ucl was collected on a filter and washed with H_2O. Recrystallization from EtOH–THF gave 2.63 g (72%) of yellow crystals, mp 166–170°.

See Table IV for additional data and other compounds prepared by this method.

1-(*m*-Aminophenyl)-4-(*p*-fluorosulfonylphenyl)butane Ethanesulfonate (24c).—A solution of 1.05 g (3.2 mmoles) of **23c** (Table III), 0.35 g (3.2 mmoles) of $EtSO_3H$, 100 ml of 95% EtOH, and 100 mg of PtO_2 was shaken with H_2 at 2–3 atm until the uv of the solution no longer showed a double bond conjugated with the ring. The filtered solution was evaporated to a thin syrup *in vacuo*, then stored at 0° until crystallization started. The mixture was slightly thinned with 2-PrOH, then filtered. The product was washed with cold 2-PrOH, then recrystallized from C_6H_6 petroleum ether (bp 30–60°); yield 0.50 g (38%), mp 114–116°. *Anal.* ($C_{18}H_{24}FNO_5S_2 \cdot C_2H_5SO_3H$) C, H, N.

Irreversible Enzyme Inhibitors. CLXI.[1,2] Proteolytic Enzymes. XIII.[3] Inhibitors of Guinea Pig Complement Derived by Quaternization of 3-Acylamidopyridines with α-Bromomethylbenzenesulfonyl Fluorides. II

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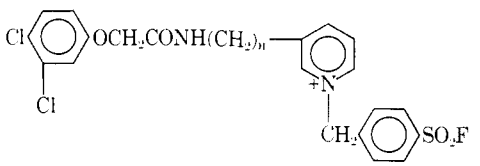
Received May 29, 1969

Twenty quaternary salts (**26**) derived from N-(3-pyridyl)- or N-(3-pyridylmethyl)-3,4-dichlorophenoxyacetamide (**24**) by reaction with substituted fluorosulfonylbenzyl bromides (**25**) were evaluated as inhibitors of the lysis of sheep red blood cells by hemolysin and complement. The most effective compound was 3-(3,4-dichlorophenoxyacetamido)-N-(6-chloro-2-fluorosulfonylbenzyl)pyridinium bromide (**16**); at 62 and 31 μ*M*, **16** showed 84 and 45% inhibition, respectively. A number of these compounds were excellent irreversible inhibitors of α-chymotrypsin; for example, **16** had an $I_{50} \sim K_i$ of 5.7 μ*M* and at this concentration gave 98% inactivation in 2 min.

Inhibition of the serum complement system has potential medical use for organ transplantation and in certain arthritic states.[5,6] One of the normal functions of the complement system, a complex mixture of at least eleven serum proteins, is for rejection of foreign cells by lysis.[6,7] Since some of the proteins of the complement system are proteases with "tryptic" or "chymotryptic" properties,[6,7] this system can be inhibited with inhibitors of trypsin[3] or chymotrypsin[3,8] when measured by complement-antibody-mediated lysis of sheep red blood cells (RBC).[3,9]

Among the inhibitors of guinea pig complement found in this laboratory are the pyridine quaternaries, **1** and **2**;[3] it was also established that the SO_2F moiety was necessary for activity.[3] For example, 0.5 m*M* **1**



1, $n = 0$
2, $n = 1$

showed 45% inhibition of complement when measured by RBC lysis. This observation has been verified by Becker;[10] 0.4 m*M* **1** could inhibit one out of two complement units in his assay system.[11] Furthermore, he observed that **1** at 0.4 m*M* was an irreversible inhibitor of the C'1s component with a half-life of 18 min.[10]

Sixteen additional variants of **1** and **2** with changes in the fluorosulfonylbenzyl moiety have now been synthesized for evaluation as inhibitors of the complement system; these have also been evaluated as irreversible inhibitors of chymotrypsin. Some of these variants are 25 times as effective as **1** or **2** as inhibitors of the complement system.

Complement Inhibition.—The data in Table I indicates the effect of a given concentration of compound on lysis of RBC catalyzed by complement, compared to a control with no compound. Any lysis of RBC by the compound in the absence of complement is expressed as a percentage of the total lysis possible, 0.7 OD unit, corrected for 0–5% lysis in the absence of compound and complement.[3]

The *p*-SO_2F quaternaries (**1** and **2**) were previously reported[3] from this laboratory to give about 50% inhibition of complement when assayed at 0.5 and 1 m*M*, respectively (Table I); when the SO_2F group was moved to the *meta* position, activity was improved less than twofold.[3] The *o*-SO_2F isomers (**5** and **6**) have now been synthesized for comparison. Activity was considerably enhanced, being about tenfold with **5** and about 25-fold with **6**; the two compounds showed 50% inhibition somewhere between 0.031 and 0.062 m*M*.

The effect of chloro substitution on the fluorosulfonylbenzyl moiety was then studied. There are two possi-

(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, E. E. Janson, and N. M. J. Vermeulen, *J. Med. Chem.*, **12**, 898 (1969).

(3) For the previous paper on complement see B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 677 (1969), paper CLVI of this series.

(4) NDEA predoctoral fellow.

(5) B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **12**, 408 (1969), paper CLII of this series.

(6) H. J. Müller-Eberhard, *Advan. Immunol.*, **8**, 1 (1968).

(7) (a) Ciba Foundation Symposium, "Complement," G. E. W. Wolstenholme and J. Knight, Ed., Little, Brown and Co., Boston, Mass., 1965; (b) P. H. Sehon and K. F. Austen, *Ann. Rev. Med.*, **19**, 1 (1968).

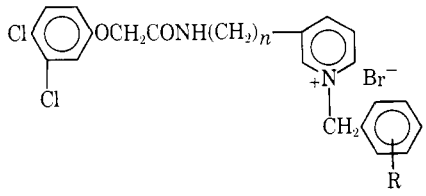
(8) B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **12**, 115 (1969), paper CLIII of this series.

(9) E. A. Kabat and M. M. Mayer, "Experimental Immunochemistry," 2nd ed, Charles C Thomas, Springfield, Ill., 1967, pp 149–153.

(10) Private communication from Dr. E. L. Becker, Walter Reed Army Medical Center.

(11) M. M. Glovsky, E. L. Becker, and N. J. Hallbrook, *J. Immunol.*, **100**, 979 (1968).

TABLE I: INHIBITION[a,b] OF GUINEA PIG COMPLEMENT BY

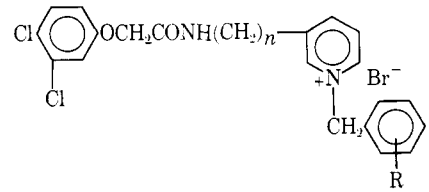


No.	n	R	Concn, mM	% inhibn	% lysis[c]
1[d]	0	p-SO_2F	0.5	45	4
			0.25	12	
2[d]	1	p-SO_2F	1	56	10
			0.5	19	
3[d]	0	m-SO_2F	0.5	58	7
4[d]	1	m-SO_2F	1	63	7
			0.5	33	
5	0	o-SO_2F	0.25	89	0
			0.125	91	
			0.062	63	
			0.031	27	
6	1	o-SO_2F	1	93	5
			0.125	89	
			0.062	68	
			0.031	40	
7	0	2-Cl-4-SO_2F	0.25[e]	71	3
			0.125	37	
8	0	3-Cl-4-SO_2F	0.125[e]	28	2
			0.062	0	
9	1	2-Cl-4-SO_2F	1	88	3
			0.5	68	
			0.25	33	
10	1	3-Cl-4-SO_2F	0.5[e]	42	3
			0.25	9	
11	0	4-Cl-3-SO_2F	0.125[e]	30	2
			0.062	11	
12	1	4-Cl-3-SO_2F	0.5[e]	51	0
			0.25	15	
13	0	3-Cl-2-SO_2F	0.25[e]	88	6
			0.125	82	
			0.062	49	
14	0	4-Cl-2-SO_2F	0.125[e]	96	0
			0.062	79	
			0.031	17	
15	0	5-Cl-2-SO_2F	0.25[e]	94	2
			0.125	90	
			0.062	68	
			0.031	30	
16	0	6-Cl-2-SO_2F	0.5	94	4
			0.125	95	
			0.062	84	
			0.031	45	
17	1	3-Cl-2-SO_2F	0.5	92	5
			0.25	88	
			0.125	67	
			0.062	42	
18	1	4-Cl-2-SO_2F	0.5	90	4
			0.25	95	
			0.125	90	
			0.062	46	
			0.031	12	
19	1	5-Cl-2-SO_2F	1	95	4
			0.125	93	
			0.062	65	
			0.031	29	
20	1	6-Cl-2-SO_2F	0.5	85	8
			0.25	88	
			0.125	90	
			0.062	56	
			0.031	22	

[a] The technical assistance of Sharon Lafler with these assays is acknowledged. [b] See ref 5 for assay of inhibition of sheep red blood cell lysis by hemolysin and guinea pig complement. The compounds were dissolved in either MeOEtOH or 4:1 MeOEtOH–H_2O for assay. [c] Lysis in the absence of complement corrected for 0–5% lysis in the absence of compound; the number is expressed as a per cent of the total lysis possible, 0.7 OD unit. [d] Data from ref 3. [e] Maximum solubility in the assay mixture.

TABLE II

INHIBITION[a] OF CHYMOTRYPSIN BY



				Irreversible		
No.	n	R	I_{50},[b] μM	Inhib, μM	Time, min	% inactvn
1[d]	0	p-SO_2F	15	15	2, 8, 30[e]	81, 89, 100
2[d]	1	p-SO_2F	16	16	0.5, 4, 8[e]	50, 96, 100
3[d]	0	m-SO_2F	51	51	2, 8, 30[e]	50, 90, 100
4[d]	1	m-SO_2F	96	96	2, 4[e]	98, 100
5	0	o-SO_2F	13	13	<2[e]	100
6	1	o-SO_2F	3.2	3.2	<2[e]	100
7	0	2-Cl-4-SO_2F	12	12	30	100
8	0	3-Cl-4-SO_2F	14	14	30	84
9	1	2-Cl-4-SO_2F	20	20	30	100
10	1	3-Cl-4-SO_2F	120	60	30	100
11	0	4-Cl-3-SO_2F	13	13	30	41
12	1	4-Cl-3-SO_2F	200	200	30	100
13	0	3-Cl-2-SO_2F	28	28	30	100
14	0	4-Cl-2-SO_2F	12	12	30	100
15	0	5-Cl-2-SO_2F	6.0	6.0	30	100
16	0	6-Cl-2-SO_2F	5.7	5.7	2, 4[e]	98, 100
17	1	3-Cl-2-SO_2F	37	37	2, 30[e]	93, 100
18	1	4-Cl-2-SO_2F	4.4	4.4	<2[e]	100
19	1	5-Cl-2-SO_2F	3.3	3.3	30	100
20	1	6-Cl-2-SO_2F	4.4	4.4	<2[e]	100

[a] The technical assistance of Julie Leseman with these assays is acknowledged. [b] Assayed with 200 μM N-glutaryl-L-phenylalanine p-nitroanilide in 0.05 M Tris buffer containing 10% DMSO as previously described by B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **10**, 1129 (1967); I_{50} = concentration for 50% inhibition which is about equivalent to K_i. [c] Inactivation performed with $\simeq 1$ μM enzyme at 24° in 0.05 M Tris buffer (pH 7.4) containing 10% DMSO, then the remaining enzyme assayed with N-benzoyl-L-tyrosine ethyl ester in pH 8.1 Tris buffer containing 0.1 M $CaCl_2$ as described by B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **12**, 118 (1969). [d] Data from ref 13. [e] From a six-point time study.

ble chloro derivatives of both **1** and **2**. The two chloro derivatives (**7**, **8**) of **1** showed enhanced inhibition by a factor of 2–4; similar results were observed with the chloro derivatives (**9**, **10**) of **2**.

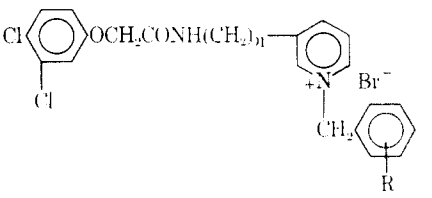
Only one each (**11**, **12**) of the possible chloro derivatives of the m-SO_2F isomers (**3**, **4**) were synthesized; less than twofold enhancement of activity was observed. Although three other chloro-m-fluorosulfonylbenzyl derivatives are possible these were not synthesized since (a) the o-SO_2F series (below) gave better inhibition, and (b) their synthesis would be quite laborious.

The four possible chloro-o-fluorosulfonylbenzyl derivatives in each series were synthesized. All four chloro derivatives (**13**–**16**) of **5** showed good potency. The 3-chloro derivative (**13**) was slightly less potent than the parent **5**, the 4-chloro (**14**) and 5-chloro (**15**) were about equipotent to **5**, and the 6-chloro derivative (**16**) was about twice as potent; thus **16** is about 15 times more potent than the first compound (**1**)[3] evaluated in this series of pyridinium quaternaries.

The four chloro derivatives (**17**–**20**) of **6** did not show increased potency over **6**. Both the 5-chloro (**19**) and 6-chloro (**20**) were about equipotent to **6**, but the 3-chloro (**17**) and 4-chloro (**18**) were about half as potent.

Table III

Physical Properties of

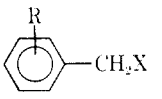


No.	n	R	Method	Yield, %	Mp, °C	Formula	Analyses
5	0	o-SO_2F	B	55[a]	202–204	$C_{20}H_{16}BrCl_2FN_2O_4S$	C, H, F
6	1	o-SO_2F	B	18[a]	160–162	$C_{21}H_{18}BrCl_2FN_2O_4S$	C, H, F
7	0	2-Cl-4-SO_2F	A	98[b]	151–153	$C_{20}H_{15}BrCl_3FN_2O_4S$	C, H, F
8	0	3-Cl-4-SO_2F	B	73[b]	228–229	$C_{20}H_{15}BrCl_3FN_2O_4S$	C, H, N
9	1	2-Cl-4-SO_2F	B	78[a]	128–130	$C_{21}H_{17}BrCl_3FN_2O_4S$	C, H, F
10	1	3-Cl-4-SO_2F	B	56[a]	217–218	$C_{21}H_{17}BrCl_3FN_2O_4S$	C, H, N
11	0	4-Cl-3-SO_2F	B	55[b]	243–246	$C_{20}H_{15}BrCl_3FN_2O_4S$	C, H, N
12	1	4-Cl-3-SO_2F	B	75[a]	205–208	$C_{21}H_{17}BrCl_3FN_2O_4S$	C, H, N
13	0	3-Cl-2-SO_2F	B	53[a]	214–216	$C_{20}H_{15}BrCl_3FN_2O_4S$	C, H, N
14	0	4-Cl-2-SO_2F	A	88[b]	210–213	$C_{20}H_{15}BrCl_3FN_2O_4S$	C, H, F
15	0	5-Cl-2-SO_2F	B	27[a]	207–208	$C_{20}H_{15}BrCl_3FN_2O_4S$	C, H, F
16	0	6-Cl-2-SO_2F	B	41[a]	189–191	$C_{20}H_{15}BrCl_3FN_2O_4S$	C, H, N
17	1	3-Cl-2-SO_2F	B	44[a]	189–191	$C_{21}H_{17}BrCl_4FN_2O_4S$	C, H, N
18	1	4-Cl-2-SO_2F	B	67[b]	186–188	$C_{21}H_{17}BrCl_3FN_2O_4S$	C, H, F
19	1	5-Cl-2-SO_2F	B	88[b]	194–196	$C_{21}H_{17}BrCl_3FN_2O_4S$	C, H, F
20	1	6-Cl-2-SO_2F	B	9[c]	142–145	$C_{21}H_{17}BrCl_3FN_2O_4S$	C, H, N

[a] Recrystallized from Me_2CO. [b] Recrystallized from EtOH. [c] Recrystallized from Me_2CO–toluene.

Table IV

Physical Properties of



No.	R	X	Method	Yield, %	Mp, °C	Bp, °C (mm)	Formula	Analyses
27	o-SO_2F	H	D[a,b]	65		33–34 (0.15)	$C_7H_7FO_2S$	
28	2-Cl-4-SO_2F	H	D[c]	67[d]	16–18	52–56 (0.05)	$C_7H_6ClFO_2S$	C, H, F
29	3-Cl-4-SO_2F	H	D[c]	63[e]	33–34	68–70 (0.20)	$C_7H_6ClFO_2S$	C, H, F
30	4-Cl-3-SO_2F	H	D[a]	70[f]	39–41	67–75 (0.20)	$C_7H_6ClFO_2S$	C, H
31	3-Cl-2-SO_2F	H	D[a]	57[b]	35–37		$C_7H_6ClFO_2S$	
32	4-Cl-2-SO_2F	H	D[f]	70[b]	30–32	44–48 (0.05)	$C_7H_6ClFO_2S$	C, H, F
33	5-Cl-2-SO_2F	H	D[g]	68[e]	41–43		$C_7H_6ClFO_2S$	C, H, F
34	6-Cl-2-SO_2F	H	D[c]	61[f]	45–58		$C_7H_6ClFO_2S$	C, H
35	o-SO_2F	Br	E	40[g]	85–87		$C_7H_6BrFO_2S$	C, H, S
36	2-Cl-4-SO_2F	Br	E	26[e]	53–55		$C_7H_5BrClFO_2S$	C, H, F
37	3-Cl-4-SO_2F	Br	E	36[f]	83–86		$C_7H_5BrClFO_2S$	C, H, F
38	4-Cl-3-SO_2F	Br	E[h]	98	Oil		$C_7H_5BrClFO_2S$	
39	3-Cl-2-SO_2F	Br	E	56[f]	91–94		$C_7H_5BrClFO_2S$	C, H
40	4-Cl-2-SO_2F	Br	E[i]	63	Oil		$C_7H_5BrClFO_2S$	
41	5-Cl-2-SO_2F	Br	E	97	Oil		$C_7H_5BrClFO_2S$	
42	6-Cl-2-SO_2F	Br	E	98[m]	48–50[f]		$C_7H_5BrClFO_2S$	C, H

[a] This compound was made by an alternate procedure by W. Davies and J. H. Dick, *J. Chem. Soc.*, 2104 (1931). [b] The starting sulfonyl chloride has been reported by L. Harding, *ibid.*, **119**, 260 (1921). [c] The starting sulfonyl chloride has been reported by W. Davies, *ibid.*, **119**, 853 (1921). [d] The compound was not recrystallized. [e] The starting sulfonyl chloride has been reported by W. A. Silvester and W. P. Wynne, *J. Chem. Soc.*, 691 (1936). [f] Recrystallized from petroleum ether (60–110°). [g] Made by N. M. J. Vermeulen of this laboratory; the starting sulfonyl chloride has been reported by W. P. Wynne and J. Bruce, *J. Chem. Soc.*, **73**, 731 (1898). [h] Recrystallized from petroleum ether (30–60°). [i] The starting sulfonyl chloride has been reported by M. W. Coombs, T. M. Sharp, and A. G. Turner, British Patent 709,992 (1954); *Chem. Abstr.*, **49**, P10379*h* (1955). [j] The starting sulfonyl chloride has been reported by R. Herz, W. Bauer, N. Steiger, E. Albrecht, and R. Dreser, German Patent 555,140 (1926); *Chem. Abstr.*, **26**, P5105 (1932). [k] The starting sulfonyl chloride has been reported by J. T. Hackmann and V. P. Pittman, British Patent 956,857 (1964); *Chem. Abstr.*, **61**, P1795*e* (1964). [l] The oil was dissolved in petroleum ether (30–60°) and cooled and the solution was decanted from the yellow residue. The crude product was obtained by evaporation of the solution *in vacuo*. [m] Crude product suitable for the next step.

The most potent compound in the series (**1**–**20**) to date is **16** which shows 84% inhibition at 0.062 mM and 45% inhibition at 0.031 mM; whether or not activity in this series can be further enhanced is currently being investigated in this laboratory.

Chymotrypsin Inhibition. Since **1**–**4** were the best active-site-directed irreversible inhibitors[12] of chymotrypsin yet reported,[13] the new analogs synthesized

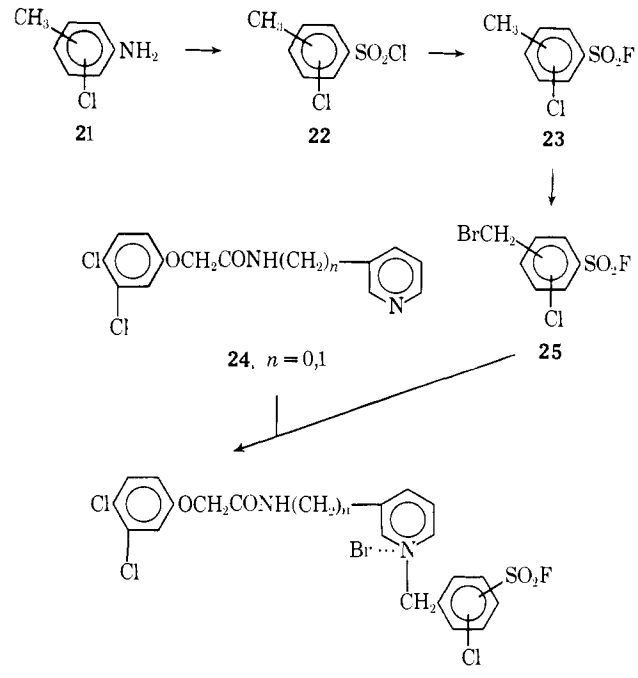
(12) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," John Wiley and Sons, Inc., New York, N. Y., 1967.

(13) B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **12**, 221 (1969), paper CL of this series.

(**5**–**20**) for inhibition of complement were investigated as inhibitors of chymotrypsin (Table II); some of the new compounds were even faster irreversible inhibitors. It is notable that the most potent compounds on complement (**16**, **5**, **6**, **17**, and **20**) were extremely rapid irreversible inhibitors of chymotrypsin when incubated at an $I_{50} \simeq K_i$[14] concentration giving 93–100% inactivation in 2 min or less; furthermore, all but **17** (I_{50} = 37 μM) had excellent I_{50}'s in the 3–13-μM range.

Chemistry.—The new inhibitors (**5**–**20**) in Table I can be generalized by **26**; these were made by quaternization of the appropriate pyridylamides (**24**) with the requisite fluorosulfonylbenzyl bromides (**25**). The necessary benzyl bromides were made by the sequence of **21** → **25** previously employed for *m*-fluorosulfonylbenzyl bromide[13] (see Scheme I).

SCHEME I



Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample had an appropriate ir spectrum and moved as a single spot on tlc on Brinkmann silica gel GF; Brinkmann MN-polyamide was used for the quaternary salts. Combustion values within 0.4% of theoretical for C, H, and N or F were obtained.

3-(3,4-Dichlorophenoxyacetamido)-N-(4-chloro-2-fluorosulfonylbenzyl)pyridinium Bromide (14) (Method A).—A solution of 0.594 g (2.0 mmoles) of N-(3-pyridyl)-3,4-dichlorophenoxyacetamide (**24**, n = 0)[13] and 2.0 g (5 mmoles) of 4-chloro-2-fluorosulfonyl-α-bromotoluene (**40**) (Table IV) in 15 ml of Me_2CO was refluxed for 18 hr. The product was collected on a filter, washed with Me_2CO and crystallized from EtOH; yield 1.03 g (88%) of white solid, mp 210–213°. See Table III for additional data and other compounds made by this procedure.

Method B was the same as method A except that only 10 ml of Me_2CO was used and the solution was stirred at room temperature for 48 hr.

2-Methyl-6-chlorobenzenesulfonyl Chloride (Method C).—To a stirred mixture of 100 g (0.70 mole) of 2-methyl-6-chloroaniline and 250 ml of 12 *N* HCl cooled in an Me_2CO–ice bath was added dropwise a solution of 52 g (0.75 mole) of $NaNO_2$ in 70 ml of H_2O at such a rate that the temperature remained between −5 and 5° (20 min). The resulting solution was added portionwise (15 min) to a mixture of 20 g of $CuCl_2$, 20 ml of H_2O, and 500 ml of glacial HOAc saturated with SO_2. The temperature was maintained at about 25° and N_2 was evolved. After the mixture was stirred an additional 15 min, it was diluted with 2000 ml of ice water and extracted with three 200-ml portions of C_6H_6. The combined extracts were washed with two 200-ml portions of H_2O, dried ($MgSO_4$), treated with activated charcoal, then evaporated *in vacuo*. Distillation yielded 53 g (34%) of colorless oil, bp 88–89° (0.05 mm).

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

The other sulfonyl chlorides used in Table IV were made by this procedure[15] in 30–73% yields. See Table IV for references to alternate methods of synthesis; in each case the same melting point or boiling point was obtained.

2-Fluorosulfonyl-4-chlorotoluene (32) (Method D).—A mixture of 40 g (0.18 mole) of 2-chlorosulfonyl-4-chlorotoluene, 50 ml of dioxane, 30 g (0.50 mole) of KF, 10 ml of DMF, and 10 ml of H_2O (added in that order with stirring) was refluxed with mechanical stirring for 30 min; 600 ml of ice-cold H_2O was added, and the product was extracted with two 200-ml portions of C_6H_6. The combined extracts were washed with two 200-ml portions of H_2O, dried with $MgSO_4$, then evaporated *in vacuo*. Distillation gave 26 g (70%) of colorless oil, bp 44–48° (0.05 mm), which quickly crystallized, mp 30–32°. A small amount was recrystallized from petroleum ether (60–110°) for analysis, mp 30–31°. See Table IV for additional data and other compounds made by this procedure.

Method E.—The substituted toluenesulfonyl fluorides were brominated with NBS in CCl_4 as previously described.[13] *These compounds are severe skin irritants and should be handled with caution.*

(15) (a) H. Meerwein, G. Dittmar, R. Göllner, K. Hafner, F. Mensch, and O. Steinfort, *Chem. Ber.*, **90**, 841 (1957); (b) B. R. Baker and J. K. Coward, *J. Heterocycl. Chem.*, **4**, 195 (1967), paper XC of this series.