Effects of C2-Alkylation, N-Alkylation, and N,N′-Dialkylation on the Stability and Estrogen Receptor Interaction of (4*R*,5*S*)/(4*S*,5*R*)-4,5-Bis(4-hydroxyphenyl)-2-imidazolines

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(4*R*,5*S*)/(4*S*,5*R*)-4,5-Bis(4-hydroxyphenyl)-2-imidazolines bearing 2,2′-H (**3a**), 2,2′-Cl (**3b**), 2,2′,6-Cl (**3c**), and 2,2′-F (**3d**) substituents in the aromatic rings were C2-alkylated (**5a**−**i**), N-alkylated (**7**, **7a**−**c**), and N,N′-dialkylated (**9a**−**c**). The synthesis started from the diastereomerically pure (1*R*,2*S*)/(1*S*,2*R*)-1,2-diamino-1,2-bis(4-methoxyphenyl)ethanes **1a**−**d**, which were cyclized to the imidazolines **2a**−**d** and **4a**−**i** with triethylorthoesters or iminoethers. Ether cleavage with BBr_3 yielded the (4*R*,5*S*)/(4*S*,5*R*)-4,5-bis(4-hydroxyphenyl)-2-imidazolines **3a**−**d** and **5a**−**i**. The N-alkylation and N,N′-dialkylation of **2b**, employed for obtaining **7a**−**c** and **9a**−**c**, were performed prior to the ether cleavage with alkyl iodine in dry THF. By use of HPLC, the influence of the substitution patterns in the aromatic rings and alkyl chains at the C2- or N-atoms on the hydrolysis rate of the imidazolines was studied under in vitro conditions. It appeared that only imidazolines with *C*2- or *N*-alkyl substituents show sufficient stability to interact as heterocycles with the estrogen receptor (ER). The resulting gene activation was monitored in a luciferase assay using ERα-positive MCF-7-2a breast cancer cells stably transfected with the plasmid ERE_{wtc}luc. It is interesting to note that C2-alkylation led to a strong reduction or even a complete loss of activity whereas N-alkylation improved the estrogenic profile. The (4*R*,5*S*)/(4*S*,5*R*)-*N*-ethyl-4,5-bis(2-chloro-4-hydroxyphenyl)-2-imidazoline **7b** has proven to be the most active compound in this structure−activity relationship study (EC_{50} = 0.015 μM).

Introduction

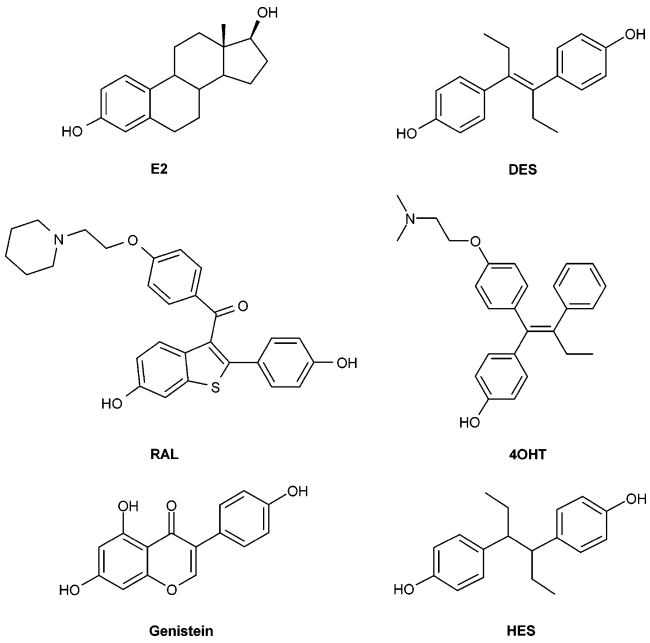
The estrogen receptors ERα and ERβ are important targets in the treatment and prevention of several diseases. The endogenous ligand estradiol (E2) not only regulates the growth, differentiation, and function of the male and female reproductive systems through these receptors but also influences bone maintenance, the central nervous system, and the cardiovascular system.[1−4]

Crystal structures of the ligand binding domain (LBD) of both ER subtypes cocrystallized with E2 or other agonists, e.g., diethylstilbestrol (DES) or genistein, as well as selective estrogen receptor modulators (SERMs), e.g., raloxifene (RAL) and 4-hydroxytamoxifen (4OHT), providing a good insight into the binding mode of drugs at the ERs (for structures, see Chart 1).[5−7]

Agonists build H-bridges to Glu353, Arg394, and His524 and van der Waals contacts to aliphatic residues of amino acids in the LBD of ERα.[5,6] A comparable binding mode was determined for ERβ with H-bridges to Glu305, Arg346, and His475.[7] These bindings lead to a conformational change of the receptor molecule followed by a dimerization of ER/drug conjugates and an interaction with the "estrogen response element" (ERE) of the DNA.

The varying tissue distribution of the ERs encourages the search for new steroidal or nonsteroidal compounds with subtype selectivity.[8] Moreover, the development of heterocyclic compounds with an unusual ER binding mode shows a lot of promise in optimizing hormonal replacement therapy.

Chart 1



In previous papers, we have described new lead structures for the design of novel hormonally active drugs.[9−11] It was demonstrated that (4*R*,5*S*)/(4*S*,5*R*)-4,5-bis(4-hydroxyphenyl)-2-imidazolines and (2*R*,3*S*)/

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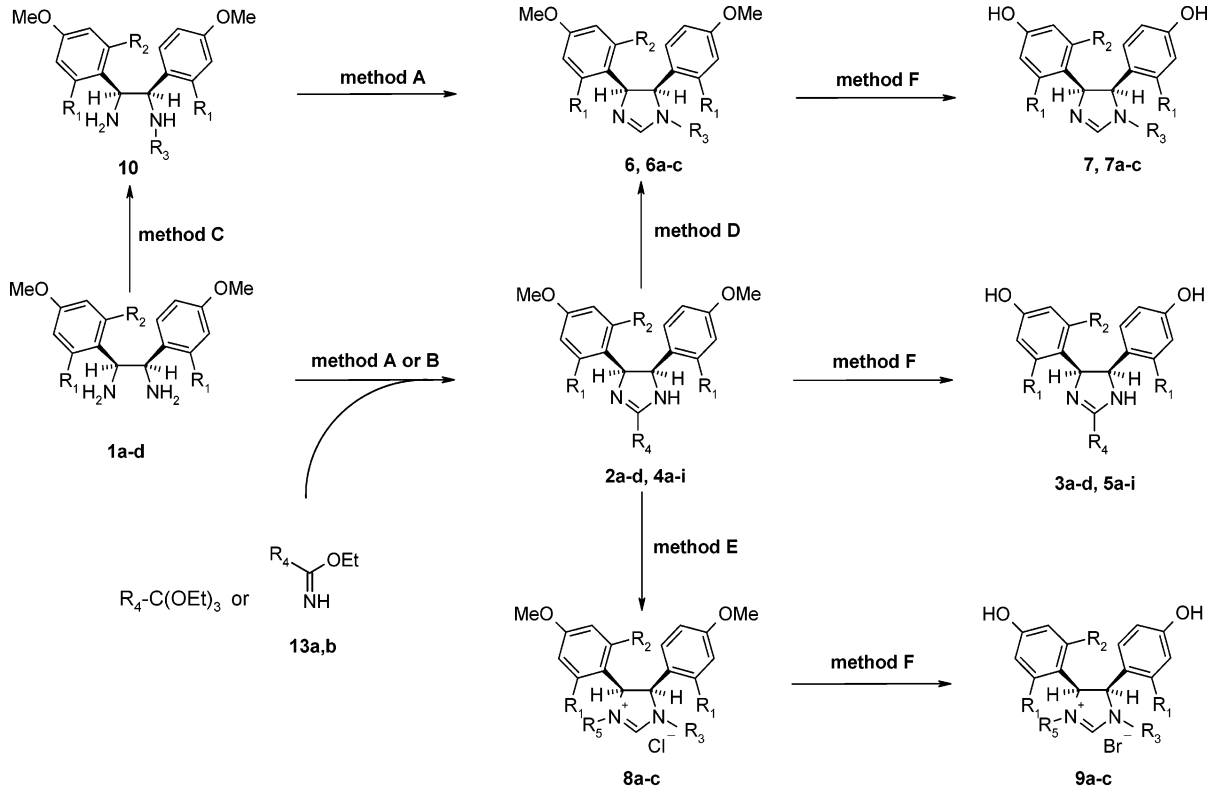
Scheme 1



compound	R_1	R_2	R_3	R_4	R_5
Non-alkylated compounds					
1a,2a,3a					
1b,2b,3b	Cl				
1c,2c,3c	Cl	Cl			
1d,2d,3d	F				
C-alkylated compounds					
4a, 5a				CH_3	
4b, 5b	Cl			CH_3	
4c, 5c	Cl	Cl		CH_3	
4d, 5d	F			CH_3	
4e,5e				C_2H_5	
4f, 5f	Cl			C_2H_5	
4g, 5g	F			C_2H_5	
4h				$C_2H_4OCH_3$	
4i	Cl			$C_2H_4OCH_3$	
5h				C_2H_4OH	
5i	Cl			C_2H_4OH	
N-alkylated compounds					
6, 7			C_2H_5		
6a, 7a	Cl		CH_3		
6b, 7b	Cl		C_2H_5		
6c, 7c	Cl		C_3H_7		
8a, 9a	Cl		CH_3		CH_3
8b, 9b	Cl		C_2H_5		C_2H_5
8c, 9c	Cl		C_3H_7		C_3H_7
10	Cl		C_2H_5		
13a				CH_3	
13b				$C_2H_4OCH_3$	

(2*S*,3*R*)-2,3-bis(4-hydroxyphenyl)piperazines activated the gene expression in ERα-containing MCF-7-2a cells despite a spatial structure that prevents the contact to His524.[9,11] These studies led to a new classification of estrogens. The essentially planar estrogens, e.g., E2 or DES, are classified as type I (class 1), whereas angular estrogens, e.g., 2,3-diarylpiperazines and 4,5-diaryl-2-imidazolines, are type II (class 2) estrogens.[9−12] Knowing the fact that imidazolines hydrolyze into formamides that can then act as type I estrogens, we investigated

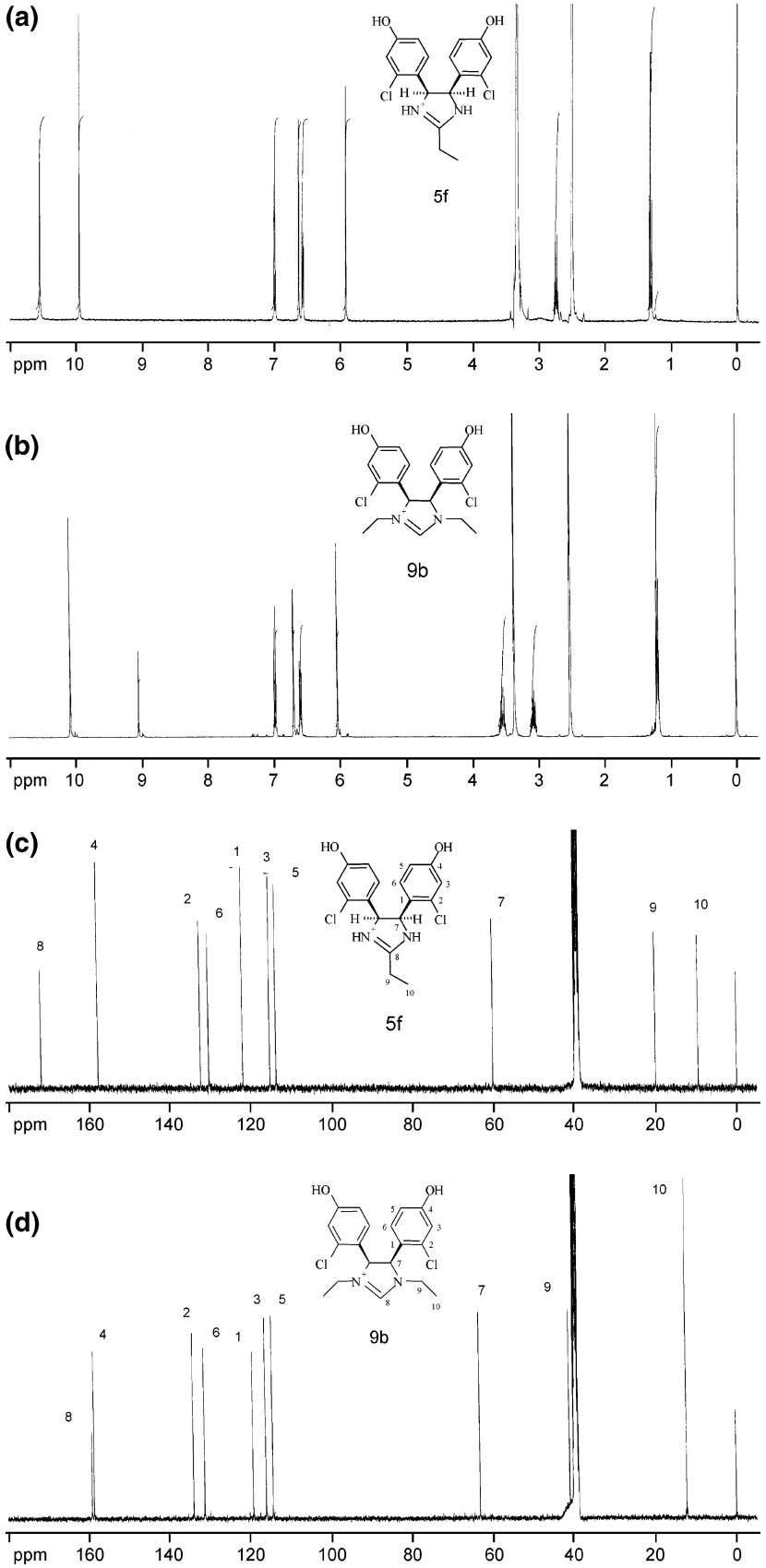


Figure 1. ^{1}H NMR (a, b) and ^{13}C spectra (c, d) of **5f** and **9b**.

the stability of (4*R*,5*S*)/(4*S*,5*R*)-4,5-bis(4-hydroxy/methoxyphenyl)-2-imidazolines under in vitro conditions and studied the influence of *C*2-alkyl, *N*-alkyl, and *N*,*N*′-dialkyl substituents on both the stability and the ER interaction.

Results

Synthesis. The synthesis of the imidazolines was performed by reaction of the respective 1,2-diamino-1,2-diarylethane (**1a**−**d**) with an excess of triethyl ortho-

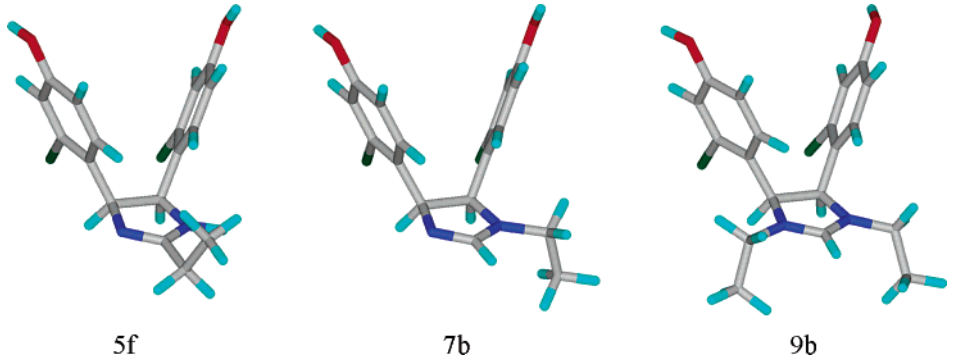


Figure 2. Low-energy conformations of **5f**, **7b**, and **9b**.

formate, triethyl orthoacetate, or triethyl orthopropionate to yield **2a**−**d**, **4a**,**b**, and **4d**−**g** or with the iminoethers **13a**,**b** to yield **4c** and **4h**,**i** (methods A and B, Scheme 1). **13a**,**b** were obtained utilizing the method of Pinner by partial hydrolysis of the respective nitrile in ethanolic HCl.[13,14]

The weak acidic character of **2b** was employed to synthesize N-alkylated imidazolines. **2b** was treated successively with butyllithium and alkyl iodine to obtain the imidazolines **6a**−**c** (method D, Scheme 1). If the imidazoline anion is reacted with an excess of alkyl iodine, the *N*,*N*′-dialkyl-2-imidazolinium salts **8a**−**c** can additionally be isolated (method E, Scheme 1). **6** was not available under these reaction conditions. Therefore, the 1,2-diamino-1,2-diarylethane **1a** had to be alkylated first (method C; compound **10**) followed by ring closure (method A, Scheme 1). All methyl ethers were cleaved using BBr_3 to yield the hydroxy-substituted compounds **3a**−**d**, **5a**−**i**, **7**, **7a**−**c**, and **9a**−**c** (method F, Scheme 1).

The 1-amino-2-formamido-1,2-bis(2-chloro-4-hydroxyphenyl)ethane **11** was obtained by hydrolysis of the imidazoline **3b** in 0.01 N NaOH.

Structural Characterization. The ^{1}H NMR spectra of C2-alkylated and N,N′-dialkylated derivatives with an identical substitution pattern in both aromatic rings (**5a**,**b**,**d**−**i**, **9a**−**c**) demonstrated only one set of signals for the aromatic protons and a singlet resonance for the benzylic protons (for the NMR spectra of **5f** and **9b**, see Figure 1). This is only possible in the case of a symmetrical heterocyclic ring with a delocalized double bond between both nitrogen atoms. To verify this, the ^{13}C NMR spectra of 4,5-bis(2-chloro-4-hydroxyphenyl)-2-imidazoline **3b** and its *C*2-ethyl (**5f**) and *N*,*N*′-diethyl (**9b**) derivative were measured. These spectra confirmed a symmetric conformation of the molecules. Compared to **3b** (eight resonances: 6 × Ar−H; 1 × C2−H; 1 × C4,5−H), there are only two additional signals of the ethyl chains detected for **5f** and **9b** (see Figure 1). This indicates a planar arrangement of the N−C═N moiety with the CH_2 groups of the alkyl chains being located in the plane of the heterocyclic ring.

The arrangement of the aromatic rings was examined by coupling constants analysis. 4-(2-Chloro-4-hydroxyphenyl)-5-(2,6-dichloro-4-hydroxyphenyl)-2-methyl-2-imidazoline **5c** and the *N*-monoalkyl derivatives (**7**, **7a**−**c**) represent asymmetric molecules with coupling constants for the benzylic protons of ^3J = 12.0−12.9 Hz. These values correlate with an arrangement of the benzylic protons in a dihedral angle of 0−15° and a planar structure of the 2-imidazoline ring with pseudo-axially oriented aromatic rings, as already described for **3a**−**d**.[10]

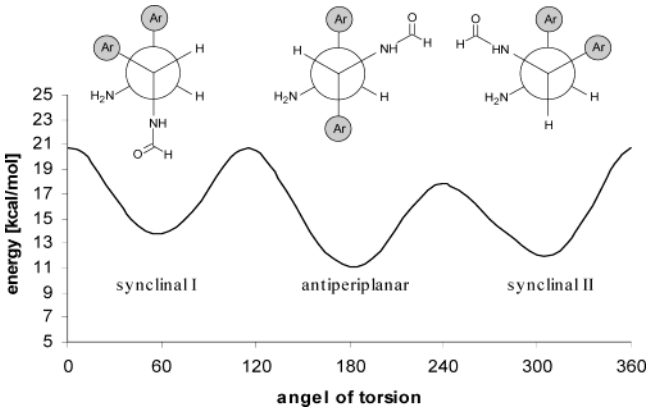


Figure 3. Energy profile for the rotation of (1*R*,2*S*)/(1*S*,2*R*)-1-amino-2-formamido-1,2-bis(2-chloro-4-hydroxyphenyl)-ethane **11**.

The orientation of the substituents at the heterocyclic ring was confirmed by theoretical calculations on the examples of **5f**, **7b**, and **9b**. As illustrated in Figure 2, the N-atoms as well as the C2-atom are partially sp^2-hybridized, with the methylene groups being located in the heterocyclic plane. The CH_3 group is oriented opposite the aromatic rings if the ethyl group is N-standing (**7b** and **9b**) and oriented toward the phenyl rings in the *C*2-ethyl derivative **5f** (see Figure 2). However, the ethyl chains are not hindered in rotation, and the energy barrier for rotation is less than 2 kcal/mol.

2-Imidazolines show temperature- and pH-dependent hydrolysis under formation of amides.[15] The coupling constant of the benzylic protons determined for the hydrolysis product **11** amounted to ^3J = 5.6 Hz. This could be an average of the coupling constants of the possible conformations during the rotation around the diarylethane bridge or could result from a preferred conformation with a dihedral angle between the benzylic protons of about 60°.

Theoretical investigations indicated three low-energy conformations of comparable energy in which the aromatic rings and the amino groups are synclinally (synclinal I, 13.72 kcal/mol; synclinal II, 12.04 kcal/mol) or antiperiplanarly (11.09 kcal/mol) oriented (see Figure 3). A conformational change is possible (activation energies: 6.73 kcal/mol for antiperiplanar → synclinal II; 9.44 kcal/mol for antiperiplanar → synclinal I).

Stability Studies. The stability of (4*R*,5*S*)/(4*S*,5*R*)-4,5-diaryl-2-imidazolines was studied under in vitro

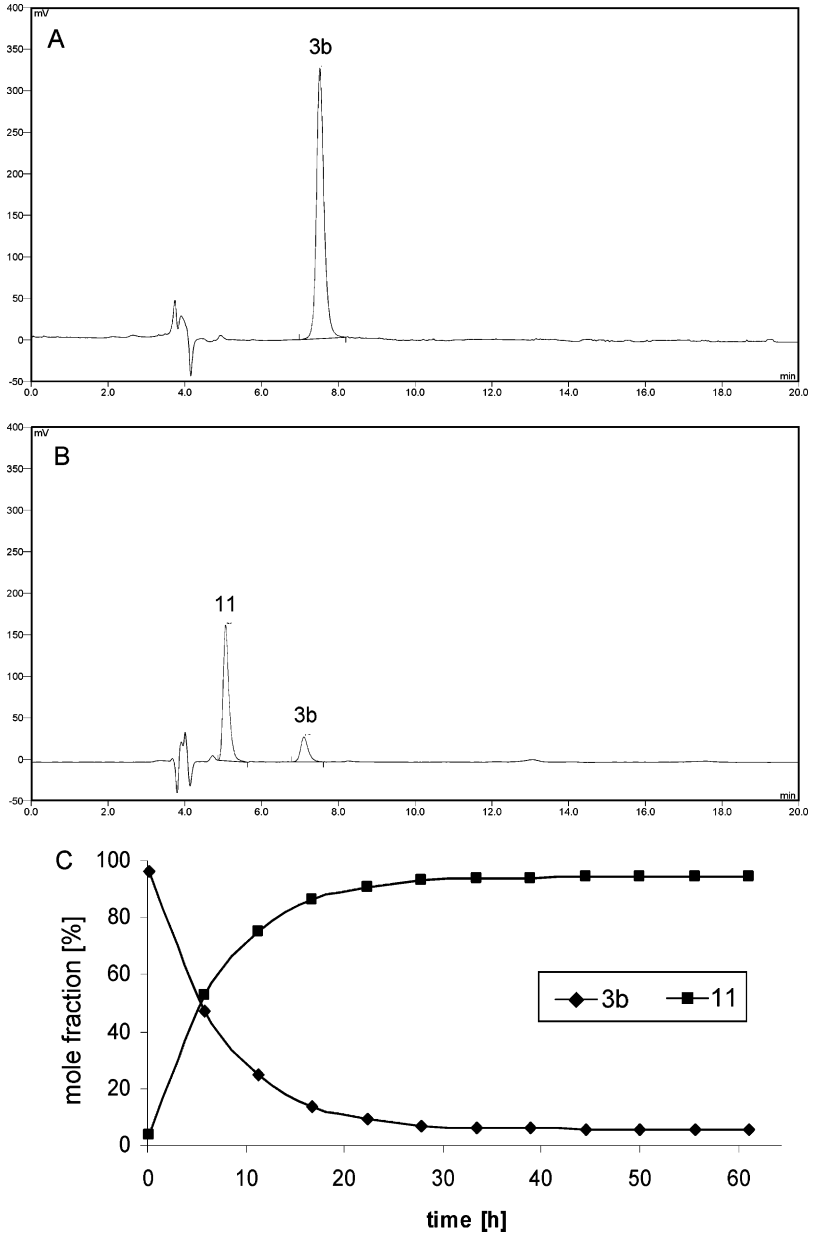


Figure 4. HPLC investigations (RP 18 material; methanol/H_2SO_4 (0.001 N, 20 mM Na_2SO_4) = 50:50; λ = 254 nm) on the stability of the 4,5-bis(2-chloro-4-hydroxyphenyl)-2-imidazoline **3b**. HPLC chromatogram at the beginning (A) and after an incubation time of 55 h (B). The kinetics of decomposition is shown in part C.

conditions by means of HPLC. In a preliminary study, we used the 4,5-bis(2-chloro-4-hydroxyphenyl)-2-imidazoline **3b** as a model. **3b** was incubated at 37 °C and pH 7.4 for 55 h. These conditions matched exactly those used in the luciferase assay to determine the hormonal activity.

The start chromatogram exhibited a well-defined peak at a retention time of t_R = 7.52 min, which was assigned to **3b** (see Figure 4A). The peak area decreased during an incubation time of 55 h, and the appearance of a hydrophilic decomposition product was observed (t_R = 5.06 min, Figure 4B). Isolation from the reaction mixture and spectroscopical characterization indicated the formation of the formamide **11**.

The hydrolysis of **3b** illustrated in Figure 4C represents a consecutive reaction with two reversible reaction steps. In the initial reaction step, the weak imidazoline base gets protonated and the ring opening takes place in the rate-determining step by nucleophilic attack of OH^- at the C2-atom (see Scheme 2). These preconditions enabled a calculation of the rate constant using KSIM (Table 1). **3b** hydrolyzed rapidly with a half-live of $t_{1/2}$ = 5.94 h and a rate constant of k_{obs} = 0.150 h^{-1}. O-methylation (**2b**) and exchange of the ortho Cl substituents (**3a**) did not significantly influence the hydrolysis rate (**2b**, $t_{1/2}$ = 4.58 h, k_{obs} = 0.198 h^{-1}; **3a**, $t_{1/2}$ = 6.36 h, k_{obs} = 0.122 h^{-1}). However, an additional 2-chlorine substituent (**3c**, $t_{1/2}$ = 13.41 h, k_{obs} = 0.052 h^{-1}) and an *N*-ethyl group (**7b**, $t_{1/2}$ = 154.03 h, k_{obs} = 0.004 h^{-1}) enhanced the stability. Interestingly, the *C*2-hydroxyethyl derivative **5i** and the *N*,*N*′-diethyl compound **9b** remained stable during the incubation time.

It should be noted that the asymmetric compounds **3c** and **7b** can form two hydrolysis products. In the case of **3c** both products were found whereby one was not formed in favor of the other. For **7b** we only detected

Scheme 2

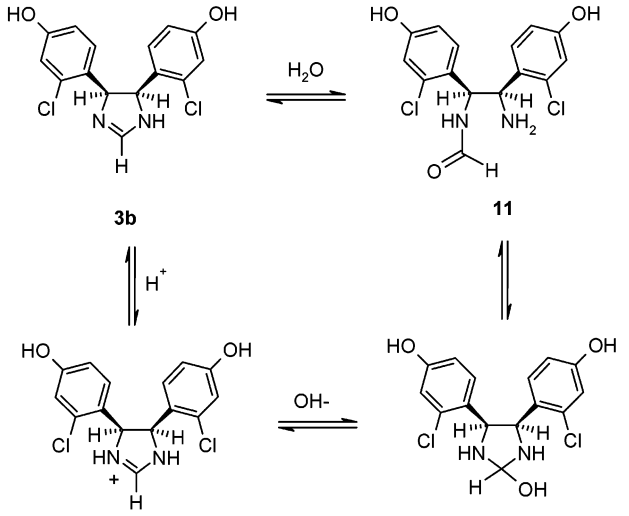
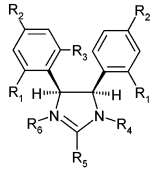


Table 1. Half Life of 2-Imidazolines under in Vitro Conditions[a]

	R_1	R_2	R_3	R_4	R_5	R_6	half-life $t_{1/2}$ [h]	k_{obs} [h^{-1}]
								
2b	Cl	OCH_3					4.58	0.198
3a		OH					6.36	0.122
3b	Cl	OH					5.94	0.150
3c	Cl	OH	Cl				13.41	0.052
5i	Cl	OH			C_2H_4OH		nd[b]	nd[b]
7b	Cl	OH		C_2H_5			154.03	0.004
9b	Cl	OH		C_2H_5		C_2H_5	nd[b]	nd[b]

[a] In vitro conditions: solvent = PBS; pH 7.4; temperature of 37 °C. [b] No decomposition was observed for **5i** and **9b** within 55 h.

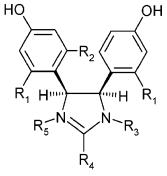
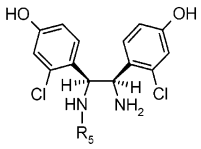
one decomposition product in the HPLC chromatograms. Theoretically, a secondary and a tertiary amide could be formed. In accordance with de Savignac et al.,[16] we propose the favored formation of the secondary amide.

Biological Properties. The interactions of the compounds **3a**−**d** with the ER were previously described[10] and are summarized in Table 2. The relative binding affinity (RBA) to the ER was determined for all compounds in a competition experiment with [^{3}H]-E2 and calf uterine cytosol. The RBA values, which are less than 0.1% (data not shown), indicate that none of the compounds can displace E2 in large amounts from its binding site. Nevertheless, we investigated the effects on the ERα-containing MCF-7-2a cell line in order to evaluate whether the imidazolines interact independently of E2 in the LBD of the ER. In this assay, the expression of luciferase correlates very well with the estrogenic potency of drugs.[17]

Despite their low RBA values, the 2-imidazolines **3b** and **3c** activated the luciferase expression. The Cl substituents in the aromatic rings enhanced the hydrophobicity and mediated a relative transcriptional potency of RTP = 0.021% (EC_{50} = 0.380 μM) and 0.123% (EC_{50} = 0.065 μM), respectively (Table 2 and ref 9).

N-monoalkylation of **3b** increased the estrogenic activity further: **3b** (*N*-H: EC_{50} = 0.380 μM; RTP = 0.021%) < **7a** (*N*-CH_3: EC_{50} = 0.300 μM; RTP = 0.027%) < **7c** (*N*-C_3H_7: EC_{50} = 0.150 μM; RTP = 0.053%) < **7b** (*N*-C_2H_5: EC_{50} = 0.015 μM; RTP = 0.533%).

Table 2. Activation of Luciferase Expression in MCF-7-2a Cells

						luciferase expression in MCF-7-2a cells		
	R_1	R_2	R_3	R_4	R_5	relative activation [%] at 1 μM	EC_{50} [μM]	RTP[a] [%]
								
3a						0		
3b	Cl					67	0.380	0.021
3c	Cl	Cl				112	0.065	0.123
3d	F					0		
5a				CH_3		2		
5b	Cl			CH_3		0		
5c	Cl	Cl		CH_3		20	5	0.002
5d	F			CH_3		12		
5e				C_2H_5		6		
5f	Cl			C_2H_5		6		
5g	F			C_2H_5		0		
5h				C_2H_4OH		0		
5i	Cl			C_2H_4OH		20		
7			C_2H_5			0		
7a	Cl		CH_3			78	0.300	0.027
7b	Cl		C_2H_5			103	0.015	0.533
7c	Cl		C_3H_7			86	0.150	0.053
9a	Cl		CH_3		CH_3	54	0.780	0.010
9b	Cl		C_2H_5		C_2H_5	60	0.500	0.016
9c	Cl		C_3H_7		C_3H_7	6		
E2						100	0.000 08	100
								
11					CHO	21	3	0.003
12					H	44	1.5	0.005

[a] Relative transcriptional potency: % RTP = [EC_{50}(E2)/EC_{50}(ligand)] × 100.

On the other hand, N,N′-dialkylation reduced the activity. **9c** was completely inactive, while **9b** (EC_{50} = 0.500 μM; RTP = 0.016%) and **9a** (EC_{50} = 0.780 μM; RTP = 0.010%) showed a luciferase expression slightly lower than **3b** (see also Figure 5).

While N-substituents positively influenced the hormonal effects, the alkyl chains at C2 reduced the activity. Only the *C*2-methyl derivative **5c** showed marginal effects (EC_{50} = 5 μM; RTP = 0.002%) that are more than 50 times lower than that of its parent compound **3c**. The hydrolysis product **11** that might be formed during the incubation of **3b** with MCF-7-2a cells significantly induced the luciferase expression only in the highest used concentration of 10 μM (Figure 5). A relative activation of 21% was measured at a concentration of 1 μM. This effect is even lower than that determined for the 1,2-diamino-1,2-bis(2-chloro-4-hydroxyphenyl)ethane **12** (44% at 1 μM).

Finally, it is noted that none of the 2-imidazolines were able to antagonize the effect of 1 nM E2.

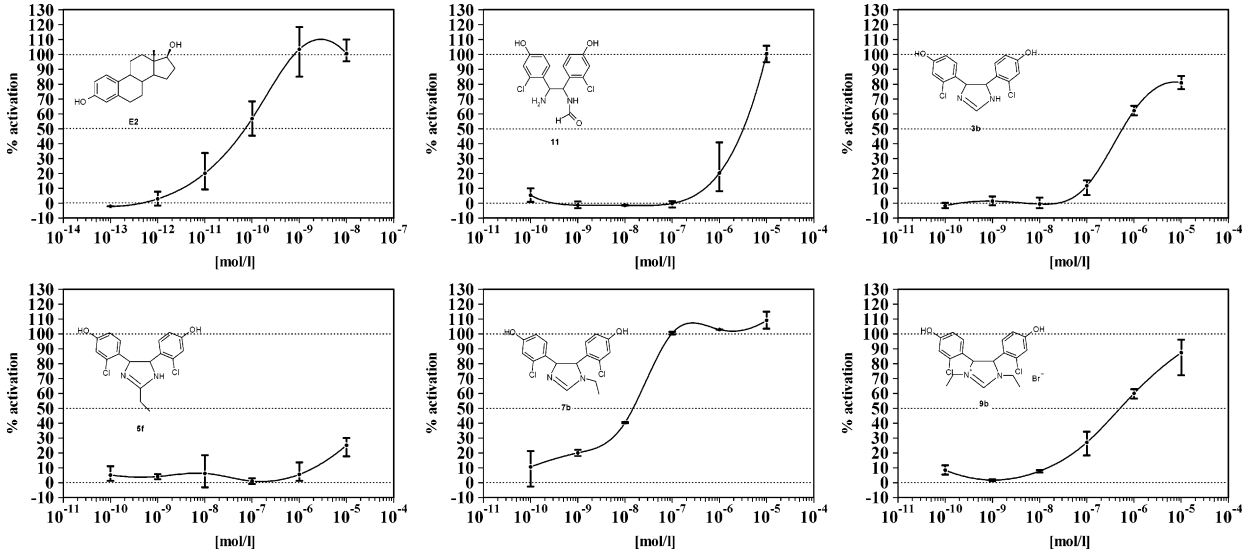


Figure 5. Luciferase expression in MCF-7-2a cells stably transfected with the reporter plasmid ERE_{wtc}luc.

Discussion

The stimulation of the ER by endogenous estrogens plays an important role in both male and female physiologies.[18] Unfortunately, E2 stimulation is also implicated in the development of breast cancer.[19] Consequently, many ER ligands were designed to prevent E2-mediated tumor growth. This led to the discovery of antiestrogens for the treatment of hormone-dependent tumors.

Throughout the 1970s, research focused mainly on optimizing the antiestrogenic properties of tamoxifen (TAM). This potent antibreast cancer agent also expressed increased estrogenic properties in other tissues, e.g., the endometrium, which limits its therapeutical use.[20] Such a diversity of pharmacological effects led to its classification as a selective estrogen receptor modulator.[21]

TAM acts as a prodrug that is activated by hydroxylation of the para position of the 1-phenyl ring, resulting in 4OHT. In both cases, the ligand recognition by the ER is achieved through a combination of specific hydrogen bonds and van der Waals interactions. Crystal structures of the LBDs of ERα and ERβ that cocrystallized with agonists and antagonists indicated that the binding of 4OHT induces a conformation of the LBD that differs in both secondary and tertiary structural organization from those driven by E2 binding.[5−7] It was concluded from these studies that agonists have to be planar, hydrophobic molecules bearing two OH groups with an O−O distance of about 12 Å.

Our studies, however, revealed that molecules with quite different spatial structures also interact with the ER. Especially *RS*/*SR*-configurated 2,3-diarylpiperazines and 4,5-diaryl-2-imidazolines activated gene expression in ERα-containing MCF-7-2a breast cancer cells.[9,10] This effect is quite remarkable because the 1,2-diarylethane pharmacophor takes a conformation with synclinally (piperazines) or pseudoaxially oriented aromatic rings (imidazolines). Compounds from the most promising 4,5-bis(4-hydroxyphenyl)-2-imidazoline series acted as full agonists despite their inability to displace estradiol in high amounts from its binding site (competition experiment with [^{3}H]-E2: RBA < 0.1%). These findings led to their classification as type II (class 2) estrogens.[9,12]

This categorization as type II estrogens cannot be done without restriction because 4,5-diarylimiodazolines may hydrolyze in aqueous solution into 1-amino-2-formamido-1,2-diarylethanes.

Under conditions comparable to those used in our in vitro assay (37 °C, pH 7.4, incubation time of 55 h), **3b** rapidly decomposed into the formamide **11** with a half-life of $t_{1/2}$ = 5.94 h (see Figure 4C). Substituents in the aromatic rings only marginally influenced the hydrolysis rate, while alkyl chains located at the heterocycle stabilized the molecule. All C2- and N-substituted as well as the N,N′-dialkylated compounds proved to be stable during the incubation time of 55 h. Only the *N*-ethyl-2-imidazoline **7c** was marginally hydrolyzed by elongating the incubation time ($t_{1/2}$ = 154 h).

The acyclic compounds can adapt three low-energy conformations with synclinally and antiperiplanarly arranged aromatic rings (for **11**, see Figure 3). The latter represents a spatial structure comparable to DES or HES. Despite this structural analogy, the activity of **11** on the MCF-7-2a cell line was distinctly lower than that of **3b** (see Figure 5 and Table 2) and the 1,2-diamino-1,2-bis(2-chloro-4-hydroxyphenyl)ethane **12** (see Table 2). Interestingly, N-ethylation and N,N′-diethylation not only enhanced the stability but also respectively increased (**7b)** or inhibited (**9b**) the activity of **3b**. This suggests that the 2-imidazolines interact with the ER without previous hydrolysis.

While the interactions of type I estrogens in the LBD of the ERs are well-known, we postulated a novel binding mode for type II estrogens at ERα based on our results on the ERα-positive MCF-7-2a cell line.

Initially, type II estrogens of the (4*R*,5*S*)/(4*S*,5*R*)-4,5-bis(4-hydroxyphenyl)-2-imidazoline series are attached to the LBD of the ERα contacting the amino acids Glu353 and Arg394. We proposed these contacts because they are documented in all crystal structures of agonists as well as antagonists. Because of the angular structure of the molecules, the second anchorage, which is essential for the gene activation, can only be achieved in the neighboring side pocket. It is very likely that they

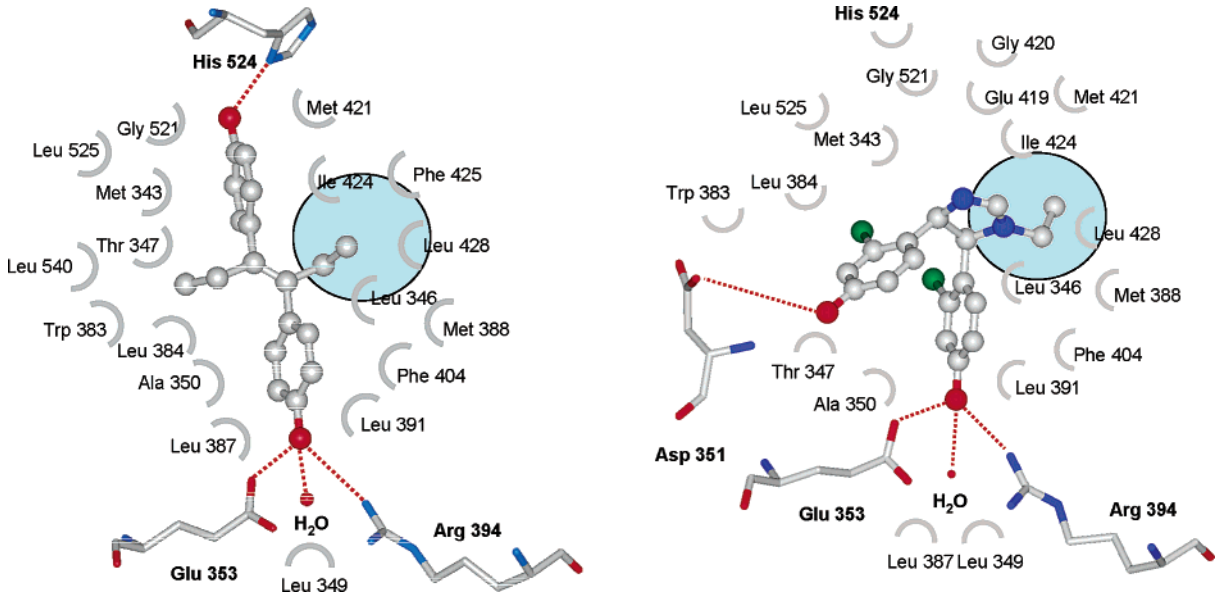


Figure 6. Interaction of DES in the LBD of ERα (left) and the postulated mode of action of the *N*-ethyl-4,5-bis(2-chloro-4-hydroxyphenyl)-2-imidazoline **7b** (right).

are H-bonded to Asp351 because this amino acid also mediated the agonistic properties of 4OHT. (The substitution of glycine for aspartate at position 351 (Asp351Gly ER) changed the activity of the 4OHT/ER complex from estrogen-like to completely antiestrogenic.[22]) Nevertheless, Thr347, as a second crucial amino acid in the hydrophobic side pocket,[23] has to be taken into consideration as we have recently demonstrated using theoretical methods.[24]

The endocrine activity of type II estrogens depends on the hydrophobicity of the molecule. 2-Imidazolines and piperazines gain activity by introducing halides into the aromatic rings and alkyl chains at the nitrogen atoms. The luciferase expression showed a maximum with 2-Cl substitution; the respective 2-F derivatives were by far less active.

Alkyl substituents at the 2-imidazolines not only increased the stability of the compounds but also increased the hydrophobicity and consequently the hormonal activity. The best effect was shown by the *N*-ethyl derivative **7b** ($EC_{50} = 0.015\ \mu M$). Reduction (*N*-methyl (**7a**), $EC_{50} = 0.300\ \mu M$) or elongation (*N*-propyl (**7c**), $EC_{50} = 0.150\ \mu M$) of the alkyl chain reduced the activity. A comparable dependence of the ER binding on the chain length was determined in the HES and DES series. Therefore, we assume that the alkyl residues at the imidazoline core interact with comparable hydrophobic parts in the LBD (see Figure 6). The decline of activity after N,N′-dialkylation can be interpreted as a steric hindrance during ER binding comparable to that indicated for 7α,11β-disubstituted E2 derivatives.[25]

Two findings cannot be explained at the moment: the inactivity of the *C*2-alkyl derivatives and the activity of the cationic N,N′-dialkyl derivatives. The positive charge of the compounds **9a**,**b** did not disturb the ER interaction, whereas the small methyl group located at C2 prevented gene activation. These have to be clarified in further studies.

Experimental Section

General Procedures. The following instruments were used to collect various spectra: IR spectra (KBr pellets), Perkin-Elmer model 580 A; ^{1}H NMR, Burker ADX 400 spectrometer at 400 MHz (internal standard, TMS); EI-MS spectra, CH-7A-Varian MAT (70 eV) and Kratos MS 25 RF (80 eV). Elemental analyses were carried out at the Microlaboratory of the Free University of Berlin. Stability studies were made on an HPLC-system with a Kontron high-pressure gradient system, HPLC pump 422, HPLC autosampler 465, HPLC−UV detector 430A, (BiotekKontron, Germany), and thermostat K 20 (Haake, Germany). As the HPLC column a Nucleosil 100-5 C_{18} 250 mm × 4 mm i.d. with Nucleosil 120-5 C_{18} precolumn 30 mm × 4 mm i.d. (Macharey & Nagel) was used. All computational graphics were built using SYBYL 6.7 (Tripos Inc. 1699 South Hanley Road, St. Louis, MO 63144). Geometry optimization was carried out utilizing the MM3 force field within the program running on an INDY workstation. The liquid scintillation counter was a 1450 Microbeta Plus (Wallac Finnland), and luminescence measurement was carried out using a Microlumat LB 96 P (EG & G Berthold, Germany).

Syntheses. The (1*R*,2*S*)/(1*S*,2*R*)-1,2-diamino-1,2-diarylethanes **1a**−**d** and **12** and the (4*R*,5*S*)/(4*S*,5*R*)-4,5-diaryl-2-imidazolines **2a**−**d** and **3a**−**d** were synthesized as described earlier.[9,10]

General Procedure for the Synthesis of 2-Alkyl-4,5-diaryl-2-imidazolines with Triethylorthoesters (Method A). An aqueous solution (1.00 mmol) of the appropriate 1,2-diamino-1,2-diarylethane with 0.25 mL of HCl_{conc} and 2.00 mL of either triethyl orthoacetate or triethyl orthopropionate was heated to reflux. The reaction mixture was cooled to room temperature and combined with 20 mL of chloroform. The pH of the solution was adjusted to 1 with 2 N HCl. The organic layer was separated, and the water phase was washed twice with chloroform. The water phase was neutralized with a saturated $NaHCO_3$ solution, and the products were extracted three times with chloroform. The combined chloroform phases were dried over Na_2SO_4, and the solvent was evaporated. Purification was performed by column chromatography on silica gel, recrystallization, or isolation as the hydrochloride by treatment with etheral HCl.

General Procedure for the Synthesis of 2-Alkyl-4,5-diaryl-2-imidazolines with Iminoethers (Method B). Amounts of 1.00 mmol of the appropriate 1,2-diamino-1,2-diarylethane and 1.30 mmol of the iminoether were dissolved in 15 mL of dry ethanol and heated to reflux. Subsequently, the solvent was evaporated and the crude product was purified by column chromatography or recrystallization.

(4*R*,5*S*)/(4*S*,5*R*)-4,5-Bis(4-methoxyphenyl)-2-methyl-2-imidazoline (4a). 4a was obtained from (1*R*,2*S*)/(1*S*,2*R*)-1,2-diamino-1,2-bis(4-methoxyphenyl)ethane **1a** (3.0 mmol, 817

mg) and triethyl orthoacetate (6.00 mL) by method A. Reaction time: 5 h. Purification: recrystallization from chloroform. Yield: 2.3 mmol (680 mg), 77% colorless powder; mp 121 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−3300 br, w (NH), 3147 m, 2901 m, 2840 m (OCH_3), 1612 s, 1584 m, 1514 s, 1469 m, 1255 s, 1173 m, 1036 m, 799 m. ^{1}H NMR (DMSO-d_6): δ 2.01 (s, 3H, CH_3), 3.60 (s, 6H, OCH_3), 5.07 (s, 2H, ArC*H*), 6.58 (*AA′BB′*, ^3J = 8.6 Hz, 4H, Ar*H*-3, Ar*H*-5), 6.81 (*AA′BB′*, ^3J = 8.6 Hz, 4H, Ar*H*-2, Ar*H*-6).

(4*R*,5*S*)/(4*S*,5*R*)-4,5-Bis(2-chloro-4-methoxyphenyl)-2-methyl-2-imidazoline (4b). 4b was obtained from (1*R*,2*S*)/(1*S*,2*R*)-1,2-diamino-1,2-bis(2-chloro-4-methoxyphenyl)-ethane **1b** (3.0 mmol, 1024 mg) and triethyl orthoacetate (6.00 mL) by method A. Reaction time: 5 h. Isolation: as hydrochloride and recrystallization from methanol/ether. Yield: 2.4 mmol (975 mg), 81% colorless powder; mp 231 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−3300 br, m, (NH), 3060 s, 2930 s, 2840 m (OCH_3), 1605 s, 1500 s, 1440 s, 1290 s, 1245 m, 1040 s, 1025 m, 855 s. ^{1}H NMR (DMSO-d_6): δ 2.43 (s, 3H, CH_3), 3.69 (s, 6H, OCH_3), 5.97 (s, 2H, ArC*H*), 6.82 (dd, ^3J = 8.7 Hz, ^4J = 2.4 Hz, 2H, Ar*H*-5), 6.87 (d, ^4J = 2.4 Hz, 2H, Ar*H*-3), 7.17 (d, ^3J = 8.7 Hz, Ar*H*-6), 10.79 (s, 2H, N*H*, exchangeable by D_2O).

(4*R*,5*S*)/(4*S*,5*R*)-4-(2-Chloro-4-methoxyphenyl)-5-(2,6-dichloro-4-methoxyphenyl)-2-methyl-2-imidazoline (4c). 4c was obtained from (1*R*,2*S*)/(1*S*,2*R*)-1,2-diamino-1-(2-chloro-4-methoxyphenyl)-2-(2,6-dichloro-4-methoxyphenyl)ethane **1c** (0.50 mmol, 224 mg) and the hydrochloride of acetimidic acid ethyl ester **13a** (0.65 mmol, 80 mg) by method B. Reaction time: 72 h. Purification: chromatography on silica gel with chloroform/methanol (4/1) and isolation as hydrochloride. Yield: 0.41 mmol (179 mg), 82% ochre powder; mp 227 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−3300 br, m (NH), 3220 m, 3064 m, 2927 m, 2699 m, 1602 s, 1556 m, 1466 m, 1432 m, 1405 m, 1285 m, 1264 m, 1243 m, 1201 m, 1180 m, 1072 m, 1040 m, 881 m, 852 m, 816 m, 789 m. ^{1}H NMR (DMSO-d_6): δ 2.36 (s, 3H, CH_3), 3.70 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 5.91 (d, ^3J = 12.8 Hz, 1H, ArC*H*), 6.37 (d, ^3J = 12.8 Hz, 1H, ArC*H*), 6.86−6.89 (m, 3H, Ar*H*-3, Ar*H*-5, Ar′*H*-5), 7.05 (d, ^4J = 2.7 Hz, 1H, Ar′*H*-3), 7.53 (d, ^3J = 9.4 Hz, 1H, Ar′*H*-6), 10.30 (s, 2H, N*H*, exchangeable by D_2O).

(4*R*,5*S*)/(4*S*,5*R*)-4,5-Bis(2-fluoro-4-methoxyphenyl)-2-methyl-2-imidazoline (4d). 4d was obtained from (1*R*,2*S*)/(1*S*,2*R*)-1,2-diamino-1,2-bis(2-fluoro-4-methoxyphenyl)-ethane **1d** (3.0 mmol, 925 mg) and triethyl orthoacetate (6.00 mL) by method A. Reaction time: 6 h. Purification: recrystallization from chloroform. Yield: 2.3 mmol (778 mg), 76% colorless powder; mp 206 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−3300 br, m, (NH), 3080 s, 2940 s, 2840 m (OCH_3), 1625 s, 1510 s, 1445 m, 1315 m, 1290 s, 1160 m, 1115 s, 1025 m, 835 m. ^{1}H NMR (DMSO-d_6): δ 2.15 (s, 3H, CH_3), 3.63 (s, 6H, OCH_3), 5.49 (s, 2H, ArC*H*), 6.52−6.55 (m, 4H, Ar*H*-3, Ar*H*-5), 6.97 (dd, ^3J = 8.4 Hz, ^{4}J(H,F) = 8.4 Hz, 2H, Ar*H*-6).

(4*R*,5*S*)/(4*S*,5*R*)-4,5-Bis(4-methoxyphenyl)-2-ethyl-2-imidazoline (4e). 4e was obtained from (1*R*,2*S*)/(1*S*,2*R*)-1,2-diamino-1,2-bis(4-methoxyphenyl)ethane **1a** (1.00 mmol, 272 mg) and triethyl orthopropionate (2.00 mL) by method A. Reaction time: 5 h. Purification: recrystallization from chloroform. Yield: 0.84 mmol (260 mg), 84% yellow powder; mp 138 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2960 m, 2835 w (OCH_3), 1610 m, 1512 m, 1260 s, 1095 s, 1025 s, 800 s. ^{1}H NMR (DMSO-d_6): δ 1.22 (t, ^3J = 7.6 Hz, 3H, CH_2CH_3), 2.34 (q, ^3J = 7.6 Hz, 2H, CH_2CH_3), 3.60 (s, 6H, OCH_3), 5.07 (s, 2H, ArC*H*), 6.58 (*AA′BB′*, ^3J = 8.7 Hz, 4H, Ar*H*-3, Ar*H*-5), 6.82 (*AA′BB′*, ^3J = 8.7 Hz, 4H, Ar*H*-2, Ar*H*-6).

(4*R*,5*S*)/(4*S*,5*R*)-4,5-Bis(2-chloro-4-methoxyphenyl)-2-ethyl-2-imidazoline (4f). 4f was obtained from (1*R*,2*S*)/(1*S*,2*R*)-1,2-diamino-1,2-bis(2-chloro-4-methoxyphenyl)-ethane **1b** (3.0 mmol, 1024 mg) and triethyl orthopropionate (6.00 mL) by method A: Reaction time: 5 h. Isolation: as hydrochloride and recrystallization from methanol/ether. Yield: 1.7 mmol (697 mg), 56% colorless crystals; mp 225 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−3300 br, m, (NH), 3055 m, 2930 s, 2690 m, 1605 s, 1570 m, 1500 s, 1460 m, 1440 m, 1290 s, 1250 s, 1040 s, 855 m. ^{1}H NMR (DMSO-d_6): δ 1.33 (t, ^3J = 7.5 Hz, 3H, CH_2CH_3), 2.77 (q, ^3J = 7.5 Hz, 2H, CH_2CH_3), 3.69 (s, 6H, OCH_3), 5.99 (s, 2H, ArC*H*), 6.77 (dd, ^3J = 8.8 Hz, ^4J = 2.5 Hz, 2H, Ar*H*-5), 6.88 (d, ^4J = 2.5 Hz, 2H, Ar*H*-3), 7.15 (d, ^3J = 8.8 Hz, Ar*H*-6), 10.75 (s, 2H, N*H*, exchangeable by D_2O).

(4*R*,5*S*)/(4*S*,5*R*)-4,5-Bis(2-fluoro-4-methoxyphenyl)-2-ethyl-2-imidazoline (4g). 4g was obtained from (1*R*,2*S*)/(1*S*,2*R*)-1,2-diamino-1,2-bis(2-fluoro-4-methoxyphenyl)-ethane **1d** (3.0 mmol, 925 mg) and triethyl orthopropionate (6.00 mL) by method A. Reaction time: 4 h. Isolation: as hydrochloride and recrystallization from methanol/ether. Yield: 2.8 mmol (1062 mg), 92%; mp 236 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−3300 m, br, (NH), 3065 m, 2940 s, 1625 s, 1510 s, 1445 m, 1315 m, 1290 m, 1270 m, 1160 m, 1115 m, 1030 m, 835 m. ^{1}H NMR (DMSO-d_6): δ 1.33 (t, ^3J = 7.6 Hz, 3H, CH_2CH_3), 2.74 (q, ^3J = 7.6 Hz, 2H, CH_2CH_3), 3.66 (s, 6H, OCH_3), 5.84 (s, 2H, ArC*H*), 6.60−6.66 (m, 4H, Ar*H*-3, Ar*H*-5), 7.10 (dd, ^3J = 8.8 Hz, ^4J(H,F) = 8.6 Hz, 2H, Ar*H*-6), 10.79 (s, 2H, N*H*, exchangeable by D_2O).

(4*R*,5*S*)/(4*S*,5*R*)-4,5-Bis(4-methoxyphenyl)-2-(2-methoxyethyl)-2-imidazoline (4h). 4h was obtained from (1*R*,2*S*)/(1*S*,2*R*)-1,2-diamino-1,2-bis(4-methoxyphenyl)ethane **1a** (3.00 mmol, 817 mg) and the hydrochloride of 3-methoxypropionimidic acid ethyl ester **13b** (3.90 mmol, 654 mg) by method B. Reaction time: 3 h. Purification: column chromatography with chloroform/methanol (9/1) and isolation as hydrochloride. Yield: 2.34 mmol (882 mg), 78% yellow powder; mp 65 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−3300 br, m (NH), 3073 m, 2935 m, 2838 m (OCH_3), 1611 s, 1583 m, 1515 s, 1252 s, 1463 w, 1300 w, 1180 m, 1114 w, 1031 m. ^{1}H NMR (DMSO-d_6): δ 2.98 (t, ^3J = 5.9 Hz, 2H, $CH_2CH_2OCH_3$), 3.40 (s, 3H, $CH_2CH_2OCH_3$), 3.63 (s, 6H, OCH_3), 3.81 (t, ^3J = 5.9 Hz, 2H, $CH_2CH_2OCH_3$), 5.62 (s, 2H, ArC*H*), 6.70 (*AA′BB′*, ^3J = 8.7 Hz, 4H, Ar*H*-3, Ar*H*-5), 6.91 (*AA′BB′*, ^3J = 8.7 Hz, 4H, Ar*H*-2, Ar*H*-6), 10.93 (s, 2H, N*H*, exchangeable by D_2O).

(4*R*,5*S*)/(4*S*,5*R*)-4,5-Bis(2-chloro-4-methoxyphenyl)-2-(2-methoxyethyl)-2-imidazoline (4i). 4i was obtained from (1*R*,2*S*)/(1*S*,2*R*)-1,2-diamino-1,2-bis(2-chloro-4-methoxyphenyl)-ethane **1b** (3.00 mmol, 1024 mg) and the hydrochloride of 3-methoxypropionimidic acid ethyl ester **13b** (3.90 mmol, 654 mg) by method B. Reaction time: 3 h. Purification: column chromatography with chloroform/methanol (9/1) and isolation as hydrochloride and recrystallization from methanol/ether. Yield: 1.41 mmol (630 mg), 47% yellow powder; mp 68 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−3300 br, w (NH), 3059 m, 2929 m, 2839 m (OCH_3), 1606 s, 1571 m, 1500 s, 1293 m, 1247 m, 1041 m, 755 m. ^{1}H NMR (DMSO-d_6): δ 3.02 (t, ^3J = 5.9 Hz, 2H, $CH_2CH_2OCH_3$), 3.40 (s, 3H, $CH_2CH_2OCH_3$), 3.69 (s, 6H, OCH_3), 3.81 (t, ^3J = 5.9 Hz, 2H, $CH_2CH_2OCH_3$), 6.01 (s, 2H, ArC*H*), 6.79 (dd, ^3J = 8.8 Hz, ^4J = 2.5 Hz, 2H, Ar*H*-5), 6.88 (d, ^4J = 2.5 Hz, 2H, Ar*H*-3), 7.09 (d, ^3J = 8.7 Hz, 2H, Ar*H*-6), 10.93 (s, 2H, N*H*, exchangeable by D_2O).

General Procedure for the Synthesis of (4*R*,5*S*)/(4*S*,5*R*)-*N*-Ethyl-1,2-diaryl-2-imidazolines (Method C). A solution of the appropriate 1,2-diamino-1,2-diarylethane (4.00 mmol) in 5 mL of dry ethanol was heated to reflux. An amount of 1.00 mmol of ethyl iodine in 2 mL of dry ethanol was added slowly to the hot solution. The solvent was evaporated, and the crude was purified by column chromatography on silica gel. *N*-Alkyl-1,2-diamino-1,2-diarylethanes can be converted into (4*R*,5*S*)/(4*S*,5*R*)-*N*-alkyl-1,2-diaryl-2-imidazolines by method A.

(1*R*,2*S*)/(1*S*,2*R*)-*N*-Ethyl-1,2-diamino-1,2-bis(4-methoxyphenyl)ethane (10). 10 was obtained from (1*R*,2*S*)/(1*S*,2*R*)-1,2-diamino-1,2-bis(4-methoxyphenyl)ethane **1a** (4.0 mmol (1089 mg)). Reaction time: 16 h. Purification: column chromatography with chloroform/methanol (9/1) under addition of 1% triethylamine. Yield: 0.6 mmol (187 mg), 60% colorless powder; mp 77 °C. ^{1}H NMR (DMSO-d_6): δ 0.84 (t, ^3J = 7.1 Hz, 3H, CH_2CH_3), 1.55 (br, 3H, N*H*, NH_2, exchangeable by D_2O), 2.18−2.27 (m, 2H, CH_2CH_3), 3.58 (d, ^3J = 6.6 Hz, 1H, ArC*H*), 3.72 (s, 6H, OCH_3), 3.85 (d, ^3J = 6.6 Hz, 1H, ArC*H*), 6.81−6.83 (m, 4H, Ar*H*-3, Ar*H*-5, Ar′*H*-3, Ar′*H*-5), 7.09−7.13 (*m*, 4H, Ar*H*-2, Ar*H*-6, Ar′*H*-2, Ar′*H*-6).

(4*R*,5*S*)/(4*S*,5*R*)-*N*-Ethyl-4,5-bis(4-methoxyphenyl)-2-imidazoline (6). 6 was obtained from **10** (1.00 mmol, 300 mg) and triethyl formate (2.00 mL) by method A. Reaction time: 6 h. Isolation as hydrochloride and recrystallization from methanol/ether. Yield: 0.88 mmol (304 mg), 88% orange oil. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−3300 br (NH), 3002 m, 2961 m, 2939 m, 2839 m (OCH_3), 1639 m, 1613 m, 1514 s, 1462 m, 1299 m, 1236 s, 1180 m, 1032 m, 838 m, 755 m. ^{1}H NMR (DMSO-d_6): δ 1.17 (t, ^3J = 7.2 Hz, 3H, CH_2CH_3), 2.98−3.03 (m, 1H, CH_2-CH_3), 3.50−3.56 (m, 1H, CH_2CH_3), 3.64 (s, 3H, OCH_3), 3.64 (s, 3H, OCH_3), 5.64 (d, ^3J = 12.1 Hz, 1H, ArC*H*), 5.75 (d, ^3J = 12.1 Hz, 1H, ArC*H*), 6.71 (*AA′BB′*, ^3J = 8.7 Hz, 4H, Ar*H*-3, Ar*H*-5, Ar′*H*-3, Ar′*H*-5), 6.93−6.96 (m, 4H, Ar*H*-2, Ar*H*-6, Ar′*H*-2, Ar′*H*-6), 8.94 (s, 1H, N=C*H*−N), 10.84 (s, 1H, N*H*, exchangeable by D_2O).

General Procedure for the Synthesis of (4*R*,5*S*)/(4*S*,5*R*)-*N*-Alkyl-1,2-bis(2-chloro-4-methoxy)-2-imidazolines (Method D). An amount of 2.20 mmol of butyllithium (1.6 M in hexane) was added to a cooled solution (−78 °C) of **2b** (1.00 mmol) in 10 mL of dry THF. After the mixture was stirred for 15 min, 1.10 mmol of alkyl iodine in 2 mL of dry ethanol was added slowly. The stirred solution was allowed to warm to room temperature overnight. Etheral HCl was added, and the solvent was evaporated. The crude product was purified by column chromatography on silica gel.

(4*R*,5*S*)/(4*S*,5*R*)-*N*-Methyl-4,5-bis(2-chloro-4-methoxyphenyl)-2-imidazoline (6a). 6a was obtained from **2b** (0.75 mmol, 291 mg) and methyl iodine (0.83 mmol, 117 mg, 51 μL). Purification: column chromatography with chloroform/methanol (9/1) and isolation as hydrochloride. Yield: 0.69 mmol (277 mg), 92% orange powder; mp 93 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−3300 br, m (NH), 3088 m, 2838 m (OCH_3), 1650 s, 1607 s, 1500 s, 1294 m, 1244 m, 1044 s. ^{1}H NMR (DMSO-d_6): δ 2.95 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 5.97 (d, ^3J = 12.3 Hz, 1H, ArC*H*), 6.06 (d, 1H, ^3J = 12.3 Hz, ArC*H*), 6.73 (dd, ^3J = 8.8 Hz, ^4J = 2.6 Hz, 1H, Ar*H*-5), 6.80 (dd, ^3J = 8.7 Hz, ^4J = 2.6 Hz, 1H, Ar′*H*-5), 6.88 (d, ^4J = 2.6 Hz, 1H, Ar*H*-3), 6.93 (d, ^4J = 2.6 Hz, 1H, Ar′*H*-3), 7.01 (d, ^3J = 8.8 Hz, 1H, Ar*H*-6), 7.18 (d, ^3J = 8.7 Hz, 1H, Ar′*H*-6), 8.94 (s, 1H, N=C*H*−N), 10.89 (s, 1H, N*H*, exchangeable by D_2O).

(4*R*,5*S*)/(4*S*,5*R*)-*N*-Ethyl-4,5-bis(2-chloro-4-methoxyphenyl)-2-imidazoline (6b). 6b was obtained from **2b** (0.70 mmol, 271 mg) and ethyl iodine (0.77 mmol, 129 mg, 67 μL). Purification: column chromatography with ether/methanol (6/1). Yield: 0.53 mmol (200 mg), 76% yellow oil. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3019 m, 2840 w (OCH_3), 1684 w, 1604 m, 1496 m, 1287 w, 1216 s, 1044 m, 874 w, 758 s. ^{1}H NMR (DMSO-d_6): δ 1.13 (t, ^3J = 7.2 Hz, 3H, CH_2CH_3), 2.76−2.87 (m, 1H, CH_2CH_3), 3.14−3.25 (m, 1H, CH_2CH_3), 3.69 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 5.51 (d, ^3J = 11.0 Hz, 1H, ArC*H*), 5.85 (d, ^3J = 11.0 Hz, 1H, ArC*H*), 6.45 (dd, ^3J = 8.7 Hz, 1H, Ar*H*-5), 6.62−6.65 (m, ^4J = 2.5 Hz, 2H, Ar*H*-3, Ar*H*-5), 6.68 (d, ^3J = 8.7 Hz, 1H, Ar*H*-6), 6.72 (d, ^4J = 2.5 Hz, 1H, Ar*H*-3), 7.17 (d, ^3J = 9.1 Hz, 1H, Ar*H*-6), 7.31 (br, 1H, N=C*H*−N).

(4*R*,5*S*)/(4*S*,5*R*)-*N*-Propyl-4,5-bis(2-chloro-4-methoxyphenyl)-2-imidazoline (6c). 6c was obtained from **2b** (0.70 mmol, 271 mg) and propyl iodine (0.77 mmol, 131 mg, 72 μL). Purification: column chromatography with chloroform/methanol (9/1), isolation as hydrochloride, and recrystallization from methanol/ether. Yield: 0.09 mmol (40 mg), 13% yellow needles; mp 103 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−3300 br (NH), 3082 m, 2941 m, 2839 w (OCH_3), 1638 s, 1607 s, 1499 s, 1294 m, 1248 m, 1083 m, 1043 s. ^{1}H NMR (DMSO-d_6): δ 0.88 (t, ^3J = 6.6 Hz, 3H, $CH_2CH_2CH_3$), 1.49−1.58 (m, 2H, $CH_2CH_2CH_3$), 2.83−2.90 (m, 1H, $CH_2CH_2CH_3$), 3.40−3.47 (m, 1H, $CH_2CH_2CH_3$), 3.69 (s, 6H, OCH_3), 6.03 (d, ^3J = 12.2 Hz, 1H, ArC*H*), 6.09 (d, 1H, ^3J = 12.2 Hz, ArC*H*), 6.69 (dd, ^3J = 8.8 Hz, ^4J = 2.6 Hz, 1H, Ar*H*-5), 6.84 (dd, ^3J = 8.6 Hz, ^4J = 2.6 Hz, 1H, Ar′*H*-5), 6.87 (d, ^4J = 2.6 Hz, 1H, Ar*H*-3), 6.94 (d, ^4J = 2.6 Hz, 1H, Ar′*H*-3), 6.98 (d, ^3J = 8.8 Hz, 1H, Ar*H*-6), 7.25 (d, ^3J = 8.6 Hz, 1H, Ar′*H*-6), 8.95 (s, 1H, N=C*H*−N), 10.95 (s, 1H, N*H*, exchangeable by D_2O).

General Procedure for the Synthesis of (4*R*,5*S*)/(4*S*,5*R*)-*N*,*N*′-Dialkyl-1,2-bis(2-chloro-4-methoxyphenyl)-2-imidazolinium Chlorides (Method E). Amounts of 1.00 mmol of **2b** and 1.20 mmol of NaH (suspended in paraffin) were dissolved in 20 mL of dry THF and heated to reflux. After the addition of 2.50 mmol of alkyl iodine in 2 mL of dry THF, the solution was heated for further 18 h. Subsequently, an amount of 20 mL of water was added at room temperature and the water phase was extracted twice with dichloromethane. The combined organic layers were dried over Na_2SO_4, and etheral HCl was added. After evaporation of the solvent, the crude product was purified by column chromatography on silica gel or recrystallization.

(4*R*,5*S*)/(4*S*,5*R*)-*N*,*N*′-Dimethyl-4,5-bis(2-chloro-4-methoxyphenyl)-2-imidazolinium chloride (8a). 8a was obtained from **2b** (0.29 mmol, 120 mg) and methyl iodine (0.73 mmol, 103 mg, 45 μL). Recrystallization from methanol/ether. Yield: 0.24 mmol (98 mg), 81% yellow needles; mp 181 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−3400 br, 2935 m, 2813 w, 1660 s, 1607 s, 1573 m, 1500 s, 1465 m, 1440 m, 1327 m, 1294 s, 1247 s, 1192 m, 1148 m, 1043 s, 871 m. ^{1}H NMR (DMSO-d_6): δ 2.97 (s, 6H, CH_3), 3.70 (s, 6H, OCH_3), 6.00 (s, 2H, ArC*H*), 6.77 (dd, ^3J = 8.7 Hz, ^4J = 2.6 Hz, 2H, Ar*H*-5), 6.92 (d, ^4J = 2.6 Hz, 2H, Ar*H*-3), 7.06 (d, ^3J = 8.7 Hz, 2H, Ar*H*-6), 8.88 (s, 1H, N=C*H*−N).

(4*R*,5*S*)/(4*S*,5*R*)-*N*,*N*′-Diethyl-4,5-bis(2-chloro-4-methoxyphenyl)-2-imidazolinium Chloride (8b). 8b was obtained from **2b** (0.70 mmol, 271 mg) and ethyl iodine (1.75 mmol, 273 mg, 141 μL). Recrystallization from methanol/ether. Yield: 0.36 mmol (158.3 mg), 51% yellow needles; mp 190 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−3500 br, 2976 m, 2839 w (OCH_3), 1647 s, 1608 s, 1502 s, 1295 m, 1262 s, 1038 m, 878 m, 849 m. ^{1}H NMR (DMSO-d_6): δ 1.19 (t, ^3J = 7.2 Hz, 6H, CH_2CH_3), 3.01−3.20 (m, 2H, CH_2CH_3), 3.49−3.58 (m, 2H, CH_2CH_3), 3.69 (s, 6H, OCH_3), 6.10 (s, 2H, ArC*H*), 6.77 (dd, ^3J = 8.7 Hz, ^4J = 2.5 Hz, 2H, Ar*H*-5), 6.93 (d, ^4J = 2.5 Hz, 2H, Ar*H*-3), 7.10 (d, ^3J = 8.7 Hz, 2H, Ar*H*-6), 8.98 (s, 1H, N=C*H*−N).

(4*R*,5*S*)/(4*S*,5*R*)-*N*,*N*′-Dipropyl-4,5-bis(2-chloro-4-methoxyphenyl)-2-imidazolinium Chloride (8c). 8c was obtained from **2b** (0.34 mmol, 140 mg) and propyl iodine (0.85 mmol, 145 mg, 79 μL). Recrystallization from methanol/ether. Yield: 0.26 mmol (117 mg), 77% yellow needles; mp 231 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−3400 br, 2964 m, 2935 m, 1648 s, 1632 s, 1608 s, 1572 m, 1498 s, 1263 m, 1441 m, 1292 s, 1243 s, 1043 s, 870 m, 851 m. ^{1}H NMR (DMSO-d_6): δ 0.89 (t, ^3J = 7.3 Hz, 6H, $CH_2CH_2CH_3$), 1.56 (m, 4H, $CH_2CH_2CH_3$), 3.01 (m, 2H, $CH_2CH_2CH_3$), 3.43 (m, 2H, $CH_2CH_2CH_3$), 3.70 (s, 6H, OCH_3), 6.13 (s, 2H, ArC*H*), 6.77 (dd, ^3J = 8.7 Hz, ^4J = 2.5 Hz, 2H, Ar*H*-5), 6.92 (d, ^4J = 2.5 Hz, 2H, Ar*H*-3), 7.10 (d, ^3J = 8.7 Hz, 2H, Ar*H*-6), 9.03 (s, 1H, N=C*H*−N).

General Procedure for Ether Cleavage with BBr_3 (Method F). A solution of the methyl ether (1.00 mmol) in 20 mL of dry CH_2Cl_2 was cooled to −60 °C. At this temperature, BBr_3 (2.25 mmol/methoxy group) in 5 mL of dry CH_2Cl_2 was added under N_2 atmosphere. Then the reaction mixture was allowed to warm to room temperature and was stirred for further 48 h. After the reaction was cooled with an ice bath, the surplus of BBr_3 was hydrolyzed three times with dry methanol. The resulting crude product (hydrobromide) was purified by recrystallization.

(4*R*,5*S*)/(4*S*,5*R*)-4,5-Bis(4-hydroxyphenyl)-2-methyl-2-imidazoline (5a). 5a was obtained from **4a** (1.00 mmol, 296.4 mg). Recrystallization from methanol. Yield: 0.91 mmol (317.2 mg), 91% yellow powder; mp 289 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−2500 br, s (OH), 1615 s, 1591 s, 1516 s, 1447 m, 1427 m, 1356 m, 1260 s, 1201 s, 1048 m, 876 m, 807 m. MS (EI, 100 °C): *m*/*z* (%) = 268 (27) [$M^{+\bullet}$], 161 (23), 147 (100), 120 (18), 106 (54). ^{1}H NMR (DMSO-d_6): δ 2.40 (s, 3H, CH_3), 5.55 (s, 2H, ArC*H*), 6.51 (*AA′BB′*, ^3J = 8.5 Hz, 4H, Ar*H*-3, Ar*H*-5), 6.79 (*AA′BB′*, ^3J = 8.5 Hz, 4H, Ar*H*-2, Ar*H*-6), 9.32 (s, 2H, O*H*, exchangeable by D_2O), 10.42 (s, 2H, N*H*, exchangeable by D_2O). Anal. ($C_{16}H_{16}N_2O_2$·HBr) C, H, N.

(4*R*,5*S*)/(4*S*,5*R*)-4,5-Bis(2-chloro-4-hydroxyphenyl)-2-methyl-2-imidazoline (5b). 5b was obtained from **4b** (1.00 mmol, 401.7 mg). Recrystallization from methanol. Yield: 0.96 mmol (408.5 mg), 96% brown powder; mp >300 °C. IR (KBr,

cm^{-1}): $\bar{\nu}$ = 3600−2500 br, s (OH), 1603 s, 1578 s, 1499 s, 1432 s, 1287 s, 1258 s, 1212 s, 1050 m, 1030 m, 896 m, 857 m, 820 m. MS (EI, 100 °C): *m*/*z* (%) = 336 (31) [M$^{+\bullet}$], 181 (100), 156 (25), 140 (42), 105 (24). ^{1}H NMR (DMSO-d_6): δ 2.40 (s, 3H, C*H*$_3$), 5.91 (s, 2H, ArC*H*), 6.57 (dd, ^3J = 8.6 Hz, ^4J = 2.1 Hz, 2H, Ar*H*-5), 6.63 (d, ^4J = 2.1 Hz, 2H, Ar*H*-3), 7.02 (d, ^3J = 8.6 Hz, 2H, Ar*H*-6), 9.94 (s, 2H, O*H*, exchangeable by D$_2$O), 10.57 (s, 2H, N*H*, exchangeable by D$_2$O). Anal. ($C_{16}H_{14}Cl_2N_2O_2$·HBr·0.5H$_2$O) C, H, N.

(4*R*,5*S*)/(4*S*,5*R*)-4-(2-Chloro-4-hydroxyphenyl)-5-(2,6-dichloro-4-hydroxyphenyl)-2-methyl-2-imidazoline (5c). 5c was obtained from **4c** (0.25 mmol, 109.1 mg). Recrystallization from methanol. Yield: 0.20 mmol (90.4 mg), 80% ochre powder; mp >300 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−2500 br, s (OH), 1602 s, 1567 m, 1501 m, 1460 w, 1429 m, 1259 m, 1212 m, 1074 m, 954 m, 898 w, 861 w, 788 w. MS (EI, 400 °C): *m*/*z* (%) = 370 (31) [M$^{+\bullet}$], 215 (41), 181 (100), 156 (20), 140 (45), 105 (20). ^{1}H NMR (DMSO-d_6): δ 2.35 (s, 3H, C*H*$_3$), 5.89 (d, ^3J = 12.9 Hz, 1H, ArC*H*), 6.32 (d, ^3J = 12.9 Hz, 1H, ArC*H*), 6.61 (d, ^4J = 2.4 Hz, 1H, Ar*H*-3), 6.64 (d, ^4J = 2.4 Hz, 1H, Ar′*H*-3), 6.67 (dd, ^3J = 8.6 Hz, ^4J = 2.4 Hz, 1H, Ar′*H*-5), 6.79 (d, ^4J = 2.4 Hz, 1H, Ar*H*-5), 7.39 (d, ^3J = 8.6 Hz, 1H, Ar′*H*-6), 10.12 (s, 1H, O*H*, exchangeable by D$_2$O), 10.54 (s, 1H, O*H*, exchangeable by D$_2$O), 10.63 (s, 1H, N*H*, exchangeable by D$_2$O), 10.81 (s, 1H, N*H*, exchangeable by D$_2$O). Anal. ($C_{16}H_{13}Cl_3N_2O_2$·HBr·H$_2$O) C, H, N.

(4*R*,5*S*)/(4*S*,5*R*)-4,5-Bis(2-fluoro-4-hydroxyphenyl)-2-methyl-2-imidazoline (5d). 5d was obtained from **4d** (0.50 mmol, 169.6 mg). Recrystallization from methanol. Yield: 0.39 mmol (165.6 mg), 78% brown powder; mp >300 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−2500 br, m (OH), 1631 s, 1606 s, 1501 m, 1460 m, 1295 m, 1253 m, 1202 m, 1152 w, 1104 m, 1042 m, 967 w, 845 w. MS (EI, 120 °C): *m*/*z* (%) = 304 (31) [M$^{+\bullet}$], 165 (100), 124 (55), 96 (17). ^{1}H NMR (DMSO-d_6): δ 2.39 (s, 3H, C*H*$_3$), 5.76 (s, 2H, ArC*H*), 6.32 (dd, ^3J(H,F) = 12.1 Hz, ^4J = 2.2 Hz, 2H, Ar*H*-3), 6.43 (dd, ^3J = 8.5 Hz, ^4J = 2.2 Hz, 2H, Ar*H*-5), 6.94 (dd, ^3J = 8.5 Hz, ^4J(H,F) = 8.5 Hz, 2H, Ar*H*-6), 9.90 (s, 2H, O*H*, exchangeable by D$_2$O), 10.56 (s, 2H, N*H*, exchangeable by D$_2$O). Anal. ($C_{16}H_{14}F_2N_2O_2$·HBr·2H$_2$O) C, H, N.

(4*R*,5*S*)/(4*S*,5*R*)-4,5-Bis(4-hydroxyphenyl)-2-ethyl-2-imidazoline (5e). 5e was obtained from **4e** (0.50 mmol, 155.2 mg). Recrystallization from methanol. Yield: 0.45 mmol (172.8 mg), 90% brown powder; mp 281 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−2500 br, s (OH), 1604 m, 1517 s, 1445 m, 1271 s, 1215 s, 1176 m, 1104 m, 1085 m, 807 m. MS (EI, 150 °C): *m*/*z* (%) = 282 (35) [M$^{+\bullet}$], 161 (100), 146 (12), 106 (43). ^{1}H NMR (DMSO-d_6): δ 1.33 (t, ^3J = 7.6 Hz, 3H, CH$_2$C*H*$_3$), 2.75 (q, ^3J = 7.6 Hz, 2H, C*H*$_2$CH$_3$), 5.56 (s, 2H, ArC*H*), 6.52 (*AA*′*BB*′, ^3J = 8.5 Hz, 4H, Ar*H*-3, Ar*H*-5), 6.79 (*AA*′*BB*′, ^3J = 8.5 Hz, 4H, Ar*H*-2, Ar*H*-6), 9.33 (s, 2H, O*H*, exchangeable by D$_2$O), 10.43 (s, 2H, N*H*, exchangeable by D$_2$O). Anal. ($C_{17}H_{18}N_2O_2$·HBr·H$_2$O) C, H, N.

(4*R*,5*S*)/(4*S*,5*R*)-4,5-Bis(2-chloro-4-hydroxyphenyl)-2-ethyl-2-imidazoline (5f). 5f was obtained from **4f** (0.50 mmol, 193.8 mg). Recrystallization from methanol. Yield: 0.44 mmol (192.7 mg), 87% beige powder; mp 308 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3500−2600 br, m (OH), 1688 s, 1602 s, 1578 s, 1500 m, 1428 m, 1303 m, 1263 s, 1169 m, 1027 m. MS (EI, 290 °C): *m*/*z* (%) = 350 (27) [M$^{+\bullet}$], 195 (100), 140 (40), 105 (17). ^{1}H NMR (DMSO-d_6): δ 1.31 (t, ^3J = 7.6 Hz, 3H, CH$_2$C*H*$_3$), 2.74 (q, ^3J = 7.6 Hz, 2H, C*H*$_2$CH$_3$), 5.92 (s, 2H, ArC*H*), 6.57 (dd, ^3J = 8.6 Hz, ^4J = 2.4 Hz, 2H, Ar*H*-5), 6.64 (d, ^4J = 2.4 Hz, 2H, Ar*H*-3), 7.00 (d, ^3J = 8.6 Hz, 2H, Ar*H*-6), 9.55 (s, 2H, O*H*, exchangeable by D$_2$O), 10.55 (s, 2H, N*H*, exchangeable by D$_2$O). Anal. ($C_{17}H_{16}Cl_2N_2O_2$·HBr·H$_2$O) C, H, N.

(4*R*,5*S*)/(4*S*,5*R*)-4,5-Bis(2-fluoro-4-hydroxyphenyl)-2-ethyl-2-imidazoline (5g). 5g was obtained from **4g** (1.00 mmol, 382.8 mg). Recrystallization from methanol. Yield: 0.95 mmol (390.2 mg), 95% red-brown powder; mp 196 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−2500 br, s (OH), 1628 m, 1609 m, 1513 w, 1461 w, 1299 w, 1100 w. MS (EI, 210 °C): *m*/*z* (%) = 318 (28) [M$^{+\bullet}$], 179 (100), 124 (44), 96 (15). ^{1}H NMR (DMSO-d_6): δ 1.31 (t, ^3J = 7.6 Hz, 3H, CH$_2$C*H*$_3$), 2.71 (q, ^3J = 7.6 Hz, 2H, C*H*$_2$CH$_3$), 5.77 (s, 2H, ArC*H*), 6.33 (dd, ^{3}J (H,F) = 12.1 Hz, 2H, Ar*H*-3), 6.45 (dd, ^3J = 8.3 Hz, 2H, Ar*H*-5), 6.95 (dd, ^3J = 8.3 Hz, 2H, Ar*H*-6), 9.90 (s, 2H, O*H*, exchangeable by D$_2$O), 10.56 (s, 2H, N*H*, exchangeable by D$_2$O). Anal. ($C_{17}H_{16}F_2N_2O_2$·HBr·0.5H$_2$O) C, H, N.

(4*R*,5*S*)/(4*S*,5*R*)-4,5-Bis(4-hydroxyphenyl)-2-(2-hydroxyethyl)-2-imidazoline (5h). 5h was obtained from **4h** (0.90 mmol, 340.4 mg). Recrystallization from methanol/ether. Yield: 0.55 mmol (217.8 mg), 61% gray powder; mp 231 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−2500 br, s (OH), 1610 s, 1517 s, 1447 m, 1340 w, 1263 m, 1210 m, 1176 m, 1064 m. MS (EI, 380 °C): *m*/*z* (%) = 147 (20), 106 (14), 94 (100). ^{1}H NMR (DMSO-d_6): δ 2.84 (t, ^3J = 5.8 Hz, 2H, C*H*$_2$CH$_2$OH), 3.88 (t, ^3J = 5.8 Hz, 2H, CH$_2$C*H*$_2$OH), 5.31 (br, 1H, CH$_2$CH$_2$O*H*, exchangeable by D$_2$O), 5.56 (s, 2H, ArC*H*), 6.50 (*AA*′*BB*′, ^3J = 8.5 Hz, 4H, Ar*H*-3, Ar*H*-5), 6.81 (*AA*′*BB*′, ^3J = 8.5 Hz, 4H, Ar*H*-2, Ar*H*-6), 9.32 (s, 2H, O*H*, exchangeable by D$_2$O), 10.45 (s, 2H, N*H*, exchangeable by D$_2$O). Anal. ($C_{17}H_{18}N_2O_3$·HBr·H$_2$O) C, H, N.

(4*R*,5*S*)/(4*S*,5*R*)-4,5-Bis(2-chloro-4-hydroxyphenyl)-2-(2-hydroxyethyl)-2-imidazoline (5i). 5i was obtained from **4i** (1.00 mmol, 445.8 mg). Recrystallization from methanol/ether. Yield: 0.82 mmol (402.1 mg), 82% ochre powder; mp 232 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−2500 br, s (OH), 1603 s, 1578 m, 1500 s, 1434 m, 1332 w, 1289 m, 1253 m, 1216 m, 1059 m, 1040 m, 911 w, 862 m, 826 w. MS (EI, 170 °C): *m*/*z* (%) = 208 (17), 128 (100), 100 (11), 65 (95). ^{1}H NMR (DMSO-d_6): δ 2.85 (t, ^3J = 5.9 Hz, 2H, C*H*$_2$CH$_2$OH), 3.87 (t, ^3J = 5.9 Hz, 2H, CH$_2$C*H*$_2$OH), 5.02 (br, 1H, CH$_2$CH$_2$O*H*, exchangeable by D$_2$O), 5.93 (s, 2H, ArC*H*), 6.64 (dd, ^3J = 8.6 Hz, ^4J = 2.4 Hz, 2H, Ar*H*-5), 6.64 (d, ^4J = 2.4 Hz, 2H, Ar*H*-3), 7.01 (d, ^3J = 8.6 Hz, 2H, Ar*H*-6), 9.94 (s, 2H, O*H*, exchangeable by D$_2$O), 10.61 (s, 2H, N*H*, exchangeable by D$_2$O). Anal. ($C_{17}H_{16}Cl_2N_2O_3$·HBr·2H$_2$O) C, H, N.

(4*R*,5*S*)/(4*S*,5*R*)-*N*-Ethyl-4,5-bis(4-hydroxyphenyl)-2-imidazoline (7). 7 was obtained from **6** (0.26 mmol, 90 mg). Recrystallization from methanol/ether. Yield 0.24 mmol (104.0 mg), 94% brown powder; mp 206 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−2500 br, s (OH), 1640 s, 1612 m, 1518 s, 1443 m, 1368 m, 1338 m, 1268 m, 1224 m, 1172 m, 1107 m, 1042 w, 878 w, 809 m. MS (EI, 135 °C): *m*/*z* (%) = 282 (30) [M$^{+\bullet}$], 150 (100), 107 (22). ^{1}H NMR (DMSO-d_6): δ 1.17 (t, ^3J = 7.2 Hz, 3H, CH$_2$C*H*$_3$), 2.98−3.07 (m, 1H, C*H*$_2$CH$_3$), 3.47−3.56 (m, 1H, C*H*$_2$-CH$_3$), 5.55 (d, ^3J = 12.0 Hz, 1H, ArC*H*), 5.65 (d, ^3J = 12.0 Hz, 1H, ArC*H*), 6.53 (*AA*′*BB*′, ^3J = 8.2 Hz, 4H, Ar*H*-3, Ar*H*-5, Ar′*H*-3, Ar′*H*-5), 6.77−6.81 (*m*, 4H, Ar*H*-2, Ar*H*-6, Ar′*H*-2, Ar′*H*-6), 8.89 (s, 1H, N=C*H*−N), 9.33 (s, 1H, O*H*, exchangeable by D$_2$O), 9.41 (s, 1H, O*H*, exchangeable by D$_2$O), 10.63 (s, 1H, N*H*, exchangeable by D$_2$O). Anal. ($C_{17}H_{18}N_2O_2$·HBr·3H$_2$O) C, H, N.

(4*R*,5*S*)/(4*S*,5*R*)-*N*-Methyl-4,5-bis(2-chloro-4-hydroxyphenyl)-2-imidazoline (7a). 7a was obtained from **6a** (0.15 mmol, 60.2 mg). Recrystallization from methanol/ether. Yield: 0.05 mmol (21.9 mg), 32% brown powder; mp 292 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−2500 br, s (OH), 1657 s, 1607 s, 1577 m, 1501 s, 1437 m, 1290 m, 1265 m, 1226 m, 1044 m, 903 m. MS (EI, 180 °C): *m*/*z* (%) = 338 (19) [M$^{+\bullet}$], 184 (13), 170 (100). ^{1}H NMR (DMSO-d_6): δ 2.95 (s, 3H, C*H*$_3$), 5.88 (d, ^3J = 12.0 Hz, 1H, ArC*H*), 5.98 (d, 1H, ^3J = 12.0 Hz, ArC*H*), 6.54 (dd, ^3J = 8.6 Hz, ^4J = 2.3 Hz, 1H, Ar*H*-5), 6.60 (dd, ^3J = 8.5 Hz, ^4J = 2.3 Hz, 1H, Ar′*H*-5), 6.63 (d, ^4J = 2.3 Hz, 1H, Ar*H*-3), 6.68 (d, ^4J = 2.6 Hz, 1H, Ar′*H*-3), 6.86 (d, ^3J = 8.6 Hz, 1H, Ar*H*-6), 7.02 (d, ^3J = 8.5 Hz, 1H, Ar′*H*-6), 8.91 (s, 1H, N=C*H*−N), 9.95 (s, 1H, O*H*), 10.02 (s, 1H, O*H*), 10.89 (br, 1H, N*H*). Anal. ($C_{16}H_{14}Cl_2N_2O_2$·HBr·2H$_2$O) C, H, N.

(4*R*,5*S*)/(4*S*,5*R*)-*N*-Ethyl-4,5-bis(2-chloro-4-hydroxyphenyl)-2-imidazoline (7b). 7b was obtained from **6b** (0.15 mmol, 60.2 mg). Recrystallization from methanol/ether. Yield: 0.50 mmol (189.6 mg), 64% colorless powder; mp 262 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−2500 br, s (OH), 1643 s, 1608 s, 1500 s, 1437 m, 1261 m, 1218 m, 1044 m, 903 m, 861 w. MS (EI, 200 °C): *m*/*z* (%) = 350 (16) [M$^{+\bullet}$], 184 (100), 141 (13). ^{1}H NMR (DMSO-d_6): δ 1.16 (t, ^3J = 7.2 Hz, 3H, CH$_2$C*H*$_3$), 2.92−3.01 (m, 1H, C*H*$_2$CH$_3$), 3.47−3.57 (m, 1H, C*H*$_2$CH$_3$), 5.96−6.02 (br, 2H, ArC*H*), 6.51 (dd, ^3J = 8.7 Hz, ^4J = 2.5 Hz, 1H, Ar*H*-5), 6.61−6.63 (m, 2H, Ar*H*-3, Ar′*H*-5), 6.69 (d, ^4J = 2.4 Hz,

1H, Ar′*H*-3), 6.84 (d, ^3J = 8.7 Hz, 1H, Ar*H*-6), 7.07−7.09 (m, 1H, Ar′*H*-6), 8.95 (s, 1H, N=C*H*−N), 9.95 (s, 1H, O*H*, exchangeable by D_2O), 10.03 (s, 1H, O*H*, exchangeable by D_2O), 10.76 (br, 1H, N*H*, exchangeable by D_2O). Anal. ($C_{17}H_{16}Cl_2N_2O_2$·HBr) C, H, N.

(4*R*,5*S*)/(4*S*,5*R*)-*N*-Propyl-4,5-bis(2-chloro-4-hydroxyphenyl)-2-imidazoline (7c). 7c was obtained from **6c** (0.07 mmol, 30.0 mg). Recrystallization from methanol/ether. Yield: 0.06 mmol (26.8 mg), 82% brown powder; mp 245 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−2500 br, m (OH), 2962 m, 2925 m, 2853 m, 1602 m, 1497 m, 1457 m, 1440 m, 1360 w, 1261 s, 1212 m, 1180 m, 1096 m, 1038 m, 902 w, 857 w, 803 m, 690 w. MS (EI, 250 °C): *m*/*z* (%) = 364 (22) [$M^{+\bullet}$], 236 (37), 198 (99), 167 (13), 156 (17), 128 (100), 105 (14). ^{1}H NMR (DMSO-d_6): δ 0.87 (t, ^3J = 7.4 Hz, 3H, $CH_2CH_2CH_3$), 1.50−1.56 (m, 2H, $CH_2CH_2CH_3$), 2.86−2.93 (m, 1H, $CH_2CH_2CH_3$), 3.41−3.50 (m, 1H, $CH_2CH_2CH_3$), 5.97 (d, ^3J = 12.1 Hz, 1H, ArC*H*), 6.03 (d, ^3J = 12.1 Hz, 1H, ArC*H*), 6.50 (dd, ^3J = 8.6 Hz, ^4J = 2.4 Hz, 1H, Ar*H*-5), 6.62−6.64 (m, 2H, Ar*H*-3, Ar′*H*-5), 6.69 (d, ^4J = 2.4 Hz, 1H, Ar′*H*-3), 6.83 (d, ^3J = 8.6 Hz, 1H, Ar*H*-6), 7.09−7.11 (m, 1H, Ar′*H*-6), 8.97 (s, 1H, N=C*H*−N), 9.96 (s, 1H, O*H*, exchangeable by D_2O), 10.05 (s, 1H, O*H*, exchangeable by D_2O), 10.81 (br, 1H, N*H*, exchangeable by D_2O). Anal. ($C_{18}H_{18}Cl_2N_2O_2$·HBr) C, H, N.

(4*R*,5*S*)/(4*S*,5*R*)-*N*,*N*′-Dimethyl-4,5-bis(2-chloro-4-hydroxyphenyl)-2-imidazolinium bromide (9a). 9a was obtained from **8a** (0.24 mmol, 100 mg). Yield: 0.22 mmol (96 mg), 85% yellow powder; mp 184 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3500−3000 br, m (OH), 2934 m, 1662 s, 1608 s, 1577 m, 1500 s, 1437 m, 1291 s, 1261 s, 1222 m, 1044 m, 902 m, 861 m. MS (EI): *m*/*z* (%) = 133 (23), 111 (26), 97 (48), 83 (50), 74 (93), 59 (100), 43 (52). ^{1}H NMR (DMSO-d_6): δ 2.97 (s, 6H, CH_3), 5.92 (s, 2H, ArC*H*), 6.57 (dd, ^3J = 8.6 Hz, ^4J = 2.4 Hz, 2H, Ar*H*-5), 6.67 (d, ^4J = 2.4 Hz, 2H, Ar*H*-3), 6.89 (d, ^3J = 8.6 Hz, 2H, Ar*H*-6), 8.89 (s, 1H, N=C*H*−N), 10.02 (s, 2H, O*H*, exchangeable by D_2O). Anal. ($C_{17}H_{17}BrCl_2N_2O_2$·H_2O) C, H, N.

(4*R*,5*S*)/(4*S*,5*R*)-*N*,*N*′-Diethyl-4,5-bis(2-chloro-4-hydroxyphenyl)-2-imidazolinium bromide (9b). 9b was obtained from **8b** (0.23 mmol, 100.0 mg). Recrystallization from methanol/ether. Yield: 0.20 mmol (90.7 mg), 88% yellow powder; mp >300 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−2500 br, m (OH), 1649 s, 1608 s, 1499 m, 1438 w, 1259 m, 1221 m, 1044 w, 901 w. MS (EI, 50 °C): *m*/*z* (%) = 350 (21), 251 (12), 222 (22), 184 (100), 141 (17), 128 (24), 105 (10). ^{1}H NMR (DMSO-d_6): δ 1.18 (t, ^3J = 7.2 Hz, 6H, CH_2CH_3), 3.02−3.10 (m, 2H, CH_2CH_3), 3.48−3.57 (m, 2H, CH_2CH_3), 6.01 (s, 2H, ArC*H*), 6.57 (dd, ^3J = 8.6 Hz, ^4J = 2.4 Hz, 2H, Ar*H*-5), 6.68 (d, ^4J = 2.4 Hz, 2H, Ar*H*-3), 6.94 (d, ^3J = 8.6 Hz, 2H, Ar*H*-6), 8.97 (s, 1H, N=C*H*−N), 10.07 (s, 2H, O*H*, exchangeable by D_2O). Anal. ($C_{19}H_{21}BrCl_2N_2O_2$·H_2O) C, H, N.

(4*R*,5*S*)/(4*S*,5*R*)-*N*,*N*′-Dipropyl-4,5-bis(2-chloro-4-hydroxyphenyl)-2-imidazolinium Bromide (9c). 9c was obtained from **8c** (0.23 mmol, 100 mg). Yield: 0.16 mmol (78 mg), 69% yellow powder; mp >300 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−3000 br, m (OH), 1643 s, 1609 s, 1499 m, 1436 m, 1264 m, 1217 m, 1044 m, 904 m, 861 m. ^{1}H NMR (DMSO-d_6): δ 0.89 (t, ^3J = 7.3 Hz, 6H, $CH_2CH_2CH_3$), 1.56 (m, 4H, $CH_2CH_2CH_3$), 3.02 (m, 2H, $CH_2CH_2CH_3$), 3.41 (m, 2H, $CH_2CH_2CH_3$), 6.04 (s, 2H, ArC*H*), 6.56 (dd, ^3J = 8.5 Hz, ^4J = 2.3 Hz, 2H, Ar*H*-5), 6.66 (d, ^4J = 2.3 Hz, 2H, Ar*H*-3), 6.94 (d, ^3J = 8.5 Hz, 2H, Ar*H*-6), 9.02 (s, 1H, N=C*H*−N), 10.02 (s, 2H, O*H*, exchangeable by D_2O) Anal. ($C_{21}H_{25}BrCl_2N_2O_2$·H_2O) C, H, N.

General Procedure for the Synthesis of the (1*R*,2*S*)/(1*S*,2*R*)-1-Amino-2-formamido-1,2-bis(2-chloro-4-hydroxyphenyl)ethane (11). An amount of 0.50 mmol (202.0 mg) of **2b** was dissolved in 100 mL of 0.01 N NaOH and stirred for 12 h at room temperature. The solution was extracted three times with ethyl acetate. The combined organic layers were then dried over Na_2SO_4. After the evaporation of the solvent, the crude product was purified by recrystallization from CH_2Cl_2. Yield: 0.38 mmol (128.1 mg), 75% yellow powder; mp 75 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 3600−2500 br, m (OH), 1668 m (C=O), 1606 s, 1541 m (NH), 1499 s, 1445 m, 1385 m, 1260 m, 1238 m, 1095 w, 1040 m, 904 m, 856 m, 815 m, 691 w. MS (EI, 80 °C): *m*/*z* (%) = 322 (11), 194 (51), 167 (12), 156 (60), 140 (10), 128 (100), 105 (10), 65 (29). ^{1}H NMR (DMSO-d_6): δ 1.61−1.96 (br, 2H, NH_2, exchangeable by D_2O), 4.43 (d, ^3J = 5.6 Hz, 1H, ArC*H*NH_2), 5.54 (dd, ^3J = 5.6 Hz, ^3J = 9.0 Hz, 1H, ArC*H*NHCHO), 6.56 (dd, ^3J = 8.6 Hz, ^4J = 2.4 Hz, 1H, Ar′*H*-5), 6.61 (d, ^4J = 2.4 Hz, 1H, Ar′*H*-3), 6.66−6.75 (m, 2H, Ar*H*-3, Ar*H*-5), 6.87 (d, ^3J = 8.6 Hz, 1H, Ar′*H*-6), 7.20 (d, ^3J = 8.6 Hz, 1H, Ar*H*-6), 7.94 (s, 1H, C*H*O), 8.43 (d, ^3J = 9.0 Hz, 1H, N*H*CHO, exchangeable by D_2O), 9.66 (br, 1H, O*H*, exchangeable by D_2O), 9.74 (br, 1H, O*H*, exchangeable by D_2O). Anal. ($C_{15}H_{14}Cl_2N_2O_3$) C, H, N.

General Procedure for the Synthesis of Iminoethers. An amount of 100 mmol of the appropriate nitrile was dissolved in a mixture of 110 mmol of dry ethanol and 100 mL of dry ether and cooled with an ice bath. Dry HCl was added until a precipitate formed. The flask was sealed and stored in a refrigerator for 24 h. The precipitate was collected by suction filtration and dried over P_2O_5.

Acetimidic Acid Ethyl Ester (13a). 13a was obtained from acetonitrile (50.0 mmol, 2.05 g, 2.59 mL). Recrystallization from dry ether. Yield: 42.0 mmol (5.63 g), 84% white powder; mp 112 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2980 br (NH_2), 1710 s, 1640 s, 1600 s, 1480 s, 1455 s, 1395 s, 1345 s, 1145 s, 1015 s, 835 s. ^{1}H NMR (DMSO-d_6): δ 1.35 (t, ^3J = 7.0 Hz, 3H, OCH_2CH_3), 2.36 (s, 3H, CH_3), 4.40 (q, ^3J = 7.0 Hz, 2H, OCH_2CH_3), 10.86 (br, 1H, N*H*, exchangeable by D_2O), 11.75 (br, 1H, N*H*, exchangeable by D_2O).

3-Methoxypropionimidic Acid Ethyl Ester (13b). 13b was obtained from 3-methoxypropionitrile (50.0 mmol, 4.26 g, 4.53 mL). Recrystallization from dry ether. Yield: 38.0 mmol (6.37 g), 76% white powder; mp 95 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2890 br (NH_2), 1640 s, 1600 s, 1440 s, 1390 s, 1130 s, 1090 m, 995 m. ^{1}H NMR (DMSO-d_6): δ 1.18 (t, ^3J = 7.1 Hz, 3H, OCH_2CH_3), 2.50 (q, ^3J = 6.1 Hz, 2H, OCH_2CH_2), 3.22 (s, 3H, OCH_3), 3.53 (q, ^3J = 6.1 Hz, 2H, OCH_2CH_2), 4.05 (q, ^3J = 7.1 Hz, 2H, OCH_2CH_3), 11.26 (br, 1H, N*H*, exchangeable by D_2O), 12.03 (br, 1H, N*H*, exchangeable by D_2O).

Stability Studies. The mobile phase was methanol/H_2SO_4 (0.001 N, 20 mM Na_2SO_4) in the range of 50/50 to 30/70. The probes were prepared as aqueous solutions (1.0 mM in MilliQ water). Prior to analysis, they were diluted with PBS to 0.1 mM, placed in the autosampler (tempered to 37 °C) and immediately injected. Stability was monitored for 55 h. Detection of the eluted probes was made with UV at 254 nm.

Biological Methods. 1. Biochemicals, Chemicals, and Materials. The following is a list of materials and the companies from which they were purchased: dextran, 17β-estradiol, L-glutamine (L-glutamine solution, 29.2 mg/mL PBS), and minimum essential medium Eagle (EMEM) from Sigma (Munich, Germany); Dulbecco's modified Eagle medium without phenol red (DMEM) from Gibco (Eggenstein, Germany); fetal calf serum (FCS) from Bio whittaker (Verviers, Belgium); gentamycin sulfate from Fluka (Deisenhofen, Germany); trypsin (0.05%) in ethylenediaminetetraacetic acid (0.02%) (trypsin/EDTA) from Boehringer (Mannheim, Germany); penicillin−streptomycin gold standard (10 000 IE penicillin/mL, 10 mg of streptomycin/mL) and geneticin disulfate (geneticin solution, 35.71 mg/mL PBS) from ICN Biomedicals GmbH (Eschwege, Germany); norit A (charcoal) from Serva (Heidelberg, Germany); cell culture lysis reagent (5×) (diluted 1:5 with purified water before use) and luciferase assay reagent from Promega (Heidelberg, Germany); Optiphase HiSafe3 scintillation liquid from Wallac (Turku, Finland); NET-317-estradiol[2,4,6,7-^{3}H-(N)] (17β-[^{3}H]estradiol) from Du Pont NEN (Boston, Maryland).

Phosphate buffered saline (PBS) was prepared by dissolving 8.0 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4·$2H_2O$, and 0.2 g of KH_2PO_4 (all purchased from Merck or Fluka) in 1000 mL of purified water. Tris-buffer (pH 7.5) was prepared by dissolving 1.211 g of trishydroxymethylaminomethane, 0.3722 g of Titriplex III, and 0.195 g of sodium azide (all from Merck or Fluka) in 1000 mL of purified water. Deionized water was produced by means of a Millipore Milli-Q water system,

resistivity of >18 MΩ. T-75 flasks, reaction tubes, and six-well plates were purchased from Renner GmbH (Dannstadt, Germany).

2. Transcriptional Binding Assay. Luciferase Assay. The pertinent in vitro assay was described earlier by Hafner et al.[17] One week before starting the experiment, MCF-7-2a cells were cultivated in DMEM supplemented with L-glutamine, antibiotics, and dextran/charcoal-treated FCS (ct-FCS, 50 mL/L). Cells from an almost confluent monolayer were removed by trypsinization and suspended to approximately 2.2×10^5 cells/mL in the growth medium mentioned above. The cell suspension was then cultivated in six-well flat-bottomed plates (0.5 mL of cell suspension and 2 mL of medium per well) under growing conditions (see above). After 24 h, an amount of 25 μL of a stock solution of the test compounds was added to achieve concentrations ranging from 10^{-5} to 10^{-10} M and the plates were incubated for 50 h. Before harvesting, the cells were washed twice with PBS, and then an amount of 200 μL of cell culture lysis reagent was added into each well. After 20 min of lysis at room temperature, cells were transferred into reaction tubes and centrifuged. Luciferase was assayed using the Promega luciferase assay reagent. An amount of 50 μL of each supernatant was mixed with 50 μL of substrate reagent. Luminescence (in relative light units, RLU) was measured for 10 s using a microlumat. Measurements were corrected by correlating the quantity of protein (quantified according to Bradford[26]) of each sample with the mass of luciferase. Estrogenic activity was expressed as percent activation of a 10^{-8} M estradiol control (100%).

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