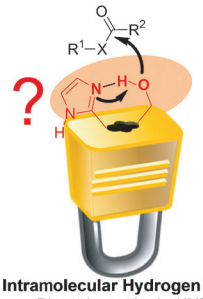
A model family: A new family of 2-hydroxyimidazoles with various linkers has been evaluated as serine–histidine bare dyad models. The presence and strength of intramolecular hydrogen bonding has been evidenced and evaluated by spectroscopic and computational methods (see figure).



Hydroxyimidazoles

J. Leclaire, M. Mazari, Y. Zhang, C. Bonduelle, O. Thillaye du Boullay, B. Martin-Vaca, D. Bourissou,* I. De Riggi, R. Fortrie, F. Fotiadu,* G. Buono* ..... ■■■■–■■■■

Bare Histidine–Serine Models: Implication and Impact of Hydrogen Bonding on Nucleophilicity

Marseille–Provence...
...is the European Capital of Culture in 2013. In this issue two articles from the Marseille-based team Chirosciences analyze the role of imidazole alcohol hydrogen bonding in the nucleophlicity of dyad models (see J. Leclaire, D. Bourissou, F. Fotiadu et al. on page ■■ ff.) and describe a facile synthesis and assemblies of tetraalkylporphyrins in two and three dimensions (see Y. Kikkawa, T. S. Balaban et al. on page ■■ ff.).

© 2013 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

CHEMISTRY
A EUROPEAN JOURNAL

ChemPubSoc
Europe

DOI: 10.1002/chem.201301275

Bare Histidine–Serine Models: Implication and Impact of Hydrogen Bonding on Nucleophilicity

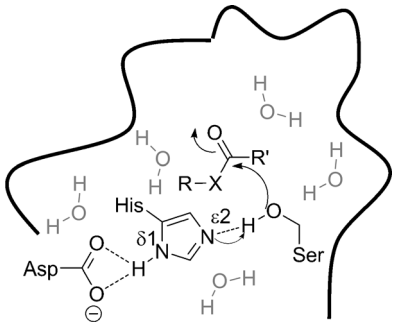
Julien Leclaire,*[a] Messaoud Mazari,[a] Yuan Zhang,[a] Colin Bonduelle,[b] Olivier Thillaye du Boullay,[b] Blanca Martin-Vaca,[b] Didier Bourissou,*[b] Innocenzo De Riggi,[a] Rémy Fortrie,[a] Frédéric Fotiadu,*[a] and Gérard Buono[a]

Abstract: A new family of 2-hydroxyalk(en/yn)ylimidazoles has been evaluated as serine–histidine bare dyad models for the ring-opening reaction of L-lacOCA, a cyclic *O*-carboxyanhydride. These models were selected to unravel the implication of intramolecular hydrogen bonding and to substantiate its influence on the nucleophilicity of the alcohol moiety, as it is suspected to occur in enzyme active sites. Although designed to exclusively facilitate the preliminary step of proton transfer during the studied ring-opening reaction, these minimalistic models depicted a measureable increase in reactivity relative to the isolated fragments. A couple of reliable experimental and theoretical methods have been developed to readily monitor the strength of the intramolecular hydrogen bond in dilute solution. Results show that the folded conformers are the most nucleophilic species because of the intramolecular hydrogen bond.

Keywords: amino acids · density functional calculations · hydrogen bonding · NMR spectroscopy · nucleophilicity

Introduction

The family of serine hydrolases, which is involved in numerous biological processes and widely used in industry,[1] has been extensively studied over several decades, thus providing a large amount of structural data. Its active site is characterized by an aspartic acid–histidine–serine (Asp–His–Ser) triad that undergoes a two-step *O*-acylation, *O*-deacylation process (Scheme 1). As aliphatic alcohols are much less nucleophilic than imidazoles, a basic question concerning serine hydrolases is how this catalytic triad imparts high nucleophilic reactivity to the serine β-OH group in the first reactive step (the formation of a covalent tetrahedral adduct between this residue and the substrate). It is commonly believed to be achieved by Asp–His and His–Ser intramolecular hydrogen bonding (IMHB) because it cannot exclusively be attributed to medium effects in the active-site cleft.



Scheme 1. Proposed mode of action of the Asp–His–Ser catalytic triad of serine hydrolases/lipases in the presence of a competing hydrogen-bond donor such as water.

The Asp–His IMHB is in fact supposed to strongly preorganize the imidazole ring in such a position that it preferably abstracts the SerO–H proton, with which it is engaged in an IMHB, rather than reacting on the substrate.[2] Although numerous experimental observations showed that this strong hydrogen bond[3] conformationally locks the imidazole ring as soon as the resting state of the enzyme is empty, direct evidence of the Ser–His interaction, which is a prerequisite to the catalytic cycle, is still lacking to date. No X-ray picture of a functional and empty active site (i.e., at a pH at which His is deprotonated and in the absence of interfering salt) clearly depicts a Ser–His hydrogen bond.[4] The only compelling, though indirect evidence of a His–Ser hydrogen bond comes from liquid-state NMR spectroscopic studies.[5]

Whereas the catalytic power of this class of hydrolytic enzymes results from a subtle combination of various sophisti-

[a] Dr. J. Leclaire, Dr. M. Mazari, Y. Zhang, Dr. I. De Riggi, Dr. R. Fortrie, Prof. F. Fotiadu, Prof. G. Buono
Centrale Marseille, Aix Marseille Université, CNRS
iSm2 UMR 7313, 13397, Marseille (France)
E-mail: julien.leclaire@centrale-marseille.fr
frederic.fotiadu@centrale-marseille.fr

[b] Dr. C. Bonduelle, Dr. O. Thillaye du Boullay, Prof. B. Martin-Vaca, Dr. D. Bourissou
University of Toulouse, UPS, LHFA
118 Route de Narbonne
31062 Toulouse CEDEX 09 (France)
and CNRS, LHFA UMR 5069
31062 Toulouse (France)
E-mail: dbouriss@chimie.ups-tlse.fr

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201301275.

 © 2013 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

These are not the final page numbers!

cated and efficient strategies,[6] a key question remains to be answered: Although so far unobserved, how might this Ser–His IMHB exist, be favored by some preorganization, and lead by itself (i.e., in the absence of any other effect) to a measureable increase in the nucleophilicity of the alcohol moiety? Answering this question formally requires one to extract the dyad from the highly complex protein shell and study it as a single isolated molecular species.

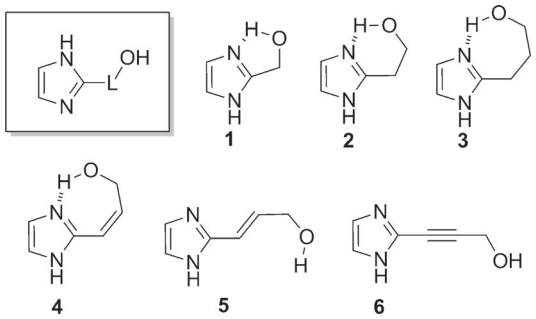
So far, only a few studies in physical organic chemistry have focused on the nucleophilicity of Ser–His bioinspired dyad[7] models, whereas various His–Asp counterparts have been synthesized and analyzed.[8] Pioneering work conducted in the 1980s revealed that the kinetics of acylation/deacylation on 2- and 4-hydroxymethylimidazoles can be modulated by the susbtituents of the heteroaromatic ring.[9] Whereas no IMHB was experimentally observed and identified as the source of the activation of such structures, it was principally concluded that the imidazole ring acted as a general base.

To address the questions of 1) the impact of preorganization on the formation an IMHB within the Ser–His dyad prior to substrate attack and 2) its potential implication on the nucleophilicity, we chose to prepare and study a series of six α,ω-2-imidazole alcohols **1–6** as new synthetic minimalistic models. The results are reported herein. Spacers of different lengths and degrees of flexibility have been incorporated between the chemically active groups. The nucleophilicity of these model dyads has been evaluated in a model acylation reaction, namely, the ring opening of an *O*-carboxyanhydride. The pK_a values of compounds **1–6** have been determined by using a pH meter and their propensity to adopt folded intramolecularly hydrogen-bonded structures in solution has been assessed by variable-temperature ^{1}H NMR spectroscopy. In addition, DFT calculations have been performed to gain more insight into the conformational landscape and to estimate the strength of the intramolecular hydrogen bond.

Results and Discussion

Synthesis of dyad models 1–6: So far, Ser–His dyad models synthesized to probe cooperative effects between the functional moieties have mostly been limited to hydroxymethylimidazoles.[9] We chose to explore the impact of the number of rotatable bonds between the primary alcohol and the N-heterocyclic base on the nucleophilicity of the resulting structures. The underpinning questions we intended to address were the influence of the degree of conformational preorganization between both groups on the strength of the potential hydrogen bond between them and the hypothetical correlation between this noncovalent bond and a kinetic effect. It is important to note here that, from the point of view of physical organic chemistry, intermolecular hydrogen bonding between an alcohol and an imidazole is unlikely to occur in the presence of many amides (all around the protein active site) and eventually also in the presence of water molecules. Indeed, amides and water are slightly better hydrogen-bond donors than alcohols, and should therefore preferentially associate with an imidazole ring as an acceptor. By using the thermodynamic semiquantitative α,β ranking recently developed by Hunter,[10a] the equilibrium constant for an intermolecular alcohol–imidazole association should reach at the maximum the modest value of $K_{inter} = 0.1$ M.[10b] From this simple analysis, one can conclude that if the Ser–His IMHB does exist, its intrinsic stability is poor and the association should clearly be favored by intramolecular effects that are usually gathered into the effective molarity. To confirm this hypothesis, α,ω-2-hydroxyimidazoles of various chain lengths and flexibilities have been synthesized and studied.

The ease of intramolecular connection of an α,ω-bifunctional structure is known to be strongly influenced by the number of single bonds in the chain, which determines the strain of the ring being formed but also the torsional entropy lost upon connection.[11] The series explored here was hence limited to potential five- to seven-membered rings (as the minimal ring strain corresponds to five or six centers) with various conformational flexibilities. Compounds **1–6** were prepared in a few steps and good overall yield (≈40%) (Scheme 2; see the Supporting Information for details). The 2-hydroxymethyl derivative **1** was obtained by direct reduction of the 2-formyl precursor[12a] according to reported procedures.[12b–e] Selective lithiation of the C2 position of N2-sulfamoyl imidazoles, followed by ethylene oxide ring opening,[13] yielded after deprotection the hydroxyethyl derivative **2**,[14a,b] as previously described. This strategy was extended to N-protected 2-methylimidazole, thereby providing a new way to access compound **3**.[14c] New dyads **4–6** were obtained through a Wittig reaction between 2-formylimidazole and ethyl(triphenylphosphoranylidene)acetate ylide (for diastereomers **4** and **5**) and through Sonogashira coupling between 2-iodo-1-[2-(trimethylsilyl)ethoxymethyl] (SEM) imidazole and propargylacetate (for compound **6**). The Wittig coupling procedure developed by McNab et al.[15] was improved by using tetrahydrofuran instead of pyridine or benzene as the solvent. This led to a shorter reaction time (48 h instead of 1 week for complete conversion), higher yields, and an equimolar mixture of *Z*/*E* compounds.



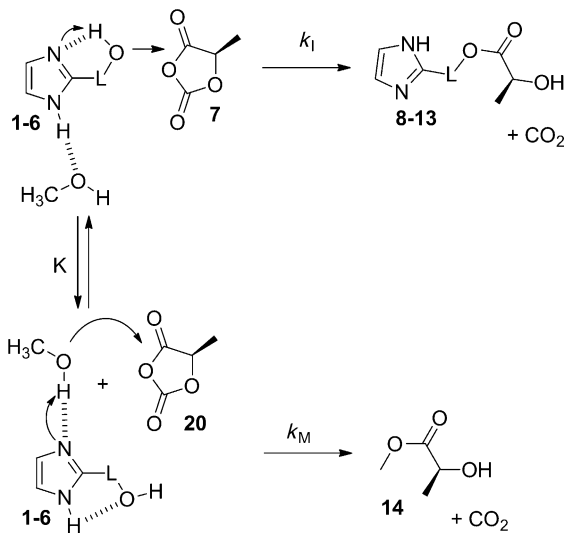
Scheme 2. 2-Hydroxyalk(en/yn)yl imidazoles **1–6** synthesized and studied as Ser–His dyad models.

 © 2013 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Hydride reduction of the N-protected diastereoisomers[16a] was preferred over hydrogenation[16b] as it was 100% chemoselective toward the ester moiety. Although dimethsulfamoyl was a suitable protecting group for the synthesis of compounds **1**–**5**, the Sonogashira coupling proceeded with higher conversion with SEM, which allowed further orthogonal O,N-deprotection reactions.

Reactivity of 1–6 in a model acylation reaction: To assess and compare the nucleophilicity of the hydroxylimidazoles **1**–**6**, we studied the ring opening of the *O*-carboxyanhydride derived from L-lactic acid (L-lacOCA) **7**.[17a] This highly reactive compound was shown to be an activated equivalent of lactide towards ring-opening polymerization (ROP). By using 4-dimethylaminopyridine (DMAP) as catalyst and an alcohol as initiator, polylactic acid (PLA) of controlled molar mass and narrow polydispersity was prepared under mild conditions.[17a,b] The mechanism of the elementary ring-opening step has been thoroughly studied computationally, and the pathway that involves basic activation of the alcohol was found to be favored over the nucleophilic activation of the *O*-carboxyanhydride.[17c] Note that lipase-mimicking compounds have also recently started to be explored as catalysts for the ROP of lactide.[17d]

Compounds **1**–**6** combine two such moieties, a primary alcohol and a basic imidazole. They were first evaluated individually in the ring opening of **7** in the presence of methanol as a competitor (Scheme 3). A control experiment showed



Scheme 3. Putative reactive complexes in rapid interconversion leading to L-lactate adducts **8**–**13**.

that no reaction occurs between methanol and L-lacOCA **7** in the absence of a base. To avoid any polymerization and limit the reaction to a single coupling, a strong excess amount of methanol (90 equiv, which in fact represents a 40:60 MeOH/CH_3CN mixture) was used relative to **7** (c_7 = 0.1 M). Comparatively, a slight excess amount of the dyad model **1**–**6** (1.5 equiv) was introduced. As methanol already plays the role of a competing hydrogen-bond donor, an aprotic polar solvent such as acetonitrile appeared suitable to potentially observe any nucleophilic behavior of the dyad. Acetonitrile, like water and amides, although a weaker hydrogen-bond acceptor than imidazole, should not allow the formation of an intermolecular imidazole–alcohol hydrogen bond ($\Delta\Delta rG° = +2$ kJ mol^{-1}).[10b] After 5 min at room temperature, ^{1}H NMR spectroscopy provided the proportion of products, which remained subsequently constant, thus indicating that the conversion of this kinetically controlled reaction was complete. Products that resulted from the attack of alcohol moieties on the anhydride of L-lacOCA were exclusively obtained. Integration of the CH_2O signals provided the proportion of products **8**–**13** relative to methyl-L-lactate **14** (Table 1).

Table 1. Distribution of products **8**–**13** relative to methyl-L-lactate **14** resulting from the nucleophilic attack on L-lacOCA **7** (c_7 = 0.1 M) in acetonitrile; kinetic effective molarity (EM); and pK_a of the dyad mimics **1**–**5** measured at 0.1–0.001 M concentrations in 1 M aqueous KCl at 300 K.

	1	**2**	**3**	**4**	**5**	**6**
LacOIm [%]	15	40	10	50 (93)[a]	0	0
8–13						
EM = k_I/k_M	3	8	2	10.1	–	–
pK_a	7.33	7.00	7.07	4.36	5.71	4.80

[a] c_7 = 0.001 M.

These preliminary experiments conducted independently on compounds **1**–**6** provided a first classification of their relative nucleophilicity with respect to methanol (Table 1). Despite the strong excess amount of methanol used, dyad adducts **8**–**13** were obtained in significant amounts. Decreasing the proportion of methanol to 10 equiv yielded these compounds as major products that could subsequently be purified by column chromatography and fully characterized (see the Supporting Information). Within the saturated series **1**–**3**, hydroxyethylimidazole **2** appeared as the most active compound, potentially involving a six-membered ring IMHB during the nucleophilic activation process. In the C_3 series **3**–**5**, a marked difference in reactivity was observed between *Z* and *E* unsaturated isomers, the former being the most reactive (five times more than the saturated analogue **3**), whereas the latter, similarly to the alkynyl model **6**, did not lead to any detectable amount of corresponding lactate adduct (Table 1). Although **5** and **6** do not benefit from any intramolecular activation, because of conformational restrictions, their total absence of reactivity in the presence of methanol provides an insight into the detrimental electronic impact of the bridging unsaturation on the nucleophilicity of the terminal alcohol moiety. The propenenyl and propargyl alcohol residues are indeed expected to be intrinsically less nucleophilic than methanol.[18]

To confirm this preliminary classification, competition experiments that involved two hydroxyimidazoles and an excess amount of methanol were conducted under identical conditions (see the Supporting Information). This second set of data confirmed the higher activity of **2** with respect to **1**

© 2013 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

These are not the final page numbers!

and **3**, and of **4** with respect to **3**, **5**, and **6**. In the saturated series, the variation in the number of carbon atoms proved to have a crucial impact on the reactivity. In the second series, although the length of the aliphatic chain linking the base to the nucleophile is fixed, the accessible conformational space notably differs, thus leading to an even greater discrimination in terms of activity. Direct confrontation between the most active derivatives **2** and **4** yielded a balanced mixture of corresponding adducts (40:40) in full agreement with individual measurements.[19]

In terms of reactive pathways (Scheme 3), the trimolecular mixture of reactants (compounds **1**–**6**, methanol, and L-LacOCA) can either react through an intramolecular activation of the dyad (rate constant k_I) to lead to **8**–**13** or through the intermolecular activation of methanol by the imidazole ring of the dyad (rate constant k_M) to lead to **14**. The first pathway involves a bimolecular reactive complex with which methanol might hypothetically interact as a hydrogen-bond acceptor through an intermolecular O···H–N bond, whereas the second route relies on a trimolecular reactive complex in which methanol acts as donor and the dyad might hypothetically be engaged in an intramolecular N–H···O bond. The two reactive complexes might interconvert through a hydrogen-bond rescrambling process with an equilibrium constant $K=[K^{inter}_{(O-H\cdots N)}\times K^{intra}_{(O\cdots H-N)}]/[K^{intra}_{(O-H\cdots N)}\times K^{inter}_{(O\cdots H-N)}]$ to reach values of 6.3 and 25 for **2** and **4**, respectively. This equilibrium roughly evaluates the difference in between intra- and intermolecular N–H···O and O–H···N hydrogen bonding (from 4.5 to 8 kJ mol^{-1} for **2** and **4**, respectively).[20] The difference in molecularity between the two pathways involved in this model could be experimentally confirmed by using a fixed stoichiometry between the three molecular partners (**4**, MeOH, and L-lacOCA) while decreasing the overall concentration ($c_7=0.1$ to 0.001 mol L^{-1}) in the reactive aliquots (Table 1). Gas-phase chromatography was used on this range of concentrations to monitor the distribution of adducts **11** and **14** in complement to ^{1}H NMR spectroscopy. The amount of methyl-L-lactate **26** was precisely calibrated and monitored in the various reaction mixtures. It yielded, in agreement with the model, a linear distribution of the product ratio [**14**]/[**11**] with increasing concentrations.[21] For millimolar concentrations, at the lowest detection limit of methyl-L-lactate by the chromatographic method, adduct **11** was obtained in 93% yield. From the product distribution previously discussed, the corresponding ratio of kinetic constants k_I/k_M could easily be extracted. This rate corresponds to the kinetic effective molarity (^{k}EM), an indicator of the relative intrinsic reactivity between the intra- and intermolecularly activated reactive states (see Table 1 and the Supporting Information). This indicator of the benefit of the intramolecular preorganization on the reactivity, which represents the hypothetical methanol concentration required for the intermolecular process to compete with the intramolecular one, varied between 2 and 10 M within the dyad series. It is one order of magnitude higher than the acceleration induced by supramolecular precomplexation of reactive partners,[22a,b] but several orders of magnitude lower than the acceleration observed during the cyclization of preorganized diester systems designed by Bruice and co-workers.[22c] In practice, methanol must indeed be used as a cosolvent to be kinetically competitive with the dyad models. This translates into a reduction of the activation energy of 1.7–5.7 kJ mol^{-1} with a barrier of 60–70 kJ mol^{-1}. Such a value underpins a noticeable effect during the first step (the activation/deprotonation of the alcohol) of a complex molecular process of addition/elimination that involves the breakage and formation of several covalent bonds. Within the dyad series, the difference in the energy of activation between **3** and **4** (the least and most reactive dyads) of 5.1 kJ mol^{-1} provides a quantitative estimate for the advantage of freezing out a rotor during the formation of an intermolecular noncovalent bond, which is in agreement with earlier reports.[23]

Physical indicators of nucleophilic activation: The pK_a values of the imidazolium salts of compounds **1**–**6** were measured by half-protonation of the conjugated neutral form in 1 M KCl aqueous solutions in a concentration range of 0.1 to 0.001 mol L^{-1}, with each point being run in triplicate. Alkenyl and alkynyl derivatives **4**, **5**, and **6** displayed lower values than the saturated series **1**–**3**, thereby revealing the influence of the connecting unsaturated bond on the basicity of the heterocyclic nitrogen center, although the lone pair and the carbon–carbon double bond are in orthogonal orbitals. Within diastereoisomers, the configuration of the carbon–carbon double bond has a dramatic impact on the acidity of the imidazolium ring: the pK_a difference between the conjugated acids of *Z* and *E* diastereoisomers **4** and **5** reaches 1.4 units, the former being the most acidic. For the saturated series **1**–**3**, an evolution of moderate amplitude is observed (0.33 pK_a units). The C_2-bridged compound **2** is the least basic, in contrast with early measurements on 2-hydroxyalkylpyridines, the basicity of which was reported to decrease with chain length.[24]

At this stage, it is interesting to compare the pK_a values of the imidazolium salts with the nucleophilicity of their conjugate base **1**–**6**, as quantified by the kinetic constants of the L-lacOCA ring opening. In his early work on hydroxymethylimidazoles, Brown et al. found a strong correlation between nucleophilicity and basicity; the more acidic the imidazolium salt was, the more reactive the conjugated base in model acylation reactions.[25] For this hydroxymethyl series, increasing the electronic density on the heteroaromatic ring increases both the intrinsic hydrogen-bond donor character of the imidazole and the basicity, whereas the degree of preorganization (and hence the effective molarity) remains constant. In contrast, in both the alkyl- and alkenyl-bridged series **1**–**3** and **4**–**6**, the most reactive compound towards L-lacOCA was found to be the least basic (Table 1). In this collection, the nature of the spacer affects the electronic density, and therefore the intrinsic donor character and the basicity of the imidazole to a certain extent, but it also strongly and independently affects the effective molarity by spatial preorganization. The decorrelation between intrinsic hydrogen-bonding character and degree of preorgani-

© 2013 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

zation hence reasonably explains the absence of general correlation between the Brønsted basicity and nucleophilicity evaluated experimentally.

Direct observation of the IMHB by ^{1}H NMR spectroscopy in highly diluted conditions: As mentioned earlier, the only experimental evidence of the IMHB in serine hydrolases to date is the downfield shift of the $N^{\varepsilon 2}$ His signal in solution-state ^{15}N NMR spectroscopy upon the chemical deactivation of the proximal β-OH catalytic group (Scheme 1), as the proton involved in the IMHB is replaced by a neutral substituent. This selective neighboring effect of the chemical mutation was therefore reported to demonstrate the existence of a N···H–O-type hydrogen bond between His and Ser in the resting state in solution.[26,27]

Direct experimental observations of O–H···N(sp^2) IMHBs remain extremely scarce, and comparatively, evidence for N–H···O(sp^2) systems is relatively abundant. Still, NMR spectroscopy stands as a method of choice for detecting hydrogen bonds[28–30]: in nonprotic and relatively dry media, NH and OH protons can easily be observed, and they usually resonate at higher chemical shifts when hydrogen-bonded.

In the present study, 2-hydroxyalk(en)ylimidazoles **1**–**6** were dissolved in [D_7]DMF at 1 mM concentration.[31] Previous studies reported that self-associations of α,ω-hydroxyalkylpyridines were characterized by molar affinities in apolar solution.[32] Therefore, noncovalent oligomeric associations between hydroxyimidazoles should be ruled out under the present analytical conditions. To further mimic the solvation state of the microenvironment of hydrolytic active sites, an equivalent of water was introduced as a potential intermolecular hydrogen-bond competitor. Spectra of **1**–**6** displayed large signals for OH and NH protons at room temperature, with the former sharpening into a well-resolved triplet t(^3J(OH,CH_2) coupling) near the freezing point of DMF (Figure 1). As expected, both types of nuclei were more deshielded at low temperatures at which hydrogen-bonded and stabilized conformers should be more populated.

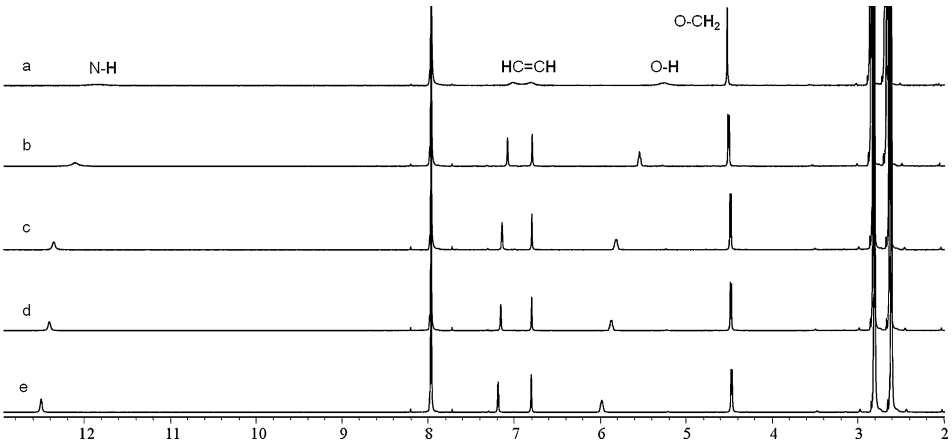


Figure 1. Variable-temperature ^{1}H NMR (400 MHz) spectra of **1** (1.0 mM) in [D_7]DMF): a) 300, b) 280, c) 235, d) 225, and e) 215 K.

In the context of hydrogen-bonded structures, temperature coefficients $d\delta/dT$ provide a finer level of analysis than the chemical shift, thus allowing one to differentiate between protons engaged or not in such interactions and also to differentiate those interacting with the solvent from those protected from the bulk solution and engaged in strong intramolecular bonds. As revealed in Table 2, while the chemical shift of the non-hydrogen-bonded protons such as CH_2OH slightly shifts downfield with increasing temperature, hydrogen-bonded ones such as NH and OH display a strong upfield variation.[33] In fact, within hydrogen-bonded protons, the pivotal value of the temperature coefficient that discriminates between intermolecularly solvent-bonded and intramolecularly solute-bonded protons was set at around -4.5 ppb K^{-1} for amides in peptides[34] and around -3 ppb K^{-1} for hydroxyl in saccharides.[35] Downfield shifts, smaller temperature coefficients, slower rates of exchange, and lower vicinal coupling constants (^3J(OH,CH_n)) are usually indicative of strong hydrogen bonds, four factors that were met only with compound **4**. The OH temperature coefficient of this active dyad model was superior to -1.5 ppb K^{-1}, whereas the other compounds displayed an average value of -8 ppb K^{-1}. The strong reactivity and low pK_a value displayed by **4** appeared to indicate a tight IMHB at 300 K, which, according to the series of spectra recorded, was reinforced at lower temperatures. In terms of statistics, the intramolecular hydrogen-bonded conformer should in fact strongly dominate the global population at this temperature and below. This picture was confirmed by theoretical calculations (see below).

Table 2. Temperature coefficients [ppb K^{-1}] between 300 and 215 K for compounds **1**–**6**.

	d[δ(OH)]/dT	d[δ(CH_2^{α})]/dT	d[δ(NH)]/dT
1	−8.18	0.45	−7.50
2	−7.75	1.63	−7.38
3	−8.43	0	−5.43
4	−1.43	0	−6.57
5	−8.16	0.34	−4.46
6	−6.82	0	−5.87

In silico conformational study of dyad models 2 and 4: The conformational populations of the most nucleophilic dyads **2** and **4** of each series (saturated/unsaturated) were estimated by DFT calculations by using four different methods and including solvent correction (see the Supporting Information). Coherent results were obtained with the different methods, and the dis-

© 2013 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

These are not the final page numbers!

cussion will be focused here on the results obtained at the M06-2X/6-31++G**+SMD level of theory, which is particularly relevant for hydrogen-bonded systems.[36] The results were compared to the variable-temperature ^{1}H NMR measurements to determine the influence of the conformational distribution on the averaged magnetic signals. The proportion of the activated conformers and the level of their calculated molecular orbitals were additionally related to the reactivity evaluated experimentally to determine the potential correlation between the degree of preorganization in the reactive states and the reactivity.

The results summarized in Figure 2 indicate that **2** and **4** do not fundamentally differ in terms of conformational landscape at 298 K, which seems at first sight to disagree with the NMR spectroscopic measurements. In fact, at room temperature, around 85% (respectively 75%) of dyad **4** (respectively **2**) adopts the O–H···N intramolecularly bonded conformation, the distribution over the minor species being broader for **2** than for **4**, however. These latter species, being either unfolded or displaying the reversed N–H···O IMHB, are destabilized by 5–7 kJ mol^{-1} for **2** (and 6–13 kJ mol^{-1} for **4**) with respect to the ground-state structure, which provides a rough estimation of the intramolecular binding constant and of the hydrogen-bond strength for both dyad models. Considering the nature of the experimental parameter that allows for the detection of the IMHB by NMR spectroscopy, the conformational excess (CE) between the N–H···O bonded species and the other species, and even more its variation with temperature, should be the most relevant indicator. And indeed, it appears that CE, which should be reasonably related to the averaged chemical shift,[37] undergoes a much stronger variation for **2** than for **4** on the temperature scale experimentally explored by NMR spectroscopy.

In light of the NMR spectroscopic data, these calculations seem to reveal a conformational landscape dominated by a folded species **4c** up to room temperature. This tendency is underpinned by a slightly stronger IMHB for **4** than for **2**. In both cases, the intramolecular binding constant should be about 5–10 kJ mol^{-1}, which matches the average value (6 kJ mol^{-1}) of the entropic penalty that has to be paid to immobilize molecular partners through noncovalent intermolecular interaction. These orders of magnitude confirm that an N···H–O hydrogen bond might only occur intramolecularly in polar and protic media and is modulated through the conformational preorganization provided by the spacing unit. In the present case, thermodynamic effective molarities can be estimated to be around 10^2 and 4×10^2 for **2** and **4**, respectively. In terms of kinetics, DFT calculations including solvent correction conducted on intramolecularly bound and unbound conformers of **2** and **4** indicate that within the framework of a natural bond orbital (NBO) analysis, the oxygen lone pair that is involved in the ring-opening reaction of L-lacOCA is significantly higher in energy in the bound than the unbound conformer. Concomitantly, the nitrogen lone pair is significantly stabilized when the IMHB

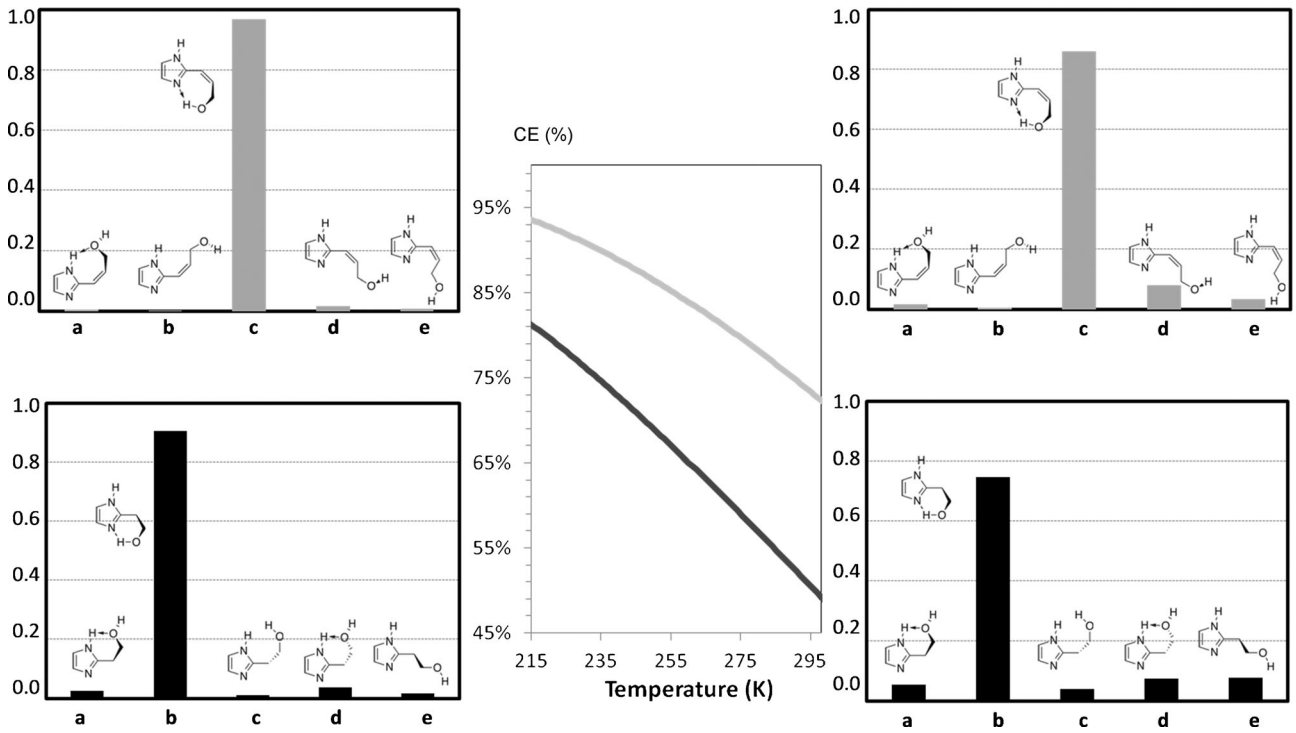


Figure 2. Analysis of the conformational population at thermodynamic equilibrium of the most active dyad models **4** (gray) and **2** (black) at 298 and 215 K. In both cases, the SMD/M06-2X/6-31++G** level of theory has been used (see text for a full description of calculations). Bold numbers stand for conformer labels and fractions. Central frame: corresponding conformational excess (CE) with respect to the temperature at the same interval for compound **2** (black) and **4** (gray).

© 2013 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

is present. As a consequence, the nucleophilicity of the hydroxyl group is expected to be significantly elevated by the IMHB in the bonded conformation.

Still, whereas the strength of the IMHB and the proportion of the folded conformer differs noticeably between **2** and **4**, the two dyad models display similar reactivity toward L-LacOCA. This observation tends to indicate that, whereas the IMHB must indeed exist to generate a productive reactive pathway, the increase in nucleophilicity is not correlated to its strength in the reactive state. This is in agreement with a Curtin–Hammet-type model in which intra- and intermolecularly bound species are rapidly interconverting, whereas the rate-limiting step remains as the addition/elimination processes (Scheme 3).

Conclusion

2-Hydroxyalk(en)ylimidazoles, which are easily accessible synthetically, have proven to be reasonable serine–histidine dyad models for detecting the postulated OH···N IMHB in solution and for evaluating its degree of implication on the nucleophilicity of the alcohol moiety. In fact, while tested as nucleophiles for the ring-opening reaction of L-lacOCA, these minimalistic models displayed higher nucleophilicity than their intermolecularly activated counterparts. This result points out that in the absence of additional physical effects that might be involved within enzyme active sites, a Ser–His-like IMHB substantially activates *O*-nucleophilicity, a phenomenon that potentially contributes to the enzymatic activity. This noncovalent bond clearly inherently facilitates the rate-limiting[17c] proton transfer but should be ineffective in accelerating the creation of the covalent bond between reactive partners. It is therefore remarkable that the resulting impact, although it remains modest and partly related to a favorable molecularity effect, is experimentally measureable. Whereas the basicity of the imidazole ring has often been evoked as an indicator of the extent of the nucleophilic activation, it has presently been proven not to correlate with the reactivity. In this series, variation in the length and flexibility of the spacers strongly biased the preference between intra- and intermolecular proton abstraction, thereby altering the pK_a and the *O*-nucleophilicity of the entities by construction. Concerning the IMBH, a couple of simple experimental and theoretical methods have been applied to easily detect the presence of IMHB in dilute solution in the presence of polar and protic competitors. The resulting data collected led to the conclusions that the intramolecular hydrogen-bonded species might only be detected when strongly dominating the conformational landscape, and that the species arises from the conformational preorganization between both moieties (herein provided by the spacing unit but presumably exerted by the Asp–His interaction in hydrolases) rather than intrinsic stability. In fact, it clearly appears that this productive geometry that involves a robust internal hydrogen bond provides a significant increase in alcohol nucleophilicity by itself.

Experimental Section

Variable-temperature NMR spectroscopic measurements on activated alcohols 1–6: NMR spectra at variable temperatures were recorded using a Bruker AVANCE DPX 400 MHz spectrometer, equipped with a 5 mm multinuclear reverse Z-gradient probe and using [D_7]DMF as the solvent. The temperature of the probe was calibrated by the methanol standard method, and a delay of 600 s was used before recording the NMR spectra at each temperature. The operating frequency was 400.13 MHz for ^{1}H. Each spectrum was run with a relaxation delay of 2 s, a 30° pulse, a time domain size of 64 K, and a real transform size of 32 K. Each NMR spectroscopic sample was prepared by using a new 5 mm tube capped with a septum and that contained a small amount of freshly activated 3 Å molecular sieves (to keep the medium as dry as possible during the overall NMR spectroscopy experiment) under an inert atmosphere. Then a solution of compound **1–5** ($\approx$1 mg) in dry [D_7]DMF (600 μL; $\geq$99.5 % D, (HDO + D_2O) $<$0.05 %) was transferred with a syringe through the septum.

General procedure for the ring-opening of 7 by a single dyad model 1–6 in the presence of methanol NMR spectroscopic monitoring: In a typical experiment, a solution of L-lacOCA in CD_3CN (20 mg; 0.17 mmol in 2 mL) was added to a solution of **1–6** in CD_3OD (0.26 mmol in 0.63 mL). The mixture was stirred at room temperature for 5 min and then analyzed by ^{1}H NMR spectroscopy.

Computational methods: Conformational studies were achieved with the Gaussian 03[38] software suite. Full scans of the rotational degrees of freedom of the alkyl chains were first performed at the AM1 level of theory, with 15° steps being used for dihedral angles. All local minima of the resulting potential-energy surface were subsequently fully optimized at the B3LYP/6-31G(d,p), B3LYP/6-31++G(d,p), B3LYP/6-31++G(d,p), and M06-2X/6-31++G levels of theory, with the nature of the stationary points being systematically tested through vibrational frequency calculations. A solvent was introduced as single-point electronic energy corrections using gas-phase optimized geometries and the C-PCM or SMD continuum model[39] (solvent: DMF,[40] radii: UAKS, tesserae: 0.2). Finally, Gibbs free energies were estimated by using a homemade Python script with the usual ideal gas/rigid rotator/harmonic oscillator approximation and the ideal solute thermodynamic reference (infinitely diluted solute with a reference concentration of 1 mol L^{-1}).

X-ray structural analysis: Detailed crystal structures, cell parameters, and *R* values for **2** and **9** are reported in the Supporting Information.

CCDC-932593 (**2**) and 932494 (**9**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Acknowledgements

Financial support from the French MENRT for M.M. and the Chinese CSC for Y.Z. is gratefully acknowledged. N. Saffon (Institut de Chimie de Toulouse) is acknowledged for the X-ray diffraction structures.

[1] a) M. J. Page, E. Di Cera, *Wiley Encycl. Chem. Biol.* **2009**, *4*, 305–312; b) P. G. Ashton-Rickardt, *Curr. Immunol. Rev.* **2009**, *5*, 187–199; c) R. Sedrani, U. Hommel, J. Eder, *Gene Fam. Targeted Mol. Des.* **2009**, 159–197; d) D. Kumar, T. N. Savitri, R. Verma, T. C. Bhalla, *Res. J. Microbiol.* **2008**, *3*, 661–672; e) C. M. Ardito, C. D. Briggs, H. C. Crawford, *Expert Opin. Ther. Targets* **2008**, *12*, 605–619; f) Y. Wang, W. Luo, G. Reiser, *Cell. Mol. Life Sci.* **2008**, *65*, 237–252; g) A. Guomundsdottir, J. Bjarnason, in *Novel Enzyme Technology for Food Applications* (Ed. R. Rastall), CPR Press, Boca Raton, **2007**, pp. 205–214.
[2] A. Frey, S. A. Whitt, J. B. Tobin, *Science* **1994**, *264*, 1927–1930.
[3] a) W. W. Bachovchin, *Biochemistry* **1986**, *25*, 7751–7759. In the liquid phase, such a lack of data can easily be attributed to the fact

 © 2013 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

These are not the final page numbers!

that the Ser–His dyad is less sequestered to solvent than the His–Asp counterpart. In the solid phase, NMR spectroscopic studies (see reference [6]) recently confirmed what X-ray snapshots (see reference [7]) seemed to point out: the Ser–His intramolecularly bonded microscopic state does not seem to be sufficiently populated to be macroscopically observed. For NMR spectroscopic studies, see: b) K. Coffman Haddad, J. L. Sudmeier, D. A. Bachovchin, W. W. Bachovchin, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 1006–1011; for X-ray studies, see: c) E. S. Radisky, J. M. Lee, C.-J. K. Lu, D. E., Jr. Koshland, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 6835–6840.

[4] In 1982, Steitz and Shulman reviewed the X-ray crystal structures and NMR spectroscopic data on serine proteases and confirmed early conclusions: the His–Ser hydrogen bond does not seem to exist in resting enzymes but must form upon complexation with substrates. Bachovchin recently addressed this question by carrying out equivalent ^{15}N NMR spectroscopic studies on enzyme crystals and found that there was no His–Ser hydrogen bond in the crystal (see reference [3]). In fact, as crystals were routinely made in high Li_2SO_4 solutions, a sulfate ion was bound in the active site between His and Ser, thereby raising the pK_a of His such that it is protonated even at high pH in crystals and therefore unable to hydrogen bond to Ser195. This result was recently confirmed by ultra-high resolution X-ray studies (see reference [3c]).

[5] a) G. Robillard, R. G. Shulman, *J. Mol. Biol.* **1974**, *86*, 519–540; b) G. Robillard, R. G. Shulman, *J. Mol. Biol.* **1974**, *86*, 541–558.

[6] Such as reducing the effective molarity by displaying various residues in optimal positions on a macromolecular flexible scaffold, activating the reactive partners through general acid–base catalysis, stabilizing various transition states with the help of an oxyanion hole, desolvating the reactive entities, and so on.

[7] Qualitatively, the fundamental mechanism of enhancing the nucleophilic power of any given oxygen atom is to enhance the electron density of the nucleophilic lone pair. This leads to two molecular strategies of generating increased electron density in the reactant state by altering the coordination and/or bonding of the nucleophile. The first one is desolvation, or more precisely the stripping of hydrogen-bond donors to the nucleophilic lone pair. The second one consists of coordinating the alcohol proton (i.e., hydrogen bonding to a general base that might ultimately lead to its ionization). In addition to these two mechanisms, an electric field generated at the active site can polarize a bond to the nucleophile, thereby enhancing its nucleophilicity. Consequently, the physical properties of the active site (dielectric constant, dipolar moment, permeability toward water, and so forth) are of crucial importance to the activation of the reactive partners. For an extensive review on this topic, see: V. E. Anderson, M. W. Ruszczycky, M. E. Harris, *Chem. Rev.* **2006**, *106*, 3236–3251.

[8] V. T. D'Souza, M. L. Bender, *Acc. Chem. Res.* **1987**, *20*, 146–152.

[9] In particular, intramolecularly activated acylation reactions were observed and monitored on 2- and 4-hydroxymethylimidazoles that bear various substituents on the heterocycle, whereas the corresponding analogues that lack the alcohol moiety failed to intermolecularly induce the acyl transfer on water or ethanol. For more details, see: a) V. Somayaji, K. I. Skorey, R. S. Brown, R. G. Ball, *J. Org. Chem.* **1986**, *51*, 4866–4872; b) D. M. Quinn, J. P. Elrod, R. Ardis, P. Friesen, R. L. Schowen, *J. Am. Chem. Soc.* **1980**, *102*, 5358–5365; c) K. I. Skorey, V. Somayaji, R. S. Brown, *J. Am. Chem. Soc.* **1988**, *110*, 5205–5206; d) K. I. Skorey, V. Somayaji, R. S. Brown, *J. Am. Chem. Soc.* **1989**, *111*, 1445–1452.

[10] a) C. Hunter, *Angew. Chem.* **2004**, *116*, 5424–5439; *Angew. Chem. Int. Ed.* **2004**, *43*, 5310–5324; b) from this model, $\Delta\Delta G^\circ = 6\ \mathrm{kJ\,mol^{-1}}$, which translates into $K_{inter} = 0.088$ M.

[11] V. M. Krishnamurthy, V. Semetey, P. J. Bracher, N. Shen, G. M. Whitesides, *J. Am. Chem. Soc.* **2007**, *129*, 1312–1320.

[12] a) S. Sahli, B. B. Stump, T. Welti, W. B. Schweizer, F. Diederich, D. Blum-Kaelin, J. D. Aebi, H.-J. Boehm, *Helv. Chim. Acta* **2005**, *88*, 707–730; b) M. L. Quan, P. Y. S. Lam, Q. Han, D. J. P. Pinto, M. Y. He, R. Li, C. D. Ellis, C. G. Clark, C. A. Teleha, J.-H. Sun, R. S. Alexander, *J. Med. Chem.* **2005**, *48*, 1729–1744; c) J. M. Lázaro Martínez, P. N. Romasanta, G. Y. Buldain, A. K. Chattah, *J. Org. Chem.* **2010**, *75*, 3208–3213; d) X, Sun, J. Qiu, S. A. Strong, L. S. Green, J. W. F. Wasley, J. P. Blonder, D. B. Colagiovanni, S. C. Mutka, A. M. Stout, J. P. Richards, G. J. Rosenthal, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5849–5853; e) direct synthesis between imidazole and formaldehyde activated by microwave: S. Lupsor, F. Aonofriesei, M. Iovu, *Med. Chem. Res.* **2012**, *21*, 3035–3042.

[13] I. M. McDonald, I. M. Buck, D. J. Dunstone, I. D. Linney, M. J. Pether, J. Spencer, K. I. M. Steel, P. Tisselli, P. T. Wright, R. A. D. Hull, C. Austin, E. A. Harper, E. Griffin, S. B. Kalindjian, C. M. R. Low *PCT Int. Appl.* **2006**, WO 2006-GB2042 20060602.

[14] a) D. J. Chadwick, R. I. Ngochindo, *J. Chem. Soc. Perkin Trans. 1* **1984**, *3*, 481–486; b) A. J. Carpenter, D. J. Chadwick, *Tetrahedron* **1986**, *42*, 2351–2358; c) adapting the procedure used for N-methylimidazole: R. J. Sundberg, P. V. Nguyen, *Med. Chem. Res.* **1997**, *7*, 123–136.

[15] H. McNab, C. Thornley, *J. Chem. Soc. Perkin Trans. 1* **1997**, 2203–2209.

[16] a) The reactive medium was subsequently neutralized at low temperature to avoid the formation of the aldehyde (resulting from double-bond migration) as the major product; b) G. Bridger, A. Kaller, C. Harwig, R. Skerlj, D. Bogucki, T. R. Wilson, J. Crawford, E. J. McEachern, B. Atsma, S. Nan, Y. Zhou, D. Schols, C. D. Smith, M. R. Di Fluri, US Patent US2004/19058 A1, **2004**.

[17] a) O. Thillaye du Boullay, E. Marchal, B. Martin-Vaca, F. P. Cossio, D. Bourissou, *J. Am. Chem. Soc.* **2006**, *128*, 16442–16443; lipases have also been shown to promote efficiently the ROP of lacOCA: b) C. Bonduelle, B. Martin-Vaca, D. Bourissou, *Biomacromolecules* **2009**, *10*, 3069–3073; c) practically all are already found in: C. Bonduelle, B. Martin-Vaca, F. P. Cossio, D. Bourissou, *Chem. Eur. J.* **2008**, *14*, 5304–5312; d) O. Coulembier, T. Josse, B. Guillerm, P. Gerbaux, P. Dubois, *Chem. Commun.* **2012**, *48*, 11695–11697.

[18] The unsaturation of the side chain is expected to alter the nucleophilicity of the alcohol moiety. In fact, measurements on primary amines have revealed the following order of nucleophilicity: $MeNH_2 > H_2C{=}CH{-}CH_2NH_2 \approx EtNH_2 > CH{\equiv}CCH_2NH_2$. For more details, see: F. Brotzel, Y. C. Chu, H. Mayr, *J. Org. Chem.* **2007**, *72*, 3679–3688.

[19] The remaining 20% correspond to higher oligomers. On the basis of *k* values, the theoretical ratio is 36:44.

[20] Whereas the intermolecular constant can be roughly evaluated by using Hunter's model detailed in reference [10a], the ratio of intramolecular binding constants was deduced from the calculations of the Gibbs enthalpy of the corresponding bonded states (see below and the Supporting Information).

[21] For the trimolecular scenario **4**+MeOH+**7**: $d[LacOMe]/c_0dt = k_M[ImOH]/c_0[MeOH]/c_0[LacOCA]/c_0$, whereas the bimolecular scenario **4**+**7** follows: $d[ImOMe]/c_0dt = k_I[ImOH]/c_0[LacOCA]/c_0$, with [MeOH] being approximately constant and roughly equal to $90c_7(t=0)$, the resulting rate gives: $d[LacOMe]/d[LacOMe] = k_M/k_I[MeOH] \times 90c_7(t=0)/c_0 = k'c_7(t=0)$. For more details, see the Supporting Information.

[22] a) E. Kassianidis, R. J. Pearson, D. Philp, *Org. Lett.* **2005**, *7*, 3833–3836; b) S. Anderson, H. L. Anderson, J. K. M. Sanders, *J. Chem. Soc. Perkin Trans. 1* **1995**, 2255–2267; c) T. C. Bruice, F. C. Lightstone, *Acc. Chem. Res.* **1999**, *32*, 127–136.

[23] H. Adams, E. Chekmeneva, C. A. Hunter, M. C. Misuraca, C. Navarro, S. M. Turega, *J. Am. Chem. Soc.* **2013**, *135*, 1853–1863.

[24] J. M. Mayer, B. Testa, *Helv. Chim. Acta* **1982**, *65*, 1868–1884. Nevertheless, such an evolution was reported to be a general trend for *ortho*, *meta*, or *para*-hydroxyalkyl structures, whereas for the latter, IMHB is clearly geometrically inaccessible. Such a tendency could therefore not be directly attributed exclusively to the IMHB strength because it is subjected to electronic parameters such as a through-bond inductive effect of the OH group.

[25] A linear relationship was actually found between $\log(k_{max})$ (with k_{max} being the kinetic constant for ring opening of a strained tricyclic anilide) and pK_a value. See reference [30d,e].

 © 2013 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

[26] a) S. O. Smith, S. Farr-Jones, R. G. Griffin, W. W. Bachovchin, *Science* **1989**, *244*, 961–963; b) W. W. Bachovchin, *Magn. Reson. Chem.* **2001**, *39*, S199–S213.

[27] The similar magnitudes of the hydrogen-bond perturbations of N1 and N3 chemical shifts as donors and acceptors indicate at least qualitatively that the strength of the Asp–His and His–Ser hydrogen bonds are not very different. For more details, see reference [4].

[28] Although infrared spectroscopy helps in differentiating free, inter-, and intramolecularly bonded OH groups, establishing a strict hierarchy between compounds on the basis of the strength of their IMHB remains tedious. All the more as one must operate under sufficiently diluted conditions to avoid the interference of associated forms. For illustrative studies on α,ω-hydroxalkylpyridines, see: a) L. P. Kuhn, R. A. Wires, W. Ruoff, H. Kwart, *J. Am. Chem. Soc.* **1969**, *91*, 4790–4793; b) M. Tissier, C. Tissier, *Bull. Soc. Chim. Fr.* **1969**, *9*, 3155–3161; c) F. Peral, E. Gallego, *J. Mol. Struct.* **1997**, *415*, 187–196.

[29] In the context of serine proteases, the observation of the ^{1}H signal of the proton shared between His and Asp was historically the first direct evidence of the nature of the interaction between the members of the triad. See: G. Robillard, R. G. Shulman, *J. Mol. Biol.* **1972**, *71*, 507–511.

[30] Such a typical low-field signal was also observed in *cis*-urocanic derivatives designed as natural and simple His–Asp dyad mimics that involve a direct covalent linkage of the side-chain functionalities through a rigid seven-membered hydrogen-bonded ring. See reference [2].

[31] This solvent appeared to gather a relevant set of physical properties because it was a hydrogen-bond acceptor and displayed a slightly higher polarity than the local microenvironment that surrounded the catalytic residues. For a reference on the local polarity within the active site of hydrolases, see: a) E. L. Mertz, L. I. Krishtalik, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 2081–2086; b) L. I. Krishtalik, *Chem. Phys.* **2005**, *319*, 316–329.

[32] NMR spectroscopic studies so far have focused on the resonance and coupling patterns of the nitrogen acceptor and, less frequently, of neighboring carbon atoms (see reference [30]). The atoms engaged in the noncovalent bond and, to a lesser extent, the adjacent carbon atoms can be used as magnetic probes, with hydrogen bonding influencing their chemical shifts (δ^{15}N, $\delta(^{13}C_{\alpha}H_n)$, $\delta(C_{\alpha}-^{1}H_n)$) and coupling constants ($^{1}J(^{15}N,^{1}H)$, $^{3}J(^{1}H-C_{\alpha},N\cdots^{1}H)$, $^{1}J(^{13}C_{\alpha},^{1}H)$) with the shared proton. Multidimensional experiments combined with long acquisition time or isotopically labeled substrates are generally required when operating under dilute conditions to minimize intermolecular effects.

[33] The amplitude of this variation depends, on the one hand, on factors that are constant for **1**–**5** such as the nature of the solvent and the nature of the XH (X = O, NH) group and, on the other hand, on individual factors such as the localization of the intra-/intermolecular accepting group.

[34] a) S. Rothemund, H. Weisshoff, M. Beyermann, E. Krause, M. Bienert, C. Mügge, B. D. Sykes, F. D. Sönnichsen, *J. Biomol. NMR* **1996**, *8*, 93–97; b) N. J. Baxter, M. P. Williamson, *J. Biomol. NMR* **1997**, *9*, 359–369.

[35] a) C. Sandström, H. Baumann, L. J. Kenne, *J. Chem. Soc. Perkin Trans. 2* **1998**, 2385–2393; b) S. Bekiroglu, L. Kenne, C. Sandström, *J. Org. Chem.* **2003**, *68*, 1671–1678.

[36] For benchmark papers that support this point of view, see: a) A. V. Marenich, C. J. Cramer, D. G. Truhlar, *J. Phys. Chem. B* **2009**, *113*, 6378–6396; b) R. F. Ribeiro, A. V. Marenich, C. J. Cramer, D. G. Truhlar, *Phys. Chem. Chem. Phys.* **2011**, *13*, 10908–10922.

[37] Unfolded states and to a lesser extent the N–H···O folded state should display a similar chemical shift for the hydroxyl proton, which is invariably engaged in intermolecular hydrogen bonding with the solvent or with water. In contrast, such an exchange is restricted in the intramolecularly bonded species, and the magnetic environment of the hydroxyl proton is strongly influenced by the imidazole donor.

[38] Gaussian 03, Revision D.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, Gaussian, Inc., Wallingford CT, **2004**.

[39] a) V. Barone, M. Cossi, *J. Phys. Chem. A* **1998**, *102*, 1995–2001; b) M. Cossi, N. Rega, G. Scalmani, V. Barone, *J. Comput. Chem.* **2003**, *24*, 669–681.

[40] E. S. Böes, P. R. Livotto, H. Stassen, *Chem. Phys.* **2006**, *331*, 142–158.

Received: April 4, 2013
Published online: ■■ ▮▮, 0000

 © 2013 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

These are not the final page numbers!