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The synthesis of conformationally constrained analogues of the ACE inhibitor Idrapril

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Abstract

A conformationally constrained analogue **3** of the ACE inhibitor Idrapril has been prepared by a five-step synthesis. The key step in the synthesis is the desymmetrization of *endo*-norborn-5-ene-2,3-dicarboxylic anhydride **4** by reaction with *t*-butyl (*S*)-prolinate.

Keywords: Amino acids and derivatives; Anhydrides; Enzymes inhibitors; Hydroxamic acids and derivatives

Introduction

Angiotensin converting enzyme (ACE, EC 3.1.15.1) is a zinc metalloprotease, a biological function of which is to cleave the two *C*-terminal amino acids (histidine and leucine) from the decapeptide angiotensin I, thus forming an octapeptide; angiotensin II. Angiotensin II is a vasoconstrictor, so increased levels of this octapeptide *in vivo* results in an increase in blood pressure which can cause hypertension, heart disease, haemorrhage and renal failure. In addition to being involved in the biosynthesis of angiotensin II, a second biological function of ACE is the degradation of bradykinin, a peptide that exhibits vasodepressing properties. The removal of bradykinin from the human body thus further increases blood pressure.[1]

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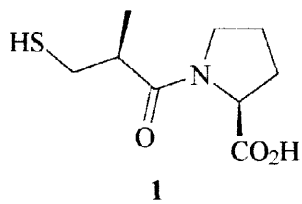
In view of the biological properties of ACE, there has been much interest in the synthesis of ACE-inhibitors as drugs for the control of human blood pressure. This resulted in the synthesis of Captopril **1**, the first orally active ACE-inhibitor to be used clinically.[1,2] Subsequently, a large number of other highly potent ACE-inhibitors have been reported, including Idrapril **2** which has been shown to have a similar potency to Captopril both *in vitro* and *in vivo*.[3] Within Idrapril, the hydroxamic acid unit binds to the zinc atom at the active site of ACE,[4] the carboxylic acid mimics the *C*-terminus of angiotensin-I, and the rest of the molecule occupies the space that would be occupied by the sidechains of the *C*-terminal histidine and leucine residues of angiotensin-I. Idrapril is a conformationally flexible molecule and has been shown to exist in solution as a mixture of conformations and in particular as *cis* and *trans* rotamers about the tertiary amide bond.[5] Conformationally constrained analogues of Idrapril might therefore be expected to show enhanced biological activity provided that the molecule is constrained to the correct conformation to fit into the active site of ACE.



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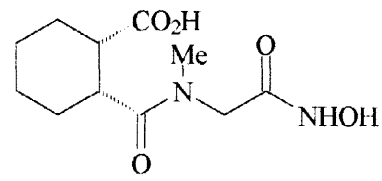

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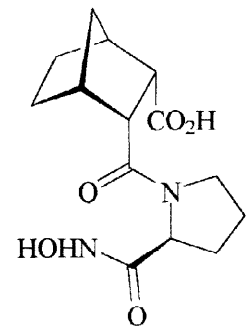


3

In recent publications, we have shown that *meso* anhydrides can be desymmetrized by proline esters, producing enantiomerically pure amido acids.[6] This methodology has been used to prepare peptide analogues which preferentially adopt β-sheet and β-turn conformations,[7] and to synthesize pseudo-peptides containing β-amino cyclopropane carboxylic acid residues.[8] In this manuscript, the use of this desymmetrization methodology in the synthesis of a conformationally constrained analogue **3** of Idrapril is reported. Within compound **3**, the cyclohexane ring of Idrapril has been constrained to a boat conformation by incorporation within a conformationally rigid norbornane ring. The effect of this is to force the two substituents attached to the ring to eclipse one another whilst in Idrapril, where the cyclohexane ring will be in a chair conformation, one substituent will be equatorial and the other axial.[5] Modification of the *N*-methyl glycine residue within Idrapril to an (*S*)-proline unit within analogue **3** prevents rotation around the CH_2-N bond and thus further reduces the conformational flexibility of the analogue.

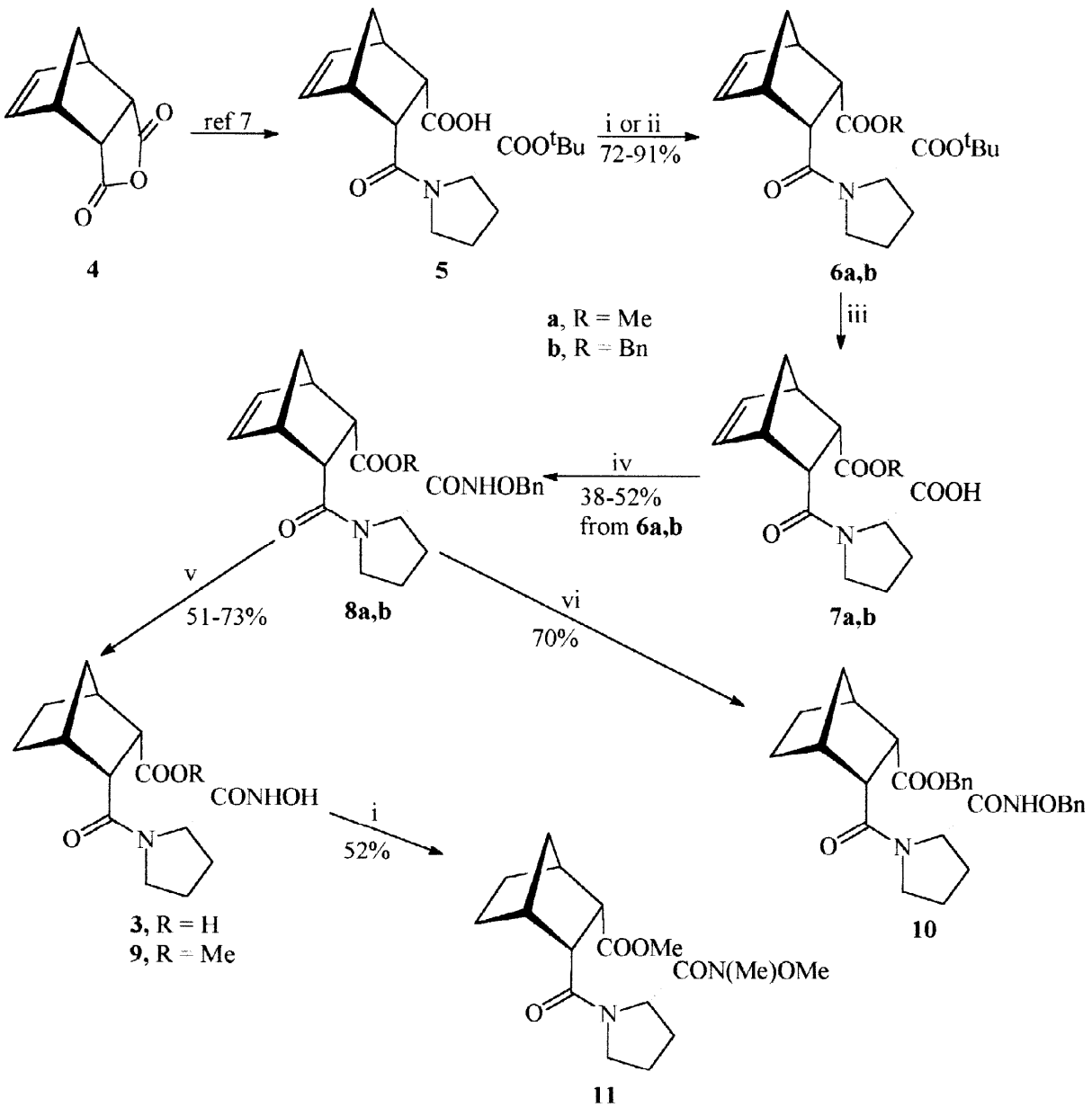
Results

Initially, it was anticipated that Idrapril analogue **3** could be prepared in just two steps by the desymmetrization of *endo*-norborn-5-ene-2,3-dicarboxylic anhydride **4** by a derivative of (*S*)-proline hydroxamate followed by hydrogenation of the resulting norbornene derivative. However, whilst the asymmetric ring-opening of anhydride **4** is known to occur cleanly and diastereoselectively with both *t*-butyl and methyl (*S*)-prolinate,[6,7] reaction of anhydride **4** with (*S*)-proline hydroxamate[9] or *O*-benzyl (*S*)-proline hydroxamate[10] gave a complex mixture of products which did not seem to include the desired adducts. It was also not possible to form compound **3** by reaction of the known amido acid[6] derived from anhydride **4** and methyl (*S*)-prolinate with hydroxylamine. Hence, a less convergent synthesis of compound **3** was developed as shown in **Scheme 1**.

Desymmetrization of anhydride **4** by *t*-butyl (*S*)-prolinate as previously reported[7] gave amido acid **5**. Esterification of amido acid **5** with dimethyl sulphate gave methyl ester **6a**, whilst esterification with benzyl alcohol in the presence of DCC and a catalytic amount of DMAP gave benzyl ester **6b**. Acidolysis of the *t*-butyl esters from compounds **6a,b** using trifluoroacetic acid gave the corresponding acids **7a,b**, which were used without purification. Conversion of acids **7a,b** to the corresponding *O*-benzyl hydroxamates **8a,b** was achieved (in 38 and 52% yield from esters **6a,b**) by reaction with *O*-benzyl hydroxylamine hydrochloride in the presence of DCC, HOBt and triethylamine. In an attempt to improve the chemical yield for the formation of compounds **8a,b**, alternative methods for activating the carboxylic acid group were investigated. However, conversion of acid **7a** to the corresponding *para*-nitrophenyl ester (with DCC and *para*-nitrophenol) followed by reaction of the *para*-nitrophenyl ester with *O*-benzyl hydroxylamine gave the desired adduct **8a** in improved chemical yield (57%) but as a 1:1 mixture of epimers at the proline stereocentre which could be separated by flash chromatography.

Treatment of compound **8a** with hydrogen in the presence of a charcoal supported palladium catalyst resulted in hydrogenolysis of the benzyl protecting group and hydrogenation of the norbornene alkene unit to form compound **9** which is the methyl ester of the desired Idrapril analogue **3**. Similar hydrogenation of compound **8b** resulted in the hydrogenolysis of both benzyl protecting groups and reduction of the alkene to give the desired compound **3** as a white powder. Attempts to saponify the methyl ester of compound **9** resulted only in decomposition, however compound **9** is a potential prodrug of Idrapril analogue **3** since enzymatic hydrolysis of the methyl ester may occur *in vivo*. Attempts were also made to cleave the benzyl protecting groups of compound **8b** without hydrogenating the alkene bond, thus allowing access to derivatives of compound **3**. However, catalytic transfer hydrogenation[11] of ester **8b** using

cyclohexene and a charcoal supported palladium catalyst gave only compound **3**, whilst hydrogenation using Lindlar catalyst[12] reduced the alkene but did not remove the benzyl groups, to give norbornane **10** as the isolated product.



Scheme 1: *Reagents;* i) Me_2SO_4 / K_2CO_3; ii) BnOH / DCC / DMAP; iii) TFA; iv) DCC / HOBt / Et_3N / $BnONH_2.HCl$; v) H_2 / Pd / C; vi) H_2 / Lindlar catalyst

Compound **3** exhibited two sets of peaks in its ^1H NMR spectrum which have been assigned to the *cis* and *trans* rotamers about the amide bond. Slow rotation around this amide bond seems to be a common feature of all norbornane (and norbornene) derivatives which possess an

endo-proline derived amide and an *endo*-carboxylic acid functionality on positions two and three of the norbornane ring. Similar rotamers are observed for compound **5**[7] and the corresponding compound derived from methyl (*S*)-prolinate.[6] Conversion of the acid into an ester as in compounds **6a,b** and **9** increases the rate of rotation around this amide bond so that only a single set of peaks are observed in the NMR spectra of the compounds. To prove the purity of compound **3** and to provide a reference compound for biological evaluation, compound **3** was exhaustively methylated with dimethyl sulphate to give ester **11** which, as expected exhibited a single set of peaks in both its ^{1}H and ^{13}C NMR spectra.

Compounds **3**, **9**, and **11** were screened for activity as inhibitors of five proteases. Disappointingly, all three compounds were completely inactive against ACE, which probably indicates that the eclipsed conformation imposed by the norbornane ring is not appropriate for the active site of this enzyme. All three compounds did however show low levels of inhibition (20, 10, and 10% respectively at a 100 μmol concentration) of fibroblast collagenase (MMP-1), compounds **3** and **11** exhibited 10% inhibition (at a 100 μmol concentration) of gelatinase-A (MMP-2), and compound **3** showed 10% inhibition (at a 100 μmol concentration) of both enkephalinase and stromelysin-1 (MMP-3).

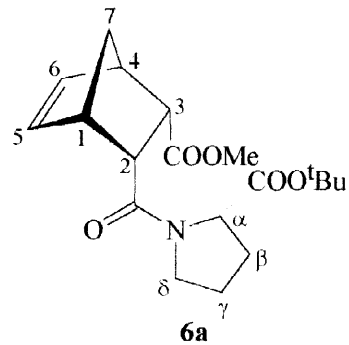
Conclusions

A concise synthetic approach to conformationally constrained protease inhibitors based on the desymmetrization of a *meso* anhydride has been developed. The compounds were designed as inhibitors of ACE, but showed no inhibition of this enzyme, and were most active against fibroblast collagenase (MMP-1). These results suggest that the eclipsed conformation of the *C*-terminal acid and metal binding site is not appropriate for the active site of ACE, but that this conformation is compatible with the active site of other proteases.

Experimental

^{1}H NMR spectra were recorded at 250MHz on a Bruker AM250 spectrometer fitted with a ^{1}H-^{13}C dual probe, and were recorded at 293K in $CDCl_3$. Spectra were internally referenced either to TMS or to the residual solvent peak, and peaks are reported in ppm downfield of TMS. Multiplicities are reported as singlet (s), doublet (d), triplet (t), quartet (q), some combination of these, broad (br), or multiplet (m). ^{13}C NMR spectra were recorded at 62.5MHz on the same spectrometer as ^{1}H NMR spectra, at 293K and in $CDCl_3$. Spectra were referenced to the solvent peak, and are reported in ppm downfield of TMS. Peak assignments for both ^{1}H and ^{13}C NMR

spectra are made using the numbering scheme illustrated below for compound **6a**. Superscripted letters indicate that assignments may be interchanged.



6a

Infra red spectra were recorded on a Perkin Elmer 1600 series FTIR spectrometer, only characteristic absorptions are reported. Low resolution mass spectra were recorded using chemical ionization with ammonia as the reagent gas on a VG Biotech Quattro II triple quadrupole spectrometer. Only significant fragment ions are reported, and only molecular ions are assigned. High resolution mass spectra were recorded using chemical ionization with ammonia as the reagent gas on a VG ZAB-E spectrometer. Optical rotations were recorded on an Optical Activity Ltd. Polar 2001 polarimeter, and are reported along with the solvent and concentration in g/100ml. Melting points are uncorrected. Elemental analyses were performed within the Chemistry department on a Carlo Erba Model 1106 or Model 1108 analyser.

Amido ester ***6a***

To a solution of amido acid **5** (5.4 g, 16.1 mmol) in acetone (160 ml) was added Me_2SO_4 (2.3 ml, 24.0 mmol), and K_2CO_3 (5.6 g, 40.3 mmol), and the resulting solution was heated at reflux for 6 hours. On cooling to room temperature, the inorganic salts were filtered and the acetone evaporated *in vacuo*. The residue was redissolved in EtOAc (100 ml) and 0.5 M aqueous ammonia (100 ml) was added. The mixture was stirred at room temperature for 1 hour, after which the separated organic layer was washed with water (3 × 50 ml), dried ($MgSO_4$), and evaporated *in vacuo* to leave amido ester **6a** (5.1 g, 91%) as a white solid. M.p 108-112 °C; (Found: C, 65.0; H, 8.0; N, 4.4. $C_{19}H_{27}NO_5$ requires C, 65.3; H, 7.8; N, 4.0%); $[\alpha]_D^{23}$ -64.4 (c 1.0, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 2980 (m), 1734 (s), 1717 (m), 1652 (s) and 1558 (m); δ_H 1.30 (1H, d, *J* 8.5 Hz, 7-CH_2), 1.44 (9H, s, $C(CH_3)_3$), 1.4-1.5 (1H, m, 7-CH_2), 1.8-2.2 (4H, m, Pro β-CH_2 and Pro γ-CH_2), 3.1-3.2 (3H, m, 1-CH, 4-CH and 2-CH^a), 3.39 (1H, dd, *J* 9.5, 3.0 Hz, 3-CH^a), 3.45-3.65 (2H, m, Pro δ-CH_2), 3.56 (3H, s, OCH_3), 4.33 (1H, dd, *J* 8.0, 4.0 Hz, Pro α-CH), 6.15-6.25 (2H, m, 2 × =CH); δ_C 24.58 (Pro γ-CH_2), 27.90 ($C(\underline{C}H_3)_3$), 29.11 (Pro β-CH_2), 46.42

(1-CH^a), 46.62 (4-CH^a), 46.85 (Pro δ-$CH_2{}^b$), 47.98 (2-CH^a), 48.35 (7-$CH_2{}^b$), 48.71 (3-CH^a), 51.34 (OCH_3), 59.37 (Pro α-CH), 80.74 ($\underline{C}(CH_3)_3$), 133.94 (=CH), 135.41 (=CH), 170.41, 171.67 and 172.86 (2 × CO_2 and CON); m/z (CI, NH_3) 350 (MH^+, 100), 294 (5) [Found: (CI, NH_3) MH^+, 350.1967. $C_{19}H_{28}NO_5$ requires *M*, 350.1967].

Amido ester ***6b***

A solution of amido acid **5** (6.0 g, 17.9 mmol), DCC (4.1 g, 19.6 mmol), benzyl alcohol (1.9 ml, 18.8 mmol) and DMAP (0.2 g, 1.8 mmol) in CH_2Cl_2 (80 ml) was allowed to stir at room temperature for 12 hours. The dicyclohexylurea by-product was removed by filtration and the filtrate was washed with water (3 × 50 ml), 1 M HCl (3 × 50 ml), saturated aqueous Na_2CO_3 (3 × 50 ml), and water (2 × 50 ml) and dried ($MgSO_4$). The solvent was evaporated *in vacuo* and the residue subjected to flash chromatography using 80% Petrol / 20% EtOAc as eluent to give (R_f = 0.20) amido ester **6b** (5.5 g, 72%) as a white solid. M.p 61-65 °C; (Found: C, 70.6; H, 7.0; N, 3.6. $C_{25}H_{31}NO_5$ requires C, 70.6; H, 7.3; N, 3.3%); $[\alpha]_D^{22}$ -64.4 (c 1.0, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 2978 (s), 1736 (s), and 1648 (s); δ_H 1.32 (1H, d, *J* 8.5 Hz, 7-CH_2), 1.4-1.5 (1H, m, 7-CH_2), 1.44 (9H, s, $C(CH_3)_3$), 1.7-2.1 (4H, m, Pro β-CH_2 and Pro γ-CH_2), 3.15-3.25 (2H, m, 1-CH and 4-CH), 3.26 (1H, dd, *J* 10.0, 3.0 Hz, 2-CH^a), 3.39 (1H, dd, *J* 10.0, 3.0 Hz, 3-CH^a), 3.55-3.7 (2H, Pro δ-CH_2), 4.24 (1H, dd, *J* 7.5, 4.5 Hz, Pro α-CH), 4.95 (1H, d, *J* 12.0 Hz, $PhCH_2$), 5.0 (1H, d, *J* 12.0 Hz, $PhCH_2$), 6.25-6.3 (2H, m, 2 × =CH), 7.3-7.4 (5H, m, ArH); δ_C 24.50 (Pro γ-CH_2), 28.01 ($C(\underline{C}H_3)_3$), 29.02 (Pro β-CH_2), 46.24 (1-CH^a), 46.42 (4-CH^a), 47.53 (7-$CH_2{}^b$), 48.47 (2-CH^a), 48.66 (Pro δ$CH_2{}^b$), 48.87 (3-CH^a), 59.34 (Pro α-CH), 66.16 ($PhCH_2$), 80.81 ($\underline{C}(CH_3)_3$), 128.02, 128.35 and 128.50 (3 × ArCH), 133.94 (=CH), 135.53 (=CH), 136.31 (*ipso* aromatic), 170.37, 171.73 and 172.26 (2 × CO_2 and CON); m/z (CI, NH_3) 426 (MH^+, 100), 370 (3) [Found: (CI, NH_3) MH^+, 426.2280. $C_{25}H_{32}NO_5$ requires *M*, 426.2280].

O-Benzyl hydroxamate ***8a*** *(Method A)*

Amido ester **6a** (2.1 g, 6.0 mmol) was dissolved in a solution of CH_2Cl_2 (10 ml) and trifluoroacetic acid (10 ml), and allowed to stir at room temperature overnight. The solvent was evaporated *in vacuo* to leave acid **7a** as a yellow oil, which was used without purification. To a cooled (0 °C) suspension of the acid in CH_2Cl_2 (80 ml), DCC (1.6 g, 7.8 mmol), HOBt (1-hydroxybenzotriazole) (1.1 g, 7.8 mmol) and *O*-benzyl hydroxylamine hydrochloride (1.2 g, 7.8 mmol) were added, followed by Et_3N (2.5 ml, 18.0 mmol). The mixture was stirred at room temperature for 16 hours, after which the dicyclohexylurea by-product was removed by filtration. The solution was washed with 1 M HCl (2 × 50 ml), saturated aqueous Na_2CO_3 (2 ×

50 ml), and water (2 × 50 ml). The organic phase was separated, dried ($MgSO_4$) and evaporated to dryness *in vacuo*. The crude product was purified by flash chromatography using 50% Petrol / 50% EtOAc as eluent to give (R_f = 0.12) hydroxamate **8a** (0.91 g, 38%) as a white solid. M.p 64-66 °C; $[\alpha]_D^{22}$ -80.5 (c 1.0, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3221 (m), 2980 (m), 2943 (m), 1737 (s), 1690 (s), and 1632 (s); δ_H 1.24 (1H, d, *J* 8.5 Hz, 7-CH_2), 1.40 (1H, d, *J* 8.5 Hz, 7-CH_2), 1.7-2.3 (4H, m, Pro β-CH_2 and Pro γ-CH_2), 2.95 (1H, br s, 1-CH^a), 3.20 (1H, br s, 4-CH^a), 3.25-3.3 (2H, m, 2-CH and 3-CH), 3.4-3.6 (2H, m, Pro δ-CH_2), 3.58 (3H, s, OCH_3), 4.30 (1H, d, *J* 7.5, Pro α-CH), 4.77 (1H, d, *J* 11.0 Hz, $PhCH_2$), 4.86 (1H, d, *J* 11.0 Hz, $PhCH_2$), 6.5-6.2 (2H, m, 2 × =CH), 7.3-7.5 (5H, m, ArH), 9.85 (1H, s, CONHO); δ_C 25.18 (Pro γ-CH_2^a), 26.52 (Pro β-CH_2^a), 42.30 (1-CH^b), 46.56 (4-CH^b), 47.23 (7-CH_2^c), 47.57 (2-CH^b), 48.58 (Pro δ-CH_2^c), 48.73 (3-CH^b), 51.52 (OCH_3), 57.52 (Pro α-CH), 77.99 ($PhCH_2$), 128.39, 128.46 and 129.07 (3 × ArCH), 133.89 (=CH), 135.45 (*ipso* aromatic), 137.77 (=CH), 169.40, 172.69 and 173.21 (2 × CON and CO_2); m/z (CI, NH_3) 399 (MH^+, 62), 293 (19), 250 (53) [Found: (CI, NH_3) MH^+, 399.1920. $C_{22}H_{27}N_2O_5$ requires *M*, 399.1920].

O-Benzyl hydroxamate ***8a*** *(Method B) and its diastereomer at* C_α

Amido ester **6a** (0.20 g, 0.57 mmol) was dissolved in CH_2Cl_2 (1 ml) and trifluoroacetic acid (1 ml), and allowed to stir at room temperature overnight. The solvent was evaporated *in vacuo* to leave acid **7a** as a yellow oil, which was used without purification. To a cooled (0 °C) suspension of the acid in CH_2Cl_2 (5 ml), DCC (0.25 g, 1.1 mmol), *para*-nitrophenol (0.15g, 1.1 mmol) and Et_3N (0.25 ml, 1.8 mmol) were added. After stirring for 16 hours, the dicyclohexylurea by-product was removed by filtration. The filtrate was washed with saturated aqueous Na_2CO_3 (5 × 10 ml), and the organic layer dried ($MgSO_4$) and evaporated to dryness *in vacuo* yielding the crude *para*-nitrophenol active ester. The ester was then redissolved in CH_2Cl_2 (10 ml), and *O*-benzyl hydroxylamine hydrochloride (0.14 g, 0.86 mmol) and Et_3N (0.25 ml, 1.7 mmol) were added at 0 °C. The solution was stirred at room temperature for 14 hours, then washed sequentially with 1 M HCl (2 × 5 ml), saturated aqueous Na_2CO_3 (2 × 5 ml), and water (2 × 5 ml). The organic phase was dried ($MgSO_4$) and evaporated to dryness *in vacuo* affording a yellow oil. Column chromatography using 50% Petrol / 50% EtOAc as eluent afforded (R_f = 0.49; EtOAc) **8a** (0.08 g, 35%) and (R_f = 0.42; EtOAc) the diastereomer at C_α (0.05 g, 22%) as white solids. Analytical data for **8a** is reported in Method A. Analytical data for the diastereomer of **8a**: M.p. 130-133 °C; $[\alpha]_D^{22}$ +79.8 (c 1.0, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3292 (m), 2957 (s), 1725 (s), 1690 (s), and 1650 (s); δ_H 1.25 (1H, d, *J* 8.5 Hz, 7-CH_2), 1.40 (1H, d, *J* 8.5 Hz, 7-CH_2), 1.8-2.35 (4H, m, Pro β-CH_2 and Pro γ-CH_2), 3.05 (1H, br s, 1-CH^a), 3.16 (1H, br s, 4-CH^a), 3.1-3.3 (2H, m, 2-CH and 3-CH), 3.35-3.5 (2H, m, Pro δ-CH_2), 3.46 (3H, s,

OCH_3), 4.43 (1H, d, *J* 7.5 Hz, Pro α-CH), 4.74 (1H, d, *J* 11.0 Hz, $PhCH_2$), 4.89 (1H, d, *J* 11.0 Hz, $PhCH_2$), 6.09 (1H, dd, *J* 5.5, 2.5 Hz, =CH), 6.31 (1H, dd, *J* 5.5, 3.0 Hz, =CH), 7.2-7.4 (5H, m, ArH), 9.96 (1H, s, CONHO); δ_C 24.64 (Pro γ-CH_2), 28.06 (Pro β-CH_2), 46.49 (1-CH^a), 46.67 (4-CH^a), 46.80 (7-CH_2^b), 47.78 (2-CH^a), 48.38 (Pro δ-CH_2^b), 48.60 (3-CH^a), 51.93 (OCH_3), 58.94 (Pro α-CH), 77.92 ($PhCH_2$), 128.27 and 129.14 (2 × ArCH), 133.38 (=CH), 135.73 (*ipso* aromatic), 136.59 (=CH), 168.88, 171.86 and 173.44 (2 × CON and CO_2); m/z (CI, NH_3) 416 ($M + NH_4^+$, 12), 399 (MH^+, 100), 293 (40), 250 (92), 248 (96) [Found: (CI, NH_3) MH^+, 399.1920. $C_{22}H_{27}N_2O_5$ requires *M*, 399.1920].

O-Benzyl hydroxamate ***8b***

Amido ester **6b** (4.0 g, 9.4 mmol) was dissolved in a solution of CH_2Cl_2 (20 ml) and trifluoroacetic acid (10 ml), and allowed to stir at room temperature overnight. The solvent was evaporated *in vacuo* to leave acid **7b** as a yellow oil, which was used without purification. To a cooled (0 °C) suspension of the acid in CH_2Cl_2 (90 ml), DCC (2.9 g, 14.1 mmol), HOBt (1.9 g, 14.1 mmol), and *O*-benzyl hydroxylamine hydrochloride (2.25 g, 14.1 mmol) were added, followed by Et_3N (3.9 ml, 28.2 mmol). The mixture was stirred at room temperature for 16 hours, after which the dicyclohexylurea by-product was removed by filtration. The solution was washed with 1 M HCl (2 × 50 ml), saturated aqueous Na_2CO_3 (2 × 50 ml), and water (2 × 50 ml). The organic phase was separated, dried ($MgSO_4$) and evaporated to dryness *in vacuo*. The crude product was purified by flash chromatography using 50% Petrol / 50% EtOAc as eluent to give (R_f = 0.15) *O*-benzyl hydroxamate **8b** (2.3 g, 52%) as a white solid. M.p 97-100 °C; (Found: C, 70.9; H, 6.3; N, 6.0. $C_{28}H_{30}N_2O_5$ requires C, 70.9; H, 6.4; N, 5.9%); $[\alpha]_D^{23}$ -84.5 (c 1.0, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3213 (m), 3012 (m), 1731 (m), 1696 (m), and 1632 (s); δ_H 1.35 (1H, d, *J* 8.5 Hz, 7-CH_2), 1.47 (1H, d, *J* 8.5 Hz, 7-CH_2), 1.5-2.5 (4H, m, Pro β-CH_2 and Pro γ-CH_2), 3.02 (1H, br s, 1-CH^a), 3.24 (1H, br s, 4-CH^a), 3.3-3.55 (4H, m, 2-CH, 3-CH and Pro δ-CH_2), 4.2-4.25 (1H, m, Pro α-CH), 4.83 (1H, d, *J* 11.0 Hz, $PhCH_2$), 4.93 (1H, d, *J* 11.0 Hz, $PhCH_2$), 5.0 (2H, s, $PhCH_2$), 6.2-6.3 (2H, m, 2 × =CH), 7.3-7.5 (10H, m, ArH), 9.91 (1H, s, NHO); δ_C 25.09 (Pro γ-CH_2^a), 26.29 (Pro β-CH_2^a), 46.37 (1-CH^b), 46.63 (4-CH^b), 47.14 (7-CH_2^c), 47.55 (2-CH_b), 48.64 (Pro δ-CH_2^c), 48.87 (3-CH^b), 57.42 (Pro α-CH), 66.26 ($CO_2\underline{C}H_2Ph$), 77.97 ($NHOCH_2Ph$), 128.45, 128.63, 128.71 and 129.09 (4 × ArCH), 133.86 (=CH), 135.46 (*ipso* aromatic), 135.75 (=CH), 135.99 (*ipso* aromatic), 169.36, 172.07 and 173.07 (2 × CON and CO_2); m/z (CI, NH_3) 475 (MH^+, 26), 369 (24), 326 (58), 324 (100) [Found: (CI, NH_3) MH^+, 475.2233. $C_{28}H_{31}N_2O_5$ requires *M*, 475.2233].

Hydroxamic acid ***9***

To a solution of *O*-benzyl hydroxamate **8a** (0.28g, 0.70 mmol) dissolved in MeOH (15 ml) was added 10% Pd / C (0.015 g). The resulting suspension was stirred at room temperature under an atmosphere of H_2, until adsorption of H_2 gas had ceased. The mixture was then filtered through Celite, the latter being washed with MeOH (10 ml). The combined organic layers were evaporated *in vacuo* and the residue subjected to flash chromatography using EtOAc as eluent to give (R_f = 0.18) hydroxamic acid **9** (0.16 g, 73%) as a white powder. M.p 85 °C (dec.); $[\alpha]_D^{22}$ -64.0 (c 1.0, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3249 (br), 2954 (s), 2878 (m), 1734 (s), 1680 (s), and 1624 (s); δ_H 1.2-2.5 (10H, m, 5-CH_2, 6-CH_2, 7-CH_2, Pro β-CH_2 and Pro γ-CH_2), 2.5-2.7 (2H, m, 1-CH and 4-CH), 2.77 (1H, d, *J* 10.0 Hz, 2-CH^a), 3.18 (1H, dd, *J* 11.5, 4.0 Hz, 3-CH^a), 3.4-3.6 (2H, m, Pro δ-CH_2), 3.61 (3H, s, OCH_3), 4.45-4.5 (1H, m, Pro α-CH), 7.50 (1H, br s, NHOH), 10.08 (1H, br s, NHOH); δ_C 23.31 (5-CH_2^a), 24.66 (6-CH_2^a), 25.15 (Pro γ-CH_2^a), 28.16 (Pro β-CH_2), 39.76 (1-CH^b), 39.91 (7-CH_2), 40.33 (4-CH^b), 45.94 (2-CH^c), 47.21 (3-CH^c), 47.45 (Pro δ -CH_2), 51.0 (OCH_3), 57.47 (Pro α-CH), 169.75, 172.50 and 173.39 (2 × CON and CO_2); m/z (CI, NH_3) 311 (MH^+, 47), 295 (55), 252 (75), 250 (100) [Found: (CI, NH_3) MH^+, 311.1607. $C_{15}H_{23}N_2O_5$ requires *M*, 311.1607].

Hydroxamic acid ***3***

To a solution of *O*-benzyl hydroxamate **8b** (0.5 g, 1.0 mmol) dissolved in MeOH (20 ml) was added 10% Pd / C (0.2 g). The resulting suspension was stirred at room temperature under an atmosphere of H_2, until adsorption of H_2 had ceased. The mixture was then filtered through Celite, the latter being washed with MeOH (20 ml). The combined organic layers were evaporated *in vacuo* and the residue subjected to flash chromatography using 90% EtOAc / 10% MeOH as eluent to give (R_f = 0.09) hydroxamic acid **3** (0.16 g, 51%) as a white powder. M.p 53-55 °C; $[\alpha]_D^{23}$ -44.2 (c 1.0, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3500-2500 (br), 3400 (m), 3216 (s), 2903 (s), 1700 (s), and 1653 (s); δ_H 0.8-2.0 (10H, m, 5-CH_2, 6-CH_2, 7-CH_2, Pro β-CH_2 and Pro γ -CH_2), 2.35-2.45 (1H, m, 1-CH^a), 2.5-2.6 (1H, m, 4-CH^a), 2.65-2.8 (1H, m, 2-CH^b), 3.15-3.25 (1H, m, 3-CH^b), 3.5-3.7 (2H, m, Pro δ-CH_2), 4.2-4.3 (1H, m, Pro α-CH), 7.02 (2H, br s, CO_2H and NHOH); δ_C 23.28 (5-CH_2^a), 24.50 (6-CH_2^a), 24.91 (Pro γ-CH_2^a), 29.43 (Pro β-CH_2), 40.10 (1-CH^b), 40.32 (4-CH^b), 42.28 (7-CH_2^c), 46.07 (2-CH^b), 47.86 (Pro δ-CH_2^c), 49.82 (3-CH^b), 57.81 (Pro α-CH), 170.24, 172.49 and 177.05 (2 × CON and CO_2); m/z (CI, NH_3) 297 (MH^+, 88), 281 (62), 279 (41) [Found: (CI) MH^+, 297.1450. $C_{14}H_{21}N_2O_5$ requires *M*, 297.1450].

O-Benzyl hydroxamate ***10***

To a solution of *O*-benzyl hydroxamate **8b** (0.20 g, 0.42 mmol) dissolved in MeOH (30 ml) was added Lindlar catalyst (0.3 g). The resulting suspension was stirred at room temperature under an atmosphere of H_2, until adsorption of H_2 had ceased (*ca.* 30 minutes). The mixture was subsequently filtered through Celite, the latter being washed with MeOH (20 ml). The combined organic layers were evaporated *in vacuo* and the residue subjected to flash chromatography using 40% EtOAc / 60% Petrol as eluent to give (R_f = 0.3) *O*-benzyl hydroxamate **10** (0.14 g, 70%) as a yellow oil. $[\alpha]_D^{22}$ -75.4 (c 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 3227 (m), 2961 (s), 2878 (m), 1735 (s), 1690 (s), and 1625 (s); δ_H 1.2-2.4 (10H, m, 5-CH_2, 6-CH_2, 7-CH_2, Pro β-CH_2 and Pro γ-CH_2), 2.4-2.6 (2H, m, 1-CH and 4-CH), 2.8-2.9 (1H, m, 2-CH^a), 3.16 (1H, dd, *J* 11.5, 4.5 Hz, 3-CH^a), 3.3-3.5 (2H, m, Pro δ-CH_2), 4.2-4.25 (1H, m, Pro α-CH), 4.84 (1H, d, *J* 11.0 Hz, $PhCH_2$), 4.91 (1H, d, *J* 11.0 Hz, $PhCH_2$), 4.99 (1H, d, *J* 11.0 Hz, $PhCH_2$), 5.08 (1H, d, *J* 11.0 Hz, $PhCH_2$), 7.2-7.5 (10H, m, ArH), 9.96 (1H, s, NHO); δ_C 23.15 (5-CH_2^a), 24.88 (6-CH_2^a), 25.23 (Pro β-CH_2^a), 26.46 (Pro γ-CH_2^a), 39.52 (1-CH^b), 40.00 (7-CH_2), 40.50 (4-CH^b), 45.75 (2-CH^c), 47.22 (Pro δ-CH_2), 47.27 (3-CH^c), 57.20 (Pro α-CH), 65.74 (Ph$\underline{C}H_2$OCO), 78.02 ($PhCH_2$ONH), 128.02, 128.24, 128.35, 128.82 and 129.07 (5 × ArCH), 135.36 (*ipso* aromatic), 136.30 (*ipso* aromatic), 169.24, 172.58 and 172.65 (2 × CON and CO_2); m/z (CI, NH_3) 477 (MH^+, 5), 387 (3) [Found: (CI, NH_3) MH^+, 477.2389. $C_{28}H_{33}N_2O_5$ requires *M*, 477.2389].

Amido ester ***11***

To a solution of hydroxamic acid **3** (0.095 g, 0.32 mmol) in acetone (2 ml) was added Me_2SO_4 (0.10 ml, 0.96 mmol), and K_2CO_3 (0.11 g, 0.80 mmol) and the resulting solution was heated at reflux for 6 hours. On cooling to room temperature, the inorganic salts were filtered and the acetone evaporated *in vacuo*. The residue was redissolved in EtOAc (2 ml) and 0.5 M aqueous ammonia (2 ml) was added. The mixture was stirred at room temperature for 1 hour, after which the separated organic layer was washed with water (3 × 2 ml), dried ($MgSO_4$), and evaporated *in vacuo* to leave ester **11** (0.056 g, 52%) as a clear oil. $[\alpha]_D^{22}$ -35.0 (c 1.0, $CHCl_3$); ν_{max} (film)/cm^{-1} 2956 (s), 2877 (s), 1734 (s), 1672 (s), and 1645 (s); δ_H 1.3-2.3 (10H, m, 7-CH_2, 6-CH_2, 5-CH_2, Pro β-CH_2 and Pro γ-CH_2), 2.5-2.55 (1H, m, 1-CH^a), 2.65-2.7 (1H, m, 4-CH^a), 2.7-2.8 (1H, m, 2-CH^b), 3.18 (3H, s, NCH_3), 3.15-3.25 (1H, m, 3-CH^b), 3.5-3.7 (2H, m, Pro δ-CH_2), 3.64 (3H, s, CO_2CH_3), 3.79 (3H, s, $NOCH_3$), 4.85 (1H, dd, *J* 8.5, 4.0 Hz, Pro α-CH); δ_C 23.24 (5-CH_2^a), 24.57 (6-CH_2^a), 24.57 (Pro γ-CH_2^a), 28.64 (Pro β-CH_2), 39.72 (1-CH^b), 39.97 (7-CH_2), 40.25 (4-CH^b), 46.24 (2-CH^b), 47.06 (Pro δ-CH_2), 47.11 (3-CH^b), 51.01 ($CO_2\underline{C}H_3$),

56.53 ($NOCH_3$), 61.23 (Pro α-CH), 170.37, 173.56 and 173.69 (2 × CON and CO_2); m/z (CI, NH_3) 339 (MH^+, 100), 309 (60), 277 (8), 250 (11) [Found: (CI, NH_3) MH^+, 339.1920. $C_{17}H_{27}N_2O_5$ requires *M*, 339.1920].

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