Synthesis of a *cis*-conformationally restricted peptide bond isostere and its application to the inhibition of the HIV-1 protease

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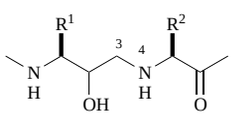
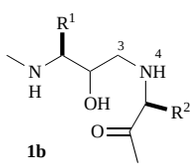
A synthesis of a new, tetrazole-based, *cis*-constrained hydroxyethylamine peptide bond isostere is reported. This has been used to produce a new class of HIV-1 protease inhibitor.

Introduction

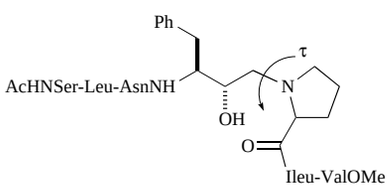
A common strategy used in drug design is to transform specific structural information contained in biologically active peptides into small, non-peptide ligands, referred to as peptidomimetics.[1] Such compounds often possess more favourable pharmacological properties while maintaining the potency and selectivity of the parent peptide. The isosteric replacement of a peptide bond, and other structural units in a peptide backbone, represents a very important and general example of the use of peptidomimetics.[1,2] Examples of peptide bond (–CONH–) isosteres include hydroxyethylamine **1a** (–CHOH–CH_2NH–),



1a



1b



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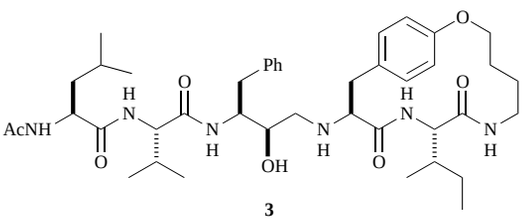

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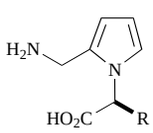

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hydroxymethylene (–CHOH–), hydroxyethylene (–CHOH–CH_2–), dihydroxyethylene (–CHOH–CHOH–) and others.[2] These general peptide bond replacements have been incorporated into oligopeptides[2] to give specific inhibitors of proteolytic enzymes,[3–4] *e.g.* JG365 **2**[3] is a potent, hydroxyethylamine-based inhibitor of the HIV protease. Here, the core isostere of the inhibitor functions as a non-hydrolysable mimic of the tetrahedral transition state which would result from enzyme catalysed cleavage of a substrate peptide bond.

Another important tool in the design of peptidomimetics is to incorporate conformationally restricted units, such as rings,[1,5] into a peptide sequence to force a ligand to adopt a known, biologically active conformation, *e.g.* peptidomimetics of the type **3**[6] have been shown to be potent and biostable inhibitors of the HIV-1 protease. A number of examples also exist in the literature whereby a peptide bond has been incorporated into an aromatic ring (*e.g.* a tetrazole[7] or a pyrrole as in **4**[8]) such that it is forced to adopt a so called *cis* geometry.[1]

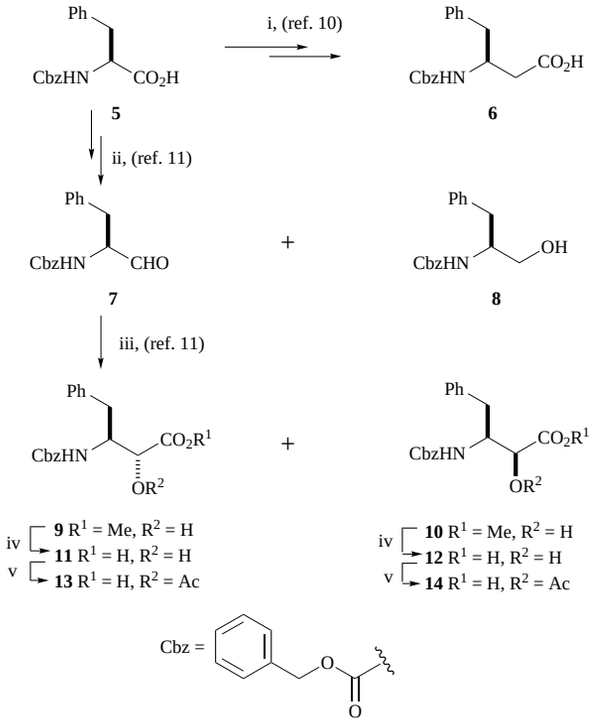
In this paper we present our initial work on the design and synthesis of the first reported example of a '*cis*' conformationally restricted isostere, which represents a combination of both the aforementioned strategies in the design of peptidomimetics, *i.e.* isosteric replacement and conformational restriction. In these peptidomimetics, *e.g.* **20**, **21** and **34**, **35**, a tetrazole has been incorporated into positions 3 and 4 of a hydroxyethylamine isostere (see structure **1a**) such that it is forced to adopt a '*cis*' geometry (see structure **1b**). Our initial results on the application of this isostere to the development of a new class of inhibitor of the HIV-1 protease are also presented. This work is part of our ongoing programme to produce a library of peptidomimetic core-structures possessing well defined conformations and reactivity.[8,9]

Results and discussion

Two main series of compounds, based on the parent hydroxyethylamine isostere **1**, were targeted for synthesis, one without a hydroxy group at C2 (Scheme 2) and one with a hydroxy group at C2 (Schemes 3 and 5). The first series provided control compounds for biological testing and the assignment of stereoisomers (*vide infra*).

N-Cbz-β-phenylalanine **6** was conveniently prepared by silver(I) oxide treatment of the α-diazo ketone derived from *N*-Cbz-phenylalanine (Scheme 1).[10] The sequence outlined in Scheme 2 began with a dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBT) catalysed coupling of *N*-Cbz-β-phenylalanine **6** with L-alanine benzyl ester to give the dipeptide analogue **15**. Treatment of **15** with phosphorus pentachloride and hydrazoic acid[7] gave the *N*- and *C*-protected tetrazole analogue **16** as a single isomer. Compound **16** was *N*-deprotected with 95% hydrobromic acid in acetic acid to give the hydrobromide **17** which was coupled with *N*-(2-quinolinylcarbonyl) (QC) protected L-asparagine, in the presence of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP), to give the tripeptidomimetic **18**. Hydrogenolysis then gave the free acid **19**, which yielded a 1 : 1 mixture of the peptidomimetics **20** and **21** on BOP catalysed coupling with an excess of *tert*-butylamine. Compounds **20** and **21**, which resulted from epimerisation of the alanine-derived residue during the final coupling step, were separable by reversed-phase HPLC [C_{18} column eluting with methanol–water (55 : 45), containing 0.1% trifluoroacetic acid].

The key starting materials for the second series, compounds

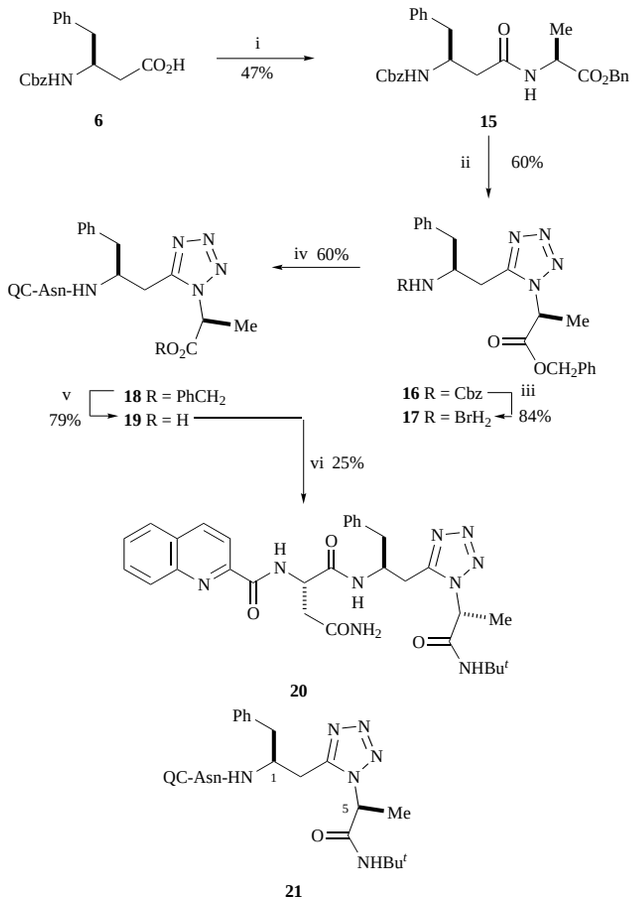


Scheme 1 *Reagents and conditions:* i, Et_3N, THF, $ClCO_2Et$ then CH_2N_2; Ag_2O; ii, Et_3N, MeONHMe·HCl, BOP, CH_2Cl_2 or 3,5-dimethylpyrazole, DCC, $CHCl_3$; $LiAlH_4$, THF; iii, KCN, EtAc, H_2O; HCl; iv, NaOH; v, Ac_2O, pyridine

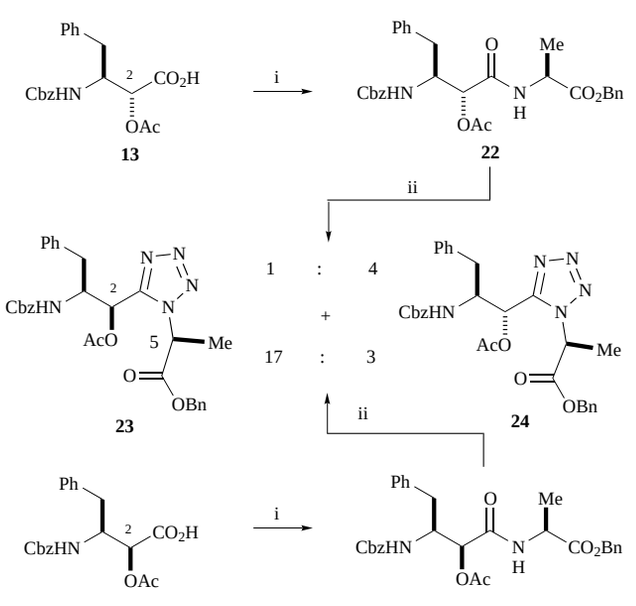
11 and **12**, were readily prepared [11,12] from *N*-Cbz-phenylalanine **5** (Scheme 1). A BOP catalysed coupling of **5** with *N*,*O*-dimethylhydroxylamine, followed by lithium aluminium hydride reduction gave the aldehyde **7** and variable amounts of the over reduced alcohol **8**. Compound **8** was re-oxidized to **7**, using Dess–Martin periodinane.[13] Reaction of **7** with potassium cyanide, followed by methanolysis of the resulting cyanohydrins, gave **9** and **10**.[12] Hydrolysis of the methyl esters, **9** and **10**, with sodium hydroxide gave **11** and **12**. Acetylation of **11** and **12** gave the corresponding acetates **13** and **14**, respectively.

A BOP catalysed coupling of **13** with L-alanine benzyl ester gave the dipeptide analogue **22** (Scheme 3), which was treated with phosphorus pentachloride and hydrazoic acid, in the presence of quinoline, to give a mixture of the *N*- and *C*-protected tetrazole-based peptidomimetics **23** and **24** (1:4 by ^{1}H NMR spectroscopy). A sample of **24** was purified from the mixture by crystallisation from ethyl acetate and light petroleum. A similar sequence starting with the C2 epimer of **13**, compound **14**, gave a 17:3 mixture of **23** and **24**, from which a sample of **23** was obtained by chromatography. The addition of quinoline [7] in the tetrazole formation step was found to mimimise epimerisation at C2 of the peptidomimetics. The intermediate imidoyl chloride, produced on reaction of the dipeptide **22** or **25** with PCl_5 (Scheme 4), is readily protonated on nitrogen such that it is very susceptible to epimerisation at C2 unless a suitable base, *e.g.* quinoline, is present. An attempted conversion of the hydroxy dipeptides **40**, rather than the acetates **22**, **25**, into the corresponding tetrazoles proved unsuccessful (Scheme 5). Compounds **40** were obtained by BOP catalysed coupling of a mixture of **11** and **12** with L-alanine benzyl ester.

A 2:3 mixture of **23** and **24** was *N*-deprotected (95% HBr in acetic acid) to give the corresponding amine salts **26** and **27** (2:3) (Scheme 5). These were coupled with *N*-QC-L-asparagine, to give **28** and **29** (2:3) which were *C*-deprotected (H_2, 10% Pd–C) to give **30** and **31**. A BOP catalysed coupling of this mixture with an excess of *tert*-butylamine gave **32**, **33**, **36** and **37** in a ratio of 1.5:1.5:1.2:1. Finally, hydrolysis with potassium carbonate in methanol and water gave a HPLC separable [C_{18} column eluting with methanol–water (60:40, containing 0.1% trifluoroacetic acid)] mixture of **34**, **35**, **38** and **39** in a ratio of 2.8:2.8:1.3:1. The conversion of **30**, **31** to give **32**, **33**, **36** and **37** resulted in epimerisation at C5 {[(5*R*)-isomers; **32**, **36**]:(5*S*)-isomers; **33**, **37**] = 2.5:2.7}, as was also the case in the preparation of **20**, **21** from **19** (Scheme 2).

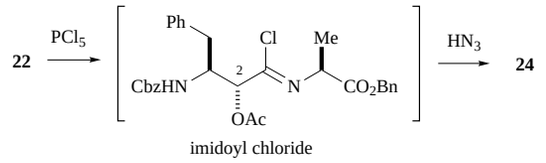


Scheme 2 *Reagents and conditions:* i, L-Ala-OBn·HCl, Et_3N, HOBT, DCC, CH_2Cl_2; ii, PCl_5, HN_3, benzene, room temp.; iii, 95% HBr, AcOH; iv, QC-L-Asn, BOP, Et_3N, CH_2Cl_2, DMF, room temp.; v, H_2, 10% Pd–C, AcOH, EtOH; vi, Bu^tNH_2, BOP, Et_3N, CH_2Cl_2, DMF, room temp.

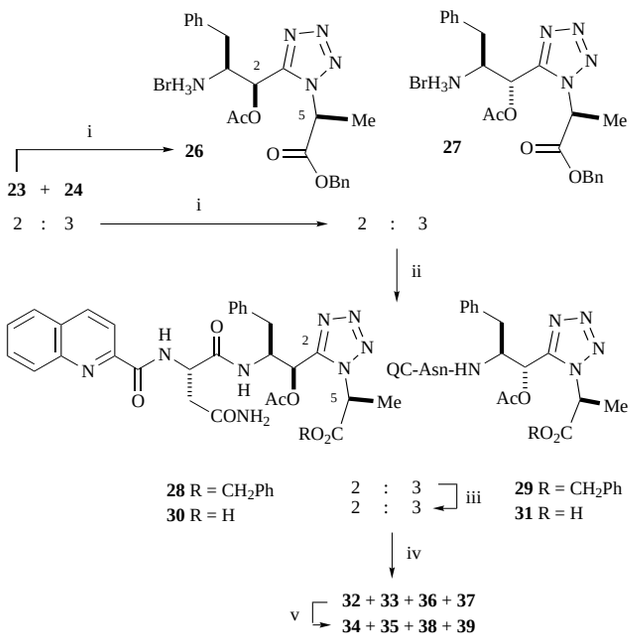


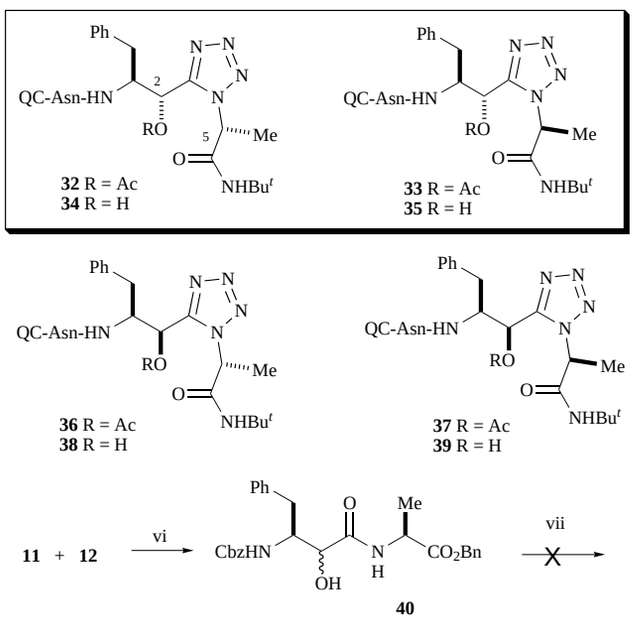
Scheme 3 *Reagents and conditions:* i, L-Ala-OBn·HCl, BOP, Et_3N, CH_2Cl_2, room temp.; ii, PCl_5, HN_3, quinoline, $CHCl_3$, room temp.

The C2 and C5 configurations of the tetrazole-based pepti-



Scheme 4





Scheme 5 *Reagents and conditions:* i, 95% HBr, AcOH; ii, QC-L-Asn, BOP, Et_3N, CH_2Cl_2, DMF, room temp.; iii, H_2, 10% Pd–C, AcOH, EtOH; iv, Bu^tNH_2, BOP, Et_3N, DMF, room temp.; v, K_2CO_3, MeOH–H_2O; vi. L-Ala-OBn·HCl, BOP, Et_3H, CH_2Cl_2, room temp.; vii, PCl_5, HN_3, quinoline, $CHCl_3$, room temp.

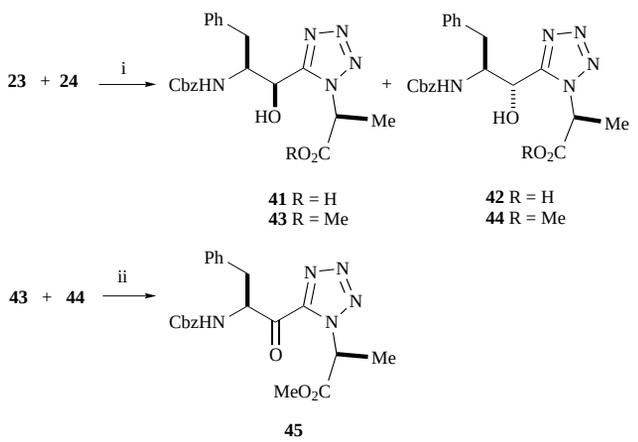
domimetics were assigned on the basis of ^{1}H NMR spectroscopy. Compounds **20** and **21** provided references for the assignment of the C5 configuration. The C5-methyl resonance of the (5*S*)-derivatives is downfield [δ_H 1.88 (**16**), 1.89 (**17**), 1.88 (**18**), 1.91 (**19**) and 1.92 (**21**)] relative to a (5*R*)-configuration [δ_H 1.74 (**20**)]. The (5*S*)-configuration corresponds to that of the starting L-alanine benzyl ester (Scheme 2). The isomeric pairs **23**, **24** and **26**, **27** were also assigned a (5*S*)-configuration on this basis (Table 1) such that, as would be expected, the original alanine configuration is still intact in these compounds. That **23**

Table 1 ^{1}H NMR spectral data

	^{1}H Chemical shift (ppm)[a]				Configuration[a]	
Compound	H1	H2	H5	Me	C2	C5
23	4.58	6.09	5.17	1.84	*S*	*S*
24	4.42	5.92	5.36	1.93	*R*	*S*
26	4.30	6.28	5.65	1.89	*S*	*S*
27	4.08	5.89	5.71	1.93	*R*	*S*
34	4.58	5.00	5.49	1.79	*R*	*R*
35	4.62	5.09	5.55	1.95	*R*	*S*
38	4.61	5.04	5.42	1.80	*S*	*R*
39	4.65	5.12	5.52	1.93	*S*	*S*
43	4.29	5.10	5.69	1.88	*S*	*S*
44	4.25	5.09	5.63	1.92	*R*	*S*
45				1.98		*S*

[a] Non-systematic substituent numbering, see Scheme 5.

and **24**, and hence **26** and **27**, differ in configuration at C2, was established by the preparation of **45** (Scheme 6). To this end,



Scheme 6 *Reagents and conditions:* i, KOH, MeOH–H_2O; ii, Dess–Martin periodinane

hydrolysis of the acetates from a mixture of **23** and **24**, followed by oxidation of the resulting C2 secondary alcohols with Dess–Martin periodinane,[13] gave a single product, **45**. The C5-Me resonance of **45** was observed at δ 1.98, a value consistent with a (5*S*)-configuration. This configuration corresponds to that of the L-alanine benzyl ester used in its synthesis. Compounds **23** and **24** must, therefore, have the same (5*S*)-configuration as the starting L-alanine benzyl ester. Compounds **23** and **24** were obtained as the major products from reactions of **13** and **14**, respectively (Scheme 3). The starting materials **13** and **14** have defined configurations at C2.

The configurations of the final products from the sequence of reactions outlined in Schemes 3 and 5 were also assigned. The (5*R*)-isomers, **34** and **38**, were readily identified on the basis of the upfield C5-Me resonances (δ 1.79 and 1.80, respectively). The corresponding resonances for the (5*S*)-isomers, **35** and **39** occurred at δ 1.95 and 1.93, respectively (Table 1). The C2-configurations of all the derivatives were also assigned on the basis of trends in the ^{1}H NMR data. In particular, the H1 and H2 resonances were downfield, and the H5 resonances upfield for a (2*S*)-configuration relative to a (2*R*)-configuration (Table 1). The C5-Me resonance is also, typically, downfield for a (2*R*)-relative to a (2*S*)-configuration.

The work presented in this paper was not only prompted by our continuing goal to produce a library of peptidomimetic core-structure possessing well defined conformations and reactivity[8,9] but also by a reported crystal structure of JG365 **2** (a potent inhibitor of the HIV-protease)[3] bound to the protease.[3] In this structure, the torsion angle, designated by τ in **2**, is close to zero (referred to here as a *cis*-like geometry). We reasoned that the tetrazole ring in peptidomimetics of the type

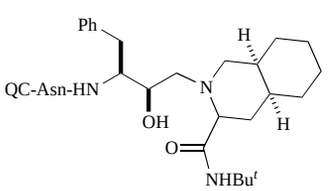
Table 2 HIV-1 protease inhibition data

Compound	IC_{50}/μM
20	300 (±10)
21	170(±20)
34	51(±3)
35	60(±10)

20, **21** (Scheme 2) and **34**, **35** (Scheme 5), would force the hydroxyethylamine isostere core into the enzyme-bound, bioactive, conformation of JG365 (see structure **1b**). The IC_{50} values (Table 2) were determined for compounds **20**, **21**, and the major isomers **34** and **35** (both have the same C2 configuration as JG365 **2**) using HIV-1 protease as described elsewhere.[14] The QC-asparagine and *tert*-butyl amide groups of the peptidomimetics were chosen based on published studies on the inhibition of the HIV-1 protease by analogues of JG365, *e.g.* **46**.[3,15]

Although the compounds tested in the current study are all considerably less potent than JG365 **2**, some preliminary structure/activity trends are evident. Firstly, it is clear that the C2 hydroxy group of the *cis*-conformationally restricted hydroxyethylamine isostere gives compounds with increased potency (compare compounds **20**, **21** with **34**, **35**, Table 2). It would also appear that there is little difference between a (5*S*)- and a (5*R*)-configuration with regards to inhibitory potency (compare **34** and **35**, Table 2).

It must be noted that the tetrazole-based peptidomimetics presented in this paper lack the extended binding sequence of amino acids (P_4-P_3')[3,16] of JG365 **2**, which is known to favour binding in the '*cis*' geometry. The mode of binding of the inhibitors, **2** and **46**, to the HIV-1 protease are quite distinct. The central hydroxyethylamine core (P_1-P_1') of **2** adopts the '*cis*' geometry upon which the current study is based (see structure **1b** and earlier for a discussion) while the equivalent backbone of **46** is thought to adopt an alternative '*trans*' arrangement.[2,3,15] The '*cis*' geometry is favoured when the inhibitor peptide sequence is extended to include P_2' and P_3' residues (Ile and Val, respectively, in **2**).[2,3,15] The *tert*-butyl amide of **46** is thought to occupy the S_2' enzyme subsite forcing it into the alternative '*trans*' arrangement. With this in mind, the peptide sequence of **34** is currently being extended in the *C*-direction. In addition, alternative amino acids to alanine, at the P_1' position, are being investigated. A large enzyme pocket is available for binding the P_1' residue. These and other studies are in progress to optimise the potency of the *cis*-conformationally constrained tetrazoles towards the HIV-1 protease and to further develop them as general peptide bond isosteres.



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Experimental

General

Melting points were obtained using a Hot Stage Microscope and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 300 spectrometer and a Varian XL-300 spectrometer, respectively, in $CDCl_3$ unless otherwise specified. Infrared spectra were obtained using a Perkin Elmer 1600 FTIR Spectrophotometer. Mass spectra were obtained on a Kratos MS80RFA magnetic sector double focusing mass spectrometer. Optical rotations were measured on a JASCO J-20C recording spectropolarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Flash chromatography was carried out on silica gel 60 (mesh 63–200 μm). Preparative chromatography was carried out using a Chromatotron (Harrison Research Inc.) using glass plates coated with Merck type 60 PF_{254} silica gel. Light petroleum refers to the fraction of bp 60–70 °C and ether refers to diethyl ether. HIV-1 protease inhibition assays were carried out as described.[14]

N-Benzyloxycarbonyl-L-phenylalaninal 7

To a stirred solution of Dess–Martin periodinane[13] (774 mg, 1.8 mmol) in dichloromethane (5 cm^3) was added a solution of the alcohol **8** (476 mg, 1.7 mmol), obtained from literature[11] preparations of **7**, in dichloromethane (5 cm^3) and the mixture was stirred at room temp. for 1 h. Ether (25 cm^3) and a solution of $Na_2S_2O_3 \cdot 5H_2O$ (2 g, 8 mmol) in saturated aqueous $NaHCO_3$ (20 cm^3) were added and the mixture was stirred at room temp. for 15 min. The organic layer was washed with saturated aqueous $NaHCO_3$ (5 cm^3), water (5 cm^3) and saturated aqueous NaCl (5 cm^3), dried and evaporated to give the aldehyde **7**[11] (468 mg, 98%).

General procedures

Method A: coupling. To a 0 °C solution of the carboxylic acid in dichloromethane (5 cm^3 mmol^{-1}) was added triethylamine (1 equiv.), 1-hydroxybenzotriazole (HOBT) (1 equiv.) and the amine (1 equiv.; alternatively, 1 equiv. of the amine·HCl or HBr salt and an extra 1 equiv. of triethylamine), and the mixture was stirred for 10 min. DCC (1 equiv.) was added, stirring was continued at 0 °C for a further 10 min and the solution was then left to warm to room temp. over 18 h. The mixture was filtered and evaporated under reduced pressure. The residue was redissolved in ethyl acetate (5 cm^3), washed with aqueous 2 M HCl (2.5 cm^3), aqueous 10% $NaHCO_3$ (2.5 cm^3) and water (2.5 cm^3), dried and evaporated. The crude product was purified by flash chromatography or recrystallisation.

Method B: coupling. Triethylamine (2 equiv.) was added to a solution of the carboxylic acid, amine (1 equiv.; alternatively, 1 equiv. of the amine·HCl or HBr salt and an extra 1 equiv. of triethylamine) and BOP (1.1 equiv.) in dichloromethane or DMF and the mixture was stirred at room temp. for 1 h. A further portion of triethylamine (1 equiv.) was added and stirring was continued at room temp. for 18 h. Saturated aqueous NaCl (3 cm^3) was added and the mixture was extracted with ethyl acetate (3 × 5 cm^3). The organic phase was washed with aqueous 2 M HCl (2.5 cm^3), aqueous 1 M $NaHCO_3$ (3 × 2.5 cm^3) and water (2.5 cm^3), dried and evaporated. The product was purified by flash chromatography.

Method C: tetrazole formation. To a stirred suspension of the amide (1 equiv.) in dry benzene (5 cm^3 mmol^{-1}) was added crystalline PCl_5 (1 equiv.). A transparent solution formed and the mixture was stirred at room temp. for 45 min. An extra portion of PCl_5 (0.2 equiv.) was added and stirring was continued for a further 45 min. A benzene (3 cm^3) solution of hydrazoic acid (10 equiv.) was added and the mixture was stirred at room temp. for 2 d. The mixture was diluted with benzene (5 cm^3) and the organic phase was washed with aqueous 1 M $NaHCO_3$ (3 × 2.5 cm^3), water (2.5 cm^3) and saturated aqueous NaCl (2.5 cm^3). The organic phase was dried and evaporated to give a mixture of the tetrazole and the unreacted starting material which were separated by flash silica chromatography.

Method D: tetrazole formation. Quinoline (2.4 equiv.) was added to a stirred solution of PCl_5 (1.2 equiv.) in dry chloroform (5 cm^3 mmol^{-1}) at room temp. to give a white precipitate. After stirring for 30 min, a solution of the amide (1 equiv.) in chloroform (5 cm^3 mmol^{-1}) was added slowly, keeping the temperature below 20 °C. A further portion of PCl_5 (0.2 equiv.) was added after 1 h and stirring was continued for 2.5 h. A benzene (3 cm^3) solution of hydrazoic acid (30 equiv.) was added and the

mixture was stirred at room temp. for 2 d. The mixture was evaporated, redissolved in ethyl acetate (10 cm^3) and washed successively with aqueous 2 M HCl (2 × 2.5 cm^3), water (2 × 2.5 cm^3) and saturated aqueous NaCl (2.5 cm^3). The solution was dried and evaporated to give a crude mixture of the tetrazole and the unreacted amide which was purified by flash silica chromatography.

Method E: removal of the Cbz protecting group. To a stirred solution of the protected amine (1 equiv.) in acetic acid (1 cm^3) was added 50% HBr in acetic acid (1.9 cm^3 $mmol^{-1}$). After 20 min at room temp., the solution was cooled to −10 °C and pre-cooled ether (10 cm^3) was added with vigorous stirring. Light petroleum (5 cm^3) was added to the resulting precipitate and the mixture was left to stand at 0 °C for 15 min. The residue was washed with light petroleum (2 × 5 cm^3) and the combined organic extracts were evaporated to dryness to give the corresponding amine hydrobromide.

Method F: hydrogenolysis of the benzyl ester. A stirred solution of the benzyl ester (1 equiv.) and acetic acid (1 to 3 drops) in ethanol (2.5–5 cm^3) was hydrogenated for 18 h at room temp. in the presence of 10% Pd–C (188 mg $mmol^{-1}$). The mixture was filtered through Celite, evaporated, redissolved in aqueous 1 M $NaHCO_3$ and washed with a small amount of ethyl acetate (2 cm^3). The aqueous phase was acidified with solid sodium bisulfite to pH 2.5 (universal indicator paper) and the free acid was extracted into ethyl acetate (3 × 10 cm^3). The combined ethyl acetate fractions were dried and evaporated to give the free acid.

(2*S*,3′*S*)-Benzyl 2-[4-phenyl-3-(benzyloxycarbonylamino)-butanoylamino]propanoate 15

N-Cbz-L-β-phenylalanine **6**[10] (1.451 g, 4.6 mmol) was reacted with L-alanine benzyl ester hydrochloride according to general coupling method A. Crystallisation of the crude product from ethyl acetate–light petroleum gave **15** as fine white crystals (1.025 g, 47%), mp 144–145 °C; ν_{max}/cm^{-1} 3427, 3030, 1713, 1670 and 1499; $[\alpha]_D^{20}$ −19 (*c* 0.04 in dichloromethane); δ_H 1.37 (3 H, d, *J* 7.4, Me), 2.33 (1 H, dd, *J* 15.2 and 5.4, CH_ACO), 2.45 (1 H, dd, *J* 14.8 and 5.2, CH_BCO), 2.80 (1 H, dd, *J* 13.4 and 8.1, CHCH_2), 2.99 (1 H, dd, *J* 13.7 and 6.4, CHCH_2), 4.14 (1 H, m, C*H*CH_2), 4.61 (1 H, m, C*H*Me), 5.07 (2 H, s, CbzCH_2), 5.16 and 5.22 (2 H, ABq, *J* 12.2, BnCH_2), 5.75 (1 H, d, *J* 7.8, NH), 6.09 (1 H, d, *J* 5.9, N*H*CBz) and 7.19–7.40 (15 H, m, arom); δ_C 18.10, 38.43, 40.13, 48.07, 50.11, 66.48, 67.19, 126.57, 127.90, 127.97, 128.17, 128.44, 128.46, 128.56, 128.62, 129.29, 135.25, 137.88, 155.86, 170.41 and 172.60 [Found: $(M - PhCH_2)^+$, 383.1606. $C_{21}H_{23}N_2O_5$ requires *m*/*z* 383.1607].

(1′*S*,2″*S*)-[1′-(Benzyloxycarbonyl)ethyl]-5-[2″-(benzyloxycarbonylamino)-3″-phenylpropyl]-1*H*-tetrazole 16

The amide **15** (978 mg, 2.1 mmol) was reacted according to general method A for tetrazole formation. Purification on a 4 mm chromatatron plate, eluting with ethyl acetate–pentane (3:15 to 2:5), gave two fractions. The first fraction contained **16** (484 mg, 47%), mp 103–104 °C (Found: C, 67.5; H, 5.7; N, 14.0. $C_{28}H_{29}N_5O_4$ requires C, 67.3; H, 5.85; N, 14.0%); $[\alpha]_D^{20}$ −51 (*c* 0.01 in dichloromethane); ν_{max}/cm^{-1} 3431, 1751, 1713 and 1510; δ_H 1.88 (3 H, d, *J* 7.3, Me), 2.87 (1 H, dd, *J* 10.2 and 7.5, CHCH_A), 2.98 (3 H, m, CH_2CN_4 and CHCH_B), 4.25 (1 H, m, C*H*CH_2), 5.01 (1 H, q, *J* 7.3, C*H*Me), 5.04 (2 H, s, CbzCH_2), 5.08 and 5.14 (2 H, ABq, *J* 12.0, BnCH_2), 5.48 (1 H, d, *J* 7.8, N*H*Cbz), 7.08 (2 H, m, arom) and 7.19–7.38 (13 H, m, arom); δ_C 16.53, 26.74, 39.03, 50.79, 55.49, 66.75, 68.27, 126.97, 128.00, 128.18, 128.26, 128.53, 128.73, 128.77, 128.82, 129.06, 134.32, 137.10, 152.76, 157.59 and 167.59 (Found: M^+, 499.2223. $C_{28}H_{29}N_5O_4$ requires *m*/*z* 499.2219). The second fraction contained starting material **15** (335 mg, 34%).

(1′*S*,2″*S*)-1-[1′(Benzyloxycarbonyl)ethyl]-5-[3″-phenyl-2″-(quinolin-2-ylcarbonyl-L-asparaginylamino)propyl]-1*H*-tetrazole 18

The tetrazole **16** (362 mg, 0.7 mmol) was reacted with 50% HBr in acetic acid, according to general method E, to give the amine hydrobromide **17** (271 mg, 84%) which was not purified further; δ_H 1.89 (3 H, d, *J* 7.4, Me), 2.99–3.31 (4 H, m, CHCH_2 and CH_2CN_4), 4.09 (1 H, m, C*H*CH_2), 5.14 (2 H, s, BnCH_2), 5.61 (1 H, q, *J* 7.3, C*H*Me) and 7.26–7.38 (10 H, m, arom) (Found: MH^+, 366.1931. $C_{20}H_{24}N_5O_2$ requires *m*/*z* 366.1929).

The amine hydrobromide **17** (218 mg, 0.49 mmol) was reacted with *N*-(2-quinolinylcarbonyl)-L-asparagine[17] (1.1 equiv.) in dichloromethane (3 cm^3) and DMF (40 μl) according to general coupling method B. Purification by flash chromatography, eluting with ethyl acetate–light petroleum (1:1 to 1:0) gave the amide **18** as an oil (186 mg, 60%); $[\alpha]_D^{20}$ +12 (*c* 0.03 in MeOH); δ_H 1.88 (3 H, d, *J* 7.3, Me), 2.71 and 2.83–3.08 (6 H, m, AsnCH_2, CHCH_2 and CH_2CN_4), 4.52 (1 H, m, C*H*CH_2), 4.96 (1 H, m, AsnC*H*), 5.14 (2 H, m, BnCH_2), 5.17 (1 H, q, *J* 7.3, C*H*Me), 5.83 (1 H, br s, NH), 6.26 (1 H, br s, NH), 7.00–7.11 (5 H, m, arom), 7.23–7.34 (5 H, m, arom), 7.59 (1 H, t, QCH), 7.73 (1 H, t, QCH), 7.82 (1 H, d, QCH), 8.12 (2 H, m, QCH), 8.22 (1 H, d, QCH) and 9.20 (1 H, d, *J* 8.3, AsnNH); δ_C 16.39, 26.98, 37.49, 39.87, 49.20, 50.28, 55.70, 68.32, 118.54, 126.76, 127.57, 128.32, 128.56, 128.68, 128.74, 129.19, 129.84, 130.28, 134.50, 136.88, 137.54, 146.34, 148.21, 153.27, 165.00, 168.00, 170.78 and 173.61 (Found: MH^+, 635.2713. $C_{34}H_{35}N_8O_5$ requires *m*/*z* 635.2730).

(1′*R*,2″*S*)- and (1′*S*,2″*S*)-1-[1-(*tert*-Butylaminocarbonyl)ethyl]-5-[3″-phenyl-2″-(quinolin-2-ylcarbonyl-L-asparaginylamino)propyl]-1*H*-tetrazole 20 and 21

The benzyl ester **18** (60 mg, 0.1 mmol) was hydrogenated by general method F to give the acid **19** (41 mg, 79%) which was not purified further; δ_H 1.91 (3 H, d, *J* 7.3, CHMe), 2.62–2.86 (2 H, m, AsnCH_2), 3.10 (4 H, m, CH_2Ph and CH_2CN_4), 4.60 (1 H, m, C*H*CH_2Ph), 4.92 (1 H, m, AsnC*H*), 5.18 (1 H, q, *J* 7.3, C*H*Me), 6.03 (1 H, br, NH), 6.28 (1 H, br, NH), 7.03 (1 H, d, *J* 7.4, NH), 7.08–7.23 (5 H, m, arom), 7.65 (1 H, t, QCH), 7.80 (1 H, t, QCH), 7.88 (1 H, d, QCH), 8.17 (2 H, m, QCH), 8.32 (1 H, d, QCH) and 9.17 (1 H, d, *J* 8.3, AsnNH); δ_H($CDCl_3$, [$^2H_6$]DMSO) 16.18, 26.62, 36.92, 38.96, 49.05, 49.65, 55.36, 118.28, 126.19, 127.31, 127.77, 128.07, 128.79, 128.87, 129.43, 129.83, 136.77, 137.06, 146.05, 148.55, 152.70, 164.06, 170.07, 170.22 and 172.81 (Found: MH^+, 545.2265. $C_{27}H_{29}N_8O_5$ requires *m*/*z* 545.2260).

The acid **19** (29 mg, 0.05 mmol) was reacted with *tert*-butylamine (1.5 equiv.) in dichloromethane according to general coupling method B. Purification by flash chromatography eluting with ethyl acetate–methanol (1:0 to 9:1) gave an epimeric mixture (8 mg, 25%; 1:1 by ^1H NMR spectroscopy) of the amides **20** and **21**. A sample of the epimeric mixture was separated by reversed-phase HPLC on a C_{18} analytical column eluting with methanol–water (55:45, 0.1% TFA). The amide **21** eluted first peak retention time t_R18:48 min; $[\alpha]_D^{20}$ +22 (*c* 0.01 in MeOH); δ_H($CDCl_3$, TFA) 1.31 (9 H, s, CMe_3), 1.92 (3 H, d, *J* 7.3, Me), 2.76–3.04 (4 H, m, CH_2CN_4 and AsnCH_2), 3.15 (2 H, d, *J* 6.4, CH_2Ph), 4.48 (1 H, m, C*H*CH_2Ph), 4.92 (1 H, m, AsnCH), 5.09 (1 H, q, *J* 7.3, C*H*Me), 6.24 (2 H, br, AsnNH_2), 6.44 (1 H, br, NH), 6.97–7.15 (3 H, m, arom), 7.13 (2 H, m, arom), 7.34 (1 H, d, *J* 7.8, NH), 7.68 (1 H, t, QCH), 7.82 (1 H, t, QCH), 7.92 (1 H, d, QCH), 8.19 (2 H, m, QCH), 8.37 (1 H, d, QCH and 9.23 (1 H, d, *J* 8.3, AsnNH); δ_C($CDCl_3$, CD_3OD) 17.85, 27.56, 28.14, 39.25, 48.73, 49.88, 51.66, 57.54, 118.53, 126.58, 127.66, 128.25, 128.40, 128.98, 129.62, 130.30, 136.66 and 137.60 (Found: MNa^+, 622.2875. $C_{31}H_{37}N_9O_4Na$ requires *m*/*z* 622.2866). The amide **20** eluted second; peak retention time t_R 20:40 min; $[\alpha]_D^{20}$ +14 (*c* 0.02 in MeOH); δ_H($CDCl_3$, CD_3OD, TFA), 1.34 (9 H, s, CMe_3), 1.74 (3 H, d, *J* 7.3, Me), 2.73 (2 H, m, AsnCH_2), 2.91–3.13 (4 H, m, CH_2CN_4 and CH_2Ph), 4.50 (1

H, m, C*H*CH$_2$Ph), 4.90 (1 H, m, AsnCH), 5.02 (1 H, q, *J* 6.8, C*H*Me), 7.10–7.20 (5 H, m, arom), 7.33 (1 H, d, *J* 7.8, NH), 7.66 (1 H, t, QCH), 7.81 (1 H, t, QCH), 7.91 (1 H, d, QCH), 8.12 (1 H, d, QCH), 8.17 (1 H, d, QCH) and 8.34 (1 H, d, QCH); δ_C(CDCl$_3$, CD$_3$OD, TFA) 17.56, 27.59, 28.09, 37.41, 38.84, 49.29, 49.82, 51.75, 57.22, 118.45, 126.74, 127.60, 128.21, 128.53, 128.89, 129.51, 130.27, 136.65, 137.56, 146.37, 148.39, 165.69 and 166.86 (Found: MNa$^+$, 622.2875. $C_{31}H_{37}N_9O_4$ requires *m*/*z* 622.2866). Unreacted starting acid was extracted into the NaHCO$_3$ wash during the workup. This phase was acidified with solid sodium bisulfite, extracted with ethyl acetate (3 × 5 cm^3), dried and evaporated to give recovered **19** (18 mg, 62%).

(2*S*,2′*R*,3′*S*)- and (2*S*,2′*S*,3′*S*)-Benzyl 2-[2′acetoxy-3′-(benzyloxycarbonylamino)-4′-phenylbutanoylamino]propanoate 22 and 25

Acetic anhydride (3 equiv.) was added to a solution of **11**[12] or **12**[12] in pyridine (3 cm^3) and the mixture was stirred at room temp. for 18 h. Saturated aqueous NaCl (3 cm^3) was added and the mixture was extracted with chloroform (4 × 10 cm^3). The organic phase was dried and evaporated to give the corresponding acetate **13** (quant.) or **14** (quant.) which were used without further purification. Acetate **13**; δ_H 2.19 (3 H, s, Me), 2.84 (1 H, dd, *J* 13.2 and 8.3, CHC*H*$_A$), 2.96 (1 H, dd, *J* 13.4 and 7.0, CHC*H*$_B$), 4.59 (1 H, m, C*H*CH$_2$), 5.03 (1 H, d, *J* 5.8, C*H*OAc), 5.00 and 5.09 (2 H, ABq, *J* 12.7, CbzC*H*$_2$), 5.32 (1 H, d, *J* 9.7, NH) and 7.18–7.32 (10 H, m, arom). Acetate **14**; δ_H 2.00 (3 H, s, Me), 2.72 (1 H, m, CHC*H*$_A$), 2.99 (1 H, m, CHC*H*$_B$), 4.43 (1 H, m, C*H*CH$_2$), 4.77 and 4.95 (2 H, ABq, *J* 12.2, CbzC*H*$_2$), 4.99 (1 H, d, *J* 5.8, C*H*OAc), 5.54 (1 H, d, *J* 8.8, NH) and 7.06–7.26 (10 H, m, arom); δ_C 21.54, 36.27, 53.19, 66.52, 77.10, 126.30, 127.67, 127.79, 128.28, 129.19, 136.30, 137.64, 156.38, 172.01 and 174.48.

The above acetate samples of **13** or **14** were each reacted with L-alanine benzyl ester hydrochloride (1.1 equiv.) in dichloromethane according to general coupling method B. Purification by flash chromatography eluting with ethyl acetate–light petroleum (3:2) gave the corresponding dipeptide analogues **22** or **25**. Compound **22** (501 mg, 51%), mp 142–144 °C (Found: C, 67.65; H, 6.0. $C_{30}H_{32}N_2O_7$ requires C, 67.65; H, 6.1%); $[\alpha]_D^{20}$ −19 (*c* 0.01 in dichloromethane); δ_H 1.37 (3 H, d, *J* 6.9, Me), 2.05 (3 H, s, COMe), 2.84 (2 H, d, *J* 7.3, CHC*H*$_2$), 4.43 (1 H, m, C*H*CH$_2$), 4.59 (1 H, m, C*H*Me), 4.98 and 5.04 (2 H, ABq, *J* 12.0, CbzC*H*$_2$), 5.14 and 5.21 (2 H, ABq, *J* 12.0, BnC*H*$_2$), 5.21 (1 H, d, *J* 3.9, C*H*OAc), 5.47 (1 H, d, *J* 9.8, N*H*Cbz), 6.68 (1 H, d, 7.3, N*H*CHMe) and 7.17–7.38 (15 H, m, arom); δ_C 17.96, 20.51, 37.75, 48.01, 53.24, 66.68, 67.28, 73.35, 126.65, 127.95, 128.36, 128.49, 128.58, 129.16, 135.07, 136.89, 155.52, 167.52 and 168.98 [Found: (M − PhCH$_2$)$^+$, 441.1661. $C_{23}H_{25}N_2O_7$ requires *m*/*z* 441.1661]. Compound **25** (218 mg, 44%); $[\alpha]_D^{20}$ −11 (*c* 0.06 in dichloromethane); ν_{max}/cm^{-1} 1713, 1686, 1506 and 1217; δ_H 1.41 (3 H, d, *J* 7.0, Me), 2.10 (3 H, s, COMe), 2.90 (1 H, dd, *J* 13.7 and 8.8, CHC*H*$_A$), 2.99 (1 H, dd, *J* 14.2 and 6.3, CHC*H*$_B$), 4.39 (1 H, m, C*H*CH$_2$), 4.59 (1 H, m, C*H*Me), 5.02 (2 H, s, CbzC*H*$_2$), 5.14 and 5.20 (2 H, ABq, *J* 12.5, CH$_2$), 5.17 (1 H, m, C*H*OAc), 5.35 (1 H, d, *J* 9.5, NHCbz), 6.95 (1 H, d, *J* 7.5, NH) and 7.22–7.34 (15 H, m, arom); δ_C 17.78, 20.74, 36.78, 48.11, 53.74, 66.67, 67.25, 74.11, 126.63, 127.74, 127.97, 128.13, 129.38, 128.46, 128.57, 129.12, 135.07, 136.94, 155.99, 167.31, 169.79 and 172.17 [Found: (M − PhCH$_2$)$^+$, 441.1660. $C_{23}H_{25}N_2O_7$ requires *m*/*z* 441.1661].

(1′*S*,1″*S*,2″*S*)- and (1′*S*,1″*R*,2″*S*)-5-[1″-Acetoxy-2″-(benzyloxycarbonylamino)-3″-phenylpropyl]-1-[1′-benzyloxycarbonylethyl]-1*H*-tetrazole 23 and 24

The amide **25** (184 mg, 0.35 mmol) was reacted according to general method D for tetrazole formation. Purification by flash chromatography, eluting with ethyl acetate–light petroleum (2:3) gave two fractions. The first fraction contained an oily mixture (17:3 by ^{1}H NMR spectroscopy) of the tetrazoles **23** and **24** (121 mg, 63%). Further chromatography gave an inseparable mixture of **23** and **24** and a pure sample of **23** (25 mg, 13%); $[\alpha]_D^{20}$ −34 (*c* 0.01 in dichloromethane); ν_{max}/cm^{-1} 1755, 1724 and 1512; δ_H 1.84 (3 H, d, *J* 7.4, Me), 1.84 (3 H, s, COMe), 2.82 (1 H, dd, *J* 14.4 and 7.6, CHC*H*$_A$Ph), 2.93 (1 H, dd, *J* 13.9 and 8.6, CHC*H*$_B$Ph), 4.58 (1 H, m, C*H*CH$_2$), 5.02 and 5.08 (2 H, ABq, *J* 11.7, CbzC*H*$_2$), 5.07 and 5.13 (2 H, ABq, *J* 12.7, BnC*H*$_2$), 5.17 (1 H, m, C*H*Me), 5.79 (1 H, d, *J* 9.3, NH), 6.09 (1 H, d, *J* 5.4, C*H*OAc), 7.01 (2 H, m, arom) and 7.21–7.34 (13 H, m, arom); δ_C 17.09, 20.13, 37.06, 53.83, 56.28, 64.43, 66.81, 68.27, 127.10, 127.86, 128.13, 128.22, 128.50, 128.67, 128.72, 128.80, 134.32, 136.22, 151.22, 156.24, 167.65 and 169.60 (Found: M$^+$, 557.2280. $C_{30}H_{31}N_5O_6$ requires *m*/*z* 557.2274). The second fraction contained unreacted amide **25** (26 mg, 14%).

In a second experiment, the amide **22** (455 mg, 0.9 mmol) was reacted according to general method D for tetrazole formation. Purification by flash chromatography as above gave two fractions. The first fraction contained a mixture (4:1 by ^{1}H NMR spectroscopy) of the tetrazoles **24** and **23** (302 mg, 63%), a sample of which (15 mg) was recrystallized from ethyl acetate–light petroleum to give fine white needles of **24** (5 mg), mp 96–98 °C (Found: C, 64.7; H, 5.8. $C_{30}H_{31}N_5O_6$ requires C, 64.6; H, 5.6%); $[\alpha]_D^{20}$ −49 (*c* 0.01 in dichloromethane); δ_H 1.93 (3 H, d, *J* 7.3, Me), 1.97 (3 H, s, COMe), 2.91 (2 H, m, CHC*H*$_2$), 4.42 (1 H, m, C*H*CH$_2$), 5.02 (2 H, s, CbzC*H*$_2$), 5.05 and 5.12 (2 H, ABq, *J* 12.2, CH$_2$), 5.36 (1 H, m, C*H*Me), 5.92 (1 H, d, *J* 5.4, C*H*OAc), 7.07 (2 H, m, arom) and 7.20–7.32 (13 H, m, arom); δ_C 16.52, 19.98, 36.38, 54.38, 56.12, 64.95, 66.68, 68.11, 126.72, 127.82, 127.97, 128.13, 129.32, 128.49, 128.53, 128.58, 128.89, 134.23, 136.01, 136.45, 152.00, 155.67, 167.60 and 169.79 (Found: M$^+$, 557.2273. $C_{30}H_{31}N_5O_6$ requires *m*/*z* 557.2274). The second fraction contained unreacted amide **22** (147 mg, 32%).

(1′*S*,1″*S*,2″*S*)- and (1′*S*,1″*R*,2″*S*)-5-(1″-Acetoxy-2″-amino-3″-phenylpropyl)-1-(1′-benzyloxycarbonylethyl)-1*H*-tetrazole hydrobromide 26 and 27

The tetrazole **23** (25 mg, 0.1 mmol) was reacted with 50% HBr in acetic acid, according to general method E, to give the amine hydrobromide **26** (17 mg, 84%); δ_H(CD$_3$OD) 1.89 (3 H, d, *J* 7.4, Me), 2.04 (3 H, s, COMe), 3.03 (2 H, m, CHC*H*$_2$), 4.30 (1 H, m, C*H*CH$_2$), 5.16 (2 H, s, CH$_2$), 5.65 (1 H, q, *J* 7.3, C*H*Me), 6.28 (1 H, d, *J* 4.4, C*H*OAc) and 7.17–7.38 (10 H, m, arom) (Found: MH$^+$, 424.1991. $C_{22}H_{26}N_5O_4$ requires *m*/*z* 424.1984). By an identical procedure, a mixture (2:3 by ^{1}H NMR spectroscopy) of the tetrazoles **23** and **24** (45 mg, 0.1 mmol) was reacted with 50% HBr in acetic acid to give a mixture (2:3 by ^{1}H NMR spectroscopy) of the amine hydrobromides **26** and **27** (33 mg, 89%); δ_H(CD$_3$OD) **27** (from the mixture) 1.93 (3 H, d, *J* 7.3, CH*Me*), 2.16 (3 H, s, COMe), 2.95 (2 H, m, CHC*H*$_2$), 4.08 (1 H, m, C*H*CH$_2$), 5.07 and 5.13 (2 H, ABq, *J* 12.2, CH$_2$), 5.71 (1 H, q, *J* 7.3, C*H*Me), 5.89 (1 H, d, *J* 4.4, C*H*OAc) and 7.17–7.38 (10 H, m, arom).

(1′*R*,1″*R*,2″*S*)-, (1′*S*,1″*R*,2″*S*)- (1′*R*,1″*S*,2″*S*,2″*S*)- and (1′*S*,1″*S*,2″*S*)-1-[(*tert*-butylaminocarbonyl)ethyl]-5-[1″-hydroxy-3″-phenyl-2″-(quinolin-2-ylcarbonyl-L-asparaginylamino)propyl]-1*H*-tetrazole 34, 35, 38 and 39

A mixture (31 mg, 0.1 mmol, 2:3 by ^{1}H NMR spectroscopy) of **26** and **27** was reacted with QC-L-Asn (1.3 equiv.) and BOP (1.3 equiv.) in dichloromethane (5 cm^3)–DMF (0.05 cm^3) for 2 d, according to general coupling method B. The crude product (36 mg) contained a mixture (approx 2:3) of **28** and **29**, and was used without further purification; δ_H 1.83 and 1.93 (each 3 H, s, COMe), 1.92 and 1.98 (each 3 H, d, *J* 7.3, Me), 2.68–3.18 (m, CHC*H*$_2$Ph and AsnCH$_2$), 4.59 (m, C*H*CH$_2$Ph), 4.86 (m, AsnCH), 5.11 (m, BnC*H*$_2$), 5.80 (m, C*H*Me), 6.12 and 6.36 (each 1 H, d, *J* 6.3, 5.4, C*H*OAc), 6.60 and 6.79 (each 1 H, d, *J* 7.8, NH), 7.02–7.36 (m, arom), 7.57–8.27 (m, QCH) and 9.07 and 9.17 (each 1 H, d, *J* 8.3 and 7.8, AsnNH).

The crude mixture of benzyl esters **28** and **29** (36 mg) was hydrogenated by general method F to give a mixture of the acids **30** and **31** (15 mg) which was not purified further; δ_H 1.84–2.00 (6 H, m, COMe and CH*Me*), 2.66–3.22 (4 H, m, CH_2Ph and AsnCH_2), 4.70–4.98 (2 H, m, C*H*CH_2Ph and AsnCH), 5.50 and 5.67 (each 1 H, q, *J* 7.3, C*H*Me), 6.31 (1 H, d, *J* 4.4, C*H*OAc), 6.82–8.30 (11 H, m, arom) and 9.12 and 9.23 (each 1 H, d, *J* 8.3, AsnNH).

The mixture of acids **30** and **31** (15 mg, 0.03 mmol) was reacted with *tert*-butylamne (5 equiv.), BOP (1.9 equiv.) and triethylamine (1.5 equiv.) in DMF (0.5 cm^3) according to general coupling method B. Purification by flash chromatography eluting with ethyl acetate–methanol (1:0 to 9:1) gave the four epimers **32**, **33**, **36** and **37** (4 mg, 24%; 1.5:1.5:1.2:1 by ^{1}H NMR spectroscopy) (Found: MH^+, 658.3104. $C_{33}H_{40}N_9O_6$ requires *m*/*z* 658.3101). Reversed phase HPLC on a C_{18} analytical column [methanol–water (55:45, 0.1% TFA)] showed four peaks with retention times of 20:46, 24:31, 28:46 and 30:46 min. A mixture of the unreacted acids **30** and **31** (10 mg, 67%) was recovered from the $NaHCO_3$ washing.

The preceeding mixture of the four acetates (4.0 mg, 0.01 mmol) and potassium carbonate (1.7 mg, 0.01 mmol) were dissolved in methanol–water (0.5 cm^3 of a 9:1 mixture) and the solution was stirred at room temp. for 18 h. Evaporation under reduced pressure gave a residue which was dissolved in ethyl acetate, washed with water, dried and evaporated to give a mixture of **34**, **35**, **38** and **39** (3.5 mg, 95%, 2.8:2.8:1.3:1). The four epimers were separated by reversed-phase HPLC on a C_{18} analytical column, eluting with methanol–water (1:1, 0.1% TFA); peak retention times (t_R) **39** 14:19 (0.39 mg), **38** 16:53 (0.51 mg), **35** 20:11 (1.11 mg) and **34** 26:14 min (1.11 mg). δ_H(**34**) 1.32 (9 H, s, Me_3), 1.79 (3 H, d, *J* 6.9, Me), 2.75 (2 H, m, AsnCH_2), 2.93 and 3.19 (each 1 H, dd, CH_2Ph), 4.58 (1 H, m, C*H*CH_2), 4.84 (1 H, m, AsnCH), 5.00 (1 H, d, *J* 5.4, C*H*OH), 5.49 (1 H, q, *J* 6.8, C*H*Me), 7.01–7.17 (5 H, m, arom), 7.67 (1 H, t, QCH), 7.82 (1 H, t, QCH), 7.93 (1 H, d, QCH), 8.14 (2 H, m, QCH) and 8.36 (1 H, d, QCH) (Found: MH^+, 616.2995. $C_{31}H_{38}N_9O_5$ requires *m*/*z* 616.2995); δ_H(**35**) 1.31 (9 H, s, Me_3), 1.95 (3 H, d, *J* 7.3, Me), 2.68 (2 H, m, AsnCH_2), 2.83 and 3.15 (each 1 H, dd, CH_2Ph), 4.62 (1 H, m, C*H*CH_2), 4.81 (1 H, m, AsnCH), 5.09 (1 H, d, *J* 5.9, C*H*OH), 5.55 (1 H, q, *J* 7.3, C*H*Me), 6.87–7.15 (5 H, m, arom), 7.68 (1 H, t, QCH), 7.82 (1 H, t, QCH), 7.94 (1 H, d, QCH), 8.15 (2 H, m, QCH) and 8.38 (1 H, d, QCH) (Found: MH^+, 616.2995. $C_{31}H_{38}N_9O_5$ requires *m*/*z* 616.2995); δ_H(**38**) 1.29 (9 H, s, Me_3), 1.80 (3 H, d, *J* 6.8, Me), 2.52 and 2.66 (each 1 H, dd, AsnCH_2), 2.97 and 3.12 (each 1 H, d, CH_2Ph), 4.61 (1 H, m, C*H*CH_2), 4.83 (1 H, m, AsnCH), 5.04 (1 H, d, *J* 5.9, C*H*OH), 5.42 (1 H, q, *J* 7.3, C*H*Me), 7.09–7.13 (5 H, m, arom), 7.68 (1 H, t, QCH), 7.81 (1 H, t, QCH), 7.93 (1 H, d, QCH), 8.17 (2 H, m, QCH) and 8.38 (1 H, d, QCH); δ_H(**39**) 1.30 (9 H, s, Me_3), 1.93 (3 H, d, *J* 7.3, Me), 2.44 and 2.70 (each 1 H, dd, AsnCH_2), 2.89 and 3.09 (each 1 H, dd, CH_2Ph), 4.65 (1 H, m, C*H*CH_2), 4.77 (1 H, m, AsnCH), 5.12 (1 H, d, *J* 5.3, C*H*OH), 5.52 (1 H, q, *J* 6.8, C*H*Me), 7.09–7.15 (5 H, m, arom), 7.67 (1 H, t, QCH), 7.81 (1 H, t, QCH), 7.93 (1 H, d, QCH), 8.17 (2 H, m, QCH) and 8.37 (1 H, d, QCH).

(2*S*,2′*R*,3′*S*)- and (2*S*,2′*S*,3′*S*)-Benzyl 2-[3′-(benzyloxycarbonylamino)-2′-hydroxy-4′-phenylbutanoylamino]propanoate 40

A mixture of the acids **11** and **12**[12] (45 mg, 0.1 mmol; 4:1 by ^{1}H NMR spectroscopy) was reacted with L-alanine benzyl ester hydrochloride according to general coupling method A. Purification by flash chromatography eluting with ethyl acetate–dichloromethane (1:9 to 1:3) gave a mixture of the epimers **40** (42 mg, 61%; 4:1 by ^{1}H NMR spectroscopy); δ_H(**40a**) (from the mixture) 1.35 (3 H, d, *J* 7.3, Me), 2.99 (2 H, m, CHCH_2Ph), 4.13 (2 H, m, C*H*CH_2 and C*H*OH), 4.57 (1 H, m, C*H*Me), 4.99 (2 H, s, CbzCH_2), 5.06 and 5.15 (2 H, ABq, *J* 12.2, BnCH_2), 5.20 (1 H, d, *J* 5.4, OH), 5.55 (1 H, d, *J* 8.8, CbzN*H*) and 7.17–7.36 (15 H, m, arom); δ_H(**40b**) (partial data from the mixture) 1.29 (3 H, d, *J* 7.4, Me); δ_C(mixture) 17.69, 17.88, 36.42, 47.74, 47.80, 55.37, 55.55, 66.69, 66.74, 66.94, 67.02, 72.61, 72.43, 126.39, 127.67, 127.78, 127.94, 128.28, 128.32, 128.46, 129.18, 135.18, 136.16, 137.71, 137.65, 156.78, 157.05, 172.16 and 172.39 [Found: $(M - PhCH_2)^+$, 399.1556. $C_{21}H_{23}N_2O_6$ requires *m*/*z* 399.1556]. Reaction of this mixture under tetrazole formation general method C gave an intractable mixture.

(1′*S*,1″*S*,2″*S*)- and (1′*S*,1″*R*,2*S*″)-5-[2″-(Benzyloxycarbonylamino)-1″-hydroxy-3″-phenylpropyl]-1-(1′-methoxycarbonylethyl)-1*H*-tetrazole 43 and 44

A mixture (121 mg, 0.2 mmol; 17:3 by ^{1}H NMR spectroscopy) of the tetrazoles **23** and **24** was dissolved in methanol–water (5 cm^3 of a 8:2 mixture) containing potassium hydroxide (2.2 mg, 0.04 mmol), and the mixture was stirred at room temp. for 18 h. The mixture was acidified with aqueous 2 M HCl and evaporated. Purification on a 1 mm chromatatron plate, eluting with a gradient of light petroleum–ethyl acetate–methanol (3:2:0 to 0:7:3), gave two fractions. The first fraction contained a mixture (7:3 by ^{1}H NMR spectroscopy) of the methyl esters **43** and **44** (19 mg, 20%). The second fraction contained an inseparable mixture (1:1 by ^{1}H NMR spectroscopy) of the free acids **41** and **42** (55 mg, 59%) which were not characterised.

The mixture of **43** and **44** was subjected to reversed-phase HPLC on a C_{18} analytical column, eluting with methanol–water (40:60, 0.1% TFA). The methyl ester **43** eluted first; peak retention time t_R 39:46 min; δ_H 1.88 (3 H, d, *J* 7.4, Me), 3.15 (2 H, m, CHCH_2), 3.72 (3 H, s, OMe), 4.29 (1 H, m, C*H*CH_2), 4.97 and 5.04 (2 H, ABq, *J* 12.4, CbzCH_2), 5.10 (1 H, d, *J* 3.9, C*H*OH), 5.45 (1 H, d, *J* 7.8, NH), 5.69 (1 H, q, *J* 7.3, C*H*Me) and 7.17–7.31 (10 H, m, arom); δ_C 17.05, 35.98, 53.16, 56.88, 67.13, 68.04, 126.80, 127.77, 128.17, 128.47, 128.64, 129.16, 135.83, 137.30, 154.72, 157.31 and 169.19 (Found: M^+, 439.1858. $C_{22}H_{25}N_5O_5$ requires *m*/*z* 439.1855). The methyl ester **44** eluted second; peak retention time t_R 41:31 min; δ_H 1.92 (3 H, d, *J* 7.4, Me), 3.09–3.18 (2 H, m, CHCH_2), 3.70 (3 H, s, OMe), 4.25 (1 H, m, C*H*CH_2), 4.97 and 5.04 (2 H, ABq, *J* 12.2, CbzCH_2), 5.09 (1 H, d, *J* 4.4, C*H*OH), 5.30 (1 H, d, *J* 8.3, NH), 5.63 (1 H, q, *J* 7.3, C*H*Me) and 7.13–7.30 (10 H, m, arom); δ_C 16.37, 36.52, 53.35, 56.62, 67.02, 67.10, 126.73, 127.71, 128.44, 128.58, 129.04, 129.14, 135.83, 136.94, 154.08, 157.28 and 169.20 [Found: $(M - CH_4O)^+$, 407.1594. $C_{21}H_{21}N_5O_4$ requires *m*/*z* 407.1593].

(1′*S*,2*S*″)-5-[2″-(Benzyloxycarbonylamino)-3″-phenylpropanoyl]-1-(1′-methoxycarbonylethyl)-1*H*-tetrazole 45

A mixture of **43** and **44** (2.7 mg, 0.01 mmol; 1:1 by ^{1}H NMR spectroscopy) and Dess–Martin periodinane[13] (14 mg, 0.04 mmol) was dissolved in dichloromethane (3 cm^3) and the solution was stirred at room temp. for 18 h. To the cloudy solution was added $Na_2S_2O_3$ (0.05 mmol) in saturated aqueous $NaHCO_3$ (2 cm^3), and the mixture was stirred at room temp. for 10 min. The mixture was washed with saturated aqueous $NaHCO_2$ (2 cm^3) and water (2 cm^3), dried and evaporated to give **45** (quant.) as a colourless oil; δ_H 1.98 (3 H, d, *J* 7.3, Me), 3.21 (1 H, dd, *J* 13.9 and 7.6, CHCH_A), 3.45 (1 H, dd, *J* 14.2 and 4.0, CHCH_B), 3.75 (3 H, s, OMe), 5.04 (2 H, s, CbzCH_2), 5.33 (1 H, d, *J* 6.8, NH), 5.59 (1 H, m, C*H*CH_2), 5.79 (1 H, q, *J* 7.3, C*H*Me), 7.10 (2 H, m, arom) and 7.24–7.37 (8 H, m, arom); δ_C(incomplete) 16.19, 37.39, 53.39, 58.31, 59.83, 67.26, 127.43, 128.03, 128.29, 128.54, 128.80, 129.36 and 164.79 (Found: M^+, 437.1701. $C_{22}H_{23}N_5O_5$ requires *m*/*z* 437.1699).

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