Supplementary Information for:

Mechanism of formation and ion mobility separation of protomers and deprotomers of diaminobenzoic acids and aminophthalic acids

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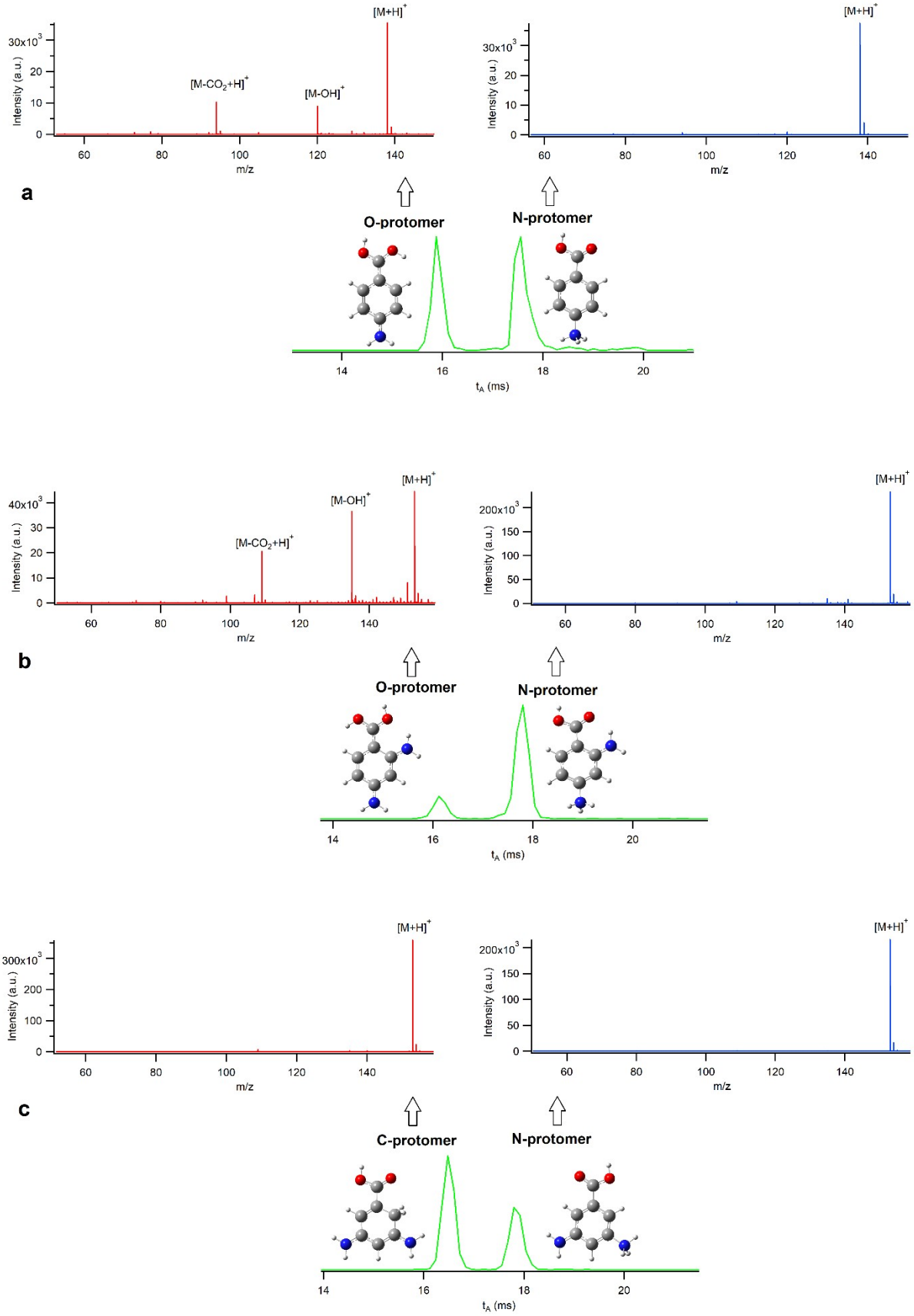
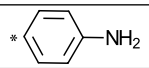
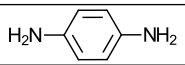
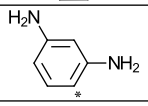
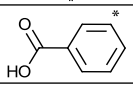
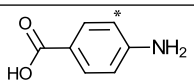
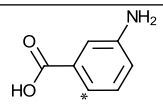
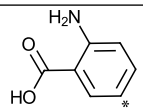
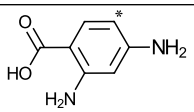
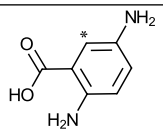
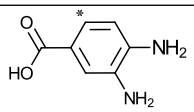
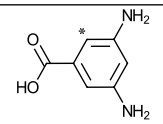
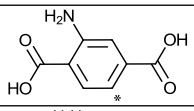
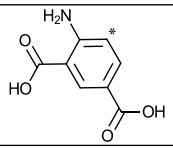
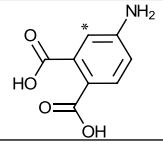
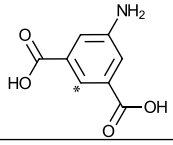
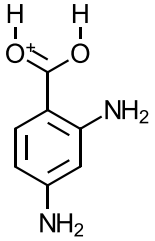
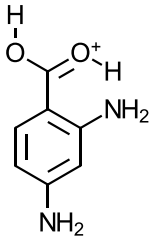


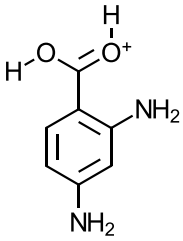
Figure S1. Comparison of ESI-MS results for protomers of (a) *p*-amino benzoic acid, (b) 2,4-DABA, and (c) 3,5-DABA acquired with standard transmission tuning.

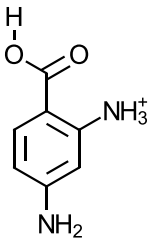
Tables S1. Calculated proton affinities (PA) and gas phase basicities (GB) of nitrogen, oxygen, and carbon sites of amino benzoic acid derivatives. The most basic site has been considered in each case (marked with an asterisk). The PA and GB values are in kJ mol^{-1}.

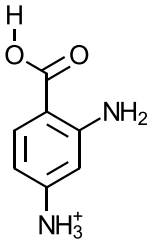
		Nitrogen atom		**Oxygen of C=O**		**Carbon of ring**	
Compound	**Structure**	PA	GB	PA	GB	PA	GB
Benzene						769.1	740.2
Aniline						892.6	861.5
p-DAB		922.5	901.2			893.1	863.2
m-DAB		906.2	881.7			961.4	930.2
Benzoic acid				833.6	803.3	747.6	718.7
p-ABA		860.1	836.4	884.5	856.2	849.6	820.2
m-ABA		870.3	842.4	852.2	824.7	870.1	839.8
o-ABA		902.7	868.9	854.6	824.5	874.5	842.5
2,4-DABA		925.1	891.8	918.8	885.4	930.0	898.6
2,5-DABA		938.9	907.2	872.9	842.2	883.6	855.9
3,4-DABA		896.3	864.7	889.4	859.9	871.5	841.6
3,5-DABA		889.9	868.4	863.9	837.2	943.9	912.9
2ATP		891.4	856.9	838.6	807.5	858.2	826.3
4AIP		876.8	842.7	870.1	840.2	835.4	804.6
4AP		849.9	823.9	940.0	903.1	837.7	808.7
5AIP		856.7	831.0	840.7	813.2	845.6	816.6



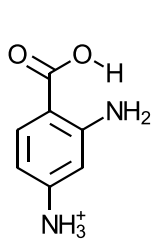


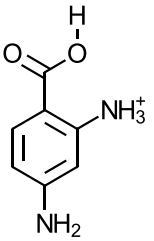


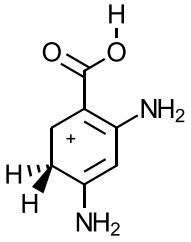


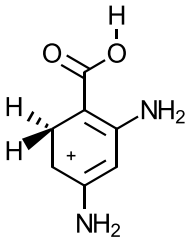


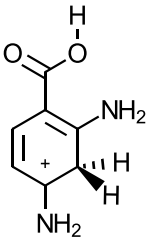
	[2,4+H]⁺-a	[2,4+H]⁺-b	[2,4+H]⁺-c	[2,4+H]⁺-d	[2,4+H]⁺-e
Gas phase	48.7	13.2	32.8	6.8	49.3
Water	51.9	NA	49.1	0.0	11.1
Methanol	52.3	NA	48.7	0.0	12.6
CCS_{N2}	**126.0**	**123.8**	**125.2**	**129.7**	**139.6**





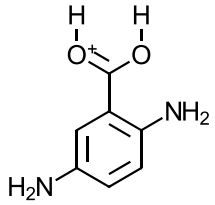


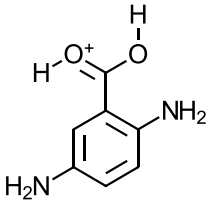


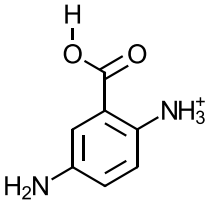


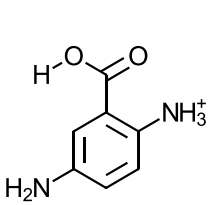
	[2,4+H]⁺-f	[2,4+H]⁺-g	[2,4+H]⁺-h	[2,4+H]⁺-i	[2,4+H]⁺-j
Gas phase	107.3	26.6	0.0	148.9	6.2
Water	35.5	11.6	10.1	154.5	7.8
Methanol	36.5	12.3	9.8	153.7	7.7
CCS_{N2}	**142.1**	**133.7**	**125.6**	**125.7**	**127.9**

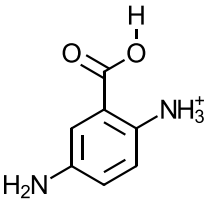
Figure S2. Relative Gibbs free energies and trajectory method-calculated CCS_{N2} values for possible protomers of [2,4-DABA+H]⁺. Tomasi's Polarized Continuum Model (PCM) was used for calculations in solvents water and methanol. Gibbs energies are in kJ mol^{-1}. Note: for NA, the protomer [2,4+H]⁺-b was converted to the most stable isomer [2,4+H]⁺-d during the structural optimization in the solvents.



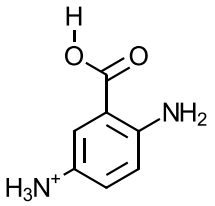


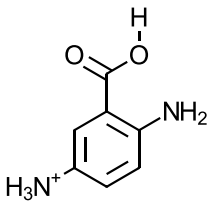


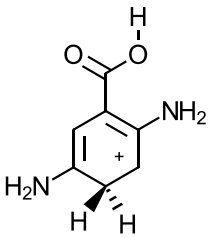


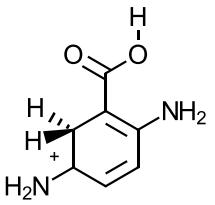


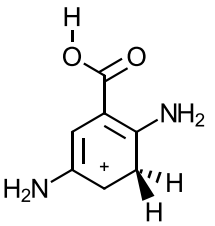
	[2,5+H]$^+$-a	[2,5+H]$^+$-b	[2,5+H]$^+$-c	[2,5+H]$^+$-d	[2,5+H]$^+$-e
Gas phase	83.3	64.9	0.0	34.8	25.1
Water	90.7	86.4	6.0	24.6	16.3
Methanol	89.4	84.8	4.8	24.2	15.7
CCS_{N2}	**128.1**	**125.0**	**129.9**	**129.2**	**132.8**





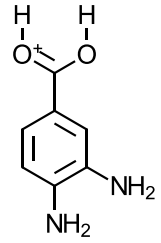


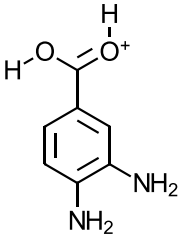


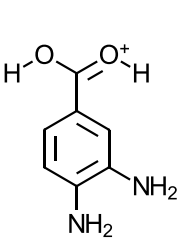


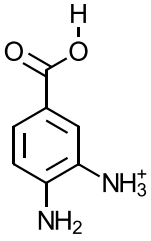
	[2,5+H]$^+$-f	[2,5+H]$^+$-g	[2,5+H]$^+$-h	[2,5+H]$^+$-i	[2,5+H]$^+$-j
Gas phase	25.6	33.2	91.2	51.3	55.4
Water	0.0	5.5	95.3	51.1	71.8
Methanol	0.0	5.2	94.8	50.4	70.3
CCS_{N2}	**137.0**	**137.7**	**128.6**	**129.7**	**125.7**

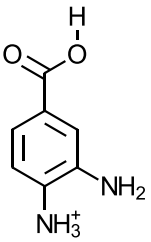
Figure S3. Relative Gibbs free energies and calculated CCS_{N2} values for possible protomers of [2,5-DABA+H]$^+$. Tomasi's Polarized Continuum Model (PCM) was used for calculations in solvents water and methanol. Gibbs energies are in kJ mol^{-1}.



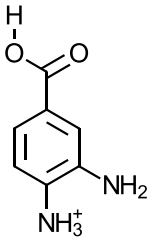


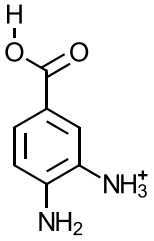


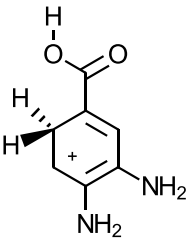


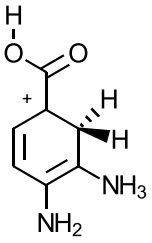


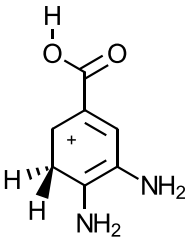
	[3,4+H]⁺-a	[3,4+H]⁺-b	[3,4+H]⁺-c	[3,4+H]⁺-d	[3,4+H]⁺-e
Gas phase	21.5	4.8	27.6	3.4	4.6
Water	59.8	55.4	65.8	2.0	8.7
Methanol	57.9	53.5	64.3	2.6	8.1
CCS_{N2}	**126.9**	**125.5**	**124.0**	**134.1**	**135.3**





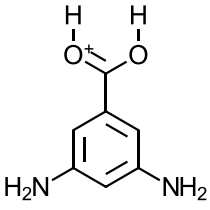


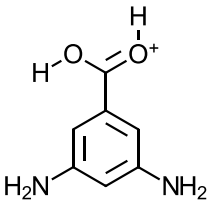


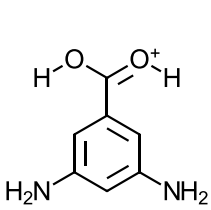


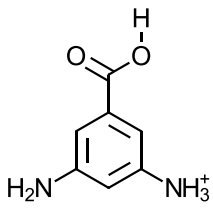
	[3,4+H]⁺-f	[3,4+H]⁺-g	[3,4+H]⁺-h	[3,4+H]⁺-i	[3,4+H]⁺-j
Gas phase	2.8	0.0	23.1	31.6	33.5
Water	7.4	0.0	58.1	57.8	58.4
Methanol	7.3	0.0	56.6	56.5	57.3
CCS_{N2}	**135.6**	**134.9**	**127.4**	**129.2**	**128.3**

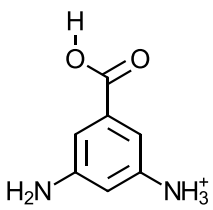
Figure S4. Relative Gibbs free energies and calculated CCS_{N2} values for possible protomers of [3,4-DABA+H]⁺. Tomasi's Polarized Continuum Model (PCM) was used for calculations in solvents water and methanol. Gibbs energies are in kJ mol^{-1}.



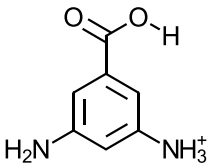


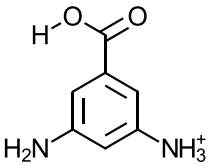


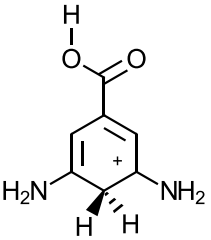


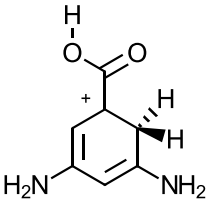


	[3,5+H]⁺-a	[3,5+H]⁺-b	[3,5+H]⁺-c	[3,5+H]⁺-d	[3,5+H]⁺-e
Gas phase	96.0	75.6	88.7	44.5	45.9
Water	93.0	85.9	92.9	10.2	11.0
Methanol	95.5	86.0	93.6	12.2	12.6
CCS_{N2}	**130.0**	**125.0**	**123.4**	**140.8**	**140.3**



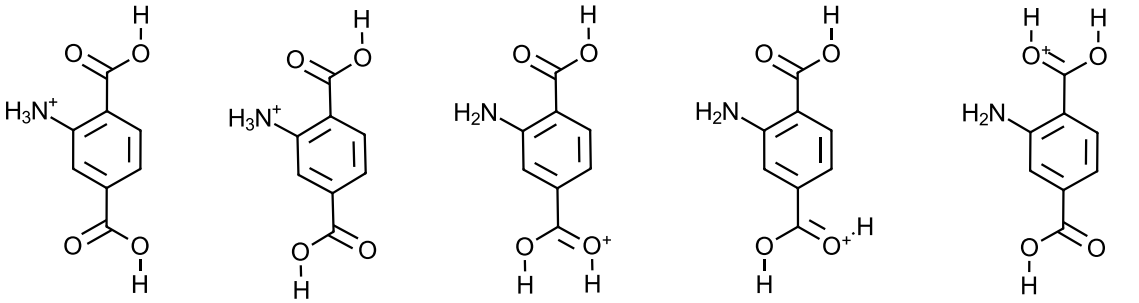




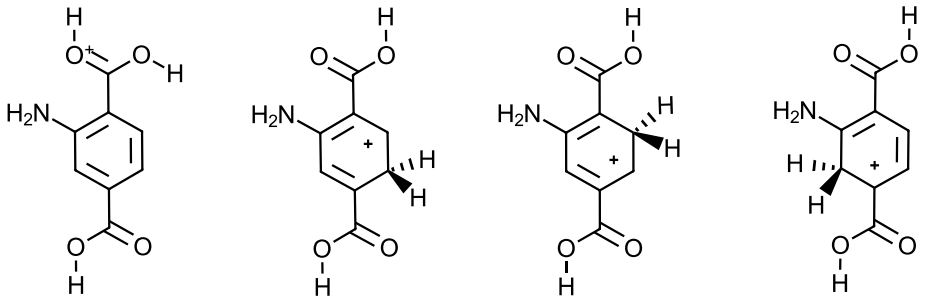


	[3,5+H]⁺-f	[3,5+H]⁺-g	[3,5+H]⁺-h	[3,5+H]⁺-i
Gas phase	97.2	86.6	31.2	0.0
Water	26.9	26.4	26.6	0.0
Methanol	29.3	28.8	27.8	0.0
CCS_{N2}	**143.9**	**141.5**	**130.0**	**129.2**

Figure S5. Relative Gibbs free energies and calculated CCS_{N2} values for possible protomers of [3,5-DABA+H]⁺. Polarized Continuum Model (PCM) was used for calculations in solvents water and methanol. Gibbs energies are in kJ mol^{-1}.

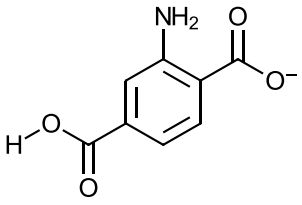


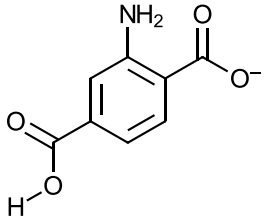
	[2ATP+H]$^+$-a	[2ATP+H]$^+$-b	[2ATP+H]$^+$-c	[2ATP+H]$^+$-d	[2ATP+H]$^+$-e
Gas phase	0.0	3.2	71.8	55.8	68.3
Water	0.0	0.0	76.4	73.2	68.9
Methanol	0.0	0.1	75.7	72.0	68.4
CCS_{N2}	**139.6**	**140.4**	**139.9**	**135.1**	**138.9**



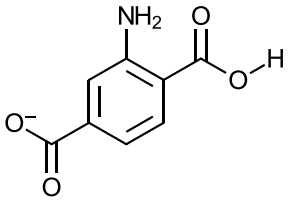
	[2ATP+H]$^+$-f	[2ATP+H]$^+$-g	[2ATP+H]$^+$-h	[2ATP+H]$^+$-i
Gas phase	49.4	30.6	135.9	42.7
Water	66.2	51.9	152.3	59.4
Methanol	65.7	51.3	151.1	59.1
CCS_{N2}	**135.9**	**134.5**	**134.3**	**136.1**

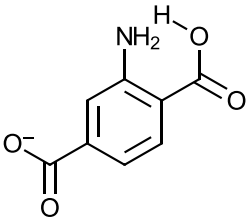
Figure S6. Relative Gibbs free energies and calculated CCS_{N2} values for possible protomers of [2ATP+H]$^+$. Polarized Continuum Model (PCM) was used for calculations in solvents water and methanol. Gibbs energies are in kJ mol^{-1}.





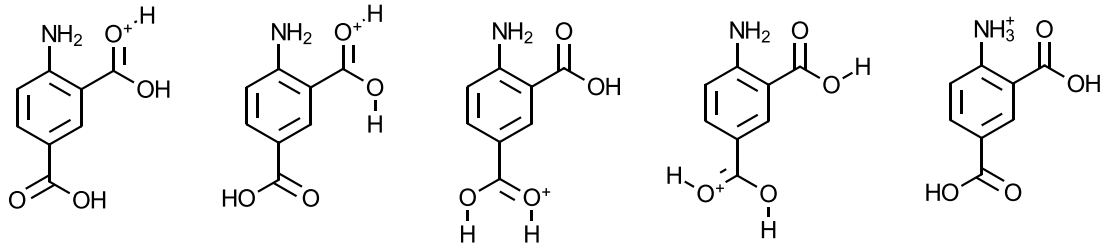
	[2ATP-H]$^-$-a	[2ATP-H]$^-$-b
Gas phase	0.8	0.0
Water	2.0	2.1
Methanol	1.9	1.8
CCS_{N2}	**144.0**	**144.2**



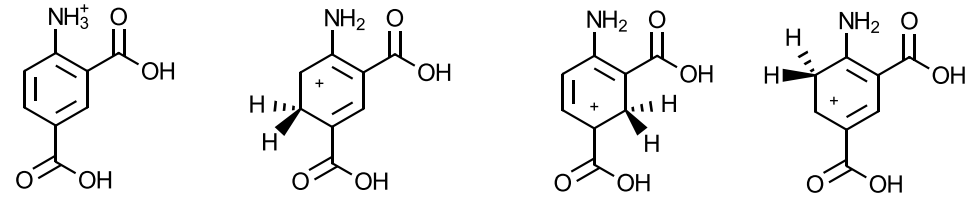


	[2ATP-H]$^-$-c	[2ATP-H]$^-$-d
Gas phase	11.5	18.5
Water	0.0	8.5
Methanol	0.0	8.9
CCS_{N2}	**147.2**	**147.3**

Figure S7. Relative Gibbs free energies and calculated CCS_{N2} values for possible deprotomers of [2ATP-H]$^-$. Polarized Continuum Model (PCM) was used for calculations in solvents water and methanol. Gibbs energies are in kJ mol^{-1}.

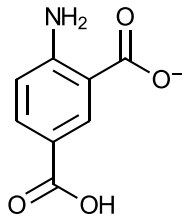


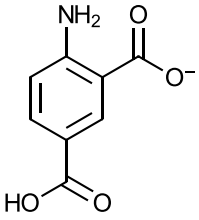
	[4AIP+H]$^+$-a	[4AIP+H]$^+$-b	[4AIP+H]$^+$-c	[4AIP+H]$^+$-d	[4AIP+H]$^+$-e
Gas phase	56.3	36.1	18.7	2.5	0.0
Water	56.1	58.2	43.2	41.8	1.0
Methanol	55.6	57.5	42.7	40.9	1.0
CCS_{N2}	**140.2**	**136.5**	**135.4**	**133.0**	**141.1**



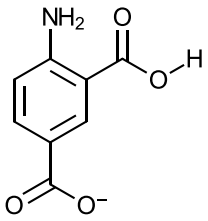
	[4AIP+H]$^+$-f	[4AIP+H]$^+$-g	[4AIP+H]$^+$-h	[4AIP+H]$^+$-i
Gas phase	0.4	146.1	135.3	38.2
Water	0.0	166.1	162.2	56.5
Methanol	0.0	166.6	161.7	56.1
CCS_{N2}	**140.4**	**133.6**	**133.7**	**135.0**

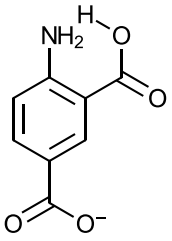
Figure S8. Relative Gibbs free energies and calculated CCS_{N2} values for possible protomers of [4AIP+H]$^+$. Polarized Continuum Model (PCM) was used for calculations in solvents water and methanol. Gibbs energies are in kJ mol^{-1}.





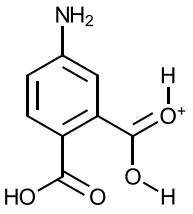
	[4AIP-H]⁻-a	[4AIP-H]⁻-b
Gas phase	0.0	4.0
Water	0.0	0.4
Methanol	0.0	0.5
CCS_{N2}	**144.0**	**144.2**

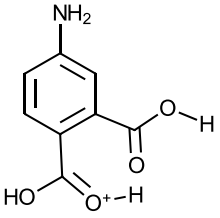


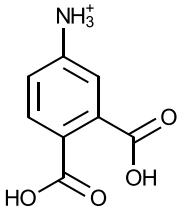


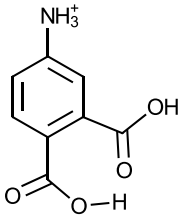
	[4AIP-H]⁻-c	[4AIP-H]⁻-d
Gas phase	31.2	49.4
Water	9.1	23.9
Methanol	9.5	24.7
CCS_{N2}	**148.5**	**149.2**

Figure S9. Relative Gibbs free energies and calculated CCS_{N2} values for possible deprotomers of [4AIP-H]⁻. Polarized Continuum Model (PCM) was used for calculations in solvents water and methanol. Gibbs energies are in kJ mol^{-1}.

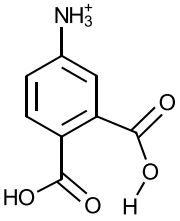


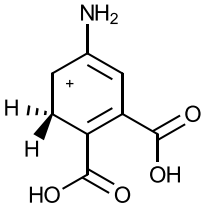


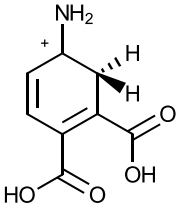


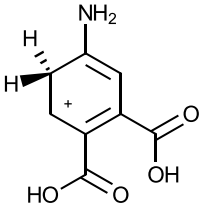


	[4AP+H]$^+$-a	[4AP+H]$^+$-b	[4AP+H]$^+$-c	[4AP+H]$^+$-d
Gas phase	68.2	0.0	79.2	105.1
Water	57.8	5.4	0.0	3.1
Methanol	53.9	2.8	0.0	4.2
CCS_{N2}	**129.6**	**128.7**	**148.3**	**149.8**



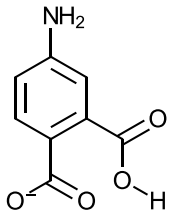


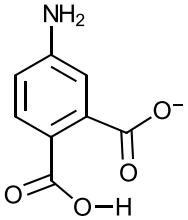




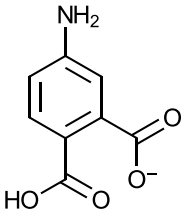
	[4AP+H]$^+$-e	[4AP+H]$^+$-f	[4AP+H]$^+$-g	[4AP+H]$^+$-h
Gas phase	94.2	182.2	94.4	97.2
Water	2.9	155.9	51.6	55.2
Methanol	2.1	154.5	50.3	54.2
CCS_{N2}	**148.3**	**134.3**	**138.2**	**138.3**

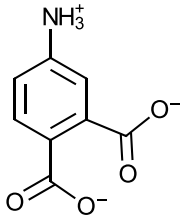
Figure S10. Relative Gibbs free energies and calculated CCS_{N2} values for possible protomers of [4AP+H]$^+$. Polarized Continuum Model (PCM) was used for calculations in solvents water and methanol. Gibbs energies are in kJ mol^{-1}.





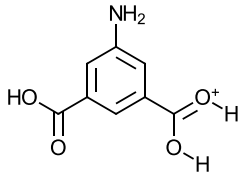
	[4AP-H]$^-$-a	[4AP-H]$^-$-b
Gas phase	81.5	0.0
Water	41.1	0.0
Methanol	42.7	0.0
CCS_{N2}	**146.8**	**139.9**

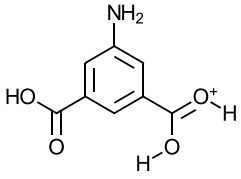


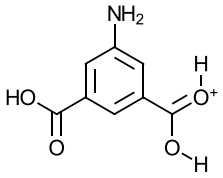


	[4AP-H]$^-$-c	[4AP-H]$^-$-d
Gas phase	77.1	377.1
Water	35.6	113.5
Methanol	37.1	120.7
CCS_{N2}	**145.8**	**180.1**

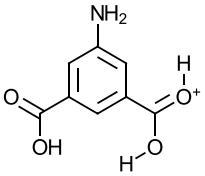
Figure S11. Relative Gibbs free energies and calculated CCS_{N2} values for possible deprotomers of [4AP-H]$^-$. Polarized Continuum Model (PCM) was used for calculations in solvents water and methanol. Gibbs energies are in kJ mol^{-1}.

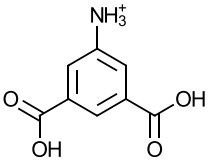


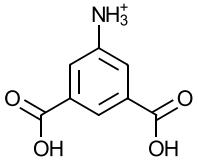




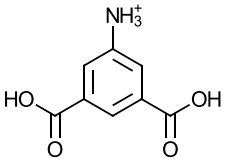
	[5AIP+H]$^+$-a	[5AIP+H]$^+$-b	[5AIP+H]$^+$-c
Gas phase	39.2	17.8	27.4
Water	78.1	75.6	76.5
Methanol	77.8	74.7	75.8
CCS_{N2}	**140.9**	**136.5**	**137.1**

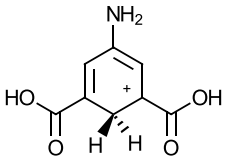


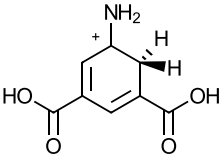




	[5AIP+H]$^+$-d	[5AIP+H]$^+$-e	[5AIP+H]$^+$-f
Gas phase	38.2	0.0	2.3
Water	86.1	1.2	0.0
Methanol	85.2	0.7	0.0
CCS_{N2}	**132.8**	**150.2**	**150.3**

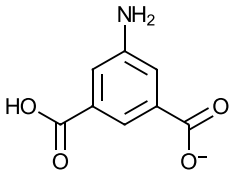


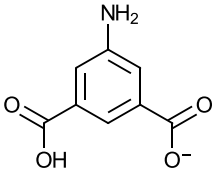


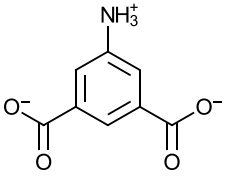


	[5AIP+H]$^+$-g	[5AIP+H]$^+$-h	[5AIP+H]$^+$-i
Gas phase	6.3	14.4	31.9
Water	1.8	54.0	64.2
Methanol	2.5	52.5	63.4
CCS_{N2}	**150.3**	**137.5**	**138.4**

Figure S12. Relative Gibbs free energies and calculated CCS_{N2} values for possible protomers of [5AIP+H]$^+$. Polarized Continuum Model (PCM) was used for calculations in solvents water and methanol. Gibbs energies are in kJ mol^{-1}.







	[5AIP-H]$^-$-a	[5AIP-H]$^-$-b	[5AIP-H]$^-$-c
Gas phase	5.3	0.0	222.8
Water	0.0	1.9	64.8
Methanol	0.0	1.2	69.2
CCS_{N2}	**151.1**	**150.7**	**176.5**

Figure S13. Relative Gibbs free energies and calculated CCS_{N2} values for possible deprotomers of [5AIP-H]$^-$. Polarized Continuum Model (PCM) was used for calculations in solvents water and methanol. Gibbs energies are in kJ mol^{-1}.

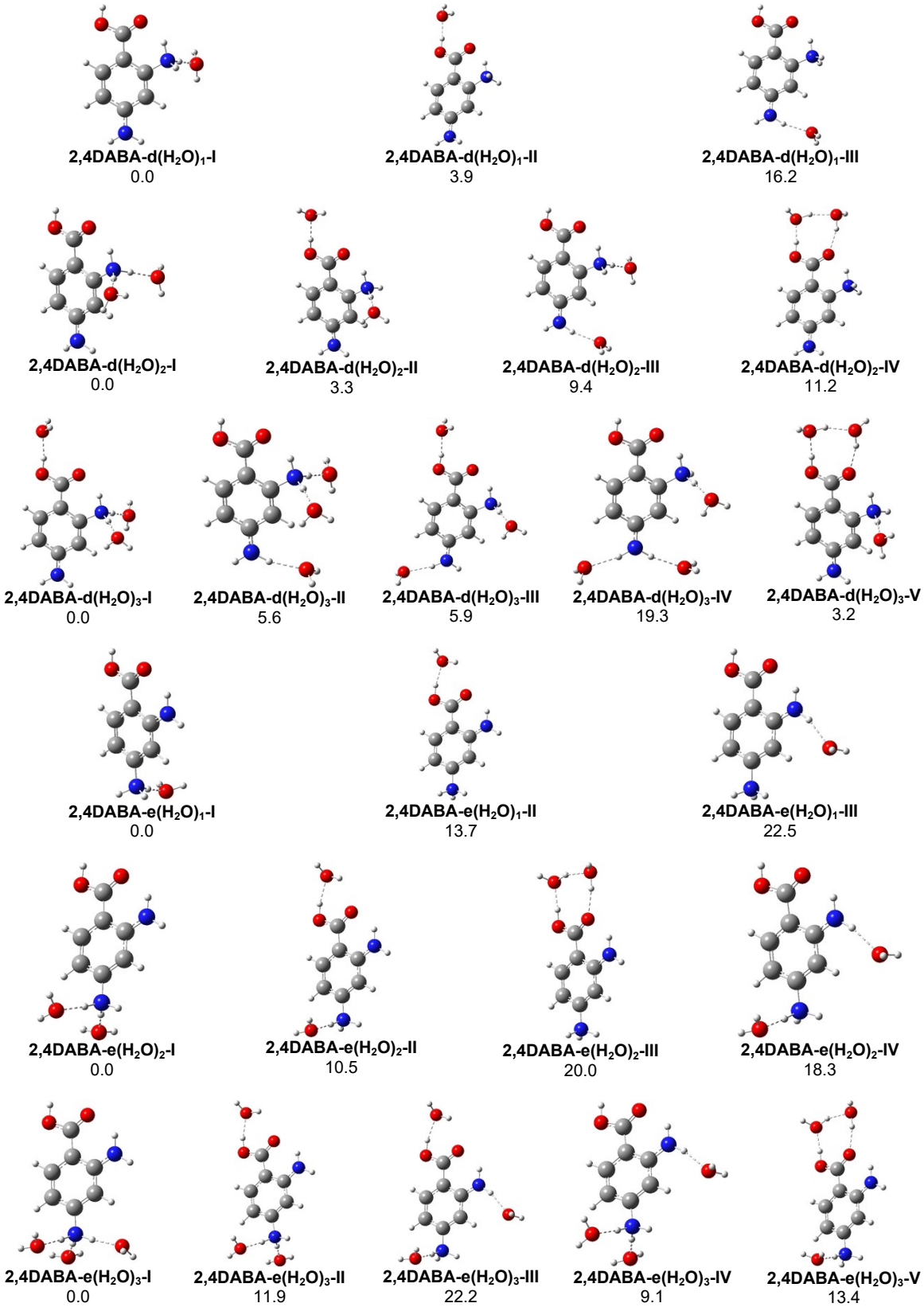


Figure S14. Comparison of the optimized structures and relative stabilities of [2,4-DABA+H]$^+$-**d**$(H_2O)_{1,2,3}$ and [2,4-DABA+H]$^+$-**e**$(H_2O)_{1,2,3}$ isomers in aqueous solution with dielectric constant of 78.25 using PCM model. The relative Gibbs energies are in kJ mol^{-1}.

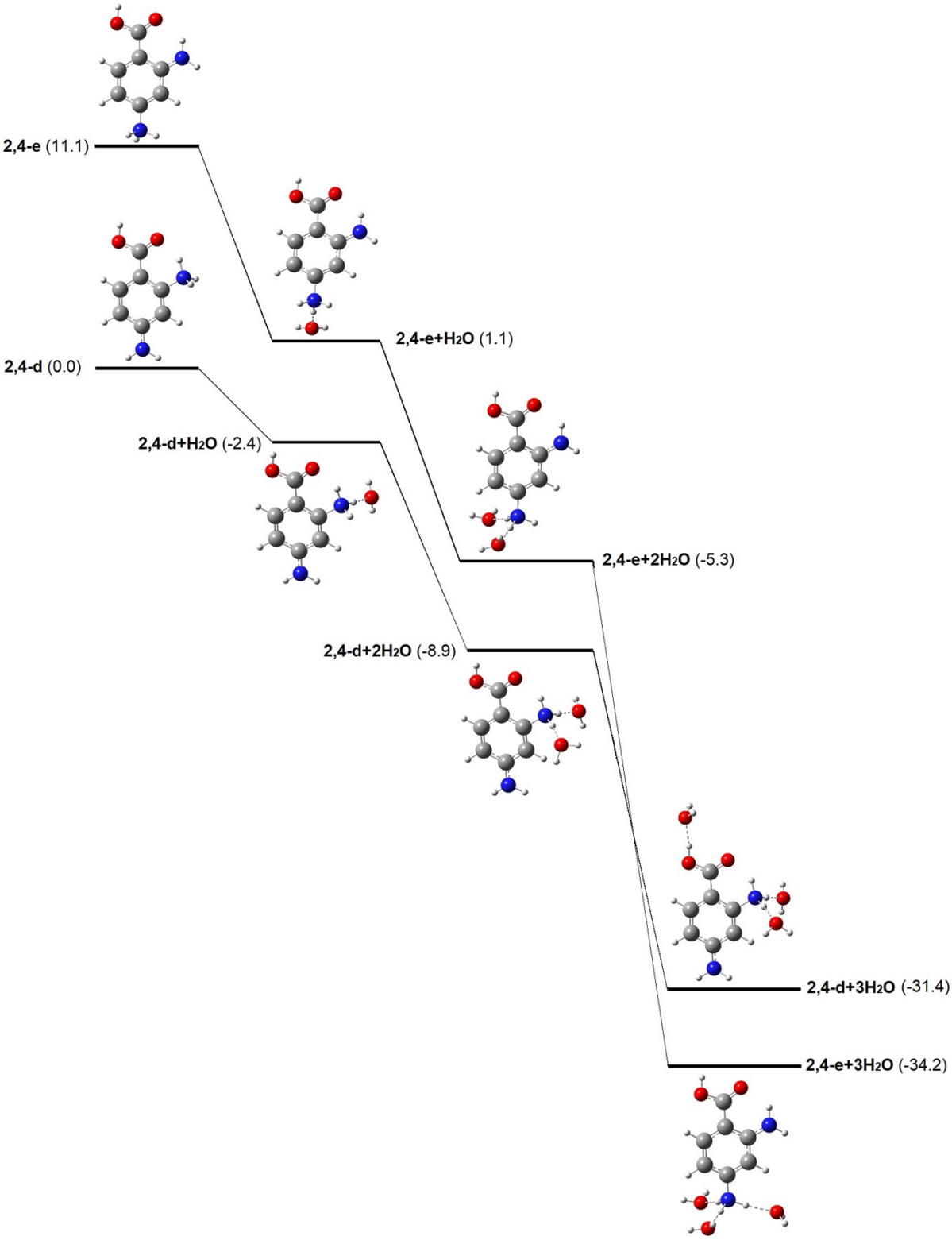


Figure S15. Stabilization of the 2,4-DABA N-protomers **d** and **e** (see **Figure S2**) due to micro-hydration (explicit hydration). The calculation in aqueous solution with implicit model of PCM (ε=78.35) shows that the isomer **d** is more stable. However, when hydrogen bonding interaction of water molecules in aqueous solution is considered, the most stable isomer is isomer **e** with the calculated CCS_{N2} of 139.6 Å^2 in good agreement with the experimental $^{DT}CCS_{N2}$ value of 140.0 Å^2. For the isomer **d**, the calculated CCS_{N2} is 129.7 Å^2. Gibbs energies in parenthesis are in kJ mol^{-1}.

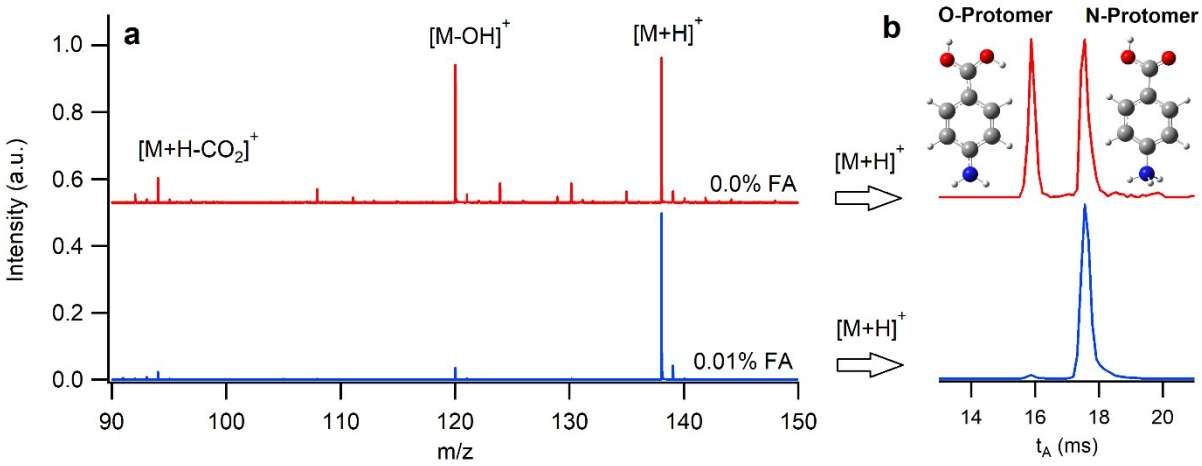


Figure S16. Experimental mass and IM spectra for *para*-amino benzoic acid (*p*-ABA) in H_2O:MeOH (50:50) with and without formic acid. With the addition of 0.01% (v/v) formic acid, the amine group is predominantly protonated in solution and very little O-protomer is observed.

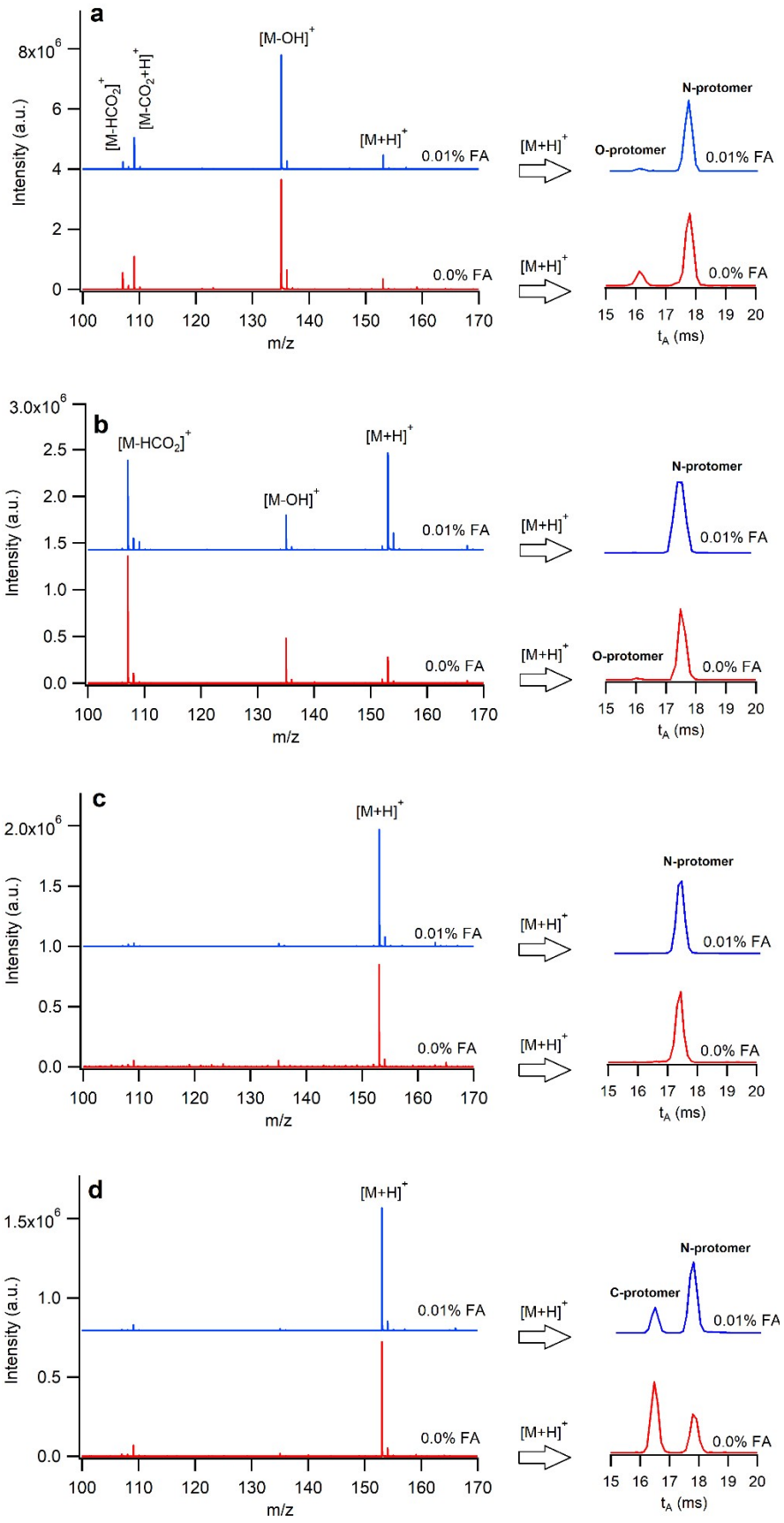


Figure S17. *m/z*-selected IM spectra and corresponding MS results for (a) 2,4-DABA, (b) 2,5-DABA, (c) 3,4-DABA, and (d) 3,5-DABA in H_2O:MeOH (50:50) without and with 0.01% FA.

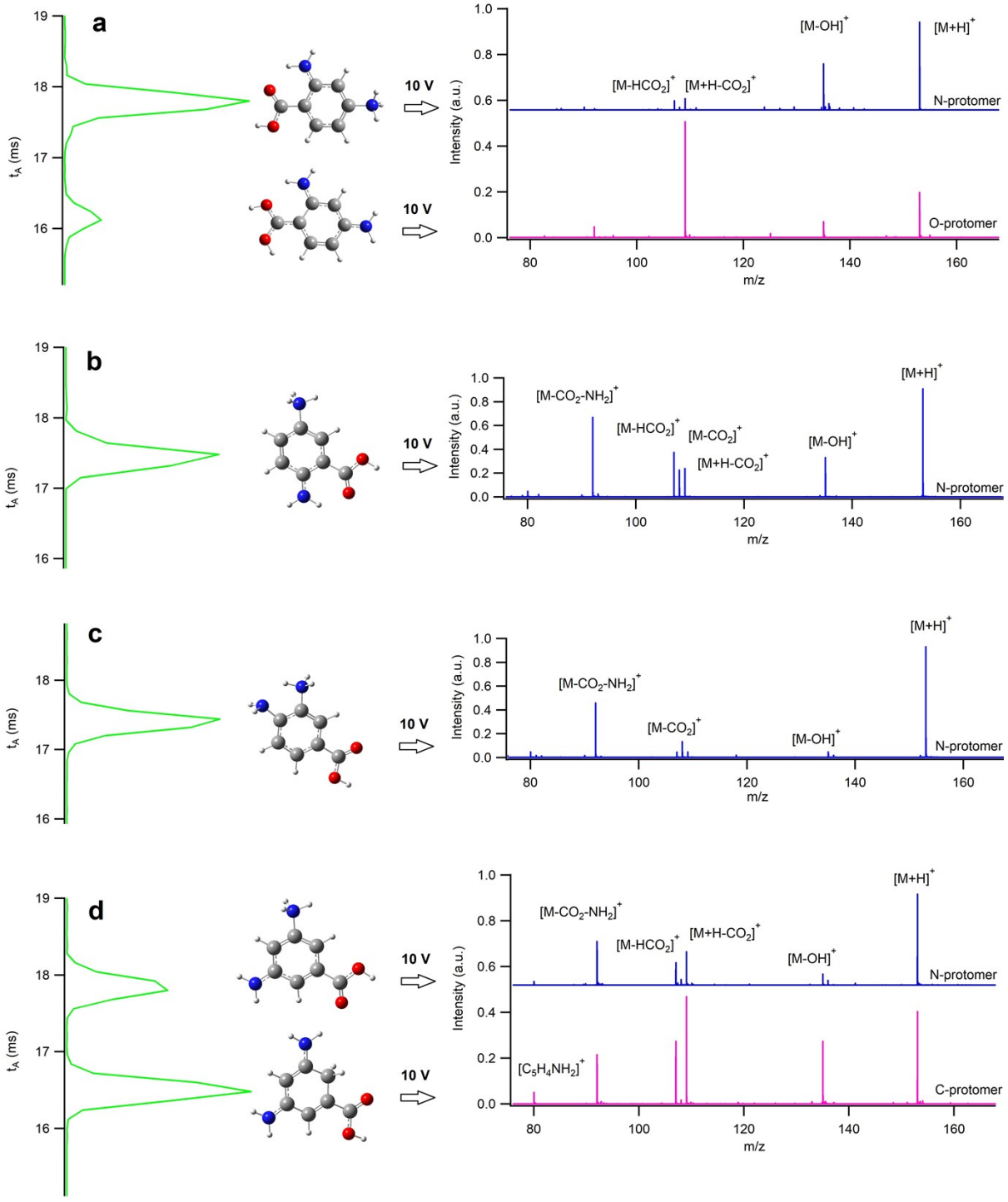
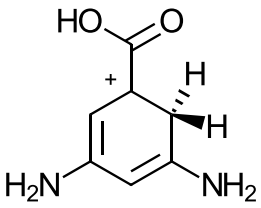
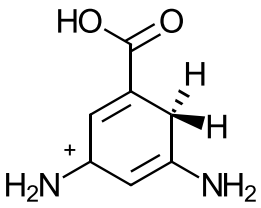


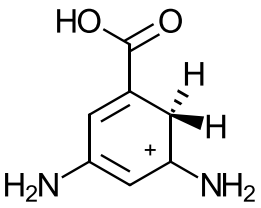
Figure S18. Comparison of HRMS fragment results for protomers of (a) 2,4-DABA and (b) 2,5-DABA (c) 3,4-DABA and (d) 3,5-DABA using a CID voltage of 10 V.



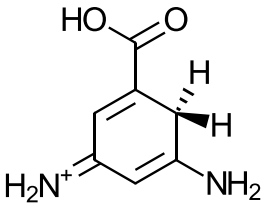
[3,5-DABA+H]$^+$-I



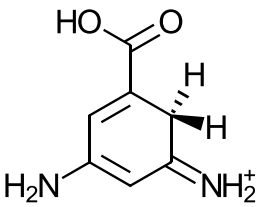
[3,5-DABA+H]$^+$-II



[3,5-DABA+H]$^+$-III



[3,5-DABA+H]$^+$-IV



[3,5-DABA+H]$^+$-V

Figure S19. Resonance stabilization of the C-protomer of 3,5-DABA. Structures **IV** and **V** show the participation of lone-pair electron of NH_2 group in the resonance of the positive charge.

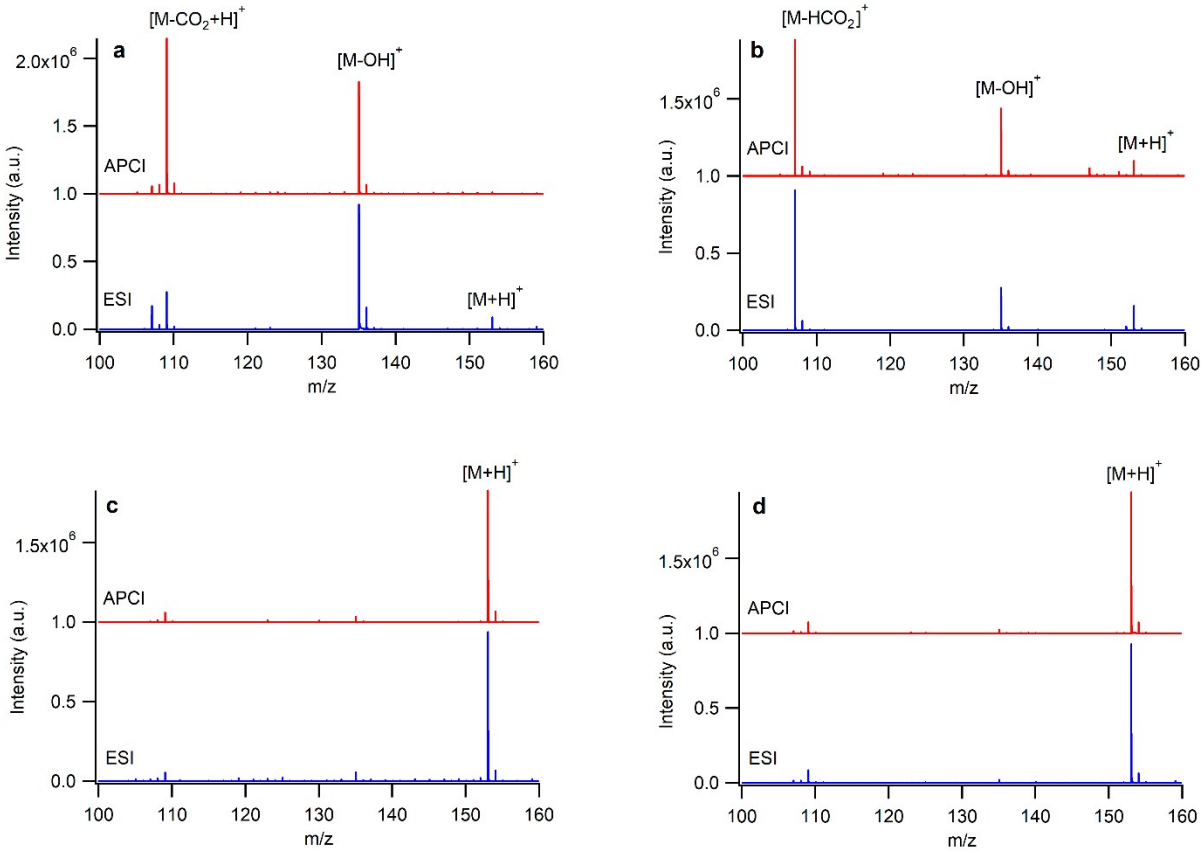


Figure S20. Comparison of mass spectra of (a) 2,4-DABA, (b) 2,5-DABA, (c) 3,4-DABA, and (d) 3,5-DABA ionized using APCI and ESI ion sources in H_2O:CH_3OH solvents.

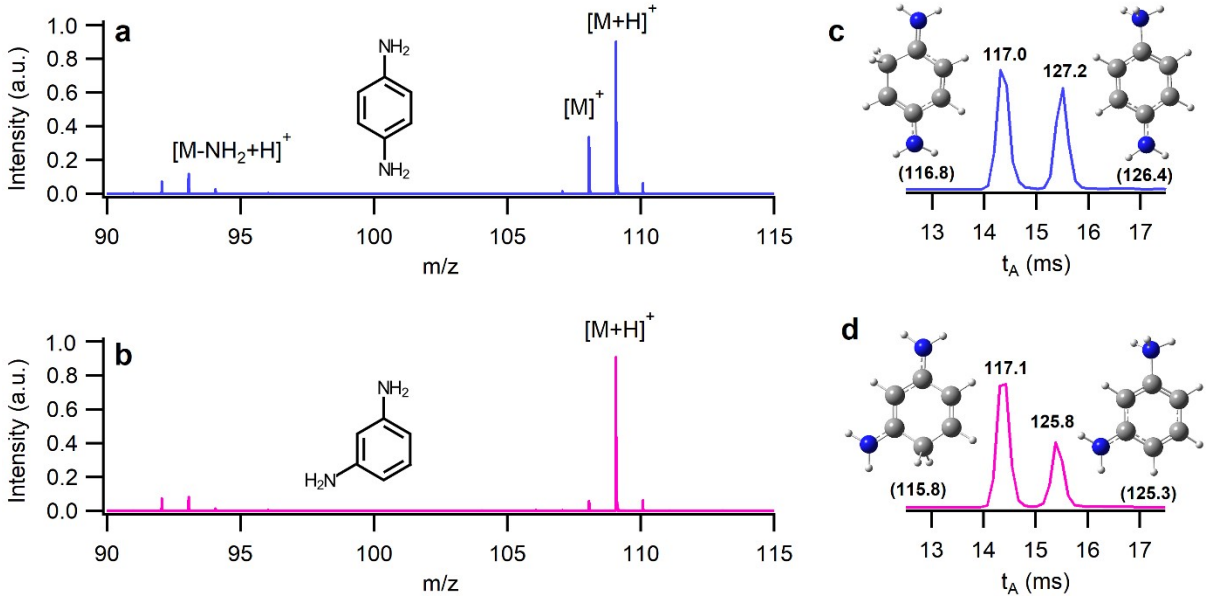


Figure S21. The mass spectra of (a) *p*-DAB and (b) *m*-DAB ionized by ESI with 0.01% FA. The *m/z*-selected IM spectra of (c) *p*-DAB and (d) *m*-DAB. The experimental and theoretical (numbers in parenthesis) CCS_{N2} values are in Å^2.

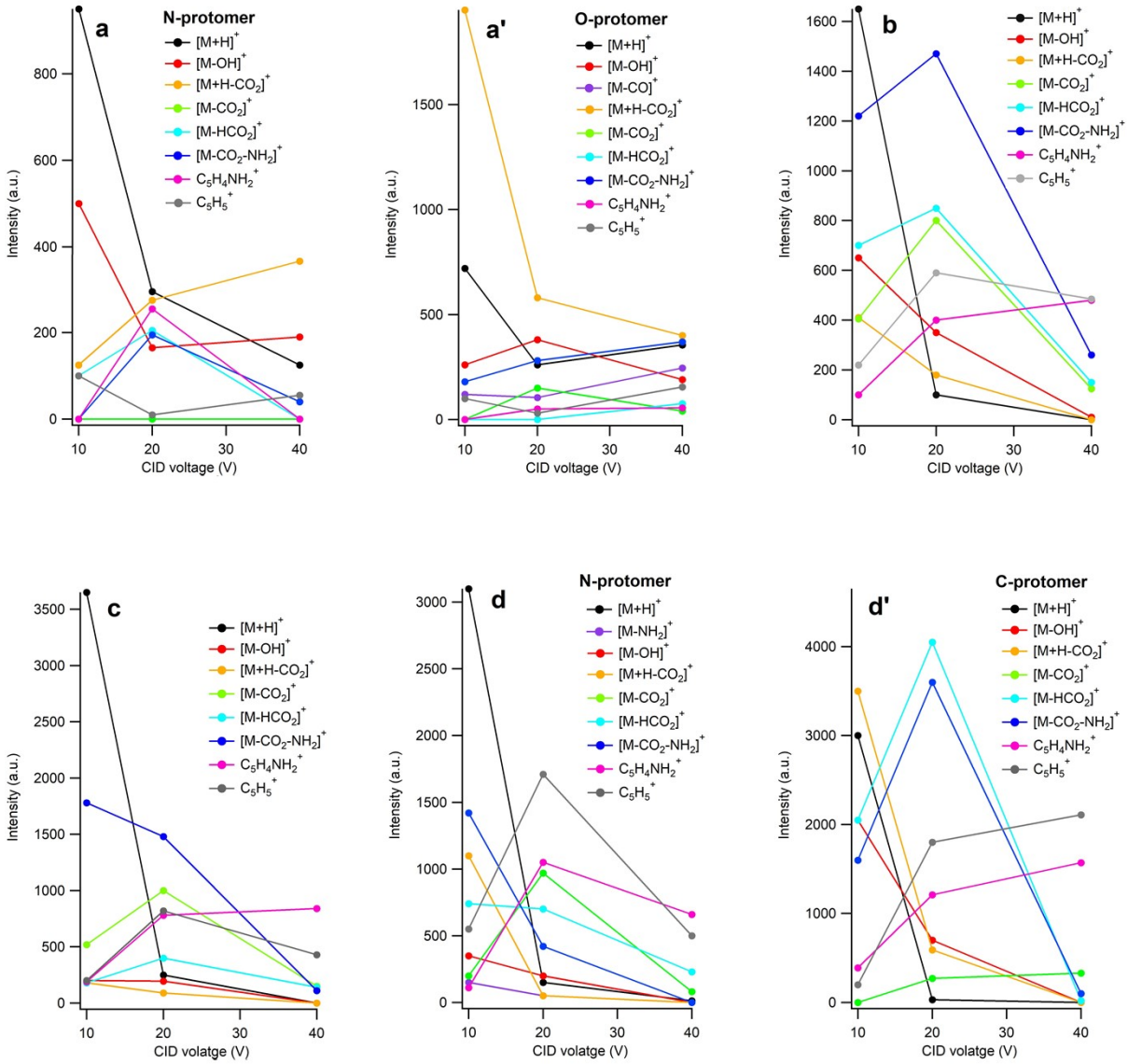


Figure S22. The intensity of fragment ions produced using post-IM CID for the protomers of (a and a') 2,4-DABA (N- and O-protomers), (b) 2,5-DABA (N-protomer), (c) 3,4-DABA (N-protomer), and (d and d') 3,5-DABA (N- and C-protomers) with respect to the applied CID voltage.

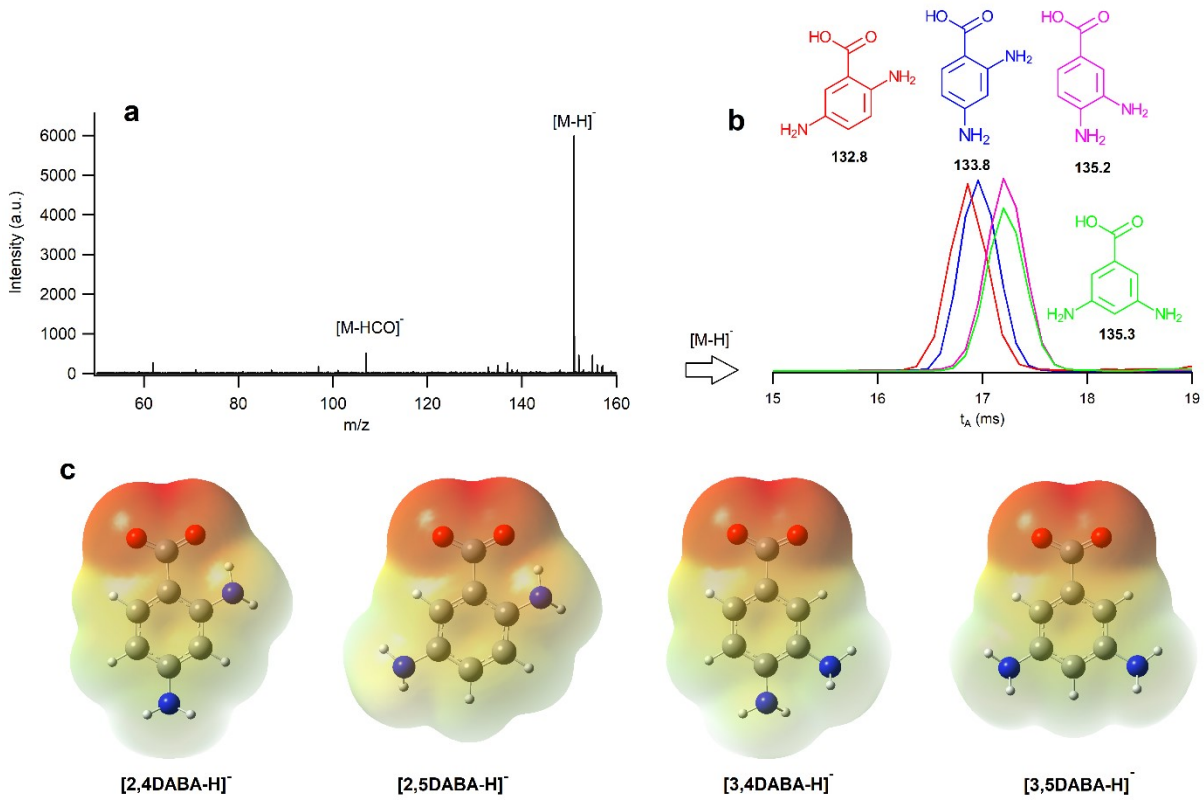


Figure S23. (a) Typical mass spectrum (3,5-DABA) for diamino benzoic acids in negative mode. (b) Experimental IM spectra for all DABA isomers. (c) The ESP maps for the $[M-H]^-$ ions of diamino benzoic acids. The numbers are experimental ${}^{DT}CCS_{N2}$ in Å^2.

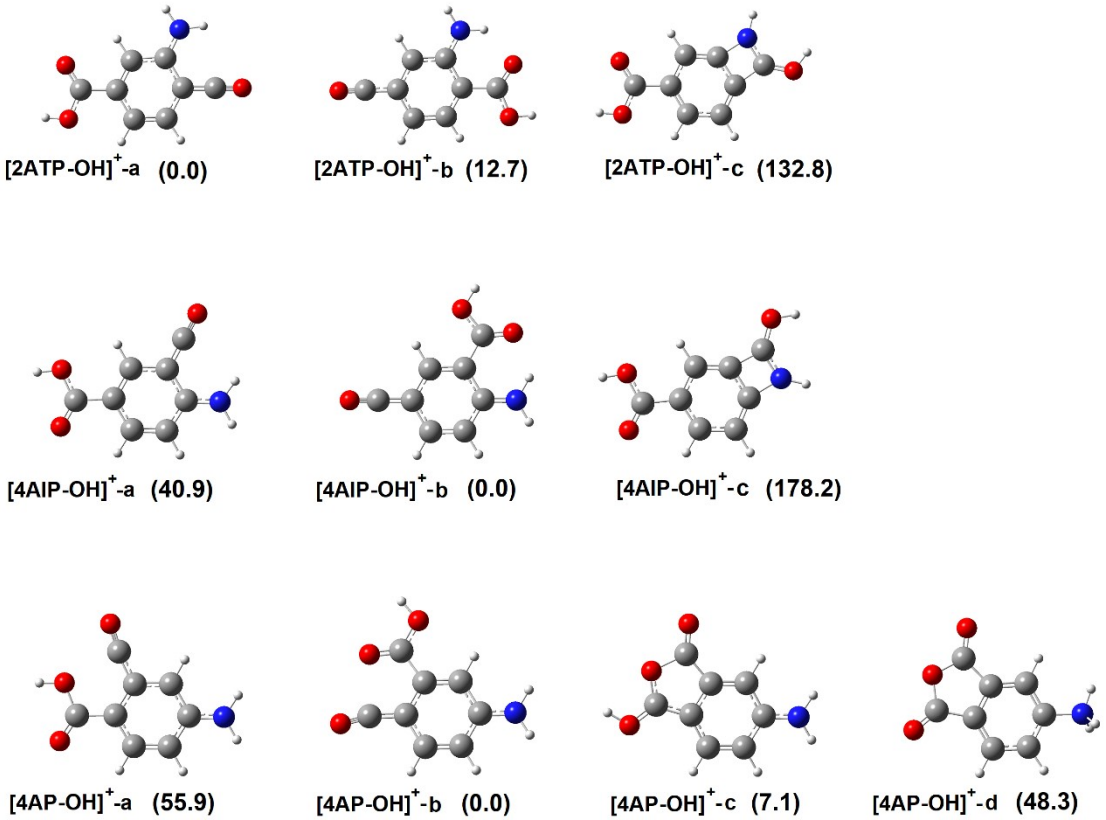


Figure S24. Comparison of relative Gibbs free energies of different isomers of $[M\text{-}OH]^+$ ions of 2ATP, 4AIP, and 4AP in the gas phase. The Gibbs energies are in kJ mol^{-1}.

Table S2. The calculated ΔH and ΔG values for deprotonation of diaminobenzoic acids (DABA) and aminophthalic acids (APA) in the gas phase, in methanol, and in water solvents. For the APA isomers, the most acidic site is reported. The energies are in kJ mol^{-1}.

	Gas phase		Aqueous solution		Methanol	
Compound	**ΔH**	**ΔG**	**ΔH**	**ΔG**	**ΔH**	**ΔG**
2,4-DABA	1437.5	1406.6	1205.3	1174.0	1210.3	1178.9
2,5-DABA	1422.7	1391.3	1196.4	1164.8	1201.2	1169.3
3,4-DABA	1442.8	1409.8	1202.4	1170.1	1207.6	1175.3
3,5-DABA	1437.9	1405.7	1196.5	1165.2	1201.7	1170.8
2ATP	1394.7	1364.5	1186.5	1155.0	1191.0	1159.5
4AIP	1395.1	1364.8	1187.7	1156.8	1192.0	1161.2
4AP	1334.2	1304.5	1147.9	1120.2	1151.5	1123.8
5AIP	1411.4	1379.1	1189.1	1155.0	1194.0	1160.4

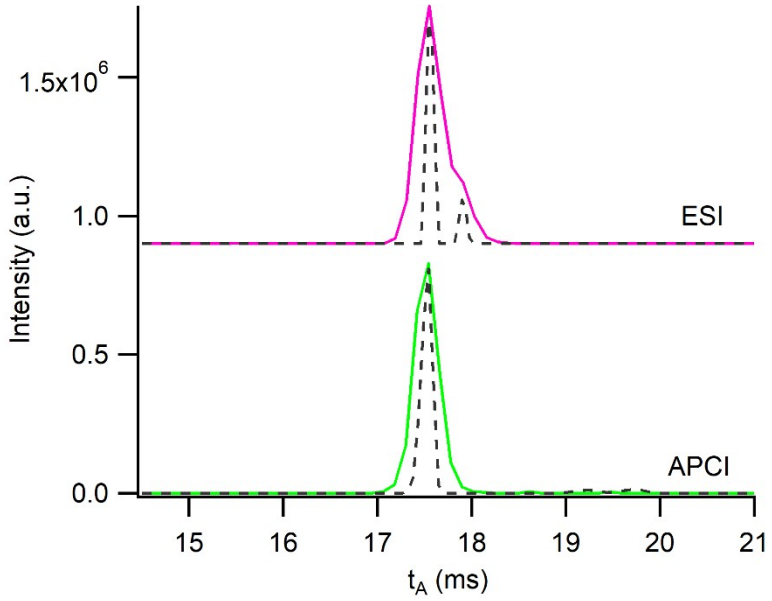


Figure S25. Comparison of the *m/z*-selected IM spectra of [2ATP-H]$^-$ ion ionized in ESI and APCI ion sources. The dash line spectra are the IM spectra achieved with high resolution demultiplexing (HRdm).