Supplementary Material: Conformation-specific spectroscopy of capped Glutamine-containing peptides: Role of a single glutamine residue on peptide backbone preferences

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1. Relaxed Potential Energy Scan-Hindered OH Rotation

A relaxed potential energy scan was performed on a simple model glycine molecule to better understand the barrier associated with the hindered interal rotation of the carboxylic acid OH. The results shown in figure S1 reveal two minima which are associated with a *cis*- and *trans*-OH rotamers separated by a ~55 kJ/mol barrier.

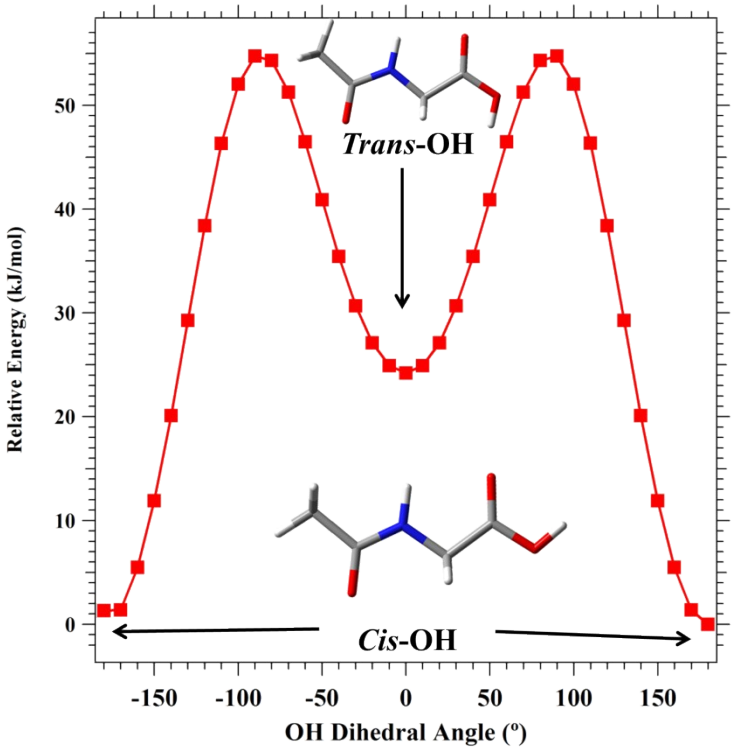


Figure S1: Relaxed potential energy scan of the hindered internal rotation of a carboxylic acid OH in a simply glycine residue showing the barrier height and relative energies of the *cis*- and *trans*-OH rotamers.

2. Table of Experimental and Calculated Vibrational Frequencies

Table S1: Experimental and Theoretical Vibrational Frequencies for the conformers of Z-Gln-OH, Z-Gln-NHMe, Ac-Gln-NHBn, and Ac-Ala-Gln-NHBn.

Structure Name	Hydrogen Bond Motif	OH Stretch		NH Stretch Frequency (cm^{-1})						C=O Stretch/Amide I Frequency (cm^{-1})		NH- and OH-Bend/Amide II Frequency (cm^{-1})					
				Asym. NH_2		Sym. NH_2		NH Stretch		C=O Stretch		NH Bend		NH_2 Bend		OH Bend	
		Exp.	Theory	Exp.	Theory	Exp.	Theory	Exp.	Theory	Exp.	Theory	Exp.	Theory	Exp.	Theory	Exp.	Theory
Z-Gln-NHMe																	
Conf.A	C5//<u>*C8*</u>			3560	3557	3442	3437	3402	3404	1740	1730	1584	1576	1605	1598		
								3351	3364	1721	1716	1510	1515				
										1709	1705						
Z-Gln-OH																	
Conf. A	C5//<u>*C8*</u> (*trans*-OH)	3142	3230	3562	3562	3443	3442	3430	3432	1801	1806	1513	1518	1613	1599	1419	1404
										1739	1725						
										1705	1700						
Conf. B	C5//<u>*C9*</u> (*cis*-OH)	3577	3578	3507/3517	3521	3371	3383	3446	3444	1792	1795	1533	1537	1614	1613		
										1741	1739						
										1731	1720						
Conf.C	C5//<u>*Stacked*</u> (*cis*-OH)	3577	3578	3524	3523	3412	3412	3458	3450	1796	1800	1532	1534	1609	1602		
										1750	1743						
										1737/ 1733	1724						
Ac-Gln-NHBn																	
Conf. A	C5//<u>*C8*</u>			3562	3563	3443	3444	3402	3419	1720	1719	1577	1573	1603	1598		
								3310	3339	1703	1699	1509	1523				
										1695	1693						
Conf B	<u>*C7/π*</u>			3529	3535	3415	3416	3457	3468	1725	1728		1527		1595		
								3354	3376	1713	1708/1706	1521	1518				
Conf. C	C7//<u>*C7/C8/π*</u>			3509	3513	3400	3401	3343	3361	1706	1705	1558	1556/ 1552		1599		
								3270	3329	1682	1692/1680						
Ac-Ala-Gln-NHBn																	
Conf. A	C10//<u>*C7/π*</u>			3520	3526	3408	3411	3470	3471	1718	1722	1546	1554	1601	1599		
								3361/ 3357	3380/ 3378	1706	1704	1531	1528				
										1694	1695	1508	1508				
										1681	1691						

3. Comparison of Energies of Ac-Gln-NHBn and Ac-Gln-NHMe

This table S2 summarizes the results of the calculated energies of selected conformational families with the –NHBn cap and with the –NHMe cap. This table shows the effect the aromatic cap plays on structural stabilization.

Table S2: Energies of Ac-Gln-NHBn under different capping schemes.

Hydrogen Bonding Pattern	Relative Energy (kJ/mol)	
	NHBn Cap	NHMe Cap
C7//*C7/C8*	0.57	5.88
Conf. C		
C7//*C7*	8.88	7.93
C5//*C9/C8*	6.25	3.23
C5//*C9/C8*	2.13	3.23
C5//*C9/C8*	2.76	1.27
C7//*C7/C8*	9.31	5.89
C5//*C9/C8*	3.99	1.26
C7/π → C7//*C7*	0.00	5.15
Conf. B		
C7//*C7*	7.32	5.15
C5//*C9/C8*	2.75	1.27
C7/π → *C7*	7.47	12.99
C5//*C8*	1.54	0.01
Conf. A		
C5//*C8*	3.32	0.00

4. Comparison of Energies of Ac-Ala-Gln-NHBn and Ac-Ala-Gln-NHMe

This table S3 summarizes the results of the calculated energies of selected conformational families with the –NHBn cap and with the –NHMe cap. This table shows the effect the aromatic cap plays on structural stabilization.

Table S3: Energies of Ac-Ala-Gln-NHBn under different capping schemes

Hydrogen Bonding Pattern	Relative Energy (kJ/mol)	
	NHBn Cap	NHMe Cap
C5/C5//*C8*	28.66	11.94
C7//*C7/C8/C12*	10.42	0.89
C5//*C12*	25.07	10.27
C5/C5//*C8*	28.73	7.66
C5/C7//*C7/C8*	28.43	16.08
C5//*C8/C12*	24.13	6.33
C7//*C12/C8*	20.13	10.10
C7//*C7/C8/C12*	10.91	0.88
C10//*C7* , Assigned Conformer	0.00	0.00
C5/C5//*Stacked*	43.19	23.23
C10//*C7/C8*	4.67	1.50

5. Additional Calculated Z-Gln-OH Structures

The Z-Gln-OH structures shown in figure S2 are calculated structures that were not experimentally assigned. These structures are either calculated low energy conformers, or are structures that were experimentally identified in the other glutamine containing molecules studied in this report.

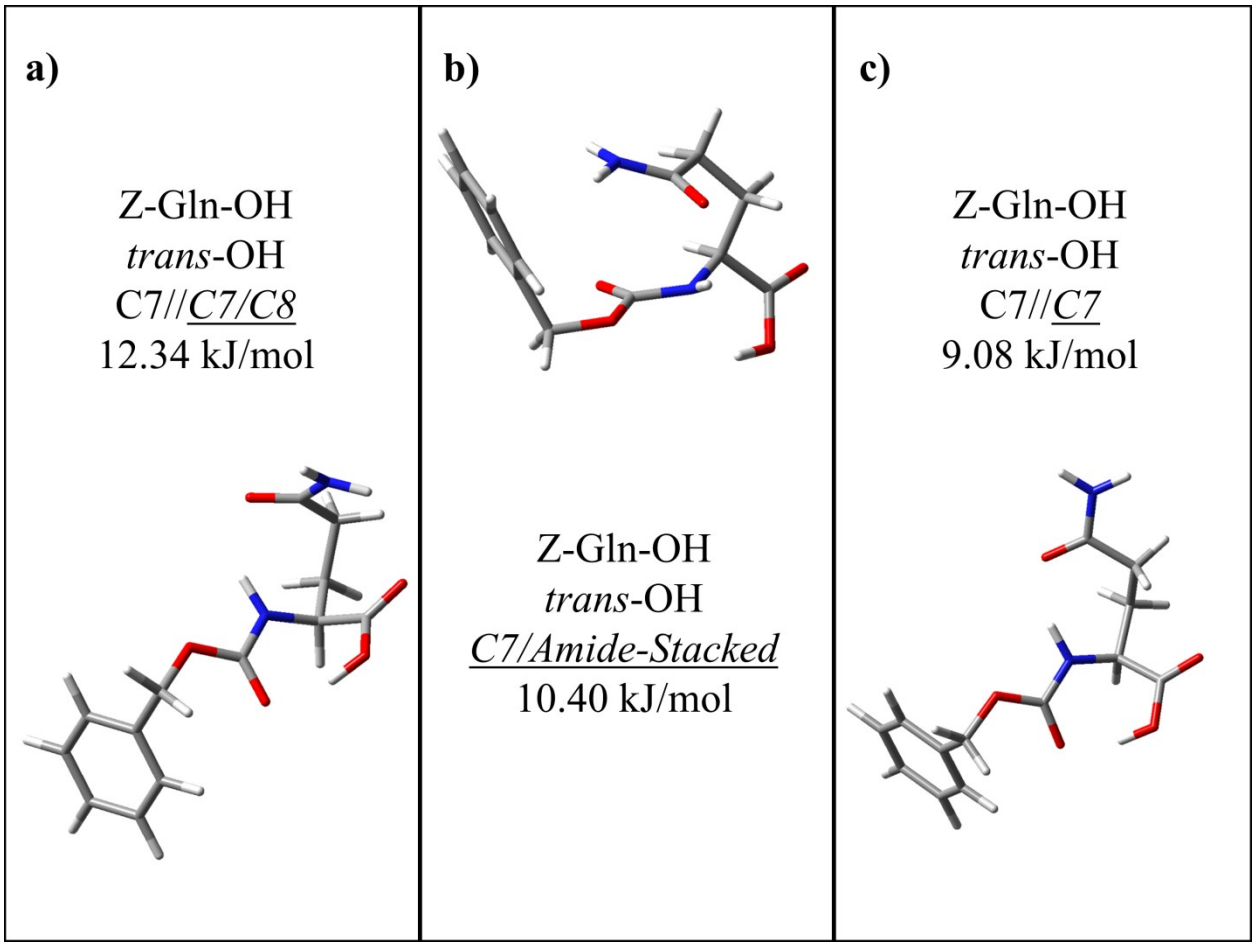


Figure S2: Low-energy and other structures of Z-Gln-OH which were not experimentally assigned: (a) a C7//*C7/C8* structure, (b) a *C7/amide-stacked* structure, and (c) a C7//*C7* structure.

6. Additional Calculated Z-Gln-NHMe Structures

The Z-Gln-NHMe structures shown in figure S3 are calculated structures that were not experimentally assigned. These structures are either calculated low energy conformers, or are structures that were experimentally identified in the other glutamine containing molecules studied in this report.

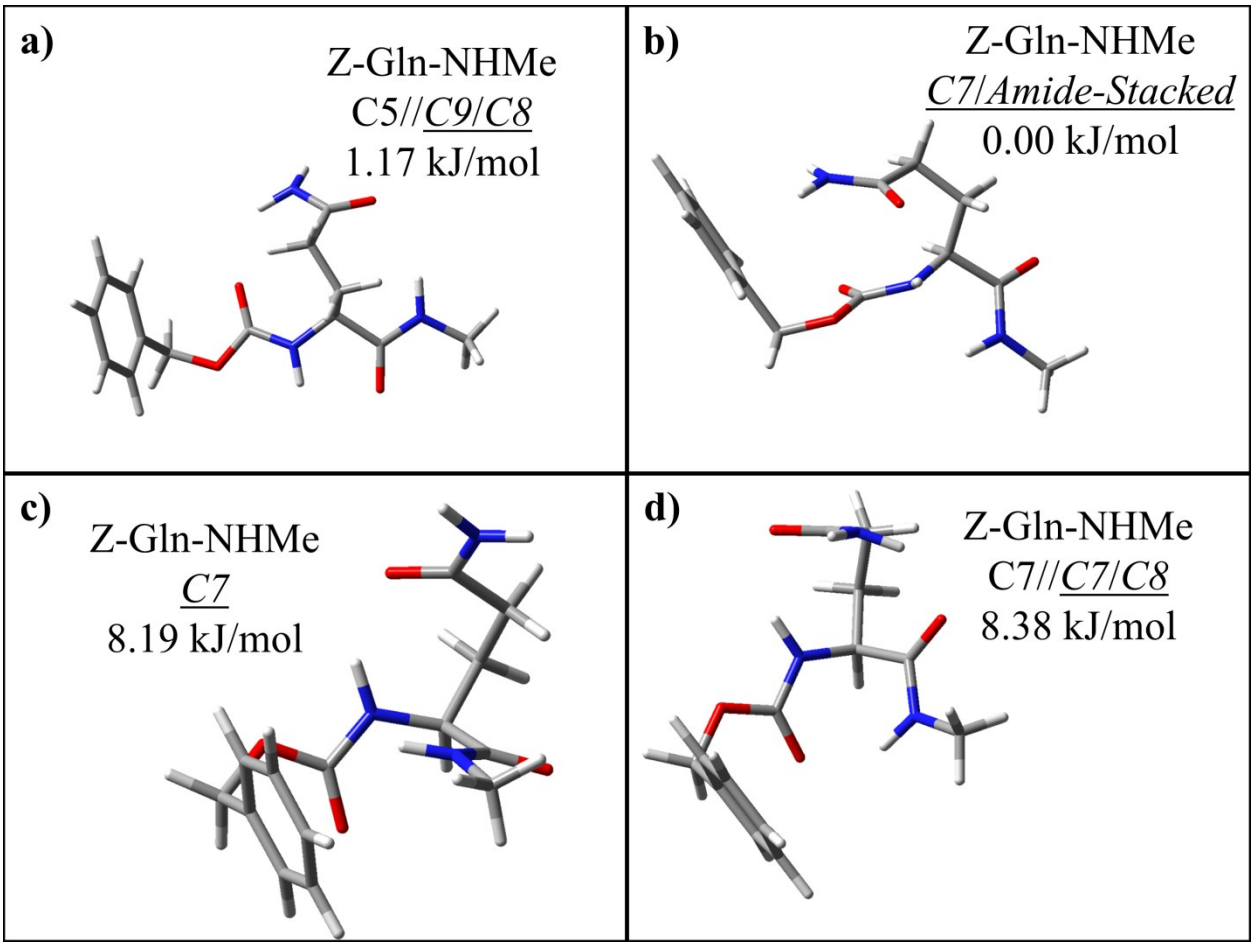


Figure S3: Low-energy and other structures of Z-Gln-NHMe which were not experimentally assigned: (a) a C5//*C9*/*C8* double bridge structure, (b) a *C7*/*amide-stacked* structure, (c) a *C7* structure, and (d) a C7//*C7*/*C8* structure.

7. Additional Calculated Ac-Gln-NHBn Structures

The Ac-Gln-NHBn structures shown in figure S4 are calculated structures that were not experimentally assigned. These structures are either calculated low energy conformers, or are structures that were experimentally identified in the other glutamine containing molecules studied in this report.

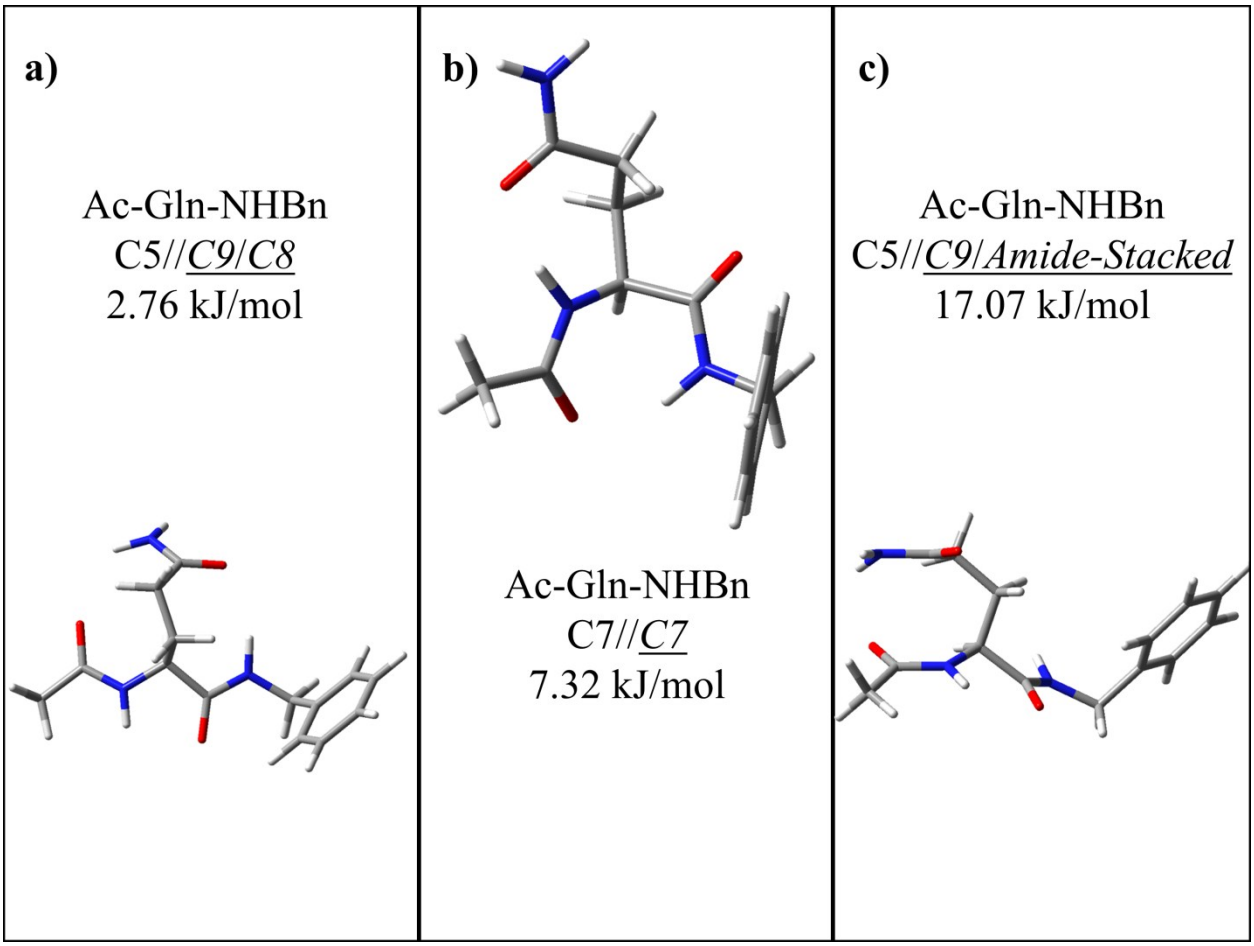


Figure S4: Low-energy and other structures of Ac-Gln-NHBn which were not experimentally assigned: (a) a C5//*C9/C8* structure, (b) a C7//*C7* structure, and (c) a C5//*C9/amide-stacked* structure.

8. Additional Calculated Ac-Ala-Gln-NHBn Structures

The Ac-Ala-Gln-NHBn structures shown in figure S5 are calculated structures that were not experimentally assigned. These structures are either calculated low energy conformers, or are structures that were experimentally identified in the other glutamine containing molecules studied in this report.

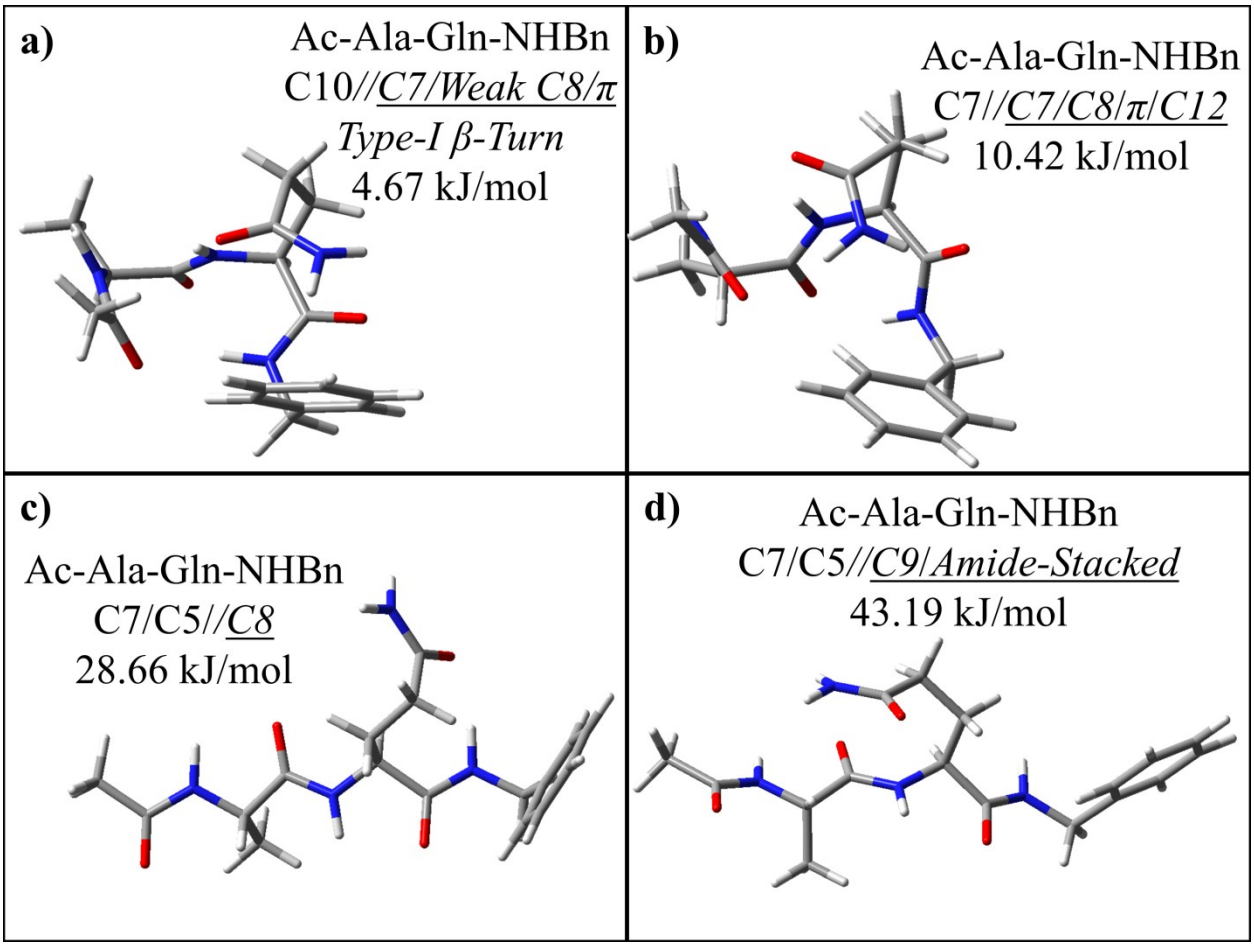


Figure S5: Low-energy and other structures of Ac-Ala-Gln-NHBn which were not experimentally assigned: (a) a C10//*C7/ Weak C8/π* type-I β-turn, (b) a C7//*C7/C8/π/C12* structure, (c) a C5/C5//*C8* structure, and (d) a C5/C5//*Amide-Stacked* structure.

9. Comparison of *C7/π* Strcutures in Ac-Gln-NHBn and Ac-Ala-Gln-NHBn

Figure S6 pictorially demonstrates the similarities in the structure adopted by the glutamine residue in conformer B Ac-Gln-NHBn and conformer A Ac-Ala-Gln-NHBn. The structure adopted by glutamine in both conformers is a *C7/π* conformation with a single, strong hydrogen bond between the sidechain and backbone. This is a a nice example of the notion of structural inheritance where by the inherent structural preferences of the single glutamine is carried over into larger glutamine containing molecules.

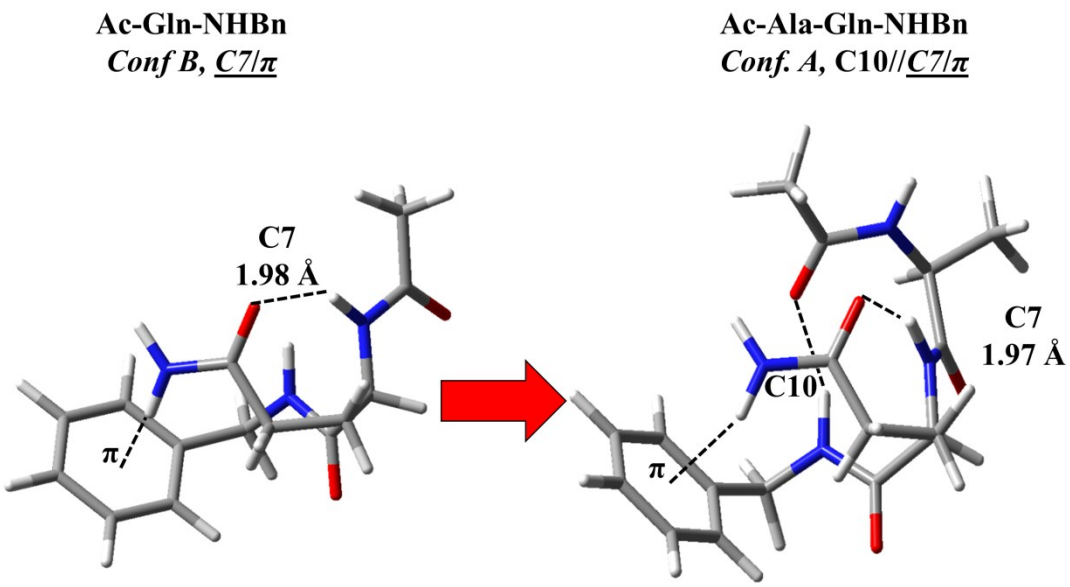
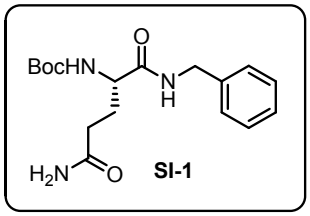


Figure S6: Comparison of the *C7/π* structure of glutamine which is found in both Ac-Gln-NHBn conformer B and Ac-Ala-Gln-NHBn conformer A

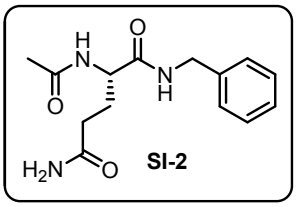
10.Synthetic Procedure for Ac-Gln-NHBn and Ac-Ala-Gln-NHBn

Boc-Gln-NHBn (SI-1):



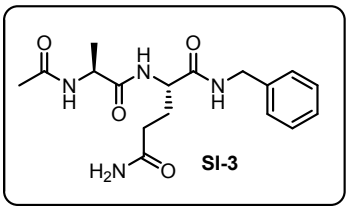
Compound **SI-1** was synthesized by adding benzylamine (1 eq., 2.217 mL, 20.30 mmol) to a stirred solution of Boc-L-glutamine (1 eq., 5.000 g, 20.30 mmol), 2,4,6-collidine (2 eq., 5.383 mL, 40.60 mmol), EDCI (1 eq., 3.892 g, 20.30 mmol), and HOAt (1 eq., 20.30 mmol) in 46 mL DMF at 0 °C. The reaction was warmed to room temperature while stirring over 17 hours. The reaction was diluted with 400 mL 1M aqueous $NaHSO_4$ and was extracted with 150 mL EtOAc three times. The EtOAc layers were combined and successively washed with 200 mL saturated aqueous $NaHCO_3$, 200 mL 5% aqueous LiCl, and 200 mL brine. The EtOAc layer was dried over $MgSO_4$, filtered, and solvent was removed *in vacuo*. The resulting white solid was purified via silica column chromatography eluting with 4% MeOH v/v and 0.2% AcOH v/v in DCM to yield 4.50g of **SI-1** as a white powder (13.4 mmol, 66% yield). **^{1}H NMR** (Methanol-d_4, 400 MHz): δ = 7.36 – 7.19 (m, 5H), 4.49 – 4.29 (m, 2H), 4.06 (dd, 1H, *J*=8.9, 4.6 Hz), 2.30 (t, 2H, *J*=7.7 Hz), 2.11 – 1.98 (m, 1H), 1.93 – 1.80 (m, 1H), 1.44 (s, 9H). **HRMS** *m/z* (ESI): calc. for $C_{17}H_{25}N_3O_4$ $[M + H]^+$ 336.1918 found 336.1909.

Ac-Gln-NHBn (SI-2):



Compound **SI-1** (1 eq., 1.000 g, 2.981 mmol) was Boc-deprotected with 6 mL 6M HCl in dioxane. The reaction mixture was stirred at room temperature for 1 hour followed by evaporation of dioxane under N_2 for 2 hours. To the resulting white solid was added 12 mL DMF and DIEA (3 eq., 1.558 mL, 8.943 mmol) followed by acetic anhydride (2 eq., 0.564 mL, 5.962 mmol). The reaction mixture was stirred at room temperature for 5 hours. The resulting solid reaction mixture was dried under N_2 and dissolved in 10% MeOH v/v in DCM. The solution was loaded onto a silica column and eluted with 10% MeOH v/v in DCM to yield 159.21 mg of **SI-2** as a white solid (0.574 mmol, 19% yield). **TLC** R_f = 0.23 (10% MeOH v/v in DCM). **^{1}H NMR** (Methanol-d_4, 400 MHz): δ = 7.36 – 7.21 (m, 5H), 4.46 – 4.34 (m, 3H), 2.31 (t, 2H, *J*=7.7 Hz), 2.18 – 2.06 (m, 1H), 2.02 (s, 3H), 1.99 – 1.87 (m, 1H).

Ac-Ala-Gln-NHBn (SI-3):



Compound **SI-1** (1 eq., 1.000 g, 2.981 mmol) was Boc-deprotected with 6 mL 6M HCl in dioxane. The reaction mixture was stirred at room temperature for 1 hour followed by evaporation of dioxane under N_2 for 2 hours. To the resulting white solid was added 6 mL DMF, DIEA (3 eq., 1.558 mL, 8.943 mmol), N-acetyl-L-alanine (1 eq., 0.3908 g, 2.981 mmol), and HOAt (1.2 eq., 5.96 mL 0.6 M in DMF, 3.577 mmol) followed by EDCI (1.2 eq., 0.686 g, 3.577 mmol). The reaction mixture was stirred at room temperature for 20 hours. The cloudy reaction mixture was diluted with 50 mL of Et_2O and filtered to yield a white solid. The solid was sequentially triturated three times with MeOH and once with 15 mL ice-cold saturated aqueous $NaHCO_3$ to yield 175.44 mg of **SI-3** as a white powder (0.504 mmol, 17% yield). **^{1}H NMR** (Deuterium Oxide, 500 MHz): δ = 7.41 (t, 2H, *J*=7.6 Hz), 7.34 (t, 1H, *J*=7.6 Hz), 7.31 (d, 2H, *J*=7.7 Hz), 4.41 (s, 2H), 4.33 (dd, 1H, *J*=9.2, 5.3 Hz), 4.25 (q, 1H, *J*=7.2 Hz), 2.43 – 2.30 (m, 2H), 2.18 – 2.09 (m, 1H), 2.06 – 1.96 (m, 4H), 1.35 (d, 3H, *J*=7.2 Hz). **HRMS** *m/z* (ESI): calc. for $C_{17}H_{24}N_4O_4$ $[M + H]^+$ 349.1871 found 349.1877.