α-Methylene tetrazole-based peptidomimetics: synthesis and inhibition of HIV protease

Barnaby C. H. May *[a,b] and Andrew D. Abell *[a]

[a] *Department of Chemistry, University of Canterbury, Private Bag 4800, Christchurch, New Zealand. E-mail: a.abell@chem.canterbury.ac.nz*
[b] *Department of Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, California 94143-0450, USA. E-mail: alchemi@itsa.ucsf.edu*

Received (in Cambridge, UK) 8th October 2001, Accepted 22nd November 2001
First published as an Advance Article on the web 14th December 2001

An α-methylene tetrazole-based dipeptidomimetic (**11**) has been prepared as a constrained and non-hydrolysable core for incorporation into peptides. A single crystal X-ray structure determination revealed that its solid-state conformation closely resembles that of the isosteric core of JG-365 bound to HIV protease. The α-methylene tetrazole isosteric unit was then incorporated into a number of peptide sequences and the resulting compounds **6**–**8** were assayed against HIV protease. The assay results suggest that the longer the *C*-terminal substitution the greater the potency, a result that reflects the interplay of the geometry of the tetrazole isostere and the *C*-terminal substituent.

Introduction

In recent years we, and others, have sort to develop peptidomimetics in which the geometry of a constituent peptide bond, or peptide bond isostere, is forced to adopt a '*cis*-like' † geometry by its incorporation into a tetrazole heterocycle (see Fig. 1).[1–5] For

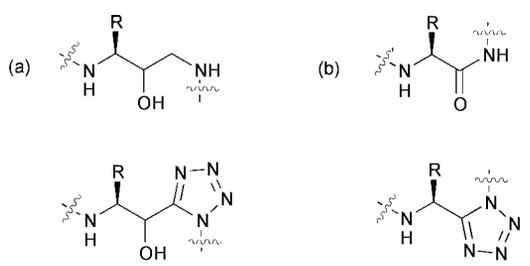
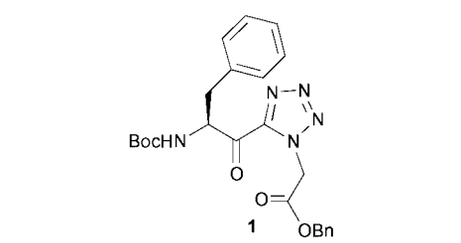
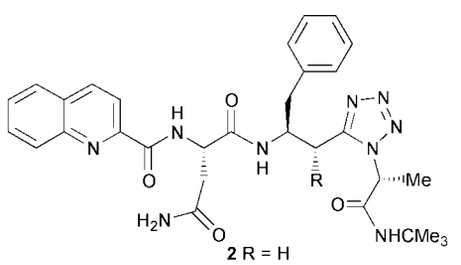


Fig. 1 '*cis*-Like' tetrazole-based peptidomimetics (below) and the parent (above).

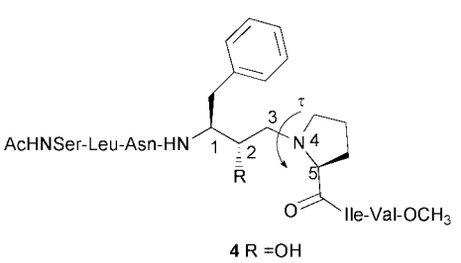
example, the 1,5-disubstituted tetrazole ring is known to be an excellent mimic of a *cis*- amide bond [Fig 1 (b)],[4] and we have recently extended this work by developing non-hydrolysable tetrazole-based amide bond isosteres [Fig 1 (a)]. Compounds that incorporate the tetrazole motif represent important examples of a general class of peptidomimetic[5] in which molecular shape is preorganised into a well defined and biologically active, and/or biostable, conformation. During the course of our work in this area we have developed syntheses of α-keto tetrazole (*e.g.* **1**),[2] α-methylene tetrazole (*e.g.* **2**)[1] and α-hydroxymethylene tetrazole (*e.g.* **3**)[1] based isosteres. The design of these core isosteres was inspired by our observation[1] that JG-365 (**4**) has a *cis*-like geometry about the central hydroxyethylamine core (see torsion angle designated by τ in structure **4**) when bound to HIV protease.[6,7] We have also recently reported some complementary work on developing a *trans*-like olefin-based isostere,[8] as a model for the biologically active, HIV protease-bound, conformations of Saquinavir,[9] and Nelfinavir.[10] We now report the synthesis of some tetrazole-based HIV protease inhibitors using general strategies (Schemes 1 and 2) for *C*-terminal extension of the peptidic sequence of α-methylene tetrazole-based ligands of type **2**.



1



2 R = H
3 R = OH



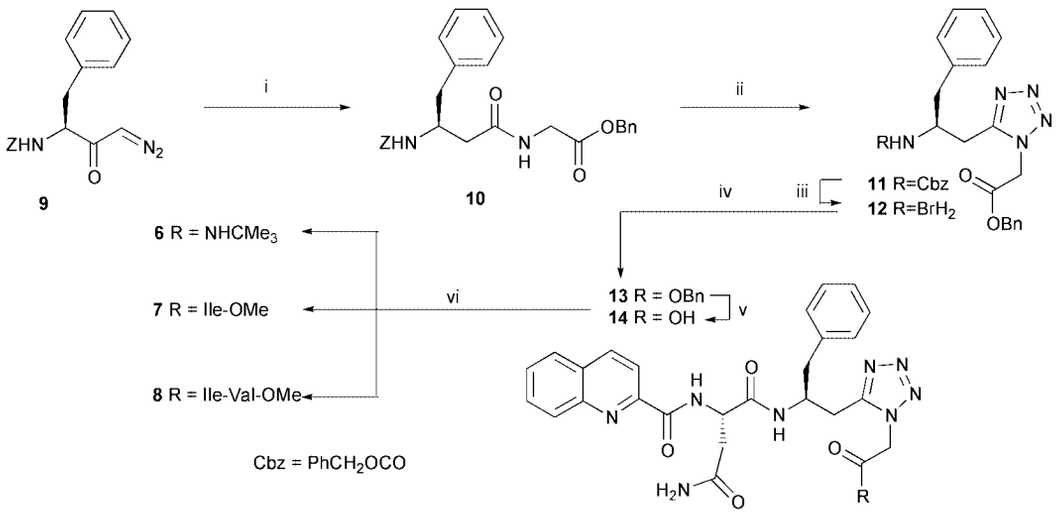
4 R =OH
5 R = H

Results and discussion

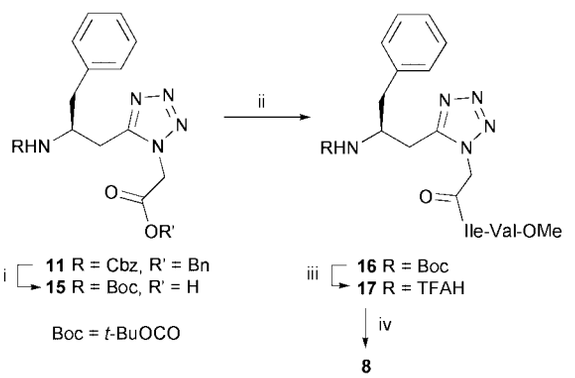
The target peptidomimetics **6**–**8** (Scheme 1) were designed with amino acid residues at positions P_2', P_3' and P_4' as found in the

† '*cis*-Like' describes the relative conformation of the peptide backbone where oxygen has no priority.

 DOI: 10.1039/b109128j



Scheme 1 *Reagents and conditions*: i, Gly–OBn, 300 nm, Et_3N, rt, 18 h; ii, PCl_5, quinoline, HN_3, rt, 2 d; iii, 50% HBr–acetic acid, rt, 30 min; iv, BOP, Et_3N, QC–L–Asn–OH, rt, 18 h; v, H_2, 10% Pd/C, 1 atm, rt, 24 h; iv, *t*-BuNH (**6**) or L–Ile–OMe (**7**) or L–Ile–L–Val–OMe (**8**) and EDCI, HOBt, rt, 18 h.



Scheme 2 *Reagents and conditions*: i, H_2, 10% Pd/C, 1 atm, Boc_2O, rt, 5 h; ii, EDCI, HOBt, DIPEA, L–Ile–L–Val–OMe, rt, 18 h; iii, 95% TFA–water, rt, 30 min; iv, EDCI, HOBt, DIPEA, QC–L–Asn–OH, rt, 18 h.

known HIV protease inhibitors JG-365[6] and Saquinavir.[9] These residues were introduced in an attempt to fill other (relative to **2** and **3**) available subsites (S_3–S_4') ‡ of HIV protease and hence to maximize binding interactions that are thought to favor the *cis*-like geometry of the central tetrazole-based isostere (see below for a discussion). The resulting structure–activity-relationship study (SAR) tests the effect of an increase in *C*-terminal substitution on HIV protease inhibitory activity.

The use of an α-methylene tetrazole isostere, as in **2**, rather than the alternative α-hydroxymethylene tetrazole, as in **3**, reduces the complexity of the synthesis without unduly compromising bioactivity. It is known that the carbon centres adjacent to the tetrazole ring are susceptible to epimerisation under the conditions of peptide coupling.[4,11] As such, the α-methylene tetrazole isostere was specifically incorporated into the dipeptidomimetic, *homo*-phenylalanine-[CN_4]-glycine **11** (Scheme 1), which then formed the core of the target compounds **6**–**8**. We reasoned that these compounds would have activity in the μM range, based on the known HIV protease inhibitor **5** (IC_{50} =3.5 μM),[12] which also lacks a hydroxy-based isostere. This was deemed acceptable since we were interested in demonstrating the methodology and in simply comparing the relative activity of the peptidomimetics with different *C*-terminal substituents. Glycine was used at P_1', rather than alanine as in **2** and **3**, for ease of synthesis and since it was not expected to significantly effect potency.[13]

‡ Note the use of Schechter–Berger nomenclature[24]–the residues on the *N*-terminal side of the peptide bond that is to be cleaved are denoted P_1–P_n, and those on the *C*-terminus are denoted P_1'–P_n'. In turn, the corresponding subsites on the enzyme are denoted S_n–S_n'.

The final point to consider about the ligand design is that the nature of the *C*-terminal substituent is known to influence the mode of binding of an inhibitor to HIV protease. A *tert*-butyl *C*-terminal substituent, as in Saquinavir,[9] induces binding with a pseudo *trans*-conformation about the non-hydrolysable isostere.[14] A solid state structure of HIV protease bound Saquinavir shows the *tert*-butyl substituent twists to occupy the S_2' binding site rather than binding in an extended conformation. Thus, it would be expected that the *C*-terminal *tert*-butyl group of our first generation of compounds (**2** and **3**)[1] is not optimum for binding in the desired *cis*-like geometry dictated by a tetrazole-based isostere. In other words, *cis*-like binding, as dictated by the tetrazole isostere, is thought to be complemented by extension of the peptide sequence in the *C*-terminal direction as in **4** and **5**, but not the *tert*-butyl group of **2** and **3**. We sort to test this hypothesis further in this paper.

Synthesis

The synthesis of the core α-methylene tetrazole-based dipeptide mimic **11** is outlined in Scheme 1. *N*-Cbz-L-phenylalanine diazoketone **9**[15] was subjected to a photochemical Wolff rearrangement, with concomitant peptide coupling to glycine benzyl ester,[16] to give the dipeptide intermediate **10** in 85% yield. The dipeptide **10** was then reacted with phosphorus pentachloride, in the presence of quinoline, according to the method of Zabrocki *et al.*[4] After 3.5 hours at room temperature the imidoyl chloride intermediate was reacted (*in situ*) with a benzene solution of hydrazoic acid.[17] The crude tetrazole was separated from unreacted starting dipeptide by flash column chromatography and a single recrystallisation, to give the desired tetrazole dipeptide mimic **11** in 62% yield. A single crystal X-ray structure of **11** (see Fig. 2 and later for a discussion) was determined at 160(2) K and was satisfactorily refined. The tetrazole **11** was then deprotected by treatment with 50% hydrobromic acid–acetic acid to give the amine hydrobromide **12**, in 99% yield (Scheme 1). The free amine **12** was coupled to *N*-(quinolin-2-ylcarbonyl)-L-asparagine (QC-L-Asn-OH),[18] using benzotriazolyl-*N*-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) in the presence of triethylamine, to give **13** in 51% after chromatography. Catalytic hydrogenation of **13** gave the key tetrapeptidomimetic **14** in 70% yield. This was finally coupled, under *N*-(3-dimethylaminopropyl)-*N'*-ethyl-carbodiimide hydrochloride (EDCI)–1-hydroxybenzotriazole (HOBt) conditions, with separate samples of *tert*-butylamine, L-isoleucine methyl ester and *N*-(L-isoleucine)-L-valine methyl ester. Each of the resulting crude products was then purified by reverse-phase HPLC to give **6**, **7** and **8** in 17%, 18% and 11% yield, respectively.

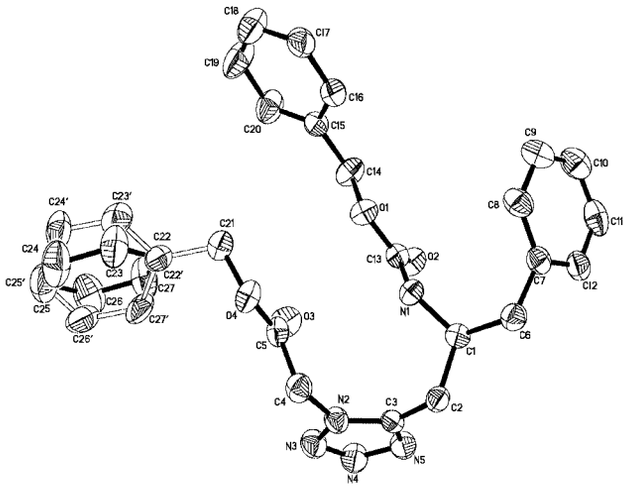


Fig. 2 An ORTEP representation of **11** showing atom labelling.

The peptidomimetic **8** was also synthesised using an alternative deprotection and coupling strategy that enabled extension of the *C*-terminus of **11** prior to *N*-terminal coupling (Scheme 2). For this approach an exchange of the *N*-protecting group was necessary as the tetrazole forming reaction (quinoline, PCl_5, HN_3, Scheme 1, ii) is not amenable to *N*-Boc protection.[4] Thus, debenzylation of **11** and an *in situ* exchange of the *N*-Cbz protecting group for *N*-Boc,[19] provided **15** in 76% yield. The free acid was then coupled with *N*-(L-isoleucine)-L-valine methyl ester hydrochloride, under standard EDCI conditions, to give **16** in 75% yield after chromatography. Treatment of **16** with 95% aqueous TFA gave **17** which was coupled directly with *N*-(quinolin-2-ylcarbonyl)-L-asparagine[18] again under EDCI conditions, to give **8** in 40% yield after chromatography.

X-Ray structure

The structural data obtained for the tetrazole heterocycle of **11** (see Fig. 2) compare well with other reported 1,5-disubstituted tetrazolyl groups.[20,23] The tetrazole ring was found to be essentially planar with the torsion angles C3–N2–N3–N4, N2–N3–N4–N5, N4–N5–C3–N2, N3–N2–C3–N5, C3–N5–N4–N3, being 0.04 (0.40)°, −0.02 (0.41)°, 0.03 (0.40)°, −0.04 (0.41)°, −0.01 (0.42)°, respectively and the ring atoms showing a mean deviation from the plane of 0.00 Å. Bond lengths of the tetrazole heterocycle are all similar, with the bonds C3–N5, N5–N4, N4–N3, N3–N2, N2–C3 being, 1.317 (5) Å, 1.364 (5) Å, 1.302 (5) Å, 1.356 (4) Å, 1.334 (5) Å, respectively. The C3–N5 and N4–N3 bonds are slightly shorter than the other ring bonds, indicative of their double bond character. The N5–N4–N3 endocyclic bond angle, 110.5 (3)°, is wider than the C3–N5–N4 and N4–N3–N2 bond angles, 106.1 (3)° and 105.9 (3)°. The phenyl ring of the *homo*-phenylalanine side chain was disordered in the crystal lattice and the structure was solved by refining the phenyl ring in two conformations, related by rotation about the C_2 axis. The solid state geometry of dipeptidomimetic **11** is further characterised by the distinct fold at the *homo*-phenylalanine-[CN_4]-glycine sequence, as defined by the torsion angles, C3–C2–C1–N1 (66.3°), N2–C3–C2–C1 (−97.1°), C3–N2–C4–C5 (98.2°), and N2–C4–C5–O4 (−172.3°).

A comparison of the geometry of the α-methylene tetrazole-based isostere (as taken from the solid-state structure of **11**, see Fig. 2 for atom numbering) and the core hydroxyethylamine isostere of HIV protease bound JG-365 (see structure **4** for atom numbering) reveals that the key torsion angles about the *cis*-like bond of both structures are in good agreement. The torsion angle, C2–C3–N2–C4, about the tetrazole ring of **11** is forced by the tetrazole annular structure to be essentially planar at 13.05 (0.59)°. This is a good approximation of the corresponding torsion angle, C2–C3–N4–C5, observed in the bound conformation of JG-365, which is only slightly more planar at 11.6°. The correct positioning of the side chain residues, P_1 and P_1', of the α-methylene tetrazole isostere is important for favorable interactions to occur with the binding subsites, S_1 and S_1', of HIV protease. The inter-residue distance of **11** (C1–C4) and JG-365 (C1–C5) are similar at 3.88 Å and 4.31 Å, respectively. The distance between the P_1' residue (C4), and the central non-hydrolysable isostere (C2) in **11** is 3.19 Å, only slightly longer than in JG-365, where the corresponding distance (C2–C5) is 2.96 Å.

Table 1 Inhibition of HIV protease.

Compound	IC_{50} (μM)
6	94(±50)
7	47(±20)
8	18(±10)

Inhibition of HIV protease

The α-methylene tetrazole-based inhibitors, **6**, **7** and **8** were tested for *in vitro* activity against HIV protease,[21] and the results are detailed in Table 1. The known inhibitor JG-365 (**4**), which provided the design basis for the current study, exhibits an IC_{50} of 6.0 nM.§ This compares to the most potent inhibitor in our series, compound **8**, which gave an IC_{50} value of 18 μM (*i.e.* 3000 times less potent). As discussed above this is expected since the α-methylene tetrazole core of **6**–**8** lacks the key hydroxy group and large S_1' residue (proline) of **4**. The α-methylene tetrazole-based inhibitor **8** is, however, similarly potent to the ethylamine [CH_2CH_2NH] based HIV protease inhibitor **5** (IC_{50} = 3.5 μM), which also lacks an α-hydroxy functionality.

The results presented in Table 1 suggest that potency may correlate with the length of the *C*-terminus. Previous literature reports detailing the binding mode of *tert*-butyl substituted HIV protease inhibitors (see above for a discussion),[14] suggest a non-complementarity of the *tert*-butyl P_2' residue and the tetrazole-based isostere towards HIV protease binding. On this basis it would be expected that **6** would possess a reduced activity compared to **7**, and more particularly, **8**. Such a trend is suggested by the results in Table 1, but further work is required to confirm this preliminary finding. The most *C*-extended and active derivative of the series, compound **8** (IC_{50} = 18 μM), was found to be slightly more potent than compound **3** (IC_{50} = 60 μM),[1] which possesses a favorable α-hydroxymethylene tetrazole isostere but the disfavored *tert*-butyl *C*-terminal.

Conclusion

We have synthesised a core dipeptidomimetic, containing a non-hydrolysable α-methylene tetrazole isostere, which is suitable for incorporation into peptidomimetics, typified by **6**–**8**. An X-ray structure determination of dipeptidomimetic **11** has shown that its solid state structure and conformation is well defined and closely resembles that of the bioactive *cis*-like conformation of the core isostere of the JG-365 (**4**), a potent inhibitor of HIV protease. Peptidomimetics **6**–**8** were assayed against HIV protease and the results suggest that the activity of these ligands is affected by the complementarity between the *cis*-like tetrazole-based isostere and the *C*-terminal substituents, where extended *C*-terminal residues gave greater activity. This result reflects the interplay of the geometry of the tetrazole isostere and the *C*-terminal substituent.

§ IC_{50} values of **4** and **5** were obtained independently of this current series of compounds (see refs. 6, 7 and 12 respectively).

Experimental

Melting points were obtained on an Electrothermal apparatus 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. Infra red spectra were obtained using a Perkin Elmer 1600 FTIR spectrometer. Mass spectra were obtained on a Kratos MS80RFA magnetic sector double focussing mass spectrometer or by a Micromass LCT. Optical rotations were measured on a Jasco J-20C recording spectropolarimeter and $[\alpha]_D^{20}$ values are give in units of 10^{-1} deg cm^2 g^{-1}. Flash column chromatography was carried out on silica gel 60 (63–200 μm). Plate layer chromatography (PLC) was carried out on aluminium-backed Merck Kieselguhr KG60F$_{254}$ silica plates. Preparative high pressure liquid chromatography (HPLC) was preformed on a Phillips PU4100 HPLC, fitted with a PU4120 diode array detector. Injections were made to either a Rainin RP-C$_{18}$ analytical or RP-C$_{18}$ preparative column.

General procedures

Method A; peptide coupling. To a solution of carboxylic acid (1.0 eq.) and 1-hydroxybenzotriazole (HOBt, 1.5 eq.) in dry *N,N*-dimethylformamide in a flame-dried flask under a nitrogen atmosphere, was added amine hydrochloride or hydrobromide (1.1 eq.) as a solution in dry *N,N*-dimethylformamide. The reaction was cooled in an ice bath with stirring and *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDCI, 1.5 eq.), as a solution in dry *N,N*-dimethylformamide was added dropwise. The reaction was maintained in an ice bath for 1 h with stirring, then warmed to room temperature and stirring continued for 24 h. The reaction mixture was diluted with ethyl acetate (20 cm^3), and washed with saturated aqueous sodium bicarbonate (3 × 20 cm^3), 1 M aqueous hydrochloric acid (3 × 20 cm^3) and saturated aqueous sodium chloride (2 × 25 cm^3). The organic phase was dried over anhydrous magnesium sulfate, filtered and evaporated. See Experimental section for detail.

(2*S*)-1-[(*tert*-Butylaminocarbonyl)methyl]-5-[3-phenyl-2-(quinolin-2-ylcarbonyl-L-asparaginylamino)propyl]-1*H*-tetrazole 6

The free acid **14** (10 mg, 0.02 mmol), was coupled to *tert*-butylamine (3 mg, 0.04 mmol) with EDCI (5 mg, 0.03 mmol) and HOBt (4 mg, 0.03 mmol) in dry *N,N*-dimethylformamide (5 cm^3), according to method A. The crude protected tripeptide was purified by HPLC (RP-C$_{18}$ preparative column, 28% water–methanol, retention time 14 min) to give the protected tripeptide **6** (2 mg, 17%); $[\alpha]_D^{20}$ +12 (c = 0.001, methanol); δ_H(300 MHz; CD_3OD) 1.30 (9H, s, $C(CH_3)_3$), 2.71 (2H, d, J = 6.3 Hz, Asn–β–H_2), 2.94 (2H, m, hPhe–γ–H_2),¶ 3.11 (2H, d, J = 6.9 Hz, hPhe–α–H_2), 4.51 (1H, m, hPhe–α–H), 4.83 (1H, m, Asn–α–H_2), 5.08 (2H, AB system, δ_A = 5.09, β_B = 5.12, d, J = 17.1 Hz, Gly–α–H_2), 6.96 (1H, t, J = 7.5 Hz, Ph-4-H), 7.08 (2H, t, J = 7.8 Hz, Ph-3-H_2), 7.21 (2H, d, J = 7.2 Hz, Ph-2-H_2), 7.69 (1H, t, J = 6.9 Hz, QC-6-H), 7.84 (1H, t, J = 7.2 Hz, QC-7-H), 8.01 (1H, d, J = 7.8 Hz, QC-3-H), 8.16 (2H, d, J = 8.7 Hz, QC-8-H & QC-5-H), 8.47 (1H, d, J = 8.4 Hz, QC-4-H); δ_C(75 MHz; CD_3OD) 28.05 (hPhe–α–CH_2), 28.86 ($C(CH_3)_3$), 37.89 (Asn–β–CH_2), 40.96 (hPhe–γ–CH_2), 49.93 (Gly–α–CH_2), 51.12 (hPhe-β–CH), 51.93 (Asn–α–CH), 53.89 ($C(CH_3)_3$), 122.73, 127.89, 129.18, 129.67, 130.63, 131.12 (ArCH), 139.18, 139.02 (ArC), 148.05 (Q-9-C), 150.63 (QC-2-C), 155.79 (CN_4), 165.96 (Gly–CO), 168.70 (QC–CO), 172.89 (Asn–CO), 175.31 (Asn–$CONH_2$); *m/z* (FAB) 610.2878 ($MHNa^+$); $C_{30}H_{37}N_9O_4Na$ requires 610.2866.

¶ hPhe = homophenylalanine.

(2*S*)-1-[(Methyl-L-isoleucinylcarbonyl)methyl]-5-[3-phenyl-2-(quinolin-2-ylcarbonyl-L-asparaginylamino)propyl]-1*H*-tetrazole 7

The free acid **14** (58 mg, 0.11 mmol), was coupled to L-isoleucine methyl ester hydrochloride (22 mg, 0.12 mmol) with EDCI (31 mg, 0.16 mmol) and HOBt (25 mg, 0.16 mmol) in dry *N,N*-dimethylformamide (5 cm^3), according to method A. The crude protected tetrapeptide was purified by HPLC (RP-C$_{18}$ preparative column, 25% water–methanol, retention time 12 min) and precipitated from ether to give the protected tripeptide **7** (13 mg, 18%); mp 105–107 °C; $[\alpha]_D^{20}$ −4 (c = 0.0028, methanol); δ_H(300 MHz; CD_3OD) 0.87 (3H, d, J = 6.3 Hz, Ile–β–CH_3), 0.88 (3H, t, J = 7.5 Hz, Ile–δ–H_3), 1.34 (2H, AB system, δ_A = 1.24, δ_B = 1.43, m, Ile–γ–H_2), 1.84 (1H, m, Ile–β–H), 2.70 (2H, m, Asn–β–H_2), 2.94 (2H, d, J = 6.9 Hz, hPhe–γ–H_2), 3.11 (2H, d, J = 6.9 Hz, hPhe–α–H_2), 3.67 (3H, s, OCH_3), 4.28 (1H, d, J = 5.7 Hz, Ile–α–H), 4.53 (1H, m, hPhe–β–H), 4.83 (1H, t, J = 6.6 Hz, Asn–α–H), 5.28 (2H, AB system, δ_A = 5.24, δ_B = 5.32, d, J = 17.1 Hz, Gly–α–H_2), 6.96 (1H, t, J = 7.2, Ph-4-H), 7.08 (2H, t, J = 7.3 Hz, Ph-3-H_2), 7.21 (2H, d, J = 7.5 Hz, Ph-2-H_2), 7.69 (1H, t, J = 7.2 Hz, QC-6-H), 7.83 (1H, t, J = 7.5 Hz, QC-7-H), 7.99 (1H, d, J = 8.4 Hz, QC-3-H), 8.15 (2H, d, J = 8.7 Hz, QC-5-H & QC-8-H), 8.46 (1H, d, J = 8.7 Hz, QC-4-H); δ_C(75 MHz; CD_3OD) 12.08 (Ile–δ–CH_3), 16.24 (Ile–γ–CH_3), 26.56 (Ile–α–CH_2), 28.77 (hPhe–α–CH_2), 38.08 (Asn–α–CH_2), 38.62 (Ile–β–CH), 41.07 (hPhe–β–CH_2), 49.93 (Gly–α–CH_2), 51.27 (hPhe–β–CH), 51.91 (Asn–α–CH), 52.91 (OCH_3), 58.91 (Ile–α–CH), 119.94 (QC-3-CH), 127.82, 129.32, 129.73, 130.66, 131.09, 131.83 (ArCH), 136.95, 139.20, 139.22 (ArC), 148.18 (QC-9-C), 150.63 (QC-2-C), 155.87 (CN_4), 166.55 (QC–CO), 167.43 (Gly–CO), 172.91 (Asn–CO), 173.66 (Ile–CO), 175.34 (Asn–$CONH_2$); *m/z* (FAB) 680.2933 ($MHNa^+$); $C_{33}H_{39}N_9O_6Na$ requires 680.2921.

(2*S*)-1-[(Methyl-L-valinyl-L-isoleucinylcarbonyl)methyl]-5-[3-phenyl-2-(quinolin-2-ylcarbonyl-L-asparaginylamino)propyl]-1*H*-tetrazole 8

Method A. The free acid **14** (50 mg, 0. 09 mmol), was coupled to *N*-(L-isoleucine)-L-valine methyl ester hydrochloride (30 mg, 0.10 mmol), with EDCI (27 mg, 0.14 mmol) and HOBt (22 mg, 0.14 mmol) in dry *N,N*-dimethylformamide (5 cm^3), according to method A. The crude protected pentapeptide was purified by HPLC (RP–C$_{18}$ preparative column, 28% water–methanol, retention time 24 min) and precipitated from ether to give the protected tetrapeptide **8** (7 mg, 11%); mp 238–240 °C; $[\alpha]_D^{20}$ −4 (c = 0.0036, methanol); δ_H(300 MHz; CD_3OD) 0.88 (6H, dd, J = 2.4, 7.8 Hz, Val–γ–H_3), 0.90 (3H, t, J = 8.1 Hz, Ile–γ–H_3), 0.93 (3H, d, J = 6.9 Hz, Ile–β–CH_3), 1.39 (2H, AB system, δ_A = 1.21, δ_B = 1.57, m, Ile–γ–H_2), 1.86 (1H, m, Ile–β–H), 2.09 (1H, m, Val–β–H), 2.69 (2H, d, J = 6.0 Hz, Asn–β–H_2), 2.93 (2H, m, hPhe–γ–H_2), 3.13 (2H, d, J = 6.3 Hz, hPhe–α–H_2), 3.68 (3H, s, OCH_3), 4.29 (1H, d, J = 6.3 Hz, Val–α–H), 4.32 (1H, d, J = 8.1 Hz, Ile–α–H), 4.53 (1H, m, hPhe–β–H), 5.27 (2H, AB system, δ_A = 5.25, δ_B = 5.30, d, J = 8.7 Hz, Gly–α–H_2), 6.92 (1H, t, J = 7.2 Hz, Ph-4-H), 7.06 (2H, t, J = 7.8 Hz, Ph-3-H_2), 7.20 (2H, d, J = 7.5 Hz, Ph-2-H_2), 7.69 (1H, t, J = 7.5 Hz, QC-6-H), 7.84 (1H, t, J = 7.2 Hz, QC-7-H), 8.01 (1H, d, J = 7.8 Hz, QC-3-H), 8.14, 8.17 (2H, d, J = 3.9 Hz, QC-5-H, & QC-8-H), 8.47 (1H, d, J = 8.4 Hz, QC-4-H); δ_C(75 MHz; CD_3OD) 11.66 (Ile–δ–CH_3), 16.03 (Ile–β–CH_3), 18.95 (Val–γ–CH_3), 19.74 (Val–γ–CH_3), 26.20 (Ile–γ–CH_2), 28.85 (hPhe–α–CH_2), 31.94 (Val–β–CH), 38.15 (Asn–β–CH_2), 38.54 (Ile–β–CH), 41.01 (hPhe–γ–CH_2), 49.49 (Gly–α–CH_2), 51.27 (hPhe–β–CH), 51.95 (Asn–α–CH_2), 52.68 (OCH_3), 59.67 (Ile–α–CH & Val–α–CH), 119.98 (QC-3-CH), 127.87, 129.34, 129.73, 130.66, 131.09, 131.122, 131.87 (ArCH), 139.40, 139.27 (ArC), 148.05 (QC-9-C), 150.63 (QC-2-C), 155.79 (CN_4), 167.25 (Gly–CO), 170.47 (QC–CO), 172.89 (Asn–CO), 173.54 (Val–CO), 174.02 (Ile–CO), 175.32

(Asn–$CONH_2$); *m/z* (FAB) 779.3611 ($MHNa^+$); $C_{38}H_{48}N_{10}O_7Na$ requires 779.3605.

Method B. The tetrazole methyl ester trifluoroacetate **17** (25 mg, 0.04 mmol) was coupled to *N*-(quinolin-2-ylcarbonyl)-L-asparagine[18] (12 mg, 0.04 mmol) with EDCI (12 mg, 0.06 mmol), HOBt (9 mg, 0.06 mmol) and DIPEA (5 mg, 0.04 mmol) in dry *N,N*-dimethylformamide (5 cm^3) according to method A. The crude coupled product was purified by plate layer chromatography (100% ethyl acetate), to give the protected pentapeptide **8** (12 mg, 40%) as a white solid. Spectral data are as recorded above.

(3*S*)-Benzyl 2-[4-phenyl-3-(benzyloxycarbonylamino)butanoylamino]ethanoate 10

A solution of glycine benzyl ester hydrochloride (1.12 g, 3.7 mmol) and triethylamine (0.75 g, 7.4 mmol) in dry acetonitrile (40 cm^3) was prepared in a flame-dried quartz reaction vessel (100 cm^3 capacity) under a nitrogen atmosphere. A solution of *N*-Z-L-phenylalanine α-diazoketone,[15] (1.20 g, 5.6 mmol) in dry acetonitrile (40 cm^3) was prepared in a flame-dried flask under a nitrogen atmosphere and then transferred, *via* syringe, to the quartz reaction vessel. The reaction vessel was irradiated at 300 nm under a nitrogen atmosphere for 24 h or until the evolution of nitrogen had ceased. Solvent was removed to ¼ of the original volume, ethyl acetate (100 cm^3) was added, and the solution was washed with 1 M aqueous citric acid (2 × 100 cm^3), saturated aqueous sodium chloride (2 × 100 cm^3), dried over anhydrous magnesium sulfate, filtered and evaporated. The crude reaction product was purified by flash column chromatography (30% ethyl acetate–dichloromethane) and a single recrystallisation (ethyl acetate–petroleum ether) to give dipeptide, **10**, as a white solid (2.19 g, 85%); mp 144–146 °C; $\nu_{max}(CDCl_3)/cm^{-1}$ 3429, 3033, 2252, 1743, 1712, 1674, 1498; $[\alpha]_D^{20} = -2.8$ (*c* = 0.0043, methanol); δ_H(300 MHz; $CDCl_3$) 2.43 (2H, m, hPhe–γ–H_2), 2.90 (2H, m, hPhe–α–H_2), 4.01 (2H, d, *J* = 5.4 Hz, Gly–α–H_2), 4.16 (1H, m, hPhe–β–H), 5.04 (2H, s, Z–H_2), 5.16 (2H, s, Bn–H_2), 5.76 (1H, d, *J* = 7.8 Hz, hPhe–NH), 6.24 (1H, br s, Gly–NH), 7.18–7.34 (15H, m, ArH); δ_C(75 MHz; $CDCl_3$) 38.56 (hPhe–α–CH_2), 40.06 (hPhe–γ–CH_2), 41.22 (Gly–α–CH_2), 50.08 (hPhe–β–CH), 66.47 (Z–CH_2), 67.22 (Bn–CH_2), 126.55, 127.88, 127.96, 128.36, 128.40, 128.51, 128.61, 129.29 (ArCH), 135.04, 136.55, 137.83 (ArC), 155.85 (Z–CO), 169.64 (Gly–CO), 171.07 (hPhe–CO); TLC (analytical, 30% ethyl acetate–CH_2Cl_2) R_f = 0.25; *m/z* (FAB) 461.2088 (MH^+); $C_{26}H_{29}N_2O_5$ requires 461.2076.

(2*S*)-5-[2-(Benzyloxycarbonylamino)-3-phenylpropyl]-1-(benzyloxycarbonylmethyl)-1*H*-tetrazole 11

A solution of phosphorus pentachloride (290 mg, 1.2 mmol) in dry chloroform (15 cm^3) was prepared in a flame-dried flask under a nitrogen atmosphere. Freshly distilled quinoline (310 mg, 2.4 mmol) was added dropwise to the stirred solution at room temperature. The white precipitate that formed was stirred for 20 min at room temperature, while a solution of dipeptide **10** (502 mg, 1.0 mmol) in dry chloroform (15 cm^3) was prepared in a flame-dried flask under a nitrogen atmosphere. The phosphorus pentachloride suspension was cooled in an ice–water bath, prior to the dropwise addition of the dipeptide **10** over 30 min. After 30 min at <10 °C the ice–water bath was removed and a further portion of phosphorus pentachloride (45 mg, 0.2 mmol) was added. The reaction was stirred at room temperature for 2.5 h then a benzene solution of hydrazoic acid (30–50 eq.),[17] pre-dried over anhydrous sodium sulfate, was added. The reaction was stirred at room temperature for 48 h, then evaporated and redissolved in ethyl acetate (30 cm^3). The organic phase was washed with 2 M aqueous hydrochloric acid (2 × 50 cm^3), water (2 × 50 cm^3) and saturated aqueous sodium chloride (2 × 50 cm^3), dried over anhydrous magnesium sulfate, filtered and evaporated. The crude reaction product was purified by flash column chromatography (30% ethyl acetate–dichloromethane) and a single recrystallisation (ethyl acetate–petroleum ether) to give tetrazole **11** (300 mg, 62%); mp 101–104 °C; $\nu_{max}(CDCl_3)/cm^{-1}$ 3315, 1749, 1693, 1535, 1452, 1272, 1257; $[\alpha]_D^{20}$ −14.6 (*c* = 0.0015, methanol); δ_H(300 MHz; $CDCl_3$) 2.89–3.04 (4H, m, hPhe–α–H_2 & hPhe–γ–H_2), 4.21 (1H, m, hPhe–β–H), 4.98 (2H, s, Z–H_2), 5.00 (2H, AB system, δ_A = 4.93, δ_B = 5.04, d, *J* = 18.1 Hz, Gly–α–H_2), 5.16 (2H, AB system, δ_A = 5.14, δ_B = 5.19, d, *J* = 12.3 Hz, Bn–H_2), 5.45 (1H, d, *J* = 15 Hz, hPhe–NH), 7.11–7.35 (15H, m, ArH); δ_C(75 MHz; $CDCl_3$) 26.67 (hPhe–α–CH_2), 39.07 (hPhe–γ–CH_2), 47.46 (Gly–α–CH_2), 50.64 (hPhe–β–CH), 66.48 (Z–CH_2), 68.16 (Bn–CH_2), 126.74, 127.97, 128.19, 128.26, 128.37, 128.46, 128.58, 128.75, 129.04 (ArCH), 134.11, 136.15, 136.95 (ArC), 153.25 (CN_4), 155.71 (Z–CO), 165.12 (Gly–CO); TLC (analytical, 30 % ethyl acetate–CH_2Cl_2), R_f = 0.40; *m/z* (FAB) 486.2131 (MH^+); $C_{27}H_{28}N_5O_4$ requires 486.2141.

X-Ray analysis of compound 11. $C_{27}H_{27}N_5O_4$, *M* 485.54, mp 101–104 °C, crystal dimensions 0.8 × 0.4 × 0.02 mm, triclinic, *a* 5.0379 (3) Å, *b* 10.1066 (7) Å, *c* 12.7865 (9) Å, *α* 83.577 (2) °, *β* 81.897 (2) °, *γ* 76.475 (2) °, *V* = 624.55(7) $Å^3$, space group *P*1, *Z* = 1, F(000) = 256, D_{calc} = 1.291 Mg m^{-3}, absorption coefficient 0.089 mm^{-1}, *θ* range for data collection 3.23 to 25.61, index ranges $-1 \le h \le 5$, $-10 \le k \le 11$, $-12 \le l \le 15$, data/restraints/parameters 1867/3/365, goodness of fit on F^2 was 1.063, final *R* indices $[I > 2\sigma(I)]$ R_1 = 0.0359, wR_2 = 0.0898, *R* indices (all data) R_1 = 0.0427, wR_2 = 0.0957, largest difference peak and hole 0.169 and = −0.151 e$Å^{-3}$. A full sphere of data was collected at 163(2) K. Of the 2198 reflections obtained, 1867 were unique (R_{int} = 0.1010) and were used in the full-matrix least-squares refinement. The structure was solved by direct methods.[22] Hydrogen atoms were fixed in idealised positions. All non-hydrogen atoms were refined with anisotropic atomic displacement parameters. Neutral scattering factors and anomalous dispersion corrections for non-hydrogen atoms were taken from Ibers and Hamilton.[23] CCDC reference number 169831. See http://www.rsc.org/suppdata/p1/b1/b109128j/ for crystallographic files in .cif or other electronic format.

(2*S*)-5-(2-Amino-3-phenylpropyl)-1-(benzyloxycarbonylmethyl)-1*H*-tetrazole hydrobromide 12

The tetrazole **11** (840 mg, 1.73 mmol) was dissolved in concentrated acetic acid (0.9 cm^3) and the solution treated with 50% hydrobromic acid–acetic acid (4.5 cm^3). The reaction was stirred at room temperature for 30 min, then diethyl ether (45 cm^3), precooled in an ice-bath, was added with vigorous stirring. Petroleum ether (20 cm^3) was added and the reaction was allowed to stand in an ice bath for 30 min. The solvent was decanted off and the brown residue washed with diethyl ether–petroleum ether (1 : 1, 3 × 30 cm^3). Solvent was removed in vacuum and the brown residue dried *in vacuo* over potassium hydroxide for 24 h. The hydrobromide salt **12** (746 mg, 99%) was used without further purification; δ_H(300 MHz; $CDCl_3$) 2.58 (2H, m, hPhe–γ–H_2), 2.75 (2H, m, hPhe–α–H_2), 3.59 (1H, m, hPhe–β–H), 4.73 (2H, s, Bn–H_2), 4.94 (2H, d, *J* = 3.0 Hz, Gly–α–H_2), 6.77–6.89 (10H, m, ArH); δ_C(75 MHz; $CDCl_3$) 26.90 (hPhe–α–CH_2), 39.81 (hPhe–γ–CH_2), 48.20 (Gly–α–CH_2), 52.30 (hPhe–β–CH_2), 69.63 (Bn–CH_2), 129.12, 129.88, 130.04, 130.08, 130.55, 130.77 (ArCH), 136.54, 136.60 (ArC), 154.50 (CN_4), 167.83 (Gly–CO); *m/z* (FAB) 352.1771 (MH^+); $C_{19}H_{22}N_5O_2$ requires *m/z* 352.1773.

(2*S*)-1-(Benzyloxycarbonylmethyl)-5-[3-phenyl-2-(quinolin-2-ylcarbonyl-L-asparaginylamino)propyl]-1*H*-tetrazole 13

A solution of **12** (630 mg, 1.49 mmol), *N*-(quinolin-2-ylcarbonyl)-L-asparagine (469 mg, 1.63 mmol),[18] benzotriazolyl-*N*-oxytris(dimethylamino)phosphonium hexafluorophosphate

(BOP reagent, 723 mg, 1.63 mmol) and dry *N,N*-dimethylformamide (0.100 cm^3), in dry dichloromethane (10 cm^3) was prepared in a flame-dried flask under a nitrogen atmosphere. The solution was stirred at room temperature and triethylamine (450 mg, 4.46 mmol) was added dropwise. The reaction was stirred for 1 h, prior to the addition of a further portion of triethylamine (150 mg, 1.48 mmol). The reaction was stirred at room temperature for 18 h and was quenched with saturated aqueous sodium chloride (5 cm^3). The reaction was diluted with ethyl acetate (100 cm^3), separated and washed with 2 M aqueous hydrochloric acid (3 × 100 cm^3), 2 M aqueous sodium bicarbonate (3 × 100 cm^3) and saturated aqueous sodium chloride (2 × 100 cm^3). The solution was dried over anhydrous magnesium sulfate, filtered and evaporated. The crude product was purified by flash column chromatography (ethyl acetate) and a single recrystallisation from methanol to give protected tripeptide **13** (470 mg, 51%); mp 187–189 °C; $\nu_{max}(CDCl_3)/cm^{-1}$ 3394, 3305, 3215, 1749, 1649, 1616, 1533, 1500, 1215; $[\alpha]_D^{20}$ + 12 (c = 0.001, methanol); δ_H(300 MHz; $CDCl_3$) 2.78 (AB system, δ_A = 2.67, δ_B = 2.89, J = 7.2, 15.6 Hz, Asn–β–H_2), 2.98 (2H, d, J = 7.5 Hz, hPhe–γ–H_2), 3.06 (2H, d, J = 6.3 Hz, hPhe–α–H_2), 4.45 (1H, m, hPhe–β–H), 4.89 (1H, m, Asn–α–H), 5.19 (2H, s, Bn– H_2), 5.21 (2H, s, Gly–α–H_2), 5.37 (1H, br s, NH), 5.80 (1H, br s, NH), 7.03–7.37 (10H, m, ArH), 7.64 (1H, t, J = 6.9 Hz, QC-6-H), 7.79 (1H, t, J = 6.6 Hz, QC-7-H), 7.89 (1H, d, J = 7.8 Hz, QC-3-H), 8.17–8.22 (2H, m, QC-8-H & QC-5-H), 8.33 (1H, d, J = 8.1 Hz, QC-4-H), 9.25 (1H, d, J = 8.1 Hz, QC-NH); δ_C(75 MHz; $CDCl_3$–DMSO-d_6) 25.91 (hPhe–α–CH_2), 36.29 (Asn–β–CH_2), 38.88 (hPhe–γ–CH_2), 46.92 (Gly–α–CH_2), 48.80 (hPhe–β–CH), 49.07 (Asn–α–CH), 66.82 (Bn–CH_2), 117.65, 125.45, 126.80, 127.10, 127.38, 127.63, 128.19, 128.69, 129.23 (ArCH), 133.61, 136.42, 136.45 (ArC), 145.36 (QC-9-C), 148.23 (QC-2-C), 152.87 (CN_4), 163.05 (QC–CO), 165.02 (Gly–CO), 169.63 (Asn–CO), 171.49 (Asn–$CONH_2$); TLC (analytical, ethyl acetate), R_f = 0.20; *m/z* (FAB) 621.2583 (MH^+); $C_{33}H_{33}N_8O_5$ requires 621.2573.

(2*S*)-1-(Carboxymethyl)-5-[3-phenyl-2-(quinolin-2-ylcarbonyl-L-asparaginylamino)propyl]-1*H*-tetrazole 14

A stirred solution of the benzyl ester **13** (382 mg, 0.62 mmol) and acetic acid (0.500 cm^3) in ethyl acetate–ethanol (4 : 1, 50 cm^3) was hydrogenated at room temperature in the presence of 10% palladium on carbon (240 mg). The mixture was filtered through a plug of Celite, evaporated and redissolved in aqueous 1 M sodium bicarbonate (50 cm^3). The aqueous phase was washed with ethyl acetate (20 cm^3) and then acidified to pH = 2 with solid sodium bisulfite. The free acid was extracted with ethyl acetate (4 × 50 cm^3), dried over anhydrous magnesium sulfate, filtered and evaporated and the solid dried *in vacuo* over potassium hydroxide for 24 h. The free acid **14** obtained as a white solid (312 mg, 70 %) was used without further purification; mp 184–185 °C; ν_{max}(KBr)/cm^{-1} 3421, 3315, 1747, 1718, 1647, 1529, 1498; $[\alpha]_D^{20}$ −36 (c = 0.0005, methanol); δ_H(300 MHz; CD_3OD) 2.72 (2H, d, J = 5.7 Hz, Asn–β–H_2), 2.94 (2H, m, hPhe–γ–H_2), 3.12 (2H, d, J = 6.6 Hz, hPhe–α–H_2), 4.52 (1H, m, hPhe–β–H), 4.83 (1H, m, Asn–α–H), 5.30 (2H, s, Gly– α–H_2), 6.95 (1H, t, J = 6.9 Hz, Ph-4-H), 7.08 (2H, t, J = 7.8 Hz, Ph-3-H_2), 7.20 (2H, d, J = 7.2 Hz, Ph-2-H_2), 7.71 (1H, q, J = 7.8 Hz, QC-6-H), 7.85 (1H, q, J = 7.2 Hz, QC-7-H), 8.00 (1H, d, J = 8.1 Hz, QC-3-H), 8.16 (2H, d, J = 8.7 Hz, QC-8-H, & QC-5-H), 8.47 (1H, d, J = 8.7 Hz, QC-4-H); δ_C(75 MHz; DMSO-d_6) 27.17 (hPhe–α–CH_2), 30.57 (Asn–β–CH_2), 37.16 (hPhe–γ–CH_2), 47.90 (Gly–α–CH_2), 49.48 (hPhe–β–CH), 50.18 (Asn–β–CH), 118.78, 125.06, 126.30, 128.30, 128.39, 129.07, 129.26, 130.83 (ArCH), 138.14, 138.23 (ArC), 146.04 (QC-9-C), 149.59 (QC-2-C), 153.99 (CN_4), 163.59 (QC–CO), 167.99 (Asn–CO), 170.33 (Asn–$CONH_2$), 171.75 (Gly–COOH); *m/z* (EI) 531.2099 (MH^+); $C_{26}H_{27}N_8O_5$ requires 351.2100.

(2*S*)-5-[2-(*tert*-Butyloxycarbonylamino)-3-phenylpropyl]-1-(carboxymethyl)-1*H*-tetrazole 15

A solution of tetrazole **11** (200 mg, 0.41 mmol) and di-*tert*-butyl dicarbonate (180 mg, 0.82 mmol) in methanol (10 cm^3) was hydrogenated at room temperature in the presence of 10% palladium on carbon (40 mg) for 5 h. The reaction mixture was filtered through a Celite plug and the solvent removed in vacuum. Precooled ether (10 cm^3) was added with vigorous stirring and the product left to stand at 5 °C for 2 h. The white solid was removed by filtration and dried *in vacuo* over potassium hydroxide for 24 h. The free acid **15** (113 mg, 76%) was thus obtained as a white solid; δ_H(300 MHz; acetone) 1.41 (9H, s, Boc–$(CH_3)_3$), 3.08 (2H, m, hPhe–α–H_2), 3.29 (2H, d, J = 6.3 Hz, hPhe–γ–H_2), 4.38 (1H, m, hPhe–β–H), 5.52 (2H, s, Gly–α–H_2), 6.26 (1H, d, J = 7.8 Hz, NH), 7.30–7.40 (5H, m, ArH); δ_C(75 MHz; acetone) 28.92 (Boc–$(CH_3)_3$), 29.60 (hPhe–α–CH_2), 41.57 (hPhe–γ–CH_2), 52.30 (hPhe–β–CH), 80.57 (Boc–$C(CH_3)_3$), 127.88, 129.76, 130.62 (ArCH), 139.64 (ArC), 157.83 (CN_4), 160.39 (Boc–CO); *m/z* (ES) 362.1814 (MH^+); $C_{17}H_{24}N_5O_4$ requires 362.1828.

(2*S*)-5-[2-(*tert*-Butyloxycarbonylamino)-3-phenylpropyl]-1-[(methyl L-valinyl-L-isoleucinylcarbonyl)methyl]-1*H*-tetrazole 16

The tetrazole free acid **15** (17 mg, 0.05 mmol) was coupled to *N*-(L-isoleucine)-L-valine methyl ester hydrochloride (14 mg, 0.05 mmol) with EDCI (13 mg, 0.07 mmol), HOBt (10 mg, 0.07 mmol) and *N,N*-diisopropylethylamine (DIPEA) (7 mg, 0.05 mmol) in dry *N,N*-dimethylformamide (5 cm^3), according to method A. The crude product was purified by flash column chromatography to give tetrazole **16** (22 mg, 75%) as a white solid; mp 158–160 °C; δ_H(300 MHz; CD_3OD) 0.84 (3H, t, J = 6.3 Hz, Ile–δ–H_3), 0.85 (6H, d, J = 5.7 Hz, Val–γ–H_3), 1.27 (9H, s, Boc–$(CH_3)_3$), 1.37 (2H, AB system, δ_A = 1.26, δ_B = 1.55, m, Ile–γ–H_2), 1.84 (1H, m, Ile–β–H), 2.11 (1H, m, Val–β–H), 2.87 (1H, d, J = 6.6 Hz, hPhe–γ–H_2), 3.02 (2H, d, J = 9.9 Hz, hPhe–α–H_2), 3.68 (3H, s, OCH_3), 4.15 (1H, m, hPhe–β–H), 4.25 (1H, d, J = 7.8 Hz, Ile–α–H), 4.33 (1H, d, J = 5.7 Hz, Val–α–H), 5.13 (2H, AB system, δ_A = 5.09, δ_B = 5.18, d, J = 17.1 Hz, Gly–α–H_2), 6.34 (1H, d, J = 8.1 Hz, NH), 7.17–7.27 (5H, m, ArH); δ_C(75 MHz; CD_3OD) δ 11.44 (Ile–δ–CH_3), 15.86 (Ile–β–CH_3), 18.66 (Val–γ–CH_3), 19.60 (Val–γ–CH_3), 25.74 (Ile–γ–CH_2), 28.82 (Boc–$(CH_3)_3$), 29.20 (hPhe–α–CH_2), 31.51 (Val–β–CH), 37.75 (Ile–β–CH), 41.15 (hPhe–γ–CH_2), 49.74 (Gly–α–CH_2), 51.36 (hPhe–β–CH), 52.67 (OCH_3), 58.92 (Val–α–CH), 59.29 (Ile–α–CH), 80.38 (Boc–$C(CH_3)_3$), 127.57, 129.42, 130.15 (ArCH), 138.70 (ArC), 155.34 (CN_4), 157.21 (Boc–CO), 166.21 (Gly–CO), 173.14 (Val–CO), 173.28 (Ile–CO); *m/z* (ES) 588.3512 (MH^+); $C_{29}H_{46}N_7O_6$ requires 588.3510.

(2*S*)-5-(2-Amino-3-phenylpropyl)-1-[(methyl L-valinyl-L-isoleucinylcarbonyl)methyl]-1*H*-tetrazole trifluoroacetate 17

Protected tetrapeptide **16** (22 mg, 0.04 mmol) was dissolved in 95% aqueous trifluoroacetic acid (5 cm^3) and stirred slowly at room temperature for 30 min. Solvent was removed by rotary evaporation and the brown residue washed with petroleum ether (3 × 10 cm^3) and dried *in vacuo* over potassium hydroxide for 24 h to give trifluoroacetate **17** (25 mg, quant.), which was used without further purification; δ_H(300 MHz; CD_3OD) 0.87 (12H, m, Val–γ–H_3, Ile–γ–H_3, Ile–β–CH_3), 1.37 (2H, AB system, δ_A = 1.22, δ_B 1.52, m, Ile–γ–H_2), 1.86 (1H, m, Ile–β–H), 2.07 (1H, m, Val–α–H), 3.12 (2H, m, hPhe–γ–H_2), 3.29 (2H, m, hPhe–α–H_2), 3.62 (3H, s, OCH_3), 4.10 (1H, m, hPhe–β–H), 4.24 (2H, m, Ile–α–H, Val–α–H), 5.31 (2H, br s, Gly–α–H_2), 7.23–7.29 (5H, m, ArH); δ_C(75 MHz; CD_3OD) 11.54 (Ile–δ–CH_3), 15.80 (Ile–β–CH_3), 18.77 (Val–γ–CH_3), 19.43 (Val–γ–CH_3), 25.61 (Ile–γ–CH_2), 26.12 (hPhe–α–CH_2), 31.29 (Val–β–CH), 37.98 (Ile–β–CH), 39.17 (hPhe–γ–CH_2), 48.95 (Gly–α–CH_2), 52.10 (hPhe–β–CH), 52.45 (OCH_3), 59.11 (Val–α–CH), 59.43

(Ile–α–CH), 128.43, 129.91, 130.40 (ArCH), 136.21 (ArC), 154.21 (CN_4), 166.52 (Gly–CO), 173.14 (Val–CO), 173.25 (Ile–CO); *m*/*z* (ES) 488.2991 (MH^+); $C_{24}H_{38}N_7O_4$ requires 488.2985.

Acknowledgements

This work is partially supported by a research grant from the Royal Society of New Zealand Marsden Fund. The authors are gratefully indebted to Zachery Beck, Scripps Research Institute, La Jolla, California, USA, for *in vitro* HIV-1 protease testing. Addditionally, the authors would like to thank Ward Robinson and Peter Steel, Department of Chemistry, University of Canterbury, New Zealand for assistance in solving the crystal structure presented here.

References

1 A. D. Abell and G. Foulds, *J. Chem. Soc., Perkin Trans. 1*, 1997, 2475.
2 B. C. H. May and A. D. Abell, *Tetrahedron Lett.*, 2001, **42**, 5641.
3 B. C. H. May and A. D. Abell, *Chem. Commun.*, 2001, **20**, 2080.
4 J. Zabrocki, G. D. Smith, J. B. Dunbar and H. Iijima, *J. Am. Chem. Soc.*, 1988, **110**, 5875; G. D. Smith, J. Zabrocki, T. A. Flak and G. R. Marshall, *Int. J. Pept. Protein Res.*, 1991, **37**, 191.
5 J. Gante, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1699.
6 D. H. Rich, J. Green, M. V. Toth, G. R. Marshall and S. B. H. Kent, *J. Med. Chem.*, 1990, **33**, 1285.
7 A. L. Swain, M. M. Miller, J. Green, D. H. Rich, J. Schneider, S. B. Kent and A. Wlodawer, *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 8805.
8 M. K. Edmonds and A. D. Abell, *J. Org. Chem.*, 2001, **66**, 3747.
9 J. C. Craig, I. B. Duncan, D. Hockley, C. Grief, N. A. Roberts and J. S. Mills, *Antivir. Res.*, 1991, **16**, 295.
10 M. Longer, B. Shetty, I. Zamansky and P. Tyle, *J. Pharm. Sci.*, 1995, **84**, 1090.
11 B. C. H. May, Conformationally Restricted Peptidomimetics, Ph.D. Thesis, University of Canterbury, NZ, 2000.
12 A. G. Tomasseli, M. K. Olsen, J. O. Hui, D. J. Staples, T. K. Sawyer, R. L. Heinrekson and C-S. Tomich, *Biochemistry*, 1990, **29**, 264.
13 G. B. Dreyer, M. B. Lambert, T. D. Meek, T. J. Carr and T. A. Tomaszek, *Biochemistry*, 1992, **31**, 6646.
14 D. H. Rich, C.-Q. Sun, J. N. V. N. Prasad, A. Pathiasseril, M. V. Toth, G. R. Marshall, M. Clare, R. A. Mueller and K. Houseman, *J. Med. Chem.*, 1991, **34**, 1225; A. Krohn, S. Redshaw, J. C. Ritchie, B. J. Graves and M. H. Hatada, *J. Med. Chem.*, 1991, **34**, 3340.
15 K. Plucinska and B. Liberek, *Tetrahedron*, 1987, **43**, 3509.
16 D. Seebach, M. Overhand, F. N. M. Kuhnle and B. Martinoni, *Helv. Chim. Acta*, 1996, **79**, 913; H. M. M. Bastiaans, A. E. Alewijnse, J. L. van der Baan and H. C. J. Ottenheijm, *Tetrahedron Lett.*, 1994, **41**, 7659.
17 Prepared fresh and used without titration according to: *Frieser and Frieser Reagents for Organic Synthesis*; John Wiley and Sons, New York, 1967, p. 446.
18 N. P. Camp, D. A. Perrey, D. Kinchington, P. C. D. Hawkins and H. Gani, *Bioorg. Med. Chem.*, 1995, **3**, 297.
19 M. Salaitani, K. Hori and Y. Ohfune, *Tetrahedron Lett.*, 1988, **29**, 2983.
20 Z. Dauter, S. A. Chawdhuri and M. A. Hamid, *Cryst. Struct. Commun.*, 1982, **11**, 999.
21 For details on assay procedure see Z. Beck, Y. C. Lin and J. H. Elder, *J. Virol.*, 2001, **75(19)**, 9458.
22 G. M. Sheldrick, *Acta Crystallogr. Sect. A. Found. Crystallogr.*, 1990, **46**, 467.
23 J. A. Ibers and W. C. Hamilton, eds. *International Tables for Crystallography*, Kynoch Press, Birmingham, 1992, Vol C.
24 I. Schechter and A. Berger, *Biochem. Biophys. Res. Commun.*, 1967, **27**, 157.