Supporting Information

Synthesis of Natural Products and Cyclic Peptides from Lignin-derived Aromatics

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General Information

Chemicals: Chemical reagents were purchased from Sigma Aldrich, Alfa Aesar, Fischer Scientific or Acros Organics and used without further purification. Unless otherwise stated, reactions were carried out in flame dried glassware under a slight positive pressure of N_2 gas.

Solvents: Unless otherwise stated, tetrahydrofuran (THF) and dichloromethane (DCM) were anhydrous and obtained from a solvent still (MBraun, SPS-800). Other anhydrous solvents were used as purchased from Fischer Scientific, Aldrich or Acros.

Thin Layer Chromatography (TLC) and Purification Technique: TLC analyses were carried out using glass-backed TLC plates. The plates were visualized under UV lamp (254/365 nm) followed by staining with aqueous potassium permanganate and dried using a heat gun. Column chromatography was carried out using silica gel (40–63 μm, Silicycle) or aluminium oxide (50-200 μm, Acros Organics) using a glass column with a tap.

Melting Points (M.p.): Melting points were measured using capillary tubes on an Electrothermal 9100 melting point apparatus. Values are quoted in ranges and to 0.1 ^{o}C.

Infra-Red (IR): Infra-red spectra were recorded using a Shimadzu IRAffinity-1 spectrometer equipped with PIKE MIRacle™ ATR (ZnSe-crystall) or Specac The Quest ATR (Diamond) and high pressure clamp to produce thin films. Only significant absorptions maxima (vmax) were quoted in wavenumbers (cm^{-1}).

Mass Spectrometer (MS): Mass spectrometric (m/z) data were delivered by EPSRC National Mass Spectrometry Service Centre in Swansea and University of St Andrews mass spectrometry service. Chemical ionisation techniques were applied and a Thermofischer LTQ Orbitrap XL spectrometer was used. Values are quoted as a ratio of mass to charge (m/z) in Das [Da]. Low-resolution mass spectra were obtained with an Agilent 6130 single quad apparatus equipped with an electrospray ionization source. High-resolution mass spectra (HRMS) were obtained with a Thermo Exactive Orbitrap mass spectrometer.

Mass Spectrometer/Mass Spectrometer (MS/MS): MSMS data were acquired using a TripleTOF 5600+. The sample was subjected to chromatography on an Acclaim PepMap 100

C18 trap and an Acclaim PepMap RSLC C18 column (ThermoFisher Scientific), using a nano-LC Ultra 2D plus loading pump and autosampler (Eksigent). The MSMS fragmentation pattern was interrogated for diagnostic peaks.

Nuclear Magnetic Resonance (NMR): NMR spectra were recorded on either a Bruker Ultrashield 700 (^{1}H 700, ^{13}C 175 MHz); Bruker Ascend 500 (^{1}H 500; ^{13}C 125 MHz) or a Bruker Advance II 400 (^{1}H 400; ^{13}C 101 MHz). ^{13}C NMR spectra were taken with a DEPTQ pulse sequence. Spectra were recorded using the deuterated solvents. In ^{1}H NMR, multiplicities of signals are denoted as follows: br (broad), s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), m (multiplet). Coupling constants (*J*) are quoted to the nearest 0.1 Hz. Chemical shifts (δ) are stated in ppm and referenced to residual solvent signals ($CHCl_3$ 7.250 ppm (s), 77.170 ppm (t); D_2O 4.790 ppm (s); $(CD_3)_2SO$ 2.50 ppm (s) or 39.52 ppm (pentet)). Signals of protons and carbons were assigned, as far as possible, by using the following two dimensional NMR spectroscopy techniques: [^{1}H, ^{1}H] COSY (Correlation Spectroscopy), [^{1}H, ^{1}H] TOCSY (Total Correlation Spectroscopy), HSQC (Heteronuclear Single Quantum Coherence), [^{1}H, ^{1}H] ROESY (Rotating-frame NOE Spectroscopy) and long range [^{1}H, ^{13}C] HMBC (Heteronuclear Multiple Bond Correlation). EXSY (Exchange Spectroscopy) experiment was used to identify equilibrium chemical exchange either at room temperature or with heating.

Optical rotation: Optical rotations were determined using a Perkin Elmer Model 341 Polarimeter with a Na/Hal lamp (Na D line, 589 nm). Calculations were done according to:

$$[\propto]_D^{20} = \frac{\alpha}{c \times l}$$

With a cell length ℓ = 1 dm, concentration c = 0.1 g/mL and optical rotation α_{obs}.

High Performance Liquid Chromatography (HPLC): The HPLC grade acetonitrile (MeCN) was purchased from VWR Ltd. Aqueous buffers and aqueous mobile-phases for HPLC were prepared using water purified with an Elga© Purelab Milli-Q water purification system (purified to 18.2 MΩ.cm). Analytical Reverse Phase-HPLC was performed on an Agilent infinity 1260 series equipped with a VWD detector and a single quadrupole MS using a Macherey-Nagel Nucleodur C18 column (10 µm × 4.6 × 250 mm). Several chromatographic systems were used; System A2: 1 ml/min flow rate with MeCN and 0.1% aqueous TFA [95% TFA (3 min),

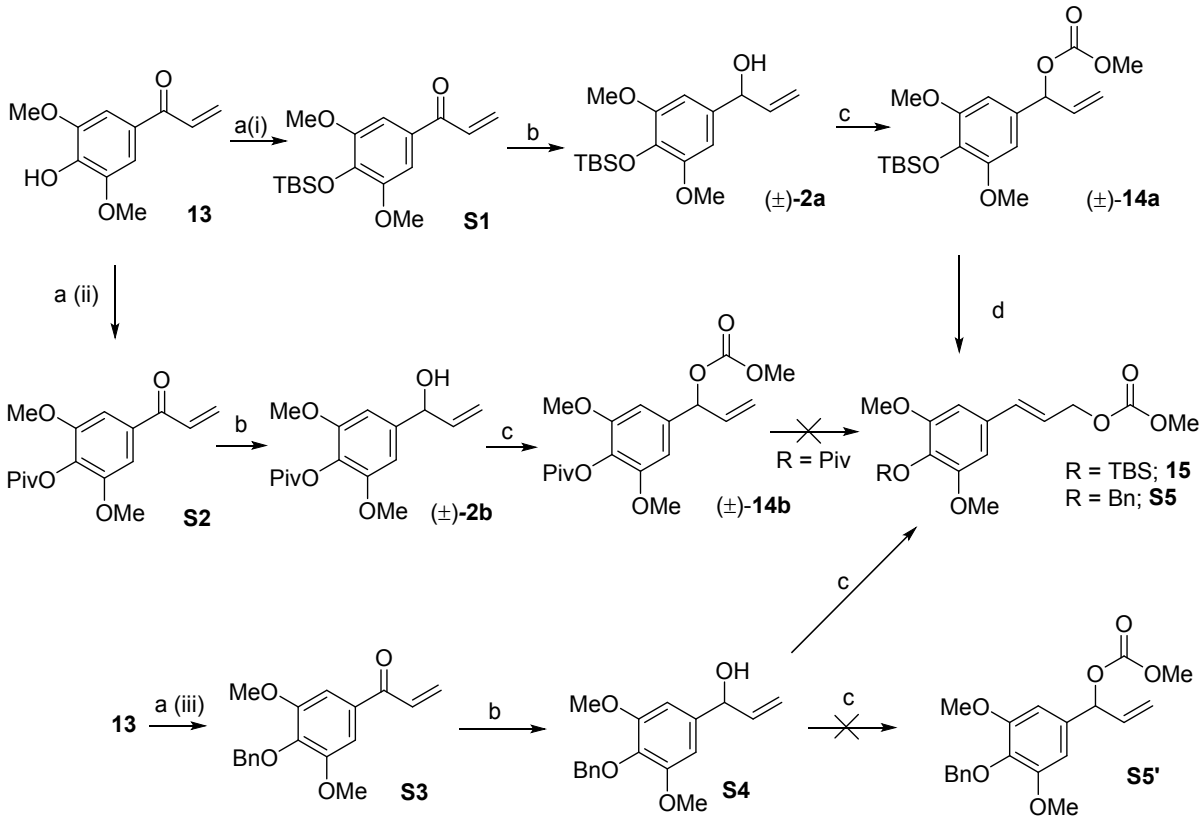
linear gradient from 5 to 100% of MeCN (45 min)] and UV detection at 220 nm. System A3: 1 ml/min flow rate with MeCN and 0.1 % aqueous TFA [95% TFA (3 min), linear gradient from 5 to 95% of MeCN (85 min)] and UV detection at 220nm. Semi-preparative HPLC was performed on an Agilent Infinity preparative scale purification 1260 series equipped with a VWD detector using a a Macherey-Nagel Nucleodur C18 column (10 µm × 16 × 250 mm). The chromatographic system used was System P1: 10 ml/min flow rate with MeCN and 0.1% aqueous TFA [95% TFA (5 min), linear gradient from 5 to 35% of MeCN (5 min), linear gradient from 35 to 37% of MeCN (20min)] and UV detection at 220 nm.

Ultra Performance Liquid Chromatography (UPLC): Analytical RP-UPLC was performed on a Waters Acquity equipped with a VWD detector using a Waters C18 column (1.7 µm × 2.1 × 50 mm). The chromatographic system used was system A1: 0.6 ml/min flow rate with MeCN and 0.1% aqueous TFA [linear gradient from 10 to 95% of MeCN (5 min)] and UV detection at 220 nm.

Enzymatic Reactions: Reactions performed in the enzymatic media were monitored using Maldi-MS acquired using a 4800 MALDI TOF/TOF Analyser (ABSciex, Foster City, CA) equipped with a Nd:YAG 355 nm laser and calibrated using a mixture of peptides. The spot was analysed in positive MS mode between 800 and 4000 m/z, by averaging 1000 laser spots. The samples, diluted in water to reduce the buffer concentration, (0.5 ml) were applied to the MALDI target along with alpha-cyano-4-hydroxycinnamic acid matrix (0.5 ml, 10 mg/ml in 50:50 acetonitrile:0.1% TFA) and allowed to dry.

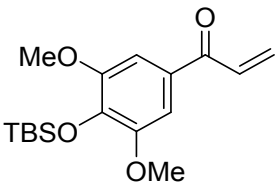
Circular Dichromism: Measurements were performed at room temperature in $CHCl_3$ in a 1mm path length cell with a Bio-Logic MOS-500 spectrometer.

Experimental procedures for the synthesis of Branched Allylic Carbonates (±)-14a and (±)-14b and Linear Allylic Carbonates 15 and S5



Scheme S1 Synthetic route to **(±)-14a, (±)-14b** and **S5**. (a)(i) TBSCl, DMAP, Imidazole, DCM, rt, 1 h, 90%; (a)(ii) PivCl, DMAP, Imidazole, DCM, 1 h, 87%; (a)(iii) BnCl, DMAP, Imidazole, DCM, 1 h, 87%; (b) $NaBH_4$, $CeCl_3.7H_2O$, MeOH, 1 h, 95% for **2a**, 91% for **2b**; (c) Methyl chloroformate, LiHMDS, THF, 1 h, -10 °C, 15% for **14a**, 89% for **14b,** 48% (over 3 steps) for **S5**; (d) degraded during chromatography.

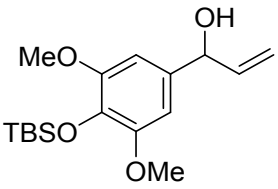
1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-one (S1)



A solution of enone **13** (0.30 g, 1.44 mmol, 1.0 eq) in DCM (4 mL) was added to a stirring solution of 4-DMAP (0.17 g, 1.44 mmol, 1.0 eq) and imidazole (0.19 g, 2.88 mmol, 2.0 eq) in DCM (10 mL), followed by the addition of TBSCl (0.25 g, 1.73 mmol, 1.2 eq). The resulting mixture was left to stir at room temperature for 1 h (TLC analysis). Afterwards, the mixture was neutralised with saturated aqueous solution of $NaHCO_3$ (50 mL) and the aqueous layer was further extracted with DCM (50 mL). Combined organic layer were washed with water (50 mL), brine (60 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography using 2-5% EtOAc in petroleum ether gave compound **S1** as light-yellow oil (0.42 g, 1.29 mmol, 90%).

IR (neat) ν_{max}/cm^{-1}: 2929, 1664, 1577, 1506, 1462, 1332, 1128; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. For $C_{17}H_{26}O_4SiNa^+$ 345.1493; found 345.1488; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.22 (s, 2H), 7.17 (dd, *J* = 17.0, 10.4 Hz, 1H), 6.43 (dd, *J* = 16.9, 1.7 Hz, 1H), 5.87 (dd, *J* = 10.5, 1.7 Hz, 1H), 3.86 (s, 6H), 1.01 (s, 9H), 0.15 (s, 6H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 189.5, 151.5, 139.8, 132.2, 129.9, 129.4, 106.3, 56.0, 25.8, 18.9, -4.4.

1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-ol ((±)-2a)

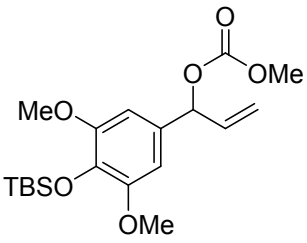


$NaBH_4$ (0.23 g, 6.21 mmol, 5.0 eq) was added, gradually, to a cooled stirring solution of $CeCl_3.7H_2O$ (0.55 g, 1.49 mmol, 1.2 eq) and compound **S1** (0.40 g, 1.24 mmol, 1.0 eq) in MeOH (12 mL). The resulting mixture was left to stir at 0 °C for 1 h. Afterwards, the mixture was quenched with saturated aqueous solution of ammonium chloride (50 mL) and extracted with ethyl acetate twice (2 x 75 mL). The combined organic layer were washed with water (40 mL), brine (50 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography using 10-20% EtOAc in petroleum ether gave allylic alcohol (±)-**2a** as white solid (0.42 g, 1.31 mmol, 95%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.57 (s, 2H), 6.07 (ddd, *J* = 17.1, 10.3, 5.6 Hz, 1H), 5.36 (dt, *J* = 17.1, 1.5 Hz, 1H), 5.21 (dt, *J* = 10.3, 1.4 Hz, 1H), 5.13 (d, *J* = 5.6 Hz, 1H), 3.81 (s, 6H), 1.03 (s, 9H), 0.14 (s, 6H).

^{1}H NMR data was consistent with previously reported data.[S1]

1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)allyl methyl carbonate ((±)-14a)

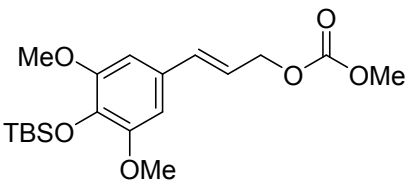


LiHMDS (2.98 mL, 2.98 mmol, 1 M in THF, 1.2 eq) was added slowly to a cooled solution of solution of (±)-**2a** (0.80 g, 2.49 mmol, 1.0 eq) in THF (32 mL) at -10 °C, and left to stir at -10 °C for 30 min followed by the addition of methyl chloroformate (0.30 g, 0.25 mL, 3.23 mmol, 1.3 eq). After the reaction had reached completion (TLC analysis), approx. 30 min, the mixture was quenched with water (40 mL). The organic layer was further washed with water (2 x 50 mL) and brine (50 mL) and dried with Na_2SO_4 and

concentrated *in vacuo.* Purification by silica gel column chromatography using 2-5% EtOAc in petroleum ether provided (±)-**14a** (0.17 g, 0.44 mmol, 15%) as a clear oil.

IR (neat) ν_{max}: 2930, 2857, 1747, 1510 cm^{-1}; **HRMS** (ESI) *m/z* $[M\text{-}C_2H_3O_3]^+$ calcd. for $C_{17}H_{27}O_3Si^+$ 307.1724; found 307.1712; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.55 (s, 2H), 6.05 – 5.97 (m, 2H), 5.36 – 5.29 (m, 1H), 5.28 – 5.21 (m, 1H), 3.79 – 3.75 (m, 9H), 1.00 (s, 9H), 0.11 (s, 6H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 155.1, 151.6, 135.9, 134.5, 130.4, 117.1, 104.4, 80.4, 55.7, 54.8, 25.8, 18.7, -4.6.

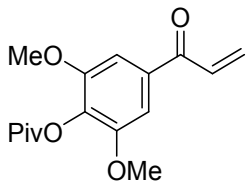
(*E*)-3-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)allyl methyl carbonate (15)



Linear carbonate **15** (65 mg, 0.17 mmol) was isolated after slow column chromatography (silica) of branched carbonate (±)-**14a** (200 mg, 0.52 mmol) in petroleum ether/EtOAc (95:5). A number of additional degradation products were visible by TLC but could not be isolated in sufficiently pure form to enable structural assignment.

HRMS (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{19}H_{30}O_6SiNa^+$ 405.1704; found 405.1688; **IR** (neat) ν_{max}: 2930, 2855, 1746, 1584, 1510 cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.65 – 6.60 (m, 3H), 6.19 (dt, *J* = 15.8, 6.7 Hz, 1H), 4.80 (dd, *J* = 6.7, 1.3 Hz, 2H), 3.84 – 3.81 (m, 9H), 1.02 (s, 9H), 0.15 (s, 6H);**^{13}C NMR** (126 MHz, $CDCl_3$) δ 155.7, 151.7, 135.5, 134.9, 128.7, 120.5, 103.9, 68.6, 55.7, 54.8, 25.7, 18.8, -4.6.

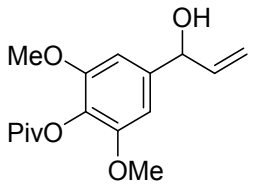
4-acryloyl-2,6-dimethoxyphenyl pivalate (S2)



Same experimental procedure was followed as described for the synthesis of compound **S1**. A solution of enone **13** (0.30 g, 1.44 mmol, 1.0 eq) in DCM (4 mL) was added to a stirring solution of 4-DMAP (0.17 g, 1.44 mmol, 1.0 eq) and Imidazole (0.19 g, 2.88 mmol, 2.0 eq) in DCM (10 mL), followed by the addition of PivCl (0.20 g, 1.73 mmol, 1.2 eq). Purification by silica gel chromatography using 2-5% EtOAc in petroleum ether gave compound **S2** as a colourless oil (0.37 g, 1.26 mmol, 87%).

IR (neat) ν_{max}/cm^{-1}: 2972, 1753, 1670, 1597, 1456, 1415, 1330, 1101; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. For $C_{16}H_{20}O_5Na^+$ 315.1203; found 315.1196; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.19 (s, 2H), 7.12 (dd, *J* = 17.0, 10.5 Hz, 1H), 6.43 (dd, *J* = 17.0, 1.5 Hz, 1H), 5.91 (dd, *J* = 10.5, 1.5 Hz, 1H), 3.84 (s, 6H), 1.37 (s, 9H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 189.9, 175.9, 152.5, 135.0, 132.1, 130.3, 105.6, 56.4, 39.2, 27.3.

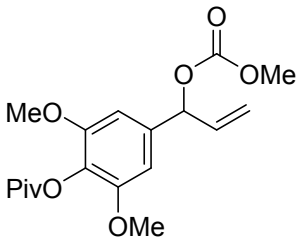
4-(1-hydroxyallyl)-2,6-dimethoxyphenyl pivalate ((±)-2b)



Same experimental procedure was followed as described for the synthesis of allylic alcohol (±)-**2a.** $NaBH_4$ (0.23 g, 6.30 mmol, 5.0 eq) was added, gradually, to a cooled stirring solution of $CeCl_3.7H_2O$ (0.56 g, 1.52 mmol, 1.2 eq) and compound **S2** (0.37 g, 1.26 mmol, 1.0 eq) in MeOH (12 mL). Purification by silica gel chromatography using 10-20% EtOAc in petroleum ether gave allylic alcohol (±)-**2b** as a light-yellow solid (0.27 g, 0.93 mmol, 91%).

M.p. 77–78 °C; **HRMS** (ESI) *m/z* $[M+NH_4]^+$ calcd. For $C_{16}H_{26}O_5N^+$ 312.1795; found 312.1807; **IR** (neat) ν_{max}/cm^{-1}: 3509, 2974, 1722, 1606, 1506, 1456, 1233, 1121; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.63 (s, 2H), 6.04 (ddd, *J* = 17.1, 10.3, 6.0 Hz, 1H), 5.38 (dt, *J* = 17.1, 1.4 Hz, 1H), 5.23 (dt, *J* = 10.3, 1.4 Hz, 1H), 5.16 (d, *J* = 6.0 Hz, 1H), 3.81 (s, 6H), 1.40 (s, 9H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 176.5, 152.3, 140.7, 139.9, 128.4, 115.5, 103.0, 75.4, 56.2, 39.1, 27.3.

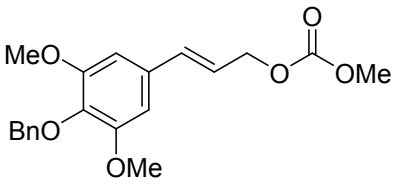
2,6-dimethoxy-4-(1-((methoxycarbonyl)oxy)allyl)phenyl pivalate ((±)-14b)



To a solution of (±)-**2b** (5.50 g, 18.7 mmol, 1.0 eq) in anhydrous THF (125 mL) under N_2 at -10 °C was added LiHMDS solution (1M in THF, 22.4 mL, 22.4 mmol, 1.2 eq) dropwise. The reaction was stirred for 15 min at -10 °C before warming to room temperature for 15 min, and cooling back to -10 °C. Methyl chloroformate (1.73 mL, 22.5 mmol, 1.5 eq) was added dropwise and the reaction was warmed to rt and stirred for 1 h, before quenching with brine (50 mL). The aqueous phase was extracted with EtOAc (30 mL x 3). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to yield the crude product as yellow oil. Purification by silica chromatography eluting with 5–30% EtOAc in petroleum ether yield the desired product (±)-**14b** as an off white solid (5.87 g, 16.7 mmol, 89%).

M.p. 73–75 ^{o}C; **HRMS** (ESI) *m/z* $[M + NH_4]^+$ calcd. For $C_{18}H_{28}O_7N^+$ 370.1849; found 370.1853; **IR** (neat) ν_{max}/cm^{-1}: 2970, 1749, 1604, 1508, 1462, 1256, 1110; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.62 (s, 2H), 5.92–6.09 (m, 2 H), 5.19–5.45(m, 2 H), 3.78–3.84 (s, 9 H), 1.39 (s, 9 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 176.3, 154.9, 152.4, 136.2, 135.5, 129.1, 117.5, 104.0, 80.1, 56.3, 54.9, 39.1, 27.2.

(*E*)-3-(4-(benzyloxy)-3,5-dimethoxyphenyl)allyl methyl carbonate (S5)



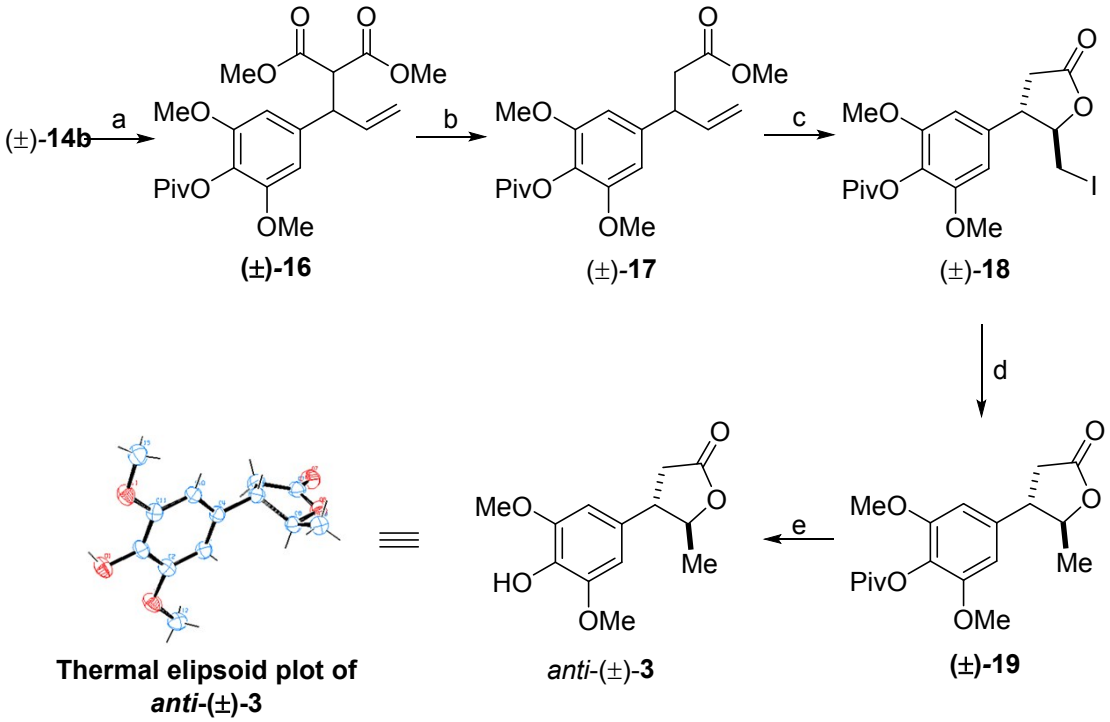
A solution of enone **13** (0.30 g, 1.44 mmol, 1.0 eq) in DCM (4 mL) was added to a stirring solution of 4-DMAP (0.17 g, 1.44 mmol, 1.0 eq) and imidazole (0.19 g, 2.88 mmol, 2.0 eq) in DCM (10 mL), followed by the addition of BnBr (0.29 g, 1.72 mmol, 1.2 eq). The crude material **S3** was used in the subsequent reaction without further purification.

$NaBH_4$ (0.25 g, 6.70 mmol, 5.0 eq) was added, gradually, to a cooled stirring solution of $CeCl_3.7H_2O$ (0.59 g, 1.60 mmol, 1.2 eq) and crude **S3** (0.40 g, 1.34 mmol, 1.0 eq) in MeOH (12 mL). The crude material (±)-**S4** was used in the subsequent reaction without further purification.

LiHMDS (1.59 mL, 1.59 mmol, 1 M in THF, 1.2 eq) was added slowly to a cooled solution of solution of crude (±)-**S4** (0.40 g, 1.33 mmol, 1.0 eq) in THF (13 mL) at -10 ^{o}C, and left to stir at -10 ^{o}C for 30 min followed by the addition of methyl chloroformate (0.15 g, 1.59 mmol, 1.2 eq). Purification by silica gel column chromatography using 2-5% EtOAc in petroleum ether provided (±)-**S5** (0.25 g, 0.69 mmol, 48% over 3 steps) as light-yellow oil. No trace of the desired compound **S5'** (Scheme S1) was observed.

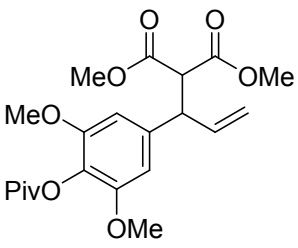
IR (neat) ν_{max}/cm^{-1}: 2954, 1745, 1581, 1504, 1452, 1417, 1259, 1124, 1008; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. For $C_{20}H_{22}O_6Na^+$ 381.1309; found 381.1301; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.45–7.48 (m, 2H), 7.27–7.35 (m, 3H), 6.61 (dt, *J* = 15.8, 1.3 Hz, 1H), 6.60 (s, 2H), 6.20 (dt, *J* = 15.7, 6.4 Hz, 1H), 5.00 (s, 2H), 4.77 (dd, *J* = 6.5, 1.2 Hz, 2H), 3.82 (s, 6H), 3.80 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 155.7, 153.6, 137.7, 137.1, 134.9, 131.8, 128.5, 128.1, 127.8, 121.8, 103.8, 75.0, 68.3, 56.1, 54.8.

Experimental procedures for the synthesis of *anti*-(±)-Descurainolide A (*anti*-(±)-3)



Scheme S2 synthesis of *anti*-(±)-Descurainolide A 3. Reaction conditions: (a) NaH, Dimethyl malonate, $RhCl(PPh_3)_3$ (5 mol%), $P(OMe)_3$ (20 mol%), THF, 40 °C, 1 h, 87% (b) LiCl, H_2O, DMSO, 140 °C, 16 h, 76% (c) I_2, MeCN, 0 °C to rt, 48 h, 70% combined yield (d) Pd/C, H_2, NaOAc, MeOH, rt, 16 h, 86% (e) 2 M HCl/1,4-dioxane (1:1), 100 °C, 12 h, 70%. Thermal elipsoid plot of *anti*-(±)-**3** at 50% ellipsoid probability is also shown (CCDC number 1505597)[S2].

Dimethyl 2-(1-(3,5-dimethoxy-4-(pivaloyloxy)phenyl)allyl)malonate (±)-16



$P(OMe)_3$ (167 µL, 1.42 mmol, 20 mol%) was added to a stirring burgundy-red solution of $Rh(PPh_3)_3Cl$ (327 mg, 0.355 mmol, 5 mol%) in degassed anhydrous THF (30 mL) under N_2 atmosphere at 40 °C. The resulting light-yellow solution was stirred for 15 minutes at same temperature. In a separate flask, dimethyl malonate (973 µL, 8.51 mmol, 1.2 eq) was added slowly to the slurry of NaH (60% wt. in mineral oil, 312 mg, 7.80 mmol, 1.1 eq) in degassed anhydrous THF (30 mL) under N_2 atmosphere and left to stir at room temperature for 15 mins and then transferred to the previously prepared the catalyst solution *via* a Teflon cannula. After 5 mins, allylic carbonate (±)-**14b** (2.50 g, 7.09 mmol, 1.0 eq) was added and the resulting mixture was heated to 40 °C for 3 hr. After the reaction had

reached completion (TLC analysis), the reaction mixture was diluted with EtOAc (70 mL) and washed with saturated aqueous solution of NH_4Cl (20 mL). The organic layer was washed with water (30 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Purification by silica chromatography using 5–30% EtOAc in petroleum ether gave (±)-**16** (2.53 g, 6.20 mmol, 87%) as a colourless oil.

HRMS (ESI) *m/z* $[M + NH_4]^+$ calcd. For $C_{21}H_{32}O_8N$ 426.2122; found 426.2116; **IR** (neat) ν_{max}/cm^{-1} 2961, 2359, 1753, 1740, 1602, 1462, 1190, 1132; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.62 (s, 2H), 5.92–6.09 (m, 2 H), 5.19–5.45 (m, 2H), 3.78–3.84 (s, 9 H), 1.39 (s, 9 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 176.3, 154.9, 152.4, 136.2, 135.5, 129.1, 117.5, 104.0, 80.1, 56.3, 54.9, 39.1, 27.2.

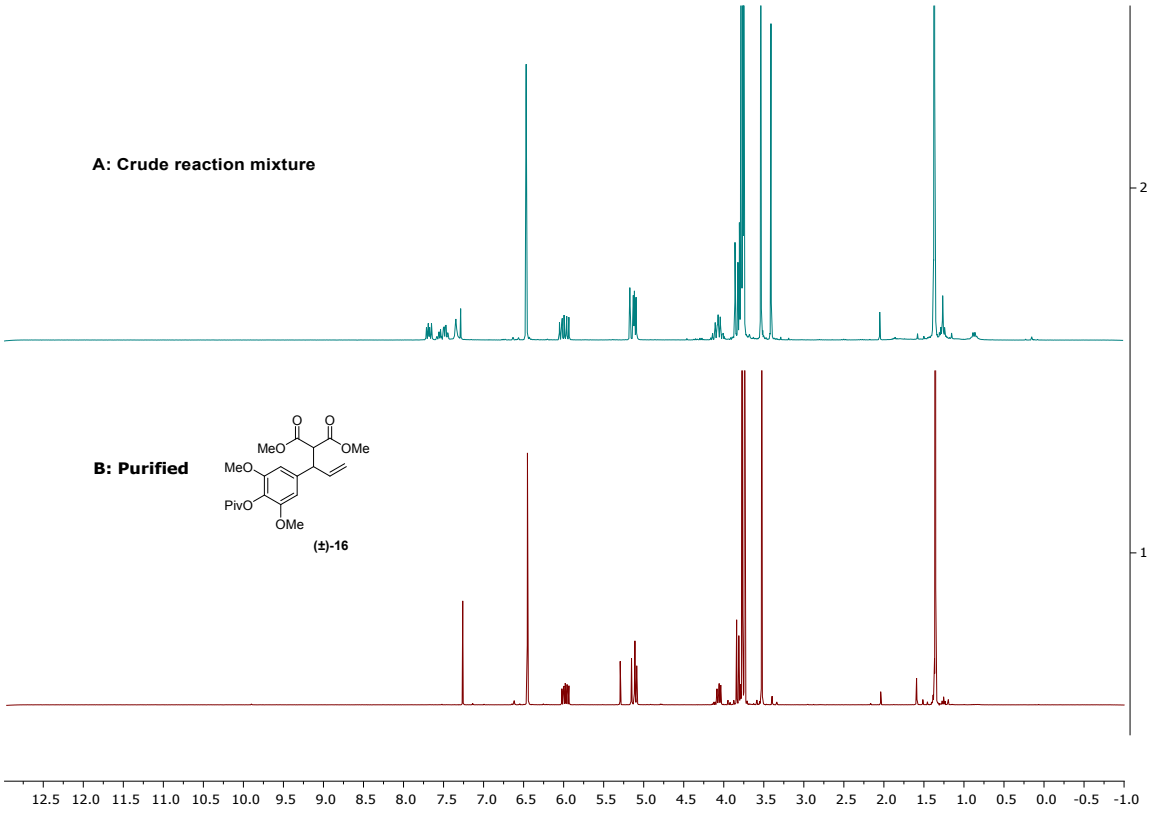
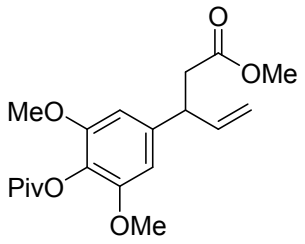


Figure S1 Crude ^{1}H NMR (spectrum **A**) of reaction to form (±)-**16** as a single regioisomer (as far as can be detected). Spectrum **B** is the ^{1}H NMR obtained for (±)-**16** after purification on silica and this was the only product recovered from the reaction.

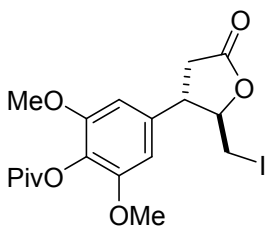
Methyl 3-(3,5-dimethoxy-4-(pivaloyloxy)phenyl)pent-4-enoate ((±)-17)



To a solution of (±)-**16** (2.20 g, 5.39 mmol, 1.0 eq) in DMSO (20 mL) was added water (612 µL, 34.0 mmol, 6.3 eq) and LiCl (1.17 g, 27.5 mmol, 5.1 eq). The resulting reaction mixture was heated to 140 °C for 16 hrs. Afterwards, thereaction was allowed to cool and then poured onto water (150 mL) and extracted with EtOAc (40 mL x 3). The combined organic layers were washed with brine (25 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification by silica chromatography using 0–30% EtOAc in petroleum ether gave (±)-**17** as an off-white solid (1.43 g, 4.09 mmol, 76%).

M.p. 82–84 °C; **HRMS** (ESI) *m/z* $[M + NH_4]^+$ calcd. For $C_{19}H_{30}O_6N^+$ 368.2068; found 368.2064; **IR** (neat) ν_{max}/cm^{-1}: 2970, 1749, 1605, 1508, 1462, 1256, 1109; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.46 (s, 2 H), 5.84–6.07 (m, 1 H), 4.95–5.19 (m, 2 H), 3.82–3.90 (m, 1 H), 3.80 (s, 6 H), 3.66 (s, 3 H), 2.66–2.82 (m, 2 H), 1.39 (s, 9 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 176.5, 172.2, 152.2, 140.5, 139.8, 127.8, 115.0, 104.3, 56.2, 51.7, 45.8, 40.2, 39.1, 27.3.

2-(iodomethyl)-5-oxotetrahydrofuran-3-yl)-2,6-dimethoxyphenyl pivalate ((±)-18)



To a solution of ester (±)-**17** (896 mg, 2.56 mmol, 1.0 eq) in anhydrous MeCN (51 mL) under N_2 atmosphere at 0 °C was added I_2 (3.25 g, 12.8 mmol, 5.0 eq). The reaction mixture was allowed to warm to room temperature over *ca*. 2 hrs and stirred for 46 hrs at rt. The reaction mixture was washed thoroughly with saturated aqueous $Na_2S_2O_3$ (70 mL) and extracted with EtOAc (25 mL x 3). The combined organic layers were washed with brine (25 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification by silica chromatography using 0–30% EtOAc in petroleum ether yielding the *anti*-(±)-**18** (827 mg, 1.79 mmol, 70%) as a pale yellow solid.

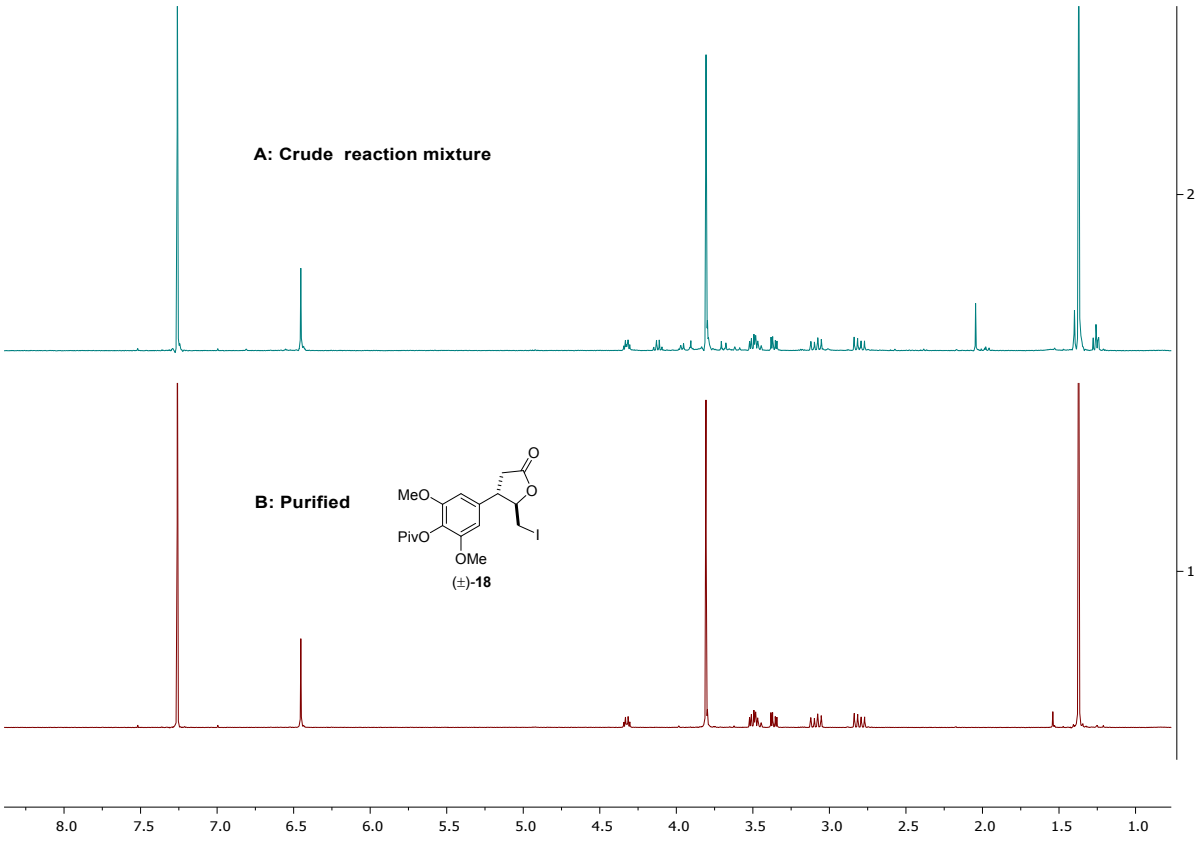
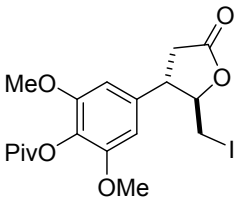


Figure S2 Crude ^{1}H NMR (Spectrum A) of the Iodolactonisation reaction. Based on this crude ^{1}H NMR, the formation of (±)-**18** was judged to be 19:1 *anti*:*syn*. Only *anti*-(±)-**18** was detected by ^{1}H NMR with reaction condition of I_2/MeCN at 0 °C for 48 h (data not shown). The ^{1}H NMR (spectrum B) of the purified compound is also shown.

***anti*-(±)-18**



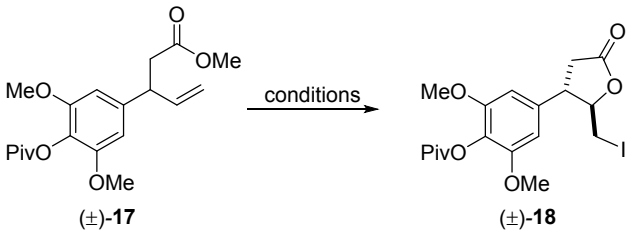
(±)-**18**

M.p. 136–137 °C; **HRMS** (ESI) *m/z* $[M + NH_4]^+$ calcd. For $C_{18}H_{27}IO_6N^+$ 480.0878; found 480.0863; **IR** (neat) ν_{max}/cm^{-1}: 2970, 1784, 1747, 1602, 1512, 1464, 1126; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.48 (s, 2H), 4.35 (dt, *J* = 7.0, 4.5 Hz, 1H), 3.83 (s, 6 H), 3.44–3.56 (m, 2 H), 3.38 (dd, *J* = 11.2, 4.5 Hz, 1H), 3.11 (dd, *J* = 18.0, 9.3 Hz, 1H), 2.83 (dd, *J* = 18.0, 9.1 Hz, 1H), 1.39 (s, 9 H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 176.4, 174.3, 152.9, 136.7, 128.8, 103.7, 84.0, 56.4, 47.7, 39.2, 37.0, 27.2, 6.7.

***Syn*-(±)-18**

M.p. 146–148 °C; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd. For $C_{18}H_{23}IO_6Na^+$ 485.0437; found 485.0420; **IR** (neat) ν_{max}/cm^{-1}: 2968, 1778, 1748, 1601, 1513, 1462, 1119; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.46 (s, 2 H), 4.95 (dt, *J* = 8.2, 5.9 Hz, 1H), 3.87 (ddd, *J* = 8.6, 5.9, 3.0 Hz, 1H), 3.82 (s, 6 H), 3.20 (dd, *J* = 10.3, 5.9 Hz, 1H), 3.10 (dd, *J* = 17.6, 8.6 Hz, 1H), 2.88 (dd, *J* = 17.6, 3.0 Hz, 1H), 2.77 (dd, *J* = 10.3, 8.2 Hz, 1H), 1.39 (s, 9 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 176.3, 175.7, 152.6, 136.7, 134.4, 103.8, 82.9, 56.3, 44.4, 39.1, 36.9, 27.2, 1.3.

Table S1: Optimisation of the Iodolactonisation of (±)-**16** to (±)-**18**

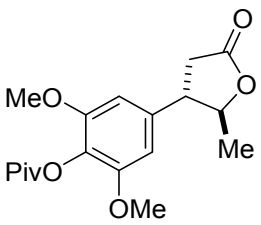


(±)-**17** (±)-**18**

Conditions	Molarity	Scale (mg)	*anti*:*syn*	Conversion (Isolated yield)
MeCN/I_2 (2 eq.)	0.1	20	4:1	72%
MeCN/I_2 (5 eq.)	0.25	20	1:1.3	100%
MeCN/I_2 (5 eq.)	0.1	20	4:1	100%
MeCN/I_2 (5 eq.)	0.05	20	4.5:1	100%
MeCN/I_2 (5 eq.)	0.05	896	19:1	100% (70%)

Reaction time: 48 h. Conversion determined by ^{1}H NMR of crude reaction mixture.

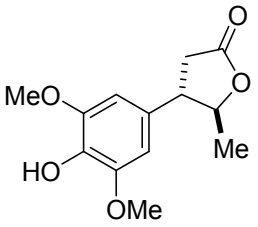
2,6-dimethoxy-4-((2,3-*trans*)-2-methyl-5-oxotetrahydrofuran-3-yl)phenyl pivalate (*anti*-(±)-19)

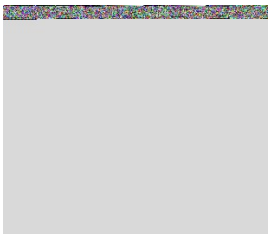


To a suspension of iodolactone *anti*-(±)-**18** (806 mg, 1.74 mmol, 1.0 eq) in MeOH (35 mL) under N_2 atmosphere was added NaOAc (658 mg, 8.02 mmol, 5.0 eq) followed by Pd/C (185 mg, 1.74 mmol, 1.0 eq, 10% by wt). The reaction mixture was stirred for 15 min then evacuated with vacuum and placed under an atmosphere of H_2 (balloon) and stirred for 16 hrs at rt. The reaction mixture was filtered through celite (washing with MeOH (50 mL) and DCM (50 mL) and concentrated *in vacuo*. Purification by silica chromatography using 10–50% EtOAc in Petroleum ether gave *anti*-(±)-**19** (505 mg, 1.50 mmol, 86%) as an off-white solid.

M.p. 164–166 °C; **HRMS** (ESI) *m/z* $[M + Na]^+$ calcd. For $C_{18}H_{24}O_6Na^+$ 359.1471; found 359.1458; **IR** (neat) ν_{max}/cm^{-1}: 2974, 1775, 1742, 1603, 1516, 1462, 1115; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.47 (s, 2 H), 4.58 (dd, *J* = 8.5, 6.1 Hz, 1H), 3.82 (s, 6 H), 3.21 (dt, *J* = 10.7, 8.5 Hz, 1H), 2.98 (dd, *J* = 17.7, 8.5 Hz, 1H), 2.78 (dd, *J* = 17.7, 10.7 Hz, 1H), 1.46 (d, *J* = 6.1 Hz, 3H), 1.39 (s, 9 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 176.4, 175.3, 152.7, 136.6, 128.6, 103.9, 83.0, 56.3, 50.0, 39.1, 37.6, 19.5.

4-(4-hydroxy-3,5-dimethoxyphenyl)-5-methyldihydrofuran-2(3*H*)-one
Descurainolide A (*anti*-(±)-3)





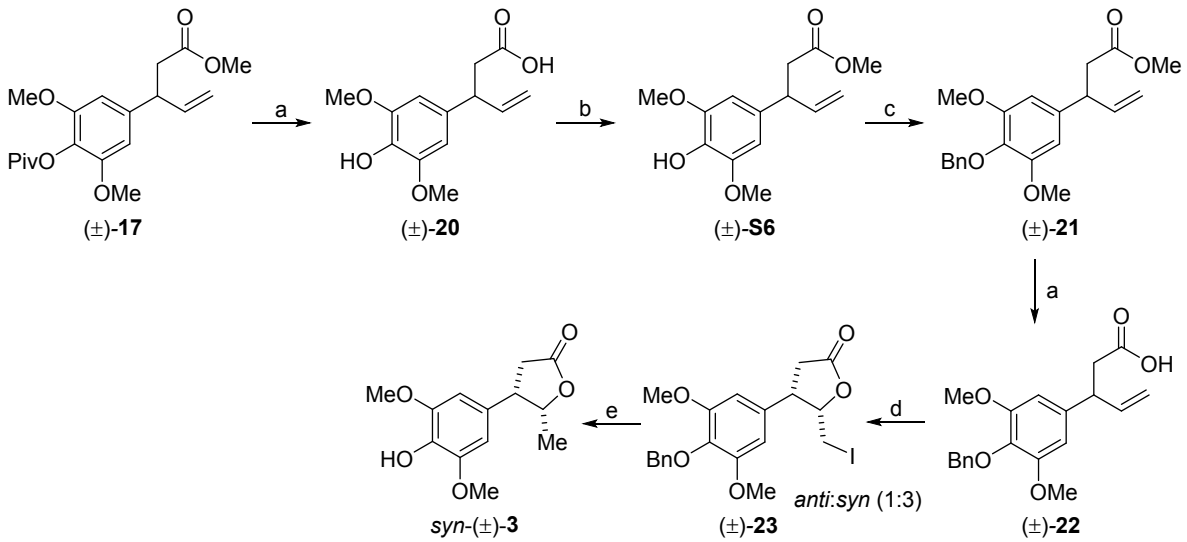
To a solution of lactone *anti*-(±)-**19** (450 mg, 1.34 mmol) in 1,4-dioxane (27 mL) was added 1 M HCl solution (27 mL). The reaction was heated to reflux for 16 h before pouring onto brine (50 mL) and extracting with EtOAc (20 mL x 3). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by silica chromatography using 0–70% EtOAc in petroleum ether to give *anti*-(±)-**3** as a light brown solid (236 mg, 0.936 mmol, 70%).

Further recrystallisation (1:1:8 EtOAc: acetone: hexane) afforded off-white needles of *anti*-(±)-**3** (142 mg, 0.563 mmol). Small molecule X-ray crystallographic analysis of *anti*-(±)-**3** was

carried out (CCDC number 1505597)[S2] and confirmed the assigned structure. The liquor was concentrated and filtered to yield a light brown solid *anti*-(±)-**3** (58 mg, 0.230 mmol).

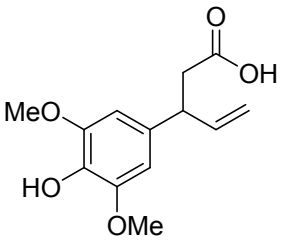
M.p. 126–127 °C (lit. 117-118 °C); **HRMS** (ESI) *m/z* [M + Na] calcd. For $C_{13}H_{16}O_5Na^+$ 275.0895; found 275.0884; **IR** (neat) ν_{max}/cm^{-1} 3391, 2934, 1780, 1610, 1522, 1456, 1204, 1111; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.29 (s, 1 H), 6.67 (s, 2 H), 4.52 (dq, *J* = 9.3, 6.1 Hz, 1H), 3.76 (s, 6 H), 3.18 (dt, *J* = 11.7, 8.7 Hz, 1H), 2.93 (dd, *J* = 17.2, 11.7 Hz, 1H), 2.77 (dd, *J* = 17.1, 8.4 Hz, 1H), 1.28 (d, *J* = 6.1 Hz, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 176.1, 148.5, 135.1, 129.0, 105.5, 82.9, 56.5, 49.5, 37.7, 19.0.

Experimental procedures for the synthesis of *syn*-(±)-3



Scheme S3: Synthetic route to *Syn*-(±)-**3**. Reaction and conditions: (a) 4 M NaOH, THF/MeOH (1:1), 100 °C, 92% for (±)-**17** and 90% for (±)-**22** (b) NaH, MeI, DMF, rt, 1 h, 95% (c) NaH, BnBr, DMF, rt, 1 h, 83% (d) I_2, $NaHCO_3$ (aq), DCM, rt, 12 h, 85% combined yield (e) Pd/C, H_2, NaOAc, MeOH, rt, 16 h, 62% for *syn*-(±)-**3**.

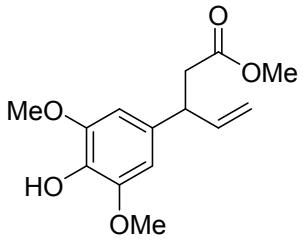
3-(4-hydroxy-3,5-dimethoxyphenyl)pent-4-enoic acid ((±)-20)



A solution of compound (±)-**17** (0.80 g, 2.28 mmol) in THF/MeOH/4 M NaOH (1:1:1) (30 mL) was heated to 100 °C for 12 hrs. After the reaction had reached completion (TLC analysis), 2 M HCl (20 mL) was added to the mixture until pH = 1 and then extracted with ethyl acetate (3 x 50 mL). The combined organic layers were washed with brine (50 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography using 2-5% MeOH in DCM afforded acid (±)-**20** (0.53 g, 2.10 mmol, 92%) as a white solid.

M.p. 105–114 °C **IR** (neat) ν_{max} 3423, 2933, 1687, 1612, 1519, 1427, 1215, 1112; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. For $C_{13}H_{16}O_5Na^+$ 275.0895; found 275.0886; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.43 (s, 2H), 5.93 – 6.01 (m, 1H), 5.11 (d, *J* = 1.3 Hz, 1H), 5.08 (dt, *J* = 7.2, 1.3 Hz, 1H), 3.86 (s, 6H), 3.75 – 3.81 (m, 1H), 2.78 (dd, *J* = 15.5, 8.0 Hz, 1H), 2.70 (dd, *J* = 15.5, 7.2 Hz, 1H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 178.1, 147.1, 140.0, 133.6, 133.3, 115.0, 104.2, 56.4, 45.3, 40.2.

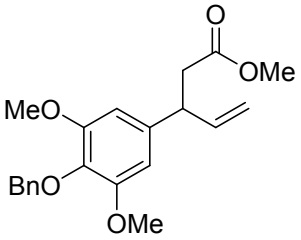
Methyl 3-(4-hydroxy-3,5-dimethoxyphenyl)pent-4-enoate ((±)-S6)



A solution of compound (±)-**20** (0.50 g, 1.98 mmol, 1.0 eq) in DMF (10 mL) was added slowly to a slurry of NaH (60% in mineral oil, 87.2 mg, 2.18 mmol, 1.1 eq) in DMF (9 mL) at room temperature and left to stir for 30 min. Afterwards, MeI (0.31 g, 2.18 mmol, 1.1 eq) was added and the resulting mixture was left to stir for a further 1 hr at room temperature. After the reaction had reached completion, it was quenched with saturated aqueous solution of NH_4Cl (100 mL), and extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with brine (50 mL), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography using 20–30% EtOAc in petroleum ether gave (±)-**S6** (0.50 g, 1.88 mmol, 95%) as a light-yellow oil.

IR (neat) ν_{max} 3439, 2951, 1730, 1612, 1517, 1450, 1353, 1209, 1109; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. For $C_{14}H_{18}O_5Na^+$ 289.1052; found 289.1046; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.40 (s, 2H), 5.94 (ddd, *J* = 17.1, 10.1, 6.8 Hz, 1H), 5.50 (s, 1H), 5.02 – 5.08 (m, 2H), 3.83 (s, 6H), 3.74 – 3.80 (m, 1H), 3.60 (s, 3H), 2.72 (dd, *J* = 15.1, 8.0 Hz, 1H), 2.65 (dd, *J* = 15.1, 7.4 Hz, 1H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 172.4, 147.0, 140.2, 133.5, 133.4, 114.7, 104.1, 56.3, 51.7, 45.5, 40.3.

Methyl 3-(4-(benzyloxy)-3,5-dimethoxyphenyl)pent-4-enoate ((±)-21)

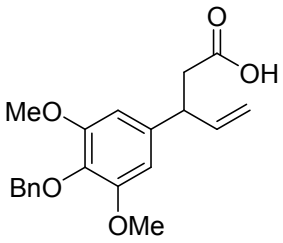


A solution of compound (±)-**S6** (1.25 g, 4.73 mmol, 1.0 eq) in DMF (15 mL) was added slowly to a slurry of NaH (60% in mineral oil, 0.21 g, 5.19 mmol, 1.1 eq) in DMF (20 mL) at room temperature and left to stir for 30 min at room temperature. Afterwards, benzyl bromide (0.89 g, 5.19 mmol, 1.1 eq) was added and the resulting mixture was left to stir for a further 1 hr at room temperature. After the reaction had reached completion, it was quenched with saturated aqueous solution of NH_4Cl (100 mL), and extracted with EtOAc (2 x 150 mL). The combined organic layers were washed with brine (100 mL), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography using 5–10% EtOAc in petroleum ether gave (±)-**21** (1.40 g, 3.91 mmol, 83%) as a colourless oil.

IR (neat) ν_{max} 1735, 1589, 1508, 1460, 1332, 1257, 1242, 1130, 1014; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. For $C_{21}H_{24}O_5Na^+$ 379.1521; found 379.1513; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.47–7.51 (m, 2H), 7.32 – 7.37 (m, 2H), 7.27 – 7.31 (m, 1H), 6.42 (s, 2H), 5.95–6.02 (m, 1H), 5.08–5.12 (m, 2H), 4.98 (s, 2H), 3.81 (s, 6H), 3.64 (s, 3H), 2.66–2.79 (m, 2H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 172.4, 153.6, 140.0, 138.3, 138.0, 135.7, 128.5, 128.2, 127.8, 115.0, 104.6, 75.1, 56.2, 51.7, 45.8, 40.3.

Note: The exposure of (±)-**21** to the reaction condition of I_2/$NaHCO_{3(aq)}$ in DCM at room temperature gave (±)-**23**; *anti*:*syn* 1:1

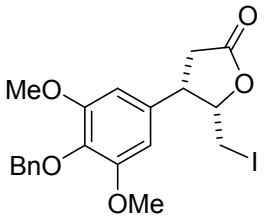
3-(4-(benzyloxy)-3,5-dimethoxyphenyl)pent-4-enoic acid ((±)-22)



A solution of compound (±)-**21** (0.30 g, 0.84 mmol) in THF/MeOH/4 M NaOH (1:1:1) (12 mL) was heated to 100 °C for 3 hrs. After the reaction had reached completion (TLC analysis), 2 M HCl (120 mL) was added to the mixture until pH = 1 and then extracted with ethyl acetate (3 x 150 mL). The combined organic layers were washed with brine (50 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography using 2-5% MeOH in DCM afforded acid (±)-**22** (0.26 g, 0.76 mmol, 90%) as a white solid.

M.p. 90–94 °C ; **IR** (neat) ν_{max} 3001, 1693, 1589, 1421, 1126; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. For $C_{23}H_{22}O_5Na^+$ 365.1365; found 365.1357; **^{1}H NMR** (500 MHz, $CDCl_3$) δ δ 7.47–7.50 (m, 2H), 7.32–7.36 (m, 2H), 7.27–7.31 (m, 1H), 6.42 (s, 2H), 5.94–6.03 (m, 1H), 5.13–5.14 (m, 1H), 5.09–5.12 (m, 1H), 3.81 (s, 6H), 2.69–2.83 (m, 2H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 178.1, 153.6, 139.8, 138.05, 137.99, 135.8, 128.5, 128.2, 127.9, 115.2, 104.6, 75.1), 56.2, 45.5, 40.2.

4-(4-(benzyloxy)-3,5-dimethoxyphenyl)-5-(iodomethyl)dihydrofuran-2(3*H*)-one ((±)-23)



To a white suspension of compound (±)-**22** (0.87 g, 2.53 mmol, 1.0 eq) in DCM/$NaHCO_3$ (1:1) (26 mL) was added a solution of I_2 (0.71 g, 5.56 mmol, 2.2 eq) in DCM (4 mL) at room temperature. The resulting pink-white suspension, which later turned to a red solution, was stirred at room temperature for 12 hrs. After the reaction had reached completion (TLC analysis), the reaction mixture was diluted with DCM and washed with saturated aqueous solutions of NH4Cl (40 mL) and $Na_2S_2O_3$ (40 mL). The combined organic layers were washed with brine (50 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography using 20-30% EtOAc in petroleum ether furnished (±)-**23** (*syn*:*anti* = 3:1) as colourless oil (1.01 g, 2.16 mmol, 85% combined yield).

IR (neat) ν_{max} 1778, 1585, 1508, 1458, 1323, 1238, 1122; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. For $C_{20}H_{21}IO_5Na^+$ 491.0331; found 491.0312; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.45 – 7.48 (m, 2H), 7.33–7.37 (m, 2H), 7.29–7.33 (m, 1H), 6.39 (s, 2H), 5.04 (s, 2H), 4.90–4.95 (m, 1H), 3.83 (s, 6H), 3.04–3.16 (m, 2H), 2.85 (dd, *J* = 17.6, 2.8 Hz, 1H), 2.71 (dd, *J* = 10.3, 7.9 Hz, 1H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 175.9, 153.8, 137.5, 136.5, 132.1, 128.7, 128.2, 128.0, 105.2, 83.1, 75.0, 56.3, 44.3, 36.9, 1.5.

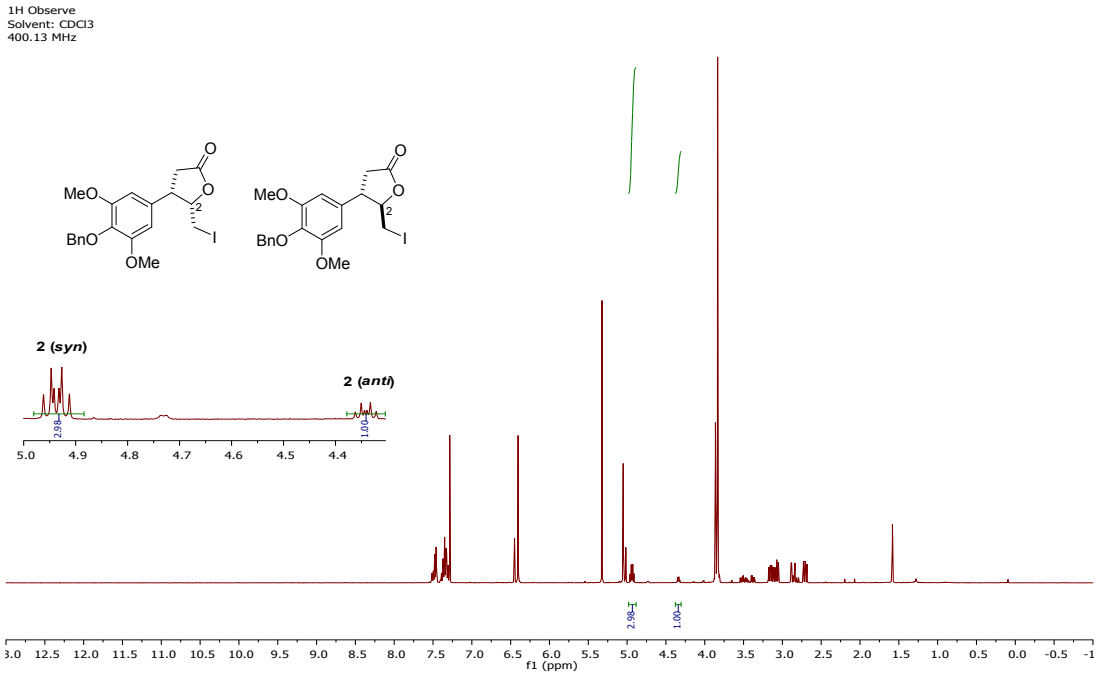
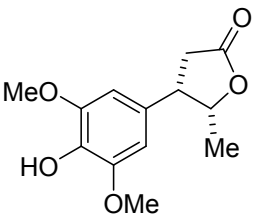


Figure S3 Crude ^{1}H NMR of the Iodolactonisation reaction for the formation of (±)-**23**. Ratio of *anti*:*syn* (1:3) was determined by the integration of the C2-H's in both *anti*:*syn* (±)-**23**. In a reaction using THF as solvent, *anti*:*syn* ratio is 1:2 was obtained (data not shown).

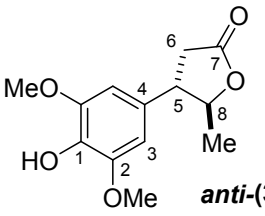
4-(4-hydroxy-3,5-dimethoxyphenyl)-5-methyldihydrofuran-2(3*H*)-one (*syn*-(±)-3)



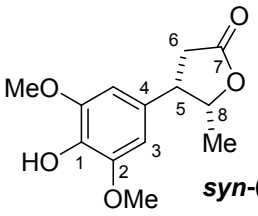
A solution of *syn*-(±)-**23** (0.95 g, 2.03 mmol, 1.0 eq) in EtOAc (5 mL) was added to a stirring black suspension of Pd/C (10% wt, 0.22 g, 2.03 mmol, 1.0 eq) and NaOAc (0.18 g, 2.23 mmol, 1.1 eq) in MeOH (15 mL). The reaction vessel was evacuated and backfilled with H_2 gas thrice. The reaction mixture was left to stir at room temperature for 12 h under H_2 atmosphere. After the reaction had reached completion (TLC analysis), it was filtered through celite and concentrated *in vacuo*. Purification by silica gel chromatography using 30-40% EtOAc in petroleum ether furnished *syn*-(±)-**3** (0.32 g, 1.25 mmol, 62%) as a light yellow oil.

IR (neat) ν_{max} 3400, 2939, 1766, 1610, 1519, 1460, 1323, 1219, 1112; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. For $C_{13}H_{16}O_5Na^+$ 275.0895 found 275.0885; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.30 (s, 1H), 6.47 (s, 2H), 4.93 – 4.85 (m, 1H), 3.79 – 3.71 (m, 7H), 2.93 (dd, *J* = 17.2, 8.3 Hz, 1H), 2.81 (dd, *J* = 17.2, 8.4 Hz, 1H), 0.90 (d, *J* = 6.5 Hz, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 176.7, 147.9, 134.6, 127.7, 105.5, 79.4, 56.1, 43.7, 33.1, 16.3.

Table S2 Comparison of the ^{1}H and ^{13}C NMR of experimental and literature Descurainolide A.[S3]



***anti*-(3)**

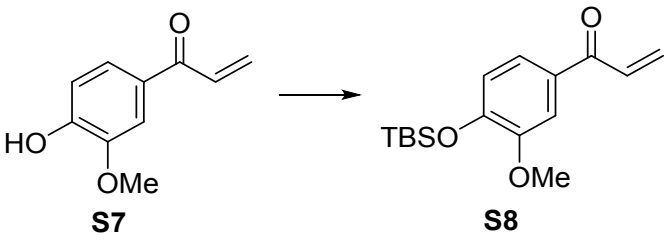


***syn*-(3)**

	^{1}H NMR (ppm, DMSO-d_6)		
	Experimental (*anti*-)-3	**Literature (*anti*-)-3**	**Experimental (*syn*-)-3**
1	-	-	-
OH	8.29 (1H, s)	8.26 (1H, s)	8.30 (1H, s)
2	-	-	-
OMe	3.76 (6H, s)	3.75 (6H, s)	3.79 – 3.71 (7H, m)- overlaps with **5**
3	6.67 (2H, s)	6.66 (2H, s)	6.47 (2H, s)
4	-	-	-
5	3.18 (1H, m)	3.17 (1H, m)	3.79 – 3.71 (7H, m) – overlaps with **OMe**
6	2.93 (1H, dd, *J* = 17.2, 11.7 Hz)	2.92 (1H, dd, *J* = 17.1, 11.4 Hz)	2.93 (1H, dd, *J* = 17.2, 8.3 Hz)
	2.77 (1H, dd, *J* = 17.1, 8.4 Hz)	2.76 (1H, dd, *J* = 17.1, 8.4 Hz)	2.81 (1H, dd, *J* = 17.2, 8.4 Hz)
7	-	-	-
8	4.52 (1H, m)	4.51 (1H, m)	4.93 – 4.85 (1H, m)
Me	1.28 (3H, d, *J* = 6.1 Hz)	1.28 (3H, d, *J* = 6.1 Hz)	0.90 (3H, d, *J* = 6.5 Hz)
	^{13}C (ppm, DMSO-d_6)		
	Experimental (*anti*-)	**Literature (*anti*-)**	**Experimental (*syn*-)**
1	135.1	134.8	134.6
OH	-	-	-
2	148.5	148.1	147.9
OMe	56.5	56.1	56.1
3	105.5	105.3	105.5
4	129.0	128.6	127.7
5	49.6	49.0	43.7
6	37.7	37.1	33.1
7	176.1	175.6	176.7
8	82.9	82.4	79.4
Me	19.0	18.6	16.3

Experimental Procedure for the Synthesis of Allylic Alcohols (±)-2c-f

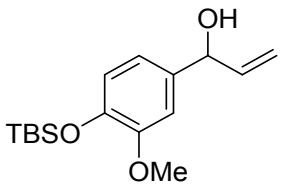
1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-one (S8)



Same experimental procedure was followed as described for the synthesis of enone **S1** but starting from enone **S7**. Purification by silica gel chromatography using 2-5% EtOAc in petroleum ether gave compound **S8** as colourless oil (0.40 g, 1.36 mmol, 81%).

IR (neat) ν_{max} 2929, 1666, 1589, 1508, 1417, 1276, 1168; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. For $C_{16}H_{24}O_3SiNa^+$ 315.1392; found 315.1382; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.54 (d, *J* = 2.0 Hz, 1H), 7.48 (dd, *J* = 8.1, 2.0 Hz, 1H), 7.17 (dd, *J* = 16.9, 10.4 Hz, 1H), 6.88 (d, *J* = 8.2 Hz, 1H), 6.41 (dd, *J* = 17.0, 1.8 Hz, 1H), 5.83 (dd, *J* = 10.5, 1.8 Hz, 1H), 3.85 (s, 3H), 0.98 (s, 9H), 0.17 (s, 6H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 189.2, 151.3, 150.2, 132.0, 131.3, 128.9, 123.1, 120.3, 111.7, 55.5, 25.7, 18.5, -4.5.

1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-ol ((±)-2c)

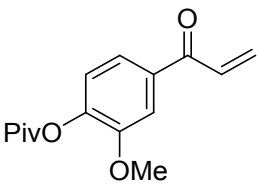


Same experimental procedure was followed as described for the synthesis of (±)-**2a**. $NaBH_4$ (0.26 g, 6.80 mmol, 5.0 eq) was added, gradually, to a cooled stirring solution of $CeCl_3.7H_2O$ (0.61 g, 1.63 mmol, 1.2 eq) and compound **S8** (0.40 g, 1.36 mmol, 1.0 eq) in MeOH (12 mL). The resulting mixture was left to stir at 0 °C for 1 h. Purification by silica gel chromatography using 10-20% EtOAc in petroleum ether gave allylic alcohol (±)-**2c** as light yellow oil (0.36 g, 1.22 mmol, 90%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.87 (d, J = 1.7 Hz, 1H), 6.78–6.80 (m, 2H), 6.03 (ddd, J = 16.2, 10.3, 5.7 Hz, 1H), 5.31 (dt, J = 17.1, 1.2 Hz, 1H), 5.17 (dt, J = 10.3, 1.2 Hz, 1H), 5.11 (d, J = 5.7 Hz, 1H), 3.79 (s, 3H), 2.12 (s, 1H), 0.98 (s, 9H), 0.14 (s, 6H).

^{1}H NMR Spectroscopic data was consistent with previously reported data.[S4]

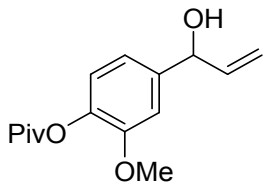
1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-one (S9)



Same experimental procedure was followed as described for the synthesis of enone **S1** but starting from **S7**. Purification by silica gel chromatography using 2-5% EtOAc in petroleum ether gave compound **S9** as colourless oil (0.40 g, 1.52 mmol, 90%).

IR (neat) ν_{max} 2972, 1755, 1670, 1597, 1506, 1413, 1274, 1101; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. For $C_{15}H_{18}O_4Na^+$ 285.1103; found 285.1092; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 1.8 Hz, 1H), 7.53 (dd, *J* = 8.0, 1.8 Hz, 1H), 7.14 (dd, *J* = 17.0, 10.5 Hz, 1H), 7.09 (d, *J* = 8.2 Hz, 1H), 6.43 (dd, *J* = 17.0, 1.7 Hz, 1H), 5.91 (dd, *J* = 10.5, 1.6 Hz, 1H), 3.86 (s, 3H), 1.36 (s, 9H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 189.8, 176.3, 151.7, 144.5 , 135.8, 132.1, 130.3, 122.8, 122.1, 112.2, 56.1, 39.4, 27.2.

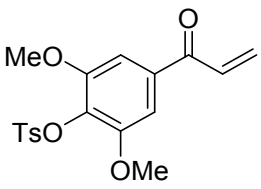
1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-ol ((±)-2d)



Same experimental procedure was followed as described for the synthesis of (±)-**2a**. $NaBH_4$ (0.28 g, 6.80 mmol, 5.0 eq) was added, gradually, to a cooled stirring solution of $CeCl_3.7H_2O$ (0.67 g, 1.63 mmol, 1.2 eq) and compound **S9** (0.40 g, 1.52 mmol, 1.0 eq) in MeOH (15 mL). The resulting mixture was left to stir at 0 °C for 1 h. Purification by silica gel chromatography using 10-20% EtOAc in petroleum ether gave allylic alcohol (±)-**2d** as light yellow oil (0.28 g, 1.06 mmol, 69%).

IR (neat) ν_{max} 3527, 2972, 11728, 1604, 1506, 1421, 1282, 1112, 1028; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{15}H_{20}O_4Na^+$ 287.1254; found 287.1250; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.91–6.93 (m, 2H), 6.85 (ddd, *J* = 8.1, 1.9, 0.6 Hz, 1H), 5.27 (dt, *J* = 17.0, 1.4 Hz, 1H), 5.13 (dt, *J* = 10.3, 1.2 Hz, 1H), 5.05 (d, *J* = 5.9 Hz, 1H), 3.74 (s, 3H), 2.85 (s, 1H), 1.35 (s, 9H) **^{13}C NMR** (101 MHz, $CDCl_3$) δ 176.8, 151.1, 141.4, 140.1, 139.4, 122.4, 118.5, 115.1, 110.4, 74.8, 55.8, 39.0, 27.2.

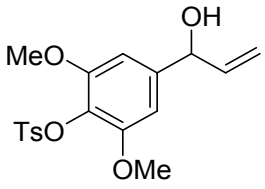
1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-one (S10)



Tosyl chloride (0.27 g, 1.44 mmol, 1.0 eq) was added to a stirring solution of enone **8** (0.30 g, 1.44 mmol, 1.0 eq) and potassium carbonate (0.37 g, 2.71 mmol, 1.88 eq) in THF/H_2O (1:1) (14 mL) at room temperature. The resulting mixture was left to stir for 3 h. After the reaction reached completion (TLC analysis), the mixture was neutralised with saturated aqueous solution of NH_4Cl (25 mL) and extracted with EtOAc (2 x 30 mL). Combined organic layers were washed with water (25 mL), brine (25 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography using 5-10% EtOAc in petroleum ether gave **S10** (0.45 g, 1.24 mmol, 86%) as a colourless oil.

IR (neat) ν_{max} 1670, 1595, 1460, 1416, 1371, 1334, 1238, 1149, 1128, 1089; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{18}H_{18}O_6SNa^+$ 385.0716 found 385.0710; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.85 (d, *J* = 8.4 Hz, 2H), 7.33 (d, *J* = 7.9 Hz, 2H), 7.13 (s, 2H), 7.08 (dd, *J* = 17.1, 10.5 Hz, 1H), 6.44 (dd, *J* = 17.0, 1.5 Hz, 1H), 5.94 (dd, *J* = 10.5, 1.5 Hz, 1H), 3.73 (s, 6H), 2.45 (s, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 189.8, 153.6, 144.9, 136.1, 134.8, 132.0, 130.9, 129.3, 128.4, 105.6, 56.3, 21.8.

1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-ol ((±)-2e)

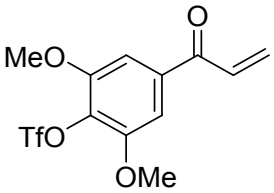


The same experimental procedure was followed as described for the synthesis of (±)-**2a**. $NaBH_4$ (0.23 g, 6.20 mmol, 5.0 eq) was added, gradually, to a cooled stirring solution of $CeCl_3.7H_2O$ (0.55 g, 1.48 mmol, 1.2 eq) and compound **S10** (0.45 g, 1.24 mmol, 1.0 eq) in MeOH (15 mL). The resulting mixture was left to stir at 0 °C for 1 h. Purification by silica gel chromatography using 10-20% EtOAc in petroleum ether gave allylic alcohol (±)-**2e** as light yellow oil (0.35 g, 0.96 mmol, 77%).

IR (neat) ν_{max} 3498, 2978, 1597, 1500, 1465, 1417, 1363, 1172, 1124, 1089, 1002; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{18}H_{20}O_6SNa^+$ 387.0878; found 387.0866; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.3 Hz, 2H), 7.28 (d, *J* = 8.3 Hz, 2H), 6.53 (s, 2H), 5.92 (ddd, *J* = 16.4, 10.3, 6.1 Hz, 1H), 5.29 (dt, *J* = 17.0, 1.3 Hz, 1H), 5.14 (dt, *J* = 10.2, 1.3 Hz, 1H), 5.04 (d, *J* = 6.0 Hz, 1H), 3.59

(s, 6H), 2.65 (s, 1H), 2.40 (s, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 153.1, 144.5, 142.5, 139.7, 134.7, 129.1, 128.2, 127.0, 115.5, 102.7, 74.9, 55.8, 21.6. **^{19}F NMR** (376 MHz, $CDCl_3$) δ -73.8.

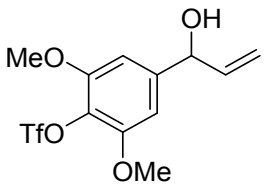
1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-one (S11)



Triflic anhydride (0.44 g, 1.58 mmol, 1.1 eq) was added to a stirring solution of enone **8** (0.30 g, 1.44 mmol, 1.0 eq) and pyridine (0.22 g, 0.23 mL, 2.88 mmol, 2.0 eq) in DCM (14 mL) at 0 °C. The resulting mixture was allowed to warm up to room temperature and left to stir for 1 h. After the reaction reached completion (TLC analysis), the mixture was neutralised using 1 M HCl (25 mL) and extracted with EtOAc (2 x 30 mL). Combined organic layers were washed with water (25 mL), brine (25 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography using 5-10% EtOAc in petroleum ether gave **S11** (0.42 g, 1.23 mmol, 85%) as a colourless oil.

IR (neat) ν_{max} 2924, 1668, 1604, 1465, 1413, 1338, 1238, 1220, 1205, 1130; **HRMS** (NSI) *m/z* $[M+Na]^+$ calcd. for $C_{12}H_{12}F_3O_6S^+$ 341.0301; found 341.0304; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.18 (s, 2H), 7.09 (dd, *J* = 17.0, 10.6 Hz, 1H), 6.46 (dd, *J* = 17.0, 1.5 Hz, 1H), 5.99 (dd, *J* = 10.6, 1.3 Hz, 1H), 3.95 (s, 6H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 189.6, 152.6, 137.2, 131.8, 131.4, 119.9, 117.4, 105.4, 56.6; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -73.6.

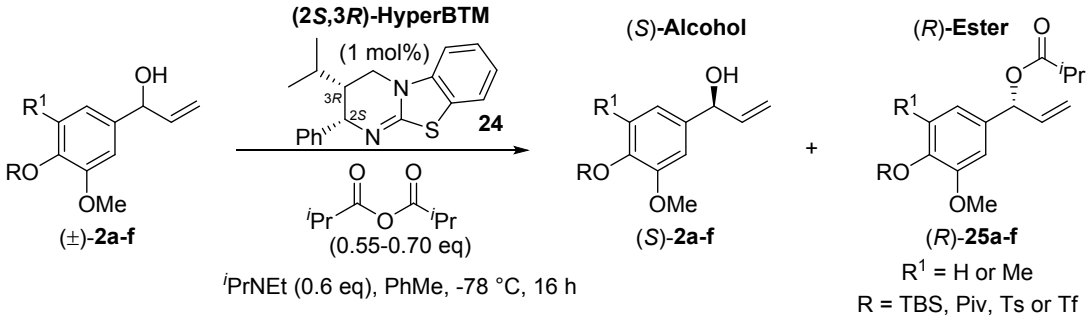
1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-ol ((±)-2f)



The same experimental procedure was followed as described for the synthesis of (±)-**2a**. $NaBH_4$ (0.23 g, 6.15 mmol, 5.0 eq) was added, gradually, to a cooled stirring solution of $CeCl_3.7H_2O$ (0.54 g, 1.47 mmol, 1.2 eq) and compound **S11** (0.40 g, 1.23 mmol, 1.0 eq) in MeOH (15 mL). The resulting mixture was left to stir at 0 °C for 1 h. Purification by silica gel chromatography using 10-20% EtOAc in petroleum ether gave allylic alcohol (±)-**2f** as light yellow oil (0.30 g, 0.87 mmol, 71%).

IR (neat) ν_{max} 3338, 1610, 1500, 1465, 1417, 1340, 1224, 1136; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{12}H_{13}F_3O_6SNa^+$ 365.0283; found 365.0271; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.63 (s, 2H), 5.94 (ddd, *J* = 16.8, 10.3, 6.5 Hz, 1H), 5.35 (dt, *J* = 17.0, 1.2 Hz, 1H), 5.21 (dt, *J* = 10.2, 1.2 Hz, 1H), 5.10 (d, *J* = 6.2 Hz, 1H), 3.85 (s, 6H), 2.39 (s, 1H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 152.4, 143.7, 139.5, 120.0, 117.4, 116.2, 102.7, 75.1, 56.3; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -73.8.

Experimental procedure for Isothiourea-catalysed Kinetic Resolution Reactions of Allylic Alcohols (±)-2a-f



General Procedure – Kinetic Resolution with HyperBTM:

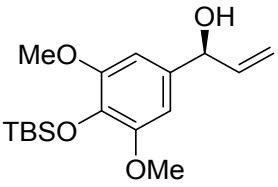
(2*S*,3*R*)-HyperBTM **24** (1 mol %, from catalyst stock solution (see below for preparation)), $^{i}Pr_2NEt$ (0.60 eq) and propionic anhydride (0.50–0.70 eq) were added sequentially to a cooled stirring solution of the appropriate alcohol (1.0 eq) in PhMe (0.35 M) at –78 °C. The resulting mixture was stirred for 16 h. Afterwards, it was quenched with 1 M HCl. The solution was then diluted with EtOAc and washed with $NaHCO_3$ (2 x 10 mL) and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The alcohol and ester were purified by column chromatography and analysed by chiral HPLC.

Note: (2*S*,3*R*)-HyperBTM enantiomer **24** was used in all the kinetic reactions, unless otherwise stated.

Preparation of catalyst stock solution[S5]

HyperBTM (50 mg) and toluene (3 mL) were placed in a 5 mL volumetric flask. Once the mixture was homogeneous toluene was added until the total volume of the mixture had reached 5 mL. The concentration of the solution was 0.032 M.

(*S*)-1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-ol ((*S*)-2a)



Following the general procedure, the alcohol (±)-**2a** (408 mg, 1.26 mmol), HyperBTM (3.9 mg, 13 µmol, 1 mol %), $^{i}Pr_2NEt$ (132 µL, 0.76 mmol) and isobutyric anhydride (133 µL, 0.82 mmol) were reacted in PhMe (15 mL) for 16 h to give the crude product. Purification by silica gel column chromatography using 20% EtOAc in petroleum ether gave resolved alcohol (*S*)-**2a** (180 mg, 0.55 mmol, 44%) and ester (*R*)-**25a** (250 mg, 0.63 mmol, 50%). X-ray crystallographic analysis confirmed the absolute configuration of (*S*)-**2a** (CCDC number 1505596)[S2]

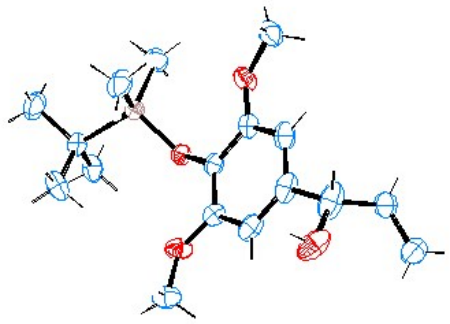


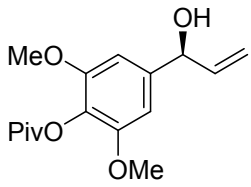
Figure S4 Thermal ellipsoid plot of (*S*)-**2a** at 50 % ellipsoid probability.[S2] The minor component of disorder has been omitted for clarity.

^{1}H NMR analysis was consistent with (±)-**2a** reported above.

Alcohol (*S*)-**2a: Specific Rotation** $[\propto]_D^{20}$ = +4.5 (*c* = 2.0, $CHCl_3$); **Chiral HPLC analysis**: Chiralpak AD-H (99:1 Hexane:IPA, flow rate 1.0 mL min^{-1}, 35 °C) t_R (*S*-enantiomer): 21.5 min, t_R (*R*-enantiomer): 31.1 min, >99% *ee*.

Ester (*R*)-**25a: Specific Rotation** $[\alpha]_D^{20}$ +19.2 (*c* = 0.5, $CHCl_3$); **Chiral HPLC analysis**: Chiralpak IA (99.8:0.2 Hexane:IPA, flow rate 1.0 mL min^{-1}, 30 °C) t_R (*R*-enantiomer): 5.2 min, t_R (*S*-enantiomer): 5.9 min, 78% *ee*.

(*S*)-4-(1-hydroxyallyl)-2,6-dimethoxyphenyl pivalate ((*S*)-2b)



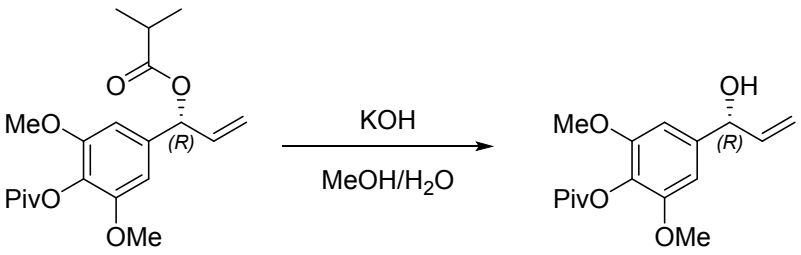
Following the general procedure, the alcohol (±)-**2b** (101 mg, 0.344 mmol), HyperBTM (108 µL from stock solution, 3 µmol, 1 mol %), $^{i}Pr_2NEt$ (42 µL, 0.24 mmol) and isobutyric anhydride (38 µL, 0.23 mmol) were reacted in PhMe (1.2 mL) for 16 h to give the crude product. Purification by silica gel column chromatography using 20% EtOAc in petroleum ether gave resolved alcohol (*S*)-**2b** (27 mg, 0.09 mmol, 30%) and ester (*R*)-**25b** (53 mg, 0.14 mmol, 52%).

^{1}H NMR analysis was consistent with (±)-**2b** reported above.

Alcohol (*S*)-**2b**: **Specific Rotation** $[\propto]_D^{20}$ = +8.0 (*c* = 0.2, $CHCl_3$); **Chiral HPLC analysis**: Chiralcel OJ-H (99:1 Hexane:IPA, flow rate 1.0 mL min^{-1}, 30 °C) t_R (*S*-enantiomer): 42.2 min, t_R (*R*-enantiomer): 49.8 min, >99% *ee*.

Ester (*R*)-**25b**: **Specific Rotation** $[\propto]_D^{20}$ = +30.5 (*c* = 1.0, $CHCl_3$), **Chiral HPLC analysis** Chiralcel OJ-H (99:1 Hexane:IPA, flow rate 1.0 mL min^{-1}, 30 °C) t_R (*R*-enantiomer): 6.2 min, t_R (*S*-enantiomer): 8.7 min, 75% *ee*.

Hydrolysis of 25b – to obtain (*R*)-2b



25b **2b**

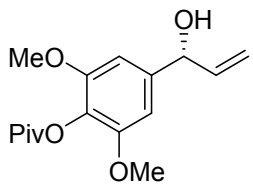
To a solution of ester **25b** (925 mg, 2.43 mmol, 76% *ee*) in MeOH (10 mL) was added dropwise a solution of KOH (134 mg, 2.38 mmol) in MeOH/H_2O (7 mL, 5:2) at room temperature and the reaction mixture was stirred for 16 h. After, H_2O was added and the mixture was extracted with CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated

under reduced pressure to produce a white solid (744 mg, 99%, 76% *ee*) without any further purification.

^{1}H NMR analysis was consistent with (±)-**2b** reported above.

Alternatively, (*R*)-**2b** could be obtained in high *ee* by using (2*R*,3*S*)-HyperBTM.

(*R*)-4-(1-hydroxyallyl)-2,6-dimethoxyphenyl pivalate ((*R*)-2b)

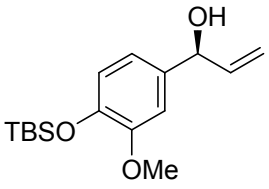


Following the general procedure, alcohol (±)-**2b** (723 mg, 2.46 mmol), (2*R*,3*S*)-HyperBTM (7.6 mg, 25 µmol, 1 mol %), ^{i}Pr$_2$NEt (64 µL, 0.37 mmol) and isobutyric anhydride (56 µL, 0.34 mmol) were reacted in toluene (7 mL) for 48 h to give the crude product. Purification by silica gel column chromatography using 20% EtOAc in petroleum ether gave resolved alcohol (*R*)-**2b** (548 mg, 1.86 mmol, 75%) and ester (*S*)-**25b'** (85 mg, 0.23 mmol, 9%).

Alcohol (*R*)-**2b**: **Specific Rotation** $[\propto]_D^{20}$ = -15.3 (*c* = 0.1, $CHCl_3$); **Chiral HPLC analysis**: Chiralcel OJ-H (99:1 Hexane:IPA, flow rate 1.0 mL min^{-1}, 30 °C) t_R (*S*-enantiomer): 42.2 min, t_R (*R*-enantiomer): 49.8 min, 92% *ee*.

Ester (*S*)-**25b': Specific Rotation** $[\propto]_D^{20}$ = −16.6 (*c* = 1.0, $CHCl_3$), **Chiral HPLC analysis** Chiralcel OJ-H (99:1 Hexane:IPA, flow rate 1.0 mL min^{-1}, 30 °C) t_R (*R*-enantiomer): 6.2 min, t_R (*S*-enantiomer): 8.7 min, 52% *ee*.

(*S*)-1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-ol ((*S*)-2c)

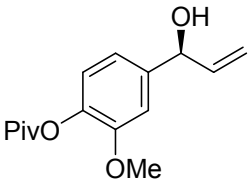


Following the general procedure, the alcohol **(±)-2c** (85 mg, 0.29 mmol), HyperBTM (90 µL from stock solution, 3 µmol, 1 mol %), ^{i}Pr$_2$NEt (30 µL, 0.17 mmol) and isobutyric anhydride (30 µL, 0.19 mmol) were reacted in toluene (0.8 mL) for 16 h to give the crude product. Purification by silica gel column chromatography using 20% EtOAc in petroleum ether gave resolved alcohol (*S*)-**2c** (33 mg, 0.11 mmol, 39%) and ester (*R*)-**25d** (44 mg, 0.12 mmol, 43%).

Alcohol (*S*)-**2c: Specific Rotation** $[\alpha]_D^{20}$ −3.2 (*c* 0.5, $CHCl_3$); **Chiral HPLC analysis:** Chiral HPLC analysis Chiralpak AD-H (99.5:0.5 hexane : IPA, flow rate 1.0 mL min^{-1}, 220 nm, 30 °C) t_R (*S*-enantiomer): 23.7 min, t_R (*R*-enantiomer): 29.1 min, 89% *ee*.

Ester (*R*)-**25c: Specific Rotation** $[\alpha]_D^{20}$ +23.4 (*c* 1.0, $CHCl_3$); **Chiral HPLC analysis**: Chiralpak IA (99.8:0.2 hexane : IPA, flow rate 1.0 mL min^{-1}, 211 nm, 30 °C) t_R (*R*-enantiomer): 5.2 min, t_R (*S*-enantiomer): 5.8 min, 81% *ee*.

(*S*)-1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-ol ((*S*)-2d)



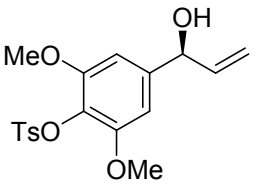
Following the general procedure, the alcohol **(±)-2d** (95 mg, 0.36 mmol), HyperBTM (112 µL from stock solution, 4 µmol, 1 mol %), $^iPr_2NEt$ (37 µL, 0.21 mmol) and isobutyric anhydride (38 µL, 0.23 mmol) were reacted in toluene (1.0 mL) for 16 h to give the crude product. Purification by silica gel column chromatography using 20% EtOAc in petroleum ether gave resolved alcohol (*S*)-**2d** (36 mg, 0.14 mmol, 39%) and ester (*R*)-**25d** (56 mg, 0.17 mmol, 47%).

Alcohol (*S*)-**2d: Specific Rotation** $[\alpha]_D^{20}$ = +8.2 (*c* = 1.0, $CHCl_3$); **Chiral HPLC analysis**: Chiralpak AD-H (95:5 Hexane: IPA, flow rate 1.0 mL min^{-1}, 30 °C) t_R (*S*-enantiomer): 11.4 min, t_R (*R*-enantiomer): 15.7 min, 97% *ee*.

^1H NMR analysis was consistent with (±)-**2d** reported above.

Ester (*R*)-**25d: Specific Rotation** $[\alpha]_D^{20}$ = +30.5 (*c* = 2.0, $CHCl_3$); **Chiral HPLC analysis**: Chiralpak AD-H (99:1 Hexane:IPA, flow rate 1.0 mL min^{-1}, 30 °C) t_R (*R*-enantiomer): 12.3 min, t_R (*S*-enantiomer): 14.7 min, 85% *ee*.

(*S*)-1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-ol ((*S*)-2e)



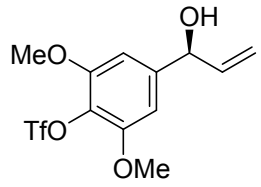
Following the general procedure, the alcohol (±)-**2e** (55 mg, 0.15 mmol), HyperBTM (47 µL from stock solution, 1 µmol, 1 mol %), $^{i}Pr_2NEt$ (16 µL, 0.09 mmol) and isobutyric anhydride (15 µL, 0.09 mmol) were reacted in THF (0.8 mL) for 16 h to give the crude product. Purification by silica gel column chromatography using 30% EtOAc in petroleum ether furnished resolved alcohol (*S*)-**2e** (20 mg, 0.05 mmol, 36%) and ester (*R*)-**25e** (27 mg, 0.06 mmol, 41%).

Alcohol (*S*)-**2e: Specific Rotation** $[\alpha]_D^{20}$ = +10.7 (*c* = 1.0, $CHCl_3$); **Chiral HPLC analysis:** Chiralpak IB (92:8 Hexane:IPA, flow rate 1.0 mL min^{-1}, 30 °C) t_R (*S*-enantiomer): 35.7 min, t_R (*R*-enantiomer): 39.9 min, 93% *ee*.

^{1}H NMR analysis was consistent with (±)-**2e** reported above.

Ester (*R*)-**25e: Specific Rotation** $[\alpha]_D^{20}$ = +22.4 (*c* = 1.2, $CHCl_3$); **Chiral HPLC analysis**: Chiralpak IB (92:8 Hexane:IPA, flow rate 1.0 mL min^{-1}, 30 °C) t_R (*R*-enantiomer): 10.5 min, t_R (*S*-enantiomer): 11.7 min, 72% *ee*.

(*S*)-1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-ol ((*S*)-2f)



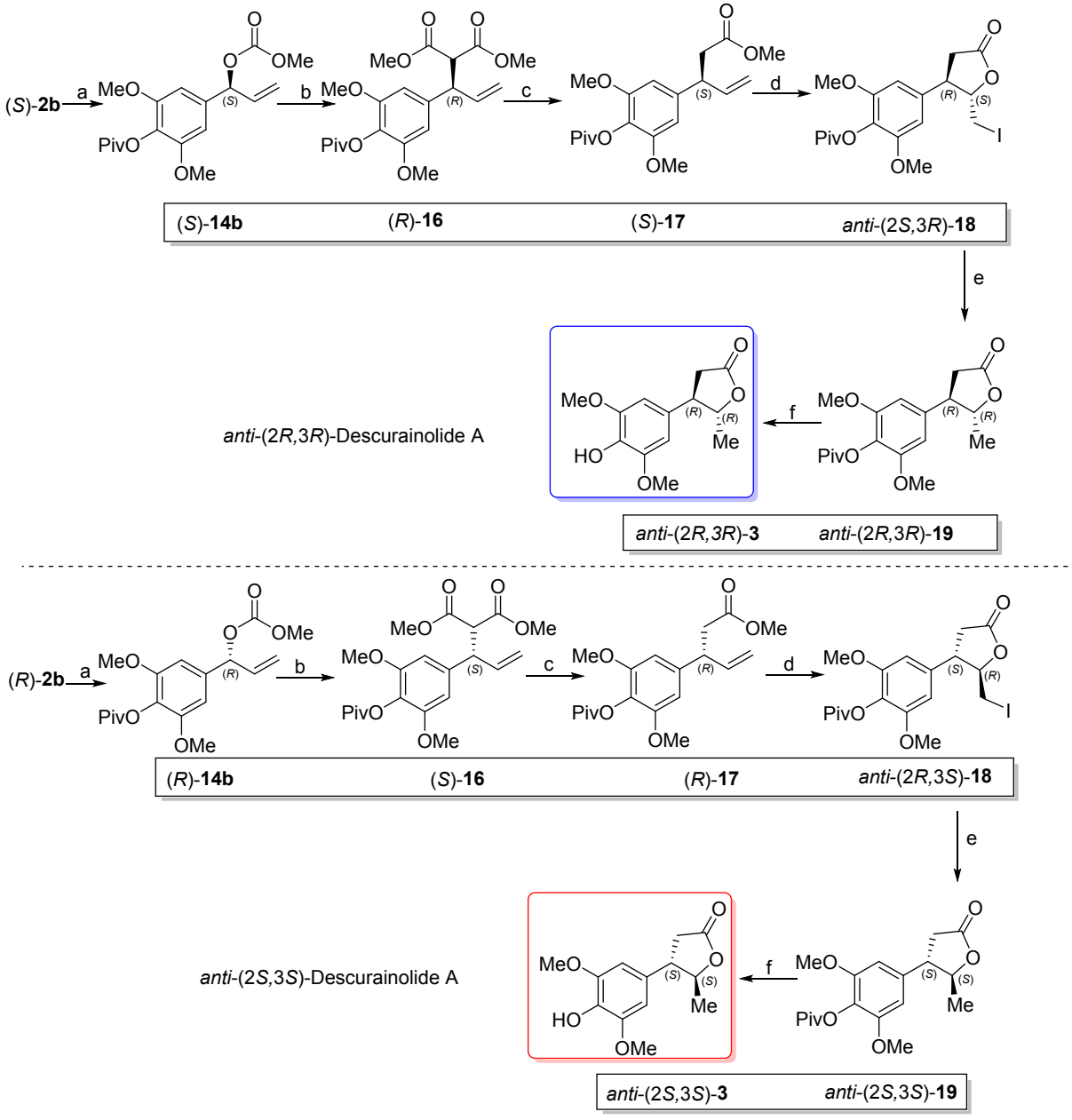
Following the general procedure, the alcohol (±)-**2f** (120 mg, 0.35 mmol), HyperBTM (109 µL from stock solution, 3 µmol, 1 mol %), $^{i}Pr_2NEt$ (36 µL, 0.21 mmol) and isobutyric anhydride (31 µL, 0.19 mmol) were reacted in toluene (1.2 mL) for 16 h to give the crude product. Purification by silica gel column chromatography using 20% EtOAc in petroleum ether furnished resolved alcohol (*S*)-**2f** (58 mg, 0.17 mmol, 48%) and ester (*R*)-**20f** (50 mg, 0.12 mmol, 35%).

^{1}H NMR analysis was consistent with (±)-**2f** reported above.

Alcohol (*S*)-**2f: Specific Rotation** $[\alpha]_D^{20}$ = +9.6 (*c* = 1.5, $CHCl_3$); **Chiral HPLC analysis**: Chiralpak AD-H (95:5 Hexane:IPA, flow rate 1.0 mL min^{-1}, 30 °C) t_R (*S*-enantiomer): 10.7 min, t_R (*R*-enantiomer): 13.3 min, 55% *ee*.

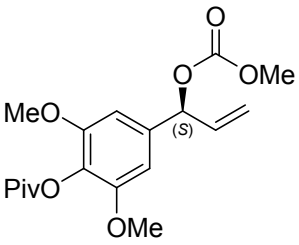
Ester (*R*)-**25f: Specific Rotation** $[\alpha]_D^{20}$ = +20.9 (*c* = 2.0, $CHCl_3$); **Chiral HPLC analysis**: Chiralpak AD-H (99:1 Hexane:IPA, flow rate 1.0 mL min^{-1}, 30 °C) t_R (*R*-enantiomer): 10.3 min, t_R (*S*-enantiomer): 14.4 min, 85% *ee*.

Asymmetric Synthesis of *anti*-(2*R*,3*R*)- and *anti*-(2*S*,3*S*)-Descurainolide A



Scheme S4 Asymmetric Synthesis of *anti*-(2R,3*R*) and *anti*-(2*S*,3*S*)-Descurainolide A

(*S*)-2,6-dimethoxy-4-(1-((methoxycarbonyl)oxy)allyl)phenyl pivalate ((*S*)-14b)



Same experimental procedure was followed as described for the synthesis of (±)-**14b**. LiHMDS (1.0 M in THF, 1.01 mL, 1.01 mmol, 1.2 eq) was added to a cooled solution of (*S*)-**2b** (0.25 g, 0.84 mmol, 1.0 eq, 99% *ee*) in THF (8 mL) at -10 °C. The resulting mixture was stirred at the same temperature for 15 min and then methyl chloroformate (95.6 mg, 1.01 mmol, 1.2 eq) was added. Purification by silica gel chromatography using 2-5% EtOAc in petroleum ether afforded (*S*)-**14b** (0.22 g, 0.62 mmol, 74% yield, 99% *ee*) as a colourless oil. X-ray crystallographic analysis confirmed the absolute configuration of (*S*)-**14b** (CCDC number 1505598).[S2]

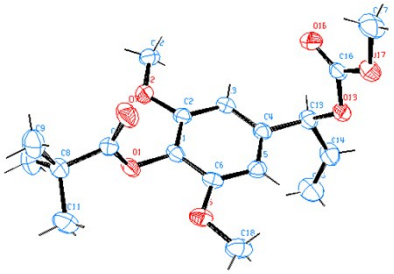
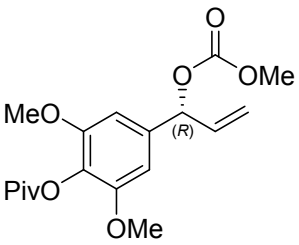


Figure S5 Thermal ellipsoid plot of (*S*)-**14b** at 50% ellipsoid probability.[S2]

^{1}H NMR analysis was consistent with (±)-**14b** reported above.

Specific Rotation $[\propto]_D^{20}$ = -36.3 (*c* = 1.0, $CHCl_3$); **Chiral HPLC analysis**: Chiralcel OJ-H (99.5:0.5) Hexane: IPA, flow rate 1.0 mL min^{-1}, 30 ^{o}C) t_R (*R*-enantiomer) = 16.4 min, t_R (*S*-enantiomer) = 19.0 min.

(*R*)-2,6-dimethoxy-4-(1-((methoxycarbonyl)oxy)allyl)phenyl pivalate ((*R*)-14b)



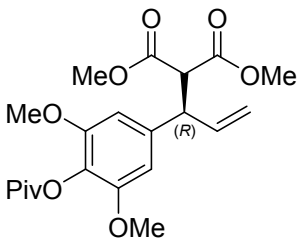
Same experimental procedure was followed as described for the synthesis of (±)-**14b**. LiHMDS (1.0 M in THF, 1.01 mL, 1.01 mmol, 1.2 eq) was added to a cooled solution of (*R*)-**2b** (0.24 g, 0.84 mmol, 1.0 eq, 92% *ee*) in THF (8 mL) at -10 °C. The resulting mixture was stirred at the same temperature for 15 min and then methyl chloroformate

(95.6 mg, 1.01 mmol, 1.2 eq) was added. Purification by silica gel chromatography using 2-5% EtOAc in petroleum ether afforded (*R*)-**14b** (0.28 g, 0.79 mmol, 95% yield, 93% *ee*) as a light-yellow oil.

^{1}H NMR analysis was consistent with (±)-**14b** reported above.

Specific Rotation $[\propto]_D^{20}$ = +33.7 (*c* = 0.4, $CHCl_3$); **Chiral HPLC analysis**: Chiralcel OJ-H (99.5:0.5) Hexane: IPA, flow rate 1.0 mL min^{-1}, 30 ^{o}C) t_R (*R*-enantiomer) = 15.8 min, t_R (*S*-enantiomer) = 20.3 min.

Dimethyl (*R*)-2-(1-(3,5-dimethoxy-4-(pivaloyloxy)phenyl)allyl)malonate ((*R*)-16)

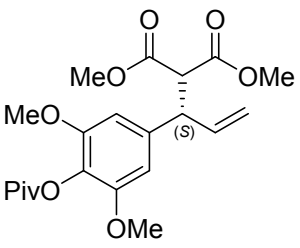


Same experimental procedure was followed as described for the synthesis of (±)-**16**. $P(OMe)_3$ (14.1 mg, 0.11 mmol, 20 mol%) was added to a burgundy-red solution of $Rh(PPh_3)Cl$ (26.4 mg, 0.03 mmol, 5 mol%) in THF (10 mL) at 40 °C. The resulting light-yellow solution was stirred at 40 °C for 30 min and then allowed to cool to room temperature. In a separate vessel, dimethyl malonate (0.15 g, 1.14 mmol, 2.0 eq) was added to the slurry of NaH (60% in mineral oil, 45.6 mg, 1.14 mmol, 2.0 eq) in THF (10 mL) at room temperature, after 30 min it was transferred *via* Teflon cannula to the vessel containing the catalyst solution at *room temperature*. A solution of allylic carbonate (*S*)-**14b** (0.20 g, 0.57 mmol, 1.0 eq, 99% *ee*) in THF (5 mL) was added to the mixture at room temperature. The resulting mixture was stirred at room temperature for 12 h. Purification by silica gel chromatography using 10-20% EtOAc in petroleum ether furnished (*R*)-**16** (0.20 g, 0.49 mmol, 85% yield, 98% *ee*).

^{1}H NMR analysis was consistent with (±)-**16** reported above.

Specific Rotation $[\propto]_D^{20}$ = +12.5 (*c* = 0.8, $CHCl_3$); **Chiral HPLC analysis**: Chiralcel OJ-H (97:3 Hexane:IPA, flow rate 0.5 mL min^{-1}, 40 ^{o}C) t_R (*R*-enantiomer) = 20.6 min, t_R (*S*-enantiomer) = 23.7 min.

Dimethyl (*S*)-2-(1-(3,5-dimethoxy-4-(pivaloyloxy)phenyl)allyl)malonate ((*S*)-16)



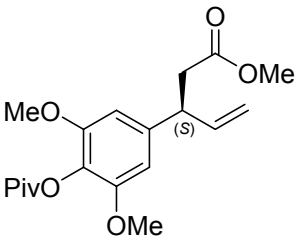
Same experimental procedure was followed as described for the synthesis of (±)-**16**. $P(OMe)_3$ (17.6 mg, 0.14 mmol, 20 mol%) was added to a burgundy-red solution of $Rh(PPh_3)Cl$ (32.8 mg, 0.04 mmol, 5 mol%) in THF (5 mL) at 40 °C. The resulting light-yellow solution was stirred at 40 °C for 30 min and then allowed to cool to room temperature. In a separate vessel, dimethyl malonate (0.18 g, 1.42 mmol, 2.0 eq) was added to the slurry of NaH (60% in mineral oil, 56.8 mg, 1.42 mmol, 2.0 eq) in THF (10 mL) at room temperature, after 30 min it was transferred *via* Teflon cannula to the vessel containing the catalyst solution at *room temperature*. A solution of allylic carbonate (*R*)-**14b** (0.25 g, 0.71 mmol, 1.0 eq, 93% *ee*) in THF (5 mL) was added to the mixture at room temperature. The resulting mixture was allowed to stir at room temperature for 12 h.

Purification by silica gel chromatography using 10-20% EtOAc in petroleum ether furnished (*S*)-**11** (0.23 g, 0.56 mmol, 79% yield, 93% *ee*).

^{1}H NMR analysis was consistent with (±)-**16** reported above.

Specific Rotation $[\propto]_D^{20}$ = -7.1 (*c* = 0.8, $CHCl_3$); **Chiral HPLC analysis**: Chiralcel OJ-H (97:3 Hexane:IPA, flow rate 0.5 mL min^{-1}, 40 ^{o}C) t_R (*R*-enantiomer) = 20.9 min, t_R (*S*-enantiomer) = 23.0 min.

Methyl (*S*)-3-(3,5-dimethoxy-4-(pivaloyloxy)phenyl)pent-4-enoate ((*S*)-17)

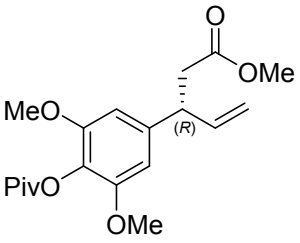


Same experimental procedure was followed as described for the synthesis of (±)-**17**. LiCl (0.12 g, 2.94 mmol, 6.0 eq) and H_2O (45 mg, 2.49 mmol, 5.1 eq) were added to a stirring solution of (*R*)-**16** (0.20 g, 0.49 mmol, 1.0 eq) in DMSO (14 mL). The resulting mixture was heated to 140 °C for 12 h. Purification by silica gel chromatography using 10-20% EtOAc in petroleum ether gave (*S*)-**17** (95 mg, 0.27 mmol, 55% yield, 99% *ee*) as a colourless oil.

^{1}H NMR analysis was consistent with (±)-**17** reported above.

Specific Rotation $[\propto]_D^{20}$ = -13.5 (*c* = 0.4, $CHCl_3$); **Chiral HPLC analysis**: Chiralcel OJ-H (97:3 Hexane:IPA, flow rate 1 mL min^{-1}, 30 °C) t_R (*S*-enantiomer) = 11.4 min, t_R (*R*-enantiomer) = 13.7 min.

Methyl (*R*)-3-(3,5-dimethoxy-4-(pivaloyloxy)phenyl)pent-4-enoate ((*R*)-17)

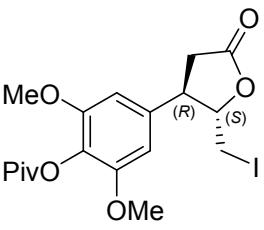


Same experimental procedure was followed as described for the synthesis of (±)-**17**. LiCl (0.19 g, 4.49 mmol, 6.0 eq) and H_2O (68 mg, 3.77 mmol, 5.1 eq) were added to a stirring solution of (*S*)-**16** (0.30 g, 0.74 mmol, 1.0 eq) in DMSO (20 mL). The resulting mixture was heated to 140 °C for 12 h. Purification by silica gel chromatography using 10-20% EtOAc in petroleum ether gave (*R*)-**17** (0.12 g, 0.35 mmol, 47% yield, 90% *ee*) as a colourless oil.

^{1}H NMR analysis was consistent with (±)-**17** reported above.

Specific Rotation $[\propto]_D^{20}$ = +14.2 (*c* = 0.4, $CHCl_3$); **Chiral HPLC analysis**: Chiralcel OJ-H (97:3 Hexane:IPA, flow rate 1 mL min^{-1}, 30 °C) t_R (*S*-enantiomer) = 11.7 min, t_R (*R*-enantiomer) = 13.7 min.

4-((2*S*,3*R*)-2-(iodomethyl)-5-oxotetrahydrofuran-3-yl)-2,6-dimethoxyphenyl pivalate ((*anti*-(2*S*,3*R*)-18))



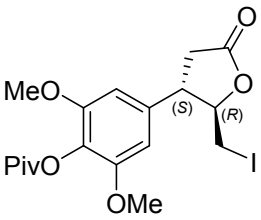
Same experimental procedure was followed as described for the synthesis of *anti*-(±)-**18**. A solution of I_2 (0.18 g, 1.43 mmol, 5.0 eq) in MeCN (3 mL) was added to a stirring solution of *anti*-(*S*)-**17** (0.10 g, 0.29 mmol, 1.0 eq) in MeCN (2 mL) at 0 °C. The resulting red solution was allowed to warm up to room temperature and left to stir for 12 h. The two diastereoisomers (10:1) were carefully separated by silica gel chromatography using 10-20% EtOAc in

petroleum ether. The desired major diastereoisomer *anti*-(2*S*,3*R*)-**18** (65 mg, 0.14 mmol, 48%, 99% *ee*) was obtained as a colourless oil**.** The remaining fractions were obtained as a mixture of diastereoisomers (25 mg, 0.05 mmol, 18%).

^{1}H NMR analysis was consistent with *anti*-(±)-**18** reported above.

Specific Rotation $[\propto]_D^{20}$ = +17.2 (*c* = 1.3, $CHCl_3$); **Chiral HPLC analysis**: Chiralpak AD-H, 95:5 IPA Hexane: IPA, flow rate 1.5 mL min^{-1}, 30 ^oC) t_R (2*S*,3*R*-enantiomer) = 17.5 min, t_R (2*R*,3*S*-enantiomer) = 20.0 min.

4-((2*R*,3*S*)-2-(iodomethyl)-5-oxotetrahydrofuran-3-yl)-2,6-dimethoxyphenyl pivalate (*anti*-(2*R*,3*S*)-18)

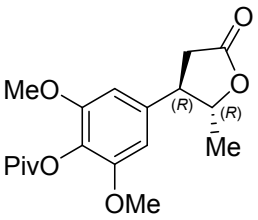


Same experimental procedure was followed as described for the synthesis of *anti*-(±)-**18**. A solution of I_2 (0.22 g, 1.75 mmol, 5.0 eq) in MeCN (5 mL) was added to a stirring solution of *anti*-(*S*)-**17** (0.12 g, 0.35 mmol, 1.0 eq) in MeCN (3 mL) at 0 °C. The resulting red solution was allowed to warm up to room temperature and left to stir for 12 h. The two diastereoisomers (10:1) were carefully separated by silica gel chromatography using 10-20% EtOAc in petroleum ether. The desired major diastereoisomer *anti*-(2*R*,3*S*)-**18** (76 mg, 0.16 mmol, 45%, 90% *ee*) was obtained as a colourless oil**.** The remaining fractions were obtained as a mixture of diastereoisomers (34 mg, 0.07 mmol, 21%).

^{1}H NMR analysis was consistent with *anti*-(±)-**18** reported above.

Specific Rotation $[\propto]_D^{20}$ = -31.5 (*c* = 0.4, $CHCl_3$); **Chiral HPLC analysis**: Chiralpak AD-H, 95:5 IPA Hexane: IPA, flow rate 1.5 mL min^{-1}, 30 ^oC) t_R (2*S*,3*R*-enantiomer) = 17.4 min, t_R (2*R*,3*S*-enantiomer) = 20.1 min.

2,6-diemthoxy-4-((2*R*,3*R*)-2-methyl-5-oxotetrahydrofuran-3-yl)phenyl pivalate ((*anti*-(2*R*, 3*R*)-19)

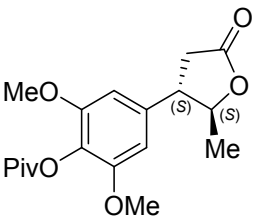


Same experimental procedure was followed as described for the synthesis of *anti*-(±)-**19**. A solution of *anti*-(2*R*,3*S*)-**18** (62 mg, 0.13 mmol, 1.0 eq) in MeOH (1 mL) was added to a black suspension of NaOAc (42.7 mg, 0.52 mmol, 4.0 eq) and Pd/C (10% wt, 14.3 mg, 0.13 mmol, 1.0 eq) in MeOH (2 mL) at room temperature. The vessel was evacuated and backfilled with H_2 gas and then left to stir at room temperature under the atmosphere of H_2 for 16 h. Purification by silica gel chromatography using 20-30% EtOAc in petroleum ether furnished *anti*-(2*R*,3*R*)-**19** (22.6 mg, 0.07 mmol, 52%, 99% *ee*) as a white solid.

^{1}H NMR analysis was consistent with *anti*-(±)-**19** reported above.

Specific Rotation $[\propto]_D^{20}$ = +3.9 (*c* = 0.3, $CHCl_3$); **Chiral HPLC analysis**: Chiralpak AD-H 97:3 Hexane: IPA, flow rate 1.0 mL min^{-1}, 30 ^{o}C) t_R (2*R*,3*R*-enantiomer) = 28.6 min, t_R (2*S*,3*S*-enantiomer) = 39.4 min

2,6-diemthoxy-4-((2*S*,3*S*)-2-methyl-5-oxotetrahydrofuran-3-yl)phenyl pivalate (*anti*-(2*S*,3*S*)-19)

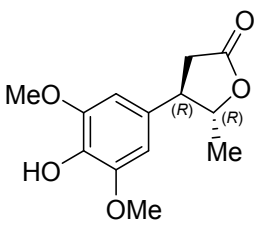


Same experimental procedure was followed as described for the synthesis of *anti*-(±)-**19**. A solution of *anti*-(2*S*,3*R*)-**18** (76.4 mg, 0.17 mmol, 1.0 eq) in MeOH (5 mL) was added to a black suspension of NaOAc (15.3 mg, 0.19 mmol, 1.1 eq) and Pd/C (10% wt, 17.6 mg, 0.17 mmol, 1.0 eq) in MeOH (5 mL) at room temperature. The vessel was evacuated and backfilled with H_2 gas and then left to stir at room temperature under the atmosphere of H_2 for 16 h. Purification by silica gel chromatography using 20-30% EtOAc in petroleum ether furnished *anti*-(2*S*,3*S*)-**19** (33.5 mg, 0.09 mmol, 59%, 91% *ee*) as a white solid.

^{1}H NMR analysis was consistent with *anti*-(±)-**19** reported above.

Specific Rotation $[\propto]_D^{20}$ = -5 (*c* = 0.3, $CHCl_3$); **Chiral HPLC analysis**: Chiralpak AD-H 97:3 Hexane: IPA, flow rate 1.0 mL min^{-1}, 30 °C) t_R (2*R*,3*R*-enantiomer) = 28.6 min, t_R (2*S*,3*S*-enantiomer) = 39.4 min.

(2*R*,3*R*)-4-(4-hydroxy-3,5-dimethoxyphenyl)-5-methyldihydrofuran-2(3*H*)-one (*anti*-(2*R*,3*R*)-3)



Same experimental procedure was followed as described for the synthesis of *anti*-(±)-**3**. 1 M HCl (2 mL) was added to a solution of *anti*-(2*R*,3*R*)-**19** (22.6 mg, 0.07 mmol, 1.0 eq) in 1,4-dioxane (2 mL). The resulting mixture was heated to 100 °C for 12 h. Purification using 30-50% EtOAc in petroleum ether furnished *anti*-(2*R*,3*R*)-**3** (10.7 mg, 0.04 mmol, 61% yield, 98% *ee*). X-ray crystallographic analysis of a sample recrystallised from EtOAc: acetone: hexane (1:1:8) confirmed the absolute configuration of *anti*-(2*R*,3*R*)-**3** (CCDC number 1510492).

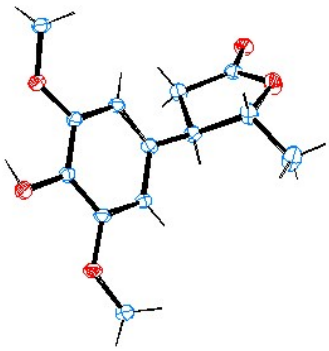
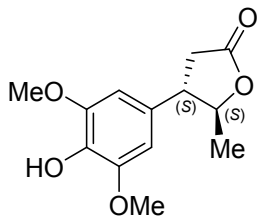


Figure S6 Thermal ellipsoid plot of *anti*-(2*R*,3*R*)-**3** at 50% elipsoid probability.[S2]

^{1}H NMR analysis was consistent with *anti*-(±)-**3** reported above.

Specific Rotation $[\propto]_D^{20}$ = +5.7 (*c* = 0.19, $CHCl_3$); on other occasions when the specific rotation was measured, this value was found to vary. **Chiral HPLC analysis**: Chiralcel OD-H (85:15 Hexane: IPA, flow rate 1.0 mL min^{-1}, 30 °C) t_R (2*R*,3*R*-enantiomer) = 40.1 min, t_R (2*S*,3*S*-enantiomer) = 52.0 min.

(2*S*,3*S*)-4-(4-hydroxy-3,5-dimethoxyphenyl)-5-methyldihydrofuran-2(3*H*)-one (*anti*-(2*S*,3*S*)-3)



Same experimental procedure was followed as described for the synthesis of *anti*-(±)-**3**. 1 M HCl (2 mL) was added to a solution of *anti*-(2*S*,3*S*)-**19** (22.6 mg, 0.07 mmol, 1.0 eq) in 1,4-dioxane (2 mL). The resulting mixture was heated to 100 °C for 12 h. Purification using 30-50% EtOAc in petroleum ether furnished *anti*-(2*S*,2*S*)-**3** (10.6 mg, 0.04 mmol, 42% yield, 91% *ee*).

^{1}H NMR analysis was consistent with *anti*-(±)-**3** reported above.

Specific Rotation $[\propto]_D^{20}$ = -5.0 (*c* = 0.7, $CHCl_3$); on other occasions when the specific rotation was measured, this value was found to vary. **Chiral HPLC analysis**: Chiralcel OD-H (85:15 Hexane: IPA, flow rate 1.0 mL min^{-1}, 30 °C) t_R (2*R*,3*R*-enantiomer) = 41.9 min, t_R (2*S*,3*S*-enantiomer) = 50.2 min.

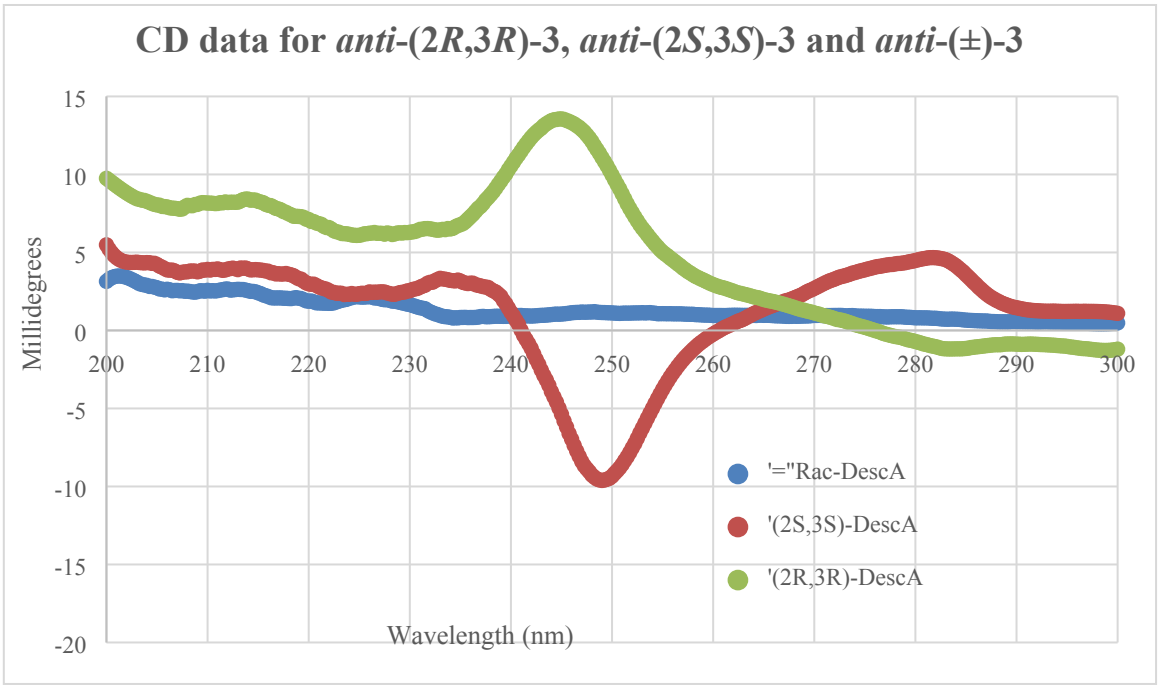
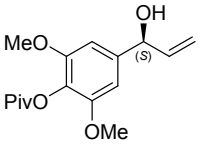
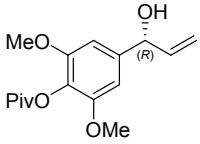
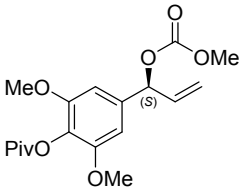
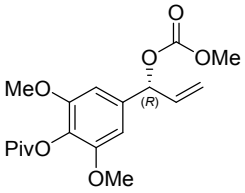
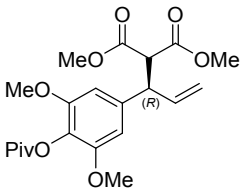
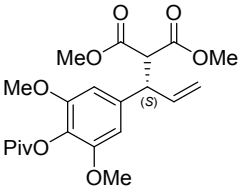
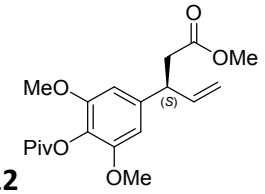
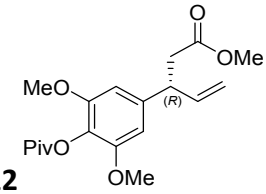
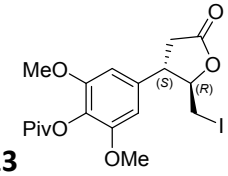
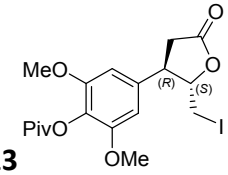
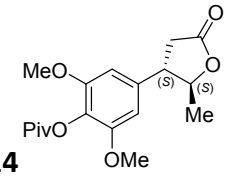
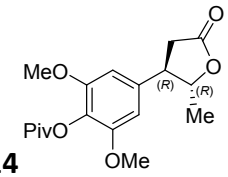
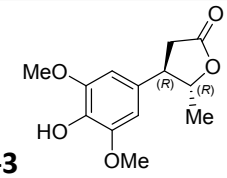
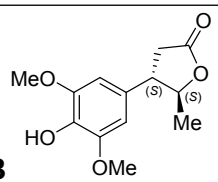


Figure S7 CD data (Millidegrees v.s. Wavelength in nm) for *anti*-(2*R*,3*R*)-**3** (green line) and *anti*-(2*S*,3*S*)-**3** (red line) between 200 – 300 nm. A spectrum for *anti*-(±)-**3** was also obtained (blue line).

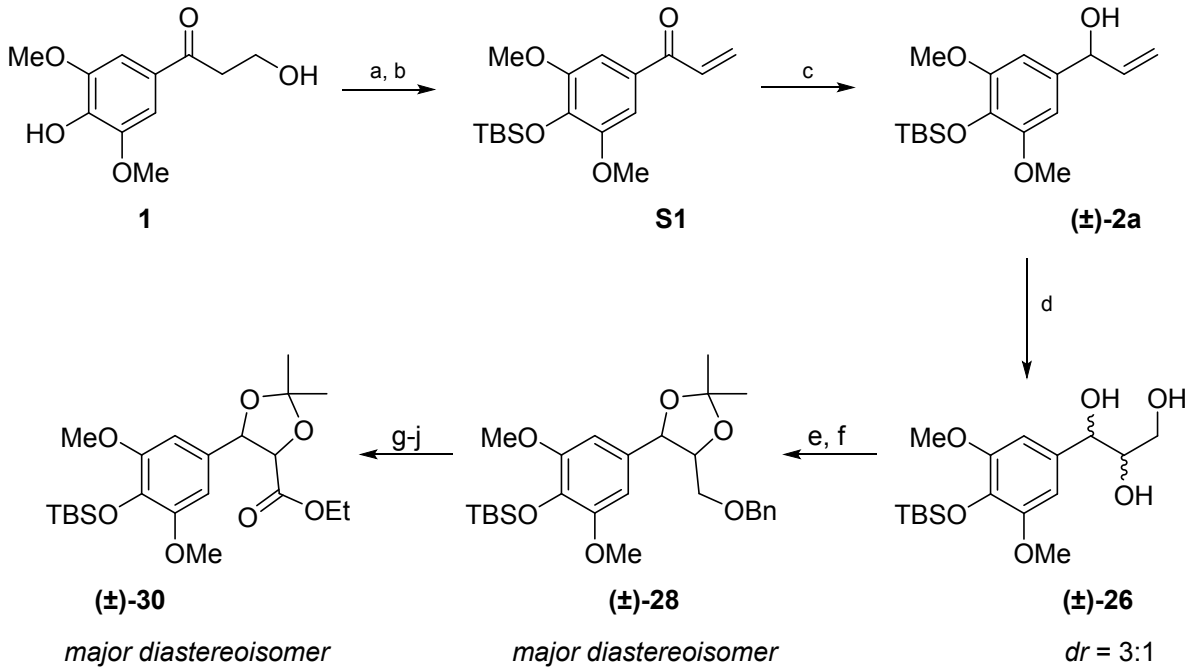
Summary of polarimetry and HPLC details for the asymmetric synthesis of Descurainolide A 3

Table S3 Polarimetry and HPLC details for the asymmetric synthesis of Descurainolide A.

Compound	**Specific Rotation** ($[\propto]_D^{20}$)	**Enantiomeric Excess (*ee*)**
 **(*S*)-2b**	+8.0 (*c* = 0.2, $CHCl_3$)	>99%
 **(*R*)-2b**	-15.3 (*c* = 0.1, $CHCl_3$)	92%
 **(*S*)-9b**	-36.3 (*c* = 1.00, $CHCl_3$)	99%
 **(*R*)-9b**	+33.7 (*c* = 0.4, $CHCl_3$)	93%
 **(*R*)-11**	+12.5 (*c* = 0.8, $CHCl_3$)	98%
 **(*S*)-11**	-7.1 (*c* = 0.8, $CHCl_3$)	93%

 **(*S*)-12**	-13.5 (*c* = 0.4, $CHCl_3$)	>99%
 **(*R*)-12**	+14.2 (*c* = 0.4, $CHCl_3$)	90%
 ***anti*-(2*R*,3*S*)-13**	-31.5 (*c* = 0.4, $CHCl_3$)	90%
 ***anti*-(2*S*,3*R*)-13**	+17.2 (*c* = 1.3, $CHCl_3$)	99%
 ***anti*-(2*S*,3*S*)-14**	-5 (*c* = 0.3, $CHCl_3$)	91%
 ***anti*-(2*R*,3*R*)-14**	+3.9 (*c* = 0.3, $CHCl_3$)	99%
 ***anti*-(2*R*,3*R*)-3**	+5.7 (*c* = 0.19, $CHCl_3$)	98%
 ***anti*-(2*S*,3*S*)-3**	-5.0 (*c* = 0.7, $CHCl_3$)	91%

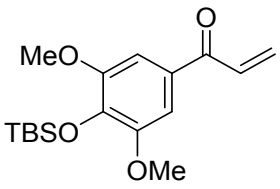
Experimental Procedures for the Synthesis of S-Phenolic Lignin-Based Unnatural Amino Acid (±)-4



Scheme S5: "S-Phenolic" is a term used to describe lignin aromatic system which contains two methoxy groups at the 3- and 5-position. Lignin extraction and depolymerisation which led to the isolation of **1** and subsequent conversion to its corresponding enone (not drawn) have been described in reference S6.

Reaction conditions for the synthesis of (±)-**30**: (a) NBu_4I, PPh_3, DDQ, DBU, DCM (see reference S1 for procedure) (b) TBSCl, DMAP, Imidazole, DCM, rt, 1 h (c) $NaBH_4$, $CeCl_3.7H_2O$, MeOH, rt, 1 h (d) OsO_4, NMO, THF/H_2O, rt, 12 h (e) K_2CO_3, BnBr, DMF, rt, 1 h (f) 2,2-dimethoxypropane, *p*TSA.H_2O (*catalytic amount*), DCM, 91% yield over 2 steps (g) Pd/C, H_2, NaOAc, MeOH, rt, 12 h (h) Dess-Martin Periodinane, DCM, rt, 2 h (i) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, ^{t}BuOH/H_2O (j) $SOCl_2$, DCM then EtOH, 78% over four-steps

1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-one (S1)

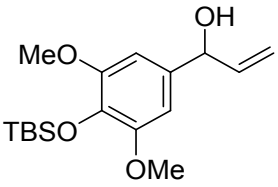


This batch of **S1** was prepared in an analogous manner to that reported above. A solution of the enone (0.30 g, 1.44 mmol, 1.0 eq.) in DCM (4 mL) was added to a stirring solution of 4-DMAP (0.17 g, 1.44 mmol, 1.0 eq.) and Imidazole (0.19 g, 2.88 mmol, 2.0 eq.) in DCM (10 mL), followed by the addition of TBSCl (0.25 g, 1.73 mmol, 1.2 eq.). The resulting mixture was left to stir at room temperature for 1 h (T.L.C control). Afterwards, the mixture was neutralised with saturated aqueous solution of NH_4Cl (20 mL) and the aqueous layer was further extracted with DCM (20 mL). Combined organic layer were washed with water (15 mL), brine (10 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel

chromatography using 2-5% EtOAc in Petroleum ether gave compound **S1** as light-yellow oil (0.42 g, 1.29 mmol, 90%).

IR (neat) ν_{max}/cm^{-1}: 2929, 1664, 1577, 1506, 1462, 1332, 1128; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. For $C_{17}H_{26}O_4SiNa^+$ 345.1600; found 345.1488; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.22 (s, 2H), 7.17 (dd, *J* = 17.0, 10.4 Hz, 1H), 6.43 (dd, *J* = 16.9, 1.7 Hz, 1H), 5.87 (dd, *J* = 10.5, 1.7 Hz, 1H), 3.86 (s, 6H), 1.01 (s, 9H), 0.15 (s, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 189.5, 151.5, 139.8, 132.2, 129.9, 129.4, 106.3, 56.0, 25.8, 18.9, -4.4.

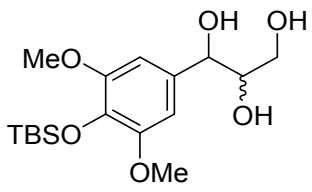
1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-ol (±)-2a



This batch of **2a** was prepared in an analogous manner to that reported above. $NaBH_4$ (0.23 g, 6.21 mmol, 5.0 eq) was added, gradually, to a cooled stirring solution of $CeCl_3.7H_2O$ (0.55 g, 1.49 mmol, 1.2 eq) and compound **S1** (0.40 g, 1.24 mmol, 1.0 eq) in MeOH (12 mL). The resulting mixture was left to stir at 0 °C for 1 h. Afterwards, the mixture was quenched with saturated aqueous solution of ammonium chloride (25 mL) and extracted with ethyl acetate twice (2 x 25 mL). The combined organic layer were washed with water (20 mL), brine (20 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography using 10-20% EtOAc in Petroleum Ether gave allylic alcohol (±)-**2a** as a white solid (0.42 g, 1.31 mmol, 95%).

M.p. 94–96 °C; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{17}H_{28}O_4SiNa^+$ 347.1655; found 347.1647; **IR** (neat) ν_{max}/cm^{-1}: 3491, 2928, 1591, 1506, 1119; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.57 (s, 2H), 6.07 (ddd, *J* = 17.1, 10.3, 5.6 Hz, 1H), 5.36 (dt, *J* = 17.1, 1.5 Hz, 1H), 5.21 (dt, *J* = 10.3, 1.4 Hz, 1H), 5.13 (d, *J* = 5.6 Hz, 1H), 3.81 (s, 6H), 1.03 (s, 9H), 0.14 (s, 6H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 151.6, 140.2, 135.1, 133.8, 115.0, 103.4, 75.5, 55.8, 25.8, 18.7, -4.6.

1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)propane-1,2,3-triol (±)-26

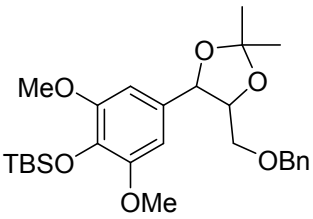


To a solution of (±)-**2a** (0.63 g, 1.94 mmol, 1 eq.) in THF/H_2O (8 mL/2 mL) was added NMO (0.38 g, 3.30 mmol, 1.7 eq.), followed by the addition of OsO_4 (2.5% wt in ^{t}BuOH, 0.19 g, 0.019 mmol, 0.01 eq.) at room temperature. The resulting mixture was left to stir at the same temperature for 12 h. After the reaction had reached completion (T.L.C control), the mixture was diluted with EtOAc (35 mL) and washed successively with $NaHCO_3$ (25 mL) and $Na_2S_2O_3$ (25 mL). The combined aqueous layers were further extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with water (25 mL), brine (25 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography using 5-10% MeOH in DCM afforded (±)-**26** (0.51 g, 1.43 mmol, 74%) as a mixture of diastereoisomers (3:1, as deduced by relative ratio between the peaks at 4.74 ppm and 4.57 ppm in the ^{1}H NMR).

IR (neat) ν_{max}/cm^{-1}: 3363, 2929, 1589, 1508; **HRMS** (ESI) *m/z* [M+NH_4^+] calcd. for $C_{17}H_{34}O_5NSi^+$ 376.2150; found 376.2148; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.55–6.53 (m, 2H), 4.74 (d, *J* = 5.3 Hz, 0.77H), 4.57 (d, *J* = 7.0 Hz, 0.23H), 3.77 (d, *J* = 2.5 Hz, 6.77H), 3.74–3.69 (m, 1H), 3.68–3.63 (m, 0.77H), 3.59–3.54 (m, 0.23H), 3.49–3.44 (m, 0.23H), 1.00–0.98 (m, 9H), 0.12–0.10 (m, 6H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 134.2, 134.0, 133.0, 132.9, 103.6, 103.2, 76.0, 75.4, 74.8, 63.4, 63.2, 55.9, 25.9, 18.8, -4.5.

These spectroscopic data are consistent with previously reported data (see reference S1)

(4-(5-((benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,6-dimethoxyphenoxy)(*tert*-butyl)dimethylsilane (±)-28



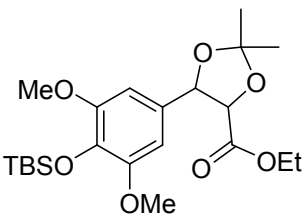
K_2CO_3 (0.38 g, 2.78 mmol, 2.0 eq.) was added to a stirring solution of triol (±)-**26** (0.54 g, 1.40 mmol, 1.0 eq., *dr* = 3:1) in DMF (15 mL) at room temperature. After all the solids had dissolved, BnBr (0.31 g, 1.81 mmol, 1.3 eq.) was added, and the resulting mixture was left to stir for 3 h at room temperature. Afterwards, the mixture was diluted with EtOAc (30 mL) and was washed with saturated aqueous solution of $NaHCO_3$ (35 mL). The organic layer

was washed with water (30 mL), brine (30 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*.

The resulting residue was then dissolved in DCM (10 mL), followed by the additions of 2,2-dimethoxypropane (0.66 g, 6.35 mmol, 5.0 eq.) and *p*TSA.H_2O (13.2 mg, 0.06 mmol, 0.05 eq.). The mixture was left to stir for a further 1 h at room temperature. After full consumption of the starting material (*ca.* 1 h), the mixture was diluted with DCM (20 mL) and neutralised with saturated aqueous solution of $NaHCO_3$ (10 mL). The organic layer was washed with water (20 mL), brine (15 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography using 2-5% EtOAc in Petroleum ether furnished (±)-**28** (0.62 g, 1.26 mmol, 91% over two steps, *dr* = 3:1) as a colourless oil. The major diastereoisomer (currently unassigned stereochemistry) was separated and after further purification was taken forward to the next reaction.

IR (neat) ν_{max}/cm^{-1} 2929, 1593, 1458, 1373, 1232, 1128; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{27}H_{40}O_6SiNa$ 511.2594, found 511.2580; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.46–7.48 (m, 2H), 7.30–7.34 (m, 2H), 7.27–7.29 (m, 1H), 6.52 (s, 2H), 5.17 (d, *J* = 6.9 Hz, 1H), 4.97 (s, 2H), 4.39 (dt, *J* = 6.9, 6.0 Hz, 1H), 3.80 (s, 6H), 3.38 (dd, *J* = 10.7, 6.2 Hz, 1H), 3.25 (dd, *J* = 10.6, 5.7 Hz, 1H), 1.62 (s, 3H), 1.45 (s, 3H), 0.78 (s, 9H), -0.09 (s, 3H), -0.14 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 153.4, 137.9, 136.4, 133.2, 128.7, 128.2, 127.9, 108.5, 104.2, 79.9, 79.2, 75.1, 63.1, 56.2, 27.4, 26.0, 24.9, 18.4, -5.36.

ethyl 5-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylate (±)-30



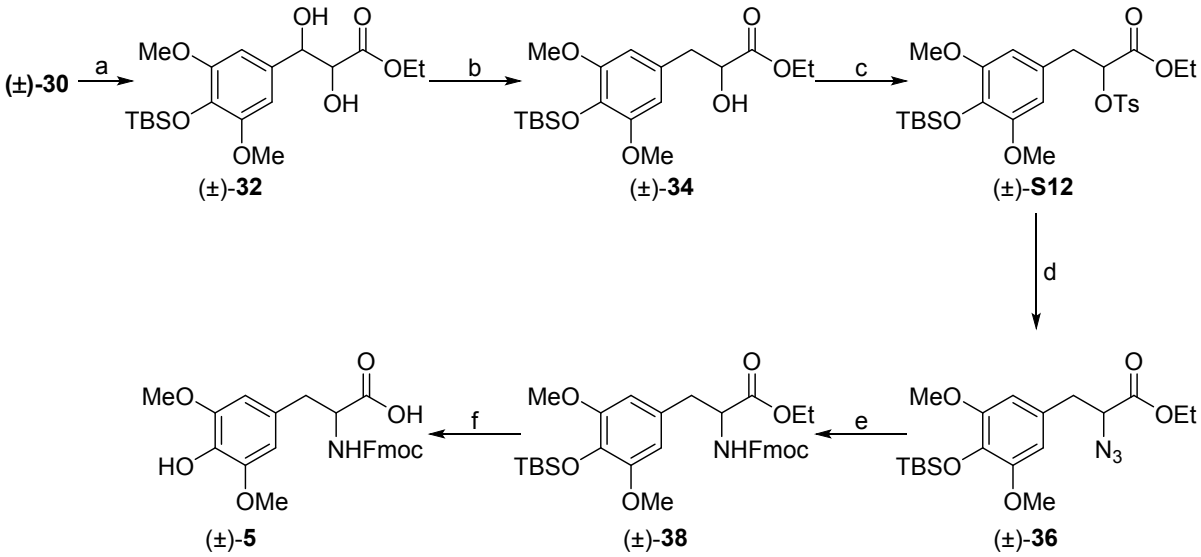
A solution of the major diastereoisomer of compound (±)-**28** (0.50 g, 1.02 mmol, 1.0 eq.) in MeOH (5 mL) was added to a black suspension of Pd/C (10% wt, 0.10 g, 1.02 mmol, 1.0 eq.) and NaOAc (91.9 mg, 1.12 mmol, 1.11 eq.) in MeOH (5 mL). The reaction vessel was evacuated with vacuum and backfilled with H_2 gas. The mixture was left to stir under the atmosphere of H_2 for 12 h. The mixture was then filtered through celite and the filtrate was concentrated *in vacuo*.

The residue was dissolved in DCM (12 mL) in a reaction vessel and Dess-Martin Periodinane (0.52 g, 1.22 mmol, 1.2 eq.) was added. The resulting mixture was allowed to stir at room temperature for 2 h, and then washed with saturated aqueous solution of $NaHCO_3$ (15 mL). The aqueous layer was extracted with DCM (2 x 25 mL) twice. The combined organic layers were washed with water (20 mL), brine (25 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*.

The crude mixture was dissolved in $^tBuOH/H_2O$ (10 mL/2 mL), followed by the addition of $NaClO_2$ (0.11 g, 1.22 mmol, 1.2 eq.), NaH_2PO_4 (0.15 g, 1.22 mmol, 1.2 eq.) and a drop of 2-methyl-2-butene. The resulting mixture was left to stir at room temperature for 12 h and then washed with 1 M HCl (15 mL). The aqueous layer was extracted with DCM (2 x 25 mL). The combined organic layers were washed with brine (35 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*.

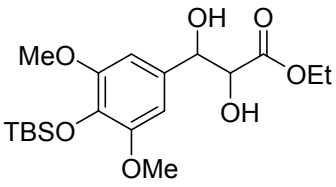
The crude material was dissolved in DCM (12 mL), followed by the dropwise addition of $SOCl_2$ (0.14 g, 1.22 mmol, 1.2 eq.) at room temperature. After 45 mins, EtOH (56 mg, 72 µl, 1.22 mmol, 1.2 eq.) was added. The reaction was then left to stir for 1.5 h and washed with a saturated aqueous solution of $NaHCO_3$ (20 mL). The aqueous layer was extracted with DCM (2 x 25 mL). The combined organic layers were washed with water (30 mL), brine (35 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography using 2-10% EtOAc in Petroleum ether furnished (±)-**30** (0.35 g, 0.79 mmol, 78% over four steps, single unassigned diastereomer) as a colourless oil.

IR (neat) ν_{max}/cm^{-1} 2929, 2358, 1755, 1591, 1512, 1462, 1421, 1375, 1332, 1246, 1128, 1101; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{22}H_{36}O_7SiNa$ 463.2230, found 463.2219; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.60 (s, 2H), 5.07 (d, *J* = 7.5 Hz, 1H), 4.32 (d, *J* = 7.4 Hz, 1H), 4.20–4.27 (m, 2H), 3.78 (s, 6H), 1.60 (s, 3H), 1.54 (s, 3H), 1.26 (t, *J* = 7.1 Hz, 3H), 0.99 (s, 9H), 0.11 (s, 6H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 170.6, 151.8, 134.5, 130.1, 111.5, 103.6, 81.3, 81.1, 61.5, 55.8, 27.1, 25.9, 25.8, 18.8, 14.3, -4.49.



Scheme S6: Synthesis of (±)-**5**. Reaction conditions (a) *p*TSA.H_2O, DCM, rt, 1 h, 77% (b) Et_3SiH, $BF_3.OEt_2$, DCM, -78 °C, 2 h, 56% (c) LiHMDS, TsCl, THF, rt, 1 h, 75% (d) NaN_3, DMF, rt, 3 h, 88% (e) Pd/C (10% wt), H_2, EtOAc, 1 h and then FmocCl, THF, 1 h, 93% (f) 2 M HCl/1,4-dioxane (1:1), 100 °C, 12 h, 72%

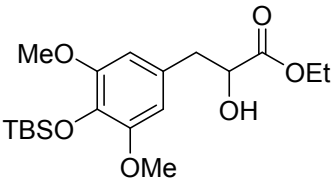
ethyl 3-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)-2,3-dihydroxypropanoate (±)-32



*p*TSA.H_2O (21.6 mg, 0.11 mmol, 0.10 eq.) was added to a stirring solution of the single unassigned diastereomer (±)-**30** (0.50 g, 1.14 mmol, 1.0 eq.) in DCM (10 mL) at room temperature and then left to stir for 1 h. After full consumption of the starting material (T.L.C control), the mixture was diluted with DCM (20 mL) and neutralised with a saturated aqueous solution of $NaHCO_3$ (20 mL). The organic layer was washed with water (35 mL), brine (20 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography using 10-30% EtOAc in Petroleum ether furnished a single unassigned diastereomer (±)-**32** (0.35 g, 0.87 mmol, 77%) as a white solid.

M.p. 80–84 °C; **IR** (neat) ν_{max}/cm^{-1} 3516, 2929, 1761, 1722, 1591, 1512, 1462, 1334, 1244, 1120; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{19}H_{32}O_7SiNa$ 423.1917; found 423.1801; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.57 (s, 2H), 4.86 (d, *J* = 3.2 Hz, 1H), 4.31 (d, *J* = 3.0 Hz, 1H), 4.23 (m, 2H), 3.78 (s, 6H), 3.11 (br s, 1H), 2.78 (br s, 1H), 1.25 (t, *J* = 7.0 Hz, 3H), 0.99 (s, 9H), 0.10 (s, 6H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.9, 151.6, 134.2, 132.4, 103.5, 74.9 (x2), 62.2, 55.9, 25.9, 18.8, 14.2, -4.4.

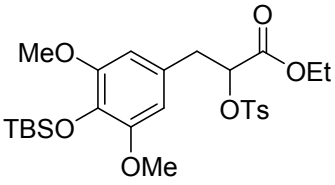
ethyl 3-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)-2-hydroxypropanoate (±)-**34**



A solution of the single unassigned diastereomer (±)-**32** (1.70 g, 4.25 mmol, 1.0 eq.) in DCM (30 mL) was cooled to -78 °C, after 5 min, $BF_3.OEt_2$ (1.21 g, 8.50 mmol, 2.0 eq.) was added, followed by the addition of Et_3SiH (0.98 g, 8.50 mmol, 2.0 eq.) at -78 °C. The resulting mixture was left to stir at -78 °C for 2 h and then MeOH was added at -78 °C. After 10 min, the cooling bath was removed. The mixture was allowed to warm up to room temperature, diluted with EtOAc (35 mL) and then quenched with a saturated aqueous solution of NH_4Cl (30 mL). The aqueous layer was further extracted with EtOAc (2 x 30 mL). The combined organic layers were washed with water (30 mL), brine (35 mL), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography using 10-30% EtOAc in Petroleum ether furnished (±)-**34** (0.92 g, 2.38 mmol, 56%) as a colourless oil.

IR (neat) ν_{max}/cm^{-1} 3468, 2927, 1716, 1589, 1508, 1463, 1344, 1278, 1242, 1126, 1103; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{19}H_{32}O_6SiNa$ 407.1968; found 407.1853; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.39 (s, 2H), 4.40 (dd, *J* = 6.3, 4.6 Hz, 1H), 4.19 (qd, *J* = 7.1, 1.7 Hz, 2H), 3.75 (s, 6H), 3.02 (dd, *J* = 14.0, 4.4 Hz, 1H), 2.89 (dd, *J* = 14.0, 6.5 Hz, 1H), 2.72 (br s, 1H), 1.26 (t, *J* = 7.1 Hz, 3H), 0.99 (s, 9H), 0.10 (s, 6H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 174.1, 151.5, 133.4, 128.7, 106.6, 71.4, 61.7, 55.8, 40.9, 25.9, 18.8, 14.3, -4.90.

ethyl 3-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)-2-(tosyloxy)propanoate (±)-**S12**

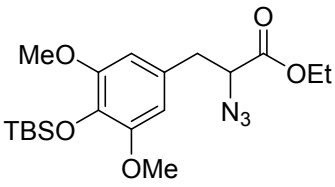


LiHMDS (1.0 M in THF, 2.86 mmol, 2.86 mL) was added to a stirring solution of (±)-**34** (0.92 g, 2.38 mmol, 1.0 eq.) in THF (18 mL) at room temperature. After 10 min, TsCl (0.54 g, 2.86 mmol, 1.2 eq.) was added. The resulting mixture was left to stir for a further 1 h, diluted with EtOAc (35 mL) and then washed with a saturated aqueous solution of NH_4Cl (35 mL). The organic layer was washed with water (30 mL), brine (35 mL), dried with

$MgSO_4$, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography using 5-10% EtOAc in Petroleum ether furnished (±)-**S12** (0.96 g, 1.78 mmol, 75%) as a white solid.

M.p. 92–96 °C; **IR** (neat) ν_{max}/cm^{-1} 2954, 1759, 1743, 1589, 1512, 1460, 1367, 1346, 1244, 1174, 1126, 1029; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{26}H_{38}O_8SSiNa$ 561.2057; found 561.1936; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.46 (d, *J* = 8.2 Hz, 2H), 7.15 (d, *J* = 8.0 Hz, 2H), 6.16 (s, 2H), 4.81 (dd, *J* = 9.4, 3.9 Hz, 1H), 4.17 (q, *J* = 7.1 Hz, 2H), 3.65 (s, 6H), 3.04 (dd, *J* = 14.2, 4.0 Hz, 1H), 2.99 (dd, *J* = 14.2, 9.6 Hz, 1H), 2.39 (s, 3H), 1.23 (t, *J* = 7.1 Hz, 3H), 1.00 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 168.7, 151.4, 145.0, 133.5, 132.7, 129.6, 127.8, 126.9, 106.0, 78.8, 62.1, 55.5, 38.7, 25.9, 21.7, 18.8, 14.1, -4.47.

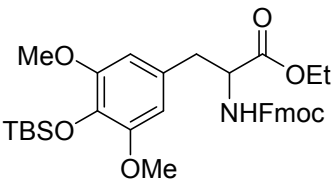
ethyl 2-azido-3-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)propanoate (±)-**36**



NaN_3 (0.58 g, 8.90 mmol, 5.0 eq.) solid was added all at once to a stirring solution of compound (±)-**S12** (0.96 g, 1.78 mmol, 1.0 eq.) in DMF (17 mL) and then left to stir at room temperature for 3 h. Afterwards, the reaction mixture was diluted with EtOAc (40 mL) and washed with a saturated aqueous solution of NH_4Cl (30 mL). The organic layer was washed with water (30 mL), brine (35 mL), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography using 5-10% EtOAc in Petroleum ether furnished (±)-**36** (0.64 g, 1.57 mmol, 88%) as a colourless oil.

IR (neat) ν_{max}/cm^{-1} 2929, 2102, 1739, 1589, 1512, 1460, 1421, 1242, 1186, 1128, 1031; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{19}H_{31}N_3O_5SiNa$ 432.2033; found 432.1914; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.40 (s, 2H), 4.20 (dq, *J* = 7.0, 2.5 Hz, 2H), 3.98 (dd, *J* = 8.6, 5.7 Hz, 1H), 3.77 (s, 6H), 3.08 (dd, *J* = 13.8, 5.5 Hz, 1H), 2.93 (dd, *J* = 13.8, 8.4 Hz, 1H), 1.26 (t, *J* = 7.0 Hz, 3H), 0.99 (s, 9H), 0.10 (s, 6H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 170.1, 151.7, 133.6, 128.3, 106.3, 63.5, 61.9, 55.9, 38.2, 25.9, 18.8, 14.3, -4.52.

ethyl 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)propanoate (±)-**38**

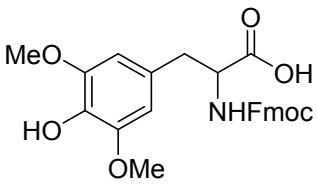


A solution of compound (±)-**36** (0.64 g, 1.57 mmol, 1.0 eq.) in EtOAc (5 mL) was added to a stirring black suspension of Pd/C (10% wt, 0.17 g, 1.57 mmol, 1.0 eq.) in EtOAc (10 mL). The reaction vessel was evacuated under vacuum and backfilled with H_2 gas three times and then left to stir at room temperature for 1 h. Afterwards, the mixture was filtered through celite, and the filtrate was concentrated *in vacuo*.

The crude mixture was dissolved in THF (10 mL) in a reaction vessel, followed by the addition of Fmoc-Cl (0.45 g, 1.73 mmol, 1.1 eq.). The resulting mixture was left to stir at room temperature for 1 h, diluted with EtOAc (30 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (20 mL). The organic layer was washed with water (25 mL), brine (25 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography using 5-10% EtOAc in Petroleum ether furnished (±)-**36** (0.89 g, 1.47 mmol, 93%) as a white solid.

M.p. 105–110 ^{o}C; **IR** (neat) ν_{max}/cm^{-1} 3284, 2956, 1753, 1676, 1552, 1510, 1452, 1344, 1249, 1213, 1124, 1049; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. $C_{34}H_{43}NO_7SiNa$ 628.2809; found 628.2685; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.6 Hz, 2H), 7.54–7.57 (m, 2H), 7.39 (d, *J* = 7.4 Hz, 2H), 7.29 (td, *J* = 7.2, 1.0 Hz, 2H), 6.31 (s, 2H), 5.26 (d, *J* = 8.7 Hz, 1H), 4.60–4.64 (m, 1H), 4.40 (dd, *J* = 10.6, 7.1 Hz, 1H), 4.34 (dd, *J* = 10.6, 7.2 Hz, 1H), 4.11–4.22 (m, 3H), 3.73 (s, 6H), 3.00–3.06 (m, 2H), 1.24 (t, *J* = 7.1 Hz, 3H), 1.00 (s, 9H), 0.11 (s, 6H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 171.1, 155.7, 151.6, 144.0, 143.8, 141.4, 128.2, 127.8, 127.2, 125.2, 125.1, 120.1, 106.3, 67.2, 61.6, 55.8, 54.9, 47.2, 38.7, 25.9, 18.8, 14.3, -4.49.

2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-hydroxy-3,5-dimethoxyphenyl)propanoic acid (±)-**5**



A 2 M aqueous solution of HCl (15 mL) was added to a solution of (±)-**23** (0.89 g, 1.47 mmol, 1.0 eq.) in 1,4-dioxane (15 mL). The resulting mixture was heated up to 100 °C and left to stir at this temperature for 12 h. The mixture was then allowed to cool to room temperature, diluted with EtOAc (15 mL) and washed with brine (20 mL). The aqueous layer was washed further with EtOAc (2 x 15 mL). The combined organic layers were dried with $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was recrystallised in Petroleum ether/EtOAc, and the residue was collected by filtration to afford (±)-**5** (0.49 g, 1.06 mmol, 72%) as a white crystalline solid.

M.p. 195–202 °C; **IR** (neat) ν_{max}/cm^{-1}: 3502, 3331, 1718, 1689, 1616, 1537, 1244, 1111; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{26}H_{25}NO_7Na$ 486.1631; found 486.1519; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 7.88 (d, *J* = 7.5 Hz, 2H), 7.66 (d, *J* = 7.5 Hz, 2H), 7.39–7.43 (m, 2H), 7.28–7.31 (m, 2H), 6.58 (s, 2H), 4.11–4.24 (m, 4H), 3.72 (s, 6H), 3.00 (dd, *J* = 13.8, 4.1 Hz, 1H), 2.76 (dd, *J* = 13.4, 10.6 Hz, 1H); **^{13}C NMR** (125 MHz, DMSO-d_6) δ 173.5, 155.9, 155.8, 147.6, 143.8, 140.6, 133.9, 127.9, 127.6, 127.0 (x2), 125.3, 120.1, 106.5, 65.6, 55.9, 55.8, 46.9, 36.6.

During the synthesis of (±)-**38**, a solution of the crude amine (±)-**40** was hydrolysed using LiOH to give a TBS-protected amino acid (±)-**41**. (±)-**41** was recrystallized to provide crystals suitable for X-ray crystallographic analysis (Figure S8).

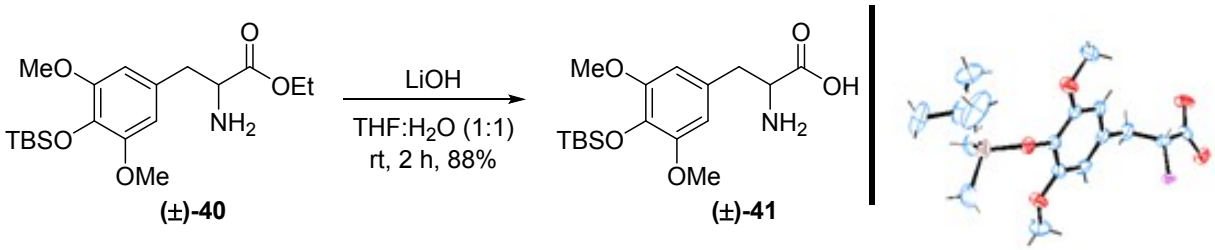
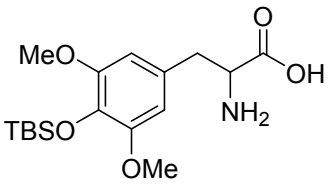


Figure S8. Synthesis and thermal ellipsoid plot (50% probability) of TBS-protected amino acid (±)-**41.**[S2]

2-amino-3-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenol)propanoic acid (±)-**41**

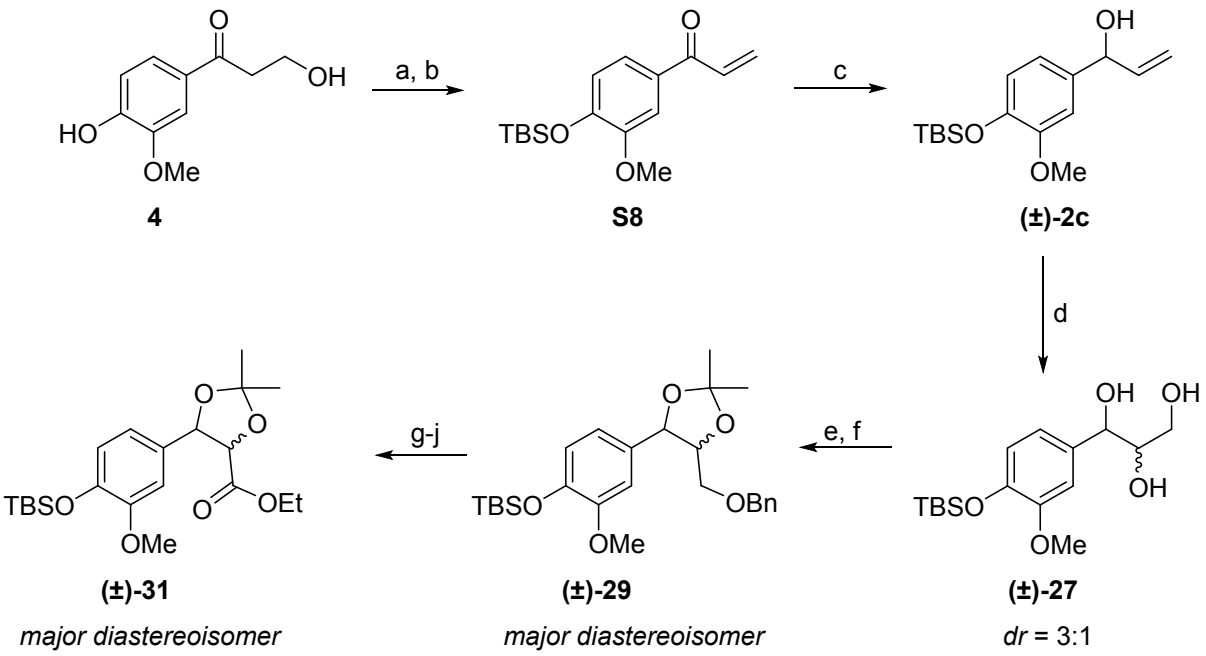


LiOH (19.2 mg, 0.81 mmol, 5.0 eq.) was added to a stirring solution of crude (±)-**40** (60 mg, 0.16 mmol, 1.0 eq.) (which was obtained from the hydrogenation of (±)-**36**) in THF:H_2O (1 mL:1 mL) at room temperature. The resulting mixture was left to stir for 2 h, neutralised with 2 M HCl (10 mL) and then extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (15 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo* produced amino-acid (±)-**41** (50 mg, 0.14 mmol, 88% crude yield) as a white powder.

Small molecule X-ray crystallographic analysis of (±)-**41** was carried out (**CCDC 1506889**) and confirmed the assigned structure.[S2]

M.p. 216–220 °C; **IR** (neat) ν_{max}/cm^{-1} 2927, 1624, 1589, 1510, 1460, 1328, 1244, 1126; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{17}H_{29}NO_5SiNa$ 378.1815; found 378.1703; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 6.54 (s, 2H), 3.71 (s, 6H), 3.32 (m, 1H), 3.11 (dd, *J* = 14.5, 3.4 Hz, 1H), 2.68 (dd, *J* = 13.8, 9.6 Hz, 1H), 0.95 (s, 9H), 0.06 (s, 6H).

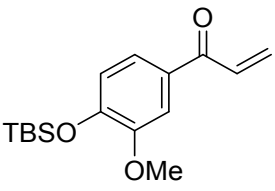
Experimental Procedures for the Synthesis of G-Phenolic Lignin-Based Unnatural Amino Acid (±)-6



Scheme S7: "G-Phenolic" is a term used to describe lignin aromatic system which contains one methoxy group at the 5-position. Lignin extraction and depolymerisation which led to the isolation of **2** have been described previously in reference S6.

Reaction conditions for the synthesis of (±)-**31**: (a) NBu_4I, PPh_3, DDQ, DBU, DCM (b) TBSCl, DMAP, Imidazole, DCM, rt, 1 h (c) $NaBH_4$, $CeCl_3.7H_2O$, MeOH, rt, 1 h (d) OsO_4, NMO, THF/H_2O, rt, 12 h (e) K_2CO_3, BnBr, DMF, rt, 1 h (f) 2,2-dimethoxypropane, *p*TSA.H_2O (*catalytic amount*), DCM (g) Pd/C, H_2, NaOAc, MeOH, rt, 12 h (h) Dess-Martin Periodinane, DCM, rt, 2 h (i) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, ^{t}BuOH/H_2O (j) $SOCl_2$, DCM then EtOH, 68% over 4-steps

1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-one (S8)



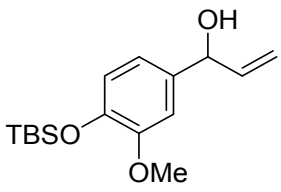
This batch of **S8** was prepared in an analogous manner to that reported above. PPh_3 (1.18 g, 4.48 mmol, 2.2 eq.) and DDQ (0.92 g, 4.08 mmol, 2.0 eq.) were dissolved in DCM (12 mL) and then NBu_4I (1.31 g, 4.08 mmol, 2.0 eq.) was added. The resulting solution was stirred at room temperature for 5 min followed by the addition of a solution of G-phenolic monomer **4** (0.40 g, 2.04 mmol, 1.0 eq.) in DCM (4 mL) at room temperature. The resulting mixture was stirred at room temperature for 4 h, and then DBU (1.24 g, 8.16 mmol, 4.0 eq.)

was added. After 2 h, the mixture was diluted with ethyl acetate (35 mL) and washed with a saturated solution of NH_4Cl (35 mL). The organic layer was washed with water (20 mL), brine (20 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*.

A solution of the crude enone in DCM (6 mL) was added to a stirring solution of 4-DMAP (0.25 g, 2.04 mmol, 1.0 eq.) and Imidazole (0.27 g, 4.08 mmol, 2.0 eq.) in DCM (15 mL), followed by the addition of TBSCl (0.37 g, 2.44 mmol, 1.2 eq.). After 1 h, the mixture was diluted with ethyl acetate (30 mL) and washed with saturated solution of NH_4Cl (30 mL). The organic layer was washed with water (20 mL), brine (20 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography using 2-5% EtOAc in Petroleum Ether gave compound **S8** as a colourless oil (0.45 g, 1.54 mmol, 76%).

IR (neat) ν_{max} 2929, 1666, 1589, 1508, 1417, 1276, 1168; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{16}H_{24}O_3SiNa$ 315.1495; found 315.1382; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.54 (d, *J* = 2.0 Hz, 1H), 7.48 (dd, *J* = 8.1, 2.0 Hz, 1H), 7.17 (dd, *J* = 16.9, 10.4 Hz, 1H), 6.88 (d, *J* = 8.2 Hz, 1H), 6.41 (dd, *J* = 17.0, 1.8 Hz, 1H), 5.83 (dd, *J* = 10.5, 1.8 Hz, 1H), 3.85 (s, 3H), 0.98 (s, 9H), 0.17 (s, 6H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 189.2, 151.3, 150.2, 132.0, 131.3, 128.9, 123.1, 120.3, 111.7, 55.5, 25.7, 18.5, -4.5.

1-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-dimethoxyphenyl)prop-2-en-1-ol (±)-2c

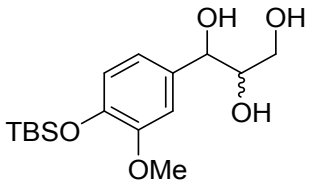


This batch of **(±)-2c** was prepared in an analogous manner to that reported above. $NaBH_4$ (0.26 g, 6.84 mmol, 5.0 eq.) was added, gradually, to a cooled stirring solution of $CeCl_3.7H_2O$ (0.61 g, 1.63 mmol, 1.2 eq.) and compound **S8** (0.40 g, 1.36 mmol, 1.0 eq.) in MeOH (12 mL). The resulting mixture was left to stir at 0 °C for 1 h. Purification by silica gel chromatography using 10-20% EtOAc in Petroleum Ether gave allylic alcohol (±)-**2c** as a light yellow oil (0.36 g, 1.22 mmol, 90%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.87 (d, J = 1.7 Hz, 1H), 6.78–6.80 (m, 2H), 6.03 (ddd, J = 16.2, 10.3, 5.7 Hz, 1H), 5.31 (dt, J = 17.1, 1.2 Hz, 1H), 5.17 (dt, J = 10.3, 1.2 Hz, 1H), 5.11 (d, J = 5.7 Hz, 1H), 3.79 (s, 3H), 2.12 (s, 1H), 0.98 (s, 9H), 0.14 (s, 6H).

^{1}H NMR Spectroscopic data was consistent with the previously reported data (see reference S1)

1-(4-((*tert*-butyldimethylsilyl)oxy)-3-methoxyphenyl)propane-1,2,3-triol (±)-27

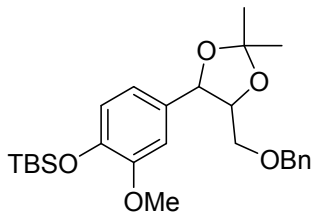


The same experimental procedure was followed as described for the synthesis of (±)-**26**. **(±)-2c** (0.33 g, 1.12 mmol, 1 eq.), NMO (0.19 g, 1.69 mmol, 1.5 eq.) and OsO_4 (2.5% wt in ^{t}BuOH, 0.11 g, 0.011 mmol, 0.01 eq.) in THF/H_2O (4 mL/1 mL). Purification on silica gel afforded (±)-**27** (0.34 g, 1.05 mmol, 94%) as a mixture of diastereoisomers (3:1) as deduced by relative ratio between the peaks at 4.77 ppm and 4.61 ppm in the ^{1}H NMR.

IR (neat) ν_{max}: 3342, 2929, 1508, 1278; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{16}H_{28}O_5SiNa$ 351.1598; found 351.1593; **^{1}H NMR** (500 MHz, $CDCl_3$) 6.90–6.87 (m, 1H), 6.85–6.82 (m, 1H), 6.81–6.78 (m, 1H), 4.77 (d, *J* = 5.5 Hz, 0.96H), 4.61 (d, *J* = 7.1 Hz, 0.32H), 3.80–3.78 (m, 3H), 3.77–3.70 (m, 1.68H), 3.68–3.65 (m, 0.96H), 3.59–3.55 (m, 0.32H), 3.49–3.45 (m, 0.32H), 0.99–0.98 (m, 9H), 0.15–0.13 (m, 6H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 151.3, 145.0, 133.9, 133.8, 121.0, 119.2, 118.8, 110.4, 110.1, 76.1, 75.8, 75.1, 74.8, 63.4, 63.2, 55.6, 25.8, 18.6, -4.5.

These spectroscopic data are consistent with previously reported data (see reference S1)

(4-(5-((benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-methoxyphenoxy)(*tert*-butyl)dimethylsilane (±)-29

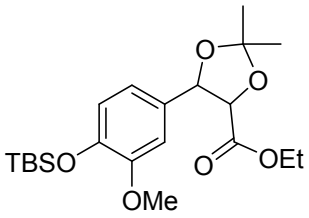


The same experimental procedure was followed as described for the synthesis of (±)-**28**. K_2CO_3 (0.34 g, 2.44 mmol, 2.0 eq.), triol (±)-**27** (mixture of diastereomers, 0.40 g, 1.22 mmol, 1.0 eq., *dr* = 3:1) in DMF (15 mL) at room temperature, and BnBr (0.27 g, 1.59 mmol, 1.3 eq.) were used. 2,2-dimethoxypropane (0.64 g, 6.10 mmol, 5.0 eq.) and *p*TSA.H_2O (11.6 mg, 0.06 mmol, 0.05 eq.) was used. Purification by silica gel chromatography using 2-5% EtOAc in Petroleum ether furnished (±)-**29** (0.50 g, 1.09 mmol, 89% over two steps, *dr* = 3:1) as a colourless oil. The major diastereoisomer was separated, after further purification, and taken forward to the next reaction.

IR (neat) ν_{max}/cm^{-1} 2927, 1514, 1456, 1373, 1255, 1213, 1165, 1134, 1076, 1026; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{26}H_{38}O_5SiNa$ 481.2489, found 481.2300; **^{1}H NMR** (500 MHz, $CDCl_3$) δ

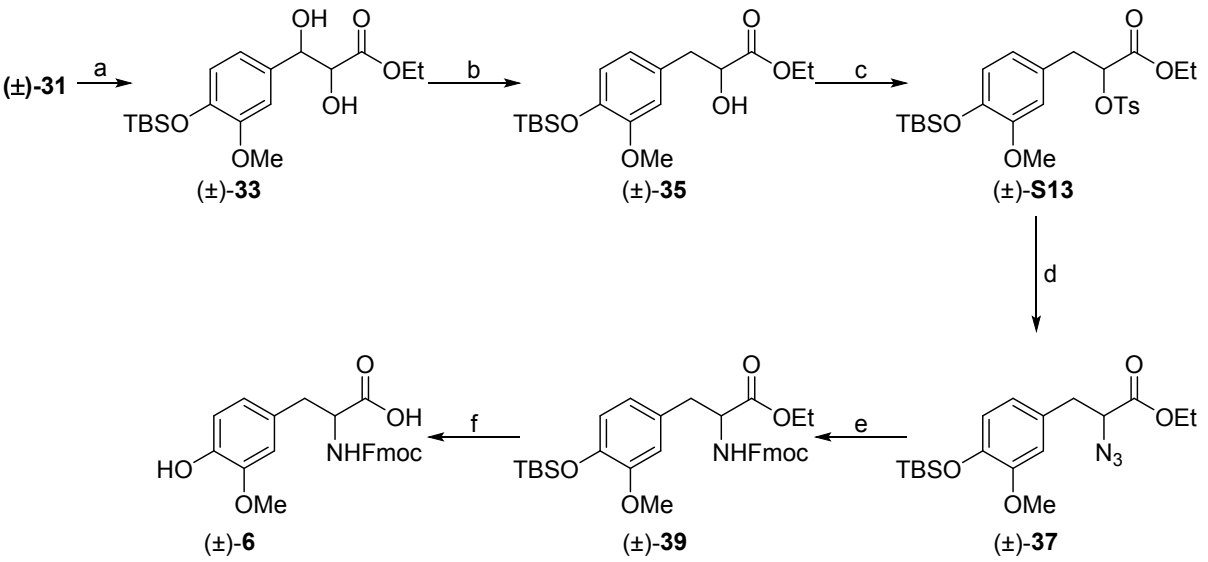
7.42 (m, 2H), 7.34 (m, 2H), 7.28 (m, 1H), 6.87 (d, *J* = 1.7 Hz, 1H), 6.81 (d, *J* = 8.1 Hz, 1H), 6.77 (dd, *J* = 8.3, 1.7 Hz, 1H), 5.18 (d, *J* = 7.0 Hz, 1H), 5.14 (s, 2H), 4.38 (q, *J* = 6.0 Hz, 1H), 3.87 (s, 3H), 3.36 (ddd, *J* = 10.6, 6.1 Hz, 1H), 3.20 (dd, *J* = 10.6 ,5.7 Hz, 1H), 1.61 (s, 3H), 1.45 (s, 3H), 0.77 (s, 9H), -0.11 (s, 3H), -0.17 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 149.4, 147.7, 137.2, 130.6, 128.6, 127.9, 127.3, 119.5, 113.8, 110.8, 108.4, 79.4, 78.9, 71.1, 63.1, 56.0, 27.4, 25.9, 24.8, 18.3, -5.42.

ethyl 5-(4-((*tert*-butyldimethylsilyl)oxy)-3-methoxyphenyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylate (±)-31



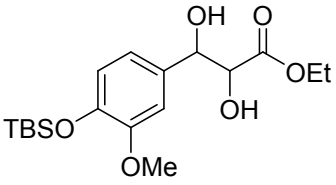
The same experimental procedure was followed as described for the synthesis of (±)-**30**. Using (±)-**29** (single, unassigned diastereomer, 0.30 g, 0.65 mmol, 1.0 eq.), MeOH (7 mL), Pd/C (10% wt, 69.0 mg, 0.65 mmol, 1.0 eq.) and NaOAc (59.2 mg, 0.72 mmol, 1.11 eq.) for the first step under the atmosphere of H_2. Dess-Martin Periodinane (0.33 g, 0.78 mmol, 1.2 eq.) and DCM (8 mL) were used for the second step. ^{t}BuOH/H_2O (5 mL/1 mL), $NaClO_2$ (70.5 mg, 0.78 mmol, 1.2 eq.), NaH_2PO_4 (93.5 mg, 0.78 mmol, 1.2 eq.) and a drop of 2-methyl-2-butene were used for the third step. Finally, DCM (8 mL), $SOCl_2$ (92.8 g, 0.78 mmol, 1.2 eq.) and EtOH (35.9 mg, 46 µl, 0.78 mmol, 1.2 eq.) were used. Purification by silica gel chromatography using 5-10% EtOAc in Petroleum ether furnished (±)-**31** (single, unassigned diastereomer, 0.18 g, 0.44 mmol, 68% over four steps) as a colourless oil.

IR (neat) ν_{max}/cm^{-1} 2929, 2856, 1751, 1516, 1456, 1375, 1280, 1257, 1163, 1099; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{21}H_{34}O_6SiNa$ 433.2125, found 433.2120; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.90 (d, *J* = 1.8 Hz, 1H), 6.85 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.82 (d, *J* = 8.1 Hz, 1H), 5.06 (d, *J* = 7.5 Hz, 1H), 4.30 (d, *J* = 7.6 Hz, 1H), 4.16–4.26 (m, 2H), 3.79 (s, 3H), 1.59 (s, 3H), 1.53 (s, 3H), 1.24 (t, *J* = 7.0 Hz, 3H), 0.97 (s, 9H), 0.13 (s, 6H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 170.4, 151.1, 145.3, 130.9, 120.9, 119.3, 111.4, 110.3, 81.3, 80.8, 61.4, 55.5, 27.0, 25.8 (x2), 18.5, 14.2, -4.55.



Scheme S8: Synthesis of (±)-**6**. Reaction conditions (a) *p*TSA.H_2O, 69% (b) Et_3SiH, $BF_3.OEt_2$, DCM, -78 °C, 2 h, 66% (c) LiHMDS, TsCl, THF, rt, 1 h, 60% (d) NaN_3, DMF, rt, 3 h, 78% (e) Pd/C (10% wt), H_2, EtOAc, 1 h and then FmocCl, THF, 1 h, 98% (f) 2 M HCl/1,4-dioxane (1:1), 100 °C, 12 h, 76%

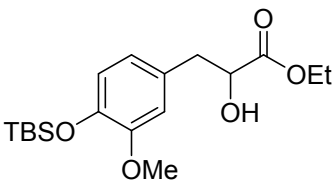
ethyl 3-(4-((*tert*-butyldimethylsilyl)oxy)-3-methoxyphenyl)-2,3-dihydroxypropanoate (±)-33



The same experimental procedure was followed as described for the synthesis of (±)-**32**. *p*TSA.H_2O (18.5 mg, 0.09 mmol, 0.10 eq.) was added to a stirring solution of (±)-**31** (single, unassigned diastereomer, 0.40 g, 0.98 mmol, 1.0 eq.) in DCM (10 mL) at room temperature and then left to stir for 1 h. Purification by silica gel chromatography using 10-30% EtOAc in Petroleum ether furnished (±)-**33** single, unassigned diastereomer, 0.25 g, 0.67 mmol, 69%) as a colourless oil.

IR (neat) ν_{max}/cm^{-1} 3400, 2929, 1732, 1514, 1463, 1282, 1251, 1155, 1033; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{18}H_{30}O_6SiNa$ 393.1812, found 393.1819; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.91 (s, 1H), 6.79 (s, 2H), 4.85 (dd, *J* = 6.3, 3.6 Hz, 1H), 4.28 (dd, *J* = 6.3, 3.5 Hz, 1H), 4.16–4.22 (m, 2H), 3.78 (s, 3H), 3.33 (dd, *J* = 6.3 Hz, 1H), 3.02 (dd, *J* = 6.3 Hz, 1H), 1.22 (dd, *J* = 7.1 Hz, 3H), 0.97 (s, 9H), 0.13 (s, 6H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.9, 150.9, 144.9, 133.4, 120.7, 118.9, 110.5, 74.9, 74.7, 62.1, 55.5, 25.8, 18.5, 14.2, -4.52.

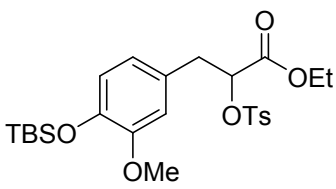
ethyl 3-(4-((*tert*-butyldimethylsilyl)oxy)-3-methoxyphenyl)-2-hydroxypropanoate (±)-**35**



The same experimental procedure was followed as described for the synthesis of (±)-**34**. A solution of (±)-**33** (single, unassigned diastereomer, 2.03 g, 5.48 mmol, 1.0 eq.) in DCM (35 mL) was cooled to –78 °C, after 5 min, $BF_3.OEt_2$ (1.56 g, 10.97 mmol, 2.0 eq.) was added, followed by the addition of Et_3SiH (1.28 g, 10.97 mmol, 2.0 eq.) at –78 °C. The resulting mixture was left to stir at –78 °C for 2 h and then MeOH was added at –78 °C. Purification by silica gel chromatography using 10-30% EtOAc in Petroleum ether furnished (±)-**35** (1.27 g, 3.59 mmol, 66%) as a colourless oil.

IR (neat) ν_{max}/cm^{-1} 3464, 1732, 1512, 1463, 1278, 1157, 1091, 1035; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{18}H_{30}O_5SiNa$ 377.1863, found 377.1860; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.75 (d, *J* = 7.9 Hz, 1H), 6.71 (d, *J* = 2.0 Hz, 1H), 6.64 (d, *J* = 8.0, 2.0 Hz, 1H), 4.39 (dd, *J* = 6.4, 4.5 Hz, 1H), 4.18 (q, *J* = 7.0 Hz, 2H), 3.77 (s, 3H), 3.03 (dd, *J* = 14.0, 4.5 Hz, 1H), 2.90 (dd, *J* = 14.1, 6.5 Hz, 1H), 1.25 (t, *J* = 7.1 Hz, 3H), 0.97 (s, 9H), 0.12 (s, 6H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 174.2, 150.8, 144.0, 129.7, 121.8, 120.8, 113.6, 71.4, 61.7, 55.4, 40.3, 25.8, 18.5, 14.3, -4.52.

ethyl 3-(4-((*tert*-butyldimethylsilyl)oxy)-3-methoxyphenyl)-2-(tosyloxy)propanoate (±)-**S13**

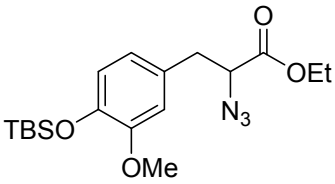


The same experimental procedure was followed as described for the synthesis of (±)-**S12**. LiHMDS (1.0 M in THF, 4.31 mmol, 4.31 mL) was added to a stirring solution of (±)-**35** (1.27 g, 3.59 mmol, 1.0 eq.) in THF (25 mL) at room temperature. After 10 min, TsCl (0.82 g, 4.31 mmol, 1.2 eq.) was added. The resulting mixture was left to stir for a further 1 h. Purification by silica gel chromatography using 5-10% EtOAc in Petroleum ether furnished (±)-**S13** (1.10 g, 2.16 mmol, 60%) as a colourless oil.

IR (neat) ν_{max}/cm^{-1} 2929, 1759, 1739, 1598, 1514, 1463, 1369, 1278, 1176, 1035; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{25}H_{36}O_7SSiNa$ 531.1951, found 531.1949; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.50 (d, *J* = 8.3 Hz, 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 6.62 (d, *J* = 7.9 Hz, 1H), 6.49 (dd, *J* = 8.0, 2.1 Hz, 1H), 6.46 (d, *J* = 1.9 Hz, 1H), 4.82 (dd, *J* = 9.0, 4.4 Hz, 1H), 4.13 (q, *J* = 7.2 Hz, 2H), 3.65 (s, 3H), 3.04 (dd, *J* = 14.3, 4.5 Hz, 1H), 2.94 (dd, *J* = 14.2, 9.1 Hz, 1H), 2.39 (s, 3H), 1.20 (t, *J* = 7.1 Hz,

3H), 0.98 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 168.6, 150.7, 144.9, 144.3, 132.8, 129.6, 127.9, 127.8, 121.6, 120.7, 112.8, 78.7, 61.9, 55.2, 38.1, 25.8, 21.7, 18.5, 14.1, -4.55.

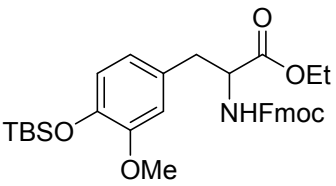
ethyl 2-azido-3-(4-((*tert*-butyldimethylsilyl)oxy)-3-methoxyphenyl)propanoate (±)-**37**



The same experimental procedure was followed as described for the synthesis of (±)-**36**. NaN_3 (0.70 g, 10.8 mmol, 5.0 eq.) solid was added all at once to a stirring solution of (±)-**S14** (1.10 g, 2.16 mmol, 1.0 eq.) in DMF (20 mL) and then left to stir at room temperature for 3 h. Purification by silica gel chromatography using 5-10% EtOAc in Petroleum ether furnished (±)-**37** (0.64 g, 1.69 mmol, 78%) as a colourless oil.

IR (neat) ν_{max}/cm^{-1} 2929, 2104, 1739, 1514, 1463, 1280, 1159, 1126, 1037; **HRMS** (ESI) *m/z* $[M+Na]^+$ calcd. for $C_{18}H_{29}N_3O_4SiNa$ 402.1927, found 402.1907; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.77 (d, *J* = 8.0 Hz, 1H), 6.71 (d, *J* = 1.8 Hz, 1H), 6.67 (dd, *J* = 7.8, 1.9 Hz, 1H), 4.20 (q, *J* = 7.0 Hz, 2H), 3.98 (dd, *J* = 8.3, 5.7 Hz, 1H), 3.78 (s, 3H), 3.09 (dd, *J* = 13.9, 5.6 Hz, 1H), 2.94 (dd, *J* = 14.0, 8.4 Hz, 1H), 1.25 (t, *J* = 7.1 Hz, 3H), 0.98 (s, 9H), 0.13 (s, 6H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 170.1, 151.0, 144.3, 129.3, 121.5, 121.0, 113.2, 63.4, 61.8, 55.5, 37.5, 25.8, 18.5, 14.2, -4.98.

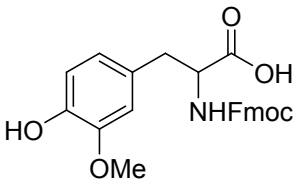
ethyl 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-((*tert*-butyldimethylsilyl)oxy)-3-methoxyphenyl)propanoate (±)-**39**



The same experimental procedure was followed as described for the synthesis of (±)-**38**. A solution of (±)-**37** (0.64 g, 1.69 mmol, 1.0 eq.) in EtOAc (5 mL) was added to a stirring black suspension of Pd/C (10% wt, 0.18 g, 1.69 mmol, 1.0 eq.) in EtOAc (10 mL). The crude mixture was dissolved in THF (10 mL) in a reaction vessel, followed by the addition of Fmoc-Cl (0.52 g, 2.02 mmol, 1.2 eq.). Purification by silica gel chromatography using 5-20% EtOAc in Petroleum ether furnished (±)-**39** (0.95 g, 1.65 mmol, 98%) as a colourless oil.

IR (neat) ν_{max}/cm^{-1} 3325, 2929, 1716, 1512, 1448, 1280, 1201, 1035; **HRMS** (ESI) *m/z* [M+Na]$^+$ calcd. for $C_{33}H_{41}NO_6SiNa$ 598.2703, found 598.2699; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.57 (d, *J* = 7.3 Hz, 2H), 7.40 (d, *J* = 7.4 Hz, 2H), 7.31 (d, *J* = 7.4 Hz, 2H), 6.77 (d, *J* = 8.0 Hz, 1H), 6.64 (d, *J* = 1.5 Hz, 1H), 6.57 (dd, *J* = 7.8, 1.3 Hz, 1H), 5.31 (d, *J* = 8.4 Hz, 1H), 4.63 (dt, *J* = 14.1, 6.1 Hz, 1H), 4.38 (d, *J* = 7.1 Hz, 2H), 4.12–4.23 (m, 3H), 3.75 (s, 3H), 3.06 (d, *J* = 5.9 Hz, 2H), 1.24 (t, *J* = 7.1 Hz, 3H), 1.00 (s, 9H), 0.15 (s, 6H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 171.7, 155.7, 150.9, 144.2, 143.9, 143.8, 141.3, 129.2, 127.8, 127.1, 125.1, 121.7, 120.9, 120.0, 113.1, 67.1, 61.5, 55.5, 54.9, 47.2, 38.1, 25.8, 18.5, 14.2, -4.53.

2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-hydroxy-3-methoxyphenyl)propanoic acid (±)-**6**



The same experimental procedure was followed as described for the synthesis of (±)-**5**. 2 M HCl (10 mL) was added to a solution of compound (±)-**39** (0.99 g, 1.72 mmol, 1.0 eq.) in 1,4-dioxane (10 mL). Crude material was recrystallised in Petroleum ether and EtOAc. Compound (±)-**6** (0.57 g, 1.31 mmol, 76%) was obtained a white solid.

M.p. 150–155 ^{o}C; **IR** (neat) ν_{max}/cm^{-1} 3332, 2949, 1693, 1591, 1514, 1448, 1336, 1259, 1151, 1031; **HRMS** (ESI) *m/z* [M+Na]$^+$ calcd. for $C_{25}H_{23}NO_6Na$ 456.1525, found 456.1520; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.67 (br s, 1H), 7.88 (d, *J* = 7.4 Hz, 2H), 7.63 (d, *J* = 7.3 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.30 (q, *J* = 7.3 Hz, 2H), 6.77 (s, 1H), 6.54–6.61 (m, 2H), 4.11–4.23 (m, 3H), 3.87 (br s, 1H), 3.66 (s, 3H), 2.99 (dd, *J* = 13.8, 4.1 Hz, 1H), 2.77 (dd, *J* = 13.7, 8.2 Hz, 1H); **^{13}C NMR** (125 MHz, DMSO-d_6) δ 173.8, 155.9, 147.2, 144.9, 143.9, 143.8, 140.7, 129.1, 127.7, 127.1, 125.3, 121.5, 120.2, 115.1, 113.4, 65.6, 56.3, 55.5, 46.6, 36.4.

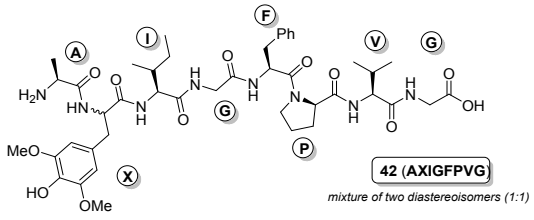
Peptide Synthesis

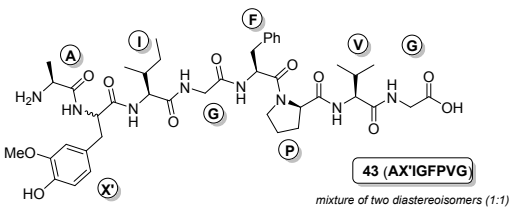
Cloning, Expression and Purification of Enzyme P*at*G$_{mac}$

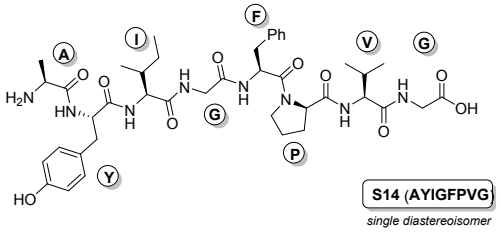
P*at*G$_{mac}$ was cloned from genomic DNA (*Prochlon sp.*) into the pHISTEV vector (a kind gift from Dr Haunting Liu) by Dr Jesko Koehnke. The enzyme was expressed in *Eschericia coli* BL21 (DE3) cells grown in auto-induction media as previously described.[S7] Enzyme purification was done as follows: Cell pellets were re-suspended in lysis buffer (20 mM Tris pH 8.0, 500 mM NaCl, 20 mM Imidazole, 3 mM BME), supplemented with 0.4 mg DNAse (SIGMA) per gram of wet cell pellets and EDTA-free protease inhibitor tablets (Roche; 1 per 50 mL re-suspension). The cells were lysed *via* passage through a cell disruptor at 207 MPA (Constant Systems) and clarified by centrifugation (40, 000 *g*, 4 °C, 20 min). The supernatant was passed through a Ni-NTA-sepharose 6 Fast Flow column (GE Healthcare) equilibrated in lysis buffer. The bound proteins were washed with lysis buffer and eluted with elution buffer (20 mM Tris pH 8.0, 500 mM NaCl, 250 mM Imidazole, 3 mM BME). The eluted proteins were dialysed (3 x 300 mL, 4 °C) into storage buffer (10 mM Bicine pH 8.5, 150 mM NaCl, 1 mM TCEP).

Experimental Procedure for the Synthesis of Linear Peptides 42 (AXIGFPVG), 43 (AX'IGFPVG), and S14 (AYIGFPVG)*

*Amino acid sequence in brackets; **A** = Alanine, **X** = Unnatural Amino Acid derived from (±)-**5**, **X'** = Unnatural Amino Acid derived from (±)-**6**, **Y** = L-Tyrosine, **I** = Isoleucine, **G** = Glycine, **F** = Phenylalanine, **P** = Proline, **V** = Valine.

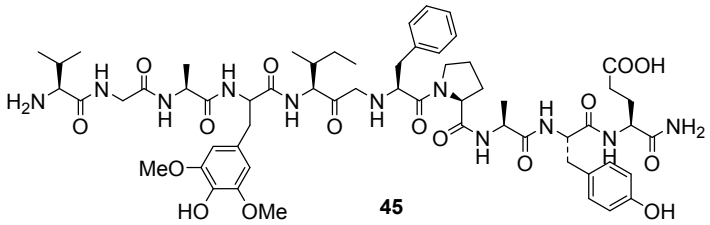






The solid-phase synthesis of **42**, **43** and **S14** was carried out on preloaded Gly-2-chlorotrityl chloride resin (0.75 mmol/g) purchased from Bachem. The synthesis was performed on an automatic peptide synthesiser (Biotage Syrowave) on a 0.15 mmol scale. Amino acids were double coupled using a 4-fold excess of amino acid (0.60 mmol) for 20 mins at 75 °C making use of DIC (0.60 mmol, 9.2 µL, 0.5 M in DMF), Oxyma (0.60 mmol, 8.5 mg, 1 M in DMF) and HBTU (0.60 mmol, 23 mg, 0.5 M in DMF), DIEA (0.60 mmol, 10 µL, 2 M in DMF) coupling protocols. Unnatural amino acids (±)-**5**, (±)-**6** were single coupled for 30 mins at 75 °C with DIC (0.60 mmol, 9.2 µL, 0.5 M in DMF), Oxyma (0.60 mmol, 8.5 mg, 1 M in DMF). Prior to adding the next amino acid, the peptide-bound resin was Fmoc deprotected with 20% piperidine/DMF (*v*/*v*, 1 mL) for 10 mins. The first Fmoc deprotection was carried out with 50% piperidine/DMF for 3 mins in order to prevent aspartimide formation. After coupling, the resin was washed with DMF (6×2 mL). The final peptide-bound resin was dried under vacuum and the peptide was cleaved from the resin with a cocktail of reagents: 20% HFIP (hexafluoro isopropanol) in DCM (v/v) (2 mL) for 30 mins for **42** and **43**; 94% trifluoroacetic acid (TFA), 4% H_2O, 2% triisopropylsilane (TIS) 2 mL) for 2 hrs for **S14**. The resin was filtered away and the filtrate was concentrated *in vacuo* to afford a white powder (yield = 52% (**42**), 45% (**43**), 48% (**S14**)).

Experimental Procedure for the Synthesis of Linear Peptide 45 (VGAXIGFPAYD)

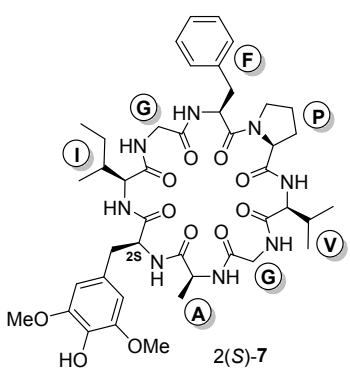


45

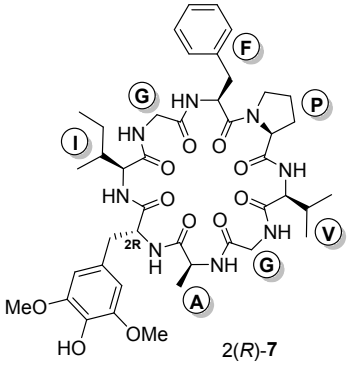
The solid-phase synthesis of **45** was carried out on rink amide chemmatrix resin (0.47 mmol/g) purchased from Iris Biotech. The synthesis was performed on an automatic peptide synthesiser (Biotage Syrowave) on a 0.047 mmol scale. Amino acids were double coupled using a 4-fold excess of amino acid (0.19 mmol) for 20 mins at 75 °C making use of DIC (0.19 mmol, 2.9 µL, 0.5 M in DMF), Oxyma (0.60 mmol, 2.7 mg, 1 M in DMF) and HBTU (0.19 mmol, 7.2 mg, 0.5 M in DMF), DIEA (0.19 mmol, 3.1 µL, 2 M in NMP) coupling protocols. Unnatural amino acid (±)-**5** was single coupled for 30 mins at 75 °C with DIC (0.19 mmol, 2.9 µL, 0.5 M in

DMF), Oxyma (0.19 mmol, 2.7 mg, 1 M in DMF). Prior to adding the next amino acid, the peptide-bound resin was Fmoc deprotected with 20% piperidine/DMF (*v*/*v*, 1 mL) for 10 mins. After coupling, the resin was washed with DMF (6×2 mL). The final peptide-bound resin was dried under vacuum and the peptide was cleaved from the resin with a cocktail of reagents: 920% HFIP (hexafluoro isopropanol) in DCM (v/v) (2 mL) for 30 mins; 94% trifluoroacetic acid (TFA), 4% H_2O, 2% triisopropylsilane (TIS) 2 mL) for 2 hrs. The resin was filtered away and the filtrate was concentrated *in vacuo* to afford **45** as a white powder (yield = 63%).

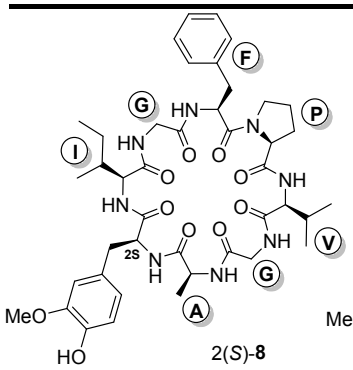
Experimental Procedure for the Synthesis of 2(*S*)-7, 2(*R*)-7, 2(*S*)-8, 2(*R*)-8 and 2(*S*)-44: Chemical Macrocyclisation of 42, 43, and S14



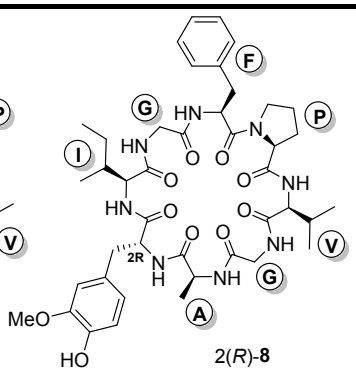
2(*S*)-**7**



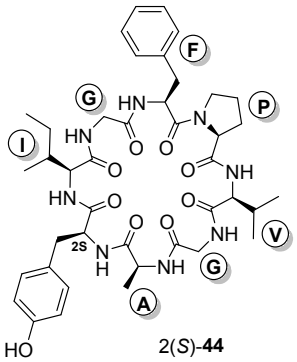
2(*R*)-**7**



2(*S*)-**8**



2(*R*)-**8**



2(*S*)-**44**

To a stirred solution of PyBOP (57 mg, 0.11 mmol), DIEA (45 µl, 0.26 mmol) in dry DMF (14 ml) at RT, a solution of the linear peptide (**42**, **43**, **S14**) (0.051 mmol) in dry DMF (3 ml) was added by syringe pump over 2 hrs. After 24 hrs the resulting mixture was concentrated *in vacuo*, diluted with DCM (20 ml) and washed with 1 M HCl (7×2 ml). The mixture was extracted with DCM (10×2 ml) and the combined organic phases were washed three times with water (10 ml), dried $MgSO_4$ and concentrated *in vacuo*. The crude residue was purified by RP-HPLC to give (8.5 mg (2(*S*)-**7**), 8.3 mg (2(*R*)-**7**), total yield = 30%; 8.4 mg (2(*S*)-**8**), 8.2 mg (2(*R*)-**8**), total yield = 41%; 15 mg (**44**), yield = 31%).

Experimental Procedure for the Chemical Macrocyclisation of 42: Small scale

To a stirred solution of PyBOP (1.6 mg, 0.003 mmol), DIEA (5 µl, 0.03 mmol) in dry DMF (2 ml) at RT, a solution of the linear peptide (**42**) (0.006 mmol) in dry DMF (1 ml) was added by syringe pump over 2 hrs. After 24 hrs the resulting mixture was concentrated *in vacuo* and freeze-dried after solubilization in H_2O/ACN 1/1 0.1% TFA. The crude was analysed by UPLC (system A1) together with a sample of authentic **42** and 2(*S*)-**7** as references.

Experimental Procedure for the Enzymatic Macrocyclisation of Linear Peptide 45 using *Pat*G_{mac}

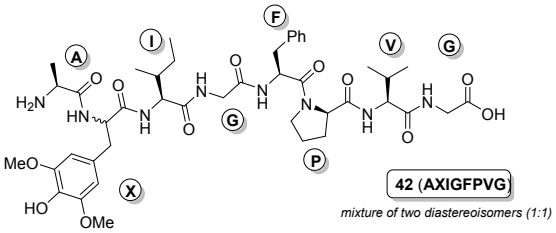
The reactions were conducted in 20 mM bicine buffer, 500 mM NaCl, and 5% DMSO solution, pH = 8.1 and incubated at 37 °C (without shaking) until full consumption of the starting peptide (MALDI monitoring) had occurred. The reaction set-up was prepared in the following order; final concentrations:

1- A solution of the linear peptide **45** (12 mg, 0.0098 mol) in DMSO; 100 µM
2- DMSO; 5%
3- 20 mM Bicine, 150 mM NaCl, pH = 8.1 buffer
4- 5 M NaCl; final concentration 500 mM
5- PatG_{mac} enzyme; 60 µM

The reaction mixture was extracted three times with ^{n}BuOH. In more detail, ^{n}BuOH (1/1, v/v) was then added to the aqueous reaction, vigorously mixed, and then centrifuged for 10 mins at high speed to help separate the two phases. The combined ^{n}BuOH fractions were evaporated under reduced pressure to dryness. The crude was solubilized in a minimum

volume of H_2O/MeOH for RP-HPLC purification giving samples of pure (2(*S*)-**7**) and (2(*R*)-**7**) (total yield = 24%).

Analytical Data for Linear peptides 42, 43, S14 and 45



42 (AXIGFPVG)

The m/z analysis was performed by ESI during LC-MS analysis. Observed mass (AXIGFPVG + H^+) = 883.4 Da, theoretical mass (AXIGFPVG + H^+) = 883.4 Da.

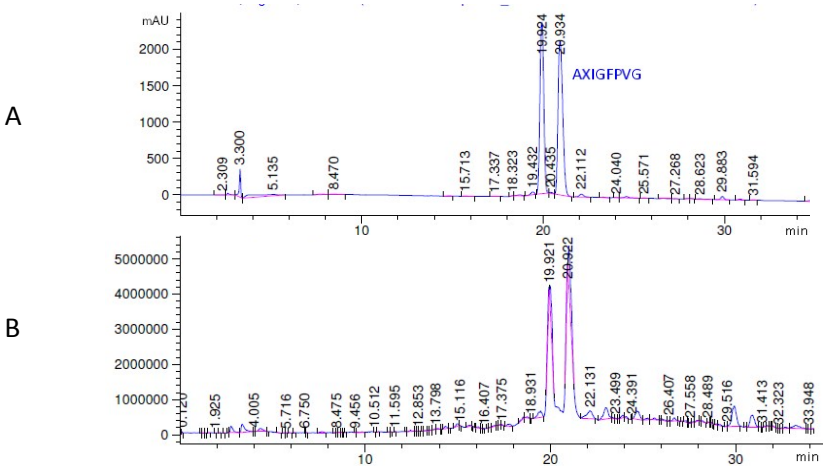


Figure S9: (A) HPLC trace (System A2) at 220 nm of **42** (*dr* = 1:1). (B) MS trace at the desired molecular weight (Single Ion Monitoring SIM mode) of **42** (*dr* = 1:1).

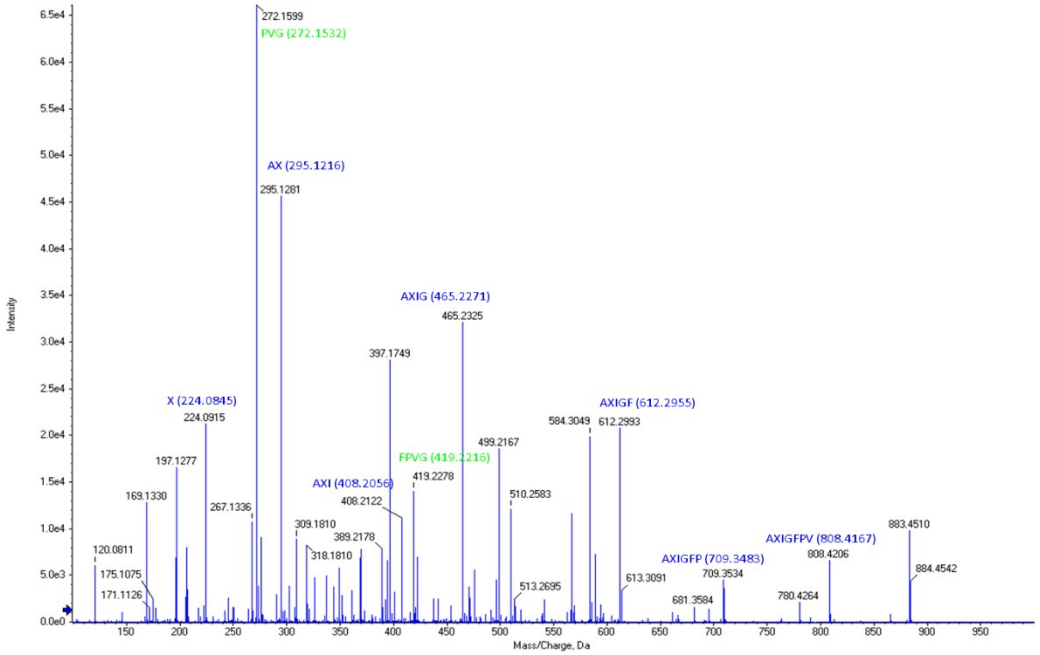
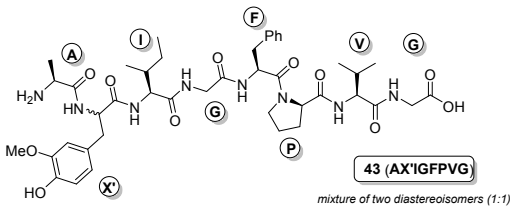


Figure S10: MS-MS fragmentation data of **42** after selection of ion with m/z = 883.4 Da. The selected fragments and their theoretical masses are shown.



AX'IGFPVG (**43**)

The m/z analysis was performed by ESI during LC-MS analysis. Observed mass (AX'IGFPVG + H^+) = 853.4 Da, theoretical mass (AX'IGFPVG + H^+) = 853.4 Da.

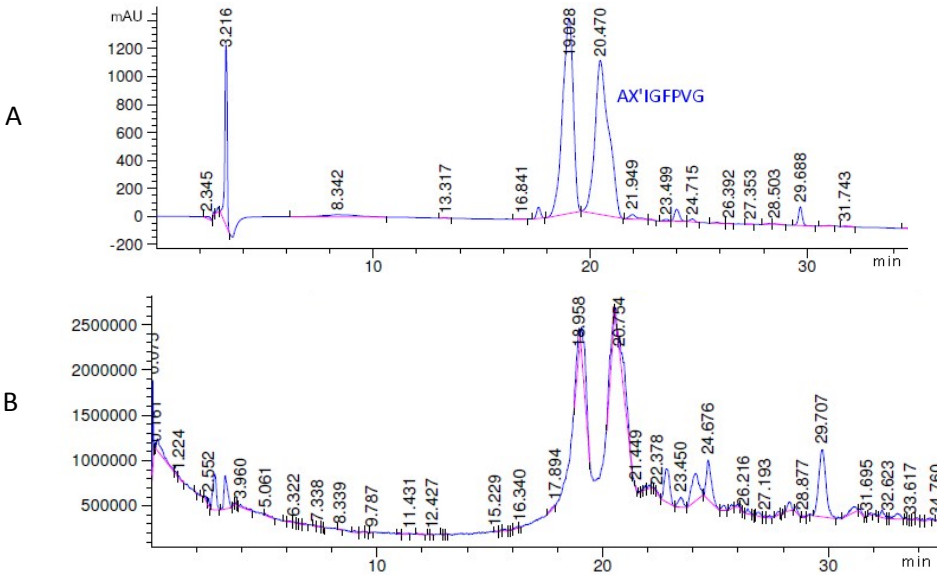
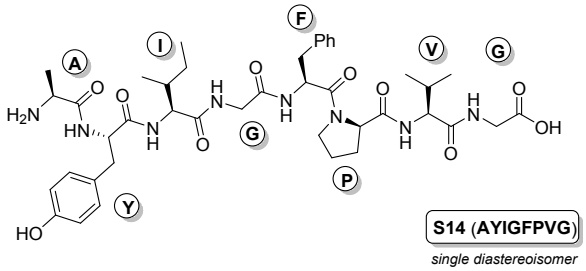


Figure S11: (A) HPLC trace (System A2) at 220 nm of **43** (*dr* = 1:1). B) MS trace at the desired molecular weight (Single Ion Monitoring SIM mode) of **43** (*dr* = 1:1).

- AYIGFPVG (**S14**)



The m/z analysis was performed by ESI during LC-MS analysis. Observed mass (AYIGFPVG + H^+) = 823.4 Da, theoretical mass (AYIGFPVG + H^+) = 823.4 Da.

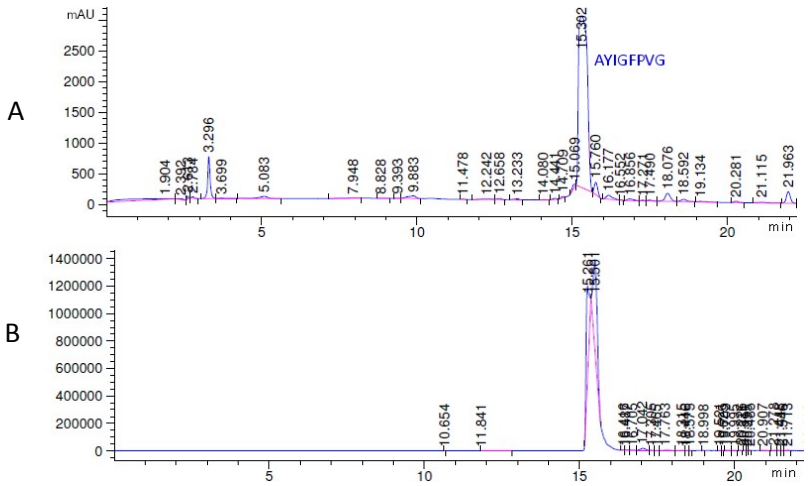
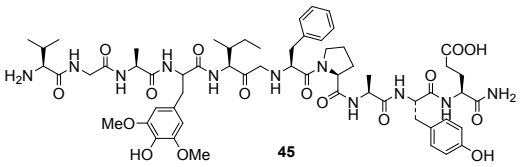


Figure S12: A) HPLC trace (System A2) at 220 nm of **S14**. B) MS trace at the desired molecular weight (Single Ion Monitoring SIM mode) of **S14**.

VGAXIGFPAYD (**45**)



The m/z analysis was performed by ESI during LC-MS analysis. Observed mass (AXIGFPVG + H^+) = 1231.6 Da, theoretical mass (AXIGFPVG + H^+) = 1231.6 Da.

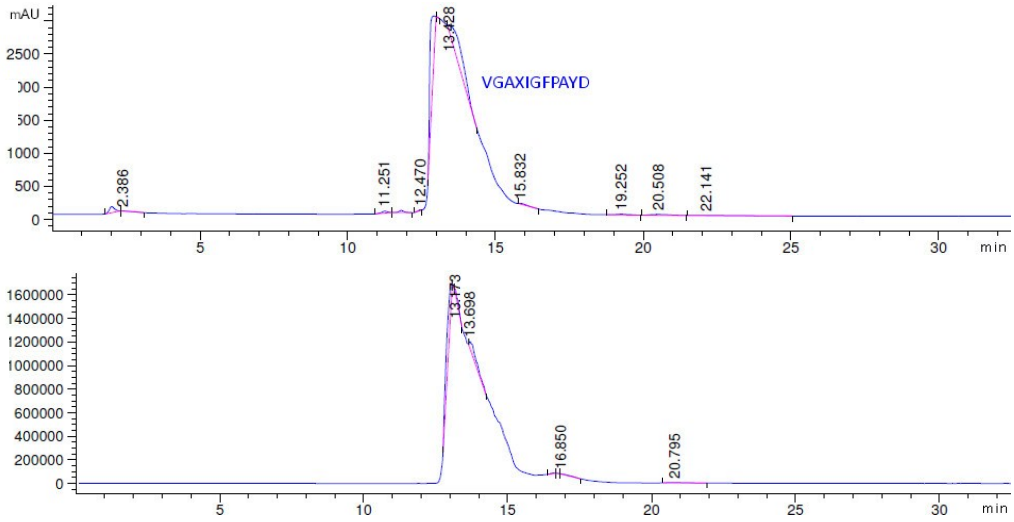
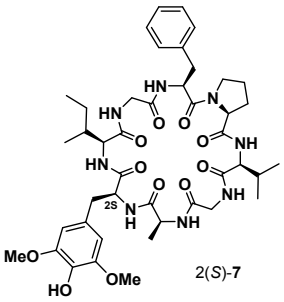


Figure S13: A) HPLC trace (System A4) at 220 nm of **45** (*dr* = 1:1). B) MS trace at the desired molecular weight (Single Ion Monitoring SIM mode) of **45** (*dr* = 1:1).

Analytical Data for Cyclic Octapeptides 7, 8, and 44

- Cyclooctapeptide (-AXIG$_a$FPVG$_b$-) 2(*S*)-**7** 2:1 (conformers)



2(*S*)-7

Yield = 15%, 8.5 mg (chemical cyclisation); Yield = 19%, 1.4 mg (enzyme-mediated cyclisation); purity = 96%; rt = 34.2 mins.

The m/z analysis was performed by HR-ESI. Observed mass (cyclo 2(*S*)-**7**) + H^+) = 865.4445 Da, theoretical mass ((cyclo 2(*S*)-**7**) + H^+) = 865.4460 Da. Observed mass ((cyclo 2(*S*)-**7**) + Na^+) = 887.4260 Da, theoretical mass ((cyclo 2(*S*)-**7**) + Na^+) = 887.4279 Da.

Melting point: 170–175 °C

Specific Rotation $[\propto]_D^{20}$ = -55 ($CHCl_3$, *c* = 1)

IR (neat) cm^{-1} = 3290, 2964, 2351, 1749, 1635, 1516, 1031.

Table S4: Assignment of ^{1}H and ^{13}C NMR (700 MHz, DMSO-d_6) 2(*S*)-**7.** Extensive 2D NMR work was carried out to achieve this assignment. NMR data for the major conformer.

Amino acid	Atom	^{1}H chemical shift	^{13}C chemical shift
Ala	NH	8.60-8.58, m	-
	α CH	3.83-3.79, m	51.0
	β CH_3	1.10, d, *J* = 7.7	17.3
	CO	-	172.8
X	NH	8.07-8.05, m	-
	α CH	4.29-4.22, m	56.4
	β CH_2	2.97-2.95, m	36.9
	γ C	-	128.1
	o-Ar	6.43, s	106.5
	m-Ar	-	147.7
	p-Ar	-	134.1
	OMe	3.72, s	56.3
	CO	-	170.4
Ile	NH	7.44, d, *J* = 7.4	-
	α CH	3.98-3.96, m	58.5
	β CH	1.65-1.62, m	36.9
	γ CH_2	1.60-1.57, m; 1.19-1.16, m	25.5
	γ CH_3	0.85-0.81, m	15.2
	δ CH_3	0.85-0.81, m	11.1
	CO	-	171.1
Gly (a)	NH	7.49-7.47, m	-
	α CH_2	4.29-4.22, m; 3.46-3.43, m	41.6
	CO	-	171.8
Phe	NH	8.67, br	-
	α CH	4.45-4.42, m	53.6
	β CH_2	2.97-2.95, m; 2.85-2.82, m	37.1
	γ-C	-	136.1
	o-Ar	7.31-7.22 (Ar)	129.1
	m-Ar	7.31-7.22 (Ar)	129.9
	p-Ar	7.31-7.22 (Ar)	129.0
	CO	-	170.8
Pro	α CH	overlapped with OMe	60.8
	β CH_2	1.92-1.88, m; 0.88-0.81, m	29.8
	γ CH_2	1.59-1.56, m; 1.30-1.26, m	21.5
	δ CH_2	3.31-3.26, m; 3.19-3.16, m	46.1
	CO	-	171.2
Val	NH	8.30, d, *J* = 9.0	-
	α CH	3.76-3.74, m	61.8
	β CH	2.00-1.97, m	29.7
	γ CH_3	0.85-0.81, m	19.5
	CO	-	171.4

Gly (b)	NH	overlapped with Ar signals	-
	α CH_2	4.14-4.10, m; overlapped with OMe	42.8
	CO	-	170.0

Table S5: Assignment of ^{1}H and ^{13}C NMR (700 MHz, DMSO-d_6) 2(*S*)-**7.** Extensive 2D NMR work was carried out to achieve this assignment. NMR data for the minor conformer.

Amino acid	**Atom**	**^{1}H chemical shift**	**^{13}C chemical shift**
Ala	NH	7.96, d, *J* = 8.6	-
	α CH	4.06-4.02, m	49.0
	β CH_3	1.16, d, *J* = 7.3	17.3
	CO	-	171.5
X	NH	overlapped with Ar	-
	α CH	4.66-4.63, m	56.4
	β CH_2	2.90-2.87, m; 2.62-2.59, m	36.9
	γ C	-	128.1
	o-Ar	6.44, s	106.5
	m-Ar	7.31-7.22 (Ar)	147.7
	p-Ar	7.31-7.22 (Ar)	134.1
	OMe	3.71, s	56.3
	CO	-	ND
Ile	NH	8.15, br	-
	α CH	3.91-3.89, m	59.1
	β CH	1.71-1.68, m	36.1
	γ CH_2	1.59-1.56, m; 1.13-1.11, m	25.5
	γ CH_3	0.85-0.81, m	15.2
	δ CH_3	0.85-0.81, m	11.1
	CO	-	171.9
Gly (a)	NH	8.60-8.58, m	-
	α CH_2	4.00-3.97, m; 3.31-3.26, m	42.4
	CO		170.3
Phe	NH	7.75, d, *J* = 8.9	-
	α CH	4.75-4.72, m	53.6
	β CH_2	2.97-2.95, m	37.1
	γ-C	-	136.1
	o-Ar	7.31-7.22 (Ar)	127.3
	m-Ar	7.31-7.22 (Ar)	129.9
	p-Ar	7.31-7.22 (Ar)	128.5
	CO	-	ND
Pro	α CH	4.29-4.22, m	61.7
	β CH_2	2.03-2.01, m; 1.92-1.88, m	28.7
	γ CH_2	1.92-1.88, m	25.0
	δ CH_2	3.62-3.60, m	47.3
	CO	-	ND
Val	NH	7.56, d, *J* = 7.5	-
	α CH	4.06-4.02, m	58.5

	β CH	2.04-2.02, m	30.1
	γ CH_3	0.85-0.81, m	19.5
	CO	-	ND
Gly (b)	NH	8.07-8.05, m	-
	α CH_2	overlapped with OMe; 3.68-3.64	42.4
	CO		170.3

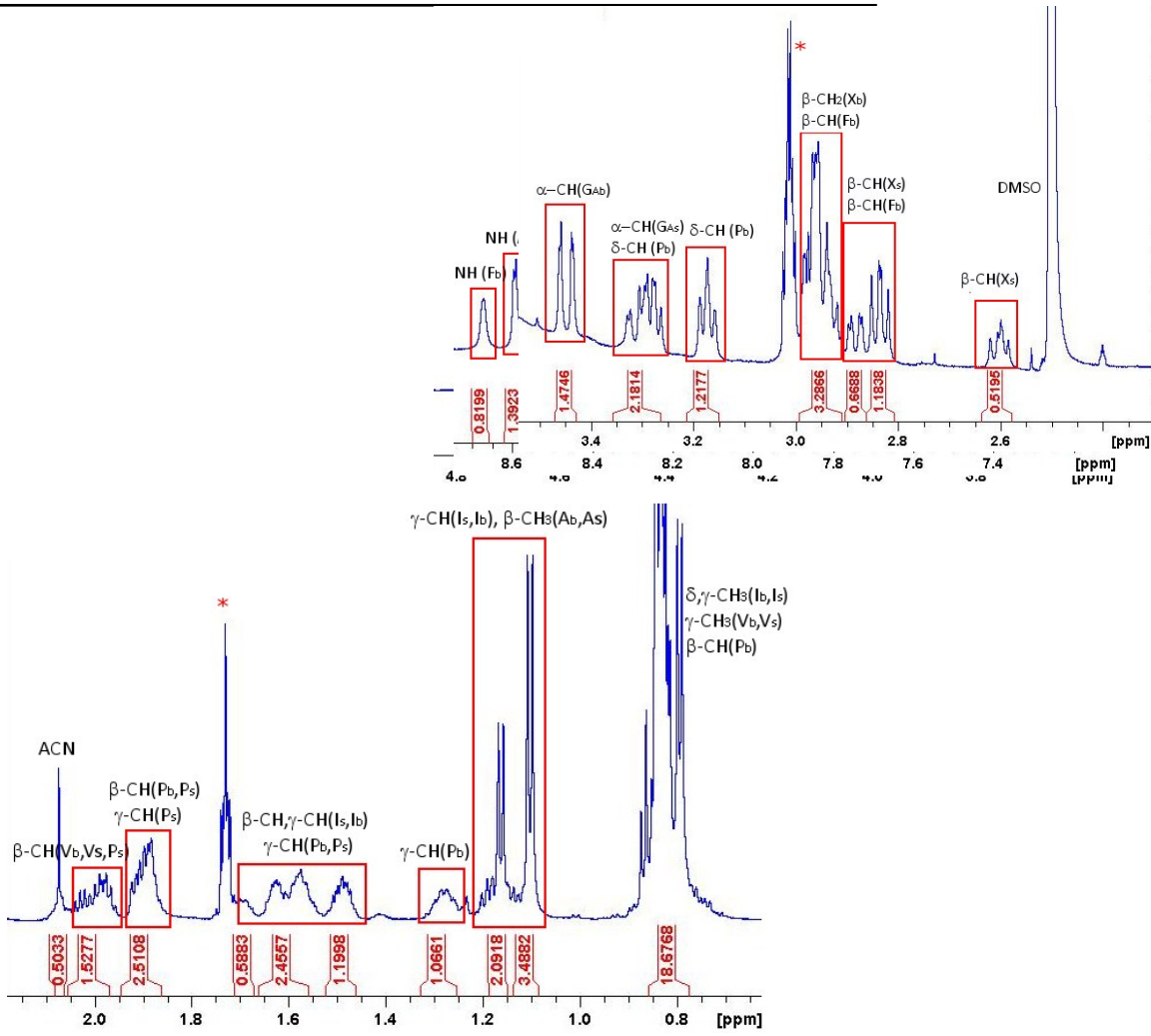


Figure S14: ^{1}H NMR spectrum (700 MHz, DMSO-d_6) of cycle 2(*S*)-**7** from the chemical macrocyclisation. Subscripts b, s are respectively referred to the major and minor conformation. ACN = acetonitrile. b = big; s = small.

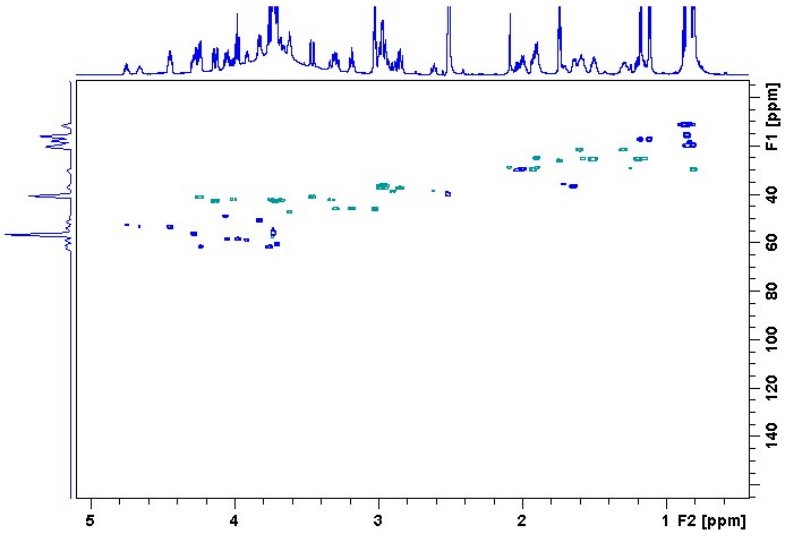


Figure S15: HSQC ^{13}C-^{1}H NMR spectrum (700 MHz, DMSO-d_6) of 2(*S*)-**7** from the chemical macrocyclisation.

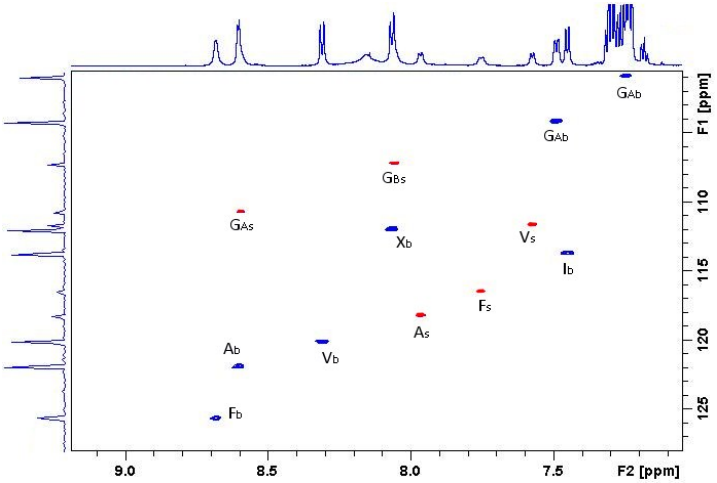


Figure S16: HSQC ^{15}N-^{1}H NMR spectrum (700 MHz, DMSO-d_6) of 2(*S*)-**7** from the chemical macrocyclisation.

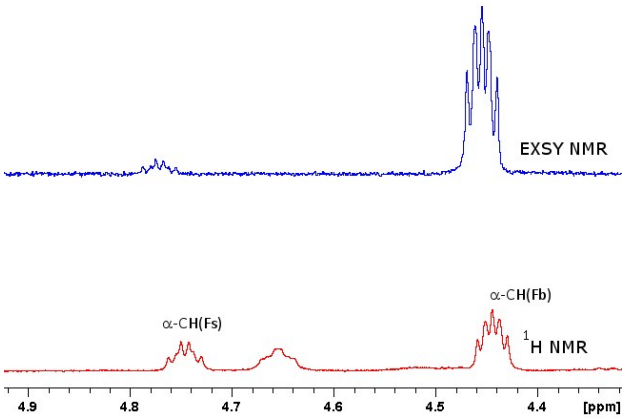


Figure S17: EXSY NMR spectrum of 2(*S)*-**7** at 35 °C. α-CH signal of Phe in the major conformation was irradiated.

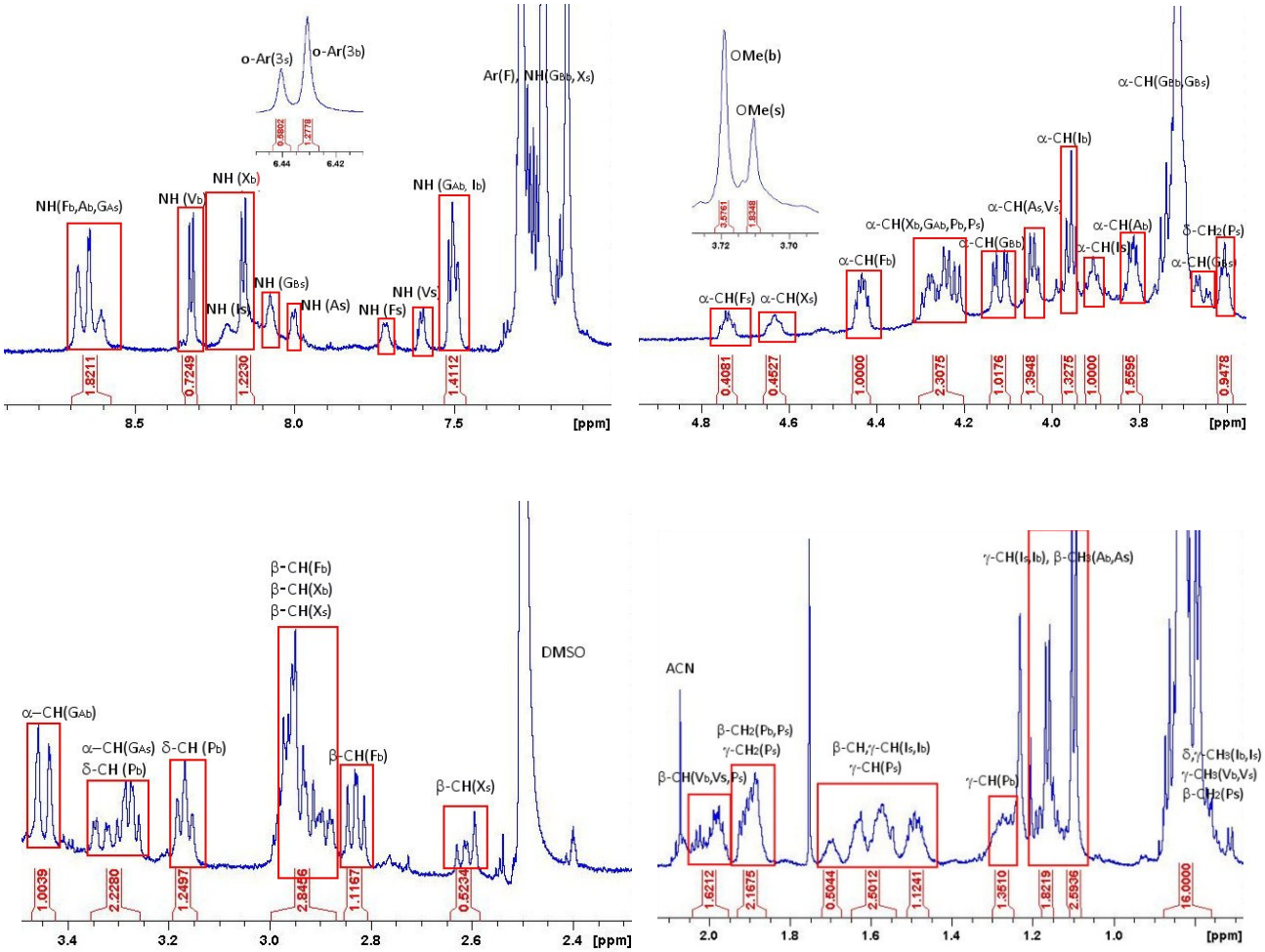


Figure S18: ^{1}H NMR spectrum (700 MHz, DMSO-d_6) of cycle 2(*S*)-**7** from the enzyme-mediated macrocyclisation.

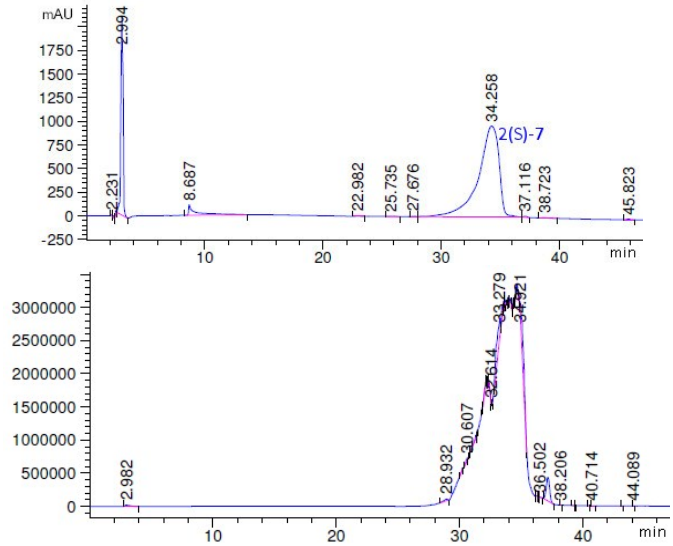


Figure S19: A) HPLC trace (System A3) at 220 nm of 2(S)-**7** from enzyme-mediated synthesis B) MS trace at the desired molecular weight (Single Ion Monitoring SIM mode) of 2(*S*)-**7**.

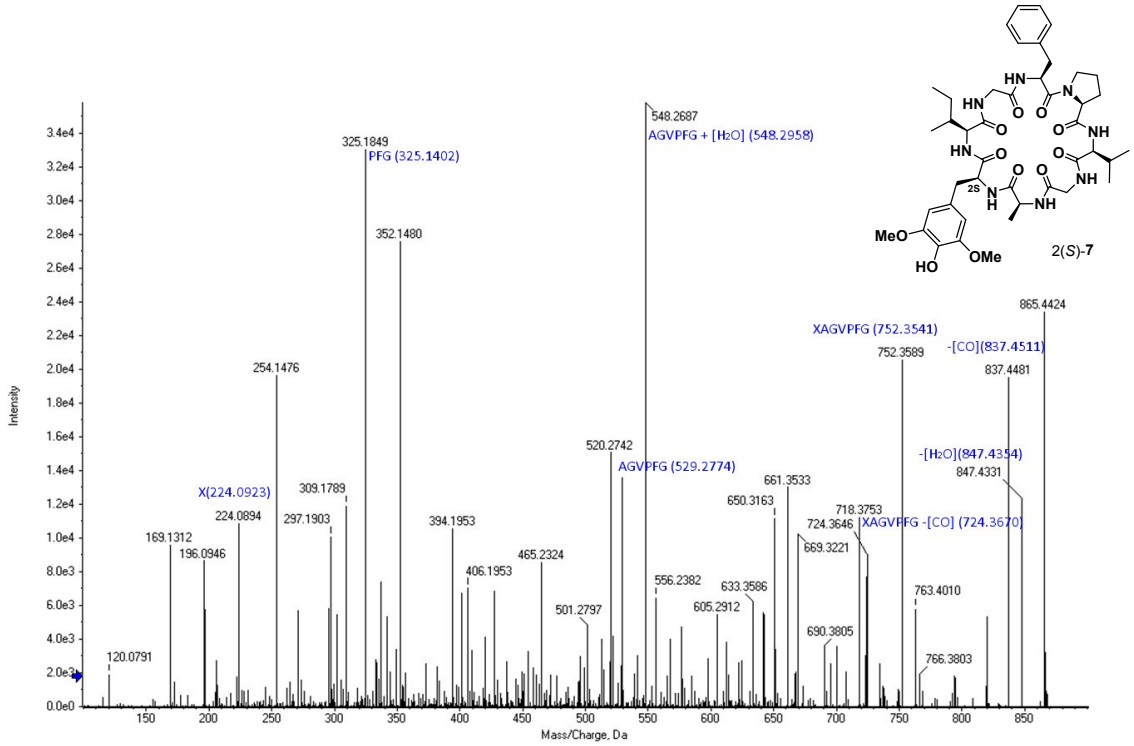


Figure S20: MS-MS fragmentation data of cycle 2(*S*)-**7** after selection of ion with m/Z = 865.4 Da. The selected fragments and their theoretical masses are shown. Fragments containing the PV bond are particularly relevant as this implies that this bond has formed as required for macrocylisation to have occurred.

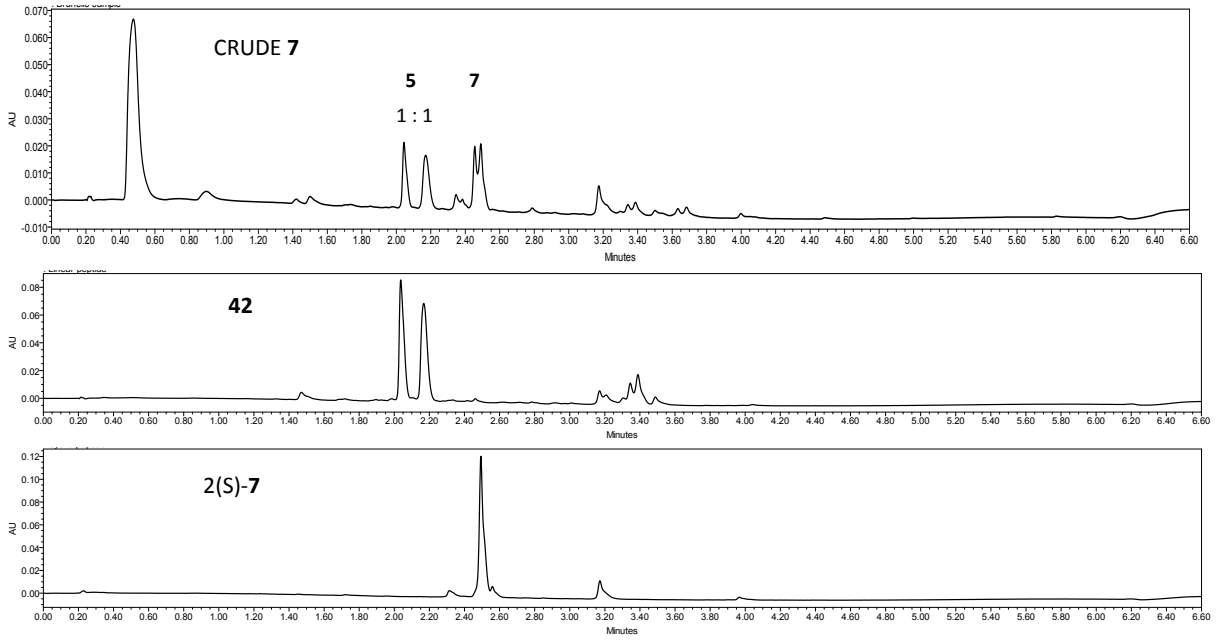


Figure S21: UPLC traces (System A1) at 220 nm of the crude sample of **7** prepared by enzyme-mediated cyclisation of **42**; the starting linear peptide **42** and authentic product 2(*S*)-**7**. This data shows that even when the enzyme-mediated reaction has not gone to completion, the approximate ratio of 2(*S*)-**7** and 2(*R*)-**7** (assigned as second peak adjacent to 2(*S*)-**7** peak) was approximately 1: 1 and that therefore there is no major difference in the rate of the enzyme-mediated formation of the two diasteromeric cyclic peptides.

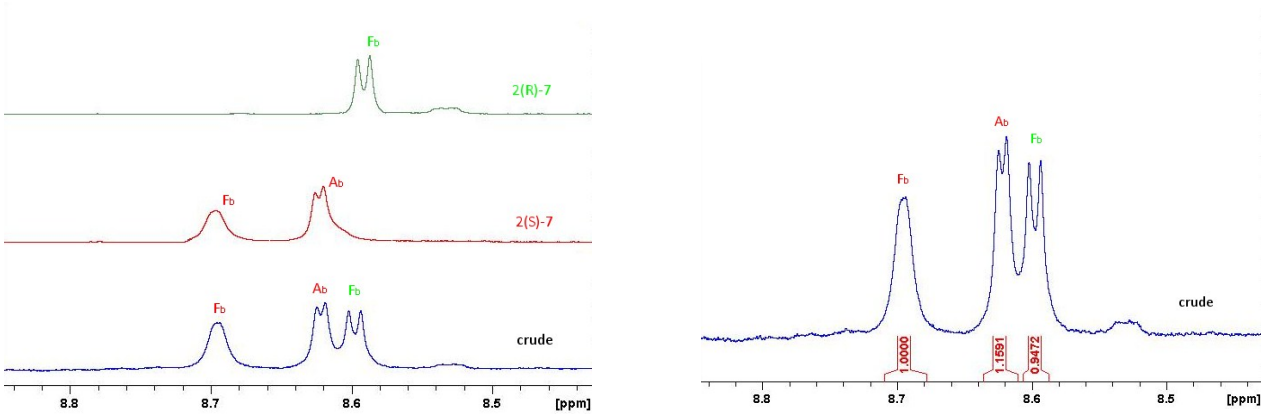
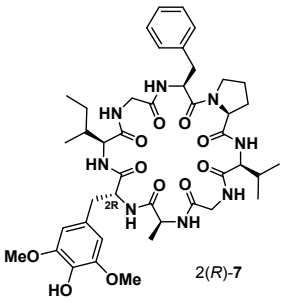


Figure S22: ^{1}H NMR spectrum (700 MHz, DMSO-d_6) of the crude mixture of 2(*S*)-**7**, 2(*R*)-**7** from the enzymatic macrocyclisation overlaid with spectra of purified 2(*S*)-**7**, 2(*R*)-**7** including an expansion of the spectrum for the crude enzyme-mediated reaction mixture.

Cyclooctapeptide (-AXIG$_a$FPVG$_b$-) 2(*R*)-**7** 7:1 (conformers)



Yield = 15%, 8.3 mg (chemical cyclisation); Yield = 21%, 0.4 mg (enzymatic cyclisation); purity = 96%; rt = 34.2 mins.

The m/z analysis was performed by HR-ESI. Observed mass (cyclo 2(*S*)-**7**) + H^+) = 865.4451 Da, theoretical mass (cyclo 2(*S*)-**7**) + H^+) = 865.4460 Da. Observed mass (cyclo 2(*S*)-**7**) + Na^+) = 887.4265 Da, theoretical mass (cyclo 2(*S*)-**7**) + Na^+) = 887.4279 Da.

Melting point: 170–175 °C

Specific Rotation $[\propto]_D^{20}$ = -78 ° ($CHCl_3$, *c* = 1)

IR: (neat) cm^{-1} = 3290, 2964, 2351, 1749, 1635, 1516, 1031.

Table S6: Assignment of ^{1}H and ^{13}C NMR (700 MHz, DMSO-d_6) 2(*R*)-**7**. Extensive 2D NMR work was carried out to achieve this assignment. NMR data for the major conformer.

Amino acid	Atom	^{1}H chemical shift	^{13}C chemical shift
Ala	NH	8.33, d, *J* = 6.5	-
	α CH	4.34-4.31, m	48.1
	β CH_3	1.16, d, *J* = 8.0	18.0
	CO	-	173.7
X	NH	8.07, br	-
	α CH	4.55-4.52, m	55.0
	β CH_2	3.23-3.20, m; 2.91-2.86, m	37.7
	γ C	-	127.3
	o-Ar	6.48, s	106.8
	m-Ar	-	148.1
	p-Ar	-	133.9
	OMe	3.72, s	56.3
	CO	-	169.1
Ile	NH	8.15-8.13, m	-
	α CH	3.90-3.88, m	59.1
	β CH	1.72-1.69, m	35.7
	γ CH_2	1.61-1.59, m; 1.35-1.32, m	21.6
	γ CH_3	0.78-0.76, m	15.7
	δ CH_3	0.78-0.76, m	11.5
	CO	-	170.8
Gly (a)	NH	7.97, t, *J* = 6.5	-
	α CH_2	overlapped with OMe -	41.4
	CO		173.1
Phe	NH	8.59, d, *J* = 6.1	-
	α CH	4.55-4.52, m	54.9
	β CH_2	2.91-2.86, m; 2.77-2.74, m	36.4
	γ-C	-	136.7
	o-Ar	7.37-7.26 (Ar)	127.3
	m-Ar	7.37-7.26 (Ar)	129.7
	p-Ar	7.37-7.26 (Ar)	129.1
	CO	-	171.1
Pro	α CH	3.47-3.45, m	60.4
	β CH_2	1.83-1.80, m; 0.88-0.85, m	30.4
	γ CH_2	1.31-1.27, m; overlapped with β CH_3 (Ala)	24.6
	δ CH_2	3.32-3.29, m; 3.23-3.20, m	46.4
	CO	-	169.0
Val	NH	8.15-8.13, m	-
	α CH	3.84-3.81, m	61.8
	β CH	2.01-1.97, m	29.7
	γ CH_3	0.81-0.76, m; 0.72, d, *J* = 7.1	19.5

	CO	-	170.7
Gly (b)	NH	7.15, t, *J* = 4.9	-
	α CH_2	overlapped with OMe	41.4
	CO		170.6

Table S7: Assignment of ^{1}H and ^{13}C NMR (700 MHz, DMSO-d_6) 2(*R*)-**7**. Extensive 2D NMR work was carried out to achieve this assignment. NMR data for the minor conformer.

Amino acid	**Atom**	**^{1}H chemical shift**	**^{13}C chemical shift**
Ala	NH	7.73, d, *J* = 7.6	-
	α CH	4.34-4.31, m	48.1
	β CH_3	1.16, d, *J* = 8.0	18.9
	CO	-	ND
X	NH	8.31, d, *J* = 8.5	-
	α CH	4.55-4.52, m	55.0
	β CH_2	3.03-3.00, m; 2.77-2.74, m	37.7
	γ C	-	127.3
	o-Ar	6.48, s	106.8
	m-Ar	-	148.1
	p-Ar	-	133.9
	OMe	3.73, s	56.3
	CO	-	168.2
Ile	NH	8.38, d, *J* = 9.1	-
	α CH	4.34-4.31, m	59.1
	β CH	1.72-1.69, m	35.7
	γ CH_2	overlapped with β CH_3(Ala); 1.09-1.04, m	19.1
	γ CH_3	0.78-0.76, m	15.7
	δ CH_3	0.78-0.76, m	11.5
	CO	-	ND
Gly (a)	NH	8.53, t, *J* = 4.7	-
	α CH_2	4.17-4.14, m; 3.23-3.20, m	41.4
	CO		ND
Phe	NH	8.15-8.13, m	-
	α CH	4.71-4.69, m	54.9
	β CH_2	2.91-2.86, m; 2.60-2.57, m	36.4
	γ-C	-	136.7
	o-Ar	7.37-7.26 (Ar)	127.3
	m-Ar	7.37-7.26 (Ar)	129.7
	p-Ar	7.37-7.26 (Ar)	129.1
	CO	-	ND
Pro	α CH	3.47-3.45, m	60.4
	β CH_2	1.24-1.22, m	29.2
	γ CH_2	1.83-1.80, m; 1.41-1.39, m	24.9
	δ CH_2	3.53-3.51, m; 3.47-3.45, m	47.1
	CO	-	169.0

Val	NH	7.80, d, *J* = 8.5	-
	α CH	4.12-4.10, m	58.2
	β CH	2.10-2.06, m	30.3
	γ CH_3	0.83-0.81, m	18.3
	CO	-	171.0
Gly (b)	NH	8.04, t, *J* = 5.7	-
	α CH_2	3.93-3.90, m	42.6
	CO		ND

Figure S23: ^{1}H NMR spectrum (700 MHz, DMSO-d_6) of cycle 2(*R*)-**7** from the chemical macrocyclisation. Subscripts b, s are respectively referred to the major and minor conformation.

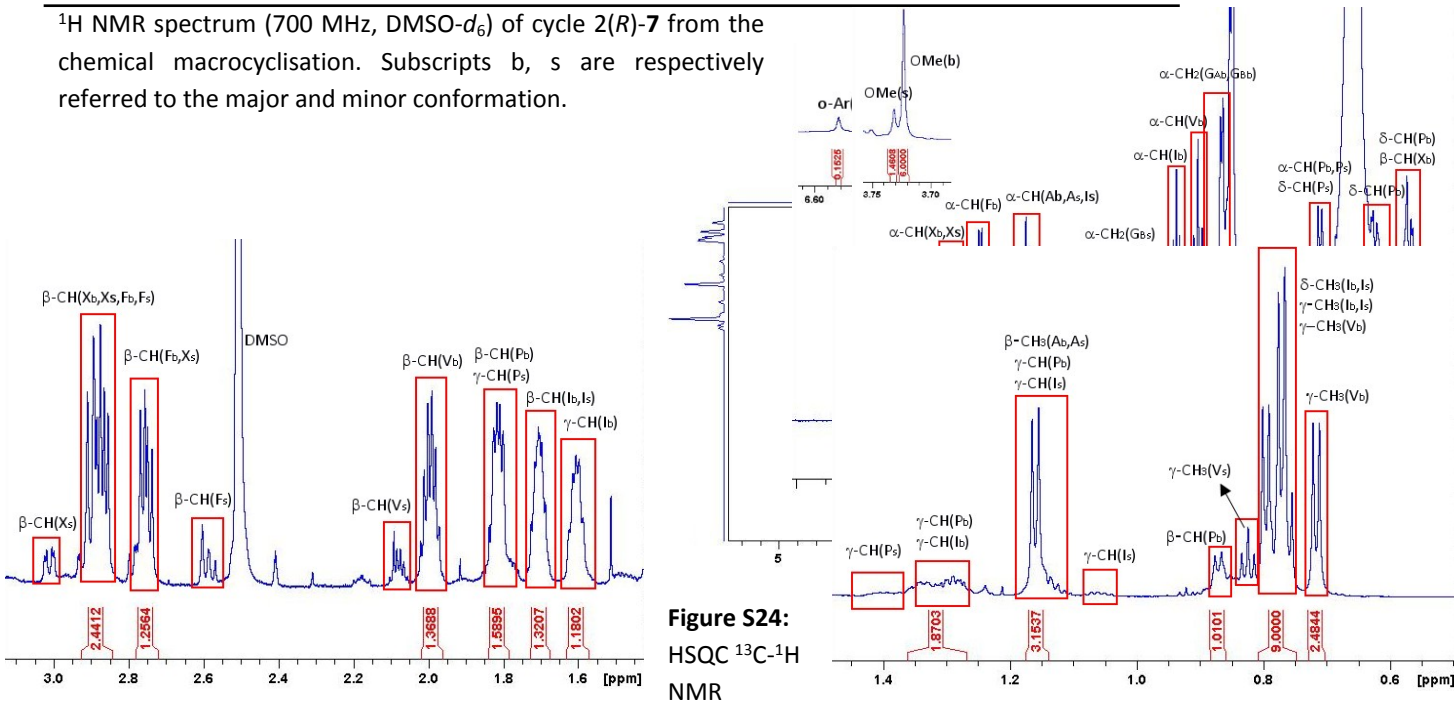


Figure S24: HSQC ^{13}C-^{1}H NMR spectrum (700 MHz, DMSO-d_6) of 2(R)-**7** from the chemical macrocyclisation.

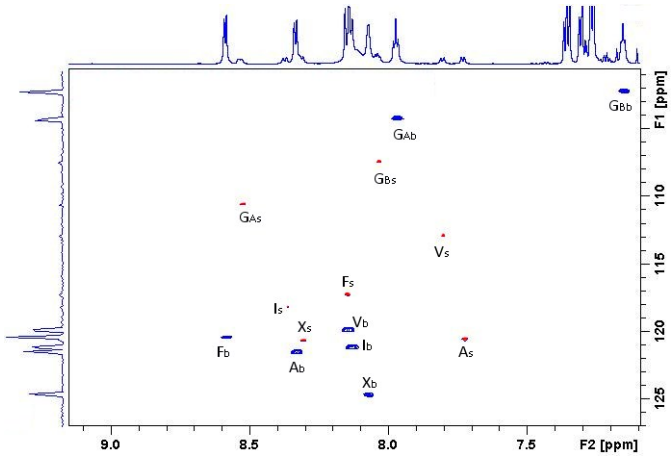


Figure S25: ^{1}H-^{15}N HSQC NMR spectrum (700 MHz, DMSO-d_6) of 2(R)-**7** from the chemical macrocyclisation.

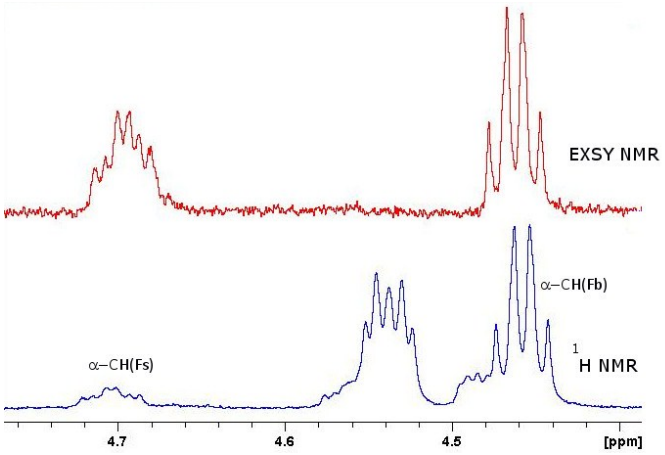


Figure S26: EXSY NMR spectrum of cycle 2(*R*)-**7** at 35 °C. α-CH signal of Phe in the major conformation was irradiated.

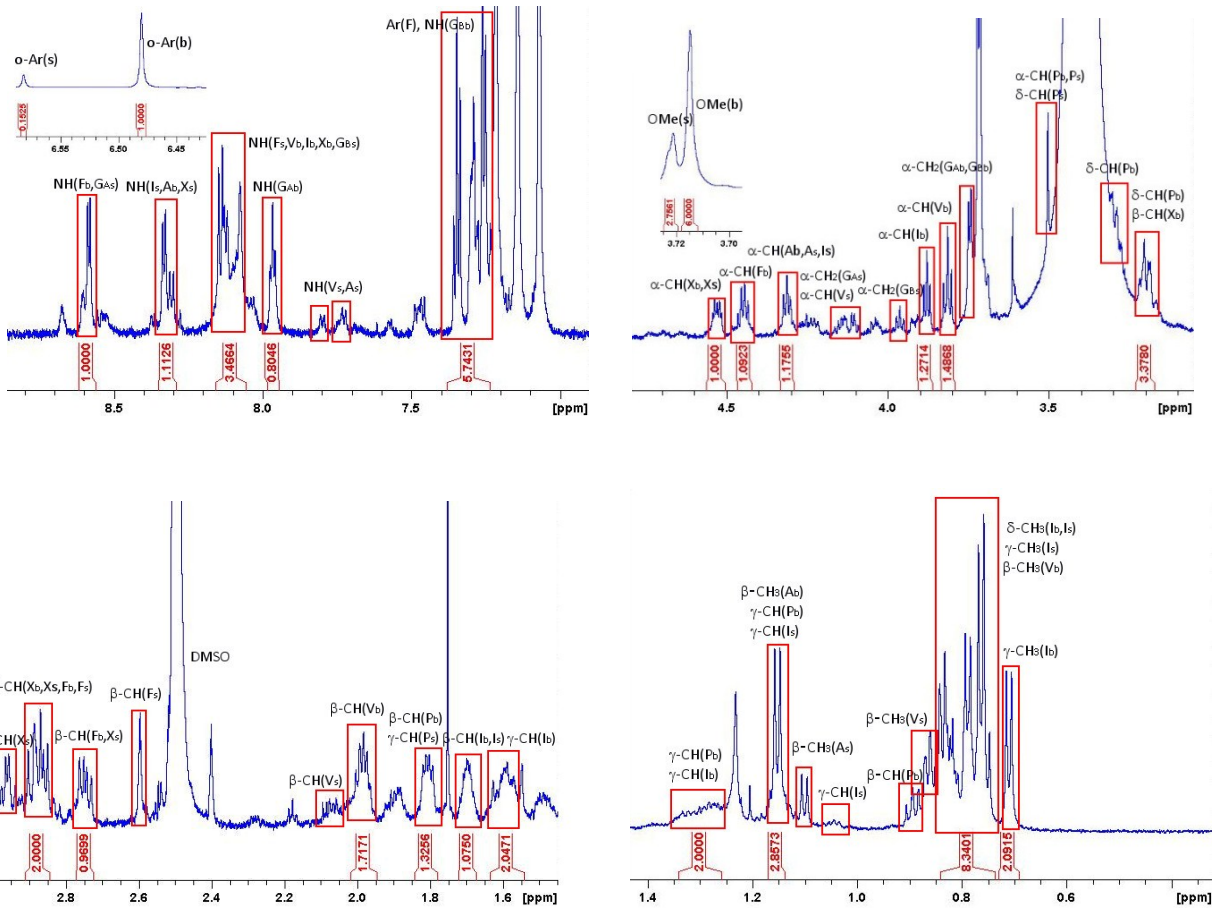
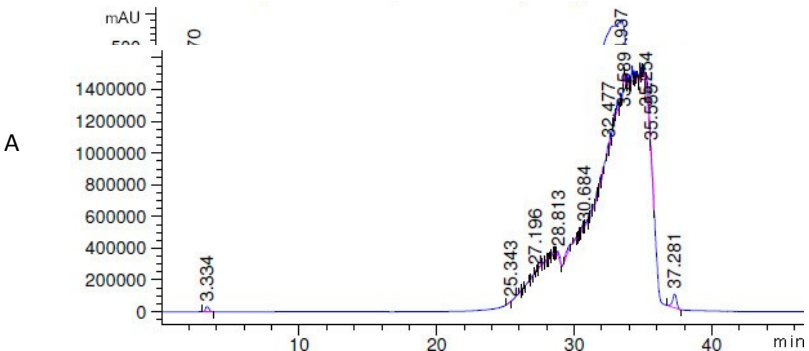


Figure S27: ^{1}H NMR spectrum (700 MHz, DMSO-d_6) of cycle 2(*R*)-**7** from the enzyme-mediated macrocyclisation.



B

Figure S28: A) HPLC trace (System A3) at 220 nm of 2(*R*)-**7**. B) MS trace at the desired molecular weight (Single Ion Monitoring SIM mode) of 2(*R*)-**7**.

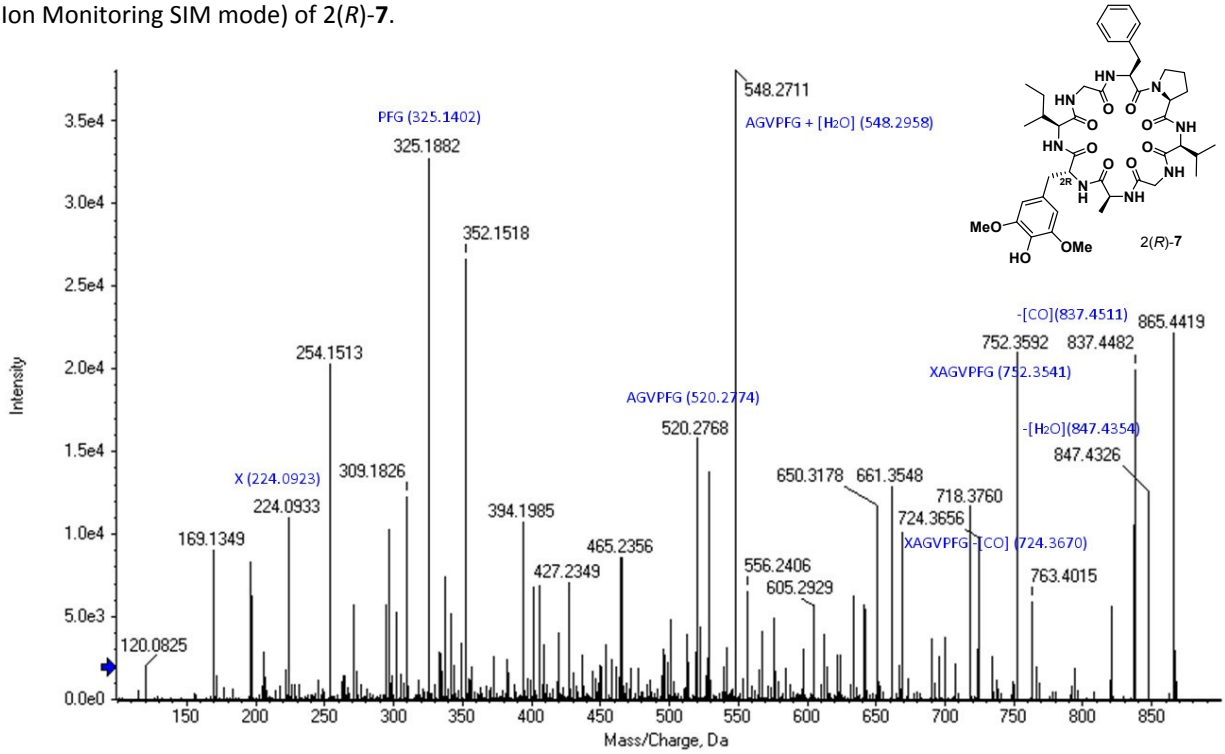
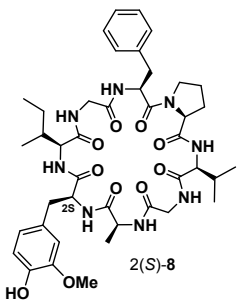


Figure S29: MS-MS fragmentation data of cycle 2(*R*)-**7** after selection of ion with m/z = 865.4 Da. The selected fragments and their theoretical masses are shown. The fragments containing the VP unit are important as they provide evidence that this bond, and hence the macrocycle, was formed.

Cyclooctapeptide (-AX'IG$_a$FPVG$_b$-) 2(*S*)-**8** 2:1 (conformers)



Yield = 15%, 8.4 mg (chemical cyclisation; purity = 96%; rt = 34.2 mins.

The m/z analysis was performed by HR-ESI.

Observed mass (cyclo 2(S)-**8**) + H^+) = 835.4341 Da, theoretical mass ((cyclo 2(S)-**8**) + H^+) = 835.4354 Da. Observed mass ((cyclo 2(S)-**8**) + Na^+) = 857.4157 Da, theoretical mass ((cyclo 2(S)-**8**) + Na^+) = 857.4174 Da.

Melting point: 175–180 °C

Specific Rotation $[\propto]_D^{20}$ = -60 ° ($CHCl_3$, *c* = 1)

IR: (neat) cm^{-1} = 3290, 2964, 2351, 1749, 1635, 1516, 1031.

Table S8: Assignment of ^{1}H and ^{13}C NMR (700 MHz, DMSO-d_6) 2(*S*)-**8**. Extensive 2D NMR work was carried out to achieve this assignment. NMR data for the major conformer.

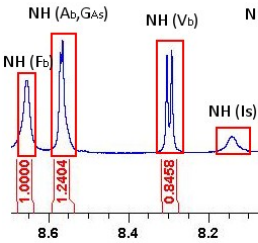
Amino acid	Atom	^{1}H chemical shift	^{13}C chemical shift
Ala	NH	8.57-8.55, m	-
	α CH	3.81-3.79, m	51.0
	β CH_3	1.10, d, J = 7.2	17.3
	CO	-	172.7
X’	NH	8.02-8.00, m	-
	α CH	4.25-4.21, m	56.3
	β CH_2	2.98-2.93, m	37.4
	γ C	-	128.4
	o-Ar	6.72, d, J = 1.5	121.5
	o’-Ar	6.55, dd, J_a = 8.0, J_b = 1.5	113.3
	m-Ar	-	145.4
	m'-Ar	6.65, d, J = 8.0	115.6
	p-Ar	-	147.7
	OMe	3.73, s	55.9
	CO	-	170.4
Ile	NH	7.44, d, J = 8.0	-
	α CH	3.96-3.94, m	58.4
	β CH	1.64-1.60, m	36.6
	γ CH_2	1.50-1.47, m; 1.20-1.17, m	25.6
	γ CH_3	0.84-0.81, m	15.4
	δ CH_3	0.84-0.81, m	11.0
	CO	-	171.1
Gly (a)	NH	7.49-7.47, m	-
	α CH_2	4.29-4.22, m; overlapped with water	41.4
	CO	-	171.8
Phe	NH	8.65, br	-
	α CH	4.45-4.42, m	53.6
	β CH_2	2.97-2.95, m; 2.85-2.82, m	37.4
	γ-C	-	136.3
	o-Ar	7.31-7.22 (Ar)	128.8
	m-Ar	7.31-7.22 (Ar)	129.6
	p-Ar	7.31-7.22 (Ar)	127.4
	CO	-	171.0
Pro	α CH	3.69-3.67, m	60.6
	β CH_2	1.92-1.87, m; 0.88-0.85, m	29.9
	γ CH_2	1.59-1.55, m; 1.30-1.26, m	21.6
	δ CH_2	3.30-3.27, m; 3.19-3.16, m	46.1
	CO	-	170.4
Val	NH	8.01, d, J = 8.4	-
	α CH	overlapped with OMe	61.8
	β CH	1.98-1.96, m	29.7
	γ CH_3	0.84-0.81, m	19.4
	CO	-	171.4
Gly (b)	NH	8.02-8.00, m	-

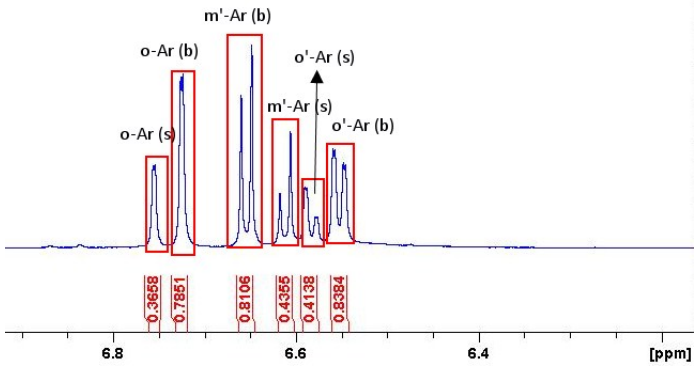
	α CH_2	4.12-4.09, m; 3.69-3.67, m	42.5
	CO		170.2

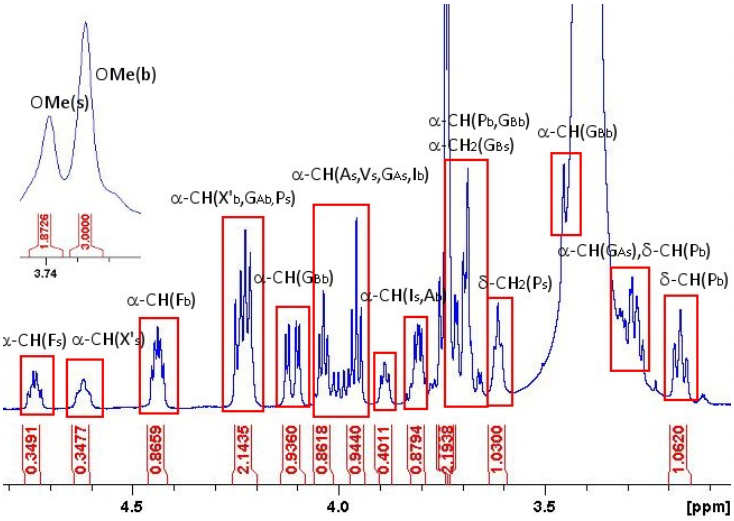
Table S9: Assignment of ^{1}H and ^{13}C NMR (700 MHz, DMSO-d_6) 2(*S*)-**8**. Extensive 2D NMR work was carried out to achieve this assignment. NMR data for the minor conformer.

Amino acid	Atom	^{1}H chemical shift	^{13}C chemical shift
Ala	NH	7.95, d, J = 7.2	-
	α CH	4.05-4.02, m	49.3
	β CH_3	1.14, d, J = 7.2	17.3
	CO	-	171.4
X’	NH	overlapped with Ar	-
	α CH	4.63-4.61, m	53.6
	β CH_2	2.89-2.86, m; 2.60-2.56, m	38.2
	γ C	-	128.4
	o-Ar	6.75, d, J = 1.5	121.7
	o’-Ar	6.58, dd, J_a = 8.0, J_b = 1.5	113.5
	m-Ar	-	145.4
	m'-Ar	6.61, d, J = 8.0	115.2
	p-Ar	-	147.7
	OMe	3.74, s	55.9
	CO	-	ND
Ile	NH	8.14, br	-
	α CH	3.90-3.87, m	59.1
	β CH	1.71-1.68, m	35.7
	γ CH_2	1.59-1.55, m; 1.12-1.10, m	25.2
	γ CH_3	0.84-0.81, m	15.4
	δ CH_3	0.84-0.81, m	11.0
	CO	-	ND
Gly (a)	NH	8.57-8.55, m	-
	α CH_2	4.01-3.98, m; 3.32-3.30, m	42.2
	CO		ND
Phe	NH	7.73, d, J = 6.9	-
	α CH	4.75-4.72, m	52.6
	β CH_2	2.97-2.95, m	37.4
	γ-C	-	136.3
	o-Ar	7.31-7.22 (Ar)	128.8
	m-Ar	7.31-7.22 (Ar)	129.6
	p-Ar	7.31-7.22 (Ar)	127.4
	CO	-	171.2
Pro	α CH	4.25-4.21, m	61.6
	β CH_2	2.08-2.06; 1.92-1.87, m	28.7
	γ CH_2	1.71-1.60, m; 1.30-1.26, m	22.1
	δ CH_2	3.62-3.60, m	47.5
	CO	-	ND
Val	NH	7.58, d, J = 7.1	-
	α CH	4.05-4.02, m	61.8
	β CH	2.05-2.02, m	30.0

	γ CH_3	0.84-0.81, m	19.4
	CO	-	ND
Gly (b)	NH	8.02-8.00, m	-
	α CH_2	3.77-3.75, m; 3.66-3.63, m	41.4
	CO	-	170.2







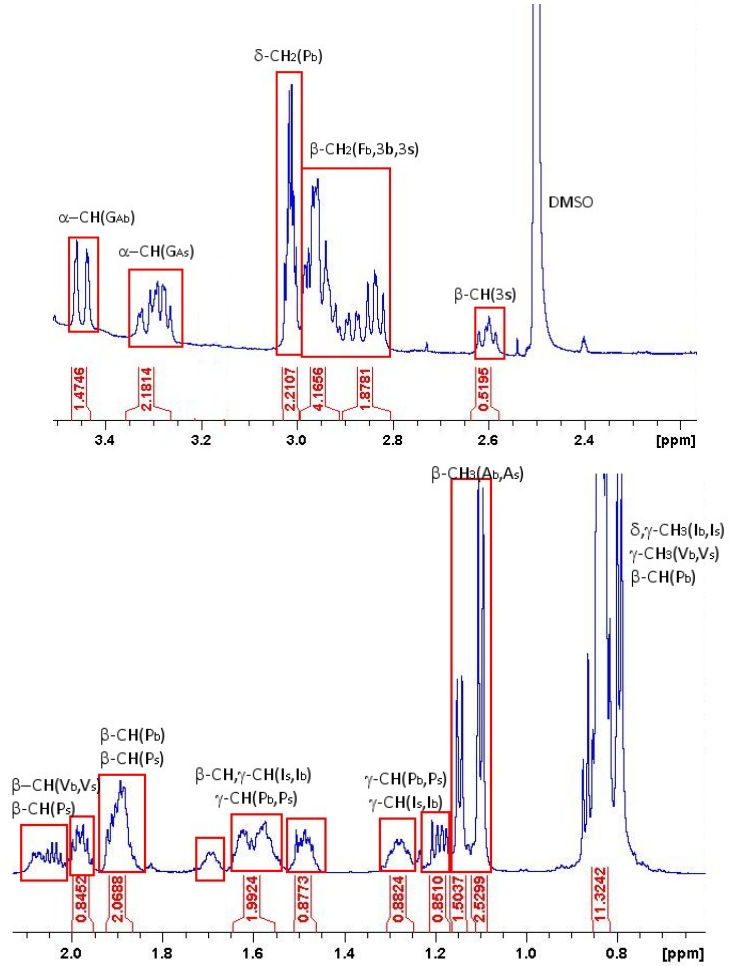


Figure S30: ^{1}H NMR spectrum (700 MHz, DMSO-d_6) of cycle 2(*S*)-**8**. Subscripts b, s are respectively referred to the major and minor conformation.

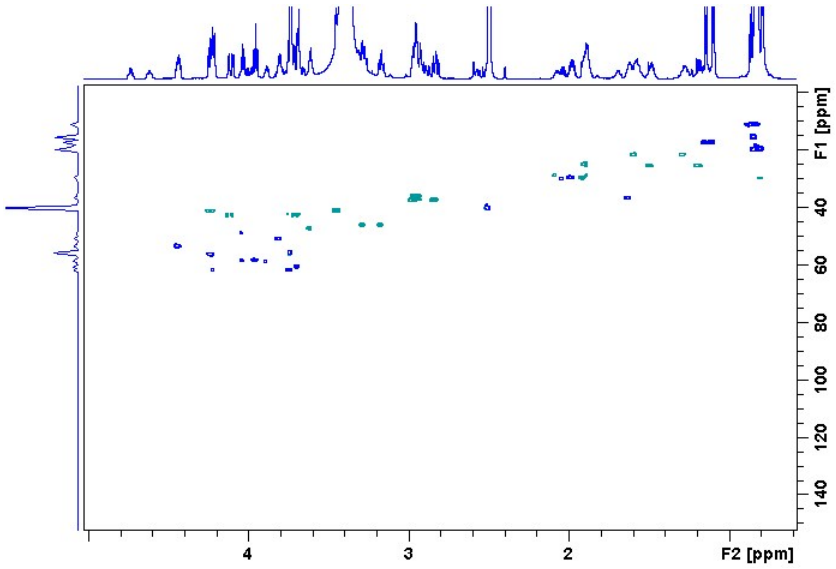


Figure S31: HSQC ^{13}C-^{1}H NMR spectrum (700 MHz, DMSO-d_6) of cycle 2(*S*)-**8**.

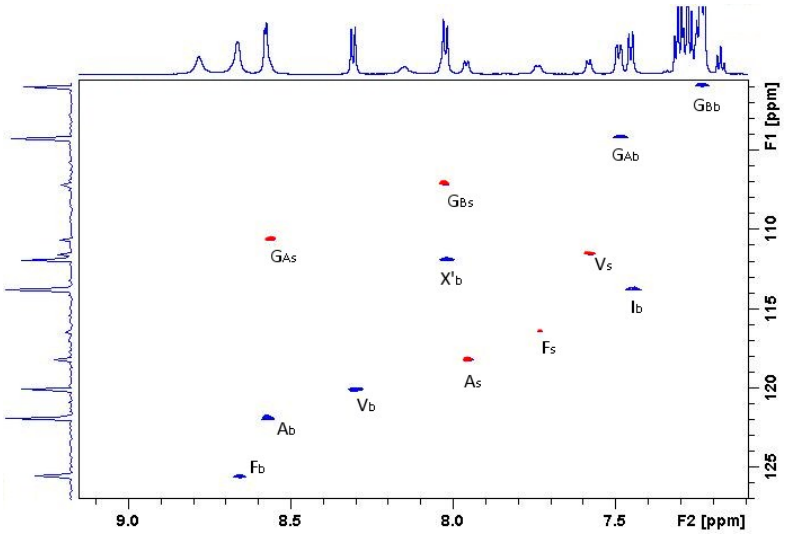


Figure S32: HSQC ^{15}N-^{1}H NMR spectrum (700 MHz, DMSO-d_6) of cycle 2(*S*)-**8**.

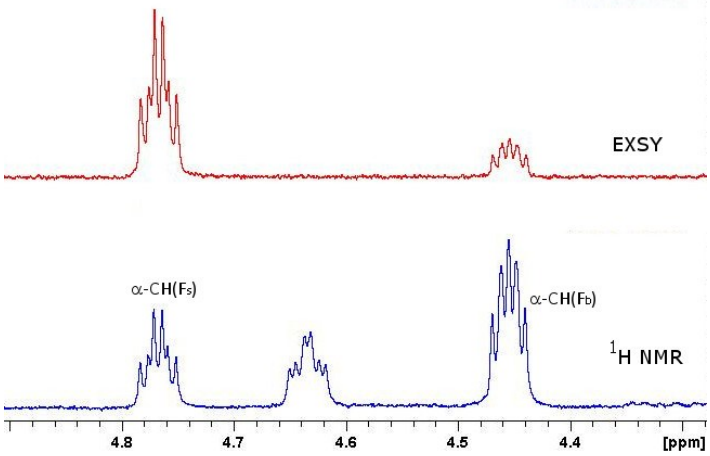


Figure S33: EXSY NMR spectrum of cycle 2*(S)*-**8** at 35 °C. α-CH signal of Phe in the major conformation was irradiated.

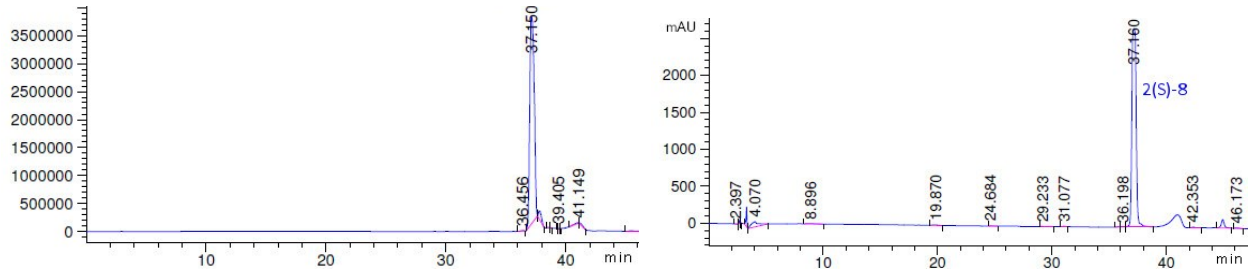


Figure S34: A) HPLC trace (System A3) at 220 nm of 2(*S*)-**8**. B) MS trace at the desired molecular weight (Single Ion Monitoring SIM mode) of 2(*S*)-**8**.

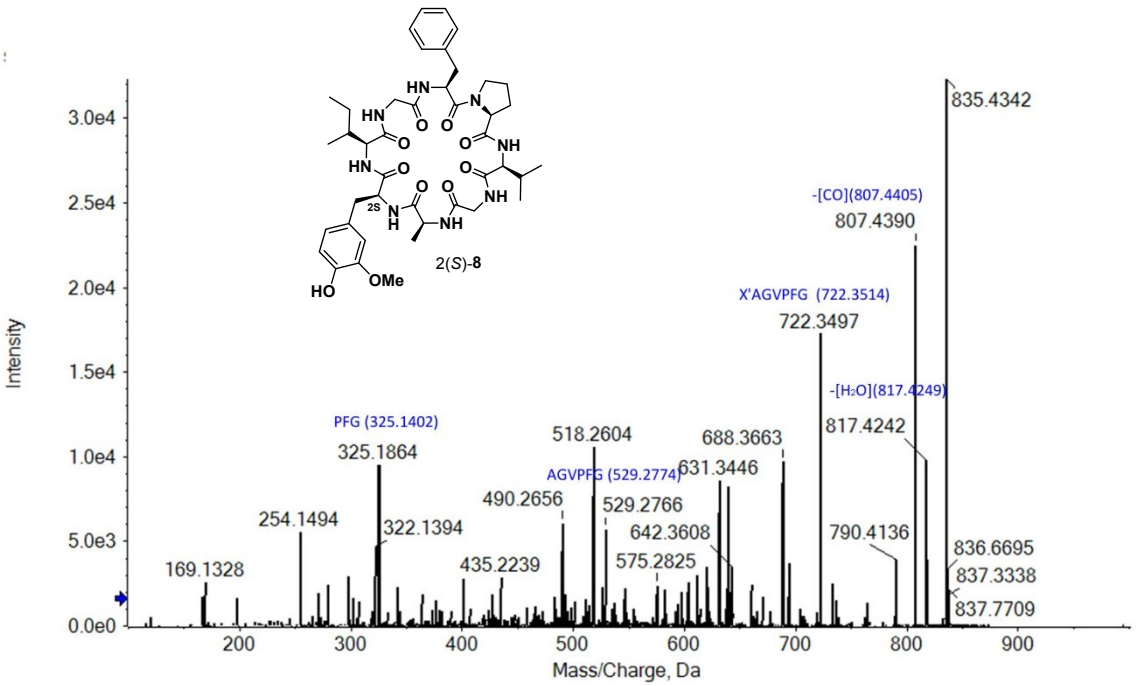
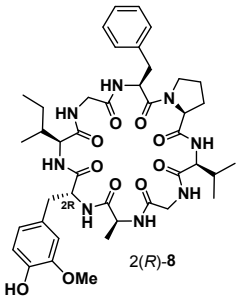


Figure S35: MS-MS fragmentation data of 2(*S*)-**8** after selection of ion with m/Z = 835.4 Da. The selected fragments and their theoretical masses are shown. Fragment containing the VP unit are important as they provide evidence that the VP bond, and hence the cycle, were formed.

Cyclooctapeptide (-AXIG$_a$FPVG$_b$-) 2(*R*)-**8** 7:1 (conformers)



Yield = 15%, 8.2 mg; purity = 96%; rt = 37.7 mins. The m/z analysis was performed by HR-ESI. Observed mass (cyclo 2(*R*)-**8** + H^+) = 835.4345 Da, theoretical mass (cyclo 2(*R*)-**8** + H^+) = 835.4354 Da. Observed mass (cyclo 2(*R*)-**8** + Na^+) = 857.41680 Da, theoretical mass (cyclo 2(*R*)-**8** + Na^+) = 857.4174 Da.

Melting point: 172–177 °C

Specific Rotation $[\propto]_D^{20}$ = -86 ° ($CHCl_3$, *c* = 1)

IR: (neat) cm^{-1} = 3290, 2964, 2351, 1749, 1635, 1516, 1031.

Table S10: Assignment of ^{1}H and ^{13}C NMR (700 MHz, DMSO-d_6) 2(*R*)-**8**. Extensive 2D NMR work was carried out to achieve this assignment. NMR data for the major conformer.

Amino acid	Atome	^{1}H chemical shift	^{13}C chemical shift
Ala	NH	8.32, d, J = 6.7	-
	α CH	4.33-4.29, m	48.3
	β CH_3	1.15, d, J = 7.1	18.1
	CO	-	173.7
X’	NH	8.07, br	-
	α CH	4.53-4.51, m	54.0
	β CH_2	3.22-3.19, m; 2.90-2.84, m	37.7
	γ C	-	128.4
	o-Ar	6.79, d, J = 1.8	122.0
	o’-Ar	6.58, dd, J_a = 8.0, J_b = 1.8	113.3
	m-Ar	-	145.3
	m'-Ar	6.63, d, J = 8.0	115.4
	p-Ar	-	147.8
	OMe	3.73, s	55.9
	CO	-	169.0
Ile	NH	8.12, d, J = 6.7	-
	α CH	3.87-3.84, m	59.1
	β CH	1.71-1.68, m	35.6
	γ CH_2	1.27-1.25, m; overlapped with β CH_3(Ala)	24.5
	γ CH_3	0.79-0.73, m	15.5
	δ CH_3	0.79-0.73, m	11.4
	CO	-	173.7
Gly (a)	NH	7.96, t, J = 6.0	-
	α CH_2	overlapped with OMe	41.4
	CO		170.6
Phe	NH	8.56, d, J = 6.0	-
	α CH	4.43-4.41, m	55.2
	β CH_2	2.90-2.84 m; 2.75-2.72, m	36.1
	γ-C	-	136.3
	o-Ar	7.36-7.25 (Ar)	128.9
	m-Ar	7.36-7.25 (Ar)	129.8
	p-Ar	7.36-7.25 (Ar)	127.7
	CO	-	169.1
Pro	α CH	overlapped with water	60.5
	β CH_2	1.82-1.79, m; 0.86-0.84, m	30.4
	γ CH_2	1.60-1.58, m; 1.34-1.31, m	21.8
	δ CH_2	3.32-3.28, m; 3.22-3.19, m	46.1
	CO	-	170.6
Val	NH	8.14, d, J = 8.9	-
	α CH	3.83-3.80, m	60.8
	β CH	1.82-1.79, m	29.2
	γ CH_3	0.79-0.73, m; 0.71, d, J = 6.9	19.5
	CO	-	171.0

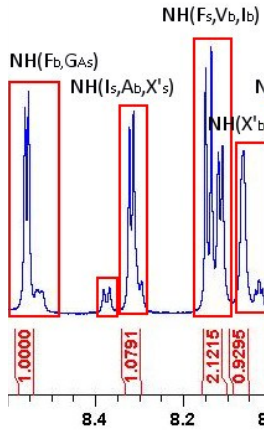
Gly (b)	NH	7.13, t, J = 4.8	-
	α CH_2	overlapped with OMe	41.4
	CO		169.1

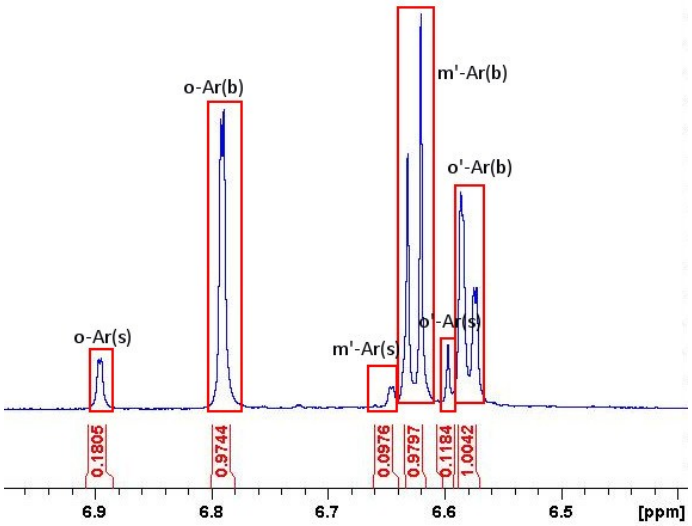
Table

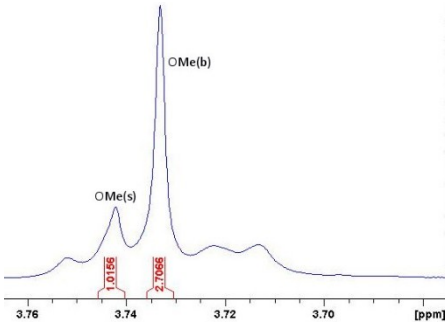
S11: Assignment of ^{1}H and ^{13}C NMR (700 MHz, DMSO-d_6) 2(*R*)-**8**. Extensive 2D NMR work was carried out to achieve this assignment. NMR data for the minor conformer.

Amino acid	**Atom**	**^{1}H chemical shift**	**^{13}C chemical shift**
Ala	NH	7.70, d, J = 7.2	-
	α CH	4.33-4.29, m	49.3
	β CH_3	1.15, d, J = 7.1	17.3
	CO	-	171.4
X’	NH	8.29, br	-
	α CH	4.53-4.51, m	53.6
	β CH_2	3.01-2.99, m; 2.78-2.75, m	37.1
	γ C	-	128.4
	o-Ar	6.90, d, J = 1.8	121.7
	o’-Ar	6.60, br	114.0
	m-Ar	-	145.1
	m'-Ar	6.66, d, J = 8.2	115.1
	p-Ar	-	147.2
	OMe	3.74, s	55.9
	CO	-	ND
Ile	NH	8.38, d, J = 9.0	-
	α CH	4.33-4.29, m	56.4
	β CH	1.71-1.68, m	35.6
	γ CH_2	1.27-1.25, m; overlapped with β CH_3(Ala)	24.5
	γ CH_3	0.79-0.73, m	15.5
	δ CH_3	0.79-0.73, m	11.4
	CO	-	ND
Gly (a)	NH	8.53, br	-
	α CH_2	4.16-4.13, m	41.4
	CO		ND
Phe	NH	overlapped with NH (V_b)	-
	α CH	4.70-4.67, m	53.9
	β CH_2	2.90-2.84, m; 2.57-2.55, m	38.6
	γ-C	-	136.3
	o-Ar	7.36-7.25 (Ar)	128.9
	m-Ar	7.36-7.25 (Ar)	129.8
	p-Ar	7.36-7.25 (Ar)	127.4
	CO	-	ND
Pro	α CH	overlapped with water	60.5
	β CH_2	1.24-1.22, m	29.2
	γ CH_2	1.83-1.80, m; 1.41-1.39, m	24.9
	δ CH_2	3.32-3.28, m; 3.22-3.19, m	46.1
	CO	-	ND
Val	NH	7.79, d, J = 9.0	-

	α CH	4.11-4.08, m	58.1
	β CH	2.09-2.05, m	30.3
	γ CH_3	0.79-0.73, m	19.5
	CO	-	ND
Gly (b)	NH	8.03, t, J = 5.5	-
	α CH_2	3.94-3.91, m	41.4
	CO	-	ND







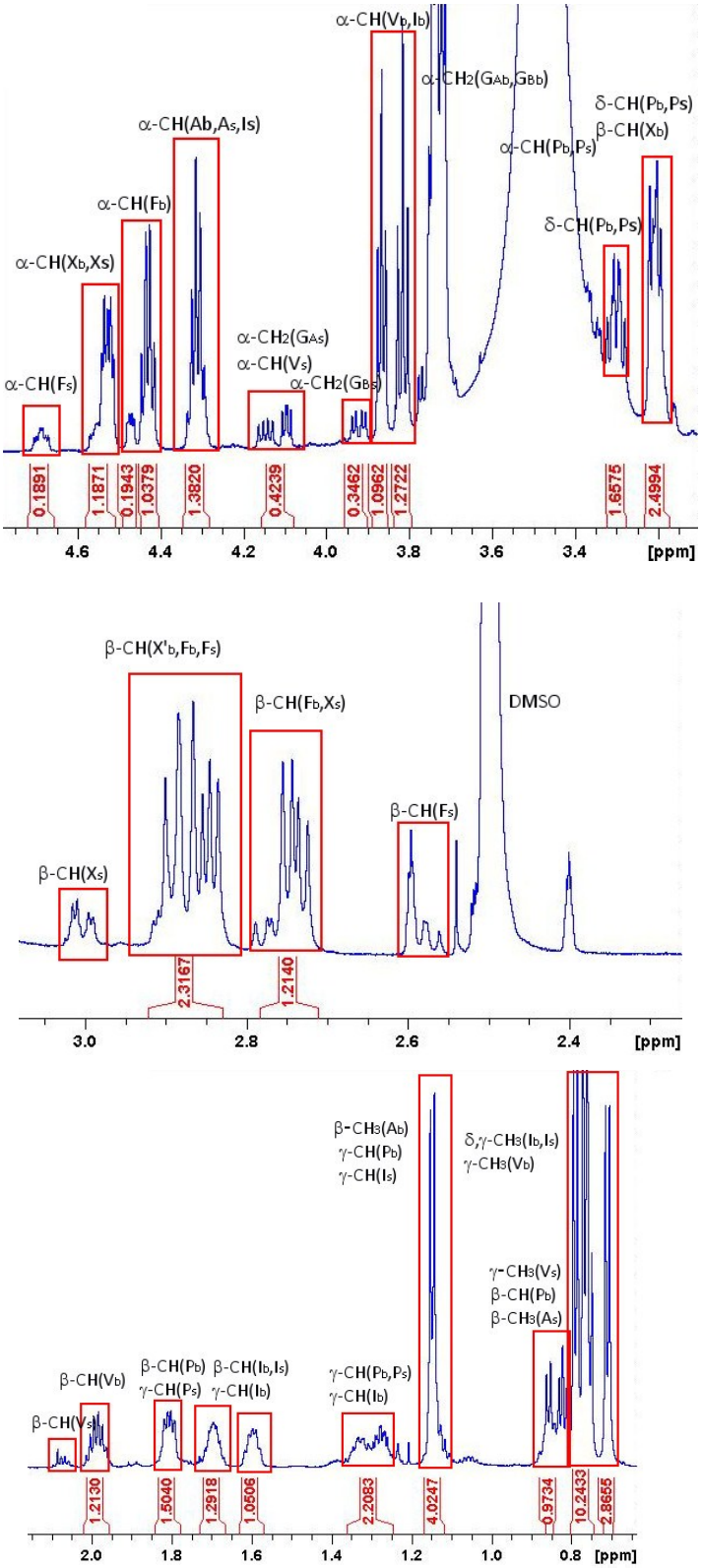


Figure S36: ^{1}H NMR spectrum (700 MHz, DMSO-d_6) of cycle 2(*R*)-**8**. Subscripts b, s are respectively referred to the major and minor conformation.

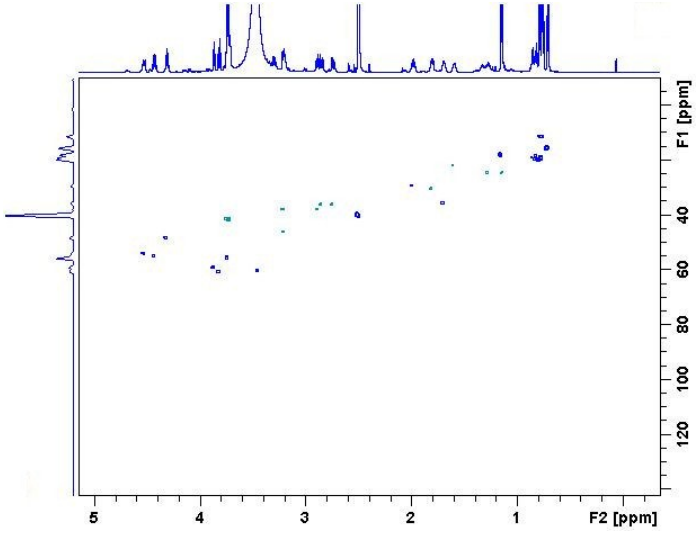


Figure S37: HSQC ^{13}C-^{1}H NMR spectrum (700 MHz, DMSO-d_6) of cycle 2(*R*)-**8**.

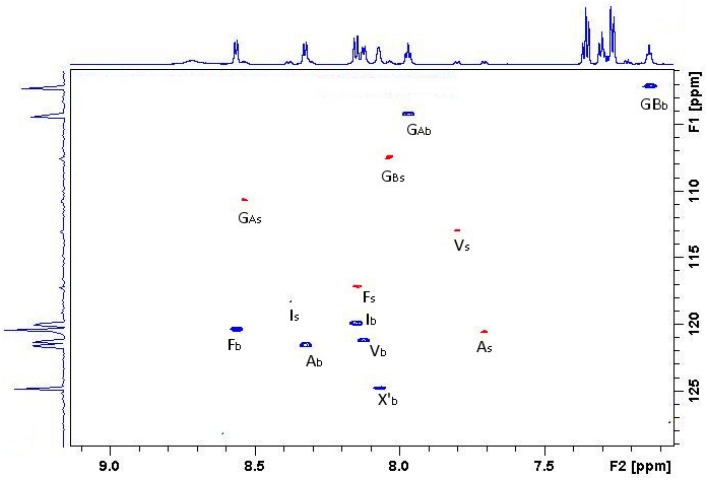


Figure S38: HSQC ^{15}N-^{1}H NMR spectrum (700 MHz, DMSO-d_6) of cycle 2(R)-**8**.

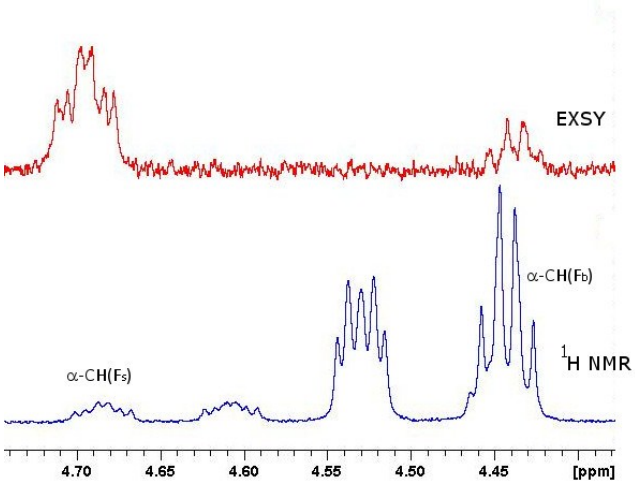


Figure S39: EXSY NMR spectrum of cycle 2(*R*)-**8** at 35 °C. α-CH signal of Phe in the minor conformation was irradiated.

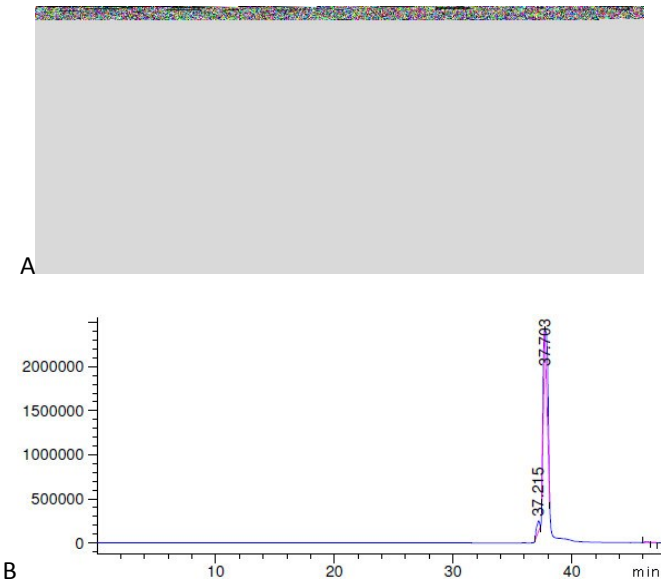


Figure S40: (A) HPLC trace (System A3) at 220 nm of 2(*R*)-**8**. (B) MS trace at the desired molecular weight (Single Ion Monitoring SIM mode) of 2(*R*)-**8**.

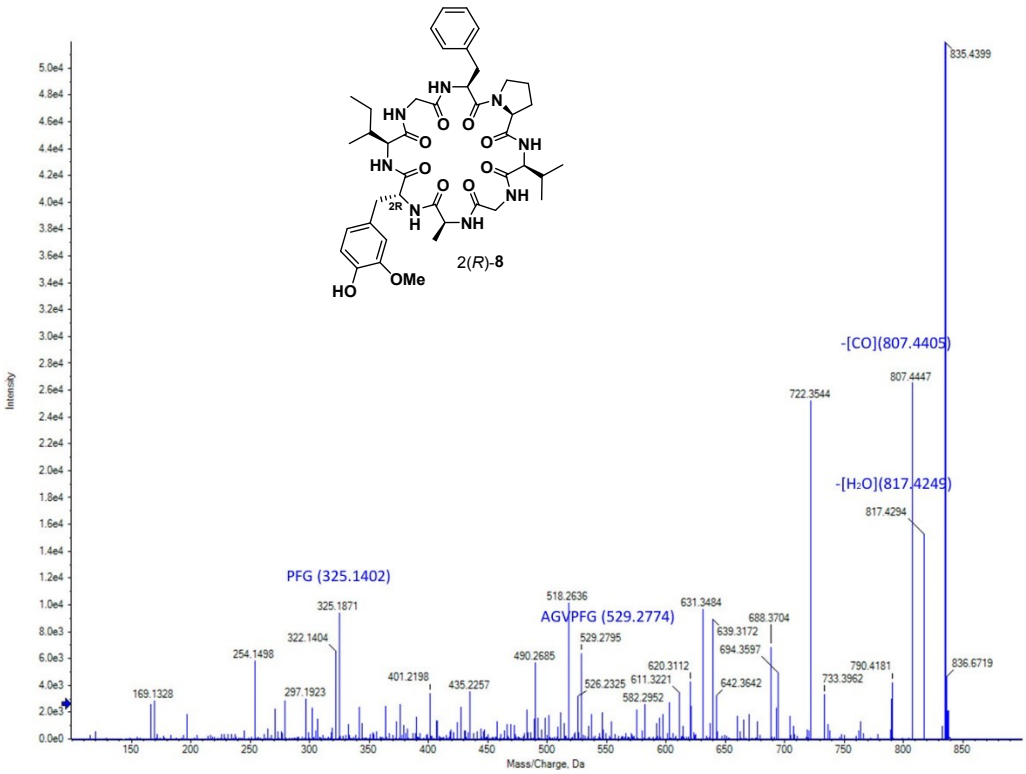
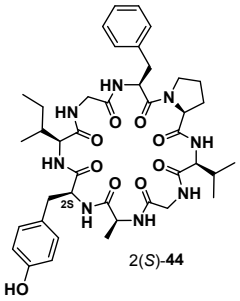


Figure S41: MS-MS fragmentation data of 2(*R*)-**8** after selection of ion with m/z = 835.4 Da. The selected fragments and their theoretical masses are shown.

Cyclooctapeptide (-AYIG$_a$FPVG$_b$-) (**44**) 2:1 (conformers)



2(*S*)-**44**

Yield = 31%, 15 mg (chemical cyclisation; purity = 96%; rt = 18.5 mins.

The m/z analysis was performed by HR-ESI.

Observed mass (cyclo 2(*S*)-**44**) + H^+) = 805.4229 Da, theoretical mass ((cyclo 2(*S*)-**44**) + H^+) = 805.4249 Da. Observed mass ((cyclo 2(*S*)-**44**) + Na^+) = 827.4041 Da, theoretical mass ((cyclo 2(*S*)-**44**) + Na^+) = 827.4068 Da.

Melting point: 181–184 °C

Specific Rotation $[\propto]_D^{20}$ = -62 ° (MeOH, *c* = 1)

IR: (neat) cm^{-1} = 3290, 2964, 2351, 1749, 1635, 1516, 1031.

Table S12: ^{1}H NMR and ^{13}C (700 MHz, DMSO). Extensive 2D NMR work was carried out to achieve this assignment. NMR data for the major conformer.

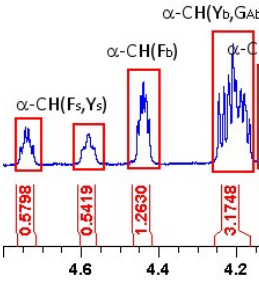
Amino acid	Atom	^{1}H chemical shift	^{13}C chemical shift
Ala	NH	8.56-8.54, m	-
	α CH	3.82-3.77, m	51.1
	β CH_3	1.10, d, J = 7.2	17.3
	CO	-	171.2
Y	NH	8.00-7.97, m	-
	α CH	4.25-4.17, m	56.5
	β CH_2	2.98-2.92, m	35.7
	γ C	-	127.8
	o-Ar	6.65, d, J = 8.2	115.2
	m-Ar	6.96, d, J = 8.2	130.1
	p-Ar	-	156.0
	OH	9.22, s	-
	CO	-	170.4
Ile	NH	7.49-7.46, m	-
	α CH	3.87-3.84, m	58.3
	β CH	1.63-1.60, m	36.8
	γ CH_2	1.51-1.47, m; 1.21-1.17, m	25.4
	γ CH_3	0.86-0.82, m	15.4
	δ CH_3	0.86-0.82, m	11.0

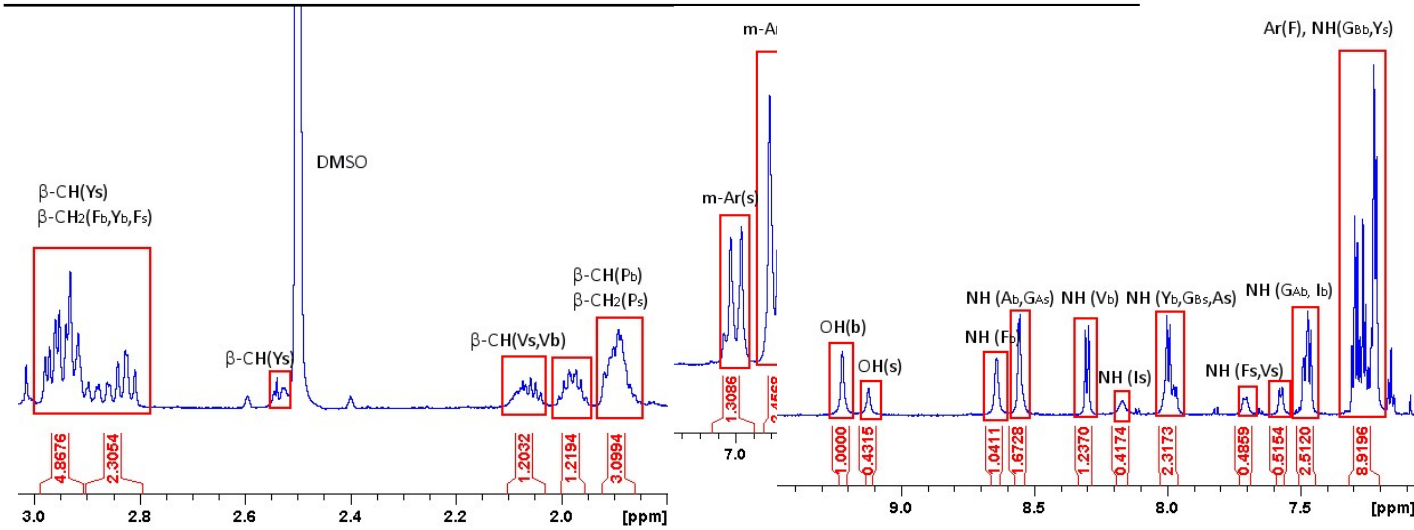
	CO	-	172.8
Gly (a)	NH	7.49-7.46, m	-
	α CH_2	4.25-4.17, m; 3.45.3.42, m	41.2
	CO		171.7
Phe	NH	8.64, br	-
	α CH	4.45-4.42, m	53.4
	β CH_2	2.98-2.92 m; 2.84-2.81, m	37.3
	γ-C	-	138.4
	o-Ar	7.29-7.21 (Ar)	128.4
	m-Ar	7.29-7.21 (Ar)	129.8
	p-Ar	7.29-7.21 (Ar)	127.2
	CO	-	171.1
Pro	α CH	3.70-3.67, m	60.6
	β CH_2	1.92-1.89, m; 0.86-0.82, m	29.7
	γ CH_2	1.59-1.56, m; 1.29-1.26, m	21.6
	δ CH_2	3.30-3.27, m; 3.18-3.15, m	45.9
	CO	-	170.4
Val	NH	8.30, d, J = 8.4	-
	α CH	3.75-3.72, m	62.0
	β CH	1.99-1.96, m	29.6
	γ CH_3	0.86-0.82, m; 0.79, d, J = 6.6	19.6
	CO	-	171.4
Gly (b)	NH	overlapped with Ar	-
	α CH_2	4.12-4.09, m; 3.75-3.72, m	42.9
	CO		170.4

Table S13: ^{1}H and ^{13}C NMR (700 MHz, DMSO). Extensive 2D NMR work was carried out to achieve this assignment. NMR data for the minor conformer.

Amino acid	Atom	^{1}H chemical shift	^{13}C chemical shift
Ala	NH	8.00-7.97, m	-
	α CH	4.01-3.97, m	49.0
	β CH_3	1.13, d, J = 7.2	17.3
	CO	-	ND
Y	NH	overlapped with Ar	-
	α CH	4.59-4.57, m	56.5
	β CH_2	2.84-2.81, m; 2.54-2.52, m	37.9
	γ C	-	127.8
	o-Ar	6.65, d, J = 8.2	115.2
	m-Ar	6.96, d, J = 8.2	130.4
	p-Ar	-	156.0
	OH	9.12, s	-
	CO	-	ND
Ile	NH	8.17, br	-
	α CH	3.88-3.86, m	58.8
	β CH	1.71-1.69, m	36.0
	γ CH_2	1.59-1.56, m; overlapped with β CH_3(Ala)	25.2
	γ CH_3	0.86-0.82, m	15.4
	δ CH_3	0.86-0.82, m	11.0

	CO	-	ND
Gly (a)	NH	8.56-8.54, m	-
	α CH_2	4.01-3.97,m; 3.82-3.77, m	42.4
	CO		ND
Phe	NH	7.71, d, J = 6.9	-
	α CH	4.76-4.72, m	52.6
	β CH_2	2.98-2.92 m	37.3
	γ-C	-	138.4
	o-Ar	7.29-7.21 (Ar)	128.4
	m-Ar	7.29-7.21 (Ar)	129.8
	p-Ar	7.29-7.21 (Ar)	127.2
	CO	-	171.3
Pro	α CH	4.25-4.17, m	61.6
	β CH_2	1.92-1.89, m	29.0
	γ CH_2	1.59-1.56, m; 1.29-1.26, m	21.6
	δ CH_2	3.62-3.60, m	47.2
	CO	-	ND
Val	NH	7.58, d, J = 7.4	-
	α CH	4.06-4.03, m	58.4
	β CH	2.07-2.03, m	30.1
	γ CH_3	0.86-0.82, m	19.6
	CO	-	171.4
Gly (b)	NH	8.00-7.97, m	-
	α CH_2	3.82-3.77, m; 3.75.3.72, m	42.6
	CO		ND





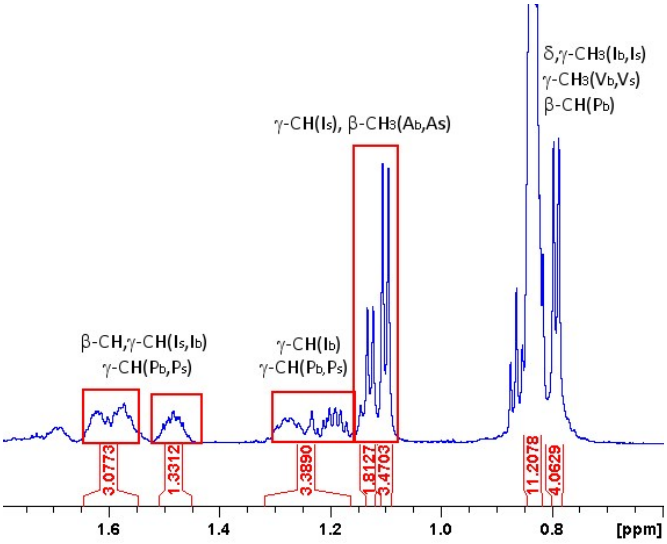


Figure S42: ^{1}H NMR spectrum (700 MHz, DMSO-d_6) of cycle **44**. Subscripts b, s are respectively referred to the major and minor conformation.

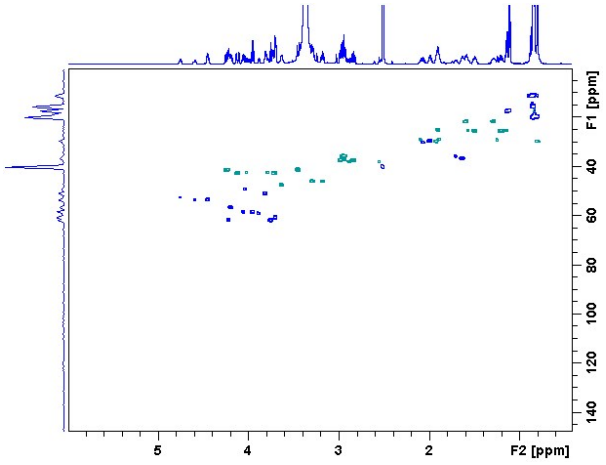


Figure S43: HSQC ^{13}C-^{1}H NMR spectrum (700 MHz, DMSO-d_6) of cycle **44**.

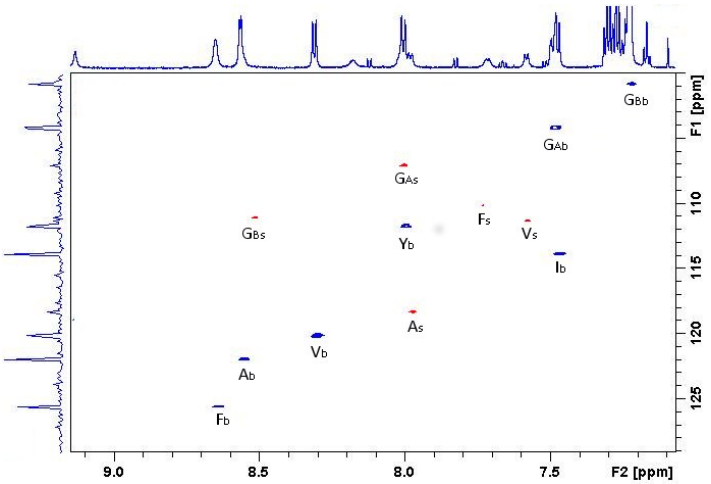


Figure S44: HSQC ^{15}N-^{1}H NMR spectrum (700 MHz, DMSO-d_6) of cycle **44**.

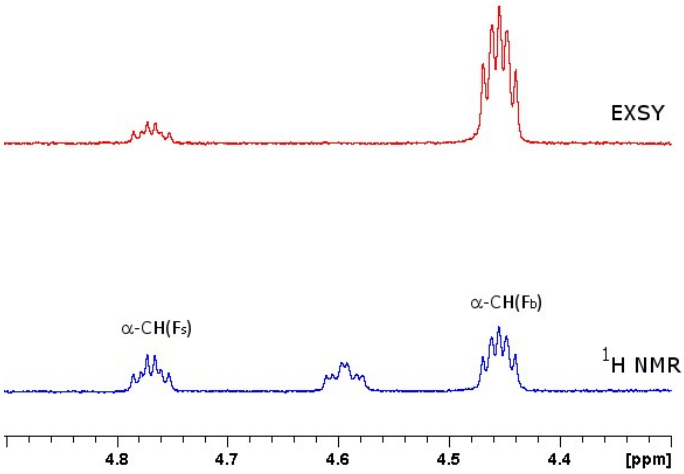


Figure S45: EXSY NMR spectrum of cycle **44** at 35 °C. α-CH signal of Phe in the minor conformation was irradiated.

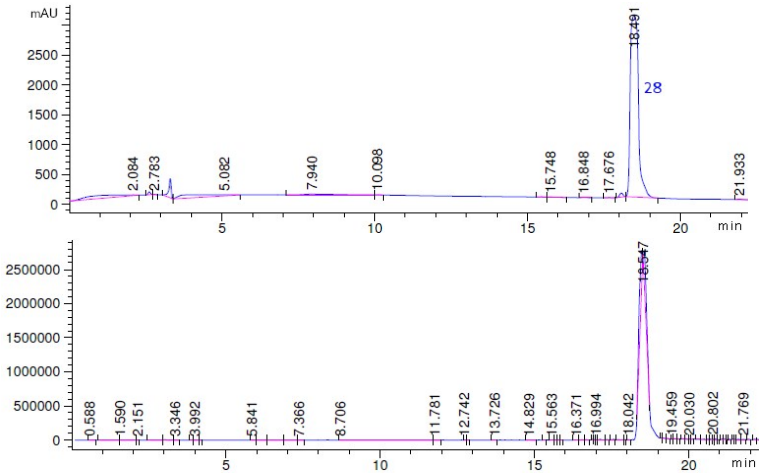


Figure S46: A) HPLC trace (System A2) at 220 nm of **44**. B) MS trace at the desired molecular weight (Single Ion Monitoring SIM mode) of **44**.

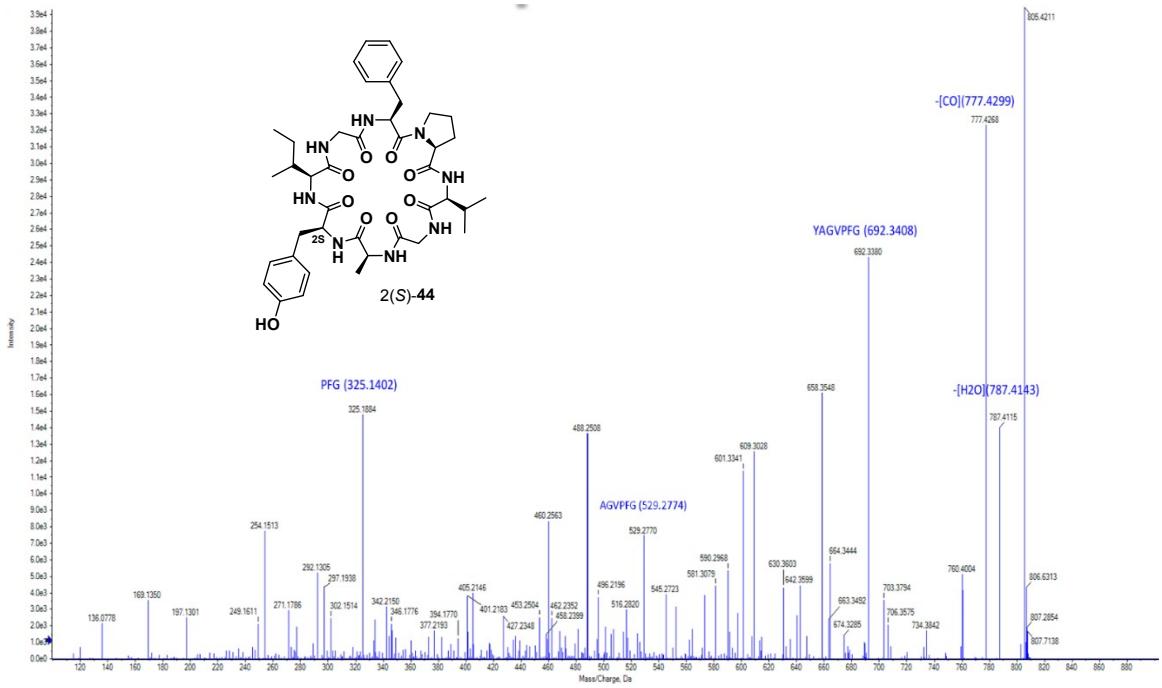


Figure S47: MS-MS fragmentation data of **44** after selection of ion with m/z = 805.4 Da. The selected fragments and their theoretical masses are shown.

Bibliography

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S2 CCDC 1505597 (*anti*-(±)-**3**), CCDC 1505596 ((*S*)-**2a**), CCDC 1505598 ((*S*)-**14b**), CCDC 1510492 (*anti*-(2*R*, 3*R*)-**3**) and CCDC 1506889 ((±)-**41**) contain the supplementary crystallographic data. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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