Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Towards Aspirin-Inspired Self-Immolating Molecules which Target the Cyclooxygenases

Supporting Information

Christopher R. Drake,[a]* Luis Estévez-Salmerón,[b,c] Philippe Gascard,[b,c] Yang Shen,[d] Thea D. Tlsty,[b,c] Ella F. Jones[a]

[a.] Department of Radiology and Biomedical Imaging, University of California San Francisco, San Francisco, CA 94107; [b.] Department of Pathology, University of California San Francisco, San Francisco, CA 94143; [c.] Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA 94143; [d.] Department of Electrical and Computer Engineering, TEES-AgriLife Center for Bioinformatics and Genomic Systems Engineering, Texas A&M University, 3128 TAMU, College Station, Texas 77843.

** Corresponding Author: chris.drake@ucsf.edu*

1. Synthetic Chemistry: Experimental Details

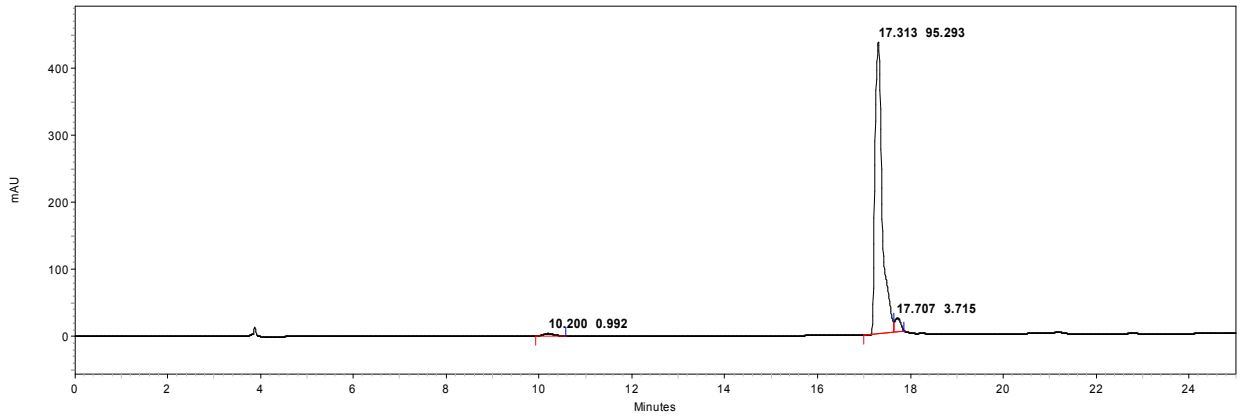
1.1. General Considerations

All commercial reagents were purchased from Sigma-Aldrich, VWR, Fisher Scientific or TCI America with purities of over 95% and, unless otherwise stated, were used as received. Anhydrous solvents were purchased from Sigma-Aldrich or VWR and used as received. Purified COX-1 (ovine) and COX-2 (human recombinant) were purchased from Cayman Chemicals. RAW264.7 murine macrophages were purchased from ATCC and cultured in DMEM + 10% heat-inactivated FBS. LPS was a kind gift from Dr. Stefanie Sowinski. Interferon-γ was purchased from Calbiochem. Media, media supplements and PBS were purchased from the University of California San Francisco's cell culture facility.

All reactions were performed in dry solvents under an inert nitrogen atmosphere and with vigorous stirring unless otherwise stated. Temperatures stated refer to the external medium. Oil baths, in combination with IKA heating mantles and thermocouples, were used to achieve elevated temperatures. Ice baths were used to cool to 0 °C. Solid CO_2 in acetone was used to cool to -78 °C. TLC was performed on EMD 60 F254 aluminum backed plates and visualized under UV light or stained with $KMnO_4$ solution. Purification over silica was achieved using EMD 60 (230-400 mesh). ^{1}H and ^{13}C NMR were recorded on a Varian 400 MHz spectrometer and processed using ACD/NMR Processor Academic Edition using residual isotopic solvent as an internal reference for organic deuterated solvents. The following abbreviations were used during assignment: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad. Mass spectra were measured by the University of Notre Dame Mass Spectrometry and Proteomics Facility. HPLC spectra were recorded on a Hitachi LaChrom Elite system using UV detection at 254 nm. UV-vis spectra were measured using a Thermo Scientific NanoDrop 2000c. Emission spectra and fluorescence data were measured using a Gemini EM fluorescence microplate reader and black-sided 96-well plates.

2. HPLC Traces:

A.



B.

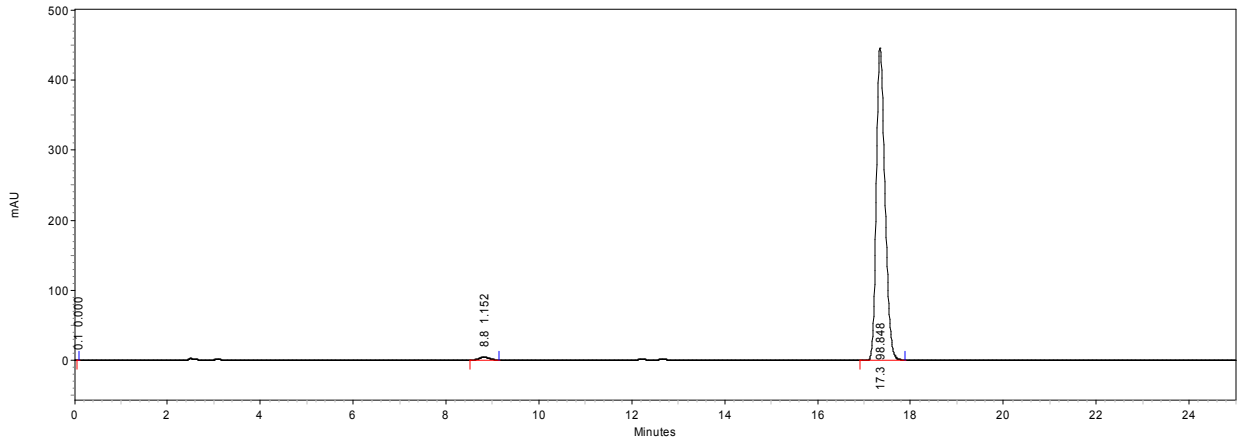


Figure S1. Measurement of Probe Purity by RP-HPLC. A. **CP-1:** The purity of **CP-1** was measured via HPLC using the following gradient of MeCN (+0.1% TFA) in H_2O (+0.1% TFA): 2-20%, 0-10 mins; 20-90%, 10-25 mins. Under these conditions 6-aminoquinoline eluted at 10.2 mins. B. **CP-2:** The purity of **CP-2** was measured via HPLC using the following gradient of MeCN (+0.1% TFA) in H_2O (+0.1% TFA): 40-60%, 0-20 mins; 60-100%, 20-25 mins. Under these conditions 6-aminoquinline eluted at 8.8 mins.

3. UV-Vis Spectra

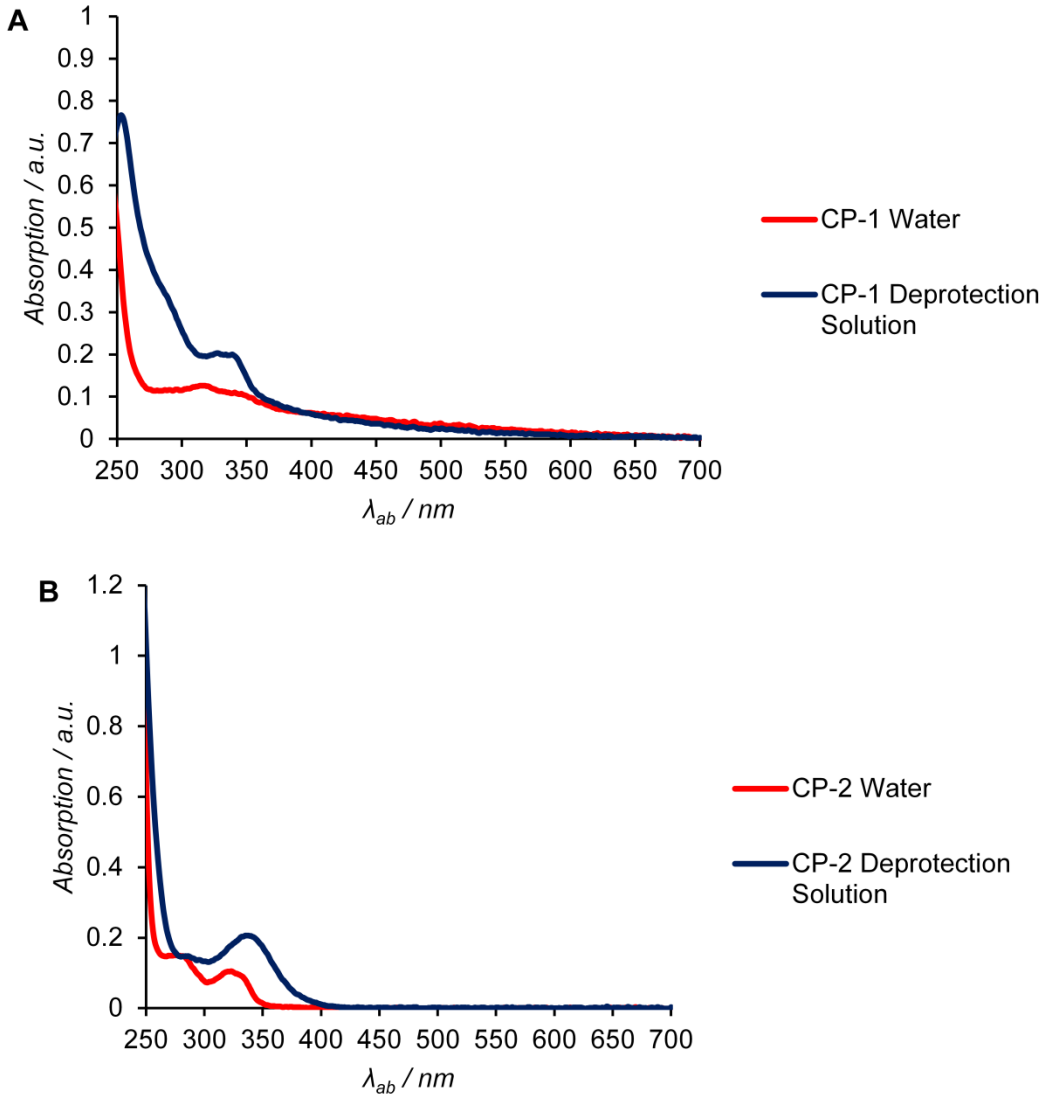
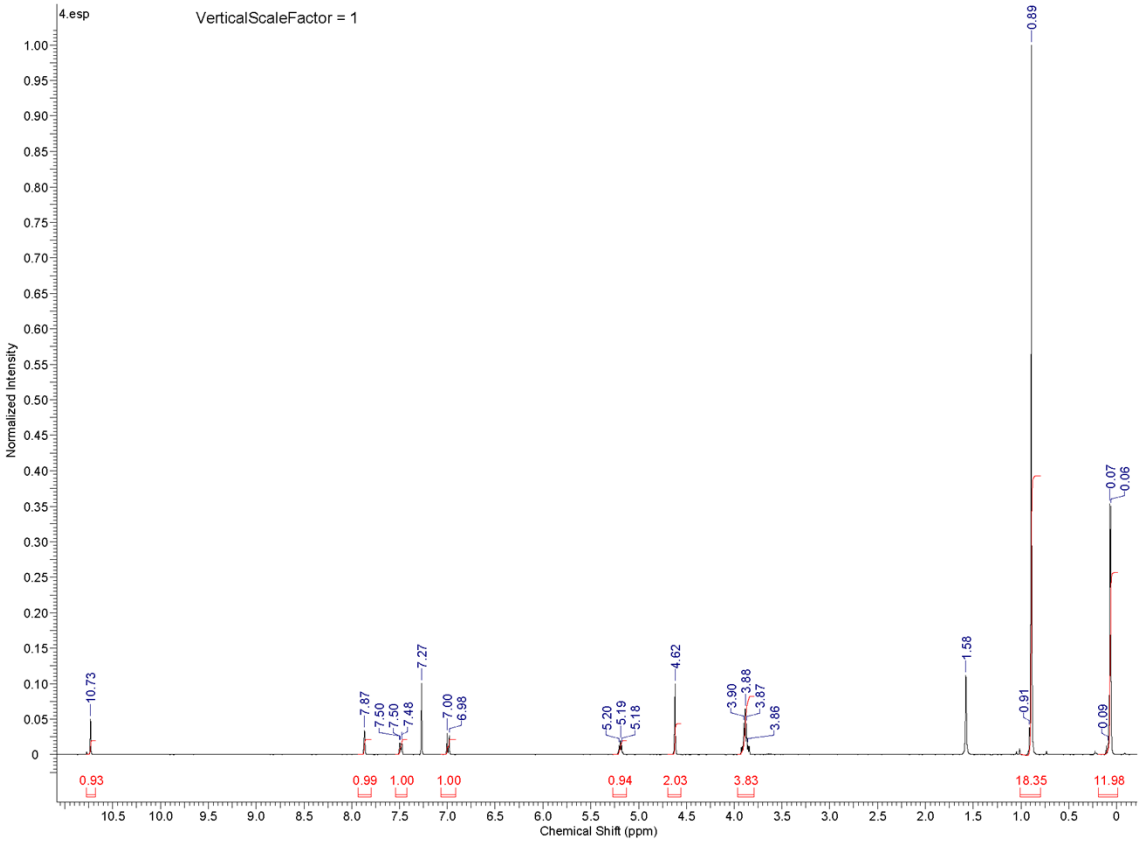
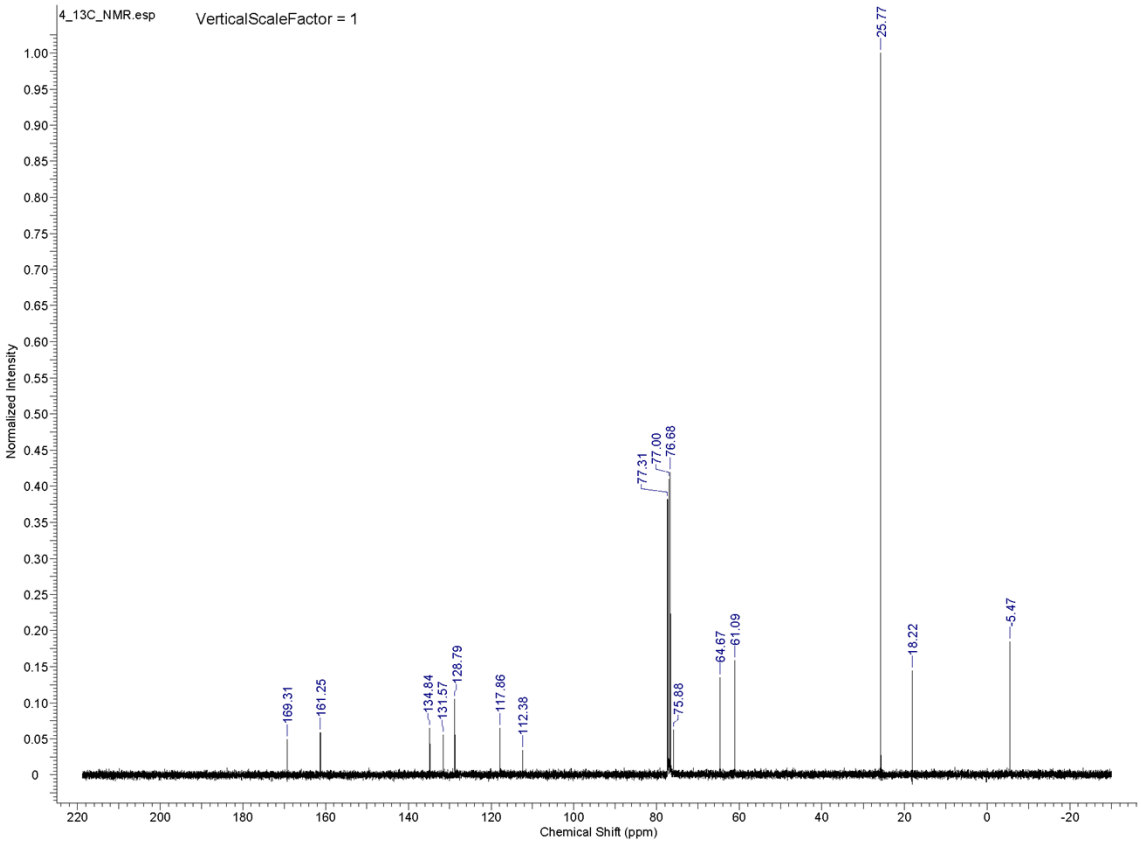


Figure S2. UV-Vis Spectra of A) **CP-1** and B) **CP-2**. Compounds were dissolved at 0.1 mgmL^{-1} in either water or deprotection buffer (2:1:1, v:v:v, methanol:water:saturated $NaHCO_3$) for 30 minutes prior to measurement of spectra.

4. NMR Spectra

1,3-Di(tert-butyldimethylsilyl)propan-2-yl 2-hydroxy-5-(hydroxymethyl)benzoate (4):

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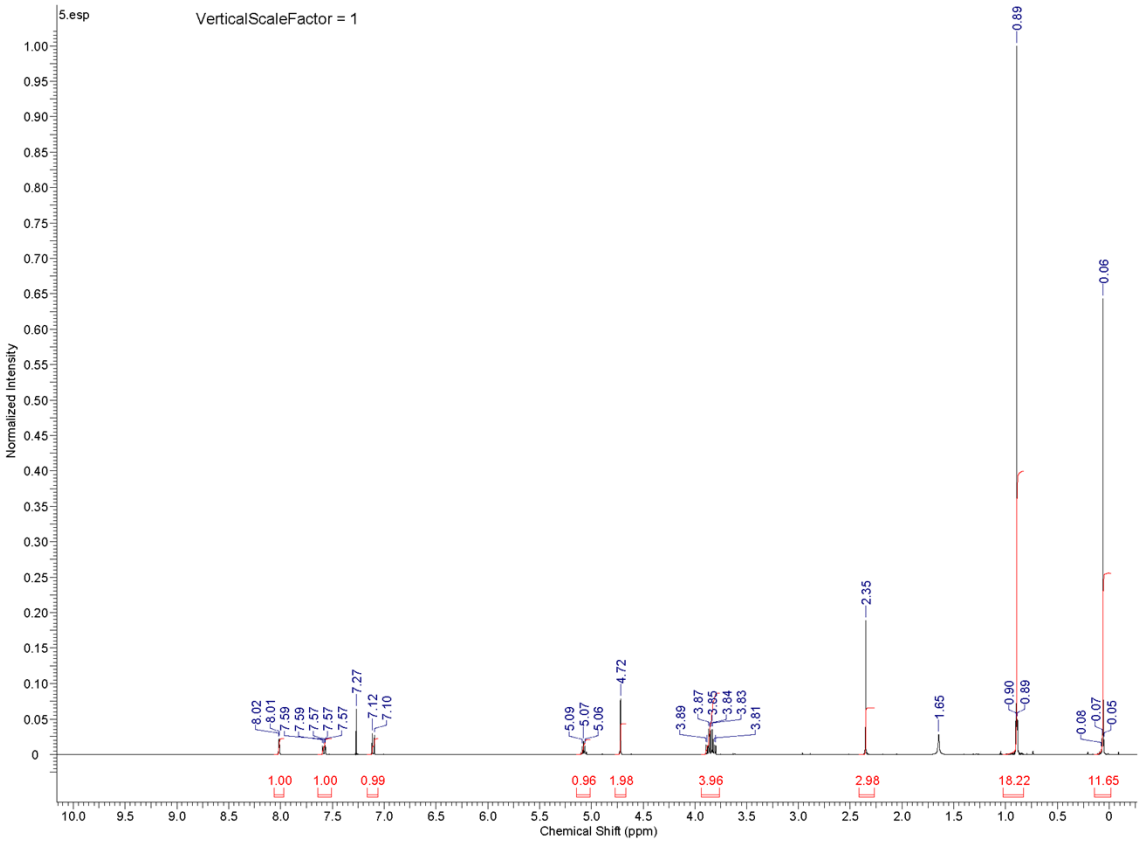
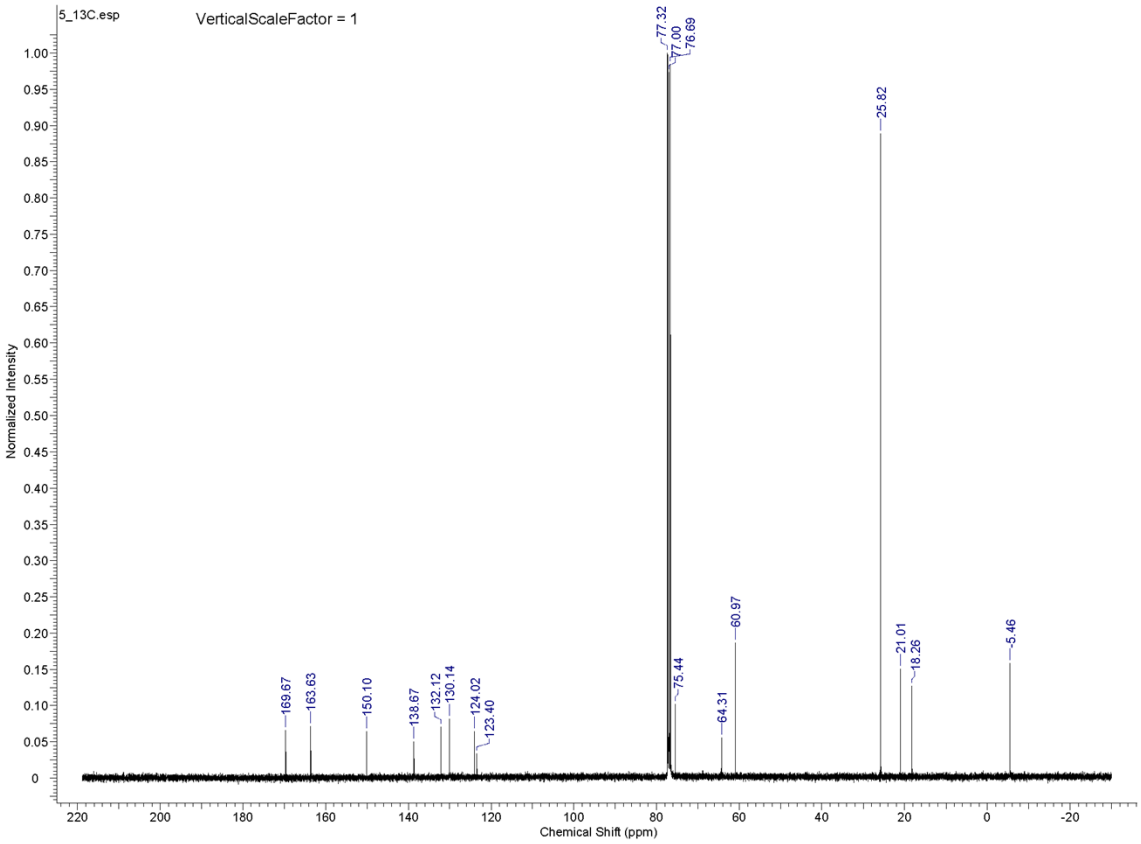
77.31
77.00
76.68
75.88
169.31
161.25
134.84
131.57
128.79
117.86
112.38
64.67
61.09
18.22
5.47

1,3-Di(tert-butyldimethylsilyl)propan-2-yl 2-(acetyloxy)-5-(hydroxymethyl)benzoate (5):

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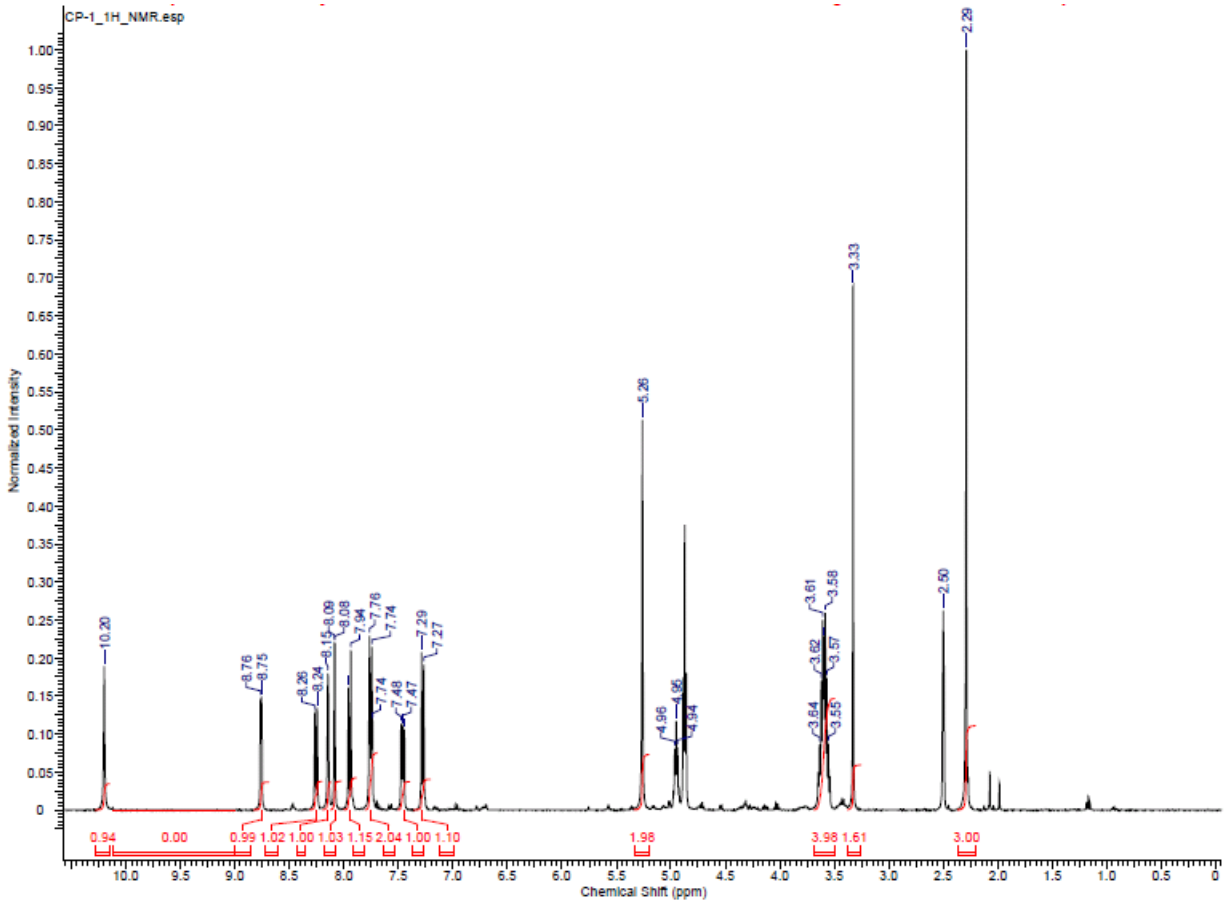
77.00
76.69
25.82
169.67
163.63
150.10
138.67
132.12
130.14
124.02
123.40
75.44
64.31
60.97
21.01
18.26
5.46
Normalized Intensity
Chemical Shift (ppm)

CP-1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Chemical Shift (ppm)
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8.75
8.26
8.24
8.15
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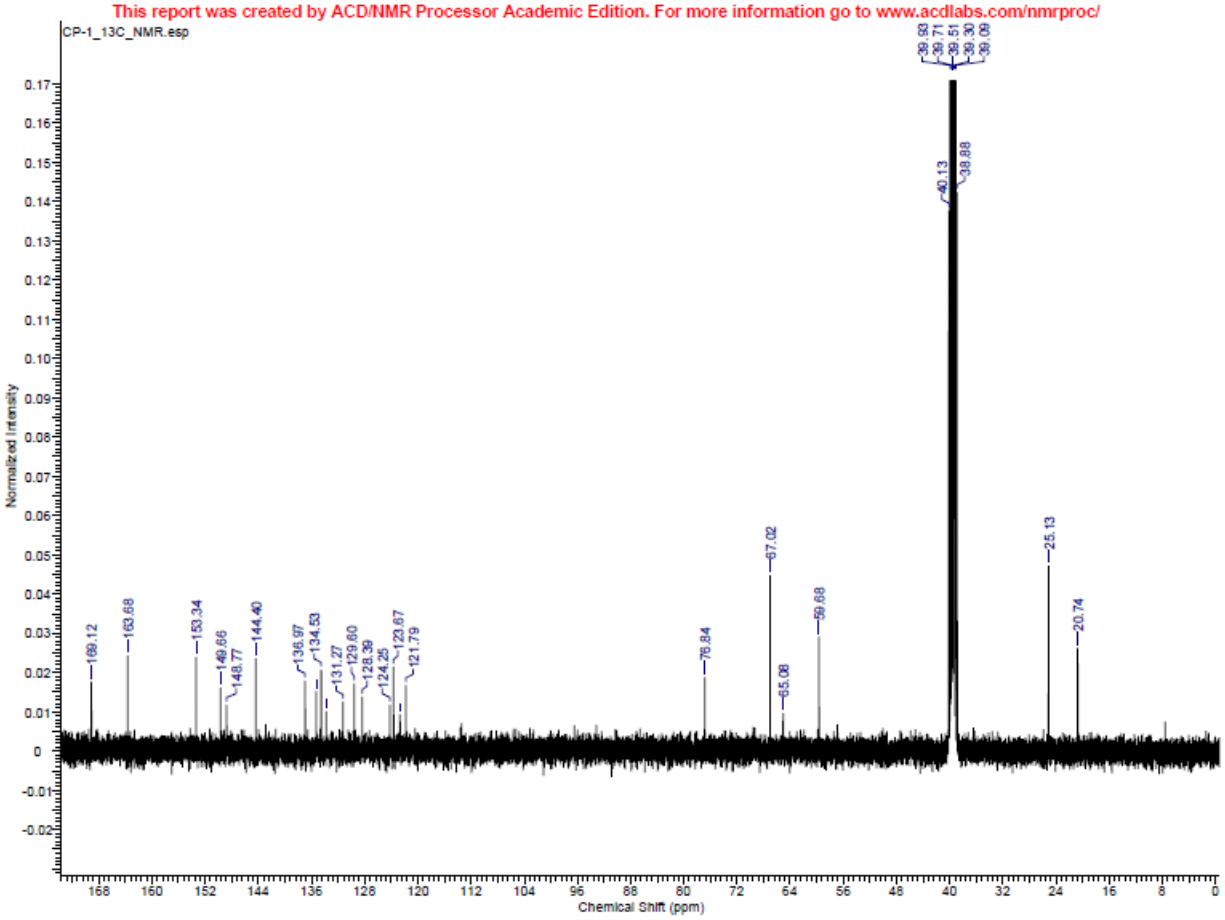
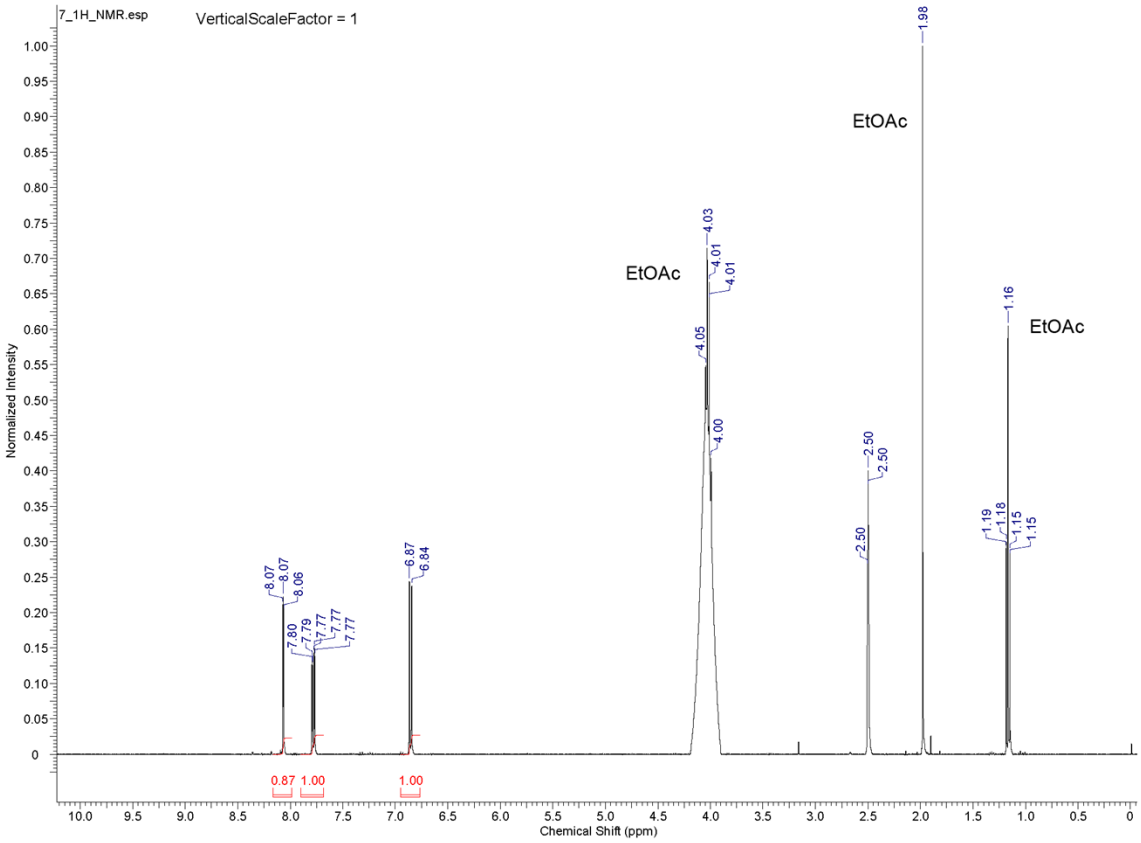
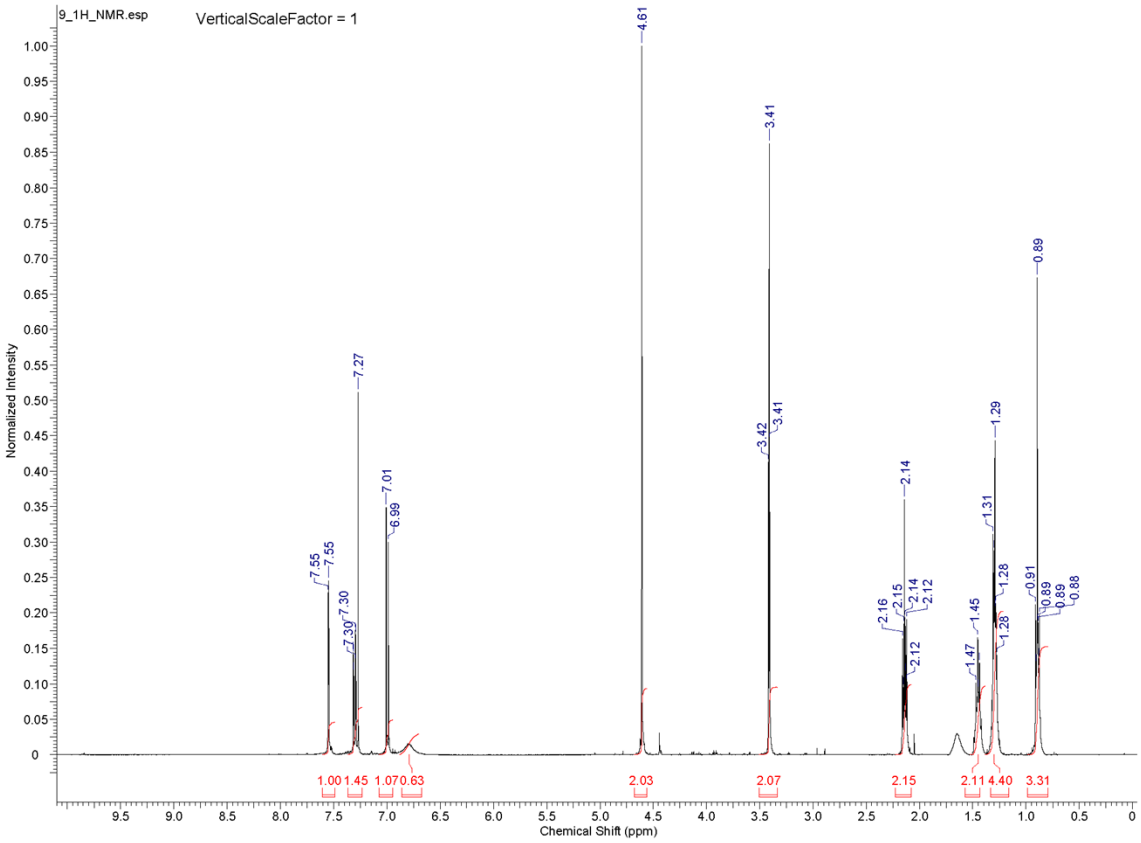
7.94
7.76
7.74
7.74
7.48
7.47
7.29
7.27
5.26
4.96
4.95
4.94
3.64
3.62
3.61
3.58
3.57
3.55
3.33
2.50
2.29
0.94
0.00
0.99
1.02
1.00
1.03
1.15
2.04
1.00
1.10
1.98
3.98
1.61
3.00

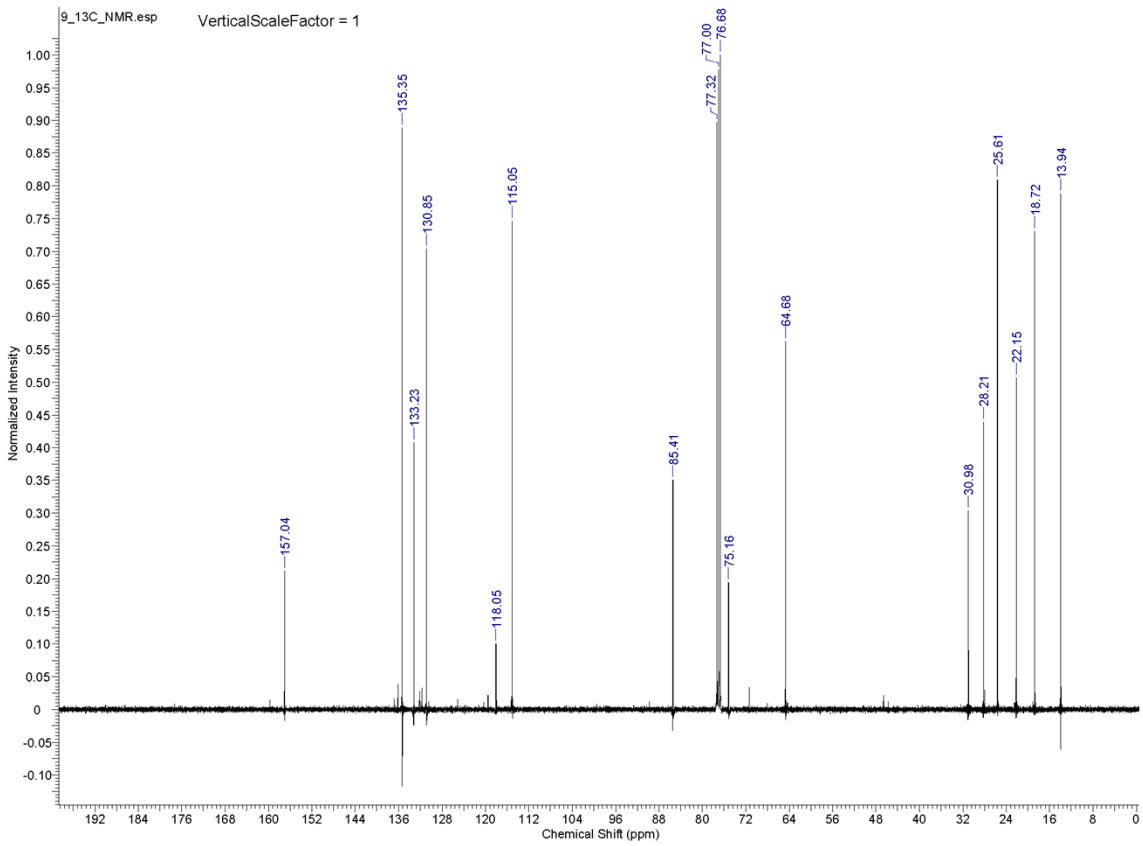


3-(Chlorosulfonyl)-4-hydroxybenzoic acid (7):



4-(Hydroxymethyl)-2-(oct-2-yn-1-ylsulfanyl)phenol (9):

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Chemical Shift (ppm)
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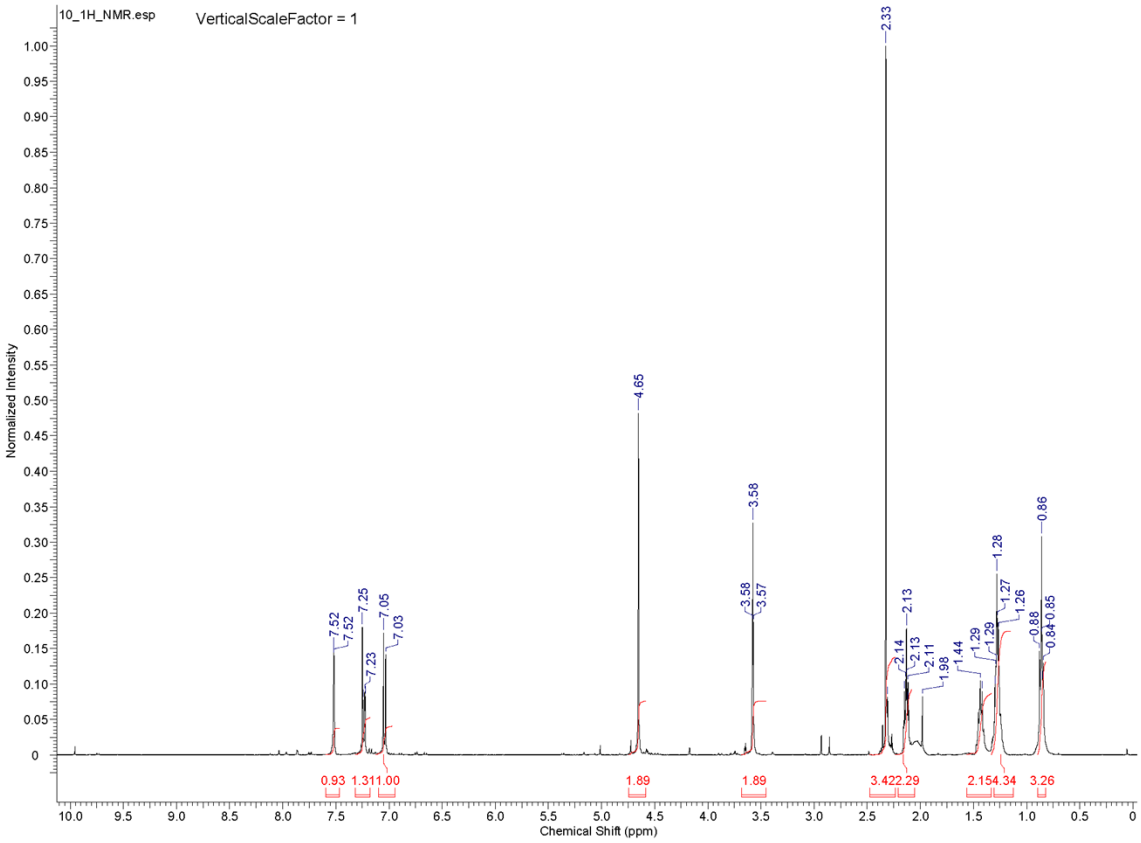
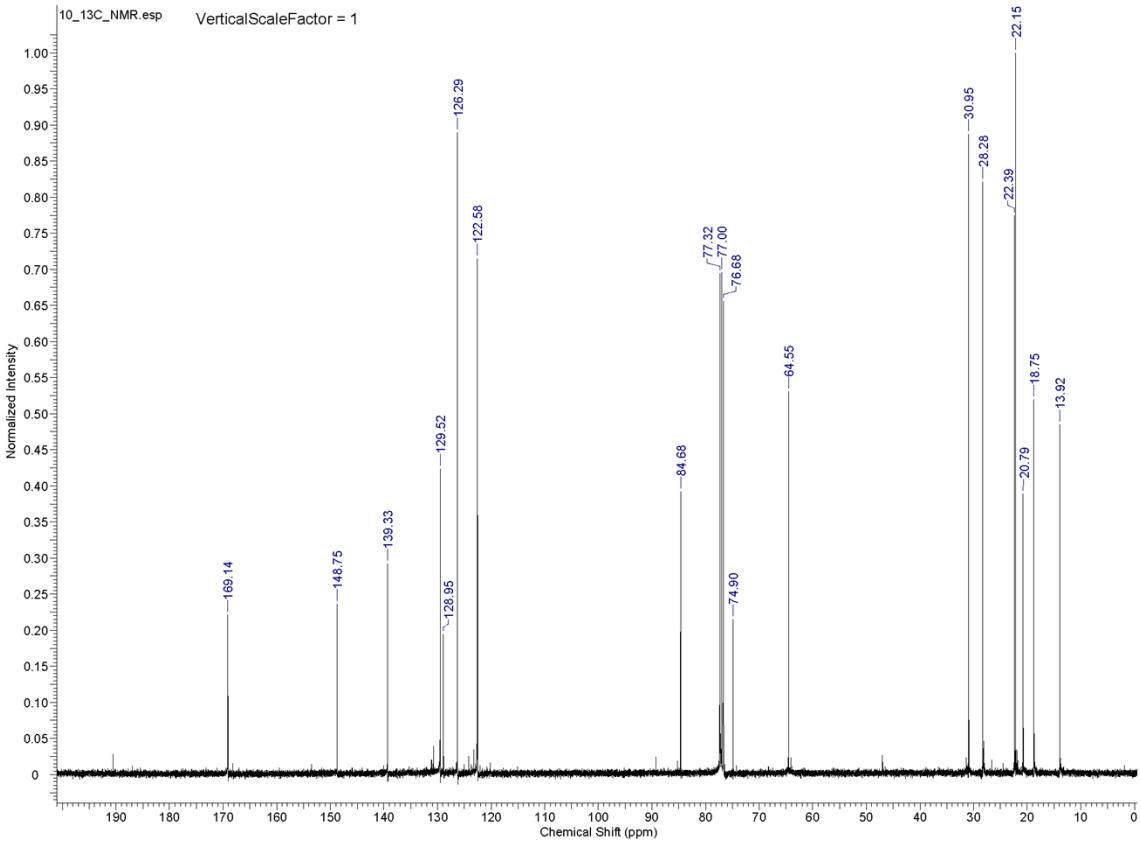
118.05
115.05
85.41
77.32
77.00
76.68
75.16
64.68
30.98
28.21
25.61
22.15
18.72
13.94

4-(Hydroxymethyl)-2-(oct-2-yn-1-ylsulfanyl)phenyl acetate (10):

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77.32
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30.95
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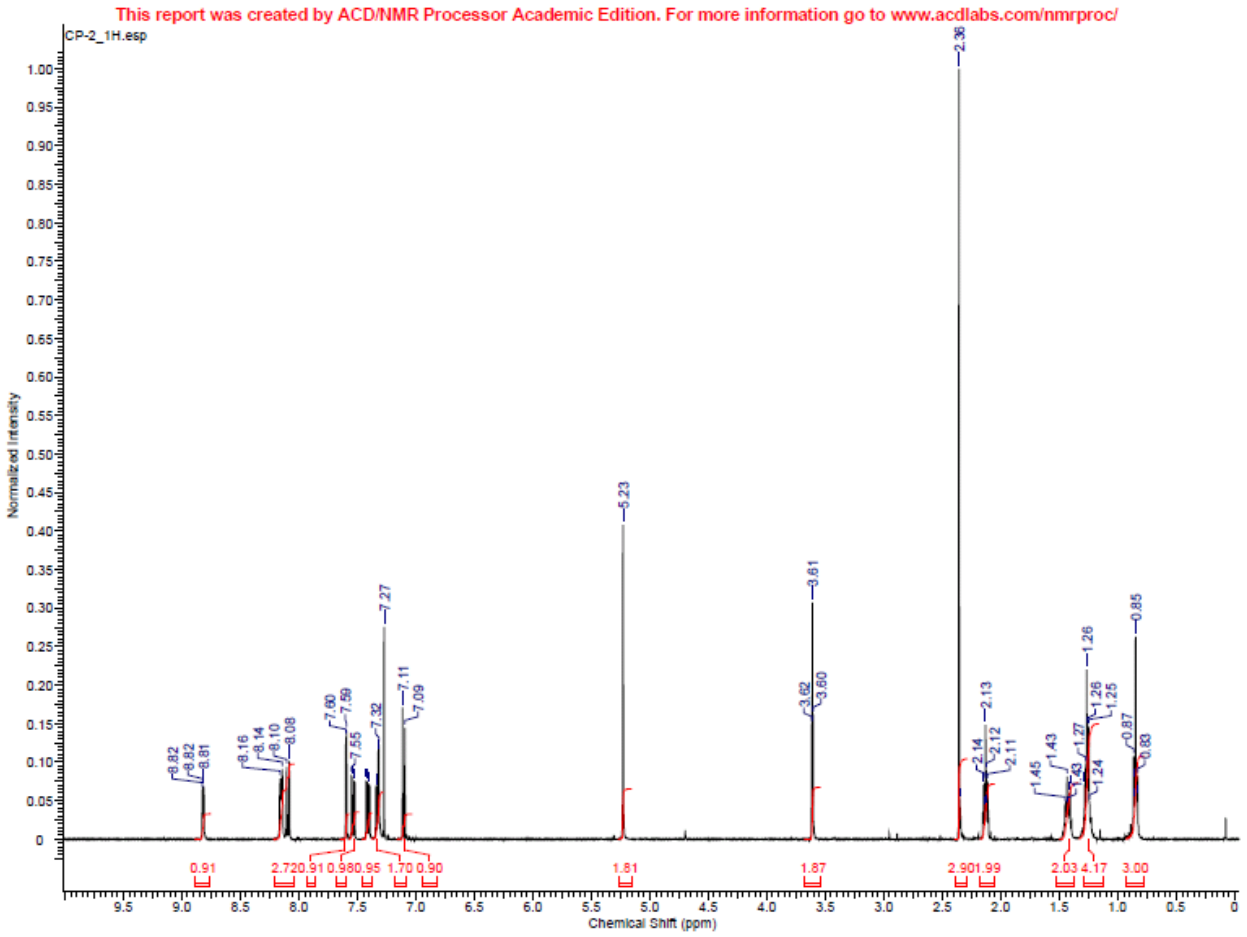
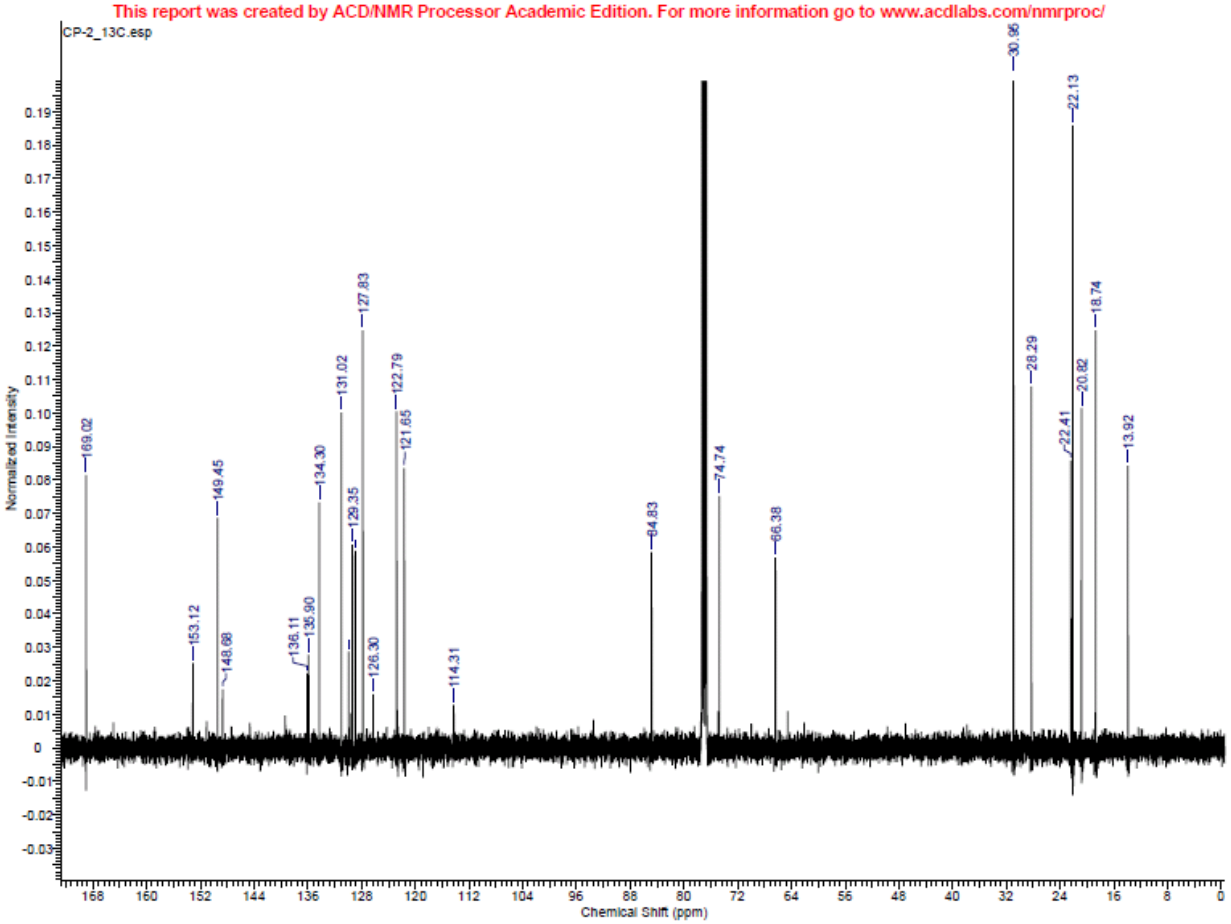
22.39
22.15
20.79
18.75
13.92

CP-2


This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
CP-2_1H.esp
Normalized Intensity
Chemical Shift (ppm)
2.36
5.23
3.61
7.27
0.85
1.26
0.91
2.72
0.91
0.98
0.95
1.70
0.90
1.81
1.87
2.90
1.99
2.03
4.17
3.00



5. Probe Stability at Various pHs:

Either **CP-1** or **CP-2** were dissolved in one of the following buffers at a concentration of 100 μM: 100 mM citrate-PBS (pH 6.0); phosphate-buffered saline (PBS, pH 7.4, 0.2 g/L KH_2PO_4, 2.16 g/L $Na_2HPO_4.7H_2O$, 0.2 g/L KCl, 8.0 g/L NaCl, 0.1 g/L $CaCl_2$, 0.1 $MgCl_2.6H_2O$); 100 mM Tris (pH 8.0), 100 mM glycine (pH 9.0). The fluorescent signal (λ_{ex} = 355 nm, λ_{em} = 535 nm) was measured and the solutions were then incubated at 37 °C for 30 mins. The fluorescent signal was measured again and the increase in fluorescence was calculated to give the data in figure S3.

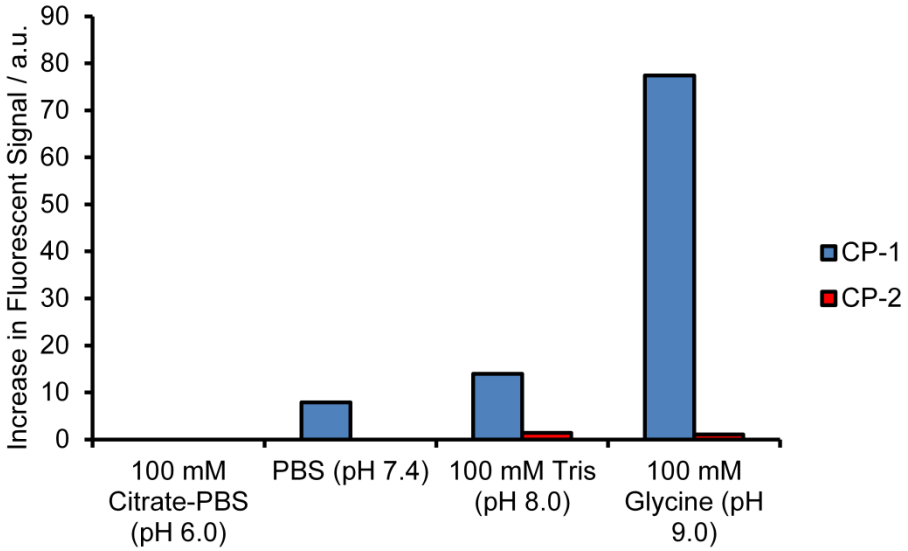


Figure S3. Stability of **CP-1** and **CP-2** against hydrolysis at various pHs.

6. Analysis of CP-1 Treated COX-2 by LC/MS/MS

All data were acquired and analyzed by MS BioWorks LLC (Ann Arbor, MI).

Data Processing: Data was searched using a local copy of Mascot with the following parameters:

Enzyme: Trypsin or none
Database: SwissProt Human (forward and reverse appended with common contaminants)
Fixed modification: Carbamidomethyl (C)
Variable modifications: Oxidation (M), Acetyl (protein N-term, KS), Deamidation (NQ)
Mass values: Monoisotopic
Peptide mass tolerance: 10 ppm
Fragment mass tolerance: 0.5 Da
Mixed mass cleavages: 3

Mascot DAT files were parsed into the Scaffold software for validation, filtering and to create a nonredundant list per sample. Data were filtered using a minimum protein value of 99.9%, a minimum peptide value of 50% (Prophet scores) and requiring at least two unique peptides per protein.

Results: The target protein, PGH2_HUMAN, was observed with 96% sequence coverage after combining the data from all three enzymes:

sp|P35354|PGH2_HUMAN (100%), 68,997.8 Da; Prostaglandin G/H synthase 2 OS=Homo sapiens GN=PTGS2 PE=1 SV=2

661 unique peptides, 1048 unique spectra, 1439 total spectra, 580/604 amino acids (96% coverage)

A.

MLARALLLCA
TRIKLFLKPT
GYKSWEAFSN
NMMFAFFAQH
QIIDGEMYPP
VLKQEHPEWG
NRIAAEFNTL
AGGRNVPPAV
YGDIDAVELY
GFQIINTASI
STEL

VLALSHTANP
PNTVHYILTH
LSYYTRALPP
FTHQFFKTDH
TVKDTQAEMI
DEQLFQTSRL
YHWHPLLPDT
QKVSQASIDQ
PALLVEKPRP
QSLICNNVKG

CCSHPCQNRG
FKGFWNVVNN
VPDDCPTPLG
KRGPAFTNGL
YPPQVPEHLR
ILIGETIKIV
FQIHDQKYNY
SRQMKYQSFN
DAIFGETMVE
CPFTSFSVPD

VCMSVGFDQY
IPFLRNAIMS
VKGKKQLPDS
GHGVDLNHIY
FAVGQEVFGL
IEDYVQHLSG
QQFIYNNSIL
EYRKRFMLKP
VGAPFSLKGL
PELIKTVTIN

KCDCTRTGFY
YVLTSRSHLI
NEIVEKLLLR
GETLARQRKL
VPGLMMYATI
YHFKLKFDPE
LEHGITQFVE
YESFEELTGE
MGNVICSPAY
ASSSRSGLDD

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DSPPTYNADY
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B.

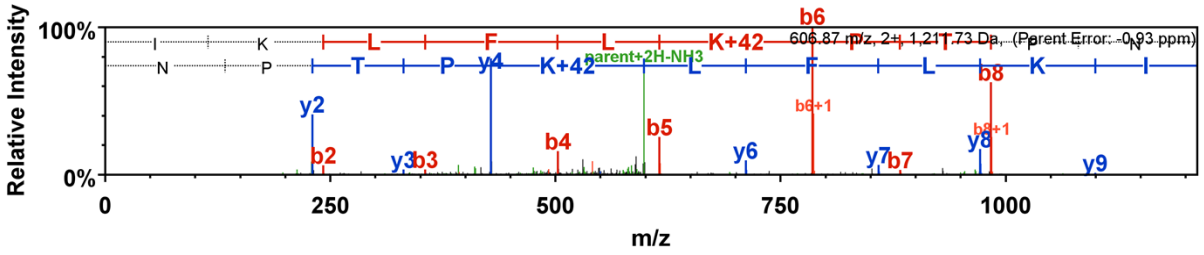

Figure S4. A. Summary of acetylation sites on **CP-1** treated COX-2, as measured via LC/MS/MS analysis following enzymatic degradation. Lysine acetylation sites highlighted in green, serine acetylation sites in light blue. Non-highlighted residues not covered by analysis. B. Typical mass spectrum for lysine acetylation site (R)IKLFLKPTPN(T).