Effects of Solvation on the Ionization and Conformation of Raclopride and Other Antidopaminergic 6-Methoxysalicylamides: Insight into the Pharmacophore

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Previous work has shown that raclopride in water at neutral pH exists in a zwitterionic form, suggesting a stereoelectronic structure largely different from that of other benzamides.[1] In the present study, the acid–base behavior of other 6-methoxysalicylamides is shown to be comparable to that of raclopride. An extensive investigation by high-temperature molecular dynamics gave insight into the conformational behavior of neutral and zwitterionic raclopride in vacuum and in water. Partitioning of raclopride and a more rigid analogue with characterization (by first-derivative UV spectroscopy) of the predominant forms in the organic phase indicated that only neutral, internally H-bonded forms partition into the organic solvent. Thus, the predominant forms of 6-methoxysalicylamides will be very different in the aqueous and organic phases. In the latter phase, and hence presumably also in the receptor phase, the drugs exist with a neutral, internally H-bonded phenolic group and are therefore stereoelectronically similar to other substituted benzamides.

Introduction

Among the atypical antipsychotic drugs, substituted benzamides[2-4] and in particular orthopramides (6-methoxybenzamides) constitute a large and well-studied class of compounds which preferentially block dopamine D_2 receptors. Variations in aromatic ring substitution and modification of the basic side chain generate a great number of compounds with characteristic biological responses. A number of quantitative structure–affinity relationships (QSARs) were developed to understand the effect of aromatic substitution and/or side-chain variation on D_2 receptor affinity.[5,6] In particular, some studies[5] pointed to the importance of a 2-hydroxy substituent not only in 4-piperidinyl derivatives[5c] but also in 1-ethyl-2-(pyrrolidinylmethyl) derivatives.[5b] These analyses suggested that orthopramides and 6-methoxysalicylamides may display different modes of binding to the D_2 receptor. It must be noted that these QSARs were developed for the neutral form of the compounds. Although no pK_a or ionization state descriptors were necessary in the correlation equations, it has been speculated that different modes of binding could be related to the unexpected conformational behavior of the zwitterionic form of some 6-methoxysalicylamides (Table I) as illustrated by our work on theoretical (gas phase) stereoelectronic features of raclopride (1).[1]

Despite the significance of its electrical states, the microscopic acid–base behavior of raclopride (Figure 1) is so far left unresolved. In the present study, we investigate the acid–base properties of a series of raclopride congeners (compounds 2–9 in Table I) in order to clarify the effects of aromatic substitution on the population of electrical forms of 6-methoxysalicylamides. The effect of side-chain variation was also examined by replacing the *N*-[(1-ethylpyrrolidin-2-yl)methyl] moiety with the relatively rigid *N*-(1-methylpiperidin-4-yl) side chain (compound 10 in Table I). The changes in conformational behavior produced by these structural modifications were examined by high-temperature molecular dynamics for neutral and zwitterionic electrical forms of raclopride (1) and the more rigid model compound 10. Finally, the conformation of raclopride and its rigid congener in media of low polarity was probed by lipophilicity and UV spectroscopy measurements in 1-octanol/buffer and *n*-heptane/buffer biphasic systems. Large solvent effects on ionization and conformation were seen which are indicative of the active form of 6-methoxysalicylamides when binding with high affinity to the D_2-receptor.

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Results and Discussion

Acid–Base Behavior of Raclopride and Analogues (Compounds 1–9). Raclopride (1) and its congeners 2–9 display two macroscopic pK_a values in the potentiometric titrations, the results being compiled in Table I. The ionization constant of the phenolic group of the unsubstituted congener (compound 6, $pK_{a1} = 8.70$) was further verified by a UV spectrophotometric method ($pK_{a1} = 8.65 \pm 0.11$). Thus, the corresponding lower pK_a values (pK_{a1}) could be assigned to the phenolic OH group, and the higher pK_a values (pK_{a2}) to the amino group, implying that all investigated compounds exist predominantly in zwitterionic forms at isoelectric pH (p*I*). At physiological pH (7.4), the proportion of the zwitterionic form varies between 96% (compounds 1 and 9) and 5% (compounds 6 and 8).

Their acid–base behavior divides raclopride derivatives 1–9 into two groups, one with higher phenol acidity ($pK_{a1} < 6$) and reduced amine basicity ($pK_{a2} < 9.2$) and the other with smaller phenol acidity ($pK_{a1} > 6.8$) and higher amine basicity ($pK_{a2} > 9.5$).

Acidity of the Phenolic Group. The acidity of phenols is of long-standing practical and theoretical interest to chemists,[7,8] and much effort has been devoted to uncover the structural factors influencing it. Among the factors considered, electronic influences such as substituent

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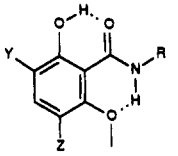
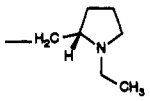
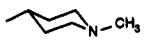
Table I. Chemical Structure of Compounds Studied and Macroscopic Dissociation Constants of 6-Methoxysalicylamides Determined by Potentiometry at 25 °C



R	Y	Z	compound	pK_{a1}[a]	pK_{a2}[b]	pI[c]	% of Z[d]
	Cl	Cl	1	5.81 ± 0.01	9.21 ± 0.01	7.51	96
	Br	Br	2	5.94 ± 0.01	9.18 ± 0.01	7.56	95
	Cl	Et	3	6.88 ± 0.01	9.60 ± 0.01	8.24	76
	Et	Cl	4	6.93 ± 0.01	9.67 ± 0.02	8.30	74
	Et	Et	5	7.28 ± 0.01	9.55 ± 0.01	8.42	56
	H	H	6	8.70 ± 0.01	9.56 ± 0.01	9.13	5
	Cl	H	7	7.13 ± 0.01	9.66 ± 0.01	8.40	65
	Et	H	8	8.71 ± 0.01	9.57 ± 0.01	9.14	5
	NO_2	H	9	5.81 ± 0.01	9.16 ± 0.01	7.49	96
	Cl	Cl	10	5.96 ± 0.01	9.12 ± 0.01	7.54	95

[a] Phenolic group. [b] Amino group. [c] The isoelectric point (pI) as the arithmetic mean of pK_{a1} and pK_{a2}. [d] Percentage of zwitterionic form at pH = 7.4.

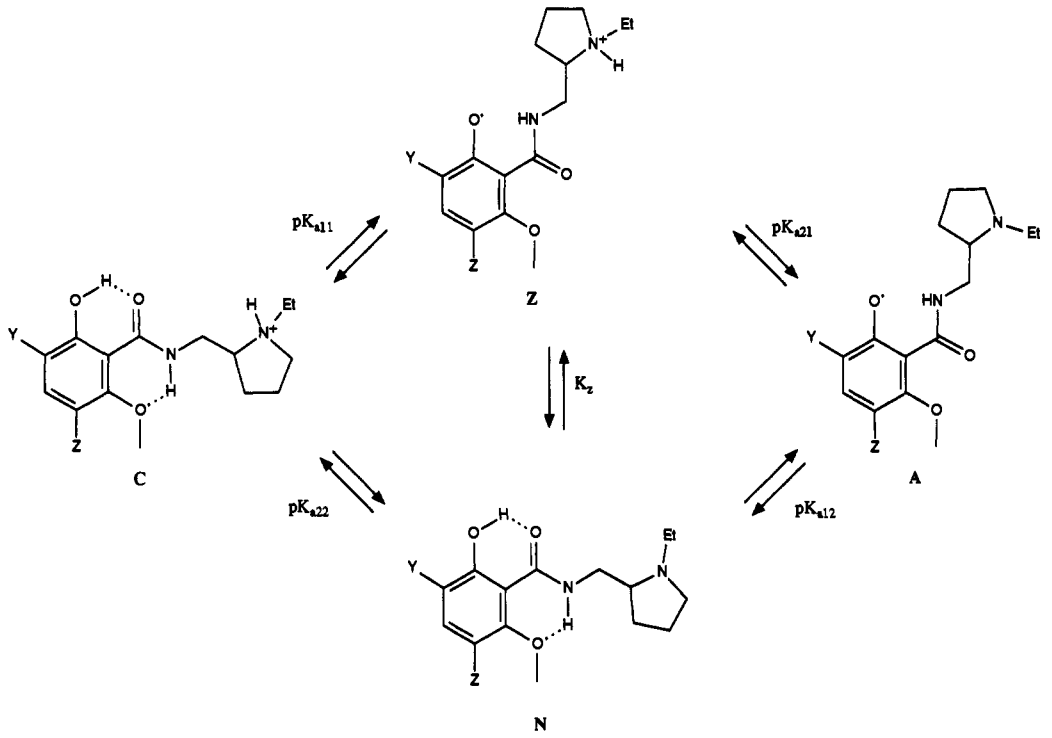


Figure 1. Microscopic acid–base behavior of raclopride and other 6-methoxysalicylamides. C: cationic form; Z: zwitterionic form; N: neutral form; A: anionic form. pK_a values are dissociation constants.

dipolar field/inductive properties, π-electron delocalization, and polarizability effects are well documented,[9,10] while steric and solvation effects are less well understood.[11]

To understand the substituents effects on the acidity of the phenolic OH group in compounds **1–9**, both the electronic effects (Hammet substituent electronic parameter σ)[12] and the steric effects (Taft substituent steric parameter modified by Hansch and Leo, E^c)[13] were considered in statistical analysis. The electronic and steric constants for the substituents investigated are listed in the Experimental Section, the most significant regression equation obtained by Partial Least Square (PLS) and multiple linear regression (MLR) being

$$pK_{a1} = -1.66\ (\pm 0.61)\sigma_o - 1.36\ (\pm 0.64)E_p^c + 7.98\ (\pm 0.42) \quad (1)$$

$$n = 9;\ r^2_{CV} = 0.80;\ N_{comp} = 2;\ r^2 = 0.92;\ s = 0.37$$

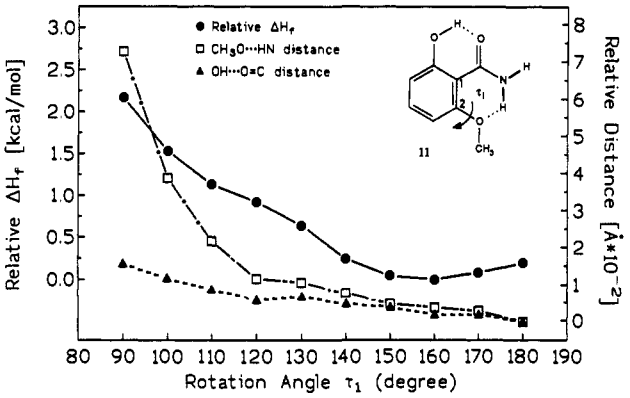


Figure 2. PM3 calculation of model compound 11 performed to investigate the influence on the hydrogen bond strength of steric effects of the substituents adjacent to the methoxy group. In the insert, $\tau_1 = 180°$.

where σ_o accounts for electronic effects ortho to the phenolic OH (3-position) and E_p^c accounts for steric

effects of the substituent para to the phenolic hydroxyl group (5-position). In eq 1, r^2_{CV} is the squared cross-validated correlation coefficient and N_{comp} is the number of significant components in PLS analysis; 95% confidence limits derived from the MLR analysis are given in parentheses. Standardization[14] of eq 1 yields

$$(pK_{a1})' = -0.78\ (\sigma_o)' - 0.61\ (E^c_p)' \qquad (2)$$

To confirm the steric effects of the para substituents, other steric parameters such as Verloop's B_1 (minimal width), B_5 (maximal width), and L (length)[13] were used to replace E^c and yielded similar results. Thus, it appears that more than 90% of the variance of the phenolic acidity in 6-methoxysalicylamides can be satisfactorily accounted for by electronic effects at the ortho position and steric effects at the para position, their influence being comparable as suggested by the coefficients in the standardized eq 2.

The regression coefficient of σ_o in eq 1 (−1.66) is suggestive of a decrease in pK_a due to electron-withdrawing ortho substituents. The phenomenon can safely be attributed to the well-known stabilization of anionic forms caused by electron-withdrawing ortho substituents.

The steric effects of para substituents are not as obvious. The size of para substituents can affect only the orientation of the neighboring methoxy group in 6-methoxysalicylamides. This group may adopt a perpendicular or coplanar orientation depending on whether the adjacent Z-substituent is bulky or not as shown by Breen et al.[15] in methoxybenzenes and by Högberg et al.[16] in salicylamides where a coplanar methoxy orientation is preferred with small neighboring groups (e.g. H, F) and an almost perpendicular orientation with larger adjacent groups (e.g. Br, Et). Although the formation of an internal hydrogen bond $CH_3O\cdots HNC{=}O$ (Table I) is still possible when the methoxy group is out of plane, its strength is nevertheless weakened.[17] This should lead to weakening of the other hydrogen bond $OH\cdots O{=}C$, an effect which increases the acidity of the OH group. This chain of effects may explain why a bulkier group adjacent to the methoxy group can increase the acidity of the OH group as shown in eq 1.

To test this hypothesis, we have examined the effect of rotating the methoxy group (rotation angle τ_1, i.e. C_1–C_2–O–CH_3 dihedral angle) on the conformational energy (relative heat of formation) and on the strength of the two hydrogen bonds (relative hydrogen bond distance) for model compound 11 (Figure 2); the geometry of calculated points, except the reaction coordinate, were optimized by PM3.[18] The results of PM3 calculations clearly show that coplanarity of the methoxy group stabilizes the hydrogen bonding interactions and hence lowers the energy. The cooperative stabilization of the two internal hydrogen bonds accompanying the rotation of the methoxy group is also suggested (Figure 2), a larger $OH\cdots O{=}C$ distance corresponding to a larger $CH_3O\cdots HNC{=}O$ distance. Although the variation of the $OH\cdots O{=}C$ distance seems modest, it is definitively larger than that calculated for the methoxy rotation in the methyl 6-methoxysalicylate. More experimental work on model molecules is needed to support the above interpretation.

Basicity of the Amine Function. The effect of aromatic substituents on the basicity of the amino group is far from obvious. The more acidic compounds (1, 2, and 9) are also the less basic ones (pK_{a2} ca. 9.2), while in compounds 3–8 the basicity remains constant (pK_{a2} ca. 9.6) despite large differences in acidity (pK_{a1} 6.9–8.7). This suggests modest inductive effects of the phenolate group acting to decrease basicity and an absence of electrostatic influences (through-space effects) between the two opposite charges. In other words, the distance between the basic protonated function and the phenolate group is so great that the charge effects is negligible, i.e. that the zwitterionic form Z (see Figure 1) exists ir more or less *extended* conformations in aqueous solution. This assumption is in contradiction with a conformational analysis of zwitterionic raclopride in vacuum,[1] which revealed a folded and twisted conformation to be the most stable one. Such a discrepancy is probably due to the neglect of solvation effect in the force-field calculation,[1] which overestimated the strength of ionic interactions. A twisted benzamide conformation is also contradicted by other studies.[6c,d] To verify this hypothesis, the conformational space for raclopride (1) in its different electrical states was reexamined.

Conformational Analysis of Electrical Forms of Raclopride (1). Due to the complexity of the raclopride molecule and the limitations of the techniques being used, our previous exploration of the conformational space was not complete. To overcome this problem, we used in this study the method of high-temperature molecular dynamics[19–21] based on the Tripos force field[22] (see Experimental Section for details). Previous studies showed that the Tripos force field can reasonably reproduce the geometry of neutral raclopride obtained from X-ray crystallography and NMR spectroscopy, while the geometries optimized by the semiempirical AM1[23] and PM3[18] methods were not as convincing due to the failure to estimate correctly rotational barriers of single bonds in a conjugated molecule.[1] For each form, two runs were performed with a different treatment of the electrostatic energy term: one *in vacuum*, i.e. with a small dielectric constant ($\epsilon = 1$), and another "*in water*", i.e. by simulating the solvent as a continuum of high dielectric constant ($\epsilon = 80$).[24]

Detailed analyses of conformers localized by high-temperature molecular dynamics are given as supplementary material, including energy distribution and geometrical parameters of most stable conformers (below a relative energy of 7.0 kcal/mol). However, to clarify the discussion, a classification is proposed (Figure 3) and the global minimum of each conformational analysis is depicted in Figures 4 and 5.

The conformational analysis of the *neutral form* of raclopride in vacuo is similar to previous studies: all the low-energy conformations are stabilized by the two internal hydrogen bonds and the stability of the *folded* (*F*), *half-folded* (*H*) and *extended* (*E*) side-chain rotamers is comparable. In contrast, in a solvent of high dielectric constant, the energy difference between the "*normal*" (*N*, two hydrogen bonds) and the "*reverse*" (*R*, one internal hydrogen bond $O{=}CNH\cdots OH$) conformations is nil; also, the folded rotamers are slightly stabilized with respect to extended forms. In both cases, nonplanar conformations (P) of the amide group with respect to the aromatic cycle were also localized. However, they are higher in energy than the planar conformations. It should be noted that the geometries presented here differ slightly from those reported in a previous paper;[1] this change is not due to a different force field (in both cases, the SYBYL 5.2 force field was used[22]) but because of limited assumptions made in the first conformational analysis.

For the *zwitterionic form* of raclopride, the effect of varying the dielectric constant is stronger. In vacuo, our

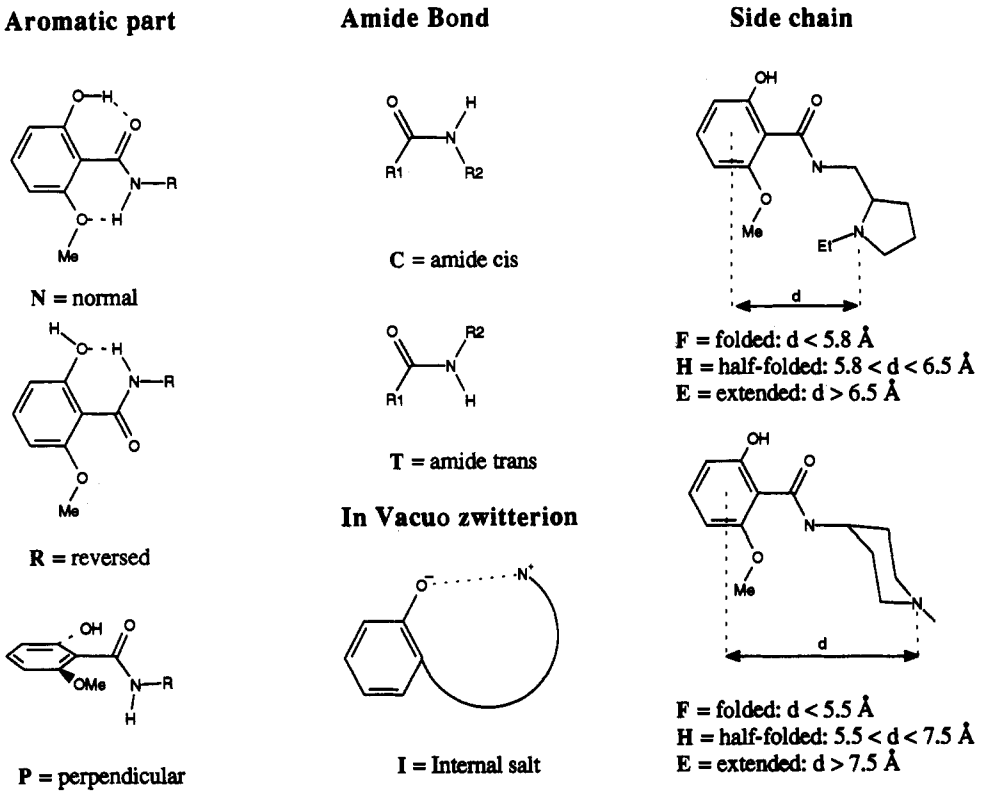


Figure 3. Schematic classification of conformers revealed by high-temperature molecular dynamics.

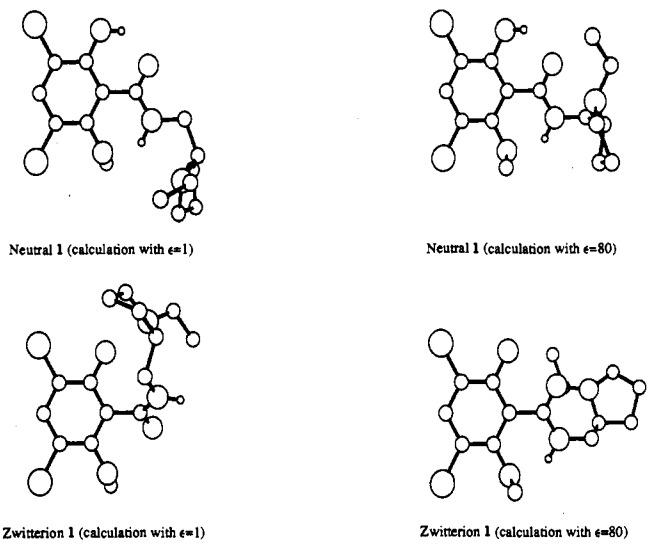


Figure 4. Representation of global minimum for each conformational run on raclopride (1).

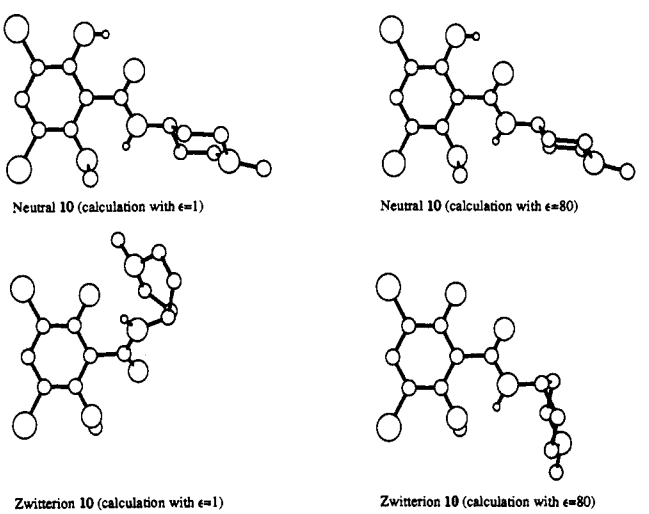


Figure 5. Representation of global minimum for each conformational run on compound 10.

previous results (stabilization of an internally ionic bonded or "*internal salt*" (*I*) conformation) were confirmed. Other internal salt conformers were also localized by the high-temperature dynamics method, in particular conformers with a *cis*-amide bond not taken into account in the previous analysis. In a high dipolar solvent, no low-energy internal salt conformations were found: the most stable conformers were folded or half-folded conformations with a "normal" (NH···OCH_3 hydrogen bond) or a "reversed" (NH···O hydrogen bond) orientation of the aromatic ring. It should be noted that some folded conformations are stabilized by a hydrogen bond between the NH^+ function and the carbonyl group of the amide function. Extended conformations were also localized: the energy difference between the first extended conformations and the corresponding folded conformations was less than 1.2 kcal/mol.

This energy difference between extended and folded side-chain conformations is very low and highly dependent on the method of calculation. In particular, simulation of water by a continuum of high dielectric constant ($\epsilon = 80$) neglects the specific interactions between water molecules and solute. Calculations with a super molecule (one molecule of raclopride in zwitterionic form surrounded by 126 molecules of water) were done with the solvent option of the SYBYL software. During the minimization process, the starting geometry (folded or extended) used for the construction of the super molecule was kept and the super molecule with extended raclopride was more stable (10 kcal/mol) than the one with folded raclopride.

Acid–Base Behavior of Raclopride Analogue 10. The above results confirm that the acid–base properties of raclopride congeners 1–9 are influenced mainly by aromatic substituents, and less by the conformational state of the side chain. To validate this conclusion, we examined the raclopride analogue 10 where the *N*-[(1-ethylpyrro-

lidin-2-yl)methyl] moiety is replaced by the more rigid *N*-(1-methylpiperidin-4-yl) side chain. Dynamics calculations of the neutral and zwitterionic form of analogue **10** confirmed its different conformational behavior *in water* relative to raclopride. The number of conformers localized by conformational analysis is much lower for the analogue **10** than for the raclopride (**1**) confirming the reduced flexibility of the latter compound (see supplementary material).

For the *neutral form* of **10**, the low-energy minima were generated by high-temperature molecular dynamics, the aryl group being either equatorial or axial to the piperidinyl ring. As for raclopride, two types of conformations of the aryl moiety were seen, one "normal" with two hydrogen bonds, and one "reversed" with only one hydrogen bond. On the whole, the half-folded and fully extended forms were the preferred ones, and showed a distance between the centroid of the phenyl ring and the piperidinyl nitrogen of ca. 7 Å and 8 Å, respectively. For the *zwitterionic form* in the gas phase, internal salt conformations with the piperidine ring in a twisted-boat or boat conformation were stabilized by electrostatic interactions between the phenolate and the protonated amine. However, in a highly dipolar solvent simulated by a continuum of dielectric constant ($\epsilon = 80$) the "internal salt" conformation was not stable. Only half-folded and extended conformations of the side chain were stable. It should be noted that, based on the distance between the protonated amine function and the center of the aromatic ring, the half-folded conformation of the piperidine side chain is topologically equivalent to the extended conformation of the pyrrolidine side chain. The global minimum of each conformational analysis is given in Figure 5.

Even with our crude simulation of water, in all stable conformations of compound **10** the intercharge distance is greater than for raclopride. Hence, if it exists, the effect of side-chain conformation on the pK_a's should be different in these two molecules. The results (see Table I) show that not only the acidity of compound **10**, but also its basicity, are nearly identical to those of raclopride. These similarities confirm that the variation in acidity and basicity in 6-methoxysalicylamides is governed mainly by their aromatic substituents.

Partitioning Behavior of Raclopride and Its *N*-(1-Methylpiperidin-4-yl) Analogue 10. As deduced from the pK_a measurements and the results of molecular dynamics experiments, the zwitterionic 6-methoxysalicylamides exist as extended or half-folded conformers in highly polar media such as water. These molecules may also exist in "internal salt" conformations in vacuo as indicated by our molecular mechanics and quantum mechanics calculations. Similarly, the existence of zwitterionic forms with folded conformations cannot be excluded in media of low polarity such as 1-octanol or *n*-heptane. To examine this possibility, we measured the distribution coefficients of raclopride (**1**) and compound **10** in 1-octanol/buffer ($\log D_{oct}$) and *n*-heptane/buffer ($\log D_{hep}$) systems at different pHs (Figures 6 and 7).

The observed bell-shaped relationships between $\log D$ and pH for both compounds again reveal their zwitterionic nature in the systems considered.[25] As we have demonstrated that the ionization behavior of raclopride (**1**) and analogue **10** is not influenced by the two distal opposite charges, the population of each electrical form (i.e. neutral, zwitterionic, cationic, and anionic) at a given pH can be calculated from eqs 3–5.[26]

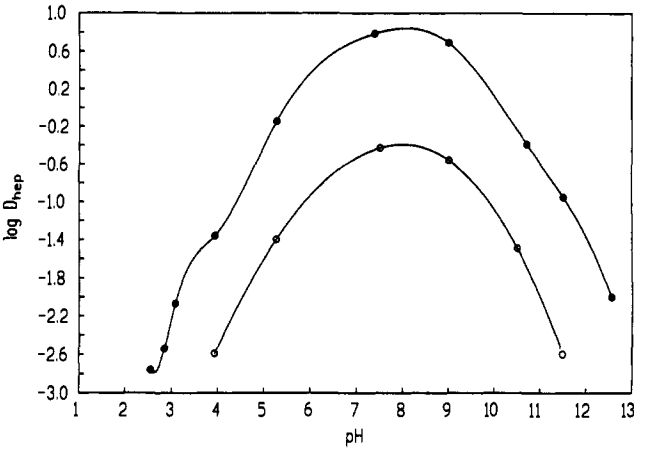


Figure 6. Distribution coefficients of raclopride (**1**) (●) and compound **10** (○) in 1-octanol/buffer at different pH ($\log D_{oct}$). The average standard deviation is smaller than 0.02 log units.

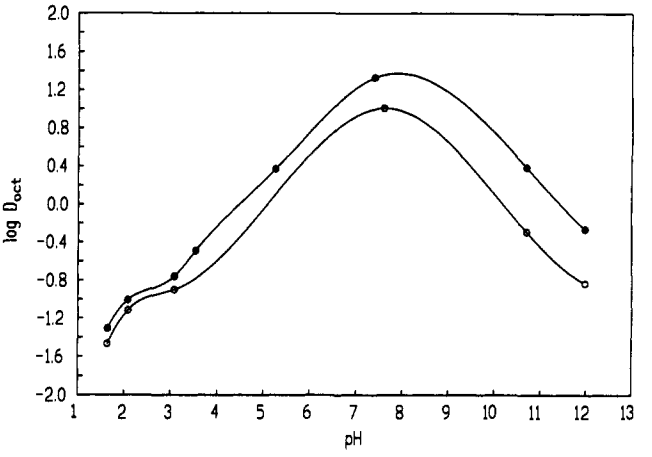


Figure 7. Distribution coefficients of raclopride (**1**) (●) and compound **10** (○) in *n*-heptane at different pH ($\log D_{hep}$). The average standard deviation is smaller than 0.02 log units.

$$K_{a1} = K_{a11} + K_{a22} \quad (3)$$

$$1/K_{a2} = 1/K_{a21} + 1/K_{a12} \quad (4)$$

$$K_z = K_{a11} \cdot 1/K_{a22} \quad (5)$$

Population calculations of the different electric forms based on $pK_{a11} = pK_{a12} = 5.81$ and $pK_{a21} = pK_{a22} = 9.21$ indicate that the zwitterionic form of raclopride (**1**) is the major form in water at pH 6–9, while the cationic and anionic forms are the major forms at pH <6 and >9, respectively. Noteworthy is the minute population of neutral forms, its maximal value being 0.04% at pH 7.4. Thus, at pH around the isoelectric point, the zwitterionic form must be the major one in the aqueous phase of a biphasic system, the contribution of the neutral form being nonsignificant (see Table II). But how much does the zwitterionic form contribute to partitioning in organic solvents?

Interestingly, the lipophilicity of raclopride is much higher than expected for a zwitterionic molecule, its $\log D_{oct}$ value being 1.33 and $\log D_{hep}$ 0.79 at pH 7.40 (Table II). Since the lipophilicity difference ($\log P - \log P^-$) between neutral and deprotonated phenols is usually smaller than 2, and ($\log P - \log P^+$) values between neutral and protonated tertiary amines are below 3.5,[27,28] it is unlikely that the $\log D$ value of the zwitterionic raclopride can be as large as 1.33 for a molecule without interaction between the two distal charges. Since the lipophilicity of

Table II. Population Percentage in Water and Lipophilicity of Raclopride (1) and Compound 10 at pH Near Their Isoelectric Point (Z: zwitterionic form; N: neutral form; C: cationic form; A: anionic form)

compound	Z	N	C	A	log D_{oct}	log D_{hep}	Δlog $D_{oct-hep}$[c]
raclopride (1)[a]	95.99	0.04	2.48	1.49	1.33	0.79	0.54
10[b]	94.88	0.06	2.17	2.89	1.01	−0.43	1.44

[a] The percentage and lipophilicity are calculated and measured, respectively, at pH 7.4. [b] The percentage and lipophilicity are calculated and measured, respectively, at pH 7.6. [c] $\Delta\log D_{oct-hep} = \log D_{oct} - \log D_{hep}$.

zwitterionic species is highly dependent on the intercharge distance (separating the opposite charges dramatically decreases lipophilicity[29]), we must assume that, if the zwitterionic form is also predominant in the organic phase, it adopts an "internal salt" conformation. The same conclusion is also true for compound 10 for which the distribution coefficients are similar to these of raclopride (log D_{oct} = 1.01 and log D_{hep} = −0.43).

At this point, it becomes indispensable to identify the species (neutral or zwitterionic) present in the organic phase. This was performed by comparing and relating the different UV spectra of neutral and anionic raclopride in the octanol phase of a octanol/buffer system, using first derivative UV/vis spectrophotometry which was shown to be far more sensitive than absorbance spectra in detecting the composition of different species.[30]

It must be noted that the phenolic group is completely ionized in highly basic solutions (0.1 M NaOH, pH 13) and hence the anionic species would be the only one partitioning into the octanol phase in the form of an ion-pair. On the other hand, the phenolic group remains neutral when the amino group is protonated in highly acidic solution (0.1 M HCl, pH 1), with the cationic or neutral species partitioning into the octanol phase. The composition of the neutral and zwitterionic form in the octanol phase of an octanol/buffer system can thus be identified from the distinct UV spectra due to the protonation/deprotonation of the phenolic group. The results are shown in Figure 8. The spectrum in the octanol phase of an octanol/buffer (pH 7.4) system corresponds to a predominantly neutral or cationic form (ca. 94%). As to the species in the heptane phase, the spectrum in the heptane phase of a heptane/buffer (pH 7.51) system shows no significant difference from that in "dry" heptane (Figure 9), the neutral form being implied in the heptane phase. The results in heptane are compatible with published[1] semiempirical AM1 and PM3 calculations which give a stabilization of the neutral form by 27.0 and 29.3 kcal/mol, respectively, with respect to the zwitterionic form.[31]

These results suggest either that the dissociation constants change dramatically in the organic phase, or that folding of the zwitterionic form is energetically unfavorable in the organic phases investigated. The first derivative UV spectra (not shown) of compound 10 is almost identical to Figure 8 and indicates that the neutral form also predominates (ca. 100%) in the octanol phase of an octanol/buffer (pH 7.4) system. Based on the knowledge of microscopic dissociation constants and distribution coefficients, the partition coefficient of the neutral raclopride (1) and compound 10 can thus be calculated as log P_{oct} being 4.7 and 4.2, respectively, and as log P_{hep} being 4.2 and 2.8, respectively.

We have previously investigated the structural information content of the parameter $\Delta\log P_{oct-hep}$, namely log P_{oct} minus long P_{hep}, which turns out to be mainly a measure

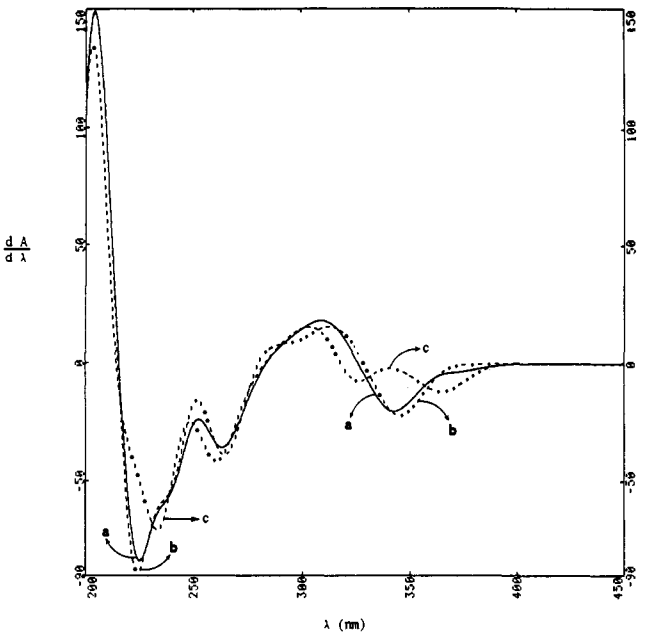


Figure 8. First derivative UV/vis spectra of raclopride (1) in the (a) octanol phase (126.7 μM) of an octanol/buffer (pH 7.4) system; (b) octanol phase (126.3 μM) of an octanol/buffer (pH 1) system; (c) octanol phase (125.6 μM) of an octanol/buffer (pH 13) system.

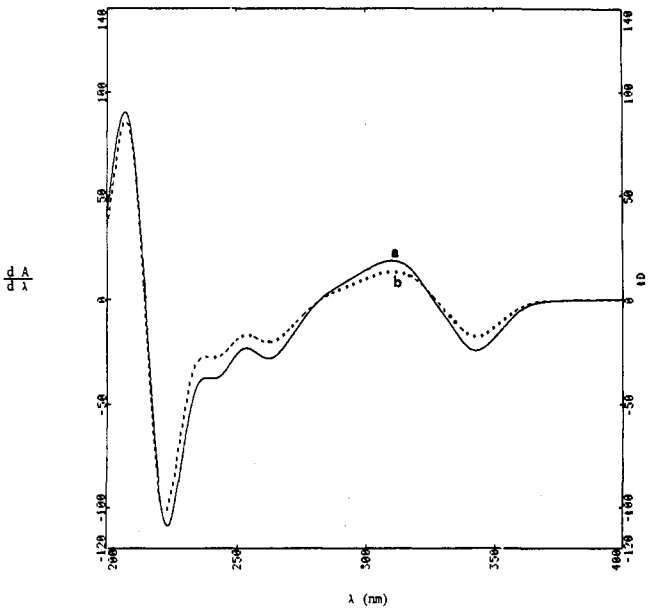


Figure 9. First derivative UV/vis spectra of raclopride (1) in (a) the heptane phase (154 μM) of a heptane/buffer (pH 7.51) system; (b) dry heptane (140 μM).

of solute hydrogen bond donating capacity.[32] Thus, the magnitude of the $\Delta\log P_{oct-hep}$ values can reveal the accessibility of the hydrogen bond donor groups (OH and CONH) in the 6-methoxysalicylamides. The $\Delta\log P_{oct-hep}$ value of raclopride (1) (0.5) and compound 10 (1.4) indicate that the phenolic and amide protons of both compounds must be poorly available for hydrogen bonding with solvents by comparison with the $\Delta\log P_{oct-hep}$ of phenol (2.28)[32] and *N*-methylbenzamide (2.55).[33] The $\Delta\log P_{oct-hep}$ value of raclopride (1) (0.5) is of similar magnitude to its $\Delta\log D_{oct-hep}$ at pH 7.4 (0.5), indicating that $\Delta\log D_{oct-hep}$ can be used directly to obtain $\Delta\log P_{oct-hep}$ in this case. A further examination on the plot of $\Delta\log D_{oct-hep}$ (i.e. log D_{oct} minus log D_{hep}) vs pH (Figure 10) as derived from Figures 6 and 7 reveals that the $\Delta\log D_{oct-hep}$ values remain constant in the pH range 5–10. Beyond this range, the

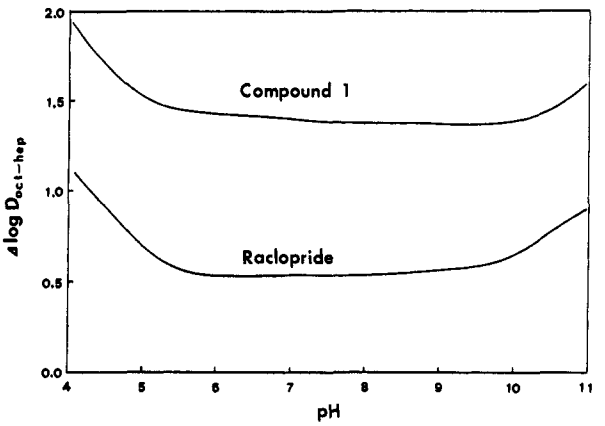


Figure 10. Plot of $\Delta\log D_{oct-hep}$ vs pH as derived from Figures 8 and 9.

$\Delta\log D_{oct-hep}$ values increase probably due to the contribution of the cationic or anionic form to $\log D_{oct}$, and not to $\log D_{hep}$.

Another analogue, eticlopride (compound 4), was also found to possess a small $\Delta\log D_{oct-hep}$ value (<0.5, yielded by $\log D_{oct} = 3.0$ (at pH 7.4) and $\log D_{hep} > 2.5$ (at pH 7.4)). This nonaccessibility of hydrogen bond donor in compounds with different ionization behavior suggests stable internal hydrogen bonds, at least in organic solvent, for all these compounds and thus a partition of the neutral form in organic solvent. The fact that the $\Delta\log P_{oct-hep}$ of raclopride is about 1 unit smaller than that of compound 10 is interesting and may be due to either or both of the following factors: (a) a difference in the inductive effect of the non-protonated amino group on the H-bond donor capacity of the amide group and (b) enhanced accessibility of the amine lone pair in the piperidine ring with respect to the pyrrolidine ring.

In summary, partition coefficient measurements and UV spectra strongly suggest that, in solvents of low polarity, 6-methoxysalicylamides are present as neutral molecules.

Conclusion

In this study, we show unambiguously that the bulkiness of the substituents para to the phenolic group of 6-methoxysalicylamides can influence the conformation of the adjacent meta and ortho substituents, and as a consequence leads to a change in the acidity of the phenolic group. This adds another evidence that substituent steric factors in addition to electronic factors can play a critical role in influencing the acidity of these phenols.

The zwitterionic nature of 6-methoxysalicylamides is of great interest in terms of stereoelectronic differences with the neutral form. The zwitterionic form adopts an extended conformation in aqueous solution due to the favorable hydration energies of the ionization sites, which is also implied by its high solubility in aqueous solution at pH 7.4.[34] However, as demonstrated by UV measurements, this form can barely partition into the octanol and heptane phases. Hence, the value of the $\log D_{oct}$ (1.33) and $\log D_{hep}$ (0.79) at physiological pH are contributed by the neutral and not the zwitterionic form. Since hydrophobic interactions play an important role in the receptor binding of dopamine antagonists,[5,35] the microenvironment of the binding site must be of low polarity; the results of this study therefore suggest that the phenolic group of raclopride and other 6-methoxysalicylamides must be neutral upon binding to the receptor.

The detailed physicochemical behavior of raclopride (1) and its analogue 10 cannot support the QSAR differences in orthopramides vs 6-methoxysalicylamides with the same *N*-[(1-ethylpyrrolidin-2-yl)methyl] side chain. We have reexamined these QSAR analyses and found that the described difference between this two series of compounds is fortuitous, principally due to some parameters used (^{13}C-NMR chemical shifts) and to a lack of balance between the number of compounds belonging to the two classes. Recent results by Norinder and Högberg[5b] demonstrate that QSAR for these two series of compounds are equivalent and suggest a common receptor binding mode for phenolic and non-phenolic benzamides with *N*-[(1-ethylpyrrolidin-2-yl)methyl] side chains.

It was shown recently that the hydrogen bond donating capacity of solutes is detrimental to their crossing of the skin[36] and blood–brain barrier.[37] Since raclopride and eticlopride were shown to readily cross the blood–brain barrier,[16] the existence of strong *internal* hydrogen bonds OH···O=C and CH_3O···HNC=O in biological lipophilic media is equally implied and is in line with their small $\Delta\log P$ values.

In summary, the triangular relationship between ionization, solvation, and conformation confers dynamic rather than static stereoelectronic features to antidopaminergic 6-methoxysalicylamides. A simplified scheme is presented in Figure 11, in which the interconversion, predominance, and conformational behavior of the neutral and zwitterionic forms of raclopride are shown. Particularly noteworthy in a pharmalogical context is the fact that the predominant forms of the drug are vastly different in aqueous and organic phases. It is suggested that the tricyclic pseudo-ring as depicted in the organic phase is a common pharmacophoric element for the antidopaminergic action of all 6-methoxysalicylamides.

Experimental Section

Chemicals. All compounds were provided by Astra Arcus AB (Södertalje, Sweden), and their synthesis can be found elsewhere.[4,6] The other reagents are from commercial sources and of purity greater than 99%.

Determination of Ionization Constants by Potentiometry. Solutions (3 mM) were prepared in distilled H_2O which had been boiled to remove O_2 and CO_2 and saturated with N_2. The ionic strength was fixed at 0.1 M using KCl. An excess of HCl was added, and the solution was back-titrated with 0.01 N NaOH using a Metrohm (Buchs, Switzerland) Model 670 titroprocessor. The temperature was 25 ± 1 °C. Titration curves were determined in triplicate and the pK_a values were calculated using a non-logarithmic linearization of the titration curve proposed by Benet and Goyan[38] and modified by Leeson and Brown[39] to overcome the problem of dilution during titration.

Determination of Ionization Constants by UV Spectrophotometry. Solutions (0.24 mM) were prepared in phosphate buffers of 10 different pH values. Spectra over the range 200–400 nm were recorded using a Philips UV spectrophotometer, and the pK_a values calculated from spectral changes using the Henderson–Haselbach equation.[40]

Measurements of Distribution Coefficient. Distribution coefficients in 1-octanol/buffer and *n*-heptane/buffer systems were measured by horizontal flow-through centrifugal partition chromatography (CPC) using a coil planet type centrifuge. The design of the instrument has been described.[41,42] The apparatus from Pharma-Tech Research Corp. (Baltimore, MD) used three columns, each of which was helically wound with five layers of PTFE tubing (3.00 mm i.d., 3.94 mm o.d.). The three columns had a total capacity of 350 mL. A Kontron Model 432 UV/vis detector coupled with a Hewlett-Packward 3392A integrator was used to detect the solutes. A Phase Separations flowmeter allowed to precisely measure flow rates.

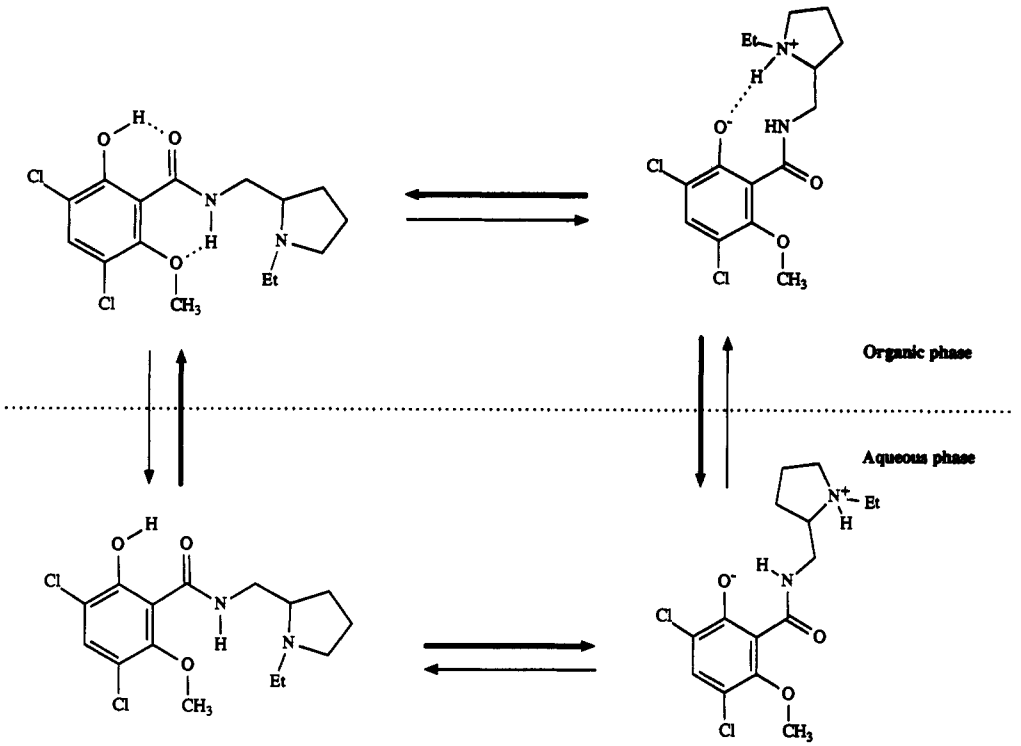


Figure 11. The interconversion, predominance, and conformational behavior of neutral and zwitterionic raclopride in a biphasic system.

For log $D < 0$, measurements began by filling up the columns with 1-octanol presaturated with 0.1 M phosphate buffer depending upon the pH used. While the columns were then revolving at a speed of 1000 rpm along the central axis, they were also rotating along their own axis in a mode of planetary motion. The mobile phase (aqueous phase) was then pumped into the columns in a "head-to-tail" mode during the rotation. As for log $D > 0$, the columns were first filled with aqueous solutions; this was then followed by pumping the mobile phase (organic phase) into the columns in a "tail-to-head" mode during the rotation. Depending upon expected distribution coefficients, the flow rate of the mobile phase was adjusted from 0.5 to 6.0 mL/min (flow rates of 0.5 mL/min for log $D < -2.3$ or > 2.3; 1 mL/min for log D between -2.3 and -1.3 or between 1.3 and 2.3; and 6 mL/min for log D between -1.3 and 0 or between 0 and 1.3). A Merck Lobar injector with a 6-way valve was used to inject samples of 200 μL (in mobile phase solution, 1–50 mM). The solute was detected at the wavelength 254 nm and all measurements performed at 25 ± 0.1 °C and in triplicate. Concentration effects were negligible, the difference in calculated log D values being smaller than 0.05 units.

Under flow rates of 0.5, 1, and 6 mL/min, ca. 310, 305, and 270 mL of the stationary phase were retained, respectively. The retention time of the solvent front (t_0) was measured by nonretained solutes (potassium dichromate, when using aqueous solution as mobile phase; biphenyl, when using octanol as mobile phase). It follows that the distribution coefficients can be calculated by eq 6:

$$\log D = \log \frac{(t_R - t_0)\cdot U}{V_t - U\cdot t_0} \text{ when using aqueous solution as mobile phase;}$$

$$\log D = \log \frac{V_t - U\cdot t_0}{(t_R - t_0)\cdot U} \text{ when using octanol as mobile phase,} \quad (6)$$

where t_R is the retention time of the solute, U is the flow rate of the mobile phase, and V_t is the total capacity of the three columns.[43]

Conformational Calculations and Statistics. The low-energy conformers explored by the high-temperature molecular dynamics method were shown to be able to reproduce those obtained by NMR spectroscopy.[18,19] The analyses are performed using the SYBYL software (versions 5.41 and 5.5) running on a Silicon Graphics Personal Iris 4D/25 workstation or a Sun Sparc 2 workstation. In this method, the molecule is first subjected to a thermalization at 2000 K during 20 ps in order to survey the conformational space. The geometry of 200 randomly selected conformers was subsequently optimized using Tripos force field including electrostatic term. The detailed procedure can be found elsewhere.[21]

In cross-validation of PLS analyses,[44] the analysis using the QSAR module of the SYBYL software is repeated by omitting one compound at a time. A cross-validated r^2_{CV} is defined as

$$r^2_{CV} = (SD - PRESS)/SD \quad (7)$$

where SD is the sum of squares of deviations of the observed values from this mean and PRESS is the prediction error sum of the squares.

The variables used in eq 1 taken from the literature[12,13] were the following: Cl ($\sigma_o = 0.68$; $\sigma_p = 0.24$, $E^c = 0.65$); Br ($\sigma_o = 0.70$; $\sigma_p = 0.22$, $E^c = 0.84$); NO_2 ($\sigma_o = 1.40$; $E^c = 2.20$); Et ($\sigma_o = -0.09$; $\sigma_p = -0.15$, $E^C = 0.38$); H ($\sigma_o = 0$; $\sigma_p = 0$, $E^c = -0.32$).

The PM3 calculations were performed with the MOPAC 5.0 software running on a Silicon Graphics Personal Iris 4D/25 workstation or a Sun Sparc 2 workstation. All the geometry optimizations were performed with the PRECISE keyword allowing similar results on both machines.[45]

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Supplementary Material Available: The results of high-temperature molecular dynamics are summarized on one scheme, two figures, and nine tables (12 pages). The corresponding geometries are available upon request to authors as SYBYL databases or in other file format. Ordering information is given on any current masthead page.

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