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, 10 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, 15 177.2 mmol, 50 equiv) was added H₂O (14 cm³) and CH₂Cl₂ (24 cm³). The flask was cooled to 0 °C, and Tf₂O (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 cm³). The combined organics 20 containing trifyl azide in CH₂Cl₂ (72 cm³) were used without further purification. To a 250 cm³ round-bottomed flask charged with L-tyrosine (0.642 g, 3.54 mmol, 1 equiv) in MeOH (24 cm³) and H₂O (12 cm³) was added K₂CO₃ (0.979 g, 7.09 mmol, 2 equiv) and CuSO₄•5H₂O (0.0442 g, 25 0.177 mmol, 0.05 equiv). Freshly prepared trifyl azide in CH_2Cl_2 (72 cm³) 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 H₂O (50 cm³) and acidified to pH 6.0 with 30 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 ($4 \times 100 \text{ cm}^3$) to remove the byproduct and then acidified to pH 2.0 with concentrated HCl (aq). The aqueous layer was extracted $_{35}$ with EtOAc (3 × 100 cm³), and the combined organics were dried over Na₂SO₄, 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. ¹H NMR (MeCN- d_3 , 400 MHz) δ 7.37 ⁴⁰ (br s, 1H, CO₂**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 \mathbf{H}_{α}), 3.10 (dd, J = 14.2 and 5.1, 1H, one of two Tyr $CH_{\alpha}CH_{2}$), 2.93 (dd, J = 14.2 and 8.1, one of two Tyr $CH_{\alpha}CH_2$) ppm. ¹³C NMR (MeCN-d₃, 100 MHz) δ 171.5, 156.9, 131.4, 128.5, 118.3,

⁴⁵ 116.1, 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 50 azido acid 5 (0.398 g, 1.921 mmol, 1 equiv) in CHCl₃ (23 cm) and methanol (12 cm³) 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 N2, and benzyl bromide (0.25 cm³, 2.113 mmol, 1.1 equiv) was 55 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 cm^3) and washed with 1N HCl (aq) $(1 \times 20 \text{ cm}^3)$. The 60 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, OCH₂**Ph**), 7.24 (d, J = 8.5, 2H, Tyr Ar**H**), 7.02 (d, J = 8.5, 2H, Tyr

⁶⁵ Ar**H**), 5.10 (s, 2H, OC**H**₂Ph), 4.18–4.23 (m, 1H, Tyr C**H**_α), 3.22 (dd, J = 14.2 and 4.9, 1H, one of two Tyr CH_αC**H**₂), 3.04 (dd, J = 14.2 and 8.1, one of two Tyr CH_αC**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 cm³ round-bottomed flask equipped with a CaCO₃ drying tube and charged with protected tyrosine

- ⁷⁵ 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
- ⁸⁰ 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 cm). The combined organics were then dried over Na₂SO₄, filtered, and concentrated in vacuo to yield a red-yellow oil. Purification via flash
- ⁸⁵ chromatography (30% EtOAc/PE) yielded N₃-Tyr(OBn)-Pro-OtBu 8 (0.3057 g, 0.679 mmol, 66%) as a pale yellow oil. ¹H NMR (CDCl₃, 400 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,
- ⁹⁰ Tyr \mathbf{H}_{α}), 3.89–3.86 (m, 1H, Pro \mathbf{H}_{α}), 3.69–3.34 (m, 3H, Pro NC \mathbf{H}_2 and one of two Pro CH_{α}C \mathbf{H}_2), 3.20–2.99 (m, 2H, Tyr CH_{α}C \mathbf{H}_2), 2.19–1.57 (m, 3H, Pro NCH₂C \mathbf{H}_2 and one of two Pro CH_{α}C \mathbf{H}_2), 1.47–1.43 (m, 9H, C(C \mathbf{H}_3)₃) ppm. ¹³C NMR (CDCl₃, 100 MHz) δ 170.7, 170.5, 168.7, 168.1, 167.7,
- 95
 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,
- ¹⁰⁰ 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 CHCl₃).

105 (S)-1-((S)-2-azido-3-(4-

(benzyloxy)phenyl)propanoyl)pyrrolidine-2-carboxylic acid (9). To a 25 cm³ round-bottomed flask charged with

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N₃-Tyr(OBn)-Pro-OtBu **8** (0.2914 g, 0.647 mmol, 1 equiv) was added TFA (3 cm³) and CHCl₃ (3 cm³), 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 cm³) to afford N₃-Tyr(OBn)-Pro **9** (0.2813 g) as a yellow oil. This oil was carried on without further purification. ¹H NMR (CDCl₃, 400 MHz) δ 10.80 (br ¹¹⁵ s, 1H, CO₂**H**), 7.45–7.10 (m, 7H, OCH₂**Ph** and Tyr Ar**H**), 6.98–6.93 (m, 2H, Tyr Ar**H**), 5.09–5.07 (m, 2H, OC**H**₂Ph), 4.62–4.41 (m, 1H, Tyr **H**_α), 3.94–3.81 (m, 1H, Pro C**H**_α), 3.64–3.04 (m, 4H, Pro NC**H**₂ and Tyr CH_αC**H**₂), 2.22–1.67 (m, 4H, Pro NCH₂C**H**₂ and Pro CH_αC**H**₂) ppm. IR 3032, ¹²⁰ 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 cm³ round-125 bottomed flask equipped with a CaCO₃ drying tube and charged with deprotected proline alkyne 10 (0.136 g, 1.035 mmol, 1 equiv) in freshly distilled CH₂Cl₂ (3 cm³) was added DIPEA (0.20 cm³, 1.138 mmol, 1.1 equiv). After 10 min of stirring, this red solution was added to a 50 cm³ 130 oven-dried flask equipped with a CaCO₃ 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 CH₂Cl₂ (10 cm³). After 16 h of stirring at rt, this solution was diluted 135 with CHCl₃ (50 cm³) and washed with H₂O (1×50 cm³), satd aq NaHCO₃ (1 \times 50 cm³), and 1N HCl (aq) (1 \times 50 cm^3). The organics were then dried over Na₂SO₄, filtered, and concentrated in vacuo to yield a brown solid. The product was purified via flash chromatography (40% 140 EtOAc/PE) to afford Boc-Val-Pro alkyne 12 (0.2071 g, 0.704 mmol, 68%) as a yellow oil. ¹H NMR (CDCl₃, 400 MHz) δ 5.25–5.21 (m, 1H, Val CH_a), 4.73–4.56 (m, 1H, Pro C**H**_α), 4.16–4.10 (m, 2H, Pro NC**H**₂), 3.66–3.32 (m, 1H, Val $CH_{\alpha}CH$), 2.34–1.86 (m, 5H, Pro NCH_2CH_2 ; Pro ¹⁴⁵ CH_αCH₂; Pro CCH), 1.47 (s, 9H, C(CH₃)₃), 1.00–0.84 (m, 6H, Val CH(CH₃)₂) ppm. ¹³C NMR (CDCl₃, 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 150 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 CHCl₃).

155

(S)-2-amino-1-((S)-2-ethynylpyrrolidin-1-yl)-3-

methylbutan-1-one triflic acid salt (13). To a 25 cm³ round-bottomed flask charged with Boc-Val-Pro alkyne 12 (0.2102 g, 0.714 mmol, 1 equiv) was added TFA (3 cm³) and CHCl₃ (3 cm³), and the mixture was stirred at rt. After 16 h, the mixture was concentrated in vacuo and subsequently coevaporated with CHCl₃ (2 × 5 cm³) and toluene (2 × 5 cm³) to afford Val-Pro alkyne 13 (0.2441 g) as a yellow oil. This oil was carried on without further ¹⁶⁵ purification. ¹H NMR (CDCl₃, 400 MHz) δ 8.13 (br s, 1H, NH₂), 4.82–3.43 (m, 6H, Val CH_α, Pro CH_α, Pro NCH₂,

Pro NCH₂C**H**₂), 2.42–2.06 (m, 4H, Pro CH_αC**H**₂; Pro CC**H**, Val CH_αC**H**), 1.22–0.88 (m, 6H, Val CH(C**H**₃)₂) ppm. IR 2973, 2882, 2238, 1654, 1510, 1453, 1398, 1368, 1267, ¹⁷⁰ 1201, 1138, 913, 834 cm⁻¹.

(S)-*tert*-butyl 2-(1-((S)-1-((S)-2-ethynylpyrrolidin-1-yl)-3methyl-1-oxobutan-2-yl)-1H-1,2,3-triazol-4-yl)pyrrolidine-1-carboxylate (18). To a 50 cm³ round-bottomed flask

- ¹⁷⁵ charged with valine azido acid 16 (0.4186 g, 2.924 mmol, 1 equiv) in MeCN (12 cm³) was added *N*-Boc-proline alkyne
 ¹⁵ (0.5710 g, 2.924 mmol, 1 equiv), DIPEA (1.02 cm³, 5.848 mmol, 2 equiv) and 2,6-lutidine (0.68 cm³, 5.848 mmol, 2 equiv). The solution was degassed with argon for
 ¹⁸⁰ 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 CHCl₃ (50 cm³) and washed with 1N HCl (aq) (1×50 cm³). The layers were separated, and the aqueous layer was extracted with CHCl₃ (3×50 cm³). The 185 combined organics were dried over Na₂SO₄, filtered, and concentrated in vacuo to provide a yellow solid (1.095 g).

To a 25 cm³ round-bottomed flask equipped with a CaCO₃ drying tube and charged with deprotected proline alkyne **10** (0.423 g, 3.216 mmol, 1.1 equiv) in freshly ¹⁹⁰ distilled CH₂Cl₂ (4 cm³) was added DIPEA (0.56 cm³, 3.216 mmol, 1.1 equiv). After 10 min of stirring, this red solution was added to a 50 cm³ oven-dried flask equipped with a CaCO₃ drying tube and charged with the yellow solid obtained above (1.095 g, 2.924 mmol, 1 equiv), EDC (0.561

- ¹⁹⁵ g, 2.924 mmol, 1 equiv), HOBt (0.395 g, 2.924 mmol, 1 equiv) and freshly distilled CH_2Cl_2 (6 cm³). After 16 h of stirring at rt, this solution was diluted with $CHCl_3$ (50 cm³) and washed with H_2O (1 × 50 cm³), satd aq NaHCO₃ (1 × 50 cm³), and 1N HCl (aq) (1 × 50 cm³). The organics were
- ²⁰⁰ then dried over Na₂SO₄, 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. ¹H NMR (CDCl₃, 400 MHz) δ 7.84–7.60
- 205 (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. $^{13}\mathrm{C}$ NMR (CDCl₃, 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,
- 210 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, 1266, 1234, 1252, 1234, 1251, 1204, 1104
- ²¹⁵ 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 CHCl₃).
- ²²⁰ (*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 cm³ 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 cm³) and CHCl₃ (2 ²²⁵ cm³), 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 ²³⁰ 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 cm^3 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

- ²⁴⁰ °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
- $_{245}$ 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**
- 250 (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

²³⁵