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Synthesis of 2-aryloxypropanoic acids analogues of clofibric acid and assignment of the absolute configuration by ^{1}H NMR spectroscopy and DFT calculations

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Abstract—A new set of optically active 2-aryloxypropanoic acids has been synthesized through a simple strategy, in good yields and excellent enantiomeric excesses. Their absolute configuration was assigned by means of a NMR-based approach consisting of the derivatization of the carboxylic acids with ethyl mandelate and the comparison of the chemical shifts of the obtained diastereomers. The effectiveness of such an approach has been tested against a larger set of chiral α-substituted-carboxylic acids and by performing high level density functional theory (DFT) calculations on a set of low energy conformations for each diastereomeric derivative. The employed computational procedure has enabled us to find a semiquantitative relationship between the experimental NMR data and the theoretically calculated energy gaps which confirms the theoretical foundations of the NMR strategy and allows to understand when and why it is most likely to fail.

1. Introduction

The NMR-based methodology for the stereochemical assignment of chiral pharmaceutical compounds has received great interest in the last years, because of its simplicity and high reliability.[1,2] Several reports have been described, in which different substrates have been investigated by NMR spectroscopy;[3] these studies are generally based on substrate derivatization with two enantiomers of a suitable chiral reagent and the comparison of the NMR spectra of the resulting pair of diastereomers. Indeed, the preference of the latter for a specific conformation leads to a variation of the chemical shift of the substituents of the asymmetric carbon of the substrate, which can be directly related to its absolute configuration. This procedure has been successfully employed for a number of chiral substrates: primary and secondary alcohols, primary amines, carboxylic acids, diols, triols, amino alcohols and thiols.[4] In some cases, theoretical studies based on molecular mechanics (MM) or semiempirical methods[5] and, very recently, on molecular dynamics simulations[6] or density functional theory (DFT) calculations[4] have supported the conformational hypothesis that the NMR strategy is based upon. Compared to other substrates, relatively few studies describing the determination of the absolute configuration of carboxylic acids by NMR spectroscopy have been reported, where the acidic function has been derivatized in ester[5,7,8] or amide[9–14] derivatives, by using chiral auxiliaries leading to an effective aromatic shielding. Riguera et al.[5] described a powerful application of this method to a series of α-chiral carboxylic acids by using a very efficient chiral reagent, ethyl 2-hydroxy-2-(9-anthryl)acetate; the use of methyl mandelate was also reported,[15] showing the ability to correlate the chemical shift with the stereochemistry of the analyzed compounds, even if with very small values of difference in chemical shift ($\Delta\delta^{RS}$), interpreted with a model where only steric considerations were taken into account.

Fibrates exert their hypolipidemic activity by the activation of PPARα, a nuclear receptor involved in lipid metabolism by regulating the transport and catabolism of fatty

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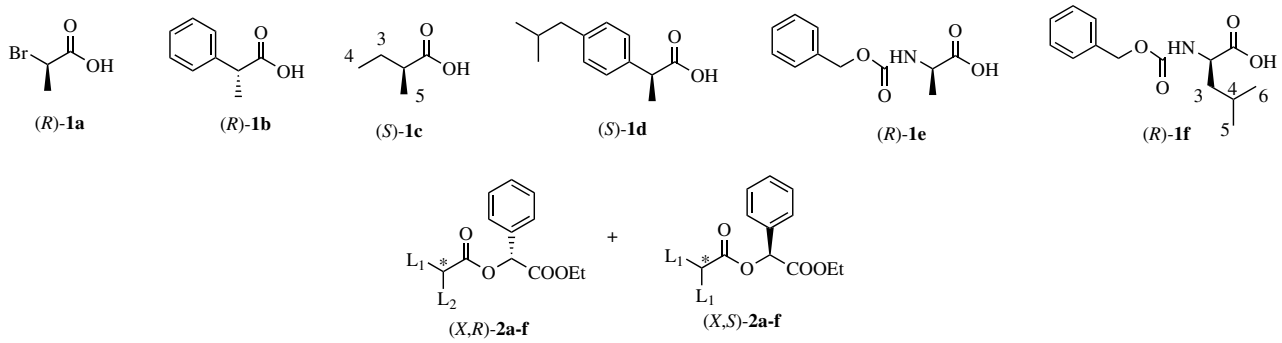


Figure 1. Training set of α-chiral carboxylic acids and general formula of their mandelic esters.

acids;[16,17] more potent and selective PPARα ligands have already been described,[18–21] with a superior clinical profile for a therapeutic intervention in dyslipidemia and other metabolic disorders. With the aim to discover new PPARα agonists, we have synthesized new optically active 2-aryloxypropanoic acids analogues of clofibric acid, the active metabolite of clofibrate. Herein, we focus on the application of an NMR-based method to our 2-aryloxypropanoic acids, with the aim of realizing a convenient and very simple determination of their absolute configuration. We decided to utilize an inexpensive alcoholic chiral reagent, easily accessible in both enantiomeric forms, the ethyl-2-hydroxy-2-phenylacetate (ethyl mandelate): despite its structural simplicity, it possesses the minimal requirements to produce the anisotropic effect. In order to test the efficacy and reliability of ethyl mandelate as a chiral reagent, we first applied this methodology to a series of commercially available α-chiral carboxylic acids with a different steric hindrance (Fig. 1). We have synthesized a series of chiral derivatives of clofibric acid (Fig. 2) with a simple reaction scheme, in good yields and excellent enantiomeric excess and applied the NMR-based approach to assign their absolute configuration.

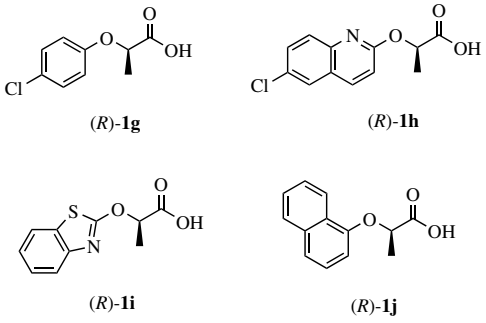


Figure 2. Selected chiral analogues of clofibric acid.

We also performed theoretical calculations on all the diastereomeric couples of (*R*)- and (*S*)-ethyl mandelate with both the first and second series of acids, so as to confirm that the sign of the $\Delta\delta^{RS}$ parameter is correlated to a conformational preference of the esters. Although the aim of such calculations is very much alike Ref. 5, the procedure we have used is much more general and accurate, since we (i) performed a conformational analysis on all the degrees of freedom; (ii) selected the most representative conformations by applying a cluster analysis; (iii) performed on these conformations a geometry optimization through quantum mechanical density functional methods which are well known to produce reliable geometries and accurate energies for organic molecules. This is particularly important because the energy differences between the conformations, responsible for the variations in the chemical shifts, that is, the $\Delta\delta^{RS}$ parameter, are reported to be within 1 kcal/mol, a value which is well below the accuracy of MM and semiempirical methods. Furthermore, besides performing DFT calculations in gas phase, we carried out geometry optimizations in solution, using $CHCl_3$ as a solvent in agreement with the experimental NMR conditions, by a Poisson-Boltzmann polarizable continuum model. The results can thus be compared to the experimental data in a much more consistent and reliable fashion.

Indeed, it has been possible to find a semiquantitative relationship between the experimental $\Delta\delta^{RS}$ and the calculated energy gaps, strongly supporting the hypothesis of a specific conformational preference underneath the present NMR strategy. Furthermore, the analysis of the stereochemistry of the diastereomers preferred conformations and of their energy differences (i.e., their different populations) has also enabled us to deepen our understanding of the applied NMR strategy for the assignment of the absolute configuration of the chiral α-substituted-carboxylic acids so as to foresee when it is expected to work best.

2. Results and discussion

In the literature,[5] the observed chemical shift for L_1/L_2 groups (directly bonded to the stereogenic carbon of the investigated carboxylic acids) upon derivatization with the (*R*)- and (*S*)-enantiomer of a chiral aryl alcohol was explained according to the preference of the acids for the antiperiplanar (*ap*) rather than the synperiplanar (*sp*) conformation of the C^*_α hydrogen respect to the C=O oxygen (Fig. 3a). This preference was shown to be maintained in the esters, and, since it is known that the alcohol part of an ester adopts a preferred conformation with the C–H synperiplanar with respect to the C=O (Fig. 3b), it leads to an antiperiplanar conformation, *ap*(*H*,*H*), for the alcoholic C–H and the acidic C_α–H, and thus to a selective shielding by the aryl group on only one of the L_1/L_2 groups

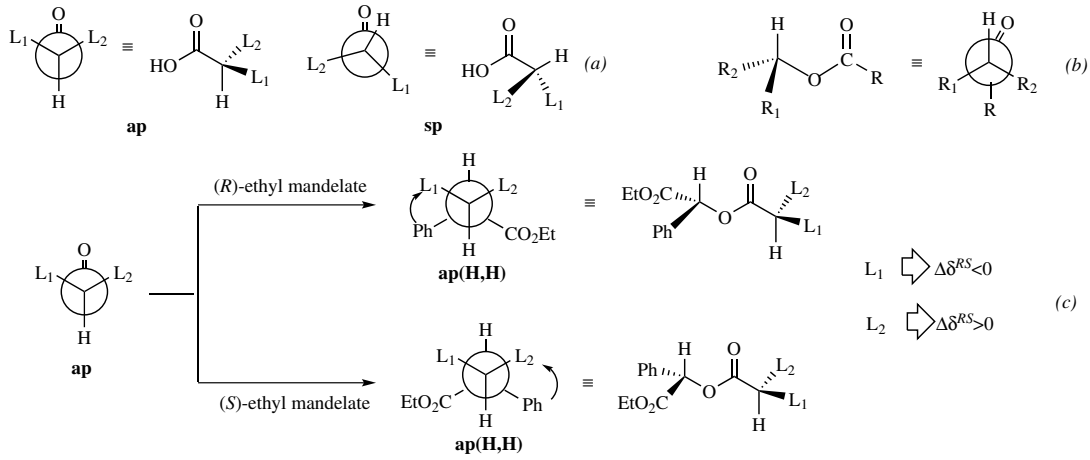


Figure 3. (a) Antiperiplanar (*ap*) and synperiplanar (*sp*) conformations of α-chiral carboxylic acids. (b) Synperiplanar conformation (alcoholic C–H and acidic C=O) preferentially adopted by the esters of α-chiral carboxylic acids with a chiral secondary alcohol. (c) Upon esterification of the *ap* conformation of the α-chiral carboxylic acids with the (*R*)- or (*S*)-enantiomers of ethyl mandelate, the alcohol C–H and the acidic C^{*}_{α}–H bonds adopt an *ap*(*H*,*H*) conformation, so that in the two diastereomers the aromatic shielding is exerted on different L_1/L_2 groups, causing a different sign for the $\Delta\delta^{RS}$ of the two groups.

in turn (Fig. 3c, with ethyl mandelate as an esterifying agent). As a consequence, hydrogens of the L_1 group will have a negative value of the $\Delta\delta^{RS}$ parameter ($\Delta\delta^{RS} < 0$), while those belonging to the L_2 group will have $\Delta\delta^{RS} > 0$, allowing a ready assignment of the absolute configuration of the original carboxylic acid by the comparison of the NMR spectra of both diastereomers. This conclusion was supported by molecular mechanics and semiempirical (AM1 and PM3) calculations on a number of carboxylic acids and their methyl esters, all showing the *ap* as the most stable conformation by 1 kcal/mol or less. The magnitude of the $\Delta\delta^{RS}$ parameter depends on the different populations of the *ap* and *sp* conformers, and thus on the energy gap between the corresponding conformations. However, the ability of the chosen chiral agent to selectively shield the signal also plays a significant role in determining the value of $\Delta\delta^{RS}$, larger aromatic cone exerted by the aryl portion of the chiral alcohol leading to more effective shielding.

Herein, we investigate the possibility to use the ethyl mandelate, which is commercially available in both enantiomeric forms and less expensive than the ethyl 2-hydroxy-2-(9-anthryl)acetate[5]; although providing a smaller aromatic shielding cone and less steric hindrance than 2-hydroxy-2-(9-anthryl)acetate, and thus leading to smaller values of $\Delta\delta^{RS}$, it is reported that a very similar chiral agent, methyl mandelate, has been successfully employed.[15] However, in that study, no real understanding of the factors determining the $\Delta\delta^{RS}$ values was attained, the results being interpreted with a model where only steric considerations were taken into account, with no reference to the acid/ester conformational preference. In order to shed light on this point and because the energy gaps between the *ap* and *sp* conformers calculated previously[5] at a low level of theory, although showing the same trend, lie below the accuracy of the employed method, we have undertaken a series of quantum mechanical DFT calculations on the considered esters. Such an approach also allowed us to find a more sound correlation and explain the possible discrepancies in their observed behaviour as well as to investigate the possibility of establishing a quantitative relationship between the energy gaps and experimental $\Delta\delta^{RS}$ values.

We first prepared the diastereomeric esters **2a**–**f** by the reaction of the commercially available α-homochiral carboxylic acids **1a**–**f** with both (*R*)- and (*S*)-ethyl mandelate, using *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP), in dichloromethane at room temperature (Fig. 1). The reaction mixtures were purified by silica gel, and protonic spectra of the corresponding diastereomeric esters **2** have been recorded (300 MHz, $CDCl_3$), with the aim of applying the NMR-based approach to assign their absolute configuration. The values of the chemical shifts and related $\Delta\delta^{RS}$ for the aliphatic protons of the L_1/L_2 substituents are reported in Table 1. Due to their overlap with the signals of the aryl protons of ethyl mandelate, the signals of the aromatic protons of the L_1/L_2 substituents could not be unambiguously assigned for all the compounds. Figure 4a shows the $\Delta\delta^{RS}$ values for those acids in the training set which could be unequivocally determined. In all the cases, the $\Delta\delta^{RS}$ sign correctly predicts the absolute configuration, although for compound **2a** its value is very close to zero. In general,

Table 1. Chemical shifts and $\Delta\delta^{RS}$ values for esters **2a**–**f**

Compd	Signal	δ (ppm) (*X*,*R*)	δ (ppm) (*X*,*S*)	$\Delta\delta^{RS}$
2a	CH_3	1.893	1.890	+0.003
2b	CH_3	1.575	1.597	−0.022
2c	CH_3 (5)	1.240	1.192	+0.048
2c	CH_2 (3)	1.515	1.566	−0.051
2c	CH_3 (4)	0.922	0.990	−0.068
2d	CH_3	1.574	1.563	+0.011
2e	CH_3	1.572	1.458	+0.114
2f	CH_2 (3)	1.851	1.704	+0.147
2f	CH (4)	1.626	1.594	+0.032
2f	CH_3 (5,6)	0.997	0.946	+0.051

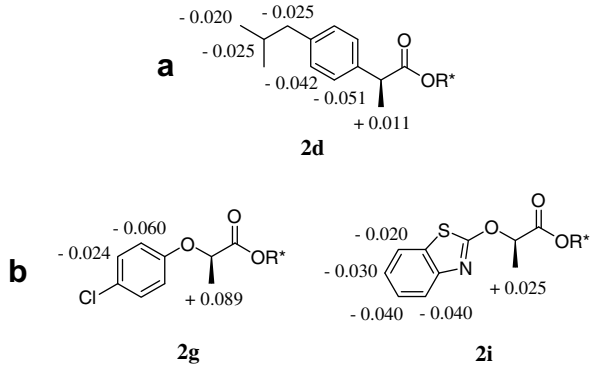
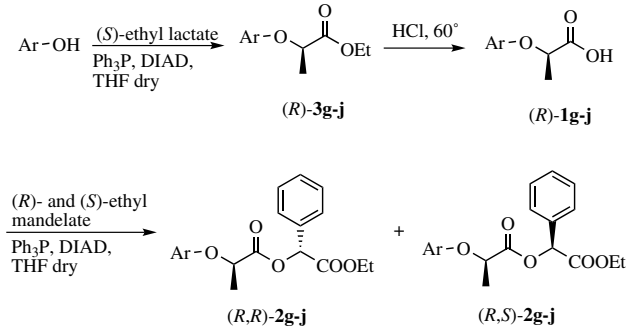


Figure 4. $\Delta\delta^{RS}$ values of aromatic and aliphatic protons determined for the diastereomeric esters **2d** of the training set (panel a), and esters **2g** and **2i** of the newly synthesized acids (panel b).

the absolute value of $\Delta\delta^{RS}$ is rather small, due, as expected, to the moderate anisotropic effect exerted by ethyl mandelate, but it is, however, larger than that obtained when using methyl mandelate.

Next, we performed the synthesis of the chiral derivatives of clofibric acid (*R*)-**1g–j** (Fig. 2): Mitsunobu coupling[22] of the appropriate commercial alcohols and (*S*)-ethyl lactate, in the presence of triphenylphosphine and DIAD, in THF dry at room temperature, furnished the (*R*)-**3g–j** esters. After hydrolysis under acidic conditions, the desired (*R*)-**1g–j** acids were obtained (Scheme 1); the enantiomeric purity of (*R*)-**1g–j** was evaluated by a capillary electrophoretic run, by using β-cyclodextrin as a chiral selector. Analyses showed enantiomeric excesses up to 98% for all the tested compounds. The (*R*,*R*)- and (*R*,*S*)-**2g–j** diastereomeric esters were afforded via Mitsunobu reaction with (*R*)- and (*S*)-ethyl mandelate, and then analyzed by NMR. The values of the chemical shifts and the related $\Delta\delta^{RS}$ for the aliphatic protons of the L_1/L_2 substituents are reported in Table 2. Figure 4b again shows the aromatic $\Delta\delta^{RS}$ values for those acids, the signals of which could be unambiguously determined. Also in this case a complete agreement between the $\Delta\delta^{RS}$ sign and the stereochemistry of substrates has been obtained. Besides, when compared with the results of the training set (Table 1), these compounds show larger values of $\Delta\delta^{RS}$.



Scheme 1.

In order to confirm the hypothesis of a relationship between the sign of the $\Delta\delta^{RS}$ values and a conformational

Table 2. Chemical shifts and $\Delta\delta^{RS}$ values for esters **2g–j**

Compd	Signal	δ (ppm) (*R*,*R*)	δ (ppm) (*R*,*S*)	$\Delta\delta^{RS}$
2g	CH_3	1.745	1.656	+0.089
2h	CH_3	1.797	1.703	+0.094
2i	CH_3	1.815	1.790	+0.025
2j	CH_3	1.898	1.814	+0.084

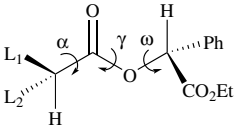


Figure 5. The three torsional angles determining the mutual position of the alcoholic C–H and the acidic C^*_α–H bonds in the esters. Distances calculated with respect to these angles were used to form clusters whose representative conformations were then minimized.

preference of the esters, we first performed a conformational search where all the degrees of freedom were taken into account; the results were clustered according to the three dihedral angles α, γ and ω (Fig. 5) determining the mutual positions of the phenyl ring and the L_1/L_2 substituents to the C^*_α of the carboxylic acid. Ten clusters were considered for each diastereomer and the leading members (i.e., the representative conformation of each cluster, chosen as the lowest energy one) were qualitatively analyzed by visual inspection. In particular, we examined the following features: (i) the relative position of the alcoholic C–H and the C=O, determined by both γ and ω, to verify if they are indeed synperiplanar; (ii) the relative position of the acidic C^*_α–H hydrogen and the C=O oxygen around the α torsional angle, to verify if they are periplanar (either *ap* or *sp*); (iii) the relative position of the acidic C^*_α–H hydrogen and the alcoholic C–H, determined by the joined values of all the three angles α, γ and ω, which is crucial for verifying the correctness of the foundations of the NMR strategy under study: the considered hydrogens have to be periplanar to lead to the aromatic shielding of the acidic L_1/L_2 groups by the phenyl ring. Throughout this analysis and in the following we will use the term periplanar in a slightly more relaxed sense than in the original Klyne and Prelog[23] acceptation[†]: here we will use synperiplanar and antiperiplanar to define a flexible dihedral angle of $0 \pm 45°$ (*syn*) or $180 \pm 45°$ (*anti*). This, however, does not diminish the generality and the conclusions of the present discussion since in spite of these less strict definitions, the aromatic cone of the phenyl ring is directed towards a well defined region, so as to selectively shield only one of the L_1/L_2 substituents of the acidic C^*_α.

The analysis of the clusters representative structures described above highlighted some important qualitative features shared by all the distereoisomers: (i) all the representative structures within 25 kJ/mol from the mini-

[†] It is noteworthy mentioning that, contrarily to its common usage which identifies it with 'coplanar', the term periplanar was introduced to define a torsional angle of $0 \pm 30°$ (*syn*) or $180 \pm 30°$ (*anti*), see, for example, Ref. 24.

mum energy structure show a synperiplanar disposition of the alcoholic C–H and the C=O group, which is in full agreement with the previous studies[5,7]; (ii) the acidic C^*_α–H hydrogen and the C=O oxygen are, within the relaxed notation underlined above, periplanar. The largest deviations from coplanarity are found for the diastereomers of acids **1e** and **1f**, which are the compounds showing the largest conformational flexibility. For most diastereomers (but not all), the minimum energy structure has an *ap* conformation in agreement with the results of the literature;[5] (iii) as a consequence of the relative positions of the groups described in the two preceding points, the alcoholic C–H and the acidic C^*_α–H hydrogens lie approximately in the same plane. Here, deviations from coplanarity, being the result of the joined deviations of three angles, are, in some cases, slightly larger than those described before, however, the relative position of the two hydrogens can always be assigned as *syn* or *anti* according to the definition given above. Since it is the mutual position of these two hydrogens to determine the direction of the aromatic shielding cone, in the following we will analyze and classify our results according to their *anti* periplanarity, *ap*(*H*,*H*), or *syn* periplanarity, *sp*(*H*,*H*). It is interesting to note that the phenyl ring responsible for the shielding, due to steric reasons, is always oriented so as to direct its aromatic cone towards the acidic moiety of the ester, the variations in the various diastereomers amount to ±15° at most and can only account for the small differences in the numerical values of the $\Delta\delta^{RS}$ (see below) passing from one system to another.

We have optimized the structures of the clusters' representative members by DFT methods both in the gas phase and in the solution. Although showing approximately the same trend, only the latter, consistent with the experimental conditions, will be discussed. Again, qualitatively, the results were similar for all the diastereomers: upon minimization, some of the structures converged to the same minimum; variations, sometimes large, from the original geometry often occurred, but always preserving a well defined *ap*(*H*,*H*) or *sp*(*H*,*H*) orientation. In many cases, especially when the conformational flexibility was higher, the energy sequence of the structures obtained from the MM conformational search changed after QM minimization. This is remarkable, as it shows that a rigorous approach to a theoretical understanding of the present NMR strategy cannot be dealt with, without the aid of accurate QM techniques and appropriate treatment of the solvent effects.

We next calculated the energy gaps, ΔE, between the lowest energy *ap*(*H*,*H*) conformation and the lowest energy *sp*(*H*,*H*) conformation within the minimized set for each diastereomer. Results are reported only for solution phase in Table 3 for the esters derived from the training set of α-chiral carboxylic acids and in Table 4 for those obtained from the analogues of clofibric acid synthesized in the present work.

Data in Table 3 show in all the cases, except for acid **1a**, that is, (*R*)-2-bromopropanoic acid, a significant preference for the *ap*(*H*,*H*) conformer, the small negative values for ester **2d** being within the accuracy of the method.

Table 3. Energy differences (in kcal/mol) in solution between the *ap*(*H*,*H*) and *sp*(*H*,*H*) conformations of the (*R*)- and (*S*)-ethyl mandelate derivatives of acids in Figure 1

Compd	ΔE^R(sol)	ΔE^S(sol)
2a	1.79	−1.31
2b	−0.15	0.96
2c	0.11	0.90
2d	2.12	−0.11
2e	2.10	1.08
2f	2.00	1.09

A positive value means that the *sp*(*H*,*H*) conformation is higher in energy.

Table 4. Energy differences (in kcal/mol) in solution between the *ap*(*H*,*H*) and *sp*(*H*,*H*) conformations of the (*R*)- and (*S*)-ethyl mandelate derivatives of acids in Figure 2

Compd	ΔE^R(sol)	ΔE^S(sol)
2g	2.72	0.71
2h	4.33	−0.23
2i	[a]	−1.23
2j	2.73	0.53

A positive value means that the *sp*(*H*,*H*) conformation is higher in energy.
[a] All the calculations converged to an *ap*(*H*,*H*) conformation.

The results for (*R*)-2-bromopropanoic acid are in agreement with the very small experimental $\Delta\delta^{RS}$ value, 0.003, and can be easily explained by examining the geometries of the most stable conformations for the (*R*,*R*)- and (*R*,*S*)-esters (Fig. 6). For both diastereomers, the most stable conformations have the large electronegative bromine atom located away from the C=O oxygen atom. In the case of the (*R*,*S*)-ester, the *ap*(*H*,*H*) conformation has instead the bromine atom close to the C=O oxygen and is thus higher in energy (more than 1 kcal/mol) than the corresponding *sp*(*H*,*H*) one. This results in a shielding effect of the aryl ring on the same group for both the (*R*,*S*)- and (*R*,*R*)-esters and in a $\Delta\delta^{RS}$ value which is practically zero.

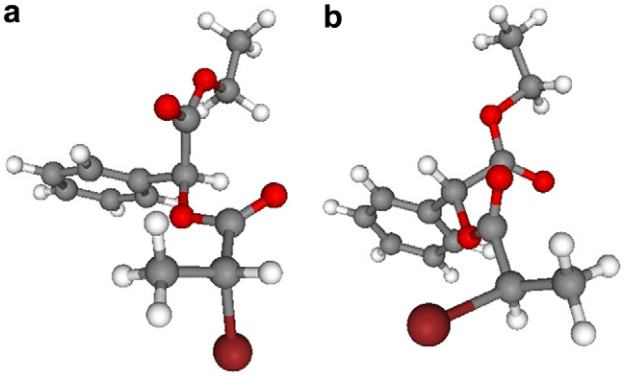


Figure 6. Most stable conformations for the (*S*)-ethyl mandelate ester (a) and (*R*)-ethyl mandelate ester (b) of the (*R*)-2-bromopropanoic acid. In both cases the preferred conformations are those with the bromine atom away from the C=O oxygen, corresponding, respectively, to a *sp*(*H*,*H*) and an *ap*(*H*,*H*) conformations.

Data in Table 4, relative to the analogues of clofibric acid, show a similar behaviour, however, a more homogeneous

pattern can be found: for all the (*R*,*R*)-esters the *ap*(*H*,*H*) conformation is much more stable—of at least 2.5 kcal/mol—than the corresponding *sp*(*H*,*H*) one, meaning that such diastereomers at room temperature can all be found quantitatively in an *ap*(*H*,*H*) conformation. For the (*R*,*S*)-esters the $\Delta\delta^{RS}$ value, on the contrary, is less than 1 kcal/mol, and in two cases, compounds **2h** and **2i**, there is a preference for the *sp*(*H*,*H*) conformation. For compound **2h**, this preference amounts to only 0.23 kcal/mol in solution (the gas phase calculation shows a slight preference for the *ap*(*H*,*H*) conformation) and does not seem to have particular consequences on the experimental $\Delta\delta^{RS}$ which is rather large, while, for compound **2i**, ΔE^{RS} values are between −1 and −2 kcal/mol, leading to a significant preference for the *sp*(*H*,*H*) conformation in the (*R*,*S*)-ester, and thus to the smallest $\Delta\delta^{RS}$ value for the set. In any case, for all the analogues of clofibric acid, the *ap*(*H*,*H*) preference in the (*R*,*R*)-esters is so overwhelming that the applied NMR strategy can always be used to correctly predict the absolute configuration of the acids.

We attempted to find an approximate semiquantitative correlation between the calculated energy differences between the *ap*(*H*,*H*) and *sp*(*H*,*H*) conformations of Tables 3 and 4 and the experimental $\Delta\delta^{RS}$ values of Tables 1 and 2, respectively.

This has been done by evaluating the difference in the population between the *ap*(*H*,*H*) and *sp*(*H*,*H*) conformers for all the compounds by using a Boltzmann distribution; the fraction of the population of the conformation *i* is thus calculated by

$$x_i = \frac{n_i}{N} = \frac{e^{-E_i/RT}}{e^{-E_{ap}/RT} + e^{-E_{sp}/RT}} \quad (1)$$

where $i = ap(H,H)$, $sp(H,H)$ and T is the temperature corresponding to the experimental conditions and R is the Boltzmann constant. As we have considered the lowest energy *ap*(*H*,*H*) and *sp*(*H*,*H*) conformers only, this is a rather crude estimate of x_i, however, one has to bear in mind that we do not expect this correlation to be fully quantitative since, as underlined above, the difference between the population of the two conformers is only one of a number of factors contributing to the numerical value of $\Delta\delta^{RS}$.

The excess of population for the *ap*(*H*,*H*) conformer is given by

$$X_{ap-sp} = x_{ap} - x_{sp} \quad (2)$$

(an excess in the population of the *sp*(*H*,*H*) conformer will thus lead to a negative value of X_{ap-sp}). Since $\Delta\delta^{RS}$ depends on the excess of population of both the (*R*)- and (*S*)-ethyl mandelate derivatives, the corresponding X_{ap-sp} values have been algebraically summed

$$X^{RS}_{ap-sp} = \frac{X^{R}_{ap-sp} + X^{S}_{ap-sp}}{2} \quad (3)$$

and the sum has been divided by 2 for normalizing purposes: a value of $X^{RS}_{ap-sp} = 1$ corresponds to the presence of the *ap*(*H*,*H*) conformer only for both diastereomers; a value of $X^{RS}_{ap-sp} = 0$ means to have equal populations of the *ap*(*H*,*H*) and *sp*(*H*,*H*) conformers in both diastereomers or to an equal excess of the *ap*(*H*,*H*) and *sp*(*H*,*H*) conformer, respectively, in the two diastereomers (in both cases this should lead to a null $\Delta\delta^{RS}$ value); a value of $X^{RS}_{ap-sp} = -1$ corresponds to the presence of the *sp*(*H*,*H*) conformer only for both diastereomers.

The obtained X^{RS}_{ap-sp} values have been linearly correlated with the experimental $\Delta\delta^{RS}$ taken with a plus sign whenever predicting the right assignment of the L_1 and L_2 groups. Since a correct correlation can be only established by comparing the signal of the hydrogens located at similar distances from the phenyl, we have considered $\Delta\delta^{RS}$ values belonging to groups in α-position with respect to the stereogenic carbon atom, that is, we have excluded lines 5, 9 and 10 in Table 1 from the correlation. The results are reported in Figure 7 for the training set, while, in Figure 8, the newly synthesized analogues of clofibric acid have been included in the set. Figure 7 shows a high correlation between the calculated X^{RS}_{ap-sp} and the experimental $\Delta\delta^{RS}$, with a R^2 value of 0.846, showing that, although the correlation is significant also in vacuum ($R^2 = 0.533$), solvent effects do play an important role and have to be included for a proper treatment. When the set of compounds is increased by the inclusion of the four analogues of clofibric acid the correlation decreases slightly to 0.748, still remaining quite large (Fig. 8). However, a close inspection of data for the latter correlation shows that the value of ΔE^{RS} for com-

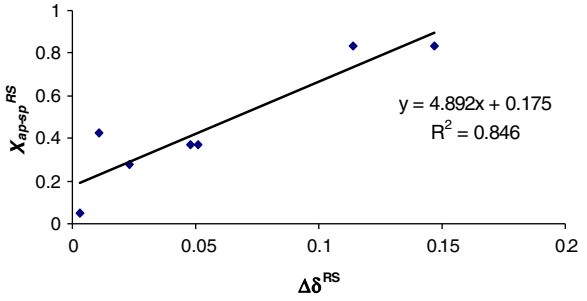


Figure 7. Linear correlation between the experimental $\Delta\delta^{RS}$ data (Table 1) and the calculated X^{RS}_{ap-sp} values for the ethyl mandelate esters of the training set of α-carboxylic acids in Figure 1.

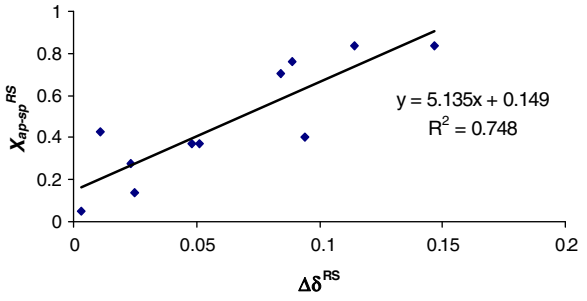


Figure 8. Linear correlation between the experimental $\Delta\delta^{RS}$ data (Tables 1 and 2) and the calculated X^{RS}_{ap-sp} values for the ethyl mandelate esters of the training set of α-carboxylic acids in Figure 1 with the addition of the analogues of clofibric acid synthesized in this work (Fig. 2).

Table 5. Chemical shifts for the substituents to the C_α of the (*S*,*R*) and (*S*,*S*) diastereomeric esters of acid **1c**, recorded at different temperatures

Temperature (°C)	Signal	δ (ppm) (*S*,*R*)	δ (ppm) (*S*,*S*)	$\Delta\delta^{RS}$
20	CH_3 (5)	1.240	1.192	+0.048
0	CH_3 (5)	1.237	1.184	+0.053
−10	CH_3 (5)	1.235	1.179	+0.056
−40	CH_3 (5)	1.231	1.162	+0.069
20	CH_2 (3)	1.515	1.566	−0.051
0	CH_2 (3)	1.506	1.561	−0.055
−10	CH_2 (3)	1.501	1.560	−0.059
−40	CH_2 (3)	1.487	1.553	−0.066
20	CH_3 (4)	0.922	0.990	−0.068
0	CH_3 (4)	0.913	0.987	−0.074
−10	CH_3 (4)	0.908	0.987	−0.079
−40	CH_3 (4)	0.895	0.982	−0.087

The absolute value of $\Delta\delta^{RS}$ becomes larger as the temperature increases.

pound **2h** is responsible for this discrepancy: the elimination of the corresponding point leads to a value of $R^2 = 0.827$, very similar to that obtained with the training set only.

To further investigate the relationship between the differences in the calculated populations of the *ap*(*H*,*H*) and *sp*(*H*,*H*) conformers in the chiral esters and the experimental values of $\Delta\delta^{RS}$ we also performed low temperature NMR experiments. Indeed, as highlighted in the literature,[5] one should expect larger absolute values for $\Delta\delta^{RS}$ as the temperature decreases, due to the increased population of the lowest energy preferred conformation. The experimental results on the diastereomeric esters (*S*,*R*)- and (*S*,*S*)-**2c** reported in Table 5 are fully coherent with this picture.

We can again use the values of X^{RS}_{ap-sp} obtained with Eqs. 1–3 and correlate them with the experimental $\Delta\delta^{RS}$ of Table 5. In this case, we should expect the correlation between the calculated X^{RS}_{ap-sp} values and the $\Delta\delta^{RS}$ of the same groups at different temperatures to be complete within the experimental and computational accuracy: the increase of $\Delta\delta^{RS}$ in this case is only due to the different populations of the considered conformations and no other effects come into play.

Figure 9 illustrates such a correlation for the three CH_n groups of the esters of compound **1c**, which is very good in each case ($0.98 < R^2 < 1$). Although the number of points to be correlated is limited, R^2 values so close to 1 indicate that the temperature dependence of the experimental $\Delta\delta^{RS}$ only depends on the different conformational populations of the diastereomers, so that such correlations could be used to quantitatively predict $\Delta\delta^{RS}$ values upon the temperature changes.

The existence of a qualitative relationship between $\Delta\delta^{RS}$ and ΔE^{RS} values and that, after some manipulations, a quantitative relationship could as well be established, underlines that the NMR strategy for the assignment of the absolute configurations of α-chiral carboxylic acids is founded on arguments based on a well defined conformational preference, as proposed.[5] Furthermore, our calculations suggest that in order for this strategy to be applied to α-chiral carboxylic acids, the related conformational constraints are not so strict: it is sufficient that the two hydrogens (the alcoholic C–H and the acidic C^*_α–H) in the esters lie approximately in an antiperiplanar position. Only in the presence of bulky and/or very electronegative groups directly linked to the C_α can the most stable conformation vary so as to determine a different aromatic shielding.

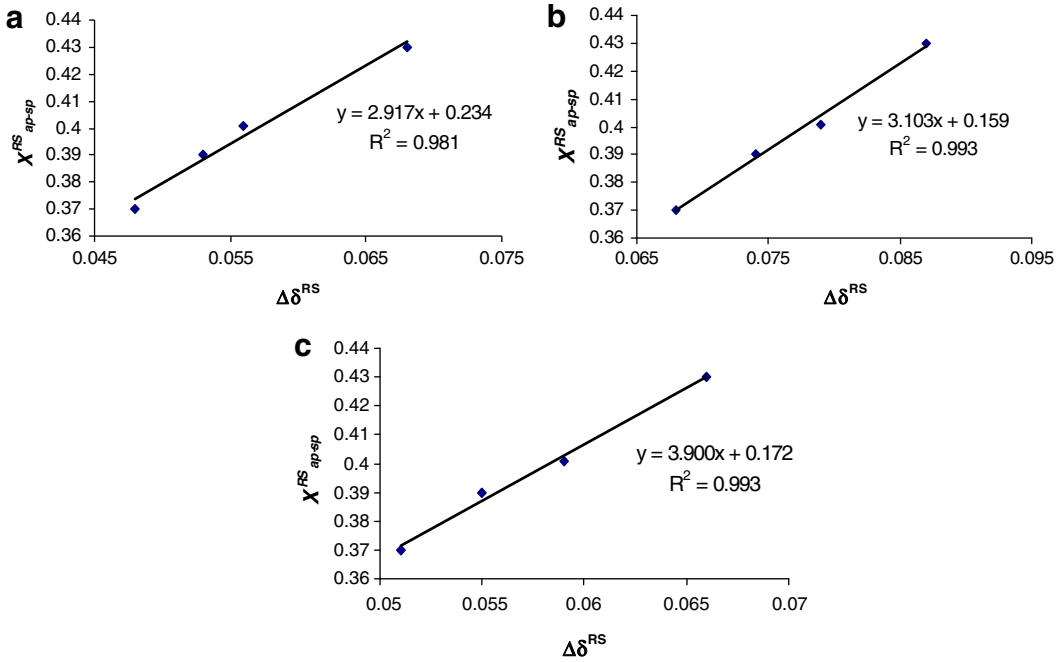


Figure 9. Linear correlation between the experimental $\Delta\delta^{RS}$ data (Table 5) for the substituents (CH_3 (5) panel (a), CH_2 (3) panel (b), CH_3 (4) panel (c)) to the C_α of the (*S*,*R*)- and (*S*,*S*)-esters of acid **1c** obtained from different temperatures NMR spectra and the corresponding theoretical X^{RS}_{ap-sp} values.

3. Conclusions

Herein, we have performed the synthesis of some chiral analogues of clofibric acid by a simple reaction scheme, in good yields and with excellent enantiomeric excess, and assigned their absolute configurations by a well-known NMR-based methodology, consisting of the derivatization of α-chiral carboxylic acids to couples of diastereomeric esters through a suitable chiral alcohol. The comparison between the diastereomers chemical shifts of the substituents to the C^*_α of the carboxylic acid should lead to the determination of the acid absolute configuration.

For that purpose we chose ethyl mandelate as an auxiliary reagent since, besides having the minimal requirements to provide a good aromatic shielding, it has the appealing benefits of being little expensive and easy to obtain in both enantiomeric forms. Although a similar reagent, methyl mandelate, had already been employed, leading to fairly good, although not excellent, results, this represents the first attempt to use this chiral agent. For this reason, we decided to thoroughly investigate the use of ethyl mandelate by testing the procedure on a set of commercially available α-chiral carboxylic acids and by performing DFT calculations on the preferred conformations of the diastereomeric esters. The success of the NMR strategy is indeed based on a well-defined conformational preference of the diastereomers leading to a selective shielding of the substituents to C^*_α in turn.

The experimental $\Delta\delta^{RS}$ sign for both the training set and the new chiral 2-aryloxypropanoic acids is in full agreement with the stereochemistry of the substrates, although with smaller absolute values than with ethyl 2-hydroxy-2-(9-anthryl)acetate, thus showing that ethyl mandelate can be successfully used, except in the case of bulky and/or very electronegative substituents to C_α.

4. Experimental

4.1. Chemistry

Melting points were determined on a Büchi B-540 apparatus and are uncorrected. Optical rotations were measured on a Perkin Elmer model 241 polarimeter. Infrared spectra were recorded on a FT-IR 1600 Perkin Elmer spectrometer. Microanalyses were carried out with an Eurovector Euro EA 3000 model analyzer; the analytical results are within 0.4% of the theoretical values. Commercial reagents were used as received from Aldrich or Fluka. THF was distilled from sodium/benzophenone.

4.1.1. General procedure for the preparation of diastereomeric esters (*X*,*R*)- and (*X*,*S*)-2a–f. A mixture of (*R*)- or (*S*)-**1a**–**f** (1.0 mmol), (*R*)- or (*S*)-ethyl mandelate (180 mg, 1.0 mmol), DCC (206 mg, 1.0 mmol) and DMAP (1.2 mg, 0.1 mmol) was dissolved in dry dichloromethane and stirred at room temperature overnight. The reaction mixture was filtered off, and after evaporation of solvent, the crude material was purified by silica gel (cyclohexane/ethyl acetate 9:1).

4.1.2. General procedure for the preparation of esters (*R*)-3g–j. A solution of diisopropylazodicarboxylate (DIAD, 607 mg, 3.0 mmol) in anhydrous tetrahydrofuran (15 mL) was added dropwise to a solution of (*S*)-ethyl lactate (260 mg, 2.2 mmol), the appropriate alcohol (2.0 mmol) and triphenylphosphine (786 mg, 3.0 mmol) in anhydrous tetrahydrofuran (50 mL). The reaction mixture was stirred at room temperature overnight, under a nitrogen atmosphere. THF was evaporated in vacuo, and the residue was purified by chromatography on silica gel (cyclohexane/ethyl acetate 95:5) to afford the desired (*R*)-**3g**–**j** esters in 36–67% yields.

4.1.3. General procedure for the preparation of acids (*R*)-1g–j. 6 M HCl (20 mmol, 3.33 mL) was added to (*R*)-**3g**–**j** (1 mmol), and the mixture was heated to 60 °C. When the reaction was complete, the mixture was diluted and extracted with diethyl ether (20 mL). The collected organic layers were dried on sodium sulfate. After solvent evaporation, crude materials were purified by crystallization to obtain (*R*)-**1g**–**j** acids in good yields and excellent enantiomeric excess.

4.1.3.1. (*R*)-2-(4-Chlorophenoxy)propanoic acid, (*R*)-1g. White needles (crystallized from petroleum ether), 52% yield; mp 115–117 °C; IR (KBr) 1712 cm^{-1}; $[\alpha]_D^{23} = +41.9$ (*c* 1.2, CH_3OH); ^1H NMR (CD_3OD) δ 1.57 (d, 3H, $J = 6.9$ Hz, CH_3), 4.78 (q, 1H, $J = 6.9$ Hz, CHCH$_3$), 6.86 (d, 2H, $J = 9.0$ Hz, CH arom), 7.24 (d, 2H, $J = 9.0$ Hz, CH arom); ^{13}C NMR (CD_3OD) δ 17.6 (*C*H$_3$), 72.4 (*C*HCH$_3$), 116.4 (*C*H arom), 126.0 (*C* arom), 129.1 (*C*H arom), 156.7 (*C* arom), 174.3 (*C*=O). Anal. Calcd for $C_9H_9ClO_3$: C, 53.88; H, 4.52. Found: C, 53.71; H, 4.53.

4.1.3.2. (*R*)-2-[(6-Chloroquinolin-2-yl)oxy]propanoic acid, (*R*)-1h. White needles (crystallized from cyclohexane), 72% yield; mp 140–143 °C; IR (KBr) 1716 cm^{-1}; $[\alpha]_D^{23} = +110.7$ (*c* 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.69 (d, 3H, CH_3CH), 5.51 (q, 1H, CH), 7.01 (d, 1H, CH arom), 7.50 (dd, 1H, CH arom), 7.68 (dd, 2H, CH arom), 7.95 (d, 1H, CH arom); ^{13}C NMR ($CDCl_3$) δ 17.6 (*C*H$_3$), 70.3 (*C*H), 113.8 (*C*H arom), 126.1 (*C* arom), 126.4 (*C*H arom), 128.9 (*C*H arom), 130.2 (*C* arom), 130.6 (*C*H arom), 138.7 (*C*H arom), 144.3 (*C* arom), 161.0 (*C* arom), 177.6 (*C*=O). Anal. Calcd for $C_{12}H_{10}ClNO_3$: C, 57.27; H, 4.01; N, 5.57. Found: C, 57.15; H, 4.02; N, 5.58.

4.1.3.3. (*R*)-2-(1,3-Benzothiazol-2-yloxy)propanoic acid, (*R*)-1i. White needles (crystallized from cyclohexane), 70% yield; mp 167–170 °C; IR (KBr) 1716 cm^{-1}; $[\alpha]_D^{19} = +35.9$ (*c* 2.0, CH_3OH); ^1H NMR (CD_3OD) δ 1.65 (d, 3H, CH_3), 5.34 (q, 1H, CH), 7.18 (m, 2H, CH arom), 7.33 (dd, 1H, CH arom), 7.54 (dd, 1H, CH arom); ^{13}C NMR (CD_3OD) δ 13.3 (*C*H$_3$), 51.4 (*C*H), 111.3 (*C*H arom), 122.3 (*C* arom), 122.8 (*C*H arom), 123.3 (*C*H arom), 126.4 (*C*H arom), 136.4 (*C* arom), 170.2 (*C* arom), 171.4 (*C*=O). Anal. Calcd for $C_{10}H_9NO_3S$: C, 53.80; H, 4.06; N, 6.27. Found: C, 53.67; H, 4.05; N, 6.26.

4.1.3.4. (*R*)-2-(1-Naphthyloxy)propanoic acid, (*R*)-1j. White needles (crystallized from cyclohexane), 36% yield; mp 126–127 °C; IR (KBr) 1706 cm^{-1}; $[\alpha]_D^{22} = -61.3$

(c 1.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.80 (d, 3H, $J = 6.9$ Hz, CH_3), 4.98 (q, 1H, $J = 6.9$ Hz, C*H*), 6.73 (d, 1H, C*H* arom), 7.31–7.35 (m, 1H, C*H* arom), 7.47–7.51 (m, 3H, C*H* arom), 7.79–7.82 (m, 1H, C*H* arom), 8.30–8.34 (m, 1H, C*H* arom); ^{13}C NMR ($CDCl_3$) δ 18.7 (CH_3), 72.5 (*C*H), 105.8, 121.7, 122.2, 125.7, 125.8 (*C*H arom), 125.8 (*C* arom), 126.9, 127.7 (*C*H arom), 134.8, 153.2 (*C* arom), 177.8 (*C*=O). Anal. Calcd for $C_{13}H_{12}O_3$: C, 72.21; H, 5.59. Found: C, 72.34; H, 5.58.

4.1.4. General procedure for the preparation of diastereomeric esters (*R*,*R*)- and (*R*,*S*)-2g–i. Diastereomeric esters (*R*,*R*)- and (*R*,*S*)-**2g**–**i** were readily obtained by Mitsunobu coupling of (*R*)-**1g**–**j** with (*R*)- or (*S*)-ethyl mandelate, as previously described for compounds **3g**–**j**.

4.2. NMR spectroscopy

^1H and ^{13}C NMR spectra were recorded on a Varian 300 MHz instrument, using tetramethylsilane (TMS) as an internal standard. All the compounds were dissolved in $CDCl_3$, (5 mg in 0.7 mL). For experiments at low temperature, samples were allowed to equilibrate for 15 min at each temperature before recording the spectra.

4.3. Computational methods

All the calculations were performed with the Schrödinger suite of programs. The conformational searches were carried out on all the diastereomeric derivatives through MACROMODEL 9.0,[25] using MMFF94s as force field and Conjugate Gradient with Polak-Ribiere first derivatives method as minimization algorithm. 3000 conformations for each diastereomer were minimized, only those within 30 kJ/mol from the global minimum were recorded. Once a conformational search was completed, the recorded conformations were subjected to a cluster analysis using XCLUSTER.[26] The conformations were clustered according to their root mean square distances with respect to the three dihedral angles determining the mutual positions of the phenyl ring and the L_1/L_2 substituents to the C^*_α of the carboxylic acid (Fig. 5). For each diastereomer, 10 clusters were obtained, and their leading members, that is, the minimum energy structure within each cluster, fully optimized by means of density functional theory calculations both in gas phase and in solution of $CHCl_3$ using JAGUAR 6.0.[27] Such calculations were performed using the B3LYP exchange correlation functional, which is known to perform particularly well for organic molecules, and the 6-31G* basis set, except for compounds derived from acid **1a**, for which an LACVP* basis set was employed, consisting of the Los Alamos effective core potential and a double-zeta valence basis set including the outermost core orbitals for Br and the all electron 6-31G* basis set for all the remaining atoms. Calculations in solutions were carried out through the Poisson-Boltzmann continuum model employing the following values for the dielectric constant and probe radius to model $CHCl_3$, $\varepsilon = 4.806$ and $r = 2.514$ Å.

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