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Stereoselective synthesis of β,ε-dihydroxy-α-amino acids by ring opening of 4,5-dihydroisoxazolyl derivatives

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ABSTRACT

Enantiomerically pure β-(4,5-dihydroisoxazol-3-yl)-substituted β-hydroxy-α-amino acids were synthesised stereoselectively by means of an addition reaction between (5,5-disubstituted-4,5-dihydroisoxazol-3-yl)-carbaldehydes and (*R*)-(+)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine (*Schöllkopf*'s reagent) as a chiral auxiliary. The addition gave mixtures of only two adducts with high diastereoselectivity. The steric configuration of the major diastereoisomer was assigned on the basis of spectroscopic data and the accepted model for the aldol condensation of the *Schöllkopf*'s reagent. The subsequent acid-catalysed hydrolysis of the pyrazine ring led to a mixture of β-(4,5-dihydroisoxazol-3-yl)-β-hydroxy-α-amino methyl esters together with a mixture of two partially hydrolysed dipeptides. The final reductive cleavage of the 4,5-dihydroisoxazole ring of these compounds made it possible to obtain the methyl esters of β,ε-dihydroxy-γ-oxo-ε,ε-disubstituted α-amino acid derivatives.

1. Introduction

β-Hydroxy-α-amino acids are the key structures of many biologically active natural products such as vancomycin[1a] or polyoxins,[1b] cyclomarins[1c] and exochelins;[1d] their highly functionalised nature also makes them very useful chiral building blocks for the synthesis of numerous compounds.[2]

Various approaches have already been developed for their stereoselective synthesis,[3] including asymmetric aldol reactions using chiral auxiliaries[4] or chiral catalysts.[5]

We had previously used '*Schöllkopf*'s reagent' (i.e., (*R*) or (*S*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine) to synthesise β-heteroaryl-substituted alanines[6a] and serines,[6b] the antibiotic azatyrosine,[6c] δ-heteroaryl-substituted β-hydroxy-γ,δ-unsaturated α-amino acids[6d] and, more recently, β-hydroxy-α-amino acids β-substituted with non-aromatic heterocycles.[6e] This reagent is a particularly attractive chiral auxiliary because both the enantiopure (*R*)- and (*S*)-forms are commercially available, and have been used in various asymmetric syntheses.[7]

Our interest in the stereoselective synthesis of new non-proteinogenic β-hydroxy-α-amino acids substituted with heterocyclic rings led us to consider the reactions between the *Schöllkopf*'s reagent (*R*)-**1** and the 4,5-dihydroisoxazole-3-carbaldehydes. The 4,5-dihydroisoxazole (2-isoxazoline) nucleus constitutes the basic skeleton of numerous compounds with broad biological activities,[8] and the particular nature of the 4,5-dihydroisoxazole ring is its versatility as a synthetic intermediate because of the ease with which it can be prepared and the numerous possible chemical transformations. Among these, reductive cleavage yields functional groups such as β-hydroxy carbonyls[9] or γ-amino alcohols[10] depending on the experimental conditions. In this way, reaction between 2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine (*R*)-**1** and the 4,5-dihydroisoxazole-3-carbaldehydes **A**, and the subsequent cleavage of the dihydropyrazine and isoxazoline rings of adducts, could allow us to obtain β,ε-dihydroxy-γ-oxo-α-amino acids **B** (Fig. 1).

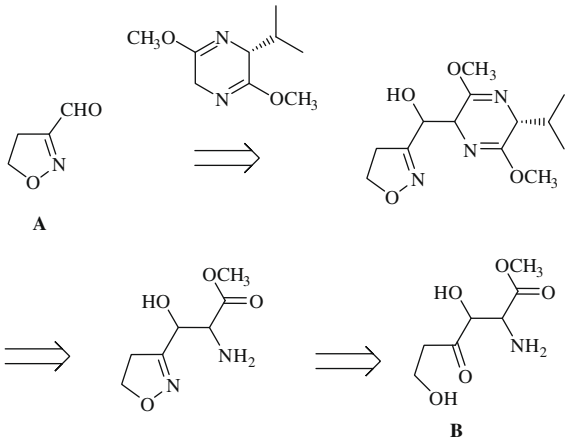

Figure 1.

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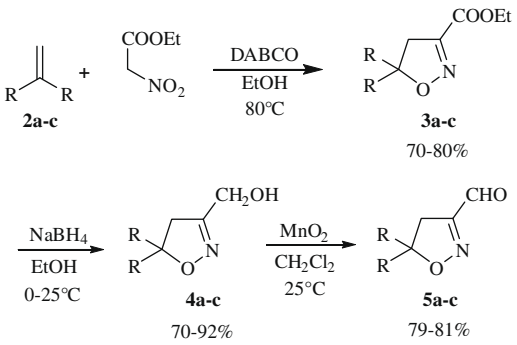
To the best of our knowledge, these polyfunctionalised α-amino acids (with a structure similar to carbohydrates) are not known, and this prompted us to take this study further.

2. Results and discussion

The absence of a racemic stereocentre in the isoxazoline ring of the aldehydes **A** allows us to minimise the total number of diastereoisomers that are derivable from the reaction with *Schöllkopf*'s reagent. As a result, we decided to use 4,5-dihydroisoxazole-3-carbaldehydes disubstituted with two identical groups at the position 5 of the ring. Given the commercial availability of alkene precursors of the isoxazoline ring, we selected aldehydes **5a–c** (Scheme 1), which were not known but were prepared by means of a base-catalysed condensation between ethyl nitroacetate and the 1,1-disubstituted alkenes **2a–c** in accordance with a recently reported method.[11] The 5,5-disubstituted-4,5-dihydroisoxazole-3-carboxylic acid ethyl esters **3a–c** were obtained in good yields and complete regioselectivity (Scheme 1). This is the first example of the application of this method to 1,1-disubstituted alkenes.



2,3,4,5a : R = Ph

2,3,4,5b : R = -$(CH_2)_5$-

2,3,4,5c : R = CH_3

Scheme 1.

Subsequent sodium borohydride reduction[12] of the ester group of **3a–c** to alcohols **4a–c** and oxidation of the latter with manganese dioxide led to aldehydes **5a–c**. The new products **3**, **4** and **5a–c** were completely characterised by means of their analytical and spectroscopic data.

In accordance with a general procedure, a solution of aldehydes **5a–c** was added to the anion of the bislactim ether (*R*)-**1** generated by *n*BuLi in THF at $T = -78$ °C. 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 integrating the pairs of doublets corresponding to the isopropyl groups in the ^{1}H NMR spectra (Scheme 2 and Table 1).

The major diastereoisomers **6a–c** 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 **6a–c** was established using the $^5J_{5H/2H}$ coupling constant value of approximately 3.5 Hz, which corresponded to a *trans*-relationship between the 5-H and 2-H protons of the pyrazine ring.[13] The (*S*)-configuration was assigned at 1′-C by comparing the chemical shifts in the ^{1}H and ^{13}C NMR spectra of diastereoisomers **6a–c** and those reported for the known 5-phenylisoxazol-3-yl and (5-*p*-tolyl-isoxazol-3-yl)-ethenyl derivatives.[6a,d] These configurations also agree with the widely accepted model for the aldol-type addition of **1** to aldehydes,[14,6e] according to which the exclusive formation of the two (2*S*)-epimers can be explained via 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 (1′*S*)-epimer comes from a more favourable transition state in which the aldehyde substituent is far from the methoxy group and from the metal atom (Fig. 2).

Table 1

5,6	R	Total yield (%)	Diastereomeric ratio[a]
a	Ph	66	93:7
b	–$(CH_2)_5$–	68	95:5
c	CH_3	64	90:10

[a] It was not possible to isolate the minor diastereoisomer whose configuration remains unknown.

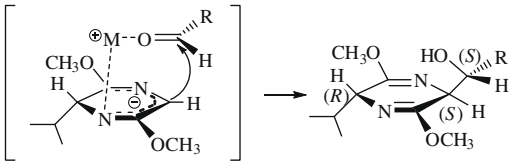
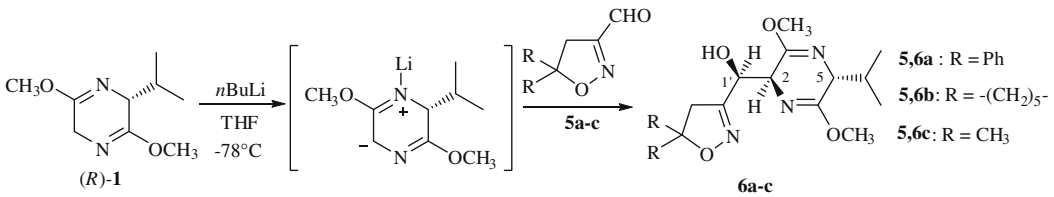


Figure 2. Favoured Transition State leading to the major diastereoisomeric adducts.

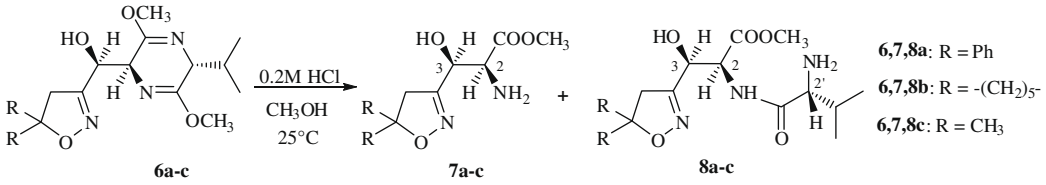
These results are interesting because the reactions were extremely diastereoselective, even though lithium was used as a counter-ion (Table 1). It is well known that the use of titanium, rather than lithium, generally makes the reaction more diastereoselective because of the tight transition state promoted by the titanium ligand.[6d,13a]

Adducts **6a–c** were hydrolysed under controlled conditions: they were treated with 3 equiv of 0.2 M HCl in methyl alcohol at room temperature for 6 h, which allowed the isolation of β-hydroxy-α-amino esters **7a–c** and dipeptides **8a–c** (Scheme 3 and Table 2).

The ^{1}H NMR spectra of the crude reaction mixtures also revealed the presence of less than 6–8% of the other possible dipeptide. The formation of these dipeptides is due to the partial hydrolysis of the pyrazine ring that often occurs with the hydrolysis reaction.[6e,15] We were unable to avoid this course even when changing the solvent (methyl alcohol, acetonitrile or THF), temperature (from $T = 0$ °C to room temperature), or the kind (HCl or TFA) or concentration of the acid (from 0.2 M to 2 M). Products **7** and **8** were easily separated by means of column chromatography, and



Scheme 2.



Scheme 3.

Table 2

6,7,8	R	Yield (%)	
		7	8
a	Ph	20	48
b	$-(CH_2)_5-$	28	42
c	CH_3	20	63

their structure was assigned using analytical and spectroscopic data. In particular, the ^{1}H NMR spectra made it possible to assign the structure of compounds **8**: these dipeptides formally derive from the formation of an amide bond between the valine carboxylic group and the amino group of amino esters **7**. For example, the ^{1}H NMR spectra of **8a** show a dd at 4.93 δ for proton 2-H with a $J_{2H/3H}$ = 3.0 Hz with the doublet at 5.00 δ relative to 3-H, and with a $J_{2H/NH}$ = 9.3 Hz with the doublet at 8.05 δ relative to a proton which integrates 1H and which exchanges with D_2O (NH amide).[15b,c]

Finally, we carried out a hydrogenolysis–hydrolysis of the 4,5-dihydroisoxazole ring of the amino esters **7** and of the dipeptides **8** using the optimum reaction conditions reported by Curran for this type of cleavage (H_2 1 atm, 5/1 MeOH/H_2O, 3 equiv of $B(OH)_3$, Raney-Ni as catalyst).[9b] Starting from products **7b–c**, we could not isolate the expected products, owing to a complete degradation of compounds **7**. The same result was observed using HCl instead of $B(OH)_3$ or Pd/C as a catalyst. Conversely, cleavage of dipeptides **8b–c** allowed us to obtain the corresponding β,ε-dihydroxy-γ-oxo α-amino acid derivatives **9b–c** in good yields (Scheme 4). In no case we were able to detect any loss of stereochemical purity.

These α-amino acid derivatives have a highly functionalised structure, which makes them extremely attractive as potential peptidomimetics.

3. Conclusion

We have reported another example of the synthesis of new, enantiomerically pure, non-proteinogenic, β-(4,5-dihydroisoxazol-3-yl)-substituted β-hydroxy-α-amino acids **7** and dipeptides **8** using the '*Schöllkopf*'s reagent' as a chiral auxiliary. The hydrogenolysis–hydrolysis of products **8** allowed us to obtain the corresponding β,ε-dihydroxy-γ-oxo α-amino acid derivatives **9**, but the hydrogenolysis of products **7** failed. However, compound **7** may be interesting because 2-isoxazoline derivatives have been used as dipeptide bioisosteres[16] and have been incorporated into biologically active compounds, such as the anti-tumour drugs acivicin.[17]

4. Experimental

4.1. General methods

Melting points were measured using a *Büchi* apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using a *Bruker AC 300* spectrometer; the chemical shifts (δ) are given in ppm relative to TMS, and all the coupling constants 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. The IR spectra (in cm^{-1}) were determined using a *Jasco FT-IR 4100* spectrometer.

(*R*)-2,5-Dihydro-3,6-dimethoxy-2-isopropylpyrazine **1**, ethyl nitroacetate and the 1,1-disubstituted alkenes **2a–c** were obtained from commercial sources.

4.2. General procedure for the preparation of compounds 3a–c

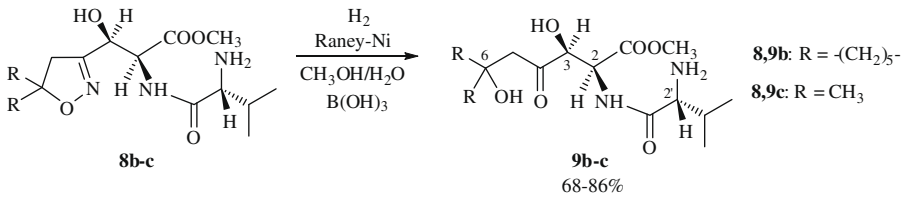
A solution of alkene **2a–c** (5 mmol, 1 equiv), ethyl nitroacetate (10 mmol, 2 equiv) and DABCO (0.5 mmol, 0.1 equiv) in ethanol (20 mL) was heated at 80 °C for five days in a sealed tube. The organic solvent was evaporated off, and the products were purified by column chromatography on silica gel (hexane/ethyl acetate = 8/2).

4.3. 5,5-Diphenyl-4,5-dihydro-isoxazole-3-carboxylic acid ethyl ester 3a[18]

Colourless solid (75%); mp 87–88 °C (Et_2O); ^{1}H NMR: δ 1.36 (3H, t, J = 7.1, CH_3); 3.85 (2H, s, 4-H); 4.33 (2H, q, J = 7.1, CH_2); 7.30–7.50 (10H, m, Ph). ^{13}C NMR: δ 13.94 (CH_3); 46.61 (4-C); 61.94 (O–CH_2); 94.63 (5-C); 125.59, 127.86, 128.54, 142.87 (C–Ph); 150.97 (3-C); 160.37 (C=O). MS-EI (m/z): 295 (M^+). Anal. Calcd for $C_{18}H_{17}NO_3$: C, 73.20; H, 5.80; N, 4.74. Found: C, 72.99; H, 5.88; N, 4.70. IR (Nujol): 1717 ($\nu_{C=O}$, C=O and $\nu_{C=N}$, C=N).

4.4. 1-Oxa-2-aza-spiro[4.5]dec-2-ene-3-carboxylic acid ethyl ester 3b[19]

Colourless liquid (70%); ^{1}H NMR: δ 1.37 (3H, t, J = 7.1, CH_3); 1.40–1.90 (10H, m, $-(CH_2)_5-$); 2.89 (2H, s, 4-H); 4.31 (2H, q,



Scheme 4.

J = 7.1, CH_2). ^{13}C NMR: δ 13.79 (CH_3); 22.82, 24.48, 35.94 (–$(CH_2)_5$–); 43.0 (4-C); 61.4 (O–CH_2); 90.36 (5-C); 150.48 (3-C); 160.77 (C=O). MS-EI (m/z): 211 (M^+). IR (Nujol): 1717 ($\nu_{C=N}$, C=N), 1740 ($\nu_{C=O}$, C=O).

4.5. 5,5-Dimethyl-4,5-dihydro-isoxazole-3-carboxylic acid ethyl ester 3c[20]

Colourless liquid (80%); ^1H NMR: δ 1.40 (3H, t, J = 7.1, CH_3); 1.49 (6H, s, 5,5-di-CH_3); 2.99 (2H, s, 4-H); 4.37 (2H, q, J = 7.1, CH_2). ^{13}C NMR: δ 13.71 (CH_3); 26.97 (5,5-di-CH_3); 45.0 (4-C); 61.61 (O–CH_2); 88.2 (5-C); 150.84 (3-C); 160.84 (C=O). MS-EI (m/z): 171 (M^+). IR (Nujol): 1722 ($\nu_{C=N}$, C=N), 1728 ($\nu_{C=O}$, C=O).

4.6. General procedure for the preparation of compounds 4a–c

A solution of ester **3a**–**c** (10 mmol, 1 equiv) in ethanol (10 mL) (ethanol/CH_2Cl_2 = 1/1 for **3a**) was added dropwise to a cold (T = 0 °C) suspension of $NaBH_4$ (26 mmol, 2.6 equiv) in ethanol (20 mL). The reaction mixture was stirred at room temperature for 6 h. The organic solvent was evaporated off, and the residue was poured into water. Acetic acid was added until pH 6, and the mixture was extracted with several portions of ethyl acetate. The combined extracts were dried over Na_2SO_4 and concentrated at reduced pressure. The crude alcohols were purified by column chromatography on silica gel (hexane/ethyl acetate = 6/4).

4.7. (5,5-Diphenyl-4,5-dihydro-isoxazol-3-yl)-methanol 4a[20]

Colourless solid (92%); mp 103–104 °C (Et_2O); ^1H NMR: δ 1.70 (1H, br, OH); 3.69 (2H, s, 4-H); 4.40 (2H, s, CH_2-O); 7.25–7.45 (10H, m, Ph). ^{13}C NMR: δ 47.84 (4-C); 57.70 (CH_2–O); 91.49 (5-C); 125.84, 127.51, 128.27, 143.68 (C–Ph); 158.84 (3-C). MS-EI (m/z): 253 (M^+). Anal. Calcd for $C_{16}H_{15}NO_2$: C, 75.87; H, 5.97; N, 12.63. Found: C, 75.82; H, 5.85; N, 12.70. IR (Nujol): 3187 (ν_{O-H}, OH), 1650 ($\nu_{C=N}$, C=N).

4.8. (1-Oxa-2-aza-spiro[4.5]dec-2-en-3-yl)-methanol 4b

Colourless liquid (89%); ^1H NMR: δ 1.46–1.82 (10H, m, –$(CH_2)_5$–); 2.54 (1H, br, OH); 2.78 (2H, s, 4-H); 4.39 (2H, s, CH_2–O). ^{13}C NMR: δ 23.81, 25.4, 36.74 (–$(CH_2)_5$–); 45.33 (4-C); 58.86 (CH_2–O); 87.47 (5-C); 158.51 (3-C). MS-EI (m/z): 169 (M^+). IR (Nujol): 3374 (ν_{O-H}, OH), 1625 ($\nu_{C=N}$, C=N).

4.9. (5,5-Dimethyl-4,5-dihydro-isoxazol-3-yl)-methanol 4c[20]

Colourless liquid (70%); ^1H NMR: δ 1.45 (6H, s, 5,5-di-CH_3); 2.40 (1H, br, OH); 2.84 (2H, s, 4-H); 4.41 (2H, s, CH_2–O). ^{13}C NMR: δ 26.93 (5,5-di-CH_3); 46.61 (4-C); 57.84 (CH_2–O); 84.57 (5-C); 158.81 (3-C). MS-EI (m/z): 129 (M^+). IR (Nujol): 3387 (ν_{O-H}, OH), 1625 ($\nu_{C=N}$, C=N).

4.10. General procedure for the preparation of compounds 5a–c

MnO_2 (5/1 = w/w) was added to a solution of alcohol **4a**–**c** (10 mmol) in CH_2Cl_2 (15 mL), and the reaction mixture was stirred at room temperature for 12 h. The MnO_2 was filtered through Celite, and the organic solvent was evaporated off. The resulting aldehydes were pure enough for the subsequent reaction.

4.11. 5,5-Diphenyl-4,5-dihydro-isoxazole-3-carbaldehyde 5a

Colourless solid (81%); mp 86–88 °C (*n*-hexane); ^1H NMR: δ 3.76 (2H, s, 4-H); 7.30–7.40 (10H, m, Ph); 9.95 (1H, s, CHO). ^{13}C NMR: δ 43.34 (4-C); 95.87 (5-C); 125.74, 128.03, 128.45, 142.57 (C–Ph); 158.71 (3-C); 185.43 (CO). MS-EI (m/z): 251 (M^+). Anal. Calcd for $C_{16}H_{13}NO_2$: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.12; H, 5.11; N, 5.71. IR (Nujol): 1698 ($\nu_{C=O}$, C=O), 1576 ($\nu_{C=N}$, C=N).

4.12. 1-Oxa-2-aza-spiro[4.5]dec-2-ene-3-carbaldehyde 5b

Colourless liquid (79%); ^1H NMR: δ 1.50–1.90 (10H, m, –$(CH_2)_5$–); 2.84 (2H, s, 4-H); 9.95 (1H, s, CHO). ^{13}C NMR: δ 23.09, 24.71, 36.28 (–$(CH_2)_5$–); 40.13 (4-C); 92.43 (5-C); 158.01 (3-C); 186.43 (CO). MS-EI (m/z): 167 (M^+). IR (Nujol): 1695 ($\nu_{C=O}$, C=O), 1573 ($\nu_{C=N}$, C=N).

4.13. 5,5-Dimethyl-4,5-dihydro-isoxazole-3-carbaldehyde 5c

Colourless liquid (80%); ^1H NMR: δ 1.51 (6H, s, 5,5-di-CH_3); 2.90 (2H, s, 4-H); 9.93 (1H, s, CHO). ^{13}C NMR: δ 27.11 (5,5-di-CH_3); 41.87 (4-C); 89.92 (5-C); 159.17 (3-C); 186.17 (CO). MS-EI (m/z): 127 (M^+). IR (Nujol): 1692 ($\nu_{C=O}$, C=O), 1572 ($\nu_{C=N}$, C=N).

4.14. General procedure for the reactions of (*R*)-1 with 5a–c

To a solution of (*R*)-**1** (0.47 mL, 2.62 mmol, 1.05 equiv) in anhydrous THF (3 mL), cooled at –78 °C, butyl lithium (1.8 mL of a 1.6 M solution in hexane, 2.88 mmol, 1.15 equiv) was added, and the mixture was stirred for 45 min. The appropriate aldehyde **5a**–**c** (2.5 mmol, 1 equiv) in THF (3 mL) was added, and the mixture was stirred at −78 °C for 1 h, and then at −18 °C for 16 h. The reaction mixture was allowed to warm to 0 °C, after which 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 **6a**–**c** were purified by means of flash chromatography on silica gel (hexane/ethyl acetate = 80/20).

4.15. (*S*)-(5,5-Diphenyl-4,5-dihydro-isoxazol-3-yl)-[(2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazin-2-yl]-methanol 6a

Colourless solid (60.5%); mp 170–171 °C (*n*-hexane); $[\alpha]_D^{20}$ = −14.4 (*c* 0.60, $CHCl_3$). ^1H NMR: δ 0.67, 1.00 (6H, 2d, J = 6.8, $CH(CH_3)_2$); 2.22 (1H, m, $CH(CH_3)_2$); 2.79 (1H, d, J = 7.7, OH); 3.51 (3H, s, OCH_3); 3.67 (3H, s, OCH_3); 3.77 (2H, s, 4-CH_2); 3.93 (1H, t, J = 3.5, 5-H); 4.19 (1H, t, J = 3.5, 2-H); 4.92 (1H, dd, J = 7.7, 3.5, 1′-H); 7.25–7.45 (10H, m, Ph); (by deuteration, the signal at 2.79 disappeared, and the signal at 4.92 turned into a doublet with J = 3.5). ^{13}C NMR: δ 16.85, 19.05 ($CH(CH_3)_2$); 32.02 ($CH(CH_3)_2$); 48.81 (4-C); 52.99 (3- and 6-OCH_3); 60.68, 61.65 (2-C and 5-C pyr.); 69.54 (1′-C); 92.16 (5-C isox.); 126.41, 127.86, 128.54, 145.05 (Ph); 160.42, 161.22, 167.51 (3-C and 6-C pyr., 3-C isox.). MS-EI (m/z): 435 (M^+), 300, 185. Anal. Calcd for $C_{25}H_{29}N_3O_4$: C, 68.95; H, 6.71; N, 9.65. Found: C, 68.9; H, 6.54; N, 9.45. IR (Nujol): 3381 (ν_{OH}, OH), 1697 ($\nu_{C=N}$, C=N).

4.16. (*S*)-[(2*S*,5*R*)-5-Isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazin-2-yl]-(1-oxa-2-aza-spiro[4.5]dec-2-en-3-yl)-methanol 6b

Colourless solid (64.6%); mp 133–136 °C (*n*-hexane); $[\alpha]_D^{20}$ = −45.0 (*c* 0.58, $CHCl_3$). ^1H NMR: δ 0.75, 1.08 (6H, 2d, J = 6.8, $CH(CH_3)_2$); 1.47–1.92 (10H, m, –$(CH_2)_5$–); 2.29 (1H, m, $CH(CH_3)_2$); 2.78 (1H, d, J = 7.5, OH); 2.86 (2H, s, 4-CH_2); 3.71 (3H, s, OCH_3); 3.79 (3H, s, OCH_3); 4.06 (1H, t, J = 3.5, 5-H); 4.22 (1H, t, J = 3.5, 2-H); 4.91 (1H, dd, J = 7.5, 3.5, 1′-H); (by deuteration, the signal at 2.78 disappeared, and the signal at 4.91 turned into a doublet with J = 3.5). ^{13}C NMR: δ 16.76, 19.01 ($CH(CH_3)_2$); 23.47, 25.08, 36.34 (–$(CH_2)_5$–); 31.84 ($CH(CH_3)_2$); 44.94 (4-C); 52.73 (3- and 6-OCH_3); 59.07, 61.00 (2-C and 5-C pyr.); 69.33 (1′-C); 87.06 (5-C isox.); 159.12, 161.03, 166.51 (3-C and 6-C pyr., 3-C isox.). MS-EI (m/z):

351 (M^+), 308, 290, 183. Anal. Calcd for $C_{18}H_{29}N_3O_4$: C, 61.52; H, 8.32; N, 11.96. Found: C, 61.47; H, 8.25; N, 11.94. IR (Nujol): 3407 (ν_{OH}, OH), 1696 ($\nu_{C=N}$, C=N).

4.17. (*S*)-(5,5-Dimethyl-4,5-dihydro-isoxazol-3-yl)-[(2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazin-2-yl]-methanol 6c

Colourless solid (57.6%); mp 86–88 °C (*n*-hexane); $[\alpha]_D^{20} = -57.2$ (*c* 0.84, $CHCl_3$). ^{1}H NMR: δ 0.75, 1.08 (6H, 2d, *J* = 6.8, CH(C*H*$_3$)$_2$); 1.46, 1.47 (6H, 2s, 5,5-di-CH$_3$); 2.31 (1H, m, C*H*(CH$_3$)$_2$); 2.81 (1H, d, *J* = 8.0, OH); 2.90 (2H, s, 4-CH$_2$); 3.72 (3H, s, OCH$_3$); 3.79 (3H, s, OCH$_3$); 4.06 (1H, t, *J* = 3.5, 5-H); 4.22 (1H, t, *J* = 3.5, 2-H); 4.91 (1H, dd, *J* = 8.0, 3.5, 1′-H); (by deuteration, the signal at 2.81 disappeared, and the signal at 4.91 turned into a doublet with *J* = 3.5). ^{13}C NMR: δ 16.63, 18.92 (CH(*C*H$_3$)$_2$); 26.91, 27.01 (5,5-di-CH$_3$); 31.70 (*C*H(CH$_3$)$_2$); 46.73 (4-C); 52.61 (3- and 6-OCH$_3$); 59.15, 60.84 (2-C and 5-C pyr.); 69.09 (1′-C); 84.69 (5-C isox.); 159.55, 160.30, 166.28 (3-C and 6-C pyr., 3-C isox.). MS-EI (*m*/*z*): 311 (M^+), 296, 253, 183. Anal. Calcd for $C_{15}H_{25}N_3O_4$: C, 57.86; H, 8.09; N, 13.49. Found: C, 57.77; H, 8.18; N, 13.36. IR (Nujol): 3430 (ν_{OH}, OH), 1697 ($\nu_{C=N}$, C=N).

4.18. General procedure for the hydrolysis of adducts 6a-c

Adducts **6a**–**c** (0.5 mmol) were dissolved in methyl alcohol (7.5 mL), and a 0.2 M solution of HCl (7.5 mL, 1.5 mmol, 3 equiv) was added. The mixture was stirred for 6 h at room temperature, and then extracted with diethyl ether in order to remove non-basic organic compounds. The mixture was diluted with $CHCl_3$ (20 mL), and 25% ammonia was added until pH 8–10 under stirring. The layers were separated, and the aqueous layer was extracted twice with $CHCl_3$ (2 × 20 mL). The combined organic layers were dried with Na_2SO_4, and the solvent was removed in vacuo. Compounds **7** and **8** were separated by flash chromatography (SiO_2, ethyl acetate:methanol = 98:2, developer: I_2 or ninhydrine).

4.19. (2*S*,3*S*)-2-Amino-3-(5,5-diphenyl-4,5-dihydro-isoxazol-3-yl)-3-hydroxy-propionic acid methyl ester 7a

Amorphous solid (20%); R_f = 0.52 (ethyl acetate:methanol = 98:2); $[\alpha]_D^{20} = +8.0$ (*c* 0.37, $CHCl_3$). ^{1}H NMR: δ 1.80–2.20 (3H, br, OH, NH$_2$); 3.73 (3H, s, OCH$_3$); 3.72, 3.83 (2H, AB-system, J_{AB} = 17.2, 4-CH$_2$-isox.); 3.99 (1H, br d, *J* = 3.2, 2-H); 4.69 (1H, br d, *J* = 3.2, 3-H); 7.3–7.5 (10H, m, Ph). ^{13}C NMR: δ 47.93 (4-C-isox.); 52.80 (OCH$_3$); 56.03 (2-C); 67.95 (3-C); 93.01 (5-C-isox.); 125.94, 126.07, 127.68, 128.40, 143.0 (Ph); 158.23, 172.55 (C=N, C=O). MS-FAB$^+$ (*m*/*z*): 341 (MH^+). IR (Nujol): 3357 (ν_{OH}, ν_{NH}, OH, NH$_2$), 1742 ($\nu_{C=O}$, C=O), 1671 ($\nu_{C=N}$, C=N).

4.20. (2*S*,3*S*,2′*R*)-2-(2-Amino-3-methyl-butyrylamino)-3-(5,5-diphenyl-4,5-dihydro-isoxazol-3-yl)-3-hydroxy-propionic acid methyl ester 8a

Amorphous solid (48%); R_f = 0.25 (ethyl acetate:methanol = 98:2); $[\alpha]_D^{20} = -1.8$ (*c* 1.13, $CHCl_3$). ^{1}H NMR: δ 0.78, 0.89 (6H, 2d, *J* = 6.8, CH(C*H*$_3$)$_2$); 1.70–2.10 (3H, br, OH, NH$_2$); 2.16 (1H, m, C*H*(CH$_3$)$_2$); 2.97 (1H, m, 2′-H); 3.62, 3.76 (2H, AB-system, J_{AB} = 17.1, 4-CH$_2$-isox.); 3.71 (3H, s, OCH$_3$); 4.93 (1H, dd, *J* = 9.3, 3.0, 2-H); 5.00 (1H, br d, *J* = 3.0, 3-H); 7.25–7.5 (10H, m, Ph); 8.05 (1H, d, *J* = 9.3, NH–CO); (by deuteration, the signals at 1.7–2.1 and 8.05 disappeared, and the signals at 2.97, 4.93 and 5.00 turned into three doublets with *J* = 3.4, 3.2 and 3.2, respectively). ^{13}C NMR: δ 16.19, 19.34 (CH(*C*H$_3$)$_2$); 30.81 (*C*H(CH$_3$)$_2$); 47.39 (4-C-isox.); 52.74 (OCH$_3$); 54.69 (2′-C); 59.73 (2-C); 69.05 (3-C); 92.58 (5-C-isox.); 125.71, 126.02, 127.67, 128.36, 143.55 (Ph); 158.56, 169.97, 174.41 (C=N, C=O ester and amide). MS-FAB$^+$ (*m*/*z*): 440 (MH^+). IR (Nujol): 3351 (ν_{OH}, ν_{NH}, OH, NH$_2$), 1743 ($\nu_{C=O}$, C=O ester), 1666 ($\nu_{C=N}$, $_{C=O}$, C=N and C=O amide).

4.21. (2*S*,3*S*)-2-Amino-3-hydroxy-3-(1-oxa-2-aza-spiro[4.5]dec-2-en-3-yl)-propionic acid methyl ester 7b

Amorphous solid (28%); R_f = 0.45 (ethyl acetate:methanol = 7:3); $[\alpha]_D^{20} = 15.4$ (*c* 0.22, CH_3OH). ^{1}H NMR: δ 2.50–2.80 (3H, br, OH, NH$_2$); 2.69, 2.86 (2H, AB-system, J_{AB} = 17.1, 4-CH$_2$-isox.); 3.78 (3H, s, OCH$_3$); 4.01 (1H, br d, *J* = 2.68, 2-H); 4.65 (1H, br d, *J* = 2.68, 3-H). ^{13}C NMR: δ 23.40, 25.00, 36.36 (–(CH$_2$)$_5$–); 44.87 (4-C-isox.); 52.55 (OCH$_3$); 56.22 (2-C); 68.80 (3-C); 87.22 (5-C-isox.); 158.50, 172.92 (C=N, C=O). MS-FAB$^+$ (*m*/*z*): 257 (MH^+). IR (Nujol): 3363 (ν_{OH}, ν_{NH}, OH, NH$_2$), 1740 ($\nu_{C=O}$, C=O), 1680 ($\nu_{C=N}$, C=N).

4.22. (2*S*,3*S*,2′*R*)-2-(2-Amino-3-methyl-butyrylamino)-3-hydroxy-3-(1-oxa-2-aza-spiro[4.5]dec-2-en-3-yl)-propionic acid methyl ester 8b

Amorphous solid (42%); R_f = 0.31 (ethyl acetate:methanol = 7:3); $[\alpha]_D^{20} = -1.7$ (*c* 0.76, $CHCl_3$). ^{1}H NMR: δ 0.89, 1.02 (6H, 2d, *J* = 6.9, CH(C*H*$_3$)$_2$); 1.30–1.80 (10H, m, –(CH$_2$)$_5$–); 2.2–2.7 (4H, br m, C*H*(CH$_3$)$_2$, OH, NH$_2$); 2.74, 2.88 (2H, AB-system, J_{AB} = 16.9, 4-CH$_2$-isox.); 3.30 (1H, br d, *J* = 3.8, 2′-H); 3.81 (3H, s, OCH$_3$); 4.92 (1H, dd, *J* = 9.3, 2.3, 2-H); 4.97 (1H, br s, 3-H); 8.23 (1H, d, *J* = 9.3, NH-CO); (by deuteration, the signals at 2.2–2.7 and 8.23 disappeared, and the signals at 3.30, 4.92 and 4.97 turned into three broad singlets). ^{13}C NMR: δ 16.11, 19.55 (CH(*C*H$_3$)$_2$); 23.32, 24.89, 36.17, 36.31 (–(CH$_2$)$_5$–); 31.08 (*C*H(CH$_3$)$_2$); 44.35 (4-C-isox.); 52.68 (OCH$_3$); 54.45 (2′-C); 60.21 (2-C); 68.90 (3-C); 87.90 (5-C-isox.); 157.83, 170.07, 175.05 (C=N, C=O ester and amide). MS-FAB$^+$ (*m*/*z*): 356 (MH^+). IR (Nujol): 3455 (ν_{OH}, ν_{NH}, OH, NH$_2$), 1738 ($\nu_{C=O}$, C=O ester), 1652 ($\nu_{C=N}$, $_{C=O}$, C=N and C=O amide).

4.23. (2*S*,3*S*)-2-Amino-3-(5,5-dimethyl-4,5-dihydro-isoxazol-3-yl)-3-hydroxy-propionic acid methyl ester 7c

Amorphous solid (20%); R_f = 0.41 (ethyl acetate:methanol = 85:15); $[\alpha]_D^{20} = +4.7$ (*c* 0.32, $CHCl_3$). ^{1}H NMR: δ 1.45, 1.47 (6H, 2s, 5,5-di-CH$_3$); 2.20–2.60 (3H, br, OH, NH$_2$); 2.81, 2.94 (2H, AB-system, J_{AB} = 16.9, 4-CH$_2$-isox.); 3.82 (3H, s, OCH$_3$); 3.99 (1H, br d, *J* = 2.8, 2-H); 4.67 (1H, br d, *J* = 2.8, 3-H). ^{13}C NMR: δ 26.81 (5,5-di-CH$_3$); 46.26 (4-C-isox.); 51.71 (OCH$_3$); 58.37 (2-C); 68.77 (3-C); 83.48 (5-C-isox.); 160.23, 173.47 (C=N, C=O). MS-FAB$^+$ (*m*/*z*): 217 (MH^+). IR (Nujol): 3332 (ν_{OH}, ν_{NH}, OH, NH$_2$), 1741 ($\nu_{C=O}$, C=O), 1676 ($\nu_{C=N}$, C=N).

4.24. (2*S*,3*S*,2′*R*)-2-(2-Amino-3-methyl-butyrylamino)-3-(5,5-dimethyl-4,5-dihydro-isoxazol-3-yl)-3-hydroxy-propionic acid methyl ester 8c

Amorphous solid (63%); R_f = 0.26 (ethyl acetate:methanol = 85:15); $[\alpha]_D^{20} = +10.9$ (*c* 1.27, $CHCl_3$). ^{1}H NMR: δ 0.91, 1.04 (6H, 2d, *J* = 6.9, CH(C*H*$_3$)$_2$); 1.40, 1.46 (6H, 2s, 5,5-di-CH$_3$); 2.05–2.15 (3H, br, OH, NH$_2$); 2.29 (1H, m, C*H*(CH$_3$)$_2$); 2.79, 2.85 (2H, AB-system, J_{AB} = 16.9, 4-CH$_2$-isox.); 3.35 (1H, br d, *J* = 3.9, 2′-H); 3.82 (3H, s, OCH$_3$); 4.97 (1H, dd, *J* = 9.2, 2.7, 2-H); 4.99 (1H, br d, *J* = 2.7, 3-H); 8.18 (1H, d, *J* = 9.2, NH–CO); (by deuteration, the signals at 2.05–2.15 and 8.18 disappeared, and the signals at 3.35, 4.97 and 4.99 turned into three sharp doublets with *J* = 3.9, 2.7 and 2.7, respectively). ^{13}C NMR: δ 16.17, 19.53 (CH(*C*H$_3$)$_2$); 27.01, 27.13 (5,5-di-CH$_3$); 31.13 (*C*H(CH$_3$)$_2$); 46.22 (4-C-isox.); 52.70 (OCH$_3$); 54.53 (2′-C); 60.26 (2-C); 69.17 (3-C); 85.57 (5-C-isox.); 158.28, 170.08, 175.12 (C=N, C=O ester and amide). MS-FAB$^+$ (*m*/*z*): 316 (MH^+).

IR (Nujol): 3423 (ν_{OH}, ν_{NH}, OH, NH_2), 1736 ($\nu_{C=O}$, C=O ester), 1654 ($\nu_{C=N}$, $_{C=O}$, C=N and C=O amide).

4.25. General procedure for the reduction of compounds 8b–c

To a solution of **8b–c** (0.4 mmol, 1 equiv) in 5/1 methanol/water (10 mL) were added boric acid (1.2 mmol, 3 equiv) and a spatula tip of Raney-Ni. The mixture was stirred vigorously under hydrogen for 6 h, and then filtered through Celite. After evaporation of the solvent, the residue was treated with water and extracted with ethyl acetate (5 × 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Compounds **9b–c** were pure enough for the spectroscopic and analytical characterisation.

4.26. (2*S*,3*S*,2′*R*)-2-(2-Amino-3-methyl-butyrylamino)-3-hydroxy-5-(1-hydroxy-cyclohexyl)-4-oxo-pentanoic acid methyl ester 9b

Amorphous solid (86%); $[\alpha]_D^{20} = +57.25$ (*c* 0.29, $CHCl_3$). ^{1}H NMR: δ 0.79, 0.95 (6H, 2d, *J* = 6.7, $CH(CH_3)_2$); 1.20–1.80 (10H, m, $-(CH_2)_5-$); 2.20 (1H, m, $CH(CH_3)_2$); 2.20–2.70 (4H, br, 2 OH, NH_2); 2.75, 2.84 (2H, AB-system, J_{AB} = 15.7, 5-CH_2); 3.20 (1H, br s, 2′-H); 3.78 (3H, s, OCH_3); 4.78 (1H, br s, 3-H); 5.20 (1H, br d, *J* = 8.4, 2-H); 7.91 (1H, br d, *J* = 8.4, NH–CO); (by deuteration, the signals at 2.20–2.70 and 7.91 disappeared, and the signals at 3.20, 4.78 and 5.20 turned into three broad singlets). ^{13}C NMR: δ 15.98, 19.49 ($CH(CH_3)_2$); 21.92, 25.36, 37.28, 38.04 ($-(CH_2)_5-$); 30.92 ($CH(CH_3)_2$); 48.80 (5-C); 52.92 (OCH_3); 53.78 (2′-C); 59.92 (2-C); 71.16 (6-C); 77.62 (3-C); 169.75, 174.89, 209.49 (C=O ester, ketone and amide). MS-FAB$^+$ (*m*/*z*): 359 (MH^+). IR (Nujol): 3347 (ν_{OH}, ν_{NH}, OH, NH_2), 1747, 1709, 1660 ($\nu_{C=O}$, C=O ketone, ester, amide).

4.27. (2*S*,3*S*,2′*R*)-2-(2-Amino-3-methyl-butyrylamino)-3,6-dihydroxy-6-methyl-4-oxo-eptanoic acid methyl ester 9c

Amorphous solid (68%); $[\alpha]_D^{20} = +6.8$ (*c* 0.28, CH_3OH). ^{1}H NMR: δ 0.81, 1.02 (6H, 2d, *J* = 6.8, $CH(CH_3)_2$); 1.26, 1.30 (6H, 2s, $C(CH_3)_2$); 2.25 (1H, m, $CH(CH_3)_2$); 2.30–2.70 (4H, br, OH, NH_2); 2.86 (2H, s, 5-CH_2); 3.21 (1H, br d, *J* = 3.3, 2′-H); 3.64 (3H, s, OCH_3); 4.76 (1H, br s, 3-H); 5.20 (1H, br d, *J* = 9.2, 2-H); 8.13 (1H, d, *J* = 9.2, NH-CO); (by deuteration, the signals at 2.30–2.70 and 8.13 disappeared, and the signals at 3.21, 4.76 and 5.20 turned into three broad singlets). ^{13}C NMR: δ 16.07, 19.40 ($CH(CH_3)_2$); 27.04, 28.14 ($C(CH_3)_2$); 31.06 ($CH(CH_3)_2$); 49.66 (5-C); 52.82 (OCH_3); 53.50 (2′-C); 60.34 (2-C); 69.94 (6-C); 77.46 (3-C); 169.67, 174.41, 209.48 (C=O ester, ketone and amide). MS-FAB$^+$ (*m*/*z*): 319 (MH^+). IR (Nujol): 3348 (ν_{OH}, ν_{NH}, OH, NH_2), 1744, 1719, 1669 ($\nu_{C=O}$, C=O ketone, ester, amide).

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18. Only the melting point of compound **3a** has been reported in Patent: US 5,516,750, 1996.
19. Compound **3b** has been mentioned in Patent: WO 2008013925, 2008, but it has not been described.
20. Compounds **3c**, **4a**, **4c** have been mentioned in: Kai, H.; Matsumoto, H.; Hattori, N.; Takase, A.; Fujiwara, T.; Sugimoto, H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1997–2000 but they have not been described.