1) Experimental details for the synthesis of starting resins **1a-g**. Experimental details and ^{1}H NMR data for the synthesis of *N*-alkyl amino acid trifluoroacetate salts **5a-g** (Table 1).

Experimental Section. General. All reagents used are commercially available and were employed without further purification. Fmoc-aminoacid Wang resins (1% divinyl benzene-polystyrene), all aminoacids, and 2,4,5-trichlorophenyl formate were purchased from Novabiochem. All glassware employed in solid-phase reactions had been silanized (treatment with 10% TMSCl/toluene for 12h) and dried. Polypropylene filter vessels were obtained from Bio-Rad. Dried tetrahydrofuran for the reductions was obtained by distillation over sodium/benzophenone ketyl. NMR spectra were recorded on Bruker AM 300, or 200 MHz instruments. Compound purity analysis was carried out by RP-HPLC on a Hewlett-Packard 1100 system using conditions described in the manuscript. Yields of crude cleaved compounds are based on the mass balance based upon the starting loading level of commercial resins.

Typical synthesis of resin-bound *N*-formyl-amino acids. *N*-formyl-phenylalanine Wang resin (1d). A sample of N-Fmoc-phenylalanine Wang resin (1.00 g, 0.40 mmol/g substitution) in a polypropylene vessel was treated with 20% piperidine/DMF (5 mL, 3 min.; then 5 mL, 25 min.). After rinsing with DMF (3 x 5mL), the resin was suspended in dry DMF (5 mL) and 2,4,5- trichlorophenyl formate (0.27 g, 1.2 mmol) was added. The reaction vessel was shaken for 2h at rt, after which time the resin was washed successively with DMF, MeOH, and CH_2Cl_2 (3 times each), and dried under high vacuum for >12h to give resin **1d** (0.86 g). A resin sample gave a negative result on a Ninhydrin test.

Typical synthesis of resin-bound *N*-acyl-amino acids. *N*-Acetyl-valine Wang resin (1f). A sample of N-Fmoc-valine Wang resin (1.00 g, 0.64 mmol/g substitution) in a polypropylene vessel was deprotected and rinsed as above. Then, it was suspended in dry DMF (5 mL), then dry Et_3N (0.2 mL) and acetic anhydride (0.5 mL) were added successively. The reaction vessel was shaken for 2h at rt, after which time the resin was washed with DMF, MeOH, and CH_2Cl_2 (3 times each), and dried under high vacuum for >12h to give resin **1f** (0.84 g). A resin sample gave a negative result on a Ninhydrin test.

Typical procedure for Diborane Reduction and Oxidative Work-Up. *N*-Ethylvaline trifluoroacetate salt (5f). A portion of *N*-Acetyl-valine Wang resin (**1f**) (0.200 g, 0.14 mmol, 0.70 mmol/g substitution) was weighed in a 10 mL silanized round bottom flask and swelled in dry THF (1.5 mL) under nitrogen. The diborane solution

(1M/THF, 0.56 mL, 0.56 mmol) was added dropwise at rt over 2 min., after which time the flask was equipped with a condenser and the suspension stirred gently at 65 °C for 12h. Upon cooling to rt, the suspended resin was rapidly transferred by pipet (silanized) to a PP vessel by using dry THF to rinse out the flask and wash the resin. Then, dry THF (2.0 mL), anhydrous diisopropylethylamine (0.4 mL) and glacial acetic acid (0.8 mL) were added successively. To the homogenized suspension was added iodine (70 mg, 0.28 mmol, as a conc. THF solution) and the vessel was shaken for 1h. The vessel was then rinsed (THF) and the resin was washed (3X each) with THF, DMF/Et_3N 3:1, MeOH, CH_2Cl_2, and dried under high vaccuum for >12h to give resin **5f** (0.194 g). A resin sample gave a positive result on a bromophenol blue test.

The bulk of resin **5f** (0.188 g, ~0.70 mmol/g) was then transferred to a small round bottom flask and stirred in a 90% TFA/CH_2Cl_2 cocktail (2 mL) for 2h. The contents were filtered through a glasswool plug, the resin was rinsed with TFA, and the filtrate evaporated and dried over high vacuum for >12h to give crude trifluoroacetate salt **5f** as a white powder (23 mg, 69%). Its purity was estimated to >95% by ^{1}H NMR and RP-HPLC.

^{1}H NMR data for all *N*-alkyl amino acid trifluoroacetate salts 5a-g (Table 1):

***N*-Me-Ala (5a).** ^{1}H NMR (300 MHz, CD_3OD) δ 3.92 (q, *J* = 7.5 Hz, 1H), 2.70 (s, 3H), 1.54 (d, *J* = 7.5 Hz).

***N*-Me-Val (5b).** ^{1}H NMR (300 MHz, CD_3OD) δ 3,77 (d, *J* = 6.0 Hz, 1H), 2.73 (s, 3H), 2.30 (m, 1H), 1.13 (d, *J* = 7.0 Hz, 3H), 1.05 (d, *J* = 7.0 Hz, 3H).

***N*-Me-Ser (5c).** ^{1}H NMR (300 MHz, CD_3OD) δ 4.01 (d, *J* = 6.0 Hz, 2H), 3.86 (t, *J* = 6.0 Hz, 1H), 2.73 (s, 3H).

***N*-Me-Phe (5d).** ^{1}H NMR (300 MHz, CD_3OD) δ 7.4-7.2 (m, 5H), 4.23 (t, *J* = 6.0 Hz, 1H), 3.3 (m, 2H), 2.70 (s, 3H).

***N*-Et-Phe (5e).** ^{1}H NMR (300 MHz, CD_3OD) δ 7.4-7.2 (m, 5H), 4.23 (t, *J* = 6.0 Hz, 1H), 3.4-3.1 (m, 2H), 3.09 (q, *J* = 7.0 Hz, 2H), 1.29 (t, *J* = 7.0 Hz, 3H).

***N*-Et-Val (5f).** ^{1}H NMR (300 MHz, CD_3OD) δ 3.76 (d, *J* = 6.0 Hz, 1H), 3.09 (q, *J* = 6.0 Hz, 2H), 2.28 (m, 1H), 1.32 (t, *J* = 6.0 Hz, 3H), 1.14 (d, *J* = 7.0 Hz, 3H), 1.05 (d, *J* = 7.0 Hz, 3H).

***N*-Pr-Val (5g).** ^{1}H NMR (300 MHz, CD_3OD) δ 3.78 (d, *J* = 6.0 Hz, 1H), 2.97 (m, 2H), 2.30 (m, 1H), 1.75 (m, 2H), 1.15 (d, *J* = 7.0 Hz, 3H), 1.05 (d, *J* = 7.0 Hz, 3H), 1.00 (t, *J* = 6.5 Hz, 3H).

2) ^{1}H NMR spectra of crude *N*-alkyl amino acid trifluoroacetate salts **5a-g** (Table 1).

Following pages

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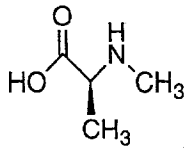

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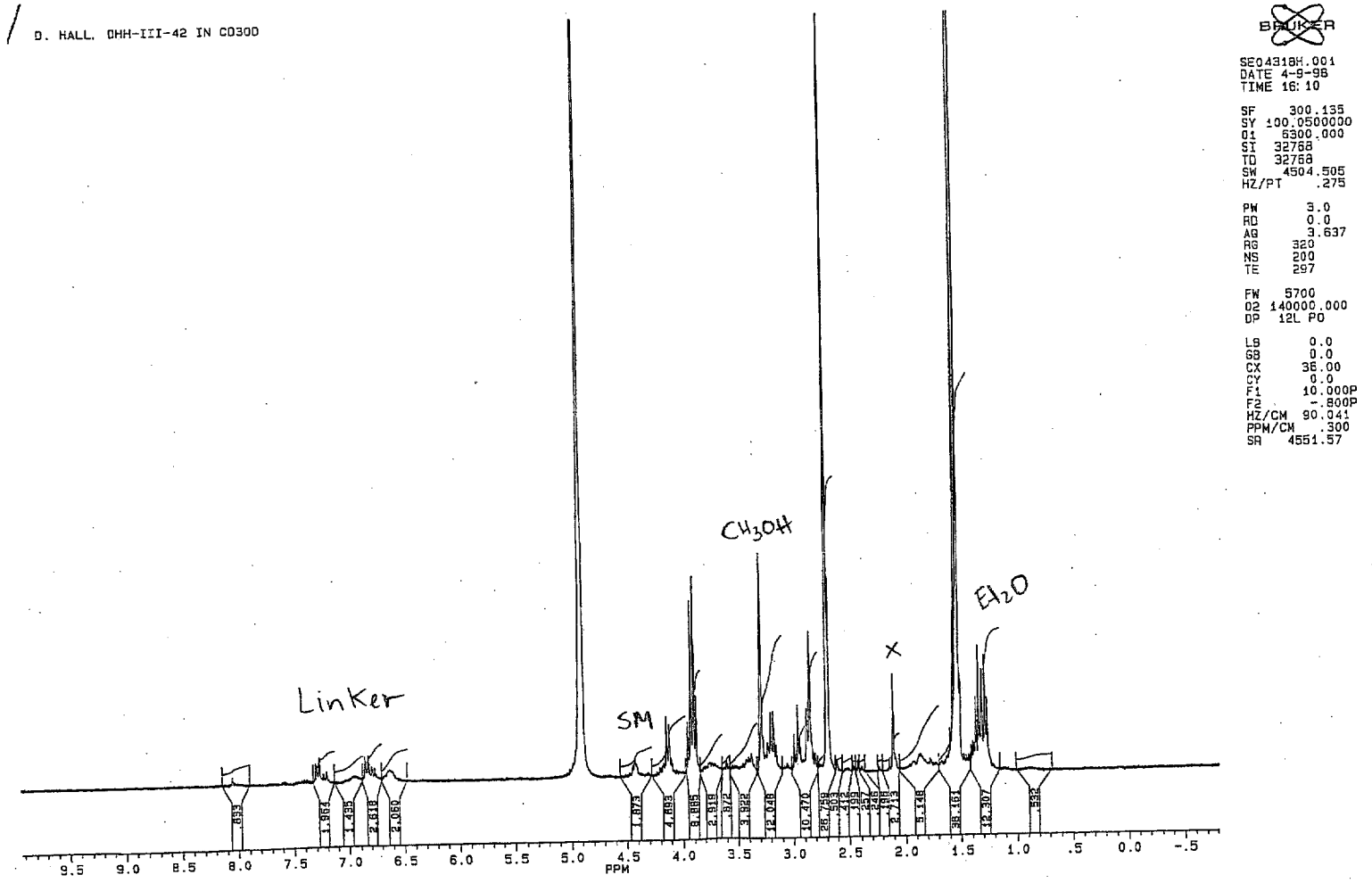

^{1}H NMR (CD_3OD)

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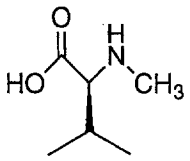

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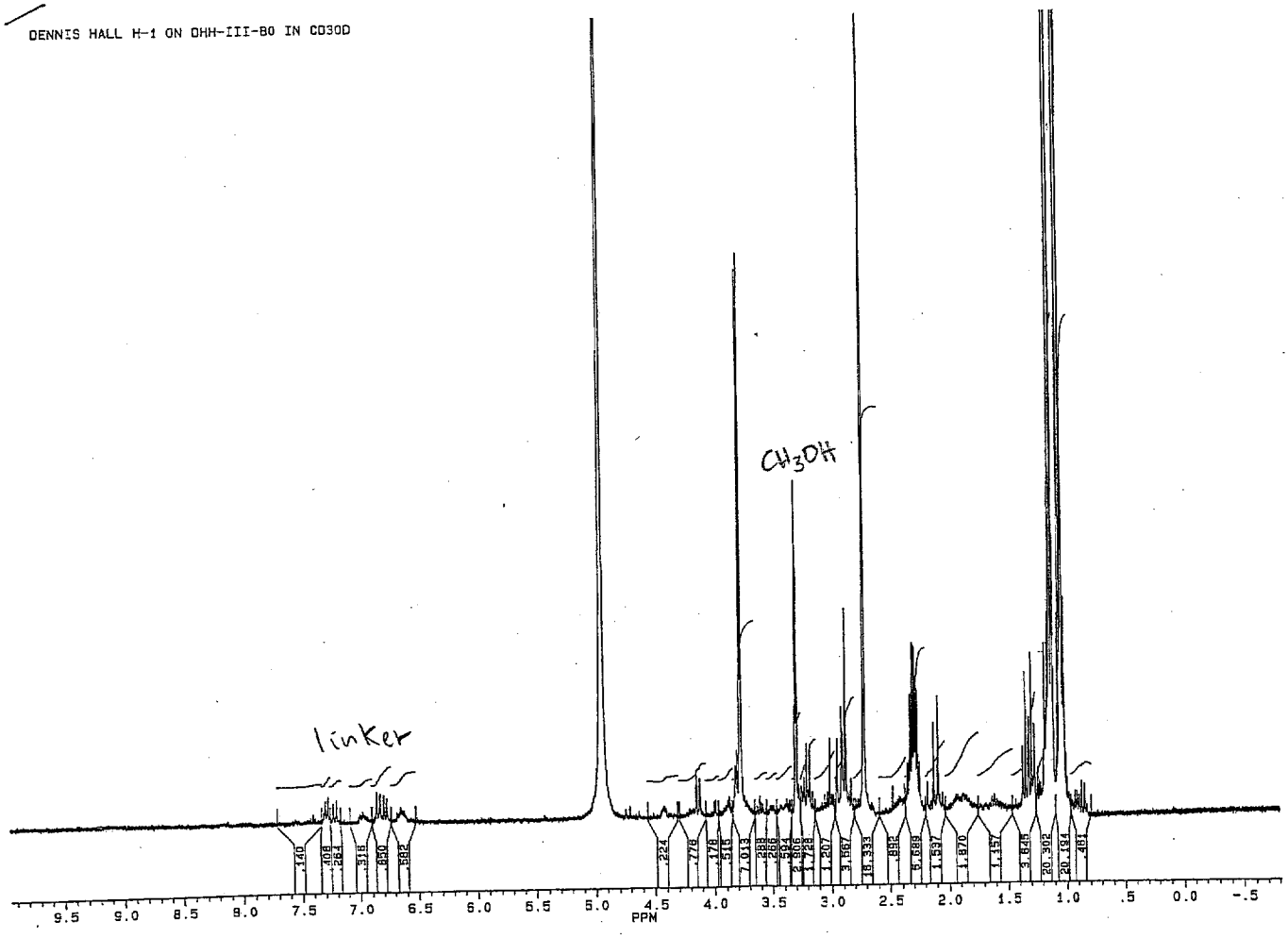

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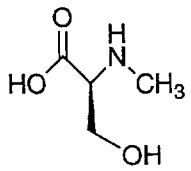
^{1}H NMR (CD_3OD)

5c (crude)



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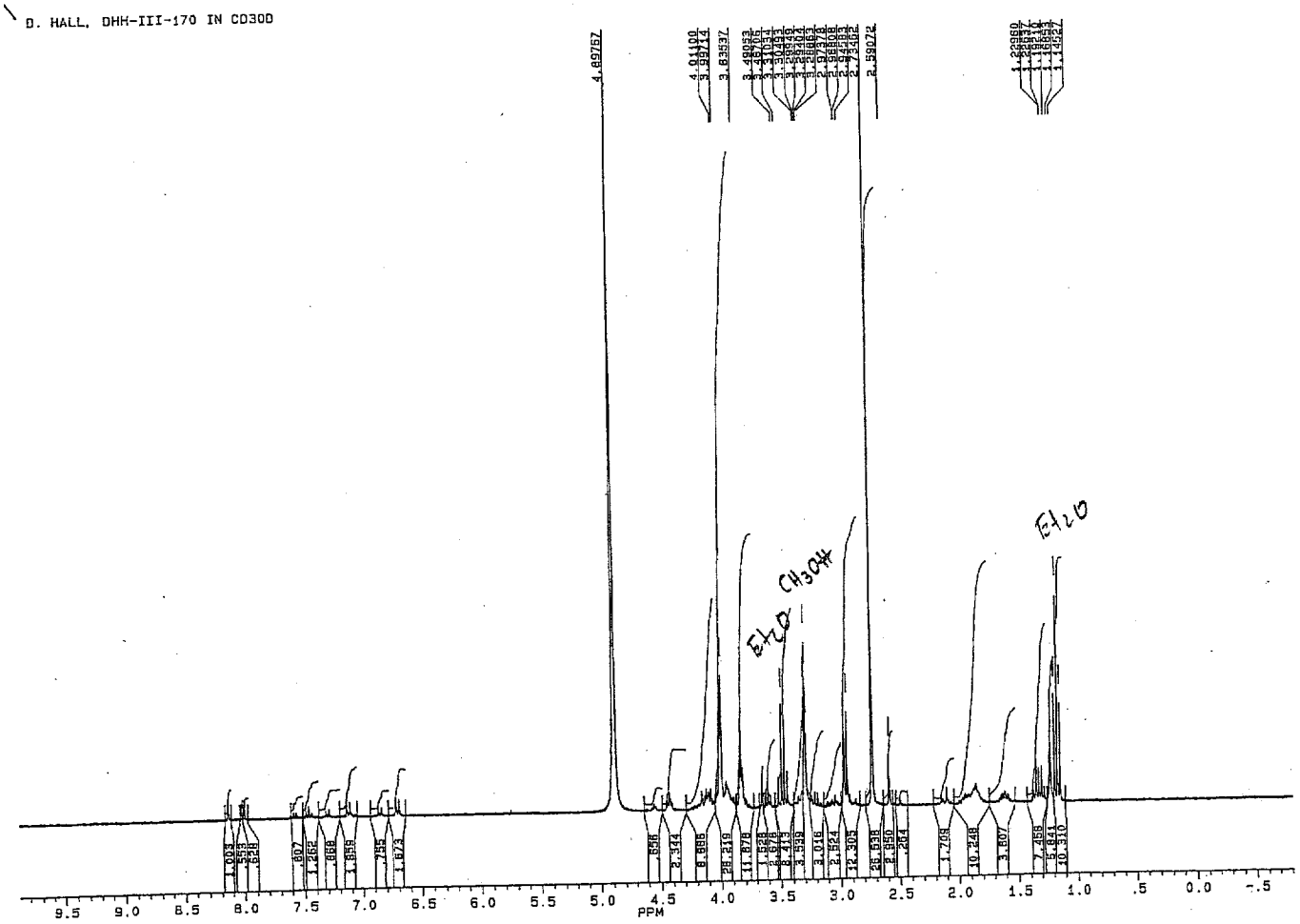

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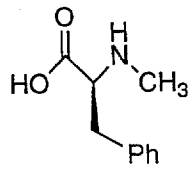
^{1}H NMR (CD_3OD)

5d (crude)



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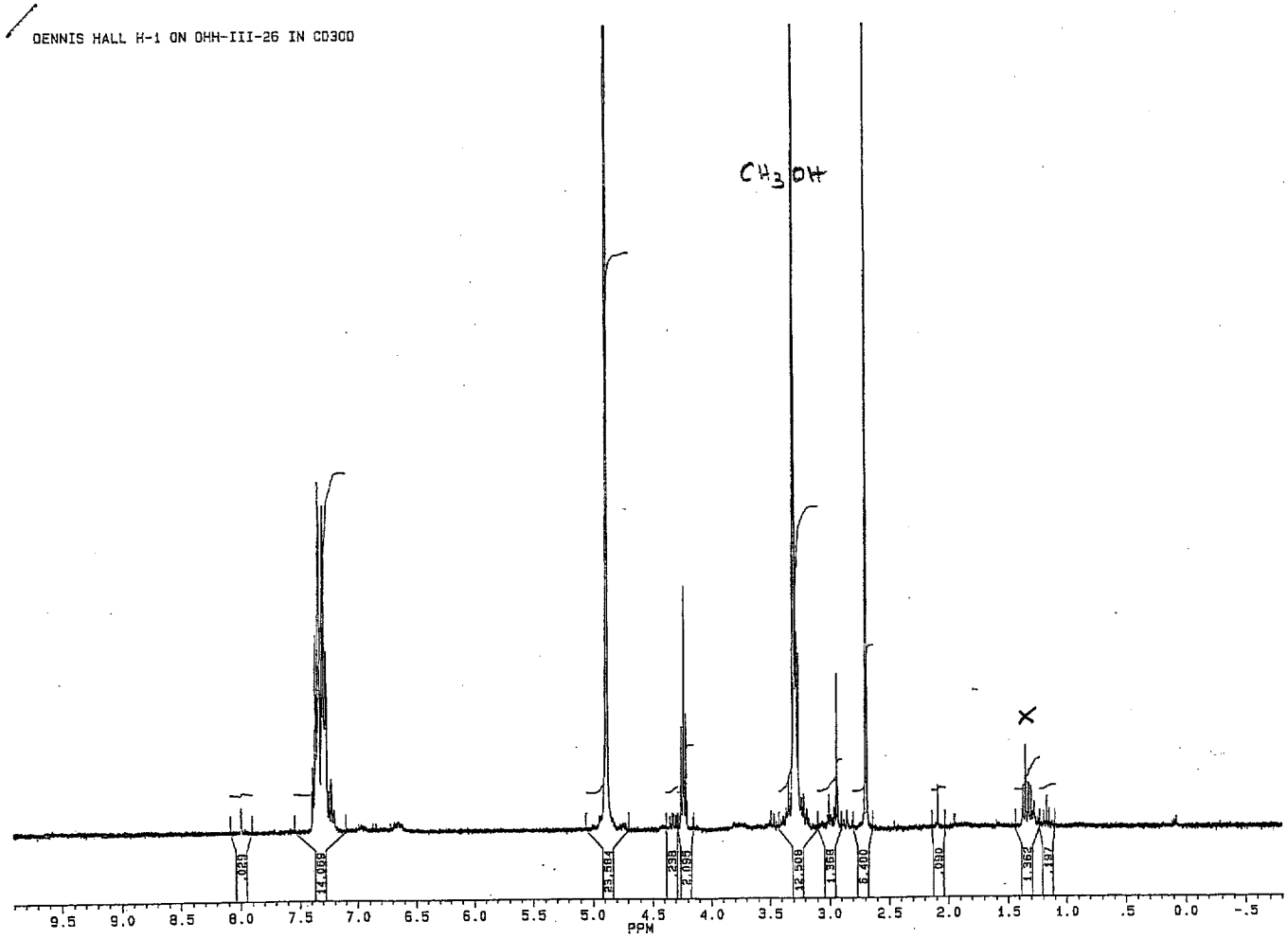

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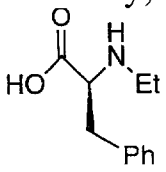
^{1}H NMR (CD_3OD)

5e (crude)



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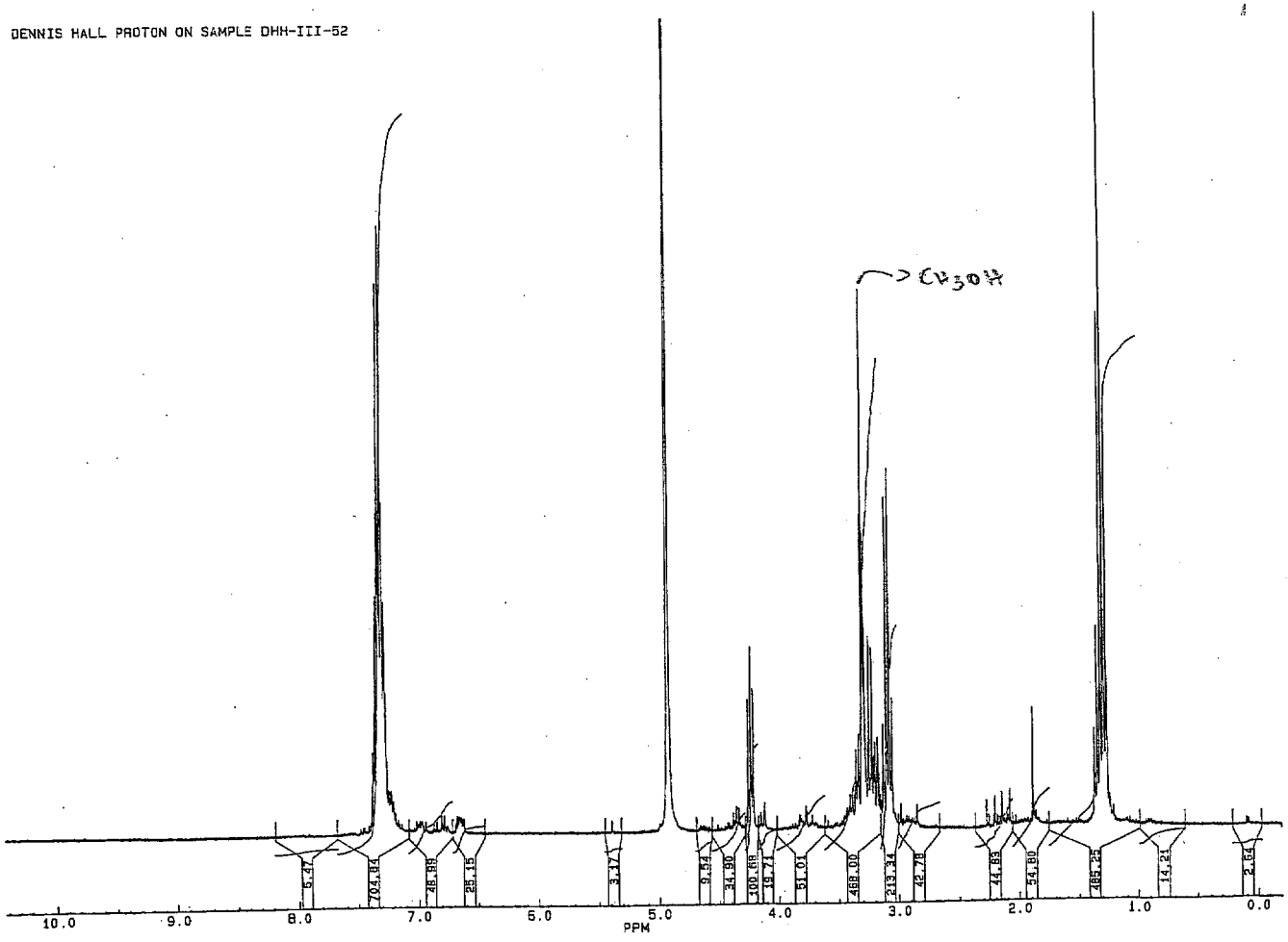

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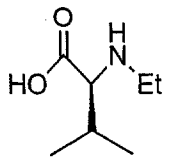
^{1}H NMR (CD_3OD)

5f (crude)



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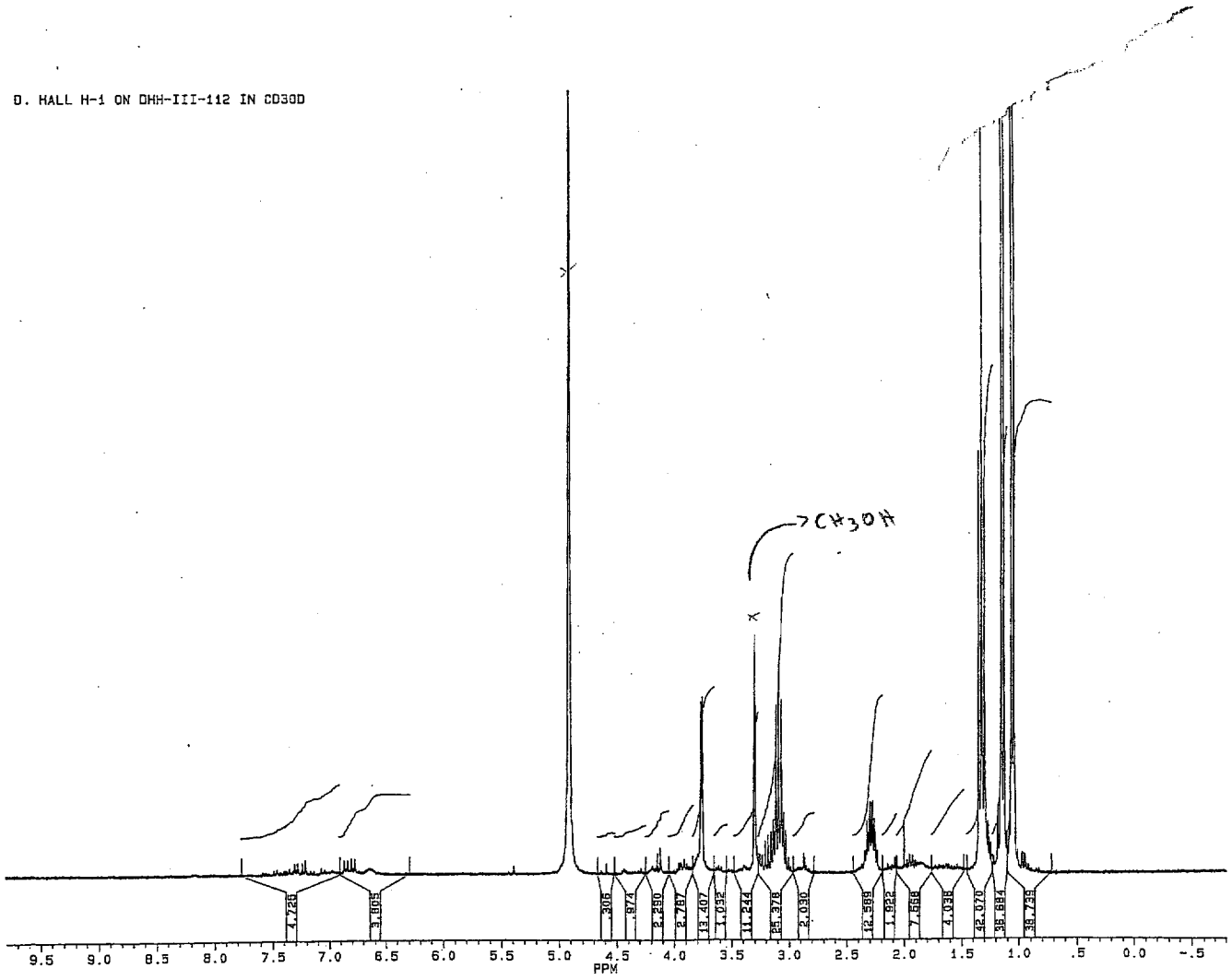

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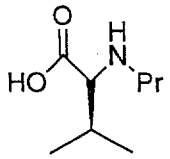

5g (crude)

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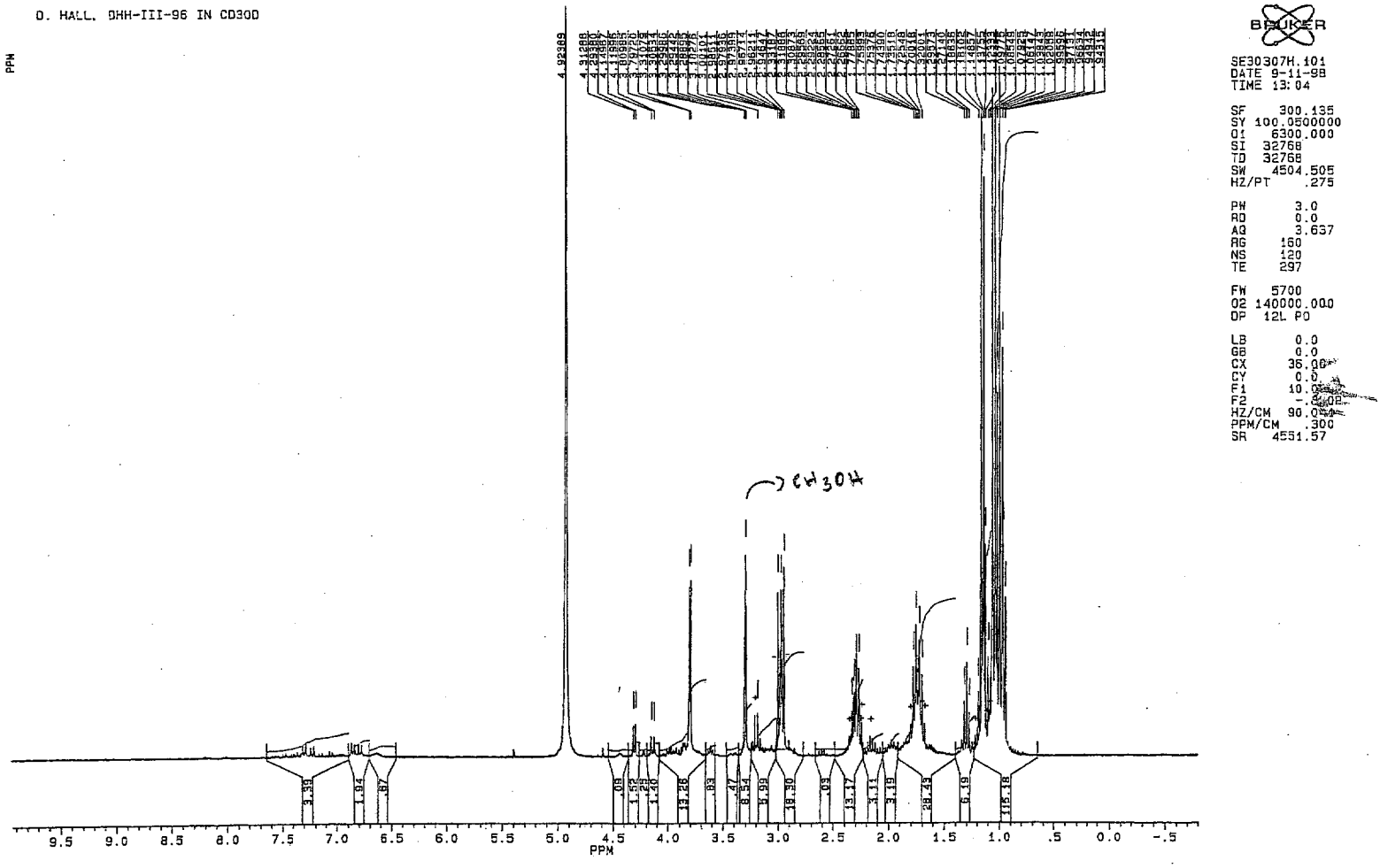

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3) Experimental details for the formation of synthetic Fmoc-MePhe-OH, its ^{1}H NMR spectra, and optical rotation measurement compared with an authentic sample.

Synthetic *N*-Fmoc-*N*-Methylphenylalanine. A sample of fresly prepared resin **4d** (0.170 g, 0.075 mmol, 0.44 mmol/g substitution) in a PP vessel was suspended in dry CH_2Cl_2 (2 mL). Diisopropylethylamine (0.075 mL, 0.45 mmol) and FmocCl (75 mg, 0.30 mmol) were added successively and the vessel was shaken 6h at rt. The resin was then washed (3X each) with CH_2Cl_2, DMF, CH_2Cl_2, and dried under high vaccuum for >12h. A resin sample gave a negative result on a bromophenol blue test.

The bulk of the resin (0.175 g) was then cleaved as above for compound **5f**. The crude synthetic sample of Fmoc-MePhe-OH was obtained as a white powder (27 mg, 88%). Although of good purity by ^{1}H NMR, it was further purified by preparative TLC on silica gel (0.5 mm, eluant: 5% MeOH/ CH_2Cl_2). The final purity was estimated to >95% by ^{1}H NMR and RP-HPLC.

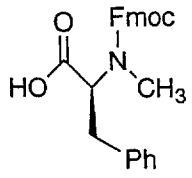
Optical rotation measurements (Perkin-Elmer 241 polarimeter, 589 nm, cell length 1 dm, at 25 °C):

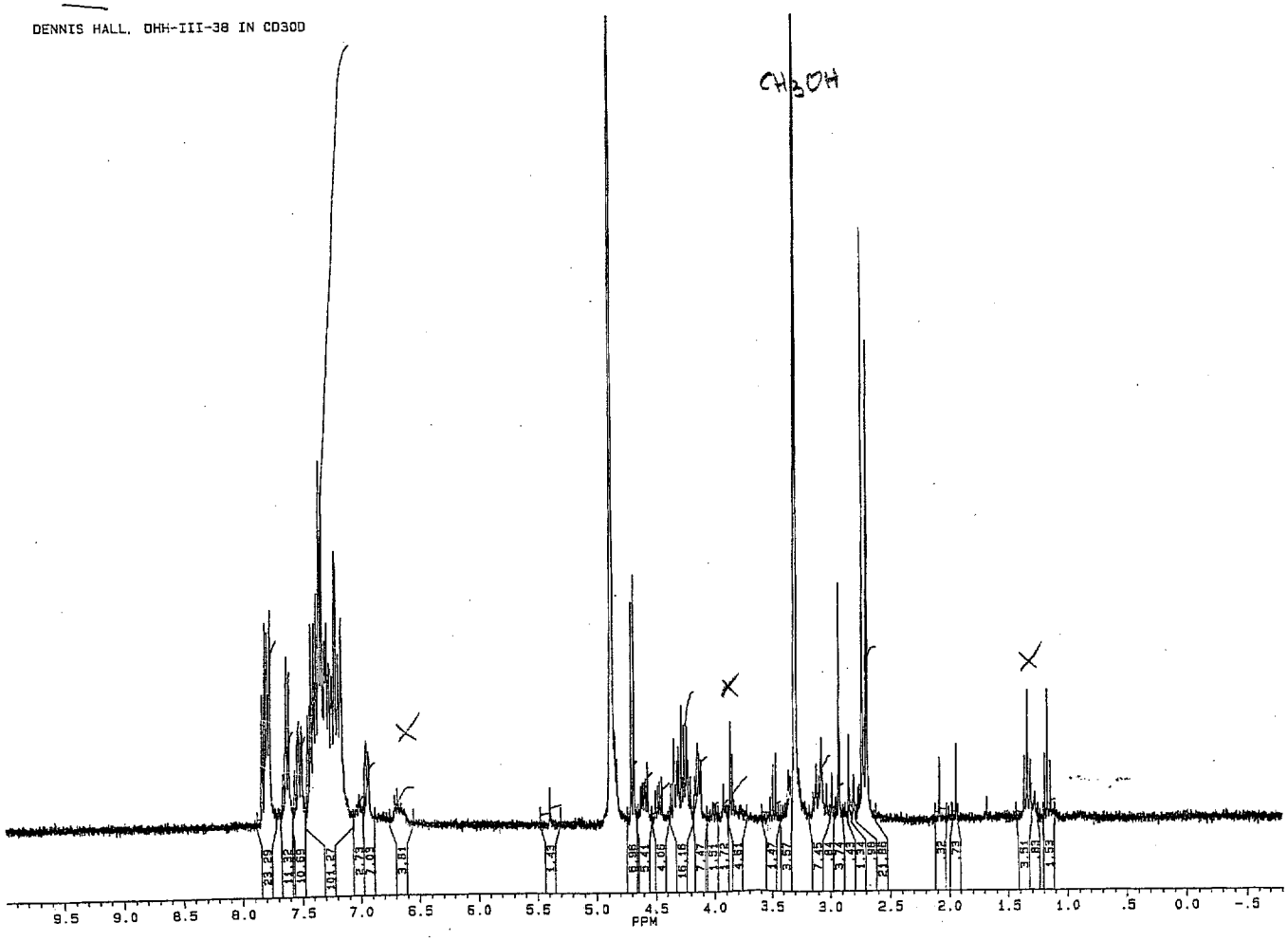
Synthetic Fmoc-MePhe-OH: 13.0 mg in 1.0 mL MeOH = -0.708°, $[\alpha]_D$ = -56°

Fmoc-MePhe-OH (Novabiochem): 15.0 mg in 1.0 mL MeOH = -0.899°, $[\alpha]_D$ = -60°

^{1}H NMR (CD_3OD)

Synthetic (crude)





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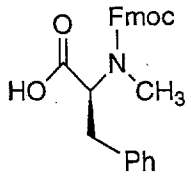
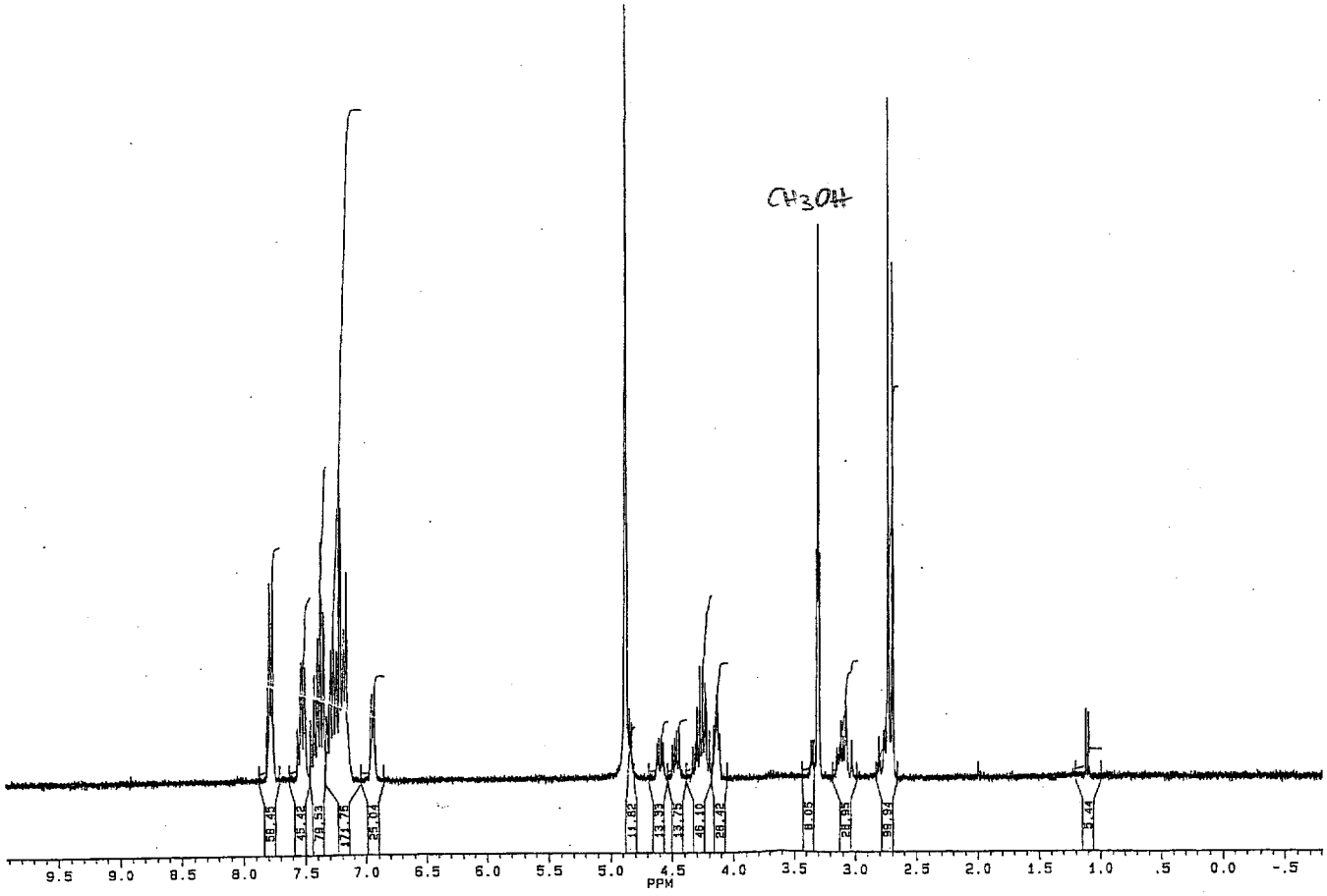
^{1}H NMR (CD_3OD)

Commercial sample



D. HALL, STANDARD IN CD3OD



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4) Experimental details and selected NMR spectral data (^{1}H, ^{13}C) for *N*-alkyl amino acid esters **4h-j** and their precursors (Table 2).

***N*-Acyl amino acid esters.** These *N*-formyl and *N*-acetyl precursors were synthesized by means similar to the solid-phase examples described above.

Typical procedure for Diborane Reduction and Oxidative Work-Up. *N*-Methylphenylalanine *t*-butyl ester (4h). The diborane solution (1M/THF, 8.9 mL, 8.9 mmols) was added at 0 °C to a solution of **1h** (0.97 g, 3.9 mmols) in dry THF (6.7 mL). The solution was stirred at reflux temperature for 4h. Upon cooling to rt, triethylamine (2 mL), glacial acetic acid (3 mL) and iodine (1.09 g, 4.3 mmols, in THF) were added successively. The mixture was stirred for 2h then transferred slowly to an extraction funnel containing conc. aqueous NaOH and aq. saturated $Na_2S_2O_3$ (10 mL). The pH was adjusted to >11 with NaOH pellets, and the solution is extracted with ether (4x). The combined organic layers were washed with brine (10 mL), dried with anhydrous $MgSO_4$, filtered and concentrated. The brown oil was then dried under high vacuum for 4h, affording crude compound **4h** (0.77 g, 84%). It was found of satisfying purity (>90%) according to ^{1}H and ^{13}C NMR analysis. Highly pure analytical samples can be obtained by flash-chromatography purification using 1-5% $MeOH/CH_2Cl_2$.

Selected ^{1}H and ^{13}C NMR spectra of compounds 4h-j are included in the following pages.

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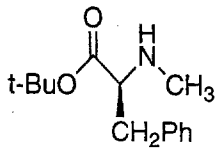

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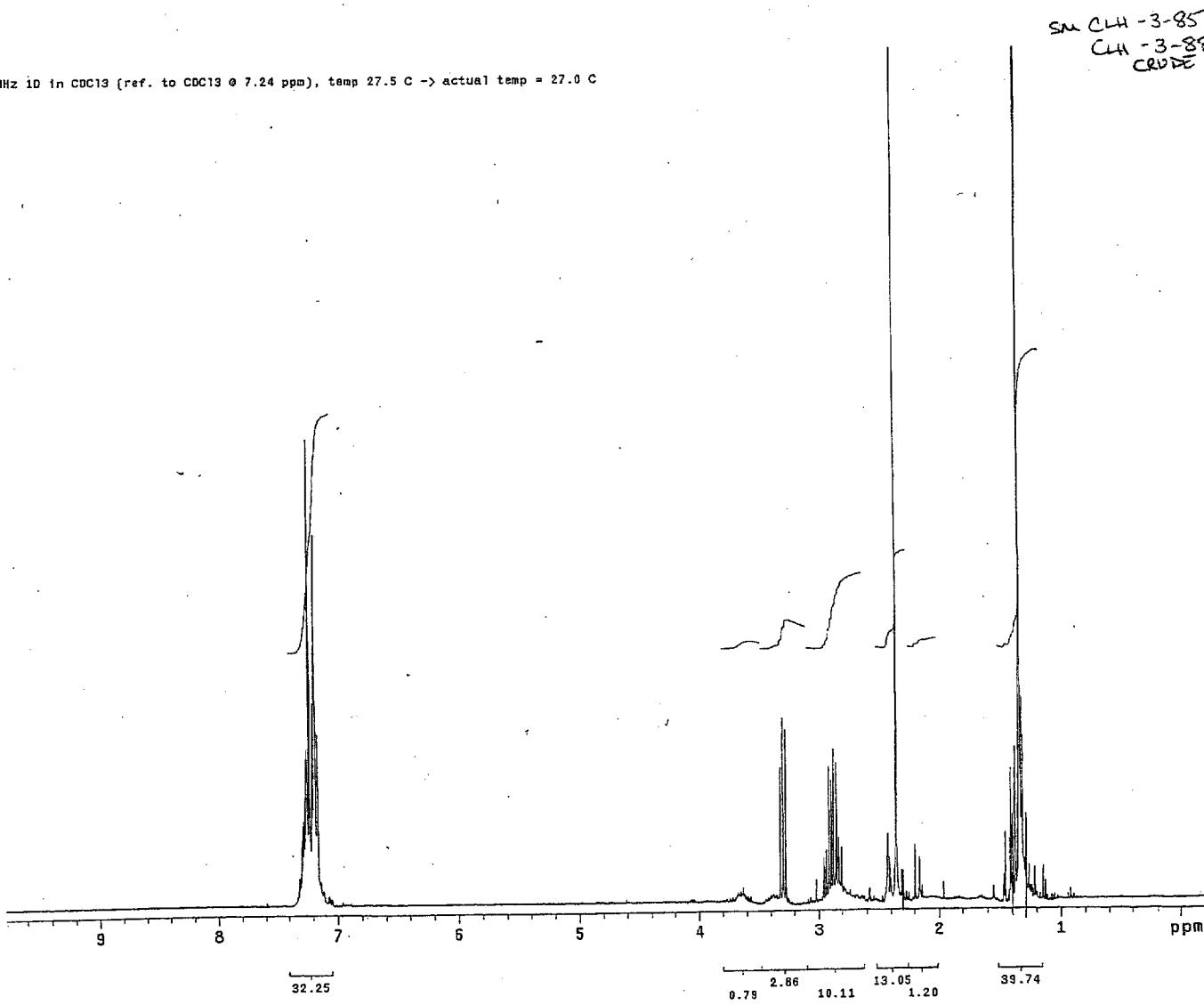

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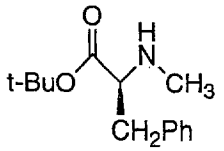

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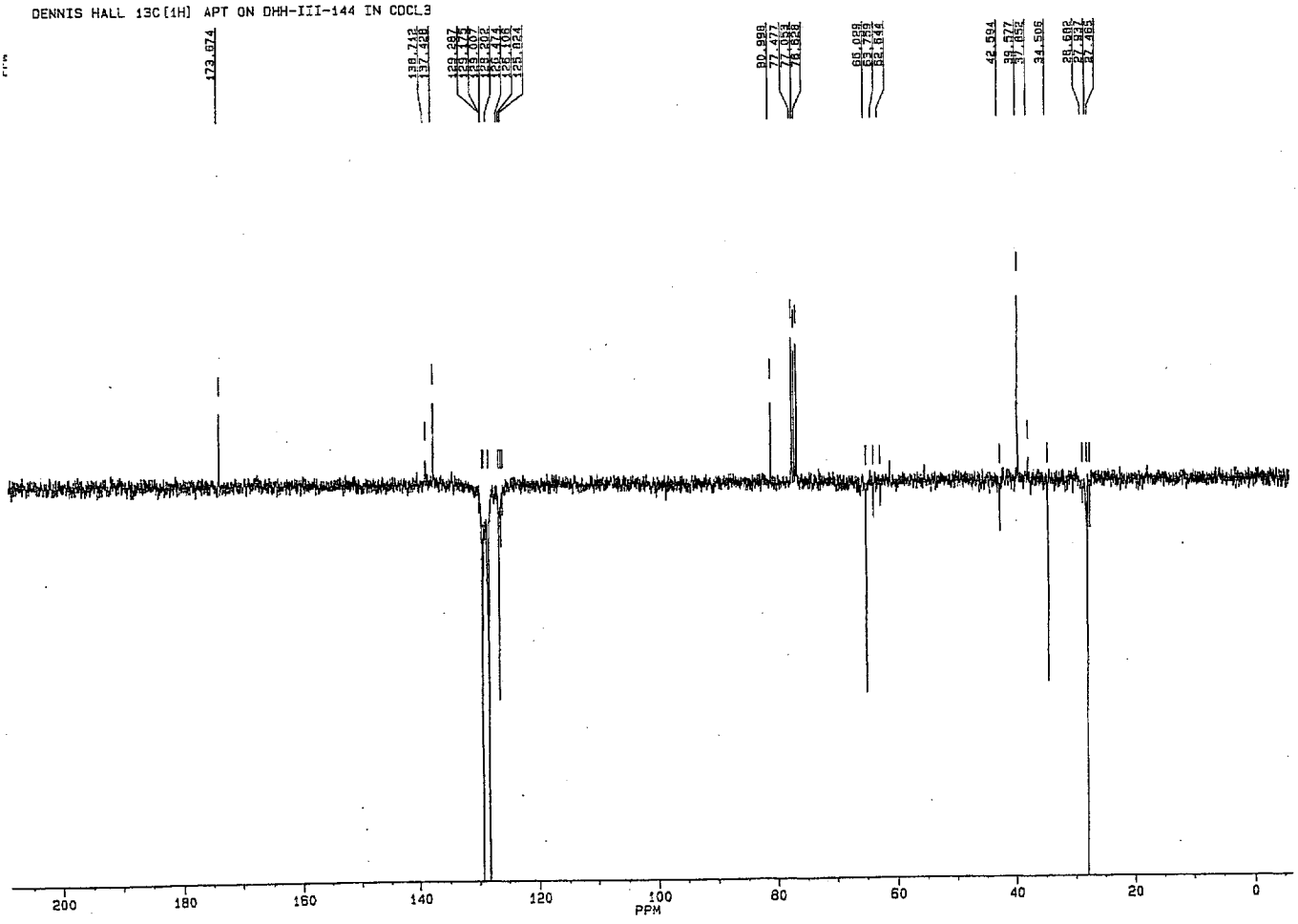

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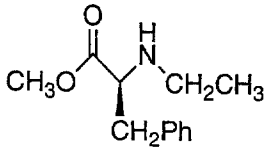
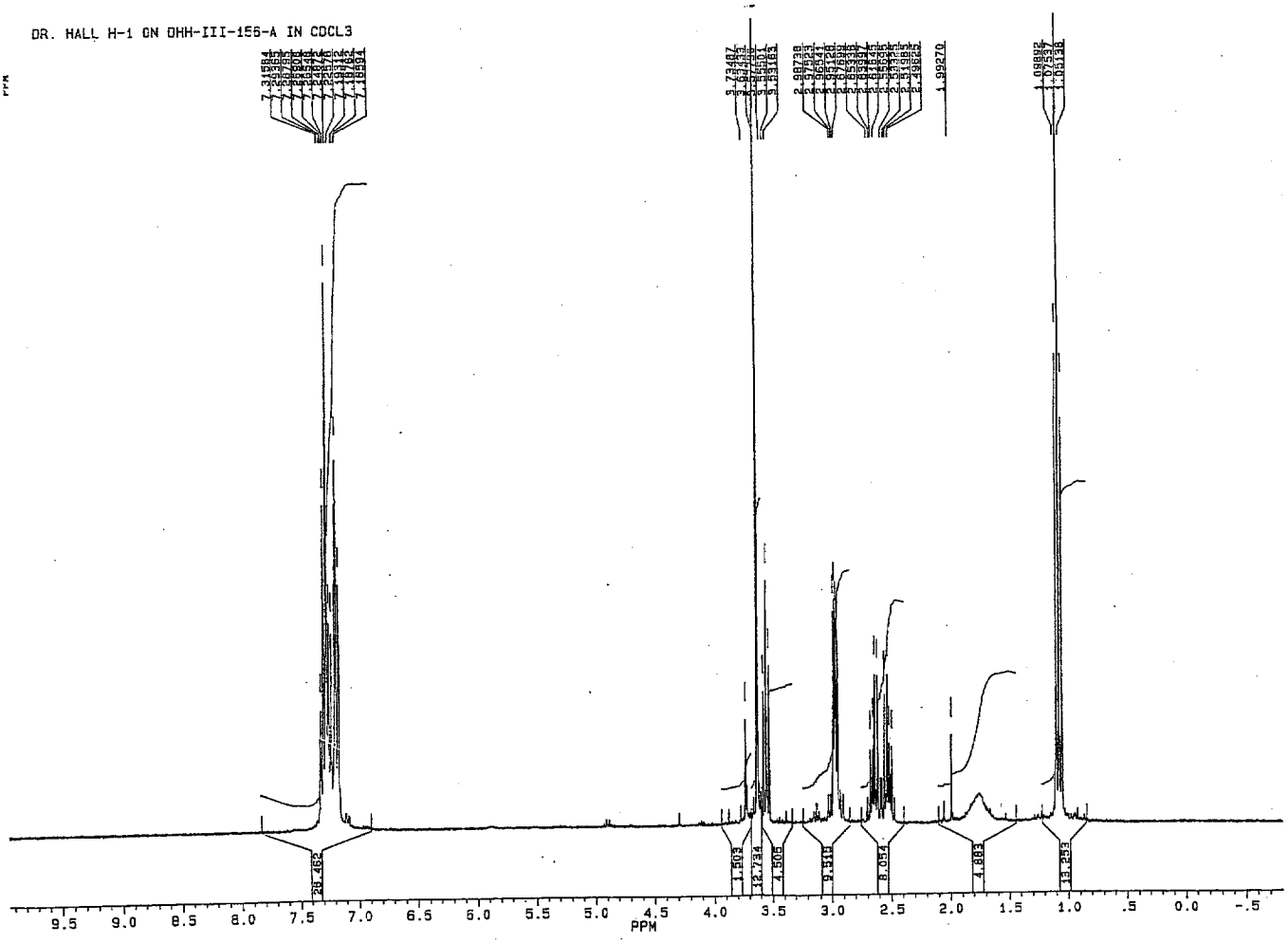
^{1}H NMR ($CDCl_3$)

4b (crude)





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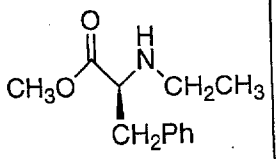
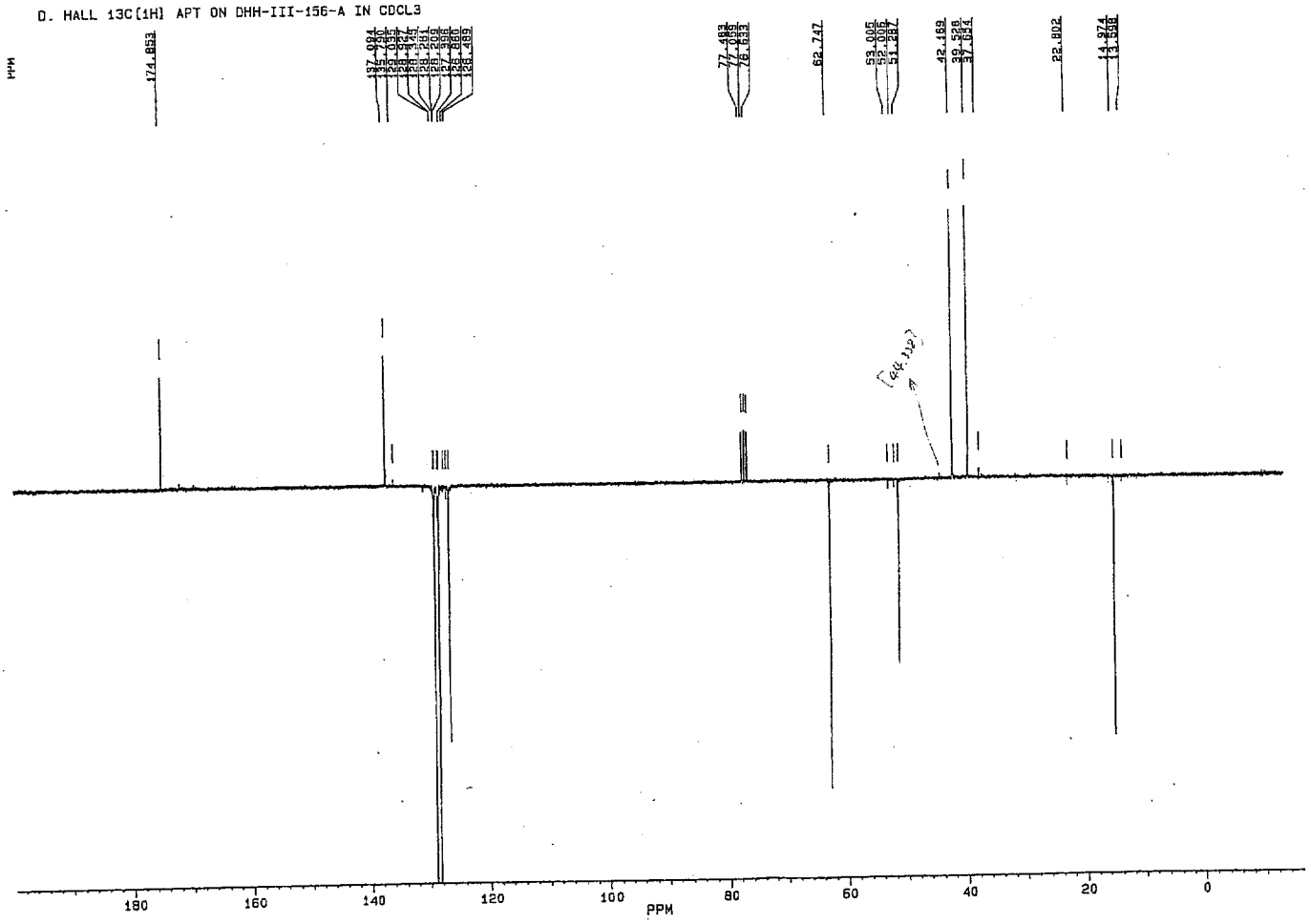


4b (crude)



BRUKER

NV02305C.001
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CAPT3.AU
DATE 2-11-98
TIME 12:24

SF 75.469
SY 112.0
O1 7600.000
SI 65536
TD 32768
SW 20000.000
HZ/PT .610

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RD 0.0
AQ .819
RG 600
NS 805
TE 297

FW 25000
O2 5000.000
DP 18H CPD

LB 1.000
GB 0.0
GX 36.00
GY 0.0
F1 200.006P
F2 -15.995P
HZ/CM 452.813
PPM/CM 6.000
SR -1393.10

D2 .0010000
S2 16H
D1 2.0000000
P9 100.00
S1 0H
P3 2.00
D5 .0040000
P1 9.00
D6 .0020000
P2 18.00
P6 8.60
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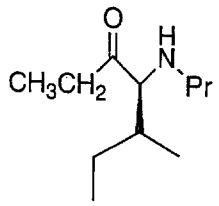
PW 0.0
DE 33.80
NS 805
DS 2

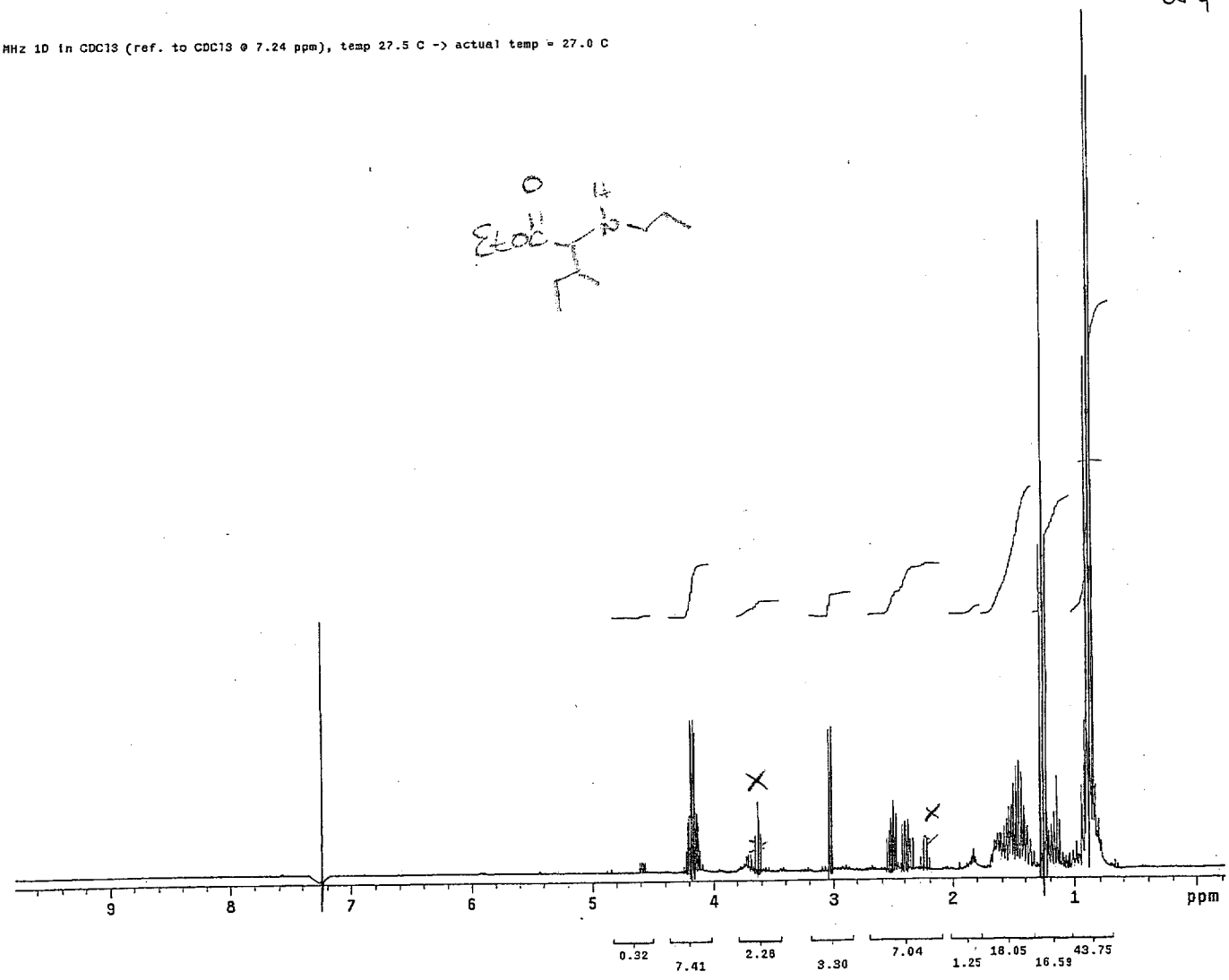
^{1}H NMR ($CDCl_3$)



4c (crude)

CLH-3-117 crude dry

300 MHz 1D in CDCl3 (ref. to CDCl3 @ 7.24 ppm), temp 27.5 C -> actual temp = 27.0 C



5) Experimental details and relevant MS and NMR spectra (^1H, ^{13}C) for the synthesis of tetraamine **8** and its triacetylated derivative **9** (Scheme 2). RP-HPLC trace of Fmoc-derivatized **9**.

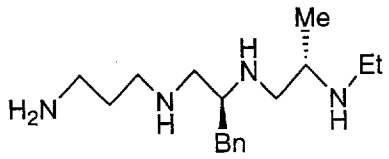
Synthesis of model tripeptide 6. It was synthesized from aminopropyl-trityl resin using standard Fmoc amino acid coupling chemistry. The *N*-terminal alanine was capped with an acetyl group using the procedure described above for **1f**. The cleaved peptide (5% TFA/CH_2Cl_2 was found of >95% purity according to ^1H NMR analysis.

Synthesis of chiral tetraamine tetrakis(trifluoroacetate) salt (8). A portion of tripeptide trityl resin **7** (0.351g, 0.259 mmol, 0.74 mmol/g substitution) was weighed in a 50 mL silanized round bottom flask. The diborane solution (1M/THF, 5.2 mL, 5.2 mmol) was added dropwise under nitrogen atmosphere at rt over 5 min., after which the flask was equipped with a condenser and the suspension refluxed gently (65 °C) for 24h. Upon cooling to rt, the suspended resin was rapidly transferred by pipet (silanized) to a PP vessel by using dry THF to rinse out the flask and wash the resin. Then, dry THF (3 mL), anhydrous diisopropylethylamine (0.7 mL) and glacial acetic acid (1.4 mL) were added successively. To the homogenized suspension was added iodine (1.32 g, 5.2 mmol, as a conc. THF solution) and the vessel was shaken for 4h. The vessel was then rinsed (THF) and the resin was washed (3× each) with THF, DMF/Et_3N 3:1, MeOH, CH_2Cl_2, and dried under high vaccuum for >12h to give free tetraamino-resin **7** (0.344 g). A resin sample gave a highly positive result on a bromophenol blue test.

A portion of fresly prepared resin **7** (0.174 g, 0.77 mmol/g) was then transferred to a round bottom flask and stirred in a 5% TFA/CH_2Cl_2 cleavage cocktail (10 mL) for 2h. The contents were filtered through a glasswool plug, the resin was rinsed with 5% TFA/CH_2Cl_2, and the filtrate was evaporated and dried over high vacuum for >12h to give crude tetraamine tetrakis(trifluoroacetate) salt **8** as a yellow oil (95 mg, 95%). Its purity was estimated to >95% as determined by ^1H and ^{13}C NMR (see following pages).

Synthesis of tetraamine triacetate trifluoroacetate salt (9). This compound was synthesized from resin **7** (0.119 g, 0.092 mmol) using a procedure similar to compound **1f**. Upon resin cleavage as above, crude compound **9** was obtained as a yellowish oil (41 mg, 96%). Its purity was estimated to >95% according to RP-HPLC analysis (see following pages) with pre-column derivatization as a Fmoc carbamate (see note 20).

^{1}H NMR (CD_3OD)



8 (crude)

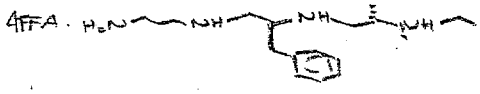
• $4CF_3CO_2H$

SMH(II)41

300 MHz 1D in CD3OD (ref. to CD3OD @ 3.30 ppm), temp 27.5 C -> actual temp =27.0 C

Solvent: cd3od
Temp. 27.5 C / 300.6 K
INOVA-300 "i300"

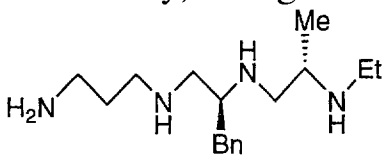
PULSE SEQUENCE
Relax. delay 3.000 sec
Pulse 77.1 degrees
Acq. time 1.994 sec
Width 3001.2 Hz
16 repetitions
OBSERVE H1, 299.9597711 MHz
DECOUPLE H1, 299.9612155 MHz
Power 3 dB
off during acquisition
on during delay
single frequency
DATA PROCESSING
FT size 65536
Total time 1 minutes

4TFA · 

Enlargement

^{1}H NMR (CD_3OD)

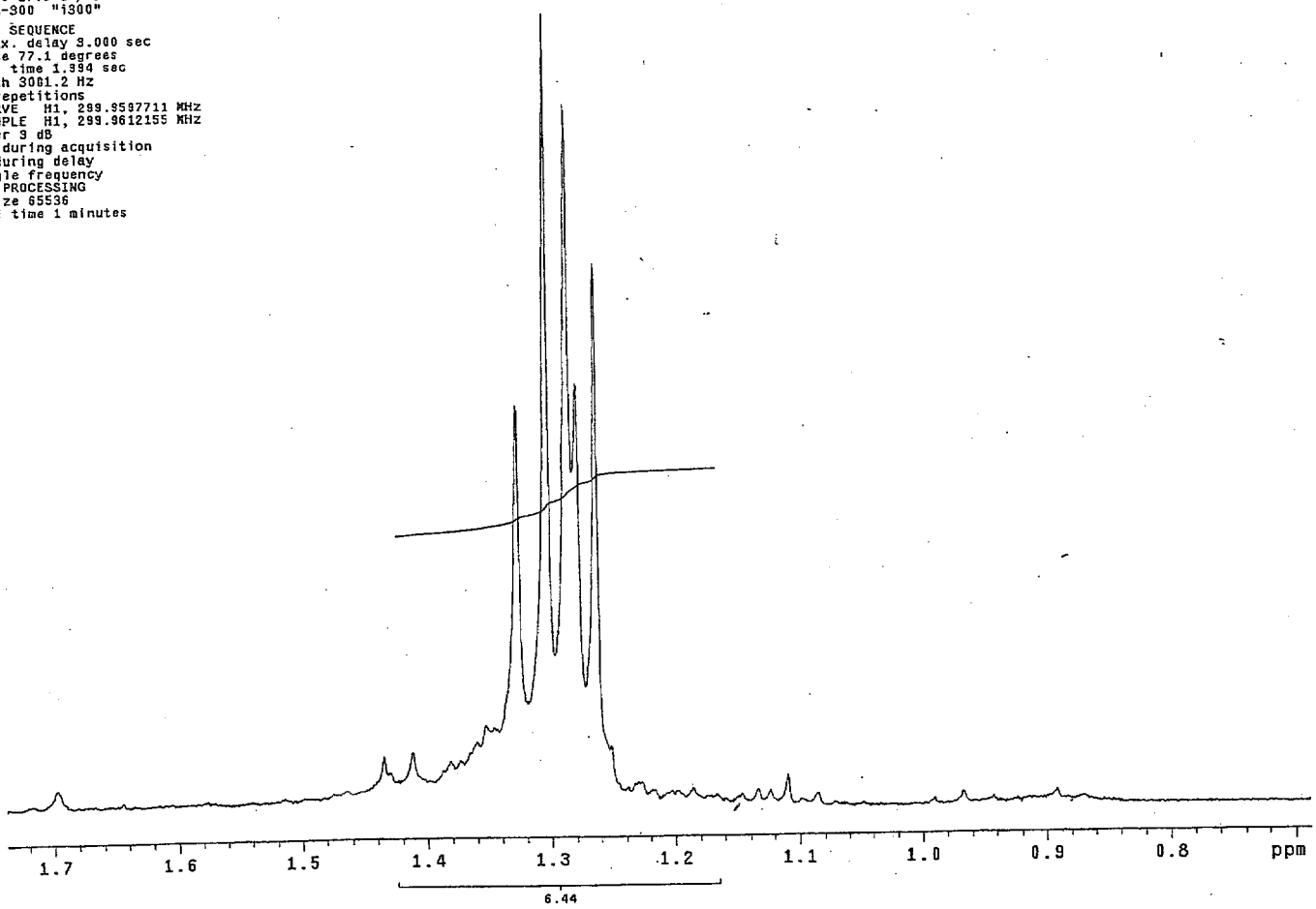
8 (crude)



• $4CF_3CO_2H$

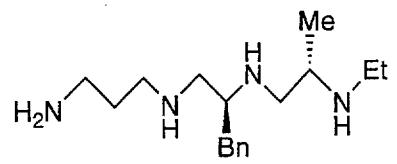
300 MHz 1D in CD3OD (ref. to CD3OD @ 3.30 ppm), temp 27.5 C -> actual temp =27.0 C

Solvent: cd3od
Temp. 27.5 C / 300.6 K
INOVA-300 "i300"
PULSE SEQUENCE
Relax. delay 3.000 sec
Pulse 77.1 degrees
Acq. time 1.994 sec
Width 3001.2 Hz
16 repetitions
OBSERVE H1, 299.9587711 MHz
DECOUPLE H1, 299.9612155 MHz
Power 9 dB
off during acquisition
on during delay
single frequency
DATA PROCESSING
FT size 65536
Total time 1 minutes

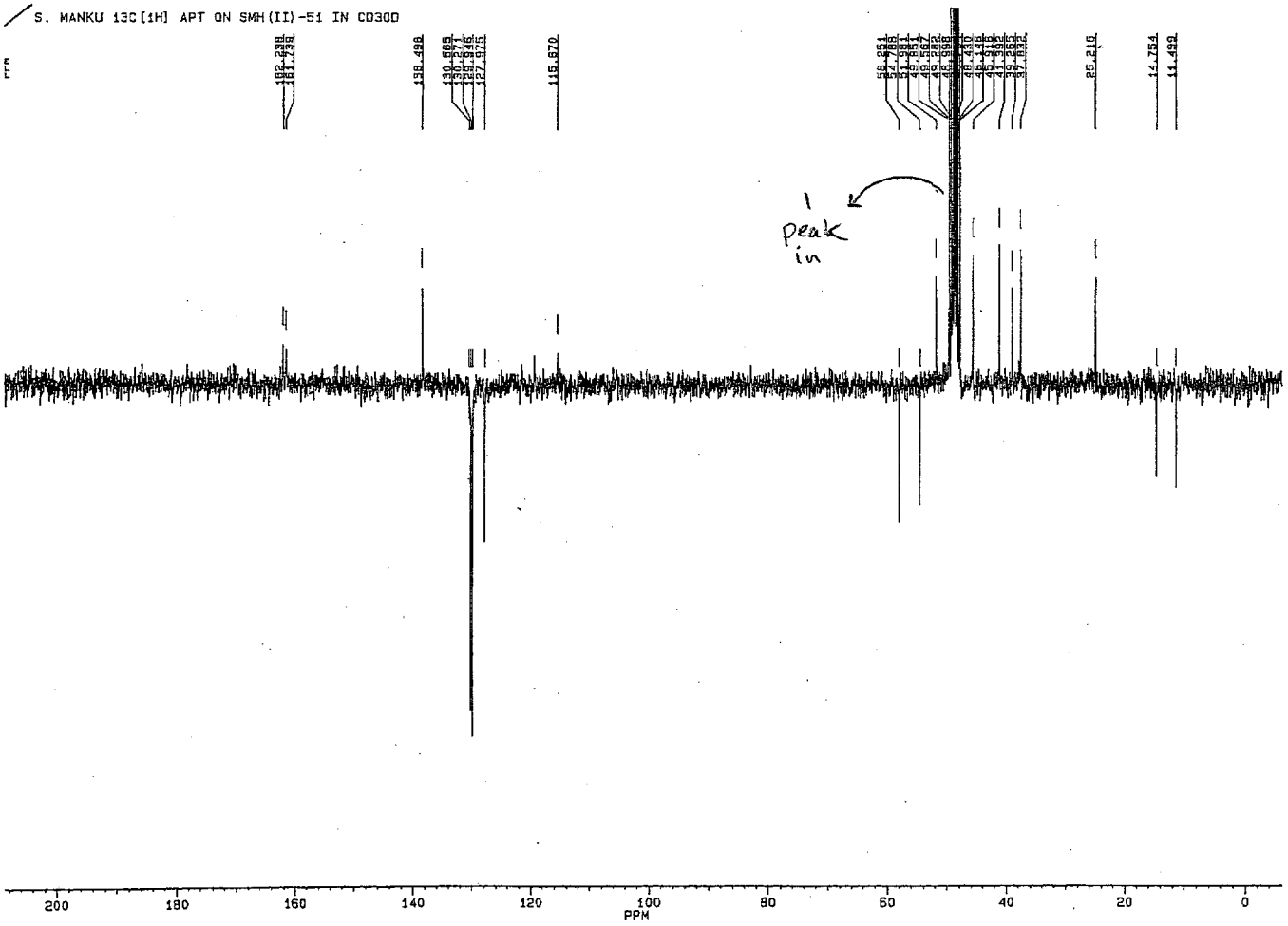


^{13}C NMR (CD_3OD)

8 (crude)



• $4CF_3CO_2H$



BRUKER

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DATE 12-11-98
TIME 21:01

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TD 32768
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HZ/PT 1.130

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RG 800
NS 6000
TE 297

FW 23200
O2 5695.000
DP 1H CPD

LB 2.000
GB 0.0
CX 36.00
CY 0.0
F1 210.016P
F2 -5.980P
HZ/CM 452.803
PPM/CM 6.000
SR -1214.68

D1 1.5000000
S1 1H
P9 100.00
D2 .0010000
S2 1H
P0 1.70
D3 .0065000
P6 8.40
D4 .0010000
RGA
RD 0.0
PW 0.0
DE 36.30
NS 6000
DS 2

Print of window 80: MS Spectrum
S. Manku

MeOH
100uL/min;nebulizer 10psi;gain 3

==========

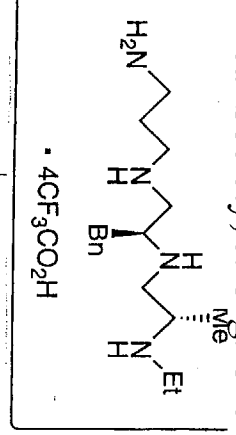
Injection Date : 11/3/98 11:41:22 AM
Sample Name : SMH(II)41
Acq. Operator : Alan

Vial : 1
Inj : 1
Inj Volume : 1 µl

Method : C:\HPCHEM\1\METHODS\MEOHPOS.M
Last changed : 11/3/98 11:38:45 AM by Alan
(modified after loading)

MeOH / H2O

(+)-ES-MS
8 (crude)



MS Spectrum

hc

Instrument 1 11/3/98 11:46:18 AM Alan

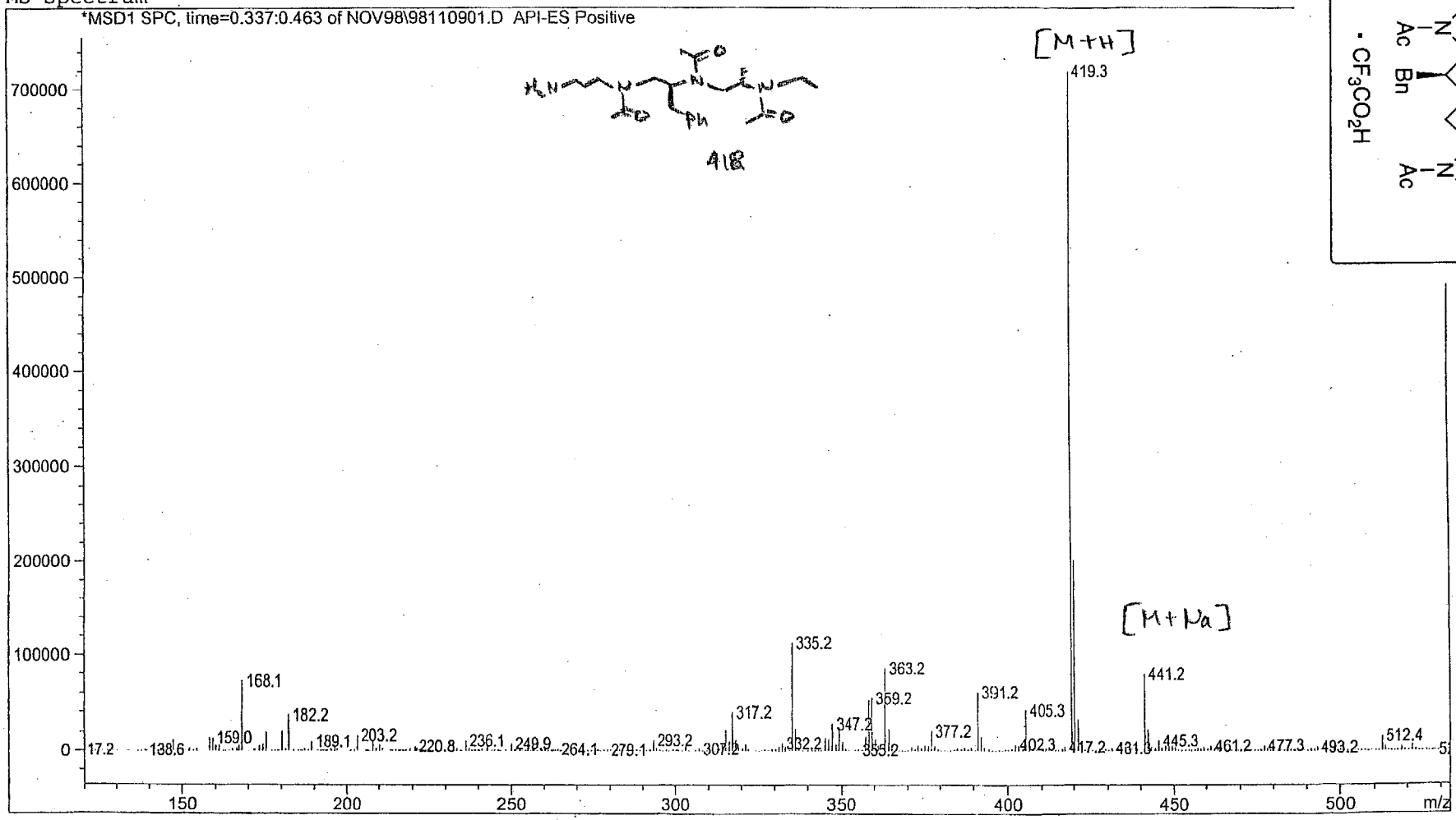
Print of window 80: MS Spectrum
S. Manku

MeOH / H2O
.00uL/min ; nebulizer 15psi; Gain 1; ramped fragmentor

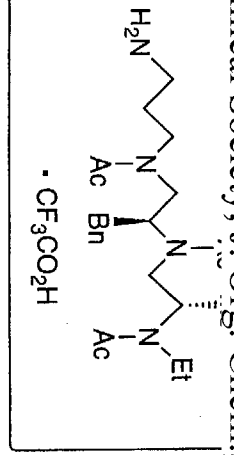
Injection Date	: 11/9/98 1:47:28 PM	Seq. Line	: 1
Sample Name	: SMH(II)47	Vial	: 81
Acq. Operator	: Don	Inj	: 1
		Inj Volume	: 1 µl
Method	: C:\HPCHEM\1\METHODS\MEOHPOS.M		
Last changed	: 11/9/98 1:35:28 PM by Don		

MeOH / H2O
.00uL/min;gain 1; ramped fragmentor;condensed

MS Spectrum



(+)-ES-MS
9 (crude)

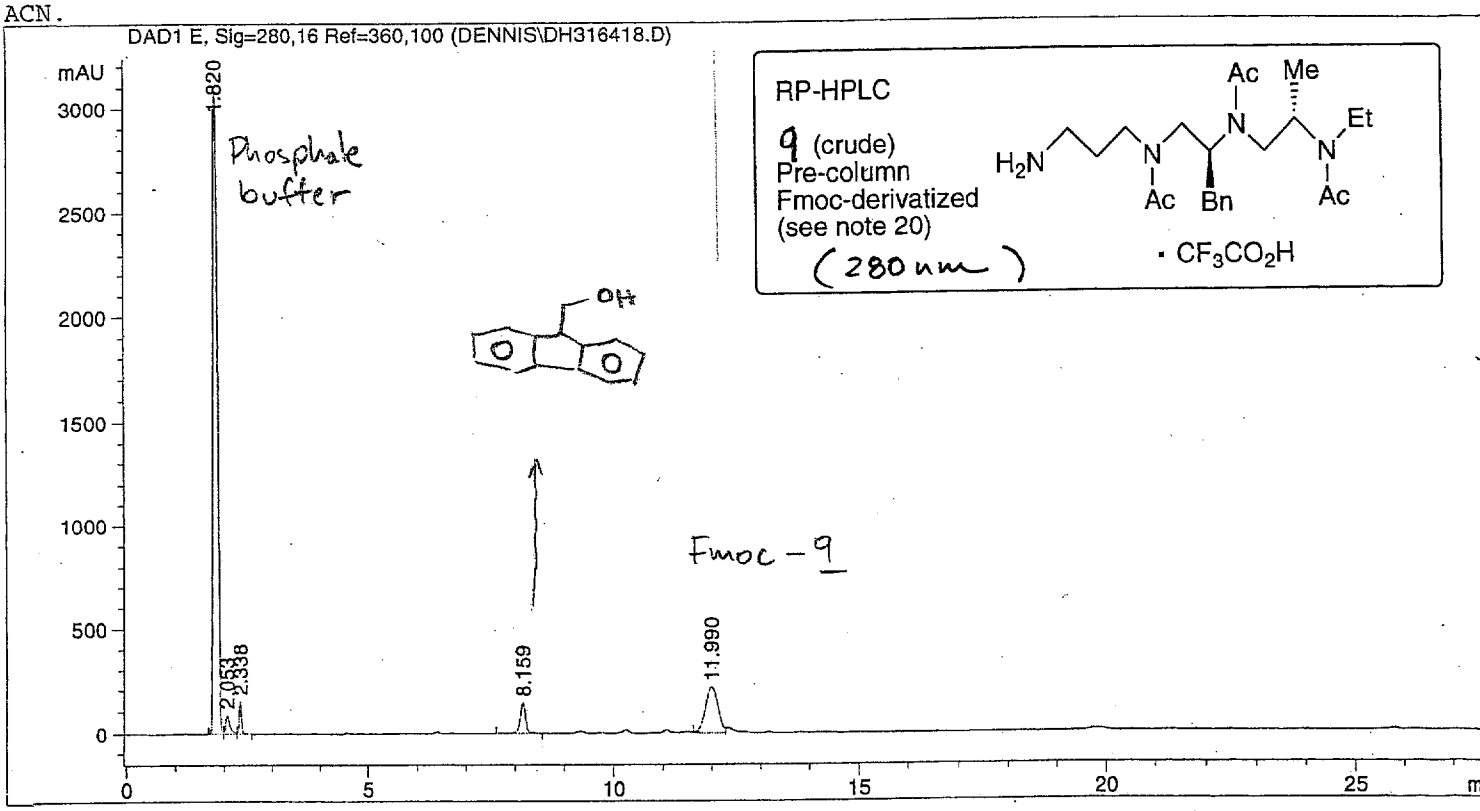


25

Sample of synthetic tetraamine triacylated and pre-co
lumn deriv. with Fmoc. General method at 280 nm UV. S

. Longer time

```
==========================================================================
Injection Date  : 11/11/98 5:46:52 PM
Sample Name     : SMH-II-47                              Vial :   1
Acq. Operator   : Dennis
                                                    Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\DENNIS\DENNIS3.M
Last changed    : 11/11/98 5:45:43 PM by Dennis
                  (modified after loading)
Analysis Method : C:\HPCHEM\1\METHODS\DENNIS\DENNIS3.M
Last changed    : 11/10/98 9:15:19 PM by Dennis
Basic method to analyze Fmoc-aminoacids and oligoamines with or without pre-column
derivatization.  Made for completing JOC manuscript on November 8 1998.  Employs 0.1% aq, TFA/
ACN.
```

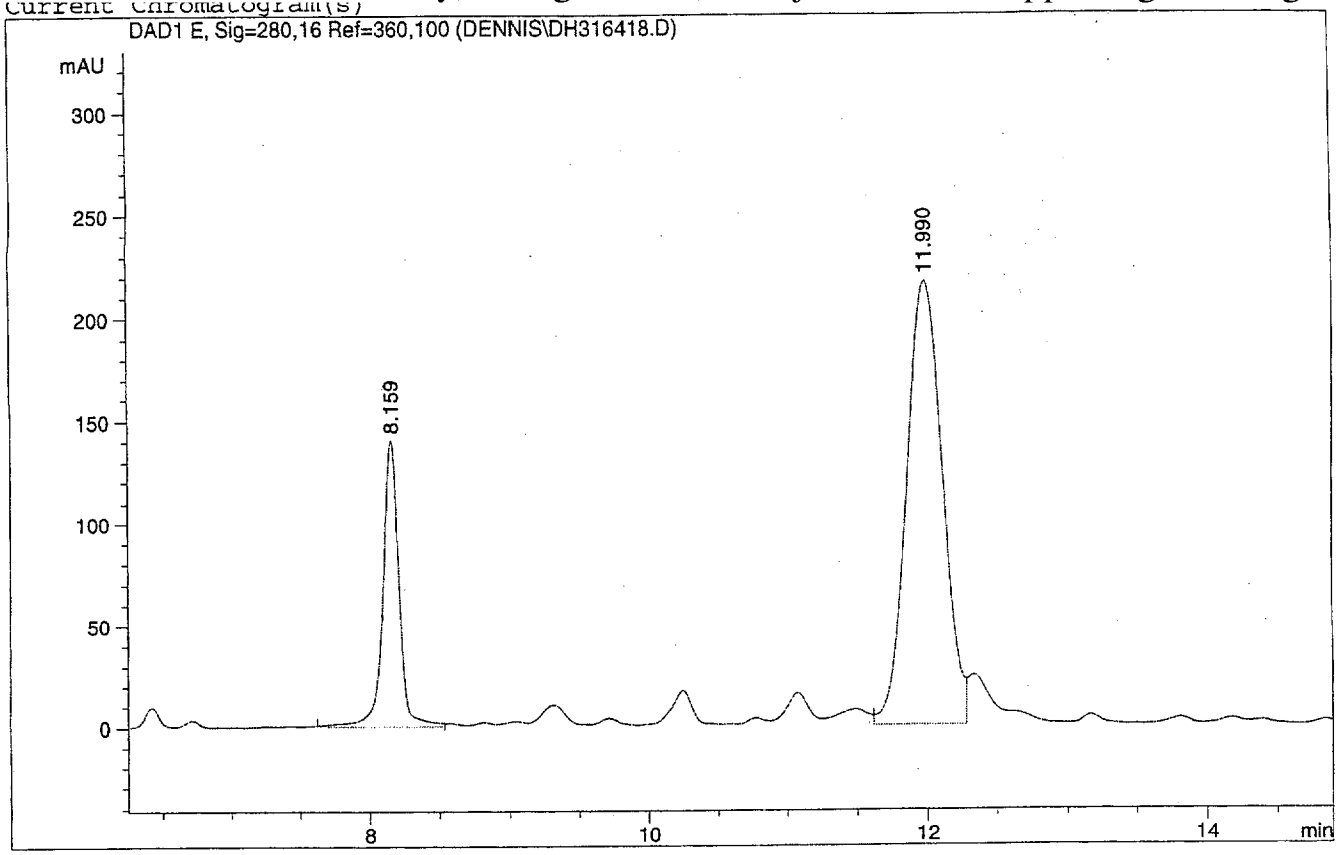



```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 E, Sig=280,16 Ref=360,100
```

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.820	BV	0.1092	1.98705e4	3083.13501	76.3780
2	2.053	VV	0.0935	551.69836	85.28393	2.1206
3	2.338	VB	0.0573	560.41656	150.98053	2.1541
4	8.159	BB	0.1233	1148.89429	140.61037	4.4161
5	11.990	VV	0.2784	3884.49683	217.45985	14.9312
Totals :				2.60160e4	3677.46969	

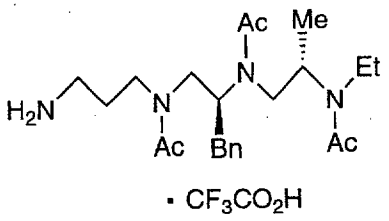
```
 Results obtained with enhanced integrator!
==========================================================================
```

Current Chromatogram(s)



RP-HPLC

9 (crude)
Pre-column
Fmoc-derivatized
(see note 20)



· CF_3CO_2H

Enlargement

27