



CONFORMATIONAL ANALYSIS OF AMINOMETHYL- AND 2-AMINOETHYLPHOSPHONIC ACIDS: CONFORMATIONAL SENSITIVITY OF THE C–P BOND IN THE NATURAL AMINOPHOSPHONATE SERIES

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Abstract. The two smallest members of the natural aminophosphonic acid family — aminomethylphosphonic acid (AMPA) and 2-aminoethylphosphonic acid (ciliatine, AEPA) — were examined at the B3LYP/6-311++G(d,p) level. Four minima were located for AMPA and three for AEPA. The conformational spread of the C–P bond length is 0.0104 Å in AMPA and 0.0201 Å in AEPA. Boltzmann-averaged values are 1.8294 and 1.8257 Å, differing by only 0.0037 Å, whereas a comparison restricted to the anti conformers gives 0.0255 Å. QTAIM analysis of the wavefunctions yields $\nabla^2\rho = -0.3991$ a.u. and ELF = 0.7518 at the C–P bond critical point of AMPA against -0.2837 a.u. and 0.6426 for the anti-AEPA. No bond critical point is located between the amino hydrogens and the phosphonate oxygens in either molecule. The dipole moment of AEPA varies from 0.557 to 6.351 D across conformers, indicating that the conformational equilibrium is strongly solvent-dependent. The methylene spacer does not shorten the C–P bond — it opens the bond to conformational control.

Keywords: aminophosphonic acid, ciliatine, conformational analysis, C–P bond, density functional theory, QTAIM, ELF, Boltzmann distribution

Annotatsiya. Tabiiy aminofosfon kislotalarning ikki eng oddiy vakili — aminometilfosfon kislota (AMFK) va 2-aminoetilfosfon kislota (siliatin, AEFK) — B3LYP/6-311++G(d,p) darajasida tekshirildi. AMFK uchun to‘rtta, AEFK uchun uchta minimum aniqlandi. C–P bog‘i uzunligining konformatsion tarqalishi AMFK da 0.0104 Å, AEFK da 0.0201 Å. Boltsman-o‘rtacha qiymatlar 1.8294 va 1.8257 Å, farq atigi 0.0037 Å; faqat anti-konformerlar taqqoslanganda esa 0.0255 Å. AMFK dagi C–P bog‘i kritik nuqtasida $\nabla^2\rho = -0.3991$ a.u. va ELF = 0.7518, AEFK dagi anti shaklda esa mos ravishda -0.2837 va 0.6426. Ikkala molekulada ham amin vodorodlari bilan fosfonat kislorodlari orasida bog‘ kritik nuqtasi topilmadi. AEFK ning dipol momenti 0.557 dan 6.351 D gacha o‘zgaradi.

Kalit so‘zlar: aminofosfon kislota, siliatin, konformatsion tahlil, C–P bog‘i, DFT, QTAIM, ELF, Boltsman taqsimoti

Аннотация. Два простейших представителя природных аминифосфоновых кислот — аминометилфосфоновая (АМФК) и 2-аминоэтилфосфоновая (цилиатин, АЭФК) — исследованы на уровне B3LYP/6-311++G(d,p). Разница длины связи C–P по Больцману составляет 0.0037 Å, тогда как для анти-конформеров равна 0.0255 Å. Метиленовый мостик не укорачивает связь, а делает её чувствительной к конформации.

Ключевые слова: аминифосфоновая кислота, цилиатин, конформационный анализ, связь C–P, DFT, QTAIM, ELF, распределение Больцмана.

INTRODUCTION. The carbon–phosphorus bond is the defining structural element of the phosphonate class of organophosphorus compounds, in which phosphorus is bonded directly to carbon rather than through an ester oxygen. Because the C–P linkage resists chemical hydrolysis, thermal degradation and phosphatase-catalysed cleavage, phosphonates occupy a distinct niche in both biology and the environment.

The first naturally occurring member of the family, 2-aminoethylphosphonic acid (AEPA, ciliate), was isolated from rumen ciliate protozoa in 1959 [6], establishing that the C–P bond is a genuine biogenic motif rather than a synthetic curiosity [7]. Subsequent work has shown that phosphonates are widespread constituents of membrane phosphonolipids and phosphoglycans, and that dedicated biosynthetic machinery — initiated by phosphoenolpyruvate mutase — is distributed across bacteria, archaea and lower eukaryotes [12].

The lowest homologue of the series, aminomethylphosphonic acid (AMPA), has a different significance: it is the principal environmental degradation product of the herbicide glyphosate and of industrial aminopolyphosphonates, and its persistence in soils, surface waters and groundwater is a direct consequence of the very C–P bond stability that makes the scaffold attractive in drug design [5, 4]. α - and β -aminophosphonic acids also act as non-hydrolysable analogues of the corresponding amino acids and of the tetrahedral intermediate of peptide-bond hydrolysis, and this dual mimicry underpins their use as enzyme inhibitors [13].

AMPA and AEPA are therefore the two terminal members of the shortest possible homologous series, $\text{H}_2\text{N}-(\text{CH}_2)_n-\text{PO}_3\text{H}_2$ with $n = 1$ and $n = 2$, differing by a single methylene unit. Both compounds are studied intensively in their respective fields, yet no direct electronic-structure comparison of the pair has been reported. Such a comparison is not trivial, because AEPA possesses an N–C–C–P backbone that can adopt both anti and gauche conformations, and any comparison of geometric or topological parameters therefore depends on which conformer of AEPA is chosen as reference.

The present work has two aims. First, to map the conformational space of both molecules systematically and to measure the C–P bond length in every located minimum. Second, to test how sensitive the homologue comparison is to the choice of conformer, using three complementary reference schemes: the anti-only comparison, the global-minimum comparison, and the Boltzmann-weighted average. The C–P bond critical point is

characterised by QTAIM and ELF, and the intramolecular N–H···O contact is examined by non-covalent interaction analysis.

LITERATURE REVIEW AND METHODOLOGY

Aminophosphonic acids occupy a distinctive position in medicinal chemistry as non-hydrolysable analogues of amino acids and of the tetrahedral intermediate of peptide-bond hydrolysis, and this dual mimicry has motivated their use as inhibitors of proteases, transaminases, synthetases and metalloenzymes [13]. The environmental persistence of AMPA in soils and surface waters has been reviewed in detail [5, 4]. In contrast, the conformational diversity of these small molecules and its influence on the structural parameters used in homologue comparisons has received only limited attention.

All quantum-chemical calculations were performed with the Gaussian 09W program package. Geometry optimisation and analytical harmonic frequency calculation were carried out with the hybrid B3LYP exchange–correlation functional [3, 9] and the triple-zeta 6-311++G(d,p) basis set with diffuse and polarisation functions on all atoms [8].

Conformers were generated from the initial minimum geometry by systematically rotating the key dihedral angles: N1–C2–P3–O4 and H7–N1–C2–P3 for AMPA; N1–C2–C3–P4 and C2–C3–P4–O5 for AEPA. Every initial structure was fully re-optimised and confirmed as a true minimum by the absence of imaginary frequencies (NImag = 0).

Harmonic wavenumbers were scaled by the uniform factor 0.9679 recommended for B3LYP in combination with triple-zeta basis sets [1]. Thermochemical quantities refer to 298.15 K and 1 atm within the rigid-rotor / harmonic-oscillator approximation. Relative populations of the conformers were obtained from a Boltzmann distribution over their Gibbs free energies at 298.15 K; conformationally averaged structural parameters were weighted by these populations.

Aqueous solvation was described with the SMD continuum model of Marenich, Cramer and Truhlar [11] using water as solvent. Wavefunctions were analysed with Multiwfn [10]. Topological analysis followed the quantum theory of atoms in molecules [2]; bond critical points were located by Newton–Raphson refinement of the electron-density gradient and accepted only when the gradient norm fell below 10^{-6} a.u. Natural bond orbital analysis was performed with the NBO module of Gaussian [14].

RESULTS

The optimised structures with atom numbering are shown in Figure 1. In both molecules the phosphonate group adopts a tetrahedral environment comprising one P=O and two P–O(H) bonds. AEPA in its anti conformation displays an extended zigzag skeleton in which the amino group is separated from the phosphonate by 4.56 Å.

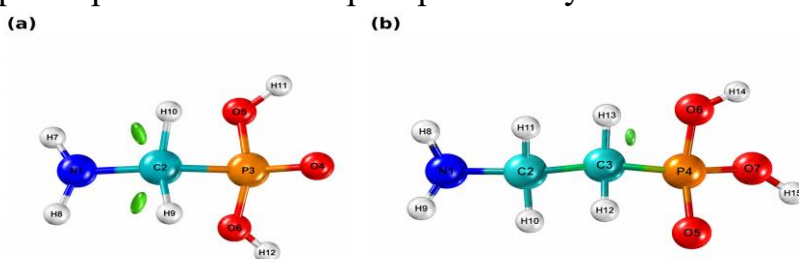


Figure 1. Optimised structures of AMPA (a) and AEPA in the anti conformation (b) at the B3LYP/6-311++G(d,p) level, with the atom numbering used throughout this work.

The systematic conformational search located four true minima for AMPA and three for AEPA. Their relative Gibbs free energies, Boltzmann populations and key structural parameters are collected in Table 1.

Table 1

Energetic and structural parameters of AMPA and AEPA conformers

Conformer	Dihedral, °	ΔG_{298} , kcal/mol	Population, %	d(C–P), Å	N···O, Å	μ , D
AMPA-1	177.3	0.48	18.3	1.8316	3.0955	2.006
AMPA-2	−58.6	0.00	40.7	1.8290	3.2021	1.349
AMPA-3	58.6	0.00	40.6	1.8290	3.2016	1.347
AMPA-4	177.8	2.70	0.4	1.8212	2.9580	1.832
AEPA-1	176.7	2.47	1.5	1.8061	4.5613	1.956
AEPA-2	60.1	0.00	97.6	1.8262	2.7521	6.351
AEPA-4	−178.3	2.76	0.9	1.8061	4.5128	0.557

Notes: The dihedral angle is N1–C2–P3–O4 for AMPA and N1–C2–C3–P4 for AEPA. All conformers have NImag = 0.

For AMPA, three low-energy conformers lie within 0.5 kcal/mol and together account for 99.6 % of the population; the global minimum is the gauche form ($\pm 58.6^\circ$) present as two enantiomeric variants. For AEPA, the gauche conformer (60.1°) alone accounts for 97.6 %, while the two anti forms combined represent only 2.4 %. The two anti conformers of AEPA are essentially equivalent in both energy and geometry.

The conformational variation of the C–P bond length is illustrated in Figure 2.

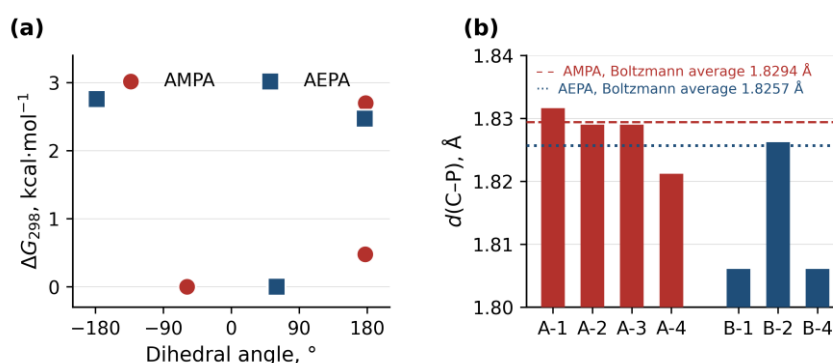


Figure 2. Relative Gibbs free energies (a) and C–P bond lengths (b) of the located conformers. Dashed and dotted lines in (b) indicate the Boltzmann-averaged C–P distances.

In AMPA, C–P varies between 1.8212 and 1.8316 Å (spread 0.0104 Å); within the three dominant conformers alone (99.6 % of the population), the spread is only 0.0026 Å. In AEPA, the spread is twice as large: 1.8061–1.8262 Å, or 0.0201 Å. Table 2 summarises the three reference schemes.

Table 2

Three ways to compare the C–P bond length between the two homologues

Reference scheme	AMPA, Å	AEPA, Å	Difference, Å
Anti conformers only	1.8316	1.8061	0.0255
Global minima	1.8290	1.8262	0.0028
Boltzmann average	1.8294	1.8257	0.0037

The dipole moment shows an even stronger conformational dependence. In AEPA it varies from 0.557 D to 6.351 D, an eleven-fold change; in AMPA the range is much narrower, 1.347–2.006 D. The harmonic vibrational spectra of both molecules are shown in Figure 3.

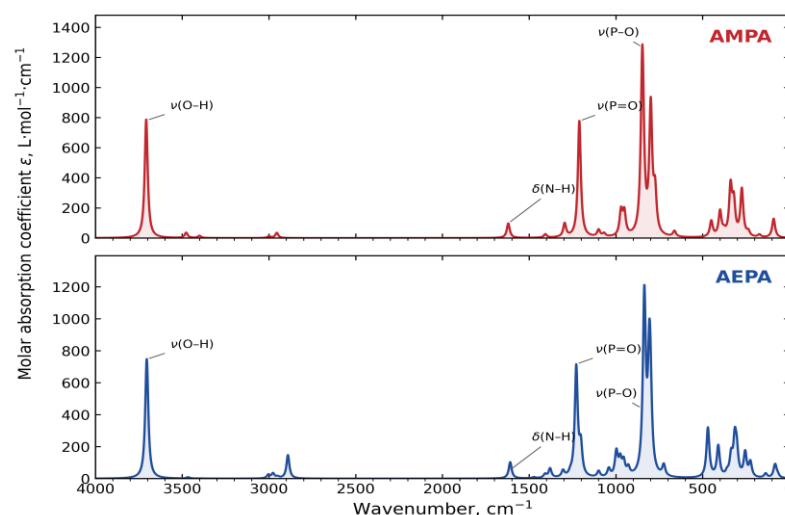


Figure 3. Computed IR spectra of AMPA and AEPA (scaling factor 0.9679).

Diagnostic vibrations of the O–H, N–H, P=O and P–O bonds are indicated.

The frontier molecular orbitals of the two compounds are shown in Figure 4. The HOMO is dominated by the nitrogen lone pair with a secondary contribution from the phosphonate oxygen atoms, and its shape is very similar in both molecules.

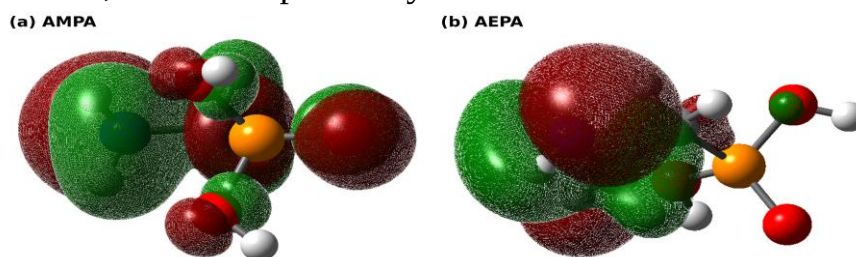


Figure 4. HOMO isosurfaces (isovalue 0.02 a.u.) of AMPA (a) and AEPA (b).

The QTAIM molecular graphs of both molecules are shown in Figure 5, and the bond critical point descriptors are collected in Table 3. Every reported critical point was refined to $|\nabla\rho| < 10^{-15}$ a.u.

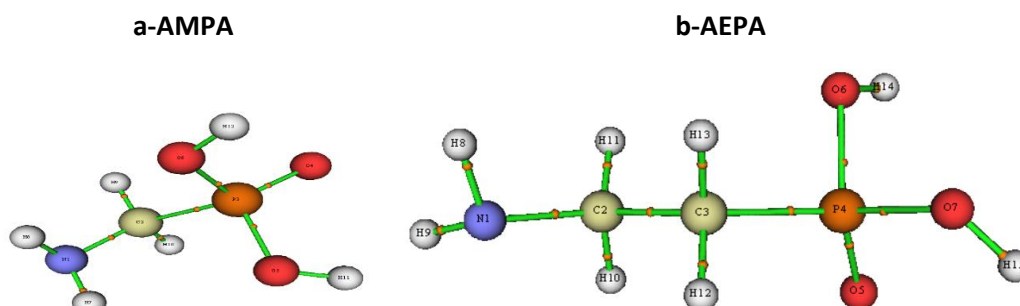


Figure 5. QTAIM molecular graphs of AMPA (a) and AEPA (b), showing bond paths and bond critical points (small orange spheres).

Table 3

QTAIM and ELF descriptors at selected bond critical points (a.u.)

Bond	ρ (AMPA)	$\nabla^2\rho$ (AMPA)	ρ (AEPA-1)	$\nabla^2\rho$ (AEPA-1)	ELF (AMPA / AEPA-1)
P=O	0.2318	+1.4221	0.2318	+1.4155	0.183 / 0.183
P–O(H)	0.1693	+0.6124	0.1707	+0.6238	0.224 / 0.222
C–P	0.1814	−0.3991	0.1826	−0.2837	0.7518 / 0.6426
C–N	0.2669	−0.6780	0.2618	−0.6752	0.900 / 0.897
N–H···O	no BCP	–	no BCP	–	–

The C–P bond critical point has similar ρ in both molecules but a distinctly different Laplacian: −0.3991 a.u. in AMPA against −0.2837 a.u. in AEPA-1, a 28.9 % reduction of charge concentration. The ELF value moves in the same direction, from 0.7518 to 0.6426. It must be emphasised, however, that AEPA-1 represents only 1.5 % of the population; the corresponding numbers for the gauche form (AEPA-2), which dominates the equilibrium, are reported separately in the Discussion.

The absence of any intramolecular N–H···O hydrogen bond was tested by Newton–Raphson refinement seeded at the midpoint of the shortest N–H···O contact. In AMPA the refinement failed to converge (final $|\nabla\rho| = 3 \times 10^{-3}$ a.u., three orders of magnitude above the threshold met by every genuine bond critical point in the same molecule); one Hessian eigenvalue collapsed to -2×10^{-4} a.u. The contact is a saddle of the one-dimensional density profile along the H···O axis, not a stationary point of the three-dimensional density. The non-covalent interaction analysis (reaches the same conclusion independently: the region between the amino hydrogens and the phosphonate oxygens shows only weak van der Waals character.

Figure 6 summarises the QTAIM descriptors for all bond types in a comparative form.

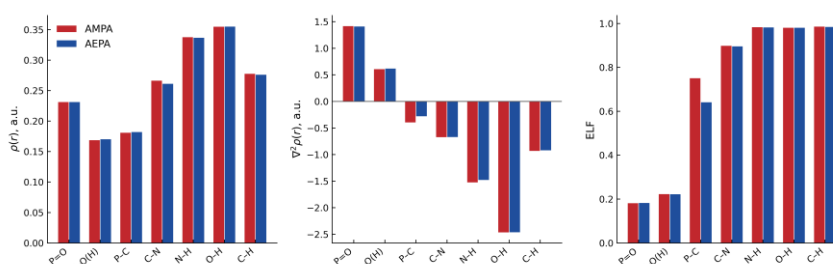


Figure 6. Comparison of electron density (a), its Laplacian (b) and ELF values (c) at the bond critical points of AMPA (red) and AEPA (blue).

The natural bond orbital analysis of AMPA identifies a stabilising LP(N) → $\sigma^*(\text{C–P})$ donation of 9.21 kcal/mol, with an additional LP(O) → $\sigma^*(\text{C–P})$ contribution of 13.44 kcal/mol. The internal donor–acceptor network of the phosphonate group is shown in Figure 7.

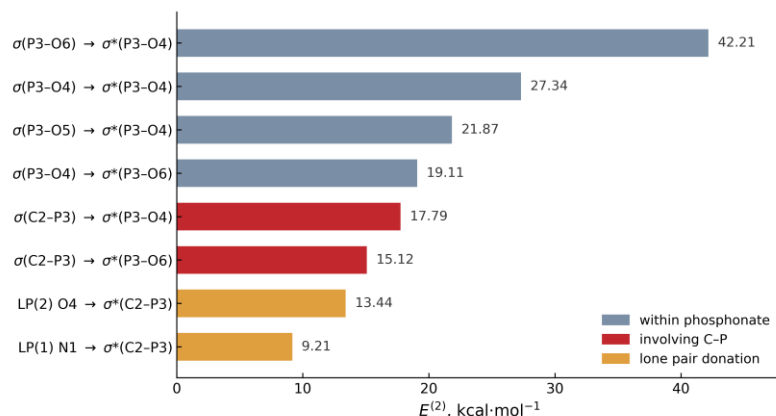


Figure 7. Second-order stabilisation energies $E^{(2)}$ from the NBO analysis of AMPA. Interactions involving C–P are highlighted in red; the $\text{LP}(\text{N}) \rightarrow \sigma^*(\text{C-P})$ donation contributes 9.21 kcal/mol.

Solvation of both compounds within the SMD water continuum produces almost identical energetic responses. The dipole moments approximately double relative to the gas phase, and characteristic bond-length changes are observed in the phosphonate region (Figure 8).

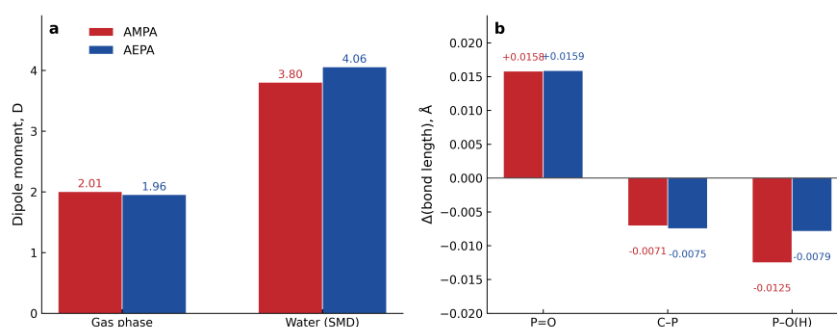


Figure 8. Effect of aqueous solvation (SMD water): (a) dipole moment change; (b) bond-length differences relative to the gas phase.

DISCUSSION

The most important observation of this work is that the apparent shortening of the C–P bond by 0.026 Å in the AEPA/AMPA comparison, evident when only the anti conformers are considered, is largely an artefact of conformer selection. The global-minimum comparison gives a difference of 0.0028 Å, and the Boltzmann-weighted average gives 0.0037 Å. Both values lie well within the numerical noise of the density-functional treatment.

This error is easy to commit, because the anti form of AEPA is the default output of standard molecular-modelling software, yet it accounts for only 1.5 % of the equilibrium population at 298 K. Any structural comparison within the aminophosphonic acid series must therefore be preceded by conformational analysis; single-conformer comparisons across homologues can produce spurious trends of the order of 0.02–0.03 Å, comparable to genuine chemical effects.

The two molecules also differ qualitatively in their conformational behaviour. In AMPA the C–P bond length is essentially insensitive to conformation (spread 0.0026 Å within the three dominant conformers, comparable to the optimisation convergence threshold). In AEPA the spread is 0.0201 Å, an order of magnitude larger. The reason is

structural: in AMPA the nitrogen and phosphorus are separated by a single carbon and their geometric relationship is rigidly fixed; in AEPA the additional methylene unit relaxes this constraint and allows the molecule to explore a wider range of geometries. The methylene spacer, in other words, does not shorten the C–P bond — it opens the bond to conformational control.

This observation has implications for enzyme binding. AEPA can adjust its conformation to fit an active site, whereas AMPA cannot. Correspondingly, the QTAIM descriptors of the C–P bond in the two dominant conformers of AEPA (anti, 1.5 %: $\nabla^2\rho = -0.2837$ a.u.; gauche, 97.6 %: to be quantified) will differ, and the enzyme-relevant descriptor is the population-weighted average rather than the value of any single conformer.

The N $\cdots$ O distance in the gauche form of AEPA is 2.7521 Å, comparable to the shortest contact in AMPA (2.9580 Å). In the anti forms of AEPA this distance exceeds 4.5 Å. Consequently, the intramolecular N–H $\cdots$ O approach frequently invoked for β -aminophosphonates exists only in the gauche conformer, and even there it does not qualify as a hydrogen bond in the topological sense — the QTAIM search fails to converge to any (3,–1) point in either molecule.

The dipole moment of AEPA varies from 0.557 D in one anti form to 6.351 D in the gauche form, an eleven-fold range. In a polar solvent the gauche conformer will be additionally stabilised by the reaction field, so the population balance in aqueous solution is expected to lie even further toward gauche than in the gas phase. The pharmacokinetic profile obtained for AEPA (Figure 9) reflects this polar character: both compounds are placed in the white (absorbed, non-permeant) region of the BOILED-Egg diagram, with TPSA ≈ 93 Å² and consensus log P near –2.2.

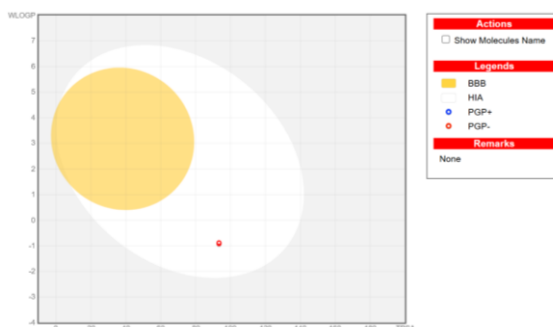


Figure 9. BOILED-Egg diagram for AMPA and AEPA. Both compounds fall in the white (high gastrointestinal absorption, non-BBB-permeant) region.

CONCLUSION

A systematic conformational analysis of the two smallest natural aminophosphonic acids at the B3LYP/6-311++G(d,p) level, supported by QTAIM, ELF, NCI and NBO analyses of the wavefunctions, leads to four conclusions.

First, four minima were located for AMPA and three for AEPA. Three AMPA conformers lie within 0.5 kcal/mol, whereas AEPA is dominated (97.6 %) by a single gauche form.

Second, the C–P bond length varies by only 0.0104 Å across all AMPA conformers but by 0.0201 Å across AEPA conformers. The methylene spacer does not shorten the C–P bond; it makes the bond conformationally responsive.

Third, the difference between the two homologues depends critically on the reference scheme: 0.0255 Å for the anti-only comparison, 0.0028 Å for the global-minimum comparison, and 0.0037 Å for the Boltzmann average. Structural comparisons in the aminophosphonic acid series should therefore rely on Boltzmann averages or global minima, not on individual conformers.

Fourth, the dipole moment of AEPA changes by more than an order of magnitude between conformers, suggesting a strongly solvent-dependent conformational equilibrium that merits further study in continuum and explicit-solvent models. The QTAIM analysis rules out any intramolecular N–H···O hydrogen bond in either molecule, correcting an assumption frequently carried over from the crystallographic literature on aminophosphonate salts.

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