

Supporting Information

4. Silk Fibroin Protein-mediated C-C Bond Formation

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1. Preliminary optimization experiments

Table S1. Determination of the ideal amount of nitromethane (**2**).^[a]

Entry	eq. MeNO ₂	Yield (%) ^[b]
1	5	38
2	10	69

[a] Reaction conditions: **1** (0.1 mmol), **2** (0.5 or 1.0 mmol), FDSF (batch 2, 10 mg), DMSO (0.5 mL), room temperature; [b] Determined by ¹H NMR analysis of the crude product.

2. Recycling study

Henry reaction

Table S2. Proof of the recyclability of FDSF-mediated Henry reaction between 4-nitrobenzaldehyde (**1**) and nitromethane (**2**).^[a]

Entry	Run	Yield 3a (%) ^[b]
1	1	95 (< 1 ^[c])
2	2	42
3	3	12
4	4	8

[a] Reaction conditions: **1** (0.1 mmol), **2** (1.0 mmol), FDSF (batch 1, 10 mg), DMSO (0.5 mL), RT, 3 h; [b] Determined by ¹H NMR analysis of the crude product; [c] Control experiment without catalyst.

Knoevenagel condensation

Table S3. Proof of the recyclability of FDSF-mediated Knoevenagel condensation between 2-nitrobenzaldehyde (**5**) and ethyl cyanoacetate (**6**).^[a]

		
Entry	Run	Yield 7 (%) ^[b]
1	1	84 (< 1 ^[c])
2	2	60
3	3	34
4	4	20

[a] Reaction conditions: **5** (0.1 mmol), **6** (0.11 mmol), FDSF (batch 1, 10 mg), DMSO (0.5 mL), RT, 3 h; [b] Determined by ¹H NMR analysis of the crude product; [c] Control experiment without catalyst.

3. Kinetic study

Table S4. Knoevenagel model reaction between 2-nitrobenzaldehyde (**5**) and ethyl cyanoacetate (**6**) mediated by FDSF.^[a]



Entry	time (min)	Yield 7 (%) ^[b]
1	0	0
2	5	8
3	10	14
4	20	21
5	30	32
6	40	46
7	60	54
8	90	64
9	120	72
10	150	80
11	180	84

[a] Reaction conditions: **5** (0.1 mmol), **6** (0.11 mmol), FDSF (batch 1, 10 mg), DMSO (0.5 mL), RT;

[b] Determined by ¹H NMR analysis of the crude product.

4. IR spectra

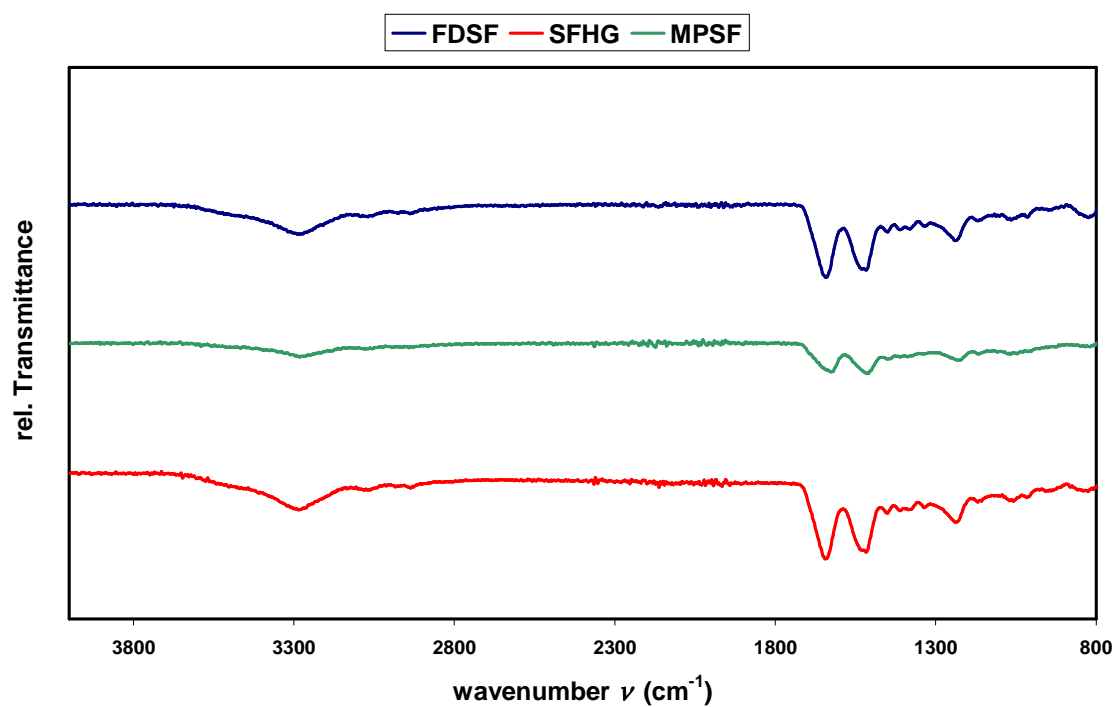


Figure S1. Comparison of the IR spectra: FDSF (blue line), MPSF (green line) and freeze-dried SFHG (red line).

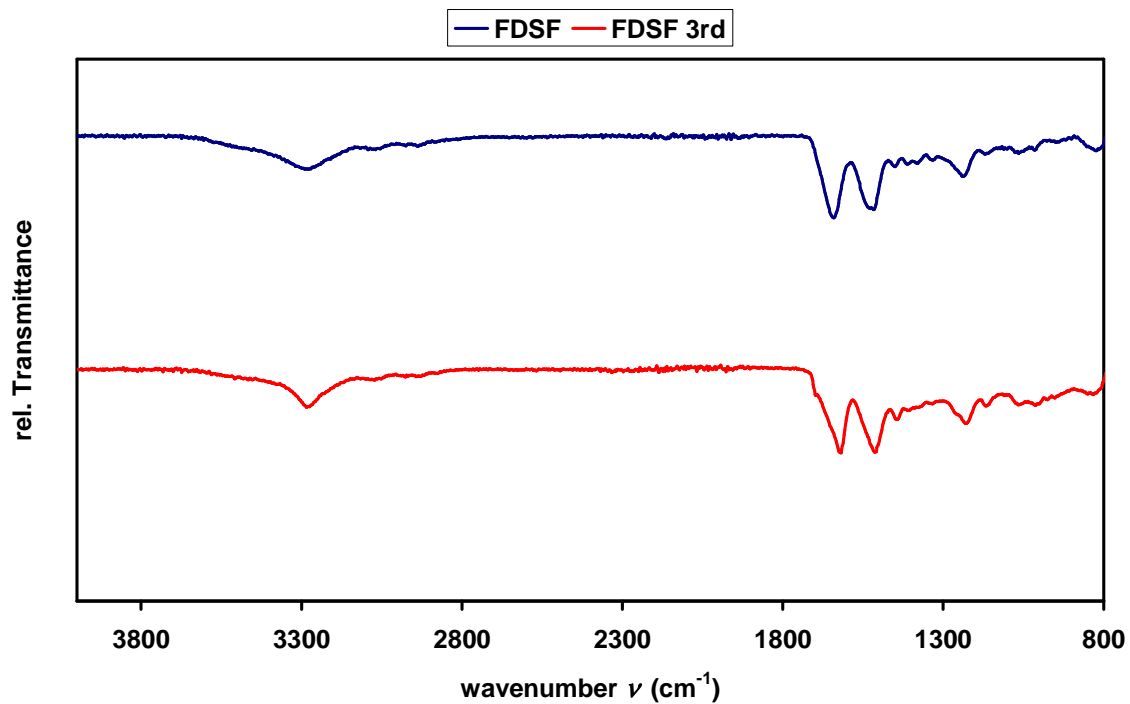


Figure S2. Comparison of the IR spectra of FDSF before (blue line) and after the 3rd cycle (red line) in the Henry reaction:

5. TGA curves

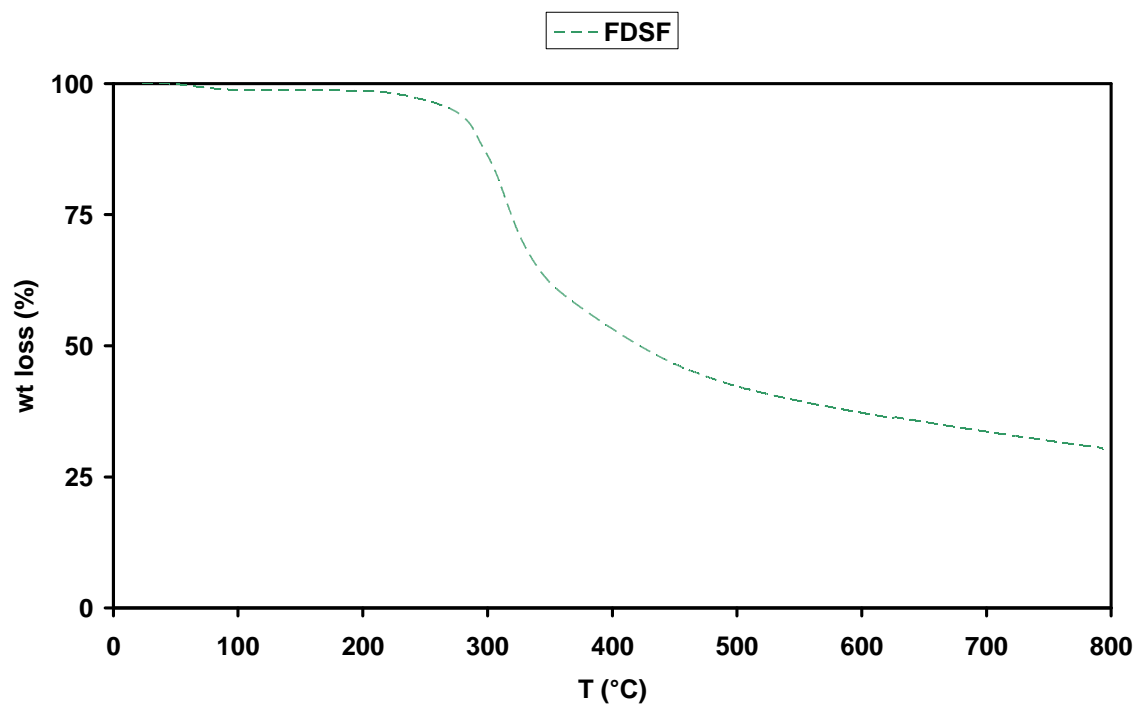


Figure S5. TGA curve of FDSF showing the highest efficacy in the tested C-C bond forming reactions.

6. Additional FESEM images

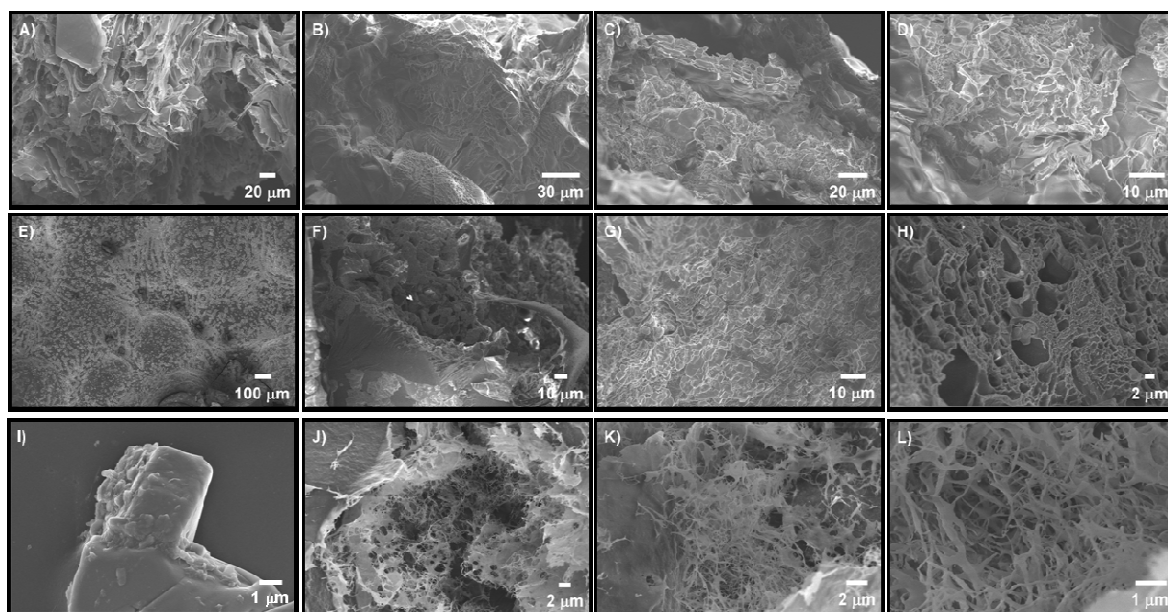


Figure S6. Additional FESEM pictures of the used catalysts: A) FDSF (mag = 310 \times); B) FDSF after 3rd cycle in the Henry reaction (mag = 510 \times); C) FDSF after 3rd cycle (mag = 608 \times); D) FDSF after 3rd cycle (mag = 1.46K \times); E) MPSF (mag = 65 \times); F) MPSF (mag = 489 \times); G) MPSF (mag = 1.01K \times); H) MPSF (mag = 1.85K \times); I) MPSF (mag = 9.28K \times); J) SFHG (mag = 2.32K \times); K) SFHG (mag = 4.38K \times); L) SFHG (mag = 12.77K \times).

7. Selected ^1H NMR spectra

Table 3, entry 1:^[2] N,N-Dimethylacetamide was used as internal standard (IS)

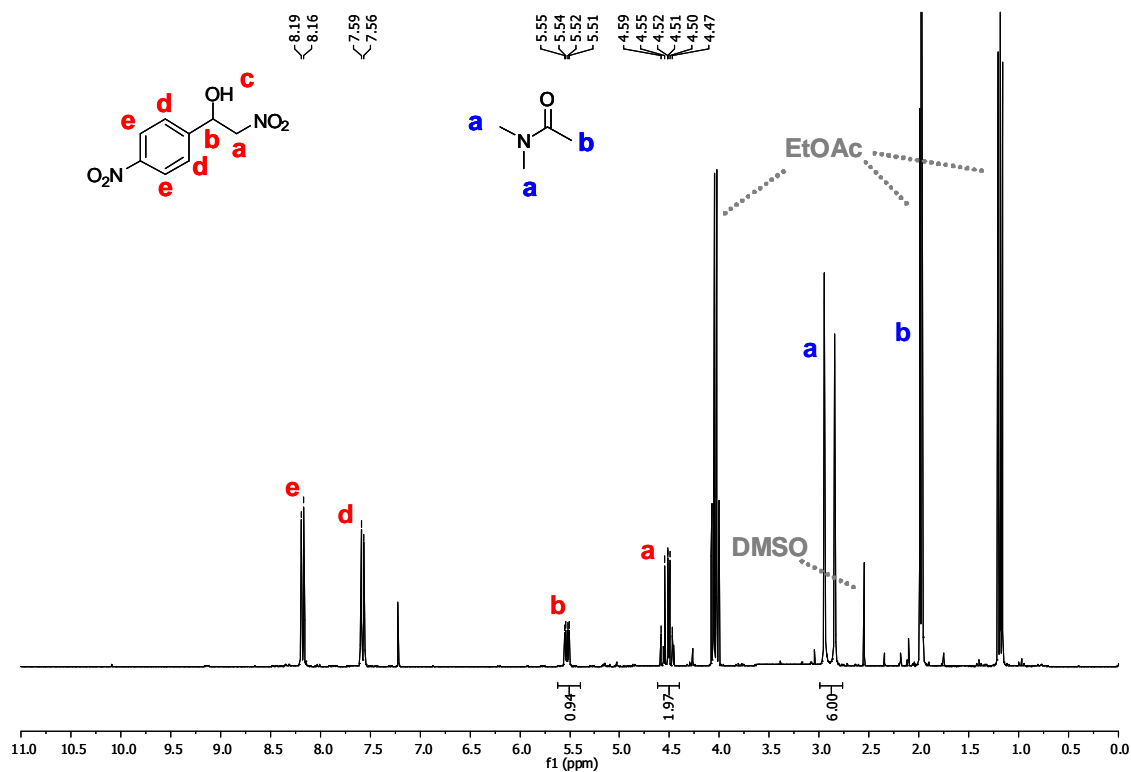


Table 3, entry 2:^[3] Diphenylmethane was used as IS

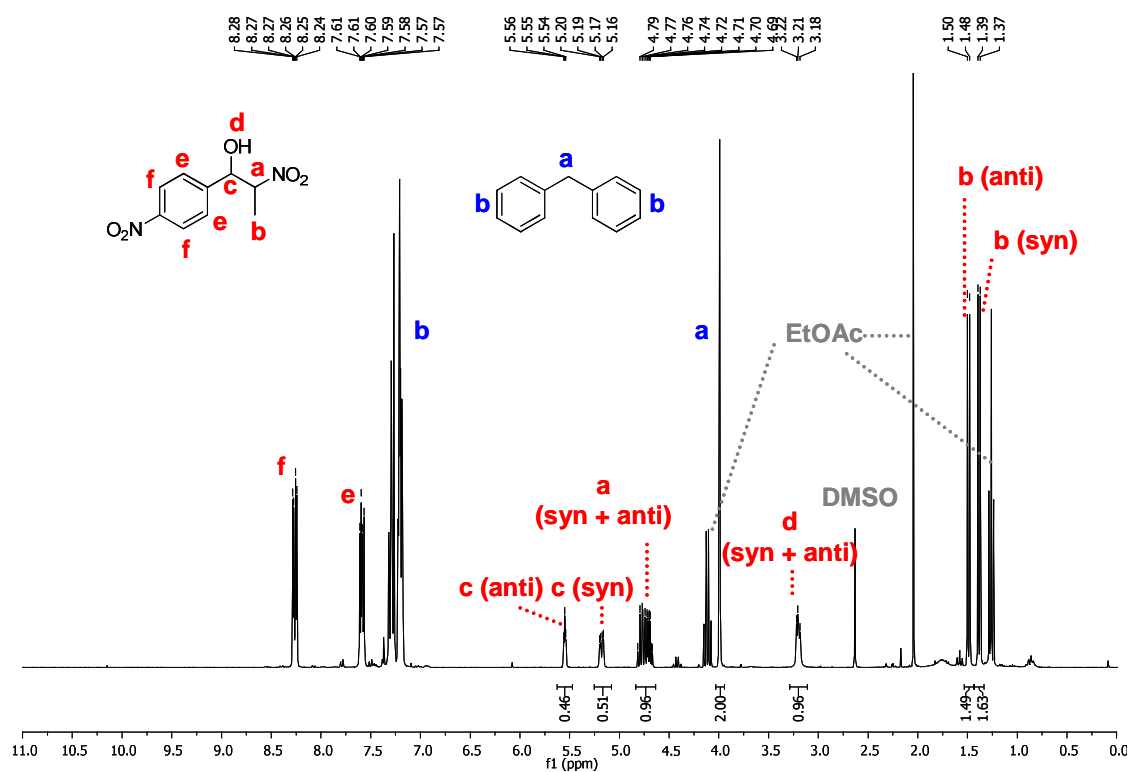


Table 3, entry 3:^[2] Diphenylmethane was used as IS

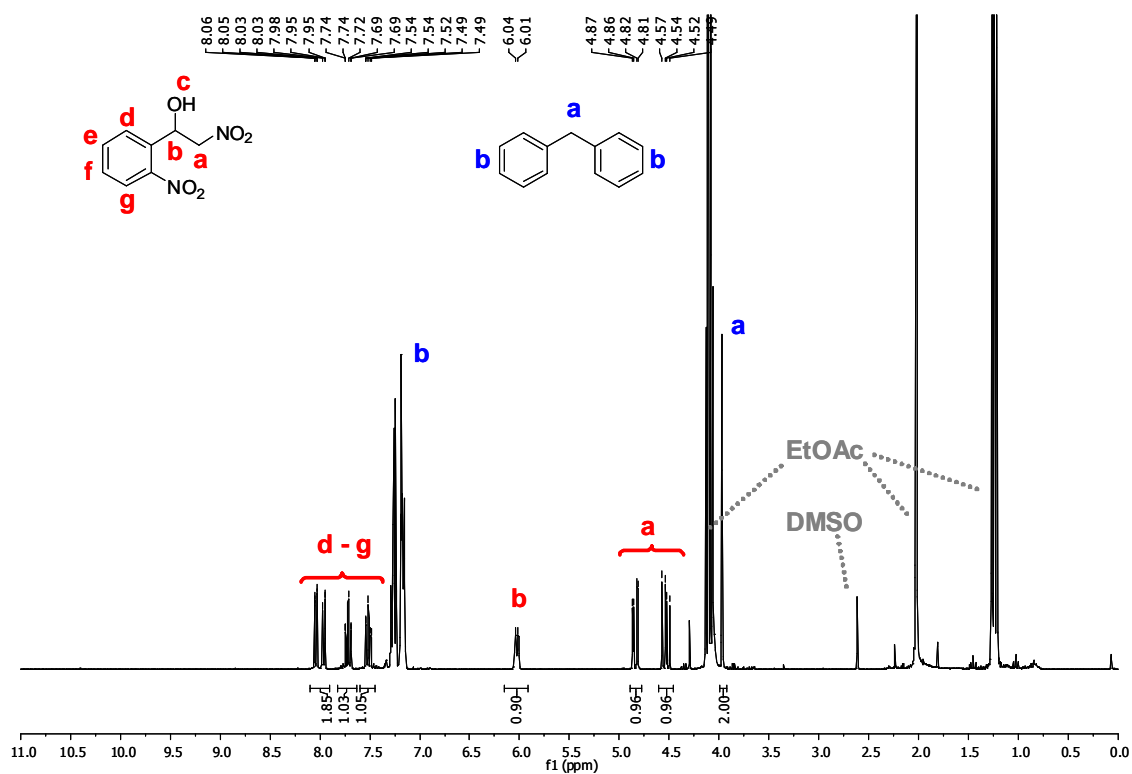


Table 3, entry 5:^[3] *N,N*-Dimethylacetamide was used as IS

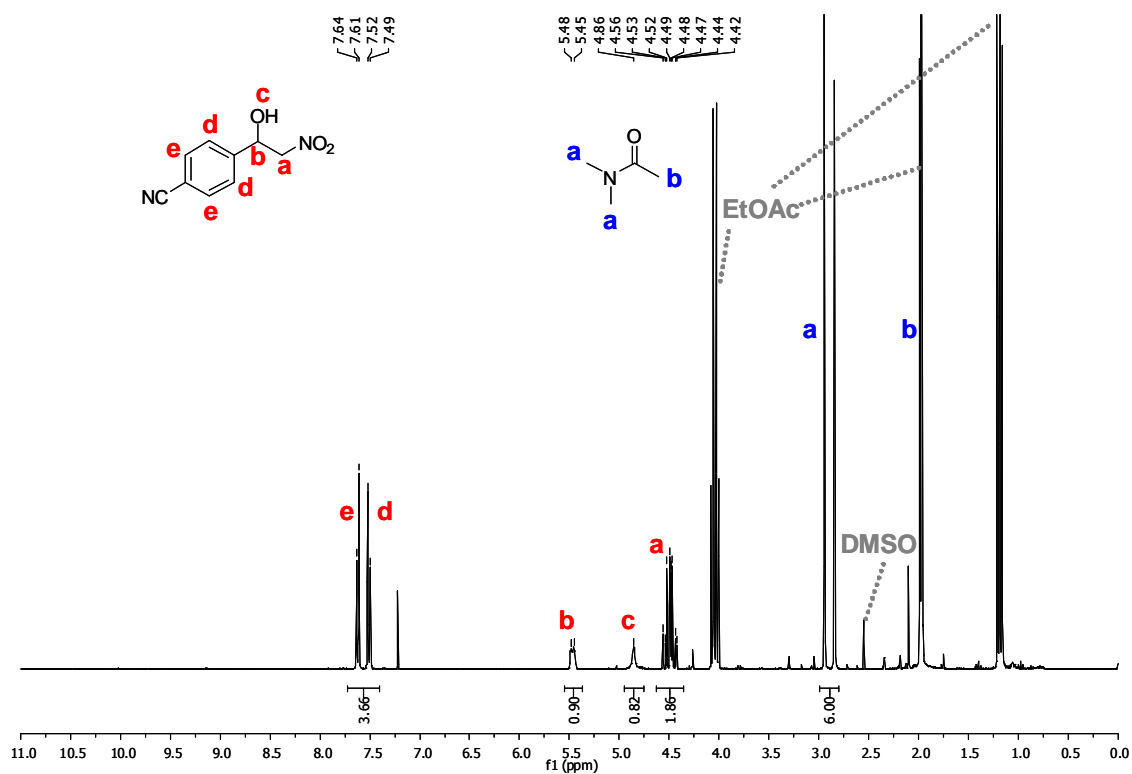


Table 3, entry 6:^[4] Diphenylmethane was used as IS

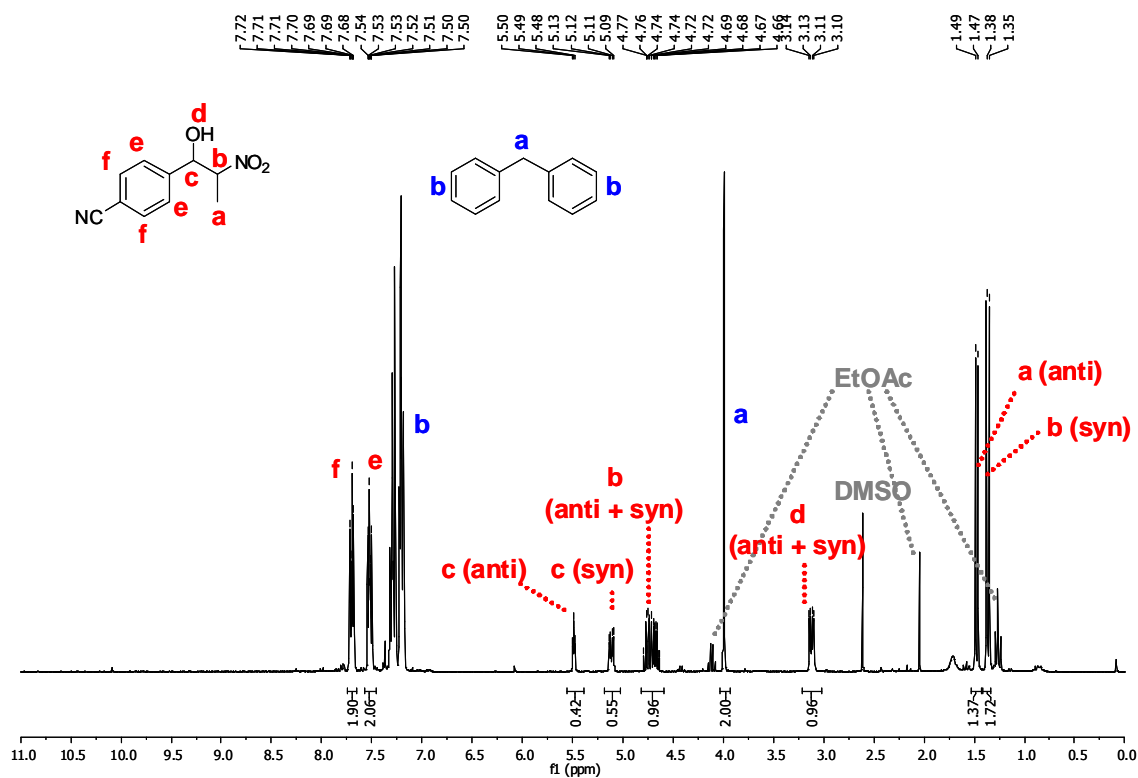


Table 3, entry 7:^[5] *N,N*-Dimethylacetamide was used as IS

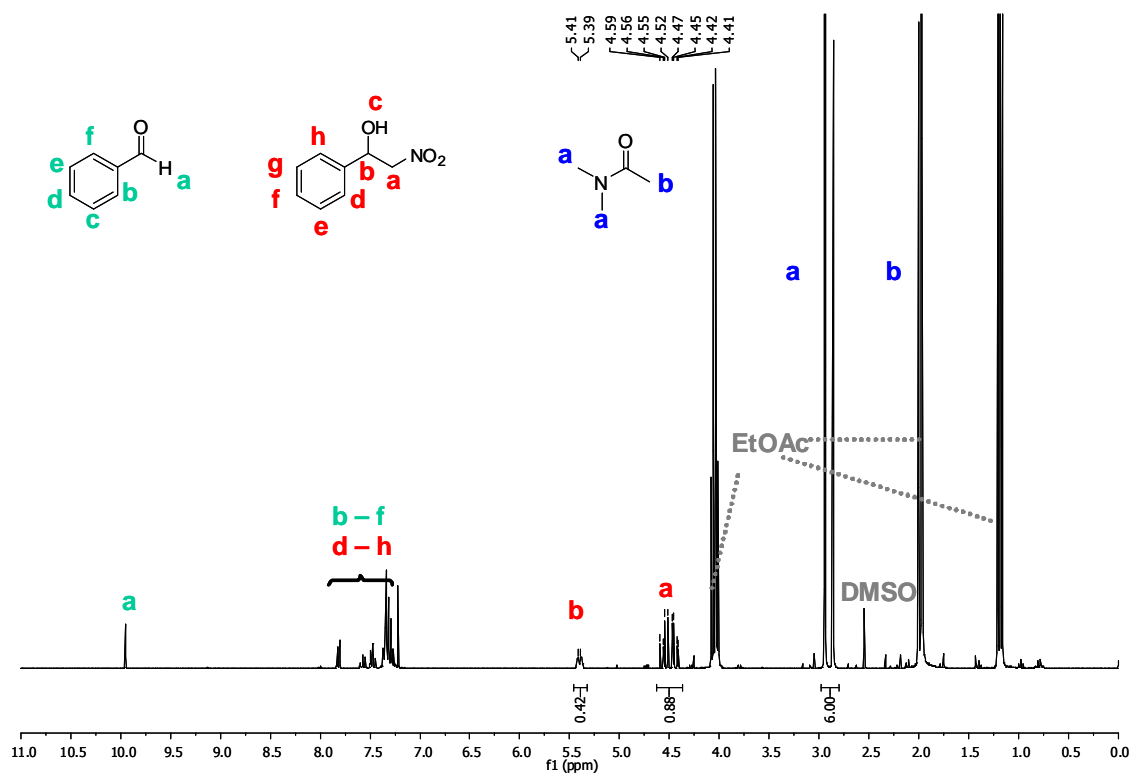


Table 3, entry 8:^[3] *N,N*-Dimethylacetamide was used as IS

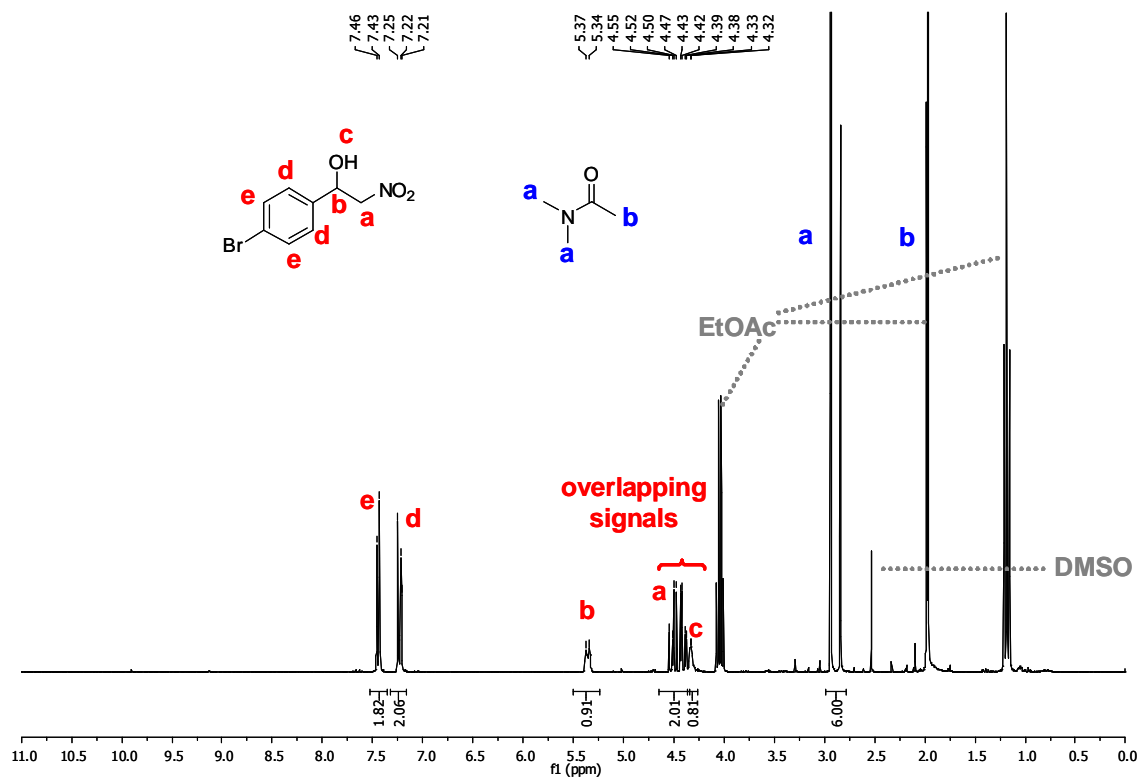


Table 3, entry 9:^[5] *N,N*-Dimethylacetamide was used as IS

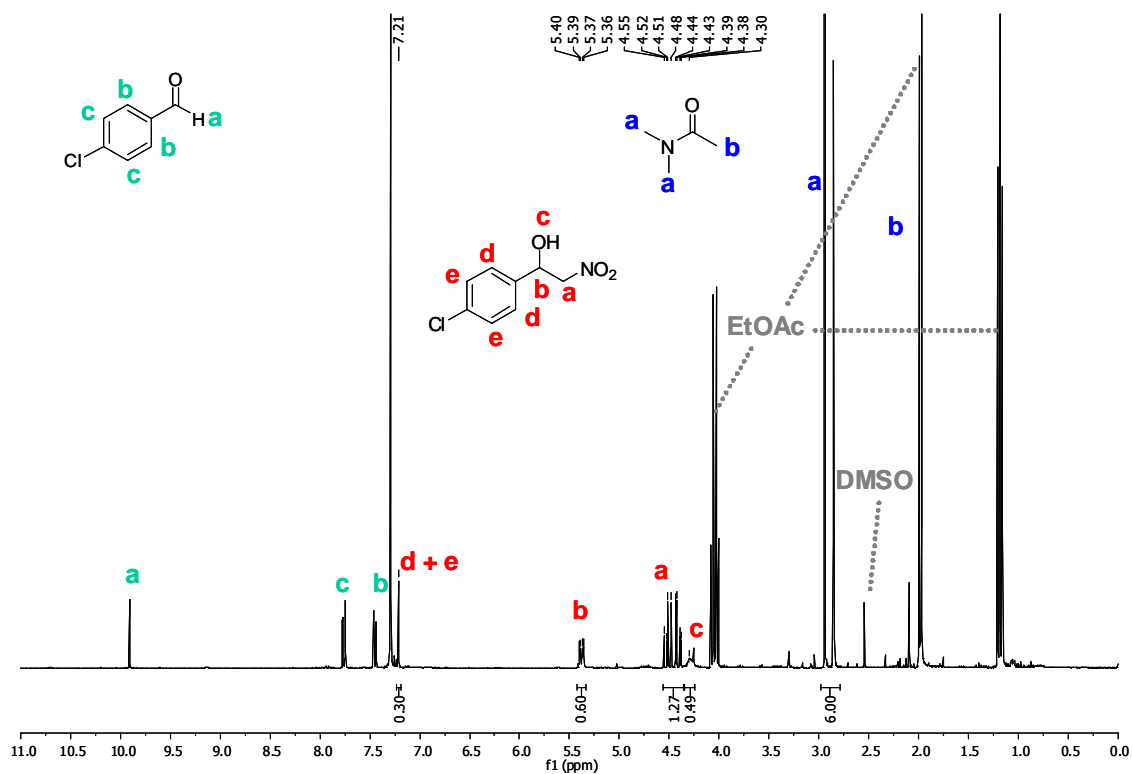


Table 3, entry 10:^[5] Diphenylmethane was used as IS

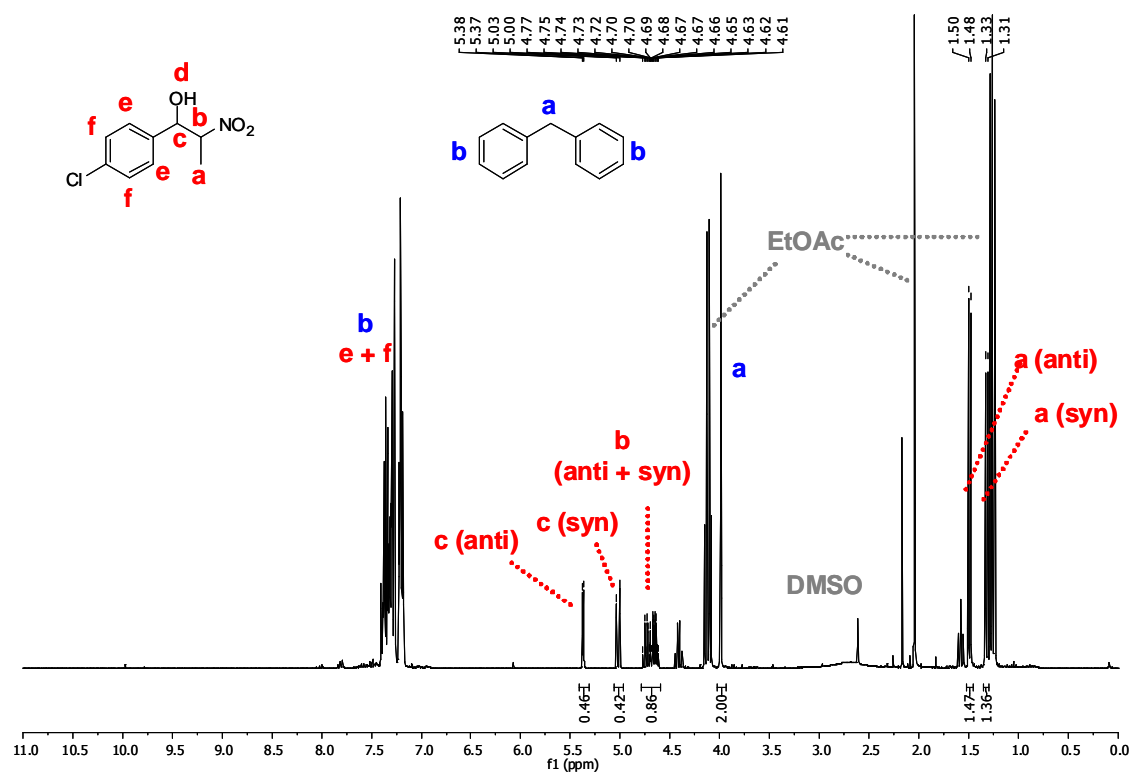
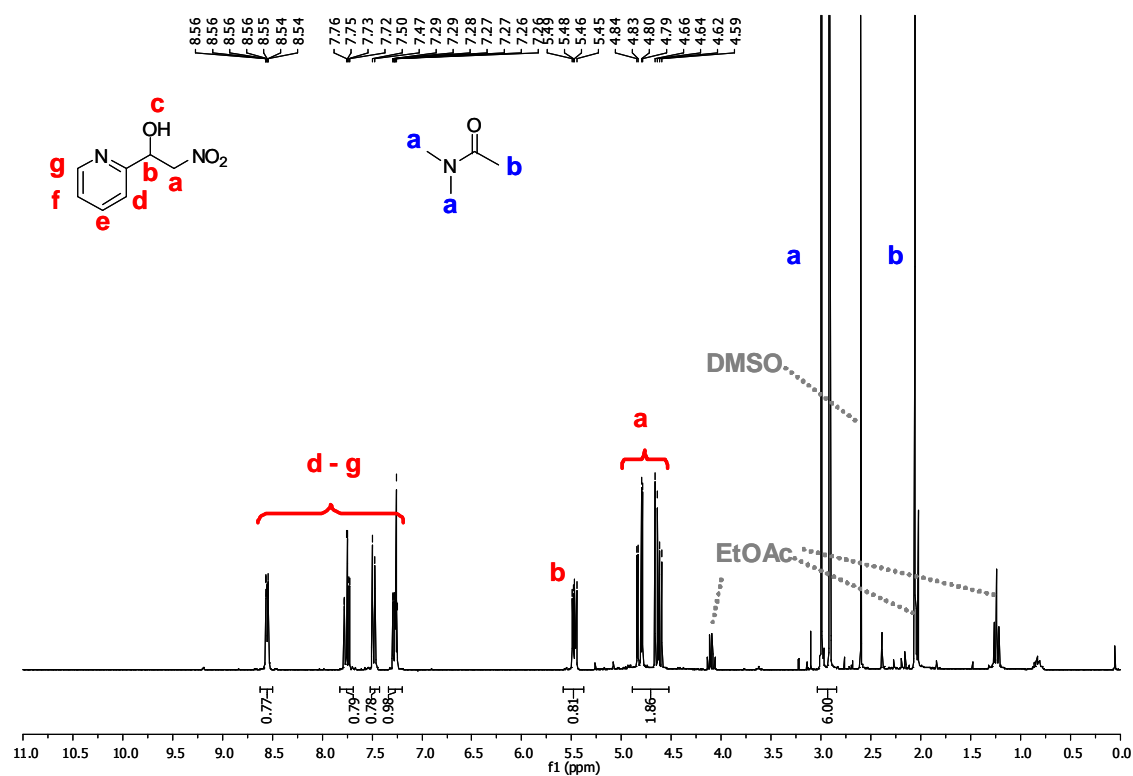
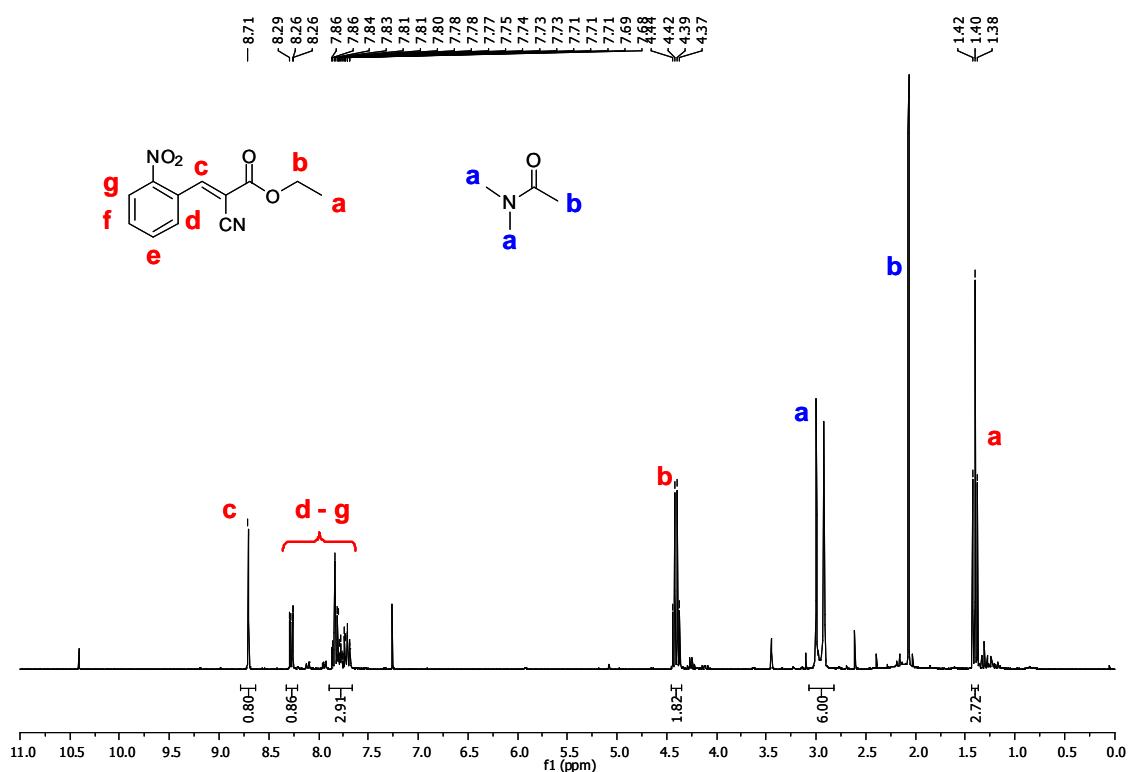


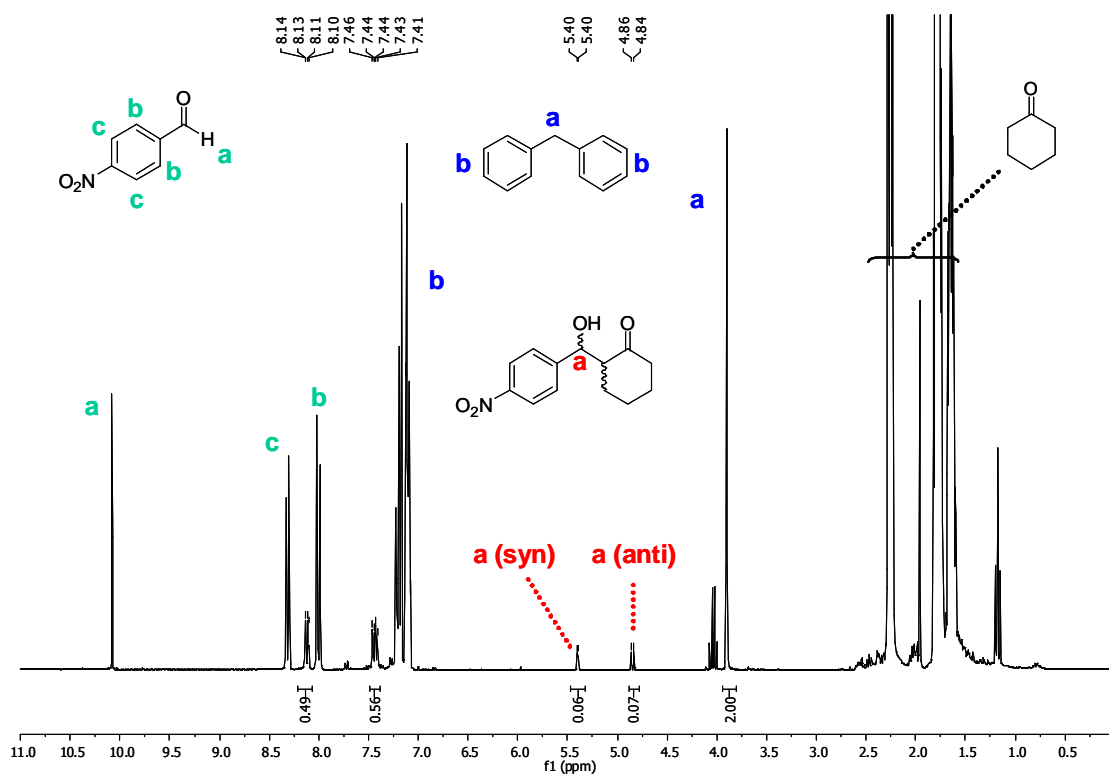
Table 3, entry 12:^[3] *N,N*-Dimethylacetamide was used as IS



Representative ^1H NMR spectra of the crude Knoevenagel condensation product **7** at 2.5 h:^[6] *N,N*-Dimethylacetamide was used as IS



Representative ^1H NMR spectra of the crude aldol product **9** in H_2O after 7 d at 37 °C (Table 4, entry 6):^[7] Diphenylmethane was used as IS



8. Typical kinetics for FDSF-mediated Henry reaction between 1a and 2

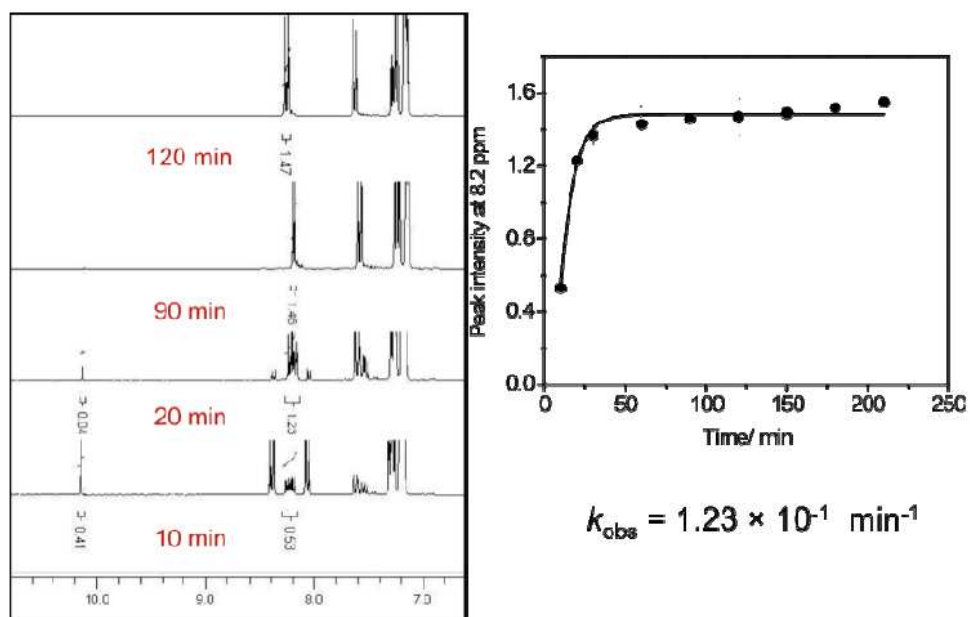
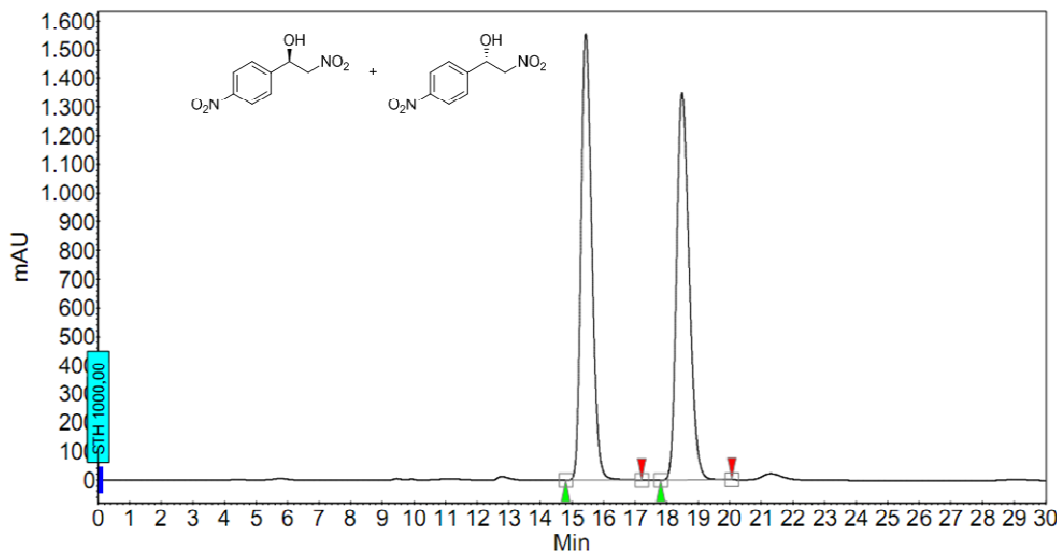


Figure S7. Kinetics for FDSF-mediated nitroaldol (Henry) reaction between **1a** and **2**.

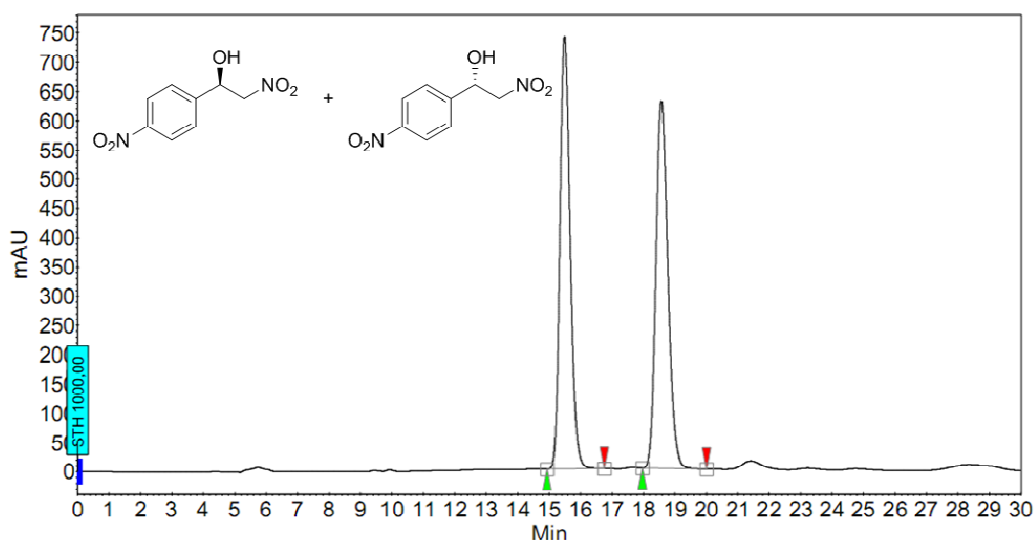
9. HPLC spectra

FDSF-mediated Henry reaction in DMSO:



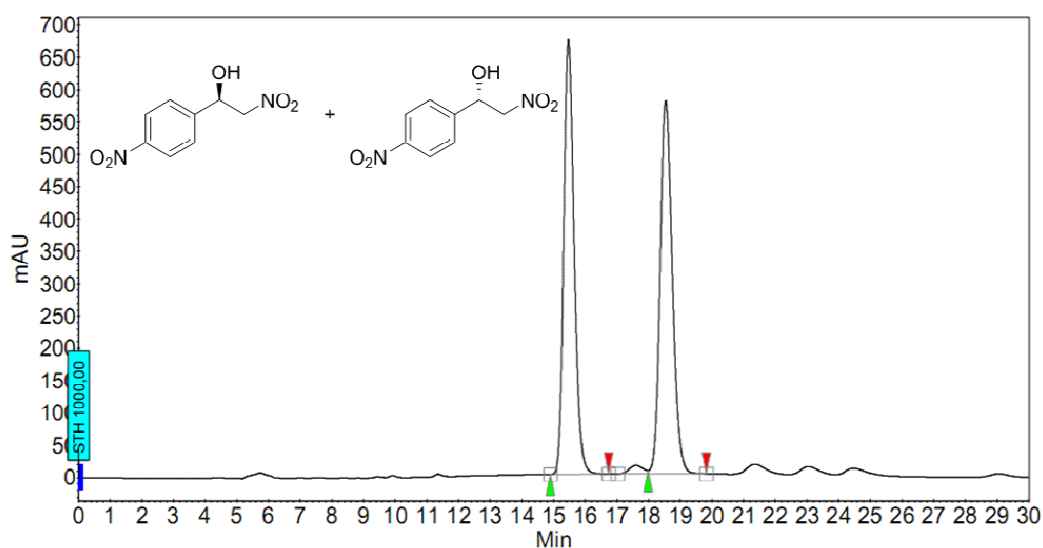
Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	15,44	49,49	1554,7	613,6	49,490
2	UNKNOWN	18,49	50,51	1350,0	626,3	50,510
Total			100,00	2904,7	1239,9	100,000

FDSF-mediated Henry reaction in H₂O:



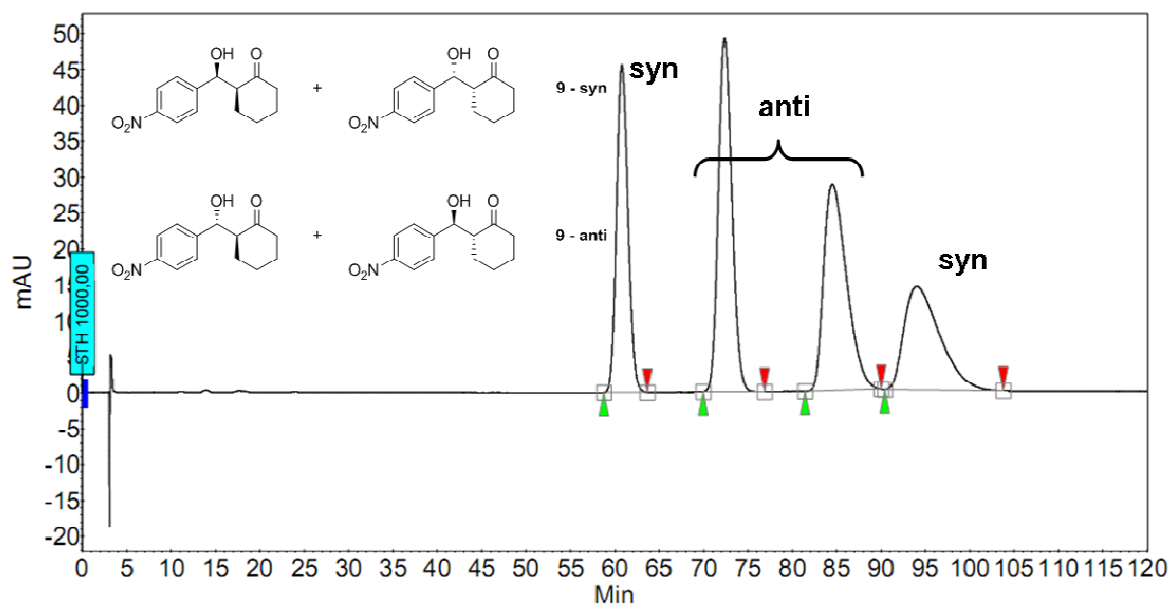
Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	15,49	49,93	739,0	277,9	49,934
2	UNKNOWN	18,57	50,07	628,9	278,6	50,066
Total			100,00	1368,0	556,5	100,000

FDSF-mediated Henry reaction in DMF:



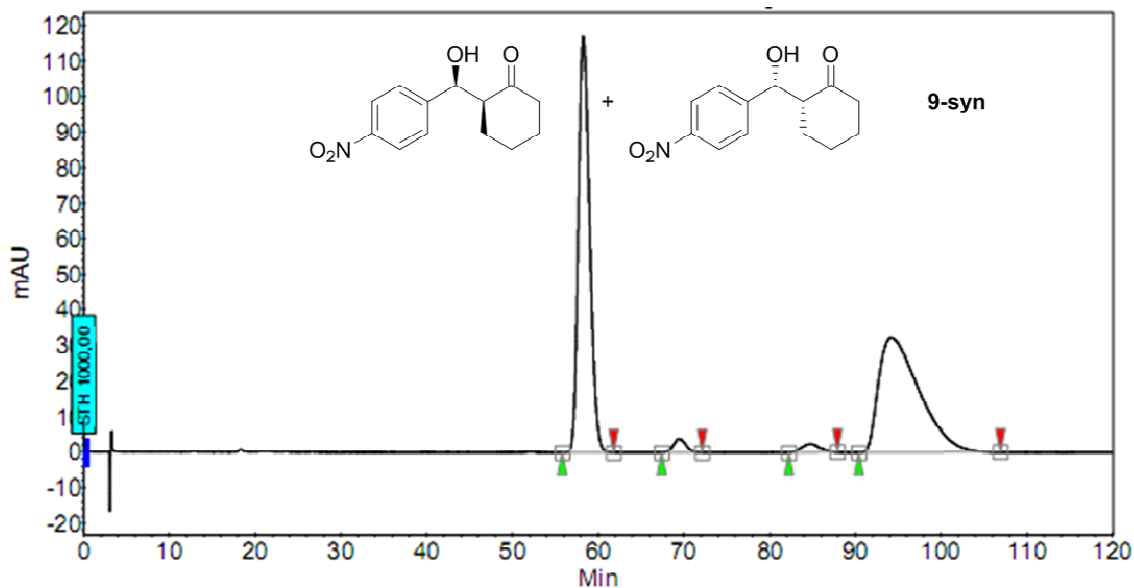
Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	15,47	49,85	672,3	255,0	49,846
2	UNKNOWN	18,54	50,15	576,0	256,6	50,154
Total			100,00	1248,3	511,6	100,000

Aldol - Racemate of 9:



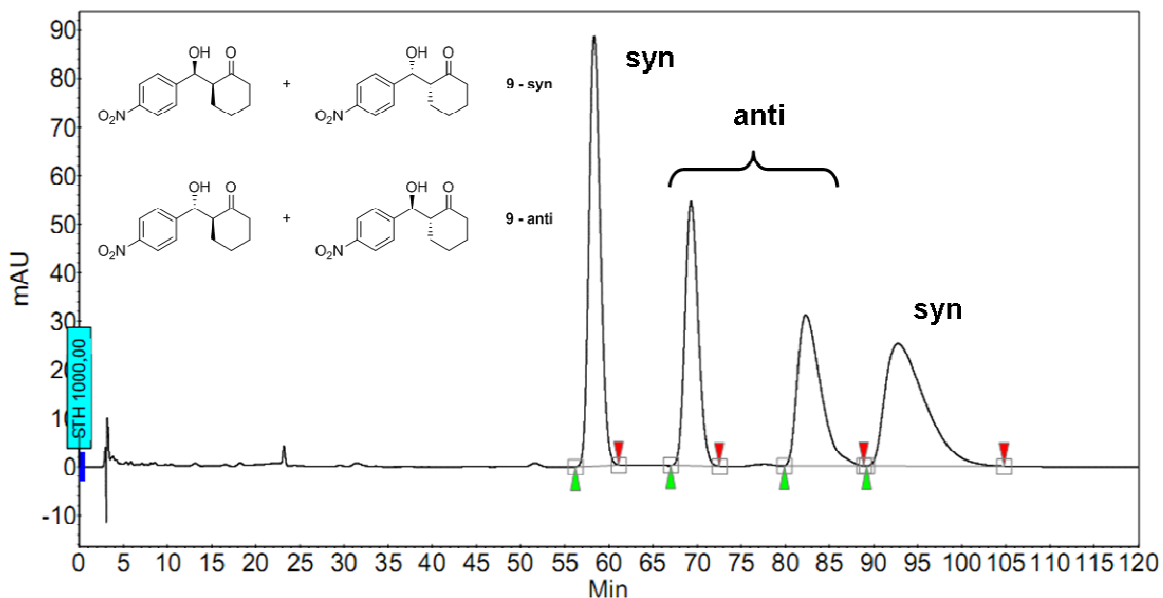
Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	60,80	22,21	45,7	68,7	22,209
2	UNKNOWN	72,37	28,27	49,3	87,4	28,267
3	UNKNOWN	84,51	27,90	28,8	86,3	27,897
4	UNKNOWN	93,98	21,63	14,3	66,9	21,627
Total			100,00	138,1	309,3	100,000

Aldol 9-syn diastereomers:



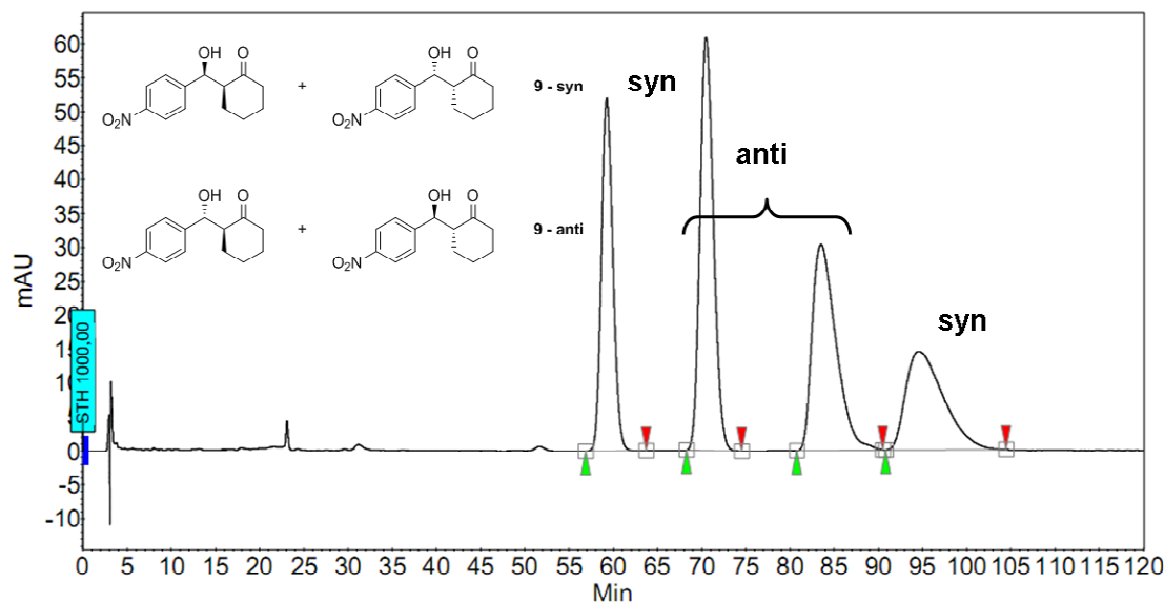
Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	58.29	48.43	117.3	179.4	48.432
2	UNKNOWN	69.53	1.79	3.9	6.6	1.791
3	UNKNOWN	84.62	1.60	2.4	5.9	1.605
4	UNKNOWN	94.11	48.17	32.4	178.5	48.173
Total			100.00	156.0	370.5	100.000

FDSF-mediated Aldol reaction in DMSO:



Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	58.34	29.25	88.6	132.3	29.246
2	UNKNOWN	69.32	20.85	54.5	94.3	20.847
3	UNKNOWN	82.32	20.88	31.0	94.4	20.880
4	UNKNOWN	92.81	29.03	25.2	131.3	29.028
Total			100.00	199.3	452.3	100.000

FDSF-mediated Henry reaction in H₂O:



10. Mechanistic considerations

The results of our studies reveal that the morphology and/or physical state of the protein plays a key role on the kinetics of the reaction, although there are no evidences that support a possible dependence with the reaction mechanism as well. Hence, the observed differences in reactivity are likely related to the diffusion-controlled nature of the reactions and/or to the accessibility of the basic catalytic sites of the protein.

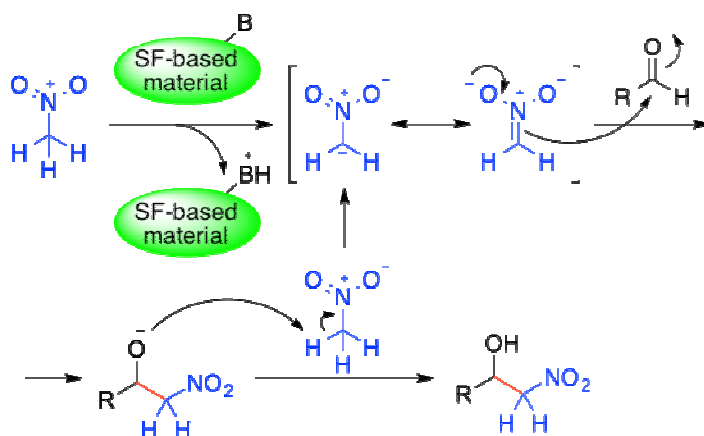
Figure S8 shows a general overview of the potential mechanisms for each model reaction. Arginine (Arg) – the most abundant (0.3%) aminoacid in silk fibroin -, lysine (Lys) and histidine (His) could participate in these reactions as basic sites (represented as “B” in Figure S8). Arginine is the strongest basic site according to its pK_a (12.10) in comparison to those of Lys (pK_a 10.67) and His (pK_a 6.04). The large excess of nitromethane in the Henry reaction (Figure S8A), as well as its lower pK_a (10.2) in comparison to Arg and Lys support the protonation of the alcoholate anion intermediate by this reactant.

In the Knoevenagel condensation (Figure S8B), the enolate intermediate is formed in first place and reacts with the aldehyde. The resulting aldol undergoes a subsequent base-induced elimination reaction. Note that, at least in the classical mechanism, the basic site could be also involved in the formation of an iminium intermediate as acceptor.

In the case of the direct aldol reaction under thermodynamic conditions (Figure S8C), a potential equilibrium imine-enamine is favoured to the imine in the presence of primary amino residues. The presence of the potential reactive enamine at low concentrations in the reaction mixture may hinder the reaction.

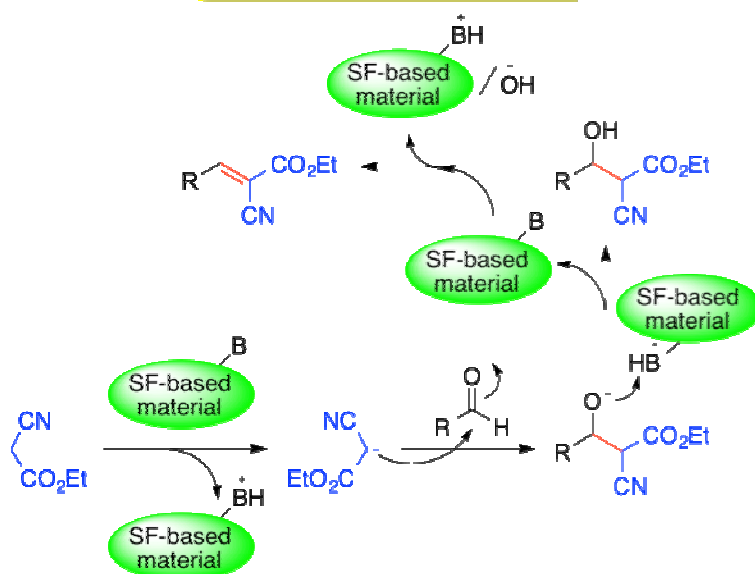
A)

Nitroaldol (Henry) reaction



B)

Knoevenagel condensation



C)

Aldol reaction

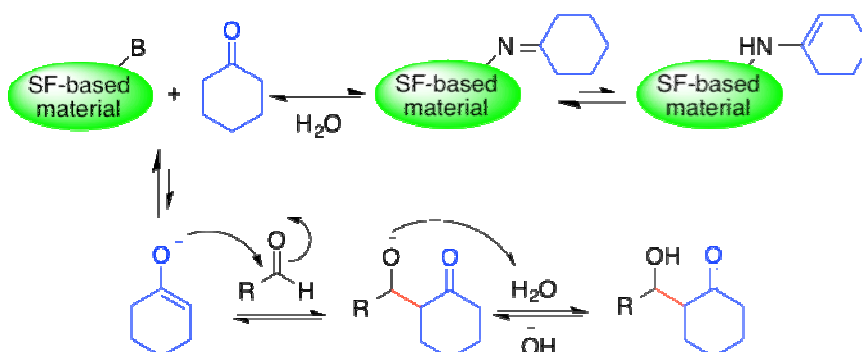


Figure S8. Plausible general mechanisms for the SF-catalyzed C-C bond formation in each model reaction.

11. Comparison with other catalysts

Henry Reaction Comparison between different catalysts and FDSF						
Entry	Catalyst	Solvent	T (°C)	Time	Yield 3 (%)	ee (%)
1 ^[a]	Et ₃ N (1 eq.)	H ₂ O	RT	3 h	86	-
2 ^[b]	D-aminoacylase from <i>Escherichia Coli</i>	DMSO	50	0.5 h	99	-
3 ^[c]	Cu(OAc) ₂ -BOX	EtOH	RT	24 h	85	78
4 ^[d]	FDSF	DMSO	RT	4 h	95	-

[a] Reference: C. L. Zhou, Y. Q. Zhou, Z. Y. Wang, *Chin. Chem. Lett.* **2003**, 14, 355-358. Reaction Conditions: **1** (1.0 mmol), **2** (1.5 mmol), H₂O (5 mL), Et₃N (1.0 mmol), isolated yield. [b] Reference: J.-L. Wang, X. Li, H.-Y. Xie, B.-K. Liu, X.-F. Lin, *J. Biotechnol.* **2010**, 145, 240-243. Reaction conditions: **1** (1M), **2** (2M), DMSO (1 mL), DA (30 mg), HPLC yield. [c] Reference: D. A. Evans, D. Seidel, M. Rueping, H. W. Lam, J. T. Shaw, C. W. Downey, *J. Am. Chem. Soc.* **2003**, 125, 12692-12693. Reaction conditions: **1** (1 mmol), **2** (10 mmol), EtOH (1.5 mL), BOX Ligand (0.055 mmol), Cu(OAc)₂ x H₂O (0.05 mmol), isolated yield. [d] Reaction conditions: **1** (0.1 mmol), **2** (1.0 mmol), FDSF (10 mg), DMSO (0.5 mL), NMR yield.

Knoevenagel Reaction Comparison between different catalysts and FDSF					
Entry	Catalyst	Solvent	T (°C)	Time	Yield 3 (%)
1 ^[a]	AcOH + Piperidine	Toluol	Reflux	1 h	74
2 ^[b]	N-functionized Starch	THF	60	1 h	96
3 ^[c]	Amine-functionalized Polysiloxanes	MeCN	RT	12 h	99
4 ^[d]	FDSF	DMSO	RT	3 h	84

[a] Reference: D. Sicker, H. Wilde, *J. prakt. Chem.* **1992**, 334, 76-80. Reaction Conditions: **1** (150 mmol), **2** (150 mmol), toluene (150 mL), Piperidine (1.0 g), AcOH (1.5 g), isolated yield. [b] Reference: A. Pourjavadi, F. Seidi, S. S. Afjeh, N. Nikoseresht, H. Salimi, N. Nemati, *Starch/Stärke* **2011**, 63, 780-791. Reaction conditions: **1** (4.8 mmol), **2** (5.0 mmol), THF (10 mL), Polymer (50 mg), isolated yield. [c] Reference: Z.-J. Zheng, L.-X. Liu, G. Gao, H. Dong, J.-X. Jiang, G.-Q. Lai, L.-W. Xu, *RSC Adv.* **2012**, 2, 2895-2901. Reaction conditions: **1** (2 mmol), **2** (2.6 mmol), MeCN (3 mL), Catalyst (10 mol%), GC yield. [d] Reaction conditions: **1** (0.1 mmol), **2** (0.11 mmol), FDSF (10 mg), DMSO (0.5 mL), NMR yield.

Note: In the case of the aldol reaction between 4-nitrobenzaldehyde and cyclohexanone, FDSF did not show any significant activity in comparison to other catalysts reported in the literature, which provided high conversions and good selectivities.^[8]

12. References

- [1] Y. Dong, W. Dong, Y. Cao, Z. Han, Z. Ding, *Catal. Today* **2011**, 175, 346-355.
- [2] D. A. Evans, D. Seidel, M. Rueping, H. W. Lam, J. T. Shaw, C. W. Downey, *J. Am. Chem. Soc.* **2003**, 125, 12692-12693.
- [3] E. Busto, V. Gotor-Fernández, V. Gotor, *Org. Process Res. Dev.* **2011**, 15, 236-240.
- [4] a) for *syn* isomer: C. Liang, D. Jiaxing, Y. Jingsong, G. Ge, L. Jingbo, *Chem. Eur. J.* **2010**, 16, 6761-6765; b) for *anti* isomer: T. Nitabaru, A. Nojiri, M. Kobayashi, N. Kumagai, M. Shibasaki, *J. Am. Chem. Soc.* **2009**, 131, 13860-13869.
- [5] Y. Qiong ji, G. Qi, Z. M. A. Judeh, *Eur. J. Org. Chem.* **2011**, 4892-4898.
- [6] D. Kühbeck, G. Saidulu, K. R. Reddy, D. D. Díaz, *Green Chem.* **2012**, 14, 378-392.
- [7] S. Paladhi, J. Das, P. K. Mishra, J. Dash, *Adv. Synth. Catal.* **2013**, 355, 274-280.
- [8] J. Kofoed, T. Darbre, J.-L. Reaymond, *Org. Biomol. Chem.* **2006**, 4, 3268-3281, and references therein.