a more detailed pharmacological and toxicological study.

Acknowledgments.—The authors wish to thank Dr. G. Sekules for performing microanalyses, Mr. E. Zugna for assistance in preparing the compounds, Miss L. Tomasi and Miss A. Franchi for carrying out the pharmacological tests, and Mrs. C. Ciani-Bonardi and Mr. E. Pavesi for help in biological investigation.

Specific Competitive Inhibition of L-Asparaginyl Transfer Ribonucleic Acid Lygase by β-Aminoamides

M. R. Harnden and T. O. Yellin[1]

Experimental Therapy Division, Abbott Laboratories, North Chicago, Illinois 60064

Received April 6, 1970

A series of β-aminoamides and closely related derivatives have been synthesized. The structural and stereochemical requirements for specific competitive inhibition of L-asparaginyl-tRNA lygase by these compounds have been investigated.

The introduction of the enzyme L-asparaginase (L-asparagine amidohydrolase) into clinical trials has been the most dramatic development in the treatment of leukemia in recent years. The ultimate usefulness of this enzyme has yet to be determined but the initial results are encouraging. In human as in animal lymphomas the effectiveness of the enzyme is correlated with a metabolic deficiency of the cancer cells, namely an inability to synthesize asparagine.[2]

Since asparaginase is relatively scarce and problems such as allergic response and development of resistance in susceptible leukemias have been encountered in asparaginase therapy,[3] we have initiated experiments designed to provide a chemotherapeutic approach to the treatment of those tumors for which L-asparagine is an essential amino acid, to be used, initially at least, with the enzyme. What we would hope for is a degree of synergism which would minimize the therapeutic dose of the enzyme, reduce its untoward effects and prevent the development of resistance and the reappearance of the disease. It is not known if a resistant form of the disease develops through a process of selection or by transformation of sensitive cells during asparaginase therapy. This question presents an intriguing theoretical as well as a practical problem of some significance but in either case some form of combination therapy should increase the likelihood of a complete cure.

Utilization of L-asparagine by the cell could be limited either by inhibition of the entry of asparagine into the cell or by interference with its essential role in cell metabolism. It is along the latter path that our efforts have been directed. The only vital function known for asparagine in mammalian cells is in protein synthesis, and so the problem defines itself as an attempt to limit the availability of asparagine in this process through specific inhibitors of L-asparaginyl-tRNA lygase. Evidence was recently obtained which indicates that such an approach might be possible.[4]

Experimental Section

Chemistry.—Melting points were taken with a Thomas-Hoover capillary apparatus and are corrected. Elemental analyses were carried out by Mr. V. Rauschel and his associates in the analytical department of Abbott Laboratories. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Ir and nmr spectra of all compounds listed here and in Table I were consistent with the structures given.

β-Aminoamide Hydrochlorides (1a, 2–8, 10, 11).—A solution of ethyl chloroformate (10.9 g, 0.1 mole) in dry PhMe (50 ml) was added over 30 min to a stirred solution of the appropriate α,β-unsaturated acid[5] (0.1 mole) and Et_3N (10.1 g, 0.1 mole) in dry PhMe (50 ml) maintained at 0°. The mixture was stirred at 0° for an additional 2 hr and then filtered. Excess NH_3 was added to the filtrate and PhMe removed at reduced pressure. The residue was dissolved in EtOH (100 ml), liquid NH_3 (50 ml) added, and the solution heated at 100° for 24 hr in a 270-ml Hastelloy autoclave. The reaction mixture was cooled, filtered, and concd under reduced pressure. The residue was dissolved in *i*-PrOH (100 ml) and the solution concentrated again. The residue was then dissolved in *i*-PrOH (50 ml) and a solution of HCl in *i*-PrOH (2.9 *N*) carefully added until pH 7 was reached. The white crystals of the β-aminoamide·HCl that deposited were filtered, washed with cold *i*-PrOH (3 portions of 20 ml), and recrystd from *i*-PrOH.

3-Amino-4,4,4-trifluorobutyramide·HCl (9), 3-Amino-4-hydroxyvaleramide·HCl (13), and 3-Amino-4-ethylphosphonobutyramide (15).—Compounds **9, 13,** and **15** were prepared by addition of NH_3 to ethyl-4,4,4-trifluorocrotonate,[6] α-angelicalactone,[7] and triethyl-4-phosphonocrotonate,[7] respectively. The same reaction conditions (EtOH, 100°, 24 hr) as described above were employed. Compounds **9** and **13** were isolated as their HCl salts in the usual manner. Compound **15** was obtained as a white crystalline solid on addition of *i*-PrOH to the residue obtained upon concentration of the reaction mixture, and was recrystd from EtOH-*i*-PrOH.

3-Amino-5-methylsulfonylvaleramide·HCl (12).—The methylthio derivative **11** (2.0 g, 0.01 mole) was suspended in AcOH (20 ml) and 80% *m*-chloroperbenzoic acid (4.3 g, 0.02 mole-equiv) added. A clear solution was obtained. The solution was allowed to remain at 20° for 20 hr and then EtOAc (100 ml) added. The product **12** was obtained as a white hygroscopic precipitate and was purified by dissolving in EtOH (50 ml) and reprecipitating with EtOAc (100 ml).

(1) To whom inquiries should be directed.

(2) J. D. Broome, *Trans. N. Y. Acad. Sci.*, **30**, 690 (1968).

(3) (a) C. M. Haskell, G. P. Cannell os, B. G. Levental, P. P. Carbone, A. A. Serpick, and H. H. Hansen, *Cancer Res.* **29**, 974 (1969); (b) J. M. Hill, E. Loeb, A. MacLellan, A. Khan, J. Roberts, W. F. Shields, and N. O. Hill, *ibid.*, **29**, 1574 (1969).

(4) T. O. Yellin, *Biochem. Biophys. Res. Commun.*, **32**, 307 (1968).

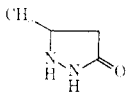
(5) The α,β-unsaturated acids were, with the exception of crotonic acid and cinnamic acid which were commercially available, prepared in 70–90% yield by reaction of the appropriate aldehyde with malonic acid in pyridine solution, as described by K. V. Auwers, *Justus Liebigs Ann. Chem.*, **432**, 58 (1923). No attempt to separate geometric isomers was made.

(6) Prepared as described by H. M. Walborsky and M. Schwarz, *J. Amer. Chem. Soc.*, **75**, 3241 (1953).

(7) Purchased from Aldrich Chem. Co., Milwaukee, Wis.

Table I

$RCHCH_2CONH_2$
|
NH_2

Compd	R	Salt	Mp, °C	Yield, %	Formula	Analyses
1a	DL-CH_3	HCl	143–144	75.4		C, H, N, Cl
b	L(−)-CH_3	HCl	133–134		$C_4H_{10}N_2O \cdot HCl$	C, H, N, Cl
c	D(+)-CH_3	HCl	133–134			C, H, N, Cl
2	CH_3CH_2	HCl	189–190	65.3	$C_5H_{12}N_2O \cdot HCl$	C, H, N, Cl
3	$(CH_3)_2CH$	HCl	213–215	29.4	$C_6H_{14}N_2O \cdot HCl$	C, H, N, Cl
4	$(CH_3)_2CHCH_2$	HCl	204–206	16.6	$C_7H_{16}N_2O \cdot HCl$	C, H, N, Cl
5	$(CH_3)(C_2H_5)CH$	HCl	190–192	24.4	$C_7H_{16}N_2O \cdot HCl$	C, H, N, Cl
6	C_6H_5	HCl	237–239	44.1	$C_9H_{12}N_2O \cdot HCl$	C, H, N, Cl
7	$C_6H_5CH_2$	HCl	214–216	52.2	$C_{10}H_{14}N_2O \cdot HCl$	C, H, N, Cl
8	$C_6H_5CH_2CH_2$	HCl	213–215	51.2	$C_{11}H_{16}N_2O \cdot HCl$	C, H, N, Cl
9	CF_3	HCl	198–200	65.3[a]	$C_4H_7F_3N_2O \cdot HCl$	C, H, N, Cl, F
10	$CH_3OCH_2CH_2$	HCl	143–145	22.1	$C_6H_{14}N_2O_2 \cdot HCl$	C, H, N, Cl
11	$CH_3SCH_2CH_2$	HCl	119–121	29.3	$C_6H_{14}N_2OS \cdot HCl$	C, H, N, Cl, S
12	$CH_3SO_2CH_2CH_2$	HCl	144–146	32.2[b]	$C_6H_{14}N_2O_3S \cdot HCl$	C, H, N, Cl, S
13	$(CH_3)(OH)CH$	HCl	118–120	28.3[c]	$C_5H_{12}N_2O_2 \cdot HCl$	C, H, N, Cl
14	L-$HOCH_2$	CF_3COOH		100[d]	$C_4H_{10}N_2O_2 \cdot CF_3COOH$	[f]
15	$C_2H_{50}P(O)(OH)CH_2$ Structure		219–221	51.2[e]	$C_6H_{13}N_2O_4P$	C, H, N, P
16	$CH_3CHCH_2CONH_2$ | $NHCH_3$	HCl	116–118	96.7	$C_5H_{12}N_2O \cdot HCl$	C, H, N, Cl
17		HCl	177–179	68.8	$C_4H_8N_2O \cdot HCl$	C, H, N, O, Cl
18	$CH_3CHCH_2CONHNH_2$ | NH_2	HCl	182–186	51.0	$C_4H_{11}N_3O \cdot 2HCl$	C, H, N, Cl

[a] From ethyl-4,4,4-trifluorocrotorate. [b] From the thioether **11**. [c] From α-angelicalactone. [d] From L-3-*tert*-butoxycarboxamido-4-hydroxybutyramide. [e] From triethyl-4-phosphorocrotonate. [f] Because of the extremely hygroscopic nature of this material a satisfactory elemental analysis could not be obtained.

L(−)-***tert*-Butoxycarboxamido-4-hydroxybutyramide.**—*N*-*tert*-Butoxycarbonyl-L-asparagine *p*-nitrophenyl ester (7.2 g, 0.02 mole) was added over 1 hr in small portions to a stirred mixture of $NaBH_4$ (15.2 g, 0.4 mole) and EtOH (300 ml) maintained at 0°. The mixture was stirred at 0° for an additional 3 hr and then neutralized with a solution of citric acid (28.0 g, 0.133 mole) in H_2O (200 ml). The EtOH was removed at reduced pressure and the aq solution remaining was extracted with $CHCl_3$ (5 portions of 300 ml). The $CHCl_3$ extracts were dried (Na_2SO_4) and coned under reduced pressure to an oil which on trituration with a mixture of EtOAc (25 ml), and Et_2O (25 ml) yielded a white solid. The solid was dissolved in *i*-PrOH (25 ml) and filtered and Et_2O (200 ml) added. A flocculent white ppt was obtained. The product (4.02 g, 92% yield) had mp 121–123°, $[\alpha]^{25}D$ 11.3° (*c* 2.0 in H_2O). *Anal.* ($C_8H_{18}N_2O_4$) C, H, N.

L(−)-**3-Amino-4-hydroxybutyramide Trifluoroacetate (14).**—L(−)-3-*tert*-Butoxycarboxamido-4-hydroxybutyramide (0.5 g) was dissolved in CF_3COOH (2 ml). An immediate evolution of gas was observed. After 5 min at 20° the CF_3COOH was removed at 1 mm and a hygroscopic oil obtained (0.8 g). In the nmr spectrum (60 MHz, D_2O) of this oil, the resonance due to the *t*-Bu grouping present in the spectrum of the starting material as a sharp singlet at δ 147 ppm, was completely absent. Tlc on silica gel indicated that the product was homogeneous. The optical activity of the product was determined by measurement of the rotation of a weighed sample of the *tert*-butoxycarbonyl derivative in CF_3COOH and correcting for the molecular weight difference; $[\alpha]^{25}D$ 25.8° (*c* 2.7 in CF_3COOH).

Resolution of DL-3-Aminobutyramide.—DL-3-Aminobutyramide·HCl (**1a**) (13.9 g, 0.1 mole) was dissolved in MeOH (200 ml) and a solution of NaOMe (5.4 g, 0.1 mole) in MeOH (50 ml) added. The resulting solution was coned under reduced pressure and the residue extracted with $CHCl_3$ (200 ml), NaCl was removed by filtration and washed with $CHCl_3$ (3 portions of 25 ml). The combined $CHCl_3$ solutions were added to a solution of D(−)-mandelic acid (15.2 g, 0.1 mole; $[\alpha]^{25}D$ 156.1° (*c* 1.0 in H_2O) in MeOH (100 ml). The solution was filtered and on cooling white crystals of L(−)-3-aminobutyramide D(−)-mandelate (10.8 g), mp 150–168°, separated. After 3 recrystallizations from *i*-PrOH the salt melted at 174–176°; $[\alpha]^{25}D$ 78.1° (*c* 1.0 in H_2O). *Anal.* ($C_{12}H_{18}N_2O_4$) C, H, N.

L(−)-3-Aminobutyramide D(−)-mandelate (8.8 g) was dissolved in H_2O (120 ml) and Na_2CO_3 (2.0 g) added. The clear solution was coned under reduced pressure and the residue extracted with boiling *i*-PrOH (200 ml). The *i*-PrOH solution was filtered and coned under reduced pressure to yield an oil (3.5 g). The oil was redissolved in *i*-PrOH (100 ml), filtered, and carefully ajusted to pH 7 with a solution of HCl in *i*-PrOH (2.9 *N*). On concentration of the solution to abou half vol and cooling, white crystals (2.7 g) were obtained. Recrystallization from *i*-PrOH afforded pure L(−)-3-aminobutyramide·HCl (**1b**), mp 133–134°; $[\alpha]^{25}D$ 23.1° (*c* 1.0 in H_2O).

D(+)-3-Aminobutyramide L(+)-mandelate, mp 174–176°, $[\alpha]^{25}D$, + 78.1° (*c* 1.0 in H_2O), was obtained from **1a** and L(+)-mandelic acid and converted into D(+)-3-aminobutyramide·HCl (**1c**) mp 133–134°; $[\alpha]^{25}D$ + 23.1° (*c* 1.0 in H_2O), by an identical procedure.

3-Methylaminobutyramide·HCl (16).—Crotonamide (8.5 g, 0.1 mole) was dissolved in a 36% aq solution of $MeNH_2$ (1 mole) and the solution allowed to remain at 20° for 3 days and then coned under reduced pressure. The oil remaining was dissolved in *i*-PrOH (100 ml) and the solution coned under reduced pressure. This process was repeated twice. The oil was dissolved in *i*-PrOH (50 ml) and a solution of HCl in *i*-PrOH (2.9 *N*) carefully added until pH 7 was reached. The white crystals that deposited were filtered, washed with cold *i*-PrOH (3 portions of 20 ml), and recrystd from *i*-PrOH to give pure **1b**, mp 116–118° (hygroscopic, melting point determination carried out in sealed tube), 96.7% yield. *Anal.* ($C_5H_{12}N_2O \cdot HCl$) C, H, N, Cl.

5-Methyl-3-pyrazolidinone·HCl (17).—Crotonamide (8.5 g, 0.1 mole), hydrazine hydrate (5.0 g, 0.1 mole), and MeOH (50 ml) were warmed until a clear solution was obtained. The solution was allowed to remain at 20° for 3 days and worked up in the same manner as described above for the preparation of **16**.

The product **17** was obtained as a white crystalline solid, mp 177–179° (lit.[8] 172–173°), 68.6% yield. *Anal.* ($C_4H_8N_2O \cdot HCl$) C, H, N, Cl.

3-Aminobutyrohydrazide·2HCl (18).—DL-3-Aminobutyramide (2.0 g, 0.02 mole, liberated from the hydrochloride **1a** with NaOMe in MeOH) and a solution of hydrazine hydrate (1.0 g, 0.02 mole) in H_2O (3 ml) were heated at reflux temperature for 16 hr. The solution was cooled, filtered, and concd under reduced pressure. This process was repeated, the remaining oil was dissolved in EtOH (25 ml), and a solution of HCl in EtOAc (0.9 *N*) was added until the solution was neutral. The white solid which pptd was filtered, dried, and recrystd from EtOH to give **18**, mp 184–186°, 63.1% yield. *Anal.* ($C_4H_4N_3O \cdot 2HCl$) C, H, N, Cl.

Enzyme Assays.[9]—The s_{100} fraction from *E. coli* was used as the enzyme source. The assay medium (total volume 1 ml) contained buffer (0.1 *M* Tris, pH 7.2), KCl (10 mM), mercaptoethanol (10 m*M*), L-aspartic acid (1 m*M*), ATP (2 m*M*), sRNA (1 mg/ml), and L-[^{14}C]asparagine (0.001 m*M*, 0.1 μCi). Test compds were dissolved in buffer and the pH adjusted with KOH (1 *M*) if necessary. After addition of the enzyme the reaction was allowed to proceed for 10 min at 37° and then stopped with ice-cold 5% CCl_3COOH (3 ml). The incubation mixture was centrifuged at 1000*g* and the precipitate was then washed once in CCl_3COOH (3 ml), once in EtOH (3 ml), and once in EtOH–Et_2O (3 ml, 3:1). Finally the residue was dried at 100° and dissolved in HCOOH (0.5 ml) and the radioactivity measured with a Packard Model 314E scintillation spectrometer.

Results and Discussion

Evidence has been presented that COOH is not essential for the binding of amino acids to their aminoacyl-tRNA lygases.[4,10] Consequently, substitution on the α-C could lead to specific inhibitors of these enzymes. Cassio, *et al.*,[10] prepared a number of amino alcohol adenylates and found them to be potent inhibitors of the aminoacyl-tRNA lygases specific for the corresponding amino acids. The amino alcohols themselves were weak competitive inhibitors with K_i's comparable to the K_m's of the parent amino acids. Here we report on the competitive inhibition of L-asparaginyl-tRNA lygase from *E. coli* by a series of β-aminoamides.

The compounds which were accessible by the synthetic routes described in this communication were not expected to provide inhibitors with a potency to merit clinical trial. The purpose of this study was primarily to gain information about the nature of asparaginyl-tRNA lygases while the synthesis of more complex and potentially more potent inhibitors (*e.g.*, asparaginol adenylate) was in progress. A similar study is planned with the mammalian enzymes.

With the exception of **12** and **14**, the β-aminoamides (Table I) were synthesized by addition of NH_3 either to α,β-unstaurated esters (**9**, **13**, **15**), or to α,β-unsaturated amides, prepared *in situ* from α,β-unsaturated acids by the chloroformate procedure[11] (**1a**, **2–8**, **10**, **11**). Compounds **1a**, **2–11**, and **13** were isolated as stable water-soluble, crystalline HCl salts. The phosphono derivative **15** was obtained as a neutral internal salt. The sulfone **12** was prepared by oxidation of the methylthio derivative **11**. Reduction of *N*-*tert*-butoxycarbonyl-L-asparagine *p*-nitrophenyl ester with a large excess of $NaBH_4$[12] afforded L-3-*tert*-butoxycarboxamido-4-hydroxybutyramide in high yield. Attempts at removal of the *tert*-butoxycarbonyl grouping with HCl in *i*-PrOH and in EtOAc invariably resulted in intramolecular alcoholysis of the primary amido function.[13] Upon treatment of the *tert*-butoxycarbonyl derivative with CF_3COOH at 20°, however, the *tert*-butoxycarbonyl grouping was rapidly removed and the desired β-aminoamide salt **14** was obtained.

In Table II the activation of L-[^{14}C]asparagine in the presence of the given concentrations of the β-aminoamides is expressed as a percentage of the activity observed in the absence of test compounds.

TABLE II

Compd	Concentration (*M*), enzyme activity (% control)
1a	10^{-2}, 0; 10^{-3}, 11; 10^{-4}, 76
b	10^{-2}, 0; 10^{-3}, 5; 10^{-4}, 36; 5×10^{-5}, 80
c	10^{-2}, 35; 10^{-3}, 72
2	10^{-2}, 25; 10^{-3}, 73
3	10^{-2}, 25; 10^{-3}, 82
4	10^{-2}, 86; 10^{-3}, 97
5	10^{-2}, 17; 10^{-3}, 70
6	10^{-2}, 98
7	10^{-2}, 102
8	10^{-2}, 102
9	10^{-2}, 99
10	10^{-2}, 63; 10^{-3}, 69
11	10^{-2}, 36; 10^{-3}, 82
12	10^{-2}, 60; 10^{-3}, 93
13	10^{-2}, 61; 10^{-3}, 79
14	10^{-2}, 0; 10^{-3}, 29; 10^{-4}, 73
15	10^{-2}, 78; 10^{-3}, 85
16	10^{-2}, 100
17	10^{-2}, 108
18	10^{-2}, 33; 10^{-3}, 87
3-Aminopropionamide·HCl[a]	10^{-2}, 23; 10^{-3}, 67
N-CBZ-L-Asparagine[b]	10^{-2}, 78; 10^{-3}, 85
D-Asparagine[b]	10^{-2}, 84; 10^{-3}, 95
L-Aspartic acid diamide[c]	10^{-2}, 0; 10^{-3}, 8; 10^{-6}, 42

[a] See ref 14 for preparation. [b] Purchased from Sigma Chemical Co., St. Louis, Mo. [c] Purchased from Cyclo Chemical Corp., Los Angeles, Calif.

Prior to its resolution (see below), the most active compound synthesized was 3-aminobutyramide (**1a**). Figure 1 shows a double reciprocal plot of enzyme activity *vs.* substrate concentration in the presence and absence of **1a**. As would be expected, the inhibition is seen to be fully competitive. From the graph we calculate the apparent K_m and K_i to be 3×10^{-6} *M* and 4×10^{-5} *M*, respectively. Resolution of **1a** was achieved by separation of the mixture of diastereomeric salts obtained with optically pure mandelic acid isomers. The *l* isomer **1b** inhibited the binding of L-asparagine to the enzyme at much lower concentrations than did the *d* isomer **1c**. **1b** is therefore assigned the L and **1c** the D configuration. **1a** (10^{-2} *M*) did not inhibit the activation of alanine, aspartic acid, glutamine, or leucine (substrates at 10^{-6} *M*).

The L-Me compound, **1b**, like L-aspartic acid diamide, inhibited the activation of asparagine much more strongly than did its decarboxylation product—3-

(8) N. P. Zapevalova and T. A. Sokolove, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 865 (1966).

(9) K. H. Muench and P. Berg, *Procedures Nucleic Acid Res.*, 375 (1966).

(10) D. Cassio, F. Lemoine, J. P. Waller, E. Sandrin, and R. A. Boissonnas, *Biochemistry*, **6**, 827, 1967.

(11) H. P. Fischer and C. A. Grob, *Helv. Chim. Acta*, **47**, 564 (1964).

(12) (a) M. S. Brown and H. Rapoport, *J. Org. Chem.*, **28**, 3261 (1963); (b) H. Seki, K. Koga, H. Matsuo, S. Ohki, I. Matsuo, and S. Yamada, *Chem. Pharm. Bull.*, **13**, 995 (1965).

(13) T. C. Bruice and F. H. Marquardt, *J. Amer. Chem. Soc.*, **84**, 365 (1962).

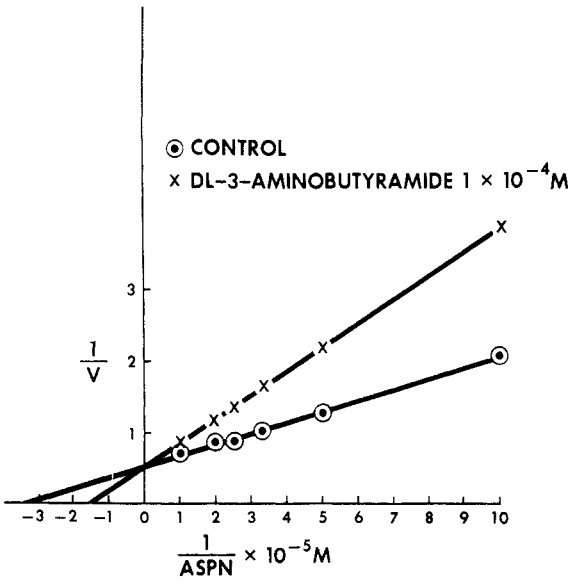


Figure 1.—Lineweaver–Burk plot showing the effect of DL-3-aminobutyramide (**1a**) on the incorporation of L-[^{14}C]asparagine (ASPN) into tRNA. See Experimental Section for assay conditions.

aminopropionamide (β-alanineamide).[14] This may indicate that in solution the latter assumes a conformation which is not favorable to binding on the lygase.[15] The CO_2H could play an "indirect" role in the binding of asparagine to the enzyme prior to activation.

Compounds possessing C_3 substituents bulkier than Me (**2**–**8**, **10**–**15**), including some of the more hydrophilic substituents, invariably had reduced activity. The inactivity of the CF_3 compound **9** may be a consequence of the greatly reduced basicity of the amino function. Replacement of α-Me of amphetamine with CF_3 has been reported to reduce the basicity of the amino function by almost 5 pK_a units.[16] The L-hydroxymethyl compound **14** showed relatively good activity, as would be expected from the work of Cassio, *et al.*[10] However, the apparent activity is diminished as a consequence of the fact that hydrolysis of the amido function is greatly facilitated in this emopound (**14**) as a result of anchimeric assistance from the OH group.[15] The rapid formation of asparticol was readily apparent in tlc on silica gel or cellulose. Even fresh solutions of **14**, therefore, also inhibited L-aspartyl-tRNA lygase under conditions similar to those described for the activation of asparagine (notation as in Table II: 10^{-2} *M*, O; 10^{-3} *M*, 62; 10^{-4} *M*, 107).

It should be noted that several of the β-aminoamides (**1**, **3**–**5**, **7**, **11**, **13**, **14**, **18**) each represent the decarboxylation product of one other natural amino acid, substituted on α-C, besides asparagine. Thus **1b** is seen as L-alanine in which CO_2H has been replaced by CH_2CONH_2. We tested the following (10^{-2} *M*) against the appropriate aminoacyl-tRNA lygase (substrates at 10^{-6} *M*): **1a** (alanine), **3** (valine), **4** (leucine), **5** (isoleucine), and **7** (phenylalanine). The results were negative. As a further point of interest, one might note that the hydrolysis of the β-aminoamides would yield carboxylic acids from which could be made the β-amino acid adenylates. These homologs may be worthy of consideration as potentially useful biochemical tools.

We have also investigated the inhibition of L-asparaginyl-tRNA lygase by several closely related compounds in order to gain further insight into the degree of structural specificity demanded for binding to the enzyme. 3-Methylaminobutyramide and 5-methyl-3-pyrazolidinone were prepared by addition of $MeNH_2$ and $(H_2N)_2$, respectively, to crotonamide, and were isolated as their HCl salts (**16**, **17**). 3-Aminobutyrohydrazide was prepared by heating **1a** with aq $(H_2N)_2$ and was isolated as its dihydrochloride (**18**). Compound **18** was considerably less potent than the corresponding amide. The methylamino compound **16** and the cyclic hydrazide **17** were completely inactive. *N*-carbobenzoxy-L-asparagine, which has been reported to inhibit the growth of an L-asparaginase-sensitive lymphoma,[17] exhibited only marginal activity.

In conclusion, further work along these lines will be required in order to see if our approach will prove fruitful. Future studies should be aided by the finding that *E. coli* tRNA can serve as acceptor to mammalian asparaginyl-tRNA lygase.[18] Attempts to prepare asparaginol adenylate as a model compound have, so far, failed. The recent discovery of a strict serine requirement in leukemic cells[19] makes it desirable to extend the search to inhibitors of serinyl-tRNA lygase as well. Also to be considered is the possible utility of such inhibitors in immunosupression[20] and microbial diseases.

Acknowledgments.—We thank Mr. Bruce Butler for technical assistance. We are grateful to Drs. E. L. Woroch and J. M. Price for their interest and encouragement.

(14) Prepared by catalytic reduction of cyanoacetamide according to F. Fusier, *Ann. Chim. (Paris)*, **5**, 882 (1950). *Anal.* ($C_3H_6N_2O \cdot HCl$) C, H, N, Cl; mp 147–149°; 45.6% yield.

(15) D. E. Koshland, Jr. and K. E. Neet, *Annu. Rev. Biochem.*, **37**, 359 (1968).

(16) R. M. Pinder, R. W. Brimblecombe, and D. M. Green, *J. Med. Chem.*, **12**, 332 (1969).

(17) M. Schlesinger, N. Grossowicz, and N. Lichtenstein, *Experientia*, **25**, 14 (1969).

(18) T. O. Yellin, unpublished.

(19) J. D. Regan, H. Vodopick, S. Takeda, W. B. Lee, and F. M. Fauleon, *Science*, **163**, 1452 (1969).

(20) R. S. Schwartz, *Nature (London)*, **224**, 275 (1969).