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Synthesis of S-(1-diazo-2,2,2-Trifluorethyl) Cysteine Dioxide : A Potential Gutamine Antagonist

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SYNTHESIS OF

S-(1-DIAZO-2,2,2-TRIFLUOROETHYL)CYSTEINE DIOXIDE:

A POTENTIAL GLUTAMINE ANTAGONIST

BY

ROBERT M. OLSON

A thesis submitted
in partial fulfillment of the requirements for the
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State University

1974

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S-(1-DIAZO-2,2,2-TRIFLUOROETHYL)CYSTEINE DIOXIDE:

A POTENTIAL GLUTAMINE ANTAGONIST

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser   Date

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At this time, I would like to extend my gratitude and appreciation to a number of people who have made this all possible.

I wish to thank my research advisor, Dr. Edwin S. Olson, for his help and interest with my project. His enthusiasm has made a lasting impression which I hope to exemplify.

Also, I wish to thank everyone associated with Shepard Hall; faculty, fellow graduate students and employees.

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Most important of all, I wish to thank my parents for the encouragement they have offered and the valuable lesson that they have taught me; never to be satisfied with the existing, to continue to strive for better things in life. Thanks, Mom and Dad.

Last, I wish to extend a special thanks to my typist. Thanks a million, Helen!

<div align="right">RMO</div>

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Specially Dedicated

to

TREVOR JAMES OLSON

No Goal is too high

if we climb with

Care and Confidence

HISTORICAL

Compounds affecting the utilization of L-glutamine (7) have a comparatively long history in the field of cancer chemotherapy. The Streptomyces antibiotics, azaserine (1) (O-diazoacetyl-L-serine) and DON (2) (6-diazo-5-oxo-L-norleucine) inhibit to varying extents those aminations in which glutamine serves as an amino group donor. This inhibition is achieved in at least one case by the glutamine antagonists alkylating the sulfhydryl group of the enzyme that catalyzes the amino group transfer of glutamine. A distinguishing feature of the diazo analogs of glutamine is the very reactive diazo group that enhances nucleophilic reactions on the adjacent carbon atom. A diazo group is represented as such:

$$\left(\underset{N=N=}{\overset{\ominus\ \oplus}{}} \right)$$
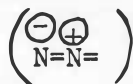
Because of the increased reactivity created by the diazo group, the diazo analogs of glutamine irreversibly inhibit the glutamine requiring enzymes.

Although having gone through exhaustive clinical tests, the L-glutamine antagonists, azaserine and DON, readily declined in interest because of their ineffectiveness in treatment of human malignancies. The recent success of L-asparaginase in the therapy of certain human neoplasms has prompted a reconsideration of compounds which can alter L-glutamine metabolism because: (1) L-glutamine is necessary in most mammalian systems, for the synthesis of L-asparagine; (2) both azaserine and azotomycin appear to be synergistic with L-asparaginase against the L5178Y mouse leukemia; and

(3) L-asparaginase from E. coli (EC-2) of the form in clinical use in the United States has intrinsic L-glutaminase activity. EC-2 preparations appear to be more active against certain tumor cells than guinea pig or agouti serum preparations that lack L-glutaminase activity. Whether this is due to the additional L-glutaminase activity remains to be proven.[1]

The purpose of making compounds which are structurally similar to the two antibiotics is threefold. These are to produce a compound which (1) is more active against the tumor, (2) is less toxic to the patient and (3) will help elucidate the mechanism and nature of its bonding to active sites of enzymes in various biological reactions.[2]

The history is divided into two parts; the first section deals with the importance of L-glutamine in metabolic and biosynthetic pathways, the second part concentrates on glutamine antagonists and their inhibitory effect with the biosynthesis or metabolic roles of the parent compound.

I. L-GLUTAMINE

In 1883, Schulze and Bosshard[3],[4] isolated pure glutamine from an aqueous extract of beet roots. However, it was not until 1932 that Chibnall's laboratory[5],[6] proved the presence of asparagine and of glutamine as normal constituents of protein. Although L-glutamine is not an essential dietary amino acid for the intact mammal, it must be present in the medium in high concentrations for the maintenance

of cells in tissues.

A very important role of glutamine is to keep the concentration of ammonia below a toxic level. This is done by the conversion of glutamate to glutamine. The formation of glutamine is also used for transient storage of part of the nitrogen in the diet. Most important of all, glutamine serves as an amide group donor for the formation of numerous compounds. This includes the synthesis of purines, pyrimidines and amino sugars found in structural polysaccharides. Interference with the metabolism of glutamine will therefore disturb a variety of metabolic pathways.

There are three positions along the biosynthetic pathway of the purines (Scheme I and II) in which glutamine acts as an amide group donor. The nitrogens in the third and ninth position of the purine ring are derived from glutamine.
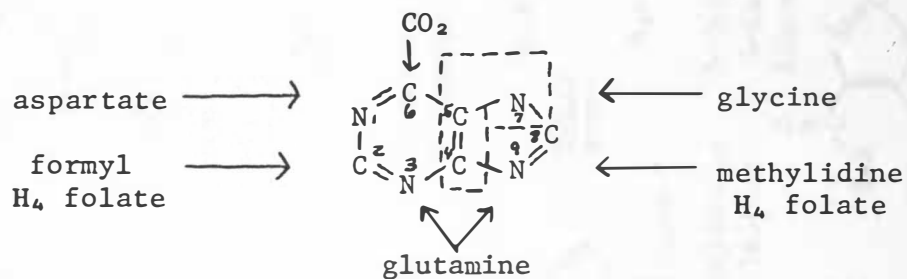
The third use of glutamine is in the formation of guanosine. In the pyrimidine pathway (Scheme III), glutamine is involved only in one reaction. The importance of glutamine's role in the biosynthetic pathways is that the formation of purines and pyrimidines are an essential part of the RNA and DNA molecules.

Biosynthesis of Inosinic Acid[7]

Scheme I

Ribose 5-P

(α-5-Phosphoribosyl-1-pyrophosphate)
(PRPP)

5-Phosphoribosyl
1-Amine

α-N-Formylglycinamidine
Ribonucleotide (FGAM)

α-N-Formylglycinamide
Ribonucleotide (FGAR)

Glycinamide
Ribonucleotide

5-Aminoimidazole
ribonucleotide

5-Aminoimidazole-
4-Carboxylic Acid
Ribonucleotide

5-Aminoimidazole-
4-N-Succinocarboxamide
Ribonucleotide

Inosinic Acid

5-Formamidoimidazole-
4-Carboxamide Ribonucleotide

5-Aminoimidazole-4-Carboxamide
Ribonucleotide

4

Biosynthesis of Adenylic and Guanylic Acid
from Inosinic Acid[7]

Inosinic Acid

+ Aspartate

GTP   GDP, P_i

Adenylosuccinic Acid

NAD$^+$

NADH

−fumarate

Xanthylic Acid

Adenylic Acid

glutamine, ATP, H_2O

glutamate, AMP, P_i

ATP, NH_3

Mg^{+2}

AMP, PP_i

Guanylic Acid
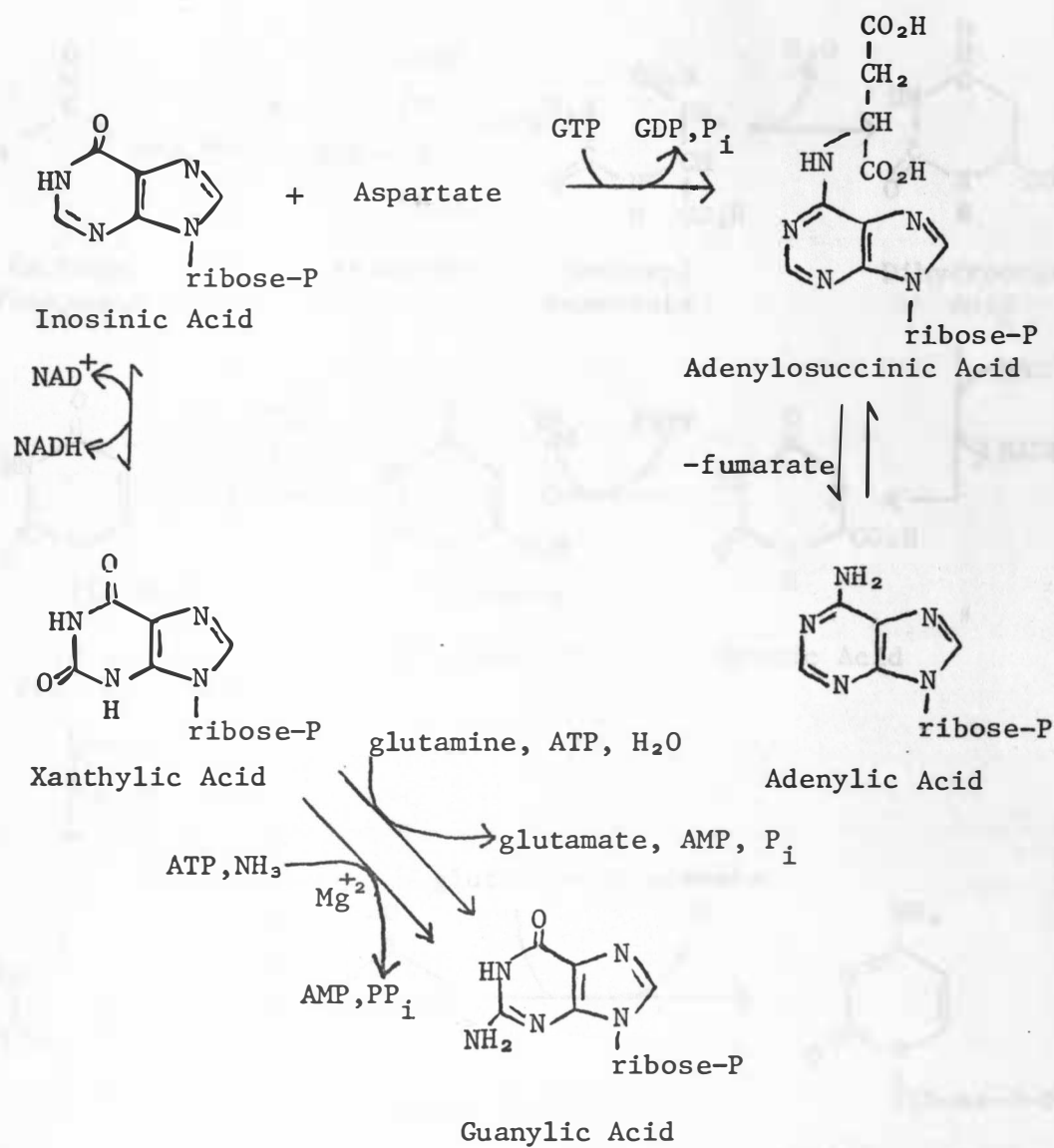
Scheme II

Biosynthesis of Pyrimidines[7]

Carbamyl Phosphate + Aspartate → Carbamyl Aspartate → Dihydroorotic Acid

Uridine-5'-Phosphate (UMP) ← Orotidine-5'-P ← Orotic Acid

Uridine-5'-Diphosphate (UDP) → Uridine-5'-Triphosphate (UTP) → Cytidine-5'-Triphosphate (CTP)
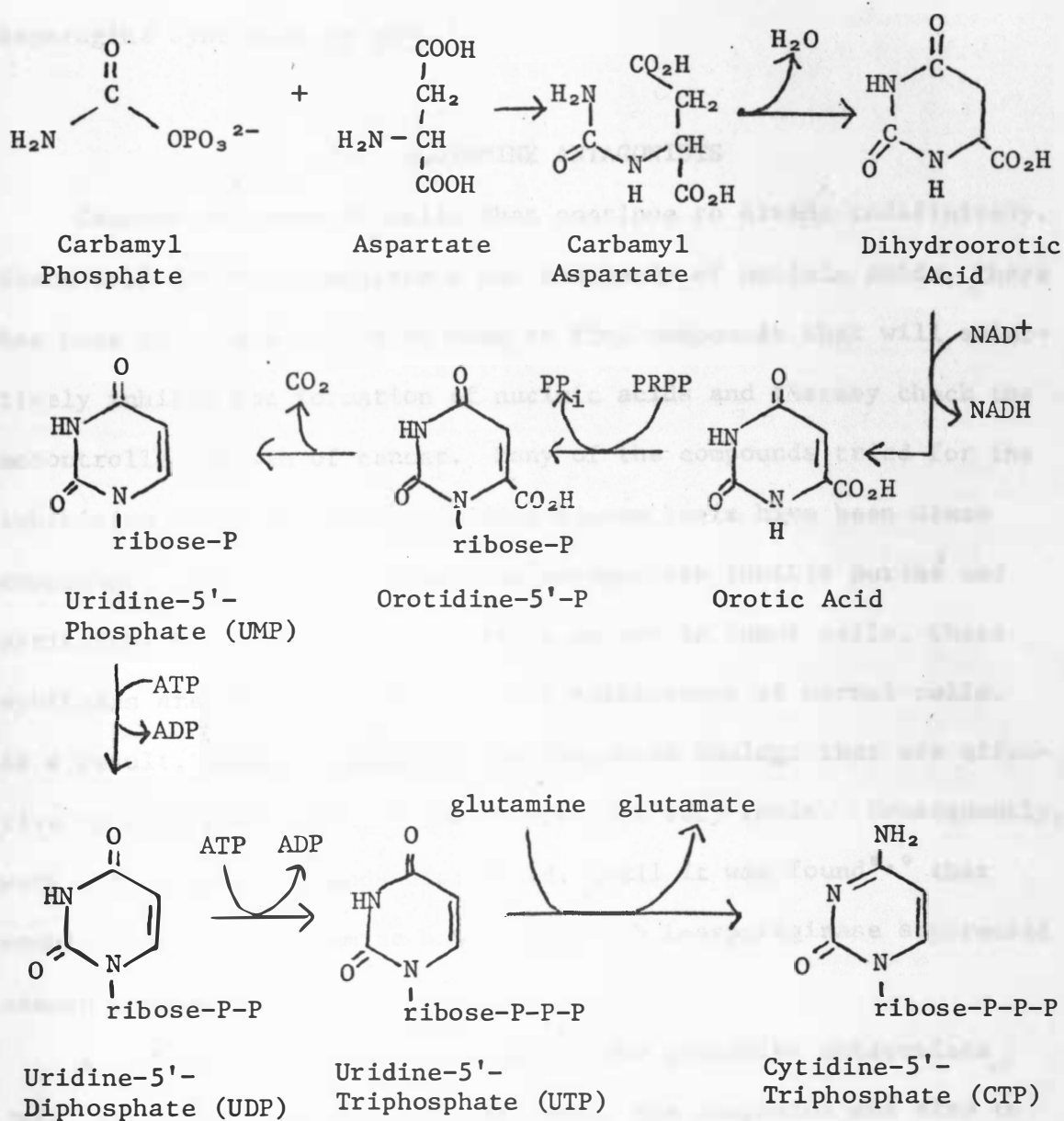
Scheme III

In summary, L-glutamine plays a vital role not only as a con-
stituent of protein but also furnishing an amido group in essential
reactions in nucleic acid, diphosphopyridine, glucosamine and L-
asparagine synthesis de novo.[1]

II. GLUTAMINE ANTAGONISTS

Cancers are made of cells that continue to divide indefinitely.
Since cell division requires a net synthesis of nucleic acids, there
has been considerable effort made to find compounds that will selec-
tively inhibit the formation of nucleic acids and thereby check the
uncontrolled growth of cancer. Many of the compounds tried for the
inhibition of purine and pyrimidine biosynthesis have been diazo
compounds. Although the glutamine antagonists inhibit purine and
pyrimidine biosynthesis to a certain extent in tumor cells, these
syntheses are also necessary for the maintenance of normal cells.
As a result, concentrations of the glutamine analogs that are effec-
tive in suppressing cancer growth are also very toxic. Consequently,
work with these compounds diminished, until it was found[8,9] that
combination of a glutamine antagonist with L-asparaginase suppressed
cancer growth.

A comparison of the structures of the glutamine antagonists
(Fig. 1) shows a marked similarity among the compounds and also to
glutamine and asparagine. Each diazo analog is discussed below as to
its effectiveness as a glutamine antagonist with major emphasis on
azaserine and DON.

Structural Formulas of Diazo Antibiotics[1]

1. $\bar{N}{=}\overset{+}{N}{=}CH{-}\overset{\overset{\textstyle O}{\|}}{C}{-}O{-}CH_2{-}\underset{\underset{\textstyle NH_2}{|}}{CH}{-}COOH$ **Azaserine (O-diazoacetyl-L-serine)**

2. $\bar{N}{=}\overset{+}{N}{=}CH{-}\overset{\overset{\textstyle O}{\|}}{C}{-}CH_2{-}CH_2{-}\underset{\underset{\textstyle NH_2}{|}}{CH}{-}COOH$ **DON (6-diazo-5-oxo-L-norleucine)**

3. $\bar{N}{=}\overset{+}{N}{=}CH{-}\overset{\overset{\textstyle O}{\|}}{C}{-}CH_2{-}\underset{\underset{\textstyle NH_2}{|}}{CH}{-}COOH$ **DONV (5-diazo-4-oxo-norvaline)**

4. $\bar{N}{=}\overset{+}{N}{=}CH{-}\overset{\overset{\textstyle O}{\|}}{C}{-}CH_2{-}CH_2{-}\underset{\underset{\textstyle NH-C-CH_3}{|}}{CH}{-}COOH$ **Duazomycin A (N-acetyl-6-diazo-5-oxo-L-norleucine**

5. $\bar{N}{=}\overset{+}{N}{=}CH{-}C{-}CH_2 \quad O \quad CH_2{-}CH_2{-}C{-}CH{=}\overset{+}{N}{=}\bar{N}$ **Azotomycin (Duazomycin B) N-(N-ɣ-glutamyl-6-diazo-5-oxo-norleucinyl)-6-diazo-5-oxo-norleucine**
   CH-NH-C-CH
   COOH   NH-C-CH₂-CH₂-CH-COOH

6. $\bar{N}{=}\overset{+}{N}{=}CH{-}\overset{\overset{\textstyle O}{\|}}{C}{-}NH{-}CH_2{-}\overset{\overset{\textstyle O}{\|}}{C}{-}NH_2$ **DGA (Diazoacetyl glycinamide)**

7. $H_2N{-}\overset{\overset{\textstyle O}{\|}}{C}{-}CH_2{-}CH_2{-}\underset{\underset{\textstyle NH_2}{|}}{CH}{-}COOH$ **L-glutamine**

8. $H_2N{-}\overset{\overset{\textstyle O}{\|}}{C}{-}CH_2{-}\underset{\underset{\textstyle NH_2}{|}}{CH}{-}COOH$ **L-asparagine**

Figure 1

Probably the most clinically tested glutamine antagonist has been azaserine. Azaserine and DON were both discovered independently by Ehrlich et al., and Stock et al. in culture filtrates of two species of Streptomyces.[10,11] Synthesis of azaserine[12] has been achieved by selective diazotization of O-glycyl-L-serine in 15-20% yields.

The most sensitive inhibition of an amination reaction by azaserine and DON is the one involving the conversion of formylglycinamide ribonucleotide (FGAR) to the corresponding amidine (FGAM).

$$
\begin{array}{c}
\text{H} \\
\text{N} \\
\text{CHO} \\
\text{O} \quad \text{NH} \\
\text{Ribose-P}
\end{array}
\quad + \quad \text{glutamine} \quad
\xrightarrow[\text{H}_2\text{O}]{\text{ATP} \quad \text{ADP} + \text{P}_i \quad \text{Mg}^{2+}}
\quad
\begin{array}{c}
\text{H} \\
\text{N} \\
\text{CHO} \\
\text{HN} \quad \text{NH} \\
\text{Ribose-P}
\end{array}
$$

α-N-formylglycinamide
ribonucleotide
(FGAR)
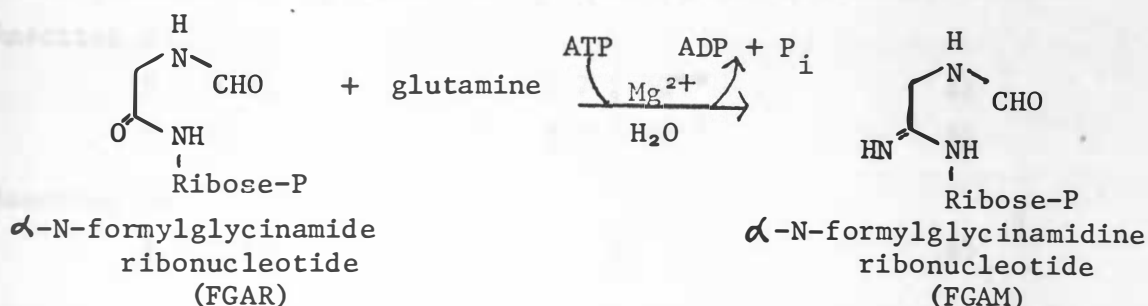
α-N-formylglycinamidine
ribonucleotide
(FGAM)

Table I shows the inhibitory effect of azaserine on three different reactions in purine biosynthesis.

Because of the inhibition of the FGAR to FGAM reaction by azaserine, mechanisms have been derived to explain the inhibition. Baker[13] in 1959 suggested that azaserine reacts with pyridoxal phosphate to form an inactive cofactor-azaserine complex which prevents pyridoxamine formation. Pyridoxal phosphate is an essential coenzyme for the transfer of the amide group from L-glutamine. The prediction by Baker that azaserine alkylates the phosphoryl group of enzymatically bound pyridoxal phosphate, does not apply to FGAR amidotransferase.[14] In 1963, French and his co-workers[15] proposed

Table I

Inhibitory Effect of Azaserine
on Purine Biosynthesis[1]

Azaserine Concentration (M)	L-Glutamine Concentration (M)	% Inhibition
Reaction 1:		
2.9×10^{-3}	2.9×10^{-3}	17
2.9×10^{-2}	2.9×10^{-3}	18
Reaction 2:		
5.7×10^{-4}	5.7×10^{-3}	61
5.7×10^{-3}	5.7×10^{-3}	95
Reaction 3:		
5.7×10^{-3}	0	40

Reaction 1: Glutamine + PRPP ⟶ 5-Phosphoribosyl + glutamate
1-amine

Reaction 2: FGAR ⟶ FGAM

Reaction 3: FGAM ⟶ 5-Aminoimidazole carboxamide
(not L-glutamine dependent) ribonucleotide

that azaserine reacts with FGAR amidotransferase [2-formamido-N-
ribosylacetamide 5'-phosphate: L-glutamine amido-ligase (adenosine
diphosphate), (EC 6.3.5.3)]. Azaserine alkylates the sulfhydryl
group of a cysteine residue of the enzyme preventing the attachment
of L-glutamine to the enzyme. The reason given for the special
reactivity of the diazo compounds with the enzyme is that the
sulfhydryl group of the enzyme is in just the right position to be
attacked by the carbon of the diazo group. Support of the latter
mechanism was made by Mizobuchi and Buchanan[16] in 1968. They pre-
sented evidence that the initial binding of glutamine to FGAR amido-
transferase involves a reversible reaction of the carboxamide carbon
with the sulfhydryl group on the enzyme to yield a γ-glutamyl thio-
ester and ammonia. Also, they showed that the azaserine-enzyme com-
plex formed involved binding at a valylcysteine dipeptide sequence.
Since azaserine does not react with free cysteine, it must be the
activation process of the active site which aids the alkylation
process. A compound similar to azaserine, ethyl diazoacetate, reacts
with water or alcohol and has a diazonium salt as an intermediate.
Ethyl diazoacetate does not react with n-butyl mercaptan but does
react with the more acidic thiophenol, presumably through a dia-
zonium salt intermediate.[17] Therefore, the reaction of azaserine
with the specific cysteine residue of the enzyme probably involves
the diazonium salt of the inhibitor. Further data[15] suggested the
reactive site of azaserine is the same as that of glutamine. Scheme
IV illustrates how a glutamine antagonist reacts with the enzyme.

Attack on FGAR Amidotransferase
by DON[15]
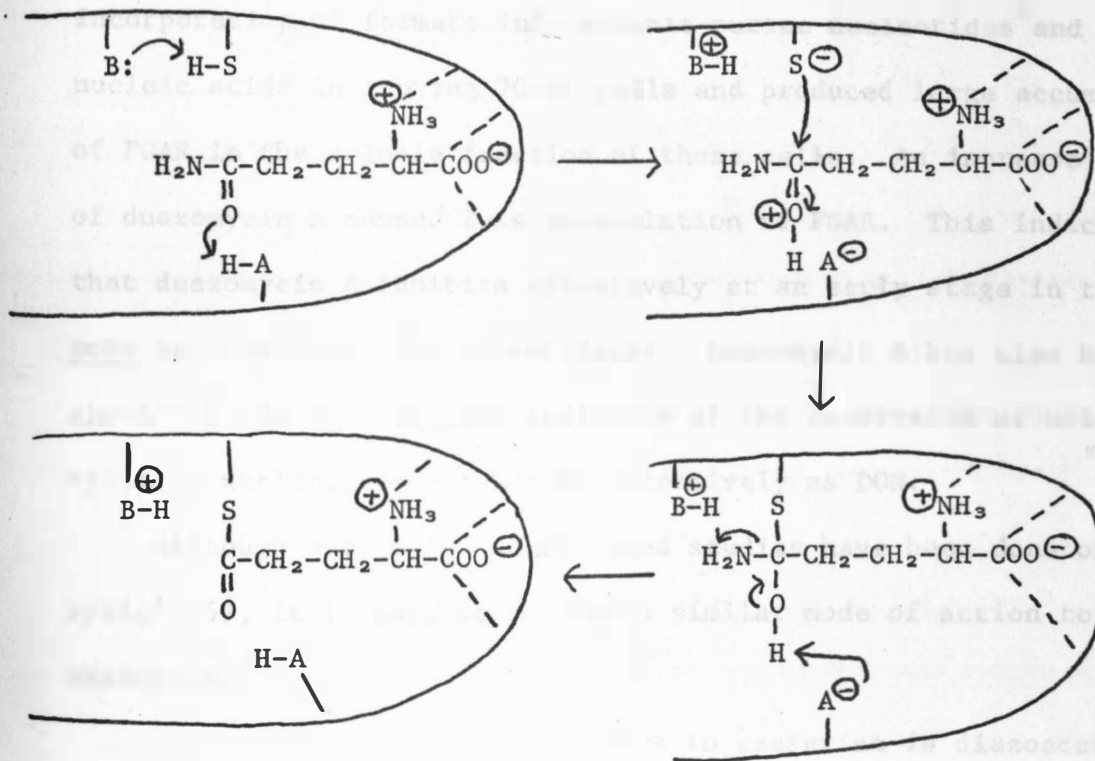

(Enzyme active site)
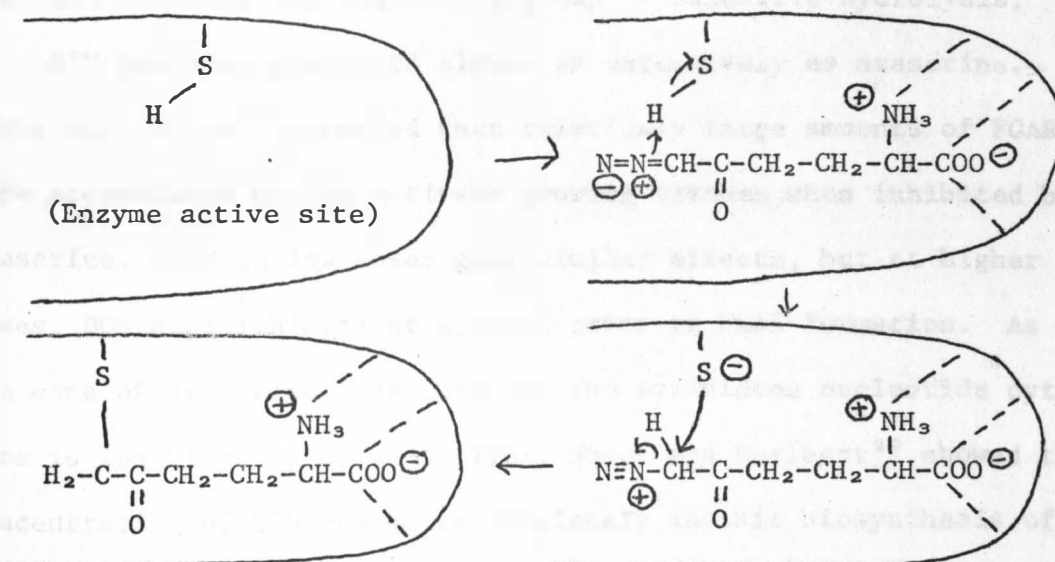
Scheme IV

By analogy to the mechanism proposed for glutamine antagonist bonding to FGAR amidotransferase, the mechanism in Scheme V is proposed for amide nitrogen transfer of glutamine.

The synthesis of DON[18] was achieved by covering the amino and α-carboxyl functions of glutamic acid with appropriate protecting groups, converting the γ-carboxyl of glutamic acid to a diazo ketone and then removing the protecting group by selective hydrolysis.

DON has been evaluated almost as extensively as azaserine. Moore and LePage[19] reported that relatively large amounts of FGAR were accumulated by the actively growing tissues when inhibited by azaserine. DON in low doses gave similar effects, but at higher doses, DON also inhibits at a point prior to FGAR formation. As in the case of azaserine, synthesis of the pyrimidine nucleotide cytidine is inhibited by DON. In 1961, Moore and Hurlbert[20] showed that concentration of DON needed to completely inhibit biosynthesis of cytidine nucleotides was at least ten times that required for biosynthesis of purine nucleotides. They also found that 4.5×10^{-3} M of DON in the presence of 3.0×10^{-5} M exogenous L-glutamine completely inhibited de novo formation of purine and cytidine nucleotides by the Novikoff tumor cell suspension, yet the cells were able to maintain synthesis of RNA at 60% of their usual rate.

DONV (3) is the next lower homolog of DON. It is classed by Handschumacher et al.[21] as an analog of L-asparagine. DONV has been successful in competition with L-asparagine for binding sites on L-asparaginase at physiological pH. In the same experiment, it

A Likely Mechanism for the Transfer
of the Amide Nitrogen of Glutamine[2]

$$B: \quad H-S$$
$$\overset{\oplus}{NH_3}$$
$$H_2N-C-CH_2-CH_2-CH-COO^{\ominus}$$
$$\underset{O}{\overset{\|}{\;}}$$
$$H-A$$

$\longrightarrow$

$$B-H \quad S^{\ominus}$$
$$\overset{\oplus}{\;}\overset{\oplus}{NH_3}$$
$$H_2N-C-CH_2-CH_2-CH-COO^{\ominus}$$
$$\overset{\oplus}{O}$$
$$H \quad A^{\ominus}$$

$\downarrow$

$$\overset{\oplus}{\;}$$
$$B-H \quad S \qquad \overset{\oplus}{NH_3}$$
$$C-CH_2-CH_2-CH-COO^{\ominus}$$
$$\underset{O}{\overset{\|}{\;}}$$
$$H-A$$

$\longleftarrow$

$$\overset{\oplus}{\;}$$
$$B-H \quad S \qquad \overset{\oplus}{NH_3}$$
$$H_2N-C-CH_2-CH_2-CH-COO^{\ominus}$$
$$\underset{O}{\overset{\|}{\;}}$$
$$H$$
$$A^{\ominus}$$

Scheme V

was shown that DONV had no effect on L-glutaminase or other L-glutamine-dependent reactions. This is in contrast to the work done by French et al.[14], who found DONV to cause 50% inhibition of the FGAR to FGAM reaction.

Anderson and Brockman[22] found that duazomycin A (4) inhibited incorporation of formate into soluble purine nucleotides and into nucleic acids in growing 70429 cells and produced large accumulations of FGAR in the soluble fraction of these cells. An increase in dose of duazomycin A caused less accumulation of FGAR. This indicates that duazomycin A inhibits effectively at an early stage in the de novo pathway of purine biosynthesis. Duazomycin A has also been shown[22] to be an effective inhibitor of the conversion of uridine to cytidine nucleotides but not as effectively as DON.

Although only a few unpublished studies have been done on azoto-mycin[1] (5), it is suppose to have a similar mode of action to DON and azaserine.

A compound similar in structure to azaserine is diazoacetyl glycinamide (DGA), (6). It displays a marked antitumor activity on the ascites forms of Sarcoma 180 and Ehrlich ascites carcinoma and on the Galliera rat sarcoma.

With a change in structure, it is interesting to note similarities and/or differences in inhibition of the same process. Aza-serine inhibits thymidine incorporation into DNA, but not that of labelled adenine. If one increases the supply of adenine in the cells, the inhibition of thymidine incorporation by azaserine is
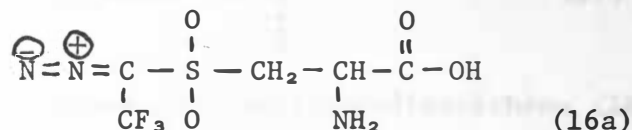
removed. DGA, on the other hand, inhibits the incorporation of labelled adenine and thymidine in DNA. Also, adenine does not modify the inhibition of thymidine incorporation by DNA. This suggests[23] that DGA causes an inhibition of DNA synthesis by acting on the final steps of purine biosynthesis. The precise nature and localization of the step at which the inhibition is exerted are under investigation.

The compounds which have received clinical trials are primarily azaserine, DON, duazomycin A and azotomycin. They have not proven effective enough as sole therapy. The feeling is now that an L-glutamine antagonist in conjunction with L-asparaginase would be the most beneficial means for treatment of malignancies.

In studies of the effectiveness of combination chemotherapy against L5178Y, Jacobs et al.[8,9] showed that combinations of L-asparaginase plus azaserine or azotomycin provided a therapeutic effect that was markedly superior to that produced by the individual drugs. A number of studies[8,9,24,25] have shown that tumors (L5178Y) with low asparagine synthetase activity generally require an extracellular source of L-asparagine and are sensitive to L-asparaginase treatment. Depletion of circulating L-asparagine by the dual process of inhibiting a precursor for its extracellular synthesis and enhancing its deamidation can lead to a combined response against a tumor with little or no asparagine synthetase activity. It is also possible that the synergistic action of glutamine analogs and L-asparaginase against L5178Y resulted from L-asparagine depletion and concurrent block of de novo purine biosynthesis.
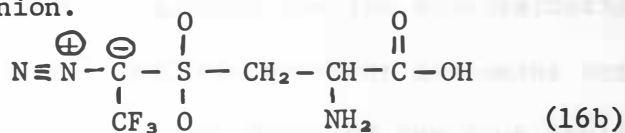
AIM OF RESEARCH

The primary goal in conducting the experimental work involved in the formulation of this thesis was to synthesize S-(1-diazo-2,2,2-trifluoroethyl)cysteine dioxide (16), a potential glutamine antagonist.

$$\overset{\ominus}{N} = \overset{\oplus}{N} = C - \overset{O}{\underset{O}{\overset{|}{S}}} - CH_2 - \underset{NH_2}{\overset{O}{\overset{\|}{CH} - C}} - OH \qquad (16a)$$
$$\underset{CF_3}{|}$$

The reason to pursue this project is the rejuvenated interest in the use of glutamine antagonists with L-asparaginase in combination chemotherapy.

The resonance structure of this compound shows the diazonium group and a carbanion.

$$N \equiv \overset{\oplus}{N} - \overset{\ominus}{\underset{CF_3}{\overset{|}{C}}} - \overset{O}{\underset{O}{\overset{|}{S}}} - CH_2 - \underset{NH_2}{\overset{O}{\overset{\|}{CH} - C}} - OH \qquad (16b)$$

Analysis of the group bonded to the carbanion shows two electron-withdrawing groups:

$$- CF_3 \quad \text{and} \quad - \overset{O}{\underset{O}{\overset{|}{S}}} -$$
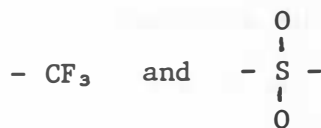
These two groups are able to stabilize the compound by dispersing the negative charge of the carbon atom. Carbanions adjacent to sulfur are stabilized by back-bonding to the sulfur atom. As the sulfur becomes more electropositive in higher coordination states, both d-orbital participation and coulombic interaction contribute to the stability of the carbanion.[26] The stabilizing effect of these two electron-withdrawing groups is illustrated by compounds that contain

the diazo group and one of the electron-withdrawing groups attached to a methine carbon. α-Diazosulfones (17) prepared by Van Leusen and Strating[27] varied in stability up to a period of four months before undergoing decomposition.

$$\overset{\ominus}{N} = \overset{\oplus}{N} = CH - \overset{\overset{\displaystyle O}{|}}{\underset{\underset{\displaystyle O}{|}}{S}} - R \qquad (17)$$
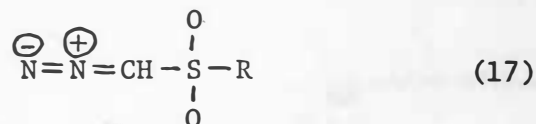
Gilman and Jones[28] showed that trifluorodiazoethane (18) was stable in solution at ordinary temperatures and an ether solution was kept for six weeks without undergoing any noticeable decomposition.

$$\overset{\ominus}{N} = \overset{\oplus}{N} = CH - CF_3 \qquad (18)$$

The stability of a compound is a very important consideration when contemplating the dose level for the administration of the drug because of the reported toxicity of other glutamine antagonists. With increased stability, lower doses of the drug could be given. Work done by Jacques and Sherman[29] suggested that an enzyme in mouse liver enzymatically degrades azaserine into ammonia and pyruvate. The reaction mechanism is probably an α, β elimination which gives the acid, pyruvate, and ammonia as end products. This was substantiated by work done by Longenecker and Snell.[30] They showed that pyridoxal and metal ions catalyze an α, β elimination reaction of azaserine which gives the same reaction products as does the enzymatic breakdown of azaserine.

The inhibitory effect of S-(1-diazo-2,2,2-trifluoroethyl) cysteine dioxide on the reaction of FGAR to FGAM is best explained by using French's mechanism (Scheme VI). Electron-withdrawing

Inhibitory Effect of
S-(1-diazo-2,2,2-trifluoroethyl)cysteine dioxide
on FGAR Amidotransferase

H—S

(Enzyme active site)

(a)

$$N=N=C-S-CH_2-CH-COO^{\ominus}$$
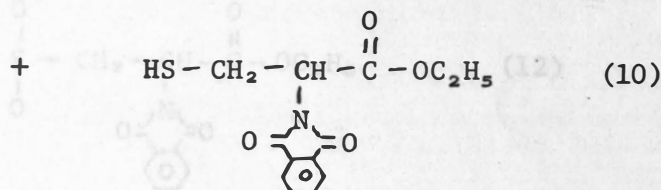
(b)

(c)

$$H-C-S-CH_2-CH-COO^{\ominus}$$

(d)
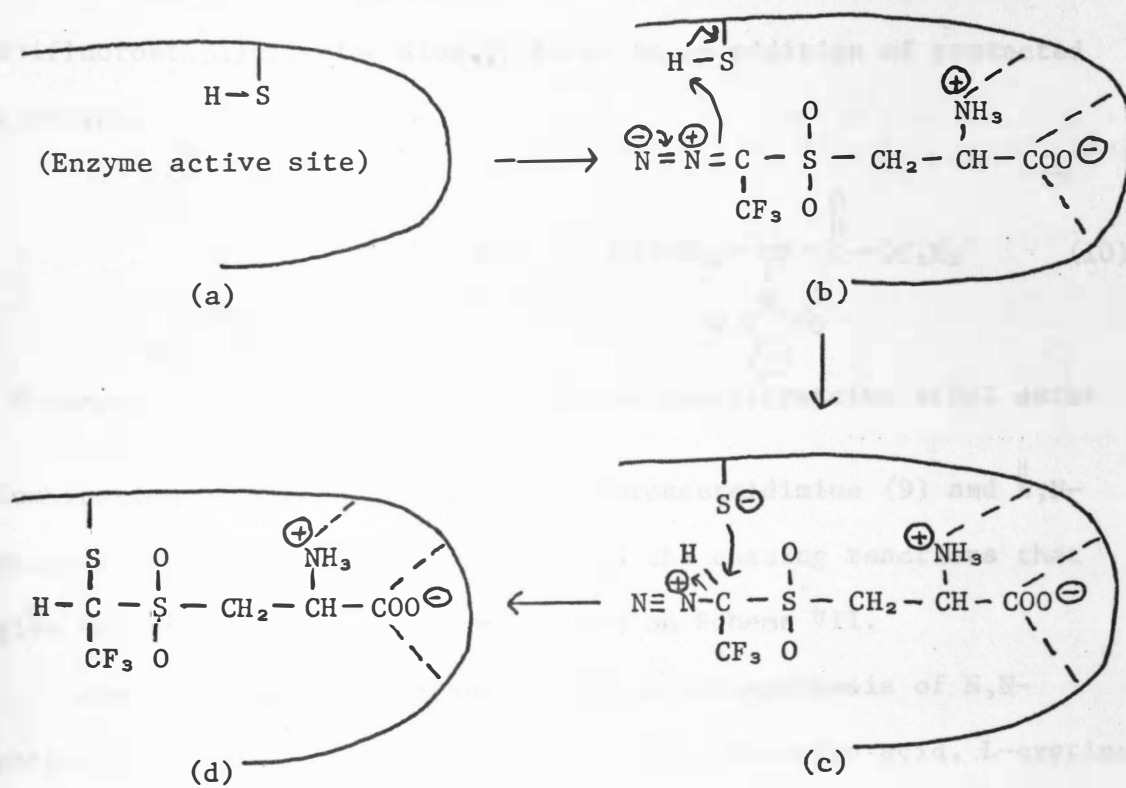
Scheme VI

groups also aid the attack of the nucleophilic sulfur on the protonated diazonium salt (see Scheme VI, fig. c). Electron-withdrawing groups stabilize the transition state in nucleophilic substitution by helping to disperse the developing negative charge.

The central reaction for the synthesis of S-(1-diazo-2,2,2-trifluoroethyl)cysteine dioxide is an imine addition of protected cysteine.
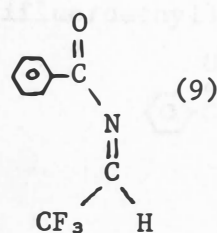
$$\underset{\substack{\text{N-benzoyl-2,2,2-trifluoro-}\\\text{acetaldimine}}}{(9)} \qquad + \qquad \underset{\text{N,N-phthaloylcysteine ethyl ester}}{HS-CH_2-CH-C-OC_2H_5 \quad (10)}$$

Combination of N-benzoyl-2,2,2-trifluoroacetaldimine (9) and N,N-phthaloylcysteine ethyl ester (10) and the ensuing reactions that give the final product can be followed on Scheme VII.

Our first approach (Scheme VIII) to the synthesis of N,N-phthaloylcysteine ethyl ester started with the amino acid, L-cystine. The amino group is protected by treatment of cystine with carboethoxy phthalimide.[31] The phthaloyl group is used for protection because of the ease with which it can be removed by treatment with hydrazine. The value of using phthalimide to protect the amino group is illustrated when the sulfone (12) is treated with nitrous acid to give the N-nitroso amide (13). If the amino group were not protected, then the nitrous acid could attack the amino or amido group giving a lower yield of the desired product (13). Very poor yields

A Synthesis of
S-(1-diazo-2,2,2-trifluoroethyl)cysteine dioxide

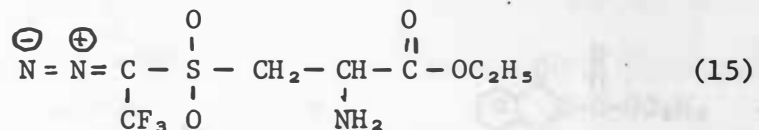1. Combination of N-benzoyl-2,2,2-trifluoroacetaldimine with N,N-phthaloylcysteine ethyl ester will give S-(1-benzamido-2,2,2-trifluoroethyl)-N,N-phthaloylcysteine ethyl ester (11).

$$\langle O \rangle - \overset{\overset{\displaystyle O}{\|}}{C} \to \overset{\overset{\displaystyle H}{|}}{N} - \overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle CF_3}{|}}{C}} - S - CH_2 - \underset{\underset{\displaystyle N}{|}}{CH} - \overset{\overset{\displaystyle O}{\|}}{C} - OC_2H_5 \qquad (11)$$

2. Oxidizing (11) will give the sulfone: S-(1-benzamido-2,2,2-trifluoroethyl)-N,N-phthaloylcysteine dioxide ethyl ester (12).

$$\langle O \rangle \overset{\overset{\displaystyle O}{\|}}{C} - \overset{\overset{\displaystyle H}{|}}{N} - \overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle CF_3}{|}}{C}} - \overset{\overset{\displaystyle O}{|}}{\underset{\underset{\displaystyle O}{|}}{S}} - CH_2 - \underset{\underset{\displaystyle N}{|}}{CH} - \overset{\overset{\displaystyle O}{\|}}{C} - OC_2H_5 \qquad (12)$$

3. Treatment of (12) with nitrous acid will form the N-nitroso amide: S-(1-N'-nitrosobenzamido-2,2,2-trifluoroethyl)-N,N-phthaloylcysteine dioxide ethyl ester (13).

$$N - \overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle CF_3}{|}}{C}} - \overset{\overset{\displaystyle O}{|}}{\underset{\underset{\displaystyle O}{|}}{S}} - CH_2 - \underset{\underset{\displaystyle N}{|}}{CH} - \overset{\overset{\displaystyle O}{\|}}{C} - OC_2H_5 \qquad (13)$$

4. Addition of sodium ethoxide to (13) will give the diazo group: S-(1-diazo-2,2,2-trifluoroethyl)-N,N-phthaloylcysteine dioxide ethyl ester (14).

$$\overset{\ominus}{N} = \overset{\oplus}{N} = \overset{}{\underset{\underset{\displaystyle CF_3}{|}}{C}} - \overset{\overset{\displaystyle O}{|}}{\underset{\underset{\displaystyle O}{|}}{S}} - CH_2 - \underset{\underset{\displaystyle N}{|}}{CH} - \overset{\overset{\displaystyle O}{\|}}{C} - OC_2H_5 \qquad (14)$$
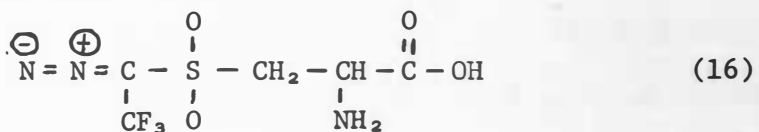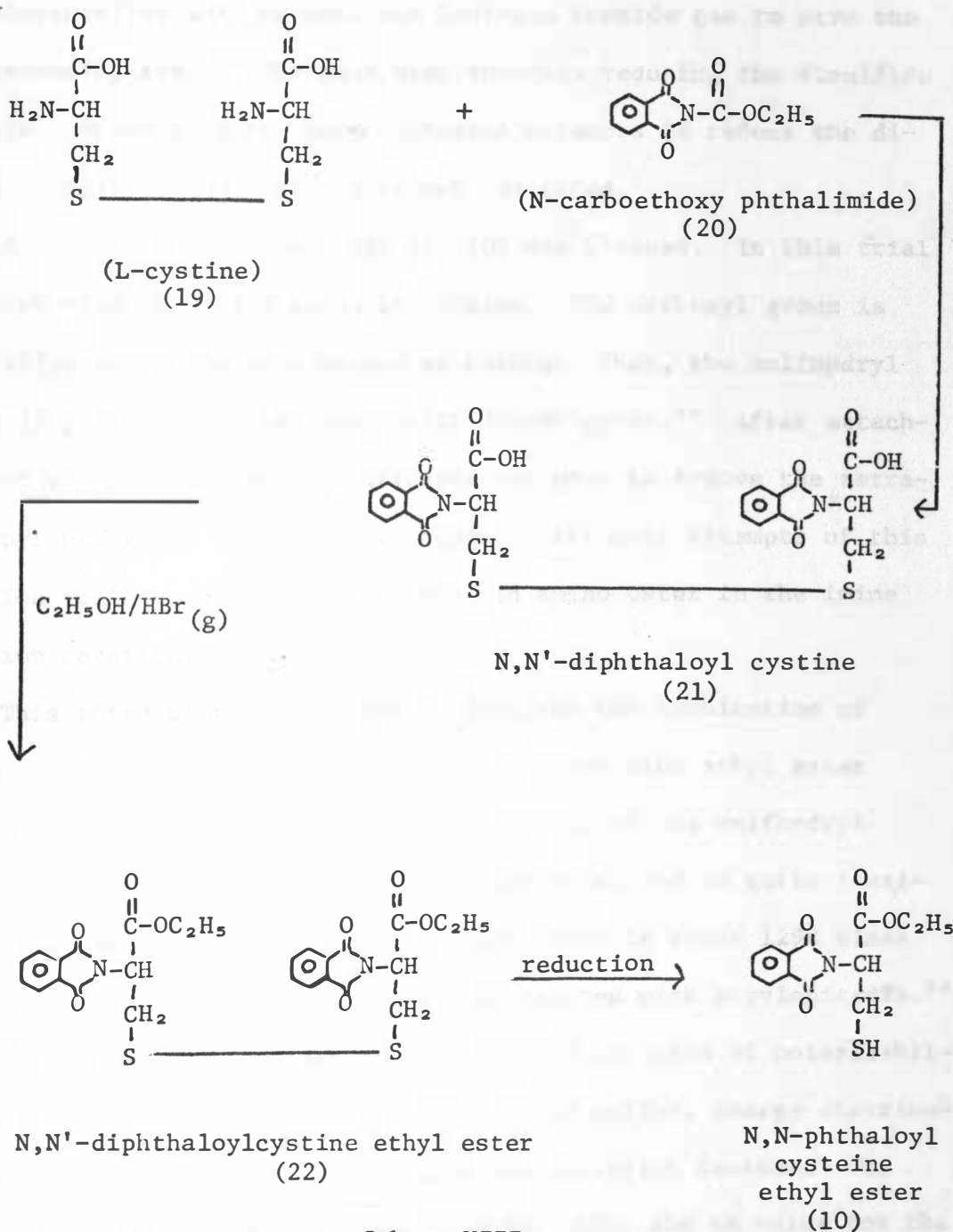
Scheme VII

(continued)

Scheme VII

5. Treatment of (14) with hydrazine will remove the phthaloyl group: S-(1-diazo-2,2,2-trifluoroethyl)cysteine dioxide ethyl ester (15).

$$\overset{\ominus}{N} = \overset{\oplus}{N} = C - \overset{O}{\underset{O}{\overset{|}{S}}} - CH_2 - \underset{NH_2}{\overset{}{CH}} - \overset{O}{\overset{||}{C}} - OC_2H_5 \qquad (15)$$
$$\underset{CF_3}{}$$

6. Addition of base and then acid to (15) will remove the ester to give S-(1-diazo-2,2,2-trifluoroethyl)cysteine dioxide (16).

$$\overset{\ominus}{N} = \overset{\oplus}{N} = C - \overset{O}{\underset{O}{\overset{|}{S}}} - CH_2 - \underset{NH_2}{\overset{}{CH}} - \overset{O}{\overset{||}{C}} - OH \qquad (16)$$
$$\underset{CF_3}{}$$

Synthesis of
N,N-phthaloylcysteine ethyl ester

(L-cystine)
(19)

(N-carboethoxy phthalimide)
(20)

$C_2H_5OH/HBr_{(g)}$

N,N'-diphthaloyl cystine
(21)

reduction

N,N'-diphthaloylcystine ethyl ester
(22)

N,N-phthaloyl
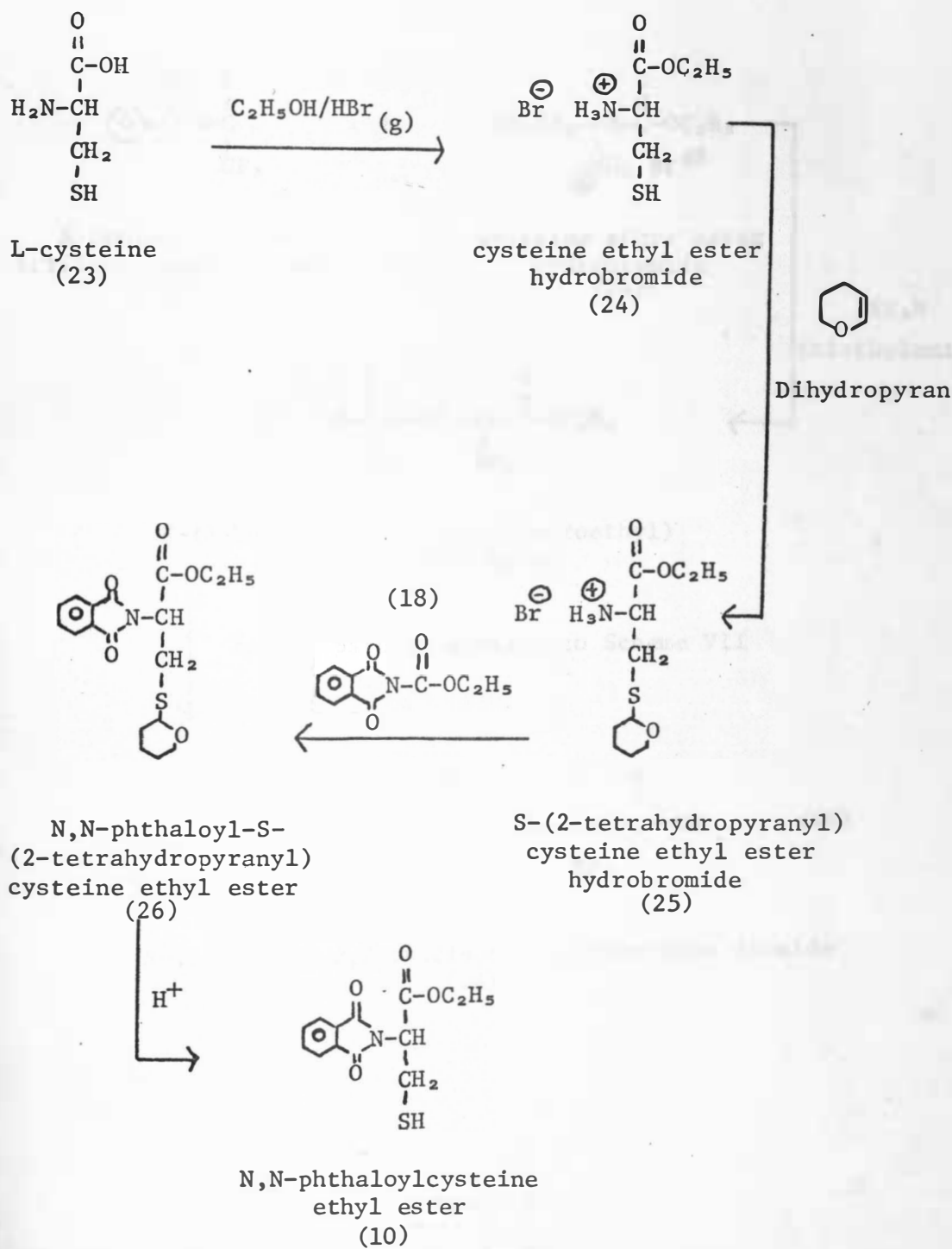cysteine
ethyl ester
(10)

Scheme VIII

of the diazo analog are obtained when the α-amino group is not pro-
tected during diazotization (15-20%) for azaserine[12] and (6%) for
DON.[18] The carboxyl group is protected by treatment of N,N'-di-
phthaloylcystine with ethanol and hydrogen bromide gas to give the
corresponding ester. The next step involves reducing the disulfide
linkage. However, after many different attempts to reduce the di-
sulfide failed, a different approach was tried.

A second route (Scheme IX) to (10) was planned. In this trial,
we start with the amino acid, L-cysteine. The carboxyl group is
esterified using the same method as before. Then, the sulfhydryl
group is protected by treatment with dihydropyran.[32] After attach-
ment of the phthaloyl group, attempts are made to remove the tetra-
hydropyranyl group from sulfur. Again, after many attempts of this
reaction failed, we used the unprotected amino ester in the imine
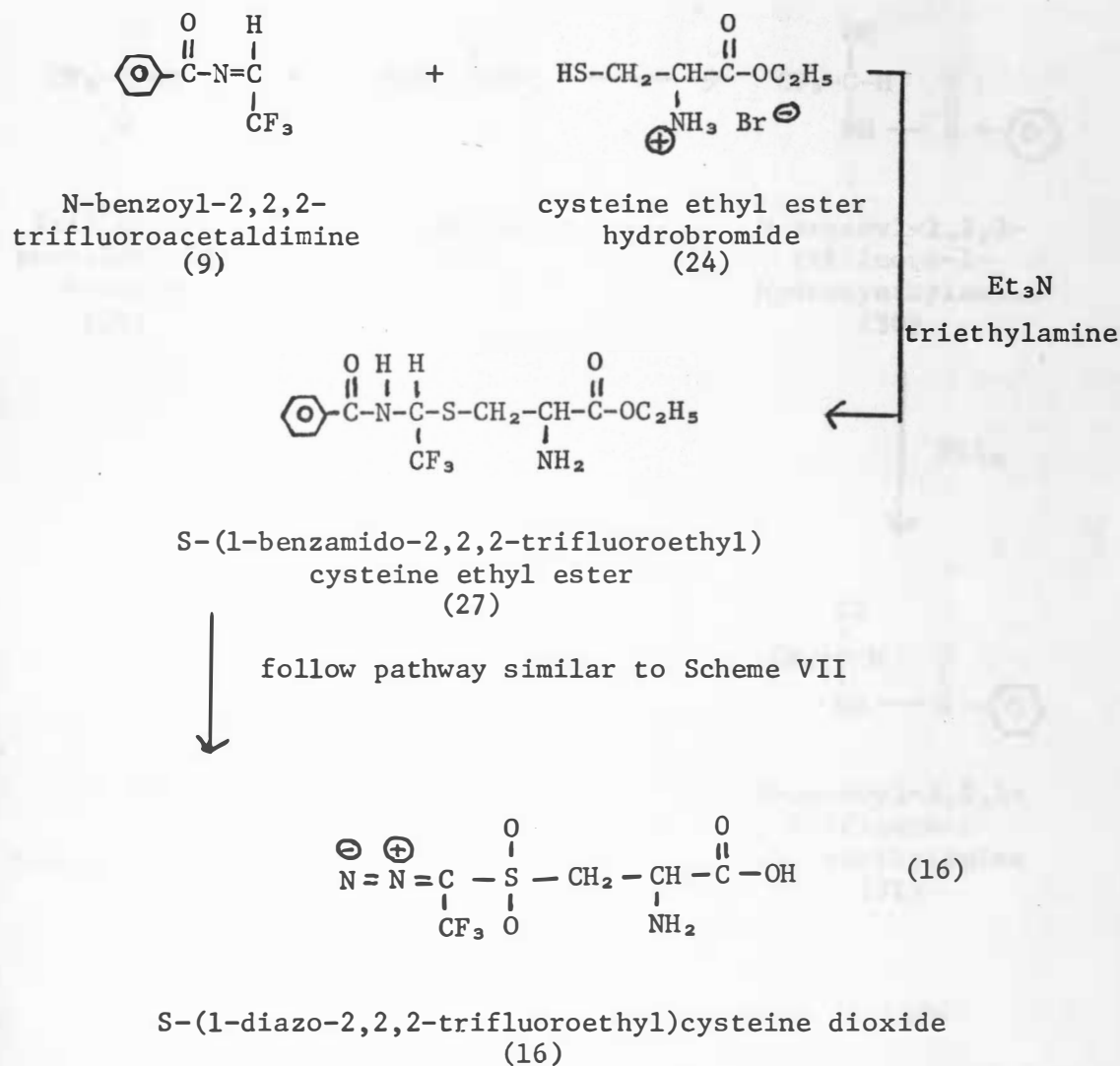addition reaction.

This third approach (Scheme X) involves the combination of
N-benzoyl-2,2,2-trifluoroacetaldimine with cysteine ethyl ester
hydrobromide. If one compares the reactivity of the sulfhydryl
group to the amino group, then this reaction should be quite feasi-
ble. The sulfur anion of cysteine ethyl ester is about 1290 times
more reactive than the amino group when reacted with acrylonitrile.[33]
This difference in reactivities is explained in terms of polarizabil-
ities of nonbonded electrons on nitrogen and sulfur, charge distribu-
tions in ground and transition states and solvation factors. The
pK value for the sulfhydryl group is 6.53, while the pK value for the
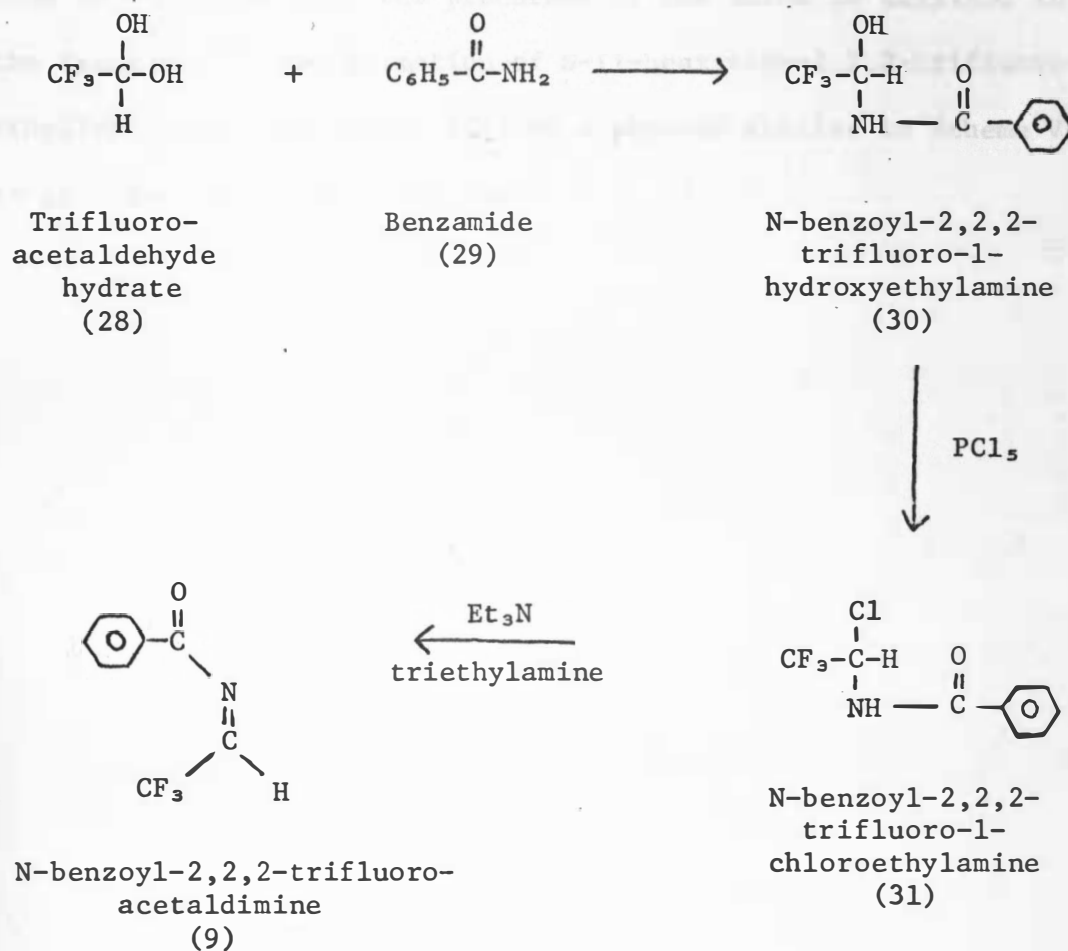
Synthesis of

N,N-phthaloylcysteine ethyl ester

$$\text{L-cysteine}\ (23) \xrightarrow{C_2H_5OH/HBr_{(g)}} \text{cysteine ethyl ester hydrobromide}\ (24)$$

L-cysteine
(23)

cysteine ethyl ester
hydrobromide
(24)

Dihydropyran

(18)

N,N-phthaloyl-S-
(2-tetrahydropyranyl)
cysteine ethyl ester
(26)

S-(2-tetrahydropyranyl)
cysteine ethyl ester
hydrobromide
(25)

H$^+$

N,N-phthaloylcysteine
ethyl ester
(10)

Scheme IX

A Synthesis of

S-(1-diazo-2,2,2-trifluoroethyl)cysteine dioxide

$$\text{Ph}-\overset{\overset{\text{O}}{\|}}{\text{C}}-\text{N}=\overset{\overset{\text{H}}{|}}{\underset{\underset{\text{CF}_3}{|}}{\text{C}}} \qquad + \qquad \text{HS}-\text{CH}_2-\underset{\underset{\overset{+}{\text{NH}_3}\ \overset{\ominus}{\text{Br}}}{|}}{\text{CH}}-\overset{\overset{\text{O}}{\|}}{\text{C}}-\text{OC}_2\text{H}_5$$

N-benzoyl-2,2,2-   cysteine ethyl ester
trifluoroacetaldimine   hydrobromide
(9)   (24)

Et₃N

triethylamine

$$\text{Ph}-\overset{\overset{\text{O}}{\|}}{\text{C}}-\overset{\overset{\text{H}}{|}}{\text{N}}-\overset{\overset{\text{H}}{|}}{\underset{\underset{\text{CF}_3}{|}}{\text{C}}}-\text{S}-\text{CH}_2-\underset{\underset{\text{NH}_2}{|}}{\text{CH}}-\overset{\overset{\text{O}}{\|}}{\text{C}}-\text{OC}_2\text{H}_5$$

S-(1-benzamido-2,2,2-trifluoroethyl)
cysteine ethyl ester
(27)

follow pathway similar to Scheme VII

$$\overset{\ominus}{\text{N}}=\overset{\oplus}{\text{N}}=\underset{\underset{\text{CF}_3}{|}}{\text{C}}-\overset{\overset{\text{O}}{|}}{\underset{\underset{\text{O}}{|}}{\text{S}}}-\text{CH}_2-\underset{\underset{\text{NH}_2}{|}}{\text{CH}}-\overset{\overset{\text{O}}{\|}}{\text{C}}-\text{OH} \qquad (16)$$

S-(1-diazo-2,2,2-trifluoroethyl)cysteine dioxide
(16)

Scheme X

Synthesis of
N-Benzoyl-2,2,2-Trifluoroacetaldimine[32]

$$CF_3-\overset{\overset{\displaystyle OH}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-OH \quad + \quad C_6H_5-\overset{\overset{\displaystyle O}{\|}}{C}-NH_2 \quad \longrightarrow \quad CF_3-\overset{\overset{\displaystyle OH}{|}}{\underset{\underset{\displaystyle NH}{|}}{C}}-H \quad \overset{\overset{\displaystyle O}{\|}}{C}-\bigcirc$$

Trifluoro-   Benzamide   N-benzoyl-2,2,2-
acetaldehyde   (29)   trifluoro-1-
hydrate   hydroxyethylamine
(28)   (30)

PCl$_5$

$$\bigcirc-\overset{\overset{\displaystyle O}{\|}}{C}\overset{\diagdown}{\underset{\diagup}{N}}\overset{\|}{\underset{\overset{\displaystyle CF_3 \quad H}{}}{C}} \qquad \xleftarrow[\text{triethylamine}]{Et_3N} \qquad CF_3-\overset{\overset{\displaystyle Cl}{|}}{\underset{\underset{\displaystyle NH}{|}}{C}}-H \quad \overset{\overset{\displaystyle O}{\|}}{C}-\bigcirc$$

N-benzoyl-2,2,2-trifluoro-   N-benzoyl-2,2,2-
acetaldimine   trifluoro-1-
(9)   chloroethylamine
                                        (31)

Scheme XI

ammonium ion group is 9.05. The sulfhydryl hydrogen is more acidic
and thus, the sulfhydryl anion is present in larger quantity under
the reaction conditions. Because N-benzoyl-2,2,2-trifluoroacetaldi-
mine is very unstable, the precursor of the imine is utilized in
the reaction.[34] The formation of S-(1-benzamido-2,2,2-trifluoro-
ethyl)cysteine ethyl ester follows a pathway similar to Scheme VII
to give the desired product (16).

RESULTS AND DISCUSSION

As previously mentioned, the synthesis of S-(1-diazo-2,2,2-trifluoroethyl)cysteine dioxide (16) by the combination of N-benzoyl-2,2,2-trifluoroacetaldimine (9) and N,N-phthaloylcysteine ethyl ester (10) was desired. The synthesis of the imine will first be discussed and the attempts to synthesize the protected cysteine will follow. Also, attempts to combine a precursor of the imine with cysteine ethyl ester hydrobromide will be discussed.

Synthesis of N-Benzoyl-2,2,2-Trifluoroacetaldimine

The precursors of the imine, N-benzoyl-2,2,2-trifluoro-1-hydroxyethylamine (30) and N-benzoyl-2,2,2-trifluoro-1-chloro-ethylamine (31) were prepared according to the procedure of Weygand, Steglich and co-workers (Scheme XI).[32]

Preparation of N-benzoyl-2,2,2-trifluoro-1-hydroxyethylamine (30) was accomplished by reacting benzamide (29) and trifluoro-acetaldehyde hydrate (28) with sulfuric acid in a sealed tube. The tube was placed in a pipe and heated for 12 hours at 80°C. The product was removed and washed with ethyl acetate and sodium bicarbonate solution. Evaporation of the ethyl acetate layer gave a 52% yield of a white crystalline solid that melted at 102-105°C. The ir spectrum of N-benzoyl-2,2,2-trifluoro-1-hydroxyethylamine (30) showed absorption at 3410 (-OH), 3310 (-NH), 1640 (amide I, $-\overset{\text{O}}{\overset{\|}{\text{C}}}-\text{NH}$)

and 1530 cm^{-1} (amide II, $-\overset{\text{O}}{\overset{\|}{\text{C}}}-\text{NH}$).

The mass spectrum showed the molecular ion at m/e 219. The cleavage of N-benzoyl-2,2,2-trifluoro-1-hydroxyethylamine (30) gave possible fragments corresponding to the m/e values and their percent abundance (Table II).

Preparation of N-benzoyl-2,2,2-trifluoro-1-chloroethylamine (31) by adding phosphorous pentachloride to N-benzoyl-2,2,2-trifluoro-1-hydroxyethylamine (30) gave a 25% yield of a light brown crystalline solid that melted at 88-91°C. Care was exercised with the addition of phosphorous pentachloride to N-benzoyl-2,2,2-trifluoro-1-hydroxy-ethylamine (30) because it was a violent reaction. It was found that best results were obtained when PCl$_5$ was slowly added to 30 in a beaker and the reagents were stirred. The ir spectrum of N-benzoyl-2,2,2-trifluoro-1-chloroethylamine (31) showed absorption at

3280 (-NH), 1660 (amide I, $-\overset{O}{\overset{\|}{C}}$-NH) and 1525 cm^{-1} (amide II, $-\overset{O}{\overset{\|}{C}}$-NH).

The mass spectrum showed the molecular ion at m/e 237. The cleavage of N-benzoyl-2,2,2-trifluoro-1-chloroethylamine (31) gave possible fragments for the corresponding m/e values and their percent abundance (Table III).

Synthesis of the imine was done in situ during its addition with the thiol for the following reasons. First, N-benzoyl-2,2,2-tri-fluoroacetaldimine (9) is unstable and not easily isolated due to its high moisture-sensitivity. Secondly, 9 is easily generated from its precursors so that it is not necessary to isolate the imine in a pure form.[34]

Table II

Mass Fragments of

$$CF_3-\overset{\overset{\displaystyle OH}{|}}{\underset{\underset{\displaystyle HN-\overset{\overset{\displaystyle O}{\|}}{C}-\bigcirc}{}}{CH}}$$
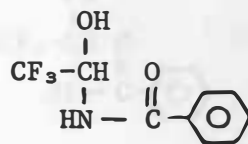
m/e	Percent Abundance	Possible Fragments [+]
69	9	CF_3
77	81	C_6H_5
105	100	$\overset{\overset{\displaystyle O}{\|}}{C}-\bigcirc$
121	54	$H_2N-\overset{\overset{\displaystyle O}{\|}}{C}-\bigcirc$
150	5	$\overset{\overset{\displaystyle OH}{|}}{\underset{\underset{\displaystyle HN-\overset{\overset{\displaystyle O}{\|}}{C}-\bigcirc}{}}{CH}}$
219	3	molecular ion

Table III

Mass Fragments of

$$CF_3-\underset{\underset{HN-\underset{\parallel}{C}-\bigcirc}{|}}{\overset{\overset{Cl}{|}}{CH}}\overset{O}{\underset{\parallel}{}}$$
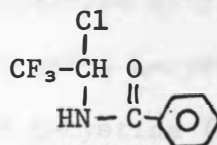
m/e	Percent Abundance	Possible Fragments [+]
69	5	CF_3
77	61	C_6H_5
105	100	$\underset{}{\overset{O}{\underset{\parallel}{C}}}-\bigcirc$
202	1	$CF_3-\underset{\underset{HN-\underset{\parallel}{C}-\bigcirc}{|}}{\overset{\overset{H}{|}}{C}}\overset{O}{\underset{\parallel}{}}$
237	3	molecular ion
239	1	M + 2

Synthesis of N,N-Phthaloylcysteine Ethyl Ester

The preparation of N,N-phthaloylcysteine ethyl ester (10) was first attempted by utilizing the amino acid, L-cystine (19) (Scheme VIII). The amino and carboxy group of L-cystine (19) were protected and then reduction of the disulfide was attempted.

Phthaloyl protection for the amino group was achieved by using the procedure of Nefkins, Tesser and Nivard.[31] Preparation of N-carboethoxy phthalimide (20) was accomplished by dissolving phthalimide in dimethylformamide and triethylamine which was treated with ethyl chloroformate at 5-10°C with vigorous stirring. After the mixture reached room temperature (about 1 hour), it was poured into water. The product was filtered and gave a 57% yield of a white crystalline solid that melted at 78-79.5°C. The ir spectrum of N-carboethoxy phthalimide (20) showed absorption at 1810 (imide,

$-\overset{\overset{O}{\|}}{C}-N-\overset{\overset{O}{\|}}{C}$), 1760 (ester, C=O), 1600 (aromatic, $-C\!\!=\!\!C-$) and 720 cm^{-1} (aromatic, C-H).

L-cystine (19) was dissolved in sodium carbonate by gentle heating and was treated with N-carboethoxy phthalimide (20). The mixture was stirred and the solution was filtered and acidified with 6N hydrochloric acid. A milky white precipitate formed which was dissolved by heating. Slow cooling gave a 52% yield of N,N'-di-phthaloyl-L-cystine (21), a white crystalline product that melted at 117-119°C. The ir spectrum of N,N'-diphthaloyl-L-cystine (21)

showed absorption at 1770 (imide, $-\overset{O}{\overset{\|}{C}}-N-\overset{O}{\overset{\|}{C}}-$), 1700 (acid, C=O), 1620

(aromatic, $-C\overset{\equiv}{=}C-$) and 720 cm^{-1} (aromatic, C-H).

The carboxyl group of (21) was protected by making the ethyl

ester following a modification of the procedure as found in Green-

stein and Winitz.[35] Problems arose in finding an appropriate acid

catalyst.

The first attempt involved suspending N,N'-diphthaloyl-L-cystine

(21) in absolute ethanol and then p-toluenesulfonic acid was added.

The mixture was refluxed for 24 hours and then filtered hot. The

filtrate was cooled and then filtered. The resulting filtrate was

evaporated in order to collect any more product. However, this pro-

cedure gave back starting material.

The second trial utilized the same procedure except that con-

centrated sulfuric acid was substituted for p-toluenesulfonic acid.

A sticky residue was collected but could not be successfully crys-

tallized.

In the third attempt, concentrated hydrochloric acid was used

and in lesser quantity than sulfuric acid. This procedure gave back

starting material.

Consequently, a stream of hydrogen bromide gas was added to a

suspension of the N,N'-diphthaloyl-L-cystine (21) in absolute ethanol.

N,N'-diphthaloyl-L-cystine (21) was immediately dissolved. The

solution was then refluxed for 24 hours and filtered hot. The fil-

trate was cooled for over 12 hours and filtered giving a 70% yield

of N,N'-diphthaloyl-L-cystine ethyl ester (22), a white crystalline

solid that melted at 87-89°C. The ir spectrum of N,N'-diphthaloyl-

L-cysteine ethyl ester (22) showed absorption at 1770 (imide, $-\overset{O}{\overset{||}{C}}-N-\overset{O}{\overset{||}{C}}-$),

1740 and 1710 (ester, C=O), 1610 (aromatic, -C==C-) and 720 cm^{-1}

(aromatic, C-H).

Reduction of the disulfide linkage to give N,N-phthaloyl-L-

cysteine ethyl ester (10) failed. Attempts to reduce the disulfide

will be discussed.

In the first attempt, N,N'-diphthaloyl-L-cystine ethyl ester (22)

was treated with sodium and liquid ammonia.[36] A test tube containing

the ester was placed in a dewar containing dry ice and acetone so

that with the addition of liquid ammonia, the mixture was cooled to

-75°C. Sodium was added and the mixture was stirred without cooling

for 1 1/2 hours. At that time, ammonium iodide was added and the

ammonia allowed to evaporate. However, this procedure gave back

starting material.

In the second attempt, the ester was dissolved in hot absolute

ethanol and mercaptoethanol[37] was added. The solution was refluxed

for 2 hours and kept under nitrogen atmosphere. The solution was

cooled but failed to give crystals. The solution was refluxed again

for 2 hours and cooled. Still, there were no crystals, so water was

added dropwise until the solution became cloudy. The solution was

cooled again and product was collected. However, analysis by ir

spectroscopy showed no -SH peak so that the disulfide linkage was not

broken.

For the third trial, the ester was dissolved in warm glacial acetic acid and zinc dust[38] was added slowly in small amounts. The mixture was boiled until colorless and diluted with two volumes of water and filtered. The filtrate was cooled to give a product. Analysis by ir showed no -SH peak so that the disulfide was not cleaved and showed that the acid had attacked the phthaloyl group.

Fourthly, the acid (21) was dissolved in 3N hydrochloric acid and tin[39] was added. The mixture was refluxed for 2 hours and then cooled. The metal was filtered off and two volumes of water were added to the filtrate. The filtrate was cooled and a precipitate was collected but failed to show a -SH peak on the ir. The product isolated was the starting material (21).

Hydrogenation[40] of the ester was then tried. The ester in dioxane was hydrogenated at room temperature in a Paar Shaker apparatus at an initial pressure of 45 psi over 5% palladium on charcoal. When the hydrogen uptake was complete (about 2 hours), the catalyst was removed by suction filtration. The filtrate was evaporated by use of a Rinco to give the product. Care was taken to make sure that the compound was not oxidized back to the disulfide by over-exposure to the air. Analysis by ir showed that the disulfide was not reduced. The reason for this failure may be due to the generally accepted statement that the efficiency of palladium or platinum as a catalyst suffers a marked diminution whenever sulfur is present.

Last of all, attempts were made to make the Bunte salt.[41] The ester was dissolved in ethanol and a 10% sodium sulfite solution was

slowly added. The solution was stirred well and placed in ice to get crystals. Analysis of the product formed by ir showed no -SH peak so that the disulfide was not cleaved.

The inability to reduce the disulfide or form the Bunte salt may be due to steric hinderance, but it cannot be proven.

Since the disulfide linkage of the N,N'-diphthaloyl-L-cystine ethyl ester (22) was not cleaved by reduction, an alternative method (Scheme IX) was needed to synthesize N,N-phthaloylcysteine ethyl ester (10). Attachment of the ester and tetrahydropyranyl groups to L-cysteine (23) was accomplished according to the procedure of Holland and Cohen.[42]

Cysteine ethyl ester hydrobromide (24) was prepared by suspending L-cysteine (23) in ethanol and the mixture saturated with hydrogen bromide gas. The mixture was stored for 12 hours at room temperature and concentrated under pressure. Difficulty was encountered with crystallization after evaporation. Addition of ether to the residue and reevaporation aided crystallization. The product formed gave a 91% yield of a white crystalline produce that melted at 94-97°C. The ir spectrum showed absorption at 2480 (-SH) and 1745 cm^{-1} (ester, C=O). A nitroprusside test was also run to prove the presence of the -SH group. Cysteine ethyl ester hydrobromide (24) gave a positive result, a red-pink color.

Treating cysteine ethyl ester hydrobromide (24) in methylene chloride with dihydropyran gave a 92% yield of S-(2-tetrahydro-pyranyl)cysteine ethyl ester hydrobromide (25), a light brown

crystalline solid that melted at 122-123°C. There was no need to re-
flux the mixture of cysteine ethyl ester hydrobromide (24) in methy-
lene chloride when dihydropyran was slowly added. Solution was imme-
diate upon addition of dihydropyran. Again, ether was added to the
syrup in order to get crystals. The ability to get crystals via this
method differs from the work of Holland and Cohen.[42] They formed the
hydrochloride methyl ester which was a residual syrup that resisted a
number of attempts at crystallization. The ir spectrum showed ab-
sorption at 1745 cm^{-1} (ester, C=O). Also, there was no -SH peak at
2480 cm^{-1}.

The attachment of the phthaloyl group followed a modification of
the procedure of Nefkins and co-workers.[31] S-(2-tetrahydropyranyl)
cysteine ethyl ester hydrobromide (25) was dissolved in triethylamine
and water. N-carboethoxy phthalimide (20) was added slowly. The
suspension was stirred and heated until completely dissolved. The
solution was cooled giving a 23% yield of N,N-phthaloyl-S-(2-tetra-
hydropyranyl)cysteine ethyl ester (26), a white crystalline solid that
melted at 200-205°C. The ir spectrum showed absorption at 1770

(imide, $-\overset{O}{\overset{\|}{C}}-N-\overset{O}{\overset{\|}{C}}-$) and 1750 cm^{-1} (ester, C=O).

The mass spectrum showed the molecular ion at m/e 363. The
cleavage of N,N-phthaloyl-S-(2-tetrahydropyranyl)cysteine ethyl
ester (26) gave possible fragments corresponding to the m/e values
(Table IV).

Table IV

Mass Fragments of

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(structure: N-phthalimido compound with C-OC₂H₅ ester and CH₂-S-tetrahydropyran side chain)
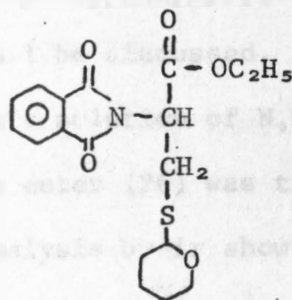
m/e	Percent Abundance *	Possible Fragments [+]
205	100	(phthalimide fragment with N–CH–CH₂–S)
290	20	(phthalimide fragment with N–CH–CH₂–S–tetrahydropyran)
363	8	molecular ion

* m/e 205 was used as the base peak because the fragments of lower
m/e value were all off scale. This was due to the fact that
difficulty was experienced in obtaining the molecular ion peak.

Attempts to remove the tetrahydropyranyl group failed. The treatment of N,N-phthaloyl-S-(2-tetrahydropyranyl)cysteine ethyl ester (26) with different reagents will be discussed.

In the first attempt, an alcoholic solution of N,N-phthaloyl-S-(2-tetrahydropyranyl)cysteine ethyl ester (26) was treated with silver nitrate solution at 0°C.[42] Analysis by ir showed that the material isolated was not the silver salt but the starting material.

Secondly, 26 was dissolved in ethanol and treated with generated H_2S gas. The solution was cooled to collect crystals. Analysis by ir showed that the material isolated was still the starting material.

In the article by Kipnis and Ornfelt,[43] they stated that the tetrahydropyranyl sulfides are in reality semimercaptals, which are unstable to acids. Consequently, attempts to remove the tetrahydropyranyl group by treatment with acids were made.

N,N-phthaloyl-S-(2-tetrahydropyranyl)cysteine ethyl ester (26) was dissolved in ethanol and treated with hydrogen bromide gas. The solution was cooled and the product collected. Analysis by ir showed that the material isolated was the starting material.

Again, an alcohol solution of N,N-phthaloyl-S-(2-tetrahydro-pyranyl)cysteine ethyl ester (26) was treated with hydrogen bromide gas. However, this time the solution was refluxed for an hour. The solution was cooled and the product collected. Analysis by ir showed that the material isolated was still the starting material.

Finally, 26 was dissolved in ethanol and was treated with 10% hydrochloric acid solution. The resulting solution was stirred and

cooled and the product collected. Analysis by ir showed that the material isolated was still the starting material.

The failure to remove the tetrahydropyranyl group from N,N-phthaloyl-S-(2-tetrahydropyranyl)cysteine ethyl ester (26) cannot be explained by this author. Although there may be steric hinderance, treatment with acid should have removed the tetrahydropyranyl group.

Because attempts to synthesize N,N-phthaloylcysteine ethyl ester (10) failed, other means to obtain 10 were tried (Scheme X) by treatment of the unprotected amino ester with the imine. As mentioned before, precursors of the imine can be utilized when treated with nucleophilic reagents.

In the first attempt, cysteine ethyl ester hydrobromide (24) was dissolved in triethylamine and methylene chloride. N-benzoyl-2,2,2-trifluoro-1-chloroethylamine (31) was slowly added.[32] Product was immediately formed upon this addition. Analysis by ir of the product formed was complicated by the presence of the triethylammonium bromide. This reaction was tried again substituting dioxane for methylene chloride as the solvent but achieved the same results.

Next, an alternative was attempted which utilizes the trifluoroacetate which eliminates to form the imine. Neither the imine nor trifluoroacetate were isolated. N-benzoyl-2,2,2-trifluoro-1-hydroxyethylamine (30) was dissolved in pyridine, cooled in ice and trifluoroacetic anhydride (TFAA) was added. Care was exercised at this point because TFAA reacts quite vigorously with the solution. After five minutes, cysteine ethyl ester hydrobromide (24) was added and
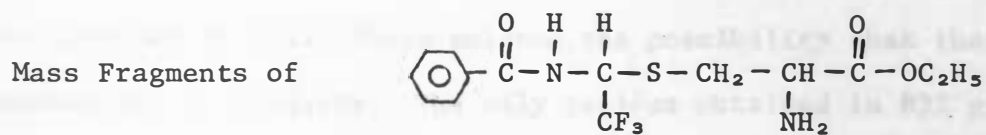
the mixture was stirred. The solution stood at room temperature for 2

hours. Ethyl acetate was added and the solution was placed in a .

separatory funnel. The ethyl acetate layer was separated upon addi-

tion of sodium bicarbonate solution. The ethyl acetate layer was

dried over anhydrous magnesium sulphate. Evaporation of ethyl ace-

tate and pyridine was done in vacuum.[44] An oily residue was left

which was very difficult to crystallize. Some success was achieved

with carbon tetrachloride. However, this procedure only gave a 4%

yield of S-(1-benzamido-2,2,2-trifluoroethyl)cysteine ethyl ester (27),

a white crystalline product that melted at 91-94°C. The ir spectrum

showed absorption at 3360 (-NH), 3170 (-NH$_2$), 1750 (ester, C=O), 1650

(amide I, $-\overset{O}{\overset{\|}{C}}$-NH) and 1580 cm^{-1} (amide II, $-\overset{O}{\overset{\|}{C}}$-NH).

The mass spectrum showed the molecular ion at m/e 350. The

cleavage of S-(1-benzamido-2,2,2-trifluoroethyl)cysteine ethyl ester

(27) gave possible fragments for the corresponding m/e values and

their percent abundance (Table V).

Because of the poor yields obtained in the preceding procedure

in attempts to crystallize the residue, synthesis of the sulphone[45]

was attempted, without crystallization.

The residue was dissolved in glacial acetic acid and was cooled

in ice. Addition of acetic anhydride and 30% hydrogen peroxide was

added dropwise over a period of thirty minutes. The solution was

allowed to stand for 4 hours in ice. The solution was heated for 1

hour at 47°C. This was allowed to stand to form crystals. An oily

Table V

Mass Fragments of

$$\underset{CF_3}{\overset{O}{\underset{\|}{C}}} - \underset{}{\overset{H}{\underset{|}{N}}} - \underset{}{\overset{H}{\underset{|}{C}}} - S - CH_2 - \underset{NH_2}{CH} - \overset{O}{\overset{\|}{C}} - OC_2H_5$$

m/e	Percent Abundance	Possible Fragments [+]
69	10	CF_3
77	69	C_6H_5
105	100	
121	11.5	
133	2	
202	3	
261	2	
277	2	
350	.35	molecular ion

residue was left which resisted numerous attempts at crystallization. Some success seemed likely when the residue was dissolved in alcohol and ether was added. Crystals formed but when filtered, the crystals turned back to an oil. There existed the possibility that there was a combination of products. The oily residue obtained in 83% yield was analyzed by ir spectroscopy and showed absorption at 1740 (ester, C=O), 1670 (amide I, $-\overset{O}{\overset{\|}{C}}-NH$), 1580 (amide II, $-\overset{O}{\overset{\|}{C}}-NH$), 1190 and 1050 cm^{-1} (sulfoxide, S=O).[46] Therefore, instead of complete oxidation to the sulfone, most of S-(1-benzamido-2,2,2-trifluoroethyl)cysteine ethyl ester (27) was converted to the sulfoxide. The lack of complete oxidation to the sulfone may be due to steric hinderance and/or the need for more vigorous conditions such as use of ammonium molybdate catalyst[47] and/or stronger heating.

With the synthesis of the sulfoxide, only one problem remains in the synthesis of the glutamine analog, that is the diazotization. Actually, the sulfoxide represents a potential glutamine analog itself and should be investigated for inhibitory properties.

EXPERIMENTAL

The work reported herein was done in residence at South Dakota State University, Brookings, South Dakota.

Description of Instrumentation Used:

Infrared spectra were obtained on a Perkin-Elmer Model 700 Infrared Spectrophotometer between sodium chloride plates. All samples were solids and run in nujol phase.

Mass spectra were run on a Finnigan 3000 Mass Spectrometer.

I.   Synthesis of N-Benzoyl-2,2,2-Trifluoroacetaldimine:

A.   N-Benzoyl-2,2,2-trifluoro-1-hydroxyethylamine: (30)

The procedure as reported by Weygand, Steglich and co-workers[32] was used to prepare N-benzoyl-2,2,2-trifluoro-hydroxyethylamine. A white crystalline product was obtained that melted at 102-105°C.

Infrared absorption bands appeared at 3410 (-OH), 3310 (-NH), 1640 (amide I, $-\overset{\text{O}}{\underset{\text{||}}{\text{C}}}$-NH) and 1530 cm^{-1} (amide II, $-\overset{\text{O}}{\underset{\text{||}}{\text{C}}}$-NH).

The mass spectrum gave a molecular ion at m/e 219. The following fragments were also noted: m/e 150, m/e 121, m/e 105, m/e 77 and m/e 69.

        Yield = 9.46 g   % Yield = 52%

B.   N-Benzoyl-2,2,2-trifluoro-1-chloroethylamine: (31)

The procedure as reported by Weygand, Steglich and co-workers[32] was used to prepare N-benzoyl-2,2,2-trifluoro-1-chloroethylamine.

A light brown crystalline product was obtained that melted at 88–91°C.

Infrared absorption bands appeared at 3280 (–NH), 1660 (amide I,

$\overset{O}{\overset{||}{-C}}$–NH) and 1525 cm^{-1} (amide II, $\overset{O}{\overset{||}{-C}}$–NH).

The mass spectrum gave a molecular ion at m/e 237. The isotopic abundance of the M + 2 peak shows the compound contains one chlorine. The following fragments were also noted: m/e 202, m/e 105, m/e 77 and m/e 69.

<div align="center">Yield = 2.47 g   % Yield = 25%</div>

II. Synthesis of N,N-Phthaloylcysteine Ethyl Ester:
Part I.

A. <u>N-Carboethoxy phthalimide</u>: (20)

The procedure as reported by Nefkins, Tesser and Nivard[31] was used to prepare N-carboethoxy phthalimide. A white crystalline product was obtained that melted at 78–79.5°C.

Infrared absorption bands appeared at 1810 (imide, $\overset{O}{\overset{||}{-C}}$–N–$\overset{O}{\overset{||}{C}}$–), 1760 (ester, C=O), 1600 (aromatic, $-C\overset{-}{=}C-$) and 720 cm^{-1} (aromatic, C–H).

<div align="center">Yield = 125.14 g   % Yield = 57%</div>

B. <u>N,N'-Diphthaloyl-L-cystine</u>: (21)

The procedure as reported by Nefkins, Tesser and Nivard[31] was used to prepare N,N'-diphthaloyl-L-cystine. A white crystalline product was obtained that melted at 117–119°C.

Infrared absorption bands appeared at 1770 (imide, $-\overset{\overset{O}{\|}}{C}-N-\overset{\overset{O}{\|}}{C}-$),
1700 (acid, C=O), 1620 (aromatic, -C=C-) and 720 cm^{-1} (aromatic,
C-H).

> Yield = 5.43 g % Yield = 52%

C. N,N'-Diphthaloyl-L-cystine ethyl ester: (22)

An adaption of the method utilized by Curtius and Goebel for
esterification of the carboxyl group as found in Greenstein and
Winitz[35] was used for preparation of N,N'-diphthaloyl-L-cystine
ethyl ester.

A stream of hydrogen bromide gas was added to the suspension of
2.0 g (0.004 moles) of N,N'-diphthaloyl-L-cystine in 100 ml of
absolute ethanol. The suspension was immediately dissolved and the
solution was refluxed for 24 hours. The solution was filtered while
hot and the filtrate was cooled for over 12 hours. The resulting
precipitate was filtered giving a white crystalline product that
melted at 87-89°C.

Infrared absorption bands appeared at 1770 (imide, $-\overset{\overset{O}{\|}}{C}-N-\overset{\overset{O}{\|}}{C}$),
1740 and 1710 (ester, C=O), 1610 (aromatic, -C=C-) and 720 cm^{-1}
(aromatic, C-H).

> Yield = 1.55 g % Yield = 70%

Part II.

D. Cysteine ethyl ester hydrobromide: (24)

The procedure as reported by Holland and Cohen[42] was used to
prepare cysteine ethyl ester hydrobromide. A white crystalline

product was obtained that melted at 94-97°C.

Infrared absorption bands appeared at 2480 (-SH) and 1745 cm^{-1} (ester, C=O).

A nitroprusside test was also run on cysteine ethyl ester hydrobromide and gave a positive result (red-pink color) indicative of a -SH group.

E. **S-(2-tetrahydropyranyl)cysteine ethyl ester hydrobromide:** (25)

Continuation of the procedure by Holland and Cohen[42] was used to prepare S-(2-tetrahydropyranyl)cysteine ethyl ester hydrobromide. A light brown crystalline product was obtained that melted at 122-123°C.

Infrared absorption band appeared at 1745 cm^{-1} (ester, C=O). Also, there was no -SH peak at 2480 cm^{-1}.

                Yield = 43.25 g   % Yield = 92%

F. **N,N-Phthaloyl-S-(2-tetrahydropyranyl)cysteine ethyl ester:** (26)

The procedure used for the attachment of the phthaloyl group was a modification of Nefkins and co-workers.[31] S-(2-tetrahydropyran-yl)cysteine ethyl ester hydrobromide [6.30 g (0.02 moles)] was dissolved in 3.0 ml (0.02 moles) of triethylamine and 30 ml of water. N-carboethoxy phthalimide [4.50 g (0.02 moles)] was added slowly. The suspension was stirred and heated until completely dissolved. The solution was cooled giving a white crystalline product that melted at 200-205°C.

Infrared absorption bands appeared at 1770 (imide, $-\overset{O}{\overset{\|}{C}}-N-\overset{O}{\overset{\|}{C}}$) and 1750 cm^{-1} (ester, C=O).

The mass spectrum gave a molecular ion at m/e 363. The following fragments were also noted: m/e 290, m/e 205.

Yield = 1.60 g   % Yield = 23%

III. Synthesis of S-(1-Benzamido-2,2,2-Trifluoroethyl)cysteine Ethyl Ester:

A. S-(1-Benzamido-2,2,2-trifluoroethyl)cysteine ethyl ester: (27)

A modification of the procedure as reported by Weygand and co-workers[44] was used to prepare S-(1-benzamido-2,2,2-trifluoroethyl) cysteine ethyl ester.

N-benzoyl-2,2,2-trifluoro-1-hydroxyethylamine [2.20 g (0.01 moles)] was dissolved in approximately 5 ml of pyridine. The solution was cooled in ice and 1.4 ml of trifluoroacetic anhydride was added with care. Cysteine ethyl ester hydrobromide [2.30 g (0.01 moles)] was added to the solution after five minutes. The mixture was stirred and the solution was left to stand for 2 hours at room temperature. Ethyl acetate was added and the solution was placed in a separatory funnel. Separation of the ethyl acetate layer was achieved upon addition of sodium bicarbonate solution and the ethyl acetate layer was dried over anhydrous magnesium sulfate. Ethyl acetate and pyridine were evaporated off in vacuum which left an oily residue (crude product) that was very difficult to crystallize. Some success was achieved with carbon tetrachloride which gave a white crystalline product that melted at 91-94°C.

Infrared absorption bands appeared at 3360 (-NH), 3170 (-NH$_2$),

1750 (ester, C=O), 1650 (amide I, $-\overset{\overset{\text{O}}{\|}}{\text{C}}$-NH) and 1580 cm^{-1} (amide II,

$-\overset{\overset{\text{O}}{\|}}{\text{C}}$-NH).

The mass spectrum showed the molecular ion at m/e 350. The

following fragments were also noted: m/e 277, m/e 261, m/e 202,

m/e 121, m/e 105, m/e 77 and m/e 69.

Yield = 3.0 g   % Yield = 86%
    (crude product)

Yield = 0.125 g   % Yield = 4%
    (crystallized product)

B. S-(1-Benzamido-2,2,2-trifluoroethyl)cysteine oxide ethyl ester:

A modification of the procedure by Weygand and Steglich[45] was

used to prepare S-(1-benzamido-2,2,2-trifluoroethyl)cysteine oxide

ethyl ester.

The crude product (3.0 g) of S-(1-benzamido-2,2,2-trifluoro-

ethyl) cysteine ethyl ester was dissolved in 50 ml of glacial acetic

acid. The solution was cooled in ice and 15 ml of acetic anhydride

was added. Hydrogen peroxide (30%) (10 ml) was added dropwise over

a period of thirty minutes. The solution was allowed to stand for 4

hours in ice and then was heated for 1 hour at 47°C. An oily residue

(crude product) was left which resisted numerous attempts at crys-

tallization. Some success seemed likely when the residue was dis-

solved in alcohol and ether was added. Crystals formed but when

filtered, the crystals turned back to oil. Some crystals formed from

water-acetone.   The product is very soluble in water and alcohol and
insoluble in other organic solvents.

Infrared absorption bands appeared at 1740 (ester, C=O), 1670

(amide I, $-\overset{O}{\overset{\|}{C}}-NH$), 1580 (amide II, $-\overset{O}{\overset{\|}{C}}-NH$), 1190 and 1050 cm^{-1}

(sulfoxide, S=O).

             Yield = 3.0 g   % Yield = 83%
             (crude product)

SUMMARY

Although the synthesis of the desired potential glutamine antagonist was not completed, much knowledge was gained in working with these compounds that warrants further investigation.

The most encouraging results occurred when the imine combined with cysteine ethyl ester hydrobromide to give S-(1-benzamido-2,2,2-trifluoroethyl)cysteine ethyl ester. Partial oxidation of this compound gave the corresponding sulfoxide which represents a potential glutamine antagonist. These compounds could be used to investigate theories about the mechanism of the amidotransferase catalyzed reactions. They may also be able to show what kind of bonding sites exist at and near the active site of the amidotransferase enzyme. This type of investigation may lead directly to an antimetabolite which is selectively active against tumor cells.

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