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Stereoselective synthesis of β-substituted-L-threonines from enantiopure 5-acetyl-2-isoxazolines

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ABSTRACT

Enantiomerically pure, 3-methyl- or 3-ethoxycarbonyl-substituted (5*S*)- and (5*R*)-5-acetyl-2-isoxazolines were obtained from the corresponding racemic mixtures by means of an enzymatic reduction with baker's yeast, followed by the separation of the enantiopure *syn*- and *anti*-alcohols and oxidation of the alcohol group. The reaction between these ketones and (2*R*)-*Schöllkopf*'s bislactim ether azaenolate was studied: using (5*S*)- and (5*R*)-3-methyl derivatives, two diastereoisomeric adducts were obtained in good yield and stereoselectivity, whereas reaction with the (5*S*)- and (5*R*)-3-ethoxycarbonyl derivatives led to a complex mixture of products. Subsequent controlled hydrolysis of the pyrazine ring led to β-(3-methyl-4,5-dihydro-isoxazol-5-yl)-L-threonines methyl ester together with the corresponding (*R*)-valine dipeptides.

1. Introduction

Optically active β-hydroxy-α-amino acids are naturally occurring compounds and also structural components of many biologically active natural products,[1] and so their analogues are very interesting as potential pharmacological tools. One of the most important means of obtaining the stereoselective synthesis of this unit[2] is to make use of asymmetric aldol reactions with chiral auxiliaries[3] or chiral catalysts.[4] Of the various classes of different chiral glycine equivalents,[5] *Schöllkopf*'s bislactim ether (i.e., (2*R*)- or (2*S*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine) is particularly attractive because it has proved to be highly diastereoselective in aldol-type reactions and is commercially available in (*R*)- and (*S*)-forms.[6]

In relation with the stereoselective synthesis of *β*-heterocyclic substituted serines by means of the reaction between *Schöllkopf*'s reagent and heterocyclic-carbaldehydes,[7] we have recently been considering 4,5-dihydro-isoxazole (2-isoxazoline) as a selected heterocycle[8] because it is easy to prepare, versatile as a synthetic intermediate of a wide range of complex natural products[9] and structurally relevant to medicinal chemistry.[10] Furthermore, as synthons, 4,5-dihydro-isoxazoles can be converted into a number of useful synthetic units, such as β-hydroxy ketones[11] or γ-amino alcohols,[12] depending on the experimental conditions used for reductive ring cleavage. Making use of the reaction between *Schöllkopf*'s reagent and 4,5-dihydro-isoxazole-3-carbaldehydes (mono- or di-substituted in position 5) followed by the cleavage of the dihydro-pyrazine and isoxazoline rings, we have obtained enantiomerically pure polyfunctionalised dipeptides (Scheme 1).[8]

With the aim of obtaining new β-hydroxy-α-amino acids, β-substituted with a 2-isoxazoline ring, that is, potentially susceptible to further transformation, and containing an asymmetric, enantiomerically pure quaternary carbon in the β position, we extended this protocol to ketones. One of the most interesting goals of organic synthesis is the asymmetric synthesis of quaternary carbon centres, and one of the most useful means of achieving it is the asymmetric addition of nucleophiles to ketones.[13] In particular, the aldol reaction between a glycine equivalent and prochiral ketones provides access to β,β-disubstituted-β-hydroxy-α-amino acids, which are of considerable interest in the synthesis of peptidomimetics because of their sterically constrained structure.[14] There are very few published examples of the reaction of *Schöllkopf*'s reagent with prochiral ketones, most of which have involved acetophenone, chloroacetone and chloroacetophenone.[15] We describe the results of a reaction between *Schöllkopf*'s reagent (2*R*)-**1** and 2-isoxazolines bearing a ketonic functionality in position 5 of the ring, such as (5*S*)- and (5*R*)-5-acetyl-3-methyl-2-isoxazoline **2a** and (5*S*)- and (5*R*)-5-acetyl-3-ethoxycarbonyl-2-isoxazoline **2b** (Fig. 1).

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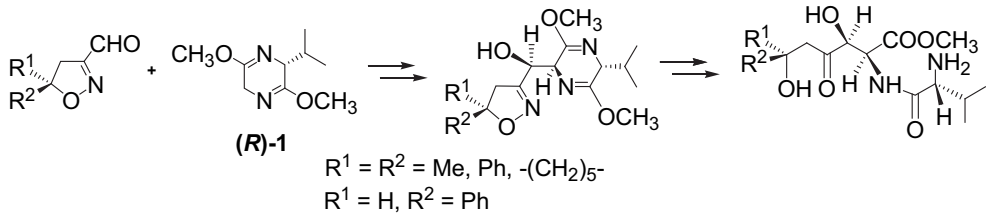


Scheme 1.

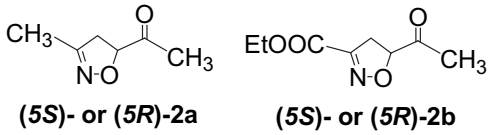


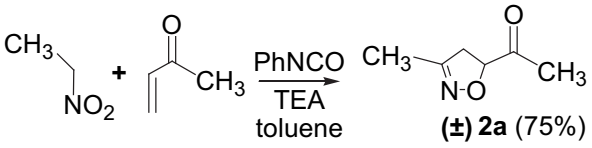
Fig. 1. Selected substrates.

The choice of a methyl ketone was suggested by the need to minimise the steric shielding of the carbonyl group already substituted with an isoxazolinic residue. Some 3-substituted-5-acetyl-4,5-dihydro-isoxazoles have been described, but little is known about enantiomerically pure compounds. With the aim of minimising the total number of diastereoisomers arising from the reaction with *Schöllkopf*'s reagent, we selected the 3-methyl and 3-carbethoxy derivatives **2a** and **2b** because the resolution of their corresponding racemate has been approximately described[16] albeit without any information about their optical rotation value and the corresponding absolute configuration. In addition to introducing another important functional group, the carbethoxy group allowed us to consider their possible competition against the reaction with *Schöllkopf*'s reagent. To the best of our knowledge, this type of reaction has not been studied.[17]

2. Results and discussion

2.1. Synthesis of (5*S*)- and (5*R*)-5-acetyl-4,5-dihydro-isoxazoles 2a,b

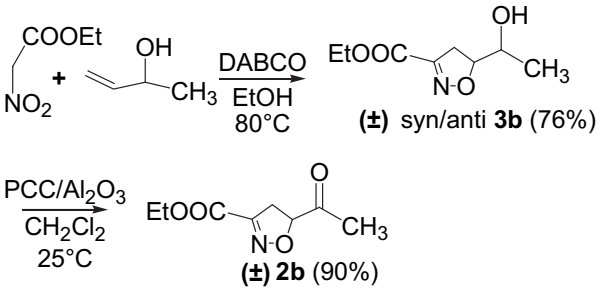
The racemic 5-acetyl-3-methyl-2-isoxazoline **2a** was regioselectively synthesised by means of the 1,3-dipolar cycloaddition of acetonitrile oxide (generated from nitroethane) with methyl vinyl ketone (Scheme 2).[18]



Scheme 2.

In the case of compound **2b**, as the analogous 1,3-cycloaddition route between ethyl nitroacetate and methyl vinyl ketone afforded the corresponding cycloadduct in very poor yield, it was necessary to use two steps. Following a recently described method,[19] the base-catalysed condensation between ethyl nitroacetate and 3-buten-2-ol afforded a mixture of *syn*/*anti* (57/43) 5-(1-hydroxyethyl)-4,5-dihydro-isoxazole-3-carboxylic acid ethyl ester **3b**, which was transformed into racemic 5-acetyl-3-ethoxycarbonyl-2-isoxazoline **2b** by oxidating the alcohol function (Scheme 3).[20]

Racemic isoxazolines **2a** and **2b** were treated with commercial baker's yeast at 35 °C, in phosphate buffer, pH 5.5–6.0, in the presence of glucose. After continuous extraction of the aqueous solution with dichloromethane, 1/1 mixtures of the corresponding *syn*/*anti* diastereoisomeric alcohols **3a**,**b** were obtained in 66–78% yield (Scheme 4).



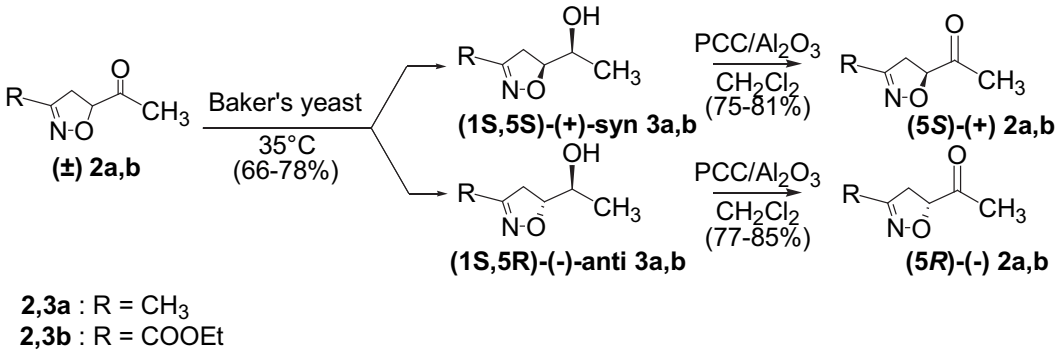
Scheme 3.

The two *syn*/*anti*-alcohols **3b** were separated by means of flash chromatography on silica gel, whereas the two *syn*/*anti*-alcohols **3a** required flash chromatography and a semi-preparative HPLC separation. In an attempt to avoid this laborious purification, we tried a stereocontrolled reduction of isoxazoline (±)-**2a** with baker's yeast in 2-propanol/water mixture in order to obtain a mixture of the enantiomerically pure ketone **2a** and alcohol **3a** directly, as previously reported in the case of a similar substrate.[21] Unfortunately, depending on the 2-propanol/water ratio used with our substrates (from 6/1 to 1/5), the reactions led either to the unreacted ketone or the completely reduced alcohol.[22] Other attempts to resolve the racemic mixture of ketone **2a** were also unsuccessful (**2a** was treated with diethyl L-tartrate or (*R*,*R*)-1, 2-diphenyl-1,2-ethanediol with the aim of obtaining a mixture of diastereoisomeric acetales, but there was no reaction in either case), as were attempts to use borane and catalytic (*S*)-(−)-*o*-tolyl-CBS-oxazaborolidine[23] to obtain enantioselective reduction to the corresponding alcohol.

The relative *syn*/*anti* configuration of compounds **3a**,**b** was assigned using ^{1}H NMR spectra from the value of the coupling constant between H-5 and H-1 (J=5.7–5.2 Hz for *syn*-**3a**,**b** and 3.2–3.3 Hz for *anti*-**3a**,**b**) in agreement with previous assignments.[21,24] The enantiomeric excess of the alcohols *syn*-**3a**,**b** and *anti*-**3a**,**b** was determined by comparing them with the racemic mixtures obtained from the reduction with $NaBH_4$ of (±)-**2a** for **3a** and from the initial cyclocondensation products (Scheme 3) for **3b**, by means of chiral HPLC analysis using a Chiralcel OD analytical column. Generally, in both cases, it was >98%.

Absolute configurations were not assigned at this stage, but were determined by means of an X-ray analysis of the adducts obtained in the next reaction with *Schöllkopf*'s reagent (see below), which allowed the assignment of configuration (1*S*,5*S*) to alcohols *syn*-**3a** and (1*S*,5*R*) to *anti*-**3a** and, by analogy, also to compounds *syn*/*anti*-**3b**.[25]

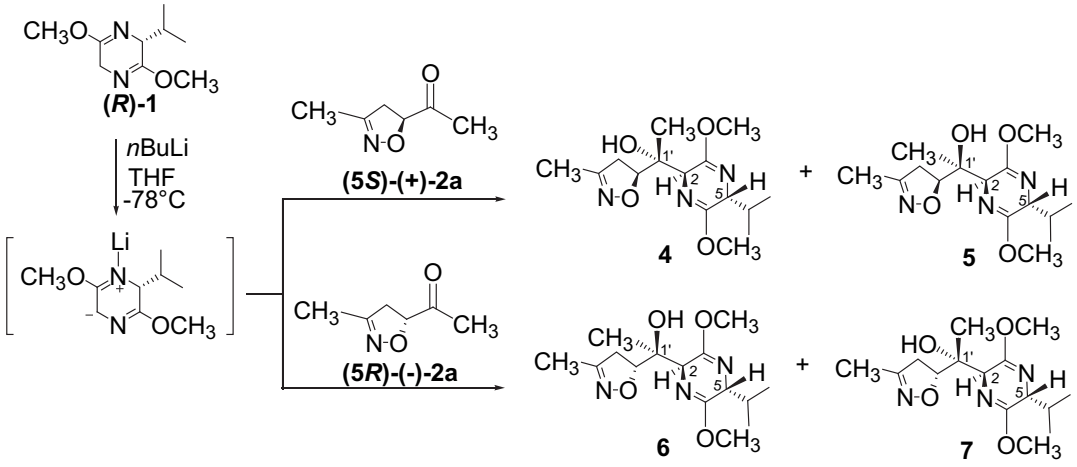
Finally, oxidation of the *syn*- and *anti*-alcohols with PCC/Al_2O_3, respectively, led to (5*S*)(+) and (5*R*)(−)-3-substituted-5-acetyl-4,5-dihydro-isoxazoles **2a**,**b** (Scheme 4). The enantiomeric excess of the final ketones **2a** and **2b** was confirmed to be, respectively, >98% and 92% by comparing them with the racemic compounds by means of chiral HPLC analysis using a Chiralcel OD analytical column for **2a**, and a Chiralcel AD analytical column for **2b**.



Scheme 4.

2.2. Addition of bislactim ether (2*R*)-1 azaenolate to ketones (5*S*)- and (5*R*)-2a,b

Various experimental conditions were examined to optimise yields and evaluate the diastereoselectivity of the addition reaction. Under the best conditions, a THF solution of ketone **2a** was added to the anion of the bislactim ether (2*R*)-**1** generated by *n*BuLi in THF at $T=-78\ ^{\circ}C$, and maintained at this temperature for 4 h; longer times or higher temperatures ($T=-20\ ^{\circ}C$) were associated with lower yields probably because of the reversibility of the addition.[8b,26] With the (5*S*)- or (5*R*)-3-methyl derivatives **2a**, the reaction gave mixtures of two diastereoisomeric adducts **4**/**5** or **6**/**7** in ratios of, respectively, 76/24 and 69/31, as estimated by integrating the doublet of the isopropyl groups in the ^1H NMR spectra of the crude reaction mixtures (Scheme 5 and Table 1).



Scheme 5.

Table 1
Total yields and ratios of compounds **4**/**5** and **6**/**7**

Ketone	Counter-ion	Total yield (%)	**4**/**5** or **6**/**7** ratio
(5*S*)-**2a**	Li^+	70	76/24
(5*S*)-**2a**	$(i\text{-PrO})_3Ti^+$	Trace	—
(5*S*)-**2a**·$TiCl_4$	Li^+	48	87/13
(5*R*)-**2a**	Li^+	65	69/31

To evaluate the influence of the counter-ion on diastereoselectivity, the lithium azaenolate was treated with triisopropoxytitanium(IV) chloride[27] to give the corresponding titanium salt before the addition of ketone (5*S*)-**2a** but a mixture of adducts **4**/**5** was obtained only in trace amounts. In a parallel experiment, in order to make the carbonyl more reactive, titanium(IV) chloride was added to a THF solution of ketone (5*S*)-**2a** before it was added to the anion of the bislactim ether. In this case compounds **4**/**5** were obtained with better diastereoselectivity (87/13) but a lower yield (48%) (Table 1).

A different result was obtained using the (5*S*)- and (5*R*)-3-ethoxycarbonyl derivative ketone **2b**: in this case, and under the best experimental conditions, the reaction led to a mixture of several compounds and varying amounts (20–40%) of unreacted ketone. The ^1H NMR spectra of this mixture revealed the presence of a pair of adducts that were isolated, but not separated, only in trace amounts after chromatographic column while the mixture of the other products was not identified. This negative behaviour may have been due to competition between ketone and carbethoxy groups, making more complicate the reactivity of **2b**.[17b]

Diastereoisomers **4**/**5** and **6**/**7** were purified by means of flash chromatography on silica gel, and their structures were confirmed on the basis of analytical and spectroscopic data. The (2*S*)-configuration of compounds **5**–**7** was established using the $^5J_{H2/H5}$ coupling constant value of approximately 3.5–4.0 Hz, which corresponds to a trans relationship between the H-2 and H-5 protons of the pyrazine ring.[28] Adducts **4** and **5** were obtained as crystalline solids and underwent X-ray crystallographic analysis, which made it possible to assign the (*S*) configuration to the C-5 of the isoxazoline ring and the pyrazine-C-2 of compound **4**, and the (*R*) configuration to the C-1′ of compound **4**; similarly, the (*S*) configuration was assigned to both the pyrazine-C-2 and the C-1′ of diastereoisomer **5** (see Supplementary data, S19 for **4** and S22 for **5**). This also allowed assigning the same (5*S*) configuration to compound (+)-**2a** and the (5*R*) configuration to (−)-**2a**.

In the case of compounds **6**/**7**, it was not possible to obtain suitable crystals for X-ray analysis, and so their absolute configurations were assigned by means of exhaustive ^{1}H NMR spectra and NOESY experiments (see Supplementary data, S25 and S28). This allowed the (*S*) configuration to be assigned to both the pyrazine-C-2 and C-1′ of compound **6**, and the (*S*) and (*R*) configurations to be, respectively, assigned to the pyrazine-C-2 and the C-1′ of diastereoisomer **7**.

2.3. Models of the addition of bislactim ether (2*R*)-1 azaenolate to ketones (5*S*)- and (5*R*)-2a

The reactions of *Schöllkopf*'s reagent with ketones have not yet been studied in detail and never using prochiral ketones containing stereocentres. These reactions always afforded mixtures of the two (2*S*)-epimers arising from the attack of the azaenolate-pyrazine from the less hindered side opposite the isopropyl group.[15] The observed stereoselectivities have been rationalised using the *Zimmerman–Traxler* six-membered ring model,[29] according to which an energetically favoured chair-like Transition State, with an equatorial disposition of the more cumbersome residue of the ketone group, should account for the prevalent formation of the 2,5-*trans*-2,1′-*syn* diastereoisomer.[15c] Our results confirm the 2,5-trans-relation in the adducts as the NMR analyses of the crude reaction mixtures never showed the presence of 2,5-*cis* diastereoisomers deriving from the attack of the azaenolate-pyrazine from the same side as the isopropyl group.

Ketones (5*S*)- and (5*R*)-**2a** afforded pairs of diastereoisomers that are only different in terms of the configuration of the alcoholic carbon atom. The reactions of *Schöllkopf*'s reagent with chiral ketone **2a** raise the question of 'double asymmetric induction'. The use of the enantiomeric forms of ketone **2a** led to both *matched* ((2*R*)-**1** and (5*R*)-**2a**) and *mismatched* ((2*R*)-**1** and (5*S*)-**2a**) situations, allowing us to evaluate the relative influence of both the carbonyl α-stereocentre (*substrate control*) and the azaenolate-pyrazine (*reagent control*) on reaction stereoselectivity. The major adducts **4** and **6**, respectively, derive from attack of the azaenolate on the *Re* and *Si* faces of the carbonyl group. In any case, taking into account that ketones contain an adjacent α-alkoxy substituent and that they were used under non-chelating experimental conditions, the observed selectivity favoured the 1′,5″-*anti* diastereoisomers. These results can be qualitatively explained on the basis of the models previously used to rationalise the stereochemical results observed in the reaction between *Schöllkopf*'s reagent and pairs of enantiomeric aldehydes.[6b,7e,30] According to the polar Felkin-Anh rule[31] for 1,2-asymmetric induction and Cornforth modification,[32] combined with the *Zimmerman–Traxler* model, it can be postulated that the Transition States **A** and **B** (Fig. 2) describe the preferential formation of adduct **4** over adduct **5**. In this case stereodifferentiation due to the chiral ketone has a greater effect than *Schöllkopf*'s pyrazine as the reaction mainly lead to the '*substrate control*' adduct **4** with good diastereoselectivity. The preferential formation of compound **6** over compound **7** from the *matched* ketone (5*R*)-**2a**, can be qualitatively explained by analysing the analogous Transition States **E**–**H** shown in Supplementary data (S2). Our results agree with computational studies recently made by Ruitz,[30c] which indicated that *trans,-syn,anti*-selectivity is most favourable for the addition of metallated bislactim ethers to matched glyceraldehyde acetonide, and *trans,-anti,anti*-selectivity most favourable for the mismatched. On the basis of this, the Cornforth-like conformations can be considered the most favourable.

2.4. Hydrolysis and hydrogenolysis of adducts into β-substituted-L-threonines

Adducts **4**–**6** were hydrolysed under controlled conditions: they were treated with 2 equiv of 0.2 N HCl in THF at room temperature for 16–24 h, which allowed the isolation of the β-substituted-L-threonines methyl esters **8**–**10** and the dipeptides **11**–**13** (Scheme 6).

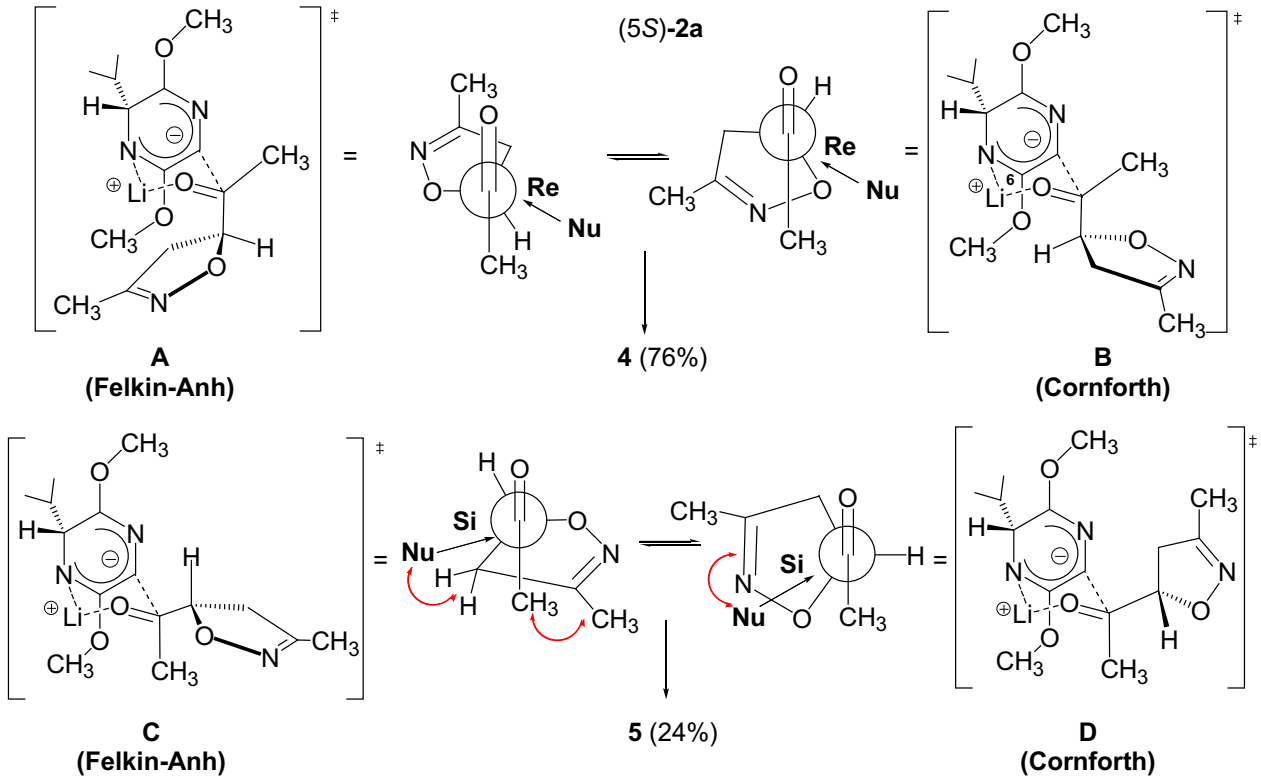
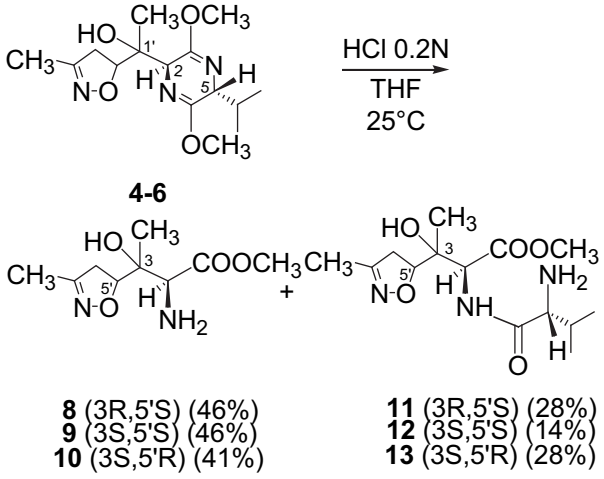


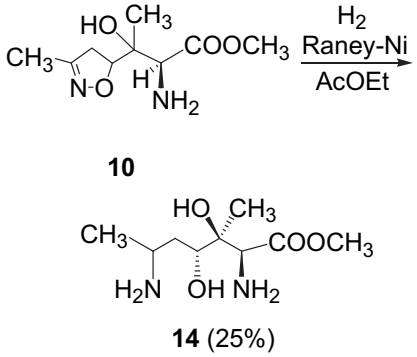
Fig. 2. Transition states **A**–**D** for the *mismatched* (2*R*)-**1** and (5*S*)-**2a** pair.

Given the partial hydrolysis of the pyrazine ring, the dipeptides formation was observed as early as during the hydrolysis reactions.[8,33] Amino esters **8**–**10** were easily separated from their corresponding dipeptides **11**–**13** by means of column chromatography and their structure was assigned using ^{1}H and ^{13}C NMR spectroscopic analysis.[8,33b,c]



Scheme 6.

Finally, several attempts to open the 4,5-dihydro-isoxazole ring of compounds **8**–**13** with hydrogen and Raney-Ni, under various experimental conditions, were made and the unique positive result was obtained only with substituted L-threonine **10** under anhydrous conditions. In this way, γ-hydroxy-ε-amino-L-threonine derivative **14** was obtained (Scheme 7). In the ^{1}H NMR spectra of crude reaction mixture it was possible to detect only one diastereoisomer that was purified by means of flash chromatography. Spectroscopic data and HRMS (FT-ICR) confirmed the structure but it was not possible to obtain suitable crystals for X-ray analysis necessary to assign the absolute configuration of the newly formed stereocentre C-6. Despite the low yield of compound **14** formation, this remains the only method to obtain this highly functionalized molecule.



Scheme 7.

3. Conclusion

The 3-methyl- or 3-ethoxycarbonyl-substituted (5*S*)- and (5*R*)-5-acetyl-2-isoxazolines were obtained by means of enzymatic resolution using baker's yeast. The subsequent reaction with *Schöllkopf*'s reagent as a chiral synthon, followed by hydrolysis of the pyrazine ring, was used to introduce an amino acid residue in order to obtain β-(2-isoxazolin-5-yl)-L-threonines. These compounds may be interesting because 2-isoxazoline derivatives have been used as dipeptide bioisosteres[34] and incorporated into biologically active compounds, such as the anti-cancer drug acivicin.[35] Hydrogenolysis of adduct **10** also allowed us to obtain γ-hydroxy-ε-amino-L-threonine derivative. Our study provides the first results relating to the reaction between *Schöllkopf*'s bislactim ether and pairs of enantiomerically pure prochiral ketones.

4. Experimental section

4.1. General

Melting points were measured using a *Büchi* apparatus. ^{1}H and ^{13}C NMR spectra were recorded using a *Bruker AC* 300 spectrometer. Chemical shifts (δ) are given in parts per million in relation to TMS; the solvent was $CDCl_3$ unless otherwise specified. All of the coupling constants (J) are in hertz. The optical rotation values were measured at 25 °C using 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. IR spectra (in cm^{-1}) were determined using a *Jasco FT-IR* 4100 spectrometer. All HPLC chromatogram are recorded at λ 214 nm. Compound (±)-**2a** was prepared as previously reported.[18]

4.2. Synthesis of (5*S*)- and (5*R*)-5-acetyl-4,5-dihydro-isoxazoles (2a,b)

*4.2.1. 1-(3-Methyl-4,5-dihydro-isoxazol-5-yl)-ethanol (mixture of (±)-syn/anti-**3a**).* To a suspension of $NaBH_4$ (0.39 mg, 10.2 mmol) in ethanol (10 mL), cooled at 0 °C, a solution of (±)-**2a**[18] (0.5 mg, 3.94 mmol) in ethanol (10 mL) was added dropwise. The reaction mixture was stirred at room temperature for 24 h. The organic solvent was evaporated off, the residue was treated with water and extracted with several portions of ethyl acetate. The combined extracts were dried (Na_2SO_4) and concentrated at reduced pressure. The ratio *syn*/*anti*=60/40 was determined by means of the integration of the multiplets relative to the H-1 protons at 3.68 and 4.05 δ, respectively, in the ^{1}H NMR spectra of the crude reaction. The mixture of diastereoisomers (±)-*syn*/*anti*-**3a** was purified and partially separated by means of flash chromatography (SiO_2, hexane/ethyl acetate=6/4). Oil (0.43 g, 85% total yield). Compounds (±)-*syn*- and *anti*-**3a** were analysed by means of chiral HPLC using a Chiralcel OD analytical column and a mixture of hexane/*i*-PrOH=98/2 with a flow rate of 1.5 mL/min, retention time (±)-*anti*-**3a**: 18.5 and 19.5 min; retention time (±)-*syn*-**3a**: 21.8 and 22.3 min.

*4.2.2. 5-(1-Hydroxyethyl)-4,5-dihydro-isoxazole-3-carboxylic acid ethyl ester (mixture of (±)-syn/anti-**3b**).* A solution of 3-buten-2-ol (1.04 mL, 12 mmol, 1 equiv), ethyl nitroacetate (2.64 mL, 24 mmol, 2 equiv) and DABCO (269 mg, 2.4 mmol, 0.2 equiv) in ethanol (30 mL) was heated at 80 °C for five days in a sealed tube. The organic solvent was evaporated off and the mixture of diastereoisomers (±)-*syn*/*anti*-**3b** was purified and partially separated by means of flash chromatography (SiO_2, hexane/ethyl acetate=3/1). The ratio (±)-*syn*/*anti*=57/43 was determined by means of the integration of the doublet relative to the methyl groups at 1.16 and 1.28 δ, respectively, in the ^{1}H NMR spectra. Oil (1.7 g, 76%). Compounds (±)-*syn*- and *anti*-**3b** were analysed by means of chiral HPLC using a Chiralcel OD analytical column: hexane/*i*-PrOH=95/5 and a flow rate of 1 mL/min, retention time (±)-*anti*-**3b**: 20.6 and 22.6 min; hexane/*i*-PrOH=98/2 and a flow rate of 1 mL/min, retention time (±)-*syn*-**3b**: 56.7 and 60.1 min.

*4.2.3. 5-Acetyl-4,5-dihydro-isoxazole-3-carboxylic acid ethyl ester (±)-(**2b**).* PCC/Al_2O_3 (3 equiv) was added to a solution of (±)-*syn*/*anti*-**3b** (1.7 g, 9.1 mmol, 1 equiv) in CH_2Cl_2 (40 mL), and the reaction mixture was stirred at reflux temperature for 24 h. The PCC was filtered through Celite, and the organic solvent was evaporated

off. The crude ketone was purified by column chromatography (SiO_2, hexane/ethyl acetate=8/2). Oil (1.5 g, 90%). Spectroscopic data of compound (±)-**2b** were in accord with those reported.[20]

*4.2.4. Reduction of (±)-(**2a**,**b**) by baker's yeast and analytical method.* Ketone (±)-**2a**[18] or (±)-**2b** (1 mmol) dissolved in the minimum amount of ethanol, was added to a suspension of commercial fermenting yeast[36] (5 g) in tap water (30 mL) containing KH_2PO_4 (60 mg), Na_2HPO_4 (30 mg), $MgSO_4$ (30 mg) and glucose (10 g). If necessary, the pH of the mixture was kept at 5.5–6.0 by addition of diluted aqueous NaOH. The reaction was carried out at 35 °C under magnetic stirring for 24 h and was monitored by TLC. The suspension was stirred with Celite at 0 °C for 15 min and then filtered. The filtered water was extracted *in continuous* with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and the solvent was evaporated at reduced pressure. The mixture of *syn*/*anti*-alcohols **3a** (78% total yield) was purified by means of flash chromatography (SiO_2, hexane/ethyl acetate=8/2) and separated by semi-preparative HPLC (Waters-Micropack, 10 μ SiO_2, hexane/*i*-PrOH=95/5, flow=7 mL/min). The mixture of *syn*/*anti*-alcohols **3b** (66% total yield) was purified and separated by means of flash chromatography (SiO_2, hexane/ethyl acetate=85/15).

*4.2.4.1. (1S,5S)-1-(3-Methyl-4,5-dihydro-isoxazol-5-yl)-ethanol ((+)-syn-**3a**).* Oil. $[\alpha]_{D^{20}}$=+148.2 (*c* 0.51, $CHCl_3$). The enantiomeric excess (>98%) was determined by means of chiral HPLC analysis using a Chiralcel OD analytical column and a mixture of hexane/*i*-PrOH=98/2 with a flow rate of 1.5 mL/min, retention time: 21.8 min. ^{1}H NMR: δ 1.22 (d, *J*=6.4, 3H, CH_3); 1.98 (s, 3H, CH_3); 2.09 (broad s, 1H, OH); 2.73 (dd, *J*=17.1, 7.4, 1H, H-4); 2.98 (dd, *J*=17.1, 10.6, 1H, H-4); 3.68 (m, 1H, H-1); 4.39 (ddd, *J*=10.6, 7.4, 5.7, 1H, H-5). ^{13}C NMR: δ 12.9 (3-CH_3); 18.7 (CH_3); 40.6 (C-4); 68.9 (C-1), 83.6 (C-5); 155.75 (C-3). IR (Nujol): 3419 (ν_{OH}, OH), 1639 ($\nu_{C=N}$, C=N). Anal. Calcd for $C_6H_{11}NO_2$: C, 55.80; H, 8.58; N, 10.84. Found: C, 55.62; H, 8.45; N, 10.78. MS-EI$^+$ (*m*/*z*): 129 (M^+).

*4.2.4.2. (1S,5R)-1-(3-Methyl-4,5-dihydro-isoxazol-5-yl)-ethanol ((−)-anti-**3a**).* Oil. $[\alpha]_{D^{20}}$=−90.0 (*c* 0.54, $CHCl_3$). The enantiomeric excess (>98%) was determined by means of chiral HPLC analysis using a Chiralcel OD analytical column and a mixture of hexane/*i*-PrOH=98/2 with a flow rate of 1.5 mL/min, retention time: 18.0 min. ^{1}H NMR: δ 1.13 (d, *J*=6.5, 3H, CH_3); 1.85 (broad s, 1H, OH); 1.97 (s, 3H, CH_3); 2.80 (dd, *J*=17.1, 10.7, 1H, H-4); 2.97 (dd, *J*=17.1, 8.6, 1H, H-4); 4.05 (m, 1H, H-1); 4.46 (ddd, *J*=10.7, 8.6, 3.2, 1H, H-5). ^{13}C NMR: δ 13.1 (3-CH_3); 17.9 (CH_3); 37.8 (C-4); 67.05 (C-1); 84.1 (C-5); 156.0 (C-3). IR (Nujol): 3420 (ν_{OH}, OH), 1641 ($\nu_{C=N}$, C=N). Anal. Calcd for $C_6H_{11}NO_2$: C, 55.80; H, 8.58; N, 10.84. Found: C, 55.70; H, 8.49; N, 10.74. MS-EI$^+$ (*m*/*z*): 129 (M^+).

*4.2.4.3. (1S,5S)-5-(1-Hydroxyethyl)-4,5-dihydro-isoxazole-3-carboxylic acid ethyl ester ((+)-syn-**3b**).* Oil. $[\alpha]_{D^{20}}$=+164.1 (*c* 0.39, $CHCl_3$). The enantiomeric excess (>98%) was determined by means of chiral HPLC analysis using a Chiralcel OD analytical column and a mixture of hexane/*i*-PrOH=98/2 with a flow rate of 1 mL/min, retention time: 53.1 min. ^{1}H NMR: δ 1.28 (d, *J*=6.5, 3H, CH_3); 1.37 (t, *J*=7.1, 3H, CH_3); 1.99 (d, *J*=6.2, 1H, OH); 3.06 (dd, *J*=17.8, 8.2, 1H, H-4); 3.24 (dd, *J*=17.8, 11.2, 1H, H-4); 3.78 (m, 1H, H-1); 4.33 (q, *J*=7.1, OCH_2); 4.67 (ddd, *J*=11.2, 8.2, 5.2, 1H, H-5). ^{13}C NMR: δ 14.1 (CH_3); 18.8 (CH_3); 35.6 (C-4); 62.1 (CH_2); 68.9 (C-1), 87.1 (C-5); 152.0 (C-3); 160.4 (C=O). IR (Nujol): 3430 (ν_{OH}, OH), 1720 ($\nu_{C=O}$, C=O), 1593 ($\nu_{C=N}$, C=N). Anal. Calcd for $C_8H_{13}NO_4$: C, 51.33; H, 7.00; N, 7.48. Found: C, 51.22; H, 6.92; N, 7.38. MS-FAB$^+$ (*m*/*z*): 188 $[M+H]^+$.

*4.2.4.4. (1S,5R)-5-(1-Hydroxyethyl)-4,5-dihydro-isoxazole-3-carboxylic acid ethyl ester ((−)-anti-**3b**).* Oil. $[\alpha]_{D^{20}}$=−134.5 (*c* 0.91, $CHCl_3$). The enantiomeric excess (95%) was determined by means of chiral HPLC analysis using a Chiralcel OD analytical column and a mixture of hexane/*i*-PrOH=95/5 with a flow rate of 1 mL/min, retention time: 22.6 min. ^{1}H NMR: δ 1.16 (d, *J*=6.5, 3H, CH_3); 1.32 (t, *J*=7.2, 3H, CH_3); 1.87 (d, *J*=3.6, 1H, OH); 3.08 (dd, *J*=17.7, 11.5, 1H, H-4); 3.22 (dd, *J*=17.7, 8.9, 1H, H-4); 4.06 (m, 1H, H-1); 4.30 (q, *J*=7.2, OCH_2); 4.68 (ddd, *J*=11.5, 8.9, 3.3, 1H, H-5). ^{13}C NMR: δ 13.9 (CH_3); 17.8 (CH_3); 32.8 (C-4); 61.95 (CH_2); 66.8 (C-1), 87.5 (C-5); 152.0 (C-3); 160.4 (C=O). IR (Nujol): 3433 (ν_{OH}, OH), 1722 ($\nu_{C=O}$, C=O), 1591 ($\nu_{C=N}$, C=N). Anal. Calcd for $C_8H_{13}NO_4$: C, 51.33; H, 7.00; N, 7.48. Found: C, 51.19; H, 6.85; N, 7.33. MS-FAB$^+$ (*m*/*z*): 188 $[M+H]^+$.

*4.2.5. Oxidation of enantiomerically pure alcohols ((+)-syn-**3a**,**b**) and ((−)-anti-**3a**,**b**).* PCC/Al_2O_3 (3 equiv) was added to a solution of alcohol (1 equiv) in CH_2Cl_2 (4 mL), and the reaction mixture was stirred at reflux temperature for 24 h. The PCC was filtered through Celite, and the organic solvent was evaporated off. The crude ketone was purified by column chromatography (SiO_2, hexane/ethyl acetate=8/2).

*4.2.5.1. (5S)-1-(3-Methyl-4,5-dihydro-isoxazol-5-yl)-ethanone (**2a**) obtained from (+)-syn-**3a**.* Oil (81%). $[\alpha]_{D^{20}}$=+177.9 (*c* 0.62, $CHCl_3$). The enantiomeric excess (>98%) was determined by means of chiral HPLC analysis using a Chiralcel OD analytical column and a mixture of hexane/*i*-PrOH=98/2 with a flow rate of 1.5 mL/min, retention time: 11.8 min.

*4.2.5.2. (5R)-1-(3-Methyl-4,5-dihydro-isoxazol-5-yl)-ethanone (**2a**) obtained from (−)-anti-**3a**.* Oil (85%). $[\alpha]_{D^{20}}$=−170.5 (*c* 0.59, $CHCl_3$). The enantiomeric excess (>98%) was determined by means of chiral HPLC analysis using a Chiralcel OD analytical column and a mixture of hexane/*i*-PrOH=98/2 with a flow rate of 1.5 mL/min, retention time: 10.5 min.

*4.2.5.3. (5S)-5-Acetyl-4,5-dihydro-isoxazole-3-carboxylic acid ethyl ester (**2b**) obtained from (+)-syn-**3b**.* Oil (75%). $[\alpha]_{D^{20}}$=+182.7 (*c* 0.45, $CHCl_3$). The enantiomeric excess (92%) was determined by means of chiral HPLC analysis using a Chiralcel AD analytical column and a mixture of hexane/*i*-PrOH=95/5 with a flow rate of 1 mL/min, retention time: 14.0 min.

*4.2.5.4. (5R)-5-Acetyl-4,5-dihydro-isoxazole-3-carboxylic acid ethyl ester (**2b**) obtained from (−)-anti-**3b**.* Oil (77%). $[\alpha]_{D^{20}}$=−187.9 (*c* 0.55, $CHCl_3$). The enantiomeric excess (92%) was determined by means of chiral HPLC analysis using a Chiralcel AD analytical column and a mixture of hexane/*i*-PrOH=95/5 with a flow rate of 1 mL/min, retention time: 15.4 min.

4.3. Addition of bislactim ether (2*R*)-1 azaenolate to ketones (5*S*)- and (5*R*)-(2a)

Butyl lithium (1.6 N solution in hexane, 1.05 equiv) was added to a solution of (2*R*)-**1** (1 equiv) in anhydrous THF (5 mL) cooled at −78 °C, and the mixture was stirred for 45 min. Ketone (5*S*) or (5*R*)-**2a** (1 equiv) in THF (4 mL) was added, and the mixture was stirred at −78 °C for 4 h. The reaction mixture was allowed to warm to −10 °C, after which a pH=7 phosphate buffer solution (10 mL) was added, and the mixture was extracted with CH_2Cl_2. The organic phase was separated and dried with Na_2SO_4, and the solvent was evaporated in vacuo. Compounds **4** and **5** and **6** and **7** were purified by means of column chromatography (SiO_2, hexane/ethyl acetate=8/2) and (hexane/ethyl acetate=7/3), respectively. They were subsequently separated by means of flash chromatography (SiO_2, Supelco—Versaflash® station, hexane/ethyl acetate=75/25).

*4.3.1. (1R)-1-[(2S,5R)-5-Isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazin-2-yl]-1-[(5S)-3-methyl-4,5-dihydro-isoxazol-5-yl]ethanol (**4**): obtained from (5S)-**2a**.* Colourless solid (53%); mp 79–81 °C

(hexane). $[\alpha]_{D^{20}}$=+75.8 (*c* 0.8, $CHCl_3$). ^{1}H NMR: δ 0.67, 1.07 (2d, *J*=6.8, 6H, CH(CH_3)$_2$); 1.2 (s, 3H, 1-CH_3); 1.95 (s, 3H, 3-CH_3); 2.29 (m, 1H, CH(CH_3)$_2$); 2.91 (dd, *J*=17.0, 10.8, 1H, H-4); 3.12 (dd, *J*=17.0, 8.9, 1H, H-4); 3.34 (broad, 1H, OH); 3.67 (s, 3H, OCH_3); 3.78 (s, 3H, OCH_3); 3.99 (broad s, 2H, H-2 and H-5 pyraz.); 4.73 (dd, *J*=10.8, 8.9, 1H, H-5 isox.). ^{13}C NMR: δ 13.1 (3-CH_3); 16.4, 19.0 (CH(CH_3)$_2$); 19.8 (1-CH_3); 31.4 (CH(CH_3)$_2$); 38.9 (C-4); 52.5 (3- and 6-OCH_3); 60.6, 61.6 (C-2 and C-5 pyr.); 75.1 (C-1); 83.5 (C-5 isox.); 155.7 (C-3 isox.); 160.9, 164.7 (C-3 and C-6 pyr.). IR (Nujol): 3435 (ν_{OH}, OH), 1692 ($\nu_{C=N}$, C=N). Anal. Calcd for $C_{15}H_{25}N_3O_4$: C, 57.86; H, 8.09; N, 13.49. Found: C, 57.67; H, 7.96; N, 13.33. MS-FAB$^+$ (*m*/*z*): 312 $[M+H]^+$. Single crystals suitable for X-ray structure determination were obtained by precipitation from hexane/ethyl acetate=1/1.

*4.3.2. (1S)-1-[(2S,5R)-5-Isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazin-2-yl]-1-[(5S)-3-methyl-4,5-dihydro-isoxazol-5-yl]ethanol (**5**): obtained from (5S)-**2a**.* Colourless solid (17%); mp 85–86 °C (hexane). $[\alpha]_{D^{20}}$=+126.1 (*c* 0.63, $CHCl_3$). ^{1}H NMR: δ 0.69, 1.02 (2d, *J*=6.7, 6H, CH(CH_3)$_2$); 0.99 (s, 3H, 1-CH_3); 1.97 (s, 3H, 3-CH_3); 2.23 (m, 1H, C*H*(CH_3)$_2$); 2.87 (dd, *J*=16.7, 11.1, 1H, H-4); 3.12 (dd, *J*=16.7, 7.9, 1H, H-4); 3.68 (s, 3H, OCH_3); 3.74 (s, 3H, OCH_3); 4.02 (t, *J*=3.7, 1H, H-5 pyraz.); 4.3 (broad, 1H, OH); 4.33 (d, *J*=4.1, 1H, H-2 pyraz.); 4.84 (dd, *J*=11.1, 7.9, 1H, H-5 isox.). ^{13}C NMR: δ 13.0 (3-CH_3); 16.7, 19.0 (CH(CH_3)$_2$); 20.9 (1-CH_3); 32.0 (C*H*(CH_3)$_2$); 39.4 (C-4); 52.6 (3- and 6-OCH_3); 59.0, 61.2 (C-2 and C-5 pyr.); 75.3 (C-1); 82.5 (C-5 isox.); 155.5 (C-3 isox.); 161.4, 164.7 (C-3 and C-6 pyr.). IR (Nujol): 3418 (ν_{OH}, OH), 1697 ($\nu_{C=N}$, C=N). Anal. Calcd for $C_{15}H_{25}N_3O_4$: C, 57.86; H, 8.09; N, 13.49. Found: C, 57.71; H, 7.94; N, 13.38. MS-FAB$^+$ (*m*/*z*): 312 $[M+H]^+$. Single crystals suitable for X-ray structure determination were obtained by precipitation from hexane/ethyl acetate=1/1.

*4.3.3. (1S)-1-[(2S,5R)-5-Isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazin-2-yl]-1-[(5R)-3-methyl-4,5-dihydro-isoxazol-5-yl]ethanol (**6**): obtained from (5R)-**2a**.* Colourless solid (45%); mp 70–72 °C (hexane). $[\alpha]_{D^{20}}$=−38.42 (*c* 0.39, $CHCl_3$). ^{1}H NMR: δ 0.66, 1.06 (2d, *J*=6.8, 6H, CH(CH_3)$_2$); 1.13 (s, 3H, 1-CH_3); 1.98 (s, 3H, 3-CH_3); 2.32 (m, 1H, C*H*(CH_3)$_2$); 2.92 (dd, *J*=16.9, 10.9, 1H, H-4); 3.13 (dd, *J*=16.9, 8.5, 1H, H-4); 3.65 (broad, 1H, OH); 3.7 (s, 3H, OCH_3); 3.73 (s, 3H, OCH_3); 3.91 (d, *J*=3.9, 1H, H-2 pyraz.); 4.00 (t, *J*=3.6, 1H, H-5 pyraz.); 4.92 (dd, *J*=10.9, 8.5, 1H, H-5 isox.). ^{13}C NMR: δ 13.1 (3-CH_3); 16.4, 19.0 (CH(CH_3)$_2$); 19.7 (1-CH_3); 31.3 (C*H*(CH_3)$_2$); 39.0 (C-4); 52.4, 52.8 (3- and 6-OCH_3); 60.6, 60.9 (C-2 and C-5 pyr.); 75.1 (C-1); 84.1 (C-5 isox.); 155.3 (C-3 isox.); 160.4, 165.3 (C-3 and C-6 pyr.). IR (Nujol): 3425 (ν_{OH}, OH), 1691 ($\nu_{C=N}$, C=N). Anal. Calcd for $C_{15}H_{25}N_3O_4$: C, 57.86; H, 8.09; N, 13.49. Found: C, 57.82; H, 7.93; N, 13.25. MS-FAB$^+$ (*m*/*z*): 312 $[M+H]^+$.

*4.3.4. (1R)-1-[(2S,5R)-5-Isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazin-2-yl]-1-[(5R)-3-methyl-4,5-dihydro-isoxazol-5-yl]ethanol (**7**): obtained from (5R)-**2a**.* Waxy solid (20%). $[\alpha]_{D^{20}}$=−36.42 (*c* 0.78, $CHCl_3$). ^{1}H NMR: δ 0.69, 1.07 (2d, *J*=6.8, 6H, CH(CH_3)$_2$); 1.06 (s, 3H, 1-CH_3); 1.97 (s, 3H, 3-CH_3); 2.3 (m, 1H, C*H*(CH_3)$_2$); 2.82 (dd, *J*=16.8, 11.0, 1H, H-4); 3.07 (dd, *J*=16.8, 8.4, 1H, H-4); 3.7 (s, 3H, OCH_3); 3.72 (broad, 1H, OH); 3.75 (s, 3H, OCH_3); 4.00 (t, *J*=3.7, 1H, H-5 pyraz.); 4.31 (d, *J*=3.9, 1H, H-2 pyraz.); 4.8 (dd, *J*=10.9, 8.5, 1H, H-5 isox.). ^{13}C NMR: δ 13.0 (3-CH_3); 16.6, 19.0 (CH(CH_3)$_2$); 20.8 (1-CH_3); 31.5 (C*H*(CH_3)$_2$); 39.0 (C-4); 52.5, 52.7 (3- and 6-OCH_3); 60.4, 61.1 (C-2 and C-5 pyr.); 75.2 (C-1); 83.0 (C-5 isox.); 155.4 (C-3 isox.); 161.5, 164.2 (C-3 and C-6 pyr.). IR (Nujol): 3446 (ν_{OH}, OH), 1698 ($\nu_{C=N}$, C=N). Anal. Calcd for $C_{15}H_{25}N_3O_4$: C, 57.86; H, 8.09; N, 13.49. Found: C, 57.76; H, 7.91; N, 13.15. MS-FAB$^+$ (*m*/*z*): 312 $[M+H]^+$.

4.4. Hydrolysis of adducts 4–6 into β-substituted-L-threonines

Aqueous HCl (0.2 N, 2.5 mL, 5.5 mmol, 2 equiv) was added to a solution of adduct **4**–**6** (0.25 mmoli, 1 equiv) in THF (1.5 mL). The mixture was stirred for 16–24 h at room temperature and then extracted with diethyl ether in order to remove non-basic organic compounds. It was then treated with 25% ammonia solution under stirring until pH=8–10, and extracted with AcOEt (4×5 mL). The combined organic layers were dried with Na_2SO_4, and the solvent was removed in vacuo. Compounds **8**–**10** and **11**–**13** were separated by means of flash chromatography (SiO_2, dichloromethane/methanol=98/2, developer: I_2 for **8**/**11** and **9**/**12**; ethyl acetate/methanol=98/2, developer: I_2 for **10**/**13**).

*4.4.1. (2S)-Amino-(3R)-hydroxy-3-[(5S)-3-methyl-4,5-dihydro-isoxazol-5-yl]-butyric acid methyl ester (**8**): obtained from **4**.* Waxy solid (46%). R_f=0.4 (dichloromethane/methanol=9/1). $[\alpha]_{D^{20}}$=+92.8 (*c* 0.9, $CHCl_3$). ^{1}H NMR: δ 1.25 (s, 3H, CH_3); 1.95 (s, 3H, 3-CH_3); 2.4 (broad, 3H, OH, NH_2); 2.91 (dd, *J*=17.6, 11.0, 1H, H-4); 3.07 (dd, *J*=17.6, 7.5, 1H, H-4); 3.41 (broad s, 1H, H-2); 3.78 (s, 3H, OCH_3); 4.5 (dd, *J*=11.0, 7.5, 1H, H-5 isox.). ^{13}C NMR: δ 12.9 (3-CH_3); 18.4 (CH_3); 39.7 (C-4); 52.4 (OCH_3); 59.7 (C-2); 73.6 (C-3); 81.6 (C-5 isox.); 155.9 (C-3 isox.); 174.35 (C=O). IR (Nujol): 3391 (ν_{OH}, ν_{NH}, OH, NH_2), 1735 ($\nu_{C=O}$, C=O), 1637 ($\nu_{C=N}$, C=N). Anal. Calcd for $C_9H_{16}N_2O_4$: C, 49.99; H, 7.46; N, 12.96. Found: C, 49.87; H, 7.28; N, 12.75. MS-EI$^+$ (*m*/*z*): 217 $[M+H]^+$.

*4.4.2. (2S)-Amino-(3S)-hydroxy-3-[(5S)-3-methyl-4,5-dihydro-isoxazol-5-yl]-butyric acid methyl ester (**9**): obtained from **5**.* Waxy solid (46%). R_f=0.21 (dichloromethane/methanol=97/3). $[\alpha]_{D^{20}}$=+123.3 (*c* 0.15, $CHCl_3$). ^{1}H NMR: δ 1.04 (s, 3H, CH_3); 1.98 (s, 3H, 3-CH_3); 2.5 (broad, 3H, OH, NH_2); 2.88 (dd, *J*=16.8, 10.9, 1H, H-4); 3.12 (dd, *J*=16.8, 8.2, 1H, H-4); 3.79 (s, 3H, OCH_3); 3.84 (broad s, 1H, H-2); 4.7 (dd, *J*=10.9, 8.2, 1H, H-5 isox.). ^{13}C NMR: δ 12.9 (3-CH_3); 20.2 (CH_3); 39.4 (C-4); 52.4 (OCH_3); 58.2 (C-2); 73.9 (C-3); 82.7 (C-5 isox.); 155.9 (C-3 isox.); 173.1 (C=O). IR (Nujol): 3379 (ν_{OH}, ν_{NH}, OH, NH_2), 1735 ($\nu_{C=O}$, C=O), 1663 ($\nu_{C=N}$, C=N). Anal. Calcd for $C_9H_{16}N_2O_4$: C, 49.99; H, 7.46; N, 12.96. Found: C, 49.90; H, 7.35; N, 12.84. MS-EI$^+$ (*m*/*z*): 217 $[M+H]^+$.

*4.4.3. (2S)-Amino-(3S)-hydroxy-3-[(5R)-3-methyl-4,5-dihydro-isoxazol-5-yl]-butyric acid methyl ester (**10**): obtained from **6**.* Waxy solid (41%). R_f=0.24 (ethyl acetate/methanol=95/5). $[\alpha]_{D^{20}}$=−48.9 (*c* 0.76, $CHCl_3$). ^{1}H NMR: δ 1.12 (s, 3H, CH_3); 1.98 (s, 3H, 3-CH_3); 2.3 (broad, 3H, OH, NH_2); 2.95 (dd, *J*=17.4, 10.9, 1H, H-4); 3.06 (dd, *J*=17.4, 8.0, 1H, H-4); 3.51 (broad s, 1H, H-2); 3.76 (s, 3H, OCH_3); 4.69 (dd, *J*=10.9, 8.0, 1H, H-5 isox.). ^{13}C NMR: δ 12.9 (3-CH_3); 18.1 (CH_3); 39.2 (C-4); 52.2 (OCH_3); 58.9 (C-2); 73.6 (C-3); 82.4 (C-5 isox.); 155.9 (C-3 isox.); 173.8 (C=O). IR (Nujol): 3305 (ν_{OH}, ν_{NH}, OH, NH_2), 1736 ($\nu_{C=O}$, C=O), 1631 ($\nu_{C=N}$, C=N). Anal. Calcd for $C_9H_{16}N_2O_4$: C, 49.99; H, 7.46; N, 12.96. Found: C, 49.89; H, 7.37; N, 12.88. MS-EI$^+$ (*m*/*z*): 217 $[M+H]^+$.

*4.4.4. (2S)-[(2R)-Amino-3-methyl-butyrylamino]-(3R)-hydroxy-3-[(5S)-3-methyl-4,5-dihydro-isoxazol-5-yl]-butyric acid methyl ester (**11**): obtained from **4**.* Waxy solid (28%). R_f=0.3 (dichloromethane/methanol=9/1). $[\alpha]_{D^{20}}$=+63.1 (*c* 0.77, $CHCl_3$). ^{1}H NMR: δ 0.85, 0.99 (2d, *J*=6.8, 6H, CH(CH_3)$_2$); 1.26 (s, 3H, CH_3); 1.96 (s, 3H, 3-CH_3); 2.28 (m, 1H, C*H*(CH_3)$_2$); 2.65 (broad, 3H, OH, NH_2); 2.97 (dd, *J*=17.7, 10.7, 1H, H-4); 3.07 (dd, *J*=17.7, 8.8, 1H, H-4); 3.34 (broad d, *J*=3.8, 1H, H-2 val.); 3.77 (s, 3H, OCH_3); 4.53 (dd, *J*=10.7, 8.8, 1H, H-5 isox.); 4.66 (d, *J*=8.6, 1H, H-2); 8.2 (d, *J*=8.6, 1H, NH). ^{13}C NMR: δ 12.9 (3-CH_3); 16.0 (CH(CH_3)$_2$); 19.6 (CH(CH_3)$_2$) and (CH_3); 30.9 (C*H*(CH_3)$_2$); 39.8 (C-4); 52.7 (OCH_3); 56.6 (C-2); 59.8 (C-2 val.); 74.8 (C-3); 83.3 (C-5 isox.); 156.4 (C-3 isox.); 171.0, 175.0 (C=O). IR (Nujol): 3415 (ν_{OH}, ν_{NH}, OH, NH_2), 1734 ($\nu_{C=O}$, C=O), 1647 ($\nu_{C=N}$, C=N). Anal. Calcd for $C_{14}H_{25}N_3O_5$: C, 53.32; H, 7.99; N, 13.32. Found: C, 53.25; H, 7.76; N, 13.21. MS-FAB$^+$ (*m*/*z*): 316 $[M+H]^+$.

*4.4.5. (2S)-[(2R)-Amino-3-methyl-butyrylamino]-(3S)-hydroxy-3-[(5S)-3-methyl-4,5-dihydro-isoxazol-5-yl]-butyric acid methyl ester (**12**): obtained from **5**.* Waxy solid (14%). R_f=0.15 (dichloromethane/

methanol=97/3). $[\alpha]_{D20}$=+99.0 (*c* 0.2, $CHCl_3$). ^1H NMR: δ 0.86, 1.0 (2d, *J*=6.8, 6H, CH(CH_3)$_2$); 1.13 (s, 3H, CH_3); 1.97 (s, 3H, 3-CH_3); 2.24 (broad m, 4H, C*H*(CH$_3$)$_2$ and OH, NH_2); 2.92 (dd, *J*=17.1, 10.8, 1H, H-4); 3.02 (dd, *J*=17.1, 8.5, 1H, H-4); 3.37 (broad d, *J*=3.7, 1H, H-2 val.); 3.79 (s, 3H, OCH_3); 4.63 (dd, *J*=10.8, 8.5, 1H, H-5 isox.); 4.85 (d, *J*=8.7, 1H, H-2); 8.18 (d, *J*=8.7, 1H, NH). ^{13}C NMR: δ 12.9 (3-CH_3); 16.3 (CH(CH_3)$_2$); 19.6 (CH(CH_3)$_2$); 20.2 (CH_3); 29.7 (*C*H(CH$_3$)$_2$); 39.6 (C-4); 52.7 (OCH_3); 56.8 (C-2); 59.9 (C-2 val.); 75.4 (C-3); 83.0 (C-5 isox.); 156.1 (C-3 isox.); 170.7, 174.2 (C=O). IR (Nujol): 3346 (ν_{OH}, ν_{NH}, OH, NH_2), 1740 ($\nu_{C=O}$, C=O), 1655 ($\nu_{C=N}$, C=N). Anal. Calcd for $C_{14}H_{25}N_3O_5$: C, 53.32; H, 7.99; N, 13.32. Found: C, 53.19; H, 7.86; N, 13.24. MS-FAB$^+$ (*m*/*z*): 316 $[M+H]^+$.

*4.4.6. (2S)-[(2R)-Amino-3-methyl-butyrylamino]-(3S)-hydroxy-3-[(5R)-3-methyl-4,5-dihydro-isoxazol-5-yl]-butyric acid methyl ester (**13**): obtained from **6**.* Waxy solid (28%). R_f=0.15 (ethyl acetate/methanol=95/5). $[\alpha]_{D20}$=−45.4 (*c* 0.83, $CHCl_3$). ^1H NMR: δ 0.87, 1.0 (2d, *J*=6.9, 6H, CH(*CH*$_3$)$_2$); 1.19 (s, 3H, CH_3); 1.98 (s, 3H, 3-CH_3); 2.15 (broad, 3H, OH, NH_2); 2.29 (m, 1H, C*H*(CH$_3$)$_2$); 3.0 (m, 2H, H-4); 3.38 (broad d, *J*=4.0, 1H, H-2 val.); 3.76 (s, 3H, OCH_3); 4.62 (m, 1H, H-5 isox.); 4.67 (d, *J*=8.3, 1H, H-2); 8.29 (d, *J*=8.3, 1H, NH). ^{13}C NMR: δ 12.8 (3-CH_3); 16.1 (CH(*C*H$_3$)$_2$); 19.1 (CH_3); 19.5 (CH(*C*H$_3$)$_2$); 30.9 (*C*H(CH$_3$)$_2$); 39.9 (C-4); 52.6 (OCH_3); 58.6 (C-2); 59.9 (C-2 val.); 74.0 (C-3); 82.6 (C-5 isox.); 156.0 (C-3 isox.); 171.4, 174.9 (C=O). IR (Nujol): 3365 (ν_{OH}, ν_{NH}, OH, NH_2), 1739 ($\nu_{C=O}$, C=O), 1658 ($\nu_{C=N}$, C=N). Anal. Calcd for $C_{14}H_{25}N_3O_5$: C, 53.32; H, 7.99; N, 13.32. Found: C, 53.22; H, 7.90; N, 13.20. MS-FAB$^+$ (*m*/*z*): 316 $[M+H]^+$.

4.5. Hydrogenolysis of adduct (10)

A spatula of Raney-Ni carefully washed with methanol and then ethyl acetate, was added to a solution of compound **10** (0.1 mmol) in ethyl acetate (4 mL). The mixture was stirred vigorously under hydrogen for 2 h, then filtered through Celite. The solvent was removed in vacuo and the residue was purified by means of flash chromatography (SiO_2, ethyl acetate/methanol=98/2, developer: I_2).

*4.5.1. (2S,3S,4R)-2,6-Diamino-3,4-dihydroxy-3-methyl-heptanoic acid methyl ester (**14**).* Waxy solid (25%). $[\alpha]_{D20}$=−33.2 (*c* 0.15, CH_3OH). ^1H NMR: δ 1.15 (d, *J*=6.0, 3H, 7-CH_3); 1.3 (broad s, 1H, H-5); 1.33 (s, 3H, 3-CH_3); 1.63 (broad, 6H, OH, NH_2); 1.87 (broad m, 1H, H-5); 2.74 (m, 1H, H-6); 3.25 (broad s, 1H, H-2); 3.31 (dd, *J*=11.4, 4.9, 1H, H-4); 3.76 (s, 3H, OCH_3). ^{13}C NMR: δ 21.1, 21.9 (3-CH_3, C-7); 39.3 (C-5); 49.6 (C-6); 52.1 (OCH_3); 65.9 (C-2); 70.2 (C-3); 73.6 (C-4); 171.3 (C=O). IR (Nujol): 3350 (ν_{OH}, ν_{NH}, OH, NH_2), 1740 ($\nu_{C=O}$, C=O). HRMS (FT-ICR)-EI$^+$ (*m*/*z*): 204.1230 $[M-NH_3+H]^+$.

4.6. Single crystal structural determination of (4) and (5)

The intensity data for **4** and **5** were collected on a Bruker Smart Apex CCD area detector using graphite-monochromated Mo Kα radiation (λ=0.71073 Å). Data reduction was made using SAINT programs; absorption corrections based on multiscan were obtained by SADABS.[37] The structures were solved by SHELXS-97[38] and refined on F^2 by full-matrix least-squares using SHELXL-97.[39] All the non-hydrogen atoms were refined anisotropically, hydrogen atoms were included as 'riding' and not refined. The isotropic thermal parameters of H atoms were fixed at 1.2 (1.5 for methyl groups) times the equivalent thermal parameter of the atoms to which corresponding H atoms are bonded.

Crystal data and results of the refinement: (i) compound **4**, colourless prism 0.31×0.20×0.18 mm, M_r=311.38, orthorhombic, space group $P2_12_12_1$, *a*=6.5252 (8) Å, *b*=6.8964 (9) Å, *c*=37.043 (5) Å, *V*=1667.0 (4) Å^3, *Z*=4, *T*=100 (2) K, μ=0.090 mm^{-1}. 25,683 measured reflections, 2408 independent reflections, 2268 reflections with $I>2\sigma(I)$, $2.20<2\theta<56.90^\circ$, R_{int}=0.0292. Refinement on 2408 reflections, 206 parameters. Flack parameter[39] for determination of the absolute configuration=−0.5 (13). Final *R*=0.0352, *wR*=0.1007 for data with $F^2>2\sigma(F^2)$, *S*=1.162, $(\Delta/\sigma)_{max}$=0.001, $\Delta\rho_{max}$=0.386, $\Delta\rho_{min}$=−0.271 eÅ^{-3}. (ii) Compound **5**, colourless prism 0.38×0.35×0.18 mm, M_r=311.38, monoclinic, space group $P2_1$, *a*=9.2885 (4) Å, *b*=8.7761 (3) Å, *c*=10.1421 (4) Å, β=90.594 (2)°, *V*=826.71 (6) Å^3, *Z*=2, *T*=173 (2) K, μ=0.090 mm^{-1}. 6145 measured reflections, 1931 independent reflections, 1749 reflections with $I>2\sigma(I)$, $4.02<2\theta<55.00^\circ$, R_{int}=0.0320. Refinement on 1931 reflections, 205 parameters, one restraint generated for floating origin. Flack parameter[39] for determination of the absolute configuration=0.3 (13). Final *R*=0.0398, *wR*=0.1018 for data with $F^2>2\sigma(F^2)$, *S*=1.085, $(\Delta/\sigma)_{max}$=0.001, $\Delta\rho_{max}$=0.269, $\Delta\rho_{min}$=−0.420 eÅ^{-3}. Crystallographic data (excluding structure factors) for **4** and **5** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 784316 (**4**) and 784317 (**5**). 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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Supplementary data

Copies of ^1H and ^{13}C NMR spectra of all compounds, NOESY experiments, HPLC chromatograms and X-ray figures. Supplementary data related to this article can be found online at doi:10.1016/j.tet.2011.02.055. These data include MOL files and InChIKeys of the most important compounds described in this article.

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