Supplementary Material

Solid-state dynamic combinatorial chemistry: reversibility and thermodynamic product selection in covalent mechanosynthesis

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Contents

1. MATERIALS

All HPLC/LCMS solvents were of LCMS or HPLC grade: LCMS grade methanol and HPLC grade (not stabilized) tetrahydrofurane (THF) were acquired from Fisher Scientific. Water was freshly collected from MilliQ Synthesis reverse osmosis unit. Formic acid was obtained from Fluka, puriss (mass spectrometry grade). HPLC grade acetonitrile for LCMS sample preparation was obtained from Fisher. Reagent-Plus 99% TFA was obtained from Sigma-Aldrich. For preparative flash chromatography an Interchim 50 um silica cartridge was used, and ethyl acetate and *n*-hexane were freshly distilled in house.

All disulfide starting materials and reagents used in the DCL experiments were purchased from commercial suppliers. p-Tolyl disulfide [103-19-5] (>97.5 % by GC) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) [6674-22-2] (>97.5 % by GC) were obtained from Acros Organics. Bis(4-chlorophenyl) disulfide [1142-19-4] (98+%) and bis(2-nitrophenyl) disulfide [1155-00-6] (98%) were purchased from Alfa Aesar. Bis(4-nitrophenyl) disulfide [100-32-3] (>95.0% by GC) was purchased from TCI Europe. Acetonitrile for solution DCL experiments was purchased from Fisher as HPLC grade

2. Preparation of dynamic combinatorial libraries (DCLs)

2.1 Preparation of mechanochemical DCLs

a) Starting from an equimolar mixture of homodimeric disulfide reagents

Equimolar quantities of each homodimeric disulfide (see Table **S1**) were selected for each reaction so that the total weight of the reaction mixture was 200 mg. the mixture was then placed in a 10 mL stainless steel grinding jar. To the jar containing the weighed reagents, two 7 mm-diameter hardened stainless steel grinding balls were added. Depending on the reaction conditions, we also added: a) nothing else; b) for blank solvent: 50 µl acetonitrile; c) for neat reaction: 2 µl (2%M) DBU; or d) for LAG reaction: 2 µl DBU + 50 µl acetonitrile. The jars were immediately closed to avoid evaporation of the reagent/grinding liquid. The mixture was then milled normally for 45 minutes at 30 Hz in a Retsch MM200 Shaker Mill unless specified otherwise. Analysis of the resulting solid product via HPLC monitored by UV and with XRPD indicated the conversion of starting materials to the heterodimeric disulfide product.

b) Starting from either (1) chromatographically purified heterodimeric disulfides or (2) from a dry solid containing the two homodimers and the corresponding heterodimer in the statistical ratio 1:1:2.

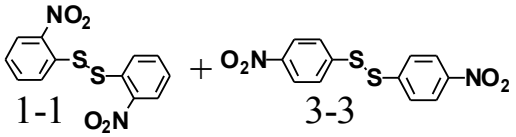
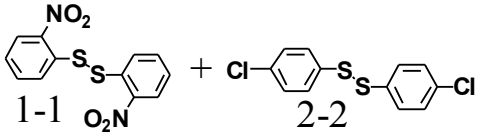
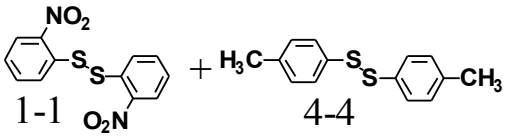
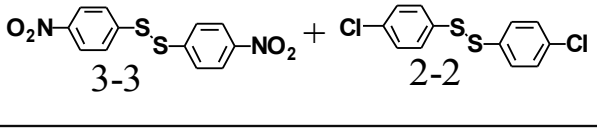
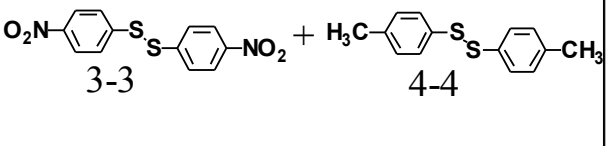
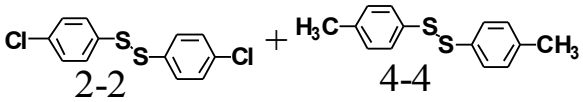
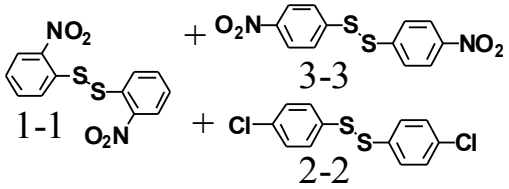
Approximately 200 mg of either (1) the single reagent or the pre-prepared mixture (2) was transferred to the 10 mL stainless steel grinding jar. The rest of procedure was as described in 2.1 a).

2.2 Preparation of solution DCLs

495 µl of a 2.02 mM freshly prepared stock solution in acetonitrile of each of the two homodimers (for a two homodimeric disulfide reaction) or 330 µl of a 3.03 mM freshly prepared stock in acetonitrile of each of the three homodimers (for a three homodimeric disulfide reaction) were added into a 2 ml HPLC vial containing a stirrer bar. Depending on the conditions: a) **blank**: 10 µl acetonitrile or b) **2 mole% DBU**: 10 µl of a freshly prepared

2mM solution of DBU in acetonitrile were added and the HPLC vials were closed with a solid screw caps to avoid evaporation. The solution was left stirring. The content of the reaction vials were analysed by HPLC at different time points and the %peak area ratio of the chromatographic peaks were used to calculate the %molar concentration of the composition of the product.

Table S1. Details of molecular weights and amounts of homodimeric disulfides used in metathesis experiments.

Reactions	Homodimer 1	Homodimer 2	Homodimer 3
	**1-1** MW:308.34 0.32 mmol 98.6 mg	**3-3** MW:308.34 0.32 mmol 98.6 mg	
	**1-1** MW:308.34 0.34 mmol 104.8 mg	**2-2** MW:287.23 0.34 mmol 97.6 mg	
	**1-1** MW:308.34 0.38 mmol 117.2 mg	**4-4** MW:246.39 0.38 mmol 93.6 mg	
	**3-3** MW:308.34 0.34 mmol 104.8 mg	**2-2** MW:287.23 0.34 mmol 97.6 mg	
	**3-3** MW:308.34 0.38 mmol 117.2 mg	**4-4** MW:246.39 0.38 mmol 93.6 mg	
	**2-2** MW:287.23 0.38 mmol 109.2 mg	**4-4** MW:246.39 0.38 mmol 93.6 mg	
	**1-1** MW:308.34 0.22 mmol 67.8 mg	**3-3** MW:308.34 0.22 mmol 67.8 mg	**2-2** MW:287.23 0.22 mmol 63.2 mg

3.1 Preparative scale synthesis of statistical solid reagent mixtures (Case 3 mixtures)

A mixture of 308.3 mg (1mmol) of **1-1** (MW=308.33) and 308.3 mg (1mmol) of **3-3** (MW=308.33) or, alternatively, a mixture of 308.3 mg (1mmol) of **1-1** (MW=308.33) and 287.2 mg (1mmol) of **2-2** (MW=287.23) were dissolved in 1000 mL of acetonitrile (Fisher HPLC grade) and stirred overnight. Subsequent HPLC analysis of the solutions confirmed that a statistical mixture (1:1:2) of both homodimers and the corresponding heterodimer has formed. The solutions were evaporated to dryness on a rotary evaporator at low temperature (< 30°C) and the solid residues were finally dried with the aid of an oil pump.To prevent changes in the metathesis equilibrium during evaporation, 50 µl of formic acid was added to each solution.

3.2 Isolation of 1-4 and 3-4

In order to isolate **1-4**, 492.7 mg (2 mmol) of **4-4** and 616 mg (2 mmol) of **1-1** (MW=308.33) were dissolved in 400 mL acetonitrile. In order to isolate **3-4**, 492.7 mg (2 mmol) of **4-4** and 616 mg (2 mmol) of **3-3** were dissolved in 400 mL acetonitrile. To each solution was added 12 µL of **dbu** (3 mol %) and left stirring. A statistical mixture was formed after several hours, as analysed by HPLC (see Table S3 and Figure S1) and TLC (SiO_2, 1:4 ethyl acetate:hexane; R_f:0.22 for **1-1**; R_f:0.42 for **3-3**; R_f:0.68 for **4-4**; R_f:0.48 for **1-4** and R_f:0.58 for **3-4**). 50 µL of formic acid was added to the solutions to quench further scrambling when concentrating the reaction and the solutions were evaporated on a rotary evaporator. The residues were poorly soluble in ethyl acetate and were therefore dissolved in dichloromethane. Silica (Breckland Silica Gel 60) was added to form a slurry which was dried on the rotary evaporator. The silica containing the statistical mixture of disulfides was added to a dry loading flash chromatography column attached to a Interchim 50 µm silica – 25 g cartridge (maximum pressure 22 bar) and fitted to the Agilent HP1100 HPLC preparative system. A gradient of 10% to 50% ethyl acetate in hexane in 15 min at 20 mL/min was run. Compounds **1-4** and **3-4** eluted as the second peak. The fractions containing pure **1-4** and pure **3-4** as per HPLC analysis were evaporated on a rotary evaporator. Any residual solvent was removed from the purified **1-4** and **3-4** using an oil pump.

3.3 DCL analysis by LCMS

HPLC-UV interfaced to MS (LCMS) was used to analyse mechanochemical and solution DCL experiments. LCMS analysis was performed using an Agilent 1100 series HPLC high pressure binary pump, autosampler and column oven with a Diode Array Detector and Agilent XCT ion-trap mass spectrometer. Analyses were performed using reversed phase HPLC columns: Zorbax SB C18 (30 mm × 2.1 mm, 3.5 µm) for LCMS method A and Kinetex PFP (50 mm × 2.1 mm, 2.6 µm) for method B, C and D. Other details of the LCMS method are listed on Table S2. Four LCMS methods were developed to analyse the DCL libraries studied. Table S3 indicates LCMS methods used to analyse the 7 DCL experiments. Typical chromatograms for the 7 DCL experiments are given in Figure S1 with a graphical description of the method.

UV detection was used to determine the composition of the product. The molar composition of the products was obtained by correlating the percent peak area ratio of their HPLC peaks as their molar percent in the product composition. A specific wavelength and bandwidth were selected experimentally for each case, so as to give as close as possible similar peak area for each of the homodimers involved in the experiment. Figure S2 tabulates

the wavelengths and corresponding bandwidths applied to determine the composition of the product for each of the 7 DCL experiments. The molar percent of the heterodimer was obtained as the difference from 100% of the composite percent of the homodimers.

Mass spectrometry data was used to confirm the identity of species in LCMS experiments. MS confirmation was not strictly necessary as the homodimers were obtained from a reputable source, and the scrambling of homodimers could only result in one heterodimer.

Alternate ion mass spectra were acquired in the standard enhanced mode using electrospray ionization (drying gas temperature: 325 ºC, nebulizer pressure: 25 psi, drying gas flow: 8 L/min, HV capillary: 3500 V; ICC target: 200 000.) The flow was split just before reaching the ion source, entering only around 50-100 µl/min into the ion source. Positive electrospray proved best for bis (2-nitrophenyldisulfide) and bis (4-nitrophenyldisulfide) starting material and for their corresponding heterodimers. However, **2-2** and **4-4** starting materials and their heterodimer **1-4** did not ionize on the electrostray ion souce.

Table S2. Details of the four LCMS methods used for analysis

LCMS Method	HPLC column	Organic Phase (B) Note 1	Gradient Note 2	Flowrate (ml/min)	Column Temp.	Highest Back Pressure	Run Time (min)
A	Zorbax SB C18	MeOH +0.1%HOFo	0-5 min 10-100% B	1.0	60ºC	230 bars	5.5 min
B	Kinetex PFP	MeOH +0.1%HOFo	0-4 min 60-90% B	0.75	50ºC	360 bars	4.0 min
C	Kinetex PFP	THF +0.1%HOFo	0-3 min 40-50% B	0.6	50ºC	230 bars	3.0 min
D	Kinetex PFP	MeOH +0.1%HOFo	0-5 min 60-70% B	0.75	50ºC	230 bars	5.5 min

Note 1: Aqueous phase for all HPLC methods is : water + 0.1%HOFo (formic acid)
Note 2: At the end of the gradient run the composition is maintained until the end of the run time
Note 3: 0.3 µl of the HPLC samples were injected into the column.

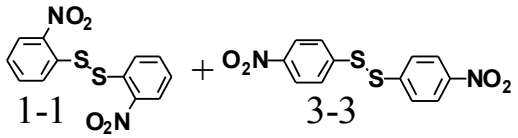
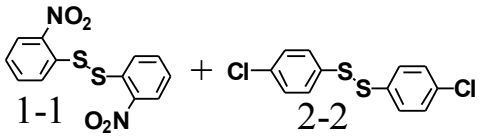
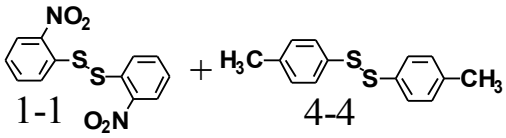
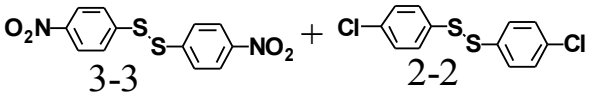
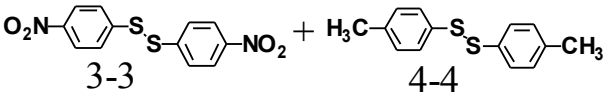
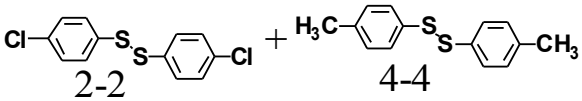
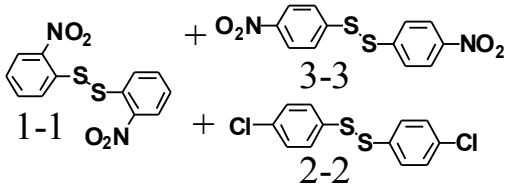
3.4 Sample preparation for LCMS analysis of mechanochemical products

Between 0.4 mg to 1.0 mg of the solid product from mechanosynthsis was weighed in a 2 ml HPLC vial. HPCL analysis solvent (freshly prepared mixture of acetonitrile and 0.2% TFA) was added to the sample to obtain a concentration of 1 mg/ml. If necessary, the sample was dissolved with the aid of sonication. For analysis, 0.3 µl of the sample solution was injected into the HPLC column.

3. 5 Sample preparation for LCMS analysis of solution products

As solution DCL samples (at 1 mM) had been prepared in 2ml HPLC vials, these vials were directly used for HPLC analysis and analysed at various time points. For analysis, 0.3 µl of the sample solution was injected into the HPLC column.

Table S3. The HPLC method, specific wavelength, corresponding bandwidth and typical chromatogram used to analyse the composition of the product.

DCL reaction experiments	LCMS Method	wavelength /bandwidth)	Typical Chromatogram Figure
	**Method B**	280 nm, 8 nm	S1-f)
	**Method A**	260 nm, 8 nm	S1-a)
	**Method A**	248 nm, 8 nm	S1-b)
	**Method A**	267 nm, 8 nm	S1-c)
	**Method A**	264 nm, 8 nm	S1-d)
	**Method C**	298 nm, 16 nm	S1-h)
	**Method D**	272 nm, 80 nm	S1-j)

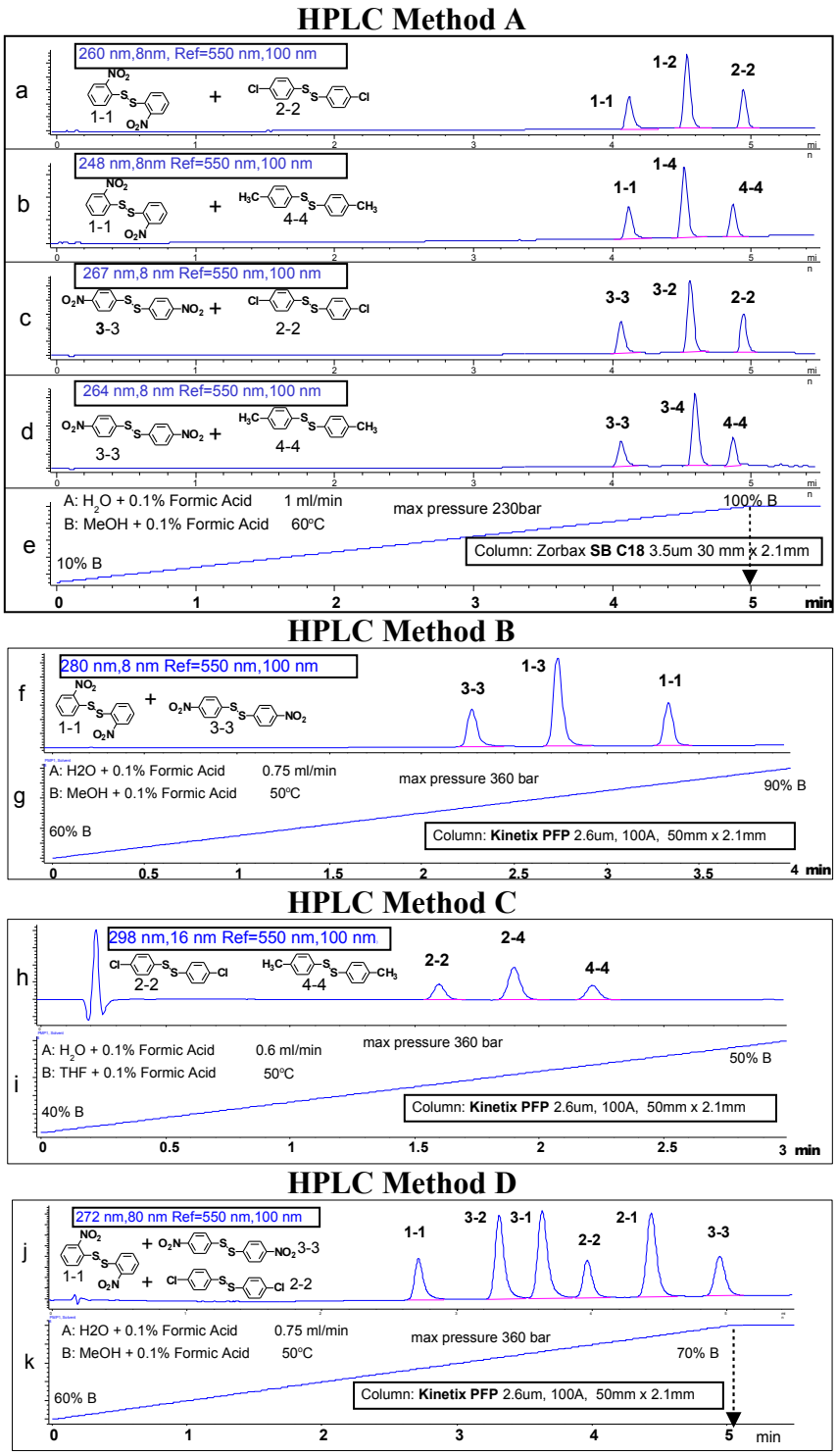


Figure S1. Typical chromatograms for different experiments with details of the HPLC method used, including the gradient range and the specific wavelength used to quantify the molar composition of the product. **Method A** [gradient and chromatographic details shown in e)] was used for reactions of: a) **1-1** with **2-2**; b) **1-1** with **4-4**; c) **3-3** with **2-2**; d) **3-3** with **4-4**. **Method B** [gradient and chromatographic details shown in g)] was used for reaction of: f) **1-1** with **3-3**. **Method C** [gradient and chromatographic details shown in i)] was used for reaction of: h) **2-2** with **4-4**. **Method D** [gradient and chromatographic details shown in k)] was used for reactions of: j) **1-1** with **2-2** and with **3-3**.

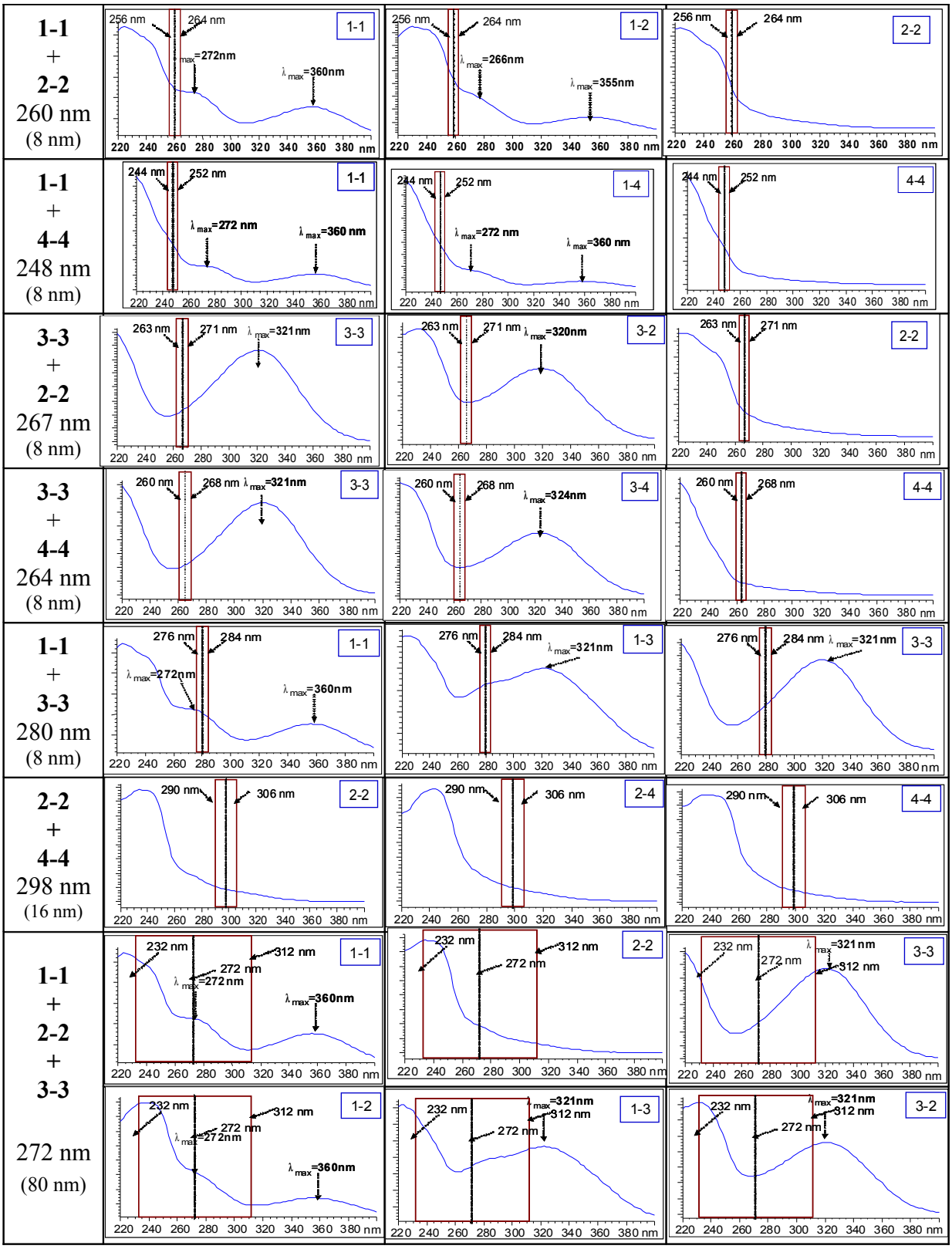


Figure S2. Typical UV spectra for the each of the homodimers and their corresponding heterodimer. For each experiment are given the wavelength and its corresponding bandwidth that gave similar peak areas for equimolar mixtures of the homodimers involved, allowing the approximate quantitative analysis of the product.

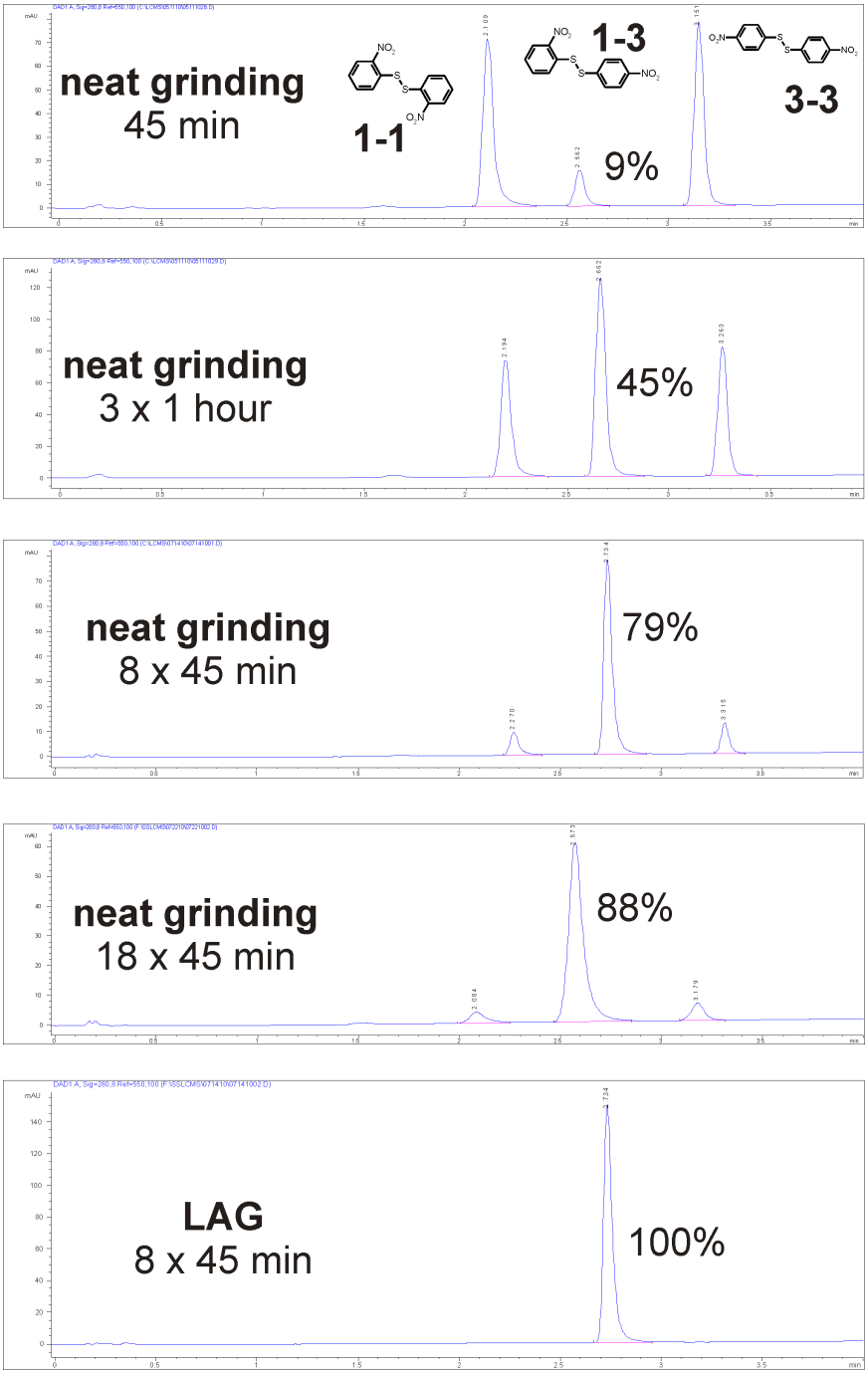


Figure S3. Comparison of the neat grinding and LAG reactivity of **1–1** and **3–3** to form **1–3**, recorded using HPLC method B monitored at 280 nm (8 nm bandwidth).

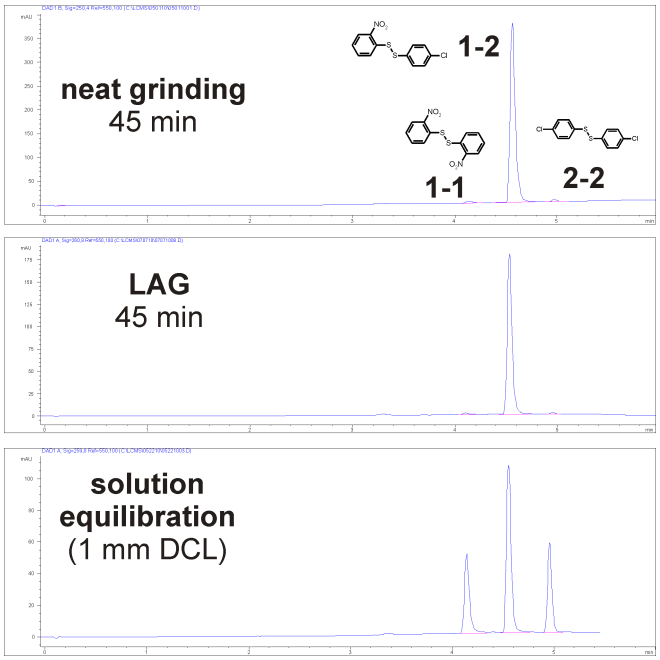


Figure S4. Comparison of 45 min neat grinding (top), 45 min LAG (middle) and 1 week solution reaction of **1–1** and **2–2** to form **1–2**, recorded using HPLC method A monitored at 260 nm (8nm bandwidth).

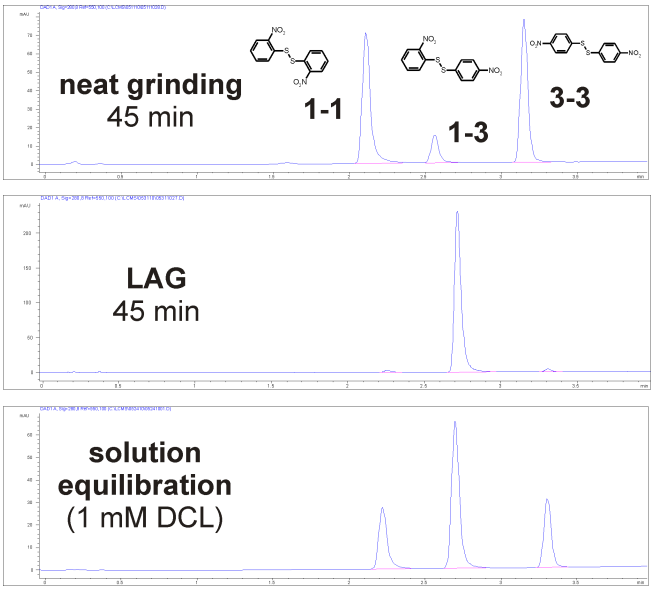


Figure S5. Comparison of 45 min neat grinding (top), 45 min LAG (middle) and 1 week solution reaction of **1–1** and **3–3** to form **1–3**, recorded using HPLC method B monitored at 280 nm (8nm bandwidth).

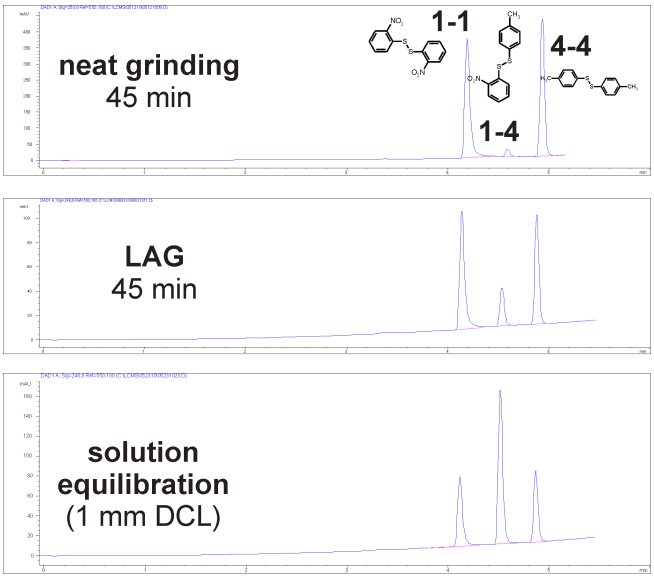


Figure S6. Comparison of 45 min neat grinding (top), 45 min LAG (middle) and 1 week solution reaction of **1–1** and **4–4** to form **1–4**, recorded using HPLC method A monitored at 248 nm (8nm bandwidth).

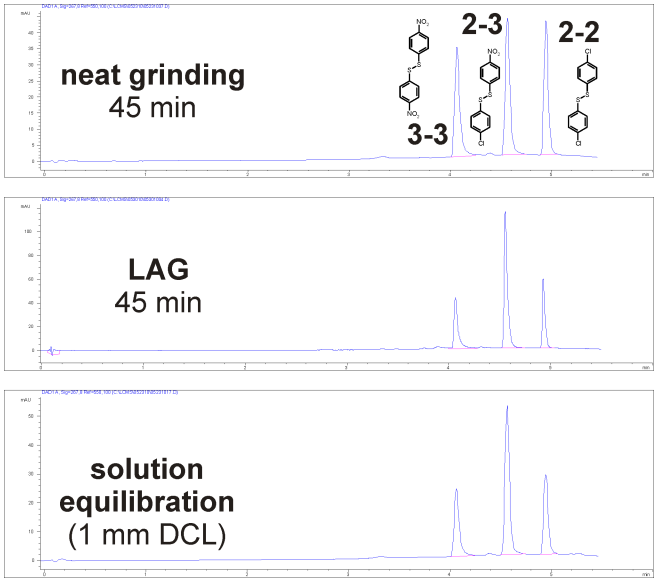


Figure S7. Comparison of 45 min neat grinding (top), 45 min LAG (middle) and 1 week solution reaction of **2–2** and **3–3** to form **2–3**, recorded using HPLC method A monitored at 267 nm (8nm bandwidth).

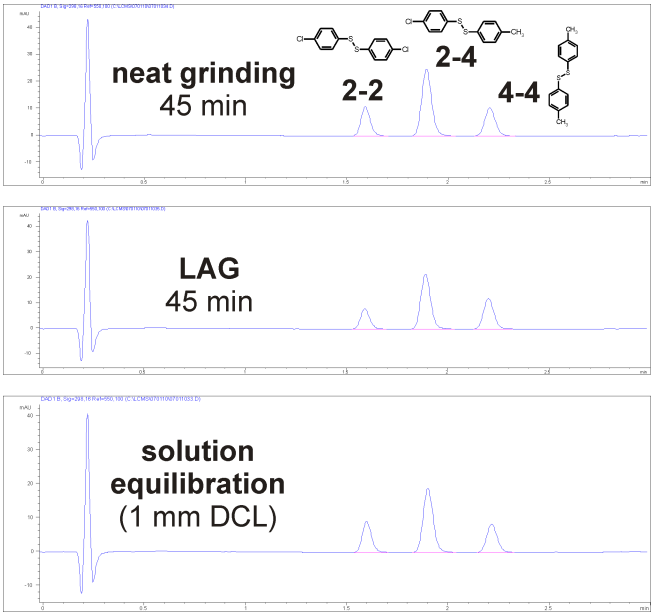


Figure S8. Comparison of 45 min neat grinding (top), 45 min LAG (middle) and 1 week solution reaction of **2–2** and **4–4** to form **2–4**, recorded using HPLC method C monitored at 298 nm (16nm bandwidth).

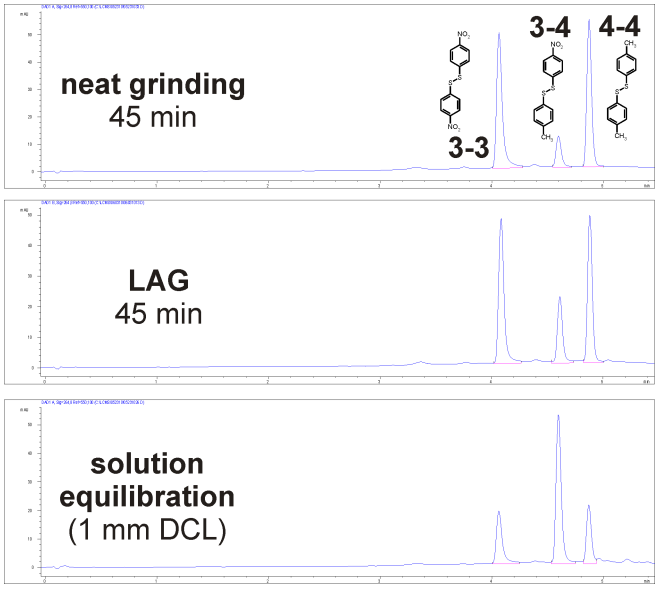


Figure S9. Comparison of 45 min neat grinding (top), 45 min LAG (middle) and 1 week solution reaction of **3–3** and **4–4** to form **3–4**, recorded using HPLC method A monitored at 264 nm (8nm bandwidth).

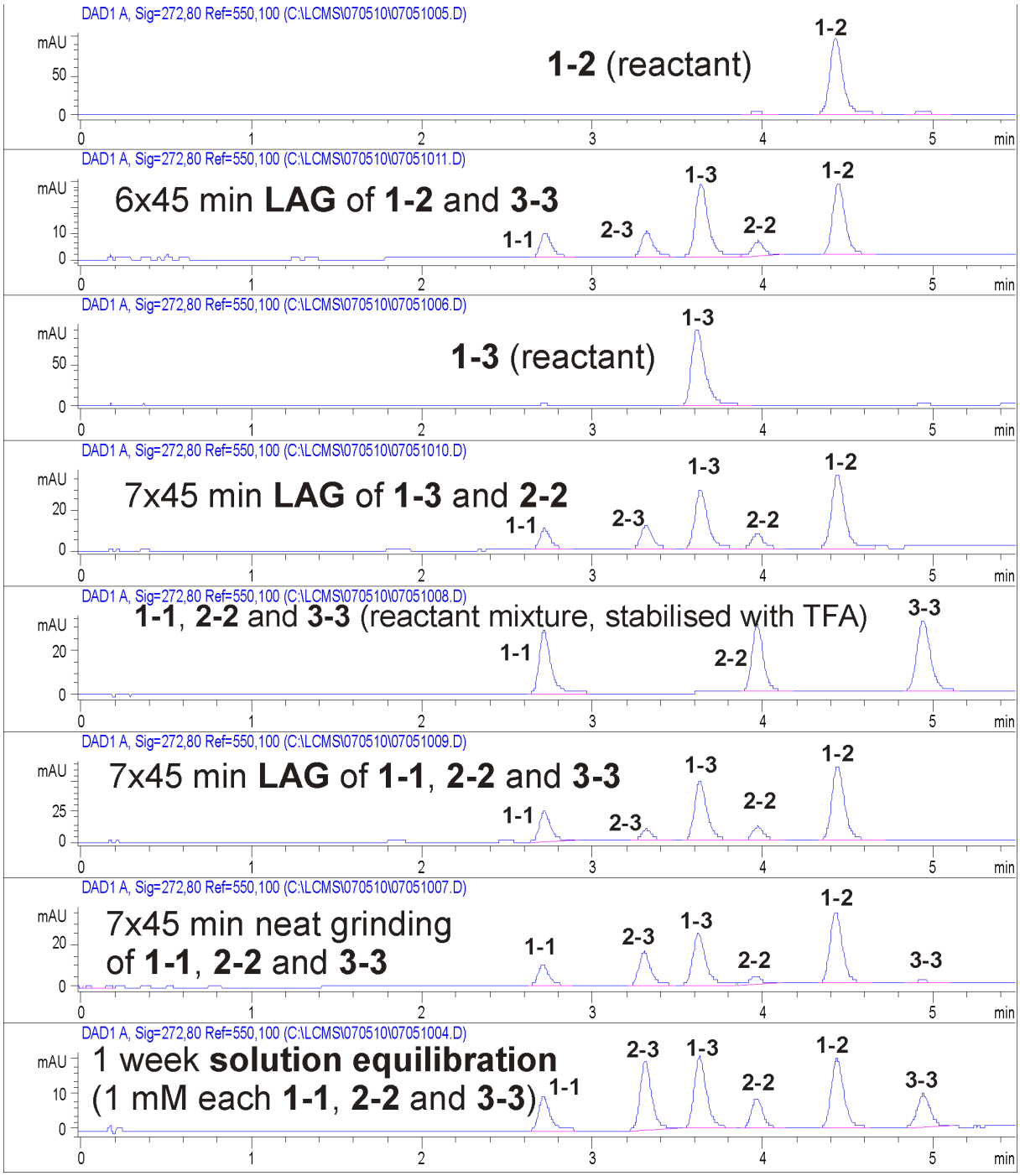


Figure S10. Comparison of HPLC chromatograms for three-component LAG reactions leading to a mixture of **1–1**, **2–2**, **3–3**, **1–2**, **1–3** and **2–3**, starting from different reactant materials. Recorded using HPLC method D monitored at 272 nm (80 nm bandwidth).

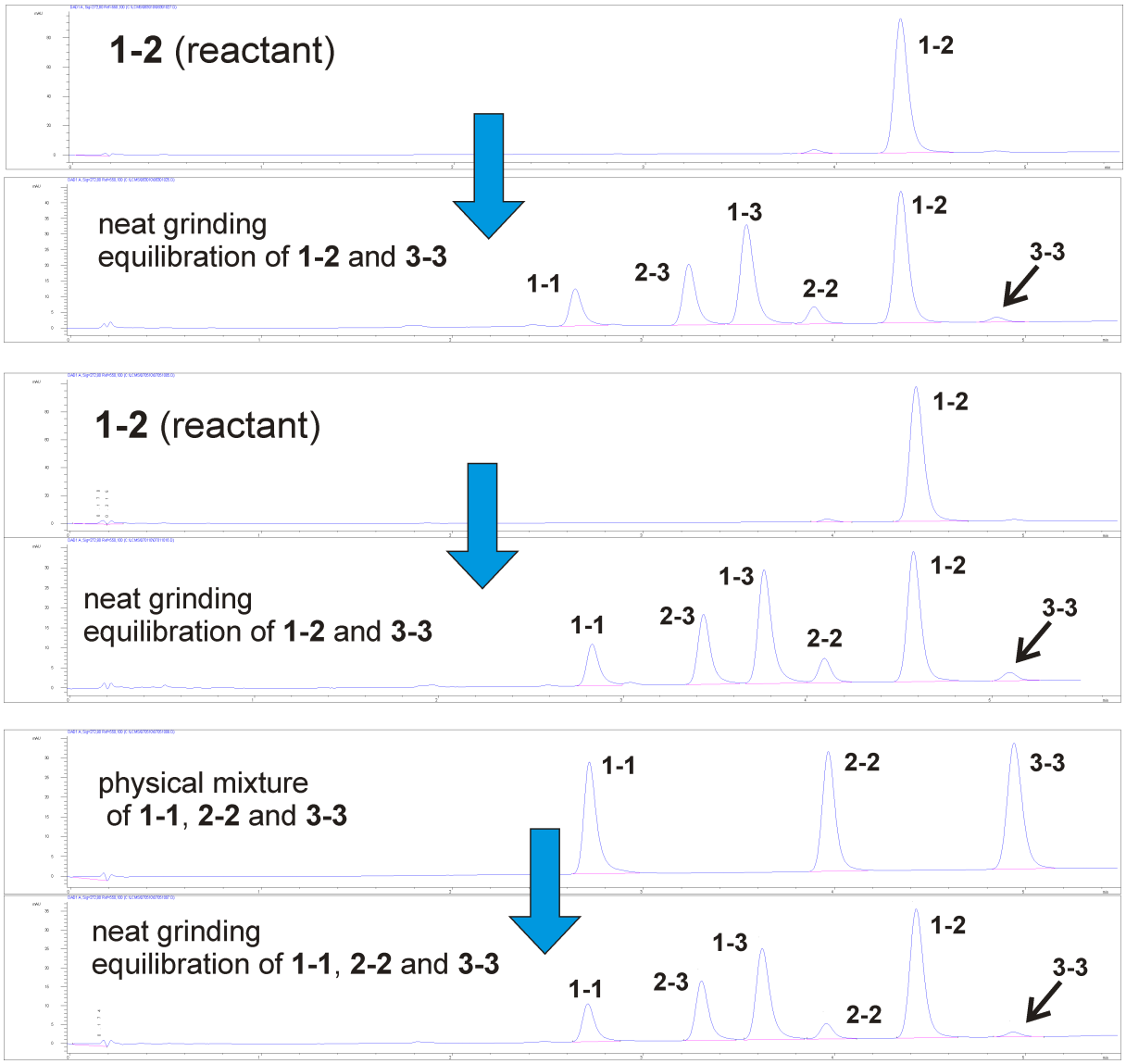


Figure S11. Comparison of HPLC chromatograms for three-component neat grinding reactions leading to a mixture of **1–1**, **2–2**, **3–3**, **1–2**, **1–3** and **2–3**, starting from different reactant materials. Recorded using HPLC method D monitored at 272 nm (80 nm bandwidth)

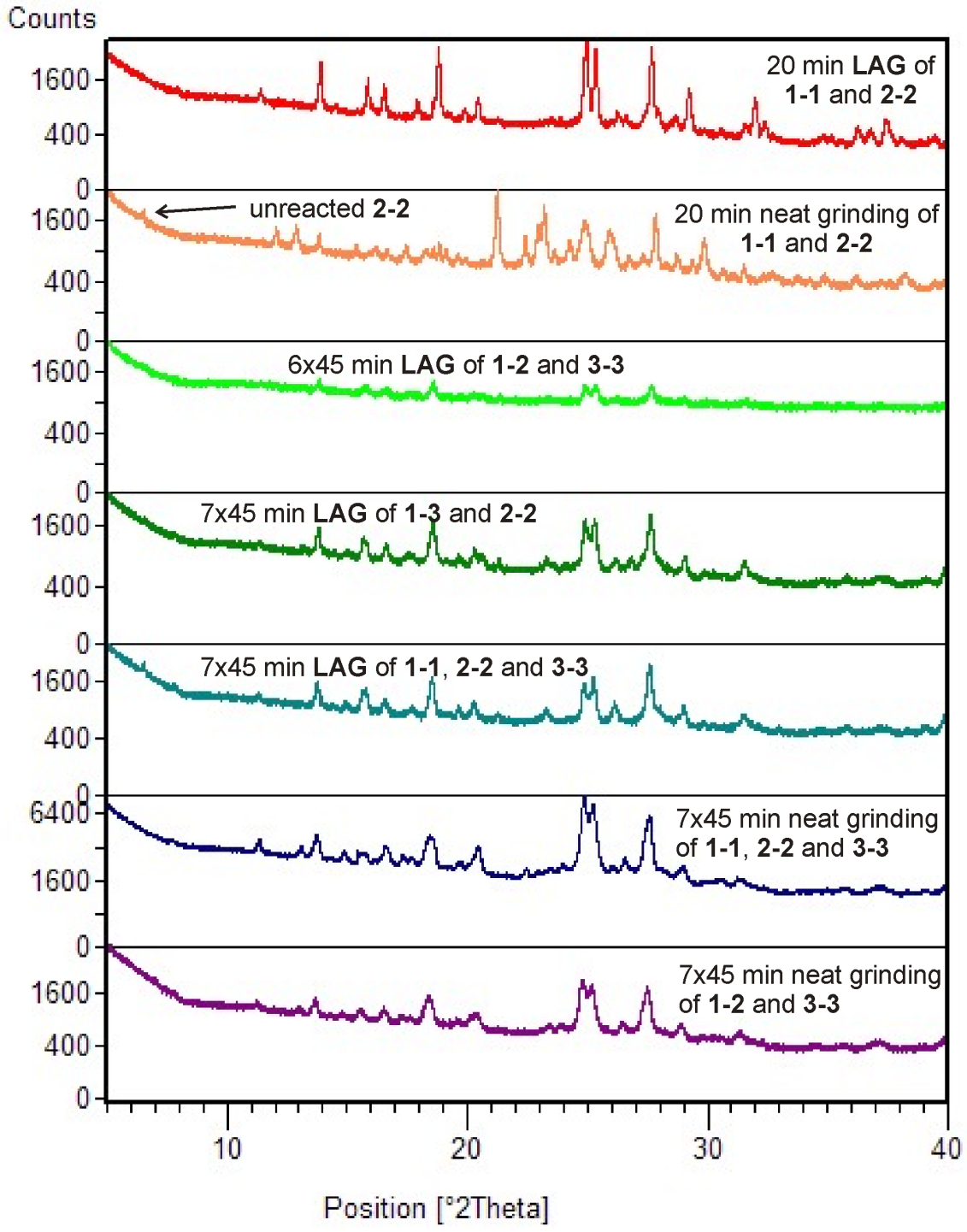


Figure S12. Comparison of PXRD patterns for the LAG and neat grinding reaction of **1–1** and **2–2** at short grinding times (20 min) and for three-component reactions leading to a mixture of **1–1**, **2–2**, **3–3**, **1–2**, **1–3** and **2–3**, starting from different reactant materials.

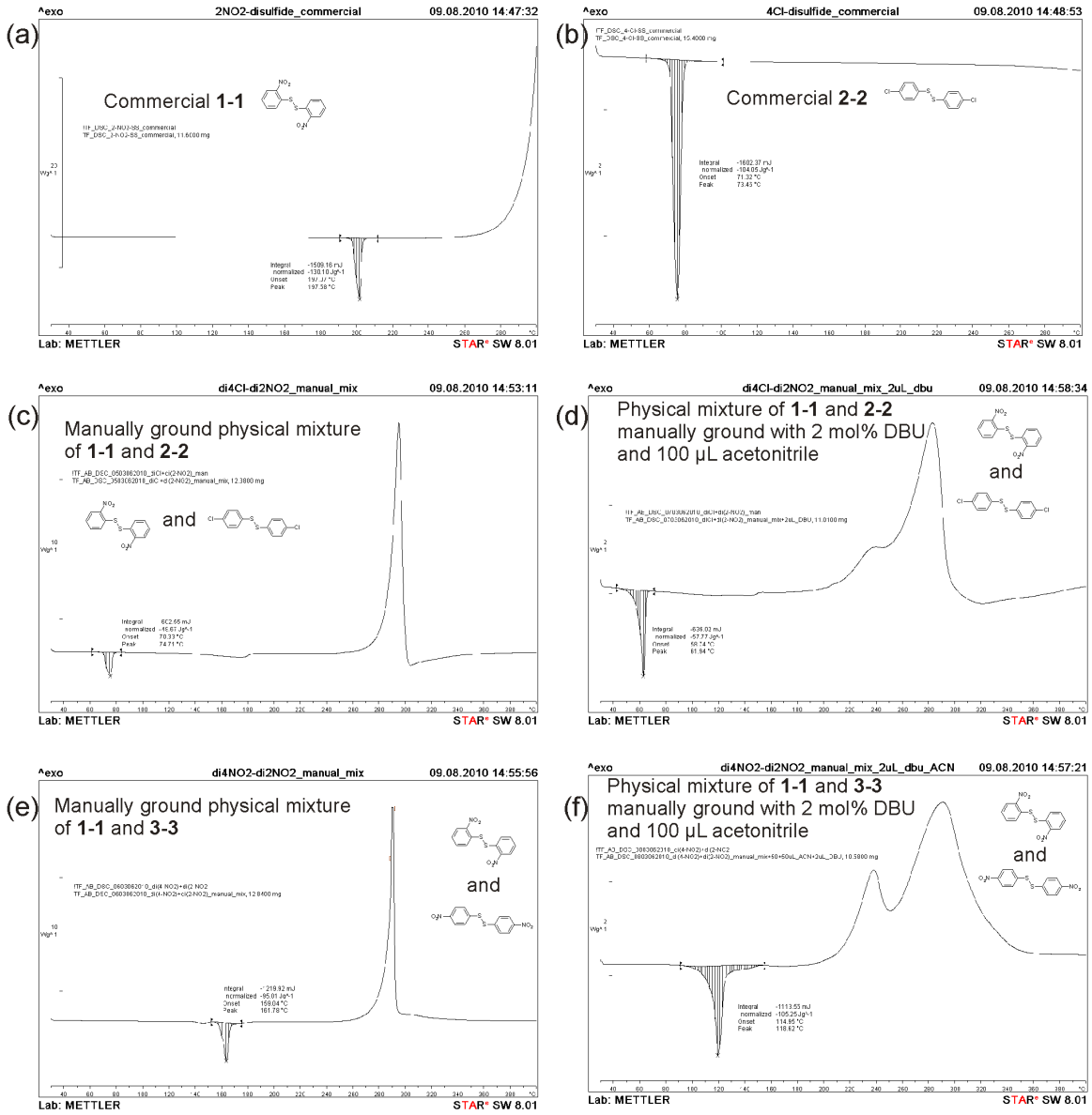


Figure S13. DSC thermograms for: (a) commercial **1–1**; (b) commercial **2–2**; (c) neat ground mixture of **1–1** and **2–2**; (d) mixture of **1–1** and **2–2** ground with 2mol% **dbu** and 100 µL acetonitrile; (e) neat ground mixture of **1–1** and **3–3** and (f) mixture of **1–1** and **3–3** ground with 2mol% **dbu** and 100 µL acetonitrile. In all cases the ground mixtures melt significantly above room temperature. The melting point of pure **1–1** is at 182 ºC, see:

COMPUTATIONAL METHODS

Molecular energies were calculated at the MP2(frozen core)/cc-pVDZ level of theory. For each molecule, full geometry optimizations were performed from a sample of starting conformations, considering several combinations of the C-S-S-C dihedral angle and the two S-S-C-C dihedral angles defining the orientation of the phenyl rings. The isolated molecule energy was taken as the lowest energy structure found for each molecule.

The intermolecular component of the crystal lattice energies were calculated using pairwise atom-atom calculations. The W99 exp-6 potential (Williams, D. E. *Journal of Computational Chemistry* **2001**, *22*, 1154-1166) was used to represent repulsion – dispersion contributions to the intermolecular potential and interactions were summed to a 15Å cutoff. Atomic multipoles were used to represent electrostatic contributions to the intermolecular potential. Atomic multipoles up to hexadecapole on each atom were generated via a Distributed Multipole Analysis of a B3LYP/6-31G** electron density. All charge-charge, charge-dipole, and dipole-dipole contributions to the lattice energy were evaluated using the Ewald summation, while higher order electrostatic interactions (up to R^{-5}) were summed to a 15 Å cutoff on entire molecules. Starting structures were prepared by extracting the molecular geometry from each crystal structure and performing a molecular optimization with selected dihedral angles constrained at observed values to maintain the conformation found in the crystal structure (constrained dihedral angles were C-S-S-C and two S-S-C-C angles for all molecules and O-N-C-C for nitro groups). The resulting molecular geometry was then placed in the original crystal structure, which was then optimized [at the MP2(frozen core)/cc-pVDZ level of theory] with respect to the position and orientation of the molecules and the lattice parameters, using the observed space group. The molecular positions and conformations were treated as rigid during lattice energy minimizations and the centre of the intermolecular interaction for hydrogen atoms was shifted by 0.1 Å along the X-H bond (X=C, N, O), as required for the W99 potential. Total crystal energies were calculated as the sum of the atom-atom intermolecular energy and the MP2 energy of the partially optimized molecular structure.

All molecular electronic structure calculations were performed using Gaussian03:
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Lattice energy calculations were performed using DMACRYS:

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Distributed multipole analyses were performed using the GDMA program, employing the original DMA algorithm:

GDMA, version 2, Stone, A. J.; University of Cambridge.
Stone, A. J. *Chem. Phys. Lett.* **1981**, *83*, 233 - 239.