## Supplementary Information

# X-Ray crystallographic signature of supramolecular triple helix formation from a water soluble synthetic tetrapeptide

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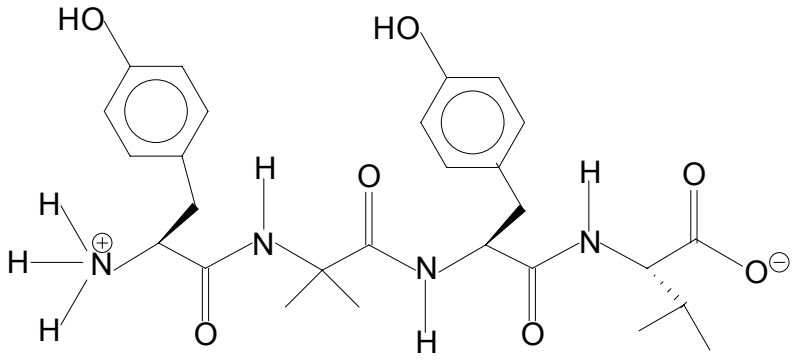


Figure S1: Chemical structure of peptide **1**

**Peptide synthesis:** The tetrapeptide was synthesized by conventional solution phase methods using racemization free fragment condensation strategy. The Boc group was used for N-terminal protection and the C-terminus was protected as a methyl ester. Couplings were mediated by dicyclohexylcarbodiimide-1-hydroxybenzotriazole (DCC/HOBt). All intermediates have been characterized by $^1$H-NMR (300MHz) and thin layer chromatography (TLC) on silica gel and used without further purification. Deprotection of methyl ester was performed using saponification method and the Boc group was deprotected by TFA. The final products were purified by column chromatography using silica (100-200 mesh size) gel as stationary phase and chloroform-methanol (6:4) as eluent. Purified final compounds have been fully characterized by 300 MHz $^1$H -NMR spectroscopy, Mass spectrometry. Crystals suitable for an X-ray diffraction study was obtained from water-methanol solution by slow evaporation.

$^1$H NMR (300 MHz, DMSO-$d_6$) δ 9.12 (br, 3H), 8.25 (s, 1H), 7.65 (d, *J* = 8.3 Hz, 1H), 7.58 (d, *J* = 8.3 Hz, 1H), 7.03 (d, *J* = 8.3 Hz, 2H), 6.98 (d, *J* = 8.3 Hz, 2H), 6.67 (d, *J* = 8.3 Hz, 2H), 6.60 (d, *J* = 8.4 Hz, 2H), 4.42-4.50 (m, 1H), 3.95-3.99 (m, 1H), 3.53 (m, 1H), 2.86-3.07 (m, 2H), 2.57-2.72 (m, 2H), 2.00-2.07 (m, 1H), 1.18 (d, *J* = 10.5 Hz, 6H), 0.81-0.89 (m, 6H).

IR (KBr) $\gamma_{max}$ 3536, 3493, 3289, 1658, 1547, 1515 cm$^{-1}$

Mass spectral data (ESI-MS) *m/z* = 529.3 $[M+H]^+$, $M_{calcd}$ = 528.

Anal.Calcd. for $C_{27}H_{36}N_4O_7$ (528): C,61.36; H,6.81 ; N,10.61

Found: C, 61.28; H, 6.80; N, 10.59

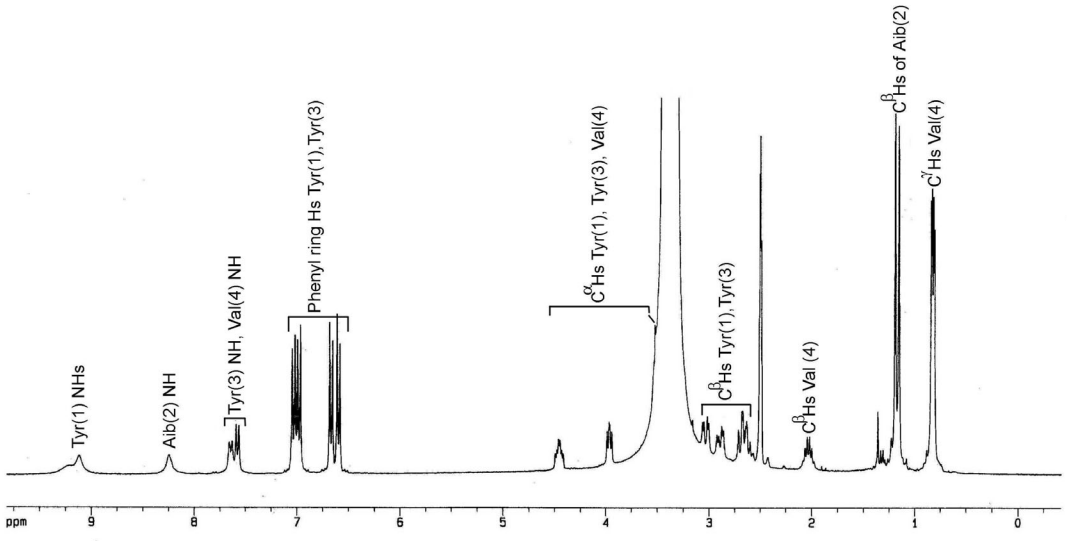


Figure S2: 300 MHz $^{1}$H NMR spectrum of peptide **1** in DMSO-$d_6$.

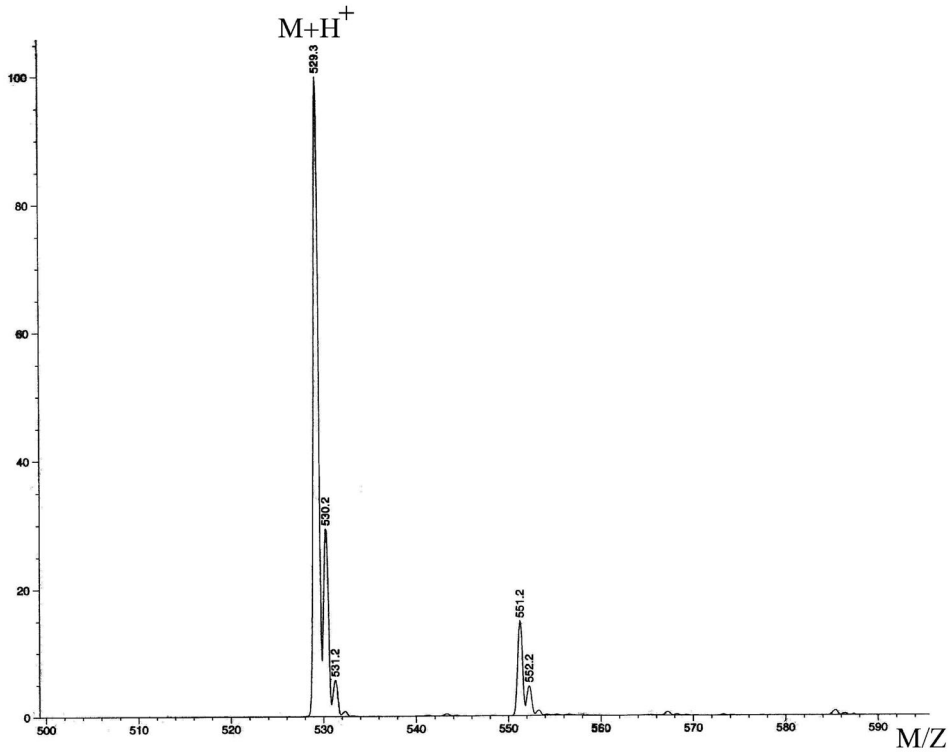


Figure S3: Typical Mass spectrum (ESI-MS) of peptide **1**.

Table S1: Final Coordinates (Å x $10^4$) and Equivalent Isotropic Displacement Parameters (Å$^2$ x $10^3$) of the non-Hydrogen atoms for peptide **1**

| Atom | x | y | z | U(eq) [Ang^2] |
|---|---|---|---|---|
| O1 | -0.0521(6) | 0.2484(6) | 0.11438(8) | 0.0766(16) |
| O1N | 0.1882(9) | 0.0964(7) | 0.00985(8) | 0.100(2) |
| O2 | 0.2139(7) | 0.7339(6) | 0.11350(7) | 0.0722(16) |
| O3 | 0.6062(5) | 0.9073(5) | 0.13473(7) | 0.0668(14) |
| O3N | 0.3553(7) | 0.7842(7) | 0.24803(7) | 0.0871(16) |
| O4 | 0.2680(5) | 1.1625(5) | 0.14990(6) | 0.0632(12) |
| O5 | 0.4908(6) | 1.3701(5) | 0.13222(7) | 0.0703(14) |
| N1 | -0.0469(6) | -0.0383(7) | 0.12827(8) | 0.0680(16) |
| N2 | 0.2045(5) | 0.3418(5) | 0.09761(6) | 0.0440(12) |
| N3 | 0.3155(5) | 0.5791(5) | 0.12972(6) | 0.0456(12) |
| N4 | 0.4478(6) | 0.9834(5) | 0.15444(6) | 0.0492(12) |
| C1' | 0.0574(7) | 0.2186(7) | 0.10724(8) | 0.0520(17) |
| C1D1 | 0.1162(14) | -0.0562(11) | 0.06312(12) | 0.099(4) |
| C1A | 0.0308(7) | 0.0356(6) | 0.10817(9) | 0.0526(14) |
| C1B | -0.0900(8) | -0.0780(7) | 0.09091(10) | 0.0727(19) |
| C1G | -0.0134(9) | -0.0267(7) | 0.06961(10) | 0.068(2) |
| C1Z | 0.1227(10) | 0.0596(9) | 0.02937(10) | 0.075(2) |
| C2' | 0.2482(6) | 0.6164(7) | 0.11324(8) | 0.0487(16) |
| C1D2 | -0.0731(9) | 0.0512(10) | 0.05619(11) | 0.076(3) |
| C2A | 0.2370(6) | 0.5185(6) | 0.09338(7) | 0.0486(16) |

| | | | | |
|---|---|---|---|---|
| C3' | 0.4807(6) | 0.8702(6) | 0.14520(8) | 0.0471(16) |
| C1E1 | 0.1800(13) | -0.0157(12) | 0.04381(13) | 0.095(3) |
| C3A | 0.3455(6) | 0.6762(6) | 0.14898(7) | 0.0424(14) |
| C3B | 0.4068(9) | 0.5948(7) | 0.16543(8) | 0.0591(19) |
| C3G | 0.3964(8) | 0.6488(7) | 0.18745(8) | 0.0557(17) |
| C3Z | 0.3687(8) | 0.7332(8) | 0.22792(8) | 0.0598(17) |
| C4' | 0.4228(7) | 1.2374(6) | 0.14362(8) | 0.0491(16) |
| C1E2 | -0.0064(10) | 0.0903(10) | 0.03588(11) | 0.082(3) |
| C4A | 0.5474(7) | 1.1727(6) | 0.15065(8) | 0.0481(16) |
| C4B | 0.6628(9) | 1.2802(8) | 0.16858(10) | 0.073(2) |
| C2B1 | 0.0989(11) | 0.5122(10) | 0.07902(10) | 0.079(3) |
| C2B2 | 0.4133(8) | 0.6170(8) | 0.08259(10) | 0.0673(19) |
| C3D1 | 0.2417(9) | 0.5718(9) | 0.19727(11) | 0.089(2) |
| C3D2 | 0.5391(8) | 0.7695(9) | 0.19846(9) | 0.0656(19) |
| C3E1 | 0.2237(10) | 0.6117(10) | 0.21773(10) | 0.086(2) |
| C3E2 | 0.5241(9) | 0.8114(9) | 0.21868(10) | 0.071(2) |
| C4G1 | 0.5561(13) | 1.2553(12) | 0.18779(11) | 0.097(3) |
| C4G2 | 0.8068(12) | 1.2328(13) | 0.17285(16) | 0.111(4) |
| O1W | 0.8438(8) | 1.6111(8) | 0.12740(12) | 0.107(2) |
| O2W | 0.0609(9) | 0.5269(11) | 0.26517(9) | 0.126(3) |

Table S2 - Bond Distances (Angstrom) for peptide **1**

| | | | | | |
|---|---|---|---|---|---|
| O1 | -C1' | 1.210(9) | C1D1 | -C1E1 | 1.342(12) |
| O1N | -C1Z | 1.358(9) | C1A | -C1B | 1.521(8) |
| O2 | -C2' | 1.214(8) | C1B | -C1G | 1.501(9) |
| O3 | -C3' | 1.194(7) | C1G | -C1D2 | 1.364(11) |
| O3N | -C3Z | 1.400(7) | C1Z | -C1E2 | 1.360(13) |
| O4 | -C4' | 1.249(8) | C1Z | -C1E1 | 1.379(13) |
| O5 | -C4' | 1.252(6) | C2' | -C2A | 1.522(7) |
| O1N | -H1N | 0.8217 | C1D2 | -C1E2 | 1.409(10) |
| O3N | -H3N | 0.8198 | C2A | -C2B2 | 1.518(9) |
| O1W | -H1W2 | 0.96(3) | C2A | -C2B1 | 1.509(11) |
| O1W | -H1W1 | 0.96(6) | C3' | -C3A | 1.537(7) |
| O2W | -H2W1 | 0.96(18) | C3A | -C3B | 1.525(8) |
| O2W | -H2W2 | 0.95(16) | C3B | -C3G | 1.519(8) |
| N1 | -C1A | 1.462(8) | C3G | -C3D2 | 1.371(9) |
| N2 | -C2A | 1.461(6) | C3G | -C3D1 | 1.340(11) |
| N2 | -C1' | 1.356(7) | C3Z | -C3E2 | 1.327(11) |
| N3 | -C3A | 1.458(6) | C3Z | -C3E1 | 1.358(10) |
| N3 | -C2' | 1.338(7) | C4' | -C4A | 1.537(9) |
| N4 | -C4A | 1.465(6) | C4A | -C4B | 1.520(9) |
| N4 | -C3' | 1.313(7) | C4B | -C4G2 | 1.545(15) |
| N1 | -H1B | 0.8904 | C4B | -C4G1 | 1.506(12) |
| N1 | -H1C | 0.8912 | C3D1 | -C3E1 | 1.398(10) |

| | | | | | |
|---|---|---|---|---|---|
| N1 | -H1A | 0.8893 | C3D2 | -C3E2 | 1.383(9) |
| N2 | -H2 | 0.8593 | C1D1 | -H1D1 | 0.9292 |
| N3 | -H3 | 0.8600 | C1A | -H1A1 | 0.92(7) |
| N4 | -H4 | 0.8603 | C1B | -H1B2 | 0.9706 |
| C1' | -C1A | 1.509(8) | C1B | -H1B1 | 0.9697 |
| C1D1 | -C1G | 1.358(15) | C1D2 | -H1D2 | 0.9291 |
| C1E1 | -H1E1 | 0.9293 | C2B2 | -H2B5 | 0.9597 |
| C3A | -H3A | 0.97(6) | C3D1 | -H3D1 | 0.9310 |
| C3B | -H3B1 | 0.9699 | C3D2 | -H3D2 | 0.9288 |
| C3B | -H3B2 | 0.9700 | C3E1 | -H3E1 | 0.9294 |
| C1E2 | -H1E2 | 0.9291 | C3E2 | -H3E2 | 0.9303 |
| C4A | -H4A | 0.9800 | C4G1 | -H4G1 | 0.9601 |
| C4B | -H4B | 0.9800 | C4G1 | -H4G2 | 0.9599 |
| C2B1 | -H2B1 | 0.9605 | C4G1 | -H4G3 | 0.9597 |
| C2B1 | -H2B2 | 0.9584 | C4G2 | -H4G5 | 0.9597 |
| C2B1 | -H2B3 | 0.9598 | C4G2 | -H4G6 | 0.9619 |
| C2B2 | -H2B6 | 0.9625 | C4G2 | -H4G4 | 0.9625 |
| C2B2 | -H2B4 | 0.9592 | | | |

Table S3 - Bond Angles (Degrees) for peptide **1**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| C1Z | -O1N | -H1N | 109.56 | C1B | -C1G | -C1D2 | 120.5(7) |
| C3Z | -O3N | -H3N | 109.56 | C1D1 | -C1G | -C1B | 121.4(7) |
| H1W1 | -O1W | -H1W2 | 105(4) | O1N | -C1Z | -C1E2 | 122.5(8) |
| H2W1 | -O2W | -H2W2 | 102(14) | C1E1 | -C1Z | -C1E2 | 115.4(7) |
| C1' | -N2 | -C2A | 122.6(5) | O1N | -C1Z | -C1E1 | 122.0(9) |
| C2' | -N3 | -C3A | 120.6(4) | O2 | -C2' | -C2A | 121.4(5) |
| C3' | -N4 | -C4A | 123.6(5) | N3 | -C2' | -C2A | 116.4(5) |
| H1A | -N1 | -H1B | 109.47 | O2 | -C2' | -N3 | 121.8(5) |
| H1A | -N1 | -H1C | 109.50 | C1G | -C1D2 | -C1E2 | 119.8(8) |
| C1A | -N1 | -H1C | 109.50 | N2 | -C2A | -C2B1 | 110.6(5) |
| H1B | -N1 | -H1C | 109.29 | N2 | -C2A | -C2B2 | 107.1(5) |
| C1A | -N1 | -H1A | 109.46 | N2 | -C2A | -C2' | 111.6(4) |
| C1A | -N1 | -H1B | 109.60 | C2' | -C2A | -C2B1 | 111.3(5) |
| C2A | -N2 | -H2 | 118.77 | C2' | -C2A | -C2B2 | 107.8(4) |
| C1' | -N2 | -H2 | 118.64 | C2B1 | -C2A | -C2B2 | 108.3(5) |
| C3A | -N3 | -H3 | 119.88 | O3 | -C3' | -N4 | 125.2(5) |
| C2' | -N3 | -H3 | 119.52 | O3 | -C3' | -C3A | 119.4(5) |
| C3' | -N4 | -H4 | 118.16 | N4 | -C3' | -C3A | 115.4(5) |
| C4A | -N4 | -H4 | 118.20 | C1D1 | -C1E1 | -C1Z | 123.5(11) |
| N2 | -C1' | -C1A | 116.7(5) | N3 | -C3A | -C3' | 109.2(4) |
| O1 | -C1' | -N2 | 123.2(5) | N3 | -C3A | -C3B | 108.9(4) |
| O1 | -C1' | -C1A | 120.1(5) | C3' | -C3A | -C3B | 112.0(5) |

C1G -C1D1 -C1E1 121.1(10) C3A -C3B -C3G 114.6(6)

N1 -C1A -C1B 110.0(5) C3B -C3G -C3D1 119.4(6)

C1' -C1A -C1B 110.9(5) C3B -C3G -C3D2 123.7(6)

N1 -C1A -C1' 107.4(5) C3D1 -C3G -C3D2 116.9(6)

C1A -C1B -C1G 114.5(5) O3N -C3Z -C3E2 118.8(6)

C1D1 -C1G -C1D2 118.2(7) C3E1 -C3Z -C3E2 120.7(6)

O3N -C3Z -C3E1 120.4(7) C1Z -C1E1 -H1E1 118.35

O4 -C4' -C4A 120.1(4) C3' -C3A -H3A 107(2)

O5 -C4' -C4A 115.0(6) N3 -C3A -H3A 107(3)

O4 -C4' -O5 124.8(6) C3B -C3A -H3A 113(3)

C1Z -C1E2 -C1D2 121.8(8) C3G -C3B -H3B1 108.63

N4 -C4A -C4B 114.5(4) C3G -C3B -H3B2 108.68

C4' -C4A -C4B 111.9(5) C3A -C3B -H3B2 108.60

N4 -C4A -C4' 109.8(5) C3A -C3B -H3B1 108.54

C4A -C4B -C4G2 110.6(6) H3B1 -C3B -H3B2 107.59

C4G1 -C4B -C4G2 110.5(7) C1D2 -C1E2 -H1E2 118.98

C4A -C4B -C4G1 111.4(7) C1Z -C1E2 -H1E2 119.18

C3G -C3D1 -C3E1 122.4(7) C4B -C4A -H4A 106.69

C3G -C3D2 -C3E2 121.7(7) N4 -C4A -H4A 106.66

C3Z -C3E1 -C3D1 118.5(8) C4' -C4A -H4A 106.74

C3Z -C3E2 -C3D2 119.9(7) C4A -C4B -H4B 108.12

C1G -C1D1 -H1D1 119.43 C4G1 -C4B -H4B 108.09

C1E1 -C1D1 -H1D1 119.44 C4G2 -C4B -H4B 108.11

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| C1B | -C1A | -H1A1 | 116(3) | C2A | -C2B1 | -H2B1 | 109.41 |
| C1' | -C1A | -H1A1 | 108(3) | H2B1 | -C2B1 | -H2B2 | 109.52 |
| N1 | -C1A | -H1A1 | 104(3) | H2B1 | -C2B1 | -H2B3 | 109.44 |
| C1A | -C1B | -H1B1 | 108.67 | H2B2 | -C2B1 | -H2B3 | 109.62 |
| C1G | -C1B | -H1B2 | 108.50 | C2A | -C2B1 | -H2B2 | 109.45 |
| C1A | -C1B | -H1B2 | 108.75 | C2A | -C2B1 | -H2B3 | 109.39 |
| C1G | -C1B | -H1B1 | 108.68 | C2A | -C2B2 | -H2B4 | 109.57 |
| H1B1 | -C1B | -H1B2 | 107.57 | H2B4 | -C2B2 | -H2B5 | 109.41 |
| C1E2 | -C1D2 | -H1D2 | 119.97 | C2A | -C2B2 | -H2B5 | 109.42 |
| C1G | -C1D2 | -H1D2 | 120.21 | C2A | -C2B2 | -H2B6 | 109.51 |
| C1D1 | -C1E1 | -H1E1 | 118.10 | H2B5 | -C2B2 | -H2B6 | 109.50 |
| H2B4 | -C2B2 | -H2B6 | 109.41 | H4G1 | -C4G1 | -H4G3 | 109.52 |
| C3E1 | -C3D1 | -H3D1 | 118.88 | C4B | -C4G1 | -H4G3 | 109.46 |
| C3G | -C3D1 | -H3D1 | 118.73 | H4G1 | -C4G1 | -H4G2 | 109.50 |
| C3G | -C3D2 | -H3D2 | 119.27 | H4G2 | -C4G1 | -H4G3 | 109.47 |
| C3E2 | -C3D2 | -H3D2 | 119.06 | C4B | -C4G2 | -H4G5 | 109.45 |
| C3Z | -C3E1 | -H3E1 | 120.78 | C4B | -C4G2 | -H4G6 | 109.44 |
| C3D1 | -C3E1 | -H3E1 | 120.72 | H4G4 | -C4G2 | -H4G6 | 109.67 |
| C3Z | -C3E2 | -H3E2 | 120.05 | H4G5 | -C4G2 | -H4G6 | 109.39 |
| C3D2 | -C3E2 | -H3E2 | 120.05 | H4G4 | -C4G2 | -H4G5 | 109.49 |
| C4B | -C4G1 | -H4G2 | 109.44 | C4B | -C4G2 | -H4G4 | 109.39 |
| C4B | -C4G1 | -H4G1 | 109.43 | | | | |