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# Deep Eutectic Solvents- a New Additive in the Encapsulation of Lipase B from *Candida antarctica*: Biocatalytic Applications

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# Supporting Information

## Detailed experimental results

Here we report the detailed results obtained in our study.

**Table S1.** Solvent screening results.

| Sol-gel code | Solvent | Substrate enantiomeric excess [%] | Conversion [%] | *E*-value* |
|---|---|---|---|---|
| **SG-1** | *n*-hexane | 23.2 ± 2.4 | 18.3 ± 2.1 | >200 |
| | MTBE | 11.0 ± 1.1 | 9.9 ± 0.8 | >200 |
| | DIPE | 17.4 ± 1.5 | 14.8 ± 1.1 | >200 |
| | 2-Methyl-THF | 4 ± 1.5 | 3.8 ± 1.4 | >200 |
| **SG-2** | *n*-hexane | 95.8 ± 0.0 | 49.0 ± 0.0 | »200 |
| | MTBE | 56.6 ± 1.6 | 36.1 ± 0.7 | >200 |
| | DIPE | 80.8 ± 6.9 | 44.6 ± 2.1 | »200 |
| | 2-Methyl-THF | 4.1 ± 1.5 | 3.9 ± 1.4 | >200 |
| **SG-3** | *n*-hexane | 90.6 ± 0.2 | 47.5 ± 0.1 | »200 |
| | MTBE | 18.5 ± 2.8 | 15.6 ± 2.0 | >200 |
| | DIPE | 85.3 ± 5.3 | 46.0 ± 1.6 | »200 |
| | 2-Methyl-THF | 5.7 ± 1.5 | 5.4 ± 1.3 | >200 |
| **SG-4** | *n*-hexane | 33.6 ± 1.1 | 25.1 ± 1.7 | >200 |
| | MTBE | 70.3 ± 1.0 | 41.3 ± 0.3 | >200 |
| | DIPE | 17.5 ± 0.1 | 14.9 ± 0.1 | >200 |
| | 2-Methyl-THF | 2.7 ± 1.4 | 2.2 ± 0.8 | 199 |
| **SG-5** | *n*-hexane | 94.3 ± 0.8 | 48.5 ± 0.3 | »200 |
| | MTBE | 76.4 ± 3.3 | 43.3 ± 1.1 | >200 |
| | DIPE | 85.7 ± 1.1 | 46.2 ± 0.3 | »200 |
| | 2-Methyl-THF | 15.5 ± 1.1 | 13.4 ± 0.8 | >200 |
| **SG-6** | *n*-hexane | 93.4 ± 0.3 | 48.3 ± 0.1 | »200 |
| | MTBE | 21.3 ± 3.6 | 17.5 ± 2.5 | >200 |
| | DIPE | 45.9 ± 3.2 | 31.4 ± 1.6 | >200 |
| | 2-Methyl-THF | 4.2 ± 0.7 | 4.1 ± 0.6 | >200 |
| **SG-7** | *n*-hexane | 73.6 ± 2.7 | 42.4 ± 0.9 | >200 |
| | MTBE | 56.7 ± 2.2 | 36.2 ± 0.9 | >200 |
| | DIPE | 83.1 ± 7.5 | 45.3 ± 2.4 | »200 |
| | 2-Methyl-THF | 8.6 ± 0.5 | 7.9 ± 0.4 | >200 |

*99.9% product enantiomeric excess

**Table S2.** Substrate: enzyme weight *ratio* screening results.

| Sol-gel code | Substrate: enzyme *ratio* [w/w %] | Substrate enantiomeric excess [%] | Conversion [%] | *E*-value* |
|---|---|---|---|---|
| **SG-2** | 20:1 | 85.9 ± 0.2 | 46.2 ± 0.1 | »200 |
| | 40:1 | 81.0 ± 4.9 | 44.7 ± 1.5 | »200 |
| | 60:1 | 79.3 ± 3.9 | 44.2 ± 1.2 | >200 |
| | 80:1 | 64.8 ± 0.8 | 39.3 ± 0.3 | >200 |
| | 100:1 | 46.1 ± 2.3 | 31.6 ± 1.1 | >200 |
| **SG-3** | 20:1 | 94.5 ± 0.7 | 48.6 ± 0.4 | »200 |
| | 40:1 | 88.0 ± 1.3 | 46.8 ± 0.4 | »200 |
| | 60:1 | 85.5 ± 0.8 | 46.1 ± 0.2 | »200 |
| | 80:1 | 81.7 ± 0.2 | 45.0 ± 0.0 | »200 |
| | 100:1 | 76.0 ± 2.7 | 43.2 ± 0.9 | >200 |
| **SG-5** | 20:1 | 95.1 ± 4.8 | 48.7 ± 1.3 | »200 |
| | 40:1 | 93.5 ± 4.8 | 48.3 ± 1.1 | »200 |
| | 60:1 | 91.1 ± 6.1 | 47.6 ± 1.7 | »200 |
| | 80:1 | 86.0 ± 4.6 | 46.2 ± 1.4 | »200 |
| | 100:1 | 75.4 ± 6.1 | 42.9 ± 2.0 | >200 |
| **SG-6** | 20:1 | 86.5 ± 0.9 | 46.4 ± 0.3 | »200 |
| | 40:1 | 81.8 ± 3.6 | 45.0 ± 1.1 | »200 |
| | 60:1 | 78.8 ± 5.7 | 44.0 ± 1.8 | >200 |
| | 80:1 | 71.8 ± 4.6 | 41.8 ± 1.5 | >200 |
| | 100:1 | 62.0 ± 0.1 | 38.3 ± 0.0 | >200 |
| **SG-7** | 20:1 | 92.4 ± 1.6 | 48 ± 0.5 | »200 |
| | 40:1 | 87.5 ± 0.8 | 46.7 ± 0.2 | »200 |
| | 60:1 | 86.9 ± 0.8 | 46.5 ± 0.2 | »200 |
| | 80:1 | 84.1 ± 3.1 | 45.7 ± 0.9 | »200 |
| | 100:1 | 76.1 ± 2.1 | 43.2 ± 0.7 | >200 |

*99.9% product enantiomeric excess

**Table S3.** Substrate: acyl donor molar *ratio* screening results.

| Sol-gel code | Substrate: acyl donor *ratio* [mol/mol %] | Substrate enantiomeric excess [%] | Conversion [%] | *E*-value* |
|---|---|---|---|---|
| **SG-2** | 1:1 | 69.7 ± 1.0 | 41.1 ± 0.3 | >200 |
| | 1:2 | 77.8 ± 6.3 | 43.7 ± 2.0 | >200 |
| | 1:3 | 80.4 ± 4.9 | 44.5 ± 1.6 | »200 |
| | 1:4 | 83.0 ± 5.1 | 45.3 ± 1.6 | »200 |
| **SG-3** | 1:1 | 73.6 ± 6.3 | 42.3 ± 2.1 | >200 |
| | 1:2 | 80.1 ± 2.1 | 44.4 ± 0.7 | »200 |
| | 1:3 | 82.0 ± 0.4 | 45.1 ± 0.1 | »200 |
| | 1:4 | 85.0 ± 2.4 | 46.0 ± 0.7 | »200 |
| **SG-5** | 1:1 | 53.4 ± 0.9 | 34.8 ± 0.4 | >200 |
| | 1:2 | 57.2 ± 1.2 | 36.4 ± 0.5 | >200 |
| | 1:3 | 59.1 ± 2.8 | 37.1 ± 1.2 | >200 |
| | 1:4 | 81.2 ± 6.7 | 44.8 ± 2.0 | »200 |
| **SG-6** | 1:1 | 79.3 ± 1.0 | 44.2 ± 0.4 | >200 |
| | 1:2 | 79.6 ± 1.2 | 44.3 ± 0.4 | >200 |
| | 1:3 | 82.0 ± 2.5 | 45.0 ± 0.8 | »200 |
| | 1:4 | 85.0 ± 0.2 | 45.9 ± 0.1 | »200 |
| **SG-7** | 1:1 | 53.4 ± 0.2 | 34.8 ± 0.1 | >200 |
| | 1:2 | 66.4 ± 0.6 | 39.9 ± 0.2 | >200 |
| | 1:3 | 67.9 ± 1.1 | 40.5 ± 0.4 | >200 |
| | 1:4 | 75.9 ± 4.0 | 43.1 ± 1.3 | >200 |

*99.9% product enantiomeric excess

**Table S4.** Temperature screening results.

| Sol-gel code | Temperature [°C] | Substrate enantiomeric excess [%] | Conversion [%] | *E*-value* |
|---|---|---|---|---|
| **SG-3** | 30 | 89.1 ± 3.4 | 47.1 ± 1.0 | »200 |
| | 40 | 58.3 ± 5.1 | 36.7 ± 2.1 | >200 |
| | 50 | 94.1 ± 4.0 | 48.4 ± 1.1 | »200 |
| | 60 | 88.8 ± 8.1 | 46.9 ± 2.3 | »200 |
| **SG-6** | 30 | 64.8 ± 24.4 | 38 ± 9.2 | >200 |
| | 40 | 93.8 ± 0.1 | 48.4 ± 0.1 | »200 |
| | 50 | 94.3 ± 1.1 | 47.3 ± 0.8 | »200 |
| | 60 | 94.8 ± 1.6 | 48.7 ± 0.4 | »200 |

*99.9% product enantiomeric excess

**Table S5.** Acyl donor screening results.

| Sol-gel code | Time [h] | Acyl donor | Substrate enantiomeric excess [%] | Conversion [%] | *E*-value* |
|---|---|---|---|---|---|
| SG-3 | 1 | Vinyl acetate | 62.4 ± 3.9 | 38.4 ± 1.5 | >200 |
| | 2 | | 76.3 ± 2.8 | 43.3 ± 0.9 | >200 |
| | 3 | | 86.0 ± 3.8 | 46.2 ± 1.1 | »200 |
| | 4 | | 97.4 ± 2.5 | 49.3 ± 0.7 | »200 |
| | 1 | Vinyl propionate | 51.1 ± 3.2 | 33.8 ± 1.4 | >200 |
| | 2 | | 59.6 ± 0.7 | 37.3 ± 0.3 | >200 |
| | 3 | | 74.7 ± 3.3 | 42.7 ± 1.1 | >200 |
| | 4 | | 91.5 ± 1.0 | 47.8 ± 0.3 | »200 |
| | 1 | Vinyl decanoate | 43.1 ± 0.3 | 30.1 ± 0.2 | >200 |
| | 2 | | 56.4 ± 1.9 | 36.1 ± 0.8 | >200 |
| | 3 | | 84.6 ± 1.7 | 45.8 ± 0.5 | »200 |
| | 4 | | 91.3 ± 0.7 | 47.7 ± 0.2 | »200 |

*99.9% product enantiomeric excess

**Table S6.** Reusability study results.

| Sol-gel code | Reaction cycle | Substrate enantiomeric excess [%] | Conversion [%] | *E*-value* |
|---|---|---|---|---|
| SG-3 | 1 | 99.9 ± 0.0 | 50.0 ± 0.0 | »200 |
| | 2 | 99.9 ± 0.0 | 50.0 ± 0.0 | »200 |
| | 3 | 94.3 ± 0.3 | 48.5 ± 0.1 | »200 |
| | 4 | 90.5 ± 3.0 | 47.5 ± 0.8 | »200 |
| | 5 | 85.2 ± 1.6 | 46.0 ± 0.5 | »200 |
| | 6 | 79.8 ± 0.2 | 44.4 ± 0.1 | >200 |
| | 7 | 75.6 ± 3.0 | 43.0 ± 1.0 | >200 |
| | 8 | 71.1 ± 2.8 | 41.5 ± 1.0 | >200 |
| | 9 | 54.8 ± 0.4 | 35.4 ± 0.2 | >200 |
| | 10 | 29.1 ± 0.3 | 22.5 ± 0.2 | >200 |

*99.9% product enantiomeric excess

**Table S7.** Substrate concentration screening results.

| Sol-gel code | Substrate concentration [mM] | Substrate enantiomeric excess [%] | Conversion [%] | *E*-value* |
|---|---|---|---|---|
| SG-3 | 25 | 79.0 ± 6.6 | 44.0 ± 2.1 | >200 |
| | 50 | 86.4 ± 3.7 | 46.3 ± 1.1 | »200 |
| | 75 | 83.8 ± 2.2 | 45.6 ± 0.7 | »200 |
| | 100 | 80.0 ± 4.5 | 44.4 ± 1.4 | »200 |
| | 250 | 65.4 ± 6.1 | 39.5 ± 2.2 | >200 |
| | 500 | 53.0 ± 6.7 | 34.5 ± 2.9 | >200 |
| | 750 | 36.1 ± 2.5 | 26.5 ± 1.4 | >200 |

*99.9% product enantiomeric excess

**Table S8.** Synergetic effect study results.

| Sol-gel code | Substrate enantiomeric excess [%] | Conversion [%] | *E*-value* |
|---|---|---|---|
| **SG-31** | 63.3 ± 6.8 | 38.7 ± 2.5 | >200 |
| **SG-32** | 79.5 ± 0.8 | 44.3 ± 0.3 | >200 |
| **SG-33** | 52.4 ± 6.6 | 34.3 ± 2.8 | >200 |
| **SG-3** | 90.2 ± 5.3 | 47.4 ± 1.5 | »200 |

*99.9% product enantiomeric excess

## Chromatographic conditions for compounds C-I

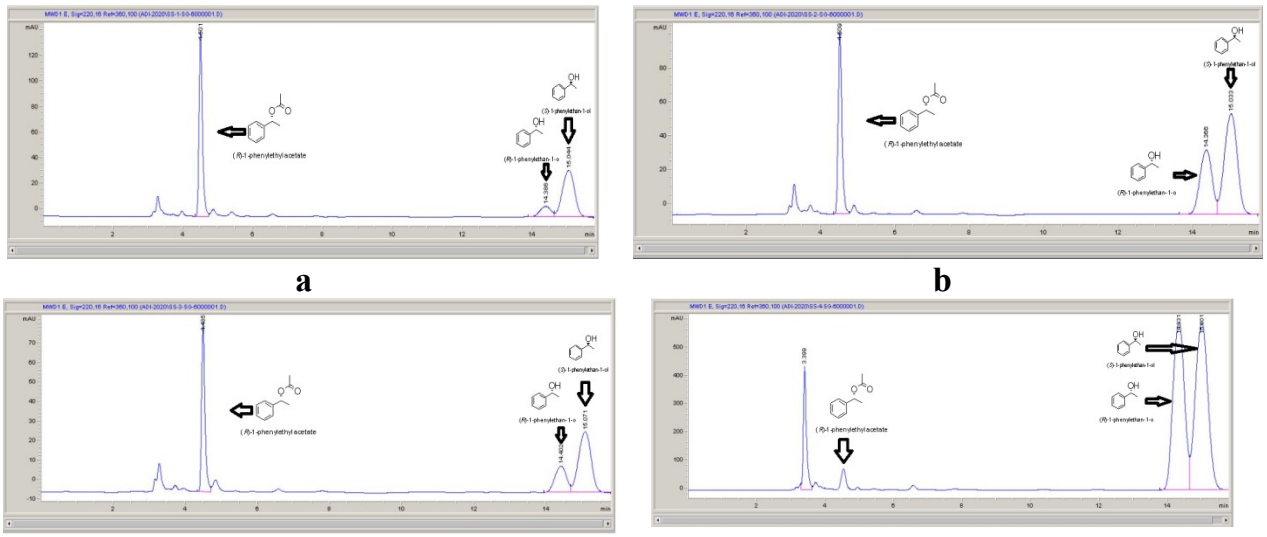
Oven temperatures and analysis time for compounds **C-I** (Figure 9) are presented in Table S9.

**Table S9.** Chromatographic conditions for the analysis of compounds **C-I**.

| Compound | Oven temperature [°C] | Analysis time [min] |
|---|---|---|
| **C** | 160 | 9.5 |
| **D** | 160 | 8.5 |
| **E** | 160 | 4.5 |
| **F** | 110 | 4.5 |
| **G** | 120 | 4.8 |
| **H** | 120 | 8.8 |
| **I** | 160 | 12.5 |

## Examples of chromatograms

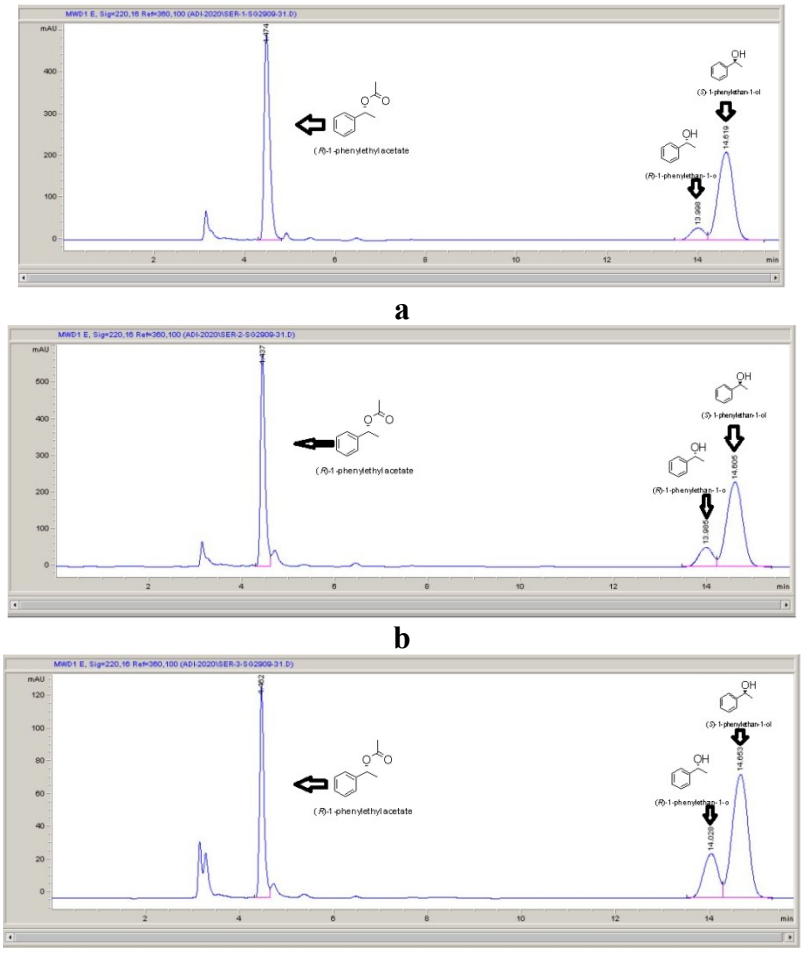
In this section we present some examples of chromatograms obtained when analyzing the experiments performed during the present study.



**a** **b**

**c** **d**

**Figure S1.** Solvent screening for biocatalyst SG-6; **a**- *n*-hexane, **b**- MTBE, **c**- DIPE, **d**- 2-Methyl-THF.



**a**

**b**

**c**

**Figure S2.** Substrate: enzyme *ratio* (w/w %) screening for biocatalyst SG-3; **a**- 20:1 weight *ratio*, **b**- 40:1 weight *ratio*, **c**- 60:1 weight *ratio*.

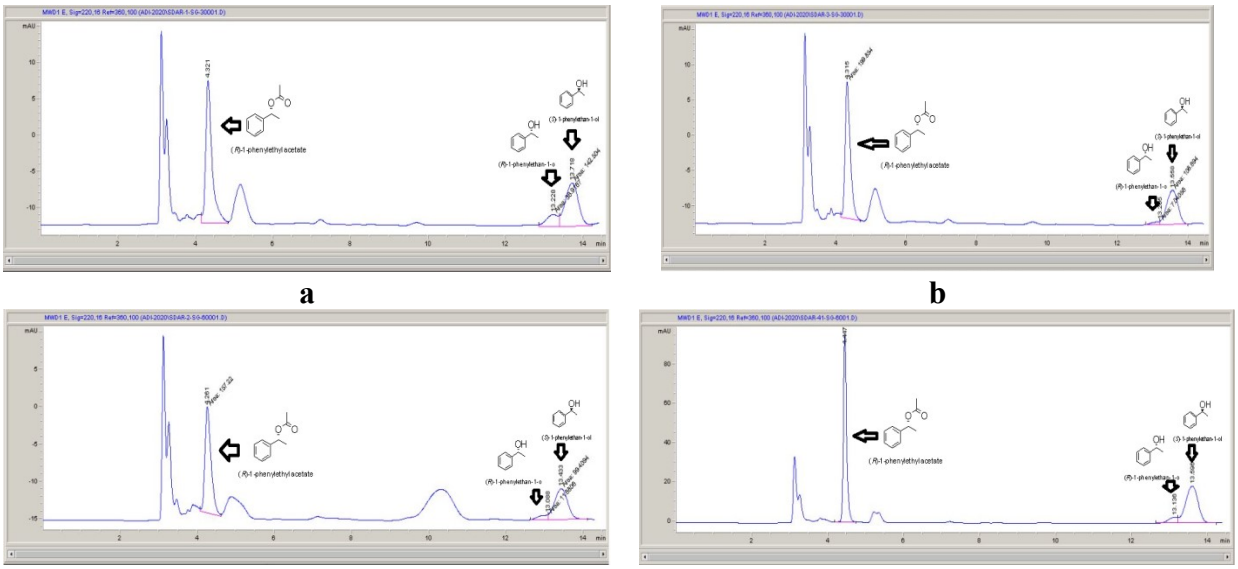


**Figure S4.** Substrate: acyl donor *ratio* (mol/mol %) screening for biocatalysts SG-3 and SG-6; **a**- 1:1 molar *ratio* for SG-3, **b**- 1:3 molar *ratio* for SG-3, **c**- 1:2 molar *ratio* for SG-6, **d**- 1:4 molar *ratio* for SG-6.

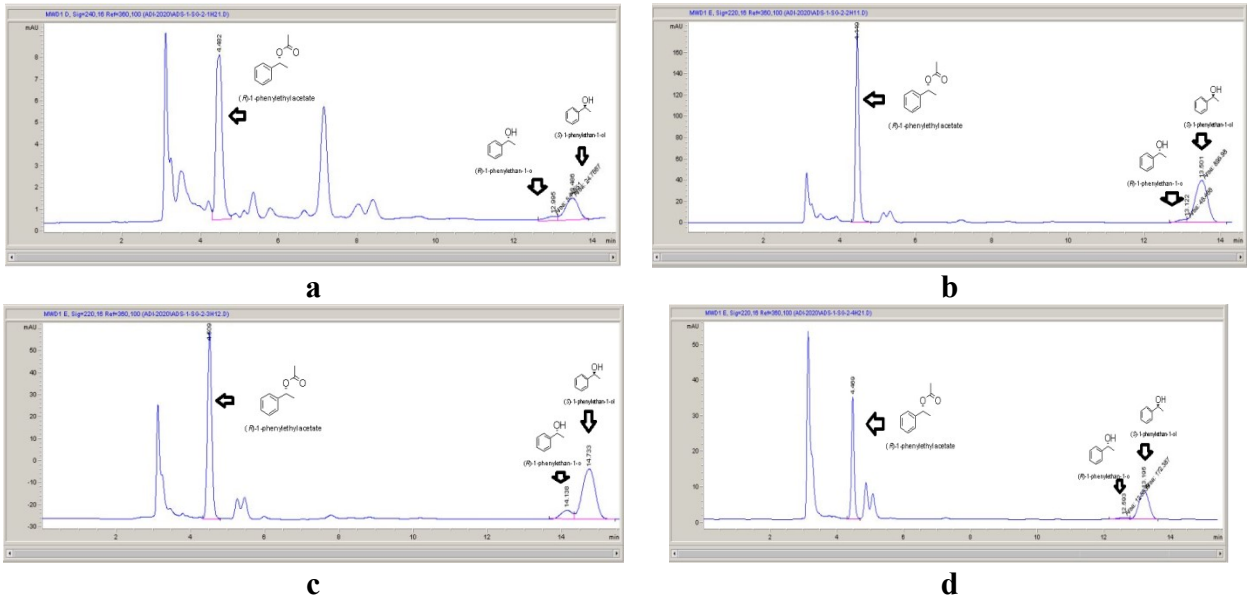
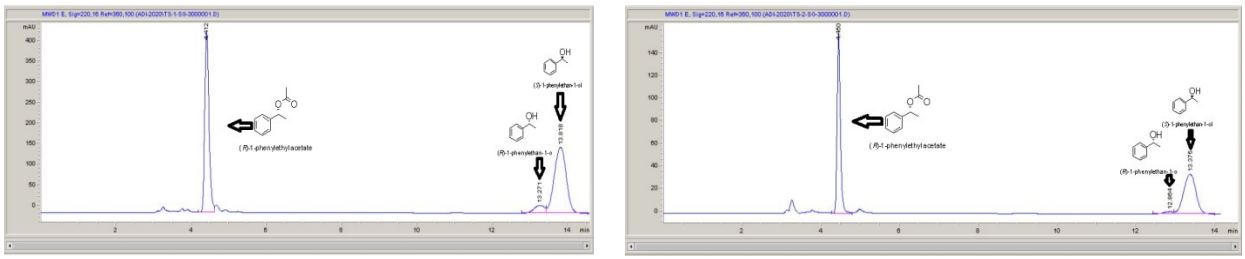
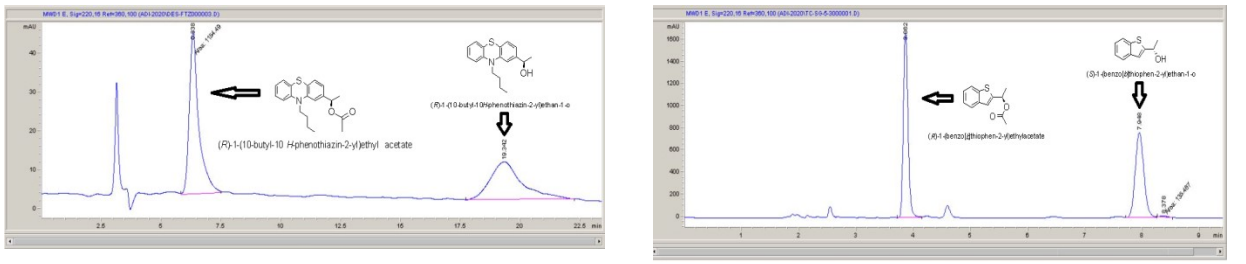


**Figure S5.** Time profile for the reaction mediated by SG-3, part of the acyl donor screening experiments; **a**- after 1 h, **b**- after 2 h, **c**- after 3 h, **d**- after 4 h.

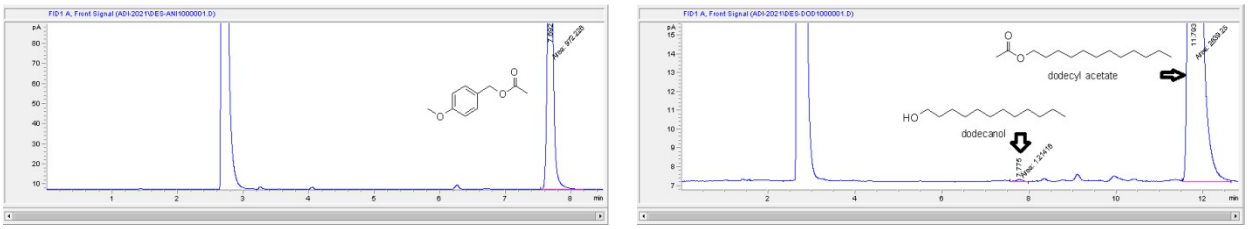


**a** **b**

**Figure S6.** Temperature screening for biocatalyst SG-3; **a**- 40 °C, **b**- 30 °C.



**a** **b**




**c** **d**

**Figure S7.** Transesterification of compounds **B** (**a**), **A** (**b**), **D** (**c**) and **I** (**d**) mediated by SG-3 using the optimal reaction conditions.