Supplementary Information

Thiol–ene coupling kinetics of *D*-limonene: a versatile '*non-click*' free-radical reaction involving a natural terpene

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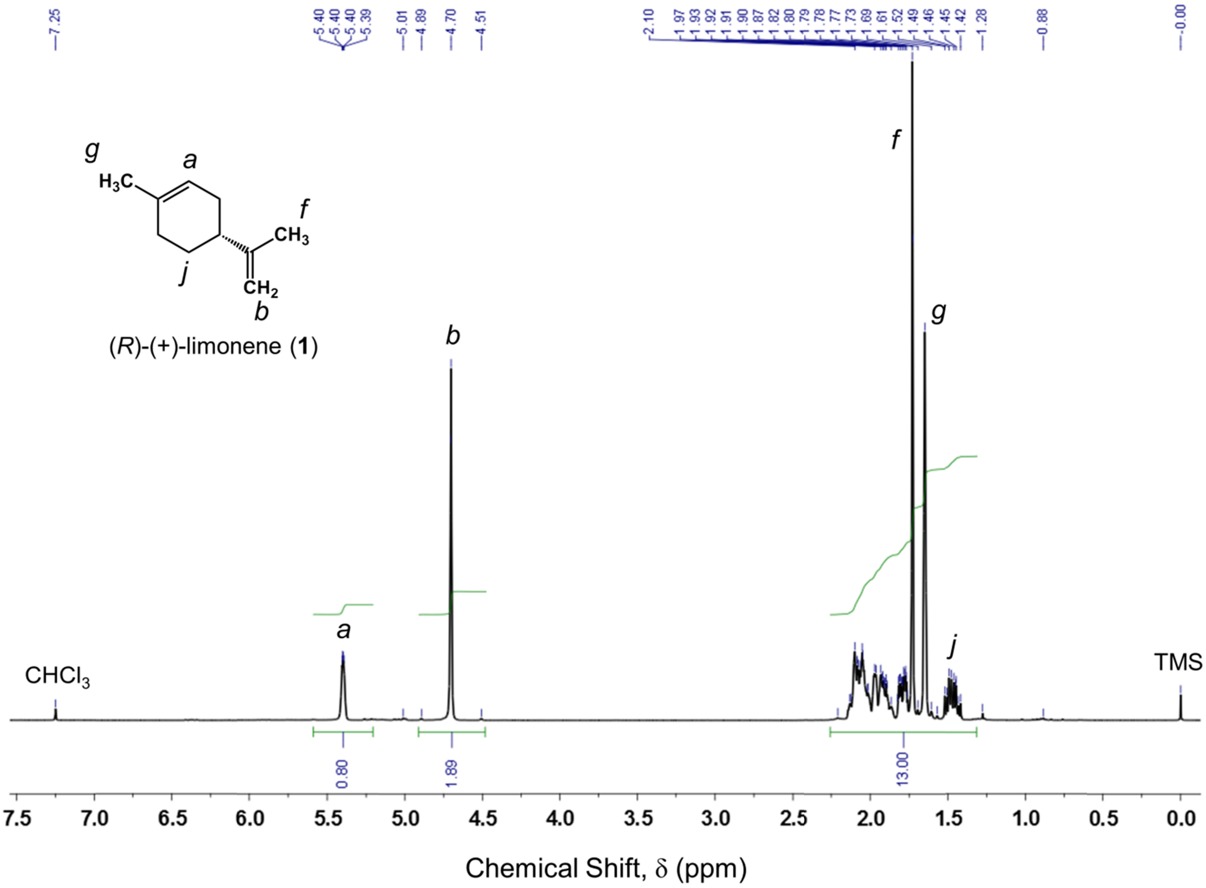
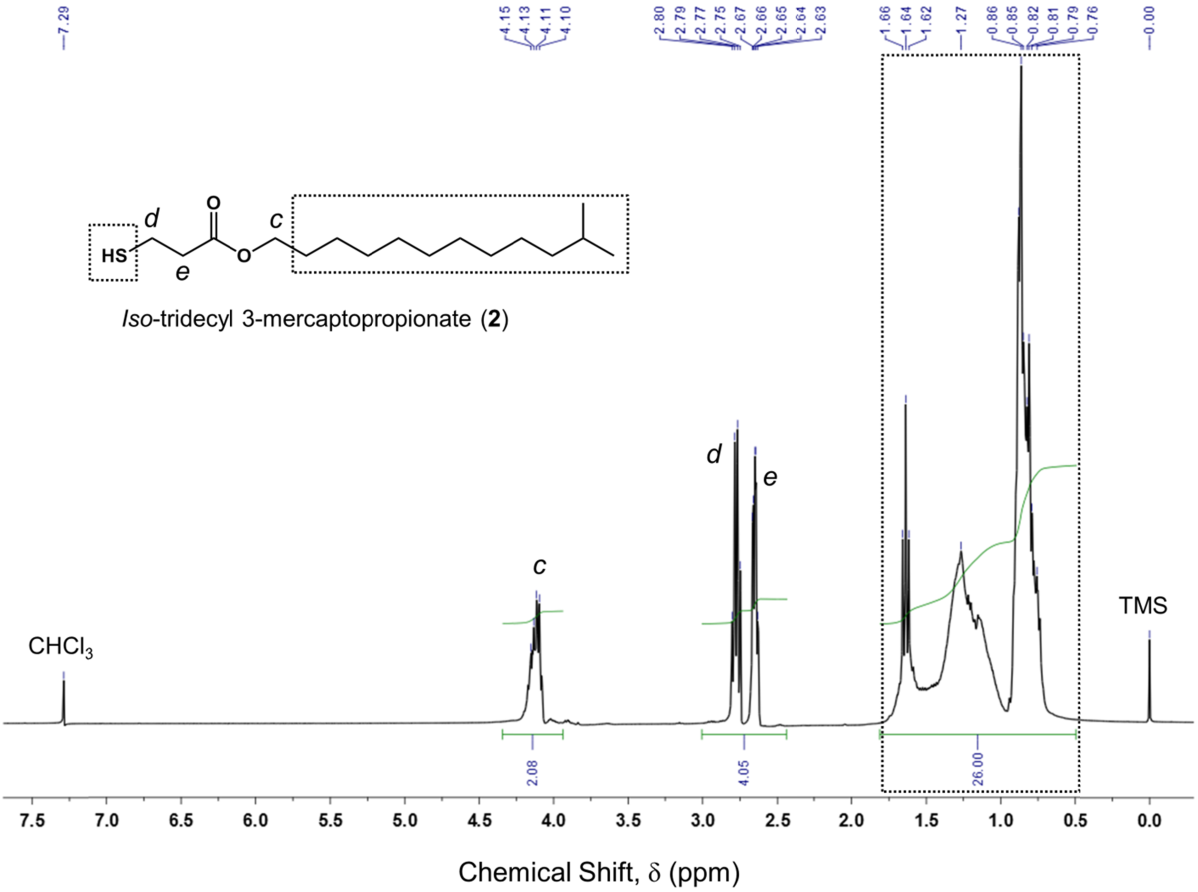
TABLE OF CONTENTS

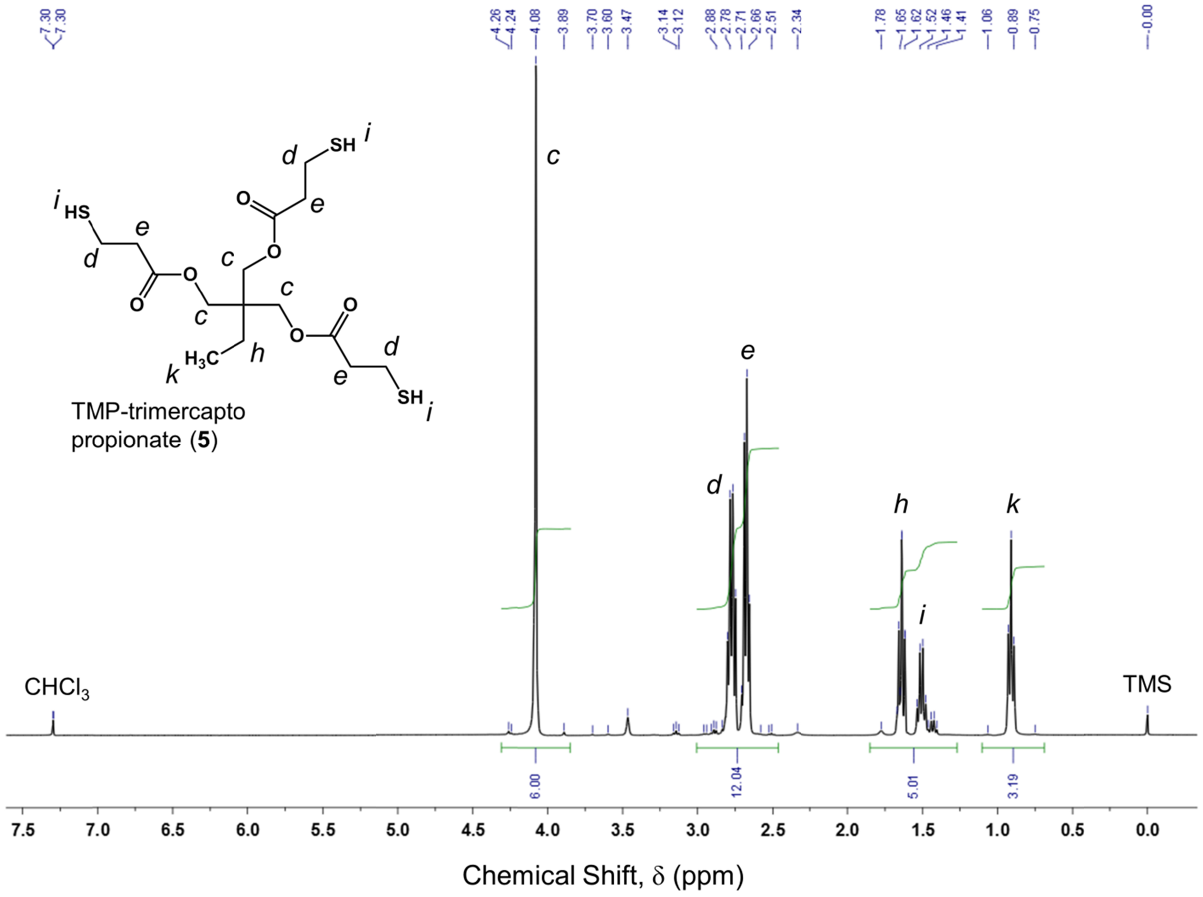
I. Figure S1. Reference ^{1}H NMR spectrum of pure *R*-(+)-limonene (**1**). Main proton signal assignments. Measured ^{1}H- integration ratio $= \frac{\text{terminal C=C bond}}{\text{internal C=C bond}} = \frac{b}{a} = 2.36$. Theoretical ^{1}H-integration ratio = 2.0.



II. Figure S2. Reference ^{1}H NMR spectrum of pure monothiol, *Iso*-tridecyl 3-mercapto propionate (C13MP, **2**)**.** Integration value of proton signal (*c*) was used as reference peak in kinetic thiol–ene spectral measurements. Main proton signal assignments.

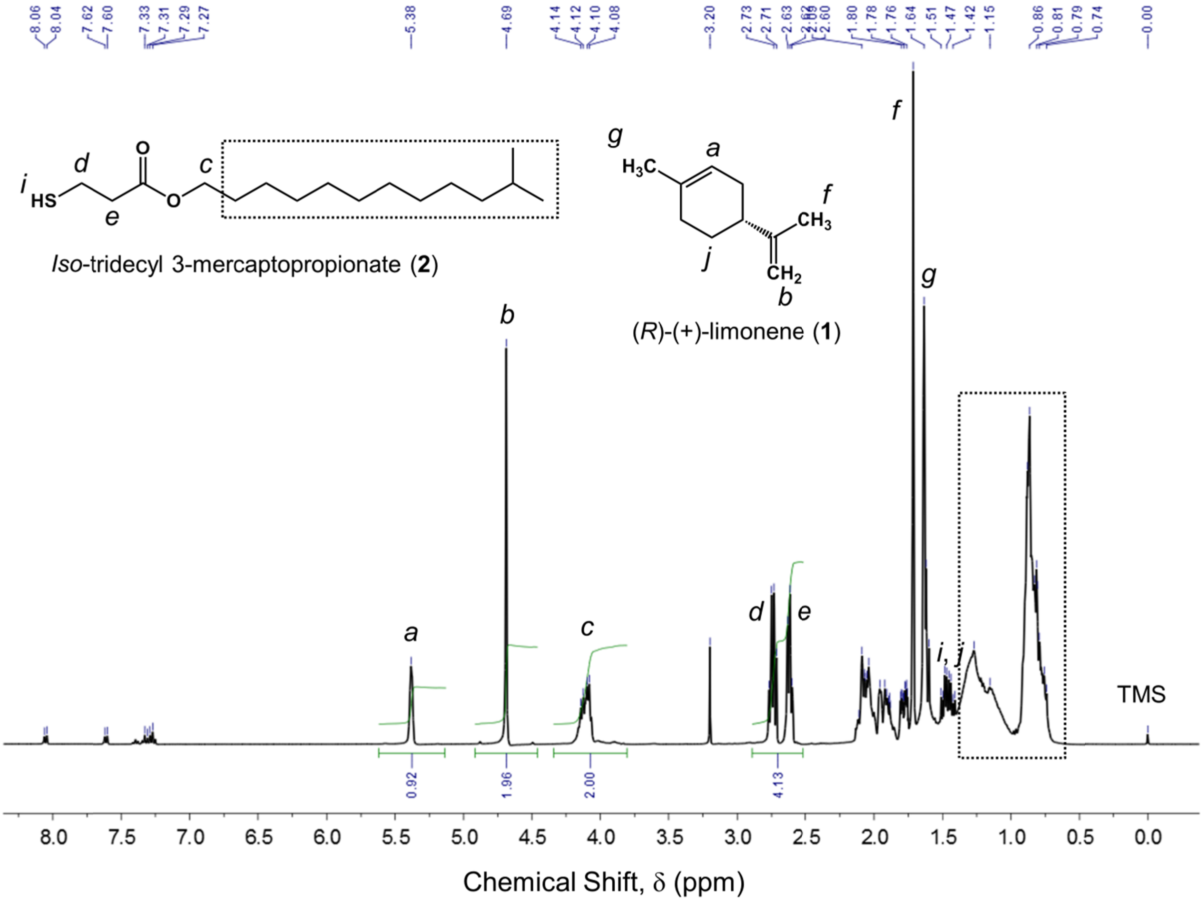


III. Figure S3. Reference ^{1}H NMR spectrum of pure TMP-trimercaptopropionate (TMPMP, **5**).



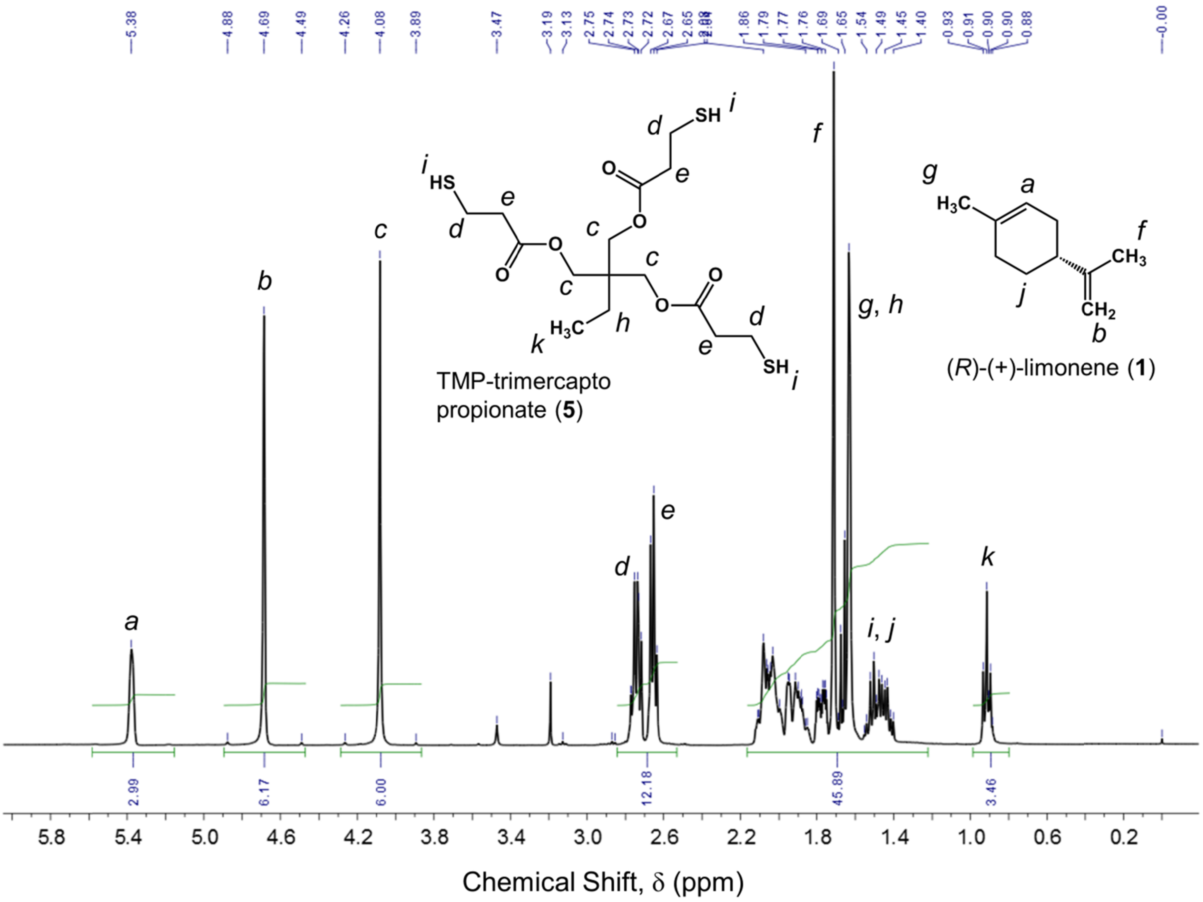
IV. Figure S4. Principal ^{1}H NMR signal assignments of thiol–ene mixture based on (*R*)-(+)-limonene (**1**) and *iso*-tridecyl 3-mercaptopropionate (**2**) before photoinduced reaction (control). Initial thiol–ene stoichiometry: 1:0.5 (1:3 thiol–terpene molecular ratio). Integration value of proton signal (*c*) was used as reference peak in kinetic thiol–ene spectral measurements.

$$\text{Initial } ^1\text{H- integration ratio} = \frac{\text{terminal C=C bond}}{\text{internal C=C bond}} = \frac{b}{a} = 2.13\,.$$



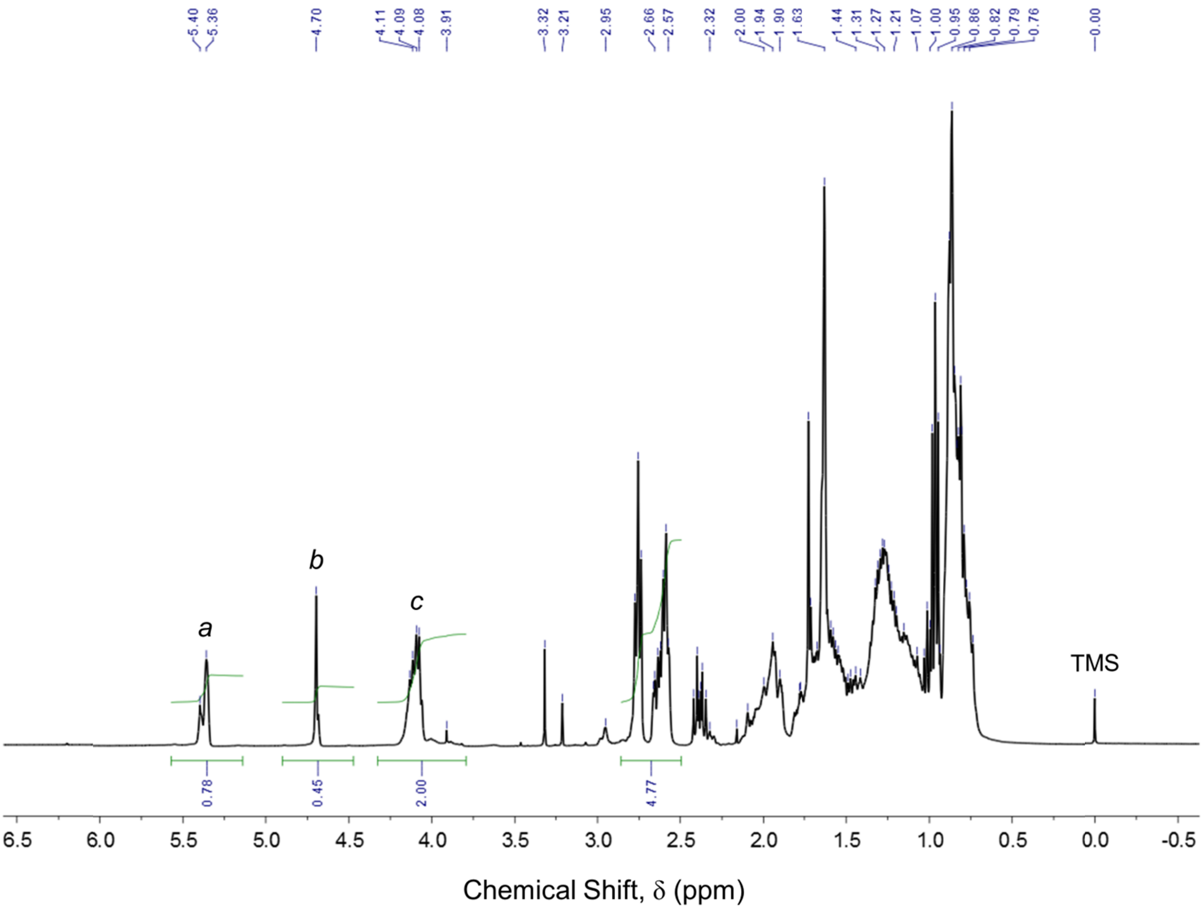
V. Figure S5. Principal ^{1}H NMR signal assignments of thiol–ene mixture based on (*R*)-(+)-limonene (**1**) and TMP-trimercaptopropionate (**5**) before photoinduced reaction (control). Initial thiol–ene stoichiometry: 1:0.5 (1:3 thiol–terpene molecular ratio). Integration value of proton signal (*c*) was used as reference peak in kinetic thiol–ene spectral measurements.

$$\text{Initial }^1\text{H- integration ratio} = \frac{\text{terminal C=C bond}}{\text{internal C=C bond}} = \frac{b}{a} = 2.06\,.$$



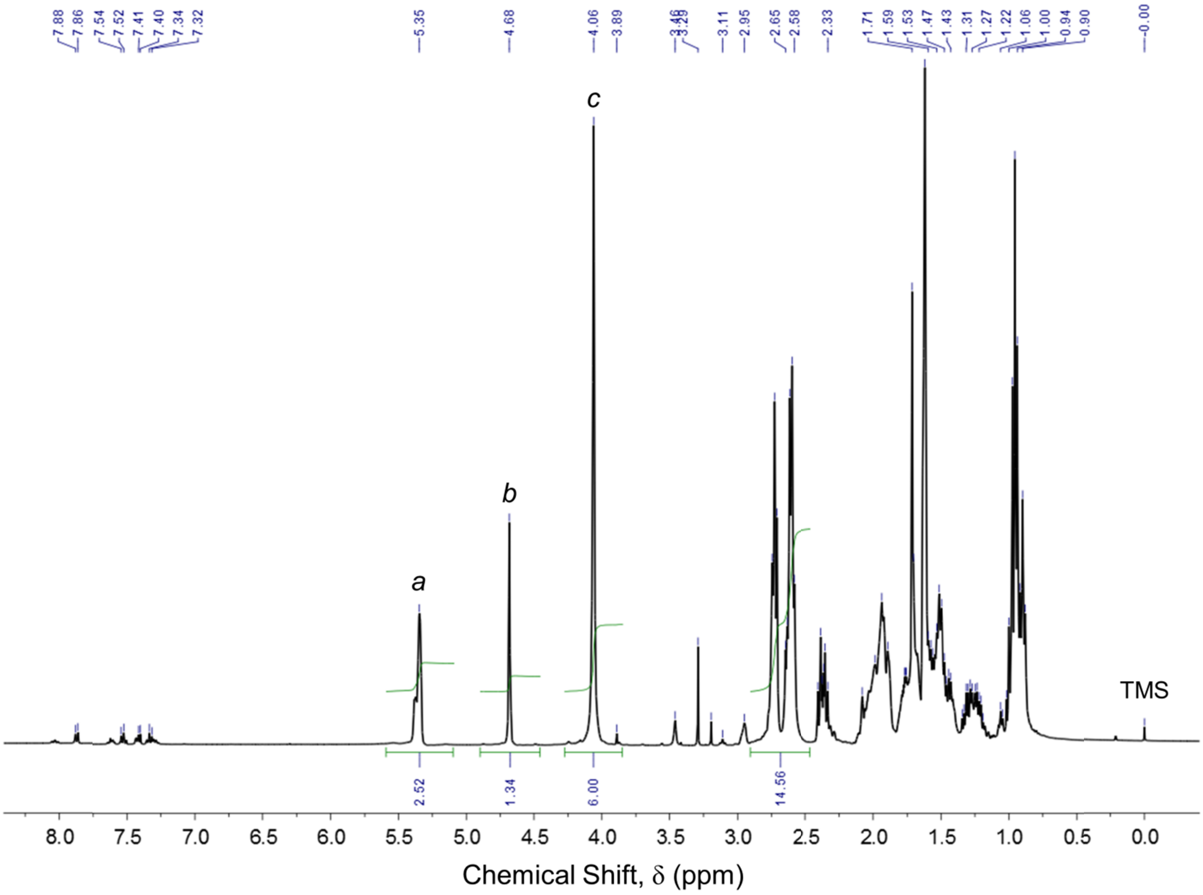
VI. Figure S6. Principal ^{1}H NMR signal assignments of thiol–ene mixture based on (*R*)-(+)-limonene (**1**) and *iso*-tridecyl 3-mercaptopropionate (**2**) after 135 min UV-irradiation (representative coupled product spectrum).

$$^{1}\text{H- integration ratio} = \frac{\text{terminal C=C bond}}{\text{internal C=C bond}} = \frac{b}{a} = 0.58$$



VII. Figure S7. Principal ^{1}H NMR signal assignments of thiol–ene mixture based on (*R*)-(+)-limonene (**1**) and TMP-trimercaptopropionate (**5**) after 180 min UV-irradiation (representative coupled product spectrum).

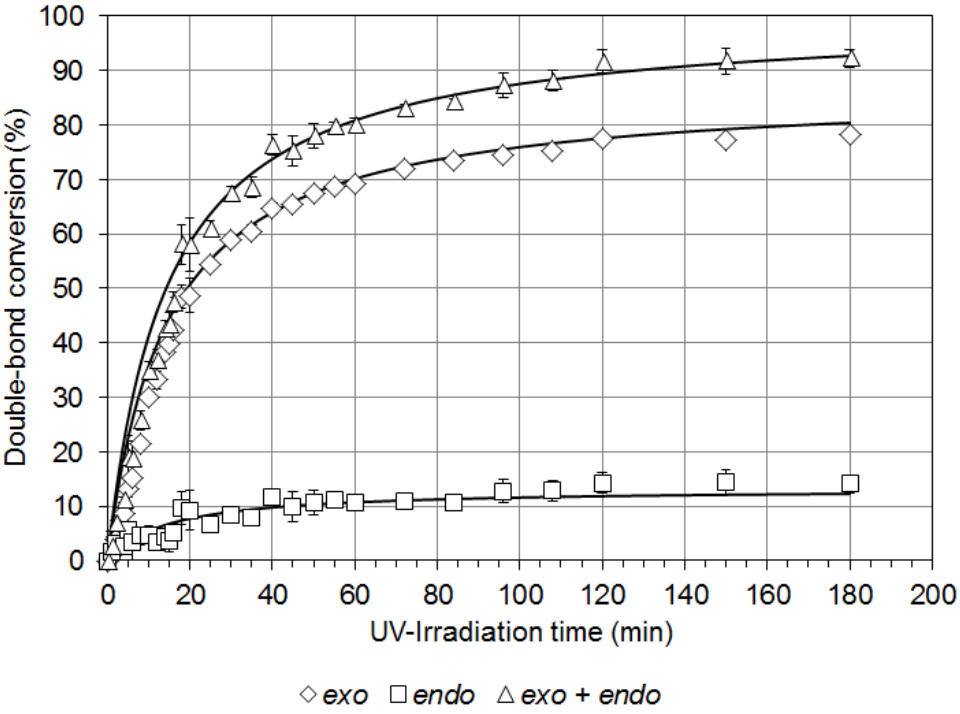
$$^1\text{H- integration ratio} = \frac{\text{terminal C=C bond}}{\text{internal C=C bond}} = \frac{b}{a} = 0.53$$



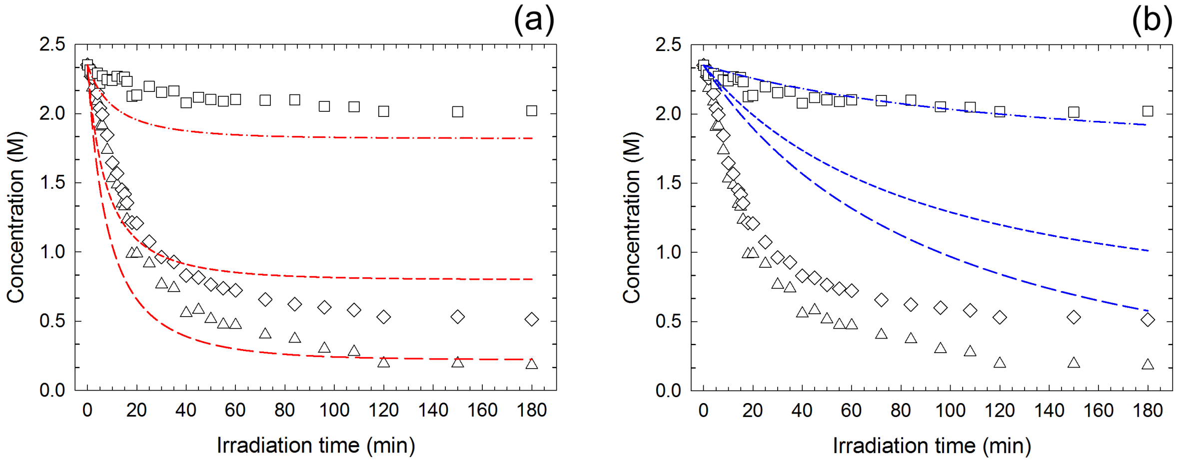
VIII. Table S1. Average kinetic conversion data obtained from ^{1}H NMR measurements for the photoinduced free-radical thiol–ene reaction between (*R*)-(+)-limonene, (**1**) and TMP-trimercapto propionate (**5**) on a 1:0.5 stoichiometry with respect to thiol–ene functional groups (1:3 thiol–terpene molecular ratio) in *d*-solvent solution and supplemented with 1.0 wt.% DMPA as photoinitiator.

	Conversion (%)			**Error (SD)**		
Time (min)	***exo -***	***endo -***	***total alkene***	***exo -***	***endo -***	***total alkene***
0	0.000	0.000	0.000	0.000	0.000	0.000
1	1.014	1.574	2.588	0.764	2.726	2.831
2	3.947	3.051	6.998	0.233	0.473	0.527
4	8.635	2.549	11.184	0.349	0.236	0.421
5	13.240	5.669	18.909	1.639	3.761	4.103
6	15.214	3.385	18.599	0.582	3.309	3.360
8	21.546	4.555	26.101	0.593	1.655	1.758
10	30.044	4.722	34.766	0.502	1.672	1.745
12	33.306	3.496	36.802	1.859	0.965	2.095
14	38.322	4.276	42.599	0.658	1.266	1.426
15	39.693	3.719	43.412	0.906	2.034	2.226
16	42.379	5.056	47.436	0.414	1.769	1.817
18	48.410	9.625	58.035	2.121	3.033	3.702
20	48.629	9.291	57.920	3.174	3.682	4.861
25	54.331	6.617	60.948	0.190	1.351	1.364
30	58.991	8.399	67.391	0.665	1.003	1.203
35	60.499	8.009	68.508	1.086	1.460	1.820
40	64.638	11.631	76.269	1.079	1.544	1.883
45	65.296	9.960	75.256	0.000	2.784	2.784
50	67.434	10.628	78.062	0.493	2.226	2.280
55	68.586	11.185	79.771	0.000	0.696	0.696
60	69.243	10.628	79.872	0.658	1.075	1.260
72	72.039	10.851	82.891	0.717	0.511	0.880
84	73.465	10.740	84.205	0.095	0.579	0.587
96	74.452	12.746	87.197	0.475	2.192	2.243
108	75.274	12.857	88.131	0.665	1.841	1.958
120	77.357	14.306	91.663	0.906	1.841	2.052
150	77.303	14.417	91.720	0.000	2.340	2.340
180	78.125	14.083	92.208	0.435	1.532	1.593

IX. Figure S8. Experimental kinetic profiles (^{1}H NMR, 400 MHz, 128 scans) obtained from thiol–ene coupling in multifunctional reaction system showing the time evolution of double-bond conversion (symbols: collected raw data from triplicate reactions). Error bars represent two-standard deviations about the mean (Table S1). The small error bars demonstrate the ability of our experimental approach to reproducibly measure conversion. Lines represent fits to the experimental data obtained from empirical determinations.



X. Figure S9. Effect of the value of k_d (colored lines) on the overall reaction kinetics from simulated model predictions when compared to the observed reaction behavior obtained from experimental data (symbols) of a thiol–ene mixture based on (*R*)-(+)-limonene (**1**) and trithiol (**5**) on a 1:0.5 thiol–ene mole stoichiometry with respect to functional groups. Concentration of: Lim/RSH (triangles), *exo*-unsaturations (diamonds) and *endo*-unsaturation (squares). For clarity error bars were not plotted. Numerically generated output curves are based on kinetic coefficients obtained from literature in regard to the *cis*/*trans*-isomerization of monounsaturated oils catalyzed by thiyl radicals.[S1] (a) $k_d = 10^{-3}$ s^{-1} (very fast, red), and (b) $k_d = 10^{-5}$ s^{-1} (very slow, blue). Lines: long dash (Lim/RSH), short dash (*exo*-unsaturation), and dash-dot (*endo*-unsaturation).



XI. Derivation of the empirical expressions for the consumption of *exo*- and *endo*-double bonds by free-radical thiol–ene coupling:

Starting equations (S1) and (S2):

$$-r_{\mathrm{Lim}} = \frac{d[\mathrm{Lim}]_t}{dt} = k_{\mathrm{obs}}[\mathrm{Lim}]_t^2 \tag{S1}$$

Relative reactivity or *selectivity*: $k_{\mathrm{r}} = \frac{r_1}{r_2} = \frac{d[exo]_t}{d[endo]_t} = \frac{m_1}{m_2} =$ constant (S2)

Since the total rate of consumption of thiol–ene reactants (R_{T}) equals the accumulation rate of one-to-one products ($R_{\mathrm{P}} = R_{\mathrm{P1}} + R_{\mathrm{P2}}$) from selective thiol–ene coupling, then:

$$R_{\mathrm{T}} = -r_{\mathrm{Lim}} = -r_{\mathrm{RSH}} = R_{\mathrm{P}} \quad \Leftrightarrow \quad -R_{\mathrm{T}} = r_1 + r_2 = R_{\mathrm{P}} \tag{S3}$$

Combining equation (S1) with equations (S2) and (S3) yields the following experimental rate expression for each alkene functional group (‘*ene*’ = *exo* or *endo*):

$$-r_{1,2} = \frac{d[ene]_t}{dt} = m_{1,2}k_{\mathrm{obs}}[\mathrm{Lim}]_t^2 \tag{S4}$$

with,

$$m_1 = \frac{k_{\mathrm{r}}}{k_{\mathrm{r}}+1} = \frac{r_1}{R_{\mathrm{T}}} \tag{S5a}$$

$$m_2 = \frac{1}{k_{\mathrm{r}}+1} = \frac{r_2}{R_{\mathrm{T}}} \tag{S5b}$$

The first-order differential equation (S4) has no explicit dependence of the dependent variable $[ene]_t$ which indicates that consumption of individual unsaturations (*exo* and *endo*) does not depend exclusively on the concentration of each respective alkene group. However, replacing in the second member of equation (S4) by the analytical solution of equation (S1) when $[\mathrm{Lim}](0)=[\mathrm{Lim}]_0$ (eqn. S6)

$$[\mathrm{Lim}]_t = \frac{[\mathrm{Lim}]_0}{1+[\mathrm{Lim}]_0 k_{\mathrm{obs}} t} \tag{S6}$$

one obtains the following analytical solution after integration:

$$[ene]_t = [ene]_0 - m_{1,2}[\mathrm{Lim}]_0 + m_{1,2}\frac{[\mathrm{Lim}]_0}{1+[\mathrm{Lim}]_0 k_{\mathrm{obs}} t} \tag{S7}$$

Simplifying equation (S7) provides the general equation describing the consumption of each alkene functional group (eqn. S8):

$$[ene]_t = [ene]_0 - m_{1,2}\left([\mathrm{Lim}]_0 - [\mathrm{Lim}]_t\right) \tag{S8}$$

Rearranging equation (S8) by making

$$\frac{[ene]_0 - [ene]_t}{[\mathrm{Lim}]_0 - [\mathrm{Lim}]_t} = \frac{\Delta[ene]}{\Delta[\mathrm{Lim}]} = \frac{d[ene]_t}{d[\mathrm{Lim}]_t} = m_{1,2} \tag{S9}$$

allows the values of the parameters $m_{1,2} = k_{1,2}/k_{\mathrm{obs}}$ to be determined from the discontinuous data points measured experimentally by plotting them as $\Delta[ene]\ vs.\ \Delta[\mathrm{Lim}]$. Also, equations (S5a) and (S5b) allow the value of k_{r} (relative reactivity) to be determined from the parameters $m_{1,2}$ *via* equation (S9) obtained from discrete experimental data.

XII. Principal Kinetic Equations Derived from the Reaction Mechanism:

1. Initiation:

$$\mathrm{PI} + h\nu \xrightarrow{k_\mathrm{d}} \mathrm{I}^\bullet \tag{1}$$

$$\mathrm{I}^\bullet + \mathrm{RSH} \xrightarrow{k_\mathrm{RSH1}} \mathrm{IH} + \mathrm{RS}^\bullet \tag{2}$$

2. Propagation / Chain-transfer 1:

$$\mathrm{RS}^\bullet + \mathit{exo} \underset{k_\mathrm{elim1}}{\overset{k_\mathrm{add1}}{\rightleftharpoons}} \mathrm{C}_1^\bullet \tag{3}$$

$$\mathrm{C}_1^\bullet + \mathrm{RSH} \xrightarrow{k_\mathrm{RSH2}} \mathrm{P}_1 + \mathrm{RS}^\bullet \tag{4}$$

3. Propagation / Chain-transfer 2:

$$\mathrm{RS}^\bullet + \mathit{endo} \underset{k_\mathrm{elim2}}{\overset{k_\mathrm{add2}}{\rightleftharpoons}} \mathrm{C}_2^\bullet \tag{5}$$

$$\mathrm{C}_2^\bullet + \mathrm{RSH} \xrightarrow{k_\mathrm{RSH3}} \mathrm{P}_2 + \mathrm{RS}^\bullet \tag{6}$$

4. Termination (homocoupling):

$$2\,\mathrm{RS}^\bullet \xrightarrow{k_\mathrm{t}} \mathrm{RSSR} \tag{7}$$

- Individual rate equations describing the propagation/chain-transfer elementary steps (3)–(6):

   1. Forward propagation reactions: $R_{\mathrm{add1,2}} = k_{\mathrm{add1,2}} \cdot [\mathrm{RS}^\bullet]_t \cdot [\mathit{ene}]_t$

      (thiyl–ene insertion)

   2. Reverse propagation reactions: $R_{\mathrm{elim1,2}} = k_{\mathrm{elim1,2}} \cdot [\mathrm{C}^\bullet_{1,2}]_t$

      (elimination by β-fragmentation)

   3. Forward chain-transfer reactions: $R_{\mathrm{CT1,2}} = -\dfrac{d[\mathrm{RSH}]_t}{dt} = k_{\mathrm{RSH2,3}} \cdot [\mathrm{C}^\bullet_{1,2}]_t \cdot [\mathrm{RSH}]_t$

      (hydrogen-transfer)

- Individual rates of propagation:

$$R_{\mathrm{p}1,2} = -\frac{d[ene]_t}{dt} = R_{\mathrm{add}1,2} - R_{\mathrm{elim}1,2} = k_{\mathrm{add}1,2} \cdot [ene]_t \cdot [\mathrm{RS}^\bullet]_t - k_{\mathrm{elim}1,2} \cdot [\mathrm{C}^\bullet_{1,2}]_t \tag{S10}$$

- General species balance of the intermediary thiyl radical:

$$\frac{d[\mathrm{RS}^\bullet]_t}{dt} = \left(k_{\mathrm{RSH}1}[\mathrm{I}^\bullet]_t + k_{\mathrm{RSH}2}[\mathrm{C}^\bullet_1]_t + k_{\mathrm{RSH}3}[\mathrm{C}^\bullet_2]_t\right)[\mathrm{RSH}]_t - \left(k_{\mathrm{add}1}[exo]_t + k_{\mathrm{add}2}[endo]_t\right)[\mathrm{RS}^\bullet]_t + k_{\mathrm{elim}1}[\mathrm{C}^\bullet_1]_t + k_{\mathrm{elim}2}[\mathrm{C}^\bullet_2]_t - 2k_{\mathrm{t}}[\mathrm{RS}^\bullet]_t^2 \tag{S11}$$

- General species balance of the intermediate carbon-centered radicals:

$$\frac{d[\mathrm{C}^\bullet_{1,2}]_t}{dt} = R_{\mathrm{formation}} - R_{\mathrm{consumption}} \tag{S12a}$$

$$\frac{d[\mathrm{C}^\bullet_{1,2}]_t}{dt} = R_{\mathrm{add}1,2} - \left(R_{\mathrm{elim}1,2} + R_{\mathrm{CT}1,2}\right) \tag{S12b}$$

$$\frac{d[\mathrm{C}^\bullet_{1,2}]_t}{dt} = k_{\mathrm{add}1,2} \cdot [\mathrm{RS}^\bullet]_t \cdot [ene]_t - \left(k_{\mathrm{elim}1,2} + k_{\mathrm{RSH}2,3} \cdot [\mathrm{RSH}]_t\right) \cdot [\mathrm{C}^\bullet_{1,2}]_t \tag{S12c}$$

In the condition of steady-state: $R_{\mathrm{i}} = R_{\mathrm{t}} \Rightarrow R_{\mathrm{p}1,2} = R_{\mathrm{CT}1,2}$, and $\frac{d[ene]_t}{d[\mathrm{RSH}]_t} = 1$, then

$$\frac{d[\mathrm{C}^\bullet_{1,2}]_t}{dt} = 0 \Rightarrow [\mathrm{C}^\bullet_{1,2}]_t = \mathrm{constant} \tag{S13}$$

$$\frac{d[\mathrm{RS}^\bullet]_t}{dt} = 0 \Rightarrow [\mathrm{RS}^\bullet]_t = \mathrm{constant} \tag{S14}$$

which means that equation (S12c)

$$\frac{d[\mathrm{C}^\bullet_{1,2}]_t}{dt} = k_{\mathrm{add}1,2} \cdot [\mathrm{RS}^\bullet]_t \cdot [ene]_t - \left(k_{\mathrm{elim}1,2} + k_{\mathrm{RSH}2,3} \cdot [\mathrm{RSH}]_t\right) \cdot [\mathrm{C}^\bullet_{1,2}]_t = 0 \tag{S15}$$

By solving equation (S15) with respect to $[\mathrm{C}^\bullet_{1,2}]_t$ one obtains

$$[\mathrm{C}^\bullet_{1,2}]_t = \frac{k_{\mathrm{add}1,2} \cdot [\mathrm{RS}^\bullet]_t \cdot [ene]_t}{k_{\mathrm{elim}1,2} + k_{\mathrm{RSH}2,3} \cdot [\mathrm{RSH}]_t} \tag{S16}$$

- when $k_{\text{elim1,2}} \gg k_{\text{RSH2,3}} \cdot [\text{RSH}]_t \Rightarrow \dfrac{[\text{C}^{\bullet}_{1,2}]_t}{[\text{RS}^{\bullet}]_t} \approx K_{1,2} \cdot [ene]_t$ (S17)

- when $k_{\text{elim1,2}} \ll k_{\text{RSH2,3}} \cdot [\text{RSH}]_t \Rightarrow \dfrac{k_{\text{add1,2}}}{k_{\text{RSH2,3}}} \approx \dfrac{[\text{C}^{\bullet}_{1,2}]_t}{[\text{RS}^{\bullet}]_t} = \text{constant}$ (S18)

  i. if $\dfrac{k_{\text{add1,2}}}{k_{\text{RSH2,3}}} \gg 1 \Rightarrow rate \propto [\text{RSH}]_t^1$, and chain-transfer is the rate-limiting step

  ii. if $\dfrac{k_{\text{add1,2}}}{k_{\text{RSH2,3}}} \approx 1 \Rightarrow rate \propto [\text{RSH}]_t^{1/2} \cdot [ene]_t^{1/2}$, and there is no rate-limiting step

  iii. if $\dfrac{k_{\text{add1,2}}}{k_{\text{RSH2,3}}} \ll 1 \Rightarrow rate \propto [ene]_t^1$, and propagation (addition) is the rate-limiting step

XIII. Computer Numerical Simulations in the Software Program COPASI:

- **Input data (model parameters):**

```
Initial time: 0 s
Initial volumes:
compartment  1 l

Initial concentrations:
PI      0.0494 mol/l
I*      0 mol/l
RSH     2.352 mol/l
IH      0 mol/l
RS*     0 mol/l
C1*     0 mol/l
P1      0 mol/l
C2*     0 mol/l
P2      0 mol/l
RSSR    0 mol/l
exo     2.352 mol/l
endo    2.352 mol/l

Reaction parameters:
1.) Photolysis of Initiator (kd)
    k1  0.0001 1/s
2.) Hydrogen abstraction 1 (kRSH1)
    k1  1e+007 l/(mol*s)
3.) Thiyl radical addition 1 (kadd1 / kelim1)
    k1  160000 l/(mol*s)
    k2  2e+007 1/s
4.) Hydrogen abstraction 2 (kRSH2)
    k1  1e+006 l/(mol*s)
5.) Thiyl radical addition 2 (kadd2 / kelim2)
    k1  290000 l/(mol*s)
    k2  1.6e+008 1/s
6.) Hydrogen abstraction 3 (kRSH3)
    k1  1e+006 l/(mol*s)
7.) Thiyl self-termination (homocoupling) (kt)
    k1  3e+009 l/(mol*s)
```

- **Output data (estimation of rate coefficients with statistics):**

```
Objective Value   Root Mean Square  Standard Deviation
0.585392          0.0473591         0.047913
Function Evaluations    CPU Time [s]      Evaluations/second [1/s]
2697                    7.48805           360.174

Parameters:
Parameter   Value Std. Deviation    Coeff. of Variation [%] Gradient
(3.) Thiyl radical addition 1 (kadd1 / kelim1)).k1
2.00346e+006 1.32417e+007           660.941             2.24262e-008
(3.) Thiyl radical addition 1 (kadd1 / kelim1)).k2
8.46784e+007 3.66383e+009           4326.75             1.82618e-010
(4.) Hydrogen abstraction 2 (kRSH2)).k1
544320       2.68489e+007           4932.55             8.25144e-008
(5.) Thiyl radical addition 2 (kadd2 / kelim2)).k1
1.39525e+006 4.27441e+007           3063.55             1.94178e-009
(5.) Thiyl radical addition 2 (kadd2 / kelim2)).k2
6.59354e+008 2.7285e+010            4138.14             7.3771e-012
(6.) Hydrogen abstraction 3 (kRSH3)).k1
595520       1.72258e+007           2892.57             2.49673e-009

Experiments:
Objective Value   Root Mean Square  Error Mean  Error Mean Std. Deviation
0.585392          0.0473591         -0.00281952 0.0472751

Fitted Values:
Objective Value   Root Mean Square  Error Mean  Error Mean Std. Deviation
[exo] 0.193586    0.0471712         -0.00985475 0.0461303
[endo]0.0285498   0.0181152         0.00803998  0.0162332
[RSH] 0.363256    0.064617          -0.0066438  0.0642746
```

```
Correlation Matrix
Rows:    Parameters
Columns: Parameters
```

Correlation Matrix	(3.) kadd1; k1	(3.) kelim1; k2	(4.) kRSH2; k1	(5.) kadd2; k1	(5.) kelim2; k2	(6.) kRSH3; k1
(3.) kadd1; k1	1	-0.89404	-0.919553	0.796323	0.894617	0.434602
(3.) kelim1; k2	-0.89404	1	0.998161	-0.825801	-0.973043	-0.515492
(4.) kRSH2; k1	-0.919553	0.998161	1	-0.832141	-0.974596	-0.510986
(5.) kadd2; k1	0.796323	-0.825801	-0.832141	1	0.715138	-0.0379189
(5.) kelim2; k2	0.894617	-0.973043	-0.974596	0.715138	1	0.671363
(6.) kRSH3; k1	0.434602	-0.515492	-0.510986	-0.0379189	0.671363	1

```
Fisher Information Matrix
Rows:    Parameters
Columns: Parameters
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Information Matrix	(3.) kadd1; k1	(3.) kelim1; k2	(4.) kRSH2; k1	(5.) kadd2; k1	(5.) kelim2; k2	(6.) kRSH3; k1
(3.) kadd1; k1	1.64E-11	-3.84E-13	5.98E-11	-1.89E-13	4.25E-16	-4.53E-13
(3.) kelim1; k2	-3.84E-13	9.01E-15	-1.40E-12	4.48E-15	-1.01E-17	1.07E-14
(4.) kRSH2; k1	5.98E-11	-1.40E-12	2.18E-10	-6.97E-13	1.57E-15	-1.67E-12
(5.) kadd2; k1	-1.89E-13	4.48E-15	-6.97E-13	3.93E-12	-8.31E-15	9.20E-12
(5.) kelim2; k2	4.25E-16	-1.01E-17	1.57E-15	-8.31E-15	1.75E-17	-1.94E-14
(6.) kRSH3; k1	-4.53E-13	1.07E-14	-1.67E-12	9.20E-12	-1.94E-14	2.15E-11

```
Parameter Estimation Task

Problem Description:
Subtask:
Time-Course Task

Problem Description:
    StepNumber: 1
    StepSize: 10800
    Duration: 10800
    TimeSeriesRequested: 1
    OutputStartTime: 0
    Output Event: 1

Method: Deterministic (LSODA)
    Integrate Reduced Model: 0
    Relative Tolerance: 1e-006
    Absolute Tolerance: 1e-012
    Max Internal Steps: 10000

List of Fitting Items:
    -inf <= (3.) Thiyl radical addition 1 (kadd1 / kelim1)).k1 <= inf; Start
Value = 160000
    Affected Experiments:
      all
    -inf <= (3.) Thiyl radical addition 1 (kadd1 / kelim1)).k2 <= inf; Start
Value = 2e+007
    Affected Experiments:
      all
    -inf <= (4.) Hydrogen abstraction 2 (kRSH2)).k1 <= inf; Start Value =
1e+006
    Affected Experiments:
      all
    -inf <= (5.) Thiyl radical addition 2 (kadd2 / kelim2)).k1 <= inf; Start
Value = 290000
    Affected Experiments:
      all
    -inf <= (5.) Thiyl radical addition 2 (kadd2 / kelim2)).k2 <= inf; Start
Value = 1.6e+008
    Affected Experiments:
      all
    -inf <= (6.) Hydrogen abstraction 3 (kRSH3)).k1 <= inf; Start Value =
1e+006
    Affected Experiments:
      all

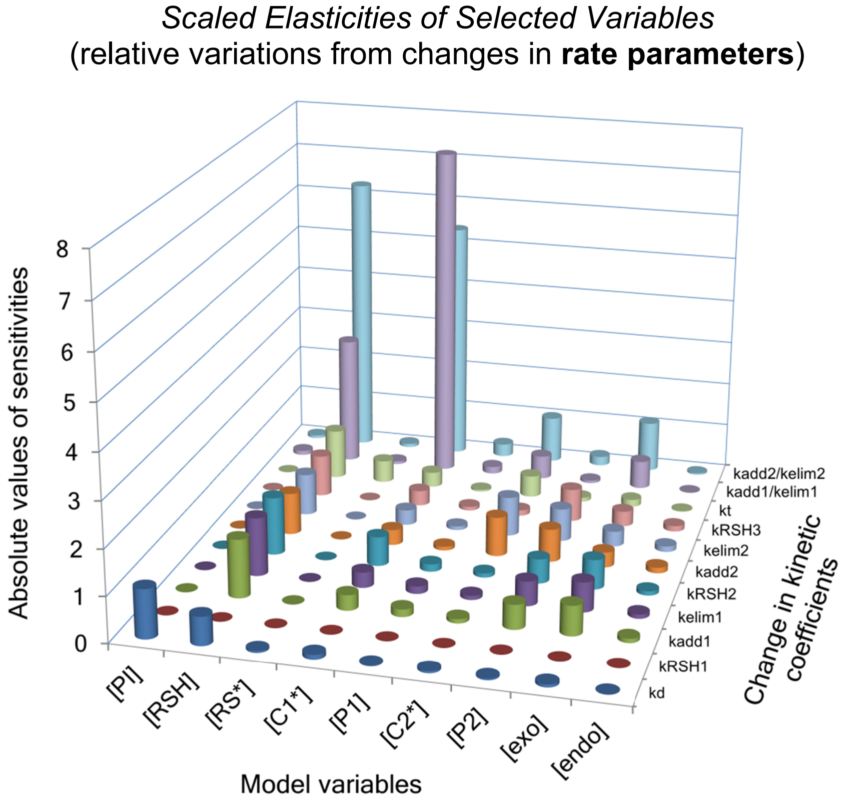
Method: Hooke & Jeeves
    Iteration Limit: 50
    Tolerance: 1e-005
    Rho: 0.2
```

- **Sensitivity Analysis**

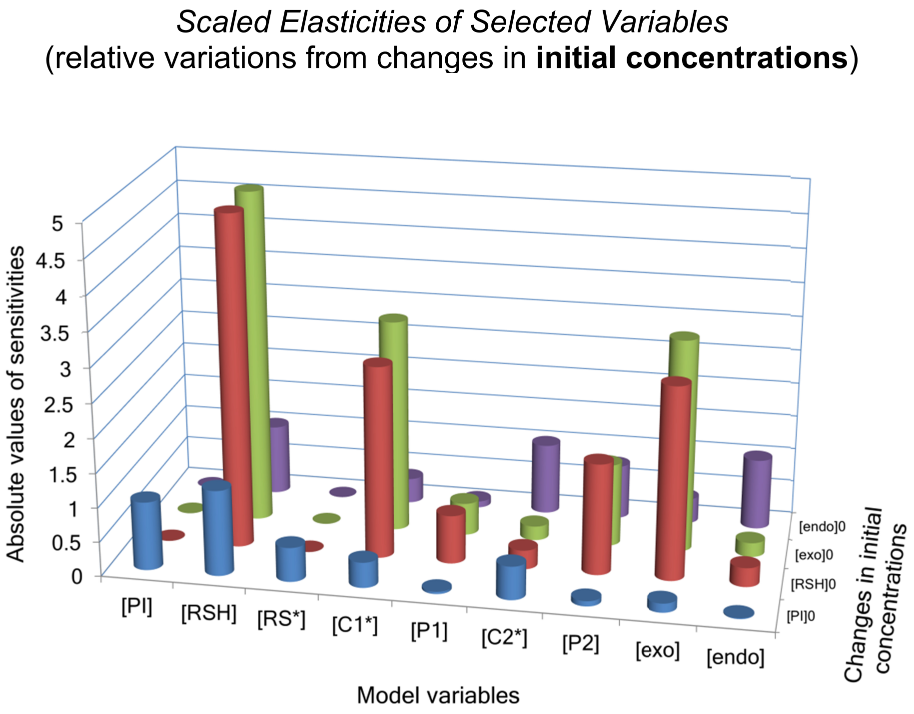
Sensitivity analysis is a tool often employed to measure the importance of a given model parameter (rate coefficient or initial concentration) in the time-course behavior of a reaction system; for example, how much the concentration of a given intermediate species depends on a specific kinetic constant. By quantifying the local robustness (sensitivities) of the system's behavior with respect to individual parameter changes is possible to assess the validity and practical value of the model.[S2]

Local sensitivities (elasticities) of the model variables corresponding to the chemical species concentrations were computed using the generic sensitivity analysis framework available in COPASI. The calculations were performed in time-course mode by setting the subtask method to '*time series*' and then selectively choosing to '*all parameter values*' (model's kinetic coefficients) or to '*initial concentrations*'. The effect of simultaneous changes in rate constants, $k_{\mathrm{add}1,2}$ and $k_{\mathrm{elim}1,2}$, in the model variables was also evaluated by independently selecting both kinetic coefficients. Sensitivities are reported in scaled units (relative terms) both as *positive* (modulus) or *positive* and *negative* values.

Compiled Results of Generic Sensitivity Analysis:



Scaled Elasticities of Selected Variables
(relative variations from changes in **rate parameters**)



Notes: although the scaled values of local sensitivities in the above graphs are expressed in absolute terms (positive values only) for better overall comparison between results, the sensitivities (also called elasticities) of each model variable (single bars) can be either *positive* or *negative* depending if the changes in individual kinetic coefficients was an increase or decrease relative to its initial value (see Tables below). The scaled values thus describe the relative changes; e.g., a scaled local sensitivity of 1.1 for [PI] indicates that if the value of k_d is increased by 10% with respect to its initial value, then [PI] drops by $1.1\times10\%=11\%$ relative to the initial condition when k_d was kept constant (i.e., when the elasticity value was zero). This way is possible to inspect which parameters affect more drastically the model in a time-course experiment by quantification of the local elasticities (i.e., how much a given variable of the model resists to changes in a specific rate coefficient independently of the others). From the graph above we can see that simultaneous changes in the $k_{\mathrm{add}1,2}/k_{\mathrm{elim}1,2}$ have profound effects on [RSH] (or [Lim]) and $[C_1^\bullet]$, whereas local variations in the value of $k_{\mathrm{RSH}1}$ does not influence any of the selected model variables too significantly. The concentration of thiyl radicals is predominantly affected by changes in the termination rate constant. In general the kinetic model is affected by changes in most of the rate coefficients. Local sensitivities of all model variables were also obtained for changes in initial concentrations of $[\mathrm{PI}]_0$, $[\mathrm{RSH}]_0$, $[exo]_0$ and $[endo]_0$.

Display of local sensitivities (unscaled) obtained from individual changes in the kinetic parameters

Selected Model Variables	k_{d}	k_{RSH1}	k_{add1}	k_{elim1}	k_{RSH2}	k_{add2}	k_{elim2}	k_{RSH3}	k_{t}	k_{add1}/k_{elim1}	k_{add2}/k_{elim2}
[PI]	-181.092	-5.92E-18	-7.05E-15	1.13E-14	2.98E-13	-4.48E-14	-1.30E-15	-1.48E-12	-2.93E-16	-9.68E-18	1.20E-18
[RSH]	-609.859	2.48E-14	-6.26E-08	1.48E-09	-2.29E-07	-6.49E-08	1.37E-10	-1.52E-07	3.59E-11	-1.65E-15	6.64E-16
[RS*]	-6.73E-06	-2.08E-21	-2.00E-20	6.93E-21	3.21E-19	-7.98E-20	-3.68E-22	-8.66E-19	-2.79E-18	-6.73E-24	1.40E-24
[C1*]	-1.92E-07	2.67E-23	3.19E-17	-7.54E-19	-2.23E-16	4.60E-17	-9.51E-20	1.07E-16	-2.11E-20	-8.22E-24	-1.10E-24
[P1]	303.195	-1.31E-13	1.56E-07	-3.67E-09	5.70E-07	-1.14E-07	2.42E-10	-2.68E-07	-2.54E-11	1.76E-15	-5.85E-16
[C2*]	-3.30E-08	-1.27E-23	3.30E-18	-7.75E-20	1.21E-17	4.46E-17	-9.43E-20	-1.48E-17	-1.15E-20	-2.22E-25	-8.06E-26
[P2]	125.572	1.06E-13	-9.32E-08	2.19E-09	-3.41E-07	1.79E-07	-3.79E-10	4.20E-07	-1.05E-11	-1.22E-16	-7.75E-17
[*exo*]	-303.195	1.31E-13	-1.56E-07	3.67E-09	-5.70E-07	1.14E-07	-2.42E-10	2.68E-07	2.54E-11	-1.76E-15	5.85E-16
[*endo*]	-125.572	-1.06E-13	9.32E-08	-2.19E-09	3.41E-07	-1.79E-07	3.79E-10	-4.20E-07	1.05E-11	1.22E-16	7.75E-17

Display of local sensitivities (scaled) obtained from individual changes in the kinetic parameters

Selected Model Variables	k_{d}	k_{RSH1}	k_{add1}	k_{elim1}	k_{RSH2}	k_{add2}	k_{elim2}	k_{RSH3}	k_{t}	k_{add1}/k_{elim1}	k_{add2}/k_{elim2}
[PI]	-1.07947	-3.53E-09	-8.42E-07	5.68E-05	9.67E-06	-3.73E-06	-5.12E-05	-5.24E-05	-5.24E-05	-0.0978866	0.0660559
[RSH]	-0.62871	2.55E-06	-1.2937	1.2928	-1.28576	-0.93315	0.928209	-0.93082	1.11137	-2.88724	6.29336
[RS*]	-0.04028	-1.25E-06	-2.40E-06	3.51E-05	1.04E-05	-6.66E-06	-1.45E-05	-3.08E-05	-0.49967	-0.06833	0.0767773
[C1*]	-0.10255	1.43E-06	0.341517	-0.34124	-0.64982	0.343117	-0.33546	0.339403	-0.33786	-7.45602	-5.41406
[P1]	0.016136	-6.98E-07	0.166148	-0.16542	0.165029	-0.08497	0.085031	-0.08497	-0.04054	0.159372	-0.286619
[C2*]	-0.04639	-1.78E-06	0.093151	-0.09231	0.09277	0.875692	-0.87458	-0.12439	-0.48394	-0.529487	-1.04413
[P2]	0.036572	3.10E-06	-0.54377	0.540068	-0.53989	0.728645	-0.72757	0.727952	-0.0921	-0.0600448	-0.207847
[*exo*]	-0.0641	2.77E-06	-0.66007	0.657189	-0.65562	0.337575	-0.33781	0.337551	0.161071	-0.632624	1.13915
[*endo*]	-0.00625	-5.30E-07	0.092951	-0.09232	0.092287	-0.12455	0.124369	-0.12443	0.015743	0.0102704	0.0354985

Display of local sensitivities (unscaled) obtained from individual changes in initial concentrations

Selected Model Variables	$[PI]_0$	$[RSH]_0$	[*exo*]$_0$	[*endo*]$_0$
[PI]	0.33958	9.38E-08	1.09E-07	-2.77E-08
[RSH]	-2.45203	0.199359	-0.20052	-0.04223
[RS*]	1.69E-07	-2.51E-13	3.42E-13	-1.78E-13
[C1*]	1.40E-09	-2.23E-10	2.47E-10	2.90E-11
[P1]	1.26598	0.563995	0.376559	-0.07323
[C2*]	7.01E-10	-8.37E-12	6.24E-12	3.13E-11
[P2]	0.525629	0.236646	-0.17604	0.115464
[*exo*]	-1.26598	-0.564	0.623441	0.073235
[*endo*]	-0.52563	-0.23665	0.176044	0.884536

Display of local sensitivities (scaled) obtained from individual changes in initial concentrations

Selected Model Variables	$[PI]_0$	$[RSH]_0$	[*exo*]$_0$	[*endo*]$_0$
[PI]	0.999953	1.31E-05	1.53E-05	-3.89E-06
[RSH]	-1.24875	4.83388	-4.8619	-1.02395
[RS*]	0.499841	-3.53E-05	4.80E-05	-2.51E-05
[C1*]	0.37055	-2.79982	3.1029	0.364812
[P1]	0.033283	0.705961	0.471345	-0.09167
[C2*]	0.487164	-0.27705	0.206584	1.0358
[P2]	0.075625	1.62105	-1.20592	0.790942
[*exo*]	-0.13223	-2.8046	3.10021	0.364176
[*endo*]	-0.01293	-0.2771	0.206137	1.03574

Note that the cell values on the matrices are either positive or negative although in the bar graphs they appear as positive values only.

XIV. Supplementary Information References

1. M. Claudino, M. Johansson and M. Jonsson, *European Polymer Journal*, 2010, **46**, 2321-2332.

2. P. Mendes, S. Hoops, S. Sahle, R. Gauges, J. Dada and U. Kummer, *Methods Mol. Biol.*, 2009, **500**, 17-59.