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

Victoria D. Bock,* Dave Speijer,* Henk Hiemstra,* and Jan H. van Maarseveen**

Supplementary Information

Procedures and spectroscopic data for compounds 5, 6, 8, 9, 12, 13, 18 and 19 and protocol for the mushroom tyrosinase activity assay.

(S)-2-azido-3-(4-hydroxyphenyl)propanoic acid (5). To a 250 cm³ round-bottomed flask containing NaN₃ (11.52 g, 177.2 mmol, 5 equiv) was added H₂O (14 cm³) and CH₂Cl₂ (24 cm³). The flask was cooled to 0 °C, and TfO (5.00 g, 17.72 mmol, 5 equiv) was added. After stirring at 0 °C for 3 h, the layers were separated, and the aqueous layer was extracted with CH₂Cl₂ (2 × 24 cm³). The combined organics containing trityl azide in CH₂Cl₂ (72 cm³) were subsequently added, and the bright blue solution was stirred at rt for 18 h. The organic solvents were removed in vacuo, and the resulting aqueous slurry was diluted with H₂O (50 cm³) and acidified to pH 6.0 with concentrated HCl (aq). The mixture was then diluted with 0.25 M phosphate buffer (pH 6.2, 50 cm³), and the aqueous layer was extracted with EtOAc (4 × 100 cm³) to remove the byproduct and then acidified to pH 2.0 with concentrated HCl (aq). The aqueous layer was extracted with EtOAc (3 × 100 cm³), and the combined organics were dried over Na₂SO₄, filtered, and concentrated in vacuo to afford protected tyrosine azido acid 5 (0.589 g, 2.843 mmol, 80% yield) as a purple solid. This solid was carried on without further purification. ¹H NMR (CDCl₃, 400 MHz) δ 7.37 (br s, 1H, CO₂H), 7.12 (d, J = 8.0, 2H, Tyr ArH), 6.78 (d, J = 8.0, 2H, Tyr ArH), 4.17–4.20 (m, 1H, Tyr CH₃), 3.10 (dd, J = 14.2 and 5.1, 1H, one of two Tyr CH₂CH₂), 2.93 (dd, J = 14.2 and 8.1, one of two Tyr CH₂CH₂) ppm. ¹³C NMR (MeCN-d₅, 100 MHz) δ 171.5, 156.9, 131.4, 128.5, 118.3, 116.1, 63.8, 37.2 ppm. IR 3283, 2114, 1719, 1614, 1516, 1445, 1235, 1107, 1017, 820 cm⁻¹.

(S)-2-azido-3-(4-benzyloxy)phenyl)propanoic acid (6). To a 250 cm³ round-bottomed flask charged with tyrosine azido acid 5 (0.398 g, 1.921 mmol, 1 equiv) in CHCl₃ (23 cm³) and methanol (12 cm³) was added K₂CO₃ (1.168 g, 8.452 mmol, 4.4 equiv). The resulting mixture was heated to reflux while flushing the system with N₂, and benzyl bromide (0.25 cm³, 2.113 mmol, 1.1 equiv) was subsequently added. After 19 h, TLC indicated consumption of the starting materials, so the mixture was cooled to rt and filtered through Celite. The filtrate was concentrated in vacuo to give a yellow solid, which was dissolved in CHCl₃ (20 cm³) and washed with 1N HCl (aq) (1 × 20 cm³). The organic phase was then dried over Na₂SO₄, filtered, and concentrated in vacuo to afford protected tyrosine azido acid 6 (0.4237 g, 1.425 mmol, 74% yield) as a yellow solid. ¹H NMR (CDCl₃, 400 MHz) δ 7.40–7.51 (m, 5H, OCH₂Ph), 7.24 (d, J = 8.5, 2H, Tyr ArH), 7.02 (d, J = 8.5, 2H, Tyr ArH), 5.10 (s, 2H, OCH₂Ph), 4.18–4.23 (m, 1H, Tyr CH₃), 3.22 (dd, J = 14.2 and 4.9, 1H, one of two Tyr CH₂CH₂), 3.04 (dd, J = 14.2 and 8.1, one of two Tyr CH₂CH₂) ppm. IR 3033, 2930, 2869, 2104, 1716, 1610, 1582, 1512, 1454, 1382, 1245, 1177, 1112, 1021, 914 cm⁻¹.

(S)-tert-butyl 1-((S)-2-azido-3-(4-benzyloxy)phenyl)propanoyl)pyrrolidine-2-carboxylic acid (8). To a 50 cm³ round-bottomed flask equipped with a CaCO₃ drying tube and charged with protected tyrosine azido acid 6 (0.3075 g, 1.034 mmol, 1 equiv) in freshly distilled CH₂Cl₂ (5 cm³) was added EDC (0.218 g, 1.138 mmol, 1.1 equiv) and HOBT (0.147 g, 1.086 mmol, 1.05 equiv). L-proline t-butyl ester (0.186 g, 1.086 mmol, 1.05 equiv) was added in freshly distilled CH₂Cl₂ (2 cm³). After 16 h, this solution was diluted with CHCl₃ (20 cm³) and washed with H₂O (1 × 30 cm³), satd aq NaHCO₃ (1 × 30 cm³), and 1N HCl (aq) (1 × 30 cm³). The combined organics were then dried over Na₂SO₄, filtered, and concentrated in vacuo to yield a red-yellow oil. Purification via flash chromatography (30% EtOAc/PE) yielded N₃-Tyr(OBn)-85-Pro-OrBu 8 (0.3057 g, 0.679 mmol, 66%) as a pale yellow oil. ¹H NMR (CDCl₃, 100 MHz) δ 7.42–7.28 (m, 5H, OCH₂Ph), 7.21–7.11 (m, 2H, Tyr ArH), 6.95–6.88 (m, 2H, Tyr ArH), 5.05–5.00 (m, 2H, OCH₂Ph), 4.47–4.41 (m, 1H, Tyr Hδ), 3.89–3.86 (m, 1H, Pro Hδ), 3.69–3.34 (m, 3H, Pro NCH₃ and one of two Pro CH₂CH₃), 3.20–2.99 (m, 2H, Pro CH₂CH₃), 2.19–1.57 (m, 3H, Pro NCH₂CH₂ and one of two Pro CH₂CH₂), 1.47–1.43 (m, 9H, C(C₃H₇)), 1.03–0.03 | [vol], 00–00 | 1

This journal is © The Royal Society of Chemistry [year]
N$_2$-Tyr(OBn)-Pro-OtBu 8 (0.2914 g, 0.647 mmol, 1 equiv) was added TFA (3 cm$^3$) and CHCl$_3$ (3 cm$^3$), and the mixture was stirred at rt. After 16 h, the mixture was concentrated in vacuo and subsequently coevaporated with CHCl$_3$ (2 x 10 cm$^3$) to afford N$_2$-Tyr(OBn)-Pro 9 (0.2813 g) as a yellow oil. This oil was carried on without further purification. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 10.80 (br S, 1H, CO$_2$H), 7.45–7.10 (m, 7H, OCH$_2$Ph and Tyr ArH), 6.98–6.93 (m, 2H, Tyr ArH), 5.09–5.07 (m, 2H, OCH$_2$Ph), 4.62–4.41 (m, 1H, Tyr $\psi$H$_3$), 3.94–3.81 (m, 1H, Pro CH$_2$O), 3.64–3.04 (m, 4H, Pro NCH$_2$H and Tyr CH$_2$CH$_2$), 2.22–1.67 (m, 4H, Pro NCH$_2$CH$_3$ and Pro CH$_2$CH$_3$) ppm. IR 3032, 2980, 2882, 2249, 2106, 1721, 1612, 1511, 1453, 1382, 1298, 1241, 1172, 1024, 910, 820 cm$^{-1}$.

**tert-butyl (S)-1-((S)-2-ethylpyrrolidin-1-yl)-3-methyl-1-oxobutan-2-ylcarbamate (12).** To a 25 cm$^3$ round-bottomed flask equipped with a CaCO$_3$ drying tube and 125 cm$^3$ to afford N$_3$-Tyr(OBn)-Pro-NH$_2$ (0.136 g, 1.035 mmol, 1 equiv) was added TFA (2 cm$^3$) and CHCl$_3$ (2 cm$^3$) to yield a brown solid. The product was purified via flash chromatography (40% EtOAc/PE to afford Boc-Val-Pro alkyne 12 (0.2071 g, 0.704 mmol, 68%) as a yellow oil. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 5.25–5.21 (m, 1H, Val CH$_2$CH$_3$), 4.73–4.56 (m, 1H, Pro CH$_2$O), 4.16–4.10 (m, 2H, Pro NCH$_2$H), 3.66–3.32 (m, 1H, Val CH$_2$CH$_3$), 2.34–1.86 (m, 5H, Pro NCH$_2$CH$_3$; Pro CH$_2$CH$_3$; Pro CCH$_3$), 1.47 (s, 9H, C(C$_6$H$_5$)$_2$), 1.00–0.84 (m, 6H, Val CH$_2$(CH$_3$)$_2$) ppm. $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 171.8, 170.8, 170.6, 155.6, 155.0, 88.1, 81.6, 79.3, 78.8, 72.7, 70.0, 68.0, 56.6, 56.5, 48.0, 47.2, 46.2, 45.5, 33.0, 32.2, 31.7, 31.6, 28.2, 24.5, 22.3, 19.4, 19.0, 17.5 ppm. IR 3309, 2973, 2934, 2876, 2245, 2116, 1709, 1644, 1503, 1428, 1391, 1366, 1341, 1310, 1248, 1172, 1092, 1043, 1017, 920, 878 cm$^{-1}$. HRMS (FAB) Calculated for C$_{16}$H$_{17}$N$_2$O$_2$(MH$^+$): 295.2023; Found: 295.2022. [$\alpha$]$_D^{20}$ = −54.3 (c 2.85 in CHCl$_3$).

**S(-)-2-amino-1-((S)-2-ethylpyrrolidin-1-yl)-3-methylbutan-1-one triflic acid salt (13).** To a 25 cm$^3$ round-bottomed flask charged with Boc-Val-Pro alkyne 12 (0.2102 g, 0.714 mmol, 1 equiv) and freshly distilled CH$_2$Cl$_2$ (6 cm$^3$) was added TFA (3 cm$^3$) and CHCl$_3$ (3 cm$^3$). After 16 h of stirring at rt, this solution was diluted with toluene (2 x 10 cm$^3$) and washed with H$_2$O (1 x 50 cm$^3$). The layers were separated, and the azequeous layer was extracted with CHCl$_3$ (3 x 50 cm$^3$). The combined organic was dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo to provide a yellow solid (1.095 g).

To a 25 cm$^3$ round-bottomed flask equipped with a CaCO$_3$ drying tube and charged with deprotected proline alkyne 10 (0.100 g, 0.714 mmol, 1 equiv) was added TFA (1 cm$^3$) and CHCl$_3$ (1 cm$^3$). After 16 h of stirring at rt, the reaction was diluted in CHCl$_3$ (50 cm$^3$) and washed with 1N HCl (aq) (1 x 50 cm$^3$). The layers were separated, and the aqueous layer was extracted with CHCl$_3$ (3 x 50 cm$^3$). The combined organic was dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo to yield a brown solid. The product was purified via flash chromatography (33% EtOAc/PE to afford Boc-Val-Pro(OBz)-CH$_2$CO$_2$H(11) 0.8882 g, 2.314 mmol, 73%) as a white solid. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 7.84–7.60 (m, 1H), 7.57–7.44 (m, 3H), 7.35–7.21 (m, 4H), 2.52–1.81 (m, 10H), 1.41–1.24 (m, 9H), 1.06–0.97 (m, 3H), 0.72–0.65 (m, 3H) ppm. $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 166.9, 166.8, 166.4, 166.1, 154.0, 151.1, 150.4, 149.9, 120.5, 119.9, 119.6, 119.1, 83.0, 82.0, 81.1, 79.1, 73.6, 72.8, 70.8, 67.0, 66.9, 66.8, 66.6, 53.4, 53.2, 53.0, 52.8, 48.5, 48.3, 47.8, 46.7, 46.7, 46.5, 46.1, 46.0, 45.9, 34.1, 33.8, 33.4, 32.9, 32.7, 32.6, 31.9, 31.7, 31.4, 28.2, 24.5, 24.4, 24.1, 23.4, 23.3, 23.2, 23.0, 22.6, 19.1, 18.8, 18.6, 18.3, 18.1 ppm. IR 3306, 3241, 2974, 2877, 2246, 1692, 1547, 1455, 1395, 1366, 1344, 1253, 1224, 1170, 1116, 1081, 1046, 918, 868, 850 cm$^{-1}$. HRMS (FAB) Calculated for C$_{16}$H$_{17}$N$_2$O$_2$(MH$^+$): 416.2663; Found: 416.2662. [$\alpha$]$_D^{20}$ = −29.1 (c 1.49 in CHCl$_3$).

**S(-)(S)-2-ethylpyrrolidin-1-yl)-3-methyl-2-(4-((S)-pyrrolidin-2-yl)-1H-1,2,3-triazol-1-yl)butan-1-one triflic acid salt (19).** To a 50 cm$^3$ round-bottomed flask charged with Boc-Val-Pro-acetone Val-Pro alkyne 18 (0.3972 g, 0.956 mmol, 1 equiv) was added TFA (2 cm$^3$) and CHCl$_3$ (2 cm$^3$), and the mixture was stirred at rt. After 16 h, the
mixture was concentrated in vacuo and subsequently coevaporated with CHCl₃ (2 × 10 cm³) and toluene (2 × 10 cm) to afford Pro-ψ(triazole)-Val-Pro alkyne TFA salt 19 (0.3254 g) as a red oil. This oil was carried on without further purification. ¹H NMR (CDCl₃, 400 MHz) δ 10.20 (br s, 1H), 8.47–8.27 (m, 1H), 5.53–4.70 (m, 3H), 3.82–3.39 (m, 4H), 2.55–1.88 (m, 10H), 1.11–1.04 (m, 3H), 0.79–0.77 (m, 3H) ppm. IR 3310, 2971, 2880, 1782, 1678, 1472, 1433, 1209, 1177, 1136, 1053, 913, 837 cm⁻¹.

Mushroom tyrosinase assay. 0.30 cm³ of a 20 mM L-DOPA solution (20 mg of L-DOPA in 5 cm³ of a 15 mM solution of phosphoric acid in water) was mixed with 1.5 cm³ of 0.1 M phosphate buffer (pH 7.0) and incubated at 25 °C for 10 min. To this mixture 0.1 cm³ of the sample solution (respective inhibitors in DMSO or neat DMSO) and 0.015 cm³ of the aqueous solution of the mushroom tyrosinase (added last) were then added. The rate of linear increase in absorbance at 470 nm was measured in 'simple kinetic mode' on an Ultrospec 2100 Pro (GE Healthcare lifesciences). The synthetic inhibitor 4-benzyloxyphenol (Sigma-Aldrich) gave an IC₅₀ value of 0.3 mM under these experimental conditions. When used as a negative control, the linear alkyne-azide precursor to cyclic pseudopeptide 2 (compound 4 in ref. 9e) gave no inhibition. All values were calculated from three independent reproducible incubations without any overlap of values obtained with different inhibitor conditions.