Supporting Information

Synthesis, Photophysical, Electrochemical, Tumor-Imaging and Phototherapeutic Properties of Purpurinimide-N-substituted Cyanine Dyes Joined with Variable Length of Linkers

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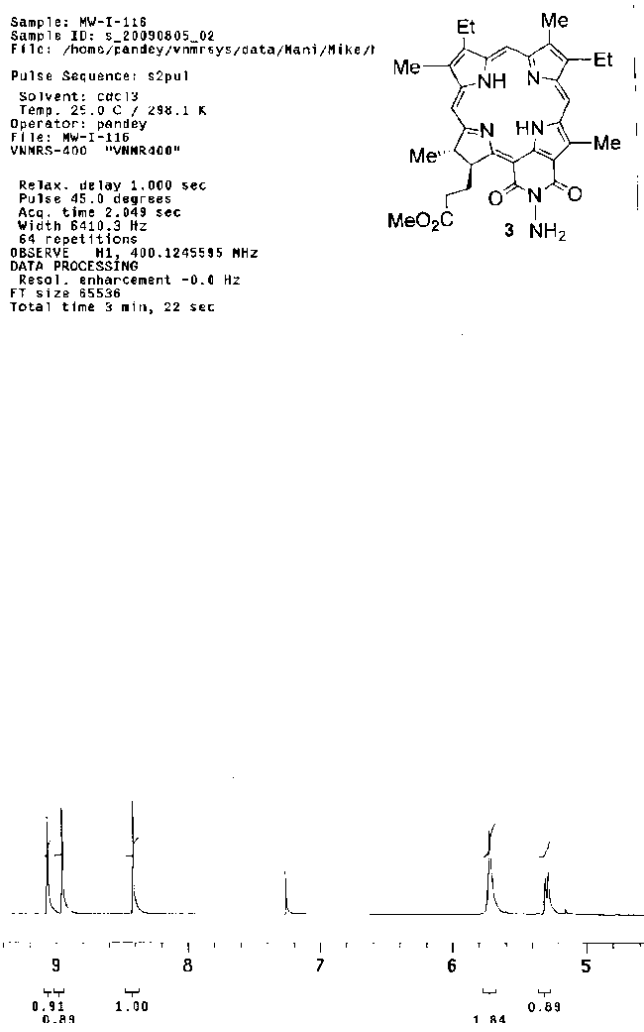
***In Vitro* Phototoxicity Assay (MTT Assay):** To determine the *in vitro* efficacy of the compounds the following procedure was performed. Colon-26 cells were grown in RPMI 1640 with 10% FCS, 2mM L-Glutamine, 50 IU penicillin/50 µg/ml streptomycin. Cells were maintained in 5 % CO_2 and 95% air at 100% humidity. For phototoxicity studies, Colon-26 cells were plated in 96 well plates at a density of 3500 cells/well in complete medium. 4 h later, compounds were added at concentrations ranging from 0.003 to 1 µM. After 24 h incubation in the dark at 37 °C, the cells were irradiated with laser light from an argon pumped dye laser using fluences of 0-2 J/cm^2 at a dose rate of 3.2 mW/cm^2 with light adjusted to the specific maximum wavelength of each drug. The light treatment is shown in Figure 13. After irradiation, the cells were incubated for 48h. After 48 h, 15 µL/well of 4 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT in PBS was added, and cells were incubated for a further 4 h. MTT is a yellow water soluble tetrazolium salt that is taken up by cells, reduced to water insoluble purple formazan crystals by metabolically active (viable) cells. The MTT-containing media was removed, and 100µl DMSO was added to solubilize the formazan crystals. The absorbance of the wells was read on a microtiter plate reader at a wavelength of 560 nm. The results were plotted as percent growth of control (untreated) cells *vs.* concentration range for each individual drug.

***In Vivo* Reflectance Spectroscopy:** *In vivo* reflectance spectroscopy is a non-invasive method of following the pharmacokinetics and measuring the concentration of a photosensitizer in an experimental animal. By measuring the light diffusely reflected from tissue containing the dye, the time at which the concentration is at its maximum can be determined and the light treatment can be delivered at the optimal tumor to normal tissue ratio, for the greatest selectivity. As described in reference 9, monochromatic light was delivered through a quartz fiber which was placed perpendicular to and touching the tissue. At a measured distance (3-5 mm) from the delivery fiber, a pick up fiber is also placed in contact with the tissue. Light is delivered at optical power levels of about 1µW to avoid any potential PDT effects during measurement. Light

$$C = \frac{1}{2 * \varepsilon}\left\{\left\{-\sqrt{\mu_s{}^2 + \frac{4}{3}\alpha_0{}^2} + \sqrt{\mu_s{}^2 + \frac{4}{3}\left(\frac{OD}{r} + \alpha_0{}^2\right)^2}\right\}\right. \qquad \text{Equation 1}$$

that enters the pickup fiber was conducted to a silicon photodiode detector that measured the photocurrent linear in power over 7-8 orders of magnitude. The wavelength was varied from 650-1000 nm and a spectrum of diffusely scattered photons was recorded. The concentration *C* was calculated using the formula in equation 1. Where ε is the molar extinction coefficient, α0 is the intrinsic tissue coefficient, OD is diffuse optical density, μ_3 is the reduced scattering coefficient, r is the distance from the detector to the pickup fiber. Molar extinction coefficient (ε) is measured using the Beer- Lambert law (A= ε lc).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Operator: pandey
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Pulse 45.0 degrees
Acq. time 2.049 sec
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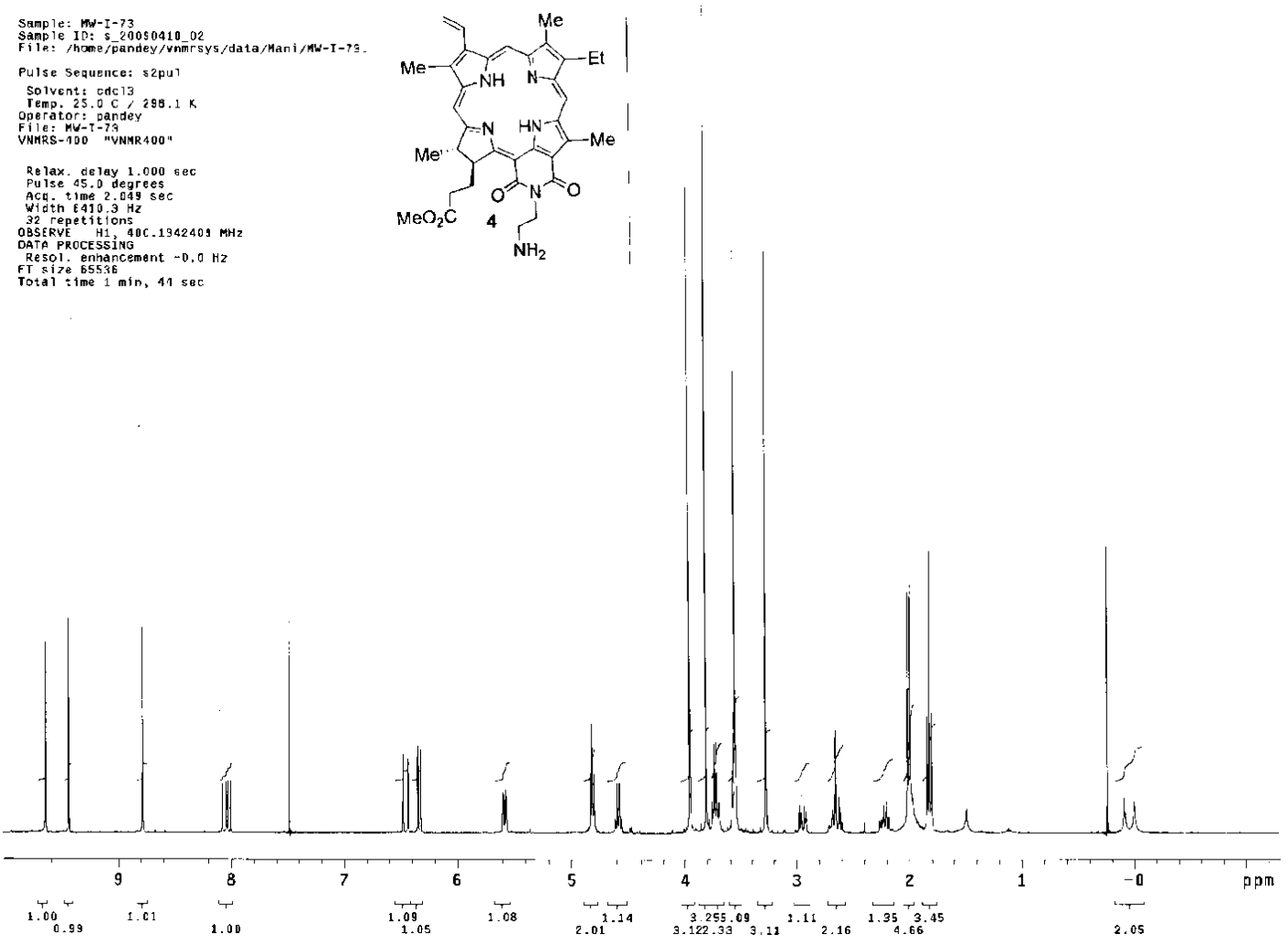
DATA PROCESSING
Resol. enhancement -0.6 Hz
FT size 65536
Total time 3 min, 22 sec
Et
Me
Me
Et
NH
N
N
HN
Me
Me
O
N
O
MeO2C
3
NH2
9
8
7
6
5
4
3
2
1
-0
ppm
0.91
1.00
0.89
1.84
0.89
0.95
5.27
2.65
3.22
2.15
3.64
4.61
2.12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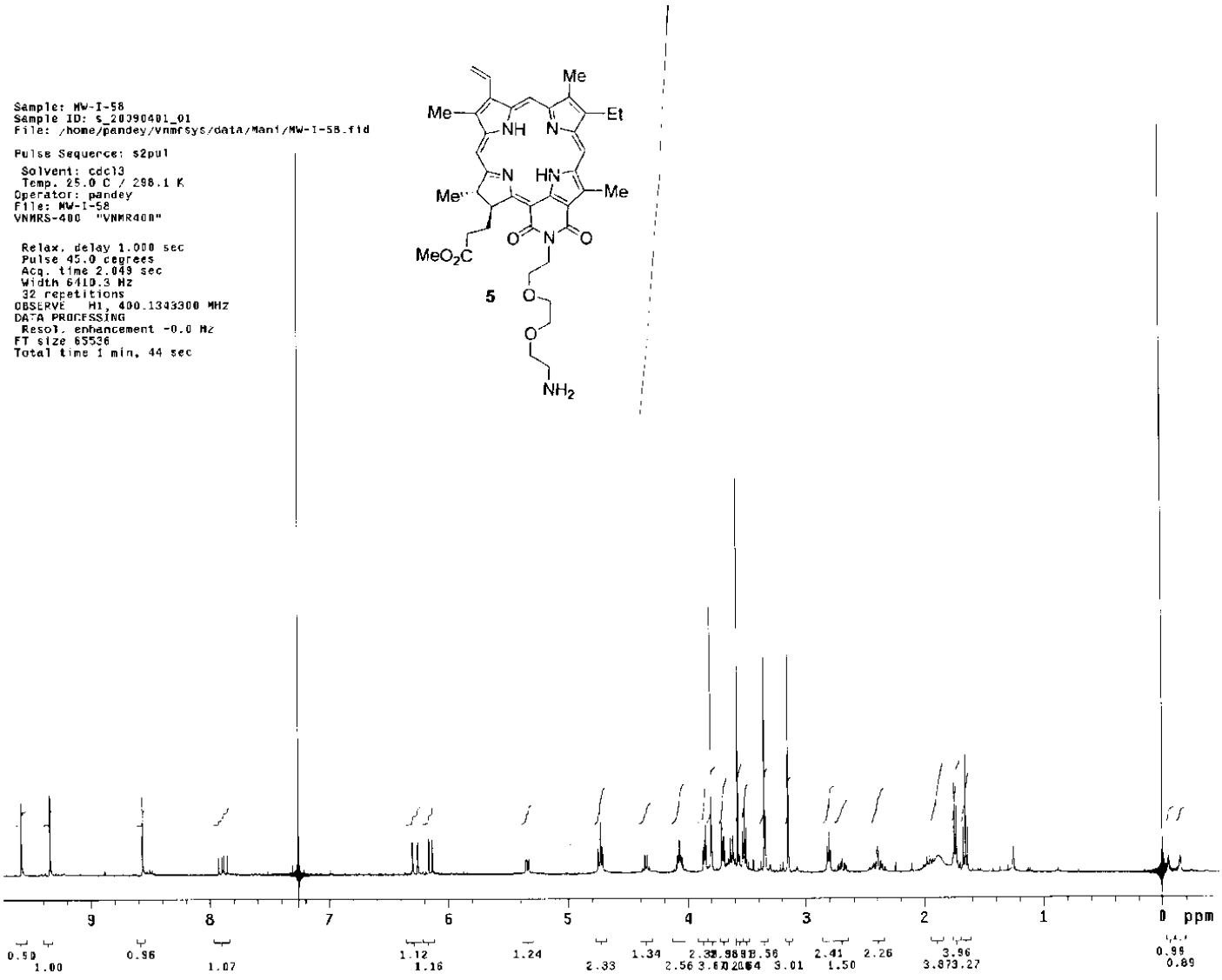
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.049 sec
Width 8410.3 Hz
32 repetitions
OBSERVE H1, 400.1942401 MHz
DATA PROCESSING
Resol. enhancement -0.0 Hz
FT size 65536
Total time 1 min, 44 sec
Me
Et
Me
NH
N
N
HN
Me
Me
O
O
N
O
MeO2C
4
NH2
9
8
7
6
5
4
3
2
1
-0
ppm
1.00
0.99
1.01
1.00
1.09
1.05
1.08
1.14
2.01
3.25
5.09
3.12
2.33
3.11
1.11
2.16
1.35
3.45
4.66
2.05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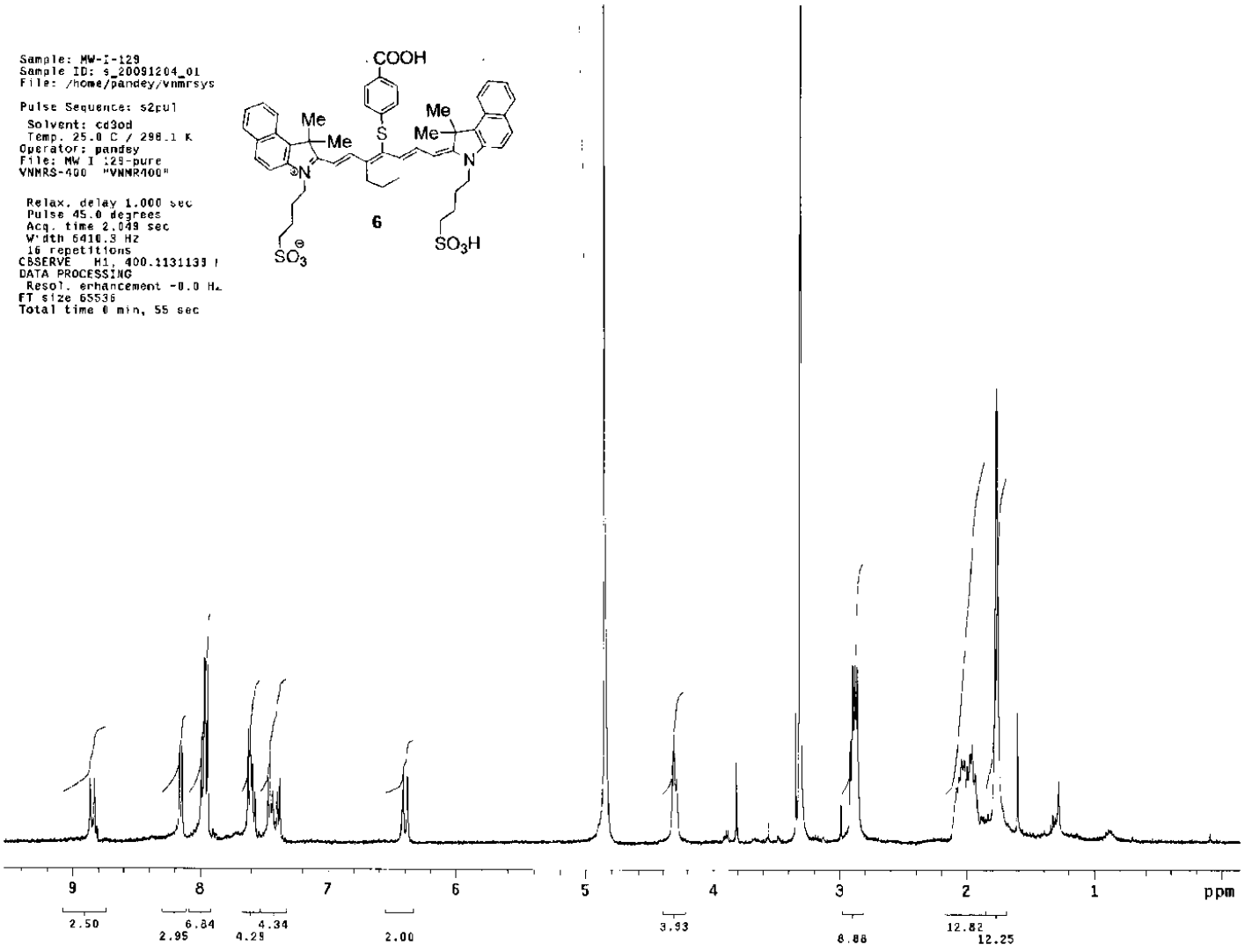
Temp. 25.0 C / 298.1 K
Operator: pandey
File: MW-I-58
VNMRS-400 "VNMR400"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.049 sec
Width 6410.3 Hz
32 repetitions
OBSERVE H1, 400.1343300 MHz
DATA PROCESSING
Resol. enhancement -0.0 Hz
FT size 65536
Total time 1 min, 44 sec
Me
Et
NH
N
HN
Me
Me
MeO2C
O
N
O
5
O
O
NH2
9
8
7
6
5
4
3
2
1
0
ppm
0.50
1.00
0.96
1.07
1.12
1.16
1.24
2.33
1.34
2.56
3.01
1.50
2.26
3.96
3.87
3.27
0.99
0.89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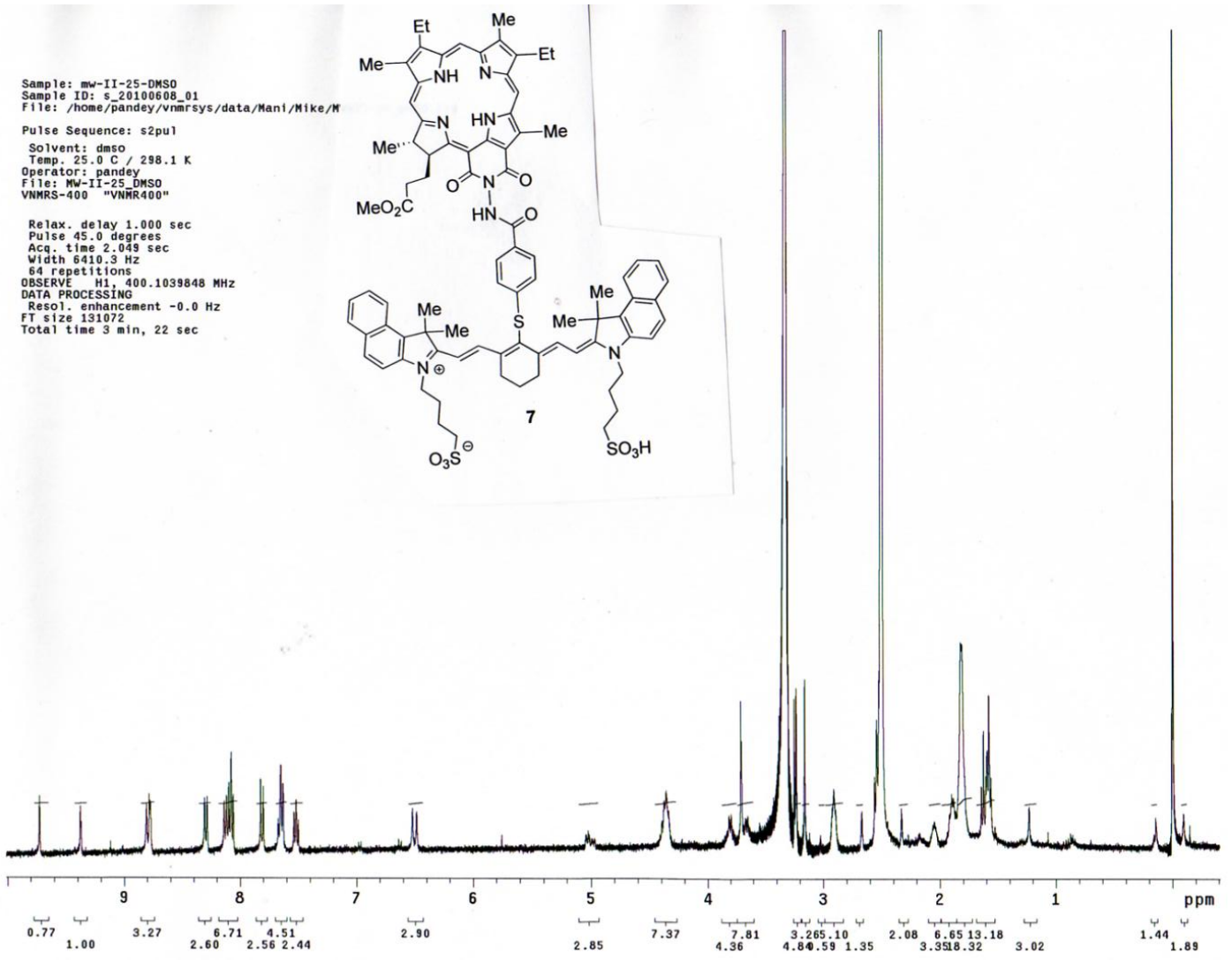
Solvent: cd3od
Temp. 25.0 C / 298.1 K
Operator: pandey
File: MW I 129-pure
VNMRS-400 "VNMR400"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.049 sec
Width 5410.3 Hz
16 repetitions
OBSERVE H1, 400.1131133 I
DATA PROCESSING
Resol. enhancement -0.0 Hz
FT size 65536
Total time 0 min, 55 sec
COOH
Me
Me
S
Me
Me
N
N
SO3
SO3H
6
9
8
7
6
5
4
3
2
1
ppm
2.50
2.95
6.84
4.29
4.34
2.00
3.93
8.88
12.82
12.25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Pulse Sequence: s2pul
Solvent: dmso
Temp. 25.0 C / 298.1 K
Operator: pandey
File: MW-II-25_DMSO
VNMRS-400 "VNMR400"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.049 sec
Width 6410.3 Hz
64 repetitions
OBSERVE H1, 400.1039848 MHz
DATA PROCESSING
Resol. enhancement -0.0 Hz
FT size 131072
Total time 3 min, 22 sec
7
9
8
7
6
5
4
3
2
1
ppm
0.77
1.00
3.27
2.60
6.71
2.56
4.51
2.44
2.90
2.85
7.37
4.36
7.81
4.84
3.26
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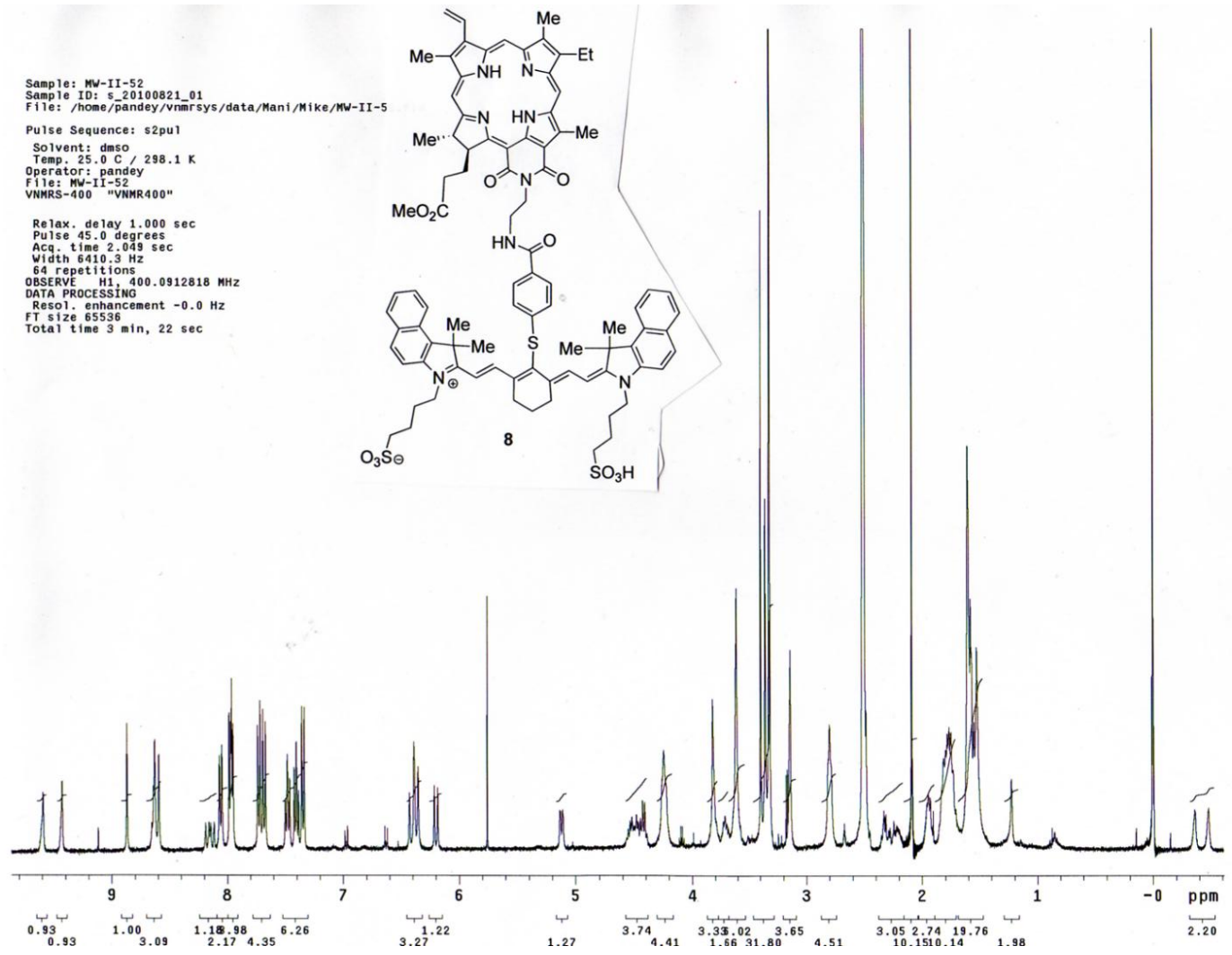
6.65
18.32
13.18
3.02
1.44
1.89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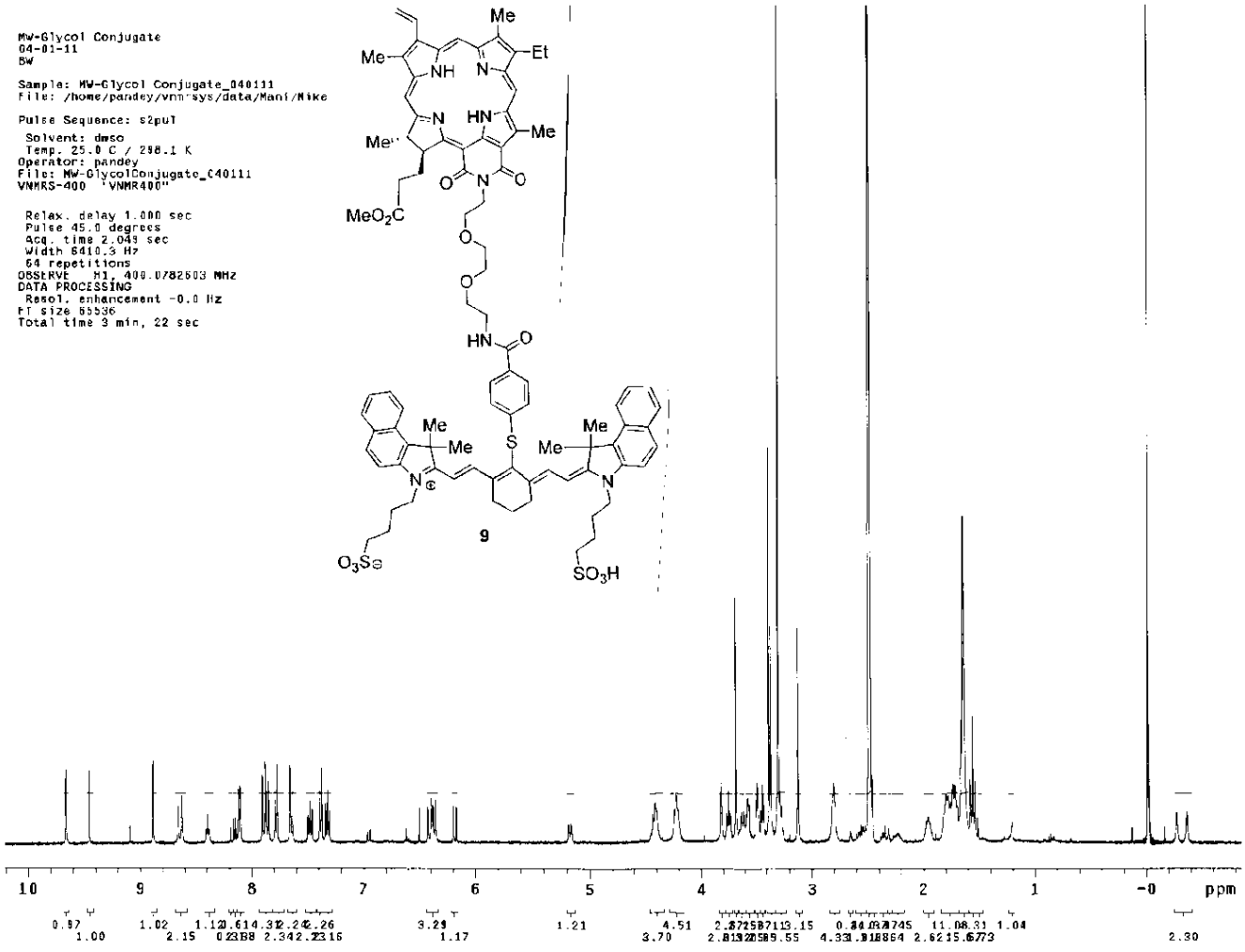
Pulse Sequence: s2pul
Solvent: dmso
Temp. 25.0 C / 298.1 K
Operator: pandey
File: MW-II-52
VNMRS-400 "VNMR400"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.049 sec
Width 6410.3 Hz
64 repetitions
OBSERVE H1, 400.0912818 MHz
DATA PROCESSING
Resol. enhancement -0.0 Hz
FT size 65536
Total time 3 min, 22 sec
8
ppm


MW-Glycol Conjugate
04-01-11
BW
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Operator: pandey
File: MW-GlycolConjugate_C40111
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Pulse 45.0 degrees
Acq. time 2.049 sec
Width 6410.3 Hz
64 repetitions
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DATA PROCESSING
Resol. enhancement -0.0 Hz
FT size 65536
Total time 3 min, 22 sec
9
ppm

HPLC Analysis

Method: HPLC analysis of conjugates # **7**, # **8**, and # **9** was carried out using a Waters Delta 600 System consisting of the 600 Controller, 600 Fluid Handling Unit and 996 Photodiode Array Detector equipped with a Waters Symmetry C18 column, 5 micron, with dimensions 4.6 x150mm. The mobile phase flow program was as follows: flow rate of 1.0 ml / min; mobile phase composition - 75% Methanol/ 25% water from 0 to 20 minutes , then a linear gradient to 100% Methanol from 20 minutes to 30 minutes, maintained at 100% Methanol from 30 minutes on.. Data collected and spectrally monitored from 300 nm to 799 nm. Percent purity of compounds based on 416 nm channel.

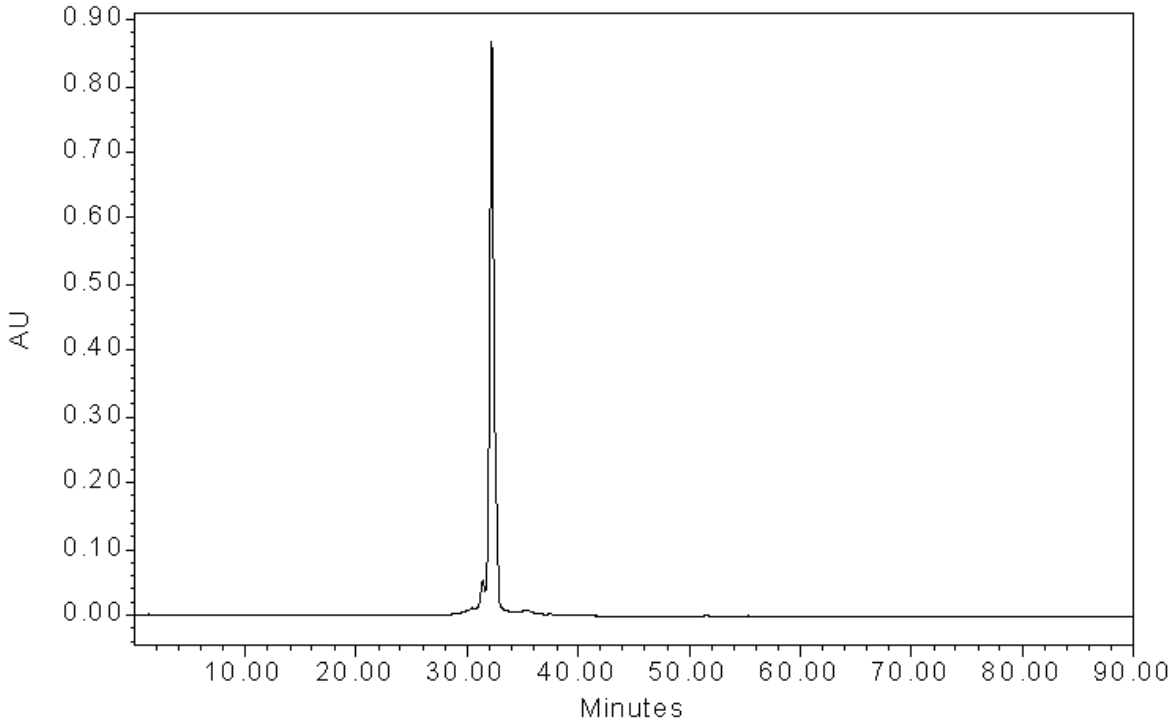

Figure S1: HPLC Chromatogram of Conjugate # **7**

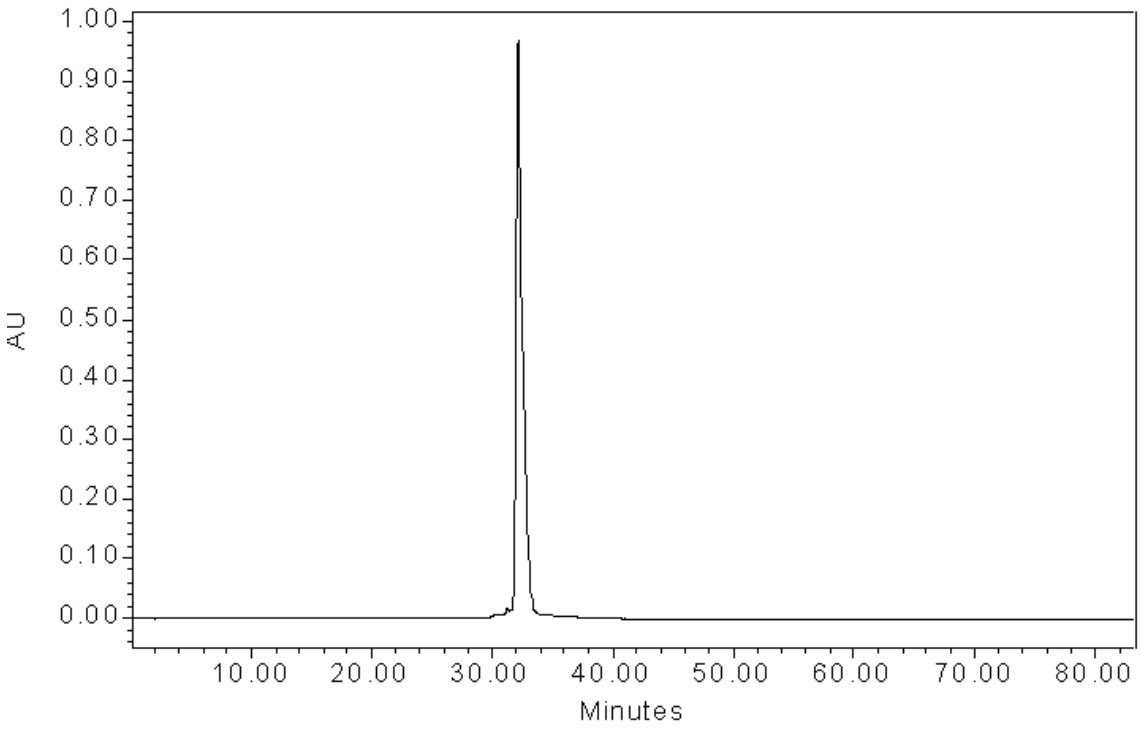

Figure S2: HPLC Chromatogram of Conjugate # **8**

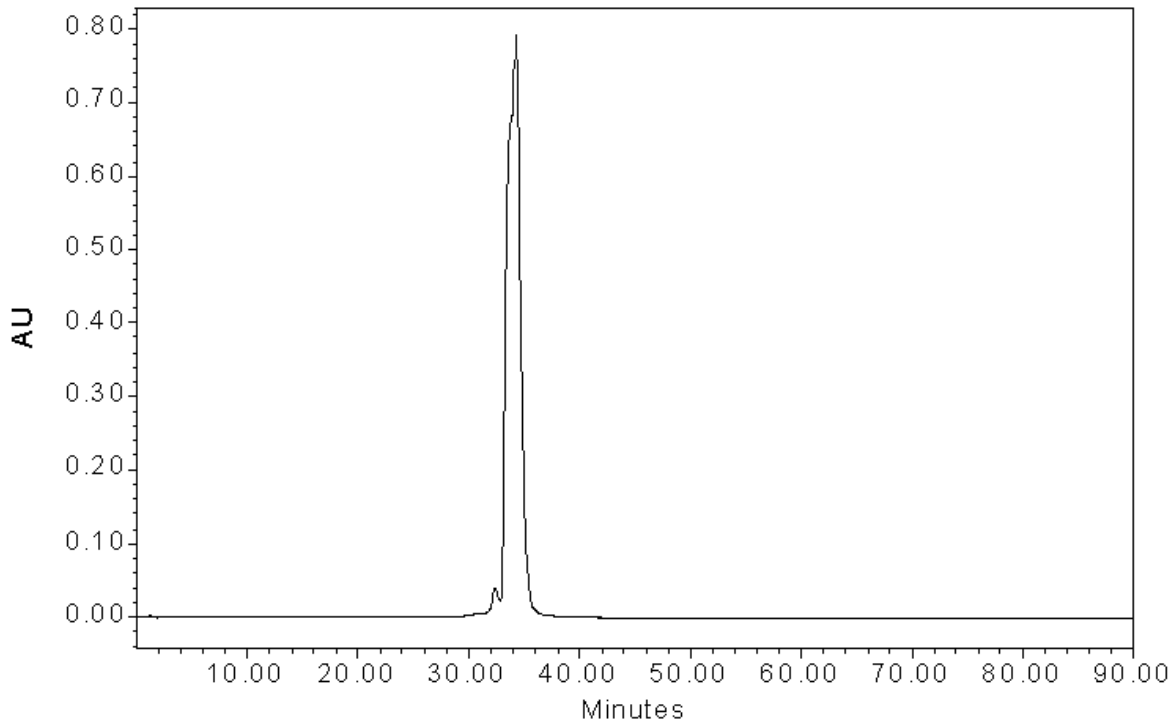

Figure S3: HPLC Chromatogram of Conjugate # **9**