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Stereoselective synthesis of β-hydroxy-α-amino acids β-substituted with non-aromatic heterocycles

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This paper is dedicated to Professor Donato Pocar on the occasion of his 70th birthday

Abstract—We have stereoselectively synthesised β-hydroxy-α-amino acids β-substituted with non-aromatic heterocycles by means of a condensation reaction between enantiomerically pure heterocyclic aldehydes and the (*R*)-(+)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine (Schöllkopf's reagent) as a chiral auxiliary. The stereocontrolled addition gave mixtures of diastereoisomers whose steric configurations were assigned on the basis of spectroscopic data and X-ray analysis. Upon controlled hydrolysis, the adducts were transformed into the corresponding methyl esters of β-hydroxy-β-heterocyclic substituted α-amino acids.

1. Introduction

β-Hydroxy-α-amino acids are an important class of amino acids because they occur naturally (serine, threonine, 3-hydroxyproline) and as components of complex natural products with wide-ranging biological properties,[1] for example, β-hydroxy-tyrosine and β-hydroxy-phenylalanine are found in clinically important glycopeptide antibiotics, anticancer and immunosuppressants. The importance of β-hydroxy-α-amino acids is also related to their usefulness as chiral building blocks in organic synthesis.[2]

The stereoselective synthesis of β-hydroxy-α-amino acids has therefore been extensively studied.[3] Amongst the many methods involving aldol condensation with chiral enolates,[4] the method enabled by 'Schöllkopf's reagent' [i.e., (*R*)- or (*S*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine] is particularly attractive since both enantiopure (*R*)- and (*S*)-forms are commercially available and have been used in various asymmetric syntheses.[5] We have previously used this chiral reagent to synthesise β-heterocyclic substituted serines,[6a] alanines,[6b] the antibiotic Azatyrosine[6c] and, more recently, β-hydroxy-γ, δ-unsaturated α-amino acids δ-heteroaryl substituted.[6d]

Continuing our studies of the stereoselective synthesis of new non-proteinogenic β-hydroxy-α-amino acids substituted with heterocyclic rings, we herein report the results of the reactions between Schöllkopf's reagent (*R*)-**1** and non-aromatic heterocyclic aldehydes **2**. In particular, we used enantiomerically pure chiral aldehydes with a stereocentre at the α-position in order to explore their possible effects on the stereochemical course of the reaction. To the best of our knowledge, there are only a few published examples of reactions of chiral aldehydes using the original[7a–d] or a modified version of Schöllkopf's reagent,[7e,f] and the only heterocycle was a 1,3-dioxolane system used to protect a 1,2-diol. These aldehydes always have a hydrogen at the α-position, but no racemisation of this position has ever been observed although the reactions were performed in a basic medium.

2. Results and discussion

Bearing in mind the heterocycles used in our previous studies, and the synthetic availability of non-aromatic

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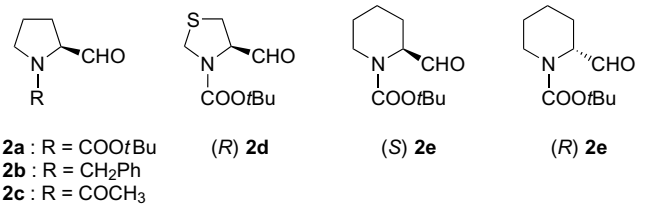
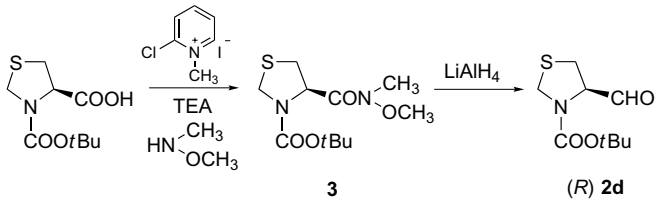


Figure 1.



Scheme 1.

heterocyclic chiral aldehydes, we chose the 2-pyrrolidinyl-, 4-(1,3-thiazolidinyl)- and 2-piperidinyl-aldehydes **2a**–**e** (Fig. 1).

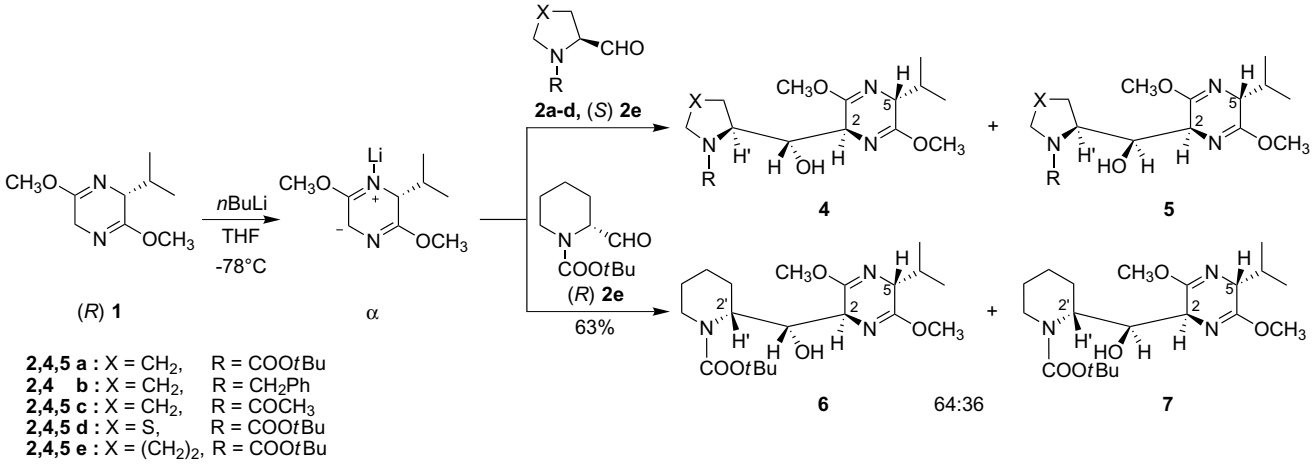
Aldehydes **2a**–**c** and **2d**, respectively, derive from the amino acids L-proline and (*R*)-1,3-thiazolidin-4-carboxylic acid, and retain the same absolute configurations, whereas aldehydes (*R*)-**2e** and (*S*)-**2e** were obtained from racemic pipecolinic acid previously resolved into the two enantiomers. In this way, we were able to test the effect of the two different enantiomers of aldehyde **2e** over the course of the reaction. The heterocyclic nitrogen needed to be protected, and so we decided to introduce some different groups on the pyrrolidinyl-substituted aldehyde, such as the *t*-butyloxycarbonyl-**2a**, benzyl-**2b** and acetyl-**2c**, in order to evaluate their different effects on the stability and reactivity of the aldehyde. To this end, we synthesised aldehydes (*S*)-**2a**,[8] (*S*)-**2b**[9] and (*S*)-**2c**[10] by means of Swern's oxidation of the corresponding (*S*)-*N*-Boc-prolinol, (*S*)-*N*-benzyl-prolinol and (*S*)-*N*-acetyl-prolinol.[11]

The preliminary condensation reactions on these aldehydes showed that the best protective group was *t*-butyloxycarbonyl- because, despite its greater steric hindrance, it enhanced the aldehyde stability and thus increased the condensation yield. Therefore we synthesised the *N*-*t*-butyloxycarbonyl-protected aldehyde (*R*)-**2d**[12] starting from the (*R*)-1,3-thiazolidin-3,4-dicarboxylic acid, 3-*tert*-butyl ester,[13] which was transformed into the Weinreb-amide **3**, and subsequently reduced to the aldehyde (*R*)-**2d** with $LiAlH_4$ (Scheme 1).

Aldehydes (*S*)-**2e** and (*R*)-**2e** were synthesised starting from the racemic pipecolinic acid, which was resolved using (*R*,*R*)- and (*S*,*S*)-tartaric acids.[14] The two enantiomers were subsequently protected as *N*-Boc derivatives,[15] reduced to alcohols using the borane–dimethylsulfide complex, and finally oxidised to the aldehydes (*S*)-**2e** and (*R*)-**2e** by means of Swern's method.[16]

In accordance with a general procedure, the α-anion of bislactim ether (*R*)-**1** was generated by *n*BuLi in THF at $T = -78$ °C to which a solution of the aldehydes **2a**–**e** was added. TLC analysis and the ^{1}H NMR spectrum of the crude reaction mixtures made it possible to establish the presence of only two of the four possible diastereoisomers, whose ratio was determined by the integration of the pairs of doublets corresponding to the isopropyl groups present in the ^{1}H NMR spectra (Scheme 2 and Table 1).

To evaluate the influence of the counter-ion on the diastereoselectivity, we performed parallel experiments in which the α-azaenolate was treated with diisopropoxytitanium(IV) dichloride[17] to give the corresponding titanium salt before the addition of aldehydes **2a**–**d** and (*S*)-**2e**: only aldehyde **2a** led to a slightly increased diastereoselectivity due to a 'tight titanium transition state'[7d] at the expense of the yield (Table 1, entries 1 and 2). In the other cases, no reaction occurred in the presence of titanium ions (Table 1, entries 4, 6 and 8), or it occurred with a decreased yield and, surprisingly, also less stereoselectivity (Table 1, entry 10). These findings differ from our previous results obtained using heteroaromatic aldehydes[6a] and β-heteroaryl-α,β-unsaturated aldehydes,[6d] probably because of



Scheme 2.

interference between the *N*-Boc-carbonyl group and the titanium salt.

Some other counter-ions (Sn, Mg or Zn) were also tested to evaluate their effect on the diastereoselectivity of the reactions with piperidinyl-aldehyde (*S*)-**2e**. The anion of (*R*)-**1** was treated with $SnCl_2$, $MgBr_2$ or $ZnCl_2$ to give the corresponding transmetalated azaenolates before the addition of the aldehyde (*S*)-**2e**. The reaction of this latter with the Sn(II) azaenolate afforded a mixture of **4e**/**5e** in almost the same yield and ratio as that obtained using the lithium salt (Table 1, entries 9 and 11). On the contrary the reactions with Mg(II) and Zn(II) azaenolates led to yields that were too low or completely failed (Table 1, entries 12 and 13).

Table 1.

Entry	**2**	Counter ion	Total yield (%)	Ratio **4**:**5**
1	**a**	Li	66	75:25
2	**a**	Ti	31	84:16
3	**b**	Li	20	100:0
4	**b**	Ti	//	//
5	**c**	Li	62	56:44
6	**c**	Ti	//	//
7	**d**	Li	56	61:39
8	**d**	Ti	//	//
9	(*S*)-**e**	Li	75	64:36
10	(*S*)-**e**	Ti	27	51:49
11	(*S*)-**e**	Sn	71	67:43
12	(*S*)-**e**	Mg	10	100:0
13	(*S*)-**e**	Zn	//	//

Except in the case of **2b**, two diastereoisomers were obtained with good chemical yields; with **2b** only one adduct was detected but at low yield, probably due to the aldehyde instability. The mixtures of diastereoisomers were separated by flash chromatography on silica gel, and their structures confirmed on the basis of analytical and spectroscopic data. Only the major adduct in the reaction of **2a** (**4a**), was obtained as a crystalline solid and underwent X-ray analysis (Fig. 2).

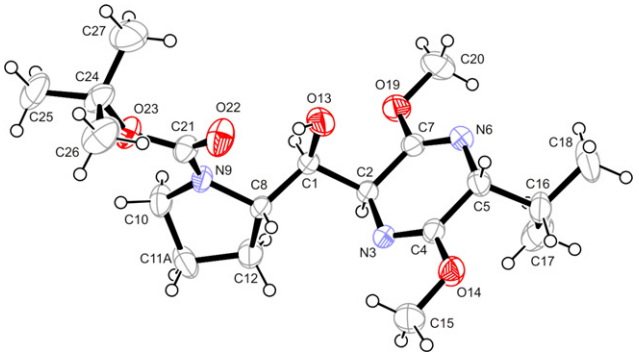


Figure 2. ORTEP plot of **4a** with atom numbering scheme. Displacement ellipsoids at 40% probability level.

In this way, the (*R*)- and (*S*)-configurations could be, respectively, assigned to the CH–OH and pyrazine-2-C of the more abundant diastereoisomer **4a**. Thereby the (*S*)- and (*S*)-configurations were assigned to the same carbon atoms of the less abundant diastereoisomer **5a**, by taking into account the generally high selectivity shown by the Schöllkopf's reagent in a large number of examples of aldol type reactions (see the discussion below).

In the adducts obtained from aldehydes **2b–d** and (*S*)-**2e**, complete correspondence was observed in the ^1H and ^{13}C NMR spectra of the major and the minor diastereoisomers in comparison with the adducts obtained from **2a**, for example, in relation to the chemical shifts of the C*H*–OH and 2′-H (or 4′-H) protons (see Section 4); consequently, the same configurations were assigned to the other diastereoisomers **4b–e** and **5c–e**. Conversely, in the case of diastereoisomers **6** and **7** derived from aldehyde (*R*)-**2e**, analysis of the ^1H NMR spectra did not allow an assignation of the absolute configurations. To this end, it was more useful to use the ^1H NMR spectra of the amino esters **8–12**, which were, respectively, obtained by means of acidic hydrolysis from the adducts **4a**, **4e**, **5e**, **6** and **7** (see below). The multiplicity of the ^1H NMR spectra of compounds **9** and **11** and **10** and **12** were similar, and almost the same C*H*–OH-2′-H coupling constants were observed in compounds **9** and **12** ($J = 9.9$ and 8.5 Hz) and **10** and **11** ($J = 10.7$ and 10.7 Hz), thus suggesting the same steric relationship. In this way the (*R*)- and (*S*)-configurations could be, respectively, assigned to the *C*H–OH and pyrazine-2-*C* of the major diastereoisomer **6**, and the (*S*)- and (*S*)-configurations to the minor compound **7**.

The observed results allow us to make some comments: the reactions of (*R*)-Schöllkopf's reagent with chiral aldehydes **2a–e** afforded pairs of diastereoisomers that are only different in terms of the configuration of the alcoholic carbon atom. The complete diastereofacial selectivity with regard to the pyrazine ring is in line with our previous results[6] and the widely accepted model for the aldol-type addition of **1** to aldehydes (Schöllkopf's mechanism),[7d] according to which the exclusive formation of the (2*S*)-epimers can be explained as a transition state in which the aldehyde attacks the azaenolate–pyrazine from the less hindered side opposite the isopropyl group. Moreover, the predominance of the (*R*)-epimer of the alcoholic carbon atom comes from an energetically more favoured transition state in which the aldehyde substituent R is far from the methoxy group and the metal atom (Fig. 3).

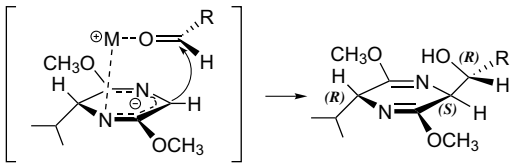


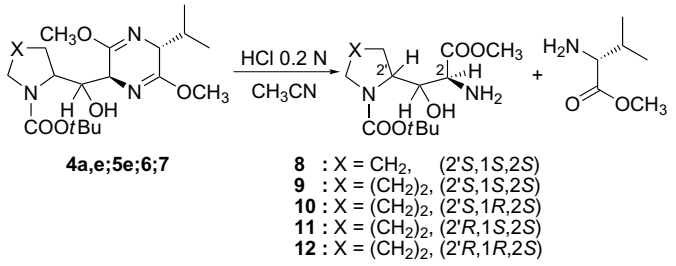
Figure 3. Favoured transition state leading to the major diastereoisomeric adducts.

As chiral aldehydes have diastereotopic carbonyl faces, the reactions of Schöllkopf's reagent with aldehydes **2a–e** raise the problem of 'double asymmetric induction'. In our case, the stereodifferentiation due to the chiral aldehyde (substrate control) clearly does not have a greater effect than

Schöllkopf's pyrazine (reagent control) as both (*R*)-**2e** and (*S*)-**2e** lead to similar stereochemical results with a *threo*:*erytro* ratio of about 1.8:1 (Table 1, entry 9 and Scheme 2), which indicates a very low level of selectivity that cannot be rationalised by taking into account the Felkin–Anh model for nucleophilic attack on an α-chiral aldehyde (or ketone).[18] In fact, the diasteroisomeric ratio obtained from aldehyde (*R*)-**2e** should actually be greater than that obtained from aldehyde (*S*)-**2e** because, in that case, both the major and minor diastereoisomers would, respectively, derive from a totally matched-pair and a totally mismatched-pair transition states, whereas the major and minor diastereoisomers of aldehyde (*S*)-**2e** both derive from 'half-matched' transition states.[7d] On the other hand, it is well-known that the lithium salts of the α-azaenolates are generally not very selective.[7a,c,d]

It is therefore very difficult to rationalise the observed stereochemical results fully, the only clear thing being the pursuit of Schöllkopf's model.

Finally, adducts **4a**, **4e**, **5e**, **6** and **7** were hydrolysed under controlled conditions: they were treated with 2 equiv of 0.2 M HCl in acetonitrile at temperatures ranging from 0 °C to rt for 24 h. In this way, the β-hydroxy-α-amino esters **8**–**12** were obtained in moderate to good yields, in addition to methyl (*R*)-valinate (Scheme 3).



Scheme 3.

The amino esters **8**–**12** were purified by means of column chromatography, and their structure was defined using analytical and spectroscopic data.

Under these experimental conditions, the *N*-Boc protection and ester functionality were retained, but the reactions also afforded mixtures of two partially hydrolysed dipeptides that reduced the yields. We therefore tried a new hydrolysis protocol using a Dowex 50 × 8 resin as an acid catalyst in a tetrahydrofuran solution of adduct **4a**. This led to complete pyrazine hydrolysis, but the simultaneous hydrolysis of *N*-Boc and the ester groups made it more difficult to separate the resulting amino acid from the (*R*)-valine.

3. Conclusion

In conclusion, we have reported another example of the synthesis of new non-proteinogenic β-hydroxy-α-amino acids β-substituted, in this case, with non-aromatic heterocyclic rings. With regard to this aspect, it has recently been found that a natural β-hydroxy-α-amino acid β-2-pyrrolidinyl substituted (Kaitocephalin) is a potent antagonist of AMPA/KA receptors.[19]

4. Experimental

4.1. General methods

Melting points were measured using a *Büchi* apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ (unless otherwise specified) on a *Bruker AC 300* spectrometer; chemical shifts (δ) are given in ppm relative to TMS and all of the coupling constants are in Hertz. Optical rotations were measured at 25 °C on a *JASCO P-1030* spectropolarimeter. The MS spectra were determined using a *VG Analytical 7070 EQ* mass spectrometer with an attached VG analytical 11/250 data system. The IR spectra were determined using a Perkin–Elmer 1725X FT-IR spectrometer, in cm^{-1}.

(*R*)-2,5-Dihydro-3,6-dimethoxy-2-isopropylpyrazine **1**, (*S*)-*N*-Boc-prolinol, (*S*)-*N*-benzyl-prolinol, L-proline, (*R*)-1,3-thiazolidin-4-carboxylic acid and pipecolinic acid were obtained from commercial sources. (*S*)-*N*-Acetyl-prolinol,[11] (*R*)-1,3-thiazolidin-3,4-dicarboxylic acid, 3-*tert*-butyl ester,[13] (*R*)-**2e** and (*S*)-**2e**[14–16] were prepared according to the reported methods.

4.2. General procedure for Swern's oxidation of (*S*)-N-protected-prolinols: synthesis of aldehydes 2a–c

Oxalyl chloride (0.66 mL, 7.55 mmol) in anhydrous CH_2Cl_2 (5 mL) was added to a solution of DMSO (1.12 mL, 15 mmol) in anhydrous CH_2Cl_2 (10 mL) cooled at −78 °C, and the mixture stirred for 1 h. A solution of (*S*)-*N*-protected-prolinol (5 mmol) in anhydrous CH_2Cl_2 (8 mL) was added and the mixture stirred at −78 °C for 3 h. The reaction mixture was treated with TEA (2.8 mL, 20 mmol) and the solution stirred at 0 °C for 3 h. After dilution with water (10 mL), the organic phase was separated, dried over Na_2SO_4 and concentrated under reduced pressure.

4.3. (*S*)-2-Formyl-pyrrolidine-1-carboxylic acid *tert*-butyl ester 2a

This compound, purified by column chromatography on silica gel (hexane/ethyl acetate = 8/2), has the same analytical and spectroscopic data reported in the literature.[20]

4.4. (*S*)-1-Benzyl-pyrrolidine-2-carbaldehyde 2b

This compound, used without purification, has the same spectroscopic data as reported in the literature.[9]

4.5. (*S*)-1-Acetyl-pyrrolidine-2-carbaldehyde 2c

This compound, purified by distillation (120–123 °C, 0.5 mm Hg), shows an ^{1}H NMR spectrum in agreement with that reported in the literature for the racemic analogues.[21] $[\alpha]_D^{20} = -37.5$ (*c* 1.07, $CHCl_3$). IR (nujol): 1733

($\nu_{C=O}$, HC=O), 1621 ($\nu_{C=O}$, N–C=O). MS-EI (*m*/*z*): 142 (M^+), 100.

4.6. (*R*)-4-[(*N*-Methoxy-*N*-methylamino)carbonyl]-3-thiazolidinecarboxylic acid, 1,1-dimethylethyl ester 3

TEA (1.65 mL, 11.8 mmol) and 2-chloro-1-methylpyridinium iodide (*Mukaiyama*'s reagent) (3.0 g, 11.8 mmol) were added to a solution of (*R*)-1,3-thiazolidin-3,4-dicarboxylic acid, 3-*tert*-butyl ester (2.5 g, 10.7 mmol) in anhydrous CH_2Cl_2 (30 mL). The reaction mixture was stirred at reflux temperature for 1 h. TEA (3.3 mL, 23.6 mmol) and *N*,*O*-dimethylhydroxylamine hydrochloride (1.15 g, 11.8 mmol) were added and the reaction mixture stirred at reflux temperature for 1 h. The mixture was cooled to room temperature and washed with 5% aqueous $NaHCO_3$ and saturated NaCl. The organic layer was dried over Na_2SO_4, concentrated and chromatographed (silica gel, toluene/ethyl acetate = 1/1) to afford a thick colourless oil (2 g, 68%). $[\alpha]_D^{20} = -117$ (*c* 0.91, CH_3OH). ^1H NMR shows the presence of rotamers: δ 1.49 (9H, s, $(CH_3)C$); 3.15 (1H, m, 5-H); 3.25 (3H, s, *N*–CH_3); 3.45 (1H, m, 5-H); 3.75 (3H, br s, O–CH_3); 4.60–4.80 (2H, m, 2-H); 4.85–5.25 (1H, m, 4-H).

4.7. (*R*)-4-Formyl-1,3-thiazolidine-3-carboxylic acid, *tert*-butyl ester 2d

To a solution of compound **3** (1.84 g, 6.67 mmol) in anhydrous THF (20 mL) cooled to −20 °C, 1 M $LiAlH_4$ (20 mL, 20 mmol) was added dropwise over 40 min. The reaction mixture was stirred for 1 h at the same temperature, then CH_3OH (2 mL), 5% aqueous NH_4Cl (15 mL) and 5% aqueous HCl (15 mL) were added. The organic solvent was evaporated off and the product extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated. The aldehyde was purified by distillation (150–160 °C, 0.5 mm Hg). Oil (1.22 g, 84%). $[\alpha]_D^{20} = -130$ (*c* 0.81, CH_3OH). ^1H NMR: δ 1.50 (9H, s, $(CH_3)_3C$); 3.25 (2H, m, 5-H); 4.40–4.70 (3H, m, 2-H, 4-H), 9.55 (1H, s, HC=O). IR (nujol): 1737 ($\nu_{C=O}$, HC=O), 1698 ($\nu_{C=O}$, N–C=O).

4.8. General procedure for the reactions of (*R*)-1 with 2a–e

To a solution of **1** (0.5 mL, 2.79 mmol) in anhydrous THF (5 mL), cooled at −78 °C, butyl lithium (2.79 mmol, 1.74 mL of a 1.6 M solution in hexane) was added and the mixture stirred for 45 min. The appropriate aldehyde **2** (2.54 mmol) in THF (5 mL) was added and the mixture stirred at −78 °C for 6 h and then at −18 °C for 12 h. The reaction mixture was allowed to warm to 0 °C and the phosphate buffer solution (20 mL) was added. The solvent was evaporated off and the residue taken up with CH_2Cl_2. The organic phase was separated and dried over Na_2SO_4. The solvent was evaporated in vacuo and the residue flash chromatographed on silica gel (hexane/ethyl acetate = 80/20 for **4/5a**, **4b** and **4/5d**; ethyl acetate/methanol = 98/2 for **4/5c**; hexane/acetone = 90/10 for **4e/5e** and **6/7**). In this way the following compounds were isolated.

4.9. (*S*)-2-[(*R*)-Hydroxy-((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazin-2-yl)-methyl]-pyrrolidine-1-carboxylic acid *tert*-butyl ester 4a

Colourless solid (50%); mp 133–135 °C (*n*-hexane); $[\alpha]_D^{20} = +13.8$ (*c* 0.98, Et_2O). ^1H NMR: δ 0.70, 1.07 (6H, 2d, $J = 6.8$, $CH(CH_3)_2$); 1.46 (9H, s, $(CH_3)_3C$); 1.75–2.15 (4H, m, 3′-H, 4′-H); 2.28 (1H, dsp, $J = 6.8$, 3.3, $CH(CH_3)_2$); 3.30 (1H, m, 5′-H); 3.55 (1H, m, 5′-H); 3.74 (6H, s, 3- and 6-OCH_3); 3.90 (1H, m, C*H*–OH); 4.00 (1H, dd, $J = 3.4$, 1.9, 5-H); 4.05 (1H, t, $J = 3.4$, 2-H); 4.41 (1H, dt, $J = 9.7$, 3.8, 2′-H); 5.00 (1H, br, OH); (by deuteration the signal at 5.00 disappeared and the signal at 3.90 turned into a doublet with $J = 9.7$). ^{13}C NMR: δ 16.49, 19.10 ($CH(CH_3)_2$); 24.06 (4′-C); 28.04 ($(CH_3)_3C$); 28.08 (3′-C); 31.10 ($CH(CH_3)_2$); 47.05 (5′-C); 52.28, 52.42 (3- and 6-OCH_3); 58.15 (2′-C); 59.05, 60.29 (2-C and 5-C); 76.58 (CHOH); 80.16 ($(CH_3)_3C$); 157.86, 161.31, 165.34 (C=O, 3-C and 6-C). MS-EI (*m*/*z*): 383 (M^+), 366, 283, 183. Anal. Calcd for $C_{19}H_{33}N_3O_5$: C, 59.51; H, 8.67; N, 10.96. Found: C, 59.59; H 8.72; N, 10.96. IR (nujol): 1655 ($\nu_{C=O}$, C=O), 1704 ($\nu_{C=N}$, C=N), 3300 (ν_{OH}, OH). Single crystals suitable for X-ray structure determination were obtained by precipitation from hexane/ethyl acetate = 80/20.

4.10. (*S*)-2-[(*S*)-Hydroxy-((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazin-2-yl)-methyl]-pyrrolidine-1-carboxylic acid *tert*-butyl ester 5a

Oil (16%); $[\alpha]_D^{20} = -6.2$ (*c* 0.84, Et_2O). ^1H NMR: δ 0.71, 1.05 (6H, 2d, $J = 6.8$, $CH(CH_3)_2$); 1.46 (9H, s, $(CH_3)_3C$); 1.70–2.15 (4H, m, 3′-H, 4′-H); 2.28 (1H, dsp, $J = 6.8$, 3.3, $CH(CH_3)_2$); 3.25 (1H, m, 5′-H); 3.30 (1H, br, OH); 3.50 (1H, m, 5′-H); 3.75 (6H, s, 3- and 6-OCH_3); 4.00–4.25 (4H, m, C*H*–OH, 2-H, 5-H, 2′-H). ^{13}C NMR: δ 16.51, 18.92 ($CH(CH_3)_2$); 23.93 (4′-C); 25.60 (3′-C); 28.60 ($(CH_3)_3C$); 31.23 ($CH(CH_3)_2$); 46.71 (5′-C); 52.29, 52.48 (3- and 6-OCH_3); 58.19 (2′-C); 58.43, 60.83 (2-C, 5-C); 72.25 (CHOH); 78.88 ($(CH_3)_3C$); 154.54, 161.96, 164.66 (C=O, 3-C, 6-C). MS-EI (*m*/*z*): 383 (M^+), 366, 283, 183. Anal. Calcd for $C_{19}H_{33}N_3O_5$: C, 59.51; H, 8.67; N, 10.96. Found: C, 59.28; H 8.44; N, 10.75. IR (nujol): 1695 ($\nu_{C=O}$, C=O), 1703 ($\nu_{C=N}$, C=N), 3408 (ν_{OH}, OH).

4.11. (*R*)-((*S*)-1-Benzyl-pyrrolidin-2-yl)-((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazin-2-yl)-methanol 4b

Oil (20%); $[\alpha]_D^{20} = +8.0$ (*c* 0.65, $CHCl_3$). ^1H NMR: δ 0.72, 1.08 (6H, 2d, $J = 6.8$, $CH(CH_3)_2$); 1.70–2.05 (5H, m, 3′-H, 4′-H, OH); 2.30 (1H, dsp, $J = 6.8$, 3.3, $CH(CH_3)_2$); 2.50 (1H, m, 5′-H); 3.00 (1H, m, 5′-H); 3.48 (1H, br t, $J = 7.0$, 2′-H); 3.61, 3.77 (6H, 2s, 3- and 6-OCH_3); 3.65 (1H, d, $J = 12.9$, *C*HPh); 3.76 (1H, m, C*H*–OH); 3.98 (1H, dd, $J = 3.5$, 2.0, 5-H); 4.05 (1H, t, $J = 3.5$, 2-H); 4.15 (1H, d, $J = 12.9$, *C*HPh); 7.35 (5H, m, Ph). ^{13}C NMR: δ 16.53, 18.99 ($CH(CH_3)_2$); 23.86 (4′-C); 28.42 (3′-C); 31.32 ($CH(CH_3)_2$); 52.18, 52.39 (3- and 6-OCH_3); 53.24 (5′-C); 57.26, 60.47 (2-C, 5-C); 60.85 (CH_2Ph); 64.79 (2′-C); 73.73 (CHOH); 126.85–138-85 (*C*-Ph); 161.87, 165.28 (3-C, 6-C). MS-EI (*m*/*z*): 373 (M^+), 356. Anal. Calcd for $C_{21}H_{31}N_3O_3$: C, 67.56; H, 8.31; N, 11.26.

Found: C, 67.25; H 8.22; N, 11.13. IR (nujol): 1703 ($\nu_{C=N}$, C=N), 3382 (ν_{OH}, OH).

4.12. 1-{(*S*)-2-[(*R*)-Hydroxy-((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazin-2-yl)-methyl]-pyrrolidin-1-yl}-ethanone 4c

Oil (35%); $[\alpha]_D^{20} = +6.9$ (*c* 0.84, $CHCl_3$). ^1H NMR: δ 0.70, 1.06 (6H, 2d, $J = 6.8$, $CH(CH_3)_2$); 1.83–2.15 (4H, m, 3′-H, 4′-H); 2.10 (3H, s, CH_3CO); 2.28 (1H, dsp, $J = 6.8$, 3.3, $CH(CH_3)_2$); 3.50 (2H, m, 5′-H); 3.73, 3.75 (6H, 2s, 3- and 6-OCH_3); 3.85 (1H, m, C*H*–OH); 3.95 (1H, dd, $J = 3.6$, 1.9, 5-H); 4.05 (1H, t, $J = 3.6$, 2-H); 4.75 (1H, dt, $J = 9.9$, 3.5, 2′-H); 4.90 (1H, br, OH); (by deuteration the signal at 4.90 disappeared and the signal at 3.85 turned into a doublet with $J = 9.9$). ^{13}C NMR: δ 16.5, 18.98 ($CH(CH_3)_2$); 22.46 (CH_3CO); 24.21 (4′-C); 27.91 (3′-C); 31.25 ($CH(CH_3)_2$); 48.47 (5′-C); 52.28, 52.50 (3- and 6-OCH_3); 58.06 (2′-C); 59.32, 60.97 (2-C, 5-C); 76.17 (CHOH); 161.17, 165.49, 172.93 (3-C, 6-C, C=O). MS-EI (*m*/*z*): 325 (M^+), 308, 185. Anal. Calcd for $C_{16}H_{27}N_3O_4$: C, 59.08; H, 8.31; N, 12.92. Found: C, 59.01; H 8.12; N, 12.90. IR (nujol): 1620 ($\nu_{C=O}$, C=O), 1701 ($\nu_{C=N}$, C=N), 3350 (ν_{OH}, OH).

4.13. 1-{(*S*)-2-[(*S*)-Hydroxy-((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazin-2-yl)-methyl]-pyrrolidin-1-yl}-ethanone 5c

Oil (27%); $[\alpha]_D^{20} = +0.3$ (*c* 1.01 $CHCl_3$). ^1H NMR: δ 0.72, 1.05 (6H, 2d, $J = 6.8$, $CH(CH_3)_2$); 1.75–2.15 (5H, m, 3′-H, 4′-H, OH); 2.10 (3H, s, CH_3CO); 2.24 (1H, dsp, $J = 6.8$, 3.3, $CH(CH_3)_2$); 3.5 (2H, m, 5′-H); 3.73, 3.76 (6H, 2s, 3- and 6-OCH_3); 4.00–4.45 (4H, m, C*H*–OH, 2-H, 5-H, 2′-H). ^{13}C NMR: δ 16.64, 18.99 ($CH(CH_3)_2$); 22.95 (CH_3CO); 24.65 (4′-C); 25.18 (3′-C); 31.35 ($CH(CH_3)_2$); 48.40 (5′-C); 52.40, 52.52 (3- and 6-OCH_3); 58.35 (2′-C); 58.62, 60.91 (2-C, 5-C); 72.10 (CHOH); 161.81, 164.65, 169.45 (3-C, 6-C, C=O). MS-EI (*m*/*z*): 325 (M^+), 308, 185. Anal. Calcd for $C_{16}H_{27}N_3O_4$: C, 59.08; H, 8.31; N, 12.92. Found: C, 58.86; H 8.05; N, 12.71. IR (nujol): 1690 ($\nu_{C=O}$, C=O), 1704 ($\nu_{C=N}$, C=N), 3350 (ν_{OH}, OH).

4.14. (*R*)-4-[(*S*)-Hydroxy-((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazin-2-yl)-methyl]-thiazolidine-3-carboxylic acid *tert*-butyl ester 4d

In the case of aldehyde **2d**, the two diastereoisomers were only partially purified by flash chromatography so that only their ^1H NMR spectra were recorded. Oil (34%); ^1H NMR show the presence of rotamers about the carbamate bond: δ 0.74, 1.09 (6H, 2d, $J = 6.8$, $CH(CH_3)_2$); 1.50 (9H, s, $(CH_3)_3C$); 1.60 (1H, m, OH); 2.28 (1H, dsp, $J = 6.8$, 3.3, $CH(CH_3)_2$); 2.95 (1H, dd, $J = 10.7$, 3.1, 5′-H); 3.35 (1H, dd, $J = 10.7$, 6.1, 5′-H); 3.75 (6H, s, 3- and 6-OCH_3); 4.05 (1H, dd, $J = 3.5$, 1.7, 5-H); 4.10 (1H, t, $J = 3.5$, 2-H); 4.15 (1H, m, *C*HOH); 4.32 (1H, d, $J = 9.4$, 2′-H); 4.80 (1H, d, $J = 9.4$, 2′-H); 4.90 (1H, m, 4′-H); (by deuteration the signal at 1.60 disappeared and the signal at 4.15 turned into a doublet with $J = 7.9$).

4.15. (*R*)-4-[(*R*)-Hydroxy-((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazin-2-yl)-methyl]-thiazolidine-3-carboxylic acid *tert*-butyl ester 5d

Oil (22%); ^1H NMR show the presence of rotamers about the carbamate bond: δ 0.71, 1.05 (6H, 2d, $J = 6.8$, $CH(CH_3)_2$); 1.47 (9H, s, $(CH_3)_3C$); 2.35 (1H, dsp, $J = 6.8$, 3.3, $CH(CH_3)_2$); 2.95 (1H, dd, $J = 10.9$, 6.4, 5′-H); 3.35 (1H, br d, $J = 10.9$, 5′-H); 3.55 (1H, br, OH); 3.77 (6H, s, 3- and 6-OCH_3); 4.10–4.25 (4H, m, 2-H, 5-H, *C*HOH, 2′-H); 4.60 (1H, t, $J = 6.9$, 2′-H); 4.85 (1H, m, 4′-H).

4.16. (*S*)-2-[(*R*)-Hydroxy-((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazin-2-yl)-methyl]-piperidine-1-carboxylic acid *tert*-butyl ester 4e

Oil (48%); $[\alpha]_D^{20} = +23.3$ (*c* 1.72, CH_2Cl_2). ^1H NMR: δ 0.74, 1.08 (6H, 2d, $J = 6.8$, $CH(CH_3)_2$); 1.47 (9H, s, $(CH_3)_3C$); 1.50–1.75 (5H, m, 3′-H (1), 4′-H, 5′-H); 1.87 (1H, br d, $J = 13.6$, 3′-H); 2.30 (1H, dsp, $J = 6.8$, 2.8, $CH(CH_3)_2$); 2.55 (1H, br, OH); 2.95 (1H, m, 6′-H); 3.77 (6H, s, 3- and 6-OCH_3); 4.04 (3H, m, 2-H, 5-H, 6′-H); 4.43 (1H, m, C*H*–OH); 4.73 (1H, t, $J = 9.7$, 2′-H); (by deuteration the signal at 2.55 disappeared and the signal at 4.43 turned into a doublet with $J = 9.7$). ^{13}C NMR: δ 16.70, 19.02 ($CH(CH_3)_2$); 19.51, 25.28, 25.42 (3′-C, 4′-C, 5′-C); 28.45 ($(CH_3)_3C$); 31.73 ($CH(CH_3)_2$); 40.20 (6′-C); 51.84 (2′-C); 52.53, 52.90 (3- and 6-OCH_3); 57.13, 60.63 (2-C, 5-C); 70.35 (CHOH); 79.64 ($(CH_3)_3C$); 154.85, 161.56, 165.83 (C=O, 3-C, 6-C). MS-EI (*m*/*z*): 397 (M^+), 298. Anal. Calcd for $C_{20}H_{35}N_3O_5$: C, 60.45; H, 8.82; N, 10.58. Found: C, 60.21; H 8.74; N, 10.33. IR (nujol): 1699 ($\nu_{C=O}$, C=O), 1745 ($\nu_{C=N}$, C=N), 3443 (ν_{OH}, OH).

4.17. (*S*)-2-[(*S*)-Hydroxy-((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazin-2-yl)-methyl]-piperidine-1-carboxylic acid *tert*-butyl ester 5e

Oil (27%); $[\alpha]_D^{20} = -3.1$ (*c* 0.62, CH_2Cl_2). ^1H NMR: δ 0.69, 1.03 (6H, 2d, $J = 6.8$, $CH(CH_3)_2$); 1.44 (9H, s, $(CH_3)_3C$); 1.50–1.75 (5H, m, 3′-H (1), 4′-H, 5′-H); 2.14 (1H, d, $J = 13.3$, 3′-H); 2.27 (1H, dsp, $J = 6.8$, 3.3, $CH(CH_3)_2$); 2.92 (1H, dt, $J = 13.3$, 2.5, 6′-H); 3.15 (1H, br, OH); 3.68, 3.77 (6H, 2s, 3- and 6-OCH_3); 3.91 (1H, m, 6′-H); 4.09 (1H, t, $J = 3.8$, 5-H); 4.14 (1H, m, 2′-H); 4.25 (1H, t, $J = 3.8$, 2-H,); 4.44 (1H, dt, $J = 10.7$, 3.7, C*H*–OH), (by deuteration the signal at 3.15 disappeared and the signal at 4.44 turned into a double doublet with $J = 10.7$, 3.7). ^{13}C NMR: δ 16.22, 18.75 ($CH(CH_3)_2$); 19.46, 24.83, 25.50 (3′-C, 4′-C, 5′-C); 28.15 ($(CH_3)_3C$); 31.25 ($CH(CH_3)_2$); 40.52 (6′-C); 49.60 (2′-C); 51.59, 53.17 (3- and 6-OCH_3); 57.76, 60.80 (2-C, 5-C); 66.61 (CHOH); 78.78 ($(CH_3)_3C$); 154.28, 161.30, 166.55 (C=O, 3-C, 6-C). MS-EI (*m*/*z*): 397 (M^+), 298. Anal. Calcd for $C_{20}H_{35}N_3O_5$: C, 60.45; H, 8.82; N, 10.58. Found: C, 60.25; H 8.64; N, 10.37. IR (nujol): 1698 ($\nu_{C=O}$, C=O), 1735 ($\nu_{C=N}$, C=N), 3440 (ν_{OH}, OH).

4.18. (*R*)-2-[(*R*)-Hydroxy-((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazin-2-yl)-methyl]-piperidine-1-carboxylic acid *tert*-butyl ester 6

Oil (40%); $[\alpha]_D^{20} = -26.95$ (*c* 1.1, CH_2Cl_2). ^{1}H NMR: δ 0.79, 1.05 (6H, 2d, $J = 6.8$, $CH(CH_3)_2$); 1.40 (9H, s, $(CH_3)_3C$); 1.43–1.75 (5H, m, 3′-H (1), 4′-H, 5′-H); 1.90 (1H, br, OH); 2.10 (1H, br d, $J = 13.4$, 3′-H); 2.24 (1H, dsp, $J = 6.8$, 3.6, $CH(CH_3)_2$); 2.90 (1H, t, $J = 12.9$, 6′-H); 3.74 (6H, s, 3- and 6-OCH_3); 3.94 (1H, d, $J = 3.6$, 5-H); 4.05 (1H, t, $J = 3.6$, 2-H); 4.1 (1H, m, 6′-H); 4.47 (2H, m, CH–OH, 2′-H). ^{13}C NMR: δ 17.19, 18.86 ($CH(CH_3)_2$); 19.22, 24.77, 25.33 (3′-C, 4′-C, 5′-C); 28.31 ($(CH_3)_3C$); 32.42 ($CH(CH_3)_2$); 39.28 (6′-C); 52.08 (2′-C); 52.48, 52.73 (3- and 6-OCH_3); 55.41, 61.46 (2-C, 5-C); 67.24 (CHOH); 79.23 ($(CH_3)_3C$); 154.98, 162.60, 166.95 (C=O, 3-C, 6-C). MS-EI (*m*/*z*): 397 (M^+), 298. Anal. Calcd for $C_{20}H_{35}N_3O_5$: C, 60.45; H, 8.82; N, 10.58. Found: C, 60.33; H 8.72; N, 10.42. IR (nujol): 1695 ($\nu_{C=O}$, C=O), 1741 ($\nu_{C=N}$, C=N), 3458 (ν_{OH}, OH).

4.19. (*R*)-2-[(*S*)-Hydroxy-((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazin-2-yl)-methyl]-piperidine-1-carboxylic acid *tert*-butyl ester 7

Oil (23%); $[\alpha]_D^{20} = +14.3$ (*c* 1.04, CH_2Cl_2). ^{1}H NMR: δ 0.74, 1.09 (6H, 2d, $J = 6.8$, $CH(CH_3)_2$); 1.49 (9H, s, $(CH_3)_3C$); 1.50–1.75 (6H, m, 3′-H, 4′-H, 5′-H); 2.33 (1H, dsp, $J = 6.8$, 3.3, $CH(CH_3)_2$); 2.90 (1H, br, OH); 2.95 (1H, m, 6′-H); 3.76, 3.79 (6H, 2s, 3- and 6-OCH_3); 3.95–4.06 (2H, m, 2-H, 5-H); 4.31–4.50 (3H, m, 6′-H, 2′-H, CH–OH). ^{13}C NMR: δ 16.95, 19.41 ($CH(CH_3)_2$); 19.79, 25.63, 26.05 (3′-C, 4′-C, 5′-C); 28.83 ($(CH_3)_3C$); 32.07 ($CH(CH_3)_2$); 40.70 (6′-C); 52.60, 53.13 (3- and 6-OCH_3); 53.24 (2′-C); 59.50, 61.28 (2-C, 5-C); 71.15 (CHOH); 79.90 ($(CH_3)_3C$); 154.75, 161.78, 165.23 (C=O, 3-C, 6-C). MS-EI (*m*/*z*): 397 (M^+), 298. Anal. Calcd for $C_{20}H_{35}N_3O_5$: C, 60.45; H, 8.82; N, 10.58. Found: C, 60.27; H 8.48; N, 10.35. IR (nujol): 1694 ($\nu_{C=O}$, C=O), 1740 ($\nu_{C=N}$, C=N), 3449 (ν_{OH}, OH).

4.20. General procedure for the hydrolysis of adducts 4a, 4e, 5e, 6 and 7

Adducts **4a**, **4e**, **5e**, **6** and **7** (2.0 mmol) were dissolved in acetonitrile (20 ml) after which the solution was cooled to 0–5 °C. A 0.2 M solution of HCl (20 ml, 4.0 mmol) was added and the mixture stirred for 24 h at room temperature. The mixture was treated with 10% ammonia until pH 8–10 after which the acetonitrile was evaporated off at reduced pressure. The product was extracted with dichloromethane (2×20 ml). The organic phase was dried with Na_2SO_4 and the solvent removed in vacuo. The residue was purified by flash chromatography (SiO_2, ethyl acetate:methanol = 98:2, developer: ninhydrin).

4.21. (*S*)-2-[(*S*)-2-Amino-(*S*)-1-hydroxy-2-methoxycarbonyl-ethyl]-pyrrolidine-1-carboxylic acid *tert*-butyl ester 8

Amorphous solid (53%); $[\alpha]_D^{20} = -51.1$ (*c* 0.86, CH_2Cl_2). ^{1}H NMR: δ 1.45 (9H, s, $(CH_3)_3C$); 1.70–2.20 (7H, m, 3′-H, 4′-H, OH, NH_2); 3.28 (1H, m, 5′-H); 3.44 (1H, br s, 2-H); 3.53 (1H, m, 5′-H); 3.75 (3H, s, OCH_3); 3.94 (1H, br d, $J = 9.1$, CH–OH); 4.17 (1H, m, 2′-H). ^{13}C NMR: δ 25.99 (4′-C); 28.52 ($(CH_3)_3C$); 29.13 (3′-C); 47.77 (5′-C); 52.54 (OCH_3); 57.25 (2′-C); 60.57 (2-C); 77.11 (CHOH); 81.31 ($(CH_3)_3C$); 159.16, 176.06 (O–C=O, N–C=O). MS-EI (*m*/*z*): 288 (M^+), 188. Anal. Calcd for $C_{13}H_{24}N_2O_5$: C, 54.17; H, 8.33; N, 9.72. Found: C, 53.99; H 8.22; N, 9.65. IR (nujol): 1660 ($\nu_{C=O}$, N–C=O), 1742 ($\nu_{C=O}$, O–C=O), 3389 (ν_{OH}, OH).

4.22. (*S*)-2-[(*S*)-2-Amino-(*S*)-1-hydroxy-2-methoxycarbonyl-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester 9

Colourless solid (69%); mp 103–104 °C (*n*-hexane); $[\alpha]_D^{20} = -30.0$ (*c* 0.5, CH_2Cl_2). ^{1}H NMR: δ 1.48 (9H, s, $(CH_3)_3C$); 1.50–1.75 (6H, m, 3′-H, 4′-H, 5′-H); 2.30 (3H, br, OH, NH_2); 2.95 (1H, m, 6′-H); 3.52 (1H, br s, 2-H); 3.80 (3H, s, OCH_3); 4.05 (1H, m, 6′-H); 4.34 (1H, br d, $J = 9.9$, CH–OH); 4.42 (1H, m, 2′-H). ^{13}C NMR: δ 19.43, 24.94, 25.36 (3′-C, 4′-C, 5′-C); 28.40 ($(CH_3)_3C$); 40.39 (6′-C); 52.37 (OCH_3); 52.60 (2′-C); 55.70 (2-C); 70.96 (CHOH); 80.11 ($(CH_3)_3C$); 154.96, 174.15 (O–C=O, N–C=O). MS-EI (*m*/*z*): 302 (M^+), 202. Anal. Calcd for $C_{14}H_{26}N_2O_5$: C, 55.63; H, 8.61; N, 9.27. Found: C, 55.29; H 8.43; N, 9.01. IR (nujol): 1682 ($\nu_{C=O}$, N–C=O), 1743 ($\nu_{C=O}$, O–C=O), 3389 (ν_{OH}, OH).

4.23. (*S*)-2-[(*S*)-2-Amino-(*R*)-1-hydroxy-2-methoxycarbonyl-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester 10

Colourless solid (65%); mp 116–118 °C (*n*-hexane); $[\alpha]_D^{20} = -54.25$ (*c* 0.92, CH_2Cl_2). ^{1}H NMR: δ 1.51 (9H, s, $(CH_3)_3C$); 1.30–1.70 (5H, m, 3′-H (1), 4′-H, 5′-H); 2.16 (1H, d, $J = 13.1$, 3′-H); 2.33 (3H, br, OH, NH_2); 2.68 (1H, t, $J = 13.1$, 6′-H); 3.65 (1H, br d, $J = 3.0$, 2-H); 3.72 (3H, s, OCH_3); 3.90 (1H, m, 6′-H); 4.15 (1H, m, 2′-H). 4.36 (1H, dd, $J = 10.7$, 3.0, CH–OH). ^{13}C NMR: δ 19.45, 24.72, 25.42 (3′-C, 4′-C, 5′-C); 28.44 ($(CH_3)_3C$); 40.79 (6′-C); 50.72 (2′-C); 51.93 (OCH_3); 57.26 (2-C); 67.96 (CHOH); 80.04 ($(CH_3)_3C$); 154.67, 172.96 (O–C=O, N–C=O). MS-EI (*m*/*z*): 302 (M^+), 202. Anal. Calcd for $C_{14}H_{26}N_2O_5$: C, 55.63; H, 8.61; N, 9.27. Found: C, 55.48; H 8.52; N, 9.12. IR (nujol): 1681 ($\nu_{C=O}$, N–C=O), 1743 ($\nu_{C=O}$, O–C=O), 3374 (ν_{OH}, OH).

4.24. (*R*)-2-[(*S*)-2-Amino-(*S*)-1-hydroxy-2-methoxycarbonyl-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester 11

Colourless solid (53%); mp 99–100 °C (*n*-hexane); $[\alpha]_D^{20} = +67.95$ (*c* 0.63, CH_2Cl_2). ^{1}H NMR: δ 1.49 (9H, s, $(CH_3)_3C$); 1.50–1.75 (5H, m, 3′-H (1), 4′-H, 5′-H); 2.16 (1H, br d, $J = 13.1$, 3′-H); 2.30 (3H, br, OH, NH_2); 2.77 (1H, t, $J = 12.4$, 6′-H); 3.57 (1H, br s, 2-H); 3.80 (3H, s, OCH_3); 4.04 (2H, m, 2′-H, 6′-H); 4.19 (1H, br d, $J = 10.7$, CH–OH). ^{13}C NMR: δ 19.04, 24.20, 25.32 (3′-C, 4′-C, 5′-C); 28.41 ($(CH_3)_3C$); 40.88 (6′-C); 51.30 (2′-C); 52.41 (OCH_3); 53.79 (2-C); 67.46 (CHOH); 79.77 ($(CH_3)_3C$); 155.70, 173.86 (O–C=O, N–C=O). MS-EI (*m*/*z*): 302 (M^+), 202. Anal. Calcd for $C_{14}H_{26}N_2O_5$: C, 55.63; H, 8.61; N, 9.27. Found: C, 55.5; H 8.36; N, 9.2. IR (nujol): 1674 ($\nu_{C=O}$, N–C=O), 1744 ($\nu_{C=O}$, O–C=O), 3387 (ν_{OH}, OH).

4.25. (*R*)-2-[(*S*)-2-Amino-(*R*)-1-hydroxy-2-methoxycarbonyl-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester 12

Amorphous solid (58%); $[\alpha]_D^{20} = +21.3$ (c 0.5, CH_2Cl_2). ^1H NMR: δ 1.44 (9H, s, $(CH_3)_3C$); 1.50–1.70 (6H, m, 3′-H, 4′-H, 5′-H); 2.55 (3H, br, OH, NH_2); 2.90 (1H, m, 6′-H); 3.61 (1H, br d, $J = 3.3$, 2-H); 3.75 (3H, s, OCH_3); 3.95 (1H, m, 6′-H); 4.08 (1H, dd, $J = 8.5$, 3.3, C*H*–OH); 4.28 (1H, m, 2′-H). ^{13}C NMR: δ 19.49, 24.95, 25.98 (3′-C, 4′-C, 5′-C); 28.35 ($(CH_3)_3C$); 40.98 (6′-C); 52.08 (2′-C); 52.42 (OCH_3); 56.90 (2-C); 73.35 (CHOH); 80.43 ($(CH_3)_3C$); 155.34, 174.41 (O–C=O, N–C=O). MS-EI (m/z): 302 (M^+), 202. Anal. Calcd for $C_{14}H_{26}N_2O_5$: C, 55.63; H, 8.61; N, 9.27. Found: C, 55.46; H 8.37; N, 9.08. IR (nujol): 1682 ($\nu_{C=O}$, N–C=O), 1740 ($\nu_{C=O}$, O–C=O), 3375 (ν_{OH}, OH).

4.26. Single crystal X-ray structural determination of 4a

The intensity data for **4a** were collected on a Bruker Smart Apex CCD area detector using graphite-monochromated Mo-Kα radiation ($\lambda = 0.71073$ Å). Data reduction was made using SAINT programs; absorption corrections based on multiscan were obtained by SADABS.[22] The structures were solved by SIR-92[23] and refined on F^2 by full-matrix least-squares using SHELXL-97.[24] All the non-hydrogen atoms were refined anisotropically, hydrogen atoms were included as 'riding' and not refined. The ORTEP-III program was used for molecular diagrams.[25]

Crystal data and results of the refinement: colourless prism $0.42 \times 0.32 \times 0.22$ mm, $M_r = 383.48$, orthorhombic, space group $P2_12_12_1$, $a = 10.413(2)$ Å, $b = 13.111(3)$ Å, $c = 15.970(3)$ Å, $V = 2180.4(8)$ Å^3, $Z = 4$, $T = 293(2)$ K, $\mu = 0.084$ mm^{-1}. 50248 measured reflections, 3252 independent reflections, 3008 reflections with $I > 2\sigma(I)$, $4.01 < 2\theta < 58.00°$, $R_{int} = 0.0338$. Refinement on 3252 reflections, 253 parameters. Flack parameter[26] for determination of the absolute configuration $= -1.2(12)$. Final $R = 0.0488$, $wR = 0.1342$ for data with $F^2 > 2\sigma$ (F^2), $S = 1.092$, $(\Delta/\sigma)_{max} = 0.001$, $\Delta\rho_{max} = 0.237$, $\Delta\rho_{min} = -0.189$ eÅ^{-3}.

Crystallographic data (excluding structure factors) for **4a** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 648502. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk).

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