Supplementary Data

Assay and Inhibition of Diacylglycerol Lipase Activity

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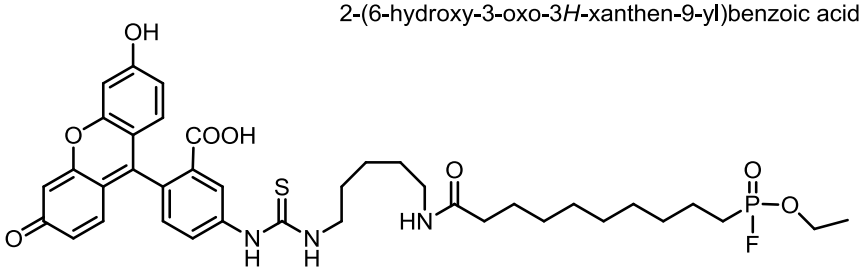
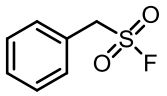
FP-fluorescein (fluorophosphoryl-fluorescein)

5-(3-(5-(10-(ethoxyfluorophosphoryl)decanamido)pentyl)thioureido)-2-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)benzoic acid



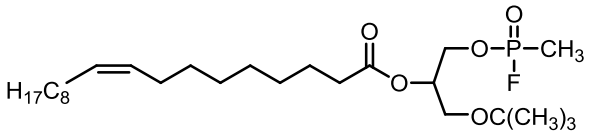
PMSF

phenylmethylsulfonyl fluoride



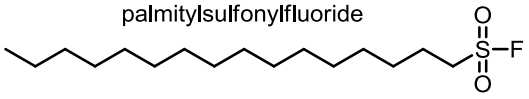
O-5596

1-(fluoro(methyl)phosphoryloxy)-3-*tert*-butoxypropan-2-yl oleate



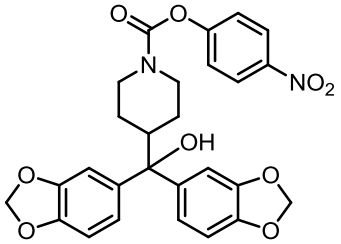
***n*-$C_{16}H_{33}SO_2F$ (SYL374)**

palmitylsulfonylfluoride



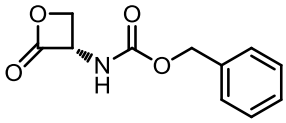
JZL184

4-nitrophenyl-4-[bis(1,3-benzodioxol-5-yl)(hydroxy)methyl]piperidine-1-carboxylate



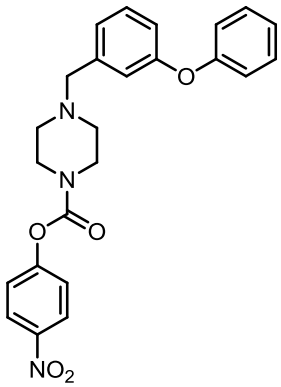
SD41

N-carbobenzoxy-L-serine β-lactone



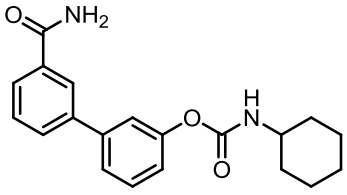
JZL195

(4-nitrophenyl) 4-[(3-phenoxyphenyl)methyl]piperazine-1-carboxylate



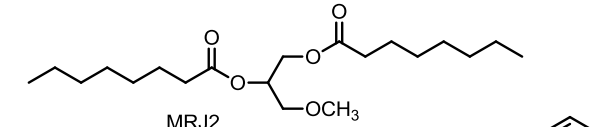
URB597

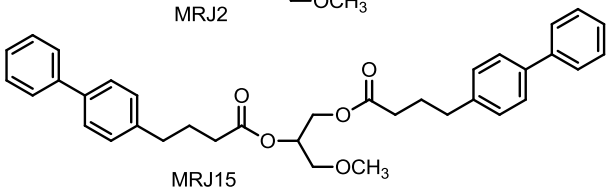
[3-(3-carbamoylphenyl)phenyl] *N*-cyclohexylcarbamate

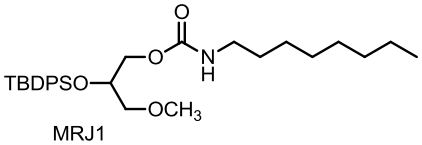


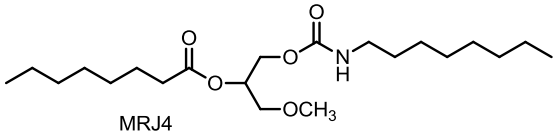
Ether lipid analogs of lead compound O-3841 **2** were synthesized including analogs that had the reactive carbamate group of RHC80267 **1**. The 3-*O*-methyl glycerol derivatives MRJ1, MRJ4, MRJ5, and MRJ6 were synthesized utilizing the tritylation, silyl ether protection, and detritylation conditions developed in the Roush laboratory (Tortosa, et al. *JACS* **2008**, *130*, 2722 and Tortosa et al. *JOC* **2008**, *73*, 9657).

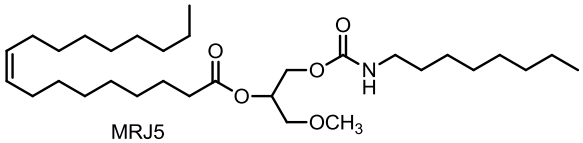
These compounds were not inhibitors of hDAGLα or mDAGLα at 10 μM. These compounds each had a Ki above 1 μM in competition binding assays for CB1 (rat brain preparation) and for CB2 (mouse or human receptor expressed in HEK293). They also did not inhibit rFAAH or hMAGL.

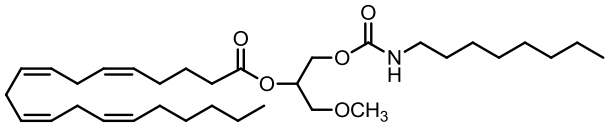

MRJ2

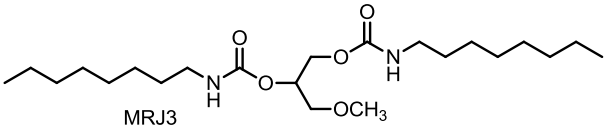

MRJ15

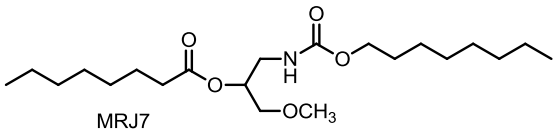

MRJ1

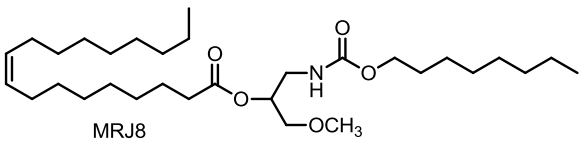

MRJ4

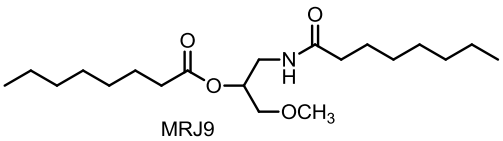

MRJ5

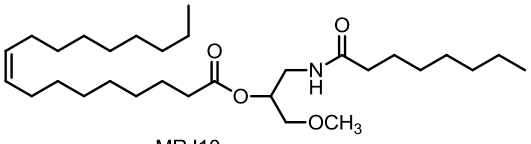

MRJ6

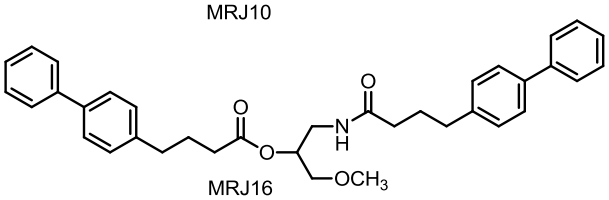

MRJ3

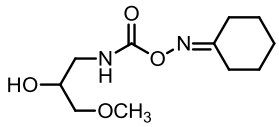

MRJ7


MRJ8


MRJ9


MRJ10


MRJ16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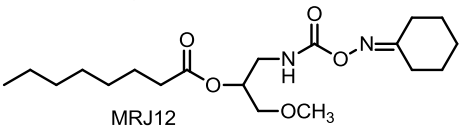
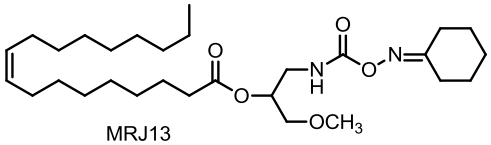
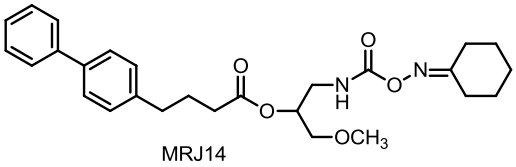
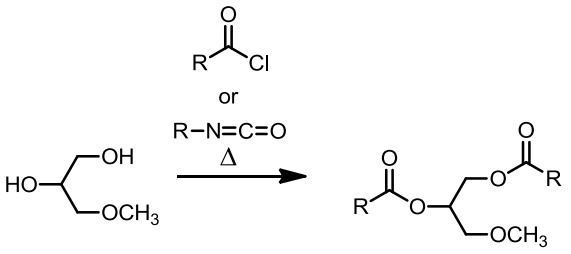
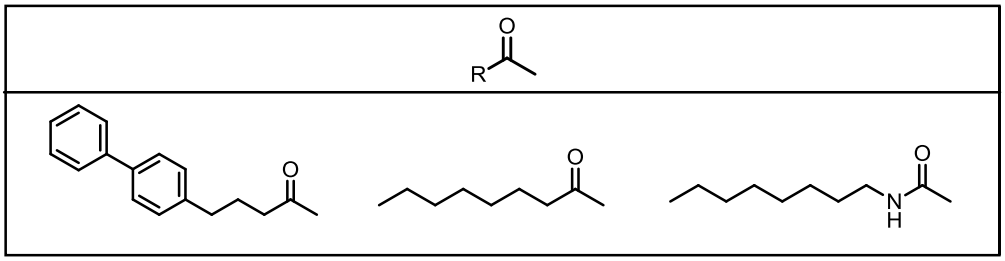
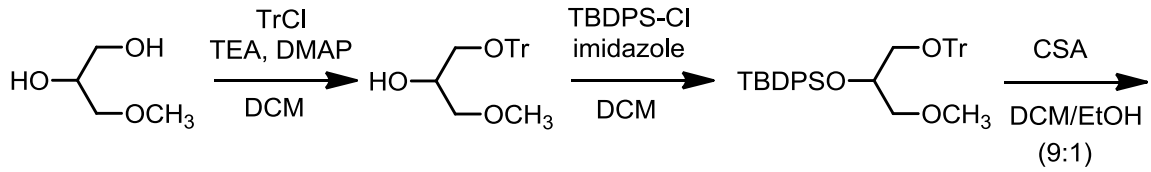

MRJ12

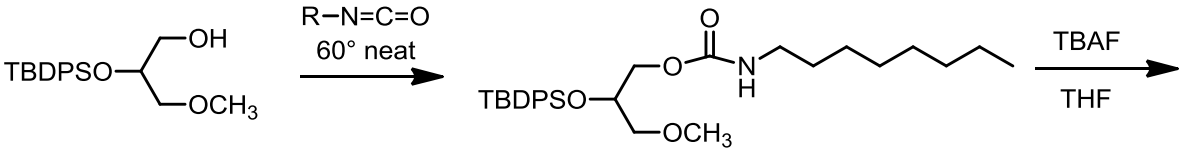

MRJ13

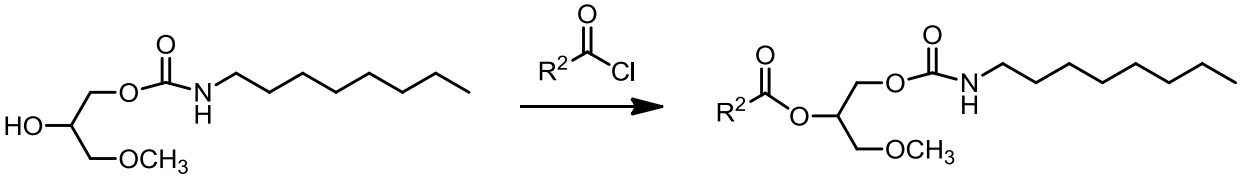

MRJ14


O
R
Cl
or
R–N=C=O
Δ
OH
HO
OCH3
O
R
O
O
R
O
R
O
OCH3


O
R
O
O
O
N
H


OH
HO
OCH3
TrCl
TEA, DMAP
DCM
OTr
HO
OCH3
TBDPS-Cl
imidazole
DCM
OTr
TBDPSO
OCH3
CSA
DCM/EtOH
(9:1)


OH
TBDPSO
OCH3
R–N=C=O
60° neat
O
O
N
H
TBDPSO
OCH3
TBAF
THF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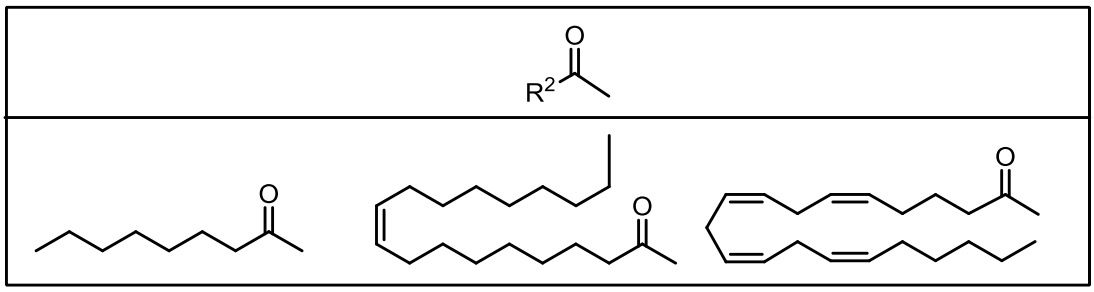
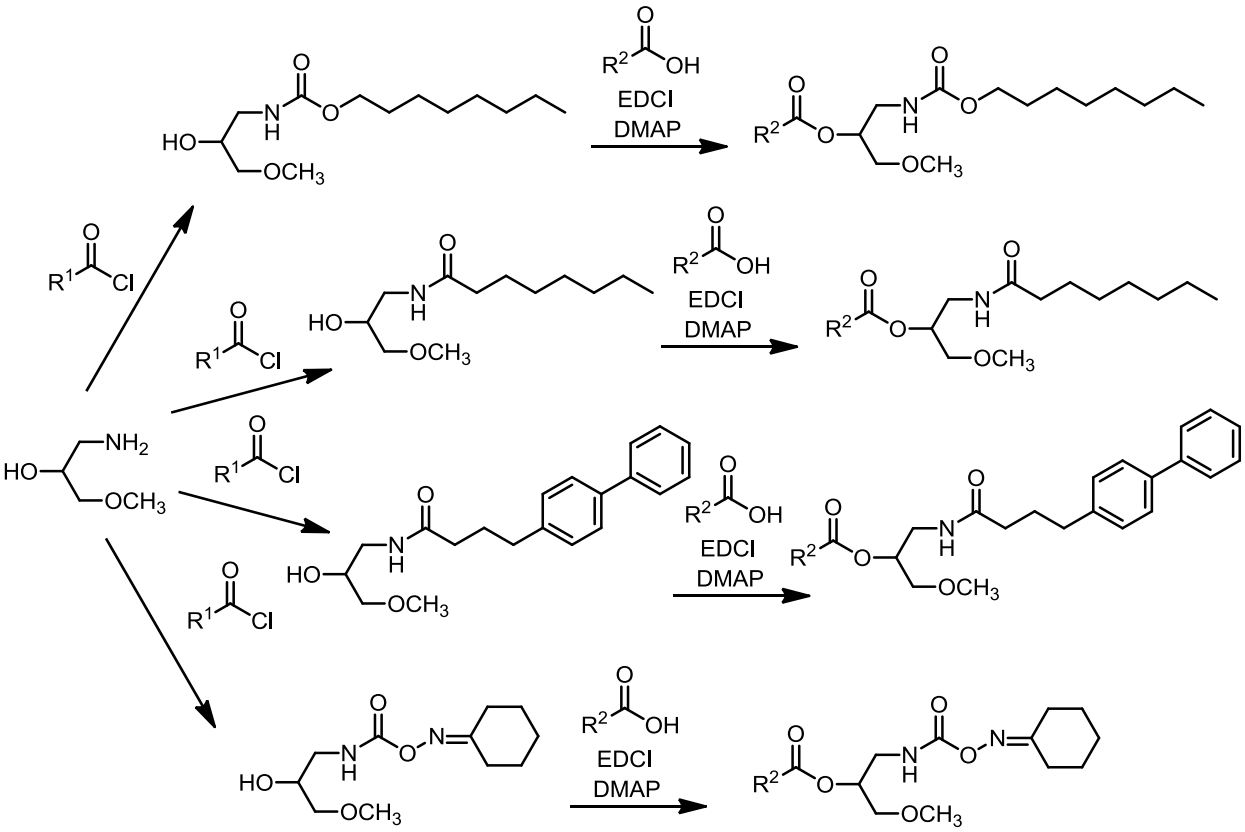
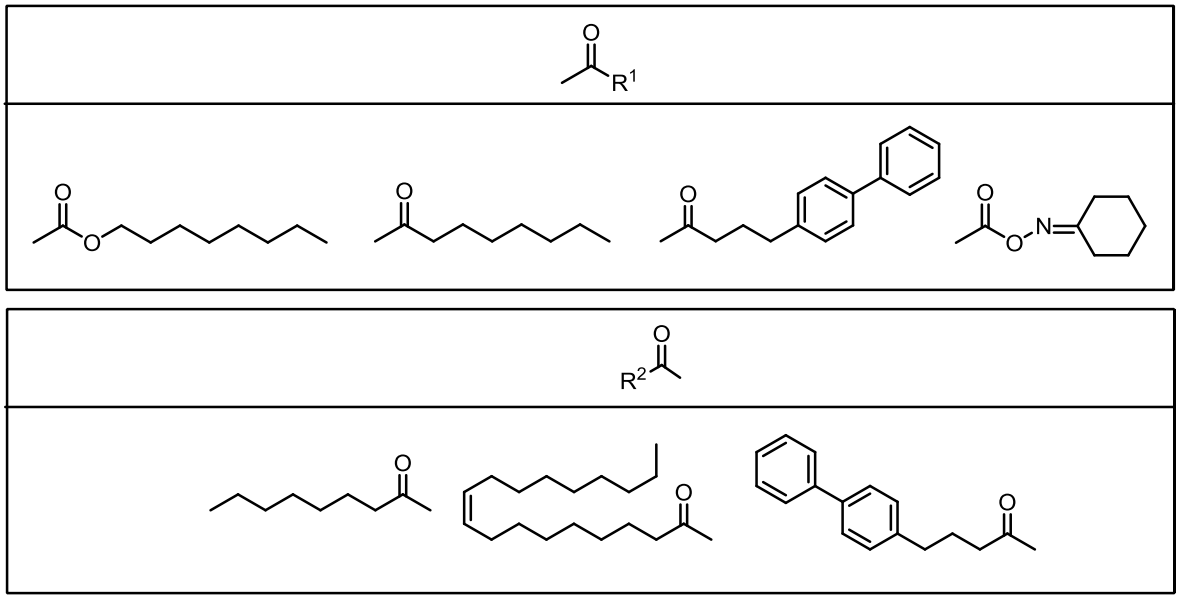
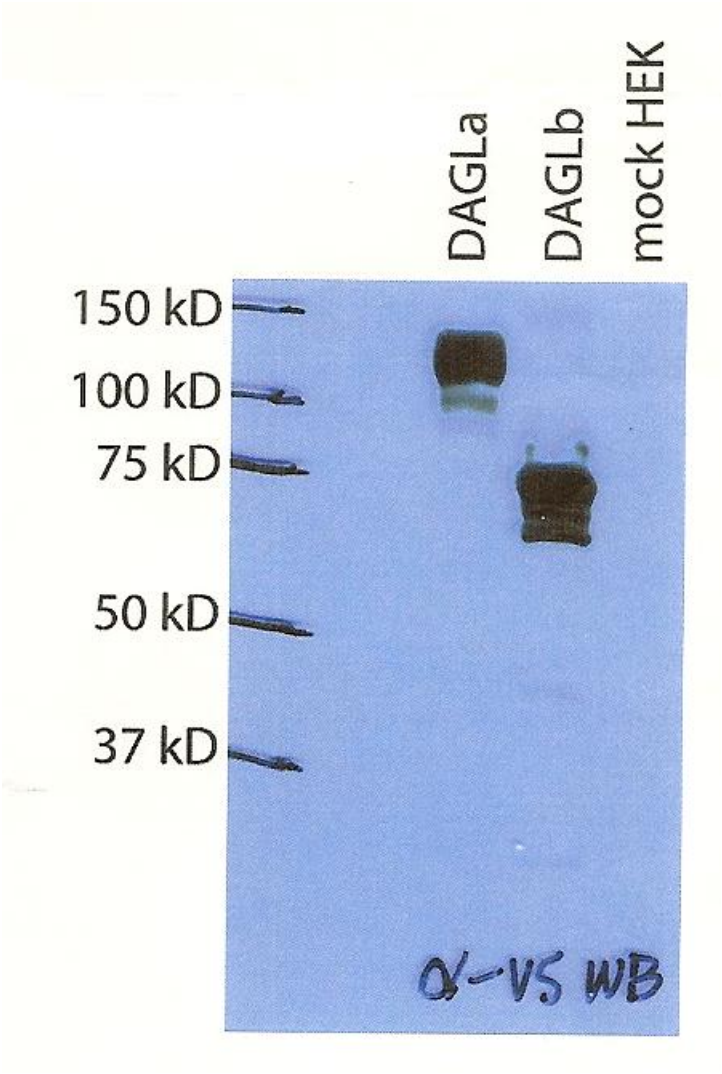
O
N
H
HO
OCH3
O
R2
Cl
O
R2
O
O
O
N
H
OCH3


O
R2
O
O
O


EDCI
DMAP
EDCI
DMAP
EDCI
DMAP
EDCI
DMAP

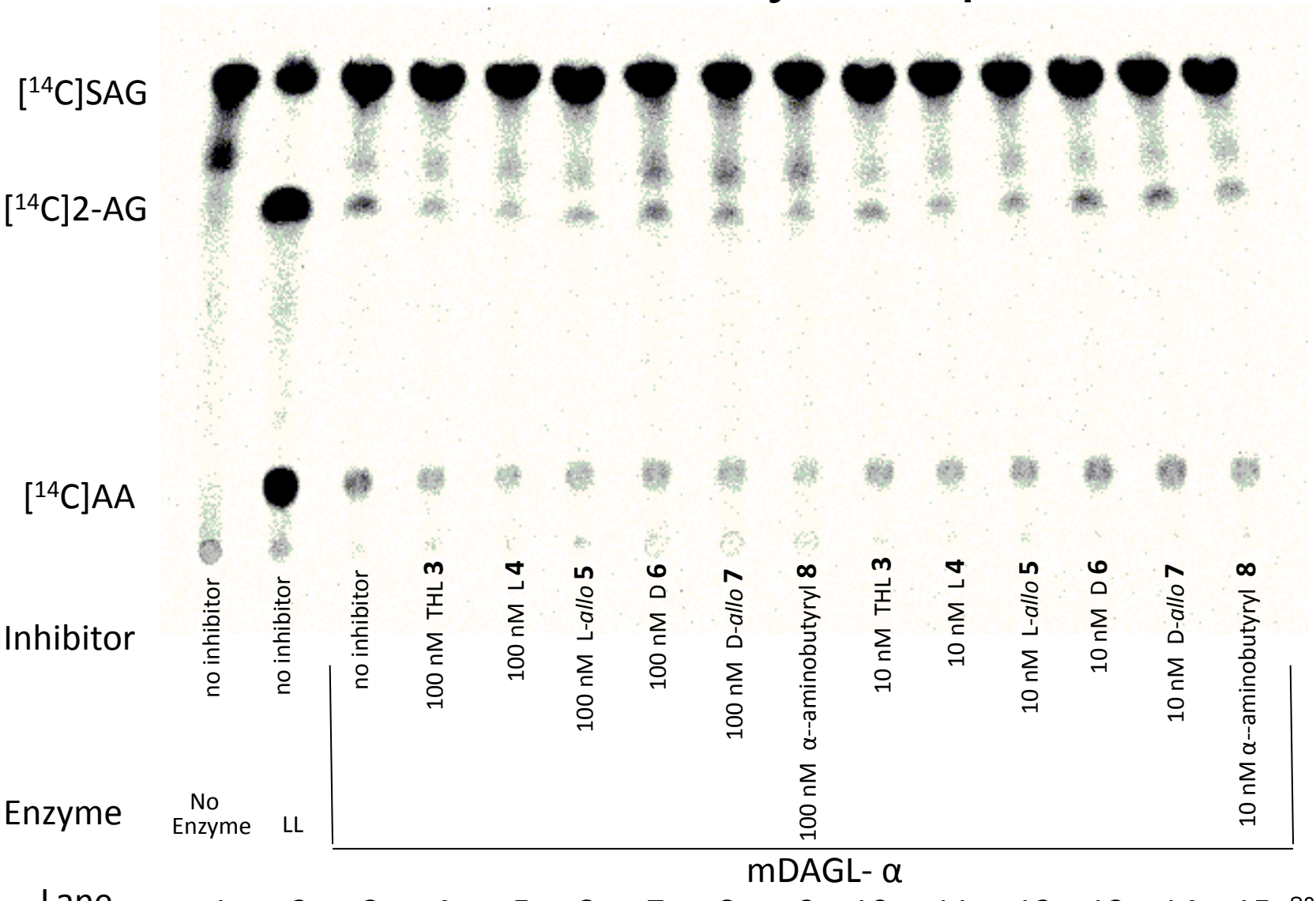


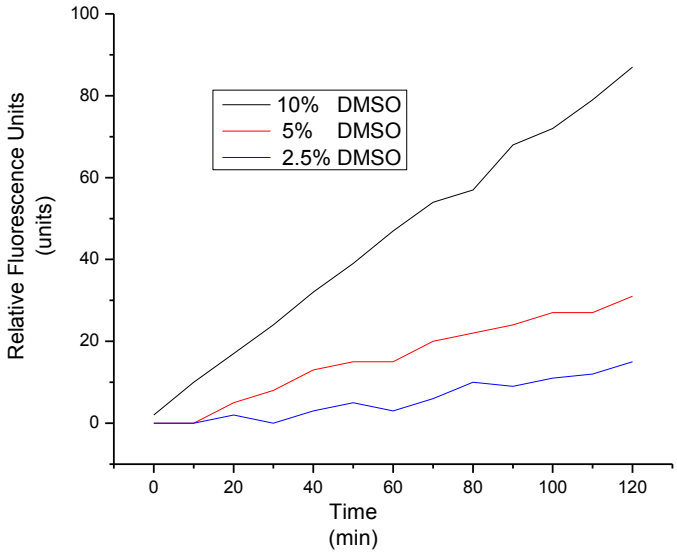


hDAGLα and mDAGLβ cDNA sequences were both subcloned into the pcDNA3.1-V5-HIS-TOPO vector. The corresponding plasmids and mock (empty pcDNA3.1) were transfected into HEK293T according to the manufacturer's protocol and cell lysates analyzed by PAGE. Western blot with anti-V5 was used by the Scripps group to confirm expression.

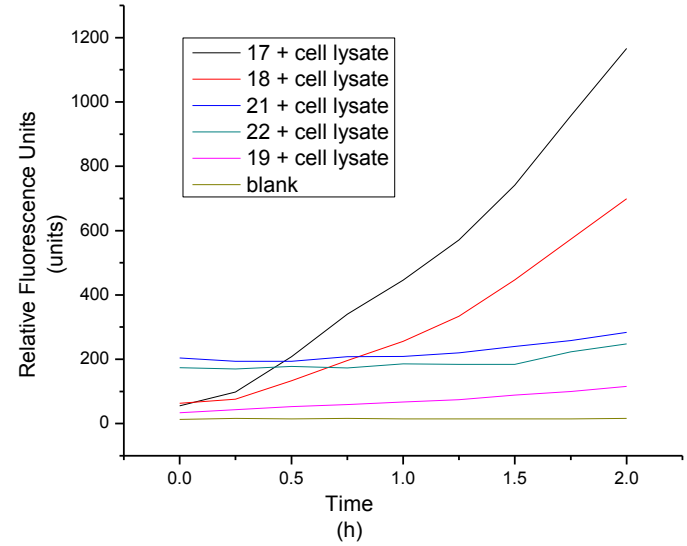
A. [1”-^{14}C]1-Stearoyl-2-arachidonoyl-*sn*-glycerol ([^{14}C]SAG) 20,000 DPM eluted (4:96 acetone/chloroform) on TLC (silica gel) with a 1 h exposure. **B.** [1”-^{14}C]1-Stearoyl-2-arachidonoyl-*sn*-glycerol ([^{14}C]SAG) 9,400 DPM eluted (4:96 acetone/chloroform) on TLC (boric acid treated silica gel plate, R_f = 0.21) with a 15 h exposure, and **C** shows the profile of this lane with less than 0.5% 1(3)-diglyceride rearrangement byproduct [1’”-^{14}C]1-stearoyl-3-arachidonoyl-*sn*-glycerol (R_f = 0.42) present upon quantitative phosphorimaging analysis.

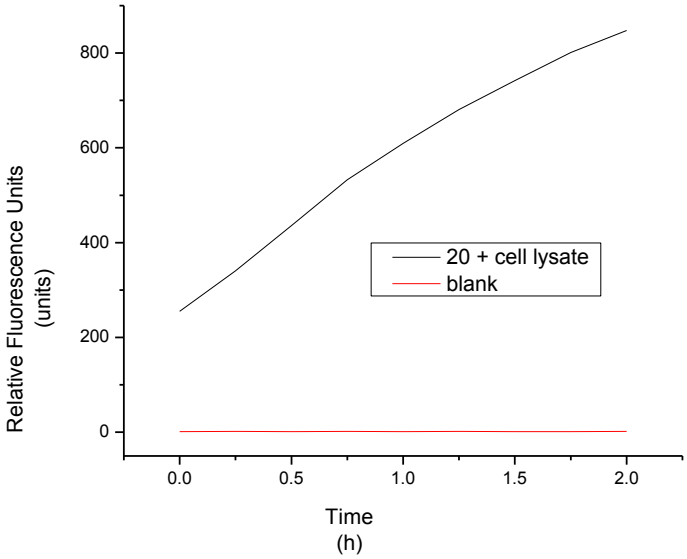
mDAGL-α Inhibition by Compounds **3-8**



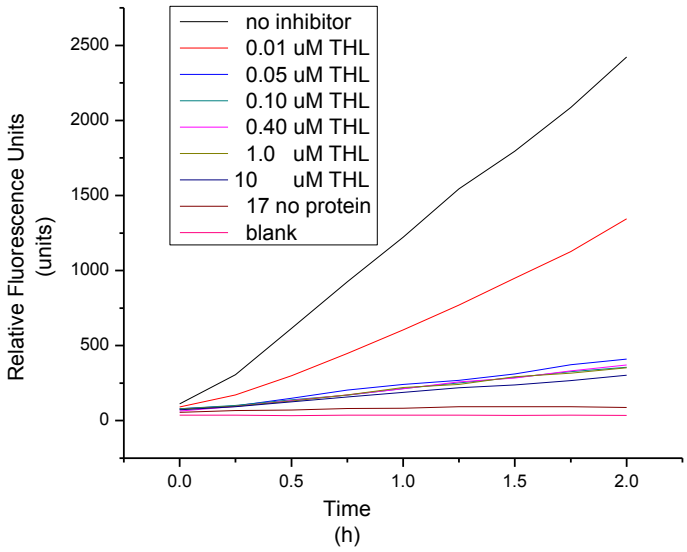


Cell lysate of hDAGLα expression with reporter compound **17** showing the effect of DMSO to solubilize the lipid substrate. No increase in activity was observed at DMSO concentrations above 10% (data not shown). The same effect on solubilizaton of substrate with DMSO for lipoprotein lipase was observed (data not shown).





Reporter compounds as substrates for hDAGLα activity with HEK293T cell lysates in the presence of 10% DMSO. Top, pyrenyl analogs **17**, **18**, **19**, **21**, and **22** (EX 320 nm, EM 400 nm). Bottom, NBD analog **20** (EX 485 nm, EM 535 nm).



Inhibition of hDAGLα activity by THL **3**. Also note the stability of reporter compound **17** under the assay conditions.

All compounds were white solids (unless otherwise noted) and had the expected IR absorbance peaks. Observed rotations were too low to be accurately determined at the 0.1 g/ 100 mL CH_2Cl_2 concentrations used. All proton NMR spectra were in $CDCl_3$ at 500 MHz. No previous publication of THL **3** to our knowledge has noted the small percentage of *trans*-*N*-formate present that does not interconvert with the *cis*-*N*-formate isomer at temperatures below the decomposition temperatures for THL and the related β-lactones. We were not able to chromatographically isolate the cis and trans formates, nor separate **D**-*allo*-isoleucyl analog **7** from the **D**-isoleucyl analog **6**.

3 THL mp 41-42 °C; ^{1}H NMR δ 0.88 (t, *J* = 6.4 Hz, 3 H), 0.89 (t, *J* = 6.4 Hz, 3 H), 0.97 (d, *J* = 5.9 Hz, 3 H), 0.98 (d, *J* = 5.9 Hz, 3 H), 1.22 - 1.50 (m, 27 H), 1.50 - 1.87 (m, 6 H), 2.00 (ddd, *J* = 15.1, 4.9, 4.9 Hz, 1 H), 2.17 (ddd, *J* = 15.2, 7.8, 7.8 Hz, 1 H), 3.22 (ddd, *J* = 7.3, 7.3, 3.9 Hz, 1 H), 4.29 (ddd, *J* = 7.8, 4.9, 3.9 Hz, 1 H), 4.70 (ddd, *J* = 9.0, 8.6, 4.9 Hz, 1 H), 5.00 - 5.06 (m, 1 H), 5.89 (d, *J* = 8.4 Hz, 1 H), 8.22 (s, 1 H). trans isomer 0.95 (d, *J* = 7.8 Hz, 0.15 H), 4.10 (ddd, *J* = 9.8, 9.7, 4.4 Hz, 0.05 H), 5.05 - 5.11 (m, 0.05 H), 5.72 (dd, *J* = 11.7, 10.3 Hz, 0.05 H), 8.06 (d, *J* = 11.7 Hz, 0.05 H).

4 L-isoleucyl analog (OMDM188) mp 59-60 °C; lit. (Ortar et al. *JMC* **2008**, *51*, 6970) mp 57-59 °C; ^{1}H NMR δ 0.88 (t, *J* = 7.3 Hz, 3 H), 0.89 (t, *J* = 7.3 Hz, 3 H), 0.96 (t, *J* = 7.8 Hz, 3 H), 0.97 (d, *J* = 7.8 Hz, 3 H), 1.10 - 1.60 (m, 28 H), 1.58 - 1.84 (m, 4 H), 1.85 - 2.00 (m, 1 H), 2.02 (ddd, *J* = 15.0, 4.5, 4.5 Hz, 1 H), 2.19 (ddd, *J* = 14.9, 7.7, 7.7 Hz, 1 H), 3.24 (ddd, *J* = 7.4, 7.4, 4.2 Hz, 1 H), 4.29 (ddd, *J* = 7.7, 4.5, 4.2 Hz, 1 H), 4.68 (dd, *J* = 8.8, 4.9 Hz, 1 H), 5.00 - 5.07 (m, 1 H), 6.05 (d, *J* = 8.8 Hz, 1 H), 8.26 (s, 1 H). trans isomer 0.99 (d, *J* = 7.8 Hz, 0.15 H), 3.97 (dd, *J* = 10.2, 4.9 Hz, 0.05 H), 5.06 - 5.11 (m, 0.05 H), 5.87 (dd, *J* = 11.7, 10.3 Hz, 0.05 H), 8.03 (d, *J* = 11.7 Hz, 0.05 H).

5 L-*allo*-isoleucyl analog (MRJ27) mp 49-50 °C; ^{1}H NMR δ 0.87 (t, *J* = 7.3 Hz, 3 H), 0.88 (t, *J* = 7.3 Hz, 3 H), 0.96 (t, *J* = 7.6 Hz, 3 H), 0.97 (d, *J* = 7.6 Hz, 3 H), 1.14 - 1.52 (m, 28 H), 1.54 - 1.88 (m, 4 H), 1.92 - 2.00 (m, 1 H), 2.02 (ddd, *J* = 15.1, 5.4, 5.4 Hz, 1 H), 2.19 (ddd, *J* = 15.1, 7.7, 7.5 Hz, 1 H), 3.23 (ddd, *J* = 7.6, 7.6, 3.9 Hz, 1 H), 4.29 (ddd, *J* = 7.6, 5.4, 3.9 Hz, 1 H), 4.77 (dd, *J* = 9.0, 3.7 Hz, 1 H), 4.96 - 5.05 (m, 1 H), 6.10 (d, *J* = 8.8 Hz, 1 H), 8.26 (s, 1 H). trans isomer 4.07 (dd, *J* = 10.2, 3.9 Hz, 0.05 H), 5.04 - 5.11 (m, 0.05 H), 6.01 (dd, *J* = 11.7, 10.3 Hz, 0.05 H), 8.00 (d, *J* = 11.7 Hz, 0.05 H).

6 D-isoleucyl analog (MRJ29) clear and colorless liquid; ^{1}H NMR δ 0.89 (t, *J* = 6.6 Hz, 6 H), 0.95 (t, *J* = 7.3 Hz, 3 H), 0.97 (d, *J* = 7.3 Hz, 3 H), 1.14 - 1.52 (m, 28 H), 1.54 - 1.88 (m, 4 H), 1.92 - 2.00 (m, 1 H), 2.03 (ddd, *J* = 14.9, 4.5, 4.5 Hz, 1 H), 2.19 (ddd, *J* = 14.9, 7.9, 6.5 Hz, 1 H), 3.24 (ddd, *J* = 7.4, 7.4, 4.2 Hz, 1 H), 4.34 (ddd, *J* = 7.9, 4.5, 4.2 Hz, 1 H), 4.66 (dd, *J* = 8.6, 4.6 Hz, 1 H), 5.01 - 5.08 (m, 1 H), 6.03 (d, *J* = 8.8 Hz, 1 H), 8.25 (s, 1

H). trans isomer 1.00 (t, *J* = 7.3 Hz, 0.15 H), 2.05 (ddd, *J* = 14.9, 4.5, 4.5 Hz, 0.05 H), 3.24 (ddd, *J* = 7.4, 7.4, 4.2 Hz, 0.05 H), 3.99 (dd, *J* = 10.3, 4.3 Hz, 0.05 H), 4.26 - 4.32 (m, 0.05 H), 5.09 - 5.17 (m, 0.05 H), 5.98 (dd, *J* = 11.7, 10.3 Hz, 0.05 H), 8.02 (d, *J* = 11.7 Hz, 0.05 H). D-isoleucine has second 5% impurity of D-*allo*-isoleucine from starting amino acid 4.77 (dd, *J* = 9.0, 3.7 Hz, 0.05 H), 5.98 (d, *J* = 8.8 Hz, 0.05 H), 8.27 (s, 0.05 H).

7 D-*allo*-isoleucyl analog (MRJ30) clear and colorless liquid; ^{1}H NMR δ 0.85 - 0.90 (m, 9 H), 0.97 (t, *J* = 7.3 Hz, 3 H), 1.16 - 1.51 (m, 28 H), 1.52 - 1.87 (m, 4 H), 1.91 - 2.01 (m, 1 H), 2.02 (ddd, *J* = 15.0, 5.0, 4.9 Hz, 1 H), 2.19 (ddd, *J* = 15.0, 7.7, 7.1 Hz, 1 H), 3.23 (ddd, *J* = 7.4, 7.3, 3.9 Hz, 1 H), 4.33 (ddd, *J* = 7.7, 5.0, 3.9 Hz, 1 H), 4.77 (dd, *J* = 9.0, 3.7 Hz, 1 H), 5.00 - 5.08 (m, 1 H), 5.96 (d, *J* = 8.8 Hz, 1 H), 8.27 (s, 1 H). trans isomer 0.96 (t, *J* = 7.3 Hz, 0.15 H), 2.13 (m, 0.05 H), 3.17 - 3.22 (m, 0.05 H), 4.08 (dd, *J* = 10.0, 3.7 Hz, 0.05 H), 4.26 - 4.32 (m, 0.05 H), 5.09 - 5.17 (m, 0.05 H), 5.87 (dd, *J* = 11.7, 10.0 Hz, 0.05 H), 8.00 (d, *J* = 11.7 Hz, 0.05 H).

8 α-Aminobutyryl analog (MRJ28) mp 43-44 °C; ^{1}H NMR δ 0.88 (t, *J* = 6.7 Hz, 3 H), 0.89 (t, *J* = 6.7 Hz, 3 H), 0.96 (t, *J* = 7.6 Hz, 3 H), 1.20 - 1.54 (m, 26 H), 1.54 - 1.90 (m, 5 H), 1.92 - 2.01 (m, 1 H), 2.02 (ddd, *J* = 15.1, 4.4, 4.4 Hz, 1 H), 2.17 (ddd, *J* = 15.1, 7.9, 7.8 Hz, 1 H), 3.23 (ddd, *J* = 7.4, 7.4, 4.2 Hz, 1 H), 4.30 (ddd, *J* = 8.0, 4.5, 4.4 Hz, 1 H), 4.64 (ddd, *J* = 7.3, 7.3, 7.3 Hz, 1 H), 5.02 - 5.09 (m, 1 H), 6.10 (d, *J* = 7.3 Hz, 1 H), 8.25 (s, 1 H). trans isomer 1.00 (t, *J* = 7.6 Hz, 0.15 H), 4.03 (ddd, *J* = 9.8, 7.8, 5.4 Hz, 0.05 H), 5.07 - 5.13 (m, 0.05 H), 5.87 (dd, *J* = 11.7, 9.8 Hz, 0.05 H), 8.08 (d, *J* = 11.7 Hz, 0.05 H).

9 β-Tridecyl-β-lactone (racemic, RID13) mp 39-40 °C; ^{1}H NMR δ 0.88 (t, *J* = 6.8 Hz, 3 H), 1.21 - 1.51 (m, 22 H), 1.69 - 1.79 (m, 1 H), 1.82 - 1.92 (m, 1 H), 3.06 (dd, *J* = 16.4, 4.2 Hz, 1 H), 3.50 (dd, *J* = 16.1, 5.9 Hz, 1 H), 4.50 (ddd, *J* = 11.5, 6.0, 6.0 Hz, 1 H).

10 *trans*-β-Lactone (racemic, MRJ18) clear and colorless liquid; ^{1}H NMR δ 0.89 (t, *J* = 6.8 Hz, 6 H), 1.20 - 1.50 (m, 18 H), 1.66 - 1.77 (m, 2 H), 1.77 - 1.95 (m, 2 H), 3.16 (ddd, *J* = 8.7, 6.4, 4.1 Hz, 1 H), 4.21 (ddd, *J* = 6.7, 6.7, 4.1 Hz, 1 H).

11 *cis*-β-Lactone (racemic, MRJ17) clear and colorless liquid; ^{1}H NMR δ 0.89 (t, *J* = 6.8 Hz, 6 H), 1.20 - 1.47 (m, 16 H), 1.45 - 1.58 (m, 2 H), 1.59 - 1.71 (m, 2 H), 1.71 - 1.85 (m, 2 H), 3.60 (ddd, *J* = 8.9, 6.8, 6.8 Hz, 1 H), 4.54 (ddd, *J* = 9.7, 6.1, 4.2 Hz, 1 H).

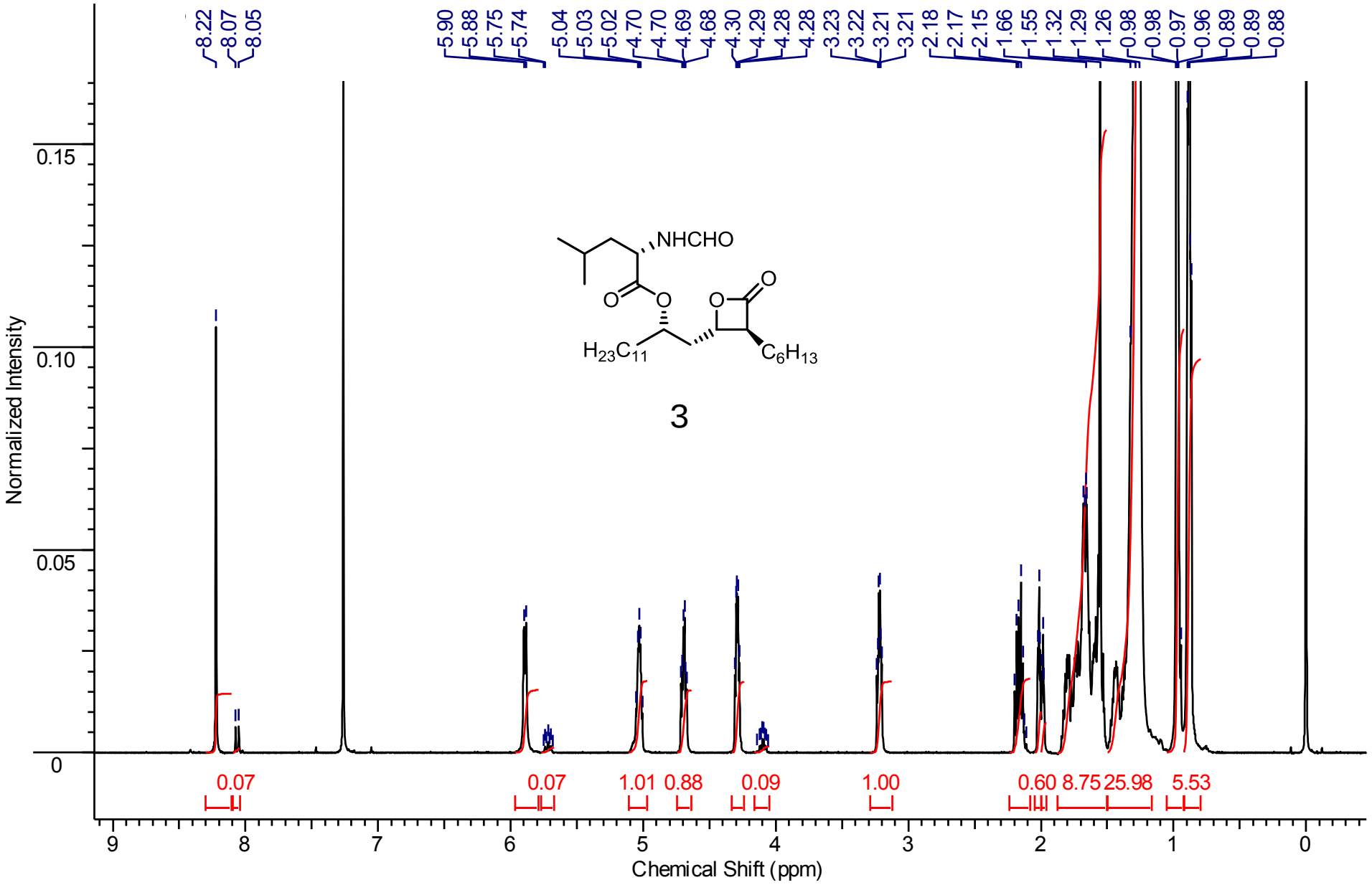
17 1-DNP-2-pyrenyl ether lipid (MRJ20) viscous yellow semi-solid; ^{1}H NMR δ 1.20 - 1.32 (m, 2 H), 1.46 (quintet, *J* =7.5 Hz, 2 H), 1.59 (quint, *J* = 7.6 Hz, 2 H), 2.14 - 2.26 (m, 2 H), 2.30 (t, *J* = 7.3 Hz, 2 H), 2.54 (t, *J* =

7.1 Hz, 2 H), 2.89 (apparent q, *J* = 7.3 Hz, 2 H), 3.39 (s, 3 H), 3.34 - 3.44 (m, 2 H), 3.55 (dd, *J* = 10.7, 5.1 Hz, 1 H), 3.58 (dd, *J* = 10.7, 5.1 Hz, 1 H), 4.22 (dd, *J* = 12.2, 7.3 Hz, 1 H), 4.44 (dd, *J*=12.0, 3.2 Hz, 1 H), 5.31-5.38 (m, 1 H), 6.37 (d, *J* = 9.8 Hz, 1 H), 7.85 (d, *J* = 7.8 Hz, 1 H), 7.90 - 8.01 (m, 4 H), 8.05 - 8.18 (m, 5 H), 8.28 (d, *J* = 9.3 Hz, 1 H), 8.89 (d, *J* = 2.9 Hz, 1 H).

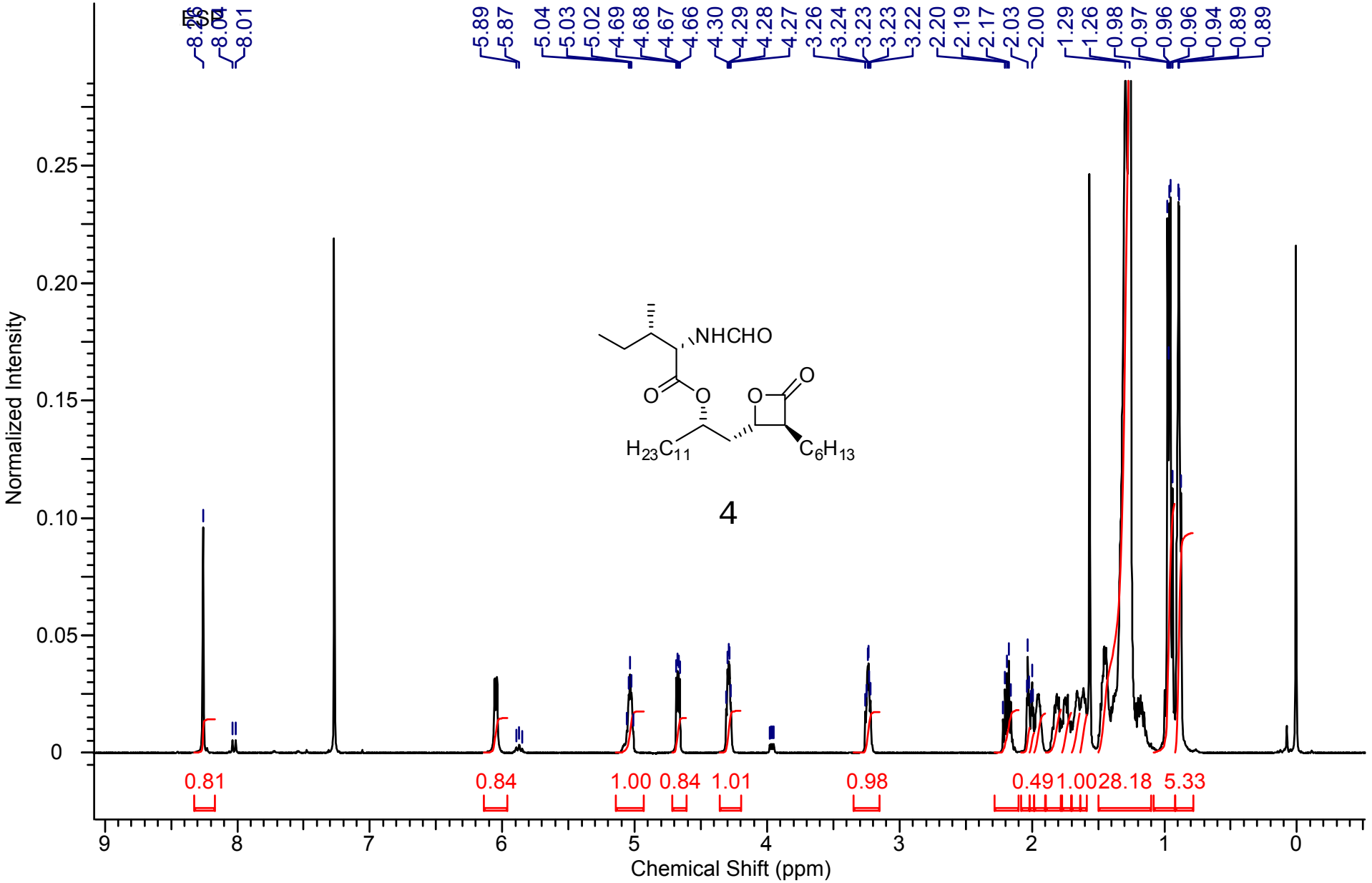
18 1-Pyrenyl-2-DNP ether lipid (MRJ21) viscous yellow semi-solid; ^{1}H NMR δ 1.21-1.30 (m, 2 H), 1.44 (quintet, *J* = 7.4 Hz, 2 H), 1.58 (quintet, *J* = 7.6 Hz, 2 H), 2.18 (quintet, *J* = 7.4 Hz, 2 H), 2.32 (t, *J* = 7.3 Hz, 2 H), 2.48 (t, *J* = 7.3 Hz, 2 H), 2.83 (dd, *J* = 13.2, 7.3 Hz, 2 H), 3.36 (s, 3 H), 3.32 - 3.38 (m, 2 H), 3.52 (dd, *J* = 10.7, 5.4 Hz, 2 H), 3.54 (dd, *J* = 10.7, 5.4 Hz, 2 H), 4.21 (dd, *J* = 12.0, 6.6 Hz, 1 H), 4.43 (dd, *J* = 12.2, 3.4 Hz, 1 H), 5.23-5.30 (m, 1 H), 6.28 (d, *J* = 9.3 Hz, 1 H), 7.82 (d, *J* = 7.3 Hz, 1 H), 7.85 (dd, *J* = 9.5, 2.7 Hz, 1 H), 7.90 - 7.97 (m, 3 H), 8.02 - 8.12 (m, 5 H), 8.23 (d, *J* = 9.3 Hz, 1 H), 8.83 (d, *J* = 2.4 Hz, 1 H).

(3) Tetrahydrolipstatin (THL)

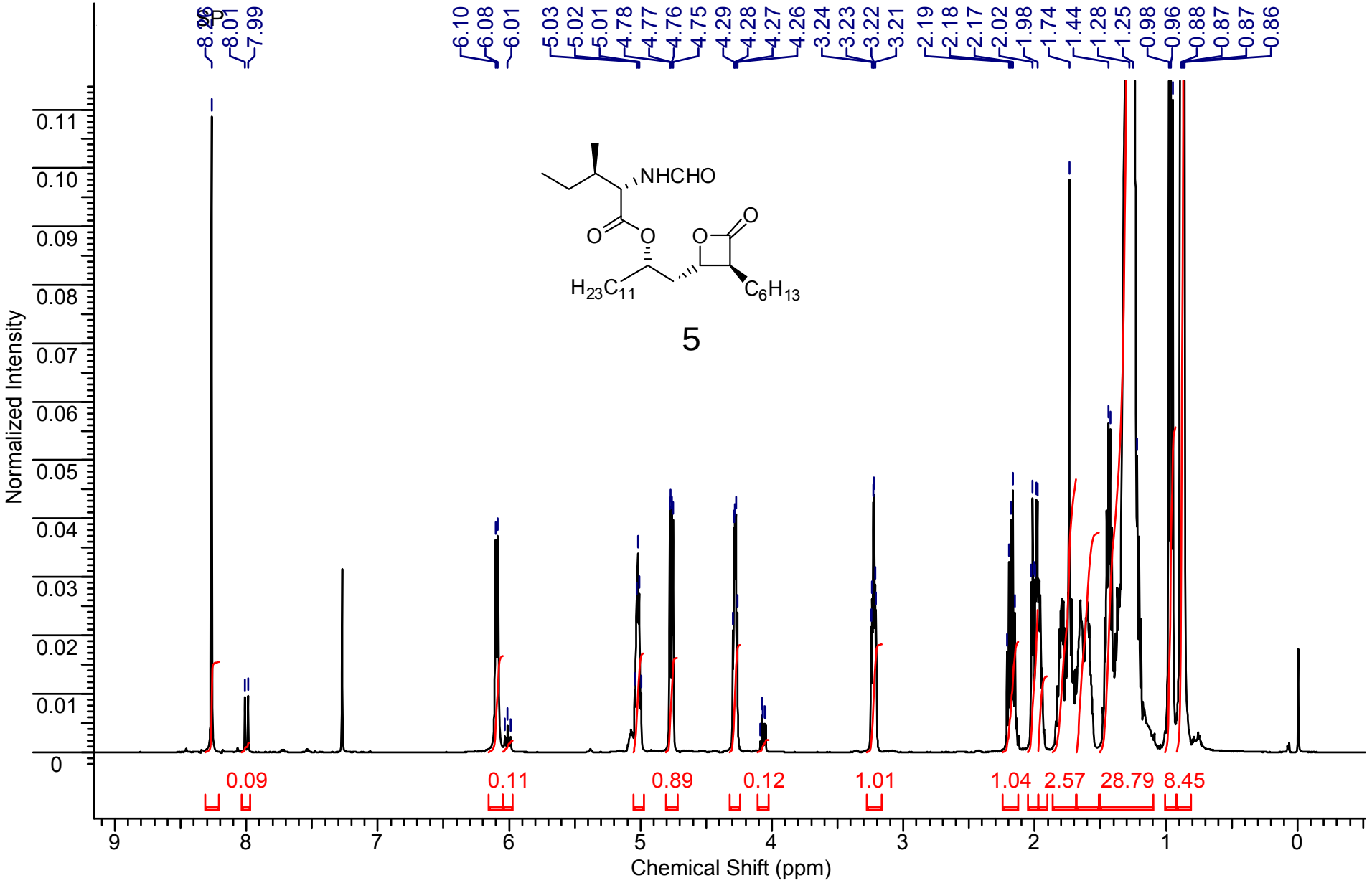
N-formyl-**L**-leucyl ester



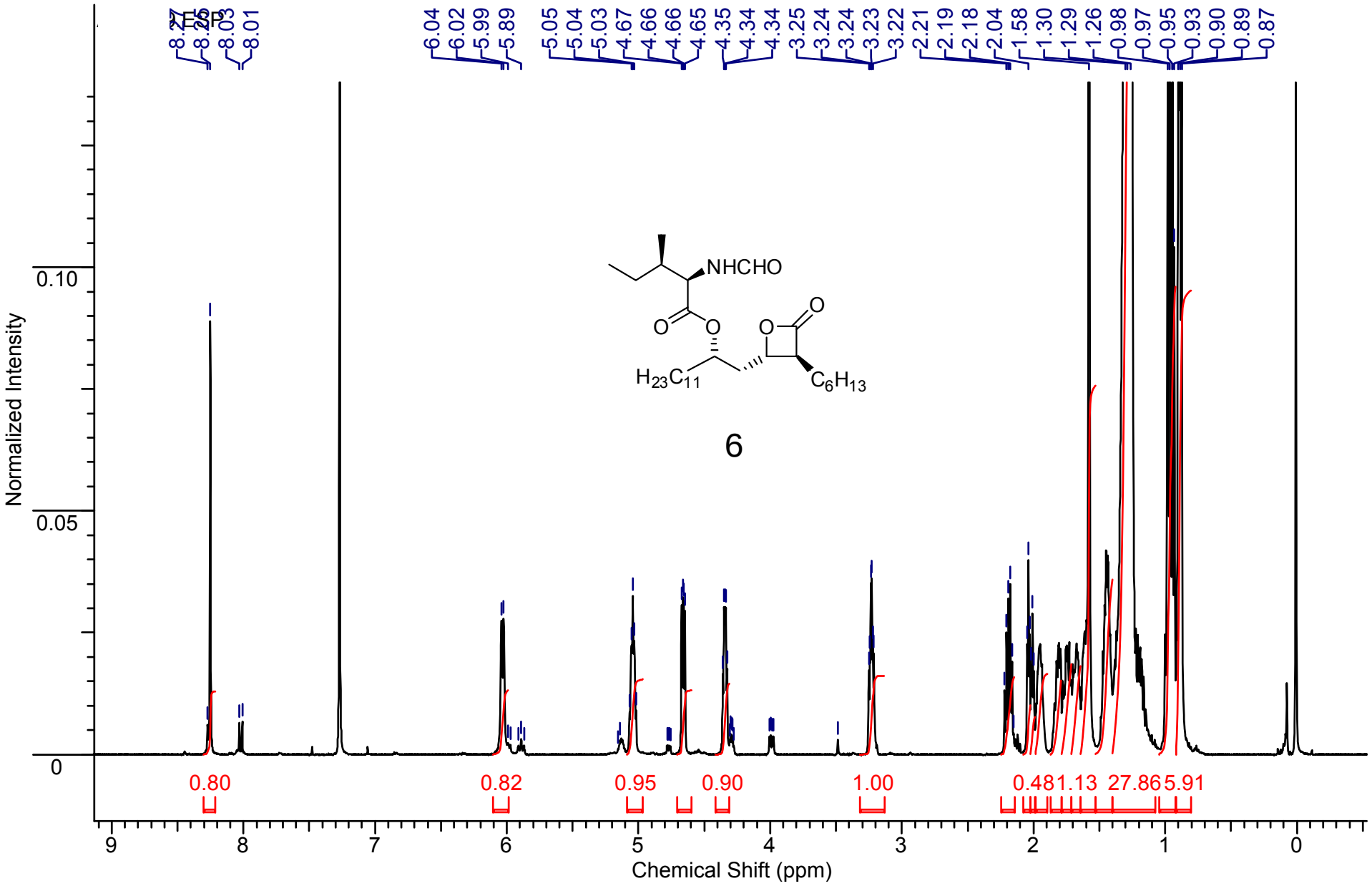
(4) *N*-formyl-**L**-isoleucyl analog



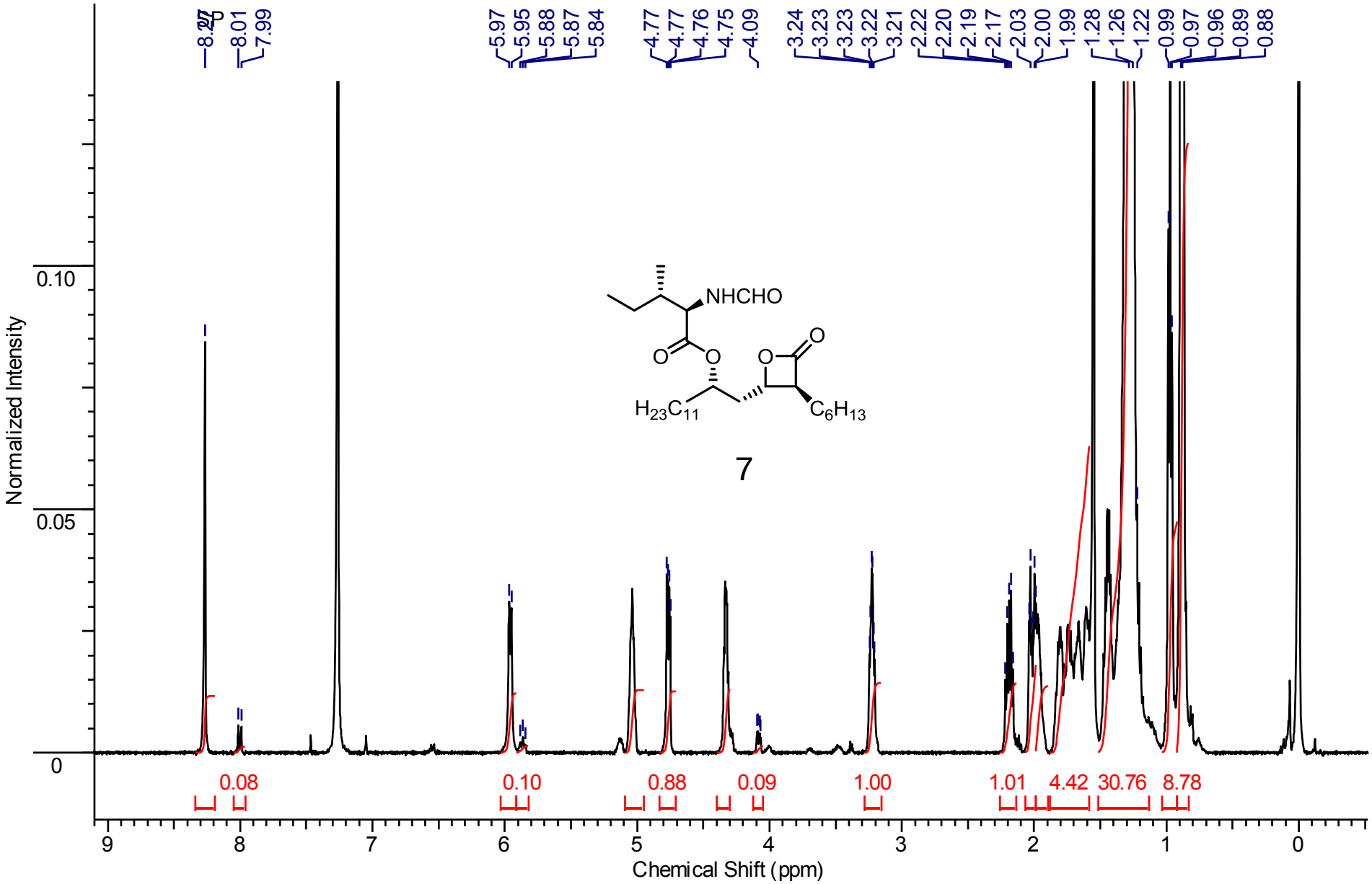
(5) *N*-formyl-**L**-*allo*-isoleucyl analog



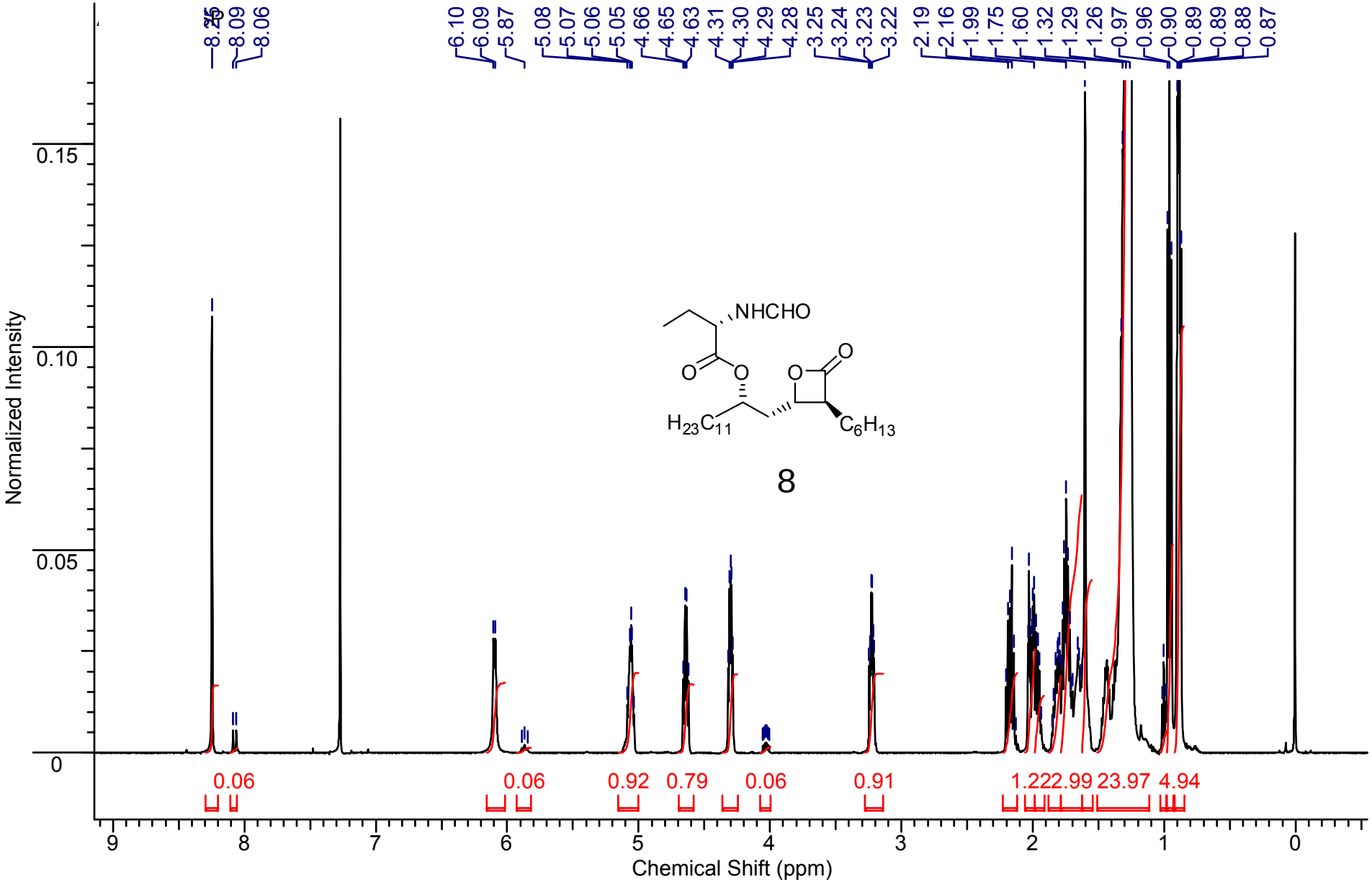
(6) *N*-formyl-**D**-isoleucyl analog



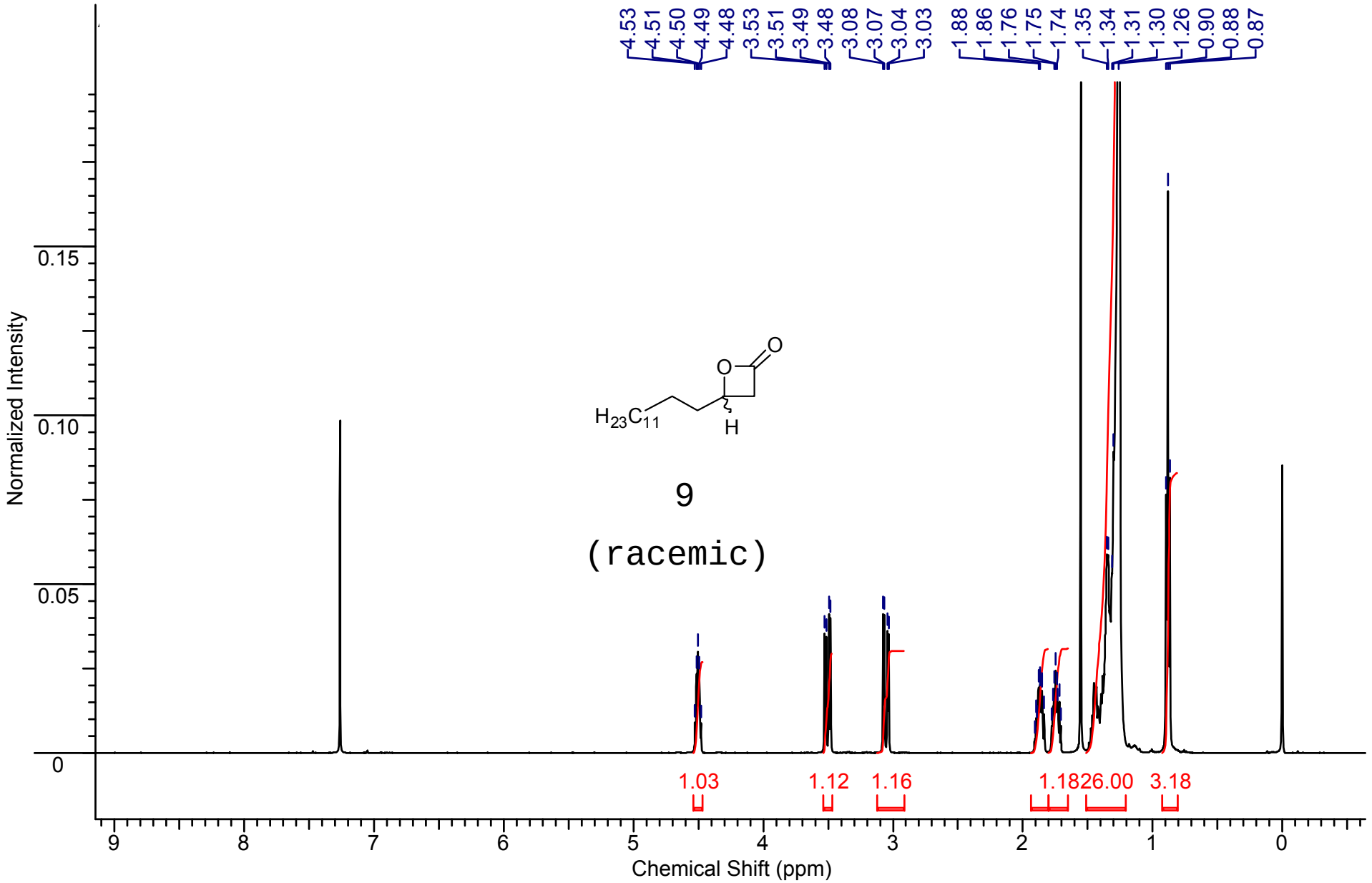
(7) *N*-formyl-**D**-*allo*-isoleucyl analog



(8) (*S*)-*N*-formyl-α-aminobutyryl analog



(9) β-lactone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4.51
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3.53
3.51
3.49
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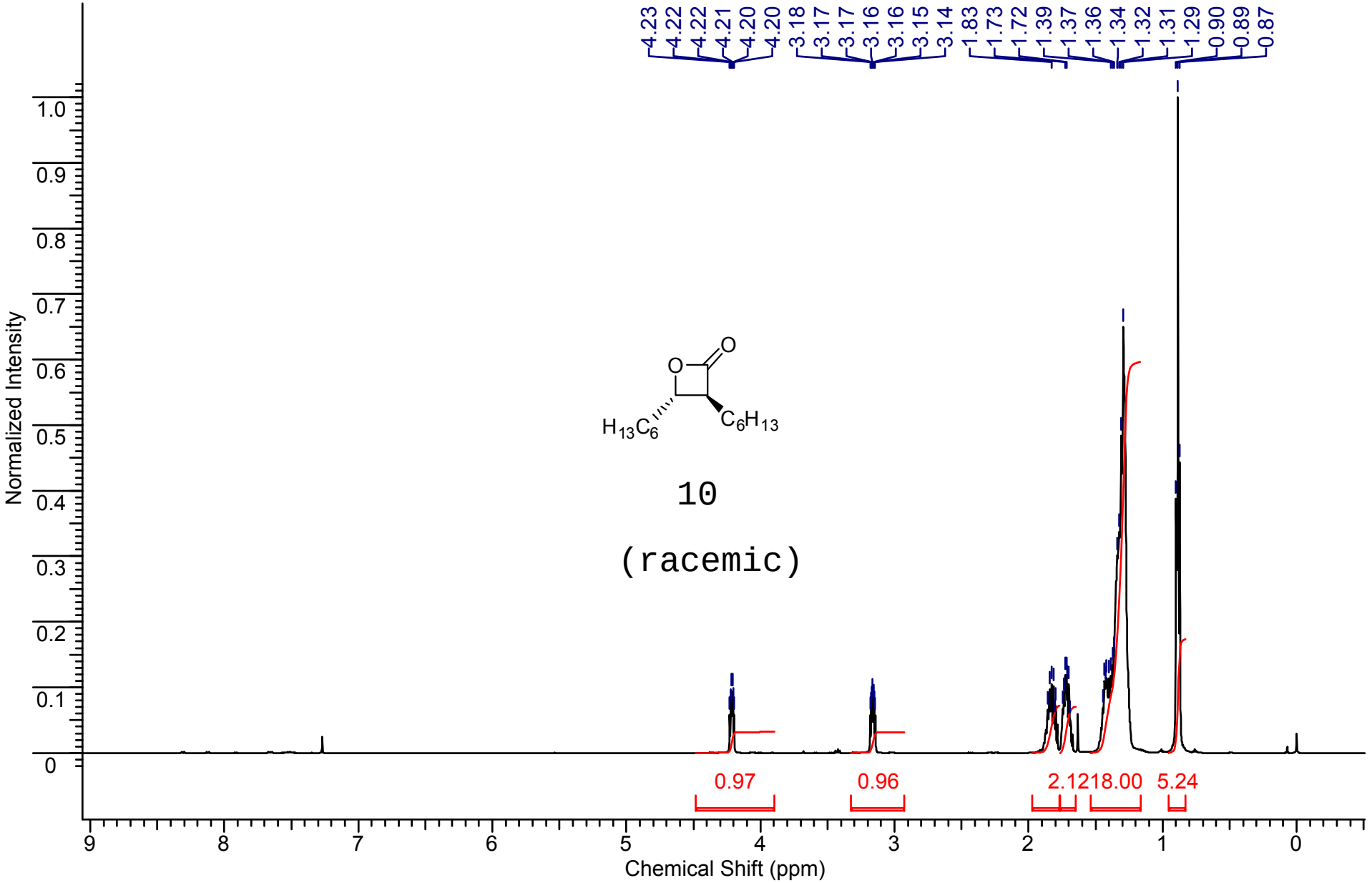
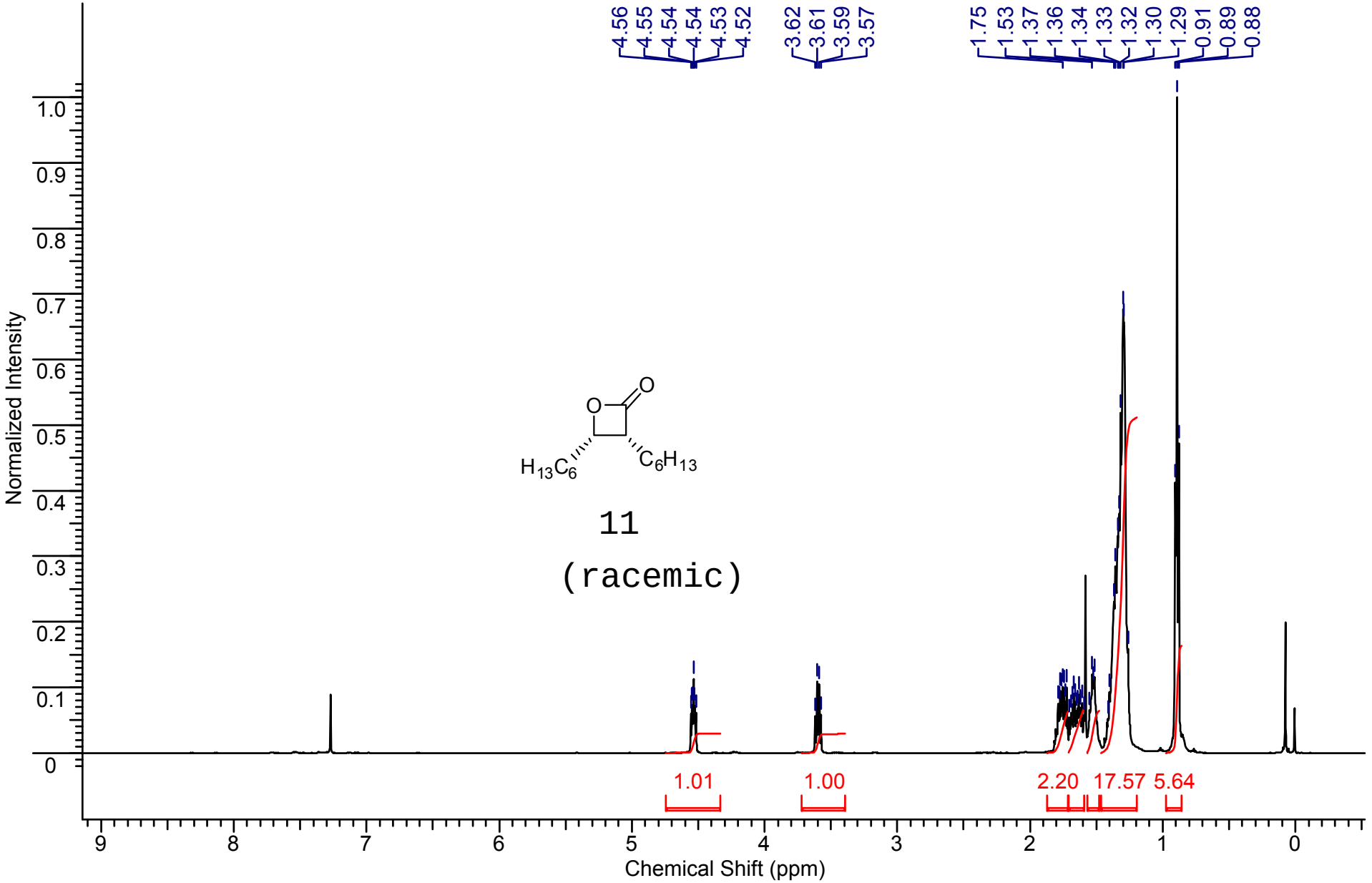
3.08
3.07
3.04
3.03
1.88
1.86
1.76
1.75
1.74
1.35
1.34
1.31
1.30
1.26
0.90
0.88
0.87
O
O
H23C11
H
9
(racemic)
Normalized Intensity
0.15
0.10
0.05
0
1.03
1.12
1.16
1.18
26.00
3.18
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

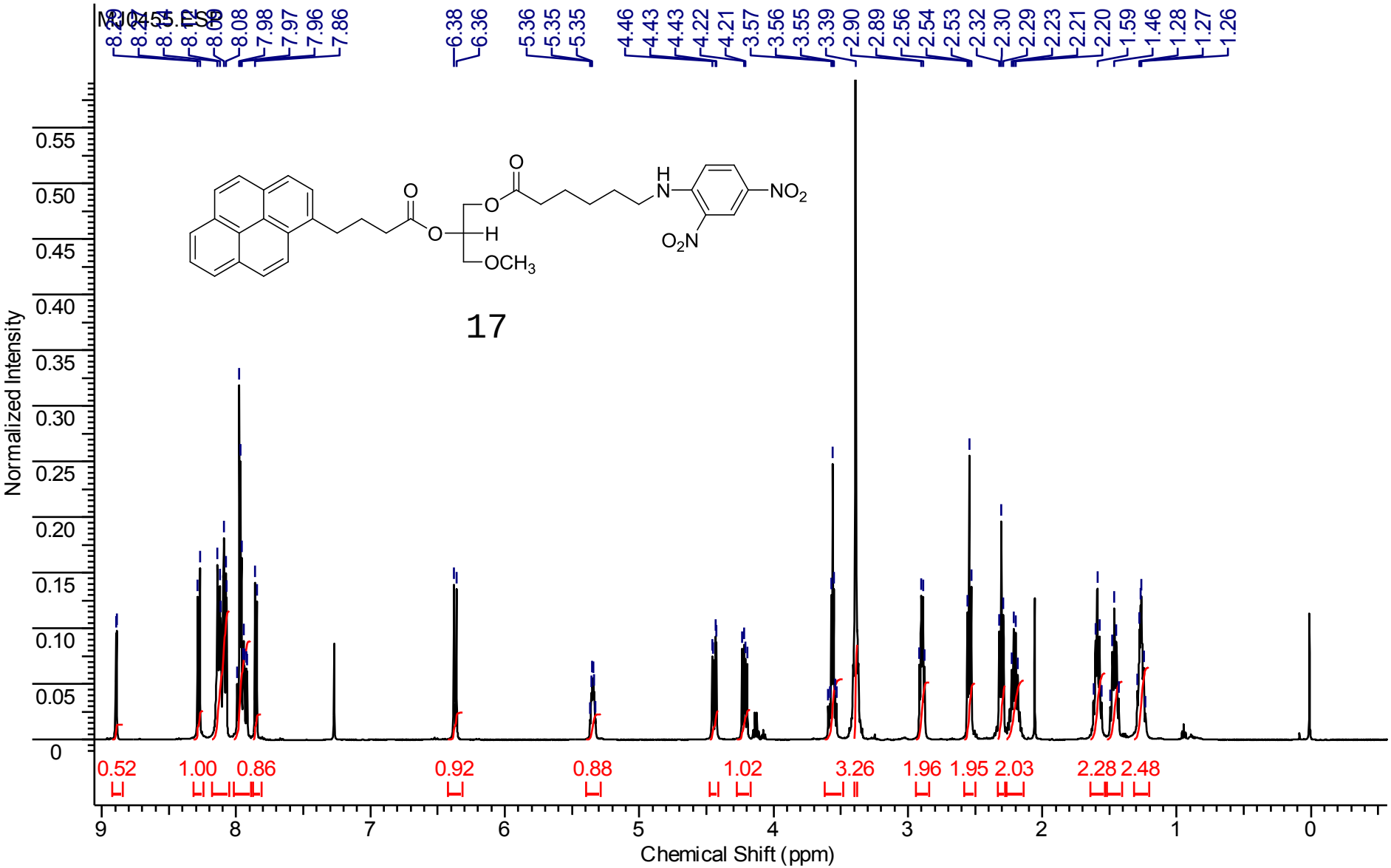
(10) trans β-lactone



(11) cis β-lactone



(17) 1-DNP-2-pyrenyl ether lipid



(18) 1-pyrenyl-2-DNP ether lipid