Supporting Information for

Structural analyses of isolated cyclic tetrapeptides with varying amino acid residues

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f) NMR characterization of **CP$_{Glu}$**

g) Mass spectrum of **CP$_{Glu}$**

cyclo[L-Tyr(Me)-D-Pro-L-Ala-D-Pro] (CP_{Ala})

a) R2PI spectrum of **CP_{Ala}**

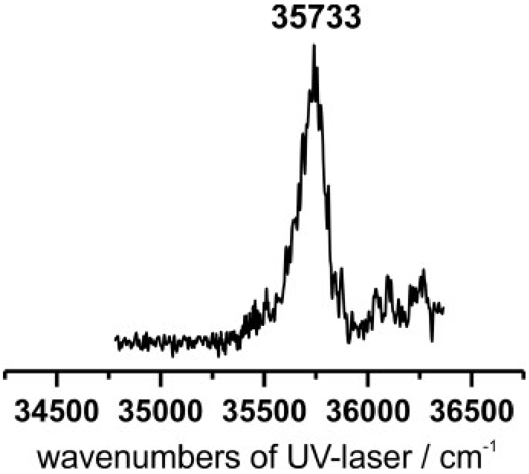


Figure S1. One-color R2PI spectrum of **CP_{Ala}** recorded in the region of 34783-36364 cm^{-1}.

b) Possible doubly hydrogen-bonded structures and calculated IR spectra of **CP$_{Ala}$** (B3LYP-D3/TZVP)

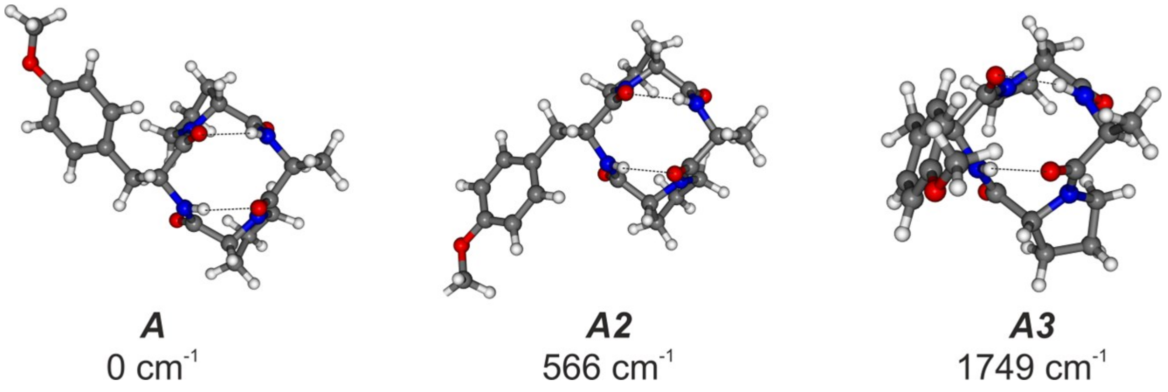


Figure S2. Possible structures of **CP$_{Ala}$** with a doubly hydrogen-bonded binding motif (***A*** type) based on DFT calculations (B3LYP-D3/TZVP). (Hydrogen bond lengths of these structures (given in Å): ***A*** (1.991 and 2.010), ***A2*** (2.00 and 1.991) and ***A3*** (2.009 and 2.031).)

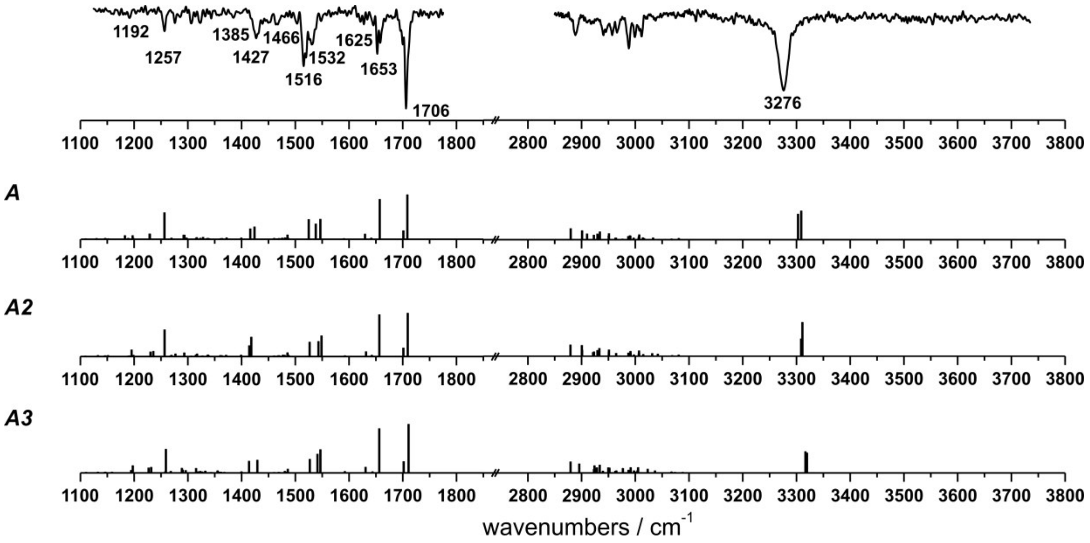
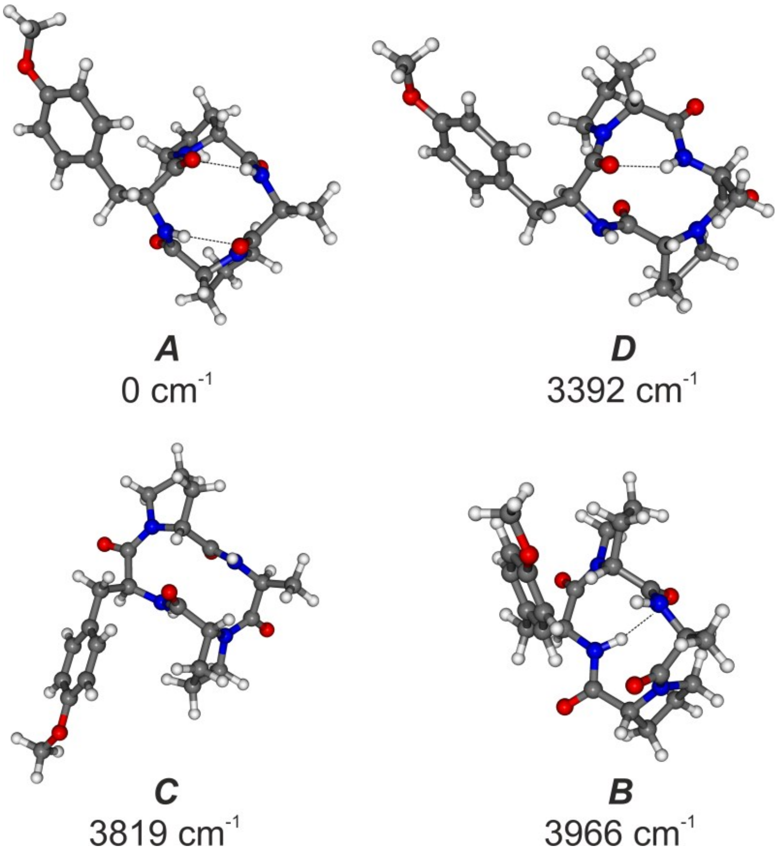


Figure S3. Comparison between the experimental IR/R2PI spectrum and calculated frequencies obtained from DFT calculations (B3LYP-D3/TZVP) of selected conformers of **CP$_{Ala}$** (*cf.* Figure S2). For a better illustration the calculated intensities in the amide A region were doubled. (Details about the used mode-specific scaling factors *cf.* main paper.)

By comparing the harmonically calculated frequencies of ***A***, ***A2*** and ***A3*** of **CP$_{Ala}$** with the experimental IR/R2PI spectrum it can be concluded that all spectra are very similar, but with respect to the NH stretching vibrations conformer ***A*** fits slightly better to the experimental frequencies than ***A2*** and ***A3***. Beside the calculated frequencies also energetic aspects have to be taken into account. Conformer ***A*** is energetically more favored than ***A2*** and ***A3*** which further supports the assignment of conformer ***A***. It should be remarked that the presence of ***A2*** and ***A3*** cannot completely be excluded. Therefore, conformers ***A2*** and ***A3*** may coexist with conformer ***A.***

c) Possible conformers of **CP**$_{\textbf{Ala}}$ and calculated IR spectra *without* dispersion corrections (B3LYP/TZVP)



A
0 cm^{-1}

D
3392 cm^{-1}

C
3819 cm^{-1}

B
3966 cm^{-1}

Figure S4. Possible structures of **CP**$_{\textbf{Ala}}$ based on DFT calculations without dispersion corrections (B3LYP/TZVP). (***A*** represents a structure with two internal hydrogen bonds (2.022 Å and 2.024 Å), ***B*** and ***D*** with one hydrogen bond (2.311 Å and 2.046 Å) and ***C*** without any hydrogen bond.)

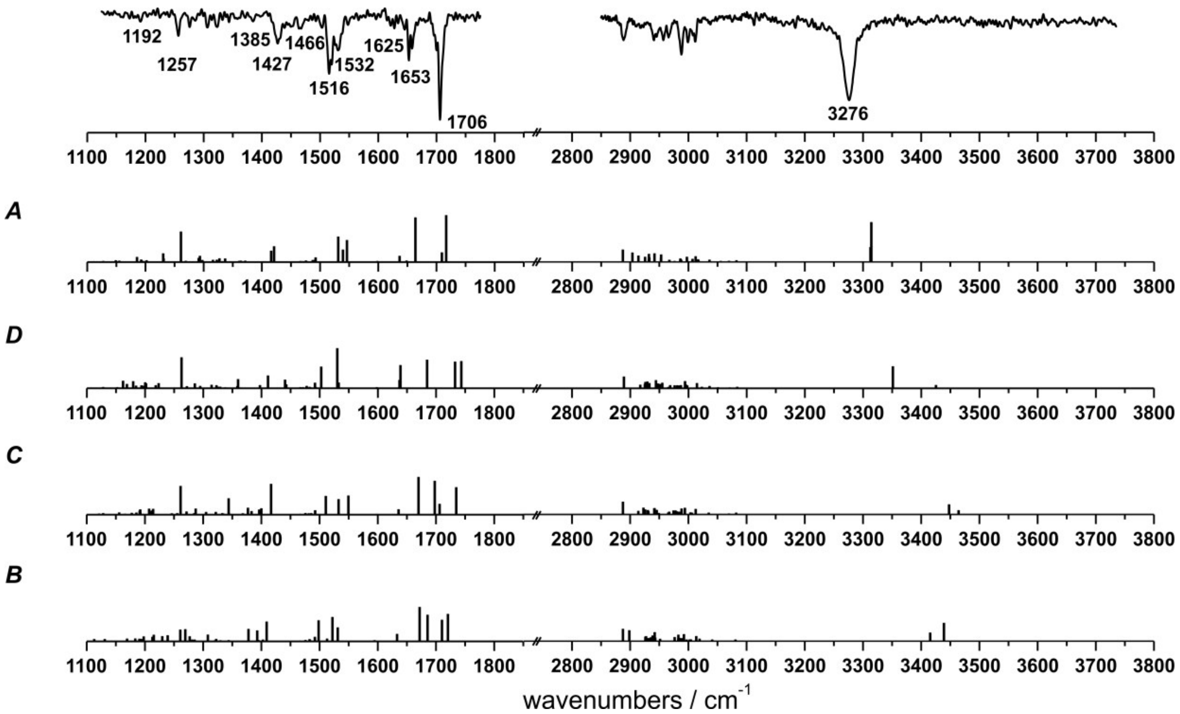


Figure S5. Comparison between the experimental IR/R2PI spectrum and calculated frequencies obtained from DFT calculations without dispersion interactions (B3LYP/TZVP) of selected conformers of $\mathbf{CP_{Ala}}$ (*cf.* Figure S4). Mode-specific scaling factors of 0.9567 for hydrogen-bonded NH stretching vibrations, 0.9613 for non-hydrogen-bonded NH stretching vibrations and 0.99 for the amide I-III region were used. For a better illustration the calculated intensities in the amide A region were doubled.

The comparison of the DFT calculations without dispersion corrections (B3LYP/TZVP) and the experimental IR/R2PI spectrum again yield the assignment of the doubly hydrogen-bonded conformer ***A*** comparable to the calculations including dispersion corrections.

d) Possible conformers and calculated IR spectra of **CP**$_{\mathbf{Ala}}$ calculated at the ri-MP2/SVP level of theory

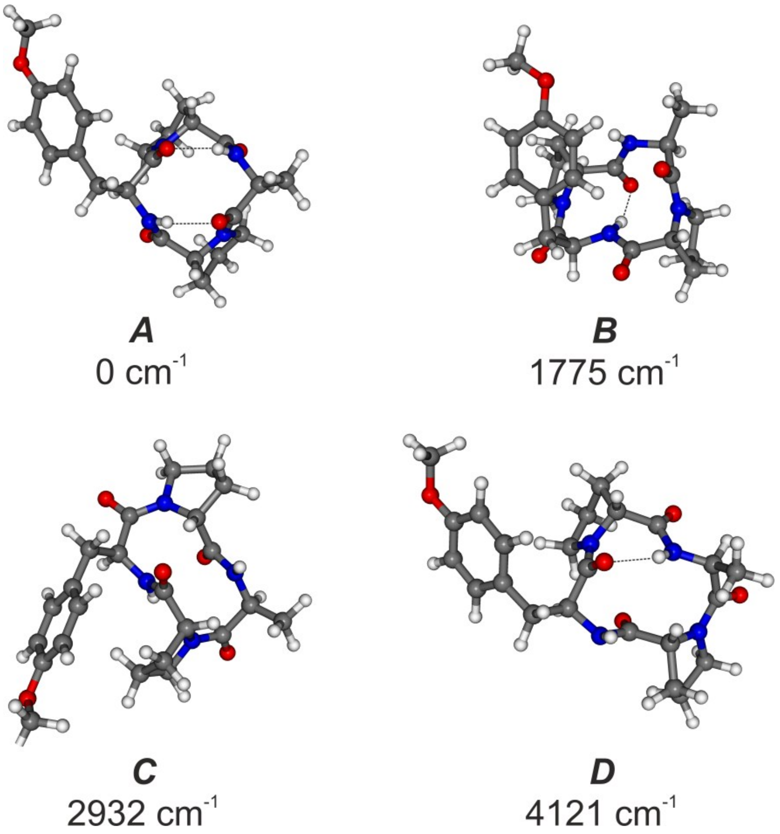


Figure S6. Possible structures of **CP**$_{\mathbf{Ala}}$ based on ri-MP2/SVP calculations. (***A*** represents a structure with two internal hydrogen bonds (1.927 Å and 1.968 Å), ***B*** and ***D*** with one hydrogen bond (2.216 Å and 1.980 Å) and ***C*** without any hydrogen bond.)

All structural binding motifs (***A***, ***B***, ***C*** and ***D***; Figure 3) obtained from the DFT calculations with dispersion corrections (B3LYP-D3/TZVP) were taken as starting geometries for the MP2 optimizations using the approximate resolution of identity (ri) methods together with a SVP basis set. These calculations were realized with Turbomole V7.1 [1,2]. The relative energies are corrected by the zero-point energy and refer to the most stable conformer.

The ri-MP2/SVP optimized structures (Figure S6) basically yield the same geometry as the DFT/B3LYP-D3/TZVP optimizations. Furthermore, a comparison of the calculated frequencies (ri-MP2/SVP) and experimental spectra of **CP**$_{\mathbf{Ala}}$ (Figure S7) again leads to the assignment of the doubly hydrogen-bonded conformer ***A*** comparable to the DFT calculations including and excluding dispersion corrections. Thus an overall agreement between different theoretical methods is obtained. This agreement is a further support that DFT calculations with dispersion corrections (Grimme D3 [3]) and a triple-zeta basis set are a very efficient tool to obtain relative energies of different conformers with reliable frequencies.

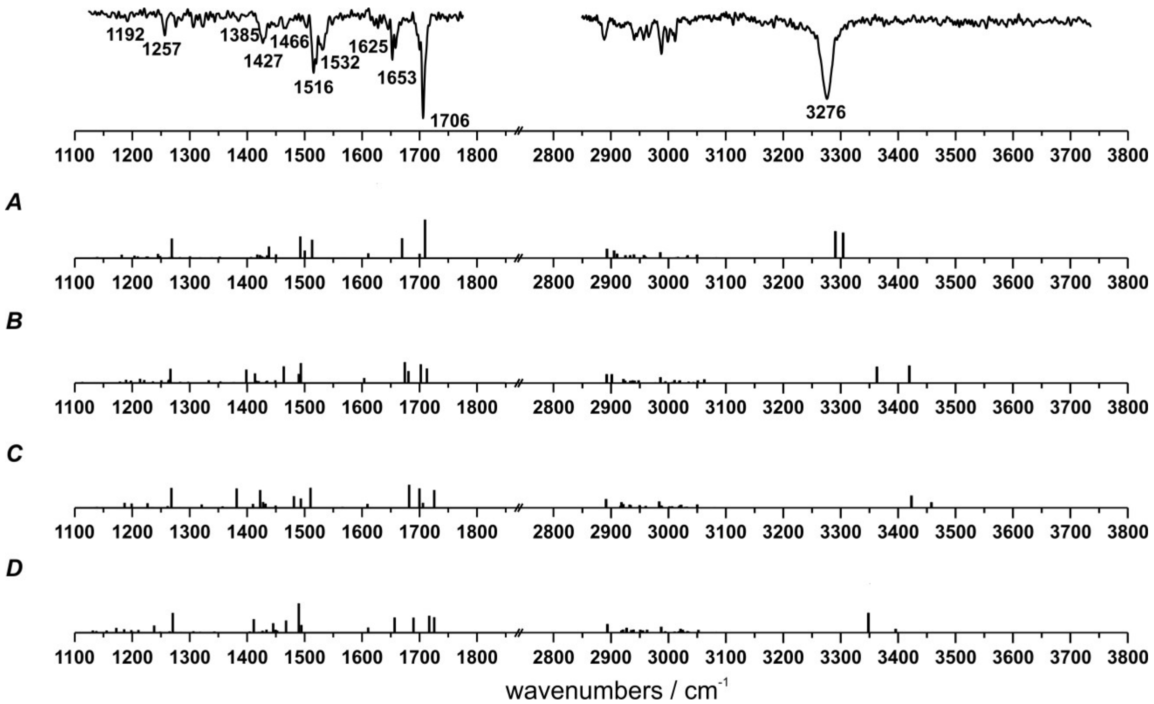


Figure S7. Comparison between the experimental IR/R2PI spectrum and calculated frequencies obtained from ri-MP2/SVP calculations of selected conformers of **CP$_{\mathbf{Ala}}$** (*cf.* Figure S6). Mode-specific scaling factors of 0.9412 for NH stretching vibrations and 0.9492 for the amide I-III region were used (for scaling factors see Ref. 4; the factor of 0.9492 is an average of the CO stretching and NH bending scaling factors of 0.9415 and 0.9568, respectively). For a better illustration the calculated intensities in the amide A region were doubled.

e) Possible conformers and calculated IR spectra of **CP$_{Ala}$** including a water solvent shell (B3LYP-D3/TZVP, ε = 78.39)

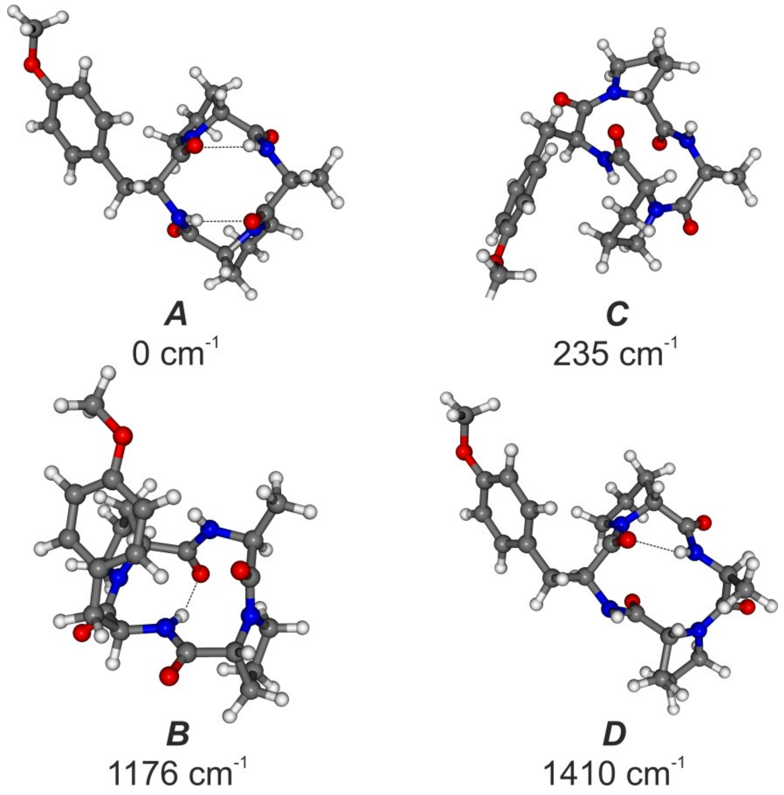


Figure S8. Possible structures of **CP$_{Ala}$** in the energetic order based on DFT calculations (B3LYP-D3/TZVP, ε = 78.39). (***A*** represents a structure with two internal hydrogen bonds (2.010 Å and 2.040 Å), ***B*** and ***D*** with one hydrogen bond (2.258 and 2.034 Å) and ***C*** without any hydrogen bond.)

Solvation effects were taken into account using the polarized continuum model (PCM) implemented in Turbomole's solvation program COSMO [5,6] (**co**nductor-like **s**creening **mo**del). This method determines the electrostatic interaction of a molecule with a solvent which treats the solvent as a continuum with a permittivity ε. These calculations were realized with a combination of Gaussian09 [7] and Turbomole [1,2] (for details see main paper section III) using the B3LYP/TZVP level of theory including dispersion corrections (Grimme D3 [6]). The energy values were corrected by the zero-point energy and refer to the most stable conformer.

For **CP$_{Ala}$** water is used as solvent with a permittivity of 78.39 (B3LYP-D3/TZVP) basically yielding the same results as without solvent effect, *i.e.* structure ***A*** turns out to be the most stable arrangement.

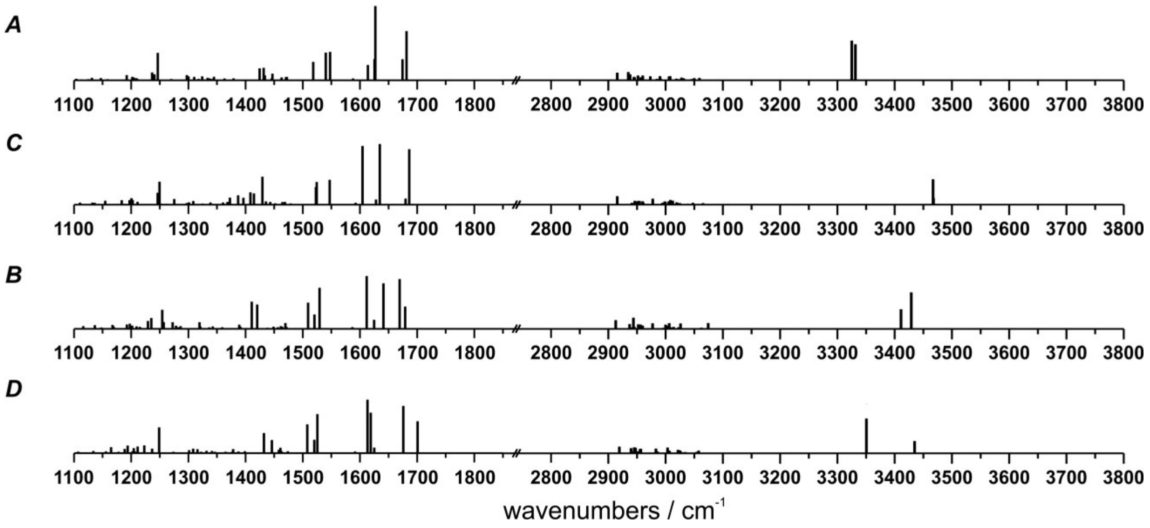


Figure S9. Calculated frequencies obtained from DFT calculations (B3LYP-D3/TZVP, ε = 78.39) of selected conformers of **CP$_{\mathbf{Ala}}$** (*cf.* Figure S8). Mode-specific scaling factors of 0.9563 for hydrogen-bonded NH stretching vibrations, 0.9608 for non-hydrogen-bonded NH stretching vibrations and 0.985 for the amide I-III region were used. For a better illustration the calculated intensities in the amide A region were doubled.

f) NMR characterization of **CP$_{Ala}$**

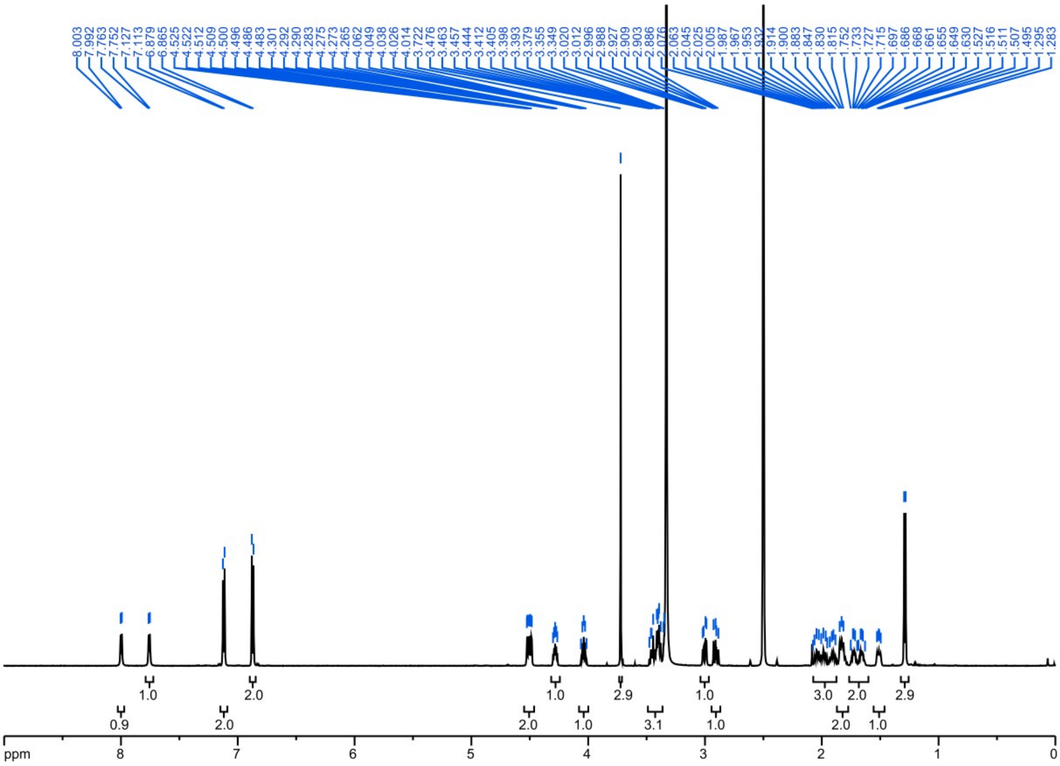


Figure S10: ^{1}H-NMR (600 MHz, DMSO-d$_6$) of **CP$_{Ala}$**.

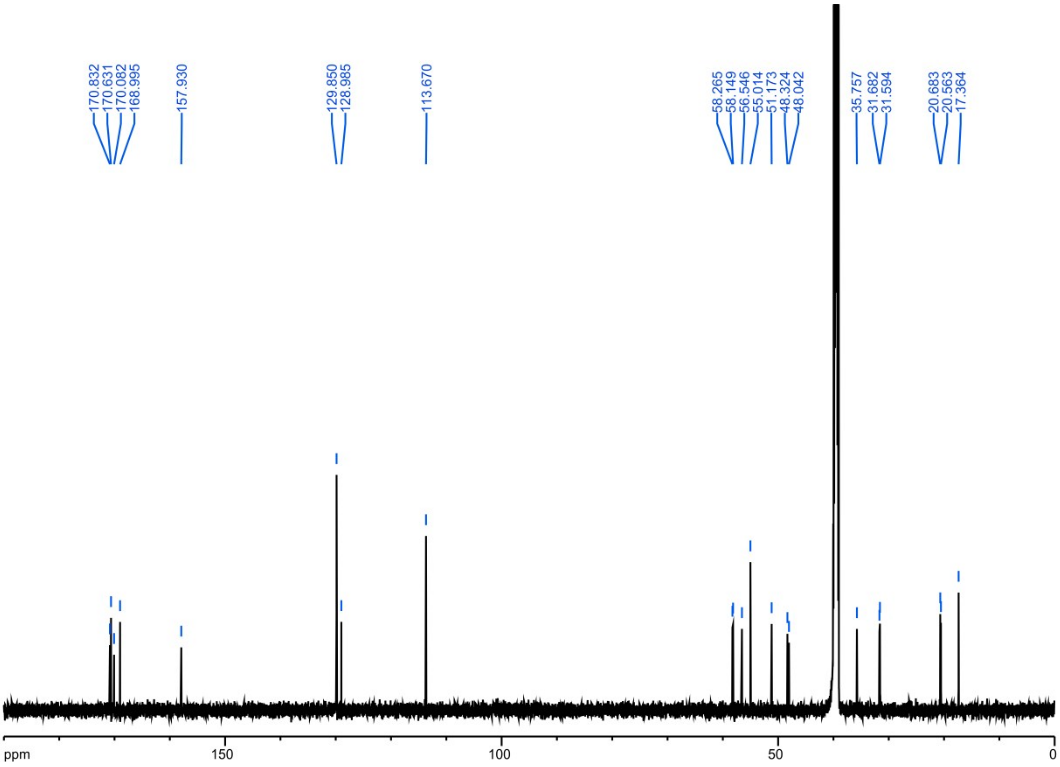


Figure S11: ^{13}C-NMR (151 MHz, DMSO-d$_6$) of **CP$_{Ala}$**.

g) Mass spectrum of $\mathbf{CP}_{\mathbf{Ala}}$

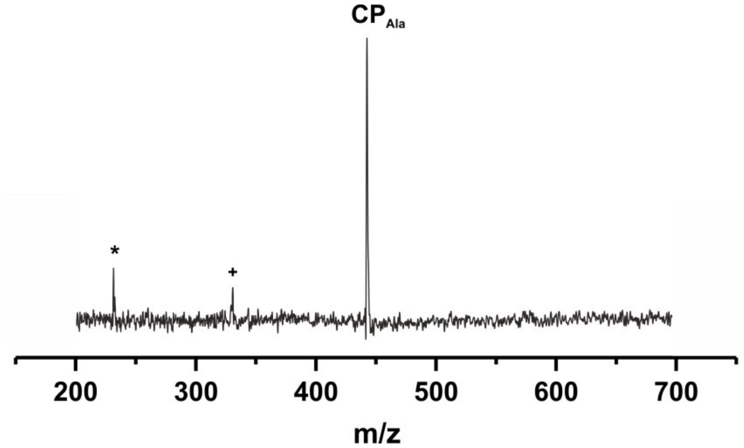


Figure S12: Mass spectrum of $\mathbf{CP}_{\mathbf{Ala}}$ with two fragments (* = 231 m/z and + = 331m/z). The parent mass signal of $\mathbf{CP}_{\mathbf{Ala}}$ obtained at a sample oven temperature of about 120 °C is constant while the IR/R2PI spectra are recorded.

cyclo[L-Tyr(Me)-D-Pro-L-Glu(Me)-D-Pro] (CP$_{Glu}$)

a) R2PI spectrum of **CP$_{Glu}$**

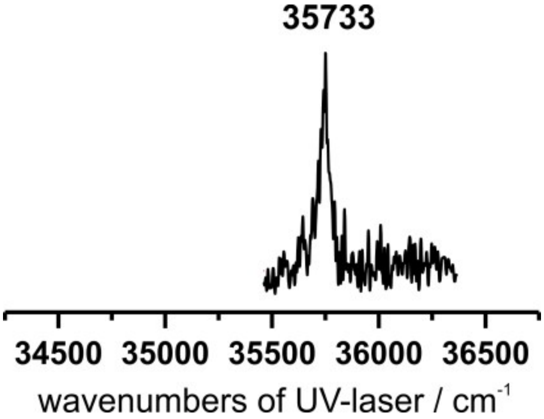
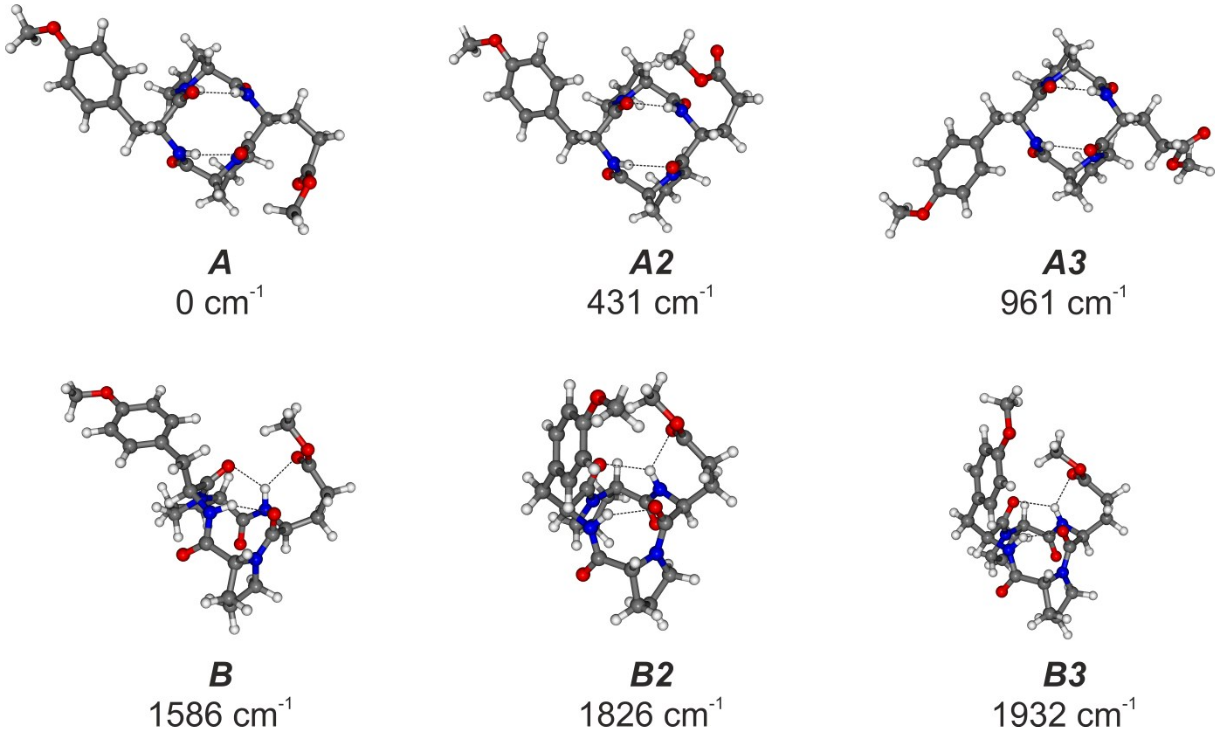


Figure S13. One-color R2PI spectrum of **CP$_{Glu}$** recorded in the region of 35461-36364 cm^{-1}.

b) Possible doubly hydrogen-bonded structures and calculated IR spectra of **CP$_{\mathbf{Glu}}$** (B3LYP-D3/TZVP)



A 0 cm^{-1}

A2 431 cm^{-1}

A3 961 cm^{-1}

B 1586 cm^{-1}

B2 1826 cm^{-1}

B3 1932 cm^{-1}

Figure S14. Possible structures of **CP$_{\mathbf{Glu}}$** with doubly hydrogen-bonded binding motifs (***A*** and ***B*** type) based on DFT calculations including dispersion corrections (B3LYP-D3/TZVP). (Hydrogen bond lengths of these structures (given in Å): ***A*** (1.988 and 2.022), ***A2*** (1.944 and 2.018), ***A3*** (1.995 and 1.997), ***B*** (1.961 and 2.264, 2.108), ***B2*** (2.267 and 1.987, 2.161) and ***B3*** (2.304 and 2.015, 2.122).)

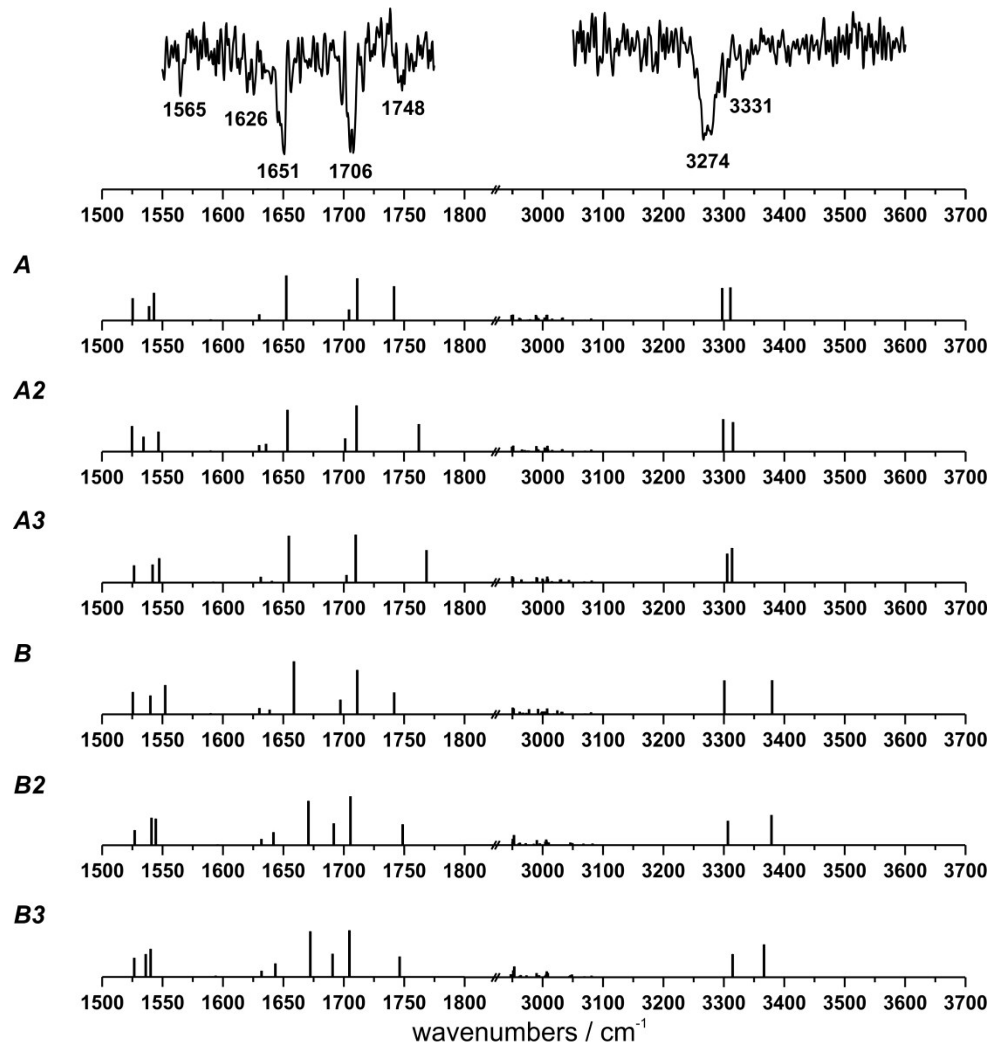
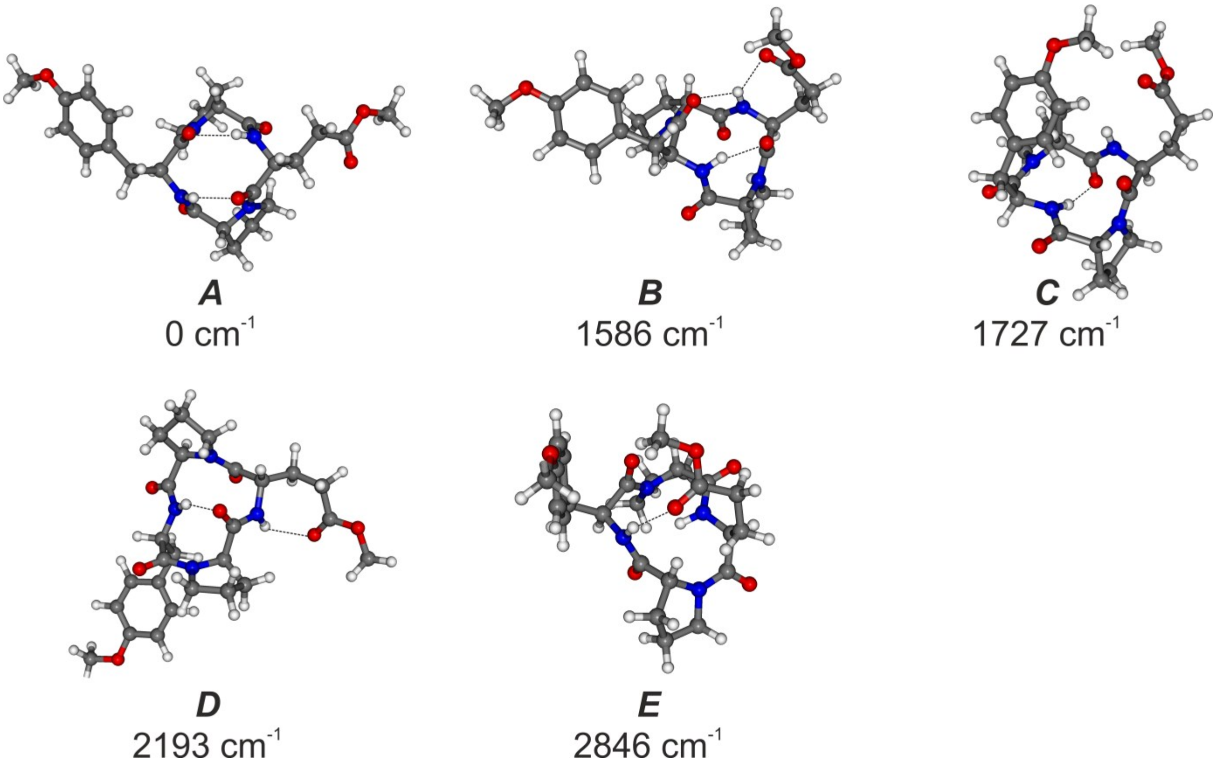


Figure S15. Comparison between the experimental IR/R2PI spectrum and calculated frequencies obtained from DFT calculations (B3LYP-D3/TZVP) of selected conformers of **CP$_{\mathbf{Glu}}$** (*cf.* Figure S14). For a better illustration the calculated intensities in the amide A region were doubled. (Details about the used mode-specific scaling factors *cf.* main paper.)

By comparing the harmonically calculated frequencies of **CP$_{\mathbf{Glu}}$** with the experimentally obtained vibrations ***A2***, ***A3***, ***B2*** and ***B3*** do not explain the IR spectrum as well as the ***A*** and ***B*** structures. In case of conformers ***A2*** and ***A3*** the calculated vibrations are above 1750 cm^{-1} (originating from the carbonyl group of the Glu side chain) and conformers ***B2*** and ***B3*** have predicted frequencies at about 1675 cm^{-1} which are not observed in the experimental spectrum. Moreover these conformers are energetically not favored.

c) Possible conformers of **CP**$_{\mathbf{Glu}}$ and calculated IR spectra *without* dispersion corrections (B3LYP/TZVP)



A
0 cm^{-1}

B
1586 cm^{-1}

C
1727 cm^{-1}

D
2193 cm^{-1}

E
2846 cm^{-1}

Figure S16. Possible structures of **CP**$_{\mathbf{Glu}}$ based on DFT calculations without dispersion corrections (B3LYP/TZVP). ***A*** and ***B*** represent structures with two internal hydrogen bonds (2.019 Å and 2.015 Å for ***A***, 2.006 Å and 2.370 Å for ***B)***, additionally ***B*** forms a further hydrogen bond to the CO side chain of the glutamic acid with a bond-length of 2.147 Å. ***C*** and ***E*** contain one hydrogen bond (bond-lengths 2.294 Å and 2.173 Å) and ***D*** has one internal hydrogen bond (2.142 Å) as well as one bond to the CO side chain of the glutamic acid (bond-length 2.241 Å).

A binding motif of **CP**$_{\mathbf{Glu}}$ without hydrogen bonds (***F*** type) was not found according to these DFT calculations without dispersion corrections within a relative energy of 6000 cm^{-1}.

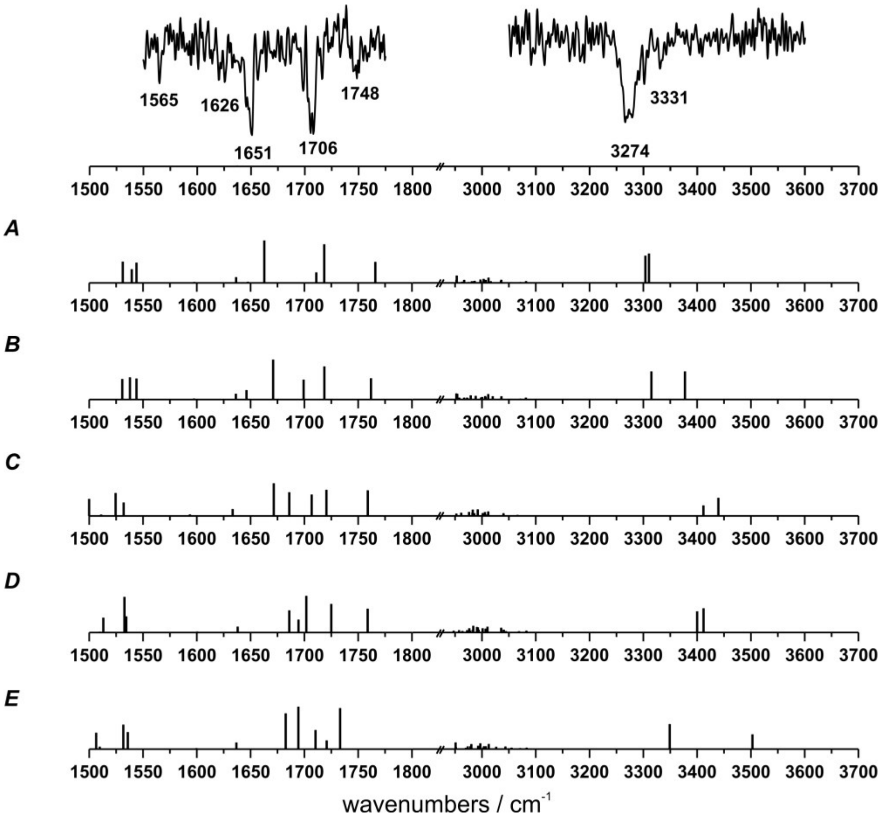


Figure S17. Comparison between the experimental IR/R2PI spectrum and calculated frequencies obtained from DFT calculations without dispersion corrections (B3LYP/TZVP) of selected conformers of **CP$_{\mathbf{Glu}}$** (*cf.* Figure S16). For a better illustration the calculated intensities in the amide A region were doubled. The following mode-specific scaling factors are used: 0.9567 for hydrogen-bonded NH stretching vibrations, 0.9613 for non-hydrogen-bonded NH stretching vibrations and 0.99 for the amide I-III region. For a better illustration the calculated intensities in the amide A region were doubled.

Comparison of the DFT calculations without dispersion corrections (B3LYP/TZVP) and the experimental IR/R2PI spectrum again lead to the assignment of the doubly hydrogen-bonded conformers ***A*** and ***B*** comparable to the calculations including dispersion corrections.

d) Possible conformers and calculated IR spectra of **CP$_{Glu}$** calculated at the ri-MP2/SVP level of theory

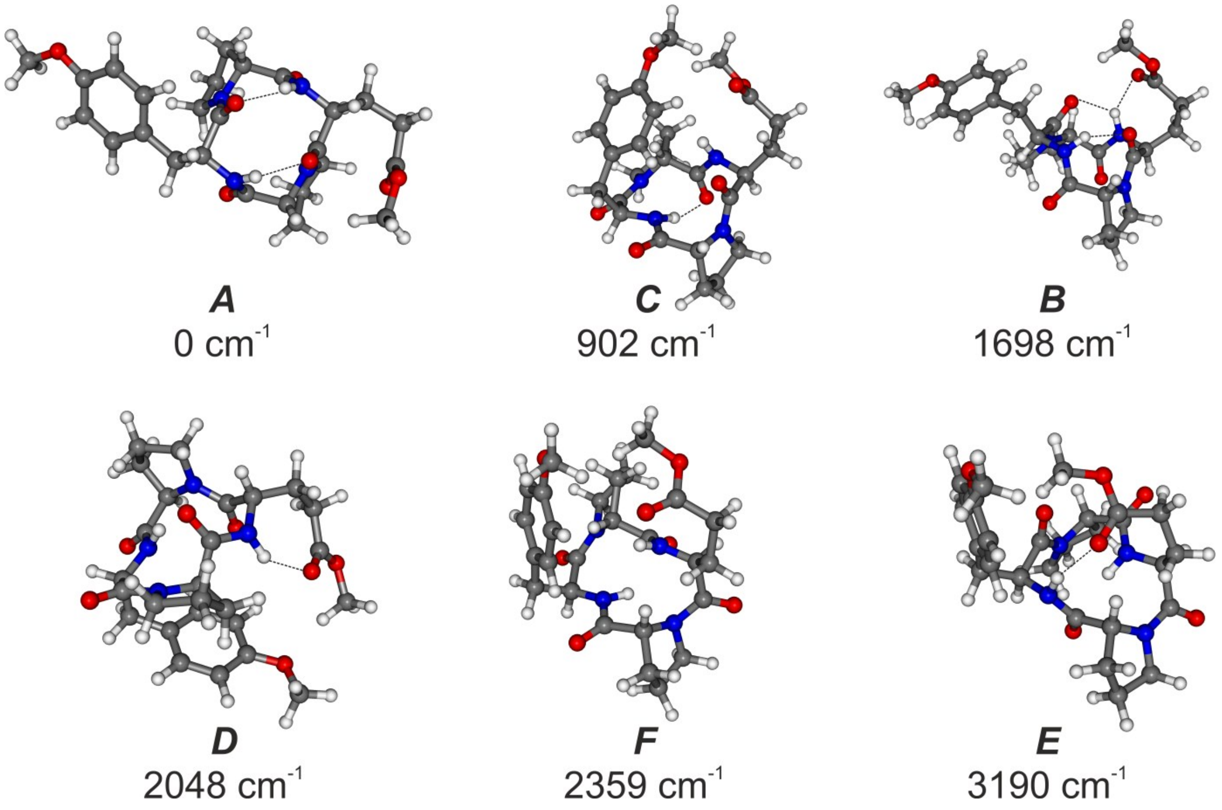


Figure S18. Possible structures of **CP$_{Glu}$** based on ri-MP2/SVP calculations. ***A*** and ***B*** represent structures with two internal hydrogen bonds (1.928 Å and 1.978 Å for ***A***, 1.949 Å and 2.276 Å for ***B)***, additionally ***B*** forms a further hydrogen bond to the CO side chain of the glutamic acid with a bond-length of 2.003 Å. ***C*** and ***E*** contain one hydrogen bond (bond-lengths 2.253 Å and 2.092 Å) and ***D*** has one internal hydrogen bond (1.984 Å) as well as one bond to the CO side chain of the glutamic acid (bond-length 1.911 Å).

All structural binding motifs (***A***, ***B***, ***C***, ***D***, ***E*** and ***F***; Figure 5) obtained from the DFT calculations with dispersion corrections (B3LYP-D3/TZVP) were taken as starting geometries for the MP2 optimizations using the approximate resolution of identity (ri) methods together with a SVP basis set. These calculations were realized with Turbomole V7.1 [1,2]. The relative energies are corrected by the zero-point energy and refer to the most stable conformer.

The ri-MP2/SVP optimized structures (Figure S18) basically yield the same geometry as the DFT/B3LYP-D3/TZVP optimizations only the relative energies change. Furthermore, a comparison of the calculated frequencies (ri-MP2/SVP) and experimental spectra of **CP$_{Glu}$** (Figure S19) again lead to the assignment of the doubly hydrogen-bonded conformers ***A*** and ***B***. Both conformers are stabilized by two internal hydrogen bonds (within the cyclic peptide ring) but conformer ***B*** additionally contains a hydrogen bond between the carbonyl group of the Glu side chain and the NH$_{Glu}$ group comparable to the DFT calculations including and excluding dispersion corrections. It should be remarked that in case of the DFT-D3 calculations the assigned conformers ***A*** and ***B*** are the most stable structures whereas in case of the ri-MP2 method structure ***C*** is more stable than conformer ***B*** although the frequencies still indicate that this structural arrangement has to be assigned to the IR spectrum (similar to the DFT calculation). Like in the case of **CP$_{Ala}$** this agreement is a further support that DFT calculations with dispersion corrections (Grimme D3 [3]) and a triple-zeta basis set are a very efficient tool to obtain relative energies of different conformers with reliable frequencies.

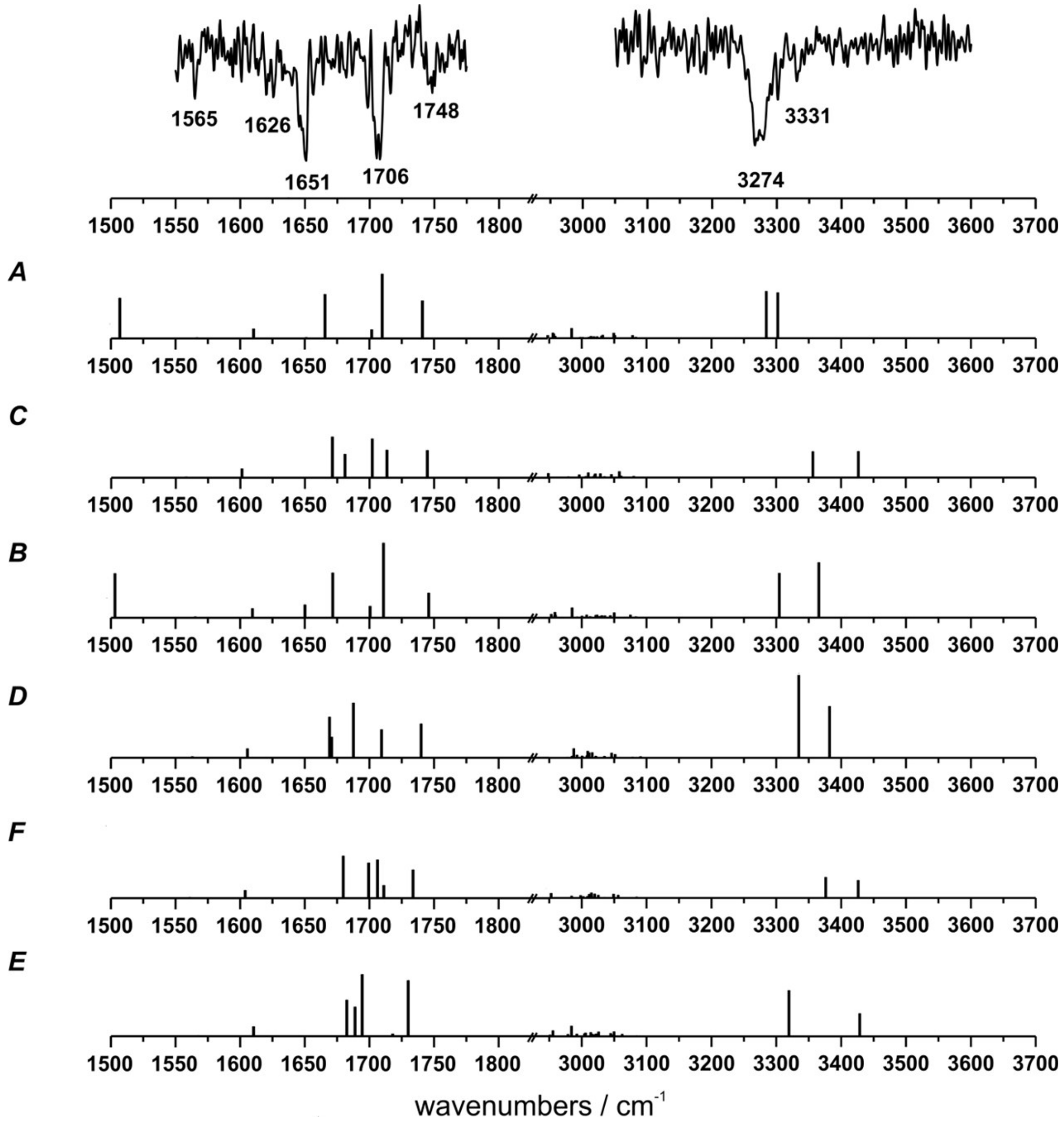
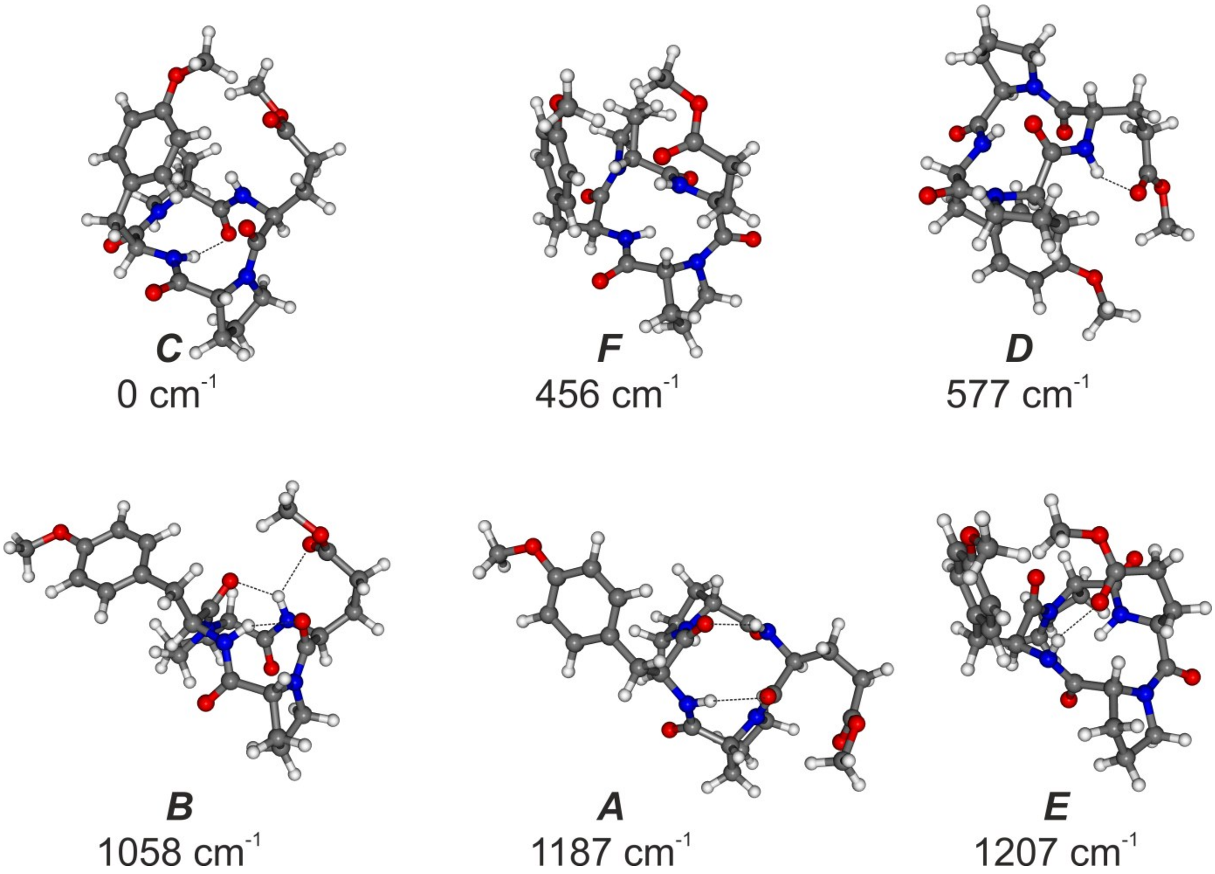


Figure S19. Comparison between the experimental IR/R2PI spectrum and calculated frequencies obtained from ri-MP2/SVP calculations of selected conformers of **CP$_{Glu}$** (*cf.* Figure S18). Mode-specific scaling factors of 0.9412 for NH stretching vibrations and 0.9492 for the amide I-III region were used (for scaling factors see Ref. 4; the factor of 0.9492 is an average of the CO stretching and NH bending scaling factors of 0.9415 and 0.9568, respectively). For a better illustration the calculated intensities in the amide A region were doubled.

e) Possible conformers and calculated IR spectra of $\mathbf{CP}_{\mathrm{Glu}}$ including a water solvent shell (B3LYP-D3/TZVP, ε = 78.39)


C 0 cm^{-1} ***F*** 456 cm^{-1} ***D*** 577 cm^{-1}

B 1058 cm^{-1} ***A*** 1187 cm^{-1} ***E*** 1207 cm^{-1}

Figure S20. Possible structures of $\mathbf{CP}_{\mathrm{Glu}}$ in the energetic order based on DFT calculations (B3LYP-D3/TZVP, ε = 78.39). ***A*** and ***B*** represent structures with two internal hydrogen bonds (2.010 Å and 2.037 Å for ***A***, 1.968 Å and 2.106 Å for ***B)***, additionally ***B*** forms a further hydrogen bond to the CO side chain of the glutamic acid with a bond-length of 2.263 Å. ***C*** and ***E*** contain one hydrogen bond (bond-lengths 2.319 Å and 2.092 Å) and ***D*** has one internal hydrogen bond (2.009 Å) as well as one bond to the CO side chain of the glutamic acid (bond-length 1.895 Å).

Similar to $\mathbf{CP}_{\mathrm{Ala}}$ solvation effects for $\mathbf{CP}_{\mathrm{Glu}}$ were also taken into account by using the PCM model implemented in Turbomole's solvation program COSMO [5,6] (for details about the calculation procedure see section $\mathbf{CP}_{\mathrm{Ala}}$/e)). Water is used as solvent with a permittivity of 78.39 (B3LYP-D3/TZVP) yielding a structure with one internal hydrogen-bond as most stable arrangement. This is in contrast to the results of $\mathbf{CP}_{\mathrm{Ala}}$ for which the most stable arrangement of the non-solvated cyclic peptide remains the most stable one upon solvation. This difference results from the additional carbonyl group in $\mathbf{CP}_{\mathrm{Glu}}$ (compared to $\mathbf{CP}_{\mathrm{Ala}}$) which can be very efficiently solvated by water molecules. Thus the variation of the side-chain can affect structural preferences significantly with respect to solvation.

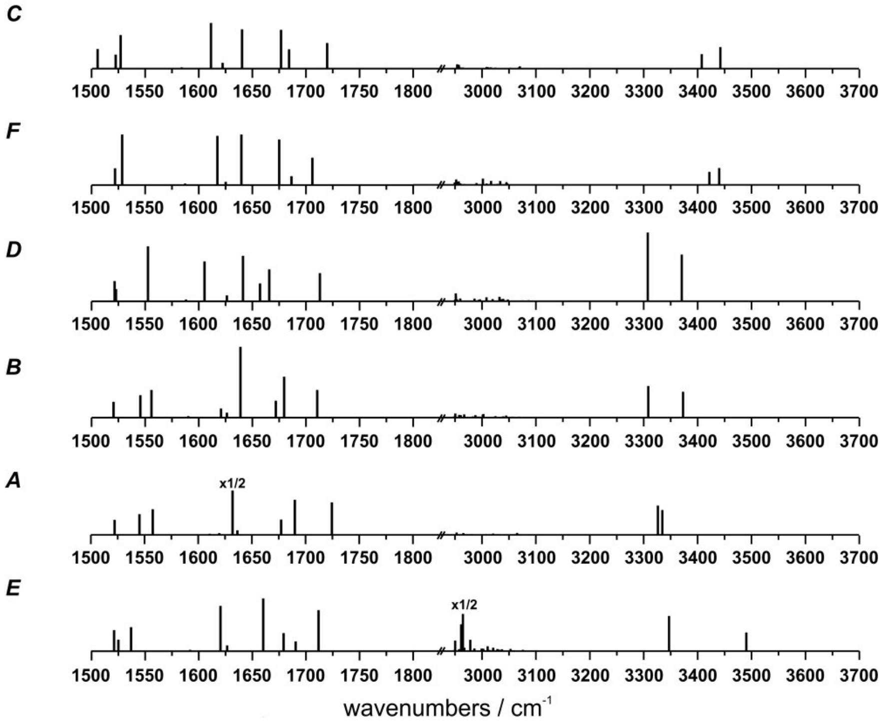


Figure S21. Calculated frequencies obtained from DFT calculations (B3LYP-D3/TZVP, ε = 78.39) of selected conformers of $\mathbf{CP_{Glu}}$ (*cf.* Figure S20). Mode-specific scaling factors of 0.9563 for hydrogen-bonded NH stretching vibrations, 0.9608 for non-hydrogen-bonded NH stretching vibrations and 0.985 for the amide I-III region were used. For a better illustration the calculated intensities in the amide A region were doubled.

f) NMR characterization of **CP**$_{\mathbf{Glu}}$

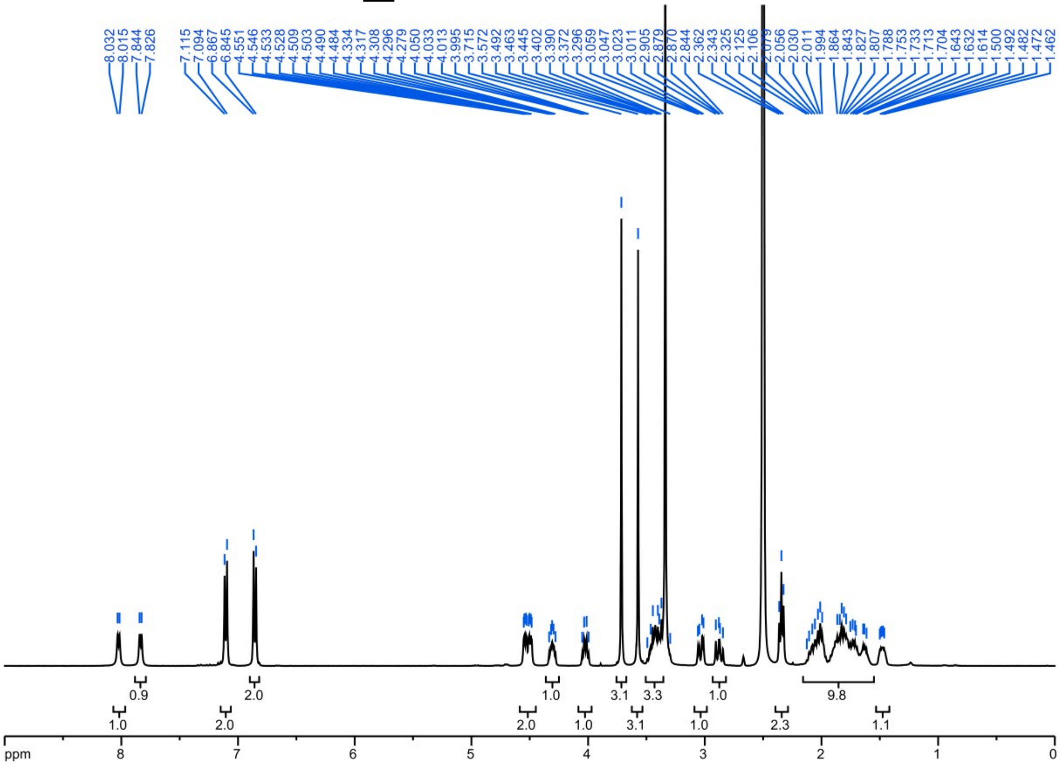


Figure S22: ^{1}H-NMR (400 MHz, DMSO-d$_{6}$) of **CP**$_{\mathbf{Glu}}$.

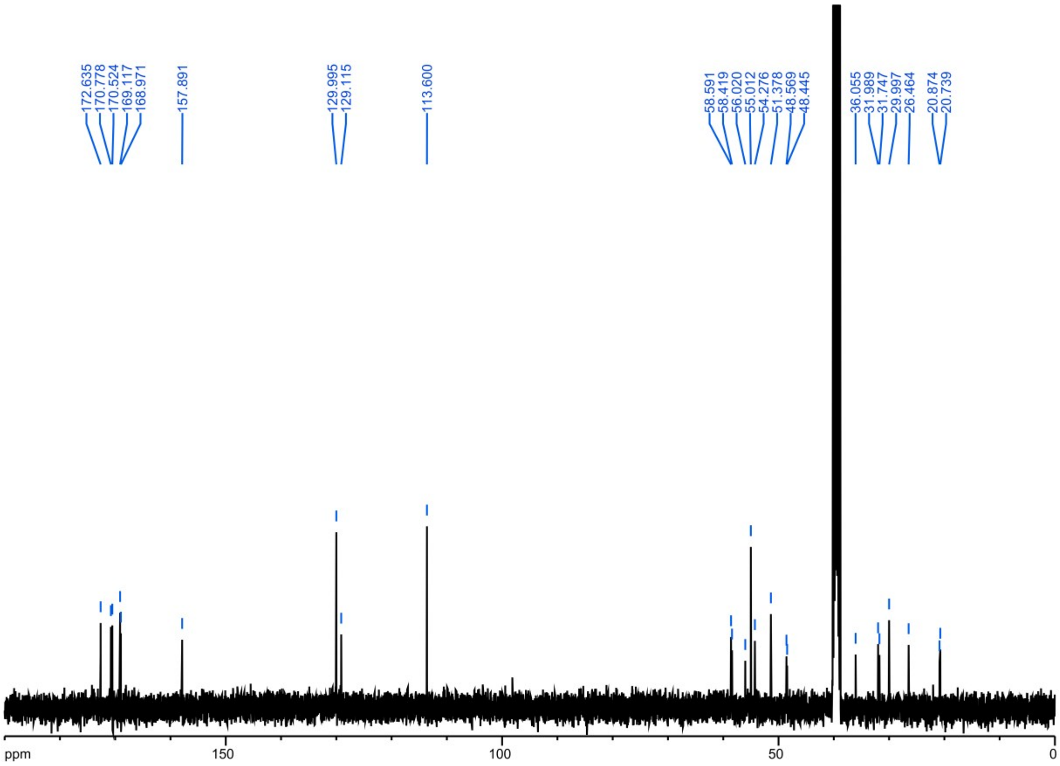


Figure S23: ^{13}C-NMR (101 MHz, DMSO-d$_{6}$) of **CP**$_{\mathbf{Glu}}$.

g) Mass spectrum of **CP$_{Glu}$**

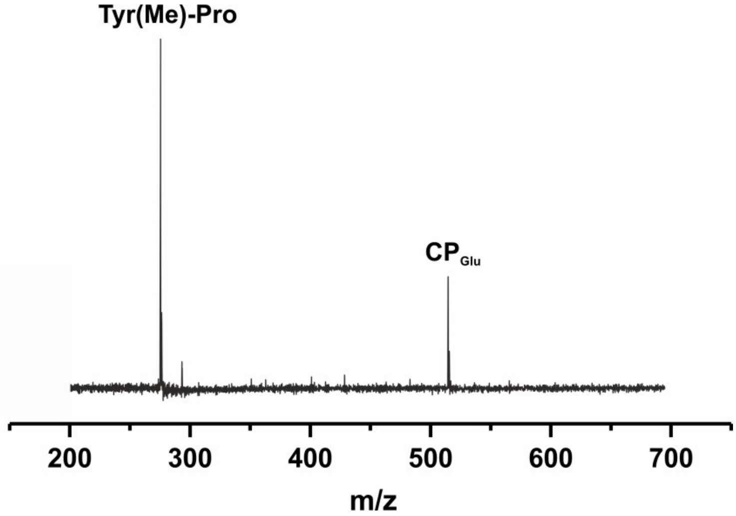


Figure S24: Mass spectrum of **CP$_{Glu}$**. The parent mass signal of **CP$_{Glu}$** obtained at a sample oven temperature of about 150 °C is constant while the IR/R2PI spectra are recorded.

References

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