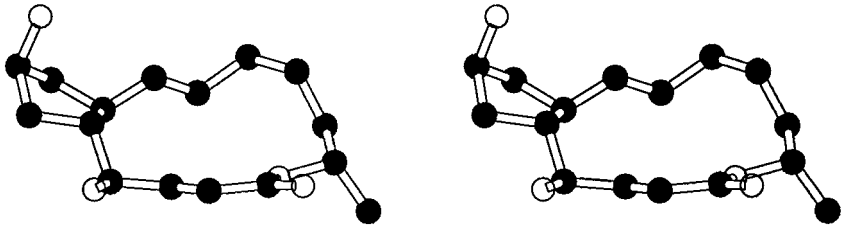


Figure 4. Stereodrawing (walleye) of the X-ray structure of brefeldin A viewed with the α,β-unsaturated carbonyl fragment "in front".

In retrospect, the direction of approach of the thiols to the α,β-unsaturated carbonyl system of brefeldin A might be understood on the basis of steric effects. When the crystal structure of brefeldin A is observed near the plane of the α,β-unsaturated carbonyl system with this moiety on the front side of the molecule (Figure 4), it can be seen that attack of the nucleophile from the "top" is hindered due to the fact that the remaining atoms of the macrocycle exist "above" the C-3 atom. In contrast, the "bottom" side of the reactive α,β-unsaturated carbonyl system appears to be much less sterically hindered, and the results presented here indicate that nucleophilic attack by the thiol occurs preferentially from this direction. This argument is also apparent when space filling models based on the crystal structure of brefeldin A are viewed.

In conclusion, the addition of thiols to brefeldin A has been found to be a highly diastereoselective reaction. On the basis of an X-ray structure of a crystalline dibenzyol derivative, the major diastereomers were assigned the 3*R* configuration.

Experimental Section

^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz instrument. HMQC, ^{1}H COSY, NOESY, and HMBC spectra were obtained on a 500 MHz spectrometer. 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. Methylene chloride and dimethylformamide were stored over 4 Å molecular sieves prior to use.

General Procedure for the Synthesis of the Michael Addition Products. 2,3-Dihydro-3(*R*)-[[(methoxycarbonyl)methyl]thio]brefeldin A (5) and 2,3-Dihydro-3(*S*)-[[(methoxycarbonyl)methyl]thio]brefeldin A (4). The experimental procedure for the reaction of (+)-brefeldin A (**1**) with methyl mercaptoacetate is representative. 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 two diastereoisomers, **5** and **4**, in 33:1 ratio and 94% total isolated yield. Adduct **5** was isolated as the major diastereoisomer in 91% yield as an oil: TLC R_f: 0.57 (10% EtOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 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($CDCl_3$) δ 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, 1280 cm^{-1}; CIMS (*m*/*z*): 387 (MH^+); HRMS: Calcd for $C_{19}H_{31}O_6S$: 387.1841. Found: 387.1832. Adduct **4**, the minor diastereoisomer, was isolated as an oil (5 mg, 2.8%): TLC R_f: 0.69 (10% EtOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.60 (m, 1 H), 5.33 (m, 1 H), 4.85 (m, 1 H), 4.32 (m, 1 H), 3.75 (s, 3 H), 3.57 (dd, 1 H, J = 7.5 and 8.2 Hz), 3.47 (dt, 1 H, J = 4.4 and 6.2 Hz), 3.44 (d, 1 H, J = 15.5 Hz), 3.30 (d, 1 H, J = 15.5 Hz), 2.74 (dd, 1 H, J = 2.8 and 3.0 Hz), 2.30−2.15 (dd and m, 4 H), 2.10−1.40 (m, 9 H), 1.20 (d, 3 H, J = 6.54 Hz); ^{13}C NMR ($CDCl_3$) δ 172.37, 170.78, 135.57, 131.47, 74.11, 72.30, 72.22, 52.77, 49.22, 47.91, 46.01, 42.35, 39.89, 36.12, 32.70, 31.74, 30.56, 25.27, 19.54; IR ($CHCl_3$): 3412, 1722, 1275 cm^{-1}; CIMS (*m*/*z*): 387 (MH^+).

All subsequently reported Michael addition reactions were also conducted on the same scale using 0.2 mmol of (+)-brefeldin A.

Preparation of Bis-lactone 7. A dry reaction flask equipped with a rubber septum and a magnetic stirring bar was charged with diastereoisomer **5** (26 mg, 0.067 mmol) and dry CH_2Cl_2 (5 mL) under N_2 at 0 °C. To this solution was added a drop of TMSOTf, and the mixture was stirred for 1 h at 0 °C and at room temperature for 2 h. The reaction progress was monitored by TLC. The reaction was quenched at 0 °C with an aqueous solution of $NaHCO_3$ (5 mL) and extracted with $CHCl_3$ (3 × 10 mL), the combined organic extract was dried over anhydrous $MgSO_4$, and the solvent was evaporated. The resulting residue was purified by silica gel column chromatography using $CHCl_3$ and then 1% EtOH/$CHCl_3$ solvent system to afford the expected product **7** (10 mg, 44%): TLC R_f: 0.59 (10% EtOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.56 (m, 1 H), 5.37 (m, 1 H), 4.92 (m, 1 H), 4.30 (m, 1 H), 4.25 (dd, 1 H, J = 1.2 and 10.3 Hz), 4.01 (bd, 1 H, J = 11.0 Hz), 3.58 (d, 1 H, J = 14.5 Hz), 3.29 (d, 1 H, J = 14.5 Hz), 2.93 (dd, 1 H, J = 1.9 and 16.4 Hz), 2.48 (dd, 1 H, J = 16.7 and

16.6 Hz), 2.40−1.90 (m, 5 H), 1.80−1.40 (m, 7 H), 1.21 (d, 3 H, J = 6.4 Hz), 1.02 (m, 1 H); ^{13}C NMR($CDCl_3$) δ 168.97, 167.52, 134.70, 130.50, 46.40, 72.15, 71.96, 44.15, 44.03, 43.00, 40.79, 37.47, 36.28, 33.33, 30.93, 25.34, 24.93, 20.38; IR ($CHCl_3$): 3407, 1692 cm^{-1}; CIMS (m/z): 355 (MH^+); HRMS: calcd for $C_{18}H_{27}O_5S$: 355.1579. Found: 355.1589.

Preparation of Bis-lactone 6. By making use of the above procedure, 3 mg of the bis-lactone **6** was prepared from diastereomer **4**: TLC R_f: 0.61 (10% EtOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.52 (m, 1 H), 5.34 (m, 1 H), 4.86 (dd, 1 H, J = 1.7 and 9.5 Hz), 4.82 (m, 1 H), 4.36 (m, 1 H), 3.51 (d, 1 H, J = 14.8 Hz), 3.40 (m, 1 H), 3.19 (d, 1 H, J = 14.5 Hz), 2.87 (dd, 1 H, J = 3.5 and 16.7 Hz), 2.70 (dd, 1 H, J = 5.7 and 16.5 Hz), 2.35 (m, 1 H), 2.40−2.15 (m, 2 H), 2.10−1.80 (m, 3 H), 1.80−1.40 (m, 6 H), 1.23 (d, 3 H, J = 6.1 Hz); ^{13}C NMR ($CDCl_3$) δ 168.97, 168.37, 135.45, 131.19, 78.39, 73.05, 72.25, 45.40, 43.48, 41.51, 38.25, 37.53, 33.80, 32.49, 29.47, 26.40, 26.38, 19.62; IR ($CHCl_3$): 3407, 1747, 1720 cm^{-1}; CIMS(m/z): 355 (MH^+); HRMS: Calcd for $C_{18}H_{27}O_5S$: 355.1579. Found: 355.1585.

Preparation of Brefeldin A 4,7-Bis-TBDMS Ether (8). A solution of **1** (0.112 g, 0.4 mmol), imidazole (0.136 g, 2 mmol), and TBDMSCl (0.241 g, 1.6 mmol) in dry DMF (2 mL) was stirred at room temperature under N_2 atmosphere for 24 h. The reaction was quenched with excess saturated aqueous $NaHCO_3$ solution and extracted with EtOAc (3 × 30 mL). The EtOAc extract was dried over anhydrous $MgSO_4$, and the solvent was removed. The resulting residue was flash chromatographed (silica gel, $CHCl_3$) to obtain the bis-TBDMS ether **8** as a colorless oil (0.150 g, 86%); TLC R_f: 0.62 ($CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.30 (dd, 1 H, J = 3.1 and 15.5 Hz), 5.85 (dd, 1 H, J = 1.8 and 15.5 Hz), 5.60 (m, 1 H), 5.25 (m, 1 H), 4.83 (m, 1 H), 4.19 (m, 1 H), 4.00 (ddd, 1 H, J = 1.9, 2.9 and 8.4 Hz), 2.20 (m, 1 H), 2.10−1.35 (m, 11 H), 1.23 (d, 3 H, J = 6 Hz), 1.90 (s, 18 H), 0.00 (s, 12 H); ^{13}C NMR ($CDCl_3$) δ 166.40, 152.49, 137.32, 129.32, 118.12, 76.41, 72.84, 71.37, 52.85, 43.87, 43.75, 42.12, 34.13, 31.86, 26.70, 25.86, 20.91, 18.05, −4.10, −4.77 and −4.87; IR ($CHCl_3$): 1715, 1472, 1256 cm^{-1}; CIMS(m/z): 509 (MH^+); HRMS: Calcd for $C_{28}H_{53}O_4Si_2$: 509.3482. Found: 509.3497.

2,3-Dihydro-3(*R*)-[(2′-hydroxyethyl)thio]brefeldin A (9). Reaction of **1** with 2-hydroxyethanethiol gave **9** as an oil (0.066 g, 93%): TLC R_f: 0.29 (10% EtOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 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 overlapping), 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 ($CDCl_3$) δ 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, 1043 cm^{-1}; HRMS: Calcd for $C_{18}H_{31}O_5S$: 359.1892. Found: 359.1999.

Bis-(3,5-dinitrobenzoate) Derivative 10. A flame-dried reaction flask was charged with the Michael addition product **9** (1.75 g, 4.88 mmol), pyridine (1.94 mL), and CH_2Cl_2 (100 mL). The reaction mixture was cooled to 0 °C, and to it was added 3,5-dinitrobenzoyl chloride (4.04 g, 17.56 mmol) in 5 min under N_2 atmosphere. The reaction mixture was then stirred at room temperature for 24 h and quenched with a saturated aqueous solution of $NaHCO_3$ (100 mL). The resulting mixture was stirred for 10 min and extracted with $CHCl_3$ (4 × 100 mL). The organic extract was washed with a saturated solution of $CuSO_4$ (2 × 100 mL) and brine (100 mL). The organic extract was dried over anhydrous $MgSO_4$, and solvent was removed under a reduced pressure. The resulting residue was purified by means of a flash column chromatography (silica gel, 1−2% EtOH/$CHCl_3$) to obtain the desired bis-(3,5-dinitrobenzoate) derivative **10** (1.07 g, 30%). It was recrystallized from EtOAc/*n*-hexanes using a solvent diffusion method: mp 110 °C; TLC R_f: 0.38 (5% EtOH/$CHCl_3$, silica gel); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.24 (m, 2 H), 9.18 (d, 2 H, J = 2.2 Hz), 9.14 (d, 2 H, J = 2.1 Hz), 5.69 (m, 1 H), 5.48 (m, 1 H), 5.37 (m, 1 H), 4.94 (m, 1 H), 4.74 (m, 1 H), 4.54 (m, 1 H), 3.78 (dd, 1 H, J = 1.7 and 8.3 Hz), 3.59 (dt, 1 H, J = 2.5 and 6.6 Hz), 3.00 (m, 2 H), 2.76 (dd, 1 H, J = 3.7 and 16.3 Hz), 2.55−1.95 (m, 8 H), 1.90−1.55 (m, 5 H), 1.27 (d, 3 H, J = 6.3 Hz); ^{13}C NMR ($CDCl_3$) δ 169.78, 162.38, 148.67, 134.77, 133.80, 133.39, 131.46, 129.50, 129.32, 122.63, 122.34, 78.49, 71.89, 65.05, 45.81, 45.55, 44.09, 40.02, 37.16, 35.12, 33.36, 31.15, 29.22, 24.86, 20.32; IR ($CHCl_3$): 3434, 2252, 1730, 1545, 1344, 1281, 1218, 1165 cm^{-1}.

2,3-Dihydro-3(*R*)-[[(2′-methoxycarbonyl)ethyl]thio]brefeldin A (12) and 2,3-Dihydro-3(*S*)-[[(2′-methoxycarbonyl)ethyl]thio]brefeldin A (11). Reaction of **1** with methyl mercaptopropionate gave two diastereoisomers, **12** and **11**, in 37:1 isolated ratio and 96% total isolated yield. The major diastereoisomer **12** was isolated as an oil (0.075 g, 94%): TLC R_f: 0.42 (10% EtOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 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 ($CDCl_3$) δ 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, 20.32; IR ($CHCl_3$): 3439, 1725, 1629, 1262 cm^{-1}; CIMS (m/z): 401 (MH^+); HRMS: Calcd for $C_{20}H_{32}O_6S$: 401.1998. Found: 401.1986. The minor diastereomer **11** was isolated as an oil (2 mg, 2.5%): TLC R_f: 0.47 (10% EtOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.53 (m, 1 H), 5.29 (m, 1 H), 4.88 (m, 1 H), 4.35 (m, 1 H), 3.75 (dd, 1 H, J = 7.8 and 8.4 Hz), 3.72 (s, 3 H), 3.27 (dt, 1 H, J = 3.6, 3.9 and 4.0 Hz), 3.00−2.80 (m, 2 H), 2.70 (d, 2 H, J = 6.6 Hz), 2.62 (m, 3 H), 2.25 (m, 2 H), 2.05−1.70 (m, 5 H), 1.60−1.30 (m, 4 H), 1.15 (d, 3 H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$) δ 180.36, 171.16, 135.48, 131.59, 73.30, 72.43, 72.09, 51.99, 49.40, 48.41, 45.86, 42.28, 40.69, 35.69, 34.42, 32.66, 30.59, 29.70, 25.98, 24.90 and 19.33; IR ($CHCl_3$): 3480, 1710, 1356, 1220 cm^{-1}; CIMS (m/z): 401 (MH^+); HRMS: Calcd for $C_{20}H_{32}O_6S$: 400.1920. Found: 400.1928.

2,3-Dihydro-3(*R*)-[(4′-methoxyphenyl)thio]brefeldin A (14) and 2,3-Dihydro-3(*S*)-[(4′-methoxyphenyl)thio]brefeldin A (13). Reaction of **1** with 4-mercaptoanisole gave two diastereoisomers **14** and **13** in 34:1 ratio and 69% total yield. The major diastereomer **14** was isolated as an oil (0.056 g, 67%): TLC R_f: 0.62 (10% EtOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 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 ($CDCl_3$) δ 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, 1488 cm^{-1}; MS (m/z): 420 (M^+); HRMS: Calcd for $C_{23}H_{32}O_5S$: 420.1970. Found: 420.1965. The minor diastereomer **13** was isolated as an oil (1.7 mg, 2%): TLC R_f: 0.71 (10% EtOH/$CHCl_3$); NMR ($CDCl_3$, 300 MHz) δ 7.45 (dd, 2 H, J = 2.9 and 8.6 Hz), 6.86 (dd, 2 H, J = 2.9 and 8.7 Hz), 5.52 (m, 1 H), 5.21 (m, 1 H), 4.88 (m, 1 H), 4.35 (m, 1 H), 3.90 (t, 1 H, J = 3.7 and 6.8 Hz), 3.79 (s, 3 H), 3.39 (dd, 1 H, J = 7.3 and 7.4 Hz), 2.64 (dd, 1 H, J = 1.9 and 4.1 Hz), 2.40−2.20 (dd and m, 4 H), 2.15−1.40 (m, 9 H), 1.20 (d, 3 H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$) δ 171.31, 159.87, 135.91, 135.20, 131.62, 114.76, 72.46, 71.96, 71.62, 55.35, 54.54, 48.68, 45.74, 42.01, 40.36, 35.00, 32.43, 30.37, 24.71, 19.08; IR ($CHCl_3$): 3421, 1722, 1593, 1494, 1243 cm^{-1}; CIMS (m/z): 420 (M^+); HRMS: Calcd for $C_{23}H_{32}O_5S$: 420.1970. Found: 420.1969.

2,3-Dihydro-3(*R*)-[(4′-hydroxyphenyl)thio]brefeldin A (16). Reaction of **1** with 4-mercaptophenol gave two diastereoisomers **16** and **15** in 35:1 ratio and 72% total yield. The major diastereomer **16** was isolated an oil (0.056 g, 70%): TLC R_f: 0.28 (10% EtOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 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); IR ($CHCl_3$): 3398, 1768, 1700, 1282, 1042 cm^{-1}; HRMS: Calcd for $C_{22}H_{30}O_5S$: 406.1814. Found: 406.1821.

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Supporting Information Available: ^1H and ^{13}C NMR spectra and X-ray data acquisition parameters for compound **10** (48 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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