

## Synthesis and Biological evaluation of histone deacetylase inhibitors that are based on FR235222: a cyclic tetrapeptide scaffold.

Erinprit K. Singh,<sup>a</sup> Suchitra Ravula,<sup>a</sup> Chung-Mao Pan,<sup>a</sup> Po-Shen Pan,<sup>a</sup> Robert C. Vasko,<sup>a</sup> Stephanie A. Lopera,<sup>a</sup> Sujith V.W. Weerasinghe,<sup>b</sup> Mary Kay H. Pflum,<sup>b</sup> and Shelli R. McAlpine<sup>a,\*</sup>

\*Department of Chemistry and Biochemistry, San Diego State University, 5500 Campanile Drive, San Diego, CA 92182-1030

### Contents:

Title page	S1
Experimental Procedures	S2
Table of compounds with text describing structural variations	S7
Spectral Data	
<i>Compound 1 NMR Dipeptide 1a2a</i>	S8
<i>Compound 1 NMR Dipeptide 3a4a</i>	S9
<i>Compound 1 NMR Linear tetramer 1a2a3a4a</i>	S10
<i>Compound 1 NMR Cyclized protected 1a2a3a4a</i>	S11
<i>Compound 1 NMR Cyclized 1a2a3a4a</i>	S12
<i>Compound 2 NMR Dipeptide 1b2a</i>	S13
<i>Compound 2 NMR Dipeptide 3a4a</i>	S14
<i>Compound 2 NMR Linear tetramer 1b2a3a4a</i>	S15
<i>Compound 2 NMR Cyclized protected 1b2a3a4a</i>	S16
<i>Compound 2 NMR Cyclized 1b2a3a4a</i>	S17
<i>Compound 2 LCMS Cyclized 1b2a3a4a</i>	S18
<i>Compound 3 NMR Dipeptide 1c2a</i>	S19
<i>Compound 3 NMR Dipeptide 3a4a</i>	S20
<i>Compound 3 NMR Linear tetramer 1c2a3a4a</i>	S21
<i>Compound 3 NMR Cyclized protected 1c2a3a4a</i>	S22
<i>Compound 3 NMR Cyclized 1c2a3a4a</i>	S23
<i>Compound 3 LCMS Cyclized 1c2a3a4a</i>	S24
<i>Compound 4 NMR Dipeptide 1b2a</i>	S25
<i>Compound 4 NMR Dipeptide 3a4b</i>	S26
<i>Compound 4 NMR Linear tetramer 1b2a3a4b</i>	S27
<i>Compound 4 NMR Cyclized protected 1b2a3a4b</i>	S28
<i>Compound 4 NMR Cyclized 1b2a3a4b</i>	S29
<i>Compound 4 LCMS Cyclized 1b2a3a4b</i>	S30
<i>Compound 5 NMR Dipeptide 1a2a</i>	S31
<i>Compound 5 NMR Dipeptide 3b4a</i>	S32
<i>Compound 5 NMR Linear tetramer 1a2a3b4a</i>	S33
<i>Compound 5 NMR Cyclized 1a2a3b4a</i>	S34
<i>Compound 5 LCMS Cyclized 1a2a3b4a</i>	S35
<i>Compound 6 NMR Dipeptide 1d2a</i>	S36
<i>Compound 6 NMR Dipeptide 3b4a</i>	S37
<i>Compound 6 NMR Linear tetramer 1d2a3b4a</i>	S38

## EXPERIMENTAL PROCEDURE

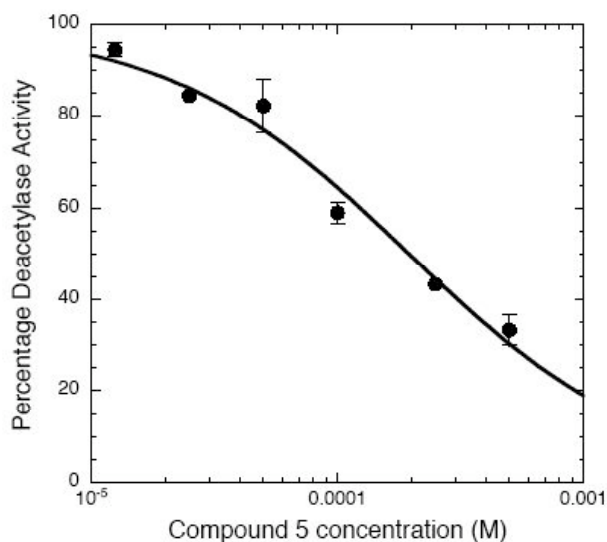
### Thymidine Uptake Assays

Proliferation of the HCT-116, HCT-15 colon cancer and WS-1 normal cell lines were tested in the presence and absence of the compounds using  $^3\text{H}$ -thymidine uptake assays. Cells treated with the compounds were compared to DMSO-treated controls for their ability to proliferate as indicated by the incorporation of  $^3\text{H}$ -thymidine into their DNA. Cells were cultured in 96 well plates at a concentration of 3,000 cells/well. The media was IMDM for HCT-116, RPMI 1640 for HCT-15 and MEM for WS-1 with L-glutamine, 10% fetal bovine serum and 0.1% penicillin-streptomycin antibiotics. After incubation for approximately 6 hours, the compounds were added. The compounds were dissolved in DMSO at a final concentration of 2 mM and tested at the concentration indicated in the manuscript. The DMSO concentration was held constant in all wells at 1%. After the cells had been incubated with the compounds for 56 hours, 1mCi  $^3\text{H}$ -thymidine per well was added and the cells were cultured for an additional 16 hours (for the cells to have a total of 72 hours with the drug), at which time the cells were harvested using a PHD cell harvester from Cambridge Technology Incorporated. The samples were then counted in a scintillation counter for 2 minutes each using ScintiVerse universal scintillation fluid from Fisher. Decreases in  $^3\text{H}$ -thymidine incorporation, as compared to controls, are an indication that the cells are no longer progressing through the cell cycle or synthesizing DNA, as is shown in the studies presented.

### HDAC Assays

The HDAC activity was measured using Fluor de Lys<sup>TM</sup> activity assay (Biomol). Briefly, HeLa lysates (25  $\mu\text{L}$ ) were incubated with or without the small molecule inhibitor for 30 min at 30  $^{\circ}\text{C}$  with shaking. Fluor de Lys substrate (25  $\mu\text{L}$ , 100  $\mu\text{M}$ ) was added and the reaction mixture was incubated at 37  $^{\circ}\text{C}$  for 45 min with shaking. Fluor de Lys developer (50  $\mu\text{L}$  of 1x) was added and incubated with shaking for 5 min. The fluorescence intensity was determined at 465 nm using a Genios Fluorimeter (Tecan). The deacetylase activity was determined by dividing the fluorescence intensity of the reaction in the presence of FR235222 derivative with the intensity in the absence of inhibitor. At least three determinations were used to calculate the mean and standard error in Figure 3.

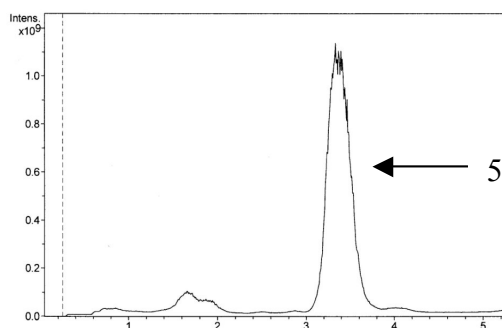
### IC<sub>50</sub> value of compound 5 in deacetylase assay.



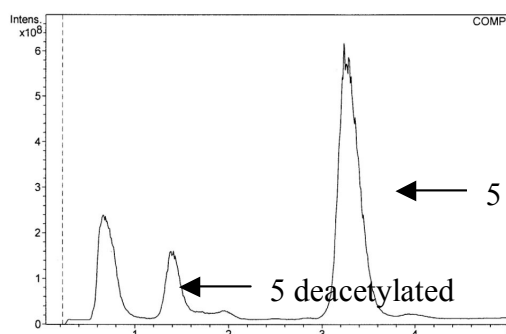
Determined IC<sub>50</sub> value for this compound was 196 ± 22  $\mu\text{M}$ .

### Deacetylase Reaction of HDACs

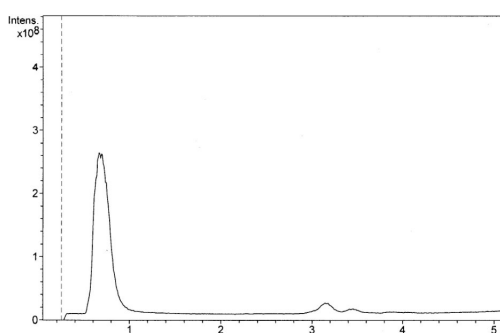
HeLa lysates (25  $\mu$ L) were incubated with or without compound 5 inhibitor for 60 min at 30  $^{\circ}$ C with shaking. TSA (1  $\mu$ M) was then added to the reaction to stop deacetylation. A second identical reaction was incubated for 60 minutes at 30 $^{\circ}$ C with shaking, following by room temperature incubation for  $\sim$ 24 hours before analysis. The amount of deacetylated compound 5 was determined using LC-MS by monitoring the peak intensity of the acetylated compound. The deacetylated compound was found in both lysate containing reactions (c and d in figure below). However, longer incubation does not lead to complete deacetylation (see figure below).



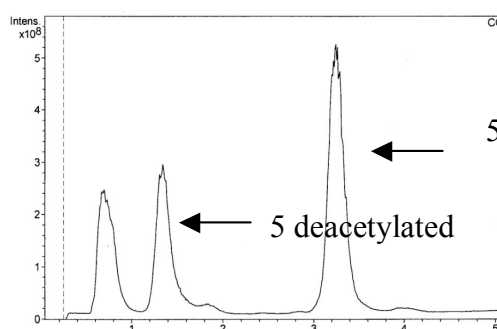
a) compound 5 only



c) compound 5 and lysate incubated 60 mins



b) lysate only



d) compound 5 and lysate incubated 24 hours

a) compound without lysate b) lysate only c) compound with lysate and stopped in 60 minutes with TSA d) compound with lysate run for  $\sim$ 24 hours

**General peptide synthesis.** All peptide coupling reactions were carried out under argon with dry solvent, using methylene chloride for dipeptide and tripeptide couplings and acetonitrile for tetrapeptide couplings. The amine (1.1 equivalents) and acid (1 equivalent) were weighed into a dry flask along with 3-6 equivalents of DIPEA and 1.2 equivalents of TBTU.\* Anhydrous methylene chloride was added to generate a 0.1M solution. The solution was stirred at room temperature and reactions were monitored by TLC. Reactions were run for 1 hour before checking via TLC. If reaction was not complete additional 0.25 equivalents were of HATU and TBTU were added. If reaction was complete then work-up was done by washing with saturated ammonium chloride. (Note: if acetonitrile was used for the reaction, methylene chloride was added to reaction upon workup and the resulting solution was washed with ammonium chloride). After back extraction of aqueous layers with methylene chloride, organic layers were combined, dried over sodium sulfate, filtered and concentrated. Acid/Base wash was used as a primary method of purification. The concentrated product was dissolved in 200mL ethyl acetate, washed with 100mL 10% HCl solution (x2), 100mL saturated sodium bicarbonate solution (x10), and 100mL saturated sodium chloride solution (x2). The product was then dried over sodium sulfate, filtered and concentrated. A secondary method of purification, flash chromatography, was performed using a gradient of ethyl acetate-hexane gave our desired peptide.

\* Some coupling reactions would not go to completion using only TBTU and therefore ~0.25 equivalents of HATU, and/or DEPBT were used. In a few cases up to 1.2 equivalents of all three coupling reagents were used.

**General Amine deprotection.** Amines were deprotected using 20% TFA in methylene chloride (0.1M) with two equivalents of anisole. The reactions were monitored by TLC, where the TLC sample was first worked up in a mini-workup using DI water and methylene chloride to remove TFA. Reactions were allowed to run for 1-2 hours and then concentrated in vacuo.

**General Acid deprotection.** Acids were deprotected using ~4 equivalents of lithium hydroxide (or until pH~11) in methanol (0.1 M). The peptide was placed in a flask, along with lithium hydroxide and methanol and stirred for about 2-3 hours for the acid to deprotect. Work-up of reactions involved the acidification of reaction solution using HCl to pH = 1. The aqueous solution was extracted three times with methylene chloride, and the combined organic layer was dried, filtered and concentrated in vacuo.

**Macrocyclization procedure (in situ).** All tetrapeptides were acid deprotected first using ~8 equivalents of lithium hydroxide (or until pH=11) in methanol (0.1M). The tetrapeptide was placed in a flask, with the lithium hydroxide and methanol stirred overnight. Within 8 hours the acid was usually deprotected. Work-up of reactions involved the acidification of reaction solution using HCl to pH=1. The aqueous solution was extracted three times with methylene chloride and the combined organic layer was dried, filtered and concentrated in vacuo. Verification of the presence of the free acid was performed via NMR and LCMS. Then, the amine of the tetrapeptide was deprotected using 25% TFA in methylene chloride (0.1M) with two equivalents of anisole. Reaction was monitored by TLC, where the TLC was first worked up in a mini-workup using DI water and methylene chloride to remove TFA. Reactions ran for 1-2 hours and then concentrated in vacuo. Verification of the presence of the free amine and free acid and disappearance of the starting linear protected pentapeptide was performed via LCMS. The crude, dry, double deprotected peptide (free acid and free amine) was then dissolved in a minimum solution of THF: acetonitrile: methylene chloride (2:2:1 ratio). Three coupling agents (DEPBT, HATU, and TBTU) were used at ~0.7 equivalents each. These coupling agents were dissolved in a calculated volume of dry 40% THF, 40% acetonitrile, and 20% methylene chloride that would give a 0.007M overall solution when included in the volume used for the deprotected peptide. The coupling agents were then added to the deprotected peptide solution. DIPEA (6 equivs or more in order to neutralize the pH) were then added to the reaction. The coupling agents are typically not very soluble in acetonitrile, which is why a combination of solvents is used.

After 1 hour, TLC and LCMS (where the LCMS sample was worked up prior to injection) indicated that a product spot was developing. The comparison R<sub>f</sub> value in the product spot on TLC was the protected linear tetrapeptide. The reactions were typically complete after 2 hours, and monitoring the starting material deprotected tetrapeptide via LCMS was the easiest method of determining completion. Upon completion, the reaction was worked up by washing with saturated ammonium chloride. After back extraction of aqueous layers with large quantities of methylene chloride, the organic layers were combined, dried, filtered and concentrated. An acid/base wash was performed, with the concentrated product dissolved in 200mL ethyl acetate, washed with 100mL 10% HCl solution (x2), 100mL saturated sodium bicarbonate solution (x10), and 100mL saturated sodium chloride solution (x2). The product was then dried over sodium sulfate, filtered and concentrated. All macrocycles were purified by initially running a crude plug of compound using an ethyl acetate/hexane gradient on silica gel, then running a column on the isolated product. Finally, when necessary reverse phase HPLC was used for additional purification using a gradient of acetonitrile and DI water with 0.1% TFA.

## Compound 1

Macrocycle 1a-2a-3a-4a (Compound 1) was synthesized following the “Macrocyclization procedure”. Utilizing 400 mg (0.51 mmols, 1.0 equivalents) of linear tetrapeptide, 540 uL (6 equivalents) of DIPEA, 116.4 mg (0.36 mmols, 0.7 equivalents) of TBTU, 138 mg (0.36 mmols, 0.7 equivalents) HATU, and 108.4 mg (0.36 mmols, 0.7 equivalents) of DEPBT. The crude reaction was purified by reverse phase-HPLC to yield the macrocycle (2.0 mg, 10% yield).

Rf: 0.5 (EtOAc: Hex 4:1)

<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ 0.8-1.0 (t, 3H), 1.4 (dd, 2H), 1.6-1.7 (m, 2H), 1.8-1.9 (m, 2H), 2.0 (m, 4H), 2.8 (m, 2H), 3.1-3.2 (d, 2H), 3.6-3.7 (m, 2H), 4.1 (m, αH), 4.2 (m, αH), 4.4 (m, αH), 4.6 (m, αH), 6.6 (m, 1H), 6.8 (m, 1H), 7.1-7.3 (m, 5H), 8.1 (m, 1H), 8.3 (m, 1H)

LCMS: m/z calcd for C<sub>24</sub>H<sub>35</sub>N<sub>7</sub>O<sub>4</sub> (M+1) = 486.28, found

### **Compound 2**

Macrocycle 1b-2a-3a-4a (Compound 2) was synthesized following the “Macrocyclization procedure”. Utilizing 361mg (0.47 mmols, 1.0 equivalents) of linear tetrapeptide precursor, 480 uL (6 equivalents) of DIPEA, 105 mg (0.33 mmols, 0.7 equivalents) of TBTU, 124 mg (0.33 mmols, 0.7 equivalents) HATU, and 98 mg (0.33 mmols, 0.7 equivalents) of DEPBT. The crude reaction was purified by reverse phase-HPLC to yield the macrocycle (1.5mg, 11% yield).

Rf: 0.5 (EtOAc:Hex 4:1)

<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ 0.8-0.9 (t, 3H), 1.2-1.3 (m, 2H), 1.5-1.6 (m, 2H), 1.8 (m, 2H), 2.0 (m, 2H), 2.2-2.3 (m, 2H), 2.7-2.8 (m, 2H), 3.0-3.1 (d, 2H), 3.5-3.6 (m, 2H), 4.1 (m, αH), 4.3 (m, αH), 4.6 (m, αH), 4.8 (m, αH), 7.1-7.3 (m, 5H)

LCMS: m/z calcd for C<sub>24</sub>H<sub>35</sub>N<sub>7</sub>O<sub>4</sub> (M+1) = 486.28, found 486.28

### **Compound 3**

Macrocycle 1b-2a-3a-4c (Compound 3) was synthesized following the “Macrocyclization procedure”. Utilizing 227.5 mg (0.29 mmols, 1.0 equivalents) of linear tetrapeptide, 303 uL (6 equivalents) of DIPEA, 65 mg (0.20 mmols, 0.7 equivalents) of TBTU, 76.9 mg (0.20 mmols, 0.7 equivalents) of HATU, and 60.5 mg

(0.20 mmols, 0.7 equivalents) of DEPBT. The crude reaction was purified by reverse phase-HPLC to yield the macrocycle (1.8 mg, 9% yield).

Rf: 0.5 (EtOAc: Hex 3:1)

$^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.9-1.0 (t, 3H), 1.3-1.4 (m, 4H), 1.5-1.6 (m, 4H), 1.9-2.0 (m, 4H), 2.8-2.9 (m, 2H), 3.0-3.1 (d, 2H), 3.5-3.6 (t, 2H), 4.2 (br, 2 $\alpha$ H), 4.4 (m, 2 $\alpha$ H), 6.7 (m, 1H), 7.0 (m, 1H), 7.2-7.3 (m, 5H),

LCMS: m/z calcd for  $\text{C}_{25}\text{H}_{37}\text{N}_7\text{O}_4$  (M+1) = 500, found 502.5.

#### Compound 4

Macrocycle 1c-2a-3a-4a (Compound 4) was synthesized following the "Macrocyclization procedure". Utilizing 514 mg (0.65 mmols, 1.0 equivalents) of linear tetrapeptide, 131  $\mu\text{L}$  (4 equivalents) of DIPEA, 105 mg (0.33 mmols, 0.5 equivalents) of TBTU, 125 mg (0.33 mmols, 0.5 equivalents) of HATU, and 98 mg (0.33 mmols, 0.5 equivalents) of DEPBT. The crude reaction was purified by reverse phase-HPLC to yield the macrocycle(2.0mg, 6% yield).

Rf: 0.5 (EtOAc: Hex 1:0)

$^1\text{H NMR}$  (500 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  0.9-1.0 (t, 3H), 1.3-1.4 (dd, 2H), 1.6 (m, 2H), 1.8 (m, 2H), 2.0-2.1 (m, 2H), 2.2 (m, 2H), 2.7 (t, 2H), 3.1 (m, 2H), 3.5 (t, 2H), 3.8 (m,  $\alpha$ H), 4.1 (m, 2 $\alpha$ H), 4.2 (s, 2H), 4.7 (m,  $\alpha$ H), 7.4-7.5 (m, 4H), 8.1 (m, 1H), 8.2 (m, 1H), 8.3 (m, 1H), 8.4 (m, 1H), 8.8 (m, 2H)

LCMS: m/z calcd for  $\text{C}_{25}\text{H}_{35}\text{N}_7\text{O}_4$  (M+1) = 498.28, found 540.7\*

\*We frequently observe a hit for plus two  $\text{Na}^+$

#### Compound 5

Macrocycle 1a-2a-3b-4a (Compound 5) was synthesized following the "Macrocyclization procedure". Utilizing 300 mg (0.59 mmols, 1.0 equivalents) of linear tetrapeptide, 620  $\mu\text{L}$  (6 equivalents) of DIPEA, 94.7 mg (0.29 mmols, 0.5 equivalents) of TBTU, 113 mg (0.29 mmols, 0.5 equivalents) of HATU, and 88.2 mg (0.29 mmols, 0.5 equivalents) of DEPBT. The crude reaction was purified by reverse phase-HPLC to yield the macrocycle(10mg, 3% yield).

Rf: 0.5 (EtOAc: MeOH 49:1)

<sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ 0.8-0.9 (t, 3H), 1.2-1.3 (dd, 2H), 1.5-1.6 (dd, 2H), 1.8 (d, 2H), 1.9-2.0 (s, 3H), 2.2-2.3 (m, 4H), 2.7-2.8 (d, 2H), 3.1-3.2 (t, 2H), 3.6-3.7 (t, 2H), 4.1 (m, αH), 4.3 (m, 2αH), 4.4 (m, αH), 4.6 (m, αH), 4.9 (m, 2H), 7.2-7.3 (m, 5H)

LCMS: m/z calcd for C<sub>26</sub>H<sub>37</sub>N<sub>5</sub>O<sub>5</sub> (M+1) = 500.28, found 500.23

### Compound 6

Macrocycle 1d-2a-3b-4a (Compound 6) was synthesized following the “Macrocyclization procedure”. Utilizing 300 mg (0.56 mmols, 1.0 equivalents) of linear tetrapeptide, 580 uL (6 equivalents) of DIPEA, 91 mg (0.28 mmols, 0.5 equivalents) of TBTU, 107 mg (0.28 mmols, 0.5 equivalents) of HATU, and 84.6 mg (0.28 mmols, 0.5 equivalents) of DEPBT. The crude reaction was purified by reverse phase-HPLC to yield the macrocycle (2mg, 2% yield).

Rf: 0.5 (EtOAc: MeOH 49:1)

<sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ 0.9-1.0 (t, 3H), 1.2-1.3 (m, 2H), 1.4-1.5 (m, 2H), 1.6-1.7 (d, 2H), 1.9-2.0 (m, 5H), 2.2-2.3 (m, 2H), 2.9 (s, 3H), 3.0 (d, 2H), 3.1-3.2 (t, 2H), 3.5 (m, 2H), 3.9 (m 2αH), 4.1 (m, 2αH), 4.5 (m, 2H), 7.2-7.3 (m,5H)

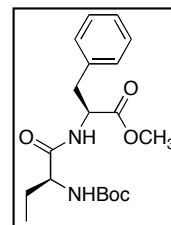
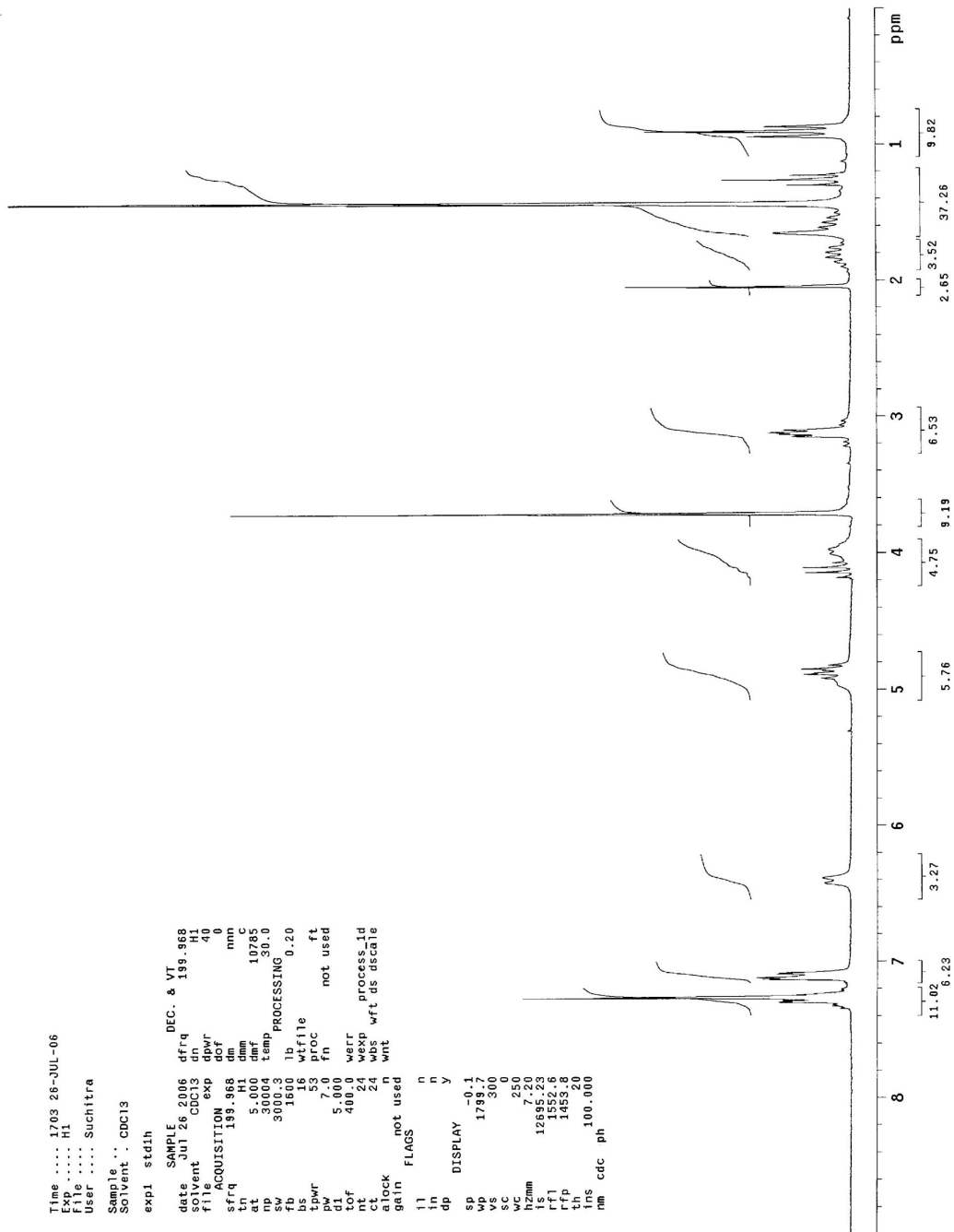
LCMS: m/z calcd for C<sub>27</sub>H<sub>39</sub>N<sub>5</sub>O<sub>5</sub> (M+1) = 514.3, found 514.2

### TABLE OF COMPOUNDS WITH TEXT DESCRIBING STRUCTURAL VARIATIONS

Comp. #	aa <sub>1</sub>	aa <sub>2</sub>	aa <sub>3</sub>	aa <sub>4</sub>	ClogP
1	Phe	Abu	Arg	D-Pro	<b>0.79</b>
2	D-Phe	Abu	Arg	D-Pro	<b>0.79</b>
3	D-Phe	Abu	Arg	D-Pip	<b>0.37</b>
4	Thq	Abu	Arg	D-Pro	<b>0.85</b>
5	Phe	Abu	Lys-Ac	D-Pro	<b>0.58</b>
6	N-Me-Phe	Abu	Lys-Ac	D-Pro	<b>0.34</b>

Phe = Phenylalanine  
 Thq = Tetrahydroquinoline  
 Abu = Aminobutyric acid  
 Arg = Arginine  
 Lys-Ac = Acetyl lysine  
 Pro = Proline  
 Pip = Piperdinyll

Time .... 1703 26-JUL-06  
 Exp ..... H1  
 User ..... Suchitra  
 Sample ..  
 Solvent . CDC13  
 exp1 std1h  
 SAMPLE DEC. & VT  
 date Jul 26 2006 dfrq 199.968  
 solvent CDCl3 d1 40  
 file 40  
 ACQUISITION exp dof 0  
 sfrq 199.968 dm mnn  
 tn 5.000 h1 10765  
 nt 30004 r1 30.0  
 sw 3000.3 Temp PROCESSING 0.20  
 fb 1600 lb  
 bs 16 wfile ft  
 spwr 5 proc not used  
 d1 7.0 fn  
 di 5.000 werr  
 nt 400.0 wepp  
 ct 24 wds  
 gain not used wft ds dscale  
 wnt  
 FLAGS n  
 l1 n  
 in n  
 dp y  
 DISPLAY -0.1  
 sp 1789.7  
 vs 300  
 sc 0  
 vc 250  
 hzmm 7.20  
 ls 12895.23  
 rf1 1552.6  
 rfp 1455.0  
 th  
 lns 100.000  
 nm cdc ph

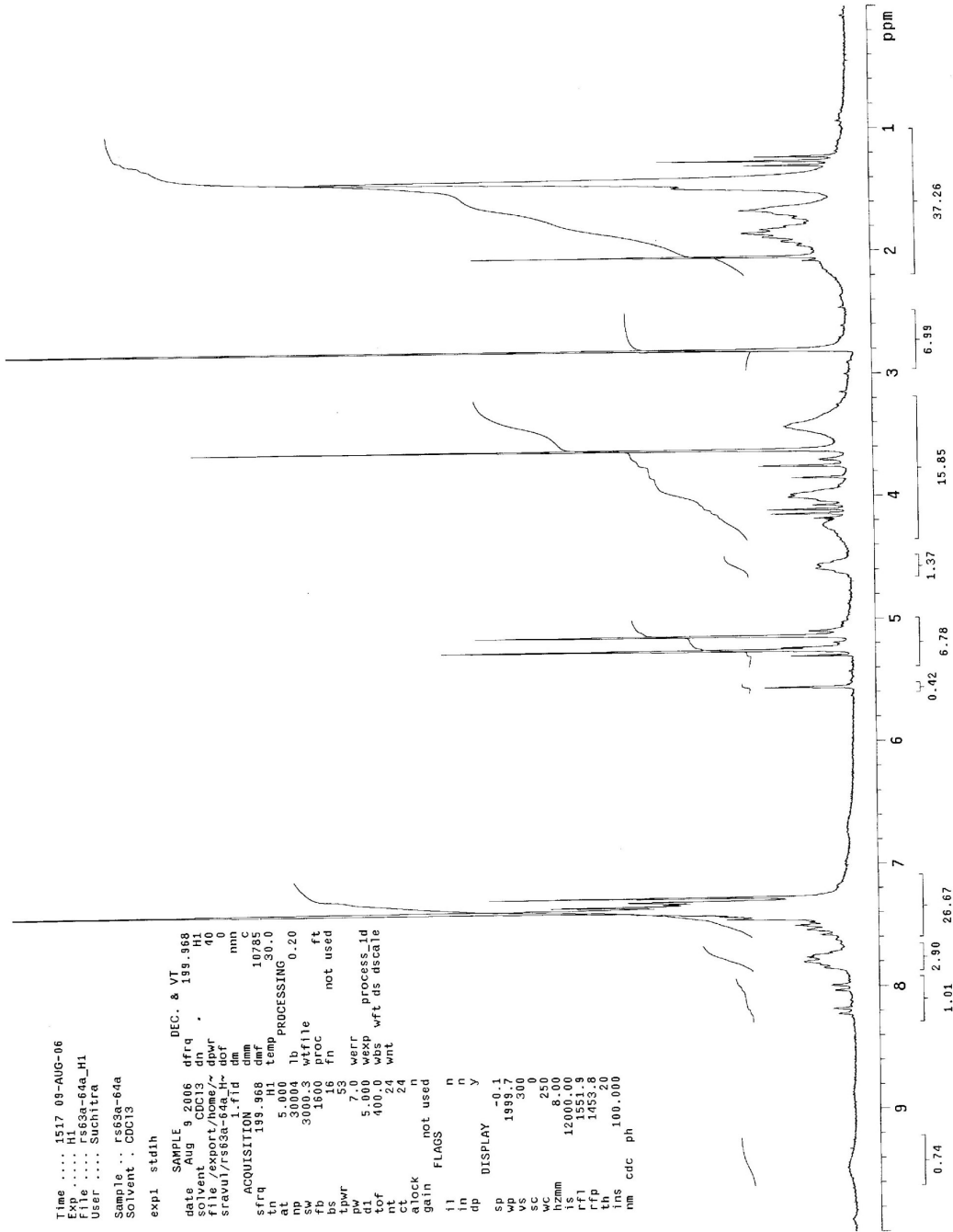


Compound 1 – NMR Dipeptide 1a2a

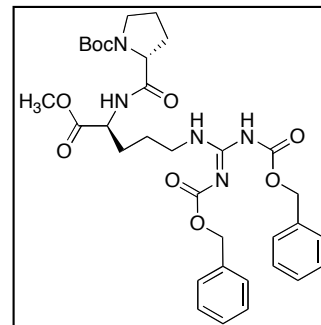


Time ..... 1517 09-AUG-06  
 Exp ..... HI  
 File ..... f663a-64a\_H1  
 User ..... Suchitra  
 Sample .. f663a-64a  
 Solvent . CDCl3

exp1 stdih  
 SAMPLE DEC. & VT  
 date Aug 9 2006 dfrq 199.988  
 solvent CDCl3 dn 40  
 file /export/64a\_H1.dbr  
 srat/f663a-64a-hr-dbr  
 1.fid dm nna  
 ACQUISITION 1876 C  
 sfrq 199.988 dmp 30.0  
 n 0  
 5.000 lb PROCESSING  
 mp 30004 wf 0.20  
 sw 3000.3 wf file ft  
 fb 1600 proc not used  
 bs 53 fh  
 pw 7.0 werr  
 d1 5.000 we xp process\_id  
 tof 400.0 wbs wft ds dscate  
 nt 24  
 24  
 alock n  
 gain not used  
 FLAGS n  
 li n  
 in y  
 dp y  
 DISPLAY  
 sp -0.1  
 wp 1999.7  
 vs 30  
 vc 0  
 wv 250  
 hzmm 8.00  
 ls 12000.00  
 rf1 1433.8  
 rf2 1433.8  
 th 1433.8  
 ins 100.000  
 nm cdc ph



Compound 1 – NMR dipeptide 3a-4a



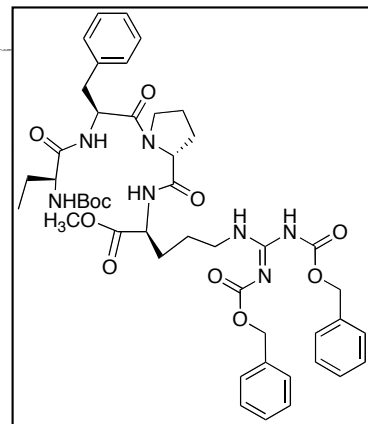
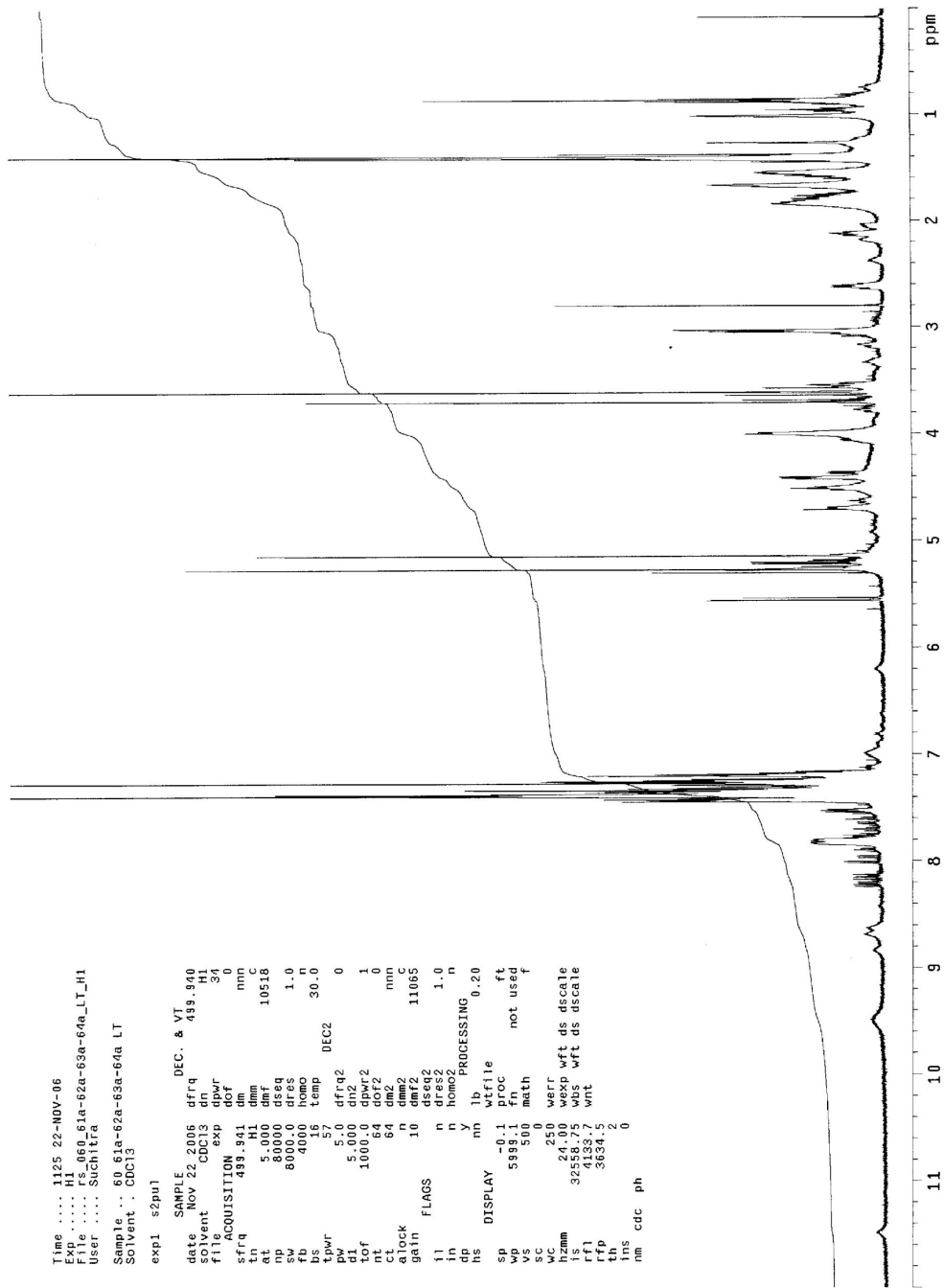
Time ..... 1125 22-NOV-06  
 Exp ..... HI 060.61a-62a-63a-64a\_LT\_H1  
 User ..... Suchitra

Sample ... 60.61a-62a-63a-64a LT  
 Solvent : CDCl3

exp1 s2pu1

```

SAMPLE_2005 DEC. & VI
date Nov 22 2005 dfrq 439.940
solvent CDCl3 dn HI
file 060.61a-62a-63a-64a_LT_H1 dpwr 3A
ACQUISITION exp dof 0
sfrq 439.941 dm nnn
in 5.000 dmp 10518
np 80000 dseq 1.0
sw 8000.0 dres 1.0
fb 4000 homo n
us 15 temp DEC2 30.0
pw 5.0 dfrq2 0
d1 5.000 dn2 1
tof 1000.0 dpwr2 1
nt 64 dor2 0
dpc 80000 dmp2 nnn
gain 10 dm2 C
flags n dseq2 11065
il n dres2 1.0
in y homo n
ds y lb PROCESSING 0.20
hs nn lb
sp 0.1 wtf file
vp 59850.0 proc not used
wc 500 math f
hzm 250 werr
f 24.00 wexp wft ds dscate
rf 32587.7 wds wft ds dscate
rfp 3634.5 wnt
th 3634.5
ins 2
nm cdc ph
  
```

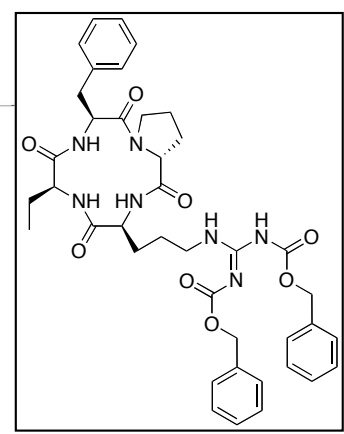
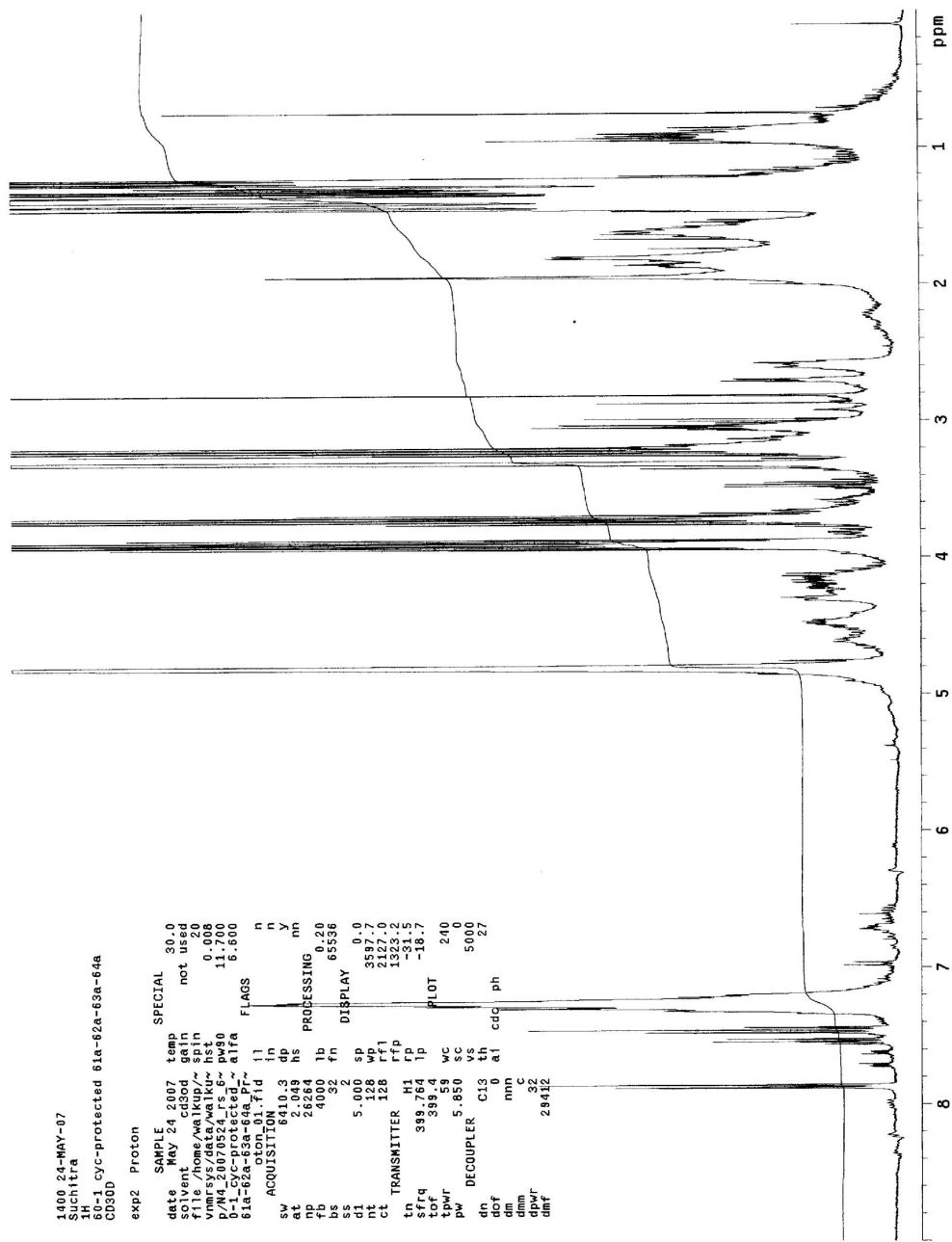


Compound 1 – NMR linear tetrapeptide 1a-2a-3a-4a

1400 24-MAY-07  
 Suchitra  
 1H  
 60-1 cyc-protected 61a-62a-63a-64a  
 CD3OD  
 exp2 Proton

```

SAMPLE          SPECIAL
date    May 24 2007    temp    30.0
file    /home/waliku/~    not us20
nmrSYS/data/waliku/~    gain
p/N4_20070524_rs_6~    hst    0.008
0-1_cyc-protected_~    pw80    11.700
61a-62a-63a-64a~    alfa    6.600
                                FLAGS
ACQUISITION
sw    6410.3    dp    11    n
at    2.049    hs    y
pp    2600    lb    PROSSING 0.20
bs    4032    fn    65536
ss    2
d1    5.000    sp    0.0
nt    128    wp1    2127.0
ct    TRANSMITTER H1    rfp    1323.2
tn    sftq    389.764    lp    PLOT -18.7
tof    399.54    wc    240
pw    DECOUPLER C13    th    5000
dn    dor    0    al    cdq    ph
dm    min
dwm    C
dppw    32
dmf    28412
  
```

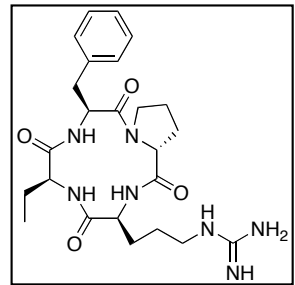
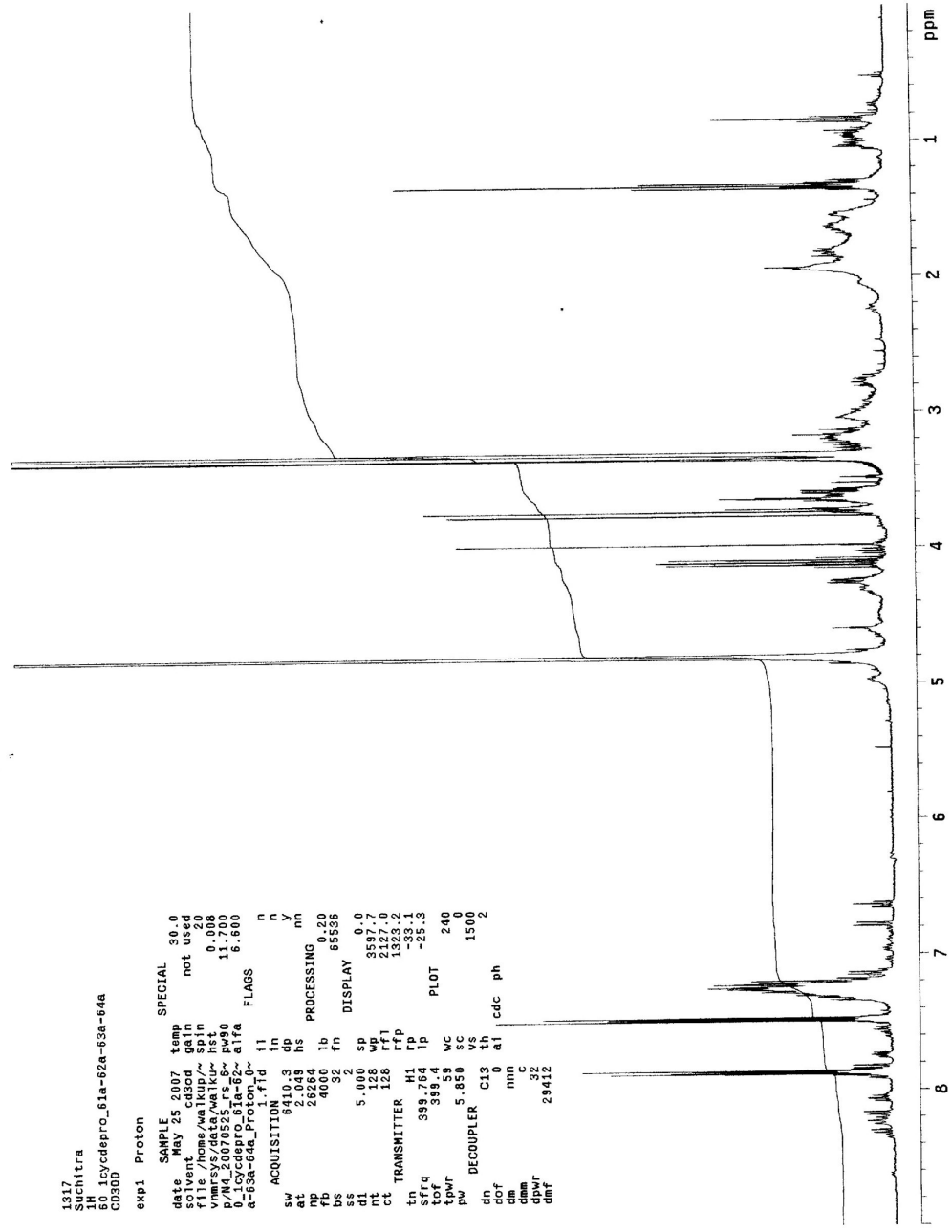


Compound 1 – NMR cyclized protected tetrapeptide 1a-2a-3a-4a

1317  
SUCHITRA  
60\_1cycdepro\_61a-62a-63a-64a  
C030D

exp1 Proton

```
SAMPLE          SPECIAL 30.0
date            may 25 0007  temp
time            0500          gpi
file            /home/walidp/~  not used
vnmr/systype   /waikup/~      spin 20
vnmr/sydata    /waikup/~      hst  0.008
p/m4_20070525_F5S~ pw90      11.700
p_1cycdepro_61a-62a-63a-64a  a1fa  6.800
a-63a-64a_Proton_1.f2d      i1      n
ACQUISITION    in          n
sw             6410.3      dp          y
at             2.049      hs          y
rf             24000      lb          0.20
bs             32        fn          85536
ss             5.000      sp          0.0
d1             128        rfd         3597.7
nt             128        rfi         2127.0
c1             128        rfp         1323.2
TRANSMITTER    H1         rp          -33.1
t1             399.764     lp          -25.3
t2             395.54      wc          240
t3             5.850      sc          1500
pw             5.850      SC          1500
DECOUPLER      C13        th          2
dn             0          al          cdc
dof            nmt
dpm            nmt
dpmc           32
dpcw           29412
dmf
```

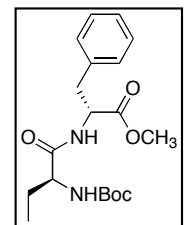
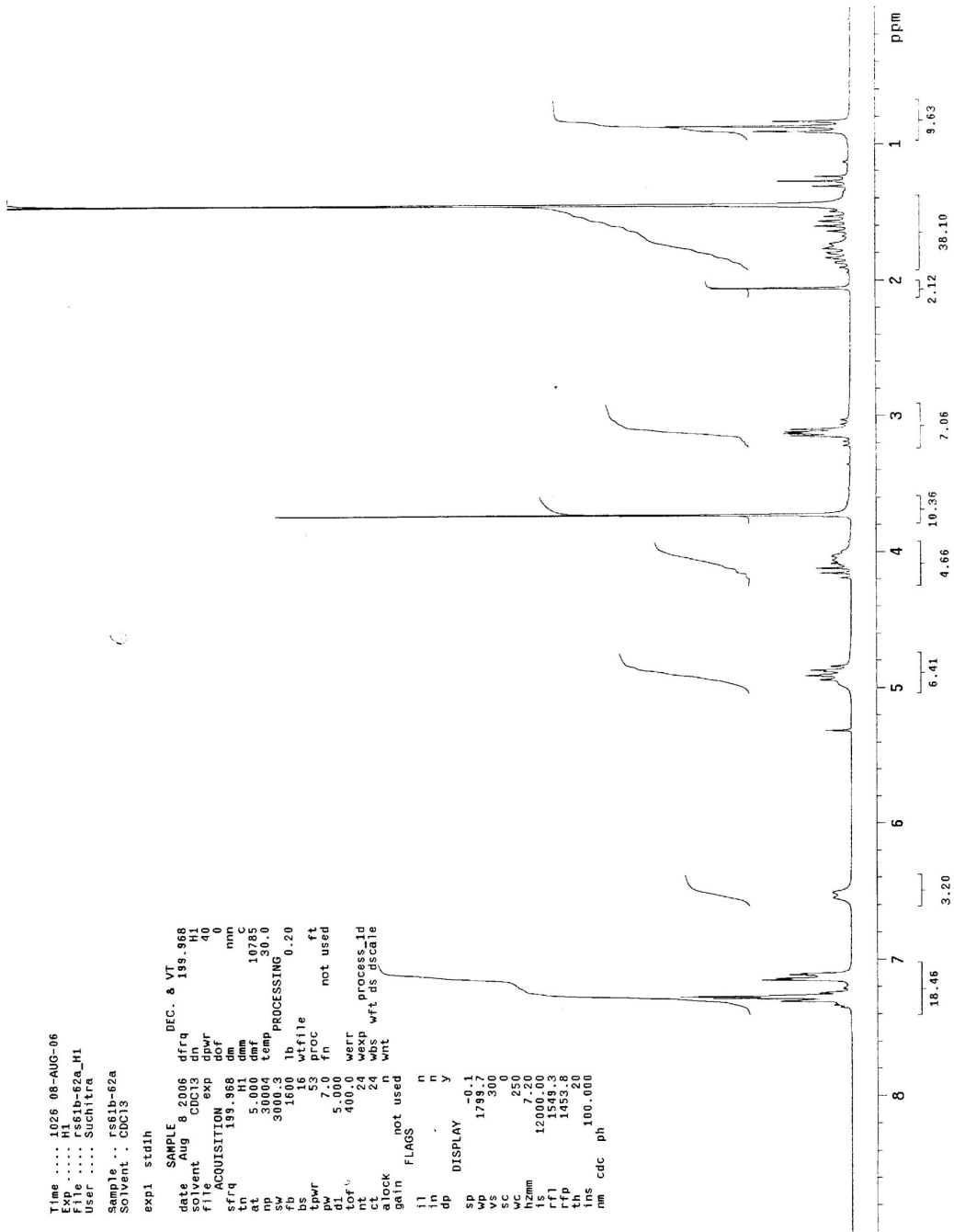


Compound 1 – NMR cyclized tetrapeptide 1a-2a-3a-4a

Time .... 1026 08-AUG-06  
 File ..... rs61b-62a\_H1  
 User ..... Suchitra  
 Sample .. rs61b-62a  
 Solvent : CDCl3

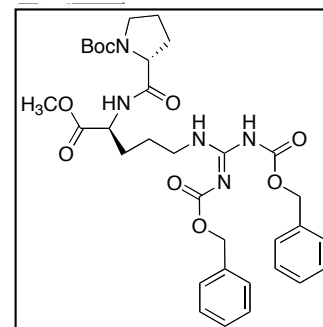
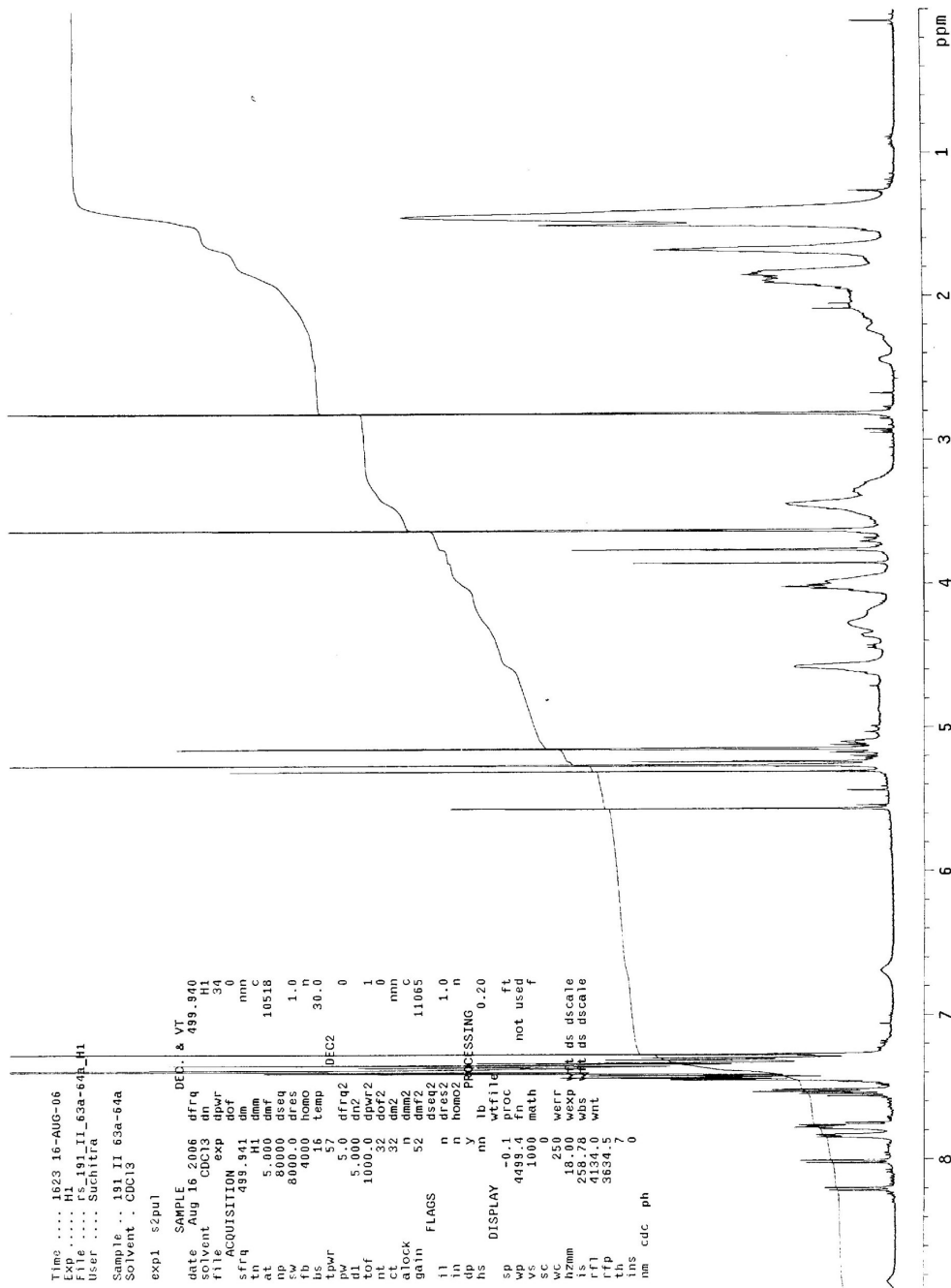
exp1 stdlh

SAMPLE DEC. & VT  
 data Aug 8 2006 dfrq 199.988  
 solvent CDCl3 dn 199.988  
 file exp 40  
 dpwr 0  
 ACQUISITION exp 40  
 sfrq 199.988 dm min  
 an 5.000 dm 10785  
 np 30004 temp 30.0  
 sw 3000.3 PROCESSING 0.20  
 fb 1600 lb wfile  
 ts 53 ft  
 pc 53 proc not used  
 pw 7.0 fn  
 d1 5.000 werr  
 tof 400.0 wepp  
 nt 24 wexp  
 clock 24 wft ds  
 gain not used  
 flags n  
 il n  
 in n  
 dp y  
 DISPLAY -0.1  
 sp 1799.7  
 vs 300  
 vc 250  
 hzmm 7.20  
 ls 12000.00  
 rf1 1549.3  
 rf2 1493.20  
 fh  
 ins 100.000  
 nm cdc ph



Compound 2 – NMR Dipeptide 1b-2a

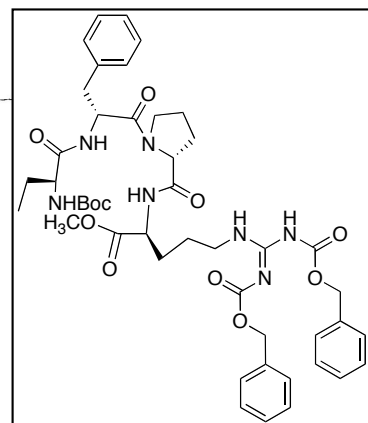
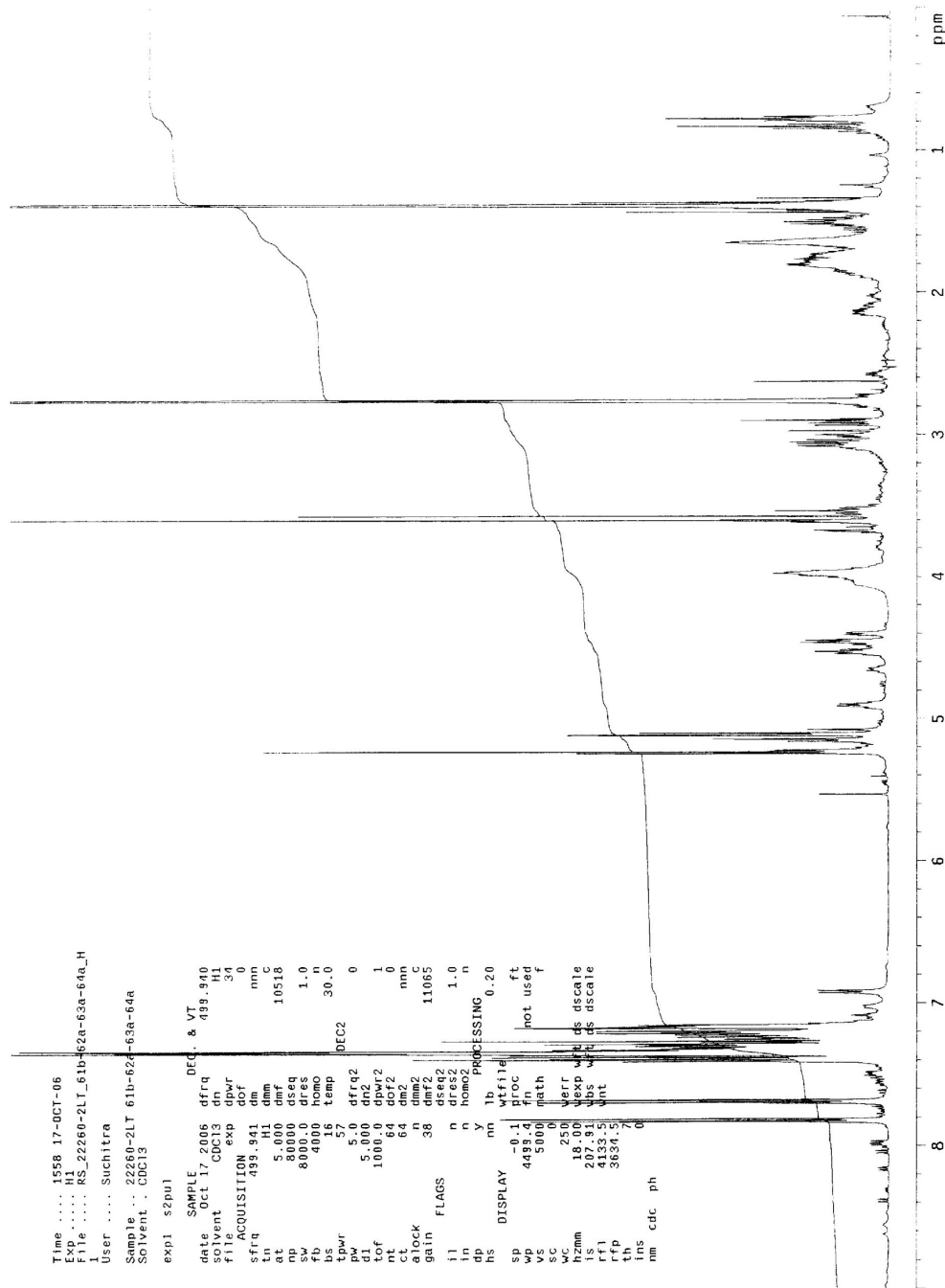
Compound 2 – NMR Dipeptide 3a-4a



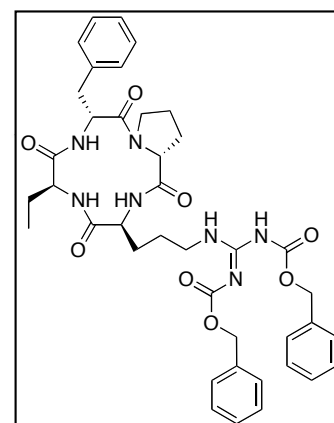
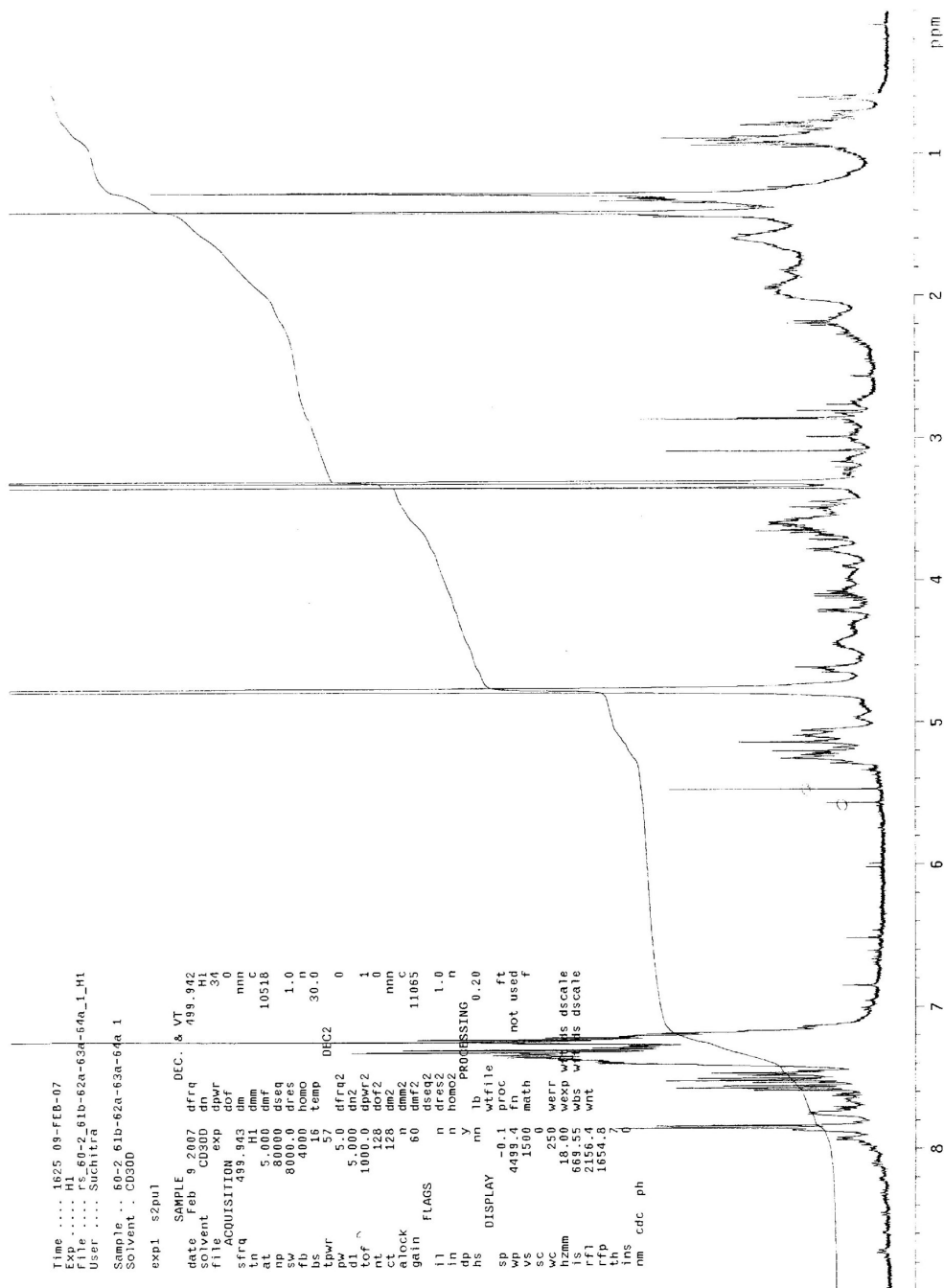
```

Time ..... 1558 17-OCT-06
Exp ..... H1
File ..... RS_22260-2LT_61b-62a-63a-64a_H
User ..... Suchitra
Sample ... 22260-2LT 61b-62a-63a-64a
Solvent : CDCl3
exp1 szpul
DEC. & VT
date Oct 17, 2006 dfrq 499.940
solvent CDCl3 dn 499.940
file exp dpwr 34
ACQUISITION exp dof 0
sfrq 499.941 dm 10518
at 5.000 dmf 10518
ap 80000 dseq 1.0
sw 8000.0 dres 1.0
fb 4000 homo n
ls 15 temp 30.0
pwr 5.0 dfrq2 0
d1 5.000 dn2 1
tof 1000.0 dpwr2 0
nt 64 dor2 0
dme 0 min C
clock gain n dmf2 11065
FLAGS n dseq2
l1 n dres2 1.0
dp y homo PROCESSING n
hs nn lb PROCESSING 0.20
DISPLAY
sp 0.1 proc not used
vp 449.000 fin
wp 5000 meth f
sc 00
wc 250 verr
hzm 18.000 exp wtd ds dscate
ls 407.500 obs wtd ds dscate
rfp 3634.5 int
th 0
ins nm cdc ph

```



Compound 2 – NMR linear tetrapeptide 1b-2a-3a-4a



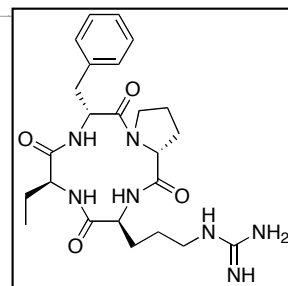
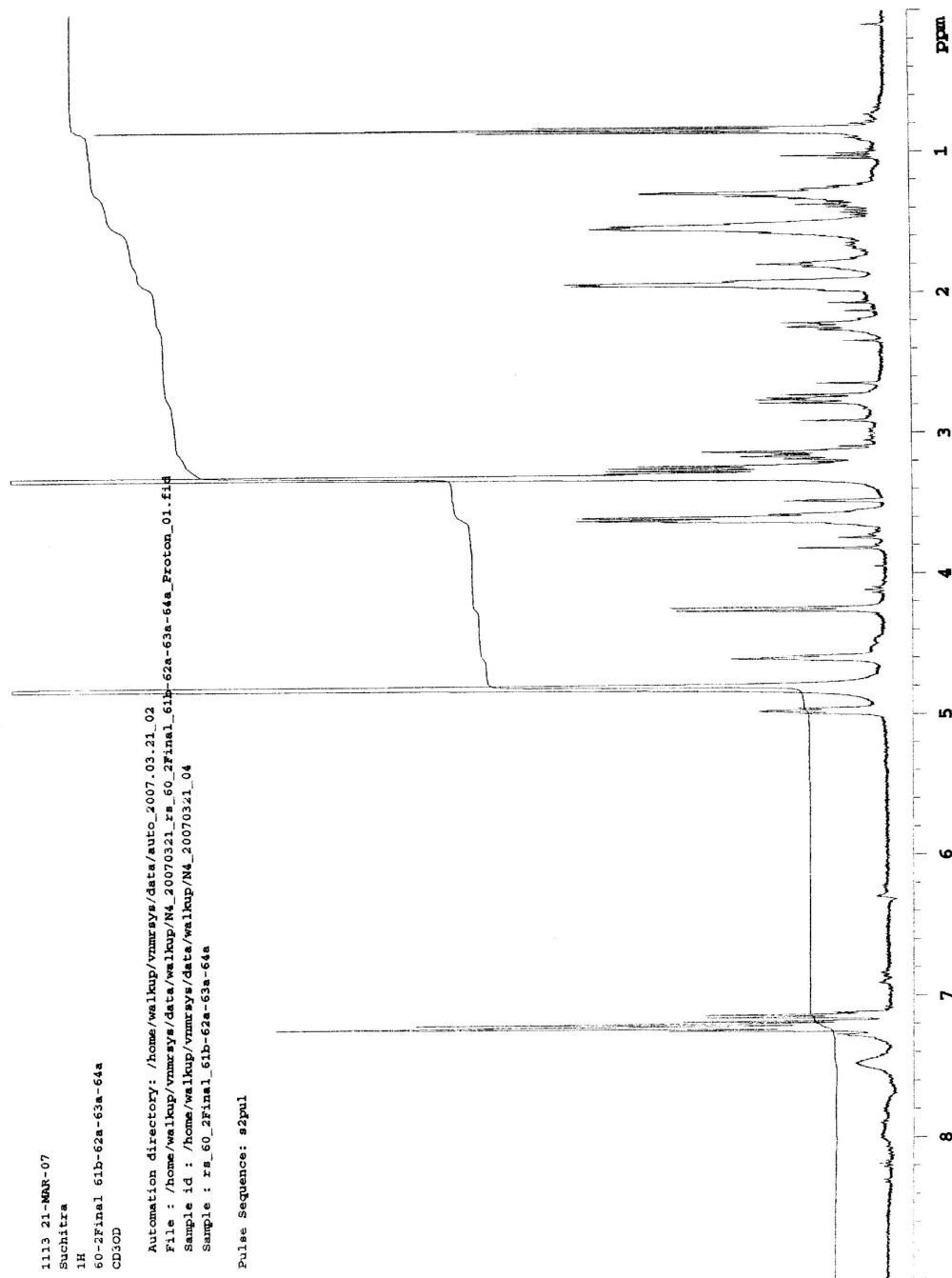
Compound 2 – NMR cyclized protected tetrapeptide 1b-2a-3a-4a



1113 21-MAR-07  
Suchitra  
1H  
60-2Final 61b-62a-63a-64a  
CD3OD

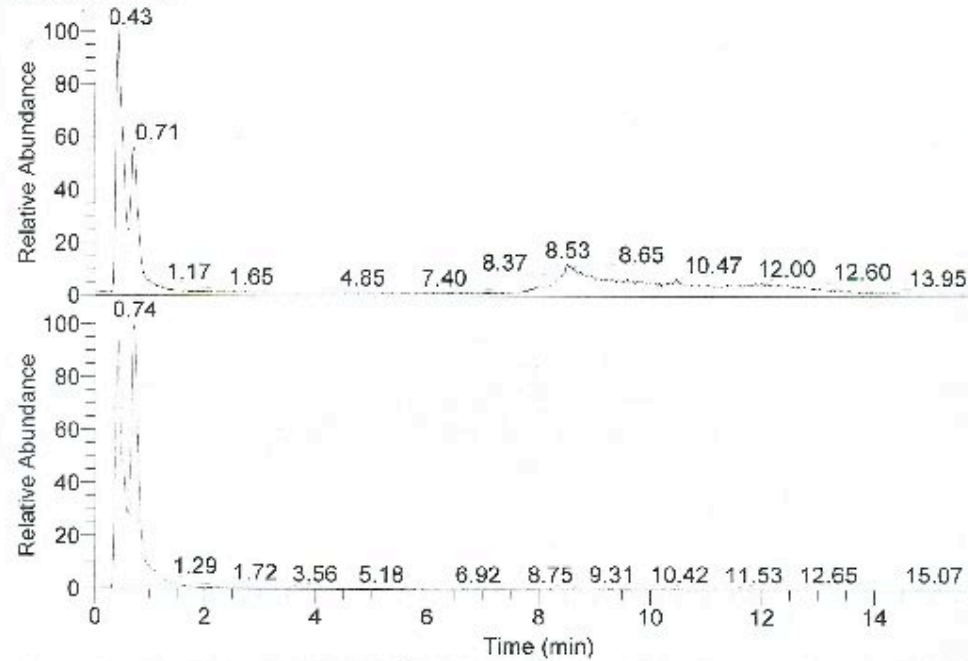
Automation directory: /home/walkup/vmrays/data/autoc\_2007.03.21\_02  
File: /home/walkup/vmrays/data/walkup/N4\_20070321\_rs\_60\_2Final\_61b-62a-63a-64a\_Proton\_01.fid  
Sample id: /home/walkup/vmrays/data/walkup/N4\_20070321\_04  
Sample: rs\_60\_2Final\_61b-62a-63a-64a

Pulse Sequence: s2pul



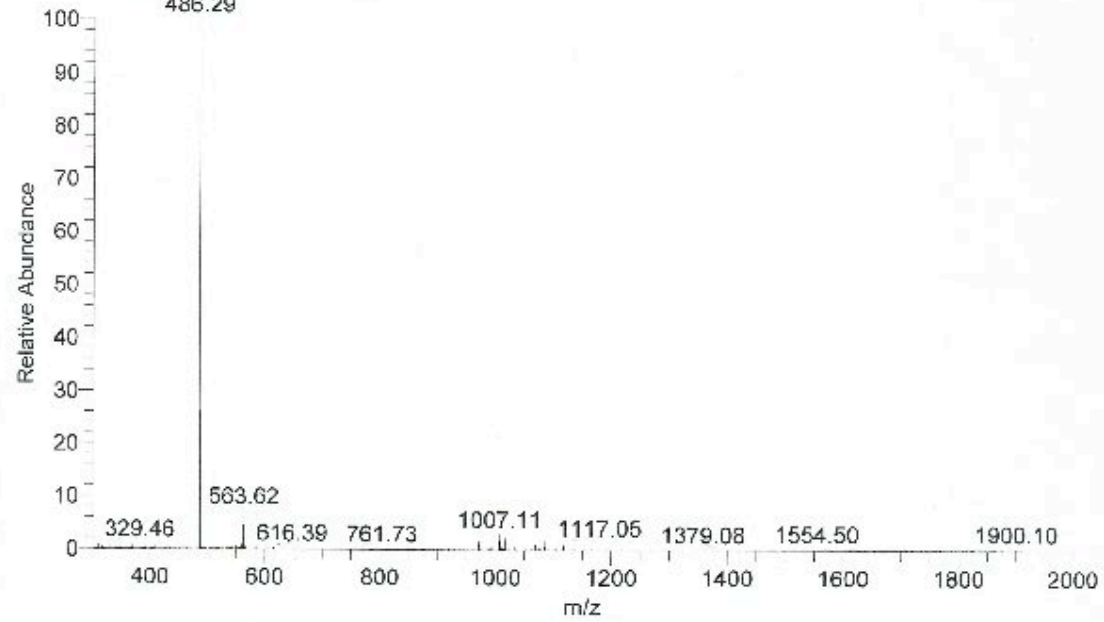
Compound 2 – NMR cyclized 1b-2a-3a-4a

RT: 0.00 - 16.00

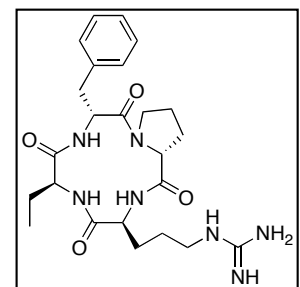


NL:  
2.89E8  
m/z=  
300.00-  
2000.00 MS  
RS\_60-2

RS\_60-2 #32 RT: 0.74 AV: 1 NL: 7.04E7  
T: + c ESI Full ms [300.00-2000.00]  
486.29



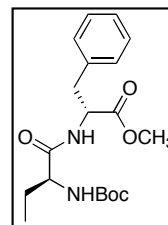
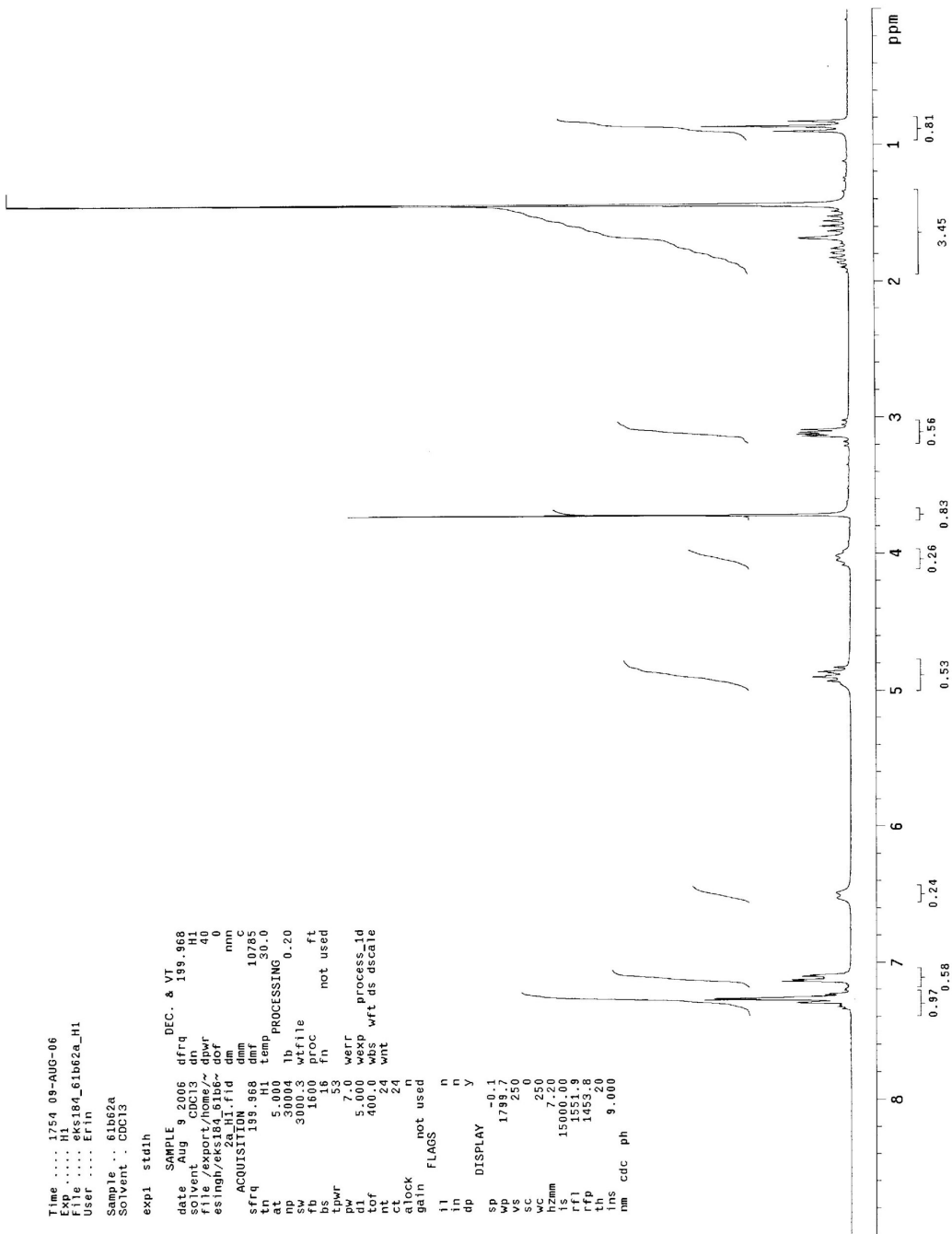
**Compound 2 – LCMS cyclized tetrapeptide**



Time ..... 1754 09-AUG-06  
 Exp ..... H1  
 File ..... eks184\_61b62a\_H1  
 User ..... ErIn  
 Sample ... 61b62a  
 Solvent ... CDCl3

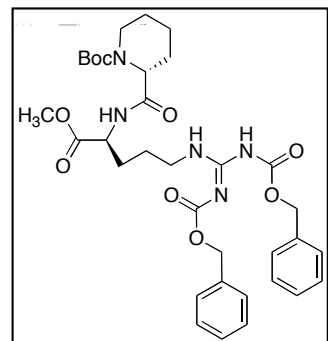
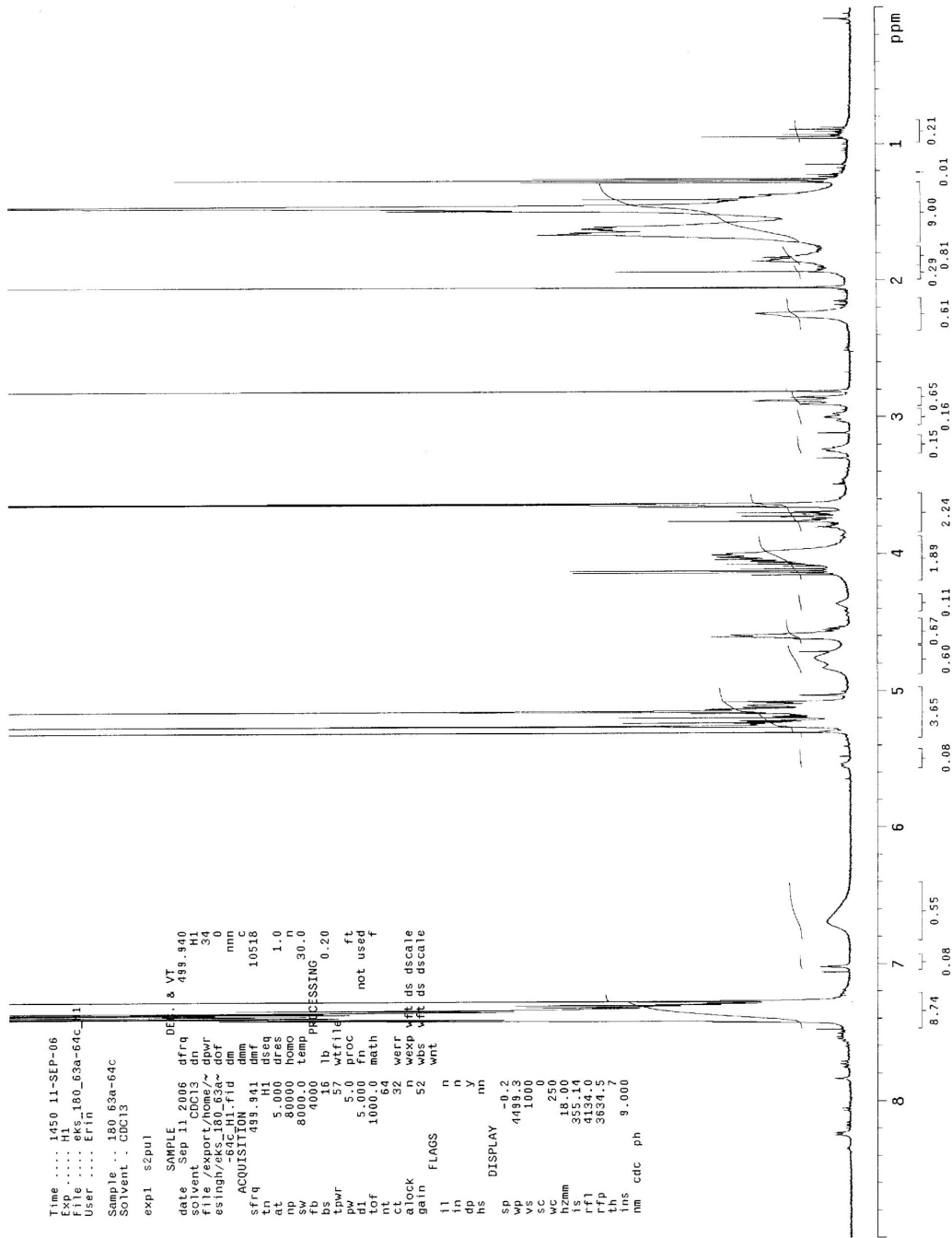
exp1 stdih

SAMPLE DEC. & VT  
 date Aug 9 2006 dfrq 199.968  
 solvent CDCl3 dn H1  
 file /export/home/~dper 40  
 es ingh/eks184\_H1 fid  
 dm nnn  
 dnm C  
 ACQUISITION 10785  
 sfrq 199.968 dmf 30.0  
 tn H1 temp  
 at 5.000 lb PROCESSING 0.20  
 sw 3000.3 wtf file  
 fb 1600 proc ft  
 bs 16 fh not used  
 tpr 7.58 werr  
 d1 5.000 wexp process\_1d  
 tof 400.0 wbs wft ds dscate  
 nt 24 wnt  
 ct 24  
 ck not used  
 gain not used  
 FLAGS  
 ll n  
 in n  
 dp y  
 sp -0.1 DISPLAY  
 wp 1799.7  
 vs 250  
 vc 250  
 hzmm 7.20  
 ls 15000.00  
 rfl 1551.9  
 tpp 1453.8  
 lns 6  
 nm cdc ph 9.000

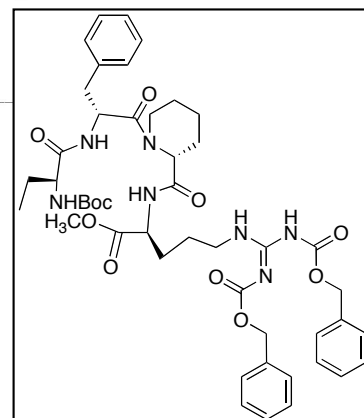
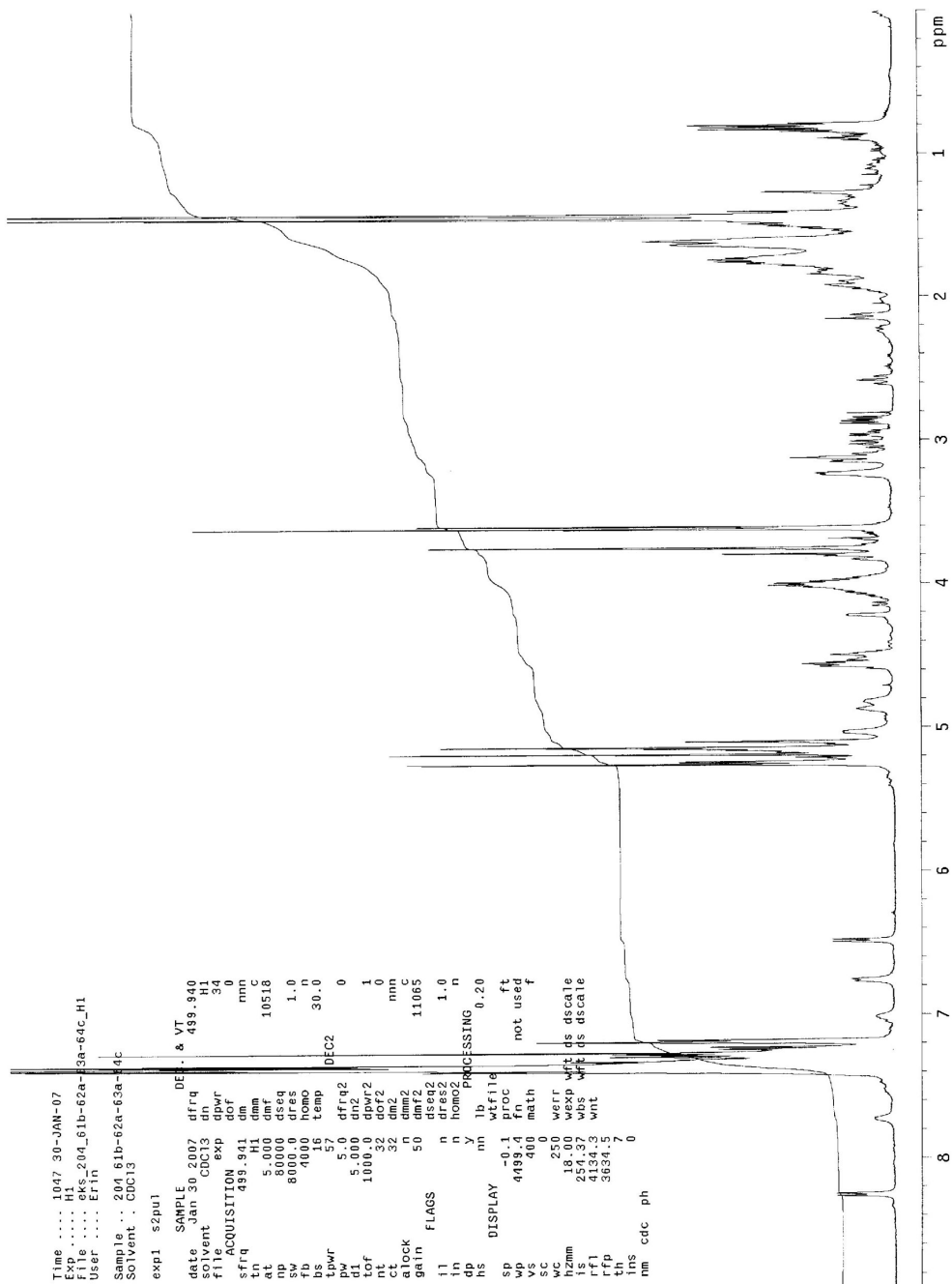


Compound 3 – NMR dipeptide 1b-2a

Time .... 1450 11-SEP-06  
 File ..... H1  
 User ..... ErIn  
 Sample .. 180\_63a-64c  
 Solvent : CDCl3  
 exp1 s2pul  
 DEPT & VT  
 date Sep 11 2006 dfrq 439.940  
 solvent CDCl3 dn H1  
 file /export/home/~ dpwr 34  
 es lgh/eks\_180\_63a~ dof 0  
 spectr H1.ftid mm  
 ACQUISITION dm 10518  
 dmf  
 sfrq 439.941  
 tn H1 dseq  
 at 5.000 dres 1.0  
 np 80000 homo 30.0  
 pb 4000 temp PREPROCESSING 0.20  
 bs 16 lb wtfile  
 tpwr 57  
 pw 5.0 proc ft  
 pr 1000.0 math not used f  
 tof 64  
 ct 32 wevr  
 alock n wexp vnt ds dscate  
 gain 52 wds vnt ds dscate  
 wnt  
 fl n  
 in n  
 dp y  
 hs mn  
 sp -0.2  
 wp 4439.3  
 vs 1000  
 sc 25.0  
 hzmm 18.00  
 ls 355.14  
 rfl 4134.0  
 rfp 3634.5  
 lrs 7  
 nm cdc ph 9.000



Compound 3 – NMR dipeptide 3a-4a

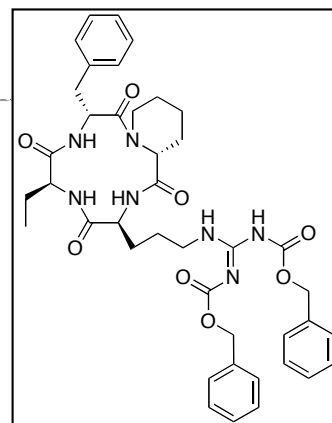
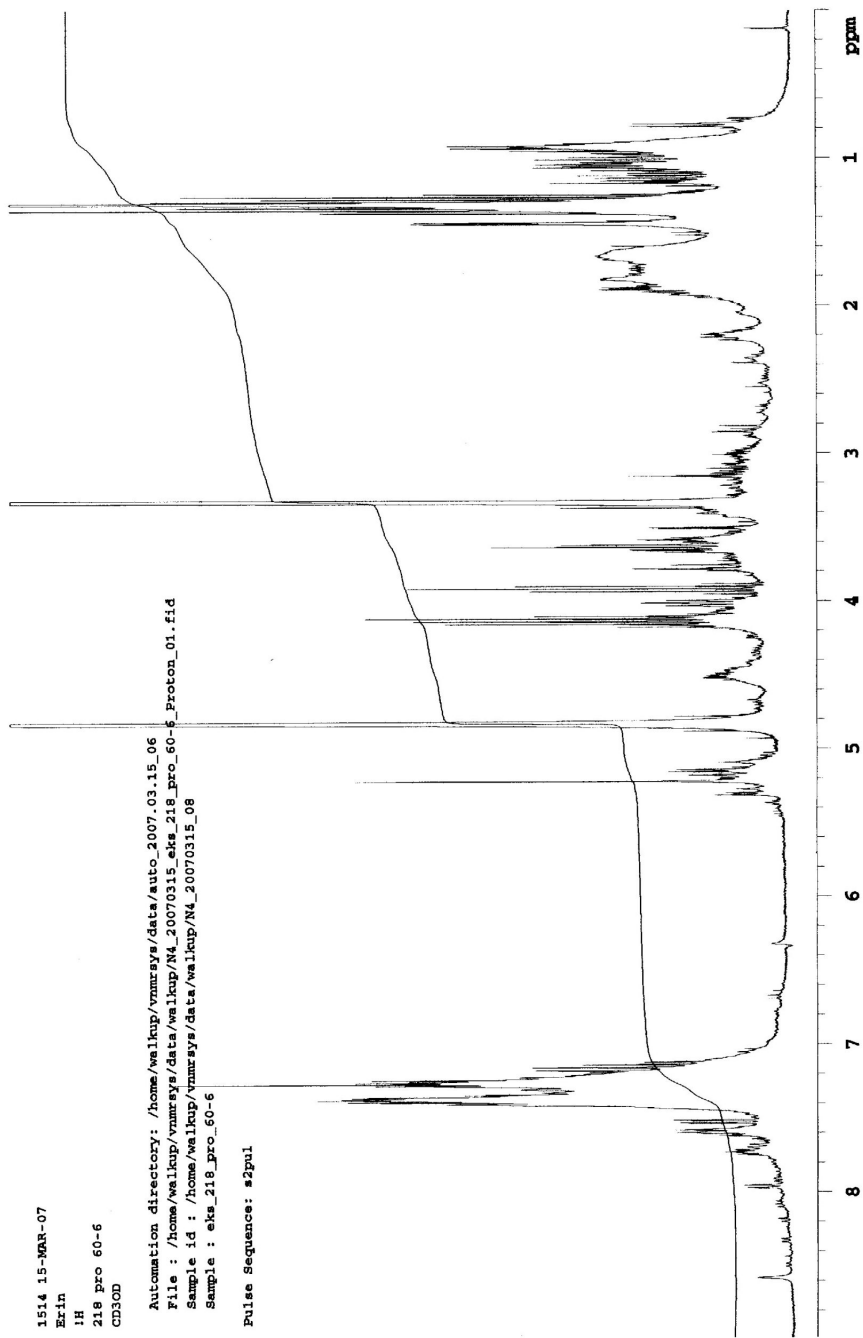


Compound 3 – NMR linear tetrapeptide 1b-2a-3a-4c

1514 15-MAR-07  
Erin  
1H  
218 pro 60-6  
CD3OD

Automation directory: /home/walkup/vnmrsws/data/auto\_2007\_03\_15\_06  
File: /home/walkup/vnmrsws/data/walkup/N4\_20070315\_eks\_218\_pro\_60-6\_Proton\_01.fid  
Sample id: /home/walkup/vnmrsws/data/walkup/N4\_20070315\_08  
Sample: eks\_218\_pro\_60-6

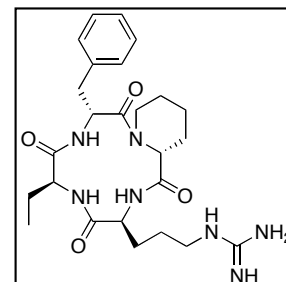
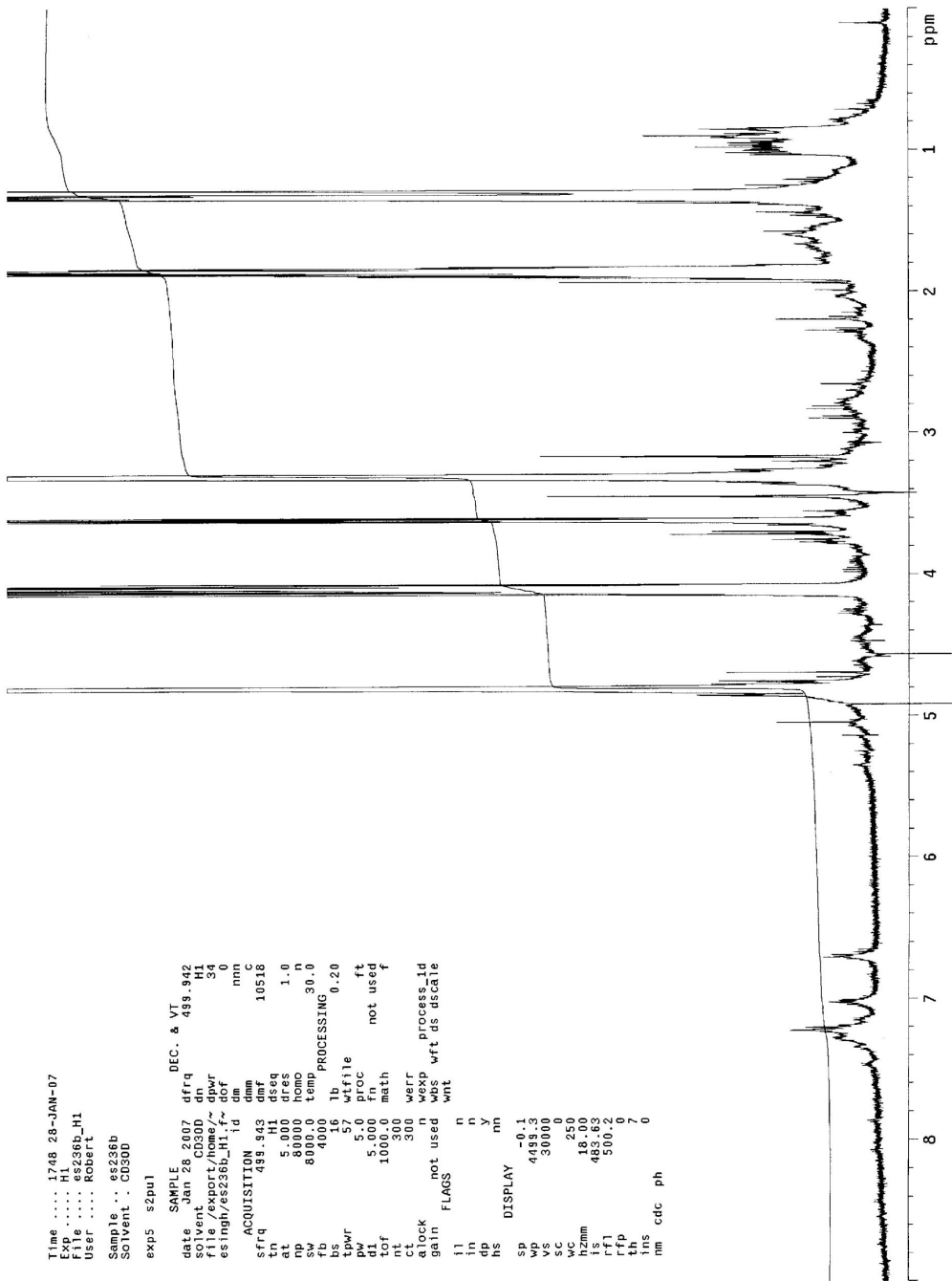
Pulse Sequence: s2pul



Compound 3 – NMR cyclized protected tetrapeptide 1b-2a-3a-4c

Time ..... 1748 28-JAN-07  
 Exp ..... H1  
 File ..... es236b\_H1  
 User ..... Robert  
 Sample ... es236b  
 Solvent ... CD3OD

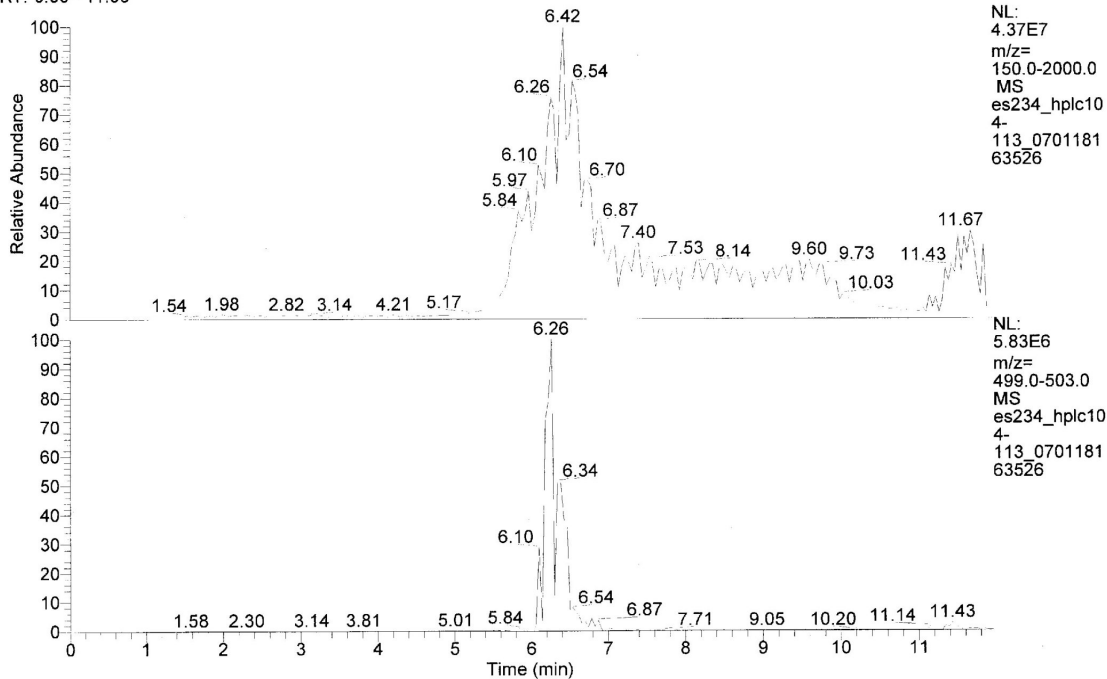
exp5 szpul  
 SAMPLE  
 date Jan 28 2007 dfrq DEC. & VT 489.942  
 solvent CD3OD dn H1  
 file /export/home/~ dpwr 34  
 es1ngh/es236b\_H1.f~ dof 0  
 chm mnc  
 id mm  
 ACQUISITION id dmf 10518  
 sfrq 489.943  
 tn H1 dseq  
 at 5.000 dres 1.0  
 sp 8000.0 homo  
 sb 8000.0 temp PROCESSING 30.0  
 fb 4000.0  
 bs 16 lb  
 tpwr 57 wfile  
 pw 500 proc ft  
 dt 1000.0 math not used f  
 nt 300  
 ct 300 werr  
 atlock n wexp process\_id  
 gain not used wts wft ds dscate  
 FLAGS  
 l1 n  
 l2 n  
 l3 n  
 l4 n  
 l5 n  
 l6 n  
 l7 n  
 l8 n  
 l9 n  
 l10 n  
 l11 n  
 l12 n  
 l13 n  
 l14 n  
 l15 n  
 l16 n  
 l17 n  
 l18 n  
 l19 n  
 l20 n  
 l21 n  
 l22 n  
 l23 n  
 l24 n  
 l25 n  
 l26 n  
 l27 n  
 l28 n  
 l29 n  
 l30 n  
 l31 n  
 l32 n  
 l33 n  
 l34 n  
 l35 n  
 l36 n  
 l37 n  
 l38 n  
 l39 n  
 l40 n  
 l41 n  
 l42 n  
 l43 n  
 l44 n  
 l45 n  
 l46 n  
 l47 n  
 l48 n  
 l49 n  
 l50 n  
 l51 n  
 l52 n  
 l53 n  
 l54 n  
 l55 n  
 l56 n  
 l57 n  
 l58 n  
 l59 n  
 l60 n  
 l61 n  
 l62 n  
 l63 n  
 l64 n  
 l65 n  
 l66 n  
 l67 n  
 l68 n  
 l69 n  
 l70 n  
 l71 n  
 l72 n  
 l73 n  
 l74 n  
 l75 n  
 l76 n  
 l77 n  
 l78 n  
 l79 n  
 l80 n  
 l81 n  
 l82 n  
 l83 n  
 l84 n  
 l85 n  
 l86 n  
 l87 n  
 l88 n  
 l89 n  
 l90 n  
 l91 n  
 l92 n  
 l93 n  
 l94 n  
 l95 n  
 l96 n  
 l97 n  
 l98 n  
 l99 n  
 l100 n  
 nm cdc ph



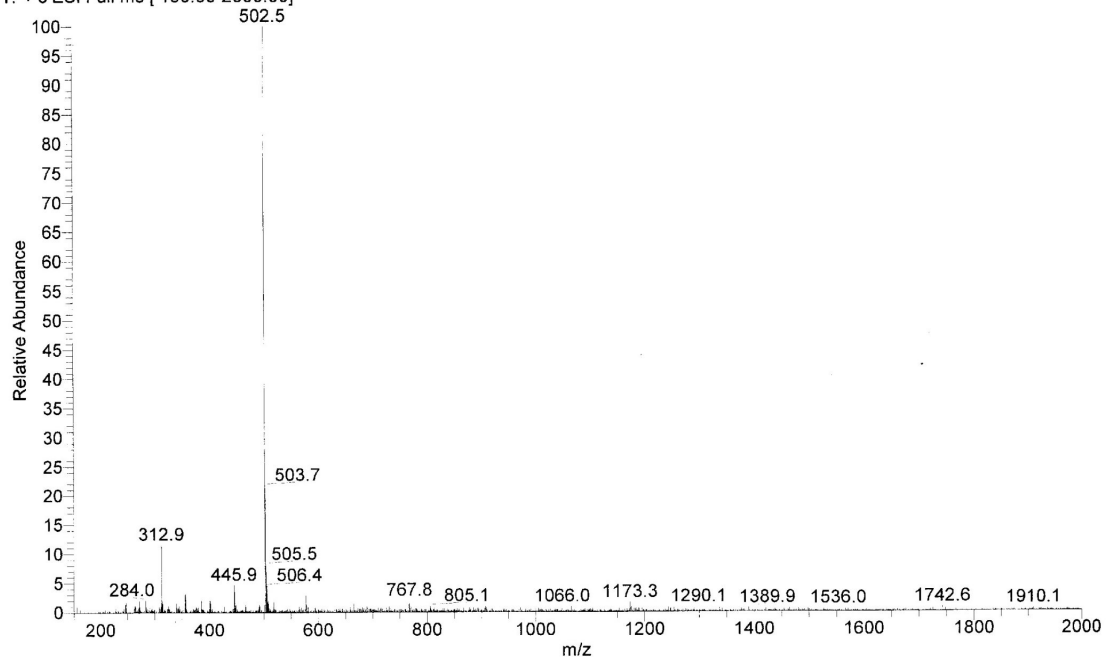
Compound 3 – NMR cyclized tetrapeptide 1b-2a-3a-4c

es234\_hplc104-113\_070118163526  
es234\_hplc104-113  
RT: 0.00 - 11.96

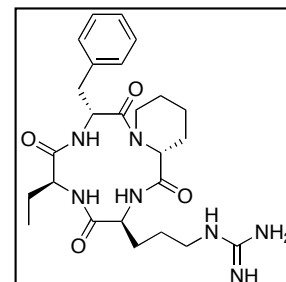
01/18/07 04:35:26 PM



es234\_hplc104-113\_070118163526 #118 RT: 6.22 AV: 1 NL: 4.56E6  
T: + c ESI Full ms [ 150.00-2000.00]



*Compound 3 – LCMS cyclized tetrapeptide*

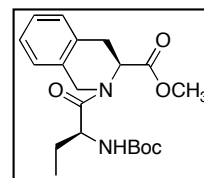
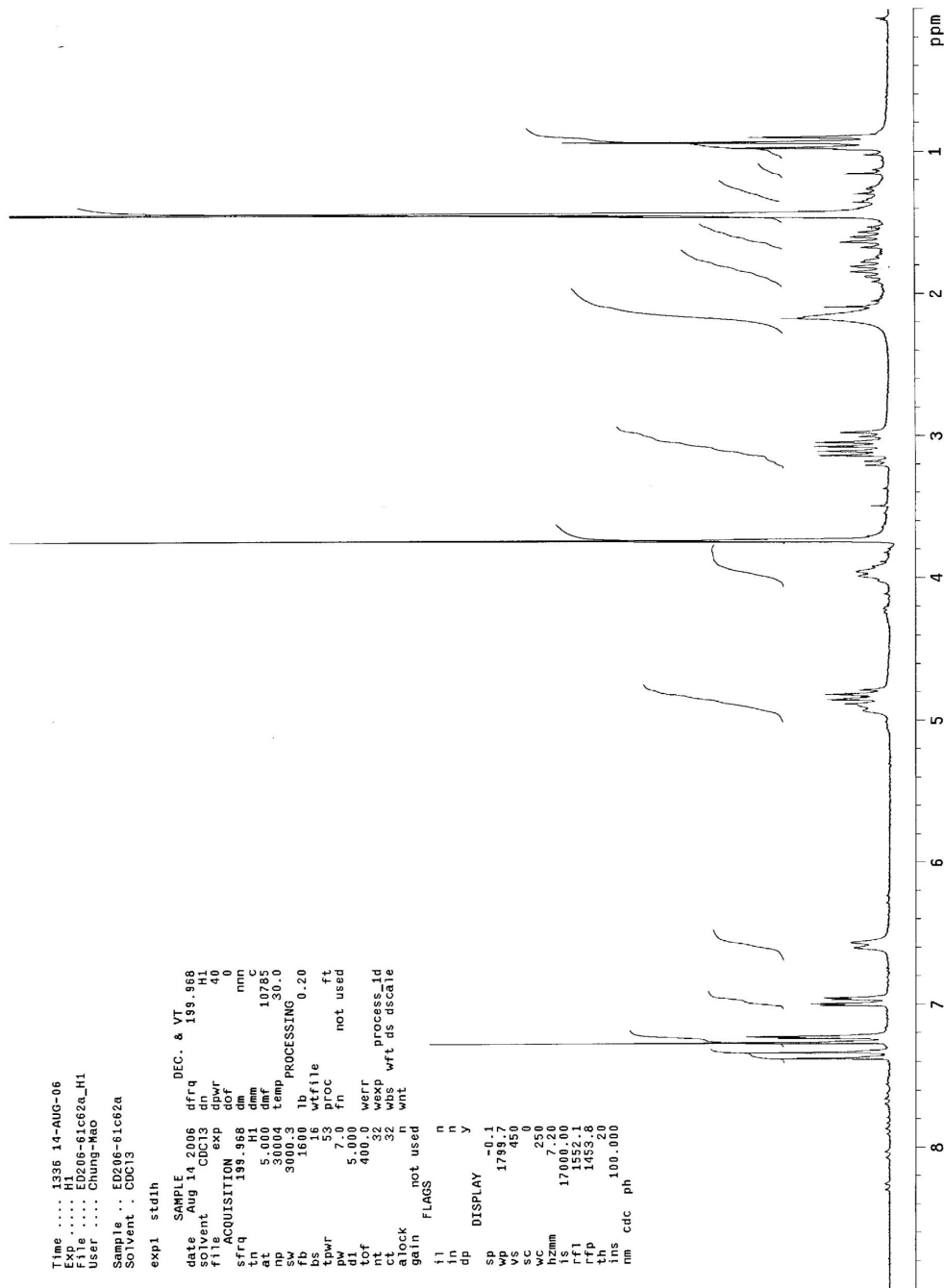




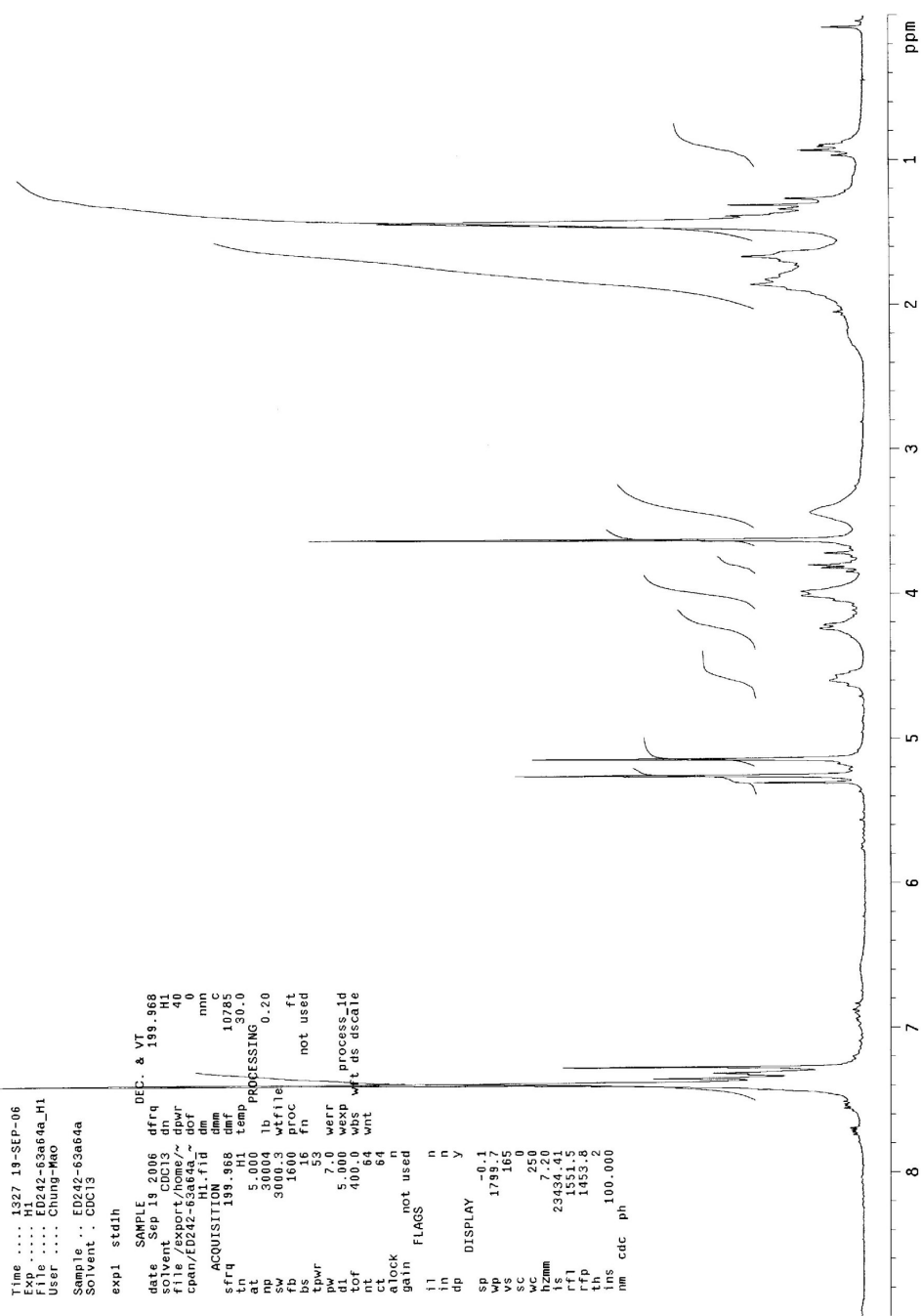
Time ..... 1335 14-AUG-06  
 Exp ..... H1  
 File ..... ED206-61c62a\_H1  
 User ..... Chung-Mao  
 Sample .. ED206-61c62a  
 Solvent .. CDCl3

```

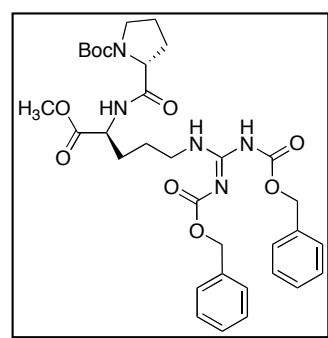
exp1 stdih
SAMPLE
date Aug 14 2006 dfrq DEC. & VT
solvent CDCl3 dn H1
file CDCl3 exp H1
ACQUISITION exp dpr 40
sfrq 199.988 dpr 40
tn H1 dnm nm
at 5.000 dmf 10785
np 30004 temp 30.0
sw 30003 lb PROCESSING 0.20
bs 16 wtf file
tpwr 53 proc ft
pw 7.0 fn not used
di 5.000 werr
df 40.32 wexp
ct 32 wbs wft ds
alock gain not used
flags n
ij n
in n
dp n
sp DISPLAY -0.1
vs 1799.7
ws 450
sc 0
wc 250
hzmm 7.20
rf 17007.20
rf1 1552.1
rfp 1453.8
th 20
ins 100.000
nm cdc ph
  
```



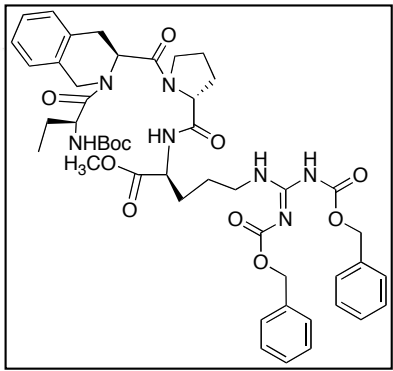
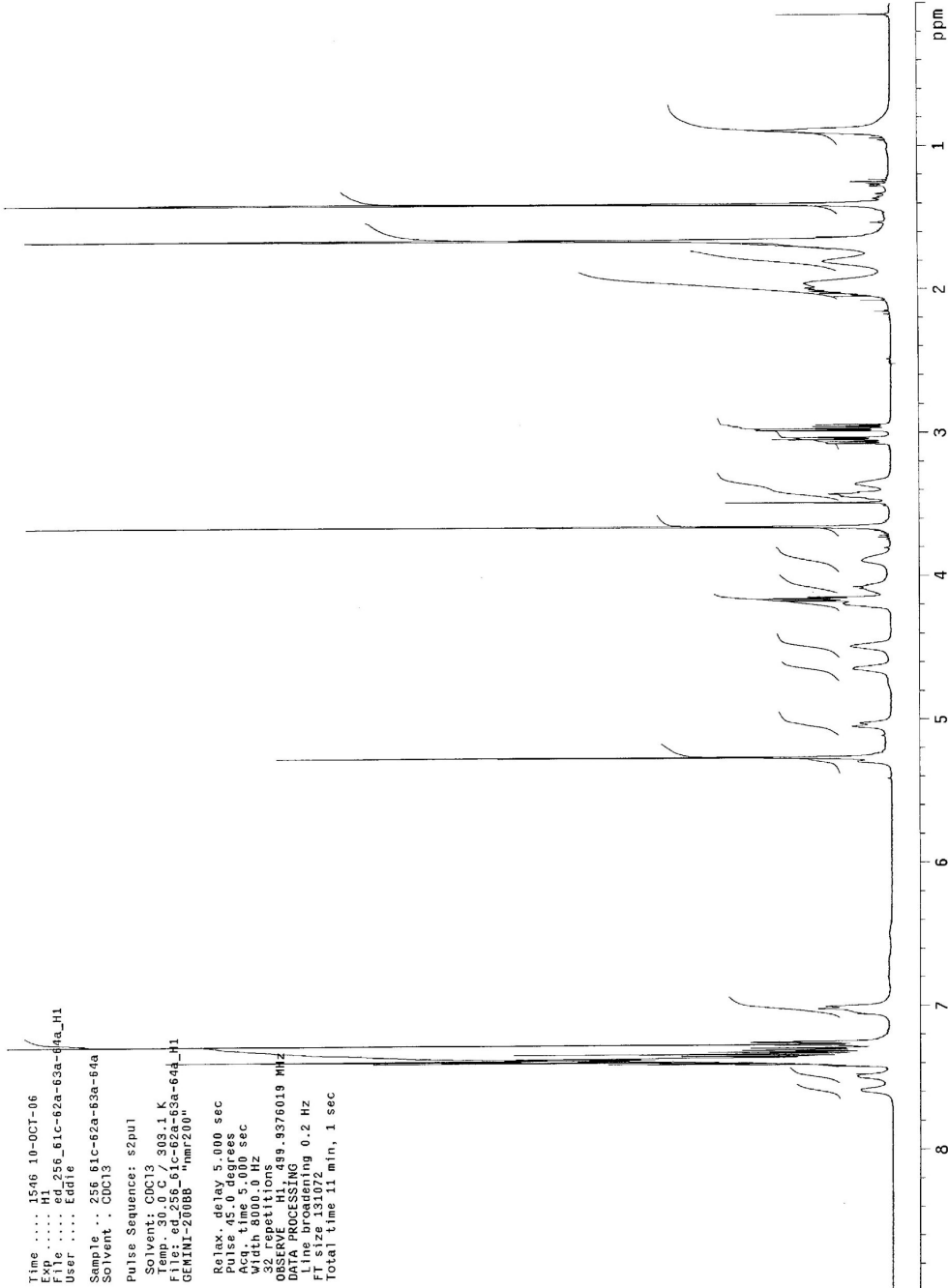
Compound 4 – NMR dipeptide 1c-2a



Compound 4 – NMR Dipeptide 3a-4a



Time .... 15:46 10-OCT-06  
 Exp ..... H1  
 File .... ed\_256\_61c-62a-63a-64a\_H1  
 User .... Eddie  
 Sample .. 256 61c-62a-63a-64a  
 Solvent . CDCl3  
 Pulse Sequence: s2pu1  
 Solvent: CDCl3  
 Temp. of C 303.1 K  
 File .. ed\_256\_61c-62a-63a-64a\_H1  
 GEMINI-200BB "hmr200"  
 Relax. delay 5.000 sec  
 Pulse 45.0 degrees  
 Acq. time 5.000 sec  
 Width 8000.0 Hz  
 F2 300.136300 MHz  
 OBSERVE H1 499.9376019 MHz  
 DATA PROCESSING  
 Line broadening 0.2 Hz  
 FT size 131072  
 Total time 11 min, 1 sec

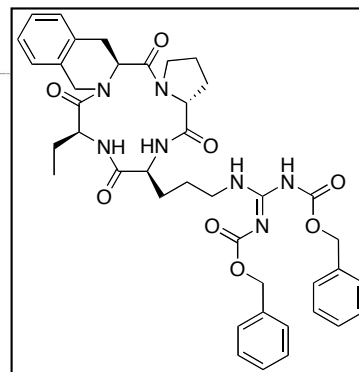
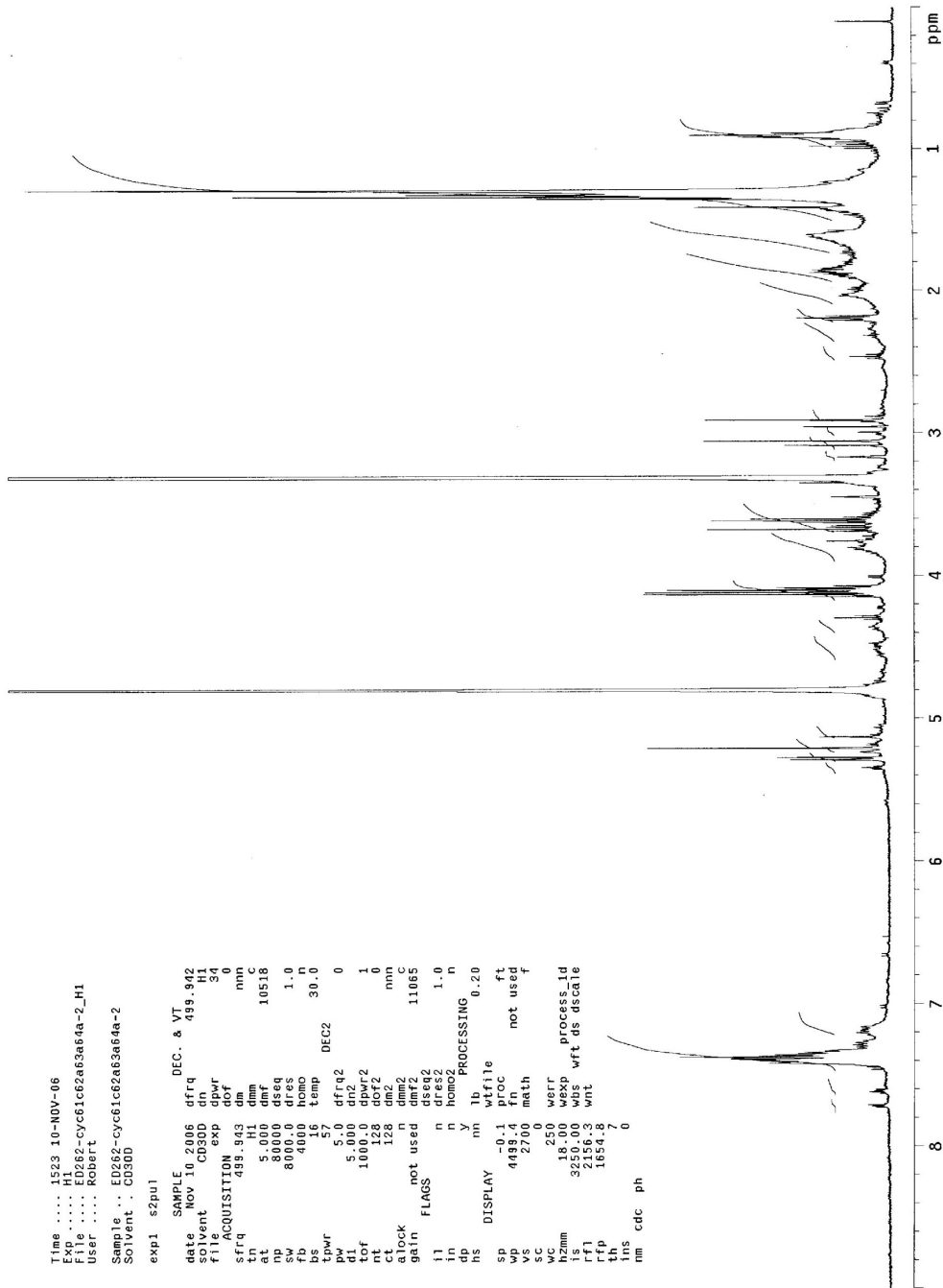


**Compound 4 – NMR linear tetrapeptide 1c-2a-3a-4a**

```

Time ..... 1523 10-NOV-06
File ..... ED262-cyc61c62a63a64a-2_H1
User ..... Robert
Sample .. ED262-cyc61c62a63a64a-2
Solvent : CD3OD
exp1 s2pul
SAMPLE
date NOV 10 2006 dfrq DEC. & VT 499.942
solvent CD3OD dn 499.942
file exp 34
ACQUISITION
sfrq 499.943 dm min
at 5.000 dmf 10518
np 8000.0 dseq
sw 8000.0 dres 1.0
fb 4000.0 homo
ts 157 temp DEC2 30.0
pw 5.0 dfrq2 0
d1 5.000 dn2
tof 1000.0 dpwr2 1
ct 128 do2
cs 128 dm
gain not used dmf2 11065
FLAGS
il n dseq2
in y dres2 1.0
dp homo
hs y PROCESSING 0.20
DISPLAY
sp -0.1 proc
ve 449.942 ft
vs 2760 math not used
wc 0
werr 250
h2mm 18.00 wexp process_id
rf 3216.03 wds wft ds dscale
rfp 1654.8 wnt
th 1654.7
ins 0
nm cdc ph

```

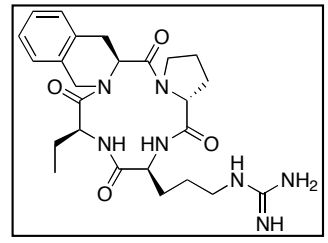
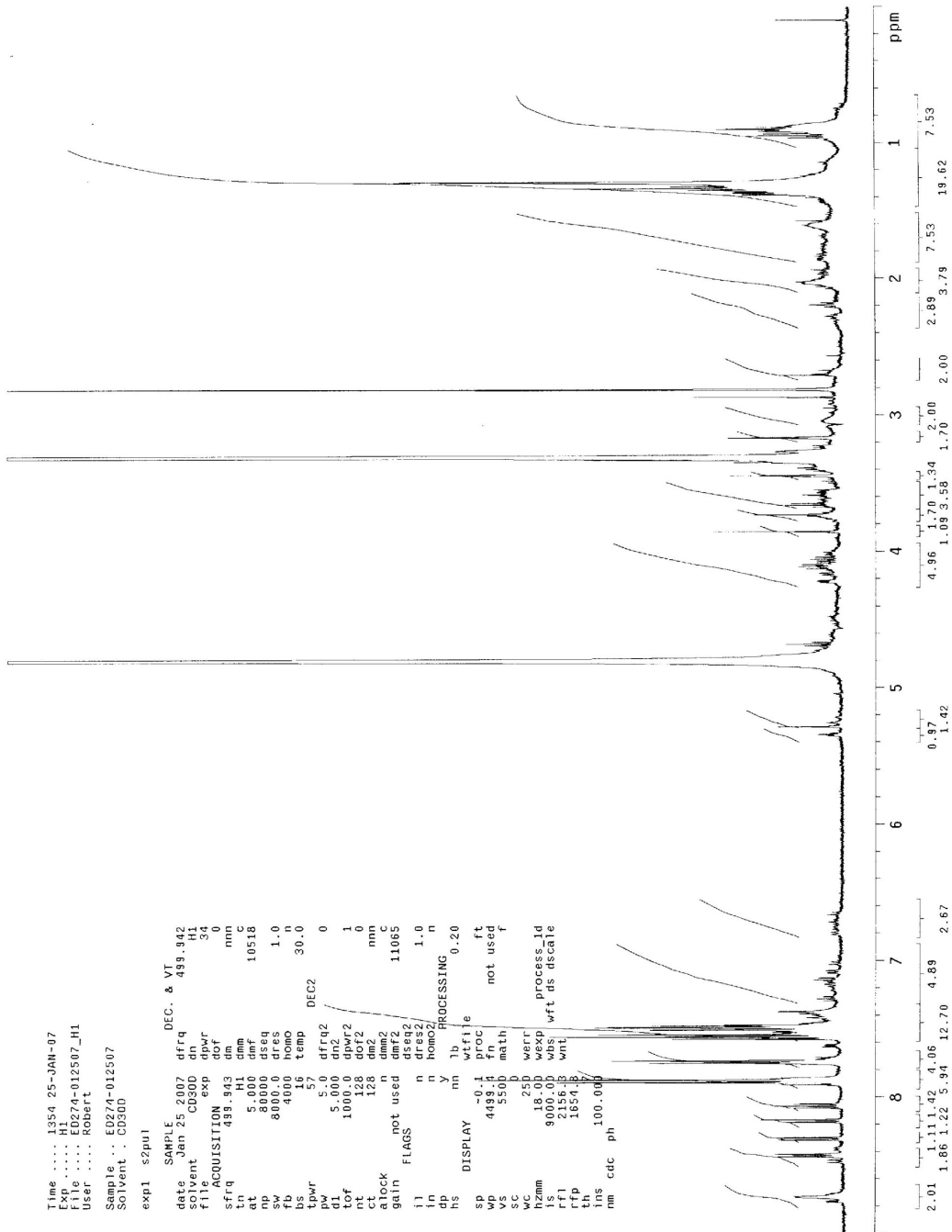


Compound 4 – NMR cyclized protected 1c-2a-3a-4a

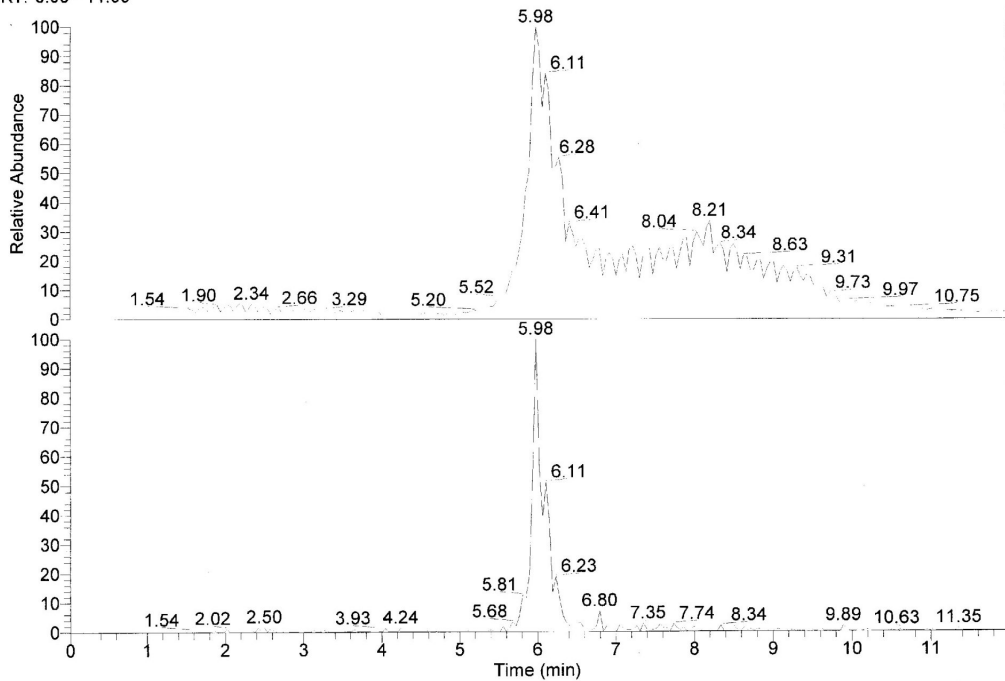
Time .... 1354 25-JAN-07  
 File ..... E0274-012507\_H1  
 User ..... Robert  
 Sample .. E0274-012507  
 Solvent . CD300

```

exp1 s2pul
SAMPLE
date      25 2007      DEC. & VT
solvent   CD300      dn      439.942
file      exp        dpwr    34
ACQUISITION
sfreq    439.943    dm      mm
at       5.000      dmf     10518
np       80000      dseq    1.0
sw       8000.0     dres    0
fb       4000      homo    30.0
tpwr     57        temp    DEC2
pw       5.0       dffrq2  0
d1       5.000     dnt     0
tof      1000.0    dpr2    1
ct       128      dor2    0
ct       128      dor2    0
alock    not used  dm2     11065
gain     not used  dmf2    11065
FLAGS
il       n         dseq2   1.0
il       n         dres2   1.0
dp       y         homo    0.20
hs      DISPLAY   nn      lb
sp      4459.1    proc    ft
vs      5500     math    not used
sc      b
wc      25D     weff
hzmm   9.18e-06  wexp
rf1    9265.8   wds
rfp    1654.8   whl
th
lms    100.000
nm     cdc     ph
  
```



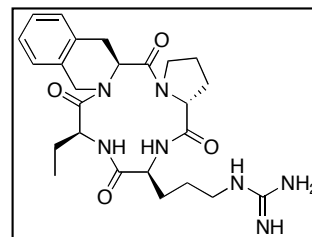
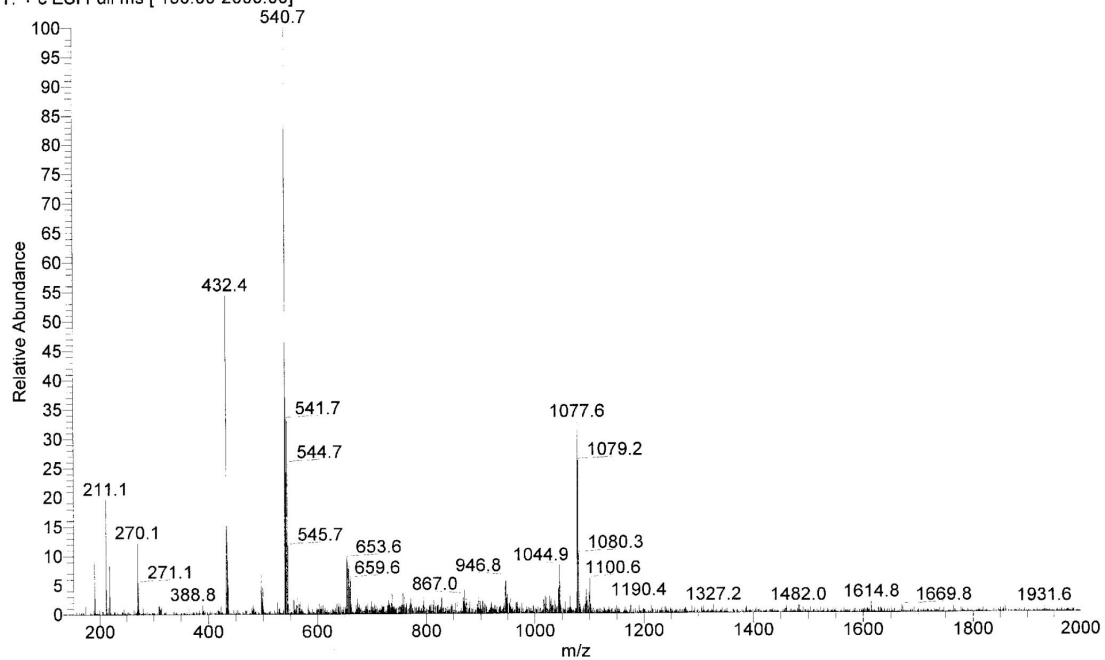
Compound 4 – NMR cyclized 1c-2a-3a-4a



NL:  
2.01E7  
m/z=  
150.0-  
2000.0 MS  
ed174\_hplc  
52

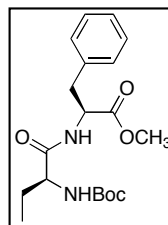
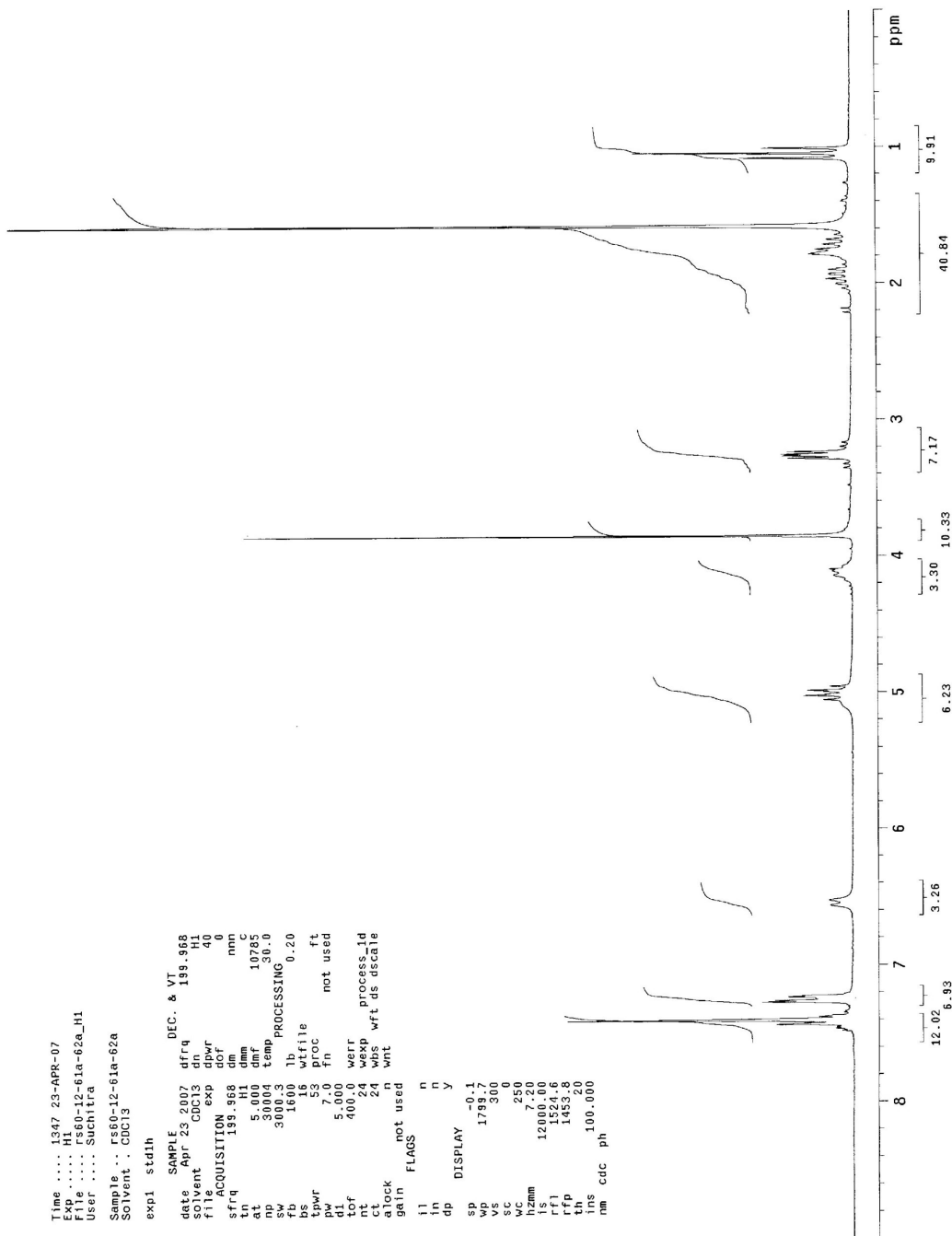
NL:  
2.16E5  
m/z=  
496.0-  
498.0 MS  
ed174\_hplc  
52

ed174\_hplc52 #113 RT: 5.98 AV: 1 NL: 1.27E6  
T: + c ESI Full ms [ 150.00-2000.00]



Compound 4 - LCMS cyclized 1c-2a-3a-4a

Time .... 1347 23-APR-07  
 File .... rs60-12-61a-62a\_H1  
 User .... Suchitra  
 Sample ... rs60-12-61a-62a  
 Solvent . CDC13  
 expl stadih  
 SAMPLE DEC. & VT  
 date Apr 23 2007 dfrq 199.968  
 solvent CDC13 dn 199.968 H1  
 file exp 40  
 ACQUISITION exp 40  
 sfrq 199.968 nmC  
 at 5.000 dmf 10785  
 np 3000.4 temp 30.0  
 sw 10.0 lb PROCESSING 0.20  
 fo 16 wf file  
 tpr 53 proc ft  
 pw 7.0 fh not used  
 dl 5.000 warr  
 tof 400.0 wexp  
 ct 24 wps process\_id  
 alock not used n wnt  
 gain not used n  
 FLAGS n n  
 in n  
 n y  
 dp DISPLAY  
 sp 0.1  
 wp 1793.0  
 wv 300  
 sc 0  
 wc 250  
 hzmm 1200.720  
 ls 102.00  
 rfp 1453.8  
 th 20  
 ins 100.000  
 nm cdc ph



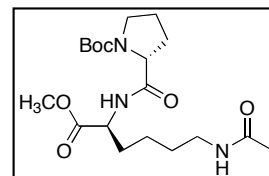
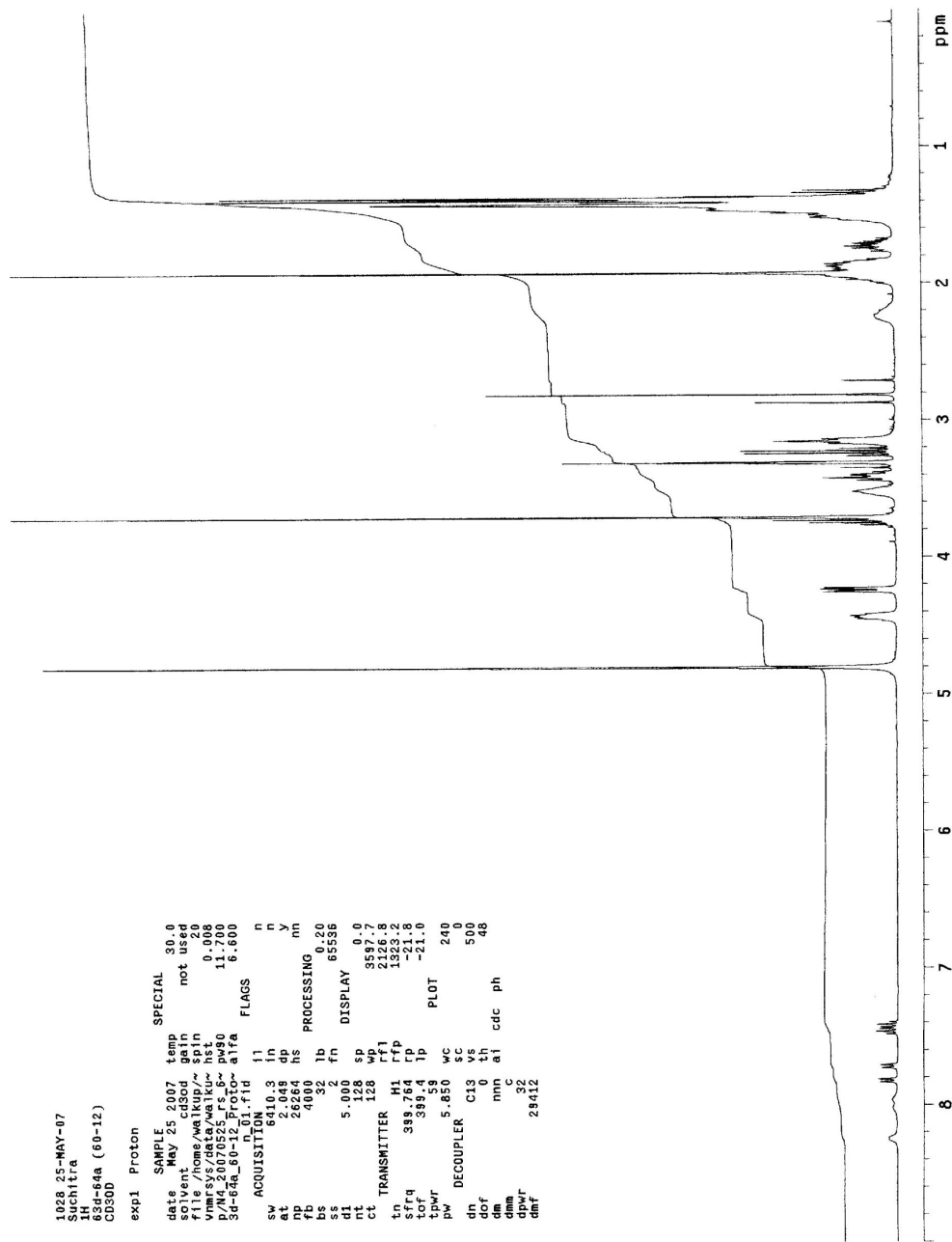
Compound 5 – NMR dipeptide 1a-2a

1028 25-MAY-07  
 Suchitra  
 3b-4a  
 CD300

exp1 Proton

```

SPECIAL 30.0
date MAY 25 2007 temp not used
solvent cd3od gain
file /home/waikup/~ spin 0.20
vmrsvs/data/waikup/hs 13.700
3b-4a_50-12 Proton- atfa 16.600
n_01.fid FLAGS
ACQUISITION 11 n
sw 6410.3 n
st 26284 n
fb 4000 hs PROCESSING mn
bs 32 1b 0.20
ss 5.002 fn DISPLAY 0.0
dl 128 sp 9597.7
ct TRANSMITTER 128 wf1 2126.8
tn H1 rfp 1323.2
frq 399.64 fp -21.0
tpwr 59 tp PLOT
pw 5.850 wc 240
dn DECOUPLER C13 sc 0
dm vs 500
dnc vs 48
dmc nnn al cdc ph
dmm C
dpwr 32
dmf 28412
  
```



Compound 5 – NMR dipeptide 3b-4a

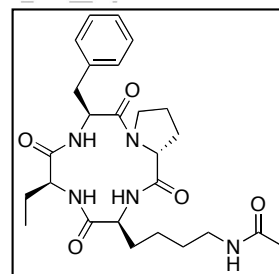
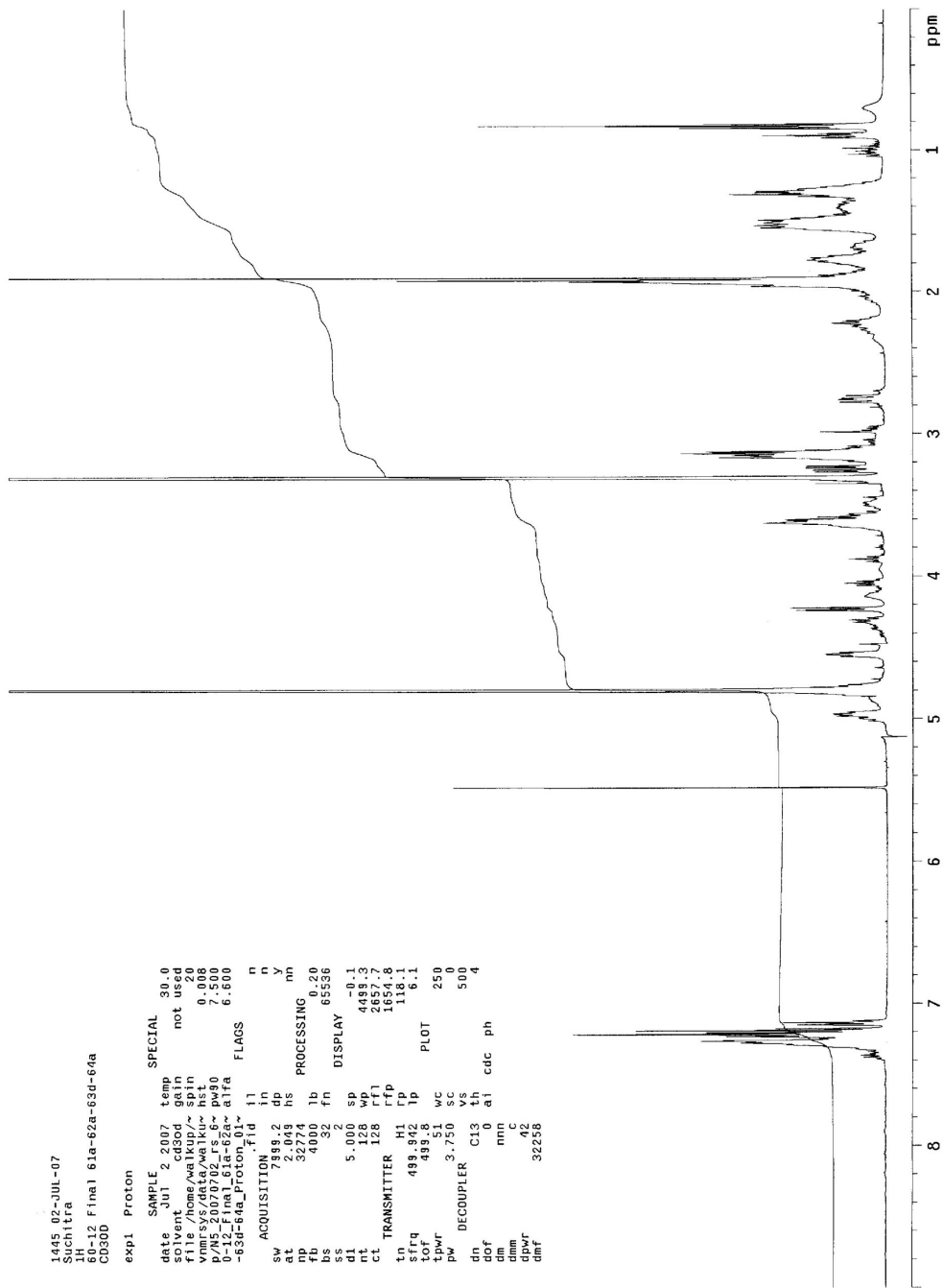




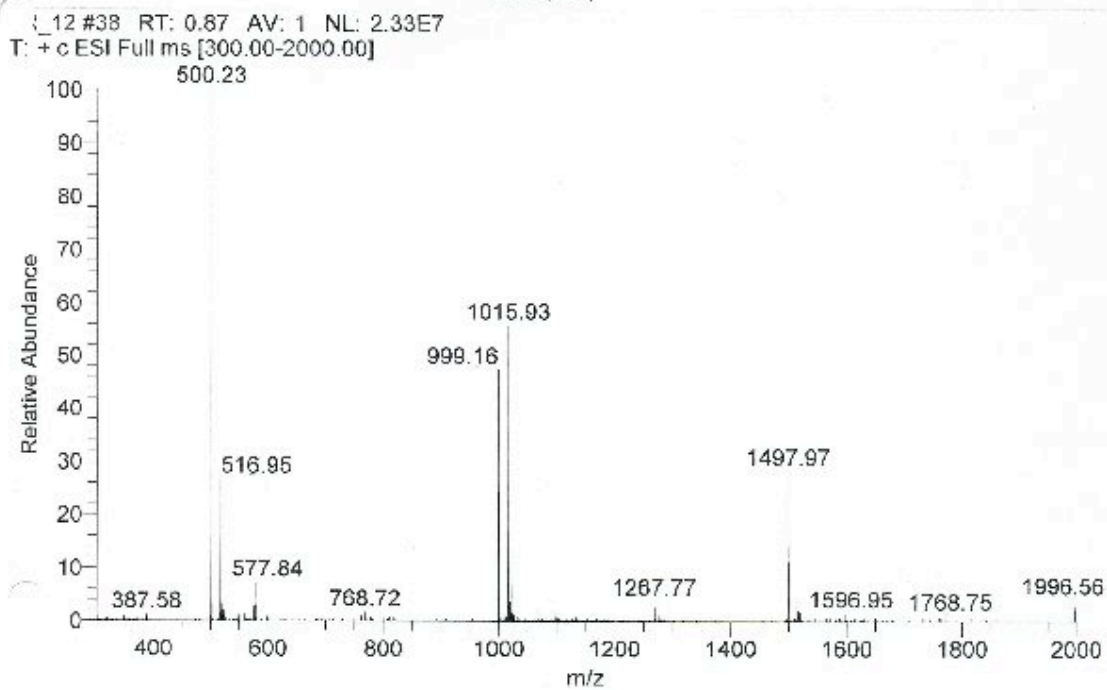
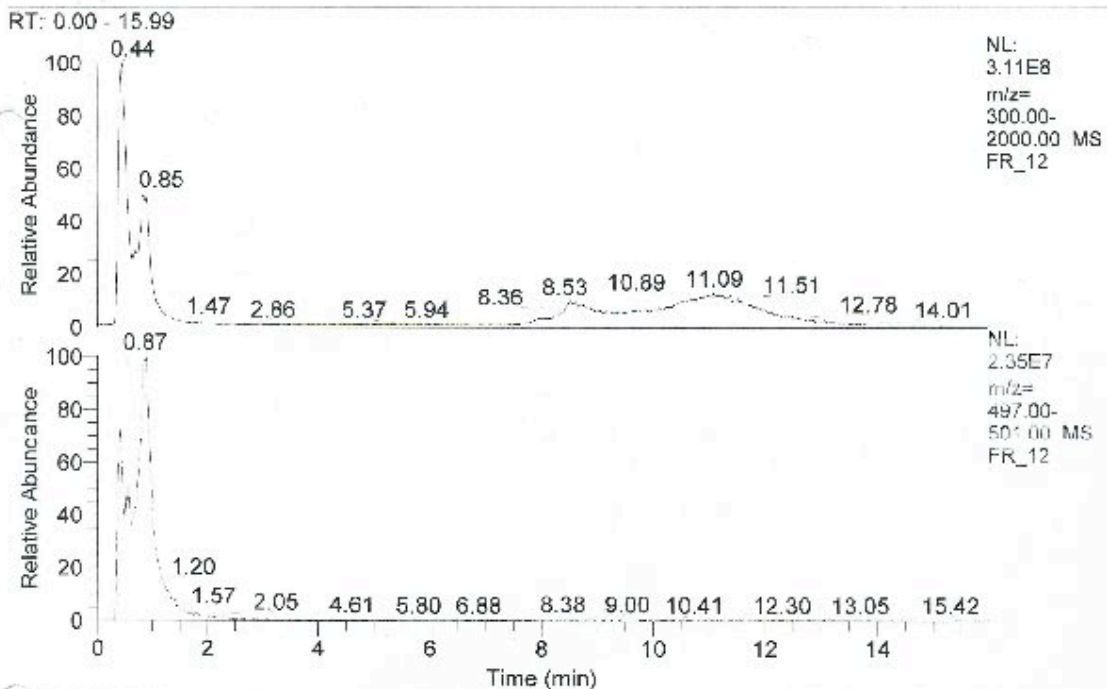
1445 02-JUL-07  
Schitra  
60-12 Final 61a-62a-63d-64a  
CD300

exp1 Proton

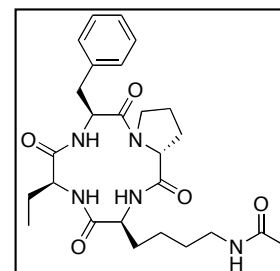
SPECIAL 30.0  
 date Jul 2 2007 temp not used  
 solvent cd300 gain  
 vmr/sv/da/ma/1k/ hst 0.008  
 p/N5\_200702\_fs\_6~ pw90 7.500  
 0-12\_Final\_61a-62a~ alfa 6.600  
 -63d-64a\_Proton\_01~ FLAGS  
 ACQUISITION f1d i1 n  
 sw 7999.2 dp y  
 at 2.043 hs  
 rp 32774 lb PROCESSING 0.50  
 bs 4092 fn 6556  
 ss 2  
 d1 5.000 sp -0.1  
 nt 128 wp 4489.3  
 ct 128 rfp 1654.6  
 tn TRANSMITTER H1  
 sfrq 499.942 tp 6.1  
 tof 499.8  
 lpwr 51 vc 250  
 pw 3.758 vs 500  
 DECOUPLER C13 th ai cdc ph  
 dn 0  
 dm rnm 42  
 dmr 42  
 dimf 32258



Compound 5 – NMR cyclized 1a-2a-3b-4a



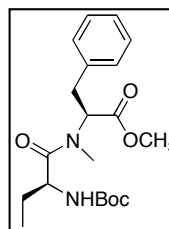
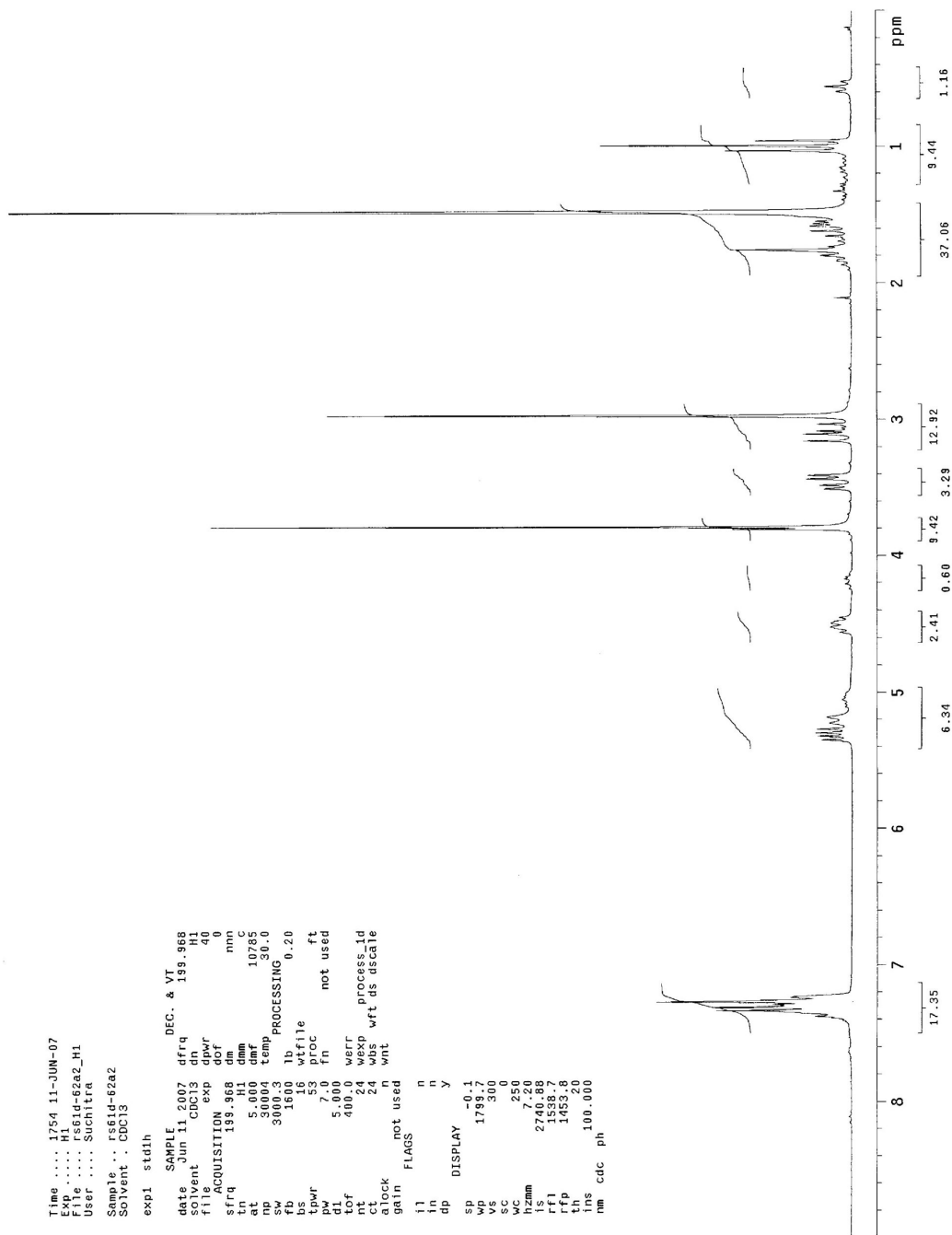
**Compound 5 – LCMS cyclized tetrapeptide**



Time .... 1754 11-JUN-07  
 File .... rs61d-62a2\_H1  
 User .... Suchitra  
 Sample .. rs61d-62a2  
 Solvent . CDCl3

```

exp1 stdih
SAMPLE          DEC. & VT
date    Jun 11 2007  dfrq   199.968
solvent  CDCl3      dn     H1
file     exp       dpwr   40
ACQUISITION  exp   dof    0
          199.968  dm     nmc
          5.000  dmf    10785
          30004  temp   30.0
          1600  lb     PROCESSING
          53    proc   0.20
          7.0  fn     not used
          5.000  werr
          400.0  wpr
          24    wds   wft ds dscle
          gain  n     wnt
          not used
          FLAGS  n
          in    n
          in    y
          dp   DISPLAY
          sp   -0.1
          wd   179300
          sc   300
          wc   250
          hzmm 7.20
          ls   2750.69
          rfp  1453.8
          th   100.000
          ins  100.000
          nm   cdc  ph
  
```

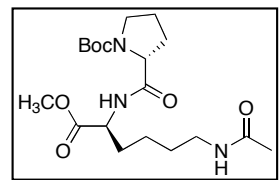
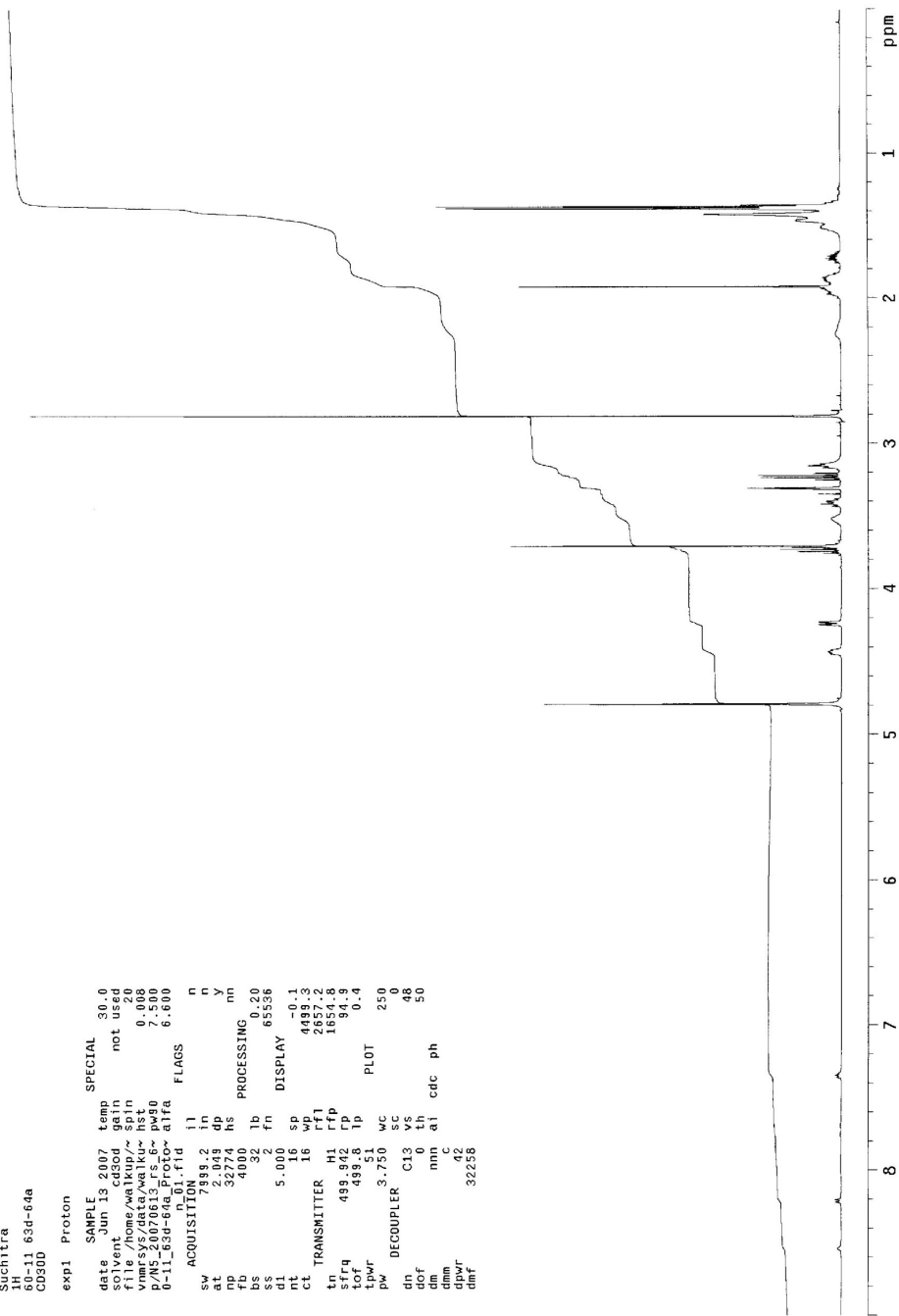


Compound 6 – NMR dipeptide 1d-2a

1144 13-JUN-07  
Schittka  
60-11 63d-64a  
CD300

exp1 Proton

```
SAMPLE          SPECIAL
date Jun 13 2007 temp 30.0
solvent none/na tdsol not used
gain 60
vmsys/da/mb/mst hst
p/MS_20070613_rs.6~ pw40 0.008
0-11_63d-64a_Proto~ alfa 7.500
001.fid i1 FLAGS 6.600
ACQUISITION
sw 7899.2 in n
at 2.049 dp y
np 32774 hs PROCESSING 0.20
tb 4000 lb
ss 32 fn 65536
d1 5.000 DISPLAY
nt 16 sp -0.1
ct TRANSMITTER H1 4491.3
tn 1654.2
sfrq 499.942 rfp 1654.8
tof 499.8 tp 0.4
tpwr 51 PLOT 250
pw DECOUPLER C13 VS 48
dn dof 0 th al cdc ph 50
dm 4
dimr 4
dntf 32256
```

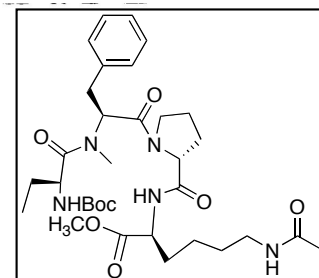
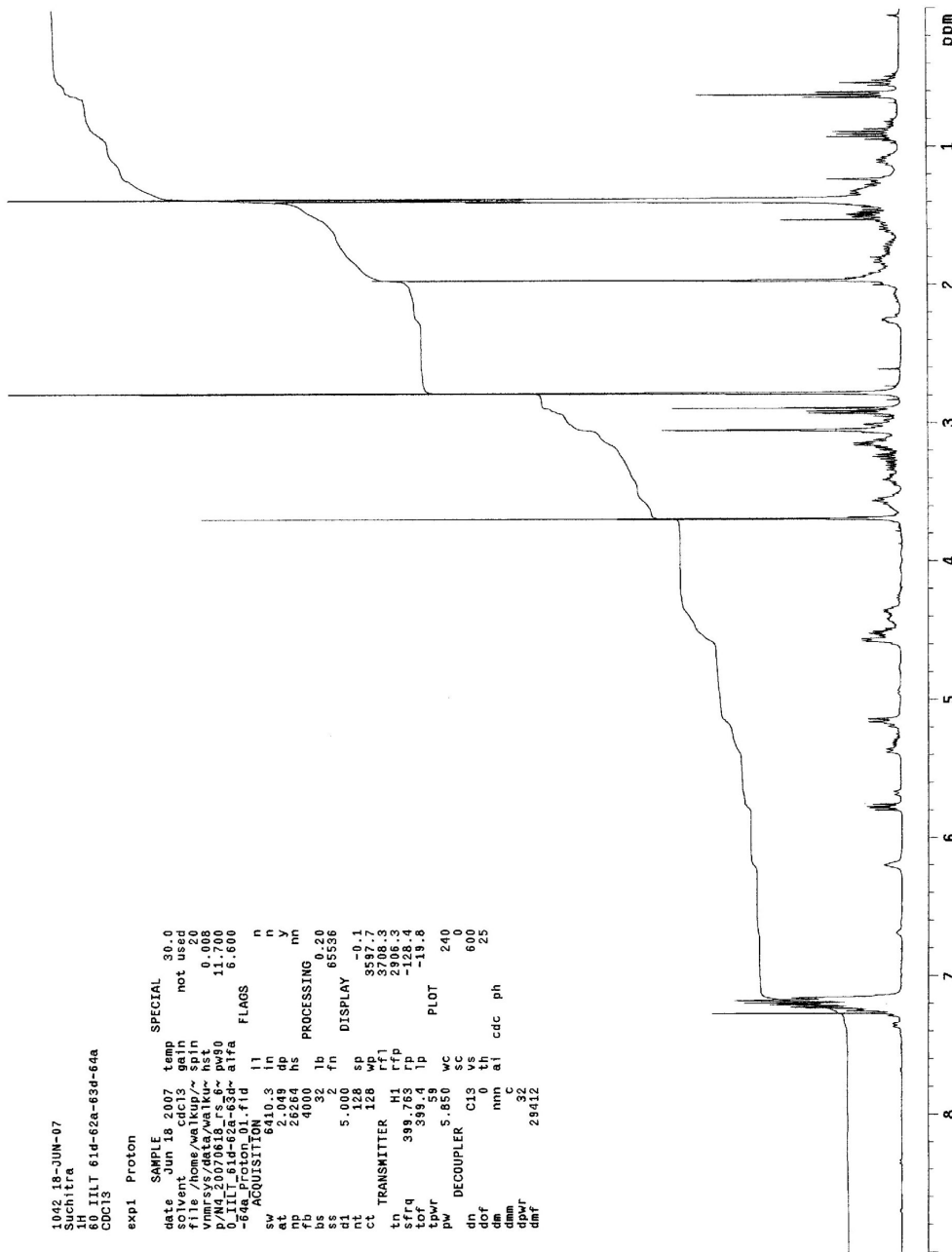


Compound 6 – NMR dipeptide 3-4a

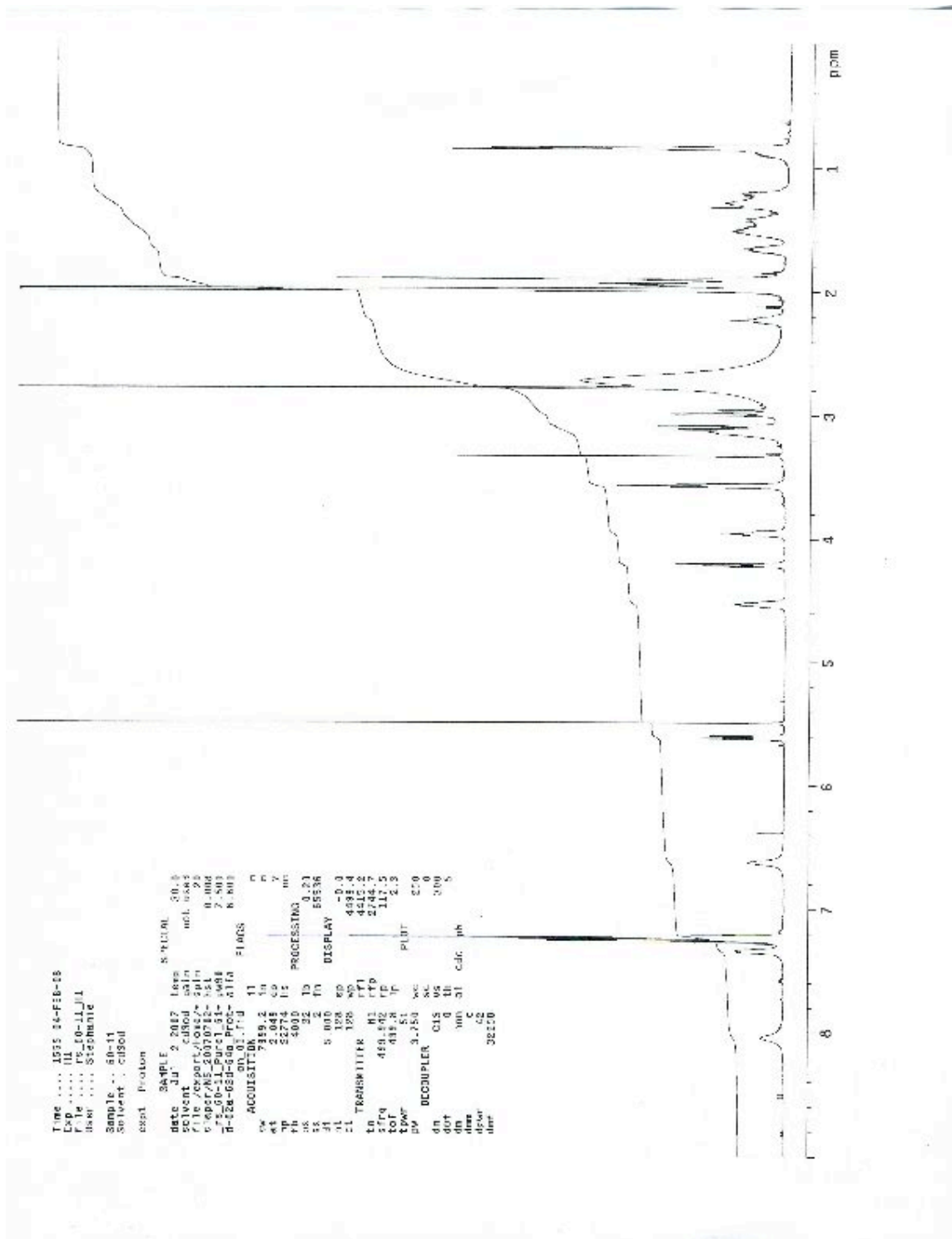
```

1002 18-JUN-07
Suchitra
60 IILT 61d-62a-63d-64a
CDC13
exp1 Proton
SAMPLE SPECIAL 30.0
date Jun 18 2007 temp 30.0
file /home/walkup/~ spin not used
vmr/sys/data/walkup/~ hst 0.008
p/MJ_20070618_1s_6~ pw90 11.700
0 IILT 61d-62a-63d-64a~ alfa 6.600
-64a~ f1d l1
ACQUISITION
sw 6410.3 in n
at 2.049 dp n
pp 26264 hs y
ss 4032 lb y
bs 2 fn 65536
d1 5.000 DISPLAY
nt 128 sp 850.1
ct TRANSMITTER H1 rfp 3768.3
tn 399.763 rfp 2906.3
sfrq 399.763 rfp -128.4
tpr 399.4 lp -19.8
pw 5.650 WC 240
DECOUPLER C13 vs 600
dof 0 th 25
dm nnn al cdc ph
dpr 32
dmf 29412

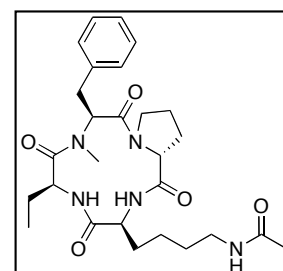
```

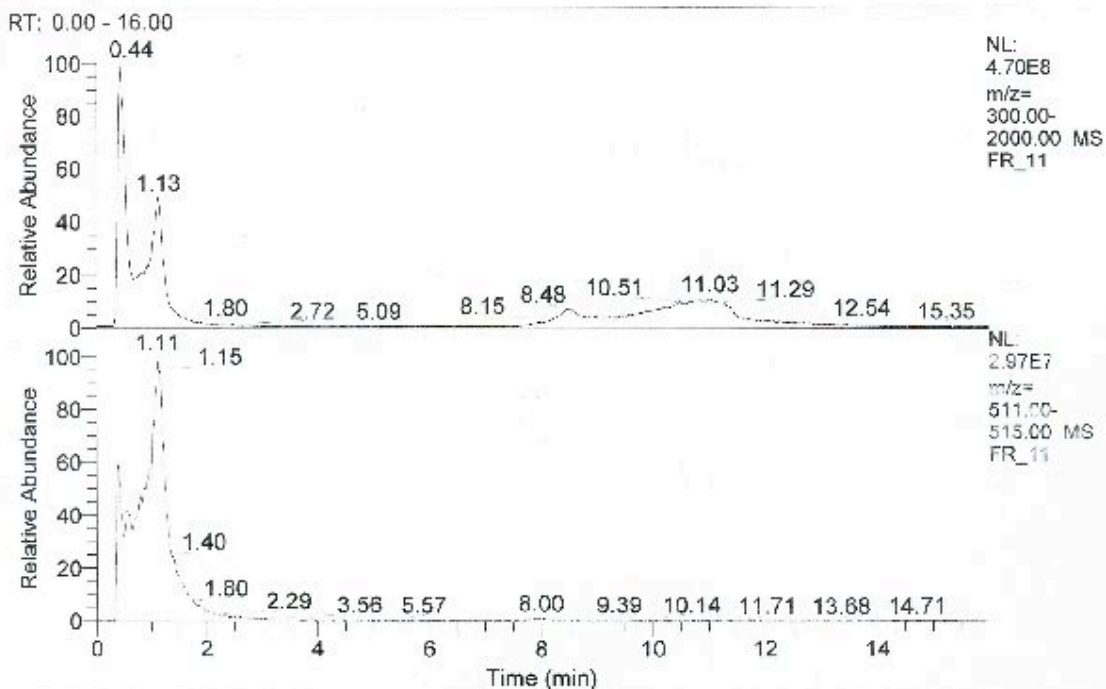


Compound 6 – NMR linear tetrapeptide 1d-2a-3b-4a

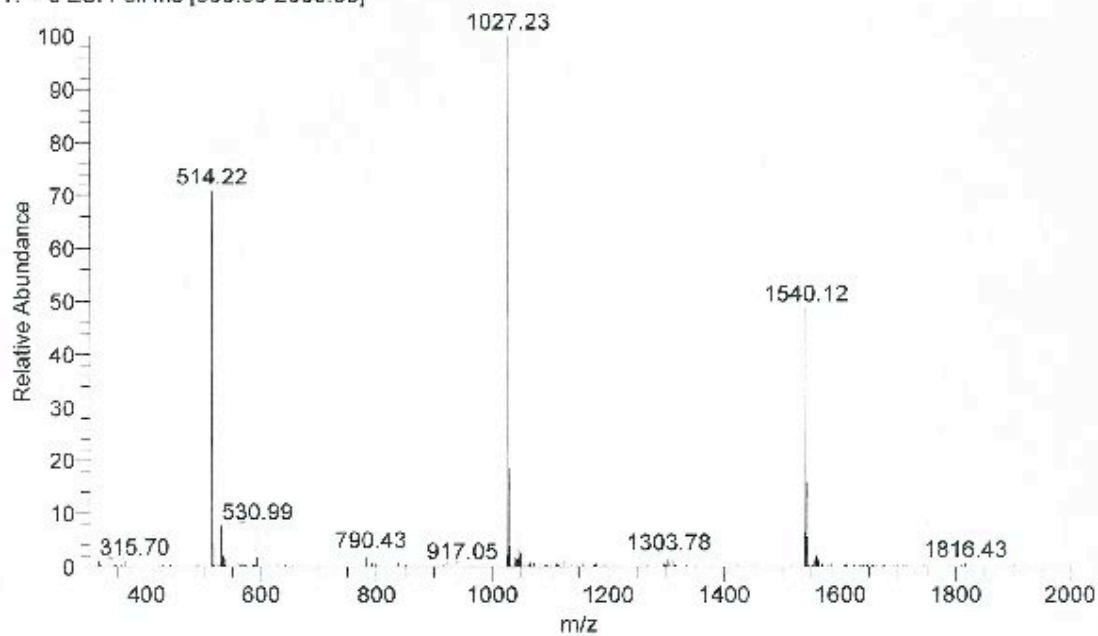


Compound 6 – NMR cyclized 1d-2a-3b-4a





FR\_11 #49 RT: 1.11 AV: 1 NL: 4.19E7  
 T: - c ESI Full ms [300.00-2000.00]



**Compound 6 – LCMS cyclized tetrapeptide**

