

Supporting Information (12 pages)

Design and synthesis of fluorescence-labeled *closo*-dodecaborate lipid: Its liposome formation and *in vivo* imaging targeting to tumor for boron neutron capture therapy

*Hiroyuki Nakamura**, *Noriko Ueda*, *Hyun Seung Ban*, *Manabu Ueno* and *Shoji Tachikawa*

*Department of Chemistry, Faculty of Science, Gakushuin University, 1-5-1 Mejiro, Toshima-ku,
Tokyo 171-8588, Japan*

E-mail: hiroyuki.nakamura@gakushuin.ac.jp

List of Contents

1. Biodistribution of FL-SBL and FL-SBL-labeled DSPC liposomes in HeLa cells	S2
2. ¹ H NMR Spectrum of (R)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl stearate	S3
3. ¹ H NMR Spectrum of compound (2)	S4
4. ¹ H NMR Spectrum of compound (3)	S5
5. ¹ H NMR Spectrum of compound (4)	S6
6. ¹ H NMR Spectrum of compound (5)	S7
7. ¹ H NMR Spectrum of compound (6)	S8
8. ¹ H NMR Spectrum of compound (8)	S9
9. ¹ H NMR Spectrum of FL-SBL	S10
10. HRMS Spectra of compounds 4-6, 8, and FL-SBL	S11
11. Size distributions of liposomes	S12

Biodistribution of FL-SBL and FL-SBL-labeled DSPC liposomes in HeLa cells

HeLa cells were treated with FL-SBL (the lipid alone) or FL-SBL-labeled DSPC liposomes. After 3 h incubation with FL-SBL or the liposomes, the cells were washed with PBS and FL-SBL or the FL-SBL-labeled DSPC liposomes were detected with a fluorescence confocal microscope. Fluorescence images of FL-SBL and the FL-SBL-labeled liposomes in HeLa cells are shown in Figure S1. Figures S1A-C show the fluorescence images of FL-SBL in plasma membrane, indicating that FL-SBLs are incorporated not into the cell cytosome but into the cell membrane. Figure S1D shows the dispersion image of the FL-SBL-labeled liposomes in PBS. As shown in Figure S1E, small fluorescence dots were observed in the cytosome and the retention of FL-SBL in the plasma membrane, which may have been a result of the degradation of liposomes, was not observed, indicating that the FL-SBL-labeled liposomes are sufficiently stable to be transported into the cells via the cell membrane.

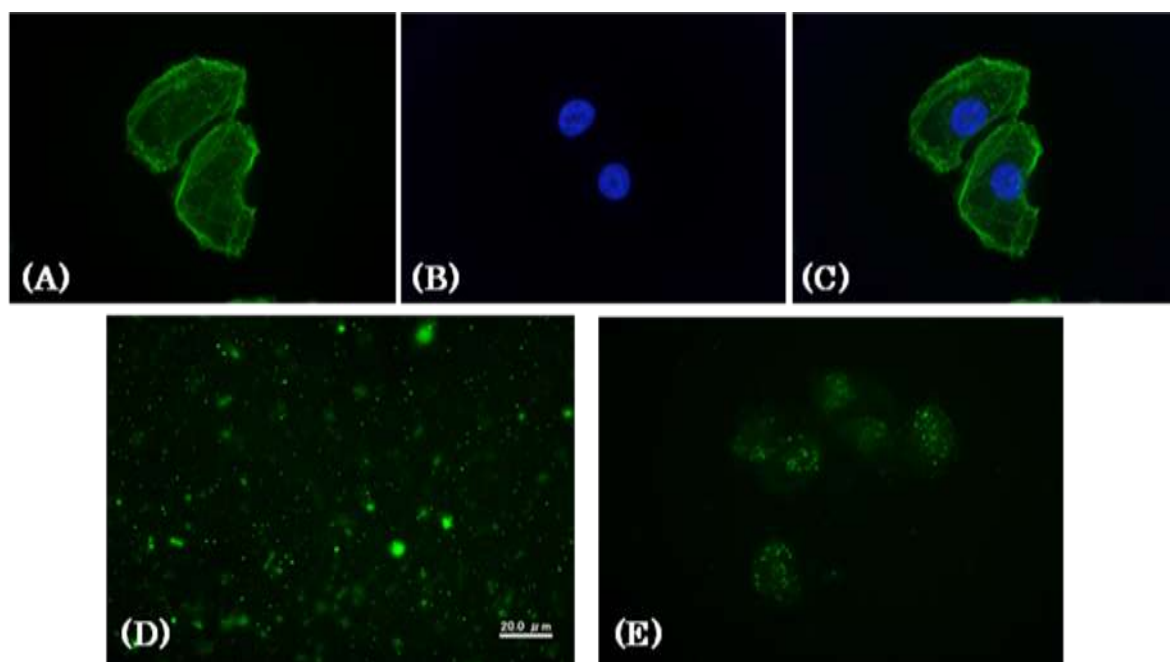


Figure S1. Confocal microscopy images of FL-SBL and FL-SBL-labeled liposomes in HeLa cells. (A) FL-SBL was incubated at 37°C for 3 h in HeLa cells. (B) Nucleuses in HeLa cells stained by Hoechst. (C) Merged image of FL-SBL (A) and Hoechst-labeled nucleus (B). (D) FL-SBL-labeled liposomes in PBS in the absence of cells. (E) Intracellular localization of FL-SBL-labeled liposomes in HeLa cells.

(R)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl stearate

$^1\text{H NMR}$ (400 MHz, CDCl_3)

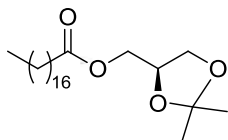
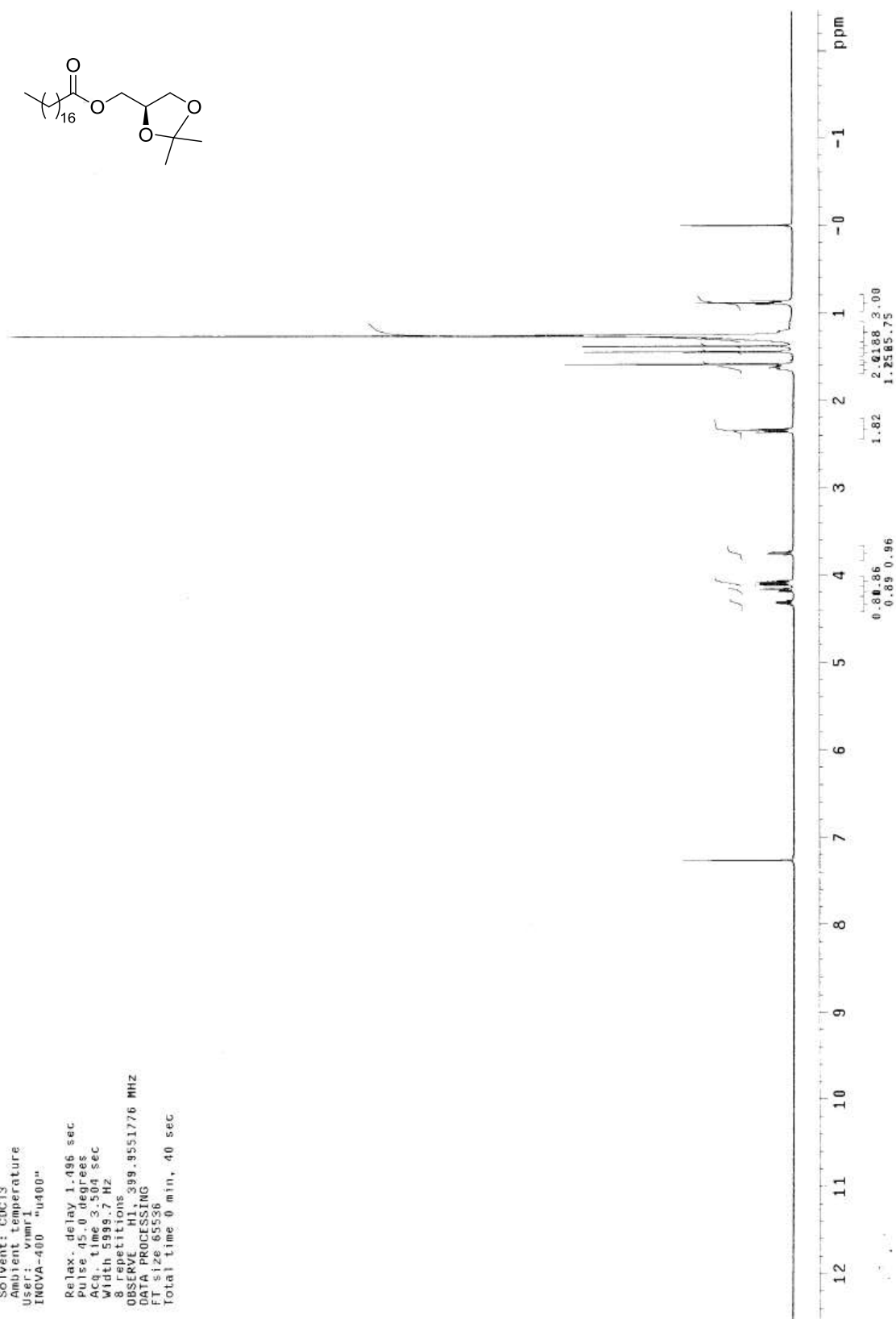
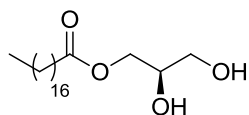


FIG. 4N
STANDARD H1 OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl_3
Ambient temperature
User: vmmr1
INOVA-400 "u400"
Relax. delay 1.496 sec
Pulse 45.0 degrees
Acq. time 3.504 sec
Width 5999.7 Hz
8 repetitions
OBSERVE H1, 399.9551776 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 40 sec

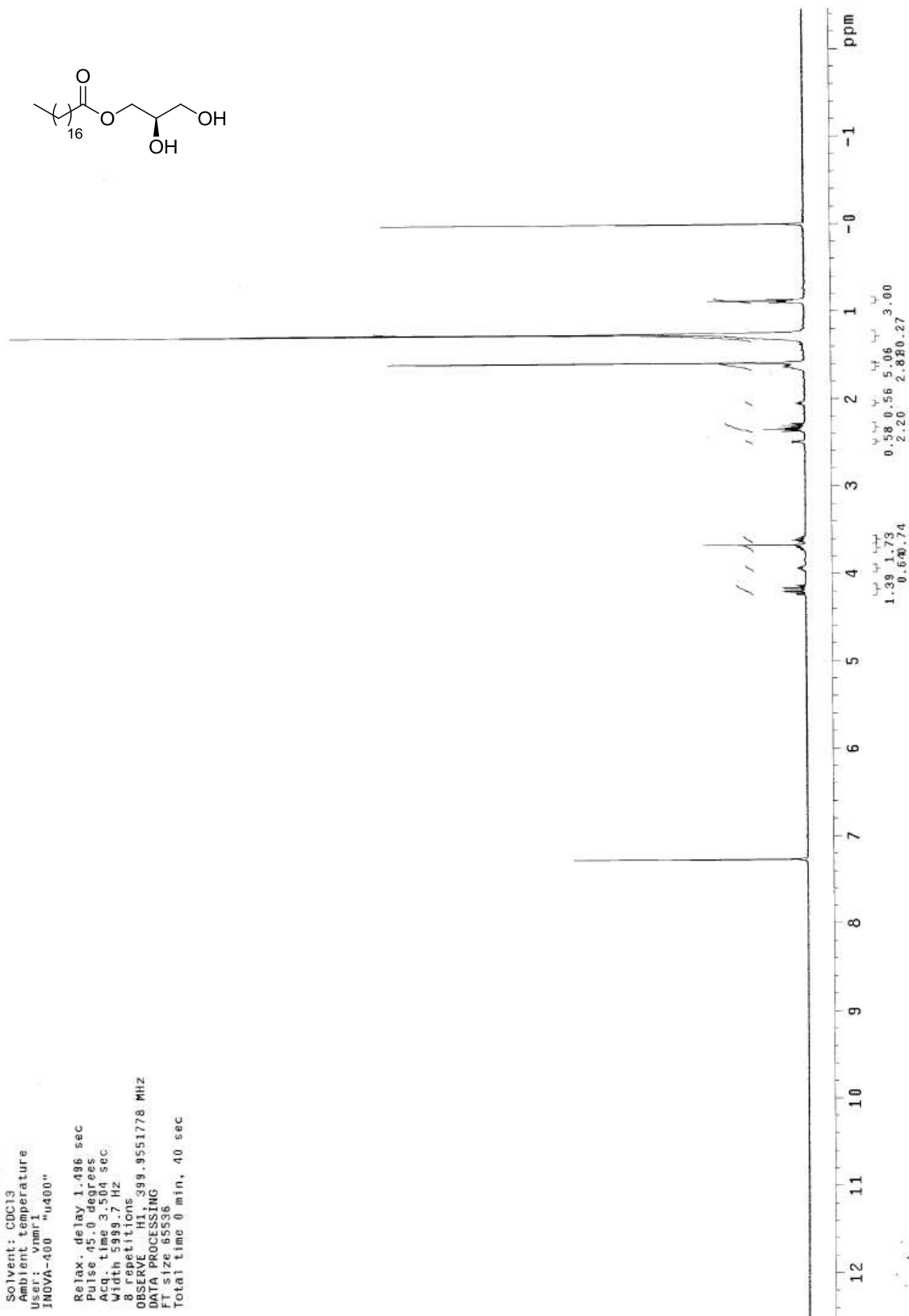


(R)-2,3-Dihydroxypropyl stearate (2)

¹H NMR (400 MHz, CDCl₃)

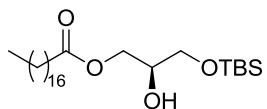


PFG 4IN
STANDARD H1 OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Unit: ppm, "u400"
INOVA-400 "u400"
Relax. delay 1.496 sec
Pulse 45.0 degrees
Acq. time 3.504 sec
Width 5999.7 Hz
8 repetitions
OBSERVE H1 399.9551778 MHz
DATA PROCESSING
F1 size 65536
Total time 0 min, 40 sec

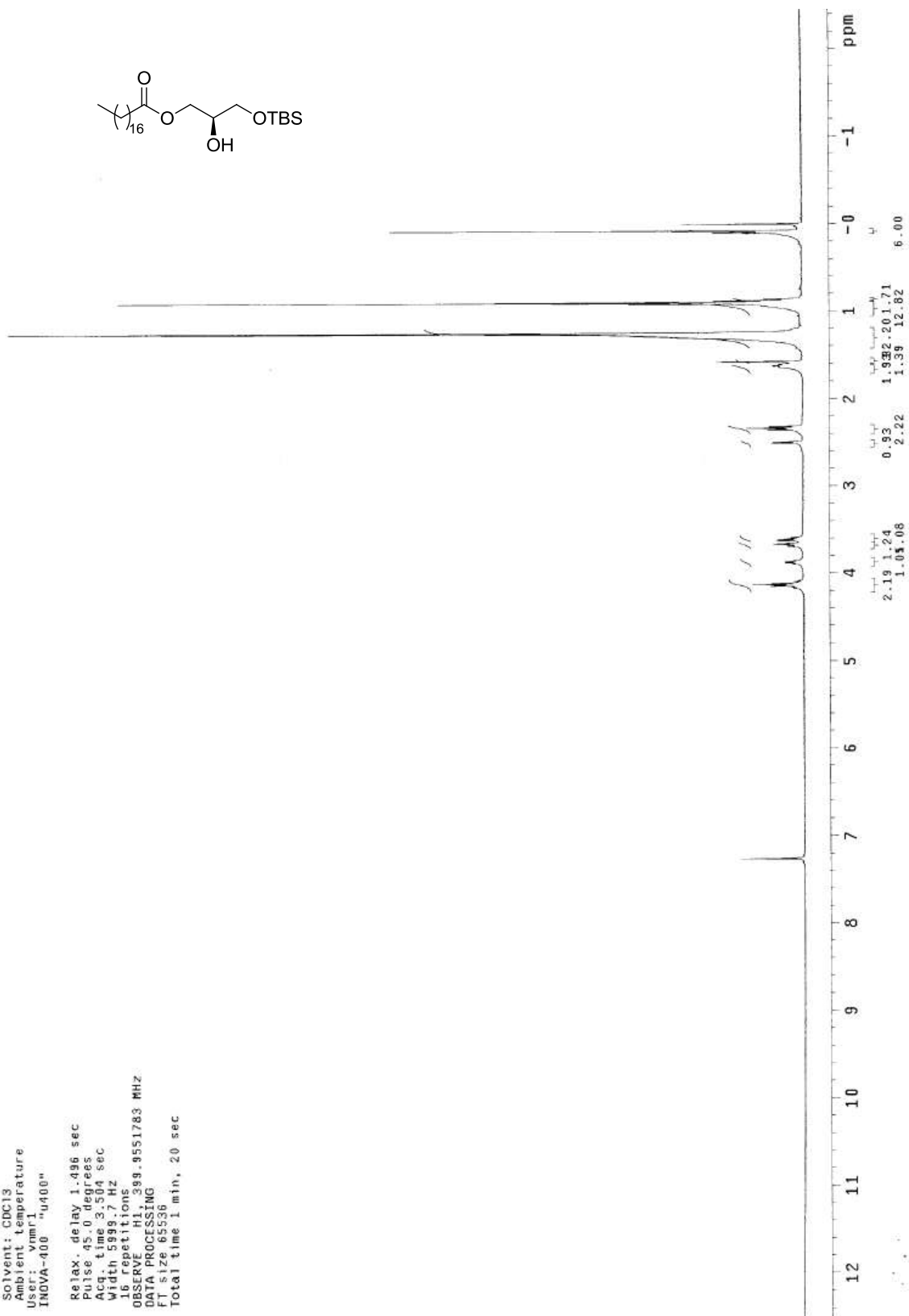


3-O-Stearoyl-1-O-(tert-butyl-dimethyl-silanyl)-sn-glycerol (3)

^1H NMR (400 MHz, CDCl_3)

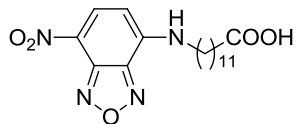


PF1_4N
STANDARD H1 OBSERVE
Pulse Sequence: s2pu1
Solvent: CDCl_3
Ambient temperature
User: vmmr1
INOVA-400 "u400"
Relax. delay 1.496 sec
Pulse 15.0 degrees
Acq. time 5.534 sec
Width 9997.442
SFO 400.146361 MHz
OBSERVE F1 399.9551783 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 20 sec

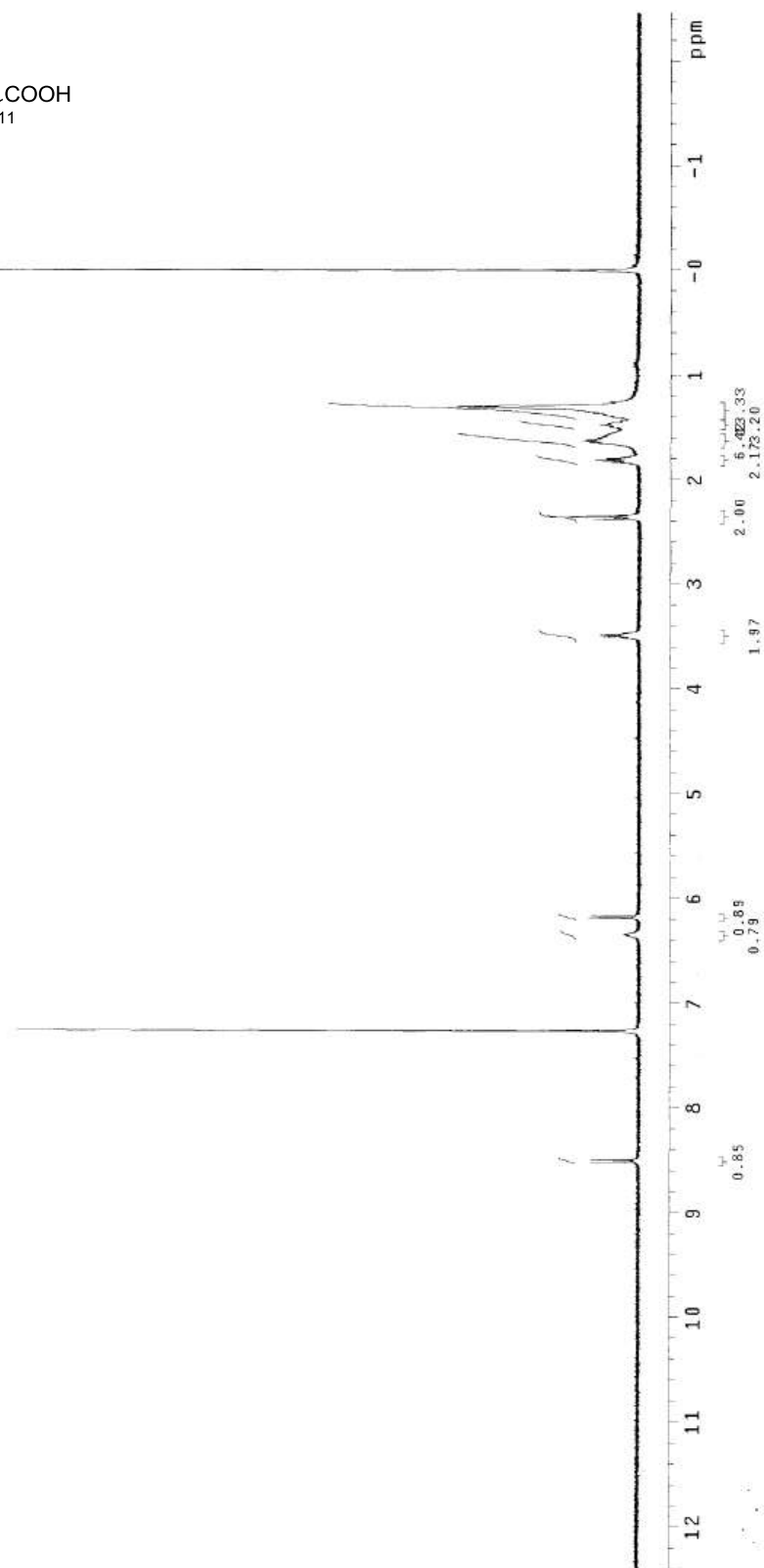


12-(7-Nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)dodecanoic acid (4)

¹HNMR (400 MHz, CDCl₃)

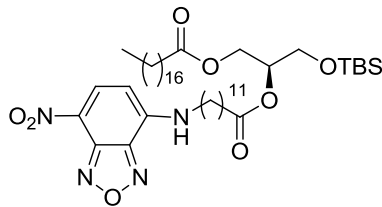


PGC 4N
STANDARD H1 OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient Temperature
User: vmmr1
INDVA-400 "u400"
Relax. delay 1.496 sec
Pulse 45.0 degrees
Acq. time 3.504 sec
Width 5999.7 Hz
8 repetitions
OBSERVE H1, 399.9551783 MHZ
DATA PROCESSING
FT size 65536
Total time 0 min, 40 sec

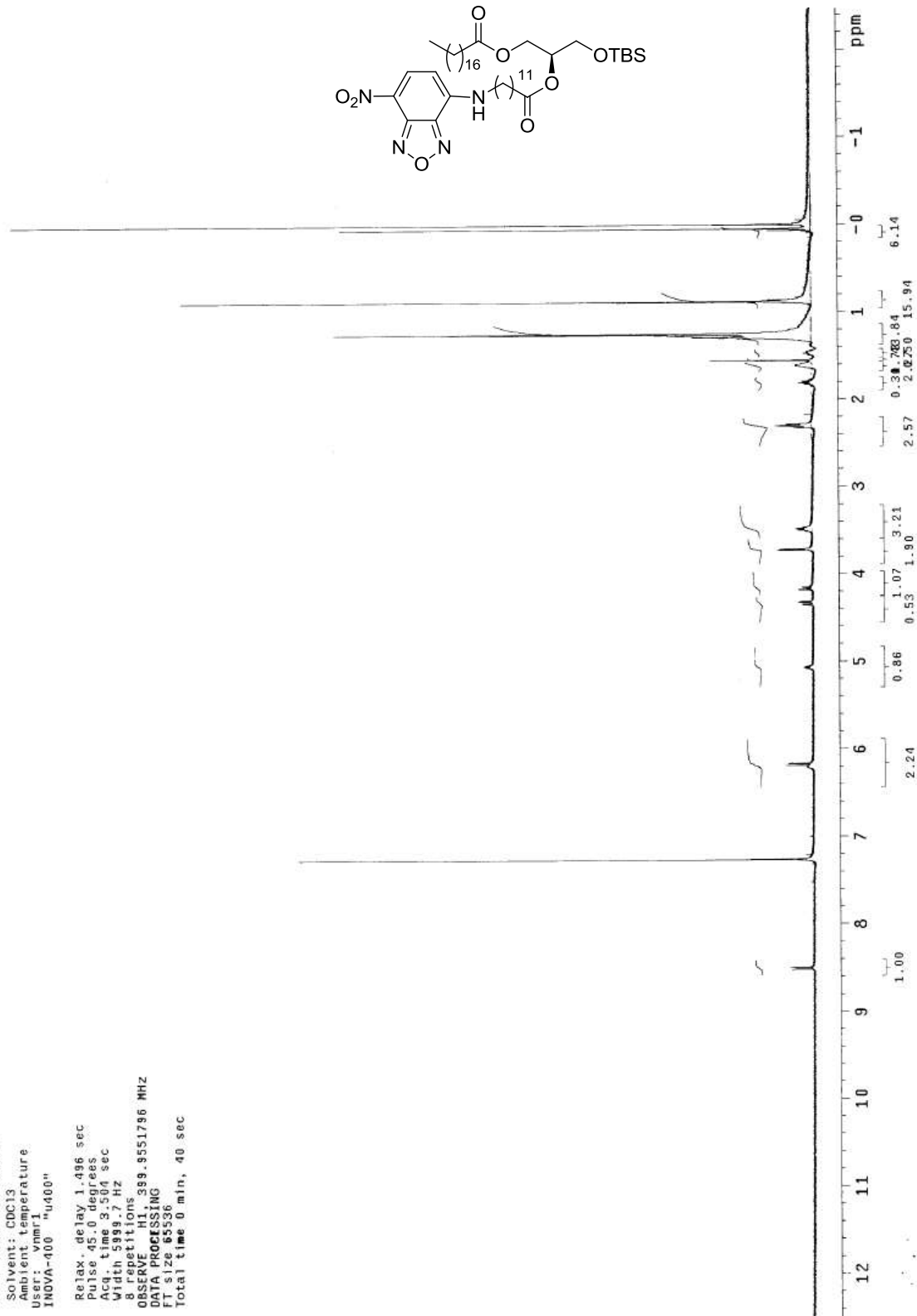


**(S)-3-(tert-Butyldimethylsilyloxy)-2-(12-(7-nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)dodecanoyl
oxy)propyl stearate (5)**

¹HNMR (400 MHz, CDCl₃)

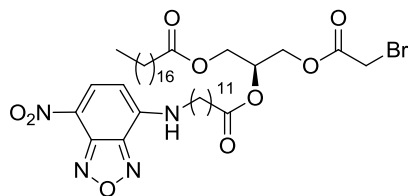


PTG_4N
STANDARD_H1_OBSERVE
Pulse Sequence: s2pu1
Solvent: CDCl3
Ambient temperature
User: vmmr1
INOVA-400 "H,400"
Relax. delay 1.496 sec
Pulse 45.0 degrees
Acq. time 3.504 sec
Width 5999.7 Hz
8 repetitions
OBSERVE_H1, 399.9551796 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 40 sec

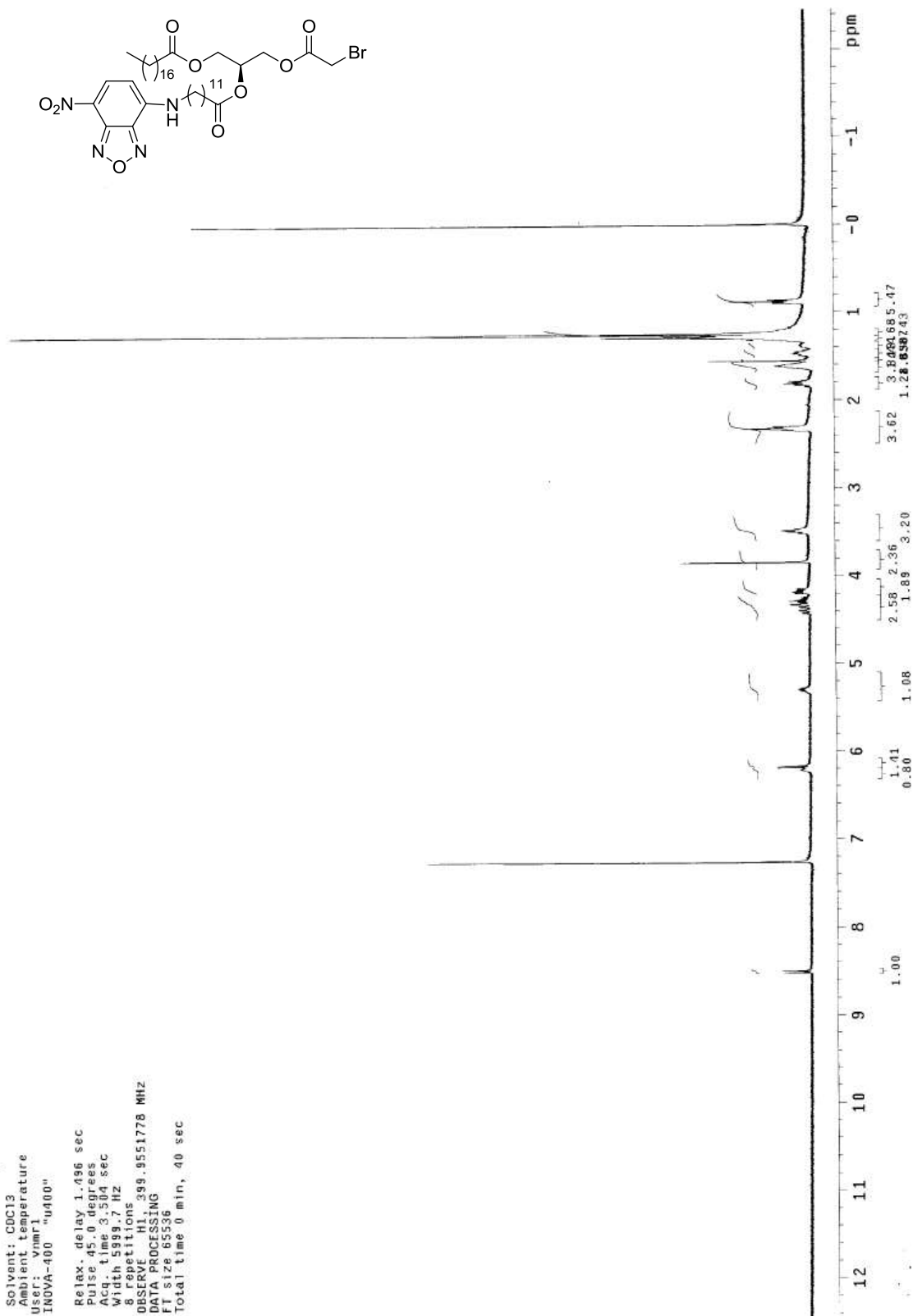


(S)-3-(2-Bromoacetoxy)-2-(12-(7-nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)dodecanoyloxy)propyl stearate (6)

¹HNMR (400 MHz, CDCl₃)

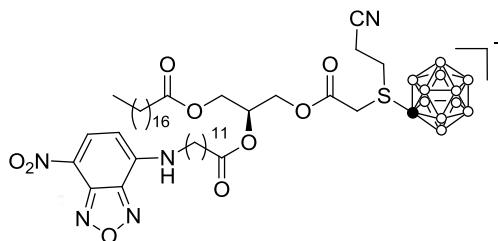


PF0_4N
STANDARD H1 OBSERVE
Pulse Sequence: s2pu1
Solvent: CDCl3
Ambient temperature
User: vnmr1
INOVA-400 "u400"
Relax.. delay 1.496 sec
Pulse 45.0 degrees
Acq. time 3.504 sec
Width 5333.7 Hz
OBSERVE time 399.9551778 MHz
DATA PROCESSING
F1 size 65536
Total time 0 min, 40 sec

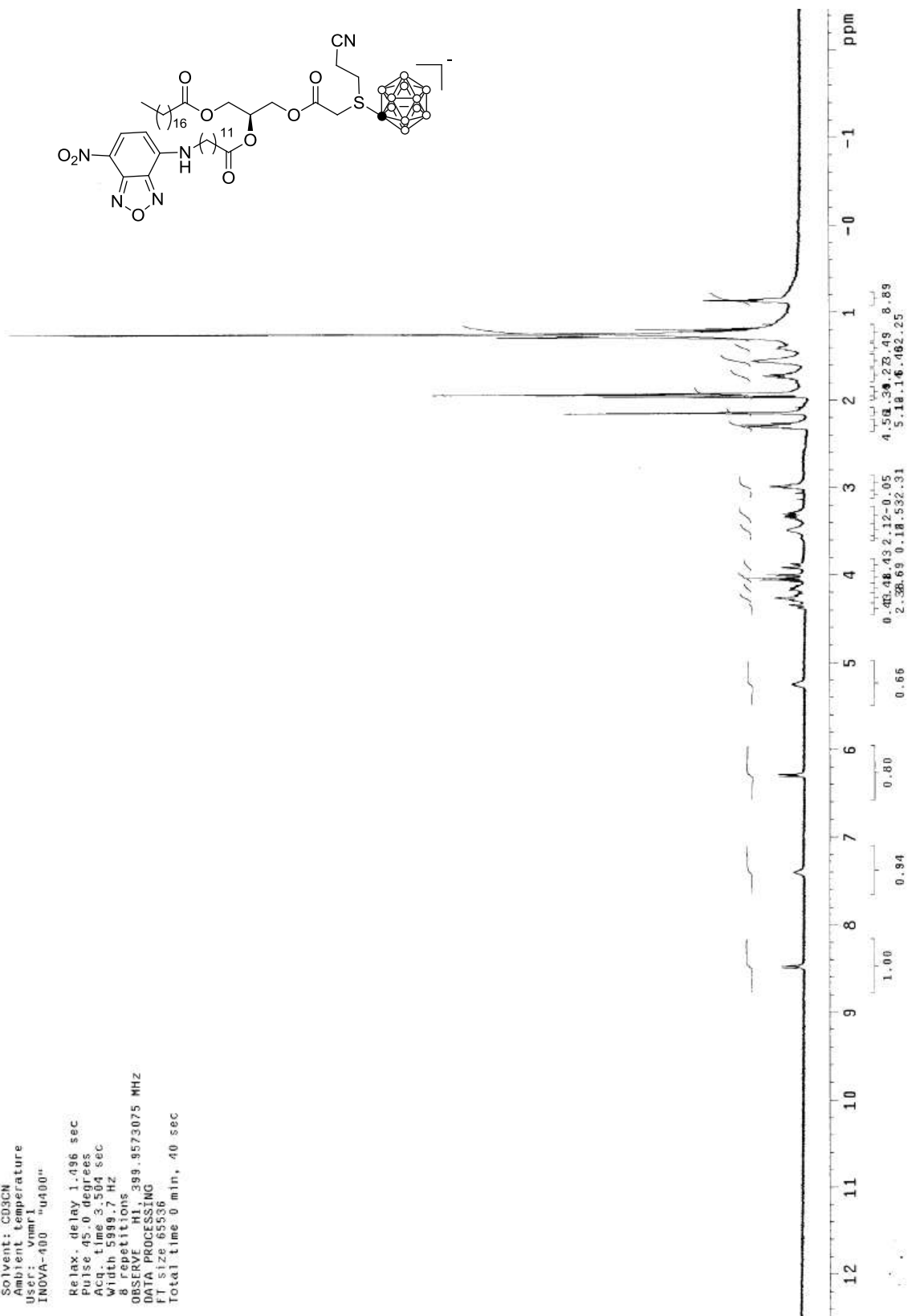


Compound 8

$^1\text{H NMR}$ (400 MHz, CDCl_3)

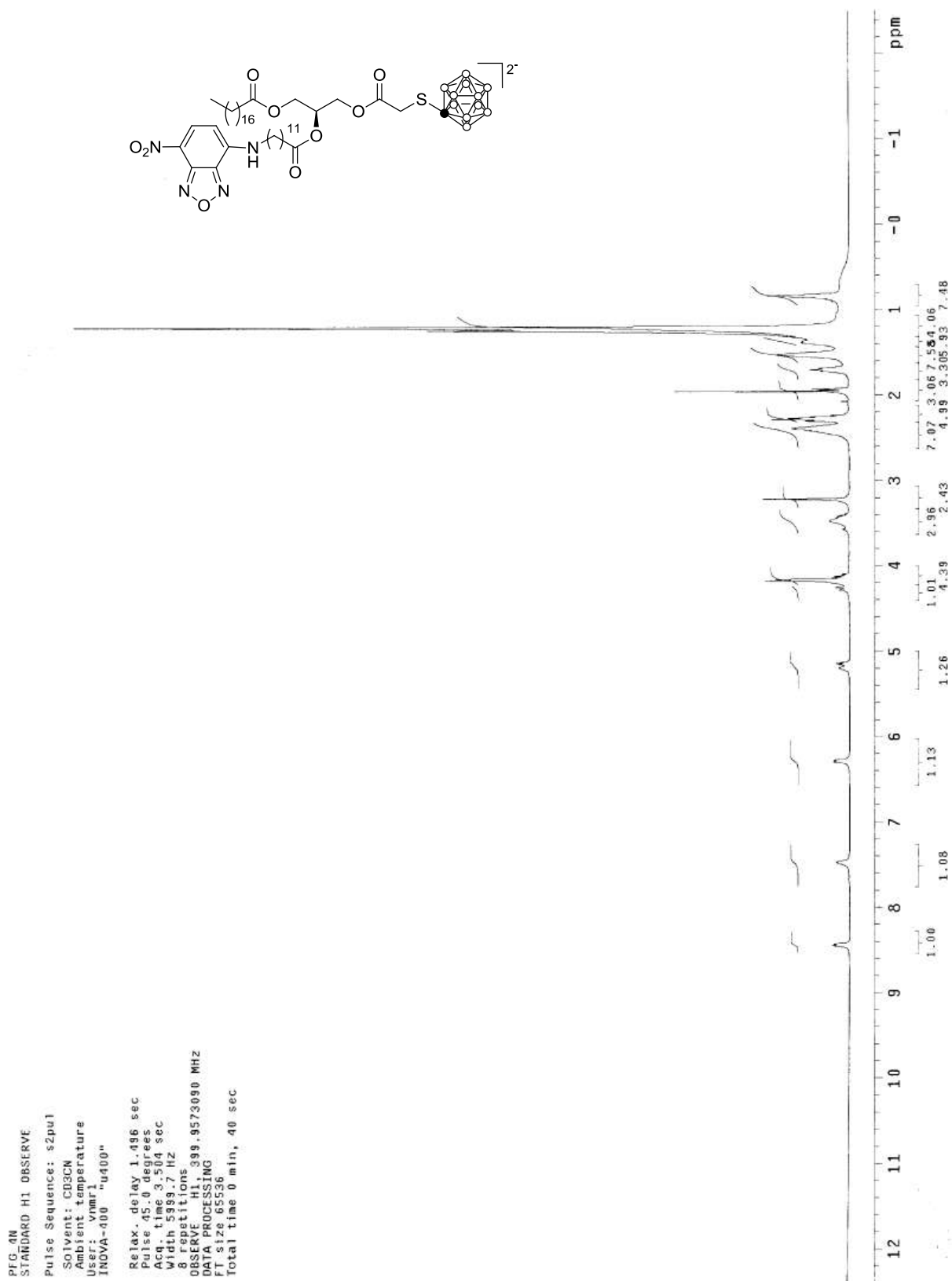


PEG_4N
STANDARD H1 OBSERVE
Pulse Sequence: s2pu1
Solvent: CD3CN
Ambient Temperature
User: vnmr1
INOVA-400 "q400"
Relax: delay 1.496 sec
Pulse: q5.0 degrees
Acq: 1.0003504 sec
Width: 5997.7 Hz
& width 5997.7 Hz
OBSERVE F1: 399.9573075 MHz
DATA PROCESSING
FT SIZE 65536
Total time 0 min, 40 sec

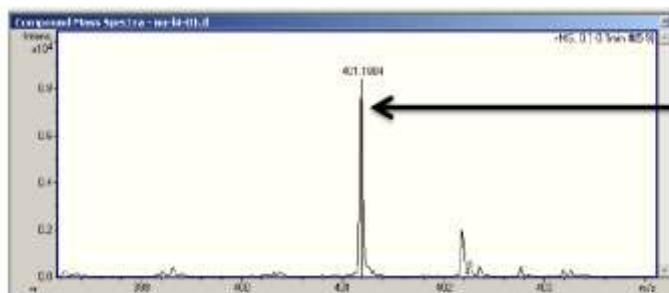


Fluorescent-labeled *closo*-dodecaborane lipid (FL-SBL).

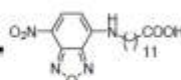
^1H NMR (400 MHz, CDCl_3)



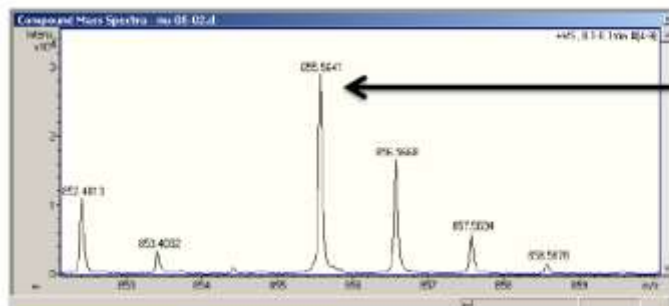
HRMS Spectra of compounds 4, 6, 8, and FL-SBL



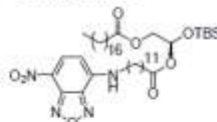
Compound 4



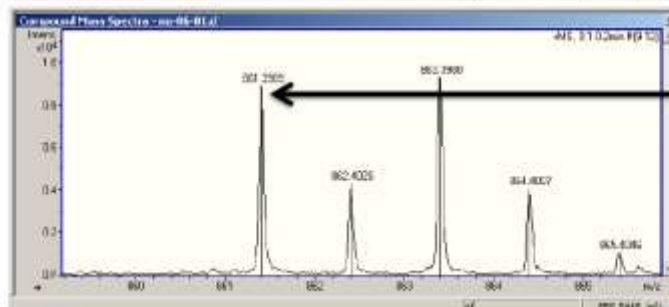
HRMS (ESI, positive) m/z calcd. for $C_{18}H_{26}N_4O_5$
 $[M+Na]^+$: 401.1801, found: 401.1804



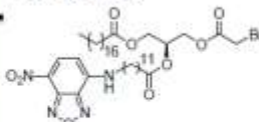
Compound 5



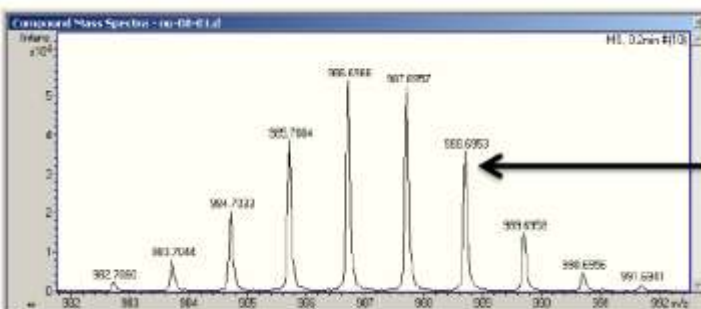
HRMS (ESI, positive) m/z calcd. for $C_{45}H_{80}N_4O_8Si$
 $[M+Na]^+$: 855.5643, found: 855.5641



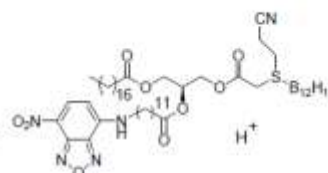
Compound 6



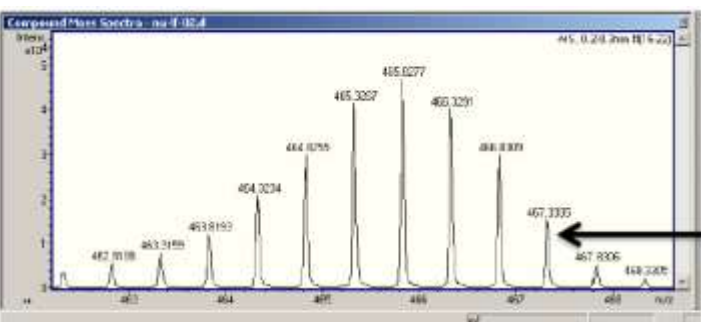
HRMS (ESI, positive) m/z calcd. for $C_{41}H_{67}BrN_4O_9$
 $[M+Na]^+$: 861.3989, found: 861.3989



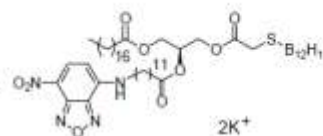
Compound 8



HRMS (ESI, negative) m/z calcd. for
 $C_{45}H_{83}B_{12}N_5O_9S$ $[M-H]^-$: 988.6950, found: 988.6953



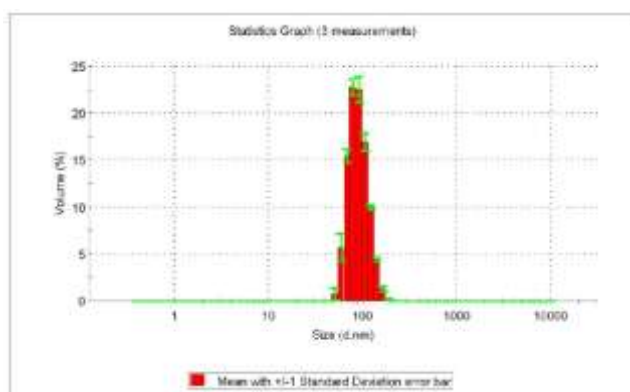
FL-SBL



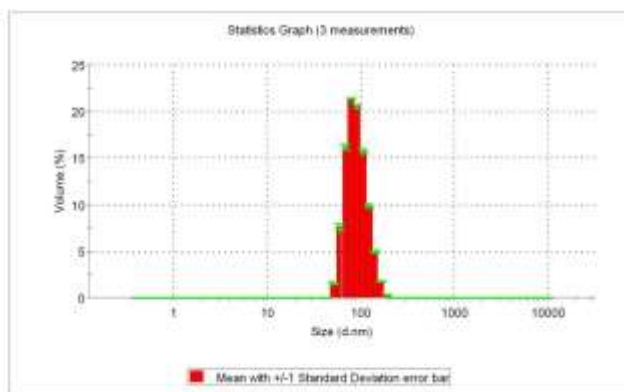
HRMS (ESI, negative) m/z calcd. for $C_{41}H_{78}B_{12}K_2N_4O_9S$
 $[(M-2K)/2]^-$: 467.3303, found: 467.3305

Size distributions of liposomes

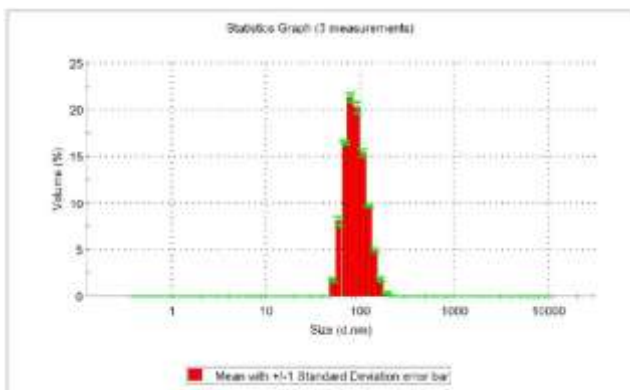
FL-SBL (0 μL , B/P = 0)



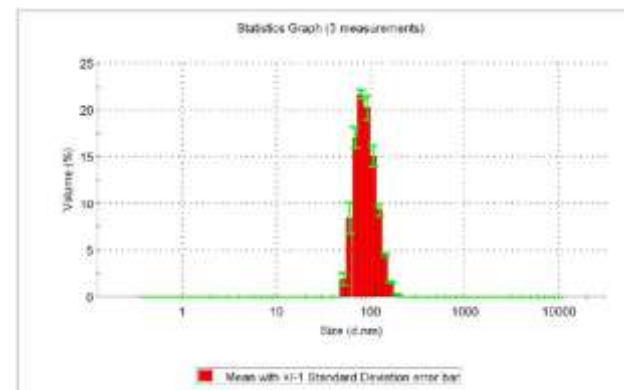
FL-SBL (10 μL , B/P = 1.9)



FL-SBL (20 μL , B/P = 3.4)



FL-SBL (30 μL , B/P = 5.4)



FL-SBL (40 μL , B/P = 7.1)

