Spiro[fluoreneisothiazolidin]one Dioxides: New Aldose Reductase and L-Hexonate Dehydrogenase Inhibitors[1]

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The first examples of spiro[fluorene-9,4′- and -9,5′-isothiazolidin]one dioxides (1 and 2) were synthesized and screened for activity as aldose reductase and L-hexonate dehydrogenase inhibitors. Compared to compounds 1, and 9,5′-compounds 2, synthesized from fluorene-9-sulfonamides by alkylation at C(9) with ethyl bromoacetate followed by cyclization, were more active, but relatively nonselective, inhibitors of aldose reductase and L-hexonate dehydrogenase, with IC_{50} values for in vitro inhibition of both enzymes on the order of 10^{-7}–10^{-8} M. However, the isomeric 9,4′-compounds 1, prepared by alkylation of fluorene-9-carboxylic acid esters with bromo- or iodomethanesulfonamide followed by cyclization, were more selective inhibitors of L-hexonate dehydrogenase with IC_{50} values of about 10^{-6} M.

Aldose reductase (AR, EC 1.1.1.21) and L-hexonate dehydrogenase (L-HDH, EC 1.1.1.19) are structurally homologous 35 kDa monomeric proteins belonging to the aldehyde reductase class (EC 1.1.1.2) of NADPH-dependent oxidoreductases.[2] Because the two enzymes have overlapping specificities for a large number of aldehyde and sugar aldose substrates, the question of distinct physiologic functions of these enzymes has not been completely resolved.[2] However, there is considerable experimental evidence that AR plays an important role in the pathophysiology of chronic experimental diabetes and galactosemia.[3] AR catalyzes the reduction of sugar aldoses to the respective polyols. After a sustained sugar insult, these polyols (sorbitol or dulcitol) accumulate rapidly in the most susceptible cell types,[3,4] thereby causing metabolic changes and increases in intracellular osmolality. These early biochemical alterations are thought to initiate the progression of debilitating functional and eventual morphological alterations associated with chronic diabetes in both humans and animal models of the disease.[3,4] The strongest evidence for a causal role of AR in diabetic complications has come from many studies demonstrating that potent inhibitors of AR can prevent not only the early elevation of intracellular sorbitol content but also later developing functional and morphological alterations in tissues such as lens, sciatic nerve, retina, and kidney of diabetic and galactosemic animals.[5] On the basis of considerable preclinical evidence, several aldose reductase inhibitors are now in clinical trials for diabetic complications.

The distinctions between AR and L-HDH include different catalytic efficiencies toward many common substrates, characteristic tissue distributions, differing susceptibilities to inhibition by various classes of compounds,[2,6] and distinct genes.[7] Regarding catalytic properties, AR has a lower K_m than L-HDH for glucose and other aldose sugars,[2] and although both enzymes have high affinity for NADPH, only AR appears to also utilize NADH at high concentrations.[8] Like many pyridine nucleotide dependent oxidoreductases, both AR and L-HDH have been shown to function as catalysts of the "reverse" reaction, that is, oxidation of an appropriate alcohol substrate to the corresponding aldehyde by $NADP^+$ (or an analogue).[9] However, the specificity of the oxidation reaction appears to be considerably more stringent than the reduction reaction. For example, dulcitol and sorbitol are very poor substrates for *oxidation* by AR[9a] and are inactive with L-HDH.[9b] L-Hexonate dehydrogenase was so designated due to its remarkable specificity for the oxidation of L-hexonic acids, such as L-gulonic, L-mannonic, and L-idonic acid, by $NADP^+$.[9b] The L-hexonic acids are oxidized to the corresponding D-hexuronic acids, which are likewise much better substrates for NADPH-supported reduction by L-HDH than by AR.[2b,8,9b] Although the physiologic role(s) of L-HDH have remained elusive, the enzyme appears not to participate significantly in polyol formation *in vivo*, since both sorbitol accumulation and sorbitol-linked functional alterations in tissues of diabetic animals are prevented very effectively by selective inhibition of AR.[2,4b,5a-d] The inhibition of L-HDH by aldose reductase inhibitors (ARIs) and the possible consequences of such inhibition over the course of chronic ARI therapy are legitimate concerns in the development of any ARI.

As part of an exploration of the effect of varying the spiro ring portion of aldose reductase inhibitors such as imirestat (AL1576/HOE843), the isomeric 9,4′- and 9,5′-spiro[fluoreneisothiazolidin]one dioxides, 1 and 2, respectively, were targeted as potential aldose reductase

(1) Presented in part at the 97th National Meeting of the American Chemical Society, Dallas, Texas, April 1989; MEDI 21.
(2) (a) Flynn, T. G. *Biochemical Pharmacol.* **1982**, *31*, 2705. (b) Wermuth, B. In *Enzymology of Carbonyl Metabolism 2. Aldehyde Dehydrogenase, Aldo-Keto Reductases, and Alcohol Dehydrogenase*; Weiner, H., Flynn, T. G., Eds.; Alan R. Liss, Inc.: New York, 1985; pp 209–230.
(3) (a) Kador, P. F.; Robison, W. G.; Kinoshita, J. H. *Annu. Rev. Pharmacol. Toxicol.* **1985**, *25*, 691. (b) Dvornik, D. *Aldose Reductase Inhibition: An Approach to the Prevention of Diabetic Complications*; Porte, D., Ed.; Biomedical Information Corp.: New York, 1987.
(4) (a) Gabbay, K. H. *N. Engl. J. Med.* **1973**, *288*, 831. (b) Kinoshita, J. H.; Fukushi, S.; Kador, P.; Merola, L. O. *Metabolism* **1979**, *28* (Suppl. 1), 462.
(5) (a) Peterson, M. J.; Sarges, R.; Aldinger, C. E.; MacDonald, D. P. *Metabolism* **1979**, *28* (Suppl. 1), 456. (b) Kikkawa, R.; Hatanaka, I.; Yasuda, A.; Kobayashi, N.; Shigeta, Y.; Terashima, H.; Morimura, T.; Tsuboshima, M. *Diabetologia* **1983**, *24*, 290. (c) Simard-Duquesne, N.; Greselin, E.; Dubuc, J.; Dvornik, D. *Metabolism* **1985**, *34*, 885. (d) Stribling, D.; Mirrlees, D. J.; Harrison, H. E.; Earl, D. C. N. *Metabolism* **1985**, *34*, 336. (e) Griffin, B. W.; McNatt, L. G.; Chandler, M. L.; York, B. M. *Metabolism* **1987**, *36*, 486.
(6) (a) Flynn, T. G. *Metabolism* **1986**, *35* (Suppl. 1), 105. (b) Sato, S.; Kador, P. F. *Invest. Ophthalmol. Vis. Sci.* **1989**, *30*, 1618.
(7) (a) Bohren, K. M.; Bullock, B.; Wermuth, B.; Gabbay, K. H. *J. Biol. Chem.* **1989**, *264*, 9547. (b) Nishimura, C.; Wistow, G.; Carper, D. In *Enzymology and Molecular Biology of Carbonyl Metabolism 2. Aldehyde Dehydrogenase, Alcohol Dehydrogenase, and Aldo-Keto Reductase*; Weiner, H., Flynn, T. G., Eds.; Alan R. Liss, Inc.: New York, 1989; pp 211–220.
(8) (a) Ward, W. H. J.; Sennitt, C. M.; Ross, H.; Dingle, A.; Timms, D.; Mirrlees, D. J.; Tuffin, D. P. *Biochemical Pharmacol.* **1990**, *39*, 337. (b) Cromlish, J. A.; Flynn, T. G. *J. Biol. Chem.* **1983**, *258*, 3583.
(9) (a) Griffin, B. W.; McNatt, L. G. *Arch. Biochem. Biophys.* **1986**, *246*, 75. (b) Mano, Y.; Suzuki, K.; Yamada, K.; Shimazono, N. *J. Biochemistry* **1961**, *49*, 618.

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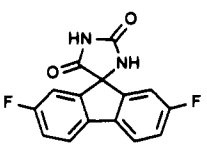
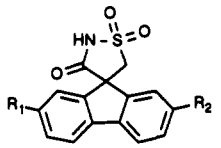


imirestat



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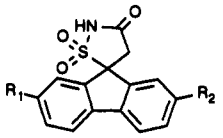


2

inhibitors. This paper describes the syntheses of the first examples of these novel classes of compounds and their activities as inhibitors of aldose reductase and L-hexonate dehydrogenase. Their relative selectivity as inhibitors of these enzymes is compared to that of the well-characterized aldose reductase inhibitors quercetin, sorbinil, imirestat, tolrestat, and ponalrestat.

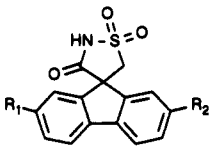
Chemistry

The spiro[9*H*-fluorene-9,4′-isothiazolidin]-3′-one 1′,1′-dioxides **1a–d** (Table I) were prepared in two steps from the fluorene-9-carboxylic acid esters **3a–d** (Scheme I). Esters **3b–d** were prepared from the corresponding fluorenes by deprotonation using *n*-butyllithium followed by reaction with carbon dioxide and esterification using methanolic hydrogen chloride.[10] Alkylation of the fluorene esters with bromo or iodomethanesulfonamide under basic conditions provided the intermediate sulfonamide esters **4a–d**. Very poor yields of **4c** and **4d** were obtained from the reaction of bromomethanesulfonamide[11] with **3c** in the presence of potassium carbonate in DMF or with **3d** using sodium methoxide in methanol. Modest improvement was seen in the preparation of **4a** and **4b** using impure iodomethanesulfonamide[12] with potassium carbonate in DMF. The sulfonamide esters **4a–d** were subsequently cyclized to the spiro[fluorene-9,4′-isothiazolidin]one dioxides upon exposure to sodium methoxide in ethanol at room temperature in yields ranging from 16 to 75%. Given the similarity of the reaction conditions for the alkylation and cyclization steps, it was surprising that none of the cyclized product was observed in the alkylation reactions.

An analogous synthetic strategy was employed for the preparation of the spiro[9*H*-fluorene-9,5′-isothiazolidin]-3′-one 1′,1′-dioxides **2a–d** (Table I and Scheme II). The fluorene-9-sulfonamides **5a–d** were first alkylated with ethyl bromoacetate at room temperature using sodium hydride in DMF to provide the sulfonamide esters **6a–d** in yields ranging from 25 to 50%. Cyclization was accomplished using either sodium methoxide in methanol in the case of **6a** or with sodium hydride in THF for **6b–d**.

Fluorene-9-sulfonamide (**5a**) was prepared in 88% yield from fluorene-9-sulfonyl chloride by reaction with ammonia. Since Paquette's method for the preparation of fluorene-9-sulfonyl chloride[13] involving the reaction of fluorene with sulfuryl chloride did not work with halo-

Table I. Spiro[fluorene-9,4′- and -9,5′-isothiazolidin]one Dioxides



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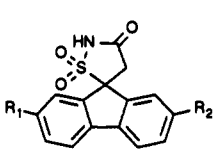

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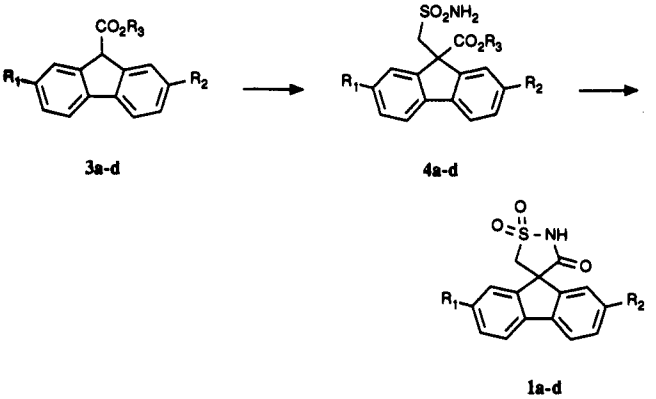
compd	R_1	R_2	mp,[a] °C	% yield	formula[c]
1a	H	H	233–237 dec[b]	30	$C_{15}H_{11}NO_3S$
1b	F	H	263–270 dec	16	$C_{15}H_{10}FNO_3S$
1c	Cl	H	266–279 dec	66	$C_{15}H_{10}ClNO_3S$
1d	F	F	260–277 dec	75	$C_{15}H_9F_2NO_3S$
2a	H	H	236–239 dec	27	$C_{15}H_{11}NO_3S$
2b	F	H	259–263 dec	55	$C_{15}H_{10}FNO_3S$
2c	Cl	H	246–248 dec	27	$C_{15}H_{10}ClNO_3S$
2d	F	F	259–262 dec	30	$C_{15}H_9F_2NO_3S$

[a] Recrystallized from ethyl acetate/hexane unless noted otherwise. [b] Recrystallized from methylene chloride/hexane. [c] All compounds had elemental analyses (C, H, N) within 0.4% of the theoretical value.

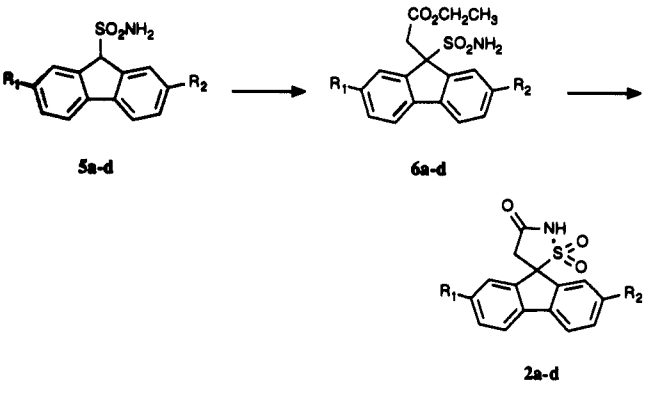
Scheme I. Synthesis of Spiro[fluorene-9,4′-isothiazolidin]one Dioxides **1a–d**



3a-d **4a-d** **1a-d**

a, $R_1 = R_2 = H$, $R_3 = Et$
b, $R_1 = F$, $R_2 = H$, $R_3 = Me$
c, $R_1 = Cl$, $R_2 = H$, $R_3 = Me$
d, $R_1 = R_2 = F$, $R_3 = Me$

Scheme II. Synthesis of Spiro[fluorene-9,5′-isothiazolidin]one Dioxides **2a–d**



5a-d **6a-d** **2a-d**

a, $R_2 = R_2 = H$
b, $R_1 = F$, $R_2 = H$
c, $R_1 = Cl$, $R_2 = H$
d, $R_1 = R_2 = F$

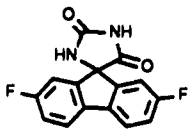
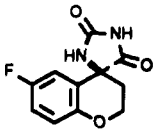
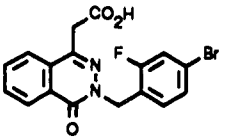
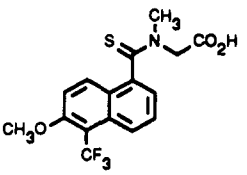
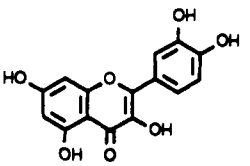
(10) York, B. M. US Patent 4,537,892, Aug 27, 1985.

(11) Bromomethanesulfonamide was prepared from bromomethanesulfonyl chloride using the procedure described for the preparation of bromoethanesulfonamide from bromoethanesulfonyl chloride, see: Carpino, L. A.; McAdams, L. V.; Rynbrandt, R. H.; Spiewak, J. W. *J. Am. Chem. Soc.* **1971**, *93*, 476. Bromomethanesulfonyl chloride was prepared using the method of Truce et al.: Truce, W. E.; Abraham, D. J.; Son, P. *J. Org. Chem.* **1967**, *32*, 990.

(12) Iodomethanesulfonamide was prepared from bromomethanesulfonamide by reaction with sodium iodide in acetone. Since we were unable to force the reaction to completion or separate the two halides, a mixture was used.

(13) Paquette, L. A.; Freeman, J. P.; Houser, R. W. *J. Org. Chem.* **1969**, *34*, 2901.

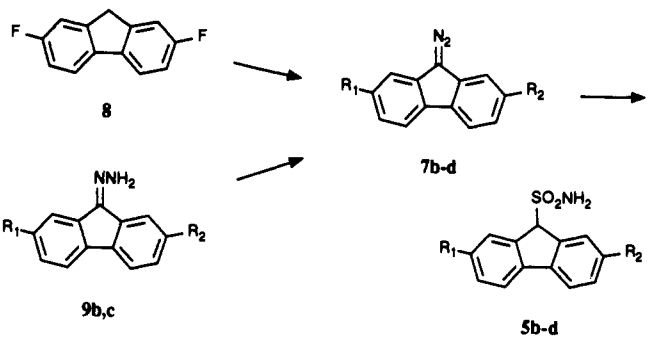
Table II. Inhibitor Activity against Rat Lens Aldose Reductase and Rat Kidney L-Hexonate Dehydrogenase

compd	structure	IC_{50}, μM RL AR	IC_{50}, μM RK L-HDH	selectivity quotient[a]
1a		>10000	4.50	>2000
1b		>10000	2.10	>5000
1c		2370	3.51	675
1d		6150	3.75	1640
2a		1.90	1.75	1.09
2b		0.169	0.151	1.12
2c		0.233	0.105	2.22
2d		0.018	0.027	0.68
10		0.005	0.009	0.56
imirestat		0.009	0.005	1.8
sorbinil		0.10	0.99	0.10
ponalrestat		0.02	2.40	0.008
tolrestat		0.002	0.95	0.002
quercetin		7.70	1.00	7.7

[a] The selectivity quotient is the ratio of IC_{50} values for RL AR to RK L-HDH. Higher numbers indicate greater activity against L-hexonate dehydrogenase.

genated fluorenes, an alternate method was developed for sulfonamides **5b–d**. Good yields of these compounds were obtained from the corresponding 9-diazofluorenes **7b–d** by reaction with sulfur dioxide and ammonia in refluxing tetrahydrofuran[14] (Scheme III). The 9-diazofluorenes were obtained by either of two routes. Compound **7d** was prepared from 2,7-difluorofluorene (**8**) by deprotonation using *n*-butyllithium followed by reaction with *p*-toluenesulfonyl azide.[15] 9-Diazofluorenes **7b** and **7c** were obtained in quantitative yield by oxidation of the hydrazone derivatives **9b** and **9c** of the corresponding fluorenones with activated manganese dioxide.[16]

Scheme III. Synthesis of 9-Fluorenesulfonamides **5b–d**



Biological Results and Discussion

Compounds **1a–d** and **2a–d**, as well as **10**, imirestat, sorbinil, ponalrestat, tolrestat, and quercetin, were evaluated for their *in vitro* inhibitory activity against both AR and L-HDH in soluble protein fractions from rat lens and rat kidney, respectively. The inhibition constants are presented in Table II. Also tabulated as the selectivity quotient is the ratio of the inhibition constant for AR to L-HDH.

As indicated in Table II, of the spiro[9*H*-fluorene-9,4′-isothiazolidin]-3′-one 1′,1′-dioxides, only **1c** and **1d** showed any activity as ARIs, but at millimolar concentrations. However, all four compounds proved to be selective, al-

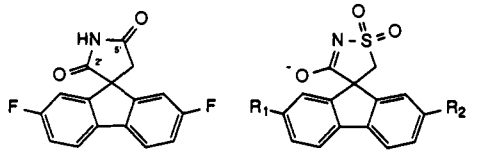
(14) This method is similar to that reported for the conversion of diphenyldiazomethane to diphenylmethanesulfonamide, see: Koosterziel, H.; Deinema, M. H.; Backer, H. J. *Recl. Trav. Chim. Pays-Bas* **1952**, *71*, 1228.

(15) For the analogous transformation of cyclopentadiene to diazocyclopentadiene, see: Doering, W. von E.; DePuy, C. H. *J. Am. Chem. Soc.* **1953**, *75*, 5955.

(16) (a) Fatiadi, A. J. *Synthesis* **1976**, *65*, 133. (b) Calf, G. E.; Fisher, B. D.; Garnett, J. L. *Chem. Commun.* **1966**, *20*, 730. (c) Barakat, M. Z.; Abdel-Wahab, M. F.; El-Sadr, M. M. *J. Chem. Soc.* **1956**, 46.

though modest, inhibitors of rat kidney L-HDH with IC_{50} values in the 10^{-6} M range, similar to that of quercetin, a well-characterized inhibitor that exhibits some selectivity for L-HDH (Table II). Interestingly, this class of L-HDH inhibitors is insensitive to halogen substitution at the 2- and 7-positions, there being no significant difference in the activities of the unsubstituted compound **1a**, the monohalo compounds **1b** and **1c**, and the difluoro compound **1d**.

On the other hand, the spiro[9*H*-fluorene-9,5′-isothiazolidin]-3′-one 1′,1′-dioxides **2a–d** are potent, nonselective inhibitors of both rat lens AR and rat kidney L-HDH with IC_{50} values for the monohalo and difluoro compounds **2b–d** in the 10^{-7}–10^{-8} M range. In terms of lack of selectivity and potency, this series is similar to the spiro[fluorenehydantoins] and spiro[fluorenesuccinimides] (e.g., imirestat and **10**, respectively). Activity against either



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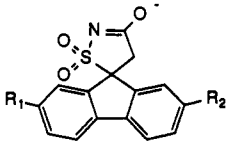

12

enzyme is sensitive to halogen substitution in this series, with the monohalo compounds being better than the unsubstituted parent and the difluoro compound being better still. This aspect also parallels the reported activity of the spiro[fluorenehydantoins] and spiro[fluorenesuccinimides].[10] Of the other ARIs evaluated, the hydantoin sorbinil was a more selective inhibitor of AR by a factor of 10, while ponalrestat and tolrestat proved to be quite selective ARIs.

Conceptually, structures **1** and **2** can be considered as analogues of spiro[fluorene-9,3′-pyrrolidine]-2′,5′-diones, a class of potent ARIs represented by compound **10** in which the 5′- and 2′-carbonyl groups, respectively, are replaced by a sulfonyl functionality. It is interesting that there is such a distinct difference in activity between the two isomers. Replacement of the 2′-carbonyl, resulting in the spiro[fluorene-9,5′-isothiazolidin]one dioxides **2**, produces a new class of inhibitor that is similar to the spiro[fluorenehydantoins] and spiro[fluorenesuccinimides] in terms of potency and selectivity toward AR and L-HDH. With the spiro[fluorene-9,4′-isothiazolidin]one dioxides **1**, however, replacement of the 5′-carbonyl with a sulfonyl group effectively eliminates ARI activity and produces a class of selective L-HDH inhibitors.

The most obvious effect of substituting either of the carbonyls by a sulfonyl group is a significant lowering of the pK_a of the proton on the adjacent nitrogen. While spiro[fluorenehydantoins] and spiro[fluorenesuccinimides] generally have a pK_a between 7.4 and 8.5, the closely related 5-phenyl-3-isothiazolidinone 1,1-dioxides are reported to have pK_as of ca. 2.4.[17] As a consequence of having a much lower pK_a, compounds **1** and **2** exist completely in their ionized forms **11** and **12** at physiological pH of 7.5, the pH at which the *in vitro* enzyme inhibition experiments are conducted. This fact offers a possible explanation as to the difference in activity between the two isomers as ARIs, assuming that these compounds interact with AR at the same site as do the spiro[fluorenehydantoins] and spiro[fluorenesuccinimides] and accepting that Kador's model of the inhibitor binding site of the enzyme[18] offers an accurate depiction of the interaction of spiroimide type inhibitors with the enzyme. Kador's model requires that the inhibitor have a polarized functionality at the position corresponding to the 2′-carbonyl of **10** that is susceptible to reversible nucleophilic attack. The sulfonyl group serves this role in the ionized form of **2**, while in **11** delocalization of the negative charge on the adjacent nitrogen into the carbonyl renders that functionality resistant to nucleophilic attack.

On the basis of its high *in vitro* activity against rat lens aldose reductase, compound **2d** was evaluated for in *vivo* activity in an acutely dosed diabetic rat model.[5a,e] In this severe model, at a dose level of 2.0 mg/kg per day, the compound inhibited sorbitol accumulation in the retina and sciatic nerve by only 15% and had no measurable effect on lens sorbitol concentration, while imirestat reduced the sorbitol level by 50% in all three tissues at doses less than 0.10 mg/kg per day. Thus, this class of compounds was dropped from further consideration.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^1H NMR spectra were obtained on either a Varian VXR-200 or (when indicated) a Varian EM-360 instrument using TMS as internal standard. Coupling constants are reported in hertz. ^{13}C NMR spectra were obtained on a Varian VXR-200 at 50 MHz using DMSO-d_6 as internal standard. IR spectra were obtained on either a Perkin-Elmer 1600 series FTIR or a Beckman IR4230 spectrometer. Mass spectra were obtained on a Finnigan TSQ46 mass spectrometer. Elemental analyses were performed by either Galbraith Laboratories (Knoxville, TN) or Oneida Research Laboratories (Whitesboro, NY) and were within 0.4% of the theoretical value for the indicated elements except where indicated. Organic extracts were dried over $MgSO_4$. Chromatography was performed on silica gel (mesh 230–400) using the indicated solvent mixture.

9-[(Aminosulfonyl)methyl]fluorene-9-carboxylic Acid Ethyl Ester (4a). A mixture of fluorene-9-carboxylic acid ethyl ester (**3a**) (11.0 g, 46.2 mmol), potassium carbonate (6.40 g, 46.2 mmol), and a 1:1 mixture of bromomethanesulfonamide and iodomethanesulfonamide in dry DMF (100 mL) was stirred under nitrogen at room temperature for 18 h. Water (200 mL) was then added and the solution was acidified with 1 M aqueous HCl (100 mL) and extracted with ethyl acetate (3 × 100 mL). After the combined organic phases were washed with brine, dried, and concentrated, the residue was chromatographed (40% ethyl acetate in hexane) to provide 4.8 g (31%) of **4a**: mp 150–156 °C; IR (KBr) 3360, 3270, 1710, 740, 725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.78–7.69 (m, 4 H), 7.50–7.32 (m, 4 H), 4.38 (bs, 2 H, exchangeable), 4.12 (q, 2 H), 4.11 (s, 2 H), 1.12 (t, 3 H). Anal. ($C_{16}H_{15}NO_4S$) C, H, N.

9-[(Aminosulfonyl)methyl]-2-fluorofluorene-9-carboxylic Acid Methyl Ester (4b). A mixture of 2-fluorofluorene-9-carboxylic acid methyl ester (**3b**) (6.3 g, 26.0 mmol) and potassium carbonate (3.60 g, 26.0 mmol) in dry DMF (200 mL) was stirred under nitrogen at 40 °C for 30 min. An 85:15 mixture of iodomethanesulfonamide and bromomethanesulfonamide (5.7 g, 26.0 mmol) was then added and the reaction was stirred at 40 °C for

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6 h and then at room temperature for 18 h. The reaction was worked up as described for **4a**. Chromatography (40% ethyl acetate in hexane) provided 2.4 g (27%) of **4b**: mp 165–179 °C; IR (KBr) 3330, 3260, 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.74–7.67 (m, 3 H), 7.52–7.31 (m, 3 H), 7.17 (ddd, 1 H, J = 2.4, 8.4, 8.9), 4.49 (bs, 2 H, exchangeable), 4.17 (d, 1 H, J = 14.8), 4.03 (d, 1 H, J = 14.8), 3.66 (s, 3 H). Anal. ($C_{16}H_{14}FNO_4S$) H, N; C: calcd, 57.31; found, 56.19.

9-[(Aminosulfonyl)methyl]-2-chlorofluorene-9-carboxylic Acid Methyl Ester (4c). A mixture of 2-chlorofluorene-9-carboxylic acid methyl ester (**3c**) (7.00 g, 27.1 mmol) and potassium carbonate (3.30 g, 23.9 mmol) in dry DMF (150 mL) was stirred under nitrogen at 40 °C for 30 min. Bromomethanesulfonamide (4.2 g, 23.9 mmol) was then added, and the reaction was stirred at 40 °C for 6 h and then at room temperature for 18 h. The reaction was worked up as described for **4a**. Chromatography (30–70% ethyl acetate in hexane) followed by recrystallization from ethyl acetate/hexane provided 600 mg (6%) of **4c**: mp 209–211 °C; IR (KBr) 3369, 3271, 1741, 1447, 1314, 1207, 1152 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.90 (m, 2 H), 7.78 (d, 1 H, J = 1.8), 7.66 (m, 2 H), 7.42 (m, 3 H), 6.96 (s, 2 H), 4.16 (d, 1 H, J = 14.4), 3.90 (d, 1 H, J = 14.3), 3.57 (s, 3 H). Anal. ($C_{16}H_{14}ClNO_4S$) C, H, N.

9-[(Aminosulfonyl)methyl]-2,7-difluorofluorene-9-carboxylic Acid Methyl Ester (4d). A mixture of 2,7-difluorofluorene-9-carboxylic acid methyl ester (**3d**) (12.4 g, 47.6 mmol) and anhydrous sodium methoxide (2.6 g, 47.6 mmol) in degassed methanol (500 mL) was heated to reflux. Once the ester had dissolved, bromomethanesulfonamide (8.2 g, 47.6 mmol) was added. After 5 h, the reaction was quenched with 1 M aqueous HCl (200 mL) and extracted with ethyl acetate (3 × 200 mL). The combined organics were washed with brine, dried, and concentrated. Chromatography (30–70% ethyl acetate in hexane) followed by recrystallization from ethyl acetate/hexane provided 900 mg (5%) of **4d**: mp 217–219 °C; IR (KBr) 3371, 3261, 1741, 1616, 1595, 1468, 814 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.86 (dd, 2 H, J = 5.1, 8.4), 7.47 (dd, 2 H, J = 2.4, 9.3), 7.25 (ddd, 2 H, J = 2.4, 8.5, 9.4), 6.94 (s, 2 H), 4.05 (s, 2 H), 3.56 (s, 3 H). Anal. ($C_{16}H_{13}F_2NO_4S$) C, H, N.

Spiro[9*H*-fluorene-9,4'-isothiazolidin]-3'-one 1',1'-Dioxide (1a). A mixture of **4a** (4.00 g, 12.1 mmol) and anhydrous sodium methoxide (4 g) in ethanol (300 mL) was stirred under nitrogen for 1 h at room temperature. The reaction was then evaporated and the residue was acidified with 1 M aqueous HCl (200 mL) and extracted with ethyl acetate (3 × 200 mL). The combined organic phases were washed with brine, dried, treated with Norit A, filtered, and concentrated. Recrystallization from methylene chloride/hexane provided 1 g (30%) of **1a**: IR (KBr) 3200–2600, 1695 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.93 (m, 2 H), 7.81 (m, 2 H), 7.50 (ddd, 2 H, J = 1.3, 7.4, 7.4), 7.40 (ddd, 2 H, J = 1.4, 7.4, 7.4), 4.35 (s, 2 H).

2-Fluorospiro[9*H*-fluorene-9,4'-isothiazolidin]-3'-one 1',1'-dioxide (1b) was prepared from **4b** in 16% yield as described for **1a** except that the reaction time was 40 min: IR (KBr) 1700, 1330 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.00–7.30 (m, 7 H), 4.43 (d, 1 H, J = 14.1), 4.31 (d, 1 H, J = 14.0); ^{13}C NMR (DMSO-d_6) δ 169.70, 164.54, 159.68, 145.86, 145.68, 143.88, 139.82, 137.28, 137.23, 129.30, 127.95, 123.92, 122.15, 121.97, 120.42, 116.52, 116.06, 112.01, 111.52, 61.10, 56.73.

2-Chlorospiro[9*H*-fluorene-9,4'-isothiazolidin]-3'-one 1',1'-dioxide (1c) was prepared from **4c** in 66% yield as described for **1a** except that the reaction time was only 15 min and a different isolation procedure was used. The ethyl acetate extract was extracted with 1 M aqueous NaOH, and the basic extract was then washed with ether, acidified, and extracted with ethyl acetate. After drying and solvent removal, the residue was recrystallized from ethyl acetate/hexane: IR (KBr) 1709, 1343, 1158 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.96 (m, 3 H), 7.84 (br dd, 1 H), 7.59–7.38 (m, 3 H), 4.47 (d, 1 H, J = 14.2).

2,7-Difluorospiro[9*H*-fluorene-9,4'-isothiazolidin]-3'-one 1',1'-Dioxide (1d). A mixture of **4d** (730 mg, 2.1 mmol) and anhydrous sodium methoxide (1 g, 18.5 mmol, 8.8 equiv) in methanol (100 mL) was stirred at room temperature under nitrogen for 30 min. The methanol was then evaporated and the residue was partitioned between 1 M aqueous NaOH (100 mL) and ether (100 mL). The basic aqueous layer was separated and the ether layer was further extracted with aqueous NaOH (2 × 100 mL). The combined basic aqueous extracts were then washed with ether (200 mL), acidified, and extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were dried, treated with Norit A, filtered, and concentrated. Recrystallization from ethyl acetate/hexane provided 500 mg (75%) of **1d**: IR (KBr) 1733, 1596, 1468, 1342 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.96 (dd, 2 H, J = 5.1, 8.5), 7.73 (dd, 2 H, J = 2.4, 9.3), 7.35 (ddd, 2 H, J = 2.4, 8.9, 8.9), 6.60 (br s, 1 H), 4.40 (s, 2 H).

2-Fluoro-9-diazofluorene (7b). A mixture of 2-fluoro-9-fluorenone hydrazone (**9b**) (6.50 g, 30.6 mmol) and activated manganese dioxide (13.0 g, 150 mmol, 5 equiv) in THF (300 mL) was stirred at room temperature for 18 h. The mixture was then filtered through Celite and concentrated to provide 6.5 g (100%) of **7a** as a red solid which was used without further purification. A sample was recrystallized from ether/hexane: mp 89–90 °C; IR (KBr) 2075, 1180 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.88–7.79 (m, 2 H), 7.49–7.26 (m, 3 H), 7.15 (br dd, 1 H, J = 2.3, 8.9), 7.01 (ddd, 1 H, J = 2.3, 8.5, 9.3); MS m/z 210 (M+), 182 (base peak).

2-Chloro-9-diazofluorene (7c) was prepared from **9c** in quantitative yield as described for **7b** and used without purification.

2,7-Difluoro-9-diazofluorene (7d). Under nitrogen, *n*-butyllithium (1 equiv, 20 mL of a 2.5 M hexane solution) was added dropwise to a stirred, −20 °C solution of 2,7-difluorofluorene (**8**) (10.0 g, 49.0 mmol) in anhydrous diethyl ether (250 mL). After 10 min, this mixture was transferred via a cannula using nitrogen pressure into a 0 °C solution of *p*-toluenesulfonyl azide (10.0 g, 50.7 mmol) in dry ether (200 mL) over 20 min. The reaction mixture was allowed to warm to room temperature over 1.5 h and was then acidified with 1 M aqueous HCl and extracted with ethyl acetate (3 × 200 mL). The combined organics were washed with brine, dried, and concentrated to provide 10 g of crude material which was leached with ether/hexane to provide 4 g (37%) of pure **7c**: mp 148–150 °C; IR (KBr) 2070, 1600, 1575, 845 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.77 (ddd, 2 H, J = 0.5, 5.0, 8.7), 7.14 (ddd, 2 H, J = 0.5, 2.5, 8.5), 7.0 (ddd, 2 H, J = 2.3, 8.5, 9.3). Anal. ($C_{13}H_6F_2N_2$) C, H, N.

2-Fluorofluorene-9-sulfonamide (5b). A solution of **7b** (5.50 g, 26.1 mmol) in THF (600 mL) was heated to reflux and treated alternately with ammonia and sulfur dioxide gas a number of times until all of the starting material was consumed as indicated by TLC (silica gel, 30% ethyl acetate/hexane). After solvent removal, the residue was diluted with water (200 mL) and extracted with ethyl acetate (3 × 200 mL). The combined extracts were dried, concentrated, and chromatographed (20% ethyl acetate in hexane) to provide 5 g (73%) of **5b**: mp 179–181 °C; IR (KBr) 3390, 3280, 1322 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.95–7.16 (m, 7 H), 5.37 (s, 1 H), 4.00 (br s, 2 H, exchangeable). Anal. ($C_{13}H_{10}FNO_2S$) C, H, N.

2-Chlorofluorene-9-sulfonamide (5c) was prepared from **7c** in 47% yield as described for **5b**: mp 190–194 °C; IR (KBr) 3388, 3280, 1541, 1325, 1148, 1133 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.93 (m, 2 H), 7.72 (m, 2 H), 7.46 (m, 3 H), 5.38 (s, 1 H), 4.01 (s, 2 H). Anal. ($C_{13}H_{10}ClNO_2S$) C, H, N.

2,7-Difluorofluorene-9-sulfonamide (5d) was prepared from **7d** in 64% yield as described for **5b**: mp 234–237 °C; IR (KBr) 3417, 3265, 2885, 1575, 805 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.67 (m, 4 H), 7.18 (m, 1 H), 5.36 (s, 1 H), 4.02 (br s, 2 H, exchangeable). Anal. ($C_{13}H_9F_2NO_2S$) C, H, N.

2-[9-(Aminosulfonyl)fluoren-9-yl]acetic Acid Ethyl Ester (6a). To a stirred, 15 °C suspension of sodium hydride (1 equiv, 12.2 mmol, 500 mg of a 60% mineral oil dispersion) in dry DMF (50 mL) was added dropwise under nitrogen a solution of fluorene-9-sulfonamide (**5a**) (3.0 g, 12.2 mmol) in DMF (100 mL) over 15 min followed by a solution of ethyl bromoacetate (2.0 g, 12.2 mmol) in DMF (20 mL) over 10 min. After 3 h at room temperature, the reaction mixture was diluted with water (100 mL), acidified to pH 5, and extracted with ethyl acetate (3 × 200 mL). The combined organic extracts were washed with brine, dried, and concentrated. Chromatography (10–30% ethyl acetate in hexane) provided 1 g (25%) of **6a**: mp 167–170 °C; IR (KBr) 3357, 3249, 1721, 1323, 1153 cm^{-1}, ^1H NMR ($CDCl_3$, 60 MHz) δ 7.8–7.2 (m, 8 H), 3.9 (br s, 2 H), 3.6 (s, 2 H), 3.5 (q, 2 H), 1.7 (t, 3 H). Anal. ($C_{17}H_{17}NO_4S$) C, H, N.

2-[9-(Aminosulfonyl)-2-fluorofluoren-9-yl]acetic acid ethyl ester (6b) was prepared from **5b** in 56% yield as described

for **6a**: mp 186–187 °C (from ethyl acetate/hexane); IR (KBr) 3350, 3250, 1710, 1158 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.81–7.67 (m, 3 H), 7.55–7.32 (m, 3 H), 7.19 (ddd, 1 H, J = 2.4, 8.7, 8.7), 3.93 (br s, 2 H, exchangeable), 3.71 (q, 2 H, J = 7.1), 3.68 (s, 2 H), 0.78 (t, 3 H, J = 7.1). Anal. ($C_{17}H_{16}FNO_4S$) C, H, N.

2-[9-(Aminosulfonyl)-2-chlorofluoren-9-yl]acetic acid ethyl ester (6c) was prepared from **5c** in 50% yield as described for **6a**: mp 189–191 °C (from ethyl acetate/hexane); IR (KBr) 3361, 3257, 1715, 1331, 1311, 1158 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.72 (m, 4 H), 7.48 (m, 3 H), 3.95 (br s, 2 H), 3.71 (m, 2 H), 0.79 (t, 3 H, J = 7). Anal. ($C_{17}H_{16}ClNO_4S$) C, H, N.

2-[9-(Aminosulfonyl)-2,7-difluorofluoren-9-yl]acetic acid ethyl ester (6d) was prepared from **5d** in 26% yield as described for **6a**: mp 215–217 °C; IR (KBr) 3357, 3250, 1710, 1160, 828 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.65 (dd, 2 H, J = 4.8, 8.6), 7.52 (ddd, 2 H, J = 2.5, 9.3), 7.20 (ddd, 2 H, J = 2.3, 8.6, 8.6), 3.97 (br s, 2 H, exchangeable), 3.75 (q, 2 H, J = 7.1), 0.85 (t, 3 H, J = 7.1). Anal. ($C_{17}H_{15}F_2NO_4S$) C, H, N.

Spiro[9*H*-fluorene-9,5′-isothiazolidin]-3′-one 1′,1′-Dioxide (2a). A mixture of **6a** (850 mg, 2.60 mmol) and anhydrous sodium methoxide (1 g, 7 equiv) in methanol was stirred at room temperature for 18 h. After the solvent was evaporated, the residue was partitioned between water (100 mL) and ethyl acetate (100 mL). The aqueous layer was separated and the organic phase was extracted with 1 M aqueous NaOH (2 × 100 mL). The combined basic aqueous layers were washed with ethyl acetate, acidified to pH 2, and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with brine, dried, and concentrated. Recrystallization from ethyl acetate/hexane provided 200 mg (27%) of **2a**: IR (KBr) 3090, 2870, 1710, 1440, 1330 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.99–7.45 (m, 9 H), 3.64 (s, 2 H).

2-Fluorospiro[9*H*-fluorene-9,5′-isothiazolidin]-3′-one 1′,1′-Dioxide (2b). A mixture of **6b** (1.2 g, 3.4 mmol) and sodium hydride (1.3 g of a 60% mineral oil dispersion, 9.5 equiv) in dry THF (300 mL) was stirred at room temperature under nitrogen for 30 min. The reaction mixture was then diluted with 1 M aqueous HCl (200 mL) and extracted with ethyl acetate (3 × 150 mL). The combined organic extracts were washed with brine, dried, and concentrated. Recrystallization from ethyl acetate/hexane provided 550 mg (55%) of **2b**: IR (KBr) 1695, 1320 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.7 (br, 1 H, exchangeable), 8.04–7.93 (m, 2 H), 7.66–8.37 (m, 5 H), 3.81 (d, 1 H, J = 17.5), 3.55 (d, 1 H, J = 17.5); ^{13}C NMR (DMSO-d_6) δ 169.45, 164.34, 159.48, 140.39, 140.21, 139.52, 139.07, 137.09, 137.04, 130.20, 128.05, 125.03, 122.46, 122.28, 120.69, 117.54, 117.08, 113.52, 113.03, 74.49, 41.90.

2-Chlorospiro[9*H*-fluorene-9,5′-isothiazolidin]-3′-one 1′,1′-dioxide (2c) was prepared from **6c** as described for **2b** except at 10 °C for 1.5 h with a different workup procedure. The reaction mixture was evaporated to dryness and the residue was partitioned between 1 M aqueous NaOH and ethyl acetate. The basic solution was separated, acidified to pH 1, and extracted with ethyl acetate. After washing with brine and drying, the ethyl acetate was evaporated and the residue was recrystallized from ethyl acetate/hexane to provide **2c** in 27% yield: IR (KBr) 3057, 2978, 1708, 1369, 1160, 1145, 722 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.99 (m, 2 H), 7.60 (m, 5 H), 3.55 (d, 1 H, J = 17.4), 3.80 (d, 1 H, J = 17.4).

2,7-Difluorospiro[9*H*-fluorene-9,5′-isothiazolidin]-3′one 1′,1′-dioxide (2d) was prepared from **6d** as described for **2b** except that the reaction time was 45 min. The workup was similar to that described for **2c**. Chromatography (10% methanol in methylene chloride) followed by recrystallization from ethyl acetate/hexane provided **6d** in 30% yield: IR (KBr) 3280–2900, 1730, 1710, 1157, 815 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.99 (dd, 2 H, J = 4.9, 8.5), 7.47 (m, 4 H), 3.72 (s, 2 H).

Biological Methods. Soluble, unfractionated preparations of aldose reductase and L-hexonate dehydrogenase were prepared from rat lens and rat kidney, respectively, using a published procedure.[9b] These tissues were selected because they contain the highest specific activity of the respective enzymes,[6a] with very low specific activity of the other enzyme of interest. Thus, there was minimal interference of one activity with the other. Unfractionated enzyme preparations were used in order to obtain more reliable inhibition data for very potent inhibitors among this group of compounds; published data indicate that purification of aldose reductase introduces modifications of the protein which actually decrease its susceptibility to inhibition by various compounds.

Inhibition constants against both enzymes for compounds **1a–d**, **2a–d**, **10**, imirestat, sorbinil, ponalrestat, tolrestat, and quercetin were obtained using sensitive fluorometric assays based on the fluorescence of NADPH, or an analogue, produced upon enzymatic oxidation of an appropriate alcohol with the respective oxidized pyridine nucleotide. These fluorescence activity assays are considerably more sensitive than various spectrophotometric assays which have been published for both enzymes. In the assay for aldose reductase,[9a] the reaction mixture contained 50 mmol/L pH 7.5 potassium phosphate buffer, 12 mmol/L benzyl alcohol, 12 μmol/L oxidized 3-acetylpyridine adenine dinucleotide phosphate, and rat lens supernatant (50–100 μg of protein) in a total volume of 1.0 mL. The assay for L-hexonate dehydrogenase was adapted from a published spectrophometric assay.[9b] The reaction mixture consisted of 50 mmol/L pH 7.5 potassium phosphate buffer, 20 mmol/L L-gulonate, 25 μmol/L $NADP^+$, and rat kidney supernatant (50–100 μg of protein) in a total volume of 1.0 mL. The enzyme-dependent rate of formation of reduced pyridine nucleotide was monitored with a fluorescence spectrophotometer set at excitation/emission wavelengths of 365/480 nm for the $NADP^+$ analogue and 365/465 nm for $NADP^+$, with 10-nm slit widths. Reactions were carried out at room temperature; blank rates with one component omitted were typically less than 10% of the rate of the complete reaction.

The IC_{50} values (inhibitor concentration producing 50% inhibition of the reaction under the standard assay conditions described) were computed by linear-regression analysis of the linear portion of the dose–response curve for each inhibitor. The reliability of the data was assured by the following procedures: (1) using the same amount of enzyme (activity) for all inhibitors; (2) demonstrating that each activity could be inhibited completely by a moderate concentration of a potent inhibitor; (3) evaluating the activity of a known inhibitor each day; and (4) generating characteristic, reproducible inhibition plots for individual inhibitors. The experimental error in the IC_{50} values was typically no greater than 25%.

Registry No. **1a**, 132606-12-3; **1b**, 132606-13-4; **1c**, 136115-35-0; **1d**, 136115-36-1; **2a**, 132627-75-9; **2b**, 132627-76-0; **2c**, 132606-14-5; **2d**, 132606-15-6; **3a**, 26878-12-6; **3b**, 97677-58-2; **3c**, 22909-50-8; **3d**, 97677-66-2; **4a**, 132606-16-7; **4b**, 132606-17-8; **4c**, 136115-33-8; **4d**, 136115-34-9; **5a**, 132606-18-9; **5b**, 132606-20-3; **5c**, 132606-22-5; **5d**, 132606-24-7; **6a**, 132606-19-0; **6b**, 132606-21-4; **6c**, 132627-77-1; **6d**, 132606-25-8; **7b**, 2069-26-3; **7c**, 81150-84-7; **7d**, 132606-23-6; **8**, 2195-50-8; **9b**, 342-99-4; **9c**, 885-41-6; **10**, 97677-42-4; bromomethanesulfonamide, 53412-78-5; iodomethanesulfonamide, 22354-13-8; ethyl bromoacetate, 105-36-2.