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Short communication

Possible factors influencing the separation mechanism of diastereomeric amino acid derivatives obtained from s-triazine type reagents

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ABSTRACT

Diastereomeric derivatives prepared from an amino acid and an amino amide using trichloro s-triazine as a coupling platform are known to produce good chromatographic diastereoselectivity for many amino acid analytes. The chromatographic diastereoselectivity of these derivatives is difficult to rationalize based on the possibility of four possible conformational isomers, which can interconvert by rotation about the C–N bonds between the amino substituents and the triazine ring. The observed diastereoselectivity implicates an unobvious but significant driving force which causes one of several conformations to be favored over the others. Several possibilities are discussed. Intramolecular hydrogen bonding between acid and amide substituents was explored using computer aided molecular modeling. While such hydrogen bonding may be geometrically possible between the amino acid and the amide substituents, it does not explain why derivatives produced from other chiral compounds which are not capable of the same hydrogen bonding interaction nevertheless exhibit substantial diastereoselectivity. Two other more general effects, steric hindrance to solvation and ion pairing, are therefore suggested as possible contributing factors to the chromatographic diastereoselectivity. Based on the conformational equilibrium behavior of related triazine compounds as reported in the literature, either one of these effects could influence the conformation of the diastereomeric derivatives even in the absence of intramolecular hydrogen bonding interactions between the two chiral substituents, and these effects may therefore be a contributing factor for the observed elution order of the diastereomers.

1. Introduction

The use of s-triazine reagents has been shown to be of significant analytical utility for the optical purity analysis of α-amino acids [1,2]. The first reported example demonstrated the effectiveness of mono-chloro s-triazine (MCT) reagents [1], which were produced from cyanuric chloride by substituting one chloride with an alkoxy or aryloxy group followed by substitution of a second chloride with an optically pure amino acid such as alanine or valine. The third remaining reactive chloride was then displaced to produce a diastereomeric derivative of an amino acid analyte. The resulting derivative afforded good diastereomeric selectivity under convenient achiral reversed-phase liquid chromatography (RP-LC) separation conditions. More recently, it was shown that the analogous dichloro s-triazine (DCT) reagents are also effective, which are similar to MCT derivatives except that no alkoxy/aryloxy group is introduced, and consequently a chloro substituent remains in the final diastereomeric derivative. The chloro triazine derivatives obtained with DCT reagents were shown to give even better diastereoselectivity than the corresponding methoxy triazine derivatives obtained with MCT reagents [2].

The diastereomeric derivatives produced by s-triazine reagents have certain similarities to derivatives produced from another reagent, 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (DNP-Ala-NH_2), also known as Marfey's reagent (MR). The use of MR for producing diastereomeric derivatives of amino acids has been known for several decades [3,4], and such derivatives are well resolved by RP-LC. In order to explain the diastereoselectivity behavior of MR derivatives, it was suggested that a planar anthracene-like ring conformation leads to complimentary hydrophobic effects for one diastereomer (D–L), which produces a derivative having both hydrophobic groups on one face of the planar system, and both polar acid/amide groups present on the other face [5]. This complimentary arrangement of the D–L diastereomer affords better adsorption to a hydrophobic stationary phase compared to L–L diastereomers, which have the non-complimentary arrangement (one polar and one non-polar group on each face) and are therefore less retained by RP-LC. Based on NMR-NOE studies, it was shown that the hydrogen atoms (H_α) attached to the alpha carbons of the amino acid/amide residues were situated in close proximity to the aromatic proton situated between the amino

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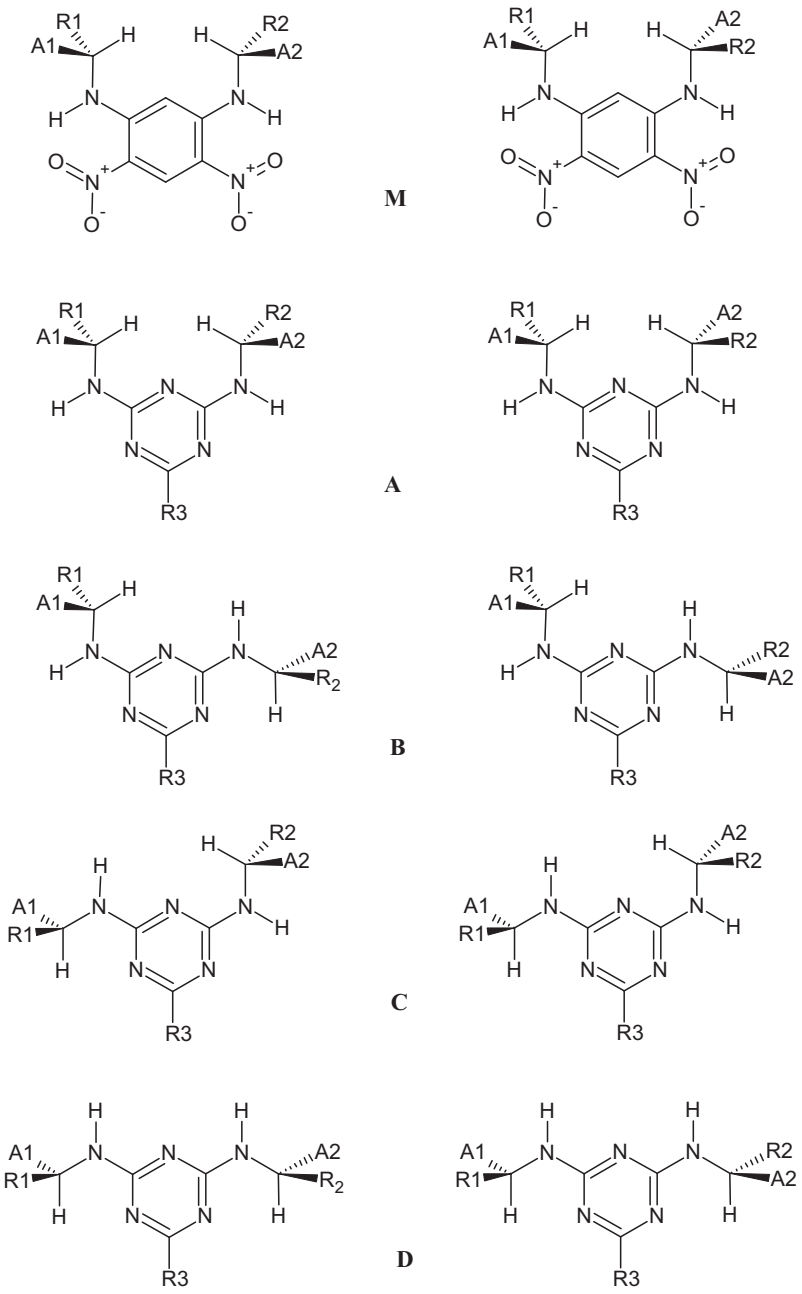

Fig. 1. Amino acid derivatives made from L-amino amide form of reagent with either L or D form of amino acid. L–D combination shown on left, and L–L on right (A1 or A2 are acid/amide groups, R1 or R2 are amino acid/amide side chains). Top shows derivatives made from MR, below are the four possible conformers (A–D) for derivatives made from s-triazine type reagent (where R3 is chloro or alkoxy substituent).

substituents, indicating a trans relationship between H_α and the amino proton within each amino acid/amide residue. This finding suggests that the amino acid residues are conformationally locked with respect to the aromatic ring, due to restricted rotation of the bonds between amino nitrogen and aromatic ring (N–$C_{(Ar)}$ bonds). The reason for the N–$C_{(Ar)}$ bond rotation restriction is presumably hydrogen bonding between one of the oxygens attached to the nitro group with the amino hydrogen atom [5].

Recently, it has been suggested that the separation mechanism for s-triazine derivatives is essentially the same as for MR derivatives [2]. This proposal is based on the same elution order, implicating that the D–L diastereomer has the same complimentary arrangement of hydrophobic groups on one face and hydrophilic groups on the opposite face, analogous to MR derivatives. It is also supported by the fact that the diastereoselectivity is largest for amino acid/amides which contain hydrophobic R-groups and becomes smaller when the R-groups become more polar. This explanation is conceptually simple and reasonable. However, this explanation requires that the molecule exists in one particular conformation. Although the assumption that the amino nitrogen atoms of the s-triazine derivatives are planar is justifiable based on NMR studies of similar compounds [6], it is important to recognize that the s-triazine derivatives are lacking the nitro substituents of MR derivatives, and are therefore lacking in any obvious intramolecular force to prohibit N–$C_{(Ar)}$ bond rotation. Thus, unlike MR derivatives where one conformation predominates, the s-triazine derivatives can exist in four conformations, two which have the complimentary adsorption aspect for D–L diastereomers (conformer types A and D, Fig. 1) and the other two complimentary for L–L (B and C, Fig. 1). Thus, with no hydrogen bonding substituent for prohibit-

ing N–$C_{(Ar)}$ bond rotation in s-triazine derivatives, the analogy to MR derivatives becomes somewhat irrelevant. If the same population exists for all conformers of s-triazine derivatives, and the most significant retention comes from conformers A and D for the D–L configuration and conformers B and C for the L–L configuration, it can be argued that the difference in retention between the two diastereomers will tend to cancel out. While this assumption neglects the possibility that the different conformers may have different chromatographic adsorption characteristics related to shape or symmetry factors, the substantial diastereoselectivity observed for s-triazine derivatives strongly suggests that the populations of the various conformers A–D are not equal. What is needed for a satisfactory explanation of the diastereoselectivity is a reason why the equilibrium between the possible conformers in Fig. 1 would favor A and/or D over B and C, even in the absence of the nitro substituents which are present in MR derivatives but absent in s-triazine derivatives. Several possible causes for the observed diastereoselectivity are considered in this brief report including intramolecular hydrogen bonding, solvation, and ion pairing effects.

2. Discussion

2.1. Limitations of hydrogen bonding effects to rationalize diastereoselectivity

When considering hydrogen bonding interactions, it is useful to use the chemically similar MR derivatives as a starting point. It was previously suggested that the acid and amide groups within MR derivatives interacted with each other to produce an intramolecular hydrogen bond [3,4]. Although this possibility was later regarded as unnecessary to explain the diastereoselectivity of MR derivatives [5], such a hydrogen bonding interaction may be a contributing factor to the diastereoselectivity of s-triazine derivatives. The geometric feasibility of such interactions can be easily demonstrated by computer aided molecular modeling (see Appendix A), indicating that s-triazine derivatives, like MR derivatives, may also be subject to a hydrogen bonding effect. If hydrogen bonding does occur, it could offer an explanation as to why conformation A is favored over the other conformations in which such a hydrogen bonding interaction is not possible. However, it is important to consider that, although a hydrogen bonding interaction is geometrically feasible, this does not necessarily mean it will become the dominant driving force in the conformational equilibrium. Although such bond formation appears geometrically possible, the likely hood of such bond rotations occurring will depend on the magnitude of energy barrier involved in the required bond rotations. This was considered on a qualitative basis by evaluating the steric interactions as shown in Fig. 2 for the optimized hydrogen bonded structures (Fig. 3). For L–L or D–D configuration, rotating each of the two C_α–N bonds by 60° leads to elimination of one eclipsing interaction (R–H) which is replaced by another (H–$C_{(Ar)}$), and a gauche type interaction occurs between the acid/amide groups (A) and the aromatic ring (Ar). Thus, the major net change is the addition of two gauche type interactions. Considering an estimated energy difference of about 0.8 kcal/mol per each gauche interaction by analogy to butane conformers [7] suggests that, although a significant population (approximately 20%) may exist in which one C_α–N rotation has occurred, the simultaneous rotation of both C_α–N bonds will likely occur statistically only in a very small population (<5%). While it has been suggested that formation of the hydrogen bond itself may stabilize the structure by several kcal/mol [4], one could also argue that the presence of a substantial amount of water in the RP-LC mobile phase will aggressively compete for hydrogen bonding sites, and

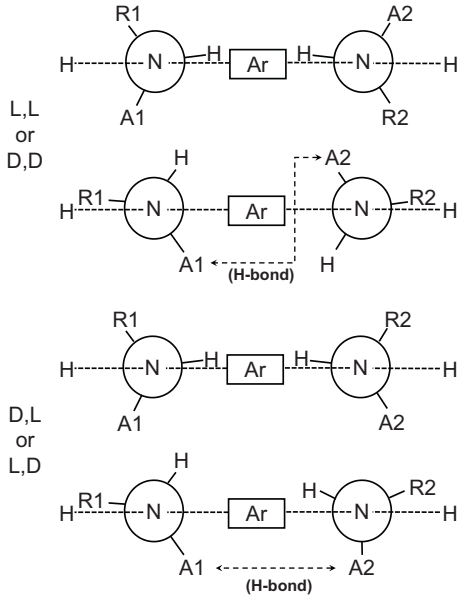

Fig. 2. Newman projections of C_α–N bonds for the two amino acid/amide residues within the structure of the derivative, showing steric interactions between aromatic ring carbon (Ar), acid/amide groups (A1 and A2) and amino acid/amide side chains (R1 and R2). Top two structures show L–L or D–D case, bottom two shows D–L or L–D case. The conformation with least steric interaction is shown (top of each pair) along with the conformations obtained by rotation of C_α–N bonds (bottom of each pair) allowing for hydrogen bonding interaction between acid/amide groups A1 and A2.

will likely destabilize any intramolecular hydrogen bonding interaction. The energy barrier may even be somewhat higher for the D–L case, due to the perpendicular conformation in which two partially eclipsed interactions are present (Fig. 2). Thus, although it appears that intramolecular hydrogen bonding is geometrically feasible, and that such an interaction may have a prohibitive effect on N–$C_{(Ar)}$ bond rotation, it also appears that the population of hydrogen bonded form will likely be quite small, meaning that the majority of molecules will remain in non-hydrogen bonded form and be free to participate in the conformational equilibria shown in Fig. 1. Therefore, the intramolecular hydrogen bonding aspect does not offer an entirely satisfactory explanation of the diastereoselectivity. Furthermore, it has been reported that, in addition to α-amino acids, s-triazine derivatives obtained from other chiral compounds such as Baclofen [8] or Mexiletine [9] are also resolved by RP-LC. Since both of these compounds are structurally different than α-amino acids, this suggests that additional more general factors are somehow stabilizing conformer A and/or D which do not involve a specific hydrogen bond interaction between the two chiral groups.

2.2. Solvation effects

Conformational studies by NMR spectroscopy of other triazine compounds have been reported in the literature [6,10,11]. In one such study, it was illustrated that for an s-triazine compound with two monalkylamino substituents and where the third substituent was a dialkylamino group, the conformation analogous to A in Fig. 1 was favored. This was noted even though bulky alkyl groups were present on the monoalkylamino substituents [10]. Such behavior is counterintuitive considering that the steric effect should favor less crowded conformer D instead of A due to the closer proximity of the groups to each other in the latter case. This unexpected tendency was attributed to the effect known as steric hindrance to solvation. However, another study utilizing bis-monoallylamino triazine compounds in which the third substituent was a

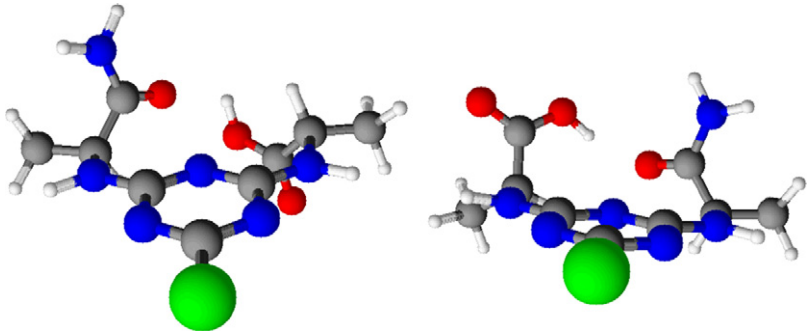

Fig. 3. 3D optimized structures for alanine derivatives showing possibility for intramolecular hydrogen bonding interactions between the hydrogen atom of acid and amide groups. Left shows L–L or D–D type structure, right shows D–L or L–D type structure.

dialkylamino group showed no clear preference for conformer A [6]. In a third study [11], of four triazine compounds tested, only one showed a preference for the conformation analogous to A in Fig. 1, while the other three showed slight tendency towards more B/C conformers (the amount of D was low or absent in all cases). However, the third study was conducted using monochloro bis-monoarylamino triazine compounds, which are probably not good analogues for the HPLC derivatives under study. The lack of consistent results obtained by these NMR studies makes it difficult to apply to the diastereomeric derivatives used in chromatography. However, the possibility of a steric hindrance to solvation effect, which does not require a hydrogen bonding interaction, offers an explanation why derivatives with substituents which are not α-amino acids [8,9] are still subject to chromatographic resolution.

2.3. Ion pairing effects

Another possible explanation for the diastereoselectivity is ion pairing effects. The mobile phase pH of about 2 produced by trifluoroacetic acid (TFA) used for the chromatographic studies [1,2] is low enough where at least partial protonation of one of the triazine nitrogens will likely occur. The presence of a population of protonated species could influence the conformational equilibria depending on the proximity of the counterion relative to the substituents on the triazine ring. This hypothesis is based on an NMR spectroscopy study which showed that the protonation of a triazine type nitrogen in the aromatic ring of a mono-dialkyl amino bis-monoalkylamino substituted s-triazine appeared to substantially alter the relative populations of conformers compared to the free base form of the same s-triazine compound [6]. This was attributed to steric influences, where the anionic counterion provides a steric hindrance to the alkyl groups. It was reported that protonation occurred at the nitrogen between the two monoalkylamino groups [6] and resulted in a major conformation which was assumed to be analogous to type D in Fig. 1. However, this effect will likely be different depending on the electron donating/withdrawing effects of the non-chiral substituent (chloro versus alkoxy) which can potentially alter the site of protonation. This hypothesis is based on calculated pK_a values (ACD pK_a DB software, v. 10.00, Advanced Chemistry Development) for model s-triazine derivatives containing alanine, alanine amide, and either chloro or methoxy as the three substituents. The calculated results indicated that the methoxy type derivative protonates on the nitrogen between the amino acid/amide groups, in contrast to the chloro type derivative in which protonation would occur at a position ortho to the chloro substituent. Thus, steric effects due to protonation/ion pairing would be different for methoxy versus chloro analogues as shown in Fig. 4. For methoxy, the pK_a value is substantially higher than the mobile phase pH of about 2 used in the chromatography studies [1,2], which indicates that it will be largely in protonated form. The position of protonation on the triazine ring is the nitrogen atom between the amino groups. Thus, the expected ion pairing effect would be to destabilize form A, which counteracts the previ-

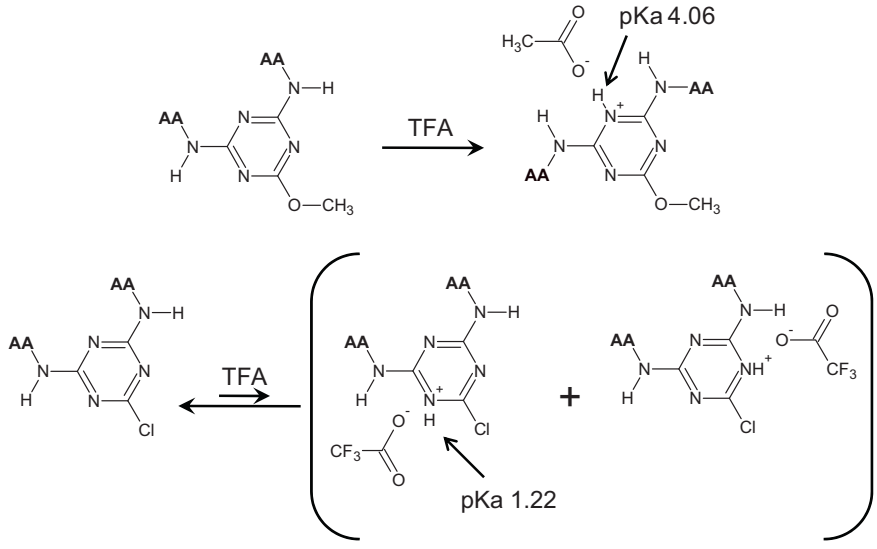

Fig. 4. Difference in ion pairing effects of the chloro versus methoxy type derivatives based on different locations of protonation within triazine ring. AA represents amino acid or amide substituents.

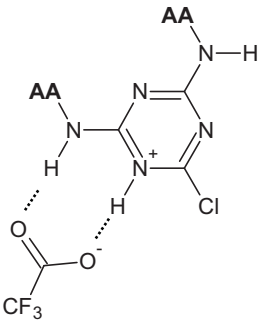

Fig. 5. Possible multiple interaction between TFA with amino hydrogen atom and protonated triazine type nitrogen in s-triazine derivative.

ously discussed steric hindrance to solvation effect, and raises the possibility that form D may predominate in the methoxy derivatives. Ion pairing effects for the chloro derivatives, on the other hand, would disfavor form D, and therefore would enhance any natural tendency towards form A produced by steric hindrance to solvation effects. Thus, ion pairing effects may offer an explanation as to why the observed diastereoselectivity of the methoxy derivatives is different (consistently lower) relative to the chloro substituted analogues [2,8,9], due to the possibility that for the methoxy case, form D is being favored instead of A as shown in Fig. 4, and that form D has an inherently lower diastereoselectivity than A, or that the ion pairing effect is not strong enough to completely eliminate the presence of conformers B and C which partially cancel the diastereoselectivity of form D. Another possibility is that, although form A still predominates in the methoxy derivatives, the ion pairing effect reduces the population of A significantly so that a substantial loss in diastereoselectivity is observed relative to the chloro derivatives.

The strength of ion pairing effects may also be enhanced further by a simultaneous hydrogen bonding interaction towards the amino nitrogen, as shown in Fig. 5. This simultaneous effect has been suggested from NMR spectroscopy studies on amino s-triazine compounds [6] and from UV/Vis spectroscopy studies on diamino prymidines [12]. Thus, although only a fraction of the chloro type derivatives will be protonated at any time under the mobile phase pH 2 mobile phase conditions (based on the calculated pK_a value of below 2, Fig. 4), this effect may nevertheless contribute to the diastereoselectivity of the chloro derivative by providing additional resistance to rotation about the N–$C_{(Ar)}$ bonds.

3. Conclusions

An attempt was made to rationalize the chromatographic diastereoselectivity of s-triazine derivatives having two amino acid substituents and a third achiral substituent. It is assumed that there must be one or more factors which influence rotation of the amino substituents about the N–$C_{(Ar)}$ bonds in such a way which favors one of the four possible conformations. For derivatives containing α-amino acid/amide substituents, molecular modeling studies suggest that intramolecular hydrogen bonding between the acid and the amide groups could in principle stabilize one of the conformations which would be consistent with the elution order, in agreement with previous proposals used to explain the diastereoselectivity of chemically similar MR derivatives. However, based on qualitative analysis of the rotational energy barrier required to produce such a hydrogen bond, as well as the expected destabilizing effect of the aqueous mobile phase used in the RP-LC separation, it is concluded that such interactions are likely weak in nature, and furthermore such an interaction cannot explain why diastereoselectivity is observed in derivatives of compounds in which one of the chiral substituents is not an α-amino acid. These considerations implicate a different driving force which influences the position of equilibrium between the various conformers. One possibility is the effect known as steric hindrance to solvation, which appears to occur in some amino s-triazine compounds to varying degrees according to literature studies in which NMR spectroscopy techniques were used to probe the position of conformational equilibrium. Another possible cause is ion pairing effects, due to steric influence and/or hydrogen bonding effects of the counterion towards the monoalkylamino substituents of the triazine derivatives, which is also suggested by spectroscopic studies of similar compounds reported in the literature. Additional experimental studies involving the effect of pH, the size and concentration of counterion, and the electron donating/withdrawing effects of the non-chiral triazine substituent on the chromatographic behavior of model triazine derivatives may be potentially useful for determining if ion pairing effects are indeed involved in the separation mechanism, but to date it does not appear that such a study has been reported.

Appendix A.

Molecular modeling studies were conducted using an s-triazine model derivative containing alanine, alanine amide, and chloro substituents. Specifically, hydrogen bonding between the acid and the amide groups was explored for conformer A, which is the only one of the four conformers A–D in which the amino acid and amide groups are close enough for mutual interaction. As a starting point, the lowest energy conformation with regard to C_α–N bond rotation was assumed to be the one in which H_α was trans to the amino hydrogen (as shown for conformer A in Fig. 1), which is analogous to the proposed conformation of MR derivatives [5]. This conformation results in the least steric interactions of the acid/amide groups and R-groups with the aromatic ring, and has a single eclipsing interaction between H_α and R-group. To probe for possible hydrogen bonding interactions, computer generated 3D optimized structures (ACD/3D Viewer software, v. 10.00, Advanced Chemistry Development, Toronto, Canada) were evaluated for C_α–N bond rotations of approximately 30° and 60° from the assumed low energy conformation. In addition to the C_α–N bond rotation, the other major consideration was rotation about the bond between C_α and the carbonyl carbon ($C_{=O}$). For each combination of C_α–N rotations, C_α–$C_{=O}$ bond rotations were adjusted so that, for one of the acid/amide substituents, the H-bond donor group (–OH or $–NH_2$) was eclipsed with H_α, and for the other acid/amide substituent, the carbonyl oxygen was eclipsed with H_α. The actual angles of rotation in the 3D optimized structures were assessed using dihedral angles $C_{=O}$–C_α–N–$C_{(Ar)}$ (for C_α–N bond rotation) and dihedral angles H_α–C_α–C=O (for C_α–$C_{=O}$ bond rotations). In all cases, rotations were within ±8° of the desired angle.

For the model s-triazine derivative under study, it was found that, for L–L or D–D configuration, 60° rotation about each C_α–N bond from the assumed low energy conformation was favorable for a hydrogen bonding interaction (both R-groups eclipsed with amino hydrogen). This is illustrated in Fig. 2 using a graphical representation similar to the one used in Ref. [5] but in which Newman projections have been added to show the interactions along the C_α–N bond. With regard to C_α–$C_{=O}$ bond rotation, interactions were favorable whenever the H-bond donor group of one acid/amide group was eclipsed with H_α and the carbonyl oxygen of the other acid/amide group was eclipsed with H_α. Thus, for L–L or D–D, two combinations were possible (amide donor/acid acceptor, acid donor/amide acceptor). For D–L or L–D, an interaction occurred for a combination of 60° rotation for one group and 30° rotation for the other group (one acid/amide group perpendicular

to aromatic plane, Fig. 2). Thus, for D–L or L–D, four combinations were possible due to conformation (eclipsed or perpendicular) and H-bonding (amide acceptor or donor) considerations. With regard to the hydrogen bonding interaction, similar results were obtained in all cases, affording values of 2.6 Å (±0.2), 1.9 Å (±0.1), and 130° (±15) respectively for O–O (or O–N) distance, H–A distance (A = acceptor), and X–HA (X = N or O) angle. These values meet all three geometric criteria (O–O distance <3.9, H–A distance <2.5, angle >90) for hydrogen bonding [13]. Example images of the 3D optimized structures are shown in Fig. 3.

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