

# Stereoselectivity in the synthesis of conformationally constrained vicinally dihydroxylated cyclic $\alpha$ -amino acids

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Stereoselective syntheses of precursors to vicinal *cis*-dihydroxy-1-aminocyclopentane- and -cyclohexane-carboxylic acid methyl esters and their methoxy analogues are described. The chiral products are isolated as pure enantiomers. The absolute configurations at the new stereogenic centres are established by X-ray analysis.

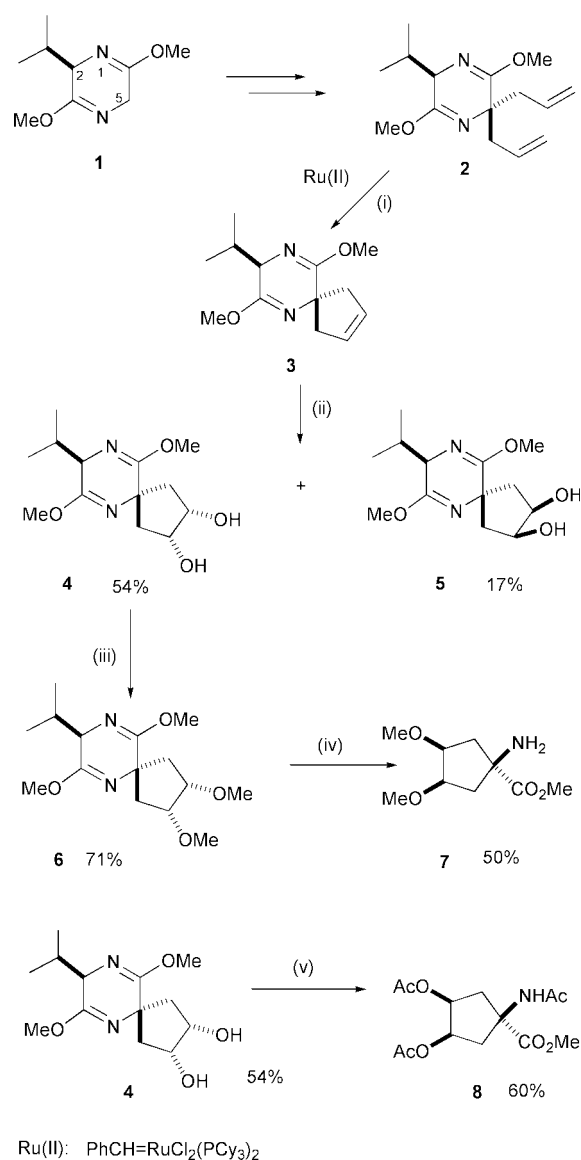
## Introduction

A wide variety of conformationally constrained amino acids are expected to be acceptable in protein biosynthesis by analogy to the findings in *Escherichia coli*.<sup>1</sup> In this organism methyl and cyclic  $\alpha,\alpha$ -disubstituted amino acids were, in general, significantly more efficiently incorporated than was D-alanine. It is also known that  $\alpha$ -substituted  $\alpha$ -amino acids are conformationally constrained and therefore, when incorporated into peptides, will constitute a powerful approach for generating structurally defined peptides as conformational probes and bioactive agents.<sup>2</sup> Such findings have stimulated work in the search for ways to prepare conformationally constrained amino acid-like structures. Our efforts have largely been directed towards the development of stereoselective methodology for the preparation of rigid, cyclic amino acids where the  $\alpha$ -carbon of the amino acid is imbedded in the ring. When the ring carries an additional substituent in the form of a hydroxy group, our target molecules have been rigidified analogues of serine and homoserine.<sup>3,4</sup> Herein we describe work for the preparation of cyclic, sterically defined *cis*-dihydroxy derivatives. The chiral information required for the preparation of these structures is available in the bislactim substrate **1**. The latter is derived from a diketopiperazine enantiomer of valine and glycine. Prior to our work, preparation of di- or higher hydroxylated, sterically pure amino acids in this series had been limited to tetrahydroxylated cyclopentane and cyclohexane  $\alpha$ -amino acids that were prepared from a spiroazidolactone.<sup>5</sup>

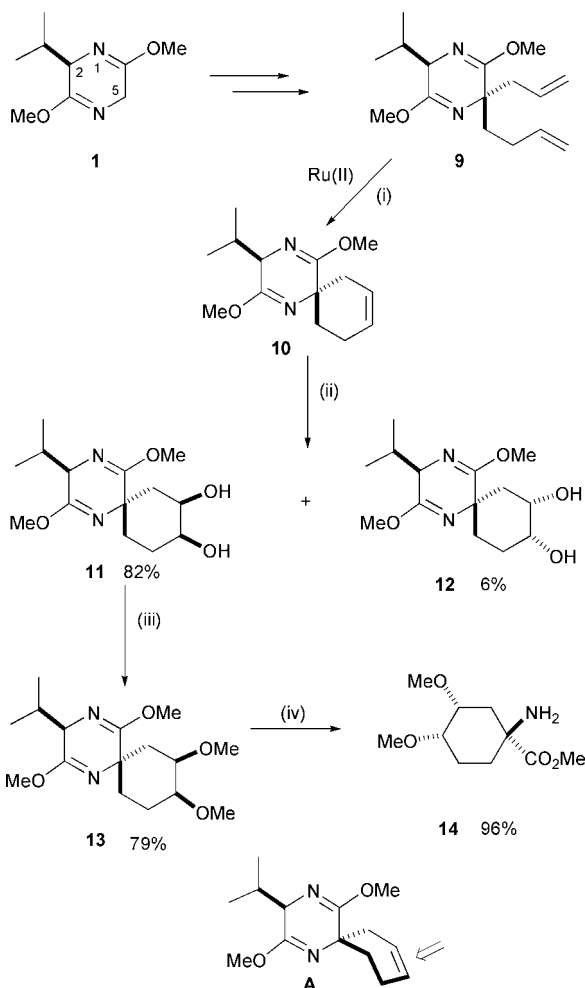
## Results and discussion

The spirocycloalkenes **3** (Scheme 1) and **10** (Scheme 2) were the substrates used for the hydroxylation studies. Their preparation involves dialkylation of the bislactim ether **1** in a stepwise and stereoselective manner (**2** and **9**) followed by a Ru(II)-catalysed ring-closing metathesis reaction as recently described.<sup>6</sup>

The vicinal dihydroxylation reaction was effected by catalytic amounts of osmium tetroxide in the presence of *N*-methylmorpholine *N*-oxide (NMO). The stereoselectivity was moderate in the dihydroxylation of the five-membered-ring structure **3**, the dihydroxy product isomer ratio **4**:**5** being 3:1. In contrast, high stereoselectivity was obtained in the oxidation of the six-membered ring **10** (Scheme 2), the ratio **11**:**12** being 14:1. The relative stereochemistry of the major isomers in the two series, however, was different between **4** and **11**. The structures were determined by single-crystal X-ray analyses. In the



**Scheme 1** Reagents and conditions: (i) 2% Ru(II), DCE; (ii) 1.0 mol% OsO<sub>4</sub>, NMO, Me<sub>2</sub>CO, H<sub>2</sub>O, 0 °C, 6 h; (iii) NaH, MeI, DMF–THF, 20 °C, 14 h; (iv) 0.1 M TFA in MeCN–H<sub>2</sub>O 1:1, 20 °C, 5 d; (v) (a) 0.1 M TFA in MeCN–H<sub>2</sub>O 1:1, 20 °C, 3 d; (b) Ac<sub>2</sub>O, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 20 °C, 2 d.



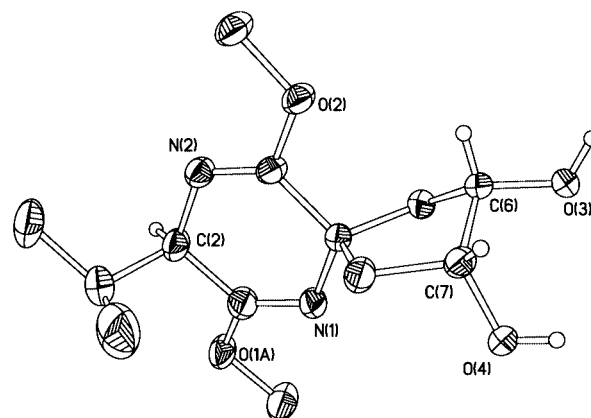
Ru(II):  $\text{PhCH}=\text{RuCl}_2(\text{PCy}_3)_2$

**Scheme 2** Reagents and conditions: (i) 2% Ru(II), DCE; (ii) 1.0 mol%  $\text{OsO}_4$ , NMO,  $\text{Me}_2\text{CO}$ ,  $\text{H}_2\text{O}$ , 0 °C, 6 h; (iii) NaH, MeI, DMF-THF, 20 °C, 14 h; (iv) 0.1 M TFA in MeCN- $\text{H}_2\text{O}$  1 : 1, 20 °C, 5 d.

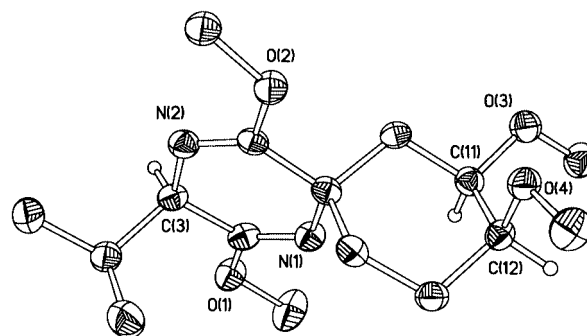
five-membered-ring series the major isomer has the vicinal *cis*-dihydroxy groups in a transfacial relationship to the 6-methoxy group in the pyrazine ring whereas a cisfacial relationship exists with the dihydroxy groups in the six-membered-ring system. Inspection of a molecular model of structure **3** indicates a high degree of shielding of the cyclopentenyl ring at the face that has the overlying pyrazine 6-methoxy group (numbering of **1**). The shielding would suggest a favoured approach to the less shielded face of the double bond, resulting in preferential formation of the transfacial structure **4** rather than stereoisomer **5**.

The six-membered ring in the spiroannulated structure **10** (Scheme 2) is more flexible and can assume conformations where the double bond is more exposed than in the spiroannulated cyclopentene. In particular, in boat-like conformations as indicated in structure **A** in Scheme 2, the face on the side of the methoxy group has reduced shielding. The other face becomes more shielded by the pyrazine 3-methoxy group and the N-4 pyrazine nitrogen (numbering of **1**). As a result, hydroxylation occurs preferably cisfacial to the 6-methoxy group, the preferential product being compound **11** rather than compound **12**.

Hydrolytic cleavage of the pyrazine ring with formation of the new amino acid as its methyl ester was effected by mild acid hydrolysis using 0.1 M TFA in aq. acetonitrile. The cyclopentane product was highly water soluble and was isolated after acylation as the triacetylated amino acid ester **8** (Scheme 1). The methoxylated analogue **7** has also been prepared. Alkylation was effected before hydrolysis. Treatment of the diol **4**



**Fig. 1** The ORTEP plot of the X-ray structure of **4**. Ellipsoids are shown at 50% probability. For clarity only hydrogens at stereogenic centres are shown. The absolute configurations at the stereogenic centres were established relative to the known chirality (2*R*) at C-2 (**4**). Crystallographic numbering scheme shown.



**Fig. 2** The ORTEP plot of the X-ray structure of **13**. Ellipsoids are shown at 50% probability. For clarity only hydrogens at stereogenic centres are shown. The absolute configurations at the stereogenic centres were established relative to the known chirality (2*R*) at C-2 (**13**). Crystallographic numbering scheme shown.

with methyl iodide and sodium hydride as base gave the dimethoxy product **6**, which was subsequently hydrolysed to its amino acid ester **7**. Compounds **7** and **8** are achiral. Chiral amino acids would require a symmetry-breaking substitution on intermediate **3**, but this was not studied because the purpose of this work was to make available methodology for the preparation of this class of conformationally constrained amino acids. Preparation of enantiomerically pure amino acid derivatives, however, has been demonstrated in the six-membered-ring series which furnished the amino acid ester analogue **14** (Scheme 2).

For stereochemical assignments, the cyclopentane diol **4** was subjected to a single-crystal X-ray analysis (Fig. 1, *vide infra*). The crystal form of the corresponding six-membered-ring diol **11** was not satisfactory under the conditions tried. In this case a single-crystal X-ray analysis was run on the dimethoxy analogue **13** (Fig. 2, *vide infra*).

In conclusion we have synthesized in a stereoselective manner dihydroxy derivatives of rigidified cyclic  $\alpha$ -amino acids and have determined their absolute configurations by single-crystal X-ray analysis.

## Experimental

$^1\text{H}$  NMR spectra were recorded in  $\text{CDCl}_3$  at 300 or 200 MHz with a Bruker DPX 300 or DPX 200 spectrometer. The  $^{13}\text{C}$  spectra were recorded in  $\text{CDCl}_3$  at 75 MHz or 50 MHz. Chemical shifts are reported in ppm using residual  $\text{CHCl}_3$  (7.24 ppm) and  $\text{CDCl}_3$  (77 ppm) as references. *J*-Values are given in Hz. Mass spectra under electron-impact conditions (EI) were recorded at 70 eV ionizing potential; ammonia was used for

chemical ionization (CI). The spectra are presented as  $m/z$  (% rel. int.). IR spectra were measured on a Nicolet Magna 550 spectrometer using ATR (attenuated total reflectance). Optical rotations were measured at ambient temperature ( $\approx 20^\circ\text{C}$ ) on a Perkin-Elmer 241 polarimeter.  $[\alpha]_{\text{D}}$ -Values are given in units of  $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$ . Dry THF was distilled from sodium and benzophenone under argon. Dry ethylene dichloride (DCE) was distilled from calcium hydride under argon. DMF was distilled from barium oxide. Solvents were degassed by bubbling argon through them.

#### X-Ray crystallographic analysis data for compounds 4 and 13†

X-Ray data were collected on a Siemens SMART CCD diffractometer<sup>7</sup> using graphite-monochromated Mo-K $\alpha$  radiation ( $\lambda = 0.71073 \text{ \AA}$ ). Data-collection method:  $\omega$ -scan, range  $0.6^\circ$ , crystal-to-detector distance 5 cm. Data reduction and cell determination were carried out with the SAINT and XPREP programs.<sup>7</sup> Absorption corrections were applied by the use of the SADABS program.<sup>8</sup> The structure was determined and refined using the SHELXTL program package.<sup>9</sup> The non-hydrogen atoms were refined with anisotropic thermal parameters; hydrogen atoms were located from difference Fourier maps and refined with isotropic thermal parameters.

Crystal data for  $\text{C}_{13}\text{H}_{22}\text{N}_2\text{O}_4$  (**4**),  $M = 270.33$ , tetragonal,  $P4_2$ ,  $a = 12.654(1)$ ,  $c = 9.215(1) \text{ \AA}$ ,  $V = 1475.5(1) \text{ \AA}^3$ ,  $Z = 4$ ,  $D_x = 1.217 \text{ Mg m}^{-3}$ ,  $\mu = 0.090 \text{ mm}^{-1}$ ,  $T = 150 \text{ K}$ , measured 16 161 reflections in  $2\theta$  range  $4.1\text{--}61.1^\circ$ ,  $R_{\text{int}} = 0.043$ . 266 Parameters refined against 4323  $F^2$ ,  $R = 0.049$  for  $I_o > 2\sigma(I_o)$  and 0.058 for all data. A disorder was observed in one of the methoxy groups.

Crystal data for  $\text{C}_{16}\text{H}_{28}\text{N}_2\text{O}_4$  (**13**),  $M = 312.40$ , monoclinic,  $P2_1$ ,  $a = 9.725(1)$ ,  $b = 8.738(1)$ ,  $c = 10.903(1) \text{ \AA}$ ,  $\beta = 109.0(1)^\circ$ ,  $V = 876.0(2) \text{ \AA}^3$ ,  $Z = 2$ ,  $D_x = 1.184 \text{ Mg m}^{-3}$ ,  $\mu = 0.085 \text{ mm}^{-1}$ ,  $T = 150 \text{ K}$ , measured 7818 reflections in  $2\theta$  range  $3.6\text{--}49.4^\circ$ ,  $R_{\text{int}} = 0.054$ . 311 Parameters refined against 2953  $F^2$ ,  $R = 0.060$  for  $I_o > 2\sigma(I_o)$  and 0.066 for all data.

#### (2*R*,3'*R*,4'*S*,5*R*)-2,5-Dihydro-5-isopropyl-3,6-dimethoxy-pyrazine-2-spirocyclopentane-3',4'-diol **4** and (2*S*,3'*R*,4'*S*,5*R*)-2,5-dihydro-5-isopropyl-3,6-dimethoxypyrazine-2-spirocyclopentane-3',4'-diol **5**

Osmium tetroxide solution (0.38 ml, 0.029 mmol; 2.5% in Bu'OH) was added to a solution of (5*R*)-2,5-dihydro-5-isopropyl-3,6-dimethoxypyrazine-2-spiro-4'-cyclopentene (676 mg, 2.87 mmol) and NMO monohydrate (427 mg, 3.16 mmol) in acetone (20 ml)–water (5 ml) at  $0^\circ\text{C}$ . The mixture was stirred at  $0^\circ\text{C}$  for 6 h before the reaction was terminated by the addition of sodium hydrogen sulfite (328 mg, 3.16 mmol). Stirring was continued for 15 min, water (20 ml) added, and the mixture extracted with methylene dichloride ( $3 \times 20 \text{ ml}$ ). The combined organic extracts were dried ( $\text{MgSO}_4$ ) and evaporated to dryness. The residual material was subjected to flash chromatography using 5% methanol in methylene dichloride to yield the isomers **4** and **5**.

Compound **4** (402 mg, 54%) was a white solid, mp  $76^\circ\text{C}$  (crude) (Found: C 57.37; H, 8.03. Calc for  $\text{C}_{13}\text{H}_{22}\text{N}_2\text{O}_4$ : C, 57.76; H, 8.20%); HRMS:  $M^+$ , 270.1569. Calc for  $\text{C}_{13}\text{H}_{22}\text{N}_2\text{O}_4$ :  $M$ , 270.1580;  $[\alpha]_{\text{D}}$   $-42.20$  ( $c$  0.87,  $\text{CHCl}_3$ );  $\nu_{\text{max}}(\text{ATR})/\text{cm}^{-1}$  3315s, 2912, 1670, 1425, 1218, 1009;  $\delta_{\text{H}}(300 \text{ MHz})$  0.53 (3 H, d,  $J$  7,  $\text{CH}_3$ ), 0.90 (3 H, d,  $J$  7,  $\text{CH}_3$ ), 1.51–1.66 (2 H, m,  $\text{CH}_2$ ), 2.04–2.34 (1 H, m, CH), 2.27–2.34 (2 H, m,  $\text{CH}_2$ ), 3.49 (3 H, s,  $\text{OCH}_3$ ), 3.53 (3 H, s,  $\text{OCH}_3$ ), 3.82 (1 H, m,  $\text{CHOH}$ ), 3.85 (1 H, d,  $J$  4, H-2), 4.10 (3 H, m, OH,  $2 \times \text{CHOH}$ );  $\delta_{\text{C}}(75 \text{ MHz})$  16.51 ( $\text{CH}_3$ ), 19.93 ( $\text{CH}_3$ ), 30.94 (CH), 45.34 ( $\text{CH}_2$ ), 45.25 ( $\text{CH}_2$ ), 52.21 ( $\text{OCH}_3$ ), 52.34 ( $\text{OCH}_3$ ), 60.29 (C-2), 63.61 (C-5), 74.38 ( $\text{CHOH}$ ), 74.53 ( $\text{CHOH}$ ), 161.25 (C), 162.96 (C);  $m/z$  (EI) 270

( $M^+$ , 1%), 227 (9), 226 (9), 209 (6), 198 (10), 184 (11), 183 (100), 154 (24).

Compound **5** (126 mg, 17%) was a white solid, mp  $104^\circ\text{C}$  (crude) (Found: C, 57.8; H, 8.3%); HRMS:  $M^+$ , 270.1572;  $[\alpha]_{\text{D}}$   $-34.60$  ( $c$  0.54,  $\text{CHCl}_3$ );  $\nu_{\text{max}}(\text{ATR})/\text{cm}^{-1}$  3315s, 2908, 1671, 1420, 1210, 1009;  $\delta_{\text{H}}(300 \text{ MHz})$  0.60 (3 H, d,  $J$  7,  $\text{CH}_3$ ), 0.98 (3 H, d,  $J$  7,  $\text{CH}_3$ ), 1.84–2.24 (5 H, m, CH,  $2 \times \text{CH}_2$ ), 3.90 (2 H, m,  $2 \times \text{OH}$ ), 3.55 (3 H, s,  $\text{OCH}_3$ ), 3.68 (3 H, s,  $\text{OCH}_3$ ), 3.90 (1 H, d,  $J$  4, H-2), 4.25 (2 H, m,  $\text{CHOH}$ );  $\delta_{\text{C}}(75 \text{ MHz})$  16.70 ( $\text{CH}_3$ ), 19.14 ( $\text{CH}_3$ ), 30.95 (CH), 46.47 ( $\text{CH}_2$ ), 46.66 ( $\text{CH}_2$ ), 52.09 ( $\text{OCH}_3$ ), 52.80 ( $\text{OCH}_3$ ), 60.77 (C-5, -2), 73.38 ( $2 \times \text{CHOH}$ ), 160.84 (C), 163.75 (C);  $m/z$  (EI) 270 ( $M^+$ , 12%), 253 (11), 228 (15), 227 (100), 209 (26), 198 (21), 195 (29), 183 (57), 154 (14).

#### (2*R*,3'*R*,4'*S*,5*R*)-2,5-Dihydro-5-isopropyl-3,3',4',6-tetramethoxy-pyrazine-2-spirocyclopentane **6**

A solution of (2*R*,3'*R*,4'*S*,5*R*)-2,5-dihydro-5-isopropyl-3,6-dimethoxypyrazine-2-spirocyclopentane-3',4'-diol **4** (404 mg, 1.50 mmol) in dry DMF (1 ml)–THF (1 ml) was added to a suspension of sodium hydride (131 mg in paraffin oil, 60%; 3.00 mmol) in DMF (10 ml)–THF (10 ml) under argon at  $0^\circ\text{C}$ . The mixture was stirred at ambient temperature for 2 h before methyl iodide (0.2 ml, 3.00 mmol) was added dropwise. The resultant mixture was stirred at ambient temperature overnight. Diethyl ether (100 ml) was added. The organic mixture was extracted with water ( $5 \times 40 \text{ ml}$ ) and washed with brine (40 ml). The ethereal solution was dried ( $\text{MgSO}_4$ ) and evaporated. The residual material was subjected to flash chromatography using hexane–EtOAc (2:1) as eluent to yield compound **6** as a colourless oil (318 mg, 71%) (Found: C, 60.57; H, 8.61. Calc for  $\text{C}_{15}\text{H}_{26}\text{N}_2\text{O}_4$ : C, 60.38; H, 8.78%); HRMS:  $M^+$ , 298.1882. Calc for  $\text{C}_{15}\text{H}_{26}\text{N}_2\text{O}_4$ :  $M$ , 298.1893;  $\nu_{\text{max}}(\text{ATR})/\text{cm}^{-1}$  2908, 1671, 1422, 1295, 1221;  $\delta_{\text{H}}(300 \text{ MHz})$  0.62 (3 H, d,  $J$  7,  $\text{CH}_3$ ), 1.00 (3 H, d,  $J$  7,  $\text{CH}_3$ ), 1.92–2.40 (5 H, m,  $\text{Me}_2\text{CH}$ ,  $2 \times \text{CH}_2$ ), 3.36 (3 H, s,  $\text{CHOCH}_3$ ), 3.37 (3 H, s,  $\text{CHOCH}_3$ ), 3.60 (3 H, s,  $\text{OCH}_3$ ), 3.64 (3 H, s,  $\text{OCH}_3$ ), 3.88–3.97 (3 H, m,  $2 \times \text{CHOCH}_3$ , H-2);  $\delta_{\text{C}}(75 \text{ MHz})$  16.80 ( $\text{CH}_3$ ), 19.22 ( $\text{CH}_3$ ), 30.99 (CH), 42.99 ( $\text{CH}_2$ ), 43.27 ( $\text{CH}_2$ ), 52.15 ( $\text{OCH}_3$ ), 52.49 ( $\text{OCH}_3$ ), 56.79 ( $\text{CHOCH}_3$ ), 56.86 ( $\text{CHOCH}_3$ ), 60.16 (C-5), 60.66 (C-2), 81.56 ( $\text{CHOMe}$ ), 81.66 ( $\text{CHOMe}$ ), 160.80 (C), 165.72 (C);  $m/z$  (EI) 298 ( $M^+$ , 2%), 283 (12), 267 (21), 255 (17), 237 (100), 226 (19), 223 (73), 197 (95).

#### Methyl 1-amino-*trans,trans*-3,4-dimethoxycyclopentane-carboxylate **7**

(2*R*,3'*R*,4'*S*,5*R*)-2,5-Dihydro-5-isopropyl-3,3',4',6-tetramethoxy-pyrazine-2-spirocyclopentane **6** (254 mg, 0.85 mmol) was dissolved in a mixture of TFA (0.2 M; 43 ml, 8.5 mmol) and acetonitrile (43 ml). The solution was stirred at ambient temperature for 5 days and evaporated almost to dryness. Water (10 ml) and methylene dichloride (20 ml) were added, the mixture shaken, and the phases separated. The aqueous phase was collected, and made alkaline by addition of conc. aq. ammonia (pH 10) before extraction with methylene dichloride ( $3 \times 10 \text{ ml}$ ). The combined extract was dried ( $\text{MgSO}_4$ ), evaporated and the residual material purified by flash chromatography using 3% methanol which gave ester **7** as a colourless oily material (86 mg, 50%). HRMS:  $M - \text{CO}_2\text{Me}$ , 144.1025. Calc. for  $\text{C}_7\text{H}_{14}\text{NO}_2$ :  $m/z$ , 144.1025;  $\nu_{\text{max}}(\text{ATR})/\text{cm}^{-1}$  3350s, 2898, 1714, 1425, 1185, 1085;  $\delta_{\text{H}}(300 \text{ MHz})$  1.85 (2 H, dd,  $J$  20, 5,  $\text{CH}_2$ ), 1.93 (2 H, br s,  $\text{NH}_2$ ), 2.33 (2 H, dd,  $J$  14, 6,  $\text{CH}_2$ ), 3.54 (6 H, s,  $2 \times \text{CHOCH}_3$ ), 3.67 (3 H, s,  $\text{OCH}_3$ ), 3.78 (2 H, t,  $J$  4.3,  $2 \times \text{CHOCH}_3$ );  $\delta_{\text{C}}(75 \text{ MHz})$  42.49 ( $2 \times \text{CH}_2$ ), 52.39 ( $\text{OCH}_3$ ), 57.31 ( $2 \times \text{CHOCH}_3$ ), 61.56 (C), 81.65 ( $2 \times \text{CHOCH}_3$ ), 176.65 (C=O);  $m/z$  (EI) 144 ( $M^+ - \text{CO}_2\text{Me}$ , 100%), 145 (14), 130 (34), 113 (17), 112 (83), 85 (20), 71 (18);  $m/z$  (CI) 204 ( $M^+ + 1$ , 100%), 205 (10), 155 (16), 144 (10,  $M^+ - \text{CO}_2\text{Me}$ ).

† CCDC reference number 207/418. See <http://www.rsc.org/suppdata/p1/b0/b001779p/> for crystallographic files in .cif format.

### Methyl 1-acetamido-*trans,trans*-3,4-diacetoxycyclopentane-carboxylate **8**

(2*R*,3'*R*,4'*S*,5*R*)-2,5-Dihydro-5-isopropyl-3,6-dimethoxy-pyrazine-2-spirocyclopentane-*cis*-3',4'-diol **4** (162 mg, 0.60 mmol) was stirred with TFA (30 ml, 6.00 mmol; 0.2 M)-acetonitrile (30 ml) at ambient temperature for 3 days. The solution was evaporated almost to dryness and water (1 ml) and methylene dichloride (10 ml) were added. The aqueous layer was collected and made alkaline by addition of conc. aq. ammonia (pH 10) before extraction with methylene dichloride (2 × 10 ml) and ethyl acetate (10 ml). The combined organic layers were dried (MgSO<sub>4</sub>), evaporated and the residual material dissolved in methylene dichloride (10 ml) with subsequent addition of 4-(dimethylamino)pyridine (DMAP) (450 mg, 3.68 mmol) and acetic anhydride (0.35 ml, 3.68 mmol). The mixture was stirred at ambient temperature for 2 days before the solvent was evaporated off. The residual material was subjected to flash chromatography using 3% methanol in methylene dichloride to yield **8** as a colourless oily material (107 mg, 60%). HRMS: M<sup>+</sup>, 301.1157. Calc for C<sub>13</sub>H<sub>19</sub>NO<sub>7</sub>: M, 301.1161; δ<sub>H</sub>(300 MHz) 1.96 (3 H, s, CH<sub>3</sub>), 2.01 (6 H, s, 2 × CH<sub>3</sub>), 2.05 and 2.10 (2 H, dd, *J* 5, CH<sub>2</sub>), 2.69 and 2.73 (2 H, dd, *J* 6, CH<sub>2</sub>), 3.69 (3 H, s, CH<sub>3</sub>O), 5.25 (2 H, m, 2 × CH), 6.30 (1 H, s, NH); δ<sub>C</sub>(75 MHz) 20.75 (2 × CH<sub>3</sub>), 22.81 (CH<sub>3</sub>), 40.09 (2 × CH<sub>2</sub>), 53.00 (CH<sub>3</sub>O), 61.25 (C-1), 72.50 (2 × CH), 169.86 (C=O), 169.93 (C=O), 173.37 (C=O); *m/z* (EI) 301 (M<sup>+</sup>, 0.2%), 183 (11), 182 (100), 140 (15), 130 (11), 122 (60), 80 (77), 43 (66).

### (2*S*,3'*R*,4'*S*,5*R*)-2,5-Dihydro-5-isopropyl-3,6-dimethoxy-pyrazine-2-spirocyclohexane-3',4'-diol **11** and (2*S*,3'*S*,4'*R*,5*R*)-2,5-dihydro-5-isopropyl-3,6-dimethoxypyrazine-2-spirocyclohexane-3',4'-diol **12**

Osmium tetroxide solution (108 μl, 0.016 mmol; 2.5% in Bu'OH) was added to a solution of (2*S*,5*R*)-2,5-dihydro-5-isopropyl-3,6-dimethoxypyrazine-2-spiro-3'-cyclohexene (402 mg, 1.61 mmol) and NMO monohydrate (240 mg, 1.77 mmol) in acetone (20 ml)-water (5 ml) at 0 °C. Sodium bisulfite (184 mg, 1.77 mmol) was added after 6 h at 0 °C and the stirring was continued for 15 min. Methylene dichloride (20 ml) was added. The aqueous layer was extracted with methylene dichloride (3 × 10 ml). The combined organic layers were dried (MgSO<sub>4</sub>) and evaporated to dryness. The two products were separated by flash chromatography using 5% methanol in methylene dichloride.

Compound **11** was a white solid (374 mg, 82%), mp 145 °C (crude) (Found: C, 59.13; H, 8.75. Calc for C<sub>14</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>: C, 59.14; H, 8.51%); HRMS: M<sup>+</sup>, 284.1728. Calc for C<sub>14</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>: M, 284.1736; [α]<sub>D</sub><sup>20</sup> +33.5 (c 0.71, CHCl<sub>3</sub>); ν<sub>max</sub>(ATR)/cm<sup>-1</sup> 3369s, 2942, 1687, 1435, 1227; δ<sub>H</sub>(300 MHz) 0.63 (3 H, d, *J* 7, CH<sub>3</sub>), 1.02 (3 H, d, *J* 7, CH<sub>3</sub>), 1.03–2.30 [7 H, m, (CH<sub>3</sub>)<sub>2</sub>CH-, 3 × CH<sub>2</sub>], 2.59 (2 H, br s, 2 × OH), 3.59 (3 H, s, OCH<sub>3</sub>), 3.63 (3 H, s, OCH<sub>3</sub>), 3.91 (1 H, d, *J* 4, H-2), 4.01 (1 H, m, CHOH), 4.14 (1 H, m, CHOH); δ<sub>C</sub>(75 MHz) 16.91 (CH<sub>3</sub>), 19.23 (CH<sub>3</sub>), 21.62 (CH<sub>2</sub>), 29.48 (CH<sub>2</sub>), 30.93 (CH), 38.50 (CH<sub>2</sub>), 52.06 (OCH<sub>3</sub>), 52.39 (OCH<sub>3</sub>), 58.44 (C-5), 60.23 (C-2), 67.77 (CHOH), 68.89 (CHOH), 160.77 (C), 164.92 (C); *m/z* (EI) 284 (M<sup>+</sup>, 7%), 267 (45), 242 (14), 241 (100), 223 (16), 212 (13), 209 (20), 167 (11).

Compound **12** was a white solid (27 mg, 6%), mp 89 °C (crude) (Found: C, 58.82; H, 8.41%); HRMS: M<sup>+</sup>, 284.1750. Calc for C<sub>14</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>: M, 284.1736; [α]<sub>D</sub><sup>20</sup> +43.70 (c 0.62, CHCl<sub>3</sub>); ν<sub>max</sub>(ATR)/cm<sup>-1</sup> 3338s, 2945, 1686, 1436, 1236; δ<sub>H</sub>(300 MHz) 0.67 (3H, d, *J* 7, CH<sub>3</sub>), 1.03 (3 H, d, *J* 7, CH<sub>3</sub>), 1.47–2.21 [7 H, m, (CH<sub>3</sub>)<sub>2</sub>CH-, 3 × CH<sub>2</sub>], 2.54 (1 H, d, *J* 10, OH), 3.57 (1 H, m, CHOH), 3.62 (3 H, s, OCH<sub>3</sub>), 3.64 (3 H, s, OCH<sub>3</sub>), 3.94 (1 H, m, CHOH), 3.97 (1 H, d, *J* 4, H-2), 5.62 (1 H, d, *J* 10, OH); δ<sub>C</sub>(75 MHz) 17.68 (CH<sub>3</sub>), 19.79 (CH<sub>3</sub>), 25.24 (CH<sub>2</sub>), 31.45 (CH), 35.94 (CH<sub>2</sub>), 39.72 (CH<sub>2</sub>), 53.00 (OCH<sub>3</sub>),

53.06 (OCH<sub>3</sub>), 58.24 (C-5), 60.82 (C-2), 71.07 (CHOH), 71.22 (CHOH), 163.16 (C), 164.34 (C); *m/z* (EI) 284 (M<sup>+</sup>, 72%), 269 (25), 241 (100), 225 (37), 223 (40), 197 (81), 196 (36), 183 (23).

### (2*S*,3'*R*,4'*S*,5*R*)-2,5-Dihydro-5-isopropyl-3,3',4',6-tetramethoxy-pyrazine-2-spirocyclohexane **13**

(2*S*,3'*R*,4'*S*,5*R*)-2,5-Dihydro-5-isopropyl-3,6-dimethoxypyrazine-2-spirocyclohexane-3',4'-diol **11** (359 mg, 1.26 mmol) in a solution of dry DMF (3 ml) and THF (3 ml) was added to a suspension of sodium hydride (138 mg in paraffin oil, ≈60% purity; 3.16 mmol) in a mixture of DMF (20 ml) and THF (20 ml) under argon at 0 °C. The resultant mixture was stirred at ambient temperature for 2 h before methyl iodide (0.2 ml, 3.16 mmol) was added dropwise. The mixture was stirred at ambient temperature overnight. Diethyl ether (100 ml) was added. The organic mixture was extracted successively with water (5 × 40 ml) and brine (1 × 40 ml), and the ethereal solution dried (MgSO<sub>4</sub>) and evaporated. The product was isolated from the residual material after flash chromatography using EtOAc-hexane 1 : 2 and was obtained as a white solid (312 mg, 79%), mp 61 °C (crude) (Found: C, 61.81; H, 8.74. Calc for C<sub>16</sub>H<sub>28</sub>N<sub>2</sub>O<sub>4</sub>: C, 61.51; H, 9.03%); HRMS: M<sup>+</sup>, 312.2034. Calc for C<sub>16</sub>H<sub>28</sub>N<sub>2</sub>O<sub>4</sub>: M, 312.2049; [α]<sub>D</sub><sup>20</sup> +0.67 (c 1.20, CH<sub>2</sub>Cl<sub>2</sub>); ν<sub>max</sub>(ATR)/cm<sup>-1</sup> 2928, 1690, 1435, 1230, 1088; δ<sub>H</sub>(300 MHz) 0.60 (3 H, d, *J* 7, CH<sub>3</sub>), 1.00 (3 H, d, *J* 7, CH<sub>3</sub>), 0.98–1.02 (1 H, m, CHH), 1.39–1.44 (1 H, m, CHH), 1.81–1.85 (2 H, m, CH<sub>2</sub>), 2.11–2.25 (3 H, m, CH and CH<sub>2</sub>), 3.27 (3 H, s, CHOCH<sub>3</sub>), 3.37 (3 H, s, CHOCH<sub>3</sub>), 3.57 (3 H, s, OCH<sub>3</sub>), 3.58 (3 H, s, OCH<sub>3</sub>), 3.63–3.70 (2 H, m, 2 × CHOCH<sub>3</sub>), 3.87 (1H, d, *J* 4, H-2); δ<sub>C</sub>(75 MHz) 16.82 (CH<sub>3</sub>), 19.20 (CH<sub>3</sub>), 22.33 (CH<sub>2</sub>), 29.88 (CH), 30.83 (CH<sub>2</sub>), 35.98 (CH<sub>2</sub>), 51.92 (OCH<sub>3</sub>), 52.20 (OCH<sub>3</sub>), 55.95 (CHOCH<sub>3</sub>), 56.63 (CHOCH<sub>3</sub>), 58.28 (C-5), 60.15 (C-2), 75.28 (CHOHMe), 77.06 (CHOMe), 160.69 (C), 165.05 (C); *m/z* 312 (M<sup>+</sup>, 8%), 297 (27), 281 (82), 269 (100), 237 (74), 211 (13), 197 (7), 153 (30).

### Methyl (1*S*,3*R*,4*S*)-1-amino-3,4-dimethoxycyclohexanecarboxylate **14**

(2*S*,3'*R*,4'*S*,5*R*)-2,5-Dihydro-5-isopropyl-3,3',4',6-tetramethoxy-pyrazine-2-spirocyclohexane **13** (192 mg, 0.62 mmol) was stirred in a solution of TFA (0.2 M; 31 ml, 6.20 mmol) and acetonitrile (31 ml) at ambient temperature for 5 days. The mixture was evaporated almost to dryness and water (5 ml) and methylene dichloride (10 ml) were added. The aqueous phase was collected, and made alkaline by addition of conc. aq. ammonia (pH 10) before extraction with methylene dichloride (3 × 10 ml). The combined extracts were dried (MgSO<sub>4</sub>), evaporated and the residual material subjected to flash chromatography using 3% methanol in methylene dichloride. The product (127 mg, 96%) was a colourless oil. HRMS: *m/z*, 158.1189. Calc for C<sub>8</sub>H<sub>16</sub>NO<sub>2</sub> (M - CO<sub>2</sub>Me): *m/z*, 158.1181; ν<sub>max</sub>(ATR)/cm<sup>-1</sup> 3510s, 2900, 1710, 1440, 1200, 1090; δ<sub>H</sub>(500 MHz) 1.22–1.39 (1 H, m, CHH), 1.52 (2 H, br s, NH<sub>2</sub>), 1.58 (1 H, dd, *J* 12.3, 2.7, CHH), 1.69–1.75 (1 H, m, CHH), 1.90–1.96 (1 H, m, CHH), 2.00–2.06 (1 H, m, CHH), 2.21 (1 H, dd, *J* 12.8, 10.3, CHH), 3.32 (3 H, s, CHOCH<sub>3</sub>), 3.36 (3 H, s, CHOCH<sub>3</sub>), 3.55–3.59 (2 H, m, 2 × CHOCH<sub>3</sub>), 3.67 (3 H, s, OCH<sub>3</sub>); δ<sub>C</sub>(50 MHz) 22.54 (CH<sub>2</sub>), 28.80 (CH<sub>2</sub>), 34.54 (CH<sub>2</sub>), 52.20 (OCH<sub>3</sub>), 56.32 (CHOCH<sub>3</sub>), 56.50 (CHOCH<sub>3</sub>), 57.33 (C), 75.86 (CHOCH<sub>3</sub>), 76.90 (CHOCH<sub>3</sub>), 177.64 (C=O); *m/z* (CI) 218 (M<sup>+</sup> + 1, 100%), 158 (5, M<sup>+</sup> - CO<sub>2</sub>Me), 126 (11).

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