Supporting Information

^{1}H, ^{13}C, ^{15}N, and ^{19}F Random Coil NMR Shifts of Trifluoromethyl-Bearing Amino Acids

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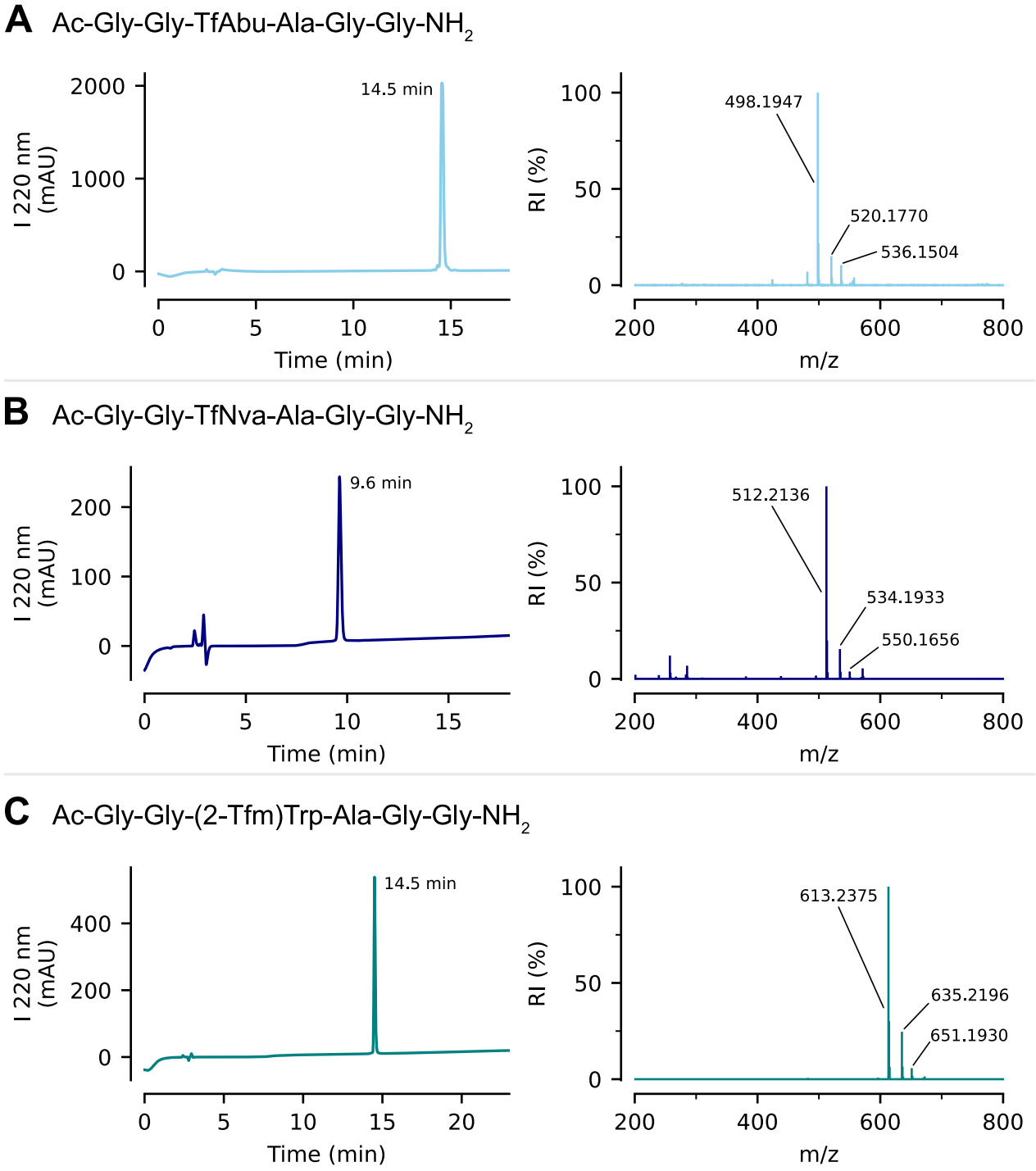

Figure S1. HPLC chromatograms and ESI-TOF MS spectra of purified random coil peptides. HPLC chromatograms were recorded on a Primaide reversed-phase analytical HPLC system (Hitachi, Ltd., Japan) equipped with a Kinetex C-18 column (250 × 21.2 mm; Phenomenex, Inc., USA). Eluent A: water containing 0.1% v/v TFA; eluent B: acetonitrile containing 0.1% v/v TFA; flow rate: 1.00 mL min^{-1}. MS Signals $(M+H)^+$, $[M+Na]^+$, and $[M+K]^+$) corresponding to target peptides are labeled in the spectra. **A** HPLC gradient: 2%B (3 min), then 2–20%B (15 min). *m/z* calcd for $[M+H]^+$ $C_{17}H_{27}F_3N_7O_7$ 498.1919, found 498.1947. **B** HPLC gradient: 5%B (3 min), then 5–35%B (15 min). *m/z* calcd for $[M+H]^+$ $C_{18}H_{29}F_3N_7O_7$ 512.2075, found 512.2136. **C** HPLC gradient: 10%B (3 min), then 10–45%B (20 min). *m/z* calcd for $[M+H]^+$ $C_{25}H_{32}F_3N_8O_7$ 613.2341, found 613.2375.

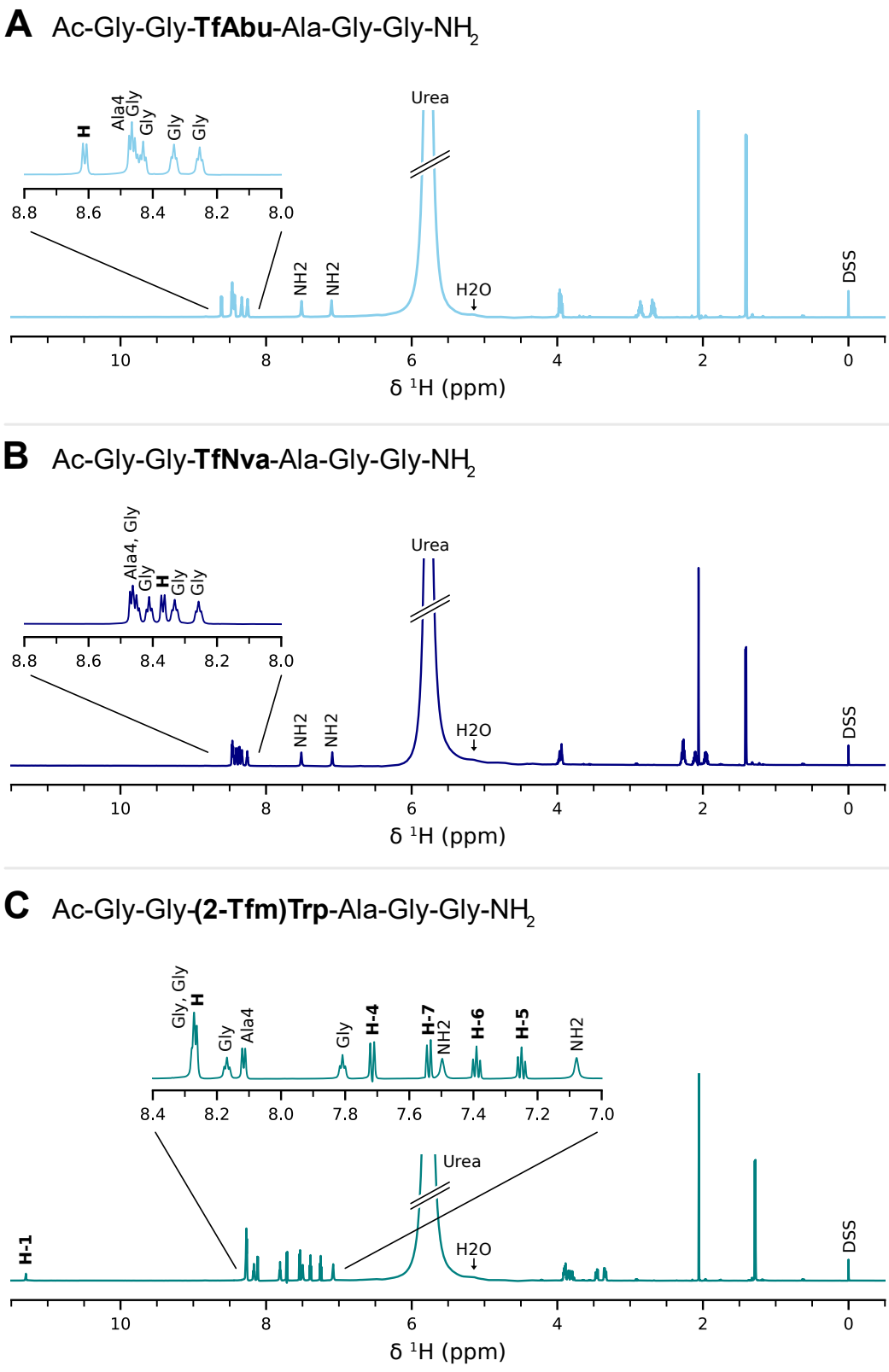

Figure S2. ^{1}H NMR spectra of random coil peptides in 10% D_2O/90% H_2O at 298 K, directly referenced to 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS). Amide and aromatic signals are labeled. Signals corresponding to the non-natural amino acids TfAbu (**A**), TfNva (**B**), and (2-Tfm)Trp (**C**) are shown in bold text.

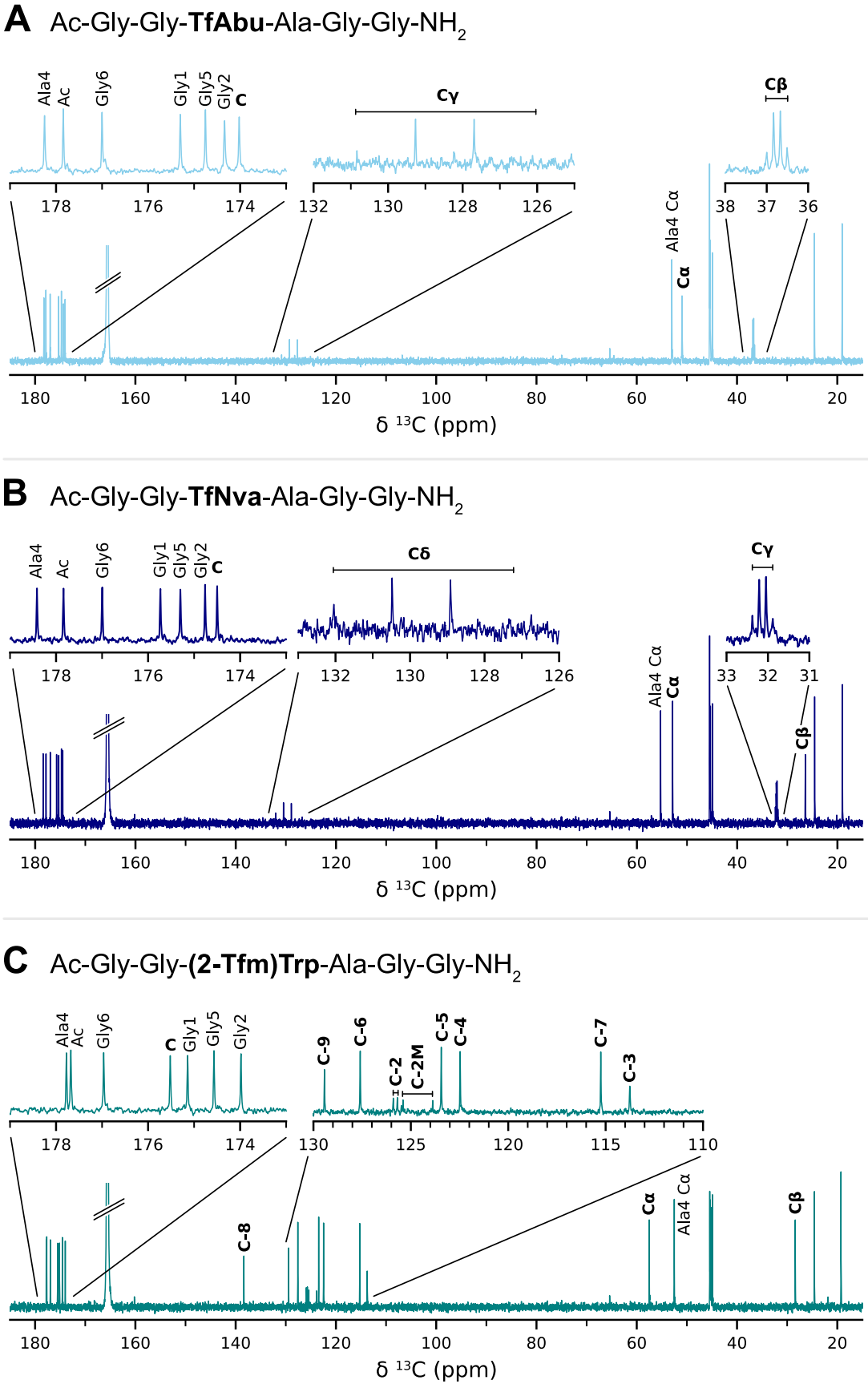

Figure S3. ^{13}C NMR spectra of random coil peptides in 10% D_2O/90% H_2O at 298 K. Carbon spectra were recorded with 30,000 (**A**), 42,000 (**B**), or 60,000 transients (**C**). Carbonyl C signals, and signals exhibiting carbon–fluorine splitting are labeled. Signals corresponding to the non-natural amino acids TfAbu (**A**), TfNva (**B**), and (2-Tfm)Trp (**C**) are shown in bold text.

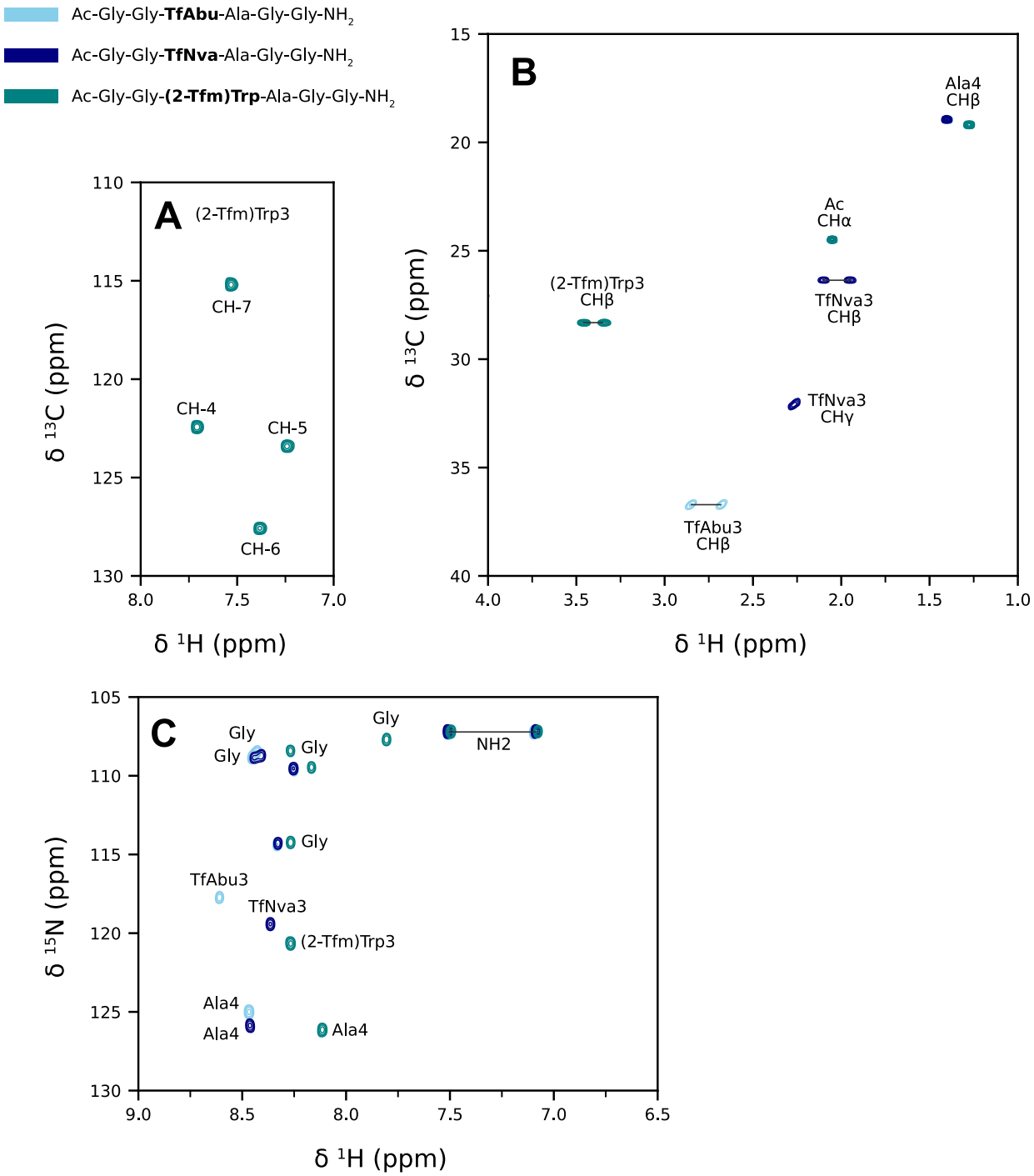

Figure S4. Overlayed 2D NMR spectra of random coil peptides. **A** ^{1}H-^{13}C HSQC aromatic region. **B** ^{1}H-^{13}C HSQC aliphatic region. **C** ^{1}H-^{15}N HSQC amide region.

Table S1. Chemical shift deviations (Δδ (ppm)) between trifluoromethyl-bearing (2-Tfm)Trp and native Trp. Δδ were calculated by subtracting the random coil values reported for Trp[1] for each atom, respectively.

Atom	δ (2-Tfm) Trp	δ Trp[a,[1]]	Δδ
H	8.27	8.25	+0.02
C	175.5	176.1	−0.6
N	120.7	121.3	−0.6
Hα	4.64	4.66	−0.02
Cα	57.5	57.5	0.0
Hβ2	3.46	3.29	+0.17
Hβ3	3.35	3.27	+0.08
Cβ	28.4	29.6	−1.2
H-1 (Hε1)	11.30	10.10[b]	+1.20
N-1 (Nε1)	127.7	— [b]	—
H-2 (Hδ1)	—	7.27	—
C-2 (Cδ1)	125.8	127.4	−1.6
C-3 (Cγ)	113.8	111.2	+2.6
H-4 (Hε3)	7.72	7.65	+0.07
C-4 (Cε3)	122.5	122.2	+0.3
H-5 (Hζ3)	7.25	7.18	+0.07
C-5 (Cζ3)	123.4	121.0[c]	+2.4
H-6 (Hη2)	7.39	7.25	+0.14
C-6 (Cη2)	127.6	124.8[c]	+2.8
H-7 (Hζ2)	7.54	7.50	+0.04
C-7 (Cζ2)	115.2	114.7	+0.5
C-8 (Cε2)	138.4	138.7	−0.3
C-9 (Cδ2)	129.5	129.5	0.0

[a] measured using a peptide with free N- and C-termini (GGWAGG)

[b] values for H-1 and N-1 were not reported in Wishart *et al.* 1995; the shift of H-1 was taken from denatured lysozyme

[c] values for C-5 and C-6 were swapped in Wishart *et al.* 1995[2]

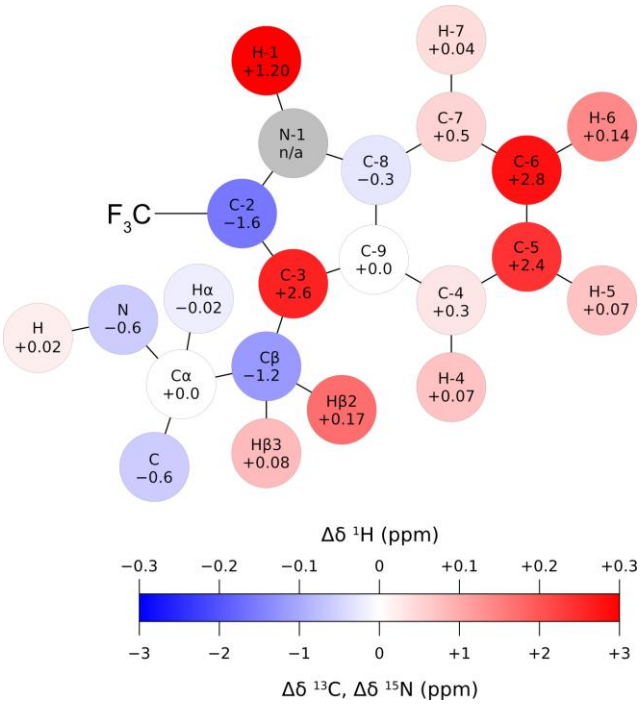

Figure S5. Visual representation of the chemical shift deviations (Δδ, Table S1) between (2-Tfm)Trp and native Trp.

Table S2. Chemical shifts observed for Ala4 when preceded by TfAbu, TfNva, or (2-Tfm)Trp. Below are the values observed for Ala4 when preceded by Ala or Trp, and the values for Ala3 as reported by Wishart *et al.*[1]

Peptide	H	C	N	Hα	Cα	Hβ	Cβ
Ac-GG(TfAbu)**A**GG-NH_2	8.47	178.2	125.0	4.35	53.0	1.41	19.0
Ac-GG(TfNva)**A**GG-NH_2	8.47	178.4	125.9	4.34	52.9	1.41	19.0
Ac-GG((2-Tfm)Trp)**A**GG-NH_2	8.12	177.7	126.1	4.22	52.5	1.29	19.3
Ac-GGA**A**GG-NH_2[1]	8.34	178.5	123.2	4.33	52.6	—	—
GGW**A**GG[1]	8.26	179.0	126.8	4.23	52.3	—	—
Ac-GG**A**AGG-NH_2[1]	8.24	177.8	123.8	4.32	52.5	1.39	19.1

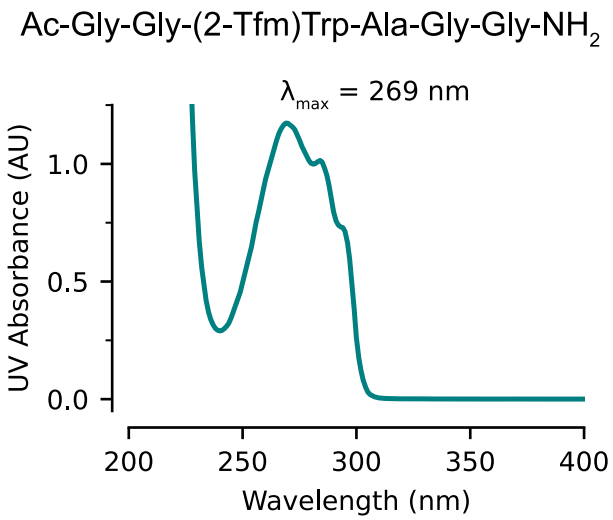

Figure S6. UV absorbance spectrum of Ac-Gly-Gly-(2-Tfm)Trp-Ala-Gly-Gly-NH_2 (0.18 mM) in water.

References

[1] D.S. Wishart, C.G. Bigam, A. Holm, R.S. Hodges, B.D. Sykes, ^{1}H, ^{13}C and ^{15}N random coil NMR chemical shifts of the common amino acids. I. Investigations of nearest-neighbor effects, *J. Biomol. NMR*, *5* (**1995**), pp. 67–81, 10.1007/BF00227471.

[2] A. Hinterholzer, V. Stanojlovic, C. Regl, C.G. Huber, C. Cabrele, M. Schubert. Identification and Quantification of Oxidation Products in Full-Length Biotherapeutic Antibodies by NMR Spectroscopy, *Anal. Chem.*, *92* (**2020**), pp. 9666–9673, 10.1021/acs.analchem.0c00965.