Asymmetric Synthesis of a Protected Dihydroxypiperazic Acid Derivative

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Abstract: A short and flexible route for the synthesis of 1,2-diisopropyl-3-methyl-(3*S*,4*R*,5*R*)-4,5-dihydroxyhexahydro-1,2,3-pyridazinetricarboxylate, from readily available acrolein, is described. This approach involves an asymmetric α-hydrazination, dihydroxylation and intramolecular cyclisation as key steps.

Key words: piperazic acid, L-proline, α-hydrazination, dihydroxylation, intramolecular cyclisation

Piperazic acids are non-proteinogenic amino acids which contain a cyclic hydrazine skeleton. They are found in several naturally occurring cyclodepsipeptides[1] which possess remarkable biological activities.[2] They are very useful synthetic intermediates for the preparation of medicinally important compounds[3] such as enzyme inhibitors,[4] antitumour and anti-HIV peptide antibiotics.[5] Piperazic acid itself inhibits γ-aminobutyric acid (GABA) uptake. Substituted piperazic acids are key components of several biologically active natural peptides including sanglifehrins,[6] GE_3[7,8] and related compounds.

The first member of this family was discovered as a component of monamycins by Hassall and co-workers.[9] Since that time, a number of piperazic acids, their C-4 oxygenated variants[10] and also 4-hydroxy-2,3,4,5-tetrahydropyridazinecarboxylic acid (HPCA), which is a key subunit in luzopeptin, have been found in natural peptides. The 4,5-dihydroxypiperidazine-3-carboxylic acids (DHPC) have been detected in a novel antifungal cyclic tetrapeptide glomecidin (Figure 1), which was produced by *Streptomyces lavendulae* H698SY2,[11] though the stereochemistry of the asymmetric carbons has not yet been determined.

Since a number of pseudopeptides or pseudomimetics containing the N–N–C–C=O fragment have great potential in medicinal chemistry,[12,13] several methods have been developed that have enabled the synthesis of the piperazic acid portion of biologically active depsipeptides.[14]

Bearing in mind the importance of unusual amino acids and in a continuation of our efforts towards the synthesis of such compounds,[15] we herein report in detail our synthetic endeavours towards the construction of piperazic acid derivative **1**, using an organocatalysed asymmetric α-hydrazination as a key reaction (Scheme 1).[16,17]

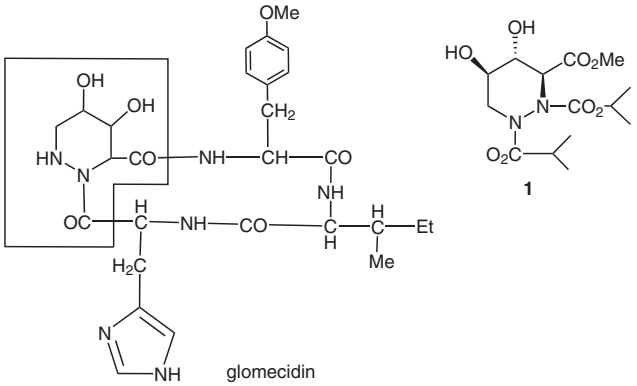
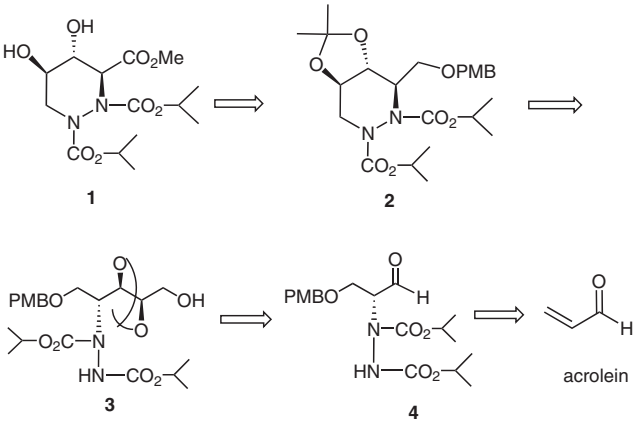


Figure 1

The synthesis began with the preparation of the three-carbon synthon, aldehyde **5**, from commercially available acrolein via Michael addition with *p*-methoxybenzyl alcohol (PMBOH; Scheme 2). Asymmetric α-hydrazination of aldehyde **5** with diisopropyl azodicarboxylate (DIAD) in the presence of a catalytic amount of L-proline (10 mol%) in acetonitrile gave the α-hydrazinated compound **4**, the optical purity of which was determined after reduction to the alcohol **4a**,[18,19] since the aldehyde **4** was configurationally unstable. The α-hydrazinated compound **4** was subjected to Wittig olefination with a two-carbon Wittig ylide, to afford **6** in 82% yield (*E*:*Z* ratio of 10:1). The resulting α,β-unsaturated ester **6** was subjected to dihydroxylation using catalytic osmium tetroxide and *N*-methylmorpholine *N*-oxide (NMO), to afford **7** and **7a** in a ratio of 4:1, respectively, in 80% yield. The two isomer-



Scheme 1

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ic diols were separated through silica gel column chromatography and subsequent synthesis of the required target compound was achieved using the major diastereomer **7**. The diol **7** was treated with 2,2-dimethoxypropane to afford **8** (88% yield). The reduction of ester functionality in compound **8** using lithium borohydride furnished the alcohol **3** in 85% yield which, on treatment with *p*-toluenesulfonyl chloride (TsCl) in the presence of triethylamine and a catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine (DMAP), afforded **9** in 92% yield. Sodium hydride assisted intramolecular cyclisation of compound **9** afforded **2** which, on PMB deprotection with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), gave the alcohol **10** in 81% yield. Compound **10** was converted into the corresponding carboxylic acid methyl ester **11**, in two steps, using bis(acetoxy)iodobenzene, followed by treatment with diazomethane, in 75% overall yield. Finally, removal of the isopropylidene group in **11** with 10-camphorsulfonic acid (CSA) in methanol, smoothly delivered the desired piperazic acid derivative **1** (Scheme 2).

Compound **7** was analysed using 1D and 2D NMR techniques such as DQF-COSY, TOCSY, NOESY and ROESY. The resonance signals of the H_6–H_{12} protons (Figure 2) were broad, which could be due to conformational flexibility of the aliphatic chain. Experiments were also carried out in order to derive the coupling constants at a range of temperatures from 5 °C to 30 °C; however, no appreciable resolution was observed. The free rotation of C–C single bond therefore made it difficult to ascertain the orientation of the hydroxyl groups at this stage, though the stereochemistry was determined through analysis of subsequent intermediates.

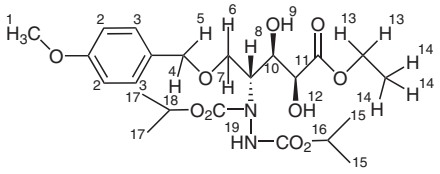
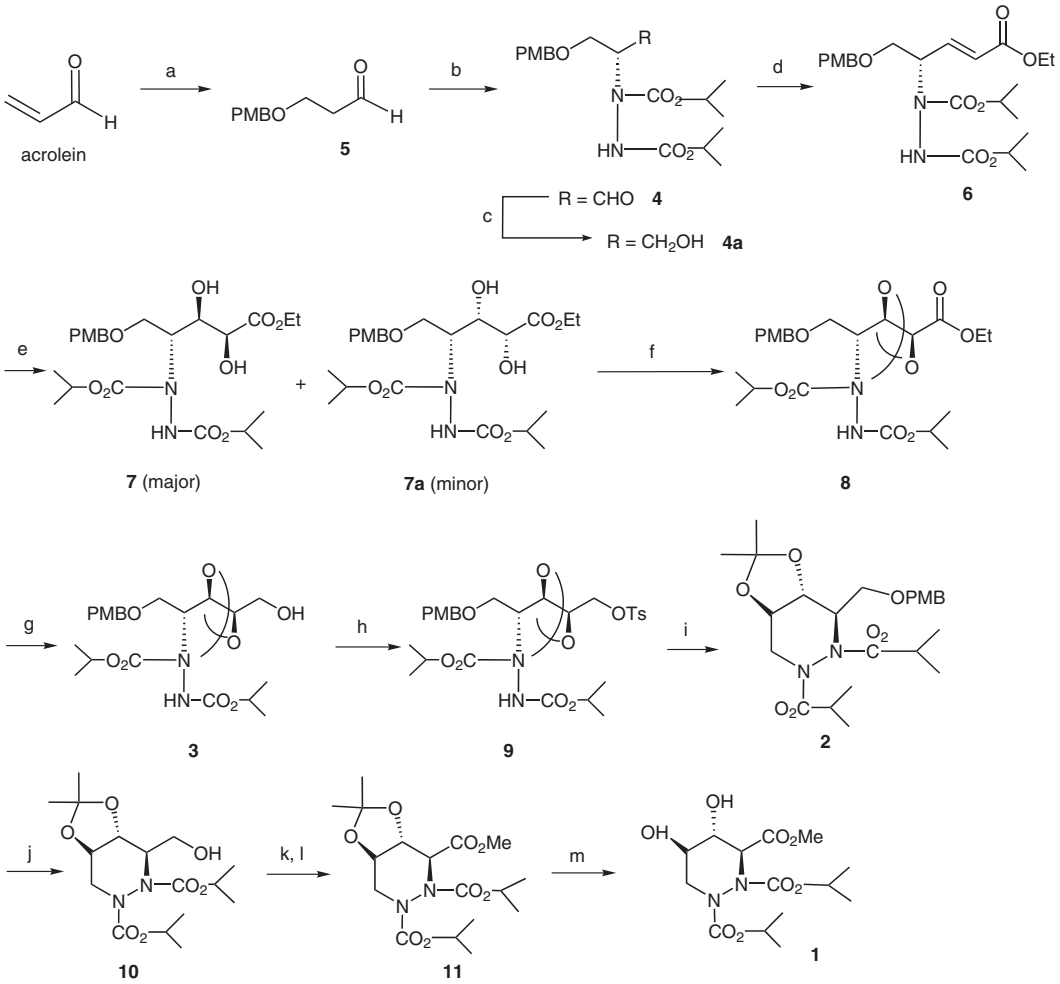


Figure 2 Atom-numbering scheme for compound **7**



Scheme 2 *Reagents and conditions*: (a) PMBOH, AcOH, $ClCH_2CO_2H$, r.t., 6 d; (b) L-proline, DIAD, MeCN, 0 °C, 14–15 h; (c) $NaBH_4$, EtOH, 0 °C, 30 min; (d) $Ph_3P{=}CHCO_2Et$, benzene, r.t., 6 h; (e) OsO_4, NMO, acetone, H_2O, r.t., 24 h; (f) (i) separation of isomers; (ii) 2,2-dimethoxypropane, CSA, acetone, r.t., 2 h; (g) $LiBH_4$, EtOH, THF, 0 °C→r.t.; (h) TsCl, Et_3N, cat. DMAP, CH_2Cl_2, 0 °C→r.t.; (i) NaH, THF, 0 °C→r.t., 2 h; (j) DDQ, CH_2Cl_2–H_2O (4:1), r.t.; (k) bis(acetoxy)iodobenzene, TEMPO, MeCN–H_2O (1:1), 0 °C→r.t.; (l) CH_2N_2, Et_2O, 0 °C→r.t.; (m) CSA, MeOH, r.t., 25–30 min.

The assignment of the structure and stereochemistry of compounds **1** and **2** was achieved after detailed NMR studies using DQF-COSY and NOESY. In compound **2** the relative configuration between the protected diol moiety and the N-substituted chiral centre is in the *trans*-conformation, as evidenced by the large coupling constants (J = 9.2 Hz and 11.5 Hz) and the presence of strong NOE cross-peaks between H_2–H_6, H_3–H_5 and H_1–H_3. The six-membered ring takes a distorted chair conformation which is characterised by the NOE cross-peaks between H_2–$H_{4'}$ and H_1–H_3 as shown in Figure 3. The large couplings observed between H_1–H_2 and between H_3–H_4 are also consistent with a distorted chair conformation.

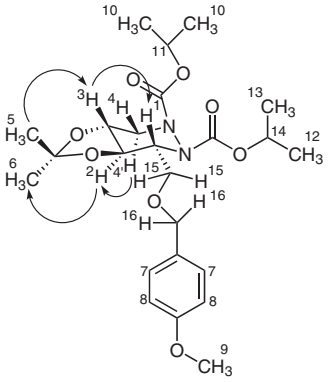


Figure 3 NOE cross-correlations observed for compound **2**

In compound **1**, the relative configuration between the diol moiety and the N-substituted chiral centre is also in the *trans*-conformation as evidenced by the coupling constant (J = 8.0 Hz). The six-membered ring also takes a distorted chair conformation, which is suggested by the NOE cross-peaks between H_2–H_4, H_1–H_2 and H_3–H_7 as shown in Figure 4. The large coupling (J = 9.7 Hz) observed between H_3–H_4 is also consistent with a distorted chair conformation.

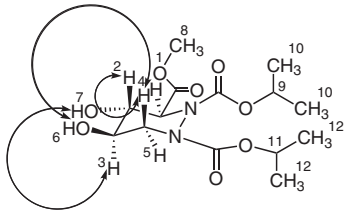


Figure 4 NOE cross-correlations observed for compound **1**

In conclusion, the synthesis of a piperazic acid derivative was achieved in reasonable yields, from readily available starting materials. The asymmetric α-hydrazination of the starting aldehyde with L-proline as catalyst and the utilisation of the chiral intermediate will be further explored in order to extend this approach to other related isomers.

Commercial reagents were used without further purification. All solvents were purified by standard techniques. Infrared (IR) spectra were recorded on a Perkin–Elmer 683 spectrometer. Optical rotations were obtained on a Jasco Dip 360 digital polarimeter. Melting points were recorded using a Fischer-Johns and are uncorrected. NMR spectra were recorded in $CDCl_3$ on a Varian Gemini 200, Bruker 300 or Varian Unity 400 NMR spectrometers. Chemical shifts (δ) are quoted in parts per million (ppm) and are referenced to tetramethylsilane (TMS) as an internal standard. Coupling constants (J) are quoted in Hertz (Hz). Column chromatographic separations were carried out on silica gel (60–120 mesh) and flash chromatographic separations were carried out using 230–400 mesh size silica gel. Mass spectra were obtained on Finnegan MAT 1020B or micromass VG 70-70H spectrometers operating at 70 eV, using a direct inlet system.

3-[(4-Methoxybenzyl)oxy]propanal (5)

Acrolein (10.2 g, 0.18 mol) was added to a solution of PMBOH (20 g, 0.14 mol), chloroacetic acid (0.82 g, 8.69 mmol) and NaOH (0.35 g, 8.69 mmol) in H_2O (1.8 mL) over a period of 5 min. Subsequently, acetic acid (3.83 g, 63.7 mmol) was added and the solution was maintained at r.t. for 6 d. The reaction mixture was diluted with CH_2Cl_2 (3 × 30 mL), washed with brine (1 × 30 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (EtOAc–hexane, 30%) to afford aldehyde **5**.

Yield: 25 g (72%); light-brown viscous oil.

IR (KBr): 1248, 1514, 1612, 1724 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 2.63–2.66 (2 H, t, J = 6.1 Hz), 3.74–3.77 (2 H, t, J = 6.1 Hz), 3.78–3.79 (3 H, s), 4.43–4.45 (2 H, s), 6.83–6.87 (2 H, d, J = 8.6 Hz), 7.21–7.24 (2 H, d, J = 8.6 Hz), 9.75–9.77 (1 H, s).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 201.2, 159.2, 129.8, 129.3, 113.7, 72.8, 63.4, 55.2, 43.8.

Diisopropyl 1-{(1*R*)-1-Formyl-2-[(4-methoxybenzyl)oxy]ethyl}-1,2-hydrazinedicarboxylate (4)

To a stirred solution of **5** (5 g, 25.77 mmol) and diisopropyl azodicarboxylate (5.07 mL, 25.77 mmol) in MeCN (50 mL) at 0 °C, was added L-proline (296 mg, 2.57 mmol, 10 mol%). The reaction mixture was stirred at 0 °C for 15 h then the solvent was removed in vacuo and the resulting mixture was extracted with EtOAc (3 × 30 mL), washed with sat. brine (30 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Since, the resulting product was unstable, the crude material was used in the next step without further purification.

Diisopropyl 1-{(1*S*,2*E*)-4-Ethoxy-1-[(4-methoxybenzyl)oxy]-methyl-4-oxo-2-butenyl}-1,2-hydrazinedicarboxylate (6)

The crude aldehyde **4** (4.5 g, 11.36 mmol) was taken in anhyd benzene (40 mL) and (ethoxycarbonylmethylene)triphenylphosphorane (4.74 g, 13.6 mmol) was added. The reaction mixture was stirred for 6 h at r.t. then the solvent was removed under reduced pressure and the resulting crude product was purified by flash column chromatography (EtOAc–hexane, 1:3) to afford ester **6**.

Yield: 4.35 g (82%); colourless viscous liquid; $[\alpha]_D^{20}$ +1.52 (*c* 1, $CHCl_3$).

IR (KBr): 821, 1035, 1249, 1386, 1714, 2982, 3301 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 1.21–1.33 (15 H, m), 3.47–3.65 (1 H, m), 3.77–3.81 (4 H, m), 4.10–4.24 (2 H, q, J = 7.0 Hz), 4.35–4.51 (2 H, m), 4.84–4.99 (3 H, m), 6.29–6.61 (1 H, m), 6.76–6.88 (3 H, m), 7.15–7.25 (2 H, d, J = 7.8 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.8, 159.3, 156.1, 142.3, 129.2, 113.8, 77.4, 72.5, 70.4, 69.7, 60.3, 55.1, 21.9, 21.8, 14.0.

HRMS (EI): *m/z* $[M + H]^+$ calcd for $C_{23}H_{35}N_2O_8$: 467.2393; found: 467.2404.

Diisopropyl 1-{(1*R*,2*R*,3*S*)-4-Ethoxy-2,3-dihydroxy-1-[(4-methoxybenzyl)oxy]methyl-4-oxobutyl}-1,2-hydrazinedicarboxylate (7)

To a solution of ester **6** (2.5 g, 5.36 mmol) in a mixture of acetone–H_2O (4:1, 40 mL) was added, sequentially, OsO_4 (cat.) and NMO (817 mg, 6.9 mmol). The resulting mixture was stirred for 24 h at r.t. then sodium metabisulfite (50 mg, 0.26 mmol) was added. After stirring the reaction mixture for an additional 30 min, the solvent was removed under reduced pressure. The reaction mixture was diluted with EtOAc (60 mL), washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification of the residue by column chromatography (EtOAc–hexane, 2:3) gave **7** (1.98 g, 74%) as a colourless liquid and **7a** as viscous liquid (450 mg, 16%).

$[\alpha]_D^{20}$ –0.47 (*c* 1, $CHCl_3$).

IR (KBr): 1109, 1249, 1717, 2983, 3313 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 1.25 (6 H, d, *J* = 6.6 Hz), 1.26 (6 H, d, *J* = 6.6 Hz), 1.29 (3 H, t, *J* = 7.0 Hz), 3.12 (1 H, br s), 3.59 (2 H, br s), 3.81 (3 H, s), 3.84 (1 H, br s), 4.03 (1 H, br s), 4.27 (2 H, q, *J* = 7.0 Hz), 4.34 (1 H, d, *J* = 11.7 Hz), 4.51 (1 H, d, *J* = 11.7 Hz), 4.64 (1 H, br s), 4.77 (1 H, br s), 4.95 (1 H, sept, *J* = 6.6 Hz), 4.97 (1 H, sept, *J* = 6.6 Hz), 6.25 (1 H, br s), 6.88 (2 H, d, *J* = 8.3 Hz), 7.21 (2 H, d, *J* = 8.3 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.3, 159.4, 129.3, 113.9, 77.4, 71.2, 70.7, 69.7, 66.0, 61.7, 55.2, 21.9, 21.8, 21.6, 14.1.

HRMS (EI): *m*/*z* $[M + H]^+$ calcd for $C_{23}H_{37}N_2O_{10}$: 501.2448; found: 501.2464.

Diisopropyl 1-{(1*R*,2*S*,3*R*)-4-Ethoxy-2,3-dihydroxy-1-[(4-methoxybenzyl)oxy]methyl-4-oxobutyl}-1,2-hydrazinedicarboxylate (7a)

$[\alpha]_D^{20}$ +5.58 (*c* 1.2, $CHCl_3$).

IR (KBr): 821, 1108, 1248, 1386, 1730, 2982, 3302 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 1.21–1.33 (15 H, m), 3.01–3.19 (1 H, m), 3.61–3.92 (5 H, m), 3.96–4.11 (1 H, m), 4.19–4.28 (2 H, q, *J* = 6.7 Hz), 4.32–4.38 (2 H, d, *J* = 11.3 Hz), 4.45–4.53 (2 H, d, *J* = 11.3 Hz), 4.59–4.65 (1 H, m), 4.86–4.97 (2 H, m), 6.21–6.27 (1 H, br s, NH), 6.79–6.85 (2 H, d, *J* = 9.0 Hz), 7.15–7.22 (2 H, d, *J* = 8.3 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.3, 159.3, 129.3, 113.9, 77.4, 71.6, 70.0, 66.7, 61.7, 57.5, 55.2, 29.6, 21.8, 21.7, 14.1.

HRMS (EI): *m*/*z* $[M + H]^+$ calcd for $C_{23}H_{37}N_2O_{10}$: 501.2448; found: 501.2434.

Diisopropyl 1-{(1*R*)-1-[(4*R*,5*S*)-5-(Ethoxycarbonyl)-2,2-dimethyl-1,3-dioxolan-4-yl]-2-[(4-methoxybenzyl)oxy]ethyl}-1,2-hydrazinedicarboxylate (8)

To diol **7** (2 g, 4.0 mmol) in acetone (30 mL) was added 2,2-dimethoxypropane (0.14 mL, 6.0 mmol) and CSA (cat.) at 0 °C. The reaction mixture was stirred for 2 h at r.t. then neutralised with sat. aq $NaHCO_3$ (20 mL). The solvent was removed under reduced pressure and the crude residue was purified by silica gel column chromatography (EtOAc–hexane, 1:4) to give **8**.

Yield: 1.9 g (88%); light-yellow liquid; $[\alpha]_D^{20}$ –0.87 (*c* 1.6, $CHCl_3$).

IR (KBr): 1108, 1248, 1383, 1716, 2983, 3417 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 1.19–1.30 (15 H, m), 1.34–1.41 (6 H, m), 3.49–3.60 (1 H, m), 3.78–3.80 (4 H, m), 4.01–4.20 (2 H, m), 4.31–4.59 (5 H, m), 4.82–4.99 (2 H, m), 6.21–6.32(1 H, br s, NH), 6.73–6.82 (2 H, d, *J* = 8.3 Hz), 7.11–7.19 (2 H, d, *J* = 8.3 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.1, 159.2, 129.0, 114.4, 110.1, 73.6, 70.8, 70.3, 66.1, 61.9, 57.1, 54.2, 27.1, 21.9, 14.1.

HRMS (EI): *m*/*z* $[M + H]^+$ calcd for $C_{26}H_{41}N_2O_{10}$: 541.2761; found: 541.2759.

Diisopropyl 1-{(1*R*)-1-[(4*R*,5*R*)-5-(Hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl]-2-[(4-methoxybenzyl)oxy]ethyl}-1,2-hydrazinedicarboxylate (3)

A solution of LiCl (423 mg, 9.99 mmol) and $NaBH_4$ (369 mg, 9.99 mmol) in EtOH (20 mL) was stirred at 0 °C for 15 min under a N_2 atmosphere in order to generate $LiBH_4$. To this, a solution of compound **8** (1.8 g, 3.33 mmol) in THF (30 mL) was added at 0 °C. The reaction mixture was stirred at 0 °C for 15–20 min then allowed to warm to r.t. with stirring overnight. The mixture was concentrated in vacuo, quenched with sat. aq NH_4Cl (20 mL) then extracted with EtOAc (3 × 20 mL). The organic layer was washed with brine (10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (EtOAc–hexane, 1:1) to afford **3**.

Yield: 1.42 g (85%); colourless liquid; $[\alpha]_D^{20}$ –2.40 (*c* 1.2, $CHCl_3$).

IR (KBr): 1052, 1109, 1683, 3019, 3459 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 1.19–1.30 (12 H, m), 1.31–1.39 (6 H, m), 1.61–1.69 (1 H, br s), 3.53–3.69 (3 H, m), 3.74–3.81 (4 H, m), 3.99–4.12 (2 H, m), 4.38–4.52 (3 H, m), 4.86–4.99 (2 H, m), 6.22–6.32 (1 H, br s, NH), 6.78–6.85 (2 H, d, *J* = 8.3 Hz), 7.16–7.21 (2 H, d, *J* = 8.3 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 159.3, 156.9, 129.7, 129.3, 115.1, 108.9, 98.0, 72.9, 70.9, 65.2, 65.9, 62.1, 55.2, 51.0, 26.9, 21.7.

HRMS (EI): *m*/*z* $[M + H]^+$ calcd for $C_{24}H_{39}N_2O_9$: 499.2655; found: 499.2639.

Diisopropyl 1-{(1*R*)-1-[(4*R*,5*S*)-2,2-Dimethyl-5-[(4-methylphenyl)sulfonyl]oxymethyl-1,3-dioxolan-4-yl]-2-[(4-methoxybenzyl)oxy]ethyl}-1,2-hydrazinedicarboxylate (9)

To a stirred solution of alcohol **3** (1.2 g, 2.40 mmol) in anhyd CH_2Cl_2 (30 mL) were added, sequentially, TsCl (549 mg, 2.88 mmol), Et_3N (0.5 mL, 3.6 mmol) and DMAP (cat.) at 0 °C under N_2. After stirring for 6 h, the reaction mixture was washed with sat. aq $NaHCO_3$ (10 mL), diluted with CH_2Cl_2 (30 mL), washed with brine (20 mL), dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography (EtOAc–hexane, 1:3) gave the pure tosylate **9**.

Yield: 1.45 g (92%); colourless oil; $[\alpha]_D^{20}$ –1.22 (*c* 1, $CHCl_3$).

IR (KBr): 983, 1109, 1514, 1614, 2984, 3415 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 1.14–1.34 (18 H, m), 2.38–2.51 (3 H, s), 3.42–3.52 (1 H, m), 3.71–3.82 (4 H, m), 3.94–4.28 (4 H, m), 4.31–4.42 (3 H, m), 4.81–4.99 (2 H, m), 6.21–6.25 (1 H, br s, NH), 6.78–6.85 (2 H, d, *J* = 6.0 Hz), 7.16–7.21 (2 H, d, *J* = 6.0 Hz), 7.24–7.36 (2 H, d, *J* = 7.5 Hz), 7.71–7.90 (2 H, d, *J* = 7.5 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 159.2, 155.6, 144.6, 133.2, 129.6, 129.2, 127.8, 113.7, 109.5, 78.0, 72.7, 71.6, 69.9, 55.0, 27.5, 21.8.

HRMS (EI): *m*/*z* $[M + H]^+$ calcd for $C_{31}H_{45}N_2O_{11}S$: 653.2744; found: 653.2743.

Diisopropyl (3*aR*,4*R*,7*aR*)-4-[(4-Methoxybenzyl)oxy]methyl-2,2-dimethylperhydro [1,3]dioxolo[4,5-*d*]pyridazine-5,6-dicarboxylate (2)

To a well stirred suspension of freshly activated NaH (61.33 mg, 1.53 mmol, 60% w/v dispersion in mineral oil) in anhyd THF (20 mL), a solution of tosylated compound **9** (1 g, 1.53 mmol) in anhyd THF (20 mL) was added dropwise at 0 °C. The reaction mixture was stirred for 2 h at r.t. then quenched with ice and diluted with EtOAc (20 mL), washed with brine (1 × 10 mL) and dried over Na_2SO_4. The reaction mixture was concentrated under reduced

pressure and the crude residue was purified by column chromatography (EtOAc–hexane, 1:3) to give cyclised compound **2**.

Yield: 600 mg (81%); yellow liquid; $[\alpha]_D^{20}$ +5.61 (*c* 1, $CHCl_3$).

IR (KBr): 828, 1104, 1382, 1514, 1613, 1706, 2983 cm^{-1}.

^1H MR (600 MHz, $CDCl_3$): δ = 1.43 (3 H, s, H-5), 1.19 (3 H, d, *J* = 6.2 Hz, H-13), 1.22 (3 H, d, *J* = 6.2 Hz, H-12), 1.25 (6 H, d, *J* = 6.2 Hz, H-10), 1.38 (3 H, s, H-6), 2.90 (1 H, t, *J* = 11.5 Hz, H-1), 3.47 (1 H, dd, *J* = 5.8, 9.2 Hz, H-3), 3.58 (1 H, dd, *J* = 5.8, 10.6 Hz, H-4), 3.72 (1 H, dd, *J* = 10.6, 9.2 Hz, H-4′), 3.75 (1 H, t, *J* = 9.2 Hz, H-2), 3.81 (3 H, s, H-9), 4.46 (2 H, m, H-16), 4.72 (2 H, dd, *J* = 4.7, 12.0 Hz, H-15), 4.91 (1 H, sept, *J* = 6.2 Hz, H-14), 4.98 (1 H, sept, *J* = 6.2 Hz, H-11), 6.87 (2 H, d, *J* = 8.4 Hz, H-8), 7.24 (2 H, d, *J* = 8.4 Hz, H-7).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.9, 154.7, 129.1, 113.6, 110.7, 72.9, 70.9, 70.2, 65.3, 58.4, 56.9, 48.4, 26.4, 21.9.

HRMS (EI): *m*/*z* $[M + H]^+$ calcd for $C_{24}H_{37}N_2O_8$: 481.2549; found: 481.2550.

Diisopropyl (3*aR*,4*R*,7*aR*)-4-(Hydroxymethyl)-2,2-dimethylperhydro[1,3]dioxolo[4,5-*d*]pyridazine-5,6-dicarboxylate (10)

To a solution of cyclised compound **2** (0.6 g, 1.25 mmol) in a CH_2Cl_2–H_2O mixture (4:1, 20 mL) at r.t., was added DDQ (340 mg, 1.5 mmol). The resulting mixture was stirred for 30 min at r.t. and then quenched with sat. aq $NaHCO_3$ (10 mL) and stirred vigourously for a further 10–15 min. The reaction mixture was diluted with CH_2Cl_2 (3 × 20 mL), washed with brine (10 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (EtOAc–hexane, 2:5) to give **10**.

Yield: 365 mg (81%); yellow liquid; $[\alpha]_D^{20}$ +3.76 (*c* 1.4, $CHCl_3$).

IR (KBr): 759, 1384, 1617, 2985, 3418 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 1.24–1.42 (18 H, m), 2.02–2.12 (1 H, br s), 2.84–2.98 (1 H, m), 3.41–3.78 (4 H, m), 4.40–4.54 (1 H, dd, *J* = 2.9, 3.6 Hz), 4.91–5.08 (3 H, m).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 154.7, 110.9, 77.2, 70.9, 70.2, 57.2, 56.7, 50.1, 48.4, 26.4, 21.9, 21.7.

HRMS (EI): *m*/*z* $[M + Na]^+$ calcd for $C_{16}H_{28}N_2O_7Na$: 383.1794; found: 383.1776.

5,6-Diisopropyl 4-Methyl (3*aR*,4*S*,7*aR*)-2,2-Dimethylperhydro[1,3]dioxolo[4,5-*d*]pyridazine-4,5,6-tricarboxylate (11)

Alcohol **10** (300 mg, 0.83 mmol) was dissolved in a mixture of MeCN (4 mL) and H_2O (4 mL) and bis(acetoxy)iodobenzene (804.67 mg, 2.49 mmol) and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO; 55.51 mg, 0.33 mmol) were successively added at 0 °C. The mixture was stirred for 1 h at r.t. then extracted with EtOAc (4 × 20 mL), washed with brine (10 mL) and the combined organic extracts were dried over Na_2SO_4 and evaporated under reduced pressure. As the crude product was unstable, the following reaction was conducted directly without further purification. The crude residue (210 mg, ~0.56 mmol) was taken in anhyd Et_2O (5 mL), CH_2N_2 (34.53 mg, 0.842 mmol) was added at 0 °C and the mixture was stirred at r.t. for 30 min. The solvent was removed under reduced pressure and the crude residue was purified by column chromatography (EtOAc–hexane, 1:3) to afford **11**.

Yield: 230 mg (71%); colourless liquid; $[\alpha]_D^{20}$ +3.38 (*c* 1, $CHCl_3$).

IR (KBr): 1106, 1180, 1385, 1714, 2927 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 1.18–1.36 (12 H, m), 1.38–1.42 (6 H, m), 2.82–2.94 (1 H, m), 3.39–3.48 (1 H, m), 3.71–3.79 (3 H, s), 4.02–4.14 (1 H, m), 4.72–4.84 (1 H, dd, *J* = 4.5, 7.5 Hz), 4.91–5.01 (2 H, m), 5.38–5.50 (1 H, m).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.9, 154.7, 111.2, 71.6, 70.1, 57.2, 52.5, 47.8, 26.9, 21.7.

HRMS (EI): *m*/*z* $[M + H]^+$ calcd for $C_{17}H_{29}N_2O_8$: 389.1923; found: 389.1915.

1,2-Diisopropyl 3-Methyl (3*S*,4*R*,5*R*)-4,5-Dihydroxyhexahydro-1,2,3-pyridazinetricarboxylate (1)

To a solution of compound **11** (80 mg, 0.206 mmol) in MeOH (15 mL) was added CSA (cat.) and the solution was stirred at r.t. for 30 min. The solvent was evaporated in vacuo and the reaction mixture was neutralised with aq $NaHCO_3$, diluted with EtOAc and purified by column chromatography (EtOAc–hexane, 3:1) to afford **1**.

Yield: 58 mg (80%); colourless liquid; $[\alpha]_D^{20}$ –5.75 (*c* 1, $CHCl_3$).

IR (KBr): 1106, 1618, 1713, 2984, 3414 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 1.23 (3 H, d, *J* = 6.3 Hz, H-12), 1.27 (6 H, d, *J* = 6.3 Hz, H-10), 2.70 (1 H, m, H-4), 3.47 (1 H, br s, H-7, OH), 3.63 (1 H, t, *J* = 8.0 Hz, H-2), 3.75 (3 H, s, H-8), 4.18 (1 H, dt, *J* = 5.7, 9.7 Hz, H-3), 4.32 (1 H, br s, H-6, OH), 4.46 (1 H, dd, *J* = 4.9, 13.6 Hz, H-5), 4.91 (1 H, sept, *J* = 6.3 Hz, H-11), 4.98 (1 H, sept, *J* = 6.3 Hz, H-9), 5.23 (1 H, d, *J* = 8.0 Hz, H-1).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.9, 154.7, 71.6, 70.1, 67.5, 58.4, 52.5, 47.8, 21.8.

HRMS (EI): *m*/*z* $[M + H]^+$ calcd for $C_{14}H_{25}N_2O_8$: 349.1610; found: 349.1606.

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