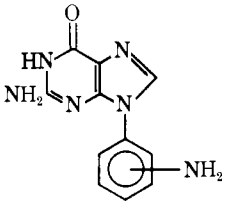
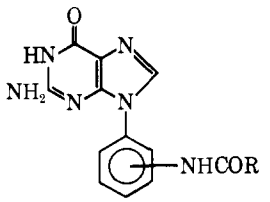


7, *meta*
8, *para*



5, R = *m*-$NHC_6H_4SO_2F$-*m*
9, R = $C_6H_5SO_2F$

Experimental Section[15]

9-[*m*-(*m*-**Fluorosulfonylphenylureido**)**phenyl**]**guanine** (**5**).—To a solution of 100 mg (0.41 mmole) of **7**[7] in 5 ml of DMF was added 92 mg (0.45 mmole) of *m*-fluorosulfonylphenyl isocyanate. After being stirred 1 hr at ambient temperature, the mixture was added to 50 ml of 0.2 *N* HCl. The product was collected on a filter and washed with H_2O. Two recrystallizations from MeOEtOH–H_2O gave nearly white crystals with a negative Bratton Marshall test for aromatic amine;[16] yield 50 mg (28%); λ_{max} (mμ) pH 1, 261; pH 13, 261. *Anal.* ($C_{18}H_{14}FN_7O_4S$) C, H, F.

9-[*p*-(*p*-**Fluorosulfonylbenzamido**)**phenyl**]**guanine** (**2**).—To a solution of **8**·HCl (0.30 mmole) in 5 ml of DMF containing 73 mg (0.72 mmole) of Et_3N was added 115 mg (0.54 mmole) of *p*-fluorosulfonylbenzoyl chloride. After being stirred 1 hr at ambient temperature, the solution gave a negative Bratton-Marshall test for aromatic amine.[16] The mixture was added to 50 ml of H_2O containing 61 mg (0.72 mmole) of $NaHCO_3$. The product was collected and washed with water, then dissolved in 5 ml of warm DMF. Addition of H_2O gave 70 mg (45%) of product as a nearly white powder: λ_{max} (mμ) pH 1, 228, 282; pH 13, 274. *Anal.* ($C_{18}H_{13}FN_6O_4S$) C, H, N.

9-[*m*-(*p*-**Fluorosulfonylbenzamido**)**phenyl**]**guanine** (**3**) was prepared in 44% yield as described for **2**: λ_{max} (mμ) pH 1, 230, 280; pH 13, 271. *Anal.* ($C_{18}H_{13}FN_6O_4S$) C, H, N.

9-[*m*-(*m*-**Fluorosulfonylbenzamido**)**phenyl**]**guanine** (**4**) was prepared in 29% yield as described for **2** except the product was recrystallized from MeOEtOH–H_2O: λ_{max} (mμ) pH 1, 267; pH 13, 270. *Anal.* ($C_{18}H_{13}FN_6O_4S \cdot 0.5H_2O$) C, H, F.

(15) All analytical samples gave combustion values within 0.3% of theoretical; each moved as a single spot on tlc with EtOH–$CHCl_3$ (3:5) on Brinkmann silica gel GF when detected under uv light and each had ir and uv spectra compatible with their assigned structures. None showed a melting point below 300°.

(16) B. R. Baker, D. V. Santi, J. K. Coward, H. S. Shapiro, and J. H. Jordaen, *J. Heterocycl. Chem.*, **3**, 425 (1966).

Irreversible Enzyme Inhibitors. CXXIV.[1,2] Active-Site-Directed Irreversible Inhibitors of Xanthine Oxidase Derived from 2- (and 8-) Benzylthiopurines Bearing a Terminal Sulfonyl Fluoride

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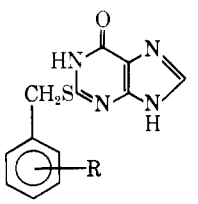
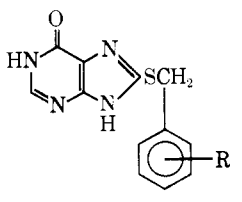
Twelve candidate irreversible inhibitors of xanthine oxidase have been synthesized and evaluated that bear a terminal sulfonyl fluoride for covalent bond formation within the inhibitor–enzyme complex. Four candidates were derived from 2-benzylthiohypoxanthine, five from 8-benzylthiohypoxanthine, and three from 8-benzylthioadenine where the sulfonyl fluoride was bridged to the phenyl moiety with a benzamido or phenylureido moiety. Of these twelve candidate irreversible inhibitors seven showed varying degrees of irreversible inhibition of xanthine oxidase and five showed none. The two best irreversible inhibitors were 8-[*m*-(*p*-fluorosulfonylbenzamido)benzylthio]hypoxanthine (**14**) and 8-[*m*-(*m*-fluorosulfonylbenzamido)benzylthio]adenine (**16**). Both **14** and **16** were reversibly complexed to xanthine oxidase about 20-fold better than the substrate. At 4×10^{-7} *M*, **16** inactivated 88% of the enzyme in 15 min with a half-life of 2 min; at the same concentration, **14** was fourfold slower since it inactivated xanthine oxidase with a half-life of 7 min.

In a previous paper of this series, 2-benzylthiohypoxanthine (**1**) and its 8 isomer (**2**) were shown to be good reversible inhibitors of xanthine oxidase, being complexed tenfold and eightfold better, respectively, than the substrate, hypoxanthine.[3] Of the four candidate active-site-directed irreversible inhibitors[4] (**3**, **7**, **9**, **13**), bearing a bromoacetamido group that were prepared in a subsequent study,[5] only **13** showed irreversible inhibition of xanthine oxidase; at a concentration of 1.5 μM ($2I_{50}$), **13** inactivated xanthine oxidase with a half-life of 50 min. With a related problem in this laboratory, it was discovered that the terminal sulfonyl fluoride group was much superior to the bromoacetamido group for effective active-site-directed irreversible inhibitors operating by the exo mechanism.[6] For example, 4,6-diamino-1,2-dihydro-1-phenyl-*s*-triazines bridged from the phenyl to a terminal sulfonyl fluoride





1, R = H
3, R = *o*-$NHCOCH_2Br$
7, R = *m*-$NHCOCH_2Br$
8, R = *m*-$NHCOC_6H_4SO_2F$-*p*

2, R = H
9, R = *o*-$NHCOCH_2Br$
13, R = *m*-$NHCOCH_2Br$
14, R = *m*-$NHCOC_6H_4SO_2F$-*p*

(1) This work was generously supported by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper of this series see B. R. Baker and W. F. Wood, *J. Med. Chem.*, **11**, 650 (1968).

(3) B. R. Baker and J. L. Hendrickson, *J. Pharm. Sci.*, **56**, 955 (1967), paper XCII of this series. In this paper is also discussed the chemotherapeutic utility for tissue-specific inhibitors of xanthine oxidase.

(4) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Enzymic Active-Site," John Wiley and Sons, Inc., New York, N. Y., 1967.

(5) B. R. Baker and J. Kozma, *J. Med. Chem.*, **10**, 682 (1967), paper XCV of this series.

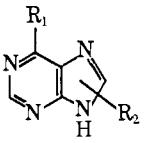
(6) The exo mechanism is defined as formation of a covalent bond within an inhibitor–enzyme complex that occurs outside the active site; in contrast, the endo mechanism is defined as covalent bond formation within the active site.[7] The active site, in turn, is defined as containing those amino acid residues responsible for complex formation with the substrate and those amino acid residues responsible for the catalytic conversion of substrate to product.[8]

(7) See ref 5, Chapter 1.

(8) See ref 5, p 188.

TABLE I

INHIBITION[a] OF XANTHINE OXIDASE BY



No.	R_1	R_2	Reversible[b] I_{50},[d] μM	Reversible[b] $([S]/[I])_{0.5}$[e]	Irreversible[c] Inhib conen, μM	Irreversible[c] Time, min	Irreversible[c] % inactn
1[f]	OH	2-$SCH_2C_6H_5$	0.75	11			
2[f]	OH	8-$SCH_2C_6H_5$	2.8	2.9			
3[g]	OH	2-$SCH_2C_6H_4NHCOCH_2Br$-*o*	0.024	340	0.025	120	0
4	OH	2-$SCH_2C_6H_4(NHCOC_6H_4SO_2F$-*p*)-*o*	30	0.27	46	60	27
5	OH	2-$SCH_2C_6H_4(NHCOC_6H_4SO_2F$-*m*)-*o*	77	0.11	80	60	30
6	OH	2-$SCH_2C_6H_4(NHCONHC_6H_4SO_2F$-*m*)-*o*	16	0.51	16	60	0
7[g]	OH	2-$SCH_2C_6H_4NHCOCH_2Br$-*m*	0.68	12	1.0	120	0
8	OH	2-$SCH_2C_6H_4(NHCOC_6H_4SO_2F$-*p*)-*m*	0.092	88	0.46	20	83
					0.092	5, 8, 60	50, 58, 58[h]
9[g]	OH	8-$SCH_2C_6H_4NHCOCH_2Br$-*o*	0.11	74	0.10	120	0
10	OH	8-$SCH_2C_6H_4(NHCOC_6H_4SO_2F$-*p*)-*o*	40	0.20	80	60	0
11	OH	8-$SCH_2C_6H_4(NHCOC_6H_4SO_2F$-*m*)-*o*	50	0.16	160	20, 60	50, 84[h]
12	OH	8-$SCH_2C_6H_4(NHCONHC_6H_4SO_2F$-*m*)-*o*	16	0.51	16	60	0
13[g]	OH	8-$SCH_2C_6H_4NHCOCH_2Br$-*m*	0.77	11	1.5	50, 120	50, 90[h]
14	OH	8-$SCH_2C_6H_4(NHCOC_6H_4SO_2F$-*p*)-*m*	0.46	18	0.92	60	90
					0.46	7, 16, 30	50, 68, 81[h]
15	OH	8-$SCH_2C_6H_4(NHCOC_6H_4SO_2F$-*m*)-*m*	0.16	51	0.70	60	100
					0.16	8, 30	25, 27[h]
16	NH_2	8-$SCH_2C_6H_4(NHCOC_6H_4SO_2F$-*m*)-*m*	0.42	19	2.1	60	100
					0.42	2, 15, 35	50, 88, 88[h]
17	NH_2	8-$SCH_2C_6H_4(NHCONHC_6H_4SO_2F$-*m*)-*m*	0.79	10	4.0	60	0
18	NH_2	8-$SCH_2C_6H_4(NHCOC_6H_4SO_2F$-*p*)-*m*	0.75	11	3.8	60	0
19[i]		$AcNHC_6H_4SO_2F$-*p*			70	60	0

[a] The technical assistance of Pepper Caseria and Maureen Baker with these assays is acknowledged. [b] Commercial xanthine oxidase from bovine milk was assayed with 8.1 μM hypoxanthine in Tris buffer (pH 7.4) containing 10% DMSO as previously described.[3] [c] Inactivation of xanthine oxidase was performed at 37° in pH 7.4 Tris buffer containing 5% DMSO as previously described,[5] except the zero point was determined by removal of an aliquot prior to addition of the inhibitor.[9] [d] Concentration necessary for 50% inhibition. [e] Ratio of concentration of substrate to inhibitor for 50% inhibition. [f] Data from ref 3. [g] Data from ref 5. [h] From time plot; see ref 5 and 9. [i] Data from ref 2.

rapidly inactivate dihydrofolic reductase;[9,10] by proper positioning of the sulfonyl fluoride, species specific[9,11] and tissue specific[12,13] irreversible inhibitors of dihydrofolic reductase were found. Therefore, nine benzylthiohypoxanthines, such as **8** and **14**, and three benzylthioadenines bearing a terminal sulfonyl fluoride were synthesized and evaluated as irreversible inhibitors of xanthine oxidase; the results are the subject of this paper.

Enzyme Results.—It was previously observed that introduction of an *o*-bromoacetamido group (**3**) on 2-benzylthiohypoxanthine (**1**) gave a 30-fold increment in reversible binding; however, **3** failed to show irreversible inhibition[5] (Table I). This increment in reversible binding by **3** was subsequently suggested to be due to hydrophobic bonding by the bromomethyl group to xanthine oxidase.[14] When the larger benzamido (**4**, **5**) or phenylureido (**6**) groups bearing a sulfonyl fluoride were introduced, a 700–3000-fold loss in reversible binding occurred compared to **3**, indicating that these larger groups were not tolerated by the contour of the enzyme surface within the enzyme–inhibitor reversible complex. A similar loss in binding was observed with these *ortho* substituents (**10**–**12**) on 8-benzylthiohypoxanthine compared to the *o*-bromoacetamido group of **9**.

Of these six sulfonyl fluorides derived from *ortho* substitution on the benzyl group of **1** and **2**, three (**6**, **10**, **12**) showed no irreversible inhibition when incubated at a concentration of I_{50}–$3I_{50}$ with the enzyme; two (**4**, **5**) showed only a poor amount of irreversible inhibition and one (**11**) was good, showing a half-life of inactivation of 20 min and 84% inactivation in 60 min. These three sulfonyl fluorides showing irreversible inhibition were considered to be too poor as reversible inhibitors to be worthy of further study. It should be noted that the rate of active-site-directed irreversible inhibition is directly dependent upon the amount of enzyme reversibly complexed, [E···I], which in turn is related to K_i and I_{50}; thus a compound with a poor I_{50} requires too high a concentration to give an effective amount of [E···I].[15] Furthermore, note that 70 μM *p*-acetamidobenzenesulfonyl fluoride (**19**) does not inactivate xanthine oxidase, indicating that a reversible enzyme–inhibitor complex is an essential

(9) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **10**, 1113 (1967), paper CV of this series.

(10) D. E. Fahrney and A. M. Gold, *J. Am. Chem. Soc.*, **85**, 997 (1963), had observed earlier that phenylmethanesulfonyl fluoride was an excellent irreversible inhibitor of chymotrypsin that operated by the endo mechanism.

(11) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 39 (1968), paper CXII of this series.

(12) B. R. Baker and R. B. Meyer, Jr., *ibid.*, **11**, 489 (1968), paper CXIX of this series.

(13) B. R. Baker and P. C. Huang, *ibid.*, **11**, 495 (1968), paper CXX of this series.

(14) B. R. Baker and W. F. Wood, *ibid.*, **10**, 1106 (1967), paper CIII of this series.

(15) For a discussion of the kinetics of irreversible inhibition, see ref 4, Chapter VIII.

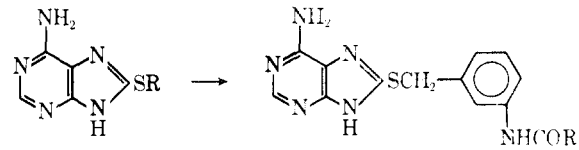
intermediate to inactivation and that inactivation by the compounds in Table I did not proceed by a random bimolecular process,[15] the latter having no specificity.

Three sulfonyl fluorides (**8**, **14**, **15**) were then investigated which were *meta*-substituted derivatives of 2-benzylthio- (**1**) and 8-benzylthiohypoxanthine (**2**). All were good reversible inhibitors of xanthine oxidase being complexed 18–88-fold better than the substrate, hypoxanthine. Furthermore all three showed 83–100% inactivation of xanthine oxidase when the compounds at a concentration of 2–5I_{50} were incubated with the enzyme. When the incubation concentration was reduced to I_{50}, **8**, **14**, and **15** showed less total inactivation in 60 min. A time study showed that **8** gave 53% inhibition in 5 min, then little further inactivation in 60 min. At an I_{50} concentration, **15** was even less ineffective, showing a maximum of 25% inactivation in 8 min, then no further inactivation. The best compound of the three was **14** which still showed 81% inactivation in 30 min at the I_{50} concentration with a half-life of 7 min.

Such nonlinear inactivations when log [E] is plotted against time had been previously observed with dihydrofolic reductase,[9] trypsin,[16] and chymotrypsin.[17,18] That such curvature was due to the enzyme-catalyzed hydrolysis of the sulfonyl fluoride to the sulfonic acid was proven in the case of chymotrypsin;[17] thus within the enzyme–inhibitor complex, either the enzyme can become inactivated due to covalent bond formation or the enzyme can catalyze hydrolysis of the inhibitor to the sulfonic acid or both. When both reactions occur, total inactivation can be achieved by using higher concentrations of inhibitor or treating with several I_{50} portions of inhibitor.[16,17]

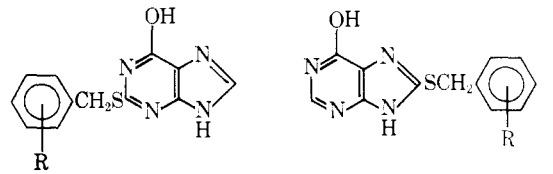
Since adenine binds to xanthine oxidase[3] slightly better than the substrate, hypoxanthine, three sulfonyl fluoride derivatives derived by *meta* substitution on 8-benzylthioadenine were synthesized for enzymic evaluation. Of these three, only **16** showed irreversible inhibition, but it was the best irreversible inhibitor in Table I; **16** complexed to the enzyme 19 times better than the substrate and at an I_{50} concentration gave 88% inactivation of the enzyme with a half-life of 2 min and at 5I_{50} gave total inactivation. When the *m*-sulfonyl fluoride group of **16** was moved to the *para* position (**18**), reversible inhibition changed only twofold, but irreversible inhibition was completely lost; a similar loss of irreversible inhibition was noted when the carboxamido bridge of **16** was increased to ureido (**17**). This sensitivity to change in the position[9] of the sulfonyl fluoride group or change of bridge[11] was previously noted with inhibitors of dihydrofolic reductase.

Note that 8-benzylthioadenine with a *p*-fluorosulfonylbenzamido group on the *meta* position (**18**) fails to inactivate xanthine oxidase, but that the identically substituted hypoxanthine (**14**) does inactivate the enzyme. This difference clearly shows that the sulfonyl fluoride group of **14** and **18** *are not identically positioned within the enzyme–inhibitor complex;* this in turn indicates that adenine and hypoxanthine may not complex to the active site of xanthine oxidase in the same manner, further supporting our earlier statement[3] that "what specific groups of the inhibitor (purine type) are complexed to the enzyme is fraught with uncertainty." Furthermore, xanthine oxidase initially oxidizes hypoxanthine at the 2 position, but initially oxidizes adenine at the 8 position,[19] thus indicating that these two



20, R = H
21, R = $CH_2C_6H_4NO_2$-*m*
22, R = $CH_2C_6H_4NH_2$-*m*

16, R = $C_6H_4SO_2F$-*m*
17, R = $NHC_6H_4SO_2F$-*m*
18, R = $C_6H_4SO_2F$-*p*



23, R = *o*-NH_2
24, R = *m*-NH_2

25, R = *o*-NH_2
26, R = *m*-NH_2

purines are complexed in different rotomeric configurations.[3] With the additional parameter of irreversible inhibition of xanthine oxidase by properly substituted hypoxanthines and adenines in this paper as well as by guanines,[2,14] pyrazolopyrimidines,[20] and other hypoxanthines,[5] a new attempt to rationalize binding of purines to xanthine oxidase will be made.[21] Further modification of the *meta*-substituted benzylthiopurines for tissue specificity, as achieved with dihydrofolic reductase,[12,13] is being pursued.

Chemistry.—8-(*m*-Aminobenzylthio)adenine (**22**) was synthesized from the 8-mercaptoadenine (**20**) by the route previously used for the benzylthiohypoxanthine derivatives (**23–26**).[5] Alkylation of **20** with *m*-nitrobenzyl chloride in 1 *N* NaOH proceeded smoothly to **21** in 72% yield. The nitro group of **21** was catalytically reduced to the amine (**22**) in the presence of a Pd–C catalyst. Reaction of the amine (**22**) with *m*- or *p*-fluorosulfonylbenzoyl chloride in DMF at 0° in the presence of Et_3N gave the candidate irreversible inhibitors, **16** and **18**, respectively. Condensation of **22** with *m*-fluorosulfonylphenyl isocyanate in DMF at room temperature afforded **17**. The remaining candidate irreversible inhibitors derived from hypoxanthine were prepared by similar methods from **23–26**.

Experimental Section[22]

8-(*m*-Nitrobenzylthio)adenine (21).—A mixture of 3.00 g (18 mmoles) of **20**, 3.08 g (18 mmoles) of α-chloro-3-nitrotoluene, and

(16) B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **11**, 245 (1968), paper CXV of this series.

(17) B. R. Baker and J. A. Hurlbut, *ibid.*, **11**, 233 (1968), paper CXIII of this series.

(18) B. R. Baker and J. A. Hurlbut, *ibid.*, **11**, 241 (1968), paper CXIV of this series.

(19) F. Bergmann, G. Lerin, H. Kwietny-Govrin, and H. Ungar, *Biochim. Biophys. Acta*, **47**, 1 (1961).

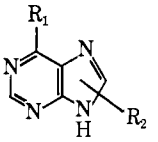
(20) B. R. Baker and J. A. Kozma, *J. Med. Chem.*, **11**, 656 (1968), paper CXXV of this series.

(21) B. R. Baker, W. F. Wood, and J. A. Kozma, *ibid.*, **11**, 661 (1968), paper CXXVI of this series.

(22) Each analytical sample gave combustion values within 0.4% of theoretical, moved as a single spot on tlc on Brinkmann silica gel GF in EtOAc–MeOH, and gave ir spectra compatible with their assigned structures. Melting points were taken in capillary tubes on a Mel-Temp block and those below 230° are corrected. Since many of the compounds had no definite melting point, they are best characterized by their uv spectra (Table II) which varied considerably.

TABLE II

PHYSICAL PROPERTIES OF



No.	R_1	R_2	Method	% yield	Mp, °C	Formula	Analyses	—λ_{max}, mμ[a]— pH 1	pH 13
4	OH	2-$SCH_2C_6H_4(NHCOC_6H_4SO_2F$-*p*)-*o*	A[b]	27	Indef	$C_{19}H_{14}FN_5O_4S_2\cdot 0.5H_2O$	C, H, N	266	275
5	OH	2-$SCH_2C_6H_4(NHCOC_6H_4SO_2F$-*m*)-*o*	A[c]	67	190–193	$C_{19}H_{14}FN_5O_4S_2\cdot 0.5H_2O$	C, H; F	266	274
6	OH	2-$SCH_2C_6H_4(NHCONHC_6H_4SO_2F$-*m*)-*o*	B[c]	56	281–283	$C_{19}H_{15}FN_6O_4S_2$	C, H; N[f]	254, 281[d]	261
8	OH	2-$SCH_2C_6H_4(NHCOC_6H_4SO_2F$-*p*)-*m*	A[c]	43	222–224	$C_{19}H_{14}FN_5O_4S_2\cdot 0.5H_2O$	C, H, N	271	275
10	OH	8-$SCH_2C_6H_4(NHCOC_6H_4SO_2F$-*p*)-*o*	A[c]	80	210–213	$C_{19}H_{14}FN_5O_4S_2$	C, H; N[g]	277	281
11	OH	8-$SCH_2C_6H_4(NHCO_6H_4SO_2F$-*m*)-*o*	A[c]	45	140–143	$C_{19}H_{14}FN_5O_4S_2$	C, H, N	276	281
12	OH	8-$SCH_2C_6H_4(NHCONHC_6H_4SO_2F$-*m*)-*o*	B[e]	53	184–185	$C_{19}H_{15}FN_6O_4S_2\cdot H_2O$	C, H, N	255, 283[d]	257, 281[d]
14	OH	8-$SCH_2C_6H_4(NHCOC_6H_4SO_2F$-*p*)-*m*	A[e]	74	238–242	$C_{19}H_{14}FN_5O_4S_2$	C, H, N	280	282
15	OH	8-$SCH_2C_6H_4(NHCOC_6H_4SO_2F$-*m*)-*m*	A[e]	37	Indef	$C_{19}H_{14}FN_5O_4S_2\cdot 0.5H_2O$	C, H, N	280	284
16	NH_2	8-$SCH_2C_6H_4(NHCOC_6H_4SO_2F$-*m*)-*m*	A[e]	74	Indef	$C_{19}H_{15}FN_6O_3S_2$	C, H, F	288	288
17	NH_2	8-$SCH_2C_6H_4(NHCONHC_6H_4SO_2F$-*m*)-*m*	B[e]	78	165–167	$C_{19}H_{16}FN_7O_3S_2$	C, H, F	261, 287	253, 287
18	NH_2	8-$SCH_2C_6H_4(NHCOC_6H_4SO_2F$-*p*)-*m*	A[b]	51	Indef	$C_{19}H_{15}FN_6O_3S_2$	C, H, F	289	289

[a] In 10% EtOH. [b] Recrystallized from DMF–H_2O. [c] Recrystallized from MeOEtOH–H_2O. [d] Inflection. [e] Recrystallized from EtOH–H_2O. [f] N: calcd, 17.0; found, 16.4. [g] N: calcd, 15.2; found, 14.5.

36 ml of 1 *N* NaOH was stirred for 2 hr at ambient temperature. The filtered solution was acidified with HOAc. The product was collected on a filter, washed with H_2O, and recrystallized from DMF–H_2O; yield 3.92 g (72%), mp 288–290° dec. *Anal.* ($C_{12}H_{10}N_6O_2S$) C, H, N.

8-(*m*-Aminobenzylthio)adenine (22).—A solution of 2.00 g (6.7 mmoles) of **21** in 200 ml of MeOEtOH was shaken with H_2 at 2–3 atm in the presence of 1.0 g of 10% Pd–C for 12 hr when reduction was complete. The filtered solution was evaporated *in vacuo* and the residue was recrystallized from MeOEtOH–H_2O; yield 1.05 g (58%), mp 268–270° dec. *Anal.* ($C_{12}H_{12}N_6S$) C, H, N.

8-[*m*-(*m*-Fluorosulfonylbenzamido)benzylthio]adenine (16) (Method A).—To a magnetically stirred mixture of 200 mg (0.73 mmole) of **22**, 3 ml of DMF, and 73 mg (0.73 mmole) of Et_3N cooled in an ice bath was added 163 mg (0.73 mmole) of *m*-fluorosulfonylbenzoyl chloride in 1 ml of DMF. After 20 min, the mixture was diluted with several volumes of H_2O. The product was collected on a filter, washed with H_2O, and recrystallized from DMF–H_2O; yield 3.92 g (72%), mp 288–290° dec. *Anal.* ($C_{12}H_{10}N_6O_2S$) C, H, N.

8-[*m*-(*m*-Fluorosulfonylphenylureido)benzylthio]adenine (17) (Method B).—To a stirred suspension of 200 mg (0.73 mmole) of **22** in 3 ml of DMF protected from moisture was added a solution of 161 mg (0.80 mmole) of *m*-fluorosulfonylphenyl isocyanate in 1 ml of DMF. After 12 hr tlc in EtOAc showed the reaction was complete. The solution was diluted with several volumes of H_2O. The product was collected on a filter, washed with H_2O, then recrystallized from EtOH–H_2O; yield 270 mg (78%), mp 165–167°. See Table II for additional data and other compounds prepared by this method.