

278221

THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.
900 THIRD AVENUE
NEW YORK, N. Y. 10022

Application for Renewal of Research Grant

First Renewal ☒

Second Renewal ☐

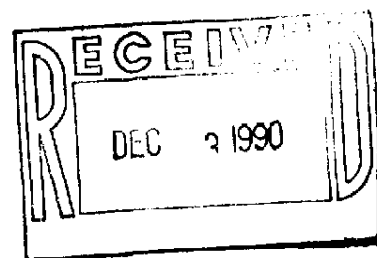
Date: 1 November 1990

1. Principal Investigator (Name, degree(s) and academic or professional title):

David W. Christianson, Ph.D., Assistant Professor of Chemistry

2. Institution and address:

University of Pennsylvania
231 South 34th Street
Philadelphia, PA 19104-6323



3. Department(s) where research will be done and/or collaboration provided;

Chemistry

4. Title of original study:

X-ray Studies of Novel Elastase-Inhibitor Complexes

5. Proposed starting date:

1 July 1991

6. How results have changed earlier specific research aims:

The specific aims of the proposal are unchanged

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7. How results to date have changed earlier working hypothesis:

The working hypothesis of the proposal is unchanged.

2782R1

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900 THIRD AVENUE
NEW YORK, N. Y. 10022

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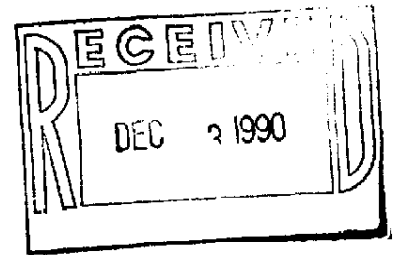
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The specific aims of the proposal are unchanged.

7. How results to date have changed earlier working hypothesis:

The working hypothesis of the proposal is unchanged.

8. Any additional facilities now required? Describe briefly:

No.

9. Any changes in personnel? Append biographical sketches of new key personnel:

No.

10. List publications or papers in press resulting from this or closely related work. See instructions concerning appending reprints and/or manuscripts.

None.

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11. Outline of experimental protocol for ensuing year.

This research concerns bioorganic and biostructural studies of both small-molecule and protein inhibitors of serine proteases. These inhibitors are of special interest since the inhibition of elastase, a serine protease, comprises a possible route toward emphysema therapy. Our support for this work by the Council for Tobacco Research began on 1 July 1990.

Our experimental protocol for the ensuing year in the small-molecule inhibitor project is as follows:

1. Complete the synthesis of the trihalo inhibitors $\text{Ac-Ala-Ala-Pro-NHC}(\text{CH}_3)\text{CX}_3$, where $\text{X} = \text{F}$ and Cl .
2. Evaluate the inhibition constant K_i for each inhibitor against elastase in solution.
3. Prepare each enzyme-inhibitor complex in the crystal, and determine the molecular structure of each complex by standard X-ray crystallographic methods.
4. Begin the synthesis of dihalo inhibitors $\text{Ac-Ala-Ala-Pro-NHC}(\text{CH}_3)\text{CHX}_2$, where $\text{X} = \text{F}$ and Cl . The only new chemistry required here will be the synthesis of the dihalo amino acid analogue, which can then be coupled via standard DCC chemistry with our stockpiled $\text{Ac-Ala-Ala-Pro-CO}_2^-$.

Our experimental protocol for the ensuing year in the X-ray crystallographic study of antichymotrypsin is as follows:

1. Determine the unit cell parameters and space group of the new crystal form of antichymotrypsin. This crystal form is novel and presents an important advantage to us since these crystals can be prepared within 1-2 weeks. The pyramidal crystal form reported in the first application to the Council takes 6-7 months to grow, so the scientific effort of crystal structure determination will certainly be advantaged by a more-readily grown crystal form of the antiprotease. If the quality of this crystal form is reproducibly good, the search for heavy atom derivatives will commence. Additionally, a structure solution may be sought via the molecular replacement method using the structure of the (non-inhibitory) serpin ovalbumin [Stein et al. (1990) Nature 347, 99] as a search probe.
2. Improve the crystal size and quality of the potential chymotrypsin-antichymotrypsin complex. Within the year, we should be able to determine the unit cell parameters of this crystal form. Additionally, analytical biochemical methods will confirm that both proteins are present with 1:1 stoichiometry in redissolved crystals. This confirmation will be an important control experiment, since we want to be sure that neither protein selectively crystallized from the solution containing both proteins.

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12. Brief summary of progress to date. Use only space provided on this page.

Grant Number 2782 PI Name CHRISTIANSON

This research concerns bioorganic and biostructural studies of both small-molecule and protein inhibitors of serine proteases. The serine protease of immediate interest is elastase, where inhibition in vivo comprises a possible route toward emphysema therapy.

In our small-molecule elastase inhibitor studies, we report that the synthesis of the halogenated inhibitors outlined in the original grant proposal is currently underway. We are synthesizing the peptidyl fragment common to the elastase inhibitors, $\text{Ac-Ala-Ala-Pro-CO}_2^-$, by standard solid-phase methods. It is our intention to "stockpile" an ample amount of this reagent in order to allow for coupling with the various halogenated amino acid analogues of interest. Our first halogenated amino acid analogues of interest in these coupling experiments are the trihalo species where the CF_3 and CCl_3 groups replace the carboxylate group of the amino acid, and we have outlined the synthesis of these analogues in the appendix material to the original proposal. We are currently embarking on the synthesis of these halogenated amino acid analogues, and we will ultimately exploit standard DCC-coupling chemistry to generate the amide linkage between our stockpiled $\text{Ac-Ala-Ala-Pro-CO}_2^-$ and the amino acid analogue.

Subsequent to the synthesis and evaluation of the inhibitory potency of these compounds, their co-complexes with elastase in the crystal will be prepared. Finally, the di- and mono-halo derivatives will be prepared and assayed in solution and in the crystal.

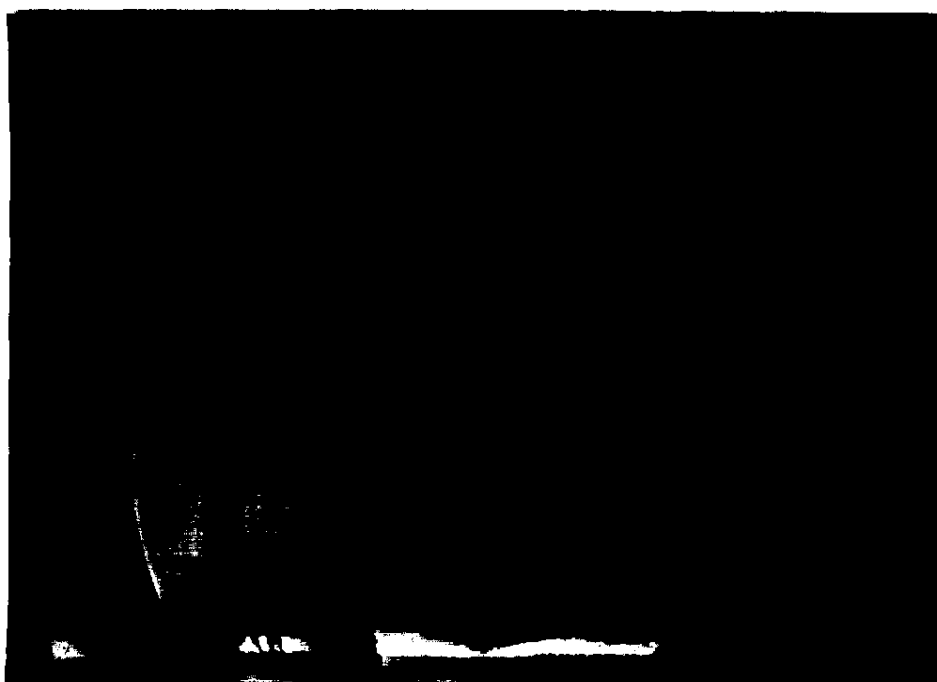
In our studies of large-molecule (i.e., protein) inhibitors of serine proteases, we now report that we have obtained a different crystal form of antichymotrypsin which is obtained after crystallization wells sit for about 1-2 weeks. Of course, this presents a tremendous advantage over those crystals which take 6-7 months to form (discussed in the original proposal), so we are exploring the diffraction qualities of this new crystal form of the antiprotease. Although we have not definitively established the space group of this new crystal form, we find that it diffracts to about 5 Å resolution in one crystal orientation in the X-ray beam, and to about 2.5 Å resolution in a perpendicular orientation. The crystal orientation which yields the higher resolution diffraction is one in which the incident X-ray beam is parallel to the long axis of the parallelepipedon-shaped crystal, so we are currently exploring crystallization conditions which will give rise to larger crystals which will diffract to higher resolution in all orientations.

Additionally, we have recently obtained small crystals of what may be the chymotrypsin-antichymotrypsin complex (we have not yet confirmed this by chemical methods). These crystals are precipitated from a 1:1 molar ratio of protease and inhibitor by polyethylene glycol.

Xeroxed photographs of these new crystals are found on the next page; original photographs are included in the original renewal application.

END OF PROGRESS SUMMARY

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New crystal form of antichymotrypsin



Crystals of a possible chymotrypsin-antichymotrypsin complex

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

GRANT NO. 2782R1

PI NAME: D.W. Christianson

13. Budget for the coming year:

% time

Amount

A. Salaries (give names or state "to be recruited")

Professional (give % time of investigator(s)
even if no salary is requested)

David W. Christianson (P.I.; 1 month)

12

\$5,000

Anzhi Wei (postdoctoral fellow)

100

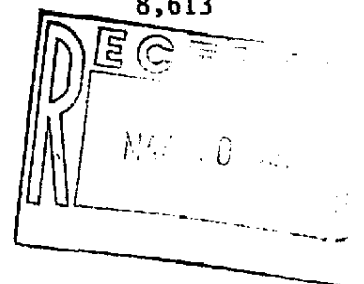
24,000

Employee benefits @ 29.7%

8,613

Technical

OK
D#7



Sub-total for A 37,613

B. Consumable supplies (by major category)

Chemicals, Solvents, photographic supplies

Sub-total for B 1,500

C. Other expenses (itemize)

Travel 1,115

Instrumentation Services 3,250

Sub-total for C 4,365

Running total of A + B + C 43,478

D. Permanent equipment (itemize)

Sub-total for D 0

E. Indirect costs (15% of A+B+C)

E 6,522

Total request 50,000

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14. Estimated future requirements (if applicable):

	<u>Salaries</u>	<u>Consumables</u>	<u>Other exps</u>	<u>Perm equip</u>	<u>Indirect costs</u>	<u>Total</u>
Year 3	39,414	1,575	4,583	-0-	6,848	52,420

*The use of CTR funds for the purchase of permanent equipment in the terminal year of a grant is not permitted.

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THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.

GRANT NO. 2782R1
PI NAME: D.W. Christianson

13. Budget for the coming year:

% time Amount

A. Salaries (give names or state "to be recruited")

Professional (give % time of investigator(s)
even if no salary is requested)David W. Christianson (P.I.; 1 month)
Anzhi Wei (postdoctoral fellow)
Employee benefits @ 29.7%12 \$5,000
100 24,000
8,613TechnicalSub-total for A 37,613

B. Consumable supplies (by major category)

Chemicals, Solvents, photographic supplies

Sub-total for B 1,500

C. Other expenses (itemize)

Travel 1,115
Instrumentation Services 3,250Sub-total for C 4,365Running total of A + B + C 43,478

D. Permanent equipment (itemize)

Sub-total for D 0

E. Indirect costs (15% of A+B+C)

E 6,522Total request 50,000R
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	<u>Salaries</u>	<u>Consumables</u>	<u>Other exps</u>	<u>Perm equip</u>	<u>Indirect costs</u>	<u>Total</u>
Year 3	39,414	1,575	4,583	-0-	6,848	52,420

*The use of CTR funds for the purchase of permanent equipment in the terminal year of a grant is not permitted.

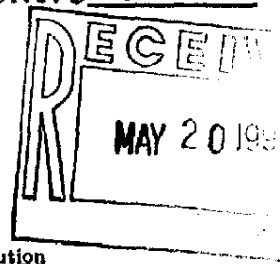
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THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.

REVISED BUDGET APPROVAL — GRANT # 2782R1 REVISED AMOUNT: \$ 50,000

It is understood that in applying for a grant the investigator and institutional officers have read and accept The Council's "Statement of Policy."



Principal Investigator

Typed Name David W. Christianson

Signature DW Christianson Date 9 May 1991

Mailing Address Dept. of Chemistry

University of Pennsylvania

Philadelphia, PA 19104-6323

Telephone (where principal investigator
can be reached during business hours):

215 898-5714

Area Code Number Extension

Responsible officer of the Institution

Typed Name SANDRA L. HOUCH

Title ASSISTANT DIRECTOR

OFFICE OF RESEARCH ADMINISTRATION

Signature UNIVERSITY OF PENNSYLVANIA

SUITE 300

Mailing Address 133 SOUTH 36TH STREET

PHILADELPHIA, PA 19104-3246

Sandra L. Houch

Telephone (215) 898-7238

Area Code Number Extension

Checks payable to:

UNIVERSITY OF PENNSYLVANIA
P.O. BOX 7777-W9535
PHILADELPHIA, PA 19175

Checks should be mailed to: (name and address of recipient)

Name and address of person responsible for providing financial information to The Council.

Name Donald J. Kearney

Address Manager, Research Accounting

327 Franklin Bldg.

3451 Walnut St.

Phila. Pa. 19104-6284

13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")

% time

Amount

Professional (give % time of investigator(s)
even if no salary is requested)

David W. Christianson (P.I.; 1 month)

12

\$ 5,000

Anzhi Wei (postdoctoral fellow)

100

24,000

Employee benefits @ 29.7%

8,613

Technical*Revised
Budget
5/27/91*Sub-total for A 37,613

B. Consumable supplies (by major category)

Chemicals, solvents

4,725

Photographic and computer supplies

1,575

Sub-total for B 6,300

C. Other expenses (itemize)

Travel

1,000

Diffractionmeter service contract

7,350

Instrumentation services

3,934

Sub-total for C 12,284Running total of A + B + C 56,197

D. Permanent equipment (itemize)

Sub-total for D 0

E. Indirect costs (15% of A+B+C)

E 8,430Total request 64,627*ok
mla*

14. Estimated future requirements (if applicable):

Salaries, supplies & other expensesPermanent equip.Indirect costsTotalYear 3: \$59,007-0-\$8,851\$67,858

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*The use of CTR funds for the purchase of permanent equipment in the terminal year of a grant is not permitted.

15. Other sources of financial support (you may continue on an additional page if necessary):

CURRENTLY ACTIVE				
List financial support from all sources, including own institution, for <u>this and related</u> research and clinical projects:				
Title of Project	Sources (give grant numbers)	Total Value of Grant	Current Annual Amount Available to You	Date of Termination of Grant
Instrumentation for Structural Biology	NSF DIR-8821184 (equipment only)	\$147,000	\$147,000	9/30/91
Biophysics of Carbon- Halogen Bonds	Searle 89-A-112	180,000	54,000	6/30/92
Structural Studies of Metalloproteins	ONR N-00014-89-J- 3037	164,400	41,000	6/30/92

Identify and describe any overlap of this application with the above grants:

None.

Indicate the total annual funds available to you this year for all research projects under your supervision: \$ 134,000

PENDING OR PLANNED				
Title of Project	Sources (give grant numbers)	Total Value of Grant	Average Annual Amount	Total Duration (Give Inclusive Dates)
Structure and Function of Carbonic Anhydrase	NIH	\$350,000	\$70,000	7/1/91- 6/30/96
Structural Studies of Proteases and their Inhibitors	Arthritis Foundation	225,000	75,000	7/1/91- 6/30/94

Identify and describe any overlap of this application with the above proposals:

This application overlaps with the proposal submitted to the Arthritis Foundation; if this renewal application is successful, the Arthritis Foundation application will be withdrawn.

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COUNCIL FOR TOBACCO RESEARCH

APPLICATION FOR RESEARCH SUPPORT—AUTHORIZING SIGNATURES

It is understood that in applying for a grant the investigator and institutional officers have read and accept The Council's "Statement of Policy."

Principal InvestigatorTyped Name David W. ChristiansonSignature DW Christianson Date 11/27/90Mailing Address Department of ChemistryUniv. of Pennsylvania, 231 S. 34th StreetPhiladelphia, PA 19104-6323Telephone (where principal investigator
can be reached during business hours):215 898-5714

Area Code Number

Extension

Responsible officer of the InstitutionTyped Name Sandra L. HouckTitle Assistant DirectorSignature Sandra L. Houck Date 11/29/90Mailing Address Sandra L. HouckAssistant DirectorOffice of Research AdministrationSuite 300133 South 26th StreetUniversity of Pennsylvania

Telephone

Philadelphia, PA 19104-3246(215) 898-7293

Area Code Number

Extension

(215) 898-7293Checks payable to: The Trustees of The University of PennsylvaniaTHE TRUSTEES OF THE
UNIVERSITY OF PENNSYLVANIA

Checks should be mailed to: (name and address of recipient)

UNIVERSITY OF PENNSYLVANIA
P.O. BOX 7777-W9535
PHILADELPHIA, PA 19178

Name and address of person responsible for providing financial information to The Council.

Name SAME AS ABOVE

Address _____

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