

SUPPORTING INFORMATION

Effect of 3-Hydroxyproline Residues on Collagen Stability

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Table S1. Data for pH Titration of 3-Hydroxyproline

Sample	pH	δ (α -proton, Hz)	Volume (μ L) of 0.10 M D_3PO_4	Volume (μ L) of 0.10 M NaOD	Volume (μ L) of 0.10 M DCl
1	1.30	2129.1	950	0	0
2	1.36	2124.2	902	48	0
3	1.42	2118.3	855	95	0
4	1.50	2111.2	807	143	0
5	1.57	2104.6	760	190	0
6	1.66	2096.2	712	238	0
7	1.78	2087.5	665	285	0
8	1.93	2077.7	617	333	0
9	2.10	2067.9	570	380	0
10	2.39	2053.5	522	428	0
11	3.05	2039.4	475	475	0
12	0.61	2166.8	0	0	950
13	0.73	2162.1	237	0	713
14	0.86	2155.5	475	0	475
15	1.05	2144.5	713	0	237

Table S2. Data for pH Titration of 4-Hydroxyproline

Sample	pH	δ (α -proton, Hz)	Volume (μ L) of 0.10 M D_3PO_4	Volume (μ L) of 0.10 M NaOD	Volume (μ L) of 0.10 M DCl
1	1.33	2290.8	950	0	0
2	1.39	2286.5	902	48	0
3	1.47	2280.5	855	95	0
4	1.52	2272.8	807	143	0
5	1.62	2264.0	760	190	0
6	1.71	2254.0	712	238	0
7	1.84	2244.5	665	285	0
8	1.99	2228.0	617	333	0
9	2.17	2214.9	570	380	0
10	2.48	2195.0	522	428	0
11	3.31	2173.1	475	475	0
12	0.60	2325.1	0	0	950
13	0.70	2321.6	237	0	713
14	0.86	2315.0	475	0	475
15	1.02	2305.2	713	0	237

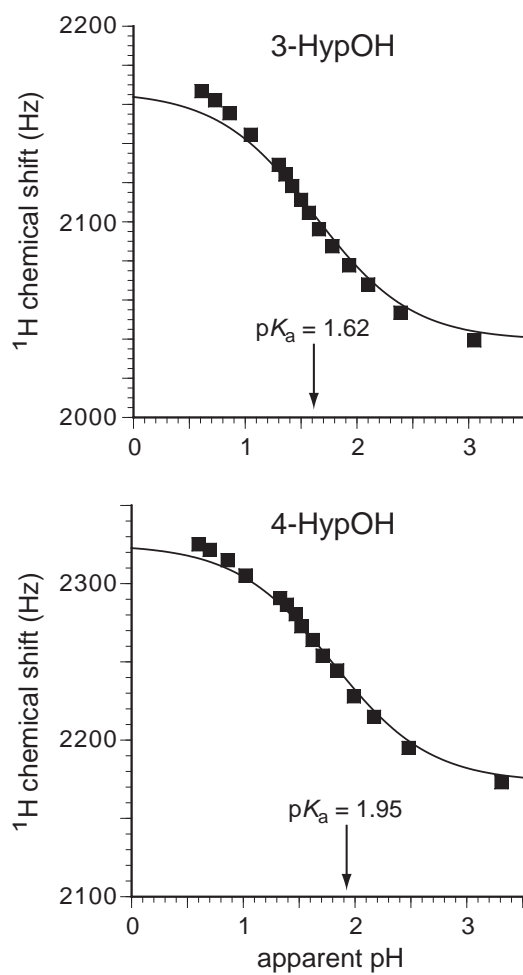


Figure S1. Effect of pH on the ^1H NMR chemical shift of 3-HypOH and 4-HypOH. Data were fitted with eq 1 to give the indicated values of $\text{p}K_a$.

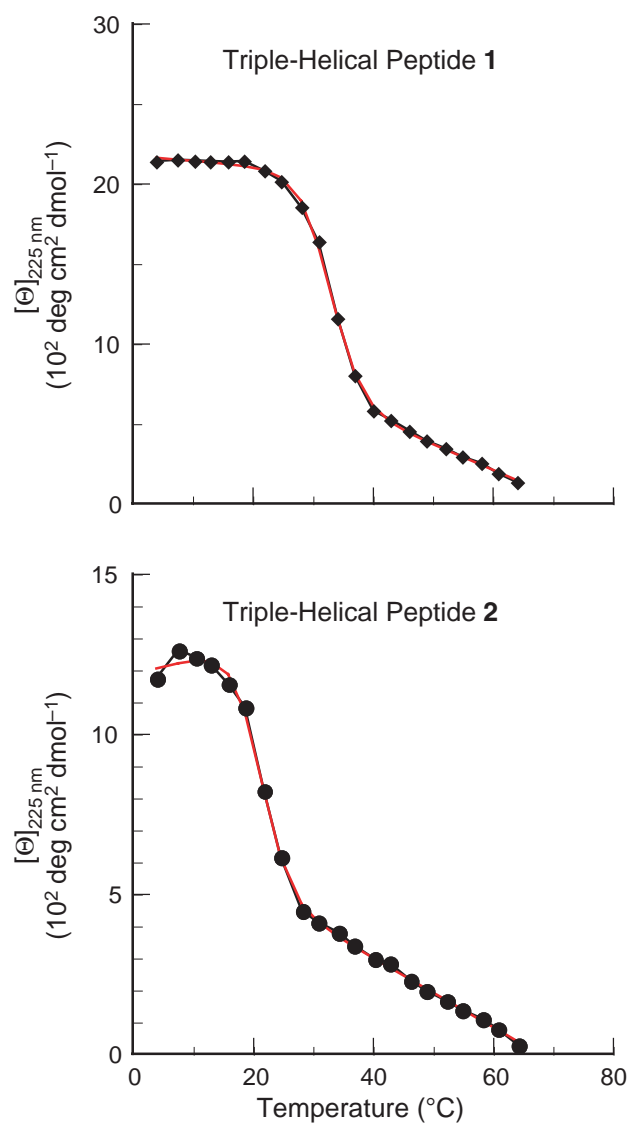


Figure S2. Effect of temperature on the ellipticity at 225 nm of triple helices of peptide 1 and peptide 2. Red lines are derived from the fit of data to a two-state model for denaturation.

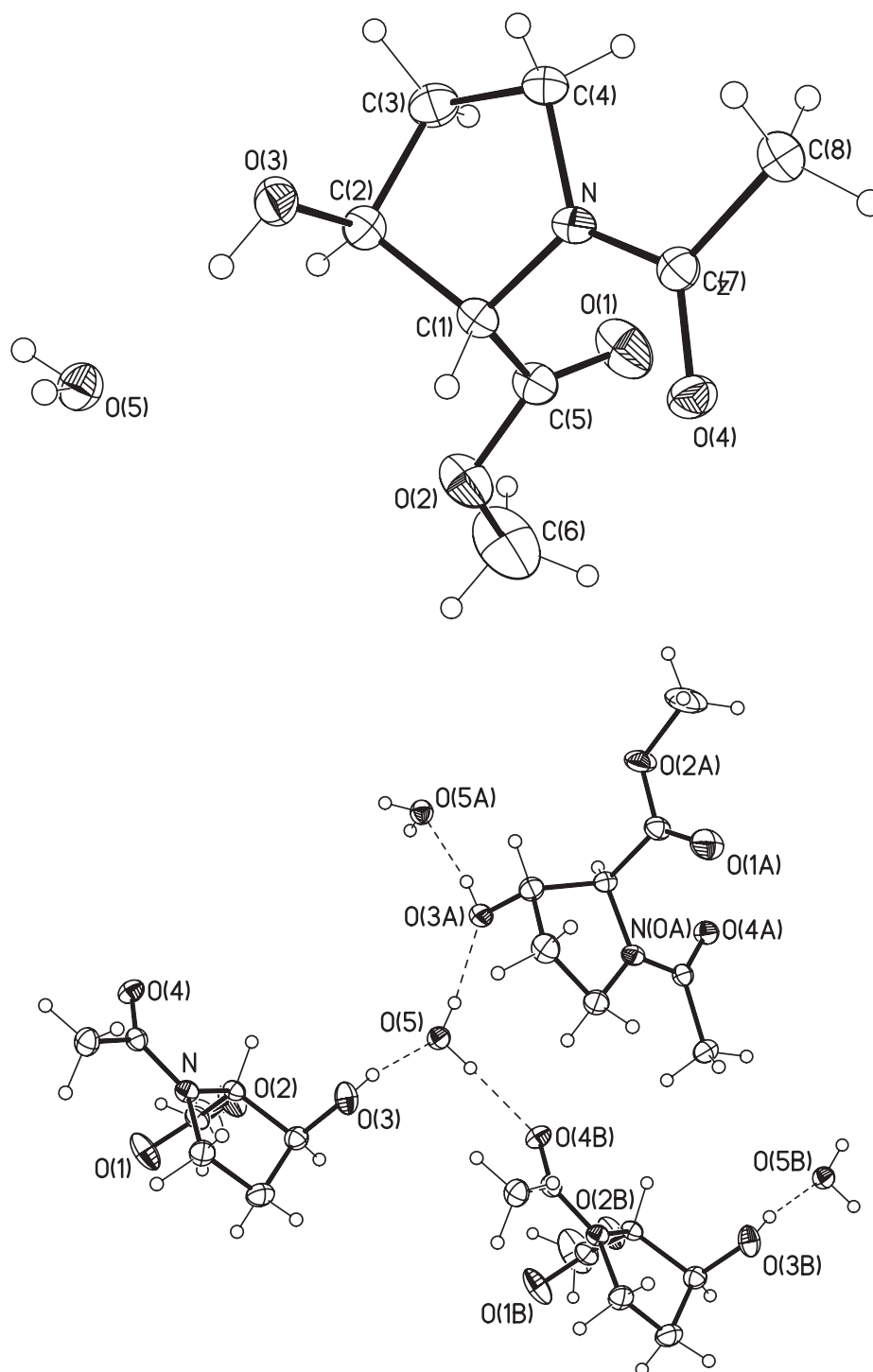


Figure S3. Ortep diagrams of crystalline N-(¹³C₂-acetyl)-3(*S*)-hydroxy-L-proline methyl ester (**8**) drawn with 30% probability ellipsoids and atom designations used in Tables S3–S9. Top, asymmetric unit. Bottom, hydrogen bonding scheme.

Table S3. Crystal Data and Structure Refinement for Amide **8**

Identification code	rai04
Empirical formula	C ₈ H ₁₃ NO ₄ ·H ₂ O
Formula weight	205.21
Temperature	173(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	P2 ₁ 2 ₁ 2 ₁
Unit cell dimensions	$a = 6.1545(6) \text{ \AA}$ $\alpha = 90^\circ$ $b = 8.3050(9) \text{ \AA}$ $\beta = 90^\circ$ $c = 20.807(2) \text{ \AA}$ $\gamma = 90^\circ$
Volume	1063.53(19) Å ³
Z	4
Density (calculated)	1.282 g/cm ³
Absorption coefficient	0.107 mm ⁻¹
F(000)	440
Crystal size	0.50 × 0.50 × 0.41 mm ³
Theta range for data collection	1.96–26.37°
Index ranges	−6 ≤ <i>h</i> ≤ 7, −9 ≤ <i>k</i> ≤ 10, −8 ≤ <i>l</i> ≤ 26
Reflections collected	2911
Independent reflections	1963 [<i>R</i> (int) = 0.0126]
Completeness to theta = 26.37°	96.7%
Absorption correction	Empirical with SADABS
Max. and min. transmission	0.9576 and 0.9486
Refinement method	Full-matrix least-squares on <i>F</i> ²
Data / restraints / parameters	1963 / 2 / 138
Goodness-of-fit on <i>F</i> ²	1.051
Final <i>R</i> indices [<i>I</i> > 2σ(<i>I</i>)]	<i>R</i> 1 = 0.0369, <i>wR</i> 2 = 0.0953
<i>R</i> indices (all data)	<i>R</i> 1 = 0.0401, <i>wR</i> 2 = 0.0975
Absolute structure parameter	N/A – assigned from synthesis
Largest diff. peak and hole	0.172 and −0.146 e.Å ⁻³

Table S4. Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($\text{\AA}^2 \times 10^3$) for Amide **8**

atom	<i>x</i>	<i>y</i>	<i>z</i>	<i>U</i> (eq)
O(1)	929(3)	6789(2)	7647(1)	65(1)
O(2)	3794(3)	5154(2)	7753(1)	52(1)
O(3)	838(2)	3244(2)	9362(1)	41(1)
O(4)	2745(2)	8452(2)	9097(1)	36(1)
O(5)	4355(2)	1427(2)	9393(1)	35(1)
N	146(2)	6601(2)	8963(1)	29(1)
C(1)	1640(3)	5476(2)	8661(1)	27(1)
C(2)	462(3)	3857(2)	8736(1)	34(1)
C(3)	-1899(3)	4333(3)	8677(1)	43(1)
C(4)	-2055(3)	5935(2)	9032(1)	38(1)
C(5)	2043(3)	5915(2)	7961(1)	35(1)
C(6)	4383(6)	5434(5)	7081(1)	92(1)
C(7)	826(3)	8039(2)	9168(1)	29(1)
C(8)	-822(3)	9093(2)	9496(1)	39(1)

$U(\text{eq})$ is defined as one third of the trace of the orthogonalized U^{ij} tensor.

Table S5. Bond Lengths [Å] and Angles [°] for amide **8**

O(1)–C(5)	1.193(2)
O(2)–C(5)	1.322(2)
O(2)–C(6)	1.463(2)
O(3)–C(2)	1.418(2)
O(4)–C(7)	1.239(2)
N–C(7)	1.336(2)
N–C(1)	1.454(2)
N–C(4)	1.470(2)
C(1)–C(5)	1.522(2)
C(1)–C(2)	1.536(2)
C(2)–C(3)	1.512(3)
C(3)–C(4)	1.524(3)
C(7)–C(8)	1.503(2)
C(5)–O(2)–C(6)	116.03(18)
C(7)–N–C(1)	120.97(15)
C(7)–N–C(4)	126.45(15)
C(1)–N–C(4)	112.53(14)
N–C(1)–C(5)	111.31(14)
N–C(1)–C(2)	102.71(14)
C(5)–C(1)–C(2)	112.61(14)
O(3)–C(2)–C(3)	109.02(16)
O(3)–C(2)–C(1)	109.36(14)
C(3)–C(2)–C(1)	102.49(14)
C(2)–C(3)–C(4)	104.44(16)
N–C(4)–C(3)	102.91(15)
O(1)–C(5)–O(2)	125.43(17)
O(1)–C(5)–C(1)	125.17(18)
O(2)–C(5)–C(1)	109.39(15)
O(4)–C(7)–N	120.56(16)
O(4)–C(7)–C(8)	122.43(16)v
N–C(7)–C(8)	117.00(16)

Symmetry transformations were used to generate equivalent atoms.

Table S6. Anisotropic Displacement Parameters ($\text{\AA}^2 \times 10^3$) for Amide **8**

atom	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
O(1)	69(1)	87(1)	37(1)	24(1)	3(1)	32(1)
O(2)	58(1)	71(1)	28(1)	8(1)	13(1)	24(1)
O(3)	48(1)	39(1)	37(1)	9(1)	13(1)	11(1)
O(4)	31(1)	34(1)	43(1)	-6(1)	3(1)	-7(1)
O(5)	38(1)	33(1)	34(1)	0(1)	-2(1)	-2(1)
N	23(1)	34(1)	29(1)	0(1)	2(1)	0(1)
C(1)	26(1)	32(1)	23(1)	-2(1)	-2(1)	3(1)
C(2)	35(1)	33(1)	32(1)	-3(1)	0(1)	0(1)
C(3)	33(1)	44(1)	53(1)	-2(1)	-4(1)	-8(1)
C(4)	25(1)	37(1)	52(1)	3(1)	3(1)	-1(1)
C(5)	34(1)	44(1)	28(1)	3(1)	1(1)	4(1)
C(6)	116(3)	127(3)	34(1)	15(1)	33(2)	51(2)
C(7)	31(1)	30(1)	25(1)	3(1)	0(1)	1(1)
C(8)	38(1)	34(1)	43(1)	-3(1)	4(1)	5(1)

The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2 a^{*2}U^{11} + \dots + 2 h k a^* b^* U^{12}]$.

Table S7. Hydrogen Coordinates ($\times 10^4$) and Isotropic Displacement Parameters ($\text{\AA}^2 \times 10^3$) for Amide **8**

atom	x	y	z	$U(\text{eq})$
H(3)	2046	2774	9372	62
H(5A)	4790(40)	1450(30)	9776(4)	59(7)
H(5B)	3860(30)	505(11)	9315(10)	40(6)
H(1)	3046	5455	8901	32
H(2)	900	3071	8396	40
H(3A)	-2853	3516	8878	52
H(3B)	-2318	4459	8220	52
H(4A)	-3150	6649	8831	45
H(4B)	-2434	5773	9489	45
H(6A)	4680	6582	7016	138
H(6B)	3180	5102	6802	138
H(6C)	5683	4807	6975	138
H(8A)	-217	10174	9554	58
H(8B)	-1188	8634	9916	58
H(8C)	-2136	9156	9231	58

Table S8. Torsion Angles [°] for Amide **8**

C(7)–N–C(1)–C(5)	–79.46(19)
C(4)–N–C(1)–C(5)	103.01(17)
C(7)–N–C(1)–C(2)	159.82(14)
C(4)–N–C(1)–C(2)	–17.72(17)
N–C(1)–C(2)–O(3)	–81.46(16)
C(5)–C(1)–C(2)–O(3)	158.71(15)
N–C(1)–C(2)–C(3)	34.10(17)
C(5)–C(1)–C(2)–C(3)	–85.72(18)
O(3)–C(2)–C(3)–C(4)	77.24(18)
C(1)–C(2)–C(3)–C(4)	–38.58(18)
C(7)–N–C(4)–C(3)	176.70(16)
C(1)–N–C(4)–C(3)	–5.92(19)
C(2)–C(3)–C(4)–N	27.71(19)
C(6)–O(2)–C(5)–O(1)	0.3(4)
C(6)–O(2)–C(5)–C(1)	179.1(2)
N–C(1)–C(5)–O(1)	–17.4(3)
C(2)–C(1)–C(5)–O(1)	97.3(2)
N–C(1)–C(5)–O(2)	163.74(16)
C(2)–C(1)–C(5)–O(2)	–81.54(19)
C(1)–N–C(7)–O(4)	0.9(2)
C(4)–N–C(7)–O(4)	178.12(16)
C(1)–N–C(7)–C(8)	–177.85(15)
C(4)–N–C(7)–C(8)	–0.7(2)

Symmetry transformations used to generate equivalent atoms: #1 $x + 1/2, -y + 1/2, -z + 2$; #2 $x, y - 1, z$

Table S9. Hydrogen Bonds for Amide **8** (Å and °)

D–H...A	d(D–H)	d(H...A)	d(D...A)	∠(D–H–A)
O(3)–H(3)...O(5)	0.84	1.81	2.6391(19)	170(1)
O(5)–H(5A)...O(3) #1	0.8400(11)	1.922(4)	2.7585(19)	174(2)
O(5)–H(5B)...O(4) #2	0.8401(11)	1.893(2)	2.7326(19)	177(2)

Symmetry transformations used to generate equivalent atoms: #1 $x + 1/2, -y + 1/2, -z + 2$; #2 $x, y - 1, z$