1,2,3-Triazoles as peptide bond isosteres: synthesis and biological evaluation of cyclotetrapeptide mimics†

Victoria D. Bock,[a] Dave Speijer,[b] Henk Hiemstra[a] and Jan H. van Maarseveen*[a]

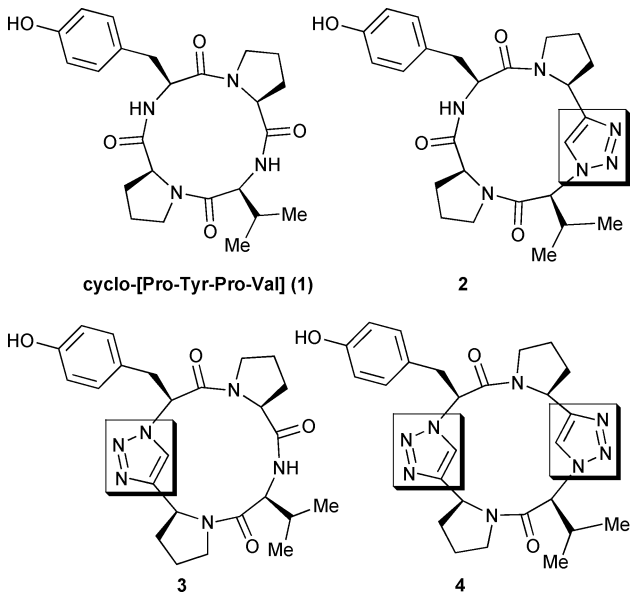
Received 15th November 2006, Accepted 30th January 2007
First published as an Advance Article on the web 6th February 2007
DOI: 10.1039/b616751a

Since the discovery of Cu^{I}-catalysed click chemistry, the field of peptidomimetics has expanded to include 1,4-connected 1,2,3-triazoles as useful peptide bond isosteres. Here, we report the synthesis of triazole-containing analogues of the naturally occurring tyrosinase inhibitor *cyclo*-[Pro-Val-Pro-Tyr] and show that the analogues retain enzyme inhibitory activity, demonstrating the effectiveness of a 1,4-connected 1,2,3-triazole as a *trans* peptide bond isostere.

Introduction

In the pursuit of potential lead compounds that bind to therapeutically relevant targets, numerous classes of peptidomimetic compounds have been developed to enhance the stability and availability of short peptide fragments *in vivo*.[1] Although the use of heterocyclic moieties in peptidomimetics has been widely reported,[2] the application of 1,2,3-triazoles to this field has occurred only recently, following the discovery of regioselective Cu^{I}-catalysed click chemistry in 2002.[3] 1,2,3-Triazoles show particular promise as amide bond isosteres, given their favourable pharmacophoric properties[4] and facile synthesis from readily available[5] azide- and alkyne-functionalised derivatives of chiral amino acids. Recently, reports have surfaced describing the incorporation of 1,2,3-triazoles into peptide nanotubes,[6] β-turn mimics,[7] protease inhibitors,[4f,8] cyclopeptide analogues[9] and peptide chain analogues.[10] Although these results suggest that triazoles have similar atom placement and electronic properties to those of a transoid peptide bond,[6a,b,11] no studies to date have analysed the effects on binding affinity or enzyme inhibition resulting from replacement of an amide bond in a natural product by a 1,2,3-triazole.

To directly assess these effects, we selected a series of three triazole analogues to the naturally occurring cyclotetrapeptide *cyclo*-[Pro-Tyr-Pro-Val] (**1**), a potent tyrosinase inhibitor isolated from *L. helveticus*[12] (Scheme 1). All reported attempts to synthesise **1** failed to yield the natural product due to the problematic ring closure step,[13] but we obtained *cyclo*-[(L)Pro-(L)Val-ψ(triazole)-(L)Pro-(L)Tyr] (**2**) in good yield *via* Cu^{I}-catalysed click chemistry-mediated macrocyclisation.[9e] Herein, we report the synthesis of two other triazole analogues (**3** and **4**, Scheme 1) and compare the inhibitory activity of the three peptidomimetic compounds to that of the natural cyclotetrapeptide.



Scheme 1

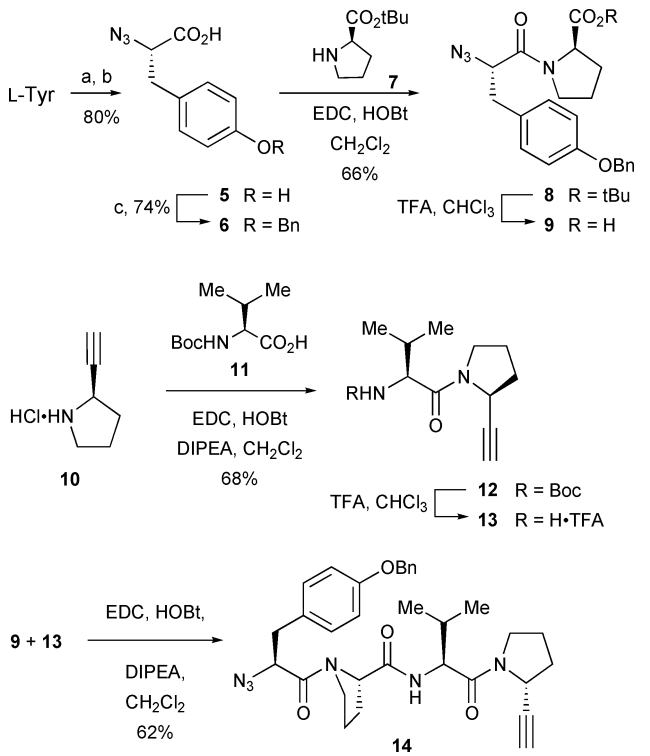
Results and discussion

Synthesis of triazole analogues **3** and **4** commenced with the preparation of the azido alkyne linear precursors *via* a modular, flexible approach based on coupling reactions between amino acids and their azido acid and amino alkyne counterparts. Construction of N_3-Tyr(OBn)-Pro-Val-Pro-C≡CH **14**, the linear precursor to cyclotetrapeptide analogue **3**, required dipeptides **8** and **12**, which could be obtained *via* EDC–HOBt-mediated peptide coupling between azido acid **6** and protected proline **7** and between proline alkyne **10**[9e] and Boc-protected valine **11**, respectively (Scheme 2). Subsequent TFA-mediated tBu–Boc removal and block coupling of the product dipeptide analogues gave linear precursor **14** in 62% yield.

Synthesis of the linear precursor to cyclotetrapeptide analogue **4**, which contains two triazole moieties in its backbone, began with Cu^{I}-catalysed alkyne–azide coupling of proline alkyne **15**[14] with valine azido acid **16**.[5a] Conveniently, this reaction required no protection for the free carboxylic acid and proceeded without difficulty to yield Boc-Pro-ψ(triazole)-Val **17** (Scheme 3). Peptide

[a]*Van't Hoff Institute for Molecular Sciences, University of Amsterdam, Nieuwe Achtergracht 129, 1018 WS, Amsterdam, The Netherlands. E-mail: jvm@science.uva.nl; Fax: +31 20 5255670; Tel: +31 20 5255671*
[b]*Department of Medical Biochemistry, Academic Medical Center, University of Amsterdam, Meibergdreef 15, 1105 AZ, Amsterdam, The Netherlands*

† Electronic supplementary information (ESI) available: Procedures and spectroscopic data for compounds **5**, **6**, **8**, **9**, **12**, **13**, **18** and **19** and protocol for the mushroom tyrosinase activity assay. See DOI: 10.1039/b616751a



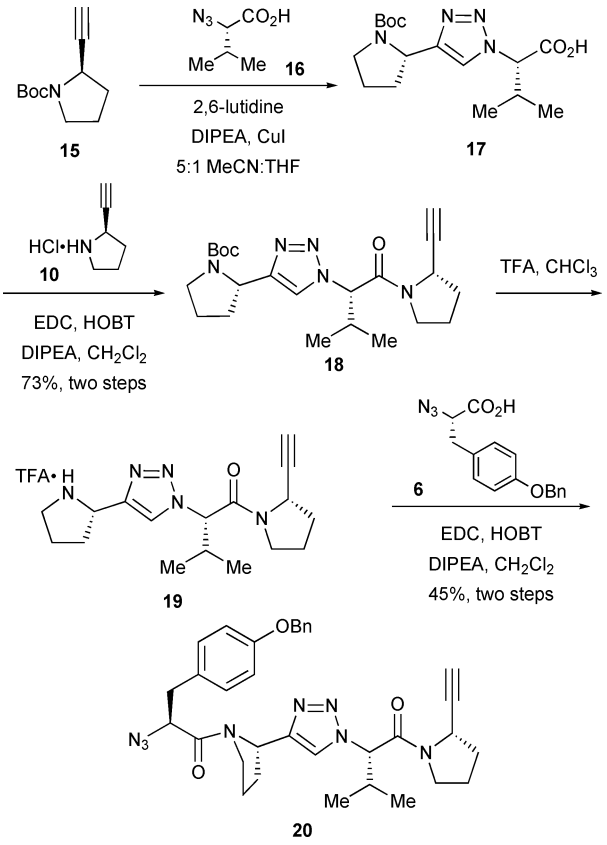
Scheme 2 *Reagents and conditions*: a) NaN_3, Tf_2O, CH_2Cl_2, H_2O, 0 °C. b) K_2CO_3, $CuSO_4$, MeOH, H_2O, rt. c) BnBr, K_2CO_3, 2 : 1 = $CHCl_3$: MeOH, reflux.

coupling of **17** with deprotected proline alkyne **10** gave Boc-Pro-ψ(triazole)-Val-Pro alkyne **18** in 73% yield over two steps. TFA deprotection and peptide coupling with tyrosine azido acid **6** provided N_3-Tyr(OBn)-Pro-ψ(triazole)-Val-Pro alkyne **20** in 45% overall yield for the two transformations due to difficulties in driving the coupling reaction to completion. Fortunately, sufficient amounts of linear precursor **20** were obtained to enable direct progression to cyclisation reactions.

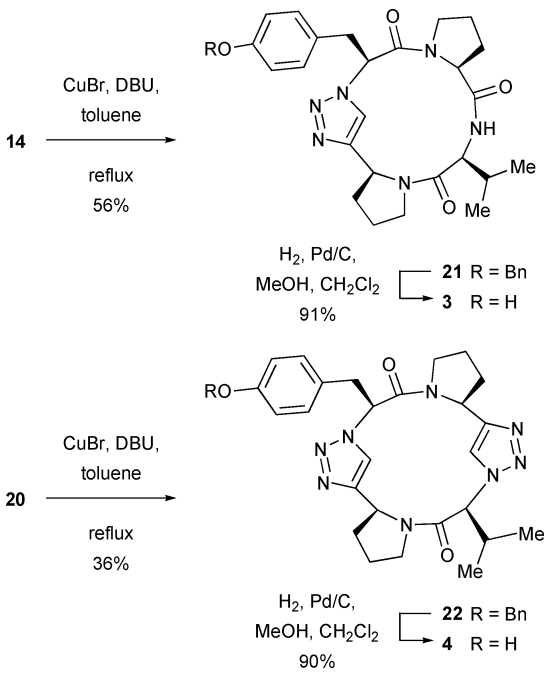
Based on the optimised Cu^I-catalysed click chemistry macrocyclisation conditions for cyclotetrapeptides,[9e] cyclisation of azido alkynes **14** and **20** provided rapid access to the two additional analogues of *cyclo*-[Pro-Val-Pro-Tyr] (Scheme 4). In the case of the cyclisation of azido alkyne **14**, purification of protected cyclotetrapeptide analogue **21** proved difficult due to low solubility, so cyclisation yields of approximately 50% were the highest obtainable.

Cyclisation of azido alkyne **20** proved more difficult. While the cyclisations leading to analogues **2** and **3** provided clean products following column chromatography, purification of the product from cyclisation of azido alkyne **20** afforded a complex mixture of compounds, forcing us to precipitate the cyclic product from an acetonitrile–water mixture, substantially lowering the yield. Overall, however, cyclotetrapeptide analogue **22** was obtained in an isolated yield of 36%.

Removal of the benzyl protecting groups in **21** and **22** *via* catalytic hydrogenation yielded the desired analogues **3** and **4** in 91% and 90% yields, respectively (Scheme 4). Although the cyclisation yields proved lower than expected from the previously described optimised cyclisation conditions,[9e] our methodology still provided an efficient route to the desired products.



Scheme 3



Scheme 4

With all the desired compounds in hand, the inhibitory effect of cyclotetrapeptide analogues **2–4** on mushroom tyrosinase activity was compared to that of the natural product[12] *via* an *in vitro* spectrophotometric assay (Table 1). Results indicate that all of the triazole analogues not only retain inhibition activity, but in fact,

Table 1 Biological activity of cyclotetrapeptide analogues compared with the natural product

Compound	Tyrosinase activity IC_{50}/mM[a]
cyclo-[Pro-Tyr-Pro-Val]	1.5[b]
Triazole analogue **2**	0.6
Triazole analogue **3**	0.5
Triazole analogue **4**	1.6

[a] Concentration for 50% reduction in tyrosinase activity from mushroom tyrosinase extract. [b] Data taken from Ref. 12

cyclotetrapeptide analogues **2** and **3** both show an approximately threefold increase in activity relative to the natural product. These IC_{50} values put cyclotetrapeptide analogues **2** and **3** in the mid-range of known naturally-occurring and synthetic inhibitors of mushroom tyrosinase.[15]

Conclusions

These results provide the strongest evidence to date that 1,4-disubstituted 1,2,3-triazoles can serve as transoid amide bond mimics in natural compounds without compromising biological activity. Although previous studies on which this work is based have elegantly shown the similar hydrogen-bonding properties of transoid amide bonds and 1,2,3-triazoles,[6a,b,11] the introduction of a triazole into a natural product to determine direct effects on biological activity provides an additional assessment of the effects of the triazole incorporation into peptide backbones. This study clearly demonstrates that analogues of an inaccessible natural product are readily available *via* Cu^{I}-catalysed alkyne–azide coupling and that these analogues show retention of biological activity when compared with a parent cyclotetrapeptide.

Experimental

General notes

Oxygen- and moisture-sensitive reactions were carried out using standard Schlenk techniques under a nitrogen atmosphere. Tetrahydrofuran and diethyl ether were freshly distilled from sodium–benzophenone. Dry DMF and CH_2Cl_2 were freshly distilled from CaH_2. All commercially available reagents were used as received, unless indicated otherwise. NMR spectra were recorded in Fourier transform mode on a Bruker ARX 400 (^1H at 400 MHz, ^{13}C at 100 MHz) magnetic resonance spectrophotometer. ^1H NMR spectra are reported as chemical shifts in parts per million (ppm) downfield from a tetramethylsilane internal standard (0.00 ppm). Spin multiplicity is described by the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, and br = broad. Coupling constants (J) are reported in Hertz (Hz). ^{13}C NMR spectra are reported as chemical shifts in ppm with the solvent resonance as the internal standard ($CDCl_3$: 77.07 ppm; MeOD: 49.00 ppm; MeCN-d_3: 118.26) and were recorded with complete heterodecoupling as APT (attached proton test) spectra. Infrared spectra were obtained from $CDCl_3$ solutions on a Bruker IFS 28 Fourier Transform spectrometer (FTIR) and are reported in wavenumbers (cm^{-1}). Fast atom bombardment (FAB) mass spectrometry was carried out using a JEOL JMS SX/SX 102A four-sector mass spectrometer, coupled to a JEOL MS-MP9021D/UPD system program. Samples were loaded in a matrix solution (3-nitrobenzyl alcohol) on a stainless steel probe and bombarded with xenon atoms with an energy of 3 keV. During the high resolution FAB-MS measurements a resolving power of 10000 (10% valley definition) was used. MALDI-TOF mass spectra were recorded on a Micromass TofSpec 2EC (Micromass, Whyttenshawe, UK). Samples (50 pmol) were dissolved in EtOAc, mixed with a concentrated solution of DHB in EtOAc and spotted directly on the stainless steel MALDI target. Silver nitrate was used for the ionisation of the molecules. Analytical TLC chromatography was performed on 250 μm silica gel 60 plates with 254 nm fluorescent indicator. The mushroom tyrosinase (EC 1.14.18.1) used for the bioassay was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Although mushroom tyrosinase differs somewhat from other sources,[16] this fungal source was used for the experiment because it is readily available. Enzyme inhibition was determined on a ULTROSPEC 2100 PRO measuring dopachrome formation at 470 nm for two minutes. All samples were first dissolved in DMSO and used at ten times dilution. Each experiment was carried out in triplicate and averaged.

(*S*)-1-((*S*)-2-Azido-3-(4-(benzyloxy)phenyl)propanoyl)-*N*-((*S*)-1-((*S*)-2-ethynylpyrrolidin-1-yl)-3-methyl-1-oxobutan-2-yl)pyrrolidine-2-carboxamide (14)

To a 25 cm^3 round-bottomed flask equipped with a $CaCO_3$ drying tube and charged with Val-Pro alkyne **13** (0.2441 g, 0.714 mmol, 1.1 equiv.) in freshly distilled CH_2Cl_2 (2.5 cm^3) was added DIPEA (0.12 cm^3, 0.714 mmol, 1.1 equiv.). After 10 min of stirring, this solution was added to a 25 cm^3 oven-dried flask equipped with a $CaCO_3$ drying tube and charged with N_3-Tyr(OBn)-Pro **9** (0.2813 g, 0.647 mmol, 1 equiv.), EDC (0.124 g, 0.647 mmol, 1 equiv.), HOBt (0.087 g, 0.647 mmol, 1 equiv.) and freshly distilled CH_2Cl_2 (2.5 cm^3). After 16 h, this solution was diluted with $CHCl_3$ (20 cm^3) and washed with H_2O (1 × 20 cm^3), satd aq $NaHCO_3$ (1 × 20 cm^3), and 1 N HCl (aq) (1 × 20 cm^3). The combined organics were then dried over Na_2SO_4, filtered, and concentrated *in vacuo* to yield a yellow solid. The product was purified *via* flash chromatography (75% EtOAc–PE) to afford N_3-Tyr(OBn)-Pro-Val-Pro alkyne **14** (0.2293 g, 0.4018 mmol, 62%) as a white solid. ^1H NMR ($CDCl_3$, 400 MHz) δ 7.41–7.26 (m, 5H), 7.18–6.87 (m, 4H), 5.02 (m, 2H), 4.97–4.44 (m, 3H), 4.00–2.91 (m, 8H), 2.38–1.94 (m, 10H), 0.99–0.86 (m, 6H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.1, 169.9, 169.8, 169.7, 169.5, 169.3, 169.2, 168.9, 168.7, 168.5, 168.4, 168.2, 168.1, 167.9, 167.6, 156.9, 156.8, 135.9, 135.8, 135.7, 130.3, 129.2, 129.1, 127.8, 127.7, 127.6, 127.5, 127.2, 127.0, 126.9, 126.7, 126.5, 126.4, 126.3, 124.8, 114.2, 114.1, 114.0, 82.7, 82.0, 81.9, 81.7, 80.8, 71.7, 71.1, 69.2, 69.1, 68.9, 68.8, 60.7, 60.6, 60.2, 59.9, 59.7, 59.4, 59.3, 59.2, 58.6, 55.5, 55.0, 54.9, 54.8, 54.7, 54.6, 47.6, 47.2, 46.9, 46.8, 46.4, 46.2, 46.0, 45.8, 45.5, 45.4, 44.9, 36.2, 36.0, 35.5, 35.4, 35.3, 35.2, 34.9, 33.2, 33.1, 31.4, 31.3, 31.2, 30.9, 30.8, 30.7, 30.6, 30.3, 30.0, 29.9, 28.6, 27.5, 27.4, 27.2, 27.1, 26.8, 26.5, 24.0, 23.8, 23.7, 23.5, 21.9, 21.5, 21.4, 18.7, 18.6, 18.3, 18.2, 17.1, 17.0, 17.09, 16.9, 16.8, 16.7, 16.4, 16.2 ppm. IR 3301, 3218, 2966, 2873, 2243, 2106, 1684, 1650, 1632, 1510, 1429, 1379, 1339, 1303, 1234, 1179, 1107, 1015, 912 cm^{-1}. $[a]_D^{20} = -64.3$ (c 1.96 in $CHCl_3$).

(*S*)-2-(4-((*S*)-1-((*S*)-2-Azido-3-(4-(benzyloxy)phenyl)propanoyl)-pyrrolidin-2-yl)-1*H*-1,2,3-triazol-1-yl)-1-((*S*)-2-ethynylpyrrolidin-1-yl)-3-methylbutan-1-one (20)

To a 50 cm^3 round-bottomed flask equipped with a $CaCO_3$ drying tube and charged with Pro-ψ(triazole)-Val-Pro alkyne TFA salt **19** (0.3254 g, 0.956 mmol, 1 equiv.) in freshly distilled CH_2Cl_2 (3 cm^3) was added DIPEA (0.17 cm^3, 0.956 mmol, 1 equiv.). After 10 min of stirring, this solution was added to a 50 cm^3 oven-dried flask equipped with a $CaCO_3$ drying tube and charged with tyrosine azido acid **6** (0.313 g, 1.051 mmol, 1.1 equiv.), EDC (0.183 g, 0.956 mmol, 1 equiv.), HOBt (0.129 g, 0.956 mmol, 1 equiv.) and freshly distilled CH_2Cl_2 (7 cm^3). After 16 h, this red–orange solution was diluted with $CHCl_3$ (50 cm^3) and washed with H_2O (1 × 50 cm^3), satd aq $NaHCO_3$ (1 × 50 cm^3), and 1 N HCl (aq) (1 × 50 cm^3). The combined organics were then dried over Na_2SO_4, filtered, and concentrated *in vacuo* to yield a brown solid. The product was purified *via* flash chromatography (33% EtOAc–PE) to afford N_3-Tyr(OBn)-Pro-ψ(triazole)-Val-Pro alkyne **20** (0.2552 g, 0.430 mmol, 45% over two steps) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.02–7.60 (m, 1H), 7.41–7.28 (m, 5H), 7.13–6.76 (m, 4H), 5.38–4.66 (m, 3.6H), 4.29–4.26 (m, 0.4H), 3.94–2.79 (m, 8H), 2.64–1.66 (m, 10H), 1.09–0.98 (m, 3H), 0.74–0.66 (m, 3H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.2, 169.1, 169.0, 167.9, 167.0, 166.6, 166.3, 166.2, 165.8, 157.9 157.8, 149.8, 149.4, 148.6, 148.1, 147.2, 137.0, 136.9, 136.8, 130.4, 130.2, 130.1, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0 127.9, 127.7, 127.5, 127.4, 122.3, 121.5, 121.1, 120.9, 120.8, 120.4, 120.1, 119.9, 119.7, 119.3, 119.1, 115.1, 115.0, 114.9, 114.8, 83.3, 83.2, 83.1, 83.0, 82.3, 82.2, 81.4, 81.2, 74.0, 73.8, 73.2, 73.1, 73.0, 71.1, 70.9, 70.7, 70.6, 70.0, 69.9, 67.2, 67.1, 67.0, 61.2, 61.1, 61.0, 60.9, 60.8, 60.6, 60.4, 54.1, 53.9, 53.7, 53.6, 53.1, 52.9, 52.9, 52.8, 48.8, 48.7, 48.6, 48.5, 48.1, 47.9, 47.8, 47.0, 46.9, 46.8, 46.7, 46.6, 46.4, 46.3, 46.2, 37.5, 37.3, 36.5, 36.4, 35.6, 34.3, 34.0, 33.9, 33.7, 33.6, 33.5, 33.3, 33.2, 33.1, 32.8, 32.7, 32.2, 32.0, 31.9, 31.8, 31.7, 31.6, 30.7, 30.6, 30.4, 29.6, 24.9, 24.8, 24.7, 24.6, 24.2, 24.0, 22.8, 22.7, 22.1, 21.9, 21.8, 19.4, 19.3, 19.2, 19.0, 18.8, 18.7, 18.6, 18.5, 18.3, 18.2 ppm. IR 3300, 2968, 2876, 2246, 2102, 1654, 1610, 1583, 1510, 1427, 1390, 1299, 1238, 1177, 1153, 1109, 1045, 1016, 911, 810 cm^{-1}. HMRS (FAB) Calculated for $C_{33}H_{39}N_8O_3$ (MH^+): 595.3147; Found: 595.3145. $[\alpha]_D^{20} = +5.1$ (*c* 0.95 in $CHCl_3$).

Bn-protected cyclic peptide 21

To a 100 cm^3 round-bottomed flask charged with alkyne azide **14** (0.033 g, 0.0578 mmol, 1 equiv.) in toluene (58 cm) was added DBU (0.026 cm^3, 0.1735 mmol, 3 equiv.). The solution was degassed with argon for thirty minutes and then heated to reflux while flushing with argon. At reflux, CuBr (1.7 mg, 0.0116 mmol, 0.2 equiv.) was added, and the solution was stirred at reflux under argon for 16 h. The mixture was then cooled to rt and poured through a 2 inch pad of Celite. The Celite pad was washed with CH_2Cl_2 (3 × 25 cm^3). The filtrate was concentrated *in vacuo* to provide a blue–green oil. The product was purified *via* flash chromatography (3% MeOH in CH_2Cl_2) to afford Bn-protected cyclic peptide **21** (0.0184 g, 0.0326 mmol, 56% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.41–7.30 (m, 5H, OCH_2Ph), 7.01 (d, J = 8.5 Hz, 2H, Tyr Ar*H*), 6.88 (s, 1H, triazole C_5H), 6.81 (d, J = 8.6 Hz, 2H, Tyr Ar*H*), 5.72–5.68 (m, 0.67H, amide N*H*), 5.43–5.41 (m, 1H, Tyr $C_\alpha H$), 5.33 (d, J = 6.9 Hz, 1H, Pro $C_\alpha H$), 5.01–4.98 (m, 2H, OCH_2Ph), 4.23 (d, J = 8.1 Hz, 1H, Pro $C_\alpha H$), 4.01 (t, J = 9.0, 1H, Val $C_\alpha H$), 3.80–3.78 (m, 1H, one of two Pro NCH_2), 3.69–3.67 (m, 2H, Pro NCH_2), 3.48–3.44 (m, 2H, one of two Pro NCH_2 and one of two Tyr $C_\alpha CH_2$), 3.35–3.32 (m, 1H, one of two Tyr $C_\alpha CH_2$), 2.46–2.41 (m, 1H, one of two Pro $C_\alpha CH_2$), 2.31–1.78 (m, 8H, Val $C_\alpha CH$, Pro $C_\alpha CH_2CH_2$, Pro $C_\alpha CH_2$), 0.85 (d, J = 6.6 Hz, 3H, one of two Val $C_\alpha CH(CH_3)_2$), 0.79 (d, J = 6.8 Hz, 3H, one of two Val $C_\alpha CH(CH_3)_2$) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.4, 168.6, 165.5, 156.9, 149.7, 135.8, 129.9, 127.5, 126.9, 126.7, 126.1, 120.8, 113.7, 68.9, 62.7, 61.2, 56.6, 54.3, 47.3, 45.4, 36.4, 36.3, 32.6, 31.1, 31.0, 28.6, 20.7, 20.5, 18.0, 17.4 ppm. IR 3208, 2966, 2874, 1657, 1616, 1512, 1434, 1386, 1302, 1246, 1231, 1178, 1094, 1048, 1016, 913 cm^{-1}. HMRS (FAB) Calculated for $C_{32}H_{39}N_6O_4$ (MH^+): 571.3035; Found: 571.3033. $[\alpha]_D^{20} = -146.1$ (*c* 0.95 in $CHCl_3$).

***cyclo*-[Pro-Tyr-ψ(triazole)-Pro-Val] 3**

To a 50 cm^3 round-bottomed flask charged with Bn-protected cyclic peptide **21** (55.0 mg, 0.0964 mmol, 1 equiv.) in MeOH (1 cm^3) and CH_2Cl_2 (9 cm^3) was added 10% Pd/C (27.5 mg). The resulting mixture was subjected to a three-cycle of vacuum–H_2 and was stirred at rt under a H_2 balloon for 16 h. The catalyst was then removed by filtration through Celite, and the filtrate concentrated *in vacuo* to afford a white solid. This solid was then dissolved in a minimal volume of CH_2Cl_2 and titrated with EtOAc. The white solid was collected by filtration, yielding cyclic peptide **3** (42 mg, 0.0877 mmol, 91% yield). ^{1}H NMR ($CDCl_3$–MeOD,[17] 400 MHz) δ 6.87–6.85 (m, 3H, triazole C_5H, Tyr Ar*H*), 6.63 (d, J = 8.4 Hz, 2H, Tyr Ar*H*), 6.14–6.10 (m, 0.4H, amide N*H*), 5.68 (t, J = 5.9 Hz, 1H, Tyr $C_\alpha H$), 5.26 (d, J = 7.2 Hz, 1H, Pro $C_\alpha H$), 4.18 (d, J = 8.2 Hz, 1H, Pro $C_\alpha H$), 3.99–3.96 (m, 1H, Val $C_\alpha H$), 3.69–3.65 (m, 3H, Pro NCH_2), 3.44–3.39 (m, 3H, Tyr $C_\alpha CH_2$ and one of two Pro NCH_2), 2.42–2.39 (m, 1H, one of two Pro $C_\alpha CH_2$), 2.13–1.77 (m, 8H, Val $C_\alpha CH$, Pro $C_\alpha CH_2$, Pro $C_\alpha CH_2CH_2$), 0.81 (d, J = 6.5 Hz, 3H, one of two Val $C_\alpha CH(CH_3)_2$), 0.74 (d, J = 6.8 Hz, 3H, one of two Val $C_\alpha CH(CH_3)_2$) ppm. ^{13}C NMR ($CDCl_3$–MeOD,[17] 100 MHz) δ 170.3, 170.1, 170.0, 166.5, 130.8, 126.3, 122.5, 115.0, 63.6, 62.2, 57.2, 55.2, 48.3, 46.6, 37.0, 33.4, 32.0, 31.7, 21.5, 21.4, 19.0, 18.1 ppm. IR 3473, 2957, 2926, 1657, 1618, 1516, 1434, 1385, 1309, 1264, 1226, 1172, 1156, 1095 cm^{-1}. HMRS (FAB) Calculated for $C_{25}H_{33}N_6O_4$ (MH^+): 481.2565; Found: 481.2563. $[\alpha]_D^{20} = -175.5$ (*c* 0.74 in $CHCl_3$).

Bn-protected cyclic peptide 22

To a 100 cm^3 round-bottomed flask charged with azido alkyne **20** (0.0269 g, 0.0452 mmol, 1 equiv.) in toluene (45 cm^3) was added DBU (0.020 ml^3, 0.1356 mmol, 3 equiv.). The solution was degassed with argon for thirty minutes and then heated to reflux while flushing with argon. At reflux, CuBr (1.3 mg, 0.0091 mmol, 0.2 equiv.) was added, and the solution was stirred at reflux under argon for 16 h. The mixture was then cooled to rt and poured through a 2 in pad of Celite. The Celite pad was washed with CH_2Cl_2 (3 × 20 cm^3). The filtrate was concentrated *in vacuo* to provide a brown solid. The product was purified *via* flash chromatography (3% MeOH in CH_2Cl_2) to afford a white solid. This solid was then washed with 50% MeCN–H_2O (5 cm^3) to yield protected cyclotetrapeptide analogue **22** (9.6 mg, 0.0161 mmol,

36% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.37–7.26 (m, 5H, OCH_2Ph), 7.10 (d, $J = 8.5$ Hz, 2H, Tyr Ar*H*), 6.95 (s, 1H, triazole C_5H), 6.84 (d, $J = 8.5$ Hz, 2H, Tyr Ar*H*), 6.45 (s, 1H, triazole C_5H), 5.56–5.53 (m, 1H, Tyr $C_\alpha H$), 5.26 (d, $J = 7.2$ Hz, 1H, Pro $C_\alpha H$), 5.06 (d, $J = 7.2$ Hz, 1H, Pro $C_\alpha H$), 4.99–4.97 (m, 3H, Val $C_\alpha H$ and OCH_2Ph), 3.73–3.31 (m, 6H, Pro NCH_2 and Tyr $C_\alpha CH_2$), 2.53–2.50 (m, 1H, Val $C_\alpha CH$), 2.31–2.28 (m, 2H, Pro $C_\alpha CH_2$), 1.97–1.76 (m, 6H, Pro $C_\alpha CH_2$ and Pro $C_\alpha CH_2CH_2$), 1.01 (d, $J = 6.5$ Hz, 3H, one of two Val $C_\alpha CH(CH_3)_2$), 0.70 (d, $J = 6.8$ Hz, 3H, one of two Val $C_\alpha CH(CH_3)_2$) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.1, 166.7, 166.2, 159.2, 150.4, 149.1, 138.2, 132.1, 129.9, 129.4, 129.3, 128.8, 120.5, 120.3, 116.2, 71.3, 69.0, 64.9, 56.6, 56.3, 48.4, 48.3, 39.4, 35.1, 35.0, 32.2, 31.0, 23.0, 22.0, 21.4, 19.6 ppm. IR 3136, 3100, 2960, 1653, 1539, 1510, 1432, 1369, 1345, 1306, 1262, 1221, 1175, 1019 cm^{-1}. HMRS Calculated for $C_{33}H_{39}N_8O_3$ (MH^+): 595.3147 (FAB) Found: 595.3145; (ESI) Found: 595.32. $[\alpha]_D^{20} = -35.9$ (*c* 0.41 in $CHCl_3$).

cyclo-[Val-ψ(triazole)-Pro-Tyr-ψ(triazole)-Pro] 4

To a 50 cm^3 round-bottomed flask charged with Bn-protected cyclotetrapeptide analogue **22** (33.2 mg, 0.0558 mmol, 1 equiv.) in MeOH (0.5 cm) and CH_2Cl_2 (4.5 cm^3) was added 10% Pd/C (50.0 mg). The resulting mixture was subjected to a three-cycle of vacuum–H_2 and was stirred at rt under a H_2 balloon for 16 h. The catalyst was then removed by filtration through Celite, and the filtrate concentrated *in vacuo* to afford cyclotetrapeptide analogue **4** (25.3 mg, 0.05022 mmol, 91% yield) as a white solid. ^{1}H NMR ($CDCl_3$–MeOD,[16] 400 MHz at 40 °C) δ 6.98 (s, 1H, triazole C_5H), 6.95 (d, $J = 8.4$ Hz, 2H, Tyr Ar*H*), 6.66 (d, $J = 8.4$ Hz, 2H, Tyr Ar*H*), 6.53 (s, 1H, triazole C_5H), 5.54–5.51 (m, 1H, Tyr $C_\alpha H$), 5.22 (d, $J = 7.2$ Hz, 1H, Pro $C_\alpha H$), 5.06 (d, $J = 7.1$ Hz, 1H, Pro $C_\alpha H$), 5.00 (d, $J = 8.9$ Hz, 1H, Val $C_\alpha H$), 3.76–3.74 (m, 2H, Pro NCH_2), 3.62–3.58 (m, 2H, Pro NCH_2), 3.37–3.34 (m, 2H, Tyr $C_\alpha CH_2$), 2.52–2.49 (m, 1H, Val $C_\alpha CH$), 2.28–2.25 (m, 2H, Pro $C_\alpha CH_2$), 2.00–1.77 (m, 6H, Pro $C_\alpha CH_2$ and Pro $C_\alpha CH_2CH_2$), 1.01 (d, $J = 6.6$ Hz, 3H, one of two Val $C_\alpha CH(CH_3)_2$), 0.70 (d, $J = 6.8$ Hz, 3H, one of two Val $C_\alpha CH(CH_3)_2$) ppm. ^{13}C NMR ($CDCl_3$–MeOD,[17] 100 MHz at 40 °C) δ 165.3, 165.0, 155.8, 148.7, 147.7, 130.3, 126.2, 119.4, 119.3, 115.2, 67.5, 63.5, 55.0, 54.8, 46.9, 46.8, 37.8, 33.5, 33.4, 30.6, 21.2, 20.4, 19.7, 17.9 ppm. IR 3106, 3059, 2962, 2925, 2875, 2244, 1731, 1633, 1538, 1511, 1432, 1379, 1344, 1299, 1260, 1177, 1086, 1021, 911, 860 cm^{-1}. HMRS (FAB) Calculated for $C_{26}H_{33}N_8O_3$ (MH^+): 505.2677; Found: 505.2676. $[\alpha]_D^{20} = -87.7$ (*c* 0.13 in DMF).

Notes and references

1 (*a*) J. Gante, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1699–1720; (*b*) A. Giannis and T. Kolter, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 1244–1267.

2 S. Borg, G. Estennebouhtou, K. Luthman, I. Csoregh, W. Hesselink and U. Hacksell, *J. Org. Chem.*, 1995, **60**, 3112–3120, and references therein.

3 (*a*) V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596–2599; (*b*) C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057–3064; (*c*) For a recent review, see: V. D. Bock, H. Hiemstra and J. H. van Maarseveen, *Eur. J. Org. Chem.*, 2006, 51–68.

4 (*a*) W. G. Lewis, L. G. Green, F. Grynszpan, Z. Radić, P. R. Carlier, P. Taylor, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 1053–1057; (*b*) L. V. Lee, M. L. Mitchell, S.-J. Huang, V. V. Fokin, K. B. Sharpless and C.-H. Wong, *J. Am. Chem. Soc.*, 2003, **125**, 9588–9589; (*c*) Y. Bourne, H. C. Kolb, Z. Radić, K. B. Sharpless, P. Taylor and P. Marchot, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 1449; (*d*) A. Krasinski, Z. Radić, R. Manetsch, J. Raushel, P. Taylor, K. B. Sharpless and H. C. Kolb, *J. Am. Chem. Soc.*, 2005, **127**, 6686–6692; (*e*) L. L. Rossi and A. Basu, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 3596–3599; (*f*) A. Brik, J. Alexandratos, Y.-C. Lin, J. H. Elder, A. J. Olson, A. Wlodawer, D. S. Goodsell and C.-H. Wong, *ChemBioChem*, 2005, **6**, 1167–1169; (*g*) R. Srinivasan, M. Uttamchandani and S. Q. Yao, *Org. Lett.*, 2006, **8**, 713–716; (*h*) D. Giguere, R. Patnam, M.-A. Bellefleur, C. St-Pierre, S. Sato and R. Roy, *Chem. Commun.*, 2006, 2379–2381; (*i*) A. Noeren-Mueller, I. Reis-Correa, Jr., H. Prinz, C. Rosenbaum, K. Saxena, H. J. Schwalbe, D. Vestweber, G. Cagna, S. Schunk, O. Schwarz, H. Schiewe and H. Waldmann, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 10606–10611.

5 For the rapid synthesis of azido acids from amino acids, see: (*a*) J. T. Lundquist, IV and J. C. Pelletier, *Org. Lett.*, 2001, **3**, 781–783; (*b*) For amino alkyne synthesis, see: G. Reginato, A. Mordini, F. Messina, A. Degl'Innocenti and G. Poli, G., *Tetrahedron*, 1996, **52**, 10985–10996; (*c*) J. R. Hauske, P. Dorff, S. Julin, G. Martinelli and J. Bussolari, *Tetrahedron Lett.*, 1992, **33**, 3715–3716.

6 (*a*) W. S. Horne, C. D. Stout and M. R. Ghadiri, *J. Am. Chem. Soc.*, 2003, **125**, 9372–9376; (*b*) W. S. Horne, M. K. Yadav, C. D. Stout and M. R. Ghadiri, *J. Am. Chem. Soc.*, 2004, **126**, 15366–15367; (*c*) J. H. van Maarseveen, W. S. Horne and M. R. Ghadiri, *Org. Lett.*, 2005, **7**, 4503–4506.

7 (*a*) J. H. Billing and U. J. Nilsson, *J. Org. Chem.*, 2005, **70**, 4847–4850; (*b*) K. Oh and Z. Guan, *Chem. Commun.*, 2006, 3069–3071; (*c*) W. J. Choi, Z.-D. Shi, K. M. Worthy, L. Bindu, R. G. Karki, M. C. Nicklaus, R. J. Fisher and T. R. Burke, Jr., *Bioorg. Med. Chem. Lett.*, 2006, **16**, 5265–5269.

8 M. Whiting, J. Muldoon, Y.-C. Lin, S. M. Silverman, W. Lindstom, A. J. Olson, H. C. Kolb, M. G. Finn, K. B. Sharpless, J. H. Elder and V. V. Fokin, *Angew. Chem., Int. Ed.*, 2006, **45**, 1435–1439.

9 (*a*) M. Roice, I. Johannsen and M. Meldal, *QSAR Comb. Sci.*, 2004, **23**, 662–673; (*b*) J. H. van Maarseveen, W. S. Horne and M. R. Ghadiri, *Org. Lett.*, 2005, **7**, 4503–4506; (*c*) S. Punna, J. Kuzelka, Q. Wang and M. G. Finn, *Angew. Chem., Int. Ed.*, 2005, **44**, 2215–2220; (*d*) Y. Angell and K. Burgess, *J. Org. Chem.*, 2005, **70**, 9595–9598; (*e*) V. D. Bock, R. Perciaccante, T. P. Jansen, H. Hiemstra and J. H. van Maarseveen, *Org. Lett.*, 2006, **8**, 919–922.

10 (*a*) N. G. Angelo and P. S. Arora, *J. Am. Chem. Soc.*, 2005, **127**, 17134–17135; (*b*) V. Aucagne and D. A. Leigh, *Org. Lett.*, 2006, **8**, 4505–4507; (*c*) O. D. Montagnat, G. Lessene and A. B. Hughes, *Tetrahedron Lett.*, 2006, **47**, 6971–6974; (*d*) A. Paul, H. Bittermann and P. Gmeiner, *Tetrahedron*, 2006, **62**, 8919–8927; (*e*) Z. Zhang and E. Fan, *Tetrahedron Lett.*, 2006, **47**, 665–669.

11 For a review, see: H. C. Kolb and K. B. Sharpless, *Drug Discovery Today*, 2003, **8**, 1128–1137.

12 H. Kawagishi, A. Somoto, J. Kuranari, A. Kimura and S. Chiba, *Tetrahedron Lett.*, 1993, **34**, 3439–3440.

13 U. Schmidt and J. Langner, *J. Pept. Res.*, 1997, **49**, 67–73.

14 H. D. Dickson, S. C. Smith and K. W. Hinkle, *Tetrahedron Lett.*, 2004, **45**, 5597–5599.

15 For reviews of tyrosinse inhibition, see: (*a*) A. Rescigno, F. Sollai, B. Pisu, A. Rinaldi and E. Sanjust, *J. Enzyme Inhib. Med. Chem.*, 2002, **17**, 207–218; (*b*) S.-Y. Seo, V. K. Sharma and N. Sharma, *J. Agric. Food Chem.*, 2003, **51**, 2837–2853; (*c*) Y.-J. Kim and H. Uyama, *Cell. Mol. Life Sci.*, 2005, **62**, 1707–1723.

16 C. W. van Gelder, W. H. Flurkey and H. J. Wichers, *Phytochemistry*, 1997, **45**, 1309–1323.

17 The product was dissolved in approximately 0.5 cm $CDCl_3$ with several drops of MeOD added to improve solubility.