that it is this α-carbon atom at which the first reaction with the enzyme occurs. Further evidence in support of this conclusion is the fact that not only benzyl- but phenethyl- and alkylamines are inhibitors with potency described by eq 7.

Comparison with Other Quantitative Structure–Activity Studies on MAO. Another quantitative structure–activity analysis of pargyline analogs has been reported recently.[12] Unfortunately, the potencies used in the calculations were not those reported in the cited source of data. The SAR analysis of other series included in that report is seriously compromised by the fact that too many variables were examined as possible predictors and compounds were arbitrarily omitted to get a better fit. Thus several of the equations might have been expected by chance.[13]

Studies on the inhibition of MAO by *N*-(phenoxyethyl)-cyclopropylamines also demonstrated electronic, hydrophobic, and steric effects on potency.[14] The dependence of lipophilicity was identical with that calculated above. The electronic and steric effects cannot be directly related between the two series.

The rate of oxidative metabolism of primary amines by rabbit liver MAO is characterized by an optimum log *P* of 2.50 and a positive dependence of pK_a.[15] Since the inhibitors in Table I have log *P*'s (log $P = 1.75 + \pi$) on both sides of the optimum for these substrates but show no optimum, the hydrophobic effects of the two series/activities/enzymes are different. The positive dependence on pK_a is also opposite to the inhibitors and the benzylamine substrate data quoted above.

In the structure–activity analysis of the relative potency of β-carbolines as monoamine oxidase inhibitor, a large influence of hydrophobicity was seen.[10,16] This is in contrast to the results presented for propynylamines. It is possible that there are hydrophobic effects on each of the steps of the enzyme–inhibitor reaction but that these effects are of similar magnitude and of opposite direction.

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Potential Inhibitors of L-Asparagine Biosynthesis. 3.[1] Aromatic Sulfonyl Fluoride Analogs of L-Asparagine and L-Glutamine†

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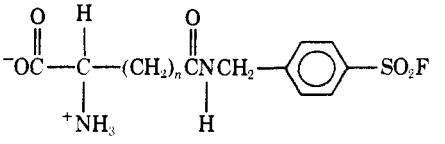
The *N*-[*p*-(fluorosulfonyl)benzyl] derivatives of L-asparagine and L-glutamine (**1a,b**) were synthesized as potential inhibitors of L-asparagine synthetase (ASase). Condensation of *p*-(fluorosulfonyl)benzylamine (**2**) with the suitably protected amino acid in the presence of dicyclohexylcarbodiimide, followed by deblocking, afforded **1a** and **1b**. Derivatives **1a** and **1b** at 10 m*M* inhibit ASase isolated from Novikoff hepatoma (rats) by 60 and 46%, respectively. Preliminary results on inhibition of Jensen sarcoma (L-asparaginase sensitive) and JA-1 sarcoma (L-asparaginase resistant) tissue cultures by 0.3 m*M* **1a** (139, 90%) and **1b** (101, 103%), respectively, are discussed.

The finding that L-asparaginase (ASNase) resistant tumors and tumors once sensitive to the deamidase exhibit an increased biosynthesis of L-asparagine via a heightened activity of L-asparagine synthetase[2-5] (ASase) has prompted the search for agents capable of inhibiting the synthetase. Such compounds could be beneficial from two standpoints. First, they could be used against the resistant line, and, second, they could be used in combination with ASNase against sensitive tumors in an attempt to prevent the development of the resistant cells.

Mammalian ASase synthesizes L-asparagine from L-aspartic acid, utilizing L-glutamine as the primary source of nitrogen.[6-8] In addition, it has been observed that the synthetase undergoes product inhibition by L-asparagine.[5,6,8-10] It was felt, therefore, that close analogs of L-asparagine and L-glutamine could possibly inhibit the biosynthesis or interfere with the utilization of L-asparagine in both L-asparaginase sensitive and resistant tumors.



1a, $n = 1$
b, $n = 2$

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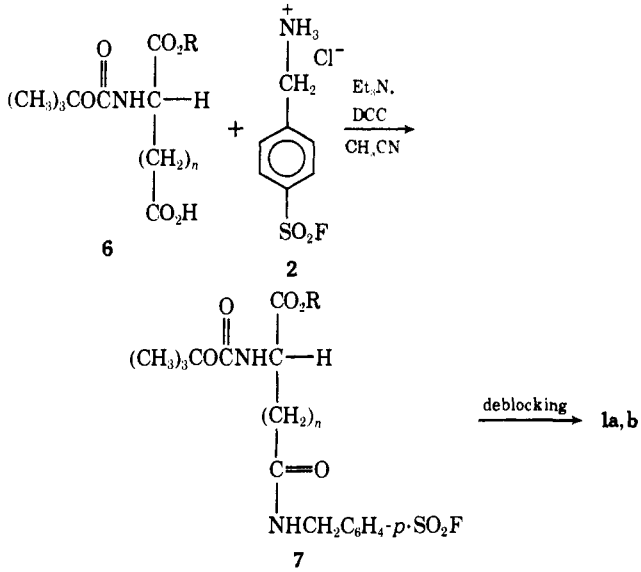
We wish to report the synthesis and biochemical testing of the *N*-[*p*-(fluorosulfonyl)benzyl] analogs of L-asparagine and L-glutamine (**1a,b**). Baker and coworkers have made extensive use of the aromatic sulfonyl fluoride group in the design of irreversible enzyme inhibitors.[11] The aromatic sulfonyl fluoride group has been shown to possess a number of desirable properties, both in its chemistry and ability to react within an enzymic-type complex.[11] Thus, aromatic sulfonyl fluorides are stable to hot acid and, in fact, to most reagents not strongly basic.[11] While stable to hot ethanol, the sulfonyl fluoride group will rapidly form a covalent bond with an alcohol group of cellulose when the sulfonyl fluoride group is attached to an appropriate carrier reversibly complexed with the cellulose.[11] Although Baker and coworkers mainly used anilinosulfonyl fluorides for incorporation of the SO_2F into their carrier compounds,[11] it was felt that this type of amino group would be too weak a nucleophile (owing to the strongly electron-withdrawing SO_2F group) to react with an activated amino acid. Instead, the next higher homolog of sulfanilyl fluoride, *p*-(fluorosulfonyl)benzylamine (**2**), would have a more reactive amino group.

Chemistry. The preparation of **1a** and **1b** was achieved via coupling of the suitably blocked L-aspartic and L-glutamic acids with **2**. Although the latter compound had been previously reported,[12] we varied their procedure by utilizing the more acid-labile *N*-benzylformamide rather than *N*-benzylacetamide, because it was both easier and afforded better yields. Benzylamine was converted in quantitative yield to *N*-benzylformamide (**3**) by heating with formamide[13] and then to *N*-[*p*-(chlorosulfonyl)benzyl]formamide (**4**) with chlorosulfonic acid. Treatment of **4** with a KF solution gave the corresponding sulfonyl fluoride **5** which could be deblocked and converted to its hydrochloride salt **2** by ethanolic HCl.

Commercial *N-tert*-butoxycarbonyl-L-aspartic acid *α-tert*-butyl ester (**6a**) was condensed with **2** in acetonitrile in the presence of dicyclohexylcarbodiimide (DCC) and triethylamine to give the asparagine derivative **7a**. Deblocking of **7a** with HBr in acetic acid gave the amino acid hydrobromide salt which was converted to the free amino acid **1a** with aqueous sodium acetate (Scheme I).

Scheme I



In the same way, commercial *N-tert*-butoxycarbonyl-L-glutamic acid *α*-benzyl ester (**6b**) was converted to the sulfonyl fluoride **7b**. Deblocking, however, was accomplished in two steps: (a) catalytic hydrogenation to remove the benzyl ester, and (b) HBr in acetic acid to cleave the *tert*-butoxycarbonyl group. The hydrobromide salt of the amino acid was neutralized with aqueous sodium acetate yielding the free amino acid **1b** (Scheme I).

Table I. Inhibition of Asparagine Synthetase[a]

Compd[b]	Enzyme fraction	Concn, m*M*	% inhibn ± SD[c]
1a	B	1	4.6 ± 5.4
1a	B	2	33.7 ± 1.1
1a	B	4	29.7 ± 4.7
1a	E	4	39.0 ± 0.7
1a	B	5	32.5 ± 4.0
1a	E	10	60.2 ± 5.2
1a[d]	E	4	22.6 ± 2.7
1a[e]	E	4	12.7 ± 1.6
1b	B	1	16.5 ± 3.8
1b	B	2	28.1 ± 2.2
1b	B	4	28.0 ± 3.2
1b	B	10	45.6 ± 0.3
N-Benzyl-L-asparagine	E	5	10.5 ± 4.2
N-Benzyl-L-glutamine	E	10	6.0 ± 2.3
N-Methyl-L-asparagine	B	10	53.6 ± 9.4
N-Methyl-L-glutamine	B	10	15.9 ± 2.9

[a] ASase purified as described in paper 2 of this series (ref 1). [b] The compounds were preincubated with ASase and substrate aspartic acid was added. The formation of L-asparagine was determined as described earlier (ref 1). [c] Percent inhibition is the mean of a triplicate run. [d] Preincubation time was extended to 30 min. [e] No preincubation, 30 min total incubation.

Biochemical Results. Rats infected with Novikoff hepatomas were utilized as the source of ASase. The initial strain of Novikoff hepatoma was kindly supplied by Dr. Manford K. Patterson, Jr. (The Samuel Roberts Noble Foundation, Inc., Ardmore, Okla. 73401). Two fractions of enzyme[1] were used: B, with a specific activity of 0.11 μmol of asparagine synthesized per milligram of protein per 30 min; and E, with a specific activity of 0.32. 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.[1]

The ability of **1a** and **1b** and their corresponding *N*-methyl and *N*-benzyl analogs to inhibit the in vitro biosynthesis of L-asparagine is shown in Table I. Results for enzyme fractions B and E appear to be correlative as evidenced by the small difference in percent inhibition for **1a** at 4 m*M*.

It had originally been intended to present enzyme inhibitions as ID_{50}'s rather than percent inhibitions, but the nature of the results precluded that. The percent inhibition for **1a** and **1b** between the concentrations of 2 and 5 m*M* plateaus and again rises at a concentration of 10 m*M* (Table I). A plot of the results would indicate a pair of sigmoid curves joined by a horizontal section where the inhibition is invariant. Although appearing anomalous at first, the results possibly could be explained as a consequence of a binary system; that is, a system composed of asparagine synthetase isoenzymes, each of which has a detectably different sensitivity to the inhibitors. Similar results have been observed for inhibiton of the monoamine oxidase system by chlorgyline.[14,15] Although it has been observed that ASase can utilize either NH_3 or glutamine as the source of nitrogen,[6,8] it has yet to be established whether one enzyme has sites for both nitrogen donors or if isoenzymes exist. It

should be noted that while fractions B and E are purified extracts, these fractions are still to be considered crude.

The efficacy of the SO_2F group in bringing about enzymic inhibition is evidenced by a comparison of the results for **1a** and **1b** with the unsubstituted *N*-benzylamino acids (Table I). The sulfonyl fluoride substituted compounds are from three- to eightfold more effective in causing in vitro inhibition than the unsubstituted analogs. This is probably not active-site specific since then the *N*-benzyl derivative should at least give reversible inhibition.

It is interesting to note that of the two *N*-methyl derivatives tested (Table I), only the asparagine analog exhibits appreciable activity. In agreement with our in vitro finding, Handschumacher and Uren have likewise found *N*-methyl-L-asparagine to be an effective inhibitor of ASase (L5178Y asparaginase resistant subline) in vitro.[16] Combination of *N*-methyl-L-asparagine with asparaginase was also seen[16] to be effective in increasing survival time of mice bearing L5178Y leukemia as well as asparaginase-resistant tumors. Surprisingly, when **1a** and **1b** were tested[17] at 10 m*M* with ASase from L5178Y/AR mouse leukemia, only 8 and 11% inhibition was observed, respectively.

Preliminary results of the inhibition by **1a** and **1b** on Jensen sarcoma (ASNase sensitive) and JA-1 sarcoma (ASNase resistant) cells in tissue culture are shown in Table II. Although **1a** at 0.3 m*M* exhibits some cytotoxicity, the effect of both compounds on 24-hr growth looks promising. That each compound affects both cell lines suggests that they may act as amino acid antagonists, inhibitors of ASase, or are nonspecific in action. Both **1a** and **1b** are presently being screened for in vivo antitumor activity by the Cancer Chemotherapy National Service Center.

Table II. Preliminary Tissue Culture Study[a]

	Concn, m*M*	Cell count × 10^{3}[b] 0 hr	1a, 24 hr	1b, 24 hr
Jensen	0	1066 ± 14	1700 ± 31	1700 ± 31
	0.03		1580 ± 202 (19)[c]	1264 ± 343 (69)
	0.30		816 ± 177 (139)	1060 ± 111 (101)
JA-1	0	616 ± 22	1176 ± 27	1176 ± 27
	0.03		1100 ± 112 (14)	806 ± 248 (66)
	0.30		670 ± 173 (90)	600 ± 198 (103)

[a] Replicate Jensen sarcoma (ASNase sensitive) and JA-1 sarcoma (ASNase resistant) cells were established in T-15 flasks with an initial inoculum of 400,000 cells in 2 ml for 24 hr: M. K. Patterson, Jr., in "Tissue Culture: Methods and Applications", P. F. Kruse, Jr., and M. K. Patterson, Jr., Ed. The medium (McCoy's 5a supplemented with 5% bovine serum) was replaced with fresh medium containing 0.03 or 0.30 m*M* L-asparagine or L-glutamine analog. Replicate cultures were counted for a zero time period. Cultures were terminated at 24 hr. Cell counts were made on a Coulter counter, Model B, in duplicate on triplicate cultures. [b] Mean ± SD. [c] Percent inhibition of growth in parentheses.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The infrared data were obtained with a Perkin-Elmer Model 267 grating infrared spectrophotometer as KBr disks, unless stated otherwise. 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. Amino acid starting materials were purchased from Cyclo Chemical or Sigma Chemical Co. L-Aspartic acid-^{14}C was obtained from Schwarz/Mann and purified as previously described.[1] Thin-layer chromatography and preparative TLC 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 had a boiling point range of 30–60°. All evaporations were performed in vacuo.

***N*-[*p*-(Chlorosulfonyl)benzyl]formamide (4).** *N*-Benzylformamide[13] (5.0 g, 37 mmol) was melted over a steam bath in a 100-ml round-bottom flask. The flask was removed and placed in an ice bath with rotation to form a thin layer of compound on the lower half of the flask. Ice-cold $ClSO_3H$ (15.9 ml, 0.24 mol) was added slowly to the cooled flask with continued rotation until all the amide was dissolved. The mixture was then allowed to warm at 50° for 3 hr, cooled in an ice bath, and then added in a dropwise manner, with stirring, to about 125 g of crushed ice. The solid which formed was collected by filtration, washed with ice-cold water, and allowed to air dry overnight, yielding 6.2 g (72.1%) of compound 4: ir ($CHCl_3$) 1690 (amide), 1380 and 1175 cm^{-1} (SO_2Cl). This compound was not analyzed but used without further purification in the preparation of 5.

***N*-[*p*-(Fluorosulfonyl)benzyl]formamide (5).** Compound 4 (6.2 g, 26.5 mmol) was allowed to stir at room temperature for 4 hr with 25 ml of 5 *M* aqueous KF which had previously been adjusted to pH 7 with 1 *N* HCl. The mixture was cooled in an ice bath, and the solid material was filtered and washed with cold H_2O, yielding 5.0 g (86.2%) of 5, mp 92–95°. Repeated recrystallization from $CHCl_3$–petroleum ether afforded an analytical sample: mp 93.5–95.5°; ir 1655 (amide), 1410 and 1210 cm^{-1} (SO_2F); NMR (acetone-d_6) δ 4.67 (d, benzyl CH_2, J = 6 Hz), 7.82, 8.15 (aromatic doublets, J = 9 Hz), 8.43 (s, CHO). Anal. ($C_8H_8FNO_3S$) C, H, S.

***p*-(Fluorosulfonyl)benzylamine Hydrochloride (2).** Amide 5 (5.0 g, 23.0 mmol) was added to 50 ml of 95% EtOH which had previously been saturated with dry HCl and the resultant mixture was allowed to reflux for 1.5 hr. The solution was decolorized with Norit, filtered with the aid of Celite, and concentrated to a solid residue. The residue was crystallized from acetone–ether, affording 4.4 g of crude 2. Recrystallization afforded 2.9 g (55.8%) of analytical material: mp 225–235° dec (lit.[12] 240° dec); ir 2970 ($^+NH_3$), 1420 and 1218 cm^{-1} (SO_2F). Anal. ($C_7H_9FNO_2S$) C, H, N.

***N*^{2}-(*tert*-Butoxycarbonyl)-*N*-[*p*-(fluorosulfonyl)benzyl]-L-asparagine *tert*-Butyl Ester (7a).** *N*-*tert*-Butoxycarbonyl-L-aspartic acid α-*tert*-butyl ester (50 mg, 0.17 mmol), hydrochloride 2 (38 mg, 0.17 mmol), and 1 ml of acetonitrile were combined and cooled in an ice bath. Triethylamine (17 mg, 0.17 mmol) was added to the mixture with stirring. Five minutes later, DCC (35 mg, 0.17 mmol) in 1 ml of acetonitrile was added in a dropwise manner. The reaction was allowed to proceed with stirring and cooling for 6 hr. The precipitated solids were removed by filtration and washed with 50 ml of ether. The organic layer was washed with 5 ml of H_2O, 5 ml of 10% citric acid solution, 10 ml of saturated $NaHCO_3$ solution, and 5 ml of H_2O and dried ($MgSO_4$). Evaporation of the ether left a residue which precipitated dicyclohexylurea (DCU) when triturated with ether. Following filtration of the DCU and evaporation of the ether, the resultant residue was crystallized from aqueous EtOH, yielding 40 mg of crude **7a**, mp range 108–133°. Two recrystallizations afforded 19 mg (24.4%) of analytically pure material: mp 135-136°; $[\alpha]^{25}D$ −2.10° (*c* 1.43, EtOH); ir 1690 (carbamate), 1650 (amide), 1415 and 1215 cm^{-1} (SO_2F). Anal. ($C_{20}H_{29}FN_2SO_7$) C, H, N. A nearly quantitative crude yield of **7a** could be obtained by carrying out the above reaction with the aid of 1 equiv of the peptide coupling catalyst 1-hydroxybenzotriazole[18] and 1 equiv of DCC.

***N*-[*p*-(Fluorosulfonyl)benzyl]-L-asparagine (1a).** To compound 7a (50 mg, 0.11 mmol) was added 1–2 ml of HBr–HOAc (Eastman, 32%). The mixture was allowed to stir for 5–10 min at room temperature and excess HBr–HOAc was removed by evaporation. The yellow residue was brought to near pH 6 with saturated sodium acetate solution. A small amount of water was added and the mixture dissolved by heating on a steam bath. Upon cooling 15 mg of free amino acid 1a was obtained. An additional crop of 2 mg was obtained by evaporation of the mother liquor to dryness and subsequent crystallization of the residue from a small amount of H_2O; the total yield was 17 mg (51.5%). One recrystallization from H_2O afforded an analytical sample of **1a**, which was isolated as the monohydrate (no definitive melting point could be obtained): $[\alpha]^{25}D$ 16.0° (*c* 1.25, 1 *N* HCl); ir 1648 (amide), 1595–1580 (CO_2^-), 1418 and 1210 cm^{-1} (SO_2F). Anal. ($C_{11}H_{13}FN_2SO_5 \cdot H_2O$) C, H, N.

N^2-(*tert*-Butoxycarbonyl)-*N*-[*p*-(fluorosulfonyl)benzyl]-L-glutamine Benzyl Ester (7b). *N*-*tert*-Butoxycarbonyl-L-glutamic acid α-benzyl ester (250 mg, 0.74 mmol), hydrochloride **2** (167 mg, 0.74 mmol), and 2 ml of acetonitrile were combined and cooled in an ice bath. Triethylamine (75 mg, 0.74 mmol) was added to the mixture and 5 min later DCC (305 mg, 1.48 mmol) in 2 ml of acetonitrile was added in a dropwise manner. After 3.5 hr the solids were removed by filtration and washed with 1–2 ml of acetonitrile and the filtrate was replaced in an ice bath. DCC (100 mg) was added to the clear solution and in several minutes more DCU precipitated. After another 3 hr the mixture was filtered, the filtrate diluted with ether (100 ml), and the solution washed successively with saturated NaCl solution, twice with 10% citric acid solution, twice with saturated $NaHCO_3$, and again with saturated NaCl. The ether layer was dried ($MgSO_4$) and evaporated to dryness. The solid remaining was triturated with ether and the insoluble DCU removed by filtration. The filtrate was evaporated and the trituration procedure repeated a second time. Crystallization of the residue was realized from aqueous methanol and 387 mg of crude product, mp 111–130°, was obtained. One recrystallization from aqueous methanol yielded 270 mg (71.8%) of product, mp 130–132°, which contained only the slightest trace of impurity as shown by TLC (94:5:1, $CHCl_3$–MeOH–HOAc). Recrystallization from aqueous methanol gave an analytical sample: mp 131–133°; $[\alpha]^{25}D$ −14.9° (*c* 0.87, MeOH); ir 1730 (ester), 1680 (carbamate), 1648 (amide), 1415 and 1214 cm^{-1} (SO_2F). Anal. ($C_{24}H_{29}FN_2O_7S$) C, H, N.

***N*-[*p*-(Fluorosulfonyl)benzyl]-L-glutamine (1b).** Blocked sulfonyl fluoride **7b** (494 mg, 0.97 mmol) in 35 ml of 20% (v/v) aqueous EtOH was hydrogenated for 25 min under atmospheric conditions in the presence of 100 mg of palladium black (Englehard). The catalyst was removed by filtration and washed with 30 ml of EtOH. The filtrate and washings were evaporated and the residue was allowed to stir for 8 min at room temperature with 10 ml of 32% HBr–HOAc. The excess acid was evaporated and the residue was mixed with 1–2 ml of H_2O, and NaOAc solution (one part saturated NaOAc and one part H_2O) was used to bring the pH to 6. The aqueous solution was filtered to remove a small amount of DCU which had been present in the starting material. The solution was then applied to three preparative cellulose plates and developed with a 3:1:2, *n*-BuOH–HOAc–H_2O solvent system. The dark uv-sensitive band was removed and the cellulose extracted with 75 ml of ice-cold water for 30 min. The mixture was filtered and the solution lyophilized, leaving 198 mg of material. Crystallization of this material from acetone–H_2O gave 100 mg (32.5%) of **1b**, 189–192°. Repeated recrystallization from the same solvent system yielded the analytical sample: mp 189–192°; $[\alpha]^{25}D$ 19.4° (*c* 1.24, 1 *N* HCl); ir 1630 (amide), 1580 (CO_2^-), 1418, 1210 cm^{-1} (SO_2F). Anal. ($C_{12}H_{15}FN_2SO_5$) C, H, N.

***N*-Benzyl-L-asparagine.** This compound was prepared from β-benzyl-L-aspartate by the general procedure of Kimura and Murata:[19] mp 275–278° dec (lit.[20] 265° dec, for racemate); $[\alpha]^{25}D$ 40.2° (*c* 1.02, 1 *N* HCl).

***N*-Benzyl-L-glutamine.** This compound was prepared from γ-methyl-L-glutamate by the general procedure of Kimura and Murata:[19] mp 213–215° dec; $[\alpha]^{25}D$ 29.7° (*c* 1.11, 1 *N* HCl) [lit.[21] mp 203–205°; $[\alpha]^{30}D$ 26.7° (*c* 5, 2 *N* HCl)].

***N*-Methyl-L-asparagine and *N*-Methyl-L-glutamine.** These compounds were prepared by the method of Kimura and Murata.[19]

Preparation of ASase, ASase Assay, and Inhibition. The preparation and assay of ASase has been described earlier.[1] In vitro inhibition studies were performed in triplicate with the percent inhibition determined from the ratio of asparagine-^{14}C synthesized in the presence of inhibitor to that in the absence of inhibitor.[1] A solution of the inhibitor dissolved in Tris solution (0.3 ml, 1.0 μmol of Tris), 0.5 ml of solution "A" (disodium ATP, 8.0 μmol; $MgCl_2 \cdot 6H_2O$, 8.0 μmol; Tris buffer, 100 μmol; pH adjusted to 8.0 with 1 *N* HCl), and 20 μ of enzyme was preincubated for 15 min at 37°. 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° for 30 min. The work-up and isolation of L-asparagine-^{14}C has been previously described.[1]

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