salts of both D-(−)- and L-(+)-tartaric acids. Crystallizations of the L-(+)-tartrate salts gave (*S*)-8 as the principal component of the less soluble diastereomeric mixture, while crystallization of the D-(−)-tartrate salts produced (*R*)-8. Freshly distilled (±)-8 (8.6 g, 53 mmol) in 500 mL of MeOH was combined with 7.9 g (53 mmol) of L-(+)-tartaric acid in 500 mL of MeOH. After 2 days, the crystals which separated were collected and recrystallized from MeOH. The mother liquor from the original crystallization was evaporated to dryness, and the free base was regenerated from the residue. This material in 400 mL of MeOH was treated with an equivalent weight of D-(−)-tartaric acid in 400 mL of MeOH. After 2 days, the crystals which separated were collected and recrystallized from MeOH. Both enantiomeric salts were repeatedly recrystallized from MeOH to constant optical rotation to provide (*S*)-8 L-(+)-tartrate [5.8 g, 70%; colorless prisms, mp 224–226 °C; $[\alpha]^{24}_D$ + 9.764° (free base, $CHCl_3$, *c* 11.88)] and (*R*)-8 D-(−)-tartrate [6.2 g, 75%; colorless prisms, mp 224–226 °C; $[\alpha]^{24}_D$ −9.647° (free base, $CHCl_3$, *c* 13.17)].

X-ray Diffraction Study of the D-(−)-Tartrate Salt of (−)-2-Amino-4-methoxyindan (8). The compound crystallizes in the noncentrosymmetric, triclinic space group, *P1*, with one molecule in a unit cell having the following dimensions: a = 9.937 Å, b = 7.479 (2) Å, c = 5.399 (1) Å, α = 77.99 (2)°, β = 104.15 (2)°, and γ = 78.35 (2)°. The density calculated for $(C_{10}H_{14}NO)^+(C_4H_5O_6)^-$, Mr = 313.3, is 1.42 g cm^{-3}. The intensities of 1106 unique reflection were measured on a computer automated four-angle diffractometer using monochromatic copper radiation. The structure was solved by direct methods (SHELXTL) and refined by the least-squares method, with anisotropic temperature factors for all non-hydrogen atoms. The hydrogen atoms were all located from a difference *E* map but were included in the final refinement at calculated positions. Figure 1 and Tables I–V (supplementary material) show an ORTEP plot of the molecule and give atom coordinates, anisotropic temperature factors, bond lengths, bond angles, and hydrogen coordinates.

(*R*)- and (*S*)-2-(*Di-n*-propylamino)-4-methoxyindan Hydrochloride (9). The free bases of (+)- and (−)-8 were alkylated by a method of Marchini et al.[12] The appropriate free base (1.3 g, 7.98 mmol) in 5 mL of dry benzene was added to a previously prepared solution of 3.0 g (79.8 mmol) of $NaBH_4$ and 19.5 g (260 mmol) of propionic acid in 50 mL of benzene (dried over 4-Å molecular sieves), and the resulting mixture was heated under reflux under N_2 for 20 h. The cooled reaction mixture was then washed with two 100-mL portions of 2 N NaOH, and the organic layer was dried (Na_2SO_4) and evaporated to leave an almost colorless oil. Treatment of this with ethereal HCl deposited a solid which was recrystallized from 2-PrOH–Et_2O. (*R*)-9·HCl: yield, 1.99 g (88%); mp 189–190 °C (lit.[1] mp for racemic mixture 189–190 °C); $[\alpha]^{23}_D$ −14.423° (HCl salt, EtOH, *c* 10.40). (*S*)-9·HCl: yield, 2.0 g (90%); mp 189–190 °C (lit.[1] mp for racemic mixture 189–190 °C); $[\alpha]^{23}_D$ +14.422° (HCl salt, EtOH, *c* 9.99).

(*R*)- and (*S*)-2-(Di-*n*-propylamino)-4-hydroxyindan Hydrobromide (1). The appropriate enantiomeric HCl salt (3.0 g, 10.6 mmol) in 30 mL of 48% HBr was heated under N_2 at 125 °C for 3 h. The reaction mixture was evaporated under reduced pressure and H_2O was removed by repeated azeotroping with benzene. The solid residue was recrystallized from 2-PrOH and then from EtOH–Et_2O. (*S*)-1·HBr: yield, 3.06 g (92%); mp 225–226 °C (lit.[1] mp for racemic mixture 204–205 °C; $[\alpha]^{23}_D$ + 13.379° (HBr salt, EtOH, *c* 10.17). (*R*)-1·HBr: yield, 3.18 g (96%); mp 224–225 °C (lit.[1] mp of racemic mixture of 204–205 °C; $[\alpha]^{23}_D$ −13.353° (HBr salt, EtOH, *c* 10.34).

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Registry No. (*R*)-1, 94843-89-7; (*S*)-1, 94843-90-0; (*R*)-1·HBr, 94843-91-1; (*S*)-1·HBr, 94843-92-2; **2**, 119-84-6; **3**, 59725-59-6; **3** (acid chloride), 59725-62-1; **4**, 59725-61-0; **5**, 40731-98-4; **6**, 13336-31-7; **7**, 24623-28-7; (*R*)-**8**, 94903-37-4; (*S*)-**8**, 94903-38-5; (*R*)-8·D-(−)-tartrate, 94903-39-6; (*S*)-8·L-(+)-tartrate, 94903-40-9; (*R*)-9·HCl, 94859-22-0; (*S*)-9·HCl, 94859-23-1; (±)-11, 94843-93-3; benzoyl chloride, 98-88-4; propionic acid, 79-09-4.

Supplementary Material Available: Figure 1 showing an ORTEP plot of (*R*)-8 and Tables I–V listing atom coordinates and temperature factors, anisotropic temperature factor, bond lengths, bond angles, and hydrogen coordinates and temperature factors (4 pages). Ordering information is given on any current masthead page.

(12) Marchini, P.; Liso, G.; Reho, A.; Liberatore, F.; Moracci, F. M. *J. Org. Chem.* **1975**, *40*, 3453.

Synthesis and β-Lactamase Inhibitory Properties of 2β-[(Acyloxy)methyl]-2-methylpenam-3α-carboxylic Acid 1,1-Dioxides

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p-Nitrobenzyl 2β-[(benzoyloxy)methyl]-2α-methylpenam-3α-carboxylate was prepared by reaction of *p*-nitrobenzyl 2-[2-oxo-3α-bromo-4-(benzothiazol-2-yldithio)azetidin-1-yl]-2-isopropenylacetate with silver benzoate in the presence of iodine. The resulting diester was oxidized to the sulfone with potassium permanganate and hydrogen peroxide, and the bromine and *p*-nitrobenzyl groups were removed by hydrogenolysis to give potassium 2β-(benzoyloxy)methyl 2α-methylpenam-3α-carboxylate 1,1-dioxide. A series of related compounds, including the pivaloyl, methoxybenzoyl, *p*-fluorobenzoyl, and *p*-aminobenzoyl derivatives, were prepared in a similar way. All of these compounds were potent β-lactamase inhibitors in vitro against the TEM β-lactamase from *Klebsiella pneumoniae* A22695 and *Bacteroides fragiles* A22695 but less active against the β-lactamase from *Staphylococcus aureus* A9606. All compounds when administered orally in a 1:1 combination with amoxicillin did not show any significant protection of mice infected with *S. aureus* A9606. 2β-(Bromomethyl)-2α-methylpenam-3α-carboxylic acid was prepared and reacted with silver nitrate to give the nitrate ester. Oxidation with potassium permanganate and catalytic reduction afforded 2β-(hydroxymethyl)-2α-methylpenam-3α-carboxylic acid 1,1-dioxide. 2β-(Bromomethyl)-2α-methylpenam-3α-carboxylic acid 1,1-dioxide was found to be a strong β-lactamase inhibitor, while the 2β-hydroxymethyl compound showed only weak β-lactamase-inhibiting properties.

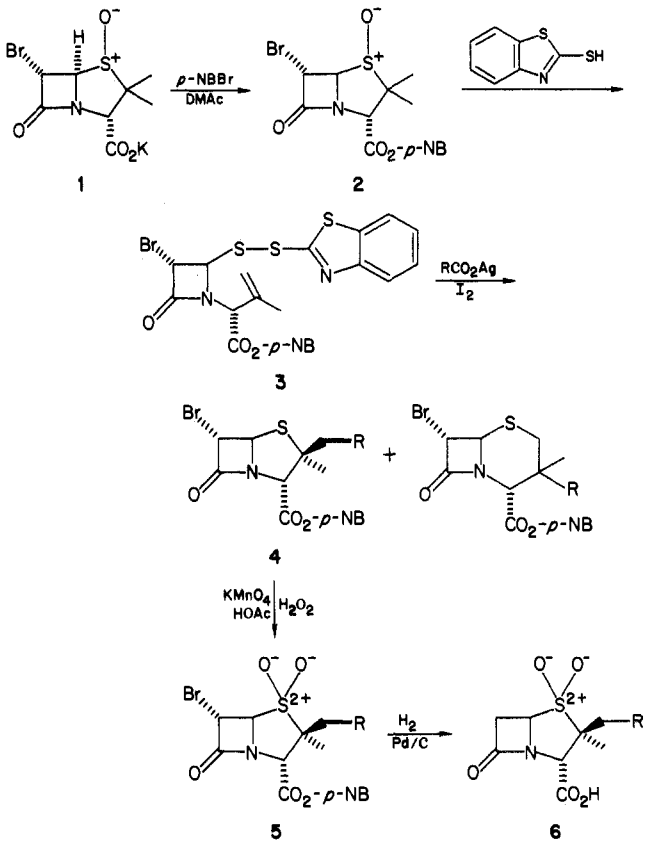
The discovery of the β-lactamase inhibitory properties of penicillanic acid sulfone (sulbactam[1]) has led us to the investigation of this activity in a number of other relatively simple semisynthetic derivatives of 6-aminopenicillanic acid.[2] Among the most active of these derivatives was 2β-(chloromethyl)-2α-methylpenam-3α-carboxylic acid

(1) Aswapokee, N.; Neu, H. C. *J. Antibiot.* **1978**, *31*, 1238.

(2) For leading references, see ref 3; Claverley, M. J.; Begtrup, M. J. *Antibiot.* **1983**, *26*, 1507. Arisawa, M.; Then, R. L. *J. Antibiot.* **1983**, *26*, 1372.

0022-2623/85/1828-0518$01.50/0

Scheme I[a]



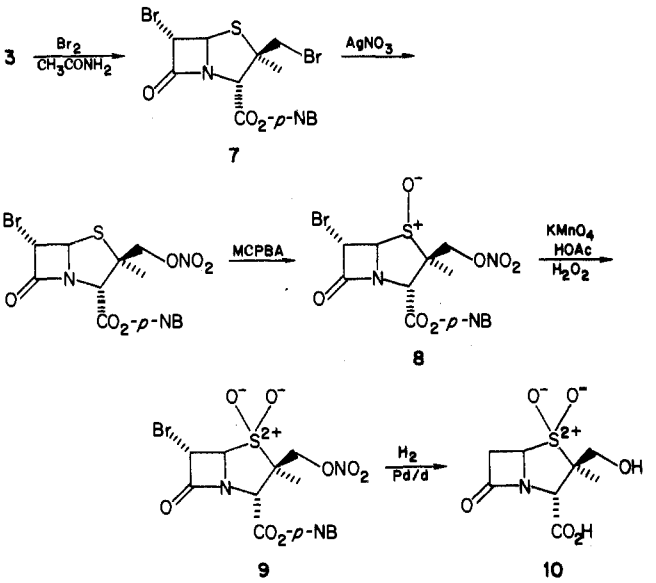
a, R = $C_6H_5C(O)O$; b, R = p-$CH_3OC_6H_4C(O)O$; c, R = p-$FC_6H_4C(O)O$; d, R = p-$H_2NC_6H_4C(O)O$; e, R = $(CH_3)_3CC(O)O$; f, R = Cl

[a] p-NB = $O_2NC_6H_4CH_2$.

1,1-dioxide.[3] We now report a series of new [(acyloxy)methyl]penam sulfones which exhibit high β-lactamase-inhibitory activity against a variety of bacterial β-lactamases and which are synergistic when used in combination with ampicillin or amoxicillin in vitro. Also tested for β-lactamase inhibitory properties were 2β-(bromomethyl)-2α-methylpenam-3α-carboxylic acid 1,1-dioxide and 2β-(hydroxymethyl)-2α-methylpenam-3α-carboxylic acid 1,1-dioxide. Although the bromo analogue possessed high β-lactamase-inhibiting properties, the corresponding hydroxy compound had much less activity.

Chemistry. 6α-Bromopenicillanic acid was prepared from 6β-aminopenicillanic acid by the method of Cignarella et al.[4] by substituting hydrobromic acid for hydrochloric acid. Conversion to the sulfoxide and p-nitrobenzoate ester has been reported in a previous communication.[3] Reaction of the β-sulfoxide with 2-mercaptobenzothiazole by the method of Kamiya[5,6] gave the disulfide **3**. Condensation of **3** with silver benzoate and iodine[7] (Simioni complex) gave a mixture of the penam **4a** and the corresponding cepham.[8] The penam was oxidized with potassium permanganate and hydrogen peroxide to the sulfone **5a**, which was purified by column chromatography. Removal of the p-nitrobenzyl blocking group and the bromine by reduction over 10% palladium on carbon afforded the ester **6a**. Other esters were prepared by using the appropriate silver salts (see Scheme I).

Scheme II



Scheme III

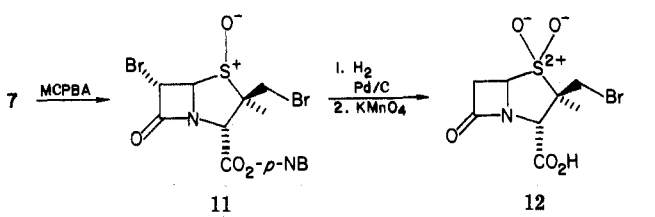


Table I. β-Lactamase Inhibitory Properties of Various Penam Sulfones against Selected β-Lactamases

compd	R	min protective concn (MPC),[a] μg/mL Kpn[b]	Sa[c]	Bf[d]
6a	$C_6H_5CO_2$	0.8	>100	6.25
6b	p-$CH_3OC_6H_4CO_2$	0.1	12.5	6.2
6c	p-$FC_6H_4CO_2$	0.2	50	3.1
6d	p-$NH_2C_6H_4CO_2$	0.2	25	1.6
6e	$(CH_3)_3CCO_2$	0.8	25	12.5
6f[e]	Cl	3.1	3.1	12.5
10	OH	6.2	>100	100
12	Br	1.6	3.1	12.5
sulbactam		1.6	12.5	12.5
clavulanic acid		0.1	0.4	63

[a] The MPC is the lowest concentration of the test compound required to protect 7-(phenylacetamido)-3-(2,4-dinitrostyryl)-3-cephem-4-carbocylic acid from β-lactamase-catalyzed hydrolysis under standard conditions (see ref 10). [b] TEM β-lactamase from *K. pneumoniae* A20634. [c] β-Lactamase from *S. aureus* A9606. [d] β-Lactamase from *B. fragilis* A22695. [e] Potassium 2β-(chloromethyl)-2α-methylpenam-3α-carboxylate 1,1-dioxide (BL-P2013).

As shown in Scheme II, 2β-(hydroxymethyl)-2α-penam-3α-carboxylate 1,1-dioxide (**10**) was prepared by reaction of the disulfide **3** with bromine in the presence of acetamide to form the 2β-(bromomethyl)penam **7**. Displacement of the bromine with silver nitrate gave the nitrate ester, which was oxidized with *m*-chloroperbenzoic acid to the sulfoxide **8**. The sulfoxide was prepared because efforts to remove the blocking groups from the sulfide were unsuccessful. The bromine, the p-nitrobenzyl group, and the nitro group were all removed simultaneously by hydrogenation with 10% palladium on charcoal to give com-

(3) Gottstein, W. J.; Crast, L. B., Jr.; Graham, R. G.; Haynes, U. J.; McGregor, D. N. *J. Med. Chem.* **1981**, *24*, 1531.
(4) Cignarella, G.; Pifferi, G.; Testa, E. *J. Org. Chem.* **1962**, *27*, 2668.
(5) Kamiya, T.; Tevaji, T.; Hashimoto, M.; Nakaguchi, O.; Oku, T. *Tetrahedron Lett.* **1973**, 300.
(6) Kamiya, T.; Tevaji, T.; Hashimoto, M.; Nakaguchi, O. U.S. Patent 3 954 732; *Chem. Abstr.* **1975**, *84*, 25687Q.
(7) Birkenbach, L.; Meisenheimer, K. *Chem. Ber.* **1936**, *69*, 723.
(8) Darby, N.; Wolfert, P. K. U.S. Patent 4 183 850; *Chem. Abstr.* **1980**, *92*, 163962 d.

Table II. Synergistic Combinations of Amoxicillin with 2-[(Acyloxy)methyl]penam Sulfones

compd	min inhibitory conc (MIC),[a] μg/mL			
	S. aureus A9606	*S. aureus* A15033	*E. coli* A20111	*E. coli* A20107
amoxicillin	32	32	>125	>125
6a	>125	>125	>125	>125
amoxicillin + **6a**[b]	4/4	8/8	16/16	125/125
6b	63	63	>125	>125
amoxicillin + **6b**	2/2	4/4	16/16	32/32
6c	>125	>125	>125	>125
amoxicillin + **6c**	2/2	2/2	8/8	125/125
6d	63	>125	>125	>125
amoxicillin + **6d**	2/2	2/2	16/16	125/125
6e	63	>125	>125	>125
amoxicillin + **6e**	2/2	2/2	8/8	63/63
clavulanic acid	32	63	63	63
amoxicillin + Clav	2/2	4/4	4/4	16/16
BL-P2013	>125	>125	>125	>125
amoxicillin + BL-P2013	4/4	8/8	4/4	16/16

[a] MIC values were obtained with use of an agar dilution method whereby organisms were deposited onto medicated agar plates with a Steers replication device; see ref 11. [b] Synergism was measured by determining the MIC for a 1:1 (w/w) mixture of the test compound with amoxicillin.

pound **10**. The preparation of compound **12** from **7** is shown in Scheme III.

Biology. The minimum protective concentration MPC) values for compounds **6a–f**, **10**, and **12** are reported in Table I and demonstrate the variation in inhibiting activity of these sulfones against the TEM, *Staphylococcus aureus*, and *Bacteroides fragilis* enzymes, using clavulanic acid, sulbactam, and compound **6f** as standards. The least active sulfone was compound **10**. Compounds **6f** and **12**, where R is a good leaving group, showed the best activity against all three organisms while all other 2-substituted esters, both aromatic and aliphatic, were uniformly weak against the *S. aureus* enzyme. Substituting the aromatic ring with a *p*-amino group did not improve the MPC against the *S. aureus* enzyme. The minimum inhibitory concentrations (MIC) of the (acyloxy)methyl sulfones **6a–e** in combination with amoxicillin were tested against two β-lactamase-producing strains of *S. aureus* and *E. coli* (Table II). The ability of these sulfones to protect amoxicillin from hydrolysis by the enzymes related well with the minimum protective concentrations reported in Table I. Orally administered combinations of compounds **6a–e** with amoxicillin (1:1, w/w) did not demonstrate significant protection of mice infected with *S. aureus* A9606.[9] Compounds **10** and **12** were not tested in mice.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The IR spectra were recorded on a Beckmann 5240 spectrophotometer using KBr pellets. NMR spectra were obtained on a Varian HA100 spectrophotometer using $(Me)_4Si$ as an internal standard.

***p*-Nitrobenzyl 2-[2-Oxo-3α-bromo-4-(benzothiazol-2-yldithio)azetidin-1-yl]-2-isopropenylacetate (3).** A solution of 10 g (0.023 mol) of *p*-nitrobenzyl 6α-bromopenicillanic acid sulfoxide[3] was heated under nitrogen at reflux in 200 mL of anhydrous dioxane with 4.2 g (0.025 mol) of 2-mercaptobenzothiazole for 3.5 h. Approximately 30 mL of dioxane was distilled off at the start of the reflux. The solution was diluted with water and extracted with EtOAc. The EtOAc was separated, washed with water, and dried over anhydrous $MgSO_4$. The solvent was evaporated at 30 °C (15 mm) to a crystalline solid. The solid was slurried with ether, collected, and air-dried to give 12.5 g (99%) of **3**: mp 108–109 °C; IR 1790 (s), 1740 (s), 1520 (s), 1350 (s), 1010 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3–8.4 (m, 8), 5.52 (d, 1), 5.32 (d, 1), 5.25 (s, 2), 2.25 (s, 1), 5.13 (s, 1), 5.03 (s, 1) 1.83 (s, 3). Anal. ($C_{22}H_{18}BrN_3O_5S_3$) C, H, N.

***p*-Nitrobenzyl 6α-Bromo-2β-[(benzoyloxy)methyl]-2α-methylpenam-3α-carboxylate 1,1-Dioxide (5a).** To a slurry of 3.7 g (0.016 mol) of silver benzoate (Aldrich) in 72 mL of benzene was added 2 g (0.008 mol) of iodine. A viscous slurry resulted. The mixture was stirred for 1 h, after which a solution of 2.3 g (0.004 mol) of the disulfide **3** in 80 mL of CH_2Cl_2 was added all at once. The mixture was stirred for 4 h at 23 °C and filtered through Celite. The filtrate was washed with a 10% aqueous solution of sodium thiosulfate until no I_2 color remained and finally with saturated brine solution. The solution was dried over anhydrous $MgSO_4$ and evaporated at 30 °C (15 mm) to yield a yellow solid which weighed 1 g (**4b**). The sulfide was dissolved in 30 mL of glacial acetic acid at 23 °C, and while the mixture was stirred, a saturated solution of potassium permanganate in H_2O was added dropwise until a pink coloration persisted for 5 min. The mixture was then treated dropwise with a 30% solution of hydrogen peroxide until the solution became colorless. The solution was diluted with 200 mL of water and a white solid was collected, washed with water, and dried in vacuum over P_2O_5 to give an amorphous solid, which was purified by silica gel column chromatography (SiliCAR CC-7) using 9:1 toluene–ethyl acetate as the eluant to give 200 mg of crystalline **5b**: mp 125 °C; IR 1780 (s), 1740 (m), 1540 (m), 1350 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.4–8.5 (m, 9), 5.42 (s, 2), 5.3 (d, 1), 4.8 (m, 2) 4.7 (d, 1), 1.6 (s, 3). Anal. ($C_{22}H_{19}BrN_2O_8S$) C, H, N.

***p*-Nitrobenzyl 6α-Bromo-2β-[[(4-methoxybenzoyl)oxy]methyl]-2α-methylpenam-3α-carboxylate 1,1-Dioxide (5b).** The procedure used for the preparation of **5a** was followed with 1.8 g (0.0069 mol) of silver 4-methoxybenzoate, 868 mg (0.0035 mol) of iodine, and 1 g (0.0017 mol) of disulfide **3** as starting material. The crystalline sulfone weighed 950 mg: mp 84 °C; IR 1815 (s), 1770 (m), 1720 (m), 1610 (s), 1525 (s), 1350 (s)8 1260 (s), 770 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.8–8.4 (m, 8), 5.35 (s, 2), 5.2 (d, 1), 4.4–5.0 (m, 4), 1.45 (s, 3). Anal. ($C_{23}H_{21}BrN_2O_{10}S\cdot 3H_2O$) H, N; C: Calcd, 42.40; found, 41.94.

***p*-Nitrobenzyl 6α-Bromo-2β-[[(4-fluorobenzoyl)oxy]methyl]-2α-methylpenam-3α-carboxylate 1,1-Dioxide (5c).** The procedure used for the preparation of **5a** was also used for the preparation of **5c** with 2.6 g (0.01 mol) of silver 4-fluorobenzoate, 1.3 g (0.005 mol) of iodine, and 1.5 g (0.0025 mol) of the disulfide **3** as starting material. A total of 320 mg of the sulfone **6d** was isolated: IR 1820 (s), 1735 (m), 1610 (m), 1530 (s), 1350 (s), 1270 (s), 770 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.0–8.5 (m, 8), 5.4 (s, 2), 5.25 (d, 1), 4.5–4.8 (m, 4), 1.4 (s, 3). Anal. ($C_{22}H_{19}FN_2O_9S\cdot {}^1/_2C_7H_8$ (toluene)) C, N; H: calcd, 5.10; found, 4.47.

***p*-Nitrobenzyl 6α-Bromo-2β-[[(4-nitrobenzoyl)oxy]methyl]-2α-methylpenam-3α-carboxylate 1,1-Dioxide (5d).** The procedure used for the preparation of **5d** with 5 g (0.018 mol) of silver *p*-nitrobenzoate, 5 g (0.011 mol) of the disulfide **3**, and 2.3 g (0.009 mol) of iodine as starting material. The crystalline sulfone was isolated from ethyl acetate and ether to yield 110 mg: mp 164–165 °C; IR 1795 (s), 1735 (s), 1550 (s), 1550 (s), 1350 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.5–8.5 (m, 8), 5.4 (s, 2), 5.26 (d, 11), 4.6–5.1 (m, 4), 1.5 (s, 3). Anal. ($C_{22}H_{18}BrN_3O_{11}S$) C, H, N.

***p*-Nitrobenzyl 6α-Bromo-2β-[(pivaloyloxy)methyl]-2α-methylpenam-3α-carboxylate 1,1-Dioxide (5e).** To a slurry of 2.1 g (0.01 mol) of silver trimethylacetate in 30 mL of toluene was added 1.3 g (0.005) of iodine. The slurry was stirred at 32 °C for 30 min and added to a solution of 1.5 g (0.0025 mol) of the disulfide **3** in 30 mL of CH_2Cl_2. The mixture was stirred at 32 °C for 4 h and filtered through Celite to remove the silver salt. The filtrate was washed with an aqueous solution of sodium

(9) Mice were treated orally with a 1:1 combination of amoxicillin and test compound at 0- and 2-h postchallenge. The experiments were terminated after 5 days and the PD_{50} was calculated by the method of Spearman and Karber "Statistical Methods in Biological Assay", 2nd ed.; Finney, D. J., Ed.; Hafner Publishing Co.: New York, 1964.

(10) 'Callaghan, C. H.; Morris, A.; Kirby, S. M.; Shingler, A. H. *Antimicrob. Agents Chemother.* **1972**, *1*, 283.

(11) Steers, E.; Foltz, E. L.; Graves, B. S. *Antibiot. Chemother.* **1959**, *9*, 307.

thiosulfate and saturated salt solution and finally dried over anhydrous magnesium sulfate. Evaporation gave 1.2 g of a crude sulfide, which was a mixture of two-thirds penam and one-third cepham. The solid was dissolved in 20 mL of glacial acetic acid and saturated aqueous $KMnO_4$ was added until a pink color persisted for 5 min. The solution was treated dropwise with 30% H_2O_2 until a colorless solution resulted. The solution was diluted with water and a white solid precipitated. The precipitate was removed by filtration and dried in vacuo over P_2O_5 to yield 1.2 g. This crude sulfone was flash chromatographed on silica gel G60 with ethyl acetate–toluene (10:90) to give 120 mg of crystalline penam **5e**: mp 155 °C dec; IR 1820 (s) 1770, 1740 (m), 1540 (m), 1355 (s), 1150, 1180 (s) cm^{-1}; ^1H NMR ($CDCl_3$) 7.4–8.5 (m, 4), 5.35 (s, 2), 5.25 (d, 1), 4.7 (d, 1), 4.6 (s, 1), 4.6–4.2 (m, 2), 1.4 (s, 3), 1.2 (s, 9). Anal. ($C_{20}H_{23}BrN_2O_9S$): C, H, N.

Potassium 2β-[(Benzoyloxy)methyl]-2α-methylpenam-3α-carboxylate 1,1-Dioxide (6a). To a suspension of 600 mg of 10% palladium on wide pore carbon (Engelhardt) in 50 mL of water was added 325 mg (0.0039 mol) of sodium bicarbonate. To this mixture was added a solution of 1.1 g (0.0019 mol) of the sulfone ester **5a** dissolved in 40 mL of ethyl acetate and the mixture was hydrogenated at 50 psi at 20 °C for 3.5 h. The catalyst was removed by filtration and the aqueous layer was separated and washed twice with 50 mL of ether. The solution was adjusted to pH 1.5 with concentrated HCl and extracted with EtOAc. The EtOAc was washed with water, dried over anhydrous $MgSO_4$, and concentrated at 30 °C (15 mm) to a volume of ca. 1–2 mL. After addition of 20 mL of Skellysolve B, the solvent was decanted and the residue was dissolved in 5 mL of acetone and treated with solid potassium 2-ethylhexanoate to pH 6 (wet pH paper). The solid was removed by filtration, washed several times with acetone, and dried for 15 h in vacuo over P_2O_5 to yield 380 mg of a colorless solid: mp 145 °C dec; IR 3400 (m), 1770 (s), 1720 (s), 1630 (s), 1390 (m), 1280 (s), 1148 (s), 710 (s) cm^{-1}; ^1H NMR (D_2O) δ 7.4–8.2 (m, 5), 5.1 (m, 1), 4.9 (m, 2), 4.5 (s, 1), 3.6 (m, 2), 1.7 (s, 3). Anal. ($C_{15}H_{14}KNO_7S \cdot H_2O$). C, N; H: calcd, 5.10; found, 4.47.

Potassium 2β-[[(4-Methoxybenzoyl)oxy]methyl]-2α-methylpenam-3α-carboxylate 1,1-Dioxide (6b). The procedure used for the preparation of **6a** was followed with use of 900 mg (0.001 mol) of sulfone **5c**. A white solid was collected, which weighed 200 mg: mp 162 °C dec; IR 3420 (m), 1780 (s), 1710 (s) 1610 (s), 1390 (m), 1280 (s), 1175 (s), 770 (m) cm^{-1}; ^1H NMR (D_2O) δ 6.8–8.1 (m, 4), 4.9–5.2 (m, 1), 4.6–5.0 (m, 2), 4.45 (s, 1), 3.85 (s, 3), 3.2–3.8 (m, 2), 1.65 (s, 3). Anal. ($C_{16}H_{17}NO_8S \cdot H_2O$) C, H, N.

Potassium 2β-[[(4-Fluorobenzoyl)oxy]methyl]-2α-methylpenam-3α-carboxylate 1,1-Dioxide (6c). The procedure used for the preparation of **6a** was followed with 1.2 g (0.002 mol) of the sulfone **5d** as starting material. The colorless solid obtained weighed 150 mg: mp 168 °C; IR 3420 (m), 1770 (m), 1625 (s), 1325 (s), 1275 (s), 760 (m) cm^{-1}; ^1H NMR (D_2O) δ 7.8–8.2 (m, 2), 7.0–7.4 (m, 2), 5.0 (m, 1), 4.6–5.0 (m, 2), 4.4 (s, 1), 3.2–3.9 (m, 2), 3.2–3.9 (m, 2), 1.6 (s, 3). Anal. ($C_{15}H_{13}FKNO_7S \cdot H_2O$) H, N; C: calcd, 42.14; found, 42.97.

Potassium 2β-[[(4-Aminobenzoyl)oxy]methyl]-2α-methylpenam-3α-carboxylate 1,1-Dioxide (6d). A solution of 1.5 g (0.0023 mol) of the ester dissolved in 50 mL of EtOAc was added to a suspension of 600 mg of 10% Pd/C and 400 mg (0.0046 mol) of $NaHCO_3$ in 50 mL of H_2O. The mixture was hydrogenated at 50 psi for 3 h. The catalyst was removed by filtration and the water layer was separated and washed twice with 50 mL of ether and was lyophilized to afford a light yellow solid, which weighed 800 mg: mp 120 °C slow dec; IR 1780 (s), 1720 (m), 1620 (s), 1600 (s), 1280 (s) cm^{-1}; ^1H NMR (D_2O) δ 1.7 (s, 3), 3.2–3.9 (m, 2), 4.4 (s, 1), 4.6 (s, 1) 5.0 (m, 2), 6.8–7.8 (m, 4).

Potassium 2β-[(Pivaloyloxy)methyl]-2α-methylpenam-3α-carboxylate 1,1-Dioxide (6e). The procedure for the preparation of **6a** was used with 1.3 g (0.0023 mol) of the sulfone **5d** as starting material. A colorless solid was isolated, which weighed 310 mg: mp 168 °C; IR 1780 (s), 1725 (m), 1625 (s), 1320 (s), 1140 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.8–8.1 (m, 4), 4.9–5.2 (m, 1), 4.6–5.0 (m, 2), 4.45 (s, 1), 3.85 (s, 3), 3.2–3.8 (m, 2), 1.65 (s, 3). Anal. ($C_{13}H_{18}KNO_7S$) C, N, H.

***p*-Nitrobenzyl 6α-Bromo-2β-(bromomethyl)-2α-methylpenam-3α-carboxylate (7).** A total of 2 g (0.0035 mol) of disulfide **3** was dissolved in 50 mL of THF and cooled in an ice bath to 5 °C. A total of 206 mg of acetamide was added followed by 552 mg of bromine. The bromine decolorized immediately. The solution was stirred for 1 h at 23 °C and poured into 500 mL of water. The aqueous suspension was extracted with ethyl acetate. The organic layer was separated, dried over anhydrous $MgSO_4$, and evaporated to yield 1.13 g of crystals: mp 123–124 °C; IR 1790 (s), 1740 (s), 1520 (s), 1350 (s), 1010 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.5 (s, 3), 3.3–3.8 (m, 2), 4.8 (d, 1), 5.2 (s, 1), 5.3 (s, 2), 5.5 (d, 1), 7.8–8.4 (m, 4). Anal. ($C_{15}H_{16}Br_2N_2O_5S$) C, H, N.

***p*-Nitrobenzyl 6α-Bromo-2β-(nitrooxymethyl)-2α-methylpenam-3-carboxylate 1-Oxide (8).** To a solution of 17 g (0.038 mol) of the disulfide **3** and 2.3 g (0.039 mol) of acetamide in 250 mL of THF was added 6.3 g (0.039 mol) of the bromine in 20 mL of CCl_4. The solution was stirred at 5 °C for 0.5 h, poured into 1 L of H_2O, and extracted with EtOAc. The EtOAc was dried over anhydrous $MgSO_4$ and evaporated to a light yellow solid. The solid was slurried with 150 mL of acetone and filtered to recover 2 g of unreacted disulfide. A total of 4 g of $AgNO_3$ was added in 50 mL of CH_3CN and the mixture was stirred at 25 °C for 15 h. The AgBr was collected by centrifugation and the solvent was evaporated under reduced pressure (32 °C) to a light yellow residue. The solid was dissolved in 50 mL of Me_2Cl_2 at 5 °C and treated with 8.4 g (0.05 mol) of *m*-chloroperoxybenzoic acid. The solution was stirred for 2 h and the Me_2Cl_2 was evaporated to an oil, which was triturated with ether to give 7 g of crude sulfoxide **8**. The crude product, which contained mainly the penam and cepham sulfoxides, was purified by flash chromatography using a solvent system of CH_2Cl_2–acetone (100:1). The fast running material was the penam. A total of 1.02 g of crystalline sulfoxide was isolated: mp 138–140 °C; IR 1805 (s), 1765 (s), 1650 (s), 1520 (s), 1350 (s), 1280 (s), 850 (m) cm^{-1}; ^1H NMR (Me_2SO) δ 7.6–8.4 (m, 4), 5.7 (d, 1), 5.25–5.6 (m, 2), 5.2 (d, 1), 5.1 (s, 1), 4.8–5.2 (m, 2), 1.2 (s, 3). Anal. ($C_{15}H_{13}BrN_3O_9S$) C, H, N.

***p*-Nitrobenzyl 6α-Bromo-2β-(nitrooxymethyl)-2α-methylpenam-3α-carboxylate 1,1-Dioxide (9).** A solution of 2.5 g (0.005 mol) of sulfoxide **8** was dissolved in 200 mL of glacial acetic acid and treated with a saturated aqueous solution of $KMnO_4$ until a pink coloration persisted for 3 min. The mixture was treated dropwise with a 30% solution of H_2O_2 until the solution became colorless. The solution was poured into 1 L of H_2O and the solid was collected by filtration and dried over P_2O_5 to yield 2.5 g: mp 155–156 °C; IR 1810 (s), 1750 (s), 1650 (s), 1520 (s), 1350 (s), 1340 (s), 1140 (s), 1280 cm^{-1}; ^1H NMR (Me_2SO) δ 7.6–8.4 (m, 4), 5.75 (s, 2), 5.4 (s, 2), 5.3 (s, 1), 4.8–5.3 (m, 2), 1.5 (s, 3). Anal. ($C_{15}H_{14}BrN_3O_{10}S$) C, H, N.

Potassium 2β-(Hydroxymethyl)-2α-methylpenam-3α-carboxylate 1,1-Dioxide (10). A solution of 800 mg (0.0016 mol) of *p*-nitrobenzyl 6α-bromo-2β-(nitrooxymethyl)-2α-methylpenam-3α-carboxylate 1,1-dioxide in 25 mL of EtOAc was added to a suspension of 800 mg of 10% Pd on wide-pore carbon (Engelhardt) and 200 mg of $NaHCO_3$ in 20 mL of water. The mixture was hydrogenated at 50 psi for 1 h. The catalyst was collected by filtration and the aqueous solution was extracted twice with EtOAc. The solution was adjusted to pH 1.6 with concentrated HCl and was extracted with EtOAc, washed with H_2O, dried over anhydrous $MgSO_4$, and evaporated at 32 °C (15 mm) to a gummy residue. The residue was dissolved in 20 mL of acetone and treated with solid potassium 2-ethylhexanoate. The precipitate was collected by centrifugation, washed twice with acetone, and dried in vacuo over P_2O_5 to yield 43.8 mg of the hygroscopic sulfone **10**: mp >120 °C slow dec; ^1H NMR (D_2O) δ 4.95 (m, 1), 4.25 (s, 1), 3.9–4.3 (m, 2), 3.2–3.8 (m, 2), 1.55 (s, 3).

***p*-Nitrobenzyl 6α-Bromo-2β-(bromomethyl)-2α-methylpenam-3α-carboxylate 1-Oxide (11).** To a solution of 552 mg (0.0011 mol) of the sulfide **7** in 50 mL of CH_2Cl_2 at 23 °C was added 240 mg of *m*-chloroperoxybenzoic acid. The solution was stirred for 1.5 h and evaporated at 30 °C (15 mm) to a gummy residue, which was triturated with ether to give 270 mg of sulfoxide **11**: IR 1800 (s), 1760 (s), 1522 (s), 1350 (s), 1055 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.4 (s, 2), 5.0–5.2 (m, 2), 4.7 (s, 1), 3.7–4.2 (m, 2), 1.4 (s, 3). Anal. ($C_{15}H_{15}Br_2N_2O_6S$) C, H, N.

Potassium 2β-(Bromomethyl)-2α-methylpenam-3α-carboxylate 1,1-Dioxide (12). A solution of 2.7 g (0.0062 mol) of sulfoxide **11** in 75 mL of EtOAc was added to a suspension of 1 g of 30% Pd on Celite and 560 mg of $NaHCO_3$ in 100 mL of H_2O. The mixture was hydrogenated at 50 psi (25 °C) for 3 h. The mixture was filtered to remove the catalyst and the filtrate

was treated with a saturated solution of $KMnO_4$ until a pink coloration persisted for 3–5 min. The mixture was adjusted to pH with $NaHCO_3$ to coagulate the MnO_2. The mixture was then filtered and the filtrate was adjusted to pH 5 with concentrated HCl. The solution was evaporated to a volume of 25 mL (32 °C (15 mm)) and adjusted to pH 1.5 with concentrated HCl. The sulfone was extracted with ether, dried, dried over anhydrous $MgSO_4$, and evaporated to an oil, which was solidified by stirring with Skellysolve B. The solid was then dissolved in 15 mL of acetone and treated with solid potassium 2-ethylhexanoate. The white crystals were collected and weighed 142 mg after air-drying: mp >140 °C slow dec; IR 2960 (s), 1795 (s), 1615 (s), 1310 (m), 1140 (s) cm^{-1}; ^1H NMR (D_2O) δ 5.05 (m, 1), 4.4 (s, 1), 3.9–4.3 (m, 2), 3.2–4.0 (m, 2), 3.67 (s, 3). Anal. ($C_8H_9BrKNO_5S$) C, H, N.

Dicesium *N*-Succinimidyl 3-(Undecahydro-*closo*-dodecaboranyldithio)propionate, a Novel Heterobifunctional Boronating Agent

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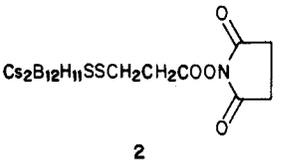
The synthesis of a novel heterobifunctional agent, dicesium *N*-succinimidyl 3-(undecahydro-*closo*-dodecaboranyldithio)propionate, is described. This structure contains an active ester component known to react rapidly under very mild conditions with amino groups of proteins, resulting in covalent linkage. With use of this boronating agent, approximately 480 boron atoms have been incorporated per molecule of a polyclonal antibody directed against human thymocytes and 1300 boron atoms per molecule were incorporated into a monoclonal antibody, 17-1A, directed against human colorectal carcinoma cells. Binding of the boronated antibodies to the corresponding target cells was demonstrated by means of membrane immunofluorescence. There was some loss in reactivity, as determined by fluorescent end point titers, but specificity remained unchanged. The data suggest that boronated antibodies potentially could be used to selectively deliver boron-10 to tumor cells in order to achieve their destruction by neutron capture.

Since Locher[1] first proposed the use of boron-10 for neutron capture therapy (BNCT) in 1936, the key limitation of this therapeutic approach has stemmed from an inability to deliver a sufficient concentration of the neutron absorber specifically and uniformly throughout the tumor.[2] The development of hybridoma technology[3] has offered the possibility of selectively delivering the capture agent to tumors by linking boron-10-containing compounds to monoclonal antibodies directed against tumor associated antigens. Toward this end, a number of protein-binding boron compounds have been synthesized and covalently incorporated into proteins.[4-11] In this study, we have utilized an established protein-linking heterobifunctional reagent,[12] *N*-succinimidyl 3-(2-pyridyldithio)propionate, **1**, as the coupler and have synthesized a polyhedral borane analogue containing an active ester function that would permit covalent incorporation of this boronating agent. The compound synthesized, **2**, is dicesium *N*-succinimidyl 3-(undecahydro-*closo*-dodecaboranyldithio)propionate.[13]



2

Reactions of structure **2** (abbreviated as SBDP) with both monoclonal and polyclonal antibodies were undertaken to evaluate the boronating potential of this agent. The reactions were carried out under very mild conditions, by allowing the reagent to react with the antibody in PBS (phosphate buffered saline, pH 7.2) for 1 h at ambient temperature. Following overnight storage at 4 °C, the boronated antibody was separated from excess reagent and byproducts by sequential passage through Sephadex G-25 columns (Pharmacia Fine Chemicals, Piscataway, NJ). The purified, conjugated antibody was analyzed for boron content by prompt-γ analysis[14] and for protein concentration by Bio-Rad Protein Assay (Bio-Rad Laboratories, Richmond, CA). With use of these data, the number of

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