Electronic Supplementary Material (ESI) for Polymer Chemistry.
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Supporting Information

for

Cholesterol Functionalized Aliphatic *N*-Substituted 8-Membered Cyclic Carbonate

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Experimental section:

Materials

All reagents were purchased from Sigma-Aldrich or TCI or Merck and unless otherwise specified, were used as received. Solvents were of ACS or HPLC grade and unless specifically mentioned, used as received. Catalyst, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), was distilled twice from CaH_2 under dry N_2 and stored in the glove box. Macroinitiators, mPEG-OH, were purchased from RAAP Polymere GmbH (Tuebingen, Germany) or Polymer Source Inc. (Quebec, Canada). Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[mPEG-5000] and (DPPE-PEG)Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) were purchased in powder form from Avanti Lipids, Inc (USA). All

monomers and reagents were dried extensively by freeze drying process under high vacuum prior to transfer into the glove box.

Methods

The ^{1}H- and ^{13}C- nuclear magnetic resonance (NMR) spectra were recorded using a Bruker Avance 400 spectrometer. Size exclusion chromatography (SEC) was recorded at 35 °C on a Waters 2695D (Waters Corporation, U.S.A.) separation module connected with an Optilab rEX differential refractometer (Wyatt Technology Corporation, U.S.A.) and data processed with Astra software (Wyatt Technology Corporation, U.S.A.; version 5.3.4.20). Tetrahydrofuran was used as the eluent (flow rate of 1.0 mL/min) and Waters HR-4E as well as HR 1 columns (Waters Corporation, U.S.A.). Transmission electron microscopy (TEM) was conducted on FEI Tecnai G2 F20 operating at an acceleration voltage of 200 keV. Samples for TEM were prepared by first placing 4.0 μL of sample onto TEM grid (200 mesh; formvar-coated copper, Ted Pella Inc., U.S.A.). One minute later, by using a wedge of filter paper, excess solution was removed (*via* capillary action). Phosphotungstic acid (2 % w/v in deionized water; 4.0 μL), the staining agent, was then added. After a minute, the excess solution of staining agent was removed *via* capillary action with a wedge of filter paper and the grid was allowed to further dry under ambient conditions.

Synthesis of cholesteryl bis(2-hydroxyethyl)carbamate (**1**):

In a round bottom flask (RBF, 500 mL), equipped with a magnetic stir bar and N_2 flow inlet, diethanolamine (5.97 g) and Na_2CO_3 (12.52 g) were dissolved in the 1:2 mixture of DI water: THF (150 mL : 75 mL) and the mixture was allowed to equilibrate in an ice-bath for about 15 – 30 min. Cholesteryl chloroformate (22.50 g) was added to the reaction mixture and the reaction was allowed to proceed in ice-cold conditions for 1-2 h, followed by ~ 14 h at room temperature. To the reaction mixture, ethyl acetate (EA, 200 mL) was added and the organic layer was isolated and to the remaining aqueous layer, saturated NaCl aqueous solution (150

mL) was added. The aqueous layer was twice extracted with EA (200 mL) and the organic layers were combined and dried over Na_2SO_4 and the volatiles were removed under vacuuo to yield **1** as a white solid (25.9 g, 99+ %).

Synthesis of cholesteryl 2-oxo-1,3,6-dioxazocane-6-carboxylate (**2**):

In a RBF (500 mL) equipped with a magnetic stir bar and N_2 flow inlet, dodecyl cholesteryl bis(2-hydroxyethyl)carbamate (**1**, 10.05 g) was THF (100 mL) were mixed and allowed to cool in ice-bath for about 15 – 30 min. To this cooled reaction mixture, ethyl chloroformate (7.5 mL) was added and was allowed to equilibrate at ice-cold conditions for another 15 – 30 min. To the cold reaction mixture, triethylamine (11.0 mL) was added through a dropping funnel (over 15 - 30 min). The reaction was allowed to proceed in ice-cold conditions for about 1 – 2 h and then allowed to proceed at room temperature for ~ 8 h. The precipitated triethylamine salts were filtered off and concentrated in vacuuo to result in crude product as off-white solid, that was recrystallized from ~ 1 : 1 dichloromethane : diethylether solvent mixture (100 mL), left overnight in 4 °C fridge, to results in shiny flaky crystals (7.83 g, 74.2 % (crop 1: 6.28 g; crop 2: 1.55 g)).

General conditions for polymer synthesis (3):

Experimental notes on block copolymer synthesis: All polymerization reactions were conducted in a 7-mL vial (with a magnetic stir bar) in a nitrogen-filled glovebox at room temperature. Typically, 5 mol % DBU with respect to monomer, was used as the catalyst. *Representative example (***3j***; Table 1):* Monomer **2** (545 mg, 1002.2 μmol, 19.6 equiv.) and mPEG-OH (macroinitiator, 5.0 kDa, 256 mg, 51.2 μmol, 1.0 equiv.) were dissolved in DCM (~ 2.0 mL). DBU (7.5 μL, 7.6 mg, 50.2 μmol., 1.0 equiv.) was added to this solution. . After about 2 hours, about 30 - 50 mg of benzoic acid was added to quench the reaction. . Polymer was purified by precipitating 3 times into diethylether: hexanes (50:50) mixture (50 mL) and

dried under vacuum until constant mass was achieved, to obtain a white powdery solid (759 mg).

General conditions for block copolymer self-assembly:

Block copolymer **3**, dissolved in THF (1.0 mg/mL) was taken in dialysis membrane (molecular weight cut-off (MWCO) of 1000 Da; Spectra/Por®; U.S.A.) and dialyzed against deionized water (1 L; at room temperature). Water was changed at 3, 18 and 21 hours. Final concentration of polymer solution was ~ 0.4 mg/mL (assuming that there is no polymer loss).

Liposome Preparation:

Liposomes were prepared using the film rehydration and extrusion procedure. A thin film of DPPC was formed by dissolving in and subsequent removal of chloroform (dry N_2 stream followed by 55 °C vacuum oven for 18 h). The thin DPPC film was then rehydrated with deionized water. The aqueous DPPC dispersions were extruded in a sequence of decreasing filter pore sizes (400, 200, and 100 nm, respectively) using an Avanti Mini-Extruder setup maintained at 55 °C. Incorporation of either DPPE-PEG or Polymer **3a** was accomplished through the post-insertion mechanism. The desired polymer was dispersed in water via stirring overnight and then the solutions were heated to 55 °C (with DPPC liposomes still maintained at 55 °C). The solutions (polymer and DPPC) were mixed at an appropriate ratio to achieve 5 % by mol polymer and held at 55 °C with regular agitation for 1 h. All liposomes were analyzed using a Malvern Nano ZS dynamic light scattering (DLS) instrument maintained at 25 °C and equipped with a 532-nm laser. Solutions were analyzed in polystyrene cuvettes and using instrument-standard backscattering detector (173°).

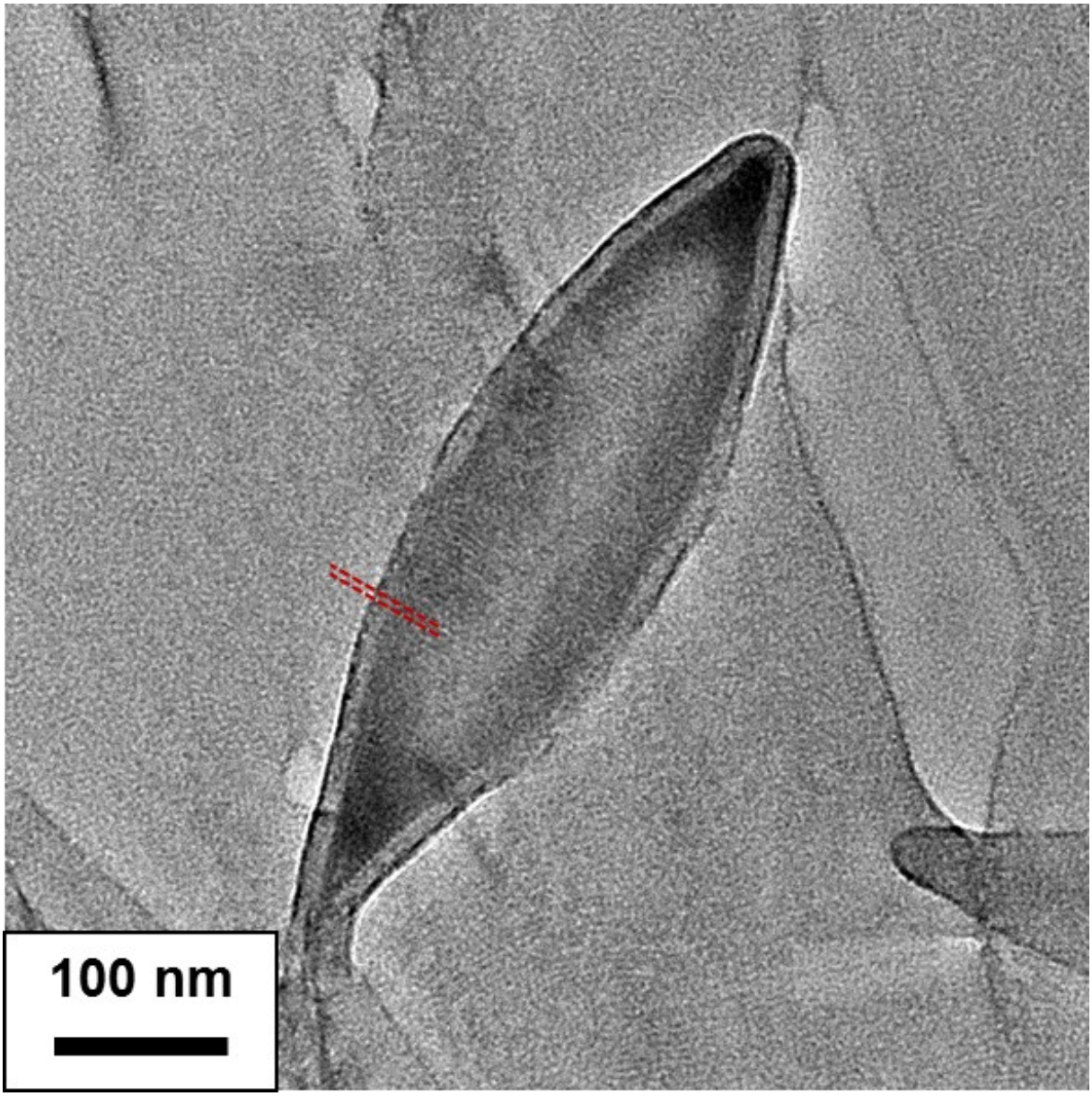
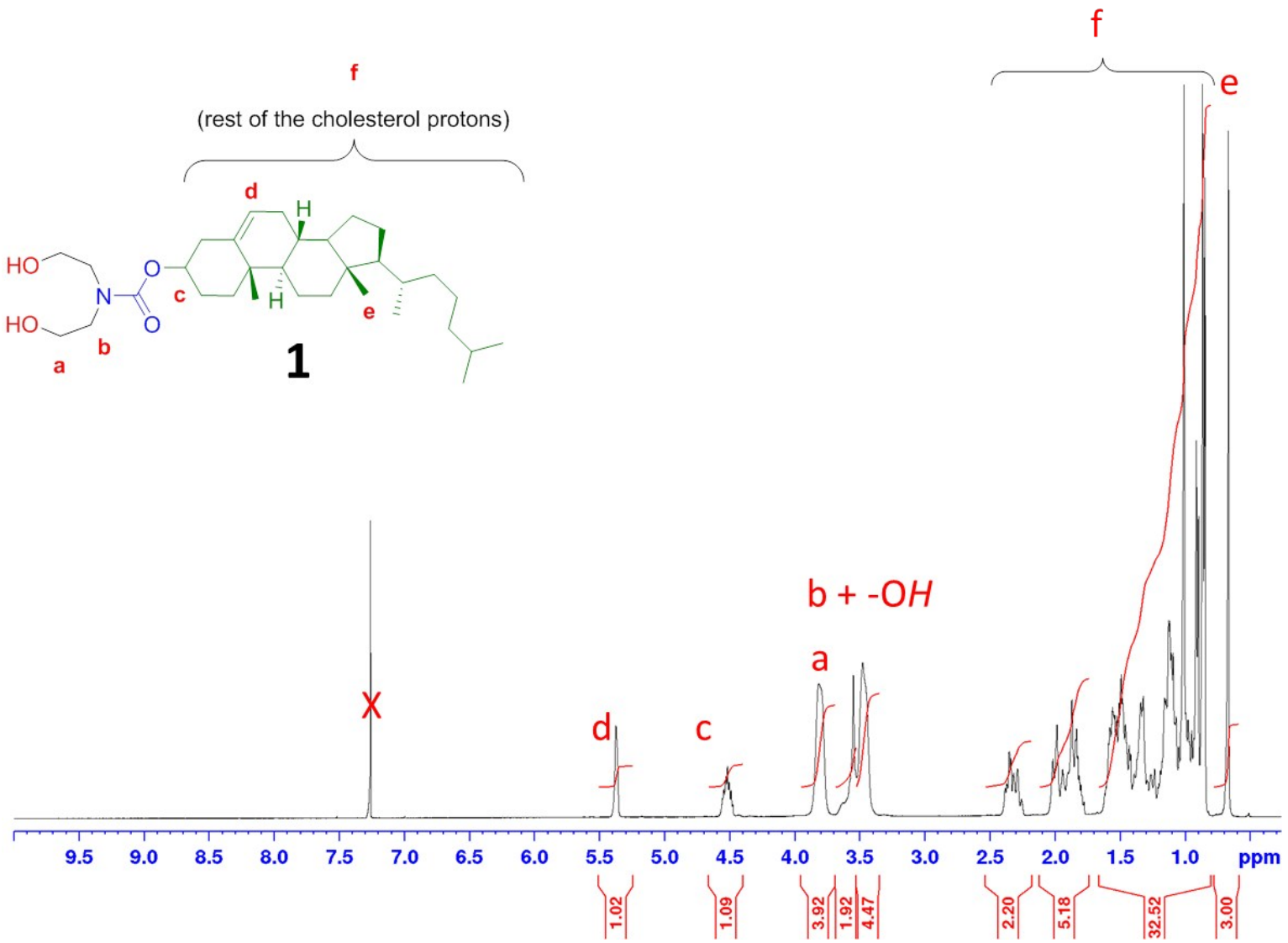
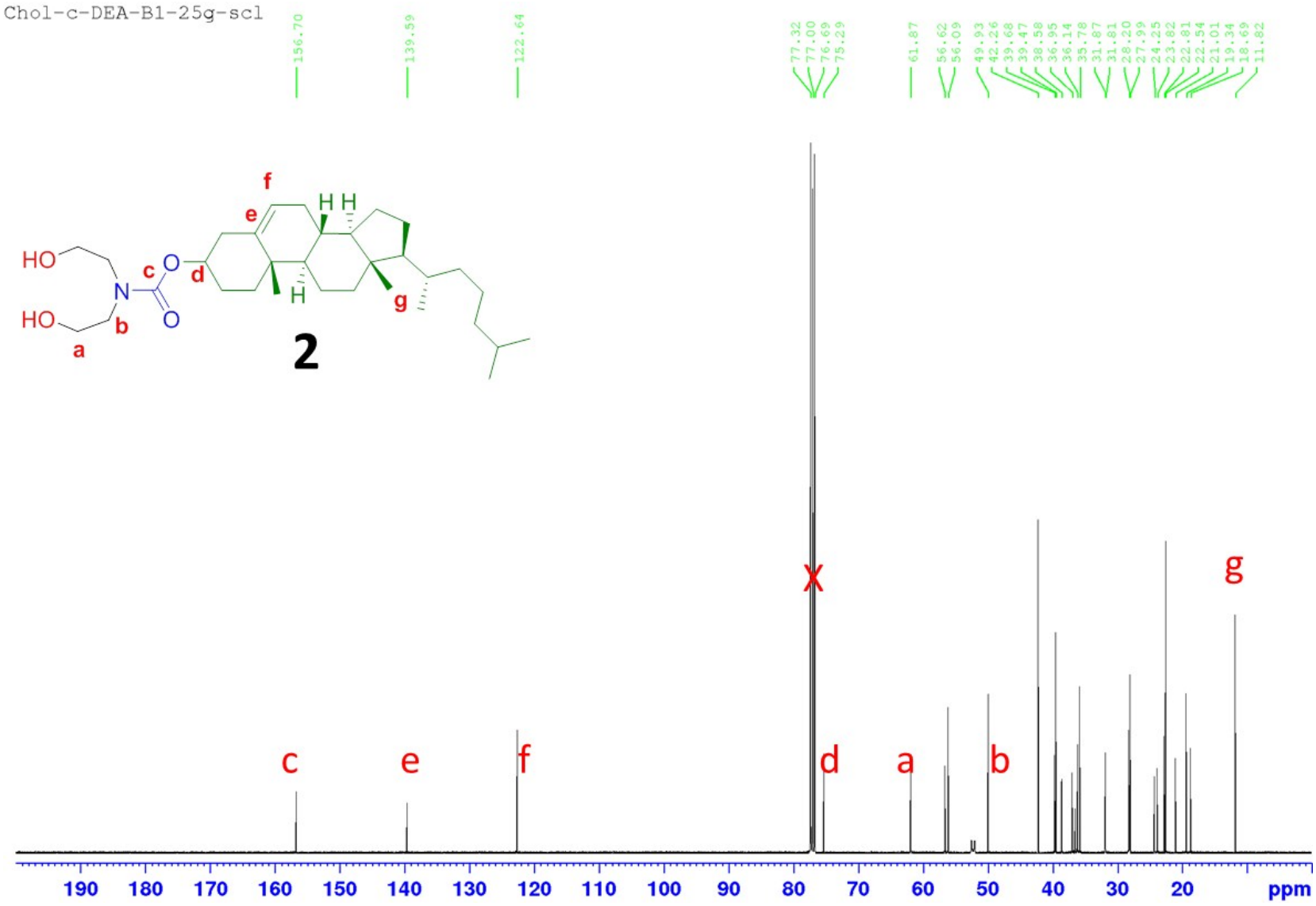
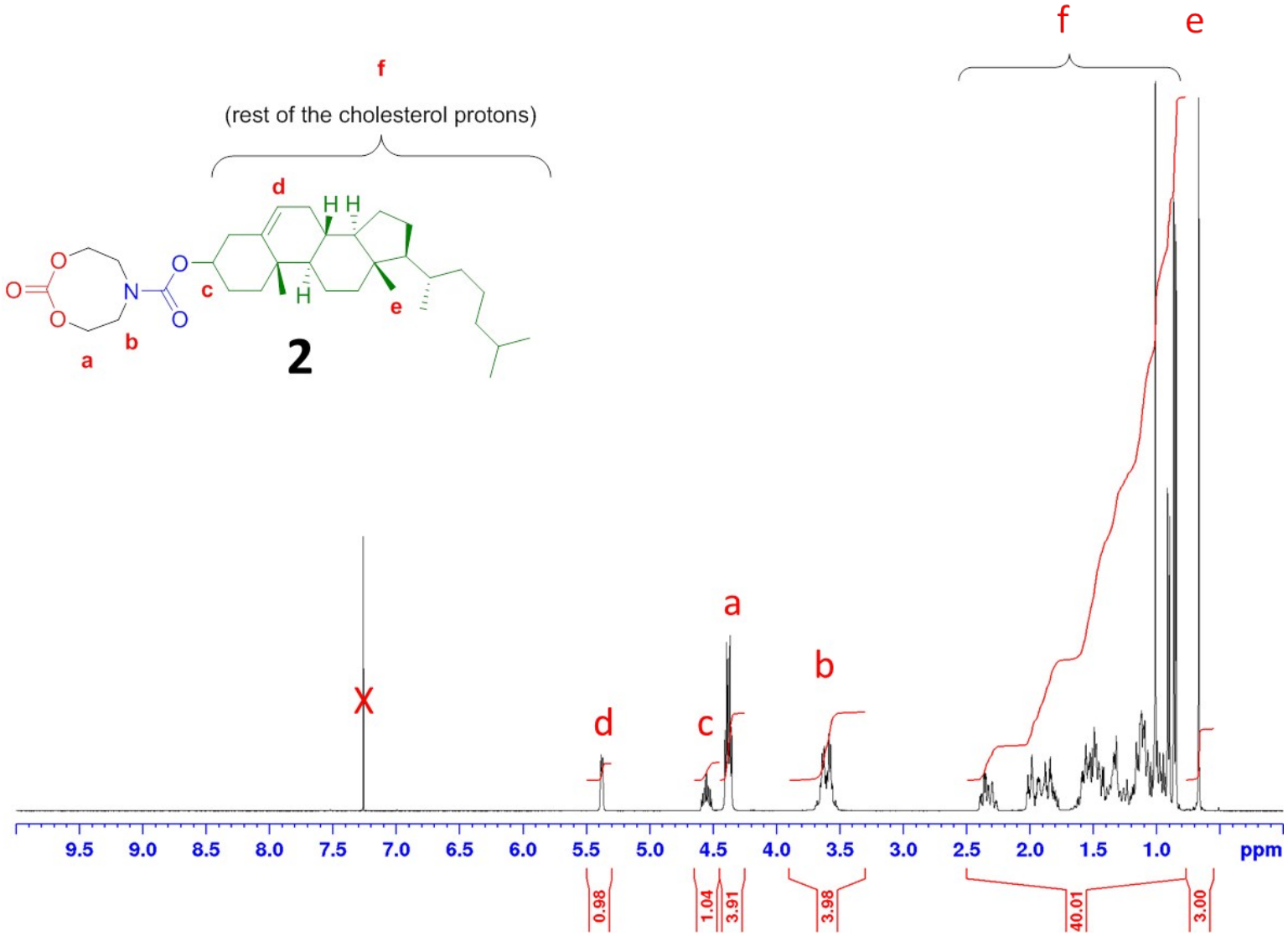


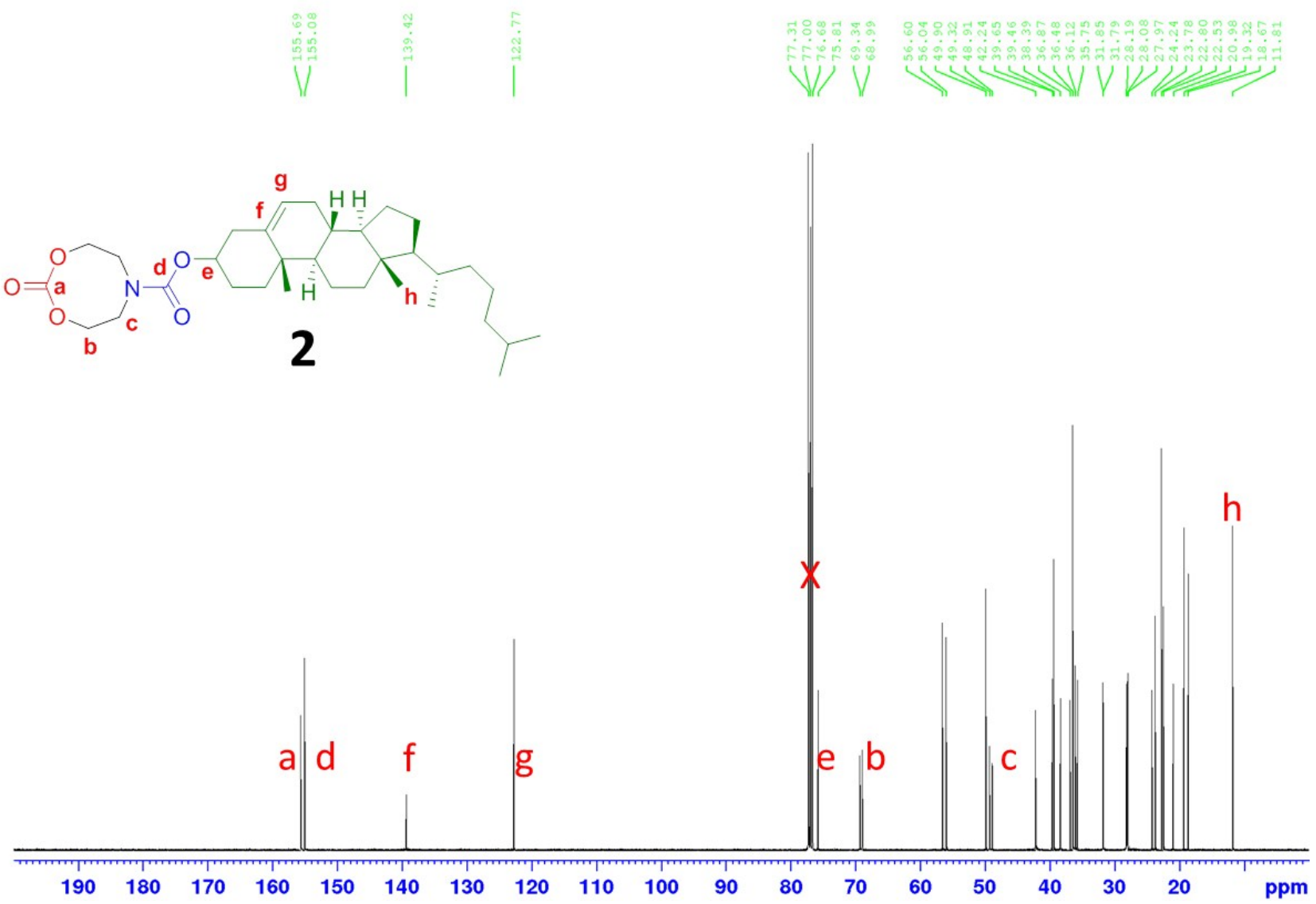
Figure S1. Higher magnification TEM micrograph of membrane-like structures, formed by polymer **3k** with internal stripped patterns (lines are guide to eye), presumably due to the ordering of cholesteryl side-chains.

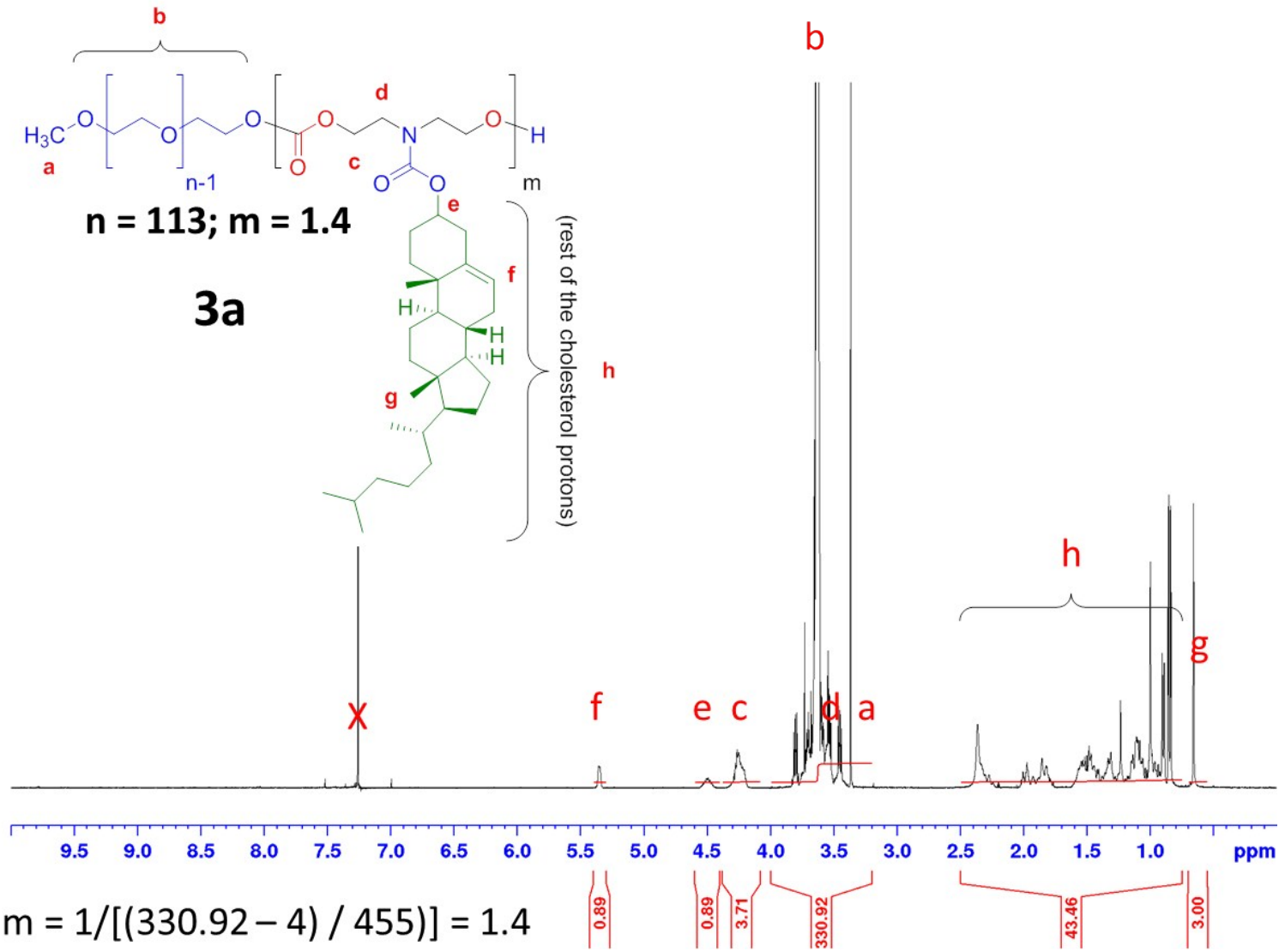
Compilation of NMR spectra and SEC chromatographs


f
(rest of the cholesterol protons)
d
H
HO
HO
N
O
O
c
H
e
b
a
1
f
e
b + -OH
a
X
d
c
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.02
1.09
3.92
1.92
4.47
2.20
5.18
32.52
3.00

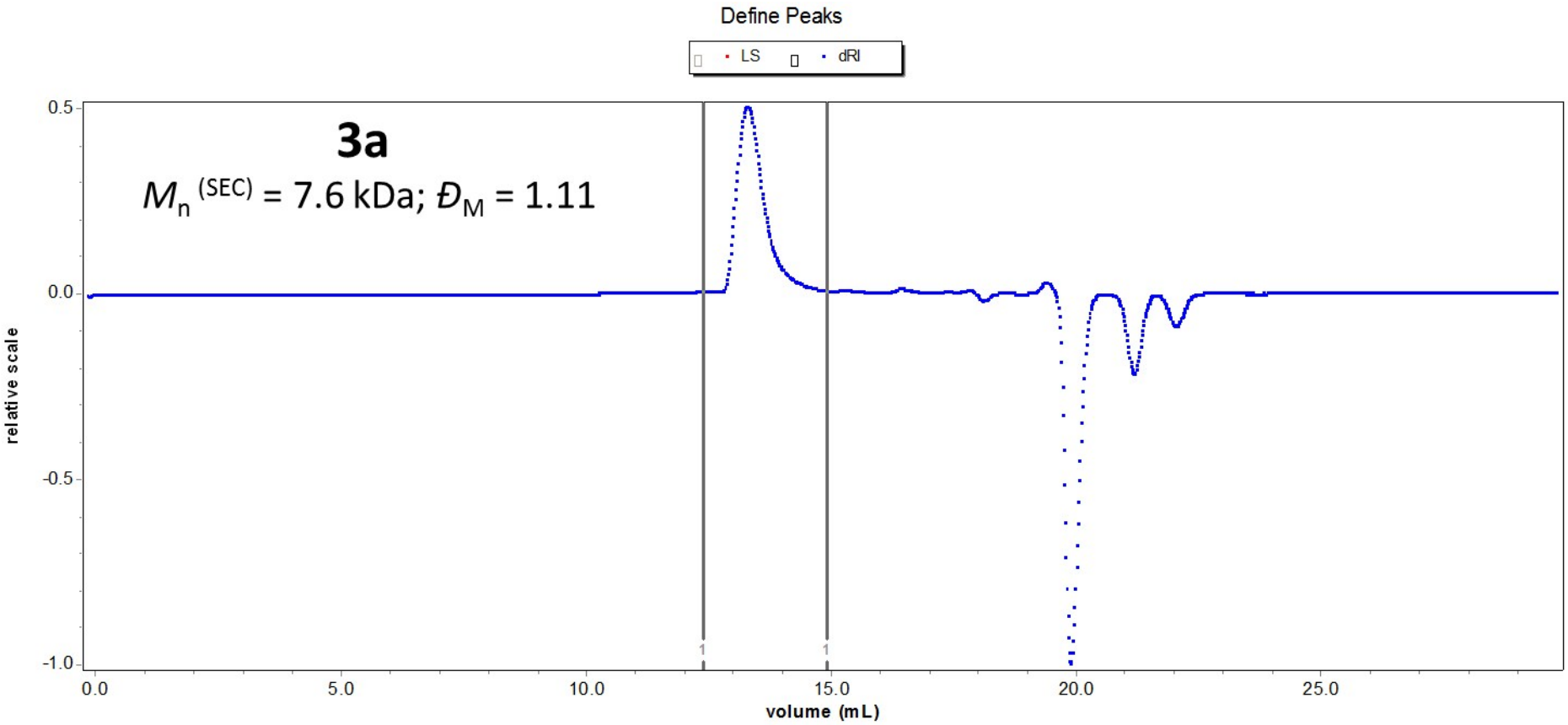

Chol-c-DEA-B1-25g-scl
156.70
139.59
122.64
77.32
77.00
76.69
75.29
61.87
56.62
56.09
49.93
42.26
39.68
39.47
38.58
36.95
36.14
35.78
31.87
31.81
28.20
27.99
24.25
23.82
22.81
22.54
21.01
19.34
18.69
11.82
HO
HO
N
O
O
a
b
c
d
e
f
g
H H
H
2
X
c
e
f
d
a
b
g
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

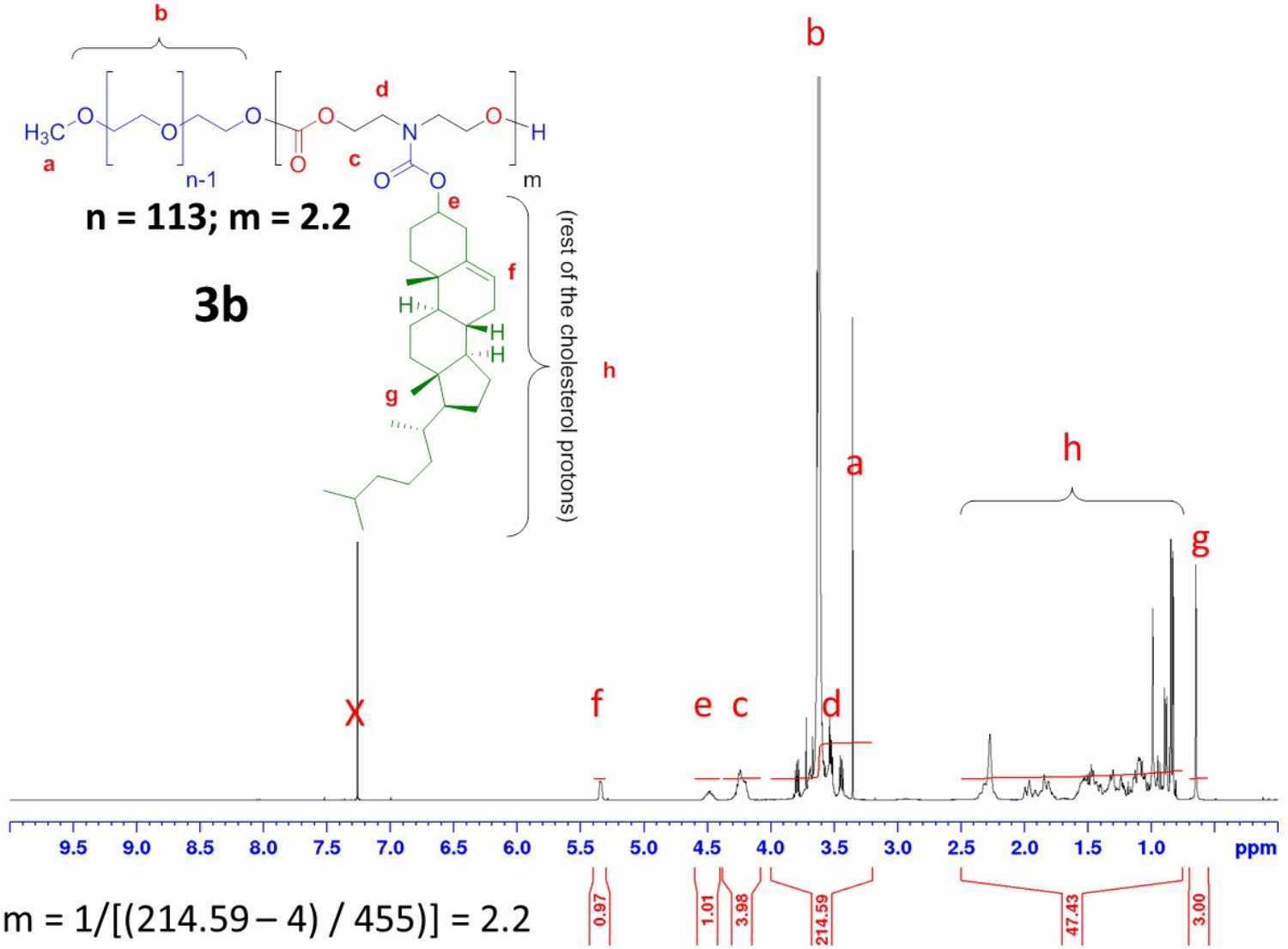

f
(rest of the cholesterol protons)
d
H H
O
O
O
N
O
c
H
e
O
a
b
2
f
e
a
b
X
d
c
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
0.98
1.04
3.91
3.98
40.01
3.00


155.69
155.08
139.42
122.77
77.31
77.00
76.68
75.81
69.34
68.99
56.60
56.04
49.90
49.32
48.91
42.24
39.65
39.46
38.39
36.87
36.48
36.12
35.75
31.85
31.79
28.19
28.08
27.97
24.24
23.78
22.80
22.53
20.98
19.32
18.67
11.81
a
b
c
d
e
f
g
h
2
X
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

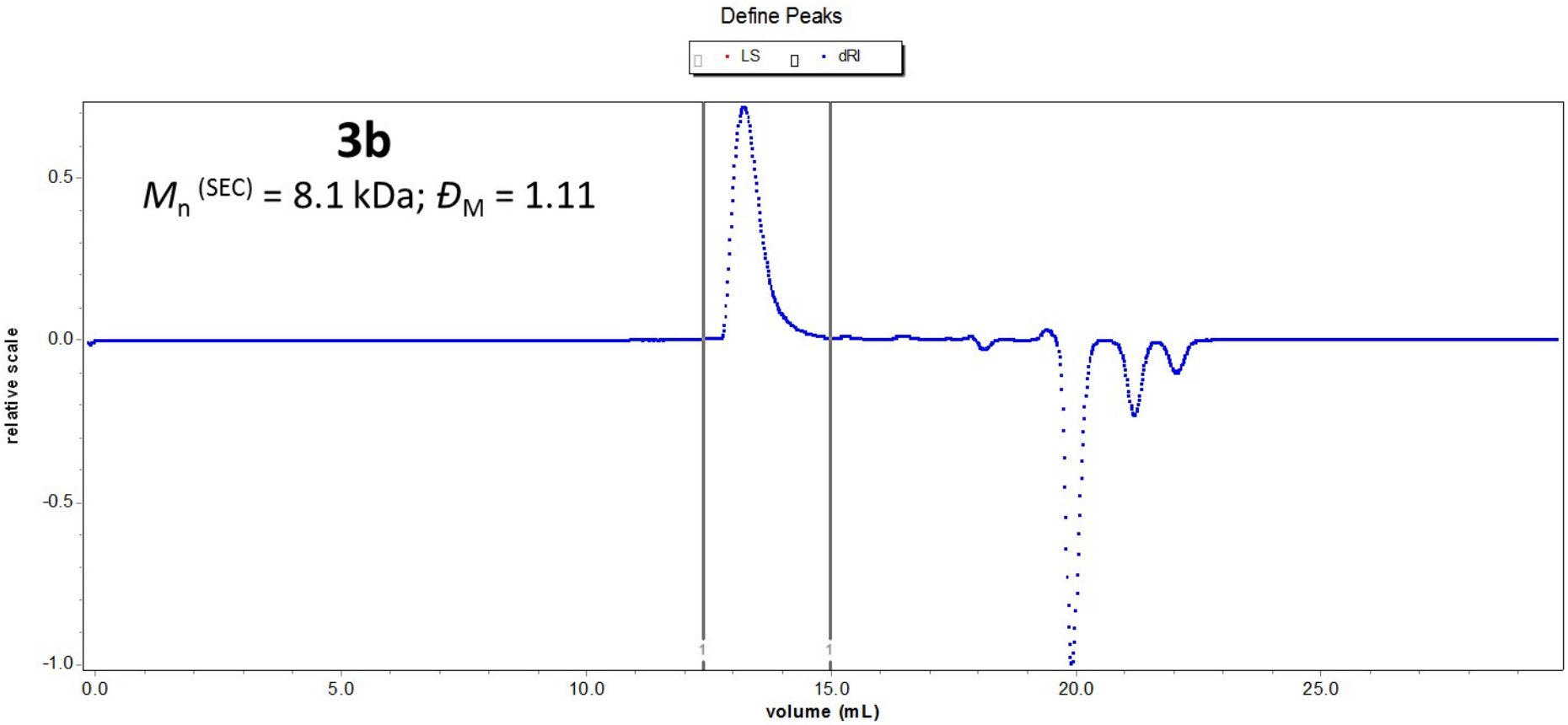


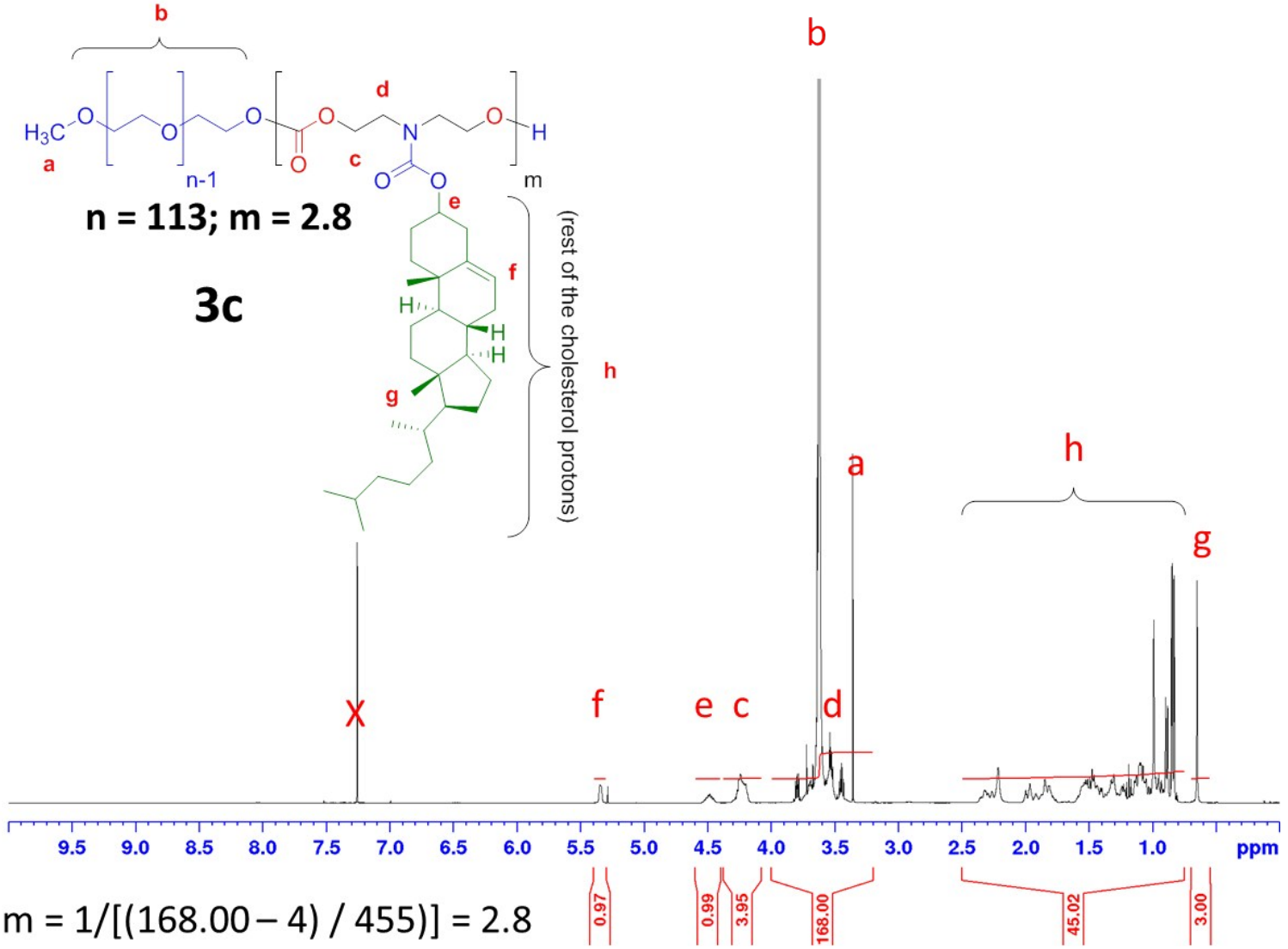
m = 1/[(330.92 – 4) / 455)] = 1.4


Define Peaks
LS
dRI
3a
M_n (SEC) = 7.6 kDa; $Đ_M$ = 1.11
0.5
0.0
-0.5
-1.0
relative scale
0.0
5.0
10.0
15.0
20.0
25.0
volume (mL)

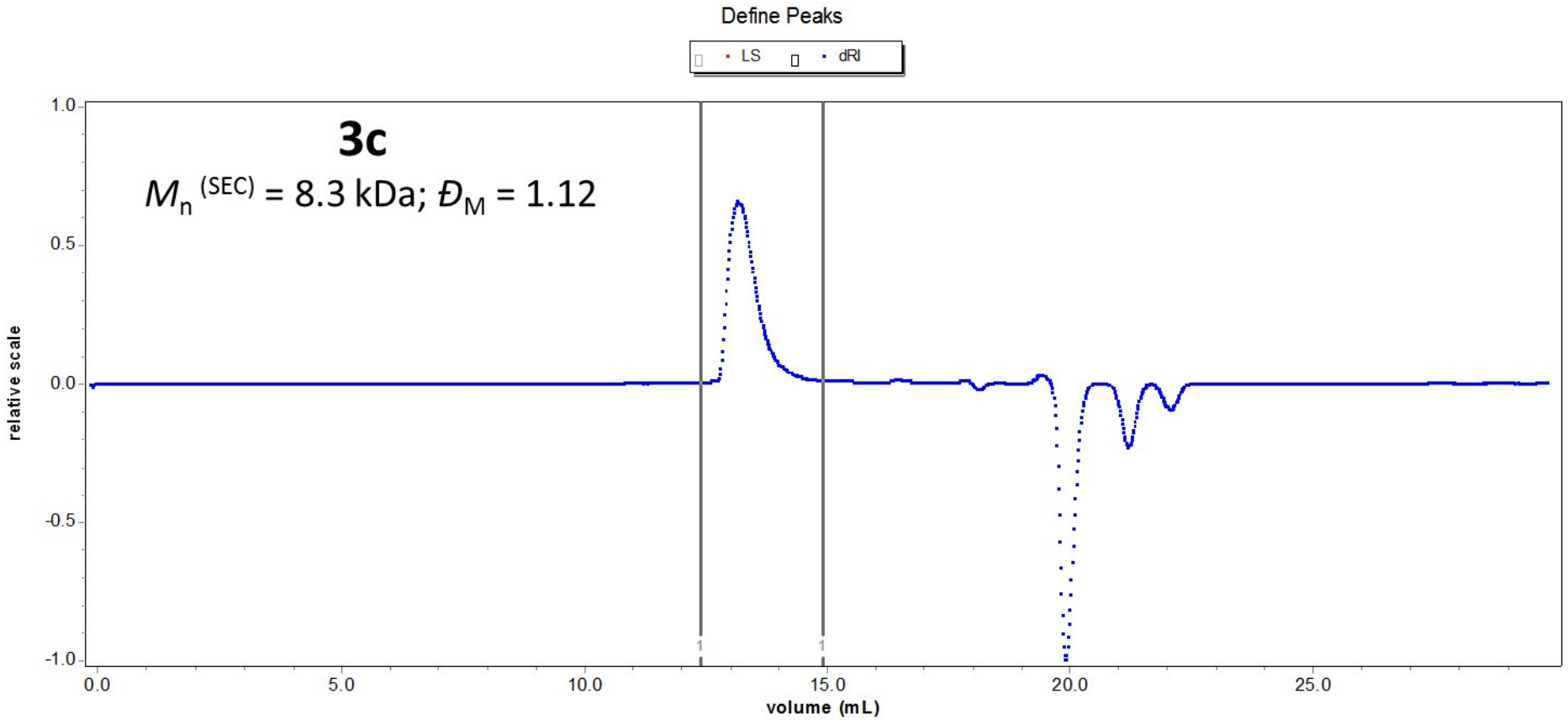


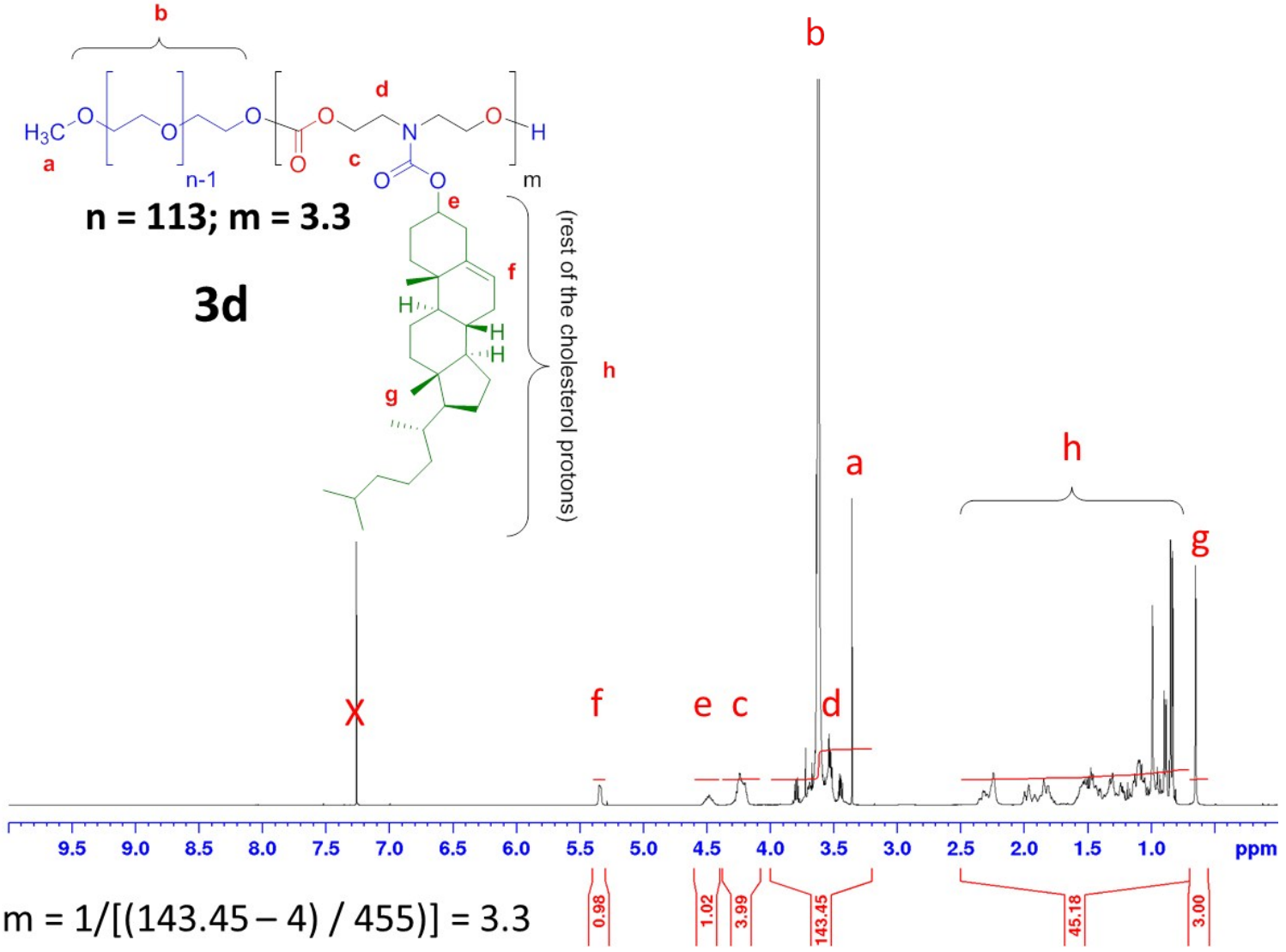
m = 1/[(214.59 – 4) / 455)] = 2.2


Define Peaks
LS
dRI
3b
M_n (SEC) = 8.1 kDa; $Đ_M$ = 1.11
0.5
0.0
-0.5
-1.0
relative scale
0.0
5.0
10.0
15.0
20.0
25.0
volume (mL)

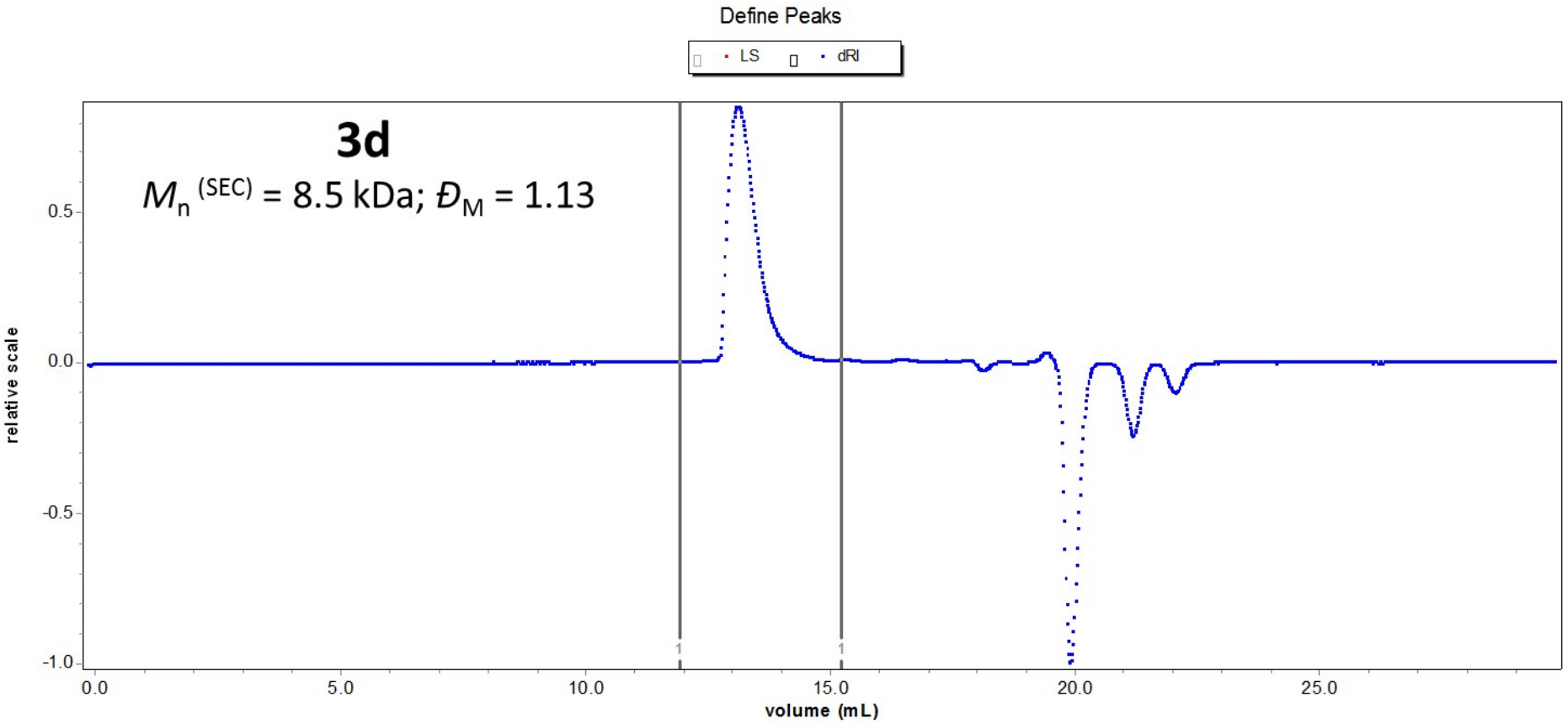


m = 1/[(168.00 – 4) / 455)] = 2.8


Define Peaks
LS
dRI
3c
M_n (SEC) = 8.3 kDa; $Đ_M$ = 1.12
1.0
0.5
0.0
-0.5
-1.0
relative scale
0.0
5.0
10.0
15.0
20.0
25.0
volume (mL)



m = 1/[(143.45 – 4) / 455)] = 3.3


Define Peaks
LS
dRI
3d
M_n (SEC) = 8.5 kDa; $Đ_M$ = 1.13
relative scale
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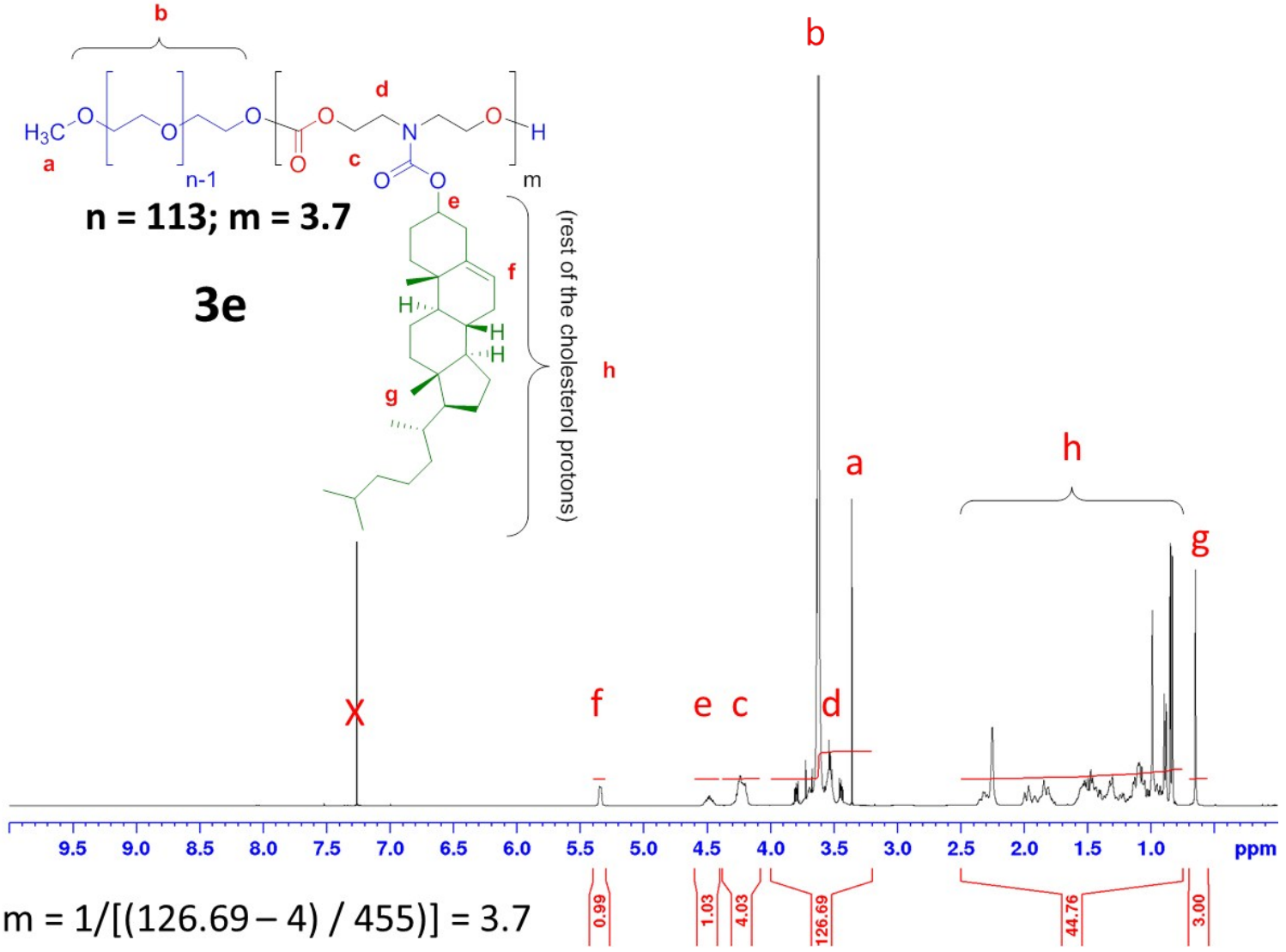
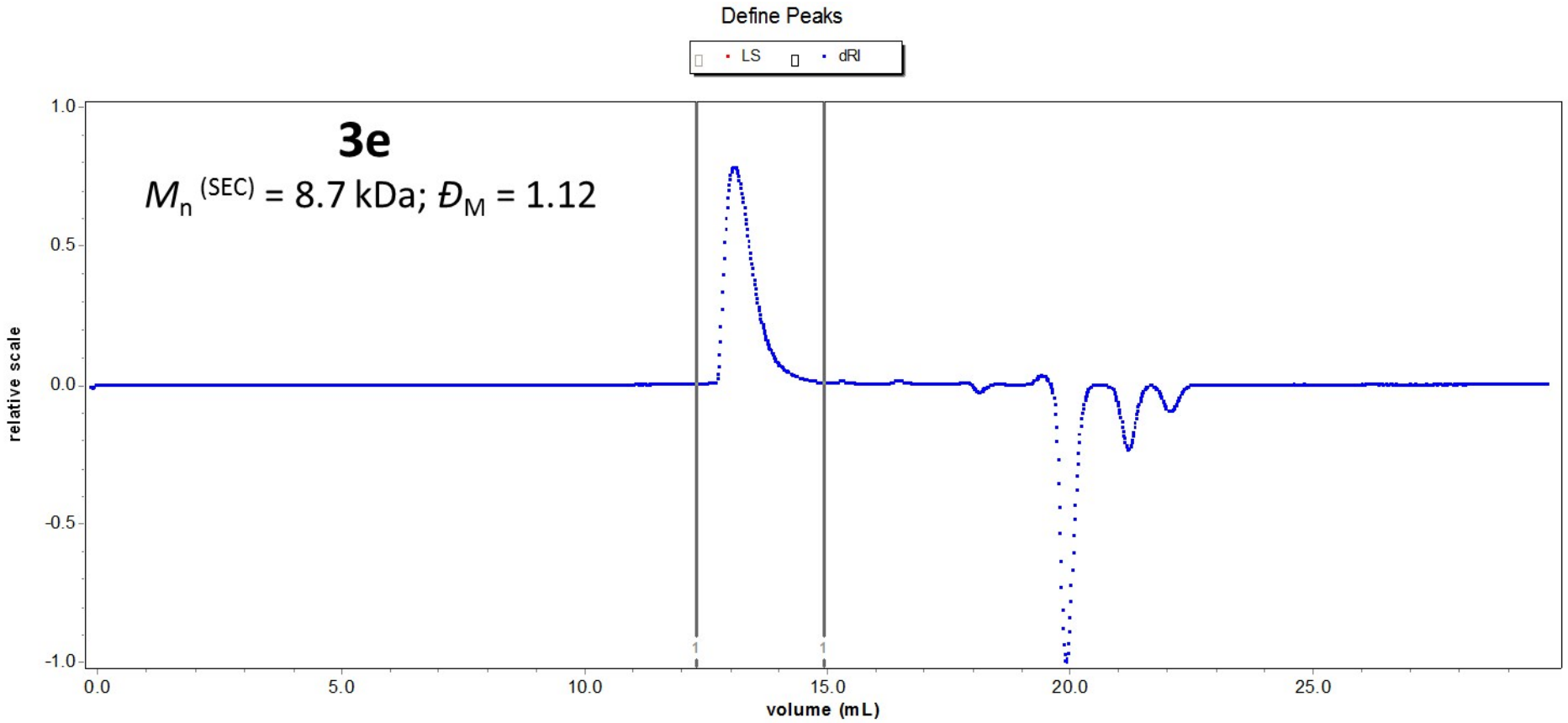
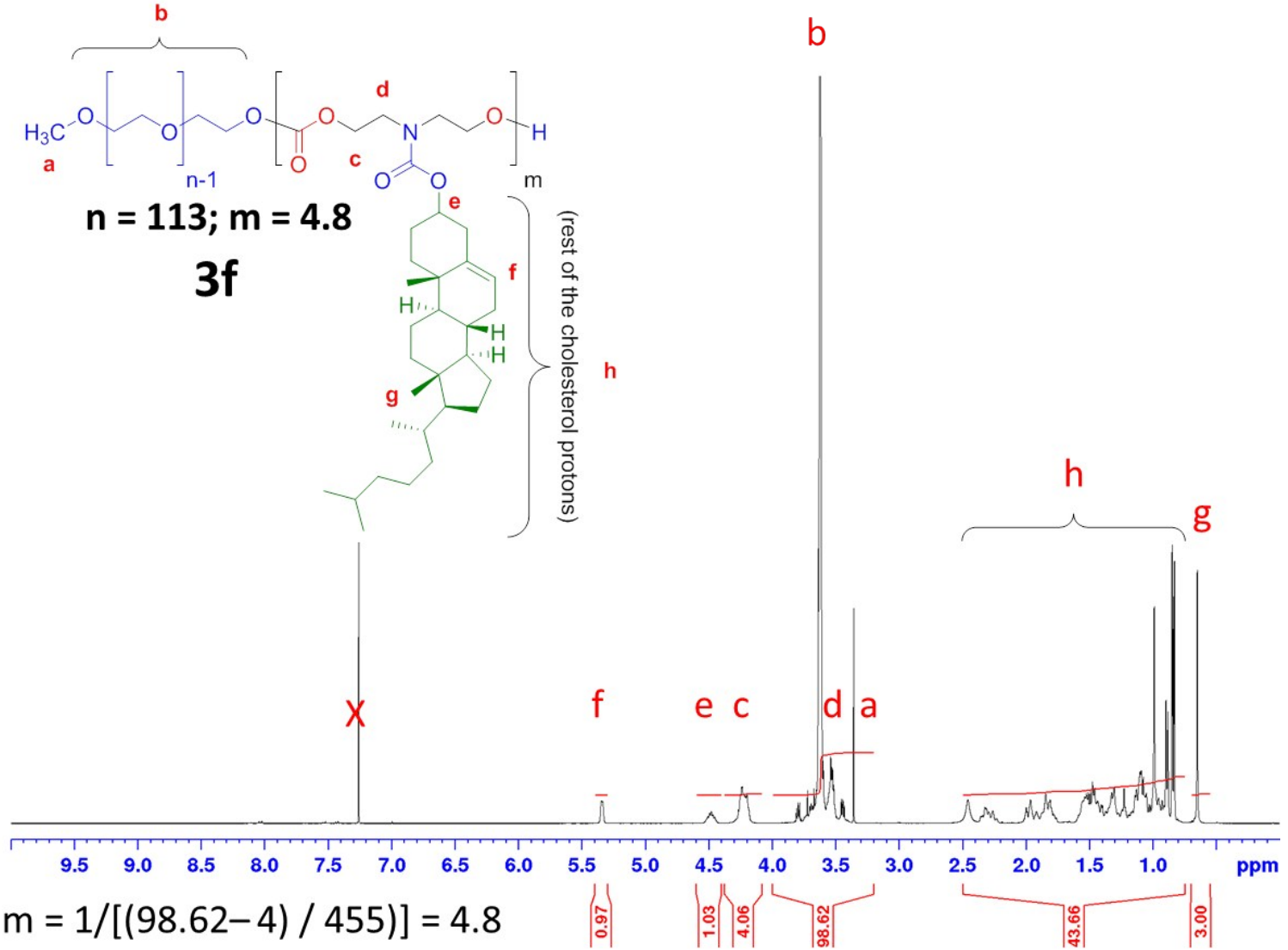
0.0
-0.5
-1.0
0.0
5.0
10.0
15.0
20.0
25.0
volume (mL)

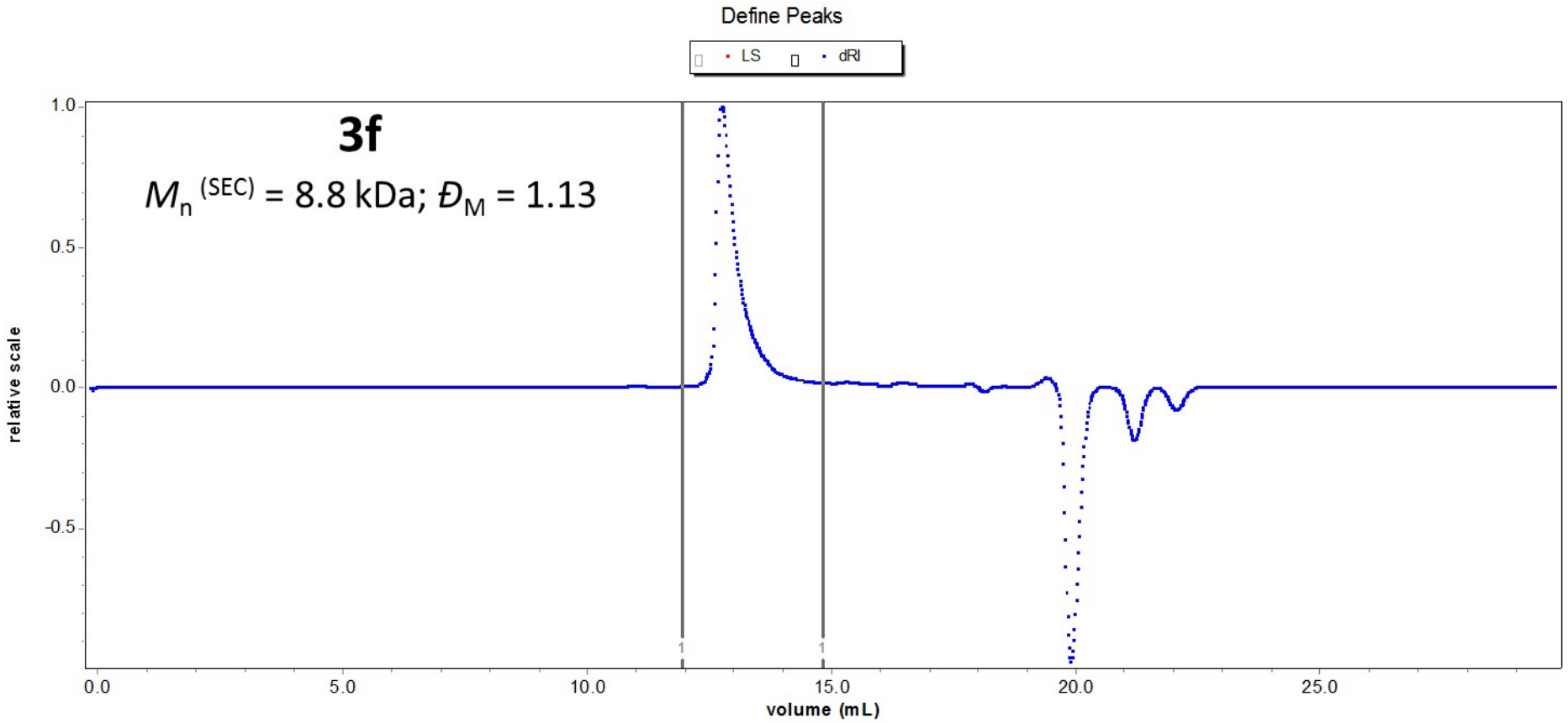


m = 1/[(126.69 – 4) / 455)] = 3.7


Define Peaks
LS
dRI
3e
M_n (SEC) = 8.7 kDa; $Đ_M$ = 1.12
1.0
0.5
0.0
-0.5
-1.0
relative scale
0.0
5.0
10.0
15.0
20.0
25.0
volume (mL)



m = 1/[(98.62– 4) / 455)] = 4.8


Define Peaks
LS
dRI
3f
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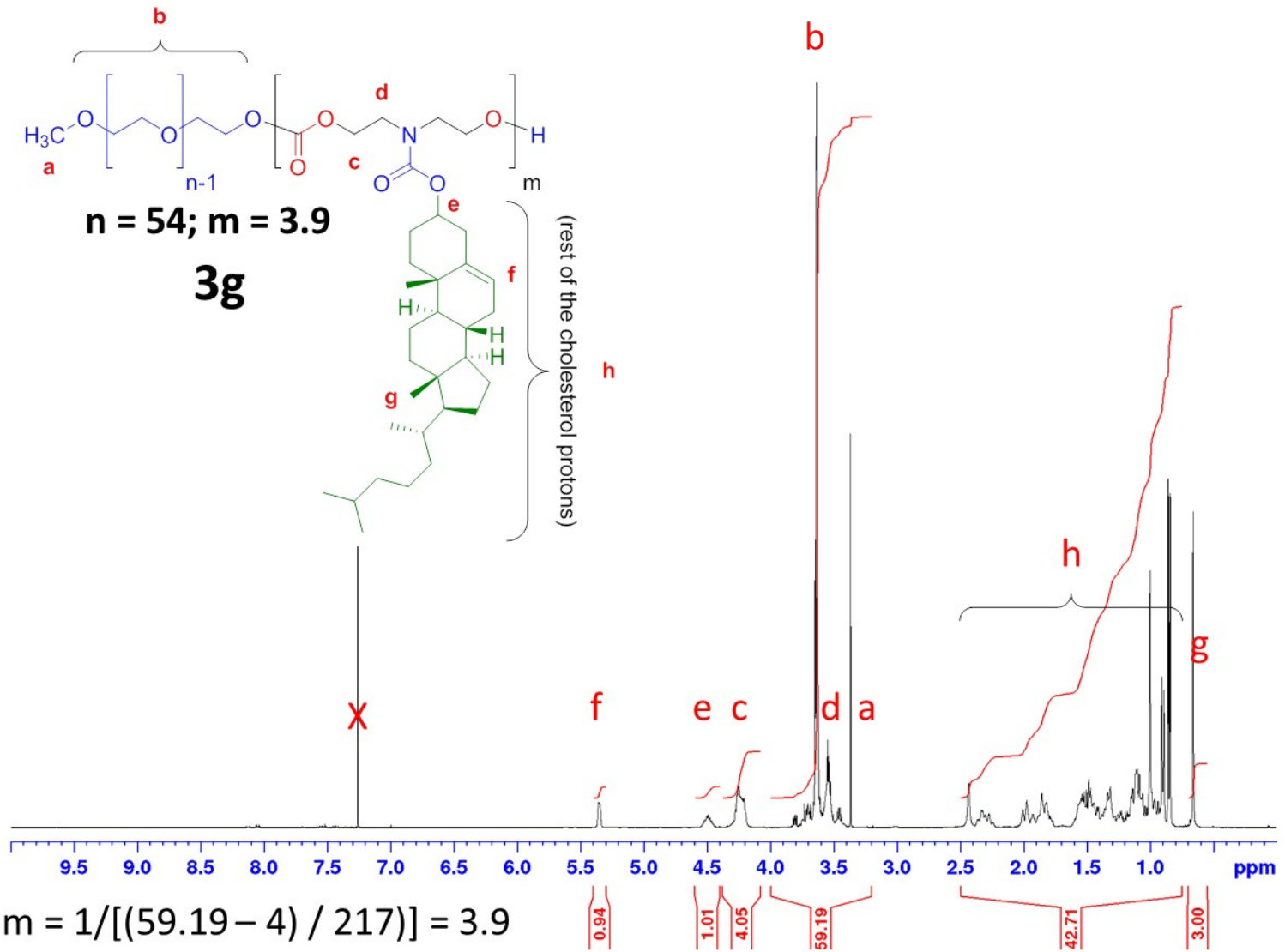
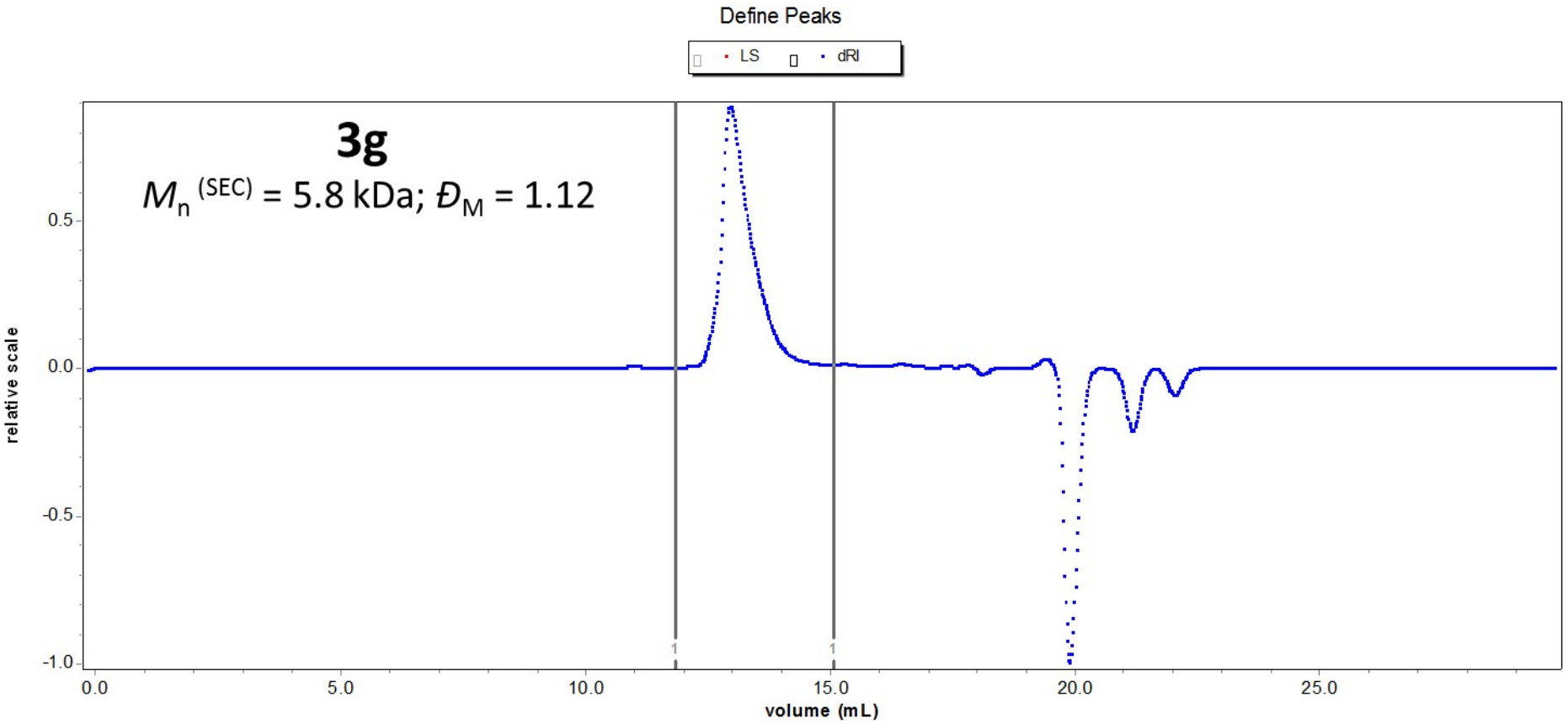
0.5
0.0
-0.5
relative scale
0.0
5.0
10.0
15.0
20.0
25.0
volume (mL)



m = 1/[(59.19 – 4) / 217)] = 3.9


Define Peaks
LS
dRI
3g
M_n (SEC) = 5.8 kDa; $Đ_M$ = 1.12
relative scale
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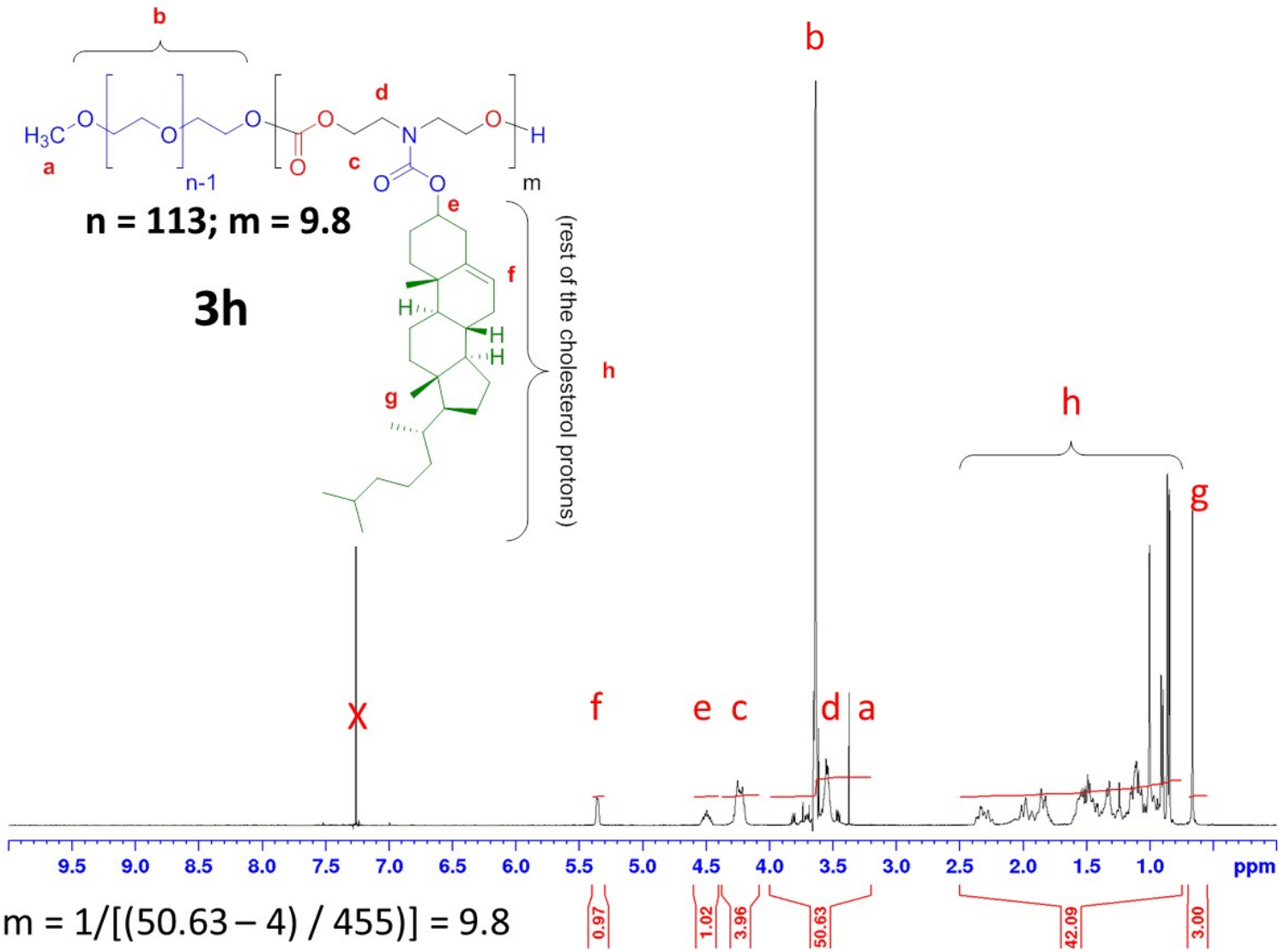
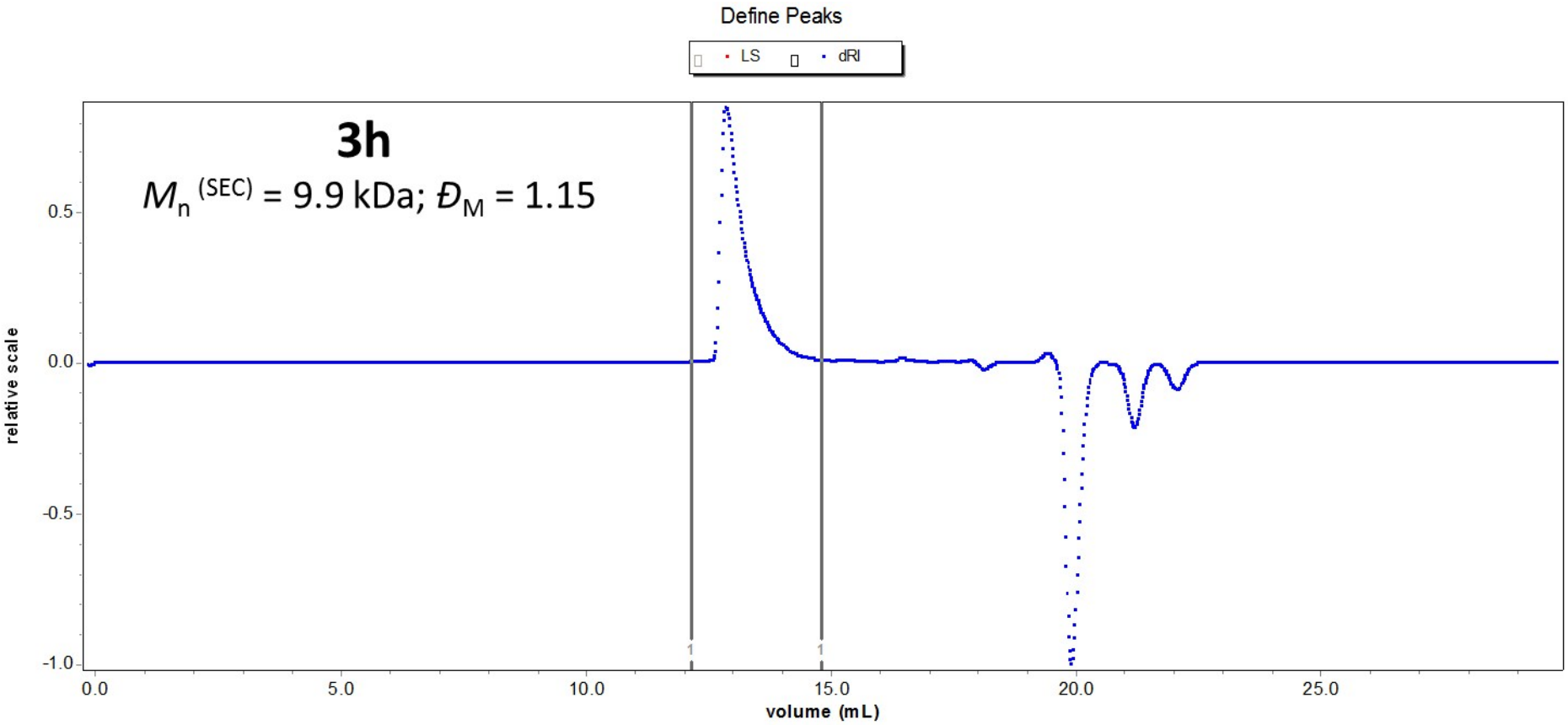
0.0
-0.5
-1.0
0.0
5.0
10.0
15.0
20.0
25.0
volume (mL)



m = 1/[(50.63 – 4) / 455)] = 9.8


Define Peaks
LS
dRI
3h
M_n (SEC) = 9.9 kDa; $Đ_M$ = 1.15
relative scale
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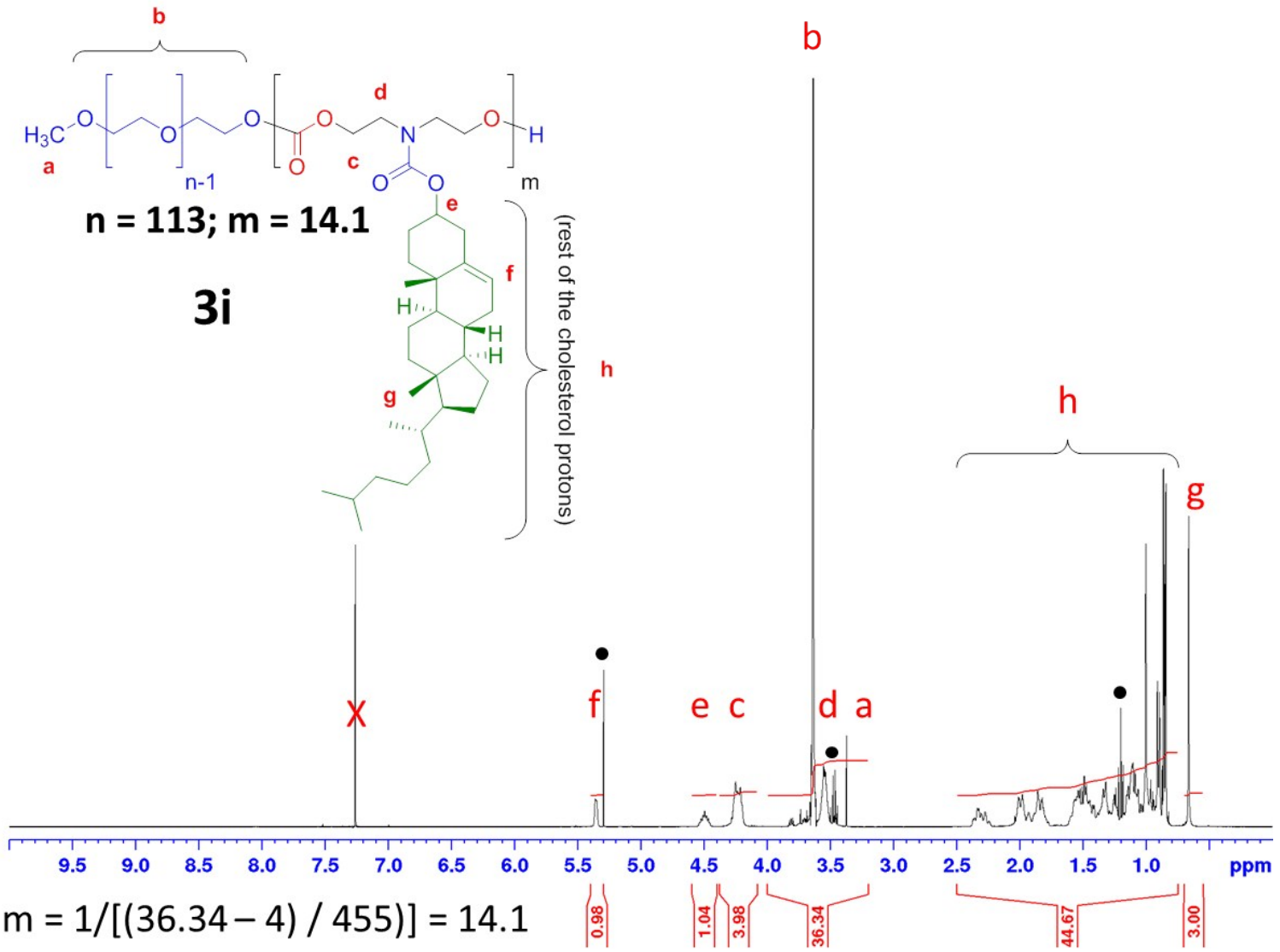
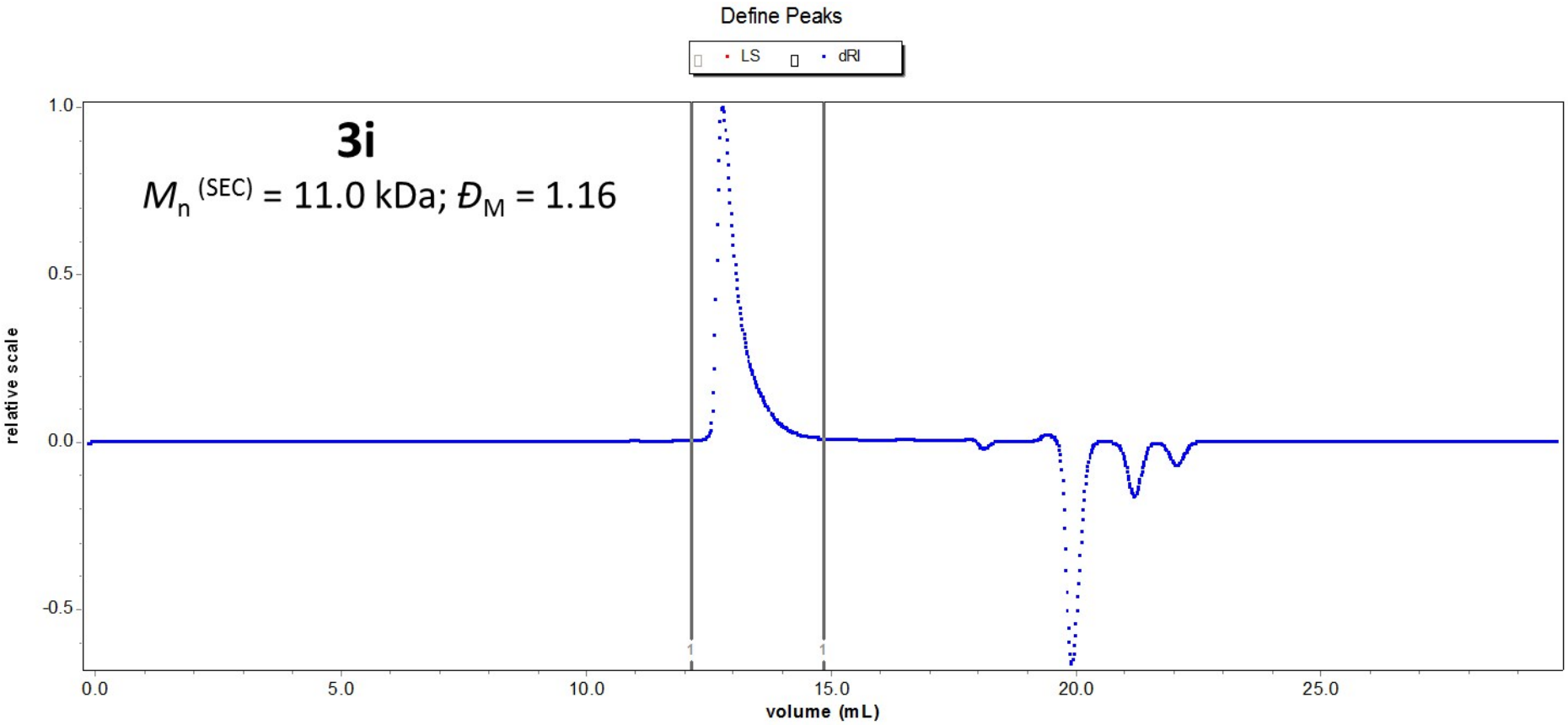
0.0
-0.5
-1.0
0.0
5.0
10.0
15.0
20.0
25.0
volume (mL)



m = 1/[(36.34 – 4) / 455)] = 14.1

• = Presence of trace residual solvents – dichloromethane and diethyl ether


Define Peaks
LS
dRI
3i
M_n (SEC) = 11.0 kDa; $Đ_M$ = 1.16
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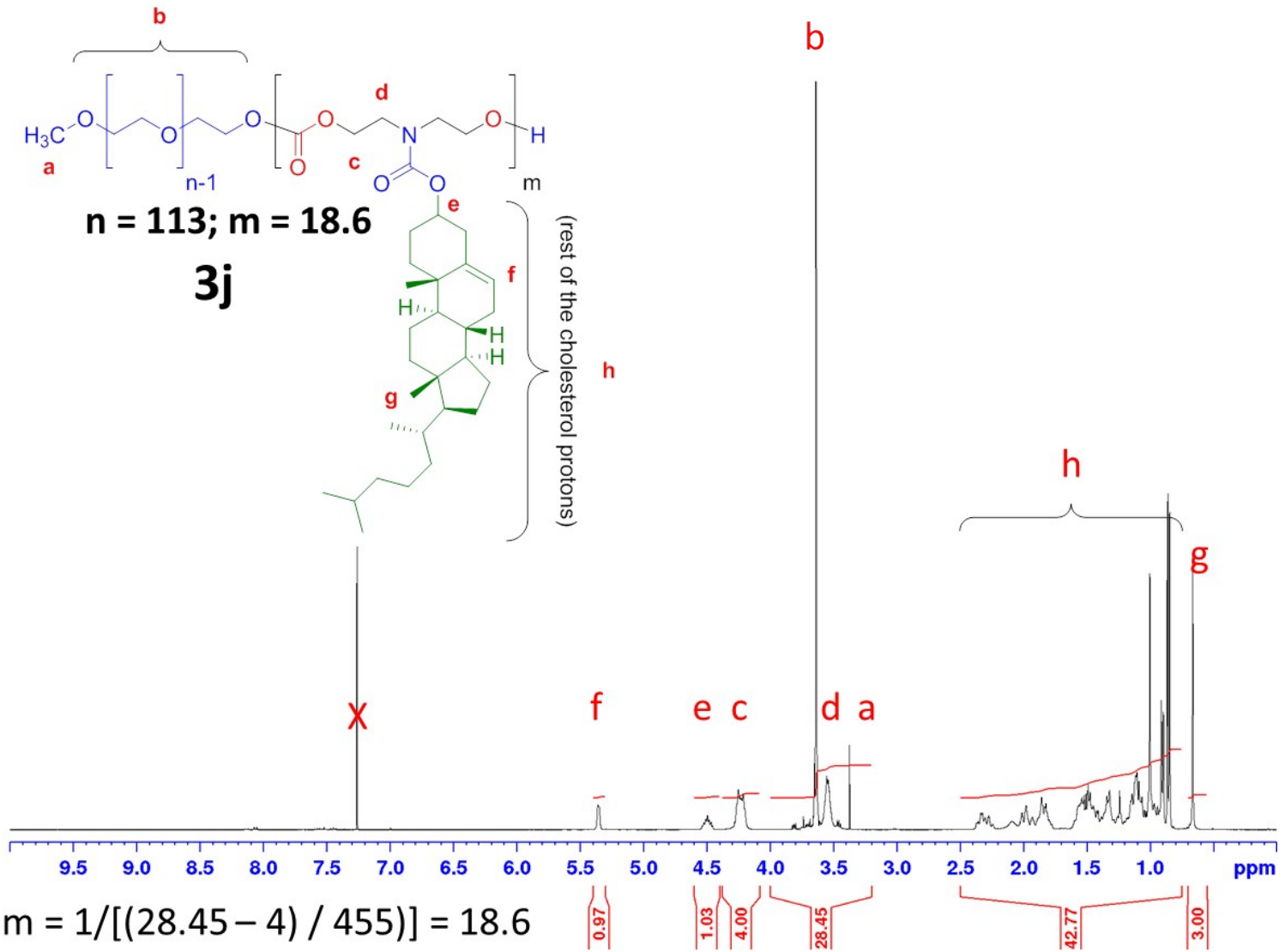
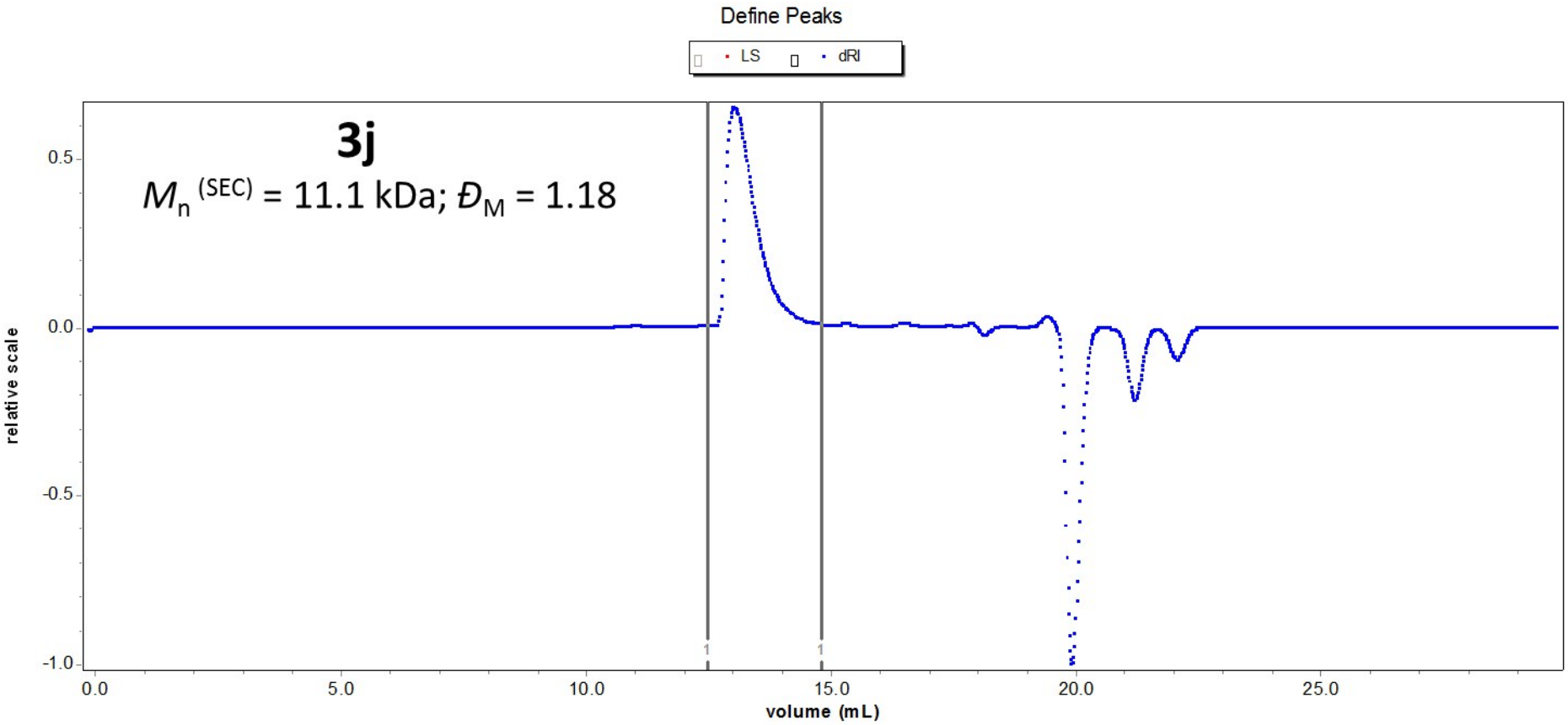
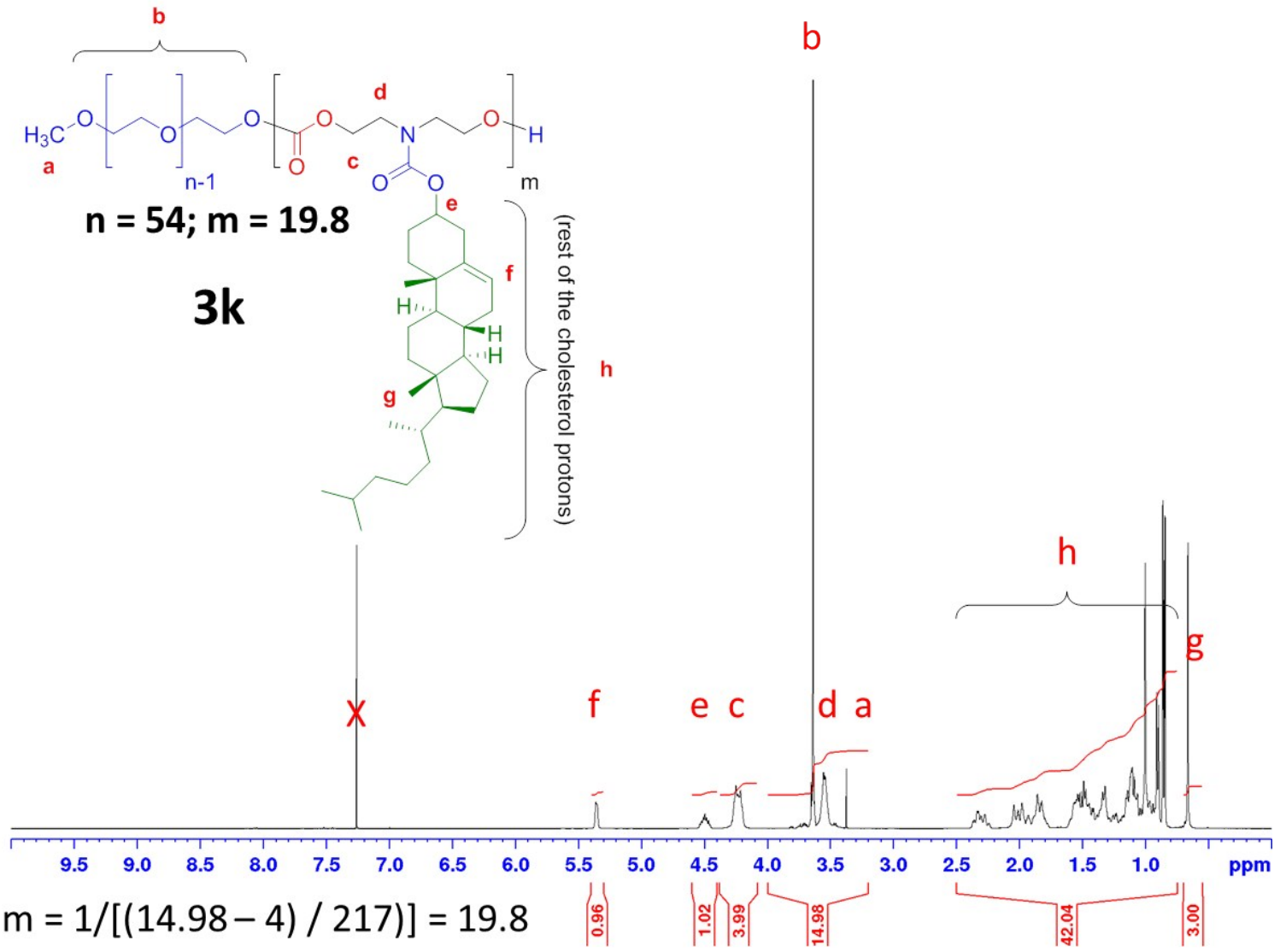
0.5
0.0
-0.5
relative scale
0.0
5.0
10.0
15.0
20.0
25.0
volume (mL)

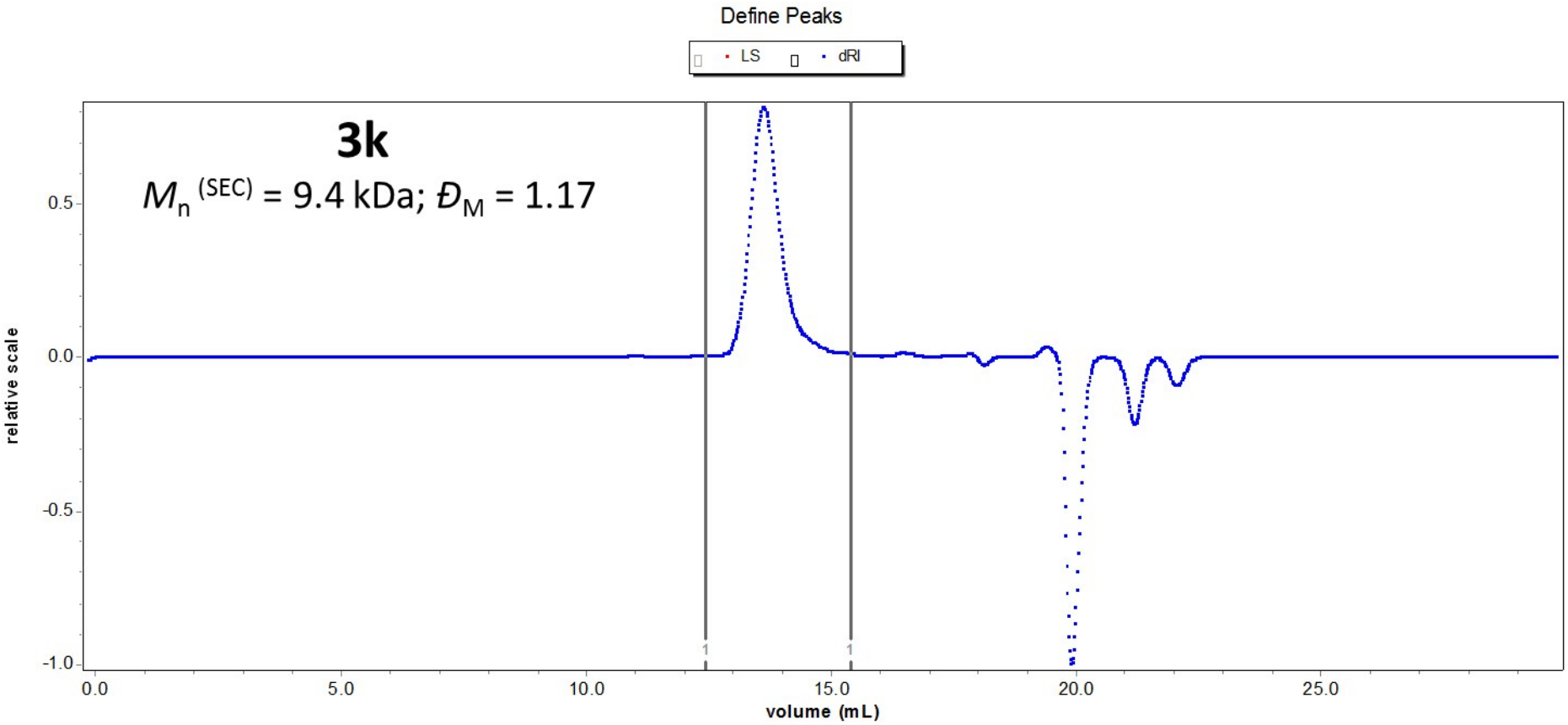


m = 1/[(28.45 – 4) / 455)] = 18.6


Define Peaks
LS
dRI
3j
M_n (SEC) = 11.1 kDa; $Đ_M$ = 1.18
0.5
0.0
-0.5
-1.0
relative scale
0.0
5.0
10.0
15.0
20.0
25.0
volume (mL)



m = 1/[(14.98 – 4) / 217)] = 19.8


Define Peaks
LS
dRI
3k
M_n (SEC) = 9.4 kDa; $Đ_M$ = 1.17
relative scale
0.5
0.0
-0.5
-1.0
0.0
5.0
10.0
15.0
20.0
25.0
volume (mL)
1
1