Potential Inhibitors of L-Asparagine Biosynthesis. 4.[1a,b] Substituted Sulfonamide and Sulfonylhydrazide Analogues of L-Asparagine

Steven Brynes, Gilbert J. Burckart,[1c] and Michael Mokotoff*

Department of Medicinal Chemistry, School of Pharmacy, University of Pittsburgh, Pittsburgh, Pennsylvania 15261.
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Several N-substituted sulfonamides and N′-substituted sulfonylhydrazides have been prepared as sulfur analogues of L-asparagine with the potential of acting as inhibitors of L-asparagine synthetase (ASase, from Novikoff hepatoma). L-Cystine was converted in known steps to *N*-carboxy-3-(sulfonylchloro)-L-alanine dibenzyl ester (1). Condensation of 1 with *O*-benzylhydroxylamine, *p*-(fluorosulfonyl)benzylamine, or monoethyl fumarylhydrazide (**9**), followed by deblocking with HF, gave 3-(hydroxysulfamoyl)-L-alanine (**3a**), 3-[*p*-(fluorosulfonylbenzyl)]sulfamoyl-L-alanine (**3c**), and 3-sulfo-L-alanine *S*-[2-[(*E*)-3-(ethoxycarbonyl)acryloyl]hydrazide] (**3e**), respectively. Similarly, 1 with 2-chloroethylamine and deblocking with H_2–Pd gave 3-[(2-chloroethyl)sulfamoyl]-L-alanine (**3b**). *tert*-Butyl carbazate was allowed to react with 1 and the *tert*-butyl group was removed with HCl. The resulting sulfonylhydrazide **7** was condensed with *p*-(fluorosulfonyl)benzoyl chloride and then deblocked with HF to give 3-sulfo-L-alanine *S*-[2-[*p*-(fluorosulfonyl)benzoyl]hydrazide] (**3d**). The inhibition of ASase by **3a–e** at 2 mM was 97, 0, 30, 43, and 37%, respectively, and **3a** was competitive with L-aspartic acid. Neither **3a** nor **3e** was effective in increasing the life span of mice bearing P-388 lymphocytic leukemia.

The resistance of tumors to L-asparagine depletion by L-asparaginase (ASNase) has been correlated with an enhanced asparagine biosynthesis by the enzyme L-asparagine synthetase (ASase).[2-4] As a result, the search for chemical agents capable of inhibiting ASase is of interest from the standpoint of cancer chemotherapy.[5] Such compounds may potentially prove most useful in combination therapy with ASNase against ASNase sensitive tumors by preventing the emergence of resistant lines which are characterized by high levels of ASase. We have noted that several amide-like analogues of L-asparagine, namely, L-β-aspartyl hydrazide,[6] L-β-aspartohydroxamic acid,[7-9] and 3-sulfamoyl-L-alanine (Table I), exhibit either some in vitro inhibitory activity of ASase or a significant ability to prolong the life of mice infected with leukemia when combined with L-asparaginase.[6,9] We have thus prepared and biochemically tested several structurally related N-substituted sulfonamides and N′-substituted sulfonylhydrazides (**3a–e**, Scheme I). With the exception of **3a** and **3b**, these analogues are potential irreversible inhibitors containing reactive groups capable of forming covalent bonds with enzymes.

Chemistry. The common intermediate required for the synthesis of compounds **3a–e** was the sulfonyl chloride **1**. In order to prepare **1**, L-cystine was converted to di-*N*-carbobenzoxy-L-cystine by the method of duVigneaud and Miller[10a] and then to its dibenzyl ester as described by Ross et al.[11] By an adaptation of the method of Douglass and Farah,[12] controlled chlorination of the dibenzyl ester afforded the sulfonyl chloride **1** in 75% yield. Although Ross et al.[11] described a procedure which reportedly gave **1** in 50% yield, we were unable to repeat their method, obtaining instead the corresponding sulfonate ester **4** (Scheme I). Condensation of **1** with the appropriate amine or hydrazide followed by deblocking afforded the desired free amino acids (Scheme I).

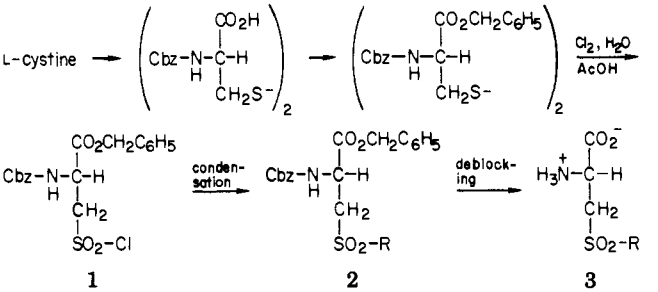
Reaction between sulfonyl chlorides and hydroxylamine in ethanol has been reported to be a general method for preparing sulfonohydroxamic acids.[13] However, the use of alcohol as solvent in the synthesis of **2a** (X = H) led predominantly to the corresponding ethyl sulfonate **4**. Sulfonohydroxamic acid **2a** (X = H) was subsequently obtained in fair yields (ca. 40%) by condensation of **1** with hydroxylamine hydrochloride in CH_3CN in the presence of $NaHCO_3$. On a small scale, removal of the benzyl-blocking groups was smoothly achieved with H_2–Pd in a 73% yield. However, on a larger scale the latter deblocking was complicated by side products. Much better overall yields of **3a** (X = H), as well as greater ease of preparation, were achieved by reaction of **1** with *O*-benzylhydroxyl-

Table I. Inhibition of Asparagine Synthetase[a]

Compd[b]	Concn, mM	% inhibn ± SD[c]
3a (X = H)	1	77.6 ± 2.8
	2	96.5 ± 0.9
3b	2	0
3c	2	29.5 ± 1.6
3d	2	43.1 ± 2.2
3e	**2**	37.3 ± 1.7
3-Sulfamoyl-L-alanine[d]	2	25.8 ± 5.3
L-β-Aspartohydroxamic acid[e]	**2**	15.8 ± 5.9

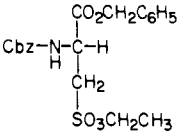
[a] ASase partially purified as described in paper 2 of this series (ref 16). [b] The compounds were preincubated with ASase and substrate aspartic acid was added. The formation of L-asparagine was determined as described earlier (ref 16). [c] Percent inhibition and standard deviation derived from an inhibition assay run in duplicate on three separate days (triplicate of duplicates) utilizing freshly prepared inhibitor solutions. [d] Prepared by the method of Ross et al.;[11] see Experimental Section. [e] Obtained from Sigma Chemical Co.

Scheme I



1 **2** **3**

2,3a, R = -NHOX; X = H, $CH_2C_6H_5$
2,3b, R = $-NHCH_2CH_2Cl$
2,3c, R = $-NHCH_2C_6H_4$-*p*-SO_2F
2,3d, R = $-NHNHCOC_6H_4$-*p*-SO_2F
2,3e, R = -NHNHCOCH=CHCO$_2$Et (trans)



4

Cbz = $C_6H_5CH_2OCO$-

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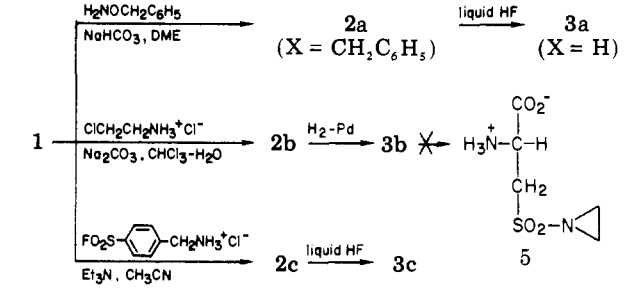
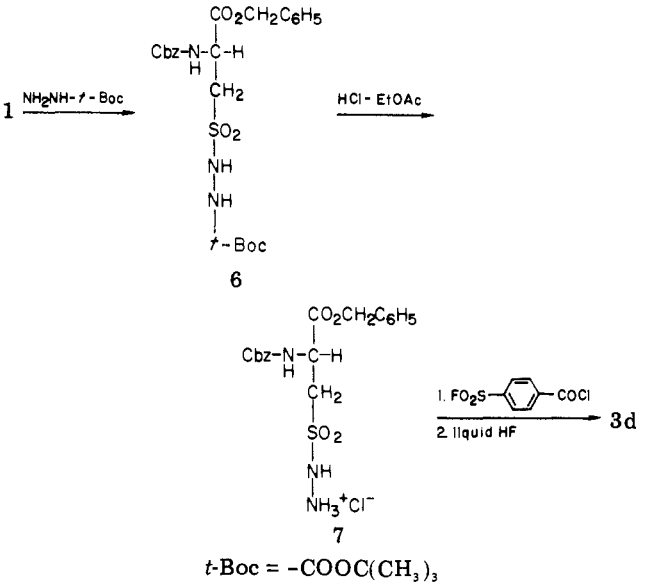
Scheme II



Scheme III



t-Boc = $-COOC(CH_3)_3$

amine to give the triply blocked compound **2a** (X = $CH_2C_6H_5$), followed by subsequent deblocking with liquid HF[14] (Scheme II).

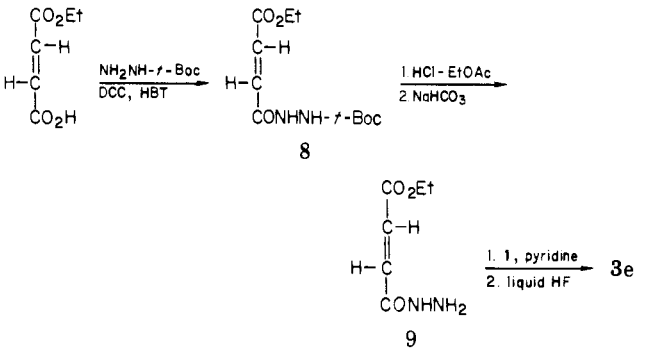
The preparation of chlorosulfonamide **2b** was also found to be quite sensitive to reaction conditions: 2-chloroethylamine hydrochloride was combined with **1** in the presence of Na_2CO_3 utilizing a two-phase solvent system ($CHCl_3$–H_2O). Single-phase systems, such as acetone–water or $CHCl_3$ alone, tended to produce low yields. Catalytic hydrogenation of **2b** afforded the corresponding free amino acid **3b** (Scheme II). Attempts at converting **3b** to its sulfonylaziridine **5**, by intramolecular base cyclization, were unsuccessful.

Substituted sulfonamide **2c** was obtained from the condensation of **1** with *p*-(fluorosulfonyl)benzylamine hydrochloride[1a] in the presence of Et_3N; the use of an inorganic base did not afford the product. The cleavage of the blocking groups of **2c** was smoothly accomplished with liquid HF,[14] affording **3c** (Scheme II).

The first approach to the synthesis of substituted hydrazides **3d,e** involved the preparation of the free hydrazide **7**, as outlined in Scheme III. The use of free hydrazine in the synthesis of **7** was avoided in order to prevent bis-product formation and hydrazinolysis of the benzyl ester. Condensation of *p*-fluorosulfonylbenzoyl chloride with **7** gave the desired blocked amino acid **2d** which was then readily deprotected with liquid HF[14] to give **3d** (Scheme III).

The attempted condensation of **7** with the *N*-hydroxysuccinimide ester of ethyl fumarate was nonproductive. A second route was then considered in which the substituted hydrazide would be reacted with sulfonyl chloride **1**. The heretofore unreported monoethyl fumarylhydrazide (**9**) was obtained by condensation of *tert*-butyl carbazate with ethyl fumarate via dicyclohexylcarbodiimide (DCC)

Scheme IV



and 1-hydroxybenzotriazole (HBT),[15] followed by the removal of the t-Boc group from **8** with HCl–EtOAc (Scheme IV). Reaction of **1** with **9** was carried out in pyridine to give **2e**, which was selectively deblocked with liquid HF[14] to afford **3e** (Scheme IV).

Biochemical Results. Rats bearing Novikoff hepatomas were utilized as the source of ASase. Excised tumors were kindly supplied by Dr. Manford K. Patterson, Jr. (The Samual Roberts Noble Foundation, Inc., Ardmore, Okla. 73401), and ASase was isolated as previously described.[16] The enzyme fraction used in the inhibition studies has a specific activity of 0.51 μmol of asparagine synthesized per milligram of protein per 30 min. L-Aspartic acid-^{14}C was incubated with L-glutamine, ASase, and other needed cofactors, and the L-asparagine-^{14}C synthesized was isolated as previously described.[16]

The ability of **3a–e** to inhibit the in vitro biosynthesis of L-asparagine is shown in Table I. With the exception of sulfonohydroxamic acid **3a**, none of the asparagine analogues exhibits strikingly high inhibitory activity. The results for **3c** (29.5%, 2 mM) are comparable to that obtained for its carbonyl analogue (33.7%, 2mM).[1a] In fact, the results for **3c–e**, as well as those for two similar SO_2F containing analogues,[1a] wherein percent inhibitions vary little, i.e., 30–40%, might well indicate that these compounds are nonspecific in action.

A notable exception, sulfonohydroxamic acid **3a** (X = H), exhibits a high inhibitory activity of 77.6% at 1 mM and 96.5% at 2 mM. Inasmuch as **3a** (X = H) does not have a chemically reactive moiety, one would expect its interaction with ASase to be of a reversible nature. As shown in Figure 1, when L-aspartic acid is the variable substrate and compound **3a** (X = H) the inhibitor, a double reciprocal plot shows competitive inhibition of ASase.

DL-β-Aspartohydroxamic acid, the carbonyl analogue of **3a**, has been shown to inhibit ASase from KB human tumor cells approximately 36% at 1 mM[8] (presumably the L form would have enhanced inhibition), whereas the L form was shown to increase the survival time of mice infected with both asparaginase sensitive and resistant leukemias by approximately 100%,[9] as well as to possess neoplasm inhibiting activity in humans.[9] Surprisingly, in our ASase system at 2 mM, L-β-aspartohydroxamic acid showed very little inhibitory activity. Apparently this compound cannot compete with L-aspartic acid for the active site of ASase as does **3a** (X = H).

Compounds **3a** (X = H) and **3b–e** were also tested[17] for their ability to inhibit ASase from L5178Y/AR mouse leukemia. In this system, with L-glutamine as the nitrogen source, **3c–e** were essentially inactive at 10 mM, **3b** inactive at 1 mM, while **3a** (X = H) inhibited the enzyme by 23 and 81% at 1 and 10 mM concentration, respectively. Under the experimental conditions described, it appears

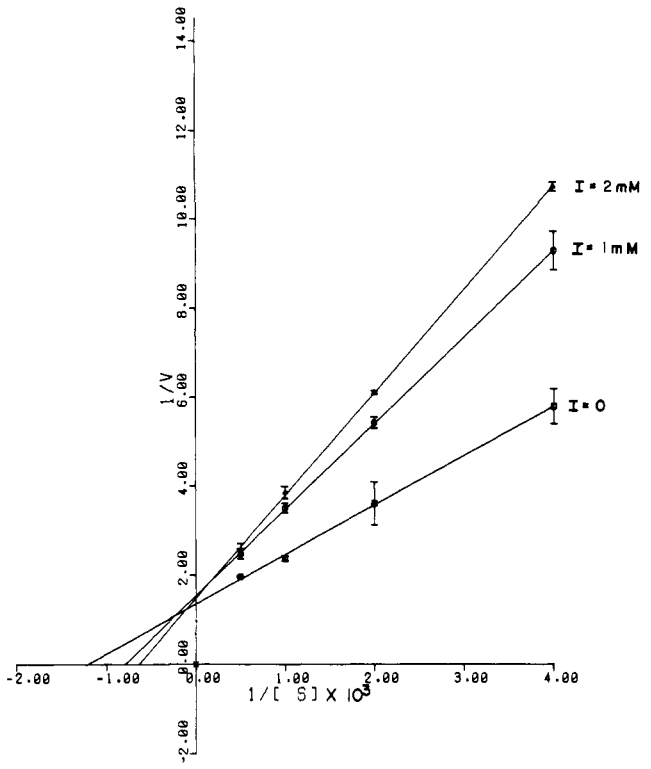


Figure 1. Inhibition of ASase by 3-(hydroxysulfamoyl)-L-alanine (**3a**, X = H). Reciprocal plots (computer drawn by least-squares regression analysis) with L-aspartic acid as variable substrate. Assay conditions as outlined in the Experimental Section except L-aspartic acid at 0.25, 0.50, 1.0, and 2.0 mM (final concentration) and L-aspartic acid-^{14}C, 2×10^6 dpm/assay tube (206 mCi/mmol). The y intercept and its standard error for I = 0, 1, and 2 mM is 1.35 ± 0.12, 1.54 ± 0.14, and 1.48 ± 0.06, respectively. Thus, within the experimental error of the data, this graph represents competitive inhibition. S = molarity; V = μmol of asparagine/mg of protein/30 min.

as if ASase from rat Novikoff hepatoma is more easily inhibited by substrate and product analogues than is the enzyme from L5178Y/AR mouse leukemia.[1a]

Both **3a** (X = H) and **3e** were submitted to the Drug Research and Development Program of the National Cancer Institute for in vivo screening in CDF_1 mice infected with P-388 lymphocytic leukemia, an ASNase resistant strain. The protocol presently in effect at NCI requires an increase in life span of 25% (T/C 125) in order to consider a compound active; both compounds submitted failed to meet this criterion and were considered inactive.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The infrared spectral data were obtained with a Perkin-Elmer Model 267 grating infrared spectrophotometer as KBr disks, unless stated otherwise. Optical rotations were determined with a Perkin-Elmer 241 or Rudolph 70 polarimeter. Radioactivity was determined with a Packard Model 3310 Tri-Carb scintillation spectrometer. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and Spang Microanalytical Laboratory, Ann Arbor, Mich., and are within ±0.4% of the theoretical values. Amino acid starting materials were purchased from Sigma Chemical Co. L-Aspartic acid-^{14}C was obtained from Schwarz-Mann and purified as previously described.[16] Thin and preparative layer chromatography were carried out with silica gel GF (Analtech, Inc.) or cellulose GF (Quantum Industries) and spots located with either UV light or by treatment of the plate with HCl fumes, followed by heating and subsequent spraying with ninhydrin. The petroleum ether used has a boiling point range of 30–60 °C. All evaporations were performed in vacuo on solutions that had been previously dried over anhydrous Na_2SO_4 or $MgSO_4$.

The apparatus for conducting the liquid HF reactions was made from linear polyethylene tubing, the stopcocks (Nalgene) were of polypropylene with a Teflon TFE plug, the reservoir bottle of linear polyethylene, and the reaction vessel of Teflon FEP. All of the plastic connections were sealed by dripping hot beeswax (white) on them and allowing it to solidify. Hydrogen fluoride from a tank was condensed (dry ice–acetone) in the reservoir bottle and then allowed to distill into the reaction vessel.

***N*,*N*-Dicarbobenzoxy-L-cystine.** This compound was prepared by the method of duVigneaud and Miller:[10a] mp 122–124 °C (reported[10a] mp 113–115 °C); $[\alpha]^{24}_D$ −84.9° (*c* 2.92, AcOH) [reported[10b] $[\alpha]^{20}_D$ −91.7° (AcOH)].

***N*,*N*-Dicarbobenzoxy-L-cystine Dibenzyl Ester.** This compound was prepared by either the method of Ross et al.[11] or Frankel and Gertner:[18a] mp 82–84 °C (reported[11,18a] mp 59–60, 79 °C); $[\alpha]^{23}_D$ +38.3° (*c* 1.20, $CHCl_3$) [reported[18b] $[\alpha]^{20}_D$ +45.6° (*c* 1.5, $CHCl_3$)].

***N*-Carboxy-3-(sulfonylchloro)-L-alanine Dibenzyl Ester (1).** *N*,*N*-Dicarbobenzoxy-L-cystine dibenzyl ester (20.0 g, 29.1 mmol) was dissolved in 185 mL of glacial acetic acid, and then 20 mL of H_2O was added. Chlorine gas was condensed (20 mL, 0.44 mol) in a large test tube by cooling in dry ice. By removing the tube from the dry ice, the chlorine slowly distilled and was introduced by way of a delivery tube just above the surface of the vigorously stirred and ice-cooled acetic acid solution. The ice bath was intermittently placed under the reaction flask in order to prevent freezing of the acetic acid solution. After all the chlorine had been introduced, the flask was flushed with N_2 gas and the contents of the vessel washed into a round-bottom flask with CCl_4. The mixture was concentrated to dryness and the solid crystallized from CCl_4, affording 18.0 g (75%) of compound 1, mp 103.5–105 °C. Recrystallization from CCl_4 gave the analytical sample: mp 111.5–112 °C (reported[11] 98–99 °C); $[\alpha]^{26}_D$ +5.2° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 1751 (ester), 1722 (carbamate), 1381 and 1170 cm^{-1} (SO_2Cl). Anal. ($C_{18}H_{18}NO_6ClS$) C, H, Cl.

***N*-Carboxy-3-(ethoxysulfonyl)-L-alanine Dibenzyl Ester (4).** When the above procedure was performed on a smaller scale and the reaction worked up with EtOH, as described by Ross et al.,[11] only the corresponding sulfonate ester **4** was obtained. The analytical sample was obtained by crystallization from benzene–petroleum ether: mp 103.5–104 °C; $[\alpha]^{26}_D$ +14.3° (*c* 0.6, $CHCl_3$); IR 1731 (ester carbonyl), 1699 (carbamate), 1349, 1328, 1177 cm^{-1} ($-SO_2OEt$); NMR ($CDCl_3$) δ 1.28 (t, 3 H, OCH_2CH_3), 4.18 (q, 2 H, OCH_2CH_3). Anal. ($C_{20}H_{23}NO_7S$) C, H, S.

***N*-Carboxy-3-sulfamoyl-L-alanine Dibenzyl Ester.** This compound was prepared by the method of Ross et al.[11] from 1 and NH_3: mp 125.5–126.5 °C; $[\alpha]^{23}_D$ −42.6° (*c* 1.01, EtOH) (reported mp 125–126 °C).

3-Sulfamoyl-L-alanine. Catalytic reduction of the above dibenzyl ester according to the method of Ross et al.[11] gave the desired compound: mp 190–194 °C dec; $[\alpha]^{26}_D$ −9.1° (*c* 1.0, H_2O) [reported[11,19] mp 197–198, 190–200 °C dec; $[\alpha]^{25}_D$ −16.3° (*c* 1.97, H_2O)].

***N*-Carboxy-3-(hydroxysulfamoyl)-L-alanine Dibenzyl Ester (2a, X = H).** Dry $NaHCO_3$ (205 mg, 2.44 mmol) and NH_2OH–HCl (85 mg, 1.22 mmol) were added to a solution of 1 (500 mg, 1.22 mmol) in 5 mL of CH_3CN. The resultant mixture was allowed to stir at room temperature overnight. The reaction mixture was transferred to a separatory funnel, diluted with 200 mL of EtOAc, and washed with two 10-mL portions of 10% citric acid and 10 mL of saturated NaCl. The organic layer was dried and evaporated. The residue was purified by applying 100-mg portions to silica gel preparatory plates and developing with 3% MeOH–$CHCl_3$. The desired band was extracted and the residue obtained on evaporation was crystallized from ether–petroleum ether, yielding 192 mg (38.6%) of **2a** (X = H), mp 120–124.5 °C. Recrystallization afforded an analytical sample: mp 121.5–123.5 °C; $[\alpha]^{27}_D$ −12.5° (*c* 1.08, EtOH); IR 3240 (NOH), 1720 cm^{-1} (br, carbamate and ester). Anal. ($C_{18}H_{20}N_2O_7S$) C, H, N.

***N*-Carboxy-3-(benzyloxysulfamoyl)-L-alanine Dibenzyl Ester (2a, X = $CH_2C_6H_5$).** Powdered *O*-benzylhydroxylamine hydrochloride (Sigma Chemicals, 2.33 g, 14.6 mmol) was dissolved in 15 mL of H_2O and made basic with solid K_2CO_3. The liberated free amine was extracted into ether and the ether layer was washed with saturated NaCl, dried, and concentrated on a steam bath to a volume of about 20 mL. Compound 1 (2.00 g, 4.86 mmol)

was dissolved in 25 mL of dimethoxyethane (purified by passage over Woelm basic alumina) and added dropwise to a stirred mixture of $NaHCO_3$ (0.41 g, 4.88 mmol) and the ethereal *O*-benzylhydroxylamine. After 2 days the solids were removed by filtration and washed with ethyl acetate, and the combined filtrate was concentrated. The oily residue was dissolved in 50 mL of ethyl acetate and extracted three times with 15-mL portions of 10% citric acid and once with saturated NaCl, dried, and concentrated. The residue which solidified was crystallized from ether–petroleum ether, affording 1.89 g of product, mp 89–91 °C. A second crop, 0.15 g, mp 93–95 °C, raised the yield to 2.04 g (84%). The analytical sample had mp 94–95 °C; $[\alpha]^{24}_D$ −8.4° (*c* 0.97, EtOH); IR 1741 (benzyl ester), 1685 (carbamate), 1340 and 1147 cm^{-1} (SO_2NH). Anal. ($C_{25}H_{26}N_2O_7S$) C, H, N.

3-(Hydroxysulfamoyl)-L-alanine (3a, X = H). (a) Sulfonohydroxamic acid **2a** (X = H, 50 mg, 0.12 mmol) was dissolved in 5 mL of 30% aqueous EtOH and hydrogenated under atmospheric conditions in the presence of 30 mg of Pd black. Following removal of the catalyst by filtration and evaporation of the filtrate, the residue was crystallized from H_2O–EtOH, yielding 16 mg (72.7%) of the free amino acid **3a** (X = H) which contained only trace impurities as evidenced by TLC on cellulose (3:1:2, BuOH–HOAc–H_2O). Recrystallization afforded an analytical sample: mp 170–174 °C dec; $[\alpha]^{26}_D$ +2.2° (*c* 0.99, AcOH–H_2O, 9:1); IR 3245 (NOH), 1140 cm^{-1} (SO_2NHOH). Anal. ($C_3H_8N_2O_5S$) C, H, N.

(b) Triply protected **2a** (X = $CH_2C_6H_5$) (1.00 g, 2.01 mmol) and anisole (1.0 mL, 9.0 mmol) were allowed to stir with 5–10 mL of liquid HF for 30 min at −78 °C in the above described apparatus. Following removal of the HF by aspiration, the residue was dissolved in 60 mL of 5% acetic acid and washed three times with 10-mL portions of ethyl acetate. The aqueous layer was lyophilized and the resulting powder dissolved in water and brought to a pH of about 6 with $KHCO_3$. After lyophilizing again, the residue was crystallized from H_2O–EtOH to give 290 mg (81%) of **3a** (X = H), identical in the IR with the analytical sample prepared above.

***N*-Carboxy-3-[(2-chloroethyl)sulfamoyl]-L-alanine Dibenzyl Ester (2b).** 2-Chloroethylamine hydrochloride (240 mg, 2.1 mmol) and Na_2CO_3 (310 mg, 2.9 mmol) were dissolved in 6 mL of H_2O and stirred vigorously for 6 h with a solution of **1** (400 mg, 0.97 mmol) in 7 mL of $CHCl_3$. The $CHCl_3$ layer was washed with 5% HCl, 5% Na_2CO_3, and saturated salt solution, dried, and concentrated. Crystallization from CCl_4 afforded 300 mg (66%) of **2b**: mp 127–127.5 °C; $[\alpha]^{23}_D$ +34.4° (*c* 0.9, $CHCl_3$); IR ($CHCl_3$) 1342 and 1139 cm^{-1} (SO_2NH). Anal. ($C_{20}H_{23}N_2O_6SCl$) C, H, N.

3-[(2-Chloroethyl)sulfamoyl]-L-alanine (3b). Compound **2b** (800 mg, 1.76 mmol) was dissolved in 40 mL of EtOH and hydrogenated under atmospheric conditions in the presence of 200 mg of Pd black. When the theoretical amount of H_2 was consumed, the catalyst was removed by filtration and the filtrate concentrated to dryness. Crystallization from H_2O–EtOH afforded 366 mg (90%) of desired product: mp 171–174 °C; $[\alpha]^{23}_D$ +7.6° (*c* 1.2, H_2O). Anal. ($C_5H_{11}ClN_2O_4S$) C, H, N.

***N*-Carboxy-3-[*p*-(fluorosulfonylbenzyl)]sulfamoyl-L-alanine Dibenzyl Ester (2c).** Triethylamine (0.21 mL, 1.50 mmol) was added to a mixture of **1** (200 mg, 0.48 mmol) and *p*-(fluorosulfonyl)benzylamine hydrochloride[18] (216 mg, 0.96 mmol) in 3 mL of dry CH_3N. After stirring for 3 h at room temperature, the mixture was filtered to remove insolubles. The filtrate was diluted to 100 mL with EtOH, washed with 1 N HCl, saturated $NaHCO_3$, and saturated NaCl, and dried. Following evaporation, the 251 mg of oily residue was applied to two silica gel preparatory plates and developed with 4% MeOH–$CHCl_3$. The appropriate bands were extracted with 7% MeOH–$CHCl_3$ and following removal of solvent the residue was crystallized from EtOAc–petroleum ether to give 154 mg of product, mp 108–110 °C; a second crop of 15 mg, mp 101–105 °C, elevated the yield to 62%. Recrystallization afforded an analytical sample: mp 110–113 °C; $[\alpha]^{27}_D$ −6.2° (*c* 1.08, EtOH); IR 1722 (benzyl ester), 1688 (carbamate), 1410 and 1219 (SO_2F), 1323 and 1141 cm^{-1} (SO_2NH). Anal. ($C_{25}H_{25}FN_2O_8S_2$) C, H, N.

3-[*p*-(Fluorosulfonylbenzyl)]sulfamoyl-L-alanine (3c). Protected sulfonyl fluoride **2b** (150 mg, 0.27 mmol) and anisole (0.06 mL, 0.54 mmol) were allowed to stir with 5–10 mL of liquid HF at 0 °C for 1 h in the above described apparatus. Following removal of the HF by evaporation at the aspirator, the residue was dissolved in 30 mL of 5% HOAc and washed twice with 15 mL of EtOAc. Storage of the aqueous layer in the refrigerator overnight in preparation for lyophilization gave rise to 36 mg of analytically pure amino acid, mp 202–207 °C dec. Lyophilization of the mother liquor, followed by crystallization of the residue from H_2O, afforded an additional 20 mg of pure material, bringing the yield to 61%: mp 202–207 °C dec; $[\alpha]^{26}_D$ +4.2° (*c* 0.62, 1 N HCl); IR 1415 and 1210 (SO_2F), 1322 and 1141 cm^{-1} (SO_2NH). Anal. ($C_{10}H_{13}FN_2O_6S_2$) C, H, N.

***N*-Carboxy-3-sulfo-L-alanine Dibenzyl Ester *S*-(2-*tert*-Butoxycarbonyl)hydrazide (6).** *tert*-Butyl carbazate (1.92 g, 14.58 mmol) was added in one portion to a solution of **1** (2.0 g, 4.9 mmol) in 15 mL of $CHCl_3$. After stirring for 5 h at room temperature, the reaction mixture was filtered and the filtrate diluted to 300 mL and transferred to a separatory funnel. The $CHCl_3$ solution was washed twice with 50 mL of 10% citric acid, saturated $NaHCO_3$, and H_2O. The $CHCl_3$ layer was dried, the solvent removed by evaporation, and the residue crystallized from EtOAc–petroleum ether, yielding 1.71 g of **5**, mp 78–80 °C; a second crop of 0.18 g, mp 76–80 °C, brought the yield to 77%. Recrystallization afforded the analytical sample: mp 79.5–82 °C; $[\alpha]^{27}_D$ −13.5° (*c* 1.01, EtOH); IR 1752 (ester), 1742 (carbazate), 1685 (carbamate), 1341 and 1140 cm^{-1} (SO_2NH). Anal. ($C_{23}H_{29}N_3O_8S$) C, H, N.

***N*-Carboxy-3-sulfo-L-alanine Dibenzyl Ester *S*-Hydrazide Hydrochloride (7).** Carbazate **6** (500 mg, 0.99 mmol) was stirred for 10 min with 3 mL of EtOAc which had been previously saturated with dry HCl. The solvent was removed; the residue was dissolved in 2–3 mL of EtOAc and triturated with 15–20 mL of petroleum ether. Stirring was continued for 10–15 min to ensure complete solidification of the gum. Collection of the white solid by filtration yielded 432 mg of **6**, mp 107–116 °C, containing a trace impurity: IR 1745 (ester), 1700 (carbamate), 1325 and 1145 cm^{-1} (SO_2NH). This compound was used without further purification, as attempted crystallization led to decomposition.

***N*-Carboxy-3-sulfo-L-alanine Dibenzyl Ester *S*-[2-[*p*-(Fluorosulfonyl)benzoyl]hydrazide] (2d).** *p*-(Fluorosulfonyl)benzoyl chloride (Aldrich, 249 mg, 1.1 mmol) was added to a cooled mixture of CuCl (10 mg), Na_2CO_3 (64 mg, 0.6 mmol), and **7** (406 mg, 1.0 mmol) in 8 mL of THF. The reaction mixture was allowed to stir for 23 h at room temperature, transferred to a separatory funnel containing 300 mL of EtOAc, and washed with 10 mL of H_2O, twice with 10 mL of 10% citric acid, twice with 15 mL of saturated $NaHCO_3$, and with 10 mL of saturated NaCl. The organic layer was dried ($MgSO_4$) and evaporated to a solid residue (437 mg). The residue was purified by preparative TLC using 3% MeOH–$CHCl_3$ and the desired product subsequently crystallized from acetone–petroleum ether, yielding 180 mg of **2d**, mp 185–187 °C; a second crop afforded 15 mg, mp 180–183 °C, bringing the yield to 33%. Recrystallization afforded an analytical sample: mp 187–189 °C; $[\alpha]^{27}_D$ −3.3° (*c* 1.10, acetone); IR 1730 (ester), 1680 (carbamate), 1665 (hydrazide), 1420 and 1215 cm^{-1} (SO_2F). Anal. ($C_{25}H_{24}FN_3O_9S_2$) C, H, S.

3-Sulfo-L-alanine *S*-[2-[*p*-(Fluorosulfonyl)benzoyl]-hydrazide] (3d). Blocked sulfonyl fluoride **2d** (150 mg, 0.25 mmol) was deprotected with 5–10 mL of liquid HF in the presence of anisole (0.06 mL, 0.54 mmol) as previously described for **3c**. After washing the aqueous layer with EtOAc and lyophilization, the residue was crystallized from water, yielding 54 mg (59%) of desired free amino acid. Repeated recrystallization provided an analytical sample: mp 207–208 °C dec; $[\alpha]^{26}_D$ +16.8° (*c* 0.57, 1 N HCl); IR 1415 and 1218 cm^{-1} (SO_2F). Anal. ($C_{10}H_{12}FN_3O_7S_2$) C, H, N.

***N'*-(*tert*-Butoxycarbonyl)monoethyl Fumarylhydrazide (8).** Dicyclohexylcarbodiimide (14.3 g, 69 mmol) was added to a cooled solution of monoethyl fumarate (10.0 g, 69 mmol), *tert*-butyl carbazate (9.1 g, 69 mmol), and 1-hydroxybenzotriazole (9.3 g, 69 mmol) in 150 mL of CH_3CN. The reaction mixture was allowed to stir for 1 h at 0 °C and 1 h at room temperature. The solvent was removed, the solid triturated with EtOAc, and the mixture filtered. The filtrate was transferred to a separatory funnel and washed with 1 N HCl, saturated $NaHCO_3$, and saturated NaCl. The solvent was evaporated leaving a quantitative yield of oil which was immediately purified by column chromatography (silica gel, 4% MeOH–$CHCl_3$). Pooling of the ap-

propriate fractions and removal of the solvent afforded an oil which crystallized from ether–petroleum ether, yielding 10.35 g (58%) of slightly impure material. An analytical sample, mp 78–80 °C with softening at 55 °C, was obtained by further purification via preparative TLC. Anal. ($C_{11}H_{18}N_2O_5$) C, H, N.

Monoethyl Fumarylhydrazide (9). Hydrazide 8 (7.35 g, 28.5 mmol) was allowed to stir for 15 min with EtOAc (100 mL) which had been previously saturated with dry HCl. Evaporation of the solvent followed by trituration of the residue with ether afforded a white precipitate. The solid was subsequently crystallized from EtOH–ether to yield 4.5 g of hydrazide salt. The salt was transferred to a separatory funnel and mixed with 200 mL of EtOAc. Saturated $NaHCO_3$ was added to the funnel with shaking until gas evolution ceased. The organic solvent was washed with saturated NaCl and dried. Evaporation of the solvent left a solid which crystallized from EtOAc–petroleum ether to give 2.27 g (50%) of **9**, mp 116–120 °C. Recrystallization afforded an analytical sample: mp 118–121 °C; IR 1700 (ester) and 1655 cm^{-1} (hydrazide). Anal. ($C_6H_{10}N_2O_3$) C, H, N.

***N*-Carboxy-3-sulfo-L-alanine Dibenzyl Ester *S*-[2-[(*E*)-3-(Ethoxycarbonyl)acryloyl]hydrazide] (2e).** Hydrazide **9** hydrochloride (648 mg, 3.33 mmol) was added to 10 mL of ice-cold pyridine, then sulfonyl chloride 1 (687 mg, 1.67 mm) was added portionwise, and the mixture was allowed to stir at 0 °C for 30 min. The reaction was stopped by the addition of 15 mL of concentrated HCl. The aqueous solution was saturated with NaCl and extracted three times with 50-mL portions of EtOAc. The organic layer was washed with saturated $NaHCO_3$ and saturated NaCl and dried. Evaporation of the EtOAc left 724 mg of an oil which was subsequently purified by preparative TLC (5% MeOH–$CHCl_3$). After extraction of the desired band, the product was crystallized from ether–petroleum ether, affording 296 mg of **2e**, mp 136–138 °C; a second crop of 17 mg, mp 130–133 °C, elevated the yield to 35%. Recrystallization yielded the analytical sample: mp 142–144.5 °C; $[\alpha]^{27}_D$ −1.9° (*c* 1.07, EtOH); IR 1685 (carbamate), 1342 and 1150 cm^{-1} (SO_2NH). Anal. ($C_{24}H_{27}N_3O_9S$) C, H, N.

3-Sulfo-L-alanine *S*-[2-[(*E*)-3-(Ethoxycarbonyl)acryloyl]hydrazide] (3e). Fumaryl hydrazide **2e** (125 mg, 0.23 mmol) was deblocked with 5–10 mL of liquid HF in the presence of anisole (0.06 mL, 0.54 mmol) as described for **3c**. The lyophilized material was crystallized from water yielding 44 mg (62%) of analytically pure material: mp 208.5–209.5 °C; $[\alpha]^{26}_D$ +17.7° [*c* 1.0, AcOH–H_2O (9:1)]; IR 1712 cm^{-1} (conjugated ester). Anal. ($C_9H_{15}N_3O_7S$) C, H, N.

Preparation of ASase, ASase Assay, and Inhibition. The preparation and assay of ASase has been described earlier.[16] In vitro inhibition studies were performed in duplicate on three separate days (triplicate of duplicates) utilizing freshly prepared inhibitor solutions. Percent inhibition was determined from the ratio of asparagine-^{14}C synthesized in the presence of inhibitor to that in the absence of inhibitor.[16] A solution of the inhibitor in 0.3 mL of H_2O (or 0.02 N HCl if necessary for solubilization), 0.5 mL of solution "A" (disodium ATP, 8.0 μmol; $MgCl_2 \cdot 6H_2O$, 8 μmol; Tris buffer, 100 μmol; pH adjusted to 8.0 with 1 N HCl), and 20 μL of ASase was preincubated for 15 min at 37 °C. Solution "B" (0.2 mL, containing L-glutamine, 20.0 μmol; L-aspartic acid, 2.0 μmol; L-aspartic acid-^{14}C, 2×10^6 dpm) was added and the mixture incubated at 37 °C for 30 min. The work-up and isolation of L-asparagine-^{14}C has been previously described.[16]

Product Analysis. That the radioactivity measured in the enzyme reaction was a consequence of L-asparagine-^{14}C formation was tested by the procedure of Holcenberg.[20] Two test tubes were charged with 0.3 mL of H_2O, 0.5 mL of stock solution "A", 0.2 mL of stock solution "B", and 20 μL of purified enzyme; a third tube containing all but enzyme served as a blank control. The tubes were incubated for 30 min at 37 °C. To one of the tubes containing enzyme was added 10 IU of L-asparaginase (Squibb Aspinase), and all three tubes were then incubated an additional 30 min. Following work-up in the usual manner,[16] the following results were obtained: blank control (no enzyme), 1080 cpm; control with ASase, 22772 cpm; enzyme (ASase) followed by L-asparaginase, 1258 cpm. These results indicate that radioactivity due to L-asparagine formation was greater than 98%.

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