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Stereoselective synthesis of both enantiomers of α-(hydroxymethyl)glutamic acid

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ABSTRACT

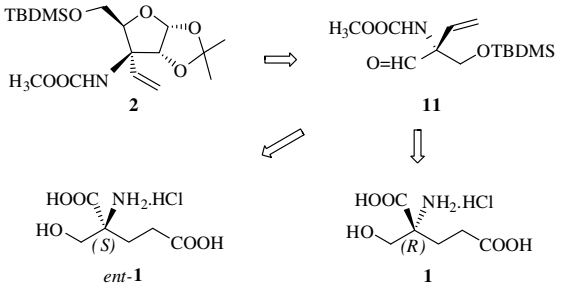
An efficient stereoselective synthesis of both enantiomers of 2-(hydroxymethyl)glutamic (HMG) acid starting from highly functionalized (2*R*)-2-(*tert*-butyldimethylsilyloxymethyl)-2-(methoxycarbonylamino)but-3-enal **11** as a suitable synthon is reported.

1. Introduction

The amino acid L-glutamate is one of the most abundant neurotransmitters found in the mammalian brain, which mediates most of the excitatory synaptic transmission. The glutamatergic pathways are involved in numerous physiological and neuropathological phenomena, such as epilepsy, ischemic brain damage, learning and memory; furthermore, they play major roles in the development of normal synaptic connections in the brain.[1]

This excitatory amino acid acts through two broad classes of the receptors: ionotropic (iGluRs) and metabotropic glutamate receptors (mGluRs). The mGlurs are coupled with G proteins inducing intracellular messenger cascades and they have been subdivided into the three functional classes based on the sequence similarity of amino acids, effector mechanisms and pharmacological relation to agonists and antagonists.[1a,g] For each of these three groups of the metabotropic glutamate receptors as mentioned above, several types of agonists and antagonists have been identified.[1,2]

It was found that the novel α-substituted α-amino acid (2*R*)-α-(hydroxymethyl)glutamic acid (HMG) **1** (Scheme 1), synthesized by Kozikowski et al.,[3] is a selective agonist of metabotropic glutamate receptor group II. Research[3] directed towards the understanding of the functions of metabotropic glutamate receptors with the goal to discover more potent selective mGluRs agonists and antagonists showed that HMG **1** acts as a selective mGluR3 agonist and is able to be a weak mGluR2 antagonist. These interesting biological findings and architecturally novel structures (α-substituted α-amino acid moiety) have interested synthetic chemists, and several total syntheses of enantiopure **1** and its antipode *ent*-**1** have been developed. For the construction of this non-proteinogenic amino acid Kozikowski et al.[3] employed a tandem Michael addition ring-closure protocol followed by the



Scheme 1.

stereoselective alkylation reaction starting from the serine derivative, Langlois et al.[4] reported the stereocontrolled synthesis of *ent*-**1** from (*S*)-pyroglutaminol, while Ohflune et al.[5a] and Ma et al.[5b] applied asymmertic Strecker syntheses for the enantioselective construction of the tetrasubstituted carbon centre of **1** and *ent*-**1**, Casiragi et al.[6] synthesized both antipodes of HMG from two intermediary lactams during the enantioselective total synthesis of a functionalized L-glutamic acid analogue, Park and Jew[7] utilized catalytic Michael addition for the enantioselective synthesis of *ent*-**1**, Hayes et al.[8] developed the synthesis of **1** using an alkylidene carbene 1,5 insertion starting from Garner's aldehyde; Miyaoka et al.[9] used a lipase catalyzed method for the preparation of a chiral oxazoline which was converted to **1** and Vassiliou et al.[10] reported the synthesis of *ent*-**1**, in which improved Schöllkopf's methodology for construction of the quaternary carbon was used.

2. Results and discussion

Our retrosynthetic analysis illustrated in Scheme 1 suggested that utilizing the functional group interconversions in the highly functionalized scaffold **11**, prepared from the protected furanose

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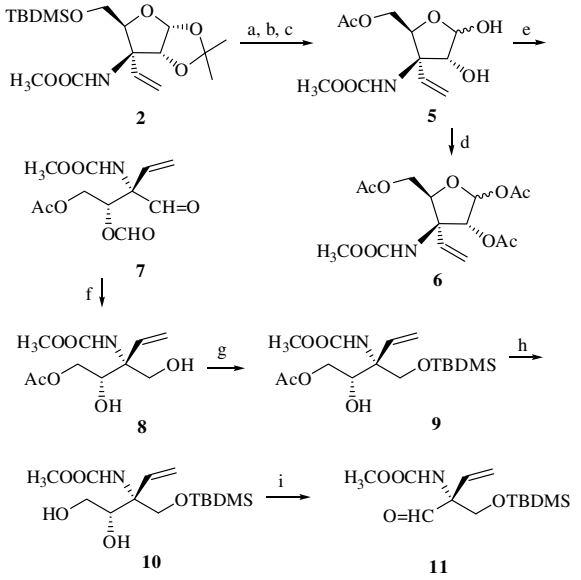
2[11] possessing a (3*S*)-configuration on the tetrasubstituted carbon stereocentre, would provide both enantiomers **1** and *ent*-**1** of 2-(hydroxymethyl)glutamic acid.

Herein, our strategy employed in the synthesis of **1** and *ent*-**1** is organized into two parts: (a) the modification of furanose derivative **2** into aldehyde **11** and (b) the functionalization of protected α-substituted scaffold **11** into **1** and its enantiomer *ent*-**1**. Modification of the furanose derivative **2** into serine building synthon **11**, followed by desilylation of the known[11] 5-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-1,2-*O*-isopropylidene-3-(methoxycarbonylamino)-3-*C*-vinyl-α-D-xylofuranose **2** using tetrabutylammonium fluoride in THF, gave the corresponding alcohol **3** in 94.5% yield. Acetylation of **3** with acetic anhydride in pyridine and in the presence of DMAP afforded acetate **4** in 95% yield (Scheme 2). Acid hydrolysis of **4** removed the acetonide protecting group to give lactol **5** (Scheme 2) as a mixture of anomers which were characterized as acetates **6a** and **6b** (Ac_2O, pyridine, DMAP, 89%; α:β = 1:2, determined by ^1H NMR spectroscopic analysis, including NOE data). Oxidative cleavage[12] of the furanose **5** with sodium metaperiodate in CH_3OH/H_2O (1:1) afforded the protected aldehyde **7**, which was used after flash column chromatography in the next reaction to avoid problems connected with its possible instability. Reduction of the aldehyde **7** with $NaBH_4$ in methanol gave diol **8** in 61% isolated yield. The primary hydroxy group in **8** was selectively silylated[13] (TBDMSCl, Et_3N, DMF and DMAP) to afford **9** in 78% yield. Deprotection of the acetyl group in **9** under basic conditions (K_2CO_3 and CH_3OH) gave no satisfactory results; we obtained a cyclic carbamate as the protecting group for both hydroxy and amino functions. Thus, deacetylation of **9** was achieved using diisobutylaluminium hydride in CH_2Cl_2 and gave partially protected aminopolyol **10** (92%, Scheme 2).

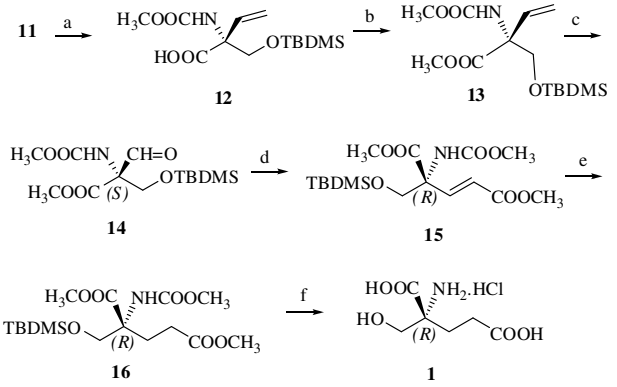


Scheme 2. Reagents and conditions: (a) TBAF, THF, 0 °C→rt, **3**, 94.5%; (b) Ac_2O, DMAP, pyridine, **4**, 95%; (c) TFA/H_2O (8:2), rt, **5**, 89%; (d) Ac_2O, DMAP, pyridine, **6**, 89%; (e) $NaIO_4$, CH_3OH/H_2O = 1:1, rt, **7**, 93%; (f) $NaBH_4$, CH_3OH, **8**, 61%; (g) TBDMSCl, Et_3N, DMF, DMAP, **9**, 78%; (h) DIBALH, CH_2Cl_2, −50 °C, **10**, 92%; (i) $NaIO_4$, CH_3OH/H_2O = 1:1, rt, **11**, 90.5%.

Oxidative fragmentation[12] of **10** with $NaIO_4$ afforded aldehyde **11** in 90.5% yield (Scheme 2). This α-substituted serinal building block **11** possesses a suitable structure for functional group manipulations, which are necessary for the construction of **1** and its antipode *ent*-**1**. The key aldehyde **11** was used in a divergent manner.

2.1. Functionalization of the protected α-substituted serinal fragment 11 into 1

In order to obtain **1**, treatment of **11** with $NaClO_2$ in CH_3CN/*tert*-butyl alcohol/2-methyl-2-butene provided carboxylic acid **12** in 99.5% yield, and subsequent esterification (CH_3I, K_2CO_3 and DMF)[14] gave the corresponding methyl ester **13** (51%, Scheme 3). The $RuCl_3/NaIO_4$ mediated oxidation[15] of the vinyl group in **13** afforded aldehyde **14** (65%, Scheme 3) which was immediately treated with the stabilized ylide ($Ph_3P{=}CHCO_2CH_3$) to give (*E*)-α,β-unsaturated ester **15** exclusively in 97% (Scheme 3). The observed coupling constant in **15** (J = 15.9 Hz) clearly assigned the *trans*-configuration of the double bond. Hydrogenation of **15** was carried out under standard H_2–Pd/C conditions to produce the corresponding saturated derivative **16** (87%). Finally, saponification of **16** ($NaOH/H_2O$, CH_3OH), followed by neutralization with IRC-76 resin (H^+ type) and subsequent reflux with 6 M HCl, afforded the HCl salt of (2*R*)-α-(hydroxymethyl)glutamic acid **1** as an off-white solid (81%, Scheme 3).

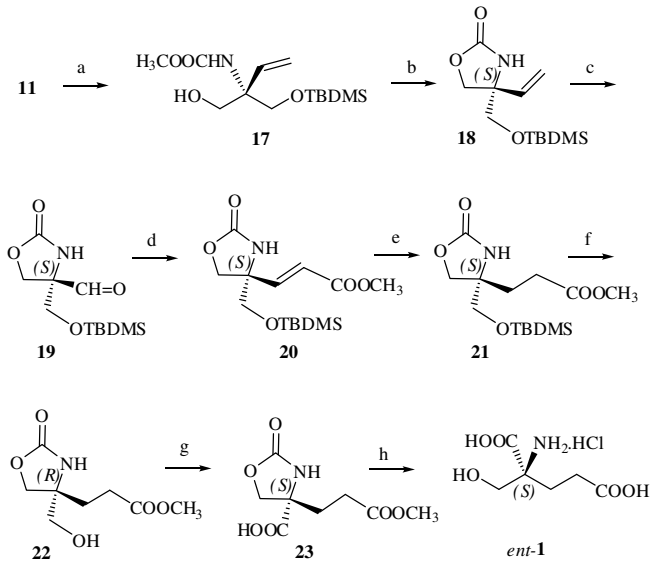


Scheme 3. Reagents and conditions: (a) $NaClO_2$, NaH_2PO_4, CH_3CN/*t*-BuOH/2-methyl-2-butene, **12**, 99.5%; (b) CH_3I, DMF, K_2CO_3, **13**, 51%; (c) $RuCl_3/NaIO_4$, $CH_3CN/CCl_4/H_2O$ = 2:2:3, **14**, 65%; (d) $Ph_3P{=}CHCOOCH_3$, CH_2Cl_2, **15**, 97%; (e) H_2, Pd/C, EtOH, **16**, 87%; (f) (i) 10% aq NaOH, CH_3OH, 80 °C; (ii) 6 M HCl, reflux, **1**, 81%.

2.2. Functional group manipulations of aldehyde 11 into *ent*-1

In order to obtain *ent*-**1**, the aldehyde **11** was reduced with $NaBH_4$ in methanol (Scheme 4). Treatment of the resulting alcohol **17** with NaH in THF induced cyclization to afford oxazolidinone **18** in 97% yield.

Ozonolysis[16] of **18** at −78 °C in methanol furnished aldehyde **19** (72%, Scheme 4), which after Wittig olefination with stabilized ylide ($Ph_3P{=}CHCO_2CH_3$, CH_2Cl_2) afforded a mixture of α,β-unsaturated esters (*E*:*Z* = 10:1.8 ratio, determined by ^1H NMR) in 94% yield. The subsequent chromatographic separation gave geometrically pure (*E*)-ester **20**. The coupling constant observed in **20** (J = 15.8 Hz) indicated the *trans*-configuration of the double bond. Hydrogenation of the Wittig adduct **20** in the presence of 5% Pd/C in EtOH provided the corresponding saturated derivative **21** (84%, Scheme 4). Deprotection of **21** using tetrabutylammonium fluoride in THF gave the alcohol **22** in 62.5% yield. The primary hydroxyl group in **22** was oxidized directly into the carboxylic acid **23** using $RuCl_3/NaIO_4$ in $CCl_4/CH_3CN/H_2O$ (2:2:3). Finally, treatment of **23** under the same conditions as for compound **16** afforded the HCl salt of (2*S*)-configured amino acid *ent*-**1** (82%, Scheme 4). The spectroscopic (^1H and ^{13}C NMR) data were fully identical with those reported[6,8] for **1** and its antipode *ent*-**1**, and the physical properties also showed good agreement with those reported[6,8] for both enantiomers.



Scheme 4. Reagents and conditions: (a) $NaBH_4$, CH_3OH, **17**, 61%; (b) NaH, THF, 0 °C→rt, **18**, 97%; (c) O_3, −78 °C, CH_3OH, Ph_3P, **19**, 92%; (d) $Ph_3P{=}CHCOOCH_3$, CH_2Cl_2, **20**, 79%; (e) H_2, Pd/C, EtOH, **21**, 84%; (f) TBAF, THF, **22**, 62.5%; (g) $RuCl_3$/$NaIO_4$, $CH_3CN/CCl_4/H_2O$ = 2:2:3, **14**, 53.5%; (h) (i) 10% aq NaOH, CH_3OH, 80 °C; (ii) 6 M HCl, reflux, *ent*-**1**, 82%.

3. Conclusion

In conclusion, this work established a novel synthetic pathway to (2*R*)-α-(hydroxymethyl)glutamic acid **1** (16 steps, overall 2.9% yield) and its enantiomer *ent*-**1** (14 steps, overall 6.6% yield) starting from the same chiral building block **11**. We have shown that the functional group interconversions of **11** also allow for the preparation of several important intermediates as **15**, **19** and **20** which possess the highly functionalized α-substituted α-amino acid moiety necessary for the construction of biologically interesting compounds such as myriocin,[17b,d,e] FTY720[17b–d] and KRP-203.[17a]

4. Experimental

4.1. General

All commercially available reagents were used without purification, and solvents were dried by distillation from standard drying agents (under N_2). Flash column chromatography was carried out using Merck Silica Gel 60 (0.040–0.063 mm). TLC was performed with Merck Silica Gel 60 F_{254} analytical plates, and the compounds were visualized by ultraviolet light (254 nm), phosphomolybdic acid solution (5% in EtOH), or KMnO4 basic solution. ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury Plus 400 FT NMR spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C) using $CDCl_3$ as the solvent and TMS as the internal reference. For ^1H δ are given in parts per million relative to TMS (0 ppm) and for ^{13}C, they are given relative to $CDCl_3$ (δ = 77.0). Infrared (IR) spectra were measured with a Perkin–Elmer 599 IR spectrometer and reported in wavenumbers (cm^{-1}). Optical rotations were recorded with a P3002 Krüss polarimeter and reported as follows: $[\alpha]_D$ (*c* in grams per 100 mL, solvent) The melting points were determined on the Kofler block, and are uncorrected.

4.1.1. 3-Deoxy-1,2-*O*-isopropylidene-3-(methoxycarbonylamino)-3-*C*-vinyl-α-D-xylofuranose 3

To a solution of compound **2**[11] (13.58 g, 35.04 mmol) in dry tetrahydrofuran (350 mL) were added activated 4 Å powdered molecular sieves (6.45 g). The suspension was treated with a 1 M solution of Bu_4NF in THF (35 mL, 35.04 mmol) at 0 °C. The resulting reaction mixture was stirred for a further 10 min at 0 °C and then for 15 min at room temperature. The solid was removed by filtration and the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (100 mL) and washed with water (120 mL). The water layer was extracted with further portions of ethyl acetate (2 × 70 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/ethyl acetate, 2:1) to afford 9.05 g (94.5%) of compound **3** as a white solid; mp 137–139 °C; $[\alpha]_D^{20} = +92.5$ (*c* 0.35, $CHCl_3$); ν_{max} (liquid film) 3353, 3007, 1710, 1693, 1076 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.33 (3H, br s, CH_3), 1.52 (3H, br s, CH_3), 2.28–2.42 (1H, m, OH), 3.63 (3H, s, CH_3O), 3.81–3.89 (2H, m, H_5, H_4), 4.04 (1H, dd, $J_{5,5}$ = 13.0 Hz, $J_{5,4}$ = 3.7 Hz, H_5), 5.07 (1H, d, $J_{2,1}$ = 3.4 Hz, H_2), 5.33 (1H, d, $J_{7trans,6}$ = 17.5 Hz, H_{7trans}), 5.37 (1H, d, $J_{7cis,6}$ = 10.8 Hz, H_{7cis}), 5.90 (1H, d, $J_{2,1}$ = 3.4 Hz, H_1), 6.06 (1H, dd, $J_{7trans,6}$ = 17.5 Hz, $J_{7cis,6}$ = 10.8 Hz, H_6), 7.22 (1H, br s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.2 (CH_3), 26.8 (CH_3), 51.9 (CH_3), 58.0 (CH_2), 68.5 (C), 78.8 (CH), 83.6 (CH), 104.7 (CH), 112.5 (C), 116.9 (CH_2), 132.2 (CH), 155.7 (C). Anal. Calcd for $C_{12}H_{19}NO_6$: C, 52.74; H, 7.01; N, 5.13. Found: C, 52.64; H, 7.08; N, 5.15.

4.1.2. 5-*O*-Acetyl-3-deoxy-1,2-*O*-isopropylidene-3-(methoxycarbonylamino)-3-*C*-vinyl-α-D-xylofuranose 4

To a solution of **3** (9.05 g, 33.12 mmol) in pyridine (250 mL) were added DMAP (0.407 g, 3.33 mmol) and acetic anhydride (4.70 mL, 49.72 mmol). Stirring was continued for 30 min at room temperature. The reaction mixture was poured into ice water (220 mL) and extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/ethyl acetate, 3:1) to give 9.92 g (95%) of compound **4** as a colourless oil; $[\alpha]_D^{20} = +56.1$ (*c* 0.30, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 1.32 (3H, br s, CH_3), 1.53 (3H, br s, CH_3), 2.10 (3H, s, CH_3CO), 3.65 (3H, s, CH_3O), 4.09–4.16 (2H, m, H_4, H_5), 4.48 (1H, dd, $J_{5,5}$ = 13.8 Hz, $J_{5,4}$ = 5.0 Hz, H_5), 5.01 (1H, d, $J_{2,1}$ = 3.6 Hz, H_2), 5.29 (1H, dd, $J_{7trans,6}$ = 17.6 Hz, $J_{7trans,7cis}$ = 0.7 Hz, H_{7trans}), 5.36 (1H, dd, $J_{7cis,6}$ = 10.9 Hz, $J_{7trans,7cis}$ = 0.7 Hz, H_{7cis}), 5.69 (1H, br s, NH), 5.88 (1H, d, $J_{2,1}$ = 3.6 Hz, H_1), 6.04 (1H, dd, $J_{7trans,6}$ = 17.6 Hz, $J_{7cis,6}$ = 10.9 Hz, H_6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.8 (CH_3), 26.2 (CH_3), 26.6 (CH_3), 52.1 (CH_3), 60.1 (CH_2), 67.8 (C), 78.0 (CH), 83.6 (CH), 104.6 (CH), 112.5 (C), 117.2 (CH_2), 131.7 (CH), 155.5 (C), 170.1 (C). Anal. Calcd for $C_{14}H_{21}NO_7$: C, 53.33; H, 6.71; N, 4.44. Found: C, 53.20; H, 6.81; N, 4.41.

4.1.3. 5-*O*-Acetyl-3-deoxy-3-(methoxycarbonylamino)-3-*C*-vinyl-D-xylofuranose 5

Compound **4** (9.00 g, 28.54 mmol) was treated with a mixture of TFA/H_2O (240 mL, 8:2) for 45 min at room temperature. Removal of the solvent gave a residue, which was purified on silica gel (hexane/ethyl acetate, 1:2) to afford 6.99 g (89%) of lactol **5** as a colourless oil; ν_{max} (liquid film) 3413, 3020, 1710, 1087 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 2.11 (3H, s, $CH_{3\alpha}CO$), 2.12 (3H, s, $CH_{3\beta}CO$), 3.68 (3H, s, $CH_{3\alpha}O$), 3.69 (3H, s, $CH_{3\beta}O$), 3.85–3.96 (4H, m, 2 × OH_α, 2 × OH_β), 4.10–4.19 (2H, m, $H_{5\alpha}$, $H_{5\beta}$), 4.25–4.45 (4H, m, $H_{4\alpha}$, $H_{4\beta}$, $H_{5\alpha}$, $H_{5\beta}$), 4.50–4.53 (2H, m, $H_{2\alpha}$, $H_{2\beta}$), 5.23 (1H, d, $J_{7trans,6}$ = 17.5 Hz, $H_{7trans\alpha}$), 5.29 (1H, d, $J_{7trans,6}$ = 17.4 Hz, $H_{7trans\beta}$), 5.35 (1H, d, $J_{7cis,6}$ = 10.8 Hz, $H_{7cis\alpha}$) 5.39 (1H, d, $J_{7cis,6}$ = 10.8 Hz, $H_{7cis\beta}$), 5.55 (2H, m, $H_{1\alpha}$, $H_{1\beta}$), 5.85 (2H, br s, NH_α, NH_β), 6.06 (1H, dd, $J_{7trans,6}$ = 17.4 Hz, $J_{7cis,6}$ = 10.8 Hz, $H_{6\beta}$), 6.11 (1H, dd, $J_{7trans,6}$ = 17.5 Hz, $J_{7cis,6}$ = 10.8 Hz, $H_{6\alpha}$).

4.1.4. 1,2,5-Tri-*O*-acetyl-3-deoxy-3-(methoxycarbonylamino)-3-*C*-vinyl-α-D-xylofuranose 6a and its β-anomer 6b

To a solution of lactol **5** (0.18 g, 0.65 mmol) in pyridine (4.9 mL) were added DMAP (16 mg, 0.013 mmol) and acetic anhydride (0.184 mL, 1.95 mmol) at room temperature. The resulting reaction mixture was stirred overnight at the same temperature, then poured into ice water (4 mL) and extracted with diethyl ether (2 × 8 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. Chromatography of the residue on silica gel (hexane/ethyl acetate, 3:1) afforded 70 mg of **6a** (30%) and 139 mg of **6b** (59%) as colourless oils.

α-Anomer **6a:** $[\alpha]_D^{20} = +81.3$ (*c* 0.31, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 2.08 (3H, s, CH_3CO), 2.08 (3H, s, CH_3CO), 2.09 (3H, s, CH_3CO), 3.64 (3H, s, CH_3O), 4.21 (1H, dd, $J_{5,5}$ = 12.5 Hz, $J_{5,4}$ = 3.8 Hz, H_5), 4.29 (1H, dd, $J_{5,5}$ = 12.5 Hz, $J_{5,4}$ = 3.3 Hz, H_5), 4.61 (1H, m, H_4), 5.19 (1H, d, $J_{7trans,6}$ = 17.3 Hz, H_{7trans}), 5.31 (1H, d, $J_{7cis,6}$ = 10.7 Hz, H_{7cis}), 5.59 (1H, d, $J_{2,1}$ = 5.1 Hz, H_2), 5.98 (1H, dd, $J_{7trans,6}$ = 17.3 Hz, $J_{7cis,6}$ = 10.7 Hz, H_6), 6.20 (1H, br s, NH), 6.43 (1H, d, $J_{2,1}$ = 5.1 Hz, H_1). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.5 (CH_3), 20.9 (CH_3), 20.9 (CH_3), 52.4 (CH_3), 62.3 (CH_2), 65.5 (C), 76.5 (CH), 81.9 (CH), 93.3 (CH), 116.2 (CH_2), 133.2 (CH), 155.6 (C), 169.3 (C), 170.1 (C), 170.5 (C). Anal. Calcd for $C_{15}H_{21}NO_9$: C, 50.14; H, 5.89; N, 3.90. Found: C, 50.20; H, 5.81; N, 3.91.

β-Anomer **6b:** $[\alpha]_D^{20} = -11.5$ (*c* 0.34, $CHCl_3$); ν_{max} (liquid film) 1723, 1213, 1080 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 2.08 (3H, s, CH_3CO), 2.09 (3H, s, CH_3CO), 2.10 (3H, s, CH_3CO), 3.65 (3H, s, CH_3O), 4.25 (1H, dd, $J_{5,5}$ = 12.3 Hz, $J_{5,4}$ = 5.8 Hz, H_5), 4.38 (1H, dd, $J_{5,5}$ = 12.3 Hz, $J_{5,4}$ = 2.9 Hz, H_5), 4.72 (1H, m, H_4), 5.23 (1H, d, $J_{7trans,6}$ = 17.2 Hz, H_{7trans}), 5.33 (1H, d, $J_{7cis,6}$ = 10.7 Hz, H_{7cis}), 5.38 (1H, d, $J_{2,1}$ = 3.5 Hz, H_2), 5.87 (1H, dd, $J_{7trans,6}$ = 17.2 Hz, $J_{7cis,6}$ = 10.7 Hz, H_6), 6.16 (1H, d, $J_{2,1}$ = 3.5 Hz, H_1), 6.47 (1H, br s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.6 (CH_3), 20.9 (2 × CH_3), 52.4 (CH_3), 62.7 (CH_2), 66.4 (C), 82.9 (CH), 84.3 (CH), 98.4 (CH), 116.3 (CH_2), 133.4 (CH), 155.7 (C), 169.7 (C), 170.3 (C), 171.3 (C). Anal. Calcd for $C_{15}H_{21}NO_9$: C, 50.14; H, 5.89; N, 3.90. Found: C, 50.10; H, 5.91; N, 3.85.

4.1.5. (2*S*,3*S*)-4-*O*-Acetyl-3-*O*-formyl-2-(methoxycarbonylamino)-2-vinylbutanal 7

To a solution of diol **5** (6.89 g, 25.03 mmol) in methanol (40.8 mL) was added an aqueous solution of sodium metaperiodate (6.42 g, 30.04 mmol) in water (40.8 mL). The mixture was stirred at room temperature for 45 min and then diluted with CH_2Cl_2. The insoluble material was removed by filtration. The aqueous layer was extracted with further portions of CH_2Cl_2 (2 × 30 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure to give 6.36 g (93%) of aldehyde **7** as a colourless oil, which was used immediately in the next step without purification. ^1H NMR (400 MHz, $CDCl_3$): δ 2.01 (3H, s, CH_3CO), 3.69 (3H, s, CH_3O), 4.22 (1H, dd, $J_{4,4}$ = 12.7 Hz, $J_{4,3}$ = 4.5 Hz, H_4), 4.47 (1H, dd, $J_{4,4}$ = 12.7 Hz, $J_{4,3}$ = 2.5 Hz, H_4), 5.36 (1H, d, $J_{6trans,5}$ = 17.6 Hz, H_{6trans}), 5.52 (1H, d, $J_{7cis,6}$ = 10.8 Hz, H_{6cis}), 5.92–6.07 (3H, m, H_3, H_5, NH), 8.13 (1H, s, OCHO), 9.47 (1H, s, CHO). Anal. Calcd for $C_{11}H_{15}NO_7$: C, 48.35; H, 5.53; N, 5.13. Found: C, 48.20; H, 5.51; N, 5.11.

4.1.6. (2*R*,3*S*)-4-*O*-Acetyl-2-(methoxycarbonylamino)-2-vinylbutane-1,3-diol 8

To a solution of crude aldehyde **7** (6.29 g, 23.02 mmol) in methanol (170 mL) was added sodium borohydride (0.96 g, 25.32 mmol) at 0 °C. The resulting mixture was stirred for 40 min at the same temperature. Then the solution was neutralized by addition of Amberlite IR-120 H^+. The resin was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (hexane/ethyl acetate, 1:1) and afforded 3.47 g (61%) of alcohol **8** as a colourless oil; $[\alpha]_D^{20} = +10.3$ (*c* 0.34, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 2.09 (3H, s, CH_3CO), 3.50–3.59 (1H, m, OH), 3.69 (3H, s, CH_3O), 3.77 (1H, dd, $J_{1,1}$ = 11.7 Hz, $J_{1,OH}$ = 6.9 Hz, H_1), 4.03 (1H, dd, $J_{1,1}$ = 11.7 Hz, $J_{1,OH}$ = 4.7 Hz, H_1), 4.07–4.15 (2H, m, H_3, H_4), 4.31 (1H, m, H_4), 4.44–4.52 (1H, m, OH), 5.27 (1H, d, $J_{6trans,5}$ = 17.6 Hz, H_{6trans}), 5.34 (1H, d, $J_{6cis,5}$ = 11.0 Hz, H_{6cis}), 5.49 (1H, br s, NH), 5.91 (1H, dd, $J_{6trans,5}$ = 17.6 Hz, $J_{6cis,5}$ = 11.0 Hz, H_5). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.9 (CH_3), 52.5 (CH_3), 62.9 (C), 65.3 (CH_2), 66.1 (CH_2), 73.7 (CH), 116.4 (CH_2), 136.2 (CH), 157.4 (C), 171.2 (C). Anal. Calcd for $C_{10}H_{17}NO_6$: C, 48.58; H, 6.93; N, 5.67. Found: C, 48.50; H, 6.89; N, 5.61.

4.1.7. (2*R*,3*S*)-4-*O*-Acetyl-1-*O*-(*tert*-butyldimethylsilyl)-2-(methoxycarbonylamino)-2-vinylbutane-3-ol 9

To a solution of alcohol **8** (3.40 g, 13.75 mmol) in dry DMF (14.4 mL) were added triethylamine (2.86 mL, 20.62 mmol), DMAP (1.68 g, 13.75 mmol) and *tert*-butyldimethylsilyl chloride (3.11 g, 20.62 mmol). The resulting mixture was stirred for 35 min at room temperature. After dilution with ice water (80 mL), the solution was extracted with diethyl ether (2 × 80 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/ethyl acetate, 5:1) and gave 3.88 g (78%) of silylated product **9** as a colourless oil; $[\alpha]_D^{20} = +18.1$ (*c* 0.32, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.05 (3H, s, CH_3), 0.06 (3H, s, CH_3), 0.88 (9H, s, 3 × CH_3), 2.08 (3H, s, CH_3CO), 3.65 (3H, s, CH_3O), 3.87 (1H, d, $J_{1,1}$ = 10.2 Hz, H_1), 4.03–4.14 (m, 2H, H_4, H_3), 4.11 (1H, d, $J_{1,1}$ = 10.2 Hz, H_1), 4.28 (1H, m, H_4), 5.17 (1H, d, $J_{6trans,5}$ = 17.8 Hz, H_{6trans}), 5.29 (1H, d, $J_{6cis,5}$ = 11.2 Hz, H_{6cis}), 5.42 (1H, br s, NH), 5.87 (1H, dd, $J_{6trans,5}$ = 17.8 Hz, $J_{6cis,5}$ = 11.2 Hz, H_5). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.7 (CH_3), −5.7 (CH_3), 18.1 (C), 20.9 (CH_3), 25.7 (3 × CH_3), 52.1 (CH_3), 61.8 (C), 65.2 (CH_2), 66.1 (CH_2), 74.5 (CH), 116.1 (CH_2), 136.5 (CH), 156.6 (C), 171.1 (C). Anal. Calcd for $C_{16}H_{31}NO_6Si$: C, 53.16; H, 8.64; N, 3.87. Found: C, 53.10; H, 8.69; N, 3.81.

4.1.8. (2*R*,3*S*)-1-*O*-(*tert*-Butyldimethylsilyl)-2-(methoxycarbonylamino)-2-vinylbutane-3,4-diol 10

To a solution of **9** (3.29 g, 9.10 mmol) in dry CH_2Cl_2 (41 mL) was added DIBAl-H (27.5 mL of 1.2 M toluene solution) at −50 °C. The resulting mixture was stirred at −50 °C for 45 min and then quenched with MeOH (6.8 mL). The mixture was allowed to warm to room temperature and poured into 30% aq K/Na-tartrate (136 mL). After being stirred for 30 min, the product was extracted with ethyl acetate (2 × 70 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (hexane/ethyl acetate, 2:1) and afforded 2.68 g (92%) of alcohol as a colourless oil; $[\alpha]_D^{20} = +16.9$ (*c* 0.32, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.06 (3H, s, CH_3), 0.07 (3H, s, CH_3), 0.89 (9H, s, 3 × CH_3), 3.63 (1H, dd, $J_{4,4}$ = 11.6 Hz, $J_{4,3}$ = 6.5 Hz, H_4), 3.64 (3H, s, CH_3O), 3.70 (1H, dd, $J_{4,4}$ = 11.6 Hz, $J_{4,3}$ = 3.6 Hz, H_4), 3.85 (1H, d, $J_{1,1}$ = 10.1 Hz, H_1), 3.89 (1H, dd, $J_{4,3}$ = 6.5 Hz, $J_{4,3}$ = 3.6 Hz, H_3), 4.13 (1H, d, $J_{1,1}$ = 10.1 Hz, H_1), 5.17 (1H, d, $J_{6trans,5}$ = 17.7 Hz, H_{6trans}), 5.27 (1H, d, $J_{6cis,5}$ = 11.1 Hz, H_{6cis}), 5.67 (1H, br s, NH), 5.82 (1H, dd, $J_{6trans,5}$ = 17.7 Hz, $J_{6cis,5}$ = 11.1 Hz, H_5). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.7 (2 × CH_3), 18.0 (C), 25.7 (3 × CH_3), 52.1 (CH_3), 62.4 (C), 62.4 (CH_2), 66.3 (CH_2), 76.0 (CH), 116.0 (CH_2), 136.4 (CH), 156.6 (C). Anal. Calcd for $C_{14}H_{29}NO_5Si$: C, 52.63; H, 9.15; N, 4.38. Found: C, 52.57; H, 9.09; N, 4.37.

4.1.9. (2*R*)-2-(*tert*-Butyldimethylsilyloxymethyl)-2-(methoxycarbonylamino)but-3-enal 11

To a solution of the diol **10** (2.68 g, 8.39 mmol) in methanol (13.6 mL) was added aqueous solution of sodium metaperiodate

(2.69 g, 12.58 mmol) in water (13.6 mL). The mixture was stirred at room temperature for 90 min and then diluted with CH_2Cl_2. The solid part was removed by filtration. The aqueous layer was extracted with further portions of CH_2Cl_2 (2 × 20 mL), the combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure to give 2.18 g (90.5%) of aldehyde **11** as a colourless oil, which was used immediately in the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.02 (3H, s, CH_3), 0.03 (3H, s, CH_3), 0.85 (9H, s, 3 × CH_3), 3.68 (3H, s, CH_3O), 3.96 (1H, d, $J_{H,H}$ = 9.9 Hz, CH_2), 4.02 (1H, d, $J_{H,H}$ = 9.9 Hz, CH_2), 5.30 (1H, d, $J_{4trans,3}$ = 17.6 Hz, H_{4trans}), 5.39 (1H, d, $J_{4cis,3}$ = 10.8 Hz, H_{4cis}), 5.73 (1H, br s, NH), 5.91 (1H, dd, $J_{4trans,3}$ = 17.6 Hz, $J_{4cis,3}$ = 10.8 Hz, H_3), 9.39 (1H, s, CHO). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.6 (2 × CH_3), 18.1 (C), 25.7 (3 × CH_3), 52.2 (CH_3), 63.9 (CH_2), 68.6 (C), 118.5 (CH_2), 132.5 (CH), 155.8 (C), 196.5 (CH). Anal. Calcd for $C_{13}H_{25}NO_4Si$: C, 54.32; H, 8.77; N, 4.87. Found: C, 54.27; H, 8.79; N, 4.80.

4.1.10. (2*R*)-2-(*tert*-Butyldimethylsilyloxymethyl)-2-(methoxycarbonylamino)but-3-enoic acid 12

A solution of $NaClO_2$ (2.91 g, 32.14 mmol) and $NaH_2PO_4.2H_2O$ (3.61 g, 23.16 mmol) in water (19.6 mL) was added dropwise to the solution of aldehyde **11** (1.00 g, 3.48 mmol) in acetonitrile/*tert*-butyl alcohol/2-methylbut-2-ene (78.3 mL, 4:4:1) at 0 °C over 10 min and then stirred at the same temperature for further 15 min. The reaction mixture was poured into brine (50 mL) and extracted with ethyl acetate (2 × 70 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was filtered through a small pad of silica gel (ethyl acetate) and gave 1.05 g (99.5%) of carboxylic acid **12** as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.04 (6H, s, 2 × CH_3), 0.85 (9H, s, 3 × CH_3), 3.66 (3H, s, CH_3O), 3.97–4.06 (2H, m, CH_2), 5.31 (1H, d, $J_{4cis,3}$ = 10.7 Hz, H_{4cis}), 5.36 (1H, d, $J_{4trans,3}$ = 17.4 Hz, H_{4trans}), 5.84 (1H, br s, NH), 6.01 (1H, dd, $J_{4trans,3}$ = 17.4 Hz, $J_{4cis,3}$ = 10.7 Hz, H_3), 8.82 (1H, br s, OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.6 (2 × CH_3), 18.1 (C), 25.7 (3 × CH_3), 52.2 (CH_3), 63.0 (C), 65.5 (CH_2), 117.5 (CH_2), 133.5 (CH), 156.0 (C), 174.2 (C). Anal. Calcd for $C_{13}H_{25}NO_5Si$: C, 51.46; H, 8.30; N, 4.62. Found: C, 51.37; H, 8.35; N, 4.70.

4.1.11. Methyl (2*R*)-2-(*tert*-butyldimethylsilyloxymethyl)-2-(methoxycarbonylamino)but-3-enoate 13

K_2CO_3 (0.68 g, 49.70 mmol) and CH_3I (0.7 g, 49.31 mmol) were added to a solution of carboxylic acid **12** (1 g, 32.96 mmol) in dry DMF (65 mL) and the reaction mixture was stirred for 1 h under nitrogen atmosphere, then diluted with ice water (50 mL) and extracted with Et_2O (2 × 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography (hexane/ethyl acetate, 7:1) to afford 0.53 g (51%) of ester **13** as a colourless oil; $[\alpha]_D^{20} = -63.7$ (*c* 0.27, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.01 (3H, s, CH_3), 0.01 (3H, s, CH_3), 0.84 (9H, s, 3 × CH_3), 3.65 (3H, s, CH_3O), 3.65 (3H, s, CH_3O), 3.94 (1H, d, $J_{H,H}$ = 9.7 Hz, CH_2), 4.02 (1H, d, $J_{H,H}$ = 9.7 Hz, CH_2), 5.28 (1H, d, $J_{4cis,3}$ = 10.7 Hz, H_{4cis}), 5.33 (1H, d, $J_{4trans,3}$ = 17.4 Hz, H_{4trans}), 5.78 (1H, br s, NH), 6.00 (1H, dd, $J_{4trans,3}$ = 17.4 Hz, $J_{4cis,3}$ = 10.7 Hz, H_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.7 (CH_3), −5.6 (CH_3), 18.1 (C), 25.6 (3 × CH_3), 52.0 (CH_3), 52.8 (CH_3), 65.6 (CH_2), 66.1 (C), 117.0 (CH_2), 133.6 (CH), 155.4 (C), 171.3 (C). Anal. Calcd for $C_{14}H_{27}NO_5Si$: C, 52.97; H, 8.57; N, 4.41. Found: C, 52.90; H, 8.51; N, 4.47.

4.1.12. Methyl (2*S*)-3-*O*-(*tert*-butyldimethylsilyl)-2-formyl-2-(methoxycarbonylamino)propanoate 14

To a suspension of ester **13** (0.32 g, 1.01 mmol) in $CCl_4/CH_3CN/H_2O$ (10.85 mL, 2:2:3) were added $NaIO_4$ (0.88 g, 4.14 mmol) and ruthenium trichloride hydrate (5.5 mg, 0.026 mmol). The reaction mixture was stirred for 3 h at room temperature and then extracted with CH_2Cl_2 (2 × 11 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by very flash chromatography (hexane/ethyl acetate, 7:1) and provided 0.21 g (65%) of aldehyde **14** as a colourless oil which was used immediately in the Wittig reaction. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.02 (3H, s, CH_3), 0.03 (3H, s, CH_3), 0.85 (9H, s, 3 × CH_3), 3.71 (3H, s, CH_3O), 3.81 (3H, s, CH_3O), 4.21 (1H, d, $J_{3,3}$ = 10.3 Hz, H_3), 4.26 (1H, d, $J_{3,3}$ = 10.3 Hz, H_3), 5.94 (1H, br s, NH), 9.55 (1H, s, CHO). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.7 (CH_3), −5.7 (CH_3), 18.0 (C), 25.6 (3 × CH_3), 52.6 (CH_3), 53.4 (CH_3), 63.0 (CH_2), 72.5 (C), 156.0 (C), 166.8 (C), 192.3 (C). Anal. Calcd for $C_{13}H_{25}NO_6Si$: C, 48.88; H, 7.89; N, 4.38. Found: C, 48.92; H, 7.93; N, 4.35.

4.1.13. Dimethyl (*E*,2*R*)-2-(*tert*-butyldimethylsilyloxymethyl)-2-(methoxycarbonylamino)pent-3-endioate 15

To a solution of aldehyde **14** (0.21 g, 0.66 mmol in dry CH_2Cl_2 (3.2 mL) was added $Ph_3P{=}CHCO_2CH_3$ (0.26 g, 0.79 mmol). The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and crude product was purified by chromatography on silica gel (hexane/ethyl acetate, 7:1) to yield 0.24 g (97%) of (*E*)-α,β-unsaturated ester **15** as a colourless oil; $[\alpha]_D^{20} = -111.1$ (*c* 0.27, $CHCl_3$); ν_{max} (liquid film) 3013, 2940, 1713, 1113, 1087 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.01 (6H, s, 2 × CH_3), 0.84 (9H, s, 3 × CH_3), 3.66 (3H, s, CH_3O), 3.73 (3H, s, CH_3O), 3.77 (3H, s, CH_3O), 3.94 (1H, d, $J_{H,H}$ = 9.6 Hz, CH_2), 3.99 (1H, d, $J_{H,H}$ = 9.6 Hz, CH_2), 5.81 (1H, br s, NH), 6.04 (1H, d, $J_{4,3}$ = 15.9 Hz, H_4), 7.07 (1H, d, $J_{4,3}$ = 15.9 Hz, H_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.7 (CH_3), −5.7 (CH_3), 18.1 (C), 25.6 (3 × CH_3), 51.7 (CH_3), 52.3 (CH_3), 53.2 (CH_3), 65.7 (C), 65.7 (CH_2), 122.6 (CH), 142.8 (CH), 155.4 (C), 166.2 (C), 170.0 (C). Anal. Calcd for $C_{16}H_{29}NO_7Si$: C, 51.18; H, 7.78; N, 3.73. Found: C, 51.15; H, 7.83; N, 3.68.

4.1.14. Dimethyl (2*R*)-2-(*tert*-butyldimethylsilyloxymethyl)-2-(methoxycarbonylamino)pentanedioate 16

To a solution of α,β-unsaturated ester **15** (0.08 g, 0.21 mmol) in absolute ethanol (1.7 mL) was added 5% palladium on carbon (14.8 mg), and the resulting mixture was stirred under a hydrogen atmosphere at room temperature for 16 h, before filtering through Celite. The Celite was further washed with ethanol and the combined filtrates were concentrated under reduced pressure. The resulting residue was subjected to flash column chromatography on silica gel (hexane/ethyl acetate, 7:1) to give 0.07 g (87%) of saturated ester **16** as a colourless oil; $[\alpha]_D^{20} = -142.8$ (*c* 0.29, $CHCl_3$); ν_{max} (liquid film) 2950, 1716, 1234, 1080 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.02 (6H, s, 2 × CH_3), 0.82 (9H, s, 3 × CH_3), 2.05–2.20 (2H, m, H_3, H_4), 2.35 (1H, td, $J_{4,4}$ = 12.0 Hz, $J_{4,3}$ = 8.6 Hz, $J_{4,3}$ = 8.6 Hz, H_4), 2.43–2.55 (1H, m, H_3), 3.63 (6H, s, 2 × CH_3O), 3.74 (3H, s, CH_3O), 3.79 (1H, d, $J_{H,H}$ = 9.8 Hz, CH_2), 4.12 (1H, d, $J_{H,H}$ = 9.8 Hz, CH_2), 5.76 (1H, br s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.7 (2 × CH_3), 18.0 (C), 25.6 (3 × CH_3), 26.5 (CH_2), 28.7 (CH_2), 51.7 (CH_3), 51.8 (CH_3), 52.8 (CH_3), 64.7 (CH_2), 64.9 (C), 155.1 (C), 172.3 (C), 173.1 (C). Anal. Calcd for $C_{16}H_{31}NO_7Si$: C, 50.91; H, 8.28; N, 3.71. Found: C, 50.85; H, 8.23; N, 3.68.

4.1.15. (2*R*)-α-(Hydroxymethyl)glutamic acid 1

To a solution of **16** (0.19 g, 0.503 mmol) in CH_3OH (8.5 mL) was added 10% aqueous NaOH solution (8.5 mL) and the resulting mixture was stirred at 80 °C for 3 h. The solution was cooled to 25 °C, then neutralized with IRC-76 resin (H^+ type). The insoluble material was removed by filtration and the filtrate was concentrated to give a residue, which was dissolved in 6 M HCl (4 mL) and heated at reflux for 6 h. The solvent was removed under reduced pressure and the crude product was purified by reverse-phased column chromatography (C_{18}, H_2O as the eluent) to give HCl salt

of **1** (87 mg, 81%) as an off-white solid; mp >250 °C browning; $[\alpha]_D^{20} = -1.4$ (*c* 0.45, H_2O). ^1H NMR (400 MHz, D_2O): δ: 2.11–2.25 (2H, m, $2 \times H_3$), 2.45–2.64 (2H, m, $2 \times H_4$), 3.80 (1H, d, $J_{H,H}$ = 12.2 Hz, CH_2), 4.06 (1H, d, $J_{H,H}$ = 12.2 Hz, CH_2). ^{13}C NMR (100 MHz, D_2O): δ 26.7 (CH_2), 28.0 (CH_2), 63.3 (C), 64.3 (CH_2), 171.8 (C), 176.0 (C). Anal. Calcd for $C_6H_{11}NO_5{\cdot}HCl{\cdot}0.5H_2O$: C, 32.37; H, 5.89; N, 6.29. Found: C, 32.19; H, 5.71; N, 6.11.

4.1.16. (2*S*)-1-*O*-(*tert*-Butyldimethylsilyl)-2-(methoxycarbonylamino)-2-vinylpropane-3-ol 17

To a solution of crude aldehyde **11** (2.08 g, 7.24 mmol) in methanol (56 mL) was added sodium borohydride (0.19 g, 5.06 mmol) at 0 °C. The resulting mixture was stirred for 30 min at the same temperature. Then the solution was neutralized by addition of Amberlite IR-120 H^+. The resin was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (hexane/ethyl acetate, 7:1) and afforded 2 g (95.5%) of alcohol **17** as a colourless oil; $[\alpha]_D^{20} = +13.4$ (*c* 0.42, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.05 (3H, s, CH_3), 0.06 (3H, s, CH_3), 0.88 (9H, s, $3 \times CH_3$), 3.66 (3H, s, CH_3O), 3.66 (1H, d, $J_{3,3}$ = 11.7 Hz, H_3), 3.70 (1H, d, $J_{1,1}$ = 9.9 Hz, H_1), 3.77 (1H, d, $J_{3,3}$ = 11.7 Hz, H_3), 3.81 (1H, d, $J_{1,1}$ = 9.9 Hz, H_1), 5.18 (1H, dd, $J_{5trans,4}$ = 17.5 Hz, $J_{5trans,5cis}$ = 0.6 Hz, H_{5trans}), 5.24 (1H, dd, $J_{5cis,4}$ = 10.9 Hz, $J_{5trans,5cis}$ = 0.6 Hz, H_{5cis}), 5.48 (1H, br s, NH), 5.83 (1H, dd, $J_{5trans,4}$ = 17.5 Hz, $J_{5cis,4}$ = 10.9 Hz, H_4). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.6 (CH_3), −5.6 (CH_3), 18.1 (C), 25.7 ($3 \times CH_3$), 52.1 (CH_3), 61.2 (C), 66.4 (CH_2), 67.1 (CH_2), 115.8 (CH_2), 136.9 (CH), 156.8 (C). Anal. Calcd for $C_{13}H_{27}NO_4Si$: C, 53.94; H, 9.40; N, 4.84. Found: C, 54.07; H, 9.35; N, 4.80.

4.1.17. (4*S*)-4-(*tert*-Butyldimethylsilyloxymethyl)-4-vinyloxazolidine-2-one 18

To a solution of **17** (2.00 g, 6.91 mmol) in dry THF (35 mL) was added NaH (0.56 g, 23.33 mmol, 60% dispersion in mineral oil, free from oil with anhydrous THF) at 0 °C. The reaction mixture was stirred for 10 min at 0 °C and for further 30 min at room temperature. The mixture was partitioned between CH_2Cl_2 (20 mL) and ice water (15 mL). The aqueous layer was then extracted with CH_2Cl_2 (2×35 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica gel (hexane/ethyl acetate, 7:1) and afforded 1.73 g (97%) of compound **18** as a white solid; mp 91–92 °C; $[\alpha]_D^{20} = +66.6$ (*c* 0.36, $CHCl_3$); ν_{max} (liquid film) 2950, 1750, 1710, 1103 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.06 (6H, s, $2 \times CH_3$), 0.88 (9H, s, $3 \times CH_3$), 3.59–3.61 (2H, m, CH_2), 4.07 (1H, d, $J_{5,5}$ = 8.4 Hz, H_5), 4.35 (1H, d, $J_{5,5}$ = 8.4 Hz, H_5), 5.28 (1H, d, $J_{7cis,6}$ = 10.8 Hz, H_{7cis}), 5.37 (1H, d, $J_{7trans,6}$ = 17.4 Hz, H_{7trans}), 5.90 (1H, dd, $J_{7trans,6}$ = 17.4 Hz, $J_{7cis,6}$ = 10.8 Hz, H_6), 6.15 (1H, br s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.6 (CH_3), −5.5 (CH_3), 18.1 (C), 25.7 ($3 \times CH_3$), 62.7 (C), 67.2 (CH_2), 72.3 (CH_2), 116.3 (CH_2), 136.7 (CH), 159.4 (C). Anal. Calcd for $C_{12}H_{23}NO_3Si$: C, 55.99; H, 9.01; N, 5.44. Found: C, 56.07; H, 9.08; N, 5.38.

4.1.18. (4*S*)-4-(*tert*-Butyldimethylsilyloxymethyl)-2-oxooxazolidine-4-carbaldehyde 19

A solution of **18** (0.38 g, 1.48 mmol) in dry methanol (50 mL) was cooled to -78 °C. Ozone was then passed through the solution under vigorous stirring. The maximum time for the ozone treatment was 10 min. This resulted in the formation of a slightly blue solution. Dry N_2 was passed through the cold solution in order to remove excess of ozone. Ph_3P (0.39 g, 1.48 mmol) and CH_2Cl_2 (22 mL) were added and the solution was allowed to warm to room temperature while stirring was continued for 1.5 h. The clear solution was concentrated under reduced pressure and the residue was purified by flash chromatography on silica gel (hexane/ethyl acetate, 1:1) to give 0.27 g (72%) of aldehyde **19** as a colourless oil which was used immediately in the next step. ^1H NMR (400 MHz, $CDCl_3$): δ 0.07 (3H, s, CH_3), 0.08 (3H, s, CH_3), 0.87 (9H, s, $3 \times CH_3$), 3.80 (1H, d, $J_{H,H}$ = 10.1 Hz, CH_2), 3.99 (1H, d, $J_{H,H}$ = 10.1 Hz, CH_2), 4.17 (1H, d, $J_{5,5}$ = 9.4 Hz, H_5), 4.43 (1H, d, $J_{5,5}$ = 9.4 Hz, H_5), 5.83 (1H, br s, NH), 9.74 (1H, s, CHO). Anal. Calcd for $C_{11}H_{21}NO_4Si$: C, 50.94; H, 8.16; N, 5.40. Found: C, 50.97; H, 8.08; N, 5.35.

4.1.19. Methyl (*E*,4*S*)-3-[4-(*tert*-butyldimethylsilyloxymethyl)-2-oxooxazolidin-4-yl]prop-2-enoate 20

To a solution of aldehyde **19** (0.27 g, 1.04 mmol, in dry CH_2Cl_2 (5.0 mL) was added $Ph_3P{=}CHCO_2CH_3$ (0.42 g, 1.25 mmol). The reaction mixture was stirred at room temperature for 1.5 h. The solvent was removed under reduced pressure. The crude product was purified by chromatography on silica gel (hexane/ethyl acetate, 3:1) to yield 0.26 g (79%) of **20** as a white solid and 0.05 g (15%) of its *Z*-isomer as a colourless oil. *E*-ester **20**: mp 69–70 °C; $[\alpha]_D^{20} = +66.7$ (*c* 0.33, $CHCl_3$); ν_{max} (liquid film) 2947, 1753, 1710, 1097 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.05 (3H, s, CH_3), 0.06 (3H, s, CH_3), 0.87 (9H, s, $3 \times CH_3$), 3.65 (2H, m, CH_2), 3.75 (3H, s, CH_3O), 4.11 (1H, d, $J_{5,5}$ = 8.6 Hz, H_5), 4.39 (1H, d, $J_{5,5}$ = 8.6 Hz, H_5), 6.12 (1H, d, $J_{3',2'}$ = 15.8 Hz, $H_{2'}$), 6.53 (1H, br s, NH), 6.94 (1H, d, $J_{3',2'}$ = 15.8 Hz, $H_{3'}$). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.6 (CH_3), −5.6 (CH_3), 18.1 (C), 25.6 ($3 \times CH_3$), 51.9 (CH_3,) 62.4 (C), 66.9 (CH_2), 71.4 (CH_2), 122.2 (CH), 145.3 (CH), 159.1 (C), 166.1 (C). Anal. Calcd for $C_{14}H_{25}NO_5Si$: C, 53.31; H, 7.99; N, 4.44. Found: C, 53.25; H, 7.89; N, 4.38.

4.1.20. Methyl (4*S*)-3-[4-(*tert*-butyldimethylsilyloxy methyl)-2-oxooxazolidin-4-yl]propanoate 21

To a solution of unsaturated ester **20** and its *Z*-isomer (0.31 g, 0.98 mmol) in absolute ethanol (7.85 mL) was added 5% palladium on carbon (68.3 mg), and the resulting mixture was stirred under a hydrogen atmosphere at room temperature for 16 h, before filtering through Celite. The Celite was further washed with ethanol and the combined filtrates were concentrated under reduced pressure. The crude product was subjected to flash column chromatography on silica gel (hexane/ethyl acetate, 2:1) to afford 0.26 g (84%) of **21** as white crystals; mp 50–51 °C; $[\alpha]_D^{20} = +15.5$ (*c* 0.31, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.06 (6H, s, $2 \times CH_3$), 0.88 (9H, s, $3 \times CH_3$), 1.85 (1H, ddd, $J_{3',3'}$ = 15.5 Hz, $J_{3',2'}$ = 8.2 Hz, $J_{3',2'}$ = 6.8 Hz, $H_{3'}$), 2.04 (1H, td, $J_{3',3'}$ = 15.5 Hz, $J_{3',2'}$ = 7.7 Hz, $J_{3',2'}$ = 7.7 Hz, $H_{3'}$), 2.33–2.39 (2H, m, $2 \times H_{2'}$), 3.54 (2H, m, CH_2), 3.68 (3H, s, CH_3O), 4.03 (1H, d, $J_{5,5}$ = 8.9 Hz, H_5), 4.20 (1H, d, $J_{5,5}$ = 8.9 Hz, H_5), 5.86 (1H, br s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.6 (CH_3), −5.6 (CH_3), 18.1 (C), 25.7 ($3 \times CH_3$), 28.2 (CH_2), 29.6 (CH_2), 51.9 (CH_3,) 60.6 (C), 67.3 (CH_2), 71.1 (CH_2), 159.0 (C), 173.4 (C). Anal. Calcd for $C_{14}H_{27}NO_5Si$: C, 52.97; H, 8.57; N, 4.41. Found: C, 53.05; H, 8.63; N, 4.35.

4.1.21. Methyl (4*R*)-3-[4-(hydroxymethyl)-2-oxooxazolidin-4-yl]propanoate 22

To a solution of saturated ester **21** (0.25 g, 0.79 mmol) in dry tetrahydrofuran (5 mL) was added a 1 M solution of Bu_4NF in THF (0.8 mL, 0.79 mmol) at 0 °C. The reaction mixture was stirred for 10 min at 0 °C and then for 50 min at room temperature. The solvent was evaporated under reduced pressure and the residue purified through a small pad of silica gel (ethyl acetate) to give 0.10 g (62.5%) of desilylated derivative **22** as a colourless oil. ^1H NMR (400 MHz, $CDCl_3$): δ 1.84 (1H, ddd, $J_{3',3'}$ = 14.8 Hz, $J_{3',2'}$ = 8.3 Hz, $J_{3',2'}$ = 6.8 Hz, $H_{3'}$), 1.97–2.06 (1H, m, $H_{3'}$), 2.42–2.48 (2H, m, $2 \times H_{2'}$), 3.50 (1H, d, $J_{H,H}$ = 11.8 Hz, CH_2), 3.59 (1H, d, $J_{H,H}$ = 11.8 Hz, CH_2) 3.70 (3H, s, CH_3O), 4.08 (1H, d, $J_{5,5}$ = 8.9 Hz, H_5), 4.32 (1H, d, $J_{5,5}$ = 8.9 Hz, H_5), 6.70 (1H, br s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.1 (CH_2), 29.7 (CH_2), 52.1 (CH_3,) 61.4 (C), 66.1 (CH_2), 71.0 (CH_2), 160.1 (C), 173.7 (C). Anal. Calcd for $C_8H_{13}NO_5$: C, 47.29; H, 6.45; N, 6.89. Found: C, 47.20; H, 6.53; N, 6.86.

4.1.22. (4*S*)-4-[2-(Methoxycarbonylethyl)]-2-oxooxazolidine-4-carboxylic acid 23

To a suspension of **22** (93 mg, 0.46 mmol) in $CCl_4/CH_3CN/H_2O$ (4.9 mL, 2:2:3) were added $NaIO_4$ (0.40 g, 1.87 mmol) and $RuCl_3{\cdot}H_2O$ (9.5 mg, 0.046 mmol). The resultant reaction mixture was stirred for 5 h at room temperature and then extracted with ethyl acetate (2 × 12 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography (dichloromethane/methanol, 5:1) to afford 53 mg (53.5%) of carboxylic acid **23** as a colourless oil; $[\alpha]_D^{20} = -31.0$ (*c* 0.49, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.10–2.18 (1H, m, $H_{1'}$), 2.27–2.32 (1H, m, $H_{1'}$), 2.41–2.47 (2H, m, 2 × $H_{2'}$), 3.68 (3H, s, CH_3O), 4.22 (1H, d, $J_{5,5}$ = 8.8 Hz, H_5), 4.68 (1H, d, $J_{5,5}$ = 8.8 Hz, H_5), 5.83 (1H, br s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.5 (CH_2), 31.9 (CH_2), 52.1 (CH_3), 63.9 (C), 72.2 (CH_2), 159.9 (C), 173.2 (C), 175.1 (C). Anal. Calcd for $C_8H_{11}NO_6$: C, 44.24; H, 5.11; N, 6.45. Found: C, 44.20; H, 5.15; N, 6.51.

4.1.23. (2*S*)-α-(Hydroxymethyl)glutamic acid *ent*-1

To a solution of **23** (44 mg, 0.202 mmol) in CH_3OH (2 mL) was added 10% aqueous NaOH solution (2 mL), and the resulting mixture was stirred at 80 °C for 3 h. The solution was cooled to 25 °C and neutralized with IRC-76 resin (H^+ type). The insoluble material was removed by filtration and the filtrate was concentrated to give a residue, which was dissolved in 6 M HCl (1 mL) and heated at reflux for 6 h. The solvent was removed under reduced pressure and the crude product was purified by reverse-phased column chromatography (C_{18}, H_2O as the eluent) giving HCl salt of *ent*-**1** (35 mg, 82%) as an off-white solid; mp >250 °C browning; $[\alpha]_D^{20} = +1.1$ (*c* 0.30, H_2O); ^{1}H and ^{13}C NMR data were identical to those reported for its enantiomer **1**.

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