Acylation of Purine-8-thione and Benzimidazole-2-thione: A Reinvestigation of the Site of Acylation (1a,b)

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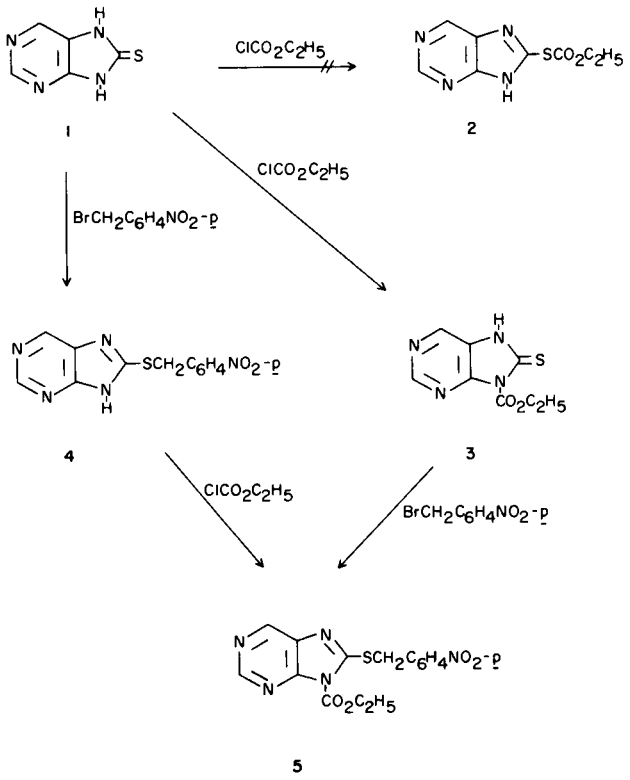
Purine-8-thione (**1**) is acylated on nitrogen, not on sulfur as was previously reported. Thus, the reactions of **1** with ethyl chloroformate and with acetic anhydride yield, respectively, ethyl purine-8-thione-9(7)-carboxylate (**3**) and 9(7)acetylpurine-8-thione (**7**) as shown by independent synthesis and spectra. In like manner, benzimidazole-2-thione (**10**) reacts with acetic anhydride, ethyl chloroformate, benzoyl chloride, and cyclohexyl isocyanate to yield the corresponding *N*-acylated derivatives. In addition, **10** yields 1,3-dibenzoylbenzimidazole-2-thione on treatment with 2 equivalents of benzoyl chloride.

Recent work (2) has shown that purine-6-thione is acylated on the 9-nitrogen of the purine ring rather than on sulfur as was previously thought (3). Since the site of acylation of purine-8-thione (**1**) had also been assigned to sulfur (3), a reinvestigation of acylation of purine-8-thione (**1**) and of benzimidazole-2-thione (**10**) was initiated.

Acylation of Purine-8-thione.

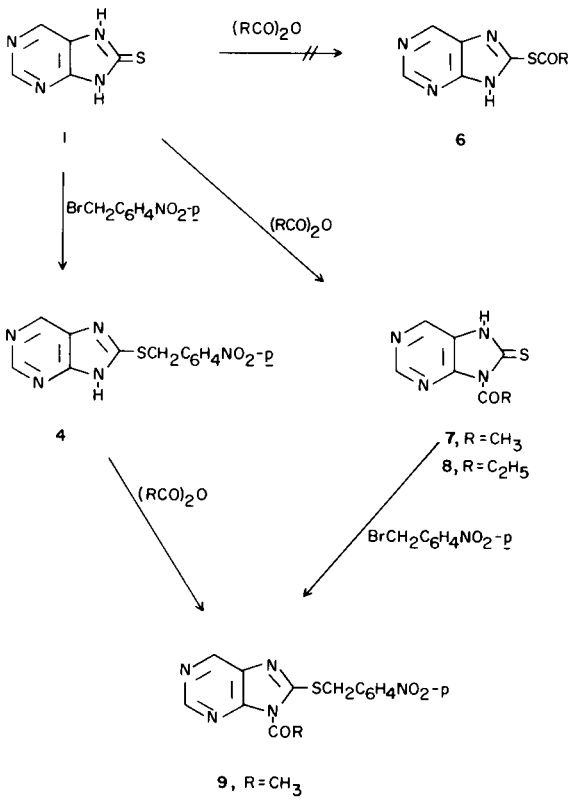
The reaction of purine-8-thione (1) with ethyl chloroformate in dimethylformamide in the presence of potassium carbonate (or triethylamine) as the acid acceptor yielded a monocarbethoxy derivative postulated by Dyer and Bender (3) to be the *S*-acyl compound **2** (Scheme I) on the basis of the supposed behavior of purine-6-thione. However, in the current work, the observation was made (Table I) that the carbethoxy derivative of purine-8-thione failed to exhibit the pronounced hypsochromic shift characteristic of the ultraviolet absorption of *S*-substituted alkyl derivatives of purine-8-thione (4). Moreover, acylation of 8-*p*-nitrobenzylthiopurine (**4**, whose structure was based on its ultraviolet spectrum), yielded the same compound (**5**) as did alkylation of the carbethoxy derivative of purine-8-thione (**3**), thus confirming that acylation had occurred on nitrogen, not sulfur. Hence, the structure of the carbethoxy derivative is **3**, ethyl purine-8-thione-9(7)-carboxylate, not **2**. It should be noted that **3** was recrystallizable from water without decomposition which is in contrast to the instability toward water of ethyl purine-6-thione-9-carboxylate (3).

Scheme I



In the current study, the reaction of purine-8-thione (**1**) with aliphatic acid anhydrides yielded monoacyl derivatives which failed to exhibit the hypsochromic shift in the ultraviolet spectrum characteristic of *S*-substituted alkyl derivatives of compound **1** (4). Moreover, *N*-acylation was demonstrated by independent synthesis since the alkylation of the acetyl derivative of **1** with *p*-nitrobenzylbromide in dimethylformamide with triethylamine as acid acceptor yielded the same product (**9**) (Scheme II) as did the acetylation of 8-*p*-nitrobenzylthiopurine (**4**). Thus the acetyl derivative of **1** is 9(7)-acetylpurine-8-thione (**7**), not **6**. Hence, the observed pattern of acylation of **1** is in contrast to that of alkylation, since alkylation results in *S*-substitution (4), while acylation results in *N*-substitution.

Scheme II



1 6 4 9, R=CH_3

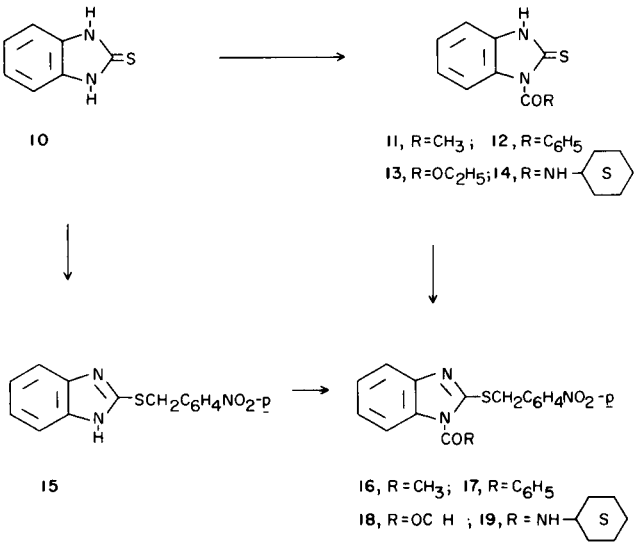
The site of acylation of purine-6-thione was shown to be the 9-nitrogen rather than the 3- or 7-nitrogen by spectral comparisons with known alkyl derivatives (2). Unfortunately, lack of suitable isomeric alkyl derivatives of **1** prevented such a comparison here and the site of acylation was arbitrarily represented at 9- in the charts with the realization that it could be the 7-position.

Acylation of Benzimidazole-2-thione.

Some confusion exists as to the site of acylation of benzimidazole-2-thione (**10**). Acylation with benzoyl chloride was reported to give an *S*-benzoyl-derivative (5) and the reaction of substituted aliphatic isocyanates with **10** was reported to yield the corresponding thiolcarbamate derivatives (6). However, the reaction of **10** with acetic anhydride yielded a monoacetyl derivative (7) later described as *N*-acetylbenzimidazole-2-thione, (**11**) (8). 1-Phenylbenzimidazole-2-thione was reported to yield an *N*-acetyl derivative (9) on the basis of the failure of the *N*-acetyl derivative to exhibit the hypsochromic shift characteristic of a modification of the thioureide structure.

In the current work, the action of a number of acylating agents on **10** under a variety of conditions was studied. None of the acyl derivatives of **10** obtained exhibited the hypsochromic shift characteristic of alkyl derivatives substituted on sulfur and *N*-acylation was shown unambiguously by the independent synthesis described in Scheme III. Thus, benzimidazole-2-thione (**10**) yielded 1-acetylbenzimidazole-2-thione (**11**) on treatment with acetic anhydride in pyridine. The reaction of **10** with one equivalent of benzoyl chloride in dimethylformamide in the presence of triethylamine yielded 1-benzoylbenzimidazole-2-thione (**12**). The melting points of **11** and **12** were in agreement with those reported by the earlier workers (5,7,8). Alkylation of **11** and **12** with *p*-nitrobenzyl bromide gave **16** and **17**, respectively, which were also obtained by acylation of **15**, a known compound (13).

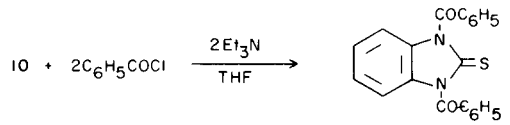
Scheme III



10 15

When **10** was treated with two equivalents of benzoyl chloride in tetrahydrofuran with two equivalents of triethylamine as acid acceptor, the product was 1,3-

dibenzoylbenzimidazole-2-thione (**20**). Assignment of structure to **20** was made on the basis of the ultraviolet spectrum (Table I).



20

The reaction of **10** with ethyl chloroformate in the presence of sodium hydride gave a monocarbethoxy derivative (**13**) whose melting point (162-163°) was not in agreement with the compound described as 1-carbethoxybenzimidazole-2-thione (92-93°) by Guha and Dutta (10) who isolated it in the course of their studies of the reaction of *o*-phenylenediamine with diethylxanthioformic ester, $EtO_2CSCSOEt$. However, no structure proof was offered by them except the formation of **10** on basic hydrolysis (10). The derivative (**13**), ethyl benzimidazole-2-thione-1-carboxylate, isolated in the present study failed to exhibit the hypsochromic shift expected of *S*-substitution on **10**, and **13** gave on alkylation compound **18**, obtained also from the known **15**. Hence acylation of **10** with ethyl chloroformate occurred on nitrogen (Scheme III).

The reaction of cyclohexyl isocyanate with **10** in dimethylformamide with pyridine as catalyst yielded

TABLE I

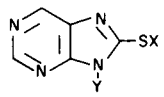
Ultraviolet Spectra of Some Purine-8-thione and Benzimidazole-2-thione Derivatives

	Cpd. No.	λ max	ε max x 10^{-3}
Purine-8-thione	1	323 (a)	—
9(7)-Acetyl-	7	323 (a)	12.5
9(7)-Propionyl-	8	323 (a)	13.5
9(7)-Carbethoxy- (c)	3	319 (b)	23.8
8-(*p*-Nitrobenzylthio)purine	4	289 (a)	20.3
9(7)-Acetyl-	9	287 (b)	24.8
9(7)-Carbethoxy-	5	296 (b)	24.2
Benzimidazole-2-thione	10	317 (a)	—
		307 (b)	—
1-Acetyl-	11	315 (a)	4.92
1-Benzoyl-	12	306 (b)	12.5
1-Carbethoxy-	13	313 (b)	9.6
1-(*N*-Cyclohexyl)carbamido-	14	313 (b)	22.1
1,3-Dibenzoyl-	20	302 (b)	16.7
2-(*p*-Nitrobenzylthio)benzimidazole (d)	15	283 (b)	19.3
		291 (b)	18.3
1-Acetyl-	16	287 (a)	17.9
1-Benzoyl-	17	263 (b)	23.6
1-Carbethoxy	18	275 (a)	18.3
		282 (e)	17.8
		293 (e)	15.4
1-(*N*-Cyclohexylcarbamido)-	19	284 (b)	19.6

(a) Methanol. (b) Dioxane. (c) Ref. 3. (d) Ref. 13. (e) Shoulder.

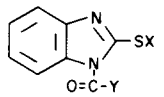
TABLE II

Derivatives of Purine-8-thione



Cpd. No.	X	Y	Method	Time, Hrs.	Yield, %	Recryst. Solv. (a)	M.p., °C (b)	Formula	Analyses. % Calcd. (Found) C	H	N
4	$CH_2C_6H_4NO_2$-*p*	H	B	12	91	E	204-206	$C_{12}H_9N_5O_2S$	50.13(50.19)	3.64(3.69)	19.49(19.47)
5	$CH_2C_6H_4NO_2$-*p*	$CO_2C_2H_5$	A	3.5	95	EA	143-144	$C_{15}H_{13}N_5O_4S$	50.12(50.03)	3.16(3.30)	24.38(24.22)
7	H (c)	$COCH_3$	C	2	78	EA	240-243	$C_7H_6N_4OS$	43.29(43.37)	3.11(3.10)	28.85(28.79)
8	H (c)	COC_2H_5	D	1.5	69	EA	248-251	$C_8H_8N_4OS$	46.14(45.93)	3.87(3.97)	26.91(26.81)
9	$CH_2C_6H_4NO_2$-*p*	$COCH_3$	D	1	87	H-EA	177-179	$C_{14}H_{11}N_5O_3S$	51.04(51.14)	3.37(3.45)	21.27(21.09)

Acyl Derivatives of Benzimidazole-2-thione



11	H (c)	CH_3	E	2	62	EA	205-206(d)	$C_9H_8N_2OS$	56.22(56.31)	4.10(4.29)	14.57(14.47)
12	H (c)	C_6H_5	F	4	82	70%E	192-195(e)	$C_{14}H_{10}N_2OS$	66.11(65.78)	3.96(4.22)	11.01(10.61)
13	H (c)	OC_2H_5	G	12	68	50%E	162-163	$C_{10}H_{10}N_2O_2S$	54.03(54.02)	4.51(4.89)	12.60(12.42)
14	H (c)										
14	H (c)	NH-(S)	H	10	87	E	187-189	$C_{14}H_{17}N_3OS$	61.05(61.11)	6.22(6.25)	15.25(15.05)
16	$CH_2C_6H_4NO_2$-*p*	CH_3	A	3	95	H-EA	140-141	$C_{16}H_{13}N_3O_3S$	58.69(58.72)	4.00(4.03)	12.83(13.02)
17	$CH_2C_6H_4NO_2$-*p*	C_6H_5	A	3	71	H	117-120	$C_{21}H_{15}N_3O_3S$	64.76(64.62)	3.88(3.93)	10.79(10.88)
18	$CH_2C_6H_4NO_2$-*p*	OC_2H_5	I	4	88	H-EA	154-156	$C_{17}H_{15}N_3O_4S$	57.12(56.74)	4.23(4.00)	11.75(12.11)
19	$CH_2C_6H_4NO_2$-*p*	NH-(S)	A	12	88	E	148-151	$C_{21}H_{22}N_4O_3S$	61.44(61.47)	5.40(5.53)	13.64(13.32)

(a) EA, ethyl acetate; E, ethanol; H-EA, 3:2-heptane-ethyl acetate; 70%E, 70% aqueous ethanol; 30%E, 30% aqueous ethanol. (b) With decomposition. (c) Drawn in thiol form for convenience. (d) Lit. (7,8), m.p. 201-202°. (e) Lit. (5), m.p. 186-187°.

1-(*N*-cyclohexyl)carbamidobenzimidazole-2-thione (**14**), which also failed to show the hypsochromic shift expected of *S*-substitution. Conversion of **14** to **19** (prepared independently from **15**) confirmed that acylation had occurred on nitrogen, not sulfur (Scheme III).

It should be noted that the pattern of *N*-acylation and *S*-alkylation established for purine-8-thione (**1**) and benzimidazole-2-thione (**10**) is in agreement with the behavior of thiourea (**11**) and imidazole-2-thione (**12**) which undergo acylation on nitrogen, but alkylation on sulfur under ordinary conditions.

EXPERIMENTAL

Melting points were determined on a Fisher-Johns apparatus and are corrected. The ultraviolet spectra were obtained on a Perkin-Elmer 202 spectrophotometer. The nmr spectra (in dimethyl sulfoxide-d_6) were obtained on a Varian A-60A spectrometer using tetramethylsilane as internal standard. Microanalyses were performed by A. Bernhardt, Mulheim (Ruhr), Germany and by M-H-W Laboratories, Garden City, Michigan.

Preparation of Compounds.

The procedures cited by letters in Table II are illustrated for typical compounds. Compounds **5**, **9**, **16**, **17**, **18**, and **19** were prepared by both Procedures A and I, *i.e.*, by alkylation of the *N*-acyl derivative and acylation of the *S*-alkyl derivative (Schemes II and III). However, the acylation of **15**, with benzoyl chloride to yield **17** was run in tetrahydrofuran with potassium carbonate as the acid acceptor.

Procedure A. Ethyl 8-*p*-Nitrobenzylthiopurine-9(7)-carboxylate (**5**).

p-Nitrobenzyl bromide (0.216 g., 1 mmole) was added to a solution of ethyl purine-8-thione-9(7)-carboxylate (**3**) and triethylamine (0.101 g., 1 mmole) in 10 ml. of anhydrous dimethylformamide and the mixture was stirred for 3.5 hours at room temperature. The mixture was poured into 20 ml. of ice water, the *p*H was adjusted to 5 with glacial acetic acid and the precipitate was collected by filtration and dried *in vacuo* to yield 0.34 g. (95%) of crude product (**5**); nmr δ 1.47 (t 3), 4.62 (q 2), 4.82 (s 2), 8.08 (q 4), 9.05 (s 1), 9.14 (s 1).

Procedure B. 8-*p*-Nitrobenzylthiopurine (**4**).

p-Nitrobenzyl bromide (4.32 g., 20 mmoles) was added to a solution of purine-8-thione (3.04 g., 20 mmoles) and triethylamine (2.02 g., 20 mmoles) in 20 ml. of anhydrous dimethylformamide. The mixture was stirred for 12 hours, poured into 100 ml. of ice water, and the *p*H adjusted to 5 with glacial acetic acid to give a sticky precipitate which yielded a granular solid upon refrigeration and scratching. The precipitate was collected by filtration, washed with water, and dried *in vacuo* to yield 5.2 g. (91%) of product (**4**), m.p. 193-195°, 204-206° after two recrystallizations from ethanol; nmr δ 4.78 (s 2), 7.96 (q 4), 8.76 (s 1), 8.90 (s 1).

Procedure C. 9(7)-Acetylpurine-8-thione (**7**).

A solution of purine-8-thione (0.5 g., 3.3 mmoles) and acetic anhydride (24 ml.) was refluxed for 2 hours. The precipitate which formed upon cooling was collected by filtration, washed with cold ether, and dried *in vacuo* over potassium hydroxide to yield 0.5 g. (78%) of product, (**7**) m.p. 240-243° d.; nmr δ 3.10 (s 3), 8.72 (s 1), 8.95 (s 1).

Procedure D. 8-*p*-Nitrobenzylthio-9(7)-acetylpurine (**16**).

A solution of 8-*p*-nitrobenzylthiopurine (13) (0.287 g., 1.0 mmole) and acetic anhydride (3 ml.) in 10 ml. of dry toluene was heated under reflux for 1 hour. Upon cooling, a precipitate formed which was collected by filtration, washed with cold ether, and dried to yield 0.17 g. of product (**16**), m.p. 176-179°. Evaporation of the mother liquor gave an additional 0.07 g. of product (87% total); nmr δ 3.0 (s 3), 4.72 (s 2), 8.09 (q 4), 9.06 (s 1), 9.16 (s 1).

Procedure E. 1-Acetylbenzimidazole-2-thione (**11**).

Benzimidazole-2-thione (5 g., 34 mmoles) was refluxed in a mixture of acetic anhydride (30 ml.) and pyridine (10 ml.) for 2 hours during which time the solid dissolved. On cooling, the solution which yielded a precipitate, was filtered, the precipitate was washed with cold ether and dried *in vacuo* to give 4.0 g. (62%) of **11** as light yellow needles, m.p. 205-207°.

Procedure F. 1-Benzoylbenzimidazole-2-thione (**12**).

To an ice-cooled solution of benzimidazole-2-thione (3.0 g., 20 mmoles) and triethylamine (2.0 g., 20 mmoles) in 20 ml. of dry dimethylformamide was added benzoyl chloride (2.8 g., 20 mmoles). The solution was stirred for 4 hours at room temperature and poured into 100 ml. of ice water. The *p*H was adjusted to 5 with glacial acetic acid and the precipitate was collected by filtration and dried *in vacuo* to yield 4.1 g. (82%) of crude product (**12**).

Procedure G. Ethyl Benzimidazole-2-thione-1-carboxylate (**13**).

Sodium hydride (0.528 g. of a 50% mineral oil suspension, 11 mmoles) was added to a stirred solution of benzimidazole-2-thione (1.5 g., 10 mmoles) in 70 ml. of anhydrous tetrahydrofuran. The suspension was stirred until the evolution of hydrogen gas had ceased, at which time ethyl chloroformate (1.2 g., 11 mmoles) was added all at once and the mixture was refluxed 12 hours, filtered while hot, and the filtrate was evaporated to dryness. The resulting residue was washed with *n*-hexane and dried *in vacuo* to yield 1.5 g., (68%) of crude product (**13**).

Procedure H. 1-(*N*-cyclohexylcarbamido)benzimidazole-2-thione (**14**).

Cyclohexyl isocyanate (5.0 g., 40 mmoles) was added all at once to a stirred solution of benzimidazole-2-thione (6.0 g., 40 mmoles) and 2 ml. of dry pyridine in 30 ml. of anhydrous dimethylformamide. The solution was stirred for 10 hours at 80-90° and poured into 100 ml. of ice water. The *p*H was adjusted to 5 with glacial acetic acid and the resulting granular solid was collected by filtration, washed with water and dried *in vacuo* to yield 9.6 g. (87%) of product (**14**), m.p. 185-190° with release of isocyanate.

Procedure I. Ethyl 2-*p*-Nitrobenzylthiobenzimidazole-1-carboxylate (**18**).

To a stirred solution of 2-*p*-nitrobenzylthiobenzimidazole (13) (1.5 g., 5.27 mmoles) and triethylamine (0.523 g., 5.27 mmoles) in 20 ml. of anhydrous dimethylformamide, was added ethyl chloroformate (0.57 g., 5.27 mmoles). A precipitate formed immediately and the suspension was stirred for 4 hours and poured into 60 ml. of ice-water. The *p*H was adjusted to 5 with glacial acetic acid, and the solid was collected by filtration and dried *in vacuo* to yield 1.7 g. (91%) of crude product (**18**), m.p. 145-146°.

1,3-Dibenzoylbenzimidazole-2-thione (**20**).

To a solution of benzimidazole-2-thione (1.50 g., 10 mmoles)

and triethylamine (2.02 g., 20 mmoles) in 40 ml. of dry tetrahydrofuran was added benzoyl chloride (2.82 g., 20 mmoles). A precipitate formed and the suspension was refluxed for 6 hours, then filtered hot through a celite pad and evaporated to dryness. The residue was washed with hexane to yield 2.9 g. (78%) of crude product, m.p. 182-184°. Recrystallization from toluene gave **20** as bright yellow needles, m.p. 187-190°. A mixture with the monobenzoyl derivative melted at 170-173°.

Anal. Calcd. for $C_{21}H_{14}N_2O_2S$: C, 70.36; H, 3.93; N, 7.81. Found: C, 70.43; H, 3.93; N, 7.75.

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(1a) This investigation was supported by the Public Health Service Research Grant No. CA-03477 from the National Cancer Institute. (b) Abstracted in part from the Ph. D. thesis of C.E.M. (1968), University of Delaware.

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