Exploring the Temperature Effect on Enantioselectivity of a Baeyer-Villiger Biooxidation by the 2,5-DKCMO Module: The SLM Approach

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1. Gas chromatography analyses

Gas chromatography (GC) analyses were carried out on a Shimadzu GC-14A with hydrogen as the gas phase in split mode and a flame ionization detector. Separation was achieved on a Cyclosil-B column (Agilent J&W GC Columns) at 398 K. Product identification was ensured by the comparison of GC retention times with those of authentic samples obtained from the well-known biotransformation of rac-1 using cyclohexanone monooxygenase CHMO.\(^1\) The following retention times were observed: (-)-(1S,5R)-1: 2.3 minutes / (+)-(1R,5S)-1: 2.5 minutes / tridecane: 5.3 minutes / (-)-(1R,5S)-2: 10.2 minutes / (+)-(1R,5S)-2: 11.2 minutes / (-)-(1S,5R)-2: 12.1 minutes / (+)-(1S,5R)-3: 12.5 minutes. Figure S1 shows a representative chromatogram of the GC analysis.

Figure S1. GC chromatogram and annotation for the enzymatic BV oxidation of racemic bicyclo[3.2.0]hept-2-en-6-one 1 by the 2,5-DKCMO.
2. Typical time-course of ketone 1 biotransformation

Figure S2. Time-course of the biotransformation of rac-1 at 287 K. ● : conversion, ■ and ▲ : remaining ketone enantiomers, ▲ and ◆ : lactone yields. The colour code is adjusted to Scheme 1. The standard deviations were calculated from duplicates.
3. The effect of the temperature on conversion and enantioselectivity

**Figure S3.** Enantioselective BV oxidation of 1 by 2,5-DKCMO from 283 K to 303 K. Conversion versus time in A, ee of ‘normal’ lactone 2 versus conversion in B.  

*Experimental conditions in duplicates: 4 mM rac-ketone 1, 25 mM AmNAH, 5.0 µM FMN in 1 mL tricine buffer (50 mM, pH 8.5) with 0.19 mg mL\(^{-1}\) 2,5-DKCMO, and 2.5 mg mL\(^{-1}\) catalase.*
4. Shape Language Modeling (SLM)

'Shape Language Modeling' (SLM, MATLAB®) tools were used to build a prescription for the 'best shaped' model of the reaction at 283 K. SLM is a method for the prescription of a curve fit using sets of shape primitives. The basic idea is the find the function, that displays the dataset most appropriately, rather than applying a mathematical model fitting best. The integration of knots at key positions allows designing functions with a general set of characteristics, which is computed between two knots individually. The results of the tested parameter variations applied to the curve "ee of substrate 1 versus conversion" are shown in Figure S4 with the 'best shaped' model in F.

Figure S4. “Shape Language Modeling” (SLM) tests using MATLAB® to identify the best model for the enantioselectivity of the reaction applying the dataset at 283.15 K.

The command slmengine was used as the driven tool for fitting the models using the prescription structures. The resulting curve is red (data set is shown as blue dots, the knots of the function are in green). All models (b) to (l) are based on the following structure:

\[ \text{slm} = \text{slmengine}(x, y, \text{'plot'}, \text{'on'}, \text{'increasing'}, \text{'on'}, \text{'leftvalue'}, 0, \text{'rightvalue'}, 100), \]

with the additions in (b) to (l) depicted below.

(a) Data set of the reaction at 283.15 K / (b) 'knots', [0, 100] / (c) 'knots', [0:50:100] / (d) 'knots', [0, 75, 100] / (e) 'knots', [0, 25, 100] / (f) 'knots', [0, 25, 75, 100] / (g) 'knots', [0, 40, 60, 100] / (h) none (default mode) / (i) 'concaveup','on' / (j) 'knots', [0:10:30 70:10:100] / (k) 'knots', [0 10 20 30 50 70 80 90 100] / (l) 'knots', [0:10:100].
The function parameters shown in Figure S4F were applied for the other datasets (287 K – 303 K) as the 'best shape' SLM function among the tested parameters. The result is shown in Figure S5.

Figure S5. Implementation of the ‘best shape’ SLM function for the plots of ee of 1 over conversion for reactions at 283 K to 303 K (A to F). The following prescriptive model (Figure S4F) was applied for the data sets at the tested temperatures: \( \text{slm} = \text{slmengine} \ (x, y, 'plot', 'on', 'increasing', 'on', 'knots', [0, 25, 75, 100], 'leftvalue', 0, 'rightvalue', 100) \). For the legend see Figure S4.

5. Enantiomeric Ratio \( E \)

The Enantiomeric Ratio \( (E) \) describes the stereoselectivity of a chemical reaction. Methodologies to determine \( E \) are divers, e.g. it can be determined directly from the \( ee \) of the substrate and the conversion \( (c) \) according to Equation 1:

\[
E = \ln \left[ \frac{[1 - c(1 + ee)]}{[1 - c(1 - ee)]} \right] \tag{2}
\]

We applied the experimental data for conversion and \( ee \) of 1 to determine \( E \) by a non-linear least-square method. A decreasing \( E \) value at higher temperatures was observed, along with a trend for a decreasing fit \( (R^2) \). Exemplary, the graphs and the results for 283 K and 303 K are show in Figure S6 and \( E \) values reported in Table S1.
Figure S6. Determination of $E$ value for reactions at 283 K and 303 K. ●: $ee$ of 1: experimental data; —: theoretical curves from regression, —: theoretical $ee$ of the product when only one product is formed. Regression analysis for the determination of $E$ was based on the experimental values (not the SLM results) gained from duplicates.

The $E$ values for the reactions at temperatures of 283 K to 303 K were also calculated using the $ees$ of 1 determined by SLM method at 25% and 50% conversion. Applying this methodology we observed an increased $E$ value during the reaction in both data sets, as shown in Table S1.

Table S1 Enantiomeric Ratio ($E$ value) for reactions from 283 K to 303 K.

| $T$ [K] | $ee$ of 1 [a] | $E$ value [b] | $E$ value (from regression) |
|---|---|---|---|
| | 25% conversion | 50% conversion | 25% conversion | 50% conversion | |
| 283 | 26.0 | 68.7 | 10.4 | 10.9 | 9.0 |
| 287 | 20.5 | 62.4 | 5.1 | 8.0 | 8.3 |
| 291 | 19.5 | 61.3 | 4.6 | 7.6 | 7.1 |
| 295 | 9.8 | 54.4 | 2.0 | 5.7 | 5.7 |
| 299 | 7.2 | 51.9 | 1.7 | 5.2 | 4.8 |
| 303 | 4.5 | 37.8 | 1.4 | 3.1 | 3.6 |

[a] SLM was applied to compute $ee$ of 1 at 25% and 50% conversion from the graphs in Figure S5. [b] $E$ values calculated from $ee$ of 1

References

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