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Original paper

Synthesis and evaluation of potential glutamine antagonists

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Summary — Ten acetylenic esters, amides and N_δ-alkynyl derivatives of serine, 2,3-diaminopropanoic acid and glutamine were prepared as potential phosphoribosyl pyrophosphate amidotransferase (EC 2.4.2.14) inhibitors as well as anti-tumor agents against experimental leukemia L1210. None of the agents were found to possess significant activity in both tests.

Résumé — **Synthèse et évaluation pharmacologiques d'antagonistes potentiels de la glutamine.** *Dix esters et amides acétyléniques de la sérine et de l'acide diamino-2,3-propanoique ainsi qu'un dérivé N_δ-alkynique de la glutamine ont été synthétisés en tant qu'inhibiteurs probables de la phosphoribosyl pyrophosphate amidotransférase (EC 2.4.2.14) et comme agents anti-tumoraux dans la leucémie expérimentale L1210 chez la souris. Ces substances se sont avérées peu actives dans les deux essais.*

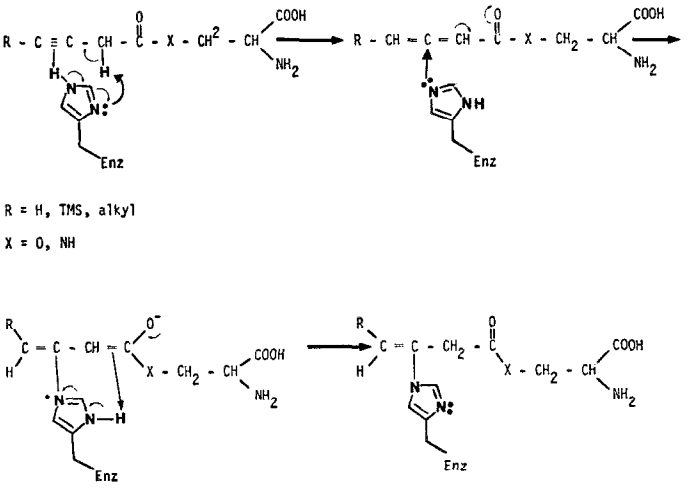
acetylenic esters / acetylenic amides / N_δ-alkynyl glutamine / serine derivatives / 2,3-diaminopropanoic acid derivatives (amino—alanine derivatives) / phosphoribosyl pyrophosphate (PRPP) amido-transferase inhibitors / anti-tumor agents

Introduction

Interest in glutamine antagonists has been renewed by the discovery of acivicin [1] (NSC-163501) and the promising results obtained with it in some experimental tumor systems [2]. However, recent reports seem to indicate that this glutamine antagonist suffers the same drawback found in azaserine and DON, that is, myelosuppression and gastrointestinal disturbances [3] in addition to a significant neurotoxicity [4]. A search for less toxic agents led us into the field of acetylenes because of their ease of allenization leading to efficient electrophilic centers [5], and somewhat reduced reactivity (in comparison with the above mentioned agents).

The goal of this research was to produce a new type of glutamine antagonist which could be active against tumoral growth. Many glutamine enzymes could possibly have been inhibited by these acetylenes, however we thought that the most interesting candidate appeared to be glutamine phosphoribosyl pyrophosphate (PRPP) amidotransferase (ATase) (EC 2.4.2.14) which is the first enzyme involved in the *de novo* synthesis of purines. It is also possibly the least sensitive to existing glutamine antagonists [6] and it was surmised that an inhibitor of this enzyme might also inactivate other key enzymes in the synthesis of purines.

Although it is known that glutamine enzymes have been targeted most successfully with the acid-activated type of drugs (*i.e.*, diazo-ketones, diazo-esters and perhaps chloro-isoxazoles), we were interested in investigating the action of base-generated potential suicide—substrates of the acetylenic class. It was thought that the base involved in glutamine hydrolysis might serve as an isomerizing agent for the title acetylenics, and/or might be alkylated by the generated electrophilic allene (Scheme 1).



Scheme 1

Unfortunately, neither the glutamine nor the ammonia active sites of human PRPP amidotransferase are known, although there is reason to believe that this enzyme shares similar characteristics with other better known glutamine utilizing enzymes from other sources [7]. Also reported are some sulfamide derivatives of 2,3-diaminopropionic acid (DAP) which are sulfamic acid derivatives of albizziine [8].

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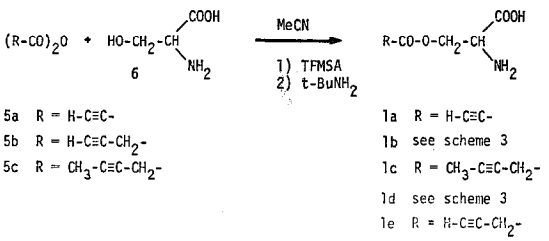
Table I. Physical, analytical and anti-tumor activity data for the acetylenic congeners of serine, 2,3-diaminopropanoic acid and glutamine.

N°	R	% Yield[a]		m.p.°C	D[b]	Formula[c]	% ILS[d]
		R—CO—O—CH_2—CH—COOH NH_2					
1a	H—C≡C—		(70)	154—8	—	e	—
1b	H_2C=C=CH—		(89)	153—4	19.7°	e	7[f]
1c	CH_3—C≡C—CH_2—	59	(79)	149—50	14.5°	$C_8H_{11}NO_4$	0
1d	TMS—C≡C—CH_2—	7	(26)	139.5—40	14.8°	$C_{10}H_{17}NO_4Si$	—
1e	H—C≡C—CH_2—	[h]		—	—	—	—
		R—NH—CH_2—CH—COOH NH_2					
2a	H—C≡C—CO—	44	(86)	177—8	— 4.6°	$C_6H_8N_2O_3 \cdot H_2O$[i]	—
2b	CH_3—C≡C—CH_2—CO—	64	(80)	202—4	— 20°	$C_8H_{12}N_2O_3$	17
2c	TMS—C≡C—CH_2—CO—	31	(50)	178.5—80	— 22.8°	$C_{10}H_{18}N_2O_3 \cdot SiH_2O$	—
3a	H_2N—SO_2—	33	(66)	196—8	— 15.4°	$C_3H_9N_3O_4S$	17
3b	$(CH_3)_2N$—SO_2—	10	(24)	197—8	— 12.8°	$C_5H_{13}N_3O_4S$[j]	—
		R—NH—CO—CH_2—CH_2—CH—COOH NH_2					
4	H—C≡C—CH_2—	47	(65)	199.5—200	racemate	$C_8H_{12}N_2O_3$	17

[a] Crude yield in parenthesis.
[b] At 23°C, c = 1, 1 N HCl.
[c] All compounds gave satisfactory analysis, unless otherwise noted.
[d] Increase in life span at optimal dose.
[e] Too unstable to be analyzed.
[f] Toxic at doses over 8 mg/kg.
[g] See [9].
[h] This compound was not obtained (see text).
[i] C,H,N; H, calculated: 5.79, found: 5.32.
[j] C,H,N; H, calculated: 6.20, found: 6.66.

These were obviously not expected to be alkylators but instead to act as competitive inhibitors at the active site.

Finally, N_δ-propargyl glutamine was prepared. This substance was presumed capable of liberating propargylamine and thus poisoning the enzyme. The compounds prepared in this work are shown in Table I. Some of these agents were screened for anti-cancer activity in the L1210 leukemia system.



Scheme 2

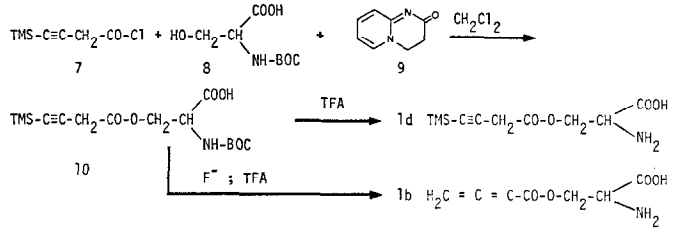
Chemistry

The general method for the preparation of the acetylenic serine esters was a modification of that of Sakami and Toennies [9]. It involved the reaction of an acid anhydride **5a—c** (1.5 equiv.) with L-serine **6** in acetonitrile solution containing 1.2 equivalents of trifluoromethanesulfonic acid (TFMSA). Neutralization by *t*-butyl amine gave the amino acids in variable yields depending upon the acetylenic chain (Scheme 2). The use of concentrated perchloric acid according to Sakami and Toennies gave similar results, but required a 4-fold greater amount of acylating agent. Acid anhydrides were prepared from the sodium salts by the oxalyl chloride method [10].

O-Pentynoyl-L-serine **1c** was obtained in a good state of purity. However, the method failed to give a pure product in the case of the propynoic ester **1a**, and an isomerization product **1b** in the case of the expected 3-butynoic ester **1e**. The propynoic ester **1a**, heavily contaminated by unknown products, was identified by its IR and NMR spectra. Purification was not attempted because of the predictable instability of the compound.

Surprisingly, the reaction of 3-butynoic anhydride with serine led only to the isolation of *O*-2,3-butadienoyl-L-serine **1b**, contaminated by what was shown to be *O*-acetoacetyl-L-serine, instead of the expected product **1e**. (NMR

spectra of a fresh solution of **1b** showed extra peaks at 2.298 (S, 0.4H, CH_3—CO) and 3.598 (s, 0.25H). The second peak disappeared on standing as would be expected for any exchangeable proton.) Therefore, it was decided to prepare **1e** as its TMS protected derivative. 4-TMS-3-butynoyl-L-serine **1d** was prepared by reaction of 4-TMS-3-butynoyl chloride **7** with BOC-L-serine **8** in the presence of a weak base, a so-called betaine **9** [11], followed by cleavage of the BOC protecting group of intermediate **10** with trifluoroacetic acid (TFA) (70% v/v) in methylene chloride (Scheme 3). Yields were low (10—20%), owing



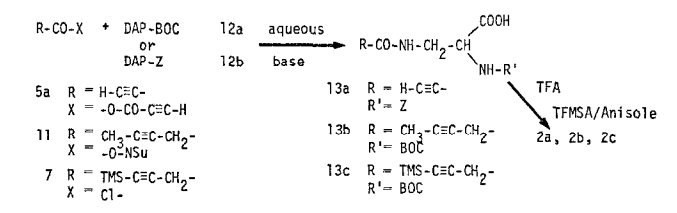
Scheme 3

to side reactions (*i.e.*, sensitivity of the TMS—butynoyl moiety to base) and possibly to partial cleavage of the TMS group in TFA. No silylated allenic component was detected in the purified compound **1d**, by either IR or NMR analysis. Attempts at the preparation of the TMS acetylenic serine derivative **1d** by the general method of Previero *et al.* [12] (acylation of serine in TFA by an acid chloride) failed to give the expected ester hydrochloride.

Similar failures in the formation of the esters were observed with 3-butynoyl and chloroacetyl chlorides, suggesting that limitations to this method might depend upon the acid. Also, no significant acetylenic ester formation took place when the acid chloride **7** was allowed to react with serine in the modified Sakami—Toennies procedure.

Cleavage of the TMS group was effected in order to gain access to the expected *O*-3-butynoyl-serine **1e**. The crude BOC-intermediate **10** was allowed to react successively with fluoride ion [13] and TFA, giving only *O*-2,3-butadienoyl-serine **1b**, with no acetylenic component being detected in IR or NMR. Seemingly, the basicity of the fluoride ion is sufficient to promote the isomerization of our compound.

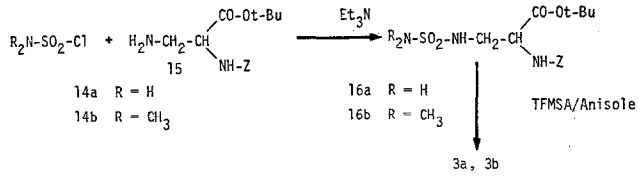
The acetylenic amides **2a—c** (Table I and Scheme 4) were obtained from either N_2-carbobenzoxy (DAP-Z) or N_2-*t*-butyloxy-carbonyl (DAP-BOC) protected 2,3-diaminopropionic acid [14] and the appropriate acetylenic acid derivative. The acylation was carried out *via* the active esters [15] in the case of compound **2b**. Preparation of the



Scheme 4

pentynoic *N*-hydroxy succinimide ester by the DCC method gave **11** as a crystalline compound. (The acylation of **12a** with 4-TMS-3-butynoyl-*N*-hydroxysuccinimide ester was also attempted but gave unsatisfactory results.) Addition of the active ester solution in THF to a solution of DAP-BOC in aqueous bicarbonate gave, after extractive work-up and treatment with 60% TFA in chloroform, good yields of 3-pentynoic derivative **2b**.

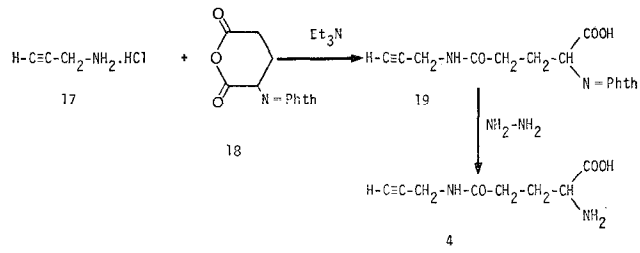
(S)-N_3(4-TMS-3-butynoyl)-amino-alanine **2c** was prepared by simple addition of 4-TMS-3-butynoyl chloride **7** to an aqueous solution of DAP-BOC and sodium bicarbonate. Work-up and acidolysis of the protecting BOC group gave an almost pure product, in 40—50% yield. Crystallization from water gave the pure amino acid. A similar procedure using N_2-carbobenzoxy-2,3-diaminopropionic acid (DAP-Z **12b**) and propynoic anhydride [16] **5a** gave good yields of (propynoyl-amino)-3-L-alanine **2a**. The cleavage of the Z group was effected with trifluoromethanesulfonic acid-anisole in methylene chloride [17]. Amide cleavage was observed to some extent, but the 2,3-diaminopropionic acid so produced could easily be removed by passage through an ion exchange resin. Attempted cleavage of the blocked intermediate with TFA/thioanisole [18] yielded only a modified compound, having a different R_f on TLC. Synthesis of the sulfamide derivatives (Scheme 5) necessitated the



Scheme 5

use of the ether soluble *t*-butyl (N_2-carbobenzoxy)-2,3-diaminopropioniate **15** prepared from DAP-Z as described by Mokotoff [19] for the N-3 isomer.

Reaction of **15** with sulfamoyl chloride **14a** [20] or *N,N*-dimethylsulfamoyl-chloride **14b** in the presence of triethylamine in ether or THF gave, after work-up and cleavage by trifluoromethanesulfonic acid/anisole/CH_2Cl_2, good yields of N_3-sulfamoylamino-L-alanine **3a**, but only low yields of the dimethyl analog **3b**. We have not yet investigated this peculiarity, which could arise from electronic factors. *N*-Propargyl-DL-glutamine **4** was obtained by reaction of propargylamine **17** on *N*-phthaloyl-DL-glutamic anhydride **18** [21] in the presence of triethylamine. Hydrazinolysis of **19** yielded the amino acid **4** (60—70% overall/yield).



Scheme 6

Enzyme inhibitory activity

The catalytic groups of the human PRPP amidotransferase (ATase) have not as yet been identified [7]. However, the amino acid sequence is known for the same enzyme of bacterial origin [22]. The bacterial ATase possesses characteristics common to other amidotransferases namely an active site cysteine, distinct NH_3 and Gln sites and glutaminase activity [23]. Assuming that conformation and identity of the functional amino acids of the active site must be retained for a given activity, we carried out a preliminary screening of our acetylenic compounds in order to probe for the presence of the putative base.

The enzyme assay is based upon phosphoribosyl pyrophosphate (PRPP) dependent hydrolysis of glutamine to glutamate, and was adapted from that of Holmes *et al.* [24, 38]. A long period of incubation of the enzyme with the potential inhibitor is used in order to facilitate the detection of time-dependent inactivation (re: suicide—substrates). A reducing incubation medium (50 mM 2-mercaptoethanol) was used, because of high sensitivity of human placental PRPP amidotransferase (EC 2.4.2.14) to oxygen inactivation. Under such conditions, any electrophilic (affinity labeling) agent generated outside the active site would be trapped by the thiol, precluding any inhibitory action.

Suicide—substrates are not affected by trapping agents, and should be easily detectable in our assay together with potent competitive inhibitors.

No significant activity was detected among our compounds at 1 mM. By comparison, azaserine (1 mM) and DON (5 μM) had inhibition values of 49% ($p < 0.01$) and 95% ($p < 0.02$), respectively.

We also tested albizziin, since it has been reported to act as a suicide—substrate for the enzyme FGAR-amidotransferase (EC 6.3.5.3) [8]. No such action was noted in our system, in which albizziin had a marginal inhibitory action of 12% ($\sigma = 4$).

Among our compounds, only 4-TMS-3-butynoyl-L-serine **1d** gave a measurable degree of inhibition (8%), which was also not significant ($0.2 < p < 0.5$).

It was felt that if any inhibitory activity was to be detected, it had to be of the suicide—substrate type, or to be the result of a powerful competitive agent under the conditions utilized in our work. Since in our mind only agents having an inhibitory potency equal to or higher than that of azaserine were to be considered for further work, the simple assay method described gave us the semi-quantitative information, that was required in the first phase of our work.

The non base-activated (**3a**, **4**) compounds were assayed by the same procedure, since the pre-incubation period does not affect competitive agents.

Anti-tumor screening

Compounds **1b**, **c**, **2b**, **3a** and **4** were submitted for *in vivo* evaluation of activity against murine L1210 leukemia. None of the agents exhibited significant anti-tumor activity, with maximum increase in life span (ILS) being in the range of 0—17% (see Table I). No definite correlation between dose and effect could be noticed. However, these agents appear to be non-toxic at daily doses of 128 mg/kg with the exception of *O*-2,3-butadienoyl-L-serine, **1b**. All ILS values and average weight changes were positive for all dosages.

Discussion

The results obtained in this study indicate that the attachment of a propargylic chain to serine or 3-amino-alanine does not lead to active compounds of the suicide—substrate type against PRPP amido transferase. This could mean that the target enzyme cannot carry out the necessary isomerization step for the action of these inhibitors or that the allene presumably formed *in situ* does not possess the proper configuration for enzymatic attack. It should be pointed out that the assay method used in the determination of enzyme inhibitory activity does not allow for the detection of an alkylating species such as the allene **1b** which could be generated from the TMS-protected compound **1d**. This reaction could take place outside the active site but remain without effect because of the presence of the thiol scavenger (2-mercaptoethanol). Therefore, one should not rule out the possibility of a different type of inactivation (*e.g.*, affinity label).

The sulfamide derivatives **3a** and **3b** were also found to be inactive, either in blocking PRPP amidotransferase or as anti-cancer agents. This implies either that no binding of the sulfamide compound to the active site occurs or that the presumably formed acyl—enzyme intermediate is unstable. The use of bulky substituents on the sulfamide nitrogen could possibly give effective inhibitors [25]. The lack of activity of *N*-propargyl-DL-glutamine might be somewhat related to the observed behavior of certain monoalkyl glutamines over those such as γ-glutamylhydroxyethylamide or γ-glutamyl-*n*-butylamide [26] which were found to be cleaved by PRPP amidotransferase. In our case, the propargylamine that would be released does not seem to inhibit the enzyme.

From the pharmacological evaluation, one can see that the amide **2b** is more potent than the serine esters **1b** or **1c** as judged by maximum ILS, indicating a possible deactivation of the latter by an O $\rightarrow$ N shift [27]. This shift may be retarded in cancer cells possessing an internal acid pH because such a shift is pH dependent, with the equilibrium under acid conditions favoring the *O*-esters. *O*-Allenyl-L-serine **1b** presents a particular case being acutely toxic at doses beyond 8 mg/kg. Since this toxicity has not been characterized, it is somewhat difficult to predict the effects of compounds **1d** or **2c** which could yield an allenic ester or amide after metabolic or chemical transformation. These considerations will be the subject of future work.

Experimental protocols

Melting points, obtained with a Buchi capillary apparatus, boiling points and temperature readings are in degrees centigrade and are uncorrected. Elemental analyses were performed by Guelph Chemical

Table II. Enzymatic inhibition assays of PRPP amidotransferase (EC 2.4.2.14) by the acetylenic congeners of serine, 2,3-diaminopropanoic acid and glutamine.[a]

Compound	% Inhibition mean ± S.E.	Values observed		N° of assays
		Min	Max	
1b	0	—		1
1c	3 ± 3	1	7	5
1d	6 ± 2	5	8	4
2a	0	—		1
2b[b]	2 ± 7	13	— 11	7
2c	1 ± 4	7	— 6	8
3a	2 ± 2	1	5	5
3b[b]	5 ± 2	3	— 7	3
4[b]	4 ± 3	3	— 8	5
Azasérine	49 ± 9	41	67	10
Albiziin	12 ± 4	9	16	3
DON	95 ± 3	93	96	2

[a] Experimental conditions given in text.
[b] Activation (possible protection against oxygen deactivation).

Table III. Anti-tumor activity of the acetylenic congeners of serine, 2,3-diaminopropanoic acid and glutamine.[a]

Compound	Dose mg/kg	m.s.t.[b]	% T/C[c]	s.a./t.a. 5[d]
1b	128	Toxic	Toxic	0/4
	32	Toxic	Toxic	0/4
	8	7.5	107	6/6
	4	7.0	100	6/6
	2	7.0	100	6/6
	1	6.0	86	6/6
	0.5	6.5	93	6/6
	0.125	6.0	86	5/6
1c	128	7.0	100	4/4
	32	7.0	100	4/4
	8	7.0	100	4/4
2b	128	6.5	108	4/4
	32	6.0	100	4/4
	8	7.0	117	4/4
	2	6.0	100	4/4
	0.5	6.0	100	4/4
	0.125	7.0	117	4/4
3a	128	6.5	108	4/4
	32	6.5	108	4/4
	8	6.0	100	4/4
	2	6.5	108	4/4
	0.5	6.5	108	4/4
	0.125	7.0	117	4/4
4	128	6.5	108	4/4
	32	6.5	108	4/4
	8	7.0	117	4/4
	2	6.5	108	4/4
	0.125	6.5	108	4/4
DON	3	7.0	117	6/6
	2	7.0	117	6/6
	1	11.5	192	6/6
	0.5	11.0	183	6/6
Control	—	6.0	100	10/10

[a] Experimental conditions given in text.
[b] Median survival time of treated animals, in days.
[c] Ratio of median survival time of treated animals over that of control group × 100.
[d] Surviving animals over total animals on day 5.

Laboratories Ltd., Guelph, Ontario, Canada. Optical rotations were determined with a Bellingham and Stanley polarimeter. Infrared spectra were recorded on a Perkin—Elmer 257 and ^{1}H NMR measurements on Perkin—Elmer R-12B and Bruker WH-90 spectrometers. These were consistent with the assigned structures. Radioactivity was determined with a three channel Nuclear Chicago liquid scintillation counter. High voltage electrophoreses were run with a Savant Instruments system (LT-48 tank and power supply). Thin—layer chromatography was performed on silica (Kieselgel 60, Merck, Darmstadt) using benzene—methanol—acetic acid 79:14:7; v:v:v (solvent A) or *n*-butanol—acetic acid—water 4:1:1; v:v:v (solvent B), and developed with either ninhydrin, or aqueous 1% potassium permanganate or detected by UV light at 254 nm.

[U-^{14}C]-L-Glutamine (40 μCi/μmol) was purchased from Amersham—Searle Corp. and purified by percolation through Rexyn CG-1 anion—exchange column (acetate) as already described [28], L-serine, L-asparagine. L-glutamic acid, 3-butyn-1-ol, propynoic acid, *N*-hydroxysuccinimide, trifluoromethanesulfonic acid, trifluoroacetic acid and *N*,*N*-dimethylsulfamoyl chloride were purchased from Aldrich and used as received. Propargylamine hydrochloride was obtained from Fluka Chemical Corp. 5-Phosphoribosyl-1-pyrophosphate (PRPP) was purchased from Sigma Chemical Co. Proteins were determined by the method of Bradford [29].

4-TMS-3-butyn-1-ol

O-ethoxyethyl-3-butynol. 3-butyn-1-ol (26.3 g, 0.375 mol) was converted into *O*-ethoxy ethyl-3-butynol in 95% yield by the procedure of Brandsma [30a]. The protected alcohol (50.6 g) had a bp = 46°C/10 Torr; n_D^{23} = 1.4167 d^{23} = 0.890; IR (CCl_4): 3330 (H—C≡C), 2960, 2150 (C≡C), 1410, 1130 (C—O) and 975 cm^{-1}; NMR (60 MHz, CCl_4) δ: 1.05 (q, 6H, CH_3, J = 6); 1.76 (t, 1H, H—C≡, J = 3); 2.5 (double t, 2H, CH_2—C≡, J_1 = 6, J_2 = 3); 3.1—3.75 (m, 4H, CH_2—); 4.52 (q, 1H, O—CH—O, J = 6).

4-TMS-3-butyn-1-ol. The title acetylenic alcohol was prepared from *O*-ethoxyethyl-3-butynol (50.6 g, 0.356 mol) by the method described by Ruden [31]. The crude reaction product was methanolyzed by the acidic ion—exchange method [32]. Filtration, concentration and distillation gave 42.7 g of 4-TMS-3-butyn-1-ol (yield 80% overall from 3-butyn-1-ol) bp = 67—68°C/5—6 Torr; d^{23} = 0.871; n_D^{23} = 1.4515; reported [35] bp = 70°C/6 Torr; d^{20} = 0.8742, n_D^{20} = 1.4507; IR (neat): 3350 (broad, OH), 2940, 2190 (C≡C), 1410, 1255, 1050 (C—O), 850 (C—Si) and 770 cm^{-1}; NMR (60 MH_2, CCl_4) δ: 0.15 (s, 9H); 2.4 (t, 2H, CH_2—C≡C, J = 6); 3.63 (broad t, CH_2—OH, J = 6).

4-TMS-3-butynoyl chloride 7

4-TMS-3-butynoic acid. 4-TMS-butynoic acid was obtained in 60% conversion yield by oxidation of 4-TMS-3-butyn-1-ol [32], mp = 57—60°C (hexanes). IR (CCl_4): 2940, 2400—3500 (COOH), 2200 (C≡C), 1725 (C≡O), 1420, 1255, 1050, 850 (C—Si); NMR (60 MHz, CCl_4) δ: 0.15 (s, 9H); 3.28 (s, 2H); 11.7 (s, 1H).

4-TMS-3-butynoyl chloride 7. This compound was prepared by the method of Adams and Ulich [10]. Addition of 4-TMS-3-butynoic acid (10.1 g, 64.6 mmol) to a solution of 18 g (2.2 eq.) of redistilled oxalyl chloride in 20 ml of dry ether, followed by refluxing (2 h) and distillation, gave 8.9 g (80%) of a clear liquid. bp = 40°C/4 Torr, d^{23} = 0.982 n_D^{23} = 1.4528 IR (neat): 2960, 2180 (C≡C), 1930 (C—C=C), 1800 (C=O), 1400, 1260, and 855 cm^{-1}. NMR (60 MHz, CCl_4) δ: 0.15 (s, 9H); 3.71 (s, 2H); 5.56 (AB quartet, 0.1H, J = 6 Hz, TMS-allenic contamination of *ca.* 5%).

Acid anhydrides

The acid anhydrides **5a**—**c** were prepared with the sodium salt of the acids by the procedure of Adams and Ulich [10]. The anhydrides were distilled once or crystallized and their identity was determined spectroscopically.

Propynoic anhydride **5a**. 50% yield. bp = 66—8°C/19 Torr. Reported [16] bp = 56°C/16 Torr.

3-Butynoic anhydride 5b

3-Butynoic acid. 3-Butynoic acid was prepared from 3-butynol according to Heilbron *et al.* [34]. The yield after recrystallization from petroleum ether (30—60°C) was 11% mp = 82—83°C; reported mp = 83—84°C [34]. NMR ($CDCl_3$) 2.32 (t, 1H, H—C≡, J = 2.5); 3.45 (d, 2H—CH_2, J = 2.5); 11.63 (S, 1H, COOH).

3-Butynoic anhydride **5b**. 51% yield. bp = 81°C/3 Torr. NMR ($CDCl_3$) 2.33 (t, 1H, *H*—C≡, *J* = 3); 3.52 (d, 2H, —CH_2—C≡, *J* = 3); 5.35—5.85 (m, 0.68H, allenic contamination of *ca.* 19%).

3-Pentynoic anhydride

3-Pentynoic acid. 3-Pentynoic acid was prepared by isomerization of 2-pentynoic acid [30b, 33]. The latter was in turn prepared from 2-pentyn-1-ol [30c] by a Jones oxidation [34]. The yield of isomerized product was 46%, mp = 100—104°C (after one recrystallization from Et_2O/petroleum ether); reported mp = 103—104°C [33].

3-Pentynoic anhydride **5c**. 70% yield. mp = 99—101°C (from ether). NMR ($CDCl_3$) 1.82 (t, 3H, CH_3—C≡, *J* = 3); 3.4 (q, 2H, CH_2—CO, *J* = 3).

N-*Hydroxysuccinimide esters*

The *N*-hydroxysuccinimide esters were prepared by the procedure of Lapidot *et al.* [15]. The 4-TMS-3-butynoyl ester proved too unstable to be isolated (loss of the TMS group was observed by NMR after evaporation of the solvents, either THF or CH_2Cl_2).

3-Pentynoic acid N-*hydroxysuccinimide ester* **7a**. 42% yield. mp = 98—101°C (from EtOH). NMR ($CDCl_3$) 1.9 (t, 3H, *J* = 3); 2.87 (s, 4H); 3.6 (q, 2H, *J* = 3).

General procedure for the synthesis of L-*serine* O-*esters (Scheme 2)*

To a cooled (4°C) solution of L-serine (465 mg, 4.4 mmol) and trifluoromethanesulfonic acid (TFMSA, 792 mg, 5.28 mmol) in 15 ml acetonitrile, was added dropwise, 6.6 mmol (1.5 eq.) of the required anhydride. The flask was closed and allowed to remain at room temperature overnight. Water (0.2 ml) was added followed in 1 h by *t*-butylamine (0.7—0.8 ml). Addition of ether precipitated the amino acid which was filtered and washed with absolute EtOH and ether. Purification was effected by treatment with activated carbon (Darco G-60) and lyophilization, followed by crystallization from 1:1 water—ethanol mixture. Physical data are given in Table I.

O-*Propynoyl*-L-*serine* **1a**. IR (KBr): 3600—2400, 3270 (H—C≡), 2120 (C≡C) 1720, 1600, 1400, 1230, 1020, 850 and 750 cm^{-1}; NMR (60 MHz, D_2O δ: 3.7 (s, 0.81 H, H—C≡); 4.1 (t, 1H, H_α, *J* = 4—5); 4.63 (d, O—CH_2—CH); 2.12 (s, 0.11H, CH_3—COCO).

O-*2,3-Butadienoyl*-L-*serine (O-allenyl*-L-*serine)* **1b**. Attempts to prepare the ester **1e** from butynoyl anhydride and L-serine by the general method above led only to the title compound. IR (KBr): 3650—2300 (NH_3^+), 1950 (C=C=C), 1715 (C=O), 1730 (O—C=O), 1600, 1500, 1400, 1330, 1160 (C—O) and 850 cm^{-1}; NMR (90 MHz, D_2O) δ: 4.091 (t, 1H, H_α, *J* = 4.1); 4.574 (d, 2H, CH_2—O, *J* = 4.1); 5.366—5.868 (m, 2.6H, allenic H); also acetoacetyl contamination, 2.298 (s, 0.4H, CH_3—CO); 3.589 (s, 0.25H, CO—CH_2CO).

O-*3-Pentynoyl*-L-*serine* **1c**. IR (KBr): 3600—2400, 1730 (O—C=O), 1600, 1400, 1225, 1170 cm^{-1}; NMR (90 MHz, D_2O) δ: 1.799 (t, 3H, CH_3—C≡, *J* = 2.5); 3.417 (q, 2H, —CH_2—C≡, *J* = 2.5); 4.078 (t, 1H, H_α, *J* = 4.1); 4.554 (d, 2H, O—CH_2—, *J* = 4.1).

O-*4-TMS-3-butynoyl*-L-*serine* ***1d*** *(Scheme 3)*

To a stirred solution of BOC-L-serine **8** (0.41 g, 2 mmol) and betaine **9** [11] (0.311 g, 2.1 mmol) in dry CH_2Cl_2 (20 ml), was added 4-TMS-3-butynoyl chloride **7** (367 mg, 2.1 mmol) dropwise and stirred overnight. The CH_2Cl_2 solution was filtered, washed successively with 1 M citric acid (2 × 10 ml), water (10 ml) and brine (10 ml) and dried over $MgSO_4$. Evaporation of the solvent gave 0.62 g (90%) of a yellowish, oily residue, which was subjected immediately to treatment by 6 ml of 80% TFA in chloroform (30 min). After evaporation of TFA and addition of ether, 0.13 g (26%) of a yellow solid, whose TLC showed contamination by polar species was obtained. The compound was purified by repeated treatment with activated carbon in water—ethanol 1:1, and the solution concentrated (yield 40 mg, 7%). IR (KBr): 2940, 3700—2400 (NH_3^+), 2190 (C≡C), 1735 (O—C=O), 1600, 1400, 1340, 1250, 1160 (C—O), 845 (C—Si) and 760 cm^{-1}; NMR (90 MHz, D_2O + TFA) δ: 0.176 (s, 9H); 3.54 (s, 2H, CH_2—C≡); 4.44 (t, 1H, H_α, *J* = 3.2); 4.65 (distorted d, O—CH_2—CH, *J* = 3.2).

Attempted synthesis of ***1e*** *from* ***10*** *(Scheme 3)*

To a solution of 0.26 g (0.757 mmol) of **10** in 20 ml of acetonitrile, was added 0.27 g (1.1 eq.) of tetra *n*-butyl ammonium fluoride trihydrate [13]. The solution was stirred for 2 h. The reaction mixture was treated as above for compound **1d**. 20 mg (15%) of white powder were obtained, whose TLC pattern was very similar to that of the allenic ester **1b** obtained previously. Infrared and NMR spectra were identical to those found for *O*-allenyl-serine **1b**.

N_2-*Protected* L-*2,3-diaminopropionic acids*

N_2-*BOC*-L-*2,3-diaminopropionic acid (DAP—BOC)* [*3-amino*-N_2-*BOC*-L-*alanine*] ***12a***. This compound was obtained in 45% yield (10 mmol scale) from BOC-L-asparagine [36] and bis-trifluoroacetoxyiodosobenzene by the method of Waki *et al.* [14]; mp = 194—195°C (dec); reported [14] mp = 198—200°C (dec).

N_2-*Z*-L-*2,3-diaminopropionic acid (DAP-Z)* [*3-amino*-N_2-*Z*-L-*alanine*] **12b**. Carbobenzoxy-L-asparagine was converted into **12b** in the same way as the BOC analogue **12a**. Purification was effected by fractional precipitation from aqueous sodium carbonate by acidification to pH 6.8, yield 70%; mp = 228.5—229.5°C (dec); reported [14] 228—230°C (dec).

N_2-*Z*-N_3-*Propynoylamino*-L-*alanine* ***13a*** *(Scheme 4)*. Propynoic anhydride **5a** (770 mg, 6.3 mmol) was added dropwise to a solution of **12b** (1 g, 4.2 mmol) in 25 ml of 0.25 M aqueous sodium carbonate. The mixture was stirred for 16 h. The solution was acidified (pH = 2) with 1 N HCl, and extracted with ethyl acetate (3 × 25 ml). The organic layer was washed with 1 N HCl, water and saturated NaCl. Drying ($MgSO_4$) and evaporation gave a yellowish oil which was pure on TLC (solvent A). The latter was triturated in benzene, and the solid so obtained (1.05 g, mp = 138—143°C) was crystallized from ethyl acetate—petroleum ether—methylene chloride. mp = 143—44°C; $[\alpha]_D^{25}$ = — 17.8° (*c* = 2.1, EtOH). Anal: Calcd. for $C_{14}H_{14}N_2O_5$: C: 57.93; H: 4.86; N: 9.65. Found: C: 57.66; H: 4.94; N: 9.51.

N_3-*Propynoylamino*-L-*alanine* ***2a*** *(Scheme 4)*. The cleavage of the Z group was effected with TMFSA (2.5 g, 4 eq.) in 60 ml of CH_2Cl_2 and 0.5 g of anisole [17]. The solid obtained was filtered, washed with absolute ethanol and ether, and dried. Yield (570 mg, 86%). Two spots were discernible in TLC (solv. B, ninhydrin), one of them corresponding to 2,3-diaminopropanoic acid (DAP). The compound was purified by ion—exchange chromatography (CG-120, pyridinium$^+$, 5 × 20 cm, 0.2 N, pH = 5.3, pyridinium formiate lyophilizable buffer). From 195 mg of crude material, 120 mg of DAP free **2a** were obtained. After crystallization from ethanol/water 10:1 at — 30°C, treatment with activated carbon and lyophilization, 10 mg of pure **2a** were obtained. The mother liquors yielded 90 mg of slightly contaminated material (44% overall yield).

IR (KBr): 3700—2600 (NH_3^+), 2110 (H—C≡), 1660 (amide I), 1600, 1400, 1535, 1340 and 1275 cm^{-1}. NMR (90 MHz, D_2O) δ: 3.525 (s, 1H, *H*—C≡); 3.668—3.981 (m, 3H, CH_2—CH—).

N_2-*BOC*-N_3*(3-pentynoyl)-amino*-L-*alanine* ***13b*** *(Scheme 4)*. The title amide was prepared by the method of Lapidot *et al.* [15] from the succinimido ester **11** (585 mg, 3 mmol) and DAP-BOC **12a** (550 mg, 2.7 mmol). The tan-colored semi-solid obtained (708 mg, 93%), almost pure on TLC (solvent A, HCl, ninhydrin), was not further purified but used directly in the next step. A sample of the semi-solid was crystallized from absolute ethanol. mp = 168.5—170.5°C; $[\alpha]_D^{26}$ = —3.2°C (*c* = 1.2, EtOH). Anal: Calcd. for $C_{13}H_{20}N_2O_5$: C: 54.92; H: 7.09; N: 9.85. Found: C: 54.43; H: 7.15; N: 9 57.

N_3*(3-Pentynoyl)-amino*-L-*alanine* ***2b*** *(Scheme 4)*. Compound **13b** (708 mg, crude) was treated with 40% TFA/$CHCl_3$ (15 ml) for 40 min. After evaporation, trituration with ether, filtration and washing with abs. EtOH ether, a yellowish solid (404 mg, 80%) was obtained. The compound was purified by treatment with activated carbon in water, lyophilization and recrystallization of the resulting white solid from ethanol/water. IR (KBr): 3320 (CO—N*H*—), 3600—2300 (NH_3^+), 1660 (amide I), 1600, 1400 (COO^-) 1330, 1235 and 1180 cm^{-1}. NMR (90 MHz, D_2O) δ: 1.1812 (t, 3H, CH_3—C≡, *J* = 2.64); 3.25 (q, 2H, —CH_2—C≡, *J* = 2.64), 3.726 (m, 2H, CH_2—N); 3.91 (m, 1H, H_α).

N_2-*BOC*-N_3*(4-TMS-3-butynoyl)amino*-L-*alanine* ***13c***. 4-TMS-3-butynoyl chloride **7** (1.3 g, 7.3 mmol) was added portion-wise to a stirred solution of **12a** (760 mg, 3.72 mmol) in 5 ml of 0.75 M aqueous sodium bicarbonate. Solid sodium bicarbonate (0.62 g) was added periodically to maintain pH at 7.5 and the reaction mixture was stirred overnight. The work-up, as described for **2b**, gave 1.56 g of **13c** as a yellow oil which was subjected to BOC cleavage.

N_3*(4-TMS-3-butynoyl)-amino*-L-*alanine* ***2c*** *(Scheme 4)*. Compound **13c** (1.56 g, crude) was stirred in 10 ml of 60% TFA/$CHCl_3$, for 1 h. The oil remaining after evaporation of the TFA was added slowly

to 100 ml of anhydrous ether. The resulting tan-colored solid was collected yielding 452 mg, 50% of **2c**. This was purified by crystallization from hot water, after treatment with activated carbon, giving white needles (280 mg, 31%).

IR (KBr): 3700—2400 (NH_3^+), 2200 (CEC), 1650 (amide I), 1600, 1400 (COO^-), 1530, 1350, 1255, 850 (C—Si) and 765 cm^{-1}. NMR (90 MHz, D_2O + TFA) δ: 0.176 (s, 9H); 3.367 (s, 2H, CH_2—C≡); 3.834 (t, 2H, —CH_2—N); 4.27 (q, 1H, H_α).

t-*Butyl*-N_2-*Z*-*2,3*-L-*diaminopropionate* ***15***. Compound **12b** (4.98 g, 21 mmol) was reacted with isobutylene (95 ml) in *p*-dioxane (90 ml) and conc. H_2SO_4 (5.8 ml) as described by Mokotoff *et al.* [19]. 4.1 g (68%) of a pale yellow oil were obtained; maleate: mp = 135—138°C; reported [19]: mp = 135—7°C $[\alpha]_D^{28}$ = — 19°C (*c* = 0.95 EtOH).

N_2-*Z*-N_3-*Sulfamoyl-amino*-L-*alanine* t-*butyl ester* ***16a*** *(Scheme 5)*. Sulfamoyl chloride **15a** (300 mg, 2.59 mmol) in 5 ml of THF was added dropwise to a stirred and cooled (0°C) solution of **15** (727 mg, 2.47 mmol) and triethylamine (262 mg, 2.59 mmol) in 40 ml of dry THF, under nitrogen. Stirring was continued for 16 h at room temperature. The solvent was evaporated *in vacuo* and replaced by 50 ml of ethyl acetate. Successive washings with 5% HCl, 5% Na_2CO_3, water, saturated NaCl, followed by drying ($MgSO_4$) and evaporation, left a clear oil (687 mg, 67%) whose TLC showed only one spot (solv. A, UV). This was used directly in the cleavage step.

N_3-*Sulfamoylamino*-L-*alanine* ***3a*** *(Scheme 5)*. Compound **16a** (687 mg, crude) was treated with TFMSA (0.99 g, 6.62 mmol), as described earlier for compound **2a**. Neutralization afforded 680 mg (66% overall) containing DAP and other contaminants as showed by TLC (solv. B, ninhydrin). Crystallization from water/EtOH 4:6, removed the higher R_f contaminants. Ion—exchange chromatography followed by recrystallization from water/EtOH 4:6 yielded pure **3a** (340 mg, 33% overall yield).

IR (KBr): 3700—2400 (very strong, NH_3^+ and H_2N—SO_2—N*H*), 1600, 1400 (COO^-) 1490, 1335, 1140 (O=S=O), 1045 cm^{-1}. NMR (D_2O) δ: 3.558 (m, 2H, —CH_2—N), 3.92 (q, 1H, H_α).

N_2-*Z*-N_3*(*N,N-*Dimethylsulfamoyl)-amino*-L-*alanine* t-*butyl ester* ***16b*** *(Scheme 5)*. A procedure similar to that described for **16a** was used, except that the reaction was run in ethyl acetate. From 1.07 g (7.47 mmol) of *N*-*N*-dimethyl-sulfamoyl chloride, triethylamine (759 mg, 7.5 mmol) and compound **15** (2 g, 6.78 mmol) in 150 ml of dry ethyl acetate, obtained 1.5 g (55%) of the intermediate were obtained. This was homogeneous as judged by TLC (solv. A, UV) and used without purification in the next step.

N_3*(*N,N-*Dimethylsulfamoyl)amino*-L-*alanine* ***3b*** *(Scheme 5)*. Treatment of the intermediate **16b** (1.5 g, crude) with TFMSA (2.2 g, 14.7 mmol) and neutralization, as described for compound **2a**, gave 343 mg (24%) of a solid. Crystallization from water/EtOH 7:3, yielded 150 mg (10%) of fine white needles.

IR (KBr): 3700—2400 (NH_3^+), 1600, 1400 (COO^-), 1500, 1340, 1140 (strong, O=S=O), 955 and 720 cm^{-1}. NMR (90 MHz, D_2O + TFA) δ: 2.823 (s, 6H, $(CH_3)_2N$); 3.707 (d, 2H, —CH_2—N); 4.235 (distorted t, 1H, H_α).

N_α-*Phthaloyl* N_δ-*propargyl*-DL-*glutamine* ***19*** *(Scheme 6)*. To a stirred suspension of N_α-phthaloyl-DL-glutamic anhydride **18** [19] (1 g, 3.85 mmol) and propargylamine hydrochloride **17** (0.43 g, 4.63 mmol) in anhydrous THF (20 ml), was added, dropwise under nitrogen, dry triethylamine (0.78 g, 7.7 mmol) and left stirrin govern ight. The TEA—HCl was filtered, the solvent removed *in vacuo* and replaced by ethyl acetate. The work-up, as for compound **12b**, gave a white solid (1 g, 83%), mp = 180—3°C (dec). This was used directly in the next step.

N_δ-*Propargyl*-DL-*glutamine* ***4*** *(Scheme 6)*. N_α-Phthaloyl-N_δ-propargyl-DL-glutamine **19** (1 g) was added to a solution of hydrazine hydrate (0.25 ml) in 25 ml of absolute EtOH, and refluxed for 2 h. The solvent was then evaporated *in vacuo*, and acetonitrile was added (20 ml), followed by conc. $HClO_4$ (0.42 ml) and heated (50°C, 15 min). Removal of the phthalylhydrazide, neutralization with *t*-butylamine and addition of ether gave a yellowship solid. This was dissolved in 15 ml of water and treated with activated carbon. The filtrate was diluted 10-fold with EtOH. 0.46 g (65%) of a white solid were obtained. Two recrystallizations from water/EtOH 1:1 gave 0.33 g (47% overall yield) of a pure compound.

IR (KBr): 3300 (H—C≡ and amine II), 3600—2500 (NH_3^+), 1635 (amide I), 1580, 1400 (COO^-), 1535 and 1330 cm^{-1}. NMR (90 MHz, D_2O) δ: 2.014—2.536 (m, 4H, —CH_2—CH_2—CO); 2.598 (t, 1H, *H*—C≡, *J* = 2.4); 3.759 (t, 1H, H_α, *J* = 5.8); 3.951 (distorted d, 2H, —CH_2—C≡, *J* = 2.4).

Preparation of PRPP-amidotransferase (EC 2.4.2.14), enzyme assay and inhibition

The enzyme was partially purified (*ca.* 12×) from human placenta, by a modification of the methods described by Holmes *et al.* [24, 37, 38] using CM-Sephadex at pH = 6 (See [33]) and deleting the passage through a DE-52 column in [20]. All buffers contained 60 mM 2-mercaptoethanol, and no sucrose. The enzyme preparation so obtained had a specific activity of 100—130 nmol/h/mg and contained 6 mg/ml of protein. Glutaminase activity accounted for less than 3% of the produced glutamate. No more than 6% of the substrate glutamine was transformed during a 30 min incubation period. The enzyme assay was conducted as described by Holmes *et al.* [38], with the following modifications: the total volume was 75 μl, containing 0.3 mg of protein, 50 mM 2-mercaptoethanol, 5 mM $MgCl_2$, 4 mM PRPP, 4 mM [U-^{14}C]-glutamine in 32 mM K_{pi} buffer. The potential inhibitor (1 μl of a 61 mM solution) was added to preincubated (15 min) enzyme solution (50 μl) and PRPP (10 μl) and the solution further incubated for 30 min. After addition of labeled glutamine (10 μl), the incubation was continued for another 30 min. The reaction was stopped by the addition of 4 μl of 0.5 N disodium EDTA. Subsequent separation and quantification of the produced glutamate was done by electrophoresis and scintillation counting. The basal activity of the enzyme was evaluated in each run, glutaminase activity being measured by omitting PRPP. The activities were compared to a blank containing no enzyme.

The amount of glutamate produced with or without an inhibitor during a 30 min incubation period, was used to determine a percentage of inhibition, according to the formula given below.

$$\% \text{ Inhibition} = \frac{\text{nmol/h/mg (control)} - \text{nmol/h/mg (with inhibitor)} \times 100}{\text{nmol/h/mg (control)}}$$

Anti-tumor activity

The tumor inoculum consisted of 10^6 ascites L1210 cells, implanted *i.p.* in female CDF_1 mice, on day 0. Drugs were administered *i.p.* (solutions in distilled water) in ascending doses (0.125—128 mg/kg), once daily, for 5 days (days 1—5). One group received DON as a comparison standard ($ILS_{(max)}$ = 92%), and the control group received saline.

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