Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.
This journal is © the Owner Societies 2015

Supporting Information for:

Serine *O*-sulfation probed by IRMPD spectroscopy

Roberto Paciotti,[1] Cecilia Coletti,[1] Nazzareno Re,[1] Debora Scuderi,[2] Barbara Chiavarino,[3] Simonetta Fornarini,[3,*] and Maria Elisa Crestoni[3,*]

[1] *Dipartimento di Farmacia, Università G. D'Annunzio, Via dei Vestini 31, I-66100 Chieti, Italy*
[2] *Laboratoire de Chimie Physique d'Orsay, Universitè Paris Sud, UMR8000 CNRS, Facultè des Sciences, Bât 350, 91405 Orsay Cedex, France*
[3] *Dipartimento di Chimica e Tecnologie del Farmaco, Università degli Studi di Roma La Sapienza, P.le A. Moro 5, I-00185 Roma, Italy*

Corresponding authors:
*Tel: +39 06 4991 3596. Fax: +39 06 4991 3602.
Email: mariaelisa.crestoni@uniroma1.it
*Tel: +39 06 4991 3510. Fax: +39 06 4991 3602.
Email: simonetta.fornarini@uniroma1.it

Contents :

. **Computational Details.** Any additional computational detail and material (xyz files of computed structures, calculated vibrational frequencies for all structures, etc.) is available from the authors upon request.

. **Fig. S1** Reproducibility of the IRMPD spectra of protonated O-phosphotyrosine (m/z 262) recorded in the 3250-3700 cm^{-1} range on two experimental set-ups: (a) a modified commercial Paul-ion trap mass spectrometer (Bruker Esquire 3000+) coupled to a tabletop IR optical oscillator /amplifier (OPO/OPA; laserVison, Bellevue, WA) pumped by an Innolas Splitlight 600 (Munchen Germany) non-seed Nd:YAG laser running at 25 Hz; (b) a modified commercial Paul-ion trap mass spectrometer (Bruker Esquire 6000+) coupled to a tabletop IR optical oscillator/amplifier (OPO/OPA; laserVison, Bellevue, WA) pumped by a Nd:YAG laser (Continuum Surelite II) running at 10 Hz. The irradiation time was 500 ms in both experiments.

. **Fig. S2** Performance of different levels of theory in the simulation of the experimental IRMPD spectrum of the **St-1** conformer of [sSer-H]$^-$ ion. Two functionals (B3LYP and M06-2X) and different basis sets were tested. The employed scale factors are reported on the right and were taken from J. P. Merrick, D. Moran, L. Radom, *J. Phys. Chem. A*, 2007, **111**, 11683-11700 for the B3LYP functional and from I. M. Alecu, J. Zheng, Y. Zhao, and D. G. Truhlar, *J. Chem. Theory Comput,* 2010, **6**, 2872-2887 for the M06-2X functional. S-O stretching modes were left unscaled. The experimental spectrum is also reported as a pale trace over the calculated spectra to enable immediate comparison.

. **Fig. S3** Mass spectrum obtained after mass selection of deprotonated O-sulfo-L-serine, [sSer-H]$^-$ (m/z 184), in a Bruker Esquire Paul trap mass spectrometer (Esquire 3000+, Bruker Inc.) before (upper trace) and after (bottom trace) irradiation with CLIO FEL IR radiation at 1206 cm^{-1}. Ion intensity is in arbitrary units.

. **Fig. S4** Mass spectrum obtained after mass selection of protonated O-sulfo-L-serine, [sSer+H]$^+$ (m/z 186), in a Bruker Esquire Paul trap mass spectrometer (Esquire 6000+, Bruker Inc.) before (upper trace) and after (bottom trace) irradiation with OPO/OPA IR radiation at 3572 cm^{-1}. Ion intensity is in arbitrary units.

. **Fig. S5** Experimental IRMPD spectrum of deprotonated L-serine-O-sulfate [sSer-H]$^-$ (bottom, red line) in the 2900-3700 cm^{-1} range, and computed IR spectra (at the M06-2X/6-311+G(d,p) level) of conformers **S*t*-1**, **S*t*-2**, **S*t*-3**, **S*t*-4**, **S*t*-5**. The experimental spectrum is also reported as a pale trace over the calculated spectra to enable immediate comparison.

. **Fig. S6** Optimized geometries (at the M06-2X/6-311+G(d,p) level) and relative enthalpy and free energy (in parentheses) values (at the MP2/aug-cc-pVTZ level) at 298 K (kJ mol^{-1}) of [sSer-H]$^-$ conformers **S*t*-7, S*t*-8**, **S*t*-9, S*t*-10**, **S*t*-11, S*t*-12**. Interatomic bond distances, marked by dashed lines, are given in Å.

. **Fig. S7** Optimized geometries (at the M06-2X/6-311+G(d,p) level) and relative enthalpy and free energy (in parentheses) values (at the MP2/aug-cc-pVTZ level) at 298 K (kJ mol^{-1}) of [sSer-H]$^-$ isomers **S*t*-1**, **ZW-1**, **S*c*-1**, **C-1**. Interatomic bond distances, marked by dashed lines, are given in Å.

. **Fig. S8** Computed IR spectra (at the M06-2X/6-311+G(d,p) level) of deprotonated L-serine-O-sulfate [sSer-H]$^-$ of conformers **S*t*-6, S*t*-7**, **S*t*-8, S*t*-9**. The experimental spectrum is also reported as a pale trace over the calculated spectra to enable immediate comparison.

. **Fig. S9** Computed IR spectra (at the M06-2X/6-311+G(d,p) level) of deprotonated L-serine-O-sulfate [sSer-H]$^-$ of conformers **S*t*-10, S*t*-11, S*t*-12**. The experimental spectrum is also reported as a pale trace over the calculated spectra to enable immediate comparison.

. **Fig. S10** Averaged spectrum of deprotonated L-serine-O-sulfate [sSer-H]$^-$ (bottom), computed by considering contributions from all thermally accessible conformers, according to their enthalpy values, and computed IR spectra (at the M06-2X/6-311+G(d,p) level) of isomers , **ZW-1**, **S*c*-1**, **C-1**. The experimental spectrum is also reported as a pale trace over the calculated spectra to enable immediate comparison.

. **Fig. S11** Averaged spectrum of deprotonated L-serine-O-sulfate [sSer-H]$^-$, computed by considering contributions from all thermally accessible conformers, according to their relative free energy values. The experimental spectrum (red line) is also reported to enable immediate comparison.

. **Fig. S12** Optimized geometries (at the M06-2X/6-311+G(d,p) level) and relative enthalpy and free energy (in parentheses) values (at the MP2/aug-cc-pVTZ level) at 298 K (kJ mol^{-1}) of [sSer+H]$^+$ isomers **N*c*-1**, **N*t*-1**, **CO-1**, **SOH-1**. Interatomic bond distances, marked by dashed lines, are given in Å.

. **Fig. S13** Averaged spectrum of protonated L-serine-O-sulfate [sSer+H]$^+$, computed by considering contributions from all thermally accessible conformers, according to their relative free energy values. The experimental spectrum (red line) is also reported to enable immediate comparison.

. **Fig. S14** Computed IR spectra (at the M06-2X/6-311+G(d,p) level) of conformers **N*c*-1**, **N*c*-2, N*c*-3,** and **N*c*-4** of protonated L-serine-O-sulfate [sSer+H]$^+$. The experimental spectrum is also reported as a pale trace over the calculated spectra to enable immediate comparison.

. **Fig S15** Computed IR spectra (at the M06-2X/6-311+G(d,p) level) of isomers **N*c*-1**, **N*t*-1**, **CO-1**,and **SOH-1** of protonated L-serine-O-sulfate [sSer+H]$^+$. The experimental spectrum is also reported as a pale trace over the calculated spectra to enable immediate comparison.

. **Table S1** Thermodynamic data for the lowest energy conformers and isomers of protonated L-serineO-sulfate [sSer+H]$^+$ and deprotonated L-serine-O-sulfate [sSer-H]$^-$ calculated at the MP2/aug-cc-pVTZ//M06-2X/6-311+G(d,p) level of theory.

. **Table S2** Selected dihedral angles (°) of low-lying conformers (**S*t*-1, S*t*-2, S*t*-3, S*t*-4, S*t*-5, S*t*-6, S*t*-7, S*t*-8, S*t*-9, S*t*-10, S*t*-11, S*t*-12**) of deprotonated L-serine-O-sulfate [sSer-H]$^-$, calculated at the M06-2X/6-311+G(d,p) level of theory.

. **Table S3** Selected dihedral angles (°) of low-lying conformers (**N*c*-1**, **N*c*-2**, **N*c*-3**, **N*c*-4**) of protonated L-serine-O-sulfate [sSer+H]$^+$, calculated at the M06-2X/6-311+G(d,p) level of theory.

. **Reference Gaussian 09**

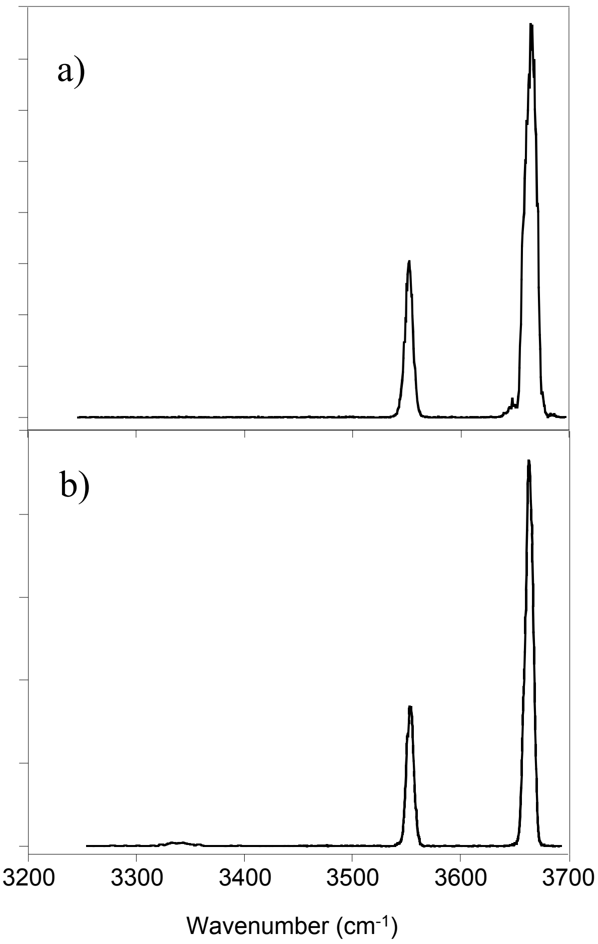


Fig. S1 Reproducibility of the IRMPD spectra of protonated O-phosphotyrosine (m/z 262) recorded in the 3250-3700 cm^{-1} range on two experimental set-ups: (a) a modified commercial Paul-ion trap mass spectrometer (Bruker Esquire 3000+) coupled to a tabletop IR optical oscillator /amplifier (OPO/OPA; laserVison, Bellevue, WA) pumped by an Innolas Splitlight 600 (Munchen Germany) non-seed Nd:YAG laser running at 25 Hz; (b) a modified commercial Paul-ion trap mass spectrometer (Bruker Esquire 6000+) coupled to a tabletop IR optical oscillator /amplifier (OPO/OPA; laserVison, Bellevue, WA) pumped by a Nd:YAG laser (Continuum Surelite II) running at 10 Hz. The irradiation time was 500 ms in both experiments.

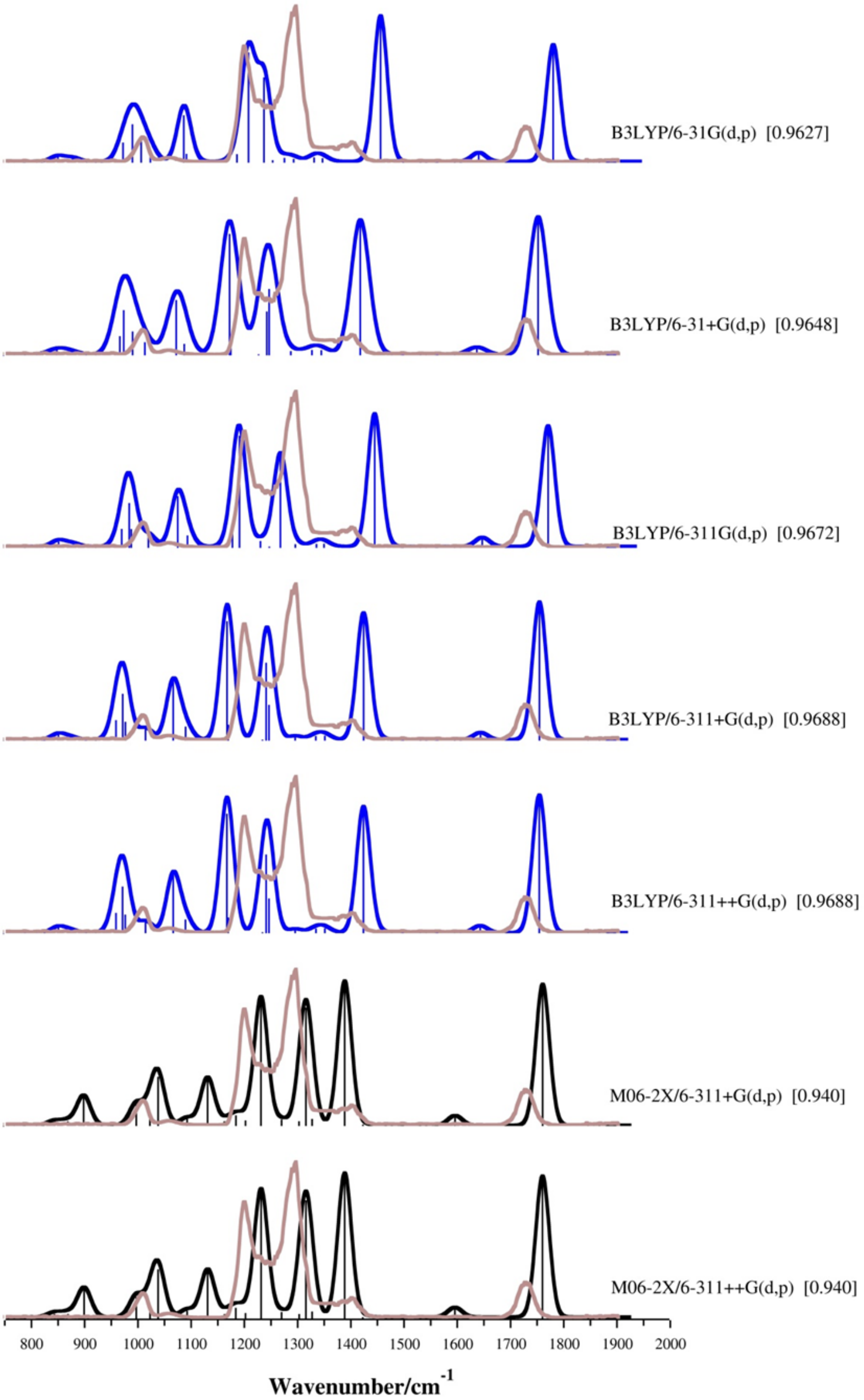


Fig. S2 Performance of different levels of theory in the simulation of the experimental IRMPD spectrum of the **St-1** conformer of [sSer-H]$^-$ ion. Two functionals (B3LYP and M06-2X) and different basis sets were tested. The employed scale factors are reported on the right and were taken from J. P. Merrick, D. Moran, L. Radom, *J. Phys. Chem. A*, 2007, **111**, 11683-11700 for the B3LYP functional and from I. M. Alecu, J. Zheng, Y. Zhao, and D. G. Truhlar, *J. Chem. Theory Comput,* 2010, **6**, 2872-2887 for the M06-2X functional. S-O stretching modes were left unscaled. The experimental spectrum is also reported as a pale trace over the calculated spectra to enable immediate comparison.

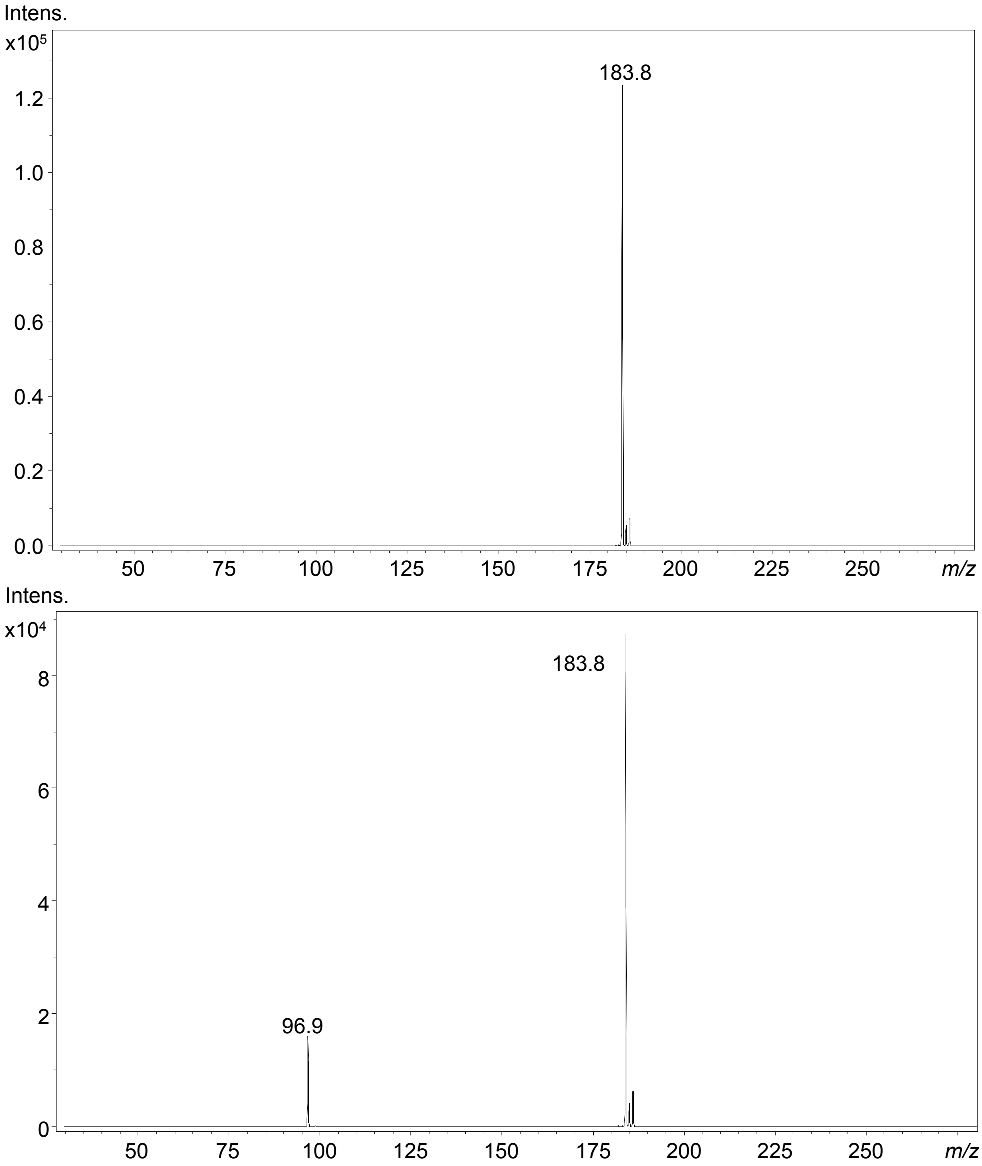


Fig. S3 Mass spectrum obtained after mass selection of deprotonated O-sulfo-L-serine, $[sSer-H]^-$ (m/z 184), in a Bruker Esquire Paul trap mass spectrometer (Esquire 3000+, Bruker Inc.) before (upper trace) and after (bottom trace) irradiation with CLIO FEL IR radiation at 1206 cm^{-1}. Ion intensity is in arbitrary units.

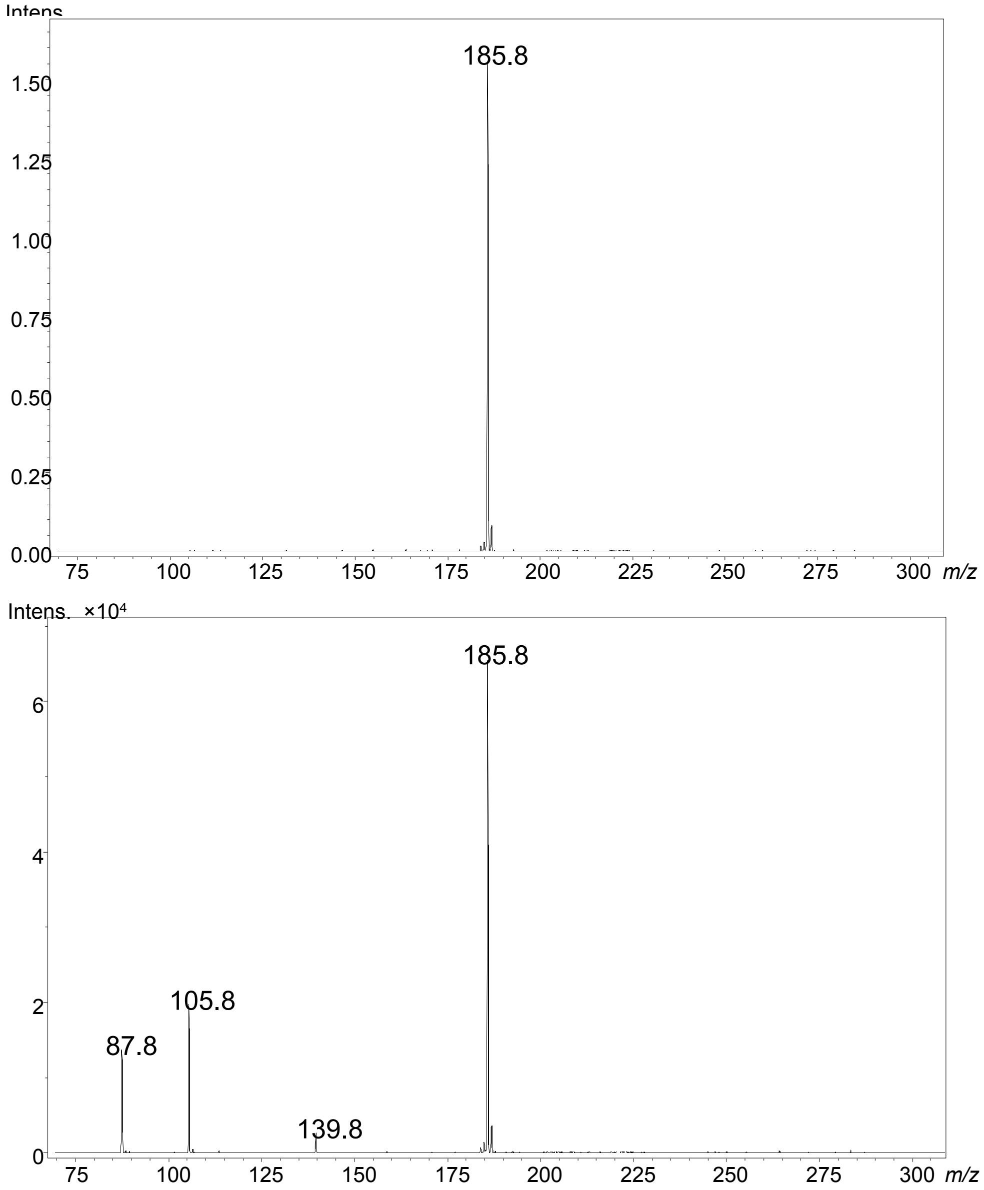


Fig. S4 Mass spectrum obtained after mass selection of protonated O-sulfo-L-serine, $[\mathrm{sSer+H}]^+$ (m/z 186), in a Bruker Esquire Paul trap mass spectrometer (Esquire 6000+, Bruker Inc.) before (upper trace) and after (bottom trace) irradiation with OPO/OPA IR radiation at 3572 cm^{-1}. Ion intensity is in arbitrary units.

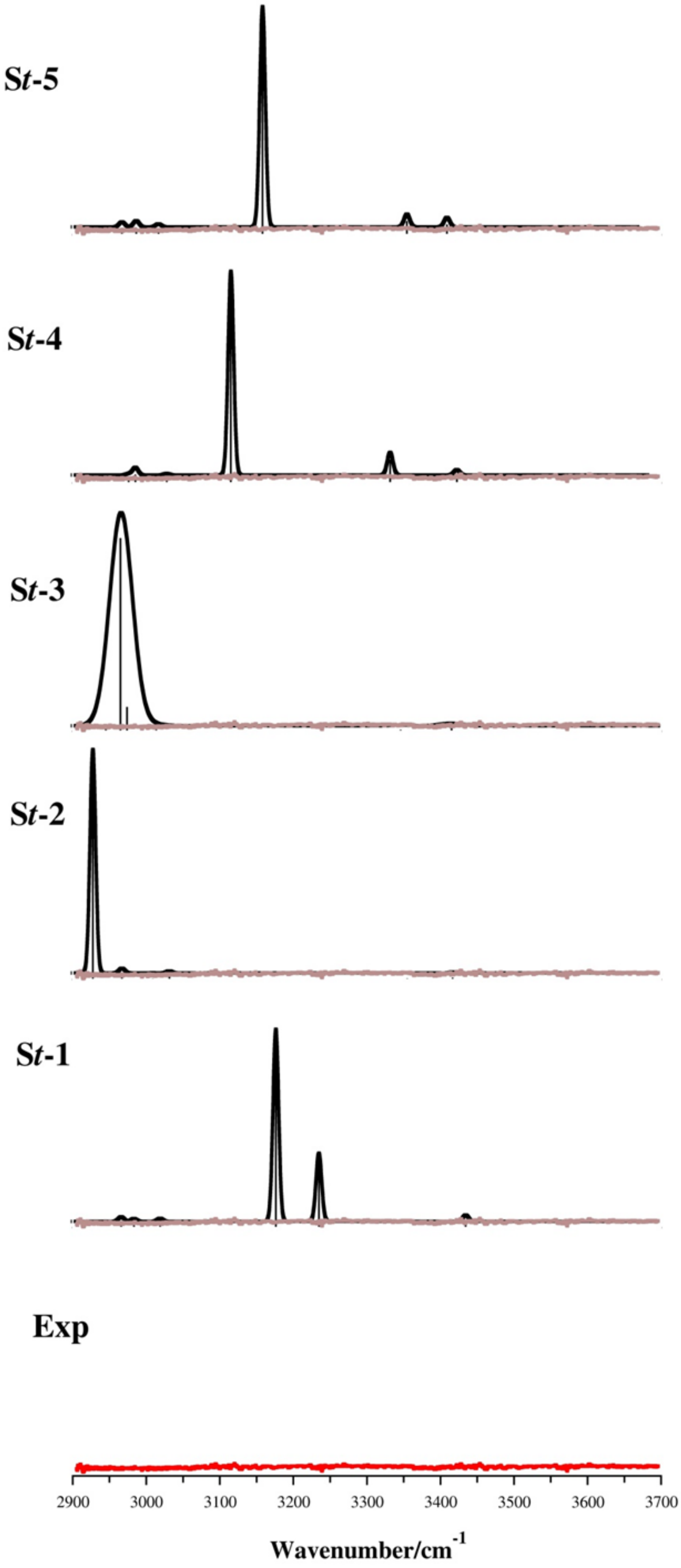
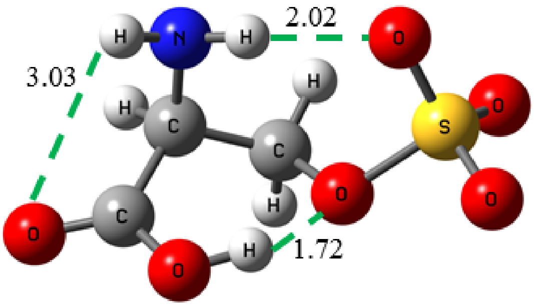
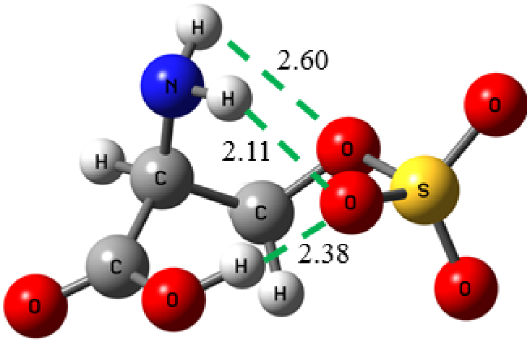


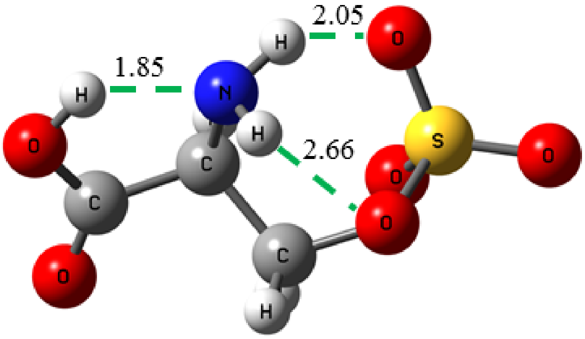
Fig. S5 Experimental IRMPD spectrum of deprotonated L-serine-O-sulfate [sSer-H]$^-$ (bottom, red line) in the 2900-3700 cm^{-1} range, and computed IR spectra (at the M06-2X/6-311+G(d,p) level) of conformers **S*t*-1**, **S*t*-2**, **S*t*-3**, **S*t*-4**, **S*t*-5**. The experimental spectrum is also reported as a pale trace over the calculated spectra to enable immediate comparison



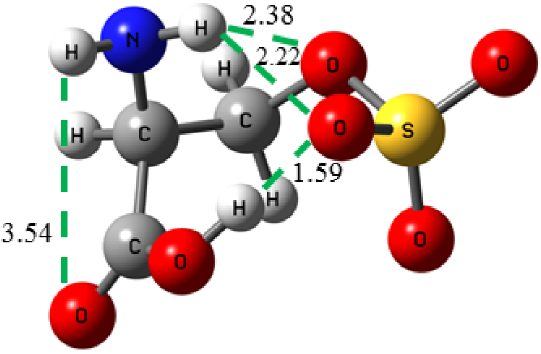
S*t*-11
12.6 (10.8)



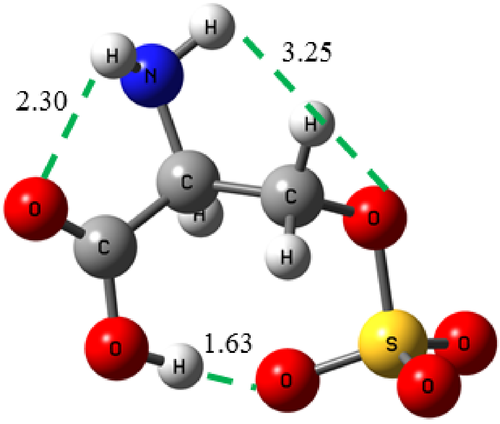
S*t*-12
9.5 (11.5)



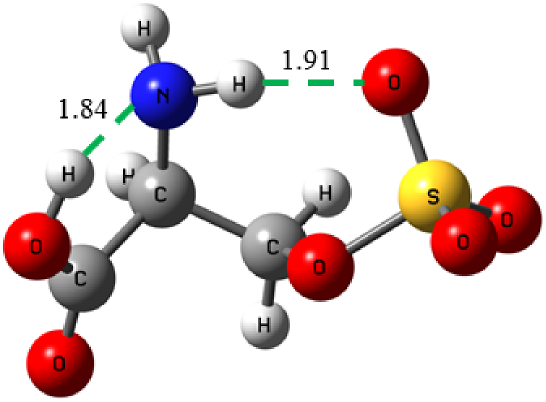
S*t*-9
6.1 (6.8)



S*t*-10
6.6 (8.9)

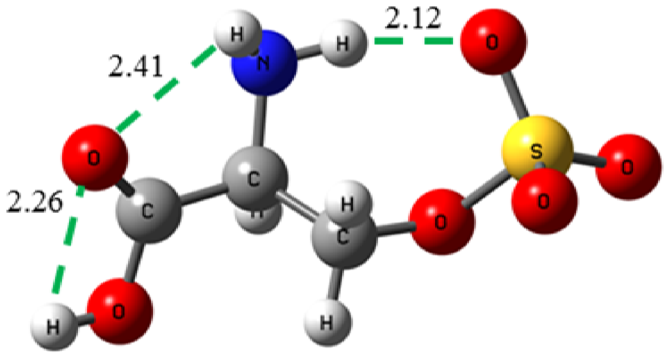


S*t*-7
4.0 (6.0)

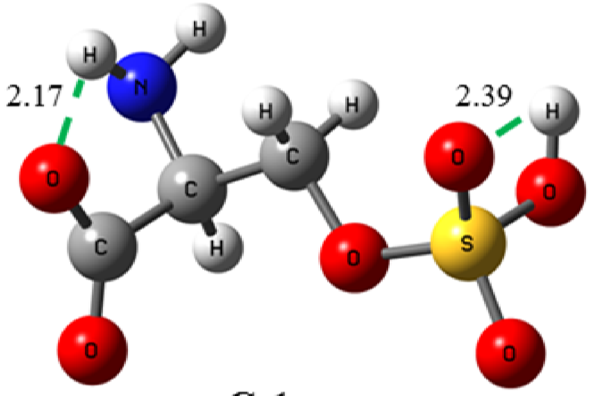


S*t*-8
7.2 (6.2)

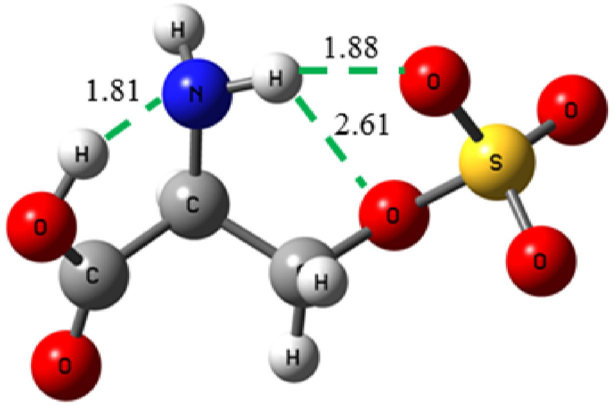
Fig. S6 Optimized geometries (at the M06-2X/6-311+G(d,p) level) and relative enthalpy and free energy (in parentheses) values (at the MP2/aug-cc-pVTZ level) at 298 K (kJ mol^{-1}) of [sSer-H]$^{-}$ conformers **S*t*-7, S*t*-8**, **S*t*-9, S*t*-10**, **S*t*-11, S*t*-12**. Interatomic bond distances, marked by dashed lines, are given in Å.



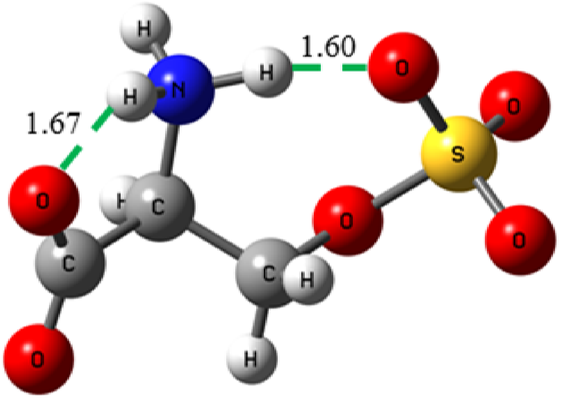
S*c*-1
35.2 (31.0)



C-1
157.6 (149.2)



S*t*-1
1.3 (0.0)



ZW-1
12.9 (14.4)

Fig. S7 Optimized geometries (at the M06-2X/6-311+G(d,p) level) and relative enthalpy and free energy (in parentheses) values (at the MP2/aug-cc-pVTZ level) at 298 K (kJ mol^{-1}) of [sSer-H]$^{-}$ isomers **S*t*-1**, **ZW-1**, **Sc-1**, **C-1**. Interatomic bond distances, marked by dashed lines, are given in Å.

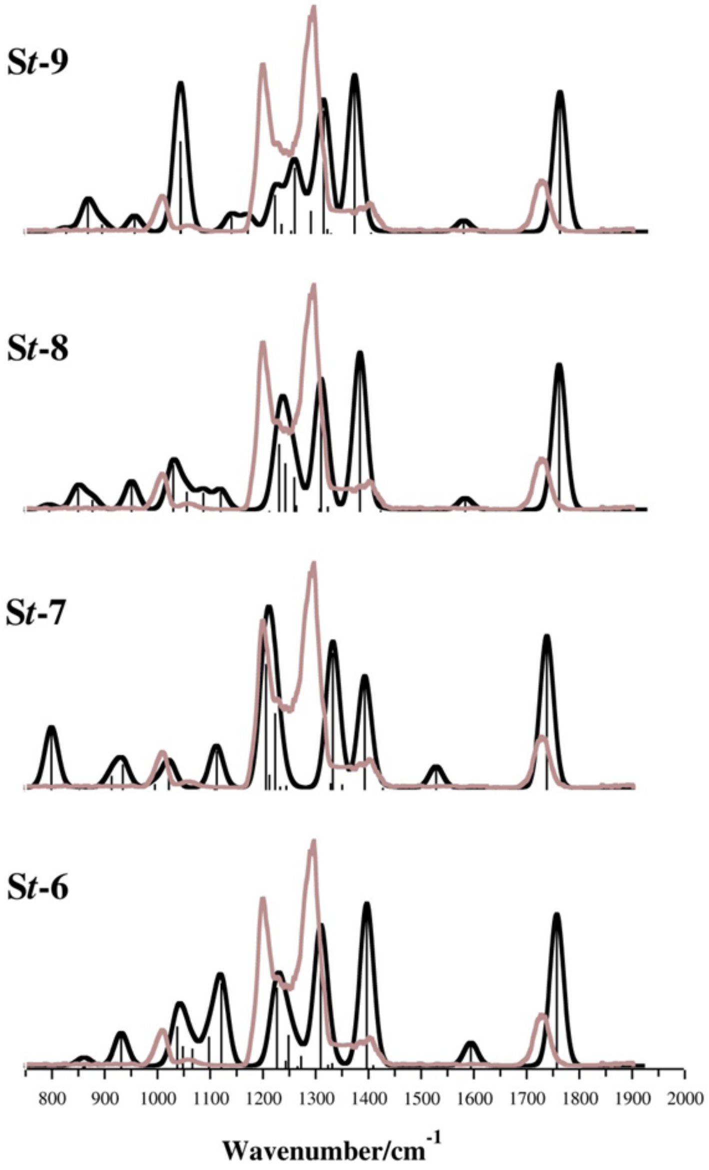


Fig S8 Computed IR spectra (at the M06-2X/6-311+G(d,p) level) of deprotonated L-serine-O-sulfate [sSer-H]$^-$ of conformers **S*t*-6, S*t*-7**, **S*t*-8, S*t*-9**. The experimental spectrum is also reported as a pale trace over the calculated spectra to enable immediate comparison.

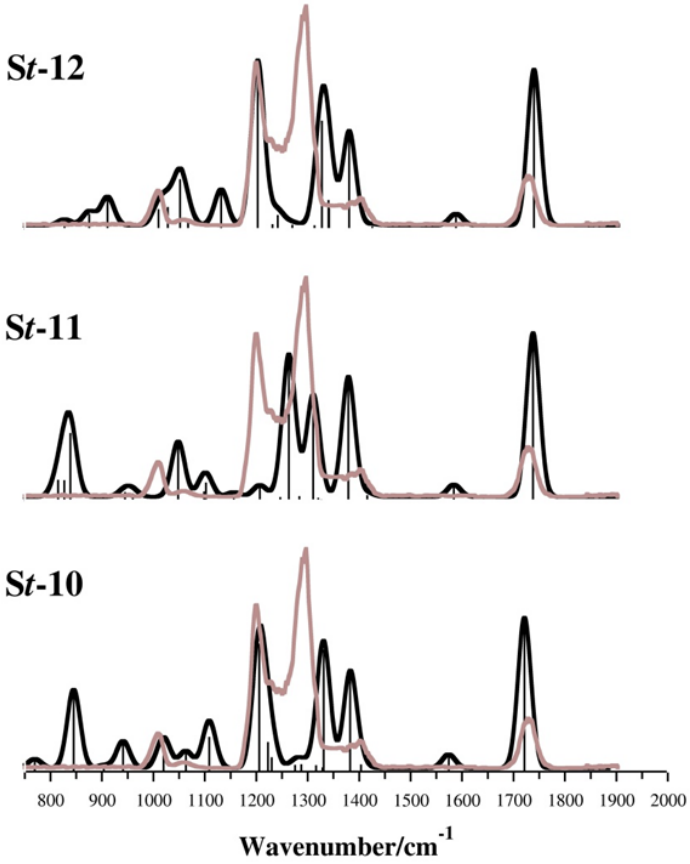


Fig. S9 Computed IR spectra (at the M06-2X/6-311+G(d,p) level) of deprotonated L-serine-O-sulfate [sSer-H]$^-$ of conformers **S*t*-10, S*t*-11, S*t*-12**. The experimental spectrum is also reported as a pale trace over the calculated spectra to enable immediate comparison.

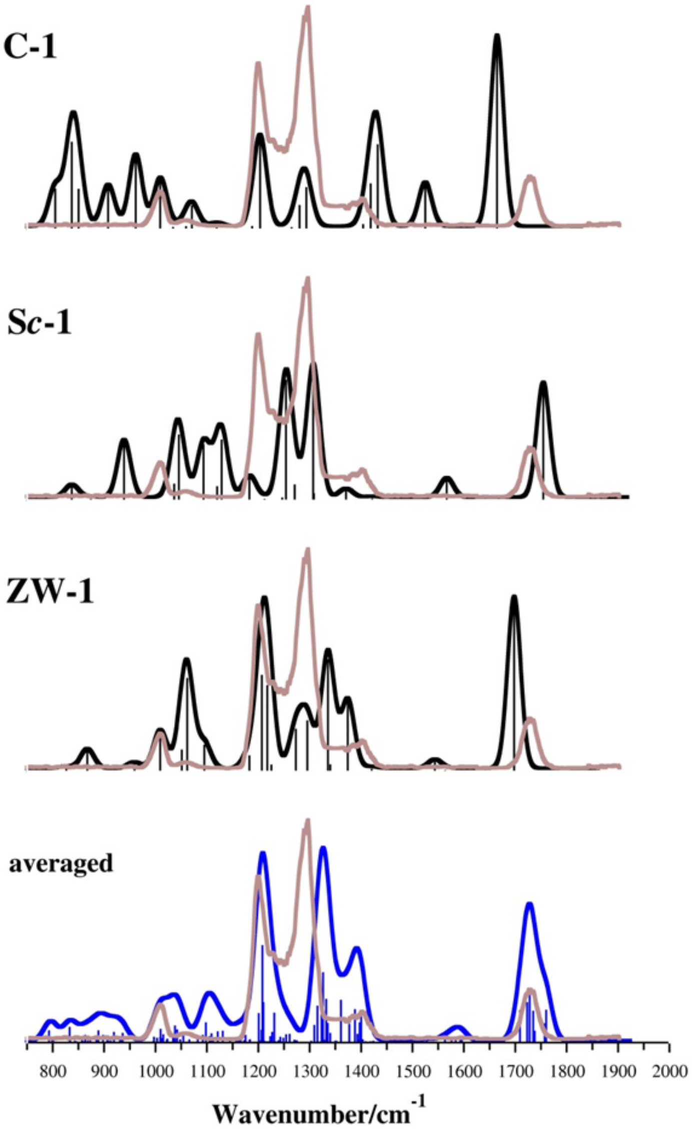


Fig. S10 Averaged spectrum of deprotonated L-serine-O-sulfate [sSer-H]$^-$ (bottom), computed by considering contributions from all thermally accessible conformers, according to their enthalpy values, and computed IR spectra (at the M06-2X/6-311+G(d,p) level) of isomers , **ZW-1**, **S*c*-1**, **C-1**. The experimental spectrum is also reported as a pale trace over the calculated spectra to enable immediate comparison.

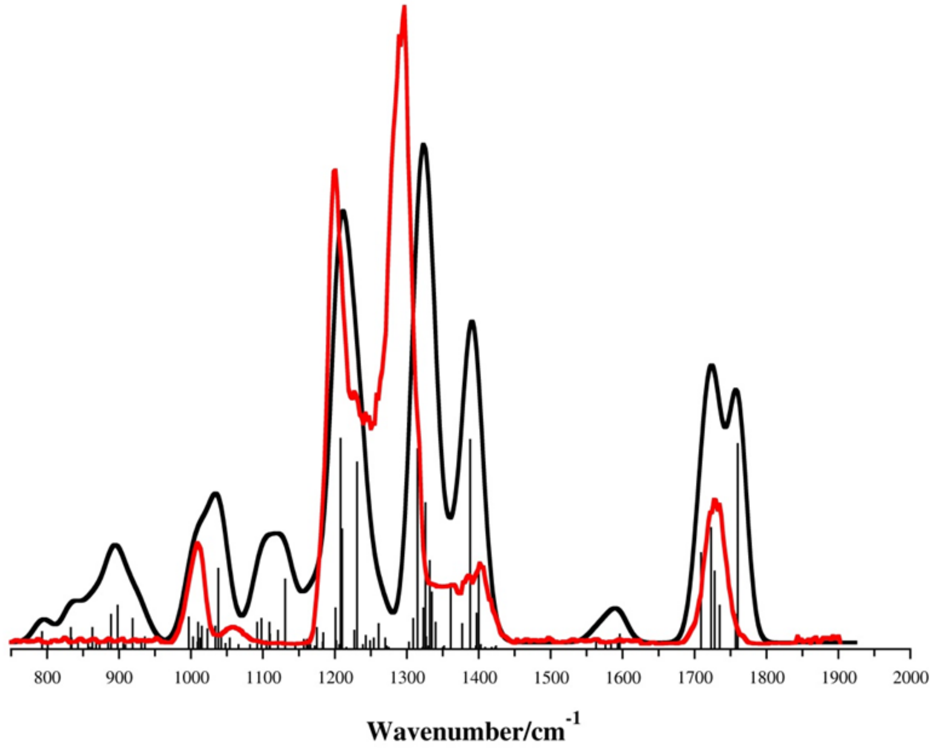
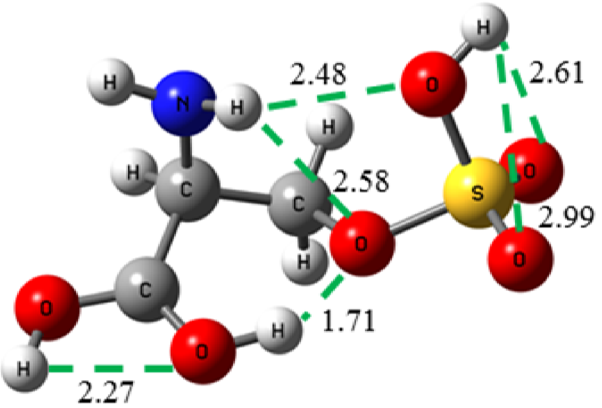
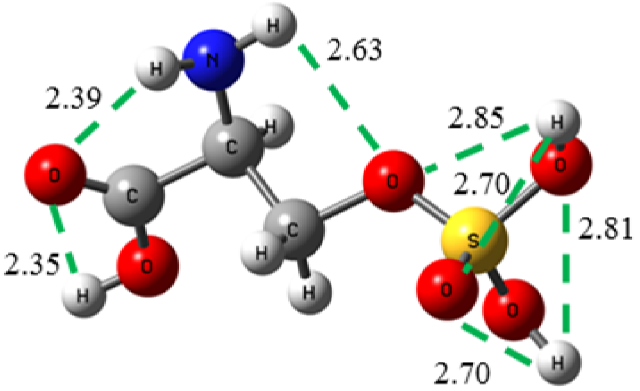


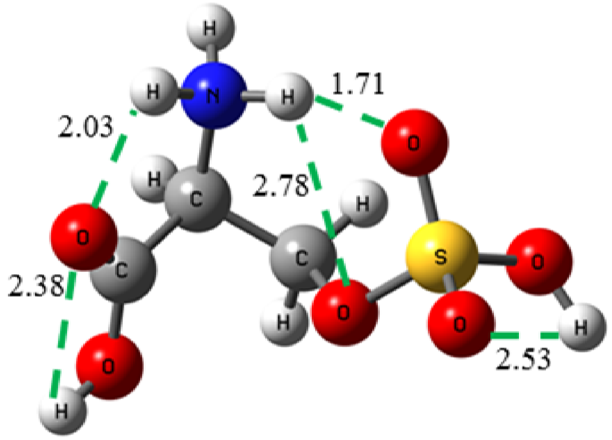
Fig. S11 Averaged spectrum of deprotonated L-serine-O-sulfate [sSer-H]$^{-}$, computed by considering contributions from all thermally accessible conformers, according to their relative free energy values. The experimental spectrum (red line) is also reported to enable immediate comparison.



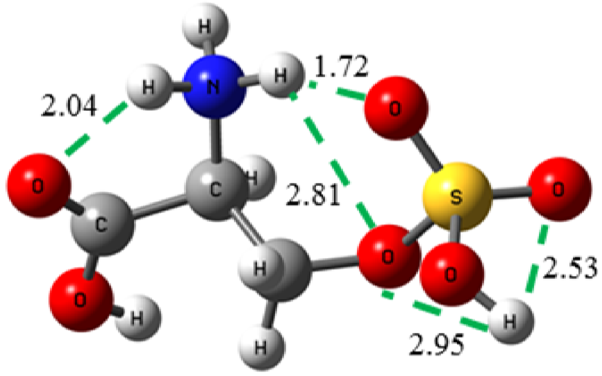
CO-1
123.1 (121.4)



SOH-1
177.8 (168.7)



N*c*-1
0.0 (0.0)



N*t*-1
40.3 (39.2)

Fig. S12 Optimized geometries (at the M06-2X/6-311+G(d,p) level) and relative enthalpy and free energy (in parentheses) values (at the MP2/aug-cc-pVTZ level) at 298 K (kJ mol^{-1}) of [sSer+H]$^+$ isomers **N*c*-1**, **N*t*-1**, **CO-1**, **SOH-1**. Interatomic bond distances, marked by dashed lines, are given in Å.

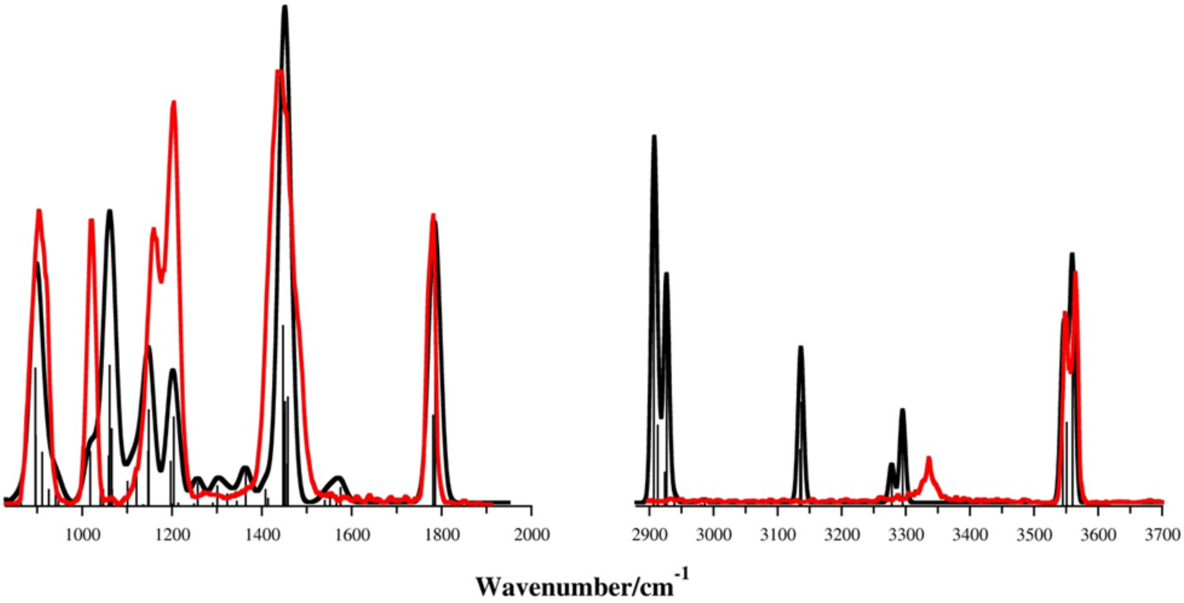


Fig. S13 Averaged spectrum of protonated L-serine-O-sulfate [sSer+H]$^+$, computed by considering contributions from all thermally accessible conformers, according to their relative free energy values. The experimental spectrum (red line) is also reported to enable immediate comparison.

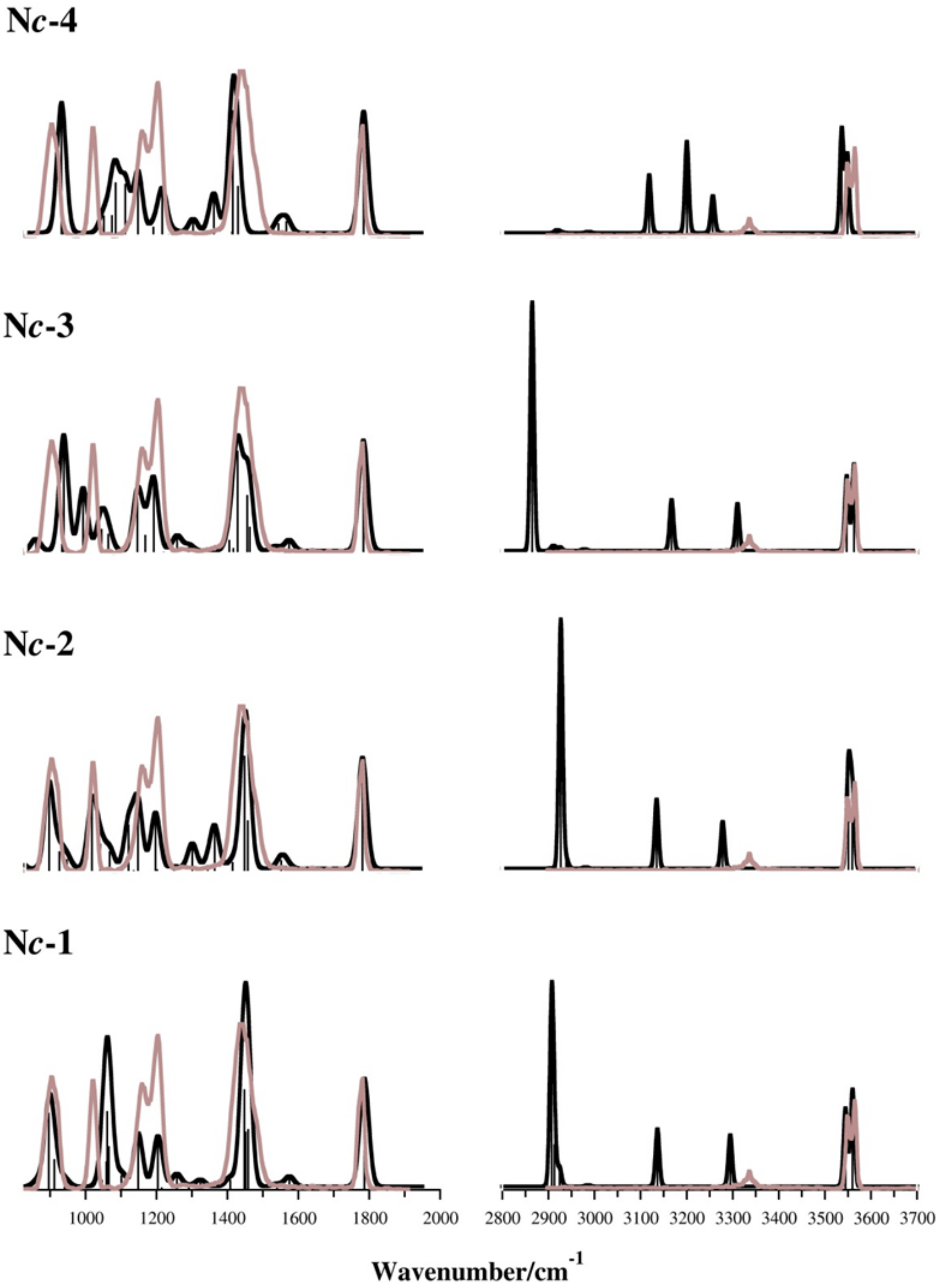


Fig. S14 Computed IR spectra (at the M06-2X/6-311+G(d,p) level) of conformers **N*c*-1**, **N*c*-2, N*c*-3,** and **N*c*-4** of protonated L-serine-O-sulfate [sSer+H]$^+$. The experimental spectrum is also reported as a pale trace over the calculated spectra to enable immediate comparison.

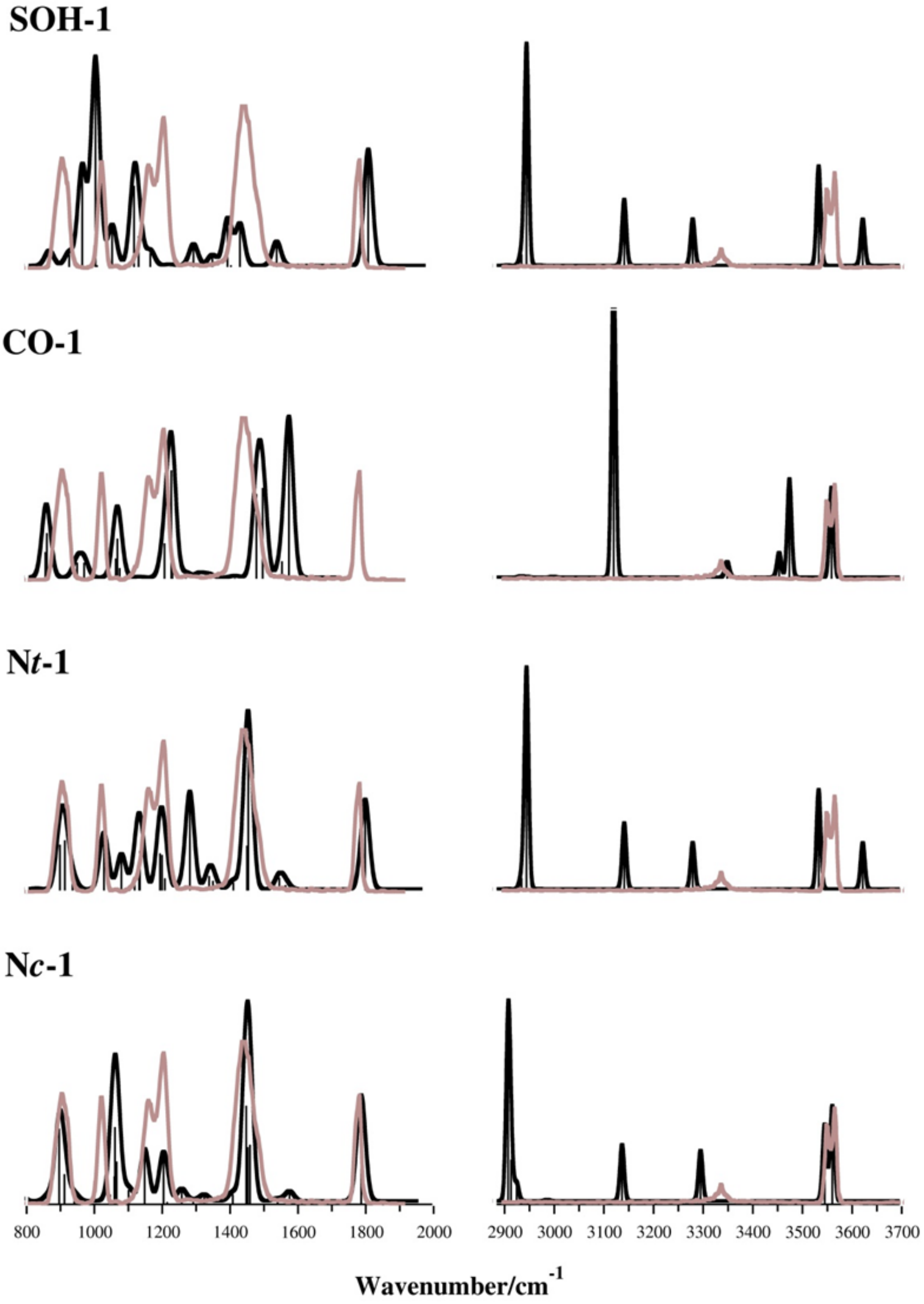


Fig S15 Computed IR spectra (at the M06-2X/6-311+G(d,p) level) of isomers **N*c*-1**, **N*t*-1**, **CO-1**,and **SOH-1**of protonated L-serine-O-sulfate [sSer+H]$^+$, . The experimental spectrum is also reported as a pale trace over the calculated spectra to enable immediate comparison.

Table S1 Thermodynamic data for the lowest energy conformers and isomers of protonated L-serineO-sulfate $[sSer+H]^+$ and deprotonated serine O-sulfate $[sSer-H]^-$ calculated at the MP2/aug-cc-pVTZ//M06-2X/6-311+G(d,p) level of theory.

Species	E[a]	$\Delta H°_{rel}$[b]	$\Delta G°_{rel}$[b]
	$[sSer-H]^-$[c]		
S*t*-1	-1020.9493352	1.3	0.0
S*t*-2	-1020.9491817	0.9	1.4
S*t*-3	-1020.9486690	1.2	1.7
S*t*-4	-1020.9495971	0.0	2.4
S*t*-5	-1020.9493780	1.3	3.8
S*t*-6	-1020.9492278	2.6	4.4
S*t*-7	-1020.9480738	4.0	6.0
S*t*-8	-1020.9470883	7.2	6.2
S*t*-9	-1020.9479653	6.1	6.8
S*t*-10	-1020.9471888	6.6	8.9
S*t*-11	-1020.9452248	12.6	10.8
S*t*-12	-1020.9463974	9.5	11.5
ZW-1	-1020.9439189	12.9	14.4
S*c*-1	-1020.9364080	35.2	31.0
C-1	-1020.8883356	157.6	149.2
	$[sSer+H]^+$[d]		
N*c*-1	-1021.7889787	0.0	0.0
N*c*-2	-1021.7875554	3.9	1.9
N*c*-3	-1021.7853213	9.6	8.2
N*c*-4	-1021.7851752	11.2	11.7
N*t*-1	-1021.7733948	40.3	39.2
CO-1	-1021.7404103	123.1	121.4
SOH-1	-1021.7179370	177.8	168.7

(a) Electronic energy at 0 K in Hartree/particle.
(b) Relative enthalpies ($\Delta H°_{rel}$) and Gibbs free energies ($\Delta G°_{rel}$) at 298 K in kJ mol^{-1}.
(c) Optimized structures depicted in Fig. 2 and Fig. S6 and S7.
(d) Optimized structures depicted in Fig 6 and Fig. S12.

Table S2 Selected dihedral angles (°) of low-lying conformers (**S*t*-1, S*t*-2, S*t*-3, S*t*-4, S*t*-5, S*t*-6, S*t*-7, S*t*-8, S*t*-9, S*t*-10, S*t*-11, S*t*-12**) of deprotonated L-serine-O-sulfate [sSer-H]$^-$, calculated at the M06-2X/6-311+G(d,p) level of theory.

	O(H)C1C2N	C2C3OS	C1C2C3O	C3OSO
S*t*-1	**-6.8°**	**114.3°**	**171.3°**	**-59.8°**
S*t*-2	**169.6°**	**-88.9°**	**128.0°**	**52.0°**
S*t*-3	**-139.2°**	**134.5°**	**-65.6°**	**134.5°**
S*t*-4	**-44.7°**	**-80.5°**	**-42.5°**	**76.1°**
S*t*-5	**-50.4°**	**-72.0°**	**-51.8°**	**84.5°**
S*t*-6	**10.3°**	**68.3°**	**158.5°**	**33.1°**
S*t*-7	**164.3°**	**-90.2°**	**131.6°**	**50.3°**
S*t*-8	**-2.8°**	**-115.5°**	**-68.7°**	**64.7°**
S*t*-9	**16.1°**	**-64.5°**	**-167.8°**	**81.7°**
S*t*-10	**-41.2°**	**77.1°**	**-99.1°**	**-78.8°**
S*t*-11	**-79.1°**	**-114.5°**	**-52.9°**	**65.7°**
S*t*-12	**-62.7°**	**94.1°**	**-124.4°**	**-55.0°**

Table S3 Selected dihedral angles (°) of low-lying conformers (**Nc-1**, **Nc-2**, **Nc-3**, **Nc-4**) of protonated L-serine-O-sulfate $[sSer+H]^+$, calculated at the M06-2X/6-311+G(d,p) level of theory.

	C2C3OS	C1C2C3O	C3OSO	OSOH
N*c*-1	**-100.5°**	**-43.8°**	**43.3°**	**-92.0°**
N*c*-2	**102.9°**	**168.4°**	**-44.4°**	**93.8°**
N*c*-3	**-103.7°**	**-48.2°**	**71.9°**	**-82.3°**
N*c*-4	**-64.0°**	**-160.9°**	**82.8°**	**83.8°**

Complete reference for Gaussian 09

Gaussian 09, Revision D.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.