Tetrahedron 66 (2010) 208–214

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Concise synthesis of both diastereomers of 3-hydroxy-L-arginine

Anke Lemke, Martin Büschleb, Christian Ducho *

Georg-August-University Göttingen, Department of Chemistry, Institute of Organic and Biomolecular Chemistry, Tammannstr. 2, 37077 Göttingen, Germany

ARTICLE INFO

Article history:
Received 7 October 2009
Received in revised form 28 October 2009
Accepted 29 October 2009
Available online 1 November 2009

Keywords:
Amino acids
Biosynthesis
Natural products

ABSTRACT

The hydroxylated amino acid 3-hydroxy-L-arginine is an intermediate in the biosynthesis of the non-proteinogenic amino acid capreomycidine and possibly also of its epimer epicapreomycidine. The novel concise synthesis of 3-hydroxy-L-arginine presented here allows the efficient preparation of both 3-epimers of this β-hydroxy amino acid. It also offers the potential to obtain suitably isotope-labelled derivatives for the elucidation of epicapreomycidine assembly in the biosynthesis of complex natural products.

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1. Introduction

Natural products with antibacterial activity are of particular interest due to emerging resistances of bacteria towards established antibiotics.[1,2] Non-proteinogenic amino acids are often characteristic structural motifs of such naturally occurring antibacterial agents. The non-proteinogenic amino acid (2*S*,3*R*)-capreomycidine ('capreomycidine') **1** is a constituent of natural products such as the tuberactinomycin peptide antibiotics,[3–7] e.g., the capreomycins[8–12] and viomycin,[13–17] which have both found clinical use as anti-tuberculosis drugs. The 3-epimer (2*S*,3*S*)-capreomycidine ('epicapreomycidine') **2** can be found as a component of the naturally occurring protease inhibitors chymostatin[18,19] and elastatinal[20,21] as well as a building block of the *Streptomyces*-produced muraymycins, a subclass of nucleoside lipopeptide antibiotics (Fig. 1).[22] Furthermore, an amino acid unit with structural similarities to **1** and **2** is a constituent of the mannopeptimycins, a novel subclass of glycopeptide antibiotics.[23–25]

The biosynthesis of **1** as part of viomycin biosynthesis in *Streptomyces vinaceus* has been elucidated (Scheme 1).[26–28] Capreomycidine assembly commences with stereoselective 3-hydroxylation of L-arginine **3**, catalysed by the non-haeme 2-oxoglutarate (2-OG) dependent Fe(II) oxygenase VioC, to give (3*S*)-3-hydroxy-L-arginine (*S*)-**4**. This β-hydroxy amino acid then undergoes ring closure to yield **1** with formal inversion of the configuration at C-3, mediated by the pyridoxalphosphate (PLP) dependent enzyme VioD. This reaction most likely proceeds via intramolecular Michael-type addition of the guanidine moiety of α,β-unsaturated intermediate **5**.

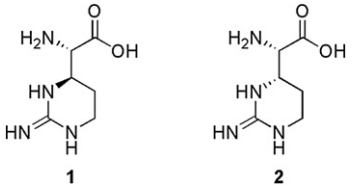
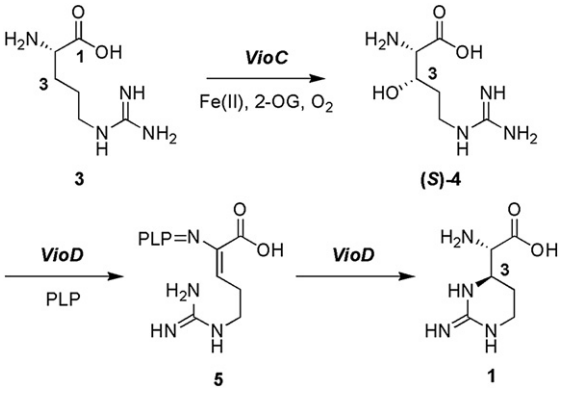


Figure 1. The non-proteinogenic amino acids capreomycidine **1** and epicapreomycidine **2**.



Scheme 1. Biosynthesis of capreomycidine **1** in *S. vinaceus*.[26–28]

* Corresponding author. Tel.: +49 (0)551 39 3284; fax: +49 (0)551 39 9660.
E-mail address: cducho@gwdg.de (C. Ducho).

0040-4020/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2009.10.102

It can be proposed that the biosynthetic assembly of epicapreomycidine **2** as part of the biosynthesis of complex natural products such as muraymycin antibiotics might occur in a similar manner. However, the stereochemical course of epicapreomycidine formation from L-arginine **3** is unclear. In addition, it cannot be ruled out that epicapreomycidine **2** is formed via enzymatically catalysed epimerisation of capreomycidine **1** at the C-3 position. There is evidence for unusual epimerisation reactions in other biosynthetic pathways in bacteria, most notably in the biosynthesis of simple carbapenems,[29,30] of thienamycin[31] and of clavulanic acid.[30] In the case of simple carbapenem β-lactam antibiotics, epimerisation at the β-lactam bridgehead position is mediated by the non-haeme 2-OG dependent Fe(II) oxygenase CarC.[32–36] The corresponding enzymes catalysing the epimerisation steps in thienamycin and clavulanic acid biosynthesis have yet to be identified.

A 3-hydroxylated arginine residue also plays a key role in the biosynthesis of clavulanic acid. Deoxyguanidino-proclavaminate, a precursor of clavulanic acid, is stereoselectively hydroxylated at its arginine moiety by the non-haeme 2-OG dependent Fe(II) oxygenase clavaminate synthase (CAS).[30,37–42] This implies that 3-hydroxy-L-arginine is not a direct precursor of clavulanic acid because β-lactam ring formation precedes arginine hydroxylation. 3-Hydroxy-arginine was also speculated to be an intermediate in the biosynthesis of the antibiotic streptothricin F, but this hypothesis was not confirmed.[43]

Studies on biosynthetic pathways employing 3-hydroxy-L-arginine as an intermediate require an efficient synthesis of both diastereomers (*R*)-**4** and (*S*)-**4** of this β-hydroxy amino acid with the potential to prepare suitably isotope-labelled derivatives (Fig. 2). So far, there is only one synthesis of 3-hydroxy-L-arginine available.[44] This established synthetic route involves a 1,3-dipolar cycloaddition reaction of a vinylglycine derivative and a nitrone as its key step. Though this approach is very elegant, it significantly limits the potential to incorporate isotope labels in a reasonable number of synthetic steps. Consequently, it has only found application in the synthesis of 5,5-dideuterated 3-hydroxy-L-arginine.[43]

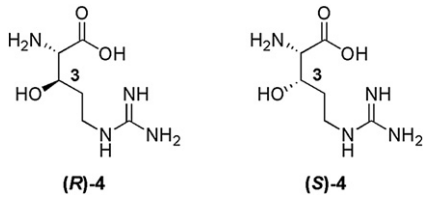


Figure 2. Target structures (*R*)-**4** and (*S*)-**4**, the two diastereomers of 3-hydroxy-L-arginine.

More detailed investigations e.g., on epicapreomycidine assembly in muraymycin biosynthesis would require additional labelled analogues of (*R*)-**4** and (*S*)-**4**, particularly doubly labelled compounds and/or derivatives with a deuterium label at the C-3 position as chemical probes for a possible epimerisation pathway (vide supra). In this work, we describe a facile synthesis of both (*R*)-**4** and (*S*)-**4** in diastereomerically pure form, bearing the potential to introduce isotope labels at several positions including C-3.

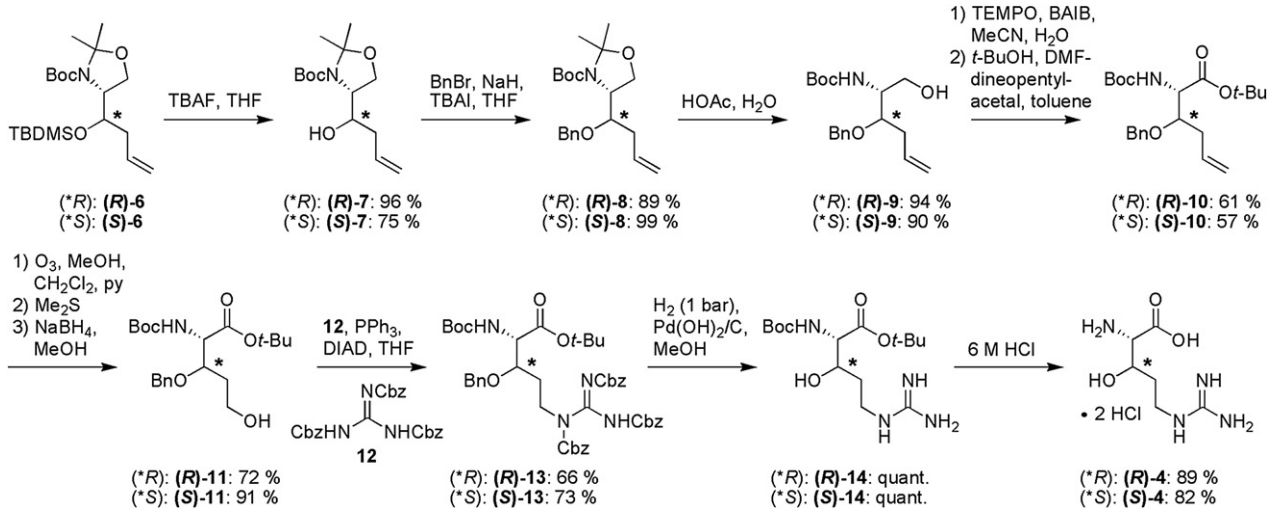
2. Results and discussion

In contrast to the known cycloaddition route, our novel approach utilises the established diastereomerically pure silylated homoallylic alcohols (*R*)-**6** and (*S*)-**6** as starting materials, which can be readily prepared from Garner's aldehyde[45,46] as previously reported (vide infra).[47] Desilylation of both (*R*)-**6** and (*S*)-**6** gave homoallylic alcohols (*R*)-**7** and (*S*)-**7** (96% and 75% yield), which were benzylated to afford (*R*)-**8** and (*S*)-**8** in yields of 89% and 99%, respectively (Scheme 2). This exchange of the alcohol protecting group was necessary as the silyl ether was concomitantly hydrolysed in the subsequent acidic acetonide cleavage, which proceeded smoothly in case of the benzylated starting material to furnish (*R*)-**9** and (*S*)-**9** (94% and 90% yield). These amino alcohols were then oxidized to the corresponding amino acids using TEMPO and bis-(acetoxy)-iodobenzene (BAIB).[48] Subsequent *tert*-butyl ester formation with the crude amino acids under Eschenmoser conditions[49] gave (*R*)-**10** and (*S*)-**10** in 61% and 57% yield, respectively, over two steps from (*R*)-**9** and (*S*)-**9** (Scheme 2). Thus, any unwanted epimerisation at the α-carbon atom, which can occur when other methods such as DCC coupling are used for *tert*-butyl esterification of carbamate-protected amino acids,[50] was not observed. Epimerisation at the α-carbon atom would have easily been detectable by NMR spectroscopy at any stage of the synthetic route due to the formation of diastereomers.

For introduction of the guanidine moiety, olefins (*R*)-**10** and (*S*)-**10** were cleaved by ozonolysis with reductive workup. In order to obtain satisfactory yields of the resulting alcohols (72% of (*R*)-**11** and 91% of (*S*)-**11**), it was essential to use a significant excess of sodium borohydride for the reduction step. The primary products of the ozonolysis reaction were derivatives of glutamate semialdehyde, and the strong preference of the cyclic hemiaminal form, which is typical for these compounds,[50] might have slowed down the reduction step. Alcohols (*R*)-**11** and (*S*)-**11** were then reacted with tris-Cbz-guanidine **12** using Goodman's guanidinylation method under Mitsunobu conditions[51] to afford protected guanidines (*R*)-**13** and (*S*)-**13** in 66% and 73% yield, respectively. The protected 3-hydroxy-L-arginines (*R*)-**13** and (*S*)-**13** could be deprotected by hydrogenolysis (products (*R*)-**14** and (*S*)-**14**, quantitative yields), followed by treatment with hydrochloric acid. Thus, diastereomerically pure 3-hydroxy-L-arginines (*R*)-**4** and (*S*)-**4** were obtained as dihydrochlorides in 89% and 82% yield, respectively, for the final deprotection step and in overall yields of 21% each over 9 steps from (*R*)-**6** and (*S*)-**6** (Scheme 2).

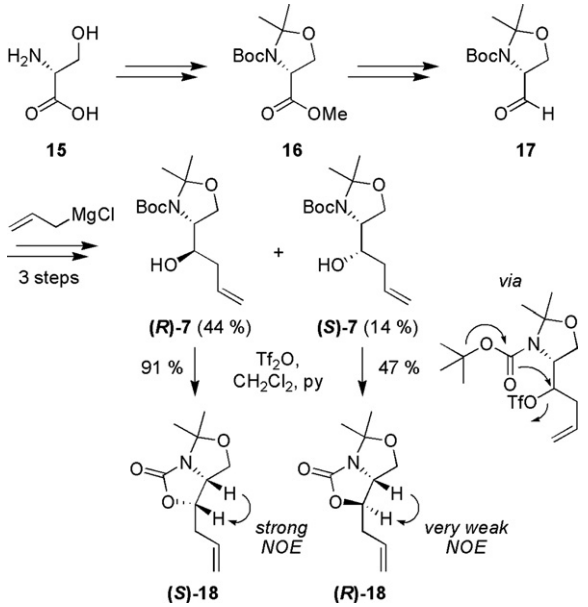
Benzyl protection of the secondary hydroxy group was essential not only for selective oxidation of the primary alcohol, but also for the guanidinylation reaction. Test reactions for the guanidinylation reaction with an *O*-unprotected analogue of (*R*)-**11** and (*S*)-**11** gave only moderate conversions and resulted in severe purification problems. Attempts to carry out the synthetic route with a diastereomeric mixture of **7** with separation of diastereomers at a late stage were unsuccessful.

In order to verify the reported stereochemical assignment and to elucidate steps for the incorporation of isotope labels, the established preparation of starting materials (*R*)-**6** and (*S*)-**6**[47] was investigated more closely. The conversion of D-serine **15** to the acetonide protected methyl ester **16** is known as is transformation of the latter to the (*R*)-configured Garner aldehyde **17**.[52,53] Aldehyde **17** can then be employed for the synthesis of starting material **6** by addition of allylmagnesium chloride and silyl protection according to the previously published procedure. The two diastereomers of **6** can be readily separated by column chromatography as reported.[47] However, the assignment of the absolute configuration at the newly formed stereocenter of the fast eluting major diastereomer (*R*)-**6** was solely based on NMR data of the Mosher ester derivative of desilylated alcohol (*R*)-**7**.[47] In order to ensure that this assignment is correct, it was envisaged to prepare cyclic analogues of both desilylated diastereomers (*R*)-**7** and (*S*)-**7** suitable for nuclear Overhauser enhancement (NOE) NMR experiments. Using a transformation similar to a previously described reaction,[54] diastereomerically pure alcohols (*R*)-**7** and (*S*)-**7** were treated with triflic anhydride (Tf_2O). The formed triflates were not stable, but underwent spontaneous intramolecular S_N2-type nucleophilic attack most likely by the carbonyl oxygen atom of the Boc group



Scheme 2. Synthesis of target compounds (*R*)-**4** and (*S*)-**4** from diastereomerically pure silylated homoallylic alcohols (*R*)-**6** and (*S*)-**6**.

with inversion of the stereochemical configuration and rupture of the *tert*-butyl-oxygen bond. However, it is unclear if the ring closure reaction occurred immediately after formation of the triflate or upon aqueous workup. Thus, cyclic carbamates (*S*)-**18** and (*R*)-**18** were formed from (*R*)-**7** and (*S*)-**7**, respectively, in isolated yields of 91% and 47% without optimisation of the reaction conditions (Scheme 3).



Scheme 3. Synthesis of carbamates **18** for NOE ^{1}H NMR studies.

The cis and trans substitution patterns of the cyclic moieties of (*S*)-**18** and (*R*)-**18** were proven by 1D NOE ^{1}H NMR studies. There was a strong NOE for the two protons attached to the fused five-membered ring of the *cis*-isomer (*S*)-**18**, but only a very weak NOE was found for the corresponding two protons of the *trans*-isomer (*R*)-**18** (Scheme 3).[55] Hence, the proposed stereochemical configuration of both cyclic carbamates (*S*)-**18** and (*R*)-**18** and therefore also of the desilylated alcohols (*R*)-**7** and (*S*)-**7** could be unambigously confirmed.

The novel synthetic route towards stereoisomerically pure 3-hydroxy-L-arginine **4** reported here permits incorporation of isotope labels for biosynthetic studies at multiple sites. Most importantly, reduction of **16** to **17** (Scheme 3) using deuterated reducing agents such as DIBAL-D or commercially available lithium aluminium deuteride (followed by Swern oxidation) would finally lead to a deuterium label at the 3-position of **4**. Application of sodium borodeuteride in the reductive workup of the ozonolysis reaction yielding intermediate **11** (Scheme 2) enables the introduction of an additional deuterium label at the 5-position of **4**. Furthermore, labelled derivatives of allyl bromide and allyl alcohol are commercially available. If a Grignard reagent was prepared from either of these compounds for the addition reaction leading to **7** (Scheme 3), introduction of deuterium or ^{13}C labels at the 4-position of **4** would be feasible.

3. Summary

In conclusion, a concise synthesis of both diastereomers of 3-hydroxy-L-arginine **4** starting from the (*R*)-configured Garner aldehyde **17** was developed. An established silylated homoallylic alcohol **6** derived from **17** was employed for conversion into β-hydroxy amino acid **10**. Further transformation of **10**, including guanidinylation under Mitsunobu conditions, led to stereoisomerically pure target compounds (*R*)-**4** and (*S*)-**4** in overall yields of 21% each over nine steps from (*R*)-**6** and (*S*)-**6** (Scheme 2). The overall yields starting from the (*R*)-configured Garner aldehyde **17** were 10% of (*R*)-**4** and 4% of (*S*)-**4**, respectively, over 11 steps. This novel synthesis of 3-hydroxy-L-arginine **4** is suitable for the preparation of multiply isotope-labelled derivatives. Since **4** is a known intermediate in the biosynthesis of the non-proteinogenic amino acid capreomycidine **1**, labelled derivatives of **4** might enable the elucidation of the biosynthetic assembly of the 3-epimer of **1**, epicapreomycidine **2**, in the biosynthesis of complex natural products such as muraymycin nucleoside antibiotics.

4. Experimental

4.1. General

Chemicals were purchased from Sigma–Aldrich, Alfa Aesar, ABCR and VWR. Diastereomerically pure starting materials (*R*)-**6** and (*S*)-**6** were synthesised as previously reported.[47] Ozone was generated using a Fischer ozone generator model 502. Reactions involving oxygen and/or moisture sensitive reagents were carried out under an atmosphere of argon using anhydrous solvents. Anhydrous solvents were obtained in the following manner: THF was dried over sodium/benzophenone and distilled, CH_2Cl_2 was dried

over $CaCl_2$ and distilled and pyridine was dried over CaH_2 and distilled. All other solvents were of technical quality and distilled prior to their use, and distilled water was used throughout.

Column chromatography was carried out on silica gel 60 (0.040–0.063 mm, 230–400 mesh ASTM, VWR) except where indicated under flash conditions. TLC was performed on aluminium plates precoated with silica gel 60 F_{254} (VWR). Visualisation of the spots was carried out using UV light (254 nm) where appropriate and/or $KMnO_4$ staining under heating (staining solution: 1 g $KMnO_4$, 6 g K_2CO_3 and 1.5 mL 5% $NaOH_{(aq)}$ (w/v), all dissolved in 100 mL H_2O). R_f values are given to the nearest 0.05.

300 MHz- and 600 MHz-^{1}H as well as 75 MHz-, 76 MHz and 126 MHz-^{13}C NMR spectra were recorded on Varian UNITY 300, MERCURY 300, INOVA 500 and INOVA 600 spectrometers. All ^{13}C NMR spectra are ^{1}H-decoupled. All spectra were recorded at room temperature except of samples in DMSO-d_6 and D_2O (standard 35 °C). Some spectra were recorded at 100 °C in order to overcome Boc rotamer formation. All NMR spectra were referenced internally to solvent reference frequencies. Chemical shifts (δ) are quoted in parts per million. Coupling constants (J) are reported in Hertz to the nearest 0.5 Hz. Assignment of signals was carried out using ^{1}H,^{1}H-COSY and HSQC spectra obtained on the spectrometers mentioned above. Low resolution ESI mass spectrometry was performed on a Varian MAT 311 A spectrometer operating in positive ionisation mode. High resolution (HR) ESI mass spectrometry was carried out on a Bruker microTOF spectrometer or a Bruker 7 T FTICR APEX IV spectrometer. Melting points (mp) were measured on a Büchi instrument and are not corrected. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter with a Na source using a 10 cm cell. Infrared (IR) spectroscopy was performed on a Perkin–Elmer Spectrum BX spectrometer with liquids and oils being measured as films on NaCl plates and solids as KBr pills. Wavenumbers (ν) are quoted in cm^{-1}. UV spectroscopy was carried out on a Perkin–Elmer Lambda-2 spectrometer. Wavelengths of maximum absorption (λ_{max}) are reported in nm with the corresponding logarithmic molar extinction coefficient ($\log \varepsilon$) given in brackets.

4.2. Syntheses

*4.2.1. N-Boc-(4R,1′R)-(1′-hydroxy-3′-butenyl)-2,2-dimethyl-1,3-oxazolidine ((R)-**7**).* To a solution of (*R*)-**6** (7.98 g, 20.7 mmol) in THF (50 mL), a solution of tetra-*n*-butyl-ammonium fluoride (16.3 g, 51.7 mmol) in THF (60 mL) was added at room temperature. After stirring at room temperature for 2 h, the reaction mixture was partitioned between water (200 mL) and Et_2O (350 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ solution (3×200 mL) and brine (1×200 mL), dried over Na_2SO_4 and the solvent removed under reduced pressure. The resultant crude product was purified by column chromatography (petroleum ether/EtOAc 3:1) to yield (*R*)-**7** (5.40 g, 96%) as a colourless oil. ^{1}H NMR (300 MHz, DMSO-d_6, 100 °C) δ 5.98–5.81 (m, 1H), 5.12–4.97 (m, 2H), 4.41 (d, J=3.5 Hz, 1H), 4.05–3.83 (m, 4H), 2.30–2.15 (m, 1H), 2.14–1.97 (m, 1H), 1.53 (s, 3H), 1.44 (s, 9H), 1.42 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6, 100 °C) δ 151.7, 136.0, 115.2, 93.1, 78.8, 69.1, 62.8, 60.2, 35.1, 27.6, 25.8, 22.9; MS (ESI) m/z 294.2 (M+Na$^+$); MS (ESI-HR) m/z calcd for $C_{14}H_{25}NNaO_4$ 294.1676 (M+Na$^+$), found 294.1678 (M+Na$^+$); $[\alpha]_D^{20}$ +25.7 (c 1.1, $CHCl_3$); IR (film) ν 3403, 2983, 2359, 1699, 1390, 1367, 1253, 1168, 1059, 850, 765; TLC R_f value 0.25 (petroleum ether/EtOAc 3:1).

*4.2.2. N-Boc-(4R,1′S)-(1′-hydroxy-3′-butenyl)-2,2-dimethyl-1,3-oxazolidine ((S)-**7**).* Isomer (*S*)-**7** was prepared in the same way as compound (*R*)-**7** with (*S*)-**6** (6.86 g, 17.8 mmol), tetra-*n*-butyl-ammonium fluoride (10.0 g, 31.7 mmol) and THF (50 mL each). The product (*S*)-**7** (3.62 g, 75%) was obtained as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6, 100 °C) δ 5.94–5.78 (m, 1H), 5.09–4.96 (m, 2H), 4.41 (d, J=6.0 Hz, 1H), 4.00 (dd, J=8.5, 2.0 Hz, 1H), 3.84 (dd, J=8.5, 6.5 Hz, 1H), 3.79–3.65 (m, 2H), 2.24–2.03 (m, 2H), 1.49 (s, 3H), 1.45 (s, 9H), 1.45 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6, 100 °C) δ 151.4, 135.6, 115.4, 92.8, 78.6, 69.6, 63.0, 60.6, 38.2, 27.6, 26.1, 23.6; MS (ESI) m/z 294.2 (M+Na$^+$); MS (ESI-HR) m/z calcd for $C_{14}H_{25}NNaO_4$ 294.1676 (M+Na$^+$), found 294.1676 (M+Na$^+$); mp 37 °C; $[\alpha]_D^{20}$ 15.7 (c 0.95, $CHCl_3$); IR (KBr) ν 3442, 2981, 2375, 1699, 1394, 1256, 1174, 1098, 913, 843, 768; TLC R_f value 0.25 (petroleum ether/EtOAc 3:1).

*4.2.3. N-Boc-(4R,1′R)-(1′-benzyloxy-3′-butenyl)-2,2-dimethyl-1,3-oxazolidine ((R)-**8**).* To a suspension of sodium hydride (60% dispersion in mineral oil, 1.03 g, 25.8 mmol) in THF (40 mL), a solution of (*R*)-**7** (5.38 g, 19.8 mmol) in THF (60 mL) was added at 0 °C, and the mixture was stirred at 0 °C for 30 min. After addition of tetra-*n*-butyl-ammonium iodide (1.46 g, 3.97 mmol) and benzyl bromide (7.1 mL, 10 g, 59 mmol), the reaction mixture was heated under reflux for 12 h and the reaction then quenched by addition of saturated aqueous NH_4Cl solution (50 mL). The aqueous layer was extracted with Et_2O (3×50 mL). The combined organics were washed with brine (2×50 mL), dried over Na_2SO_4 and the solvent removed under reduced pressure. The resultant crude product was purified by column chromatography (petroleum ether/EtOAc 20:1) to yield (*R*)-**8** (6.36 g, 89%) as a yellowish oil. ^{1}H NMR (300 MHz, DMSO-d_6, 100 °C) δ 7.40–7.21 (m, 5H), 5.97–5.81 (m, 1H), 5.14–4.99 (m, 2H), 4.67–4.51 (m, 2H), 4.15 (ddd, J=6.5, 4.5, 2.5 Hz, 1H), 4.00 (dd, J=9.5, 2.5 Hz, 1H), 3.93 (dd, J=9.5, 6.5 Hz, 1H), 3.86 (ddd, J=8.5, 4.5, 4.0 Hz, 1H), 2.40–2.29 (m, 1H), 2.28–2.15 (m, 1H), 1.54 (s, 3H), 1.43 (s, 3H), 1.43 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6, 100 °C) δ 151.3, 138.3, 135.4, 127.5, 126.8, 126.7, 115.6, 93.2, 78.9, 77.7, 71.1, 62.8, 57.2, 33.1, 27.5, 25.8, 22.7; MS (ESI) m/z 384.2 (M+Na$^+$); MS (ESI-HR) calcd for $C_{21}H_{31}NNaO_4$ 384.2145 (M+Na$^+$), found 384.2145 (M+Na$^+$); $[\alpha]_D^{20}$ +17.7 (c 1.1, $CHCl_3$); IR (film) ν 3395, 2980, 2345, 1703, 1455, 1387, 1256, 1171, 1090, 854, 737, 698; UV (MeCN) λ_{max} ($\log \varepsilon$) 258 (1.42), 204 (3.04); TLC R_f value 0.45 (petroleum ether/EtOAc 5:1).

*4.2.4. N-Boc-(4R,1′S)-(1′-benzyloxy-3′-butenyl)-2,2-dimethyl-1,3-oxazolidine ((S)-**8**).* Isomer (*S*)-**8** was prepared in the same way as compound (*R*)-**8** with (*S*)-**7** (3.50 g, 12.9 mmol), sodium hydride (60% dispersion in mineral oil, 671 mg, 16.8 mmol), tetra-*n*-butyl-ammonium iodide (953 mg, 2.58 mmol), benzyl bromide (4.6 mL, 6.6 g, 39 mmol) and THF (30 mL and 50 mL, respectively). The product (*S*)-**8** (4.61 g, 99%) was obtained as a yellowish oil. ^{1}H NMR (300 MHz, DMSO-d_6, 100 °C) δ 7.40–7.21 (m, 5H), 5.92–5.76 (m, 1H), 5.16–5.00 (m, 2H), 4.60 (d, J=11.5 Hz, 1H), 4.53 (d, J=11.5 Hz, 1H), 4.05–3.87 (m, 4H), 2.40–2.15 (m, 2H), 1.47 (s, 3H), 1.45 (s, 12H); ^{13}C NMR (75 MHz, DMSO-d_6, 100 °C) δ 151.3, 138.2, 134.5, 127.5, 126.9, 126.7, 116.2, 93.0, 78.8, 76.9, 71.4, 62.5, 59.1, 35.7, 27.6, 25.6, 23.9; MS (ESI) m/z 384.2 (M+Na$^+$); MS (ESI-HR) m/z calcd for $C_{21}H_{31}NNaO_4$ 384.2145 (M+Na$^+$), found 384.2151 (M+Na$^+$); $[\alpha]_D^{20}$ +43.4 (c 1.0, $CHCl_3$); IR (film) ν 3483, 2979, 2361, 1698, 1454, 1366, 1257, 1096, 858, 763, 698; UV (MeCN) λ_{max} ($\log \varepsilon$) 258 (1.29), 204 (2.99); TLC R_f value 0.45 (petroleum ether/EtOAc 5:1).

*4.2.5. N-Boc-3-O-benzyl-(2R,3R)-2-amino-hex-5-en-1,3-diol ((R)-**9**).* A solution of (*R*)-**8** (6.25 g, 17.3 mmol) in acetic acid (130 mL) and H_2O (26 mL) was stirred at room temperature for 48 h. The solvent was removed under reduced pressure. The resultant crude product was purified by column chromatography (petroleum ether/EtOAc 2:1) to yield (*R*)-**9** (5.24 g, 94%) as a colourless oil. ^{1}H NMR (300 MHz, C_6D_6) δ 7.21–7.03 (m, 5H), 5.87–5.70 (m, 1H), 5.16–4.95 (m, 3H), 4.35 (d, J=11.5 Hz, 1H), 4.17 (d, J=11.5 Hz, 1H), 3.99–3.86 (m, 1H), 3.66–3.47 (m, 3H), 2.39–2.19 (m, 2H), 1.40 (s, 9H); ^{13}C NMR (75 MHz, C_6D_6) δ 156.5, 138.7, 134.6, 128.6, 128.1, 127.9, 117.8, 79.1, 77.8, 72.4, 63.4, 54.4, 35.9, 28.4; MS (ESI) m/z 344.2 (M+Na$^+$); MS (ESI-HR) m/z

calcd for $C_{18}H_{27}NNaO_4$ 344.1832 ($M+Na^+$), found 344.1839 ($M+Na^+$); $[\alpha]_D^{20}$ −4.5 (*c* 1.1, $CHCl_3$); IR (film) ν 3417, 2978, 2359, 1697, 1499, 1367, 1057, 917, 748, 699; UV (MeCN) λ_{max} (log ε) 258 (1.48), 204 (2.93); TLC R_f value 0.25 (petroleum ether/EtOAc 2:1).

*4.2.6. N-Boc-3-O-benzyl-(2R,3S)-2-amino-hex-5-en-1,3-diol ((S)-**9**).* Isomer (*S*)-**9** was prepared in the same way as compound (*R*)-**9** with (*S*)-**8** (4.57 g, 12.6 mmol), acetic acid (100 mL) and H_2O (20 mL). The product (*S*)-**9** (3.66 g, 90%) was obtained as a colourless oil. ^{1}H NMR (300 MHz, C_6D_6) δ 7.27–7.02 (m, 5H), 5.84–5.66 (m, 1H), 5.19 (d, *J*=8.0 Hz, 1H), 5.08–4.94 (m, 2H), 4.32 (d, *J*=11.5 Hz, 1H), 4.16 (d, *J*=11.5 Hz, 1H), 3.88–3.73 (m, 2H), 3.66–3.47 (m, 2H), 2.36–2.04 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, C_6D_6) δ 156.1, 138.7, 134.6, 128.6, 128.0, 127.9, 117.6, 80.4, 79.1, 72.4, 62.1, 54.0, 35.9, 28.4; MS (ESI) *m*/*z* 344.2 ($M+Na^+$); MS (ESI-HR) *m*/*z* calcd for $C_{18}H_{27}NNaO_4$ 344.1832 ($M+Na^+$), found 344.1832 ($M+Na^+$); $[\alpha]_D^{20}$ +35.7 (*c* 1.1, $CHCl_3$); IR (film) ν 3439, 2972, 2439, 1693, 1497, 1367, 1170, 1058, 913, 738, 698; UV (MeCN) λ_{max} (log ε) 258 (1.24), 204 (2.94); TLC R_f value 0.25 (petroleum ether/EtOAc 2:1).

*4.2.7. N-Boc-O-benzyl-(2S,3R)-2-amino-3-hydroxy-5-hexenoic acid tert-butyl ester ((R)-**10**).* A mixture of (*R*)-**9** (5.17 g, 16.1 mmol), bis-(acetoxy)-iodobenzene (BAIB, 11.4 g, 35.4 mmol) and TEMPO (755 mg, 4.83 mmol) in MeCN (50 mL) and H_2O (50 mL) was stirred at room temperature for 3 h, and then Et_2O (200 mL) was added. The reaction mixture was extracted with saturated aqueous $NaHCO_3$ solution and the aqueous layer acidified with 2 N HCl (200 mL) and extracted with EtOAc (4×200 mL). The combined organics of this extraction were dried over Na_2SO_4 and the solvent removed under reduced pressure. The resultant crude product (5.25 g of a brown oil) was used for the subsequent esterification reaction without purification. A solution of this crude product (5.11 g) and *tert*-butanol (19 mL, 15 g, 0.20 mol) in toluene (100 mL) was heated to reflux, and then *N*,*N*-dimethylformamide dineopentylacetal (12 mL, 9.9 g, 43 mmol) was added dropwise under reflux over 30 min. After further stirring for 5 h under reflux, the reaction mixture was cooled to room temperature, washed with saturated aqueous Na_2CO_3 solution (2×100 mL) and H_2O (2×100 mL), dried over Na_2SO_4 and the solvent removed under reduced pressure. The resultant crude product was purified by column chromatography (petroleum ether/EtOAc 12:1) to yield (*R*)-**10** (3.82 g, 61% over two steps from (*R*)-**9**) as a yellowish oil. ^{1}H NMR (300 MHz, C_6D_6) δ 7.26–7.02 (m, 5H), 5.83–5.67 (m, 1H), 5.42 (d, *J*=10.0 Hz, 1H), 5.13–4.96 (m, 2H), 4.69 (dd, *J*=10.0, 2.0 Hz, 1H), 4.38 (d, *J*=11.5 Hz, 1H), 4.31 (d, *J*=11.5 Hz, 1H), 4.01 (ddd, *J*=8.0, 6.0, 2.0 Hz, 1H), 2.42–2.21 (m, 2H), 1.41 (s, 9H), 1.29 (s, 9H); ^{13}C NMR (75 MHz, C_6D_6) δ 170.7, 156.2, 138.5, 134.1, 128.5, 128.2, 127.8, 118.3, 81.3, 79.8, 79.3, 72.4, 57.0, 35.9, 28.3, 27.9; MS (ESI) *m*/*z* 414.0 ($M+Na^+$); MS (ESI-HR) *m*/*z* calcd for $C_{22}H_{33}NNaO_5$ 414.2251 ($M+Na^+$), found 414.2252 ($M+Na^+$); $[\alpha]_D^{20}$ +16.2 (*c* 1.2, $CHCl_3$); IR (film) ν 3451, 2980, 2357, 1722, 1497, 1368, 1154, 1074, 919, 740, 698; UV (MeCN) λ_{max} (log ε) 258 (1.61), 204 (2.98); TLC R_f value 0.20 (petroleum ether/EtOAc 12:1).

*4.2.8. N-Boc-O-benzyl-(2S,3S)-2-amino-3-hydroxy-5-hexenoic acid tert-butyl ester ((S)-**10**).* Isomer (*S*)-**10** was prepared in the same way as compound (*R*)-**10** with (*S*)-**9** (3.55 g, 11.1 mmol), BAIB (7.83 g, 24.3 mmol), TEMPO (518 mg, 3.31 mmol), MeCN (30 mL), H_2O (30 mL), *tert*-butanol (14 mL, 11 g, 0.15 mol), *N*,*N*-dimethylformamide dineopentylacetal (8.5 mL, 7.0 g, 30 mmol) and toluene (80 mL). The product (*S*)-**10** (2.49 g, 57% over two steps from (*S*)-**9**) was obtained as a yellowish oil. ^{1}H NMR (300 MHz, C_6D_6) δ 7.32–7.03 (m, 5H), 5.97–5.81 (m, 1H), 5.45 (d, *J*=8.5 Hz, 1H), 5.15–4.99 (m, 2H), 4.82 (dd, *J*=8.5, 3.5 Hz, 1H), 4.51 (d, *J*=11.5 Hz, 1H), 4.34 (d, *J*=11.5 Hz, 1H), 3.78 (ddd, *J*=6.5, 6.5, 3.5 Hz, 1H), 2.46–2.29 (m, 2H), 1.39 (s, 9H), 1.27 (s, 9H); ^{13}C NMR (75 MHz, C_6D_6) δ 169.7, 155.4, 138.9, 134.9, 128.4, 128.1, 127.9, 117.6, 81.7, 80.4, 79.3, 72.0, 56.5, 36.0, 28.3, 27.9; MS (ESI) *m*/*z* 414.0 ($M+Na^+$); MS (ESI-HR) *m*/*z* calcd for $C_{22}H_{33}NNaO_5$ 414.2251 ($M+Na^+$), found 414.2255 ($M+Na^+$); $[\alpha]_D^{20}$ +10.1 (*c* 0.96, $CHCl_3$); IR (film) ν 3379, 2972, 2345, 1717, 1498, 1367, 1155, 1100, 913, 732, 698; UV (MeCN) λ_{max} (log ε) 258 (1.31), 204 (2.94); TLC R_f value 0.20 (petroleum ether/EtOAc 12:1).

*4.2.9. N-Boc-3-O-benzyl-(2S,3R)-2-amino-3,5-dihydroxy-valeric acid tert-butyl ester ((R)-**11**).* A solution of (*R*)-**10** (3.71 g, 9.48 mmol) in MeOH (175 mL), CH_2Cl_2 (20 mL) and pyridine (3.1 mL) was cooled to −78 °C, and ozone was bubbled through this solution at −78 °C for 1 h. After the addition of dimethyl sulfide (8.0 mL, 6.7 g, 0.11 mol), the reaction was allowed to warm to room temperature overnight. The solvent was removed under reduced pressure, and the resultant crude aldehyde was immediately dissolved in MeOH (50 mL). Sodium borohydride (5.76 g, 152 mmol) was added portionwise to this solution at 0 °C, and the reaction mixture was stirred at room temperature for 5 h and the reaction monitored by TLC (petroleum ether/EtOAc 2:1). Following complete conversion, the reaction was quenched by addition of saturated aqueous NH_4Cl solution, and the aqueous layer was extracted with EtOAc (3×150 mL). The combined organics were washed with H_2O (2×100 mL), dried over Na_2SO_4 and the solvent removed under reduced pressure. The resultant crude product was purified by column chromatography (petroleum ether/EtOAc 2:1) to yield (*R*)-**11** (2.69 g, 72%) as a yellowish oil. ^{1}H NMR (300 MHz, C_6D_6) δ 7.30–7.02 (m, 5H), 5.54 (d, *J*=9.5 Hz, 1H), 4.73 (dd, *J*=9.5, 2.0 Hz, 1H), 4.42 (s, 2H), 4.25 (ddd, *J*=8.5, 6.5, 2.0 Hz, 1H), 3.51 (t, *J*=5.5 Hz, 2H), 1.86–1.58 (m, 2H), 1.38 (s, 9H), 1.29 (s, 9H); ^{13}C NMR (126 MHz, C_6D_6) δ 170.5, 156.5, 138.6, 128.5, 128.3, 127.9, 81.6, 79.6, 78.3, 72.6, 59.5, 57.7, 34.2, 28.5, 28.1; MS (ESI) *m*/*z* 418.2 ($M+Na^+$); MS (ESI-HR) *m*/*z* calcd for $C_{21}H_{33}NNaO_6$ 418.2200 ($M+Na^+$), found 418.2200 ($M+Na^+$); $[\alpha]_D^{20}$ +27.1 (*c* 1.1, $CHCl_3$); IR (film) ν 3433, 2972, 2359, 1717, 1498, 1368, 1155, 1069, 847, 748, 698; UV (MeCN) λ_{max} (log ε) 204 (2.98); TLC R_f value 0.15 (petroleum ether/EtOAc 2:1).

*4.2.10. N-Boc-3-O-benzyl-(2S,3S)-2-amino-3,5-dihydroxy-valeric acid tert-butyl ester ((S)-**11**).* Isomer (*S*)-**11** was prepared in the same way as compound (*R*)-**11** with (*S*)-**10** (2.38 g, 6.08 mmol), MeOH (100 mL), CH_2Cl_2 (12 mL), pyridine (2.0 mL), dimethyl sulfide (4.5 mL, 3.8 g, 61 mmol), sodium borohydride (4.14 g, 109 mmol) and MeOH (30 mL). The product (*S*)-**11** (2.18 g, 91%) was obtained as a yellowish oil. ^{1}H NMR (300 MHz, C_6D_6) δ 7.31–7.02 (m, 5H), 5.59 (d, *J*=8.0 Hz, 1H), 4.85 (dd, *J*=8.0, 3.0 Hz, 1H), 4.62 (d, *J*=11.5 Hz, 1H), 4.32 (d, *J*=11.5 Hz, 1H), 4.02 (ddd, *J*=10.0, 5.5, 3.0 Hz, 1H), 3.61 (t, *J*=5.5 Hz, 2H), 1.86–1.63 (m, 2H), 1.38 (s, 9H), 1.28 (s, 9H); ^{13}C NMR (126 MHz, C_6D_6) δ 169.6, 155.8, 138.8, 128.5, 128.3, 127.7, 82.0, 79.7, 78.5, 72.2, 59.5, 56.7, 34.2, 28.5, 28.0; MS (ESI) *m*/*z* 418.2 ($M+Na^+$); MS (ESI-HR) *m*/*z* calcd for $C_{21}H_{33}NNaO_6$ 418.2200 ($M+Na^+$), found 418.2211 ($M+Na^+$); $[\alpha]_D^{20}$ +2.8 (*c* 1.0, $CHCl_3$); IR (film) ν 3395, 2972, 2377, 1715, 1499, 1368, 1155, 1054, 847, 738, 698; UV (MeCN) λ_{max} (log ε) 257 (1.42), 204 (2.94); TLC R_f value 0.15 (petroleum ether/EtOAc 2:1).

*4.2.11. N^2-Boc-N^5,N^7,$N^{7'}$-Tris-Cbz-3-O-benzyl-(3R)-3-hydroxy-L-arginine tert-butyl ester ((R)-**13**).* To a solution of (*R*)-**11** (2.59 g, 6.55 mmol) in THF (80 mL), tris-Cbz-guanidine **12**[51] (9.06 g, 19.7 mmol) and triphenyl phosphine (2.58 g, 9.83 mmol) were added at room temperature. DIAD (1.93 mL, 1.99 g, 9.83 mmol) was added dropwise at 0 °C at such a rate that the reaction mixture was completely colourless before addition of the next drop. The reaction mixture was allowed to warm to room temperature and was stirred overnight at room temperature. The reaction was then quenched by addition of H_2O (30 mL), and the solvent was removed under reduced pressure. The resultant crude product was purified by column chromatography (petroleum ether/EtOAc 3:1) to yield (*R*)-

13 (3.63 g, 66%) as a colourless viscous oil. ^{1}H NMR (300 MHz, C_6D_6) δ 11.43 (s, 1H), 7.45–6.93 (m, 20H), 5.43 (d, J=9.5 Hz, 1H), 5.29–4.71 (m, 4H), 4.90 (s, 2H), 4.74 (dd, J=9.5, 2.0 Hz, 1H), 4.52 (d, 1H, J=11.5 Hz), 4.44 (d, J=11.5 Hz, 1H), 4.21 (ddd, J=6.5, 6.5, 2.0 Hz, 1H), 4.14–4.01 (m, 1H), 3.99–3.88 (m, 1H), 2.21–2.07 (m, 1H), 2.03–1.87 (m, 1H), 1.40 (s, 9H), 1.29 (s, 9H); ^{13}C NMR (126 MHz, C_6D_6) δ 170.5, 156.3, 154.7, 152.3, 138.7, 135.4, 128.8, 128.7, 128.6, 128.6, 128.5, 128.5, 128.3, 128.1, 127.9, 127.6, 81.4, 79.4, 77.8, 72.2, 69.0, 68.2, 57.5, 45.1, 30.7, 28.5, 28.1; MS (ESI) m/z 861.4 (M+Na$^+$); MS (ESI-HR) m/z calcd for $C_{46}H_{54}N_4NaO_{11}$ 861.3681 (M+Na$^+$), found 861.3690 (M+Na$^+$); $[\alpha]_D^{20}$ +5.3 (c 1.3, $CHCl_3$); IR (film) ν 3433, 2978, 2274, 1720, 1614, 1497, 1455, 1369, 1212, 1048, 750, 698; UV (MeCN) λ_{max} (log ε) 204 (3.65); TLC R_f value 0.30 (petroleum ether/EtOAc 3:1).

*4.2.12. N^2-Boc-N^5,N^7,$N^{7'}$-tris-Cbz-3-O-benzyl-(3S)-3-hydroxy-L-arginine tert-butyl ester ((S)-**13**).* Isomer (*S*)-**13** was prepared in the same way as compound (*R*)-**13** with (*S*)-**11** (2.05 g, 5.18 mmol), tris-Cbz-guanidine **12**[51] (7.17 g, 15.5 mmol), triphenyl phosphine (2.04 g, 7.77 mmol), DIAD (1.53 mL, 1.57 g, 7.77 mmol) and THF (60 mL). The product (*S*)-**13** (3.19 g, 73%) was obtained as a colourless viscous oil. ^{1}H NMR (300 MHz, C_6D_6) δ 11.39 (s, 1H), 7.46–6.94 (m, 20H), 5.37 (d, J=8.5 Hz, 1H), 5.22–4.73 (m, 4H), 4.93 (dd, J=8.5, 3.5 Hz, 1H), 4.86 (s, 2H), 4.70 (d, 1H, J=11.0 Hz), 4.36 (d, J=11.0 Hz, 1H), 4.07–3.91 (m, 3H), 2.05–1.79 (m, 2H), 1.36 (s, 9H), 1.30 (s, 9H); ^{13}C NMR (126 MHz, C_6D_6) δ 169.6, 155.6, 154.8, 151.8, 139.0, 135.4, 128.7, 128.6, 128.5, 128.5, 128.4, 128.3, 128.3, 127.5, 82.1, 79.4, 77.8, 71.7, 69.0, 68.2, 56.2, 45.5, 30.0, 28.5, 28.0; MS (ESI) m/z 861.4 (M+Na$^+$); MS (ESI-HR) m/z calcd for $C_{46}H_{54}N_4NaO_{11}$ 861.3681 (M+Na$^+$), found 861.3681 (M+Na$^+$); $[\alpha]_D^{20}$ +4.3 (c 0.88, $CHCl_3$); IR (film) ν 3379, 2972, 2359, 1717, 1610, 1454, 1366, 1210, 1048, 750, 697; UV (MeCN) λ_{max} (log ε) 204 (3.64); TLC R_f value 0.30 (petroleum ether/EtOAc 3:1).

*4.2.13. N^2-Boc-(3R)-3-hydroxy-L-arginine tert-butyl ester ((R)-**14**).* To a solution of (*R*)-**13** (3.51 g, 4.18 mmol) in MeOH (50 mL), 20% Pd(OH)$_2$/C (Pearlman's catalyst, 586 mg, 0.840 mmol) was added, and the reaction mixture was stirred under an atmosphere of hydrogen (1 bar) at room temperature for 19 h. It was subsequently filtered through Celite and the Celite washed thoroughly with MeOH. The solvent of the filtrate was removed under reduced pressure to yield (*R*)-**14** (1.47 g, quant.) as a colourless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.00–5.80 (br s, 5H), 3.98–3.84 (m, 2H), 3.26–3.10 (m, 2H), 1.69–1.49 (m, 2H), 1.40 (s, 9H), 1.39 (s, 9H); ^{13}C NMR (76 MHz, DMSO-d_6) δ 169.9, 156.9, 155.6, 80.4, 78.3, 68.1, 59.2, 37.5, 33.2, 28.0, 27.6; MS (ESI) m/z 347.2 (M+H$^+$); MS (ESI-HR) m/z calcd for $C_{15}H_{31}N_4O_5$ 347.2289 (M+H$^+$), found 347.2290 (M+H$^+$); mp 125 °C; $[\alpha]_D^{20}$ +1.0 (c 1.0, MeOH); IR (KBr) ν 3412, 2978, 2357, 1682, 1528, 1369, 1245, 1156, 1070, 838, 705; TLC R_f value 0.45 (CH_2Cl_2/MeOH 2:1).

*4.2.14. N^2-Boc-(3S)-3-hydroxy-L-arginine tert-butyl ester ((S)-**14**).* Isomer (*S*)-**14** was prepared in the same way as compound (*R*)-**14** with (*S*)-**13** (3.03 g, 3.61 mmol), 20% Pd(OH)$_2$/C (Pearlman's catalyst, 506 mg, 0.720 mmol) and MeOH (40 mL). The product (*S*)-**14** (1.34 g, quant.) was obtained as a colourless solid. ^{1}H NMR (300 MHz, D_2O) δ 4.17–4.07 (m, 1H), 4.05–3.94 (m, 1H), 3.41–3.29 (m, 2H), 1.88–1.69 (m, 2H), 1.49 (s, 9H), 1.45 (s, 9H); ^{13}C NMR (126 MHz, D_2O) δ 173.4, 166.2, 159.3, 85.6, 84.1, 71.0, 62.1, 40.3, 34.2, 30.3, 29.9; MS (ESI) m/z 347.2 (M+H$^+$); MS (ESI-HR) m/z calcd for $C_{15}H_{31}N_4O_5$ 347.2289 (M+H$^+$), found 347.2291 (M+H$^+$); mp 120 °C; $[\alpha]_D^{20}$ −9.4 (c 0.95, MeOH); IR (KBr) ν 3417, 2978, 2362, 1686, 1454, 1368, 1251, 1156, 1108, 842; TLC R_f value 0.45 (CH_2Cl_2/MeOH 2:1).

*4.2.15. (3R)-3-Hydroxy-L-arginine dihydrochloride ((R)-**4**).* A solution of (*R*)-**14** (1.07 g, 3.09 mmol) in 6 M aqueous HCl (30 mL) was stirred at room temperature for 3 h. The solvent was subsequently removed under reduced pressure. The resultant crude product was purified by column chromatography (RP silica gel 90 C_{18}, H_2O) to yield (*R*)-**4** dihydrochloride (720 mg, 89%) as a slightly yellowish viscous oil. ^{1}H NMR (300 MHz, D_2O) δ 4.37–4.29 (m, 1H), 4.10 (d, J=4.0 Hz, 1H), 3.42 (t, J=7.0 Hz, 2H), 2.09–1.96 (m, 1H), 1.95–1.81 (m, 1H); ^{13}C NMR (126 MHz, D_2O) δ 172.6, 159.3, 69.0, 60.4, 40.4, 34.7; MS (ESI) m/z 191.1 (M+H$^+$); MS (ESI-HR): m/z calcd for $C_6H_{15}N_4O_3$ 191.1139 (M+H$^+$), found 191.1138 (M+H$^+$); $[\alpha]_D^{20}$ +20.4 (c 1.1, H_2O); IR (film) ν 3329, 2357, 1735, 1661, 1505, 1406, 1246, 1097, 573; TLC R_f value 0.10 (*i*-PrOH/H_2O/AcOH 5:2:1 as saturated NaCl solution).

*4.2.16. (3S)-3-Hydroxy-L-arginine dihydrochloride ((S)-**4**).* Isomer (*S*)-**4** was prepared in the same way as compound (*R*)-**4** with (*S*)-**14** (147 mg, 0.424 mmol) and 6 M aqueous HCl (4 mL). The product (*S*)-**4** dihydrochloride (91 mg, 82%) was obtained as a colourless viscous oil. ^{1}H NMR (300 MHz, D_2O) δ 4.31–4.22 (m, 1H), 4.19–4.11 (m, 1H), 3.52–3.32 (m, 2H), 2.08–1.80 (m, 2H); ^{13}C NMR (126 MHz, D_2O) δ 172.3, 159.4, 69.5, 60.7, 40.6, 33.5; MS (ESI) m/z 191.1 (M+H$^+$); MS (ESI-HR) m/z calcd for $C_6H_{15}N_4O_3$ 191.1139 (M+H$^+$), found 191.1145 (M+H$^+$); $[\alpha]_D^{20}$ −1.2 (c 1.0, H_2O); IR (film) ν 3285, 2339, 1737, 1659, 1503, 1406, 1251, 1061, 584; TLC R_f value 0.10 (*i*-PrOH/H_2O/AcOH 5:2:1 as saturated NaCl solution).

*4.2.17. (S)-Configured cyclic carbamate ((S)-**18**).* To a solution of (*R*)-**7** (115 mg, 0.424 mmol) in CH_2Cl_2 (8 mL) and pyridine (2 mL), trifluoromethanesulfonic acid anhydride (Tf_2O, 239 mg, 0.849 mmol) was slowly added at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and monitored by TLC (petroleum ether/EtOAc 1:1) for complete consumption of the starting material. The reaction mixture was then washed with 1 M HCl (10 mL) and H_2O (10 mL), dried over Na_2SO_4 and the solvent removed under reduced pressure. The resultant crude product was purified by column chromatography (petroleum ether/EtOAc 5:1) to yield (*S*)-**18** (76 mg, 91%) as a yellowish oil. ^{1}H NMR (600 MHz, DMSO-d_6) δ 5.77 (ddt, J=17.0, 10.5, 6.5 Hz, 1H), 5.22–5.08 (m, 2H), 4.73 (td, J=8.0, 6.0 Hz, 1H), 4.42 (ddd, J=8.5, 8.0, 6.5 Hz, 1H), 3.87 (dd, J=8.5, 6.5 Hz, 1H), 3.74 (t, J=8.5 Hz, 1H), 2.47–2.36 (m, 2H), 1.59 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 151.7, 136.0, 115.2, 93.1, 78.8, 69.1, 62.8, 60.2, 35.1, 27.6, 25.8, 22.9; MS (ESI) m/z 220.1 (M+Na$^+$); MS (ESI-HR) m/z calcd for $C_{10}H_{15}NNaO_3$ 220.0944 (M+Na$^+$), found 220.0944 (M+Na$^+$); $[\alpha]_D^{20}$ +28.9 (c 1.1, $CHCl_3$); IR (film) ν 2989, 1747, 1644, 1374, 1314, 1260, 1160, 1113, 1037, 812, 770; TLC R_f value 0.50 (petroleum ether/EtOAc 1:1).

*4.2.18. (R)-Configured cyclic carbamate ((R)-**18**).* Isomer (*R*)-**18** was prepared in the same way as compound (*S*)-**18** with (*S*)-**7** (36 mg, 0.13 mmol), Tf_2O (75 mg, 0.26 mmol), CH_2Cl_2 (4 mL) and pyridine (1 mL). The product (*R*)-**18** (12 mg, 47%) was obtained as a yellowish oil. ^{1}H NMR (600 MHz, DMSO-d_6) δ 5.78 (ddt, J=17.0, 10.5, 7.0 Hz, 1H), 5.17 (ddt, J=17.0, 10.5, 1.5 Hz, 2H), 4.48 (td, J=6.0, 5.5 Hz, 1H), 4.09 (ddd, J=7.5, 6.5, 5.5 Hz, 1H), 4.02 (dd, J=8.0, 6.5 Hz, 1H), 3.65 (dd, J=8.0, 7.5 Hz, 1H), 2.52–2.46 (m, 2H), 1.58 (s, 3H), 1.34 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.2, 131.9, 118.8, 93.6, 76.7, 67.4, 62.2, 38.0, 27.3, 23.0; MS (ESI) m/z 220.1 (M+Na$^+$); MS (ESI-HR) m/z calcd for $C_{10}H_{15}NNaO_3$ 220.0944 (M+Na$^+$), found 220.0944 (M+Na$^+$); $[\alpha]_D^{20}$ +39.9 (c 0.90, $CHCl_3$); IR (film) ν 2989, 1760, 1368, 1244, 1152, 1048, 990, 924, 816, 768; TLC R_f value 0.45 (petroleum ether/EtOAc 1:1).

Acknowledgements

We thank the Fonds der Chemischen Industrie (FCI) for financial support. Donation of laboratory equipment by the BASF SE is gratefully acknowledged.

Supplementary data

^{1}H and ^{13}C NMR spectra of all compounds and 1D NOE ^{1}H NMR spectra of compounds (*R*)-**18** and (*S*)-**18**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.10.102.

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55. A more detailed discussion of the 1D NOE ^{1}H NMR experiments is given in the Supplementary data.