Supporting Information

Multidecagram Scale Synthesis of an Endoperoxide, Precursor of Anti-malarial and Anti-leishmanial Agents, *via* Free-Radical [2 + 2 + 2] Annulation with Molecular Oxygen

Davide Lardani,^a Roger Marti,^{a,*} Arianna Quintavalla,^{b,*} Marco Lombardo,^b Claudio Trombini^b

^a Institut ChemTech, HES-SO, Hochschule für Technik und Architektur, Boulevard de Pérolles 80, CH-1700 Freiburg, Switzerland.

^b Alma Mater Studiorum - University of Bologna, Department of Chemistry "G. Ciamician", Via Selmi 2, 40126 Bologna, Italy.

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Reproducibility

The laboratory set-up used for the small-scale experiments (Figure 2 in the manuscript) was applied to the reactions reported in the following sections of the manuscript:

- Reproducibility
- Solvents screening
- Reaction time optimization
- Design of Experiment (DoE)

To optimize the temperature control and to conduct multiple parallel reactions, an immersion cooler Huber TC50E was used.

After 3 hours the reaction was quenched with NaOH (see Experimental Section of the manuscript for details) until neutral pH. Then, the mixture was extracted with AcOEt. Under these conditions, we observed a suspension of a white solid mainly corresponding to Mn(OH)₂ (solubility in water: 0.00034 g/100 mL at 18 °C). The possibly present Mn(OAc)₂ is much more soluble (solubility in water: 700g/L at 20 °C for tetrahydrate). Therefore, a large amount of manganese salts could be recovered by filtration, but as hydroxides and not as catalytically active species.

<u>Solubility data</u>: Lide, D. R., ed. (2006). CRC Handbook of Chemistry and Physics (87th ed.). Boca Raton, FL: CRC Press. ISBN 0-8493-0487-3.

Solvent Screening

LRA model

The parameters used to evaluate the best solvent were:

- Health hazard (A), based on Globally Harmonized System of Classification and Labelling of Chemicals (GHS) pictograms
- Volatility (B), expressed as boiling point
- Volume (C)

The parameter (C) was considered as a constant, since the same volume was used for all solvents (4 mL). Each parameter was associated to a value based on three scores: low = 1, medium = 2 and high = 3.

Health hazard (A)	Volatility (B)	Volume (C)	Score
	Boiling point > 150 °C	$V \le 10 \text{ mL}$	1
	50 °C \leq Boiling point \leq 150 °C	$10 \text{ mL} < \text{V} \le 100 \text{ mL}$	2
	Boiling point < 50 °C	V ≫ 100 mL	3

 Table S1.
 LRA parameters and associated values.

Final score for each solvent is obtained by multiplying parameters A x B x C (Table **S1).** Then, solvents costs were also evaluated. The degree of purity chosen as reference is *"for synthesis"* and prices are expressed on 1 L scale (\notin /L). Data provided are available on *Sigma-Aldrich* and *VWRTM part of avantor* (Table S2).

Solvent	Final score	€/L
TFE	6	229
HFIP	4	1497
AcOH	4	35.8
TFA	4	382
НСООН	6	24.8
Propionic acid	2	40.2
MsOH	1	247

 Table S2.
 LRA final scores and costs of the screened solvents.

Optimization of Catalysts Loading, Temperature and Reagents Molar Ratio

Model response and statistical analysis

The DoE software package MODDE 9 was used for data processing and statistical analysis. It allowed us to verify the influence of individual factors on the response and to evaluate the model validity. Our model can be explained through a polynomial multivariate approach:

$$Y = k + (a \times T) + (b \times \% E) + (c \times Cat) + (d \times T^2)$$
 Eq. 1

Where :

- Y is the model response, that is the $q^{1}H$ NMR-Y %
- k is a constant
- T, %E and *Cat* are the factors, namely temperature, excess of β -ketoester and catalysts load (expressed as equivalents)
- *a*, *b*, *c* and *d* are the respective factor coefficients
- T^2 is the square of the temperature

More in detail, it is possible to see their real values in **Table S3**.

Factors	Coeff. SC.	Std. Err.	Р	Conf. Int. (±)
k	58.9835	2.24891	4.32E-18	4.66398
Τ	29.3222	1.53128	3.30E-15	3.17569
% E	3.86667	1.53128	0.0192776	3.17569
Cat	-3.57778	1.53128	0.0289782	3.17569
T^2	-23.739	2.74701	1.60E-08	5.69699

 Table S3. Scaled and centered coefficients of the response model.

Results collected in **Table S3** are graphically shown through a coefficient plot (**Figure S1**). This plot displays multi linear regression (MLR) with confidence intervals. It is used to interpret the coefficients weight, and their size represents the change in the response. The coefficient is significant, different from the noise, when the

confidence interval does not cross zero. In **Figure S1** it is apparent that temperature (T) has a major impact, compared to other factors, on the yield. As refers to the reagents ratio (%E, excess of β -ketoester) and the amount of catalysts (Cat), their signals are smaller than their associated errors. For this reason, MODDE predicts an influence on the response, but it is small and has a lot of variance. In addition, the temperature square (T*T) has been considered. According to this model, increasing the temperature, too, leads to a decrease of the yield. It means that this type of reaction has an optimum range of temperatures within it must be run (**Figure 3** in the manuscript).





Figure S2 shows the normal probability plot of the residuals (standardized or studentized) on a double Log scale. This plot allowed us to detect outliers and assess normality of the residuals.



Figure S2. Normal probability plot of the residuals

CombiFlash

Figure S3 shows an automated chromatographic system (CombiFlash) which was used to purify the 18 crude mixtures (among 27) showing acceptable yields in a standardized and reproducible way, limiting operator errors.



Figure S3. Automated chromatographic system (CombiFlash)

An 80 % cyclohexane / 20 % AcOEt mobile phase was used with 80 g normal phase silica RediSep commercial chromatography column, which has a diameter of 3 cm and 20 cm high. This single use column (there is the possibility of reuse if the column was purged with solvent and then dried) has an average particle size from 40 to 60 μ m, mesh from 230 to 400 and average pore size of 60 Å. The instrument, thanks to the use of two different wavelengths (red: 254 nm, violet: 280 nm; see **Figure S4**), is able to detect the output analytes in real time. 20 mL Test-tubes were used to collect the fractions and 375 mL of mobile phase were used as the calibration volume for the column over 3 minutes. With a flow rate of 50 mL/min the total time to perform the purification (without the calibration time) was 12 min.

Figure S4-(A) shows a typical chromatogram obtained by CombiFlash. Since **7** is not visible at 254 or 280 nm, its R_t was calculated by spraying the corresponding TLC plate with KMnO₄. The chromatogram also shows the presence of two by-products (**A**: methyl 5,5-dibutyl-4,5-dihydrofuran-3-carboxylate; **B**: not identified compound derived from ketoester degradation). These two compounds, containing conjugate double bonds, are effectively detected at 254 and 280 nm, in contrast to endoperoxide **7**. This behavior is also evident in the TLC analysis (**Figure S4-(B)**). However, the ¹H-NMR spectrum demonstrated that the amount of by-products **A** and **B** in the crude mixture was low (**A**: less than 5 %, **B**: depends on the excess of ketoester **1**).



Figure S4. (A): Chromatogram of crude methyl 6,6-dibutyl-3-hydroxy-3-methyl-1,2-dioxane-4-carboxylate (7); (B): TLC of the crude mixture.

(B)





Multidecagram Preparation of Endoperoxide 7

The set-up and the laboratory equipment used for the 1 L scale-up was depicted in the manuscript (Figure 5).

An oxygen cylinder has been installed, keeping it fixed inside the laboratory fume hood. In order to control the outgoing flow of oxygen, a mass flow regulator has been connected to the cylinder (**Figure S5**).

Figure S5. Oxygen mass flow regulator (on the left) - fritted glass tube (on the right).





Figure S6 shows the laboratory equipment used for the 3 L scale-up. This type of reactor has an important feature: a double layer, or jacket, which allows a uniform distribution for a heating/cooling system. It is divided into three main parts: the central body, wrapped by the jacket, the head space with five necks and the metal components which keep the whole airtight. Magnetic stirring cannot be used for this reactor, so a mechanical stirrer was preferred. To improve mixing, a pitched-blade helical impeller with axial flow pattern was used (**Figure S6**).

Figure S6. 3 L jacketed reactor (on the left), pitched-blade helical impeller (on the right).





The entire 3 L set-up was depicted in the manuscript (Figure 6). A refrigerated/heating circulator Julabo F25 has been connected to the reactor, to control the jacket temperature. It works by using a mixture of water/ethylene glycol, that flows inside the jacket from bottom to top at the target temperature.

Calorimetric Studies

The calorimetric studies were performed using an EasyMaxTM 102 (**Figure S7**).



Figure S7. EasyMaxTM 102.

Thanks to this instrument, it is possible to compute the temperature difference which will be observed in an adiabatic system during a reaction (ΔT_{ad}). The theory behind ΔT_{ad} can be expressed through the concept of accumulation:

$$ACC = IN - OUT + RX$$
 Eq.2

(*IN*) and (*OUT*) are terms referred to the power gained or lost from the system and (*RX*) is the power generated by the reaction. EasyMaxTM 102 is able to measure the different elements composing of the terms *ACC* (q_{acc}), *IN* (q_{flow}) and *OUT* (q_{loss} , q_{dos}). Therefore from **Eq.2** it is possible to calculate the *RX* term, which corresponds to q_r in **Eq.3**:

$$q_r = q_{acc} + q_{loss} - q_{flow} + q_{dos} [W]$$
 Eq.3

where:

• $q_{flow} = UA (T_j - T_r);$

U is the heat transfer coefficient for the reactor wall, A is the heat exchange surface, T_j is the reactor jacket temperature and T_r is the reaction mixture temperature

• $q_{acc} = m_r \cdot c_{pr} \cdot \frac{dT_r}{dt} = c'_{pr} \frac{dT_r}{dt}$;

 $m_r [kg]$ is the reaction mixture mass, $c_{pr} \left[\frac{J}{K}\right]$ is the heat capacity of the reactive mixture (the energy to supply to the substance to increase its temperature by 1 K) and $c'_{pr} \left[\frac{J}{K \cdot kg}\right]$ is the specific heat capacity of the reactive mixture (it is calculated by an EasyMaxTM 102 probe, which heats-up and supplies energy to the reaction mixture for a precise interval time, computing the induced ΔT)

- $q_{loss} = \alpha \cdot (T_r T_{amb})$; T_{amb} is the ambient temperature and α heat lost coefficient
- $q_{dos} = \frac{dm}{dt} \cdot cp \cdot (T_r T_{dos});$

 T_{dos} is the temperature of dosed reagent.

Integrating **Eq.3** we obtain:

$$Q'_{r} = \int q_{acc} + \int q_{loss} - \int q_{flow} + \int q_{dos} [J] \qquad Eq.4$$

where Q'_r is the energy generated by the reaction. Now it is possible to determine ΔT_{ad} as in **Eq.5**:

$$\Delta T_{ad} = \frac{-\Delta_r H^0}{\rho \cdot c'_{pr}} = -\frac{Q'_r}{c'_{pr}} [K]$$
 Eq.5

where:

- ρ is the mixture density
- Q'_r is the specific reaction heat
- $-\Delta_r H^0$ is the reaction enthalpy

Figure S8. Power (q'_r) generated by the [2+2+2] reaction over time.



Table S4 summarizes the reaction severity levels, in case of a reaction runaway,leading to the worst-case scenario.

$\Delta T_{ad} [K]$	Severity
> 400	catastrophic
200 - 400	critical
50 - 200	medium
< 50	negligible

Table S4. Severity of worst case scenario based on ΔT_{ad} ranges.

An isothermal DSC (Differential Scanning Calorimetry) was measured at 90 °C (reaction temperature + 50 °C) to establish the decomposition behavior of the endoperoxide **7** under these conditions (**Figure S9**). The obtained results ensured a safe process.



Figure S9. Isothermal DSC analysis of endoperoxide 7 at 90 °C.

Spectra NMR 2-butyl-1-hexene (2) ¹H NMR



¹³C NMR



NMR Methyl 6,6-dibutyl-3-hydroxy-3-methyl-1,2-dioxane-4-carboxylate (7) ¹H NMR





¹³C NMR



¹³C-APT



¹H-¹H COSY 45













ESI-MS 2-butyl-1-hexene (2)



Methyl 6,6-dibutyl-3-hydroxy-3-methyl-1,2-dioxane-4-carboxylate (7)

