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

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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 cm^3 round-bottomed flask containing NaN_3 (11.52 g, 177.2 mmol, 50 equiv) was added H_2O (14 cm^3) and CH_2Cl_2 (24 cm^3). The flask was cooled to 0 °C, and Tf_2O (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 CH_2Cl_2 (2 × 24 cm^3). The combined organics containing trifyl azide in CH_2Cl_2 (72 cm^3) were used without further purification. To a 250 cm^3 round-bottomed flask charged with L-tyrosine (0.642 g, 3.54 mmol, 1 equiv) in MeOH (24 cm^3) and H_2O (12 cm^3) was added K_2CO_3 (0.979 g, 7.09 mmol, 2 equiv) and $CuSO_4$•$5H_2O$ (0.0442 g, 0.177 mmol, 0.05 equiv). Freshly prepared trifyl azide in CH_2Cl_2 (72 cm^3) was 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 H_2O (50 cm^3) 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 cm^3), and the aqueous layer was extracted with EtOAc (4 × 100 cm^3) to remove the byproduct and then acidified to pH 2.0 with concentrated HCl (aq). The aqueous layer was extracted with EtOAc (3 × 100 cm^3), and the combined organics were dried over Na_2SO_4, filtered, and concentrated in vacuo to yield tyrosine azido acid **5** (0.589 g, 2.843 mmol, 80% yield) as a purple solid. This solid was carried on without further purification. ^{1}H NMR (MeCN-d_3, 400 MHz) δ 7.37 (br s, 1H, CO_2**H**), 7.12 (d, *J* = 8.0, 2H, Tyr Ar**H**), 6.78 (d, *J* = 8.0, 2H, Tyr Ar**H**), 4.17–4.20 (m, 1H, Tyr C**H**$_\alpha$), 3.10 (dd, *J* = 14.2 and 5.1, 1H, one of two Tyr CH$_\alpha$C**H**$_2$), 2.93 (dd, *J* = 14.2 and 8.1, one of two Tyr CH$_\alpha$C**H**$_2$) ppm. ^{13}C NMR (MeCN-d_3, 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 cm^3 round-bottomed flask charged with tyrosine azido acid **5** (0.398 g, 1.921 mmol, 1 equiv) in $CHCl_3$ (23 cm) and methanol (12 cm^3) was added K_2CO_3 (1.168 g, 8.452 mmol, 4.4 equiv). The resulting mixture was heated to reflux while flushing the system with N_2, and benzyl bromide (0.25 cm^3, 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 $CHCl_3$ (20 cm^3) and washed with 1N HCl (aq) (1 × 20 cm^3). The organic phase was then dried over Na_2SO_4, filtered, and concentrated in vacuo to afford protected tyrosine azido acid **6** (0.4237 g, 1.425 mmol, 74% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40–7.51 (m, 5H, OCH$_2$**Ph**), 7.24 (d, *J* = 8.5, 2H, Tyr Ar**H**), 7.02 (d, *J* = 8.5, 2H, Tyr Ar**H**), 5.10 (s, 2H, OC**H**$_2$Ph), 4.18–4.23 (m, 1H, Tyr C**H**$_\alpha$), 3.22 (dd, *J* = 14.2 and 4.9, 1H, one of two Tyr CH$_\alpha$C**H**$_2$), 3.04 (dd, *J* = 14.2 and 8.1, one of two Tyr CH$_\alpha$C**H**$_2$) 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-carboxylate (8). To a 50 cm^3 round-bottomed flask equipped with a $CaCO_3$ drying tube and charged with protected tyrosine azido acid **6** (0.3075 g, 1.034 mmol, 1 equiv) in freshly distilled CH_2Cl_2 (5 cm^3) 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 CH_2Cl_2 (2 cm^3). After 16 h, this solution was diluted with $CHCl_3$ (20 cm^3) and washed with H_2O (1 × 30 cm^3), satd aq $NaHCO_3$ (1 × 30 cm^3), and 1N HCl (aq) (1 × 30 cm). The combined organics were then dried over Na_2SO_4, filtered, and concentrated in vacuo to yield a red-yellow oil. Purification via flash chromatography (30% EtOAc/PE) yielded N_3-Tyr(OBn)-Pro-O*t*Bu **8** (0.3057 g, 0.679 mmol, 66%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.42–7.28 (m, 5H, OCH$_2$**Ph**), 7.21–7.11 (m, 2H, Tyr Ar**H**), 6.95–6.88 (m, 2H, Tyr Ar**H**), 5.05–5.00 (m, 2H, OC**H**$_2$Ph), 4.47–4.41 (m, 1H, Tyr **H**$_\alpha$), 3.89–3.86 (m, 1H, Pro **H**$_\alpha$), 3.69–3.34 (m, 3H, Pro NC**H**$_2$ and one of two Pro CH$_\alpha$C**H**$_2$), 3.20–2.99 (m, 2H, Tyr CH$_\alpha$C**H**$_2$), 2.19–1.57 (m, 3H, Pro NCH$_2$C**H**$_2$ and one of two Pro CH$_\alpha$C**H**$_2$), 1.47–1.43 (m, 9H, C(C**H**$_3$)$_3$) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.7, 170.5, 168.7, 168.1, 167.7, 157.8, 136.9, 136.9, 136.8, 130.2, 130.1, 128.7, 128.4, 128.09, 127.9, 127.8, 127.3, 127.2, 115.0, 114.9, 82.4, 81.2, 69.8, 69.7, 61.7, 61.4, 60.8, 59.7, 59.6, 46.7, 46.6, 46.3, 37.3, 36.4, 36.0, 30.8, 28.8, 27.98, 27.8, 27.7, 24.6, 24.3, 22.1 ppm. IR 2978, 2877, 2104, 1737, 1656, 1610, 1512, 1448, 1429, 1367, 1296, 1244, 1224, 1152, 1018, 914, 844 cm^{-1}. HMRS (FAB) Calculated for $C_{25}H_{31}N_4O_4$ (MH^+): 451.2347; Found: 451.2345. $[\alpha]_D^{20}$ = –24.7 (*c* 1.00 in $CHCl_3$).

(*S*)-1-((*S*)-2-azido-3-(4-(benzyloxy)phenyl)propanoyl)pyrrolidine-2-carboxylic acid (9). To a 25 cm^3 round-bottomed flask charged with

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N_3-Tyr(OBn)-Pro-O*t*Bu **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 × 10 cm^3) and toluene (2 × 10 cm^3) to afford N_3-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) δ 10.80 (br s, 1H, CO_2**H**), 7.45–7.10 (m, 7H, OCH_2**Ph** and Tyr Ar**H**), 6.98–6.93 (m, 2H, Tyr Ar**H**), 5.09–5.07 (m, 2H, O**C**$\mathbf{H}_2$Ph), 4.62–4.41 (m, 1H, Tyr **H**$_\alpha$), 3.94–3.81 (m, 1H, Pro C**H**$_\alpha$), 3.64–3.04 (m, 4H, Pro NC$\mathbf{H}_2$ and Tyr $CH_\alpha C\mathbf{H}_2$), 2.22–1.67 (m, 4H, Pro $NCH_2C\mathbf{H}_2$ and Pro $CH_\alpha C\mathbf{H}_2$) ppm. IR 3032, 2980, 2882, 2249, 2106, 1721, 1612, 1511, 1453, 1382, 1298, 1241, 1177, 1112, 1024, 910, 820 cm^{-1}.

***tert*-butyl (*S*)-1-((*S*)-2-ethynylpyrrolidin-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 charged with deprotected proline alkyne **10** (0.136 g, 1.035 mmol, 1 equiv) in freshly distilled CH_2Cl_2 (3 cm^3) was added DIPEA (0.20 cm^3, 1.138 mmol, 1.1 equiv). After 10 min of stirring, this red solution was added to a 50 cm^3 oven-dried flask equipped with a $CaCO_3$ drying tube and charged with Boc-Val-OH (0.236 g, 1.086 mmol, 1.05 equiv), EDC (0.198 g, 1.035 mmol, 1 equiv), HOBt (0.140 g, 1.035 mmol, 1 equiv) and freshly distilled CH_2Cl_2 (10 cm^3). After 16 h of stirring at rt, this 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 1N HCl (aq) (1 × 50 cm^3). The 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 (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) δ 5.25–5.21 (m, 1H, Val C**H**$_\alpha$), 4.73–4.56 (m, 1H, Pro C**H**$_\alpha$), 4.16–4.10 (m, 2H, Pro NC$\mathbf{H}_2$), 3.66–3.32 (m, 1H, Val $CH_\alpha C\mathbf{H}$), 2.34–1.86 (m, 5H, Pro $NCH_2C\mathbf{H}_2$; Pro $CH_\alpha C\mathbf{H}_2$; Pro CC**H**), 1.47 (s, 9H, $C(C\mathbf{H}_3)_3$), 1.00–0.84 (m, 6H, Val $CH(C\mathbf{H}_3)_2$) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 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}. HMRS (FAB) Calculated for $C_{16}H_{27}N_2O_3$ (MH^+): 295.2023; Found: 295.2022. $[\alpha]_D^{20}$ = –54.3 (*c* 2.85 in $CHCl_3$).

(*S*)-2-amino-1-((*S*)-2-ethynylpyrrolidin-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) 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 × 5 cm^3) and toluene (2 × 5 cm^3) to afford Val-Pro alkyne **13** (0.2441 g) as a yellow oil. This oil was carried on without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.13 (br s, 1H, N$\mathbf{H}_2$), 4.82–3.43 (m, 6H, Val C**H**$_\alpha$, Pro C**H**$_\alpha$, Pro NC$\mathbf{H}_2$, Pro $NCH_2C\mathbf{H}_2$), 2.42–2.06 (m, 4H, Pro $CH_\alpha C\mathbf{H}_2$; Pro CC**H**, Val $CH_\alpha C\mathbf{H}$), 1.22–0.88 (m, 6H, Val $CH(C\mathbf{H}_3)_2$) ppm. IR 2973, 2882, 2238, 1654, 1510, 1453, 1398, 1368, 1267, 1201, 1138, 913, 834 cm^{-1}.

(*S*)-*tert*-butyl 2-(1-((*S*)-1-((*S*)-2-ethynylpyrrolidin-1-yl)-3-methyl-1-oxobutan-2-yl)-1H-1,2,3-triazol-4-yl)pyrrolidine-1-carboxylate (18). To a 50 cm^3 round-bottomed flask charged with valine azido acid 16 (0.4186 g, 2.924 mmol, 1 equiv) in MeCN (12 cm^3) was added *N*-Boc-proline alkyne 15 (0.5710 g, 2.924 mmol, 1 equiv), DIPEA (1.02 cm^3, 5.848 mmol, 2 equiv) and 2,6-lutidine (0.68 cm^3, 5.848 mmol, 2 equiv). The solution was degassed with argon for 30 min, and CuI (1.11 g, 5.848 mmol, 2 equiv) was subsequently added. After 16 h stirring under argon, the reaction was diluted in $CHCl_3$ (50 cm^3) and washed with 1N HCl (aq) (1 × 50 cm^3). The layers were separated, and the aqueous layer was extracted with $CHCl_3$ (3 × 50 cm^3). The combined organics were dried over Na_2SO_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** (0.423 g, 3.216 mmol, 1.1 equiv) in freshly distilled CH_2Cl_2 (4 cm^3) was added DIPEA (0.56 cm^3, 3.216 mmol, 1.1 equiv). After 10 min of stirring, this red solution was added to a 50 cm^3 oven-dried flask equipped with a $CaCO_3$ drying tube and charged with the yellow solid obtained above (1.095 g, 2.924 mmol, 1 equiv), EDC (0.561 g, 2.924 mmol, 1 equiv), HOBt (0.395 g, 2.924 mmol, 1 equiv) and freshly distilled CH_2Cl_2 (6 cm^3). After 16 h of stirring at rt, this 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 1N HCl (aq) (1 × 50 cm^3). The 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 Boc-Pro-ψ(triazole)-Val-Pro alkyne **18** (0.8812 g, 2.314 mmol, 73%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.84–7.60 (m, 1H), 5.37–4.64 (m, 3H), 3.75–3.21 (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) δ 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, 70.5, 67.0, 66.9, 66.8, 66.6, 53.4, 53.2, 53.0, 52.8, 48.5, 48.3, 47.8, 47.6, 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}. HMRS (FAB) Calculated for $C_{22}H_{34}N_5O_3$ (MH^+): 416.2663; Found: 416.2662. $[\alpha]_D^{20}$ = –29.1 (*c* 1.49 in $CHCl_3$).

(*S*)-1-((*S*)-2-ethynylpyrrolidin-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-Pro-ψ(triazole)-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 $CHCl_3$ (2×10 cm^3) 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 ($CDCl_3$, 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 cm^3 of a 20 mM L-DOPA solution (20 mg of L-DOPA in 5 cm^3 of a 15 mM solution of phosphoric acid in water) was mixed with 1.5 cm^3 of 0.1 M phosphate buffer (pH 7.0) and incubated at 25 °C for 10 min. To this mixture 0.1 cm^3 of the sample solution (respective inhibitors in DMSO or neat DMSO) and 0.015 cm^3 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 IC_{50} 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