Electronic Supplementary Information

Biomimetic epoxidation in aqueous media catalyzed by cyclic dipeptides

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General information

Oxime resin, coupling reagents and *N*-Boc-protected amino acids were purchased from Matrix Innovation (Québec City, QC, Canada). Resin with a substitution level of 1.12 mmol/g of oxime group was used. All chemicals were purchased from commercial sources and used directly, unless indicated otherwise. Nuclear magnetic resonance (NMR) spectra were recorded using Varian Inova 400 MHz spectrometer. Chemical shifts are reported in parts per million downfield from TMS. Splitting patterns are designated as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), br (broad singlet) and m (multiplet). Mass spectra were obtained on an Agilent 6210 LC Time of Flight Mass Spectrometer in direct injection mode. HPLC data were recorded on an Agilent 1260 Infinity instrument with a C18 column (GraceVydac) and a DAD detector. Retention time is in minute followed by the percentage integration of the total chromatogram. All solvents were degassed and gradient of A (99.9% H₂O /0.1% TFA) B (69.95% CH₃CN/29.95% H₂O/0.1% TFA) in 45 min was used. Optical rotations were measured at ambient temperature on a Jasco DIP-360 digital polarimeter using a sodium lamp.

Preparation of 2,5-diketopiperazines (3-5)

4.2 Preparation of 2,5-diketopiperazines (3-5)

General procedure

4.2.1. Coupling of the first *N*-Boc protected α -amino acid on oxime resin

A desired quantity of oxime resin (1.12 mmol/g) was added to a peptide synthesis vessel. The resin was treated three times with CH₂Cl₂. Amino acid (3.0 eq) and HOBT (3.0 eq) were dissolved in DMF in a 100 mL flask and the mixture was stirred for few

minutes at 0 °C. DIC (3.0 eq), DIEA (3.0 eq) and DMAP (0.1 eq) were added and the mixture was introduced into the peptide synthesis vessel and stirred mechanically for three hours. The mixture was filtered under vacuum and the resin was washed [DMF (3 x 100 mL), MeOH (3 x 100 mL), DMF (3 x 100 mL), MeOH (3 x 100 mL)] and dried under reduced pressure.

4.2.2 Acetylation of unreacted sites on oxime resin

The resin was treated three times with CH₂Cl₂ (3 x 50 mL). A solution of 50% v/v DMF/acetic anhydride (80 mL) and DIEA (1 mL) were added to the peptide synthesis vessel and shaken for one hour. Then, the mixture was filtered under vacuum and the resin was washed [DMF (3 x 100 mL), MeOH (3 x 100 mL), DMF (3 x 100 mL), MeOH (3 x 100 mL)] and dried under reduced pressure.

4.2.3 Removal of the Boc protecting group

The resin was treated three times with CH₂Cl₂ (100 mL). A 50% v/v solution of trifluoroacetic acid (TFA) in CH₂Cl₂ was added to the peptide synthesis vessel and shaken for 30 minutes. Then, the mixture was filtered under vacuum and the resin was washed with DMF (3 x 100 mL), MeOH (3 x 100 mL), DMF (3 x 100 mL), MeOH (3 x 100 mL) and with a solution of 10% v/v DIEA in CH₂Cl₂ (100 mL).

4.2.4 Coupling of the second Boc protected α -amino acid

The amino acid (3.0 eq) was dissolved in DMF in a 100 mL flask. The solution was cooled to 0 °C, then HBTU (3.0 eq) and HOBt (3.0 eq) were added. The mixture was poured into the peptide synthesis vessel in which the resin has been previously treated with CH₂Cl₂. DIEA (6.0 eq) was also added to the vessel and the mixture was shaken for three hours. After filtration under vacuum, the resin was washed [DMF (3 x 100 mL), MeOH (3 x 100 mL), DMF (3 x 100 mL) and MeOH (3 x 100 mL)] and dried reduced under pressure. The Kaiser nihydriin test was performed to monitor the efficiency of the coupling, and the coupling procedure was repeated if needed.

4.2.5. Cyclization/cleavage from the resin

First, Boc group was removed using procedure described in (4.2.3), but without the 10% v/v DIEA/CH₂Cl₂ washing step. After drying, CH₂Cl₂ and DIEA (2.5 eq) were added to the peptide synthesis vessel and the mixture was shaken for two minutes. Acetic acid (5.0 eq) was then added and the content was further shaken for 24 h. Then the filtrate was collected and the resin was rinsed several times with CH₂Cl₂ and MeOH. All the filtrates were combined and evaporated, and the resulting solid was dissolved in CH₂Cl₂. Amberlite IR-120 was introduced to the solution to remove remaining traces of DIEA. The mixture was stirred for a few minutes and filtered. The filtrate was evaporated to

give compounds **3** to **5**. Trituration in a minimum of cold ether was performed and led to desired compounds with satisfying purity.

Characterization of 2,5-diketopiperazines 3-5:

(3S,6S)-3,6-diisobutylpiperazine-2,5-dione¹ (3): $[\alpha]_D^{22}$ -44.2° (*c* 0.1 in MeOH) (lit.,² -46.4° (*c* 0.1 in MeOH)); White powder (88%). ¹H-NMR (400 MHz, DMSO-*d*₆): δ = 0.81 (d, *J* = 6.5 Hz, 6H), 0.84 (d, *J* = 6.5 Hz, 6H), 1.32-1.46 (m, 2H) 1.50-1.56 (m, 2H) 1.60-1.80 (m, 2H), 3.60-3.73 (m, 2H) 8.15 (s, 2H). ¹³C NMR (101 MHz, DMSO-*d*₆): δ = 22.1, 23.5, 24.0, 44.0, 53.0, 168.8. HRMS (ESI-TOF, *m/z*): calcd for C₁₂H₂₃N₂O₂ (M+H)⁺ = 227.1754, found 227.1762. HPLC (retention time, purity): 23.41 min, 97 % .

(3R,6R)-3,6-diisobutylpiperazine-2,5-dione (4). $[\alpha]_D^{22}$ +44.1 (*c* 0.1 in MeOH); White powder (87%). ¹H-NMR (400 MHz, DMSO-*d*₆) : δ = 0.82 (d, *J* = 6.5 Hz, 6H), 0.85 (d, *J* = 6.5 Hz, 6H), 1.35-1.48 (m, 2H) 1.51-1.57 (m, 2H) 1.68-1.84 (m, 2H), 3.64-3.71 (m, 2H) 8.16 (s, 2H). ¹³C-NMR (101 MHz, DMSO-*d*₆): δ = 22.1, 23.5, 24.0, 44.1, 53.1, 168.9. HRMS (ESI-TOF, *m/z*): calcd for C₁₂H₂₃N₂O₂ (M+H)⁺ = 227.1754, found 227.1764. HPLC (retention time, purity): 23.20 min, 99 %

(3R,6S)-3,6-diisobutylpiperazine-2,5-dione³ (5). White powder (83%). ¹H-NMR (400 MHz, DMSO-*d*₆): δ = 0.83 (t, *J* = 6.3 Hz, 12H), 1.39-1.64 (m, 4H), 1.70-1.85 (m, 2H), 3.66-3.77 (m, 2H) 8.06 (s, 2H). ¹³C-NMR (101 MHz, DMSO-*d*₆): δ = 22.4, 23.3, 24.0, 41.7, 52.8, 169.2. HRMS (ESI-TOF, *m/z*): calcd for C₁₂H₂₃N₂O₂ (M+H)⁺ = 227.1754, found 227.1762. HPLC (retention time, purity): 21.42 min, 95%.

Preparation of linear dipeptide **6** L-Leu-L-Leu

N-Fmoc-L-Leu (5 equiv.) was activated with HBTU/HOBt in DMF, and then added to the Wang resin swollen in DMF, followed by addition of 1.5 equiv. of DIEA. The mixture was shaken mechanically for 1 h at room temperature. The resin was filtered and washed thoroughly with DMF (3 X 50 mL), MeOH (3 X 50 mL), DMF (3 X 50 mL), MeOH (3 X 50 mL) and then dried in vacuum. The completion of coupling reaction was monitored by the ninhydrin test. When necessary, a second coupling was performed under the same condition. The Fmoc group was deprotected by two consecutive 10 min treatments with 20% piperidine/DMF. *N*-Fmoc-L-Leu was coupled for 1h. Then, the resin was filtered and washed as usual. After removal of *N*-Fmoc, peptide cleavage was performed using a 95% TFA/water solution for 1 h. Filtration and evaporation gave the crude peptide carboxylate **6** purified by trituration, lyophilized and characterized by HPLC and mass spectrometry.

(S)-2-((*S*)-2-amino-4-methylpentanamido)-4-methylpentanoic acid⁴ (**6**). White powder (91%). HRMS (ESI-TOF, *m/z*): calcd for C₁₂H₂₅N₂O₃ (M+H)⁺ = 245.1860, found 245.1868. HPLC (retention time, purity): 15.62 min, 91%.

General procedure for the epoxidation of enones :

The catalyst (0.01 mmol, 0.1 equiv.) was added to a solution of enones (0.1 mmol) in 0.3 mL of hexanes and stirred at room temperature for 30 minutes. Then, 0.2 mL of a solution of 25% NaOH in 30% hydrogen peroxide (0.25g/mL) was added and the triphasic mixture was stirred in the dark at room temperature for 72h. 25 μ L of hydrogen peroxide were added to the solution after each 24 hour period. The conversion and the enantiomeric excess of the corresponding epoxychalcone were determined by chiral HPLC using a Hewlett Packard series 1050. A lower concentration of *trans*-chalcone in hexanes led to slightly lower enantioselectivities.

The absolute configuration of epoxide **2a-2i** was determined by comparison of the HPLC retention times with literature values.

HPLC analysis

trans-(2*R*,3*S*)-Epoxy-1,3-diphenylpropan-1-one (**2a**).⁵ HPLC (Chiralcel OD-H): λ 254 nm, hexane/*i*-PrOH 95/5, flow rate 0.6 mL/min, $t_R(2S,3R)$ = 18.74 min, $t_R(2R,3S)$ = 20.53 min.

trans-(2*R*,3*S*)-Epoxy-3-(2-nitrophenyl)-1-phenyl-1-propan-1-one (**2b**). HPLC (Chiralcel OD-H): λ 254 nm, hexane/*i*-PrOH 95/5, flow rate 0.6 mL/min, $t_R(2S,3R)$ = 45.74 min, $t_R(2R,3S)$ = 52.30 min.

trans-(2*R*,3*S*)-Epoxy-3-(3-nitrophenyl)-1-phenyl-1-propan-1-one (**2c**). HPLC (Chiralcel OD-H): λ 254 nm, hexane/*i*-PrOH 95/5, flow rate 0.6 mL/min, $t_R(2S,3R)$ = 61.48 min, $t_R(2R,3S)$ = 68.47 min.

trans-(2*R*,3*S*)-Epoxy-3-(4-nitrophenyl)-1-phenyl-1-propan-1-one (**2d**).⁵ HPLC (Chiralcel OD-H): λ 254 nm, hexane/*i*-PrOH 95/5, flow rate 0.6 mL/min, $t_R(2S,3R)$ = 81.03 min, $t_R(2R,3S)$ = 88.00 min.

trans-(2*R*,3*S*)-Epoxy-3-(4-chlorophenyl)-1-phenyl-1-propan-1-one (**2e**).⁶ HPLC (Chiralcel OD-H): λ 254 nm, hexane/*i*-PrOH 95/5, flow rate 0.6 mL/min, $t_R(2S,3R)$ = 21.85 min, $t_R(2R,3S)$ = 23.42 min.

trans-(2*R*,3*S*)-Epoxy-3-(4-fluorophenyl)-1-phenyl-1-propan-1-one (**2f**).⁷ HPLC (Chiralcel AD-H): λ 254 nm, hexane/*i*-PrOH 95/5, flow rate 0.6 mL/min, $t_R(2S,3R)$ =28.94 min, $t_R(2R,3S)$ =31.46 min.

trans-(2*R*,3*S*)-2,3-epoxy-3-phenyl-1-(4-fluorophenyl)propan-1-one (**2g**).⁸ HPLC (Chiralcel OD-H): λ 254 nm, hexane/*i*-PrOH 95/5, flow rate 0.6 mL/min, $t_R(2S,3R)$ =22.75 min, $t_R(2R,3S)$ =24.54 min.

trans-(2*R*,3*S*)-Epoxy-3-(4-methoxyphenyl)-1-phenyl-1-propan-1-one (**2h**).⁶ HPLC (Chiralcel OD-H): λ 254 nm, hexane/*i*-PrOH 95/5, flow rate 0.6 mL/min, $t_R(2S,3R)$ =27.01 min, $t_R(2R,3S)$ =29.42 min.

trans-(2*R*,3*S*)-2,3-epoxy-1-(4-methylphenyl)-3-(4-fluorophenyl)propan-1-one (**2i**).⁹ HPLC (Chiralcel AD-H): λ 254 nm, hexane/*i*-PrOH 95/5, flow rate 0.6 mL/min, $t_R(2S,3R)$ =37.76 min, $t_R(2R,3S)$ =45.13min.

*Epoxidation of each enones have been done in triplicate. Conversions and enantiomeric excesses reported are the averages of the separate experiments.

HPLC chromatograms with catalyst 3 :

trans-(2*R*,3*S*)-Epoxy-1,3-diphenylpropan-1-one 4 (**2a**)

trans-(2*R*,3*S*)-Epoxy-3-(2-nitrophenyl)-1-phenyl- 1-propan-1-one (**2b**)

trans-(2*R*,3*S*)-Epoxy-3-(3-nitrophenyl)-1-phenyl- 1-propan-1-one (**2c**)

trans-(2*R*,3*S*)-Epoxy-3-(4-nitrophenyl)-1-phenyl- 1-propan-1-one (**2d**).

trans-(2*R*,3*S*)-Epoxy-3-(4-chlorophenyl)-1-phenyl- 1-propan-1-one (**2e**)

trans-(2*R*,3*S*)-Epoxy-3-(4-fluorophenyl)-1-phenyl- 1-propan-1-one (**2f**).

trans-(2*R*,3*S*)-Epoxy-3-(4-methoxyphenyl)-1-phenyl-1-propan-1-one (**2g**).

trans-(2*R*,3*S*)-2,3-epoxy-3-phenyl-1-(4-fluorophenyl)propan-1-one (**2h**).

trans-(2*R*,3*S*)-2,3-epoxy-1-(4-methylphenyl)-3-(4-fluorophenyl)propan-1-one (**2i**).

HPLC chromatogram with catalyst 4 :

trans-(2*R*,3*S*)-Epoxy-1,3-diphenylpropan-1-one (**2a**)

HPLC chromatogram with catalyst 5 :

trans-(2*R*,3*S*)-Epoxy-1,3-diphenylpropan-1-one 4 (**2a**)

HPLC chromatogram with catalyst 6

trans-(2*R*,3*S*)-Epoxy-1,3-diphenylpropan-1-one 4 (**2a**)

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NMR spectra

Catalyst 3 (3*S*,6*S*)-3,6-diisobutylpiperazine-2,5-dione

Catalyst 4 (3*R*,6*R*)-3,6-diisobutylpiperazine-2,5-dione

Catalyst 5 (3*S*,6*R*)-3,6-diisobutylpiperazine-2,5-dione