

08:53:29

OCA PAD INITIATION - PROJECT HEADER INFORMATION

04/25/88

Active

Project #: G-41-679  
Center #: R6481-OA0

Cost share #: G-41-372  
Center shr #: F6481-OA0

Rev #: 0  
OCA file #:  
Work type : RES  
Document : GRANT  
Contract entity: GTRC

Contract#: 1 R29 GM39779-01  
Prime #:

Mod #:

Subprojects ? : N  
Main project #:

Project unit: PHYSICS Unit code: 02.010.152  
Project director(s): HUANG T-H ICS

Sponsor/division names: DHHS/PHS/NIH / NATL INSTITUTES OF HEALTH  
Sponsor/division codes: 108 / 001

Award period: 880401 to 890331 (performance) 890630 (reports)

Sponsor amount	New this change	Total to date
Contract value	95,866.00	95,866.00
Funded	95,866.00	95,866.00
Cost sharing amount		17,211.00

Does subcontracting plan apply ? : N

Title: STRUCTURE, DYNAMICS AND FUNCTION OF DIHYDROFOLATE REDUCTASE

PROJECT ADMINISTRATION DATA

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Security class (U,C,S,TS) :  
Defense priority rating :  
Equipment title vests with: Sponsor

ONR resident rep. is ACO (Y/N): N  
supplemental sheet  
GIT X

Administrative comments -  
INITIATION





9-71-679

SECTION IV PROGRESS REPORT SUMMARY		GRANT NUMBER 1R29GM39779-01	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Huang, Tai-huang		PERIOD COVERED BY THIS REPORT	
APPLICANT ORGANIZATION Georgia Institute of Technology		FROM April 1, 1988	THROUGH March 31, 1989
TITLE OF PROJECT (Repeat title shown in item 1 on first page) Structure, Dynamics and Function of Dihydrofolate Reductase (SEE INSTRUCTIONS)			

1. Plans for next year : no change.

2. Studies conducted during the current year :

This is the first year of this new project. Substantial effort was devoted to train new personnels ( one post doctoral fellow and two graduate students ) and initial setup. In addition the following tasks were accomplished:

(i) Worked out detailed procedures for isolating two types of dihydrofolate reductases (DHFR) from E. coli.

Approximately 200mg of E. coli chromosomal DHFR were isolated from 200g cells using methotrexate(MTX) and DEAE columns. MTX column was prepared by first couple diaminoethyl arm to the sepharose column. MTX was then attached to the arm group. 20 grams of MTX-sepharose resin was prepared. DEAE column was used to wash out protein-bound folic acid which was used to elute out DHFR from the affinity column. Scale up production for the R67 plasmid DHFR proved to be more difficult and time consuming because of the need to go through four columns. It takes roughly one month to process through one batch. At present we have 100mg of this protein. The poor solubility of both dihydrofolate and trimethoprim (TMP) also cause some problem in protein assay and cell growth. All of this has been resolved. Along the way we have purchased several equipments which facilitate the isolation process. These include a sonicator, a tangential flow concentrator, a freez-dryer and a UV detector system.

(ii) Isotopic labelling of TMP and NADP<sup>+</sup>:

We have synthesized one gram of (3',4'-d<sub>6</sub>)TMP. This was accomplished by converting 5-bromovanillin to syringaldehyde-d<sub>3</sub> with CD<sub>3</sub>ONa in the presence of a copper catalyst. The deuterated syringaldehyde was methylated with dimethyl sulfate d<sub>6</sub> to yield the hexadeuterated 3,4,5-trimethoxybenzaldehyde. Reaction of the 3,4,5-trimethoxybenzaldehyde with Beta anilinoacrylonitrile produced the aldol condensation product which was converted to hexadeutero trimethoprim by reaction with guanidine HCl. This compound will be bound to DHFR for study.

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(SEE INSTRUCTIONS)		

Labelling of NADP<sup>+</sup> and NADPH proved to be harder. High temperature H-D exchange method caused decomposition of NADP<sup>+</sup> and NADPH. A modified method using activated platinum in the presence of deuterium gas resulted in a complete exchange of C<sub>2</sub>, C<sub>4</sub>, C<sub>5</sub> and C<sub>6</sub> proton as determined by proton NMR. However, approximately 20 % of the pyridyl ring was reduced. We are still experimenting with exchange conditions and purification method to optimize the procedure.

(iii) NMR spectroscopy :

(a) Solution conformation of NADPH :

In order to study the conformation of NADPH in DHFR complexes we decided to determine the solution conformation of NADPH and to verify the resonance assignments by 2-D NMR. 2-D COSY experiments confirmed the previous assignments in the literature (Fig. 1 in Appendix). However the improved resolution at 400 and 500 MHz also allowed us to assign more resonances which were not resolved at low field reported in the literature. The conformation deduced from our data did not agree totally with that reported in the literature. Specifically 2-D NOE data suggested that the sugar moiety is 2'-exo for adenyly moiety and 2'-endo for the pyridyl moiety at 30°C. There seems to be a temperature dependent conformational change. We are currently conducting time dependent NOE in order to quantify the internuclear distances.

(b) Determination of C<sup>13</sup> tensors of trimethoprim :

We have constructed an automated single crystal probe for determining tensor orientation using fast switching stepper motor which is under the direct control of the spectrometer pulse programmer. Fig. 2 shows some representative spectra. We are currently analyzing these data. However the complexity of the molecule and the broad linewidth present some ambiguities in determining the rotation plot. We will perform 2-D exchange experiments of Grant et al in order to resolve these ambiguities. The newly constructed probe is capable of performing these experiments.

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(c) Dynamics of trimethoprim :

Fig. 3 shows the temperature dependent deuterium NMR spectra of amorphous and polycrystalline (3',4'-d<sub>6</sub>)TMP. The breadth of the spectra confirms the presence of the well known methyl group rotation. No evidence of large amplitude benzyl ring motion can be seen. No difference between the amorphous and polycrystalline material can be found.

3. Human subjects : Not applicable.

4. Vertebrate animals : Not applicable.

5. Publications : No work related to this project has been published. However we are preparing one manuscript on the conformation of NADPH in solution.

CHECKLIST  
INDIRECT COST CALCULATION

GRANT NUMBER  
1R29GM39779-01

Check the appropriate boxes and provide the information requested. Make this page the last page of the signed original of the application. Do not attach copies of this page to the duplicated copies of the application.

Indicate the applicant organization's most recent indirect cost rate established with the appropriate DHHS Regional Office, or, in the case of for-profit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office. Indirect costs will not be paid on foreign grants, construction grants, grants to Federal organizations and grants to individuals, and usually not on conference grants. Follow any additional instructions provided for Research Career Development Awards, Institutional National Research Service Awards, and specialized grant applications.

DHHS Agreement Dated: \_\_\_\_\_  No Indirect Costs Requested  
 No DHHS Agreement, but rates established with ON R DATE 7/1/88

\*CALCULATION

Enter proposed budget period:

Amount of Base \$ 66,160 × Rate Applied \*60.0 % = Indirect Costs \$ 39,696  
Add to total direct costs from page 2 and enter new total on FACE PAGE, Item 10b

\*Check appropriate box(es)

Salary and wage base  Modified total direct costs base  Other base (Explain below)  
 Off-site, other special rate, or more than one rate involved (Explain below)

Explanation (Attach separate sheet, if necessary)

\* use site 02 in NIH database. Resident instruction and all other units of Georgia Tech.

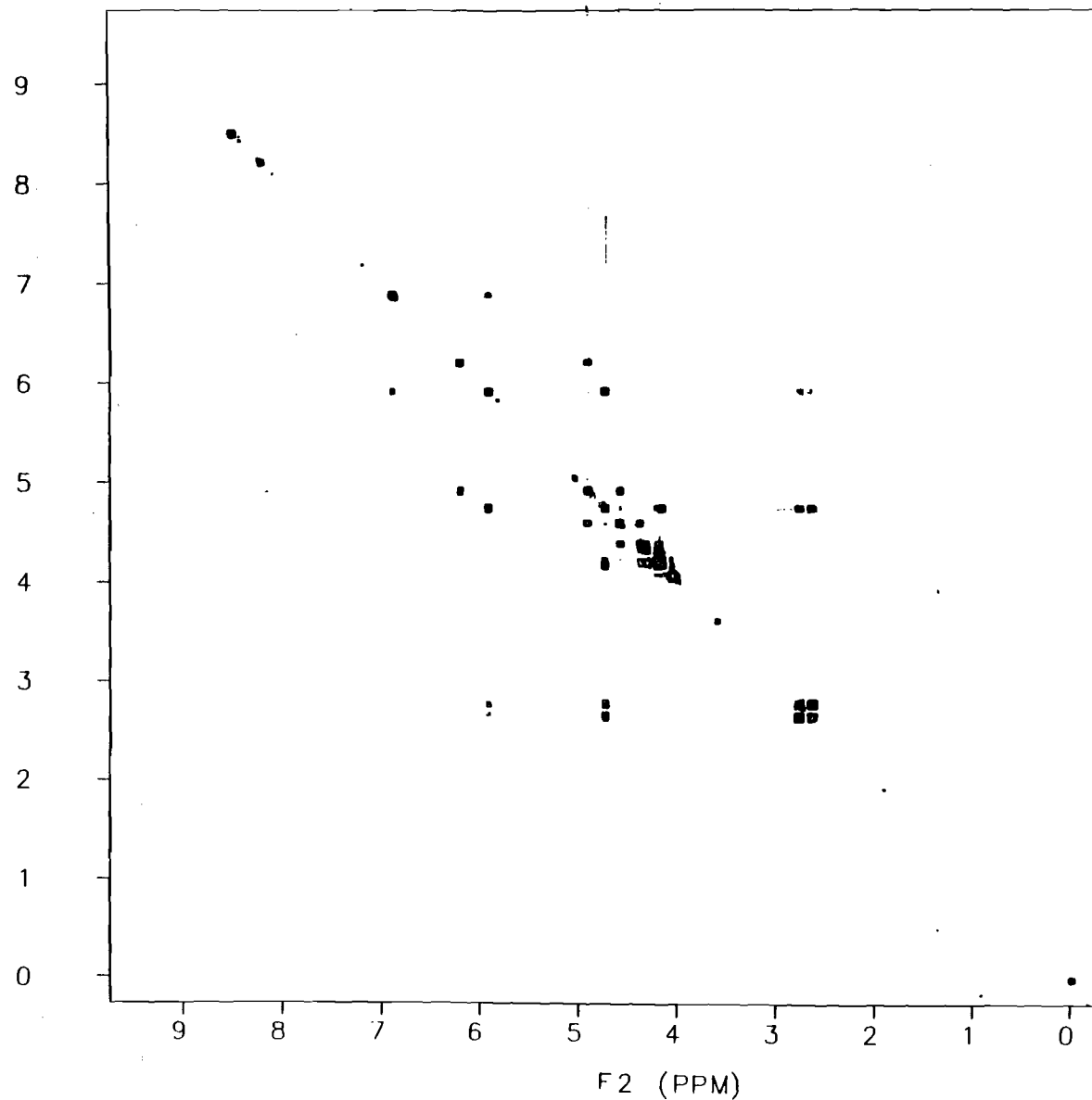
Appendices:

Fig. 1. 2-D NMR spectra of NADPH

Fig. 2. Rotational variation of the  $^{13}\text{C}$  NMR spectra of single crystal TMP

Fig. 3. Deuterium NMR spectra of (3', 4', - d6) TMP at various temperatures.

F1 (PPM)



COSY 5°C

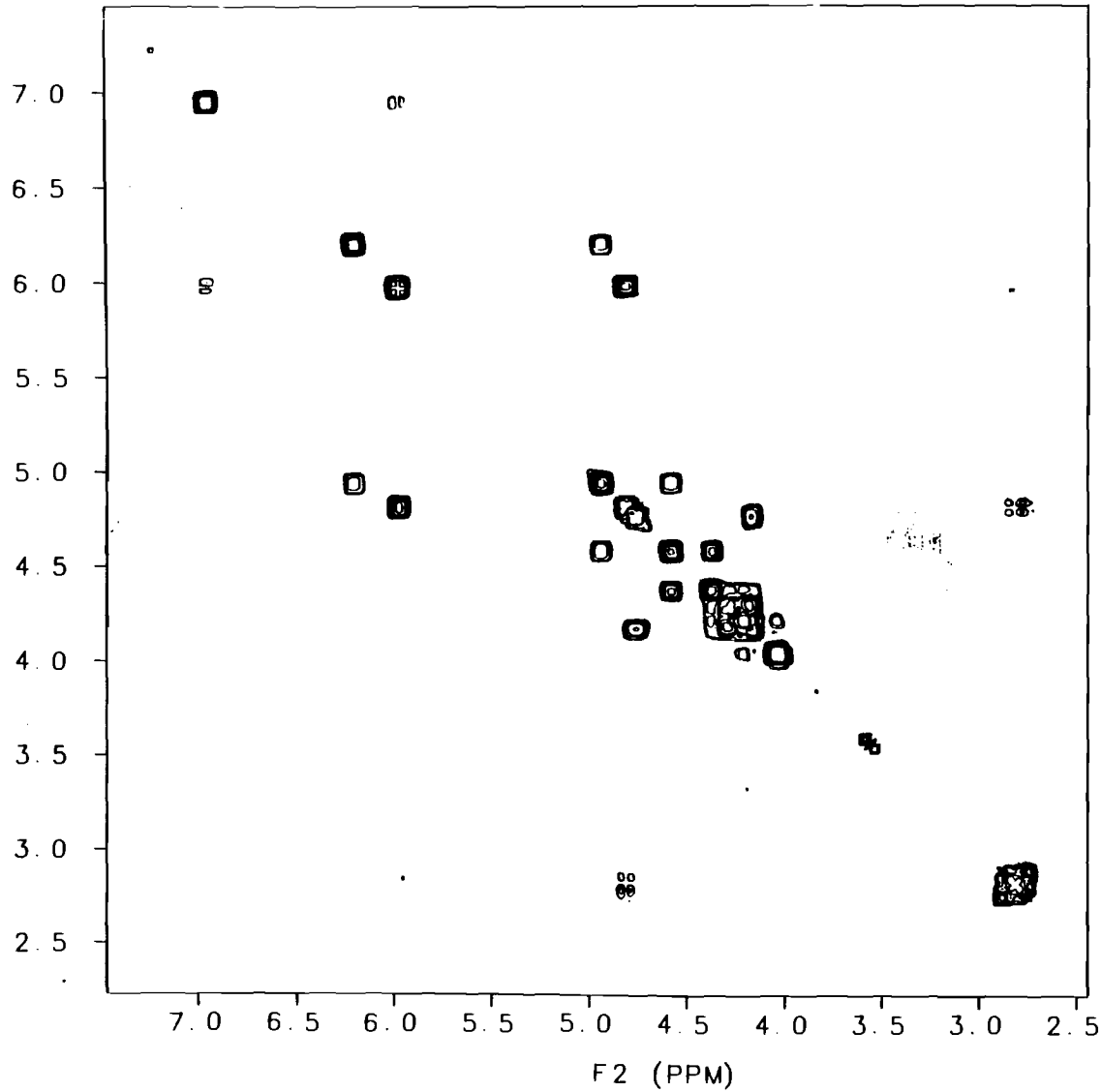
NADPH AT 5 DEG PH=9.1?

EXP5 PULSE SEQUENCE: COSY  
 DATE 10-19-88  
 SOLVENT D2O  
 FILE H10192

ACQUISITION		DEC. & VT	
TN	1.500	DN	1.500
SW	4000.0	DO	-481.0
AT	0.256	DM	YNN
NP	2048	DLP	
PW	14.5		
P1	14.5	PROCESSING	
D1	2.000	RE	0.016
D2	0	FN	2048
TO	-600	AF	0.064
NT	16	MATH	F
CT	16	FN2	2048
TEMP	0	RE2	0.004
PW90	14.5	AF2	0.016
SW2	4000.0		
NI	256	DISPLAY	
D3	0	SP	-105.6
BS	16	WP	4000.0
SS	2	VS	10
IL	N	SP2	-105.6
IN	N	WP2	4000.0
DP	Y	SC	15
HS	NN	WC	200
GAIN	0	IS	831
		RFL	105.5
		RFP	0
		TH	-
		SC2	1
		WC2	200
		INS	1.000
		RFL2	105.5
		RFP2	0
		AI	DC
			AV

Fig. 1a. 2-D COSY spectrum of NADPH at 5°C

F1 (PPM)



COSY 30°C

NADPH AT 30 DEG PH=9.12

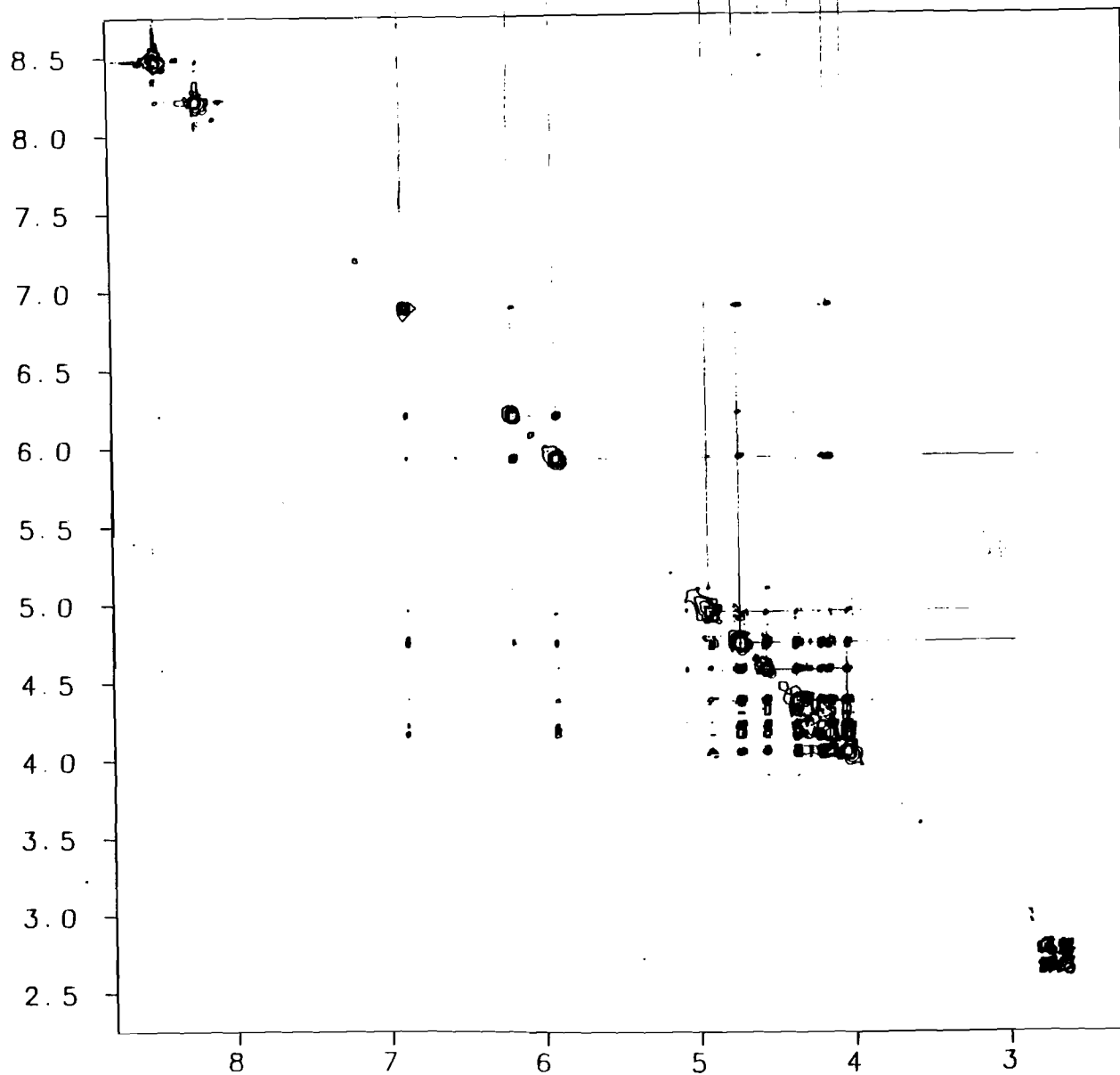
EXPT5 PULSE SEQUENCE: COSY  
 DATE 10-11-88  
 SOLVENT D2O  
 FILE H10112

ACQUISITION		DEC. & VT	
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SW	4000.0	DO	-477.6
AT	0.256	DM	YNN
NP	2048	DLP	20
PW	14.5		
P1	14.5	PROCESSING	
D1	3.000	RE	0.0
D2	0	FN	2048
TO	-600	AF	0.030
NT	16	MATH	F
CT	16	FN2	2048
TEMP	30.0	RE2	0.005
PW90	36.0	AF2	0.017
SW2	4000.0		
NI	256	DISPLAY	
D3	0	SP	977.5
BS	16	WP	2005.9
SS	2	VS	100
IL	N	SP2	891.5
IN	N	WP2	2091.9
DP	Y	SC	15
HS	NN	WC	200
		IS	1191
		RFL	239.9
		RFP	0
		TH	7
		SC2	135
		WC2	200
		INS	1.0
		RFL2	239.9
		RFP2	0
		AI	DC
			AV

Fig. 16: 2-D COSY spectrum of NADPH at 30°C

NOESY 5°C

F1 (PPM)



F2 (PPM)

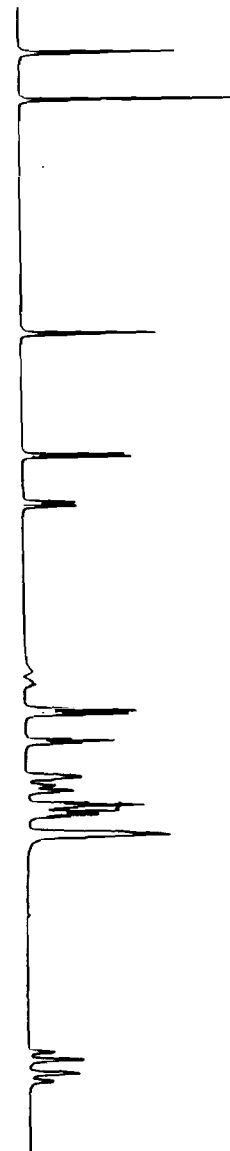


Fig. 1c : 2-D NOE  
spectrum of NADPH  
at 5°C

NMR 30°C

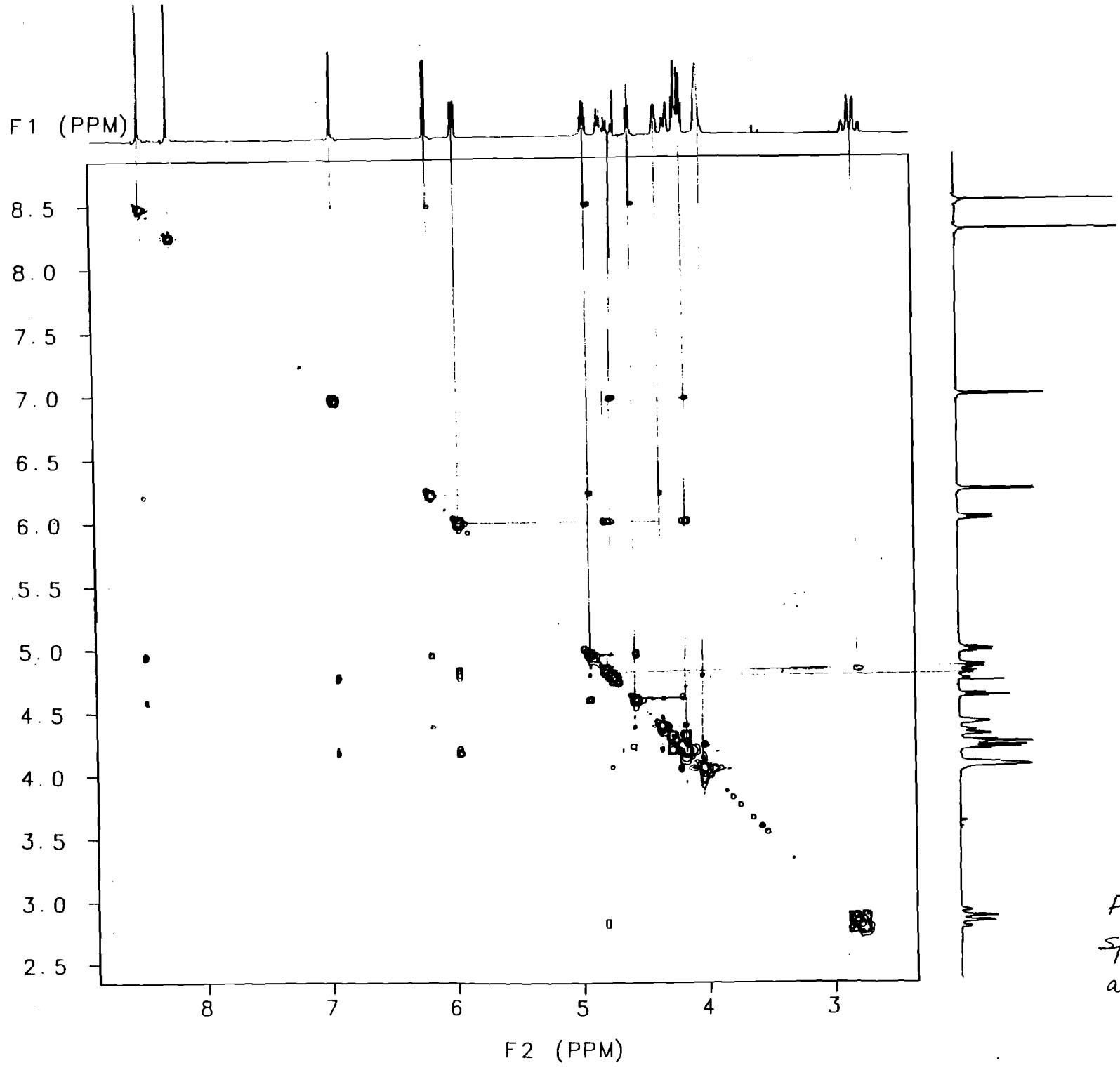


Fig. 1d: 2-D NDE spectrum of NADPH at 30°C

Fig. 2. Rotational variation of the  $^{13}\text{C}$  NMR spectra of single crystal TMP

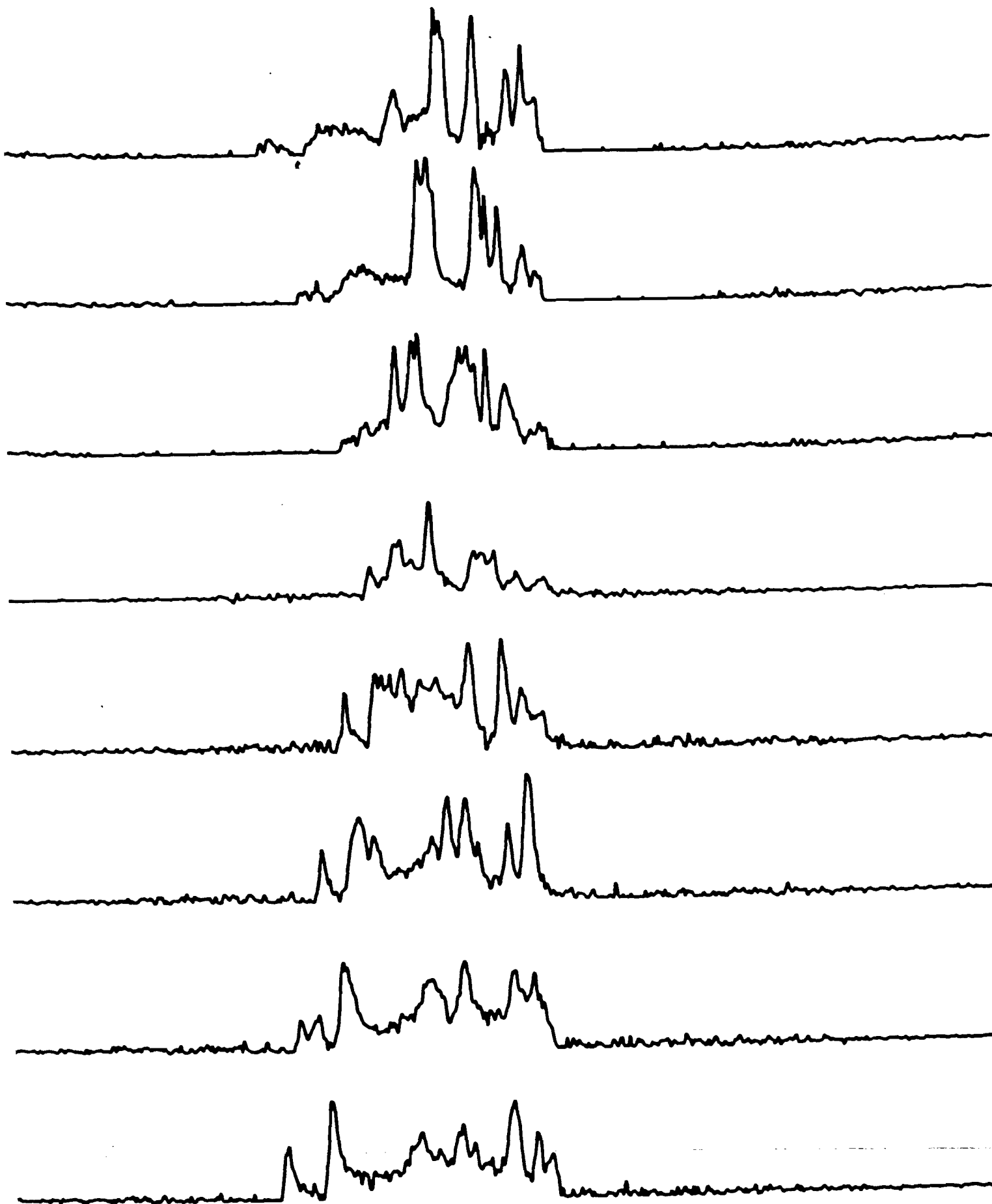


Fig. 3. Deuterium NMR spectra of (3', 4', - d6) TMP at various temperatures.

