ELECTRONIC SUPPORTING INFORMATION

Polymer-protein conjugation via a 'grafting to' approach – A comparative study of the performance of protein-reactive RAFT chain transfer agents

N.Vanparijs,[a$] S. Maji,[b$] B. Louage,[a] L. Voorhaar,[b] D. Laplace,[c] Q. Zhang,[2] Y. Shi,[d] W. E. Hennink,[d] R. Hoogenboom,[b*] B. G. De Geest[a*]

[a] *Department of Pharmaceutics, Ghent University, Ottergemsesteenweg 460, 9000 Ghent, Belgium*

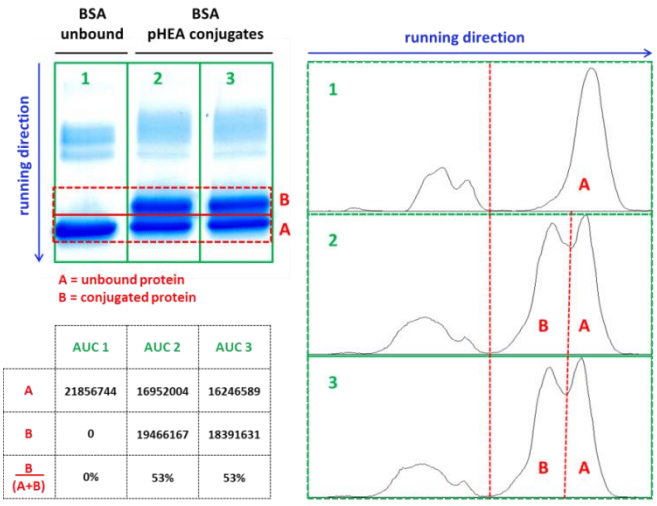
[b] *Supramolecular Chemistry Group, Department of Organic and Macromolecular Chemistry, Krijgslaan 281 S4, 9000 Ghent, Belgium*

[c] *Laboratory for Organic Synthesis, Department of Organic Chemistry, Krijgslaan 281 S4-bis, 9000 Ghent, Belgium*

[d] *Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, David de Wiedgebouw, Universiteitsweg 99, 3584 Utrecht, The Netherlands*

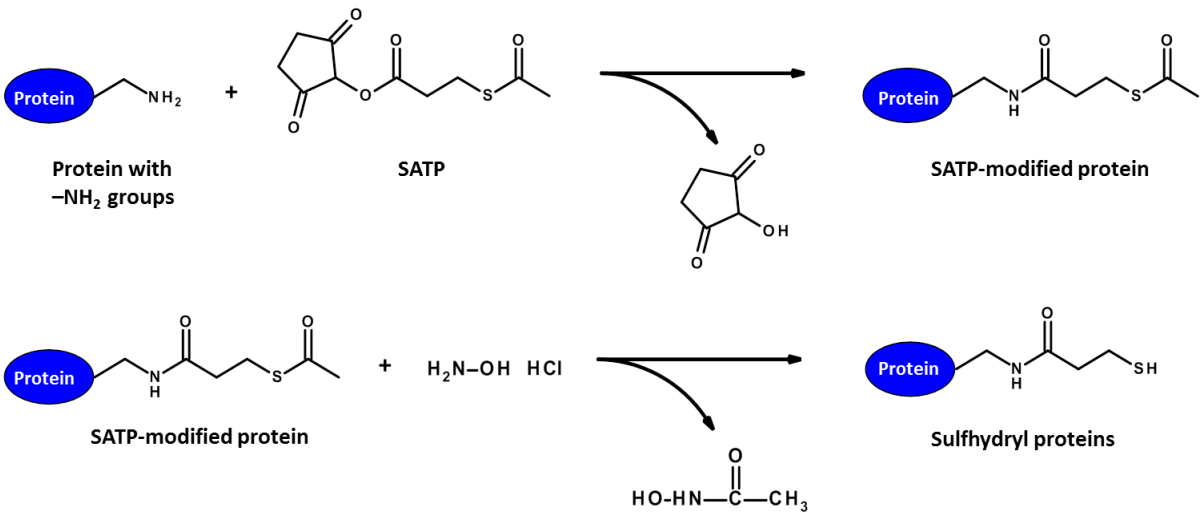
$ *These author contributed equally to this work.*

* br.degeest@ugent.be; richard.hoogenboom@ugent.be



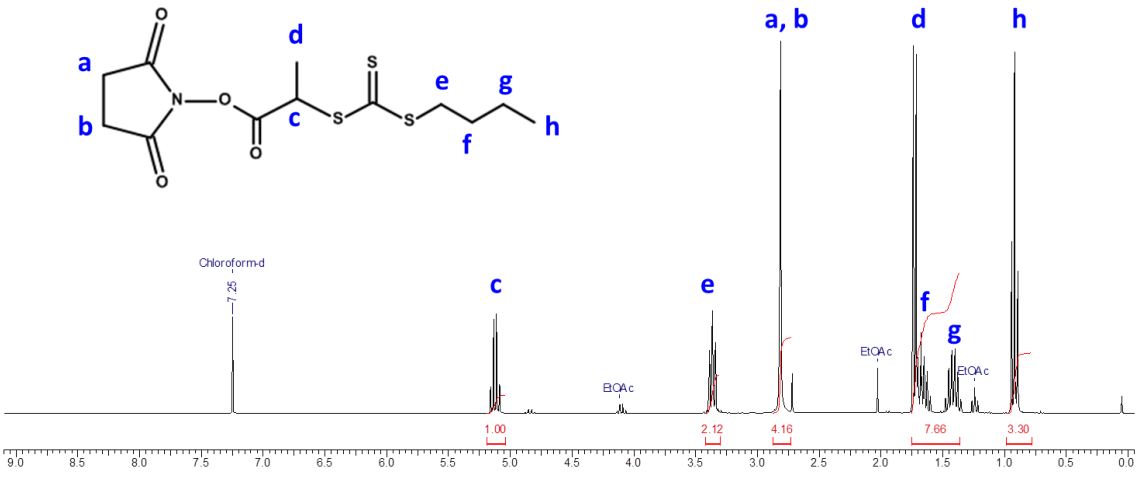
	AUC 1	AUC 2	AUC 3
A	21856744	16952004	16246589
B	0	19466167	18391631
$\frac{B}{(A+B)}$	0%	53%	53%

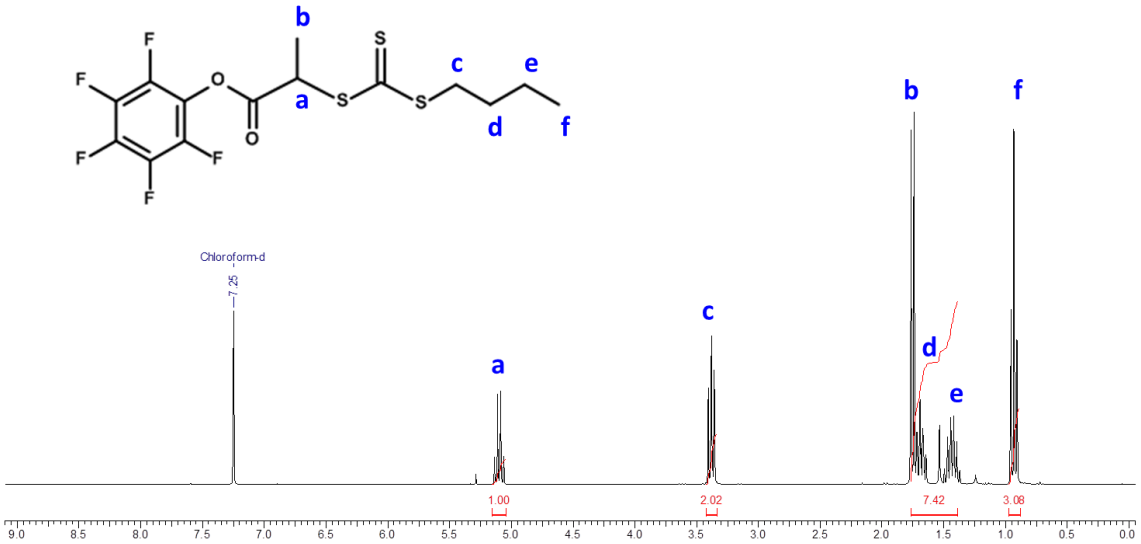
Figure S1. Quantification of protein conjugation by automated integration of optical density by ImageJ software and calculating the ratio of conjugated protein (B) to total protein content per lane (A+B). As BSA forms high molecular weight aggregates, these bands were excluded from the calculations. For OVA, aggregation did not occur.

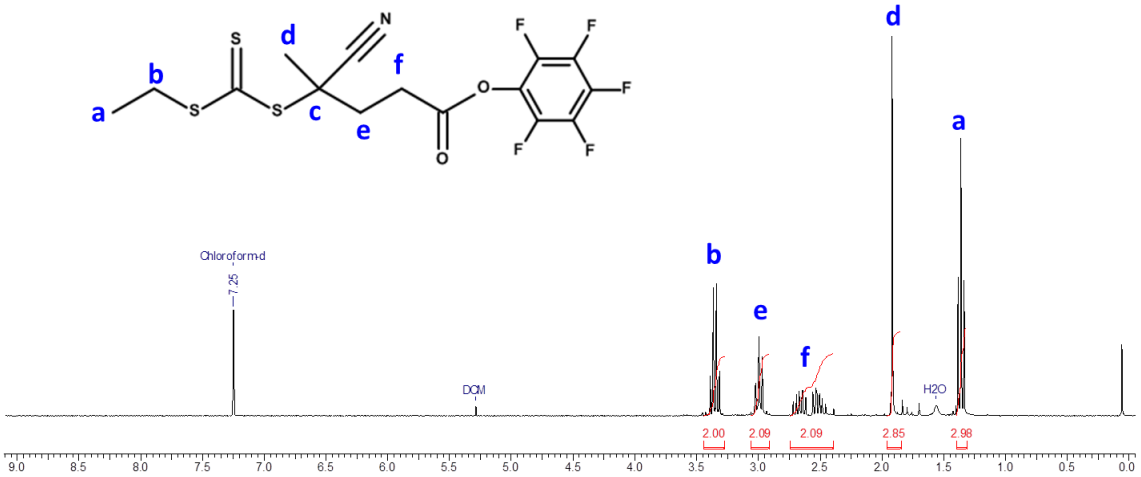


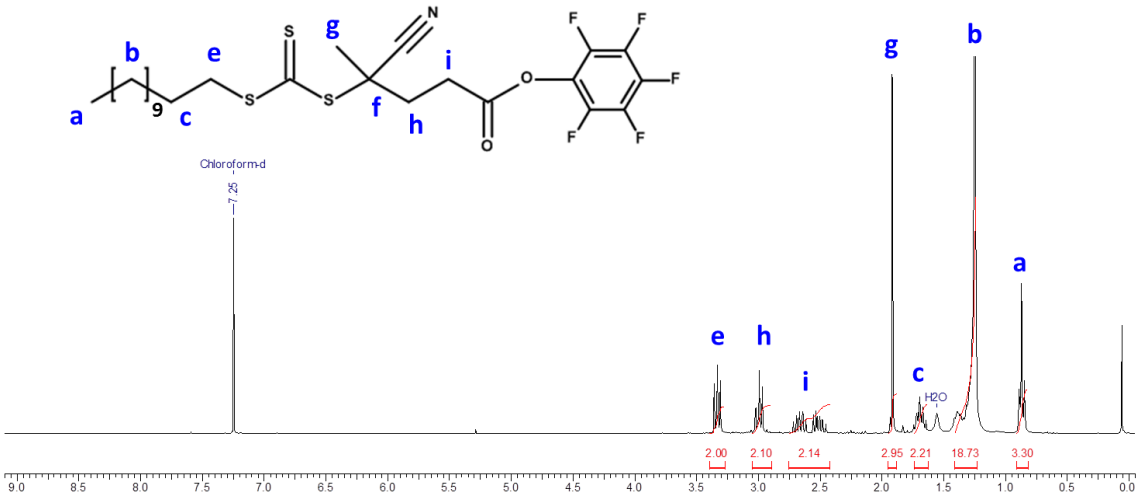
Scheme S1. Reaction scheme for the modification of protein amino groups with SATP. First, primary amines on the protein react and form an amide bond with N-succinimidyl-S-acetylthiopropionate (SATP), which contains a protected sulfhydryl. Next, hydroxylamine is used to deacetylate the sulfur and yield a sulfhydryl group.

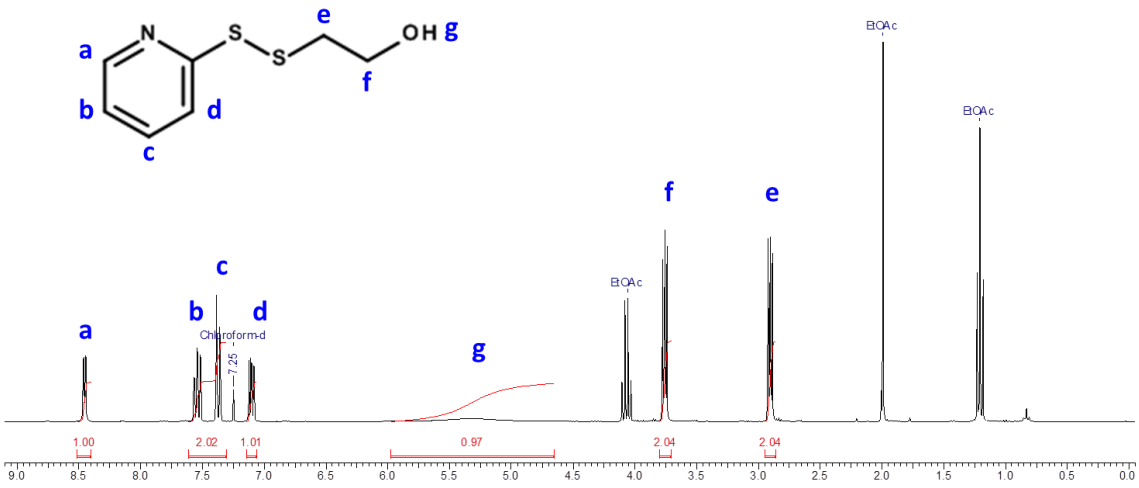
^{1}H-NMR spectra

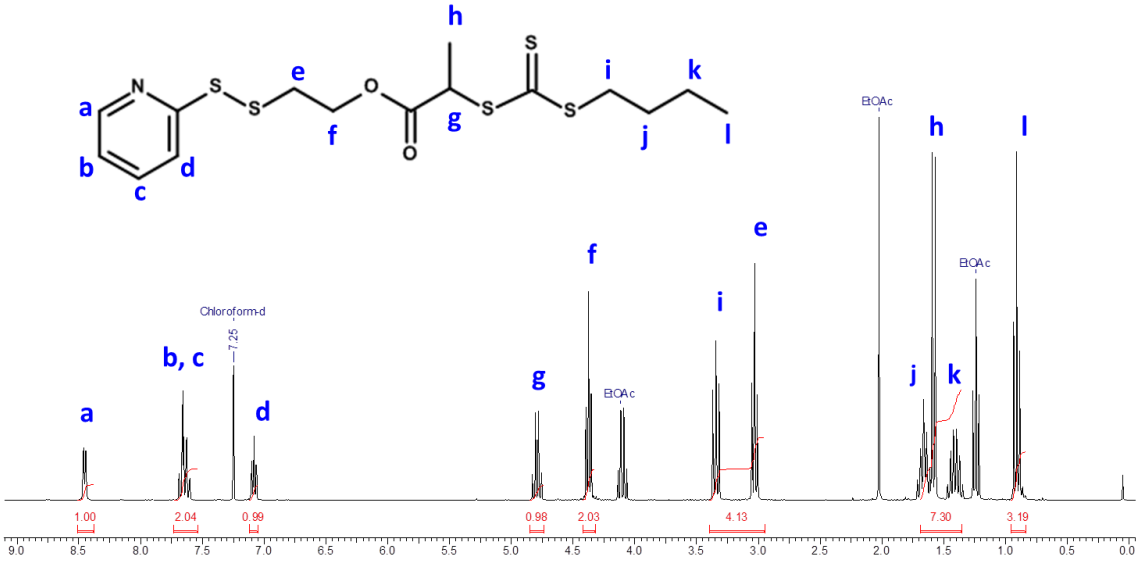


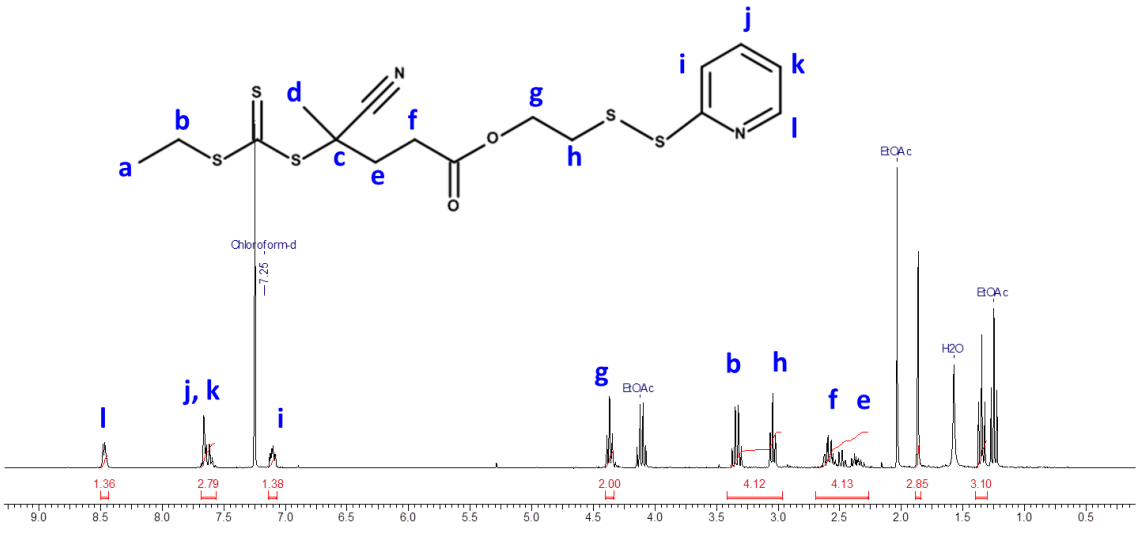

Chloroform-d
7.25
1.00
2.02
7.42
3.08

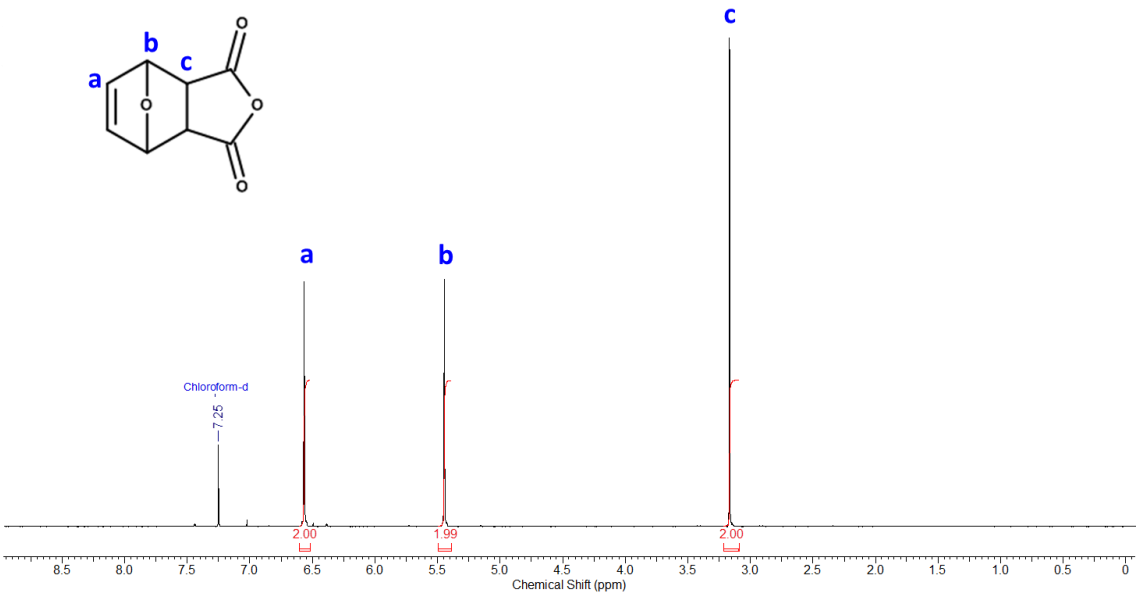

Chloroform-d
7.25
DCM
H2O
2.00
2.09
2.09
2.85
2.98

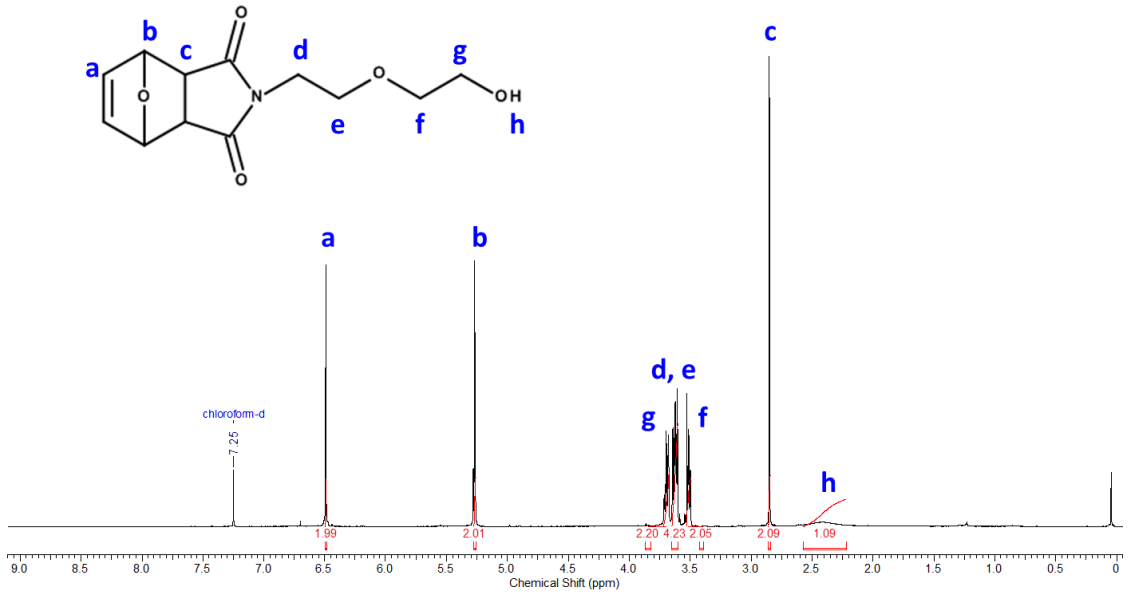

Chloroform-d
7.25
H2O
2.00
2.10
2.14
2.95
2.21
18.73
3.30

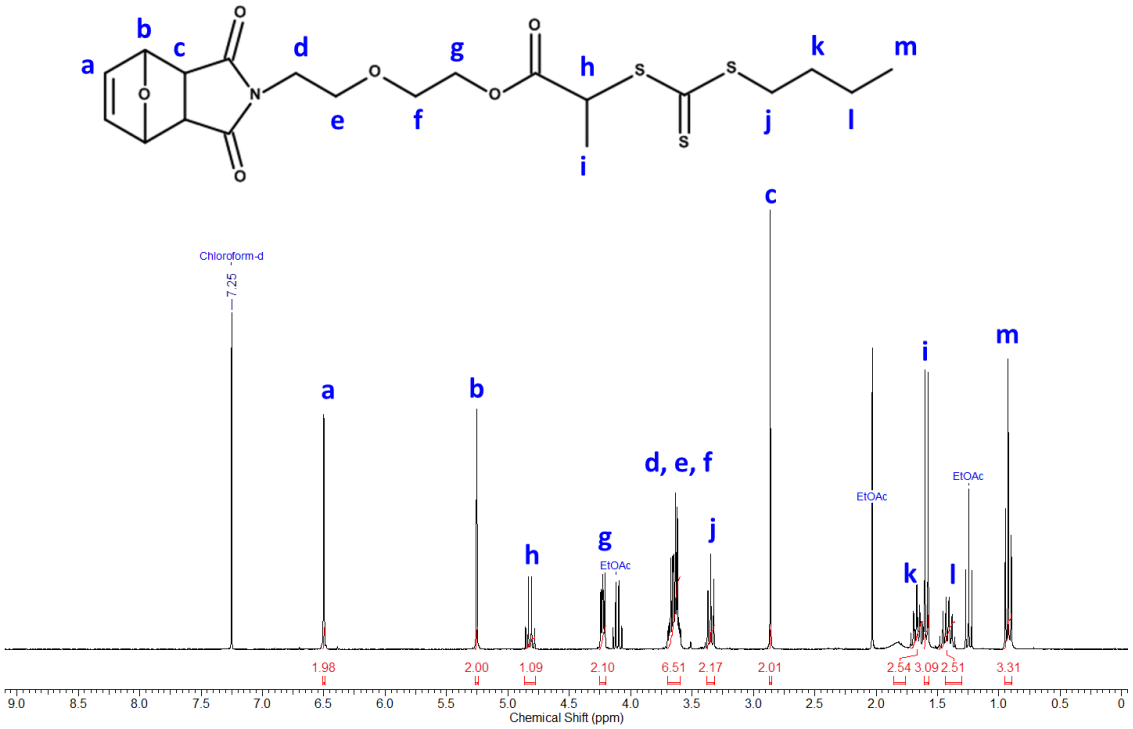

a
b
c
d
e
f
g
OH
N
S
S
EtOAc
Chloroform-d
7.25
1.00
2.02
1.01
0.97
2.04
2.04


a
b
c
d
e
f
g
h
i
j
k
l
N
S
S
S
S
O
O
EtOAc
Chloroform-d
7.25
1.00
2.04
0.99
0.98
2.03
4.13
7.30
3.19


a
b
c
d
e
f
g
h
i
j
k
l
N
S
S
S
S
O
O
EtOAc
Chloroform-d
7.25
H2O
1.36
2.79
1.38
2.00
4.12
4.13
2.85
3.10


a
b
c
O
O
O
O
c
a
b
Chloroform-d
7.25
2.00
1.99
2.00
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
0.5
0
Chemical Shift (ppm)


a
b
c
d
e
f
g
h
O
N
OH
c
a
b
chloroform-d
7.25
d, e
g
f
h
1.99
2.01
2.20
4.23
2.05
2.09
1.09
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
0.5
0
Chemical Shift (ppm)


a
b
c
d
e
f
g
h
i
j
k
l
m
O
N
S
Chloroform-d
7.25
EtOAc
1.98
2.00
1.09
2.10
6.51
2.17
2.01
2.54
3.09
2.51
3.31
Chemical Shift (ppm)