SUPPORTING INFORMATION

The Chemical Basis of Thiol Addition to Nitro-Conjugated Linoleic Acid, a Protective Cell-Signaling Lipid*

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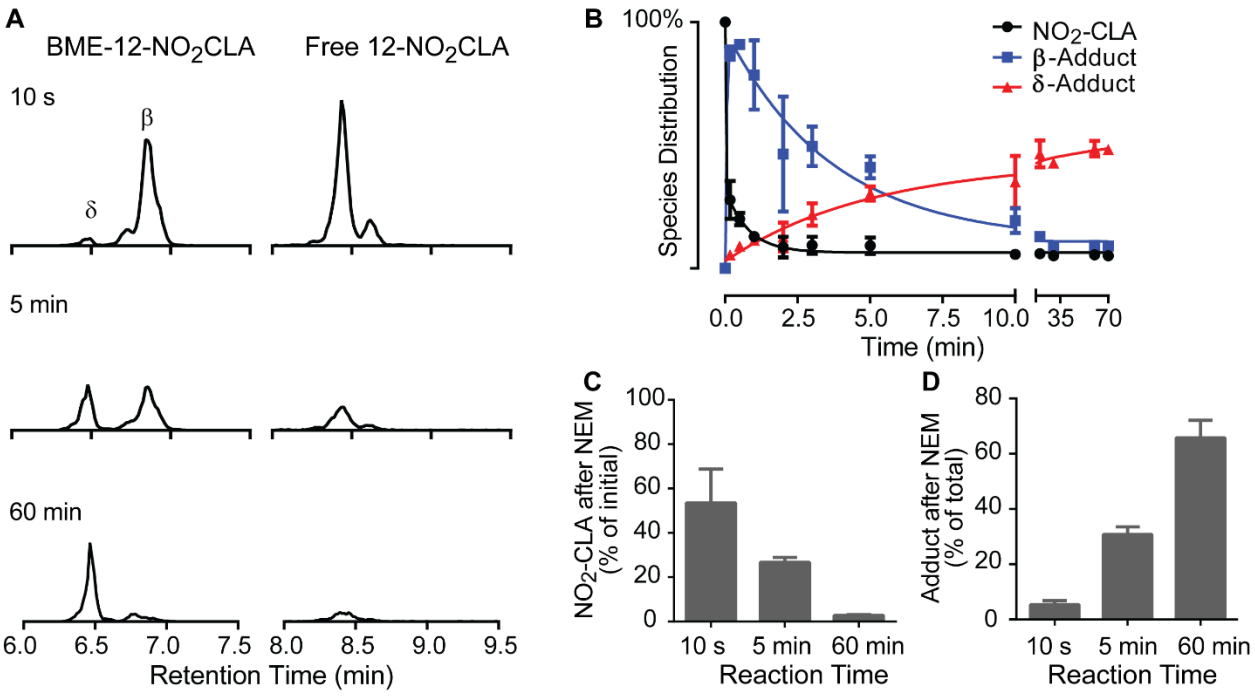

FIGURE S1. LC-MS/MS analysis of the reaction between 12-NO_2-CLA and BME. (A) 12-NO_2-CLA (10 μM) was reacted with BME (1.76 mM) and aliquots obtained at 10 s (top), 5 min (middle) and 60 min (bottom) for analysis of free 12-NO_2-CLA (right) and BME-NO_2-CLA adducts (left). (B) Representative time-course for 12-NO_2-CLA reaction with BME. (C-D) Aliquots collected at the indicated times were incubated with NEM (100 mM); the percentage of free NO_2-CLA with respect to that at time zero (C) and the percentage of BME-NO_2-CLA adduct with respect to NEM-untreated controls (D) were determined. Data are representative from 3 independent experiments.

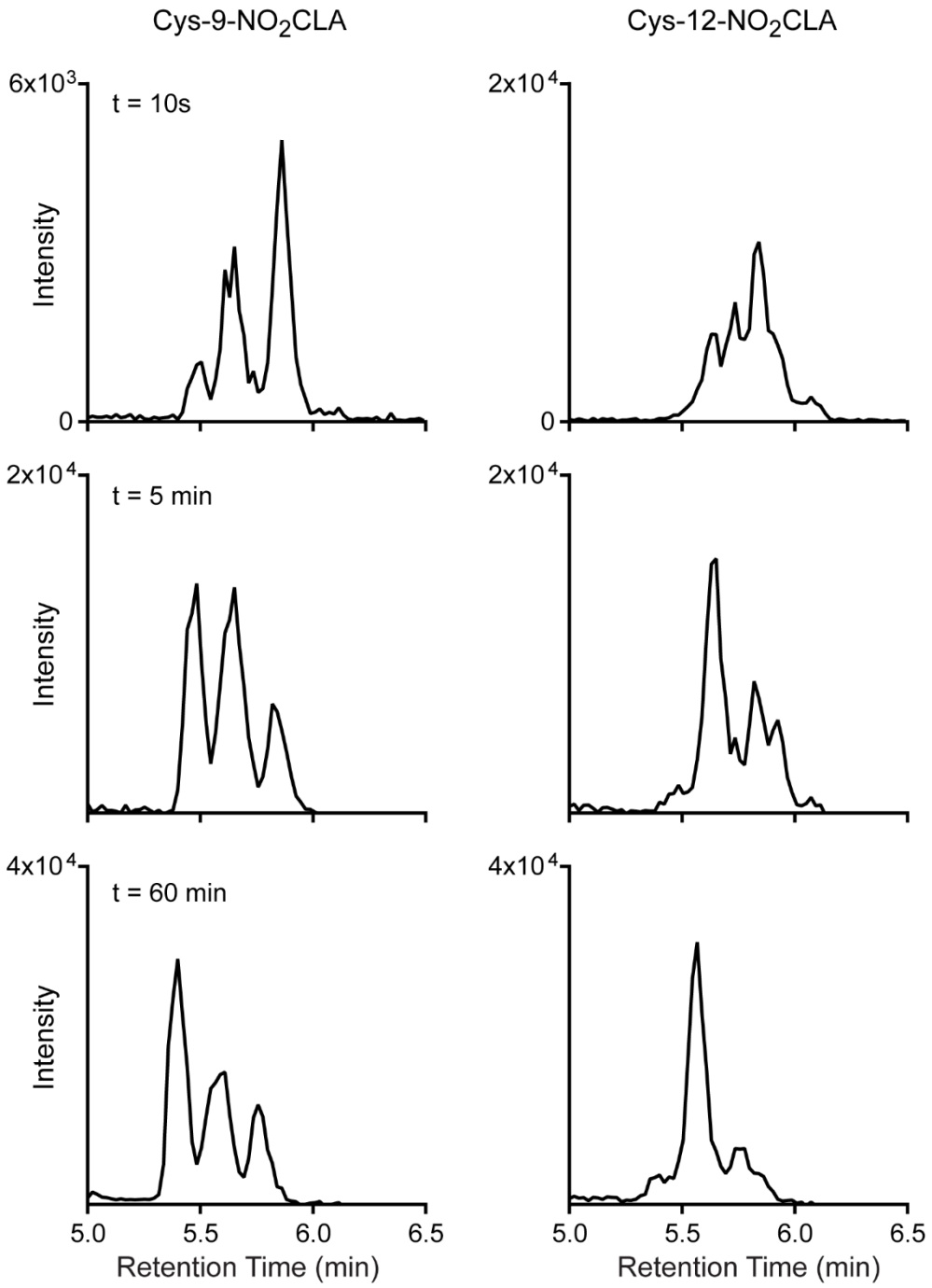

FIGURE S2. LC-MS/MS analysis of the reaction between NO_2-CLA and cysteine. 9- or 12-NO_2-CLA (10 μM) was reacted with cysteine (500 μM) and aliquots obtained at 10 s (top), 5 min (middle) and 60 min (bottom) for Cys-NO_2-CLA adduct analysis.

TABLE S1. Structural data. Calculated structural data (in Å) and energies (in Hartrees) for all the species taking part in the thiolate β-/δ-adduction by a conjugated nitroalkene model compound and further protonation. Data corresponds to structures in aqueous solution fully optimized at the PCM(IEF,water)-ωB97X-D/6-31+G(d,p) level of theory, using an ultrafine pruned (99.590) grid for numerical calculations.

RC_{β} - E_{el} =-877.2285238 au

1	6	0.000000	0.000000	0.000000
2	6	0.000000	0.000000	1.348484
3	8	2.327078	0.000000	0.029158
4	16	1.165473	-3.688697	1.651632
5	6	1.082458	-3.416196	-0.164808
6	1	0.964731	0.001235	1.845084
7	1	2.051081	-3.107376	-0.564737
8	1	0.782244	-4.328529	-0.685424
9	1	0.355615	-2.637116	-0.409007
10	6	-1.169357	0.001540	-0.924965
11	1	-1.162324	0.883245	-1.568253
12	1	-1.165227	-0.883282	-1.564566
13	1	-2.088546	0.003999	-0.341596
14	7	1.295045	-0.000208	-0.643460
15	8	1.316011	-0.002119	-1.875547
16	6	-1.167725	0.020084	2.197278
17	1	-2.155872	-0.005285	1.747513
18	6	-1.040310	0.082649	3.534594
19	1	-0.036716	0.108300	3.955052
20	6	-2.168791	0.117334	4.505053
21	1	-3.137878	0.075253	4.005015
22	1	-2.092136	-0.721827	5.202589
23	1	-2.121526	1.028593	5.108568

TS_{β} - E_{el} =-877.2209917 au

1	6	-0.030175	-0.001457	-0.023696
2	6	-0.039640	0.035700	1.359718
3	8	2.268643	0.140072	0.017484
4	16	0.410435	-2.467576	2.041122
5	6	0.866557	-3.070086	0.385925
6	1	0.896266	0.275385	1.846151
7	1	1.945280	-3.205722	0.284551
8	1	0.371594	-4.016133	0.156892
9	1	0.546150	-2.339699	-0.366413
10	6	-1.214356	-0.183850	-0.918534
11	1	-1.290286	0.621978	-1.652084
12	1	-1.150211	-1.126242	-1.469916
13	1	-2.129589	-0.197438	-0.329716
14	7	1.209304	0.064971	-0.648966
15	8	1.252318	0.055278	-1.902879
16	6	-1.248035	0.235142	2.145733
17	1	-2.205902	-0.008720	1.695157
18	6	-1.213101	0.734351	3.389976
19	1	-0.246981	0.966218	3.835012
20	6	-2.418866	0.997003	4.230260
21	1	-3.339867	0.753175	3.697020
22	1	-2.380730	0.403258	5.148658
23	1	-2.456638	2.046137	4.537768

Anionic I_{β} - E_{el} =-877.2460934 au

1	6	-0.073638	-0.004986	-0.032449
2	6	-0.045932	0.010292	1.463341
3	8	2.222444	0.061392	-0.108344
4	16	-0.656023	-1.600847	2.164271

5	6	0.354220	-2.771642	1.221522
6	1	0.985346	0.124483	1.791499
7	1	1.412560	-2.639001	1.446942
8	1	0.043425	-3.775469	1.510783
9	1	0.188238	-2.637338	0.151533
10	6	-1.353020	-0.116920	-0.795023
11	1	-1.464764	0.693253	-1.520791
12	1	-1.401345	-1.058414	-1.352984
13	1	-2.201684	-0.086651	-0.110276
14	7	1.060306	0.005289	-0.696624
15	8	1.058098	-0.035436	-1.997617
16	6	-0.892873	1.099480	2.059334
17	1	-1.922485	1.169313	1.711986
18	6	-0.441161	1.957206	2.977484
19	1	0.590760	1.865074	3.313984
20	6	-1.245626	3.058945	3.594936
21	1	-1.297164	2.939267	4.681065
22	1	-0.779192	4.029890	3.403499
23	1	-2.263903	3.080233	3.200845

Neutral O-Protonated I_β (aci-Nitro form) - E_{el} =-877.7069732 au

1	6	-0.017085	-0.015473	-0.007226
2	6	0.008716	0.047546	1.492478
3	8	2.280875	0.120542	0.000654
4	16	-0.634005	-1.530412	2.201197
5	6	0.455567	-2.721184	1.379586
6	1	1.032123	0.164599	1.842693
7	1	1.500209	-2.502534	1.602286
8	1	0.202414	-3.704995	1.774030
9	1	0.295399	-2.714772	0.300784
10	6	-1.281402	-0.157693	-0.783743
11	1	-1.373682	0.640731	-1.523560
12	1	-1.290391	-1.109754	-1.321699
13	1	-2.138283	-0.127999	-0.113013
14	7	1.082694	0.009071	-0.690838
15	8	1.201777	-0.050413	-1.954614
16	6	-0.833211	1.175862	2.022766
17	1	-1.855237	1.246747	1.656345
18	6	-0.377707	2.048633	2.923795
19	1	0.647829	1.946001	3.275527
20	1	2.930494	0.111079	-0.720314
21	6	-1.170895	3.175614	3.506537
22	1	-1.235235	3.076615	4.593878
23	1	-0.684149	4.133322	3.301289
24	1	-2.183873	3.206316	3.100630

Neutral C_α-Protonated I_β (unsaturated Nitroalkane form) - E_{el} =-877.7202186 au

1	6	-0.007805	0.072095	0.021788
2	8	0.075726	0.244197	2.978449
3	16	1.750066	0.008412	-0.504966
4	6	2.520571	-0.851976	0.894961
5	1	-0.204315	-0.845317	0.582258
6	1	1.968456	-1.761435	1.133040
7	1	3.526958	-1.119028	0.573722
8	1	2.590868	-0.212638	1.773729
9	6	-0.142509	2.640694	0.312666
10	1	-0.320520	3.427178	1.045158
11	1	0.866505	2.740255	-0.087481
12	1	-0.858568	2.759382	-0.500772
13	7	0.427605	1.130693	2.212269
14	8	1.383517	1.861402	2.417978
15	6	-0.882772	0.098383	-1.200585
16	1	-0.742621	0.925846	-1.893220
17	6	-1.803187	-0.832907	-1.453958
18	1	-1.925197	-1.650192	-0.744884
19	6	-0.355665	1.276935	0.929843
20	1	-1.387736	1.145238	1.255690
21	6	-2.705790	-0.839508	-2.647954
22	1	-2.509226	0.010807	-3.303852
23	1	-2.577200	-1.760868	-3.223290
24	1	-3.753314	-0.804636	-2.335810

RC_{δ} - E_{el} =-877.2281082 au

1	6	0.055903	0.102084	0.027721
2	6	0.025665	0.116385	1.471004
3	6	1.161923	0.110287	2.190985
4	16	2.144564	-3.685580	1.609583
5	6	0.676462	-3.504974	0.517603
6	1	-0.932020	0.135833	1.981387
7	1	2.113093	0.089615	1.662758
8	1	0.911589	-3.788680	-0.510801
9	1	-0.146836	-4.135740	0.861072
10	1	0.320794	-2.470884	0.507717
11	1	1.034375	0.069148	-0.440907
12	6	-1.024573	0.129521	-0.778943
13	7	-0.768000	0.116180	-2.203303
14	8	-1.743514	0.155852	-2.954664
15	8	0.386532	0.066199	-2.631540
16	6	-2.465605	0.170382	-0.394987
17	1	-3.012153	-0.667912	-0.830317
18	1	-2.558594	0.114989	0.688171
19	1	-2.942450	1.092634	-0.733010
20	6	1.226446	0.140033	3.679476
21	1	0.232251	0.148089	4.129196
22	1	1.779832	-0.727860	4.049693
23	1	1.774540	1.026550	4.012977

TS_{δ} - E_{el} =-877.2191037 au

1	6	0.006212	-0.025848	0.011560
2	6	0.000693	-0.034170	1.418136
3	6	1.185567	-0.069602	2.121356
4	16	1.862511	-2.639213	2.355308
5	6	1.145100	-3.145119	0.763694
6	1	-0.937668	-0.032202	1.964389
7	1	2.105174	0.014817	1.553674
8	1	1.914187	-3.325397	0.010014
9	1	0.538960	-4.047730	0.862224
10	1	0.492858	-2.342194	0.392440
11	1	0.979074	-0.041300	-0.471193
12	6	-1.091508	0.032049	-0.821916
13	7	-0.850781	0.063992	-2.195504
14	8	-1.833731	0.124542	-2.967214
15	8	0.312564	0.030063	-2.658856
16	6	-2.524750	0.056806	-0.399374
17	1	-3.085041	-0.767030	-0.847485
18	1	-2.592767	-0.036410	0.683709
19	1	-3.016387	0.988060	-0.692355
20	6	1.267992	0.234997	3.581549
21	1	0.360796	-0.068252	4.106919
22	1	2.128667	-0.245656	4.045129
23	1	1.383952	1.318430	3.703803

Anionic I_{δ} - E_{el} =-877.2561252 au

1	6	0.031575	-0.048783	0.005699
2	6	-0.006345	0.036036	1.347994
3	6	1.216539	-0.040290	2.204255
4	16	1.331820	-1.700402	3.010336
5	6	1.620451	-2.723099	1.546952
6	1	-0.950098	0.130702	1.881348
7	1	2.111062	0.051604	1.581847
8	1	2.590432	-2.493548	1.103807
9	1	1.602932	-3.765355	1.863463
10	1	0.834218	-2.549490	0.809715
11	1	0.998981	-0.140748	-0.478443
12	6	-1.108103	-0.024018	-0.887907
13	7	-0.893822	-0.026352	-2.195847
14	8	-1.894536	-0.007458	-3.018509
15	8	0.299338	-0.045923	-2.707389
16	6	-2.526886	0.003061	-0.415056
17	1	-3.104194	-0.816577	-0.851323

18	1	-2.569072	-0.092049	0.669841
19	1	-3.029412	0.934570	-0.693991
20	6	1.232392	1.019595	3.300191
21	1	0.360375	0.921961	3.952360
22	1	2.133524	0.944961	3.912256
23	1	1.204924	2.014125	2.848866

Neutral O-Protonated I_{δ} (aci-Nitro form) - E_{el} =-877.7158051 au

1	6	0.011816	0.011314	0.008246
2	6	0.004542	-0.018465	1.349084
3	6	1.257009	0.015215	2.163810
4	16	1.634983	-1.680095	2.779280
5	6	1.945836	-2.532761	1.214729
6	1	-0.922879	-0.096910	1.910340
7	1	2.102140	0.319507	1.540752
8	1	2.803982	-2.097456	0.701340
9	1	2.156486	-3.575635	1.451212
10	1	1.068113	-2.477156	0.569220
11	1	0.963012	0.078787	-0.507358
12	6	-1.179877	-0.034131	-0.824928
13	7	-1.059531	-0.011598	-2.121866
14	8	-1.994849	-0.026795	-2.980396
15	8	0.221566	0.029393	-2.650807
16	6	-2.581074	-0.081531	-0.312090
17	1	-3.121933	-0.919263	-0.757093
18	1	-2.595828	-0.188898	0.769592
19	1	-3.112551	0.835133	-0.581219
20	6	1.135772	0.942497	3.370226
21	1	0.310512	0.632061	4.016351
22	1	2.057353	0.944584	3.954998
23	1	0.938829	1.961361	3.030444
24	1	0.049215	0.040715	-3.605889

Neutral C_{α}-Protonated I_{δ} (unsaturated Nitroalkane form) - E_{el} =-877.7243552 au

1	6	0.003831	-0.008805	0.012334
2	6	0.033649	-0.046921	1.344122
3	6	1.301164	0.015738	2.138381
4	16	1.670242	-1.633470	2.864647
5	6	1.959736	-2.594248	1.359891
6	1	-0.886584	-0.129038	1.918244
7	1	2.137989	0.266954	1.481269
8	1	2.863685	-2.257149	0.851445
9	1	2.082448	-3.634710	1.659448
10	1	1.106884	-2.507246	0.684905
11	1	0.933050	0.077625	-0.544631
12	7	-1.167011	-1.081419	-1.871870
13	8	-1.778792	-0.894016	-2.913830
14	8	-0.534027	-2.097497	-1.632886
15	6	-2.558422	-0.191389	-0.065860
16	1	-3.395357	-0.179333	-0.763638
17	1	-2.556702	-1.132195	0.486143
18	1	-2.683102	0.629912	0.639822
19	6	1.215817	1.027477	3.277576
20	1	0.407723	0.771838	3.968274
21	1	2.152614	1.066027	3.836267
22	1	1.013606	2.020555	2.870969
23	6	-1.245799	0.006608	-0.812995
24	1	-1.291381	0.919007	-1.408913

Neutral C_{γ}-Protonated I_{δ} (Nitroalkene form) - E_{el} =-877.7253594 au

1	6	-0.248169	-0.017546	-0.041080
2	6	-0.070527	-0.120793	2.485243
3	16	1.759216	-0.194898	2.568914
4	6	2.243093	1.237839	1.568305
5	1	-0.337170	0.939182	2.529877
6	1	1.721685	2.132443	1.911174
7	1	3.315365	1.373937	1.709055
8	1	2.040177	1.077609	0.509611
9	1	-0.378065	1.060080	-0.039521

10	6	0.235518	-0.581720	-1.153074
11	7	0.557070	0.330404	-2.252770
12	8	0.869959	-0.174421	-3.327266
13	8	0.519653	1.545749	-2.081393
14	6	0.490016	-2.021249	-1.452941
15	1	1.547749	-2.195822	-1.659191
16	1	0.194964	-2.628789	-0.600150
17	1	-0.079715	-2.344386	-2.325193
18	6	-0.631376	-0.836792	3.710723
19	1	-0.283710	-1.872956	3.751622
20	1	-0.334004	-0.332732	4.631491
21	1	-1.722616	-0.850009	3.658726
22	6	-0.649869	-0.734142	1.202099
23	1	-0.401945	-1.796342	1.160495
24	1	-1.741328	-0.653621	1.284068

Isolated fragments

Conjugated nitroalkene model (2-nitro-hexa-2,4-diene) - E_{el} =-439.0230288 au

1	6	0.000000	0.000000	0.000000
2	6	0.000000	0.000000	1.347816
3	8	2.328755	0.000000	0.029273
4	1	0.966042	0.000354	1.842261
5	6	-1.169111	0.000113	-0.925273
6	1	-1.163395	0.882993	-1.566935
7	1	-1.164001	-0.883055	-1.566542
8	1	-2.088361	0.000637	-0.341899
9	7	1.296899	-0.000079	-0.643704
10	8	1.317491	-0.000092	-1.875010
11	6	-1.164365	0.001093	2.200708
12	1	-2.154566	-0.003111	1.754758
13	6	-1.030504	0.009149	3.539109
14	1	-0.024991	0.013911	3.955863
15	6	-2.153998	0.012150	4.515663
16	1	-2.085762	-0.859298	5.173293
17	1	-2.091846	0.893245	5.160952
18	1	-3.125958	0.005433	4.019419

Thiolate model (methanethiolate) - E_{el} =-438.2073199 au

1	6	-0.647053	0.505795	0.008339
2	1	-0.297970	-0.528948	-0.018505
3	1	-0.298851	1.001349	-0.900897
4	1	-1.738948	0.491280	-0.019953
5	16	-0.033294	1.374509	1.510728