Stabilization of Zwitterions in Solution: GABA Analogues

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The solution-phase structures of a number of conformationally restricted γ-aminobutyric acid (GABA) analogues are investigated at the MP2/6-31+G* level of theory, using both explicit water molecules and the conductor-like screening solvation model (COSMO) to model solvation. GABA analogues constrained in a cis conformation by either a double bond or cyclopropane ring have the potential to attain either folded, intramolecularly hydrogen-bonded, or partially folded conformations in solution. Systems constrained in a cis conformation by a cyclopentane or cyclopentene ring are more conformationally restricted and exist only in a folded, intramolecularly hydrogen-bonded form. GABA analogues constrained in a trans conformation by either a double bond or cyclopropane ring have the potential to adopt either partially folded or fully extended conformations in solution. Due to a lack of conformational flexibility, analogues that are constrained in a trans conformation by a cyclopentane or cyclopentene ring attain only partially folded conformations. Like GABA, conformationally flexible GABA analogues possess a large number of stable rotamers, and may exist in any or all of these conformations in aqueous solution. The structures of these analogues provide an essential foundation for subsequent structure−activity analysis of ligand binding at GABA receptors and transporters. This work is therefore expected to facilitate the design and development of new biologically active GABA analogues to treat GABA-related neurological disorders.

Introduction

As the major inhibitory neurotransmitter in the mammalian central nervous system (CNS), γ-aminobutyric acid (GABA) plays a vital role in maintaining normal neuronal activity by regulating the equilibrium between neuronal excitation and inhibition.[1] Consequently, drugs that act at GABA receptors are indicated for use in a variety of neurological disorders including epilepsy,[2,3] anxiety,[3,4] schizophrenia,[3,5] and stiff-person syndrome.[3,6] A knowledge of the low energy solvated structures of GABA and its conformationally restricted analogues will facilitate the design and development of drugs targeting the GABA binding site of these receptors.

Possessing both an amino group and a carboxylic acid group, GABA and its conformationally restricted analogues can exist in either zwitterionic or nonzwitterionic forms, depending on the environment.[7−11] GABA is known to be zwitterionic in the aqueous phase, and a number of different low energy conformers are accessible through zero-point motion.[8,9] Conformationally restricted analogues of GABA are also expected to be zwitterionic in aqueous solution, although the number of low energy conformations they can attain will be limited. To accurately model the behavior of GABA and its analogues in the predominantly aqueous in vivo environment, it is necessary to account for solvent effects. Furthermore, the GABA analogues that possess a degree of conformational flexibility may attain a number of low energy conformations. As it is unclear which conformation is biologically active, it is necessary to identify all stable aqueous-phase rotamers.

The solvated GABA molecule has previously been studied using semiempirical ab initio methods,[12−15] Hartree−Fock theory,[11−16] density functional theory,[16] and Møller−Plesset perturbation theory.[11,16] A number of conformationally restricted analogues of GABA have also been previously studied using semiempirical ab initio methods.[12,13]

The most sophisticated solvation model employed to date involved performing Monte Carlo simulations, embedding a GABA molecule within a box of water molecules described by the TIP4P potential.[11] However, this work was based on a limited exploration of configuration space of dihydrated GABA, carried out at a relatively low level of theory (HF/6-311++G**). Further, the zwitterionic conformers thus identified were then stripped of their associated water molecules before their internal energies were calculated at MP2/6-311++G** and subsequently embedded as the solute molecule in a Monte Carlo simulation. This is not necessarily a physically reasonable approach, as zwitterionic geometries that are local minima on a dihydrate potential energy surface are known to be unstable on the gas-phase surface.[16]

The most extensive exploration of configuration space of the solvated GABA zwitterion to date was carried out at MP2/6-31+G*.[16] Configuration space was explored by rotation about all rotatable bonds in the GABA molecule. A number of solvation models were investigated, including a dielectric continuum model, a discrete solvation model with two explicit water molecules, a discrete solvation model with five explicit water molecules, and a "supermolecule" continuum solvation model with two explicit water molecules and a surrounding dielectric continuum. All four models concurred that the zwitterionic structures were significantly lower in energy than the nonzwitterionic structures in the aqueous phase. The structures of the GABA analogues studied here are sufficiently similar to the structure of the parent GABA molecule that the computational protocol established for GABA will also be

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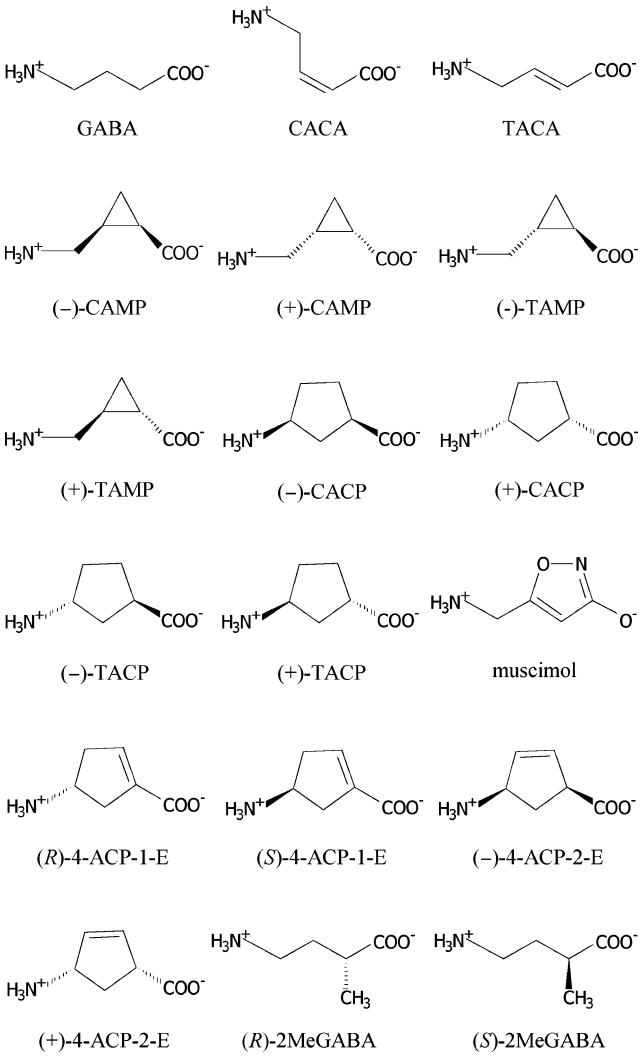

Figure 1. Schematic diagrams of GABA and GABA analogues.

applicable for identifying the stable solution-phase conformers of the GABA analogues. Further, the conclusion that the bulk solvated zwitterionic conformers are sufficiently lower in energy than the nonzwitterionic conformers so as to exist exclusively in aqueous solution will also apply to the GABA analogues studied here.

In this paper, we utilize the computational protocol developed for solvated GABA[16] to determine the likely solution-phase structures of the conformationally restricted GABA analogues shown in Figure 1. The full chemical names of these compounds and the abbreviations adopted here can be found in Table 1. These analogues have been chosen for investigation because they have been experimentally synthesized and characterized for biological activity at $GABA_C$ receptors.[17−23] However, no quantitative structure−activity relationship (QSAR) studies have been carried out to date, as the determination of the three-dimensional structures of solvated zwitterions poses a significant computational challenge. It is expected that the structures generated in this work will provide a high-quality foundation for future QSAR studies.

Computational Methods

Short-range solvent interactions were modeled by optimizing the geometry of each molecule in the presence of two explicit water molecules using second-order many-body Møller−Plesset perturbation theory (MP2)[24−27] with a 6-31+G* basis set.[28−32]

TABLE 1: Full Chemical Names and Abbreviations of the Compounds Studied

abbreviation	chemical name
GABA	4-aminobutanoic acid
CACA	*cis*-4-aminobut-2-enoic acid
TACA	*trans*-4-aminobut-2-enoic acid
(−)-CAMP	(−)-*cis*-2-aminomethylcyclopropane-1-carboxylic acid
(+)-CAMP	(+)-*cis*-2-aminomethylcyclopropane-1-carboxylic acid
(−)-TAMP	(−)-*trans*-2-aminomethylcyclopropane-1-carboxylic acid
(+)-TAMP	(+)-*trans*-2-aminomethylcyclopropane-1-carboxylic acid
(−)-CACP	(−)-*cis*-3-aminocyclopentane-1-carboxylic acid
(+)-CACP	(+)-*cis*-3-aminocyclopentane-1-carboxylic acid
(−)-TACP	(−)-*trans*-3-aminocyclopentane-1-carboxylic acid
(+)-TACP	(+)-*trans*-3-aminocyclopentane-1-carboxylic acid
muscimol	5-aminomethyl-3-hydroxyisaxazole
(*R*)-4-ACP-1-E	(*R*)-4-aminocyclopent-1-ene-1-carboxylic acid
(*S*)-4-ACP-1-E	(*S*)-4-aminocyclopent-1-ene-1-carboxylic acid
(-)-4-ACP-2-E	(1*S*,4*R*)-4-aminocyclopent-2-ene-1-carboxylic acid
(+)-4-ACP-2-E	(1*R*,4*S*)-4-aminocyclopent-2-ene-1-carboxylic acid
(*R*)-2MeGABA	(*R*)-2-methyl-4-aminobutanoic acid
(*S*)-2MeGABA	(*S*)-2-methyl-4-aminobutanoic acid

The 6-31+G* basis[28−32] was used in all calculations, as it represents the smallest possible basis set that adequately accounts for electron delocalization and orbital polarization due to hydrogen-bonding interactions. Thus this basis set represents a reasonable compromise between computational expense and chemical accuracy. Møller−Plesset perturbation theory has previously been shown to be the minimum level of theory required to qualitatively describe solvated zwitterionic compounds.[16]

Long-range solvent interactions were modeled using the conductor-like screening solvation model (COSMO)[33−35] with water as solvent. The explicit water molecules were removed to decrease the dimensionality of the system, thereby decreasing the computational cost required to explore configuration space. This approach has previously been validated for the identification of low energy structures, although it introduces a degree of error into the relative energies.[16] Configuration space was explored by sequential rotation about each rotatable bond from the minimum energy conformation. This conformational exploration was carried out within the dielectric continuum solvation model. Coupling between the torsional modes was crudely accounted for by identification of the minimum energy conformations resulting from bond rotation and subsequent rotation about the remaining rotatable bonds, starting from these conformations. To more accurately account for coupling between torsional modes, the local minimum energy conformations thus identified were further optimized at MP2/6-31+G* within the COSMO solvation model with a dielectric constant of $\epsilon = 78.39$ chosen to mimic the dielectric constant of water.

All calculations reported here were carried out using the Gaussian 03 program suite[36] on the computing facilities at the School of Chemistry, University of Sydney, and at the Australian Partnership for Advanced Computing (APAC) National Facility based at the Australian National University.

Results and Discussion

Energetic and selected geometric data for all compounds investigated are presented in Table 2, and all minimum energy structures identified by optimization within the COSMO reaction field are illustrated in Figures 2−12. The optimized dihydrate structures are not depicted here, but energetic and geometric data for the dihydrated compounds are available in the Supporting Information. Likewise, the full set of geometric data for the GABA analogues optimized within the COSMO reaction field is also available as Supporting Information. In all figures

TABLE 2: Energetic and Selected Geometric Data for the Stable Aqueous-Phase Conformers of a Range of Conformationally Restricted GABA Analogues

	N−C−C−C (deg)	O−C−C−C (deg)	C−C−C−C (deg)	energy (kJ/mol)
CACA 1	−65.4	34.0	−2.5	0.0
CACA 2	65.5	−34.7	2.5	0.0
CACA 3	118.7	42.3	−1.9	21.9
CACA 4	−125.1	−40.8	2.1	21.5
TACA 1	0.4	175.2	−179.5	5.1
TACA 2	−118.0	−168.5	−177.5	0.1
TACA 3	117.0	172.4	179.9	0.0
(−)-CAMP 1	−61.1	5.1	0.3	0.9
(−)-CAMP 2	149.0	−49.0	−0.2	12.3
(−)-CAMP 3	63.7	−38.0	−5.5	0.0
(+)-CAMP 1	−148.9	49.7	0.4	12.3
(+)-CAMP 2	−63.7	38.0	5.5	0.0
(+)-CAMP 3	59.1	0.0	0.0	1.0
(−)-TAMP 1	−152.0	−34.8	−140.5	1.1
(−)-TAMP 2	−42.0	−35.7	−136.8	15.9
(−)-TAMP 3	78.3	−36.3	−141.1	0.2
(+)-TAMP 1	−78.5	35.3	141.5	0.0
(+)-TAMP 2	151.8	34.0	140.7	1.0
(+)-TAMP 3	42.9	34.7	136.7	15.9
(−)-CACP	−80.3	−54.1	101.7	0.0
(+)-CACP	80.3	54.7	−101.6	0.0
(−)-TACP 1	162.8	−92.9	81.1	4.2
(−)-TACP 2	78.3	−47.8	147.8	2.6
(+)-TACP 1	−93.7	51.0	−163.4	0.0
(+)-TACP 2	−163.7	72.9	−87.4	4.1
muscimol 1	−110.8	179.7	179.5	0.0
muscimol 2	110.4	−179.7	−179.5	0.0
(−)-4-ACP-2-E	−91.1	−40.0	93.7	0.0
(+)-4-ACP-2-E	91.0	41.7	−94.0	0.0
(*S*)-4-ACP-1-E	−89.4	9.1	167.7	0.1
(*R*)-4-ACP-1-E	89.3	−11.5	−167.8	0.0
(*R*)-2MeGABA 1	−46.2	86.8	−45.0	0.3
(*R*)-2MeGABA 2	−73.0	−54.5	79.2	0.0
(*R*)-2MeGABA 3	−93.5	−53.5	−176.6	35.1
(*R*)-2MeGABA 4	−179.7	−46.9	−59.3	15.7
(*R*)-2MeGABA 5	−179.9	80.8	59.3	21.6
(*R*)-2MeGABA 6	−172.8	2.7	70.1	22.7
(*R*)-2MeGABA 7	176.1	−50.5	−173.4	21.2
(*R*)-2MeGABA 8	89.8	−14.2	−59.5	0.4
(*R*)-2MeGABA 9	54.4	−78.7	32.1	9.3
(*R*)-2MeGABA 10	69.4	−52.5	−169.6	24.9
(*S*)-2MeGABA 1	−90.2	16.6	58.2	0.5
(*S*)-2MeGABA 2	−71.3	−70.1	84.0	1.8
(*S*)-2MeGABA 3	−56.3	77.4	−30.3	8.8
(*S*)-2MeGABA 4	−69.6	47.0	169.7	24.8
(*S*)-2MeGABA 5	−176.0	58.5	173.9	21.2
(*S*)-2MeGABA 6	179.6	46.6	58.9	15.6
(*S*)-2MeGABA 7	179.7	−82.2	−59.7	21.5
(*S*)-2MeGABA 8	72.8	56.1	−78.9	0.0
(*S*)-2MeGABA 9	46.0	−86.8	45.1	0.4
(*S*)-2MeGABA 10	88.0	77.9	175.6	35.4

presented in this paper, the atoms are represented as grayscale spheres. The hydrogen atoms are white, the carbon atoms are dark gray, the oxygen atoms are black, and the nitrogen atoms are light gray. In all cases, all the structures identified as local minima within the solvation model were zwitterionic. It can be observed from Table 2 that the relative energies of the stable conformers fall within 36 kJ/mol of the global minimum energy structure, for all compounds surveyed. Previous work on benchmarking methods for modeling solvated zwitterions has shown that the neglect of short-range solvent interactions and deficiencies in both the size of the basis set and the treatment of electron correlation can introduce an error of up to 100 kJ/mol in relative energies.[16] The largest errors occurred for extended conformers, where the binding of the zwitterionic GABA molecule to explicit solvent molecules significantly reduced the overall dipole moment. Hence, considering the "dehydrated" molecule as an isolated solute within a continuum solvation model artificially overestimated the stability of these conformers. As a result, for each compound considered here, all identified stable conformers can be considered approximately isoenergetic in aqueous solution. The structural and energetic features of the low energy conformers of each compound will be discussed in more detail below. The minimum energy structures of the solvated conformationally restricted GABA analogues will then be compared to the minimum energy structures of solvated GABA.

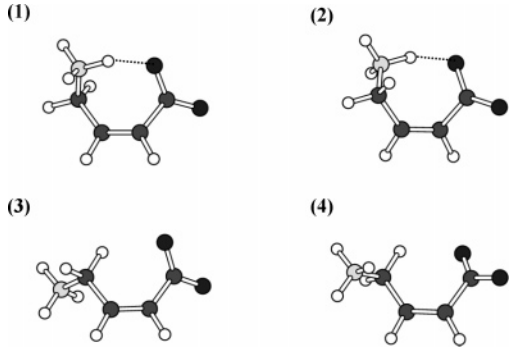

Figure 2. Minimum energy CACA conformers optimized at MP2/6-31+G* in the COSMO reaction field ($\epsilon = 78.39$). Intramolecular hydrogen bonds are indicated by dotted lines.

CACA. The lowest energy structure obtained for the CACA dihydrate was zwitterionic, in a folded, intramolecularly hydrogen-bonded conformation. Each explicit water molecule was hydrogen-bonded to both the amino and carboxylate groups, with one molecule positioned above and one molecule positioned below the plane of the cyclic CACA molecule. We have not attempted to depict the geometries for the dihydrated compounds here; full details are supplied in the Supporting Information. Upon "dehydration" and rotation about the N−C−C−C and O−C−C−C dihedrals within the COSMO solvation model, a further three low energy solvated structures were identified. Further optimization within the COSMO solvation model resulted in the identification of the four low energy structures illustrated in Figure 2. From this figure, it is observed that the local minima on the bulk solvated CACA surface correspond to the molecule being in either a folded, intramolecularly hydrogen-bonded conformation (rotamers 1 and 2) or a partially folded conformation (rotamers 3 and 4).

TACA. The structure corresponding to the global minimum energy on the TACA dihydrate potential energy surface was found to be a partially folded zwitterion. The water molecules were arranged to form a two-molecule bridge between the amino and the carboxylate moieties. This involved one water molecule bound to the amino moiety, the other water molecule bound to the carboxylate, and the two waters hydrogen-bonded to each other. Conformational exploration resulted in the identification of an additional two extended rotamers, both of which were predicted by the COSMO model to be approximately 5 kJ/mol lower in energy than the partially folded conformer. All three local minima are illustrated in Figure 3.

CAMP. The CAMP dihydrate optimized to folded, intramolecularly hydrogen-bonded zwitterionic conformers for both the (+) and (−) isomers. Each water molecule formed a single-molecule bridge between the amino and carboxylic moieties, with each water molecule hydrogen bonded to both groups, analogous to the positioning of the water molecules around the CACA solute. Following "dehydration", rotation about each rotatable bond, and optimization in the COSMO reaction field, both CAMP isomers were found to possess three stable

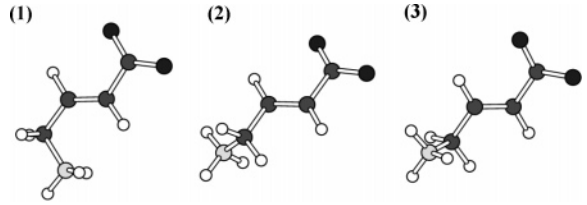

Figure 3. Minimum energy TACA conformers optimized at MP2/6-31+G* in the COSMO reaction field ($\epsilon = 78.39$).

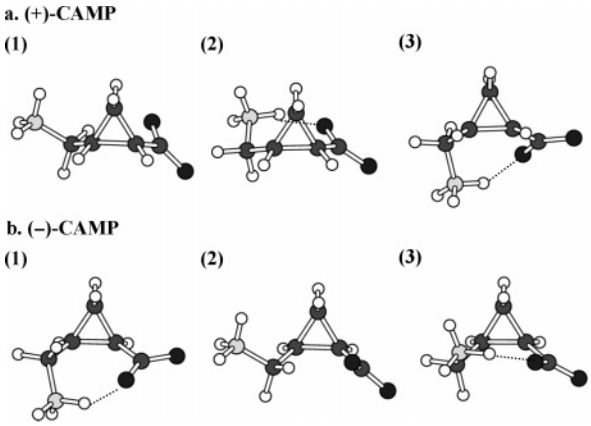

Figure 4. Minimum energy CAMP conformers optimized at MP2/6-31+G* in the COSMO reaction field ($\epsilon = 78.39$). Intramolecular hydrogen bonds are indicated by dotted lines.

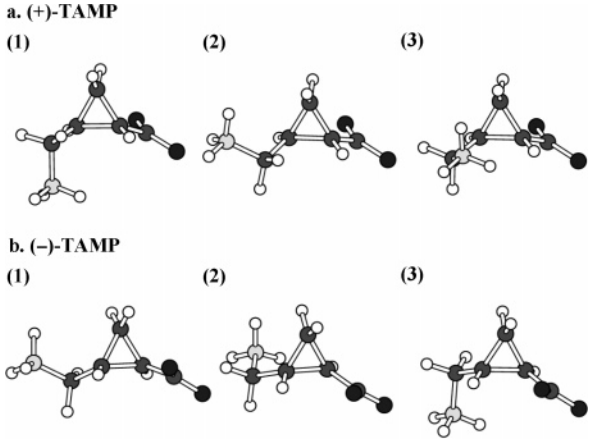

Figure 5. Minimum energy TAMP conformers optimized at MP2/6-31+G* in the COSMO reaction field ($\epsilon = 78.39$).

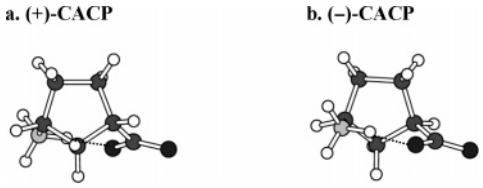

Figure 6. Minimum energy CACP conformers optimized at MP2/6-31+G* in the COSMO reaction field ($\epsilon = 78.39$). Intramolecular hydrogen bonds are indicated by dotted lines.

structures: two folded, intramolecularly hydrogen-bonded conformers and one partially folded conformer. These structures are illustrated in Figure 4. As (+)- and (−)-CAMP are enantiomers of one another, each stable conformer identified for one isomer had a corresponding mirror image which was a stable conformer of the other isomer. The energies of these mirror images were the same to within numerical uncertainty.

TAMP. The optimized (−)-TAMP dihydrate structure was a partially folded zwitterion, with two molecules of water forming a bridge between the amino and carboxylate termini. The lowest energy structure identified for the (+)-TAMP dihydrate was fully extended and zwitterionic, also with two water molecules bridging between the amino and carboxylate groups. However, despite these initial differences in structure, the results of the conformational exploration revealed that both the (+) and (−) isomers possessed three local minima on the bulk solvated TAMP surface. Two of these local minima corresponded to partially folded conformers, and the other corresponded to an extended conformer. The structures of all six rotamers are shown in Figure 5. From Figure 5 and Table 2, it can be seen that each stable conformer identified for one isomer had a corresponding mirror image that was a stable conformer of its enantiomer.

CACP. The lowest energy structure obtained for the CACP dihydrate was zwitterionic, in a folded, intramolecularly hydrogen-bonded conformation. The two explicit water molecules collectively formed a bridge between the carboxylate and amino moieties. Conformational exploration yielded no further stable rotamers. The minimum energy structures of (−)- and (+)-CACP are shown in Figure 6. As the CACP molecule is highly constrained, it is likely that the single conformer identified for each solvated isomer is also the biologically active conformation.

TACP. The lowest energy conformations of both the (+) and (−) isomers of the TACP dihydrate were partially folded and zwitterionic, with two molecules of water forming a bridge which spanned the length of the molecule, connecting the amino and carboxylic acid groups. However, the alignment of the carboxylic and amino acid moieties relative to the cyclopentane ring was different for each isomer. For the (−) isomer, the amino group was in the plane of the cyclopentane ring and the carboxylate was out of plane. For the (+) isomer, the inverse situation occurred: the amino group was out of plane and the carboxylate group was in plane. Bond rotation and reoptimization within the COSMO solvent model yielded only the two structures identified as dihydrates. However, as (+)- and (−)-TACP are enantiomers, it was expected that each stable conformer would possess a corresponding mirror image that was a stable conformer of the other isomer. Therefore, the geometries of both isomers were reoptimized within the COSMO solvation model starting from geometries corresponding to the mirror image of the alternate isomer. Each isomer was thus shown to have two low energy conformations: one conformation with the amino group in the plane of the ring and the carboxylate group out of plane and the other conformation with the carboxylate group in the plane of the ring and the amino group out of plane. The structures of all stable conformers are shown in Figure 7.

Muscimol. Geometry optimization of the muscimol dihydrate system located a partially folded, zwitterionic structure. The two water molecules were found to form a two-membered bridge between the amino and carboxylate groups. "Dehydration" and rotation about the N−C−C−C dihedral resulted in the identification of a second symmetry-equivalent rotamer of energy identical to that of the original. The structures of these two muscimol rotamers are depicted in Figure 8.

4-ACP-1-E. Both the (*R*) and (*S*) isomers of 4-ACP-1-E optimized to partially folded, zwitterionic structures with two water molecules bridging between the amino and carboxylate termini. Conformational exploration involved rotation about the O−C−C−C dihedral angle; however, no further stable structures were identified. The minimum energy structures of each isomer are illustrated in Figure 9. As observed from Figure 9 and Table 2, these two structures are mirror images of one another, with identical energies. As 4-ACP-1-E possesses a high

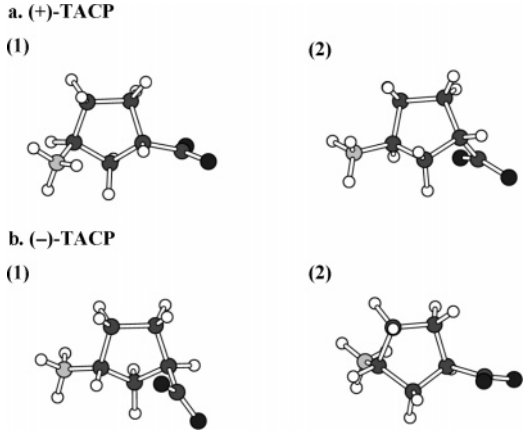

Figure 7. Minimum energy TACP conformers optimized at MP2/6-31+G* in the COSMO reaction field ($\epsilon = 78.39$).

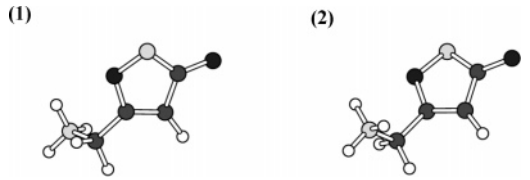

Figure 8. Minimum energy muscimol conformers optimized at MP2/6-31+G* in the COSMO reaction field ($\epsilon = 78.39$).

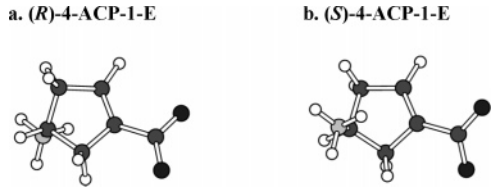

Figure 9. Minimum energy 4-ACP-1-E conformers optimized at MP2/6-31+G* in the COSMO reaction field ($\epsilon = 78.39$).

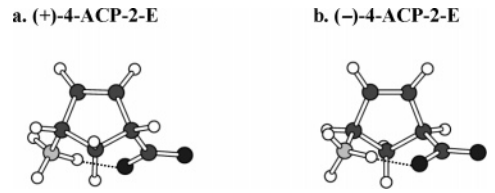

Figure 10. Minimum energy 4-ACP-2-E conformers optimized at MP2/6-31+G* in the COSMO reaction field ($\epsilon = 78.39$). Intramolecular hydrogen bonds are indicated by dotted lines.

degree of conformational rigidity, it is likely that the low energy solvated conformers identified here are also the biologically active conformations.

4-ACP-2-E. The 4-ACP-2-E dihydrate optimized to folded, intramolecularly hydrogen-bonded zwitterionic conformers for both the (+)- and (−) isomers. The two water molecules were arranged to collectively form a bridge between the amino and carboxylate moieties. As for 4-ACP-1-E, conformational exploration involved rotation about the O−C−C−C dihedral, but no further local minima on the bulk solvated 4-ACP-2-E surface were identified. The low energy conformers (+)-4-ACP-2-E and (−)-4-ACP-2-E identified here were found to be mirror images of one another (Figure 10 and Table 2) with identical energies (Table 2). Due to the lack of conformational flexibility of the 4-ACP-2-E molecule, these low energy solvated conformers are also likely to correspond to the biologically active conformations.

2-Methyl-GABA. Both the (*R*) and (*S*) enantiomers of the 2-methyl-GABA dihydrate optimized to give folded, intramolecularly hydrogen-bonded zwitterionic structures. The two water molecules were positioned to form a bridge between the amino and carboxylate groups. Like GABA, its methylated derivative 2-methyl-GABA also has a high degree of conformational flexibility. As a consequence, a large number of stable solution-phase structures were identified through rotation about the O−C−C−C, C−C−C−C, and N−C−C−C dihedrals. These conformers are illustrated in Figures 11 and 12. Rotamers 1, 2, 8, and 9 represent folded, intramolecularly hydrogen-bonded structures for both the (*R*) and (*S*) isomers of 2-methyl-GABA. Rotamers 3, 4, 5, 6, 7, and 10, on the other hand, correspond to partially and fully extended configurations. As all 10 rotamers are approximately isoenergetic, it is impossible to predict which conformation or conformations of 2-methyl-GABA will be biologically active.

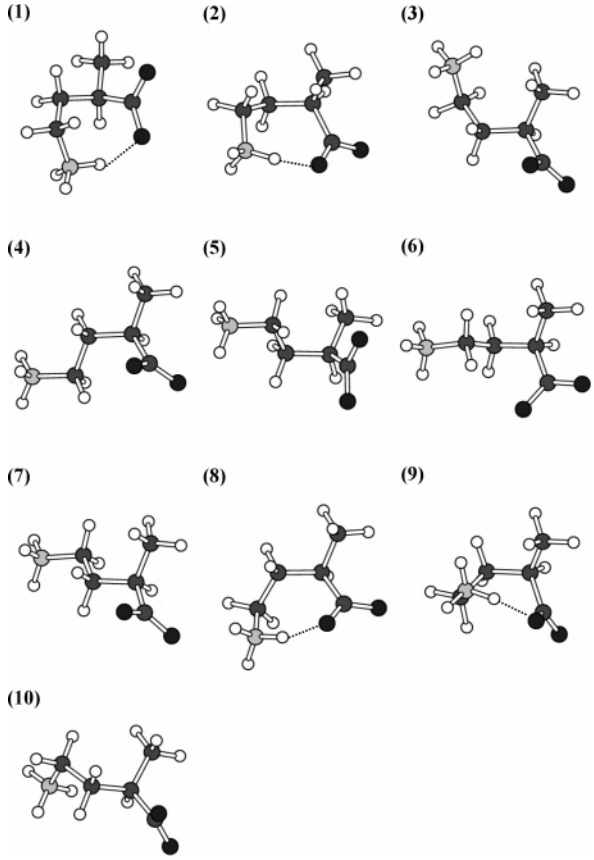

Figure 11. Minimum energy (*R*)-2MeGABA conformers optimized at MP2/6-31+G* in the COSMO reaction field ($\epsilon = 78.39$). Intramolecular hydrogen bonds are indicated by dotted lines.

Comparison with GABA. Previous studies of solvated GABA identified nine stable rotamers in solution.[16] For clarity and comparative purposes, Figure 3 of ref 16 has been reproduced here as Figure 13. The GABA analogues studied here can be broadly classified into three structural subtypes: highly flexible, partially constrained, and highly constrained. The degree of conformational flexibility was found to have implications for the number of stable conformations the molecule could possibly attain and the torsional structure of these minimum energy conformations.

The highly flexible 2-methyl-GABA was found to possess 10 stable conformers. Of these 10 conformers, nine corresponded to previously identified solvated GABA structures,[16] with similar N−C−C−C, C−C−C−C, and C−C−C−O dihedral angles. However, one extra stable conformer of 2-methyl-GABA was also identified for both the (*R*) and (*S*) enantiomers, and these structures are labeled as conformers 3 and 10 in Figures 11 and

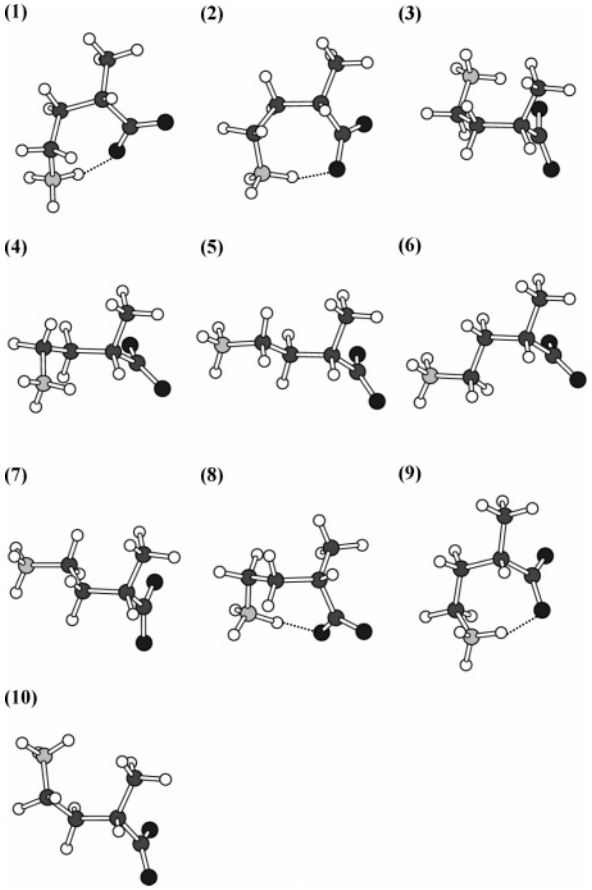

Figure 12. Minimum energy (*S*)-2MeGABA conformers optimized at MP2/6-31+G* in the COSMO reaction field ($\epsilon = 78.39$). Intramolecular hydrogen bonds are indicated by dotted lines.

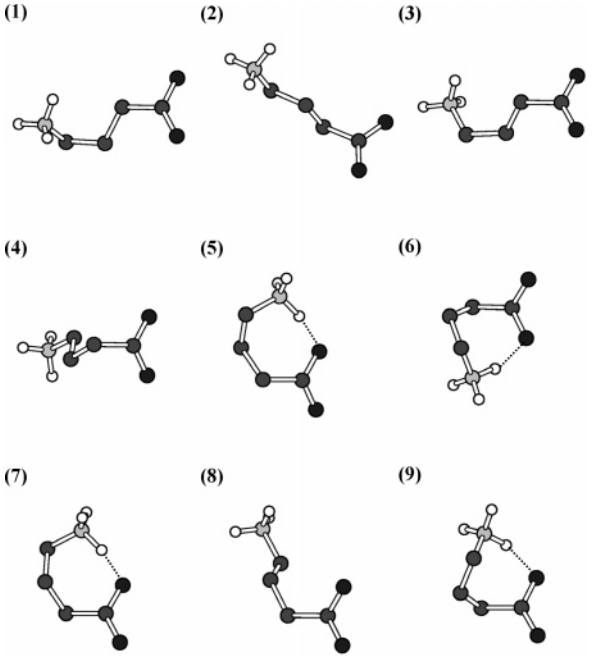

Figure 13. Minimum energy GABA conformers optimized at B3LYP/6-31+G* in the COSMO reaction field ($\epsilon = 78.39$), reproduced from ref 16. Intramolecular hydrogen bonds are indicated by dotted lines. For clarity, only terminal hydrogen atoms are displayed.

12, respectively. However, these were the least stable 2-methyl-GABA conformers identified, at around 35 kJ/mol from the lowest energy conformer.

The partially constrained CACA, TACA, CAMP, and TAMP were found to possess conformers with a pharmacophoric pattern similar to previously identified solvated GABA conformers.[16] However, each molecule studied here had a reduced number of stable conformers, and these stable conformers corresponded to a subset of the stable conformers identified for the more conformationally flexible parent GABA molecule. In particular, the cis-constrained CACA and CAMP possessed conformers that were similar to the folded and partially extended GABA conformers 1, 3, 5, 6, 7, and 9 (Figure 13), while the trans-constrained TACA and TAMP possessed conformers that were similar to the partially and fully extended GABA conformers 1, 2, 3, 4, and 8 (Figure 13).

The remaining molecules considered here are all highly constrained due to the presence of a five-membered-ring system. These compounds are distinguished from one another by variation in the hybridization of the carbon atoms and in the positioning of the five-membered ring relative to the carboxylate and amino termini. As a result, the stable conformers of each of these molecules correspond to different subsets of the stable conformers of the parent GABA molecule.[16] For example, TACP, with the amino and carboxylate termini bound to sp^3 hybridized carbon atoms on the five-membered ring and also constrained in a trans configuration, was found to attain stable structures corresponding to partially extended conformers of the parent GABA molecule. The low energy conformers of (−)-TACP were found to correspond to GABA conformers 3 and 8 (Figure 3), whereas the low energy conformers of (+)-TACP were found to correspond to GABA conformers 1 and 4 (Figure 13). In contrast, CACP and 4-ACP-2-E, also with amino and carboxylate termini bound to sp^3 carbon atoms on the five-membered ring, but constrained in a cis configuration, possessed stable conformers that corresponded exclusively to folded, intramolecularly hydrogen-bonded GABA conformers. (+)-CACP and (−)-CACP were found to correspond to GABA conformers 5 and 7, respectively. (+)-4-ACP-2-E and (−)-4-ACP-2-E also corresponded to GABA conformers 5 and 7, respectively. The minimum energy conformers of both enantiomers of 4-ACP-1-E, with the carboxylate terminus attached to the five-membered ring through an sp^2 hybridized carbon, were found to differ from the previously identified GABA conformers. This occurs as a result of the difference in the hybridization of this carbon atom relative to sp^3 hybridization of the carbon atom at the analogous position in the parent GABA molecule. Similarly, muscimol, again with the carboxylate bound through an sp^2 hybridized carbon atom, but also with the plane of the carboxylate terminus constrained by the five-membered ring, possessed two stable conformers that did not directly correspond to any of the stable GABA conformers shown in Figure 13. However, the minimum energy conformers of both 4-ACP-1-E and muscimol can be categorized as partially extended conformers.

Overall, a high degree of structural diversity can be observed among all the conformationally restricted GABA analogues considered here, from compounds that exist exclusively in a folded, intramolecularly hydrogen-bonded conformation to analogues that possess only partially and fully extended conformers. Given that all the compounds considered here, with the exception of (*S*)-4-ACP-1E, are biologically active at $GABA_C$ receptors,[17,19−21,23] either as agonists or competitive antagonists, this suggests that the GABA binding site must be relatively flexible. Interestingly, the conformationally restricted analogues that preferentially attain partially and fully extended conformations[17,20,21] generally exhibit lower EC_{50} and IC_{50}

values than the analogues that preferentially attain folded, intramolecularly hydrogen-bonded conformations.[19,20,23] This means that trans-constrained analogues generally have higher affinities for the $GABA_C$ binding site than cis-constrained analogues, and implies that it is likely to be the partially or fully extended GABA rotamers that are the most biologically active forms.

Summary

GABA analogues constrained in a cis configuration by either a double bond or a cyclopropane ring have been shown to possess both folded, intramolecularly hydrogen-bonded and partially folded conformations of approximately equal energy in aqueous solution. Trans-constrained analogues with either a double bond or a cyclopropane ring, on the other hand, have stable rotamers that are either partially folded or fully extended. GABA analogues constrained in a cis configuration by a cyclopentane or cyclopentene ring system are the most conformationally restricted. As a consequence, only one stable folded, intramolecularly hydrogen-bonded conformer could be identified in aqueous solution. It is not unreasonable to expect that this stable aqueous-phase conformer is similar to the biologically active conformation, as the mammalian body is a predominantly aqueous environment. Cyclopentane analogues constrained in a trans configuration, however, possess two stable partially folded conformers, which are equally likely to be the biologically active conformations. Unconstrained GABA analogues, like GABA itself, possess a large number of stable rotamers in solution—from folded, intramolecularly hydrogen-bonded conformers, through partially folded conformers, to fully extended conformers. Each of the identified stable conformers is approximately isoenergetic, within the limitations of the solvation model and level of theory used. Therefore, each of these low energy solvated conformers is a candidate for the biologically active conformation or conformations, as it is currently unknown which conformer is present in vivo and at the receptor binding site. However, existing experimental data suggest that trans-constrained analogues have a higher affinity for the $GABA_C$ receptor. It is therefore postulated that the partially or fully extended GABA rotamers are likely to be the biologically active forms.

This work represents a systematic and accurate ab initio investigation into the aqueous-phase structure of conformationally restricted GABA analogues. These results provide a high-quality starting point for subsequent quantitative structure−activity relationship analysis, which is expected to provide insight into which conformers of GABA and its conformationally restricted analogues are biologically active. This will, in turn, aid in the design and development of drugs that selectively target specific GABA receptors and transporters.

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Supporting Information Available: Geometries and energies of all structures described in this work. This material is available free of charge via the Internet at http://pubs.acs.org.

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