Bleomycin Analogs. Synthesis and Proton NMR Spectral Assignments of Thiazole Amides Related to Bleomycin A_2 (1)

James M. Riordan and Ted T. Sakai (2)

Comprehensive Cancer Center and the Department of Biochemistry,
University of Alabama in Birmingham, Birmingham, Alabama 35294

Received June 29, 1981

The synthesis of a series of 2-substituted thiazole-4-carboxamides of 3-aminopropyldimethylsulfonium chloride is described and the proton nmr spectral assignments of these derivatives are made. The compounds, which are fragments and analogs of the DNA-binding portion of the antitumor antibiotic bleomycin A_2, will serve as probes of structure-activity relationships in this family of drugs.

J. Heterocyclic Chem., **18**, 1213 (1981).

The bleomycins, a group of antitumor antibiotics (Figure 1), cause the degradation of DNA in a process which requires iron(II) and dioxygen (3,4). This degradation is thought to be responsible, at least in part, for the biological activity of these compounds. We have previously demonstrated (5) that the DNA-binding portion of bleomycin A_2 (the most common congener) resides almost entirely in the terminal "dipeptide" (**1**) comprised of the bithiazole moiety and the cationic amine. This portion interacts minimally with the rest of the drug molecule (5,6), in particular, the residues responsible for binding the metal ion cofactor (Figure 1). In this paper, we

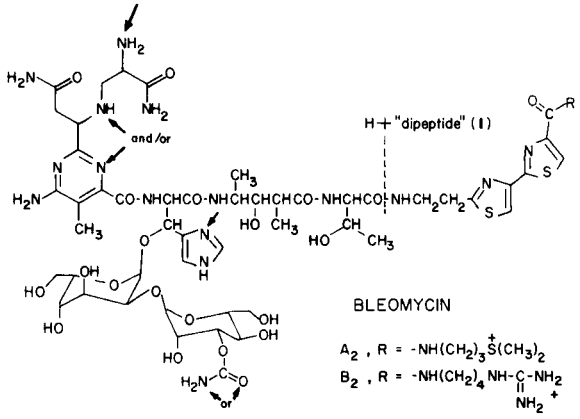
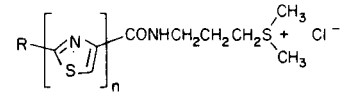


Figure 1. Structure of the bleomycins and the terminal "dipeptide" (**1**). Arrows indicate some of the ligands involved in binding the metals ions.

describe the preparation of a series of derivatives of the DNA-binding region of bleomycin A_2 and report the proton nmr spectral assignments for these compounds.

These derivatives contain 3-aminopropyldimethylsulfonium chloride as the cationic terminus and have varying numbers of thiazole rings. They include, in addition to the acetyldipeptide described previously (5), 2,4'-bithiazole analogs structurally resembling the dipeptide, a 2,4':2',4"-terthiazole derivative, and two monothiazole



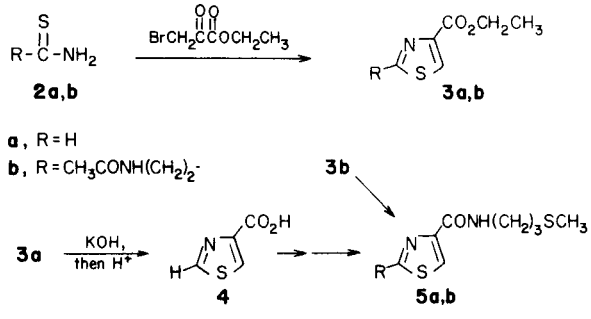
Compound No.	n	R
17a	1	H-
17b	1	$CH_3CONHCH_2CH_2$-
18a	2	H-
18b	2	$CH_3CONHCH_2CH_2$-
18c	2	CH_3-
18d	2	H_2N-
19	2	$CH_3CONHCH_2CH_2CH_2CH_2$-
20	3	H-

Figure 2. Structures of synthetic thiazole derivatives related to bleomycin A_2.

analogs. The structures of the derivatives are shown in Figure 2. The analytical and spectral data for the derivatives synthesized are summarized in Tables 1-3.

Monothiazole derivatives were prepared by extension of literature methods and by use of materials employed in the synthesis of the bithiazole system (5) (Scheme 1). Reaction of thioformamide (**2a**), prepared by the method of Kurkjy and Brown (7), with ethyl bromopyruvate gave the monothiazole ethyl ester (**3a**) in 55% yield (8). Alkaline

Scheme I



0022-152X/81/061213-09$02.25

Table I
Analytical Data

Compound No.	% Yield(a)	Recrystallization Solvent	M.p.(b)	Formula	Calcd. % C	Calcd. % H	Calcd. % N	Found % C	Found % H	Found % N
3b	65	ethyl acetate-petroleum ether	81-83°	$C_{10}H_{14}N_2O_3S$	49.57	5.82	11.56	49.49	5.84	11.51
5a	82	distilled	65-67°	$C_8H_{12}N_2OS_2$	44.42	5.59	12.95	44.43	5.60	12.91
5b	61	——	oil	$C_{12}H_{19}N_2O_2S_2$	47.82	6.35	13.94	48.00	6.39	13.86
7a	67	ethyl acetate-petroleum ether	180-181°	$C_8H_6N_2O_2S_2$	42.47	2.67	12.38	42.33	2.72	12.29
7c	83	ethanol	155-156.5°	$C_9H_8N_2O_2S_2$	44.99	3.36	11.66	44.98	3.38	11.66
7d	80	ethanol-water	209-210°	$C_8H_7N_3O_2S_2$	39.82	2.92	17.42	39.88	2.95	17.38
7e	77	ethyl acetate	180.5-181.5°	$C_{20}H_{17}N_3O_4S_2$	56.19	4.01	9.83	56.14	4.01	9.79
7f	95	ethanol	115-117°	$C_{17}H_{14}N_2O_4S_2$	54.53	3.77	7.48	54.55	3.81	7.45
8a	67	dichloromethane-petroleum ether	81-82°	$C_{11}H_{13}N_3OS_3 \cdot 0.5H_2O$	42.84	4.58	13.62	42.87	4.58	13.59
8c	82	ethanol	101-102°	$C_{12}H_{15}N_3OS_3$	45.98	4.82	13.41	46.08	4.82	13.42
8d	77	ethyl acetate-petroleum ether	159.5-161°	$C_{11}H_{14}N_4OS_3$	42.02	4.49	17.82	42.11	4.51	17.77
9	81	ethanol	216-217°	$C_{19}H_{15}N_3O_4S_2$	55.19	3.66	10.16	55.11	3.67	10.11
10	97	chloroform-petroleum ether	118-120°	$C_{23}H_{24}N_4O_3S_3$	55.18	4.83	11.19	55.12	4.88	11.15
11	57	ethyl acetate-petroleum ether	126.5-128°	$C_{17}H_{24}N_4O_2S_3$	49.49	5.86	13.58	49.62	5.86	13.55
12	97	ethanol-water	152-153°	$C_{10}H_{10}N_2O_3S_2$	44.43	3.73	10.36	44.49	3.75	10.35
13	82	ethyl acetate	218.5-219.5°	$C_{10}H_8N_2O_3S_2$	44.76	3.01	10.44	44.80	3.01	10.41
16	90	ethanol	126.5-128.5°	$C_{14}H_{14}N_4OS_4$	43.96	3.69	14.65	44.15	3.77	14.57
17a	40	(c)	(d)	$C_9H_{15}ClN_2OS_2 \cdot 2.5H_2O$	34.66	6.46	8.98	34.65	6.49	8.96
17b	71	(c)	(d)	$C_{13}H_{22}ClN_3O_2S_2 \cdot 2H_2O$	40.25	6.76	10.83	40.25	6.79	10.77
18a	69	(c)	(d)	$C_{12}H_{16}ClN_3OS_3 \cdot H_2O$	39.17	4.93	11.42	39.14	4.98	11.40
18c	54	(c)	(d)	$C_{13}H_{18}ClN_3OS_3 \cdot H_2O$	40.88	5.28	11.00	40.86	5.29	11.01
18d	86	(c)	(d)	$C_{12}H_{17}ClN_4OS_3 \cdot 3.5H_2O$	33.68	5.65	13.09	33.88	5.70	13.01
19	70	(c)	(d)	$C_{18}H_{27}ClN_4O_2S_3 \cdot 1.5H_2O$	44.11	6.17	11.43	44.06	6.17	11.34
20	72	(c)	(d)	$C_{15}H_{17}ClN_4OS_4 \cdot 2H_2O$	38.41	4.51	11.94	38.44	4.51	11.93

(a) Values are overall yields. (b) Values are for analytical samples recrystallized from the listed solvent. (c) Sulfonium salts were obtained as amorphous solids after lyophilization. (d) Melting points were not determined for sulfonium salts because of their hygroscopic nature.

Table 2
Infrared Spectral Data (a)

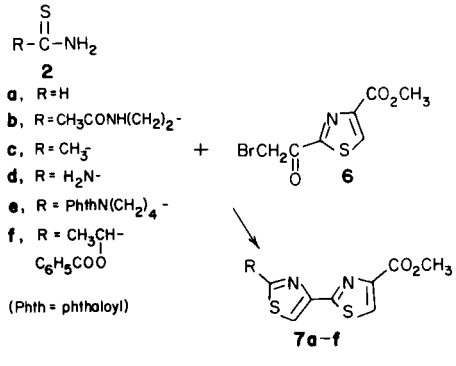
Compound No.	Absorption bands, cm^{-1}
3b	3350(NH), 1720(C=O), 1665(amide I), 1540(amide II)
5a	3270(NH), 1650(amide I), 1540(amide II)
5b	(neat) 3300(NH), 1665, 1650(amide I), 1560, 1545(amide II)
7a	(KBr) 1740(C=O)
7c	(KBr) 1725(C=O)
7d	3420, 3300(NH), 1710(C=O), 1640(amide I), 1555(amide II)
7e	1770(imide C=O), 1720(ester, imide C=O)
7f	1740, 1730(ester C=O)
8a	3250(NH), 1655(amide I), 1550(amide II)
8c	3325(NH), 1665(amide I), 1540(amide II)
8d	3390, 3320(NH), 1660, 1630(amide I), 1540, 1525(amide II)
9	1770, 1720(imide C=O), 1695(acid C=O)
10	3450(NH), 1770, 1720(imide C=O), 1645(amide I), 1555(amide II)
11	3300(NH), 1658, 1645(amide I), 1565, 1540(amide II)
12	3290(OH), 1725(C=O)
13	1725(ester C=O), 1690(ketone C=O)
15	1725(ester C=O)
16	3325(NH), 1670(amide I), 1540(amide II)

(a) Except where noted, spectra were obtained as Nujol mulls on sodium chloride plates.

hydrolysis of **3a** gave the acid **4** which, after conversion to the acid chloride, was reacted with 3-(methylthio)propylamine to provide the amide **5a** in 82% yield.

Reaction of 3-acetamidothiopropionamide (**2b**) (5) with ethyl bromopyruvate gave a 65% yield of the 2-substituted ester **3b**. This material could be converted in 61% yield to the amide **5b** by direct aminolysis with 3-(methylthio)-propylamine.

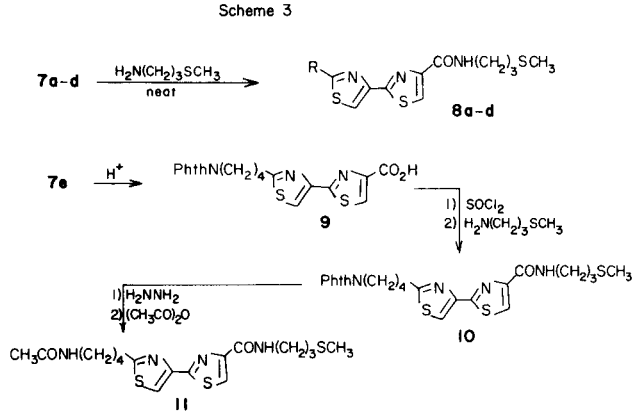
Scheme 2



The 2,4'-bithiazole derivatives were prepared by procedures analogous to those described previously (5) for the acetyldipeptide (**18b**) (Scheme 2). The common starting material was methyl 2-(2-bromoacetyl)thiazole-4-carboxylate (**6**) which was reacted with the desired thioamide (**2a-f**) to give the corresponding bithiazole ester (**7a-f**). Thus, the 2'-unsubstituted-, 2'-methyl and 2'-amino-derivatives (**7a,c** and **d**) were prepared by using thioformamide, thioacetamide and thiourea, respectively. For these derivatives, direct amidation of the ester gave the desired amide (**8a**, and **d**).

Thioamide **2e** reacted with **6** in 77% yield to give the methyl ester of the phthalimidobutyl-substituted bithiazole (**7e**). The conversion of this derivative to the acetamido derivative required a more circuitous route than described for the other derivatives (Scheme 3). Acid hydrolysis of **7e** in aqueous 1,2-dimethoxyethane removed the ester function in 81% yield and the resulting acid (**9**) was converted to the corresponding amide **10** in 97% yield *via* the acid chloride which was not isolated. Hydrazinolysis of **10** specifically removed the phthaloyl group and acetylation with acetic anhydride containing a catalytic amount of pyridine gave amide **11** in 57% yield.

Scheme 3



Formation of the 2,4':2,4"-terthiazole system involved extension of the scheme used to prepare the bithiazole derivatives (Scheme 4). The bromoketone **6** was reacted with *O*-benzoylthiolactamide (**2f**) (which had also been used in the preparation of **6** (5)), to give methyl 2'-[1-(benzoyloxy)ethyl]-2,4'-bithiazole-4-carboxylate (**7f**) in 95% yield. This product was transesterified with sodium methoxide in methanol to the 2'-[1-(hydroxy)]ethyl derivative **12** in 97% yield. Oxidation of **12** with activated manganese dioxide gave the ketone **13** (82%) and bromination of **13** with pyridinium bromide perbromide gave a 9:1 mixture of the bromoketone **14** and unreacted **13**. This mixture was sufficiently pure for use in the next reaction. Compound **14** was reacted with thioformamide (**2a**) to provide the terthiazole methyl ester **15** in 93% yield. This compound could be converted to amide **16** in 90% yield by treatment with 3-(methylthio)propylamine.

All of the thioether derivatives except **8d** could be methylated to the respective sulfonium iodide derivatives using iodomethane in methanol at room temperature (Scheme 5). The amino bithiazole derivative **8d** showed a tendency to methylate on the 3'-nitrogen under the same conditions. Attempts to protect the ring by trifluoroacetyl-

Table 2
Infrared Spectral Data (a)

Compound No.	Absorption bands, cm^{-1}
3b	3350 (NH), 1720 (C=O), 1665 (amide I), 1540 (amide II)
5a	3270 (NH), 1650 (amide I), 1540 (amide II)
5b	(neat) 3300 (NH), 1665, 1650 (amide I), 1560, 1545 (amide II)
7a	(KBr) 1740 (C=O)
7c	(KBr) 1725 (C=O)
7d	3420, 3300 (NH), 1710 (C=O), 1640 (amide I), 1555 (amide II)
7e	1770 (imide C=O), 1720 (ester, imide C=O)
7f	1740, 1730 (ester C=O)
8a	3250 (NH), 1655 (amide I), 1550 (amide II)
8c	3325 (NH), 1665 (amide I), 1540 (amide II)
8d	3390, 3320 (NH), 1660, 1630 (amide I), 1540, 1525 (amide II)
9	1770, 1720 (Imide C=O), 1695 (acid C=O)
10	3450 (NH), 1770, 1720 (imide C=O), 1645 (amide I), 1555 (amide II)
11	3300 (NH), 1658, 1645 (amide I), 1565, 1540 (amide II)
12	3290 (OH), 1725 (C=O)
13	1725 (ester C=O), 1690 (ketone C=O)
15	1725 (ester C=O)
16	3325 (NH), 1670 (amide I), 1540 (amide II)

(a) Except where noted, spectra were obtained as Nujol mulls on sodium chloride plates.

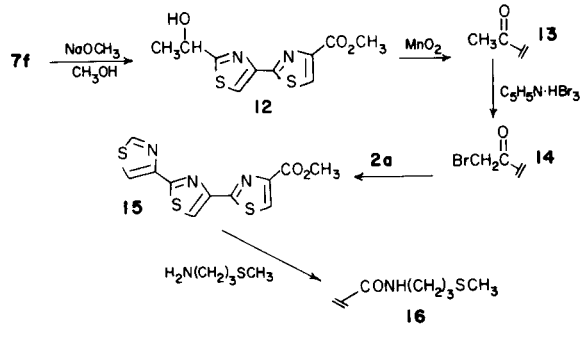
ation of the amino group were unsuccessful as the blocking group could be removed by dissolution of the compound in methanol. However, preferential methylation of the alkyl sulfur atom could be achieved by carrying out the methylation at $-15°$ for 6 days.

Because of the tendency of the iodide salts to discolor, they were converted to the chloride derivatives by passage of their aqueous solutions through columns of Dowex 1X8 (chloride form). All of the chloride salts gave combustion analyses consistent with their existence as hydrates.

The assignments of the resonances of the 2′-substituents and the 4-carboxyl substituents were made by inspection and comparison with unsubstituted derivatives and by the use of straightforward decoupling techniques. The assignments arrived at are summarized in Table 3. Characteristically, all of the (methylthio)propylamide derivatives showed very similar chemical shifts for the hydrogens in the 4-carboxyl side chain, as did all the sulfonium compounds. The acetamido side chains showed a similar tendency.

The aromatic resonances were readily assigned for the monothiazole derivatives. For the 2-substituted analogs, the assignments are trivial. Comparison then with the unsubstituted monothiazole shows the C_2H resonance to be

Scheme 4



the lower field resonance of the two aromatic resonances, with a four-bond coupling constant between the two of 2.2 Hz.

The coupled $C_2'H$ and $C_5'H$ resonances of the bithiazole derivatives **7a**, **8a** and **18a** could be assigned in a similar manner, again assigning the lower field resonance showing coupling as being that of the hydrogen located between the ring nitrogen and sulfur atoms. This leaves the remaining aromatic resonance as the C_5H. The resonances of the third (″) ring of the terthiazole system could be made analogously.

Table 3

Proton NMR Spectral Data

Compound No.	Solvent	Chemical Shift, ppm (a)
3a	deuterochloroform	8.95(d,H-2,$J_{2,5}$=2.5Hz,1H), 8.27(d,H-5,1H), 4.36(q,CH_2,2H), 1.43(t,CH_3,3H).
3b	"	8.08(s,H-5,1H), 6.22(broad,NH,1H), 4.42(q,-OCH_2,2H), 3.71(q,NCH_2,2H),3.26(t,CH_2,2H), 1.98(s,CH_3CO, 3H), 1.43(t,CH_3,3H).
4	deuterodimethylsulfoxide	9.15(d,H-2,$J_{2,5}$=1.5Hz,1H), 8.51(d,H-5,1H).
5a	deuterochloroform	8.75(d,H-2,$J_{2,5}$=2.2Hz,1H),8.17(d,H-5,1H), 7.53(broad,NH,1H), 3.58(q,NCH_2,2H), 2.60(t,SCH_2,2H),2.12(s,CH_3,3H),1.96(m,internal CH_2,2H).
5b	"	7.99(s,H-5,1H), 7.48(broad,propylene NH,1H),5.84(broad,ethylene NH,1H), 3.71(q,eth.NCH_2,2H), 3.57(q,prop.NCH_2,2H), 3.21(t,eth.CH_2,2H), 2.61(t,SCH_2,2H), 2.12(s,SCH_3,3H), 1.99(s, CH_3CO, 3H), 1.94(m, prop.internal CH_2,2H).
7a	"	8.87(d,H-2',$J_{2',5'}$=1.95Hz,1H),8.24(d,H-5',1H),8.22(s,H-5,1H), 3.99(s,-OCH_3,3H).
7b(b)	"	8.20(s,H-5,1H), 8.06(s,H-5',1H), 6.23(s,NH,1H), 3.98(s,-OCH_3,3H), 3.74(m,NCH_2,2H), 3.24(t,CH_2,2H), 2.01(s,CH_3CO,3H).
7c	"	8.18(s,H-5,1H),8.00(s,H-5',1H),3.98(s,-OCH_3,3H), 2.77(s,CH_3,3H).
7d	"	8.15(s,H-5,1H),7.42(s,H-5',1H), 4.03(s,NH_2,2H), 3.95(s,-OCH_3,3H).
7e	"	8.16(s,H-5,1H),8.00(s,H-5',1H),7.77(m,phthaloyl CH,4H), 3.97(s,-OCH_3,3H), 3.76(t,NCH_2,2H), 3.09(t,2'-α-CH_2,2H), 1.86(m,internal CH_2CH_2,4H).
7f	"	8.20(s,H-5,1H),8.15(s,H-5',1H),8.12(m,phenyl o-CH,2H), 7.62(m,phenyl p-CH,1H), 7.49(m,phenyl m-CH,2H), 6.45(q,CH,1H), 3.98(s,-OCH_3,3H),1.88(d,CH_3,3H).
8a	"	8.88(d,H-2',$J_{2',5'}$=1.95Hz,1H),8.13(s,H-5,1H), 8.04(d,H-5',1H), 7.55(broad,NH,1H),3.59(q,NCH_2,2H), 2.61(t,SCH_2,2H), 2.13(s,SCH_3,3H), 1.97(m,internal CH_2,2H).
8b(b)	"	8.10(s,H-5,1H), 7.87(s,H-5',1H), 7.53(broad,prop.NH,1H),6.23(broad,eth.NH,1H), 3.75(m,eth.NCH_2,2H),3.59(m,prop.NCH_2,2H), 3.25(t,eth.CH_2,2H), 2.61(t,SCH_2,2H), 2.13(s,SCH_3,3H), 2.00(s,CH_3CO,3H),1.96(m,prop.internal CH_2,2H).
8c	"	8.09(s,H-5,1H), 7.81(s,H-5',1H), 7.54(broad,NH,1H), 3.58(q,NCH_2,2H), 2.78(s,CH_3,3H),2.61(t,SCH_2,2H), 2.13(s,SCH_3,3H), 1.96(m,prop.internal CH_2,2H).
8d	"	8.06(s,H-5,1H),7.53(broad,NH,1H), 7.25(s,H-5',1H), 5.07(s,NH_2,2H), 3.57(q,NCH_2,2H), 2.61(t,SCH_2,2H), 2.13(s,SCH_3,3H), 1.95(m,prop.internal CH_2,2H).
9	deuterodimethylsulfoxide	8.45(s,H-5,1H),8.18(s,H-5',1H), 7.85(m,phthaloyl CH,4H), 3.64(m,NCH_2,2H),3.08(t,2'-α-CH_2,2H),1.75(m,internal CH_2CH_2,4H).
10	deuterochloroform	8.08(s,H-5,1H),7.82(s,H-5',1H),7.55(t,NH,1H),3.76(t,phthalimido-NCH_2,2H), 3.58(q,NCH_2,2H), 3.11(t,2'-α-CH_2,2H), 2.61(t,SCH_2,2H), 2.13(s,SCH_3,3H), 1.96,1.88(m,internal CH_2,CH_2CH_2,6H).
11	"	8.10(s,H-5,1H),7.84(s,H-5',1H),7.55(broad,prop.NH,1H),5.58(broad,acetyl NH,1H),3.59(q,prop.NCH_2,2H),3.32(q,butylene NCH_2,2H), 3.09(t,2'-α-CH_2,2H), 2.62(t,SCH_2,2H), 2.13(s,SCH_3,3H), 1.99,1.96(s,m,CH_3CO,prop.internal CH_2,5H), 1.89(m,2'-β-CH_2,2H), 1.66(m,2'-γ-CH_2,2H).

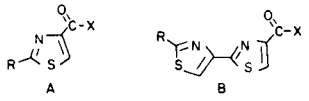
Table 3 (continued)

Compound No.	Solvent	Chemical Shift, ppm (a)
12	deuterochloroform	8.20(s,H-5,1H),8.12(s,H-5',1H),5.20(q,CH,1H), 3.98(s,-OCH_3,3H), 2.80(broad, OH,1H),1.70(s,CH_3,3H).
13	"	8.46(s,H-5,1H), 8.26(s,H-5',1H), 4.00(s,-OCH_3, 3H),2.78(s,CH_3CO,3H).
14	"	8.55(s,H-5,1H), 8.27(s,H-5',1H),4.78(s,$BrCH_2$,2H), 4.00(s,-OCH_3,3H).
15	"	8.89(d,H-2", $J_{2",5"}$=1.95Hz,1H), 8.23(s,H-5,1H),8.19(s,H-5',1H), 8.16(d,H-5",1H),4.00(s,-OCH_3, 3H).
16	"	8.89(d,H-2", $J_{2",5"}$=1.85Hz,1H), 8.18(d,H-5",1H); 8.14(s,H-5,1H), 8.01(s,H-5',1H), 7.58(t, NH,1H), 3.61(q,NCH_2,2H), 2.63(t,SCH_2,2H), 2.14(s,SCH_3,3H), 1.98(m, internal CH_2,2H).
17a	deuterium oxide	9.04(d,H-2, $J_{2,5}$=1.95 Hz,1H),8.28(d,H-5,1H),3.60(t,NCH_2,2H), 3.39(t,SCH_2,2H),2.92(s,$S(CH_3)_2$,6H),2.17(m,internal CH_2,2H).
17b	"	8.14(s,H-5,1H), 3.61,3.60(2t, prop.,eth.NCH_2,4H), 3.41(t, SCH_2, 2H), 3.25(t,2-α-CH_2,2H), 2.92(s,$S(CH_3)_2$,6H), 2.17(m,prop.internal CH_2,2H), 1.95(s,CH_3CO,3H).
18a	"	9.13(d,H-2', $J_{2',5'}$=1.95Hz,1H), 8.30(d,H-5',1H), 8.28(s,H-5,1H),3.64(t,NCH_2,2H), 3.42(t,SCH_2,2H), 2.94(s,$S(CH_3)_2$,6H), 2.20(m,internal CH_2,2H).
18b (b)	"	8.23(s,H-5,1H), 8.10(s,H-5',1H),3.61(t,eth.NCH_2,2H),3.60(t,prop.NCH_2,2H), 3.39(t,SCH_2, 2H), 3.27(t,2'-α-CH_2,2H), 2.92(s,$S(CH_3)_2$,6H), 2.18(m,prop.internal CH_2,2H), 1.95(s,CH_3CO,3H).
18c	"	8.23(s,H-5,1H), 8.04(s,H-5',1H), 3.63(t,NCH_2,2H), 3.41(t,SCH_2,2H),2.93(s,$S(CH_3)_2$,6H),2.77(s,CH_3,3H), 2.20(m,internal CH_2,2H).
18d	"	8.14(s,H-5,1H), 7.34(s,H-5',1H),3.61(t,NCH_2,2H),3.41(t,SCH_2,2H),2.94(s,$S(CH_3)_2$,6H), 2.19(m,internal CH_2,2H).
19	"	8.25(s,H-5,1H),8.10(s,H-5',1H),3.63(t,prop.NCH_2,2H), 3.41(t,SCH_2,2H),3.21(t,butylene NCH_2,2H), 3.12(t,2'-α-CH_2,2H), 2.93(s,$S(CH_3)_2$,6H), 2.20(m,prop.internal CH_2, 2H),1.97(s,CH_3CO,3H),1.86(m,2'-β-CH_2,2H),1.62(m,2'-γ-CH_2,2H).
20	"	9.07(d,H-2", $J_{2",5"}$=1.95Hz,1H), 8.27(d,H-5",1H), 8.21(s,H-5,1H), 8.17(s,H-5',1H), 3.61(t,NCH_2,2H), 3.42(t,SCH_2,2H), 2.94(s,$S(CH_3)_2$,6H), 2.20(m,internal CH_2,2H).

(a) Chemical shifts are referenced to internal tetramethylsilane for organic solvents and internal sodium 4,4-dimethyl-2,2,3,3-tetradeutero-4-silapentanoate for aqueous solutions. (b) Data for compounds **7b**, **8b**, and **18b** were taken from reference 5.

Table 4

Chemical Shift Behavior of Thiazole Derivatives



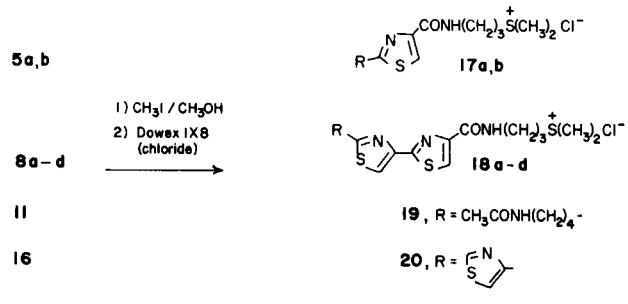
A B

Structure (a)	R	δ(amide) − δ(ester)		δ(salt) − δ(amide)	
		C_5H	$C_5'H$	C_5H	$C_5'H$
A	H-	-0.10	—	+0.12	—
A	$CH_3CONH(CH_2)_2$-	-0.10	—	+0.14	—
B	H	-0.09	-0.20	+0.17	+0.24
B	H_2N-	-0.09	-0.17	+0.08	+0.09
B	$CH_3CONH(CH_2)_2$-	-0.09	-0.19	+0.13	+0.23
B	CH_3-	-0.09	-0.19	+0.14	+0.23
B	$PhthN(CH_2)_4$-	-0.09	-0.18	—	—
B	$CH_3CONH(CH_2)_4$-	—	—	+0.13	+0.28
B		-0.09	-0.18	+0.07	+0.16

(a) Ester, X = -OCH_3, -OCH_2CH_3; amide, X = -$NH(CH_2)_3SCH_3$; salt, X = -$NH(CH_2)_3\overset{+}{S}(CH_3)_2Cl^-$

Assignment of the aromatic resonances of the 2′-amino derivatives takes advantage of the fact that this substitution allows the ready exchange with solvent of $C_5'H$ of the sulfonium derivative **18d** leaving only the C_5H resonance. The assignments of the aromatic resonances of the other aminobithiazole derivatives can be made on the assumption that the C_5H and $C_5'H$ resonances maintain their relative positions in the respective spectra.

Scheme 5



The assignment of the aromatic resonance of bithiazole ring systems in which the 2′-position is substituted (including the terthiazole derivatives) is somewhat more tenuous. However, we have noticed consistent trends in the behavior of these resonances as the structures are changed from the respective esters to the (methylthio)-propylamides to the sulfonium salts. We have used as controls the 2′-unsubstituted and 2′-aminobithiazole derivatives and the monothiazole derivatives, the resonances of which can be assigned unambiguously. In both the mono- and bithiazole systems, the C_5H resonances show upfield shifts of about 0.10 ppm in going from the ester to the amide and downfield shifts of the order of 0.15 ppm in going from the amide to the sulfonium salt (Table 4). The shifts of the $C_5'H$ resonances of the two control bithiazoles are about 0.20 ppm upfield as the structure is changed from the ester to the amide; however, the shifts seen in going to the sulfonium salt are not uniform, although the trend is downfield.

By using these observations, the chemical shift behavior of the other derivatives may be analyzed (Table 4). There is clearly one aromatic resonance in each basic bithiazole structure which moves upfield by about 0.10 ppm and one resonance which moves upfield by about 0.20 ppm in going from the ester to the amide derivative. The former resonances generally move back downfield by about 0.15 ppm upon conversion to the sulfonium salt; the latter set moves back downfield by more than 0.22 ppm. On the basis of these trends and by comparison with the control derivatives, we have assigned the resonance which moves less in each instance to C_5H and the one which moves more to $C_5'H$.

It is clear that the more consistent trends occur when the basic compound in question is changed from the ester to the amide rather than when the amide is converted to the sulfonium salt. The inconsistency apparent in the latter case may reflect the fact that chemical shifts in chloroform (amides) are being compared to chemical shifts in water (sulfonium salts) and that solvent effects are present, whereas comparisons of esters and amides are in the same solvent. We have assumed to some extent that the behavior of the $C_5'H$ resonance of the 2-amino derivatives presents a special case because of the probable existence of tautomeric imino structures which would be expected to affect this resonance and that the behavior of $C_5'H$ resonances is more likely to be exemplified by the unsubstituted control. In the case of the terthiazoles, we have used the consistent shift behavior of the resonances in going from the ester to the amide to make the assignments. This assumption results in the assignment of the $C_5'H$ resonance as that one which moves more in going from the amide to salt, in spite of the relatively small changes seen compared to the other derivatives.

It is noted that the relative positions of the aromatic resonances (*i.e.*, higher or lower field) do not necessarily have a direct relationship to whether they are the C_5H or $C_5'H$ resonances, although in most instances the C_5H resonance occurs to lower field. Also, it appears that the $C_5'H$ resonances of the bithiazole systems are more sensitive to changes in the 4-carboxyl group than are the closer C_5H resonances as indicated by the larger shifts experienced by the former. In this regard, the apparently less consistent behavior of the $C_5'H$ resonances may also reflect sensitivity of this position to electronic effects from substituents at the 2′-position on the same ring, as in the case of the 2′-amino derivatives.

These compounds, together with the nmr spectral properties, are being used to study the mode of interaction of bleomycin A_2 with synthetic polynucleotides. It is hoped that the structure-activity information obtained using these derivatives will allow the determination of the geometry of complexes between the parent drug and the target DNA. The data may ultimately permit the design and preparation of synthetic derivatives of these drugs having modified activities and toxicities.

EXPERIMENTAL

Melting points were determined on a Laboratory Devices Mel-Temp apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer Model 710B spectrophotometer. Proton nmr measurements were made on a Bruker WH-400 spectrometer. Chemical shifts are referenced to internal tetramethylsilane (organic solvents) or sodium 4,4-dimethyl-2,2,3,3-tetradeutero-4-silapentanoate (aqueous solvent). Both reference compounds were obtained from Stohler Isotope Chemicals (Waltham, MA). Thin-layer chromatography was run on precoated silica gel F_{254} plates (0.25 mm thickness, Eastman Kodak, Rochester, NY). All analytical samples were found to be homogeneous upon chromatography using ethyl acetate and chloroform:methanol (3:1, by volume). Preparative tlc was performed on precoated silica gel GF plates (1.0 mm thickness, Analtech, Newark, NJ). Combustion analyses were performed by Atlantic Microlabs, Atlanta, GA. All commercially available chemicals were reagent grade or were purified prior to use.

Thioformamide (**2a**) was prepared by the method of Kurkjy and Brown (7). Ethyl thiazole-4-carboxylate (**3a**) was prepared as described by Erne, *et al.*, (8) by reacting thioformamide with ethyl bromopyruvate in 1,4-dioxane. Thiazole-4-carboxylic acid (**4**) was prepared by alkaline hydrolysis of **3a** as described by Erlenmeyer and Morel (9).

5-Phthalimidopentanenitrile (prepared by the reaction of sodium cyanide with 4-phthalimidobutyl bromide (10)) was converted to 5-phthalimidothiopentanamide (**2e**) by treatment with hydrogen sulfide in *N,N*-dimethylformamide (5,11). *O*-Benzoylthiolactamide (**2f**) was prepared as described previously (5). The synthesis of compounds **7b**, **8b** and **18b**, the nmr properties of which are described in part here, was published previously (5).

For all procedures described below, the analytical data, including yields, recrystallization solvents, melting points, and combustion analyses are given in Table 1. All ir and nmr spectral properties are summarized in Tables 2 and 3.

Methyl 3-(Thiazole-4-carboxamido)propyl Sulfide (**5a**).

A mixture of thiazole-4-carboxylic acid (**4**) (527 mg, 4.1 mmoles) and 10 ml of thionyl chloride was refluxed for 2.5 hours. Excess thionyl chloride was removed *in vacuo* giving a quantitative yield of the acid chloride which was used without further purification. The acid chloride was dissolved in 10 ml of dichloromethane and treated with a solution of 3-(methylthio)propylamine (1.05 g, 10 mmoles) in 2 ml of dichloromethane. The solution was stirred at room temperature for 2 hours and then extracted with 25 ml of 1 *N* hydrochloric acid followed by 25 ml of saturated sodium chloride. The organic phase was dried over magnesium sulfate and then evaporated to dryness, giving 720 mg (81%) of crude **5a**, m.p. 64-66.5°. A portion of this material was distilled at 0.05 mm Hg and a bath temperature of 120° giving an analytical sample of **5a**, m.p. 65-67°.

Ethyl 2-(2-Acetamidoethyl)thiazole-4-carboxylate (**3b**).

A solution of 3-acetamidothiopropionamide (**2b**) (665 mg, 5.03 mmoles) (5) and ethyl bromopyruvate (980 mg, 5.03 mmoles) in 3 ml of *N,N*-dimethylformamide was stirred at 60° for 3 hours. The solution was concentrated *in vacuo* and the residue was dissolved in 15 ml of dichloromethane and washed with two 15 ml portions of water. The dichloromethane fraction was dried with magnesium sulfate and evaporated to dryness. Upon standing at room temperature, the residue crystallized, giving 800 mg (65%) of **3b**, m.p. 75-79.5°. A portion of this material was recrystallized from ethyl acetate-petroleum ether, giving an analytical sample, mp 81-83°.

3-[2-(2-Acetamidoethyl)thiazole-4-carboxamido]propyl Methyl Sulfide (**5b**).

A solution of **3b** (833 mg, 3.64 mmoles) in 2 ml of 3-(methylthio)-propylamine was heated at 80° for 4 hours. The solution was dissolved in 25 ml of dichloromethane and washed with 20 ml of cold 1*N* hydrochloric acid followed by 10 ml of sodium bicarbonate and then water. The dichloromethane fraction was dried with magnesium sulfate, decolorized with charcoal and evaporated to give 605 mg (61%) of **5b** as a brown oil. Preparative tlc on silica gel using ethyl acetate as solvent gave an analytical sample as an oil.

General Procedure for Preparing 2'-Substituted 2,4'-Bithiazole-4-carboxylic Acid Methyl Esters (**7a-f**).

Methyl 2-(2-bromoacetyl)thiazole-4-carboxylate (**6**) was treated with a 10% molar excess of the appropriate thioamide (**2a-f**). The solvent for **2a** was 1,4-dioxane and the reaction was carried out for 16 hours at room temperature. The solvent for **2c** was methanol and for **2b,d,e** and **f** was *N,N*-dimethylformamide. The reactions of **2b-e** were carried out at 60-65° for 2 to 3 hours; that of **2f** was carried out at room temperature for 24 hours. Compounds **7c,e** and **f** crystallized upon cooling the reaction mixtures. Compounds **7a,b** and **d** were isolated by evaporation of the solvents *in vacuo* (see Tables 1-3).

General Procedure for Preparing 3-(Methylthio)propylamides of 2'-Substituted 2,4'-Bithiazole-4-carboxylic Acids (**8a-d**).

Compounds **8a-d** were prepared from esters **7a-d** by dissolution in neat 3-(methylthio)propylamine with heating as described above for the conversion of **3b** to **5b**. The properties of these derivatives are summarized in Tables 1-3.

2'-(4-Phthalimidobutyl)-2,4'-bithiazole-4-carboxylic Acid (**9**).

A solution of **7e** (837 mg, 1.96 mmoles) in a mixture of 1,2-dimethoxyethane (30 ml) and 4 *N* hydrochloric acid (15 ml) was refluxed for 48 hours. Upon cooling, the solution deposited 460 mg of **9**, m.p. 216-217°. The remaining solution was concentrated to dryness *in vacuo* and the residue was recrystallized from ethanol giving an additional 200 mg of product, m.p. 216-217°, 81% overall yield.

Methyl 3-[2'-(4-Phthalimidobutyl)-2,4'-bithiazole-4-carboxamido]propyl Sulfide (**10**).

A mixture of **9** (901 mg, 2.18 mmoles) and 8 ml of thionyl chloride was refluxed for 3 hours. The resulting solution was concentrated *in vacuo* to give a quantitative yield of the acid chloride which was used without further purification. The acid chloride was dissolved in 50 ml of dichloromethane and the solution was cooled to 0°. To this was added a solution of 3-(methylthio)propylamine (275 mg, 2.62 mmoles) in 20 ml of dichloromethane containing triethylamine (363 mg, 3.6 mmoles). The mixture was stirred at 0° for 2 hours and then was washed successively with 25 ml each of 1 *N* hydrochloric acid and saturated sodium chloride. The dichloromethane fraction was dried with magnesium sulfate and evaporated to dryness giving 1.06 g (97%) of crude **10**. Crystallization of a portion of this material from chloroform-petroleum ether gave an analytical sample, m.p. 118-120°.

3-[2'-(4-Acetamidobutyl)-2,4'-bithiazole-4-carboxamido]propyl Methyl Sulfide (**11**).

A suspension of **10** (1.06 g, 2.11 mmoles) in 6 ml of ethanol was treated with 6 ml of a solution of hydrazine (150 mg, 4.5 mmoles) in ethanol. The mixture was heated at 60° for 3 hours after which time it was concentrated *in vacuo*. The residue was treated with 25 ml of 6 *N* hydrochloric

acid and the crude phthalhydrazide was removed by filtration. The filtrate was concentrated to dryness, the residue was dissolved in water and the solution was made basic (*p*H ~ 12) with sodium hydroxide. The alkaline solution was extracted with two 25 ml portions of dichloromethane and the combined extracts were dried with magnesium sulfate. Removal of the solvent gave a residue which was treated with 20 ml of acetic anhydride containing 0.5 ml of pyridine. The mixture was allowed to stir for 3 hours at room temperature after which time it was concentrated to an oil. The oil was treated with 50 ml of water and the mixture was taken to *p*H ~ 3 with 1 *N* hydrochloric acid. After cooling, the product was filtered and dried under vacuum, giving 500 mg (57%) of **11**, m.p. 105-109° (resolidifies), remelts at 122-125°. Recrystallization of this material from ethyl acetate-petroleum ether gave material with m.p. 126.5-128°.

Methyl 2'-[1-(Hydroxy)ethyl]-2,4'-bithiazole-4-carboxylate (**12**).

A solution of **7f** (1.39 g, 3.72 mmoles) and sodium methoxide (336 mg, 6.22 mmoles) in 10 ml of anhydrous methanol was allowed to stand at room temperature for 5 hours. The solution was neutralized with Dowex 50X8 (hydrogen form) and the resin was removed by filtration and washed with methanol. The combined filtrate and washings were evaporated to a semi-crystalline mass which was filtered and washed with carbon tetrachloride, giving 970 mg (97%) of crude **12**. Recrystallization from ethanol-water gave 617 mg (61%) of analytically pure **12**, m.p. 152-153°.

Methyl 2'-Acetyl-2,4'-bithiazole-4-carboxylate (**13**).

A solution of **12** (345 mg, 1.28 mmoles) in 25 ml of dichloromethane was stirred with 1 g of activated manganese dioxide (12) for 3 days at room temperature. The mixture was filtered through a Celite pad and the cake was washed with dichloromethane. Evaporation of the combined filtrate and washings gave 280 mg (82%) of **13**, m.p. 211-214°. Recrystallization of the material from ethyl acetate gave analytically pure **13**, m.p. 218.5-219.5°.

Methyl 2'-(2-Bromoacetyl)-2,4'-bithiazole-4-carboxylate (**14**).

To a solution of **13** (242 mg, 0.901 mmole) in 22 ml of acetic acid was added pyridinium bromide perbromide (322 mg, 1.00 mmole). The resulting mixture was heated at 80° for 0.5 hour after which time 25 ml of water was added. The mixture was extracted with two 20 ml portions of dichloromethane. The combined extracts were dried with magnesium sulfate and evaporated to dryness, giving 309 mg of crude **14**. Nmr spectra showed the material to be a 9:1 mixture of monobrominated ketone and starting material which was satisfactory for use without purification.

Methyl 2,4';2',4"-Terthiazole-4-carboxylate (**15**).

A solution of crude **14** (343 mg, 0.89 mmole of bromoketone) in 10 ml of 1,4-dioxane was treated with 30 mmoles of thioformamide in 1,4-dioxane for 2 hours at room temperature with stirring. Removal of the solvent gave an oil which solidified upon treatment with water. The solid was filtered and dried under vacuum giving 257 mg (93%) of **15**, m.p. 220-225° dec (darkens at 218°). Recrystallization from ethanol gave 208 mg (73%), m.p. 233-234° dec, lit m.p. 233-234° dec (13).

Methyl 3-[(2,4':2',4"-Terthiazole-4-carboxamido]propyl Sulfide (**16**).

Compound **16** was prepared by direct aminolysis of **15** with 3-(methylthio)propylamine as described for the preparation of derivatives **8a-d**. Product was obtained in 90% yield, m.p. 111.5-117°. Recrystallization from ethanol gave an analytical sample, m.p. 126.5-128.5°.

General Method for Preparing Sulfonium Chlorides (**17a,b, 18a-d, 19, 20**).

A solution of the methylthioether (**5a,b, 9a-c, 11, 16**) (1 mmole) in 1 ml of a 1:1 mixture of iodomethane and methanol was allowed to stand at room temperature in a sealed tube for 12 to 15 hours. Removal of the solvent gave iodide salts generally as yellow, hygroscopic materials. The reaction of **8d** was carried out at −15° for 6 days to minimize alkylation of the 3'-nitrogen of the bithiazole ring system.

The iodide derivatives were converted to the respective chloride salts by passage of aqueous solutions through a column (1 cm × 10 cm) of Dowex 1X8 (chloride form). Lyophilization of the eluate gave good yields of the dimethylsulfonium chlorides in analytically pure form as hydrates which tended to be hygroscopic.

Acknowledgement.

We acknowledge the use of the Nuclear Magnetic Resonance Facility of the Comprehensive Cancer Center, University of Alabama in Birmingham, which is supported by U.S. Public Health Service Grant CA-13148.

REFERENCES AND NOTES

(1) This work was supported by U. S. Public Health Service Grant GM-27900 to T. T. S.

(2) To whom to address correspondence.

(3) See articles in (a) S. K. Carter, S. T. Crooke, and H. Umezawa, Eds., "Bleomycin. Current Status and New Developments", Academic Press, New York, N.Y., 1978, 365 pp; (b) S. M. Hecht, ed., "Bleomycin. Chemical Biochemical and Biological Aspects", Springer-Verlag, New York, N.Y., 1979, 351 pp.

(4) See recent reviews by (a) R. M. Burger, J. Peisach and S. B. Horwitz, *Life Sci.*, **28**, 715 (1981); (b) A. P. Grollman and M. Takeshita, "Advances in Enzyme Regulation", Vol. 18, G. Weber, ed., Pergamon Press, Oxford, 1979, p 67.

(5) T. T. Sakai, J. M. Riordan, T. E. Booth and J. D. Glickson, *J. Med. Chem.*, **24**, 279 (1981).

(6) J. D. Glickson, R. P. Pillai, and T. T. Sakai, *Proc. Nat. Acad. Sci. U.S.A.*, **78**, 2967 (1981).

(7) R. P. Kurkjy and E. V. Brown, *J. Am. Chem. Soc.*, **74**, 5778 (1952).

(8) M. Erne, F. Ramirez and A. Burger, *Helv. Chim. Acta.*, **34**, 143 (1951).

(9) H. Erlenmeyer and C. J. Morel, *ibid.*, **25**, 1073 (1942).

(10) W. S. Jones, R. S. Stander and J. White, *J. Org. Chem.*, **16**, 708 (1951).

(11) C. Io-Fon and Z. Shi-Yun, *Zh. Obshch. Khim.*, **28**, 1492 (1958); *Chem. Abstr.*, **53**, 13126 (1959).

(12) S. Ball, T. W. Goodwin and R. A. Morton, *Biochem. J.*, **42**, 516 (1948).

(13) P. Brookes, R. J. Clark, A. T. Fuller, M. P. V. Mijovic, and J. Walker, *J. Chem. Soc.*, 916 (1960).