Supporting Information

Accelerating Biphasic Biocatalysis through New Process Windows

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# Supporting Information

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**S1**
1. General methods and materials

All $^1$H and $^{13}$C NMR spectra were obtained on either a Bruker Avance 300 ($^1$H: 300 MHz, $^{13}$C: 76 MHz) or a Bruker Avance 400 ($^1$H: 400 MHz, $^{13}$C: 100 MHz) spectrometer at 25 °C in the solvent stated. Chemical shifts are reported in parts per million (ppm) and all coupling constants, J, are quoted in Hz. Multiplicities are reported with the following symbols: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and multiples thereof.

Mass spectrometric measurements were performed by R. Jenkins, R. Hick, T. Williams and S. Waller at Cardiff University on a Water LCR Premier XEtof. The molecular ion peaks values quoted for molecular ion plus hydrogen [M+H]$^+$ or molecular ion plus sodium [M+Na]$^+$. Infrared spectra were recorded on a Shimadzu iRAffinity-1 Fourier Transform ATIR spectrometer as thin films using a pike miracle ATR accessory. Characteristic peaks are quoted ($v_{max}$/ cm$^{-1}$).

Room temperature (rt) refers to 20 - 25 °C. Temperatures of 0 °C were obtained using ice/water baths. All reactions involving heating were carried out using DrySyn blocks and a contact thermometer. In vacuo refers to the use of a rotary evaporator under reduced pressure. Dry solvents such THF, diethyl ether and toluene were obtained after passing these previously degassed solvents through activated alumina columns (MBraun, SPS-800). Dry dichloromethane was obtained using phosphorous pentoxide, and then distilling when needed. All other solvents and commercial reagents were used as supplied without further purification unless stated otherwise. Thin-layer chromatography (TLC) was performed on pre-coated aluminium sheets of Merck silica gel 60 F254 (0.20 mm) and visualised by UV radiation (254 nm). Manual column chromatography was performed using silica gel 60 (Merck, 230-400 mesh) under increased pressure (Flash Chromatography), while automated column chromatography and reverse phase purifications were performed on a Biotage® Isolera Four. The solvents were used as laboratory grade.

The HPLC measurements were carried out on a Shimadzu apparatus. The different modules were SIL-10ADVP (autoinjector), LC-10ATVP (liquid chromatograph), FCV-10ALVP (pump), DGU-14A (degasser), CTO-10ASVP (column oven), SCL-10AVP (system controller) and SPD-M10A (diode array detector). The solvents used were n-hexane and 2-propanol and were of an HPLC grade. The chiral column used for the separation of the enantiomers were (Regis (RR) Whelk-01 column (0.46 cm Ø x 25 cm).

A prestained protein size marker (14.4-116.0) kDa was used to identify proteins by 12% SDS-gel. The Amicon-YM30 membranes were used for protein concentration. For synthetic procedures, all chemicals were purchased from Sigma-Aldrich, Acros Chemicals or Alpha Aesar unless otherwise stated. Anhydrous acetone, acetonitrile and THF were used as supplied without further purification unless stated otherwise. Thin layer chromatography (TLC) was performed on pre-coated aluminium sheets of Merck silica gel 60 F254 (0.20 mm) and visualised by UV radiation (254 nm). Manual column chromatography was performed using silica gel 60 (Merck, 230-400 mesh) under increased pressure (Flash Chromatography), while automated column chromatography and reverse phase purifications were performed on a Biotage® Isolera Four. The solvents were used as laboratory grade.

Gas chromatography coupled with mass spectrum (GC-MS) was performed on a Perkin Elmer Clarus 680 GC fitted with a Perkin Elmer Elite-1 column (30 m x 0.25 mm internal diameter) and a Perkin Elmer Clarus SQ 8 C mass spectrometer. The program uses an injection port temperature of 100 °C; split ratio 19:1; initial temperature 80 °C hold 2 min, ramp of 8 °C/min to 280 °C hold 3 min.

Gas chromatography with flame ionisation detector (GC-FID) chiral analysis was performed on an Agilent 7890A GC system fitted with a Restek Rt-bDEXx50m column (30 m x 0.25 mm internal diameter). GC FID method A: uses an injection port temperature of 200 °C, 5 μL was injected with a 1:19:1 split. The oven temperature was held at 100 °C for 1 min and then rose at 8 °C/min to 200 °C and then held for 2 min. GC FID method B uses an injection port temperature of 200°C; 5 μL, split ratio 10:1; initial temperature 80 °C hold 1 min, ramp of 8 °C/min to 150 °C hold 2 min. The GC FID method C uses an injection port temperature of 200 °C; 5 μL, split ratio 10:1; initial temperature 80 °C hold 10 min, ramp of 8 °C/min to 100 °C hold 2 min.

Ion exchange resin DOWEX 40-W was received from Aldrich in H$^+$ form. The resin was converted into ammonium form by washing with concentrated NH$_4$OH, then deionised water until the pH drops to 7, finally equilibrated with ion-exchange buffer (25 mM NH$_4$HCO$_3$ containing 2% i-PrOH).

All high performance counter current chromatography (HPCCC) experiments were performed using a were performed on a Dynamic Extractions Spectrum instrument (Slough, UK) which was fitted with an analytical scale column with a volume of 22 mL (0.8 mm I.D. PTFE tubing) and a revolution radius of 85 mm and a semi-preparative scale column with a volume of 132 mL (1.6 mm I.D. of 1.6 mm PTFE tubing) and a revolution radius of 85 mm. β-value range of 0.52–0.86. The Spectrum HPCCC was connected to two HPLC pumps (ECOM ECP 2010), manually controlled fluidic and sample injection valves.
2. HPCCC methodology

The high-performance counter current chromatography (HPCCC) is a liquid-liquid purification apparatus where the two liquids are immiscible. One liquid is stationary in the column (the stationary phase) and the other is pumped through it (the mobile phase). Centrifugal fields are used to maintain the first liquid phase stationary. There are currently several types of CCC instruments but only the J-type machine has been used in this work. The HPCCC is made of two bobbins containing tubing wrapped in coil. The bobbins (hence the coil) are rotated in a planetary motion that creates a radial centrifugal force. The liquids are therefore pushed in the opposite direction to the central solar axis. In CCC, two terms are important to define: the "head" which is where the liquid is pushed out of the coil and the "tail" in opposition to the "head". Depending on the rotation direction, head and tail position will change. During a run, the lighter phase (here the pentane) will always move toward the "head" while the heavy phase will remain at the "tail". Therefore, when choosing the lighter phase as the mobile phase, the liquid needs to be pumped through the tail so it goes through the heavy phase therefore causing it to be unable to exit the column and stays stationary on the column. This is called tail-to-head direction also normal phase mode of the HPCCC. It is also possible to use the heavier phase as mobile phase, in this case head-to-tail direction is used (reverse mode). In this paper, only normal phase was used for all experiments. The HPCCC system used was Dynamic Extractions Spectrum instrument (Slough, UK) containing two columns, one analytical (22 mL) and one preparative (135 mL) with an ID of 0.8 mm.

3. Asymmetric alkylation

3.1. General batch procedure for the asymmetric alkylation of N-(diphenylmethylene)glycine tert-butyl ester

In a 10 mL round bottomed flask, benzyl bromide (0.12 mL, 1 mmol) was added to a mixture of N-(diphenylmethylene)glycine tert-butyl ester (59 mg, 0.2 mmol) and chiral catalyst 3a or 3b (0.02 mmol) in toluene/chloroform (7:3, 0.75 mL). 50% aqueous KOH (0.25 mL) was then added, and the reaction mixture was stirred at room temperature until the starting material had been consumed (7 h). The suspension was diluted with ethyl acetate (20 mL), washed with water (2 x 5 mL), dried over MgSO₄, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (n-hexane/ethyl acetate) to afford the target product (48 mg, 62% yield) as a colourless oil. The enantioselectivity (66 – 87% e.e.) was determined by chiral HPLC (Regis (RR) Whelk-01 column, 2-propanol / n-hexane (5:95), 1 mL/min, 23 °C, 254 nm, retention times: S (minor) 5.3 min, R (major) 10.0 min. The absolute configuration was assigned according to literature.¹

3.2. Flow setup and general flow procedure for the asymmetric alkylation of N-(diphenylmethylene)glycine tert-butyl ester

A solution of N-(diphenylmethylene)glycine tert-butyl ester (116 mg, 0.4 mmol), benzyl bromide (0.24 mL, 2 mmol) and chiral catalyst 3b (26 mg, 0.04 mmol) in toluene (8.5 mL) was loaded into syringe A. 50% aqueous KOH (8.5 mL) was loaded into syringe B. Syringe A and B were then connected to the microflow system (PTFE) through a designated inlet using a T-connector. Tubing length 24 m, residence time = 23.5 min, internal diameter = 0.5 mm. The solutions were then delivered into the microchannel at 0.1 mL/min (combined 0.2 mL/min), in a continuous segmented flow manner using KD Scientific syringe pumps. The reaction product was quenched in water and diluted with EtOAc. The organic layer was washed with water (2 x 5 mL), dried over MgSO₄, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (n-hexane/ethyl acetate) to afford product 4 (50% yield, 89% e.e.) as a colourless oil.

Figure S1: Flow setup for the asymmetric alkylation of N-(diphenylmethylene)glycine tert-butyl ester.
3.3. HPCCC setup and general procedure for the asymmetric alkylation of \(N\)-(diphenylmethylene)glycine tert-butyl ester

Equilibration of the HPCCC

This reaction was performed under normal phase HPCCC conditions (50% aq. KOH employed as stationary phase, tail-to-head mode). Firstly, mobile phase (toluene) is pumped through the column to displace any aqueous solvent used in the washing of the column. This allows easy detection of stationary phase elution, and also stops the stationary phase being diluted by the water. To fill the HPCCC column with stationary phase, a solution of KOH dissolved in deionised water (50% aq. solution) was pumped through the column until the aqueous stationary phase was observed at the outlet. Upon filling of the machine, the stationary phase pump was switched off and the HPCCC was spun at 2100 rpm. The mobile phase (toluene) was then pumped at 0.7 mL/min to allow equilibration of the stationary phase. Once stationary phase stopped eluting from the column, equilibration had been achieved.

Sample injection

A solution of \(N\)-(diphenylmethylene)glycine tert-butyl ester (29 mg, 0.1 mmol), benzyl bromide (0.06 mL, 0.5 mmol) and chiral catalyst 3b (6.5 mg, 0.01 mmol) in toluene (3 mL) was loaded into a 3 mL syringe. The sample was injected into the mobile phase using a syringe pump at the required flow rate. The reaction product was quenched in water and diluted with ethyl acetate. The organic layer was washed with water (2 x 5 mL), dried over MgSO\(_4\), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (n-hexane/ethyl acetate) to afford the target product (25 mg, 65% yield, 86% e.e.) as a colourless oil.

3.4. Characterisation of alkylation products

\((R)\)-tert-Butyl \(-\left[(\text{diphenylmethylene})\text{amino}\right]3\)-phenylpropanoate (2):

\[
\begin{align*}
\text{Ph} & \quad \text{N} & \quad \text{CO}_2\text{Bu} \\
\text{Ph} & \quad \text{N} & \quad \text{CO}_2\text{Bu} \\
\end{align*}
\]

Performed according to the “HPCCC General Procedure”. Compound obtained as a colourless oil (25 mg, 65%).

\([\alpha]_D^{20} = +139.2^\circ\) (c = 0.5, CHCl\(_3\))

\(^1\)H NMR (500 MHz, CDCl\(_3\)): 6 = 7.60 - 7.55 (m, 2H, Ar-H), 7.39 - 7.24 (m, 6H, Ar-H), 7.21 - 7.13 (m, 3H, Ar-H), 7.07 - 7.03 (m, 2H, Ar-H), 6.60 (br d, J = 6.0 Hz, 2H, Ar-H), 4.10 (dd, J = 9.3, 4.3 Hz, 1H, NC-H), 3.23 (dd, J = 13.4, 4.3 Hz, 1H, CH\(_2\)), 3.16 (dd, J = 13.4, 9.3 Hz, 1H, CH\(_2\)), 1.44 (s, 3H, CH\(_3\)) ppm.

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)): 6 = 170.9, 170.3 (C=N, C=O), 139.6, 138.4, 136.4, 130.1, 129.9, 128.7, 128.2, 128.1, 128.1, 127.9, 127.7, 126.1, 81.1 (C-O), 68.0 (C-N), 39.6 (CH\(_2\)), 28.1 (CH\(_3\)) ppm.
The enantioselectivity (86% e.e.) was determined by chiral HPLC (Regis (RR) Whelk-01 column, 5:95 2-propanol / n-hexane, 1 mL/min, 25 °C, 254 nm, retention times: S (minor) 5.3 min, R (major) 10.0 min. Absolute configuration was assigned according to literature. Spectroscopic data are in agreement with literature.[1,2]

(R)-tert-Butyl 2-[(diphenylmethylene)amino]-3-[4-(trifluoromethyl)phenyl]propanoate (4a)

![Chemical structure of 4a]

Performed according to the “HPCCC General Procedure”. Compound obtained as a white solid (30 mg, 67%), m.p.: 109 - 112 °C.

\[\alpha\]_D^20 = +146.9° (c = 0.6, CHCl₃)

\(^1\)H NMR (500 MHz, CDCl₃): \(\delta = 7.54\ (d, J = 7.5\ \text{Hz}, 2\text{H}, \text{Ar-H}), 7.41\ (d, J = 8.0\ \text{Hz}, 2\text{H}, \text{Ar-H}), 6.59\ (d, J = 5.8\ \text{Hz}, 2\text{H}, \text{Ar-H})\), 4.09 (dd, \(J = 9.1, 4.1\ \text{Hz}, 1\text{H}, \text{NC-H}\)), 3.27 - 3.13 (m, 2H, \(\text{CH}_2\)), 1.41 (s, 3H, \(\text{CH}_3\)) ppm.

\(^{13}\)C NMR (100 MHz, CDCl₃): \(\delta = 170.7, 170.4\ (\text{C=N}, \text{C=O}), 142.7, 142.7\ 139.3, 136.1, 130.1, 128.7, 128.4, 128.2, 128.0, 127.5, 125.0 (q, \(J_{\text{C-F}} = 4\ \text{Hz}\)), 81.5 (\(\text{C-O}\)), 67.5 (\(\text{C-N}\)), 39.3 (\(\text{CH}_2\)), 28.0 (\(\text{CH}_3\)) ppm.

The enantioselectivity (87% e.e.) was determined by chiral HPLC (Regis (RR) Whelk-01 column, 5:95 2-propanol / n-hexane, 1 mL/min, 22 °C, 200 nm, retention times: S (minor) 4.7 min, R (major) 10.6 min. Absolute configuration was assigned according to literature. Spectroscopic data are in agreement with literature.[3]

Methyl-(R)-4-(3-(tert-butoxy)-2-[(diphenylmethylene)amino]-3-oxopropyl)benzoate (4b)

![Chemical structure of 4b]

Performed according to the “HPCCC General Procedure”. Compound obtained as a white solid (25 mg, 57%), m.p.: 102–106 °C.

\[\alpha\]_D^20 = +107.3° (c = 0.3, CHCl₃).

\(^1\)H NMR (500 MHz, CDCl₃): \(\delta = 7.87\ (d, J = 8.4\ \text{Hz}, 2\text{H}, \text{Ar-H}), 7.58 - 7.54\ (m, 2\text{H}, \text{Ar-H}), 7.41 - 7.26\ (m, 6\text{H}, \text{Ar-H}), 7.14\ (d, J = 8.2\ \text{Hz}, 2\text{H}, \text{Ar-H}), 6.64\ (d, J = 5.8\ \text{Hz}, \text{Ar-H})\), 4.14 (dd, \(J = 9.1, 4.5\ \text{Hz}, 1\text{H}, \text{NC-H}\)), 3.89 (s, 3H, O-\(\text{CH}_3\)), 3.31 - 3.19 (m, 2H, \(\text{CH}_2\)), 1.44 (s, 9H, \(\text{CH}_3\)) ppm.

\(^{13}\)C NMR (100 MHz, CDCl₃): \(\delta = 170.7, 170.4\ (\text{C=N}, \text{C=O}), 167.2 (\text{C=O}), 144.1, 139.4, 136.2, 132.4, 130.3, 130.1, 129.9, 129.7, 129.4, 129.4, 128.7, 128.4, 128.3, 128.1, 128.0, 127.6, 81.4 (\(\text{C-O}\)), 67.4 (\(\text{C-N}\)), 52.0 (\(\text{OCH}_3\)) 39.3 (\(\text{CH}_2\)), 28.0 (\(\text{CH}_3\)) ppm.

HRMS (ESP): \([\text{C}_{28}\text{H}_{30}\text{NO}_4]^+ = [\text{M+H}]^+\): calc 444.2177, found 444.2175.

IR (neat): \(\nu_{\text{max}}\): 3055, 2976, 2932, 1721, 1611, 1574, 1435, 1416, 1368, 1277, 1148, 1103 cm\(^{-1}\).

The enantioselectivity (87% e.e.) was determined by chiral HPLC (Regis (RR) Whelk-01 column, 5:95 2-propanol / n-hexane, 1 mL/min, 25 °C, 254 nm, retention times: S (minor) 12.6 min, R (major) 22.4 min. Absolute configuration was assigned by analogy with previous substrates.

tert-Butyl-(R)-2-[(diphenylmethylene)amino]-3-(4-nitrophenyl)propanoate (4c)
Performed according to the "HPCCC General Procedure". Compound obtained as a pale-yellow oil (26 mg, 60%).

$[\alpha]^2_{D} = +107.4^\circ$ (c = 0.7, CHCl$_3$).

$^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 8.01 - 7.97 (m, 2H, Ar-H), 7.51 - 7.47 (m, 2H, Ar-H), 7.34 - 7.28 (m, 2H, Ar-H), 7.27 - 7.21 (m, 4H, Ar-H), 7.20 - 7.16 (m, 2H, Ar-H), 6.64 (br d, $J = 6.8$ Hz, 2H, Ar-H), 4.10 (dd, $J = 8.4$, 5.0 Hz, 1H, NC-H), 3.27 - 3.17 (m, 2H, CH$_2$), 1.37 (s, 9H, CH$_3$) ppm.

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 171.0, 170.1 (C=N, C=O), 146.6, 146.5, 139.1, 136.0, 130.7, 130.5, 128.7, 128.6, 128.3, 128.1, 127.5, 123.3, 81.7 (C-O), 67.0 (C-N), 39.4 (CH$_2$), 28.1 (CH$_3$) ppm.

HRMS (ESP): [C$_{26}$H$_{27}$N$_2$O$_4$]$^+$ = [M+H]$^+$: calc 431.1971, found 431.1971.

IR (neat) $V_{\text{max}}$: 3057, 2978, 2931, 1730, 1622, 1599, 1518, 1490, 1447, 1285, 1148, 847, 696 cm$^{-1}$.

The enantioselectivity (81% e.e.) was determined by chiral HPLC (Regis (RR) Whelk-01 column, 5:95 2-propanol / n-hexane, 1 mL/min, 25 °C, 277 nm, retention times: S (minor) 9.6 min, R (major) 18.0 min. Absolute configuration was assigned according to literature. Spectroscopic data are in agreement with literature.$^{[4]}$

tert-Butyl-(R)-3-[(1,1'-biphenyl)-2-yl]-2-[(diphenylmethylene)amino]propanoate (4d)

Performed according to the “HPCCC General Procedure”. Compound obtained as a colourless oil (34 mg, 74%).

$[\alpha]^2_{D} = +206.7^\circ$ (c = 0.06, CHCl$_3$).

$^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 7.50 - 7.45 (m, 2H, Ar-H), 7.34 - 7.06 (m, 12H, Ar-H), 7.01 - 6.97 (m, 1H, Ar-H), 6.80 - 6.73 (m, 2H, Ar-H), 6.45 (br d, $J = 4.3$ Hz, 2H, Ar-H), 3.85 (dd, $J = 9.8$, 3.8 Hz, 1H, NC-H), 3.24 (dd, $J = 13.5$, 3.5 Hz, 1H, CH$_2$), 3.05 (dd, $J = 13.5$, 9.8 Hz, 1H, CH$_2$), 1.24 (s, 9H, CH$_3$) ppm.

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 170.9, 170.1 (C=N, C=O), 142.8, 141.4, 139.6, 136.4, 135.8, 132.4, 131.2, 130.3, 130.1, 130.0, 129.2, 129.1, 128.8, 128.3, 128.2, 128.1, 128.1, 127.9, 127.8, 127.0, 126.6, 126.1, 80.8 (C-O), 66.9 (C-N), 36.8 (CH$_2$), 28.0 (CH$_3$) ppm.

HRMS (ESP): [C$_{32}$H$_{32}$NO$_2$]$^+$ = [M+H]$^+$: calc 462.2435, found 462.2433.

IR (neat) $V_{\text{max}}$: 3057, 2978, 2932, 1728, 1661, 1622, 1597, 1479, 1447, 1368, 1148 cm$^{-1}$.

The enantioselectivity (81% e.e.) was determined by chiral HPLC (Regis (RR) Whelk-01 column, 5:95 2-propanol / n-hexane, 1 mL/min, 25 °C, 277 nm, retention times: S (minor) 9.6 min, R (major) 22.7 min. Absolute configuration was assigned by analogy with previous substrates.$^{[4]}$

tert-Butyl-(R)-2-[(diphenylmethylene)amino]-3-(2-iodophenyl)propanoate (4e)
Performed according to the "HPCCC General Procedure". Compound obtained as a pale-yellow oil (31 mg, 60%).

\[\alpha\] D \text{ } 20 = +186° (c = 0.03, CHCl₃).

\(^1\)H NMR (500 MHz, CDCl₃): \(\delta = 7.72\) (dd, \(J = 7.7, 1.1\) Hz, 1H, Ar-H), 7.61 - 7.56 (m, 2H, Ar-H), 7.40 - 7.23 (m, 6H, Ar-H), 7.21 (m, 2H, Ar-H), 6.90 - 6.82 (m, 2H, Ar-H), 6.55 (br d, \(J = 3.4\) Hz, 2H, Ar-H), 4.33 (dd, \(J = 9.9, 3.8\) Hz, 1H, NC-H), 3.40 (dd, \(J = 13.5, 3.8\) Hz, 1H, CH₂), 3.28 (dd, \(J = 13.5, 9.9\) Hz, 1H, CH₂), 1.47 (s, 9H, CH₃) ppm.

\(^1^3\)C NMR (100 MHz, CDCl₃): \(\delta = 170.8, 170.6\) (C=N, C=O), 140.8, 139.4, 139.2, 136.2, 132.1, 130.1, 128.8, 128.3, 128.2, 128.1, 127.9, 127.7, 101.4 (Ph-I), 81.3 (C-O), 65.2 (C-N), 43.9 (CH₂), 28.1 (CH₃) ppm.

The enantioselectivity (87% e.e.) was determined by chiral HPLC (Regis (RR) Whelk-01 column, 5:95 2-propanol / n-hexane, 1 mL/min, 25 °C, 254 nm, retention times: S (minor) 5.9 min, R (major) 15.9 min. Absolute configuration was assigned according to literature. Spectroscopic data are in agreement with literature. \[5\]

tert-Butyl-(R)-2-[(diphenylmethylene)amino]-3-(naphthalen-2-yl)propanoate (4f)

Performed according to the “HPCCC General Procedure”. Compound afforded as a pale-yellow oil (23 mg, 53%).

\[\alpha\] D \text{ } 20 = +124° (c = 0.05, CHCl₃).

\(^1\)H NMR (500 MHz, CDCl₃): \(\delta = 7.79 - 7.74\) (m, 1H, Ar-H), 7.70 - 7.64 (m, 2H, Ar-H), 7.58 - 7.53 (m, 2H, Ar-H), 7.51 (s, 1H, Ar-H), 7.44 - 7.38 (m, 2H, Ar-H), 7.37 - 7.33 (m, 1H, Ar-H), 7.33-7.26 (m, 3H, Ar-H), 7.22 - 7.14 (m, 3H, Ar-H), 6.54 (br d, \(J = 5.5\) Hz, 2H, Ar-H), 4.25 (dd, \(J = 9.3, 4.3\) Hz, 1H, NC-H), 3.44 - 3.28 (m, 2H, CH₂), 1.45 (s, 9H, CH₃) ppm.

\(^1^3\)C NMR (100 MHz, CDCl₃): \(\delta = 170.9, 170.4\) (C=N, C=O), 139.6, 136.3, 135.9, 133.4, 132.1, 130.1, 128.7, 128.4, 128.2, 128.0, 127.9, 127.7, 127.6, 127.5, 125.8, 125.2, 81.3 (C-O), 67.9 (C-N), 39.8 (CH₂), 28.1 (CH₃) ppm.

The enantioselectivity (81% e.e.) was determined by chiral HPLC (Regis (RR) Whelk-01 column, 5:95 2-propanol / n-hexane, 1 mL/min, 25 °C, 254 nm, retention times: S (minor) 7.6 min, R (major) 18.1 min. Absolute configuration was assigned according to literature. Spectroscopic data are in agreement with literature. \[2, 6\]

tert-Butyl-(R)-2-[(diphenylmethylene)amino]-5-methylhex-4-enoate (4g)

Performed according to the “HPCCC General Procedure”. Compound afforded as a colourless oil (22 mg, 61%).

\[\alpha\] D \text{ } 20 = +68.9° (c = 0.3, CHCl₃).

\(^1\)H NMR (500 MHz, CDCl₃): \(\delta = 7.65 - 7.61\) (m, 1H, Ar-H), 7.61 - 7.47 (m, 2H, Ar-H), 7.17 - 7.13 (m, 2H, Ar-H), 5.02 (tt, \(J = 7.6, 1.4\) Hz, 1H, C=CH), 3.95 (dd, \(J = 7.8, 5.5\) Hz, 1H, NC-H), 3.89 (s, 3H, O-CH₃), 2.62 - 2.48 (m, 2H, CH₂), 1.65 (s, 3H, C=C-CH₃), 1.56 (s, 3H, C=C-CH₃), 1.44 (s, 9H, CH₃) ppm.
chloride

The enantioselectivity (81% e.e.) was determined by chiral HPLC (Regis (RR) Whelk-01 column, 5:95 2-propanol / n-hexane, 1 mL/min, 25 °C, 294 nm, retention times: S (minor) 4.6 min, R (major) 6.0 min. Absolute configuration was assigned according to literature. Spectroscopic data are in agreement with literature.6

** tert-Butyl-(R)-2-[(diphenylmethylene)amino]pent-4-ynoate (4h)**

Performed according to the "HPCCC General Procedure". Compound afforded as a pale-yellow oil (13 mg, 40%).

[a]_D^20 = +77.5° (c = 0.03, CHCl₃).

1^H NMR (500 MHz, CDCl₃): 8 = 7.68 - 7.63 (m, 2H, Ar-H), 7.51 - 7.37 (m, 4H, Ar-H), 7.36 - 7.31 (m, 2H, Ar-H), 7.28 - 7.24 (m, 2H, Ar-H), 4.17 (dd, J = 8.1, 5.2 Hz, 1H, NC≡C), 5.32 - 4.84 (m, 2H, CH₂), 1.45 (s, 9H, CH₃) ppm.

13^C NMR (100 MHz, CDCl₃): 8 = 171.4, 169.6 (C=N, C=O), 139.6, 136.3, 130.4, 129.0, 128.6, 128.4, 128.3, 128.0, 81.6, 81.3, 77.3 (C≡C), 75.9 (C≡C) 66.4 (C=CH), 28.0 (CH₃), 23.4 (CH₂) ppm.

The enantioselectivity (81% e.e.) was determined by chiral HPLC (Regis (RR) Whelk-01 column, 5:95 2-propanol / n-hexane, 1 mL/min, 25 °C, 294 nm, retention times: S (minor) 4.8 min, R (major) 6.0 min. Absolute configuration was assigned according to literature.7

** tert-Butyl-(R)-2-[(diphenylmethylene)amino]hex-4-ynoate (4i)**

Performed according to the "HPCCC General Procedure". Compound afforded as a pale-yellow oil (9 mg, 25%).

[a]_D^20 = +24.7° (c = 0.6, CHCl₃).

1^H NMR (500 MHz, CDCl₃): 8 = 7.68 - 7.63 (m, 2H, Ar-H), 7.49 - 7.30 (m, 6H, Ar-H), 7.26 - 7.21 (m, 2H, Ar-H), 4.12 (dd, J = 8.2, 5.3 Hz, 1H, NC≡C), 2.83 - 2.60 (m, 2H, CH₂), 1.73 (t, J = 2.5 Hz, 3H, C≡C-CH₃), 1.45 (s, 9H, CH₃) ppm.

13^C NMR (100 MHz, CDCl₃): 8 = 171.0, 170.0 (C=N, C=O), 139.8, 136.5, 130.3, 130.1, 129.0, 128.6, 128.3, 128.2, 128.0, 81.3 (C-O), 77.3 (C≡C), 75.9 (C≡C) 65.5 (C=N), 28.0 (CH₃), 23.7 (CH₂), 3.8 (CH₃) ppm.

HRMS (ESP): [C₂₄H₂₈NO₃]⁺ = [M+H]⁺: calc 348.1965, found 348.1964.

IR(neat) Vmax: 3059, 2976, 2928, 1732, 1622, 1447, 1368, 1285, 1254, 1150, 698 cm⁻¹.

The enantioselectivity (81% e.e.) was determined by chiral HPLC (Regis (RR) Whelk-01 column, 5:95 2-propanol / n-hexane, 1 mL/min, 22 °C, 254 nm, retention times: S (minor) 5.1 min, R (major) 6.7 min. Absolute configuration was assigned by analogy with previous substrates.

3.5. Synthesis of the chiral catalyst

(2R, 5R, 1’S)-1-(9-Anthracenyl)methyl-5-ethylen-2-[1-hydroxy-1-(quinol- 4-yl)]methyl-1-azoniabicyclo[2.2.2]octane chloride
A mixture of cinchonine (500 mg, 1.70 mmol) and 9-chloromethylanthracene (390 mg, 1.72 mmol) was heated at reflux in toluene (20 mL) under argon overnight (16 h). The solution was then cooled to room temperature and the resulting precipitate filtered. The residue was recrystallized from chloroform/petroleum ether to give the title product (531 mg, 60%) as a yellow solid, m.p.: 165–168 °C.

$^1$H NMR (500 MHz, CDCl$_3$): $\delta = 9.22$ (d, $J = 8.7$ Hz, 1H), 8.90 (d, $J = 8.2$ Hz, 1H), 8.87 - 8.80 (m, 1H), 8.44 (d, $J = 9.0$ Hz, 1H), 8.07 - 8.00 (m, 1H), 7.89 (t, $J = 8.6$ Hz, 2H), 7.48 (d, $J = 8.3$ Hz, 1H), 7.31 - 7.17 (m, 3H), 7.15 - 7.03 (m, 2H), 7.00 - 6.86 (m, 3H), 6.49 (d, $J = 13.7$ Hz, 1H), 5.64 - 5.54 (m, 1H), 5.03 (d, $J = 10.5$ Hz, 1H), 4.87 (d, $J = 17.2$ Hz, 1H), 4.77 - 4.60 (m, 1H), 4.48 - 4.38 (m, 1H), 4.25 (t, $J = 11.6$ Hz, 1H), 2.55 - 2.45 (m, 1H), 1.95 (t, $J = 12.8$ Hz, 1H), 1.78 - 1.60 (m, 3H), 1.55 - 1.49 (m, 1H), 1.40 - 1.31 (m, 1H), 0.70 - 0.58 (m, 1H) ppm.

$^{13}$C NMR (125 MHz, CDCl$_3$): $\delta = 149.4, 147.0, 145.5, 135.5, 133.1, 132.7, 132.7, 130.9, 130.3, 130.0, 129.0, 128.5, 128.4, 128.2, 127.6, 127.3, 126.8, 124.9, 124.9, 124.6, 124.1, 120.0, 118.1, 117.5, 67.6, 66.8, 57.5, 54.2, 54.0, 38.1, 26.3, 24.1, 22.6 ppm.

Spectroscopic data are in agreement with literature.[8]

(2R, 5R, 1’S)-1-(1-Anthracenyl)methyl-5-ethylene-2-[1-benzyloxy-1-(quinol-4-yl)]methyl-1-azoniabicyclo[2.2.2]octane bromide (3b)

Aqueous sodium hydroxide 50% (0.25 mL, 0.325 mmol) was added to a solution of the N-anthracene salt (0.5 g, 0.95 mmol) and benzyl bromide (0.325 mL, 2.75 mmol) in dichloromethane (8 mL) and the mixture was stirred vigorously for 5 h (1500 rpm). Water was then added, and the aqueous layer extracted with dichloromethane (3 x 10 mL). The combined organics were dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to give the title product (0.40 g, 65%) as a yellow solid, m.p.: 125–127 °C.

$^1$H NMR (500 MHz, CDCl$_3$): $\delta = 9.90 - 9.60$ (m, 1H), 9.39 - 8.92 (m, 2H), 8.59 (s, 1H), 8.23 - 8.13 (m, 1H), 8.10 - 7.93 (m, 3H), 7.90 - 7.69 (m, 4H), 7.63 - 7.47 (m, 6H), 7.39 - 7.30 (m, 2H), 6.85 - 6.58 (m, 2H), 5.97 - 5.39 (m, 4H), 5.31 - 5.18 (m, 1H), 5.03 - 4.81 (m, 3H), 4.40 - 4.27 (m, 1H), 3.03 - 2.87 (m, 1H), 2.68 - 2.33 (m, 2H), 2.12 - 1.98 (m, 3H), 1.65 - 1.52 (m, 1H), 1.27 - 1.21 (m, 1H) ppm.

$^{13}$C NMR (125 MHz, CDCl$_3$): $\delta = 149.2, 148.7, 139.7, 136.4, 135.8, 134.0, 133.4, 132.4, 131.5, 130.9, 130.0, 129.2, 128.8, 128.7, 128.6, 128.5, 127.5, 127.5, 127.3, 127.1, 126.9, 126.1, 125.4, 124.9, 123.3, 118.9, 117.9, 117.6, 75.7, 71.3, 66.0, 57.2, 55.2, 54.9, 37.7, 26.4, 23.9, 23.1 ppm.

HRMS (ESP): $[C_{41}H_{39}N_2O]^+ = [M+Br]^+$: calc 575.3073, found 575.3062.

Spectroscopic data are in agreement with literature.[8]
3.6. Optimisation of the asymmetric alkylation reaction in segmented flow

Table S1: Optimisation of the asymmetric alkylation reaction in segmented flow.

![Chemical structures](image)

| Entry | Flow rate (mL/min) | Reactor volume (mL) | Residence time (min) | ID (mm) | Temp. (°C) | Solvent | Yield * (%) | ee (%) |
|-------|--------------------|---------------------|----------------------|---------|------------|---------|-------------|--------|
| 1     | 0.2                | 2.5                 | 12                   | 0.8     | rt         | Toluene / CHCl₂ - 7:3 | 1       | -        |
| 2     | 0.1                | 3.6                 | 36                   | 0.8     | rt         | Toluene | 8          | -      |
| 3     | 0.3                | 8.9                 | 29                   | 0.8     | rt         | CH₃Cl₂ | 53         | 58     |
| 4     | 0.2                | 8.9                 | 43                   | 0.8     | rt         | CH₃Cl₂ | 52         | 59     |
| 5     | 0.2                | 4.75                | 23.5                 | 0.5     | rt         | CH₃Cl₂ | 73         | 56     |
| 6     | 0.3                | 4.75                | 15.7                 | 0.5     | rt         | CH₃Cl₂ | 69         | 50     |
| 7     | 0.1                | 4.75                | 47                   | 0.5     | rt         | CH₃Cl₂ | 65         | 52     |
| 8     | 0.2                | 4.75                | 23.5                 | 0.5     | 40         | CH₃Cl₂ | 22         | 48     |
| 9     | 0.2                | 4.75                | 23.5                 | 0.5     | 0          | CH₃Cl₂ | 15         | 56     |
| 10    | 0.25               | 4.75                | 18.8                 | 0.5     | rt         | CH₃Cl₂ | 52         | 59     |
| 11    | 0.2                | 6.7                 | 33.4                 | 0.5     | rt         | CH₃Cl₂ | 59         | 56     |
| 12    | 0.25               | 6.7                 | 27                   | 0.5     | rt         | CH₃Cl₂ | 69         | 59     |
| 13    | 0.32               | 6.7                 | 21                   | 0.5     | rt         | CH₃Cl₂ | 77         | 56     |
| 14    | 0.4                | 6.7                 | 17                   | 0.5     | rt         | CH₃Cl₂ | 47         | 52     |
| 15    | 0.5                | 6.7                 | 13.4                 | 0.5     | rt         | CH₃Cl₂ | 37         | 52     |

* yield determined by quantitative ¹H NMR, using 1,3,5-trimethoxybenzene as an internal standard. * Pump A flow rate = 0.15 mL/min, Pump B flow rate = 0.10 mL/min.

3.7. Optimisation of the asymmetric alkylation reaction with HPCCC

Table S2: Optimisation of the asymmetric alkylation reaction with HPCCC.
The effect of flow rate was evaluated between the range of 0.4 - 1.0 mL/min. Up until 0.7 mL/min, an increase in percentage yield was observed. However, exceeding this flow rate resulted in a decrease in yield. These results suggest that increasing the flow rate increases the efficiency of mixing in the HPCCC. This is a well-established phenomenon observed in both laminar flow and segmented flow chemistry. Increasing the flow rate further then 0.7 mL/min led to a large amount of stationary phase being lost from the column during equilibration, leading to a decrease in the percentage yield.

### 4. Enzymatic reactions

For the transesterification of alcohol, the column is first filled with the enzyme in buffer (V_{C,aq}), then rotation is turned on and substrates in heptane is pumped from tail-to-head until the system is equilibrated. At equilibrium, V_C = V_H of aqueous phase has been displaced. (Figure S3a) The volume of the organic phase (V_H) will determine the residence time of the reaction. For the biochemical conversion of FDP into terpene products the HPCCC was used in normal phase, therefore, the stationary phase was the aqueous phase containing the enzyme and the substrate in catalysis buffer. The mobile phase was pentane, an organic solvent. To set up the reaction, the column is filled firstly with pentane (V_{c,P}) (Step 1) followed by V_{aq} mL of aqueous buffer (Step 2). At this stage the tubing contains two segments as depicted in Fig. S3b. Then rotation of the bobbin is turned on and pentane is pumped from tail-to-head to equilibrate the system that is to say to form a true stationary phase and a mobile phase. (V_{c,P} = V_{aq} mL of injected aqueous phase) will exit the column before the system is equilibrated. We define this time as the equilibration phase. Then any pentane that exits the column will have undergone intense mixing with the stationary phase and is therefore collected for analysis.
4.1. Synthetic procedures

Synthesis of 10: (2E,6E)-farnesyl diphosphate was synthesized from commercial (2E,6E)-farnesol using the method described by Davisson. Farnesal was synthesized as described by Masuda et al.. 12-OH farnesyl diphosphate was synthesized from commercial (2E,6E)-farnesol using the method of Demiray et al..

4.2. Preparation of recombinant enzymes

Recombinant aristolochene synthase from Penicillium roqueforti, amorphadiene synthase from Artemisia annua and (S)-germacrene D synthase from Solidago canadensis were expressed in E. coli BL21 (DE3)RP, E. coli BL21 (DE3), E. coli BL21 (DE3), respectively and purified as previously described. Protein concentrations were estimated by the Bradford method using commercial bovine serum albumin as the calibration standard and aliquots of enzyme were stored in 10% glycerol at –20 °C until further use.

4.3. Study of the stationary phase retention factor using water-pentane system

The HPCCC analytical column was first filled with the stationary phase (water containing fixed amount of glycerol). A specified centrifugal field was applied to the column; the mobile phase was pumped in normal phase at a specified flow rate, while the displaced stationary phase was collected at the exit. The measured volume of displaced stationary phase was used to calculate the stationary phase retention (Sf) using the following equation:

\[
S_f (\%) = \frac{V_c - (V_d - V_{ec})}{V_c} \times 100
\]

where \(V_c\) is the volume of the column, \(V_d\) is the measured displaced volume at the exit, \(V_{ec}\) is the volume resulting from the tubing linking the two bobbins, valves or tubing connecting the column to the pumps (for this system is was determined to 1 mL).

**Table S3:** Influence of glycerol content in the incubation buffer and the pentane flowrate on \(V_{ec}\).

| Pentane flowrate (mL-min\(^{-1}\)) | 0.5 | 1 | 2 |
|-----------------------------------|-----|---|---|
| 0% glycerol                       | 6   | 8 | 12|
| 10% glycerol                      | 6.5 | 9 | 13|
| 15% glycerol                      | 7   | 10| 14|
| 20% glycerol                      | 7.5 | 12| 16|
| 30% glycerol                      | 8   | 15| 18|

**Table S4:** Influence of rotation speed of the coil and the pentane flowrate on \(V_{ec}\).
5. Sesquiterpene synthesis

Same incubation buffers were used when comparing HPCCC, batch and flow segmented systems. Glycerol content was lowered to a maximum to obtain at least 50% S at a flowrate of 2 mL min⁻¹ for the analytical column unless otherwise stated.

Incubation buffer for aristolochene synthase: 20 mM Tris base, 5 mM 2-mercaptoethanol, 3 mM MgCl₂, pH = 7.5.

Incubation buffer for amorphadiene synthase: HEPES (25 mM), dithiothretol (1 mM), MgCl₂ (5 mM), pH = 7.5.

Incubation buffer for germacrene D synthase: 20 mM Tris, 10 mM MgCl₂, 5 mM 2-mercaptoethanol, 5% Glycerol pH 7.5.

Incubation buffer for amorphadiene synthase with 12-OH FDP: Glycine (25 mM), dithiothretol (1 mM), MgCl₂ (5 mM), pH 9.4.

5.1 Using the HPCCC

Method 1:

Aqueous and organic were pumped using the two HPLC pumps, temperature was regulated by a chiller to 28 ºC. The column was initially filled with pentane (HPLC grade, Sigma-Aldrich) at 6 mL min⁻¹ from tail periphery to head-center. The analytical column was then loaded through a loop with 10 mL AS incubation buffer (20 mM Tris base, 5 mM 2-mercaptoethanol, 10% glycerol, 3 mM MgCl₂, pH = 7.5) containing 6 μM AS and 0.35 mM FDP at a flow rate of 0.5 mL min⁻¹ resulting in 10 mL of pentane being displaced out of the column. The instrument was rotated at 1600 rpm and pentane was pumped at the same time from tail periphery to head-center at a flow rate of 0.5 mL min⁻¹. The pentane was collected in 5 mL fractions and analyzed by GC FID (method A). The yield was calculated by comparing peak areas of the product to a calibration curve using α-humulene.

\[
Conversion = \frac{Area_{product}}{1123.4} \times \frac{0.005}{0.0035} \times 100
\]

Table S5: Results from the three replicates of aristolochene synthase (6 μM) with FDP (0.35 mM) using method 1.

| Volume equivalent (mL) | Time (min) | Area product | Conversion (%) | Added yield (%) |
|------------------------|------------|--------------|----------------|-----------------|
| 5                      | 10         | 0            | 0              | 0.00 0.00 0.00  |
| 10                     | 20         | 0            | 0              | 0.00 0.00 0.00  |
| 15                     | 30         | 727          | 681 693.9      | 92 87 88 92 87 88 |
| 20                     | 40         | 30.6 30.1    | 29.1 4 4 4     | 96 90 92 96 90 92 |
| 25                     | 50         | 12.5 25.4    | 12.2 2 3 2     | 98 94 93 98 94 93 |
| 30                     | 60         | 10.4 23.0    | 10.1 1 3 1     | 99 97 95 99 97 95 |
| 35                     | 70         | 8.2 18 7.8   | 1 2 1          | 100 99 96 100 99 96 |

Table S6: Results from aristolochene synthase (6 μM) with double concentration of FDP (0.7 mM) using method 1.

| Time (min) | Area product | Conversion (%) | Added yield (%) |
|------------|--------------|----------------|-----------------|
| 10         | 0            | 0              | 0.00 0.00 0.00  |
| 20         | 0            | 0              | 0.00 0.00 0.00  |
| 30         | 1187.4       | 75             | 75              |
| 40         | 285.3        | 18             | 94              |
| 50         | 45.8         | 3              | 97              |
| 60         | 22.1         | 1              | 98              |
| 70         | 15.8         | 1              | 99              |
Method 2:
Aqueous and organic were pumped using the two HPLC pumps, temperature was regulated by a chiller to 28 °C. The column was initially filled with pentane (HPLC grade, Sigma-Aldrich) at 6 mL•min⁻¹ from tail periphery to head-centre. The analytical column was then loaded through a loop with 10 mL of incubation buffer containing a set concentration of protein and 0.35 mM FDP at a set flow rate resulting in 10 mL of pentane being displaced out of the column. The instrument was rotated at 1600 rpm and pentane was pumped at the same time from tail periphery to head-center at a set flow rate. The first 10 mL of pentane were discarded and then two fractions (respectively 20 mL and 10 mL) were collected and analyzed by GC FID (method A). The yield was calculated by comparing peak areas of the product to a calibration curve using α-humulene.

Table S7: Results from aristolochene synthase (6 µM) with FDP (0.35 mM) using different pentane flowrates (method 2).

| Flowrate (mL•min⁻¹) | Area product[a] | Conversion (%) |
|---------------------|-----------------|---------------|
| 0.5                 | 192.0 189.0 189.0 | 97            |
| 1                   | 204.5 194.3 194.8 | 101           |
| 2                   | 190.8 206.0 175   | 97            |

[a] Each injection was repeated three times on the GC FID

Table S8: Results from the three replicates of amorphadiene synthase with FDP using method 2.

| Replicate | Area product[a] | Area side products[a] | Yield (%)[b] | Conversion (%)[c] |
|-----------|-----------------|-----------------------|--------------|------------------|
| 1 (Fraction 1) | 170.3 169.4 168.1 | 21.1 23.9 20.9 | 89            | 100              |
| 1 (Fraction 2) | 10.7 10.7 10.7    | 0 0 0               | 88            | 97               |
| 2 (Fraction 1) | 167.8 147.6 167   | 19.9 17.5 19.9     | 83            | 92               |
| 2 (Fraction 2) | 21.5 21.9 26.7    | 0 0 0               | 83            | 92               |
| 3 (Fraction 1) | 158.3 144.8 151   | 18.7 17.2 17.2     | 83            | 92               |
| 3 (Fraction 2) | 21 23.5 23.2      | 0 0 0               | 83            | 92               |

[a] Each injection was repeated three times on the GC FID
[b] Calculated based on the average area of product in fraction 1 and 2
[c] Calculated based on the sum of the average area of product and side products in fraction 1 and 2

Table S9: Results from the three replicates of (S)-germacrene D synthase with FDP using method 2.

| Replicate | Area product[a] | Area side products[a] | Yield (%)[b] | Conversion (%)[c] |
|-----------|-----------------|-----------------------|--------------|------------------|
| 1 (Fraction 1) | 190.5 187.7 188.3 | 11.9 11.9 11.9 | 96            | 102              |
| 1 (Fraction 2) | 0 0 0            | 0 0 0                | 94            | 100              |
| 2 (Fraction 1) | 182.1 182.6 146.9 | 11.3 11.5 6.9      | 103           | 109              |
| 2 (Fraction 2) | 0 0 0            | 0 0 0                | 103           | 109              |
| 3 (Fraction 1) | 202.3 203.7 201.2 | 16.2 8.1 12.6      | 103           | 109              |
| 3 (Fraction 2) | 0 0 0            | 0 0 0                | 103           | 109              |

[a] Each injection was repeated three times on the GC FID
[b] Calculated based on the average area of product in fraction 1 and 2
[c] Calculated based on the sum of the average area of product and side products in fraction 1 and 2
Table S10: Results from the three replicates of amorphadiene synthase with 12-OH FDP using method 2.

| Replicate | Area product (R+S) [a] | Area products [b] | Yield (%) [b] | Conversion (%) [c] |
|-----------|------------------------|-------------------|--------------|-------------------|
| 1 (Fraction 1) | 168.8 | 125 | 169 | 44.7 | 29 | 45 | 72 | 90 |
| 1 (Fraction 2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 (Fraction 1) | 167.6 | 165.7 | 136 | 43 | 42.4 | 34.2 | 73 | 92 |
| 2 (Fraction 2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 (Fraction 1) | 124.4 | 148.6 | 124.8 | 36.9 | 38.5 | 32.3 | 62 | 79 |
| 3 (Fraction 2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[a] Each injection was repeated three times on the GC FID  
[b] Calculated based on the average area of product in fraction 1 and 2  
[c] Calculated based on the sum of the average area of product and side products in fraction 1 and 2

Method 3

Aqueous and organic were pumped using the two HPLC pumps; temperature was regulated by a chiller to 28 °C. The preparative column was initially filled with pentane (HPLC grade, Sigma-Aldrich) at 10 mL•min⁻¹ from tail periphery to head-center. The column was then loaded through a HPLC pump with 65 mL of incubation buffer containing a set concentration of protein (6 µM for AS) and 0.7 mM FDP at 5 mL•min⁻¹ resulting in 65 mL of pentane being displaced out of the column. The instrument was rotated at 1600 rpm and pentane was pumped at the same time from tail periphery to head-center at a set flow rate. The first 70 mL of pentane were discarded and then 70 mL of pentane (approximately 0.5 CV) were collected and analyzed by GC FID (method A). The yield was calculated by comparing peak areas of the product to a calibration curve using α-humulene.

Table S11: Results from the three replicates of aristolochene synthase with FDP on the preparative column using method 3.

| Volume equivalent (mL) | Time (min) | Area product | Added yield (%) |
|------------------------|------------|--------------|-----------------|
| 5                      | 1          | 2740.6       | 2584.7          | 2579.9          | 27±1          |
| 10                     | 2          | 3791.7       | 3815.6          | 3528.3          | 64±2          |
| 15                     | 3          | 1780.5       | 1765.1          | 1751.5          | 81±1          |
| 20                     | 4          | 885.6        | 748.5           | 681             | 89±1          |
| 25                     | 5          | 226.0        | 216.2           | 510.1           | 92±3          |
| 30                     | 6          | 68.8         | 71.9            | 174.5           | 93±3          |
| 35                     | 7          | 31.4         | 27.8            | 26              | 93±3          |
| 40                     | 8          | 19.4         | 18              | 16.3            | 93±3          |
| 45                     | 9          | 14.6         | 14.4            | 12.4            | 94±3          |
| 50                     | 10         | 12.6         | 9.7             | 10.1            | 94±3          |
| 55                     | 11         | 7.0          | 7.2             | 5.8             | 94±3          |
| 60                     | 12         | 7.0          | 6.7             | 4.6             | 94±3          |
| 65                     | 13         | 7.0          | 6.7             | 6.4             | 94±3          |
| 70                     | 14         | 6.0          | 6.2             | 4.8             | 94±3          |
Figure S5: Aristolochene synthase (6 µM) with FDP (0.7 mM) on the preparative column of the HPCCC (method 3).

Preparative scale with the HPCCC (isolated yields):
Aqueous and organic were pumped using the two HPLC pumps; temperature was regulated by a chiller to 28 °C. The preparative column was initially filled with pentane (HPLC grade, Sigma-Aldrich) at 10 mL/min⁻¹ from tail periphery to head-center. The column was then loaded through a HPLC pump with 65 mL of incubation buffer containing a set concentration of protein (6 µM for AS, 12 µM for GDS, 10 µM for ADS) and FDP (0.7 mM, 0.045 mmol) at 5 mL/min⁻¹ resulting in 65 mL of pentane being displaced out of the column. The instrument was rotated at 1600 rpm and pentane was pumped at the same time from tail periphery to head-center at a set flow rate. The first 70 mL of pentane were discarded and then 100 mL (20 min) of pentane were collected.

Preparative scale with the HPCCC (isolated yields):

Aqueous and organic were pumped using the two HPLC pumps; temperature was regulated by a chiller to 28 °C. The preparative column was initially filled with pentane (HPLC grade, Sigma-Aldrich) at 10 mL/min⁻¹ from tail periphery to head-center. The column was then loaded through a HPLC pump with 65 mL of incubation buffer containing a set concentration of protein (6 µM for AS, 12 µM for GDS, 10 µM for ADS) and FDP (0.7 mM, 0.045 mmol) at 5 mL/min⁻¹ resulting in 65 mL of pentane being displaced out of the column. The instrument was rotated at 1600 rpm and pentane was pumped at the same time from tail periphery to head-center at a set flow rate. The first 70 mL of pentane were discarded and then 100 mL (20 min) of pentane were collected. Experiment was repeated four times and organic phase was pooled together, dried (Na₂SO₄), filtered, solvent was removed by distillation then under reduced pressure to give a colorless compound.

(+)-Aristolochene (11)

FD P (10) was incubated with AS as described above to give aristolochene as a colorless oil (35 mg, 94%).

\[ \text{H NMR (500 MHz, CDCl}_3\): } \delta = 5.32 (dt, J = 5.5, 1.9 Hz, 1H), 4.74 – 4.69 (m, 2H), 2.27 – 2.09 (m, 2H), 2.06 – 1.96 (m, 2H), 1.93 – 1.82 (m, 1H), 1.77 (ddd, J = 8.5, 5.4, 3.1 Hz, 1H), 1.75 – 1.74 (m, 3H), 1.74 – 1.67 (m, 1H), 1.48 – 1.21 (m, 5H), 1.19 (dt, J = 18.6, 6.9 Hz, 1H), 0.97 (d, J = 0.6 Hz, 3H), 0.95 (d, J = 6.7 Hz, 3H) ppm.

\[ \text{C NMR (126 MHz, CDCl}_3\): } \delta = 150.6 (C=CH), 144.5 (C=CH), 118.8 (C=CH), 108.3 (C=CH), 44.2 (CH₂CH₂CH₂CH₂), 43.3 (CHCH₂), 38.8 (CHCCH₂), 37.8 (CH₂=CHCH₃), 32.6 (CH₂CH₂=CH), 31.3 (CH₂=CHC), 31.1 (CH₂CH₃), 27.8 (CH₃CH₂CH₂CH₂), 20.9 (CH₂=CHCH₃), 18.1 (CH₃C), 15.7 (CH₃CH₃) ppm.

(S)-Germacrene D (12)

FD P (10) was incubated with GDS as described above to give (S)-germacrene D as a colorless oil (34 mg, 92%).

\[ \text{H NMR (500 MHz, CDCl}_3\): } \delta = 5.71 (d, J = 15.9 Hz, 1H, H₂C=CH=CH), 5.18 (dd, J = 15.8, 9.9 Hz, 1H, H₂C=CH=CH), 5.06 (dd, J = 11.0, 4.8 Hz, 1H, CH₂=CH=CH), 4.72 (dd, J = 2.3, 0.8 Hz, 1H, C=CH₂), 4.67 (d, J = 2.3 Hz, 1H, C=CH₂), 2.30 – 2.21 (m, 10H, CH₂C=CH₂=CHCH₂CH₂, (CH₂)₂CHCHCH₂), 0.86 – 0.78 (m, 6H, (CH₃)₂CH) ppm.

\[ \text{C NMR (126 MHz, CDCl}_3\): } \delta = 149.0 (C=CH₂), 135.6 (CH=CHC=CH₂), 134.1 (CH₂=CH₂), 133.7 (CH=CHC=CH₂), 129.8 (CH₂=CH₂), 109.2 (CH₂=CH₂), 53.1 (CHCCHCH=CH), 40.9 (CH₂CH₂C=CH), 34.5 (CH₂CH₂=CH₂), 32.1 (CHCHCH=CH), 29.6 (C=CH₂CH₂CH₂), 25.0 (CH₂CH₂=CHC), 22.9 (CH₂=CHCH), 19.5 (CH₂CH₂CH), 16.1 (CH₃CH₃) ppm.

Amorpha-4,11-diene (13)

FD P (10) was incubated with ADS as described above and purified on silver (5%) impregnated silica (hexane:EtOAc 95:5) to give amorpha-4,11-diene as a colorless oil (31 mg, 84%).

\[ \text{H NMR (500 MHz, CDCl}_3\): } \delta = 5.07 (dd, J = 8.8, 1.4 Hz, 1H, CH=CH₂), 4.90 – 4.85 (m, 1H, C=CH₂), 4.64 (s, 1H, C=CH₂), 2.64 – 2.51 (m, 1H, CH₂CCHCH₂), 2.01 – 0.92 (m, 11H, (CH₂)₂CHCH(CH₂)₂CH), 1.74 (dd, J = 3.6, 3.0 Hz, 3H, CH₂=CH₃), 1.60 (s, 3H, CH=CH₂), 0.88 (t, J = 5.6 Hz, 3H, CH₃CH₂CH₂) ppm.

\[ \text{C NMR (126 MHz, CDCl}_3\): } \delta = 148.2 (CH₂=CH₂), 134.8 (CH₂=CH₂), 120.7 (CH₂=CH₂), 109.9 (CH₂=CH₂), 47.8 (CH₂=CH₂), 42.0 (CH₂CH₂CH₂CH₂), 37.8 (C=CHCH), 35.6 (CH₂CH₂CH₂), 28.0 (CH=CH₂CH₂), 26.6 (CH₂CH₂), 26.2 (CH₃CH₂), 26.0 (CH₂CH₂CH₂CH₂), 23.8 (CH₂=CH₂), 22.7 (CH₂=CH₂), 20.0 (CH₂CH₂CH₂) ppm.

Dihydroartemisinic aldehyde (14) (mixture of 11-R and 11-S epimers)
12-OH FDP was incubated with ADS as described above. The crude oil was purified by flash chromatography on silica gel (Gradient 0 to 25% dichloromethane in pentane) to obtain compound 15 as colorless oil (30 mg, 70%).

\(^{1}\)H NMR (500 MHz, CDCl\(_3\)): \(\delta = 9.63\) (d, \(J = 4.0\) Hz, 1H, CHO, (11S)), 9.58 (d, \(J = 3.9\) Hz, 1H, CHO, (11R)), 5.27 (d, \(J = 1.1\) Hz, 1H, CH\(_2\)=CH=CH, (11R)), 5.13 (s, 1H, CH\(_3\)=CH=CH, (11R)), 2.52 – 2.44 (m, 1H, 2 x C=CHCH\(_2\), (11R) and (11S)), 2.42 – 2.37 (m, 1H, 2 x OCH\(_2\)CH=CHCH\(_2\), (11S) and (11R)), 1.96 – 1.19 (m, 20H, 2 x (CH\(_3\)=CH)\(_2\)CH=CHCH\(_2\), (11R)) and (11S)), 1.73 (dd, \(J = 8.4, 3.6\) Hz, 6H, 2 x CCH\(_3\)), 1.05 (d, \(J = 6.8\) Hz, 3H, OCHCH\(_3\)), (11R)), 1.05 (d, \(J = 2.2\) Hz, 3H, OCHCH\(_3\)), (11S)), 0.99 – 0.95 (m, 1H, CH\(_2\)CHCH\(_3\)), 0.87 (d, \(J = 6.5\) Hz, 6H, 2 x CHCH\(_3\)), (11R) and (11S)) ppm.

\(^{13}\)C NMR (126 MHz, CDCl\(_3\)): \(\delta = 206.2\) (CHO, (11R)), 206.0 (CHO, (11S)), 136.2 (CH=CHH, (11R)), 136.1 (CH=CHH, (11S), 119.7 (CH=CHH, (11S)), 119.7 (CH=CHH, (11R)), 48.6 (OCH=CH, (11R)), 48.0 (OCH=CH, (11S)), 43.6 (CH\(_3\)=CHCH=CH, (11S)), 42.0 (CH\(_3\)=CHCH=CH, (11R)), 41.95 (OCH=CHCH, (11S)), 41.6 (OCH=CHCH, (11R)), 39.3 (C=CHCH, (11S)), 36.7 (C=CHCH, (11R)), 35.6 (CH\(_3\)=CHCH=CH, (11S)), 35.4 (CH\(_3\)=CHCH=CH, (11R)), 27.9 (CH\(_3\)=CHCH=CH, (11S)), 27.8 (CH\(_3\)=CHCH=CH, (11R)), 27.5 (CH\(_3\)=CHCH=CH, (11R)), 26.7 (CH\(_3\)=CHCH=CH, (11S)), 26.6 (CH\(_3\)=CHCH=CH, (11S)), 25.9 (CH\(_3\)=CHCH=CH, (11R)), 25.7 (CH\(_3\)=CHCH=CH, (11S)), 25.3 (CH\(_3\)=CHCH=CH, (11S)), 24.0 (CH\(_3\)=CHCH=CH, (11R)), 20.0 (CH\(_3\)=CHCH=CH, (11S)), 19.8 (CH\(_3\)=CHCH=CH, (11R)), 12.7 (CH\(_3\)=CHCH=CH, (11S)), 11.9 (CH\(_3\)=CHCH=CH, (11R)) ppm.

5.2. Using flow segmented systems

General procedure for biocatalysis in segmented flow:

The flow reactor (2 mL, 0.5 mm ID tubing) was constructed from PTFE tubing (Diba, Kinesis Ltd). The two liquid solutions (aqueous and organic) were loaded in glass syringe and injected to the flow reactor through a T-piece by using two syringe pumps (Fusion 100 Touch infusion syringe pump, KR Analytical Ltd), and the reaction mixture was collected in a glass vial at exit. For all experiments, the aqueous solution (5 mL) was prepared by dissolving DFP (0.35 mM, 0.175 μmol) and enzyme in the incubation buffer. The organic solvent is pentane with α-humulene (35 μM) or farnesal (70 μM) as internal standard (IS). The reaction mixture was collected after reaching steady state (third reactor volume), the organic layer was directly analyzed by GC FID (method A).

The yield was calculated by comparing peak areas of product and internal standard.

Table S12: Results from the three replicates of aristolochene synthase with FDP by flow (ratio pentane:aqueous = 1, t = 90 min).

| Replicate | Area product[a] | Area products[a] | Area internal | Yield (%)[b] | Conversion (%)[c] |
|-----------|-----------------|-----------------|--------------|-------------|-----------------|
| 1         | 390.6           | 366.3           | 447          | 47.6        | 100             |
| 2         | 382.7           | 332.7           | 371          | 39.9        | 95              |
| 3         | 341.3           | 338             | 298.4        | 32.1        | 95              |

[a] Each injection was repeated three times on the GC FID
[b] Calculated based on the average area of product
[c] Calculated based on the sum of the average area of product and side products

Table S13: Results from the three replicates of amorphaadiene synthase with FDP by flow (ratio pentane:aqueous = 1, t = 70 min).

| Replicate | Area product[a] | Area products[a] | Area internal | Yield (%)[b] | Conversion (%)[c] |
|-----------|-----------------|-----------------|--------------|-------------|-----------------|
| 1         | 205.6           | 164.1           | 172.8        | 27.8        | 75              |
| 2         | 185.7           | 180.6           | 233.9        | 37.5        | 72              |
| 3         | 183.8           | 183.5           | 171.1        | 29.6        | 69              |

[a] Each injection was repeated three times on the GC FID
[b] Calculated based on the average area of product
[c] Calculated based on the sum of the average area of product and side products

Table S14: Results from the three replicates of (S)-germacrene D synthase with FDP by flow (ratio pentane:aqueous = 1, t = 100 min).

| Replicate | Area product[a] | Area products[a] | Area internal | Yield (%)[b] | Conversion (%)[c] |
|-----------|-----------------|-----------------|--------------|-------------|-----------------|
| 1         | 186.5           | 264.8           | 252.7        | 38.7        | 67              |
| 2         | 227.5           | 262.4           | 227.5        | 35.2        | 67              |
| 3         | 383.8           | 422.44          | 339.1        | 64.8        | 78              |

[a] Each injection was repeated three times on the GC FID
[b] Calculated based on the average area of product
[c] Calculated based on the sum of the average area of product and side products
Table S15: Results from the three replicates of amorphadiene synthase with 12-OH FDP by flow (ratio pentane:aqueous = 0.5, t = 90 min).

| Replicate | Area product[a] | Area side products[a] | Area internal standard[a] | Yield [%][b] | Conversion [%][c] |
|-----------|-----------------|-----------------------|--------------------------|--------------|------------------|
| 1         | 729.9           | 715.7                 | 642.2                    | 187.6        | 184.6            |
|           |                 |                       |                          | 167.3        | 134.4            |
|           |                 |                       |                          | 132          | 119.1            |
| 2         | 507.6           | 576.8                 | 527.8                    | 131.7        | 124.5            |
|           |                 |                       |                          | 135.4        | 93.2             |
|           |                 |                       |                          | 89.3         | 97               |
| 3         | 427.3           | 486.5                 | 688.5                    | 109.3        | 75.9             |
|           |                 |                       |                          | 75.9         | 178.7            |
|           |                 |                       |                          | 77.7         | 89               |
|           |                 |                       |                          | 126.1        | 55               |

[a] Each injection was repeated three times on the GC FID
[b] Calculated based on the average area of product
[c] Calculated based on the sum of the average area of product and side products

5.3. Using traditional batch reactor

General procedure for biocatalysis in batch:

The enzyme was added to the incubation buffer to a set concentration at room temperature with gentle stirring then FDP or 12-OH FDP (0.35 mM, 0.35 mol) was added and the incubation (Vtot = 10 mL) was overlaid with pentane (5 mL) and stirred at room temperature for 36 h. The pentane overlay was removed and the aqueous layer was further extracted with pentane (2 x 5 mL) by gentle swirling and slow separation. The combined organic extracts were pooled together in a 20 mL volumetric flask (grade A+) and volume was adjusted with fresh pentane to a total volume of 20 mL. The solution was analyzed by GC FID (method A). The yield was calculated by comparing peak areas of the product to a calibration curve using α-humulene.

Table S16: Results from the three replicates of aristolochene synthase with FDP by batch.

| Replicate | Area product[a] | Area side products[a] | Yield [%][b] | Conversion [%][c] |
|-----------|-----------------|-----------------------|--------------|------------------|
| 1         | 71.6            | 63.5                  | 62.4         | 0                |
|           |                 |                       | 0            | 0                |
|           |                 |                       | 3            | 33               |
| 2         | 62.5            | 59.8                  | 69.4         | 0                |
|           |                 |                       | 0            | 0                |
|           |                 |                       | 3            | 33               |
| 3         | 50.6            | 52.4                  | 67           | 0                |
|           |                 |                       | 0            | 0                |
|           |                 |                       | 2            | 29               |

[a] Each injection was repeated three times on the GC FID
[b] Calculated based on the average area of product
[c] Calculated based on the sum of the average area of product and side products

Table S17: Results from the three replicates of amorphadiene synthase with FDP by batch.

| Replicate | Area product[a] | Area side products[a] | Yield [%][b] | Conversion [%][c] |
|-----------|-----------------|-----------------------|--------------|------------------|
| 1         | 108.4           | 90.3                  | 119.4        | 4.4              |
|           |                 |                       | 18.2         | 41               |
|           |                 |                       | 54           | 74               |
| 2         | 93.4            | 96.4                  | 84           | 47.1             |
|           |                 |                       | 48.2         | 68.1             |
|           |                 |                       | 46           | 65               |
| 3         | 67.3            | 56.3                  | 57.6         | 3.3              |
|           |                 |                       | 0            | 2.8              |
|           |                 |                       | 31           | 32               |

[a] Each injection was repeated three times on the GC FID
[b] Calculated based on the average area of product
[c] Calculated based on the sum of the average area of product and side products

Table S18: Results from the three replicates of (S)-germacrene D synthase with FDP by batch.

| Replicate | Area product[a] | Area side products[a] | Yield [%][b] | Conversion [%][c] |
|-----------|-----------------|-----------------------|--------------|------------------|
| 1         | 54.5            | 55.3                  | 51.0         | 0                |
|           |                 |                       | 0            | 0                |
|           |                 |                       | 27           | 27               |
| 2         | 48.7            | 44.7                  | 47.3         | 0                |
|           |                 |                       | 0            | 0                |
|           |                 |                       | 24           | 24               |
| 3         | 56.9            | 57.0                  | 60.2         | 0                |
|           |                 |                       | 0            | 0                |
|           |                 |                       | 30           | 30               |

[a] Each injection was repeated three times on the GC FID
[b] Calculated based on the average area of product
[c] Calculated based on the sum of the average area of product and side products

Table S19: Results from the three replicates of amorphadiene synthase with 12-OH FDP by batch.

| Replicate | Area product[a] | Area side products[a] | Yield [%][b] | Conversion [%][c] |
|-----------|-----------------|-----------------------|--------------|------------------|
| 1         | 40.9            | 44.4                  | 45.2         | 5.9              |
|           |                 |                       | 10.7         | 6.6              |
|           |                 |                       | 20           | 24               |
| 2         | 46.4            | 49.1                  | 50.6         | 6.7              |
|           |                 |                       | 12.0         | 12.2             |
|           |                 |                       | 23           | 28               |
6. Transesterification of alcohols by CalB lipase

The lipase activity was determined spectrophotometrically by following the hydrolysis of p-nitrophenyl butyrate (pNPB) at 400 nm. The initial lipase activity was 10514 U/mL (45 U/mg). One unit of lipase activity (U) is defined as the amount of lipase required to release 1 nmol of pNPB per minute at pH 7.2 at 37 °C using p-nitrophenylbutyrate as substrate. Under optimised conditions, a concentration of 1 mg/mL of enzyme was used equivalent to 450 U.

6.1. Octanol transesterification in batch

A solution of octanol (30 mM) and vinyl acetate (x mM depending on the ratio alcohol: vinyl acetate) in heptane was added to a solution of Cal B lipase (1 mg/mL) in aqueous buffer (20 mM phosphate buffer, pH 7.2). The solution was stirred at 400 rpm at 25°C and organic layer was sampled at regular interval of time to determine the conversion of octanol to octyl acetate. The solution was analyzed by GC FID (method B). The conversion was calculated by comparing peak areas of the product to a calibration curve using octyl acetate.

![Figure S6: Influence of the ratio alcohol:vinylacetate on the conversion of octanol to octyl acetate by Cal B lipase in batch.](image)

6.2. Octanol transesterification using the HPCCC

General procedure: Aqueous and organic were pumped using the two HPLC pumps, temperature was regulated by a chiller. The analytical column was initially filled with the incubation buffer (20 mM phosphate buffer, pH 7.2) containing CalB lipase at a set concentration at 6 mL•min⁻¹ from tail periphery to head-centre. The bobbin was then rotated at 1200 rpm and a solution of known concentration of octanol and vinyl acetate in heptane (HPLC grade, Sigma-Aldrich) was pumped through the column at 2 mL•min⁻¹. After 1 CV, the rotation speed and the flowrate were readjusted to the desired reaction condition. The solution at the exit of the HPCCC was then collected after 3 CV and analyzed by GC-FID (method B). The conversion was calculated by comparing peak areas of the product to a calibration curve using octanol.

Octyl acetate (7)

\[
\text{H NMR (500 MHz, CDCl}_3\text{): } \delta = 4.05 \text{ (t, J = 6.8 Hz, 2H, CH}_2\text{O)}, \ 2.04 \text{ (s, 3H, COOC}_3\text{H}_3\text{)}, \ 1.70 - 1.53 \text{ (m, 2H, CH}_2\text{)}, \ 1.40 - 1.20 \text{ (m, 10H, 5 x CH}_2\text{), 0.88 \text{ (t, J = 6.8 Hz, 3H, CH}_3\text{CH}_2\text{)}} \text{ ppm.}
\]
\( ^{13} \text{C NMR (126 MHz, CDCl}_3) \): \( \delta = 171.4 \) (CO), 64.8 (CHOCO), 31.9 (CH\(_2\)CHO), 29.4 (CH\(_2\)), 29.3 (CH\(_3\)), 28.8 (CH\(_2\)), 26.1 (CH\(_2\)), 22.8 (CH\(_3\)), 21.2 (CH\(_3\)CO), 14.2 (CH\(_3\)CH\(_2\)) ppm.

**Table S20:** Face centered DOE results with all parameters constant except temperature, rotation speed and residence time. [Cal B (1 mg·mL\(^{-1}\)), octanol (100 mM), vinyl acetate (100 mM)].

| Temperature | Rotation speed (rpm) | Residence time (min) | Replicate | Conversion (%) | Conversion mean (%) | Standard deviation |
|-------------|----------------------|----------------------|-----------|----------------|---------------------|--------------------|
| 17 °C       | 1200                 | 7,5                  | 1         | 57%            |                     |                    |
|             |                      |                      | 2         | 58%            | 58%                 | 2%                 |
|             |                      |                      | 3         | 60%            |                     |                    |
| 17 °C       | 1200                 | 30                   | 1         | 59%            |                     |                    |
|             |                      |                      | 2         | 59%            | 59%                 | 0%                 |
|             |                      |                      | 3         | 58%            |                     |                    |
| 37 °C       | 1200                 | 7,5                  | 1         | 53%            |                     |                    |
|             |                      |                      | 2         | 54%            | 53%                 | 0%                 |
|             |                      |                      | 3         | 53%            |                     |                    |
| 37 °C       | 1200                 | 30                   | 1         | 54%            |                     |                    |
|             |                      |                      | 2         | 53%            | 54%                 | 1%                 |
|             |                      |                      | 3         | 55%            |                     |                    |
| 27 °C       | 1200                 | 15                   | 1         | 56%            |                     |                    |
|             |                      |                      | 2         | 57%            | 56%                 | 1%                 |
|             |                      |                      | 3         | 56%            |                     |                    |
| 17 °C       | 1400                 | 15                   | 1         | 65%            |                     |                    |
|             |                      |                      | 2         | 68%            | 67%                 | 1%                 |
|             |                      |                      | 3         | 67%            |                     |                    |
| 27 °C       | 1400                 | 7,5                  | 1         | 66%            |                     |                    |
|             |                      |                      | 2         | 69%            | 67%                 | 1%                 |
|             |                      |                      | 3         | 66%            |                     |                    |
| 27 °C       | 1400                 | 30                   | 1         | 54%            |                     |                    |
|             |                      |                      | 2         | 51%            | 52%                 | 2%                 |
|             |                      |                      | 3         | 51%            |                     |                    |
| 37 °C       | 1400                 | 15                   | 1         | 54%            |                     |                    |
|             |                      |                      | 2         | 62%            | 60%                 | 4%                 |
|             |                      |                      | 3         | 62%            |                     |                    |
| 17 °C       | 1600                 | 7,5                  | 1         | 71%            |                     |                    |
|             |                      |                      | 2         | 70%            | 71%                 | 0%                 |
|             |                      |                      | 3         | 71%            |                     |                    |
| 27 °C       | 1600                 | 15                   | 1         | 69%            |                     |                    |
|             |                      |                      | 2         | 68%            | 69%                 | 1%                 |

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| Temperature °C | Reaction Time  | Conversion % | Run |  |  |  |
|---------------|----------------|--------------|-----|-----|-----|-----|
| 37            | 1600           | 69           | 3   | 69% | 3   | 69% |
| 17            | 1600           | 59           | 2   | 56% | 2   | 56% |
| 37            | 7,5            | 59           | 3   | 58% | 3   | 58% |
| 37            | 1400           | 65           | 1   | 65% | 1   | 65% |
| 27            | 1400           | 63           | 2   | 63% | 2   | 63% |
| 27            | 15             | 67           | 3   | 67% | 3   | 67% |

**Figure S7:** DOE analysis of the influence of the temperature using Design-Expert® software.
Figure S8: DOE analysis of the influence of the rotation speed using Design-Expert® software.

Figure S9: DOE analysis of the influence of the residence time using Design-Expert® software.

Table S21: Influence of various parameters on the conversion of octanol to octyl acetate by CalB using the HPCCC. All experiments were set up with 12 mL stationary phase.
| Ratio (alcohol:acetate) | Octanol (mM) | Temperature (°C) | Rotation speed (rpm) | Residence time (min) | Enzyme (mg/mL) | Conversion (%) |
|------------------------|--------------|------------------|----------------------|----------------------|----------------|----------------|
| 1:1                    | 30           | 25               | 1600                 | 6                    | 1              | 34%            |
| 1:3                    | 30           | 25               | 1600                 | 6                    | 1              | 73%            |
| 1:6                    | 30           | 25               | 1600                 | 6                    | 1              | 98%            |
| 1:9                    | 30           | 25               | 1600                 | 24                   | 1              | 98%            |
| 1:1                    | 30           | 25               | 1600                 | 12                   | 1              | 32%            |
| 1:1                    | 30           | 20               | 1600                 | 6                    | 1              | 42%            |
| 1:1                    | 30           | 30               | 1600                 | 6                    | 1              | 34%            |
| 1:1                    | 30           | 25               | 1600                 | 6                    | 2              | 30%            |
| 1:1                    | 30           | 25               | 1600                 | 6                    | 3              | 35%            |
| 1:1                    | 30           | 25               | 1600                 | 6                    | 0.5            | 50%            |
| 1:1                    | 30           | 25               | 1600                 | 6                    | 0.25           | 24%            |
| 1:1                    | 30           | 25               | 1600                 | 6                    | 0.5            | 36%            |
| 1:6                    | 50           | 25               | 1600                 | 6                    | 1              | 96%            |
| 1:6                    | 100          | 25               | 1600                 | 6                    | 1              | 94%            |
| 1:6                    | 500          | 25               | 1600                 | 6                    | 1              | 42%            |
| 1:1                    | 100          | 25               | 1600                 | 6                    | 1              | 60%            |
| 1:3                    | 100          | 25               | 1600                 | 6                    | 1              | 94%            |
| 1:1                    | 200          | 25               | 1600                 | 6                    | 1              | 47%            |
| 1:1                    | 200          | 25               | 1600                 | 24                   | 1              | 56%            |
| 1:1                    | 100          | 25               | 1600                 | 24                   | 1              | 49%            |
| 1:1                    | 50           | 25               | 1600                 | 6                    | 1              | 49%            |
| 1:1                    | 150          | 25°C             | 1600                 | 6                    | 1              | 54%            |
| 1:1                    | 300          | 25°C             | 1600                 | 6                    | 1              | 42%            |

6.3 Recycling efficiency of Cal B

General procedure: Aqueous and organic were pumped using the two HPLC pumps, temperature was regulated by a chiller. The analytical column was initially filled with the incubation buffer (20 mM phosphate buffer, pH 7.2) containing Cal B lipase (1 mg·mL⁻¹) at 6 mL·min⁻¹ from tail periphery to head-centre. The bobbin was then rotated at 1200 rpm and a solution of known concentration of octanol and vinyl acetate in heptane (HPLC grade, Sigma-Aldrich) was pumped through the column at 2 mL·min⁻¹. After 1 CV, the rotation speed was readjusted to 1600 rpm. The solution at the exit of the HPCCC was then collected at regular intervals and analyzed by GC-FID (method B). The yield was calculated by comparing peak areas of the product to a calibration curve using octyl acetate.
Figure S10: Recycling efficiency of CalB for the transesterification of octanol with vinyl acetate in HPCCC (Octanol 100 mM (1:3 ratio, alcohol:acetate), CalB (1 mg•mL$^{-1}$), 20°C, 1600 rpm, 6 min residence time).

6.4. Stereoselective transesterification of rac-2-pentanol by HPCCC

Aqueous and organic were pumped using the two HPLC pumps, temperature was regulated by a chiller. The analytical column was initially filled with the incubation buffer (20 mM phosphate buffer, pH 7.2) containing CalB lipase (1 mg•mL$^{-1}$) at a set concentration at 6 mL•min$^{-1}$ from tail periphery to head-centre. The bobbin was then rotated at 1600 rpm and a solution of rac-2-pentanol (100 mM) and vinyl acetate (300 mM) in pentane (HPLC grade, Sigma-Aldrich) was pumped through the column at 2 mL•min$^{-1}$. The solution at the exit of the HPCCC was then collected after 3 CV and solvent was removed under reduced pressure. The crude product was purified using flash chromatography (5% EtOAc in hexanes) to give (R)-2-pentylacetate as a colorless liquid (49%, >99% e.e.). The enantiomeric excess was determined by GC-FID (method C) using synthesized rac-2-pentylacetate from the following procedure: Pentanol (1 mL, 9.2 mmol) was added to a solution of 4-dimethylaminopyridine (2.2 g, 18.4 mmol) and glacial acetic acid (1 mL, 18.4 mmol) in dichloromethane (50 mL). Dicyclohexyl carbodiimide (3.8 g, 18.4 mmol) was subsequently added and the solution was stirred overnight at room temperature. The precipitated solids were filtered off, the filtrate was concentrated in vacuo and purified by silica flash chromatography (ethyl acetate/pentane 1:4) to yield a colorless liquid (0.85 g, 6.5 mmol, 70%).

rac-Pentan-2-yl acetate

$^1$H NMR (500 MHz, CDCl$_3$): δ = 4.96 – 4.85 (m, 1H, CHOCO), 2.02 (s, 3H,CH$_3$CO), 1.63 – 1.27 (m, 4H, 2 x CH$_2$), 1.20 (d, J = 6.3 Hz, 3H, CH$_3$CH), 0.91 (t, J = 7.3 Hz, 3H, CH$_2$CH$_2$) ppm.

$^{13}$C NMR (126 MHz, CDCl$_3$): δ = 171.2 (CO), 71.2 (CHOCHO), 38.5 (CH$_2$CHO), 21.8 (COCH$_3$), 20.4 (CHCH$_3$), 19.0 (CH$_3$CH$_2$), 14.3 (CH$_3$CH$_2$CH$_2$) ppm.
7. GC-MS and calibrations curves

Figure S11: Calibration curve for flame ionization detection response of α-humulene.

Figure S12: Calibration curve for flame ionization detection response of farnesal.
Figure S13: Example of a calibration curve for flame ionization detection response of octanol.

Data File: C:\CHEM32\DATA\FH\FHFC_182 2018-12-19 14-43-09\102F0501.D
Sample Name: FH_182_run2

Area Percent Report

Signal 1: FID1 A, Front Signal

### Peak RetTime Type Width Area Height Area

| #  | (min) | (min) | (pA*s) | (pA) | %     |
|----|-------|-------|--------|------|-------|
| 1  | 1.312 | BB    | 0.0532 | 6.39093e6 | 1.45566e6 | 99.99543 |
| 2  | 1.760 | BB    | 0.0936 | 63.88154 | 11.37526 | 0.00100 |
| 3  | 2.285 | BB    | 0.0599 | 35.20429 | 10.03268 | 0.00055 |
| 4  | 12.058| BB    | 0.0332 | 193.11363 | 89.56389 | 0.00302 |

Totals: 6.39122e6 1.45577e6

*** End of Report ***
Figure S15: Flame ionization detection response of ADS with FDP by HPCCC using method 2.
Figure S16: Flame ionization detection response of GDS with FDP by HPCCC using method 2.
Figure S17: Flame ionization detection response of ADS with 12-OH FDP by HPCCC using method 2. (11-R and 11-S epimer at 15.571 min and 15.567 min).
Figure S18: Total ion chromatogram of authentic sample of FDP with aristolochene synthase by HPCCC on the preparative scale method. Inset: mass spectrum of peak at 12.68 min.
Figure S19: Total ion chromatogram of authentic sample of FDP with amorphadiene synthase by HPCCC on the preparative scale method. Inset: mass spectrum of peak at 12.53 min.
Figure S20: Total ion chromatogram of authentic sample of FDP with germacrene D synthase by HPCCC on the preparative scale method. Inset: mass spectrum of peak at 12.63 min.
Figure S21: Total ion chromatogram of authentic sample of 12-OH FDP with amorphadiene synthase by HPCCC on the preparative scale method.
**Figure S22:** Mass spectrum of peak at 15.12 min of total ion chromatogram of authentic sample of 12-OH FDP with amorphadiene synthase by HPCCC on the preparative scale method.

**Figure S23:** Mass spectrum of peak at 15.10 min of total ion chromatogram of authentic sample of 12-OH FDP with amorphadiene synthase by HPCCC on the preparative scale method.
Figure S24: Flame ionization detection response of octanol.
Figure S25: Flame ionization detection response of octyl acetate.
Figure S26: Example of a flame ionization detection response of the enzymatic transesterification of octanol with vinyl acetate by Cal B lipase (octanol at 7.744 min and octyl acetate at 8.812 min).
Figure S27: Flame ionization detection response of chemically synthesised racemic mixture of 2-pentyl acetate.
Figure S28: Flame ionization detection response of the enzymatic transesterification of rac-2-pentanol with vinyl acetate by Cal B lipase [(R)-2-pentylacetate at 4.131 min].
8. Spectroscopic data

(R)-tert-Butyl 2-[(diphenylmethylene)amino]-3-phenylpropanoate (2)

$^1$H NMR

$^{13}$C NMR
(R)-tert-Butyl 2-[(diphenylmethylene)amino]-3-(4-(trifluoromethyl)phenyl)propanoate (4a)

$^1$H NMR:

$^13$C NMR:
Methyl-(R)-4-(3-(tert-butoxy)-2-((diphenylmethylene)amino)-3-oxopropyl)benzoate (4b)

$^1$H NMR:

$^{13}$C NMR:
**tert-Butyl-(R)-2-((diphenylmethylene)amino)-3-(4-nitrophenyl)propanoate (4c)**

**$^1$H NMR:**

![Proton NMR spectrum](image)

**$^{13}$C NMR:**

![Carbon NMR spectrum](image)

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**tert-Butyl-(R)-3-([1,1'-biphenyl]-2-yl)-2-((diphenylmethylen)amino)propanoate (4d)**

**1H NMR:**

![1H NMR spectrum](image1)

**13C NMR:**

![13C NMR spectrum](image2)
HRMS:

IR:
tert-Butyl-(R)-2-((diphenylmethylene)amino)-3-(2-iodophenyl)propanoate (4e)

$^1$H NMR:

$^13$C NMR:
**tert-Butyl-(R)-2-(((diphenylmethylene)amino)-3-(naphthalen-2-yl)propanoate (4f)**

$^1$H NMR:

![Proton NMR spectrum](image1)

$^{13}$C NMR:

![Carbon NMR spectrum](image2)
**tert-Butyl-(R)-2-((diphenylmethylene)amino)-5-methylhex-4-enoate (4g)**

$^1$H NMR:

![Proton NMR spectrum](image)

$^{13}$C NMR:

![Carbon NMR spectrum](image)
tert-Butyl-(R)-2-((diphenylmethylene)amino)pent-4-ynoate (4h)

$^1$H NMR:

$^{13}$C NMR:
tart-Butyl-(R)-2-((diphenylmethylene)amino)hex-4-ynoate (4i)

$^1$H NMR:

$^{13}$C NMR:
(2R, 5R, 1'S)-1-(9-Anthracenyl)methyl-5-ethylene-2-[1-hydroxy-1-(quinol-4-yl)]methyl-1-azoniabicyclo[2.2.2]octane chloride

$^1$H NMR:

$^{13}$C NMR:
(2R, 5R, 1'S)-1-(1-Anthracenyl)methyl-5-ethylene-2-[1-benzyloxy-1-(quinol-4-yl)]methyl-1-azoniabicyclo[2.2.2]octane bromide (3b)

$^1$H NMR:

$^{13}$C NMR:
(±)-Aristolochene (11)

$^1$H NMR (400 MHz, CDCl$_3$):
Germacrene D (12)

$^1$H NMR (500 MHz, CDCl$_3$):
Amorpha-4,11-diene (13)

\(^1\)H NMR (400 MHz, CDCl\(_3\)):
Dihydroartemisinic aldehyde (14)

$^1$H NMR (500 MHz, CDCl$_3$):
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