Supporting Information

Conformationally Constrained Peptidomimetic Inhibitors of Signal Transducer and Activator of Transcription 3.

Pijus K. Mandal,[1**] Donald Limbrick,[5**] David R. Coleman IV,[4] Zhiyong Ren,[2] J. Sanderson Birtwistle,[1] Chiyi Xiong,[3] Xiaomin Chen,[2] James M. Briggs,[5] John S. McMurray[1]*

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General Procedures

N^{α}-protected amino acids were purchased from Advanced Chemtech, NovaBiochem, ChemImpex, or AnaSpec. HOBt was from ChemImpex. Rink amide resin from Advanced Chemtech, loaded between 0.7 mmol/g or 1.2 mmol/g. Anhydrous DMF for amino acid solutions was from Aldrich. Other solvents were reagent grade and were used without further purification. NMR spectra were obtained on either a Bruker DPX 300 MHz spectrometer or a Bruker DRX 500 MHz spectrometer. Fluorescence polarization assays were carried out as described in Coleman et al (2005).

General Procedure for the synthesis of peptides and peptidomimetics. Solid phase syntheses were carried out manually using commercially available Rink resin. Resin, 0.1 – 0.2 gm, was placed in a manual reactor and swollen and washed with 5 × 10 mL of DMF/CH_2Cl_2. Fmoc groups were removed with 3 × 6 mL of 20% piperidine/DMF for 5 min each. For coupling, three-fold excesses of Fmoc-amino acids, DIC, and HOBt were used in 8-10 mL of DMF/CH_2Cl_2 and were allowed to proceed until resin samples tested negative with ninhydrin tests. For Fmoc-Haic and its analogues, stereoisomers of Fmoc-ABN, and phosphorylated cinnamic acid derivatives, couplings were performed with 1.5 equivalents each of acid, DIC and HOBt in DMF/CH_2Cl_2 overnight. After coupling and deprotection steps, resins were washed with 3 ×10 mL of DMF/CH_2Cl_2. On completion of the peptide chain, resins were washed with CH_2Cl_2 3 ×10 mL and were treated with TFA:TIS:H_2O (95:2.5:2.5) (Pearson et al., 1989) (3 × 5 mL) for 15 min each. The combined filtrates sat at rt for 1-2 hr and the volumes were reduced in vacuo. Peptides were precipitated in ice cold Et_2O, collected by centrifugation, and washed 2 × more with the same solvent and centifiged. After drying, peptides were purified by reverse phase HPLC on a Rainin Rabbit HPLC or a Varian HPLC using a Vydac 2.5 × 25 cm C18

Peptide and Protein column or a Phenomenex Luna C18 2.5 × 25 cm column. Gradients of ACN in H_2O (both containing 0.1% TFA) or ACN in 0.01 M NH_4OAc (pH 6.5) at 10 mL/min were employed. Peptides were tested for purity by reverse phase HPLC on a Hewlett Packard 1090 HPLC or an Agilent 1100 HPLC using a Vydac 4.6 × 250 mm C18 peptide/protein column or a Phenomenex Luna 4.6 × 250 mm C18 in two systems, one with 0.1% TFA in both H_2O and ACN and the other with a gradient of ACN in 0.01 M NH_4OAc, pH6.5. Gradients were 0 – 40% ACN/30 min. Peptides were dried in vacuo over P_2O_5 at 37° for 24 hr prior to use.

General procedure for the phosphorylation of resin-bound peptides. Synthesis of Ac-Tic(OPO_3H_2)-Leu-Pro-Gln-Thr-NH_2, 9

Solid phase synthesis of Fmoc-Tic(OH)-Leu-Pro-Gln(Trt)-Thr(OtBu)-NHRink Resin was carried out manually, with 0.20 gm of Rink resin (1.2 mmol/g) following the solid phase peptide synthesis methodology described above. After assembly of the amino acid chain, the resin was washed in dry CH_2Cl_2 and suspended in 10 mL of the same solvent. Dibenzyl-*N*,*N*-diisopropylphosphoramidite (0.4 mL, 1.2mmol) and tetrazole (0.2 g, 2.4mmol) were added and agitated gently with N_2 for 3 hr. The resin was drained, washed with DMF (2 × 5mL), and treated with 5-6 M *tert*-butylhydroperoxide in decane (0.5 mL, 2.5 mmol) diluted with 5mL of DMF, for 1h. The resin was washed with DMF (3 × 5mL) and the Fmoc group was removed as above. After washing the peptide was acetylated with acetic anhydride (1 mL) and 0.2mL of DIPEA in 5 mL of DMF/CH_2Cl_2. On completion of the coupling the resin was drained and washed with DMF/CH_2Cl_2 (3 ×) followed by CH_2Cl_2 (3 ×). The phosphorylated peptide was cleaved from the resin by treatment of TFA:TIS:H_2O (95:2.5:2.5) as described above. After drying the solid crude peptide (83mg) was purified by reverse phase HPLC on a Phenomenex

Luna 2.5 × 20 gm column using a gradient of ACN in H_2O (both containing 0.1%TFA) giving 36 mg of product. ESI-MS Calcd (M+H) for $C_{32}H_{49}N_7O_{12}P$: 754.3177, found: 754.1904.

REFERENCES

Coleman, IV, D.R.; Ren, R.; Mandal, P.K.; Cameron, A.G.; Dyer, G.A.; Muranjan, S.; Chen, X.; McMurray, J.S. Investigation of the binding determinants of phosphopeptides targeted to the SH2 domain of Stat3. Development of a high affinity peptide inhibitor. *J. Med. Chem.* **2005**, *48*, 6661-6670.

Pearson, D.A.; Blanchette, M.; Baker, M.L.; Guindon, C.A Trialkylsilanes as scavengers for the trifluoroacetic acid deblocking of protecting groups in peptide synthesis *Tetrahedron Lett.* **1989**, *30*, 2739-2742.

Table S1. Mass spectral analysis of phosphopeptide inhibitors of Stat3.

	Compound	M+H calc	M+H Found
5	pCinn-Leu-Pro-Gln-Thr-NH_2	683.2806	683.3
6	pInd-Leu-Pro-Gln-Thr-NH_2	696.2758	696.3
7	2-phospho-7-carbonyl-naphthyl-Leu-Pro-Gln-Thr-NH_2	707.2806	707.26
8	6-phosphoindole-3-acetic acid-Leu-Pro-Gln-Thr-NH_2	710.2915	710.30
9	N-Ac-7-phosphoryloxyTic Leu-Pro-Gln-Thr-NH_2	754.1904	754.3177
10	3-pCinn-Leu-Pro-Gln-Thr-NH_2	683.2806	683.1765
11	Ac-pTyr-(3*S*,6*S*,9*S*)-ABN-Gln-Thr-NH_2	712.2707	712.2700
12	Ac-pTyr-(3*S*,6*R*,9*R*)-ABN-Gln-Thr-NH_2	712.2707	712.2700
13	Ac-pTyr-Haic-Gln-Thr-NH_2	760.2707	760.30
14	Ac-pTyr-(R)Haic-Gln-Thr-NH_2	760.2707	760.28
15	Ac-pTyr-Leu-Indoline-Gln-Thr-NH_2	790.3177	790.5
16	pHcinn-Haic-Gln-Thr-NH_2	703.2493	703.3
17	pCinn-Leu-Pro-Gln-NHBn	671.2720	671.3500
18	pCinn-(3*S*,6*S*,9*S*)-ABN-Gln-NHBn	642.2329	642.1000
19	pCinn-(3*S*,6*R*,9*S*)-ABN-Gln-NHBn	642.2329	642.2000
20	pCinn-(3*S*,6*R*,9*R*)-ABN-Gln-NHBn	642.2329	642.1000

21	pCinn-Haic-Gln-NHBn	690.2329	690.1551
22	pCinn-ΔHaic-Gln-NHBn	688.2094	688.2184
23	pInd-Haic-Gln-NHBn	703.2203	703.23
24	pCinn-Haic-Gaba	557.1723	557.20
25	pCinn-Haic-pyrrolidine acetamide	583.1880	583.1000
26	pCinn-Haic-Gln-OH	601.1700	601.0845
27	pCinn-Haic-Gln-OMe	615.1856	615.1725
28	pCinn-Haic-Gln-NH_2	600.1859	600.1233
29	pCinn-Haic-Gln-NHMe	614.2016	614.1485
30	pCinn-Haic-Gln-NH*i*Pr	642.2329	642.2718
31	pCinn-Haic-NH_2	472.1195	472.1324
32	pCinn-Haic-Ala-NH_2	543.1567	543.1830
33	pCinn-Haic-Nle-NH_2	585.2036	585.2200
34	pCinn-Haic-Met(O)-NH_2	619.1628	619.0628
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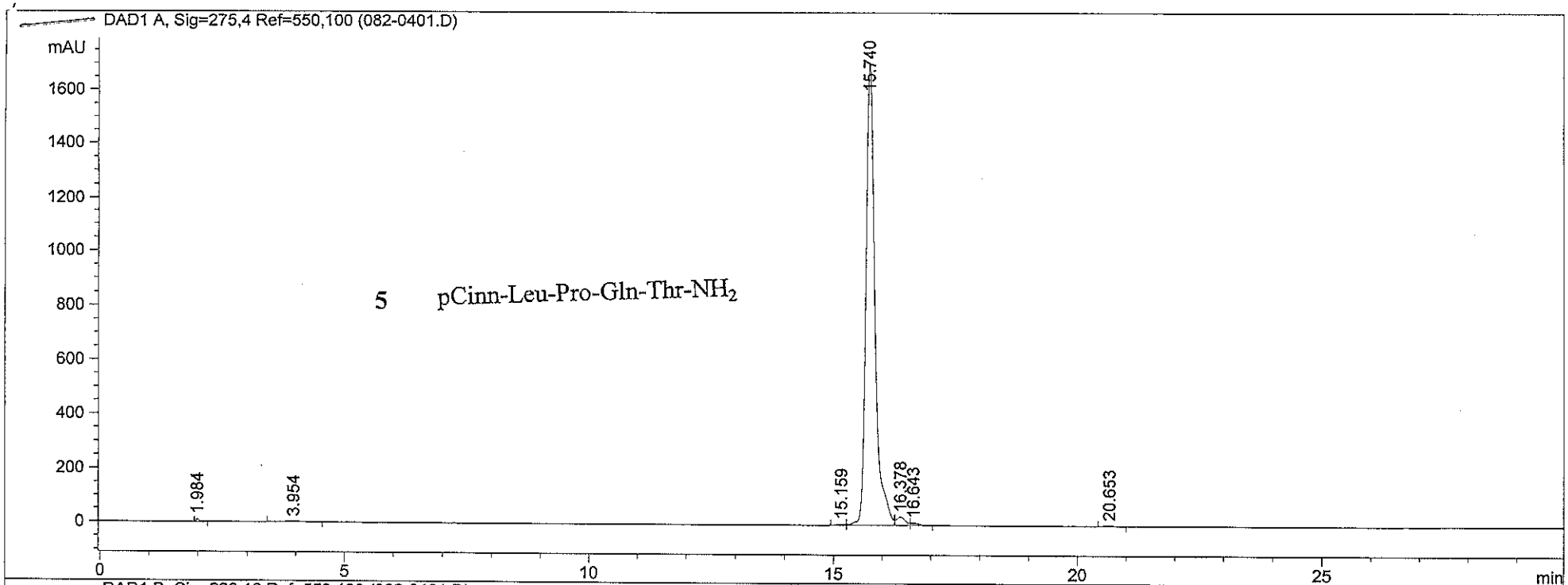

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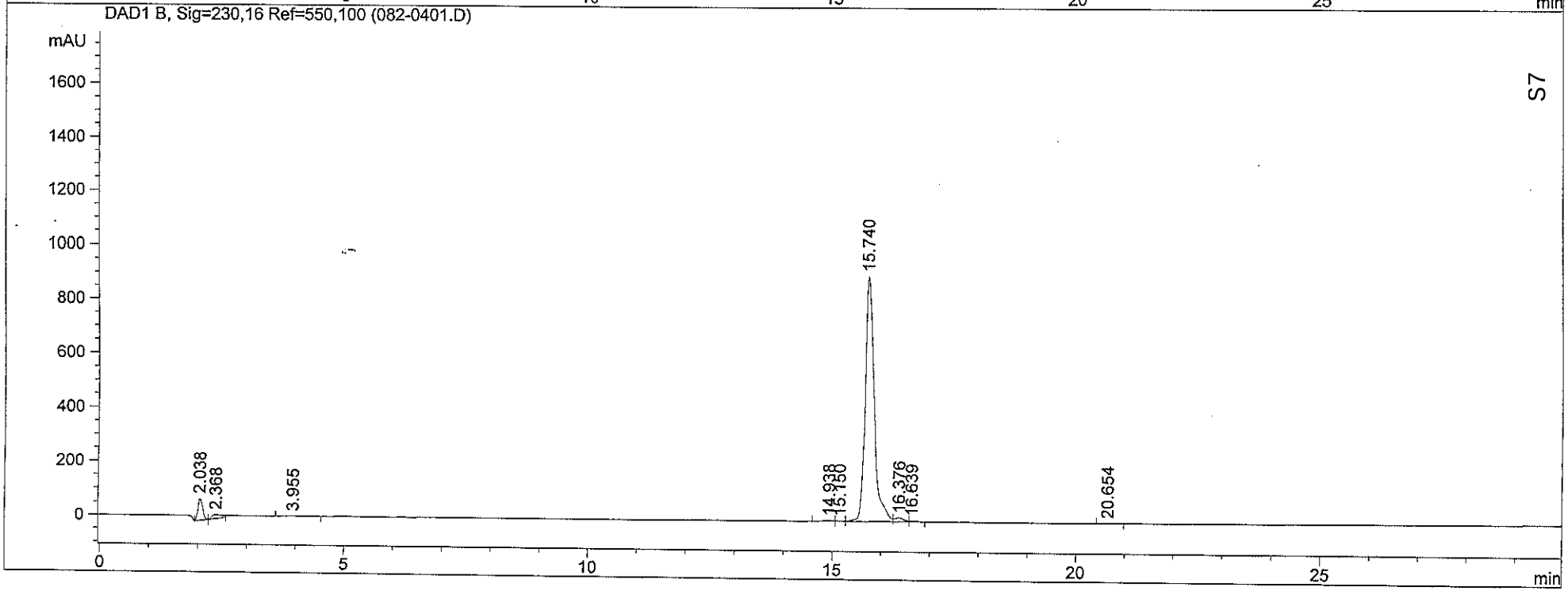

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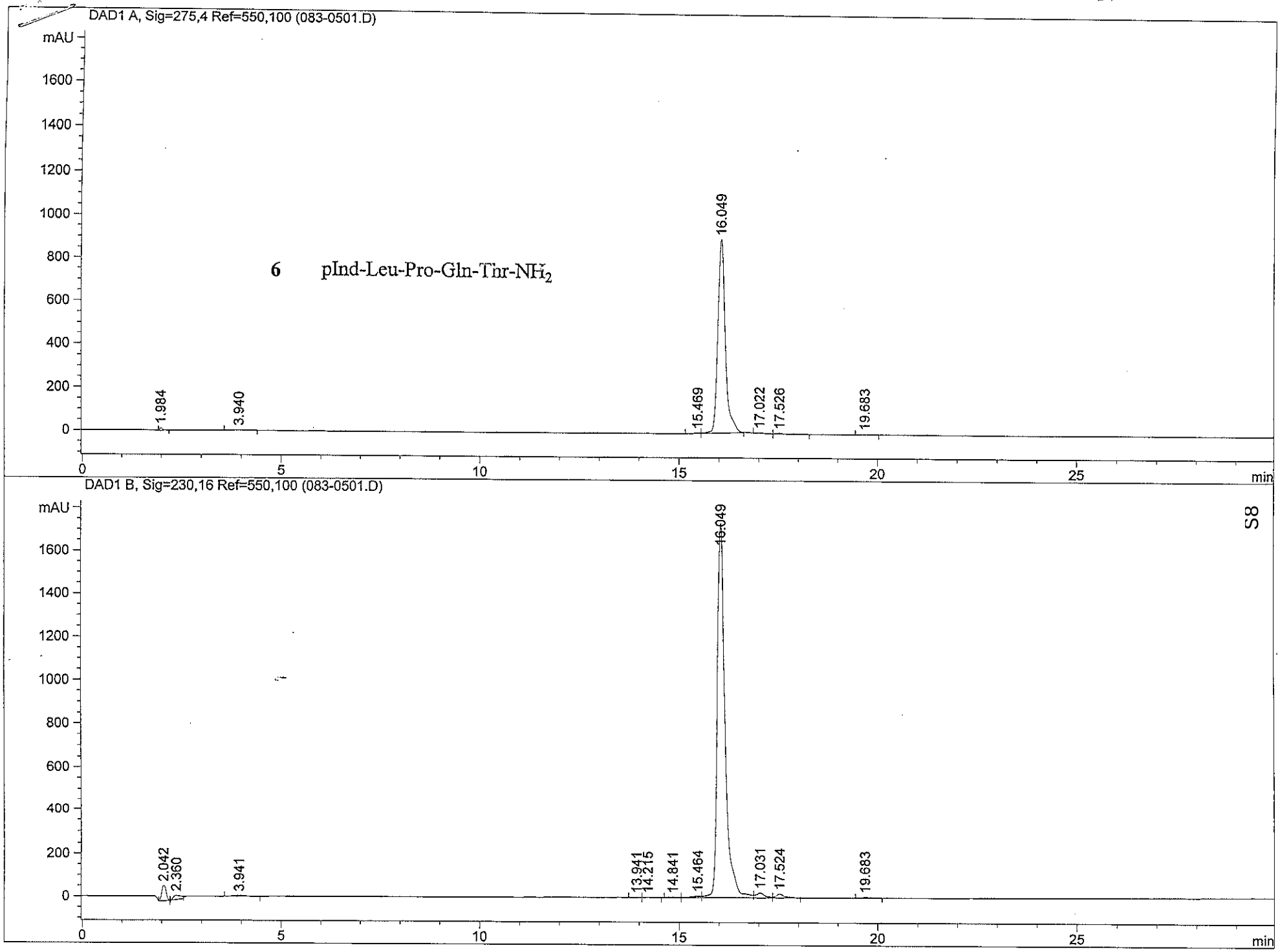
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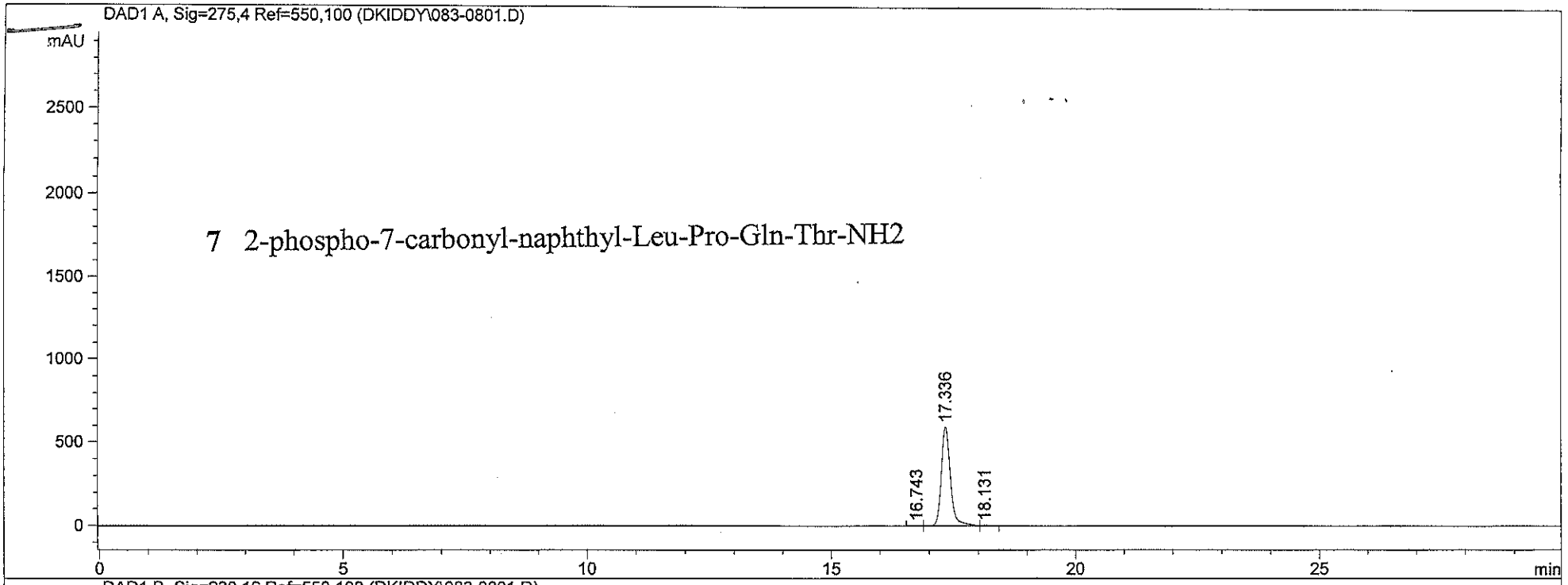
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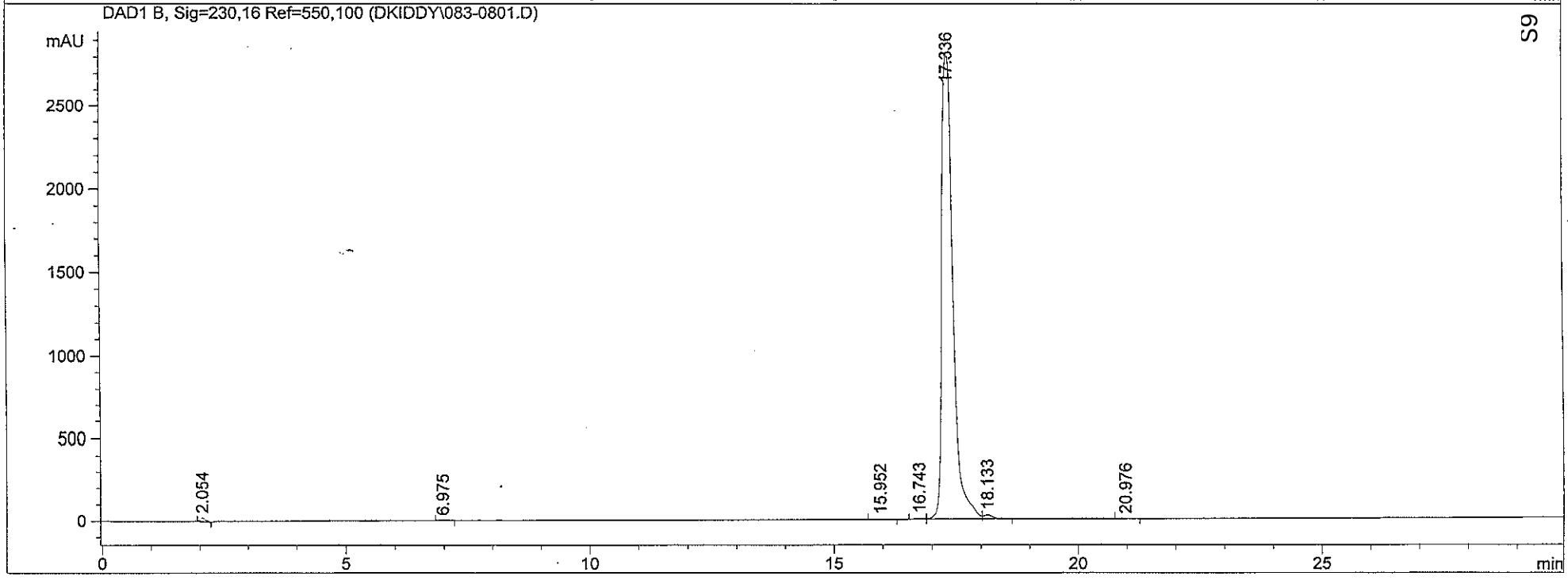
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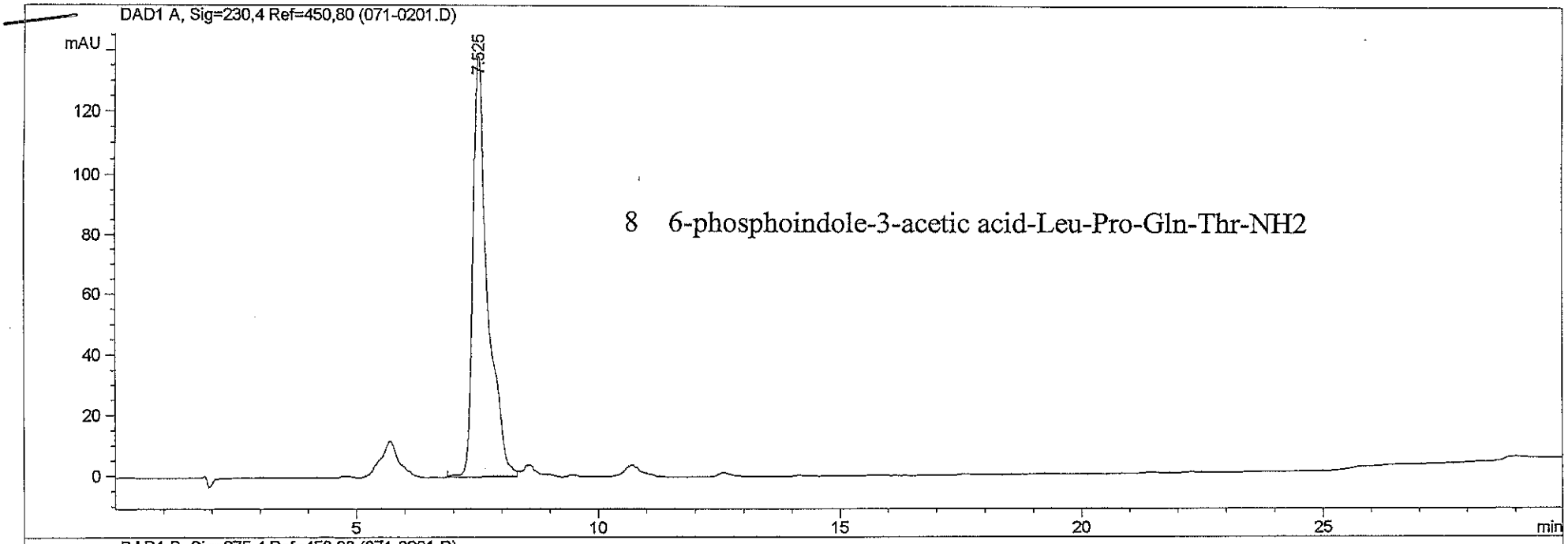

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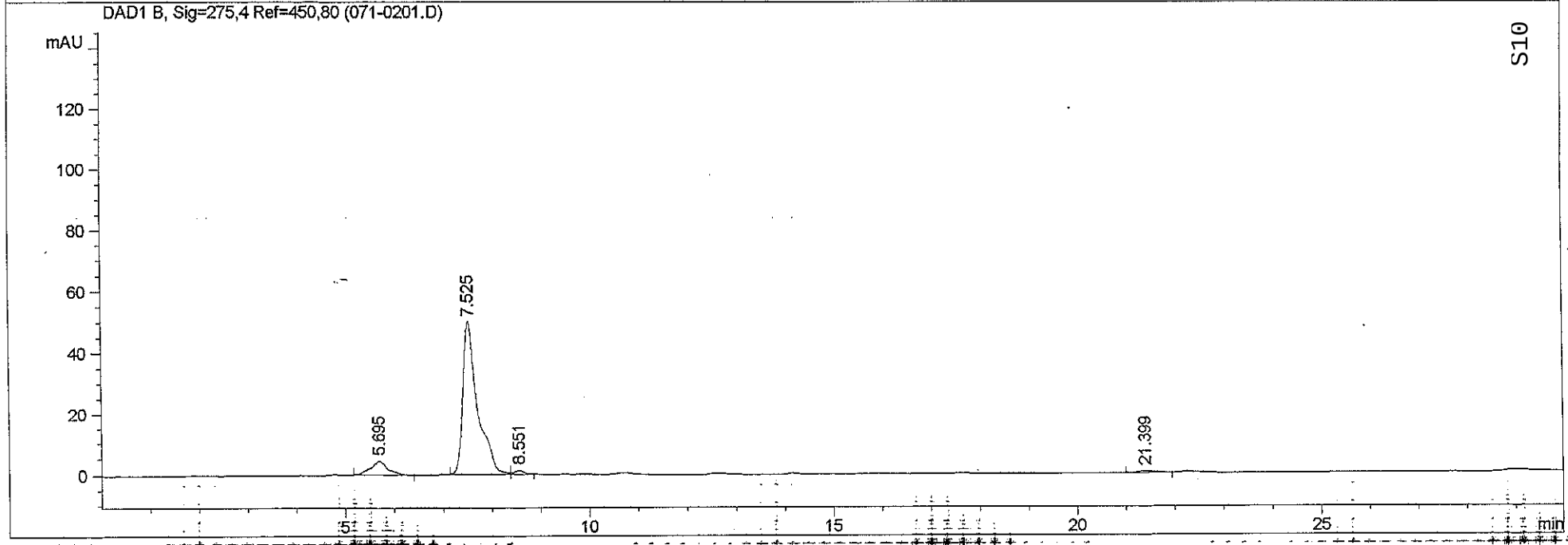

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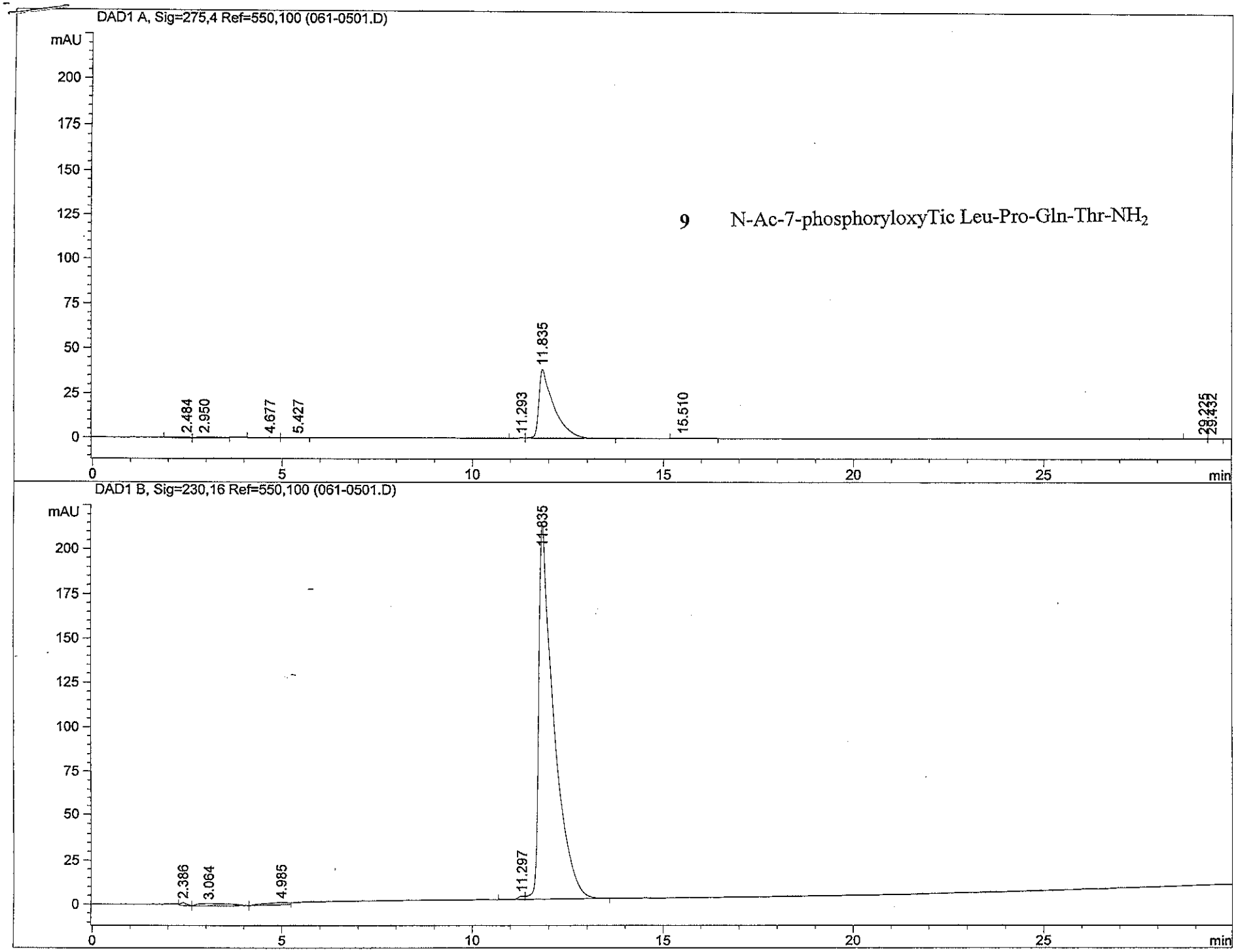

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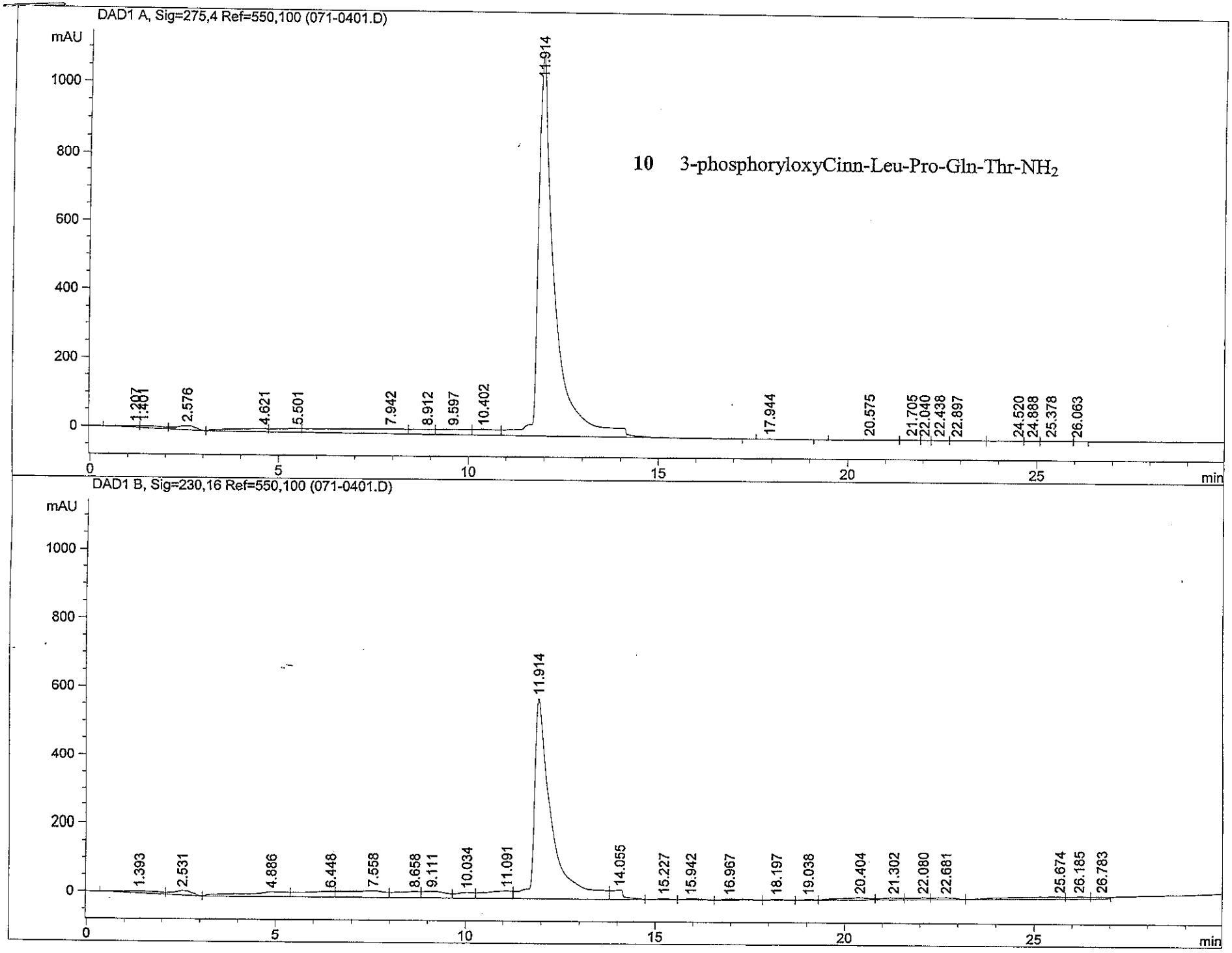
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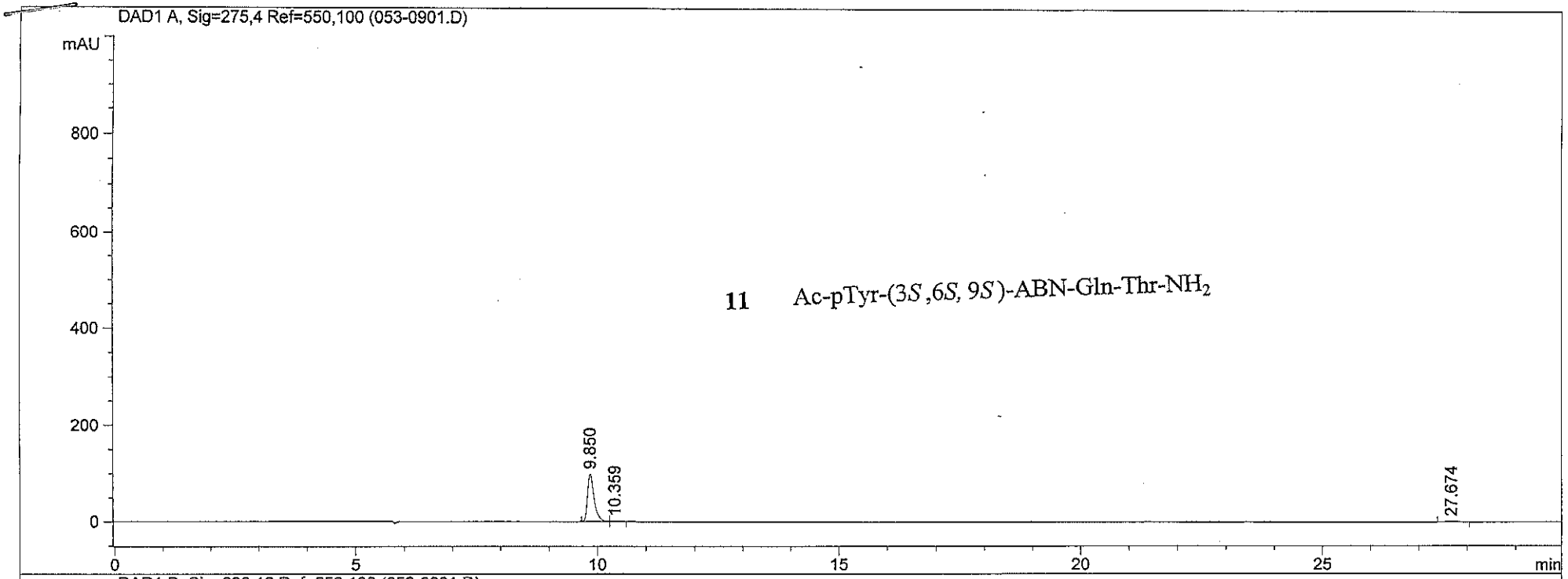

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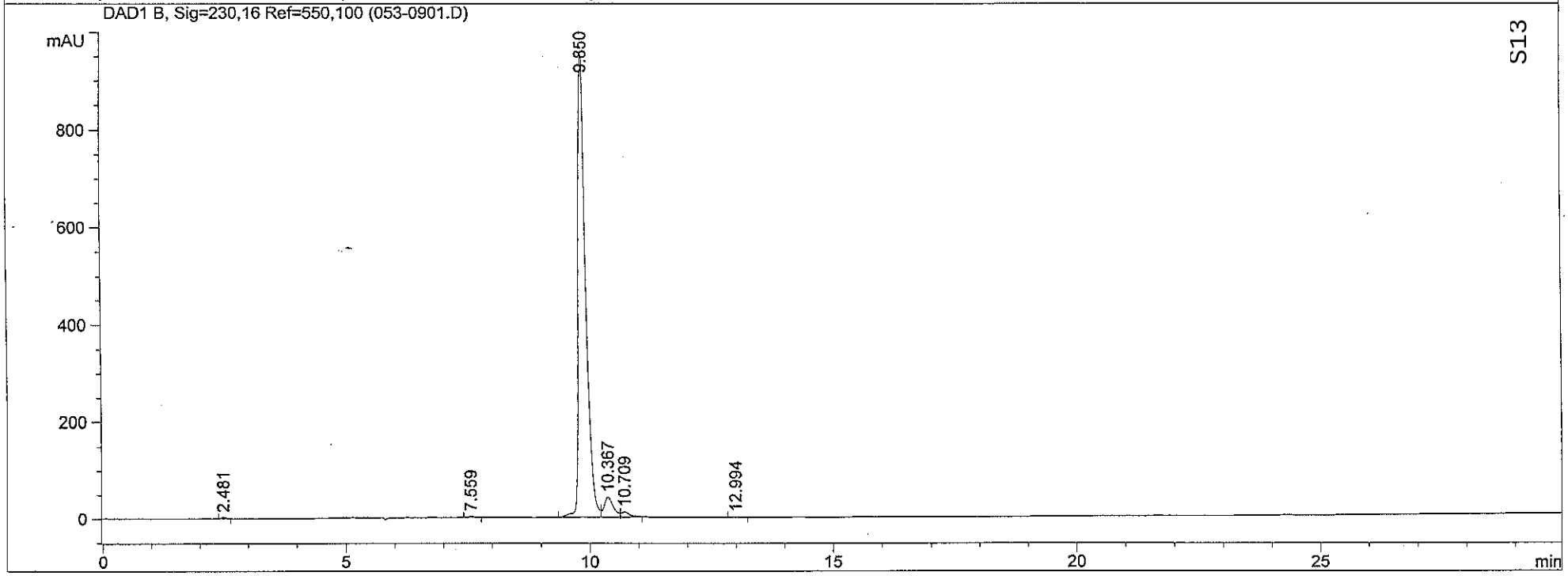

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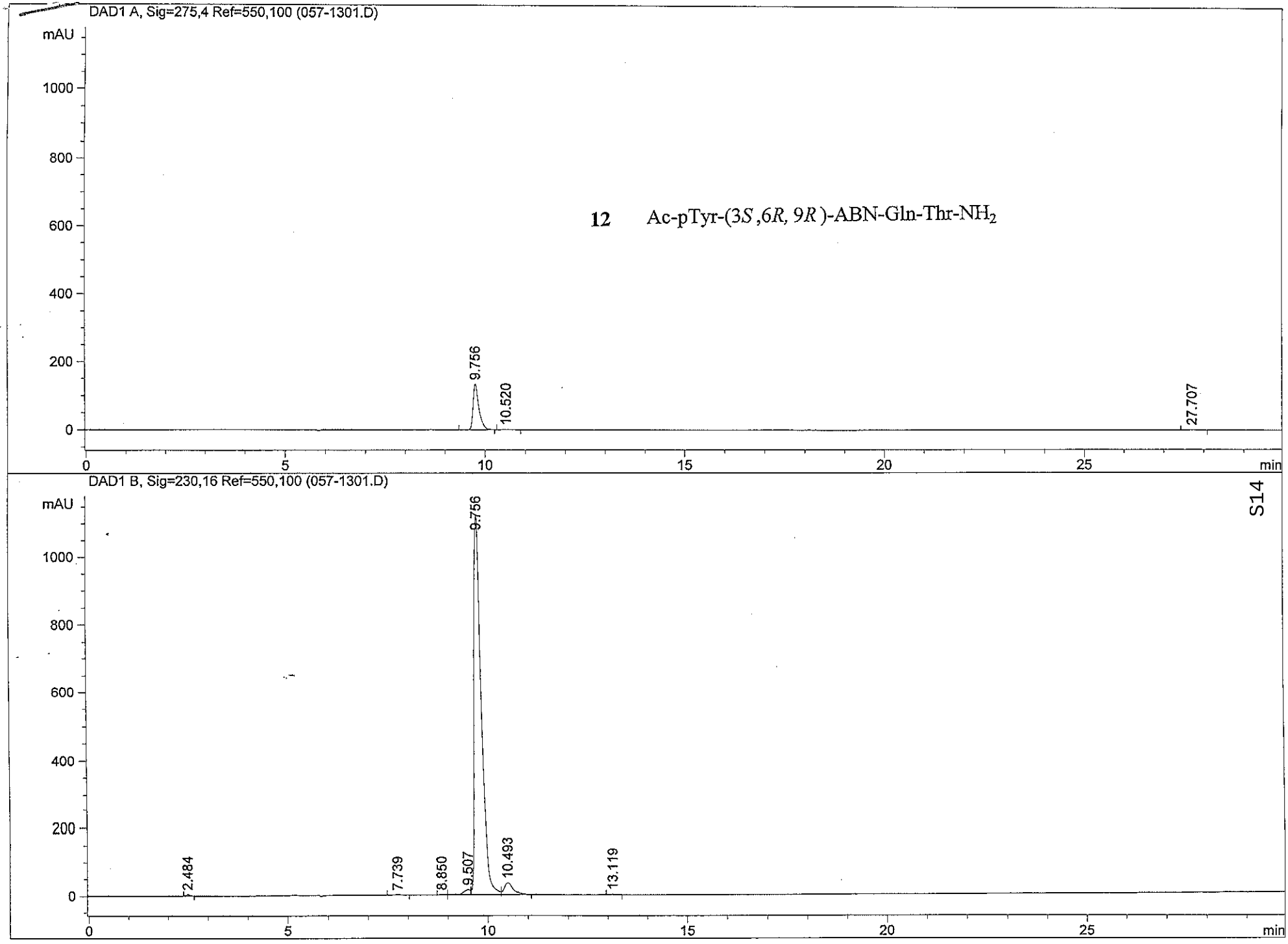

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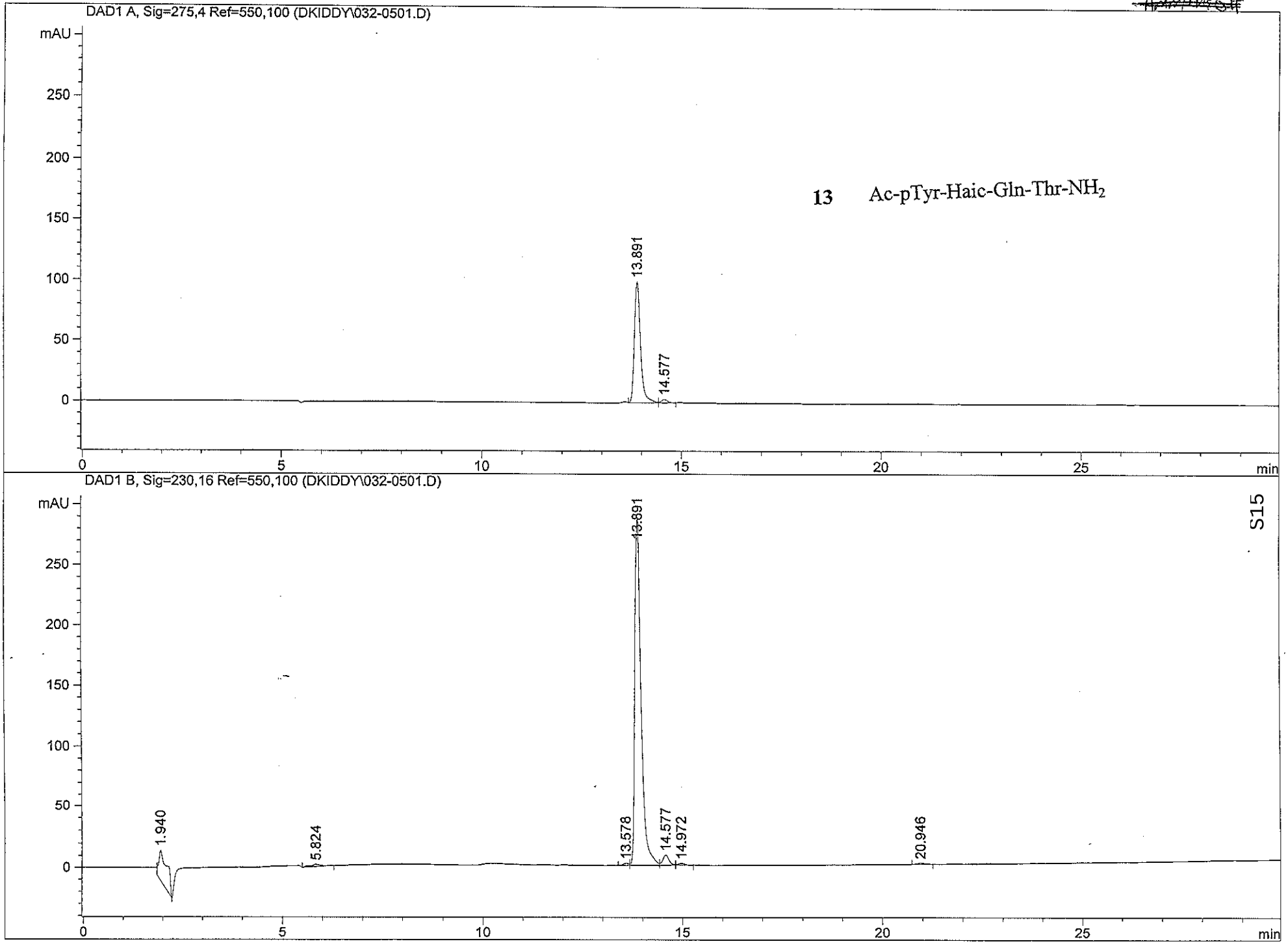
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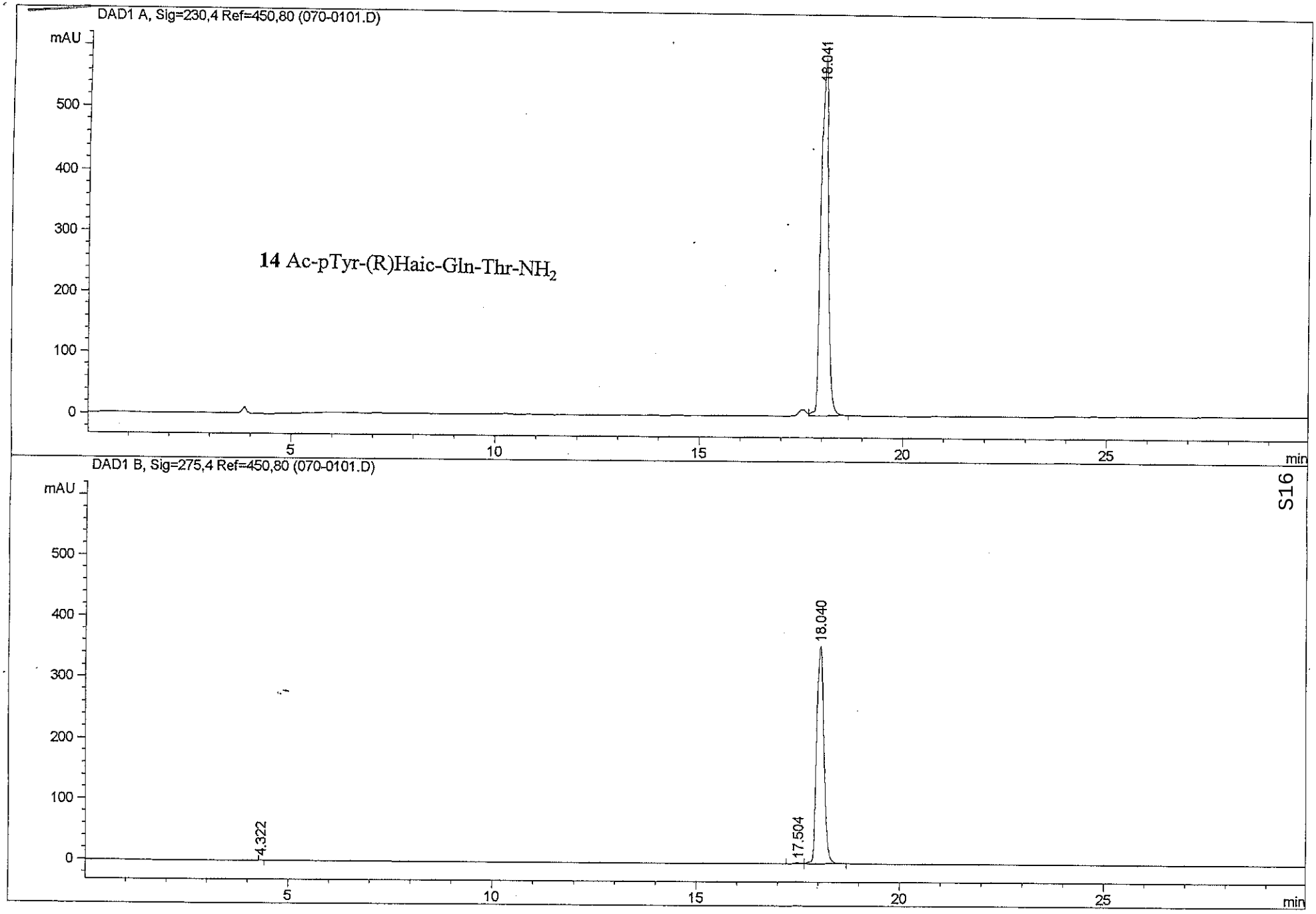
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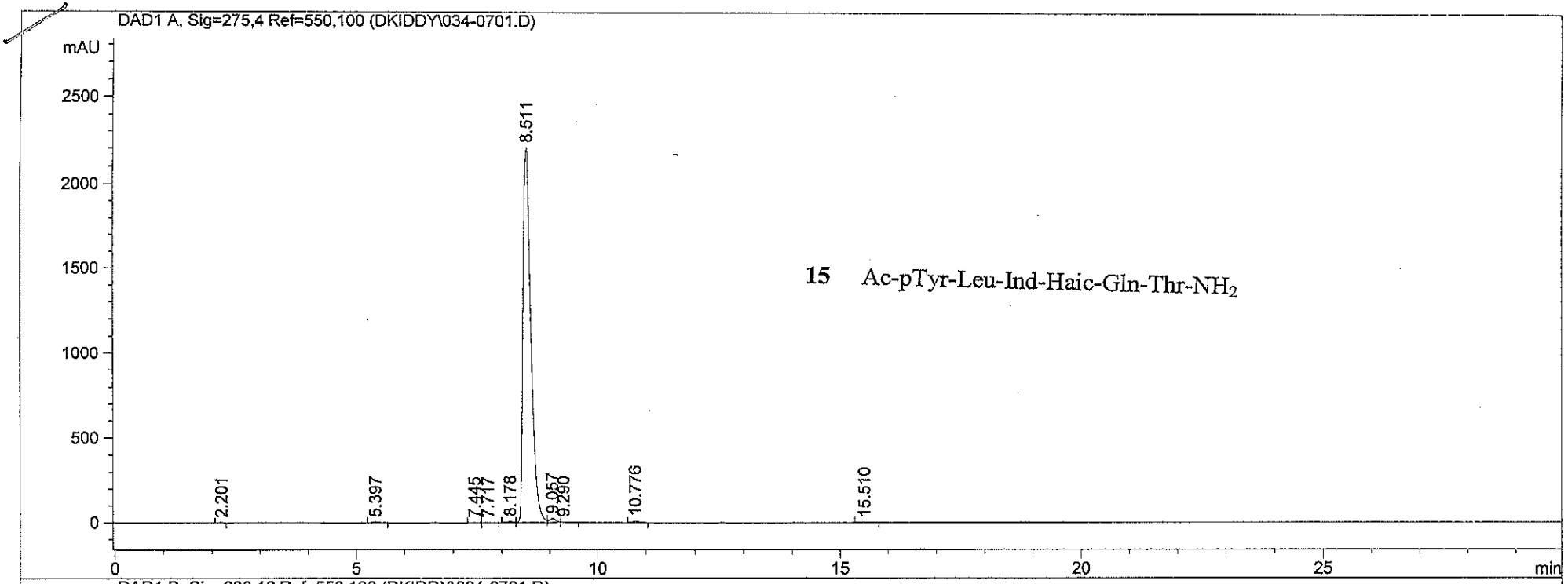

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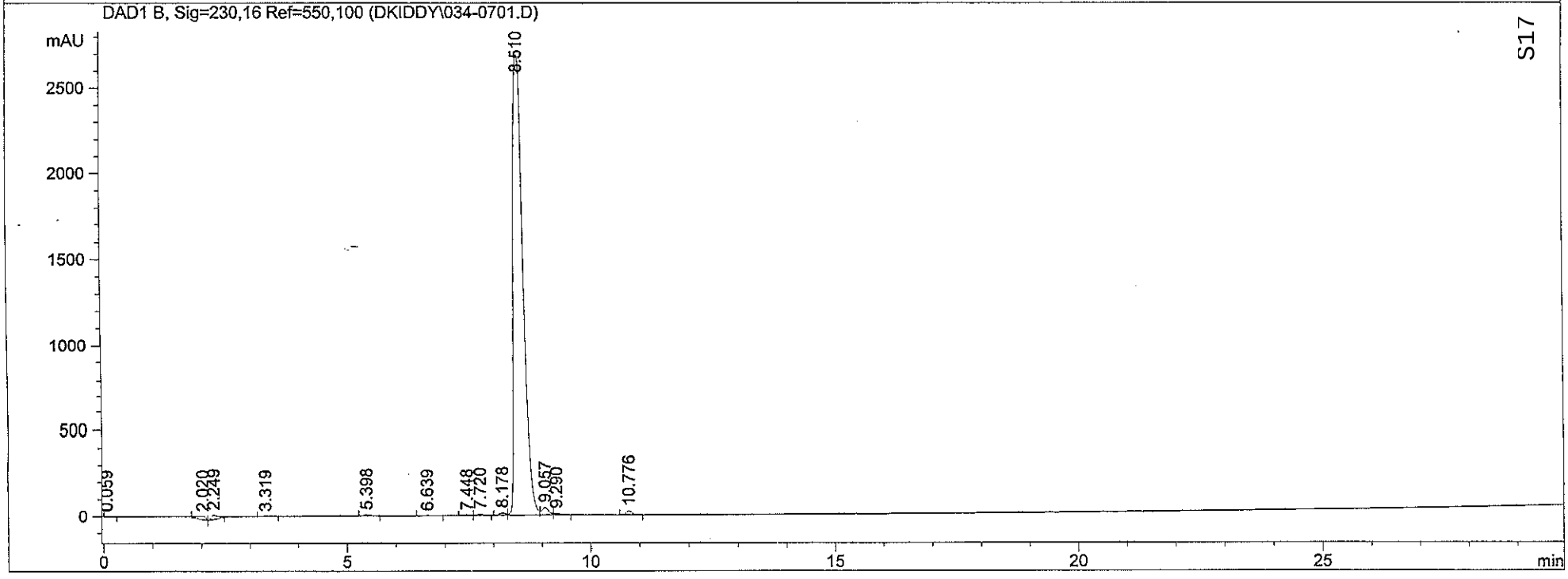

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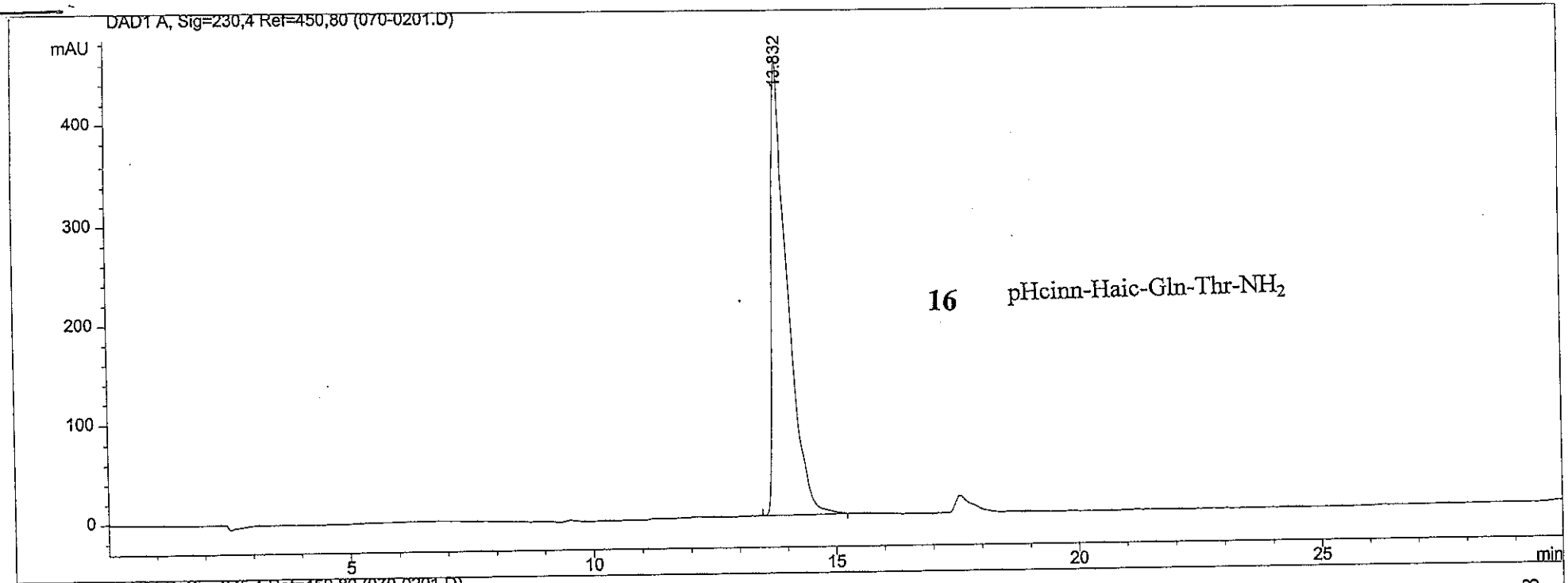

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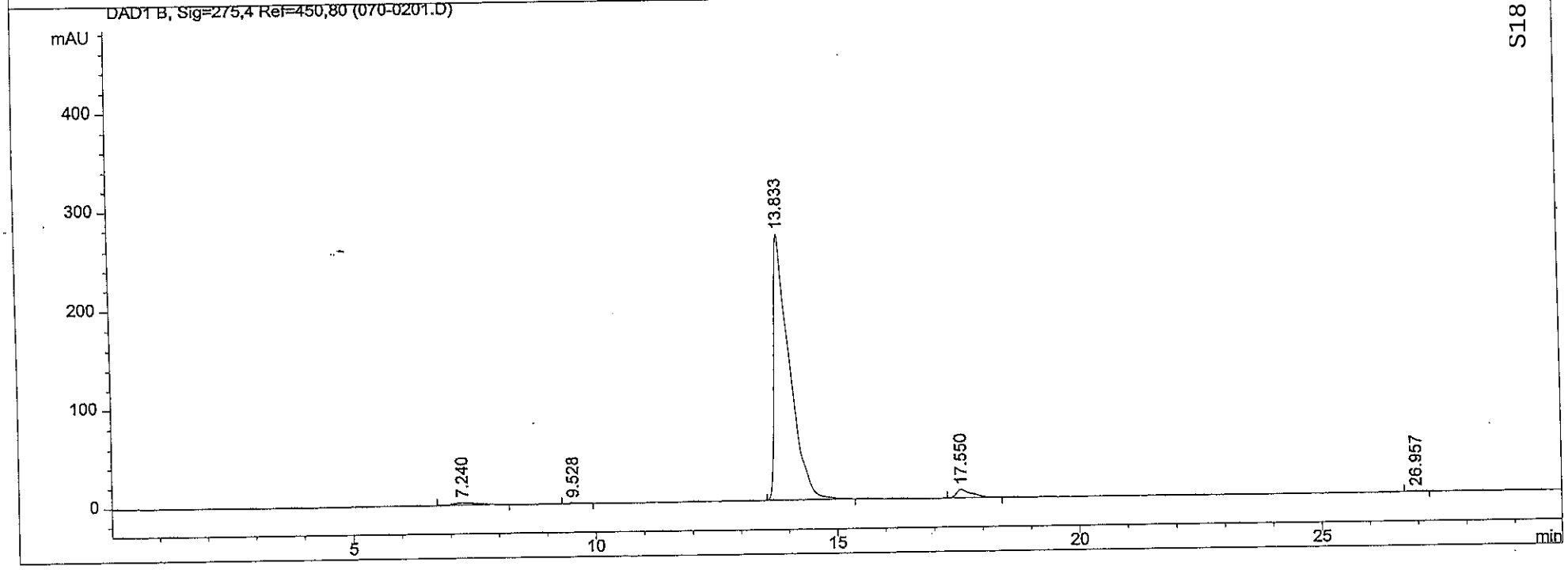

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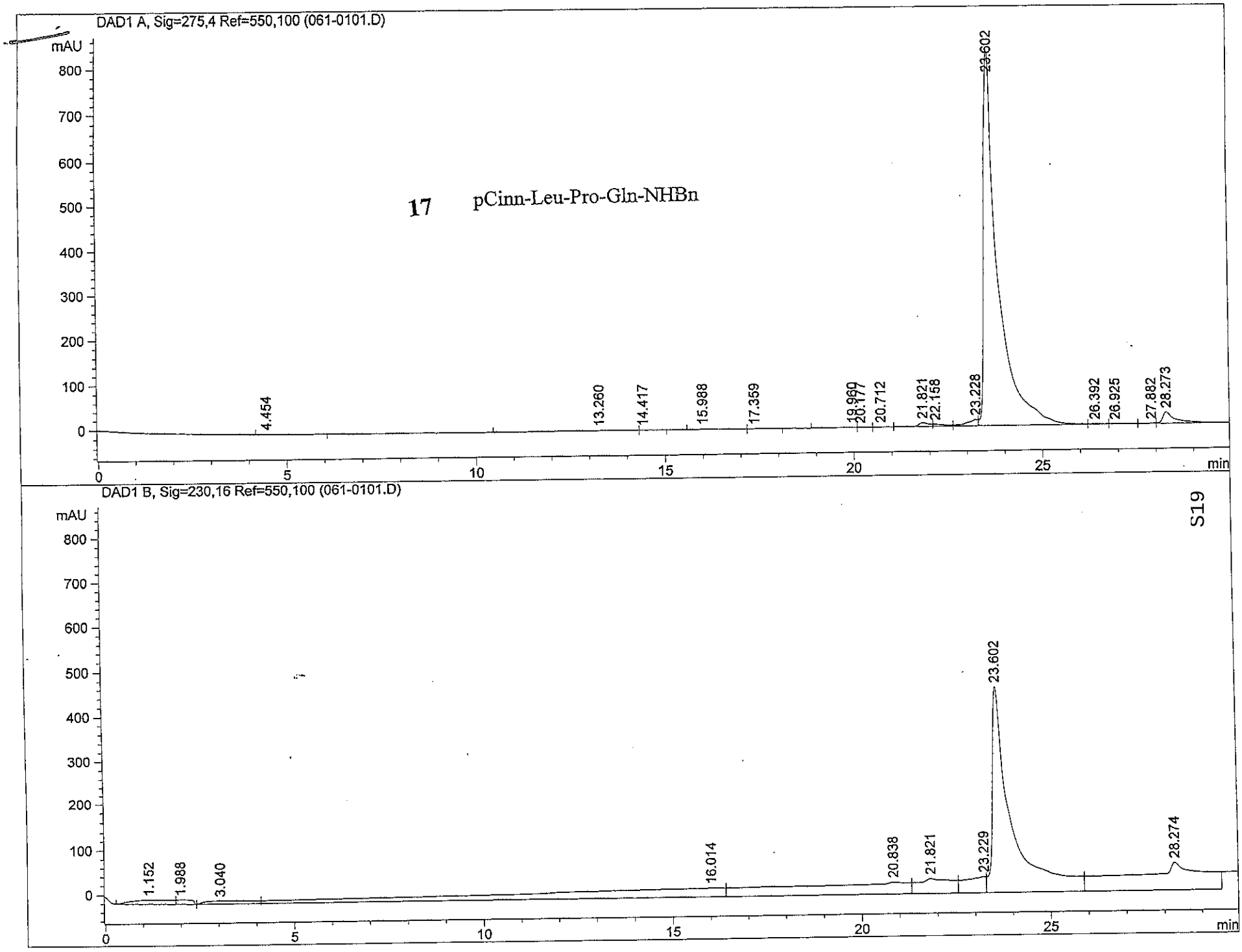
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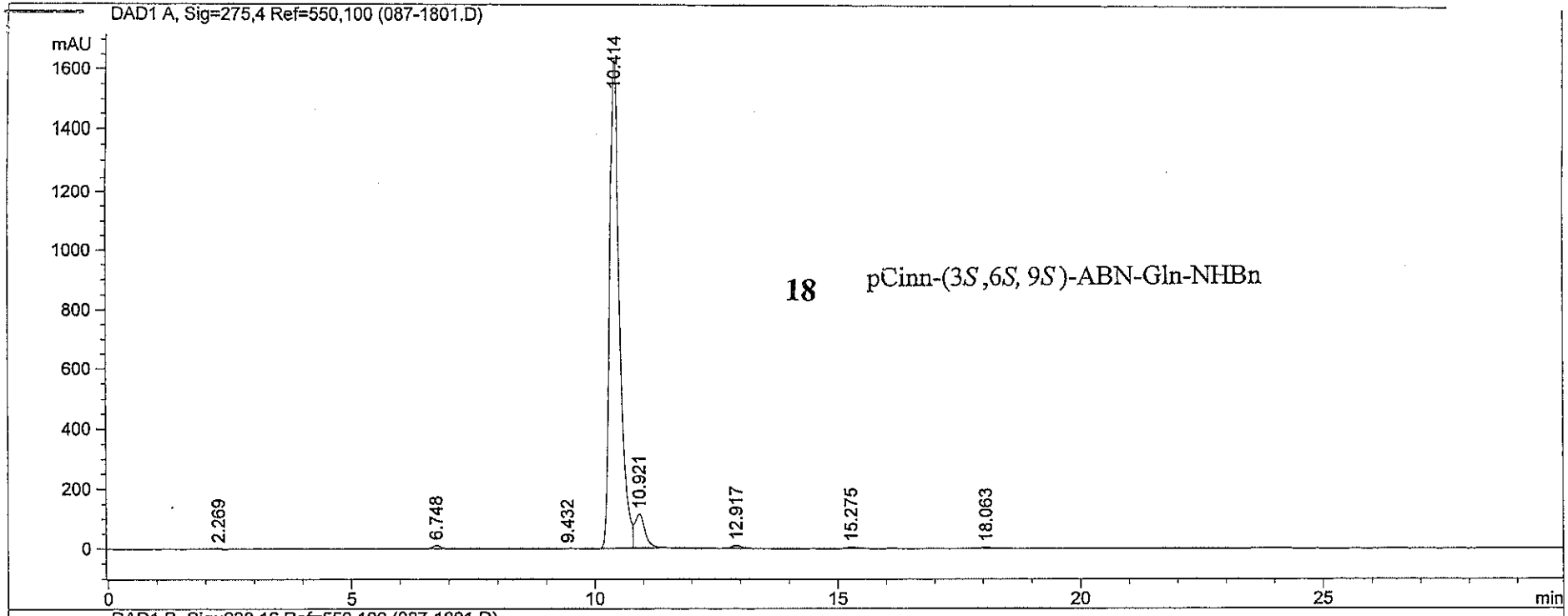

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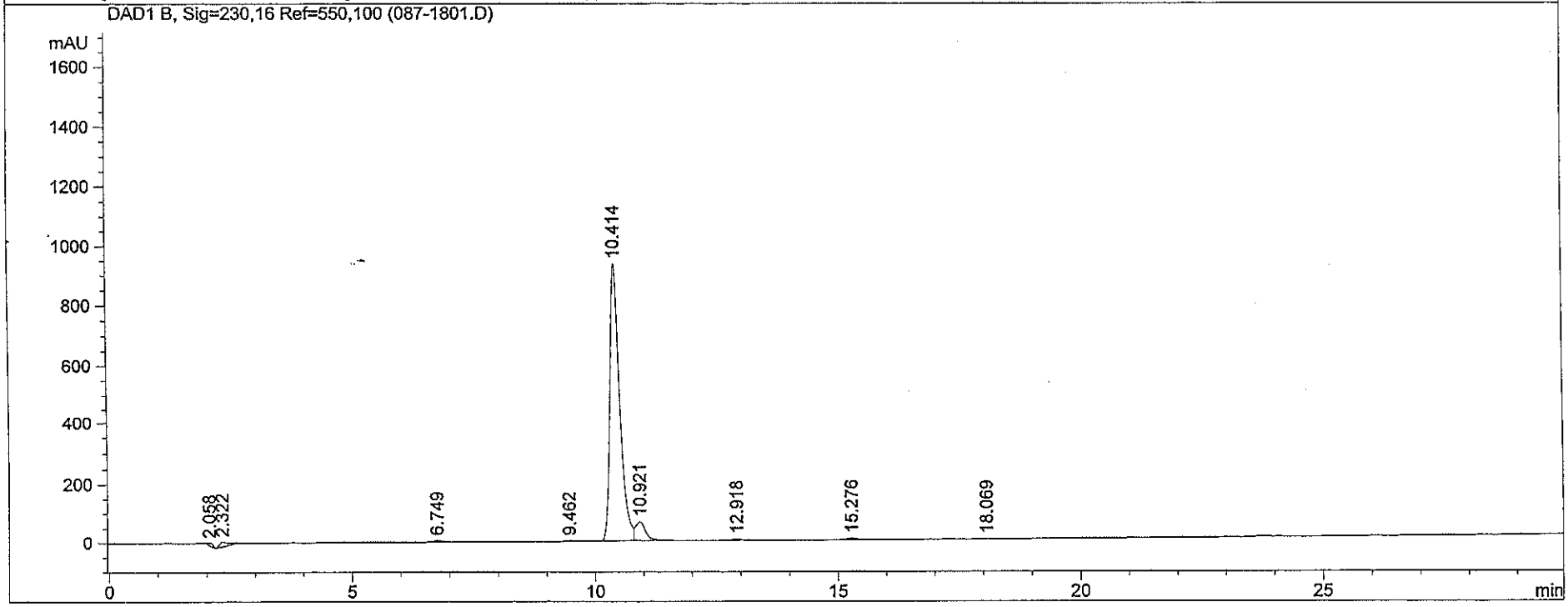

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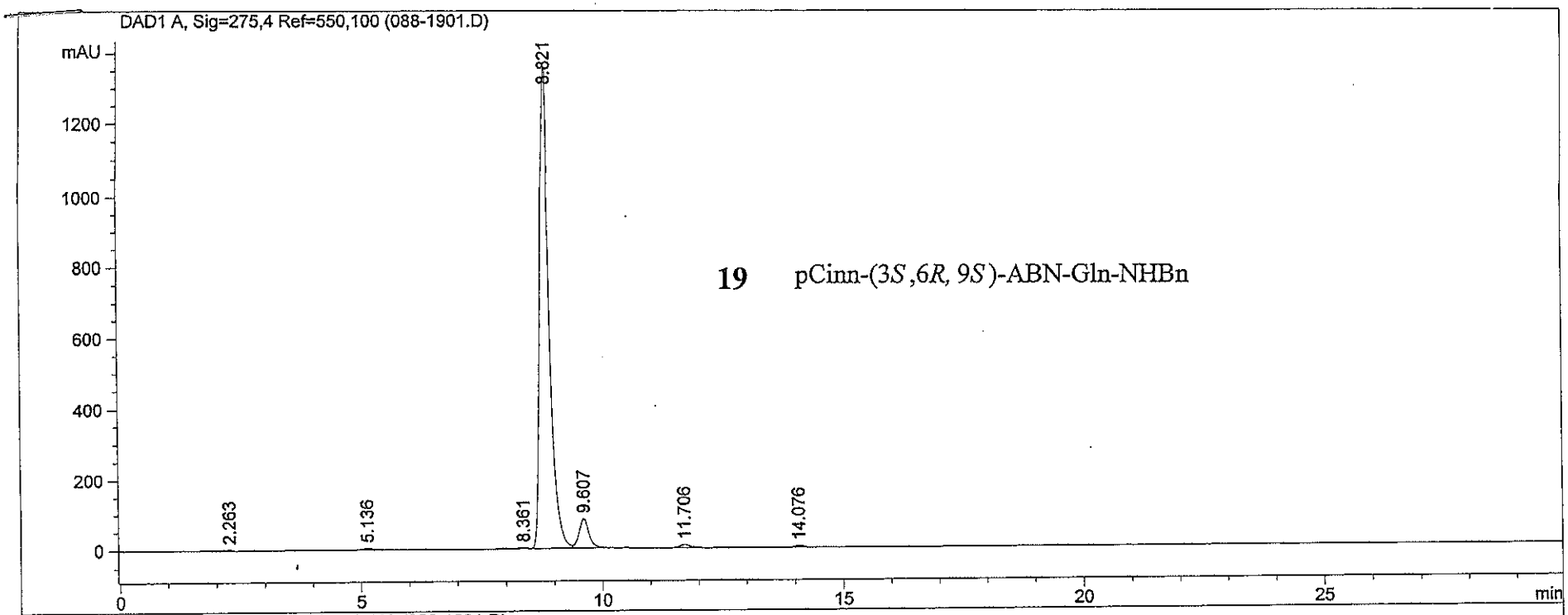

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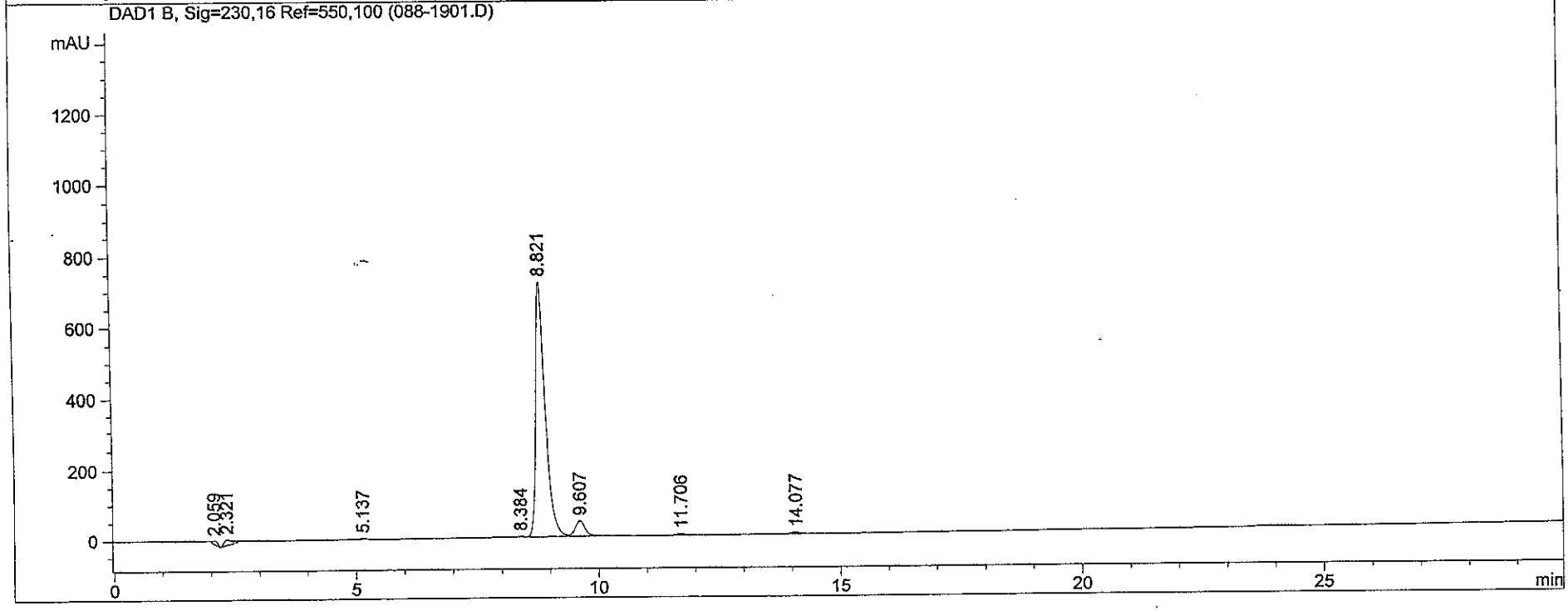

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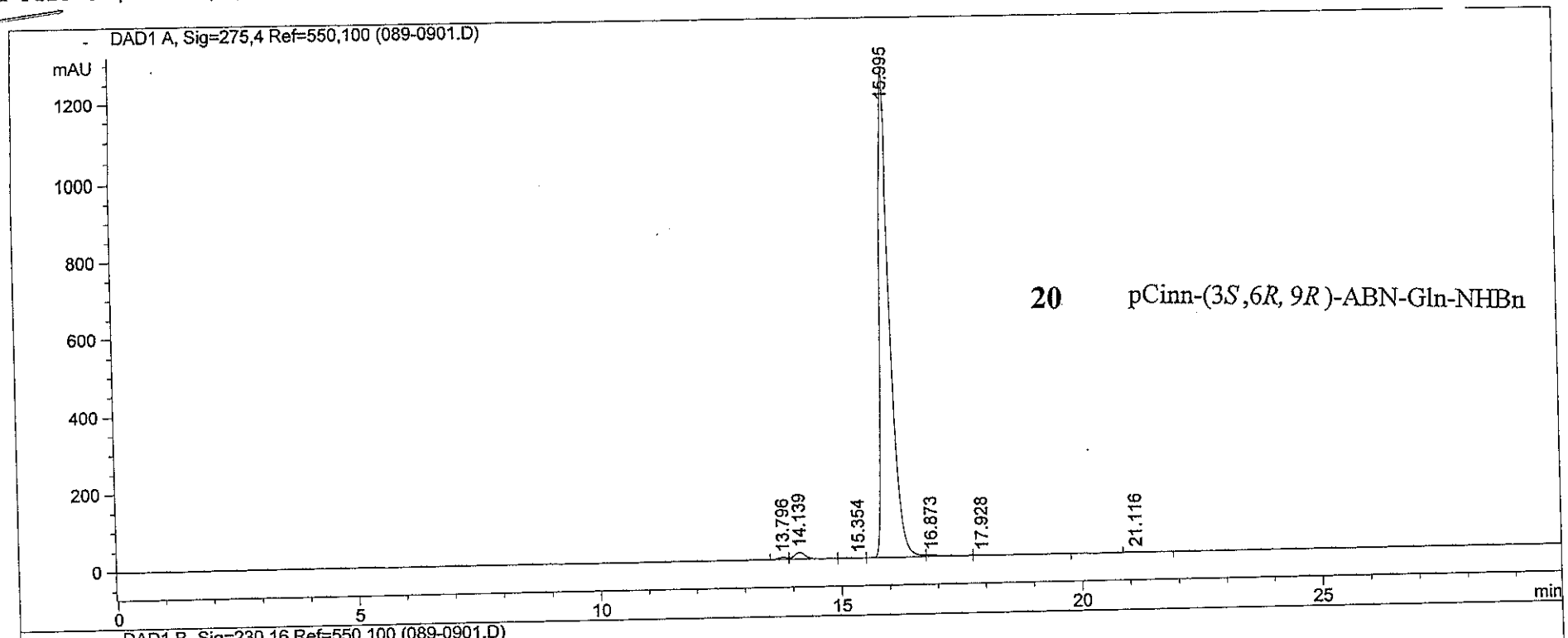

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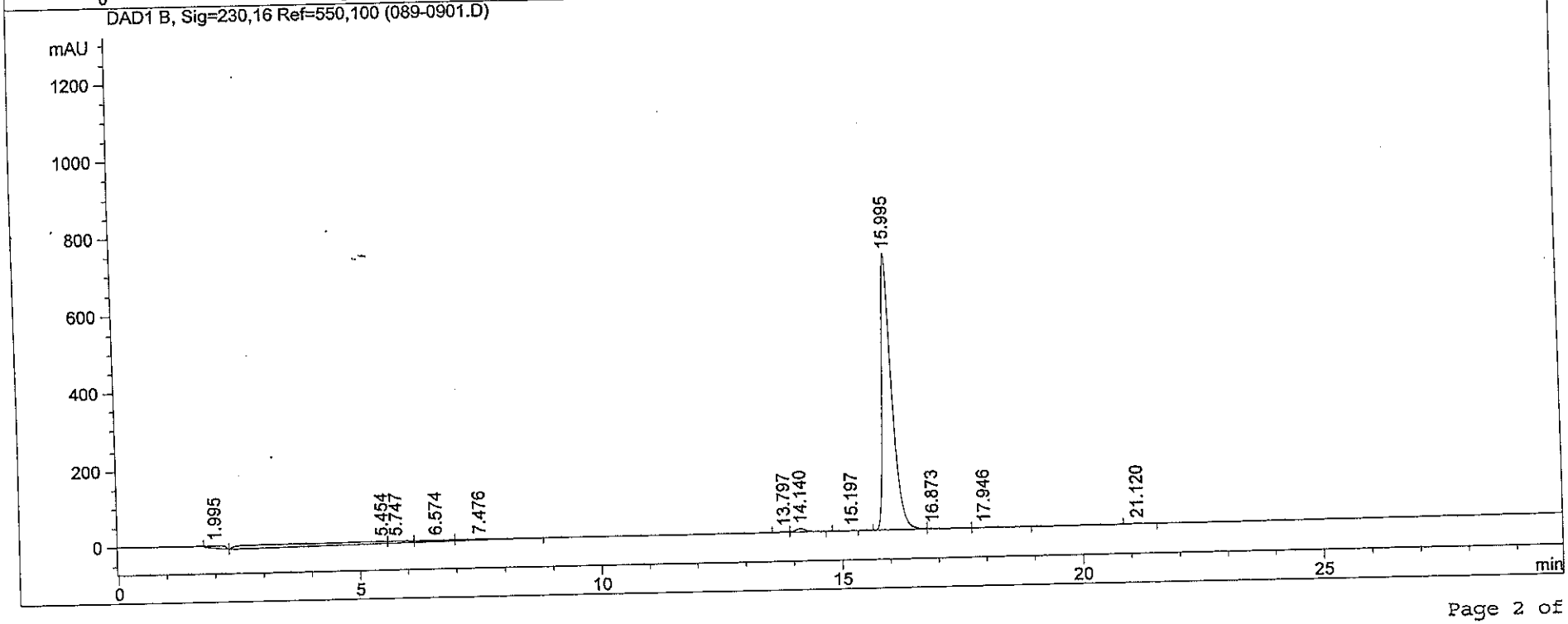

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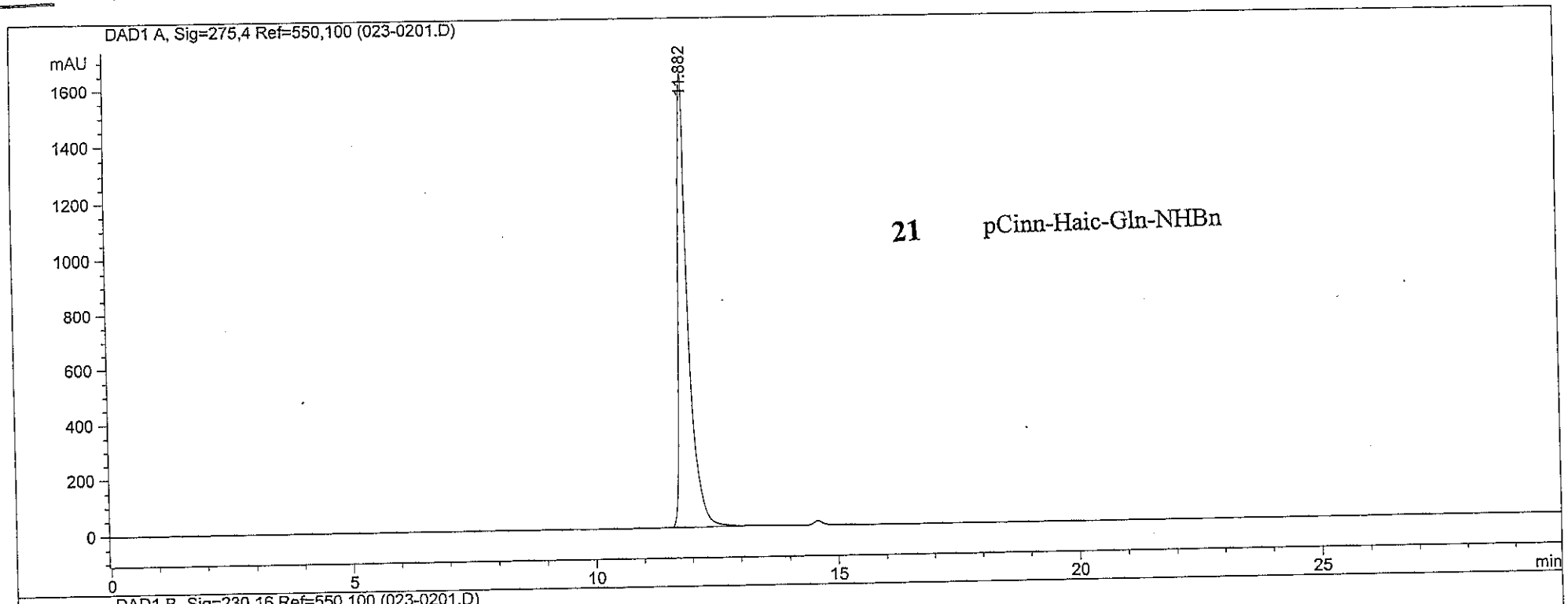

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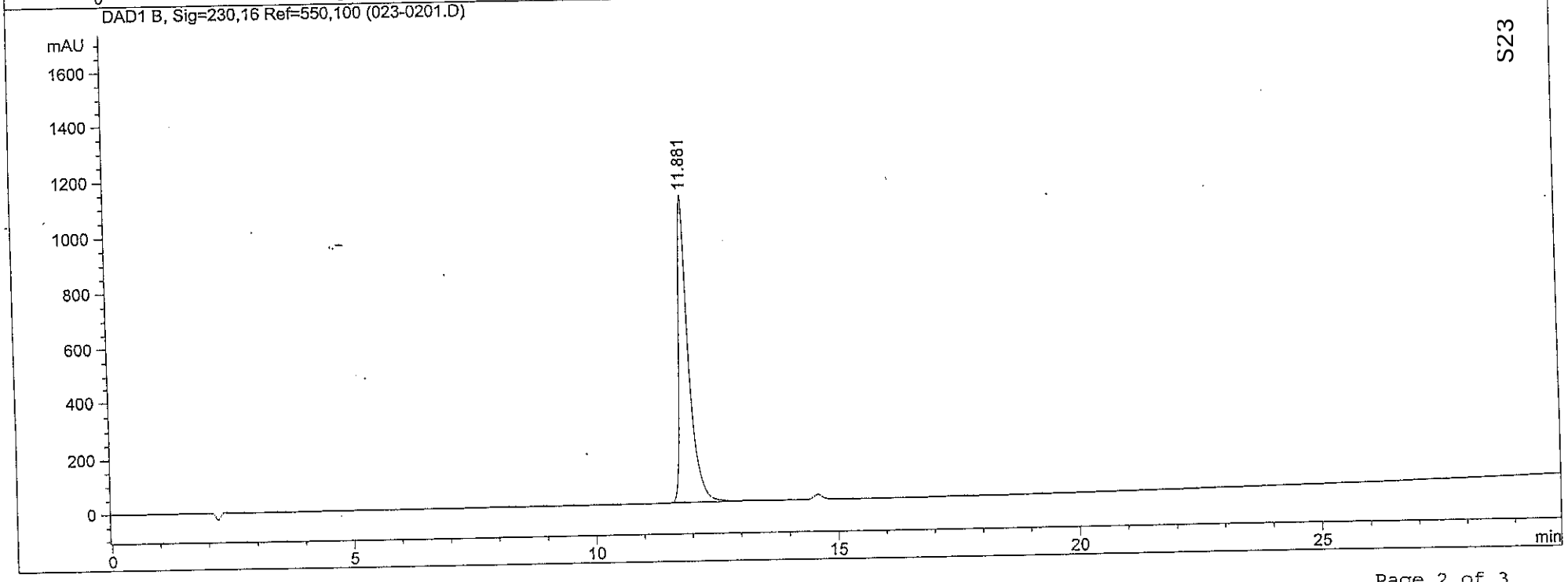

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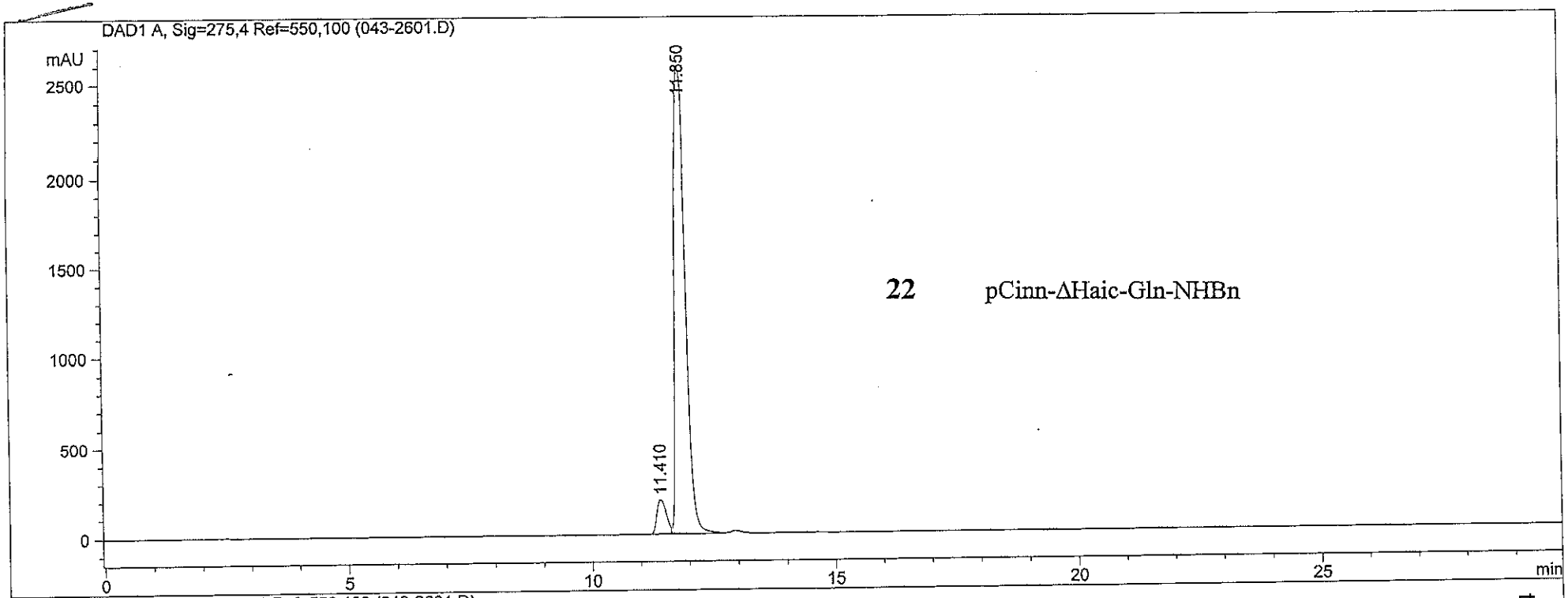

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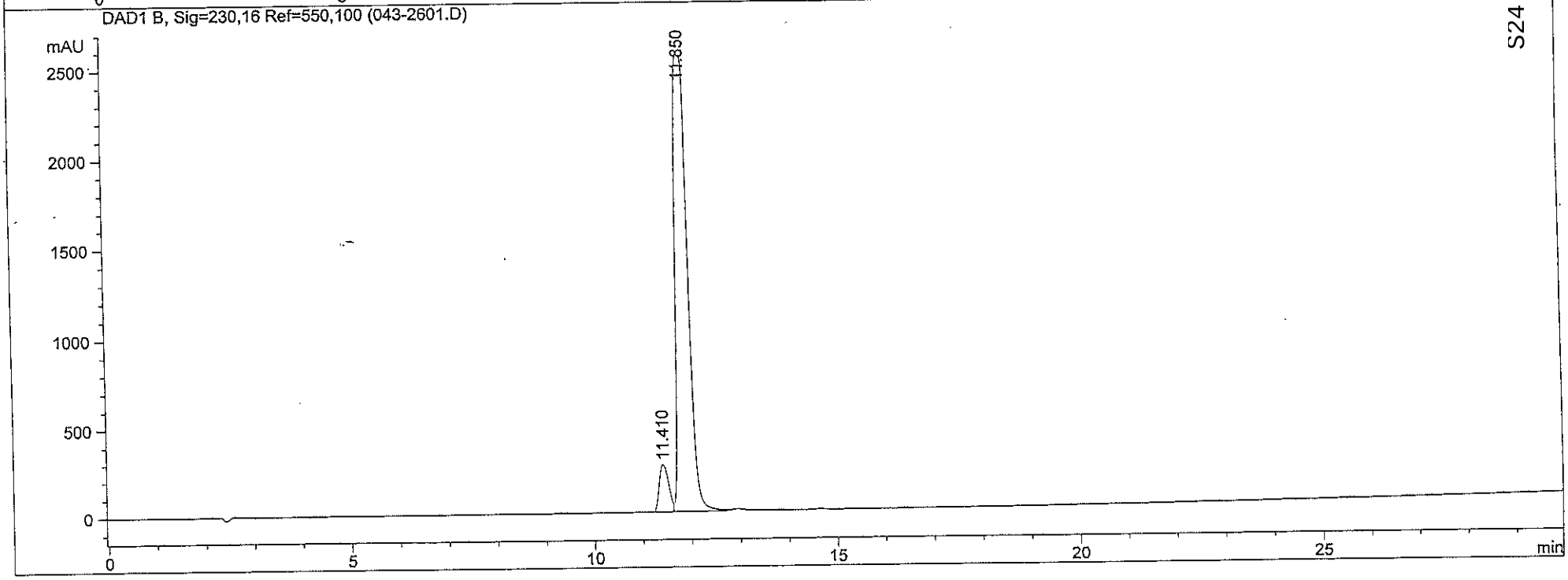
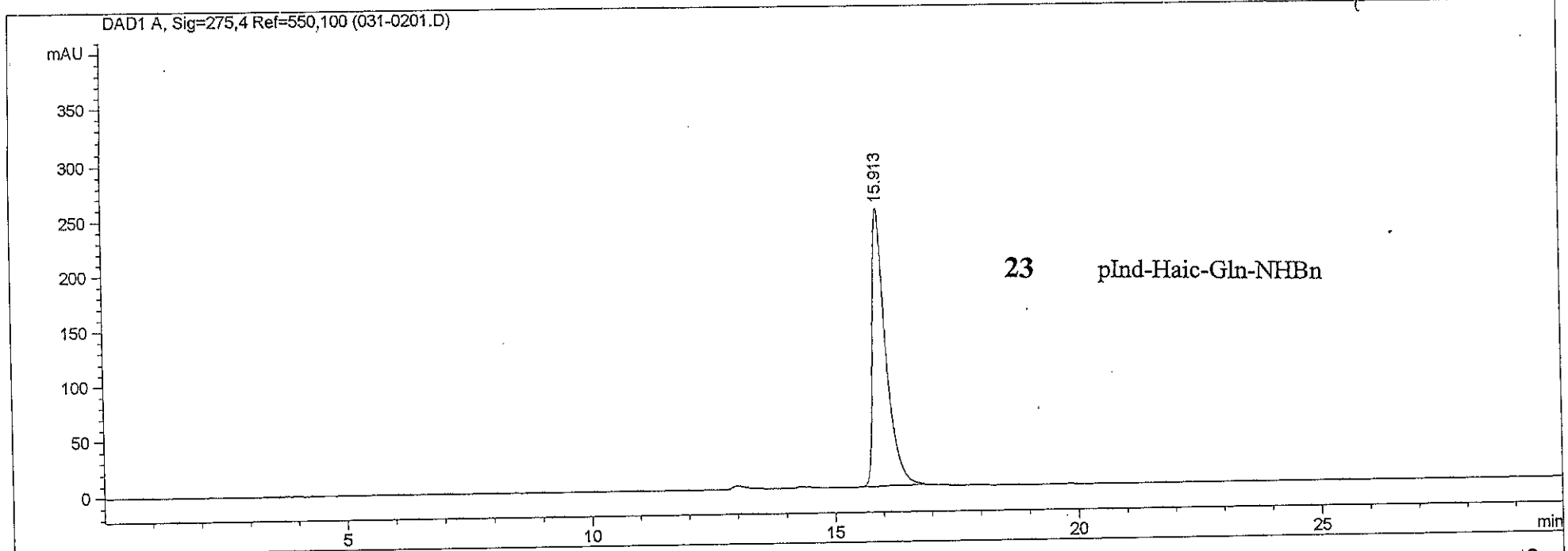
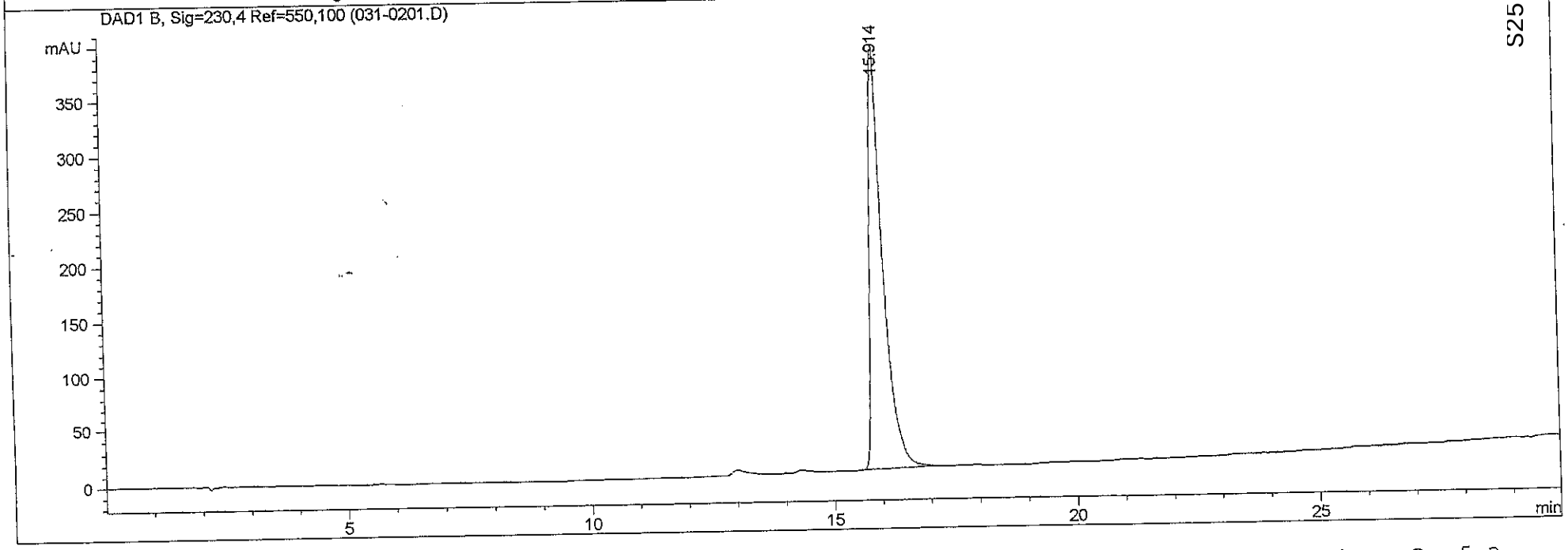


Data File C:\HPCHEM\1\DATA\031-0201.D

Sample Name: DKC1-152
AmmAc/MeOH

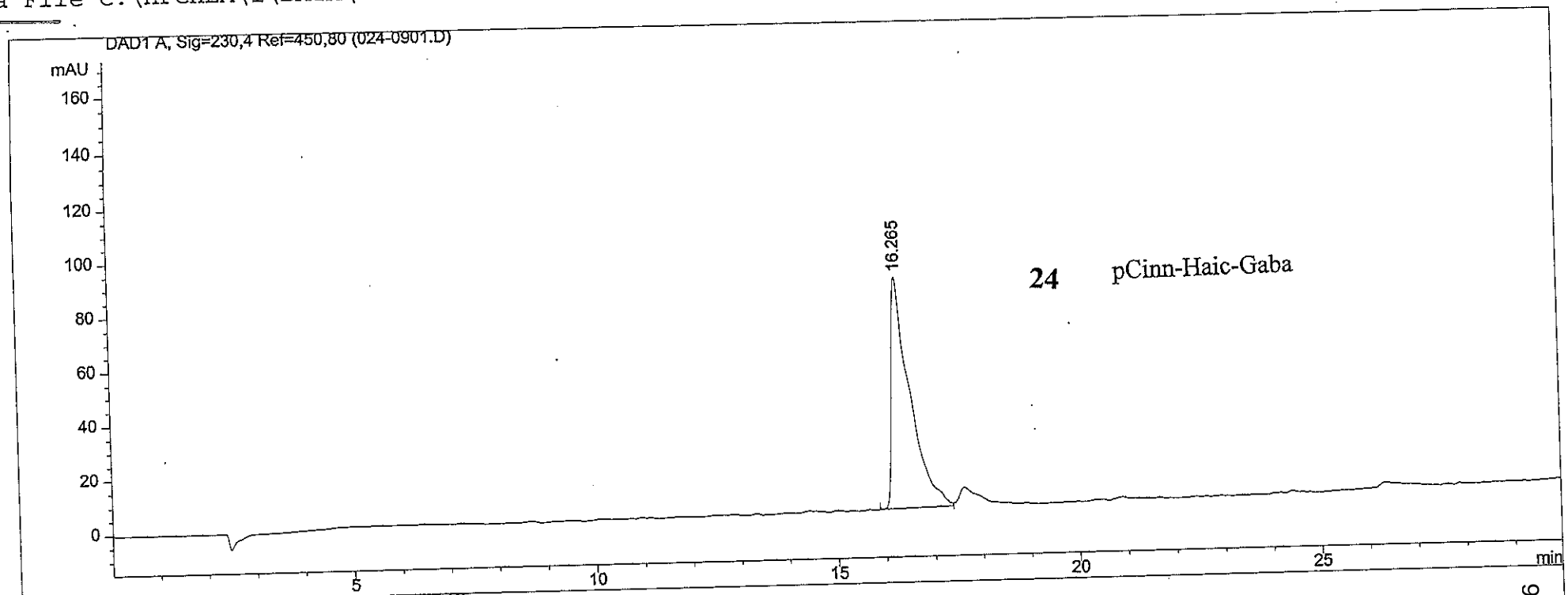


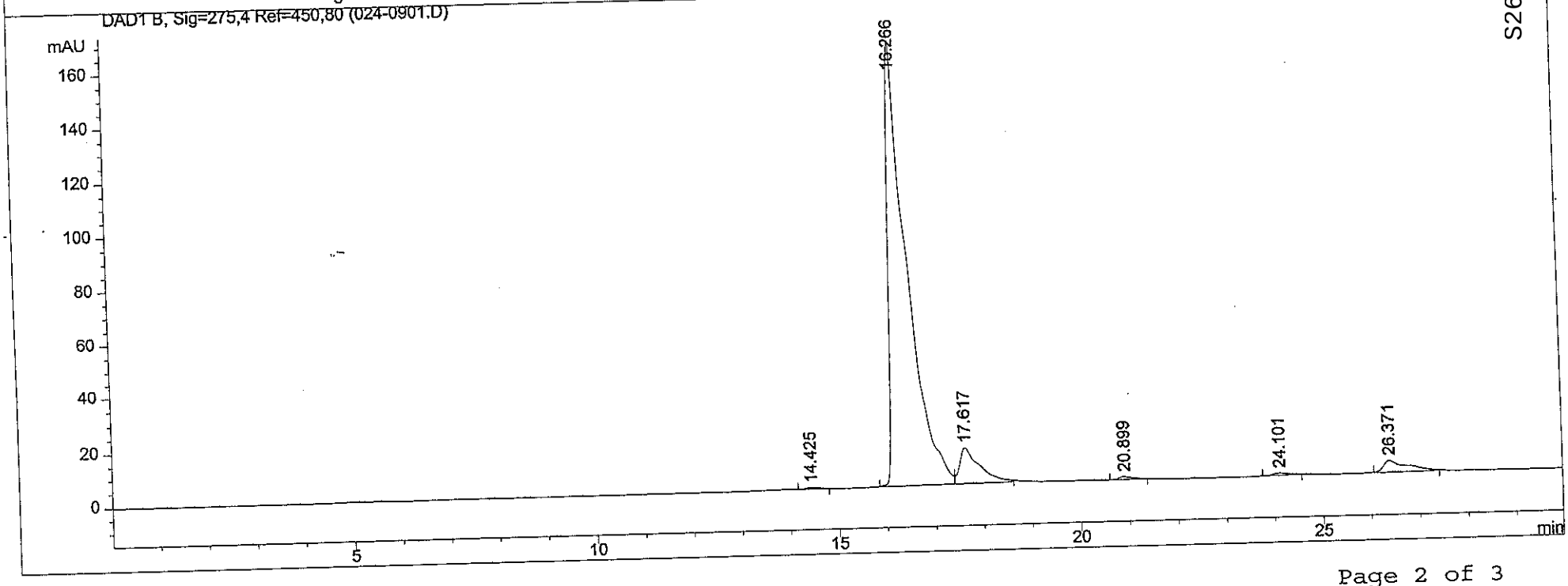
23 pInd-Haic-Gln-NHBn



S25

Data File C:\HPCHEM\1\DATA\024-0901.D





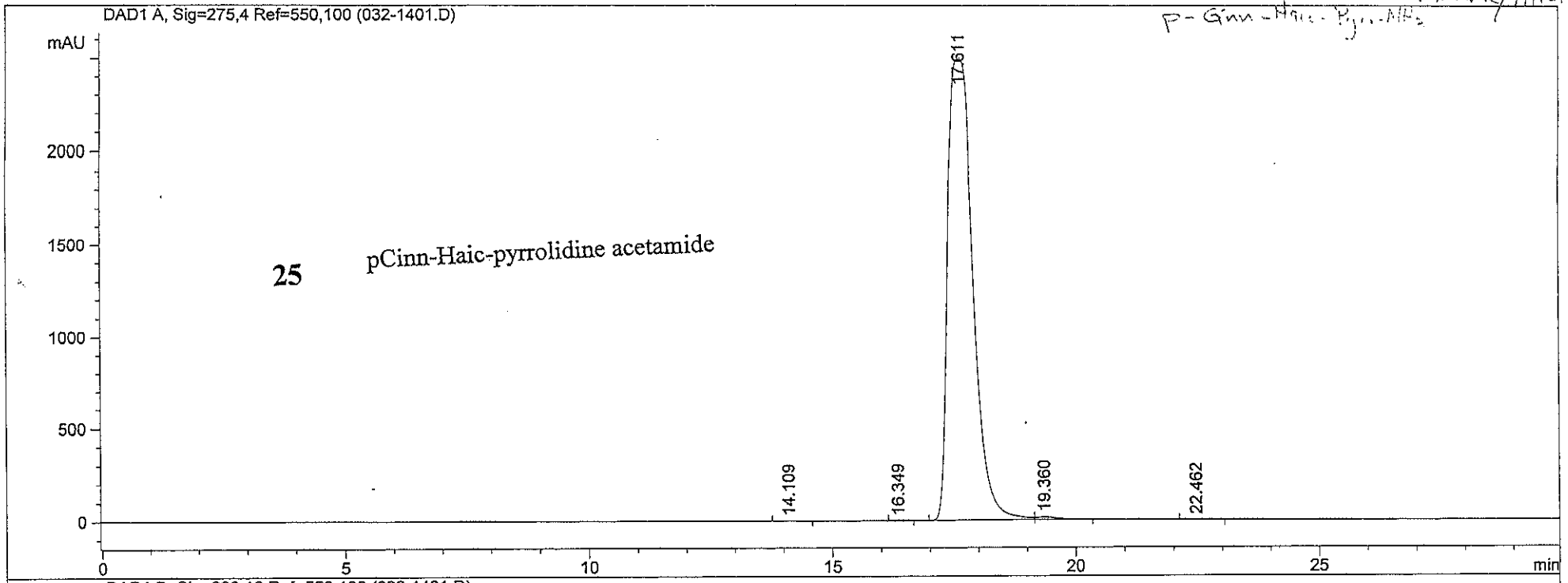
S26

Instrument 1 4/12/2007 1:36:53 PM John

AmmAc/MeOH

P-Cinn-Haic-Pyr.-NH2

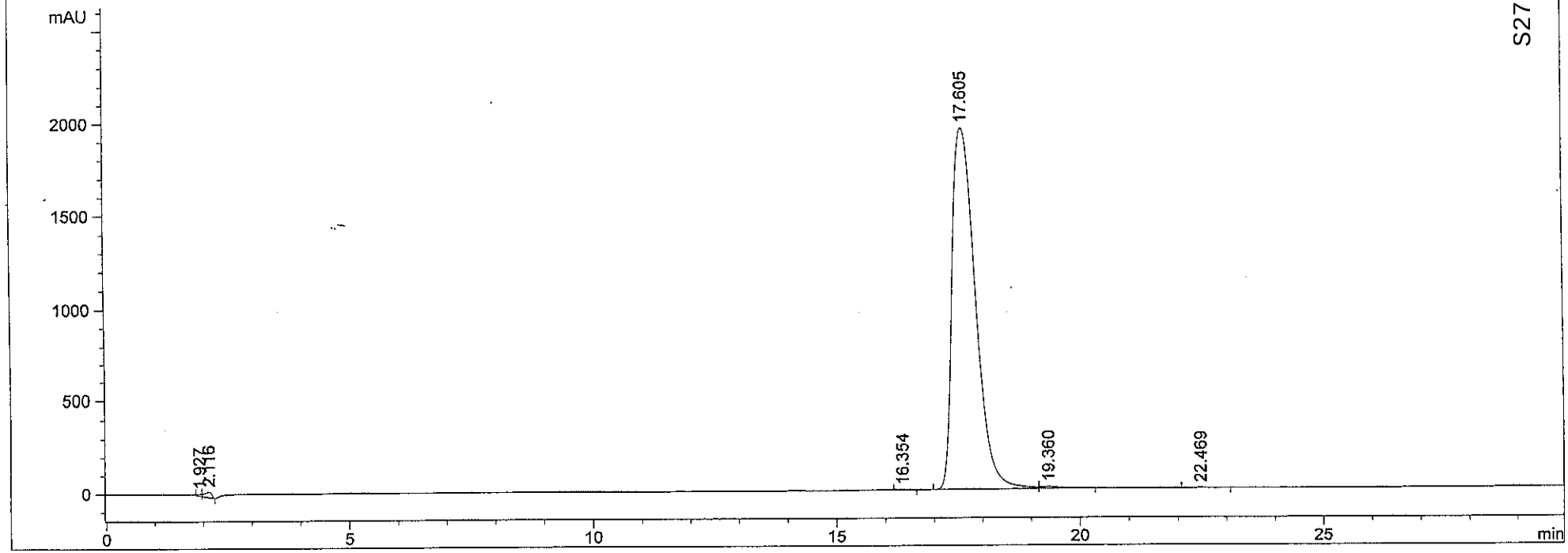
DAD1 A, Sig=275,4 Ref=550,100 (032-1401.D)

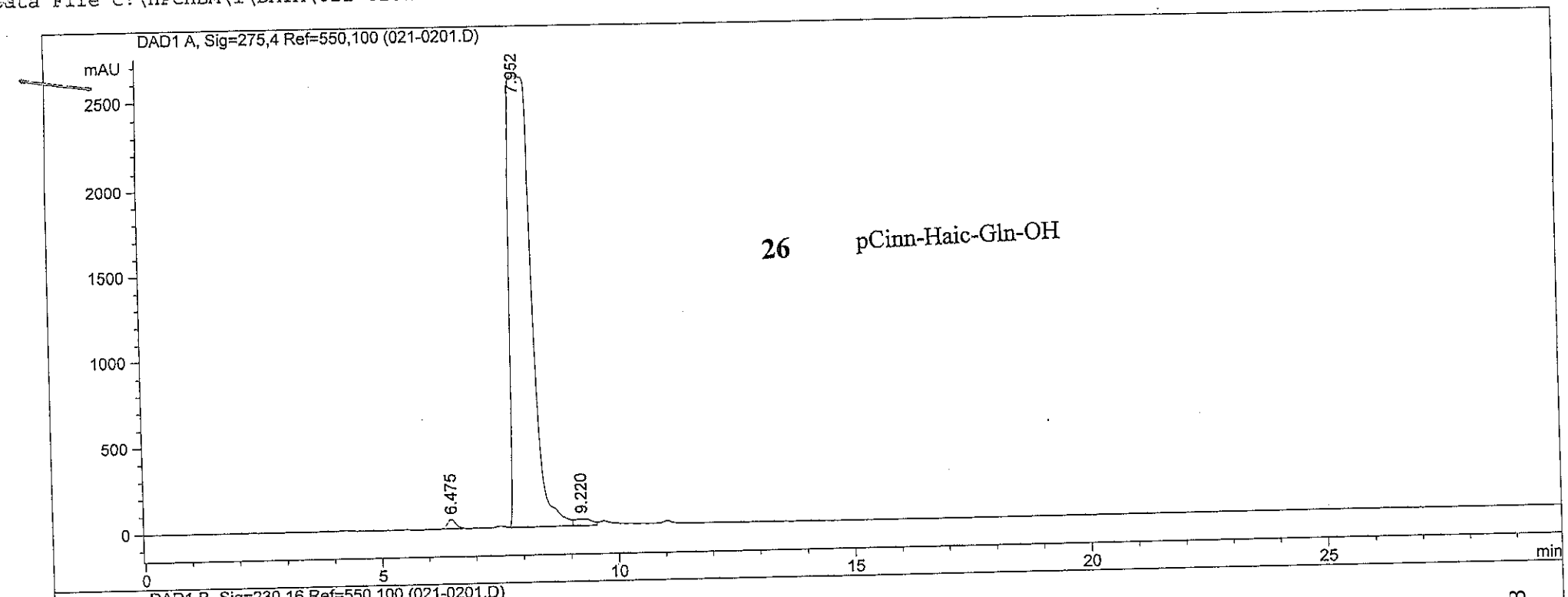


25 pCinn-Haic-pyrrolidine acetamide

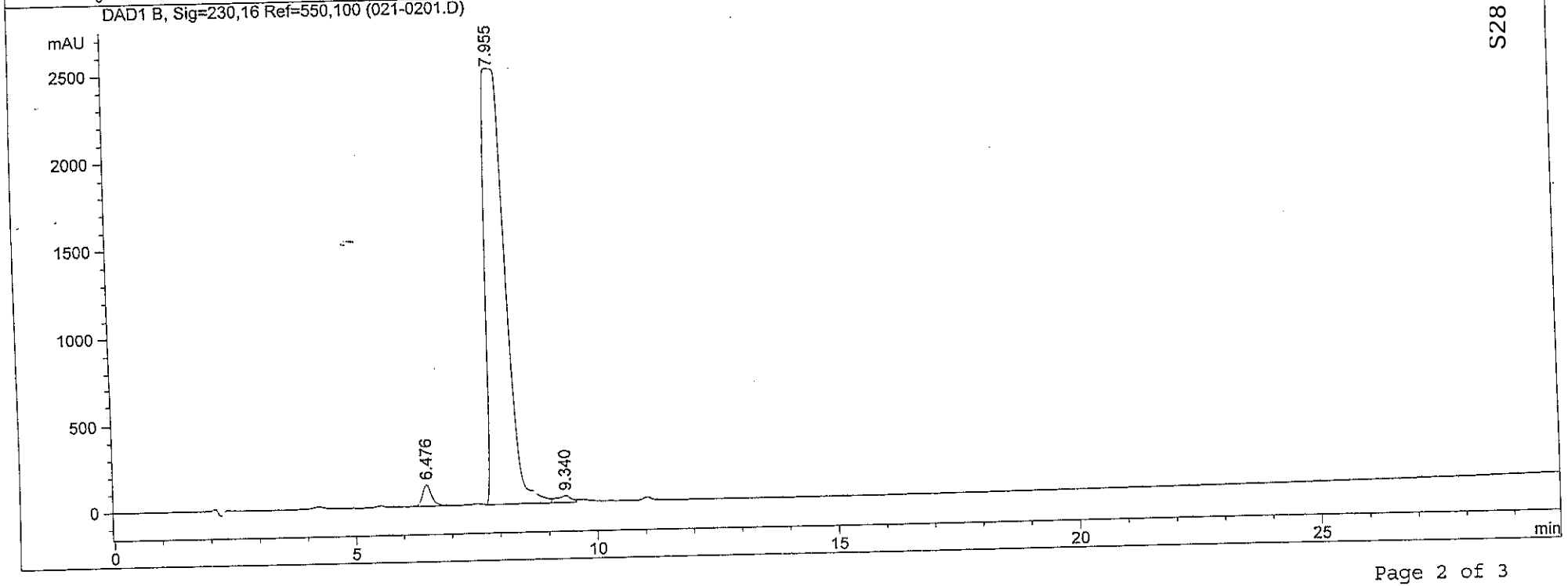
DAD1 B, Sig=230,16 Ref=550,100 (032-1401.D)

S27

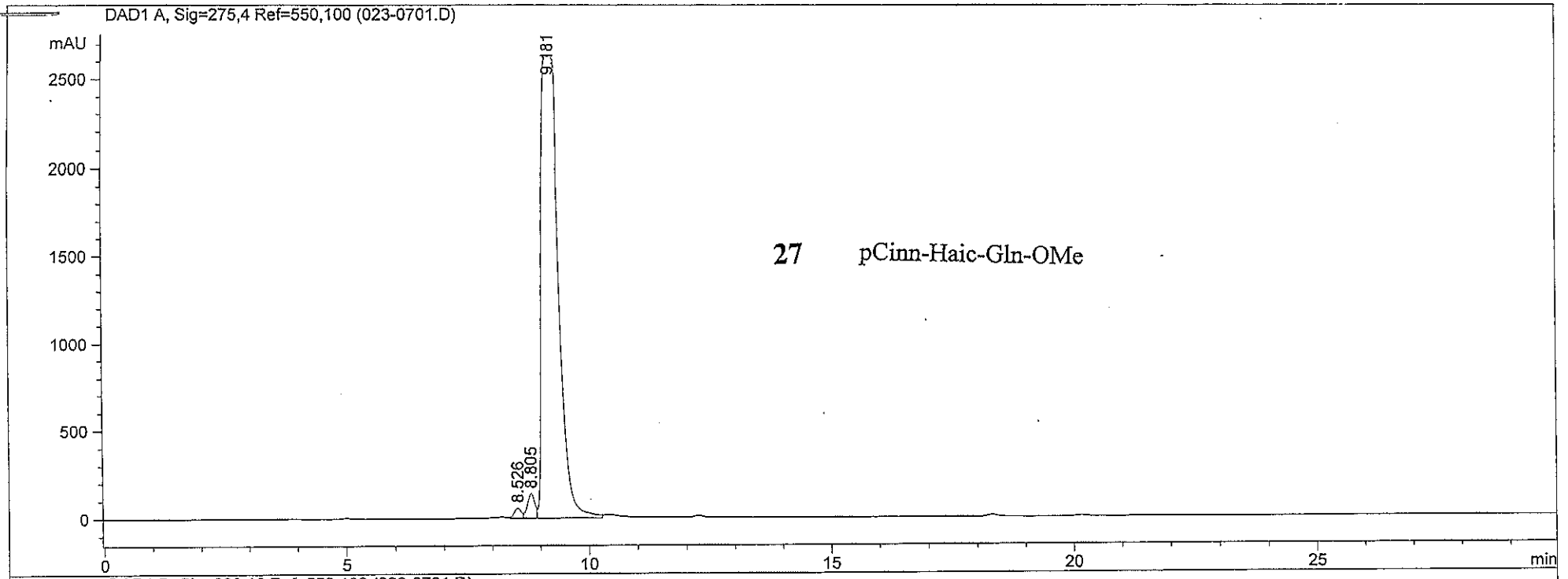




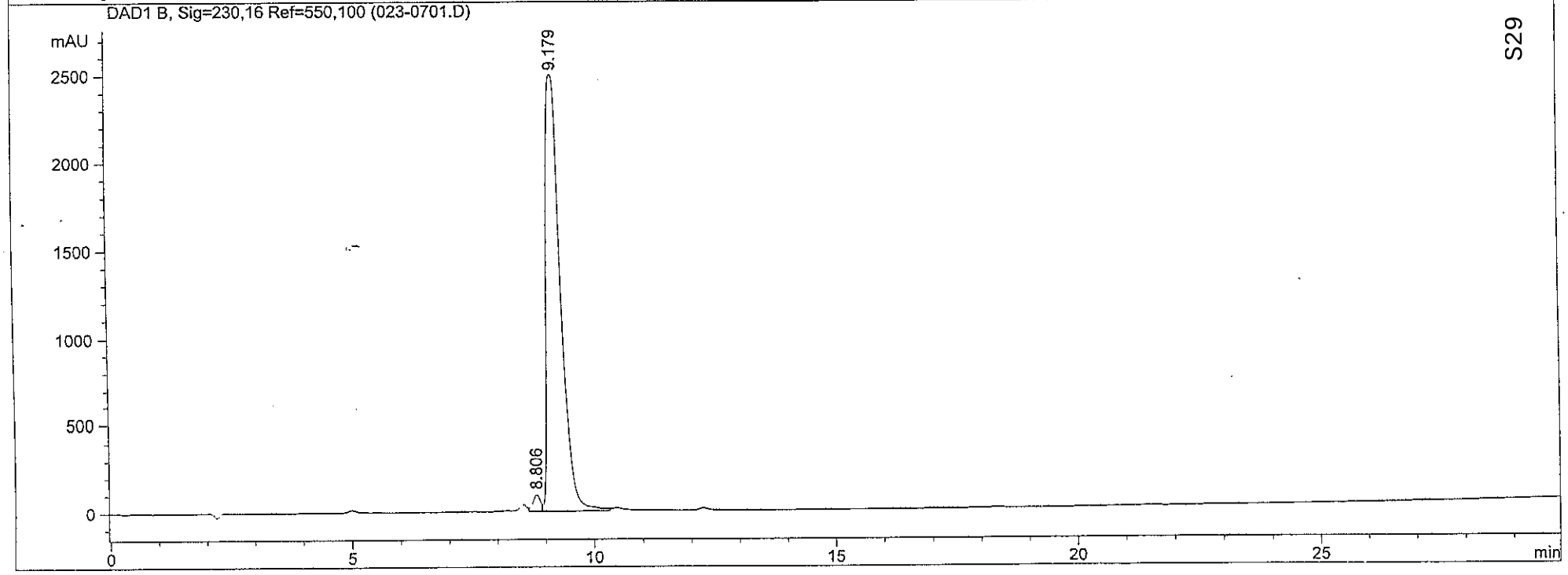
26 pCinn-Haic-Gln-OH



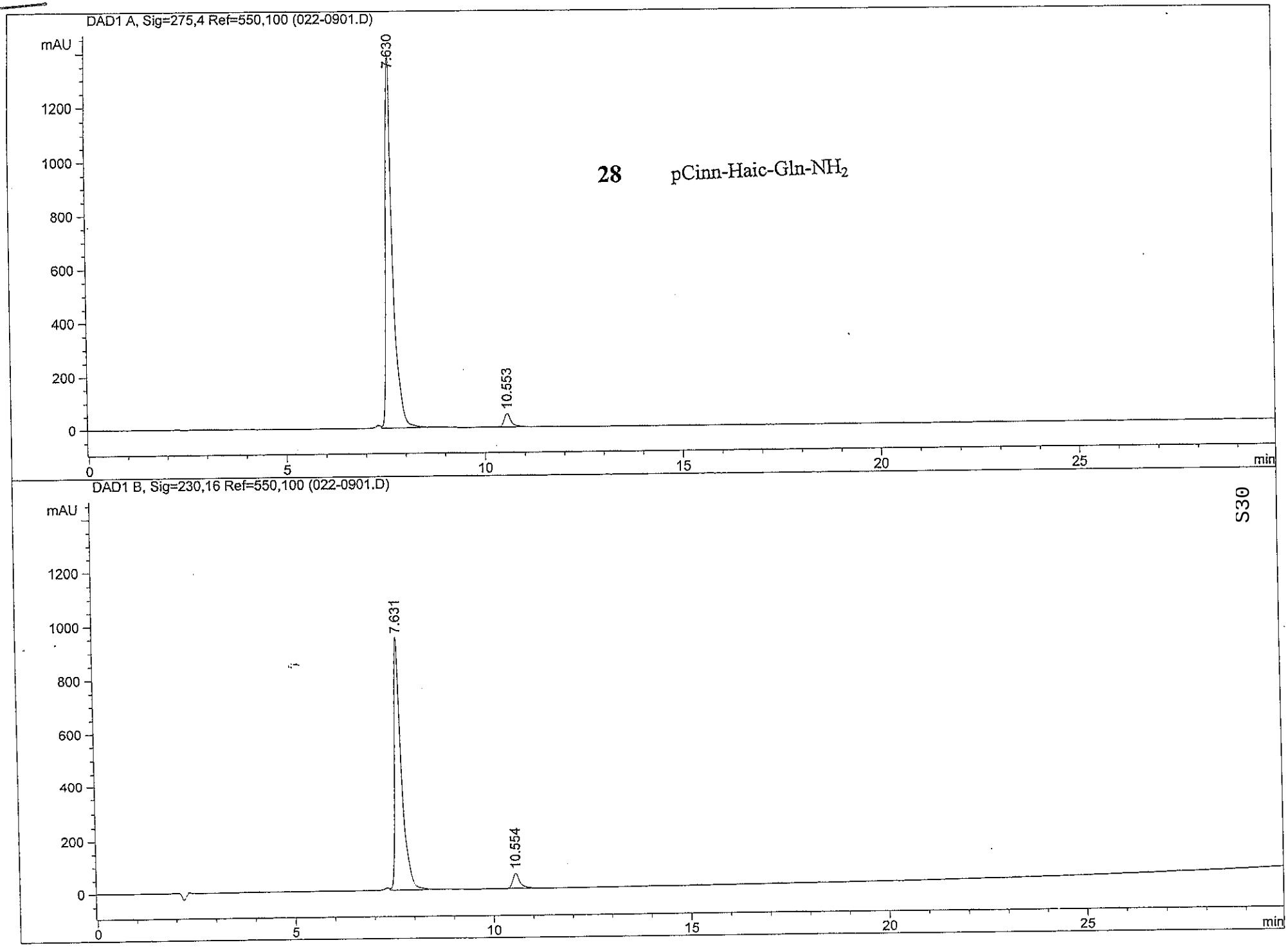
S28



27 pCinn-Haic-Gln-OMe

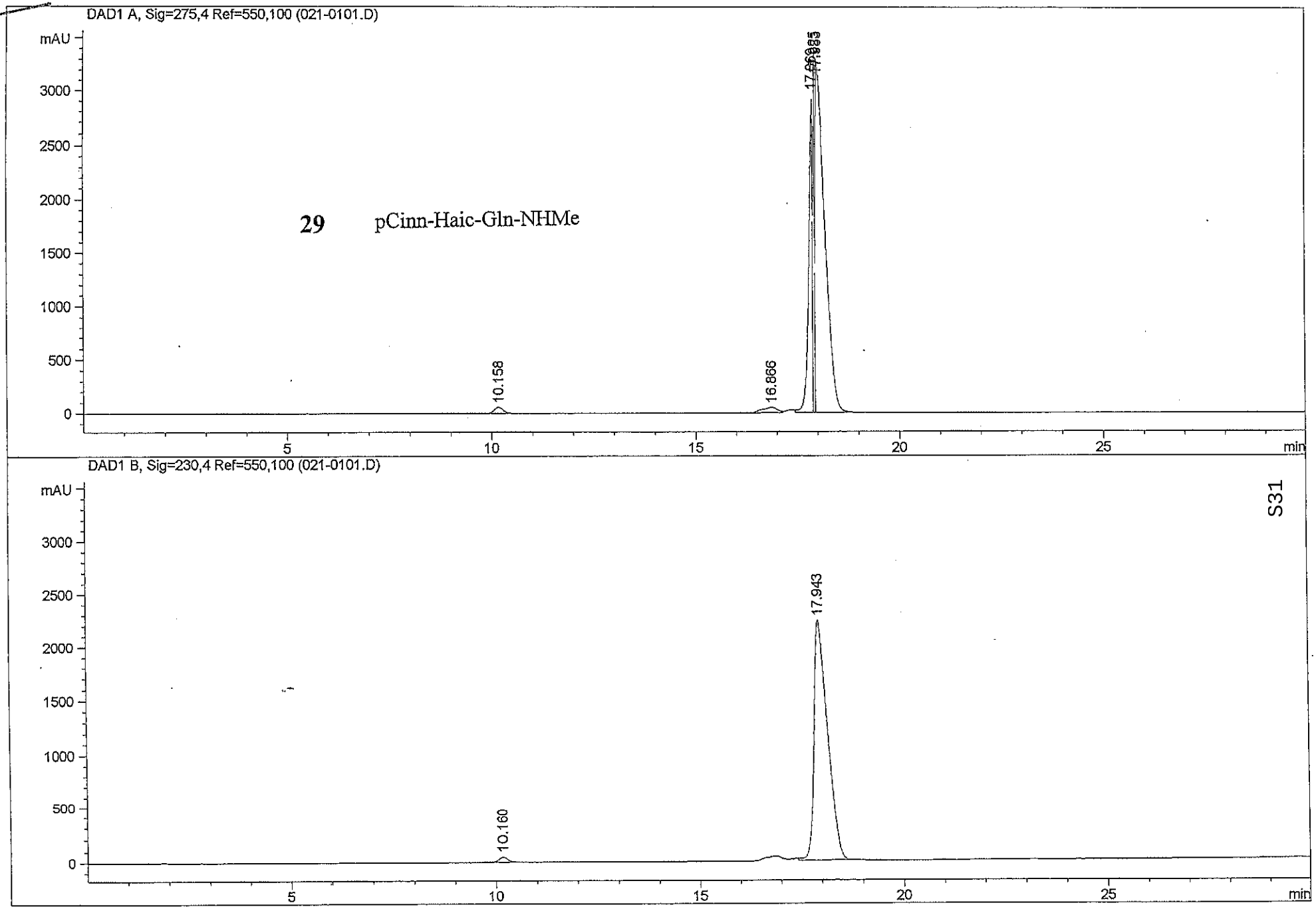


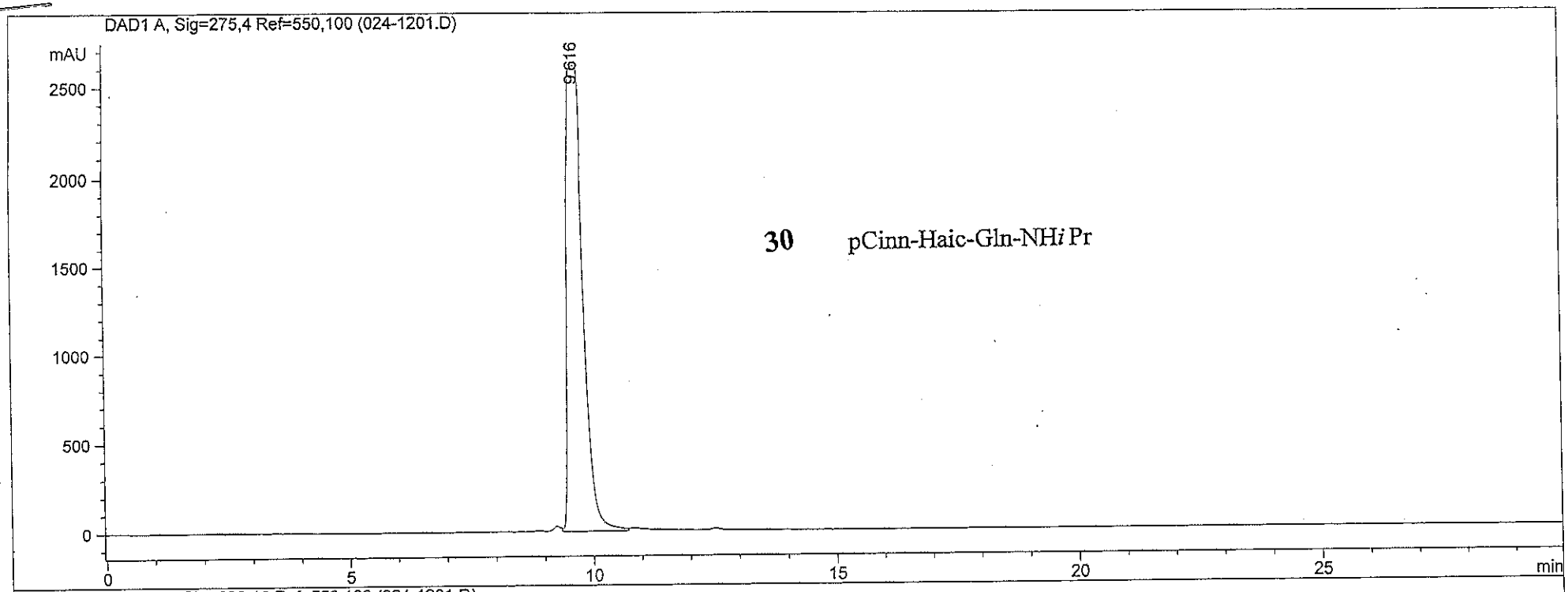
S29



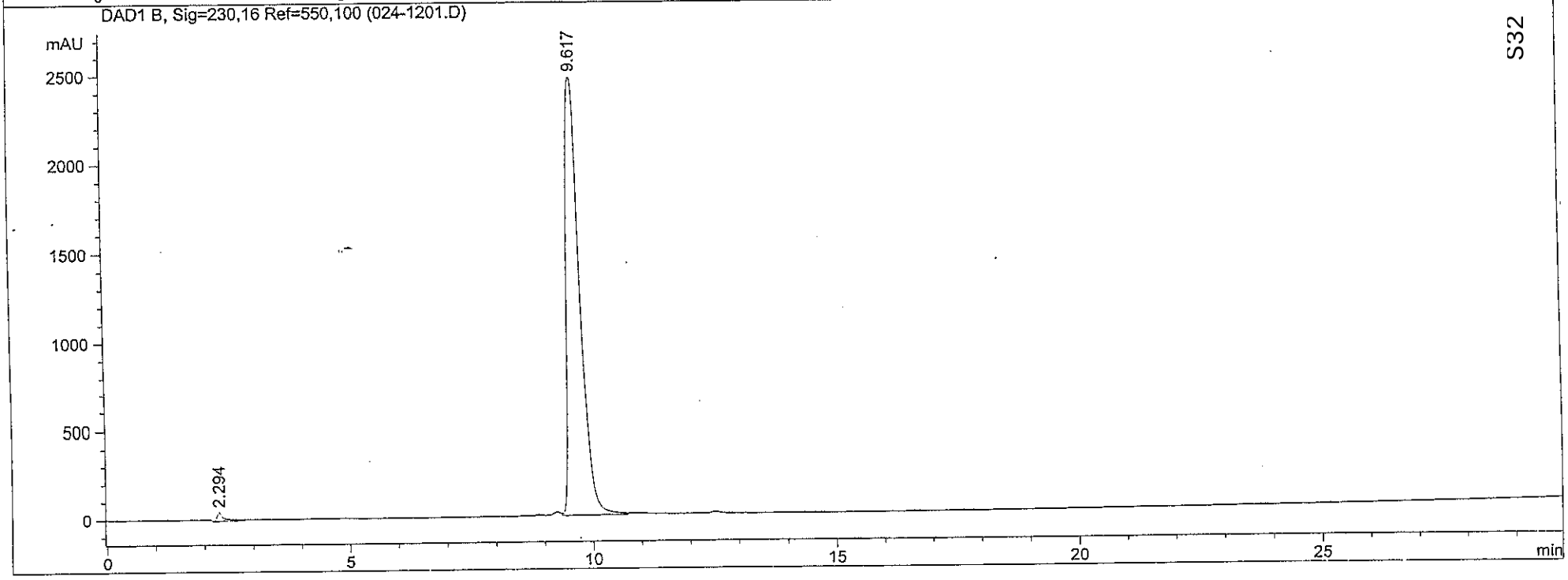
28 pCinn-Haic-Gln-NH_2

S30

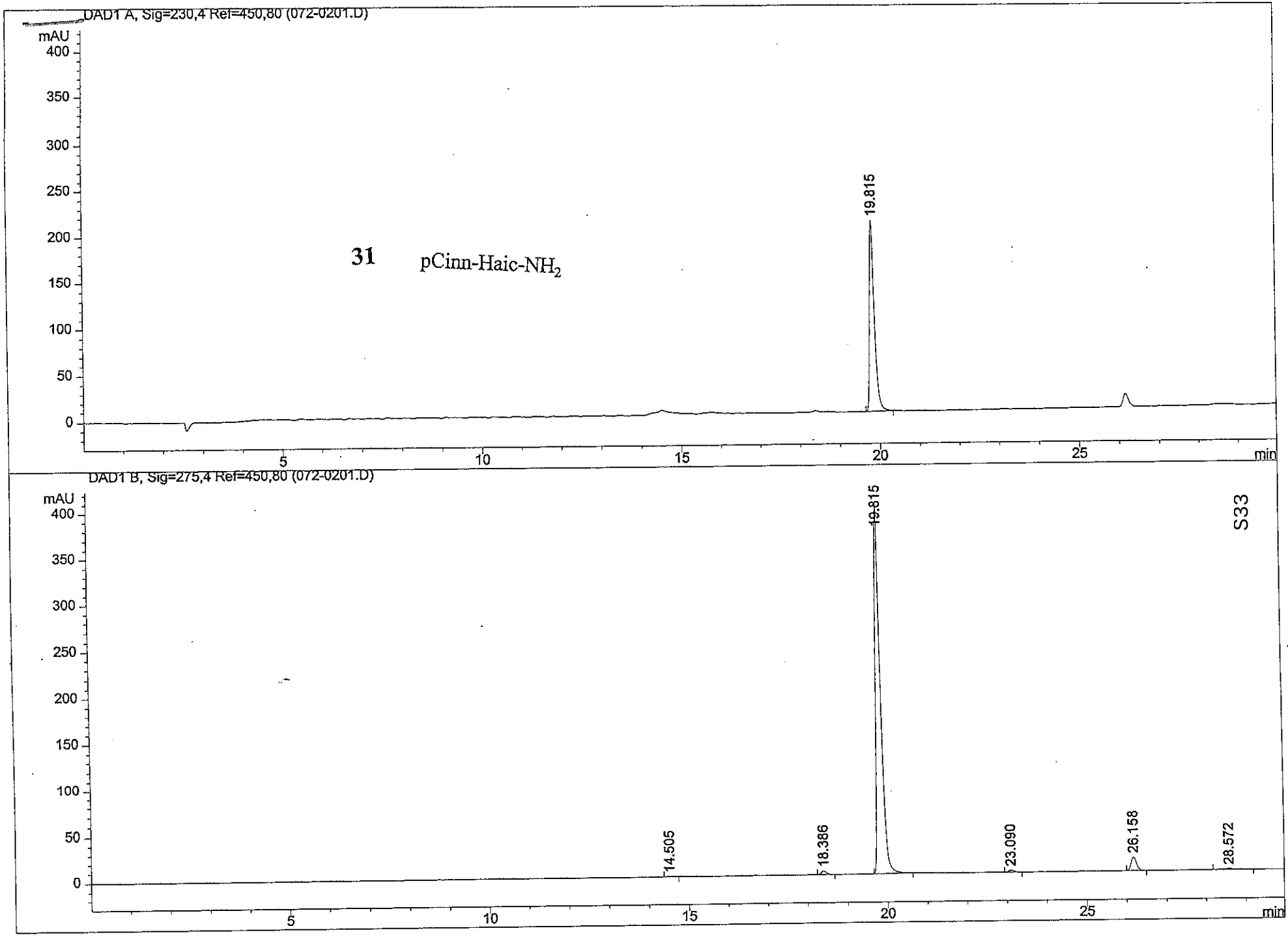

DAD1 A, Sig=275,4 Ref=550,100 (021-0101.D)
mAU
3000
2500
2000
1500
1000
500
0
29
pCinn-Haic-Gln-NHMe
10.158
16.866
17.943
5
10
15
20
25
min
DAD1 B, Sig=230,4 Ref=550,100 (021-0101.D)
mAU
3000
2500
2000
1500
1000
500
0
10.160
17.943
5
10
15
20
25
min
S31

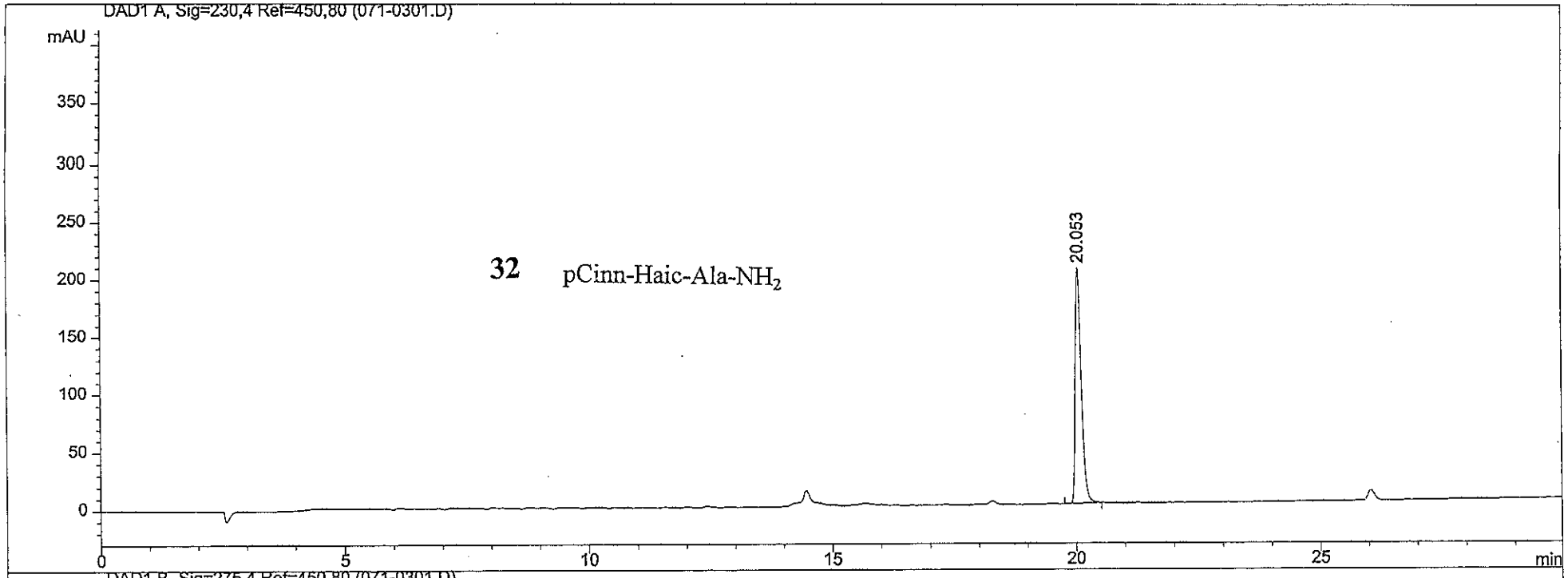


30 pCinn-Haic-Gln-NH*i* Pr

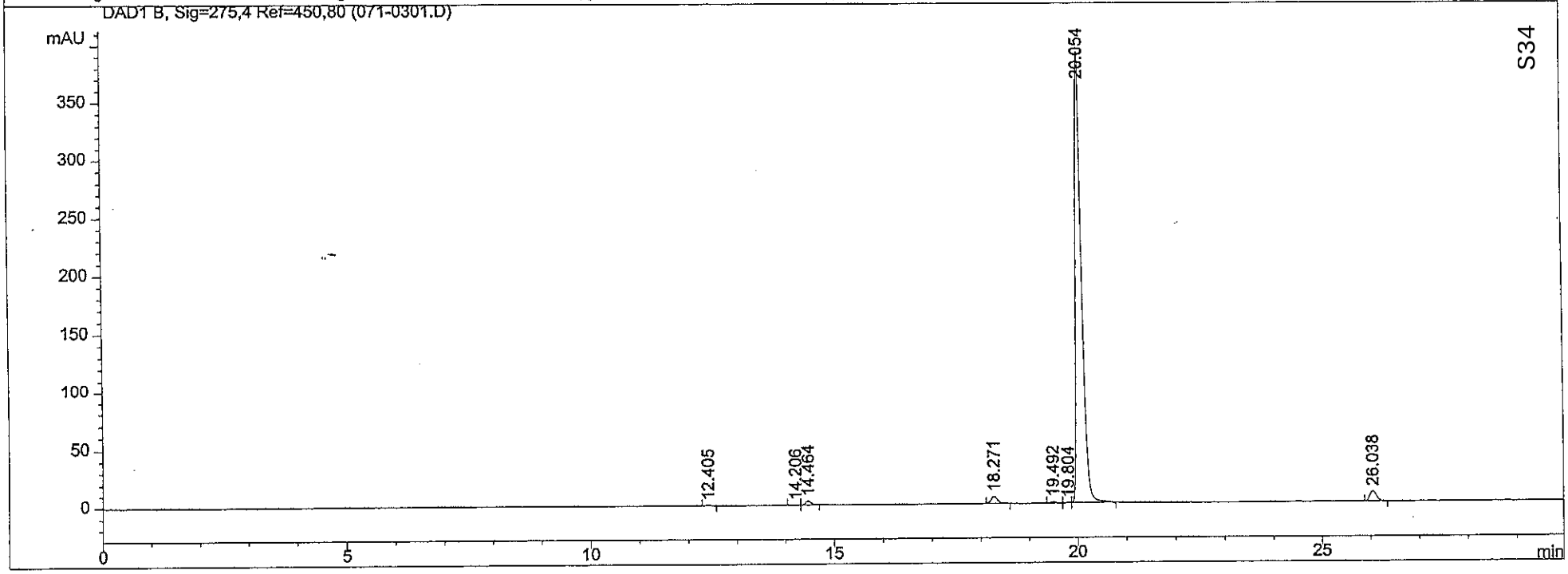


S32

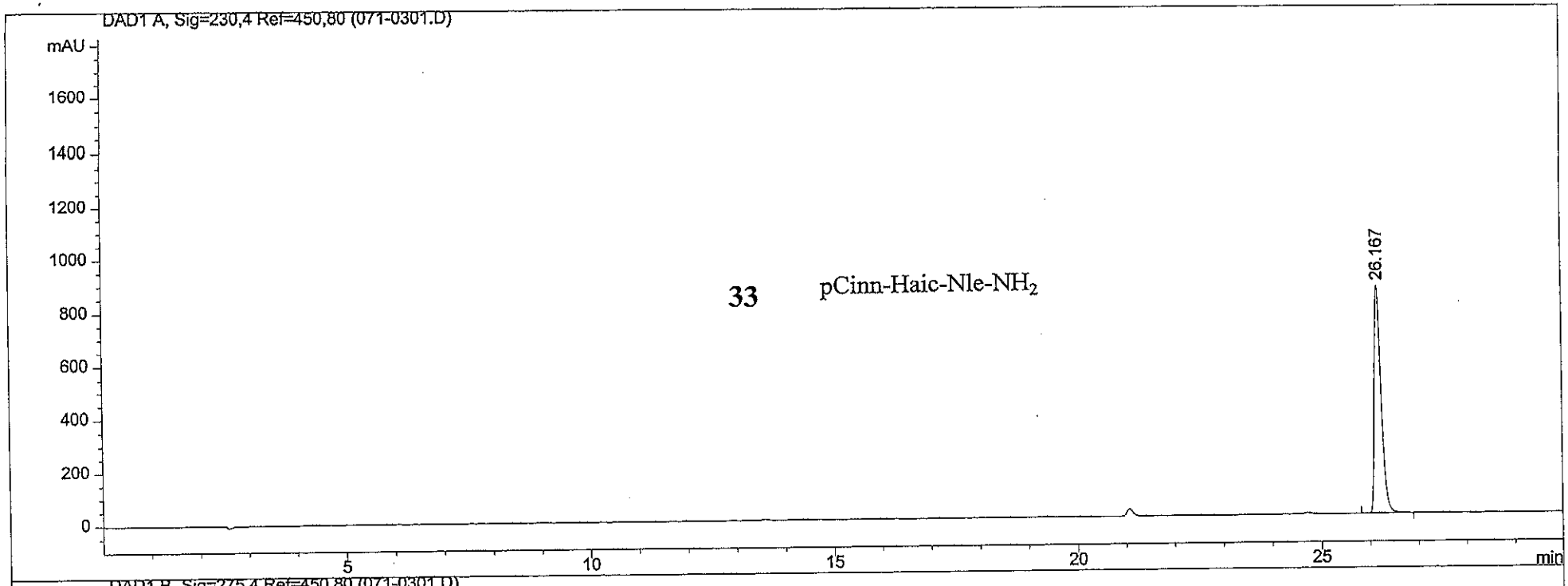

DAD1 A, Sig=230,4 Ref=450,80 (072-0201.D)
mAU
400
350
300
250
200
150
100
50
0
19.815
31 pCinn-Haic-NH2
5
10
15
20
25
min
DAD1 B, Sig=275,4 Ref=450,80 (072-0201.D)
mAU
400
350
300
250
200
150
100
50
0
19.815
S33
14.505
18.386
23.090
26.158
28.572
5
10
15
20
25
min



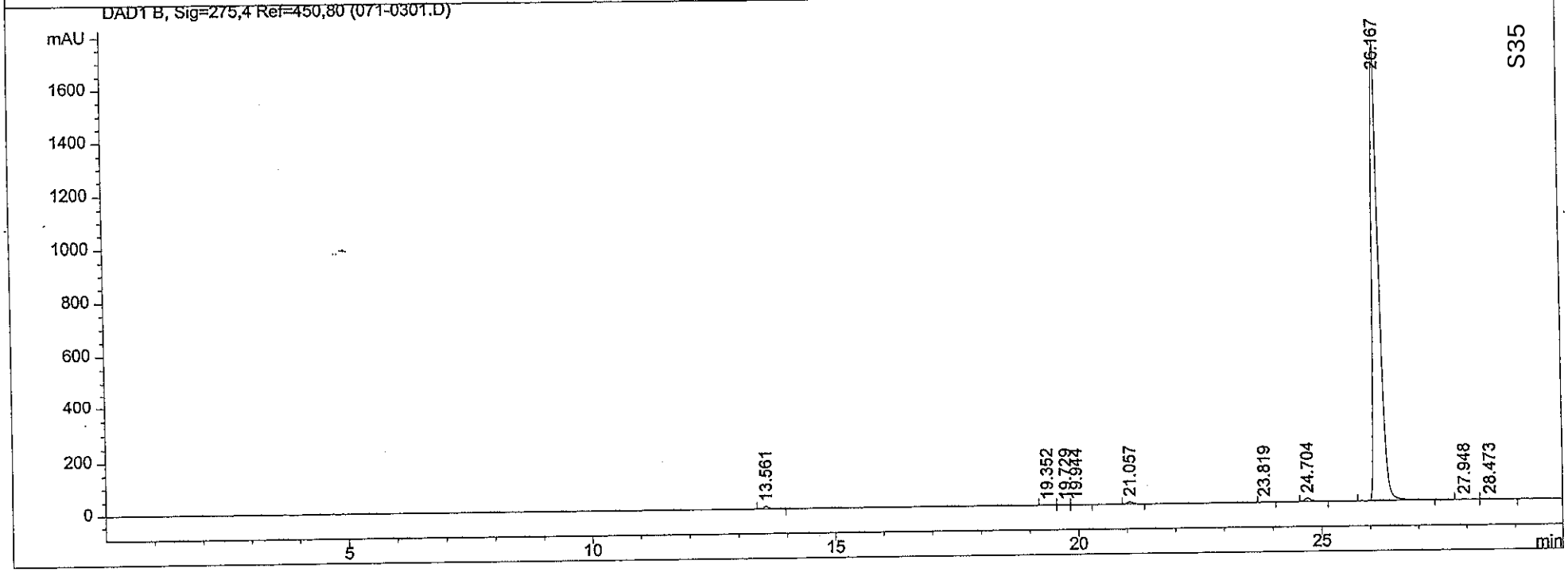
32 pCinn-Haic-Ala-NH_2



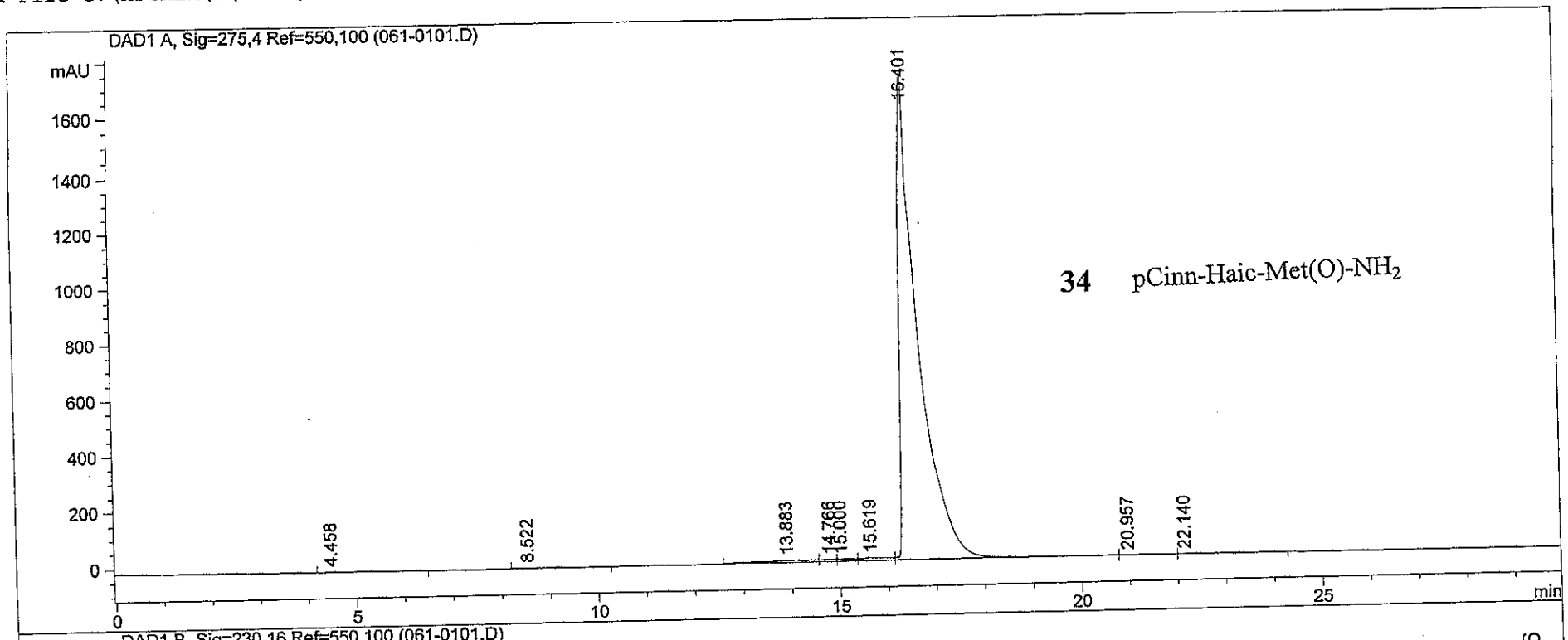
S34



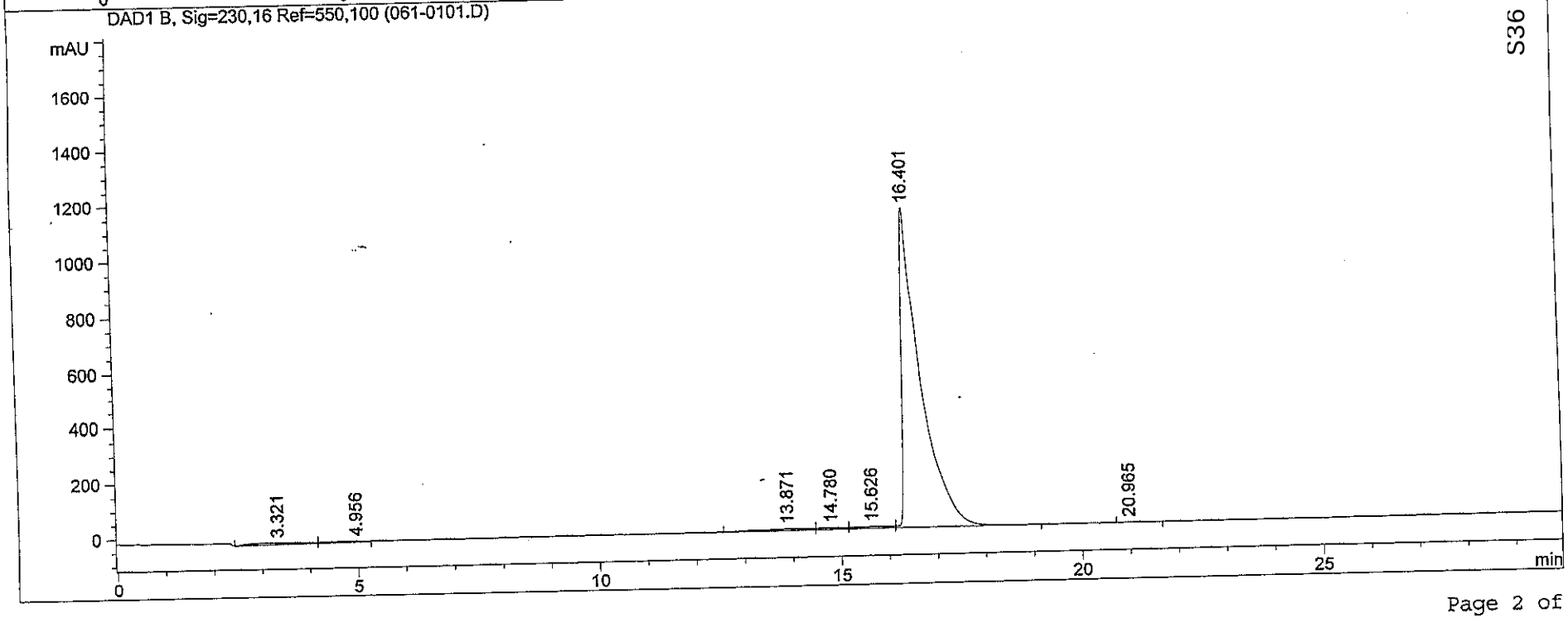
33 pCinn-Haic-Nle-NH_2



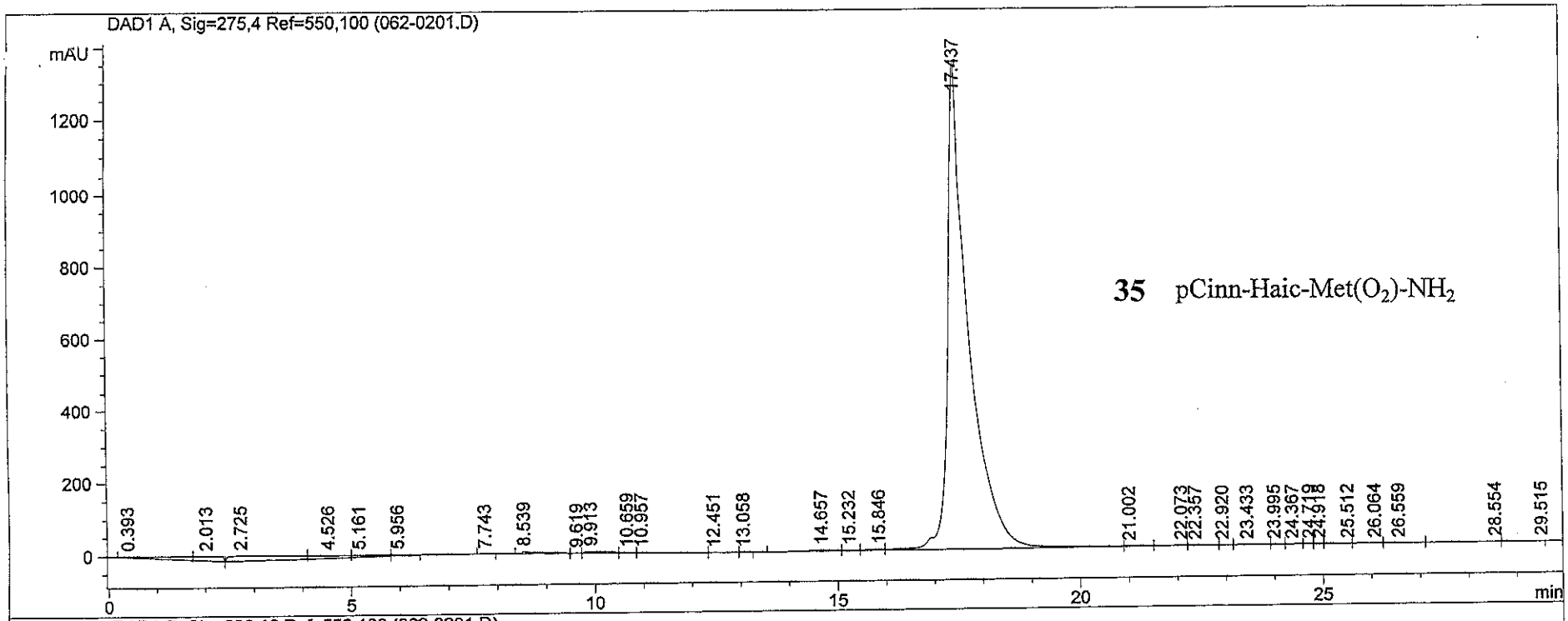
S35



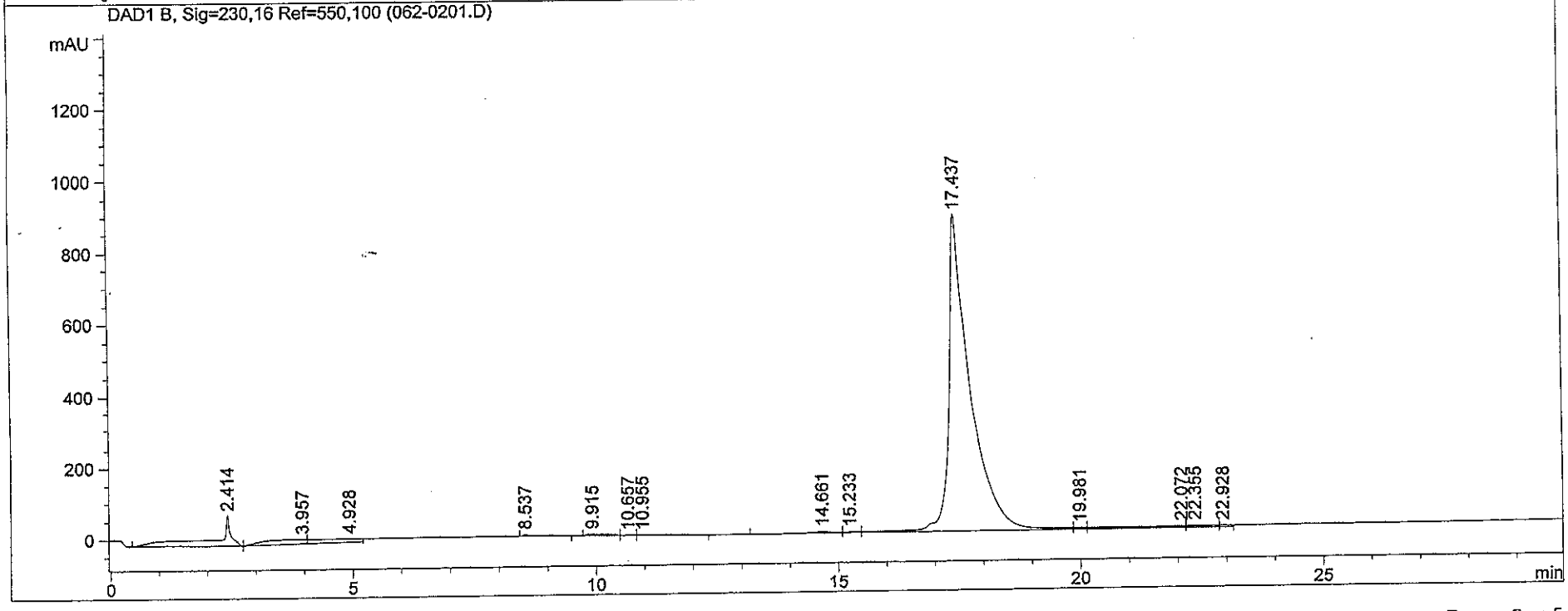
34 pCinn-Haic-Met(O)-NH_2



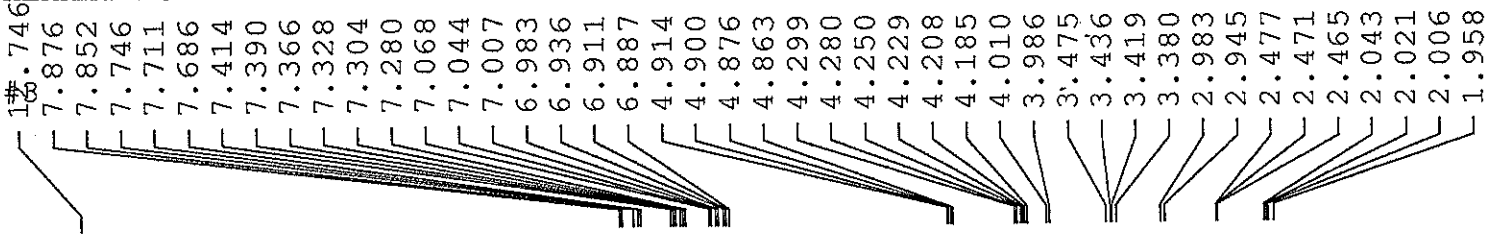
S36

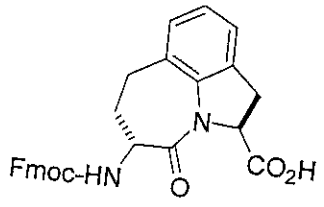


35 pCinn-Haic-Met(O_2)-NH_2



(R)-4-OXO-5-(FMOC-AMINO)-1,2,4,5,6,7-
HEXAHYDRO-AZEPINO-INDOLE-2-CARBOXYLIC ACID





45

Current Data Parameters

NAME	JSB-II-108-F15-65
EXPNO	4
PROCNO	1

F2 - Acquisition Parameters

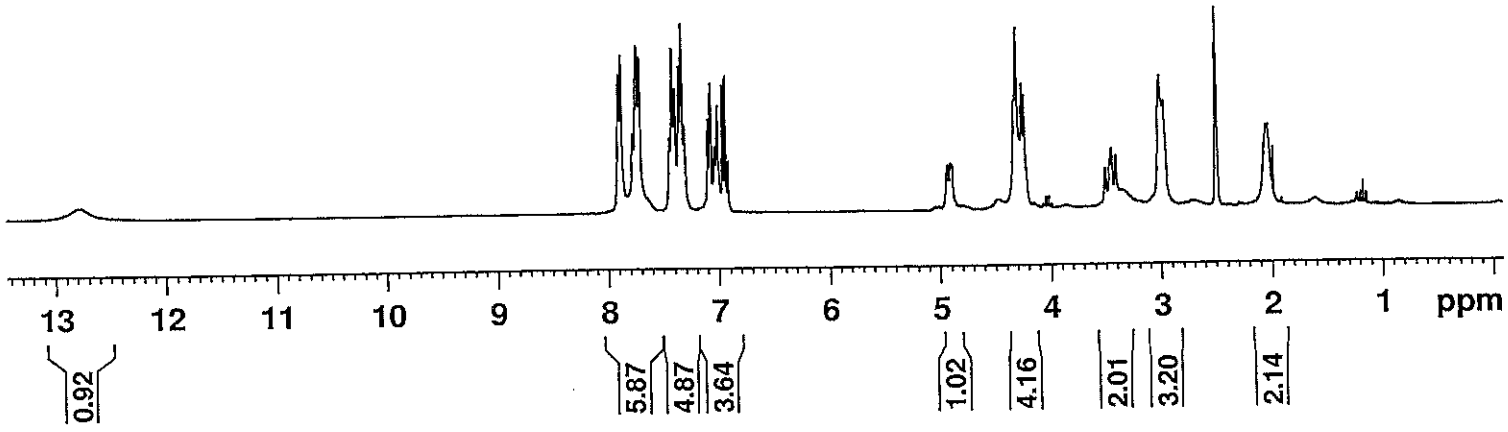
Date_	20080826
Time	13.42
INSTRUM	spect
PROBHD	5 mm QNP 1H/19
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	6172.839 Hz
FIDRES	0.094190 Hz
AQ	5.3084660 sec
RG	256
DW	81.000 usec
DE	6.00 usec
TE	683.2 K
D1	1.00000000 sec
TD0	1

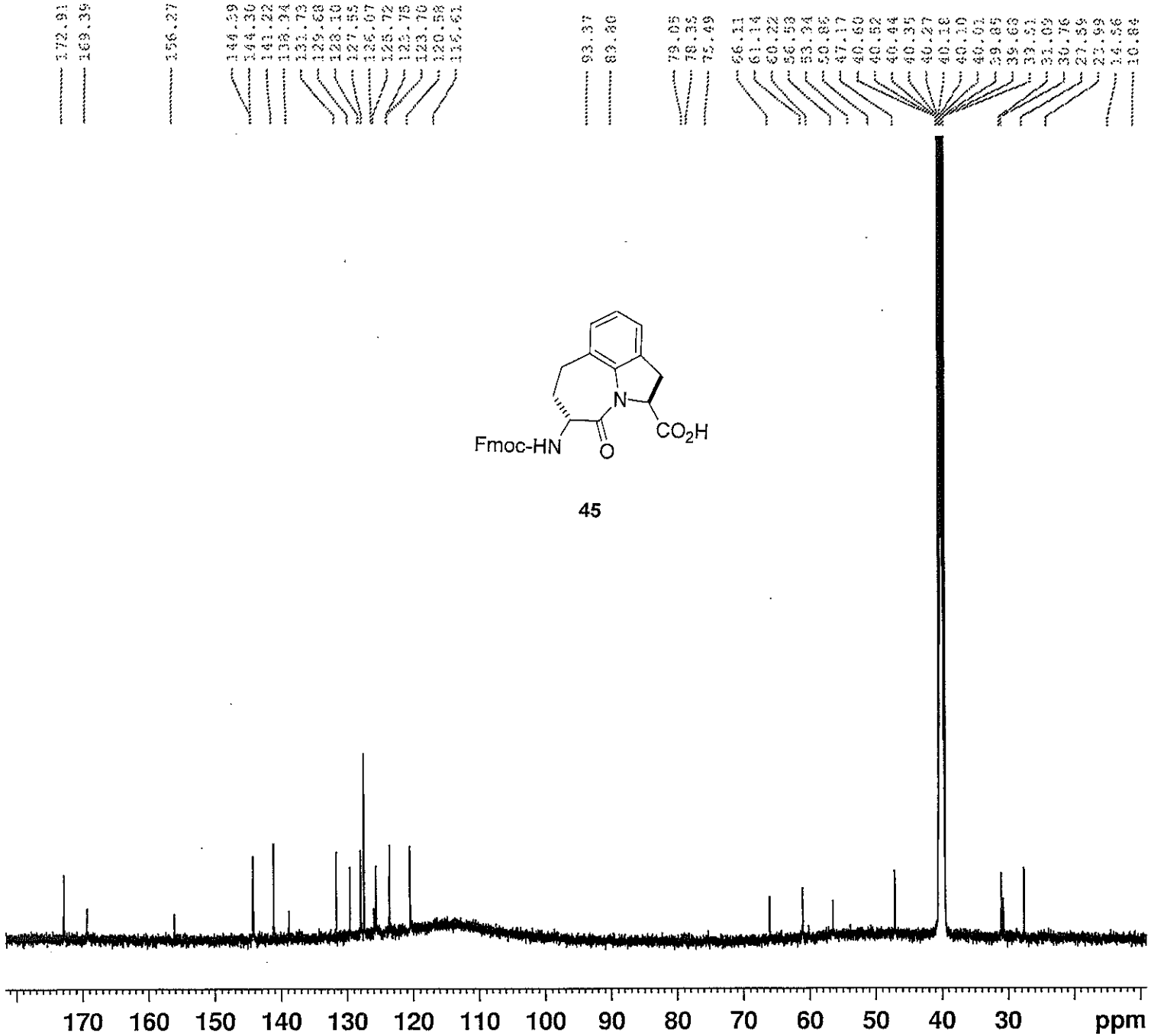
======== CHANNEL f1 ========

NUC1	1H
P1	10.25 usec
PL1	-1.00 dB
SFO1	300.1318534 MHz

F2 - Processing parameters

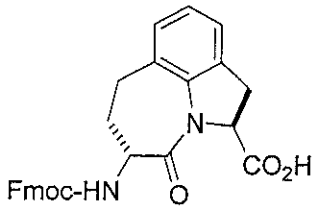
SI	32768
SF	300.1300011 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



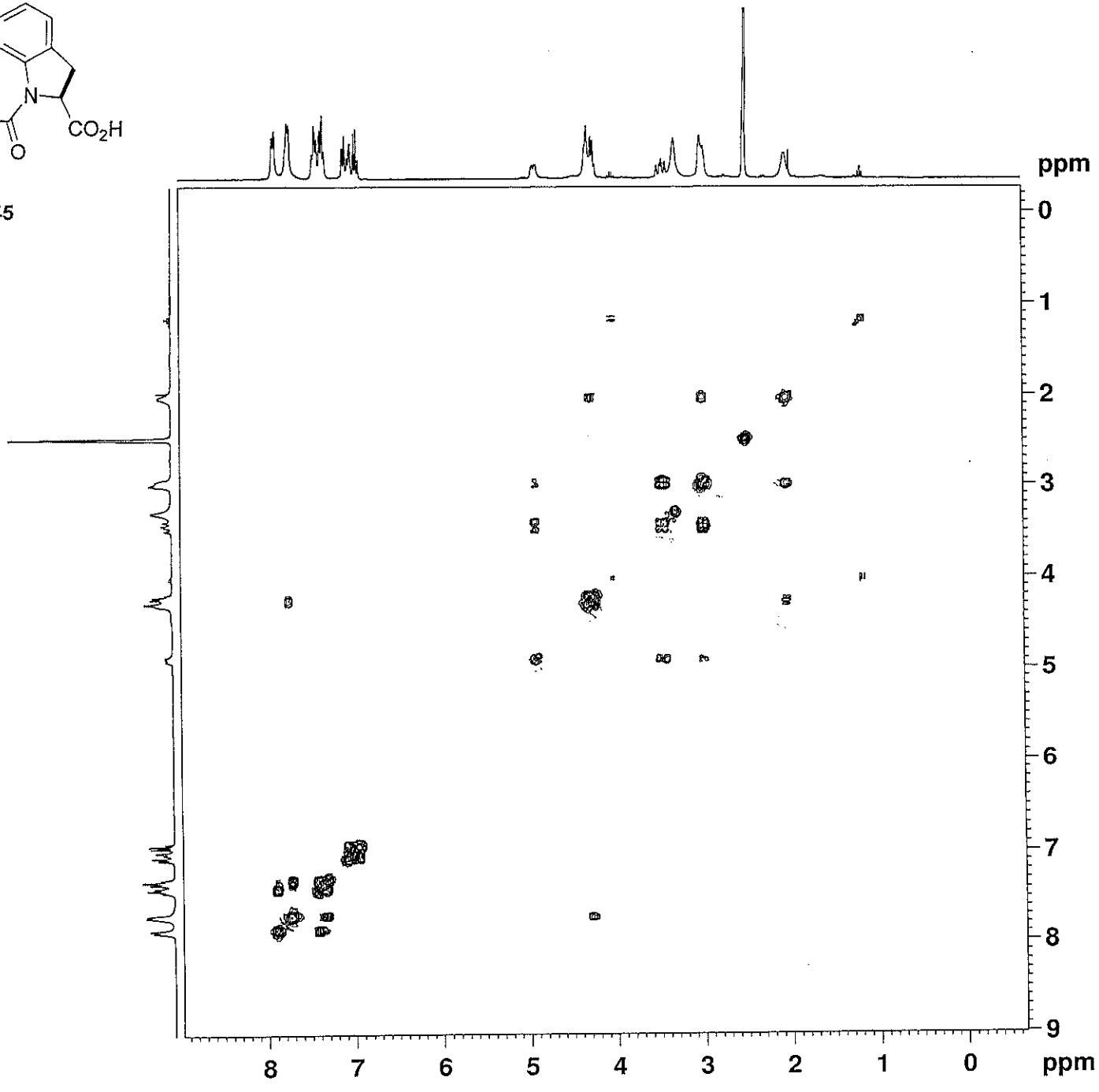



```
Current Data Parameters
NAME      JSB-II-108-F15-68
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_              20080507
Time                 17.48
INSTRUM              spect
PROBHD   5 mm TXI 1H/D-
PULPROG             zgpg30
TD                   65536
SOLVENT               DMSO
NS                   20480
DS                       4
SWH            30030.029 Hz
FIDRES          0.458222 Hz
AQ             1.0912410 sec
RG                  5792.6
DW                16.650 usec
DE                 7.50 usec
TE                300.2 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                      1

========== CHANNEL f1 ==========
NUC1                   13C
P1                  12.00 usec
PL1                 -5.50 dB
SFO1         125.7703643 MHz

========== CHANNEL f2 ==========
CPDPRG2            waltz16
NUC2                    1H
PCPD2               90.00 usec
PL2                 -2.00 dB
PL12                18.50 dB
PL13                20.00 dB
SFO2         500.1320005 MHz

F2 - Processing parameters
SI                   32768
SF           125.7577890 MHz
WDW                     EM
SSB                      0
LB                  1.00 Hz
GB                       0
PC                   1.40
```

R

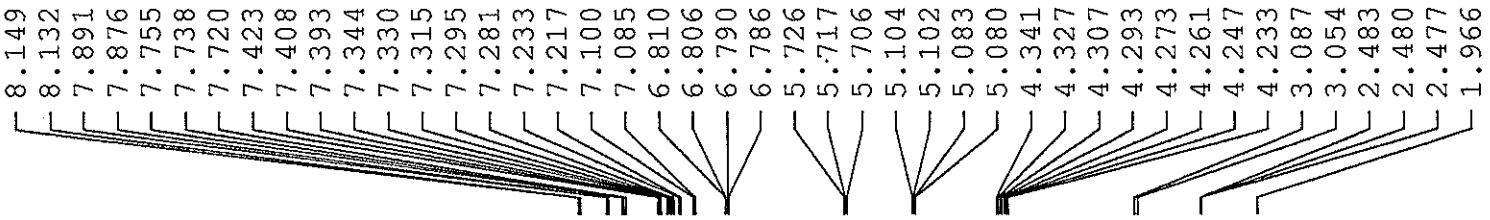


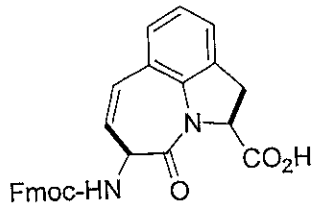
45



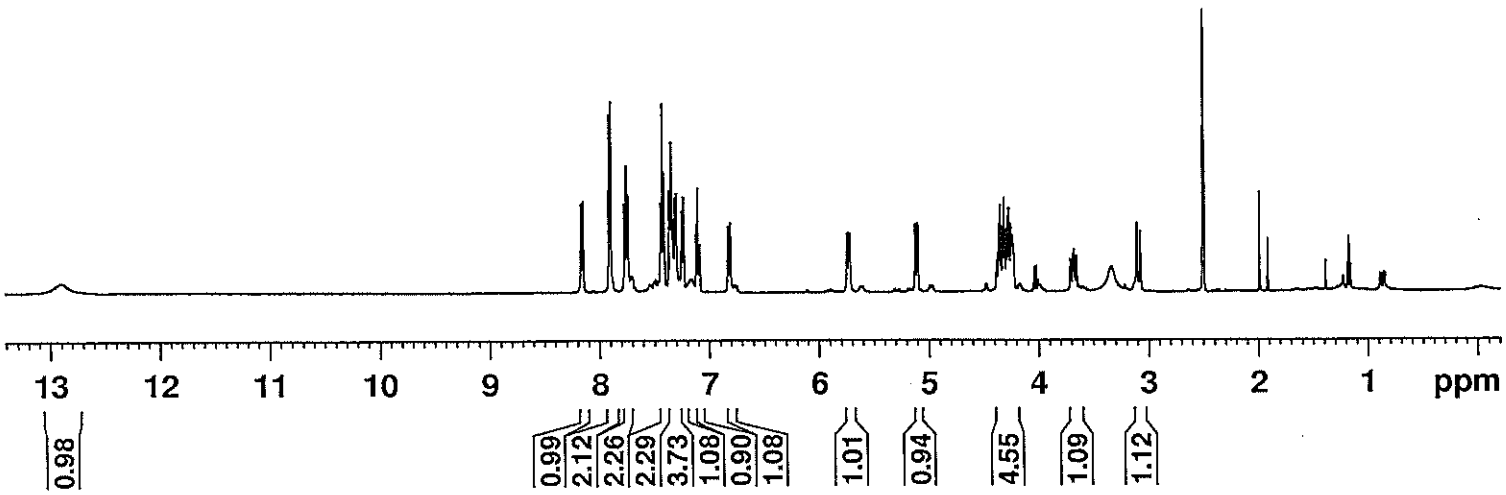

```
Current Data Parameters
NAME      JSB-II-108-F15-68
EXPNO                    7
PROCNO                   1

F2 - Acquisition Parameters
Date_              20080416
Time                 14.27
INSTRUM              spect
PROBHD   5 mm QNP 1H/19
PULPROG          cosygpqf
TD                   2048
SOLVENT              DMSO
NS                      2
DS                      8
SWH               4006.410 Hz
FIDRES            1.956255 Hz
AQ                0.2556404 sec
RG                    1024
DW                 124.800 usec
DE                   6.00 usec
TE                  301.2 K
d0             0.00000300 sec
D1             1.48689198 sec
d13            0.00000400 sec
D16            0.00020000 sec
IN0            0.00024960 sec

======== CHANNEL f1 ========
NUC1                   1H
P0                  10.25 usec
P1                  10.25 usec
PL1                 -1.00 dB
SFO1          300.1318044 MHz

======= GRADIENT CHANNEL =====
GPNAM1           SINE.100
GPNAM2           SINE.100
GPZ1                10.00 %
GPZ2                10.00 %
P16               1000.00 usec

F1 - Acquisition parameters
ND0                     1
TD                    128
SFO1            300.1318 MHz
FIDRES         31.300079 Hz
SW                13.349 ppm
FnMODE                 QF

F2 - Processing parameters
SI                    1024
SF            300.1300000 MHz
WDW                 SINE
SSB                    0
LB                  0.00 Hz
GB                     0
PC                  1.00

F1 - Processing parameters
SI                    1024
MC2                    QF
SF            300.1300000 MHz
WDW                 SINE
SSB                    0
LB                  0.00 Hz
GB                     0
```

(S0-9-OXO-8-(FMOC-AMONO)-2,8,9,9a-TETRRAHYDRO-BENZO-AZULENE-1-CARBOXYLIC ACID

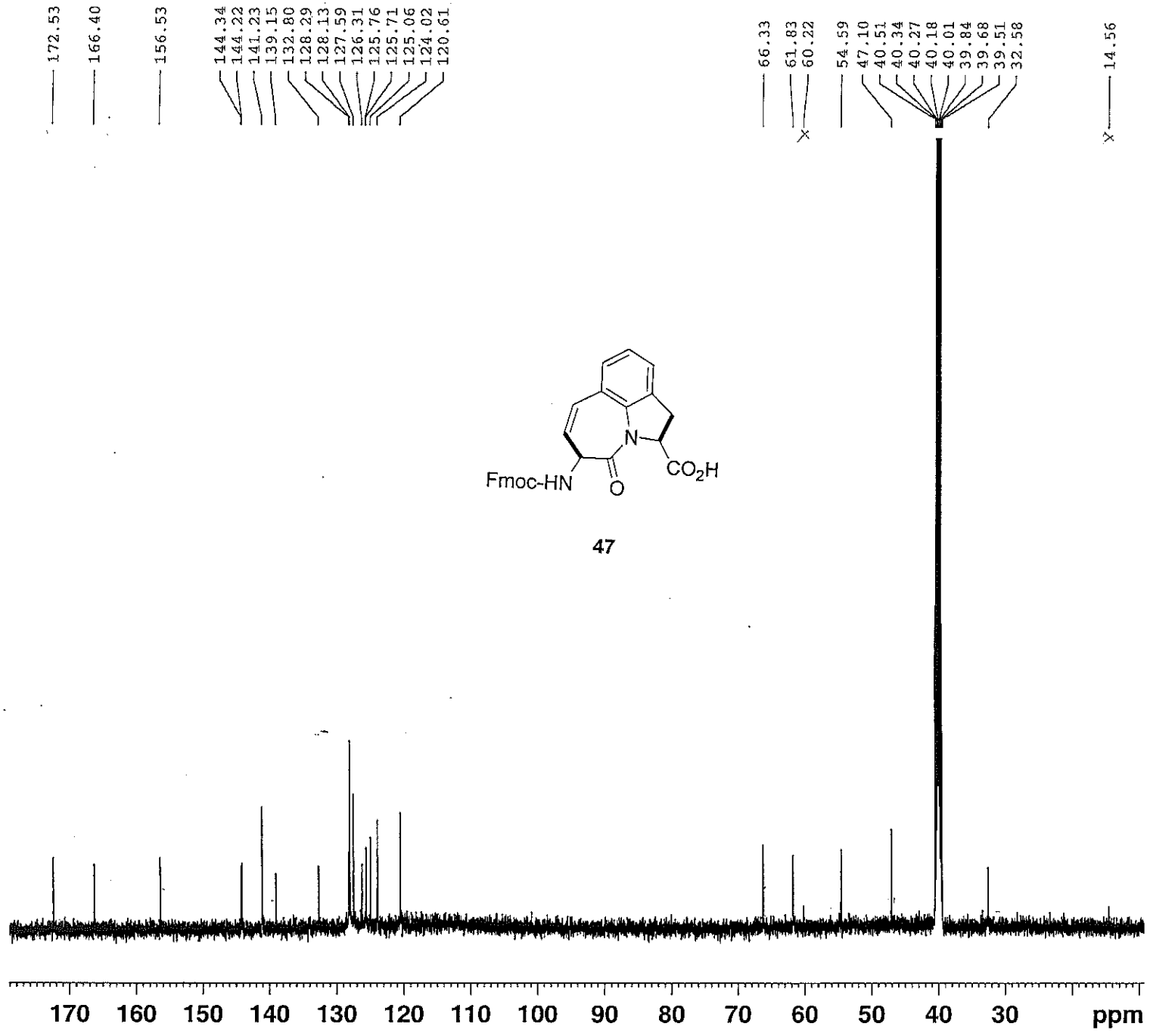




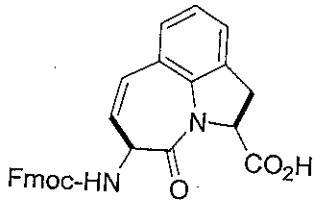
47




```
Current Data Parameters
NAME      JSB-II-101-F28-30
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_              20080520
Time                  14.26
INSTRUM               spect
PROBHD   5 mm TXI 1H/D-
PULPROG                zg30
TD                    65536
SOLVENT                DMSO
NS                       16
DS                        2
SWH               10330.578 Hz
FIDRES             0.157632 Hz
AQ                3.1720407 sec
RG                       64
DW                  48.400 usec
DE                   7.50 usec
TE                   301.2 K
D1              1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                     1H
P1                    8.50 usec
PL1                  -2.00 dB
SFO1          500.1330885 MHz

F2 - Processing parameters
SI                    32768
SF            500.1300051 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.00
```




```
Current Data Parameters
NAME      JSB-II-101-F28-30
EXPNO                     2
PROCNO                    1

F2 - Acquisition Parameters
Date_              20080520
Time                 14.41
INSTRUM              spect
PROBHD    5 mm TXI 1H/D-
PULPROG             zgpg30
TD                   65536
SOLVENT              CDCl3
NS                     956
DS                       4
SWH            30030.029 Hz
FIDRES          0.458222 Hz
AQ             1.0912410 sec
RG               14596.5
DW                16.650 use
DE                 7.50 use
TE                301.2 K
D1            2.00000000 sec
d11           0.03000000 sec
DELTA         1.89999998 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                   13C
P1                   12.00 use
PL1                  -5.50 dB
SFO1         125.7703643 MHz

======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                   1H
PCPD2                90.00 use
PL2                  -2.00 dB
PL12                 18.50 dB
PL13                 20.00 dB
SFO2         500.1320005 MHz

F2 - Processing parameters
SI                   32768
SF           125.7577890 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.00
```



47

JSB-II-111-f28-30 7 1 /opt/topspin mcmurray