Supporting Information (SI)

Revealing the Conformational Preferences of Proteinogenic Glutamic Acid Derivatives in Solution by ^{1}H NMR Spectroscopy and Theoretical Calculations

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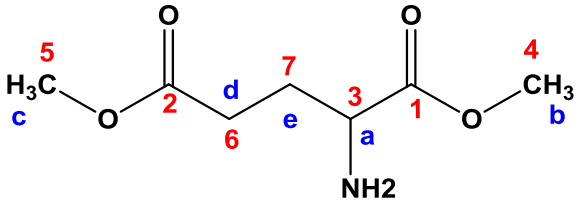
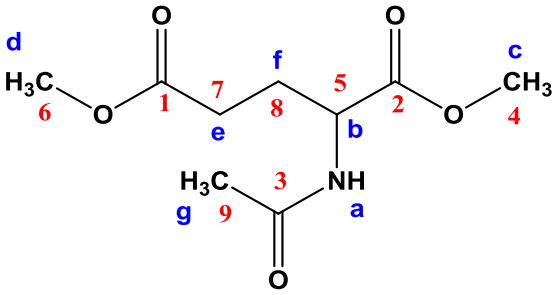
Procedures for the Synthesis of the Compounds

L-Glutamic Acid Dimethyl Ester (GluOMe)



Activated zinc dust (100 mg) was added to a suspension of the L-glutamic acid dimethyl ester hydrochloride (175.1 mg, 1.0 mmol) in 10 mL of dichloromethane. The reaction mixture was stirred for 3 h at room temperature. Then, the mixture was filtered, and the solvent was evaporated *in vacuo*. The amino acid ester free amine was obtained as a colorless liquid (191.5 mg, 0.8 mmol, 79.7 % yield), which was used without any further purification. **^{1}H NMR** (600.17 MHz, CD_3CN, 25 °C, TMS): δ (ppm) = Ha: 3.79 (t, ^{3}J= 6.4Hz); Hb: 3.73 (s, 3H); Hc: 3.63 (s, 3H); Hd: 2.49 (ddd, ^{3}J= 6.9 and 8.3 Hz, ^{2}J= 16.9 Hz, 1H); Hd': 2.42 (ddd, ^{3}J= 6.8 and 8.0 Hz, ^{2}J= 16.9 Hz, 1H); He: 2.08 (m, 2H).**^{13}C NMR** (150.91 MHz, CD_3CN, 25 °C, TMS): δ (ppm) = C1: 173.45; C2:172.64; C3: 53.12; C4: 52.19; C5: 51.01; C6: 29.07; C7: 27.74. **HRMS** (TOF-ES+): *m/z* $[M+H]^+$ calculated for $C_7H_{14}NO_4$: 176.0878; found: 176.0919.

***N*-acetyl-L-glutamic acid dimethyl ester (AcGluOMe)**



Thionyl chloride (0.7 mL, 9.6 mmol) was carefully added to a stirred solution of *N*-acetyl-L-glutamic acid (1.0 g, 5.2 mmol) in 10 mL of anhydrous MeOH. The reaction mixture was stirred for 3 h at 0 ºC and then at room temperature for further 24 h. The solvent was removed under reduced pressure, and 5 mL of water was added to the resulting residue followed by the washing with a saturated aqueous solution of $KHCO_3$. The aqueous layer was extracted with dichloromethane (3 x 5 mL), and the combined organic layers were dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to afford *N*-acetyl-L-glutamic acid dimethyl ester (453.5 mg, 2.1 mmol, 40.4 % yield) as a white solid. **^{1}H NMR** (600.17 MHz, CD_3CN, 25 °C, TMS): δ (ppm) = Ha: 6.71 (s, 1H); Hb: 4.37 (ddd, ^{3}J= 5.3, 7.9 and 8.7 Hz, 1H); Hc: 3.66 (s, 3H); Hd: 3.62 (s,3H); He: 2.37 (m, 2H); Hf: 2.07 (m, 1H); Hf': 1.87 (m, 1H); Hg: 1.89 (m, 3H). **^{13}C NMR** (150.91 MHz, DMSO-d_6, 25 °C, TMS): δ (ppm) = C1: 174.34; C2: 173.69; C3: 171.28; C4: 53.13; C5: 52.94; C6: 52.55; C7: 31.03; C8: 27.87; C9: 23.12. **HRMS** (TOF-ES+): *m/z* $[M + H]^+$ calculated for $C_9H_{16}NO_5$: 218.0984; found: 218.1003.

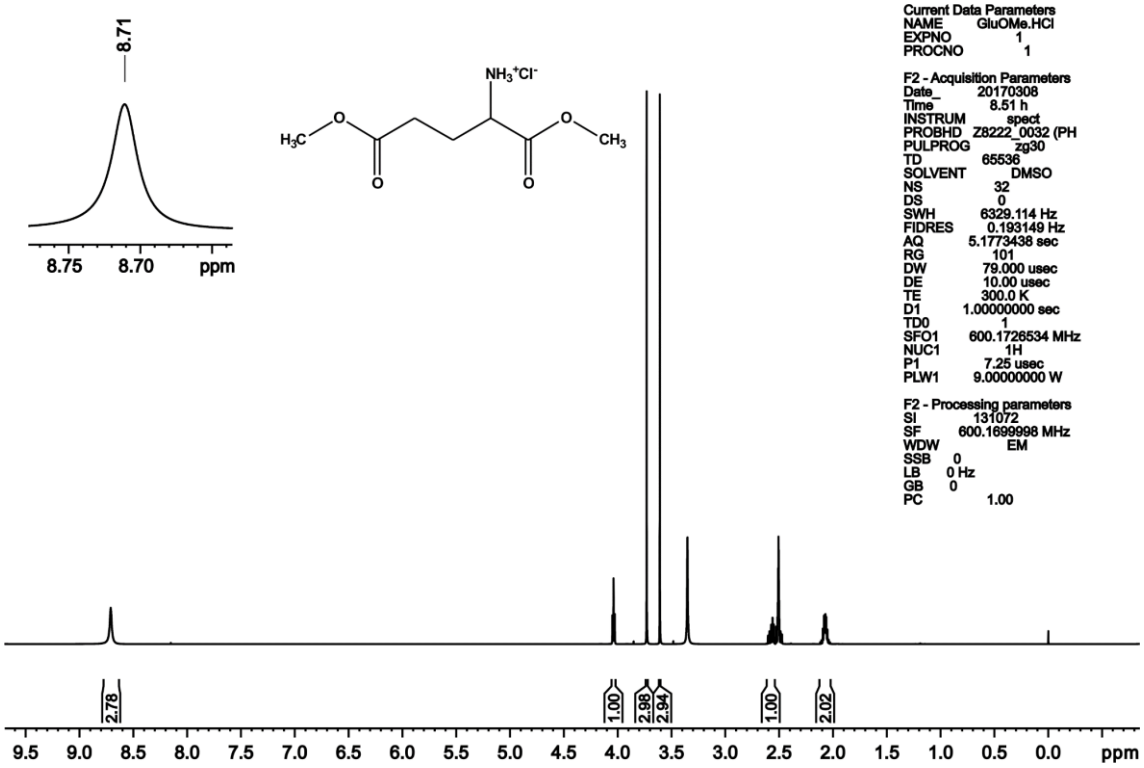


Figure S1 ^{1}H NMR spectra of GluOMe.HCl in DMSO-d_6.

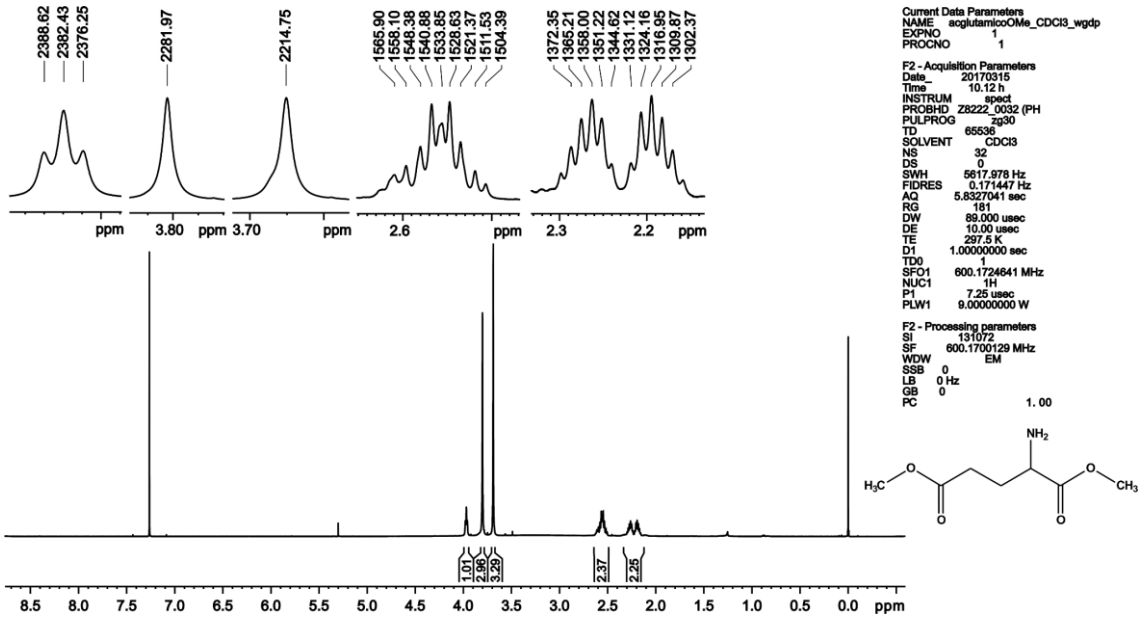


Figure S2 ^{1}H NMR spectra of GluOMe in $CDCl_3$.

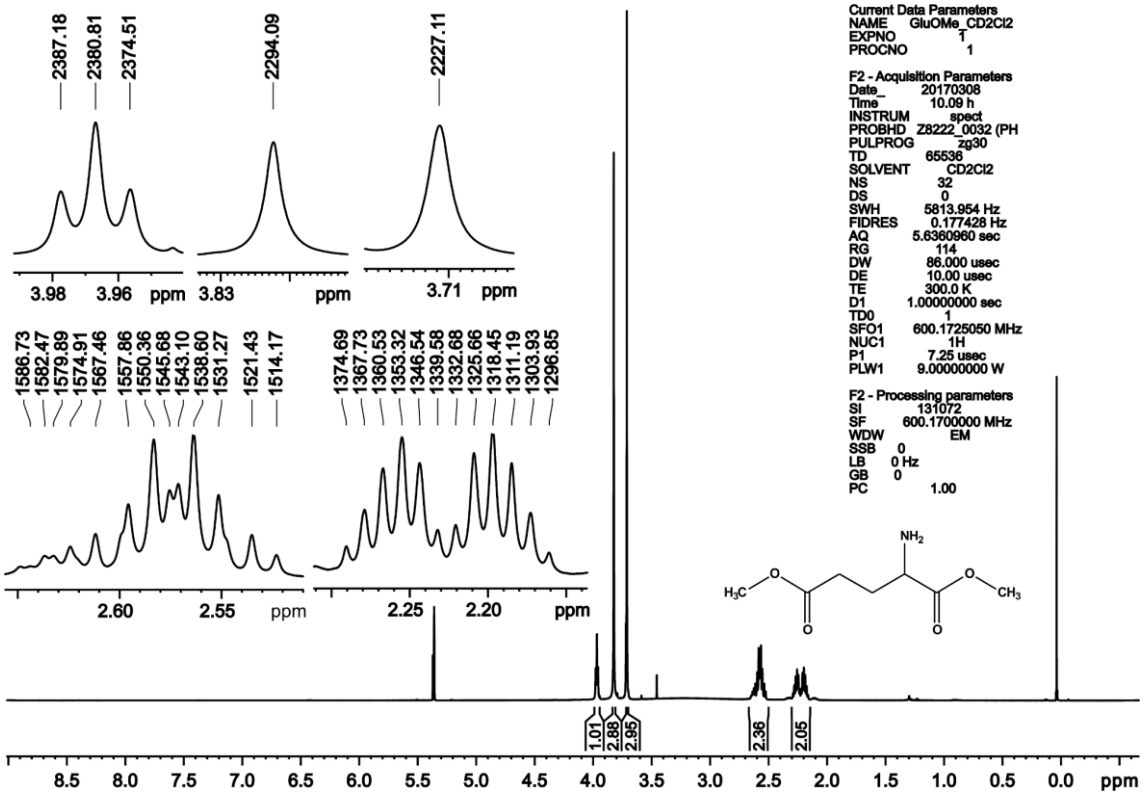


Figure S3 ^{1}H NMR spectra of GluOMe in CD_2Cl_2.

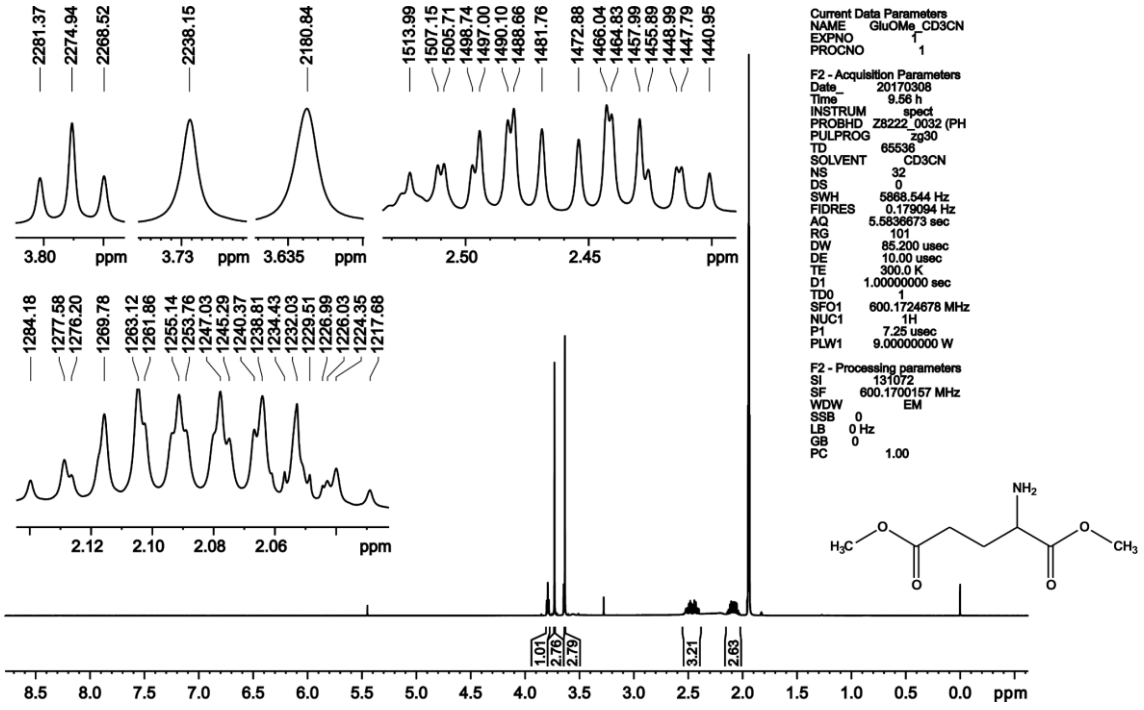


Figure S4 ^{1}H NMR spectra of GluOMe in CD_3CN.

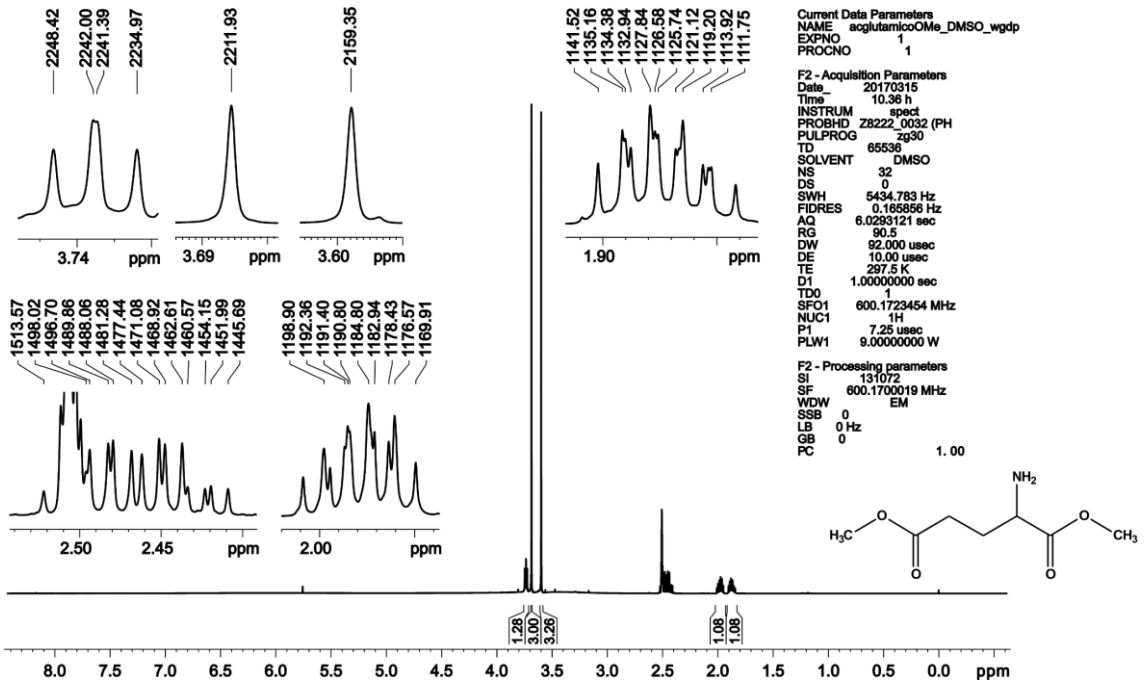


Figure S5 ^{1}H NMR spectra of GluOMe in DMSO-d_6.

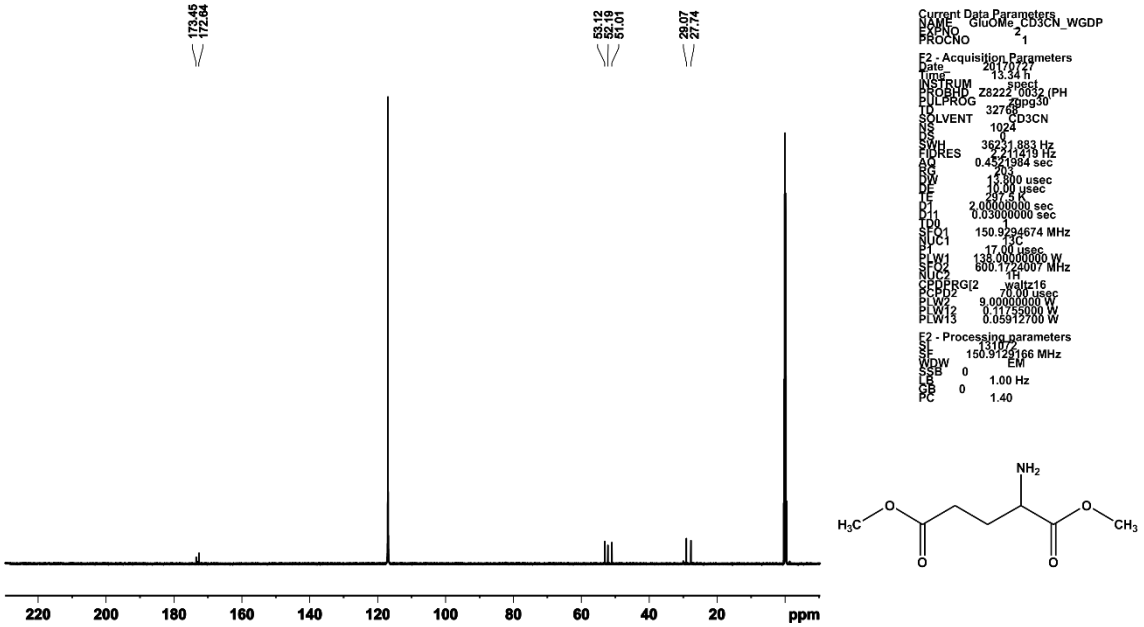


Figure S6 ^{13}C NMR spectra of GluOMe in CD_3CN.

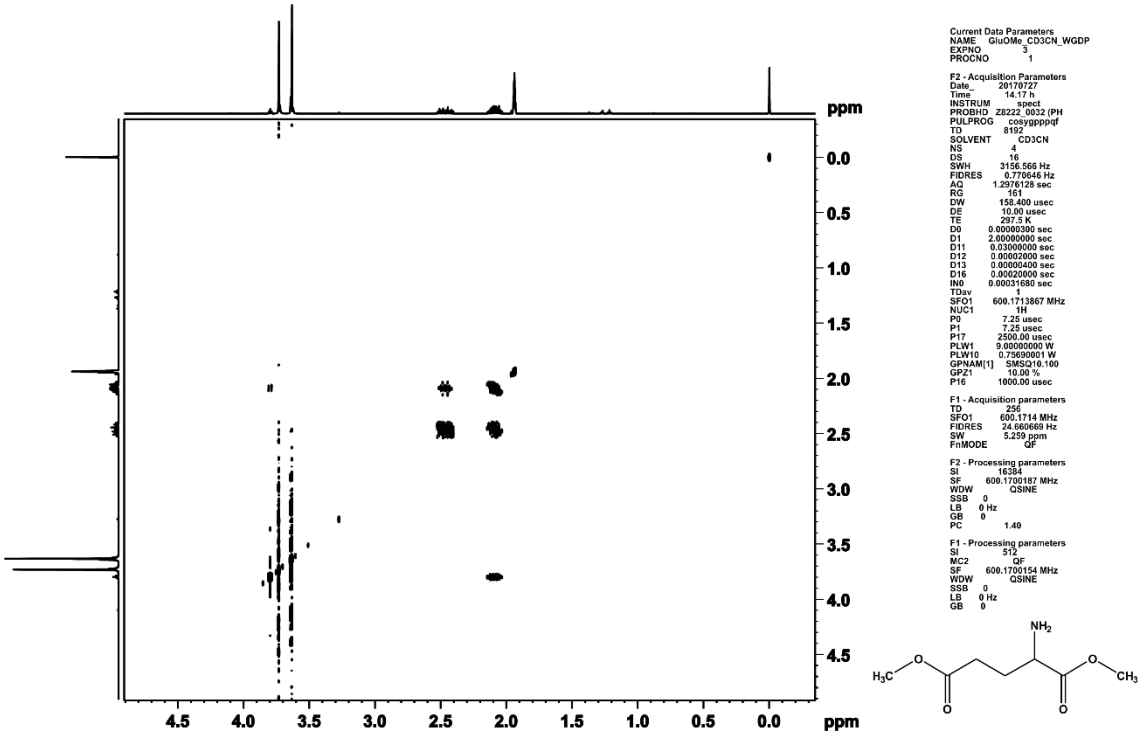


Figure S7 COSY contour plot of GluOMe in CD_3CN.

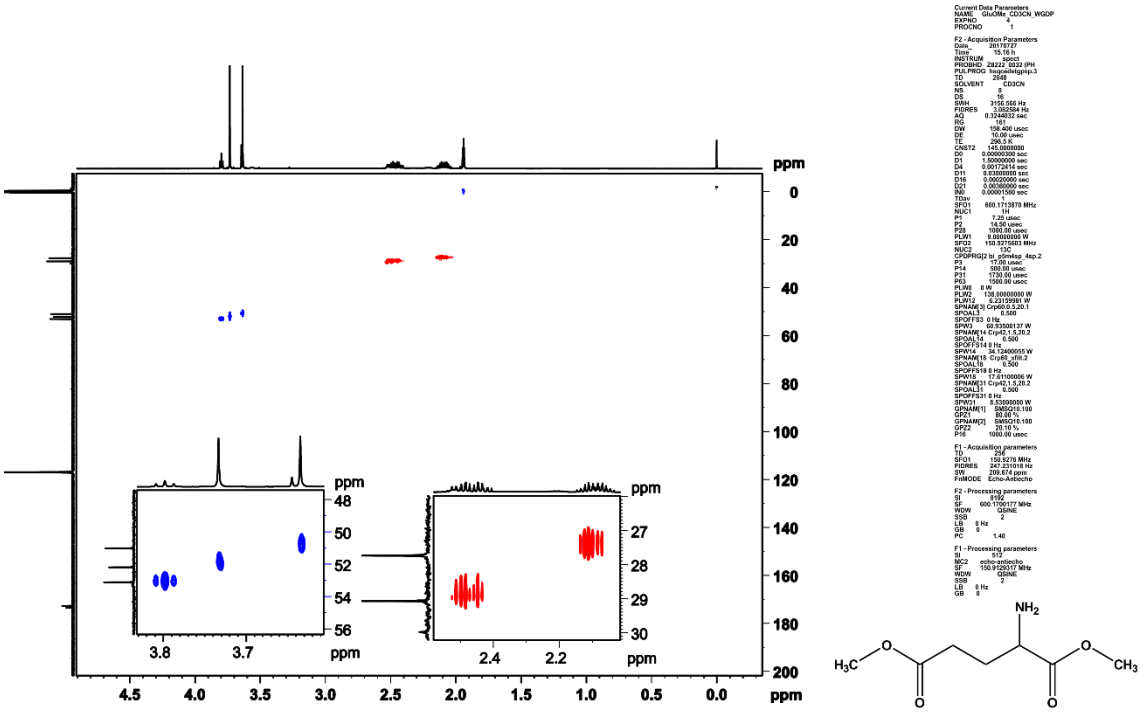


Figure S8 HSQC contour plot of GluOMe in CD_3CN (red cross peaks refer to CH_2 and blue cross peaks refer to CH or CH_3).

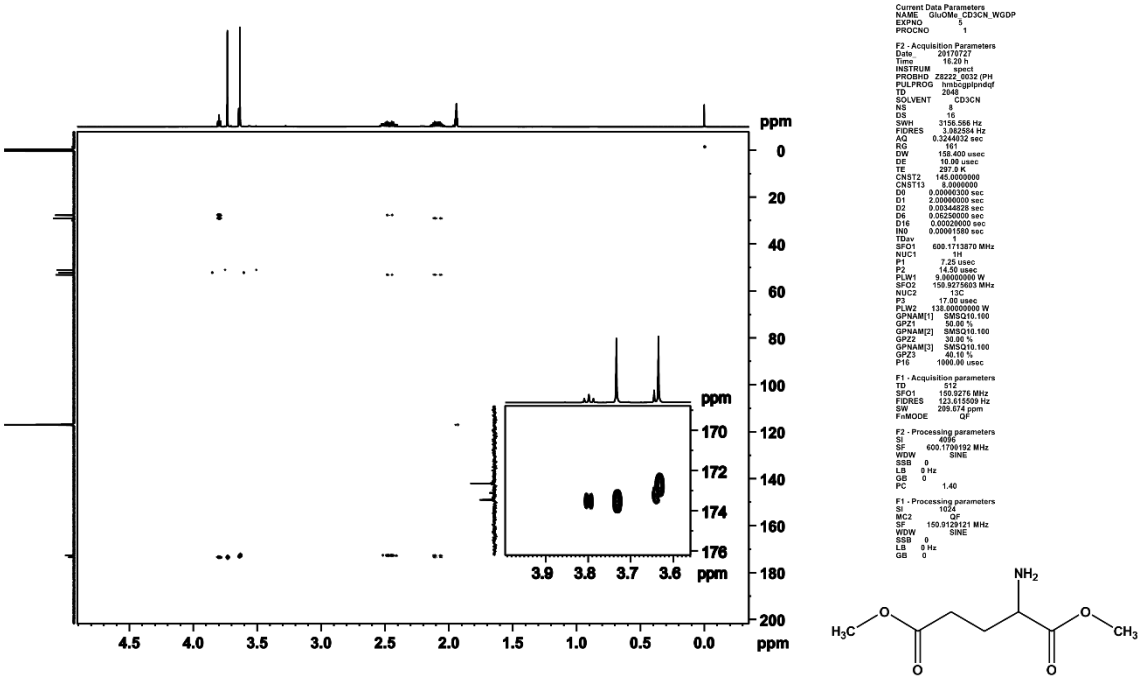


Figure S9 HMBC contour plot of GluOMe in CD_3CN.

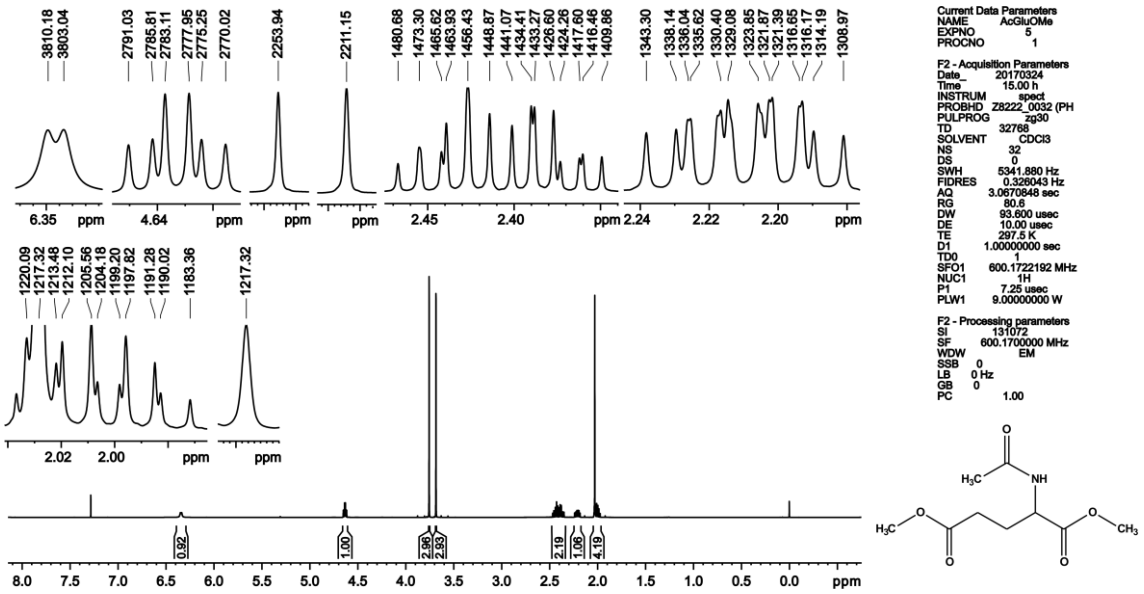


Figure S10 ^1H NMR spectra of AcGluOMe in $CDCl_3$.

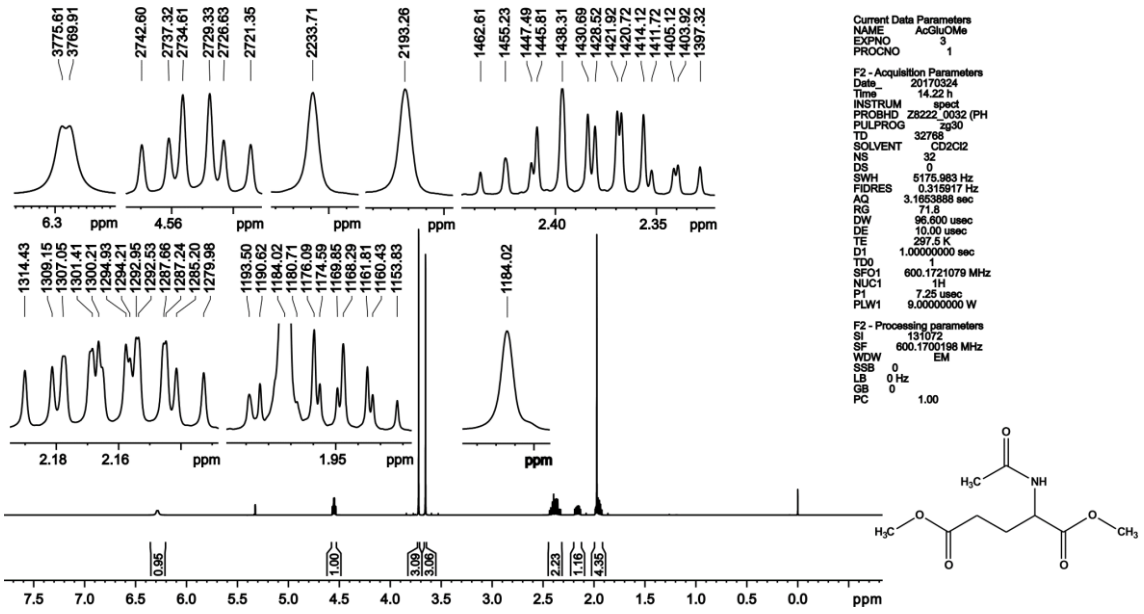


Figure S11 ^{1}H NMR spectra of AcGluOMe in CD_2Cl_2.

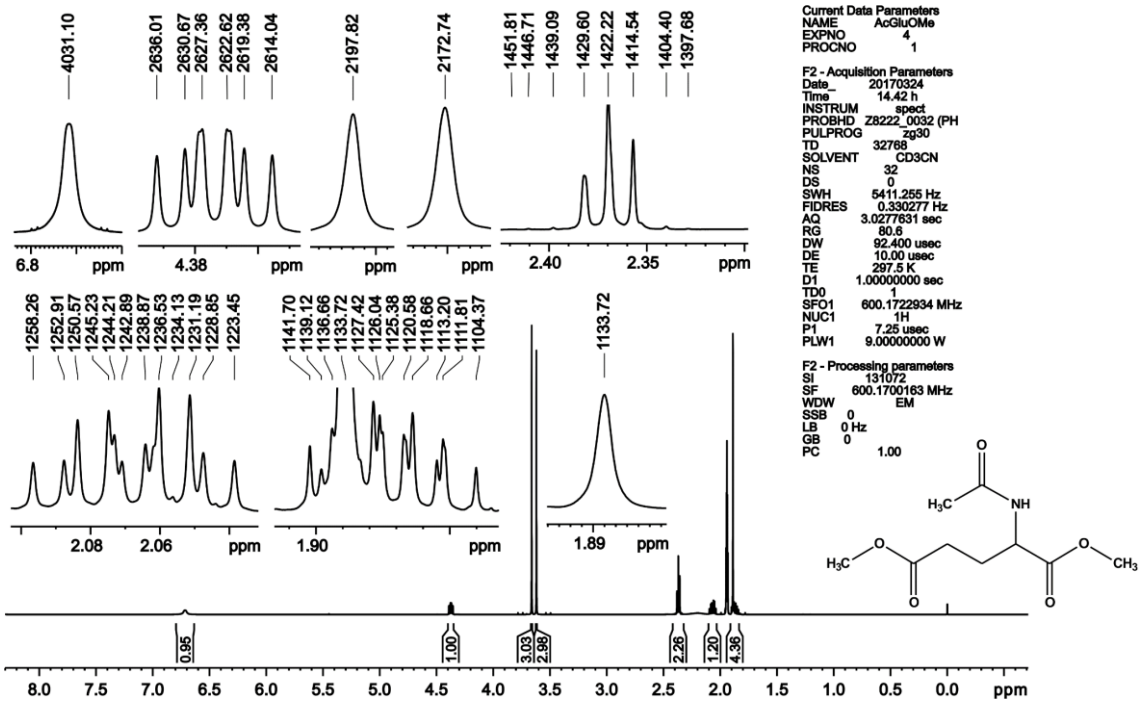


Figure S12 ^{1}H NMR spectra of AcGluOMe in CD_3CN.

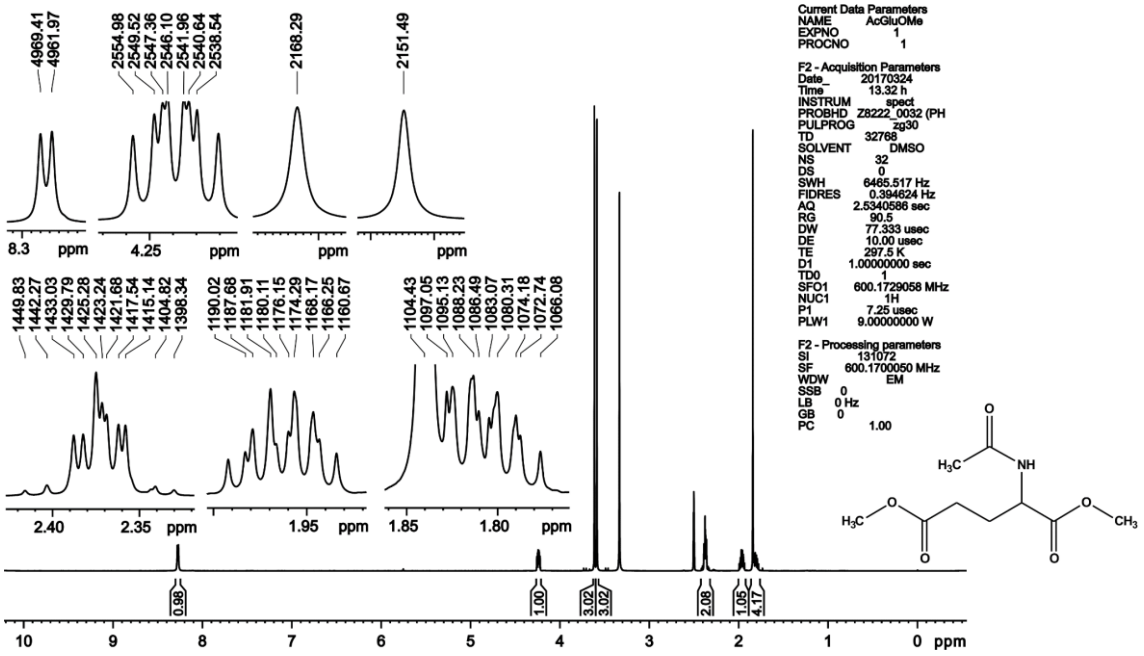


Figure S13 ^{1}H NMR spectra of AcGluOMe in DMSO-d_6.

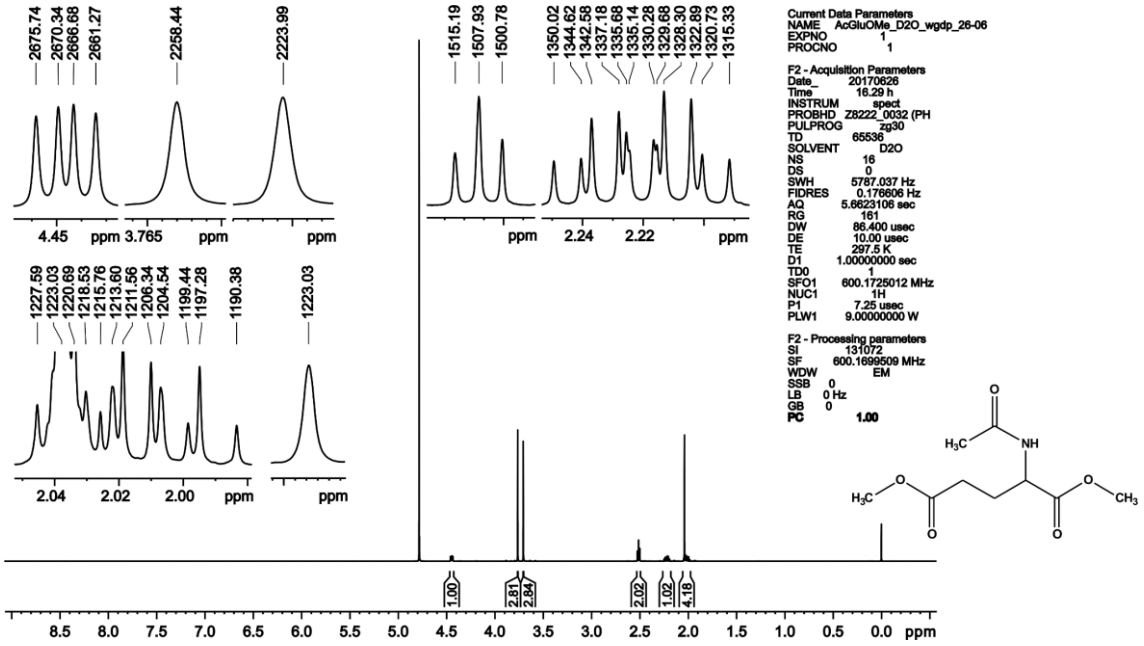


Figure S14 ^{1}H NMR spectra of AcGluOMe in D_2O.

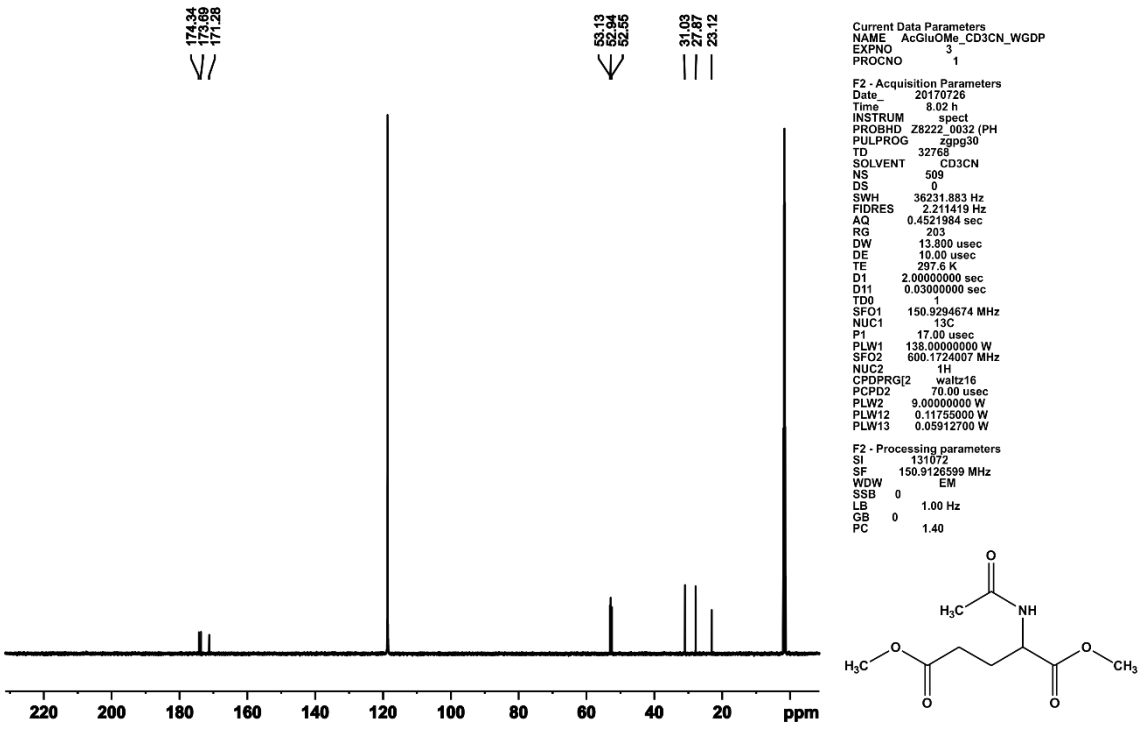


Figure S15 ^{13}C NMR spectra of AcGluOMe in CD_3CN.

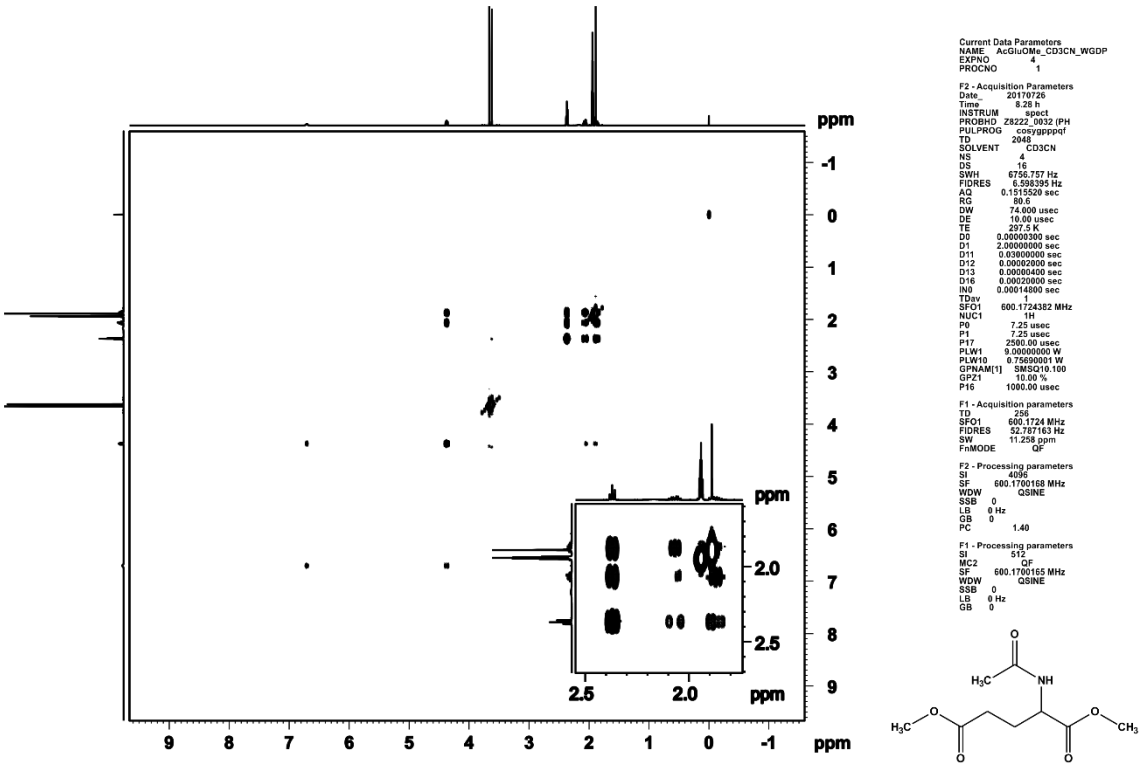


Figure S16 COSY contour plot of AcGluOMe in CD_3CN.

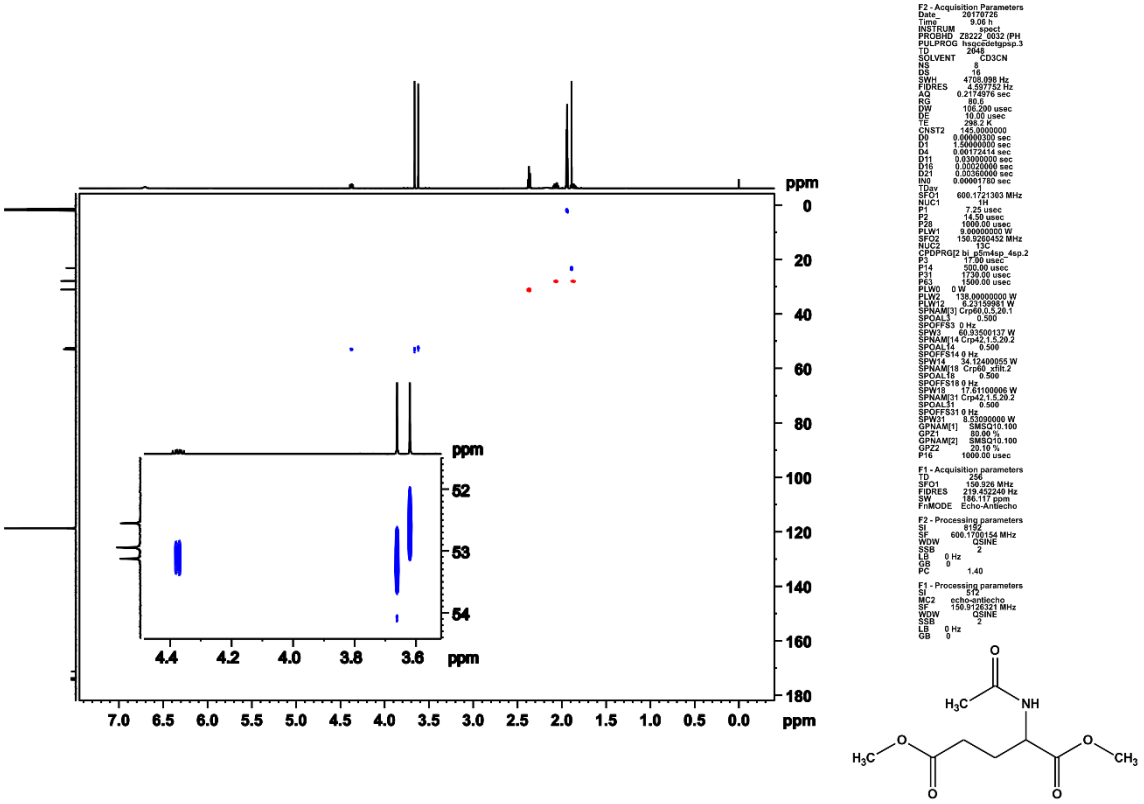


Figure S17 HSQC contour plot of GluOMe in CD_3CN (red cross peaks refer to CH_2 and blue cross peaks refer to CH or CH_3).

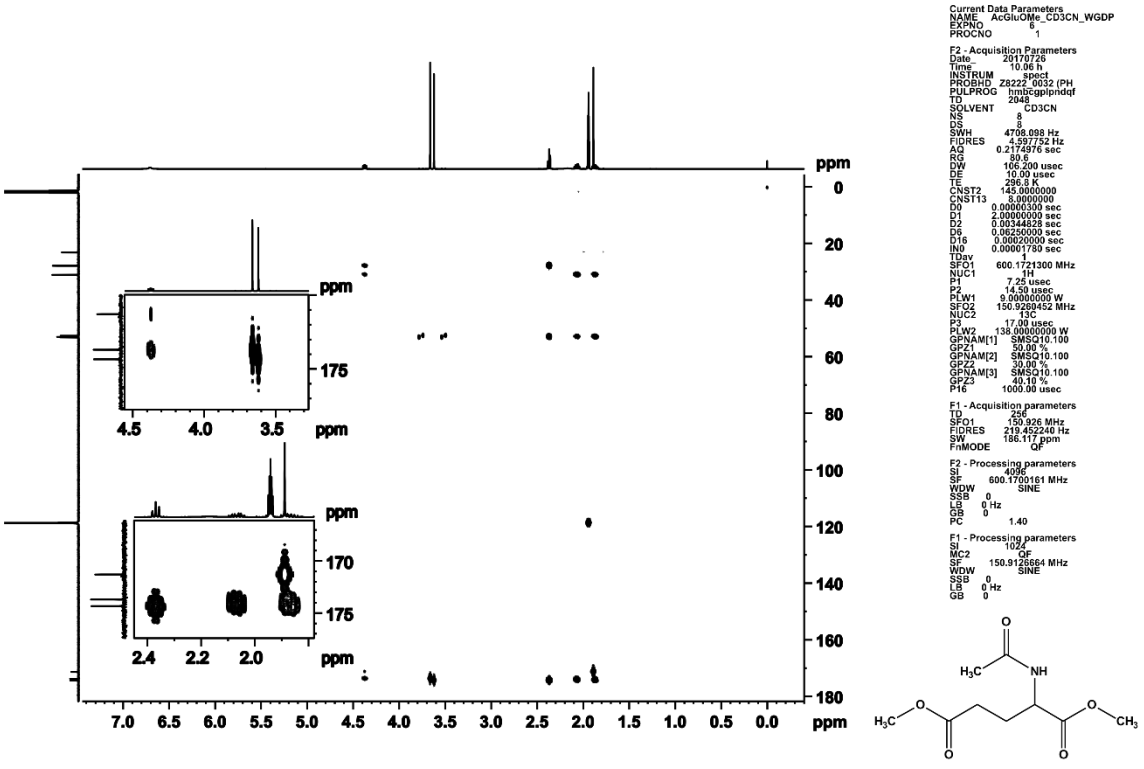


Figure S18 HMBC contour plot of GluOMe in CD_3CN.

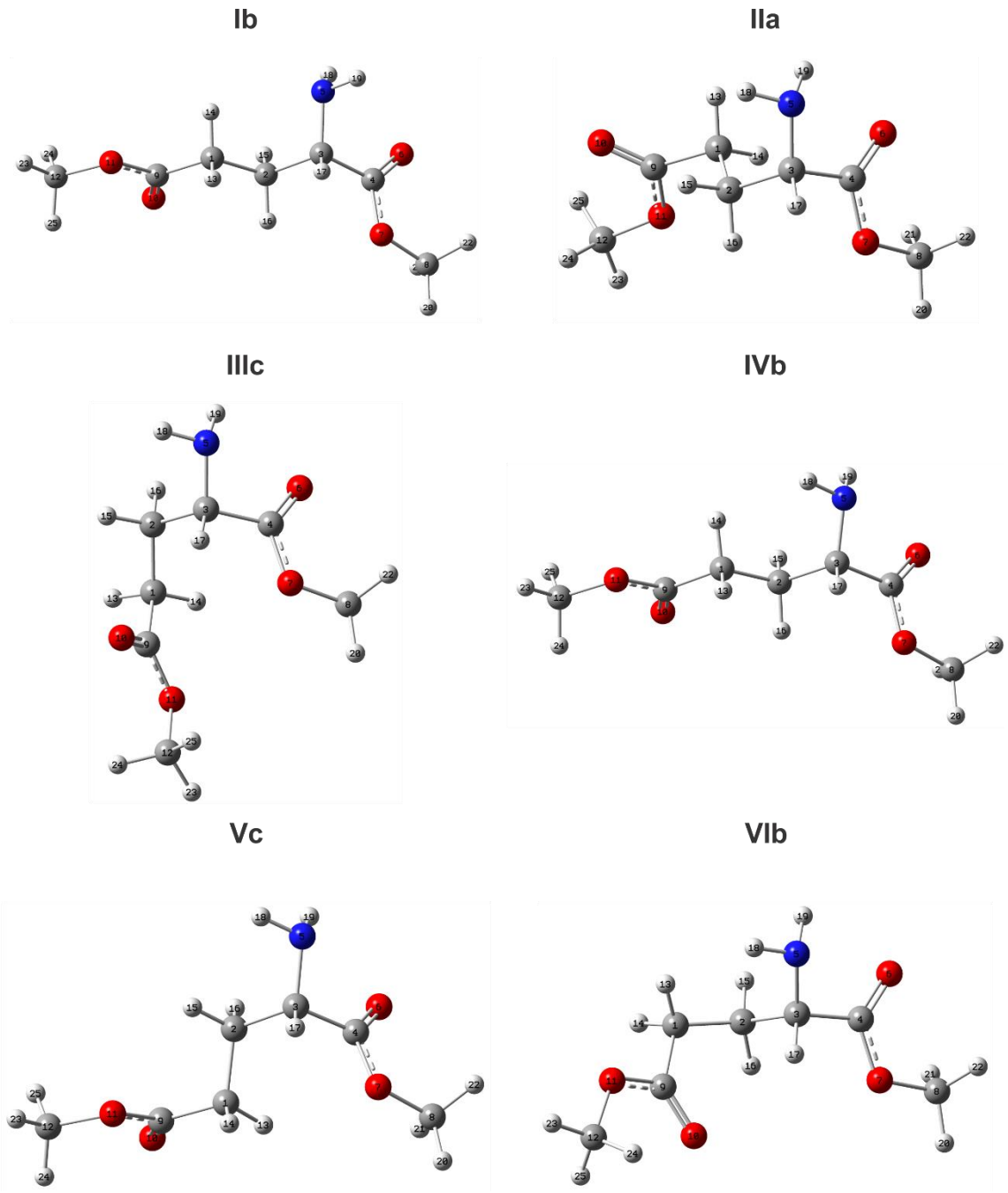


Figure S19 Conformers of GluOMe obtained theoretically at the ωB97X-D/aug-cc-pVTZ level of theory.

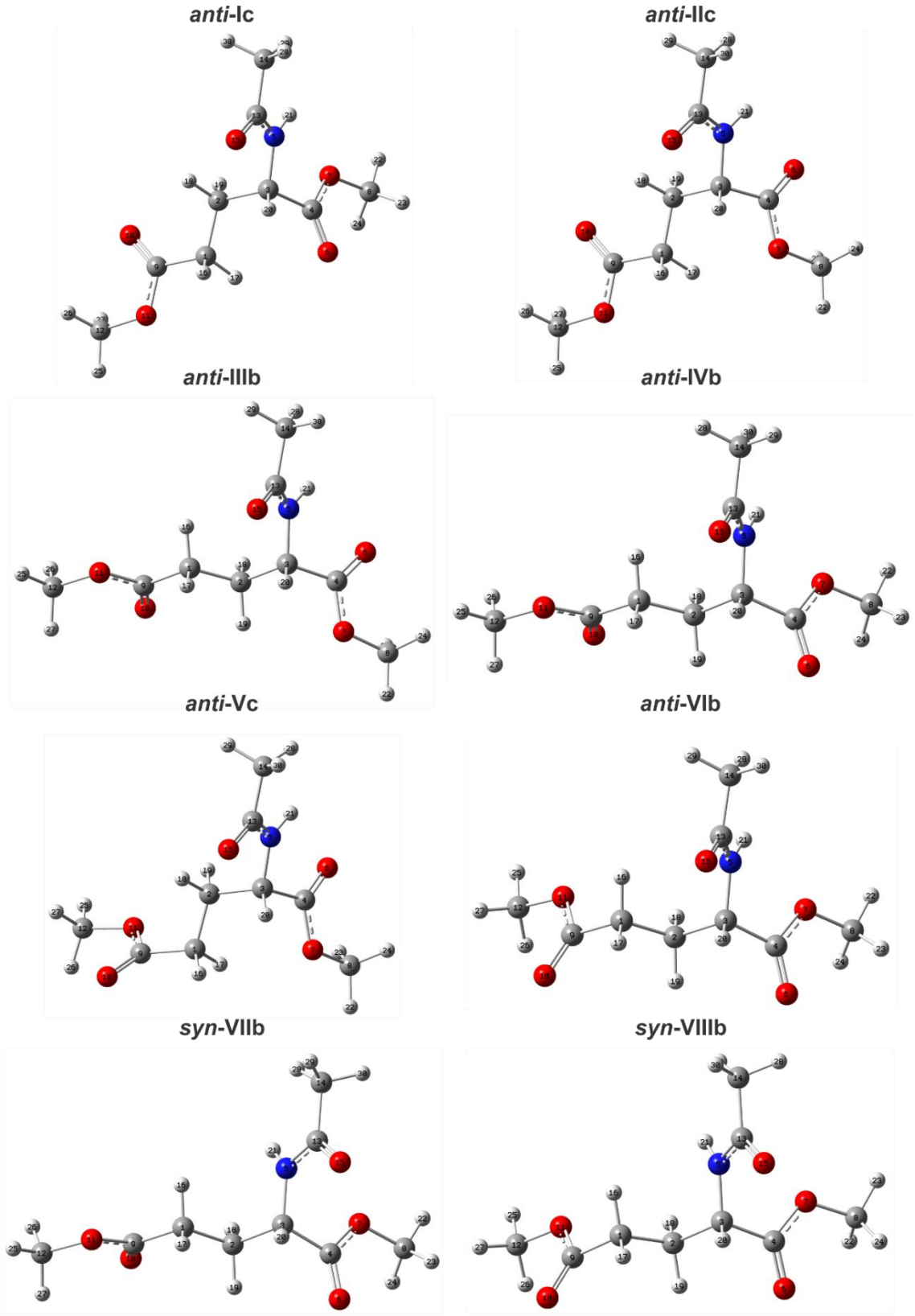


Figure S20 Conformers of AcGluOMe obtained theoretically at the ωB97X-D/aug-cc-pVTZ level of theory.

Table S1 Geometric parameters calculated at the ωB97X-D/aug-cc-pVTZ level of theory for the conformers of GluOMe both in isolated phase and in solution (IEF-PCM). Relative Gibbs free energies are given in kcal mol^{-1}, populations P in % and dihedral angles in degrees.

	Isolated		$CHCl_3$		CH_2Cl_2		CH_3CN		DMSO		Dihedral angle	
Conf.	**ΔG**	**P**	**ΔG**	**P**	**ΔG**	**P**	**ΔG**	**P**	**ΔG**	**P**	**Ha—C—C—H_{b1}**	**Ha—C—C—H_{b2}**
Ib	0.00	79.1	0.00	72.9	0.00	71.5	0.00	63.2	0.00	62.6	-66.90	176.67
IIa	—	—	1.36	7.3	1.59	5.0	1.64	4.0	1.65	3.9	63.46	-52.60
IIIc	1.22	10.1	1.38	7.0	1.45	6.2	1.36	6.3	1.35	6.4	174.82	59.31
IVb	1.98	2.8	1.52	5.6	1.34	7.4	0.97	12.3	0.94	12.8	-64.09	179.30
Vc	2.34	1.5	1.75	3.8	1.46	6.1	1.37	6.2	1.42	5.7	-179.20	64.88
VIb	1.48	6.5	1.81	3.4	1.73	3.8	1.22	8.0	1.17	8.6	-64.37	178.58

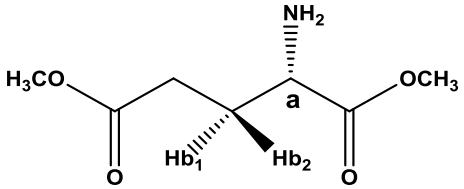
Table S2 Geometric parameters calculated at the ωB97X-D/aug-cc-pVTZ level of theory for the conformers of AcGluOMe both in isolated phase and in solution (IEF-PCM). Relative Gibbs free energies are given in kcal mol^{-1}, populations P in % and dihedral angles in degrees.

	Isolated		$CHCl_3$		CH_2Cl_2		CH_3CN		DMSO		Dihedral angle	
Conf.	**ΔG**	**P**	**ΔG**	**P**	**ΔG**	**P**	**ΔG**	**P**	**ΔG**	**P**	**Ha—C—C—H_{b1}**	**Ha—C—C—H_{b2}**
***anti*-Ic**	1.3	5.4	0.0	28.6	0.9	5.9	1.0	6.0	1.3	4.4	-174.15	70.34
***anti*-IIc**	0.0	47.5	0.0	27.4	0.0	29.2	0.0	32.6	0.3	27.1	-174.30	70.58
***anti*-IIIb**	0.2	36.3	0.5	11.8	0.9	6.6	0.9	7.0	1.0	8.2	-62.94	-179.52
***anti*-IVb**	1.5	3.8	0.5	11.4	0.0	28.9	0.0	30.5	0.0	41.8	-67.85	175.62
***anti*-Vc**	1.2	6.1	0.7	8.2	1.0	4.9	1.1	5.0	1.4	4.2	-175.25	69.11
***anti*-VIb**	2.3	0.9	0.9	6.6	0.7	9.0	1.3	3.7	1.3	4.8	-66.17	176.62
***syn*-VIIb**	—	—	1.2	4.0	0.5	12.8	1.0	6.4	1.0	7.7	69.10	174.67
***syn*-VIIIb**	—	—	1.5	2.1	1.4	2.7	0.8	8.9	1.8	1.9	-67.77	175.58

Table S3 Calculated populations (ωB97X D/aug-cc-pVTZ) of conformers **a**, **b** and **c** in the conformational equilibrium of GluOMe in both isolated phase and in different solvents ($CHCl_3$, $CH2Cl_2$, CH_3CN and DMSO).

Conformers	Isolated	$CHCl_3$	CH_2Cl_2	CH_3CN	DMSO
a	0.0	7.3	5.0	4.0	3.9
b	88.4	81.9	82.7	83.5	84.0
c	11.6	10.8	12.3	12.5	12.1

Table S4 Experimental and theoretical ^{1}H NMR data obtained for GluOMe in the different studied solvents. Chemical shifts (δ) and $^{3}J_{HH}$ coupling constants are given in ppm and Hz, respectively. ε is the solvent dielectric constant.



Solvent	ε	δH_a	$^{3}J_{HaHb1,calc}$	$^{3}J_{HaHb2,calc}$	**Average**	$^{3}J_{HaHb1,obs}$[α]	$^{3}J_{HaHb2,obs}$[α]
$CDCl_3$	4.8	3.99	3.3	10.3	6.8	6.2	6.2
CD_2Cl_2	9.1	3.96	3.3	10.2	6.7	6.3	6.3
CD_3CN	37.5	3.82	3.2	10.1	6.6	6.4	6.4
DMSO-d_6	46.7	3.74	3.2	10.2	—	6.4	7.0

[α] Error in measurements of $J = \pm 0.1$ Hz.

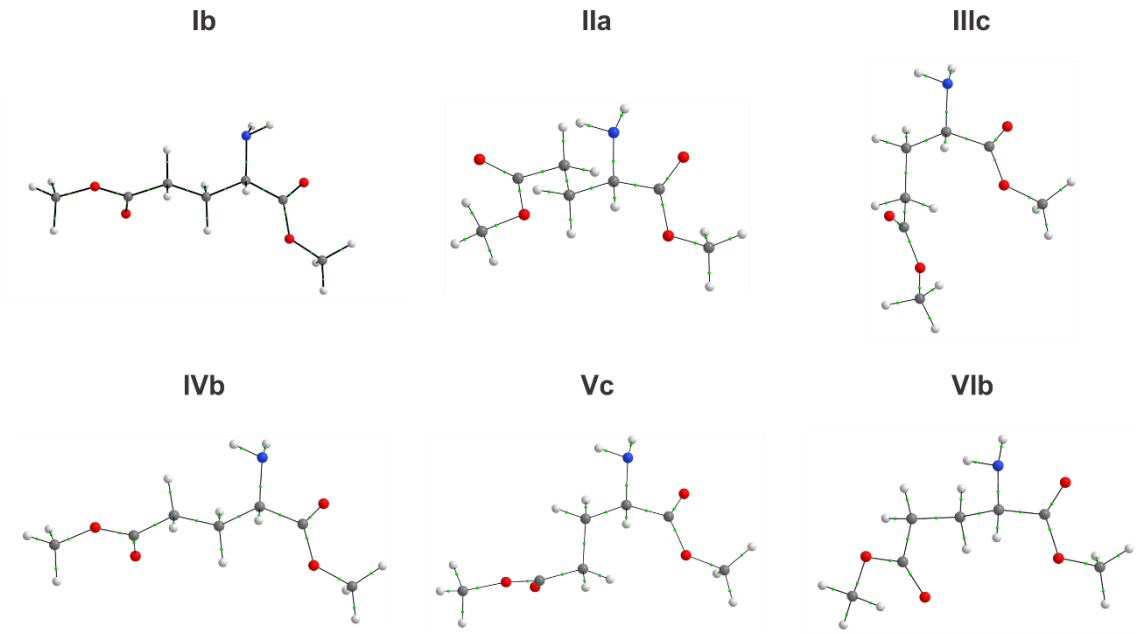


Figure S21 QTAIM molecular graphs for the conformers of GluOMe.

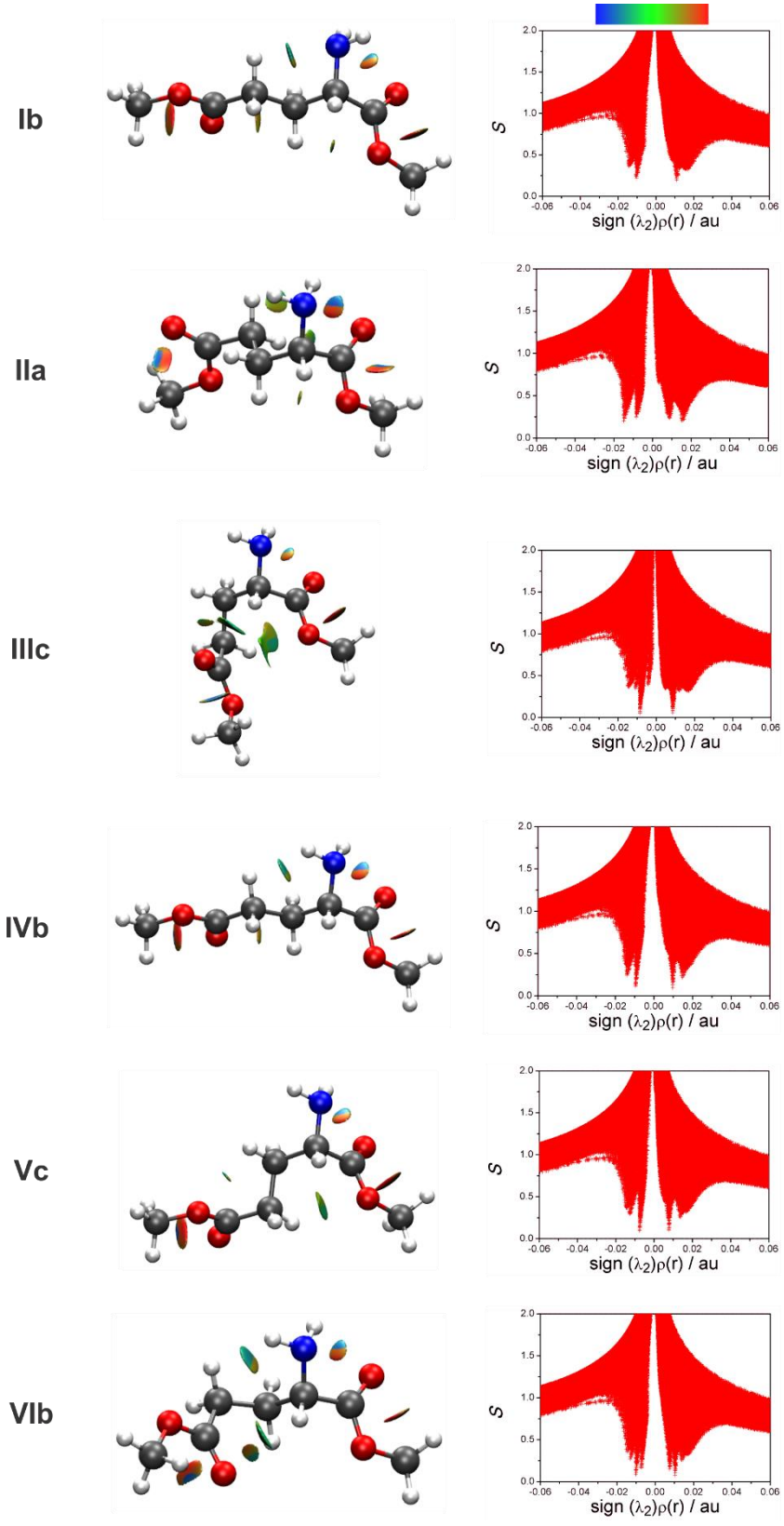


Figure S22 NCI isosurfaces generated with S = 0.5 au and blue-green-red scaling from -0.02 < $(\lambda_2)\rho(r)$ < 0.02 au, and NCI plots of the reduced density gradient *S* versus sign $(\lambda_2)\rho(r)$ for the conformers of GluOMe.

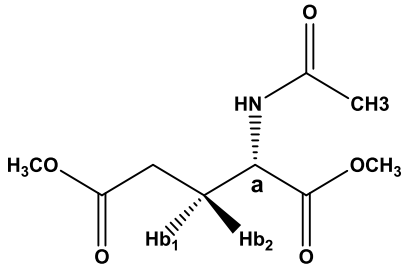
Table S5 NBO parameters obtained at the ωB97X-D/aug-cc-pVTZ level for the conformers of GluOMe. Lewis, hyperconjugative and second order perturbation $LP_2(O)\rightarrow\sigma^*_{NH}$ energies are given in kcal mol^{-1}. The energy threshold for the interactions is equal to 0.05 kcal mol^{-1}.

Conformer	Isolated		$CHCl_3$		DMSO			
	E_{Lewis}	E_{Hyper}	E_{Lewis}	E_{Hyper}	E_{Lewis}	E_{Hyper}	$LP_2(O)\rightarrow\sigma^*_{N5H18}$[a]	$LP_2(O)\rightarrow\sigma^*_{N5H19}$[a]
Ib	0.00	2.52	0.74	2.87	0.82	2.60	0.14	—
IIa	5.54	7.07	6.42	7.75	6.54	7.71	0.11	0.21
IIIc	6.92	7.56	5.81	6.51	4.28	5.13	0.19	0.30
IVb	—	—	4.06	4.52	3.52	3.87	0.27	0.36
Vc	0.15	0.00	0.00	0.00	0.00	0.00	0.11	0.35
VIb	4.33	4.73	2.60	3.17	1.59	2.28	0.20	0.39

Table S6 Calculated populations (ωB97X-D/aug-cc-pVTZ) of conformers **b** and **c** in the conformational equilibrium of AcGluOMe in both isolated phase and in different aprotic solvents ($CHCl_3$, CH_2Cl_2, CH_3CN and DMSO).

Conformers	**Isolated**	**$CHCl_3$**	**CH_2Cl_2**	**CH_3CN**	**DMSO**
b	41.0	35.9	60.0	56.5	64.4
c	59.0	64.1	40.0	43.5	35.6

Table S7 Experimental and theoretical ^{1}H NMR data obtained for AcGluOMe in the different studied solvents. Chemical shifts (δ) and $^{3}J_{HH}$ coupling constants are given in ppm and Hz, respectively. **ε** is the solvent dielectric constant.



Solvent	**ε**	**δH$_a$**	**$^{3}J_{HaHb1,obs}$**[α]	**$^{3}J_{HaHb1,calc}$**	**$^{3}J_{HaHb2,obs}$**[α]	**$^{3}J_{HaHb2,calc}$**
$CDCl_3$	4.8	4.63	7.9	8.2	5.2	6.7
CD_2Cl_2	9.1	4.55	5.3	5.9	8.0	8.8
CD_3CN	37.5	4.37	5.3	6.3	8.7	8.5
DMSO-d_6	46.7	4.24	5.4	5.6	8.9	9.1
D_2O	78.5	4.36	5.3	—	9.1	—

[α] Error in measurements of $J = \pm 0.2$ Hz.

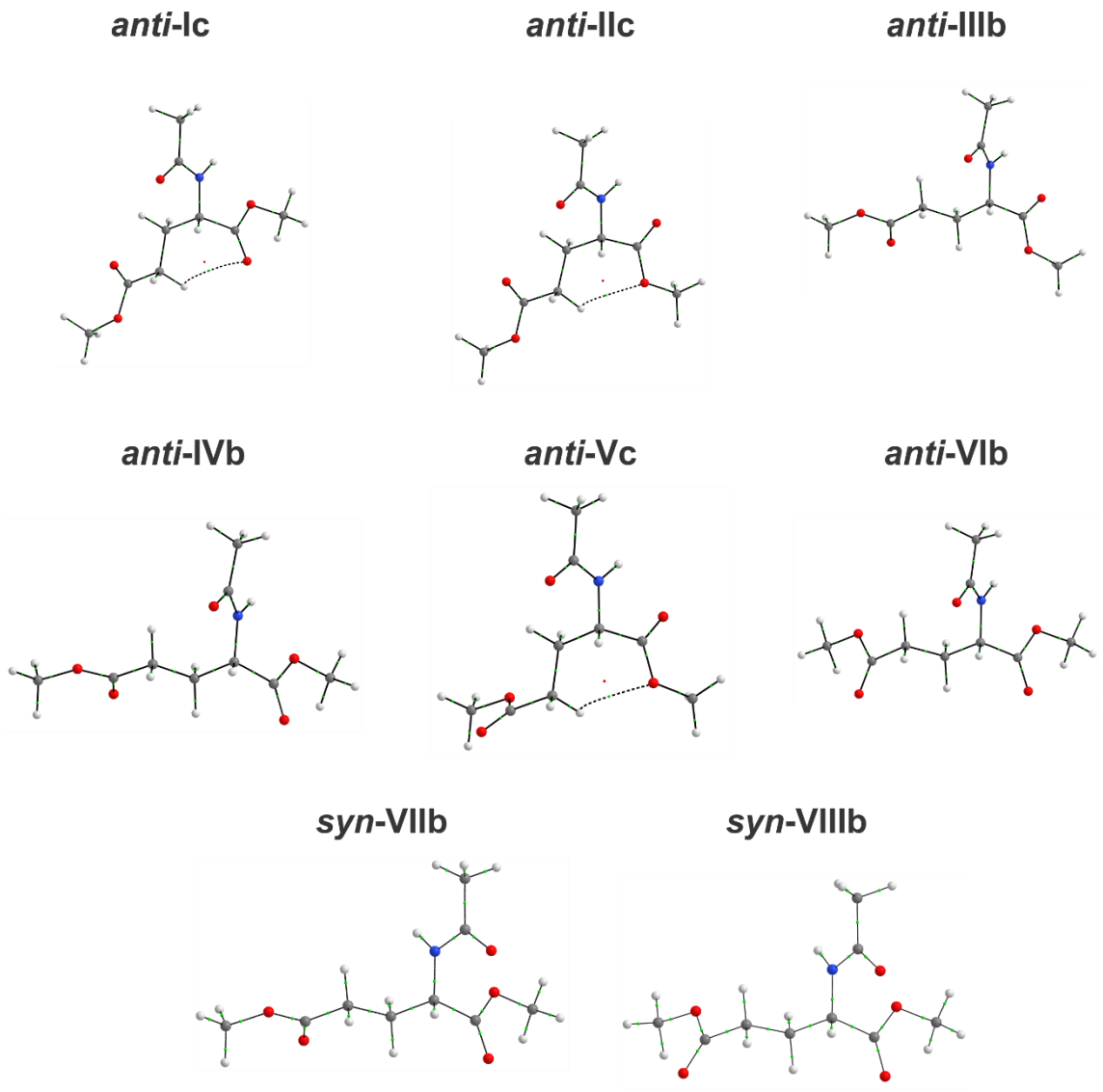
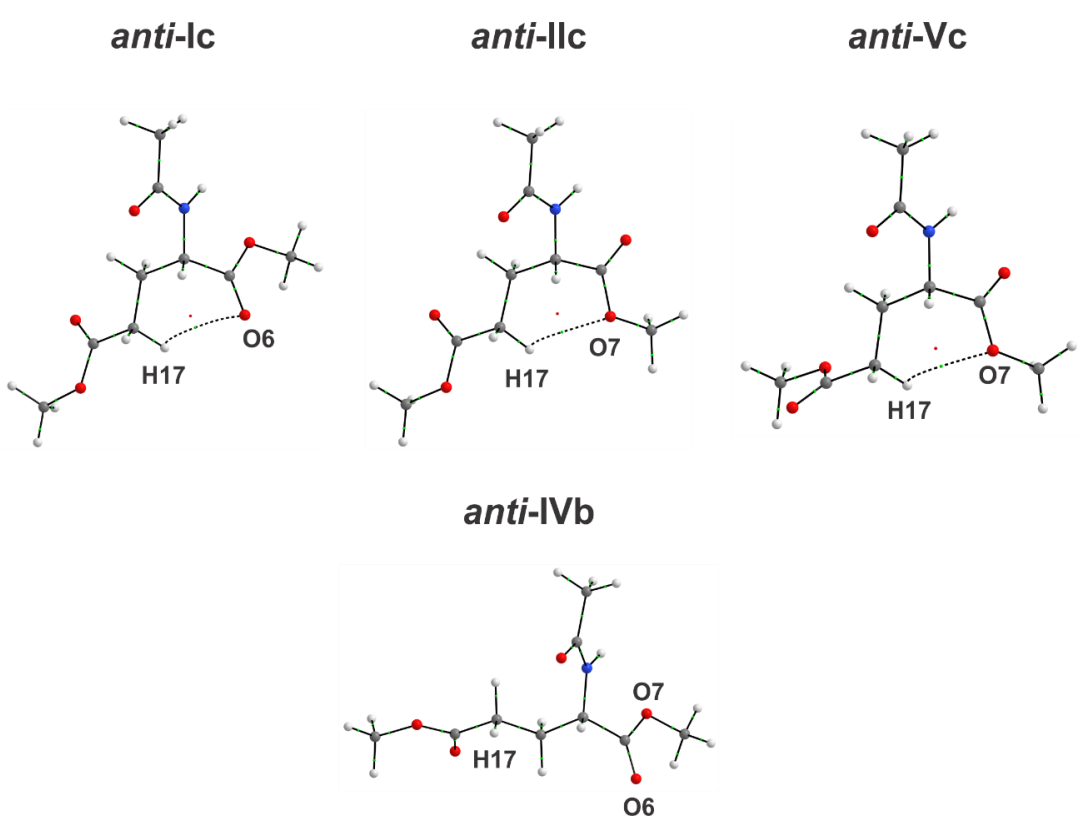


Figure S23 QTAIM molecular graphs for the conformers of AcGluOMe.

Table S8 QTAIM parameters (in au) for the conformers *anti*-Ic, *anti*-IIc, anti-Vc and *anti*-IVb (reference).

Parameter	***anti-Ic***		***anti-IIc***		***anti-Vc***		***anti-IVb***
ρ(r)	+0.0098		+0.0106		+0.0102		—
$\nabla^2\rho(r)$	+0.0367		+0.0399		+0.0382		—
ε	+0.7271		+0.2233		+0.2695		—
	H17	**O6**	**H17**	**O7**	**H17**	**O7**	**H17**
***q*(H)**	+0.0499	—	+0.0389	—	+0.0278	—	+0.0412
***M*$_1$(H)**	+0.1232	—	+0.1237	—	+0.1269	—	+0.1306
***E*(H)**	-0.6058	—	-0.6125	—	-0.6170	—	-0.6093
***V*(H)**	+43.01	—	+43.21	—	+44.09	—	+45.85
***r*^{0}**	2.53	3.28	2.53	3.37	2.53	3.37	—
r	2.04	2.79	1.97	2.70	2.05	2.73	—
Δ*r*	0.46	0.50	0.53	0.64	0.45	0.60	—

ρ(r) = electron density; $\nabla^2\rho(r)$ = laplacian of the electron density; ε = ellipticity; *q*(H) = atomic electric charge; *M*$_1$(H) = atomic first dipole moment; *E*(H) = atomic energy; *V*(H) = atomic volume; r^0 = van der Waals radius of the atom when involved in a hydrogen bond; r = van der Waals radius of the atom when not involved in a hydrogen bond; $\Delta r = r^0 - r$.



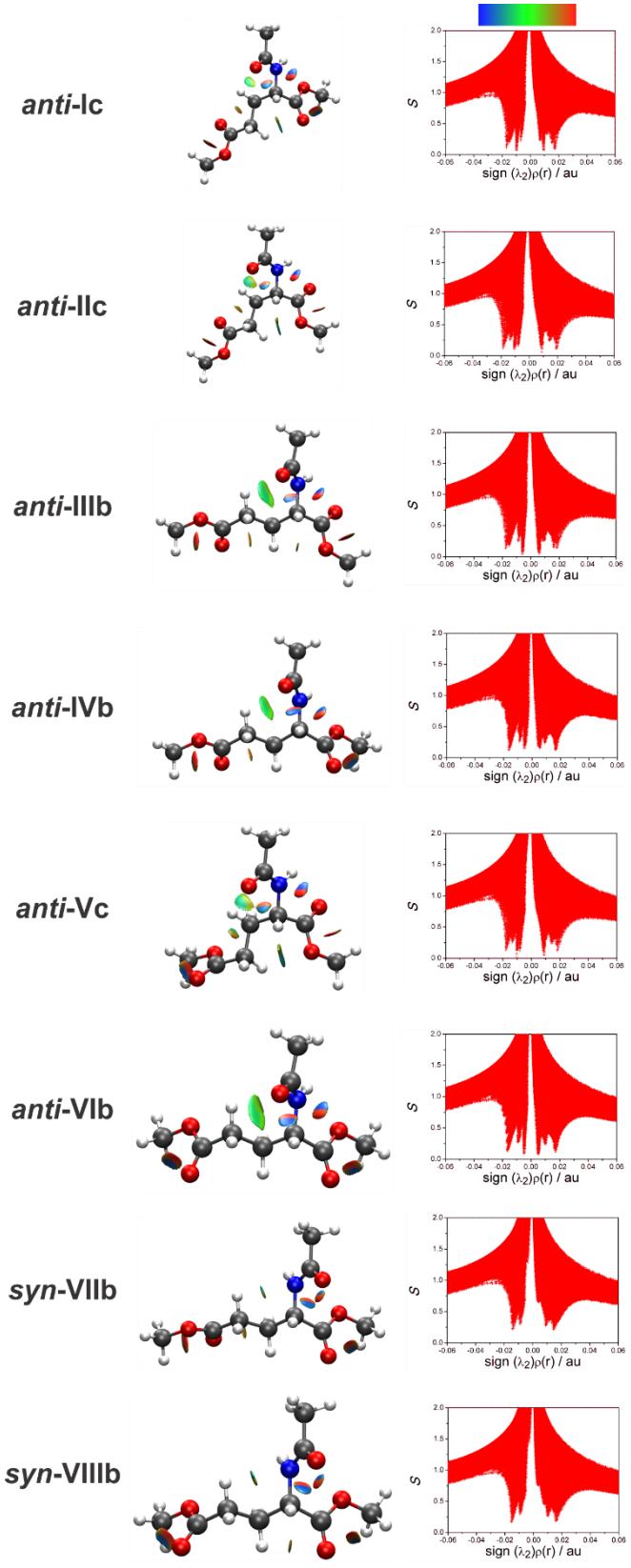


Figure S24 NCI isosurfaces generated with S = 0.5 au and blue-green-red scaling from -0.02 < (λ_2)ρ(r) < 0.02 au, and NCI plots of the reduced density gradient *S* versus sign (λ_2)ρ(r) for the conformers of AcGluOMe.

Table S9 Hyperconjugative interactions, in kcal mol^{-1}, for the conformers of AcGluOMe obtained through the NBO calculations at the ωB97X-D/aug-cc-pVTZ level of theory. The energy threshold for the interactions is equal to 0.05 kcal mol^{-1}.

Conf.	$LP_1(O6) \rightarrow \sigma^*_{N5H21}$	$LP_2(O6) \rightarrow \sigma^*_{N5H21}$	$LP_1(O7) \rightarrow \sigma^*_{N5H21}$	$LP_2(O7) \rightarrow \sigma^*_{N5H21}$	$LP_1(O) \rightarrow \sigma^*_{C1H17}$	$LP_2(O) \rightarrow \sigma^*_{C1H17}$
***anti*-Ic**	—	—	0.59	—	0.08	0.09
***anti*-IIc**	0.29	1.00	—	—	0.26	0.14
***anti*-IIIb**	0.21	0.81	—	—	—	—
***anti*-IVb**	—	—	0.39	—	—	—
***anti*-Vc**	0.25	0.88	—	—	0.05	0.16
***anti*-VIb**	—	—	0.42	—	—	—
***syn*-VIIb**	—	—	—	—	—	—
***syn*-VIIIb**	—	—	—	—	—	—

Table S10 NBO parameters obtained at the ωB97X-D/aug-cc-pVTZ level for the conformers of AcGluOMe. Lewis and hyperconjugative energies are given in kcal mol^{-1}.

Conf.	Isolated		$CHCl_3$		DMSO	
	$E_{rel,Lewis}$	$E_{rel,Hyper}$	$E_{rel,Lewis}$	$E_{rel,Hyper}$	$E_{rel,Lewis}$	$E_{rel,Hyper}$
***anti*-Ic**	0.69	1.38	0.64	0.91	0.00	0.00
***anti*-IIc**	5.02	7.22	4.42	5.87	3.30	4.28
***anti*-IIIb**	6.43	9.93	5.53	8.00	3.40	5.26
***anti*-IVb**	3.36	4.49	2.81	3.67	2.63	3.40
***anti*-Vc**	2.72	3.84	2.50	3.06	1.86	1.99
***anti*-VIb**	0.00	0.00	0.00	0.00	1.30	1.32
***syn*-VIIb**	—	—	2.75	3.66	3.64	4.76
***syn*-VIIIb**	—	—	0.39	0.40	0.02	0.24